Paper Cryoembedding

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Abstract

The article describes a unique method of face-down tissue embedding for frozen section in freezing-temperature well bars. Tissue is first placed face down in a precise position on a piece of lens paper. The tissue is then transferred in the exact position to an embedding well. The block is completed while the tissue remains face down on the paper. The paper is removed during the initial trimming of the block, leaving the tissue in its original position and orientation. The technique accomplishes precise embedding of extremely delicate and deformable tissues and arrangements of tissues. We believe this technique will facilitate a variety of difficult embedding tasks in areas of surgical pathology, Mohs surgery, and research settings. (*The J Histotechnol* 26:173, 2003)

Submitted August 5, 2003; Accepted August 7, 2003

Key words: frozen section, cryoembedding, paper cryoembedding, embedding well bars

Introduction

In the practice of frozen section pathology, we are often faced with the task of embedding tissues that are extremely delicate because of the nature of the consistency and structure of the tissue. This situation is a daily occurrence in Mohs surgery, where the technologist is faced with delicate slivers of skin with the goal of embedding this tissue precisely flat and completely visualizing the epidermis and margin. During this process the epidermis can easily be turned under, placing it at risk of being inadvertently trimmed away.

We are commonly given large flat specimens that need to be assessed by embedding the tissue on edge. Examples include ovarian cysts and endometriosis; membranous tissues, such as pleura, peritoneum, and synovium; and broad flat margins encountered in tumor resections from a variety of sites. These samples are often quite large, and our accuracy hinges on our ability to adequately sample these tissues.

This article describes a method of accomplishing these most delicate embedding tasks by face-down embedding in freezing-temperature steel wells (1,2). The technique first requires the precise positioning of the tissues on lens paper. The tissue is then transferred to the embedding well on the lens paper without disturbing the original positioning. The lens paper is eliminated during the initial trimming of the block.

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Materials and Methods

The technique is performed using steel embedding well bars, steel chucks, and vinyl dispensing slides from Precision Cryoembedding System, Pathology Innovations, Wyckoff, NJ (1), a small of piece lens paper (Fischer Scientific), and a cryoembedding medium (Tissue Tek OCT compound, Sakura Finetek, Torrance CA.).

The first example (Figure 1a) illustrates this technique using a very thin portion of skin measuring less than 1 mm in thickness. This thin sliver of skin must be embedded perfectly flat with complete visualization of the epidermis and entire margin.

Technique

1. Cut a rectangle of lens paper 3–4 mm longer than the tissue. Place a small drop of embedding medium on the dispensing slide (Figure 1b).
2. Wet the paper by passing one side over the drop of embedding medium, flip the paper over, and then place the paper on top of the drop of embedding medium on the dispensing slide (Figure 1c).
3. Press away any excess embedding medium and bubbles from under the lens paper using the back of a forceps and pull the paper over the edge of the slide so that a tab of 2–3 mm overhangs the edge (Figure 1d).
4. Place the tissue in the desired position on the paper at the edge of the slide (Figure 1e).
5. Turn the slide and look at the tissue from the side and bottom checking that the epidermis is in contact with the paper over its entirety. Make any adjustments necessary (Figure1f).
6. Touch the wetted overhanging tab of paper to the floor of the well (Figure 1g). It will adhere.
7. Pull the dispensing slide out from under the paper in an even motion (Figure 1h). The paper will drop to the

