Chapter 2
Gross Examination of Tissues in the Frozen Section Room

Stephen R. Peters

Abstract The chapter discusses gross examination of tissues in the setting of surgical pathology intraoperative consultation; *the frozen section room*. The process of gross examination is discussed in nine important steps from verifying of labeling and review of clinical information through gross examination, inking, sectioning, and cytologic preparations. These steps provide a checklist of considerations, as we perform this detailed and important part of our frozen section room task under the constraints of time. Emphasis is placed on the importance of understanding and correctly inking resection margins. The chapter offers a number of technical approaches to inking, and sectioning, as well as pointing out potential sources of error. The importance of cytology specimens and techniques for preparations are discussed.

Keywords Cytology preparations • Crush preparation • Scrape preparation • Touch preparation • Dissecting • Lymph node adipose tissue • Gross examination • Gross sectioning of tissue • Inking resection margins • Application of ink • Multiple sections • Perpendicular margin sections • Resection margin false resection margin • True resection margin • Inking resection margins • Sampling errors • Shave margin section • The sausage trick

When initiating a new resident to frozen section technique I like to start by asking the question "*What is the most important thing we do in the frozen section room*?" The answer is "*The gross*." Possibly a slight over statement, it serves to emphasize the importance of the gross examination. Our gross diagnostic acumen will never serve us better than in the frozen section room. A thorough gross examination and sampling of a wide range of tissues is our best defense against sampling errors. Recognizing important gross features of a pathologic process can strongly aid in the differential diagnosis we are considering microscopically.

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Anatomic details of complex specimens must be well understood before we can hope to make educated judgments about the extent of the patient's condition and need for further resection.

Unfortunately in our performance of intraoperative consultation there is one true limitation to the process we will employ. This limitation is *time*. And with this limitation we may sometimes feel a *bit* of pressure. We are asked to grossly examine, dissect, ink, and section a gross specimen, embed the tissue, freeze the tissue, section and retrieve the tissue, stain and cover slip the slide, and microscopically interpret the section in a period of 20 min in uncomplicated specimens. We must do our best to fight our urges to rush through any step of this process, as doing so will only put us at risk for errors. Rather we should strive to become efficient and technically skilled in all aspects of our complex task. When faced with large complex specimens or multiple specimens that will take a bit more time to properly examine I will let the surgeon know that it may take a few minutes longer. I will feel less pressured and he will find better use of the time. What follows is a detailed discussion of nine important steps in the gross examination process. Let this information serve as a checklist and fund of considerations and technical advice to best accomplish this task.

2.1 Gross Examination

The process of grossing should be performed in a meticulous and systematic fashion including all of the following steps:

2.1.1 Verify Specimen Labeling and Patient Identification

In a busy operating room situation with multiple specimens being handled at a time it is not difficult for samples and tissues to become confused both outside and inside the frozen section lab. It is wise to create and obey strict protocols in the way we verify, label, and handle multiple samples from multiple patients. Following a familiar routine is the best way to avoid errors of omission. When we deviate from routines we are more at risk of leaving out an important step.

2.1.2 Review Clinical Information

Our final diagnosis must be based on a compilation of all clinical, gross, and microscopic findings. When all three point to a common diagnosis we can proceed with confidence. In contrast, when our clinical picture or gross findings are very unusual for our suspected microscopic diagnosis it should be a warning that we should take a step back, reevaluate our possibilities, and proceed with caution.

2.1.3 Examine and Palpate All External Surfaces of the Specimen Carefully

Make sure that you understand the anatomy of the specimen. Check that all organs described in the specimen labeling are present. Examine and palpate all surfaces noting the following: color, texture, consistency, nodules, defects, adherent tissues, marking sutures, anastamotic lines, and any deviations from normal anatomy.

2.1.4 Understand the Resection Margins

The resection margins of a specimen are those surfaces that precisely represent the final cut surface of the tissue removed at an operation. *It is where the specimen ends and the patient begins*. When a resection margin contains tumor under the microscope, it signals the surgeon that the tumor has not been completely excised at that location and further resection is necessary if possible. The final status of the resection margins is an integral factor in the staging of the patient's tumor. The accuracy of the designation of these margins is critical to the patient's therapeutic outcome and treatment planning. Similarly our surgical colleagues often need to know if an inflammatory or infectious process involves a resection margin or simply if tissue at the margin is viable. Without strict attention to the anatomy involved, misinterpretations can be made by both surgeon and pathologist. Complex multiorgan resections can sometimes be difficult to orient and may contain subtle bits of anatomy such as small pieces of adjacent organs or portions of adherent surfaces that are critical for staging. For example, if a piece of parietal pleura is adherent to a lung resection, we must determine if the tissue has been infiltrated by tumor and if tumor extends to the resection margin of the pleural tissue. Both will be critical to accurate staging. Figure 2.1 shows an example of a colectomy specimen with a large tumor infiltrating neighboring organs. The illustration highlights all the resection margins that need to be considered to assess adequacy of excision and accurately stage the patient's disease. One must be aware of what represents a radial margin where the colon and other organs become retroperitoneal. All such landmarks should be identified prior to inking and dissection of the specimen.

I find that examining the specimen in anatomic position allows me to best maintain my sense of orientation of the specimen relative to the patient. Specimens such as complex head and neck resections can be quite challenging to orient and have many complex surfaces that represent resection margins. I try to imagine the patient lying on his back and visualize the specimen in position in the body. When turning the specimen to examine all of the sides, continually refer back to anatomical position to keep your orientation. Left, right, superior, inferior, medial, lateral, anterior, and posterior should be established. Pay careful attention to any sutures or clips with designations left by the surgeon to orient the specimen. For example, our surgeons often place a long suture to designate the lateral margin and a short suture to designate superior. If there is any question of what represents the true margin ask your surgeon to come to the frozen section

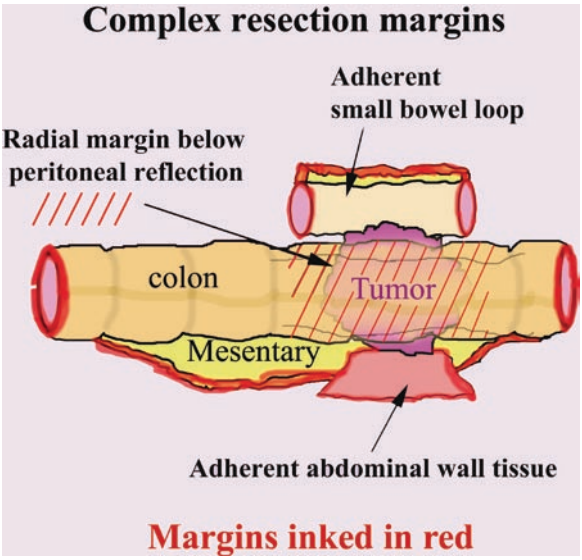

Fig. 2.1 Complex resection margins. The illustration depicts a distal colectomy specimen with a large tumor (*purple*), which has infiltrated through the colonic wall into adjacent tissues requiring the surgeon to resect a loop of small bowel and a portion of tissue from the abdominal wall. The resection margins of the specimen have been inked in *red*. An area of radial margin where the surface is retroperitoneal is indicated by the *diagonal red lines*

room to help orient the tissue prior to inking the margins. I have suggested to our surgeons, when inadvertently incising a specimen margin during the surgery, to stitch together the false margin on removal so that it will not masquerade as a true margin.

