# Atlas of Mohs and Frozen Section Cutaneous Pathology

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Editors

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Springer

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ISBN 978-0-387-84799-3 e-ISBN 978-0-387-84800-6
DOI 10.1007/978-0-387-84800-6
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2009930369



Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

*To Kerry, Poochie and Bozie for your unconditional love and acceptance of the time and effort I have spent away from you in this endeavor*

Michael B. Morgan, M.D.

*To Glicia and James, thank you for your love and encouragement*

James Spencer, M.D.

*I would like to thank my mentor and friend in Medical School, Robert Bookmyer M.D. from my Dermatology training at the University of Chicago, I would like to thank, Alan Lorincz, M.D. for inspiring me to think creatively, Maria Medenica, M.D. and David Fretzin, M.D. for Dermatopathology and Keyoumaris Soltani M.D. for Dermatological Surgery. I also want to thank Frederick Mohs, M.D. for being so generous and sharing his expertise with me. Most importantly, I want to thank my wife Sue and my children John, Sarah, Amy and Gregory for their support and encouragement.*

John Robert Hamill, Jr. M.D.

# Preface

This atlas is intended for practitioners in the fields of dermatologic surgery including Mohs cutaneous surgeons, pathologists who examine frozen section specimens derived from the skin and dermatopathologists, respectively. This book will serve as a reference pictorial atlas detailing both common and challenging cutaneous neoplasms. It will also serve as a review for physicians-in-training preparing for certifying examinations in the fields of dermatology, dermatologic surgery, Mohs surgery, pathology and dermatopathology.

The central theme of the atlas entails the microscopic analysis, diagnosis and discrimination of common and problematic cutaneous neoplasms as encountered by the dermatologist, cutaneous surgeon or pathologist employing the frozen section technique. The book includes coverage of: (1) microscopic anatomy of the various cutaneous and mucosal sites of the body; (2) diagnosis of basic/routine dermatologic entities including basal cell carcinoma and its variants as well as squamous cell carcinoma and its variants; (3) the discrimination of these foregoing neoplasms from benign epidermal-derived or adnexal derived neoplasms; (4) diagnosis and distinction of rare and/or deadly neoplasms from benign entities such as dermatofibrosarcoma protuberans and merkel cell carcinoma; (5) troubleshooting and dealing with quality control of the frozen section technique including cutting and staining; (6) new techniques including immunohistochemistry and molecular analysis.

The underlying premise of this atlas is to provide its reader with a single reference atlas dealing with the frozen section microscopic diagnosis of cutaneous neoplasms. As these malignant entities are capable of presenting in a variety of microscopic guises potentially confused with benign mimics or in a subtle fashion easily missed by the examiner, it is important that pathologists or clinicians who interpret their own biopsies are appraised of this risk.

This book should provide a shelf-reference for dermatologic surgeons, Mohs cutaneous surgeons, pathologists who perform frozen section analysis of cutaneous specimens and dermatopathologists. This book should also serve as a potential study source for dermatologists, pathologists and dermatopathologists preparing for board examinations.

Tampa, Florida
March 2008

Michael B. Morgan, M.D.
John R. Hamill, M.D.

# Prologue

Skin cancer has reached epidemic proportions in the United States, and there is no evidence that this trend will decrease any time soon. Basal cell and squamous cell carcinomas, collectively referred to as non-melanoma skin cancer, make up the vast majority of the estimated 1.5 million skin cancers seen annually in this country. There are many ways non-melanoma skin cancer may be treated, ranging from topical medications for early thin tumors, destructive techniques such as cryosurgery or curettage & electrodessication, radiation therapy, surgical excision, and lastly excision utilizing the Mohs technique. Of all these techniques, the highest cure rates currently possible are with the Mohs technique, which relies on optimal preparation and interpretation of frozen sections. Therefore, frozen section analysis has become the gold standard for skin cancer therapy.

When surgical excision is chosen as the treatment, frozen section analysis allows histologic information to become part of therapy, rather than preceding therapy (in the case of a biopsy) or confirming an already finished procedure (permanent sections read days after the surgery is over). Frozen sections may be utilized to sample a portion of a conventional surgical excision, or they may be used to examine all the exterior surface of the excised tumor during the Mohs technique. Cure rates with either conventional surgery or the Mohs technique can only be as good as the quality and interpretation of the frozen sections.

Frozen section analysis is fundamentally different than permanent sections. Details from individual cells are difficult to assess, and pattern recognition becomes more important. Traditional permanent sections have vertical cuts, and thus structures of the skin are seen vertically oriented. Slides prepared as part of the Mohs technique produce sections with horizontal and tangential cuts on the same slide, and thus familiar structures are now altered in their appearance. Experience in reading vertically oriented permanent sections does not translate to expertise in reading frozen sections. In my opinion, the most difficult part in mastering Mohs surgery is not the excision or reconstruction, but rather developing expertise in reading horizontally and tangentially oriented frozen sections.

It is our hope this book provides a scholarly reference text to the student of frozen sections for skin cancer therapy. The authors include pathologists and dermatologists practicing Mohs surgery. Mike Morgan, a dermatopathologist, has been the lead author and editor who has carried the lion's share of getting this book done, and deserves our thanks. Hopefully, dermatopathologists reading frozen sections, as well as practicing Mohs surgeons, will find this text a useful and handy reference to keep in the lab.

Clearwater, Florida
June 2008

James M. Spencer, M.D., M.S.

# The Early Days of Mohs Surgery

Mohs surgery is an extremely effective method for eradicating skin cancers. The unique feature of the technique is that it incorporates instant pathology while the patient waits. The value of the laboratory in producing frozen sections within a short period of time enables the physician to determine if all of the tumor has been removed. Upon microscopic examination of excised tissue, the physician is able to pinpoint its exact location on the patient.

Initially, the availability of cryostats was limited, and the freezing microtome stage was fed by a supply of $CO_2$ gas that was stored in large containers. The gas was allowed to pass through narrow tubing to reach the microtome stage and freeze the tissue. The $CO_2$ containers were often large and bulky, requiring substantial storage space. Furthermore, the dependence on timely deliveries of the $CO_2$ led to many inconveniences in attempting to process the tissue obtained from Mohs surgery. Shortly after, a new type of microtome was developed utilizing an electrical unit that provided a supply of cold air to freeze the specimen on the stage. In subsequent years, cryostats such as Leica became more practical and affordable and are among the most used in Mohs surgery practices today.

