Chapter 4
Cutting the Frozen Section

Stephen R. Peters

Abstract The chapter covers a detailed method of cutting frozen sections on a cryostat using brush technique in continuous motion. The text covers all steps of the cutting process including trimming, cutting and retrieving. The goal is to develop great skill and sensitivity with the brush so that we can cut repeated sections in continuous motion without hesitation. This original approach presents the cutting process in a simple step wise fashion and strives to maximize our skill and dexterity through optimal hand and body positioning. The trimming process is discussed in detail. Potential sources of error such as malorientation, under trimming, and consequences of rotating the block are addressed. The chapter includes the techniques for making and cleaning frozen section brushes. Retrieving tissues form the stage and block are described. A method of teaching and passing continuous motion technique to students is described.

Keywords Antiroll devise • Artifacts • Brush-brush • Cryostat • Blade • Frozen section • Brush technique • Body position • Hand position • Gouge • Handle • Mature face • Orientation • Premature face • Rotating the frozen section block • Frozen section brush • Frozen section of minute specimens • Trimming the frozen section block • *X–Y* axis

Now we have embedded our tissue and it is time to cut a frozen section from the prepared block. In this chapter, I will share a method for cutting the frozen sections on a cryostat using brush technique in continuous motion. The chapter covers all steps of the cutting process including trimming, cutting and retrieving. Our goal is to develop great skill and sensitivity with the brush so that we can cut repeated sections in continuous motion without hesitation. This original approach presents the cutting process in a simple step wise fashion and strives to maximize our skill and dexterity through an ergonomic approach to hand and body positioning.

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4.1 Taking Sections on the Cryostat: Brush or Antiroll Device?

With each turn of the wheel the clockwork like mechanism of the microtome advances the block forward an increment equal to the thickness setting on the cryostat. At the same time with each rotation of the cryostat wheel, our block will be shaved over the blade creating our frozen section. Our task is to guide the newly cut section onto the cryostat stage while coaxing it into a flat sheet that can be retrieved from the stage. Working contrary to our best efforts is the natural tendency for the tissue to curl as it is being cut. Our task can be accomplished in either of two ways. Using the antiroll device designed into the particular brand of cryostat or by using brush technique.

Modern cryostats offer a variety of antiroll devises for the sectioning process. Many consist of a small glass or plastic plate hovering over the blade edge. This plate is precisely adjusted so the forming section flows under the plate which guides it into a flat shape onto the stage (Fig. 4.1).

More recently, vacuum anti roll devices have been designed to apply a vacuum in front of the forming section, pulling the section into position on the stage. Figure 4.5 shows such a vacuum antiroll devise positioned behind the stage of this late model Leica 1950 cryostat.

These antiroll devises work well under most circumstances provided the device is properly adjusted and in a working state. Unfortunately in practices where cryostats are being used by a number of operators, things have a way of being left in various states of adjustment. The user must understand how to quickly make these adjustments when they do not work properly.

It is my own feeling that all students must first be trained in brush technique. A well developed skill with the frozen section brush will serve the cryotomist through any situation without relying on the mechanical devices. Starting a student with an antiroll device would be like putting a child on crutches before learning to walk.

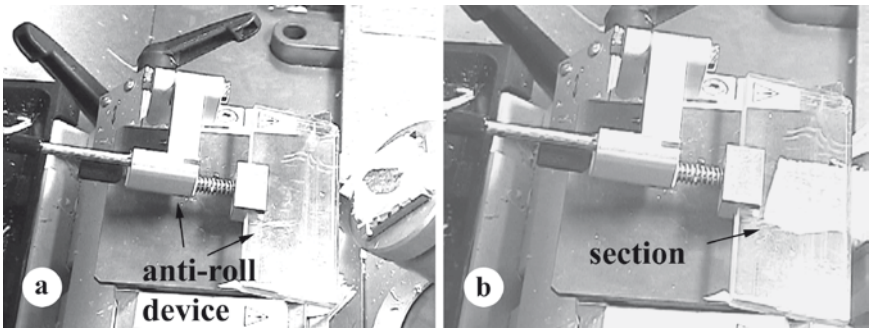

Fig. 4.1 Antiroll device. (**a**) The antiroll device (*arrows*) is in position precisely overlapping the blade edge. (**b**) As the section is cut (*arrow*) it passes under the antiroll device helping the section to remain flat as it is deposited on the stage

This chapter will concentrate on a method of frozen section brush technique that focuses on developing a high level of skill in the brush hand much like playing a musical instrument. The brush is no different than the bow of a violin or the fingernails of the guitarist. Using precise technique, practiced repetitively over time, one can develop a surprising level of skill and finesse. By optimizing our dexterity through proper ergonomics, hand positioning and conservation of motion it is possible to become a *virtuoso* with the frozen section brush.