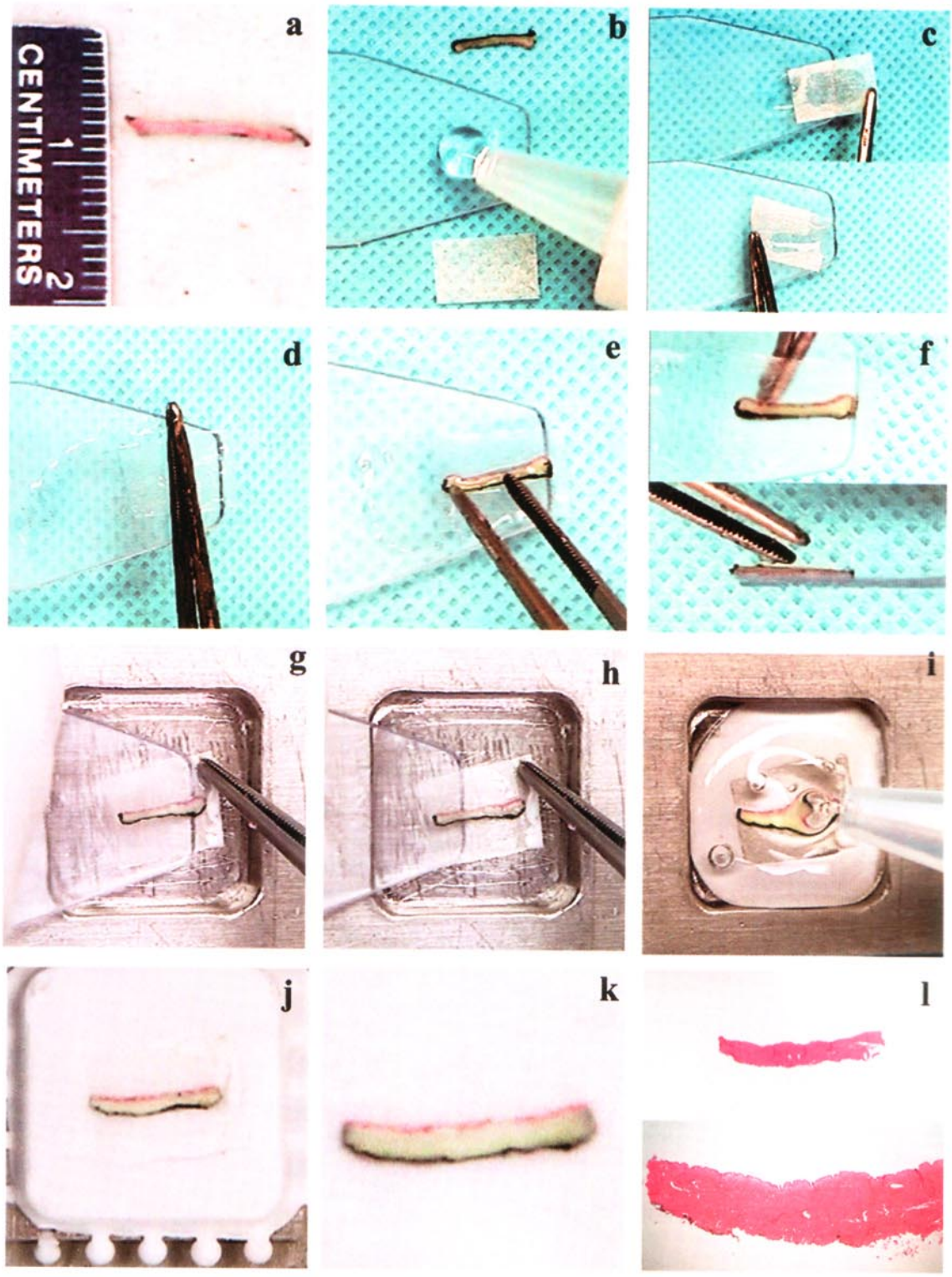

Figure 1. Paper embedding of skin specimen measuring less than 1 mm in thickness. (a) Skin sample looking at epidermal surface, measuring approximately 0.8 mm thickness. (b) A drop of embedding medium is placed on a dispensing slide. Skin specimen viewed from the side. The deep margin is inked black. A portion of lens paper (c) The lens paper is wet on one surface by passing it over the drop of embedding medium (top); Lens paper is flipped over and placed over the drop of embedding medium wetting the other side (bottom). (d) Excess medium is pressed out from under the paper using the back if the forceps. Tissue over hangs the slide leaving a tab of 2–3 mm. (e) The tissue is placed in on the lens paper and positioned. (f) A final adjustment of tissue is made by looking at the tissue from below through the slide(top) and from the side (bottom).(g) The tab of lens paper is touched to adhere to the floor of the embedding well. (h) The dispensing slide is pulled out from under the lens paper, which falls to the floor. (i) The well is filled with embedding medium. (j) Completed block before trimming. The outline of paper is visible on the surface of the block. (k) Trimmed block showing a complete section with entire epidermis (red) and Inked margin (black) visible. (l) Photograph of slide (top) and 20× micrograph of slide showing complete well-oriented section.

floor of the well. The edge of the dispensing slide or forceps can be *gently* run over the tissue to assure complete flattening.