Before the 1970's, the Mohs technique incorporated the application of a zinc chloride paste and was thus known as microscopically controlled chemosurgery. The final patented formula contained 45% zinc chloride by weight, with 40 g of stibnite antimony, 10 g of blood root (*Sanguinaria Canadensis),* and a 34.5 ml zinc chloride saturated solution. The stibnite antimony acted as a granular support material, and the bloodroot kept the zinc chloride in suspension so that it could freely move between the particles, yet not settle to the bottom. The product was not FDA approved and was prepared by University of Wisconsin pharmacy, where at the time, it could only be purchased under the authority of Fred Mohs.

The zinc chloride paste was effective in fixing the tissue in situ. It was applied in a thin layer over the involved area and could not penetrate the skin unless keratin was removed. This was accomplished using dichloracetic acid. It turned the affective area white due to precipitation of the proteins in the epidermis. Using the zinc chloride paste, Dr. Mohs created Z squares in which he impregnated a piece of gauze with the paste and cut into 1 $cm^2$ pieces. Theses gauze pieces were then applied to the Mohs defect site to prevent the area from drying out. This entire process came to be known as the fixed tissue technique.

Although Dr. Mohs had published work on the fresh tissue technique in the late 1950s, it was not until the mid-1970s that it became the favored method in Mohs surgery. In 1970, Dr. Tromovitch presented a paper at a chemosurgery meeting, reporting a 99% cure rate with close to a five-year follow-up. The advantage of using the fresh tissue technique was that many stages of Mohs surgery could be performed in one day, and the defect could be repaired immediately following completion of the surgery. Today, there are some Mohs surgeons who continue to use the zinc chloride paste to treat malignant melanoma. They believe that the paste plays a role in killing melanocytes; however, this has not yet been

substantiated. Therefore, the fresh frozen technique has become the preferred technique in the vast majority of Mohs surgery practices.

In the early days, the favorite stain for basal cell carcinoma was toluidine blue. It caused the mucopolysaccharides to stain purple revealing the presence of tumor cells. The use of toluidine blue was less popular for squamous cell carcinoma as it was more difficult to differentiate tumor from normal tissue. Toluidine blue was taken off the market in its initial formulation as it was found to be carcinogenic at higher concentrations. Hematoxylin and eosin became the standard for both squamous cell and basal cell carcinomas as well as various tumors for which Mohs surgery is utilized as treatment. The toluidine blue used today is at a much lower concentration and is preferred by many Mohs surgeons for visualizing basal cell carcinoma. However, its perceived advantage over hematoxylin and eosin is simply a matter of personal choice.

Ritu Saini, M.D.
Perry Robins, M.D.

# Contents

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# Part I
# Introduction

# Chapter 1
# Mohs and Frozen Section Overview

Michael B. Morgan and Terri Bowland

The evaluation of frozen section prepared tissues derived from the skin constitutes a burgeoning area of hospital- and outpatient-based pathology practice. Frozen section cutaneous pathology encompasses a diverse array of techniques for preparing the skin specimen and incorporates a variety of diagnostic methodologies. This book will principally address the histologic interpretation of the various cutaneous neoplasms encountered with the Mohs micrographic frozen method and traditional fresh-frozen pathology. Unusual diagnostic applications of frozen sections such as frozen section immunopathology and unconventional topics such as perineural pathology and quality assurance with technique trouble-shooting will also be covered.

Understanding that the vast majority of cutaneous neoplasms can be successfully removed in the outpatient setting without the aid of frozen section examination or treated without examination of removed tissues (e.g., photodynamic therapy, topical immune response agents (Imiquimod) or palliative measures (e.g., radiotherapy or intralesional chemotherapy), the principal discussion will revolve around the frozen-section determination of both the more common non-melanoma skin cancers, unusual malignancies of the skin (e.g., merkel cell carcinoma), simulants of cutaneous cancer, as well as discuss the important differential diagnoses and pitfalls that arise in the preparation and interpretation of these specimens. The first chapters will entail an in-depth examination of the normal epithelium, dermis and subcutaneous fat. Important age-related and/or benign degenerative changes such as solar elastosis will be discussed. Each of the topics to be considered will be preceded by a brief synopsis or *précis* of the entity entailing its epidemiologic, definitional, pathogenic, clinical and pathologic features. This will be followed by a traditional text document pertaining to the précis and finally, high-quality color photomicrographs taken at low, medium and high powers of magnification. The photomicrographs will be prepared from frozen section material and will be presented in a contrasting format with the most important differential diagnosis presented adjacent to the topic headings. The margins of each photo will contain the most important diagnostic points useful in distinguishing the entity. Each chapter will be followed by a concise bibliography.

## Indications for Frozen Sections of the Skin

The principal application of frozen section consultation is to assure the complete removal of a non-melanoma skin carcinoma (NMSC). The goal is not only to completely remove the abnormal tissue but to assure that as minimal amount of normal tissue is removed for cosmetic or functional purposes. The functional concerns entail preservation of as much of the normal anatomy as possible in highly-functional tissues such as the peri-ocular adnexae, eyelids and around the mouth or nares. In the removal of larger specimens that require complicated closures with the aid of tissue flaps or grafts, assurance of negative tumor margins is essential. Frozen section examination is also commonly employed in circumstances where the tumor has recurred or excessive post-operative scarring or radiotherapy complicates the clinical determination of tumor borders. The final indications involve the determination of various cutaneous dermatoses such as toxic epidermal necrolysis versus the staphylococcal scalded skin syndrome. As both conditions show considerable clinical overlap, portend a grave prognosis and involve vastly different modes of therapy, rapid frozen section determination between these entities can become necessary.