Our goal is to be able to cut the tissue in a *continuous uniform motion* while appraising the forming sections over multiple revolutions until we visually recognize a quality section to retrieve. Similar to cutting paraffin blocks, it seems to take several passes of the knife until an equilibrium of temperature and consistency is established which allows the sections to become uniform in thickness and quality. With skilled brush technique, we have the ability to finesse our best sections onto the stage in the most difficult settings.

Given the wide variety of backgrounds and training of cryotomists, it is not surprising to see a considerable variation in techniques used to prepare frozen sections. I have seen a variety of different styles of holding and cutting using a brush. Many cryotomists are stopped or hesitate at the beginning of the section, slowly grabbing the tissue and then accelerating through the section. Others have developed technique to the point in which they are able to cut with little or no hesitation.

As I became more comfortable with the brush, it became obvious that the quality of my sections was related to the uniformity and the fluidity with which the tissue was being cut. It also became obvious that not every section that managed to make it to the stage was optimal. As I said, it seems to take several turns of the wheel until the quality sections appear. When you are cutting in continuous motion, you can cut and evaluate the section, discarding the poor ones and take the first good one to come along. The advantages of cutting without hesitation are most obvious when dealing with our most difficult tissues. In this section, I will offer simple method of learning and teaching continuous motion frozen section brush technique. We will start by learning the elements of basic brush technique. In Chap. 5 we will learn to recognize the deviations in quality in our sections as they are being cut and to make the necessary adjustments to correct the problem. In order to do this, we will learn the many variables which play a role in the behavior of the tissue.

4.2 Continuous Motion Frozen Section Brush Technique

4.2.1 Insert the Chuck and Check the Cryostat

First, the block must be *tightly* secured in the chuck holder. Check that all of the clamping knobs, levers, or screws securing of the knife, knife holder, chuck, chuck holder and microtome are tight and free of any debris that could cause movement.

It takes very little movement to affect the thickness of a section cutting as thin as 5 microns. See *chatter* Chap. 5.

4.2.2 The Frozen Section Brush

The purpose of the brush is to catch the edge of the section as it is being cut and to maneuver the newly cut section onto the cryostat stage. Unless the temperature is ideal, a cold section will tend to curl up and pull away from the brush. For this reason, I find a brush with stiff bristles and a fairly wide gripping surface to be more functional than the more flimsy camel hair brushes used traditionally. I suggest 3/16 (#1) and 1/4 inch (#2) flat or bright bristle brushes. These can be found at art supply stores for about $3–$4. Cut the brush at an angle so that when held at an angle, the brush meets the tissue flat like an angled broom meets the floor. Cut off any excess brush handle on long artist brushes (Fig. 4.2).

4.2.2.1 Keep the Brush Clean

Sections sticking to the brush can become a nuisance when cutting in continuous motion, causing us to pause and pick up a gauze pad to wipe the brush. Fatty tissues are notorious for sticking to the brush. It pays to have several brushes available.