2.1.5 Inking Resection Margins

Proper application of marking ink or dye is essential to the pathologist's role in recognizing involved resection margins under the microscope. When the pathologist looks at a microscopic section in which resection margins have been inked, tissue surfaces coated with ink are interpreted as resection margins. When a pathologist sees tumor extending to an inked surface it is reported to the surgeon as a positive margin and the surgeon is obliged to remove more tissue from that site if possible to assure a complete excision of the tumor. It is imperative that this ink be applied with the utmost accuracy.

Traditionally black India ink was used to mark the resection margins. Inking with one color is best suited for tissue received without orientation. It is very easy to quickly ink the tissue in one color and interpret the state of the *global* resection margin as involved or not. Using a single color ink has its drawbacks in specimens that are received oriented as commonly encountered in excisional biopsies of skin

and breast. Our surgeons are expecting the resection margins examined and reported specifically for each surface, i.e., anterior, posterior, superior, inferior, medial, and lateral. Using a single color ink will require that each slide containing margin tissue slides must be designated by orientation to identify the specific margins. In storing the tissue the gross specimen must be either kept intact and oriented (risking under examination) or any unused margin tissue must be separately wrapped and labeled for holding so that the various margin tissues do not become mixed. Problems arise when a tissue that is not intentionally taken as a margin and therefore not designated as a specific margin turns up to have tumor involving an inked surface. There is no way to identify the specific site of involvement.

The practice of applying black India ink to all of the different margins of a specimen is quickly being replaced by use of sets of multiple colored inks, which are commercially available from a number of manufactures. There is a great convenience in using multiple colors. In a specimen inked in multiple colors the specific margins will be easily recognized by their color both grossly and microscopically and tissues margins do not need to be wrapped separately by orientation.

In my experience, no matter which of these media are used, great care must be used in applying these inks. When over applied, surfaces not intended as margins become falsely labeled as ink leaks into crevices and tissue planes and colors become smeared and blended into one another. The instructions supplied with the inks often call for a drying period. Unfortunately in a frozen section room situation, few have quite the patience to let a specimen dry for a prolonged period. A quick application of a 5% acetic acid to the inked surface will act as a mordant to set the ink to the tissue. The mordant acts by converting the ink into an insoluble compound with the tissue and will help prevent spread of the ink onto undesired surfaces.

If inking the specimen is done in a less than meticulous application, there are many possible causes for misinterpretation. For the purpose of this discussion I will define the terms *true margins, false negative margins*, and *false positive margins*.

2.1.5.1 True Positive Margin

Tumor extends to the true surgical resection margin. The microscopic section shows tumor extending into a correctly identified and inked resection margin (Fig. 2.2).

2.1.5.2 False Negative Margin

Failure to accurately apply dye to a true resection margin creates an opportunity for false negative interpretation by the pathologist. We may see tumor extending to a tissue edge but will not recognize it as a margin if ink has not been applied. If we fail to recognize tumor involvement of a true margin on frozen section we miss an opportunity to advise the surgeon to take additional tissue from the involved site. This will increase the likelihood of a future recurrence of the patient's tumor (Fig. 2.3).

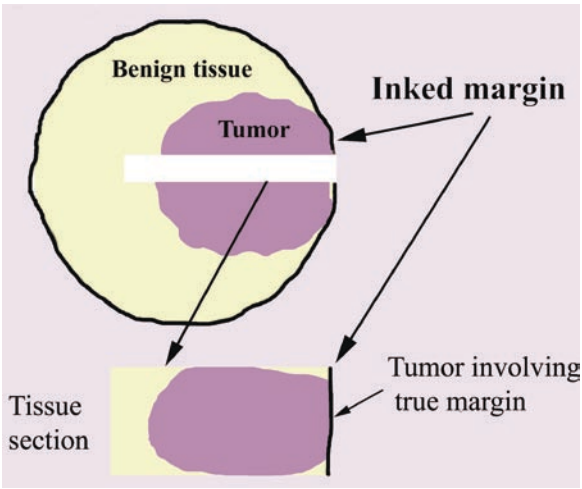

Fig. 2.2 True resection margin .The diagram shows a mock-up of a portion of breast tissue (*yellow*) with tumor (*purple*) extending into the inked margin (*black*). The white slice out of the specimen represents the section prepared into the slide below. The slide shows the tumor extending to the inked margin

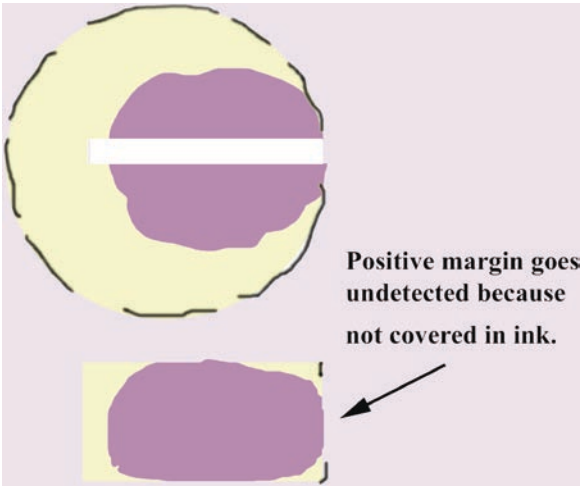

Fig. 2.3 False negative margin because of failure to apply black ink to the entire margin surface. The slide below shows that the positive margin will go undetected because it is not covered by ink where the tumor meets the surface

2.1.5.3 False Positive Margins

Surfaces that are falsely interpreted as a positive resection margin under the microscope because of the following causes:

- *Non-margin surface falsely dyed* due to misinterpretation of the anatomy or smearing or leakage of dye. If the dye is applied too thick without proper drying or use of acetic acid mordant it can be easily smeared onto other surfaces with our gloves, paper towel, or scalpel blade, creating false margins or mislabeled margins. Dye applied too heavily will allow dye to seep into crevices, along loosely held interstices and tissue planes creating false margins. One must be careful to recognize surgical cuts into the true margin of the specimen, which would create surfaces that can be carelessly dyed creating false margins. Breast lumpectomy specimens are particularly prone to this error. The surgeon may find that he is closer to the lump than first predicted and take a wider course around his target creating a slice in the specimen. Typically being soft and deformable, the defect in the lumpectomy specimen may be overlooked and inked as a margin. This is especially problematic when following the dreadful practice of dunking a specimen into a jar of ink. Similarly at the time of grossing it is possible to create false margins if the tissue is incised or planes of tissue are opened up and ink is misapplied (Figs. 2.4 and 2.5).
- *Mislabeled true margin*. A true margin mislabeled with the wrong color dye due to misinterpretation of the specimen orientation, smearing or leakage of dye (Fig. 2.6).