M. Morgan et al. (eds.), *Atlas of Mohs and Frozen Section Cutaneous Pathology*, DOI 10.1007/978-0-387-84800-6_1,

## Histologic Prerequisites to Frozen Section Evaluation

Several practices should be adopted prior to frozen section examination of the skin. One of the most important exercises to routinely employ is the pre-procedural review of permanent tissue sections obtained by prior biopsy of the lesion scheduled to be removed. In some instances, the original interpretation rendered is in error or may involve histologic subtleties usefully remembered in the interpretation of the subsequent specimen. Familiarity with the normal histology and its key variations is assumed. Dermatopathology text review, literature search and/or web image review can also be resorted to with planned removal of unusual entities. Among the more varied pathologic nuances potentially encountered that pose considerable challenge during frozen section interpretation are morpheaform basal cell carcinoma, microcystic adnexal carcinoma, dermatofibrosarcoma protuberans and simulants of malignancy such as psuedoepitheliomatous hyperplasia, basaloid follicular hamartoma and dense/obscuring inflammatory infiltrates, which will be subsequently discussed.

## Handling of the Specimen/Frozen Technique

Adequate preparation of the glass slides to be examined and the tissue chucks to be utilized are prerequisite to the handling of the skin specimen. Glass slides should be prepared with adequate patient identification, employing alcohol fast labeling, typically with leaded pencil marking. If possible, a technique employing a redundant patient identification mechanism, i.e., patient name and surgical or operative number should be considered. Cryostat tissue section chucks should be mounted with OCT embedding compound prior to tissue receipt. Once frozen, the OCT should be planned to ensure a flattened surface. This can be accomplished by simply rubbing the surface of the frozen chucks over a clean, firm, smooth surface. The undersurface of the chucks may be labeled with a colored wax pencil or pencil-lead.

Upon receipt of the specimen, assurance should be made as to the origin and correct identification of the specimen, ascertained by matching the specimen jar or sample container with the requisition form. The time of specimen receipt should also be recorded for quality assurance and turn-around-time determinations. Special attention should be given to the size, shape and particularly identifying marks, contrasting inks or suture ties orientating the specimen. The anatomic location, dimensions (three planes), the number of visible lesions, their dimension, surface attributes (e.g., keratotic, ulcerated, etc.), color of the lesion and orientating features should also be recorded. Anatomic orientation should be maintained if possible in the preparation of the specimen. Orientation may be arbitrarily assigned to clock positions (e.g., 12 o'clock) representing either an anatomically cephalad or superior orientation or corresponding to a tip of an ellipse and recorded with the aid of a diagram. Generally, skin specimens are configured in an elliptical or oval silhouette as to allow cosmetically-acceptable closure of the wound (Figs. 1.1 and 1.2). Rarely, triangular (often from the ear or lip) or oblong specimens will be received pending anatomic considerations or the extent of tumor extension. Typically, oval or elliptical specimens measuring less than 1.0 cm in length can be sectioned and submitted entirely in a single cassette. Multiple blocks may need to be prepared for larger specimens.

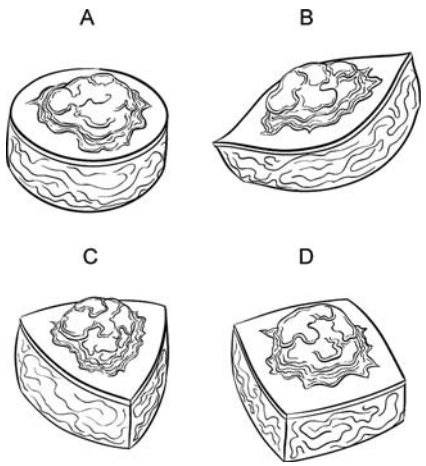


**Fig. 1.1** Round specimens **A** represent a minority of specimens submitted for frozen sections. An ellipse **B** is the most common with the tips taken to assure cosmetic closure of the defect. Triangular shaped **C** and rhomboid-shaped **D** specimens are most often removed in preparation for closing the defect with a local skin flap

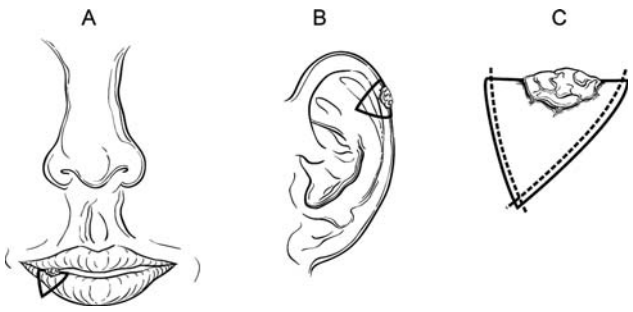


**Fig. 1.2** Wedge-shaped biopsies are obtained from free margin anatomic locations such as the lip **A.,** or ear **B.** Sections are cut parallel to the margins and embedded on edge **C**

## Orientation

Specimens should be received with accompanying orientation marks including a notch, contrasting edge or surface ink(s) and/or sutures (Fig. 1.3). The latter is preferred, and usually, a single suture is all that is necessary. An anatomically oriented sketch or diagram corresponding to the designated orientation is preferable as well. In most instances, the suture or mark may be assigned if not previously by the surgeon, to 12 o'clock with the remaining positions corresponding to a clock-face. Complicated resections may require in situ examination or clinical photographs of the outlined specimen margins prior to removal by the pathologist. Sutures should be tied off in a loose loop configuration to assure complete and efficacious removal of the suture material. Retained suture within the specimen or inadvertent slicing of the excision by the pathologist may follow tight knotting of the suture to the resected specimen.

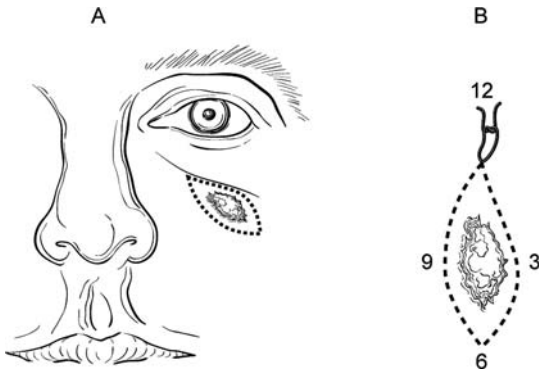


**Fig. 1.3** A suture placed at a designated point (e.g., 12 o'clock) is the most common way for the surgeon to orient the specimen

## Inking

Prior to cutting of the specimen, all surgical margins of the tissue should be painted with contrasting inks to assure microscopic delineation of orientation and tumor extent (Fig. 1.4). The ink can be applied with the aid of a toothpick or similarly configured wooden or plastic applicator to the surgical margins of the specimen. To assure steadfast ink adherence to the specimen, thorough drying of the specimen edges with a paper towel should precede ink application.