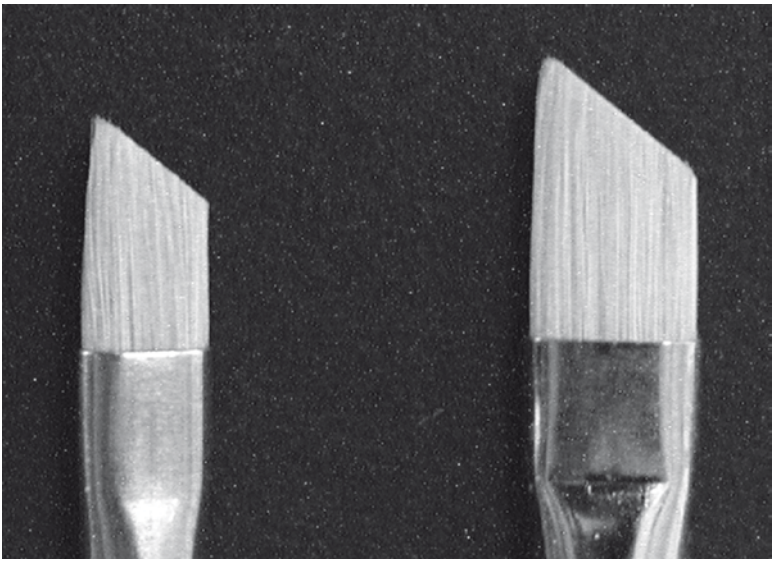

Fig. 4.2 *Left* – 3/16 inch brush; *Right* – ¼ inch brush; Brushes made of white bristle provide a stiff but flexible action. Brushes are cut at approximately 45 degree angle so when the brush is held at 45 degrees, it will meet the tissue flat like the edge of a broom meets the floor

Cleaning the brushes will reduce sticking and is essential if one is attempting to place multiple sections on the slide (cutting ribbons). It is worth taking a minute at the start of each day to clean the brushes. Using the procedure below, you can clean several brushes in less than a minute.

4.2.2.2 Quick Brush Cleaning Procedure

- Soap and water then quick dry with gauze
- Dip in ETOH and quick dry with gauze
- Dip in xylene and a quick dry with gauze
- Cool the brush by pressing it to a cold surface in the cryostat for a few seconds.

4.2.2.3 Make a Brush-Brush

This useful tool can be made by wedging or taping a piece of gauze or small brush in the cryostat as close as possible to the left of the brush hand. I call this the "brush-brush". It allows the operator to quickly wipe the brush clean against the brush-brush while cutting without hesitation. I have fashioned a rudimentary brush from a few folded biopsy bags by stapling, trimming and cutting them into a brush and taping it to the antiroll device in our cryostats. Without losing step, I can slightly veer my brush to the left to wipe the brush in the brush-brush and be back in the cycle for the next pass of the block. The brush- brush allows me to cut and wipe at any speed in continuous motion. One can experiment with a variety of comb and brush like objects attached to whatever spot is available to the left of the stage in your particular cryostat (Fig. 4.3).

4.2.3 The Blade

In the surgical pathology setting, I believe every patient deserves a new disposable blade. You will always get the best quality section with a new sharp blade. The cost of a blade is nominal when compared with the disposables being used in the operating room. Some tissues such as tough collagenous tissues or calcified tissues can quickly dull the blade. The blade should be changed when the section quality begins to fall. I have occasionally found myself getting mediocre sections with a brand new blade and changed it to find the tissue cutting easily with the second blade. Changing the blade on every case is also an important safety measure. If you cut yourself on a new blade, you will have minimized your risk of transmittable disease. Investigating an exposure takes the review of only a single chart. If a blade has been in a cryostat for multiple cases, investigating any exposure becomes a futile task with little reassurance for the injured operator.

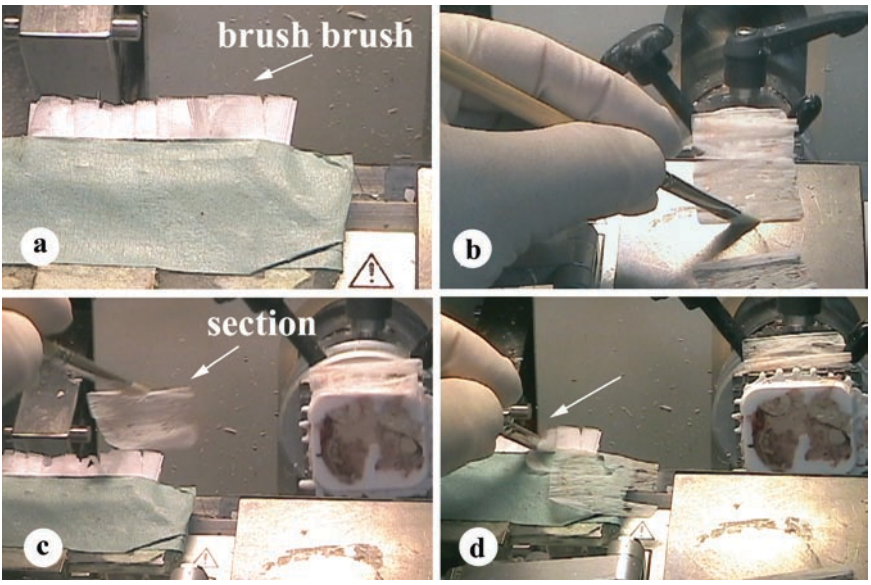

Fig. 4.3 (**a**) A small brush (*arrow*) fashioned of stapled, cut and trimmed biopsy bags is taped to the antiroll device in this cryostat. (**b**) A newly cut frozen section to be discarded is sticking to the frozen section brush. (**c**)–(**d**) The section (*arrows*) to be discarded is moved to and wiped against the *brush-brush* while maintaining continuous motion