8. Fill the well and complete the block as described previously.
9. The paper will be visible on the surface of the completed block (Figure 1j).Trim the block until the tissue is completely visualized. At this point, the paper will have been completely trimmed away from the tissue (Figure 1k). Figure 1l shows a photograph and micrograph of the completed section.

Comments

A. Use a well large enough to easily accommodate the paper.

B. Once the paper and slide have been prepared, place the tissue in the well without delay so that drying does not cause the paper it to adhere to the dispensing slide.

C. In placing the tissue in the well, make an effort to adhere the entire width of the tab evenly to the well floor. This will facilitate the paper being pulled evenly from the slide.

D. Avoid having the tissue on the tab. The desired effect is to create a downward hinge like action on the paper weighted with the specimen as the slide is pulled from under the paper.

E. Lens paper is an extremely fine paper that resists tearing and becomes translucent when wet. These properties make it very suitable for this technique. I have noticed little effect of the paper on the sharpness of the blade. If this is of concern the block and paper can be trimmed with one half the blade and the final sections taken with the other half.

F. Once the block is positioned and trimmed, any imperfections in the block face can be filled by plastering technique (1). A small drop of embedding medium is applied to a slide or any convenient applicator. The medium is lightly spread over the face of the block and quickly pressed flat and frozen with an overchuck freezing block.

This second example will demonstrate a common application of paper embedding in surgical pathology. We are often faced with large flat specimens that need to be embedded on edge. A variety of cystic lesions of the ovary and other organs; flat sheets of membranous tissue, such as those lining body cavities and joints; and broad flat margins that need to be visualized on edge are just a few common examples. Our accuracy hinges on our ability to adequately sample the tissue and view it on edge. Our ability to find the critical diagnostic area will be enhanced by examining a large area of tissue. Paper embedding technique allows us to place a large volume of this thin walled tissue on edge in a single block.

Technique

1. Prepare a rectangle of lens paper the width of the dispensing slide (25 mm) with the length about 3–4 mm. longer than the width (Figure 2a). The dispensing slide can be used as a guide and straight edge to cut the paper with a scalpel.
2. Slice the tissue at 3-mm intervals and no longer than the width of the paper (Figure2b).
3. Wet the tissue slices with embedding medium (Figure 2c).
4. Apply embedding medium to the dispensing slide, wet both sides of the paper, and remove any excess medium as in example 1 (Figures b–d).
5. Place the strips of tissue on the paper and stand them on edge by leaning them together to create a "book" of the strips. Organize the strips into the desired position. Leave a tab of paper overhanging the dispensing slide.
6. Press the tab of paper to the floor of the well and pull the slide out from under the tissue as described in the first example (Figure 2e). Complete the block.

Figure 2f–i shows the untrimmed block, the trimmed block, photograpghs, and photomicrographs of the prepared slide.

Comment

A. I used skin as an example because as a demonstration it is easy to visualize the on edge orientation as a completed microscopic slide. I do not recommend putting this much skin so close together in a single block when examining tumor margins. There is a natural tendency for embedding medium to pull away from the epidermis when sectioning skin by any method. This separation is an indication that the block is trimmed to a level that includes the entire epidermis. This property is useful in recognizing when skin has been trimmed to the desired level. This phenomenon of epidermis pulling away from the embedding medium creates a tendency for the end of the skin, which first hits the blade to curl away from the medium. This initial curl can be best avoided by warming the block to a point where there is less curling. This separation and curling phenomenon is familiar to most experienced operators. When cutting one piece of skin in a block, obtaining a complete section takes experience. In the second example, perfect sectioning of this many pieces of back to back in a block is a formidable task. For the accuracy demanded in skin tumor resections, I recommend using a more widely spaced positioning by direct placement in the well (1) or by paper embedding, or using frozen block cryoembedding technique as described in a previous publication (2).