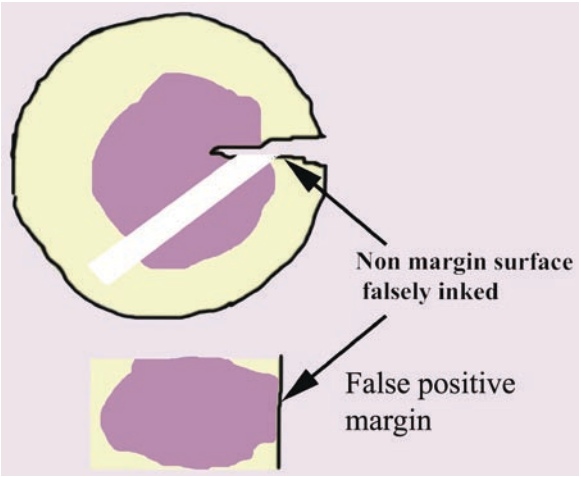

Fig. 2.4 False positive margins. The true margin is inadvertently incised during surgery or at the grossing bench and covered by ink. The slide below will be falsely interpreted as tumor extending into the inked resection margin under the microscope. Ink applied to any nonmargin surface will create potential for false positive interpretation of the margins of the specimen

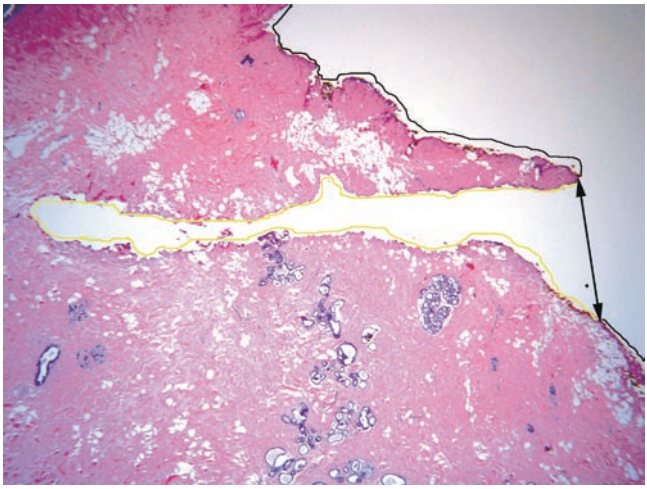

Fig. 2.5 Incised resection margin. Breast tissue micrograph showing an inadvertent incision into the true resection margin (*outlined in black*) creating a potential false margin (*outlined in yellow*). If ink is applied to the lining of the incision it will be falsely interpreted as a resection margin. ×20 magnification

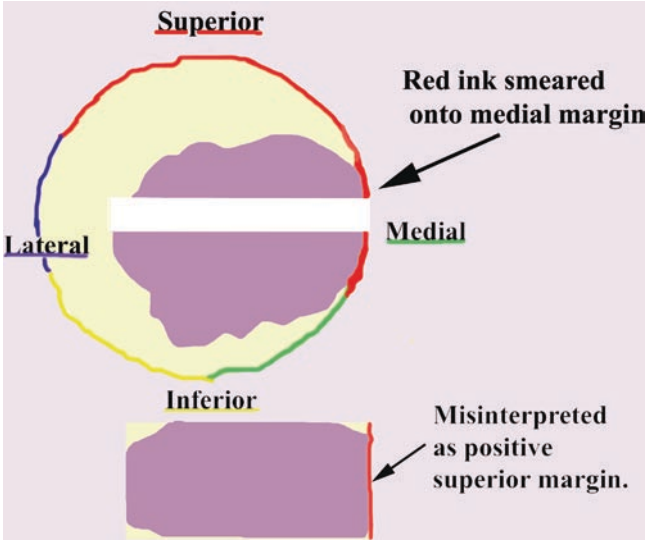

Fig. 2.6 Mislabeled margin. The red ink from the superior margin is smeared onto the medial margin. The slide below taken from the medial margin shows tumor extending into the red ink and will be misinterpreted as a positive superior margin

2.1.6 Application of Ink

There is a fine line between applying two little and too much ink. This is compounded by the fact that the thickness of the dye will vary with the age of the bottle and how well it has been sealed to prevent evaporation between uses. We need to see the inked surface under the microscope but this can be accomplished with a relatively thin uniform application. We do not need to be able to see the ink when we hold the slide up to the light! There should be constant attention to the proper filling and blotting of the applicator before applying dye to avoid overapplication. A cotton swab immersed in a bottle of dye should first be wiped on the edge of the bottle or tamped on the paper towel. If a heavily filled swab of runny ink is applied the ink will run into any available crevices and penetrate loose tissue planes creating false margins. A swab filled with ink applied to the surface of a small piece of skin will cover it in a puddle and will contaminate surfaces you intended to dye another colors. Heavy application of thick dyes will leave a thick coating that will be easily spread to unintended surfaces during the following cutting and handling. When using multiple colored inks, neatness and well-controlled application is even more critical. If treated casually, smearing and mixing colors will invariably ensue.

2.1.6.1 Suggestions for Inking Specimens

First dry the tissue and place the tissue on a dry paper towel. Any blood or liquid on the surface will cause the ink to run. A wetted towel will encourage the ink to run.

Always blot the surface of a freshly inked color before continuing to the next color. This should be done very gently in a controlled manner with gauze or other absorbent material to avoid contaminating adjacent surfaces with the excess ink. If you have a puddle of ink precisely applied over a defined surface such as a fraction of a skin ellipse, if quickly blotted it can wash over to contaminate other surfaces before the liquid is fully absorbed. Rather than grossly blotting the tissue you are really using the gauze as a vacuum to pull away the excess ink using the capillary action of the absorbent material. This can be done with very gentle pressure using a small corner of the gauze or the end of cotton swab. When blotting a multicolored surface, it must be done with a single gentle press of the gauze. Repeated blotting can spread the colors and cross contaminate margin surfaces.

When rolling or changing position of the specimen to apply additional colors, avoid touching and cross contaminating surfaces with previous colors present on the paper towel. It is best to move to a clean section of towel or new towel whenever the specimen has to be moved on the towel.

Be very conscious of where dyes have gotten onto your gloves during the dying process. I suggest a change of gloves between the dying and cutting of the tissue to avoid contamination. I often change gloves during the inking process when I get messy, especially when using multiple colors.

2.1.6.2 Designating Margin Colors

When using multiple colors to dye margins, it is imperative that the specific colors be designated for each margin in the gross dictation so that the pathologist reading the slides can recognize a given margin. As a pathologist reading the sections, it can be cumbersome to constantly refer back to the gross to recall the color of each designated margin. By initiating a conventional labeling scheme to be used on all applicable specimens it allows the pathologist to immediately know the meaning of a dye color under the microscope. A simple scheme used in our department is as follows: RED is a SUPERIOR color; YELLOW is an INFERIOR color; GREEN has an E sound like MEDIAL; BLUE has an L and should be opposite green. LATERAL; BLACK is on the back-POSTERIOR; ORANGE is ANTERIOR. (A pregnant woman carries her PUMPKIN in front.) Give this convention, when I see a margin inked red I know it is superior and a margin inked green is medial without repeatedly referring to the gross dictation. In specimens in with left and right are margin designations then colors must be individually designated in the gross description.

2.1.6.3 Applicators for Inking

Ink is commonly applied with some form of applicator sticks or cotton swabs (Fig. 2.7a). These work best for many tasks but fall short on very large surfaces and very fine tasks. For small specimens such as skin biopsies that need to be inked in multiple colors, a simple cylindrical applicator stick can be imprecise. For detailed multicolor dying, a more accurate set of application tools can be fashioned by carving the wooden end of an applicator swab for each color. With a few strokes of the scalpel, carve a flattened end similar to a fine flathead screwdriver. Next cut the flattened tip at an angle to arrive at the applicator shown in Fig. 2.7b. The swab end can then be used for blotting after application. A set for each color can be made in a few minutes and they can be wiped clean and used for an extended period. I suggest hiding them from your colleagues.