4.2.3.1 Listen to the Blade

Get to know the sound of a good section coming off the blade. There is almost no sound at all. When a block is too cold, there is a distinct sound as the blade scratches the icy block. The blade will make a variety of grating or vibrating sounds when it is at the wrong blade angle, showing movement or out of position because of a bit of medium in the blade holder.

The importance of blade angle is discussed in detail in Chap. 9.

4.2.4 Body Position

I recommend sitting as comfortable as possible when cutting the frozen sections. Most cryostats are at a height that requires many users to bend at the waist when cutting while standing. I hope you learn to use the brush as an articulate fine instrument. Why would we want to do this hunched over with our neck hyper extended? When cutting a frozen section, we need to be relaxed and comfortable in order to have maximum control of the left hand. I suggest sitting on an adjustable stool, at a height that allows your arms to most comfortably drape your hands to the stage, best allowing your left hand to assume the hand position discussed below (Figs. 4.4 and 4.5).

Fig. 4.4 Body position. The cryotomist sits in a comfortable ergonomically correct position with arms draped to the cryostat stage. This late model Leica1950 cryostat designed for user ergonomics allows the user to sit comfortably with an adjustable foot rest. The sloping front wall seen in Fig. 4.7 allows the arms to be positioned with minimal interference

4.2.5 Holding the Brush

Hold the brush like a pen in the left hand and stabilize the hand by gently resting the side of the fifth finger on the stage or where ever is most suitable in your brand of cryostat. The operator will use the fine motor skills of the fingers much like writing with a pen. Focus on developing your dexterity so you can control the brush like a fine instrument. Try writing your name across the bottom of the block. These are the muscles you will use to take a section (Fig. 4.6).

The brush is cut at an angle which approximates the angle in which the brush is held in the hand. This results in the brush meeting the tissue flat over its 1/4 '' length like an angle cut broom. The brush is held at approximately a 45 degree angle to the block face and a 45 degree angle to the stage. The ultimate position will be dictated by what is comfortable for the operator in a particular cryostat.

Fig. 4.5 Illustrates proper body position. Sitting with arms draped as comfortable as possible to achieve correct hand position. The arrow shows that there is still a degree of hyperextension at my wrist in this model cryostat. There are limitations to what positions are achievable in any given cryostat design. The deeper the cryostat stage below the front of the cryostat the more difficult it will be to achieve a comfortable position. Adjusting the stage to a higher position may relieve some hand stress

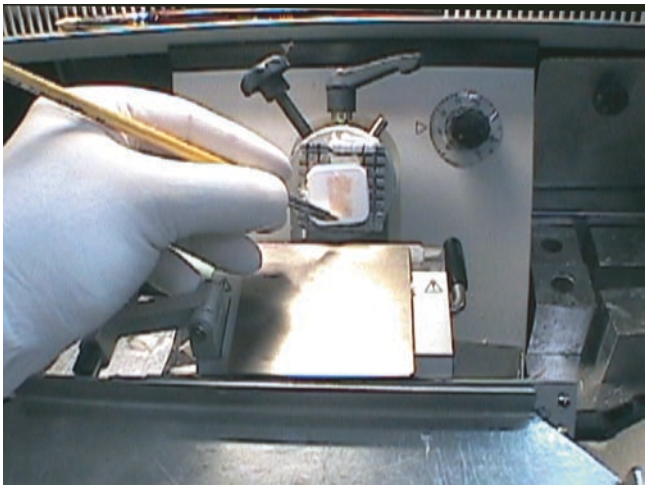

Fig. 4.6 Illustrates holding the brush. The left hand gently rests the fifth finger where convenient. The brush is being held like a pen between the thumb and first two fingers. The brush is held at approximately a 45 degree angle to the stage and a 45 degree angle to the block

Fig. 4.7 Leica 1950 cryostat; one of several versatile inside views. The black arrow shows a hand rest for brush users. The white arrow points to the embedding shelf. The tube is connected to the vacuum antiroll devise on the stage which also doubles as a vacuum cleaning device for the cryostat

Figure 4.7 shows the inside of a Leica 1950 cryostat. This late model instrument was designed to maximize the ergonomics of the cutting process. The stage is high up and slightly to the right and the front slopes toward the box so that the users arm will drape freely to the stage alleviating the uncomfortable bend at the wrist seen in Fig. 4.5. There is a padded finger rest to the left of the stage offers a suitable resting point which will not transfer much less heat than metal so the resting fingers do not get as cold. As a testimony to the value of embedding in well bars, a movable embedding shelf was designed into the front wall.