B. The second example describes a situation where we can examine a large volume of tissue in a single block. These large samples should be cut at the warmest possible temperature that sectioning is possible to reduce stress on the system. Cutting large tough pieces of tissue by any method may tax the limits of the ability of the cryostat and blade. For example, if one tried to cut a rock in the cryostat something would have to give. Similarly with very hard and tough tissues in large volume one may experience chatter (thin

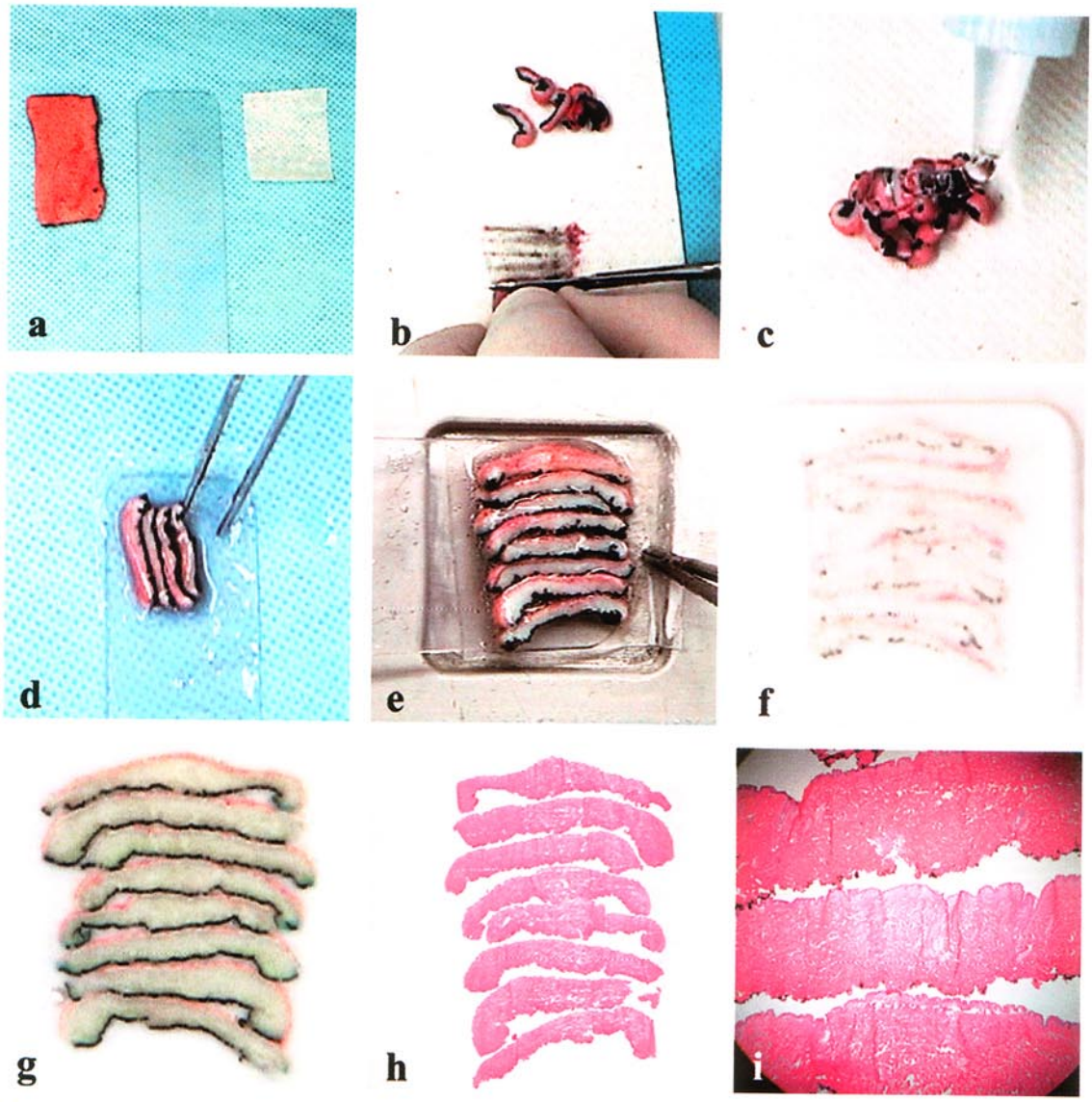

Figure 2. Embedding multiple slices of a large flat specimen on edge. (a) Skin sample 4 × 2 cm and 2–3mm thick, dispensing slide, and square of lens paper. (b) The tissue is sliced in 3-mm strips. (c) The strips of tissue are wetted with embedding medium. (d) The tissue strips are placed in a side-to-side arrangement and stood on edge by leaning against each other. (e) The tissue-filled paper is placed into well. The paper tab is touched to the well floor and the dispensing slide is pulled out from under the paper. (f) Completed block before trimming. (g) Trimmed block. Epidermis is stained red; deep surface is inked black. (h) Photograph of prepared slide. (i) Micrograph showing strips of skin embedded on edge.

parallel wavy lines on the block face), or thick and thin sections. These artifacts may also be a sign that maintenance is due or that clamping knobs are not fully tightened. Skin and very fibrotic cyst walls are examples of very tough collagenous tissue.

C. Making Jelly Rolls

When confronted with membranous tissue that is so thin it is difficult to stand the tissue on edge, a useful alternative is to make a "jelly roll". First, cut the membrane into 3 mm wide strips. These strips are easiest to work with if cut into 3–4 cm lengths. Wet the strips with embedding medium. Prepare the lens paper as above. Start the roll on the paper by grasping the end of the first strip with the forceps and rotating the forceps to form a small roll. The following strips can now be leaned against the central roll which will support the strips while standing it on edge. Continue adding to the roll as desired. The roll can now be placed in the well as described above. (Illustrations available on website)

