MOHS SURGERY AND HISTOPATHOLOGY: BEYOND THE FUNDAMENTALS

MOHS SURGERY is a highly effective technique for the surgical removal of most types of cutaneous and oral pharyngeal cancers. The procedure allows for the precise and complete removal of cancers while maximizing the preservation of surrounding normal tissue. Through the presentation and orientation of the specimens' complete surgical margin on pathology slides, the location of tumor foci and other relevant findings can be correlated with their locations on the surgical wound. The ability to create perfect slides for histological examination lies at the core of effective Mohs surgery. This procedure has the highest cure rate among alternative cancer treatment modalities for the cancers for which it is utilized. This book describes the methods the Mohs surgeon-pathologist and Mohs technician use to optimize the Mohs technique and produce the highest-quality slides and highest cure rates possible, and it breaks new ground in describing techniques that the Mohs technician uses in the lab.

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I hope this book will eventually find its way to the bookshelf equivalent of the "dustbin of history," as targeted immunotherapy and other evolving cancer treatments replace the surgical model employed today. Even a procedure as elegant as Mohs surgery will find its rightful place alongside other outdated surgical techniques. I hope the transformation happens in my lifetime.

To Ruth Gross and Edith Chepin: two peas in a pod enjoying their tenth decade of life.

KGG

To Barry Goldsmith, for patiently and thoughtfully teaching me Mohs surgery.

To the many Mohs surgery course participants for showing me how to better practice and teach our craft.

And most assuredly to Robert and Madeline, now gone, and Diedre and our sons, Adam and Steven, sine qua nons, for their boundless love, encouragement, support, and humor, which gives foundation, perspective, joy, and contentment to my life.

HKS

Our dear friend and colleague, Terry O'Grady, who died during the preparation of this book, will be greatly missed.

KGG and HKS

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MOHS SURGERY AND HISTOPATHOLOGY

PART ONE

MICROSCOPY AND TISSUE PREPARATION

CHAPTER 1

Introduction

Ken Gross and Howard K. Steinman

MOHS SURGERY will remain the gold standard for the treatment of skin cancer until immunotherapy or other nondestructive modalities replace current surgical treatments. It allows skin cancer removal with higher cure rates and greater sparing of normal tissues than other excisional techniques. Mohs surgery accomplishes this in an office setting and at reasonable cost when practiced in an optimal fashion.

There are some common misconceptions about Mohs surgery that may stand in the way of optimizing the technique and that may unnecessarily increase its cost.

MISCONCEPTION 1

Mohs surgery is first and foremost about tissue sparing. It is not; Mohs surgery's first goal is complete cancer removal. A focus on tissue sparing leads some Mohs surgeons to excise specimens with very narrow surgical margins even from areas where taking wider margins would not compromise function or closure. There are clearly many situations where excising wider margins would allow fewer stages of surgery, lower surgical costs, and would not substantively change the type of closure or lead to any cosmetic or functional degradation. In addition, if tissue sparing were the main goal of treatment, other modalities such as cryotherapy, radiation, and in selected cancers, presently available immunotherapy such as interferon alfa-2b and imiquimod would spare tissue to a greater extent while compromising cure rates by less than 5–10%.

MISCONCEPTION 2

Specimens need to be divided into many subsections (blocks) to allow optimal processing. This leads to relatively small specimens being divided into more than five tissue blocks for processing, which increases the cost and complexity of the procedure and the potential for processing and interpretation errors. It is more beneficial to process the largest blocks your technician and equipment allow and produce more high-quality wafers from each block, thus allowing easier orientation and interpretation at lower cost.

MISCONCEPTION 3

Because Mohs surgery presumably allows for precise examination of approximately 100% of the tissue margins and precise localization of tumor foci, only minimal overlap of areas adjacent to positive findings needs to be excised. While Mohs surgery has more built-in precision than other cancer surgical modalities, there is plenty of room for small errors, which can be additive. For this reason, a wider margin around positive foci is sometimes rewarding to the surgeon and beneficial to the patient.

MISCONCEPTION 4

"Good enough is good enough." The strength of Mohs surgery is that it examines approximately 100% of the true surgical margin. If complete base and epithelium (when available) are not represented on the slides, then 100% margin assessment has not been done and more sectioning through the block or obtaining more tissue from the patient is necessary. This is also an argument against doing vigorous curettement prior to the first stage of Mohs surgery: it may disrupt the peripheral epidermis, leading to incomplete peripheral margin on the slides.

MISCONCEPTION 5

Mohs surgery is difficult to perform and requires extensive training. Mohs surgery differs from other types of skin cancer excisional surgery only in a few aspects:

1. It is highly organized and system dependent, requiring excision of tissue to allow optimal processing of the complete, contiguous surgical margin and a highly skilled technician to prepare quality slides.

2. It requires accurate pathologic interpretation of horizontally cut tissue (see Chapter 11) in contrast to vertically oriented tissue normally reviewed on pathologic and dermatopathologic slides.
3. It requires the surgeon to assess the pathologic slides as well as perform the surgery.

The greatly advanced surgical skill set of many dermatologists, improved surgical training opportunities, modern surgical instruments, better local anesthetics, improved agents and devices for hemostasis, and better patient monitoring and resuscitation equipment make the surgeon's job easier and faster. Modern cryostats, chromacoding inks and stains, improved automated tissue processing, and better microscopes have all combined to make slide preparation faster, quality better, and interpretation easier than ever before.

It is therefore reasonable to assume that a higher degree of accuracy is possible in utilizing the Mohs technique today than has been in the past. In assessing the relative value of more surgical training versus more pathology training for a dermatologist (or any other physician) wishing to perform Mohs surgery, an additional year of pathology study might be of more value than an additional year of surgical training – although this is viewed with reservations, as one of the justifications for a Mohs fellowship is to allow the pathologic and clinical review of hundreds of Mohs cases. In either case, additional training is of value. We would argue that dermatologic residency training usually does, and always should, provide the training in skin surgery and skin pathology that allows the performance of a basic level of Mohs surgery within a dermatologic practice.

Mohs surgery is a cancer removal modality and defect repair may be done by the Mohs surgeon or another surgeon, and wounds may be allowed to heal by second intention.

MISCONCEPTION 6

Mohs surgery is like a religion with inviolable precepts dictating who may perform it and how it must be performed.* The only absolute precept is that, as closely as possible, 100% of the true surgical margin must be accurately assessed to ensure complete removal of the cancer. There is no specific way in which the technique must be performed. Mohs surgery obviously cannot deal with discontiguous tumors, cancer cells that have already left the surgical field, or occult satellite and/or in-transit metastasis. Whichever reliable and reproducible method the Mohs surgeon selects to accomplish the goal of 100% margin assessment and complete cancer removal should be accepted, politics aside. Although sparing normal tissue is an important virtue of Mohs surgery, the one true goal of Mohs surgery is curing the cancer. It is the ability of properly performed Mohs surgery to achieve a high cure rate that allows it to be the premier technique for the removal of skin cancer. How exactly to optimize Mohs surgery techniques that allow the Mohs surgeon to reproducibly and consistently produce these high cure rates is just detail. These details are what this book is about. We assume that the reader of these pages has a basic understanding of Mohs surgery or has read *Mohs Surgery: Fundamentals and Techniques.*[1]

REFERENCE

1. Gross KG, Steinman HK, Rapini RP. *Mohs Surgery: Fundamentals and Techniques*. St. Louis, Mo: CV Mosby Co; 1998. (The book may be ordered from ASMS (800) 616-ASMS.)

* The requirement that the Mohs surgeon must also be the Mohs pathologist is arbitrarily based only on CPT regulations. No other cancer surgery excludes the participation of pathologists or dermatopathologists from helping interpret pathology specimens.

CHAPTER 2

How to Excise Tissue for Optimal Sectioning

Ken Gross

THE GOAL OF MOHS surgery is to cure skin cancer. Optimization of Mohs surgery ensures that the high cure rates available with this technique are achieved in practice. Production of the highest-quality Mohs slides makes possible the most accurate interpretation of the surgical margins represented on those slides. The Mohs surgeon, by optimizing tissue excision at the operative table, allows the Mohs technician to produce high-quality slides that present complete surgical margins of all excised tissue.

A masterful Mohs technician may be able to salvage tissue excised with poor surgical technique, and a poor technician can make garbage from an exquisite surgical specimen. In this chapter we focus on issues of surgical technique that will help the competent Mohs technician prepare better slides and allow faster and more cost-effective Mohs surgery. Optimizing surgical technique allows for the most favorable slide preparation. The Mohs surgeon, when switching hats and becoming the Mohs pathologist, will then have the best chance of making accurate surgical margin assessments.

HOW TO EXCISE TISSUE FOR OPTIMAL SECTIONING

Even before making the first incision, the Mohs surgeon can increase the chance for complete cancer removal in as few stages as possible. The clinical margins of the tumor should be assessed with bright light and magnification. Use of an episcope and Wood's light may help define the margins of some cancers, especially pigmented lesions. Reassess the clinical margins after injection of anesthesia, as tumor margins may become more distinct after injection. Small ink dots can be drawn on the skin around the cancer to define the clinical cancer margins. Three decisions must then be made:

1. Should curettage be done to help further define the margins?
2. Should the cancer be debulked?
3. How much surgical margin past the clinical tumor should be removed?

Curettage prior to stage I cancer removal may help define the tumor margin and also debulks the cancer, but the downside of curettage is possible removal of some of the epithelial edge, especially in older patients with fragile skin. This makes margin assessment more difficult and may require a wider surgical margin around the disrupted epithelium.

Debulking is primarily done in two situations:

1. When there is a large bulky exophytic tumor, debulking the tumor makes tissue processing easier. As this is done sharply and should not extend to the specimen margins, there should be no loss of epithelial edges. If the Mohs surgeon debulks using a surgical scalpel, the blade must be wiped thoroughly free of tumor fragments, or changed, before excising stage I tissue. The Mohs technician may also debulk tissue in the laboratory, also thoroughly wiping the blade or using a separate blade before processing the specimen, to prevent artifactual tumor "floaters" from appearing on the slides.
2. To produce a vertically oriented slide of the tumor pathology when a previous biopsy is unavailable or has not been done. Having the tumor pathology available is very helpful for accurate slide interpretation.

Since Mohs surgery is most often performed following a diagnostic biopsy, there is frequently a "scab" on the cancer site. In large or deep cancers this scab is of no consequence, but in small and relatively shallow tumors it will interfere with the production of high-quality slides. It should be removed by the surgeon or technician prior to processing the tissue.

The issue of how much margin to take past clinically evident tumor is influenced by several factors:

1. Will removal of margin past the clinically clear margin cause a functional defect?

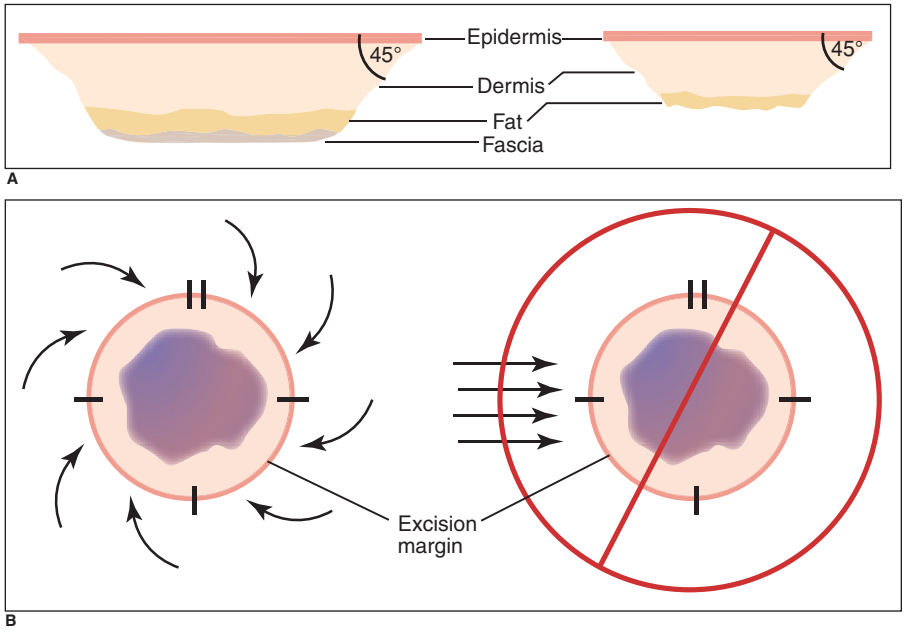


FIGURE 2.1: ***(A) The bevel is continued to the level at which the horizontal base of the specimen is to be cut, or to the level at which closure will be done, if that is deeper. On the arm, the Mohs surgeon would carry the bevel to the level of the fascia, as undermining and repair will usually be done at that level*** **(left)**. ***On most areas of the face, the bevel would be cut to superficial fat*** **(right)**, ***unless it is clinically obvious that the tumor is deeper, because that is the level at which undermining and closure will be done. (B) The specimen should be undermined from all edges toward the center and not from one edge through to the other side. Curved arrows*** **(left)** ***indicate that the Mohs surgeon is correctly cutting the specimen from all sides toward the center. Straight arrows*** **(right)** ***indicate that the Mohs surgeon is incorrectly cutting the specimen from the 9 o'clock side straight through to the other side. This is likely to lead to an unevenly cut specimen base and an irregularly beveled peripheral margin.***

2. Will removal of additional peripheral margin increase the difficulty or morbidity of the closure?
3. Will removal of additional deep tissue margin compromise the function of a motor nerve or other important underlying structure?

If a smaller margin is taken around the tumor for any of these reasons, the cure rate is not compromised because any positive margin will be removed in a subsequent stage.

The primary purpose of Mohs surgery is to achieve a high cancer cure rate. When necessary, it also has the ability to spare tissue. But this ability is subject to abuse. A small but poorly clinically demarcated sclerosing basal cell in the mid-to-lateral cheek can be removed to below mid-fat with little risk of damage to underlying structures; and a peripheral surgical margin of 5 mm or more, as opposed to 1–2 mm, will be unlikely to cause closure or cosmetic problems. This would not be true for the excision of the same tumor on the lip or eyelid. Here, the ability of Mohs surgery to spare tissue shares equal importance with its ability to achieve a high cure rate.

The Mohs technique usually requires that the edges of the specimen(s), which in stage I are epidermal or mucosal, be flattened into the same plane as the base during processing (see Chapter 6 through 8). This allows the entire deep and peripheral margins to be represented contiguously within the tissue wafers. To allow the technician to more easily flatten the tissue into a single plane for sectioning, the Mohs surgeon generally excises specimens at an approximately 45-degree angle (bevel). In the excision of large specimens, this angled cut continues only to the deepest plane of excision. At the deepest plane, the rest of the excision is horizontal (Figure 2.1A). To ensure as uniform a bevel and as flat a base as possible, the specimen should be cut from all sides toward the center and not from one edge continuously through to the other side (Figure 2.1B).

Although a 45-degree bevel is often stated to be ideal, many thin tissue areas such as the eyelid, genitalia, neck, and mucosa require little or no beveling. Thick, stiffer tissue areas, such as the back, may require more of a bevel (an angle of 30 to 40 degrees). As excisions progress deeper, the scalpel traverses first the epithelium and dermis, then fat, fascia, muscle, and periosteum. These tissues have differing abilities to flatten during tissue processing; thus, the amount of bevel needed to produce optimal slides will also change. Specimens of smaller diameter may be more difficult for the technician to flatten than larger specimens

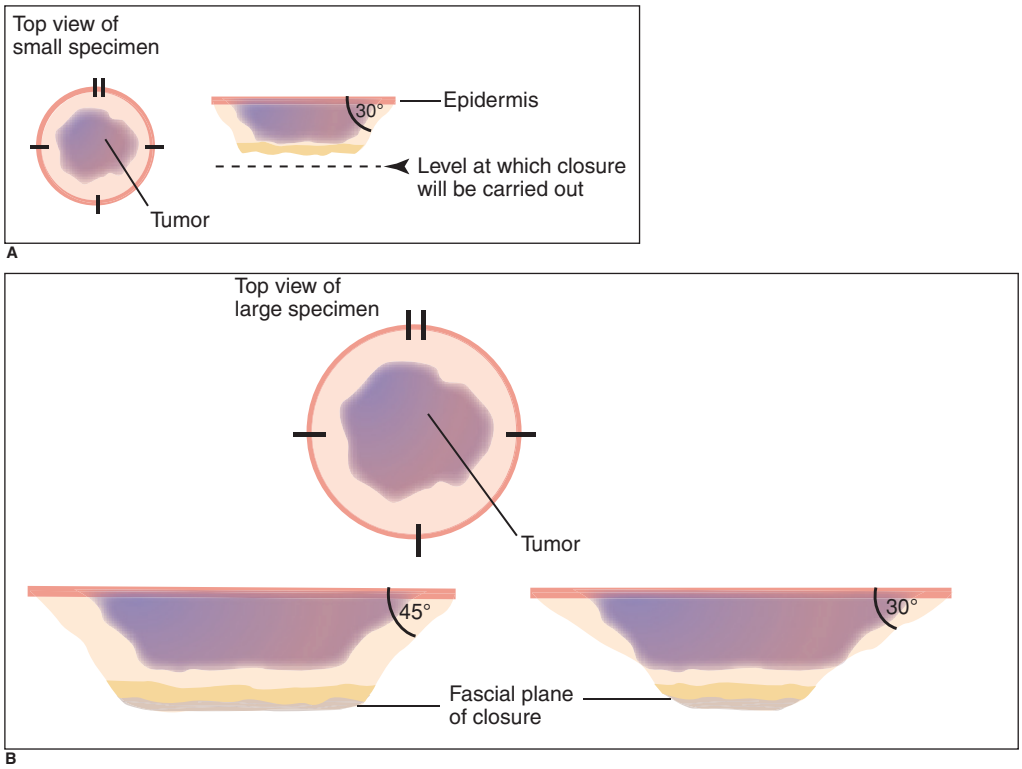


FIGURE 2.2: ***(A) Because the specimen is small, a beveling angle of 30 degrees instead of 45 degrees may be necessary to allow the technician to flatten the edges and base of the specimen into a single plane for processing, and the excision may be carried down only to a level above the level at which the defect would be undermined for closure. (B) The excision of a larger-diameter cancer may allow a steeper bevel that can be extended all the way down to the plane of eventual closure. If the cancer is obviously deeper than this plane, the excision may be carried even deeper. "Standard" surgical technique of cancer uses vertically cut specimens. Mohs surgery uses cuts made at a bevel; this bevel allows the technician to flatten the edges of the specimen into the same plane as the base so that the entire peripheral and deep margin can be completely assessed pathologically. The usual bevel is 45 degrees from the standard vertical cut and yields a specimen whose sides are cut at 45 degrees from the horizontal surface of the specimen*** **(left)**. ***A larger bevel would produce a specimen whose sides are cut at a smaller number of degrees from the flat surface of the specimen*** **(right)**. ***The 30-degree bevel makes it easier for the Mohs technician to flatten the specimen into a single plane for processing but also increases the chances that the bevel cut will transect cancer.***

and may require more of a bevel; either a wider surgical margin is required or they may not be able to be excised as deeply (Figure 2.2). This is a small disadvantage of Mohs, as opposed to standard excision technique.

When possible, the first stage in Mohs surgery should be cut to the depth of eventual closure (Figure 2.1A). This is limited by the surface dimensions of the excised specimen. A small-diameter specimen cut at a bevel of 45 degrees may not be able to be adequately flattened into a single plane by the technician and would likely reach a depth less than the probable final plane of wound closure before the base of the specimen is completely cut. To initially cut a large specimen above the depth that will be utilized for closure increases the chance of leaving cancer at the deep margin, increases surgical time and cost, and does not spare tissue, as undermining and closure in the proper plane will likely require removal of this deep tissue that was "spared" during the Mohs procedure. Thus, for example: scalp excisions should be carried to subgalea, and extremity excisions to muscle fascia.

In some situations, a sufficient bevel to allow optimal processing of the specimen may not be achievable. This is frequently true of deep but narrow alar crease tumors. If specimens require deep tissue removal, but the specimen is too narrow to allow an adequate bevel, the specimen can be taken with little or no bevel and instead prepped by the technician using the "open book" technique (Figures 2.3 and 2.4A–C).

This technique may also be used when re-excising a surgical scar after permanent section pathology has shown a positive margin. The entire scar needs to be removed to a deeper plane than the previous surgery and the peripheral margin needs to be cut as widely as the area was previously undermined. The open book technique

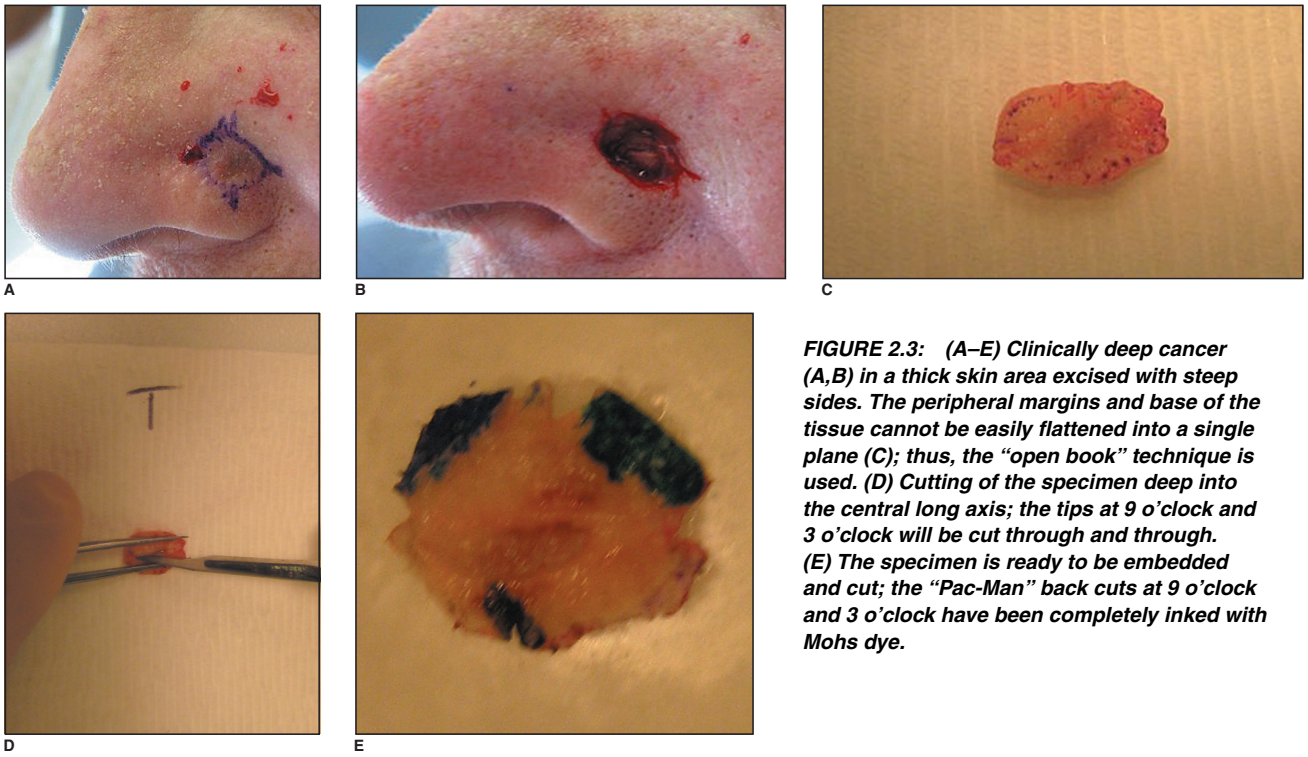


FIGURE 2.3: *(A–E) Clinically deep cancer (A,B) in a thick skin area excised with steep sides. The peripheral margins and base of the tissue cannot be easily flattened into a single plane (C); thus, the "open book" technique is used. (D) Cutting of the specimen deep into the central long axis; the tips at 9 o'clock and 3 o'clock will be cut through and through. (E) The specimen is ready to be embedded and cut; the "Pac-Man" back cuts at 9 o'clock and 3 o'clock have been completely inked with Mohs dye.*

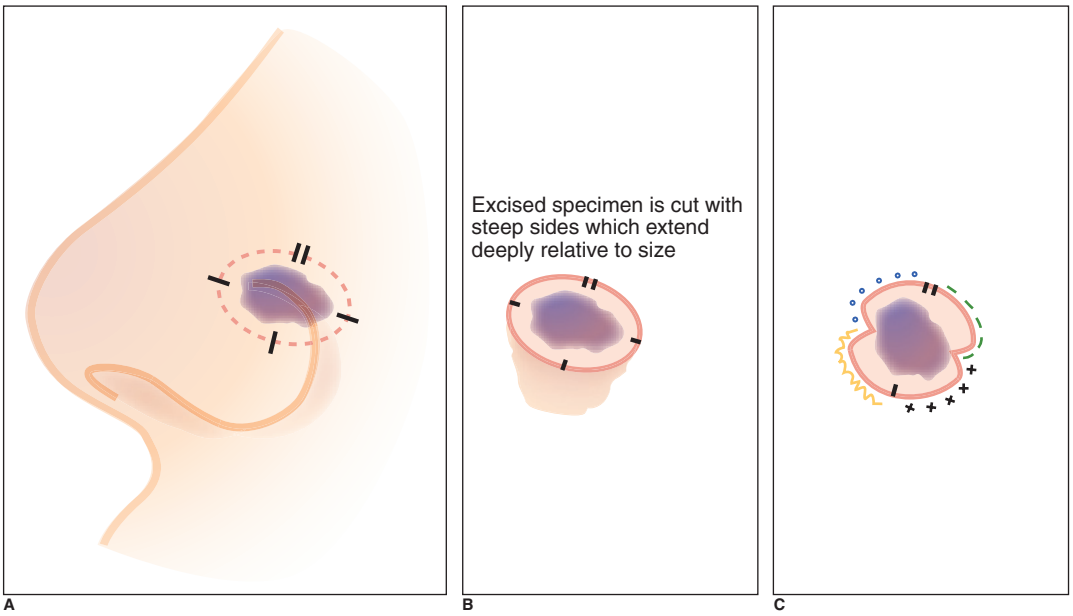


FIGURE 2.4: *Diagrammatic illustration of the same type of excision as in Figure 2.3. The inking diagram shows complete inking of the ends of the specimen, opened to allow flattening using the open book technique. (A) Diagrammatic illustration for a cancer similar in type and location as in Figure 2.3. Excision for this cancer cannot be easily done with standard beveling technique because, although clinically small, the cancer is deep and located in a thick skin area. (B) The excised tissue has very steep edges and cannot be flattened into a single plane using standard tissue relaxing incisions. Standard relaxing incisions would not be enough to get the edges and base to lie in a single plane for sectioning. (C) "Pac-Man" cuts along the long axis of the specimen (at 3 o'clock to 9 o'clock in this example) allow the specimen to "open like a book" and all the edges to be flattened into a single plane for processing. The tips are cut all the way through to further allow the specimen to lay flat. Tumor extending to the edges of the Pac-Man cuts is not at a margin because this is an artificial edge produced by the technician. The true margins include the entire base and the peripheral epithelial edges. The entire cut tips must be chromacoded so that the Mohs surgeon-pathologist has a way to ensure that the tissue at these cut tips is completely represented.*

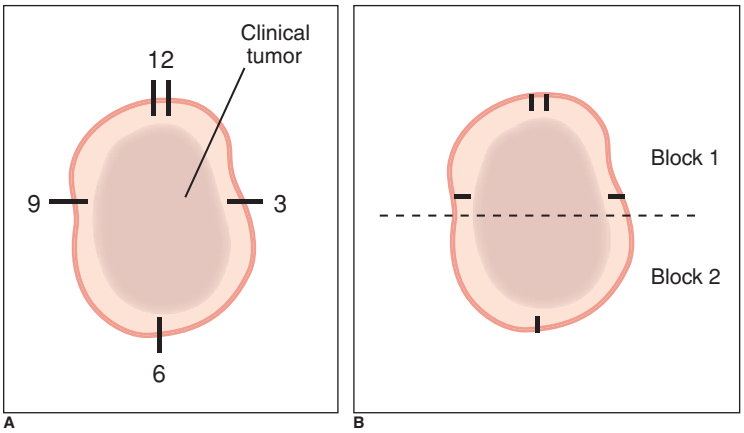


FIGURE 2.5: ***(A) A large specimen is cut. The Mohs surgeon should have but did not place the 3 o'clock and 9 o'clock reference nicks at the midpoint of the long axis of the specimen. (B) The technician has subdivided the specimen into two equally sized blocks but not at the 3 o'clock and 9 o'clock marks cut by the surgeon. This makes it harder for the Mohs surgeon-pathologist interpreting the findings on the slides to transfer them to the proper location within the excision defect on the patient because the 3 o'clock and 9 o'clock reference nicks on the patient do not correspond to where the technician subdivided the specimen into two blocks.***

works well for preparation of first-stage excisions of these specimens.

The open book technique requires special care on the part of the technician to ensure that in making cuts through the specimen, tumor is not inadvertently carried by the blade into the margins. Both ends of the specimen must also be completely inked so that the Mohs surgeon-pathologist is certain that complete surgical margins are represented on the slides.

The surgeon should choose distances between reference nicks (hatch marks) that attempt to correspond to the size of the blocks the technician is able to process in a microtome (Figure 2.5); this allows easier translation of the exact location of the tumor at a margin from the microscope to the patient. The technician should attempt to subdivide the surgical specimen at the hatch marks placed in the tissue by the surgeon, even if this results in not dividing the specimen into blocks of equal dimensions (Figures 2.5 and 2.6).

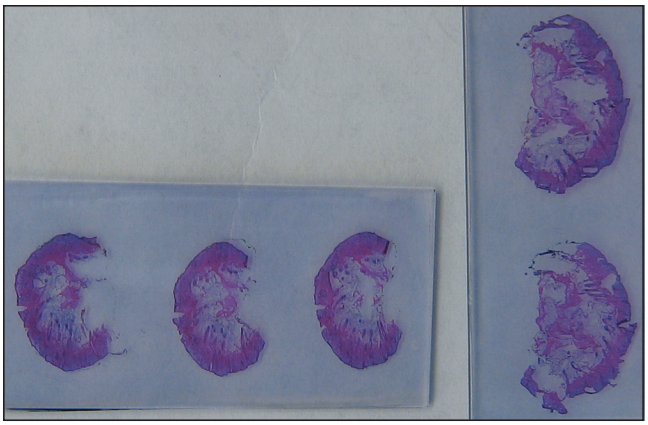


FIGURE 2.6: ***This photo illustrates how the technician actually processed the two blocks. The blocks are not of equal size; but were subdivided by the technician at the reference nicks placed on the specimen and on the patient by the surgeon. This will make it easier for the Mohs surgeon-pathologist to translate findings from the slides to the patient's wound.***

When dividing the specimen into multiple blocks, the technician must ensure that tumor is not artifactually carried to a margin where it could be misread as a positive margin. This probability may be reduced by debulking obvious tumor from the top of the specimen, by cutting from the specimen edges toward the center, and by wiping the blade after each cut (see Chapters 6, 7, and 8). Likewise, the surgeon should wipe the scalpel blade frequently when making excisions to preclude carrying cancer from a clinically occult area of the tumor to a tumor-free area. In Mohs surgery, the surgeon only knows in retrospect that the area being cut is cancer-free.

The Mohs surgeon must determine where to designate "12 o'clock" on the excised specimen. How each surgeon does this is not as important as consistency in a chosen method, so that even if a reference nick is not seen at the edges of the wound when the patient is brought back for additional cancer excision stages, the Mohs surgeon still has at least a general idea of where the 12 o'clock reference nick might have been. This may allow excision of that Mohs stage with only slightly greater than usual overlap (Figure 2.7). In some practices, 12 o'clock may always point superiorly, medially, and/or posteriorly. Other surgeons may always orient 12 o'clock toward the tip of the ear lobule on the side of the body where the cancer is located. Many other methods are equally valid. A digital photo (Figure 2.8) taken of the ink outline and reference nicks of the area to be excised can be viewed later on the camera, or printed, if confusion exists in the surgeon's mind.

As well as choosing where 12 o'clock will be on the tissue specimen, the Mohs surgeon must also ensure that clear orientation of the specimen is maintained from the operative table to the technician's inking station. This can be done in a number of ways:

1. Prior to making the final cut of the base of the specimen, the surgeon-pathologist should visually double check that the reference nicks can be clearly seen on the specimen and corresponding wound edges.

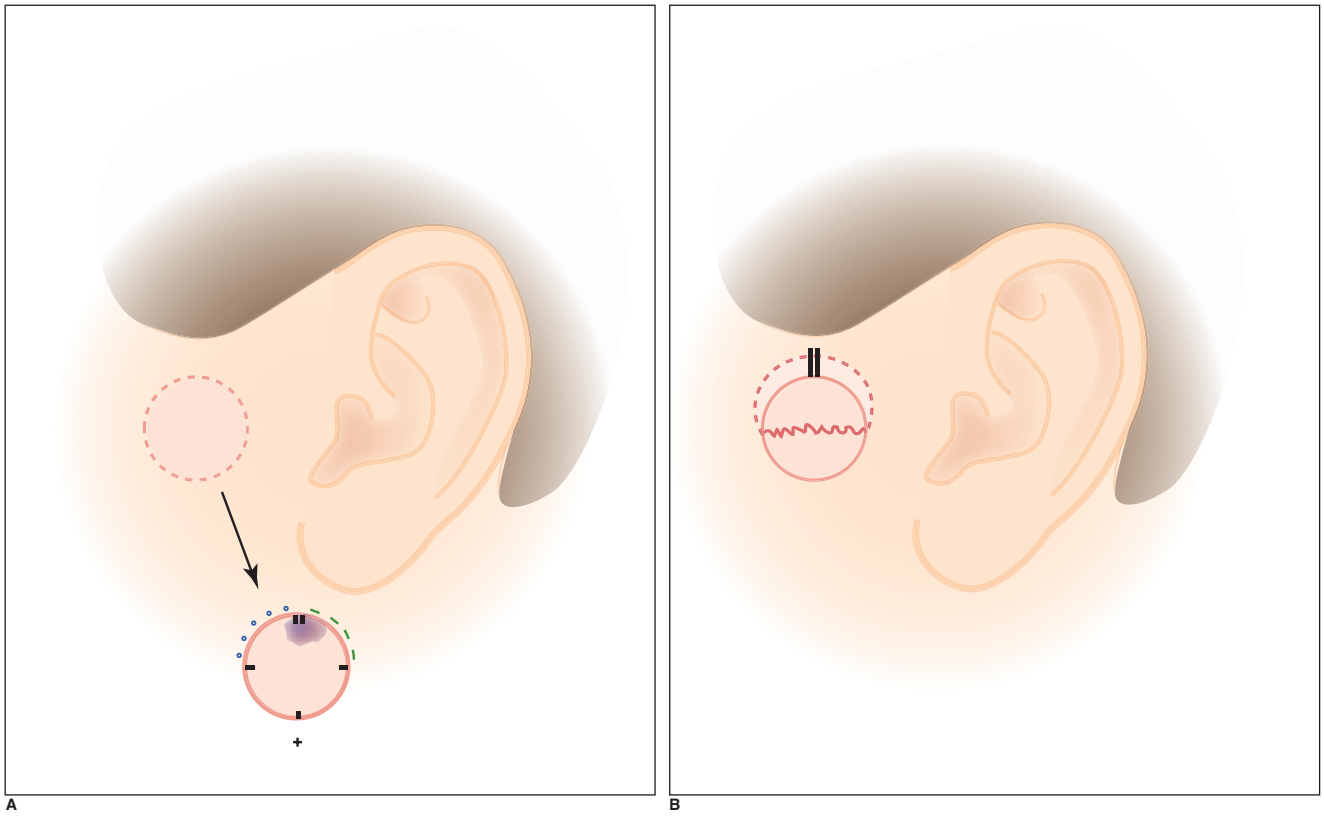


FIGURE 2.7: *(A) Mohs specimen cut with a 12 o'clock superior orientation. Cancer is noted at the 12 o'clock margin on the Mohs slide from this excision, but, unknown to the surgeon, the reference nicks did not "show" on the patient's skin after the specimen was removed. (B) No reference marks were visible when the surgeon-pathologist returned to the operative table and viewed the patient's wound. This surgeon always uses 12 o'clock as superior orientation and has a preop digital photo of the area with the tumor outlined and reference nicks demarcated with ink; therefore, in this example, the correct margins are easily ascertained by the surgeon-pathologist and a correct stage II excision easily determined. It is not always this easy.*

2. An arrow from the sterilization indicator strip can be cut and the specimen placed on the arrow so that 12 o'clock lies in the same direction as the tip of the arrow (Figure 2.9).

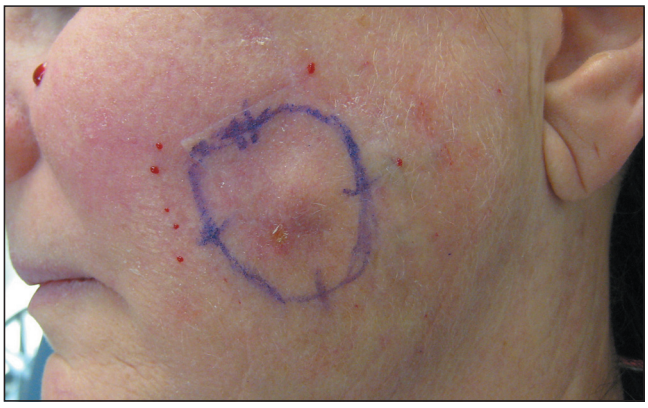


FIGURE 2.8: *Photo of a large cancer with the reference nicks drawn; the subdivision of the tissue into smaller blocks for processing will be done at these hatch marks.*

3. A blood and/or ink dot on a corner of the transfer gauze can be used to designate the 12 o'clock margin.
4. For large and/or complex specimens, digital photographs should be taken and printed of the specimen before lifting it from the wound, and again with the specimen removed but placed next to the defect (Figure 2.10); this will show the new shape of both the specimen and defect, both of which change shape after the specimen is removed. Because the Mohs map is intended to depict the patient's wound after the removal of the tissue specimen, some Mohs surgeons don't draw their maps until after the specimen is excised.

When excising cartilage, including some noncartilaginous tissue at one or more of the specimen edges will help the technician prevent the cartilage from "floating" off the slides during processing. Even if this results in a slightly larger defect, it can be so helpful in producing better-quality slides that the net result is usually worthwhile. (See also Chapter 6 for techniques for slide preparation of tissue containing cartilage.)

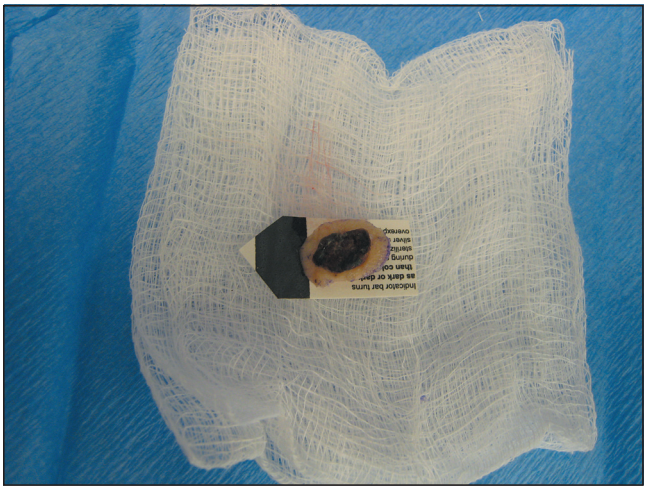


FIGURE 2.9: ***A piece of sterilization indicator strip cut as an arrow with the specimen is laid upon the "arrow" so that the arrow point and the specimen's 12 o'clock reference nick are oriented in the same direction.***

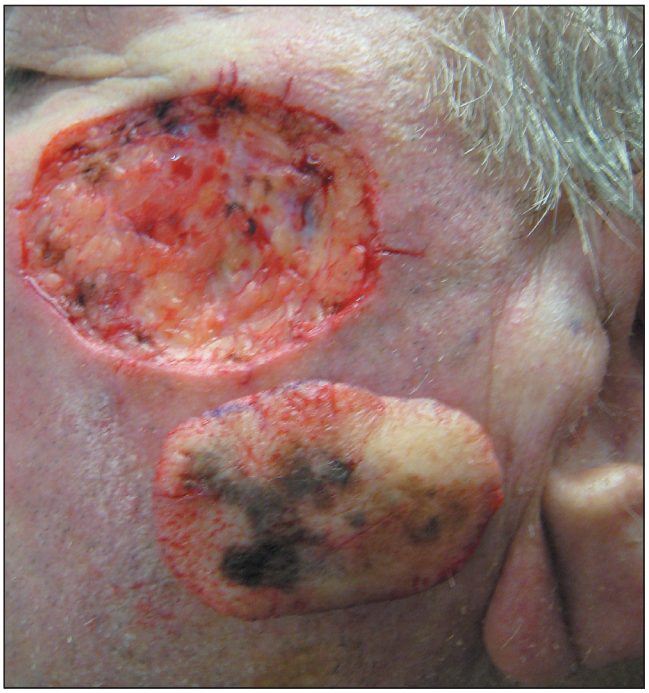


FIGURE 2.10: ***A large specimen is photographed next to the wound from which it was cut. This allows the Mohs surgeon-pathologist to easily see the relationship between the specimen and the wound, both of which change shape after excision.***

When excising large specimens from the vermillion or helix, it is sometimes best not to bevel. Cutting straight through the tissue without beveling allows these cut ends to be processed more easily (Figure 2.11).

When excising a positive margin, the surgeon should significantly overlap beyond the diagrammed extent of the positive margin unless this will cause significant functional postoperative problems.

It is critical to understand that sometimes the location of a deep positive tumor margin noted within a Mohs wafer on a slide, and then depicted on the two-dimensional (2D) Mohs map may be incorrectly located within the three-dimensional (3D) wound when the Mohs surgeon-pathologist returns to the surgical table. A deep positive margin in a 2D flattened specimen may actually lie on or partially on the wall of the 3D wound, not only at its base. This will require the removal of both additional peripheral and deep margins within the patient's wound (see Chapter 9).

Tumor in a nerve at the deep/central margin of a specimen requires excision of additional peripheral margin as well as deep margin because nerves may run in any direction and at any angle from vertical to horizontal. Furthermore, additional nerve must be seen on slides from the next Mohs stage (and be assessed as free of cancer) for the margins to be interpreted as clear. If no nerve tissue is present on the slides, that stage of surgery cannot be considered clear even

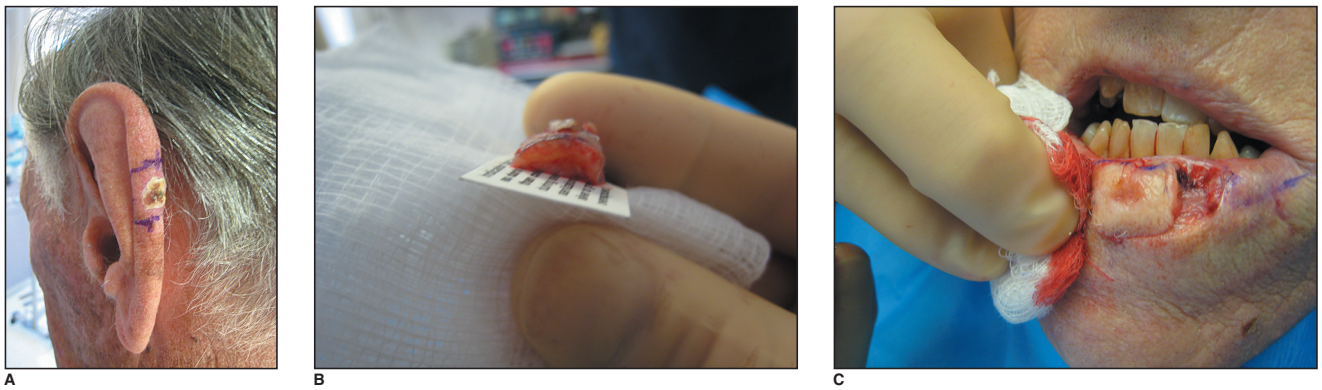


FIGURE 2.11: ***This specimen from the helix of the ear has been cut at both ends without a bevel. The technician will have to cut both ends across their short axes in a partial "bread loaf" technique to allow the ends to lie in the same plane as the base. The specimen from the lip has been excised similarly.***

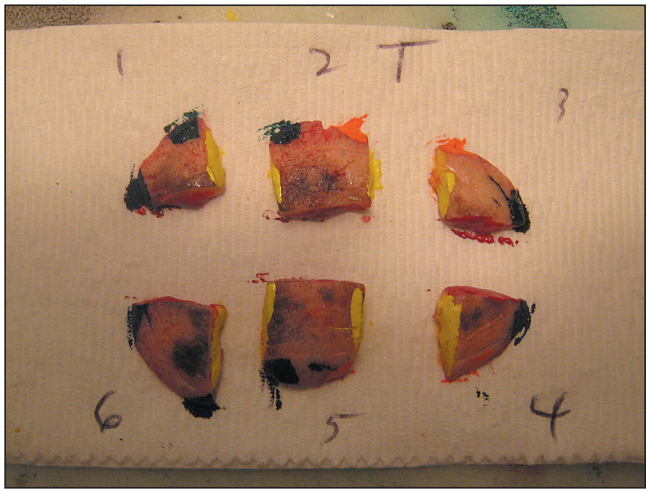


FIGURE 2.12: ***Inking pattern of a large complex specimen. The inking was done on towel paper, which is saved until the case is completed. Both the photo and the paper towel will show exactly how the chromacoding was performed by the technician, and may be used to resolve later chromacoding issues.***

if no cancer or perineural inflammation is seen (see Chapter 17).

Ensuring the integrity of chromacoding is critical, especially when large and/or complex specimens are taken (see Chapter 4). The surgeon must play an active role in this process:

1. For very large specimens, the specimen may be inked on a clean piece of white paper or gauze, which is then saved until slide evaluation is completed. The paper or gauze depicts the chromacoding pattern to safeguard against disagreement during slide review between the Mohs map and the chromacoding on the slides. When done properly, the chromacoding on the slides (when looking through the microscope) is identical to that depicted on the map.
2. A digital photograph of the specimen taken immediately after inking but before any further processing may also be used to resolve later chromacoding issues (Figure 2.12).

Some Mohs surgeons do the chromacoding in the operating room, while others allow the technician to do the chromacoding (see Chapter 4). On large deep cancers requiring multiple stages, and for complex-shaped specimens, the Mohs surgeon should employ techniques to ensure that the location of all pathologic findings noted on the slides can be translated accurately to the defect so that any areas with positive margins are accurately and completely removed in further stages.

Digital photography produces instant documentation of the tissue to be excised before it is completely removed from the patient. A second photo taken of the resulting defect with the excised tissue held next to the defect allows the surgeon to clearly see the relationships between the areas

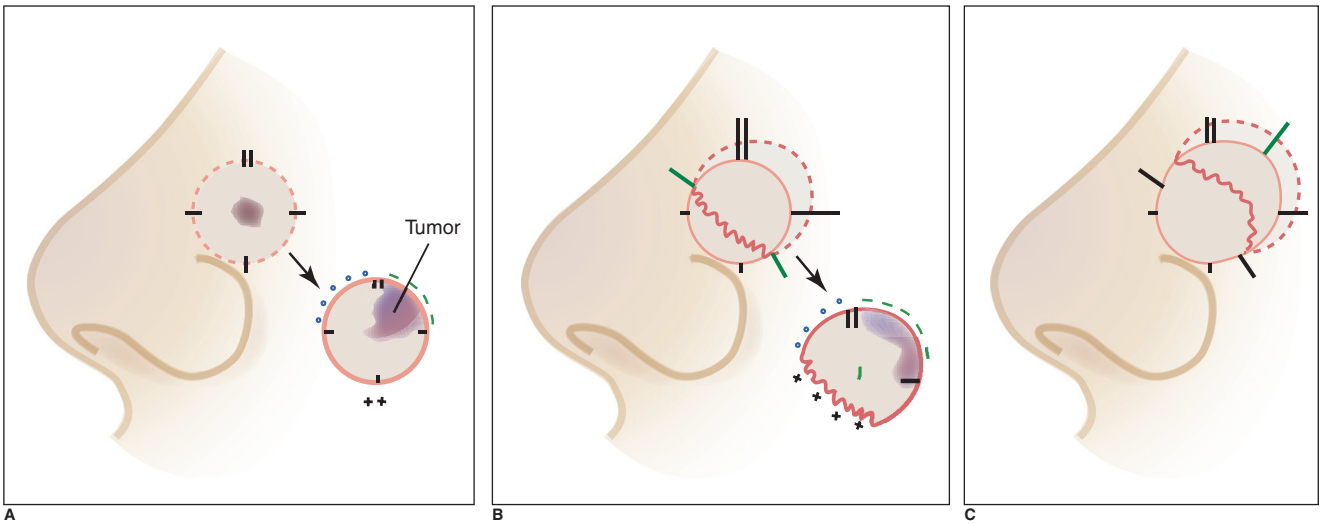
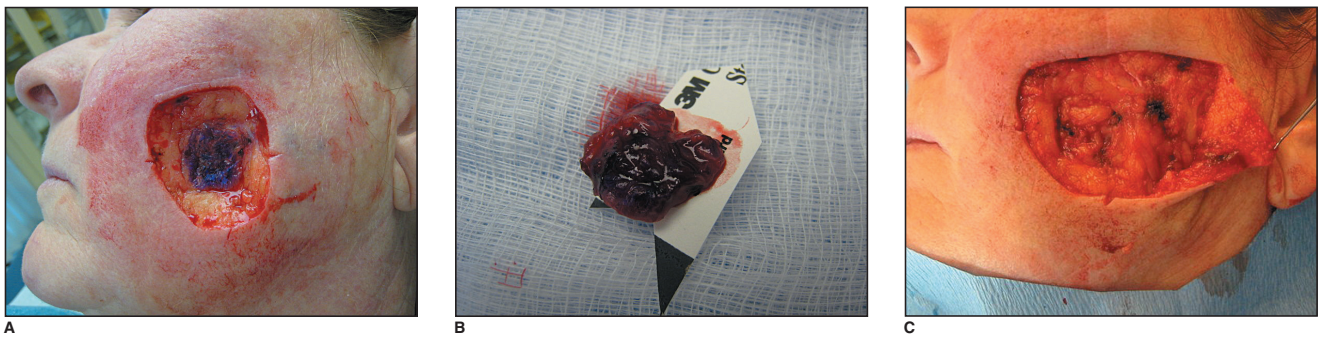


FIGURE 2.13: ***(A) Stage I specimen with reference nicks at 12–3–6–9 o'clock. (B) Stage II specimen overlaps the positive margin. The 12 o'clock and 3 o'clock reference nicks remain, but new reference nicks (green) at 10:30 and 4:30 mark the extent of stage II excision. The two new reference nicks are on the patient and not the specimen because they mark only the extent of the new stage II excision and could not be seen on the specimen. The squiggly line seen in (B) is how this Mohs surgeon represents a nonepithelial, surgically cut edge on the Mohs map. Others may represent this differently but it is important to annotate on the Mohs map where a surgical margin does not contain epithelium. (C) Stage III specimen with a new reference nick between 12 o'clock and 3 o'clock (green). The nick was placed at this point because without it, the increasing distance between the existing 12 o'clock-to-3 o'clock reference nicks would have made the Mohs surgeon-pathologist's job of translating the exact location of a positive margin in this area from the slides to the patients' wound more difficult and less accurate. This new reference nick is on the specimen and the patient's skin. Notice that the positive margins are significantly overlapped in each subsequent stage.***



A B C

FIGURE 2.14: ***The large deep specimen is positive at the central deep margin. To ensure that a complete deep margin is taken without inadvertent "holes" in the specimen, the top of the stage II specimen is inked (A) with Mohs dye (or gentian violet) prior to removal. Notice the "Pac-Man" cut into the specimen (B) by the Mohs surgeon to delineate 12 o'clock in a specimen with no other orienting features. The top side of the specimen is easily discernable because it is inked, which ensures that the specimen will not be processed "upside down," leading to a false-positive margin. The final wound (C) in the patient is checked for ink to ensure that there were no holes or missed areas when the specimen was excised.***

of remaining cancer and the defect when planning the next Mohs stage (Figure 2.10).

The surgeon may place sutures or staples in the specimen and perilesional tissue to act as reference marks. After inking and before or after specimen subsectioning, the technician removes the sutures and/or staples before proceeding further with tissue processing. New reference nicks may be added to mark the ends or midpoints in the margins of new, large, and/or complex additional stages of surgery (Figure 2.13).

During Mohs surgery of large deep tumors, when fat or muscle is excised, the surgeon should take 4–5 mm thick specimens to allow adequate tissue processing and ensure complete removal of the deep tissue layer without "holes" in the tissue. To ensure that a complete layer is removed in deep soft tissues, the wound surface may be painted with nonvital Mohs ink before excision. The wound is examined after excision for ink remaining on the deep tissue, which might indicate a hole or holes in the specimen, with the ink localizing the area(s) where complete tissue was not taken (Figure 2.14).

Occasionally, when taking deep tissue layers, there may be no features to help with orientation of the specimen. The Mohs surgeon may use staples or sutures to delineate the specimen orientation or place a "Pac-Man" cut at 12 o'clock (Figure 2.14). This is easily done with tissue scissors.

Many Mohs surgery patients take one or more anticoagulants. The Mohs surgeon is wise to ask the patient to discontinue nonprescribed anticoagulants such as alcohol, vitamin E, and herbal remedies for one week before surgery. Aspirin, warfarin, and the platelet inhibitor clopidogrel are best discontinued or modified only with the prescribing doctor's permission, which the patient should be asked to obtain before surgery. It is helpful to ask the patient's internist to adjust the warfarin dosage so that the international normalized ratio (INR) measurement is less than "2.5." The use of epinephrine in the local anesthetic diluted to a concentration of 1:400,000 decreases stress on the patient's cardiovascular system without compromising the vasoconstrictive effect of the epinephrine. Control of bleeding during and after surgery is important. One aspect of bleeding control that is extremely important in multistage Mohs surgery cases is the minimizing of electrical artifacts that can affect interpretation of slides during subsequent stages. Electrosurgical coagulation may:

1. Change the appearance of adnexa and make their differentiation from cancer cells more difficult.
2. Produce a dermal artifact that makes overall slide quality poorer and increases the chances of folds and tears in the tissue wafers.
3. Induce inflammation, which may hide tumor and be interpreted as a sign of margin involvement in tumors such as squamous cell carcinoma.

There are multiple strategies the Mohs surgeon can employ to decrease electrical artifacts:

1. Carefully localize the source of the bleeding and employ pinpoint hemostasis.
2. Use splinter forceps to pinch the bleeder and touch the electrical surgical tip to the forceps, or use bipolar coagulation.
3. Avoid using the tip of the electrical coagulation needle to sweep back and forth in the wound to stop bleeding.
4. Avoid using jewelers' forceps to grab bleeders for coagulation; the tips are too sharp and the forceps may adhere to the tissue after coagulation, resulting in tearing and further bleeding when pulling the instrument off the tissue.
5. For general oozing, pressure-dress the wound and have the patient or staff apply firm continuous pressure for 30 minutes between stages. This is good patient training

for dealing with bleeding at home after surgery and provides good hemostasis between stages without requiring excessive cauterization.

6. Set the electrosurgical unit at the lowest effective power setting that will produce rapid and adequate coagulation, but not at a setting that is so low that the use of prolonged current (with increased thermal damage) is needed to obtain hemostasis.
7. If the wound resembles charcoal after coagulation, the surgeon probably failed to find the source of the bleeding; furthermore, "charcoal always bleeds after midnight." If you are unable to find the source of the bleeding, use one of the following strategies:
   a. Apply pressure for a few minutes and look again.
   b. Look at the highest level of the wound; liquid flows down.
   c. Look at the apex of any tissue in a triangular shape.
   d. Do additional undermining of the bleeding area to look for the source of the bleeding past the cut edge.
   e. Tie a suture ligature at the bleeding area: if it helps, leave it; if not, remove it.
   f. If significant bleeding occurs from the dermal plexus, use a hook or tissue forceps to turn the tissue edges up and look for bleeding high up within the dermal plexus.

Pooled blood on the base of the excised specimen should be blotted and removed prior to processing. If pooling persists after blotting, ask the technician to cut extra wafers to ensure the bloody area does not obscure the tissue margins depicted on the slides. Blood on the slide without the presence of both cancer and the normal expected tissue is not a negative margin because blood is not a tissue margin.

Close communication between the Mohs surgeon-pathologist and the Mohs technician is important in the production of optimal slides. Having the technician in the procedure room to pick up the tissue specimen(s) allows the surgeon to point out areas of tissue that might require special handling and discuss potential orientation problems that may result from site complexity, such as uneven beveling.

Pearls

1. Wipe the scalpel blade after every cut during Mohs cancer removal.
2. Remove scabs on small shallow specimens before processing.
3. Always orient the specimen's 12 o'clock margin using the same basic technique.
4. Place reference nicks where the excision specimen is likely to be subsectioned by the Mohs technician, who should try to subsection at the reference nicks.
5. Ink the top of deep tissue before excision to ensure that thorough removal can be visually assessed.
6. Tissue conservation is a benefit of Mohs surgery but is only critical when preserving function and/or preventing cosmetic deformity.
7. Use pinpoint coagulation of bleeders.

CHAPTER 3

Optimizing the Mohs Microscope

Ken Gross

THE MICROSCOPE is an essential tool of the Mohs surgeon-pathologist and should be equipped to make the job of cancer-margin assessment as easy and as accurate as possible. In this chapter, those microscope features deemed essential, helpful, and generally unnecessary will be enumerated and the proper technique for optimizing performance will be discussed. The last section will elaborate on setting up the Mohs slide reading area.

Of the many microscope manufacturers, the Leica and Olympus models are widely available and similar in quality. The choice between these microscopes should be based on price, comfort, and ease of use. Microscope base design and control placements vary among brands. Sit down with each microscope and see how the controls "fit."

The following are highly desirable features for a Mohs microscope:

1. Trinocular microscope with binocular dual (teaching) heads (Figure 3.1), focusable and wide-angle ocular lenses (Figure 3.2), and a nose piece that holds five objective lenses. (Figure 3.3)
2. A 1×–2.5× objective lens (Figure 3.4) with a substage swing-out condenser (Figure 3.5). A slide-out condenser is acceptable, if sturdily designed.
3. Middle-quality objective lenses: companies generally offer three levels of lens quality. Suggested objective lenses are 1×–2.5×, 4×, 10×, 20×, and 40× (Figure 3.6).
4. Lighted teaching pointer.
5. High-quality color-balancing filter that sits between the substage lighting and the objective lens to allow the proper light quality and color for optimal slide viewing.
6. Halogen substage lighting (Figure 3.7). (Purchase a backup bulb.)

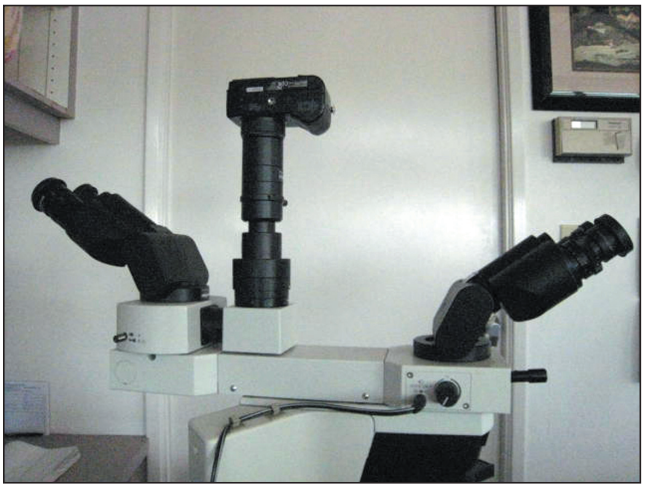


FIGURE 3.1: Trinocular microscope with a binocular dual (teaching) head.

FIGURE 3.2: Wide-angle, high-quality focusable ocular lens.

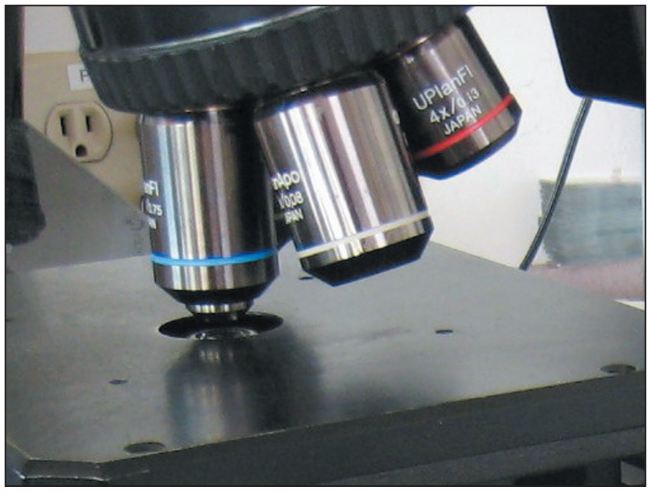


FIGURE 3.3: Nose piece holds five objective lenses.

FIGURE 3.5: Swing-out condenser.

A teaching head (binocular microscope) allows the surgeon-pathologist to review slides with the technician. In order to constantly improve their slide quality, technicians must regularly see the slides and be shown what needs improvement as well as what is already wonderful.

A flip-out condenser is required when using 1×–2.5× objectives. The aperture of these objectives is bigger than the area lighted by a standard condenser (Figure 3.8). The flip-out feature allows the small top lens of the condenser to move ("flip") out of the field so the larger condenser lens (seated below the swing-out lens) may illuminate the larger viewable field of the wide angle 1×–2.5× objective (Figures 3.9A and 3.9B).

A 1×–2.5× lens allows the Mohs pathologist to quickly see the chromacoding pattern and assess the location of positive margins or other pathologic findings that must be marked on the Mohs map. It permits fast orientation between wafers on the slide and keeps the Mohs pathologist from getting "lost" while looking at large specimens.

FIGURE 3.4: 2× lens. Using a 1×–2.5× lens for low-power viewing is essential for efficient Mohs surgery.

Wide-angle and focusable eye pieces on all four oculars of a dual-headed microscope allows viewers of different visual acuities to focus clearly, enabling optimal slide reading with or without eyeglasses. Focusable eye pieces also allow the surgeon-pathologist to easily adjust the microscope so all the objective lenses are par focal.

The following features are useful, but not essential for a Mohs microscope:

1. Tilt option for one or both heads, to minimize neck and back strain (Figures 3.10A and B).
2. Trinocular head for a camera mount, to enable digital photography (Figure 3.1).
3. Stage micrometer, to allow measurements of tumor thickness.
4. Polarizing filters.

The following features are not generally helpful for a Mohs microscope:

1. Extending (as opposed to tilt) heads.
2. X-Y mechanical stage adapter (moveable slide holder that mounts on the Microscope stage). Some Mohs pathologists may like this feature, but this author feels that it slows down slide reading, gets "glued" up, can inadvertently move the not-yet firmly adherent cover slip, and is not necessary for the lens powers used by the Mohs surgeon. This comes as standard equipment with all microscopes and can be easily removed or left in place (Figure 3.11A and B).
3. "Slide-out" (as opposed to "swing-out") condenser. A condenser with a slide-out top condenser lens should have a sturdily made mechanism for moving the top lens

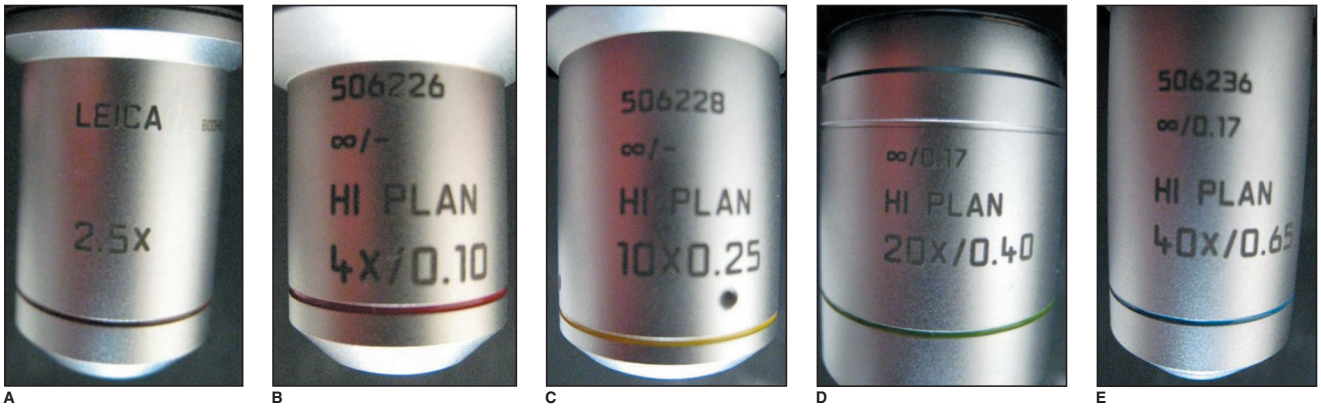


FIGURE 3.6: Suggested objectives for the Mohs surgery microscope.

out of the field, as it is moved constantly while reading Mohs slides.

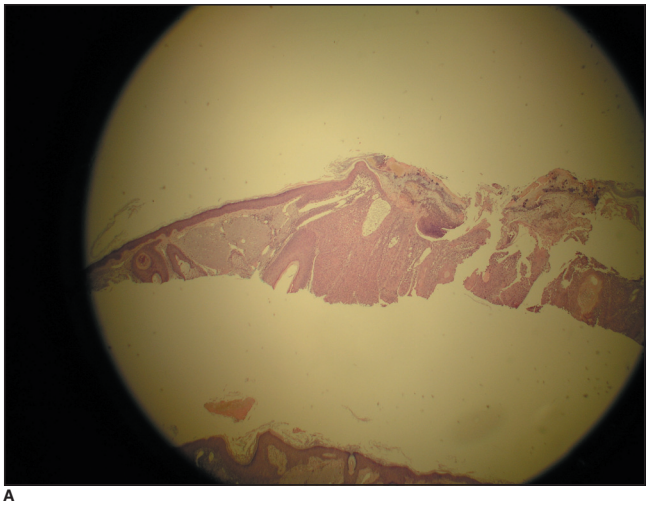
Before reading slides, the Mohs surgeon-pathologist should set up the microscope in the following manner:

1. Clean any dirty ocular, objective, and condenser lenses.
2. Set a comfortable light intensity. When the light intensity on a microscope cannot be set, it is usually because the scope is set for photo microscopy; the switch that locks the light intensity at a preset illumination for photo microscopy should be switched off to allow alteration of the light intensity.
3. Adjust the separation of the ocular lenses to your intraocular distance to allow binocular vision (one image with both eyes open).
4. Using the focusing ring on your ocular lens, set all the focusable ocular lenses to "zero" and select the 20× objective to view any available pathology slide. Using the knobs on the microscope body, coarse-focus and then fine-focus the microscope. If you have only one focusable ocular, first focus the nonadjustable ocular (with the other eye closed) using the microscope knobs. Then close the eye on the nonfocusable side and focus the image using the focusing ring on the focusable ocular. Then continue as described.
5. Change from the 40× to the 1×–2.5× objective lens, swing or slide out the top lens of the condenser, and refocus the image using only the focusing rings on the ocular lens. This will ensure that the microscope is par focal for all your objective lenses; if this is done correctly, the microscope will require only minimal fine focusing when switching between objective lenses.
6. Next, set up (focus) your microscope's condenser. Using the 20× objective with the swing-out condenser swung in and the field diaphragm mostly closed down (the field diaphragm sits above the light and below the condenser), you should now be able to visualize a 10-sided figure; you are now viewing the leaves of the condenser (Figures 3.12A and 3.12B). Adjust the condenser up and down until the 10-sided figure is sharp (Figures 13A and 13B). The condenser is now in focus and should not be moved up and down further while reading slides. Once the condenser is focused, it should remain in focus for the entire session.
7. If the 10-sided figure is not centered in your field of vision, center it using the two centering screws on the condenser. The third screw on the condenser is used only to secure the condenser in its mount, and almost never needs to be touched (Figure 3.14).
8. The microscope is now completely set up for reading your Mohs slides in the optimal fashion. Open the field diagram and start reading your slides.

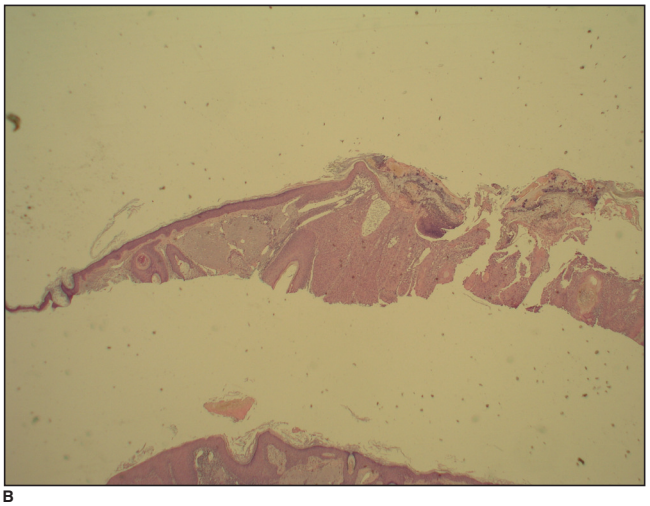
FIGURE 3.7: Halogen substage lighting. Having a spare bulb available is essential.

This author follows those simple steps before every Mohs session. Done routinely, the entire process takes about 1 minute but saves the surgeon-pathologist many

FIGURE 3.8: This 2× lens has a bigger opening (aperture) than the aperture of the top lens of a standard condenser.



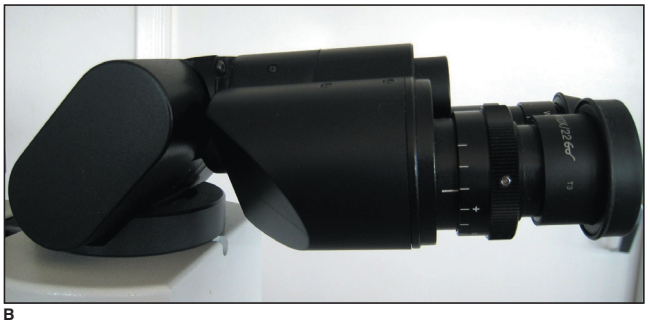
A



B

FIGURE 3.9: (A) View with swing-out condenser in place. (B) The swing-out top lens of the flip-out condenser is swung out to allow enough light through the condenser to light the entire 2× field.

A



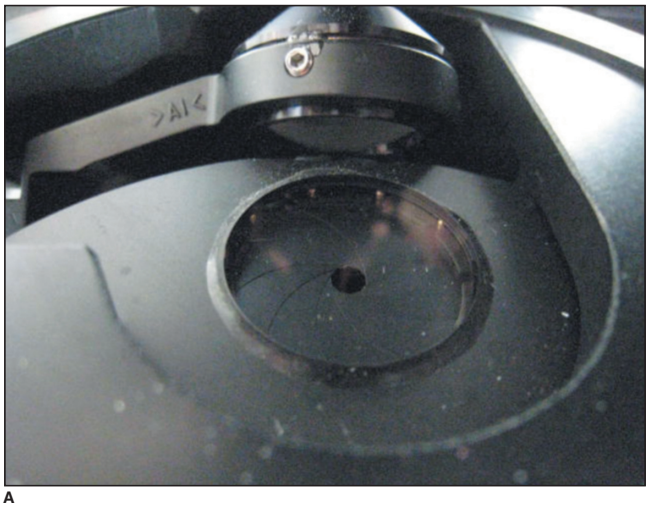
B

FIGURE 3.10: (A) Tilt-head binocular focusable wide-angle oculars with the tilt-head tilted up. (B) Tilt-head tilted down to accommodate a shorter-stature surgeon-pathologist.

A

B

FIGURE 3.11: (A) Microscope stage with X-Y mechanical stage adapter. (B) Microscope stage with X-Y mechanical stage adapter removed. Removal is easily done and makes viewing slides easier and faster.



A

B

FIGURE 3.12: *(A) Condenser with leaves closed down. (B) Condenser leaves are wide open.*

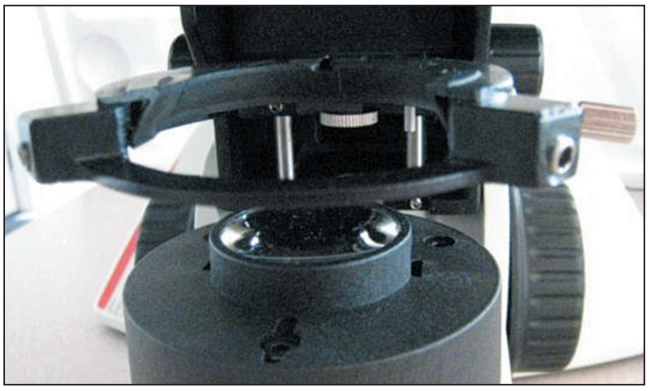
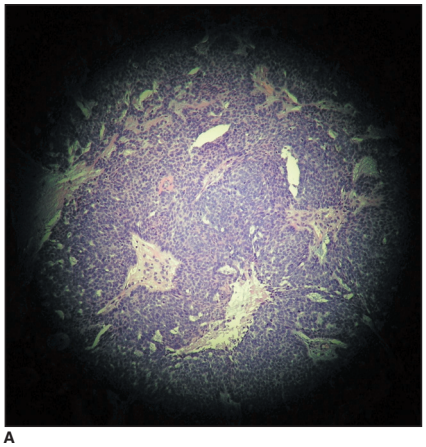


FIGURE 3.14: *If the 10-sided figure is not centered in your field of vision, center it using the two centering screws on the condenser. The actual adjustment screws are not in place in this photo but lie at the ends of the two rectangular blocks in the ring that holds the condenser. The third screw on the condenser (silver screw on the right side of the photo) is used only to secure the condenser in its mount, and almost never needs to be touched.*

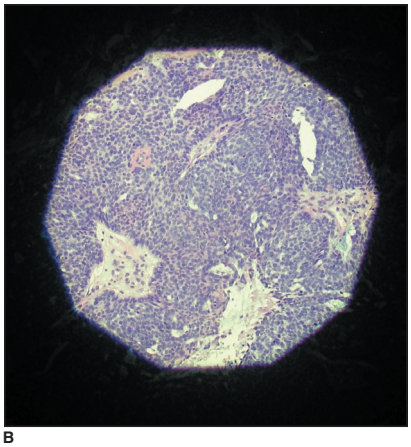
minutes during the hours of reading slides. As objective lenses are changed during reading of the slides, the condenser diaphragm opening may be adjusted to increase or decrease contrast, but this is seldom necessary in actual practice; some microscopes have a color-coded setting on the condenser diaphragm that matches the different colors on the objective lens to facilitate this (Figure 3.15).

By purchasing a microscope optimized for Mohs surgery and then optimizing its viewing features before each use, the Mohs surgeon-pathologist can work efficiently and attain maximum accuracy. It is advisable to have the microscope professionally cleaned and adjusted every 6 to 12 months.

The Mohs slide reading area (Figure 3.16) should have a table that allows two people to view slides together from opposite sides of the table; alternatively, microscopes



A



B

FIGURE 3.13: *(A) Although the microscopic image is focused, the 10-sided figure (Figure 3.11B) is not. (B) Condenser is now focused and should not be moved up and down during the entire Mohs session.*

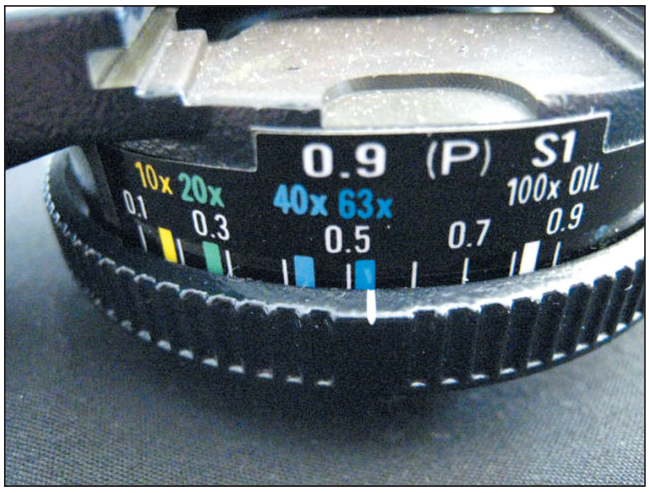


FIGURE 3.15: As objective lenses are changed during reading of the slides, the condenser diaphragm opening may be adjusted to increase or decrease contrast, but this is seldom necessary in actual practice; some microscopes have a color-coded setting on the condenser diaphragm that matches the different colors on the objective lens to facilitate this.

can be purchased with an adapter to allow side-by-side viewing if the room configuration does not allow across-the-table viewing. The microscope can also be put on a large Lazy Susan (Figures 3.16 and 3.17) to allow either user to "drive" without changing seats. There must be enough desk/counter space for multiple trays of slides and a large enough writing surface to allow for viewing and marking the Mohs maps. A phone should be readily accessible for prompt communication with the Mohs lab.

FIGURE 3.16: Mohs slide reading area.

FIGURE 3.17: Lazy Susan under microscope.

Glass-marking pens (e.g., Pilot pens) should be available to mark slides. Reading chairs should be comfortable and adjustable.

Pearls

1. Working without a 1×–2.5× objective lens (which requires a swing-out or slide-out condenser) and working without wide-field oculars is like working with a 20-year-old computer: it works, but not well or efficiently.
2. Using a mechanical stage adapter to move Mohs slides around the microscope stage is fine if you frequently use very high magnification objectives or an oil immersion lens, but slows down the process of slide interpretation and gets "glued" up during the reading of Mohs slides. This opinion is not shared by the co-editor of this book.
3. When purchasing a microscope, compare "apples to apples." Lenses can be purchased in three or more quality levels based on their color correction, how well they flatten the image, and how much light they allow through the lens (numerical aperture), etc. Any or all of these factors vary among lenses of different quality. Better quality costs more money. The higher the numerical aperture, the more expensive the lens. You will pay more for wide-angle oculars that enlarge the field of view and for focusable oculars that permit people of different visual acuities, with or without glasses, to more easily view slides concomitantly. However, these one-time costs buy decades of efficiency, accuracy, and comfort.
4. Not ensuring that the microscope is par focal before each use wastes time and energy because Mohs pathology requires constantly switching between objective lenses.
5. The microscope is not a good place to economize when setting up a Mohs practice.

CHAPTER 4

Tissue Preparation and Chromacoding

Howard K. Steinman

PROBLEMS RELATED to Mohs surgery tissue preparation and chromacoding usually result from orientation errors, specimen damage, poor quality slides, incorrect or incomplete margin assessment, misidentification of specimens, and errors in map notations.

TISSUE PREPARATION

Meticulous tissue preparation is essential for producing high-quality pathology slides. Good preparation may improve the histologic quality of slides from poorly excised specimens. Poor processing techniques can lower the quality of slides from properly excised tissue, and good processing techniques can improve the slide quality derived from suboptimally excised tissue. Care is necessary to preserve orientation, especially when multiple tissue pieces are manipulated and moved for chromacoding and embedding.

Tissue preparation involves three general phases (Table 4.1). First, perform a global assessment to ensure that specimens are properly oriented and correctly drawn on the map, and that their surgical margins appear intact. Then, if necessary, subdivide specimens into pieces suitable for microtome processing and slide preparation. Finally, manipulate and alter specimens so their surgical margins can be placed in a single plane for embedding.

TABLE 4.1: Essentials of Tissue Preparation

Global inspection of specimens
Presence of fat or cartilage
Right-side-up position, and oriented
Shapes correspond to map
Reference marks are visible and correspond to map
Surgical margins are intact
Subdivide larger specimens, as needed
Tissue manipulation
Reposition torn or separated pieces
Remove exophytic surface components, if needed
Score (place relaxing incisions), if needed

The global assessment involves examining specimens' orientation, shape, and structure. The technician must know when specimens contain significant amounts of fat or cartilage (Figure 4.1), as these require specialized embedding and slide preparation techniques (see Chapters 6 and 8). It must also be ensured that specimens have not been accidentally rotated or turned upside down. This is especially important for specimens lacking epithelial margins. Reference marks must be visible and the specimens oriented toward a defined clinical reference point. The shapes and reference marks must approximate their drawings on the map (Figure 4.2). If there is a discrepancy concerning the orientation, shape, or reference marks, the map may have been incorrectly drawn or the specimen may not be from the case depicted on the map.

Specimens' surgical margins must be intact, without tears or separated pieces. Peripheral epithelial edges must not be folded, and folded edges should be repositioned before proceeding. Tears must be reapproximated, and any

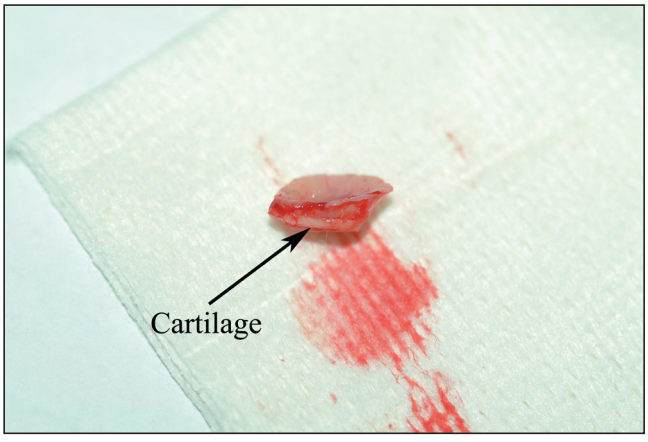


FIGURE 4.1: ***Specimen* (side view) *containing cartilage. Tissues containing cartilage require specialized processing. They may also require relaxing incisions to ensure that the surgical margin can be placed in one plane.***

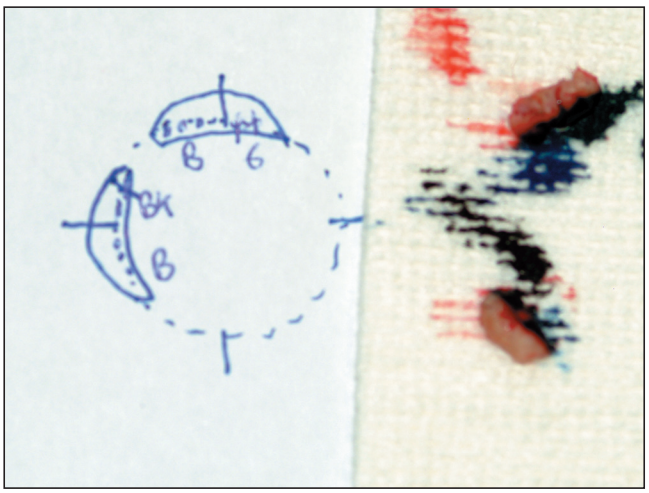


FIGURE 4.2: ***Specimen orientation, shape, reference mark location, and inking patterns must be depicted correctly on the map.***

separated pieces must be repositioned, preserving their relative orientations, before embedding.

Map discrepancies and problems with a specimen's structure must be resolved before chromacoding and embedding. Failure to ensure that specimens are correctly oriented, right side up, contiguous, unfolded, and properly depicted on the map may result in errors in slide interpretation and tumor localization, which might result in incomplete tumor extirpation.

Next, manipulation of specimens may be necessary to ensure complete margin representation on the slides after tissue embedding and microtome sectioning. The unneeded surface portions of exophytic specimens should be debulked, taking care not to disrupt tissue near the surgical margins (Figure 4.3). Exophytic tumors are ideally debulked before excision (see Chapter 2).

The technician must ensure that the entire surgical margin can be placed in one plane for complete margin assessment (see Chapters 2, 6, and 8). Steeply angled and thick specimens and those containing cartilage may require relaxing incisions (also called "scoring") or may need to be cut into smaller pieces to permit necessary movement (relaxation) of the specimen margins.

Relaxing incisions are best placed through only the surface portions of the specimen. Excessively deep cuts may disrupt the surgical margins and appear on the pathology slides. Relaxing incisions may be a planned component for excisions in which more vertically angled peripheral margins are desired. This may occur when performing Mohs surgery on incompletely excised lesions (Figure 4.4). Planned relaxing incisions through these specimens permit tissue sparing and proper embedding.

The final phase of tissue preparation is to determine whether specimens must be subdivided. Specimen subdividing significantly increases the time needed for slide preparation and slide interpretation. It also increases the potential for errors during tissue inking, embedding, slide preparation and labeling, microscopic interpretation, and notating findings on the map. Moreover, deeper (nonmarginal) portions of the specimen at the lines of subdivision become artifactual surgical margins, as they are pressed flat with the true surgical margins during embedding. This may result in false-positive findings during slide interpretation. It is thus recommended that specimens be processed as one piece when possible (Figure 4.5), and in as few tissue pieces as possible when subdividing is required. The prime factors in determining when specimen subdivision (subsectioning) is necessary are the sizes of microscope slides, microtome object holders, and freezing and embedding technologies available to the technician.

Tissue subdivisions are best cut along the specimens' reference lines to preserve orientation and simplify correlation of findings from the slides to the map (Figure 4.6). It is important that surgeons anticipate the need for subdividing when planning large excisions and draw and place enough

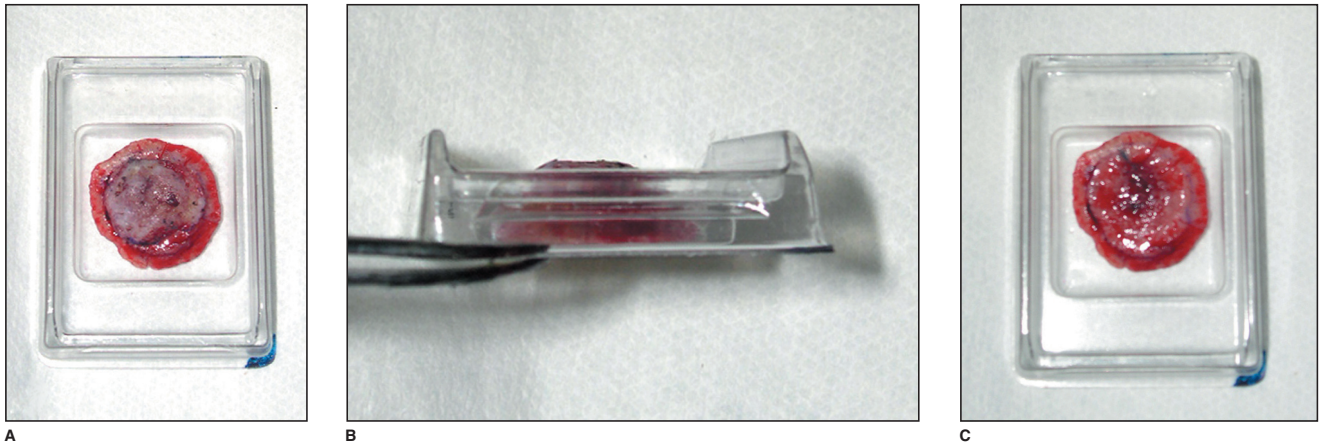


FIGURE 4.3: ***(A and B) Specimen with excessive superficial (nonmarginal) tissue impairing efficient embedding. (C) Specimen after surface debulking.***

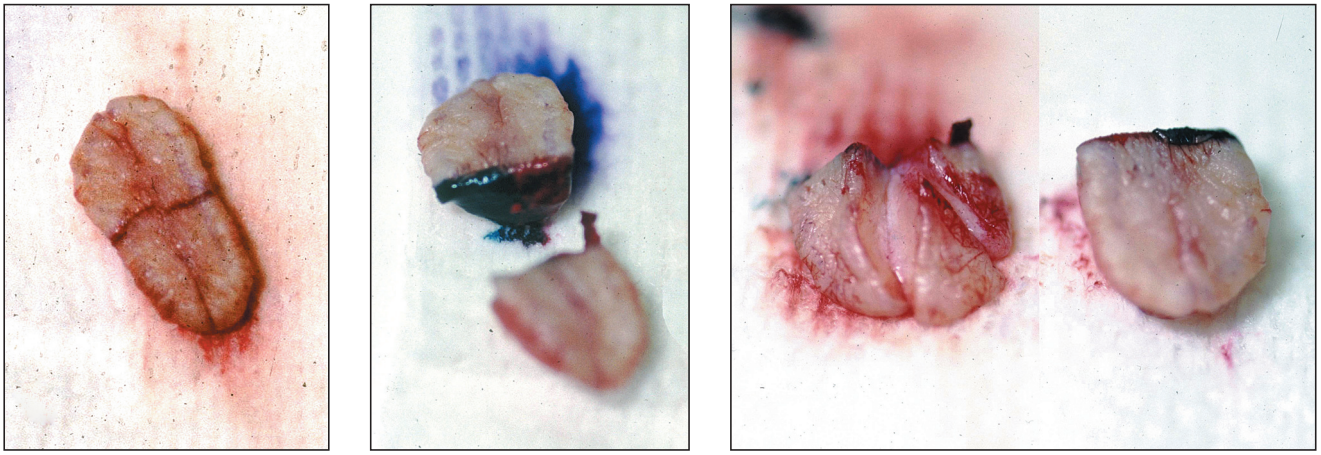


FIGURE 4.4: *(A) A steeply angled Mohs specimen from an incompletely excised skin cancer. Note scar in center of specimen. (B) After bisecting specimen, edges cannot be placed in one plane for embedding. (C) Relaxing incisions placed to put surgical margins in one plane for block 1* **(left).**

reference marks preoperatively. It is also vital for the distance between reference lines not to exceed the usable width of available slides and microtome tissue holders (Figure 4.7A and B).

Compression artifact may occur when cutting tissue. Very sharp, large blades (such as #10 and #20 scalpels) are recommended for subdividing specimens (Figure 4.8). Gentle tissue handling with forceps is required to prevent tissue compression.

CHROMACODING

Chromacoding is the use of inks to mark specimens and the recording of these markings on the map. It is usually performed by touching wooden sticks coated with inks to specimens' edges and reference marks. Chromacoding is necessary to ensure that complete surgical margins are represented on the slides, to preserve orientation during slide interpretation, and for correlating findings from the slides to the map. It is very important for indicating possible errors in tissue processing, embedding and slide preparation, and map drawing and marking.

Chromacoding is needed to differentiate among pieces from subdivided specimens. It can also be used to distinguish between multiple tumors excised from one patient, and to uniquely identify specimens from different patients during a surgery session (Table 4.2).

The complete surgical margin must be represented on Mohs surgery slides. Epithelium (epidermis or mucosa) is

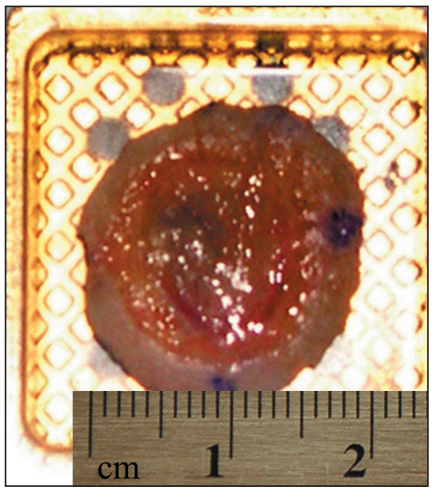


FIGURE 4.5: *Many specimens, even relatively large specimens, can be processed as one piece. This saves preparation and interpretation time and minimizes risk of errors.*

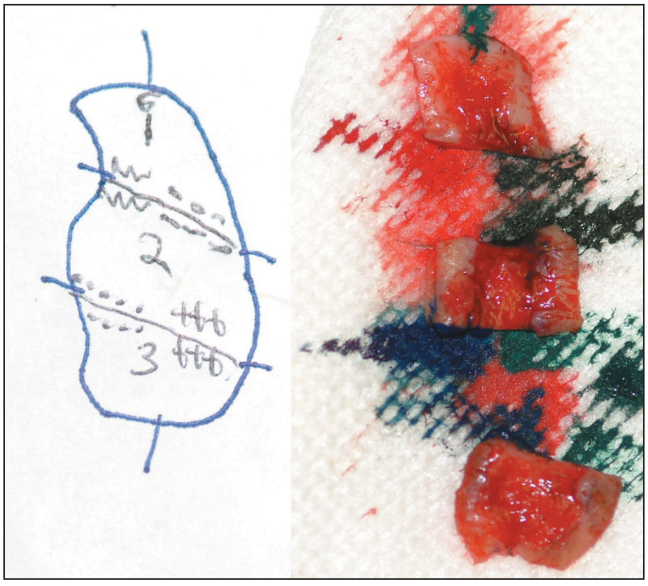
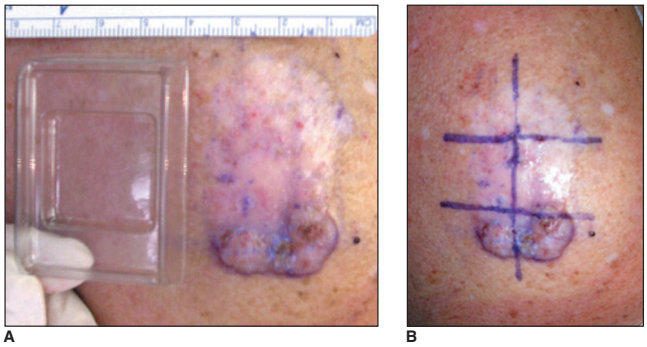


FIGURE 4.6: *Specimen subdivided (and inked) along its reference lines. Note that adjacent cut edges have been inked in the same color to simplify slide interpretation.*



A B

FIGURE 4.7: (A) Largest available tissue mold (corresponding to largest microtome chuck) next to tumor. (B) Appropriate number of reference lines placed before excision in anticipation of specimen subsectioning. (See also Figure 5.6.)

a good indicator of a peripheral surgical margin. Specimen edges lacking epithelium require tissue inking to ensure that the peripheral margin (edge) is completely present on the slides. Proper technique is required to prevent ink from flowing onto the deep tissue margins, as errantly applied ink will appear on successive slide tissue wafers, falsely indicating a peripheral surgical margin. Correct technique is to apply a minimal amount of ink to only the cut edge of the specimen to prevent deeper ink migration. After dipping sticks into ink, most ink should be removed by first wiping the stick against the ink bottle opening and then rolling it on gauze or paper towel.

One common problem is when an inked edge is not visible on the slide tissue wafers. This may occur when ink has been placed on the top of the specimen edge and specimen surface, with insufficient or no ink placed at the surgical margin. (Proper inking techniques are discussed in greater detail in Chapter 7.)

Edges composed of epithelium do not require inking, although inking the reference nicks is useful for orientation.

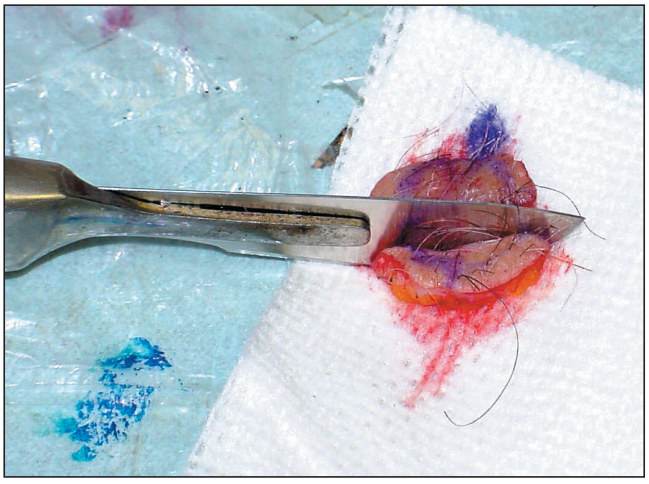


FIGURE 4.8: Large scalpels, such as the #20 blade depicted here, are useful to cut tissue cleanly, with less risk of compressing artifact.