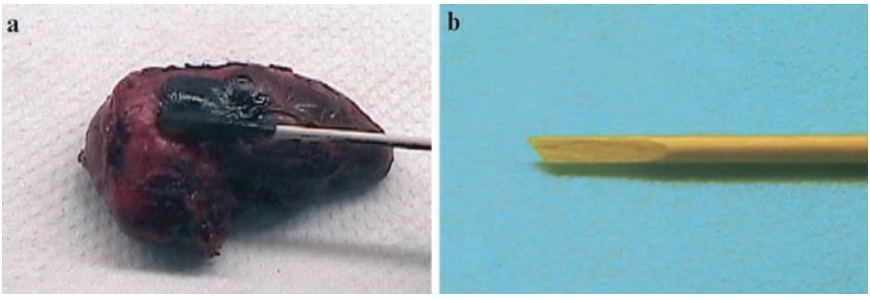

Fig. 2.7 (**a**) Cotton tipped swab being used to apply black India ink to the margins of a thyroid lobectomy specimen. (**b**) Simple home-made carved inking tool for fine applications such as in inking of smaller skin lesions

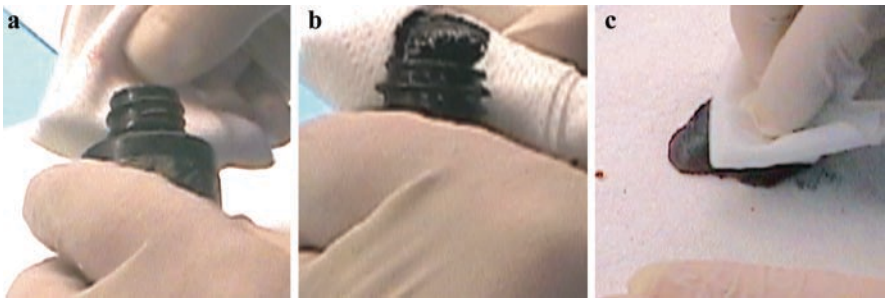

Fig. 2.8 Inking with a gauze pad. Fold or gather a gauze pad and briefly turn over the ink bottle to fill the pad. Apply ink in a blotting fashion to cover he specimen

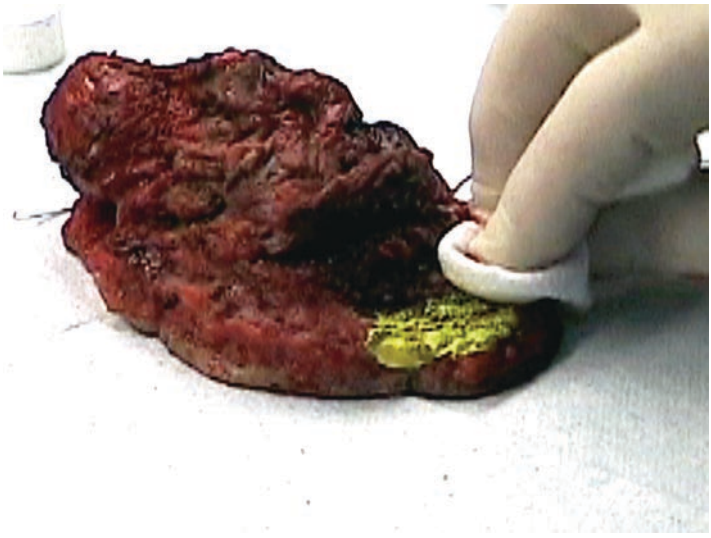

Fig. 2.9 Applying ink with a gauze pad to a large sarcoma resection specimen

For very large areas such as the deep side of a mastectomy specimen, use one or two 4×4 gauze pads gathered or folded to form a pad. Fill the pad by holding the pad over the mouth and briefly turning over the bottle. This can then be used to apply dye in a blotting or gentle wiping fashion. This method is particularly valuable when applying dye to irregular surfaces with crevices. By lightly filling the pad, ink can be lightly applied in a *dry brush* fashion onto the surface desired without ink running into every crack (Fig. 2.8 and 2.9).

2.1.7 Dissecting and Sectioning the Specimen

Large complex specimens should be opened completely and carefully examined in an orderly fashion using standard grossing techniques. Try not to let the constraints of time lead you to rush through a proper dissection process. On the other hand there is always the need to preserve the anatomy and relationships for the formal gross examination and dictation often performed at a later time. In many hospital settings the formal gross exam and dictation will be performed by a different person from the one who performed the gross at the time of frozen section. In these settings we must be do our best to preserve the gross findings in the specimen and convey any important gross findings that were disturbed in sectioning during the intraoperative consultation.

On opening fresh bowel or stomach there is often considerable luminal contents and adherent mucus obscuring our examination. In order to properly examine the mucosal surface some rinsing is often necessary. A gentle approach is to rinse with saline while gently wiping adherent secretions and debris. On occasion we are called upon to find some minute biopsy site or tumor on the mucosal surface of the stomach or bowel. In order to find such minute gross findings as a previous biopsy site or small superficial lesion, it is necessary to thoroughly clean the mucosal surface. I was taught it is taboo to run water onto fresh mucosal surfaces because of the damaging effects of hypotonic water on the cells. Unfortunately, in these situations the important question needs to be answered during the surgery. Resorting to a thorough cleaning by running the mucosal surface under water in these situations may be a necessary evil in order to find the gross lesion. I cannot recall an instance where having done so has affected my ability to interpret the section microscopically on H&E. I imagine more sophisticated studies might be affected by this practice.

For simple specimens such as breast biopsies and thyroids, the specimen should be bread-loafed completely at 3-mm intervals. Typically biopsied for a palpable nodule or radiologic finding, these specimens can harbor small occult lesions peripheral to the target lesion. A few slices in an intact specimen that remains held together like a book is not an adequate examination. *The process of bread loafing and lying out of the tissue slices should be considered separate processes, each to be completed before beginning to examine the cut sections.* The 3mm sections should be laid out on a paper towel so that all cut faces are visible. Take care in lying out the sections so that consecutive cut surfaces are visible. If two mirror image surfaces are laid face up, one of the two consecutive faces will be face down and subject to overlooking.

Dissect off any unessential fat. A typical example here is frozen section of lymph nodes. When examining lymph nodes it pays to be meticulous about removing all of the fat from the surface and medulla if present. Figure 2.10 illustrates a simple scalpel technique for *peeling away the remaining fat* from a lymph node.

- Start by palpating the node to define its borders and removing the gross fat from the node by any means of smearing , squashing, cutting, and scraping the gross fat off of the node using fingers or scalpel.