Typically, contrasting inks of red and blue are employed for the peripheral margins and black for the base of the specimen. Additional inks may be applied to assess tips or oblong peripheral margins. Excess ink should be blotted from the surface of the painted specimen prior to each additional ink application. Following inking, the specimen is prepared for cutting.

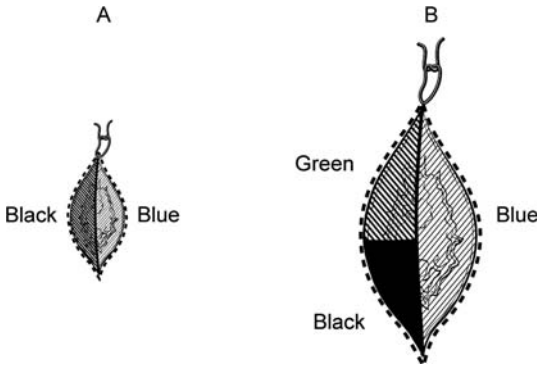


**Fig. 1.4** Inking an ellipse. A small ellipse (less than 1 cm) should be painted with at least two contrasting ink colors **A**. Larger ellipses **B** can be painted with at least three inks

## Cutting the Specimen

Typically, the specimen is bread-loafed perpendicularly to the long axis at nickel-thick intervals as to assess the peripheral extent of the tumor microscopically (Fig. 1.5). Exceptions to this rule exist however. Larger specimens (greater than 3 cm in length) may be cut parallel to the surgical margins and embedded in different cassettes. Wedge or triangular-shaped specimens should be handled

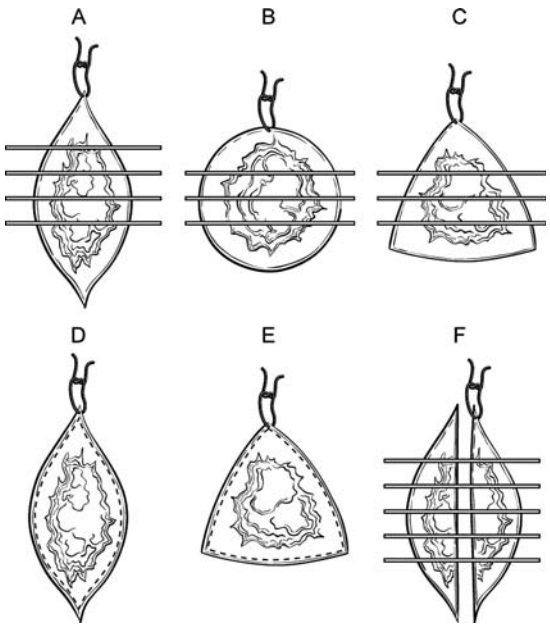


**Fig. 1.5** Recommended ways to cut specimens. In each instance smaller (less than 3 cm) elliptical **A.,** round **B.,** or triangular **C.** specimens single side is painted with a single or preferably two contrasting inks corresponding to the 12 o'clock to 3 o'clock and 3 o'clock to 6 o'clock margins, respectively, with the entire 6 to 12 o'clock margin painted in a third color. The base is typically painted in black. The specimens are bread-loafed along the short axis. Larger elliptical specimens **D.,** eliptical **E.,** or triangular **F.** can be prepared with parallel sections taken along the surgical margins

as follows: Each of the mucosal or cutaneous surgical margins should be assessed by taking parallel sections along the surgical margins to the apex with the remainder of the specimen bread-loafed entirely.

## Embedding

Embedding of the cut-tissue specimens is of particular concern. Anatomic orientation should be maintained for all specimens with the epidermal surface of each specimen arranged to first meet the knife edge upon sectioning (Fig. 1.6). Generally, no more than four specimens should be placed upon a single block as it is difficult to assure complete and uniform facing of the block with each of the cut specimens when this number is exceeded. The tips of elliptical or rhomboid shaped specimens may be deferred to permanents as they rarely possess carcinoma. Typically, the true surgical margin in parallel sections is mounted deep to assure its preservation with sectioning. Cryostat sections should be approximately 4 microns thick, and efforts should be made to ensure that the tissue sections are not folded and that immediate fixative immersion is performed once this tissue is firmly affixed to the slide. Sections should not be obtained for staining until the tissue is uniformly frozen and completely surrounded by OCT medium. Re-excision specimens may occasionally pose some quandary in preparation and cutting. Typically, wider excisions will incorporate a central defect with an intact base allowing uniform breadloafed sections. Occasionally, the specimen will possess a through-and-through central defect. Care must be exercised in properly orienting the halfed peripheral portions obtained from the central defect area.

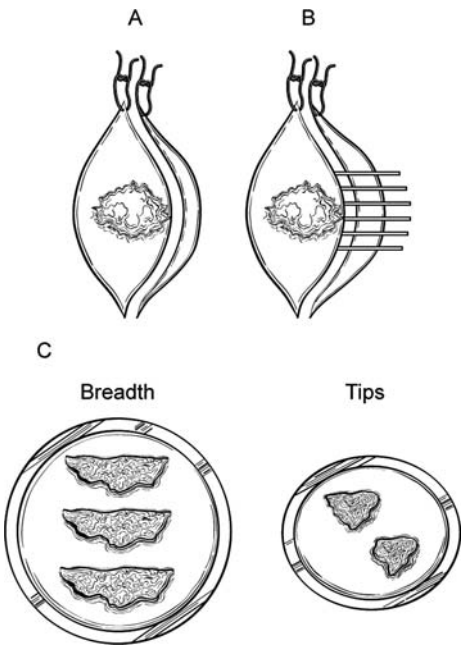


**Fig. 1.6** Placing tissue in the block maintaining orientation. The pieces are placed sequentially, so that if a positive margin is obtained, the site of involvement can be determined (**A** and **B**). Peripheral portions of central area should be placed in register with flanking sections (**C**)