TABLE 4.2: Purposes for Chromacoding

Ensure that complete margins are on slides
Preserve orientation during slide interpretation
Differentiate among subdivided specimen pieces
Correlate findings from slides to the map
Evaluate for indicators of tissue mishandling and map marking errors
Distinguish between multiple tumors from one patient
Uniquely identify specimens from different patients

Ink may also be placed in the center of the deep surgical margin, particularly when working with new histotechnicians. Some surgeons want this ink to be visible on the first slide wafers placed on the microscope slides to guard against overfacing (removing excessive tissue during microtome cutting before placing tissue wafers on the slide).

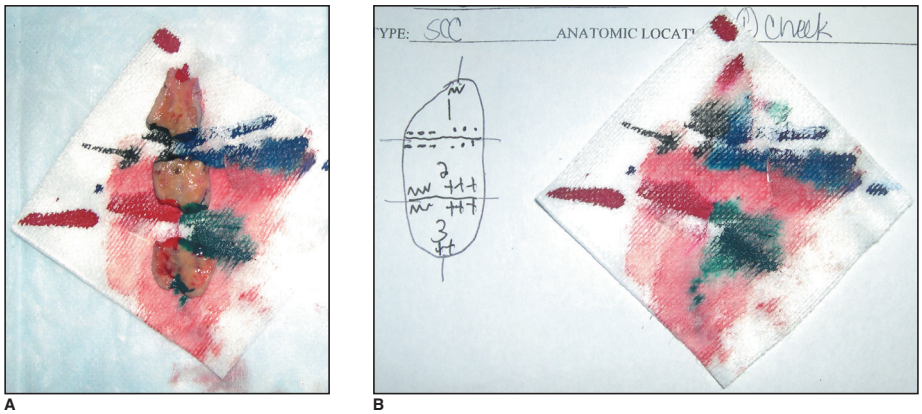
A serious problem occurs when the slide inking patterns do not correspond to those on the map. This error may be minimized by inking all tissue edges before recording the inking patterns on the map. Errors may also be minimized by not removing any tissue pieces for embedding until all have been inked. Maps may be corrected, while ink cannot be removed from tissue edges.

It is also useful to place specimens on a piece of gauze or paper (rather than directly on a cutting board) for chromacoding and to retain this material until all slides have been interpreted and their pathology findings marked on the map with confidence. The material will retain ink patterns and may aid in resolving situations where mismarking of the map is suspected (Figure 4.9A and B).

When applying two different-colored inks to a tissue edge, it is best to approach the edge from the side being marked (i.e., approach and mark the left half from the left side and the right half from the right side). This will prevent inking errors, should the wooden stick touch the wrong tissue edges (Figure 4.10).

An effective method to enhance the accuracy of slide interpretation and marking of findings on the map is to use inks to clearly demonstrate reference lines on the slides. Epithelial margins may be marked simply by placing small ink dots into the reference nicks (Figure 4.11A). These colored points are readily visible on the pathology slides. This technique is especially useful when scoring (relaxing incisions) has introduced additional nick-like disruptions of a specimen's periphery, as is shown in Figure 4.4C. Reference marks on nonepithelial edges are often not visible on slides, especially if the specimen was subdivided. Since nonepithelial edges require inking of their full lengths, an effective way to denote their reference lines is to mark edge segments with different colors that meet at the reference line (Figure 4.11B).

Because of the methods used to embed, section, microtome, and transfer tissue wafers to slides, and as a result of



A B

FIGURE 4.9: *(A) Specimens inked on gauze. All pieces were inked before any were removed for embedding. (B) Inking patterns retained on the gauze may be used to substantiate tissue inking patterns on slides and map. In this case, gauze corroborates the pattern written on the map.*

microscope optical properties, slides' chromacoding patterns under the microscope should be identical to those on the map. If the patterns are different, it is vital to resolve the discrepancy. Possible solutions are that (1) the ink patterns on the map were drawn differently than the tissue was inked; (2) the wrong tissue section's slide is being reviewed; (3) the slide is mislabeled; (4) the slide is from another Mohs case; and (5) the tissue was turned upside down before processing (Table 4.3). A review of other slides from the case, the slide label, and the gauze or paper on which the tissue was inked will likely help resolve the problem.

The patterns used for chromacoding are discussed in more detail in Chapter 10. Those particularly useful in solving and preventing problems during Mohs surgery are now briefly described.

It is useful to ink edges between neighboring specimen pieces the same color when chromacoding subdivided surgery specimens. During slide review, tumor and other foci will often be identified at or near the edge of a subdivided specimen. Coding apposing cut edges the same color

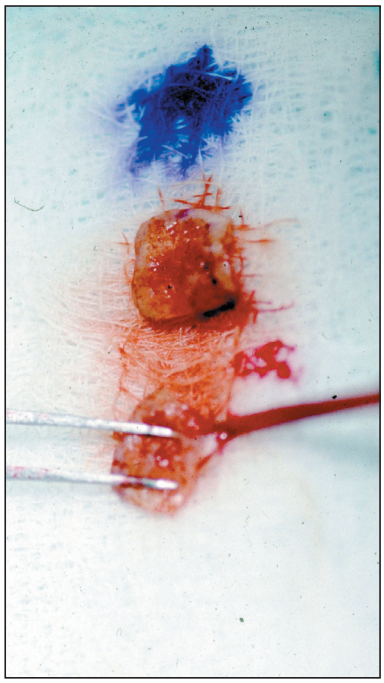
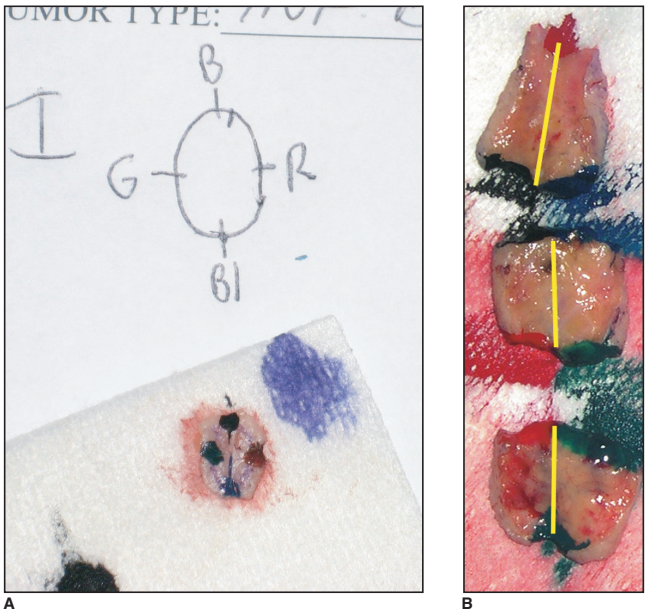


FIGURE 4.10: *When applying different-colored inks to nearby tissue edges, it is best to approach the edge from the side being marked (i.e., approach and mark the left half from the left side and the right half from the right side). This helps prevent ink contamination of the tissue edges.*



A B

FIGURE 4.11: *(A) First-stage specimen with complete epithelial margins may be inked with dots in reference nicks. (B) Close-up of tissue shown in Figure 4.9A. Yellow lines denote vertical reference line. Note that each cut nonepithelial edge is marked with two colors that meet at the reference line.*

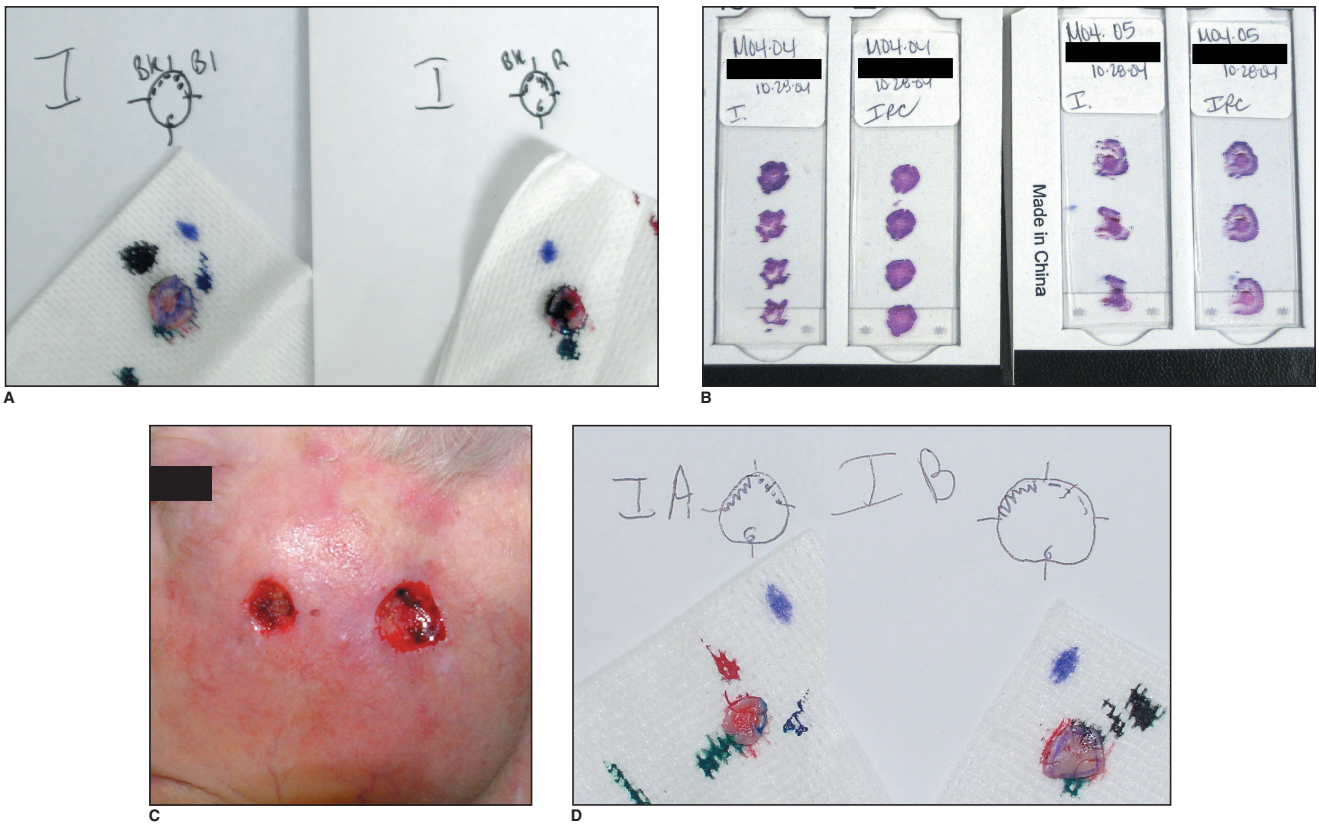
Table 4.3: Possible Reasons for Discrepancy between Inking Patterns on Slides and the Mohs Map

The map pattern was mistakenly drawn differently than the tissue was inked
The wrong slide from the case is being reviewed
The slide is mislabeled
The slide is from another Mohs case
The tissue was turned upside down before processing

allows the surgeon to more easily find and review slides for the relevant location on both edges (on different slides) by simply looking for the correct ink color.

Subdividing larger specimens often creates symmetric pieces, which will appear identical, absent proper chromacoding, on the slides. When inking opposing cut edges the same color, two or more pieces will often have similar shapes and identically inked edges. To prevent confusion during slide review, it is advisable to mark one of the pieces with a third color.

Finally, first-stage surgery specimens are frequently circles or ovals of similar size. When treating several patients at once, the surgeon will be presented with multiple slide trays and maps containing similarly appearing tissue wafers and diagrams. Some surgeons will therefore chromacode each first-stage tumor of similar size differently, to guard against working with the incorrect slides or map (Figure 4.12A,B). This possibility is of particular concern when treating more than one tumor from the same patient in the same session. The first-stage slides and maps from the patient will likely be presented to the surgeon nearly simultaneously, and chromacoding the cases differently minimizes the risk of error (Figure 4.12C and D).



A B C D

FIGURE 4.12: (A and B) The shapes of specimens from sequential patients are similar clinically and on slides. They are differentiated by inking patterns to minimize error risk. (C and D) For the same reasons, using different inking patterns is also effective when excising two specimens from the same patient.

CHAPTER 5

Embedding Techniques

Edward H. Yob

THE EMBEDDING technique is the first step in Mohs tissue processing for achieving high-quality slides. Once tissue is harvested from a patient undergoing Mohs surgery, it begins its journey through the lab, ending in the preparation of the final slides. After the specimen is mapped, divided, and chromacoded, it is covered with embedding media while still maintaining its precise orientation. The technician then freezes the tissue in the block adfixed to the chuck. It is then secured in the object holder located on the microtome (Figure 5.1); the microtome is the instrument within the cryostat that actually cuts the tissue into microthin wafers that can be evaluated microscopically.

This procedure allows the tissue to be precisely oriented throughout the freezing process, resulting in finished slides that include the entire lateral and deep margins of the tissue removed by the surgeon.

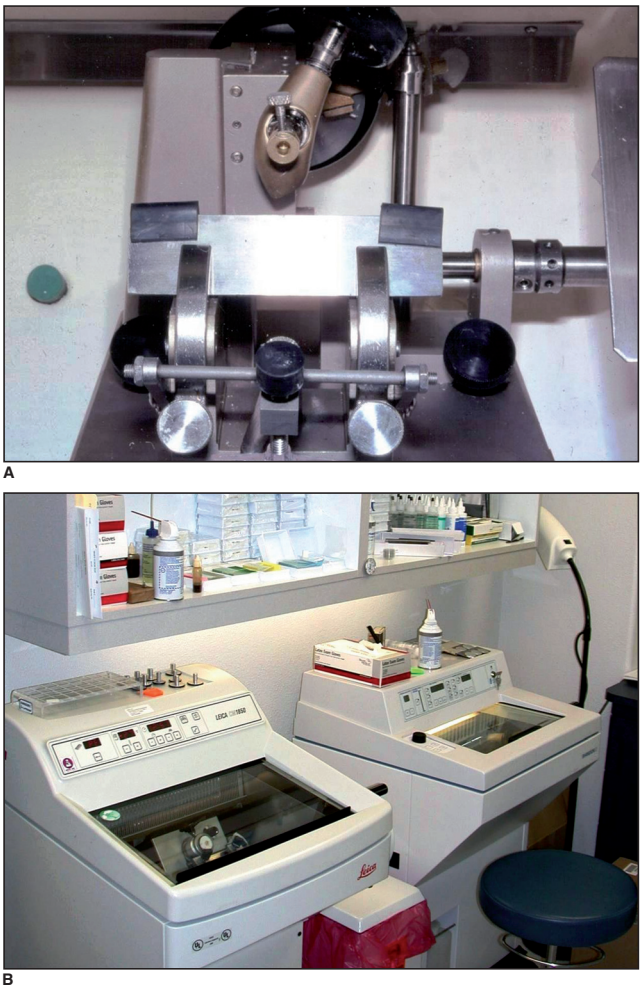


FIGURE 5.1: (A) View of microtome. (B) View of cryostat.

FREEZING AND MOUNTING SPECIMENS

There are many methods to freeze and mount specimens in the Mohs surgery lab. A method should be considered acceptable if it results in the production of thin wafers with complete margins represented. Working together, the Mohs surgeon and Mohs technician should find the most efficient methods to achieve their desired results.

All methods of freezing and preparing tissue involve adhering the tissue to a chuck (also referred to as an "object"

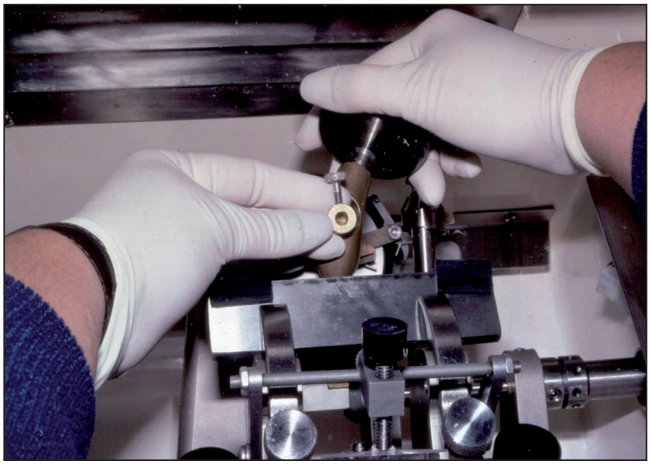


FIGURE 5.2: Ball and socket joint.

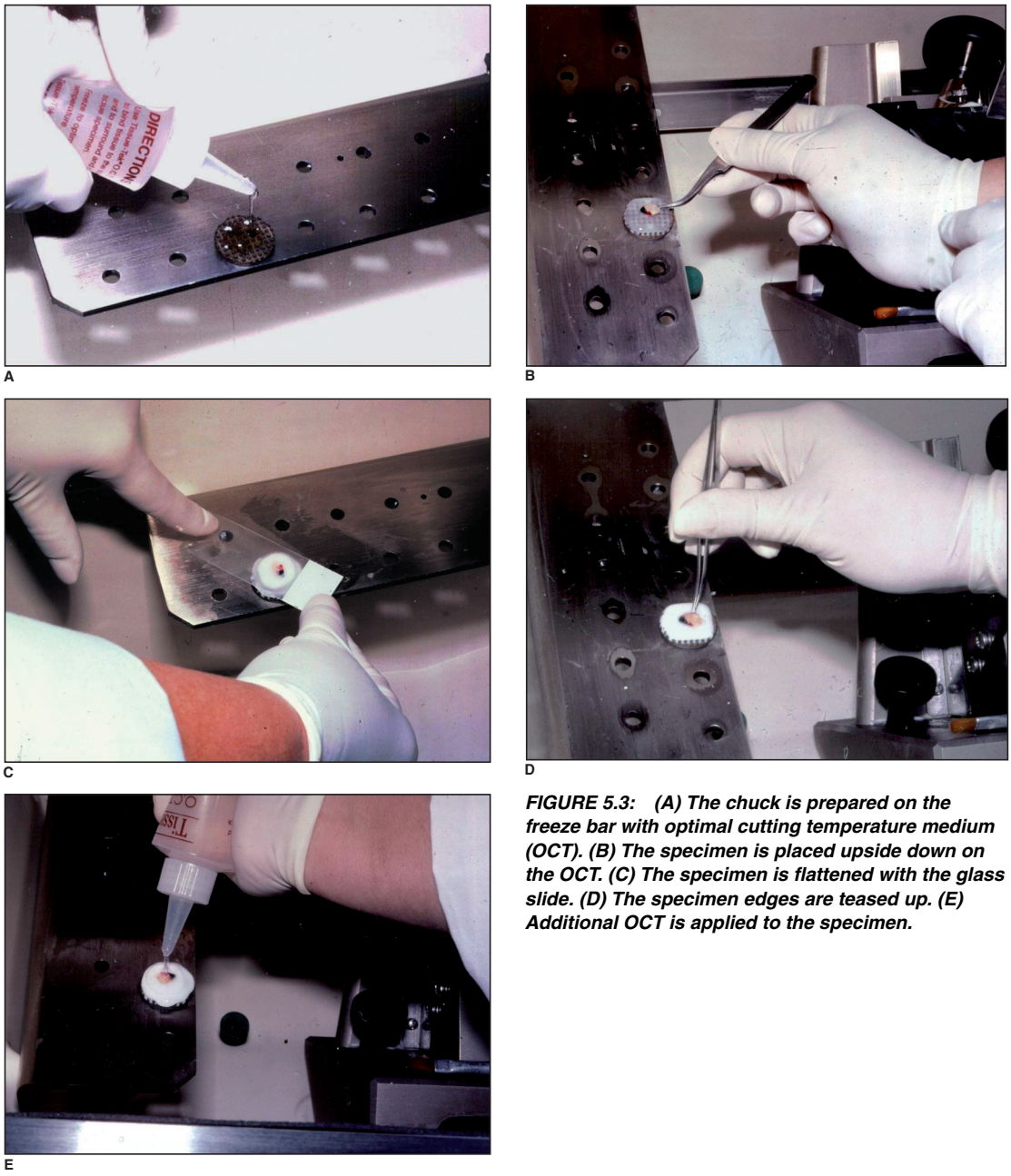


FIGURE 5.3: *(A) The chuck is prepared on the freeze bar with optimal cutting temperature medium (OCT). (B) The specimen is placed upside down on the OCT. (C) The specimen is flattened with the glass slide. (D) The specimen edges are teased up. (E) Additional OCT is applied to the specimen.*

or "microtome stage"). The chuck is a round or square metallic stage with a center post that is secured in the microtome by a clamp called the object holder. With rare exception, the object holder has an adjustable ball and socket joint (Figure 5.2) to allow for minor adjustments in specimen's alignment with respect to the blade. Some older cryostats have a fixed object holder, which means that once the specimen is frozen its alignment is set and cannot be modified.

The coldest part of a cryostat is the freeze bar. This area is 20°–30°C colder than the main part of the cryostat, permitting faster freezing of the embedded tissue. All the embedding methods discussed in this chapter utilize the freeze bar. A heat sink, which can dramatically speed up freeze times, can be used with most of the methods described below.

Direct Technique

The direct technique (Figure 5.3), or basic method, is little used today but serves as an excellent teaching tool for understanding the process.

1. A clean chuck is placed on the freeze bar and coated with optimal cutting temperature medium (OCT), or optimal

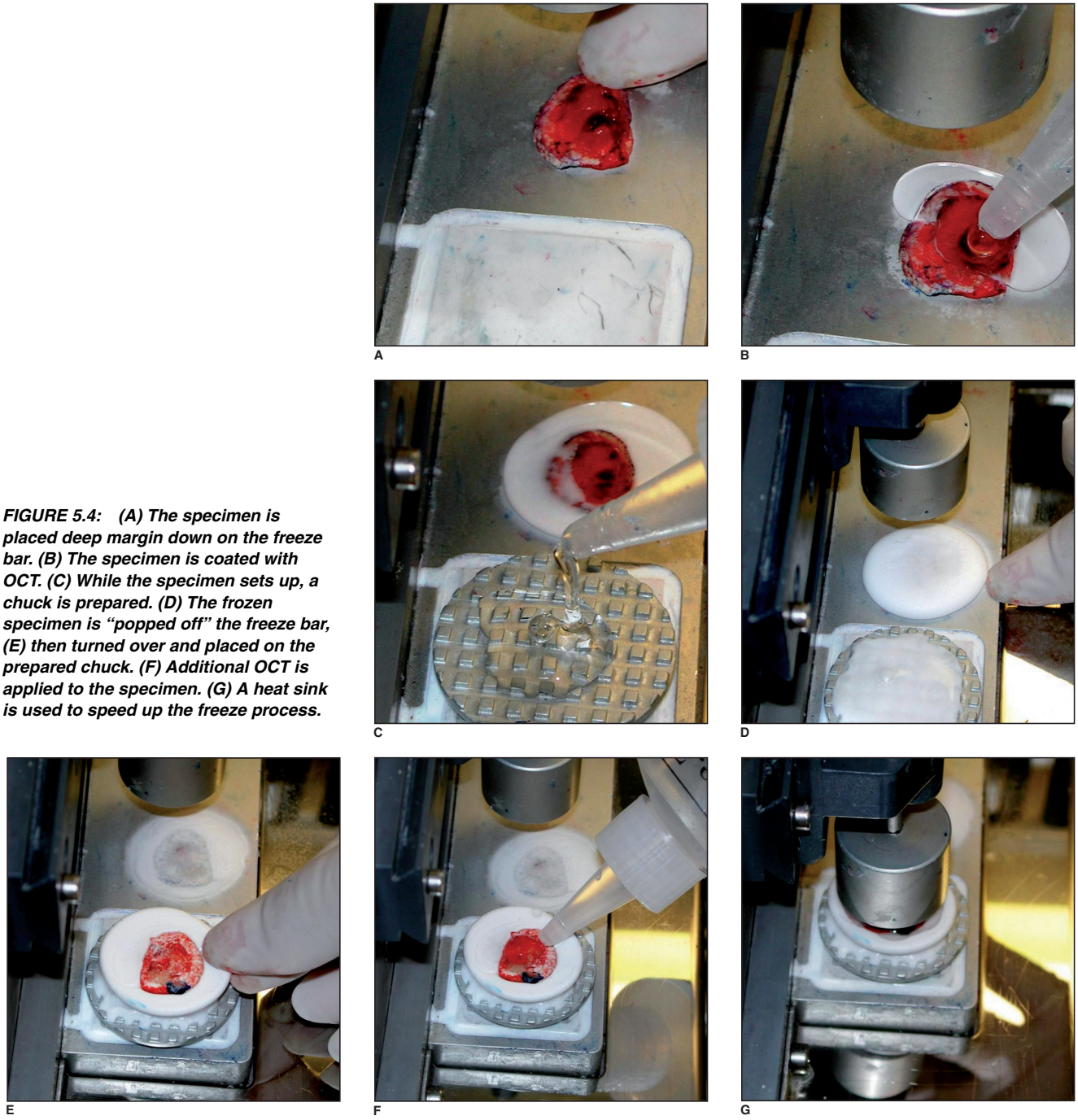


FIGURE 5.4: ***(A) The specimen is placed deep margin down on the freeze bar. (B) The specimen is coated with OCT. (C) While the specimen sets up, a chuck is prepared. (D) The frozen specimen is "popped off" the freeze bar, (E) then turned over and placed on the prepared chuck. (F) Additional OCT is applied to the specimen. (G) A heat sink is used to speed up the freeze process.***

cutting temperature medium, the embedding medium. While there are various brands, the term OCT is used here as a generic term for all types of embedding media.

2. As the OCT begins to set up (embedding media turns from a clear gel to opaque as it freezes or solidifies), the Mohs specimen is placed upside down on the semisolid OCT. The deep (surgical) margin of the specimen should be face up, as this is the surface that must be examined on the finished slide.
3. The specimen is flattened with a glass slide or other flat object.
4. The edges are checked to ensure that any edge not in plane with the surface is gently teased up or down with a fine pair of forceps or other delicate instrument.

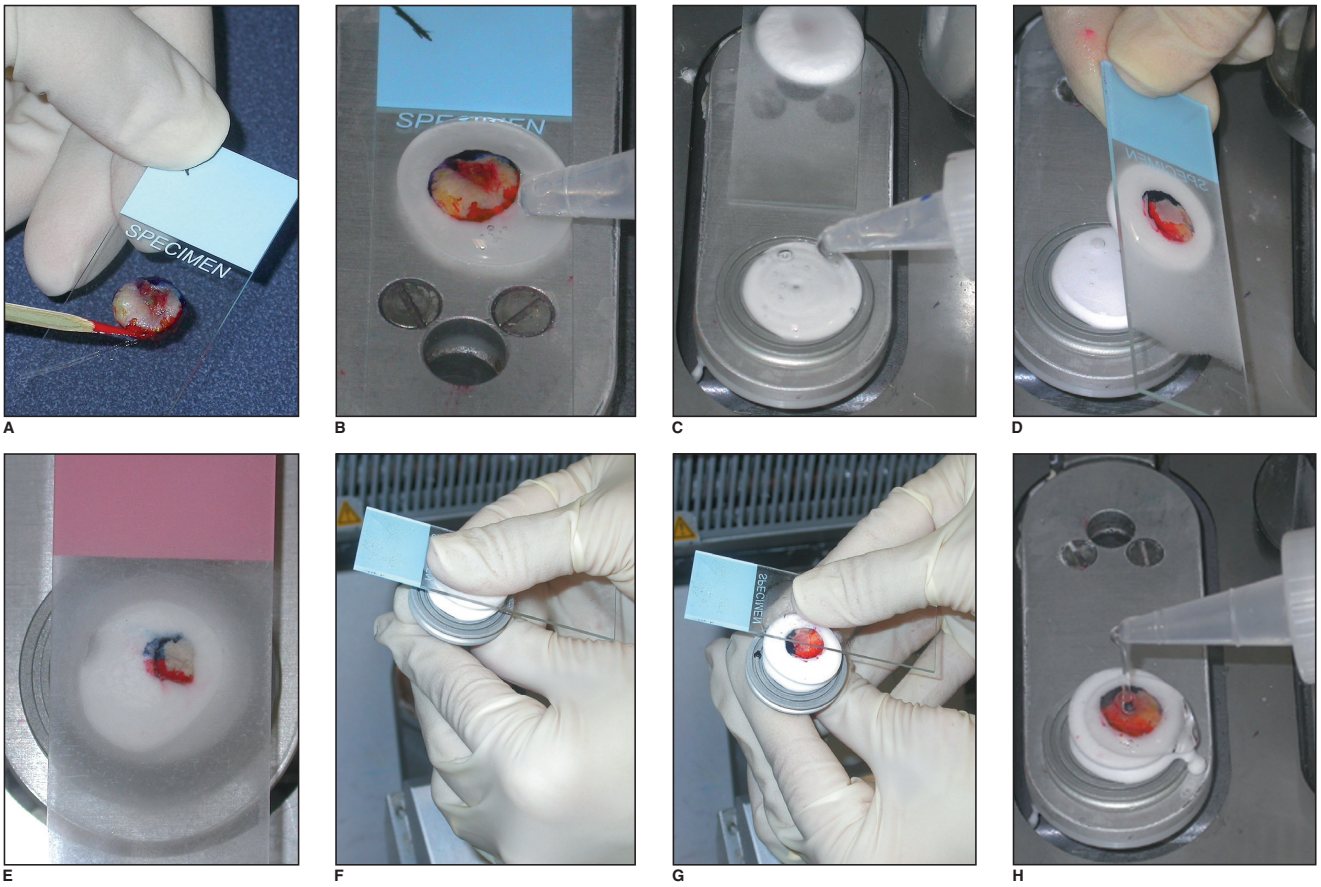


FIGURE 5.5: *(A) The specimen is received in the lab on a glass slide. (B) The slide is placed on the freeze bar and the specimen is coated with OTC. (C) As the OCT sets up, a chuck is prepared. (D) The slide is turned over and adfixed to the chuck. (E) The slide is frozen to the chuck. (F) A gloved finger is used to warm the back of the slide. (G) The slide is slipped off the specimen. (H) Additional OCT is applied to the specimen.*

5. Additional OCT is applied and allowed to freeze. The addition of OCT is common to all methods. It allows final orientation adjustments to be made through this OCT during the cutting phase without sacrificing tissue at the surgical margin.

The Freeze Bar Technique

The freeze bar technique (Figure 5.4) is a method that is relatively easy to learn and offers consistent and fairly rapid results. It requires a clean freeze bar.

1. The specimen is placed deep margin down directly on the freeze bar. The lateral edges are gently pushed down to assure a complete lateral margin.
2. The specimen is coated with OCT. As this OCT sets up, OCT is also applied to a chuck.
3. The specimen is "popped off" the freeze bar, turned over, and immediately placed on the semisolid OCT-covered chuck, and both are allowed to freeze together.
4. The surface of the specimen is then coated with additional OCT.

Glass Slide Technique

The glass slide technique (Figure 5.5) can be used with all types of specimens, but has proven particularly useful for very small or fragile specimens, such as those that involve tumors around the eyelids. In our laboratory, we maintain sterilized glass slides for use as our transfer media when a specimen is particularly fragile. This allows the specimen to be placed directly on the slide at the operating table, minimizing handling of the specimen and disruption of the tissue margins.

1. The specimen is placed deep side down on a glass slide. (If the specimen is thin, it can be chromacoded directly on the slide.)
2. The slide is placed specimen side up on the freeze bar and the specimen is coated with OCT.

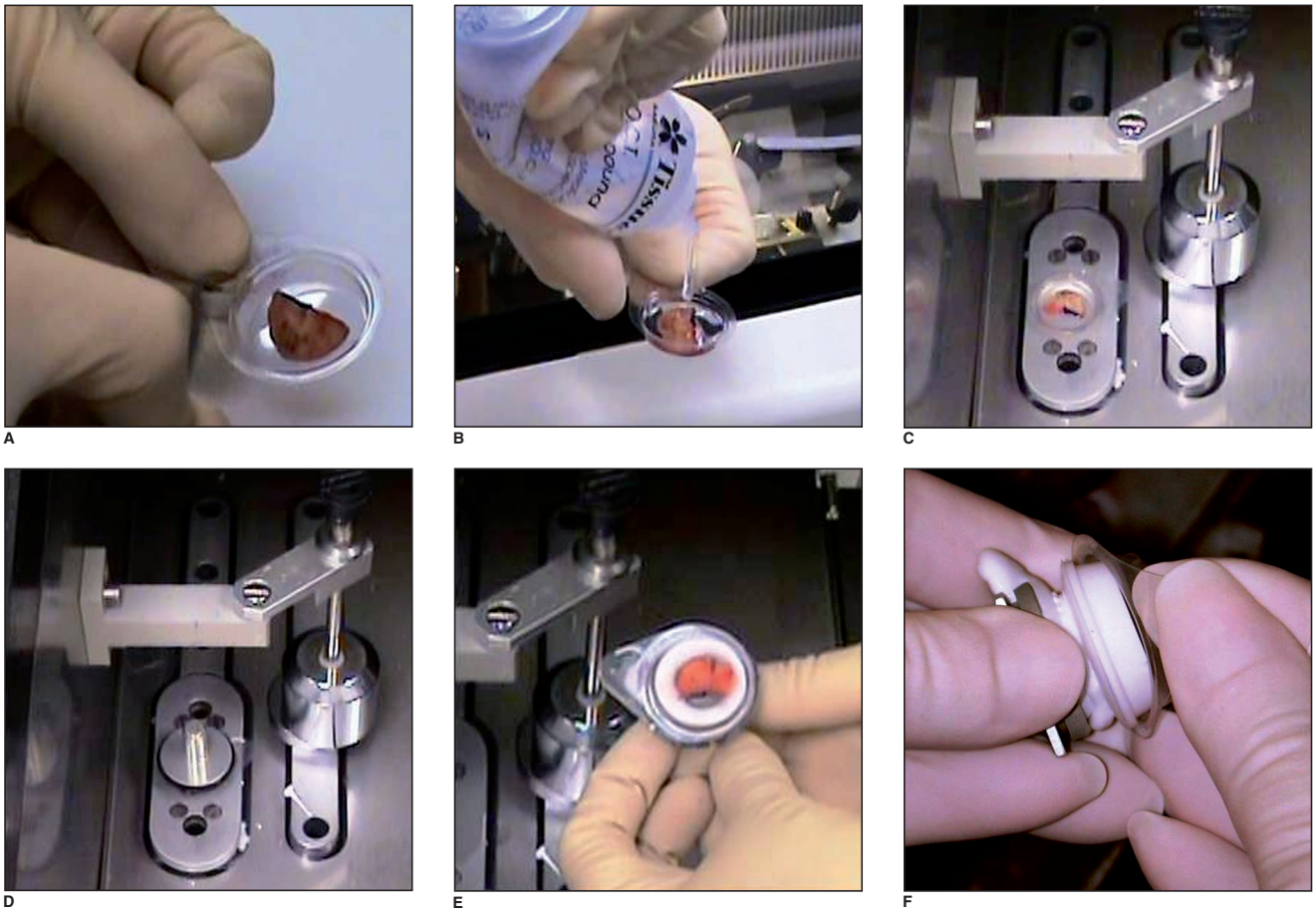


FIGURE 5.6: *(A) The specimen is placed deep side down in the cryomold. (B) The mold is partially filled with OCT (C), then placed on the freeze bar. (D) More OCT is added and a chuck is adfixed. (E) The cryomold and chuck are frozen together. (F) The mold is peeled away from the specimen.*

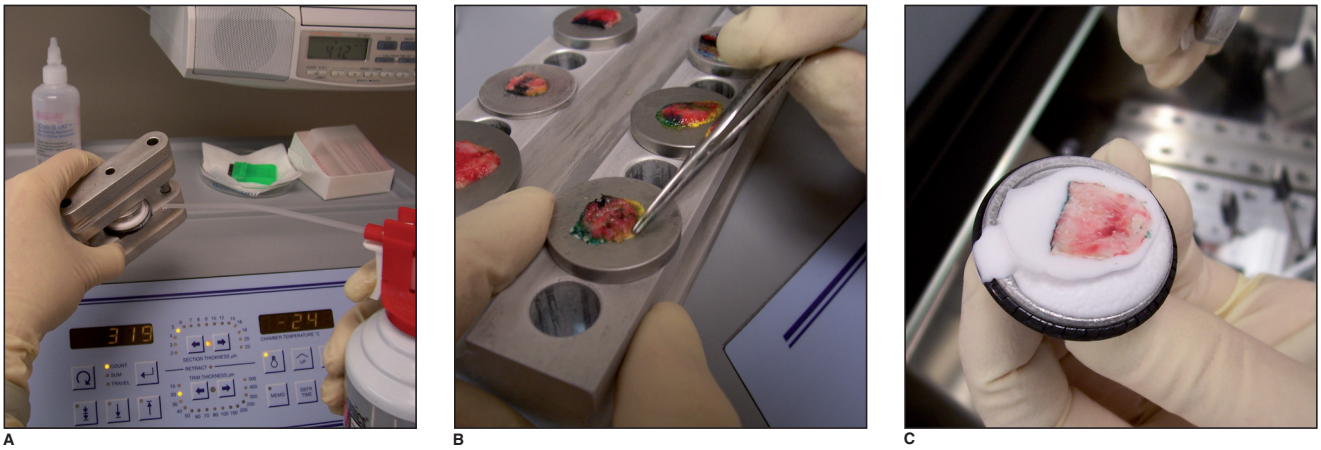


FIGURE 5.7: *(A) The cryoembedder. (B) Specimens are applied to chucks in the cryoembedder. (C) Tissue processed with the cryoembedder.*

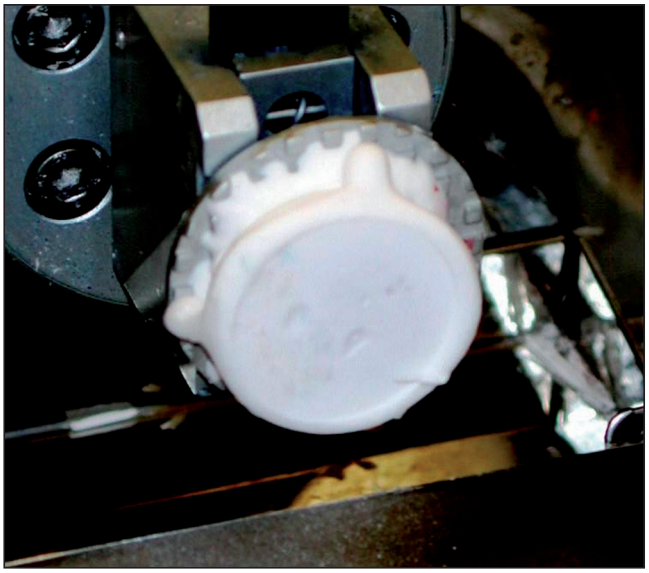


FIGURE 5.8: The frozen specimen on the chuck is placed in the object holder.

3. As the OCT is setting up, a chuck is prepared on the freeze bar with OCT.
4. When the OCT on the chuck begins to set, the slide is turned over and placed on the chuck OCT to OCT where they are allowed to freeze together.
5. After the slide and chuck are firmly adfixed, the slide is removed. This can be accomplished by placing a gloved finger on the back of the slide, which quickly warms it up, allowing easy separation of the slide from the specimen.
6. The specimen is coated with an additional layer of OCT.

FIGURE 5.9: "Facing the block."

FIGURE 5.10: Final alignment adjustments are made.

Cryomold Technique

The cryomold technique (Figure 5.6) utilizes a plastic disposable mold that, when used properly, aligns the specimen and the chuck in a consistent manner. This method is strongly preferred by technicians working with a cryostat that does not have an object holder with an adjustable ball and socket joint.

1. The specimen is placed deep side down on the bottom of a room-temperature cryomold. Care must be taken to assure the lateral margins are pushed down and are in the same plane as the deep margin.
2. A small amount of OCT is placed in the mold, which is then placed on the freeze bar.

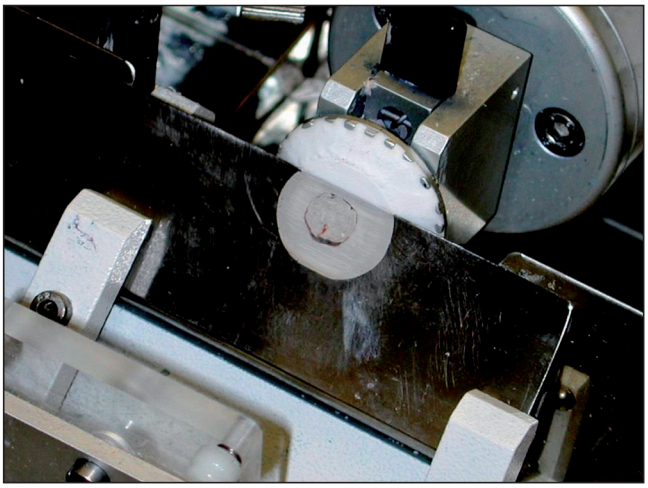


FIGURE 5.11: The specimen is cut and is ready for "pick up" onto a slide.

3. As the OCT in the mold begins to freeze, more OCT is added and a chuck is placed on top of the mold.
4. Once frozen, the mold is peeled away and the specimen is ready for processing. In many labs, additional OCT is placed on the specimen to allow for final alignment in the microtome.

Other Methods

Through the years, creative Mohs surgeons and inventive Mohs histotechnicians have developed various devices and methods using liquid nitrogen and/or metals that exchange heat at a faster rate and speed up the freeze process. An example of a device in use today is the cryoembedder (see Figure 5.7.)

Cutting the Tissue

The chuck with the attached tissue is placed in the object holder and the process of cutting the tissue begins (Figure 5.8). The basic steps include:

1. The cryostat is set to the proper temperature (usually –20°C to –29°C) based on the type of tissue to be cut. It is important to gauge the temperature at the microtome blade, which may be determined by placing a thermometer on the blade and comparing the results with the readings on the cryostat. Although fat is optimally cut at colder temperatures than cartilage, it is not necessary to reset the cryostat temperature for each type of tissue being cut. Instead, when cutting fat, the technician may spray the embedded fat-containing specimen with a cryogen to lower its temperature before cutting.
2. The specimen is aligned in the object holder at 90 degrees to the blade.
3. The micrometer is set to a thick cut as the outer layer of OCT is pared away or rough-cut. This is called "facing the block" (Figure 5.9).
4. As soon as tissue appears in the OCT, it is time for final alignment (Figure 5.10). This is the adjustment of the chuck to ensure a flat plane across the entire specimen.
5. At this point, the micrometer is reset for final cutting thickness, usually 4–10 microns. Epidermis and mucosa may be cut thinner, while fat may require thicker sections.
6. As tissue wafers are cut (Figure 5.11), they are placed on the microscope slides and are ready for the staining process. (See Chapter 7.)

In most Mohs labs, the techniques used by the histotechnician have evolved over time and depend upon the skills and preferences of the surgeon and the skills and experience of the Mohs technician. Achieving final slides with thin, well-stained specimens featuring complete margins is the goal. To reach this goal, a team approach with meticulous attention to detail and a professional cooperative atmosphere between the Mohs surgeon and the Mohs histotechnician is essential.

PEARLS

- Brushes, gauze, and all other implements used by the technician to process tissue should be stored within the cryostat to keep them cold. If they are warmer than the specimen, the specimen wafers may melt and adhere to their surfaces.
- Blades must be clean and sharp.
- A clean, polished freeze bar is essential.
- Use blade guards for protection. Most cryostat injuries are cuts sustained while attempting to process tissue quickly.
- The microtome must be properly maintained and lubricated. Even the slightest imperceptible vibrations and movements can result in artifacts on the slide.
- Consistency is essential when placing tissue wafers on the slide. The first cut wafer should always be placed either nearest to the label or farthest from the label.
- Specimen (wafer) orientation on the slides should be maintained. This enables the surgeon-pathologist to quickly go from wafer to wafer on a slide without losing orientation to the microanatomy within the wafers.
- The same universal precautions followed in the operating room should be followed in the lab. All histologic debris should be treated as contaminated.

PART TWO

INTRODUCTION TO LABORATORY TECHNIQUES

Alex Lutz

AFTER EXCISION, the Mohs specimen is normally placed on gauze or other media having a defined and visible 12 o'clock reference mark that corresponds to that of the specimen and the Mohs map. The specimen is left whole or subdivided into smaller pieces, based on its size and surgeon preference. It is then left to lay flat, chromacoded by the surgeon or technician, and processed into pathology slides by frozen sectioning. The slides are stained with hematoxylin and eosin (H&E) or toluidine blue and delivered along with the map to the Mohs surgeon who performed the surgery.

As the popularity of Mohs surgery has grown, so has the demand for concrete knowledge of practical, efficient, and effective Mohs tissue processing techniques. Chapters 6, 7, and 8 are intended to serve as a reference for making high-quality Mohs slides. They will also discuss structural changes that occur when tissue is processed and how knowledge of these phenomena can improve the slides produced. The techniques and pearls discussed are based on the author's personal experiences over the past 15 years.

Of all the assets at a Mohs technician's disposal, the most important is "frame of mind." When processing tissue, technicians must assume that anything that might go wrong to yield a negative outcome is in their control and is a direct result of their handling of the specimen or equipment. Keep an open mind and assume self-fault to constantly improve. The idea that some specimens just dislodge from the tissue freezing medium (TFM) or that some epidermis is impossible to lay flat is incorrect leads to persistently poor results and limits the ability to improve.

CHAPTER 6

Lab Pearls: Making Great Slides

Alex Lutz

MOHS TECHNICIANS have one primary goal: the production of perfect slides. Consistently making high-quality slides has as much to do with problem solving as with technical skill. Problem-solving ability comes with experience. This chapter will discuss techniques and problem-solving skills that the author has found aid in producing excellent slides.

LAB EQUIPMENT

High-quality slide production requires well-functioning and well-maintained lab equipment. It is surprising how poorly the cryostats in some laboratories cut tissue. This is usually the result of years of poor maintenance. Cryostat refrigeration and microtome components must be serviced at least every six to 12 months to cut tissue properly. Without a properly cutting cryostat, it is nearly impossible to make excellent slides. When working with a poorly maintained cryostat, the technician can only partially compensate for the "play" in the microtome. Such cryostats will usually alternately cut thick and thin wafers. When this happens, the technician often places the thick wafers on the slides and discards the thin ones. When presented with a cryostat in need of repair, the technician should arrange for microtome maintenance before the next Mohs surgery session.

SPECIMEN RETRIEVAL

During specimen transfer from the patient to the Mohs laboratory, many things can go wrong: specimens may be misoriented on the transfer medium, become lost, or assigned to the wrong map. These errors can be minimized by establishing a specimen retrieval protocol in which each step of specimen transfer to the Mohs laboratory is addressed. All involved personnel should review and "sign off" on the protocol, which should then be strictly enforced.

Details to consider when writing a specimen retrieval protocol:

1. Who will record patient information (such as name and site) on the Mohs map?
2. Where will the map be placed in the surgery room?
3. Will the specimen outline be drawn before or after the excision?
4. Where and on what type of transfer media will the specimen be placed after removal from the patient?
5. Will the patient's chart accompany the map to the Mohs laboratory?
6. How will the 12 o'clock specimen margin be denoted on the transfer medium?
7. Will the specimen and transfer medium be placed in a covered Petri dish?
8. Will saline solution be placed on the specimen and transfer medium before transfer to the Mohs lab?
9. Who will take the specimen to the Mohs lab (the physician, nurse, or Mohs technician)?
10. Will the physician or technician decide whether and how to divide the tissue?

There is no "best" answer to these questions, but all of these factors must be considered and a standard, repeatable protocol developed in each office performing Mohs surgery.

SPECIMEN PREPARATION

Prepping Board

This author (A.L.) prefers a 9 × 12 × 1/4-inch–thick white plastic chopping board as the prepping board. The large grossing boards sold by laboratory supply companies are too bulky, and wood cutting boards can trap tissue and blood, making them difficult to clean. A permanent 12 o'clock designation should be drawn on the board with a Sharpie pen. When processing tissue, place the specimen on the board so its 12 o'clock hatch mark is aligned

FIGURE 6.1: Prepping/staining work area.

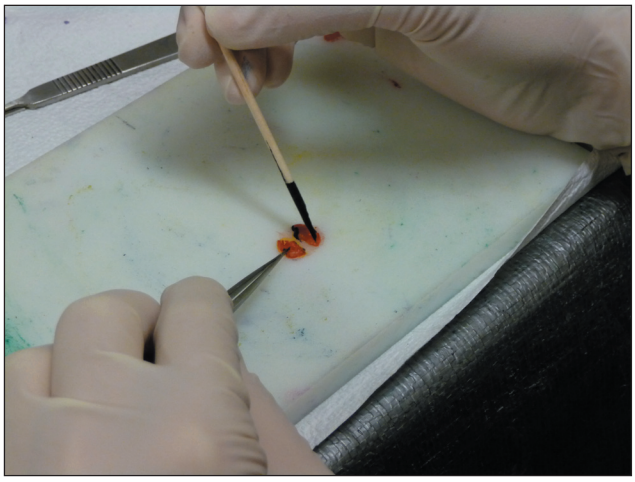


FIGURE 6.3: Specimen inked with 12 o'clock orientation aligned.

with the 12 o'clock mark on the prepping board. The small prepping board can then be turned in any direction while maintaining orientation. It is better to turn the prepping board than to pick up and rotate the specimen. This feature is also important, because the technician must often attend to other tasks before completing work on a specimen on the prepping board. When the technician returns to complete preparation, specimen orientation is still maintained (Figures 6.1–6.4).

Processing Specimens Whole

Mohs surgeons differ in how they prefer to process first-stage specimens. Many prefer to embed first-stage tissue as a single, whole block. When the technician receives the specimen, surface tension will have drawn the superficial tissue edges toward the center. This may require correction using relaxing incisions. To place these incisions, first verify orientation and transfer the specimen to the prepping board, aligning the specimen's 12 o'clock mark with that on the cutting board. Then cut a relaxing incision parallel to the specimen's entire epidermal edge, positioned at 1/4–1/3 of the specimen's radius in from its edge. Bevel the incision about 30 degrees from horizontal and incise through 1/2–2/3 of the full thickness of the specimen. The depth and angle of the relaxing incisions are important to permit the epidermal edge to fillet without creating a false margin. If the incisions are made too shallow, they are ineffective; if they are cut too deeply, they can potentially disrupt the deep margin on later wafer cuts. Additional vertical relaxing incisions through the center of the specimen are made as necessary. Wipe the blade frequently while making these incisions so tumor is not carried from one area of the specimen to another on the knife blade.

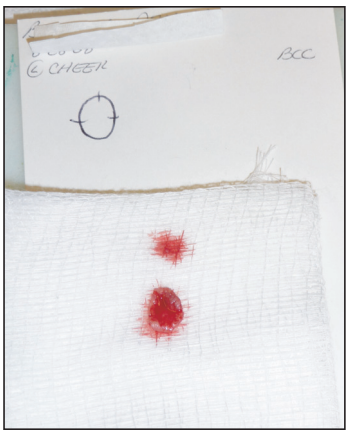


FIGURE 6.2: Specimen with map.

For specimens processed whole, extend and deepen hatch marks made by the surgeon to expose the dermis. This aids adherence of the outer edge of the specimen to the tissue freezing medium (TFM) and helps prevent epidermal folding ("roll-ups"), particularly on the edge nearest the microtome blade. Position the specimen so a hatch mark is the first area to contact the microtome blade. Because TFM holds more firmly to the exposed dermis in the hatch mark than the epidermal edge, this helps prevent the epithelial edge from separating from the TFM and rolling up or folding.

Subdividing Specimens

Within any specimen, the surgeon's goal is for tumor cells to be in the superficial/central portions only, not at the deep surgical margin. Thus, when subdividing specimens into smaller pieces for processing, it is important to use proper technique to prevent small foci of tumor from dislodging and moving to the margins. Always use a sharp blade to prevent tissue from being dislodged and carried to

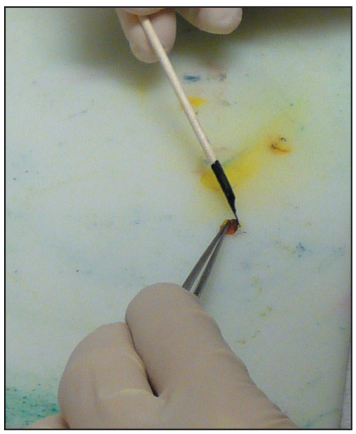


FIGURE 6.4: Whole specimen being inked.

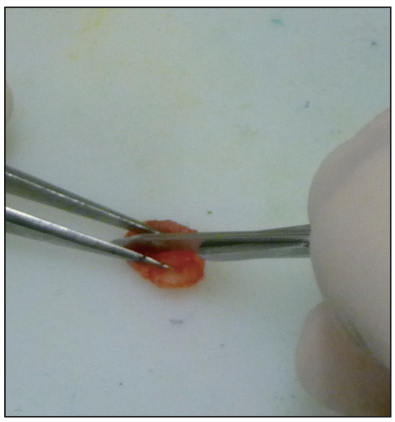


FIGURE 6.6: Scalpel blade should be in contact with cutting board prior to bisecting.

a margin, and frequently wipe the blade. Either make two cuts from the peripheral edge toward the center on opposite sides of the specimen, thus bisecting it, or cut from one peripheral edge across the specimen to the opposite edge in one continuous motion. Ensure that the blade is in contact with the cutting board prior to its contacting the specimen and remains so during the entire cut. Avoid any downward motion as cuts are incised. This will tend to force any dislodged tissue fragments laterally and minimize the chance of superficial tumor cells moving downward into the deep, "true" margin (Figures 6.5 and 6.7).

Prepping the Specimen to Lay Flat

One of the most important and essential aspects of properly prepping the specimen is to ensure that its surgical margin can be laid flat for Mohs tissue processing. This is the foundation of Mohs histology slides. If the specimen is not prepped to lie flat, it is impossible to achieve 100% representation of the contiguous surgical margins without cutting excessively into the tissue block.

If the Mohs specimen is properly prepped, initial tissue wafers containing complete margins can be produced by cutting no more than 2–5% into the block. In total, no more than 10–20% of the block need be cut to produce the "step-sections" most Mohs surgeon-pathologists require. No matter how well the tissue is prepped, the first wafer placed on the slide seldom contains the complete surgical margin. Because this first wafer is closest to the surgical margin, it is important to have it on the slide even if it is not "complete." Indeed, some surgeons prefer that this first wafer be "incomplete" to ensure that the technician has not cut excessively into the tissue block before placing wafers on the slides.

Technicians employ many different strategies for flattening the specimen, from "squashing and freezing" the specimen to incising perpendicular cuts deeply into the specimen, creating a "blooming onion" effect. The concern with squashing and freezing is that it creates tension in the specimen that remains after it has been frozen. This may cause the specimen to recoil out of the TFM and roll up into an unusable shape when it is cut on the microtome. The deeply cut, "blooming onion" technique has a very high chance of creating a false-positive margin if cuts are made too deeply into the specimen and these cuts appear on deeper tissue wafers. When a specimen is excised, natural tissue tension may prevent the peripheral, epidermal edge from laying flat. When the specimen is excised, its intrinsic skin tension is released and the peripheral edges lift and

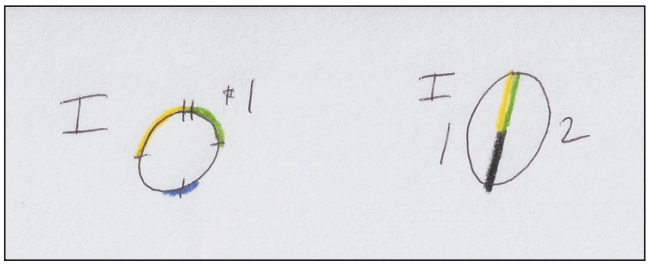


FIGURE 6.5: Map examples of whole vs. bisected specimens.

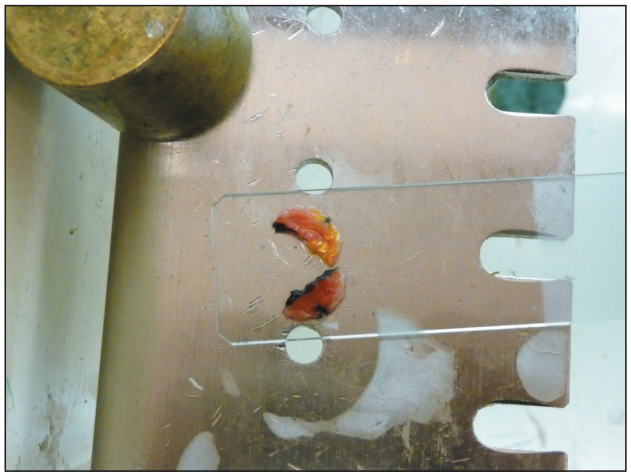


FIGURE 6.7: Bisected specimen ready for embedding.

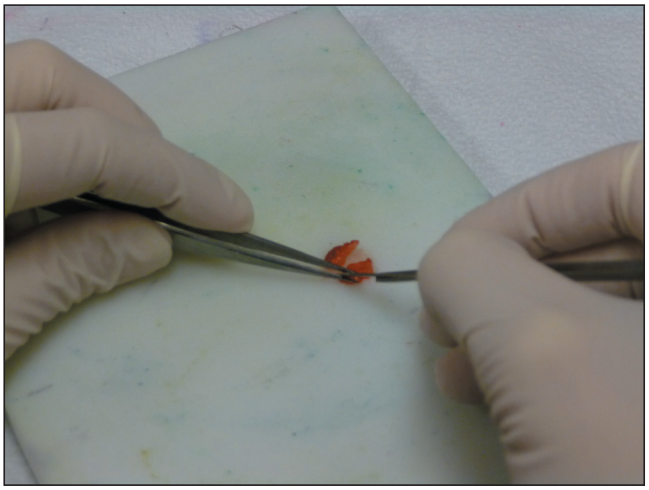


FIGURE 6.8: Prepare specimen to lay flat with incisions parallel to the epidermis.

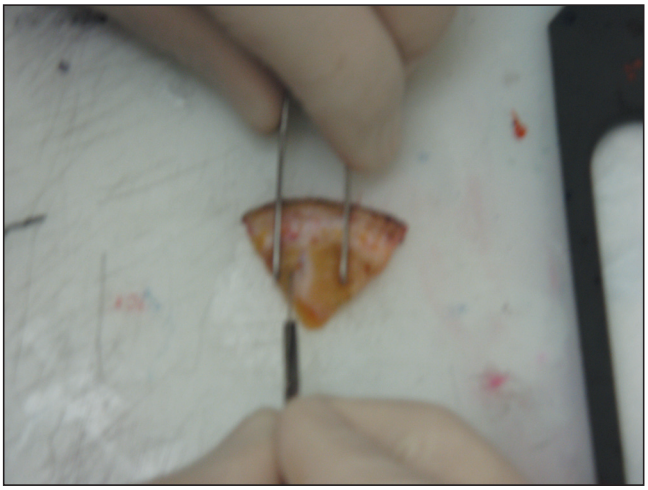


FIGURE 6.10: Vertical relaxing incisions to help the specimen "accordion out."

retract centrally. This surface tension must be relaxed for the specimen to lay flat. This is achieved by making relaxing incisions at a bevel of 20–45 degrees, parallel to the epidermal edge, between the epidermal edge and the center (Figures 6.8–6.10). These relaxing incisions are often critically necessary and can be used on all specimens regardless of whether they are processed whole or subdivided into multiple blocks and are circular, triangular, square, or another shape.

Additional vertical superficial incisions may be placed within "problem areas" to further ensure that the specimen lies completely flat. Together, these superficial vertical and beveled incisions permit the specimen to "accordion out," and place the peripheral and deep margin in the same plane. Dumbbell or infinity symbol–shaped specimens are best relaxed with lengthwise cuts.

Sharply angled corners often cause problems. The best technique for these is to incise vertical relaxing incisions through their apices. The technician must document the location of these incisions on the Mohs map and alert the Mohs surgeon-pathologist.

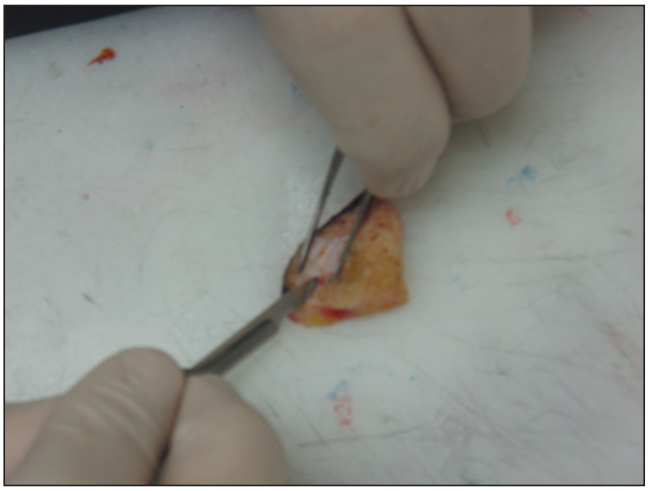


FIGURE 6.9: Relaxing excisions (30 degrees from the horizon and parallel with the epidermal edge).

"Pac-Man"–shaped, through-and-through, vertical incisions may be used to relax circular and oval incisions. The cut surfaces must be completely inked to allow the Mohs surgeon-pathologist to ascertain whether the entire area is represented on the slides.

Prepping Specimens with Fat

Many Mohs surgeons and technicians do not place enough importance on complete fat representation on the slides. Skin cancer generally grows in the path of least resistance and may extend into fat. Often, only the fat margin may contain residual tumor. It is essential that all margins, even those with abundant fat, are represented 100% on the slides to ensure complete removal of the cancer.

Many technicians have difficulty preparing slides with complete deep margin representation from tissues containing fat. This problem can be solved if the correct lower tissue-freezing temperature is achieved, because fat has a lower freezing point than dermis or epidermis. When the technician processes a fatty, multiple-block specimen and embeds all pieces at the same time, fat on blocks processed later will present better than fat processed earlier, because the fat in the blocks processed later has had more time to freeze. One method to cut fat better is to allow the tissue to sit and freeze in the cryostat for 20 to 30 minutes. Time constraints and surgeon demands, however, often require that slides be processed more rapidly.

More rapid freezing can be achieved by spraying specimens with liquid nitrogen. Liquid nitrogen should be used exclusively because canned freeze sprays will not cool fat sufficiently to be cut effectively. Canned freeze spray contains 1,1,1,2-tetrafluoroethane with a freeze temperature

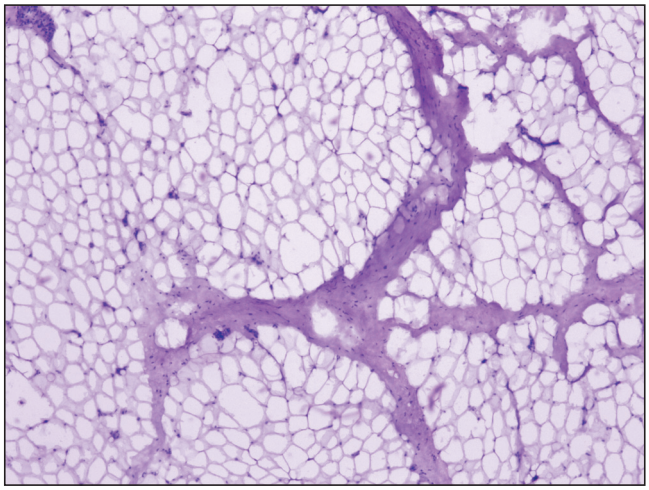


FIGURE 6.11: Fat with connective tissue.

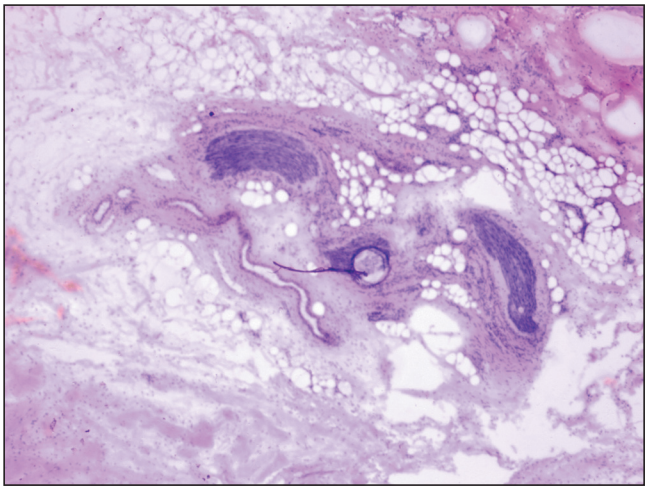


FIGURE 6.12: Nerve in fat.

of −51°C. Liquid nitrogen has a freeze temperature of −197°C, and spraying the fat directly with it while cutting will help get better sections of fat. It is vital that airborne-pathogen precautions be followed when spraying tissue in the cryostat to prevent bacteria, viruses, and other debris inside the cryostat from blowing into the technician's lungs. A safer, alternative technique is to spray liquid nitrogen on the tissue block outside the cryostat and then promptly return the tissue to the specimen holder. Another technique is to pour liquid nitrogen into an insulated cup and apply it to the face of the block with a large cotton swab. If all else fails, thicker wafers can be prepared just for representation of the fat. The technician should first produce thin cuts (6 microns) for representation of the epidermis and dermis and then take one or two thick cuts at 16–40 microns to represent the fat.

The Mohs surgeon-pathologist requires thin cuts of the epidermis and dermis to achieve a "mono-layer" of cells for optimal pathology interpretation. Thicker wafers are 3–4 cells thick and make pathology evaluation for cancer much more difficult. Thick cuts, however, are an option (a poor option, from the pathologist's point of view) for fat to obtain complete representation and permit reasonably accurate pathology interpretation. All Mohs technicians should be expected to provide thin wafers for epidermal and dermal pathology interpretation and, if necessary, thicker wafers with complete representation of the margins containing fat (Figures 6.11 and 6.12).

Prepping Specimens with Cartilage

Specimens with cartilage must be processed differently than other tissues. Cartilage specimens are prepped using the same relaxing incisions previously described. In some cases, when these techniques are inadequate to produce a flat specimen, the "squash and freeze" technique may be the only one that will allow the specimens to lay flat.

With the squash and freeze technique, the specimen is sandwiched between a glass slide and the prepping board, with the deep margin in contact with the slide. The slide is then sprayed with liquid nitrogen until the deep portion of the specimen is frozen in the desired flat position. The specimen's edges are then teased and pushed down with forceps while the base/deep margin remains frozen and in contact with the glass slide. The specimen is then covered with TFM and embedded before it can thaw and unflatten.

Alternately, the specimen can be placed on the frozen steel of a heat sink or freeze bar until it freezes. Then the edges are teased down until they contact the metal and freeze in place. A bead of TFM is then placed over the specimen to freeze and encapsulate the specimen. The frozen specimen-containing bead is popped off the steel and placed bottom side up into a bed of gelatinous TFM that has been placed on a warm microtome chuck. The bead, TFM, and chuck are then frozen together as one block. The use of this technique is not optimal but may be the only method available. Without using one of these techniques, the technician may need to cut through the entire specimen to obtain full representation of all the tissue margins on the slides. It is helpful for the Mohs surgeon to inform the technician when specimens contain cartilage to make the technician's job of preparing quality slides easier (Figures 6.13 and 6.14; see also pages 52–56).

Prepping Periosteum

Specimens containing periosteum are most commonly encountered during excisions of deeply penetrating squamous cell carcinomas of the scalp. The periosteum is usually excised separately from other scalp tissues in hope that it will be free of tumor, indicating no bony invasion. Periosteal tissue is thin, highly vascular, with a great deal of elasticity. This gives it a tendency to roll under and stick to itself. Because periosteum is only 1–2 mm thick, it requires

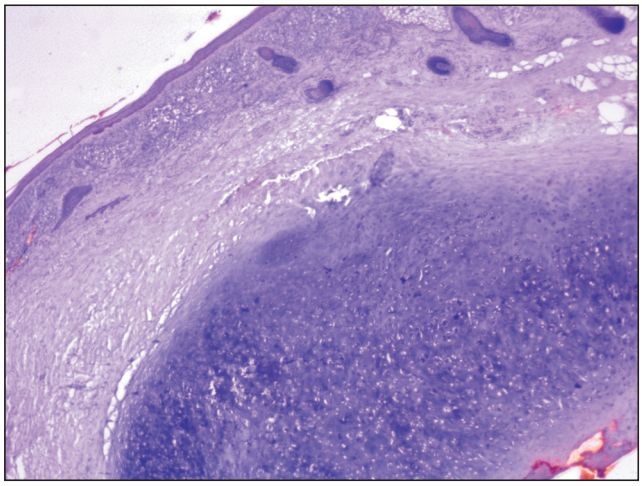


FIGURE 6.13: Cartilage example.

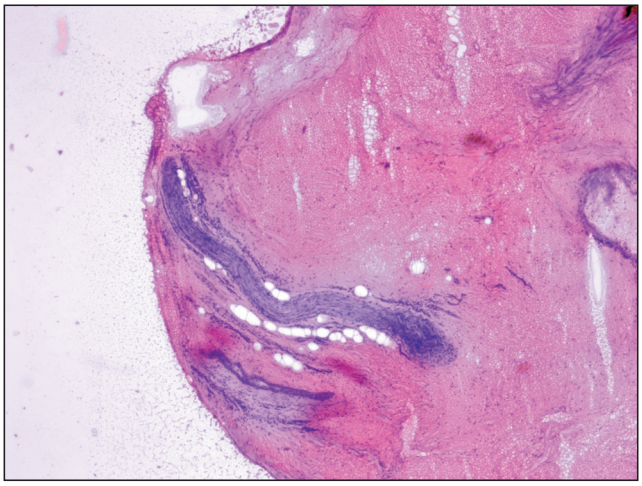


FIGURE 6.15: Periosteum with nerve.

very careful processing because there is no extra tissue to "waste."

When processing periosteum, ensure that the lateral edges are lying flat and are fully unfolded. Folds may produce a false-negative margin if tumor resides and is hidden from view within the fold. The technician may not section through the thicker fold, erroneously thinking a complete margin is already represented on the slides when it is not. Periosteum must be carefully teased until no edges are folded and any air gaps are noted and removed. It must be accurately mapped, inked, and embedded using a slide or cryomold, carefully inspecting for full deep and lateral margin exposure.

The technician must be extra diligent in adjusting the specimen holder/ball joint so that the flat face of the specimen is perfectly parallel and equidistant from the microtome blade. If this is done correctly, the block should "face off" within 30 microns, and wafers can be placed on slides immediately. Very thin wafers must be cut because there is very little available tissue to process. All periosteum specimens should have sections taken until the entire block of tissue is completely cut through (Figures 6.15 and 6.16).

Prepping Complex Mucosal Tissues: Eyelid, Oral, and Genital

Eyelid, oral, and genital tissues are sites where tumor frequently crosses epithelial boundaries from epidermis to mucosa. To properly process these specimens, the technician must have a complete understanding of the margin's composition. If an eyelid specimen contains conjunctival mucosa as a significant percentage of the peripheral margin, the physician must inform the technician of it before the tissue is processed. Otherwise, the technician could easily mistake the conjunctival epithelial edge for dermis and process the specimen as though that portion of the peripheral margin lacked epithelium.

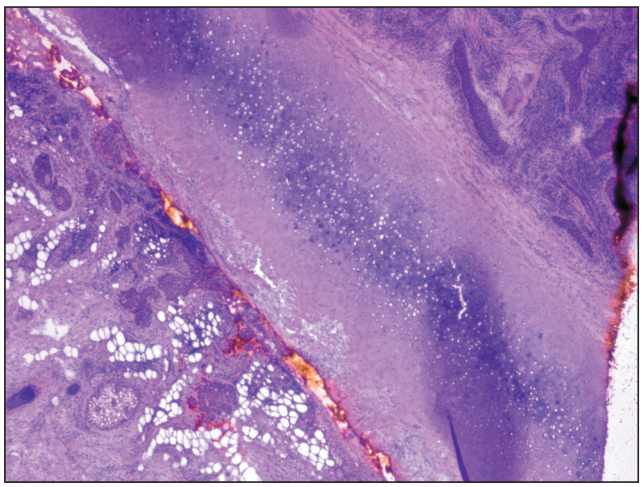


FIGURE 6.14: Cartilage surrounded by basal cell carcinoma.

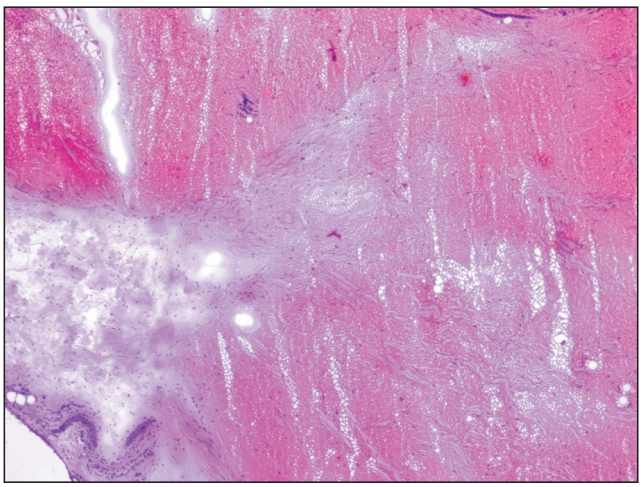


FIGURE 6.16: Periosteum with blood.

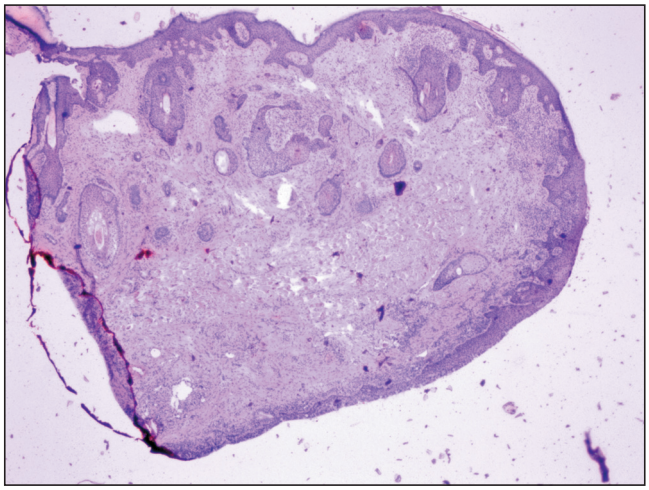


FIGURE 6.17: Section representing eyelid conjunctival epithelium/epidermal junction.

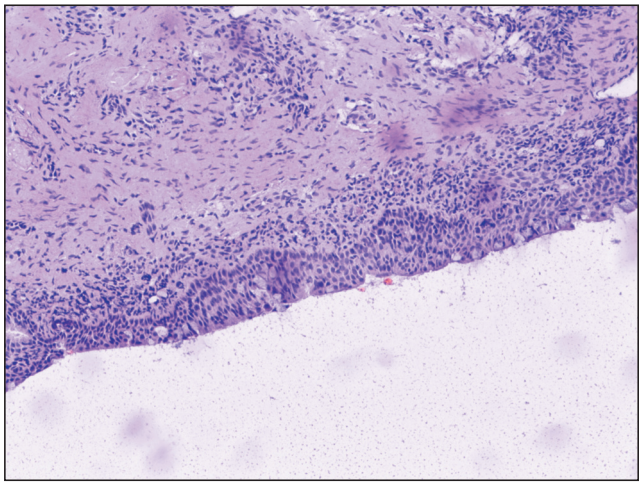


FIGURE 6.19: Conjunctival epithelium (magnified).

From the technician's standpoint, site locations involving oral or conjunctival mucosa tend to be very elastic and sticky. For this reason, the technician needs to ensure that the outer edges are teased and laying flat and the outer rim of epithelium is present (Figures 6.17–6.19).

Embedding the Specimen

The specimen is embedded by gluing it to the metal chuck (specimen disc) with TFM, which is designed to bond to both tissue and metal. Mohs specimens are best embedded at close to room temperature. Maximal surface area is critical to proper embedding. Nearly all chucks used in Mohs surgery are sandblasted or chemically treated to increase their surface area and give the TFM more surface area for adherence. Because of specimens' high water content, water-based TFM is used. The polyvinyl glycol component of most TFMs keeps the medium softer at cryostat temperatures to avoid "chatter" and "shattering" on the blade during cutting. The best technique is to embed a room temperature specimen onto a hand-warmed chuck. This will help prevent the specimen from popping out of the TFM and the TFM from separating from the chuck. If TFM is applied to an already frozen specimen or chuck, it will freeze on contact and not "seep in" and take advantage of all available surface area. If specimens consistently separate from the chuck, the technician should check to see what detergent is being used to clean the chucks. Detergent residue can interfere with proper binding of TFM to the chuck.

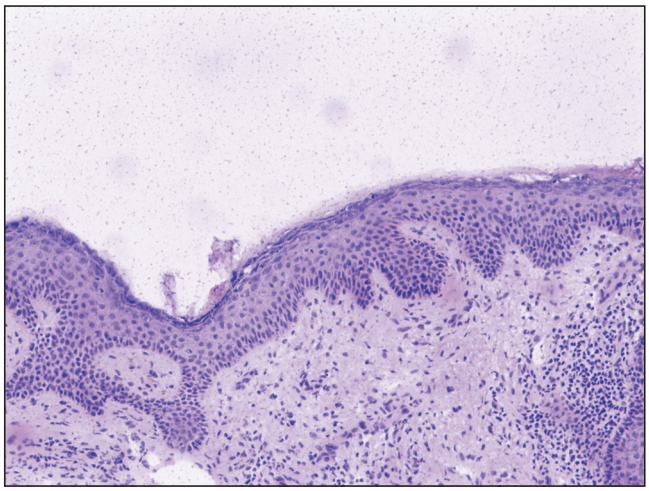


FIGURE 6.18: Eyelid epidermis (magnified).

Embedding Techniques (See Also Chapter 5). Many effective embedding techniques have been developed over the years. A brief overview of these techniques follows, concluding with those this author (A.L.) feels are best for tissue embedding.

Direct Mount Technique. The oldest technique is the direct mount technique. The specimen is placed bottom side up onto a frozen chuck/specimen holder and surrounded with TFM. The specimen is covered completely with medium until it resembles a small frozen ice cream cone. After embedding, the block is "faced" (cut into) until excess surface TFM is removed and the technician feels that the specimen has been completely exposed. Wafers are then cut and placed onto slides while continuing to cut deeper and deeper to get full tissue margin representation.

Freeze Bar/Blade Holder Technique. The specimen is placed bottom side down onto a flat, frozen metal part of the cryostat, usually the blade holder or freeze bar. The specimen freezes on contact and is then covered with TFM, which also freezes. The "bead" containing the specimen is then popped off the metal surface with forceps and placed bottom side up onto a chuck on the freeze bar and covered with TFM. The chuck/specimen/TFM unit is then allowed to freeze together into a solid block.

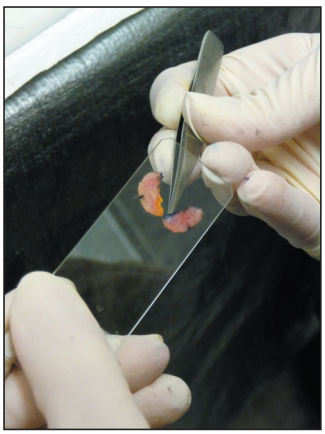


FIGURE 6.20: Ensure the specimen margin is fully in contact with the glass by pressing using fine forceps.

Reverse Slide Technique/Cryomold Technique. The specimen is placed bottom side down onto a glass slide or cryomold. A chuck and the specimen on the slide or cryomold are both covered with TFM. The slide is inverted and placed over the chuck so that both liquid TFM mounds meld. Freeze spray is applied to the top of the slide until the TFM begins to set up. A heat sink is placed onto the slide to help the block freeze from both top and bottom. The slide is then gently popped off or, in the case of the cryomold, the mold is peeled off. When done properly, 85–95% of the specimen margin is exposed, which allows production of nearly complete wafers without facing deeply into the block.

Helpful Hints for Embedding

1. Place the specimen on the slide and into the cryostat for no more than 10 to 20 seconds. The goal is to freeze only the specimen's deep side where it comes into contact with the slide, not to have the specimen freeze solid.
2. Warm the chuck in your hand prior to placing it on the freeze bar. Immediately cover the chuck with TFM and then cover the specimen and slide with TFM. Flip the slide and "sandwich" the two TFM layers together. There are two reasons for doing this: First, the chucks have multiple indentations to increase their surface area; take advantage of that. If the TFM is placed on a warm chuck, it will flow into these small indentations. Second, melding the two TFM layers (on slide and chuck) and freezing them avoids fracture planes. If you pour liquid TFM onto frozen TFM and freeze them, the liquid TFM retracts slightly from the frozen TFM mound as it freezes. When cutting, there is a higher likelihood of the specimen separating at the fusion of the melded TFM layers and shattering on the blade (Figures 6.20–6.25).

FIGURE 6.22: Chuck covered with tissue freezing medium.

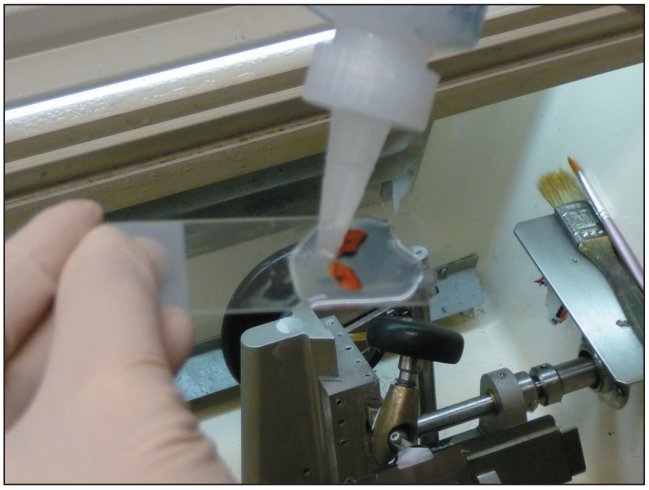


FIGURE 6.21: Bisected specimen on mounting slide covered with tissue freezing medium.

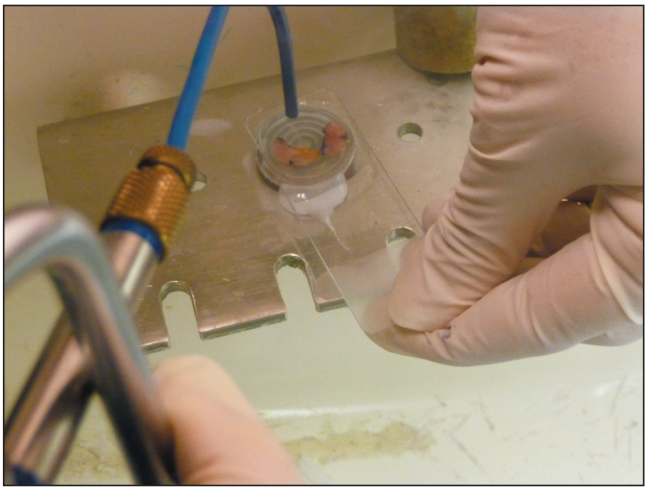


FIGURE 6.23: Specimen/chuck being frozen as one contiguous block.

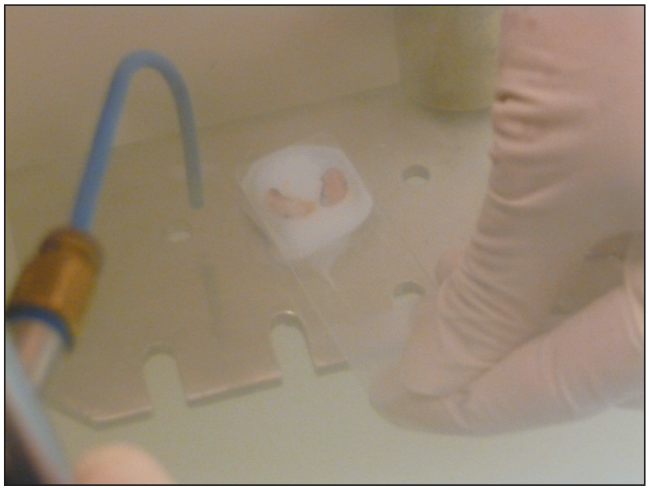


FIGURE 6.24: Specimen/chuck being frozen as one contiguous block.

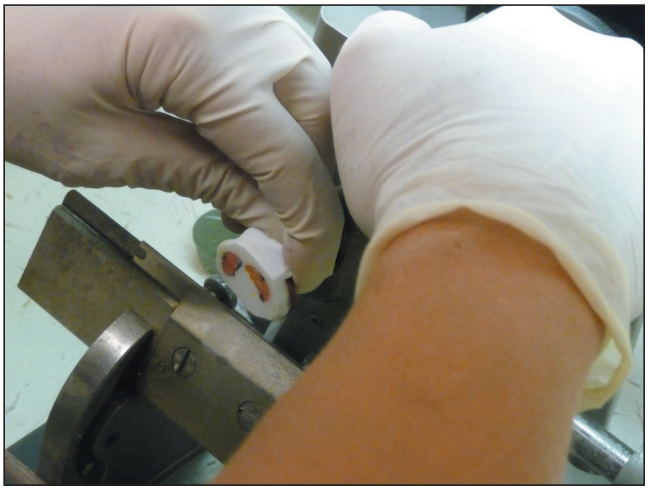


FIGURE 6.26: Specimen block with initial angle adjustment.

Angling the Specimen Block

With the exception of the Ames Tissue Tek II, all available cryostat microtomes have some type of pivoting ball-joint mechanism to which the specimen block attaches, which permits the block to be angled properly for cutting. Angling is a precise technique that requires practice to perfect. The goal is to orient the block parallel and equidistant from the microtome blade edge as the entire block passes by the blade. When performed properly, the specimen surface is "faced off" quickly, usually in less than 30–40 microns, and very little tissue is wasted. The technician must determine the best way to hold and move the specimen block around the joint mechanism. Holding the block and ball joint freely in one hand while loosening the ball joint lock with the other is too imprecise and takes far too long to orient the block precisely to the blade. A better technique is to find a place on the microtome, typically behind where the joint meets the microtome body, then hold the block and pivot joint with the same hand. This permits use of much finer motor-skill movements and allows more accurate and quick angle adjustment (Figures 6.26–6.27).

Sectioning in the Cryostat

Taking sections is in many ways the most difficult part of preparing Mohs slides. Sectioning technique determines whether all of the hard work of prepping and embedding leads to excellent or suboptimal slides. Of the two ways to draw and cut the specimen across the blade – the antiroll plate technique and the free-hand brush technique – the free-hand brush method is far superior. Antiroll plates are delicate and require precise adjustments for the wafer to slide between the blade and the antiroll plate without distortion. Antiroll plates are also susceptible to ice and debris buildup and must be cleaned regularly as cutting progresses. Also, the antiroll plate is not adaptable; as the specimen is cut, the stationary plate cannot move to adjust to the wafer coming off the blade. Finally, after every cut,

FIGURE 6.25: Specimen/chuck being frozen as one contiguous block.

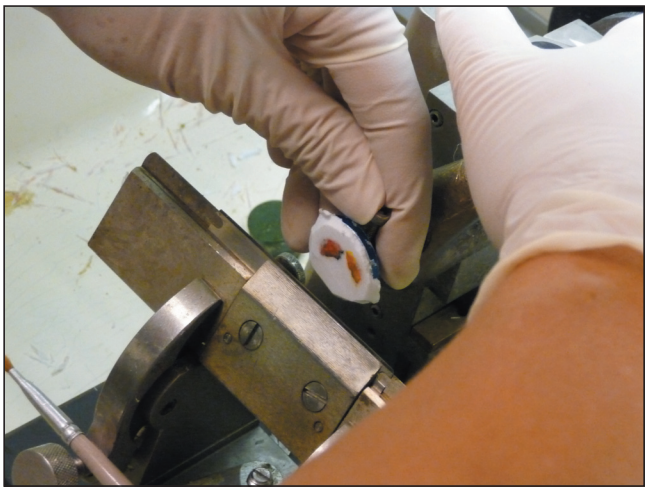


FIGURE 6.27: Block angle being adjusted.

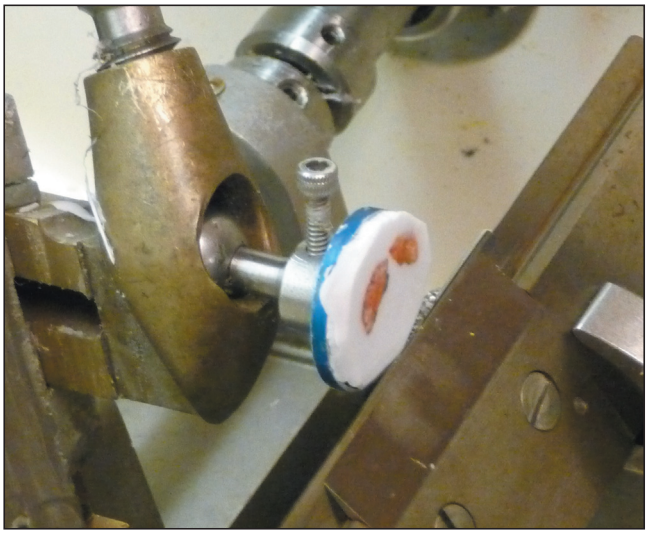


FIGURE 6.28: Brush technique for cutting.

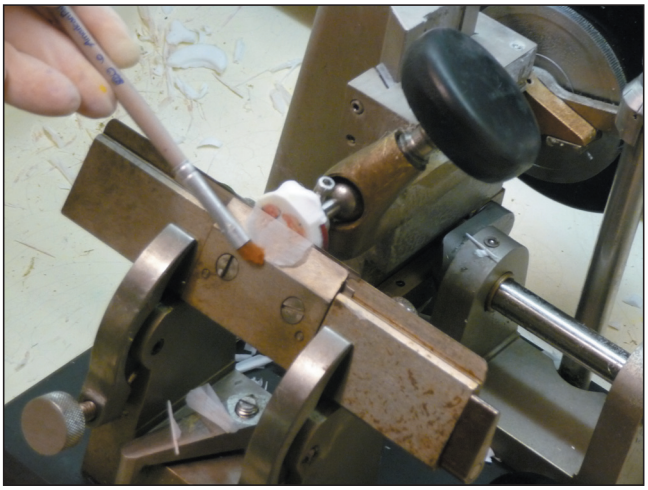


FIGURE 6.30: Brush technique for cutting.

the antiroll plate must be lifted and the wafer often must still be "teased out" with brushes to eliminate any folding or undulation. Nevertheless, antiroll plates are capable of working reasonably well, and the newer, glass antiroll plates are far superior to the earlier plastic ones. Antiroll plates are slower and less adaptable than the free-hand brush technique, but are perfectly acceptable for use by technicians who prefer them.

With the brush technique, the antiroll plate is usually removed from the microtome and the technician cuts into the block just far enough to engage the bottom edge of the TFM with the brush without touching tissue. Because the circumference of the specimen is surrounded by a minimum of 3 mm of TFM during embedding, the wafer can be engaged without touching actual tissue. As cutting continues, the brush then guides the wafer as it hits and "floats up" along the surface of the blade (Figures 6.28–6.32).

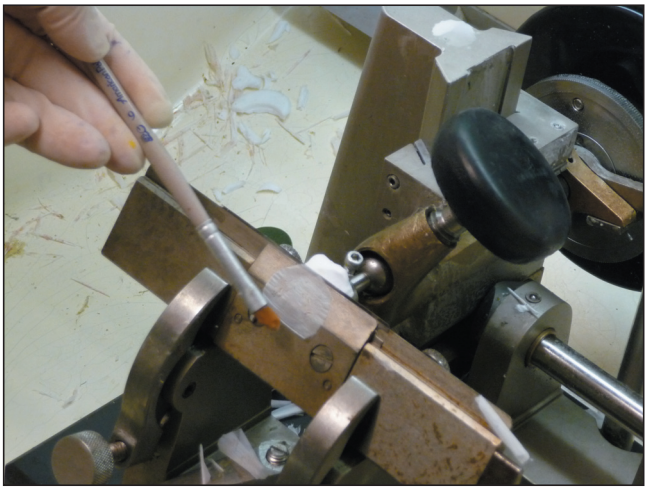


FIGURE 6.31: Brush technique for cutting.

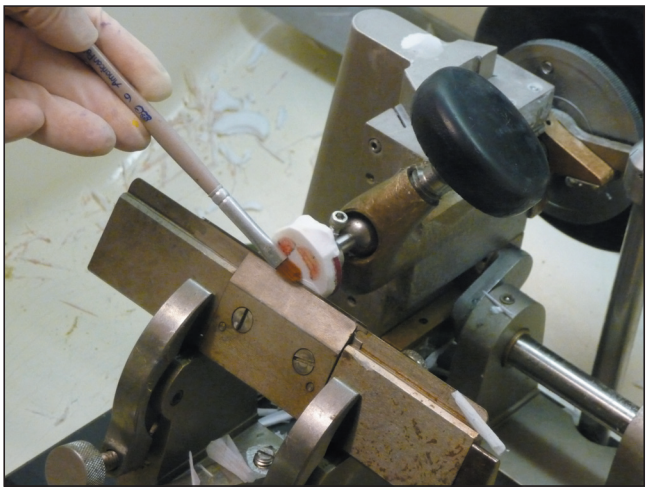


FIGURE 6.29: Brush technique for cutting.

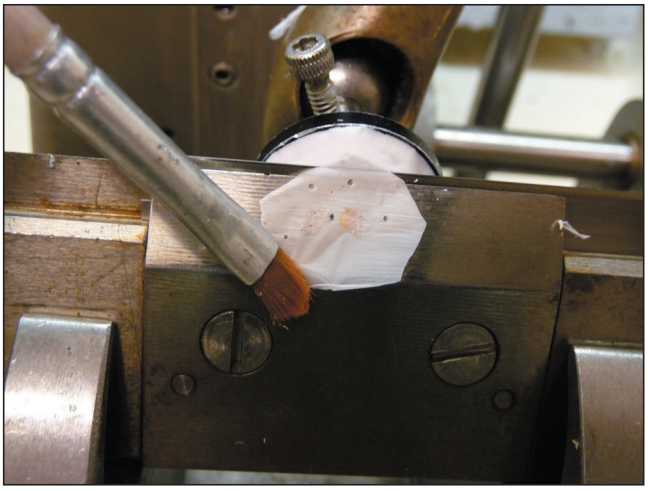


FIGURE 6.32: Brush technique for cutting.