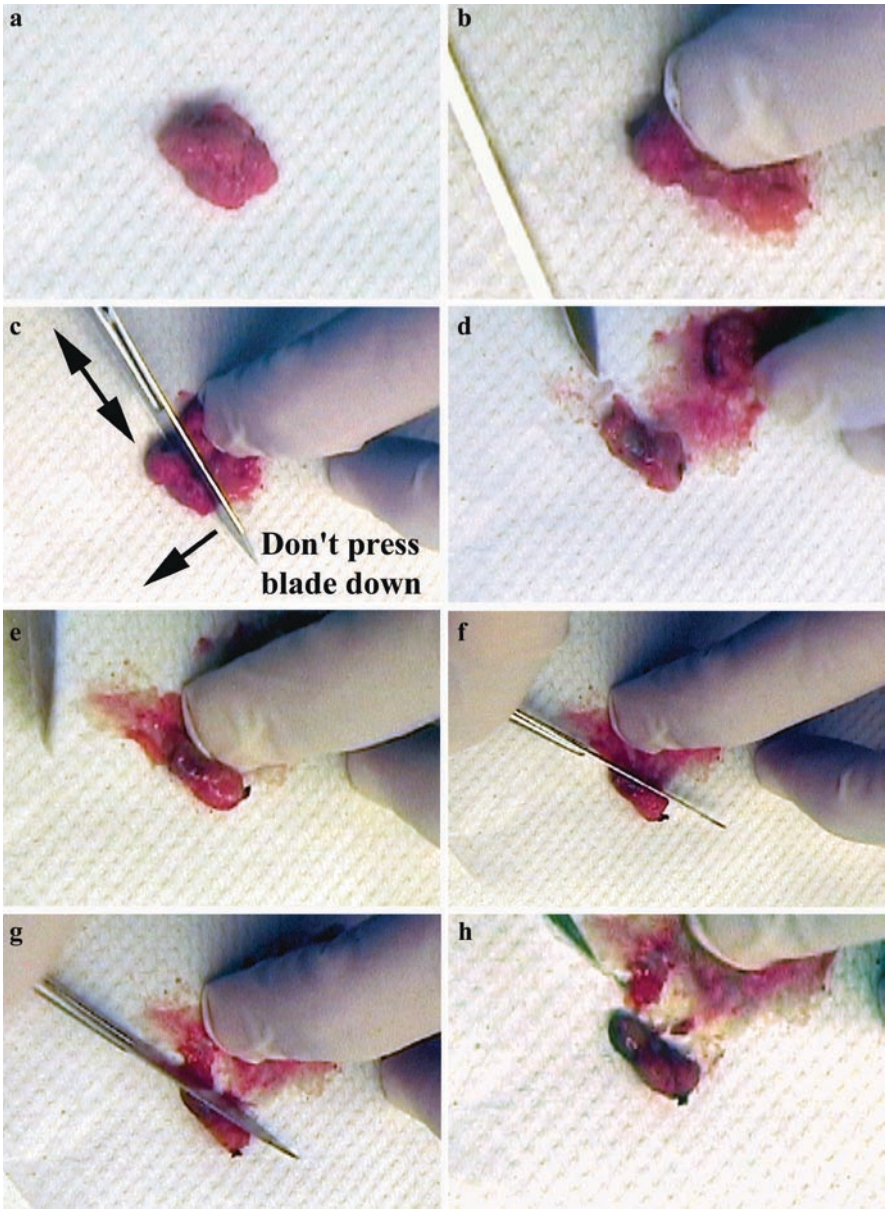

Fig. 2.10 Removing fat from a lymph node capsule. (**a**) Small lymph node covered in a layer of fat on a dry paper towel; (**b**) Smear the fatty tissue to the paper towel while pushing the node to one side with the finger tip; (**c**) Simultaneously move the scalpel back and forth (*double-headed arrow*), push the lymph node to the side with the blade (*single-headed arrow*) and press very lightly down with the scalpel so that it does not cut through the fat. The fat adhering to the towel will be cut away in the plane that forms between the towel and the rolling node. (**d**) Lymph node with most of the fat removed; (**e–h**) Repeat the process to remove any remaining fat

- Next slowly roll the node on a dry paper towel. As the remaining fat adheres to the towel, a plane of cleavage stretches between the adherent fat and the node.
- Cut through this plane by pressing very lightly down with the scalpel so that it does not cut through the fat while simultaneously moving the scalpel back and forth and pushing the lymph node to the side with the side of the blade. The fat adhering to the towel will be cut away in the plane that forms between the towel and the rolling node.
- Repeat the process to remove any remaining fat.
- With careful dissection one can remove the fat without incising the lymph node capsule. Some pathologists may criticize this technique suggesting that removing this fatty tissue may cause failure to identify tumor in perinodal fat. It is my rational that I have a much better chance of finding a positive node with a intact well-prepared section than in a suboptimal section that is cut with great difficulty due to the presence of fat.

2.1.7.1 The "Sausage Trick"

This technique allows you to take soft, difficult to cut tissue and make it firm and easy to cut by rolling the tissue in a paper towel or absorbent pad. This technique also helps prevent the knife blade from dragging ink into the tissue. This works particularly well with fresh fatty breast specimens received for frozen section. To roll the tissue, use a dry high-quality paper towel or absorbent pad. Some of the higher quality folded towels work fine. If you only have low-quality paper towels use two together and first dry the tissue. If wet, these low-quality towels will not firm up quite the same and shreds of paper will tear away when you unwrap the tissue. I find these sturdier absorbent pads to work very best (Fig. 2.11).

2.1.8 Examining the Cut Specimen

The tissue slices should be examined meticulously in the order they are laid out using both our eyes and palpating with our fingers, slice by slice. Tissues should be examined as closely as our eyes will allow us to focus. A hand magnifier can be a useful aid. When examining these cut sections I encourage the examiner to *look away from the pathology.* If one jumps quickly to the obvious nodule in one of the central sections, surely one day they will miss the 3-mm nodule at the edge. If we focus our attention on the large obvious bowel tumor we can easily overlook other important findings. Large complex specimens should be examined by anatomic regions in an orderly and meticulous fashion. An experienced examiner should be able to recognize the gross architectural elements corresponding to the histological features seen microscopically. For example, we should be able to recognize the depth of gross tumor infiltration relative to the layers of the bowel wall or recognize that our breast tumor is grossly infiltrating into the overlying nipple.

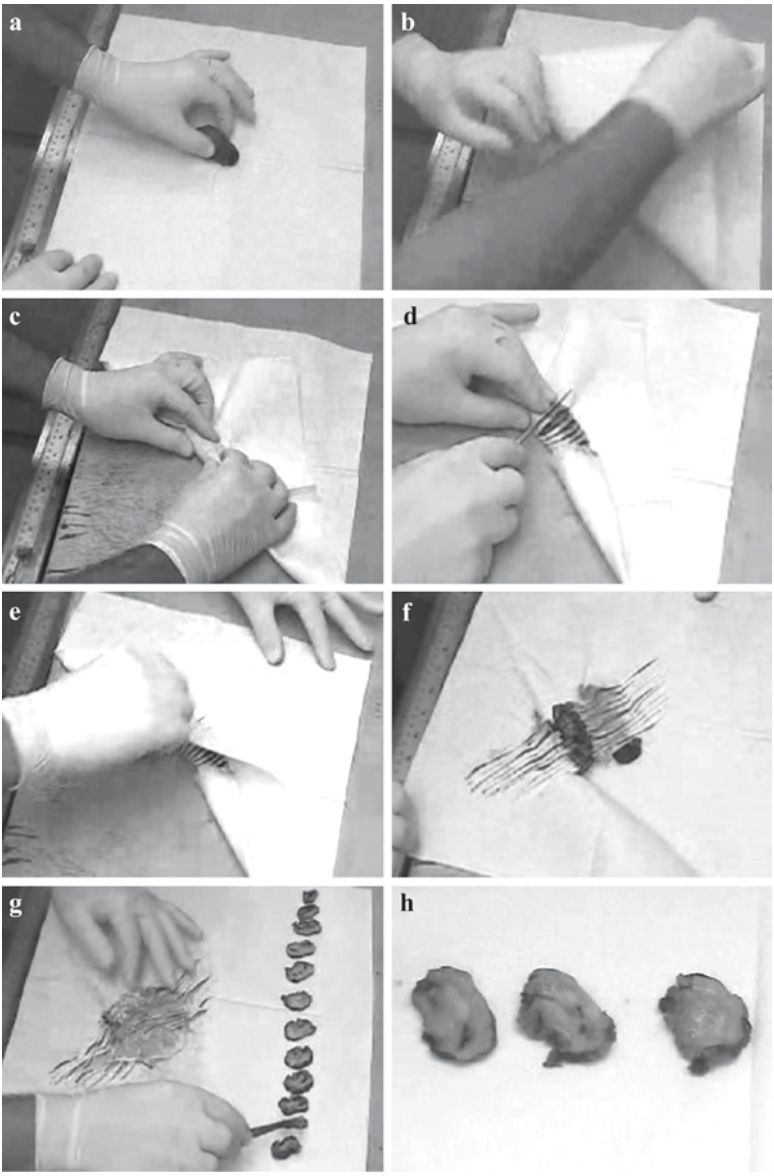

Fig. 2.11 The sausage trick. (**a**) Place the specimen on the diagonal in the center of a strong dry paper towel or absorbent pad. (**b**) Fold the towel diagonally across the tissue. (**c**) Crimp the towel firmly to the tissue and roll the towel and tissue into a firm sausage. (**d**) Slice the sausage into 3-mm slices. (**e**) Unroll the towel by gently pulling the corner backward. (**f**) The sliced specimen is still mostly intact. (**g**) Lay out the specimen entirely in an orderly fashion. (**h**) Examine each piece visually and by palpation