## Mohs Technique

The Mohs technique was developed by Dr. Frederic Mohs in Wisconsin over 50 years ago as a means of extirpating non-melanoma skin cancer among patients who either failed traditional surgical means of removal or were deemed potentially inoperable on the basis of the tumor dimensions or anatomic location. The technique incorporates an alternative means for securing and preparing the tissue specimens for rapid histologic interpretation. The principal difference lies in how the tissue sections are cut prior to interpretation. Due to the emphasis upon conserving as much normal tissue as possible, orientation of the specimen is at a premium and is accomplished with the aid of meticulous use of color coordinated sketches. The first specimen taken termed level one is first examined to confirm a tissue diagnosis followed by the removal of successively wider slivers of involved-margin tissues termed levels. The first level is typically excised round with a 45° angle to assure that the specimen can be easily manipulated and is typically no thicker than 4 millimeters in thickness. In such fashion, the epithelium is vertically oriented with the dermis and subcutaneous fat oriented in a horizontal fashion allowing for the epithelium to be circumferentially visualized with the dermis. The process can be imagined with the aid of an orange (Figs. 1.7–1.10).

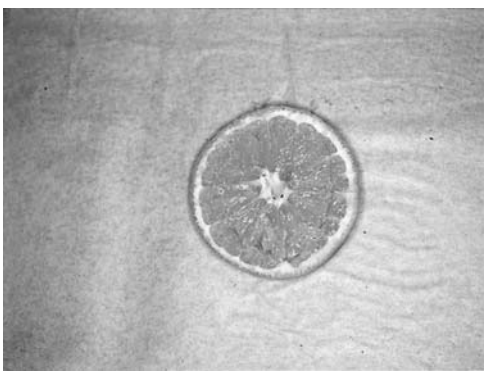

**Fig. 1.7** The meaty substance of the orange representing the tumor/dermis and the peel the external margin

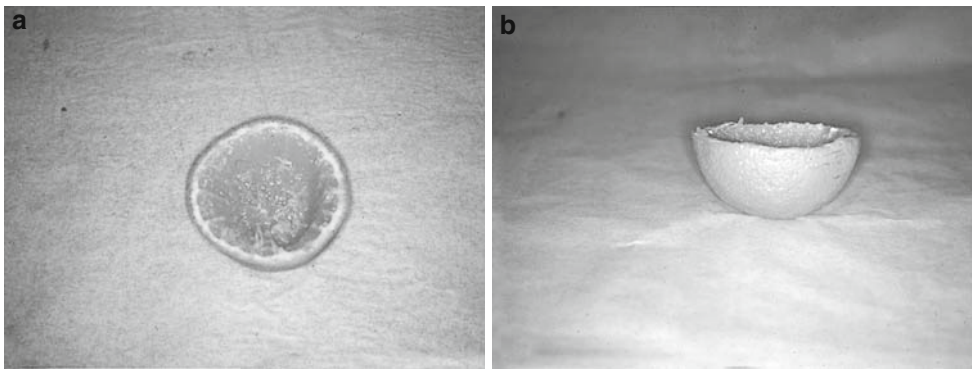


**Fig. 1.8a and b** The tumor (orange) is debulked with a curette and then beveled

**Fig. 1.9** Notice how the peel is unfolded so that 100% of the exterior surface is examined in a single plane following sectioning

bread-loafing of the skin specimen, this technique involves successive longitudinal cuts emanating from the epicenter of the tumor basin. This technique is ideally suited for non-melanoma cutaneous carcinomas such as basal cell carcinoma and squamous cell carcinoma or low-grade cutaneous sarcomas such as atypical fibroxanthoma or dermatofibrosarcoma protuberans that represent low-grade malignancies that tend to recur locally, rarely metastasize and involve little risk to the patient should a narrow margin of resection in an effort to preserve normal tissue result in incomplete removal and recurrence of the original tumor. The application of this technique or traditional frozen sectioning to high grade malignancies such as merkel cell

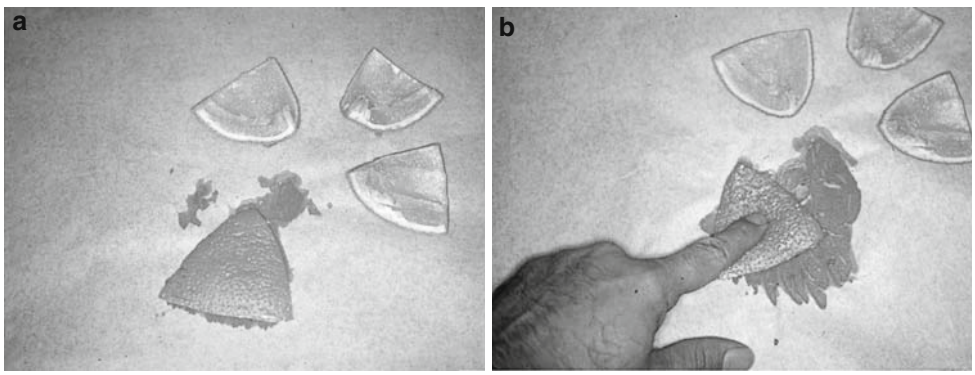


**Fig. 1.10a and b** Next, the specimen is placed deep-side down upon a cold bar or chuck without OCT, and the surface edges are pushed down to allow adherence to the cold bar forming a crowning-contact of the entire epidermal margin circumference

Sections so prepared will show the deep margin with the entire peripheral aspect of the epithelium. The successive levels are divided into roughly equal color delineated quadrants or as slivers if only focal margin positivity is encountered, each examined separately following tissue freezing and staining. The specimens are prepared for examination in a radial fashion in which the soft tissue margins are preferentially examined in a successive manner to permit the sparing of as much normal tissue as possible. Unlike traditional carcinoma or melanoma constitutes a more controversial area as these tumors possess a high propensity to metastasize and if allowed to recur and or remain incompletely removed following initial excision, are associated with a poorer prognosis. The Mohs technique is typically employed in the outpatient setting by surgeons specially trained in this technique as dermatologists or plastic/ENT surgeons. The principal utilities of this technique include the efficiency of the technique as a single physician is involved in the removal and

interpretation of the specimen and that it can be performed in a outpatient setting. The principal disadvantage of this technique is the time involved in examination and preparation of the tissues compared to routine outpatient-based excision as well as the expertise required by the operator. The success rate of this method as determined by the recurrence rate is at least comparable to traditional frozen-section methods with some series showing a superior recurrence rate.