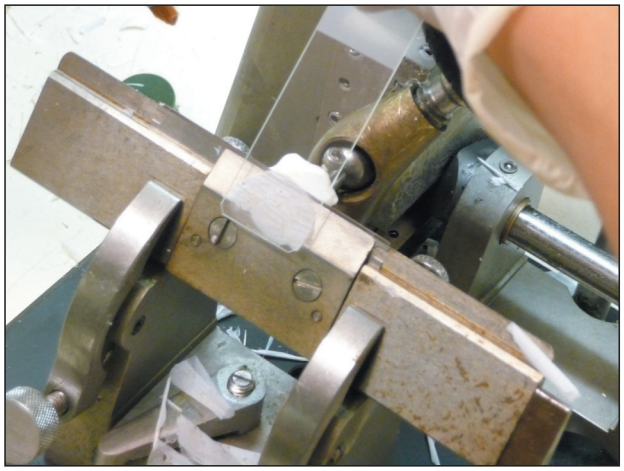


FIGURE 6.33: ***Specimen pickup onto the slide. Pick up specimen wafer from the bottom edge.***

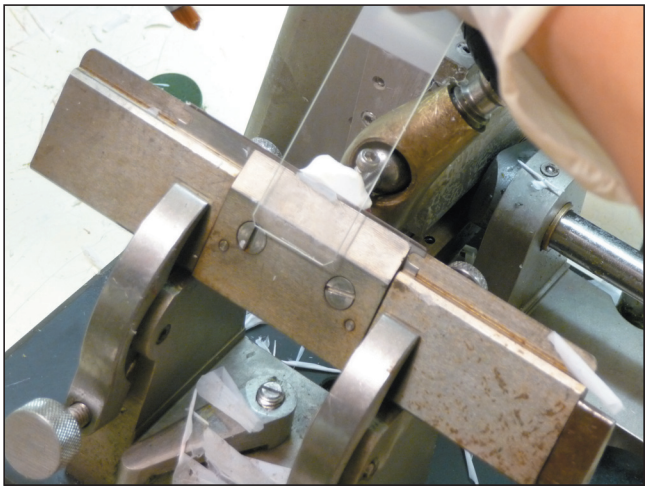


FIGURE 6.35: ***Wafer pickup onto the slide. Hold the slide steady until the wafer is completely picked up onto the slide.***

Once the wafer has been cut, it is allowed to adhere to the slide. One technique that makes the wafer easier to handle is to pause before cutting completely through the wafer. By stopping just shy of the edge, the technician has to deal with only one side instead of two sides of the wafer rolling up. This is another reason to surround the specimen with a 3-mm border of TFM; the 12 o'clock edge of the wafer (the last edge of the wafer to be cut) contains only TFM, while the tissue within the wafer has been cut but is not directly handled and is ready for "pickup" onto the slide. The technician then holds down the 6 o'clock wafer margin with the brush and puts a room temperature slide in contact with this 6 o'clock portion of the wafer. The cut tissue specimen and surrounding TFM will melt onto and adhere to the slide (Figures 6.33–6.35).

During this "pickup," it is critical to hold the slide with a steady hand. As the specimen wafer melts onto the slide, the technician must prevent wrinkling. This is best accomplished by picking up the specimen from bottom to top (6 o'clock edge to 12 o'clock edge), permitting a "sheeting action" so that any wrinkles present are stretched out during pickup. "Anchoring positions" help keep this part of the process simple: the technician anchors the hand holding the slide by resting it on a fixed object to allow greater control and a better chance of attaining a clean pickup. The cut tissue within the wafer must be in direct contact with the slide after pickup. The TFM edges may overlap the TFM, but tissue may not overlap the TFM. This is because the TFM will be washed off in the first water bath of the staining process. If tissue overlaps TFM, it will wash off the slide during staining.

Do not place too many cuts on each slide. Use additional slides as needed for re-cuts. Processing additional re-cuts is not a problem, but incomplete margin representation is unacceptable.

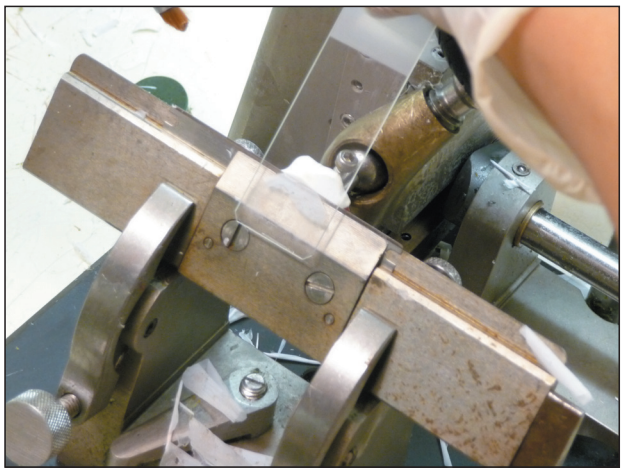


FIGURE 6.34: ***Wafer pickup onto slide. Hold the slide steady and adjust to eliminate wrinkles as the wafer melts onto the slide.***

Tips for Better Brush Techniques

Pay attention to the wafer edges. Often, one side will tend to wrinkle up more than the other. If this happens, hold the wrinkled side of the leading edge coming off the blade with the brush. The added tension on the wafer from the brush should reduce or eliminate wrinkles, giving a crisper specimen to pick up.

Tissue separating from the TFM as the wafer comes off the blade may be pulled and guided with the brush. There is no taboo preventing the technician from occasionally touching the tissue with the brush. Although there is a legitimate concern about transferring unrelated tissue from the brush to the wafer, if the brush is kept clean and the technician has good dexterity, this risk is minimal. Touching the brush to tissue that is separating may save it before it rolls up or pulls out.

FIGURE 6.36: Whole specimen prepped and inked.

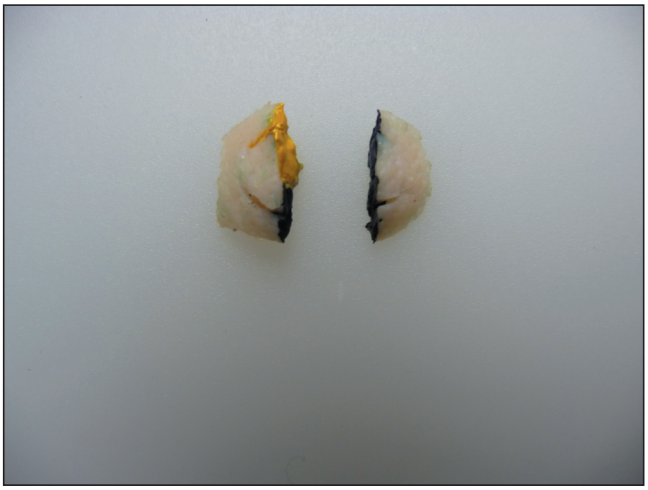


FIGURE 6.38: Bisected specimen prepped and inked.

Oddly Shaped Specimens

Shape usually determines how specimens are subdivided into smaller pieces. Most specimens requiring subdivision are bisected along their long axes. A subdivided specimen is then oriented in the block so the long axis of the cut edge is 30–45 degrees relative to the blade and the cut edge contacts the blade first, before the epidermis. Thus, it is actually the deep margin that the blade cuts first. The blade encounters the softest part of the specimen first (fat or dermis) and the more dense epidermis last. This minimizes epidermal rolling. If epidermis contacts the blade first, there is an increased chance that it will pull out of the TFM and fold over upon itself, because it does not adhere to the TFM as well as most other types of tissue.

Specimens Processed Whole

First-stage specimens processed whole are inked along their epidermal edges. As discussed above, the technician should increase the length and depth of the hatch marks before processing. Carefully orient the block prior to cutting so that one of these deepened hatch marks is the first area to hit the blade. This will help minimize epidermal rollovers, because the dermis in the hatch mark adheres better to the TFM (Figures 6.36 and 6.37).

Bisected Specimens

When bisecting a specimen, the technician should ensure that chromacoding ink is applied only to the cut edge, not the underside, and covers the entire cut edge. At the junction of two ink colors, both inks should touch.

Orient the specimen in the block so that it is offset 30–45 degrees from the blade edge, then angle the block so that it is equidistant from the blade and begin to "face off" the block (Figures 6.38 and 6.39).

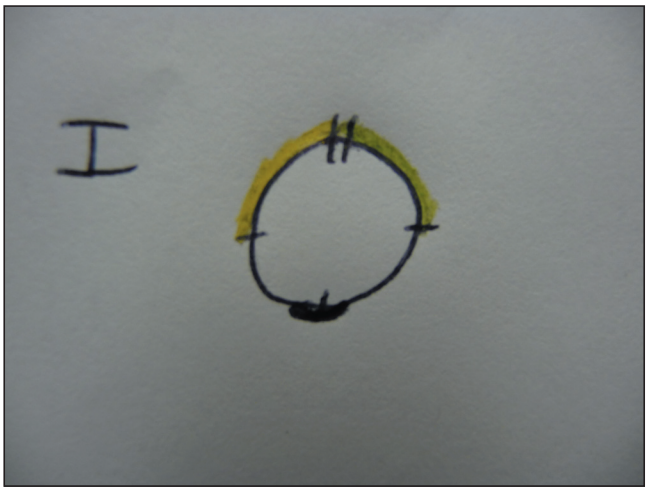


FIGURE 6.37: Whole specimen-corresponding map.

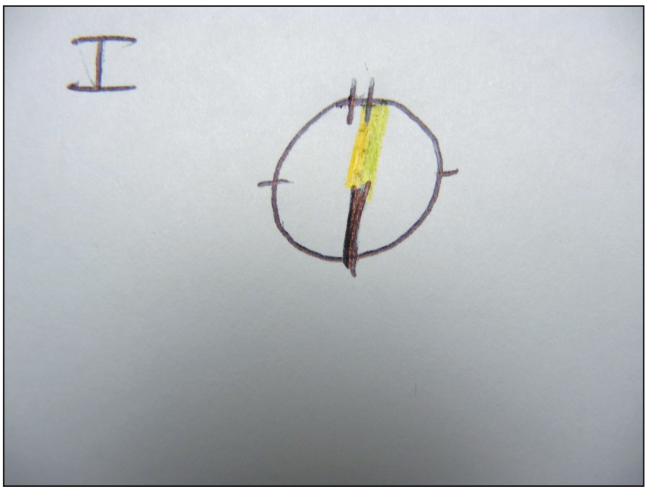


FIGURE 6.39: Bisected specimen-corresponding map.

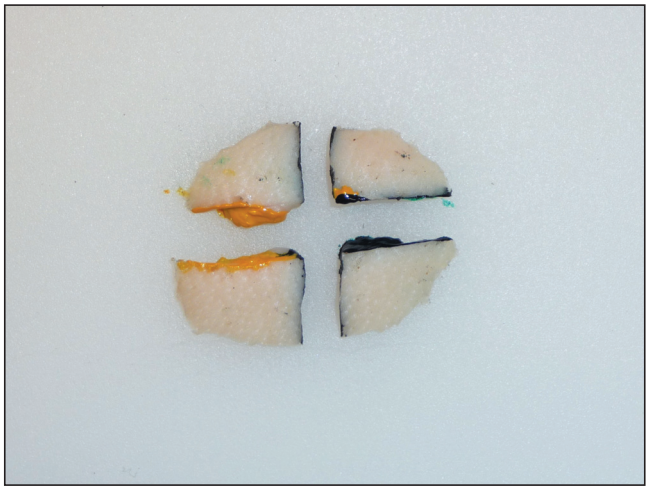


FIGURE 6.40: Stage I specimen quadrasected and inked.

FIGURE 6.42: Stage I specimen trisected and inked.

Quadrasected Specimens

When quadrasecting a specimen, the four pieces must be chromacoded so that each is uniquely inked without duplication. Use three or more colors: one for the entire 12 o'clock-to-6 o'clock vertical edge, one for the 9 o'clock-to-center (left horizontal) cut edge, and a third color for the 3 o'clock-to-center (right horizontal) cut edge (Figures 6.40 and 6.41).

Mohs surgeon-pathologists may have their own color schemes for chromacoding. If the surgeon draws the color codes on the Mohs map, the technician should follow the color code meticulously or discuss possible changes with the surgeon before implementing them.

Triangular-Shaped Specimens

Ink the specimen so that each cut edge from apex to center has a different color (Figures 6.42 and 6.43).

Square-Shaped Specimens

Triangles and squares have apices that are extremely difficult to flatten. Bisect the specimens from their apices to the center, cutting through each apex. Ink one color per cut, four in all. Cutting through the apices produces flatter specimens (Figures 6.44 and 6.45).

Infinity-Shaped Specimens

Infinity-shaped specimens occur when the surgeon is excising two adjacent cancers. These specimens are best bisected along their long axes, making the specimen easier to prepare and cut in the microtome.

Large Specimens

Large specimens need considerable organization and attention to detail when subdividing and chromacoding. Because the technician generally has only six colors available for

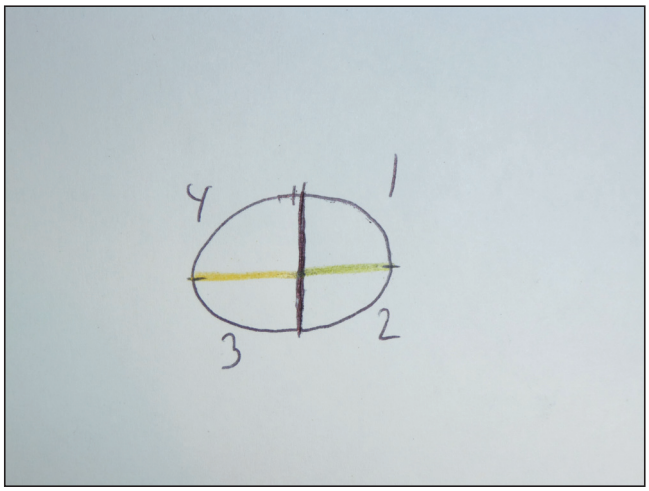


FIGURE 6.41: Quadrasected specimen's map.

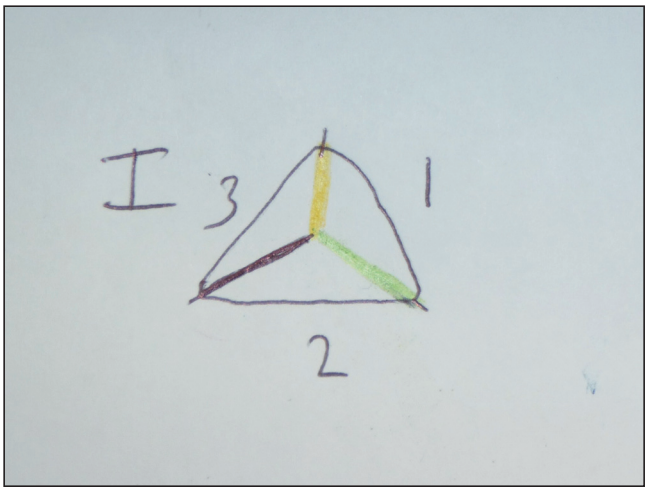


FIGURE 6.43: Trisected specimen's map.

FIGURE 6.44: Square cut stage I quadrasected and inked.

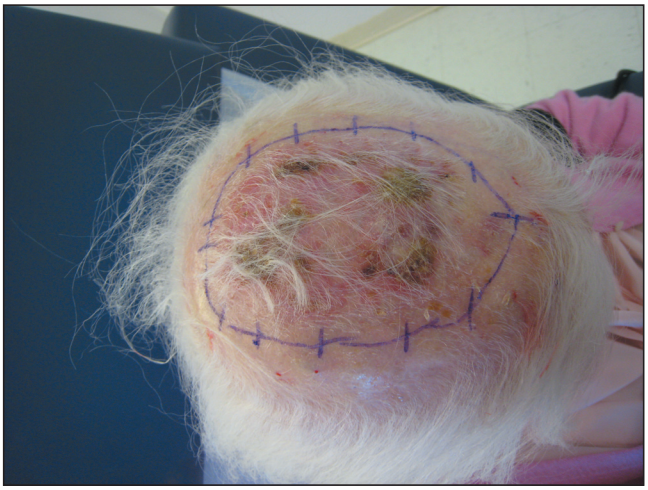


FIGURE 6.46: Large case, preop.

chromacoding, another method of differentiating cut edges may be needed. After running out of primary colors, one method is to ink one half of some cut edges with one color and the other half with a second color. All apposing cut edges from subdividing are best inked with the same color or colors. When the Mohs surgeon-pathologist finds tumor along an inked edge, the adjacent edge (on a different slide) will have the same color, making evaluation for tumor between subdivided specimen pieces easier and less confusing (Figures 6.46–6.48).

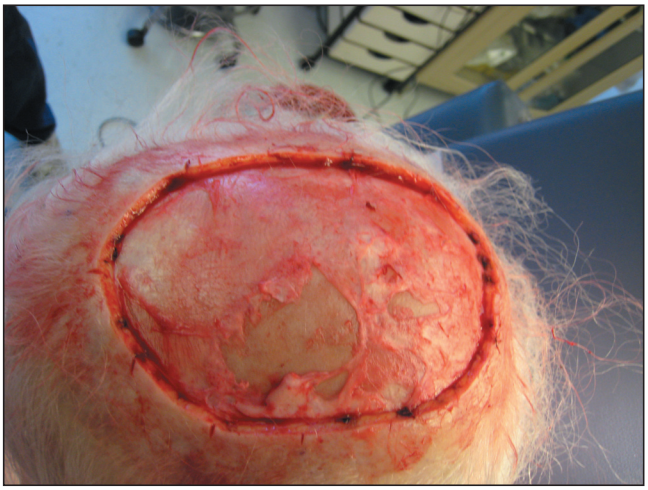


FIGURE 6.47: Large case, postop.

Subsequent Stages

After the first stage, subsequent-stage specimens may come in a variety of sizes and shapes. Some specimens may be bounded by epithelium on all sides; others may have no epithelium. Any poorly beveled specimens should be prepped until they lie flat. Debulking of specimens should

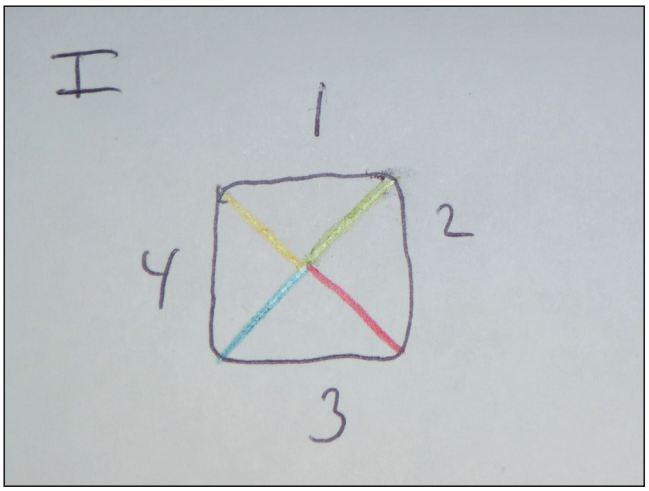


FIGURE 6.45: Square cut specimen's map.

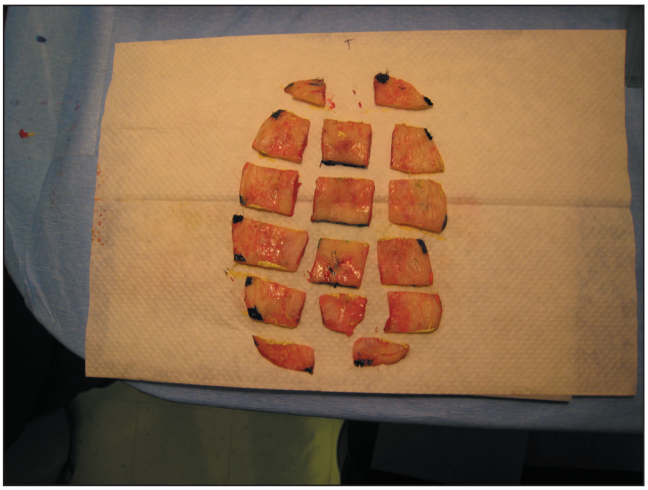


FIGURE 6.48: Large case, prepped and inked.

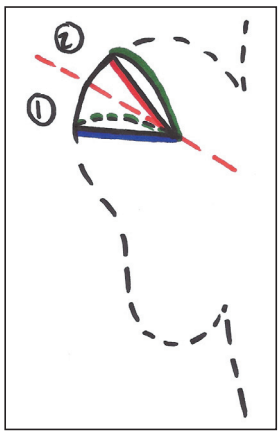


FIGURE 6.49: ***Two-dimensional (2D) map showing how ear wedge was divided and inked.***

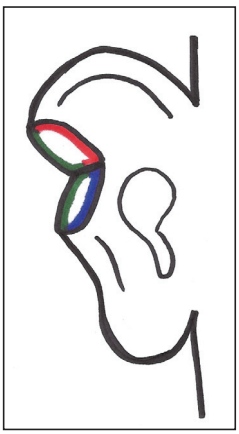


FIGURE 6.51: ***Three-dimensional (3D) map showing inking with reference to the defect area.***

only be done if necessary. Processing should always follow these fundamental precepts: Get the specimen oriented, get it to lay flat, get it mapped and inked, get it cut and placed on slides.

Wedges

Surgeons excise wedge-shaped specimens when excising skin cancers on free anatomic edges, which have eroded from one epithelial side through to the opposite side. This may occur on ear helices, eyelid margins, and lips. These wedge-shaped specimens have epithelium on both sides: either epidermis on both sides (e.g., an ear wedge) or epidermis and mucosa (e.g., lip or eyelid wedges). Close collaboration between surgeon and technician is required for all cases involving a wedge.

Mapping is difficult because the more complex three-dimensional shape of the specimen and its chromacoding are not accurately depicted in the x or y axes of the two-dimensional (2D) map. Depiction of the third dimension, the z axis, must be depicted on the paper. This confusing challenge can be remedied by drawing two maps. The first is a (2D) overhead view, showing subdivision patterns and chromacoding. The second is a three-dimensional (3D) drawing showing the specimen as it exists

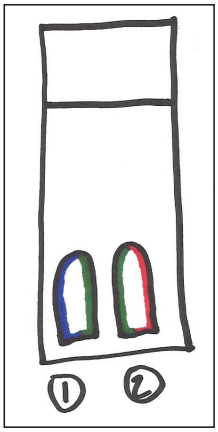


FIGURE 6.52: ***Hand-drawn depiction of inked specimen on the mounting slide.***

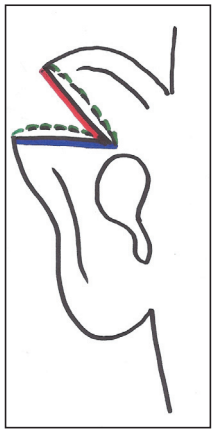


FIGURE 6.50: ***Two-dimensional map showing inking with reference to defect area.***

FIGURE 6.53: ***Ear wedge example inked.***

FIGURE 6.54: Ear wedge example inked, divided, and placed true margin side down.

in space. Instead of the differential chromacoding representing the 2D superior/inferior and lateral/medial areas of the specimen, the chromacoding also must represent the anterior/posterior sides of the specimen (Figures 6.49–6.54).

CONCLUSION

Mohs tissue processing comprises many techniques which, when performed sequentially, lead to the production of high-quality Mohs slides. This chapter did not describe all the techniques available to the technician, but rather those techniques that this author has found useful.

PREPARING SLIDES WITH CARTILAGE

Michael Shelton

GROSSING

Cartilage is one of the most challenging tissues from which the Mohs technician is asked to make quality slides. It is challenging to gross the specimen, challenging to cut, and even challenging to stain because cartilage easily washes off the slides during the staining process. For many years, the following techniques have been helpful to this author in producing high-quality slides of tissues containing varying amounts of cartilage.

Cartilage strongly tends to retain its shape and therefore needs to be processed in a way that will ensure the specimen will stay flat, with the epidermal edges down, as the specimen is embedded, cut, and stained. While using forceps to forcefully push the epidermal edges down on two sides (Figure B.1), use a scalpel to make relaxation incisions into the center of the specimen (Figure B.2), which is under considerable tension. These incisions will cause the pressure at the center of the specimen to be released and allow the edges to begin to flatten. This is repeated throughout the specimen wherever the tissue "refuses" to relax (Figure B.3). These relaxing incisions need not be restricted to the center of the specimen. Use caution while making these relaxing incisions to avoid cutting at the peripheral edges, creating additional reference nicks (hatch marks).

When necessary, the hatch marks cut into the specimen by the surgeon may be widened by the technician, giving additional play to the tissue and helping it to flatten further. In a situation where the specimen is removed with the cartilage extending beyond the epidermal edges, a situation which Mohs surgeons should try to avoid (see Chapter 2), a few extra steps are needed to ensure that the epithelial edges overlap the cartilage. On the epithelial surface, "open" the existing hatch marks by cutting a vertical incision from the 12 o'clock-to-6 o'clock hatch marks (Figure B.4) and then cut another incision from the 9 o'clock-to-3 o'clock hatch marks (Figure B.5). The hatch marks will be opened

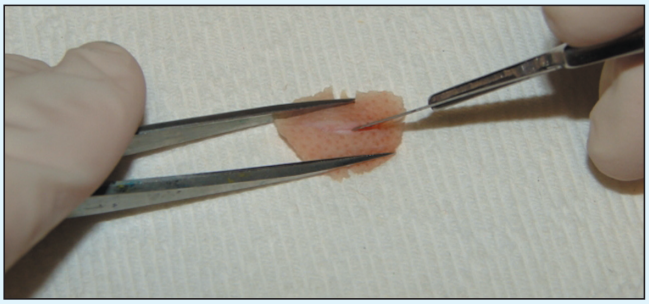


FIGURE B.1: Forceps are used to forcefully push the epidermal edges down on two sides.

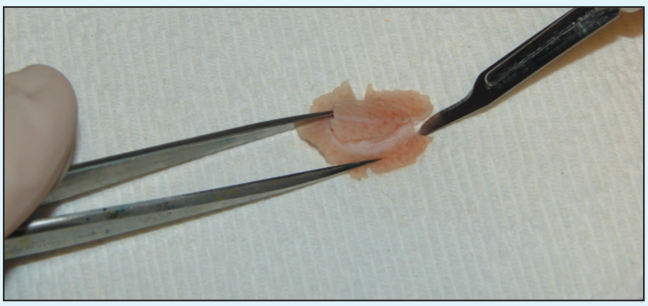


FIGURE B.2: While keeping the specimen under tension with forceps, use a scalpel to make relaxing incisions in the central area of the specimen.

significantly and the epidermis, still attached to the cartilage, will be separated into four quadrants. The cartilage remains intact, because the incisions made by the technician are not through and through. Each quadrant's epidermal edge can then be slid down to the base of the specimen (Figure B.6); this allows the epithelial edge to extend past the cartilage and become the peripheral margin of the specimen, and allows these peripheral epithelial edges to lie in the same plane as the base of the cartilage. The specimen is then inked per office protocol. The cut edges of the hatch marks must be inked if they have been widely opened; this will allow the Mohs pathologist to determine if complete margin representation has been achieved.

While freezing the tissue (Figure B.7) on a set surface (slide, freeze bar, cryomold, or other similar surface), the epidermal edges can be further manipulated to lie flat. When freezing the prepared specimen to a surface, first start with the most difficult edge to lie flat; once that edge is frozen in place, the rest of the epidermal edges can be more easily flattened to the base of the specimen. A stubborn edge that will not stay down can be coerced into position. Using forceps (or a cotton-tip applicator), hold the edge down and spray it

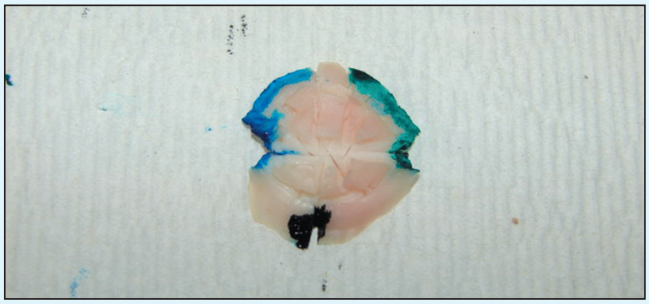


FIGURE B.3: *Continue to make relaxing incisions until the specimen is fully flattened. Relaxing incisions that cut through the edge of the epidermis should only be made at the hatch marks (reference nicks).*

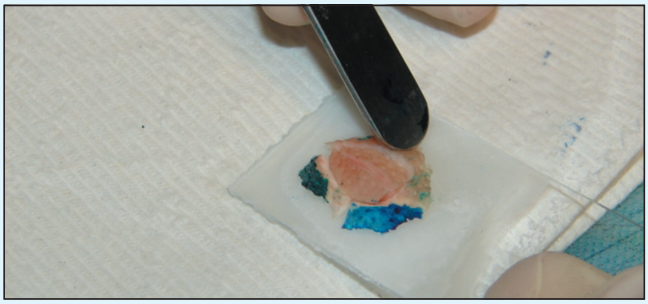


FIGURE B.6: *The four quadrants of epidermis can be slid outward and down to the same plane as the base of the specimen.*

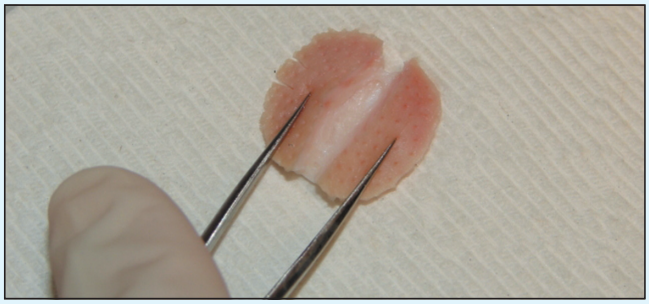


FIGURE B.4: *At the epithelial surface, open the existing hatch marks (reference nicks) from 9 o'clock to 1 o'clock.*

FIGURE B.7: *While freezing the specimen, continue to further push the edges of the specimen flatter.*

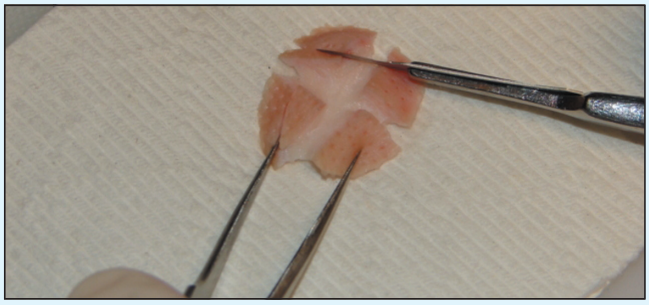


FIGURE B.5: *At the epithelial surface, open the existing hatch marks (reference nicks) from 12 o'clock to 6 o'clock. Do not cut all the way through the cartilage.*

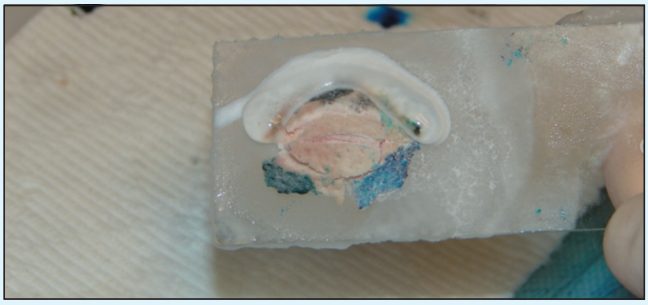


FIGURE B.8: *To further aid the edges in adhering to the slide, add dabs of optimal cutting temperature medium as needed.*

with liquid nitrogen to freeze it into the desired position. To further aid in adhering this edge to the surface, place a dab of optimal cutting temperature medium (OCT) on it (Figure B.8) to "glue" it into place. The specimen is then prepared in its OCT mold, as the technician prefers, and cut in the usual way (Figure B.9).

In the case of a specimen too large to process as a whole, the specimen can be bisected or more as needed. Stress can be a difficult factor to work through; take care to work within the technician's abilities. This will help with processing the best-quality slides.

CUTTING

Cartilage is temperature sensitive and will cut better at a higher temperature than subcutaneous fat, which needs to be cut at a lower temperature. Cartilage can be optimally cut at a range of −16° to −22°. The technician needs to be mindful while cutting if the temperature is too cold, because fat needs to be cut also and the cartilage may shatter along the blade. The cartilage can be warmed slightly by placing it on a warm object. Some technicians use a gloved thumb or finger. This should be avoided because the unintentional need for stitches can make a stressful day worse. An acceptable and functional alternative is to keep a couple of cotton tipped applicators on the slide warmer. The applicators can be touched to the cartilage without warming the rest of the specimen.

The cartilage will cut beautifully, and the difficulty encountered the most is the cartilage separating from the connective tissue. Keep the connective tissue cold while cutting, with the connective tissue in contact with the cartilage. It will be a great asset to keeping the cartilage on the slide.

In the case where there is more cartilage than epidermis on the specimen, connective tissue will not be on the base of the specimen. This will be a cause of concern during the staining process. Connective tissue cannot be relied on to keep the cartilage in place. Staining the slide carefully is a must.

STAINING

After completing the hard work of prepping and cutting the cartilage-containing specimen, improper staining can still ruin the Mohs technician's day. Cartilage "dislikes" being stained. As soon as the slides are immersed in the first water wash, wafers tend to come loose and fall from the slides. The following staining technique will ensure that the cartilage adheres to the slide with the least probability of losing wafers.

Before cutting, buy and use adhesive slides (cartilage slides), such as: plus slides, A-slides, or positive slides. These slides are specially treated to hold a static charge, thus allowing better adhesion of the wafers to the slides. Good-quality slides are a must, a bad-quality slide will be a nightmare when trying to stain beautifully cut sections. Most technicians know the disappointment of good slides gone bad. Experimenting with different brands of slides can be of benefit to ensure the cartilage will remain on the slides during your staining procedure. Once the sections are cut, place them onto a "slide warmer" (Figure B.10) and warm the slides before staining. Satisfactory slide warmers include a candle warmer, coffee cup warmer, or other similar device. Allow the sections to warm up and dry out for 30 to 60 seconds before staining.

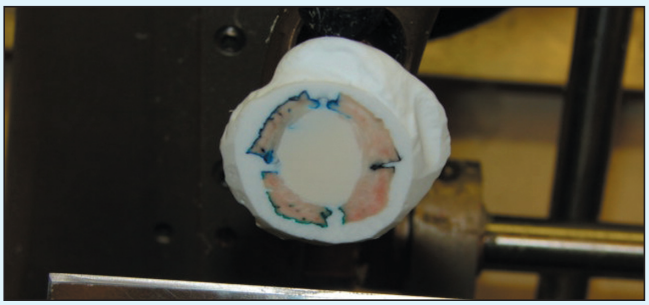


FIGURE B.9: Cut the specimen in the usual method.

FIGURE B.10: Place the cut slides on a "slide warmer" to warm and dry for 30 to 60 seconds before staining.

Staining of Mohs slides without cartilage is done in our laboratory using a Linistain SLS and standard hematoxylin and eosin (H&E) technique, per office protocol*:

Alcohol	1 minute
Water wash	Until slide is clear of debris
Hematoxylin	1 minute
Water wash	Until excess hematoxylin is removed
Bluing	30 seconds
Eosin	5 dips
Alcohol wash	As many as needed to remove excess eosin
Clearing agent	10 dips

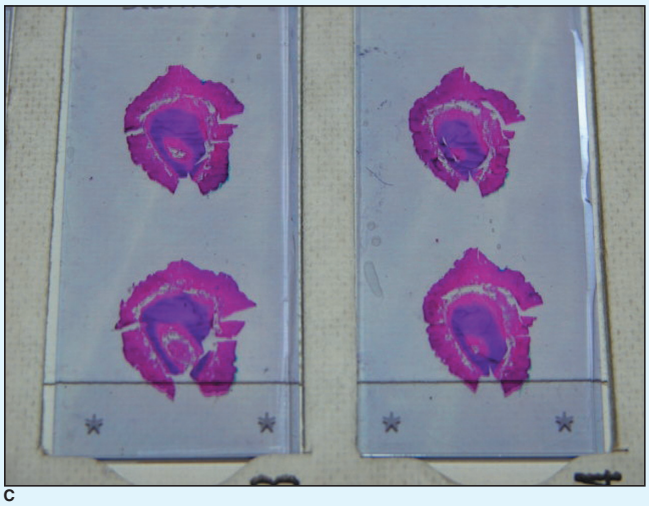
* The time and number of chemical stations can be adjusted to meet desired results.

This technique does not work well for staining cartilage because it does not achieve optimal adhesion of the cartilage-containing wafers to the slides. To achieve better adhesion of the wafers to the slides, the use of a stronger fixative is added to the staining procedure. Before the slides are immersed into the first chemical station (alcohol) they are first dipped in acetone, which fixes the cartilage more quickly than alcohol and promotes better specimen adherence to the slides. The use of acetone is a standard procedure in many pathology labs working with immunohistochemistry and special stains. Do not substitute the first alcohol station with acetone; instead, add the acetone to the beginning of the staining process.

FIGURE B.11: *Staining begins with 1 minute of acetone prior to the first alcohol bath. The "Linistainer" water pressure is "turned down" before staining cartilage.*

A

B



C

FIGURE B.12: *(A–C) Pig's ear cartilaginous specimens cut for illustration purposes.*

Our laboratory's staining protocol for cartilage is*:

Acetone	1 minute
Alcohol	1 minute
Water wash	Until the slide is clear of debris
Hematoxylin	1 minute
Water wash	Until excess hematoxylin is removed
Bluing	30 seconds
Eosin	5 dips
Alcohol wash	As needed to remove excess eosin
Clearing agent	10 dips

* The time and number of chemical stations can be adjusted to meet desired results.

Throughout the entire staining process, proceed slowly and gently. The faster you rush through the stains, the easier it is to wash the cartilage from the slides. Strong, heavy, or fast agitation in the chemicals will only be damaging to all the work put into grossing and cutting sections. The water pressure on automated stainers can be regulated to reduce or increase the strength of the water wash; turn down the water pressure (Figure B.11).

Using these techniques, the difficulties affecting cartilage processing may become less of a burden even if not less of a challenge. The epidermis can be aligned with the base of the cartilaginous specimen, and cartilage will remain on the slide after staining. Figure B.12 shows pig ear specimens cut for illustration purposes.

CHAPTER 7

Lab Pearls: Staining, Inking, and Coverslipping

Alex Lutz

INKING, STAINING, and coverslipping are the most correctable Mohs laboratory processes in improving slide quality. This chapter will discuss improving tissue ink appearance, enhancing hematoxylin and eosin (H&E) staining, color, contrast, and quality, and preventing air-bubble formation and glue seepage after coverslipping.

INKING (CHROMACODING)

Proper inking (chromacoding) of specimens is critical to the complete removal of skin cancer. Inking performs the dual roles of specimen orientation and specimen margin representation. Every processed piece of tissue must have epithelium or ink around its complete circumference. These indicate to the surgeon-pathologist that all peripheral margins are represented on the slides.

When inking subdivided specimens, the entire cut edges must be inked. It is important to avoid allowing any ink to flow onto the underside of the specimen. The inked edge functions as a surrogate for epithelium, denoting when the full margin is represented on the tissue wafers. If a specimen's underside is inadvertently inked, the technician seeing the ink may mistakenly believe the wafer's complete edge has been reached, indicating that the full margin is represented, and would therefore not cut additional wafers. This would leave the true margin within the tissue freezing medium (TFM), and any tumor in this unrepresented area would be left untreated. Ink accidentally spreading to the underside of the specimen is called "ink bleeding." Techniques to prevent ink bleeding include:

1. Careful and deliberate inking.
2. Placement of the specimen on an absorbent pad or gauze during chromacoding.
3. Increasing the inks' viscosity.

Thicker inks appear more prominently under the microscope. Blue and black ink are the most likely to bleed due to their low viscosity. Green, red, yellow, and orange inks are thicker and bleed less easily, provided they are shaken well prior to use. Green ink is a vital dye and must be handled carefully because it will permanently stain counters, equipment, and clothing.

There are several techniques for thickening inks. If bottles of ink sit for a few days, their components will separate. Colored liquid settles to the bottom of the bottle and clear liquid rises to the top. Drawing out some of this separated clear liquid with a syringe and discarding it will thicken the residual ink. Adding a small amount of talcum powder or corn starch significantly thicken inks without adversely affecting staining. Finally, ink bottles may be left uncapped for 2 to 3 weeks to thicken their contents, but this method is harder to control and could be messy if an ink bottle spilled (Figures 7.1–7.3).

STAINING THE SPECIMEN

Hematoxylin and eosin staining is a two-step differentiation staining in which the hematoxylin stains the cell nuclei blue and the eosin stains the cytoplasm pink-to-orange

FIGURE 7.1: Davidson ink set.

FIGURE 7.2: Close-up of ink junction (bisected specimen).

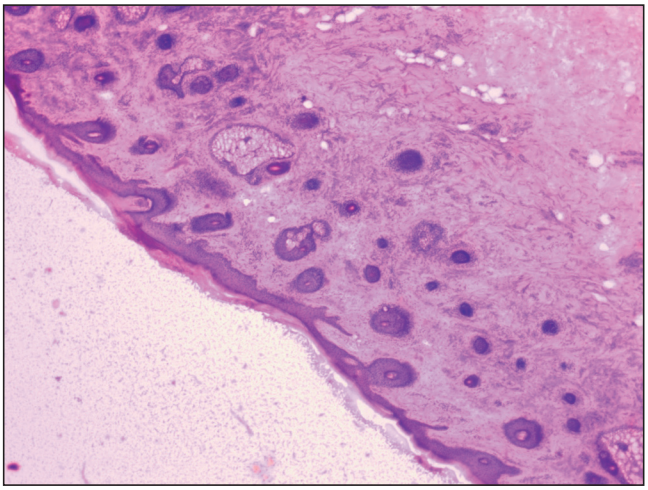


FIGURE 7.4: Too eosinophilic.

(Figures 7.1–7.3). Staining problems are grouped into three main categories: chemical incompatibility, chemical decomposition, and cross-contamination. All of these problems are caused by human error.

Chemical incompatibility occurs most often when chemicals that must work together are purchased from different manufacturers. All staining chemicals must be purchased from the same supplier! Chemicals produced by Medical Chemical Corporation (MCC) have been particularly reliable in this author's experience. MCC manufactures high-grade chemicals for scientific use. Their products are often relabeled and sold by different suppliers. Clearing reagents and coverslipping media should also be purchased from the same supplier, as they are used together to clear slides and secure coverslips. Use of incompatible clearing reagents and coverslipping media usually results in the formation of a milky film, which may interfere with accurate slide interpretation.

Chemical decomposition usually results from reagents being exposed to air and light. Hematoxylin is photosensitive and, if exposed to light, including fluorescent light, will decompose over time, causing it to stain too lightly. Hematoxylin is in a supersaturated solution, so its storage container must be shaken to ensure proper mixing before it is transferred to staining trays. Eosin contains 70% ethanol or other alcohols, which will evaporate over time when exposed to air. This will cause the eosin to become increasingly concentrated as it is topped off in the staining trays. Over a few months, eosin can double or more in strength, overpowering the hematoxylin and causing the slides to look eosinophilic (pink). Coverslipping glues contain volatile components, such as toluene, that will evaporate if left open to air, causing the glue to become thicker and less effective. Eventually, this thicker medium will become a "goopy mess" and will cause air bubbles to form on the slides.

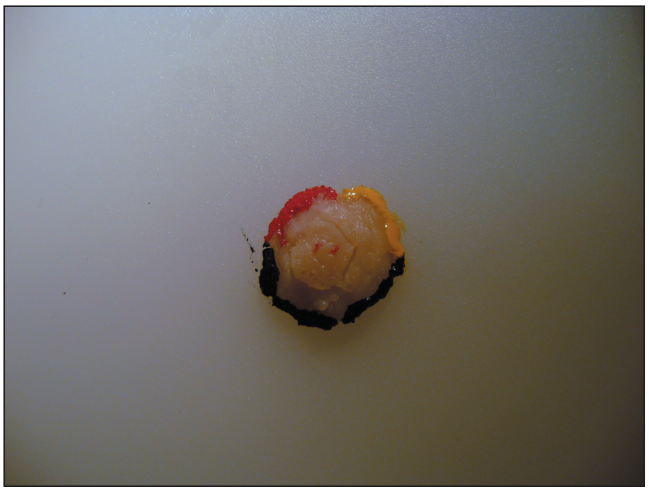


FIGURE 7.3: Close-up of specimen inked whole.

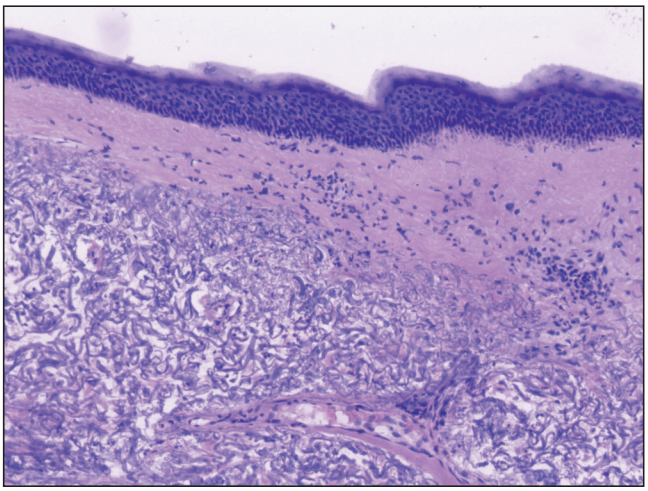


FIGURE 7.5: Properly stained section.

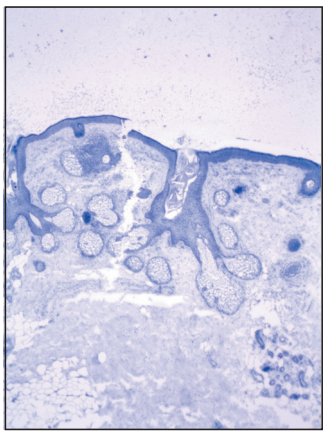


FIGURE 7.6: Too hematoxyphilic.

Cross-contamination occurs when slides are removed from one staining liquid and not blotted of excess staining liquid before being placed in the next chemical or rinse. Avoiding cross-contamination is most critical when slides are moved from the first water bath to the hematoxylin and from the last water bath to the eosin, because the move directly affects the chemicals responsible for tissue staining. Hematoxylin must be slightly acidic to penetrate cell nuclei and fix to the nuclear material and produce an even nuclear stain. When slides continually carry water into the hematoxylin, the hematoxylin eventually becomes neutralized and ineffective. If water from the last water bath is continually carried into the eosin, the eosin's 70% alcohol composition will be diluted and it will stain the cell cytoplasm too lightly or not at all. Great effort should be made to avoid carryover cross-contamination by blotting the slides and staining rack before slides are placed in each solution tray. Although known as "cross-contamination," this would be best referred to as "dilution" or "neutralization," as that is actually what happens when water dilutes or neutralizes a solution.

Staining quality may be improved by using a timer to accurately control how long slides remain in each solution during the staining process, rather than estimating the time. Staining strength is time-dependent and best not left to guesswork (Figures 7.4–7.6).

Use rinses adequately by dipping slides 10 to 20 times in each rinse or gently agitating them in the rinse for 10 to 20 seconds, replacing rinsing reagents as necessary. If water rinses significantly change color from clear to blue for water rinses of hematoxylin, or clear to orange for alcohol rinses of eosin, promptly replace them.

The most common mistake of busy technicians is not leaving slides in clearing reagent rinses long enough to adequately clear them. If not completely cleared, the eosin stain will not remain in the tissue and will pull out after coverslipping, creating an orange halo around the now blue-colored wafers. The slides must be left in clearing reagent rinses for a minimum of two minutes. Consider using a coverslipping medium, such as Surgipath's Clearium, that has a clearing reagent as part of its composition.

If eosin or hematoxylin is overstaining tissue, the staining strength and differentiation can be corrected by varying the staining times of either or both the hematoxylin and eosin. Increased staining times of one staining agent and/or decreasing times of the other should yield the desired result. However, if the eosin continually overstains the hematoxylin, muddying differentiation, eosin can be diluted by adding 90% (or higher) alcohol until the eosin weakens enough to allow better staining contrast. Dilute the eosin first by 25%, then incrementally by up to 50% as necessary.

Even if the chemicals are consistently purchased from the same, reputable supplier, reagent chemicals are made in large batches that will vary from one another. It is therefore essential to regularly monitor staining and quickly address any changes that occur to ensure consistency in the clarity and differentiation of the Mohs slides (Figures 7.7–7.9).

This author's personal preference for H&E staining is Gill's 3 hematoxylin and 1% Eosin Y in 70% ethanol. One hundred percent alcohol is used as fixative. Tap water is used to dissolve the TFM. Slides are placed in the Gill's 3 hematoxylin for 1 to 2 minutes, rinsed with four water baths (blotting the edge of the slides between each transfer), then allowed to "blue up" in the last water bath for about 20 seconds. This is followed by a quick dip of a few seconds into the Eosin, then rinsing with four alcohol baths, blotting the slides between each transfer. Finally, a coverslip is applied with generous amounts of Clearium. Clearium allows for skipping the reagent step at the end of the staining process, as the clearing reagent is inherent in the Clearium coverslipping medium.

COVERSLIPPING

Coverslipping is the last, but still important, step in the slide-making process. Even if everything is done perfectly

FIGURE 7.7: Staining station with timer.

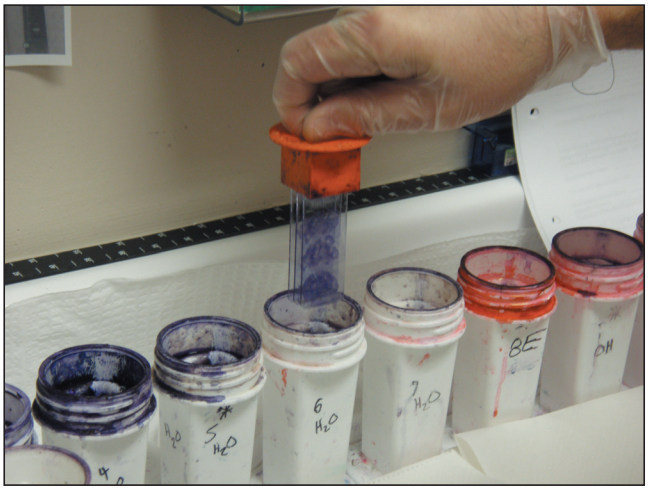


FIGURE 7.8: Posthematoxylin water rinses.

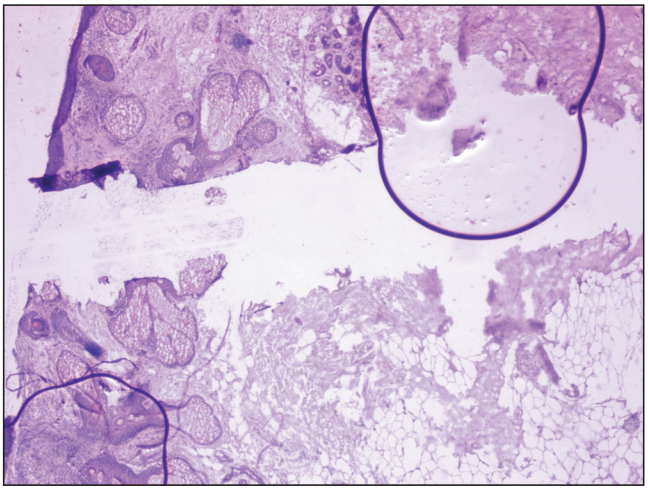


FIGURE 7.11: Air bubbles from insufficient coverslipping medium.

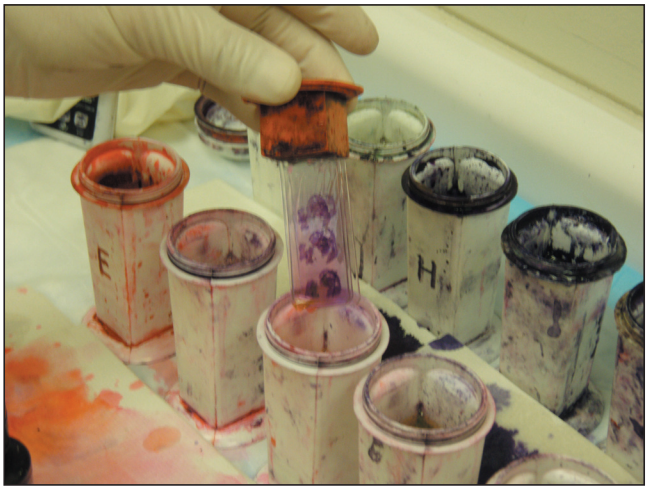


FIGURE 7.9: Post eosin alcohol rinses.

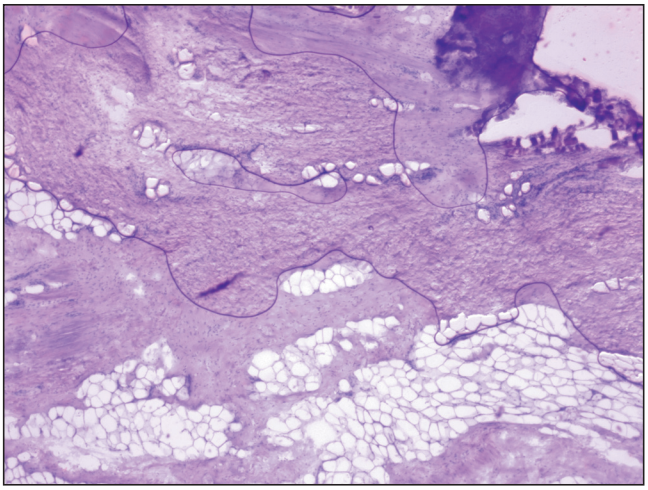


FIGURE 7.12: Air bubbles from poor coverslipping technique.

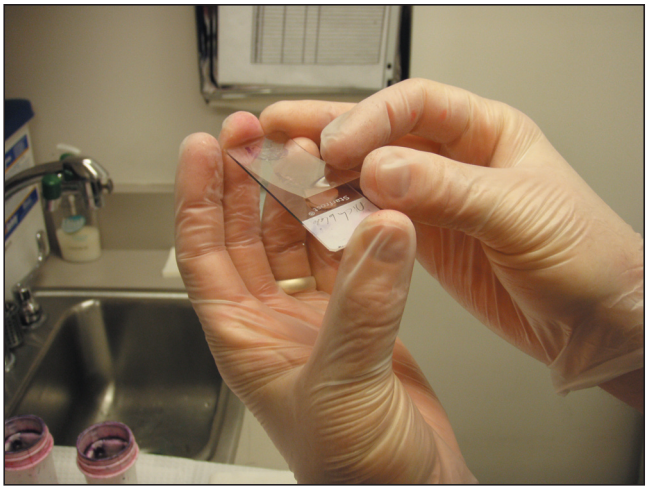


FIGURE 7.10: Flexing coverslip to avoid air bubbles.

FIGURE 7.13: Slide being coverslipped.

up to this point, poor coverslipping can ruin the slides. Manual dexterity and control of both the slide and coverslip are critical to prevent damage to one or more wafers by incorrect coverslip placement.

Techniques for coverslipping include:

1. The author's preferred technique is to carefully place the coverslip lengthways onto the slide while flexing it slightly. Several coverslips may be broken while learning this technique. The coverslip is flexed so that any bubbles in the coverslipping medium are "popped out" and new bubble formation is avoided as the coverslip straightens (Figure 7.10).
2. The medium (glue) is placed on the slide and the slide is turned upside down onto the adjacent coverslip lying next to it on the counter.
3. The coverslip is placed lengthways over the slide and dropped gently onto the slide at an angle, so one coverslip edge contacts the slide before the other.

When the coverslip is resting on the slide, its "frosted side edge" will extend past the side edge of the slide. If there are any residual air bubbles, the coverslip's corner may be lifted again, allowing the bubbles to "pop" with the changing surface tension on the medium. Adequate medium must be placed on the slide before coverslipping or the slide will dry out in just a few days, and tissue exposed by any air bubbles will degrade. Once the slide is coverslipped and bubble free, the overhanging coverslip edge should be pushed until it is flush with the edges of the slide, and the back of the slide and its edges should be wiped. The slide is now ready for reading (Figures 7.10–7.13).

CHAPTER 8

Lab Pearls: Troubleshooting Slide Quality

Alex Lutz

POOR SLIDE quality is the most common complaint of Mohs surgeons about the work of Mohs technicians. This chapter addresses quality concerns in Mohs slides – assessment, troubleshooting, and tracing deficiencies to the specific technical errors that caused them.

Mohs technicians must be able to continually assess the quality of their slides. Familiarity with the optimal setup and use of a microscope is of paramount importance for technicians and is discussed in detail in Chapter 3. Ideally, the Mohs surgeon-pathologist should use a high-quality, two-headed binocular microscope and review slide quality on a regular basis with the technician (Figures 8.1, 8.2, and 8.3).

Mohs technicians should be responsible for checking the quality of their work, as they, more than the surgeon-pathologist, have the expertise to remedy any problems discovered. The best Mohs technicians are proactive and do not wait for complaints about slide quality. The technician should initially scan slides at low magnification to assess overall quality, then at higher power, as necessary. With the slide on the stage, each cut should be checked for complete tissue representation, adequate chromacoding, and imperfections within the wafers. Ideally, the microscope should have a 1×–2.5× scanning objective to assess for gross imperfections. Using the lowest available magnification permits detection of folding, wrinkling, and tissue loss. These may be caused by wafers freezing to the blade or being pulled or stretched to the point of tearing during pickup.

Next, magnification should be increased to 4×, and each cut should be checked again for complete tissue representation as well as quality of chromacoding. Assessment should be made for good differentiation between the cellular details stained with hematoxylin and those stained with eosin, and for whether the specimen wafers are evenly stained or areas of the wafers are unstained or understained (see Chapter 7). This information should be used to correct any problems noted.

FIGURE 8.1: Microscope oculars.

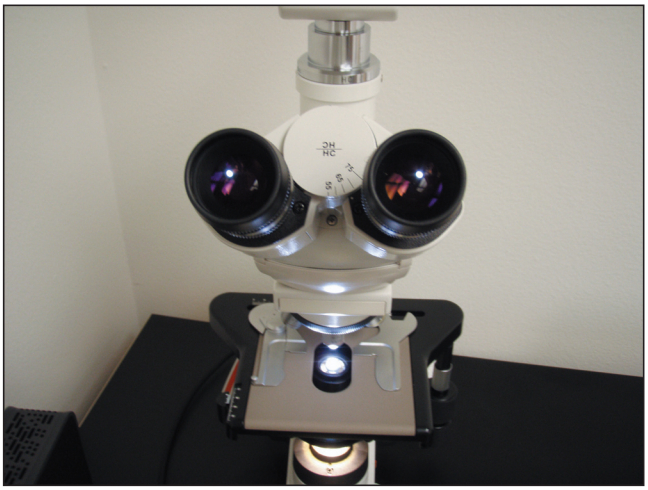


FIGURE 8.2: Microscope.

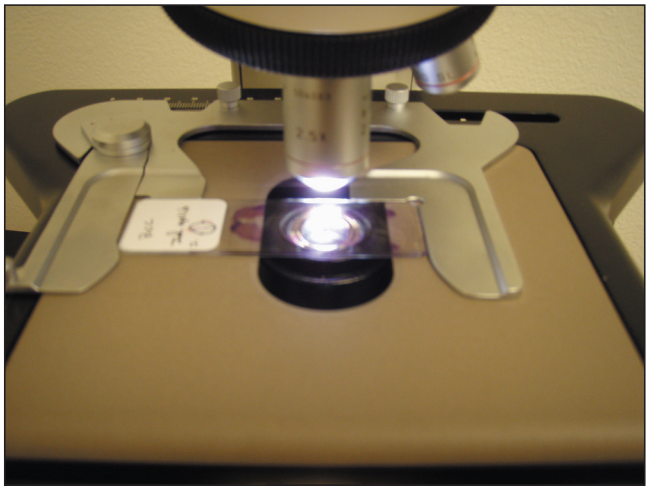


FIGURE 8.3: 2.5× scanning objective.

Problems noted microscopically usually fall into three categories:

1. Margin completeness.
2. Wrinkling and folding.
3. Staining.

MARGIN COMPLETENESS

Resolution of the issue of incomplete margins involves retracing steps to determine where the error occurred. Technicians must have the professional integrity and humility to critically look for errors and learn from those errors to continually improve slide quality.

If part of the deep or peripheral margin is incomplete, deeper sections should be cut until full representation is achieved. Then it should be determined why margins were incomplete despite initially placing multiple wafers on the slides: Was the specimen perfectly flat before it was embedded? Did it remain perfectly flat during the embedding process, or did it dislodge in some way and fall back into the tissue freezing medium (TFM) during freezing? After facing the block, was the block sufficiently sectioned into until full representation of the specimen was present on each wafer?

Determine if missing margin(s) appear on re-cuts. If a missing margin does not appear after cutting through the block, a common cause is improper tissue flattening. The result of this is a high area of tissue that was cut away and forever lost when the block was "faced."

An important skill all Mohs technicians should have is the ability to check their unstained slides for epithelium. If technicians can assess whether complete epithelium is present on the wafers they are cutting before these wafers are stained, they can ensure that peripheral specimen margins are fully represented while still cutting tissue. The technician should assume the tissue being cut has a complete epithelial margin. To evaluate peripheral margin epithelium, the unstained slides should be held up to a light and examined closely. If the wafers are cut whole, there should be epithelium along the entire circumference. If bisected, there should be marking inks along the entire cut edges and epithelium along the entire outer edges. Epithelium will appear as a thin, translucent line or ribbon at the outer edge of the wafer. This is distinguished from the unstained dermis, which appears opaque and more frosted. Adipose, usually in the deep margin, is also opaque with a jelly-like appearance. If a portion of most of the wafers has no translucent rim where epithelium is expected, or incomplete tissue inking, there is a strong chance that the peripheral margins are not adequately present and immediate additional re-cuts should be prepared. Practice and microscopic confirmation after staining sharpens the eyes and increases confidence in the prestaining assessment of peripheral margins.

WRINKLING AND FOLDING (Figures 8.4–8.7)

Nearly all problems with wrinkling and folding arise when drawing the specimen across the blade and when picking up the wafer for placement onto the slide. When the specimen is being cut, the technician must ensure that the cut wafer is properly retrieved. The technician must guide the wafer across the blade with the brush at the exact same speed as the specimen wafer is being cut across the blade edge. The wafer must be guided in the air as it floats over the blade holder. Heavy-handed brush technique will push the wafer against the blade holder as it is drawn across the blade and may cause "fall-out areas" (pits) or "compression," which results in wrinkling of the wafer. When picking up the specimen wafer onto the slide, the top edge of the wafer (the last portion to be cut) must be allowed to remain attached to the top of the specimen block. This holds the top edge in place, keeps it from curling, and allows the technician to

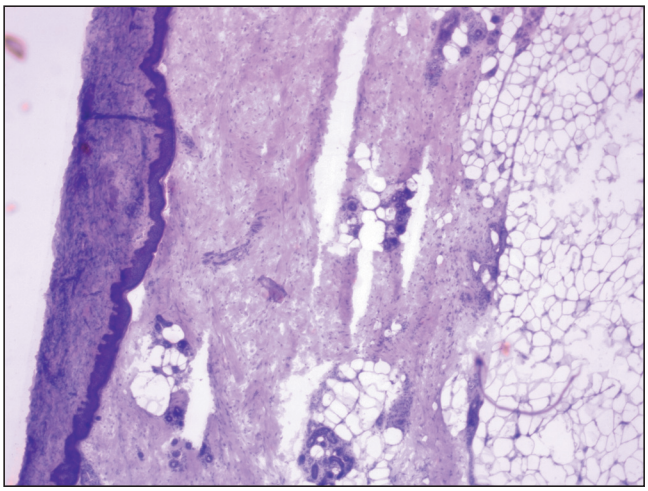


FIGURE 8.4: Folded epidermis.

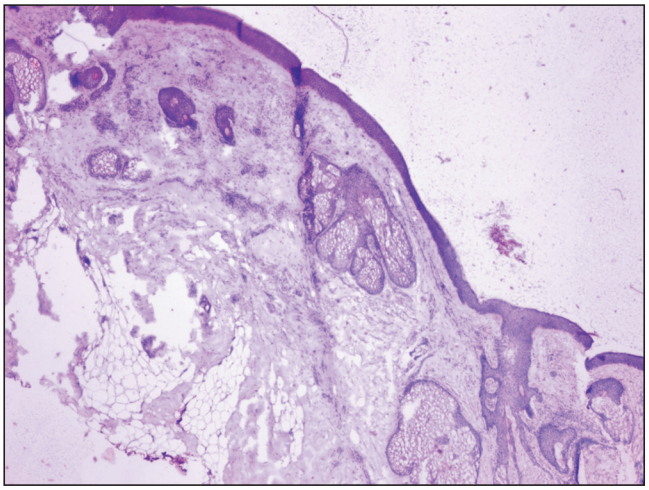


FIGURE 8.5: Folding from pickup.

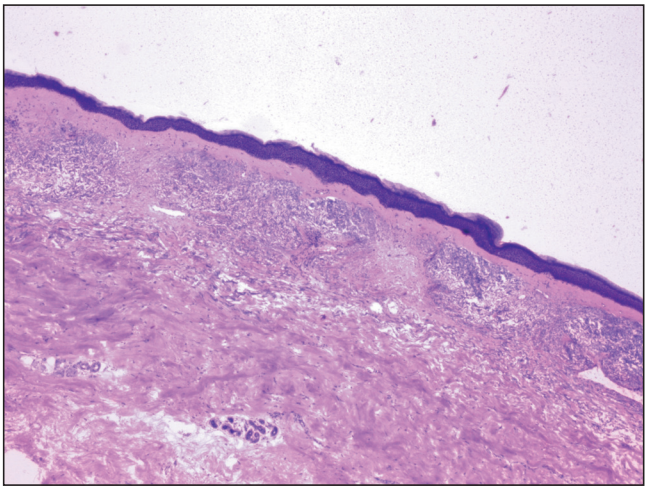


FIGURE 8.7: Optimal section.

just hold the bottom edge with the brush to control curling and folding. The specimen is then picked up onto the slide from the bottom to the top in a "sheeting action." This is the difficult part. The slide must be held steady and at the correct angle to allow the specimen to melt onto the slide without wrinkling or stretching. Wrinkling may occur if the angle is too shallow and stretching if the angle is too steep. Many technicians pick up the wafer using a "slap technique," plopping the slide down onto the top of the wafer and thus allowing the entire wafer to melt onto the slide at the same time. With this technique, great care must be taken to pull out any wrinkles before the pickup, because the method offers no opportunity for pulling out wrinkles during wafer placement onto the slide. Because pulling out every wrinkle with brushes is time-consuming and technically very difficult with the slap technique, this author prefers the "draw" pickup method (Figures 6.33–6.35).

Picking up wafers using the draw method is more efficient. It also permits greater control over the pickup, which is essential for making high-quality slides. Many technicians are averse to changing the settings of the blade holder adjustment lock. This adjustment lock determines the angle at which the blade contacts the specimen. The microtome blade angle is usually set by the installation service that initially set up the cryostat. Histotechnicians should have or acquire the confidence and training to make any necessary adjustments. If the blade holder adjustment lock is improperly set up, the technician may cut substandard slides for years. Because this angle directly affects the ability to produce high-quality slides, technicians must learn how to recognize when it is necessary to make adjustments. Typically, when a blade holder is improperly adjusted, the specimen wafer reacts in one of two ways: If the blade angle is too steep, the wafer flips up, skittering across the face of the block as it is cut. If the blade angle is too shallow, the block pushes against the bottom of the blade with the first turn of the crank, producing an erratic and uneven cut with the second or third turn of the crank. An angle somewhere in

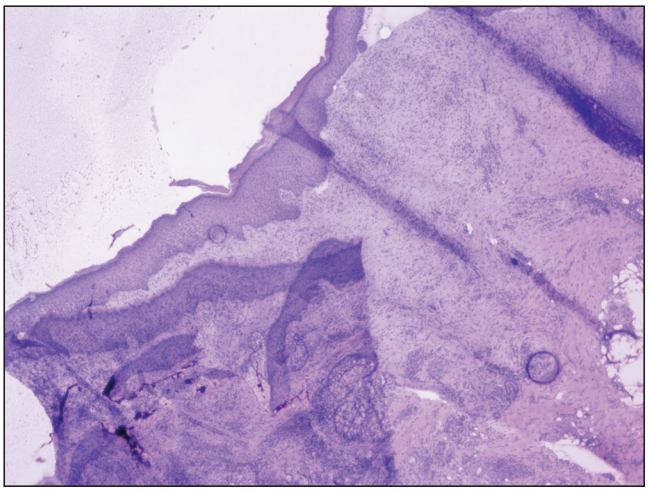


FIGURE 8.6: Multiple folds in section.

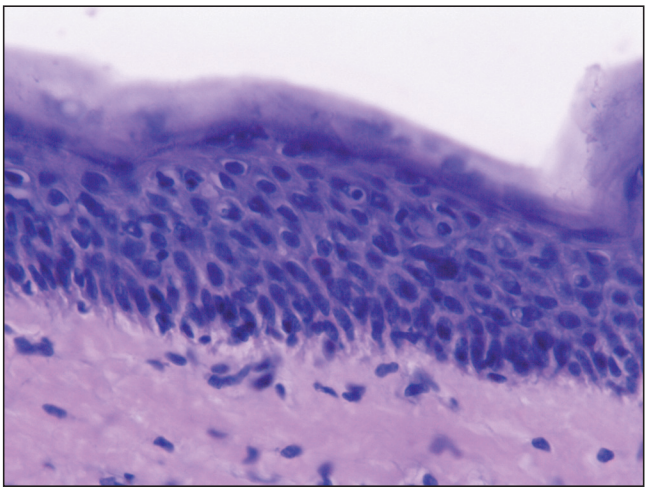


FIGURE 8.8: Good differentiation.

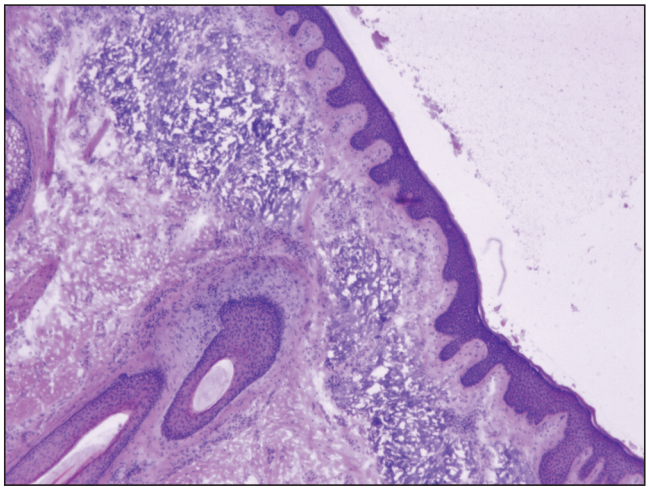


FIGURE 8.9: ***Section with solar elastosis.***

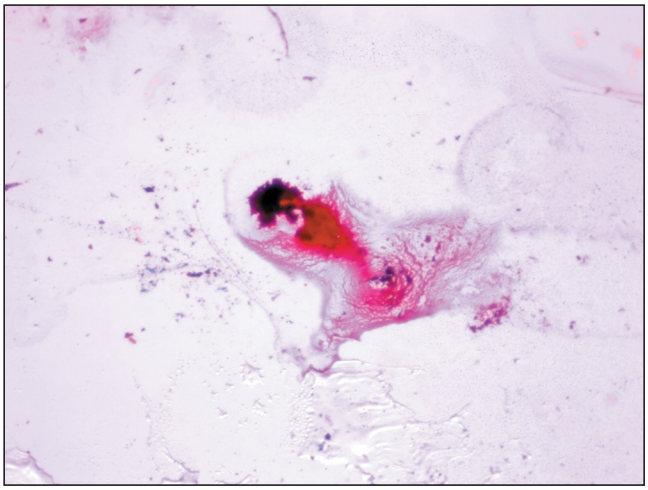


FIGURE 8.11: ***Pooling eosin from incomplete clearing.***

the middle is the "sweet spot." The wafer will contact the blade edge and float over its surface at the same angle as the beveled edge of the blade. If after initial adjustment of the blade holder the specimen wafer comes up at a 45-degree angle relative to the blade holder, the edge should still be considered too steep and the blade holder should be readjusted shallower until the optimal angle is attained. The same is true if the angle is too shallow. Typically, if the blade angle is too shallow but is steep enough to cut sections, the leading edge of the wafer will come off the blade but hit the blade holder ridge, the edge of the clamp that actually holds the disposable blade in place. This indicates that the blade angle should be increased. All blade angle adjustments should be made with a tissue-free chuck so that TFM, but not tissue, is wasted. It is best not to make these adjustments in the middle of a surgery session, unless it is absolutely necessary. In summary: Do not assume that the blade angle in the cryostat or that specified in the cryostat manual is the optimal blade angle; check the blade angle, and find the "sweet spot" for yourself.

STAINING (Figures 8.7–8.12)

Staining problems are common and are easily remedied. Corrections do not require manual dexterity or complex technique. Use a timer when staining to eliminate timing errors.

Common staining problems include:

1. **Erratic slide staining.** The wafers are stained unevenly, with halos in some areas around the TFM of adjacent wafers. This is typically a result of TFM overlap. Wafers were placed close enough to one another that the TFM around one is partially or completely covering that of the adjacent wafer. This problem is usually eliminated by agitating the slide in the water bath that comes after

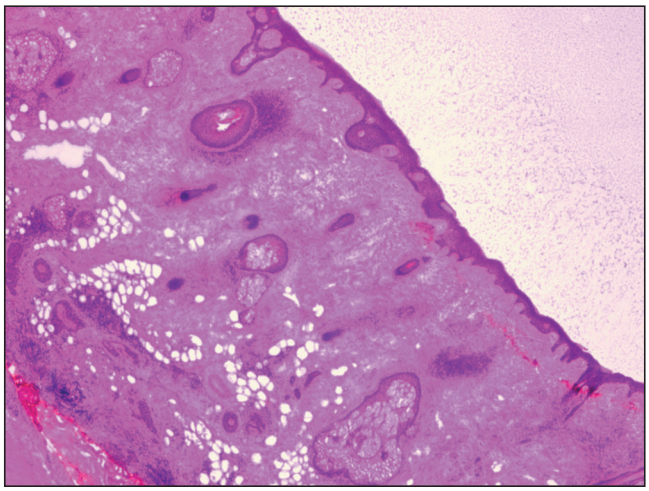


FIGURE 8.10: ***Too eosinophilic.***

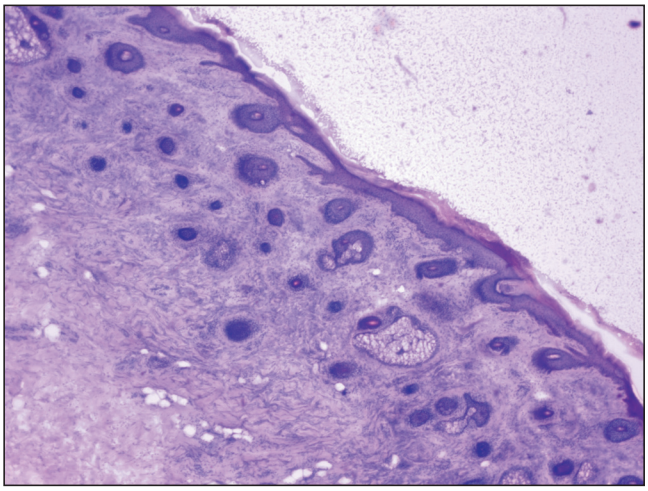


FIGURE 8.12: ***Eosin pulled out of section from incomplete clearing.***

the fixative, ensuring that all of the TFM is dissolved and removed from the slide.

2. **Staining is too eosinophilic (pink).** Part of the technician's job is to ensure that all of the staining chemicals are compatible. Depending upon the manufacturer, some eosin and some hematoxylin may be made stronger or weaker than others. Thus it may be necessary to adjust the initial stain (hematoxylin) to the counter stain. This can be done by adjusting the time the slide is left in the hematoxylin/eosin and/or by diluting the eosin with 100% alcohol to lessen the stain's intensity. Chemicals made by Medical Chemical Corporation (MCC) of Torrance, California, are sold and distributed under many different distributors' labels. This author has found the stains manufactured by MCC to be very reliable.
3. **Eosin is "pulling out" of the tissue wafers after coverslipping.** This is usually a result of improper "clearing" of the tissue using xylene substitute or another clearing reagent. Generally, changing the clearing reagent and/or increasing the time allotted for clearing the tissue corrects this problem (Figures 8.11 and 8.12).
4. **Issues related to hematoxylin.** There are several different types of hematoxylin stains that differ in strength. Moreover, the strength of specific types of hematoxylin differs among manufacturers.

Types of hematoxylin include:

1. **Harris Hematoxylin.** Among the oldest of the hematoxylin stain compositions. This stain was the standard for many years. Harris Hematoxylin is a nuclear stain that penetrates the nuclear membrane of most cells to stain them. In order for nuclear penetration to occur, the stain must be slightly acidic. This is why nearly all commercial-grade, non–special-order hematoxylin stains contain acetic acid. The stain requires slight buffering for proper coloration. This is the reason many staining protocols include a "bluing solution" after the hematoxylin step. When hematoxylin staining is too strong and shortening the staining time does not eliminate the excessive color density, the technician may use a differentiating agent immediately after the hematoxylin, but before the bluing reagent. The differentiating agent is slightly acidified water and destains the tissue. This step, however, may cause additional problems because the differentiating agent is hard to control and may destain the tissue too much, or too little.
2. **Mayer's I and II Hematoxylin.** These stains are similar in strength to the Harris Hematoxylin, but formulated to form fewer crystals in solution, decreasing the amount of hematoxylin crystal artifact on the slides.
3. **Gill's 1, 2, and 3 Hematoxylin.** Gill's is the newest hematoxylin formulation and comes in three different strengths, the choice of which depends upon the application. For Mohs surgery, Gill's 2 or 3 is preferred, depending upon the manufacturer's stain strength and the strength of eosin being used.

Slide troubleshooting requires that the Mohs technician constantly attend not only to what is happening in the cryostat, but to how the staining process is affecting the final slide product. It is vital that the technician continually and critically analyze the work product and look for ways to improve it.

CHAPTER 9

Mohs Slides Organization and Standardization for Effective Interpretation

Ken Gross

MOHS SURGEONS often do multiple cancer excisions concomitantly. This may involve multiple patients and sometimes multiple sites on these patients. Organization is the way the Mohs surgeon-pathologist approaches and optimizes the excision of the cancer, processes and interprets the tissue margins, and translates these findings back to the patient's surgical wound in an efficient manner. Standardization allows the Mohs method to be reproducible and reliably accurate.

SLIDE ORGANIZATION

When performing multiple simultaneous Mohs surgeries for skin cancer, the Mohs surgeon-pathologist must have the slides organized in a way that ensures that the correct patient's slides are being read from the deepest wafers (closest to the true margin) to the shallowest wafers (deepest into the block); that each stage is clearly differentiated from the preceding and following stages; that the chromacoding is accurately done and interpretable on the slides; that multiple tumors on the same patient can be distinguished; and that the pathologic findings can be related to the patient's defect(s).

EVALUATING AND INTERPRETING MOHS SLIDES EFFECTIVELY

Over the course of hours, the Mohs technician should produce high-quality slides that will demonstrate approximately 100% of the true margins of the excised tissue. Before attempting to interpret these slides, the Mohs surgeon-pathologist should follow several steps:

1. The microscope should be set up for optimal performance (see Chapter 3).
2. The biopsy slides for each patient and cancer site should be at the microscope along with the Mohs frozen section slides for each site.
3. The first frozen section Mohs slide produced each day should be assessed for quality, and any problems immediately addressed with the Mohs technician. An entry should then be made in a Mohs log documenting the quality of the slides and any corrective actions taken to rectify slide quality deficiencies.
4. The names and accession numbers on the slides should be checked to be sure that they correspond to the names and accession numbers of the Mohs maps on which the interpretation of these slides is to be entered. Ensure that the slides are in order, so that the first slide corresponds to the deepest margin (first cuts off the block) and subsequent slides are also examined in the correct order. Check that the number of blocks into which the tissue was subdivided is represented on the slides and that the general shape and size of the tissue on the slides

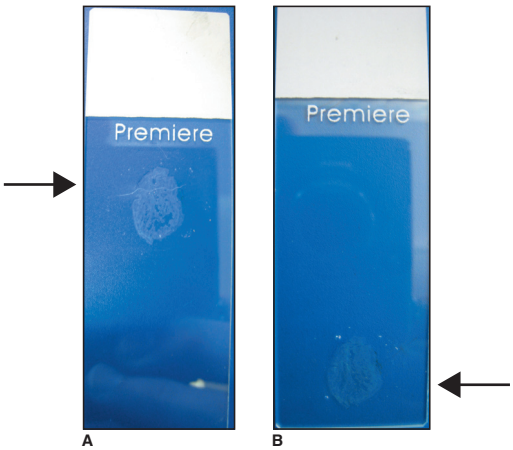
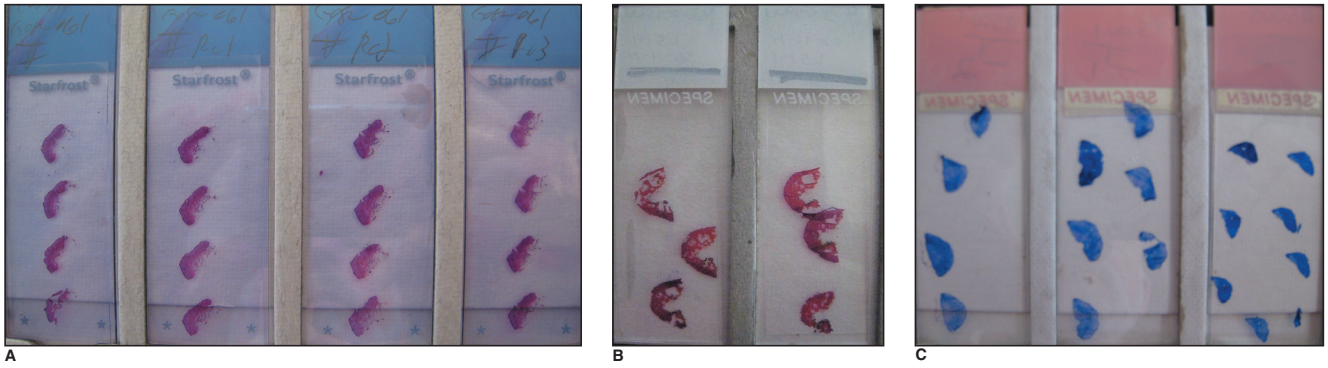


FIGURE 9.1: ***(A) The first wafer is adjacent to the label and each adjacent wafer from progressively deeper cuts into the block will be placed progressively on the slide farther from the label end. (B) The first wafer is placed adjacent to the end of the slide away from the label; each adjacent wafer is from progressively deeper cuts into the block and is placed along the slide progressively closer to the label end of the slide.***



A B C

FIGURE 9.2: ***(A) Slides where each wafer is placed in the same orientation and laid out on the slides in a straight line. (B,C) Wafers randomly placed on the slides, regardless of orientation. In (B) the wafers are at least in the same orientation although not lined up but in (C) they are randomly scattered about the slides, making assessment by the surgeon-pathologist very difficult.***

corresponds to the general shape and size of the processed tissues.

Only after ensuring that the correct slides are being read in the correct order should the Mohs surgeon-pathologist begin to interpret the slides. Each Mohs office should have an established protocol for where the first-cut wafer is placed on the slide: toward the label, or at the end of the slide away from the label (Figure 9.1).

The wafers should be placed in the same orientation and laid out on the slide in a straight line (Figure 9.2A). Wafers placed haphazardly on the slide (Figure 9.2B,C) suggest a poorly trained, lazy, or incompetent Mohs technician and make reading the slides more difficult, more time-consuming, and more prone to error. In assessing the completeness of the deep and peripheral margins, it is often necessary to evaluate more than one and even more than a few wafers; this process is expedited by having the wafers all oriented in the same direction and lined up on the slides. Once the Mohs surgeon-pathologist determines where the 12–3–6–9 o'clock reference nicks are for one wafer, it is easier to maintain the orientation if it remains the same on all the wafers (Figure 9.3).

The Mohs surgeon-pathologist and the Mohs technician should have an established protocol for how thin (in microns) the tissue should be cut. It is easier to read high-quality slides cut at 4–6 microns than more thickly cut tissue.

Established protocol should also determine how much total tissue is represented on the Mohs slides and how much tissue is wasted between wafers (Figure 9.4; see also Chapter 11). This information is critical in interpreting the pathologic findings. Each Mohs surgeon-pathologist must decide how much tumor-free tissue, in microns or millimeters, constitutes "clear margin" (1,000 microns = 1 mm).

If standard office protocol called for 5-micron-thick wafers, which a good technician with a sharp blade and well-adjusted cryostat at proper temperature should easily be capable of producing, the first wafer on the first slide examined would be approximately 15 microns into the block and away from the actual surgically cut base of the tissue. This first wafer is almost always incomplete; if all first-cut wafers are complete, the technician is "facing the block" too deeply, which may have untoward consequences), including:

1. A false-positive margin, because too much base was cut away to get other areas to "fill in" in order to make the first wafer on the slide complete.
2. An epithelial edge may never show up on the slide because it was cut away when the technician was over-facing the block to produce a complete first wafer.

In this author's office, the Mohs surgeon-pathologist typically examines nine wafers cut from each block. The wafers are each cut with the cryostat set at about 5 microns, but the thickness that a cryostat cuts may vary slightly from the indicated setting. If the technician takes five turns

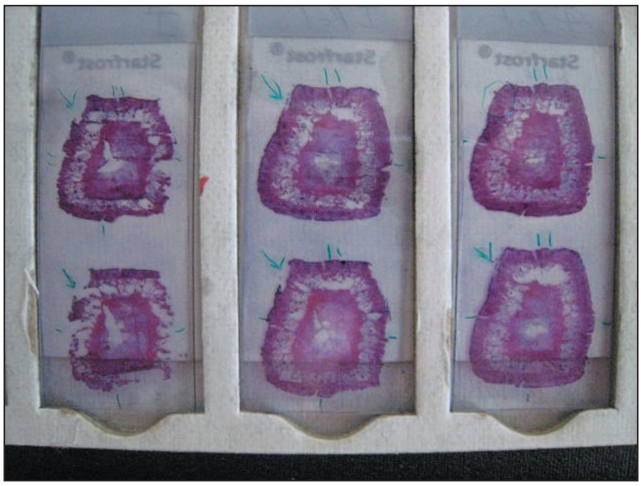


FIGURE 9.3: ***Reference nicks are easily seen on this specimen and are oriented identically on all wafers from this same block.***

FIGURE 9.4: ***There are three slides depicted in this picture. The first wafer cut from the block is at the nonlabeled end of the left-hand slide and a total of nine wafers are cut and placed sequentially on three slides. The first wafer is approximately 15 microns into the block because the TFM and a small amount of tissue were "faced" off the block and an early first wafer was placed on the slide. The second wafer is placed on the slide after five turns of wasted tissue. Five turns times 5 microns of wasted tissue equals 25 microns before wafer 2 is placed on the slide. The third and last wafer on the slide may be calculated to be approximately 75 microns from the "true base" of the surgically cut specimen. Ten turns of wasted tissue is taken between each wafer on the second slide, and the last (sixth) wafer on this slide is approximately 240 microns from the true base of the surgically cut specimen. Twenty turns of wasted tissue is taken between each wafer on the third slide (if there is a third slide), and the last (ninth) wafer is approximately 555 microns from the true base of the surgically cut specimen. These calculations probably underestimate the wasted tissue because the technician may unintentionally waste wafers that are poorly cut or have other technical deficiencies.***

between wafers for the first slide, 10 turns between wafers for the second slide, and 20 turns between wafers for the third slide, the Mohs surgeon-pathologist can calculate the approximate total amount of tissue cut away from the true base of the excised cancer tissue and also how much tissue has been cut away before any area of cancer begins to show up on the slides. The Mohs surgeon-pathologist can then decide if there is enough of a clear margin to call the area clear, or if any area requires further re-excision The Mohs surgeon-pathologist can be certain that slightly more than 0.5 mm of tissue from the surgical "true base" is represented on the slides illustrated in Figure 9.4. If an area of cancer was seen within the deep margin of wafer 6 and evaluation of the preceding three wafers showed that this same general area is both represented on these wafers and free of cancer, the Mohs surgeon-pathologist knows with a high degree of certainty that approximately 115 microns of tumor-free tissue is present between the true surgically cut margin and the area of cancer seen on the slide. There is no way to know whether the cancer was in the wasted 50 microns of tissue between wafers 5 and 6. Each Mohs surgeon-pathologist must decide how much clear tissue represents a clear margin. This may vary depending on several clinical and histologic factors: whether the cancer is primary or recurrent; what type of cancer is being excised; how much inflammation is present; and the quality of the slides. Other factors may also have to be taken into consideration. Undifferentiated squamous cell carcinoma or recurrent sclerosing basal cell carcinoma deserves different criteria than primary nodular basal cell carcinoma.

There are several considerations for the Mohs surgeon-pathologist when interpreting the deep margin. If cancer is seen within a wafer deep into the block, the Mohs surgeon-pathologist must start evaluating each preceding wafer to determine the following information:

1. Is the tissue area within which cancer cells are seen represented on previously cut wafers? Tissue not represented on previous wafers may not be interpreted as a clear margin (Figure 9.5).
2. Is fibrosis and/or inflammation present on wafers cut earlier than the wafer where cancer cells are first noted? If cancer shows up after fibrosis and/or inflammation in the same location on earlier wafers, then the wafer

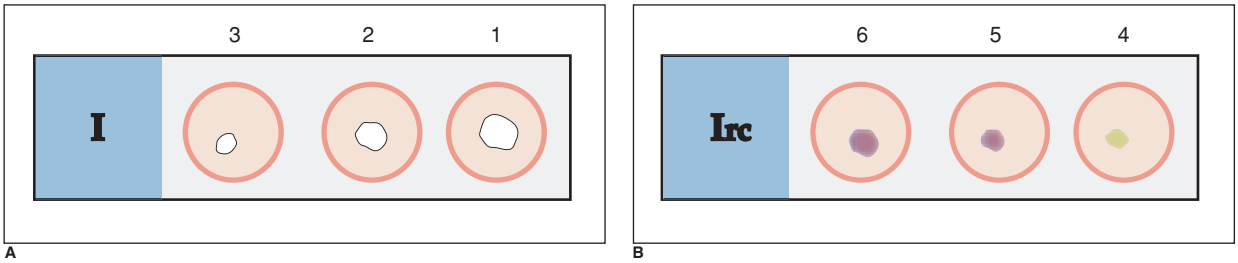


FIGURE 9.5: ***Wafers 5 and 6 show tumor (blue). To determine whether this is a true positive cancer margin or cancer seen because the technician has cut more deeply into the block and therefore away from the true margin, the Mohs surgeon-pathologist reads backwards through the wafers. Wafer 4 has complete tissue represented and some inflammation (yellow), but no cancer. Wafers 1, 2, and 3 do not have complete tissue represented in the area that was positive for cancer in wafer 5 because there is missing tissue (incomplete base, colored white) where the cancer subsequently was noted on wafers 5 and 6. Therefore, wafers 1, 2, and 3 are not clear of cancer (because they are not complete) and wafer 5 represents cancer at the true margin and requires re-excision.***

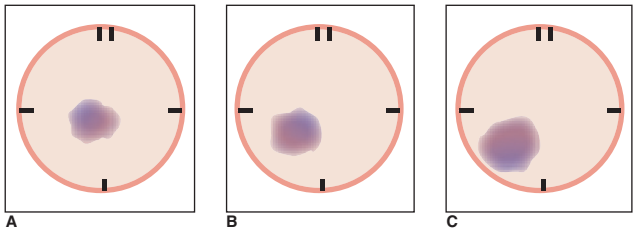


FIGURE 9.6: *(A) Tumor seen in the first wafer is within the deep margin. (B) and (C) This tumor progresses toward a peripheral margin on subsequent wafers as the technician cuts deeper into the block. Even though the cancer does not extend all the way to the epithelial edge, an additional peripheral rim of tissue and additional deep base should be re-excised to ensure adequate tumor margin overlap (see also Figures 9.8–9.10).*

where the fibrosis inflammation first shows up must be considered the first wafer with the positive margin. It is better to overinterpret than to underinterpret these findings.

3. Even if complete peripheral margins (epithelial margin on the first stage) is seen on the slides, and even if all these peripheral margins are clear, is there a progression of tumor toward one or more peripheral margins as you examine the wafers sequentially (Figure 9.6)? If so, the Mohs surgeon-pathologist should strongly consider taking an additional rim of peripheral tissue from 5 o'clock to 10 o'clock as well as additional deeper tissue.
4. If cancer is seen at a deep margin, close to but clearly not involving the peripheral margin epithelium, the Mohs surgeon-pathologist must be very careful to understand where this positive cancer margin is located within the surgical wound.

Figure 9.7A,B shows a hypothetical cancer on a patient's skin, viewed from above, before (A) and after (B) excision. The discussion that follows will illustrate the importance of three-dimensional (3D) interpretation of the two-dimensional (2D) Mohs slides to arrive at the correct area for re-excision of a positive cancer margin.

It is important that the Mohs surgeon-pathologist understands what happens to a 3D piece of tissue cut from a patient when the technician flattens the specimen into a single plane for sectioning. Figure 9.8A shows a side view of the tissue before flattening and Figure 9.8B shows the same tissue after flattening.

In Figure 9.9A, the area between the epithelial peripheral edge and the flat base appears on the slide as a positive margin within the deep base. The Mohs surgeon-pathologist must understand that this cancer is located within the 12 o'clock-to-3 o'clock base in the 2D tissue wafer but that upon returning to the patient's wound, this base is located within, or partially within, the area of beveled-cut tissue (the wall of the excision) as well as within the flat base of the wound (Figure 9.9B,C,D). The reason for this potential confusion is illustrated in

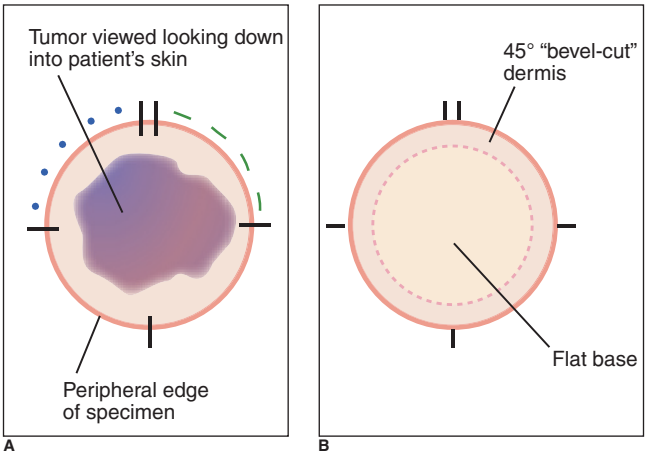


FIGURE 9.7: *(A) Patient's cancer viewed from above and marked for excision. (B) Defect after stage I excision, also viewed from above. The length of the bevel cut running at about a 45-degree angle from the surface of the patient's skin to the flat base is variable depending on how deeply the wound was cut from the surface to the flat base.*

Figure 9.9B and C where the tissue flattening has moved the tumor from a position on the excision wall (B) to the base of the flattened specimen (C) in preparation for cutting the tissue into slides. The area of residual cancer in the patient lies partially within the sidewall of the defect, not just in the base, as visible in the patient's wound (Figure 9.9D) when the Mohs surgeon-pathologist returns to the operating table and examines the wound. It is critically important that the Mohs surgeon-pathologist understand the relationship between the positive deep margin on the slide (Figure 9.9A) in this situation and the location of this margin within the patient's wound. It is necessary to take an additional peripheral margin as well as partial deep base to properly excise and overlap the positive margin (Figure 9.10).

Another source of confusion and potential error in effectively interpreting the deep margin occurs when there is perineural or intraneural inflammation or cancer. The involved nerve may lie within the base area of the wafer but may traverse the wound in any direction; while it may go to a greater depth within the patient's wound, it is even more likely to extend horizontally. Therefore, the next stage of excision must include both peripheral and deep tissue, and the nerve must be visualized in the subsequent stage before that margin can be called clear (see Chapter 17). If the next stage shows no cancer but also no nerve tissue, the cancer cannot be considered completely excised.