Important information can even be gathered looking carefully at even the tiniest specimens. In a 1-mm speck of brain tissue, the difference between the opaque tan white appearance of a normal brain and the translucent gray of a glioblastoma is quite obvious. The difference in the colors brown vs. gray in a liver core biopsy immediately helps point the pathologist in the right direction.

2.1.9 Taking the Sections for Frozen Sectioning

Many specimens consist of small pieces of tissue without orientation. These can be dealt with by simply taking a piece or cutting a slice of the tissue for embedding. However, in many cases recognizing the pathology in the gross state will require us to grossly cut our sections in a most specific manner to best demonstrate the findings. Samples cut to demonstrate involvement of margins must be sectioned and embedded in a precise manner. In many cases there may be several possible approaches. One must foresee how the tissue will be oriented and displayed after cutting, embedding, and sectioning. Some tissues can be very challenging to cut in the fresh state. For example, taking a section of an unfixed polyp attached to its underlying tissue can be a challenging task. Figure 2.12 illustrates an approach to precisely section a polyp through its stalk and attachment. This approach can be applied to many of our difficult sectioning tasks.

- *Start with a sharp scalpel*. When approaching our most difficult tasks always start with a new blade. This is most important in cutting friable tissues, which will crumble easily when assaulted with a dull blade. Scalpels can become dull more quickly than we sometimes realize. Just because you put on a new blade a

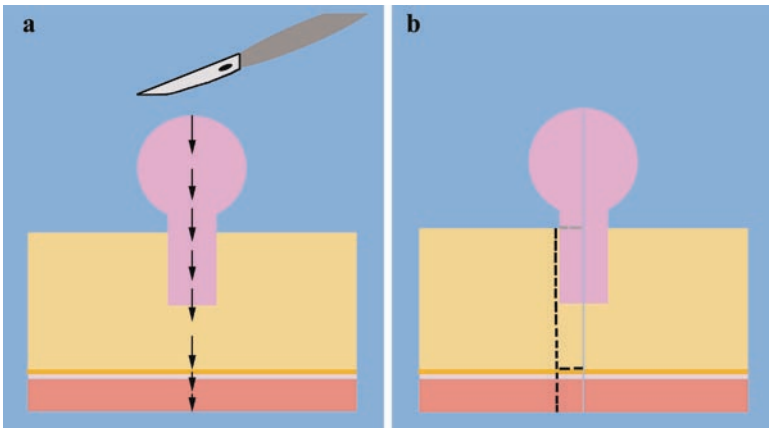

Fig. 2.12 Sectioning a polyp. (**a**) Using long very gentle strokes of the scalpel blade make the first cut (*arrows*) through the head of the polyp while aiming to continue through the stalk and remaining wall. (**b**) The remaining cuts will excise the remainder of the section (*dotted lines*)

few minutes ago and only cut a couple of small specimens it does not mean that the blade is still sharp enough.

- *Make the first pass of the scalpel through the plane you wish to see under the microscope*. The initial cut represents the face to be embedded and cut for our frozen section. The following cuts will excise the remainder of the tissue section and trim the other five sides. In our example taking a section of an unfixed polyp attached to its underlying tissue the first cut should be made bisecting the polyp and preserving its stalk attachment. The split polyp can then be excised from the specimen and taken to a flat surface for trimming.
- *The knife blade should begin its cut at the most difficult and critical aspect of the tissue*. Approach the task by beginning the cut at the critical cutting surface where it is most difficult to cut across without crumbling. In the example of bisecting the polyp and preserving its stalk attachment, the knife should begin on the head of the polyp and continue through aiming for the stalk. In taking a section of uterus, start by incising the softer more deformable endometrium to define the thickness of the section and then continue to cut across the underlying myometrium.
- *Use minimal scalpel pressure to cut the most delicate tissues*. To split the friable soft head of a polyp, using very little downward pressure with long continuous backward or forward motion of the scalpel will result in the cleanest cut with least crumbling. Using any type of knife or blade we will obtain cleanest, most uniform cuts by using long continuous backward and forward strokes of the blade. *The more delicate the tissue, the more gently we need to press down with the blade and rely on the sharpness of the blade to cleave the tissue without crumbling.* It is helpful resting the polyp on a paper towel when cutting a polyp while attached in an intact organ. It may be easier to first excise a block of tissue containing the polyp before bisecting it. This may make it easier to get the tissue into a position to make such a fine controlled cut.

Beware of cysts! It is wise to exert minimal pressure with the fingers and scalpel when cutting tissue that is grossly cystic or may contain small cysts. When we squeeze tissues with our hands and press firmly with a scalpel, we raise the pressure in any cystic components. As the scalpel blade encounters these cysts under pressure the contents will squirt out of the tissue like a pin hole in a water balloon. This can put the operator at risk for eye contamination and transmittable disease. Protective eyewear is common sense in any grossing situation.

Sections can be taken from resection margins either perpendicular to the margin or parallel to the margin, also called a shave margin. When one sections a margin perpendicular to the margin, the section will demonstrate how close the tumor is to the margin (Fig. 2.13a). The disadvantage in sectioning margins perpendicularly is that you are actually only examining a 5-μm-wide portion of the entire surface. Even if a 30-mm-wide margin is entirely examined in 10 perpendicular sections of 3-mm thickness, the pathologist will only see ten 5 μm widths of tissue spaced 3 mm apart or a total of 50 μm. This rather exhaustive study of ten margin sections will only have actually examined 1/600th of the tissue. In tumors such as breast cancer