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# Chapter 2
# Quality Assurance

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The outcome of Mohs micrographic surgery relies heavily on the abilities of the histotechnician. The duties of the Mohs histotechnician require more precision than those of the general histotechnician. Central to this are the understanding and skill set required in preparing sections so the surgeon can assess the entire peripheral margin. Mohs histotechnology training can take place at standard histotechnology education programs or can be learned on the job. It is the experience of the authors that most people who have been trained to cut routine histopathology can be trained to cut Mohs sections.

This chapter will review the Mohs histotechnology process and the salient aspects of maintaining high quality sections.

## Tissue Preparation

The work of the histotechnician begins at the point the surgeon harvests the tissue. The surgeon has many ways of marking the tissue for orientation and cutting into discrete tissue blocks. The most common methodology is to create extended hash marks at each of the points where the tissue will be cut. Most frequently, a nick is created at the six and 12 o'clock or three and nine o'clock points in anticipation of a bisected specimen. Some individuals will place additional hash marks on one half of a specimen to create asymmetry if they do not have a meticulous way of guaranteeing the tissue will not be rotated from the time it leaves the patient to the time it is brought into the laboratory. A double hash mark at one point can also be employed to serve the same purpose. On very small specimens, as will be discussed below, the specimen can be maintained as one piece with a "pacman" or butterfly configuration.

Our surgeons dissect and gross the specimen in the procedure room, though this is an issue of personal preference. Some feel grossing by the histotechnician under magnified light allows the technician to prepare specimens optimally for cutting. Regardless, one should try to create the least number of blocks for a given specimen. In creating tissue blocks, each cut edge represents an area of thickness that can rotate or roll toward or away from the blade. In principle and in practice, each edge represents an additive potential for false positives or false negatives. Thus, the optimal Mohs stage is a very thin one that is cut into as few pieces as possible.[1]

In our practice, pre-printed diagrams of the face and body are used for mapping. The mapping itself is done by the surgeon with the histotechnician marking in the dyed areas. A drawn representation of the initial specimen is made by the surgeon, with the orientation and size maintained as best as possible. A map that is drawn true to the tissue specimen allows the surgeon to easily superimpose persistent areas of tumor on the slide to the map and, ultimately, to the surgical site (Fig. 2.1).

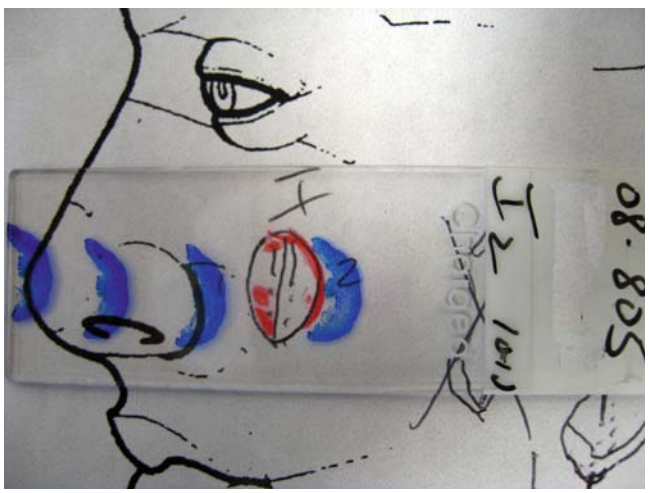

**Fig. 2.1** Areas of positive involvement are marked on the map and can be correlated closely to the cut section and surgical site

A variety of commercial dyes is available for the inking of specimens. The Davidson Dye System and the Delasco tissue stains are popular choices among many Mohs labs

M. Morgan et al. (eds.), *Atlas of Mohs and Frozen Section Cutaneous Pathology*, DOI 10.1007/978-0-387-84800-6_2,

and provides a wide variety of color options. Classically, Dr. Mohs used mercurichrome as his red dye and concentrated laundry bluing as his blue dye. While choice of dye is one of personal preference, it is important that the dye be applied sparingly as dye bleeding from one area to another may lead to confusion and inability to differentiate how the specimen should be correlated to the surgical site. In that unfortunate situation, if persistent tumor is noted, one is obligated to treat both the area felt to be positive and its mirror image so that the chance of leaving tumor behind is eliminated. In our practice, inking is typically done by the histotechnician, though in some practices it is done by the surgeon.

Some specimens, such as those that are cut thickly or with tall 90° edges, do not lie flat on their own. In these cases, relaxing incisions can be used to facilitate the complete flattening of the tissue and its marginal surface. These incisions on the non-marginal surface are cut partially through the thickness of the specimen, taking special care not to cut through to the marginal aspect. These incisions can take on several configurations including: a cross-hatch pattern, or concentric cuts parallel to the epidermal margin and transected by radial incisions.[2] Debulking of the central portion of the specimen can also facilitate flattening. These techniques can be performed in-vivo or ex-vivo and work well on most soft tissues. Incisions and debulking performed in-vivo minimize the risk of tissue margin disruption that can occur artificially in the lab. Relaxing incisions do not work as well on cartilage, however. It is our experience that slides prepared with neither albumin nor commercially available charged slides significantly help cartilage stay in place. The most useful way to keep cartilage in the final tissue sections is to take the stage so as to maintain the cartilage's attachment to soft tissue. This tissue acts as a tether or hinge so that the cartilage stays in place and will not float or "chunk" away during sectioning and staining. Special care must be taken to minimize agitation of the tissue during the staining process to keep it in place.

## Sectioning

There are many commercially available cryostats, with the majority today manufactured by Leica, TBS and Microm (Zeiss). The choice of cryostat is one of personal preference, particularly as it relates to the important features of tissue advancement and tissue cutting. It is the experience of the authors that automated tissue advancement can be a significant timesaver. However, automatic cutting of tissue for frozen sections does not allow for the precise control that the manual hand wheel offers in working with difficult specimens, though this again is an issue of personal preference.