On deep tissue stages beyond stage I where fat, fascia, muscle, and other tissues are involved, the entire peripheral margin should be chromacoded; three or four different colors may be used to make orientation and interpretation of the slides easier. Additionally, the amount of wasted tissue examined and found to be clear of cancer before any cancer shows up on a subsequent wafer should be greater than for

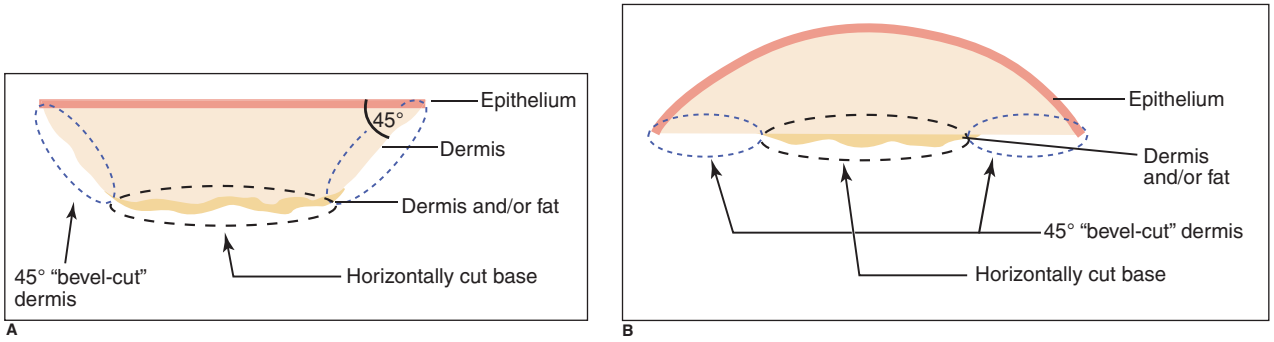


FIGURE 9.8: *(A) A side view of a hypothetical specimen cut from a Mohs patient in Figure 9.10 before the tissue is flattened into a single plane for sectioning. (B) The epithelial edges and base are now flattened into a single plane for sectioning. The technician is processing the tissue, not yet seen by the Mohs surgeon-pathologist because slides are not yet cut. No cancer is depicted in Figure 9.8A,B because the figure illustrates what happens to the cut tissue as it is flattened by the technician into a single plane for processing.*

stage I slide interpretation before calling this deeper stage "cancer free" at the margins. This is because deep tissue excisions have a greater chance of handling, orienting, and processing errors. The thickness of the excised tissue may be less uniform, and it is harder to be sure that 100% of the specimen edge lies in the same plane as the base plane of the excision. This deep tissue may be more difficult for the Mohs technician to process and cut. It is advisable to have

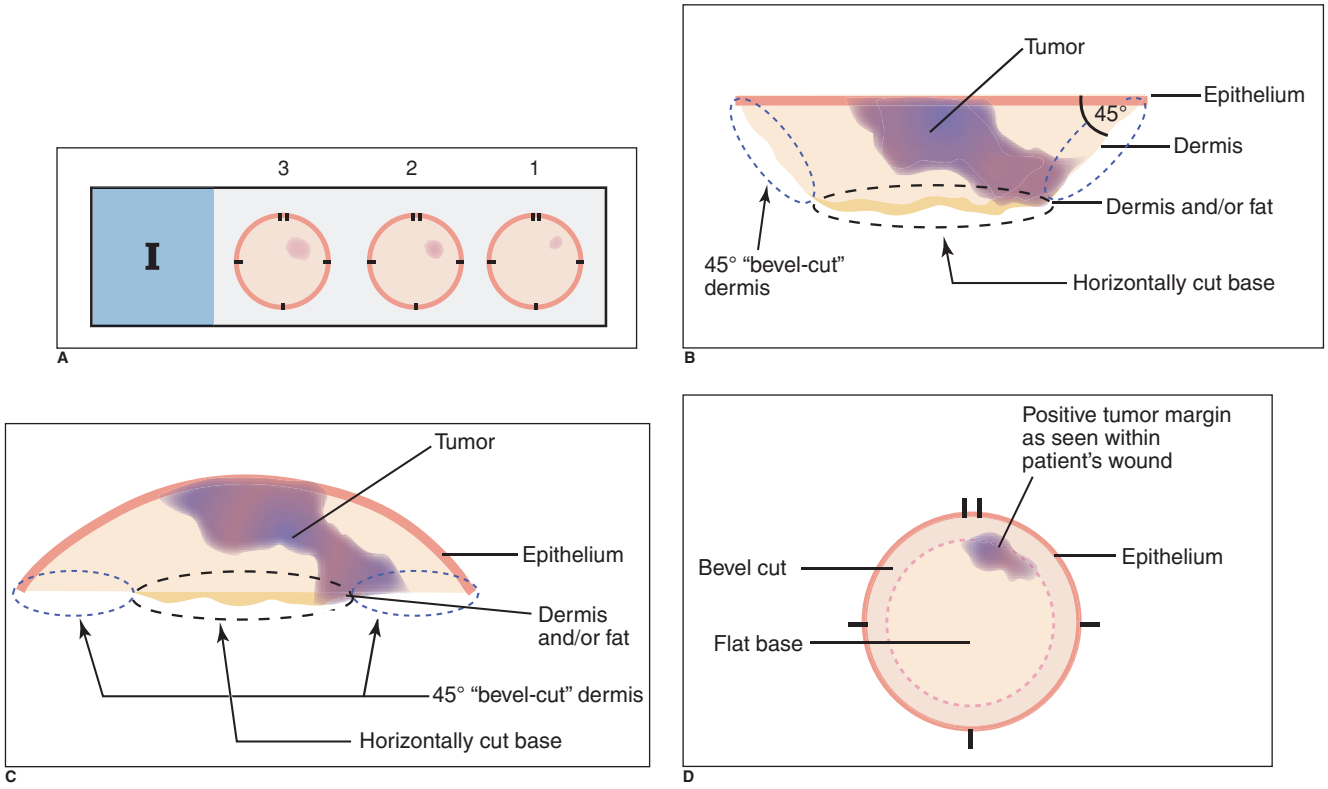


FIGURE 9.9: *(A) Mohs slide produced from tissue excised in Figure 9.7A,B and processed by the technician, showing a positive cancer margin in the "deep" 12 o'clock-to-3 o'clock margin in the wafer. Note that the cancer seen on the wafer does not extend to the wafer's epithelial edge on wafer 1, and on subsequent step-cuts into the block it still doesn't extend to the epithelial edge. (D) depicts this cancer as it might appear within the patient's surgical wound when the surgeon-pathologist looks down at the wound in preparation for taking a stage II excision. Note that the cancer in (A) is in the "base" of the 2D wafer but as seen in (B–D) also extends onto the wall of the 3D surgical defect. (B) Based on the interpretation of the slide in (A), the cancer is shown in side view approximately as it might appear in the excised tissue before the technician has flattened the tissue into a single plane for cutting and (C) after the technician has flattened the specimen into a single plane in preparation for cutting.*

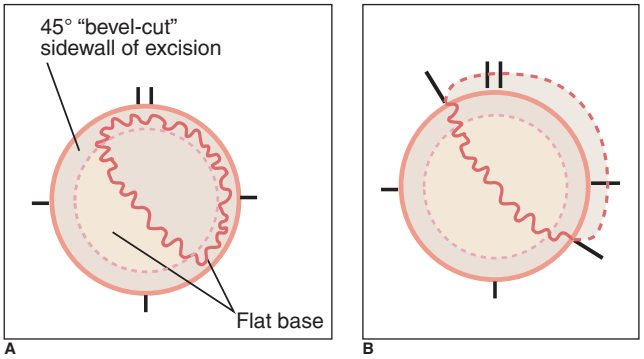


FIGURE 9.10: ***(A) Incorrect stage II re-excision of the positive margin depicted in Figure 9.9A–D. The Mohs surgeon-pathologist has failed to recognize that more than the flat base seen in the patient's wound. has residual cancer. (B) Correct stage II re-excision overlapping the positive cancer margin, which involves the flat deep base and the beveled sidewall of the stage I wound. This re-excision correctly interprets the location of the tumor in the patient's wound, based upon the slide depicted in Figure 9.9A; heavy dashed line represents a tissue excision margin taken with an epithelial margin and wavy lines represent margins without epithelium. How margins with and without epithelium are depicted on the Mohs map varies among Mohs surgeons but must be depicted to correctly interpret the completeness of the surgical margins on the slides.***

the technician cut extra slides or even to "step through" the entire block of tissue. Deep margins with "laked blood" that persists on deeper wafers cannot be interpreted as clear margins; laked blood is not a tissue margin. Deep margins with persistent "holes" that do not fill in when additional wafers are cut cannot be interpreted as clear margins; a hole cannot represent a tissue margin.

When examining the peripheral epithelial margin, a different set of problems confronts the Mohs surgeon-pathologist:

1. Can the chromacoding be ascertained, does it agree with the Mohs map, and can the specimen wafers be oriented to the patient's wound?
2. Is 100% of the epithelial (the term epithelium encompasses both epidermis and mucosa) margin represented on the stage I excision slides? Stages after stage I may or may not have any epithelium. Nonepithelial peripheral margins are completely inked so that the ink allows determination of complete margin representation.
3. Is 100% of a nonepithelial or mostly nonepithelial margin represented on the wafers of the tissues taken for stages beyond stage I?
4. Is the margin without folds, and thus able to be interpreted?
5. For stages beyond stage I, are there new reference nicks (on the patient and depicted on the Mohs map) and is it clear where the tissue represented on the slide(s) orients to the patient's changing wound?

Some Mohs surgeon-pathologists feel it is "blasphemy" to use more chromacoding colors than the absolute minimum number of colors necessary to ascertain orientation. Since we all live in a less than perfect world, chromacoding areas with additional colors is easily done and is small insurance that enough of the chromacoding will be seen on the slides for clear orientation.

When excising and processing more than one cancer site on the same patient, the use of slightly different chromacoding for each cancer may prevent the accidental mislabeling of the slides from being undetected (see Figure 9.11 and Chapters 4 and 7).

Verify that 100% of the epithelial margins are represented on the tissue wafers. This may require looking at multiple wafers if 100% of the epithelium is not seen on any one wafer. Folded epithelial edges should be interpreted with caution; tumor can hide in the folds. If any area is persistently folded, the Mohs technician must be told or shown which edges are folded and asked to cut additional slides. It may be necessary for the technician to "turn the block" in an attempt to produce wafers without folding. If the block is turned, it should be noted on the Mohs path worksheet, and any positive cancer margins seen on subsequent wafers should be carefully oriented to be sure the "turned block" is taken into account. If the Mohs technician produces consistently folded wafers, a change should be made in the method used to place the wafers on the slides (see Chapters 6 and 8). If there are areas of missing epithelium, each Mohs surgeon-pathologist must decide how much missing epithelium is acceptable; the answer should be little or none. If the Mohs surgeon-pathologist discovers that there is confusion about the chromacoding or orientation that cannot be clearly resolved, an additional margin of tissue around the entire area of confusion should be re-excised (Figure 9.12).

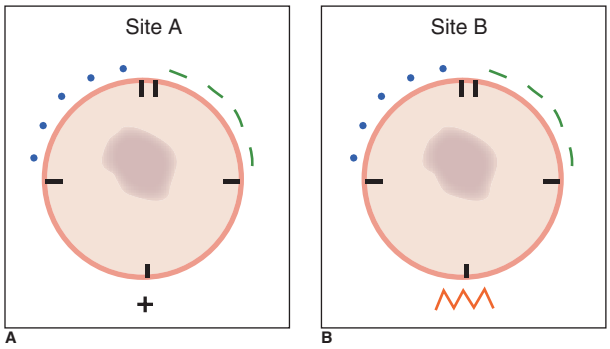


FIGURE 9.11: ***(A and B) A different 6 o'clock chromacode color is used to differentiate the wafers from otherwise identical cancer incisions from two different sites on the same patient. This author always tries to process stage I excisions in a single whole block (if size permits) and apply the chromacoding as depicted to the epithelial peripheral edges. Some Mohs surgeons chromacode the reference nicks and some divide the tissue and chromacode the cut faces.***

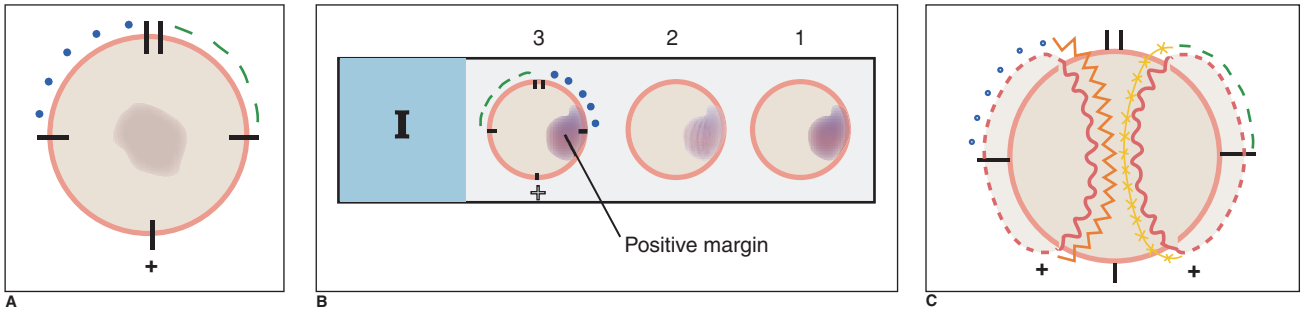


FIGURE 9.12: *(A) Chromacode pattern as depicted on the Mohs map. (B) Chromacode pattern seen on the tissue wafers disagrees with the Mohs map. The Mohs surgeon cannot be 100% certain whether the positive cancer margin is at 3 o'clock or 9 o'clock because either the technician inked the specimen incorrectly or the map depiction of the chromacoding was drawn in error. Theoretically, this could also be caused by the technician flipping all three wafers before placing them on the slide, but this would be extremely unlikely. (C) Patient's wound showing the subsequent re-excision of the positive margin. Because of the uncertainty of exactly where the positive margin is located the Mohs surgeon-pathologist is doing the re-excision to encompass all possible locations of the positive tumor margin.*

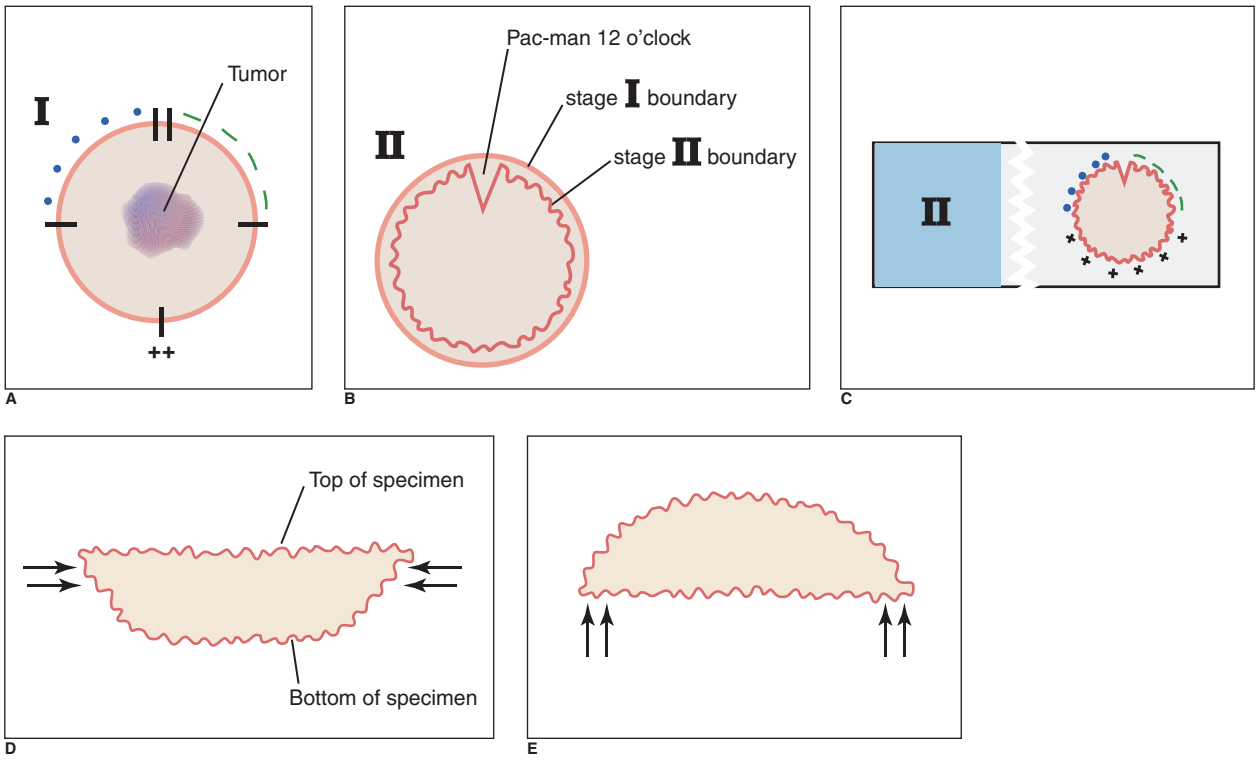


FIGURE 9.13: *(A) Mohs map depicting a central deep positive cancer margin. (B) Mohs map depicting the stage II re-excision of this positive margin with a "Pac-Man" cut at 12 o'clock to help ensure the proper orientation of a specimen with no epithelium at any edge. (C) Diagrammatic illustration of the slide showing one tissue wafer from the specimen depicting the chromacoding of the entire peripheral margin. Seeing ink on all the tissue margins, the Mohs surgeon-pathologist can be fairly certain that all excised tissue is represented on the slide wafers. (D) Side view of the stage II deep tissue excision (B) (before flattening the specimen) shows the chromacoding of the upper half of the peripheral tissue edges (arrows) to ensure that when the Mohs surgeon-pathologist reads the slides, it can be ascertained whether the entire edge of the specimen has been flattened into the same plane as the tissue base and is represented on the slide wafers. Note that the top and bottom of the specimen may be indistinguishable and that all the peripheral edges are also identical without the "Pac-Man" cut. (E) The tissue has been flattened and is ready for cutting. The upper half of the chromacoded peripheral margin should be seen at the outer edges of the wafers if the tissue is completely represented. This is directly analogous to the complete epithelial edge seen in a correctly processed first-stage excision.*

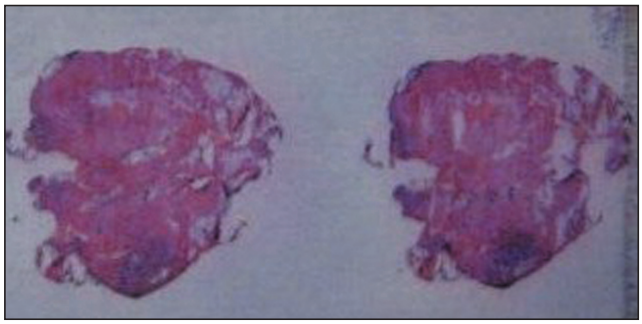


FIGURE 9.14: An easily seen "Pac-Man" back cut into the 12 o'clock margin of a deep stage II re-excision containing no epithelium. The specimen is oriented on the slide so that 12 o'clock faces to the left.

When evaluating tissue specimens without epithelium, the only way the Mohs surgeon-pathologist has of verifying that complete peripheral margins are represented on the slides is by noting that the chromacoding ink is seen at all the margins. The ink should be applied to the upper half of the tissue edge to ensure that the entire edge is represented (Figures 9.13). A "Pac-Man" back cut into the tissue can be helpful in orienting deep tissue specimens without any other orienting features (Figure 9.14). One hundred percent of the deep tissue peripheral margin should be inked (Figure 9.13C, 9.15).

GENERAL RULES FOR EFFECTIVELY READING AND INTERPRETING MOHS SLIDES

1. When ready to begin the evaluation of the slides, start with the first wafer on the first slide and evaluate each wafer and each slide in the order in which it was cut from the block.
2. When evaluating the pathologic findings on the Mohs slides: ascertain the chromacode pattern; use the established chromacoding pattern to determine the orientation on the Mohs map. Then determine if the orientation of the tissue wafers matches the map orientation and chromacode. If cancer is noted within any wafer, determine if it represents a true positive cancer margin by going back through the wafers. Relate any positive findings back to the Mohs map and mark them on the map.
3. Relate the findings to the patient's surgical wound and determine how much tissue should be removed at the next Mohs stage.
4. Verify that 100% of the epithelial edge is represented on stage I slides and that complete peripheral margins are represented for other stages.
5. Verify that complete base is represented on the slides.
6. Look for the cancer on the wafers; if the cancer is seen, work backwards through the wafers to determine if the cancer is at a true surgical margin.

Other criteria, such as intense inflammation for squamous cell carcinoma, should also be evaluated. Mark the map appropriately to include any significant noncancerous findings. If slides are subsequently reviewed at a later time, or if there is a medicolegal issue at a later time, it will be important to have all the findings annotated on the map. Never assume that if the last wafer on the last slide that was cut is clear of cancer, the cancer has been cleared. The tumor that was biopsied may dive below the plane of excision in some areas of your excision, and the tissue directly above these areas may be negative for cancer (Figure 9.16).

When taking stages beyond stage I to encompass and remove a positive tumor area, overlap the positive margin at all sides and depth. When looking at the slides from these subsequent excisions, the second- or additional-stage slides can be held up against the stage I slide to allow the Mohs surgeon-pathologist to visually note if the size of the subsequent excision is grossly large enough to encompass the entire positive margin (Figure 9.17). This is expedited by use of a Pilot pen to outline the positive margin on the stage I slide containing the positive margin, and to mark the 12–3–6–9 o'clock orientation on the slide wafer containing this margin (Figures 9.3 and 9.17).

The Mohs technician makes the task of reading and interpreting the slides easier by dividing the tissue into the largest subsections that can be processed and that will fit on a standard microscope slide (Figure 9.18). Because the technician is processing fewer slides (but with larger tissue wafers), the slides are produced more quickly. The technician has more time to cut additional wafers, making interpretation of the slides easier. Confusing anatomy such as hair follicles can be followed as they develop and are

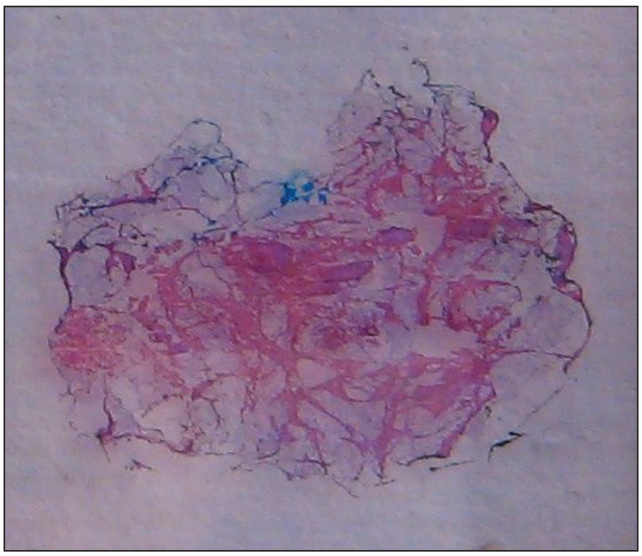
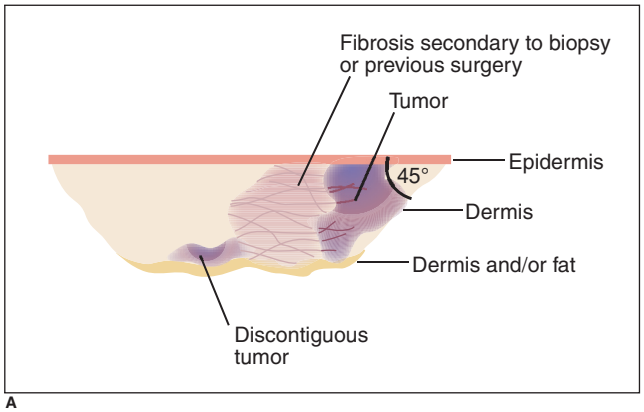
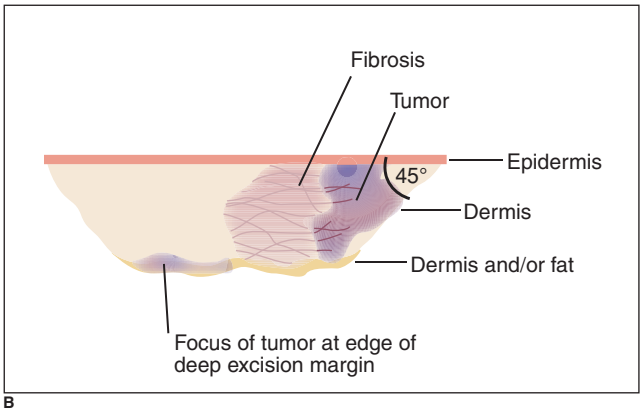


FIGURE 9.15: Chromacoding of the entire peripheral margin of a stage II specimen and "Pac-Man" 12 o'clock back cut. If the chromacode colors are not seen on the initially cut wafers, additional re-cuts are obtained until the entire inked edges are seen or the entire block is cut through.

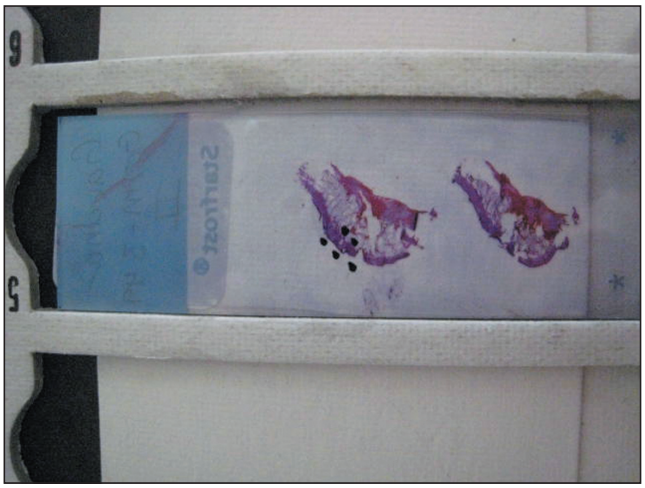


A



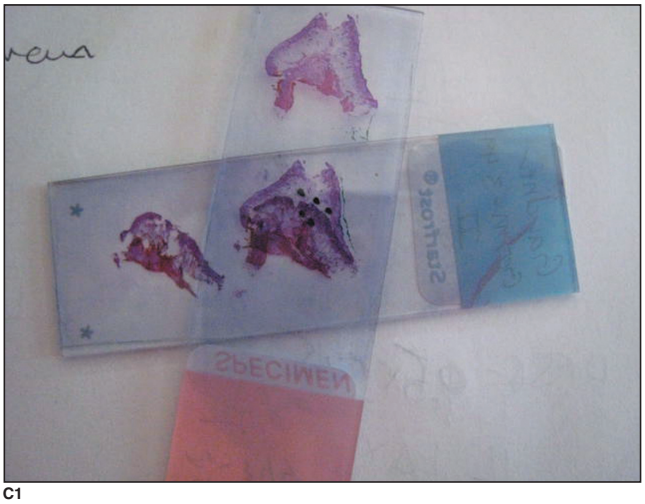
B

FIGURE 9.16: *Patient has discontiguous areas of tumor with scar tissue from a biopsy or previous surgery. (A) The excised specimen depicted in side view shows a discontiguous focus of tumor, but the focus is at the same depth as the deep extension of the contiguous tumor on the right side of this figure. The Mohs surgeon-pathologist would be unlikely to miss this discontiguous tumor focus. (B) Depicts a discontiguous tumor focus that lies deeper than the contiguous tumor on the right side of the figure. This focus could easily be missed by the pathologist-surgeon if the earliest-cut wafers were not carefully examined, or if the technician faced too much tissue from the block before taking the first wafer. It could also be easily missed if the surgeon-pathologist did not view all the wafers sequentially in the mistaken belief that if the last (deepest-cut) wafer is clear of cancer, then the stage margins must also be clear of cancer. Scar adjacent to tumor should be interpreted as a positive margin, and in this situation would prevent such a discontiguous tumor focus from being missed.*

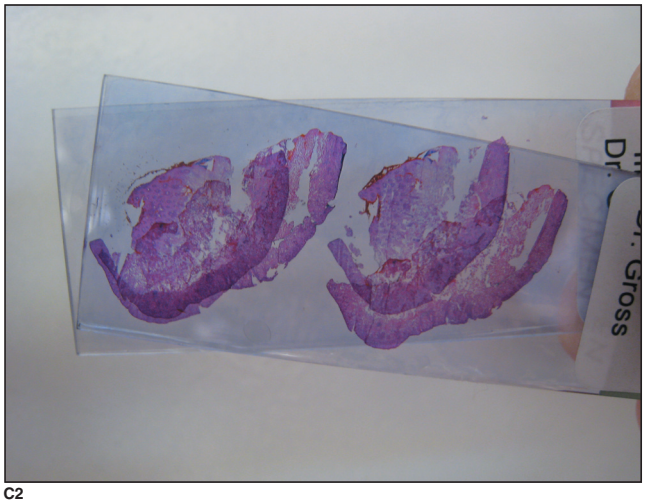


A

B



C1



C2

FIGURE 9.17: *(A) The cancer area on the wafer from a stage I slide and the 12–3–6–9 o'clock reference nicks on the same wafer are outlined with a Pilot pen. (B) The 12–3–6–9 o'clock reference nicks on the re-excision slide from stage II are marked with a Pilot pen. (C) The two slides are held together and overlapped to ascertain whether the stage II re-excision is grossly large enough to have encompassed the positive stage I cancer area. Notice that the slides from stage I and stage II have different colors at the label end. This makes organization of the slides easier. The example labeled (C1) shows the slides from (A) and (B) being overlapped as described. (C2) is a second example from another case.*

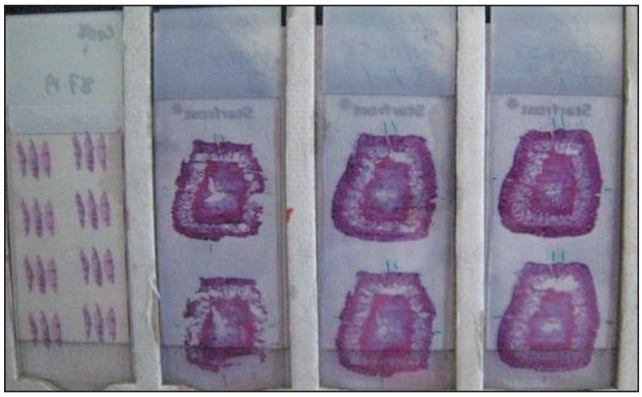


FIGURE 9.18: A large stage I wafer with the original biopsy slide on the tray to the left of the Mohs slides. The slides are turned upside down so patient information on the slides is not shown. By processing specimens in large blocks (rather than subdividing the tissue into many smaller blocks), the Mohs surgeon-pathologist's job of interpretation is made easier, and with less work, the Mohs technician can cut more wafers from each block rather than more blocks from the same tissue specimen.

examined through multiple wafers, thereby easily distinguishing them from cancer. The Mohs surgeon-pathologist has slides from fewer blocks to read and can more easily orient the tissue to the chromacode and to the patient's wound. There are fewer opportunities for error throughout the process.

If a decision cannot be made regarding whether a margin is positive or negative, the Mohs surgeon-pathologist has several options:

1. Stop the Mohs procedure until further pathologic consultation is obtained.
2. Cut additional slides (deeper cuts).
3. Obtain "special stains" using either frozen or permanent technique, depending on availability. Often the "best special stain" is obtained simply from additional step cuts into the block.
4. Take more tissue overlapping the area in question.

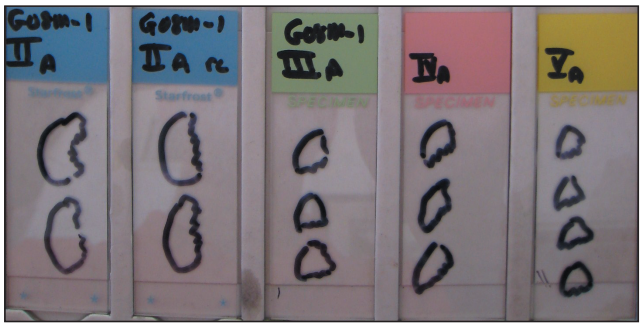


FIGURE 9.19: Using different-colored slide labels for different stages is a further aid for the Mohs surgeon-pathologist in preventing confusion and decreasing the error rate. The stage I slide that is not depicted here has a clear labeled end.

FIGURE 9.20: Slides from one Mohs session. Notice that each stage has its own color-coded slides. The biopsy slide is to the left of each case and the slides are in left to right order from earliest to deepest cuts.

If there are technical problems producing high-quality slides, such as areas of missing epithelium or holes in the base of the tissue, it may be necessary to excise and process additional tissue.

There are slides manufactured with "label" areas (Figure 9.19) in different colors, which can be used for different stages, or the technician can use imbedding media of different colors for different stages. Using these different colors for different stages is a further aid for the Mohs surgeon-pathologist in preventing confusion and decreasing the error rate

The Mohs technician should place the prepared and labeled Mohs slides into slide containers, which hold up to 20 slides, and should place them in the order in which they will be read: left to right, from the first- to last-cut slides. A biopsy slide should be available and placed into the slide container just to the left of the first slide (Figures 9.18 and 9.20). The Mohs slides should all be clearly labeled before the Mohs technician takes them to the microscope for interpretation. The Mohs technician should label all the slides with a Sharpie pen before beginning to cut tissue. This will prevent incorrect labeling of the slides and subsequent placing of the slides in other than the order in which they were cut. The Mohs surgeon-pathologist should make sure to have the correct biopsy slide(s), Mohs slides, and Mohs worksheet (name, site, path accession numbers) before beginning to read and interpret the slides. Pilot pens in one or more colors should be accessible to the Mohs surgeon-pathologist for marking areas of interest and/or importance on the glass slides.

There are many ways to label slides, and no single method is necessarily better than any other. However, once a method is established, that method should always be used, without fail and without deviation. The method used by this author is shown in Figure 9.21. The slides must be reproducibly and identically organized to distinguish patient(s), site(s), block(s), stage(s), and deeper re-cut(s).

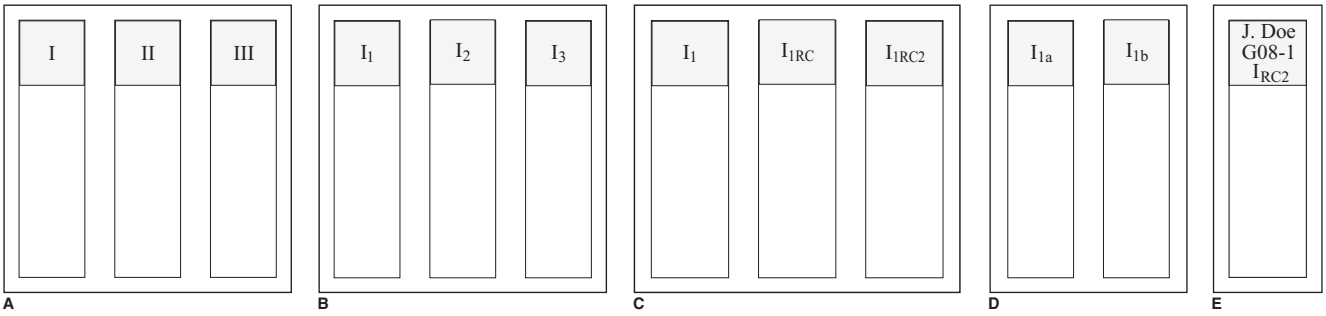


FIGURE 9.21: *Roman numerals depict stages (A) and Arabic numerals depict tissue blocks (subsections) (B). "RC" is the first re-cut slide, and subsequent re-cut slides are depicted with RC followed by an Arabic number indicating the order of the re-cut slides (C). Different cancer sites on the same patient are distinguished by a, b, c, etc. (D). Mohs accession numbers may or may not run independently of biopsy accession numbers in the office log books. The slides usually have the year followed by the case accession number (G08-xxx) and in some offices an "M" is added to follow the year (G08M-xxx) to distinguish Mohs accession numbers from non-Mohs path accession numbers. The slide label depicted in (D) is the first case of 2008 and is a stage I case with one block and is the second re-cut slide.*

FIGURE 9.22: *Unstained slides (every third slide) are cut during processing of the Mohs case and will be stained for immunohistochemistry (IHC) if it is felt to be necessary. The IHC can be done by permanent or frozen section.*

If immunohistochemistry (IHC) is planned for a Mohs case, either during the Mohs procedure or later in another pathology laboratory, alternate slides should be cut and left unstained for IHC. Cutting slides later from the tissue left in the block will lead to erroneous results (Figure 9.22).

Cases with clear margins are returned to the Mohs technician for drying before filing and final labeling. Computer-generated labels are easily produced. Difficult cases may be set aside for quality assurance (QA) review. Cases should be pulled out of the files 4 to 6 months after completion to verify the slide quality is still excellent. Bubbles on the slides or dried-up slides seen at the 6-month "look back" may mean that the coverslipping technique was poor or that the glue used was incompatible with the imbedding material or clearing material, or that there was excess water on the slide before coverslipping. If slide quality doesn't hold up after filing, corrective action should be taken (see Chapters 6 through 8).

CHAPTER 10

Mohs Mapping

Howard K. Steinman

MOHS SURGERY requires meticulous recording of wound and specimen details and pathology findings on a diagrammatic Mohs map. The map is vital as a safeguard against orientation errors and for correlating findings from pathology slides to the wound.

The map is also a component of the operative report, the pathology report, and a medicolegal document. Properly completed, it will accurately depict what was performed and why. Mohs maps may be drawn on preprinted anatomic diagrams, blank paper sheets, or photographs. All surgery stages may be depicted on one map sheet, or each stage may have its own.

Map notation occurs in three settings (Table 10.1). *Before surgery*, maps must contain patient demographic data, the surgery date, and the tumor type and anatomic location. *Before tissue processing*, maps must accurately depict (1) specimen shape and orientation, (2) reference marks, (3) patterns of any specimen subdivision into smaller tissue sections, (4) section numbering, and (5) the tissue inking patterns. *After slide interpretation*, maps must precisely depict the location of tumor foci and other relevant findings. These findings may include incomplete surgical margins, foci of dense inflammation and scar tissue; unrelated benign tumors (e.g., nevi or keratoses), and any orientation errors.

TABLE 10.1: Data for Mohs Maps

Before surgery
Patient name
Patient account or chart number
Surgery date
Mohs surgery case number
Biopsy slide accession number
Tumor type
Tumor anatomic location
Before tissue processing
Diagram (or photograph) of specimen shape and orientation
Reference marks
Specimen subdivision patterns
Section numbering
Tissue inking patterns
After slide review
Location of tumor foci
Location of other relevant pathology findings, including:
Incomplete surgical margin, foci of dense inflammation, scar tissue, unrelated benign tumors, orientation errors, incidental additional findings

MAP SHAPES

The map depicts the shape of the surgical margin. The wound usually expands after the excision while the specimen margin contracts, often asymmetrically (Figure 10.1). The relevant shape for map drawing is the wound base, where residual tumor will be located. Map shapes may be precise or representational depictions of the wound. Drawing the diagram larger than the wound permits more precise correlation of slide findings to the wound.

DESIGNATING EPITHELIAL MARGINS

During slide interpretation, the presence of epithelium is the most reliable indicator that the peripheral surgical

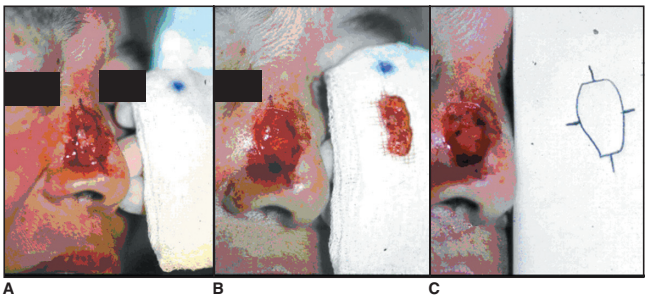
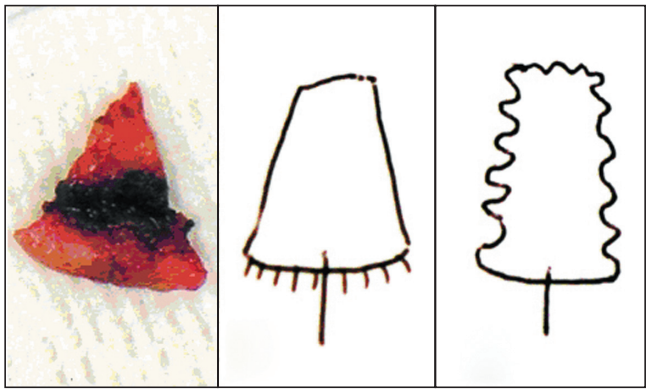


FIGURE 10.1: Excised stage I specimen within wound (A) and after placement on transfer gauze (B). Note contraction of the specimen. Histologic findings recorded on the Mohs map must be correlated with the wound, thus the relevant shape for the Mohs map is the surgical defect (C).



A B C

FIGURE 10.2: ***Mohs specimen with epithelium only at one edge (A). The epithelial portion may be designated by radiating lines (B) or by "squiggly" lines for edges lacking epithelium (C). Other methods of differentiating epithelial from nonepithelial edges are equally acceptable.***

margin is present. Second and subsequent Mohs stage specimens often lack epithelium on some or all of their periphery. Tissue ink on these edges serves as a surrogate for epithelium. To ensure complete margin assessment, it is important to depict on the map where specimen edges lack epithelium and what color ink has been placed at these edges. Assessment of nonepithelial tissue edges is not adequate, unless ink on these edges is visible microscopically. Two reliable techniques for depicting peripheral nonepithelial margins are to (1) draw short radiating lines from areas with epithelium or (2) draw edges with epithelium with a smooth line and nonepithelial margins with sinusoidal lines (Figure 10.2).

SPECIMEN INKING

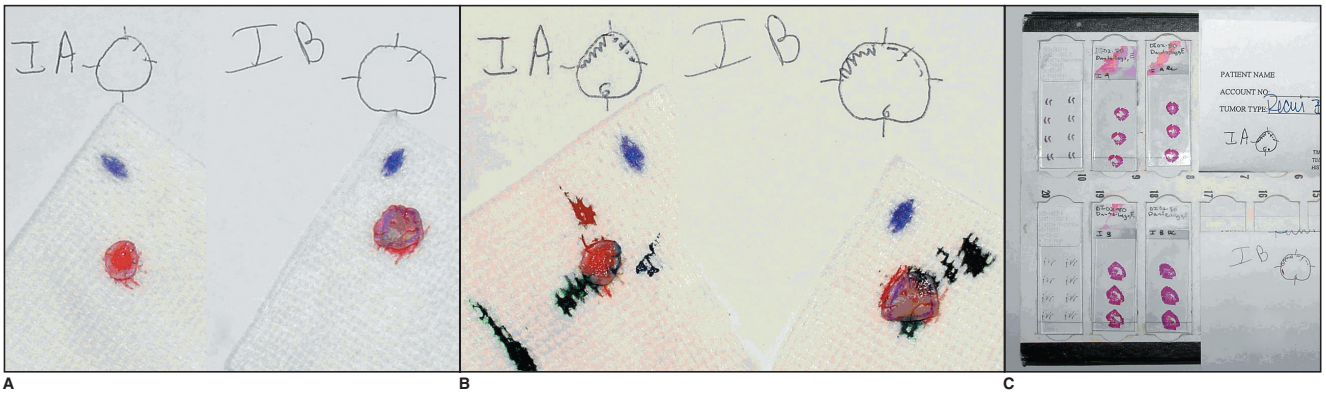
Specimen inking is the primary means of preserving orientation of tissue wafers on the slides to the map and then to the wound. It is also important for ensuring that subdivided tissue specimens are properly labeled on their separate pathology slides. Each subdivided tissue piece should have a distinct inking pattern, especially when several pieces have the same size and shape (Figures 10.3 and 10.4; Chapter 4, Figure 4.12). Effective inking is usually accomplished by using 2 or 3 colors. In rare instances, for very large or complex-shaped specimens, four colors may be required. Stemming from Dr. Frederick Mohs' methods, red has traditionally been used as a tissue ink color. Many find red ink more difficult to differentiate from nonepithelial tissues. Thus, blue, black, and green are now commonly used.

It is important to select a unique map code for each ink color and use it consistently for all cases. All Mohs surgeons working in the same practice are advised to agree on one coding scheme. Printing the inking codes on the Mohs map sheets and writing them on the ink bottles will enhance consistent code use (Figure 10.5).

Tissue inks must be placed on the cut edges of subdivided specimens and on all other nonepithelial margins. This is necessary not only to ensure complete margin assessment but to preserve specimen orientation. Care must be taken to prevent migration of the ink past the specimen edges or the ink will not represent the true peripheral margin microscopically.

First-stage specimens usually have complete epithelial margins but still require tissue inking to preserve orientation. Ink placement on these specimens may be along portions of the peripheral margin (Figure 10.3) or simply within some or all of the reference nicks (Chapter 4, Figure 4.11). Wooden sticks containing scant amounts of ink should be used to apply inks and should be directed toward the specimen from the side that is to be inked to prevent accidental placement of ink on undesired portions of the tissue (Chapter 4, Figure 4.10). Once applied, there is no method for removing misapplied ink and the map must reflect its presence.

Many first-stage specimens can be processed as one block of tissue (Chapter 4, Figure 4.5). In surgical pathology



A B C

FIGURE 10.3: ***Two similarly sized and shaped lesions from the same patient (A). Different inking patterns were used (red-blue-green and red-black-green) (B). Note the very similar slides and Mohs maps (C). The different inking patterns help prevent confusion between the two Mohs cases.***

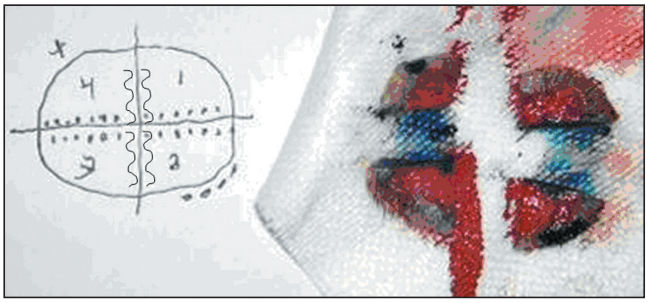


FIGURE 10.4: ***Subdivided specimens with adjacent edges inked the same color may result in identical-looking sections. In this example, sections 1 and 3 and sections 2 and 4 have the same ink colors in the same orientation. A third color on the epithelial edges of sections 2 (black) and 4 (green) more clearly differentiate all subsections.***

terminology, this is termed "total embedding 1" or TEx1. TEx1 processing greatly speeds slide preparation and interpretation. It also prevents unnecessary disruption of the surgical margins, which, when cut edges are pressed flat for embedding, could move nonmarginal tissue, including deeper tumor foci, into the same plane as the true margin. This author's current preferred inking method for TEx1 of first-stage specimens is to simply place inks within the reference nicks. One can also use a different inking scheme for each first-stage case processed in a single surgery session. First-stage slides often look grossly similar, and several trays of slides may be near the microscope. Use of different inking schemes helps ensure that the surgeon is reviewing the correct slides and that the slides are properly labeled (Figure 10.3 and Chapter 4, Figure 4.12).

Inking adjacent cut edges of subdivided specimens with the same ink color facilitates more efficient evaluation of nearby tissue on opposite sides of cut edges. The surgeon-pathologist can then place slides of different sections on the microscope and rapidly find the relevant location by searching for the same color ink (Chapter 4, Figure 4.6) Many surgeons prefer to use the same inking schemes for all cases. For example, blue might be used for the superior and left sides of cut edges while black is used for the inferior and right sides.

A third ink color is often desirable on subdivided specimens. Division of large specimens often results in pieces of equal size and shape. The resulting, often symmetric, tissue pieces would look identical without a third color on one of every two identically inked pieces, especially when adjacent cut edges are inked the same color. One effective method to differentiate these pieces is to apply a small dot of a third ink to only one of them (Figure 10.4).

Surface Inking of Deep Excision Specimens

The superficial deep orientation of specimens containing epithelium is relatively easy to maintain. Specimens composed exclusively of nonepithelial tissue, such as fat, fascia, muscle, cartilage, and periosteum, require more careful handling to ensure that they are not accidentally turned over during excision, specimen transfer, and tissue processing. They also may require alternatives to tissue nicking, such as sutures or staples, for reference marking. Specimens without epithelium may look grossly identical on both surfaces. To maintain orientation after excision, it is advisable to mark the surface with gentian violet before excision (Figure 10.6).

MARKING HISTOLOGIC FINDINGS

The process for marking relevant histologic findings is discussed in Chapter 17. Both tumor foci and other significant histologic findings must be recorded on the map. The map is the pathology report and may be scrutinized by clinicians, administrators, and legal experts long after case completion. It is important to document both foci that will be excised and those that will be ignored; the surgeon's reasoning must be evident simply by reviewing the map. Findings that require documentation include incomplete peripheral and deep margins, dense inflammation, and unrelated malignant tumor foci (e.g., superficial basal cell carcinoma foci noted while excising a squamous cell carcinoma). Other findings that may merit documentation include scar tissue, actinic and seborrheic keratoses, basaloid budding, nevi, and benign adnexal tumors.

Tumor foci are commonly drawn in red ink. All other findings are usually drawn in black ink, with a written explanation of each focus' characteristics. It is important to provide an explanation for all nontumor foci, leaving only tumor foci without labels. Mohs maps are often photocopied or scanned in gray scale, so the red color will be lost. On copies, foci without labels may then be assumed to represent tumor (Figure 10.7). One effective technique for more clearly depicting the reasoning for how and why additional tissue was excised is to draw the outline of the previous stage's wound shape in dashed lines on the map

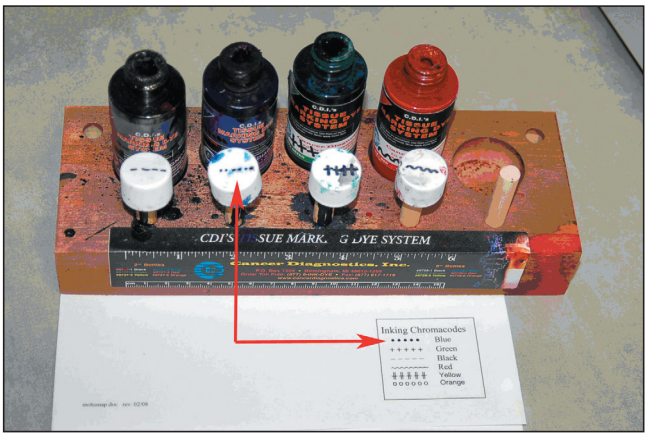
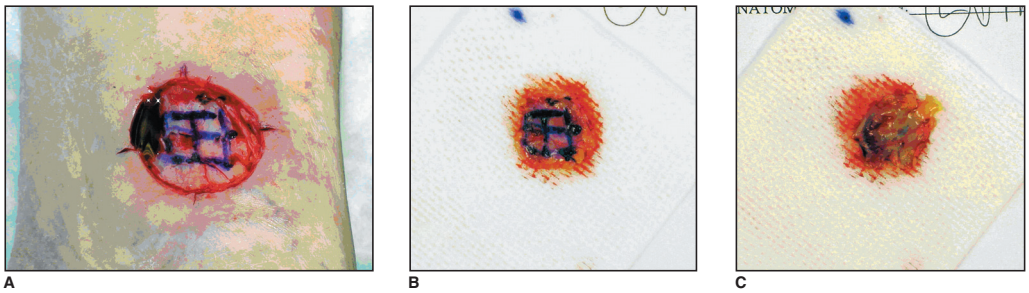


FIGURE 10.5: ***Mohs inking codes are preprinted on the Mohs map and written on the ink bottles and bottle caps.***



A B C

FIGURE 10.6: *(A) Forearm wound with central residual tumor. Fascial surface is marked with gentian violet. (B) Excised specimen is properly oriented, showing gentian violet (GV) markings on the surface. (C) Specimen turned downside up, showing absence of GV markings.*

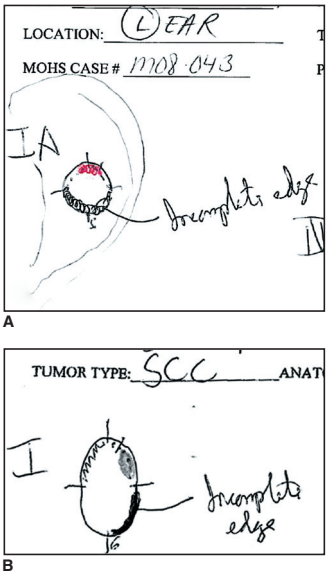
(Chapter 4, Figure 4.2). This technique clearly delineates which foci were excised and which were ignored.

ORIENTATION ERRORS

If inking, processing, and mapping are performed correctly, inking patterns will appear the same on the map and on the slides when the slides are viewed through the microscope. If the inking patterns are not the same: (1) the inking pattern drawn on the map did not match that on the tissue; (2) tissue sections were incorrectly labeled during slide preparation; (3) the specimen was accidentally turned upside down before embedding; or (4) the wrong slides (other slides from the same case or slides from another lesion or patient) are being reviewed.

If tumor or any other foci are noted that require an additional stage of Mohs surgery, any discrepancy between the map and slides must be resolved so that the location of relevant slide findings can be correlated with the wound base with certainty. If all sections from a stage are free of tumor, and there is no suspicion of tissue having been inverted, the orientation error may be noted on the map and no further tissue excised.

To resolve suspected orientation errors, first check that the map and slides are from the same patient, the same tumor (if more than one tumor is being excised from that patient), the correct Mohs stage, and the correct tissue subsection. Next, examine all other slides from the Mohs stage. Some sections may be inked correctly and/or identifiable by their size, shape, or amount of epithelium. By this process of elimination, it may be possible to elucidate the errors in



A B

FIGURE 10.7: *Maps showing tumor foci and areas of incomplete skin edge. (A) Tumor foci are traditionally marked with red ink and all other significant findings in black ink. It is important to provide an explanation for all nontumor foci, leaving tumor foci without labels. Mohs maps are often photocopied or scanned in gray scale, so the red color will be lost. On copies, foci without labels may be assumed to represent tumor. (B) It is evident where the (unlabeled) tumor focus is located, despite the lack of color distinction.*

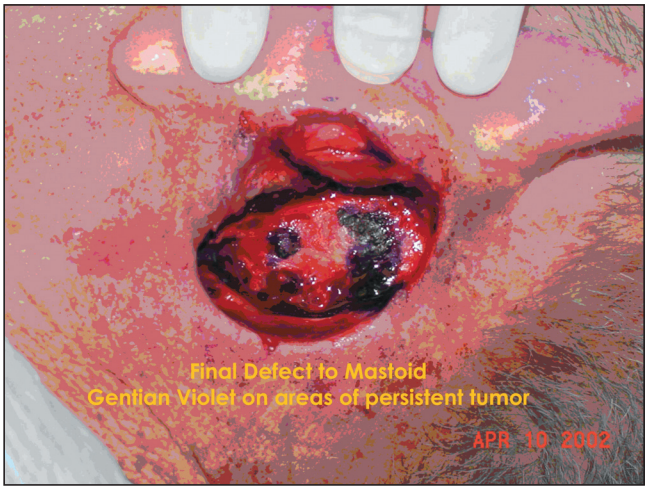


FIGURE 10.8: *Patient with tumor extension into mastoid bone. Areas of tumor involvement were marked with GV and the lesion photographed. Digital editing software was used to add a caption, and the picture was e-mailed to the surgeon continuing the case.*

processing and labeling. If uncertainty persists, examine the gauze or paper on which the tissue was inked. The retained ink patterns may indicate how the tissue was actually inked and reveal how the map was mismarked or that a tissue piece was inverted (Chapter 4, Figure 4.9). If all methods to elucidate the orientation error and permit accurate slide interpretation fail, the Mohs surgeon is obligated to excise an additional layer of tissue around all areas of the wound where orientation is uncertain.

WHEN TUMOR CANNOT BE COMPLETELY EXCISED

Situations may arise when the Mohs surgeon cannot completely extirpate the tumor and the patient must be referred to another surgeon. In some cases, this is the expected result. The patient is known to have a deeply invasive tumor, and the Mohs surgeon is been tasked with clearing the peripheral margins and the deep margins to the greatest extent possible. In other cases, due to unexpected tumor invasion or poor patient selection and planning, Mohs surgery cannot be continued. Reasons for this can include invasion of tumor through the skull, into the orbit, bony ear canal, nasal sinuses, parotid gland, or other vital structures. In these instances, it is incumbent upon the Mohs surgeon to provide very detailed information to the next surgeon on the location of residual tumor.

An excellent technique is to mark areas of residual tumor directly on the wound with sterile gentian violet and photograph these markings. This is especially useful for residual tumor in bone. Another technique is to take high-resolution operative photographs and mark the location of tumor on the photographic prints. Photo editing software may also be used for this purpose. The resulting digital photographs or scanned prints may then be e-mailed to the next surgeon for review and planning, even before the patient arrives (Figure 10.8).

PART THREE

MICROANATOMY AND NEOPLASTIC DISEASE

CHAPTER 11

Normal Microanatomy: Vertical and Horizontal

John B. Campbell

THE MAJORITY of cancers removed using the Mohs technique are located on sun-damaged skin. This is reflected pathologically as a background of epidermal atrophy, basaloid hyperplasia, intraepidermal actinic dysplasia (disorderly maturation with cellular atypia), and atypical melanocytosis, all of which may act in concert to confuse the Mohs surgeon-pathologist trying to assess the pathologic material for the presence or absence of cancer cells (Figures 11.1–11.7).

Excisions are traditionally cut by Mohs surgeons at an approximately 45-degree angle (bevel) to allow the Mohs technician to prepare sections in which the epithelial edge

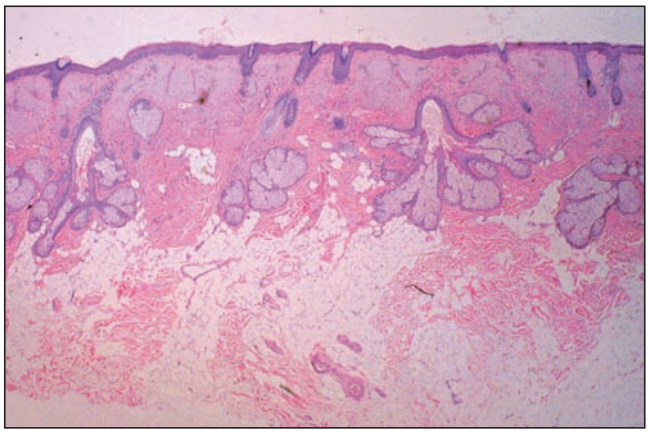


FIGURE 11.1: Sun-damaged skin.

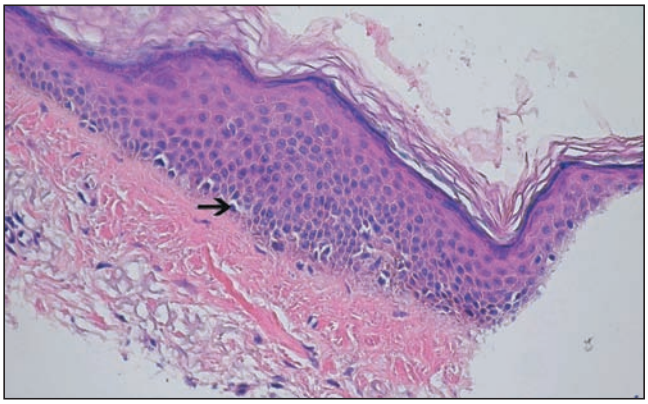


FIGURE 11.3: Atypical melanocytosis.

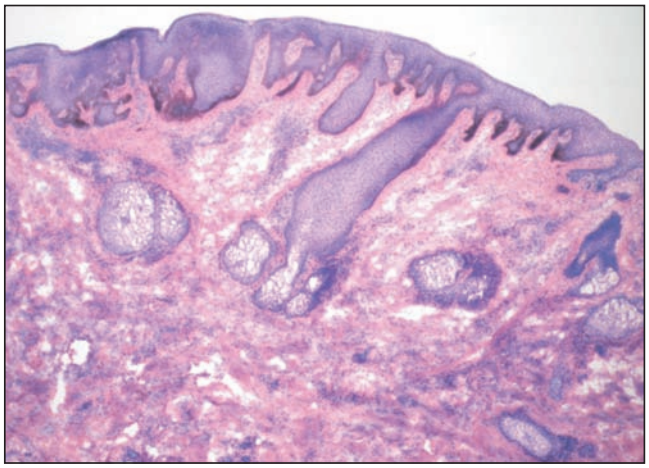


FIGURE 11.2: Epidermal lentiginous hyperplasia.

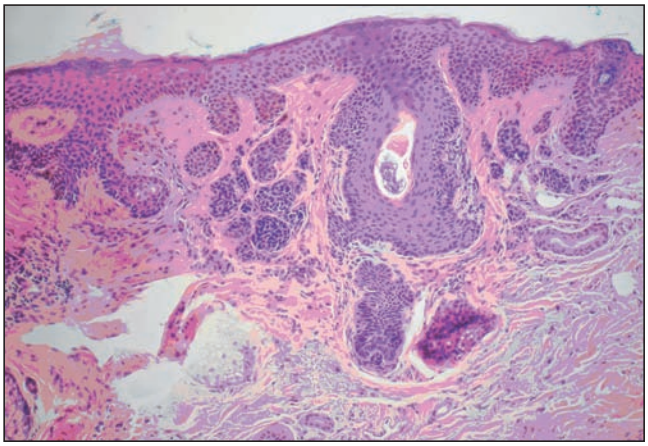


FIGURE 11.4: Basaloid hyperplasia.

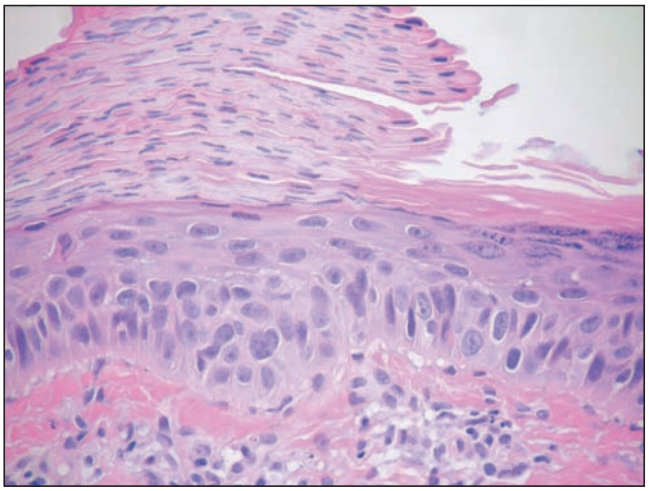


FIGURE 11.5: Moderate actinic dysplasia. Compare with severe degree in 11.6. Notice disorderly maturation, cytologic atypia, and parakeratosis (viewer may compare with adjacent epithelium in actual sections).

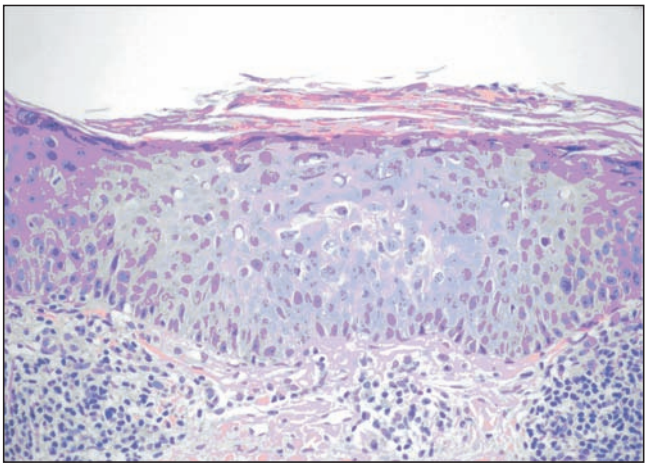


FIGURE 11.6: Severe actinic dysplasia. Compare with moderate degree in 11.5. Notice disorderly maturation, cytologic atypia, and parakeratosis (viewer may compare with adjacent epithelium in actual sections).

and deep base lie in the same plane. This results in tissue being cut in three planes relative to the epithelial surface: vertical, horizontal, and tangential. The Mohs surgeon-pathologist must then view tissue in these three different planes: a horizontal relationship to the skin surface, particularly when wafers are cut from near the epidermal surface or the central base; a vertical or perpendicular orientation, particularly near the edges of specimens "rolled" by the technician to present a flat plane for sectioning; and a tangential orientation for those wafers cut between horizontal and vertical (Figure 11.8; see also Chapter 9, Figure 9.8). This is a potential source of confusion for a novice Mohs surgeon-pathologist. In practice, most sections demonstrate "tangential" relationships among skin appendage structures and epithelium. As shown in Figure 11.8, sections from the midbase are viewed predominantly in a plane parallel to the central overlying epidermis (horizontal). At various levels in the specimen, the epithelium may be viewed in a perpendicular cross-section (100 microns and 300 microns), tangential (500 microns and 700 microns), and may again be predominately horizontal near the skin surface.

The various pilosebaceous structures, as well as vessels, nerves, and epithelium, will all be present in different, three-dimensional (3D) orientations in the various sections examined. These structures present within the skin are variable in number and spacing. Typically, there is more of a sebaceous hyperplasia on the nose, and less on the temple and other facial sites. There will be many fewer appendage structures on the neck, back, or arm. When the pilosebaceous apparatus is viewed in horizontal section, there is a flowerlike arrangement of sebaceous lobules around the central follicular structure. In areas where sebaceous units are prominent, these horizontal sections may appear very busy, making it difficult to discern small islands of basal cell carcinoma, particularly when small atretic follicles are viewed in cross-section. The Mohs surgeon must become familiar with the appearance of these normal structures and their variants in frozen section preparations (Figures 11.9 and 11.10).

Numerous interpretative artifacts are inherent to Mohs surgery frozen section tissue preparation, including stromal compression, stromal fractures, thick and thin sections on the same slide, and staining artifacts. Many of these artifacts can be eliminated with good technique and experience; for this reason a novice Mohs surgeon should try to work with an experienced Mohs technician. Further interpretive problems may be caused by difficulty in recognition of biopsy site changes, including scars, regenerative and reparative changes of the pilosebaceous apparatus, active acute and chronic inflammatory infiltrates, folliculitis, suture material, and the appearance of granulomas (Figure 11.11).

Architectural and spatial factors play a role in the assessment of margins as well as in the recognition of the presence or absence of tumor. In the following paragraphs, we will explore these relationships and discuss interpretation accuracy as a function of the attainment of truly complete margins, the review of sufficient tissue (step-sections) to ensure accuracy of interpretation, and the recognition of commonly seen pathologic alterations and orientation issues.

The typical tissues that the Mohs surgeon-pathologist evaluates may exhibit slightly atrophic epidermis (5 to 7 cell-layers thick, 50–100 microns), subjacent dermis exhibiting a prominent elastotic band 100–200 microns beneath the epidermal surface, and subjacent fat (Figure 11.12). Follicular structures may vary from 50 or 60 microns up to 200 or 300 microns in greatest dimension, depending on whether they are proliferative, resting, or involuting

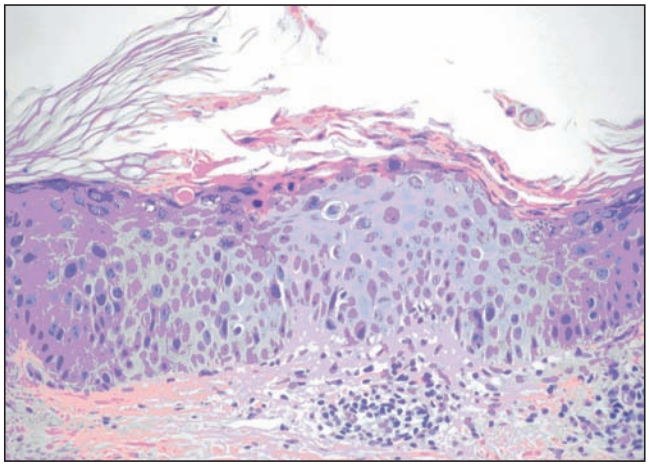


FIGURE 11.7: ***The next step in progression of dysplastic pathway is squamous cell carcinoma (SCC) in situ, with full-thickness maturation defect centrally, and much more atypia.***

(Figures 11.13 and 11.14). In vertically oriented sections, the pilosebaceous apparatus appears thin and elongated, whereas in horizontally oriented sections, the pilosebaceous apparatus appears more bulbous (Figure 11.15). In general, the follicles are confined to the dermis, but there may be occasional hair bulbs and sebaceous lobules extending into the subcutaneous fat. The presence of numerous proliferative pilosebaceous apparatuses gives a busy appearance to the sections, which may be distracting when evaluating for presence or absence of malignancy. The same follicular structures may exhibit basaloid hyperplasia, squamous metaplasia, and/or small areas of atypical basaloid follicular proliferations typical of sun-damaged skin. These changes are more frequently seen on the nose and face. Eccrine ducts can also be proliferative and may look especially ominous in inflammatory foci or when undergoing regeneration and repair. Such changes may be part of the postbiopsy healing process.

VERTICAL AND HORIZONTAL SECTIONS

Understanding orientation at various levels of the Mohs sections involves two additional issues. First, very few pilosebaceous structures are oriented perpendicular to the epithelium; most are at variably acute angles and may curve slightly as they traverse their fibrous streak from anagen to catagen phases. Second, many appendage structures are

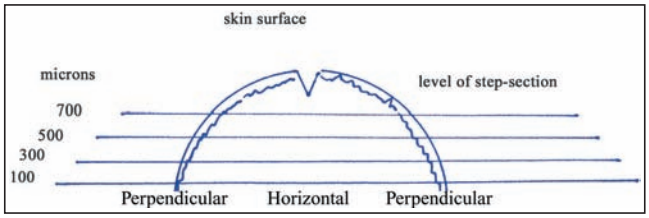


FIGURE 11.8: ***Cross-sectional diagram of planes of section.***

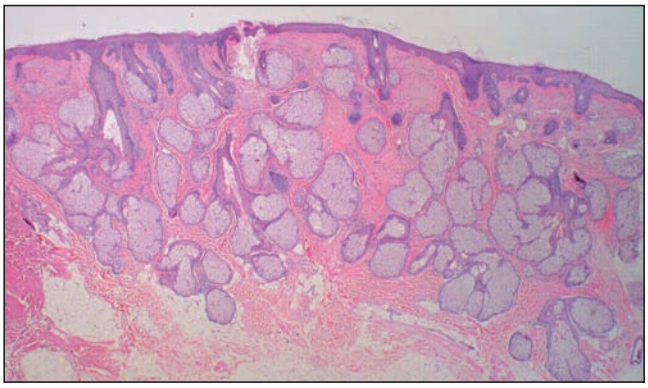


FIGURE 11.9: ***Sebaceous hyperplasia, perpendicular section.***

3D, and it may be impossible to discern their orientation with respect to the epidermal surface without the help of surrounding landmarks; consider eccrine lobules, isolated sebaceous lobules, cartilage, subcutaneous fat, and other structures that may look the same in any plane of section. Other structures, including blood vessels, pilar muscle fibers, and nerves, may appear to go in every direction and are of no help with orientation. We can therefore conclude that a rigid concept of purely horizontal or purely vertical sections does not exist microscopically, but only exists at a macroscopic level, where one can visualize how a tissue specimen is mounted and sectioned. In fact, as seen diagrammatically above, a curvilinear or tangentially excised excision is flattened in the lab to produce an artifactual "horizontal" section.

The most helpful algorithm for the Mohs surgeon is viewing multiple step-sections of tissue at predictable, periodic intervals (Figure 11.16). This allows visualization of the 3D architecture in many planes, as well as multiple views of appendage structures, and allows evaluation of tumor as it progresses from the closest surgical margin to the most superficial plane of tissue in the wound site. Initial tissue wafers will be in a plane approximating the closest

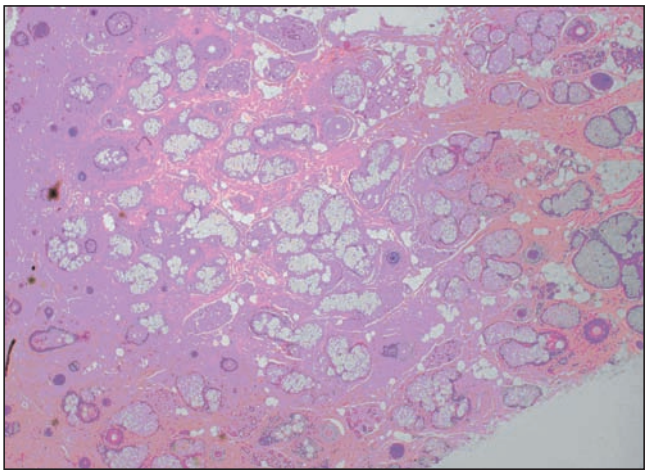


FIGURE 11.10: ***Sebaceous hyperplasia, horizontal section.***

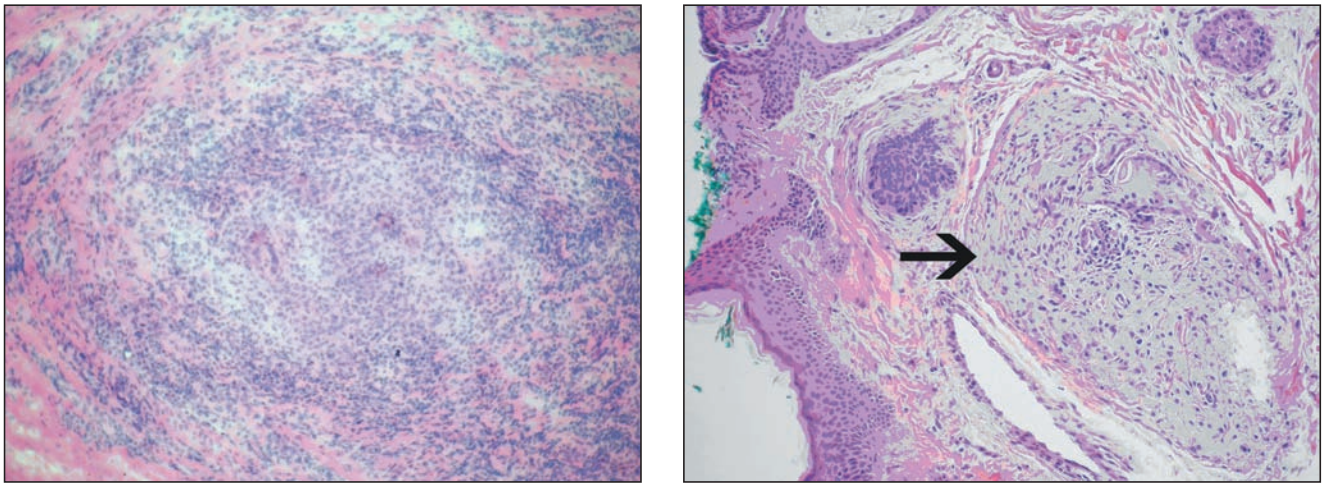


FIGURE 11.11: ***"Loose" granuloma (A); Discrete granuloma (B,*** **arrow*****).***

surgical margin, whereas deeper sections into the tissue will reveal planes of section placed away from the margin at predictable intervals. In practice, it is very helpful to be able to review structures at various levels in order to assess their pathologic importance and to determine if the tissue being viewed is benign or malignant. Multiple step-sections of 3D tissues provide a level of safety and comfort for the reviewer that is unparalleled, in contrast to the non-Mohs surgical excisional approach, regardless of whether permanent pathology or frozen sections assessment of the tissue is performed.

Typically, malignancies present in the skin grow in contiguous fashion; that is, one cell divides and makes two, two cells divide and make four, and the neoplasm proliferates by direct cell-to-cell contact. Architecturally, the presentation may be as sheets, nodules, or infiltrating strands of tumor cells. In evaluating the Mohs sections, we take advantage of this contiguous growth pattern. When tumors exhibit "skip areas," the effectiveness of Mohs surgery is decreased, as it is in recurrent cancer or when immune mechanisms or pretreatment interventions (such as fluorouracil [5-FU] and/or imiquimod) result in skip areas via a mechanism of partial tumor clearance. Small additive errors in the chain of events from the excision of the tumor to the production of slides and their pathologic interpretation can also adversely affect the Mohs cure rate.

For these reasons, I advocate reviewing multiple step-sections (Figure 11.16 and Chapter 9) to encompass a clear tissue margin of at least 1 mm to assure tumor-free margins and produce the lowest recurrence rate possible. From a macroscopic standpoint, 1 mm is a very narrow margin, but it may comprise many histologic sections: Mohs wafers are generally cut at 5–7 microns, and there are 1,000 microns in a millimeter. This concept follows the commonsense rule that a narrow surgical margin of one or two tumor-free wafers will eventuate in a higher recurrence rate than a slightly wider tumor-free margin. I have found in practice that after obtaining a few early wafers, subsequent wafers should optimally be spaced at approximately 200 microns. But there is room for other protocols in other Mohs practices, as long as consistency is maintained. Another way of stating this is that the Mohs technician should waste about 200 microns of tissue between wafers placed on the slide. Problematic histologic structures will appear and disappear in between these 200-micron steps, whereas tumor aggregates, which are usually much larger in dimension than this 200-micron step-section spacing, will persist and reveal themselves.

Evaluation of margins can be further complicated by the appearance of scar tissue, which is typically depleted of normal cutaneous microanatomy and/or tumor, and which in some presentations can be difficult to recognize (Figure 11.17A). Blood within the tissue wafers occupies space,

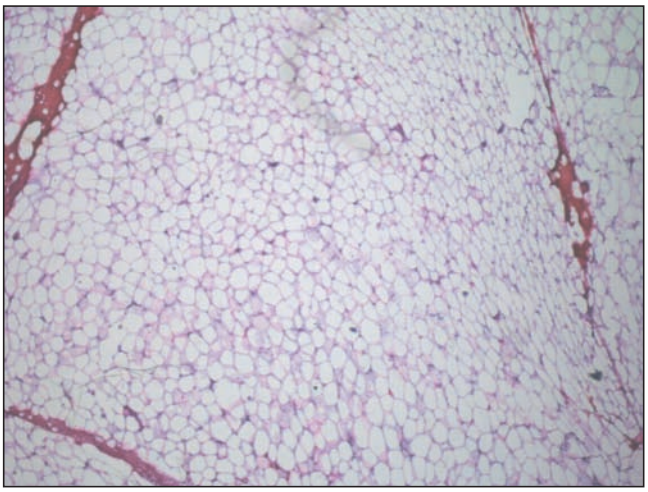


FIGURE 11.12: ***At 100-micron level (see Figure 11.8): Horizontal section, cut from midbase.***

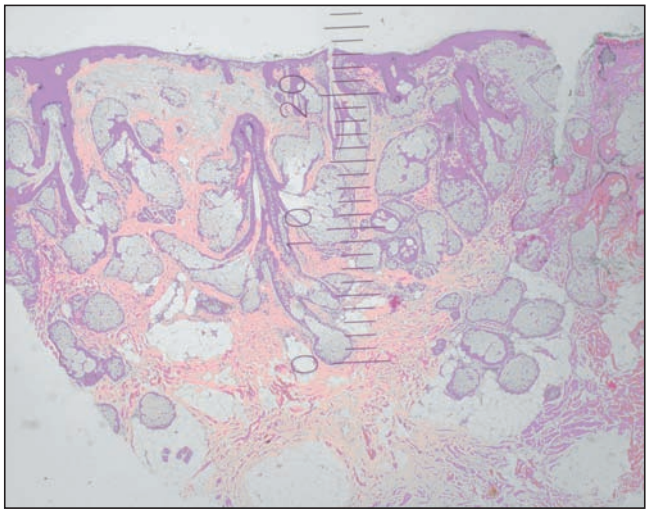


FIGURE 11.13: *Size of follicular structures. Each small hatch mark is 100 microns (1,000 microns = 1 mm).*

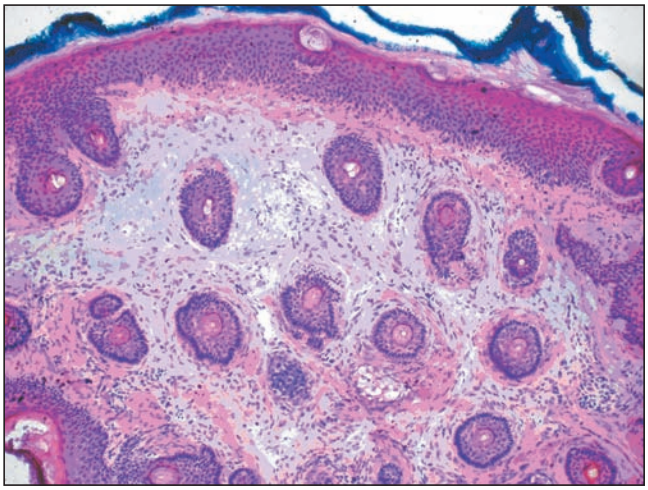


FIGURE 11.15: *Perpendicular orientation of epidermis and cross-sections (horizontal) of hair follicles on the same wafer.*

giving the Mohs surgeon-pathologist a false sense of security in regard to the surgical margin; blood can never represent a margin (Figure 11.17B). It is important to evaluate Mohs slides for scar and blood; it is generally inadvisable to call a margin "clear of cancer" when the tissue planes bounding the surgical site have pathologically altered tissue planes.

Stromal Scars

The recognition of stromal scars (Figures 11.18 and 11.19) is important in the evaluation of surgical margins because they may indicate the presence of discontiguous ("skip") tumor. Stromal scars generally exhibit overlying flattened epidermis (regenerative or reparative), and the subjacent dermis exhibits an arborizing vascular network devoid of pilosebaceous apparatuses. The scar may be infiltrated by numerous inflammatory cells, or active inflammation may be absent. Scar tissue is typically seen in the area of the skin cancer biopsy site or as an indication of previous treatment. The scar may bound the tumor or lie within the tumor. When the scar bounds the tumor, its relationship to the surgical margin is of greater importance.

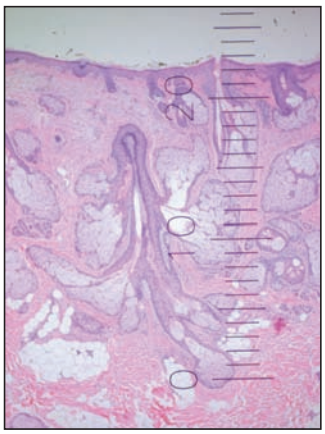


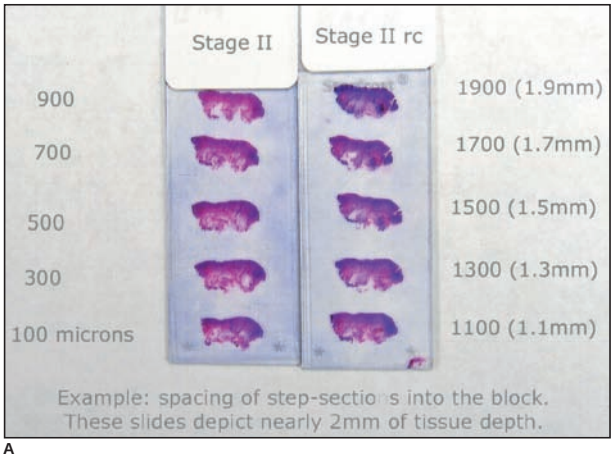
FIGURE 11.14: *Size of follicular structures. Each small hatch mark is 100 microns (enlarged view of 11.13; 1,000 microns = 1 mm).*

Differentiation of Basal Cell Carcinoma from Follicles

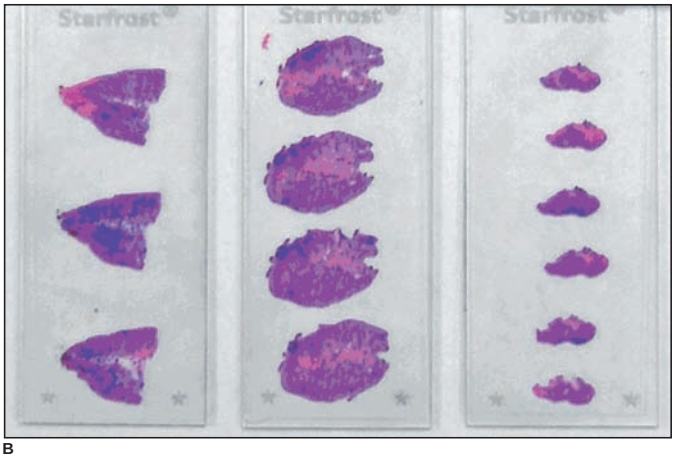
Differentiation of the basaloid cells of basal cell carcinoma (BCC) from follicular structures may be difficult. Basal cell carcinoma may arise from or involve many appendage structures. The recognition of follicular structures does not exclude the coexistence of BCC. Basal cell carcinoma will generally exhibit, at least focally, peripheral palisading, clefting, and mucin production, which is responsible for this artifactual clefting. The basal cells lining follicles and the epidermis may undergo proliferation and present as unusual basaloid structures surrounding follicles and beneath the epidermis. These proliferative processes are presumably secondary to ionizing ultraviolet radiation and/or inflammation, which affect sensitive areas such as the follicular "bulge." These irregular aggregates may sometimes appear organoid and nodular, while in other areas they may appear more sheetlike and diffuse. In general, they do not persist as multiple step-sections are reviewed, although they may be fairly widespread in locations such as the dorsal nose. They may be differentiated from BCC by their lack of persistence in the same area in the wafers where multiple step-sections are reviewed, as well as by the absence of clefting, mucin production, or peripheral palisading (Figures 11.20–11.23).