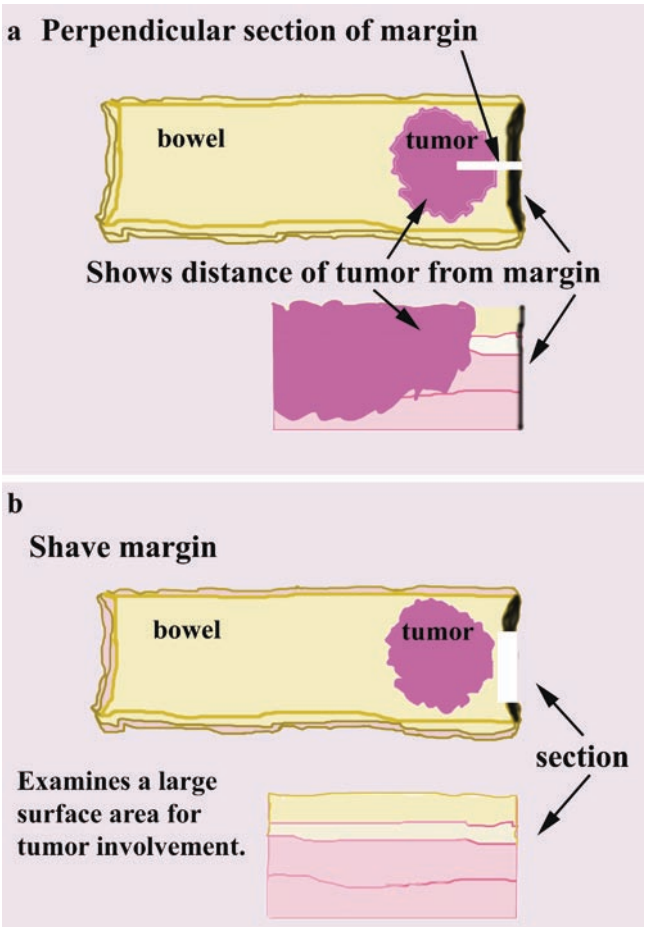

Fig. 2.13 Illustration of segment of bowel with a tumor (*purple*) close to the margin on the right. The slide made from the tissue section removed is pictured below in each frame. (**a**) Shows a section taken perpendicular to the margin clearly demonstrating the distance of the tumor from the inked margin. (**b**) An example of a section taken as a shave off the margin. The section demonstrates the absence of tumor but offers no information as to the proximity of tumor to the resection margin. The information gathered varies considerably

and visceral tumors where lymphatic and perineural invasion are commonplace, a specimen may be considered incompletely excised if it comes to within a specific distance to the margin. These situations will be better served taking sections perpendicularly despite this fractional sampling. Here again is where out meticulous attention to the gross pathologic findings will allow us to raise our odds of finding the positive margin. Perpendicular margin sections should always be taken at points where the tumor is visibly closest to the margin. In situations where no tumor is visible I have found that a little common sense can help us raise our odds beyond that of randomly taking samples. For example, given a re-excision breast biopsy

specimen where there is a biopsy cavity and no grossly visible tumor, I will pay most attention to the areas where the biopsy cavity walls come closest to the margin. This is where tumor *used to be* closest to the margin. Taking multiple sections perpendicularly will help to reduce false negatives due to sampling error.

A shave margin will demonstrate if tumor is present anywhere along the entire face of the resection margin (Fig. 2.13b). *In actuality, the ultimate resection margin will be trimmed away when surfacing the block prior to sectioning*; the distance from the ultimate margin will depend on how much tissue is needed to be trimmed away before taking the complete section. In fact, if a thin portion of tissue is embedded anything less than flat and trimmed in much less than perfect *x-y* orientation it is very likely that a portion of that margin may be trimmed through and lost for interpretation before reaching the entire tissue face. With a shave margin examination, if the tumor is not present in the tissue, the microscopist has no way of determining how close the tumor is to the margin microscopically. The goal of therapy in Mohs surgery is complete local excision of a skin tumor with minimal tissue loss. This is accomplished by examining shave margins across the entire resection margin of a skin specimen. As we will discuss in Chap. 8, it is critical that Mohs surgery specimens are embedded in a flat plane with the tissue face parallel to the chuck face (*parallel faces*). See Chap. 8 (p. 6).

Take multiple sections to reduce sampling errors. In dealing with very large tumors such as soft tissue tumors and large ovarian cystic lesions to name a few, we often rely on specific microscopic criteria to make the diagnosis of malignancy. The more widely we sample these tissues the better our odds of making a correct determination. Using the embedding techniques described in the next chapter one can take multiple tumor samples from areas of diverse gross appearances, prepare them in one or two large blocks, and raise our odds of finding the necessary features to make the correct diagnosis (Fig. 2.14).

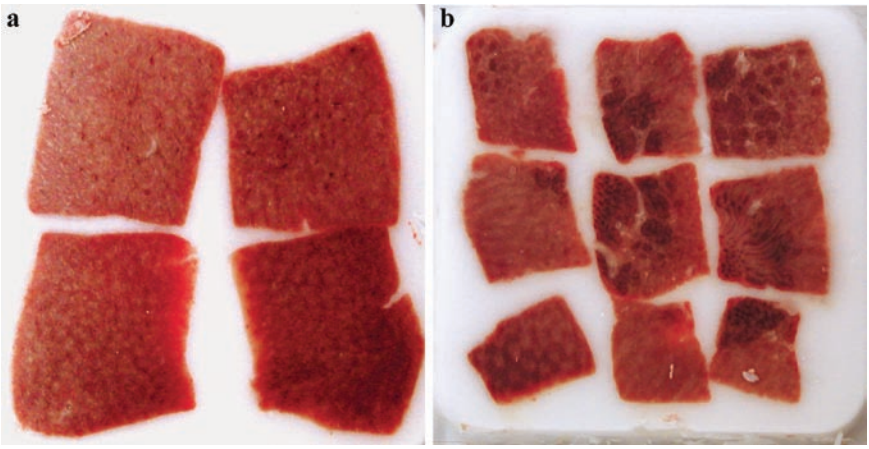

Fig. 2.14 Examples of frozen section blocks prepared by embedding multiple samples in a single block. Examining multiple samples will reduce sampling errors in large specimens

2.1.9.1 Margins on Small Specimens Removed Piecemeal

In cases where surgeons are removing small tumors that are difficult to remove in a single piece such as a locally excised small malignant laryngeal lesion that is removed in several fragments, I suggest that the surgeons take what they believe is the entire lesion and place it in a single container. In a second container I ask them to remove another thin layer of tissue from the base to be examined as the final resection margin. If this final margin tissue is free of tumor it can be reported as a negative final margin. The same applies for tissues submitted intact and oriented that have a positive margin. If by the nature of the location it is not possible to orient the new margin, I suggest that they remove what they think is adequate margin tissue and place it in one container and then remove an additional thin layer of tissue as the final margin. If the first specimen showed tumor in the margin and a single poorly oriented specimen was removed as additional margin one would expect tumor to be present somewhere in the tissue even if it was not seen on the frozen section. Possibly it is on the opposite side of the tissue and was not visualized because the tumor was buried in the block. By examining an additional thin layer of tissue we will be more confident that the final margin is negative.

2.1.10 Cytology Preparations

These simple techniques provide extremely useful information to contrast with the findings in the frozen section, particularly when examining neoplastic tissues. Smears provide a dimension of information that cannot be seen in sectioned tissue either on frozen section or paraffin-embedded samples. A well-made smear will provide optimal nuclear detail to compare with any artifacts seen on the frozen section preparation. Cytoplasmic and cell membrane characteristics are sharply visible. In brain biopsy cases, crushing and smearing a minute portion of tissue will demonstrate important clues such as the fibrillary cytoplasm of a glioma, the cohesive groups of a metastatic carcinoma, the absence of cohesion of a lymphoma, and the histiocytes of demyelinating disorders. By air drying slides and performing the Diff-Quick stain an entire new compendium of cytoplasmic details appear including nuclear and cytoplasmic vacuoles and cytoplasmic and extracellular substances such as mucin and thyroid colloid. Cytologic features are discussed in more detail in Chap. 7.

Cytologic preparations can be either fixed or air dried. Fixation should be carried out as quickly as possible for optimal cytologic detail. Air-dried slides should be made with a thin layer of cells and quickly dried for optimal preparation. It is worth waving the slide in the air for a moment to quickly dry the cells. Bloody slides with too much material will not dry quickly and result in an unsatisfactory, marginally readable preparation.

The most suitable type of cytologic preparation will vary with the type of tissue.

Touch prep – Suitable for lymph nodes and other very cellular tissues (Fig. 2.15).

Scrape prep – Suitable for firm or hard specimens. It is particularly useful in yielding cells in hard scirrhous tumors and poorly cellular and fibrous tumors such as spindle cell tumors (Fig. 2.16).