A third example will serve to demonstrate the ability to embed a very thin, extremely flimsy membranous tissue flat over its entirety. This application of paper embedding may find utility in the research laboratory setting. Figure 3 shows a 1-cm square of fetal membrane that measures 0.5 mm in thickness, is embedded flat, and is completely sectioned. This tissue was first cut in a square then spread upon the lens paper. Thinner, more delicate tissues could be picked up onto the paper by laying the paper onto the tissue to retrieve the tissue.

Discussion

Frozen section is an extremely important means of histologic preparation in the practice of surgical pathology and is used daily in most hospitals for rapid diagnosis during intra operative consultation. Frozen section also plays an important role in preparation of tissues for a variety of clinical and research applications not suitable for routinely processed tissues. Our accuracy is dependent on precise positioning of the tissue during the embedding process. Historically, the conventional frozen section embedding method entails placement of tissue face up on a chuck coated with embedding medium. This is often followed by flattening the tissue by a weighted heat extractor. This technique is considered by many to be considerably less precise and predictable than the positioning possible by conventional face down paraffin embedding technique. This lack of precision can be considered a significant disadvantage of the frozen section technique when compared to paraffin embedding. The difficulty in achieving a precisely flat tissue orientation by conventional frozen section embedding methods can demand considerable trimming through the tissue to get a complete section. Excessive trimming leads to tissue waste and at times the final goal is not achievable because of trimming through the tissue or aborting the attempt in fear of wasting the entire sample.

The methods described in this article use a system of apparatus and techniques that accomplish face-down embedding of tissue for frozen section. The system, which was described in a previous publication (1), requires stainless-steel well bars and chucks kept at freezing temperature in the cryostat. Tissue is placed into the wells by sliding the tissue into the wells with precise positioning using a thin vinyl plastic dispensing slide. This system has the advantages of speed, precision and reduced tissue wastage when compared with conventional cryostat methods using face up embedding directly on chucks. The system offers a level of versatility that in practice provides a fast and easy solution to frozen section embedding tasks.