Common Findings in Mohs Surgical Specimens

Granulomatous inflammation and discrete granulomas are sometimes difficult to recognize (Figure 11.11). In some

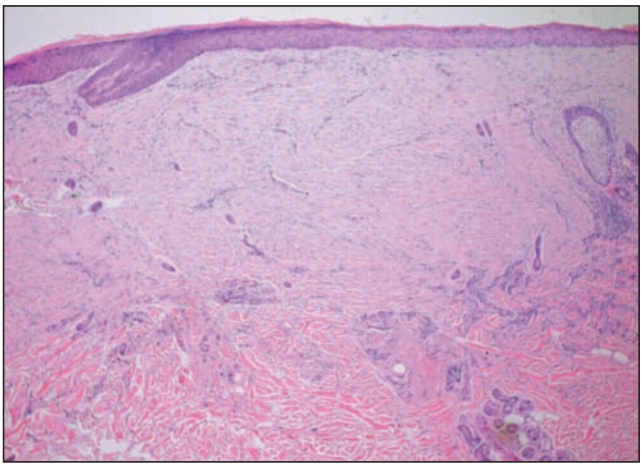


A

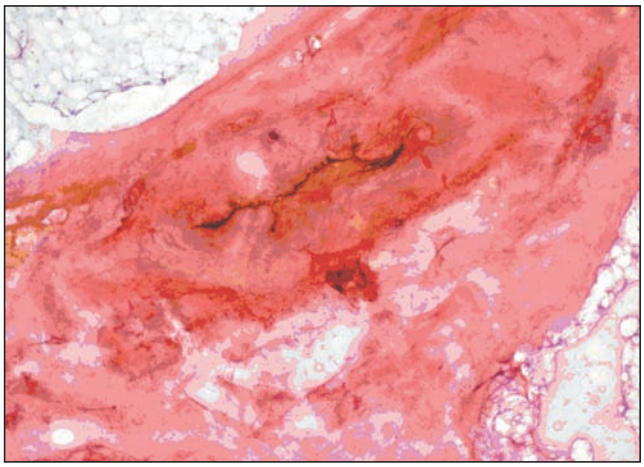


B

FIGURE 11.16: *(A) Examples of step-sections at 200-micron intervals. The first section is placed at 100 microns in this case to ensure early sampling. (B) The various step-sections may be placed three, four, or more to a slide, depending on their sizes.*

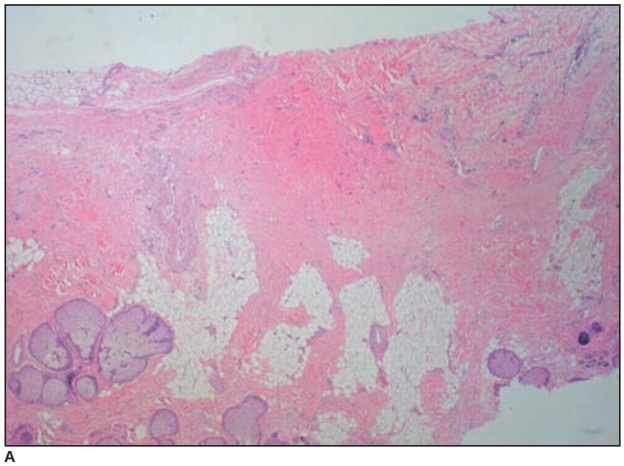


A



B

FIGURE 11.17: *Recognizable pathologic alterations. (A) Scar tissue and (B) pooled blood.*



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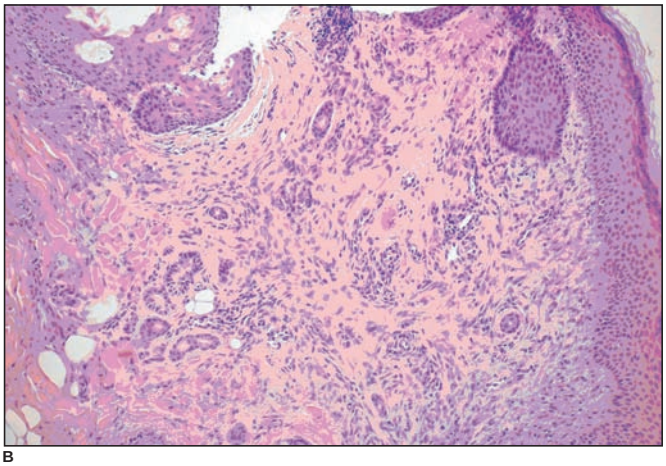
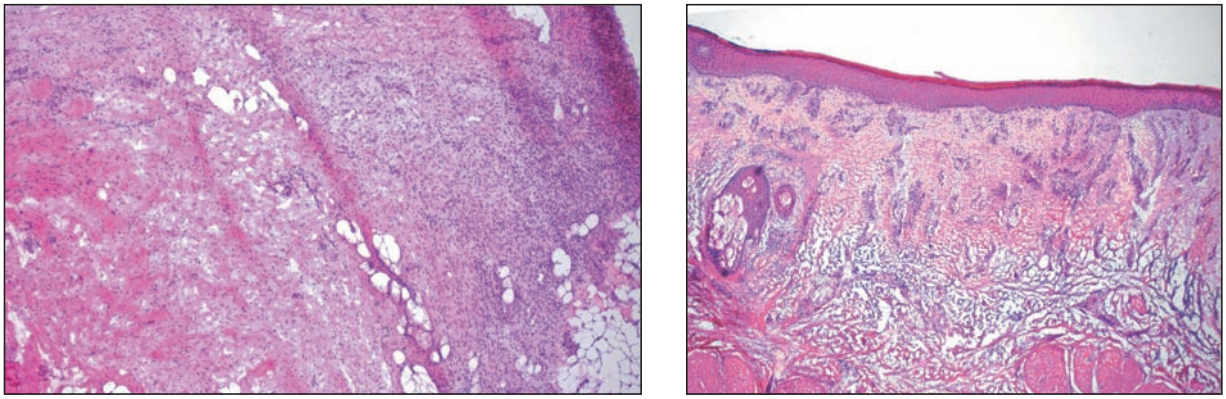


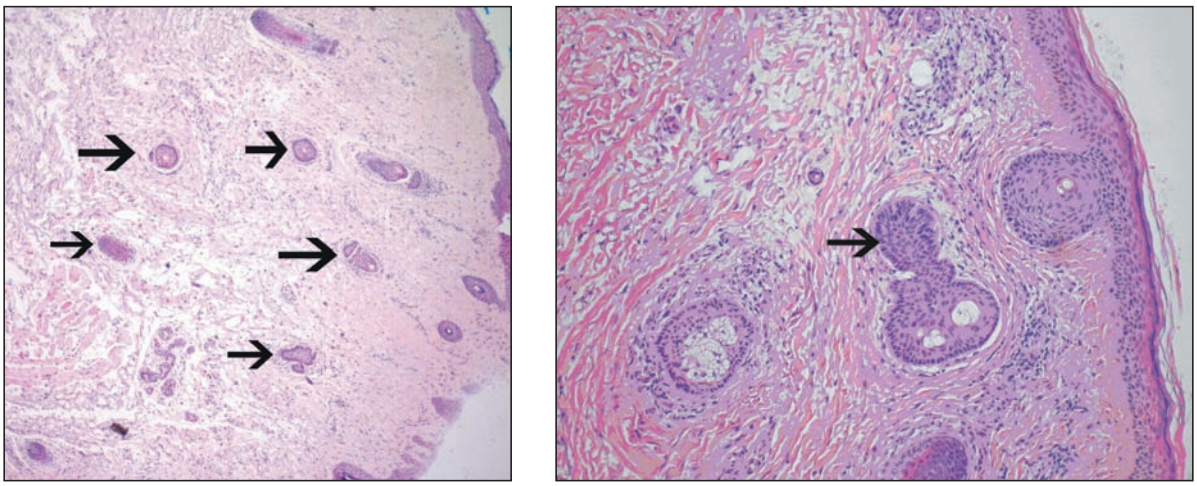
B

FIGURE 11.18: *Would you recognize stromal scarring? (A) More mature fibroblastic stroma (redder stain) and (B) more immature, inflamed stroma, with flat regenerative and/or reparative overlying epithelium.*



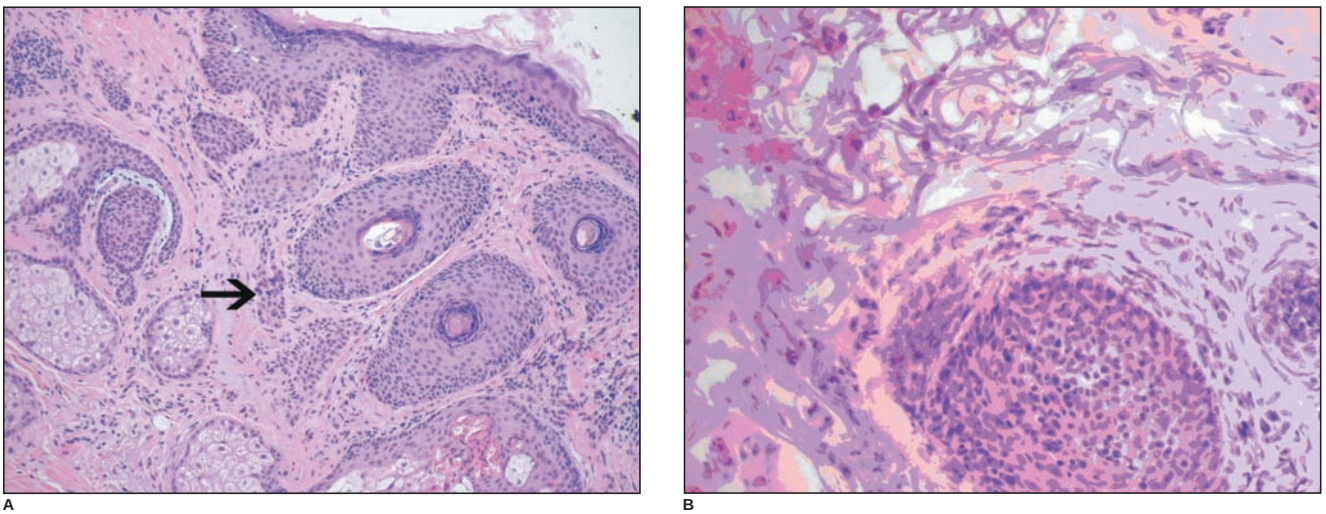
A B

FIGURE 11.19: *Stromal scarring on the left side of the section with dermatofibrosarcoma on the right superior side of the same section (A); note the difference in cellularity. Lower-extremity "stasis" scar (B).*



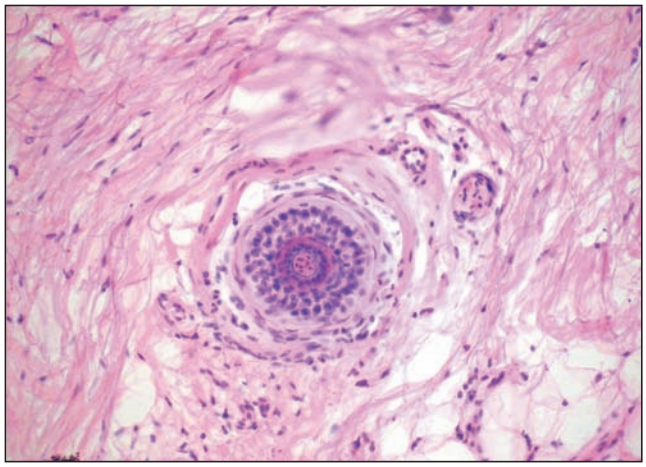
A B

FIGURE 11.20: *Atretic follicles (A); follicular basaloid hyperplasia (B).*



A B

FIGURE 11.21: *Follicular basaloid hyperplasia (A); follicular bulb with vague "pink sheath" (B).*



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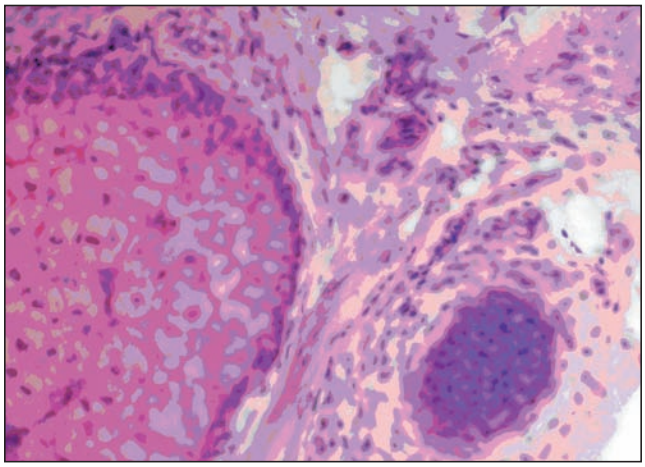
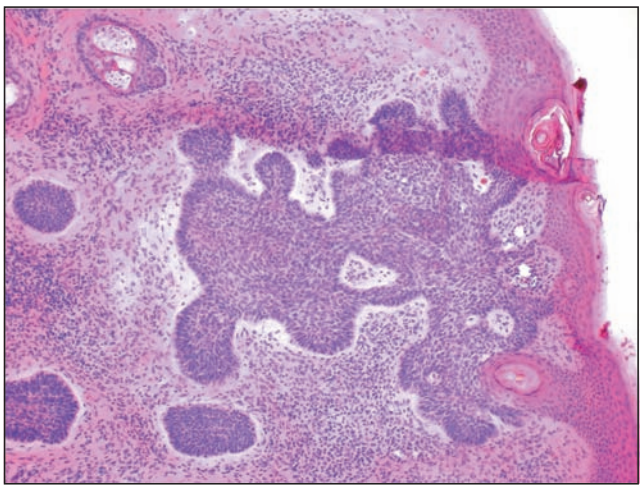


B

FIGURE 11.22: *Portions of normal follicles, with histologic layers demonstrated on the left. Both (A) and (B) show a vague surrounding "pink sheath," which is diagnostically helpful in frozen sections.*



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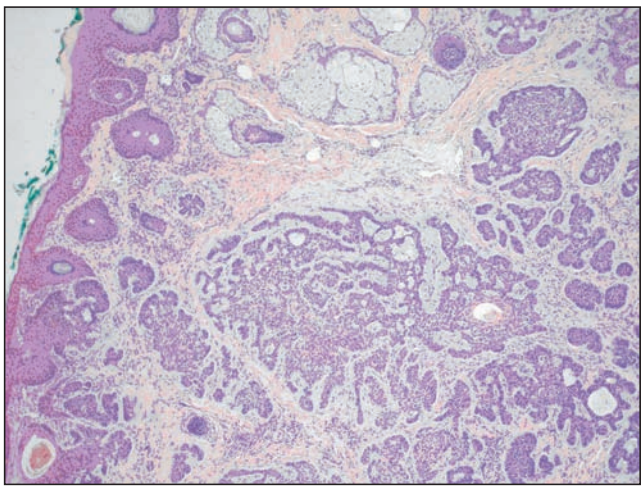
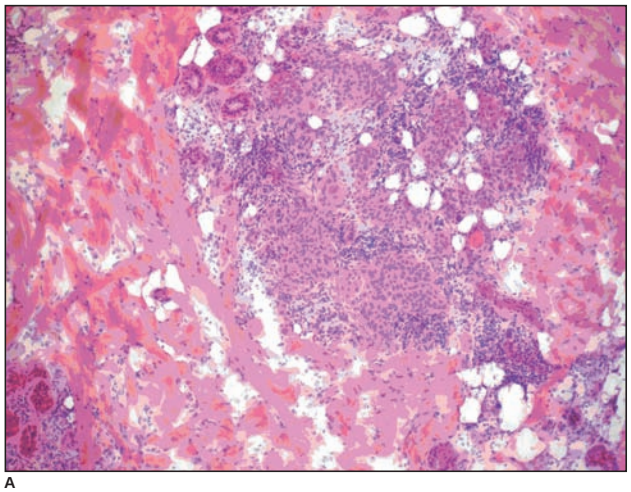


B

FIGURE 11.23: *Contrast the benign follicular structures (shown in Figures 11.20–11.22) with BCC above (A) and (B), which exhibit peripheral palisading, mucin production, clefting, and the absence of typical follicular architecture. Basal cell carcinoma will persist in the same general location as multiple step-sections are reviewed.*



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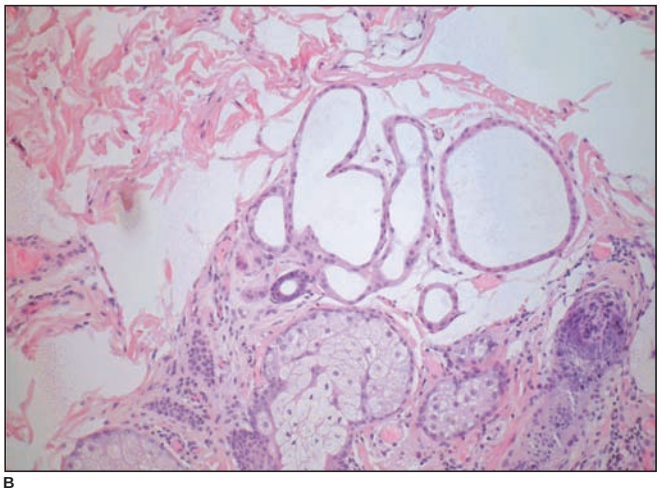
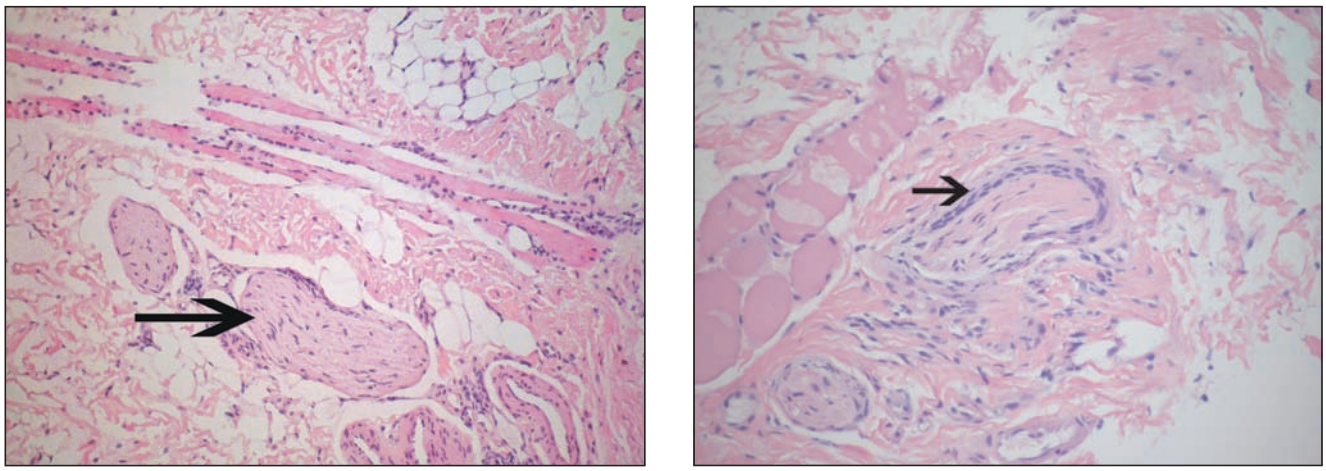


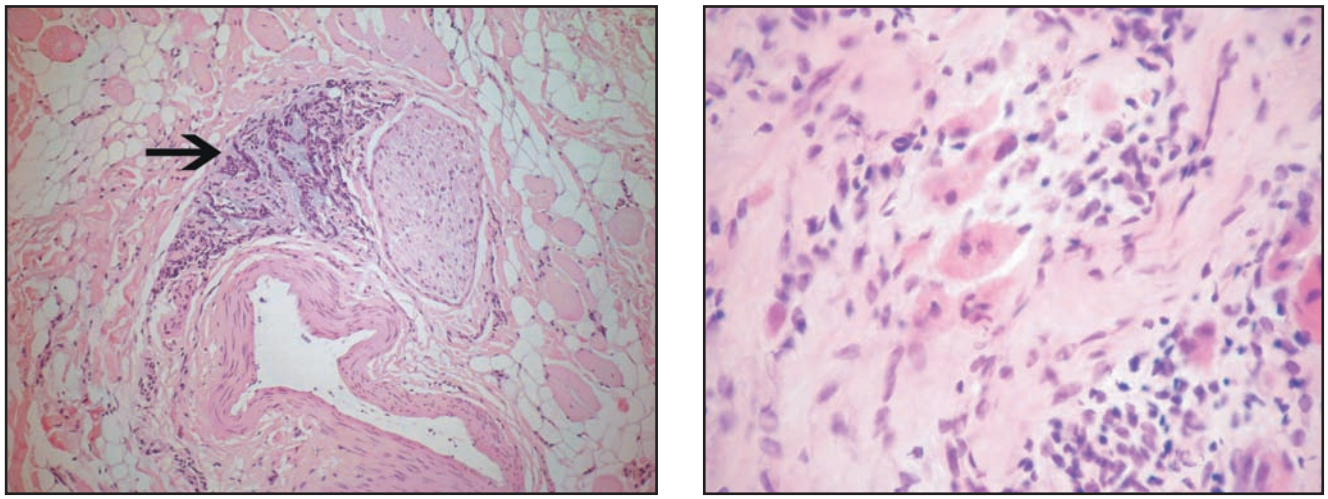
B

FIGURE 11.24: *Eccrine squamous metaplasia simulating SCC (A). Note the nodular and lobular arrangement, with varying degrees of eccrine differentiation in this Mohs slide from a BCC case. Dilated eccrine ducts (B).*



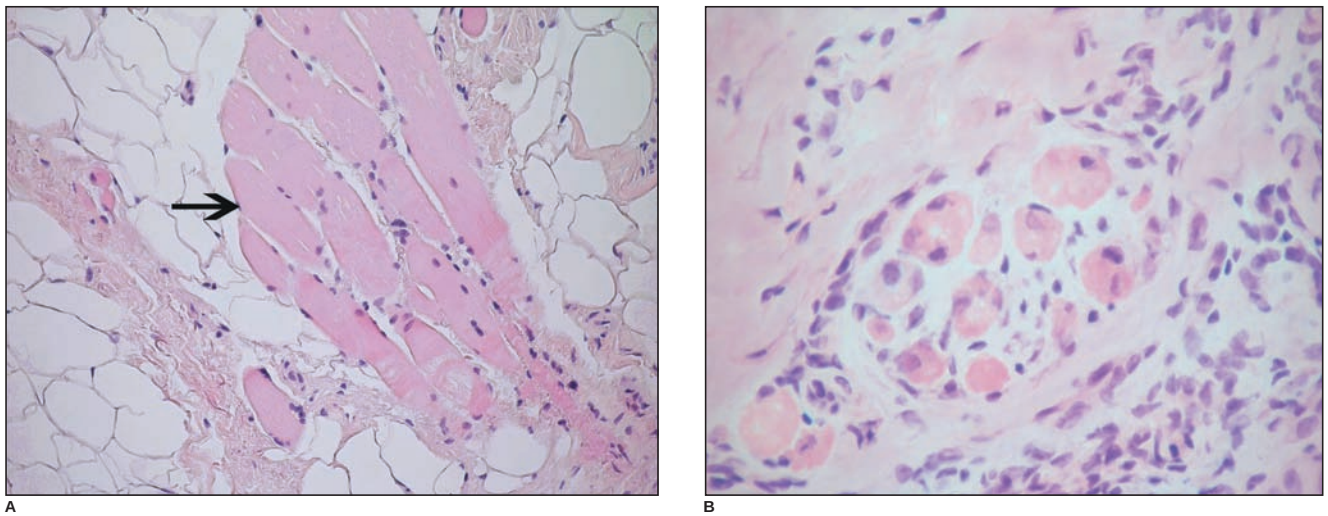
A B

FIGURE 11.25: *Peripheral nerve. Normal, with thin perineural membrane (A); a collaret of dark-staining, basophilic cells representing perineural invasion by a poorly differentiated carcinoma (B). Less prominent infiltration is also seen around surrounding nerve twigs.*



A B

FIGURE 11.26: *Basal cell carcinoma in the adventitia of the neuromuscular bundle but not in the perineurium (A); step-sections can help determine if actual perineural invasion is present. Eosinophilic individual muscle fibers from the lip surround a nerve and may be mistaken for SCC, unless multiple sections are reviewed to ascertain their origin (B); look for a continuum from bundled fibers to single cells. Just above center, pathologically altered muscle fibers simulate "tadpole" cells and, on the far right, simulate keratinization normally associated with SCC. This figure is from a Mohs case for BCC.*



A B

FIGURE 11.27: *Muscle bundles in longitudinal view (A) and cross-section view (B). Muscle cells may also be seen singly, as in Figure 11.28.*

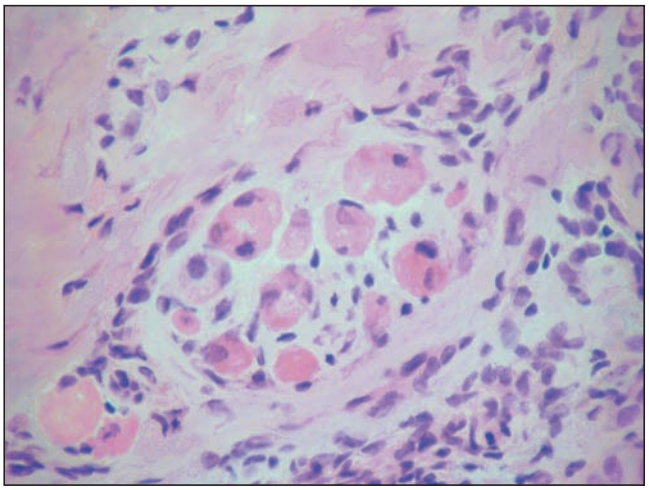


FIGURE 11.28: Pyknotic muscle fibers in an area of inflammation may simulate SCC, especially when viewed singly. Note the preserved lobular architecture.

"loose" granulomatous areas, the inflammation consists of poorly defined aggregates of loosely scattered lymphohistiocytic cells, stroma exhibiting mild fibroplasia, and derangement of the usual organoid or follicular structures. These structures may contain multinucleated giant cells and, on occasion, eosinophils. They may also exhibit polarizable material, such as remnants of suture, for which it is helpful to have polarizing plates or lenses on hand (Figure 11.11(A)).

Discrete granulomas may also be seen anywhere in the dermis or fat. A discrete granuloma is an architecturally well-demarcated aggregate of lymphocytes and histiocytes with occasional multinucleated giant cells and collagen (Figure 11.11(B)).

Eccrine ductal arrangements may sometimes be difficult to differentiate from squamous cell carcinoma (SCC) (Figure 11.24). In general, the eccrine ducts are grouped together as lobules that communicate with a single duct

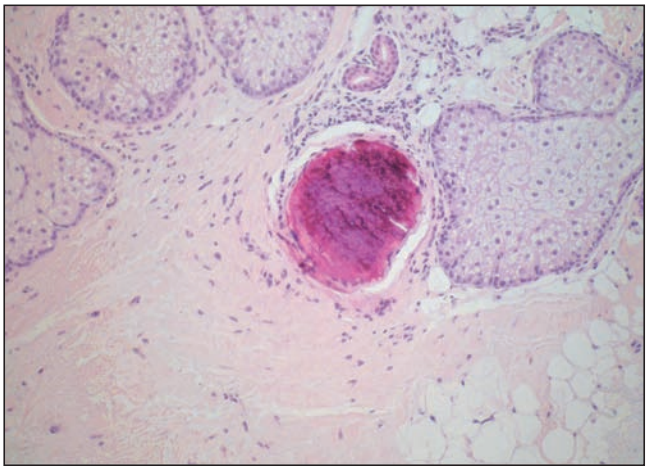


FIGURE 11.29: A calcific focus.

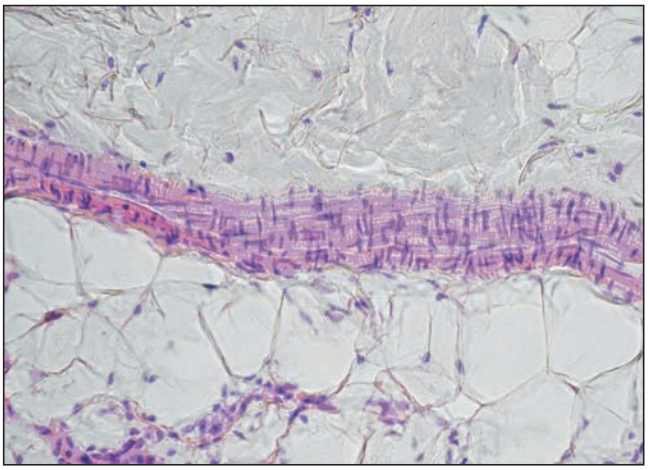


FIGURE 11.30: Arteriole with typical caterpillarlike cross-hatching.

leading to the epidermal surface. Attendant inflammation, granulomatous changes, and squamous metaplasia can sometimes make it difficult to recognize the process as benign. In general and in contrast to typical skin malignancies, these atypical regenerative or reparative eccrine structures, due to their size, tend not to persist from section to section as step-sections are reviewed.

Vascular structures and nerves (see Chapter 17) may present in cross-section, tangentially, or longitudinally. In any of these orientations, the neural structures are composed of small fibers (neuropil) with occasional thin, small, wavy, and bipolar Schwann cells within the myelin sheath itself. The nerve bundles are surrounded by a perineurium,

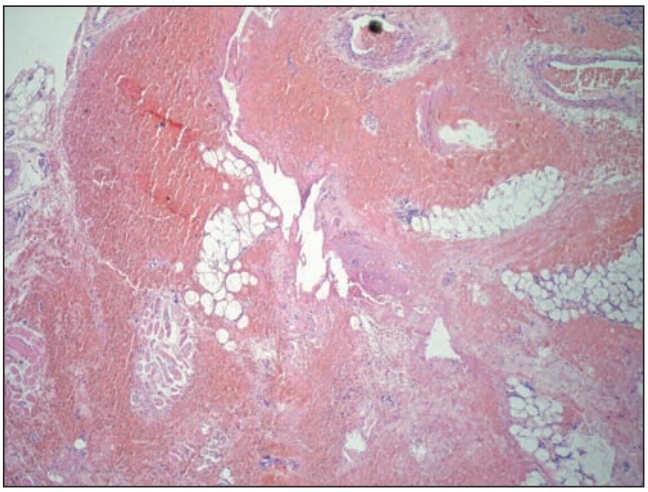


FIGURE 11.31: Stromal hemorrhage has pushed aside and replaced preexisting stromal elements; areas which should be visible in the plane of section are not. When encountered during sectioning or during reading, the Mohs technician must take multiple step-sections until the hemorrhagic area is cleared to ensure that no malignancy is present in normal tissue approximating the surgical margin.

which is a very thin membrane containing only small, spindled cells. Any thickening of this membrane by larger hyperchromatic cells is cause for concern because it may represent perineural invasion by tumor (Figures 11.25 and 11.26).

Striated muscle fibers may appear singly or in small bundles. The central portion of an individual fiber is anucleate. There are usually one or two cuboidal or elongated nuclei at the edge of the fiber's membrane (Figure 11.27).

Pyknotic muscle fibers (Figure 11.28) in an area of inflammation may simulate squamous cell carcinoma, especially when viewed singly.

Calcific foci (Figure 11.29) may occur, particularly in areas of prior biopsy or folliculitis. The calcific material in hematoxylin and eosin (H&E)-stained preparations typically stains a reddish-blue hue, although some may be totally blue or totally red. In more mature, ossified sections, there may actually be woven or cancellous bone and bone marrow elements. Because calcified material may present sectioning problems for the Mohs technician, a wider margin around any clinically ossified areas is recommended. Similarly embedded sutures, foreign material, and bone fragments scrapped from the outer table of normal bone are not easily processed by the cryostat.

Vascular structures contain endothelial and muscle cells that can be markedly atypical. One helpful diagnostic feature to aid in recognition of these benign structures is the typical crosshatching in arterioles (Figure 11.30), a finding especially prominent in frozen sections.

Hemorrhage

During surgery, when blood extravasates into the surrounding stroma, it pushes existing structures aside and forms a space-occupying mass. If this hemorrhage occurs in approximation to the margin and pushes tumor aside, it may be misinterpreted as a clear margin. It is best to operate in as bloodless a surgical field as possible, and to gently blot away any clotted and hemorrhagic material from the surgical margin prior to processing the tissue (Figure 11.31).

CHAPTER 12

Basal Cell Carcinoma: Vertical and Horizontal

A. Neil Crowson and Carlos Garcia

BASAL CELL CARCINOMA (BCC) is the most common malignant neoplasm of humans. Although eminently curable when diagnosed early, BCC constitutes an enormous financial burden for the health care system.

Basal cell carcinomas occur on both sun-protected and sun-exposed skin, but often have a different biology and morphology in these locations. Tumors occur typically in the fourth decade of life and beyond, although exceptions occur, in particular in the setting of specific genodermatoses or in immunocompromised patients. As sun exposure plays a role in the development and transformation of BCCs, patients with light-skin phenotypes, blue eyes, red hair, and easy freckling are particularly predisposed, as well as those whose occupational or leisure activities lead them to pronounced and prolonged sun exposure. Additional risk factors include exposure to arsenic, coal-tar derivatives, and irradiation, although, by far, ultraviolet light is the most important factor. Basal cell carcinoma may arise in the setting of scars, draining sinuses, ulcers, burn sites, and foci of chronic inflammation. The role of immune compromise in BCC may reflect impairment of the immune surveillance of oncogenic viruses. Genodermatoses with enhanced risk for BCC include xeroderma pigmentosum, Rasmussen syndrome, Rombo syndrome, Bazex-Dupré-Christol syndrome, albinism, and Darier's disease. These syndromes either decrease epidermal pigmentation and thus enhance the risk of ultraviolet light–induced oncogenic transformation, or promote epidermal keratinocytic genotypic instability.

Basal cell carcinoma has been associated with a variety of other lesions and/or neoplasms in the same or a nearby anatomic location, such as desmoplastic trichilemmoma, which is associated with coexistent atypical basaloid neoplasms including BCC in up to 19% of cases in our experience. Other lesions associated with coexistent BCCs include acantholytic processes, warts, porokeratosis, neurofibromata, nevus sebaceous and epidermal nevi, condylomata acuminata, hemangiomas, cysts of hair follicle derivation, pilomatricomas, and a variety of common banal neoplasms such as seborrheic keratoses and melanocytic nevi. Many of these are so common as to make their coexistence with BCC in any given patient a random event. All may be confounding variables at frozen section. Basal cell carcinomas have also been reported in collision with dermatofibroma. As in the nevus sebaceous, the basaloid epidermal proliferation overlying a dermatofibroma often shows follicular stromal induction, suggesting a recapitulation of hair follicle growth. Whether a component of a hamartoma or as a response to proplastic cytokines, it is not clear that such basaloid proliferations have a significant propensity to eventuate in a malignant neoplasm. The clinical characteristics of BCC and their corresponding histologic expressions reflect the pathogenesis of these neoplasms, which have only recently been elucidated.

Lesions that recur after radiotherapy may infiltrate widely prior to becoming clinically apparent; this, plus the fact that radiotherapy is often reserved for aggressive growth tumors, ought to enhance the suspicion of perineural infiltration in BCC recurrent after irradiation. Metastases are rare and most are said to more closely correlate to the size and depth than to the histologic subtype of the original tumor. The incidence of metastases and/or death is said to correlate to size over 3 cm in diameter. Patients with such tumors are said to have a 1–2% risk of metastases that increases to up to 20–25% in lesions greater than 5 cm (so-called "giant BCC") and up to 50% in lesions greater than 10 cm in diameter.

NEVOID BASAL CELL CARCINOMA (BASAL CELL NEVUS) SYNDROME

Described originally by Howell and Caro in 1959, the nevoid BCC syndrome is also known as the Gorlin-Goltz syndrome and is inherited as an autosomal dominant trait, with some 30–50% of cases representing sporadic mutations. Typically, the syndrome is expressed in young adulthood, but on occasion, children as young as two years of

age manifest disease expression, characterized by the presence of multiple, sometimes thousands, of BCCs, cysts of the skin and jaws, and many other abnormalities.

HISTOPATHOLOGY

Some authorities believe that the histology of most BCCs encountered in the nevoid BCC syndrome is similar to those seen in patients with sporadic BCCs. In our experience, the infundibulocystic form (see below) in a younger patient tends to correlate with nevoid BCC syndrome; we have seen skin-taglike polypoid growths in such patients.

HISTOPATHOLOGY OF BASAL CELL CARCINOMA

Traditionally, BCCs have been classified as solid (or undifferentiated), versus those tumors that manifest differentiation along eccrine, sebaceous, or other cell lines. The only proven histologic prognosticator of biologic behavior, and therefore a major determinant of what constitutes an appropriate therapeutic approach, is the architectural growth pattern and the stromal reaction to the tumor.[1] It is the architectural and the stromal morphology, therefore, that is the critical issue, while the differentiation patterns should be considered only insofar as they must be recognized as part of the histologic spectrum of BCC, as they mimic native elements of the skin and affect differential diagnosis. Misidentification of BCC as Merkel cell carcinoma or as sebaceous, eccrine, or follicular neoplasia is a diagnostic pitfall that carries with it a risk of over- or undertreatment.

Shave and punch biopsy specimens have an intrinsic error rate of approximately 20% in predicting classification of BCC subtypes when compared to excisions at the same anatomic location. This is not surprising, as the morphologic features that indicate biological transformation of BCC tend to be seen, in our hands, at the base and edges of the growing neoplasm.

UNDIFFERENTIATED BASAL CELL CARCINOMAS

There are two fundamental biological forms of BCC: indolent-growth and aggressive-growth subsets. The indolent-growth variants include the superficial and the nodular BCC. The aggressive-growth tumors include infiltrative BCC, metatypical BCC (also termed by us "basosquamous carcinoma"), and morpheaform or sclerosing BCC. Micronodular BCC may be considered a transition step between nodular and infiltrative growth tumors, with a biological behavior that is intermediate between the two. In one large retrospective series of 1,039 consecutive BCCs, 21% were nodular, 17.4% were superficial, 14.5% were micronodular, 7.4% were infiltrative, and 1.1% were morpheaform. Roughly one third of all tumors showed an admixture of patterns.

SUPERFICIAL BASAL CELL CARCINOMA

Superficial BCC consists of a proliferation of atypical basaloid cells that form along an axis parallel to the epidermal surface, and demonstrate slitlike retractions of the palisaded basal cells from the subjacent stroma (Figure 12.1). The resulting cleftlike spaces often contain alcian blue–positive mesenchymal mucins that are a presumed product of the stromal cells. Tumor cells may colonize the hair follicle and rarely the eccrine adnexal structures, and often take origin from the follicular bulges. Mitoses are infrequent and apoptic cells rare in this form of BCC, reflecting its biologic derivation from immortalized epithelial progenitor cells. Some cases manifest melanin pigmentation of the epithelium and of histiocytes in the subjacent stroma. Pigmented basal cell carcinomas are thought to be most often superficial BCCs in some series, although, in our experience, nodular BCCs constitute the most frequent form of pigmented BCC. Superficial BCCs often show a dense band–like lymphoid infiltrate. When this is seen in the setting of a biopsy or Mohs section for superficial BCC, such a lymphoid infiltrate should prompt a careful search for tumor through multiple levels.

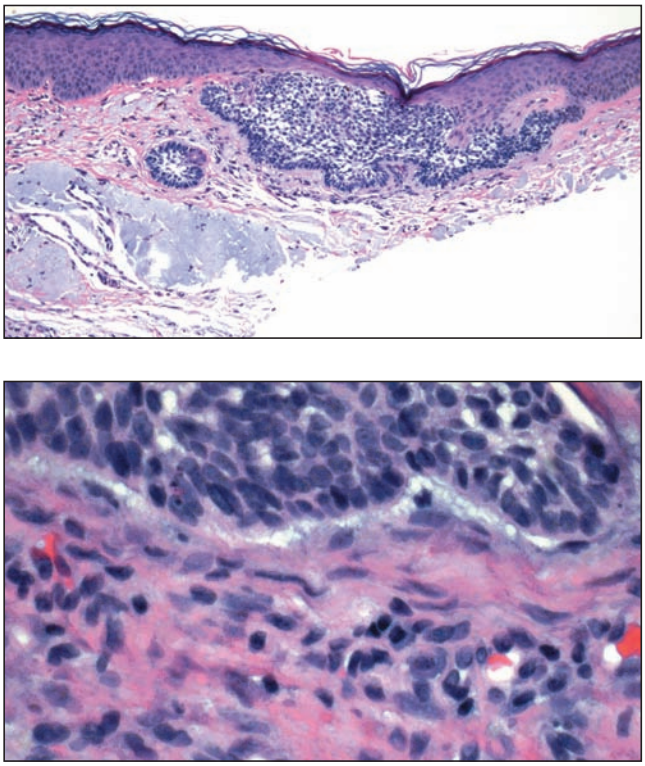


FIGURE 12.1: ***Superficial basal cell carcinoma (BCC). An atypical basaloid proliferation parallel to the long axis of the epidermis with a slitlike retraction beneath it.***

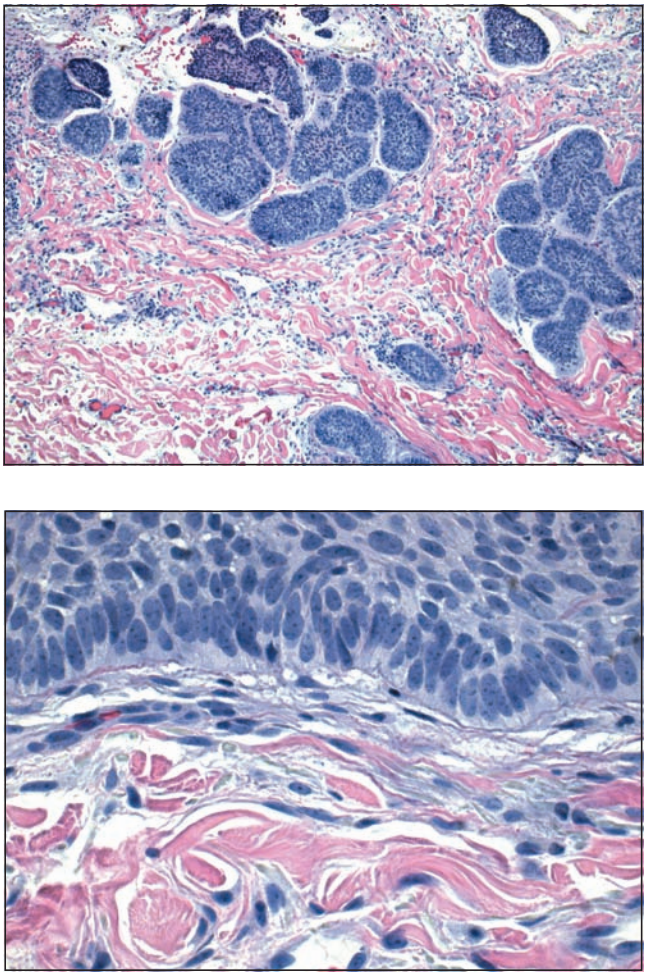


FIGURE 12.2: ***Nodular BCC. Pushing lobules of atypical basaloid cells in the dermis that are not associated with a sclerosing stromal response.***

NODULAR BASAL CELL CARCINOMA

Nodular BCC is the most common form of BCC in our experience, and is also referred to as nodulocystic BCC by some observers, although this term is not employed by us. The nodular form of BCC is characterized by discrete large or small nests of basaloid cells in either the papillary or reticular dermis, accompanied by slitlike retraction from a stroma whose fibroblasts do not appear to be plump or proplastic (Figure 12.2). Any of the differentiated elements (eccrine, sebaceous, etc.) may be seen in nodular tumors, and roughly one third of cases have a coexistent superficial component from which they derive. Superficial and nodular BCCs are often seen in sun-exposed or sun-protected skin; the dermis may or may not show solar elastosis. The surrounding stroma shows myxoid change, is rarely fibrotic, and may show calcification in tumor or adjacent stroma. Mitoses and individual cell necrosis are infrequently found. The presence of abundant slitlike retractions may cause tumor nests to drop out from the stroma during processing, yielding empty spaces with a rounded contour in the mid or deep dermis. This is an important clue to the diagnosis of nodular- and/or infiltrative- growth–pattern BCCs. A significant proportion of nodular BCCs manifest a variable admixture of superficial and/or micronodular morphologies. Melanin pigmentation of tumor cells and adjacent stromal histiocytes may be seen, as may be transition to micronodular and other aggressive-growth forms.

MICRONODULAR BASAL CELL CARCINOMA

Micronodular BCC appears as a plaquelike indurated lesion with margins that are difficult to assess clinically. This causes it to have a high recurrence rate when treated by non-Mohs modalities. Micronodular BCC has tumor nests with roughly the same shape and contour as nodular BCC, but these nests are smaller, widely dispersed, and often have an asymmetric distribution extending deeper into the dermis and/or subcutis (Figure 12.3). These monotonous, small round tumor nests are accompanied by stromal

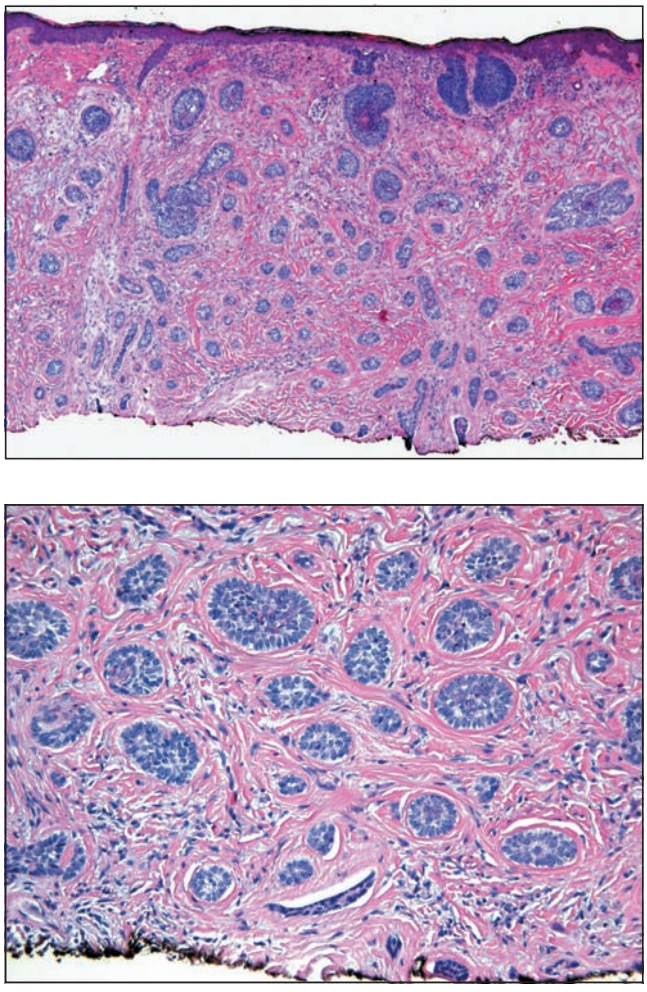


FIGURE 12.3: ***Micronodular BCC. There is widespread dermal involvement by a tumor, in which all the nodules of tumor are of similar, small size; there is a fibroproliferative stromal reaction.***

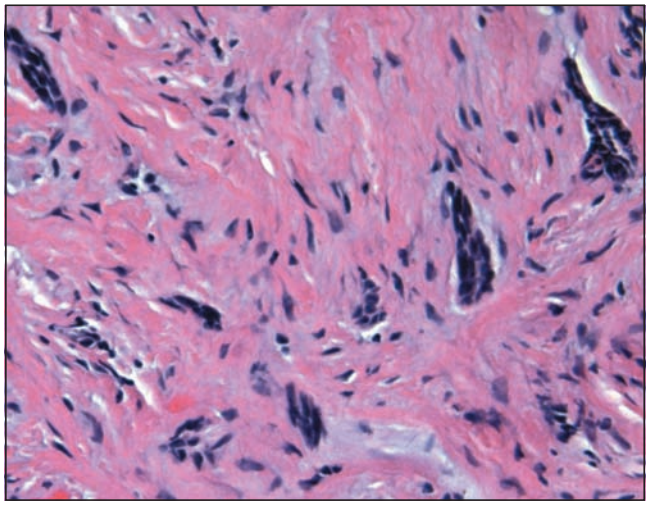


FIGURE 12.4: Morpheaform BCC has narrow columns of tumor in a sclerotic stroma.

proliferation like that of the infiltrative growth BCC. Retraction spaces are not common and the surrounding stroma shows either a myxoid or collagenized morphology.

AGGRESSIVE-GROWTH BASAL CELL CARCINOMAS

Aggressive-growth BCCs are the prototypic morpheaform BCC, infiltrative-growth BCC, and metatypical BCC (basosquamous carcinoma).

MORPHEAFORM BASAL CELL CARCINOMA

Morpheaform BCC is characterized by columns of basaloid cells, one to two cells thick, enmeshed in a densely collagenized stroma containing proplastic fibroblasts (Figure 12.4). Individual cell necrosis and mitotic activity is brisk, despite relatively low tumor volume, and the neoplasms are poorly demarcated, showing widespread invasion of the reticular dermis and penetration of subcutaneous tissue. Slitlike retraction from stroma is less common than for the nodular and superficial variants. These neoplasms may coexist with other aggressive-growth morphologies. Morpheaform BCCs represent roughly 1–5% of all BCCs and clinically present as white or yellow depressed fibrotic scars that rarely ulcerate or bleed, and occur mainly in a sun-exposed distribution. Although typically one to two cells in thickness, tumor cords up to five cells in thickness may be present; the architecture shows sharply angulated cell groups with pronounced stromal fibroplasia and fibrosis surrounding tongues of tumor. By electron microscopy, there is no delimiting basal lamina.

INFILTRATIVE-GROWTH BASAL CELL CARCINOMA

Infiltrative-growth BCC manifests irregularly sized and shaped nests of tumor cells. The nests show sharp angulation of their peripheral contours, occasional foci of slitlike retraction, frequent mitotic activity, and individual cell necrosis of the neoplastic cells (Figure 12.5). The stroma is frequently fibrotic, with plump proplastic stromal fibroblasts. Roughly one third of tumors show an admixed nodular component from which the lesions are thought to derive (Figure 12.5). Like morpheaform BCCs, these tumors are poorly circumscribed and may invade subcutis, muscle, nerve, and other structures. Perineural infiltration is as much a risk in this variant as in morpheaform BCC. Like morpheaform BCC, these tumors present clinically as a depressed yellowish or fibrotic plaque that typically lacks a rolled border or a nodular pearly component (Figure 12.6).

METATYPICAL BASAL CELL CARCINOMA

The metatypical BCC (basosquamous carcinoma) is, in our view, a form of aggressive-growth BCC with infiltrating jagged tongues of tumor cells, some of which manifest an abortive peripheral palisade and clear-cut basaloid morphology, admixed with other areas that show intercellular bridge formation and/or cytoplasmic keratinization (Figure 12.7). The presence of a coexistent classic nodular or superficial BCC component confirms the diagnosis. We distinguish these neoplasms from keratotic BCC, which is most often a nodular BCC with central squamous differentiation, and from the mixed basal cell–squamous cell carcinomas that represent a collision between two clonally distinctive and geographically separate neoplasms in the same tissue sample. Metatypical BCC is a subtype of BCC that can be confused with squamous cell carcinoma; its classification is controversial because it shows both basal cell and squamous cell carcinoma differentiation in a continuous fashion. In our view, the terms "metatypical BCC" and "basosquamous carcinoma" are a semantic distinction.

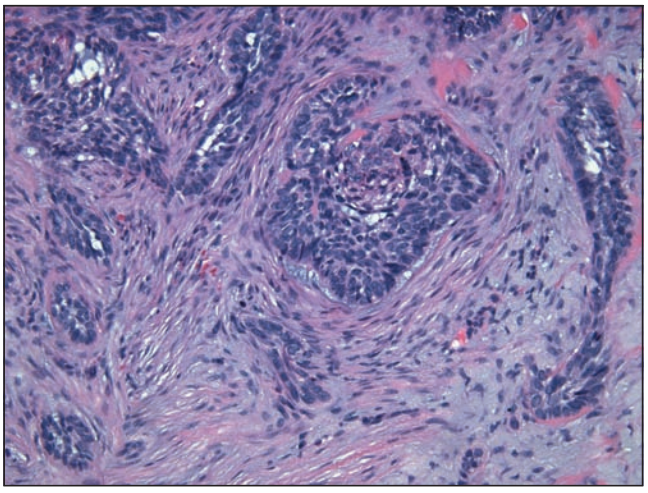
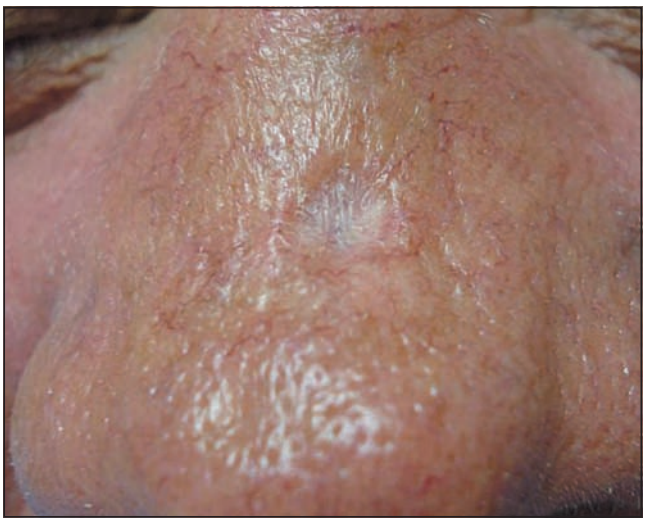


FIGURE 12.5: Mixed nodular and infiltrative growth BCC. Superimposed upon a nodular growth component with a rounded, pushing contour are irregular tongues of tumor embedded in a sclerotic stroma. It is the latter component that confers aggressive biologic characteristics on this neoplasm.



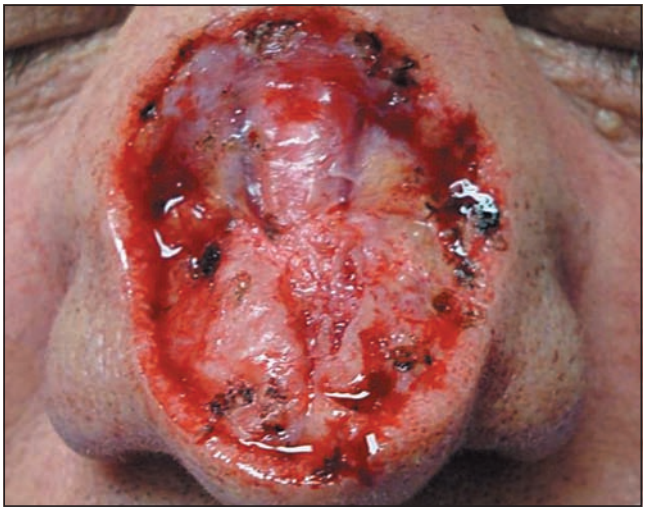


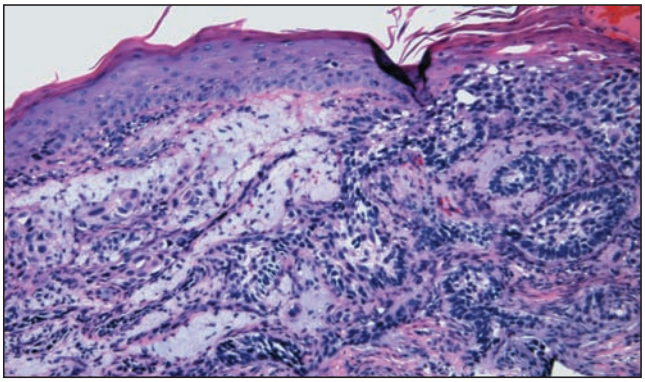
FIGURE 12.6: Infiltrative-growth BCC. Clinical morphology. It is this and other forms of aggressive-growth BCC that often prove much more widely invasive at time of Mohs surgery than is clinically suspected.

DIFFERENTIATED BASAL CELL CARCINOMAS

Basal cell carcinomas may show a variety of specific cell lineage differentiation features, which do not impact prognosis. These differentiated BCCs include tumors such as keratotic BCC; follicular BCC, which may share features with pilomatricomas; BCC with sebaceous differentiation (Figure 12.8); BCC with eccrine differentiation (Figure 12.9); BCC with trichilemmal differentiation (Figure 12.10); fibroepithelioma of Pinkus (Figure 12.11); infundibulocystic BCC (Figure 12.12); pleomorphic BCC (Figure 12.13); and BCC with myoepithelial differentiation.

KERATOTIC BASAL CELL CARCINOMA

Also known as pilar BCC, because it appears to differentiate along pilosebaceous lines, the keratotic BCC manifests



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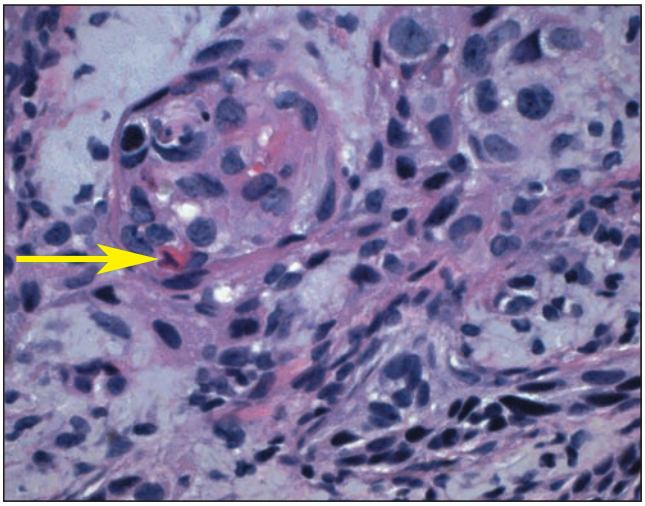

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FIGURE 12.7: Basosquamous carcinoma/"metatypical" carcinoma. These are aggressive-growth tumors with infiltrative growth architecture (A). Intercellular bridge formation and keratinization (arrow) *define the squamous component (B). That this is in fact a BCC is attested to by the presence of conventional superficial and nodular growth areas (A).*

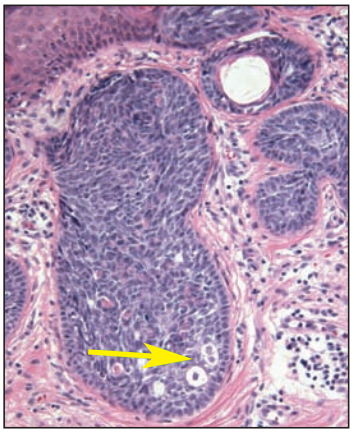


FIGURE 12.8: Basal cell carcinoma with sebaceous differentiation ("sebaceous epithelioma"). Like nodular basal cell carcinoma, the tumor nests have a rounded contour with a pushing margin. There is sebaceous differentiation characterized by multivesicular sebocytes (arrow).

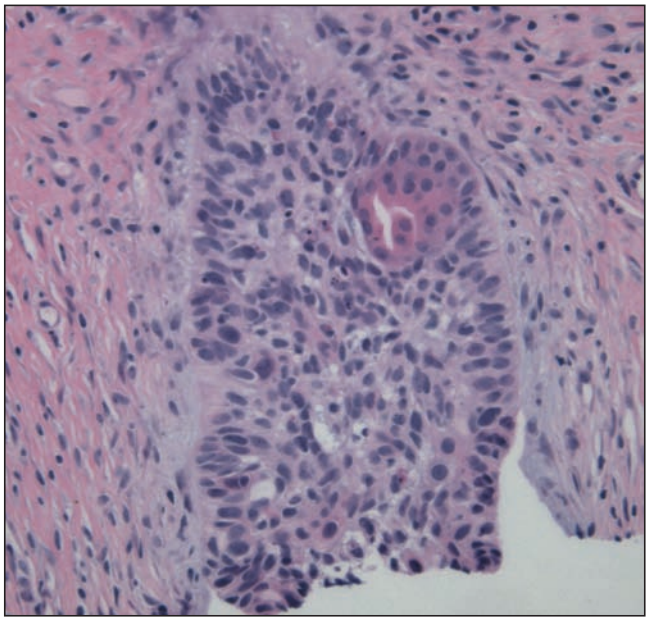


FIGURE 12.9: ***Basal cell carcinoma with eccrine differentiation. The presence of eccrine tubules in tumor nests is not uncommon and does not impact prognosis in our view.***

large basaloid tumor nests that are rounded and show central keratinization. We deem these to represent a variant of nodular BCC. The central cysts typically lack a granular cell layer and are filled with keratin and parakeratotic debris; a granular cell layer is present in some cases and the cysts may show central calcification surrounded by the basaloid tumor cells. As with other nodular BCCs, the stroma is neither proplastic nor highly collagenized. Mitotic activity is minimal, as is the presence of individual cell necrosis. True hair production is absent.

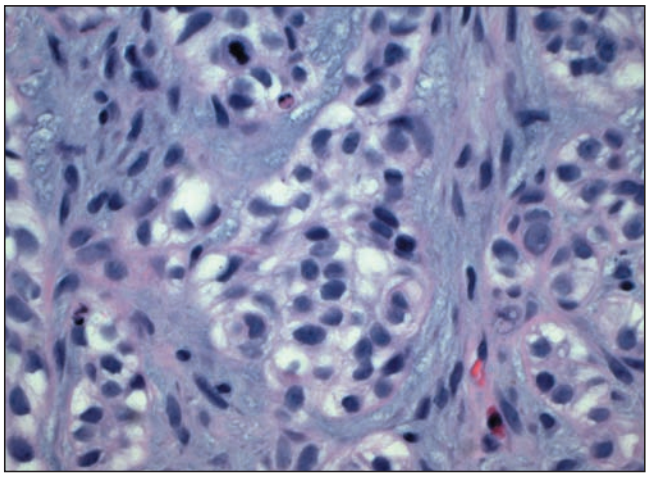
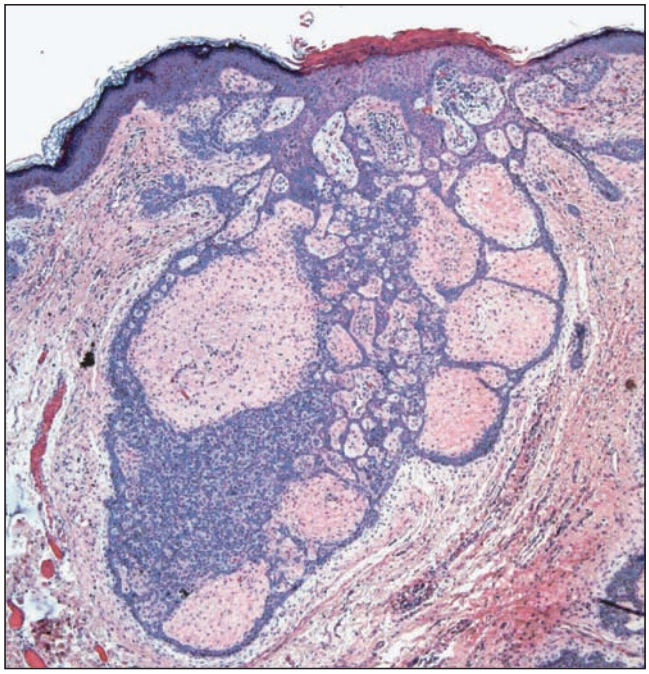


FIGURE 12.10: ***Basal cell carcinoma with trichilemmal differentiation. Trichilemmal differentiation is characterized by clear cell change and reflects cytoplasmic glycogen, as can be demonstrated with special stains.***

INFUNDIBULOCYSTIC BASAL CELL CARCINOMA

Infundibulocystic BCC has basaloid cells, typically in continuity with the overlying epidermis, which proliferate as oblong and rounded nests surrounding keratin-filled structures lined by a stratified squamoid epithelium with a granular cell layer. Follicular bulbs, dermal papillae, and papillary mesenchymal bodies typical of true follicular differentiation (seen in trichoepithelioma) are typically absent, as is true hair shaft production. The surrounding stroma contains plump cells of presumed fibroblastic lineage and shows minimal myxoid change, while the



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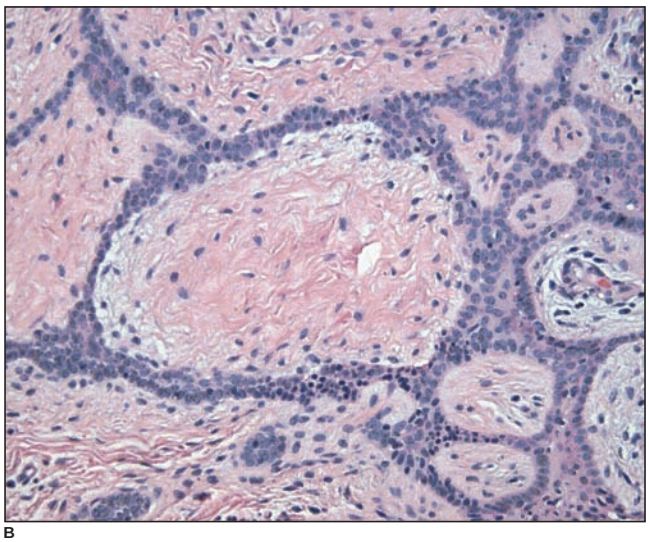

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FIGURE 12.11: ***(A and B) Fibroepithelioma of Pinkus. This is an indolent-growth tumor that has fine anastomosing strands of basaloid cells embedded in a stroma rich in plump fibroblasts.***

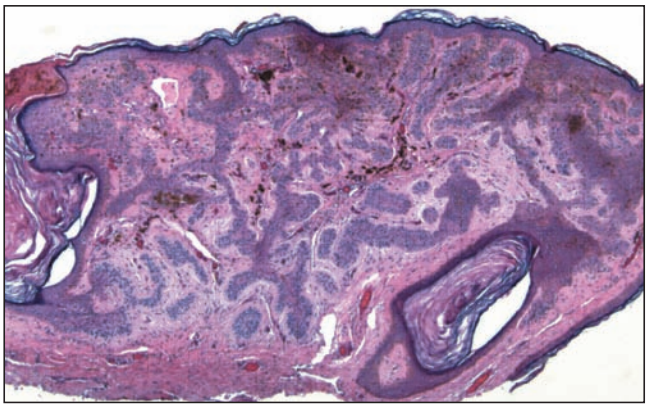


FIGURE 12.12: Infundibulocystic BCC. Basaloid tumor cells connect to the epidermis at multiple points and show infundibular cysts with central keratin but no hair shaft production. This example is a pigmented BCC with abundant melanin in tumor cells and in stromal melanophages.

circumscribed, demarcated, differentiated stroma of a trichoepithelioma is not present and there is no desmoplastic stromal reaction as seen in infiltrative-growth BCC. This tumor must be differentiated from basaloid follicular hamartoma, which manifests abortive hair papillae, typically centered around a folliclelike structure in continuity with the epidermis. These lesions are typically no more than 1–2 mm in diameter.

FOLLICULAR BASAL CELL CARCINOMA

On occasion, a BCC will show matrical differentiation comprising shadow cells adjacent to islands of proliferating basaloid cells mimicking a pilomatricoma. The shadow cells are anucleate, with eosinophilic cytoplasm, often with zones of calcification; mitoses, apoptic cells, and high-grade nuclear atypia are absent. The term "follicular squamous cell carcinoma" has been used to describe squamous cell carcinoma arising in the wall of an epidermal cyst; BCC arising in this setting is rare but not unique (personal observation).

PLEOMORPHIC BASAL CELL CARCINOMA

Some BCCs show strikingly enlarged giant hyperchromatic nuclei with amorphous nucleoplasms either scattered individually through tumor lobules or clustered, suggesting that they are components of a similar clone; these are pleomorphic BCC, or basal cell epithelioma with "monster cells" (Figure 12.13). All cases evaluated by static image analysis cytometry are aneuploid; paradoxically, mitoses, although present with similar frequency as typical nodular BCCs, are seldom atypical. These pleomorphic monster cells impart no prognostic significance and have no enhanced biological aggressiveness; we speculate that these tumors reflect senescent atypia.

BASAL CELL CARCINOMA WITH SWEAT DUCT DIFFERENTIATION

In about 1% of our cases of nodular BCC, there are areas of otherwise typical eccrine, and sometimes apocrine, differentiation. Tubules lined by cuboidal epithelium with an internal eosinophilic cuticle are centrally disposed in otherwise typical basaloid tumor cell aggregates. The internal eosinophilic cuticle stains with immunohistochemical stains for carcinoembryonic antigen (CEA) and epithelial membrane antigen (EMA).

BASAL CELL CARCINOMA WITH SEBACEOUS DIFFERENTIATION

Basal cell carcinoma with sebaceous differentiation is distinguished from sebaceous adenoma by virtue of a germinative cell component that occupies more than 50% of the transverse diameter of the tumor lobules. Typically, the

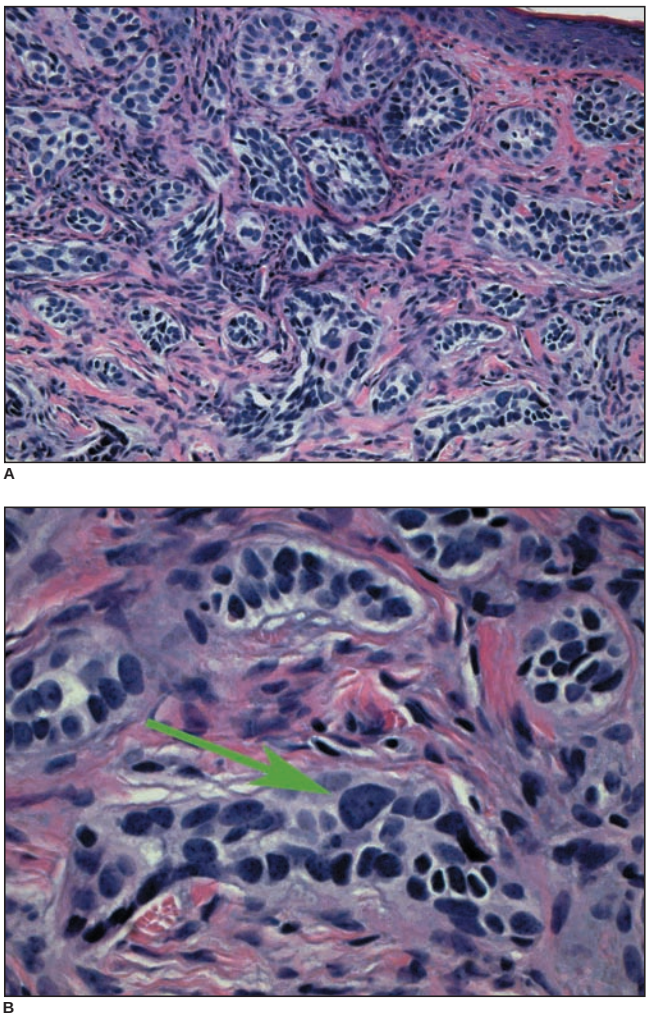


FIGURE 12.13: (A) Pleomorphic BCC. Senescent atypia is the probable origin of the "monster cells" (arrow) *that do not affect prognosis (B).*

lobules have a rounded morphology with areas of slitlike retractions, mitoses, and apoptotic debris. Such lesions are differentiated from sebaceous carcinoma by the absence of pagetoid spread in the overlying epidermis, haphazard infiltrative growth, desmoplastic stroma, or invasion of adjacent structures. Sebaceous adenoma is characterized by a lining of germinative cells less than 50% of the diameter of the neoplastic lobule, while sebaceous hyperplasia comprises a peripherally disposed layer of germinative cells one to two cells thick. In contrast is the sebaceoma, in our view, a form of hamartoma manifesting a haphazard array of germinative epithelium admixed with sebocytes and structures that recapitulate sebaceous ducts. Problematic are the sebaceous neoplasms of Muir-Torre syndrome, which sometimes defy precise classification.

FIBROEPITHELIOMA OF PINKUS

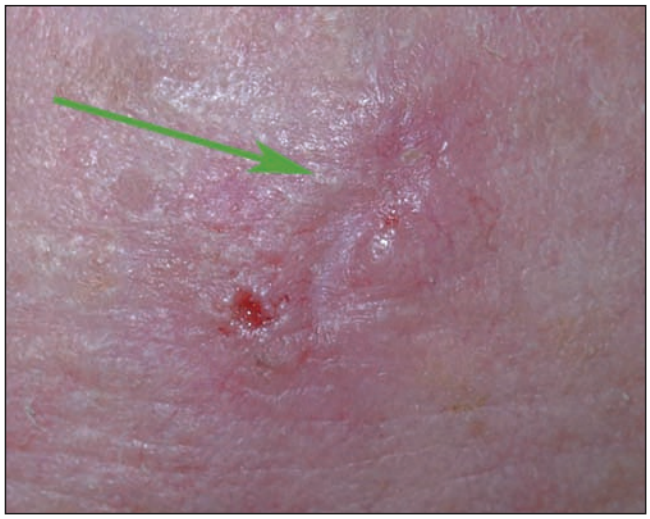
These tumors typically arise above the natal cleft or on the lower trunk as a pink or flesh-colored nodule with a constricted inferior margin, clinically mimicking a seborrheic keratosis. Histologically, elongated basaloid epithelial strands with slitlike retractions from the stroma are enmeshed in a myxoid matrix or a background of proliferating spindle cells with abundant collagen (Figure 12.11). If the lesion is completely excised, there is a well-demarcated inferior and lateral margin; the tumor frequently connects to the overlying epidermis at multiple points. The important differential diagnostic consideration is the eccrine syringofibroadenoma of Mascaro, which is usually acral and comprises elongated basaloid strands containing central eccrine ductal cells and a well-defined cuticle that connects to the undersurface of the epidermis at multiple points. Deeper levels through the paraffin block will show central eccrine ductal differentiation and a cuticle, unlike the solid undifferentiated basaloid epithelial columns of a fibroepithelioma.

RECURRENT BASAL CELL CARCINOMA

Approximately 10% of BCCs treated by conventional management recur (Figure 12.14). This recurrence rate is a result of positive margins after attempted surgical excision and varies by anatomic location and histologic subtype, being highest for the aggressive-growth variants (26.5% for infiltrative BCCs) and lowest for the indolent-growth variants (6.4% and 3.6% for nodular and superficial BCC, respectively). The recurrence rate for Mohs surgery is considerably lower than is achieved by non-Mohs modalities. Most recurrences occur within three years following the original operative procedure; however, 20% of recurrences occur between 6 and 10 years after the original surgery. Any conventionally managed recurrent lesion has a further enhanced risk of recurrence, reflecting difficult anatomical locations and the disruption of preexisting architecture by scarring. The histomorphology of recurrent BCC reflects the character of the neoplasm: more often an aggressive-growth than an indolent-growth variant, the presence of scar formation that disrupts the native anatomy, a generally greater depth of infiltration, and usually no connection to the overlying epidermis or to preexisting follicular structures (Figure 12.14).

PATHOGENESIS

The pathogenesis of BCC impacts its histomorphology and biologic behavior. Basal cell carcinoma is thought to derive from basaloid epithelia located in the follicular bulges, in the anagen hair bulbs and follicular matrix cells, and in specific basaloid cells of the interfollicular epidermis. These are pluripotent progenitor epithelia in adults or epithelial germ cells in the case of those neoplasms arising in childhood such as linear basal cell nevi. Key to our understanding of the pathogenesis of BCC has been the



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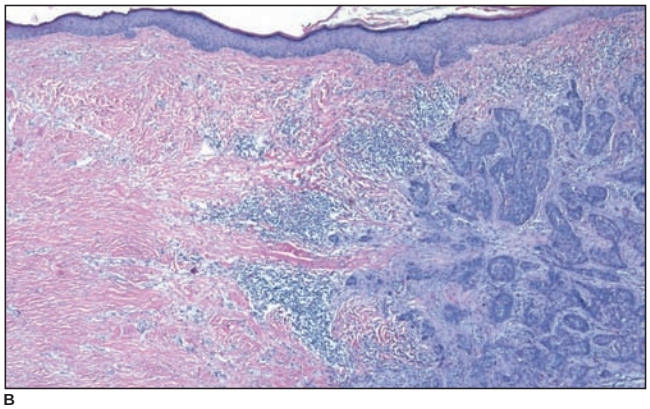


B

FIGURE 12.14: ***Recurrent BCC. In the scar from prior surgery, a flesh-colored pebbled plaque has developed (A). The presence of a scar is the clue histologically (B). (Case courtesy Dr M. Wilkerson, University of Texas Medical Branch, Galveston.)***

unraveling of the molecular basis of the nevoid basal cell carcinoma syndrome (Gorlin-Goltz), an autosomal dominant heredofamilial syndrome with expression in late childhood or young adulthood. In most patients, the abnormality involves a mutation in the human homologue of the Drosophila patched gene *(PTCH)*, a tumor suppressor gene sited on chromosome 9 q22-q31; mutations of *PTCH* eventuate in cell proliferation and play a secondary role in the initiation of BCC through activation of the *BCL2* gene. At the point of overexpression of bcl-2 protein, the sporadic and familial forms of BCC follow a similar pathway. Preferential overexpression of bcl-2 has been shown in the indolent-growth forms of BCC (superficial and nodular BCC). We propose that immortalization of progenitor epithelia of the hair follicle and the interfollicular epidermis by bcl-2 predisposes to subsequent ultraviolet light (UVL)-induced mutagenic "hits." Mutagenesis of p53 appears preferentially in the aggressive-growth, versus the indolent growth variants of BCC; enhanced expression of p53 occurs in the absence of concomitant upregulation of p21 expression.

UVL-INDUCED MUTAGENESIS AND BIOLOGIC TRANSFORMATION

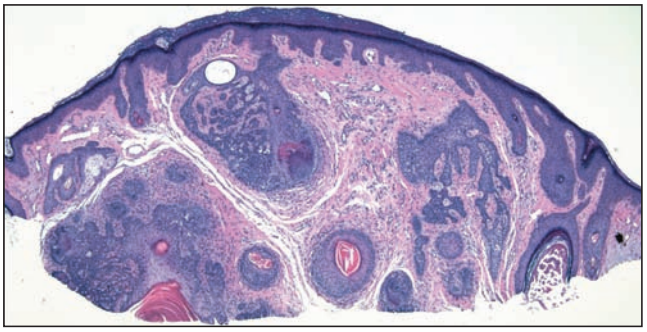
Basal cell carcinomas express p53 protein and do so preferentially in aggressive-growth variants. Mutations of p53 have been documented in up to 40% of BCCs; most mutations bear the signature of UVL induction. The aggressive-growth variants of sporadic BCC are associated with stromal fibroplasia and overexpression of mutant p53. It is this stromal response to tumor that makes the aggressive-growth BCC less amenable to non-Mohs local therapy. The interaction between tumor and stroma is critical to lesional pathogenesis. Loss of basement membrane material around individual tumor cell nests occurs with progression from indolent- to aggressive-growth neoplasms, likely reflecting the activation of matrix metalloproteinases that, in the process of transformation, digest basal lamina around tumor nests and promote the elaboration and/or release of proplastic cytokines which then become bioavailable to the rapidly proliferating aggressive-growth neoplasms. Work done in Winnipeg, Canada, and confirmed by Australian workers has shown that the indolent-growth variants, are widely distributed on both sun-exposed and sun-protected skin, while the aggressive-growth variants, such as infiltrative and morpheaform BCC, are more frequent in sun-exposed skin, with the majority of these tumors occurring on the head and neck and about 25% of cases occurring on the nose. Less prevalent in more darkly pigmented races, the histologic types of BCC seen in Africans and Hispanics are similar in histomorphology to those seen in Caucasians.

An amyloidlike substance held to derive from degenerating epithelia is present in and around some indolent-growth BCCs, but mucin deposition is a far more common finding. These mesenchymal mucins, likely products of stromal cells, consist of hyaluronic acid and dermatan sulfate. Cylinderlike inclusions of hyalin material resembling those seen in cylindroma are seen in some nests of nodular BCCs and appear to be composed of intermediate filaments including vimentin, keratin, and myosin. In rare cases, needle-shaped fibers comprising collagenous crystalloids are identified and contain types I and III collagen, reflecting degeneration of extracellular matrix components.

Ultrastuctural examination gives some clues to the pathogenesis and transformation characteristics of BCC. Both the superficial and nodular variants of BCC are surrounded by a continuous basement membrane zone comprising collagens type IV and V admixed with laminin, while the aggressive-growth variants, such as the morpheaform, metatypical, and infiltrative-growth subtypes, show an absent basement membrane and pronounced stromal desmoplasia. The percolation of malignant cells through the stroma, and their location within a desmoplastic collagen table, necessitates a surgical approach to treatment of aggressive-growth variants of BCC, with Mohs surgery especially useful for their treatment. Other ultrastructural features seen at the light microscope level include decreased numbers of hemidesmosomes along the margins of nodular and superficial BCCs, compared with the normal adjacent epidermis. In concert with the absence of type VII collagen anchoring fibrils, this is a potential explanation for the retraction spaces seen at the light microscopic level between the nodules of tumor cells and the adjacent stroma.

DIFFERENTIAL DIAGNOSIS

Different forms of BCC have different sets of differential diagnostic possibilities. The often demarcated clones of basaloid cells in superficial BCC call to mind Bowen's disease and sometimes other clonal proliferations, including clonal seborrheic keratosis or intraepidermal eccrine porocarcinoma in situ. Peripheral palisading along the dermal–epidermal junction in concert with slitlike retraction of the stroma helps establish the diagnosis of BCC. Eccrine cancers often have true luminal margins or intracytoplasmic lumina reflecting glandular differentiation. Bowen's disease shows pronounced intercellular bridge formation and cytoplasmic keratinization in concert with frequent and atypical mitotic figures uncommon in superficial BCC. Seborrheic keratoses may be difficult to distinguish from both superficial and nodular BCC; helpful is the plaquelike architecture of seborrheic keratoses, whereby a straight line drawn between the native epidermis at the shoulders of the lesion lies beneath the plane of the proliferation. Actinic keratoses occasionally mimic BCCs in the formation of downward buds of atypical basaloid cells in sun-damaged skin, but



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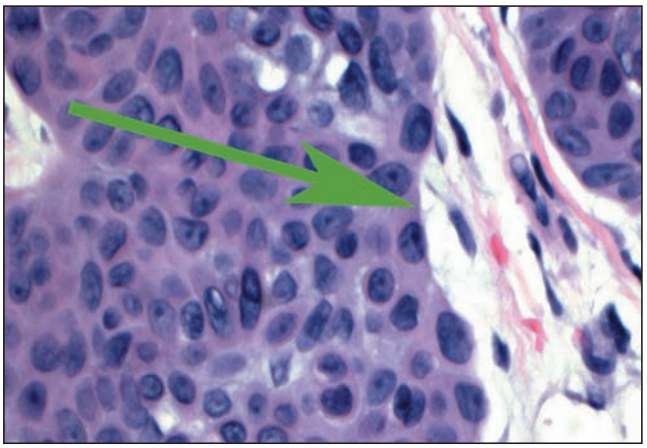

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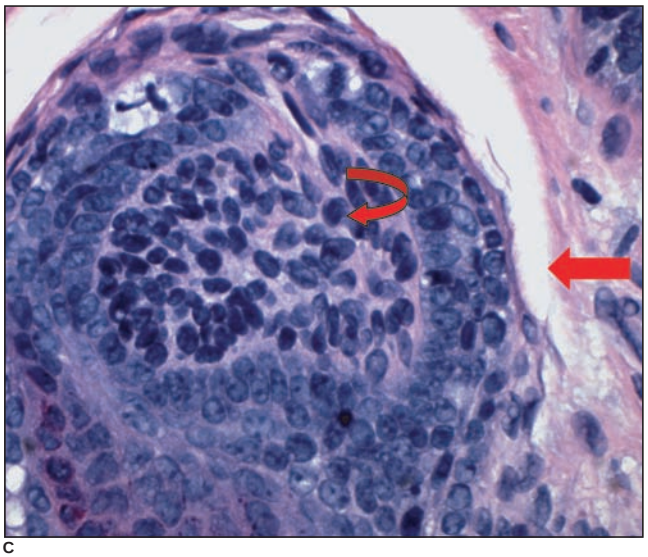


C

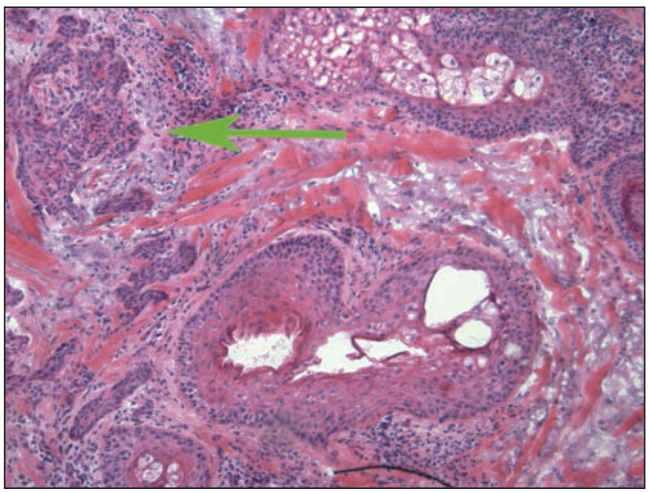
FIGURE 12.15: ***Trichoepithelioma. The tumor is sharply circumscribed (A) and carries with it a specialized stroma, which, like that of the Pinkus tumor, has plump fibroblasts that we suspect in this case are a component of a follicular hamartomatous process. Unlike BCC, where slitlike retractions separate stroma from the epithelial islands*** **(green arrow)** ***(B), the retraction artifact of trichoepithelioma produces clefts between stromas*** **(red arrow)** ***(C); the papillary mesenchymal body is a critical clue to the diagnosis as well*** **(yellow arrow).**

these are surmounted by parakeratosis and typically lack slitlike retraction from the subjacent mucinous stroma.

The differential diagnosis of nodular BCC includes the nodular intradermal eccrine proliferations such as clear cell hidradenoma, cylindroma, and eccrine spiradenoma. Clear cell hidradenoma and eccrine spiradenoma show cuboidal lumenal cells, reflecting eccrine differentiation. These neoplasms usually have few mitoses and usually do not connect to the overlying epidermis; in addition, peripheral palisading, stroma mucinosis, and individual cell necrosis are unusual. Cylindromas and eccrine spiradenomas arise in the dermis and lack connection to the epidermis. Their respective characteristic appearance of a "jigsaw puzzle" pattern, or of "blue balls in the dermis," aids in their diagnosis. Neither tumor exhibits peripheral palisading or clefting. Trichoepitheliomas show abortive hair papilla formation and papillary mesenchymal bodies in basaloid nests, which are in turn encompassed by a distinctive stroma, populated by plump stromal cells sharply demarcated from the adjacent native dermis. Trichoepitheliomas have no mitotic activity, no individual cell necrosis, and only infrequently have melanin pigment within tumor nests, while the critical cells frequently radiate around central keratin-filled cystic structures. Trichoepithelioma may be difficult to differentiate from micronodular BCC, but lacks mucin or stromal retraction. Trichoepitheliomas frequently show foreign body granulomas, fibrous stroma, papillary mesenchymal bodies, and multiple horn cysts (Figure 12.15). The more differentiated BCCs such as the infundibulocystic basal cell carcinoma, or those BCCs with abortive hair papilla formation, may be confused with trichoepithelioma. We typically do not need to resort to immunostains, but CD34 is expressed in the stroma of trichoepitheliomas and not that of BCCs; bcl-2 is expressed diffusely in indolent growth BCCs, but only around the periphery of trichoepitheliomas. The differentiated forms of nodular BCC, such as BCC with sebaceous, eccrine, or adnexal differentiation, can be distinguished in the former instance by criteria described above, and in the latter by the presence of slitlike retraction, individual cell necrosis, and mitotic activity; when present in BCC, the eccrine components are only a minor component of the overall tumor volume. Some BCCs show clear cell differentiation comprising intracytoplasmic glycogen accumulation recapitulating outer root sheath (trichilemmal) differentiation, This raises a consideration of sebaceous carcinoma, which stains with antibodies to low-molecular-weight keratins (CAM 5.2), unlike basal and squamous cell carcinomas, SCCs which are typically negative for CAM 5.2. The Thompsen-Freidenreich (T) antigen is expressed in sebaceous neoplasms but not by most basal and SCCs. Although this may be a useful diagnostic tool, it has proven problematic in our and others' hands, because even though it is strongly expressed in native sebaceous epithelium, it is not expressed in cancers of sebaceous glands. The

demonstration of cytoplasmic glycogen in large quantities (using an alcian blue periodic acid–Schiff (PAS) and PAS-diastase staining combination) typifies epithelial neoplasms with trichilemmal but not sebaceous differentiation; sebaceous carcinomas tend to be more locally aggressive and have a higher risk of metastasis than BCC. Some sebaceous tumors, including low grade carcinomas and adenomas, manifest nesting but typically lack peripheral palisading and clefting. Additionally, they include sebocytes with vacuolated cytoplasm and scalloping of the nuclei. The presence of balloon cell change in a melanocytic proliferation can mimic clear cell and sebaceous differentiation in BCCs; immunohistochemical expression of antibodies to S-100 protein, A103 (Melan-A), and gp100 protein (HMB-45) makes it possible to distinguish between these entities. Metastatic renal cell and thyroid cancers can also have clear cell morphology. Metastatic renal cell carcinoma is glycogen rich and can therefore cause diagnostic concern, but typically has a highly vascular stroma associated with abundant hemorrhage. Renal cell carcinomas also express CD10, CD15, AMCAR, and vimentin, in addition to keratins. Metastatic breast carcinoma mimics infiltrating or morpheaform BCC but without connection to the epidermis. Characteristic is the "Indian file" arrangement in lobular carcinoma of the breast, along with more marked nuclear pleomorphism. Thyroid carcinomas stain with antibodies to thyroid transcription factor (TTF), and also with thyroglobulin, if follicular differentiation is present. Merkel cell carcinoma can occasionally be confused with a solid undifferentiated form of BCC. However, Merkel cell carcinomas express cytokeratin 20, typically as droplike aggregates in a perinuclear pattern, representing Golgi complex accentuation, in addition to staining with the neuroendocrine markers synaptophysin and chromogranin. Basal cell carcinoma must also be distinguished from other basophilic staining tumors, such as ameloblastomas and cloacogenic carcinomas, which have features similar to BCC but occur in the mouth and perianal region, respectively.

With respect to the aggressive-growth BCCs, morpheaform BCC must be distinguished from desmoplastic trichoepithelioma and microcystic adnexal carcinoma. Some desmoplastic trichoepitheliomas show a downward indentation of the epidermal surface (an epidermal "dell"), accompanied by proliferating basaloid cells, superficially situated keratin cysts, and very rare mitoses in the tumor cell population. Dermal mucin production and apoptosis are absent, although calcification may be present. Microcystic adnexal carcinoma is a widely invasive tumor that typically is comprised of columns of vertically oriented atypical cells manifesting individual cell necrosis and mitotic activity. It may show cytologically banal tumor cells arranged in ducts and strands that mimic infiltrative or morpheaform BCC. It can invade deeply, often shows neurotropism, and can show differentiation into eccrine or follicular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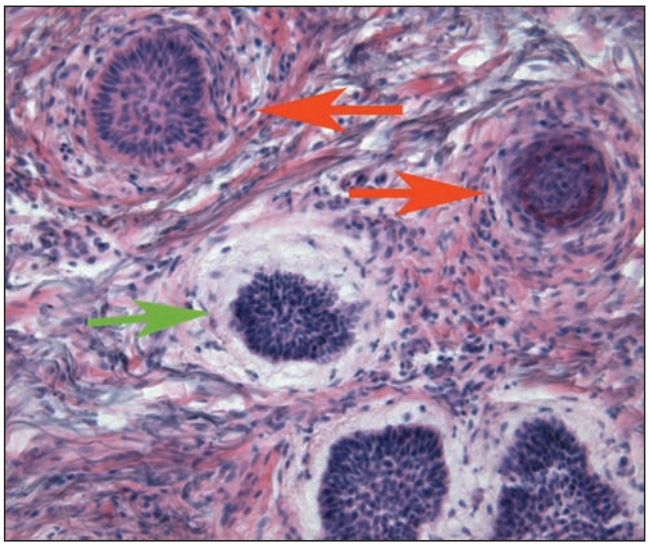
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FIGURE 12.16: ***Hair appendage structures can mimic BCC. The tumor nests*** **(green arrow)** ***are less regular and show no differentiated elements such as pilosebaceous structures or a central cuticle (A); native hair follicles lack the slitlike stromal retraction of the basal cell cancer nests*** **(green arrow)** ***and possess their own specialized mesenchymal investiture*** **(red arrow)** ***(B).***

structures. Microcystic adnexal carcinoma generally occurs around the midface, typically in middle-aged or elderly women, and frequently recurs when treated with non-Mohs modalities.

Adenoid cystic carcinoma has a characteristic cribiform pattern with fine bridges separating glandular spaces. Lobular carcinoma cells often have discrete endocytoplasmic lumina, sometimes with signet ring morphology at high power, while ductal carcinoma shows true common luminal margin formation and, in paraffin embedded tissues, expresses e-cahderin, which can be detected with commercially available monoclonal antibodies. Squamous cell carcinoma is sometimes histologically similar to

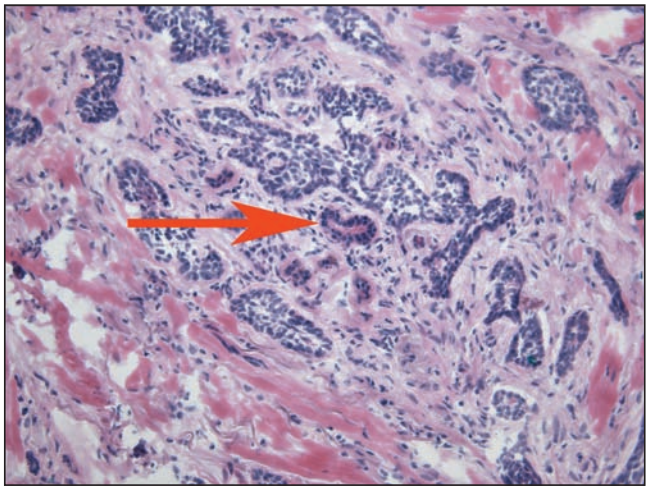

FIGURE 12.17: ***Sweat glands*** **(red arrow)** ***mimic BCC.***

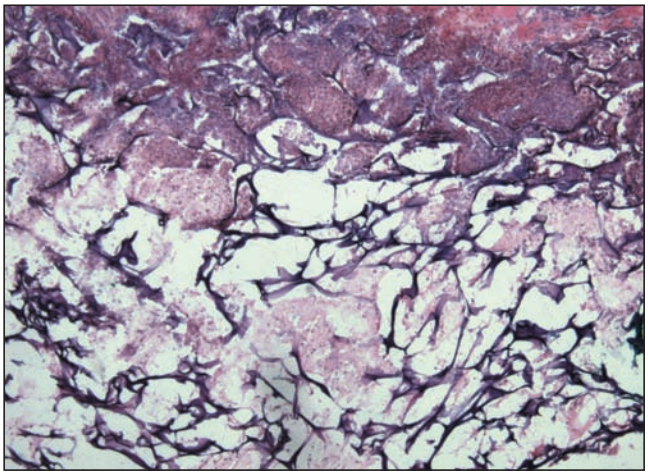

FIGURE 12.19: ***Gel foam mimics cauterized BCC.***

BCC. The cells in BCC are more basophilic and smaller, while in SCC they tend to be larger, more anaplastic, and often show frequent abnormal mitotic figures. Intercellular bridges or desmosomes are evident in SCC. The presence of keratinization is not a very useful feature, as both BCC and SCC can exhibit this feature. When two distinct populations of cells showing BCC and SCC are identified but in continuity in a single tumor, a diagnosis of basosquamous carcinoma may be rendered. Sebaceous carcinomas also manifest greater nuclear atypia, but frequently have areas of squamoid and basaloid differentiation.

HISTOLOGIC CONSIDERATIONS WITH RESPECT TO MOHS SURGERY

The histopathologic features of BCC seen with frozen section microscopy[2] are similar to those described above with permanent-section microscopy (see Chapter 11 for a description of vertical-versus-horizontal histopathology). In frozen sections stained with hematoxylin and eosin, irregularly shaped tumor strands and lobules with marked basophilic staining can be seen extending from the epidermis. Peripheral palisading and clefting are present, but cytologic details may not be as clear as in paraffin sections. Fortunately, the diagnosis of BCC does not usually require cellular detail, and diagnoses can usually be made on scanning magnification. Some Mohs surgeons use toluidine blue to stain sections of BCC (see Chapter 19). The stroma of BCC tumors is rich in mucopolysaccharides, which stain pink with toluidine blue, contrasting well with the blue background. This color difference helps the Mohs surgeon-pathologist detect residual tumor, but similar metachromasia may also appear in areas of inflammation, scar, and around pilosebaceous structures (Figure 12.16). Sweat glands (Figure 12.17) and salivary glands (Figure 12.18) can also mimic BCC. The higher cure rates reported for Mohs surgery are directly related to the quality of the frozen sections produced by the Mohs technician, the ability of the Mohs surgeon to differentiate BCC from

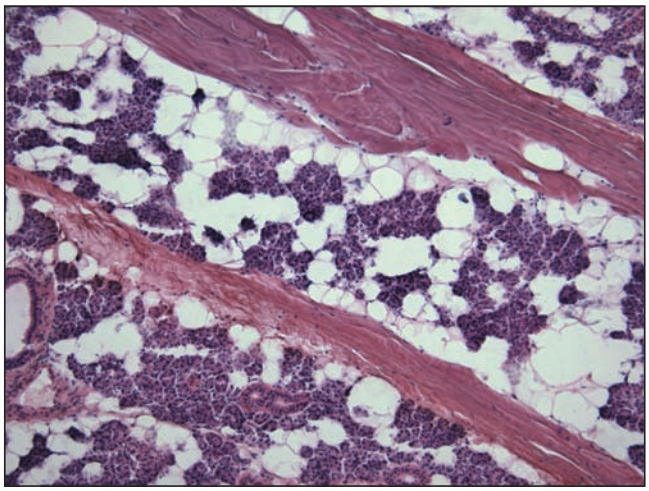

FIGURE 12.18: ***Salivary glands mimic BCC.***

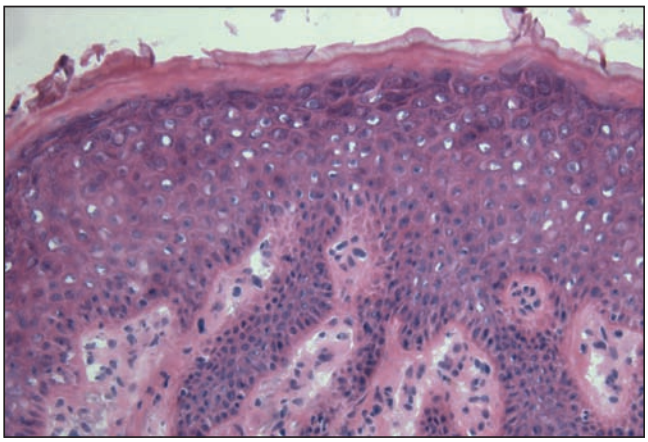

FIGURE 12.20: ***Freezing artifact can mimic pagetoid spread.***

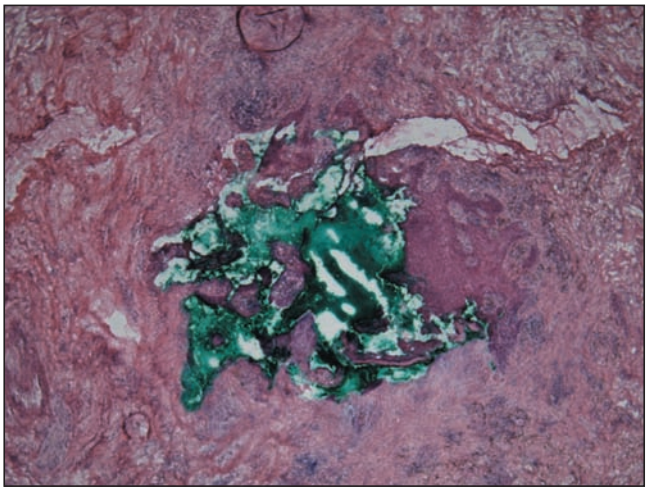

FIGURE 12.21: Transposed epidermis. In this illustration, the transposed epidermis is marked with green ink.

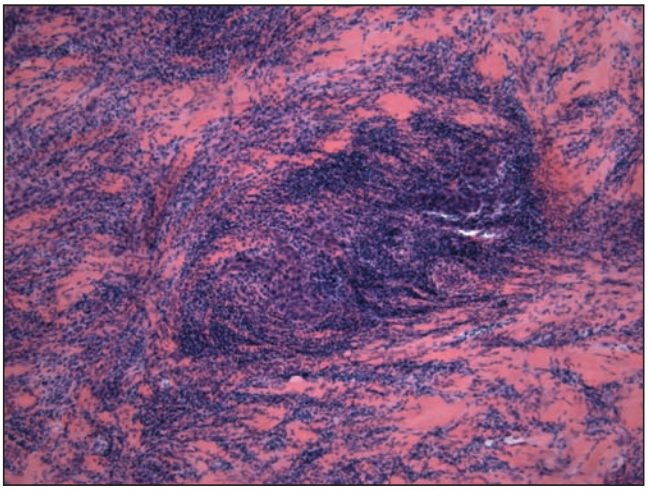

Figure 12.22: Intense inflammation is the clue to the presence of tumor in this field.

common imitators, and the ability of the Mohs technique to examine 100% of the surgical margin.

Frozen section artifacts are common and must be identified to prevent the Mohs surgeon-pathologist from labeling a surgical margin falsely positive. Careful surgical technique and tissue processing can decrease the frequency of false-positive margins, but they will still occur. Underbeveling or overbeveling during surgical excision of the cancer may cause (among other things) a thin-appearing or missing epidermis (see Chapter 2). "Streaming" epidermal cells result from the polarization of cells secondary to electrocautery. Cauterization causes coagulation of proteins, decreased cellular detail, and an amorphous appearance of normal structures that can mimic BCC. It can also produce exaggerated stromal clefting and amorphous coagulated basal cells. Hemorrhage mimics red dye. Monsel's solution can look like melanin or hemosiderin in the dermis, as well as produce granulomatous inflammation. Remnants of suture material can also produce granulomatous inflammation. Gelfoam, which was historically used for hemostasis during Mohs surgery, simulates cauterized BCC (Figure 12.19). Excessive freezing of tissue produces vacuolization of epidermal cells, mimicking pagetoid spread (Figure 12.20). Overfreezing fat may lead to shattering of tissue and incomplete sectioning. Overbeveling or overflattening of tissue during processing may result in tangential cuts that lend a psoriasiform appearance to the epidermis; trapped dermal papillae are diagnostic of this phenomenon. Tissue folds and coverslip bubbles can obscure underlying tumor. Faulty mounting of tissue may displace parts of the epidermis. Transposed epidermis may mimic tumor within the dermis (Figure 12.21). Floaters may be caused by loose tissue in staining baths or by displacement during flattening of specimens. They can resemble tumor, but lie outside the inked margin and usually are not inked themselves. Overdyeing the tissues with hematoxylin causes overstaining of inflammatory cells, which may obscure the epidermis and hair follicles. Calcium deposits mimic clumped dye or amorphous BCC. Dense inflammation in Mohs specimens makes small nests of tumor harder to detect (Figure 12.22). Deeper cuts into the tissue block or the use of immunohistochemical stains may be needed to define the exact surgical margin. Ber-EP4 and bcl-2 immunostains are useful in the separation of BCC from SCC, but cannot reliably distinguish BCC from trichoepithelioma.

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CHAPTER 13

Squamous Cell Carcinoma: Vertical and Horizontal

A. Neil Crowson and Edward H. Yob

Squamous cell carcinoma (SCC) represents the second most common human cancer after basal cell carcinoma (BCC), accounting for roughly 20% of all skin cancers. It is the most common cutaneous malignancy in African Americans. Cumulative lifetime risk is approximately 10% in Caucasians, and its increase in prevalence over the last several decades may be attributable to increased ultraviolet light exposure. Risk factors include sun exposure, increased age, arsenical exposure, immune suppression, and genetic instability syndromes including xeroderma pigmentosum. Other etiologic factors associated with the development of SCCs include infection with human papillomavirus (HPV) type 16, exposure to aromatic hydrocarbons, aerodigestive tract SCCs associated with smoking and use of chewing tobacco, and the presence of long-standing inflammation in the context of burn wounds, chronic dermatoses, and draining sinus tracts.[1]

Certain dermatoses can provide a background leading to SCC. These include lichen planus, lupus erythematosus, porokeratosis, lichen sclerosis, erythema ab igne, nevus sebaceus, and epidermal nevi. Squamous cell carcinomas may arise from chronic infections including granuloma inguinale, acne conglobata, lymphogranuloma venereum, chronic dissecting cellulitis, hidradenitis suppurativa, and chronic deep fungal infections. Epidermolysis bullosa is associated with SCCs of skin, aerodigestive tract, and esophagus.

Pathogenesis

The pathogenesis of SCCs arising in sun-exposed skin of elderly individuals reflects ultraviolet light–induced mutations of p53 and other tumor suppressor genes, in which a specific CC→TT "fingerprint" mutation is demonstrated. The cases caused by HPV type 16 involve deregulation of cell cycle control through the inactivation of the retinoblastoma gene product (pRB) through binding of a specific protein product of HPV (HPV-E6) to an internal pocket on pRB. The chronic dermatoses that lead to SCC may reflect an alternate mechanism, such as upregulation of bcl-2 expression, as a result of the inflammatory cytokine interleukin 2 elaborated by Th-1 lymphoid cells in sites of chronic inflammation. By immortalizing squamous epithelia in a fashion similar to that proposed for sporadic basal cell cancer, the risk of subsequent mutagenic "hits" by ultraviolet light or other factors could be enhanced as is postulated for human BCC. This particular mechanism might be a factor in the development of SCC within Marjolin's ulcer, as well as SCC developing in a chronic draining fistula, sinus tract, ulcer, or other chronic inflammatory site; such lesions have a 30% risk of metastasis.