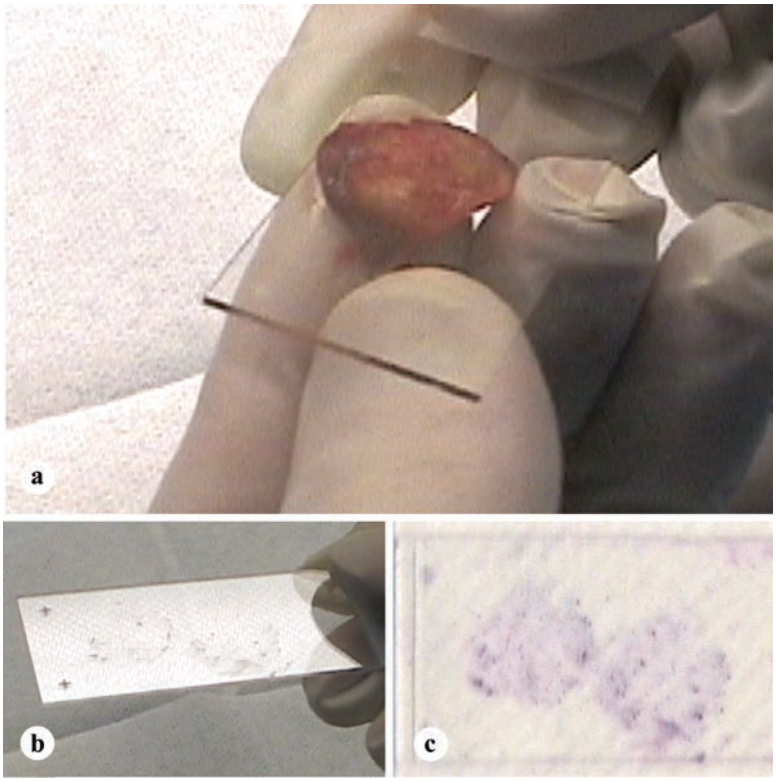

Fig. 2.15 Touch preparation (**a**) after blotting the excess blood from a freshly cut surface; touch the tissue to the slide and immediately fix the slide in 95% EtOH or Air dry. (**b**) The slide with visible imprint. (**c**) Stained slide (Diff-Quick stain)

Crush preps – Preparations can be made from tiny soft specimens that cannot be scraped or manipulated for a touch prep and are particularly valuable in the interpretation of brain biopsies where cytoplasmic processes and discohesive cells offer important clues. A small speck of a core biopsy can be sampled for adequacy using a crush preparation. Crush preps yield very little in hard fibrous tissues. While crushing the tissue, do it in a controlled manner and observe the ease with which the tissue crushes. The tissues will exhibit properties ranging from nearly that of a liquid to pasty to firm, leathery, rubbery, or bony. These properties can give you insight into the diagnostic categories. For instance, when faced with the a differential diagnosis of schwannoma vs. menengioma, I have found that most menengiomas (with the exception of more fibrotic variants) will smear to some degree where as most schwannomas were like smearing a rubber band. With rubbery tissues you can try crushing it harder but it will often have very low yield (Fig. 2.17).

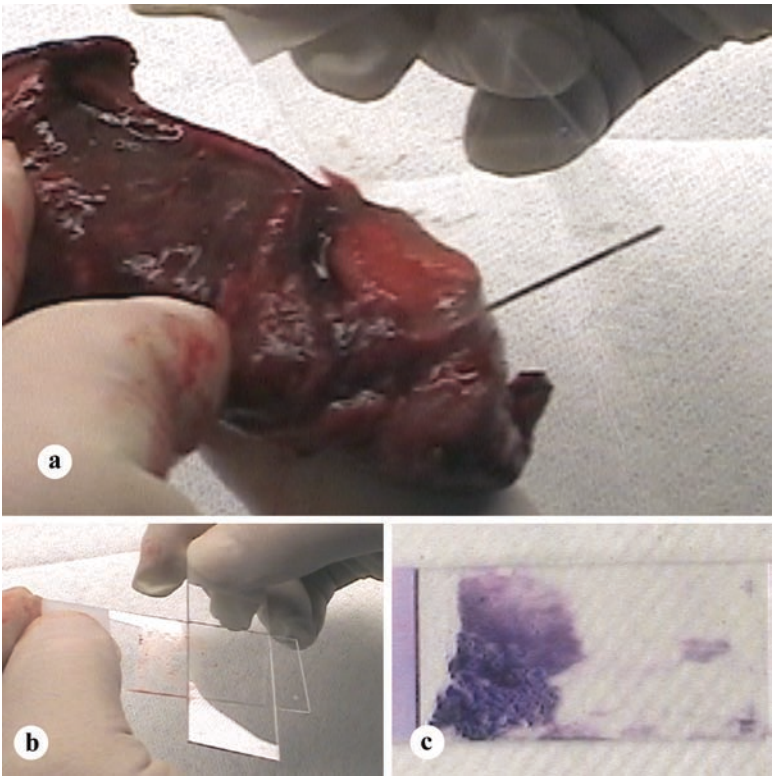

Fig. 2.16 (**a**) Scrape the edge of the slide on the tissue face. (**b**) Smearing the scrapings on a second slide. (**c**) Stained preparation (Diff-Quick stain)

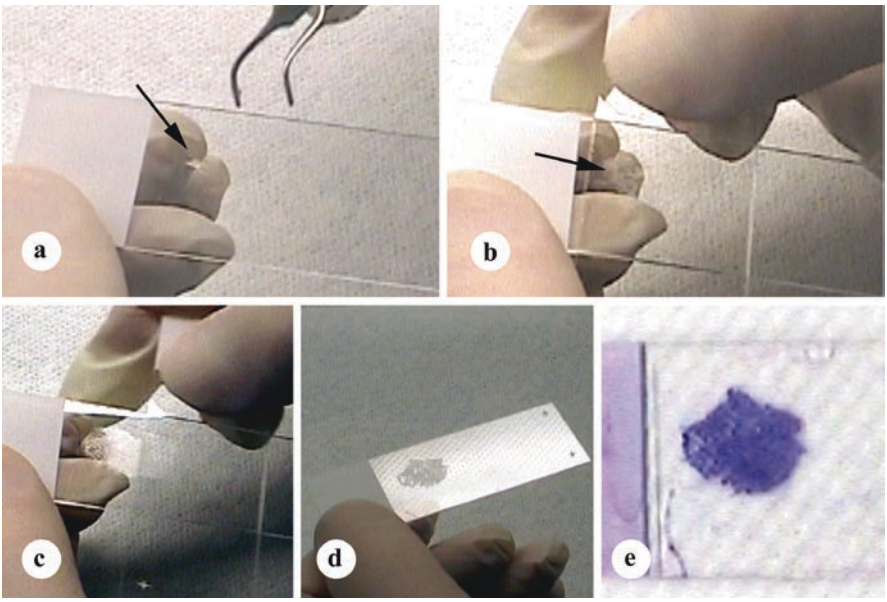

Fig. 2.17 Crush preparation. (**a**) Place a sample of tissue on the slide. Tissue can be less than a millimeter in diameter and not larger than 2 mm. (**b**) Place the second slide in smearing position. Gently apply slight pressure to the tissue watching the effect of the crushing on the tissue and being careful not to overcrush the tissue. (**c**) Smear the tissue and immediately fix the slide in 95% EtOH. (**d**) Smeared tissue on the slide. (**e**) Stained preparation (Diff-Quick stain)