The method of paper embedding described in this article accomplishes precise face-down embedding in a variety of extremely delicate samples that are too deformable to be easily pulled in position from the dispensing slides.

We conceived of this technique as a modification of a technique commonly used by technologists preparing sections during Mohs surgery. A common practice in preparing Mohs specimens is to freeze the tissue into position after precisely placing the tissue on a glass slide or other flat surface. This preparation is then inverted onto a prepared flattened media covered chuck. By using fine lens paper for the positioning surface, tissue can be placed into position, transferred to the embedding well, and directly prepared face down. The advantage of this method is a fast, one-step freezing process and a flat block face that is approximately parallel to the chuck. Each block will be very similar in the thickness and plane of embedding resulting in a faster trimming process and less adjustment of cryostat X-Y axis. At present these methods are being used in several Mohs practices and have had encouraging success as a reliable and easy to learn methodology.

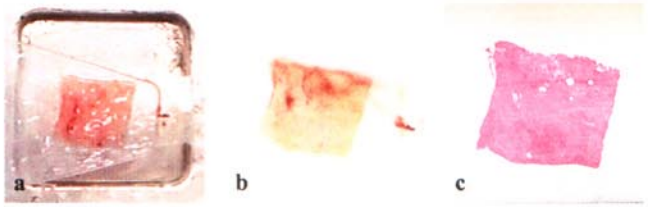

Figure 3. Very thin flat tissue embedded on face (fetal membrane). (a) Square of fetal membrane (1.0 × 1.0cm; 0.5 mm thickness) spread in position on a piece of lens paper. The paper tab overhangs the dispensing slide. (b) Trimmed block face showing entire surface visible. (c) Photograph of prepared slide showing complete section.

The concept of cutting through the lens paper in the trimming of the block may seem a bit intimidating. One's first impression might be that the paper would interfere with the ability to properly section the sample. If placed properly flat with attention to pressing out the medium from beneath the paper, the paper will be in a different plane than the tissue. The paper will have already been removed when it is time to take the section. In our experience, this fine grade of paper has little effect on the dulling of the blade. As mentioned earlier, if dulling of the blade is a concern, the trimming process can be performed on one half of the blade, which can be moved presenting the other half for the final sectioning.

The Lead Author's Comment on Conservation of Cryostat Blades

I would like to make a last comment on conservation of cryostat blades. I have had considerable exposure to a variety of frozen section laboratories. There are many operators that consider a blade should be used to cut many blocks over the course of a day. In our experience a blade looses its optimum performance after cutting considerably fewer blocks than many might realize. A tough or hard consistency of the tissue can considerably speed the process. Our duty to our patient is to provide an optimal preparation for accurate interpretation. By comparison, the cost of a disposable blade is a very small fraction of the cost of the other disposables used in a typical operation. Our guess is that most patients would probably want to pay the extra dollar or so to have their slide cut with a sharp blade.

A second consideration is our increased awareness of transmissible diseases. We are being asked to use a variety of blades, needles, and products designed to protect us against transmissible diseases. If we are accidentally cut on a blade that has been used for a multitude of patients, we are exposed to potential infection from all of these patients. Reviewing the records and history of this group could be a tedious and frightening task. If we are cut with a new blade, there is a considerably smaller risk of infection, which can also be emotionally reassuring.

In our practice I use a new portion of the blade for each new patient. I will change the blade as soon as there is any loss of quality in the sections. As pathologists both cutting and reading the slide, it is quite obvious when it should have been changed sooner.

Conclusion

This article offers a method of face-down embedding in freezing temperature wells by first positioning the tissue on

lens paper. The technique accomplishes precise embedding of extremely delicate and deformable tissues and arrangements of tissues in a flat plane. Complete sections of very thin tissues can be obtained with minimal wastage. We believe this technique will facilitate a variety of the most difficult embedding tasks in areas of surgical pathology, Mohs surgery, and research settings.

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