### *Temperature*

The ambient environment plays an important role in the cutting of tissue. High ambient temperatures can make it difficult to maintain cold temperatures in the cryostat. It is not impractical to have separate air conditioning controls for the Mohs laboratory to maintain a cooler temperature. High humidity in the room can lead to curling of specimens. Condensation that accumulates in humid conditions results in ice crystal formation that can cause cracks and fragments as the tissue is being cut.

Cutting temperature within the cryostat of approximately −24° to −26° C is ideal for most soft tissue. One exception is fat, which tends to cut better at colder temperatures of −28° to −32°. If the histotechnician cannot cool the tissue down by either using an external freeze spray or liquid nitrogen, another option is to cut double- or triple-thick sections. This will likely make some epidermal features unreadable, but will give intact and readable fatty tissue.

### *Embedding*

Many methods are employed in the critical stage of embedding tissue for Mohs sectioning. Regardless of the method, the clear objective is to embed a complete and flattened marginal plane on the mounting disc that will facilitate level sectioning (Fig. 2.2).

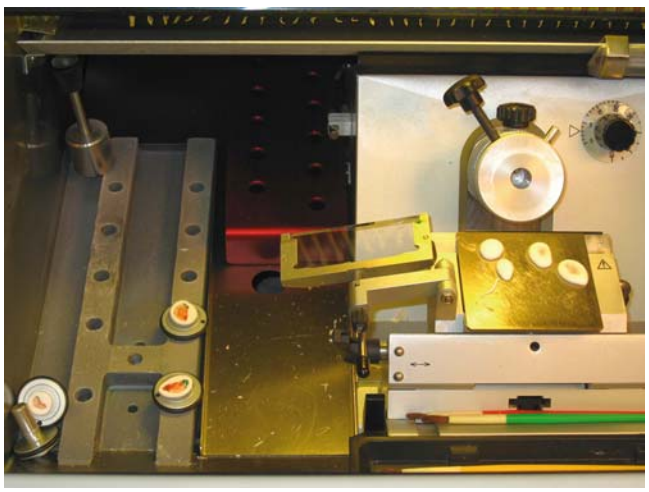

**Fig. 2.2** Here the tissue is flattened directly onto the stage as embedding media is applied. Once complete, mounting discs are placed on the freeze bar (as seen on the *left*) before sectioning

In the *direct or floating technique*, embedding media is placed on a mounting disc. The specimen, with the marginal surface facing outward, is embedded into the semi-solid media. Care is taken to tease the epidermal edges up

to create a level plane. A glass slide or heat extractor can be laid across the face to facilitate this. This method has lost popularity with the wider use of heat extractors and direct use of the freeze bar.

In contrast with the *heat extractor method*, the marginal aspect of the specimen is placed down directly onto the heat extractor. The specimen is manipulated to lie flat before embedding media is applied. Once sufficiently frozen, the specimen is flipped and placed on the mounting disc in a level manner, and allowed to freeze. The frozen specimen may not come easily off the heat extractor, and in these cases, many recommend that Teflon tape be applied to the face of the heat extractor before the tissue is first applied to prevent sticking. The heat extractor method has the advantage that the extractor can be taken out of the crytostat, and tissue manipulation can be done comfortably in open space. The *freeze bar method* is similar in principle to the heat extractor method, except that the freeze bar is a fixed area in the cryostat, and all work is accomplished within those confines.

With the *glass slide technique*, the marginal aspect of the specimen is laid down flat on a glass slide. The glass slide's transparency allows the specimen to be teased while the marginal surface is directly visualized. Once this is achieved, the slide is placed on the freeze bar, and embedding media is placed atop the specimen. Embedding media is placed across the face of the mounting disc. Once they both reach a near frozen state, the slide is flipped and placed in a level manner atop the mounting disc. Warmth from the histotechnician's fingers or thenar eminence will release the specimen from the glass slide and allow level mounting on the mounting disk.

Freezing should be done as quickly as possible to minimize ice crystal formation. The Miami special clamps were adapted and devised, in part, to facilitate embedding in the hot and humid environs of Florida[3] (Fig. 2.3).

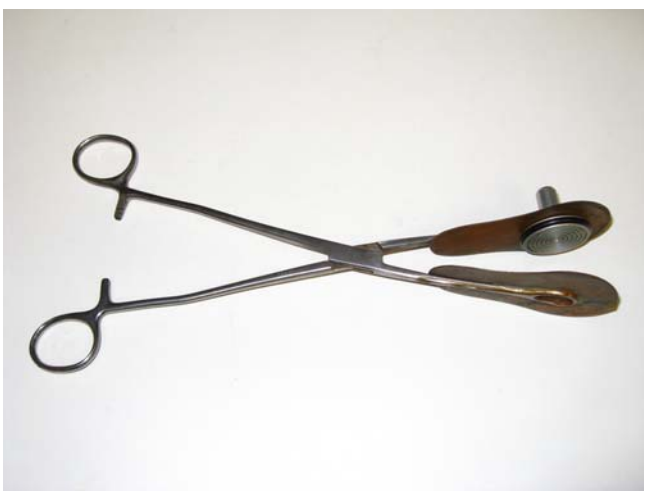

**Fig. 2.3** Modified obstetric clamps, shown with mounting disc inserted

These modified obstetric clamps allow the specimen to be secured onto a glass slide while being immersed in liquid nitrogen for quick freezing. A hole in one plate of the clamp allows a mounting disc to be introduced and clamped onto the specimen. This apparatus works very well for small specimens, though for larger specimens, the specimen cannot achieve true leveling due to the angle and pivot of the clamp's plates.

## Cutting

Cutting blades are generally categorized as permanent or disposable and may be specific to the type of cryostat used. Permanent blades may be sharper than disposable blades and can be resharpened as needed. We use disposable blades which are safe, efficacious and cost-effective. They also appear to be just as sharp as permanent blades when initially used. When disposable blades are used, the blade is moved along the blade holder over the course of the day. This prolongs the blade's sharpness as different parts of the blade interface the tissue block as the day progresses.