4.2.6 Trimming the Block

We have embedded our tissue with a goal to view the microanatomy in a particular plane and orientation. Our next task is to trim the block face until we reach the desired depth and landmarks. When trimming or surfacing the block, our goal is to first quickly shave away the superficial face of the block with whatever coarse advance mechanism is offered by the cryostat. Most of the newer cryostats have electronic controls for coarse and fine advance. Older cryostats may have a wheel attached to the microtome to coarsely advance the block.

We start by turning the cryostat wheel while operating the coarse advance button or mechanism and brushing away the shavings until we begin to see the landmarks that tell us we are close to our desired plane. Landmarks may be appearance of a

particular part of the microanatomy such as epidermis, mucosal surface or inked margin or simply evidence that a small speck of tissue is nearing the block surface. From the point where we are seeing most of the required tissue face the final trimming is done using a finer advance control and/or simply by turning the cryostat wheel. With each turn of the cryostat wheel our block will advance only the thickness of the section; that is, if we are cutting at a section thickness of 5 microns, the block will advance 5 microns with each turn of the wheel. In very thin specimens once we start to see the tissue it is best to advance tissue only by turning the cryostat wheel. This task must be approached with whatever caution is deemed necessary by the size of the sample. The cryotomist must understand how the advance mechanisms operate on their individual cryostat and have a clear sense of how much the tissue advances (and is wasted) with each press of the button or turn of the wheel. If we do not trim the tissue deep enough, our section will not include some critical feature. This will require a second section and lost time. If we trim the tissue too much, there will be unnecessary wastage of tissue and potential loss of irreplaceable specimen.

4.2.6.1 Reading the Block

The cryotomist must learn to grossly recognize the anatomy and landmarks visible on the block face and have a good idea of how the trimmed tissue face relates to the anatomy that the pathologist will be focusing on. I refer to this as *reading the block.* The trimmed block offers a tiny, highly detailed view of the gross, with landmarks such as the inked margins, mucosal surfaces, and epidermis recognizable as a very low power view of the histology. One must be acutely aware when these structures come to the surface of the block. When trimming, as one approaches the landmark, there is a period where one can be fooled into thinking you are at the correct depth. At this premature stage, it is like looking at the structure through a slightly frosted glass. One will think a structure is present but it will not appear in the section. We must be able to distinguish this *premature face* from the *mature face*, where all of the necessary landmarks have been reached. The mature face will be distinctly visible and lines and colors will be sharp and at their brightest. Comparing the block face with the section coming off the blade can sometimes offer a clue that the block is under trimmed. Get in to the habit of looking at the section as you pick it up on a slide. You will develop an ability to recognize if the complete section is present (Fig. 4.8).

4.2.6.2 *X–Y* Axis and Orientation

In order to be able to trim the block to a level where all of the required elements of the section are present without unnecessary wastage of tissue, two conditions must be met. The tissue must be embedded in a flat plane and the block must be correctly oriented in the *X–Y* axis. Using traditional face up embedding technique, structures

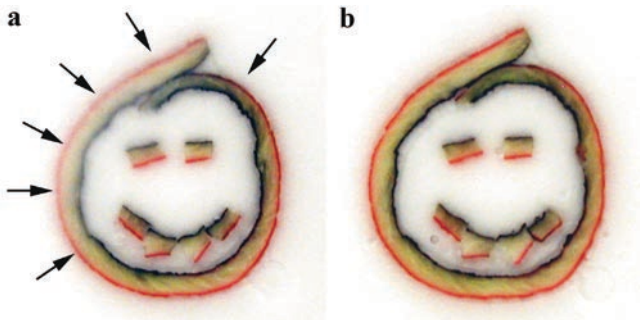

Fig. 4.8 Reading the block. The pictures illustrate a trimmed frozen section block of skin embedded as a *block face*. Figure (**a**) shows a *premature face.* The arrows show an area from 7:00 to 2:00 which has not yet been reached by the knife. The haziness of the overlying embedding medium is clearly visible from 9:00 to 12:00, but much less apparent at the periphery of these zones. Figure (**b**) shows the *mature face*. The block is fully trimmed. All of the tissue is clearly visible and margins are sharp opaque lines