Clinical Features

Squamous cell carcinoma most commonly presents as a tumor nodule, often exophytic, with an adherent scale or crust overlying a frequently skin-colored lesion that may have shades of red or brown coloration with telangiectasia. These indurated crusted plaques may ulcerate. Larger tumors may show subcutaneous nodules. The adjacent skin shows dermatoheliosis, as would be expected of a tumor usually arising in a sun-exposed site. When the mucosa of the lower lip is involved, tumors often arise in a background of actinic cheilitis, smoking, and use of chewing tobacco; extension across the vermillion border is common in this situation. Most SCCs are asymptomatic unless there is perineural invasion. Such tumors are frequently greater than 2 cm in diameter and may be associated with lymphadenopathy. Perineural infiltration may cause compromise of nerve function, including dysesthesia, anesthesia, or eventually muscle weakness. Perineural invasion affects roughly 5–10% of SCCs of the skin, with cranial nerves VI and VII (mandibular and maxillary divisions) the most frequently involved. Perineural infiltration may be associated with direct intracranial extension, increased morbidity, and death.

Metastases are uncommon in cutaneous SCCs, typically in the 2% range. Lesions involving the mucosal surface of

the lip may metastasize in about 10–15% of cases. Lesions of the ears are also associated with a higher metastatic rate, perhaps reflecting peculiarities of the vascular supply of the dermis, which derives directly from perichondrial vessels. Squamous cell carcinoma with a high rate of metastases includes inadequately treated lesions and large lesions of long duration. There is a correlation between increased thickness of SCC and recurrent and metastatatic disease. This leads some authors to measure tumor thickness with a micrometer in a fashion identical to that done for cutaneous melanoma. Squamous cell carcinomas greater than 2 cm in diameter are twice as likely to recur and three times as likely to metastasize as smaller lesions.

Histopathology

Traditionally, SCC has been classified into four major histologic types: the classic or conventional form, spindle cell SCC, acantholytic SCC, and verrucous carcinoma. Classification based upon degree of differentiation (intercellular bridges and keratinization of tumors) was proposed in the 1920s by Broders. Based upon the degree of differentiation, a grade I lesion comprises less than 25% undifferentiated cells, grade II lesions are 26–50% undifferentiated cells, grade III are 51–75% undifferentiated cells, and grade IV have greater than 75% undifferentiated cells. We simplify this scheme using cutoffs of 25%, 26–75%, and greater than 75% differentiation to indicate poorly, moderately, and well-differentiated SCCs of the conventional type. For SCCs of this type, Bowen's disease is frequently present (SCC in situ) and is in continuity with the invasive tumor by virtue of tongues or lobules of neoplastic cells that penetrate or push from the epidermis into the papillary and reticular dermis. Because SCC, like BCC and melanoma, can elaborate its own basement membrane material, we do not use periodic acid–Schiff (PAS) stains to identify the limiting basement membrane zone. In fact, the basement membrane does not constitute a barrier to invasion, but may play a role in tumor-stromal signaling. There are several specific histologic subtypes of SCC, which are important to recognize because they may mimic other forms of neoplasia leading to diagnostic misinterpretation.

SPINDLE CELL SQUAMOUS CELL CARCINOMA

Spindle cell SCC shows anastomosing bands of fully transformed, malignant spindle-shaped cells with hyperchromatic nuclei and modest quantities of tapered cytoplasm often showing granular eosinophilia; cells are arranged in both an end-to-end and a side-to-side fashion, with only very rare identifiable intercellular bridges (Figure 13.1). Mitoses are frequent and often atypical (Figure 13.2). Typically, these lesions arise in the sun-damaged skin of the head and neck of the elderly but are also seen in the setting of prior radiotherapy or less commonly of local trauma.

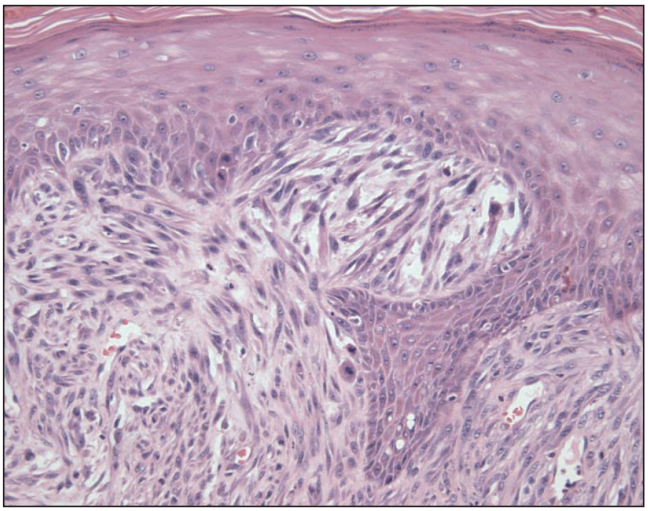


FIGURE 13.1: Spindle cell squamous cell carcinoma (SCC) characterized by anastomosing bands of spindle cells with hyperchromatic nuclei.

On occasion, there is myxoid stromal alteration, as well as scattered multinucleated neoplastic giant cells. While these lesions can be identified as SCCs by ultrastructural demonstration of desmosomes, in practice this is not attempted in the modern era. Instead, immunohistochemical methods are employed to identify cytokeratins, using antibodies such as DAKO's AE1/3 pancytokeratin; these lesions are S-100 protein negative, do not contain melanocytes, and are negative for the lineage-specific markers gp200 or A103 (HMB-45 or Melan-A). In an earlier era, the diagnosis of atypical fibroxanthoma was one of exclusion, but we now have markers for atypical fibroxanthoma such as procollagen I or CD68. Some spindle cell SCCs are so poorly differentiated that keratin expression is difficult to detect; when antibodies to pancytokeratins are negative we rely

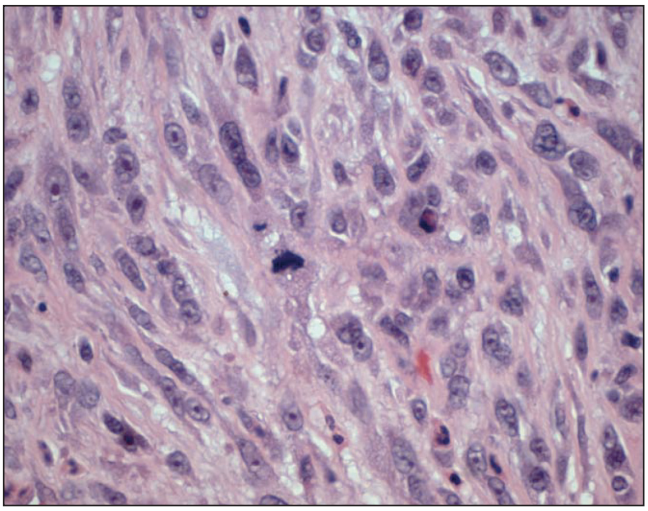


FIGURE 13.2: Spindle cell SCC. Malignant cytology and atypical mitoses with 40× objective magnification.

upon a second tier of anticytokeratin antibodies to confidently exclude a spindle cell SCC, including those that recognize both high- and low-molecular-weight cytokeratins. The use of antibodies to vimentin has a useful function in proving that tissue remains antigenic postfixation, but most of the aforementioned neoplasms express vimentin, making it of limited use in distinguishing between different diagnostic possibilities. Other mesenchymal neoplasms, including leiomyosarcoma, can mimic spindle cell SCC. Therefore, when the previously discussed markers are negative, evaluation for antibodies to actin, desmin, or myosin may be used. Some spindle cell squamous carcinomas represent true metaplastic carcinomas analogous to those of breast and other sites, by virtue of showing gradients of dedifferentiation with keratin expression in some areas and its absence in others. This reflects the multipotent capability of various cell lineages, including the squamous epithelium of the epidermis.

ACANTHOLYTIC SQUAMOUS CELL CARCINOMA

Also called pseudoglandular SCC, adenoid SCC, carcinoma segregans, or adenoacanthoma, these lesions typically are seen on the head and neck as nodules or ulcers in elderly males. The so-called "pseudovascular adenoid" SCC is classed with these lesions. Like the spindle cell SCC, these tumors may be recurrent or occur after irradiation; some observers feel that they have a more indolent biologic behavior than conventional SCC. Neoplastic cells are arranged in a lobular-growth pattern, with abundant cell-to-cell separation; they form rounded structures analogous to corps ronds to fashion lumenlike structures in which keratinocytes appear to lie free (Figure 13.3). As with all SCCs, cytoplasmic glycogen may be demonstrated with a PAS stain. Some authors lump the "signet ring" SCC with this group, but the latter is said to have a more aggressive biologic course. When hemorrhage occurs within a lesion of acantholytic SCC, the lumenlike structures can mimic vascular channels (pseudovascular adenoid SCC). On any given tissue section, some fields may give an impression that the tumor nests are in continuity with vascular elements, but this may represent either blood vessel invasion or artifact. These neoplasms do not stain with antibodies to CD31, CD34, or factor VIII.

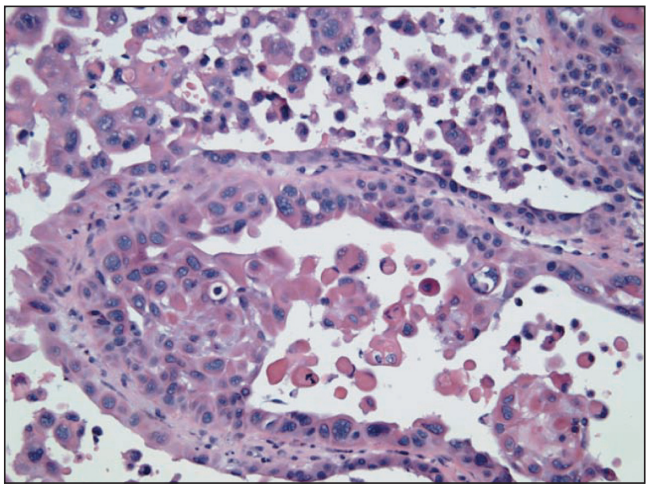


FIGURE 13.3: *Acantholytic SCC with loss of intercellular attachments showing rounded cell structures containing eosinophilic, refractile cytoplasms.*

PAPILLARY SCC

More common in elderly women, the papillary SCC is also seen in immunosuppressed patients. These are predominantly exophytic tumors, in which atypical cells covering fibrovascular stalks with invasion of the underlying dermis and widespread mitoses are seen (Figure 13.4).

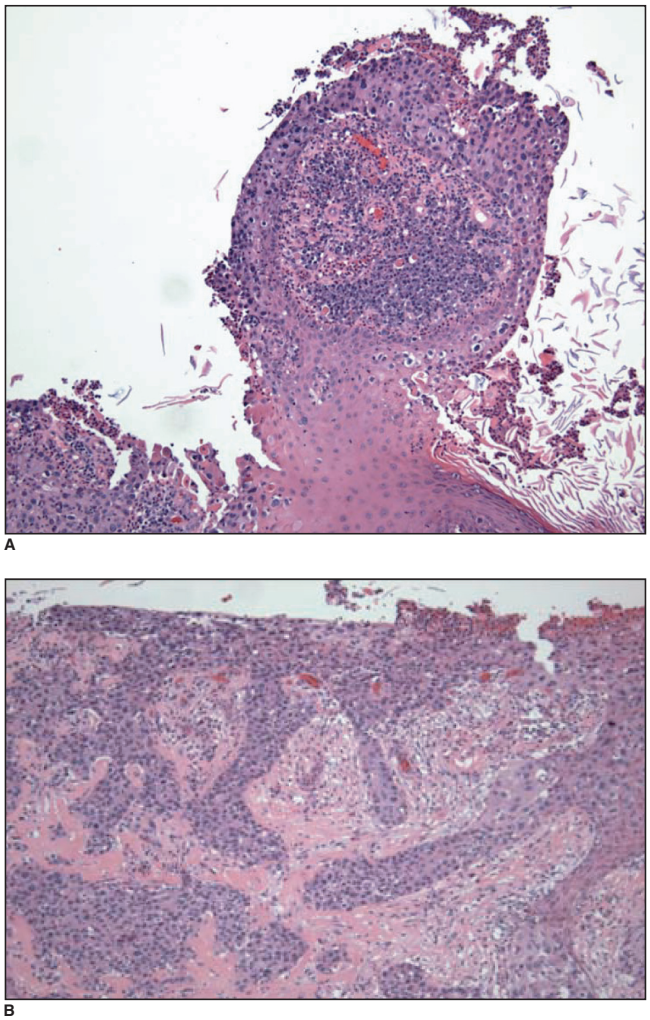


FIGURE 13.4: *(A) Papillary SCC. A papillary structure whose core contains plasma cells is covered by malignant squamous epithelia. The patient is a 93-year-old woman with a verruciform lesion of the cheek. (B) Papillary SCC showing stromal invasion by malignant squamous epithelia in the core of the papillary structure (same patient as in A).*

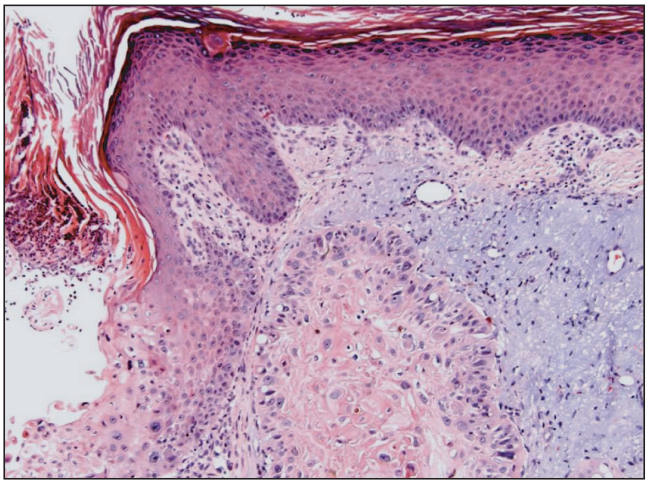


FIGURE 13.5: Follicular SCC. The tumor has typical features of SCC, but arises from the wall of a hair follicle. There is malignant transformation of the follicular lining epithelia.

FOLLICULAR SQUAMOUS CELL CARCINOMA

Arising from hair follicle walls, follicular SCC may show no suggestion of significant keratinocytic atypia in the overlying epidermis (Figure 13.5). Most such lesions occur on the head and neck of elderly patients. Consequentially, biopsies of such lesions may mimic metastases from elsewhere, including the aerodigestive tract, prior SCCs of the head and neck, or other sites (Figures 13.6 and 13.7). Avoiding misdiagnosis of this entity requires an awareness of its existence and a search for the precursor hair follicle structure that is usually in continuity with it.

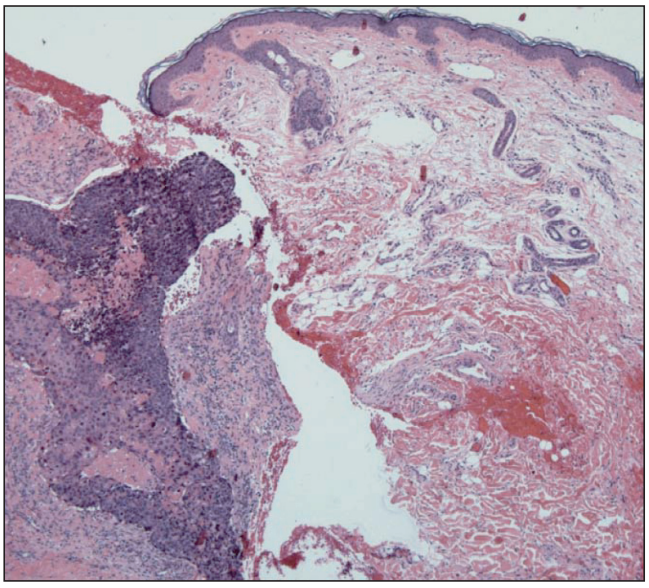


FIGURE 13.6: Metastatic SCC. Nests of malignant squamous epithelial cells are present in the dermis. There is no connection to the overlying epidermis.

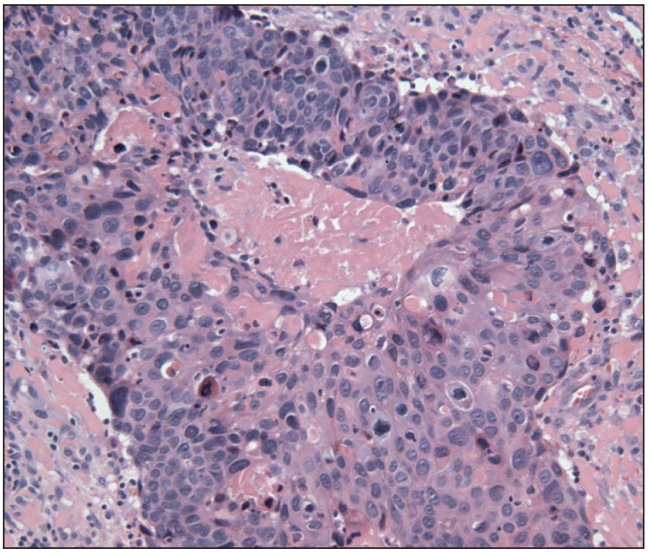


FIGURE 13.7: Metastatic SCC. Overtly malignant cytology is present in this isolated dermal tumor nest.

SUBCUTANEOUS SQUAMOUS CELL CARCINOMA

On occasion, SCCs may rapidly traverse the dermis after having arisen from the epidermis by a thin stalk that then separates. Such lesions result in a subcutaneous nodule that shows no connection to the overlying epidermis or to adnexal structures from which it has presumably arisen. Because perineural infiltration and metastases are more common in these neoplasms, they have a poorer prognosis. These lesions are difficult to distinguish from metastatic disease.

VERRUCOUS CARCINOMA

Verrucous carcinoma is a characteristic tumor with a mixed exophytic and endophytic pattern of growth, deceptively banal cytology, specific stromal features, and a colorful history. The three fundamental forms of verrucous carcinoma are those arising on palmo-plantar surfaces, the giant condyloma of Buschke and Lowenstein, and verrucous carcinoma of the upper aerodigestive tract. The latter lesion has a storied history in that the presence of such a tumor in the upper aerodigestive tract of Kaiser Wilhelm, repeatedly misdiagnosed by the great German pathologist Virchow, is said to have had a profound impact on the European pathology community in the mid-1800s. Recently, the work of Peter van Nostrand and others from the University of Toronto has helped to reawaken interest in this entity. Verrucous carcinoma of the oral cavity, also known as panoral verrucous carcinoma or florid oral papillomatosis, was first described in 1948 and is a rare neoplasm representing less than 10% of oral SCC. It is associated with the use of chewing tobacco or betel nut, poor oral hygiene, or poorly fitted dentures. Some are linked to radiotherapy and chronic inflammation. Verrucous carcinoma of plantar

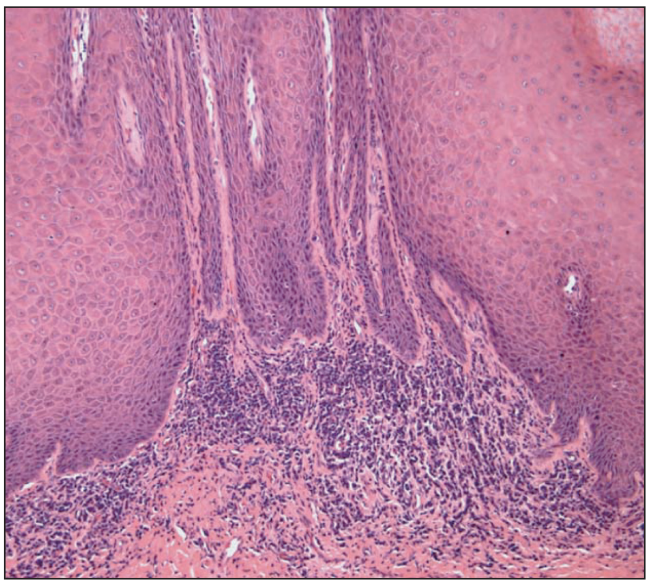


FIGURE 13.8: ***Verrucous carcinoma. There is pronounced elongation of the rete ridge pattern. The cytoplasms of the keratinocytes appear eosinophilic and glassy.***

surfaces, also known as epithelioma cuniculatum, plantar verrucous carcinoma, carcinoma cuniculatum, or papillomatosis cutis carcinoides, reflects the numerous openings of these tumors to the cutaneous surface in a fashion that mimics a rabbit burrow or cuniculatum. All of these neoplasms are frequently locally recurrent and aggressive, but they have a very low rate of metastasis. Like giant condyloma, proliferating cell nuclear antigen studies show that these lesions have low proliferation rates, and for this reason most are considered low-grade, highly differentiated SCC. The Buschke Lowenstein tumor was first described in 1925 in anogenital mucosa; in some series, most express HPV type 6 or 11 antigens. In the larynx, HPV types 16 and 18 are more common. This is a more common malignancy of the penis and represents about 10–20% of all penile cancers.

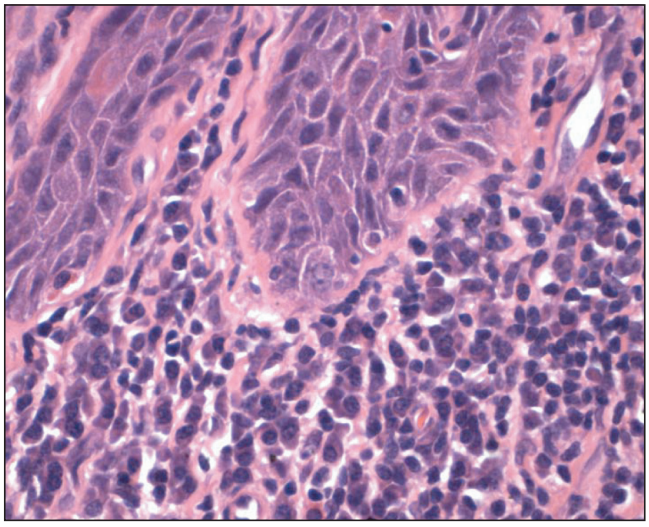


FIGURE 13.9: ***Verrucous carcinoma. Plasma cells surround the tips of bulbous rete ridges. There is no stromal desmoplasia; its presence would signal invasion, and thus a diagnosis of well-differentiated SCC of nonverrucous type.***

Clinical Features

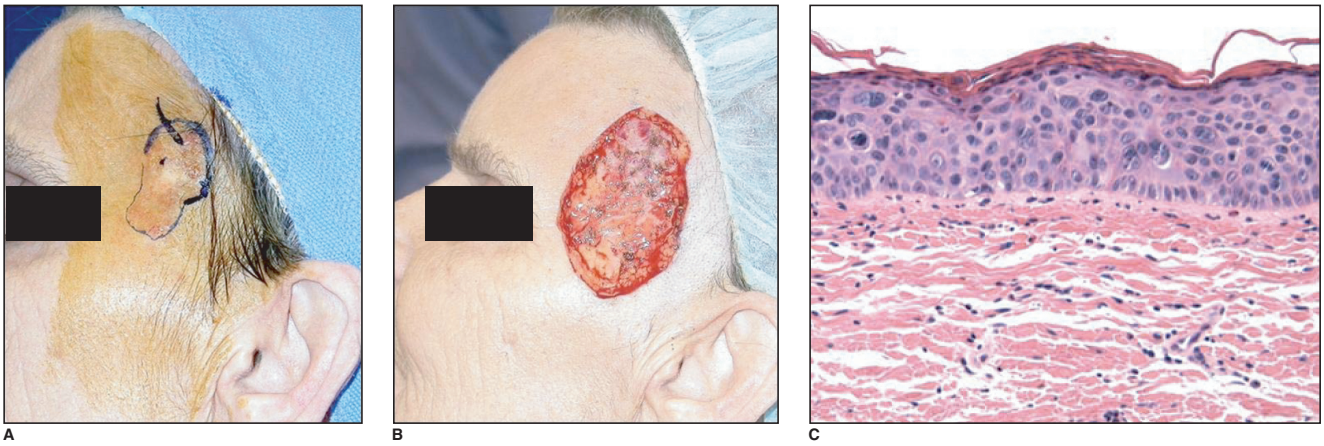
Verrucous carcinoma of the aerodigestive tract presents as a slowly growing, gray-white growth in the oral cavity or larynx of elderly males and is associated with slowly progressive invasion of adjacent structures, including cartilage and bone. Verrucous carcinoma of plantar surfaces presents as a slowly growing, exophytic, polypoid tumor on the heel or ball of the foot, the toes, and the web spaces, and occasionally on other sites. These lesions are pink or flesh in color, with well-circumscribed borders, and are often tender. Penetration into bony structures has been described. Sinuses within these lesions contain keratin and may have a foul aroma. The giant condyloma of Buschke and Lowenstein shows a polypoid excrescence on the glans penis or anogenital tract in patients between 18 and 87 years of age, with occasional ulceration and/or fistula formation.

Histopathology

Verrucous carcinoma at any site has a similar histologic morphology, consisting of a mixed exo- and endophytic squamous epithelial proliferation composed of cells with abundant quantities of glassy or eosinophilic cytoplasm and low-grade nuclear atypia (Figure 13.8). These cells show bulbous rete ridges extending into the deep reticular dermis in the plantar surfaces or deep into the submucosa in the aerodigestive tract. Typically, these broad, bulbous rete ridges are surrounded by a mixed inflammatory infiltrate dominated by mononuclear cells and are rich in plasma cells (Figure 13.9). The neoplastic epithelial lobules have a pushing, as opposed to a spiky or obviously invasive, contour and there is little in the way of stromal fibrosis or desmoplasia. Mitoses are scattered throughout the lesion

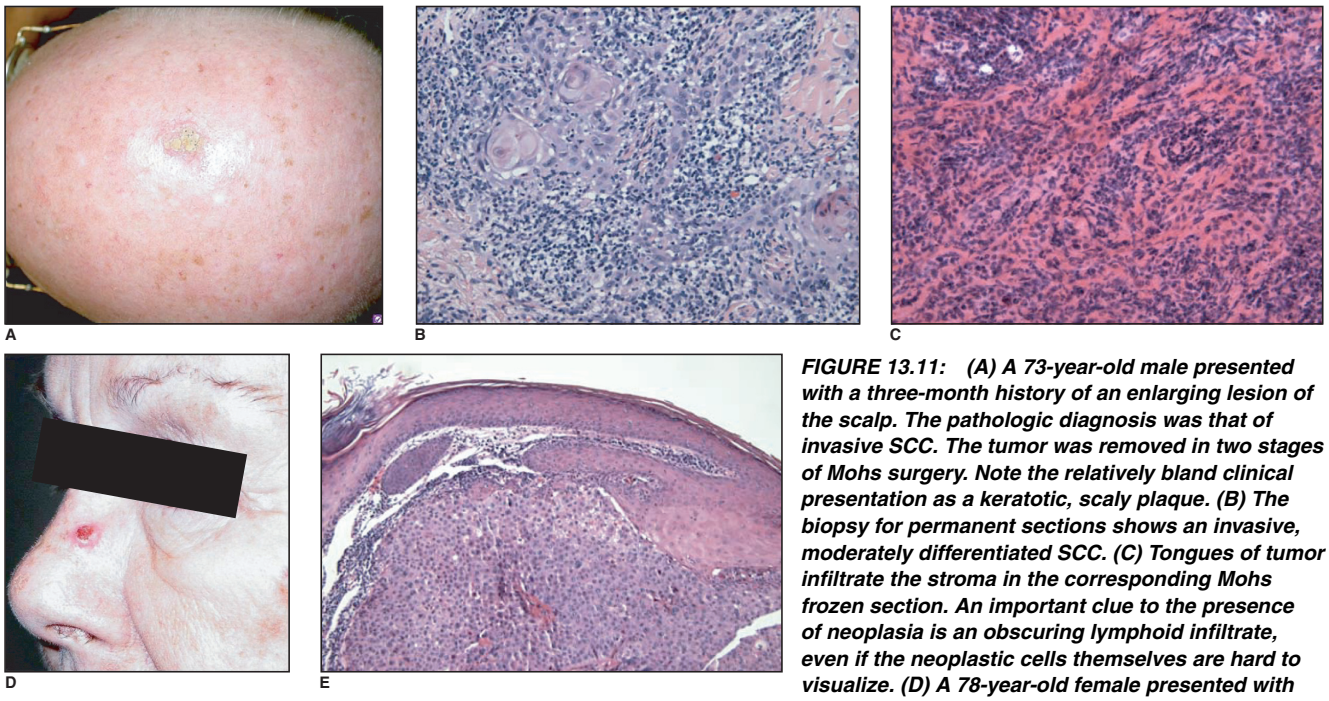
TABLE 13.1: Characteristics of SCC of the Skin Associated with an Increased Risk of Metastatic Spread

Size greater than 2 cm (Figure 13.10)
Tumor invasion to a depth greater than 4 mm
Poorly differentiated tumors (Figure 13.11)
Rapid clinical growth (Figure 13.12)
Recurrence
Perineural involvement (Figure 13.12)
Prior radiation at the site
SCC located on the lips or ears (Figure 13.13)
Immunocompromised or immunosuppressed patients (Figure 13.10)
Aggressive histology (Figure 13.11)



A B C D

FIGURE 13.10: (A) A 73-year-old woman, 15 years after liver transplant and on immunosuppressive therapy, developed a plaque of Bowen's disease, proven in permanent sections. (B) The tumor, measuring 6.5 × 4.0 cm, was removed with three stages of Mohs surgery. Note how much smaller the tumor appeared clinically. (C) The biopsy for permanent sections showed SCC in situ, with full-thickness, severe keratinocytic dysplasia. (D) The corresponding frozen section at time of Mohs surgery demonstrates identical features to those shown in (C). Notice how nuclear detail may be obscured when frozen sections are cut thick.



A B C D E

FIGURE 13.11: (A) A 73-year-old male presented with a three-month history of an enlarging lesion of the scalp. The pathologic diagnosis was that of invasive SCC. The tumor was removed in two stages of Mohs surgery. Note the relatively bland clinical presentation as a keratotic, scaly plaque. (B) The biopsy for permanent sections shows an invasive, moderately differentiated SCC. (C) Tongues of tumor infiltrate the stroma in the corresponding Mohs frozen section. An important clue to the presence of neoplasia is an obscuring lymphoid infiltrate, even if the neoplastic cells themselves are hard to visualize. (D) A 78-year-old female presented with a three-month history of an enlarging nodule on her left nasal sidewall. The histology revealed a poorly differentiated invasive SCC with clear cell differentiation, which was subsequently removed by one stage of Mohs surgery. (E) Permanent sections show a clear cell neoplasm with squamous differentiation.

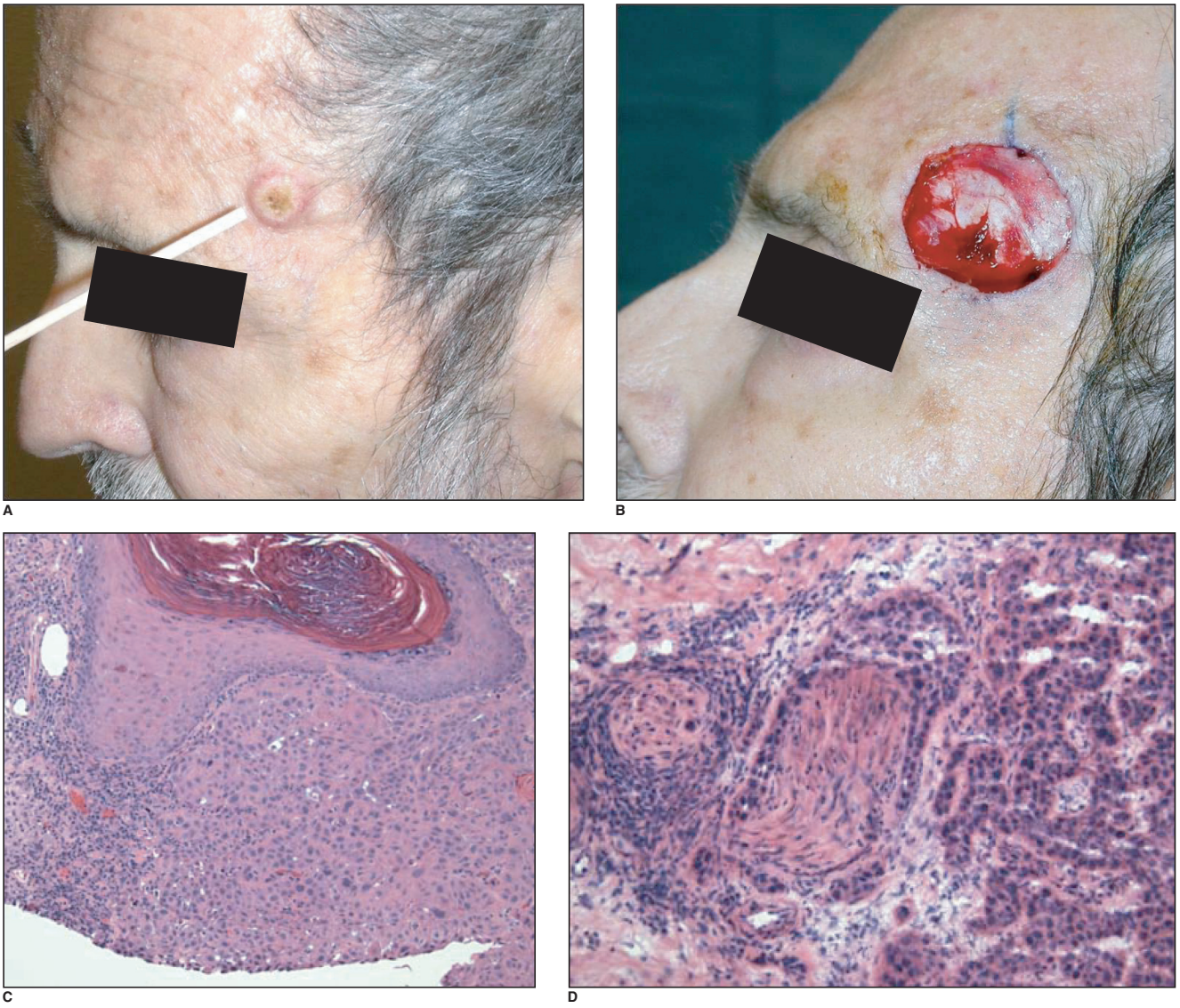


FIGURE 13.12: ***(A) A 62-year-old male presented with a rapidly growing lesion of three weeks' duration on the left temple. (B) Perineural invasion was noted on the first Mohs stage (Figure 13.12D). The tumor was removed by two stages of Mohs surgery. (C) Permanent sections of the skin biopsy showed a moderately differentiated SCC, characterized by infiltrating sheets of tumor cells; by Broders classification scheme, more than 25%, but less than 75%, of the tumor cells must show intercellular bridges or cytoplasmic keratinization to be classified as moderately differentiated SCC. (D) Perineural infiltration was noted in frozen section material at time of Mohs surgery.***

but are not frequent. The tumors are highly differentiated, and therefore produce abundant keratin, which extrudes to the surface and sometimes into the stroma, where it may lead to a foreign-body reaction. These pushing lobules may show epithelial lined crypts in the deep reticular dermis, and are filled with keratin and parakeratotic debris. Infiltration of adjacent structures including bone, muscle, and cartilage may be seen. The giant condyloma of Buschke and Lowenstein is more exophytic than the verrucous carcinoma and has an architecture resembling a condyloma, sometimes accompanied by classic koilocytic alteration. The granular cell layer may be prominent, and these changes may closely mimic a condyloma.

As any of the aforementioned tumors may show transformation to an overtly malignant neoplasm with metastatic capability, multiple tissue samples should be submitted and multiple steps through the blocks may be indicated to exclude this possibility.

Differential Diagnosis

All three of these entities have overlapping morphologies. Their differential diagnoses include: pseudoepitheliomatous hyperplasia, distinguished by the presence of terminal differentiation (keratohyalin granular formation) in concert with a lower density of plasma cell infiltration;

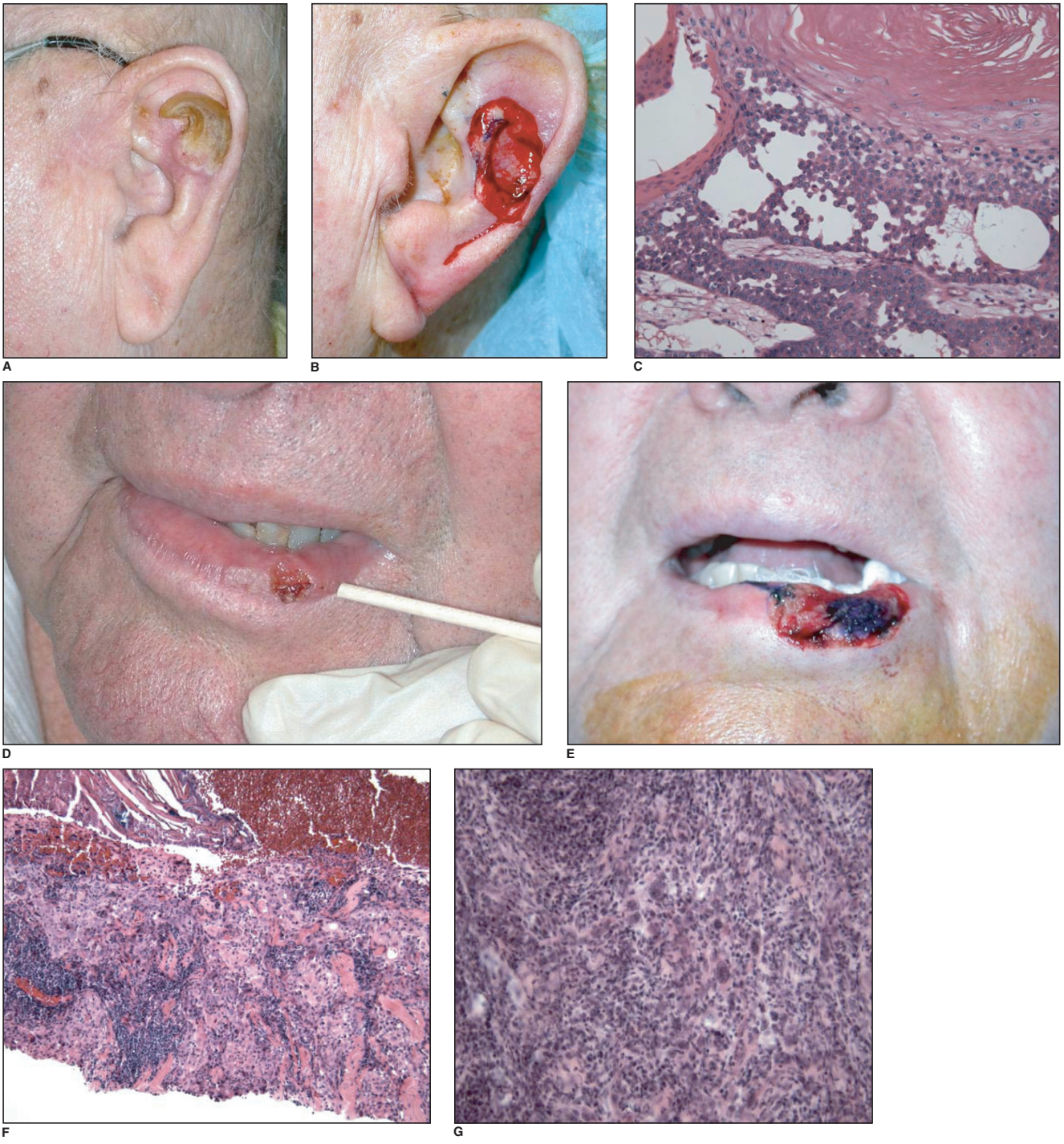


FIGURE 13.13: (A) An 85-year-old male presented with a cutaneous horn, present on the left ear for two years. (B) This moderately differentiated SCC was removed in two stages of Mohs surgery. (C) Surmounted by a prominent column of keratinization, the epidermis showed full thickness malignant transformation with dermal invasion. (D) An 83-year-old male with an ulcerated lesion on the lower lip, present for about two months. (E) The lesion, a moderately differentiated SCC (Figure 13.13F), was removed in two stages of Mohs surgery. (F) Permanent sections of the original shave biopsy showed a moderately differentiated SCC of the lower lip. (G) The corresponding Mohs frozen section.

overt classical SCC, which shows fully transformed malignant features and a more invasive pattern of growth; and verrucae. In addition, SCCs often show substantial numbers of apoptotic bodies, greater mitotic activity including atypical mitoses, more pronounced cytologic atypia, and squamous eddy formation, all of which are absent in prototypic verrucous carcinoma. Changes of viral cytopathic effect may be seen in the giant condyloma of Buschke and Lowenstein. Verrucae show characteristic stromal vascular alterations, mounds of parakeratin as surmounting tiers over the crests of papillomatous elevations, prominent bizarre keratohyalin granules, and lateral in-drawing of rete ridges at the peripheral margins of the lesion.

THE TREATMENT OF SCC BY MOHS SURGERY

While the techniques used in the Mohs laboratory to process tissue containing SCC are identical to those used for BCC, most Mohs surgeons agree that the interpretation of Mohs frozen sections is more difficult when dealing with SCC than when dealing with BCC. There are higher metastatic and recurrence rates with SCC than with BCC, underscoring the need for diagnostic accuracy and attention to detail when dealing with SCC. Even SCC in situ, which has a low potential for metastatic spread, can be highly destructive when it occurs in areas in close proximity to vital or cosmetically important structures.

When evaluating slides, the Mohs surgeon must be aware of several common areas of confusion encountered with SCC. Some of these pitfalls include irritated seborrheic keratosis, pseudoepitheliomatous hyperplasia, squamous metaplasia of eccrine ducts, and tangential cuts. While the possibility of metastatic spread resides in all SCCs, there are several characteristics that, when present, markedly increase the probability for spread (Table 13.1).

The treatment of SCC by Mohs surgery can be extremely challenging but is of enormous benefit to the patients. The Mohs surgeon must always keep in mind the seriousness of this diagnosis, including its potential for morbidity and mortality if not adequately and aggressively treated.

Pearls

1. If the frozen section evaluation is indeterminate, excise an additional layer.
2. If the tumor is particularly aggressive and the location allows, take an additional 3–4 mm of tissue.
3. If unsure of the histologic evaluation, consider an intraoperative dermatopathology consultation.
4. Keratoacanthoma is a subtype of SCC and may need to be treated as an SCC.
5. If dense inflammation is seen on the slide, precluding an accurate evaluation, consider sending a layer for permanent sections with immunohistochemistry and delaying the closure until final tumor clearance can be established, or take a deeper layer.

REFERENCE

1. Boyd AS. Tumors of the epidermis. In: Barnhill R, Crowson AN, eds. *Textbook of Dermatopathology*. 2nd ed. New York: McGraw-Hill; 2004:575–633.

CHAPTER 14

Unusual Tumors: Vertical and Horizontal

Terence O'Grady

INTRODUCTION

When Dr. Frederick Mohs developed his technique to improve the examination of surgical margins, he initially applied it for treatment of cutaneous neoplasms. Although basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) made up the bulk of these tumors, melanomas were soon added to the list, as were other neoplasms and even infectious processes.

The technique has since been utilized to ensure complete examination of surgical margins of an ever-expanding list of proliferations. Some examples include: BCC, SCC, melanoma, melanoma in situ (lentigo maligna), merkel cell carcinoma, verrucous carcinoma, sebaceous carcinoma, Bowen's disease (squamous cell carcinoma in situ), microcystic adnexal carcinoma, dermatofibrosarcoma protuberans, extramammary Paget's disease, malignant fibrous histiocytoma, leiomyosarcoma, angiosarcoma, and benign adnexal tumors.

The challenges of utilizing the Mohs techniques for tumors other than those commonly treated surgically, such as basal or SCCs, include the larger size of some of these tumors and the bland cytology that can resemble scar tissue or normal structures, as well as the need to use additional techniques or laboratory studies (e.g., immunohistochemistry stains).

This section does not represent an exhaustive list of applications of the Mohs technique to ensure complete marginal clearance of every tumor for which it has been utilized, but rather examines representative lesions and the techniques that can be used.

MERKEL CELL CARCINOMA

Merkel cell carcinoma, also called a "small blue cell tumor," is an aggressive primary neoplasm of the skin. It has a very high incidence of metastasis and a mortality rate higher than that of melanoma. It occurs primarily on the sun-exposed head and neck regions and less frequently on the trunk and extremities. Like other nonmelanoma skin cancers, it occurs more frequently in patients who are chronically immunosuppressed.

The clinical appearance is nonspecific, usually a rapidly growing cutaneous nodule. The differential diagnosis includes other nonmelanoma skin cancers, melanoma, lymphoproliferative disorders, and most importantly, metastatic small cell carcinomas, including oat cell carcinoma of the lung and other noncutaneous neuroendocrine tumors. Merkel cell carcinoma is a rare tumor, with fewer than 2,000 cases reported in the literature.

The histology of the tumor is that of sheets and cords of small basophilic cells with a very high nuclear to cytoplasmic ratio (Figure 14.1). The tumor is located in the dermis, but may also show subcutaneous extension as well as intraepithelial, pagetoid spread (Figure 14.2). Immunohistochemistry staining can accurately identify the tumor and separate it from other small blue cell tumors. These include cytokeratin 20, cytokeratin 7, and thyroid transcription factor 1 (TTF-1). Cytokeratin 20 staining shows

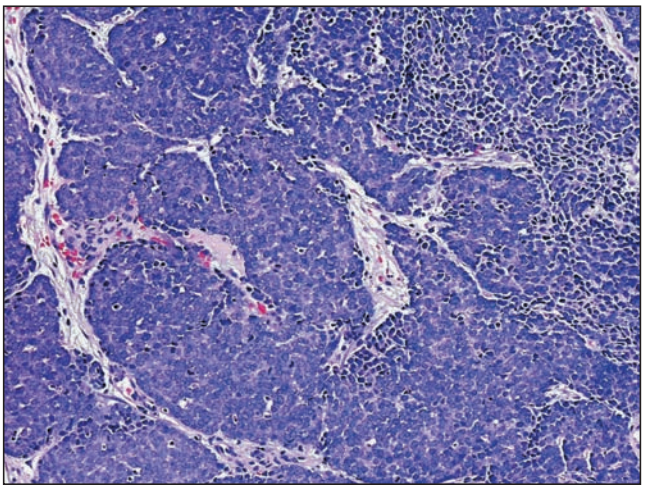


FIGURE 14.1: Merkel cell carcinoma.

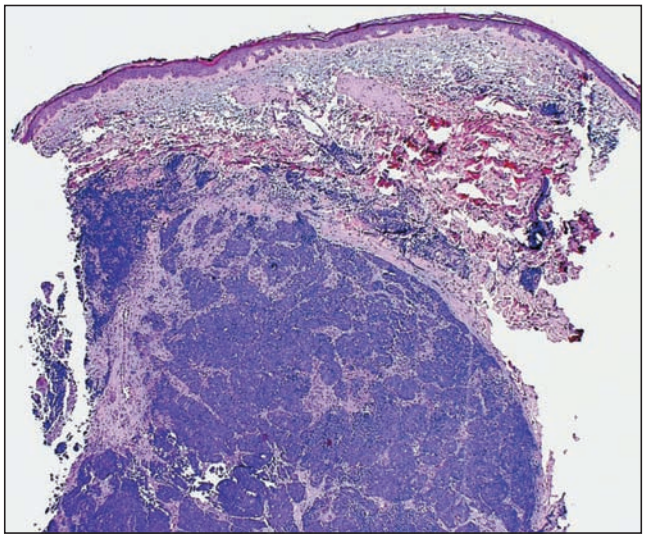


FIGURE 14.2: Merkel cell carcinoma with subcutaneous extension.

a characteristic perinuclear (Golgi) staining pattern (Figure 14.3).

The treatment consists primarily of surgery and adjunct therapies, especially radiation. The surgical options include:

- Excisional surgery with 1–2 cm margins, carried to fascia
- Mohs technique with frozen sections
- Modified Mohs technique: debulking of major tumor mass using frozen sections, then final margin evaluation using paraffin-embedded tissue

Sentinel lymph node biopsy has been used to more accurately assess regional nodal involvement, but the low number of cases makes it difficult to evaluate its effectiveness in prolonging survival.

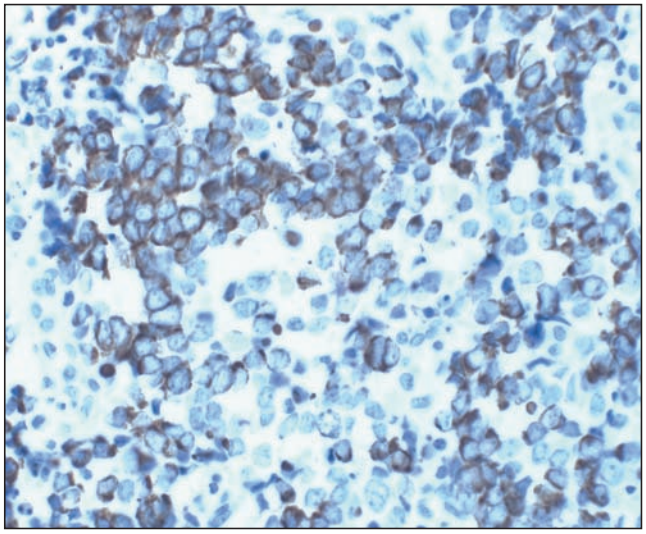


FIGURE 14.3: Merkel cell carcinoma with CK 20 immunostaining.

Adjunctive radiation therapy has been used to decrease local recurrence, as well as to improve overall survival, although reports have been conflicting. The focus of radiation therapy depends on whether lymph node involvement is ascertained. If no lymph node involvement is found (or no sentinel node exam performed), only the primary site is irradiated. If lymph node involvement is shown, irradiation is performed to the primary site and affected nodal basins.

Chemotherapy has been used with and without radiation therapy and surgery.

SEBACEOUS CARCINOMA

Sebaceous carcinoma is a malignant tumor composed of cells of sebaceous gland differentiation. Over three-quarters of the cases are found in ocular tissues, including meibomian or Zeiss glands of the eyelids, and less commonly in extraocular locations. The lesions are frequently misdiagnosed as chalazions and/or conjunctivitis. Both ocular and extraocular forms can be associated with Muir-Torre syndrome, and thus clinical correlation is important. Histologically, the tumor can range from well-differentiated lobules of basaloid cells admixed with sebocytes to poorly differentiated squamous carcinomas with few identifiable sebocytes (Figure 14.4). The ocular tumors frequently demonstrate pagetoid spread of tumor cells in the overlying epidermis (Figure 14.5). The histologic differential is primarily BCC. Histochemical (Oil Red O) and immunohistochemistry (epithelial membrane antigen [EMA]) can be used to differentiate sebaceous carcinoma from other basaloid tumors. Oil Red O is used on fresh tissue such as the frozen sections used in Mohs surgery. Epithelial membrane antigen demonstrates a microvascular pattern in paraffin-embedded tissues (Figure 14.6) and may be used in conventional surgery or modified Mohs ("slow-Mohs") sections. Mohs

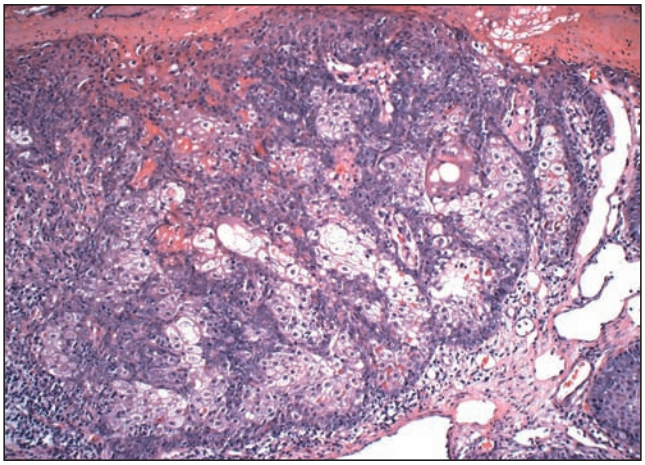


FIGURE 14.4: Sebaceous carcinoma.

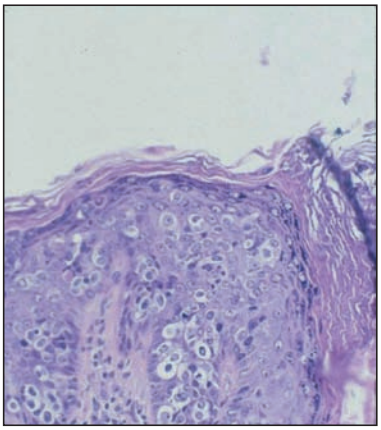


FIGURE 14.5: Sebaceous carcinoma with pagetoid extension into the epidermis.

micrographic surgery allows evaluation of the complete surgical margins of the tumor and decreases the recurrence rate of these tumors in both ocular as well as extraocular sites.

MICROCYSTIC ADNEXAL CARCINOMA

Microcystic adnexal carcinoma (or sclerosing sweat duct carcinoma) is an uncommon neoplasm that can demonstrate extensive involvement of superficial and deep dermal tissues. Although it has low malignant potential, its local invasiveness leads to persistence and need for frequent re-excisions if incompletely removed. Mohs surgery thus offers a decreased rate of recurrence for this tumor.

The tumor is frequently located on the face and can be clinically subtle, with the actual tumor more extensive than the clinical lesion ("tip of the iceberg"). It is frequently misdiagnosed clinically. Histologically, the tumor displays features that differ from the surface to the deeper tissues (Figure 14.7). In the superficial dermis, there are small cystic structures that can resemble syringomas (Figure 14.8), as well as structures resembling eccrine ducts that mimic the basaloid cords seen in desmoplastic trichoepitheliomas. As the tumor extends into the deeper tissues, it is formed of single cells and small strands of basaloid cells that resemble morpheaform or infiltrative BCCs (Figure 14.9). Perineural invasion is frequently seen. The tumor cells stain with cytokeratins and epithelial membrane antigen.

Because of the clinical banality of the lesion and extensive dermal involvement with histologically indistinct tumor cells, Mohs micrographic surgery offers an excellent way to ensure complete surgical clearance.

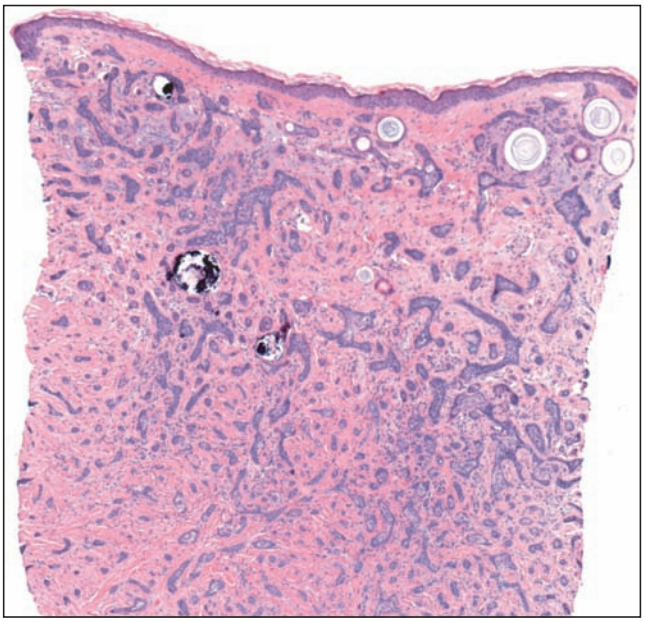


FIGURE 14.7: Microcystic adnexal carcinoma.

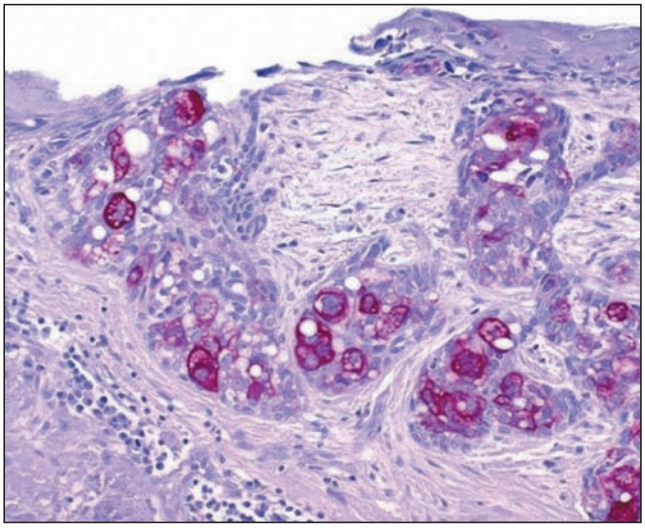


FIGURE 14.6: Sebaceous carcinoma with epithelial membrane antigen (EMA) immunostaining.

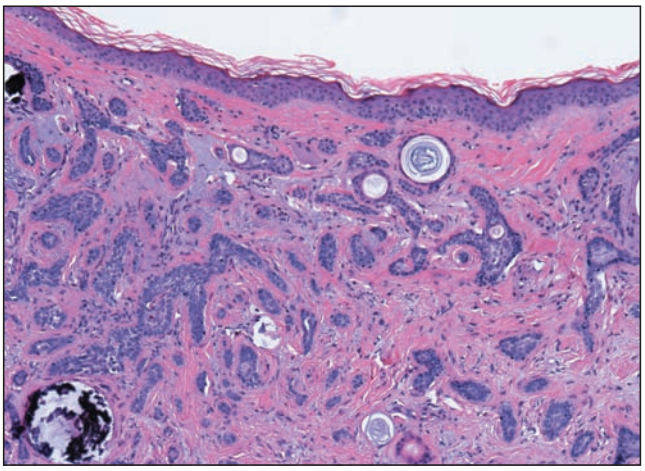


FIGURE 14.8: Microcystic adnexal carcinoma.

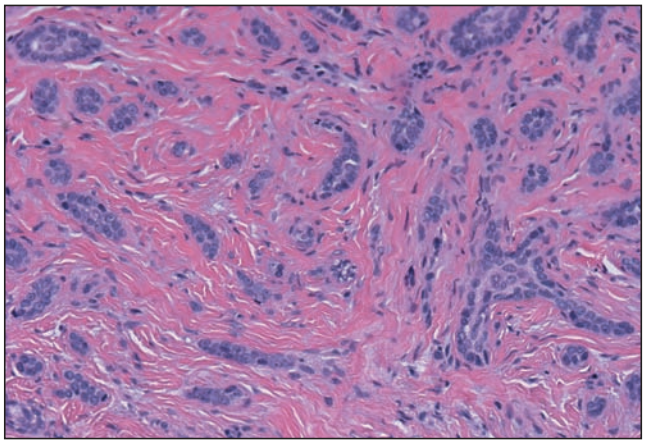


FIGURE 14.9: Microcystic adnexal carcinoma resembling basal cell carcinoma (BCC).

EXTRAMAMMARY PAGET'S DISEASE

Extramammary Paget's disease is a rare neoplasm of apocrine gland bearing regions of skin, particularly the groin, genital, and perianal areas. Clinically, the lesions can resemble dermatitis, leading to misdiagnosis and mistreatment (Figure 14.10). The disease can also be associated with internal malignancies, which must be searched for before assuming the disease is primarily cutaneous; associated malignancies include bladder, urethral, prostate, uterine, ovarian, and colorectal carcinomas.

Histologically, the tumor shows intraepidermal (pagetoid) spread of large epithelioid cells (Figure 14.11). The cells can be stained with histologic stains such as periodic acid–Schiff (PAS) (Figure 14.12), alcian blue, or mucicarmine. Immunohistochemistry stains, including

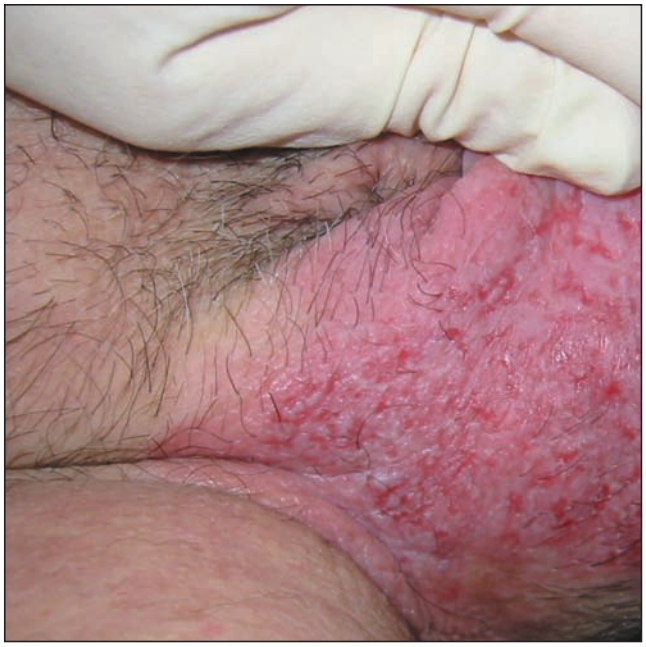


FIGURE 14.10: Extramammary Paget's disease resembling dermatitis.

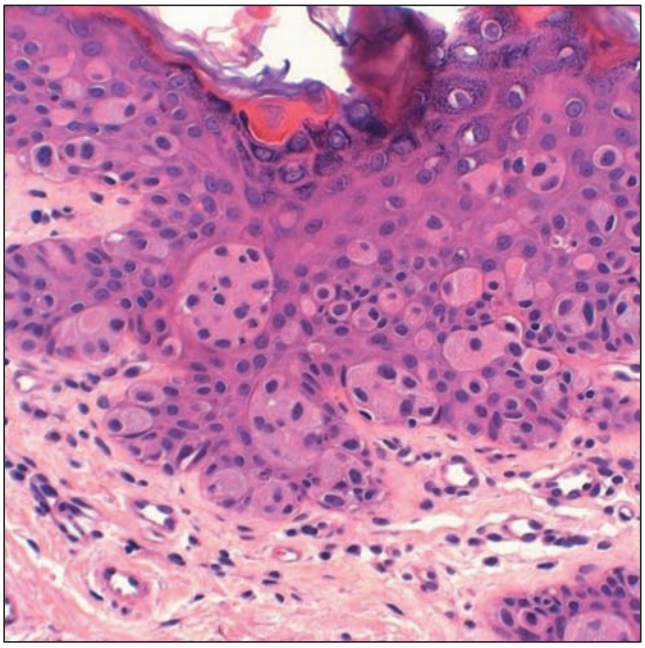


FIGURE 14.11: Extramammary Paget's disease with pagetoid extension into the epidermis.

cytokeratin 7, low-molecular-weight cytokeratins, and carcinoembryonic antigen (CEA), are also useful. These lesions can progress to become invasive carcinomas, and thus should be completely excised.

Treatment options include conventional surgical excision, Mohs micrographic surgery, or modified Mohs surgery, as well as topical agents, including fluorouracil and/or imiquimod. Because these tumors may have indistinct clinical extensions, some surgeons pretreat with these topical agents to "map" the tumor before surgical excision. Standard surgical techniques include wide local excision as

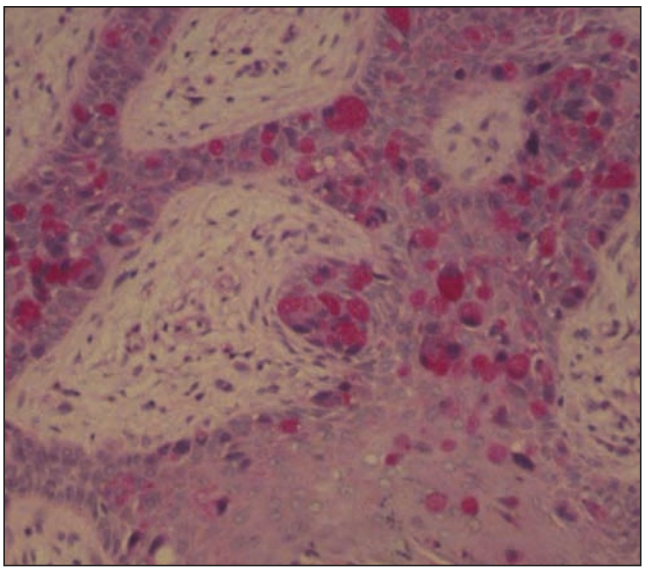


FIGURE 14.12: Extramammary Paget's disease in epidermis with periodic acid–Schiff (PAS) stain.

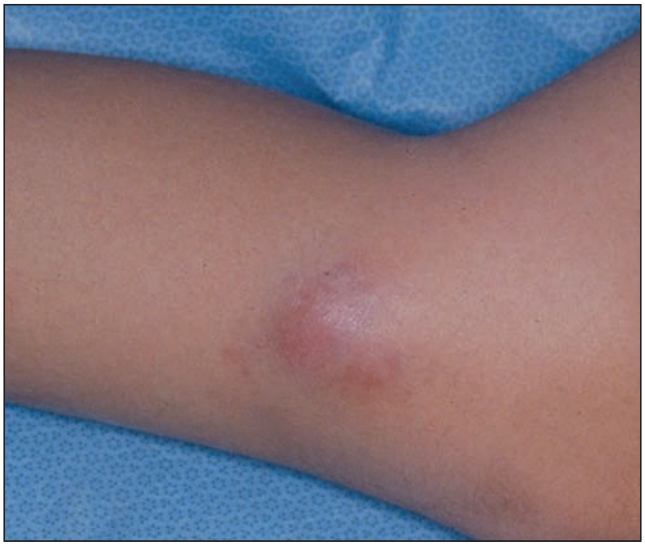


FIGURE 14.13: Dermatofibrosarcoma (DFSP) protuberans; clinical presentation.

well as vulvectomy and/or abdomen perineal resection, with high attendant morbidity and, unfortunately, a high recurrence rate. Mohs micrographic surgery allows a higher tumor clearance rate, and thus a much lower recurrence rate of these lesions. Because of the frequently large size of these lesions, some surgeons excise the central portion of the tumor using permanent-section pathology, and then evaluate the peripheral margins using the Mohs technique.

SOFT-TISSUE NEOPLASMS

In addition to the carcinomas arising in the epidermis, the Mohs technique has been utilized to examine surgical margins of many soft-tissue neoplasms. There are special challenges in approaching these tumors, including large size, indistinct borders, and bland cytology, all of which can make the histological identification of margins difficult.

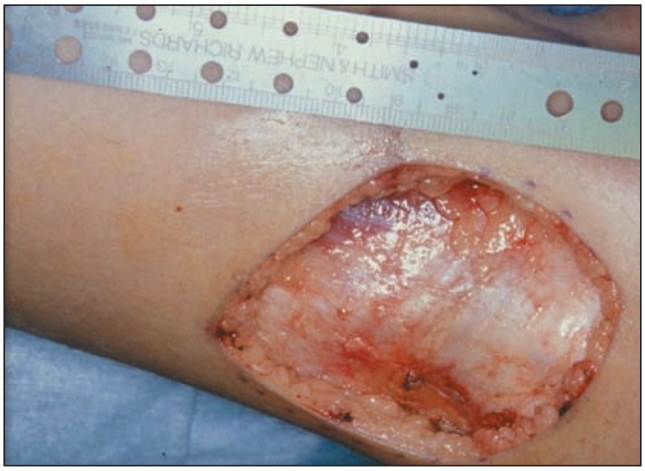


FIGURE 14.14: White streaks representing DFSP in multiple lobules away from the central area of clinical presentation.

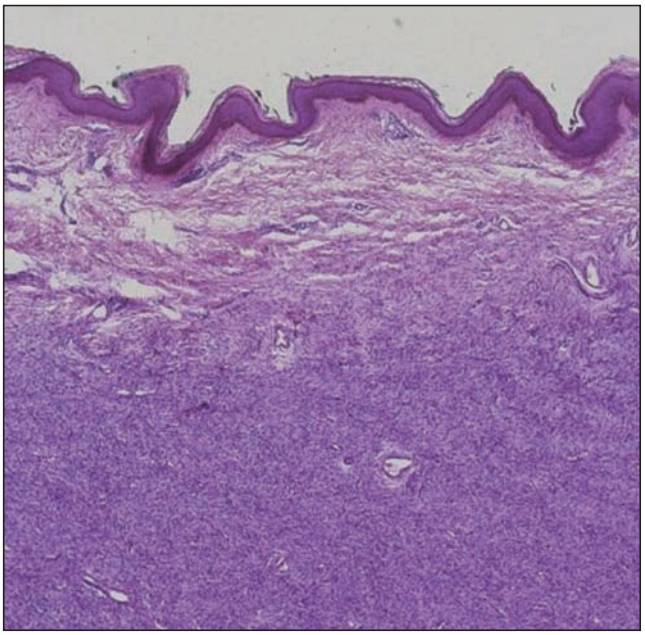


FIGURE 14.15: DFSP; nodular, subepidermal portion.

These are tumors that may require modified techniques, such as a combination of frozen sections and paraffin-embedded sections, use of immunohistochemistry staining, and a multispecialty approach for surgical repair; adjunct treatment, such as radiation and/or chemotherapy, may be required.

DERMATOFIBROSARCOMA PROTUBERANS

Dermatofibrosarcoma protuberans (DFSP) is a rare soft-tissue tumor of low-grade malignant potential. Although they metastasize rarely, these tumors recur frequently after standard excision, even with clinical margins up to 5 cm. Mohs micrographic surgery is therefore considered to be an important treatment option for these tumors.

The clinical findings can be deceiving, as the nodules or plaques on the cutaneous surface first noted by the clinician or patient may represent only the "tip of the iceberg" (Figure 14.13), with tumor extending widely and deeply into subcutaneous tissues (Figure 14.14). The lesions generally present few symptoms and grow slowly. The trunk and upper extremities are the most frequent sites of involvement.

The histology of the tumor is characteristically that of a dense spindle cell tumor with monomorphous cytology (Figure 14.15). The spindle cells are arranged in radiating fascicles resembling spokes on a wheel ("storiform" pattern). The cells also extend into and replace the fat, leaving residual adipocytes in a "honeycomb" pattern (Figure 14.16).

The CD34 immunohistochemistry stain will strongly stain the tumor; this becomes important in clearing the margins after debulking (Figure 14.17). A caveat is that

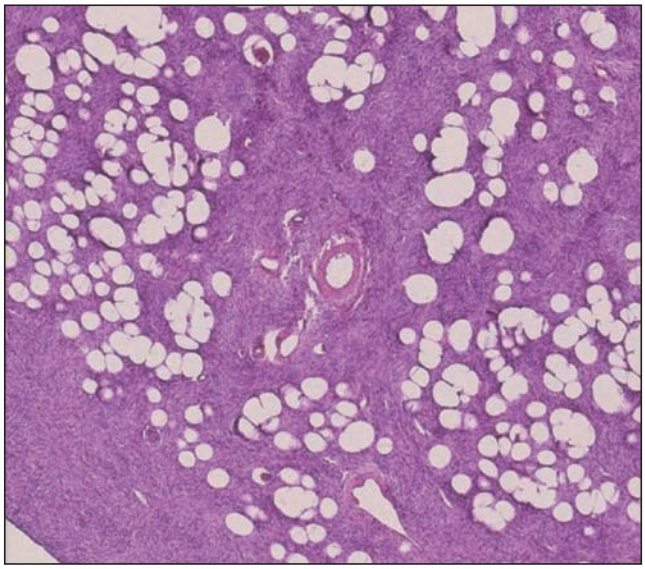


FIGURE 14.16: DFSP; deep, infiltrative pattern.

blood vessels and some dermal dendrocytic cells can also stain with CD34 (Figure 14.18).

Due to the large size of the lesion and the use of immunohistochemistry staining of residual tumor, a useful approach is to debulk the central portion of the tumor using standard Mohs with frozen sections. The dense cellularity of the sarcoma makes it readily visible on these sections. The remaining deep and peripheral margins can then be examined using paraffin-embedded sections and staining with CD34. If there is uncertainty of the cells staining with CD34 (i.e., vessels or dermal dendritic cells) a small portion of noninvolved skin can be stained as a "background" control.

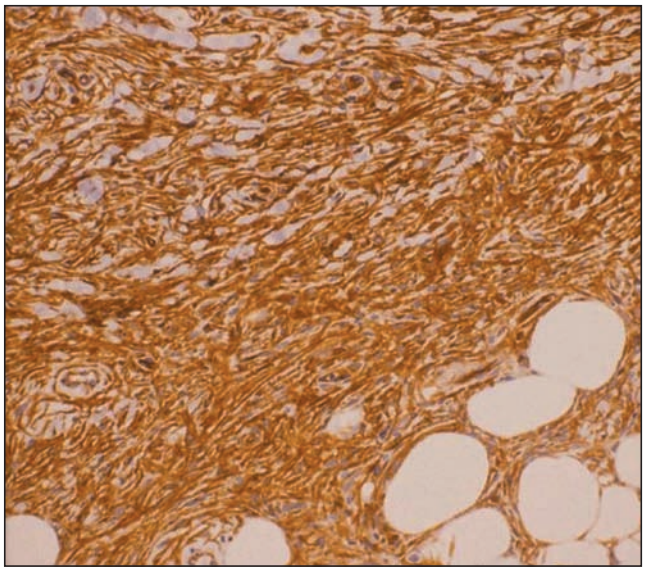


FIGURE 14.17: DFSP with CD34 immunostaining.

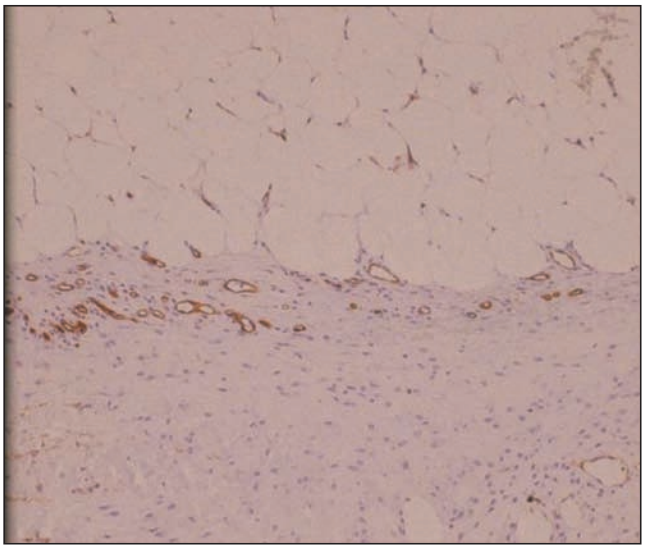


FIGURE 14.18: CD34 immunostain also highlights vascular structures.

GIANT CELL FIBROBLASTOMA

Giant cell fibroblastoma (GCF) is a rare soft-tissue tumor sharing clinical and histologic features with DFSP. The lesions, however, occur almost exclusively in children. As with DFSP, the clinical appearance of the lesions is nodules or plaques, which can represent a small portion of a larger, more deep-seated tumor (Figures 14.19 and 14.20). And as with DFSP, the tumor rarely metastasizes, but will frequently recur if incompletely excised. Also as with DFSP, Mohs micrographic surgery offers an excellent method to ensure complete excision. Because the tumors can be large, a modified approach utilizing frozen and paraffin-embedded sections may be useful.

Although the two tumors are similar, the histology of GCF differs from that of DFSP by being much less monomorphous cytologically. Two patterns are seen, a dense spindle cell proliferation (Figure 14.21), as well as a less dense proliferation in a myxoid stroma (Figure 14.22)

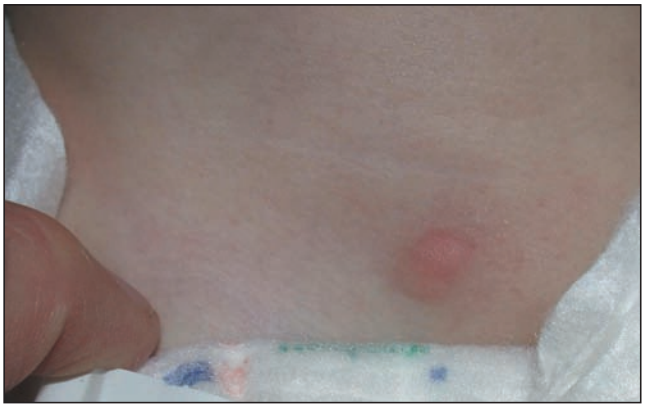


FIGURE 14.19: Giant cell fibroblastoma (GCF); clinical presentation.

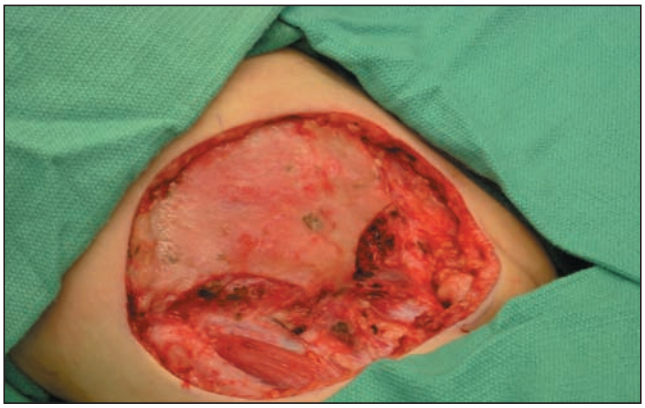


FIGURE 14.20: GCF; whitish tumor nodules more extensive than evident clinically.

with occasional floret-type giant cells (Figure 14.23) and other areas showing angiectoid or vessel-like spaces, lined with the tumor cells. The tumor cells are strongly CD34 positive (Figure 14.24), which can be used in evaluating the tissue margins (with the same caveats mentioned previously). Both DFSP and GCF share a common genetic translocation, t(17;22)(q22;q13), which can be analyzed by molecular techniques. The large size of the tumor and age group of the patient may require a multispecialty approach to the care of these patients.

ATYPICAL FIBROXANTHOMA

Atypical fibroxanthoma (AFX) is a locally aggressive soft-tissue neoplasm commonly found on the head and neck of elderly patients. The tumor has a high recurrence rate but a low rate of metastasis.

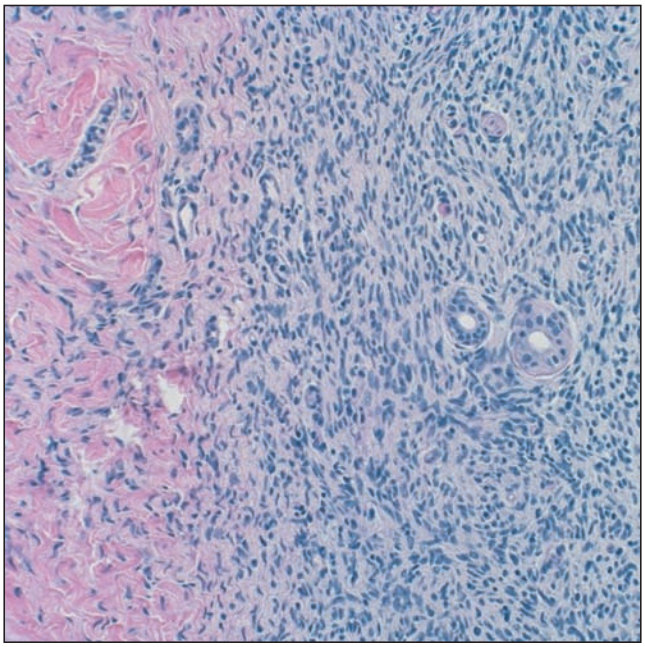


FIGURE 14.21: GCF; densely cellular area.

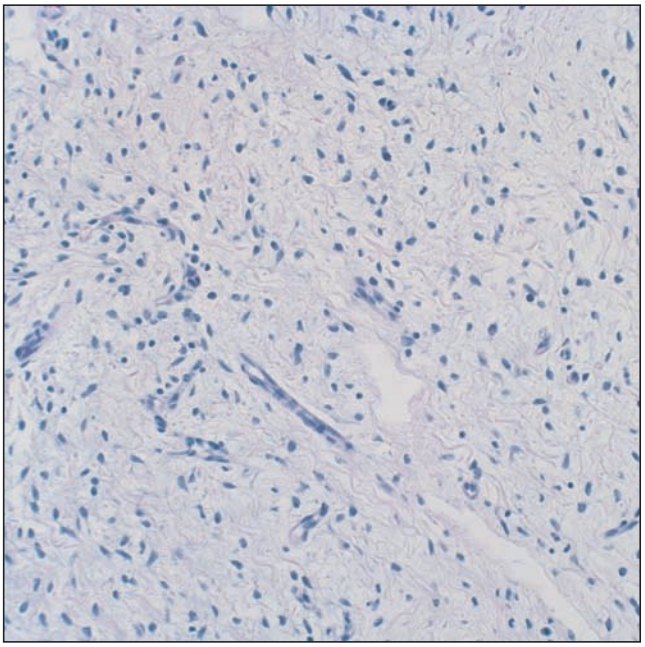


FIGURE 14.22: GCF; myxoid area.

The tumor is composed histologically of spindled and markedly atypical-appearing epithelioid cells (Figure 14.25). Mitotic figures are numerous and frequently atypical in appearance (Figure 14.26). The pleomorphism and mitotic rate of the superficial lesion can overlap with other spindle cell neoplasms such as spindle cell SCC and spindle cell melanoma. Immunohistochemistry staining is usually required to confirm the diagnosis. The histology of AFX is identical to that of malignant fibrous histiocytoma

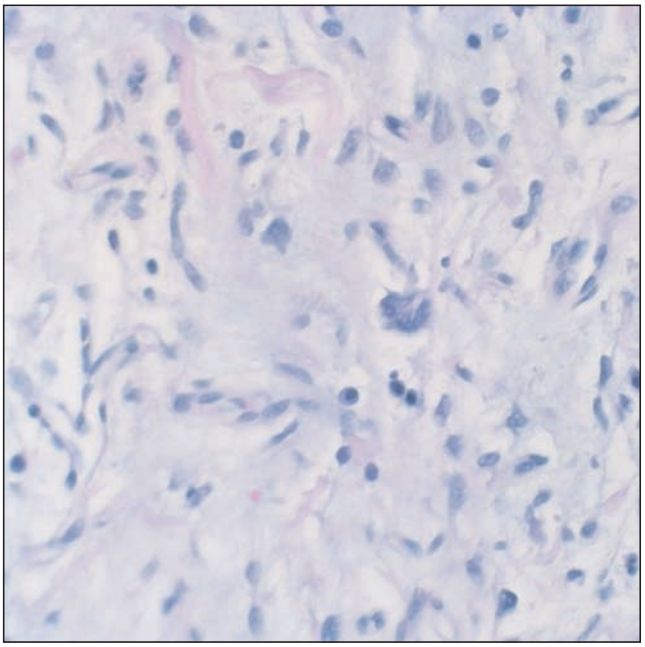


FIGURE 14.23: GCF; myxoid area with floret-type giant cells.

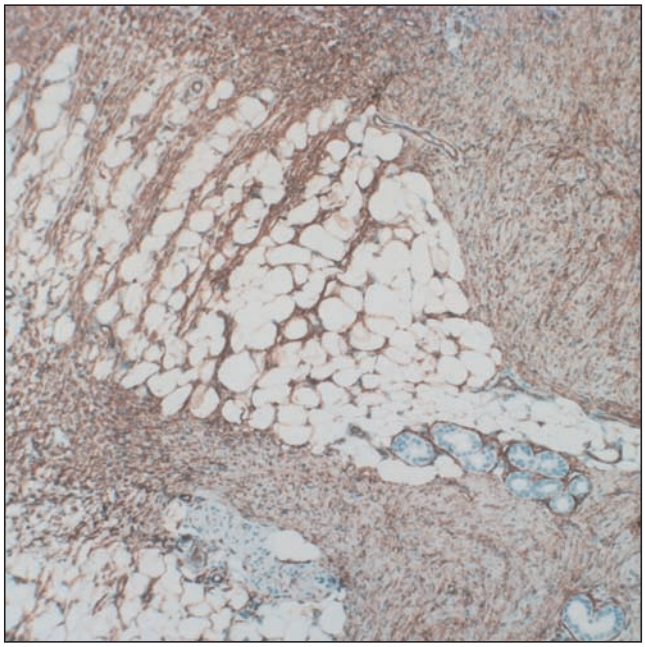


FIGURE 14.24: *GCF; CD34 immunostain is positive.*

(MFH), a deep-seated sarcoma considered a high-grade tumor. Although some consider AFX to be a superficial variant of MFH, the two tumors vary significantly in biologic behavior and are treated very differently. The superficially located AFX is well suited for use of Mohs micrographic surgery, while MFH may require a much more involved process.

ANGIOSARCOMA

Angiosarcoma represents a malignant proliferation of blood vessels that can occur in several clinical settings. A common presentation is large plaquelike lesions occurring

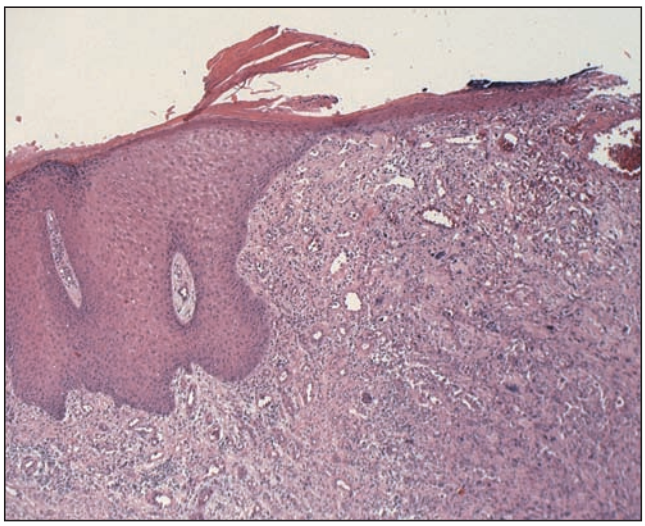


FIGURE 14.25: *Atypical fibroxanthoma (AFX); tumor in ulcerated area.*

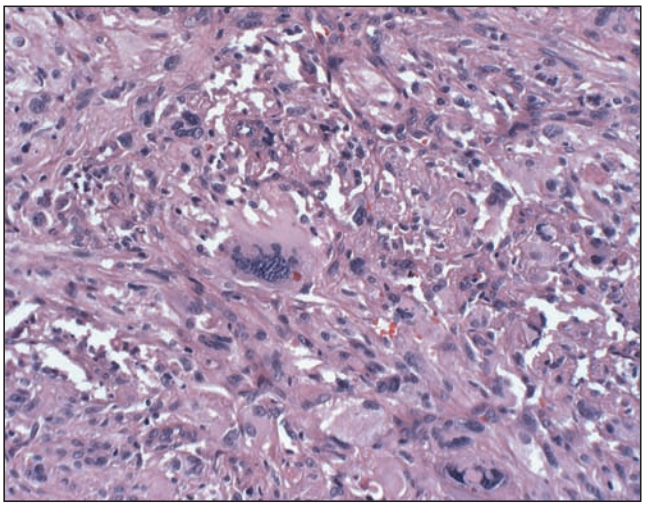


FIGURE 14.26: *AFX; bizarre cytologic characteristics.*

on the scalp of elderly patients. The clinical appearance can resemble bruises, although papular or nodular lesions can also occur. Scalp lesions are frequently noted by hairdressers or barbers. The other presentation occurs in the setting of chronic lymphedema, such as seen in postmastectomy patients (Stewart-Treves Syndrome).

Histologically, the tumor occurs in the dermis, but can extend into the subcutaneous tissues, frequently to fascia (Figure 14.27). Numerous thin-walled vascular spaces dissect through collagen, lined with pleomorphic endothelial cells (Figure 14.28). The tumor cells stain strongly with vascular stains such as CD34, CD31, and factor VIII. The

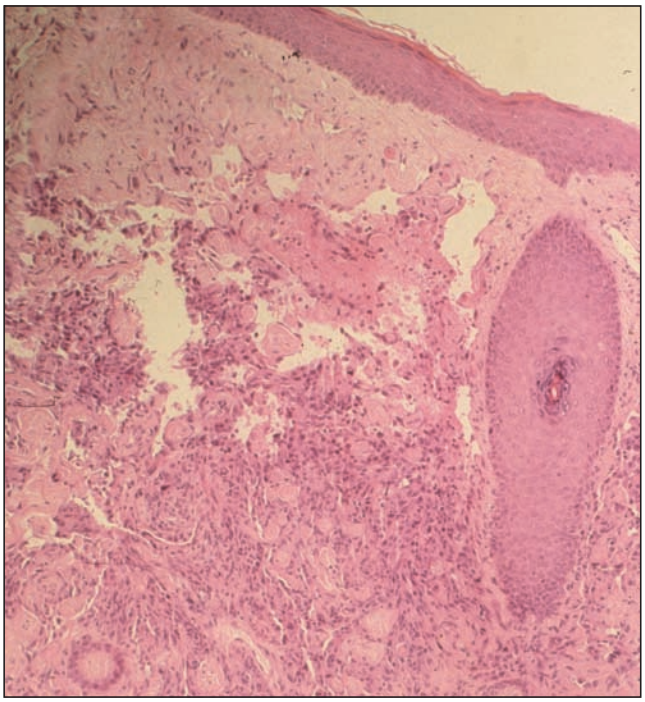


FIGURE 14.27: *Angiosarcoma; superficial and deep.*

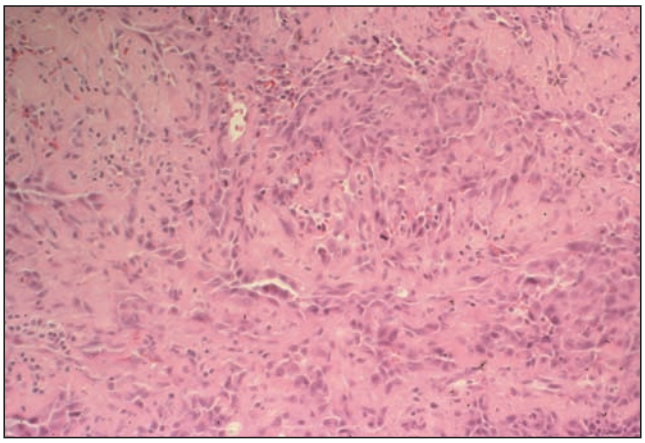


FIGURE 14.28: Angiosarcoma; higher power.

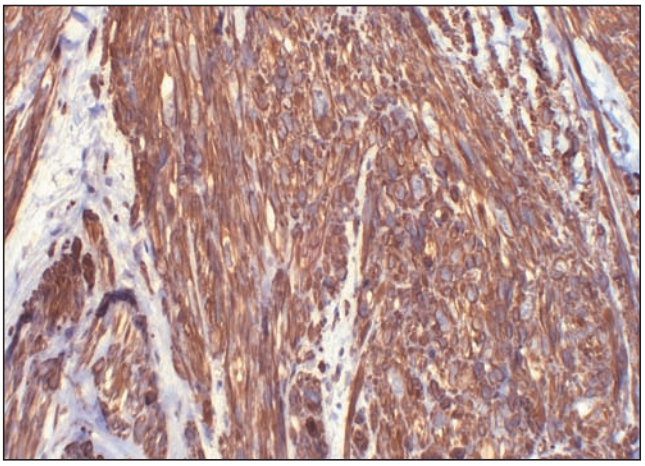
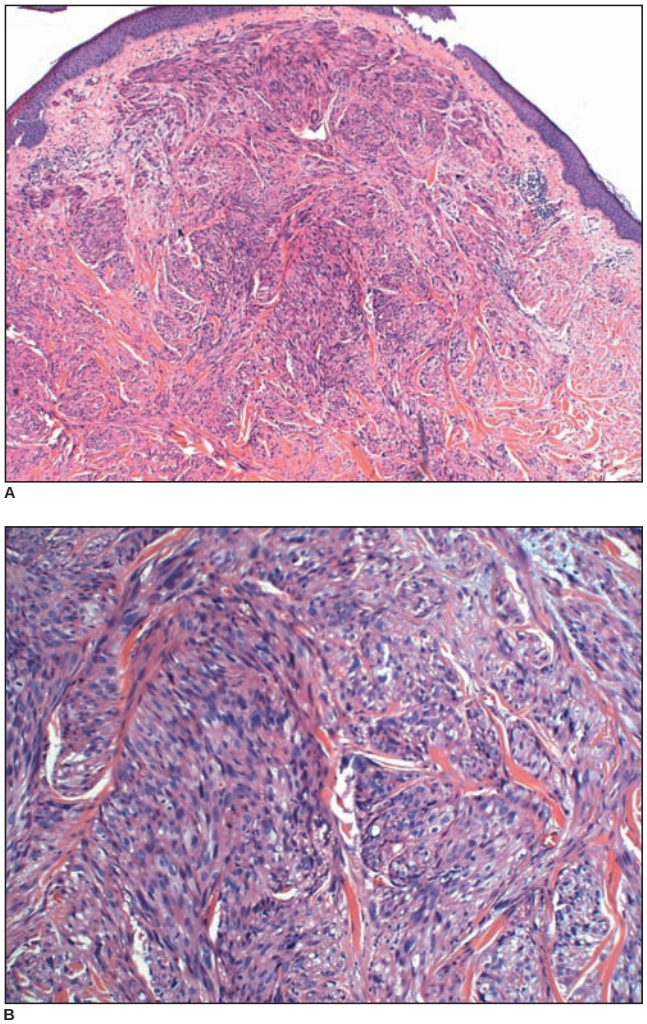


FIGURE 14.30: Leiomyosarcoma; smooth muscle actin (SMA) immunostain is positive.



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B

FIGURE 14.29: (A) Leiomyosarcoma showing marked cellular pleomorphism. (B) Leiomyosarcoma at higher power; the mitotic rate is generally greater than three per high-power field.

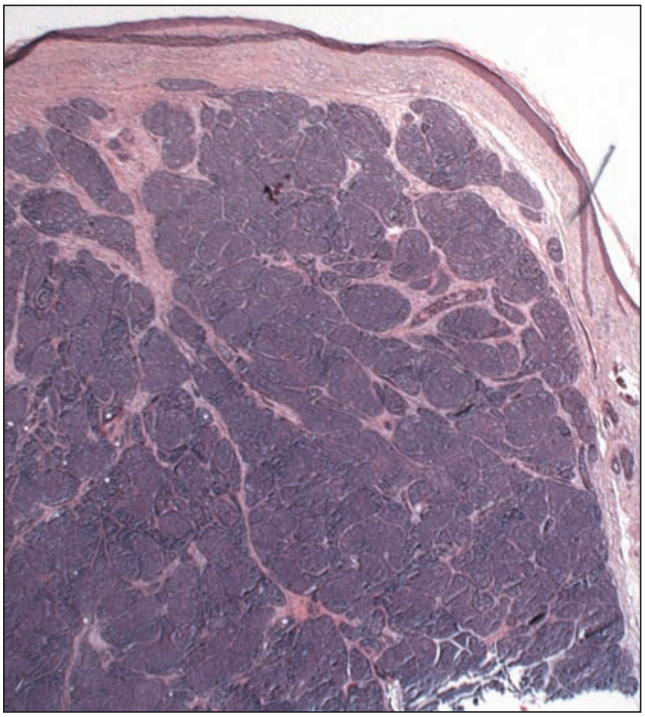
stains can help identify residual tumor in sections; however, normal vasculature will also stain, making this a more challenging task. Comparison with normal skin samples can be helpful. Recurrence is common, and even with excellent margin control, complete removal may be difficult.

LEIOMYOSARCOMA

Leiomyosarcomas occur in the skin infrequently and are more common in deeper tissues. The cutaneous tumors arise from the smooth muscle found in erector pilorum muscle (piloleiomyomas), vascular muscle coats (angioleimyomas), or genital smooth muscle. The tumors are composed of fascicles of smooth muscle cells filling the dermis. Malignant smooth muscle tumors show marked cellular pleomorphism (Figure 14.29). The mitotic rate is generally greater than three per high-power field. The cells stain strongly with smooth muscle actin (SMA) (Figure 14.30).