For frozen sectioning, one should try to achieve the thinnest sections possible. Technically it is difficult to get sections thinner than 3 to 4 microns. Sections that are thicker (above 5 to 6 microns) can often be difficult to read as cellular structures do not show very clearly. As mentioned before, thicker sections may be necessary for facilitating good sections of fat, but doing so will compromise evaluation of epidermal aspects. Alternating thicker and thinner sections on a slide is one way to get the best of all worlds.

In the process of cutting, when using a manual system, there are those that feel that a rapid turn of the cutting wheel followed by capture of the specimen on an anti-roll bar or on a chilled camel hair brush is optimal. Others work with a slow, deliberate turn of the hand. In this case, a steady hand is required so that the effect of chatter, ratcheting, or thick-thinning is avoided. The anti-roll bar is a piece of glass that is at the same temperature as the cryostat. When used, it allows a section to slide under as the blade cuts through the block. The use of the bar is a matter of personal preference, and skilled technicians generally feel the anti-roll bar slows them down.

Some histotechnicians have a microscope near the cryostat so they may evaluate unstained sections. With the substage condenser set to a low position to increase contrast, this setup allows the histotechnician to evaluate section quality before staining. This can minimize the need for deeper cuts after the mounting disc is removed from the microtome.

## Staining

The most popular stains used in Mohs surgery are hematoxylin and eosin (H&E) and toluidine blue. Maintaining quality on H&E stains can be difficult, and they are subject to pH changes with narrow tolerance ranges over time. Toluidine blue staining is more forgiving, but slightly more time is involved in setting up and, in our experience, must account for variations in local water supply conditions. A well-executed toluidine blue stain is rewarding in that metachromasia of mast cells serves as a built-in positive control. Mucin, when present, stains bright red and attracts the eye to potential tumors. Basal cell carcinomas stain an intense blue (Fig. 2.4) while squamous cell carcinomas will exhibit the greenish hue of prekeratin very clearly in many cases (Fig. 2.5).

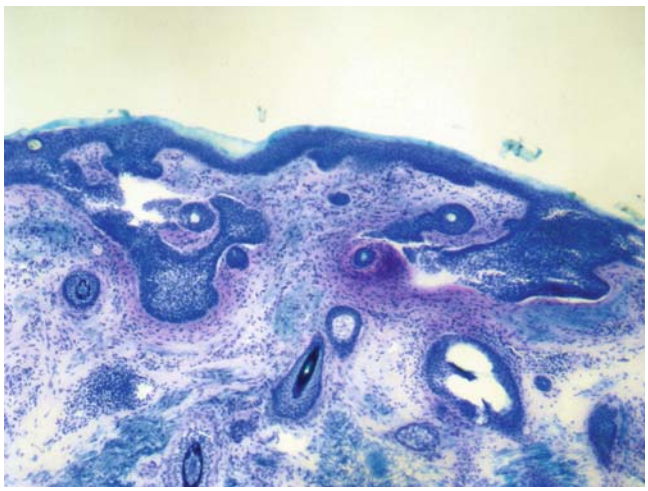

**Fig. 2.4** Mucin in the reactive stroma stains a characteristic red and helps in localizing basaloid aggregates of tumor

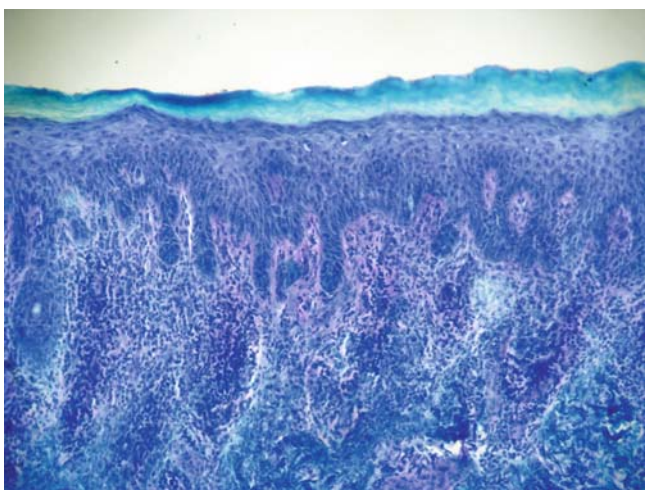

**Fig. 2.5** Mucin surrounds large cells with obvious nuclear atypia

Staining is typically done on slides with a sequence that involves the cutting of tissue and the fixation of the tissue in absolute alcohol. This is followed by the removal of excess embedding media and the application and dilution of various stains. Dehydration steps are accomplished with increasing concentrations of alcohol while the clearing of the specimens is classically done with xylene.

Because of xylene's toxic and flammable properties, many xylene substitutes are available. We find that the Limonene xylene replacement is a good substitute that does not affect slide quality and eliminates the risk of carcinogens. Despite this, our histotechnicians still work under a filtered hood to minimize the risk of excess inhalation of any of these volatile substances.

Automatic linear stainers can be time savers and allow the staining process to move smoothly in a busy practice. One concern is that autostainers can be subject to maintenance issues and breakdown. The choice of manual or automatic stainers should be a function of volume and the needs of a particular laboratory.

## Trouble Shooting / Quality Assurance

Suboptimal sections arise for myriad reasons. Maintaining communication and feedback between the surgeon and the histotechnician is integral in obtaining optimal slides for evaluation. With a multiheaded microscope, histotechnicians are able to directly correlate their techniques with what the surgeon sees and interprets (Fig. 2.6). For example, missing epidermis on sections can be brought to the attention of the histotechnician and identified. Then by changing the axes of the block holder, specific areas of the tissue block can be focused on for deeper sectioning.

The quality assurance process must occur as needed on a case by case basis and with regulatory bodies as part of a scheduled process by which slides are pulled and reviewed. Meticulous logs should be kept and reviewed with regard to changing of stains and reagents, crytostat maintenance and microscope calibration (See Table 2.1).

The following issues are commonly encountered and can be easily addressed:

Tears can result from the histotechnician flattening the specimen aggressively, or from the surgeon cutting inapparent notches. This problem often arises when the surgeon obliquely cross-cuts the base of the specimen while obtaining the Mohs layer. Tears can compromise visualization of the entire margin, and a concerted effort should be made to avoid them.

Chatter, or the "vertical blinds" effect, is likely a result of inadequate tightening and lubrication of the