BENIGN ADNEXAL TUMORS

Adnexal neoplasms are relatively common tumors, generally occurring on the head and neck. They can range in size from several millimeters in diameter to several centimeters. Most present as indistinct papules or nodules, whose diagnosis may not be suspected clinically. These neoplasms are formed of elements resembling the cutaneous adnexa: hair, eccrine, or apocrine glands and sebaceous glands. Since the adnexal structures arise from the epidermis, the tumors contain basaloid and squamous keratinocytes. Thus many of the neoplasms resemble BCCs (Figure 14.31). The differentiation of the tumors can be made by identifying features of the adnexa, such as eccrine ducts (Figure 14.32), sebocytes (Figure 14.33), apocrine decapitation secretion (Figure 14.34), and hair follicle structures such as infundibular cysts (Figure 14.35), papillary



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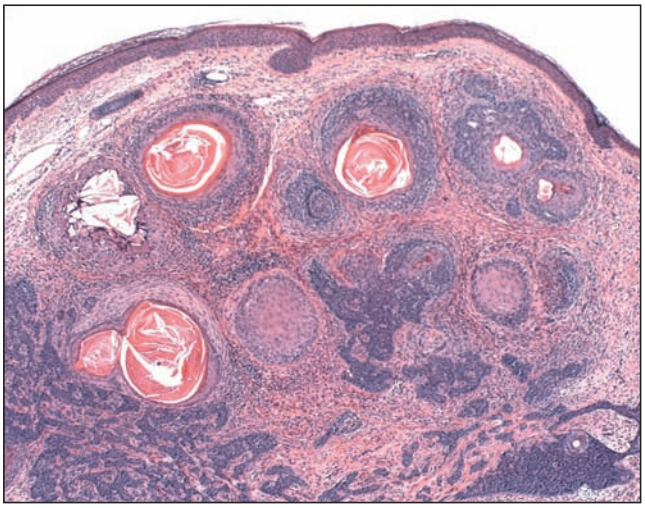


B

FIGURE 14.31: *(A) Cylindroma resembling BCC. (B) Trichoepithelioma resembling BCC.*

mesenchymal bodies (Figure 14.36), etc. The size and anatomic location of the tumors can be similar to those of BCCs, thus making Mohs micrographic surgery a logical surgical alternative for their removal. There are malignant variants of the adnexal tumors, usually larger in size and with histologic features of malignancy such as an infiltrative growth pattern, high mitotic rate, and areas of necrosis.

SUMMARY

The Mohs technique for examination of surgical margins has been used for a wide variety of neoplasms of both

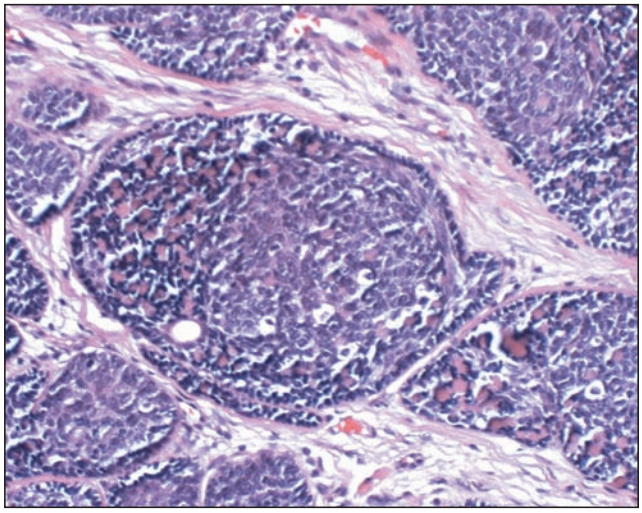


FIGURE 14.32: *Benign adnexal tumor; eccrine ductal differentiation.*

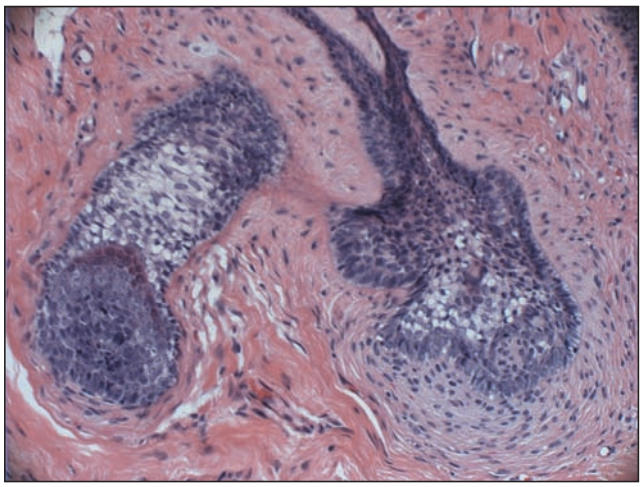


FIGURE 14.33: *Benign adnexal tumor; with sebocytes.*

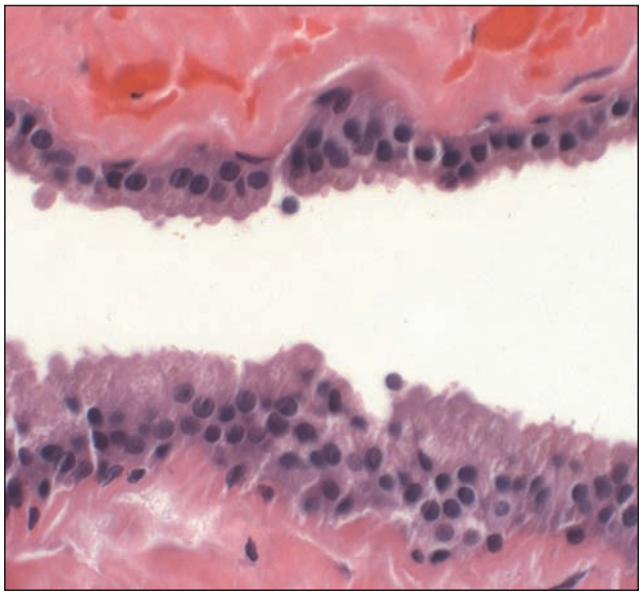


FIGURE 14.34: *Benign adnexal tumor; with decapitation secretion.*

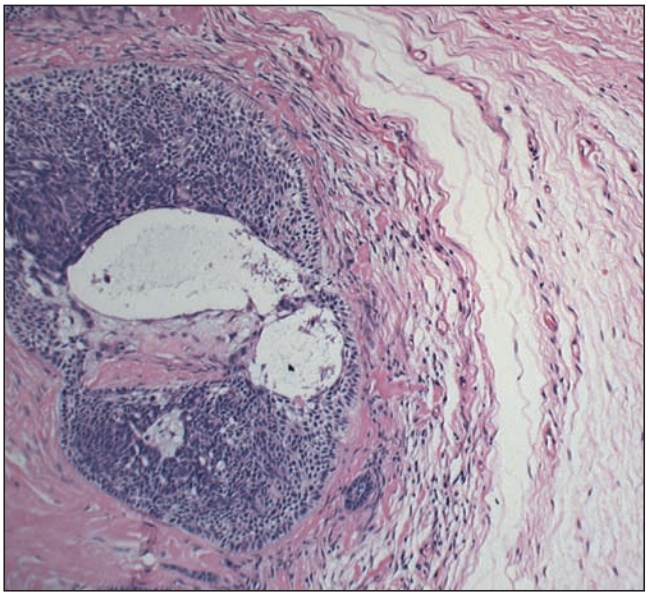


FIGURE 14.35: Benign adnexal tumor; infundibular cyst.

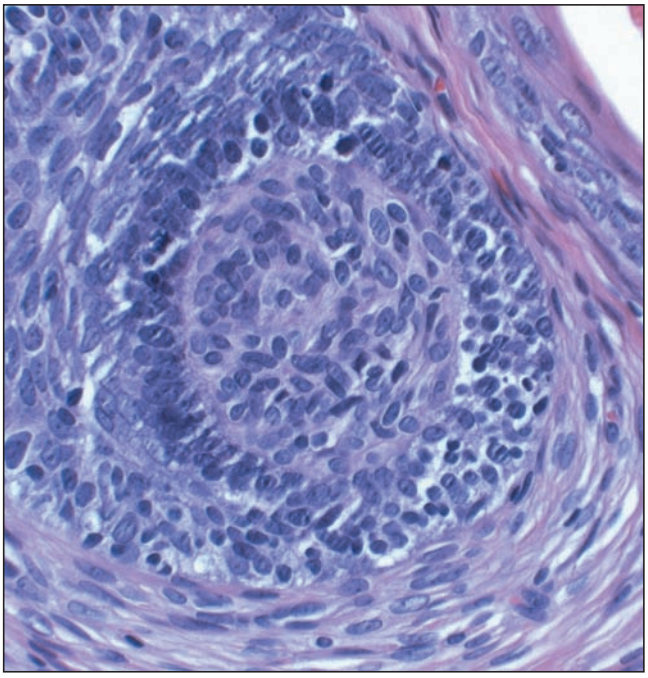


FIGURE 14.36: Benign adnexal tumor; papillary mesenchymal body.

epidermal and mesenchymal origin. The unique characteristics of these tumors can require modification of the basic Mohs technique to include the use of both frozen and paraffin-embedded sections; utilization of immunohistochemistry stains that can be applied to both frozen or formalin-fixed tissues; use of larger microscope slides and devices to examine whole mount Mohs horizontal sections; alteration of mapping of the tumor to encompass the larger size of these lesions; and the collaboration with other specialists for anesthesia, surgery, and postoperative repair. The success of this Mohs technique in treating these tumors has led to increased cure rates and decreased recurrence rates.

Chapter 15

Mohs for Melanoma

Adam J. Mamelak and Arash Kimyai-Asadi

BASIC CONCEPTS

Mohs micrographic surgery may be considered the best local treatment option for cutaneous melanoma. The key benefits of Mohs surgery for the treatment of melanocytic lesions are its ability to completely assess the tumor margins and its ability to spare normal tissue when necessary. The first benefit is particularly relevant when treating lentigo maligna and lentigo maligna melanoma, lesions that often possess poorly defined clinical margins and a high local recurrence rate when treated by standard excisional surgery. The second benefit is relevant to specific anatomic areas where removal of standard surgical margins would be associated with significant cosmetic and/or functional morbidity such as eyelid margin, nasal alar rim, and other similar areas. Other clinical scenarios where Mohs surgery for the treatment of melanoma may be valuable include locally recurrent tumors, large-diameter tumors, poorly circumscribed tumors, amelanotic melanoma, and desmoplastic melanoma.

Developments in staining and immunohistochemical techniques have allowed better visualization of melanocytes in frozen skin sections. Modifications of the Mohs technique, including "slow-Mohs" and "wide-Mohs," have been advocated by some as useful for the treatment of cutaneous melanoma. While controversies surrounding appropriate surgical margins and the adequacy of serial cross-sectioning of pathologic specimens continue, the need for complete en face examination of the surgical margins of melanocytic lesions is gaining recognition. The use of Mohs surgery does not negate the use of standard surgical margins (if desired) or further staging procedures. The micrographic technique can be utilized purely for margin control, with the goal of significantly reducing the local recurrence rate (Figure 15.1A and B).

DETAILS

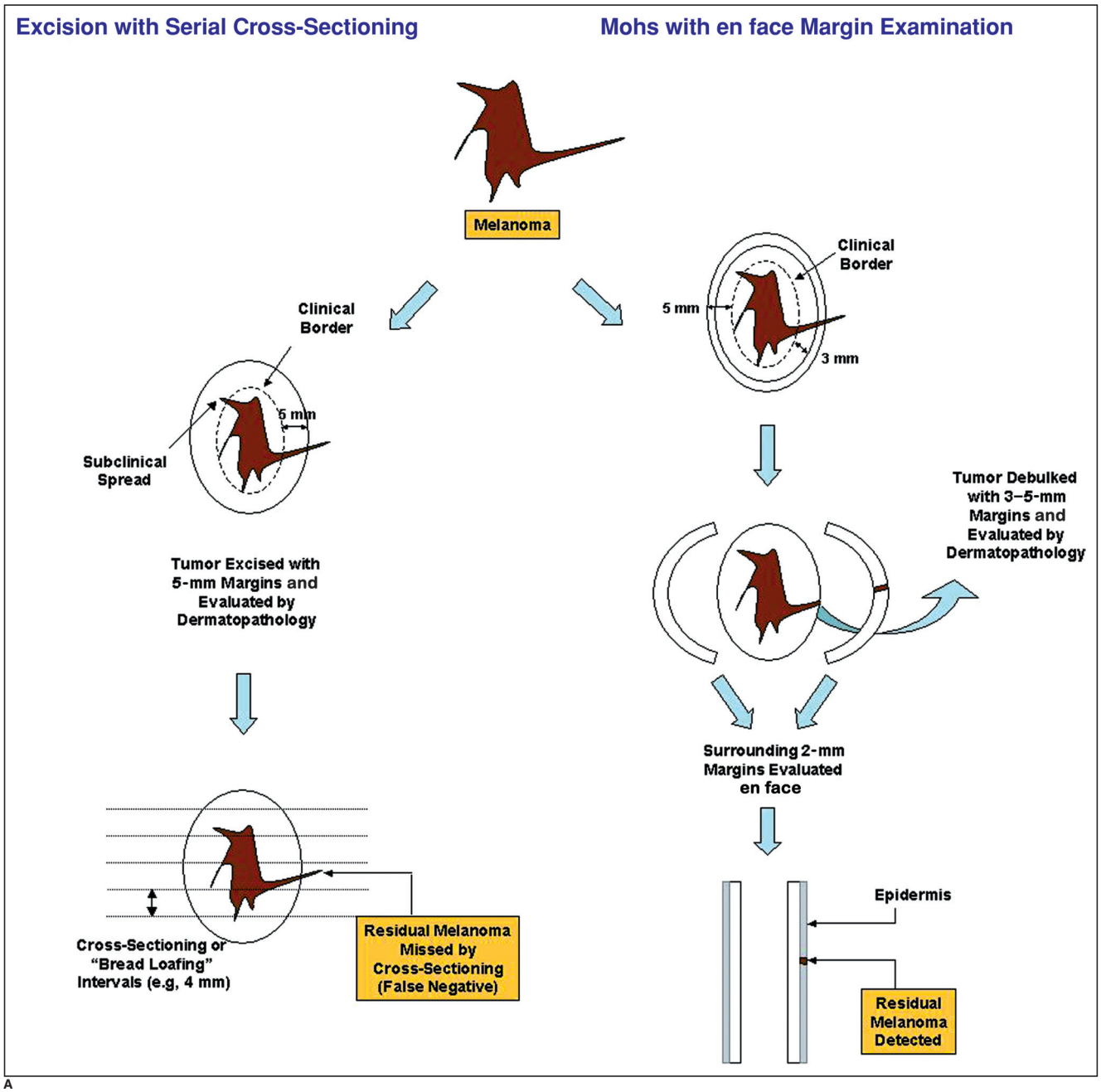
Clinical Evaluation

All patients with melanoma require a thorough history with a focus on pertinent risk factors and should undergo a complete cutaneous examination as well as clinical evaluation of the regional lymph node. Wood's lamp and/or dermatoscopic examination of atypical pigmented lesions can aid in determining the clinical tumor margins, particularly in severely sun-damaged skin or when treating tumors that are poorly circumscribed. Clinical evaluation of the regional lymph node basins should also be performed preoperatively, and management options, including stage-appropriate laboratory investigations, imaging studies, sentinel lymph node biopsy, and appropriate consultations, should be considered.

Lentigo Maligna and Lentigo Maligna Melanoma

Lentigo maligna melanoma (LMM) is one of the four main subtypes of melanoma, accounting for 5–15% of these tumors. It classically presents in elderly individuals on chronically sun-exposed skin, most commonly on the head and neck. Lentigo maligna melanoma usually presents clinically as a slowly growing, asymmetric, brown-to-black macule with color variegation and irregular borders. Extensive subclinical spread is common in these tumors (Figure 15.2), making Mohs surgery the optimal surgical treatment.

Lentigo maligna (LM) refers to the precursor lesion of LMM and is considered a melanoma in situ of sun-damaged skin that possesses only an in situ radial growth phase, although significant extension along adnexal epithelium may be present. This in situ extension along adnexal epithelium may carry the LM as deeply into the skin as the adnexal structures themselves extend. The in situ growth phase can



A

FIGURE 15.1: *(A) Treatment of melanoma in situ by standard excision and serial cross-sectioning compared to Mohs with en face margin examination. Clinically apparent tumor borders* (dashed line) *often miss subclinical disease spread. Residual tumor can be left behind when excised with standard 5-mm margins* (left side)*, as pathologists examine the specimens by cross-sectioning or "bread loafing" the tissue, typically at 4-mm intervals or wider. However, this approach can lead to false-negative readings and higher recurrence rates. Only Mohs surgery* (right side) *allows the entire surgical margin to be examined en face, thereby allowing evaluation of the entire margin and hence the detection of very small foci of residual tumor. Here, a debulking excision with 3-mm margins is performed and submitted for paraffin-embedded permanent-section pathology for staging purposes. This is followed by an excision of a 2-mm "donut" of peripheral normal-appearing skin for en face histologic margin evaluation. (B) Rates of finding melanoma at the margins using different cross-sectioning intervals after standard excision. "Bread-loaf" cross-sections through excised melanoma specimens are inherently unreliable for detecting residual melanoma at the surgical margins. "Bread-loafing" at 1-, 2-, 4-, and 10-mm intervals would have a 58%, 37%, 19%, and 7% chance of finding the positive margins detected by en face sectioning, respectively. (Modified from: Kimyai-Asadi et al.* Dermatol Surg. *2007;33:1434–1439.)*

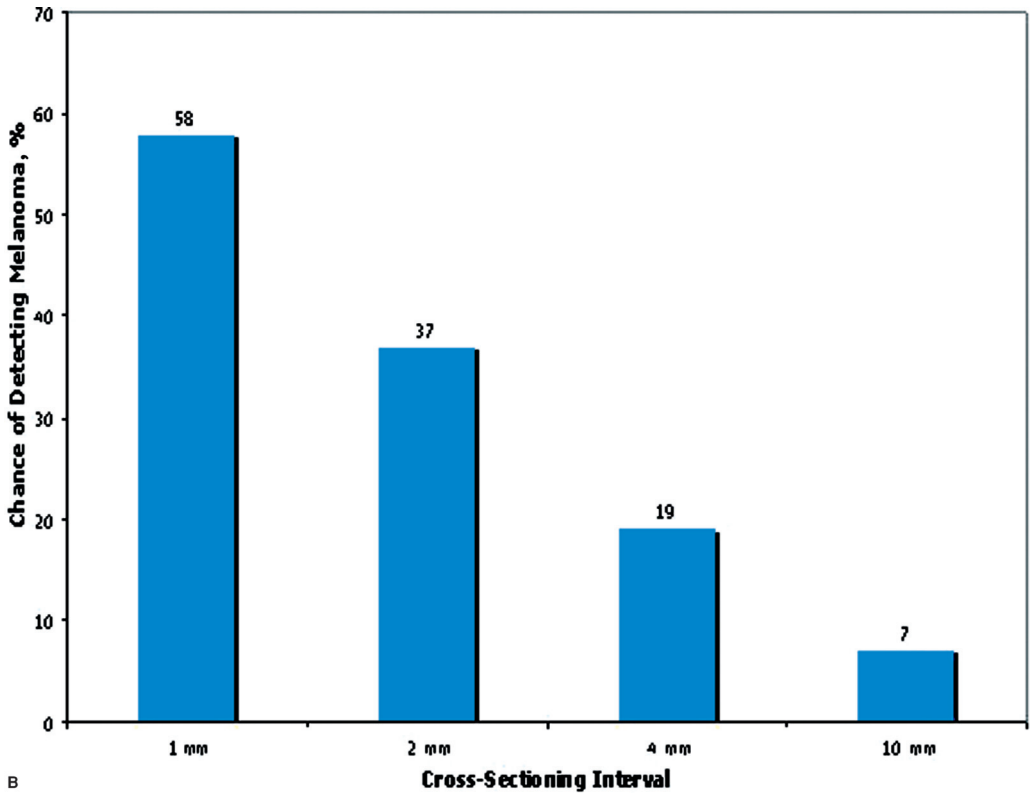


FIGURE 15.1: (cont.)

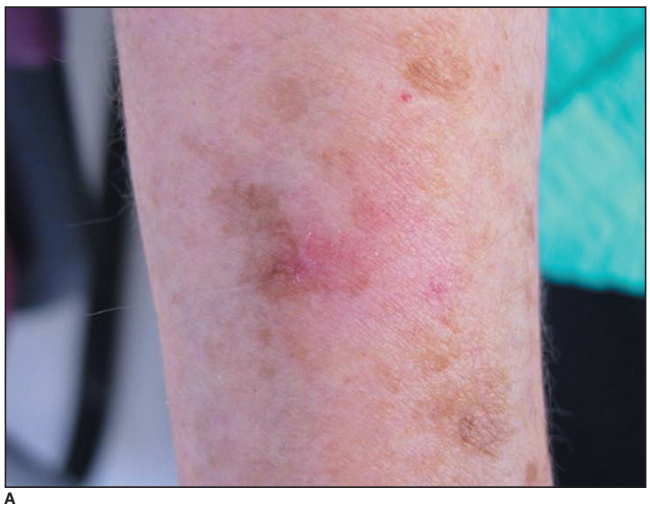
continue for a long time, potentially exceeding 10 or 20 years. In contrast, LMM is used to describe those lesions with dermal invasion. The development of a papule or nodule within a hyperpigmented patch of lentigo maligna may be indicative of invasive disease. Although no longitudinal, prospective studies have been performed, some have estimated the incidence of transformation from LM to LMM to be as high as 33–50%. Histologically, contiguous atypical basilar melanocytes with suprabasilar or pagetoid spread, as well as infiltration of melanocytes along appendageal structures, are observed in LM. Lentigo maligna melanoma shows atypical melanocytes within the dermis in addition to the features mentioned above.

Mohs Surgery and Melanoma

The goal of treatment of primary cutaneous melanoma is the complete removal of all cancer cells from the surgical field, thus preventing recurrence due to locally persistent disease. Excision with appropriate surgical margins remains the most common treatment for melanoma. Surgical margins are necessary because melanoma cells may extend an unknown distance beyond the clinical visible tumor margins, and the exact depth of the tumor is not known preoperatively. The American Academy of Dermatology (AAD) has recommended specific margins based on trials performed by the World Health Organization (WHO) that correlate with the current American Joint Committee on Cancer (AJCC) melanoma staging system. The AJCC staging system classifies melanoma into local, regional, and distant disease and is strongly correlated with survival. When staging localized disease, further consideration is given to Breslow depth, Clark level of invasion, and microscopic ulceration because these factors may affect prognosis.

The current guidelines for wide local excision of primary melanoma are: excision margins of 0.5 cm for in situ melanoma; 1 cm for invasive melanomas with Breslow depth less than 2.01 mm; 2 cm for Breslow depths of 2.01–4 mm; and 2–3 cm for Breslow depth greater than 4 mm.[1] These guidelines are often difficult to apply, particularly when confronted with tumors in functionally and/or cosmetically sensitive areas such as the head and neck, mucous membranes, and digits. Many advocate that the depth of excision extend to the underlying deep fascia; however, trials comparing this approach to excision to the deep subcutaneous fat are still lacking.

Zitelli et al. noted that the margins required to remove melanomas on the head, neck, hands, and feet were wider than those on the trunk and extremities. In addition, margins required to remove melanomas 2–3 cm in diameter were wider than those for smaller melanomas. They recommended a 10-mm surgical margin for the excision of melanoma or melanoma in situ on the trunk and proximal extremities smaller than 2 cm in diameter, and a



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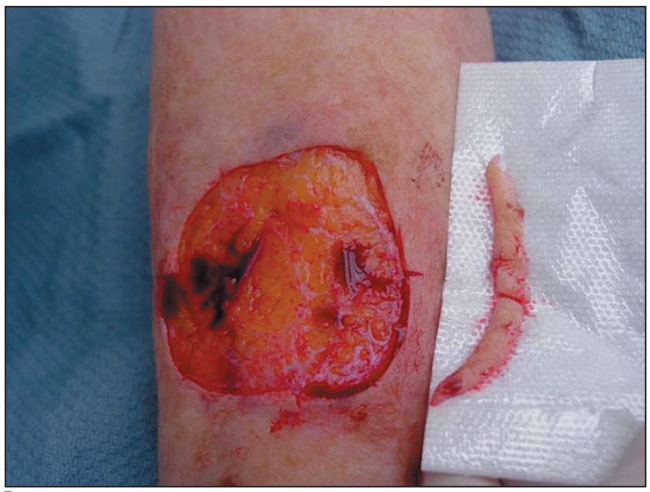


B

FIGURE 15.2: Clinical presentation of a lentigo maligna with poorly defined borders on the arm of a Caucasian individual (A) and the defect after the tumor was removed by Mohs micrographic surgery (B). The size of the defect reflects the extensive subclinical spread that could not be detected on physical examination.

15-mm margin for tumors larger than 2 cm in diameter. For melanomas on the head, neck, hands, and feet, a minimum surgical margin of 15 mm was recommended and a margin of 25 mm was found to be necessary for tumors larger than 3 cm in diameter.[2]

In a related study by Zitelli et al. examining 535 patients with 553 primary cutaneous melanomas treated by Mohs surgery with frozen section examination of the margin, the five-year survival and metastatic rates were better than historic controls treated by standard wide-margin surgery. Satellite metastases were not more common in patients treated by Mohs surgery, and local recurrence from inadequate excision of the primary tumor was 0.5% compared with the 9% recurrence rate often quoted for LM treated by standard surgical excision. Furthermore, the majority of melanomas in this study were successfully excised with a narrow margin; 83% required a 6-mm margin.[3]

In a similar study examining 625 patients with cutaneous head and neck melanoma treated with Mohs micrographic surgery, investigators found that the 5-year local recurrence rates, metastasis rates, and disease-specific survival rates were comparable to or better than historic controls based upon the AJCC criteria after Breslow thickness stratification. The authors found that the size of surgical margins required for complete excision was significantly related to tumor thickness but not tumor size or specific location.[4]

In addition, these authors noted that the margins recommended for extirpation of head and neck melanomas by the WHO were inadequate, especially for in situ disease. In order to achieve 97% complete excision rates with conventional excision for head and neck melanoma, they recommended: 9-mm margins for melanoma in situ (MIS) and melanomas with less than 1.01-mm Breslow depth; 12-mm margins for melanomas with a Breslow depth of 1.01–4.0 mm; and at least 12-mm margins for melanomas with a Breslow depth greater than 4.0 mm.[4] Others have corroborated the need for wider margins than are currently recommended, particularly for the complete excision of LM and LMM.

Mohs micrographic surgery is an excellent alternative to standard excision of melanoma, especially considering the variations in recommended margins depending on tumor location, size, and depth, as well as the comparable if not improved recurrence and survival rates. Our approach to the treatment of melanoma with Mohs surgery involves an initial debulking stage in which the clinically evident tumor is excised with a 3-mm margin through the dermis into the subcutaneous tissue plane prior to our first Mohs stage. This debulking stage is submitted for step-sectioning by standard paraffin histopathologic technique to determine maximum Breslow thickness and to assess for invasive foci in LM. In this way, the depth of tumor invasion can be assessed in a traditional manner and the patient can be appropriately staged. Our first Mohs stage is then excised as a 2-mm "donut" of surrounding normal skin to provide an initial 5-mm margin, although narrower margins are occasionally used based upon significant anatomic considerations. Excisions must be carried out into the deep subcutaneous tissue, as it is imperative that all the follicular and eccrine structures underlying the melanoma are excised. It is not rare for in situ melanoma to appear in the deep dermis or superficial subcutaneous tissue due to tracking along follicular or eccrine epithelium despite the absence of invasion; this adnexal spread may serve as a source of recurrence despite histologically clear peripheral margins.

Frozen Mohs sections (4 μm thick) prepared from the outside margins ("donut" excision) are stained with hematoxylin and eosin (H&E). To better visualize the malignant melanocytes, additional frozen sections are prepared for immunohistochemical staining. The slides are evaluated to

ensure that no tumor cells are present at the margins, and if residual tumor is detected, the involved area is re-excised using standard Mohs technique and the process is repeated until tumor-free margins are achieved (Figure 15.1A and B). For each re-excision, a 5-mm margin is typically used around the affected area. Tissue from subsequent staged excisions is processed and evaluated similarly.

A number of modifications have been made to the Mohs technique, specifically for treating melanoma. "Slow-Mohs" involves the staged, margin-controlled excision of the tumor with rush permanent sections. The stages are typically taken 24 hours apart, allowing for en face permanent sections to be made. A similar period is needed between the last stage and the final repair. The "square procedure" and staged, vertical-edge excision with radial sectioning are variations of this technique that also employ rush permanent sections. "Wide-Mohs" describes the technique in which an additional 5 mm of tissue is removed beyond the "tumor-free" margins after the tumor is cleared by Mohs; this tissue is processed using permanent section technique and might provide the surgeon with further assurance that margins are clear if there was any doubt about the adequacy or interpretation of the frozen section margins. In our practice, "slow-Mohs" and "wide-Mohs" are not utilized, as the authors feel comfortable with the quality of their frozen sections slides, confident of their ability to routinely detect melanoma at the surgical margins, and pleased with the very low recurrence rate they achieve utilizing the Mohs technique. We are well aware of the difficulties inherent in identifying melanoma at surgical margins regardless of the surgical or pathologic methods used.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry (IHC) refers to a technique used to localize proteins in a tissue specimen by exploiting the ability of antibodies to specifically recognize and bind certain antigens in that tissue. Visualization of the antibody-antigen interaction is accomplished most commonly by conjugating the antibody to an enzyme that can catalyze a color-producing reaction. This is known as an immunoperoxidase reaction.

A number of immunohistochemical stains can be used to identify melanoma based upon the antigens expressed by the malignant cells. Most commonly, these include HMB-45, MEL-5, MART-1 (melanoma antigen recognized by T-cells; also know as Melan-A), and S-100. MART-1 has been claimed to be the most consistently crisp and most easily interpreted stain. It should be noted that MART-1 is present in normal melanocytes and melanocytic nevi, as well as in melanoma. Dermal dendritic and inflammatory cells often stain with MART-1 as well, although this rarely creates difficulties in slide interpretation. The primary antibodies available for MART-1 have been found to be more sensitive and specific than those available for HMB-45. With the notable exception of spindle cell and desmoplastic melanomas, the sensitivity of MART-1 for primary cutaneous melanoma can approach 100%. There are also studies supporting the use of Mel-5 IHC staining in the treatment of LM and LMM.

Given the ability of immunohistochemical staining to aid in the detection of melanocytes at the surgical margin, many Mohs surgeons use immunohistochemistry as an aid in evaluating the tumor margins of melanomas in conjunction with hematoxylin-eosin stained frozen sections. It should be noted that these stains not only improve detection of residual melanoma at the margins, but also help reduce the need for unnecessary additional resection when the Mohs surgeon encounters areas with nonmelanocytic clear-cell proliferations, such as pagetoid actinic keratosis, or areas with significant spongiosis or freeze artifact where differentiation of melanocytes from keratinocytes may be difficult.

STAINING PROTOCOL

Evaluation of melanoma has historically been performed using hematoxylin and eosin. For the detection of melanoma, high-quality thin sections (4 μm) are critical, as thicker sections severely impair the ability to visualize melanocytes and may account for the inability of some Mohs surgeons and investigators to reliably detect melanoma on frozen sections. Protocols for H&E staining of frozen sections are readily available (Table 15.1). For Mohs surgery, this procedure can rapidly and effectively be accomplished with the help of an automated linear stainer. Standard reagents are also available for this purpose. We use Gill Hematoxylin-3 Formula (Stat Lab. Medical Products, Lewisville, TX; Cat. # SL-95) and Scotts Bluing Reagent (Stat Lab. Medical Products, Lewisville, TX; Cat. # SL-99) (see also Chapter 7).

TABLE 15.1: Hematoxylin and Eosin Staining Protocol

1.	Absolute alcohol	30 seconds
2.	Running water	1 minute
3.	Hematoxylin	30 seconds
4.	Hematoxylin	30 seconds
5.	Running water	30 seconds
6.	Running water	30 seconds
7.	Bluing (mix 3 parts H_2O with 1 part bluing agent)	30 seconds
8.	Running water	30 seconds
9.	Alcoholic eosin	30 seconds
10.	95% Alcohol	30 seconds
11.	95% Alcohol	30 seconds
12.	Absolute alcohol	30 seconds
13.	Absolute alcohol	30 seconds
14.	Absolute alcohol	30 seconds
15.	Clearant	30 seconds
16.	Clearant	30 seconds
17.	Clearant	30 seconds

TABLE 15.2: 20-Minute MART-1 Staining Protocol

1. Cut frozen sections 4 μm in thickness
2. Place on hot plate (60°C) for 1 minute
3. Immediately fix in acetone for 1 minute
4. Air-dry for 10–15 seconds
5. Rehydrate in tris-HCl buffer for 2 minutes
6. Place slide in humidity chamber
7. Add protein block for 1 minute
8. Blot
9. Add MART-1 antibody for 5 minutes
10. Swish gently in tris-HCl buffer for 10 seconds
11. Add horseradish peroxidase for 5 minutes
12. Swish gently in tris-HCl buffer for 10 seconds
13. Apply diaminobenzidine chromogen for 2 minutes
14. Rinse in H_2O for 10 seconds
15. Dip in hematoxylin 10 seconds
16. Rinse in running water
17. Dip in Scotts bluing reagent for 5 seconds
18. Rinse in running water
19. Rinse in 100% ethanol 15 seconds
20. Dry on hot plate for 15 seconds
21. Place in xylene or xylene substitute for 30 seconds
22. Coverslip

For IHC, many employ a 60-minute MART-1 staining protocol. Tissue preparation and mounting (20 minutes) is followed by a 40-minute IHC staining procedure. The antibody detection system used, ultrastreptavidin, is a very sensitive indirect labeling technique that relies upon avidin-biotin chemical bonding. It is used with a horseradish-peroxidase label and diaminobenzidine (DAB) as the chromogen. Hematoxylin can also be employed as a counterstain.

We have modified this technique by shortening incubation times so that the entire protocol takes only 20 minutes (Table 15.2). Despite this shortened protocol, the stain reliably highlights normal background melanocytes, as well as melanocytic hyperplasia and malignant melanoma, while providing a more rapid method that improves the efficiency of the Mohs laboratory and significantly reduces patient and physician waiting times. In our experience, there is no difference in slide quality between the 20-minute protocol and more time-intensive ones.

Interpretation

Recognizing the normal and abnormal distribution of melanocytes in the skin is critical for the interpretation of frozen sections from these tumors. On average, in photoprotected skin, one of every five to ten cells in the basal layer is a melanocyte; however, this number varies with body region and the amount of photodamage (Figure 15.3). The number of melanocytes increases with repeated exposure to ultraviolet light and the concentration is highest in chronically exposed areas (Figure 15.4). Increased melanocytes can also be seen in the genital region.

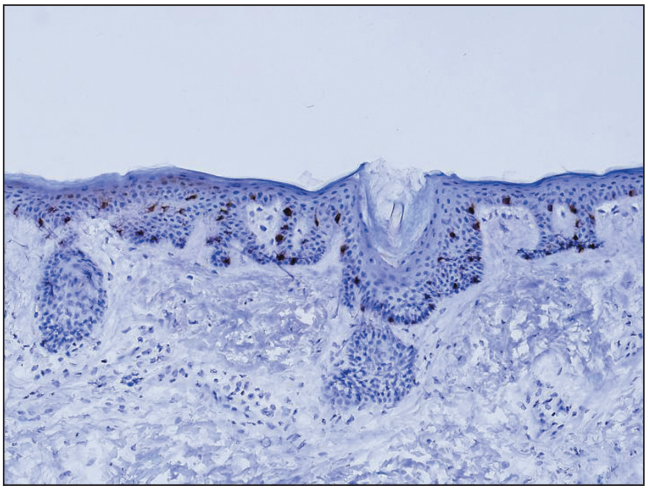


FIGURE 15.3: MART-1–stained frozen section of normal skin. Melanocytes are evenly distributed along the basal layer and occur in a melanocyte:keratinocyte ratio of 1:5 to 1:10.

The diagnosis of melanoma on histologic examination relies upon the arrangement of melanocytes in the tissue, as well as cellular morphology. Positive margins for melanoma and/or in situ melanoma sections are defined as meeting one of the following criteria: (1) contiguous basilar melanocytes; (2) nests of at least three atypical melanocytes; and (3) melanocytes above the dermoepidermal junction in conjunction with confluence or near-confluence of basilar melanocytes (Figure 15.5). Atypical melanocytes may be enlarged or multinucleated, may contain pleomorphic nuclei, or may have an atypical shape. Other suspicious histologic findings include extension of atypical, crowded

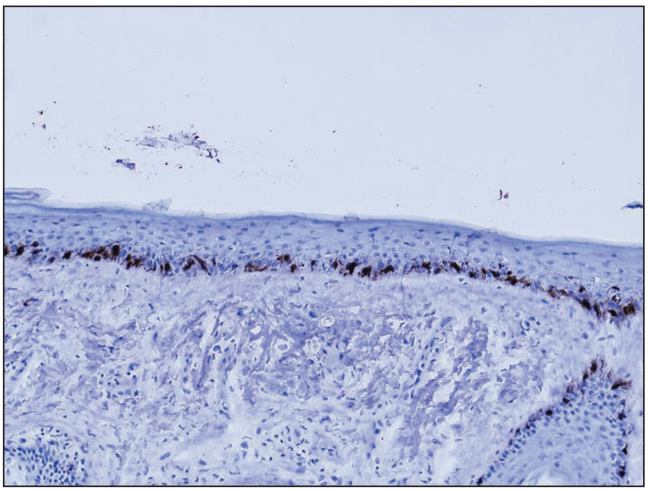
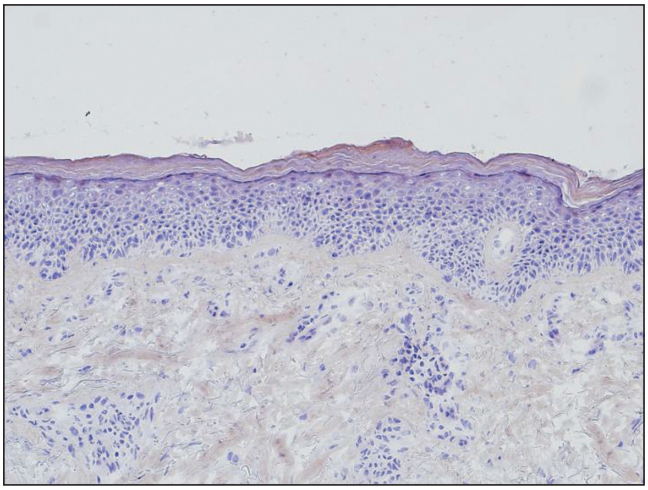


FIGURE 15.4: MART-1–stained frozen section of photodamaged skin. Melanocytes are denser, though limited to the basal layer. Although they approach confluence in some areas, true confluence is absent, as keratinocytes can be seen between potentially adjacent-appearing melanocytes. Solar elastosis is noted in the upper dermis.



A

B

FIGURE 15.5: ***(A) H&E-stained frozen section of melanoma in situ demonstrates the lentiginous spread of atypical melanocytes and bridging of melanocytic nests along the dermoepidermal junction. (B) In the MART-1–stained frozen section, crowding of melanocytes along the basement membrane, nests of atypical melanocytes along the dermoepidermal junction and migrating above the basal layer, are better and more effectively highlighted when this immunohistochemical stain is employed.***

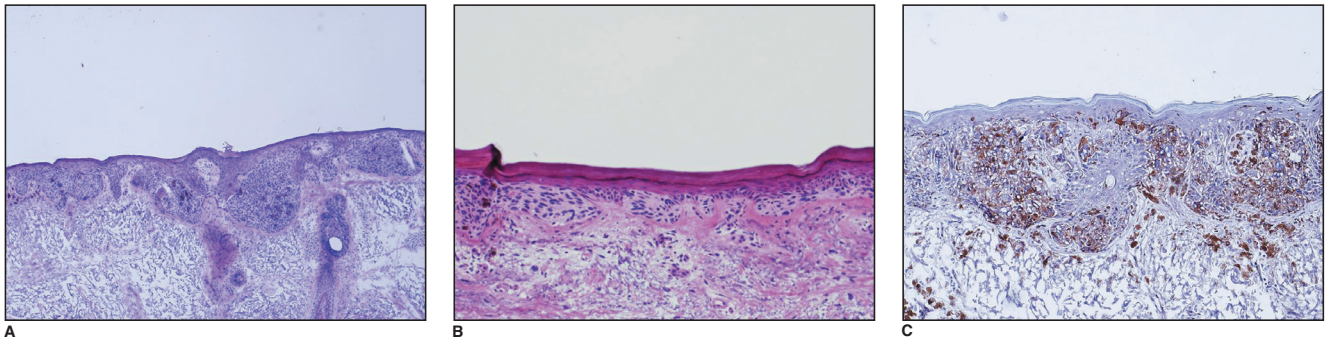
melanocytes far down adnexal structures, nonuniform distribution of pigment, excessive number of melanophages, and a brisk inflammatory response (Figures 15.6 and 15.7).

Isolated atypical melanocytes and melanocytic hyperplasia are common in sun-damaged skin and do not mandate further excision. The melanocytes in melanocytic hyperplasia appear small and nearly confluent in the lower layers of the epidermis. However, high-powered examination typically shows that true confluence is absent, as keratinocytes can be seen wedged between near-confluent basilar melanocytes. In contrast, melanocytes in LM are typically larger, more pleomorphic, and crowded, and true confluence is typically present. Of course, there will be occasional cases in which distinguishing between severe hyperplasia and in situ melanoma will be difficult regardless of the type of pathologic processing and staining utilized. Excising and processing an additional margin should be strongly considered in such cases.

POTENTIAL PROBLEMS

Many regard Mohs surgery for the treatment of melanoma as controversial. The main arguments against Mohs surgery include unreliable features on frozen histology and the potential for discontiguous tumor growth. Critics question the accuracy of detecting atypical melanocytes in frozen sections, claiming that melanocytes are often subtle and difficult to distinguish from keratinocytes. Furthermore, melanocytic cytology may not be consistently identifiable on frozen sections. For example, studies have found sensitivities as low as 59% and specificities as low as 68% when interpreting pathologic changes of melanoma on frozen sections compared with permanent sections. These studies must be contrasted with those showing 100% sensitivity and 90% specificity on frozen sections for melanoma. Clearly, the quality of the frozen sections and the skill of the physician interpreting the slides are critical. One pitfall of these studies may be the failure to cut alternate slides for IHC comparison with the H&E Mohs slides, rather than cutting slides for IHC from the tissue left in the block at the completion of the case. Finally, melanocytic hyperplasia in sun-damaged skin is difficult to differentiate from actual melanoma. This problem, however, is not specific to frozen sections. The use of immunohistochemical stains can increase the histologic sensitivity in Mohs, even though none of the immunohistochemical stains available is absolutely specific for melanoma. For example, pigmented solar keratoses can be highlighted by MART-1 and are often in the surgical field of these tumors, where they may serve as a source of false-positive surgical margins. "Slow-Mohs" with rush permanent sections after each stage is one potential solution to these problems, but the reality is that there is significant discordance among pathologists interpreting melanoma margins regardless of whether frozen sections or permanent sections are used; many of the histologic issues cited for frozen sections are present in permanent sections as well. Studies consistently show recurrence rates with Mohs surgery utilizing frozen sections to be far lower than those with traditional excision. As such, the Mohs technique works, and the criticisms merely point out its difficulty and the high degree of expertise required to utilize it.

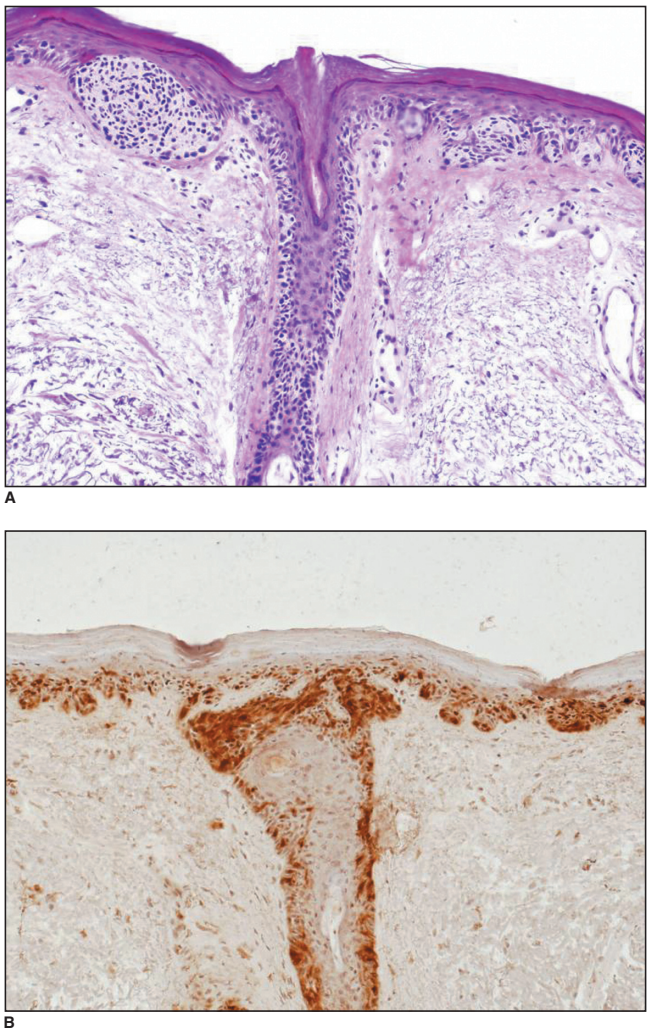
Some dermatologic surgeons, especially when dealing with a tumor that has a high capacity to metastasize, are made uncomfortable by the fact that Mohs surgery may involve margins narrower than the published guidelines for melanoma. However, discontiguous tumor growth is rather rare, as demonstrated by the fact that virtually any tumor treated with Mohs surgery has a lower recurrence rate than



A B C

FIGURE 15.6: H&E-stained frozen section of melanoma in situ. Atypical melanocytes are seen in nests and in a lentiginous pattern along the dermoepidermal junction (A,B). MART-1–stained frozen section highlights the atypical melanocytes in the epidermis (C).

if treated with wide local excision. It is much likelier that a positive margin will be surgically and histologically missed by standard wide excision than it is for the tumor to be discontiguous and missed by Mohs surgery. Based upon the results of a previous study of ours showing that melanoma present at the surgical margins typically occurs in small nests, the chance of detecting this on "bread-loaf" sections is very low (Figure 15.1B). As such, the utility of "bread-loaf" sections on excisional specimens is highly questionable and en face sectioning should be strongly preferred. Although cure rates utilizing Mohs surgery are the gold standard for the excision of high-risk tumors, it might theoretically improve cure rates if standard margin excisions, performed even by non-Mohs surgeons, had their entire surgical margins evaluated en face by permanent-section pathology.



A B

FIGURE 15.7: H&E-stained (A) and MART-1–stained (B) frozen section of melanoma in situ with follicular involvement. Atypical melanocytes are seen tracking down the hair follicle.

CONCLUSIONS

Mohs surgery should be considered a first-line surgical treatment for melanoma, particularly for lentigo maligna and lentigo maligna melanoma of the head and neck. Mohs surgery can provide significantly lower local recurrence rates than traditional excision, and provides the surgeon the ability to utilize narrower surgical margins when dictated by anatomic considerations. Additional tumor resection can be performed in the same surgical session, eliminating the need for repeat patient visits. Thin, high-quality frozen sections and a Mohs surgeon trained in interpretation of melanoma frozen sections are mandatory, and the use of immunohistochemical stains may augment the sensitivity and specificity of routine hematoxylin-eosin staining when treating melanoma with Mohs surgery.

Pearls

- A Wood's lamp and/or dematoscope may be used to highlight clinical tumor margins.
- Immunohistochemical stains may increase the histologic sensitivity of detecting melanocytes on frozen section during Mohs surgery.
- Unlike standard excision, Mohs surgery offers the possibility of complete margin examination, followed by step-sectioning by standard paraffin-embedded histopathologic technique for melanoma staging purposes.
- Tumors in anatomic and cosmetically sensitive areas are best treated by Mohs surgery when tissue sparing is of utmost importance.

- If there is any concern about whether a margin is positive or not, one should consider further resection.
- Mohs excisions must be carried out well into the subcutaneous tissue.

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CHAPTER 16

Taking Stages beyond Stage I

Tri H. Nguyen

THE HIGH CURE rate of Mohs surgery rests on one word: accuracy. A Mohs surgeon must be accurate in frozen section diagnosis and mapping tumor location. Once tumor is noted and mapped in stage I, additional layers/stages are taken in the positive areas until negative margins are obtained. Failure in this latter step will undermine the cure rate and tissue-sparing benefits of Mohs surgery. Errors are especially likely in situations that involve multiple sites, multiple stages, difficult tumors, and convoluted anatomic areas. Before touching the scalpel, a number of questions must be addressed. It is the purpose of this chapter to present a systematic approach for minimizing errors in Mohs surgery beyond stage I. The ensuing discussion assumes that in stage I, there is correct orientation, complete tissue integrity (entire epidermis present, deep tissue present without gaps, inked margins present), adequate staining, and thin sections.

DO I HAVE THE CORRECT MOHS MAP?

Wrong site, wrong patient, and wrong surgery are all sentinel errors that harm patients. Correct patient and correct site are fundamental obligations of all Mohs surgeons. For severely actinically damaged patients, identifying the correct biopsy site is a challenge unless photographs are used with measurements from indelible anatomic landmarks. Mohs maps must contain several identifiers: patient's full name, date of birth, medical record number (unique to that practice), and unique Mohs case number. Patients with the same last names should not be scheduled for Mohs on the same day, if at all possible. For same-day Mohs surgery on multiple sites, it is imperative to use distinctive mapping that cannot be confused, especially if within similar facial subunits. The majority of Mohs surgeons use either hand-drawn figures or blank maps and tissue gauze in a Petri dish for transfer of the excised tissue. While this may be adequate for single-site, single-stage tumors, it becomes less than accurate with multiple sites, challenging anatomic areas, or multiple stages. Many missteps may occur during Mohs tissue processing, such as incorrect orientation or mislabeling of tissue. These blunders may be avoided by using standardized anatomic maps and transfer cards, as outlined below.

At MD Anderson (MDA), preprinted anatomic maps and transfer cards are used. These maps facilitate site distinction for multiple-site surgery. For example, the patient presents with two skin cancers on the left nose. The anterior nasal map may be used for one site and the left nasal profile may be used for the second site. By having two distinct and separate Mohs maps, the chance of site mislabeling or confusion is minimized. Preprinted transfer cards (TCs) that correspond to the anatomic maps are used to transfer the tissue from the patient to the lab. Made of absorbent card paper, these TCs preserve the orientation of the tissue during transfer and absorb excess tissue fluid. Sections are processed and inked on these cards and the TCs are kept until all margins are clear. These TCs then serve as "backup" records of which section was which and what color inks were used.

HAVE I MAPPED THE TUMOR ACCURATELY ON THE MOHS MAP?

This question raises multiple issues in Mohs mapping: tissue marking, tissue orientation, and map marking. To enhance excisional accuracy, this Mohs surgeon applies techniques such as tissue nicks (TNs), extra TNs, differential ink colors, map annotation, and angulated resections.

Tissue Nicks

The first issue is the method of marking and orienting the tissue, about which there are two camps of practice. The first group uses gentian violet (GV) or other ink markers to orient the tissue and the patient. The second group places TNs on the removed tissue and the patient. The advantage of GV is that the ink is easily visible; it leaves no permanent

marks on the skin and does not require skill. Disadvantages are stark: it can be smudged and unclear as the skin is prepped or bleeds, it requires added motion that is separate from excising the layer (dip the ink and draw on the skin), and it is often used unsterile (potential source of infection). At MDA, GV is used in lieu of surgical markers. However, the GV is prepared in individual centrifuge tubes that are autoclaved sterile. In comparison, TNs are unequivocally the most precise method of orienting the tissue and the patient. Anything less places the patient at risk for mapping and resection inaccuracies. The advantages of properly placed TNs for orientation include indelibility and good visibility. TNs do not require added motion, as they are placed as part of removing the Mohs layer. The use of TNs has its disadvantages. It requires a soft touch to lightly score the skin. If TNs are deep, gapes occur that may interfere with closure. An ideal TN extends no further than the lower papillary dermis. If placed too closely together (<5 mm apart), TNs may contribute to tissue folds and tears. This is especially true if one nick is adjacent to the edge of a divided section. Even with TNs, there is variation in the number of TNs placed. Most Mohs surgeons utilize four single TNs, which are placed at 12–3–6–9 o'clock. In addition, this author places an extra tissue nick (ETN) between 9 and 12 o'clock to optimize orientation and mapping accuracy. An ETN has multiple uses, including tissue orientation, section identification, tumor mapping, and resections beyond stage I.

Tissue Orientation

If consistently placed (in the same location), TNs maintain specimen orientation throughout the Mohs process. Consider the circular tissue removed, in which four single TNs are placed in the usual position. As the tissue is being transferred to the Mohs laboratory, a shift occurs and the tissue is rotated. The Mohs surgeon is now unsure which TN represents the correct 12 o'clock position. Although uncommon, dropping tissue during transfer (from forceps or gauze) may also occur. In both circumstances, if an ETN is consistently placed between the 9 and 12 o'clock positions, then, regardless of the tissue displacement, accurate reorientation is always possible. The ETN also plays a role in section identification.

Section Identification

The second issue in accurate Mohs mapping is how sections are distinguished (inked and labeled) during processing. *The ideal Mohs section is a single section. Tissue loss and processing errors are directly proportional to the number of divided sections.* Some tissues, however, cannot lie completely flat as a single section, and division into two or more tissue blocks becomes necessary. With two or more sections, it is imperative to unequivocally distinguish each section. Most Mohs surgeons use differential inking (i.e., section 1 inked red and blue, section 2 inked green and blue). If a histotechnician mislabels section 1 as 2 and vice versa, then the error is evident by comparing the section inking with the map. Occasionally, the ink color is not visible from processing loss, which may result in tumor mapping to the wrong section. By using an ETN, the two sections are further distinguished; section 1 has two TNs and section 2 has only one TN. By using both differential inking and TNs, the two systems serve as redundant techniques for orientation should one method fail. Further, TNs are more readily visible than ink colors on low-power microscopy. TNs should be used *in addition* to differential inking. Errors may occur regardless of any system without constant queries for accuracy. Therefore, a compulsive Mohs surgeon must have two thoughts before evaluating for tumor:

1. Am I reading the correct slides for the correct patient and the correct site?
2. Do the sections I am reading correlate with the Mohs map?

Tumor Mapping

An ETN aids immensely in tumor mapping for long, large sections. These long sections appear unending under microscopy, and localization on the Mohs map can be difficult. An ETN allows a midway marker that will isolate better the tumor on the map. The caveat is that TNs should be used as aids to tissue mapping or orientation and not as barriers to tissue resection. For example, a tumor that comes close to a TN should be excised beyond that nick to allow for adequate tissue overlap.

Staged Resections

When multiple (>3) Mohs stages have been excised, it can become difficult to identify the positive margin. Convoluted topography like the nasal tip/ala/vestibule or areas with minimal landmarks (bald scalp) may lead to disorientation. The accumulation of multiple single TNs or GV markers after multiple stages adds to this confusion. As a solution, two closely placed (5 mm apart) TNs may be used at the leading edge of subsequent resections. These *double TNs* (DTNs) act as a visual focus for the positive margin. The use of DTNs, therefore, more easily identifies the leading edge of the last positive margin, especially when combined with angulated margins (discussed below).

Map Annotation

Annotating the Mohs map in regard to tissue planes of tumor is essential for subsequent resection. A dermal nest of cancer may be marked in two ways, with and without annotation. The annotated map reminds the surgeon to overlap

the tissue plane involved and precludes the error of inadequate tissue excision. This annotation is indispensable for deeply penetrating tumors such as dermatofibrosarcoma protuberans (DFSP), high-risk squamous cell carcinoma (SCC), microcystic adnexal carcinoma (MAC), and others.

Angulated Resections

Most Mohs layers are taken as circles or semicircles. There are at least three instances in which Mohs excisions should have angulated shapes such as triangular, square, rhomboidal, pentagonal, or hexagonal. The first application is when two tumor sites (A and B) are located within the same facial subunits. Even with distinct maps, circular excisions for both sites predispose for error in returning to the incorrect site for resections beyond stage I. However, if site A is excised as a circle and site B is removed as a rhomboid, then positive tumor on the rhomboid-shaped defect can easily be identified, or vice versa, as each shape is unique. The distinct shapes in addition to the distinct maps are additive in accuracy.

Angulated resection is also useful for stages II and beyond. The angulated corners on the lateral ends of the excised tissue clearly mark the extent of peripheral resection. When combined with double TNs at the leading edge, angulated resection margins maximize excision accuracy beyond stage I.

Finally, angulated resections are useful for larger tumors (>2–3 cm). The circular Mohs resection of a 4 cm tumor with 3-mm margins yields a large circle greater than 4.6 × 4.6 cm (tissue retraction). Locating the positive margin after stage I for a large defect, even with indelible TNs, can be challenging. If a large circle was a rhomboid with TNs, then the defect shape and sides are visually distinct and the positive margin may be more easily pinpointed.

A precaution with angulated Mohs sections is the need to flatten the corners of the layer for complete margin evaluation. If unexamined, these corners may contribute to false-negative margins. Angulated excisions in Mohs surgery do result in more tissue excision than circular layers. However, when judiciously applied for the excision of high-risk tumors, such as those involving multiple sites, large size, or multiple positive margins, they ensure the priority of oncologic cure by enhancing accurate tumor resection. More is sometimes less.

HAVE I BROUGHT THE CORRECT MAP TO THE CORRECT ROOM?

A typical Mohs schedule consists of multiple Mohs patients having their surgeries on the same day. Some practices use the same room for two or more patients at a time, with the trays changed and room cleaned between patients. A single-room, single-patient arrangement is optimal for avoiding identification errors: patient A uses room 1 until patient A is cleared and discharged. Whatever system is used, ensure that the correct map for the correct patient is present before Mohs layers are taken beyond stage I.

Can I Definitively Identify the Positive Area on the Patient?

Referring to the map and accurately identifying the same area on the patient appears straightforward. However, after three or more Mohs stages, and/or when operating upon tumors in such difficult anatomic locations as the nose, ear, or genitalia, disorientation easily occurs that may result in a Mohs layer taken from the wrong area. Disorientation can be minimized with the judicious use of angulated Mohs layers, application of double nicks, and the use of angulated borders at the leading edge of resections. Marking GV into the TNs also adds to the visual distinction of the last margin. What becomes most useful is a photographic print of the defect following the last margin. An 8- × 10-inch print of a digital photograph of the wound ensures anatomic accuracy and perspective. This photograph may be used as the Mohs map itself. This author considers such photographic adjuncts in the following situations:

1. When excising beyond stage IV.
2. When operating in the perineum/genitalia.
3. When dealing with deep or excavated defects.

Have I Marked Appropriate Margins in the Area to Be Excised?

The next challenge after accurately identifying the positive margin(s) is to decide how much additional tissue to excise. Caveats relevant to this decision include:

1. The Mohs map is a two-dimensional (2D) depiction of a three-dimensional (3D) wound.
2. What constitutes adequate tissue overlap of positive tumor areas?
3. "More is less" when dealing with high-risk tumors.

3D Defect Mohs processing flattens 3D tissue, sections it in a 2D plane, and annotates the findings on a 2D Mohs map. In other words, if the Mohs layer were a bowl, it would lie as flat as a plate after processing. Therefore, a tumor present near the base of a Mohs section may not just represent a positive base but also a positive periphery. A Mohs surgeon must visualize the 3D tumor location based on the 2D map and resect with adequate overlap, as discussed below. The manner in which the Mohs map is drawn can remind one of the 3D topography. The Mohs surgeon excises the specimen as a single piece in an angulated hexagonal shape. The

periphery of this specimen is then separated from the center in six sections, with the central deep fat as the seventh section. Sections 1 through 6 represent the outer superficial and outer deep margin and section 7 is the central deep margin. The vertical height and depth of these peripheral walls are reflected on the Mohs map as rectangular extensions from the central fat. By more accurately mirroring the depth and width of the tissue planes, and when combined with map annotations, this "origami" section diagram maximizes mapping accuracy.

Adequate Tissue Overlap Mohs mapping, albeit accurate, is not a global positioning system, and precise pinpointing is not realistic. Tissue loss is inevitable if a Mohs layer is divided into smaller sections. As much as possible, a Mohs layer should be processed as a single section to avoid processing errors and tissue loss. Given these potential errors, adequate tissue overlap in the excision of both the peripheral and deep margin is critical to compensate for the relative accuracy of Mohs surgery. If tumor is near a tissue nick, then it is wise to excise beyond the tissue nick. If a tumor is in the lower dermis, then it is prudent to include fat beyond the lower dermis. If a tumor is near muscle, then muscle must be in the resection plane. If a scar is present, then, whenever possible, remove tissue beyond the scar. If cancer is seen at the level of eccrine glands, then the subsequent layer must include at minimum the fat below the eccrine glands. Simply stated, superficially located tumor must include deeper tissue and deeper tumor must include more superficial levels of resection. Generally, a 2–3 mm margin lateral and deep to the involved tissue plane will ensure an adequate excision. More overlap is desirable for poorly differentiated, perineural, infiltrative, and recurrent tumors.

When Mohs layers are bisected and tumor is present near the base of only one section, the subsequent excision should overlap to the base of the other side as well. This not only ensures sufficient tissue excision but also creates a flat wound base that facilitates reconstruction. Map annotation is immensely helpful in ensuring sufficient tissue overlap. Noting the plane of tumor involvement on the Mohs map reminds one to remove beyond the involved tissue.

"More Is Less" When dealing with high-risk, potentially metastatic cancers, this author believes that generous Mohs layers (4-mm margins or more) are desirable. A 1–2 mm Mohs layer margin for a basal cell carcinoma (BCC) on the eyelid is ideal but becomes ridiculous for a DFSP or a poorly differentiated, recurrent SCC on the face. Tissue-sparing benefits are secondary to the priority of oncologic cure.

Have I Excised What I Intended to Excise?

The continuum of accurate orientation, processing, interpretation, mapping, and marking must be intraoperative. During excisions beyond stage I, the deep tissue is often compromised and fragmented for a variety of reasons, including the surgeon hurrying to remove the layer, visualization difficulties due to bleeding, fear of injuring deeper structures, and so on. To ensure adequate margin depth, this author often scores the deep margin before the peripheral margin. Doing so optimizes the connection between the superficial and the deep margin and allows removal of the intended layer with minimal fragmentation. This technique is valuable for tumors located in the lower dermis or deeper. Another method is to paint with GV the entire inner margin of excision. Removing the GV-inked area ensures a complete excision. For thin layers that are easily perforated, the GV technique is especially helpful. Smearing of the GV marking may occur in wounds lacking adequate hemostasis. Comparing the shape and size of the removed section is the final clinical checkpoint. The excised tissue should be wider and deeper than the positive tumor focus. Microscopically, the Mohs surgeon must see tissue planes above and below the level of tumor in the previous stage.

SUMMARY

"To err is human." (Editor's note: "To forgive is a medicolegal decision.") Errors increase as Mohs cases become more challenging for reason that include "aggressive" or extensive cancer, multiple stages, and/or complex anatomy. Regardless of the case complexity, the Mohs surgeon must preserve the accuracy of tissue orientation, processing, mapping, and subsequent excision. The techniques presented in this chapter are not necessary for every case. However, they are useful adjuncts and become indispensable as excisions continue beyond stage I. These methods have in common the theme of redundancy. Each technique is a redundant backup of another method to maintain precision should one system falter. Just as an air bag backs up a seat belt, adjunctive techniques serve one another to achieve the promised benefits of Mohs surgery: optimal cure rate and tissue conservation.

CHAPTER 17

Perineural Tumors

Alexander Miller

PERINEURAL TUMORS

Perineural invasion consists of tumor growth along, around, or in a nerve. The tumor must follow a nerve path to be considered perineural. Simply having a nerve incidentally sitting within a tumor mass does not qualify as perineural invasion. Perineural invasion generally begins in the small cutaneous nerves. Consequently, it most commonly occurs where there is a dense network of cutaneous nerves such as on the head and neck.

Incidence

The incidence of perineural invasion is somewhat difficult to quantify, as most studies on perineural invasion have been done by Mohs surgeons and/or in referral centers on select, high-risk population groups. The overall incidence of perineural invasion has been quoted as 5%.[1–3] Note that published incidences of perineural invasion are typically calculated from a select Mohs surgical or referral center patient population base. Consequently, the incidence values seem inflated, given that the majority of skin cancers are not treated with Mohs surgery and are therefore not included as part of the denominator when the incidence of perineural invasion is computed. A large population study from Australia calculated an incidence of perineural involvement of 2.7% of basal cell carcinomas (BCCs) in patients treated by Mohs surgery.[4] Others have determined an incidence of less than 1% to as high as 10%.[5–7]

Significance of Perineural Invasion

Why is perineural invasion important to recognize and treat appropriately? Subclinical perineural invasion can create a broader and deeper tumor burden than anticipated. This can result in greater surgical morbidity and reconstructive challenges. Tumors with perineural invasion have higher recurrence rates, which lead to additional morbidity. Since perineural invasion can follow nerves intracranially, there is a risk of debility and death from neglected or unrecognized tumors (Figure 17.1). Death can also result from metastasis, which is a risk particularly from aggressive squamous cell carcinomas (SCCs). Overall, perineural invasion is rare and, because of its rarity, may not always be carefully sought out and recognized.

Anatomy of Peripheral Nerves

Peripheral nerve fibers composed of axons and investing Schwann cells cluster together in fascicles of varying thickness permeated and surrounded by collagenous connective tissue. Larger nerves are composed of three basic structural components: endoneurium, perineurium, and epineurium. The endoneurium contains nerve fibers along with thin, intervening connective tissue strands, fibroblasts, scattered macrophages, and occasional capillary blood vessels. The ropelike endoneurium is surrounded by concentric layers of fibroblasts and collagen comprising the perineurium. Nerve trunks contain groups of nerve fascicles, perineurium, and a longitudinally oriented, outer connective lining of epineurium. The thicknesses of the

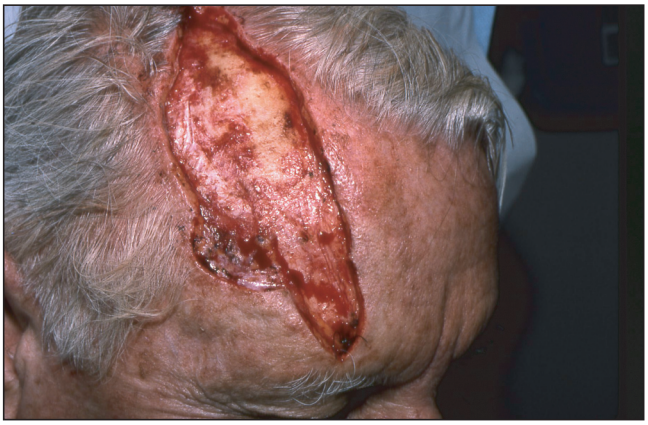

FIGURE 17.1: Perineural invasion of the supraorbital nerve (squamous cell carcinoma [SCC]).

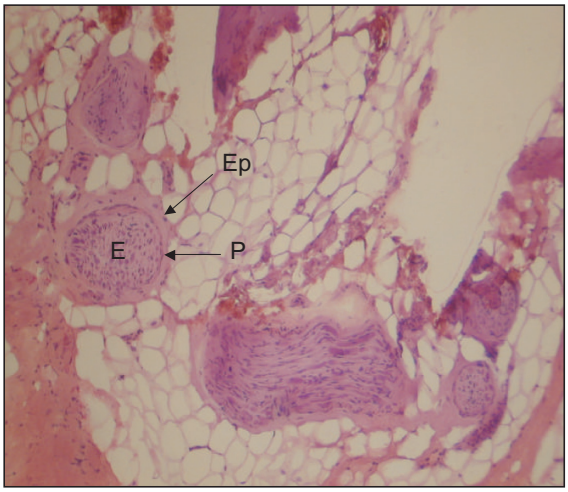

FIGURE 17.2: Epineurium (Ep); perineurium (P); endoneurium (E).

epineurium and perineurium vary with the nerve diameter. Smaller nerves have no epineurium and may have a very thin-to-indistinct perineurium (Figure 17.2). Peripheral nerves are fed by arterial branches that follow the nerve trajectory and freely anastomose around the nerve.

Perineural Invasion: Mechanism and Behavior

Why certain tumors invade in and around nerves, and the exact mechanism for this invasion, are not totally clear. It is felt that a natural cleavage plane underlies the perineurium and that this may facilitate the spread of tumor once it has penetrated into that potential space. Perineural invasion, from a practical standpoint, refers to tumor growth along or within the nerve. This is usually visualized as single or clustered tumor cells sitting along the edge of a nerve in longitudinal section or partially or completely encircling it on cross-section (Figure 17.3). Invasion of the endoneurium is rare.

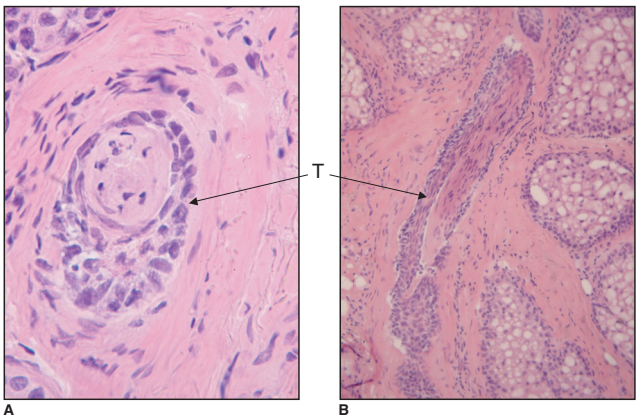

FIGURE 17.3: Perineural tumor (T) encircling nerve cross-sectioned (A) and longitudinally sectioned (B).

A tumor that has an affinity for nerves is not limited to invading a single nerve. Multiple nerve fibers, nerve branches, and even unrelated, separate nerves can be invaded by the same tumor. Perineural tumor can spread both proximally and distally along a nerve. Proximal invasion can lead to tumor extension into deeper structures, bony foramina, and intracranially. Distal spread can facilitate the extension of tumor into broad areas of dermis and subcutaneous tissue beyond the main body of the tumor. While growing along a nerve, the tumor may fully encircle it or may wind only along a limited edge of the nerve. An understanding of these properties facilitates optimal patient treatment and proper histologic evaluation of excised tissue. Most of the time, tumor spreads perineurally beyond the main tumor mass for a limited, short distance. A study of perineural invasion in SCCs found that most tumors extended 1 cm or less perineurally, and that extension of 2 cm or more was rare.[8]

Approach to the Patient

Sixty to seventy percent of patients with perineural tumor invasion have no signs or symptoms of nerve impairment. Perineural invasion is often not suspected clinically and is first identified when Mohs slide sections are examined. Symptoms or signs typically indicate advanced tumor and/or tumor enlargement in a tight space, such as a foramen, where nerve compression can result in impairment of function. Symptoms from sensory nerve involvement typically consist of paresthesias: tingling, burning, lancinating pains, poorly characterized aching, or anesthesia. Motor nerve invasion can lead to fasciculations, paresis, or paralysis of the affected musculature.

Pertinent physical findings should be sought out when perineural invasion is suspected. Patient examination should include a neurologic examination as well as palpation of named nerve sites underlying or bordering the tumor. In particular, the supraorbital nerve, when enlarged, is more easily palpated than other trigeminal or facial nerve branches (Figure 17.4). If intracranial or orbital extension of perineural tumor is suspected, appropriate imaging studies should be done. It is also essential to have in place a referral network of specialist physicians so that, when needed, a multidisciplinary approach to tumor extirpation can be activated. Such may be the case when orbital or intracranial spread of tumor is tracked.

Perineural Tumors: Types and Characteristics

The most common skin cancers, BCCs and SCCs, are also those that are most typically encountered invading perineurally. Perineural invasion is prevalent in microcystic adnexal carcinoma and has been described in other tumors (Table 17.1). Mohs surgery is the treatment of choice for nonmelanocytic cutaneous tumors with perineural

TABLE 17.1: Cutaneous Tumors with Potential for Perineural Invasion

- Basal cell carcinoma
- Squamous cell carcinoma
- Microcystic adnexal carcinoma
- Trichoepithelioma
- Glandular tumors:
  - Eccrine epithelioma
  - Adenoid cystic carcinoma
- Neurotropic malignant melanoma

invasion, and can also be useful in treating melanocytic tumors showing perineural invasion.

Basal Cell Carcinoma The incidence of perineural invasion reported in the largest series of Mohs surgical patients is 2.7%.[4] Perineural invasion by BCC is most prevalent on the head and neck, where BCC is most commonly located and where nerves densely populate the dermal and subcutaneous tissues. About 66% of perineural BCCs are located in the midfacial area: nose, cheek, maxilla, and forehead. Recurrent tumors, large tumors, and those with infiltrating, morpheaform, and metatypical (basosquamous) histologic patterns generate the highest incidence of perineural invasion. There is a slight predominance of males versus females affected with perineural invasion[1,2,4,9–11] (Table 17.2). Excision of perineural tumors generally requires more Mohs stages than similar tumors without perineural invasion. The rate of recurrence of BCCs following Mohs surgery is three times greater than that of BCCs lacking perineural invasion.[4]

Squamous Cell Carcinoma The incidence of perineural invasion has been reported to range from 2.5–14%, with the largest study to date finding a 6% incidence in Mohs surgically treated patients.[12,13] Perineural invasion is most commonly found in the midface and auricular areas, and is more common in men than in women. There is a slight preponderance of perineural invasion in recurrent tumors, and a definite association with diminishing tumor differentiation: perineural invasion predominates in moderately to poorly differentiated SCCs.

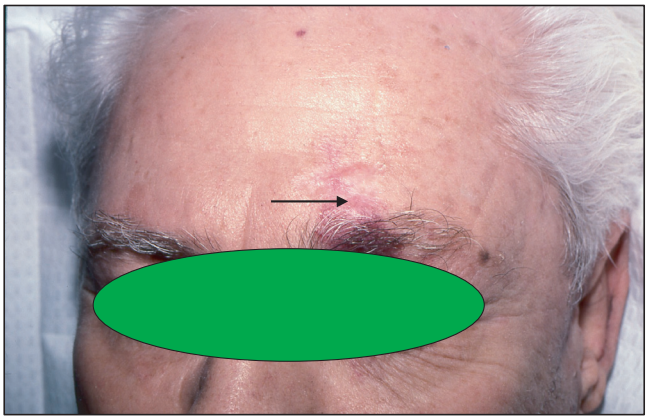

FIGURE 17.4: Recurrent deep basal cell carcinoma over trajectory of supraorbital nerve; nerve palpable (arrow).

TABLE 17.2: Basal Cell Carcinoma: Characteristics of Perineural Invasion

- Risk enhanced by:
  - Recurrent tumor
  - Large tumor size
  - Infiltrating, morpheaform, metatypical growth pattern
- Location: about 66% midfacial: nose, cheek, maxilla, forehead

Larger and deeper tumors also pose an increased risk of perineural spread. Perineural invasion is also associated with a higher rate of metastasis, in part related to the breadth of perineural tumor invasion, but also as a consequence of the aggressiveness of the tumor biology (Table 17.3).

Microcystic Adnexal Carcinoma The incidence of perineural invasion in microcystic adnexal carcinoma was 18% in a large study. Others have reported an incidence of up to 80%. Perineural invasion is particularly expected in recurrent microcystic adnexal carcinoma, where it has been identified in 86% of cases.[14] Mohs surgery is clearly the treatment of choice for these tumors.

Histology and Behavior

Common tumors that invade perineurally have similar microscopic appearances regardless of the primary tumor's histologic characteristics. Thus, basal cell, squamous cell, and microcystic adnexal carcinoma all look similar when encountered in the perineural space (Figure 17.5). Uncommonly, SCC will show perineural foci of spontaneous keratinization. The tumor cells may invade as a single-cell layer, as a thick cluster of perineural tumor cells, or as variable thicknesses of cells aligned around a given nerve. The tumor cells do not necessarily encircle the nerve. Rather,

Table 17.3: Squamous Cell Carcinoma: Incidence and Characteristics of Perineural Invasion

- Perineural invasion in about 6% of all Mohs-treated squamous cell carcinomas
- Men more commonly affected
- Slight preponderance in recurrent tumors
- Distribution: midface and auricular area
- Differentiation: moderately and poorly differentiated tumors predominate
- Larger preoperative tumor size
- Increased risk of metastasis

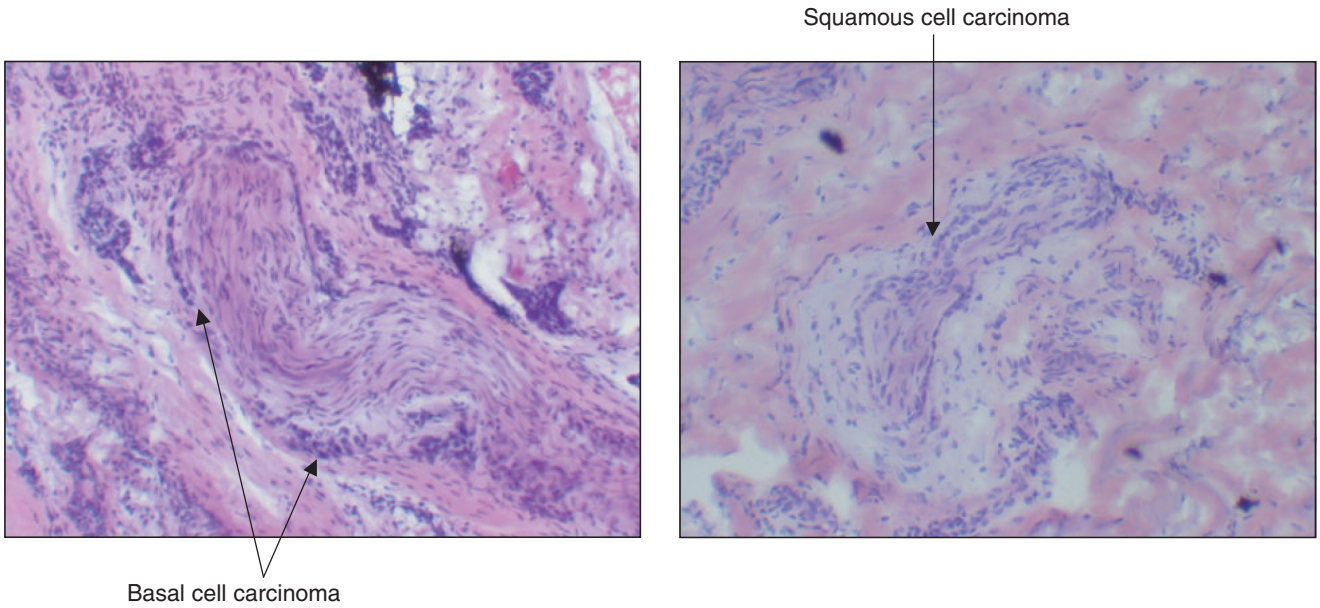

FIGURE 17.5: Histology of perineural invasion: similar cellular characteristics regardless of tumor type.

there may be areas where the invasion is concentric around a nerve and others where only a portion of the nerve circumference is invaded. The potential for partial as well as segmental perineural invasion creates challenges in identifying invasion, especially when there is only a single-cell layer of tumor cells present perineurally (Figure 17.6). One should also be aware that multiple and distinct, separate nerves may be involved within the same tissue specimen. Thus, the identification of perineural invasion in one area of tissue should lead to an intensified search for perineural tumor elsewhere within the same tissue block as well as in other tissue blocks (Figure 17.7).

Chronic inflammatory cells following the trajectory of a nerve are a valuable clue to the presence of perineural tumor and should lead one to carefully examine multiple sections looking for tumor. The tumor may appear obvious on some sections and may be absent or obscured by a dense inflammatory cell infiltrate in others (Figure 17.8).

Fibrous sleeves, also called "peritumoral fibrosis," can both mimic perineural invasion by resembling nerves and serve as markers for increased probability of perineural invasion. Fibrous sleeves are a concentric fibrosis that encircles a tumor strand (usually BCC). The fibrosis can create an illusion of a nerve that is invaded by tumor. The presence of such structures can be associated with a broad subclinical spread of tumor.

There are no specific special stains for identifying perineural invasion. Nerves are usually readily identifiable on hematoxylin and eosin (H&E) staining alone. S-100 antibody staining can be done to enhance the visibility

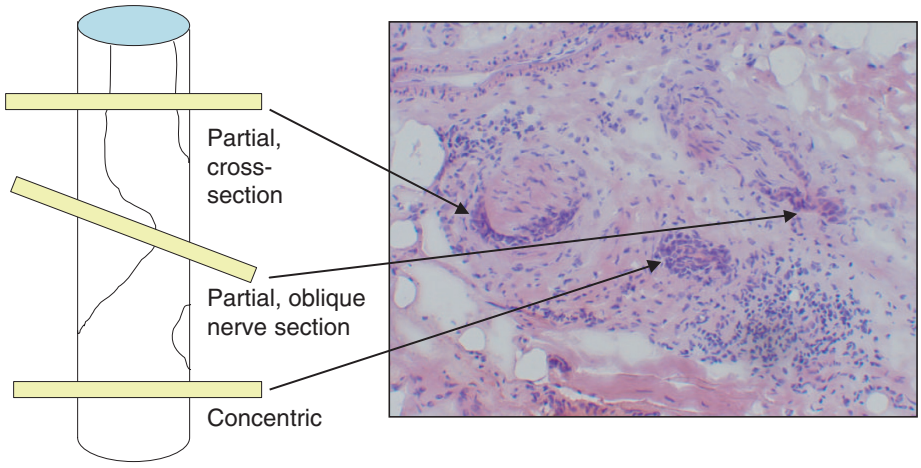

FIGURE 17.6: Partial to concentric perineural growth patterns.

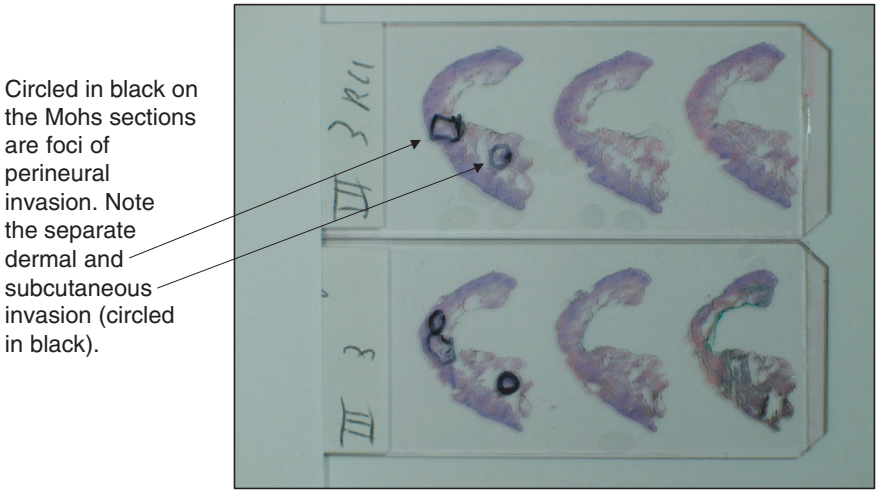

FIGURE 17.7: Multifocality of perineural invasion.

of nerves. A p75 nerve growth factor receptor (NGFR) immunoperoxidase stain has been used to identify some perineural SCCs.[15] The usefulness of this along with any other immunoperoxidase stains will remain limited in routine Mohs surgery, unless a stain that is brilliant, highly specific, easy and quick to use, and inexpensive is developed (Table 17.4).

TABLE 17.4: Histologic Characteristics of Perineural Invasion

- Rather uniform histology despite different tumor types
- Clusters to single cells aligned perineurally
- Partial to concentric perineural growth
- Multifocality of invasion
- Inflammation: clue to perineural spread
- Special stains: S-100; p75(NGFR) (for squamous cell carcinoma)

Keys to Patient and Slide Evaluation

A tumor with a high likelihood of perineural invasion (such as microcystic adnexal carcinoma), the location of a deeply invasive tumor over a foramen or along the expected trajectory of a named nerve, or recurrent tumor should all lead one to evaluate a patient preoperatively and intraoperatively for perineural invasion. Once perineural invasion is suspected or identified, the Mohs surgical excision should be adequately broad and deep. Subcutaneous perineural invasion can be missed if the tissue is excised superficially, above the level of the involved nerve. The subcutaneous

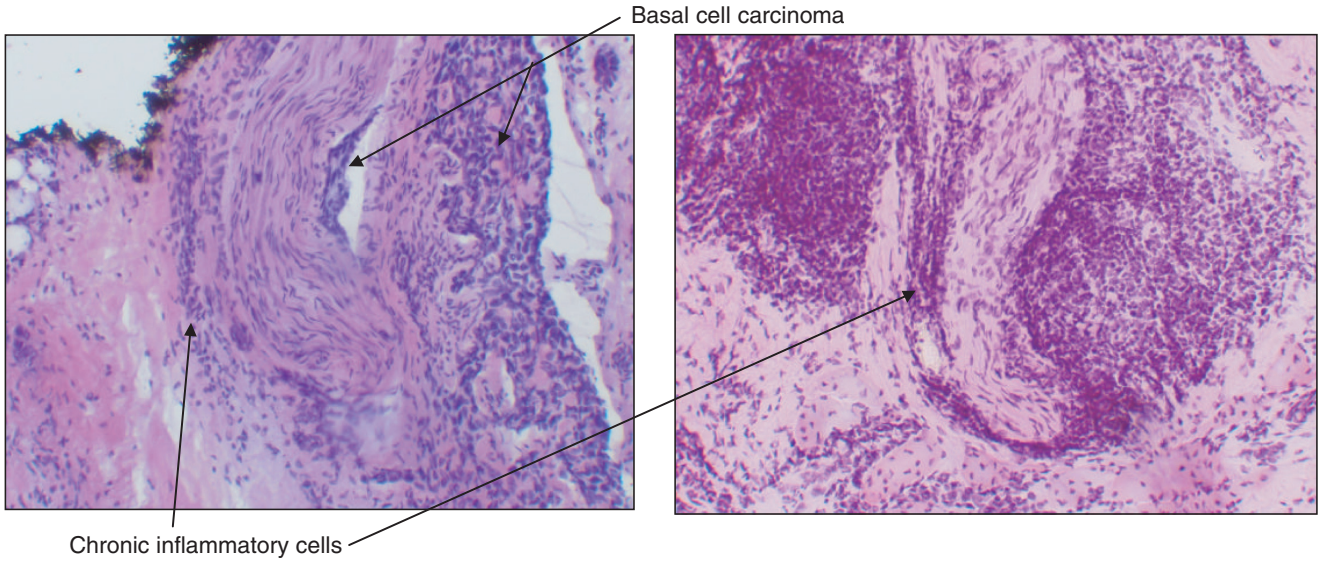

FIGURE 17.8: Perineural inflammation and perineural invasion.

TABLE 17.5: Keys to Patient and Slide Evaluation

- Pay attention to tumor type, location, recurrence.
- Evaluate tumor depth and excise deeply.
- Examine all sections on a slide; consider two or more slides per specimen.
- Ensure that true margins are visible on the slides.
- Check for slide tissue loss (fat fallout).
- Look for perineural inflammation and follow it.
- Pay attention to nerve orientation and the pitfalls that it presents.

fat should be adequately preserved on the microscopic slide to retain its architecture. Areas of missing or compressed subcutaneous fat can make perineural invasion unrecognizable.

Because there may be multiple nerves invaded on the same block of tissue and skip areas or partial invasion of the nerve circumference can happen, it is easy to overlook or ignore perineural invasion. It is therefore helpful to examine all of the sections on a given slide, even if the deeper cuts within the block are clear of tumor. Examination of multiple tissue wafers is helpful in making the diagnosis of perineural invasion. In order to ensure that true tissue margins are being observed, attention should be paid to visualizing the inking of the margins (see Chapter 10). Any persistent loss of tissue or margins on a slide should prompt one to ask for the technician to cut additional slides.

Perineural inflammation, as already mentioned, should be followed on several tissue sections, carefully searching for perineural tumor invasion. When evaluating tissue, particularly tissue in which perineural invasion is already known to be present, nerves that are sectioned tangentially or longitudinally should be thoroughly examined on several sections. This will enhance the possibility of identifying tumor that is tracking longitudinally along only one side of a nerve. The trajectory of an invaded nerve that is sectioned and the orientation of the sections through that nerve will determine whether tumor will be seen on a nerve in any given wafer as well as the way the tumor presents on any involved nerve(s) (Table 17.5).

SUMMARY: MAJOR POINTS

1. Highest risk for perineural invasion: recurrent tumor, microcystic adnexal carcinoma, deep tumor lying over a named nerve, undifferentiated tumor.
2. Perineural invasion can broadly involve multiple separate nerves or nerve branches.
3. Invasion patterns around a nerve vary: concentric, winding, linear.
4. When you see perineural inflammation, search for tumor invasion.
5. Maintain adequate depth of tissue excision.
6. Examine all sections on a slide.

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PART FOUR

SPECIAL TECHNIQUES AND STAINS

CHAPTER 18

Fixed-Tissue Mohs

Laura T. Cepeda, Daniel M. Siegel, and Norman A. Brooks

ZINC CHLORIDE paste (ZCP) is an escharotic and a tissue fixative. Its use in Western medicine was first popularized by Dr. Frederic Mohs for in situ tissue fixation as part of chemosurgery for cutaneous malignancies. Similar formulas were used by other less mainstream healers before Dr. Mohs, and some are still promoted and sold as herbal or natural cancer cures.

Dr. Mohs described the fresh-tissue technique of margin-controlled microscopic surgery without ZCP on carcinoma of the eyelid mucosa, and the routine use of ZCP during Mohs procedures lost favor after Tromovich and Stegman showed similar cure rates for basal cell cancer using fresh frozen tissue technique.

Zinc chloride paste still has utility as an adjuvant to surgery in treating melanoma and in certain situations in contemporary Mohs surgery where tissue fixation can be advantageous. In a retrospective review of patient data, Dr. Mohs had 1.5 times improved survival ($P < .003$) in patients with significantly thicker melanomas treated with ZCP prior to excision than a similar contemporaneous cohort of patients with thinner melanomas excised at Massachusetts General Hospital without prior pasting. Based on those data, Kalish et al. performed an animal study to determine if pretreating melanoma with ZCP prior to surgical excision improved outcomes. The study compared melanoma recurrence rates in mice when a tumor was either simply excised or first pasted with ZCP followed by excision and then rechallenged with injected melanoma cells. The data demonstrated that there was a decrease from 67% to 33% of new melanoma development in the mice upon tumor rechallenge when tumors from an immunogenic melanoma cell line were pretreated with ZCP prior to excision. The study indicated that ZCP stimulates antitumor immunity.

Because of these findings, we recommend the routine application of ZCP 1 to 7 days prior to excision of any melanoma thicker than 1 mm, followed by excision with standard or Mohs margins. It is an economical, simple procedure that offers a potentially significant survival advantage. There is no delay in closure of the wound; in fact, the only disadvantage is that it may add a day or two to treatment if excision is delayed due to ZCP application. One author (NAB) believes antitumor immunity may increase with time and therefore often delays standard margin excision up to 3 or 4 weeks after ZCP application without problems.

The formula for ZCP is the same formula originally described by Mohs:

Stibnite (80 mesh sieve) 40 g

Sanguinaria canadensis 10 g

Zinc chloride, saturated solution 34.5 cc

This can be prepared by a compounding pharmacy. One batch can last for years and does not expire. It may settle to bottom of container and require stirring before application. The container must be kept closed when not in use; the paste is a hygroscopic solution and will absorb ambient water from air if left open.

TECHNIQUE FOR UTILIZATION OF ZCP

Application

There are a few things to consider for successful application. First, the application of ZCP is painful, and second, it cannot penetrate intact stratum corneum. If there is concern that there is extension of subclinical tumor, a Wood's lamp may be used to help delineate the clinical margins. The biopsy site and any residual pigment are outlined with a marker.

Local prepaste anesthesia with 1% lidocaine with epinephrine is infiltrated into the outlined area. A superficial infiltration to achieve an "orange peel" appearance of the surface is all that is necessary (Figures 18.1 and 18.2). As ZCP will not penetrate intact stratum corneum, we remove this barrier using 50% trichloroacetic acid (TCA) or bichloracetic acid applied with cotton-tipped applicators until a definite white frost is achieved (Figures 18.3 and 18.4). Do

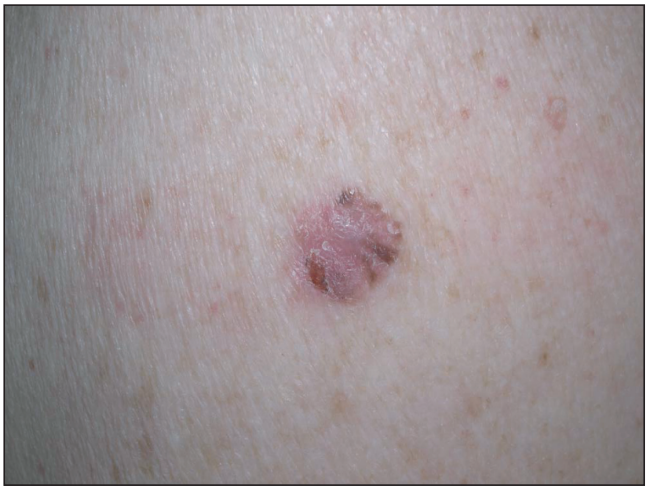

FIGURE 18.1: Biopsy-proven invasive melanoma of upper arm. Initial biopsy showed depth of 0.45 mm extending to base of specimen. Reexcision showed residual tumor to depth of 1.15 mm.

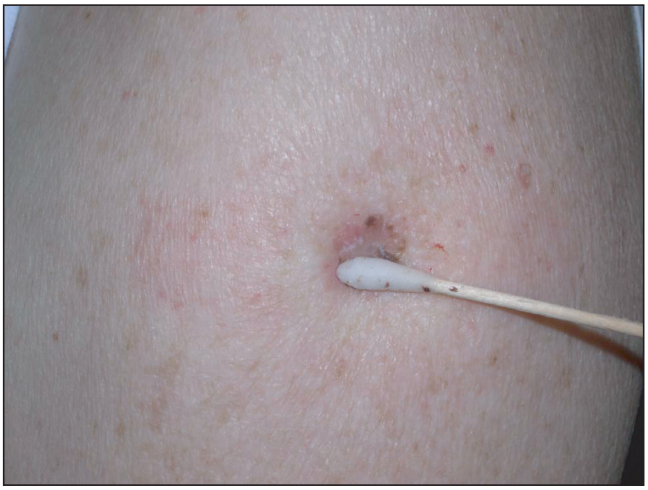

FIGURE 18.3: Application of 50% trichloroacetic acid (TCA) after anesthesia to deepithelialize tumor, being careful not to drip acid on noninvolved skin.

not trail saturated swabs or open containers of these acids over the patient; they are painful to apply, and unintended application is best avoided.

After TCA application, the ZCP is applied to the tumor in one or several layers with either end of a cotton-tipped applicator. The ZCP should be applied only to the tumor and not to the surrounding skin (Figure 18.5). Total thickness is usually 2–3 mm over the entire tumor, but for thicker tumors, more can be applied to achieve deeper penetration. Bandage the wound with a cotton ball on top of the ZCP to keep it from migrating to uninvolved skin. Cover the cotton ball with Tegaderm or OpSite for a waterproof dressing. Nothing further needs to be done to the site, and the dressing is left in place for 24 hours. The area needs no other special care. On the face, the dressing may be left in place until surgery to minimize the risk for ZCP inadvertently getting into the eye.

The patient may experience warmth, itching, and pain at the site of application as the local anesthetic wears off. Erythema or vesiculation of the site and a centimeter or more of surrounding tissue may occur, and fever or flulike symptoms may be seen with larger lesions. We offer narcotic pain relief to the patient if needed, although most patients report they do not need it. Regional lymph node tenderness may also be noted, along with some nodal enlargement, so a good nodal exam should be done preoperatively. This enlargement is transient and abates within a few days.

Excision may be performed from 1 to 7 days or more after ZCP application. Repair can be done immediately after excision. Zinc chloride paste does not interfere with

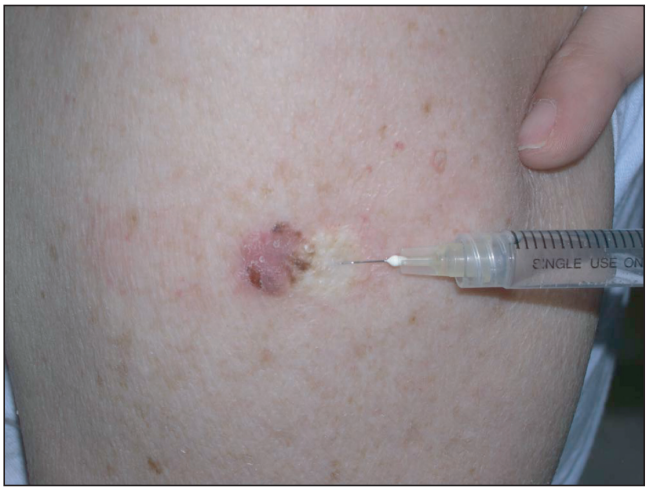

FIGURE 18.2: Superficial injection of local anesthesia to achieve "orange peel" effect.

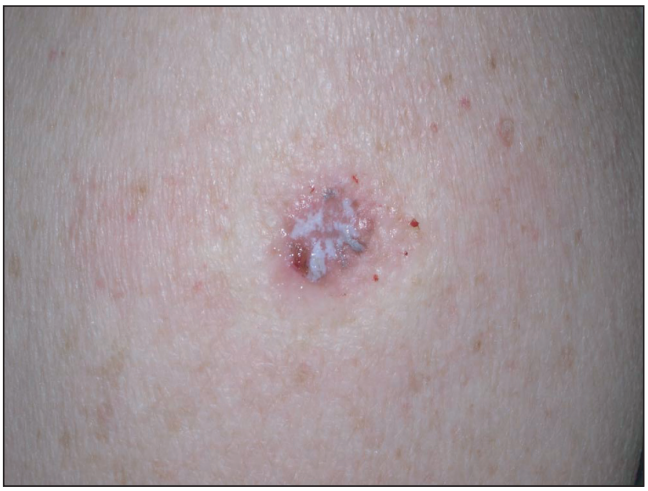

FIGURE 18.4: Partial frost over lesion after TCA application; TCA will be reapplied until thorough frost over entire lesion is achieved.

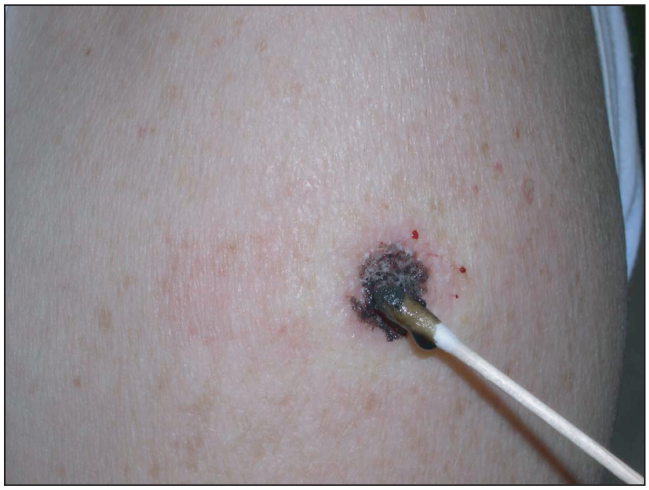

FIGURE 18.5: Application of ZCP as thin layers with cotton-tipped applicator.

sentinel lymph node biopsy. It has been reported that sentinel lymph nodes excised after ZCP application are larger than those in traditional excision, suggesting an immune-stimulating effect of the ZCP. If surgery is delayed beyond a week or so, spontaneous tissue separation and loss of the specimen may occur (Figures 18.6 and 18.7). If it does, the patient should be instructed to retain the specimen, which can still be submitted for histopathologic examination. One author (NAB) often delays standard margin surgery and routinely performs a small, fixed-tissue biopsy the day following ZCP application. If a patient declines surgery or is not a candidate for surgery for medical reasons, the site can be pasted and a suture placed for orientation for margin assessment; when the specimen separates, pathology with proper orientation is then still possible. Zinc chloride paste

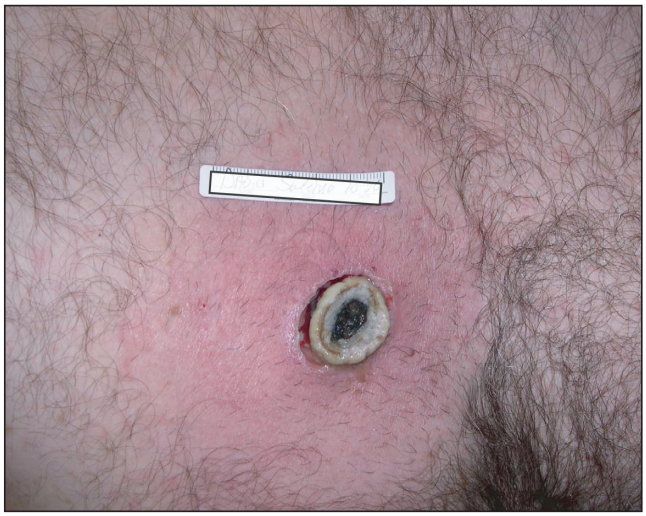

FIGURE 18.6: Subcutaneous nodule of metastatic melanoma, 10 days after ZCP application. Spontaneous tissue separation beginning to occur. Note erythema several centimeters around nodule and red granulation tissue at base.

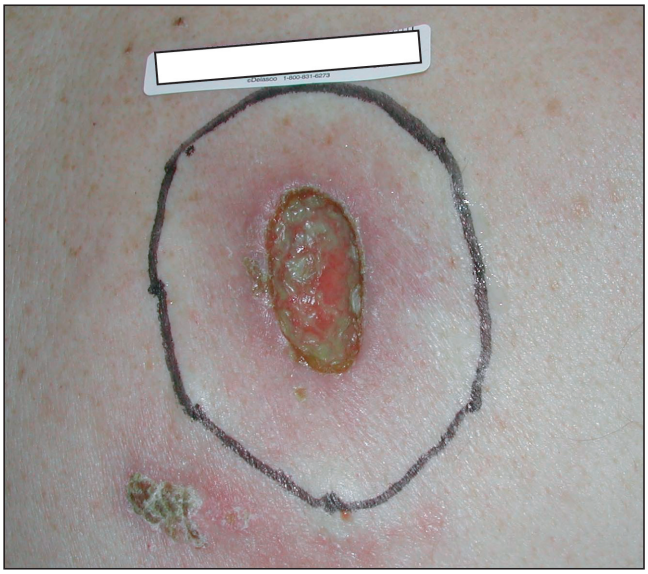

FIGURE 18.7: Malignant melanoma, 12 days after ZCP application. Specimen separated spontaneously prior to final excision.

application does not interfere with S-100 or other special stains.

In certain situations, ZCP can be applied to bone or soft tissue as part of an otherwise fresh-tissue session of Mohs surgery. Zinc chloride paste can be applied to any skin cancer with periosteum, bone, or tendon involvement. If the periosteum is microscopically involved or the bone shows pitting, a common sign of gross bony invasion, you can apply ZCP directly to it. This area of bone can be chipped away several weeks after paste application, or the bone can be allowed to spontaneously separate from viable tissue in 4 to 6 weeks. This separation can sometimes be expedited with the use of a periosteal elevator if the edges have a clear zone of separation. Healthy red granulation tissue is invariably present at the base after separation. After decalcification, the bone can be processed for routine histology to assess for residual tumor.

Another possible use of ZCP is in a patient who is prone to bleeding, especially if infection is a concern. Zinc chloride paste is a very effective hemostatic; intravascular thrombi are seen in the fixed tissue, and surgery on fixed tissue is essentially bloodless. Patients with any cutaneous malignancy who are poor surgical candidates may still be candidates for ZCP application and classic fixed-tissue Mohs surgery, with removal of fixed tissue 6 to 24 hours after paste application. Alternatively, the Mohs surgeon can wait for spontaneous separation of the tissue, as described above. If the latter route is taken, the tumor can be marked pre-ZCP application with a suture at the superior margin or similar landmark. In 1 to 3 weeks, the tissue will spontaneously separate from the base. The patient should be instructed to save the tissue, and it can be processed for permanent sections to check for margin

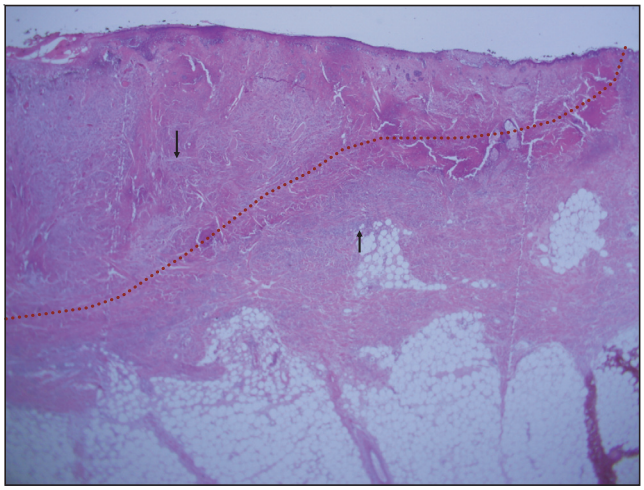

FIGURE 18.8: ***Broad, wedge-shaped zone of coagulative necrosis with dashed line demonstrating demarcation between fixed*** **(down arrow)** ***and nonfixed tissue*** **(up arrow)*****, altered collagen, and preservation of architecture.***

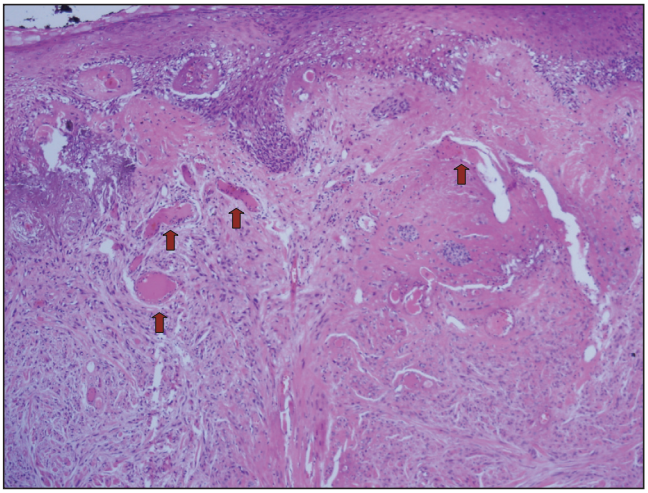

FIGURE 18.9: ***Higher magnification demonstrating fixation with preservation of architecture of specimen; note thrombi in vessels allowing for bloodless surgery*** **(red arrows).**

involvement of tumor. When the tissue separates, there is little or no blood. Also, the tissue is already fixed, so there is no concern of specimen preservation. The resultant wound, if found to be free of tumor, can be allowed to continue to heal by second intention.

Lesions of the external genitalia are treated no differently than lesions elsewhere, though the surgeon must remember that the edema that follows ZCP application in the vicinity of the urethra may result in temporary urinary retention. If this is a possibility, the need for catheterization, either intermittent or indwelling, should be discussed with the patient and involvement of an urologist in the patient's care should be considered.

Lesions of the oral mucosa pose a particular concern, as keeping the ZCP localized can be difficult. A thin layer of ZCP applied to the area of treatment, followed by the application of gauze held in place by the patient as long as tolerated, then followed by tap water swish and spit, is one approach that is functional but suboptimal. Lesions close to the lips may be exteriorized by placement of anchoring sutures for a few hours, but this is also suboptimal.

Lesions of the vaginal vault can be treated by application of paste, followed by coverage with cotton balls, which can be left in place for a few hours and then removed and the area irrigated with saline. Prolonged use of this technique could theoretically increase the risk of toxic shock syndrome.

Histology

There is necrosis of the stratum corneum and superficial epidermis secondary to the initial application of TCA. A wedge-shaped zone of altered collagen is also seen, with the base of the wedge at the surface of the specimen, corresponding to fixation of tissue by ZCP. This deepest part of the wedge is typically at the center of the tumor, which corresponds to the area of the thickest application of the ZCP (Figure 18.8). There will be some degree of inflammation between the fixed and unfixed tissue. Intravascular thrombi are often seen, hence the bloodless field Mohs originally achieved (Figure 18.9).

Complications

Complications relate to inadvertent application of ZCP to permeable surfaces such as mucosa. If this is avoided, complications are rare. One author (DMS) had a patient with an SCC involving the superior orbital rim bony structures in the early 1990s. The patient did not return in 24 hours, as instructed, for removal of the dressing and any loose paste; he returned three days later with a tender proptotic eye. Ophthalmic exam and local lubrication resulted in complete resolution without sequelae. Complications relating to necrosis deeper and/or beyond what is desired can be minimized by applying only a thin layer of ZCP when using the paste for nonmelanoma treatment. For melanoma, the surgeon may receive greater comfort with rigorous fixation, but there are no data on just how much fixation is optimal.

Contraindications

Allergy to any of the components of the paste would be a relative contraindication, not an absolute one. Allergy to bloodroot is rare; we have never seen acute allergic reactions.

CHAPTER 19

Toluidine Blue Stain for Mohs Micrographic Surgery

Ofer Arnon, Adam J. Mamelak, and Leonard H. Goldberg

BASIC CONCEPTS

Since the advent of Mohs surgery and the introduction of frozen sections into the micrographic technique, dermatologic surgeons have experimented with different histopathologic staining methods to highlight neoplastic tissue and ensure clear margins. Currently, hematoxylin and eosin (H&E) represents the most common method for staining frozen sections employed by Mohs surgeons. However, toluidine blue (T-blue) remains a fast and effective stain for Mohs micrographic surgery, especially when treating basal cell carcinoma (BCC).[1]

T-blue is a basic dye and stains acidic matter.[2] The stain is actually a complex of different dyes of slightly different colors.[3] During routine histopathology, it is often utilized to highlight amyloid and mast cells in tissue. These appear blue and red-purple in color, respectively.

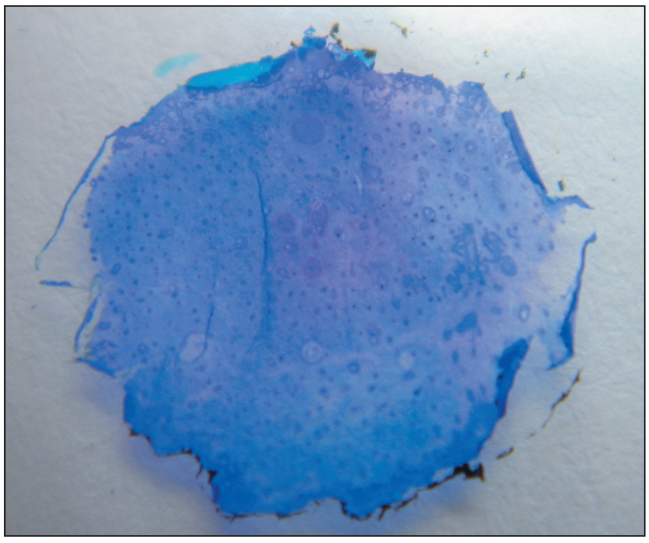

FIGURE 19.1: Mohs section stained with T-blue. The tissue appears as a single blue color; islands of BCC are stained orthochromatically with a deep blue color. The surrounding stroma highlights the tumor with characteristic pink or magenta color (center).

Grossly, the stain appears as a single blue color and can behave as a single dye, coloring cells deep blue. However, proteoglycans appear red when stained with T-blue. This change of color is called metachromasia (Figures 19.1 and 19.2).

There is still considerable uncertainty as to the exact nature of the phenomenon known as metachromasia.[2,4–6] The phenomenon itself seems to be quite simple: a pure basic dye in aqueous solutions absorbs light in a distinct manner and so appears to be colored.

When the dye reacts with tissue element to produce a color similar to that of the dye, it stains orthochromatically. T-blue may stain some tissue components orthochromatically; however, it can produce a different color, or metachromasia, with other components (Figures 19.2 and 19.3).

T-blue is a "progressive stain." That is, the tissue is stained to the desired color without using differentiation steps. Hematoxylin, on the other hand, is a regressive

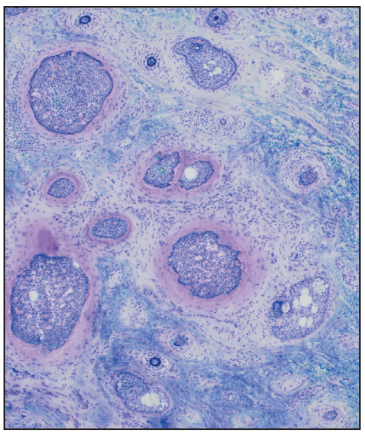

FIGURE 19.2: Basal cell carcinoma. Islands of tumor are stained orthochromatically and appear deep blue or even purple. Characteristic retraction artifact and peripheral palisading of nuclei are observed. The surrounding stroma highlights the tumor with characteristic pink or magenta color (T-blue, 100× magnification).

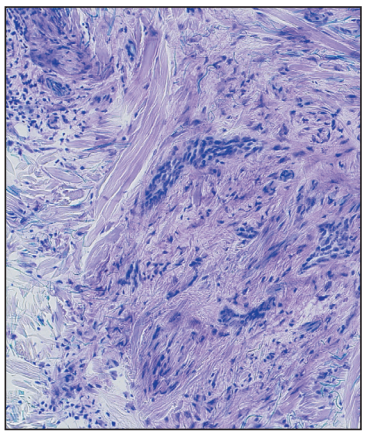

FIGURE 19.3: Sclerosing basal cell carcinoma (BCC). Angulated nests and thin strands of basaloid cells surrounded by pink stroma in the dermis are observed (T-blue, 400× magnification).

stain that involves initially overstaining tissues followed by "decolorizing" for structure differentiation.

The major advantage of T-blue staining over H&E in Mohs surgery is seen with BCC. Islands of BCC tumor are stained orthochromatically and appear deep blue. However, the surrounding stroma highlights the tumor with a characteristic pink or magenta color (Figures 19.2 and 19.3).

T-blue also highlights the dyskeratosis that is observed in the neoplastic cells comprising squamous cell carcinoma (SCC). Here, malignant squamous cells are stained turquoise in color (Figures 19.4–19.9). This is in contrast to basal cells that stain dark blue, thus allowing differentiation between the two (Figures 19.2 and 19.3).

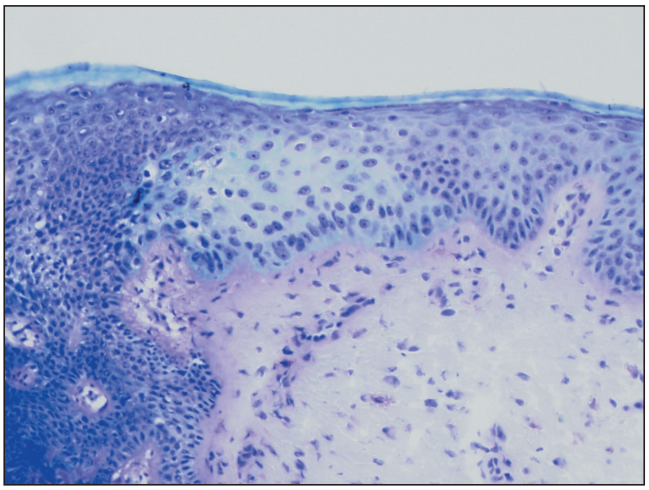

FIGURE 19.4: Bowen's disease. Aberrant epidermal maturation and a turquoise hue imparted on the atypical squamous cells are observed compared with the adjacent normal epidermis. A "wind-blown" appearance is observed among the malignant cells (T-blue, 100× magnification).

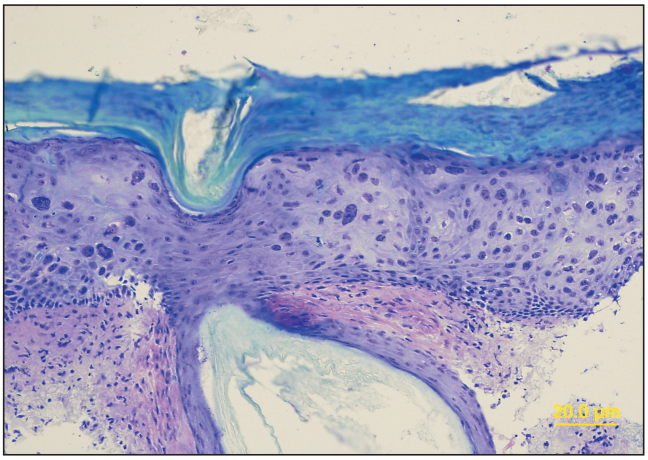

FIGURE 19.5: Squamous cell carcinoma in situ. Nuclear details of tumor cells are sharp and clear. Nuclear shape, nucleoli, chromatin clumping, and the presence of mitoses are easily observed. These features are critical when differentiating squamous cell carcinoma from benign epidermal changes (T-blue, 400× magnification).

SETUP AND STAINING PROCEDURE

Tissue is excised from patients using standard Mohs micrographic surgical techniques. Specimens are imbedded in optimal cutting temperature (OCT), and frozen sections are cut from 4–6 µm in thickness and mounted on glass microscope slides. These slides are then stained for analysis.

The T-blue staining method is simple and produces high-quality slides in only 6 to 7 minutes. Staining requires

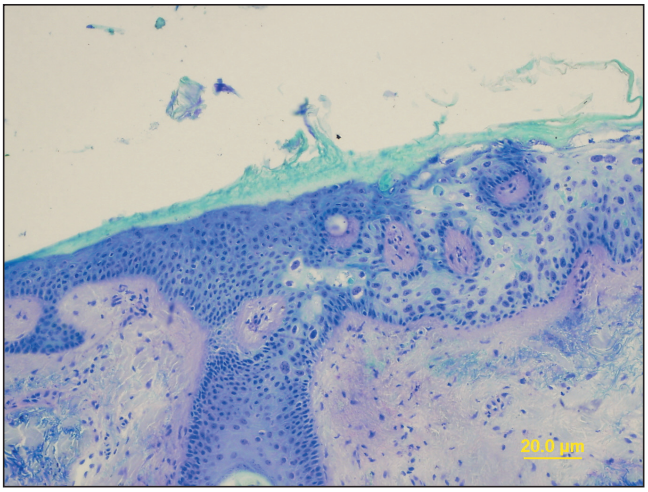

FIGURE 19.6: Squamous cell carcinoma in situ. Crowding of the basal layer, aberrant maturation of the epidermal layers, and nuclear pleomorphism are observed. A turquoise hue highlights the dyskeratosis in the malignant epidermal cells. These features differentiate the squamous cell carcinoma (SCC) from the adjacent benign epidermis to the left (T-blue, 100× magnification).

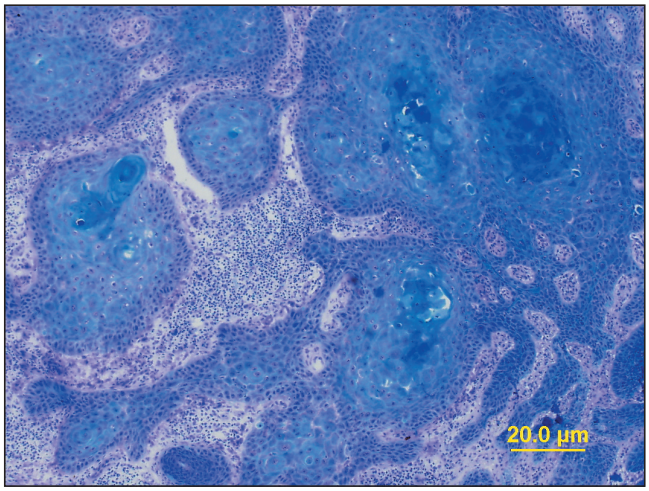

FIGURE 19.7: ***Invasive SCC. The dyskeratosis in the malignant cells stains turquoise in color (T-blue, 100× magnification).***

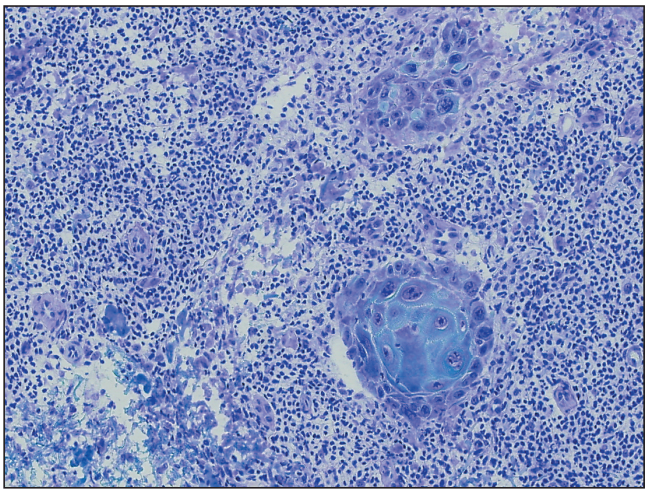

FIGURE 19.8: ***Invasive SCC surrounded by a dense lymphocytic inflammatory infiltrate. The dyskeratosis in the malignant cells stains a turquoise color. Nuclear pleomorphism can also be observed (T-blue, 400× magnification).***

the progressive submersion of the slides in seven different steps:

1. Fixation in absolute alcohol for 30 seconds
2. Running water for 60 seconds
3. T-blue 0.5% for 60 seconds
4. Running water for 60 seconds
5. 95% alcohol for 30 seconds
6. Absolute alcohol for 90 seconds
7. Clearant for 90 seconds

These steps can be performed manually or more easily with an automatic linear stainer. Following this, the slides are coverslipped using mounting media and glass coverslips.

COMMENTS

Considerable work has been done to evaluate the utility of T-blue staining in Mohs frozen sections. A study comparing T-blue and H&E,[7] as well as 26 years of the senior author's nearly daily experience with this method, has allowed a number of benefits of T-blue to be recognized (Table 19.1). Staining time is shorter for T-blue–versus H&E-stained sections (7 vs. 9 minutes). This translates into greater efficiency in the clinic and shorter patient wait times.

Histologically, the nuclear details of tumor cells are subjectively sharper and clearer in T-blue–stained sections. Specifically, the nuclear shape, nucleoli, chromatin clumping, and presence of mitoses can all be better appreciated with T-blue (Figures 19.6, 19.8, 19.10). These features are critical when evaluating SCC and benign epidermal changes.[7]

Both T-blue and H&E easily identify individual cell keratinization and necrosis present in SCC. Extracellular keratin and collagen are also identified equally well with both stains. Keratin in the stratum corneum, like intracellular keratin, is characteristically eosinophilic with H&E and aqua or turquoise in color with T-blue (Figures 19.6 and 19.11). Both stains also illustrate peripheral palisading and the retraction artifact at the periphery of BCC tumor lobules equally well. However, T-blue further identifies

TABLE 19.1: Comparison of T-blue and H&E Staining in Mohs Frozen Sections

T-Blue	H&E
One-color stain	Most common stain used in Mohs surgery
Metachromatic/mucopolysaccharides-pink	More prominent visibility of individual cell keratinization and necrosis
Used routinely in the senior author's lab and 16.8% of surveyed Mohs surgeons[7]	More time-consuming than T-blue (9-minute stain)
Rapid stain (7 minutes)	
Reliable and durable for >30 years	
Can be rinsed off and stained with H&E if necessary	

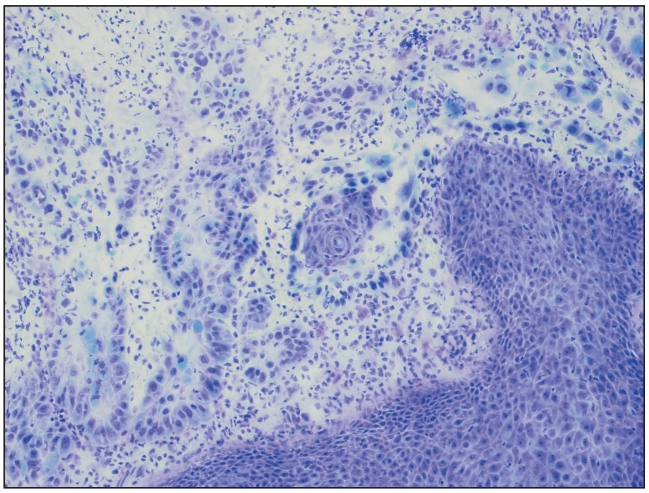

FIGURE 19.9: Invasive SCC. Turquoise color highlights the dyskeratosis produced by the invasive neoplastic cells in the dermis (T-blue, 100× magnification).

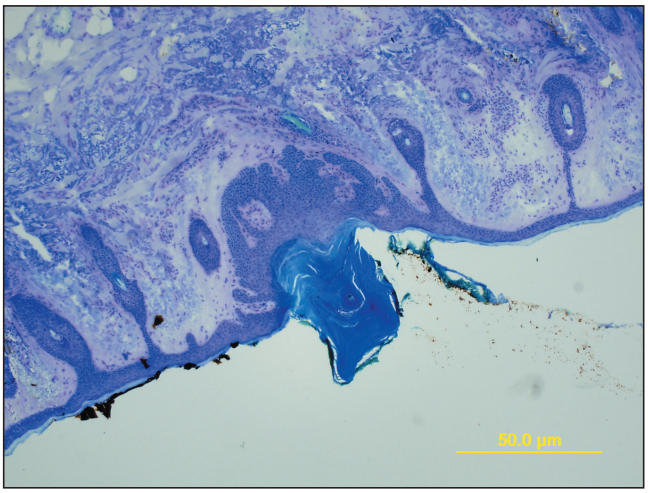

FIGURE 19.11: Cutaneous horn. A mound of hyperkeratosis stains turquoise atop a benign epidermal proliferation (T-blue, 400× magnification).

mucopolysaccharides surrounding the tumor lobules (Figures 19.1–19.3). This is not appreciated in H&E-stained sections. This stromal pattern is intense around BCC lobules but can also be seen surrounding SCC, although fainter. We have previously described the "setting-sun sign" in T-blue–stained BCC sections. This sign represents the intense pink staining of the mucopolysaccharides and is extremely useful in evaluating tumor clearance.[8]

As mentioned, T-blue is one of the stains of choice for highlighting mast cells in tissue, readily identifiable by their bright purple metachromatic granules. These cells are abundant in the stroma of BCC, compared with tumor-free tissue, and can also sometimes be seen in the stroma surrounding SCC. In contrast, mast cell granules are only occasionally seen on H&E-stained sections. This is important, as abundant mast cells, like the presence of mucopolysaccharides, may be indicative of the presence of residual tumor.[7] Appendages and soft-tissue structures are easily identified on Mohs frozen sections regardless of stain. Hair follicles are easily seen with both T-blue and H&E; however, if the follicle is cut transversely or tangentially, the inner root sheath may appear deep blue with T-blue staining. This is an important feature, as the basaloid cells surrounding hair follicles can be difficult to distinguish from BCC on some frozen sections. Perifollicular and perineural SCC can be readily identified in T-blue–stained sections (Figures 19.12–19.15). In general, it is easier to detect the isolated nests and perineural invasion of microcystic adnexal carcinoma in Mohs surgical sections stained with T-blue than with H&E.[9] Other benign and malignant tumors can also be readily and comparably identified with T-blue and H&E (Figures 19.16 and 19.17). Fat, which is

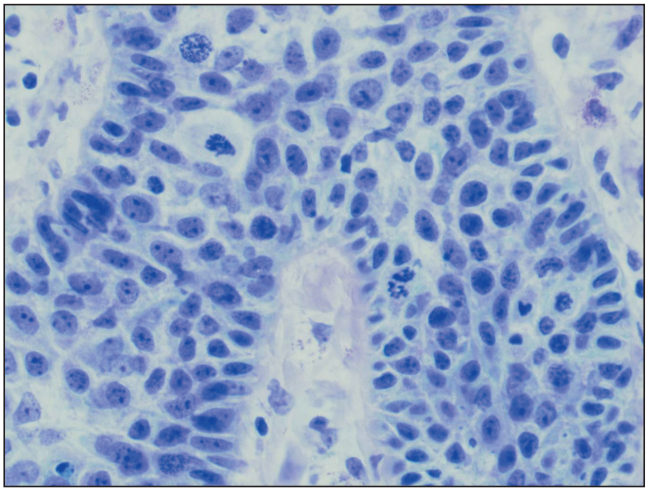

FIGURE 19.10: Squamous cell carcinoma. Pleomorphic nuclei, chromatin clumping, and mitoses are easily observed (T-blue, 400× magnification).

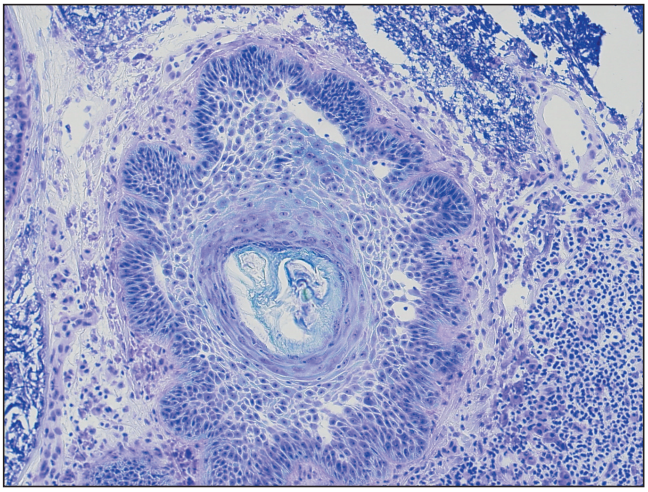

FIGURE 19.12: Atypical squamous cells encircling and tracking down a hair follicle can be observed in SCC and proliferative actinic keratoses (T-blue, 100× magnification).

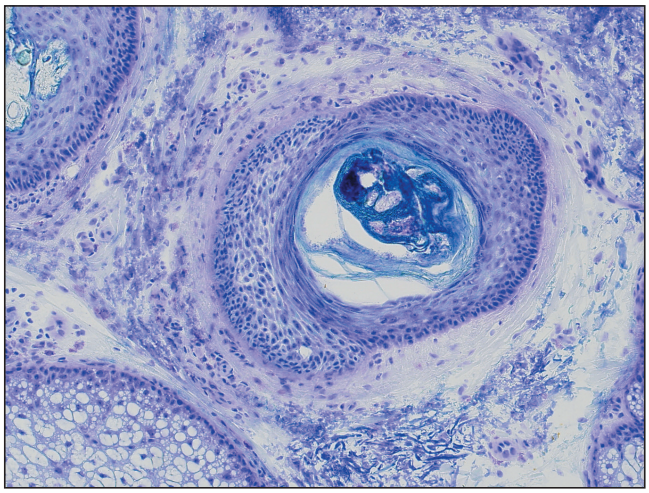

FIGURE 19.13: *Atypical squamous cells encircling and tracking down a hair follicle often seen in SCC and proliferative actinic keratoses (T-blue, 100× magnification).*

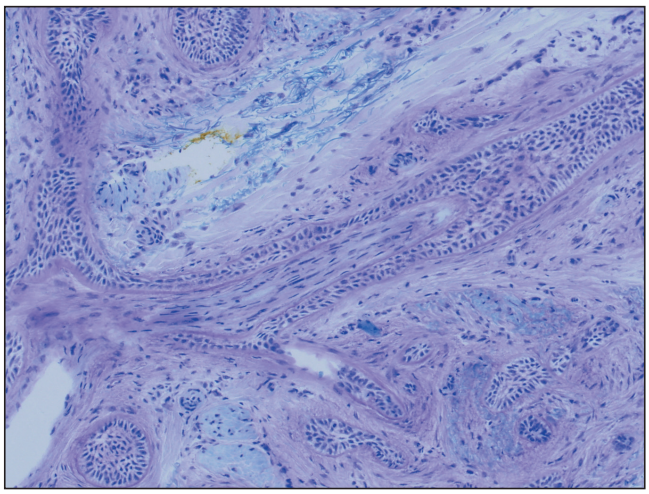

FIGURE 19.14: *Squamous cell carcinoma with perineural invasion. Malignant keratinocytes are seen tracking along the nerve fiber (T-blue, 100× magnification).*

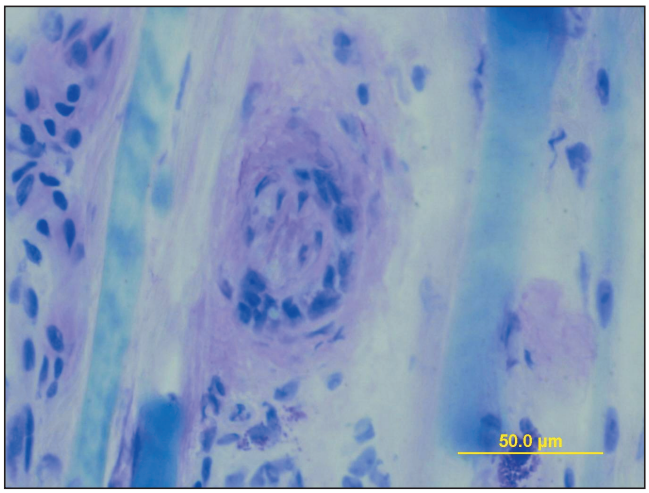

FIGURE 19.15: *Squamous cell carcinoma with perineural invasion. Malignant keratinocytes are seen surrounding a nerve fiber (T-blue, 400× magnification).*

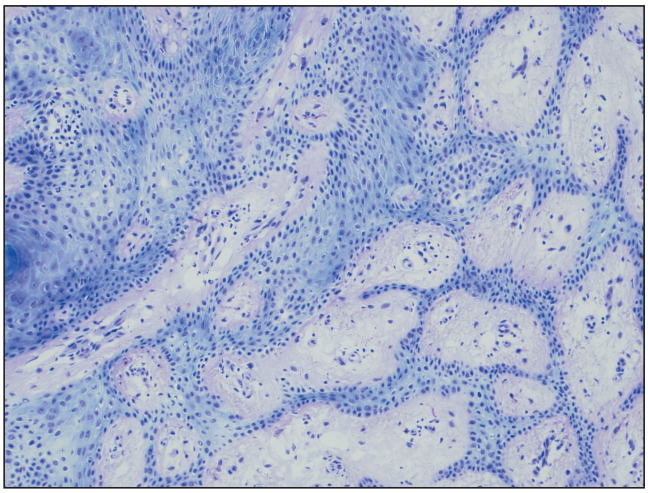

FIGURE 19.16: *Seborrheic keratosis. Reticulated strands of benign epidermal cells are observed. There is some dyskeratosis as indicated by the turquoise hue. However, no nuclear pleomorphism, mitoses, or other atypical features are observed in this benign lesion (T-blue, 100× magnification).*

difficult to cut on frozen section, is easily identified with T-blue and H&E. In addition, cartilage, muscle, and nerves are easily identified with both stains.

PROBLEMATIC AREAS

T-blue represents a fast and effective method for staining Mohs frozen sections. However, H&E is currently the most common stain employed for this purpose. Because of this, new Mohs surgeons are often faced with a time-consuming learning curve to appreciate the histopathologic details in T-blue sections. A counterstain to highlight extracellular components is also lacking in T-blue sections. Finally, melanocytes can also be appreciated with T-blue staining, although melanoma evaluation has historically been performed with H&E. The likelihood of missing a melanoma with T-blue, however, is not very great.

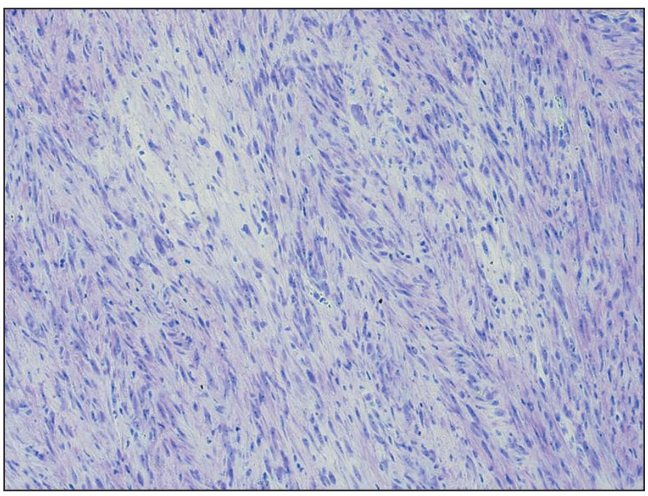

FIGURE 19.17: *Atypical fibroxanthoma. A spindle cell proliferation with cellular atypia and nuclear pleomorphism is seen throughout the dermis (T-blue, 100× magnification).*

Pearls

- The quality of T-blue staining improves when the stain is aged for more than one month.
- T-blue is often used as a counterstain for histologic and cytologic specimens, but it is used as a direct stain for Mohs micrographic section.
- The staining of mucopolysaccharides can be an important visual clue to residual tumor in sections where tumor cells are sparse.
- T-blue staining can be rinsed off and the tissue stained with H&E, if necessary.

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9. Wang SQ, Goldberg LH, Nemeth A. The merits of adding toluidine blue–stained slides in Mohs surgery in the treatment of a microcystic adnexal carcinoma. *J Am Acad Dermatol*. 2007;56:1067–1068.

CHAPTER 20

Forms and Templates for Mohs Surgery

Ken Gross and Howard K. Steinman

MOHS SURGERY is a highly orchestrated procedure performed in a logical and predictable sequence in patient after patient and session after session. It thus lends itself to the use of preprinted forms and templates. These can be adapted to the individual methods of each surgeon to serve as a checklist before, during, and after each Mohs procedure. This ensures that the entire sequence of interrelated steps of each Mohs case is performed accurately and efficiently.

There is nothing as frustrating as having a patient present for surgery with neither the patient nor the surgeon able to identify the exact location of the cancer site. Figures 20.1 and 20.2 documents the initial patient encounter, recording referral information and history and physical findings of importance for a patient who will undergo a multihour procedure using local anesthesia. The diagram may show the location of the lesion with cross-measurements from several easily referenced anatomic landmarks (Figure 20.2). A digital photograph of the site may also be taken at the first visit to document the cancer site. Some surgeons take a photograph and document cross-measurements giving the Mohs surgeon-pathologist two methods of localizing the correct cancer site on the day of surgery.

Form 2 (Figure 20.3) is a short referral form that can be completed quickly and returned to the referring physician. There are areas in which to document when the patient was seen, the treatment plan, tumor type and anatomic site, and the referring doctor's biopsy number. This serves to ensure that both the referring physician and surgeon know on which cancer site Mohs surgery will be performed.

Form 3 (Figure 20.4) is used to document informed consent. The front of the form lists the treatment options discussed and includes a diagram to briefly illustrate how the procedure will be performed, how long the patient should expect to be in the office, the expected range of cure rates for the chosen treatment and tumor type, and the potential complications of the selected treatment (Mohs surgery). Informed consent and permission for photography are documented on the back of the form (Figure 20.5). Equally important on the back is the area in which to record that the patient should be seen for follow-up after surgical healing by the referring physician.

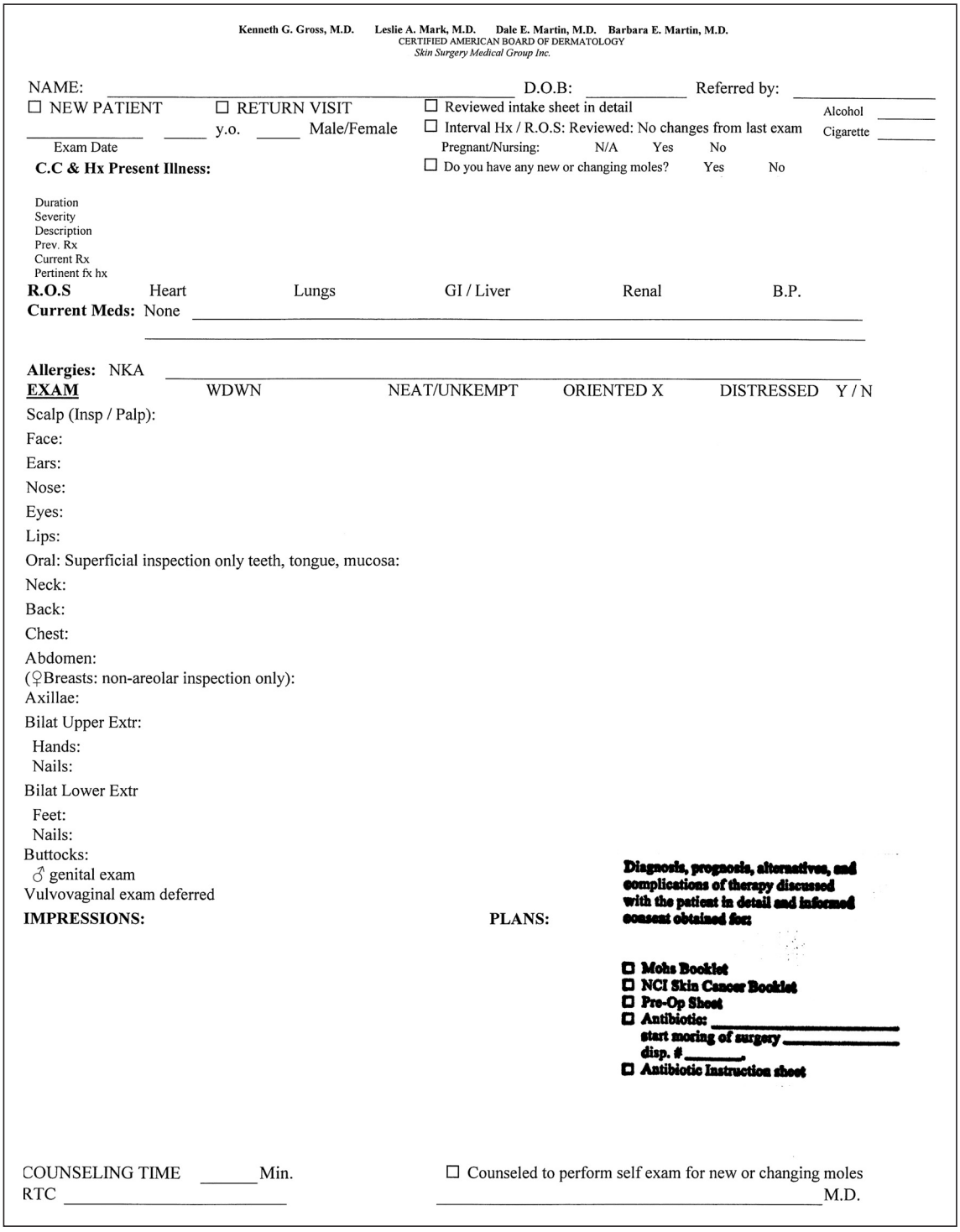
Form 4 (Figure 20.6) is a preoperative instruction sheet, given to the patient with an antibiotic prescription if prophylactic antibiotics are to be used (Figure 20.7), a Mohs booklet (Figure 20.8), and a booklet from the National Cancer Institute (NCI) titled "Skin Cancer" (Figure 20.9). The patient's cancer diagnosis can be written on the front of this booklet (Figure 20.10). Many surgeons alternatively provide skin cancer and Mohs surgery patients with information materials available for purchase from the American Academy of Dermatology, the American Society for Mohs Surgery, the American College of Mohs Surgery, and the American Society for Dermatological Surgery.

Form 5 is an intraoperative Mohs worksheet (Figure 20.11 [front] and Figure 20.12 [back]). The top part of the form may be completed before the day of surgery so that the Mohs surgeon wastes no time during the busy Mohs evolution. If there is a referring physician, preoperative, intraoperative, and postoperative pictures are recorded and sent to that physician documenting what was done and thanking him or her for the referral. Pictures may be taken for many other reasons, and the form documents when these were taken. The operative note is dictated from this completed form.

Form 6 (Figure 20.13) shows the Mohs pathology worksheet. Notice that the form registers the tissue inking "color codes" used as well as the location and outline of the first stage of surgery.

Form 7 (Figure 20.14) shows a three-page dictation outline. Using the Mohs worksheet and this outline, a complete operative report for even the most complex case can be dictated in approximately two minutes.

Crossing out errors on the Mohs path report form and Mohs worksheet could cause confusion that might lead to significant errors in the location of reference marks, cancer,



Kenneth G. Gross, M.D. Leslie A. Mark, M.D. Dale E. Martin, M.D. Barbara E. Martin, M.D.
CERTIFIED AMERICAN BOARD OF DERMATOLOGY
Skin Surgery Medical Group Inc.

NAME: _____ D.O.B: _____ Referred by: _____

☐ NEW PATIENT ☐ RETURN VISIT ☐ Reviewed intake sheet in detail Alcohol _____

_____ _____ y.o. _____ Male/Female ☐ Interval Hx / R.O.S: Reviewed: No changes from last exam Cigarette _____

Exam Date Pregnant/Nursing: N/A Yes No

C.C & Hx Present Illness: ☐ Do you have any new or changing moles? Yes No

Duration
Severity
Description
Prev. Rx
Current Rx
Pertinent fx hx

R.O.S Heart Lungs GI / Liver Renal B.P.

Current Meds: None _____

_____

Allergies: NKA _____

EXAM WDWN NEAT/UNKEMPT ORIENTED X DISTRESSED Y / N

Scalp (Insp / Palp):
Face:
Ears:
Nose:
Eyes:
Lips:
Oral: Superficial inspection only teeth, tongue, mucosa:
Neck:
Back:
Chest:
Abdomen:
(♀Breasts: non-areolar inspection only):
Axillae:
Bilat Upper Extr:
Hands:
Nails:
Bilat Lower Extr
Feet:
Nails:
Buttocks:
♂ genital exam
Vulvovaginal exam deferred

IMPRESSIONS:

PLANS:

Diagnosis, prognosis, alternatives, and complications of therapy discussed with the patient in detail and informed consent obtained for:

- ☐ **Mohs Booklet**
- ☐ **NCI Skin Cancer Booklet**
- ☐ **Pre-Op Sheet**
- ☐ **Antibiotic: _____**
  start morning of surgery _____
  disp. # _____
- ☐ **Antibiotic Instruction sheet**

COUNSELING TIME _____Min. ☐ Counseled to perform self exam for new or changing moles

RTC _____ _____M.D.

FIGURE 20.1: Front side of the initial patient intake, physical, and history form. The stamp on the bottom right is a checklist of items used for each Mohs surgery patient at the initial visit.

and other findings. For this reason, the use of a correction fluid, or white-out, by the surgeon-pathologist on these forms is now acceptable, as stated on one office manual in our practice, and was approved in a recent Institute for Medical Quality (IMQ) inspection.

With the increasing use of computers, many of these forms will become obsolete. But for those not yet fully computerized, they can be very helpful.

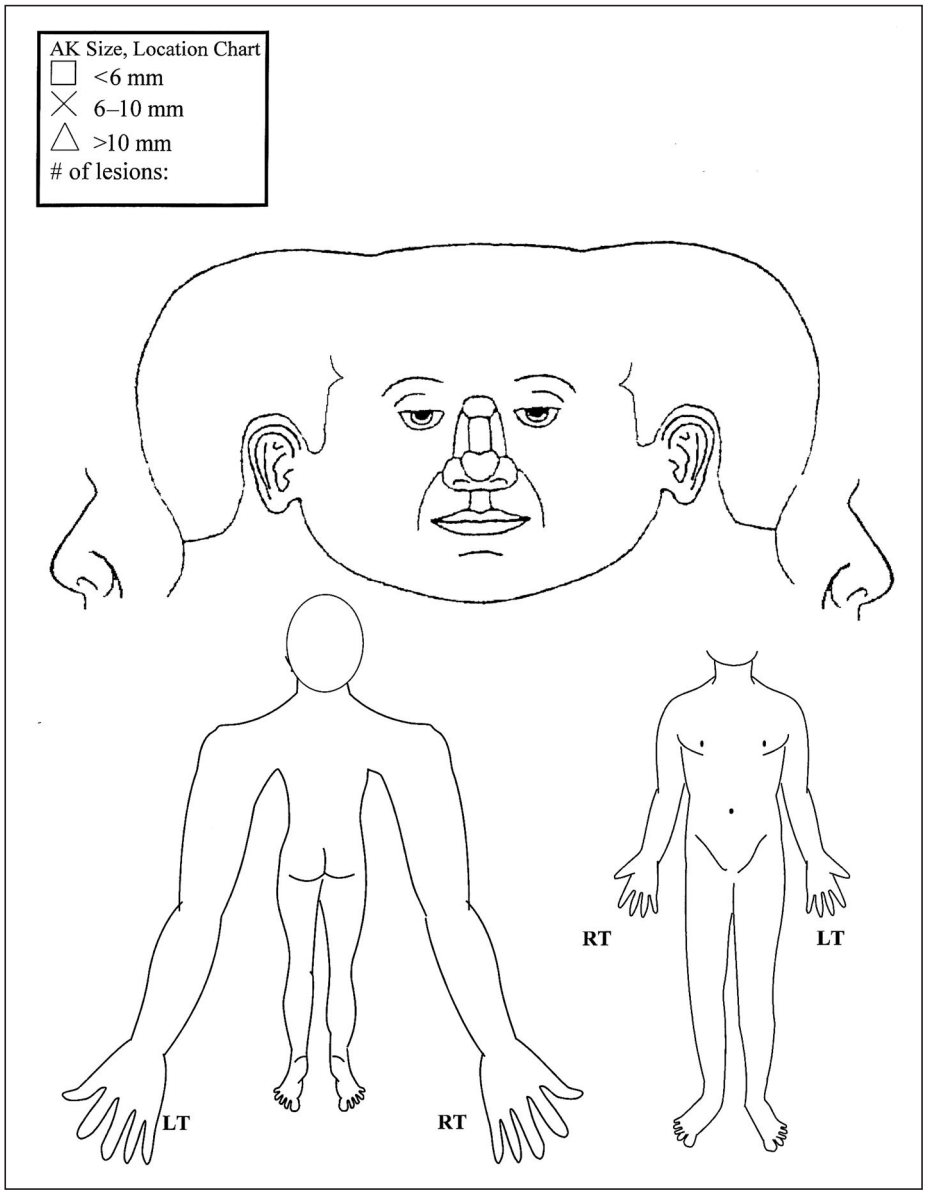


FIGURE 20.2: ***Back of the form in Figure 20.1. The diagram allows cross-referenced measurements of the cancer site location.***

KENNETH G. GROSS, M.D.
5222 Balboa Avenue, 6th Floor
San Diego, California 92117
(858) 292-5101 • Fax: (858) 292-1915

REFERRAL REPORT

Patient's Name Date of First Visit

Diagnosis

Evaluation & Treatment

FIGURE 20.3: Referral report form sent to the referring physician.

Skin Surgery Medical Group Inc.
Kenneth G. Gross, M.D. Leslie A. Mark, M.D.
Dale E. Martin, M.D. Barbara Martin, MD
5222 Balboa Avenue 5th & 6th Floor San Diego, CA 92117
24 Hour Phone : (858) 292-5101 Backup Phone: (866)560-7831

NAME: DATE:

MOHS Excision of
Closure as necessary.

SURGERY TO BE PERFORMED

- All human beings heal by permanent scar formation.
- Scar Tissue is red for 3-6 months or longer, then usually fades to white. Sunlight exposure may cause a scar to darken. Blood Vessels (telangiectasia) can form around a surgical site.
- The appearance of a surgery scar usually continues to improve for 6-12 months as the scar "matures". The tissues around the surgical site often remain swollen for about one month. The surgery scar is usually STRONG BY 25 DAYS.
- Scars overlying the active muscle areas tend to widen (stretch) with time. This cannot always be prevented.
- Scars can heal thick (keloid or hypertrophic scar) or can heal thin (atrophic scar). How they heal depends in part upon their location on the body and the healing process of the patient.
- The final appearance of a scar depends upon many factors including the SIZE, DEPTH, and LOCATION of your cancer. Chances for a good result can be estimated for a given procedure, but can NEVER BE GUARANTEED.
- If a surgical site is injured before healing is complete, the scar may gape open, the wound may bleed, and the scar may become more obvious.
- A change of feeling (sensation) often occurs around a scar. In some areas of the body there is a risk of motor nerve damage:

_____

- Infection or bleeding can occur after surgery.
- Insignificant, serious, or life threatening reactions may occur to any medicine.
  Anesthesia:
  - ❑ 2:1:1 mix: 0.75% marcaine + 1% plain lidocaine + 1% lidocaine with epi 1:100,000
  - ❑ other:
- Sometimes more than one surgical procedure is necessary to remove a large lesion, to remove a lesion in a difficult area, or to obtain the best possible repair of the surgical wound and the best possible cosmetic result.

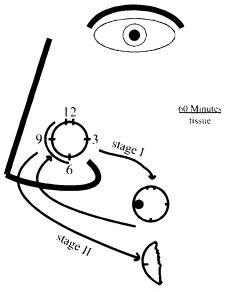
The above statements have been discussed with me. I understand them and have no further questions.

SIGNATURE OF PATIENT OR GUARDIAN DATE:

Your cancer is:

Treatment Options
X-ray
ED&C
Cryosurgery
Standard Excision
Interferon
Laser
Mohs excision
Other

Example:



Tumor free
↓
Repair as necessary
↓
Go home

- How long will it take
- Cure rate
- Read Mohs

FIGURE 20.4: Front of the informed consent form.

NAME: DATE:

MOHS Excision of
Closure as necessaary

SURGERY TO BE PERFORMED

_____ (init) I have discussed my proposed surgery in detail with Dr. Gross and he has explained the alternatives of therapy and complications of the procedure selected.

(init) I have no further questions.

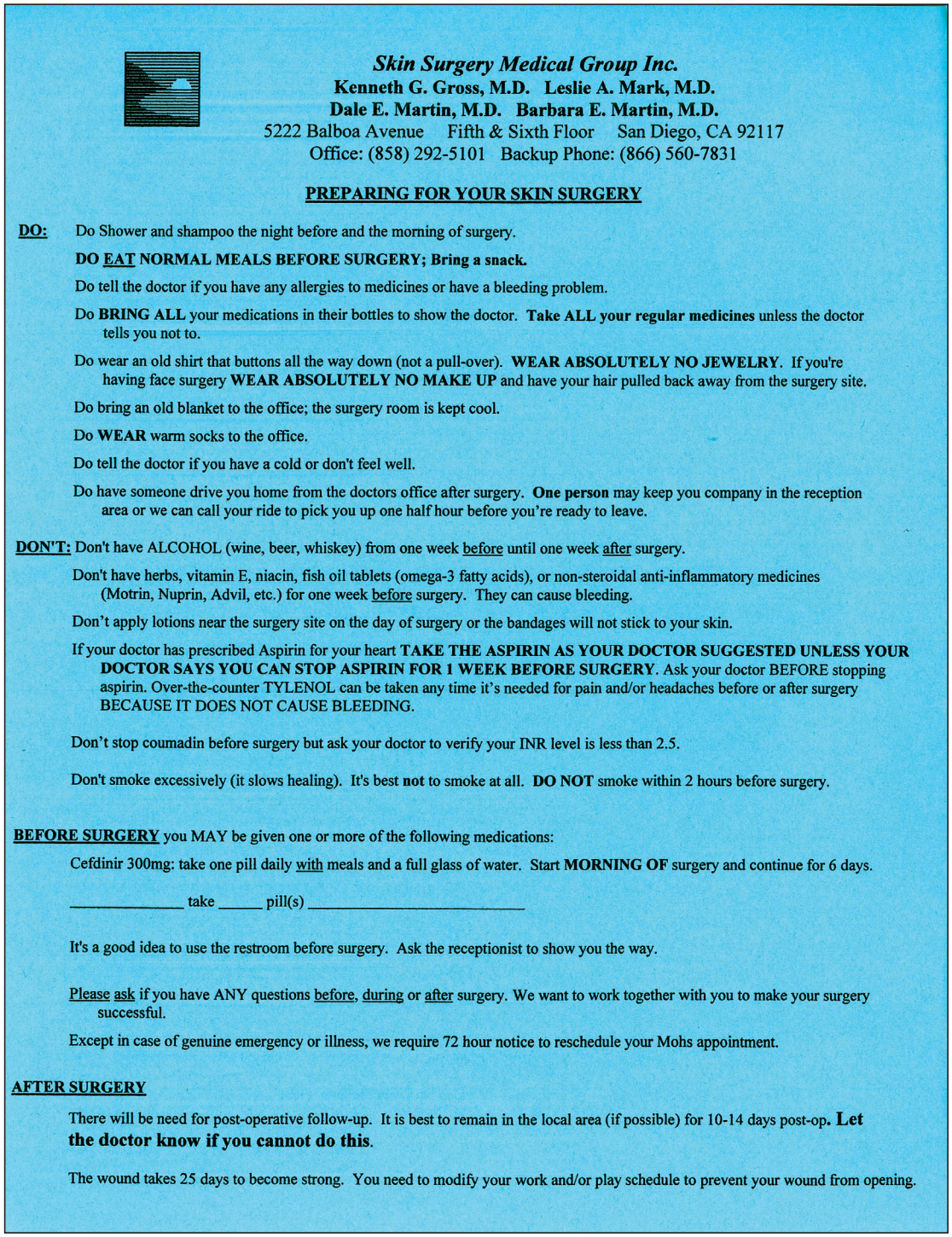
_____

I understand that NO GUARANTEE has been made and I ask that the surgery be performed by Dr. Gross as discussed and as outlined above. After surgery healing I understand the need to have long term follow-up care by _____new, recurrent or metastasis cancer.

DATE:

SIGNATURE OF PATIENT OR GUARDIAN : DATE:

FIGURE 20.5: Back of the informed consent form.



Skin Surgery Medical Group Inc.
Kenneth G. Gross, M.D. Leslie A. Mark, M.D.
Dale E. Martin, M.D. Barbara E. Martin, M.D.
5222 Balboa Avenue Fifth & Sixth Floor San Diego, CA 92117
Office: (858) 292-5101 Backup Phone: (866) 560-7831

PREPARING FOR YOUR SKIN SURGERY

DO: Do Shower and shampoo the night before and the morning of surgery.

DO EAT NORMAL MEALS BEFORE SURGERY; Bring a snack.

Do tell the doctor if you have any allergies to medicines or have a bleeding problem.

Do **BRING ALL** your medications in their bottles to show the doctor. **Take ALL your regular medicines** unless the doctor tells you not to.

Do wear an old shirt that buttons all the way down (not a pull-over). **WEAR ABSOLUTELY NO JEWELRY**. If you're having face surgery **WEAR ABSOLUTELY NO MAKE UP** and have your hair pulled back away from the surgery site.

Do bring an old blanket to the office; the surgery room is kept cool.

Do **WEAR** warm socks to the office.

Do tell the doctor if you have a cold or don't feel well.

Do have someone drive you home from the doctors office after surgery. **One person** may keep you company in the reception area or we can call your ride to pick you up one half hour before you're ready to leave.

DON'T: Don't have ALCOHOL (wine, beer, whiskey) from one week before until one week after surgery.

Don't have herbs, vitamin E, niacin, fish oil tablets (omega-3 fatty acids), or non-steroidal anti-inflammatory medicines (Motrin, Nuprin, Advil, etc.) for one week before surgery. They can cause bleeding.

Don't apply lotions near the surgery site on the day of surgery or the bandages will not stick to your skin.

If your doctor has prescribed Aspirin for your heart **TAKE THE ASPIRIN AS YOUR DOCTOR SUGGESTED UNLESS YOUR DOCTOR SAYS YOU CAN STOP ASPIRIN FOR 1 WEEK BEFORE SURGERY**. Ask your doctor BEFORE stopping aspirin. Over-the-counter TYLENOL can be taken any time it's needed for pain and/or headaches before or after surgery BECAUSE IT DOES NOT CAUSE BLEEDING.

Don't stop coumadin before surgery but ask your doctor to verify your INR level is less than 2.5.

Don't smoke excessively (it slows healing). It's best **not** to smoke at all. **DO NOT** smoke within 2 hours before surgery.

BEFORE SURGERY you MAY be given one or more of the following medications:

Cefdinir 300mg: take one pill daily with meals and a full glass of water. Start **MORNING OF** surgery and continue for 6 days.

_____ take _____ pill(s) _____

It's a good idea to use the restroom before surgery. Ask the receptionist to show you the way.

Please ask if you have ANY questions before, during or after surgery. We want to work together with you to make your surgery successful.

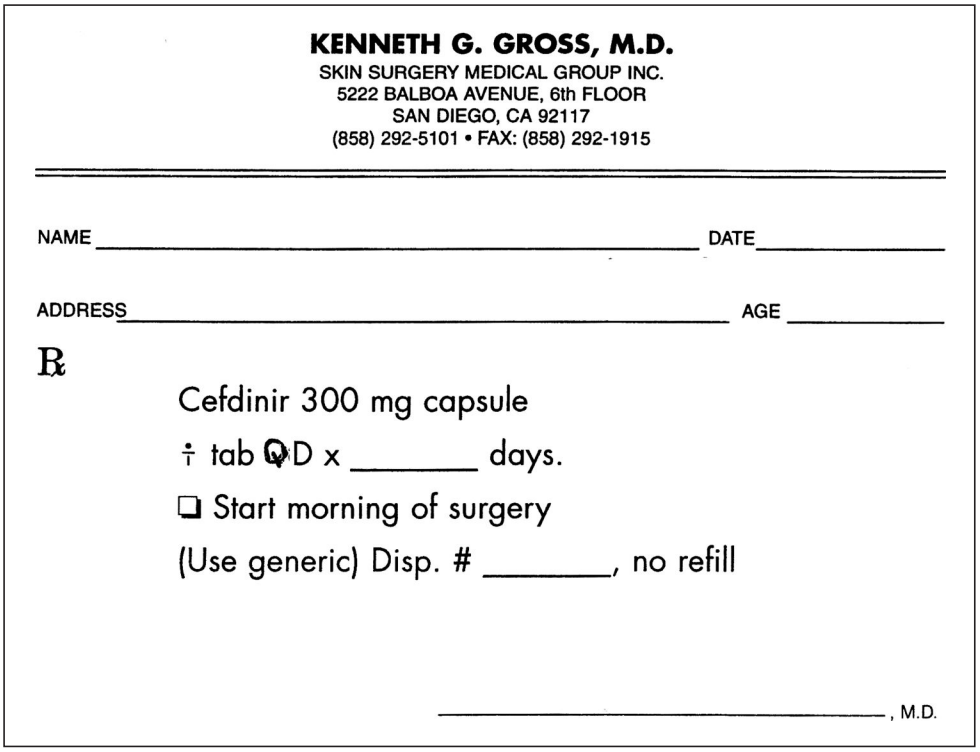
Except in case of genuine emergency or illness, we require 72 hour notice to reschedule your Mohs appointment.

AFTER SURGERY

There will be need for post-operative follow-up. It is best to remain in the local area (if possible) for 10-14 days post-op. **Let the doctor know if you cannot do this.**

The wound takes 25 days to become strong. You need to modify your work and/or play schedule to prevent your wound from opening.

FIGURE 20.6: Preoperative instruction sheets.



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SAN DIEGO, CA 92117
(858) 292-5101 • FAX: (858) 292-1915

NAME _____ DATE _____

ADDRESS _____ AGE _____

℞

Cefdinir 300 mg capsule

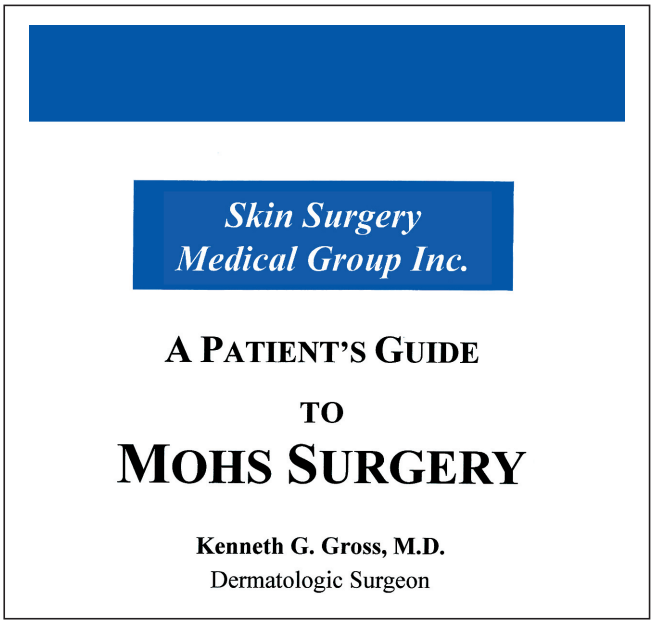
i tab QD x _____ days.

❑ Start morning of surgery

(Use generic) Disp. # _____, no refill

_____, M.D.

FIGURE 20.7: Preprinted antibiotic prescription.



Skin Surgery
Medical Group Inc.

A PATIENT'S GUIDE
TO
MOHS SURGERY

Kenneth G. Gross, M.D.
Dermatologic Surgeon

FIGURE 20.8: Upper half of the cover of the 22-page Mohs booklet that explains the Mohs procedure in simple terms and provides general postoperative instructions, a brief CV, and written and map directions to the office.

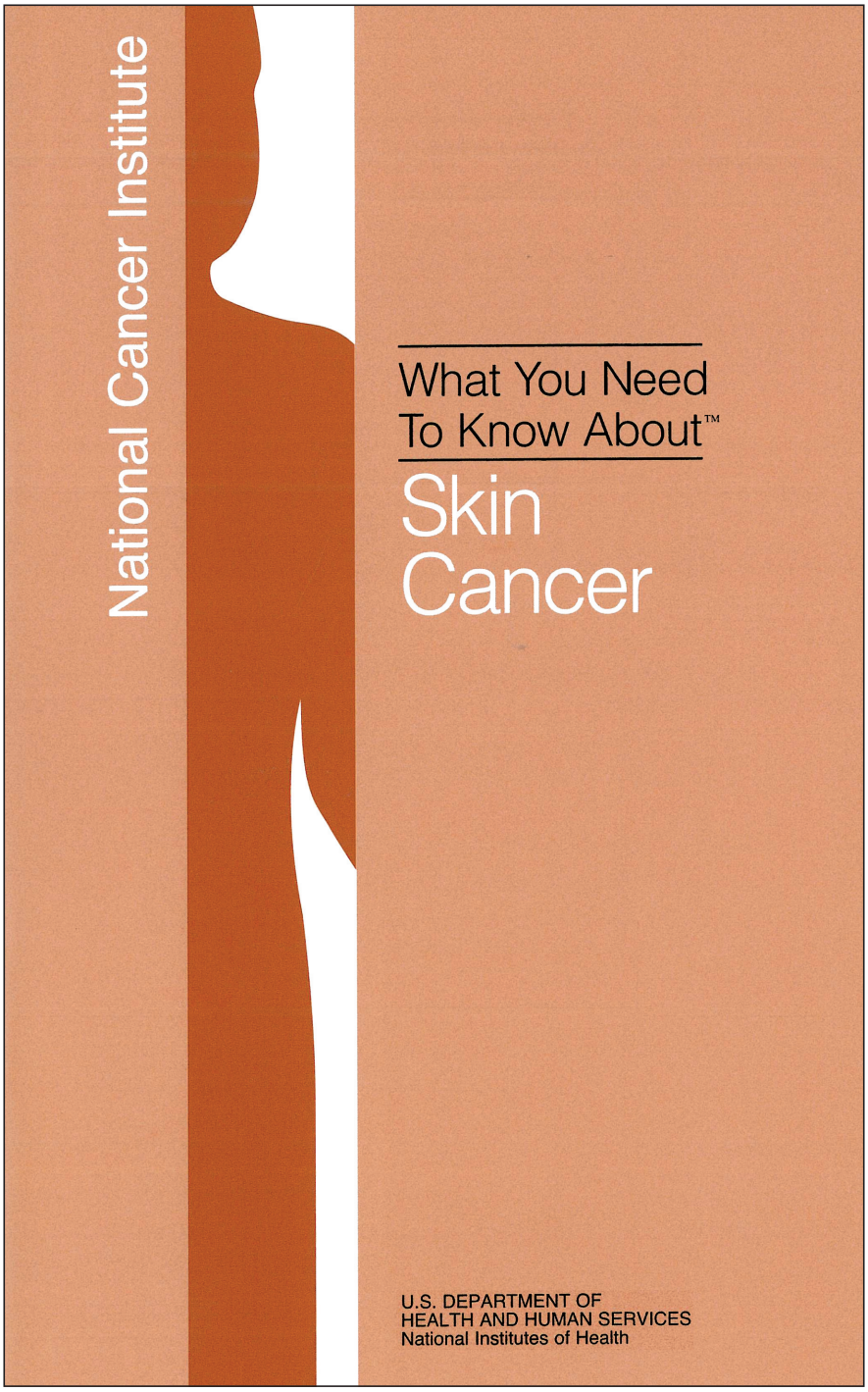
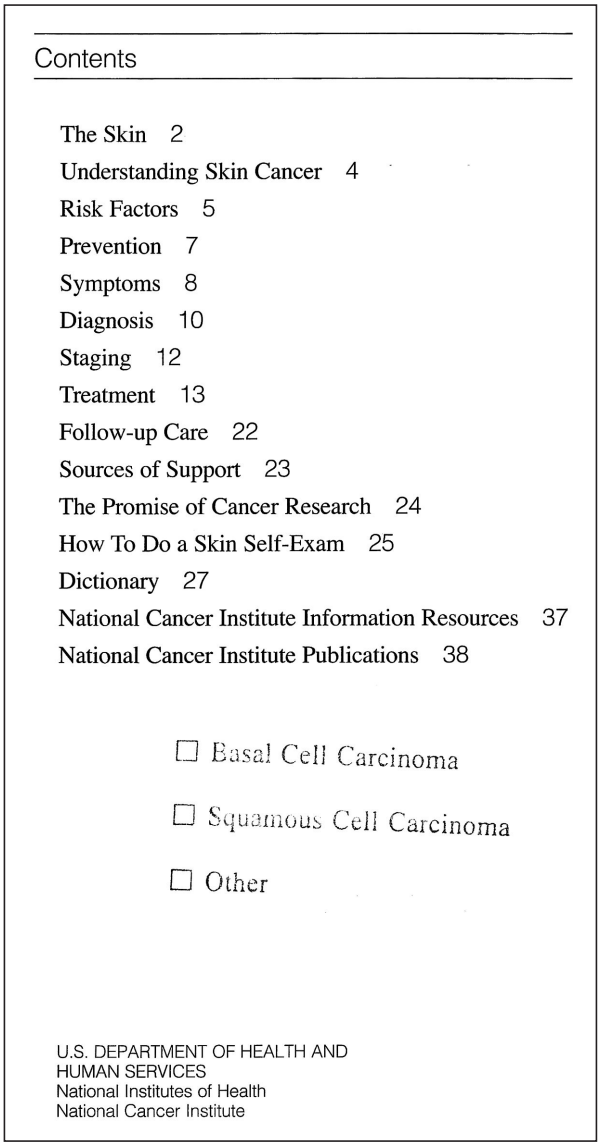


FIGURE 20.9: ***Front cover of the 40-page National Cancer Institute booklet that discusses skin cancer. Our office purchases these booklets in bulk to hand out to patients. Notice that in Figure 20.1, on the right lower area of the intake/physical exam form, there is a box to check indicating that the Mohs booklet and NCI cancer booklet were given to the patient.***



Contents

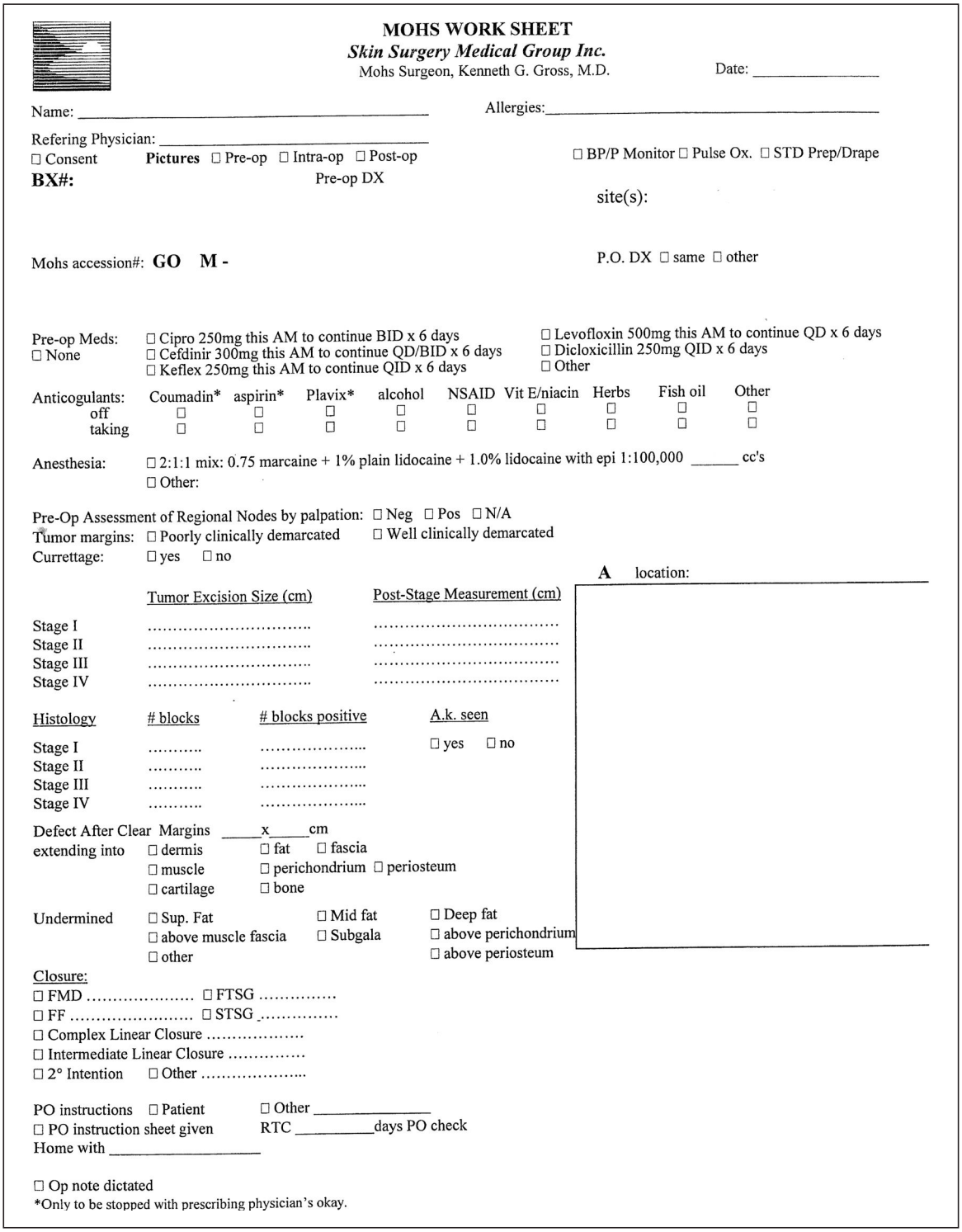
☐ Basal Cell Carcinoma

☐ Squamous Cell Carcinoma

☐ Other

U.S. DEPARTMENT OF HEALTH AND
HUMAN SERVICES
National Institutes of Health
National Cancer Institute

FIGURE 20.10: First page of the National Cancer Institute booklet, with a customized stamp that allows the surgeon to check off the type of cancer being evaluated for treatment.



MOHS WORK SHEET

Skin Surgery Medical Group Inc.

Mohs Surgeon, Kenneth G. Gross, M.D.

Date: _____

Name: _____ Allergies: _____

Refering Physician: _____

☐ Consent **Pictures** ☐ Pre-op ☐ Intra-op ☐ Post-op ☐ BP/P Monitor ☐ Pulse Ox. ☐ STD Prep/Drape

BX#: Pre-op DX site(s):

Mohs accession#: **GO M -** P.O. DX ☐ same ☐ other

Pre-op Meds: ☐ Cipro 250mg this AM to continue BID x 6 days ☐ Levofloxin 500mg this AM to continue QD x 6 days
☐ None ☐ Cefdinir 300mg this AM to continue QD/BID x 6 days ☐ Dicloxicillin 250mg QID x 6 days
☐ Keflex 250mg this AM to continue QID x 6 days ☐ Other

Anticogulants:	Coumadin*	aspirin*	Plavix*	alcohol	NSAID	Vit E/niacin	Herbs	Fish oil	Other
off	☐	☐	☐	☐	☐	☐	☐	☐	☐
taking	☐	☐	☐	☐	☐	☐	☐	☐	☐

Anesthesia: ☐ 2:1:1 mix: 0.75 marcaine + 1% plain lidocaine + 1.0% lidocaine with epi 1:100,000 _____ cc's
☐ Other:

Pre-Op Assessment of Regional Nodes by palpation: ☐ Neg ☐ Pos ☐ N/A
Tumor margins: ☐ Poorly clinically demarcated ☐ Well clinically demarcated
Currettage: ☐ yes ☐ no

A location:

	Tumor Excision Size (cm)	Post-Stage Measurement (cm)
Stage I	.....	.....
Stage II	.....	.....
Stage III	.....	.....
Stage IV	.....	.....

Histology	# blocks	# blocks positive	A.k. seen
Stage I	.....	.....	☐ yes ☐ no
Stage II	.....	.....	
Stage III	.....	.....	
Stage IV	.....	.....	

Defect After Clear Margins _____x_____cm
extending into ☐ dermis ☐ fat ☐ fascia
☐ muscle ☐ perichondrium ☐ periosteum
☐ cartilage ☐ bone

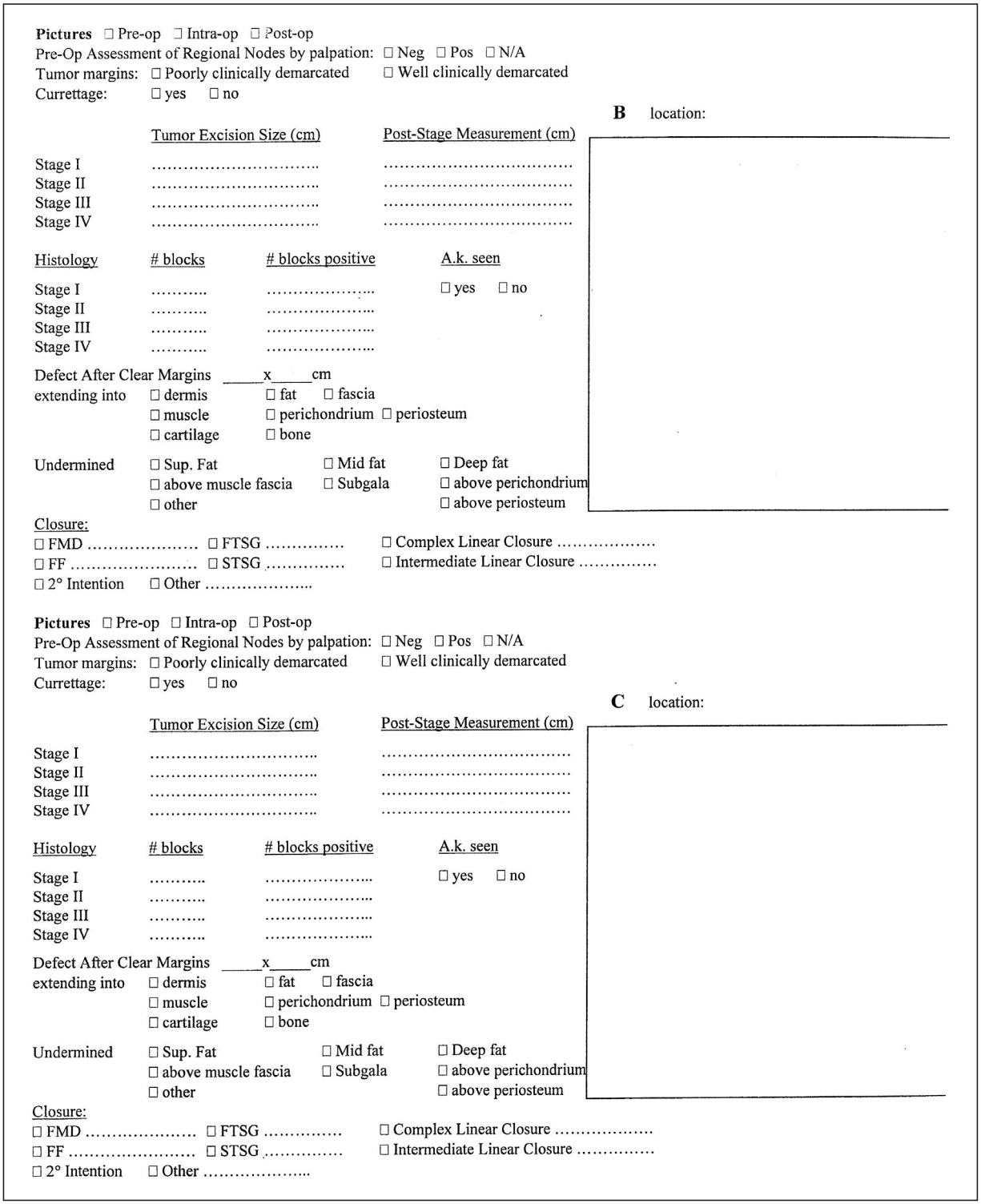
Undermined ☐ Sup. Fat ☐ Mid fat ☐ Deep fat
☐ above muscle fascia ☐ Subgala ☐ above perichondrium
☐ other ☐ above periosteum

Closure:
☐ FMD ..... ☐ FTSG .....
☐ FF ..... ☐ STSG .....
☐ Complex Linear Closure .....
☐ Intermediate Linear Closure .....
☐ 2° Intention ☐ Other .....

PO instructions ☐ Patient ☐ Other _____
☐ PO instruction sheet given RTC _____days PO check
Home with _____

☐ Op note dictated
*Only to be stopped with prescribing physician's okay.

FIGURE 20.11: Front of the Mohs worksheet.



Pictures ☐ Pre-op ☐ Intra-op ☐ Post-op
Pre-Op Assessment of Regional Nodes by palpation: ☐ Neg ☐ Pos ☐ N/A
Tumor margins: ☐ Poorly clinically demarcated ☐ Well clinically demarcated
Currettage: ☐ yes ☐ no

B location:

	Tumor Excision Size (cm)	Post-Stage Measurement (cm)
Stage I	.....	.....
Stage II	.....	.....
Stage III	.....	.....
Stage IV	.....	.....

Histology	# blocks	# blocks positive	A.k. seen
Stage I	.....	.....	☐ yes ☐ no
Stage II	.....	.....	
Stage III	.....	.....	
Stage IV	.....	.....	

Defect After Clear Margins _____x_____cm
extending into ☐ dermis ☐ fat ☐ fascia
☐ muscle ☐ perichondrium ☐ periosteum
☐ cartilage ☐ bone

Undermined ☐ Sup. Fat ☐ Mid fat ☐ Deep fat
☐ above muscle fascia ☐ Subgala ☐ above perichondrium
☐ other ☐ above periosteum

Closure:
☐ FMD ..... ☐ FTSG ..... ☐ Complex Linear Closure .....
☐ FF ..... ☐ STSG ..... ☐ Intermediate Linear Closure .....
☐ 2° Intention ☐ Other .....

Pictures ☐ Pre-op ☐ Intra-op ☐ Post-op
Pre-Op Assessment of Regional Nodes by palpation: ☐ Neg ☐ Pos ☐ N/A
Tumor margins: ☐ Poorly clinically demarcated ☐ Well clinically demarcated
Currettage: ☐ yes ☐ no

C location:

	Tumor Excision Size (cm)	Post-Stage Measurement (cm)
Stage I	.....	.....
Stage II	.....	.....
Stage III	.....	.....
Stage IV	.....	.....

Histology	# blocks	# blocks positive	A.k. seen
Stage I	.....	.....	☐ yes ☐ no
Stage II	.....	.....	
Stage III	.....	.....	
Stage IV	.....	.....	

Defect After Clear Margins _____x_____cm
extending into ☐ dermis ☐ fat ☐ fascia
☐ muscle ☐ perichondrium ☐ periosteum
☐ cartilage ☐ bone

Undermined ☐ Sup. Fat ☐ Mid fat ☐ Deep fat
☐ above muscle fascia ☐ Subgala ☐ above perichondrium
☐ other ☐ above periosteum

Closure:
☐ FMD ..... ☐ FTSG ..... ☐ Complex Linear Closure .....
☐ FF ..... ☐ STSG ..... ☐ Intermediate Linear Closure .....
☐ 2° Intention ☐ Other .....

FIGURE 20.12: Back of the Mohs worksheet.

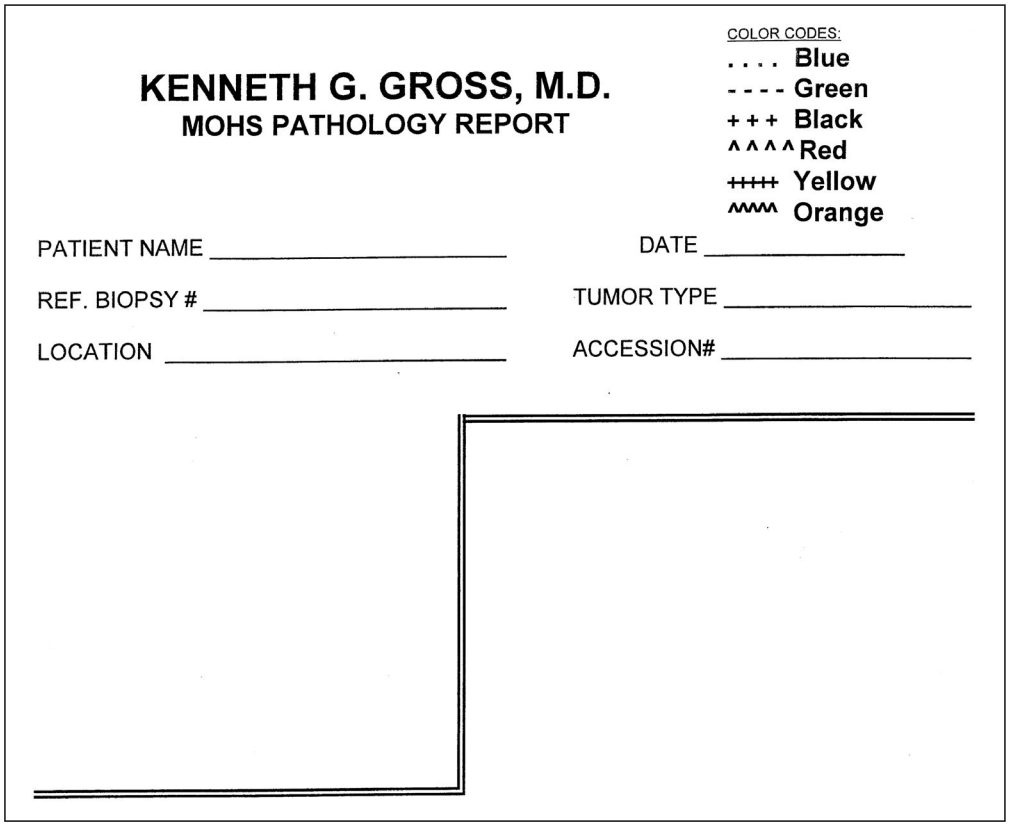


FIGURE 20.13: Upper half of the Mohs pathology worksheet.

MOHS MICROGRAPHIC SURGERY REPORT

Patient: **(1)** **Date:** **(2)**

Surgeon: **(3)** **Assistant:** **(4)**

Anatomic Location: **(5)**
Biopsy #: **(6)**
Pre-operative Diagnosis: **(7)**
Post-operative Diagnosis: **(8)**

Regional nodes: **(9)**

Procedure: Mohs micrographic surgery, fresh tissue technique completed in **(10)** stage. Repair of Mohs defect by **(11)**

Informed consent: Diagnosis, prognosis, alternatives of therapy, and complications have been explained to the patient and informed consent obtained for this procedure.

Indication (s) for Mohs surgery: **(12)**

Medical history, current medicines and allergies are in the patient's chart and have been reviewed pre-operatively.

Pre-operative meds: **(13)**

Anti-coagulants: **(14)**

The patient was brought to the procedure room; BP, pulse and O2 saturation were monitored throughout the procedure; standard prep & drape were used.

The lesion was outlined with sterile gentian violet pen under bright light with magnification and the tumor measured **(15)**

Anesthesia:
Local infiltration is carried out with a 2:1:1 mix of .75% Marcaine, 1% lidocaine, 1% plain lidocaine, 1:100,000 epinephrine, a total of **(16)** cc's initially and **(17)** cc's subsequently.

Procedure: Hatch marks were placed for orientation of the Mohs specimen.

Picture (s): **(18)**

Debulk: **(19)**

FIGURE 20.14: Operative report outline.

(2)

Stage I Mohs excision: The marked area of clinical tumor with a small rim of clinically normal surrounding skin was removed using Mohs technique with beveling of the edges. Excision was carried out to **(20)** The defect measured **(21)** cm. A Mohs map was prepared. After hemostasis, a temporary sterile dressing was placed over the wound and the patient was escorted to the surgery waiting area.

The specimen was taken to the Mohs laboratory where it was chromacoded and processed in **(22)** block (s) under (G09M- **(23)**) Dr. Gross's supervision. Frozen section slides were prepared with serial tissue sections and stained and evaluated histopathologically by Dr. Gross for interpretation of deep and peripheral margins.

Path interpretation: (24) and the Mohs map was marked accordingly by Dr. Gross.

Stage II: The patient returned to the procedure room, the dressing removed, the tumor area reprepped and draped, and anesthesia assessed and augmented as necessary. A layer of tissue **(25)** cm around the positive margin (s) was removed and the defect measured **(26)** . The tissue was processed in an identical fashion as for stage I in **(27)** block (s) under Dr. Gross's supervision. After hemostasis a temporary dressing was applied and the patient was escorted to the surgery waiting area.

Path interpretation: (28) and is identified in the margin (s) and the Mohs map was marked accordingly by Dr. Gross.

Dermpath frozen section report summary:

Stage I (29) # Blocks #Positive #Negative
II (30)

Tumor free margins were obtained.

After **(31)** stage the Mohs procedure was considered complete.

POST MOHS DEFECT REPAIR BY SURGICAL CLOSURE:

The patient was returned to the procedure room.
Dr. Gross having discussed wound closure options previously, now briefly rediscussed them with the patient.

The temporary dressing was removed, the area prepped and draped in the standard manner, and additional local anesthesia infiltrated for closure as necessary.

The wound measured **(32)** cm and extended in depth to **(33)**. Closure was undertaken using **(34)**

Undermining: (35)

Hemostasis: (36)

FIGURE 20.14: (continued)

(3)

Closure: (37)

Final measured defect: (38)

Final closure: (39)

Dressing: The wound is dressed with **(40)**

Post-operative instructions were given in writing and reviewed with the patient and home with **(41)** Follow-up appointment in **(42)** days or PRN sooner. The patient tolerated the procedure well and left the procedure room in good condition.

_____
Kenneth G. Gross, M.D.

KGG/mmw

FIGURE 20.14: (continued)

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