may be in a variety of planes making it difficult to achieve a section containing all of the desired landmarks and requiring considerable trimming and tissue wastage. Using the face down embedding techniques described in Chap. 4, it is simple to prepare blocks in a flat plane allowing us to achieve the complete desired tissue face with very little trimming. But to trim the desired face in a single plane, the plane of the block face must be in the same plane as the blade. Another term for this orientation is the *x–y axis* of the block referring to the *x–y* plane defined by the block face and its relationship to the plane defined by the plane of the blade. See Chap. 1. Figure 4.9 is an example of a block which has been partially trimmed in poor *x–y* orientation. In this example, the lower right corner of the block was considerably forward and the upper left is backward of the plane of the blade. To correct this, the upper right corner must be tilted forward along the diagonal line defined by the arrows.

Adjusting the orientation of the block needs to be done in very fine increments. Unfortunately, the means of adjusting orientation available in many cryostats is limited if present at all. If the orientation of the block is not in the plane of the blade, the block will begin to shave away an edge or corner rather than being well centered. The operator must understand whatever means of orientation your particular cryostat offers. High end models may have separate adjustment knobs for each plane allowing a fine controlled adjustment. Some cryostats offer a single knob for simply loosening the *x–y* adjustment so that the block can be freely adjusted to the required plane by hand. This requires a delicate touch and a bit of trial and error. I approach this by loosening the knob so that the chuck holder moves with a slight resistance. Adjustments must be made in infinitesimal movements, as grossly visible movements will have drastic results. *When making an x–y adjustment always take the block backward before starting to trim and approach the tissue very slowly to see where the block will just begin to shave the block.*

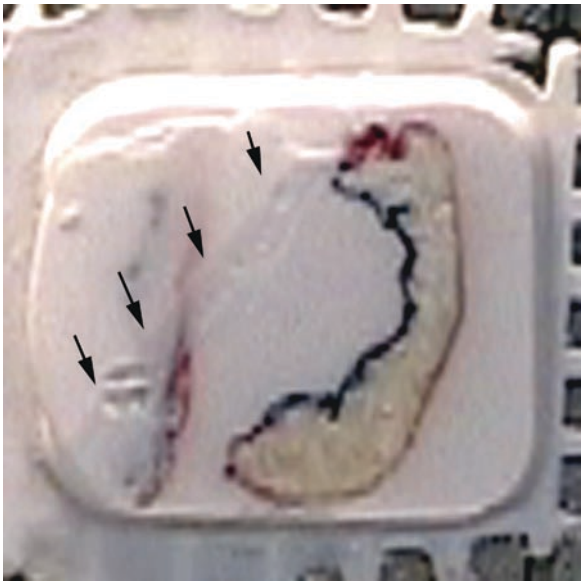

Fig. 4.9 Poor *x–y* axis orientation. The block was trimmed on a cryostat in which the *x–y* axis of the block is far from the plane of the blade. This block has been shaved deeply into the lower right corner without yet reaching the upper left side. To correct this, the upper left corner must be **slightly** tilted forward to meet the blade along the axis of the diagonal defined by the arrows

A well oriented block will first meet the blade at the center of the block. If not in the center, the process must be repeated. When making *x–y* adjustments, the part of the block which is being shaved first must be moved backward while the portion not yet reached the needs to come forward. It is best to practice adjusting orientation on insignificant tissues or blank embedding medium blocks before attempting this on patient specimens.

4.2.6.3 Rotating the Block

Many cryostats provide a means of rotating the block 360 degrees. This allows the operator to orient the tissue in any position relative to the blade. Before beginning to trim the tissue, careful consideration should be given to how the various elements of the tissue will contact the blade. See Chap. 3. After considering the questions you are trying to answer and the properties of the tissues you are cutting, begin by orienting the block relative to the knife to best achieve your goals. In practice, despite our best intentions, when we begin trimming, unexpected fatty or calcified elements may appear or we may find our tissue *curling away*.(See below) In these situations, we can benefit by rotating the block to a position that will result in the mischievous elements hitting the blade last. When rotating the block in most cases you will be changing the *x–y* orientation of the block relative to the blade to some

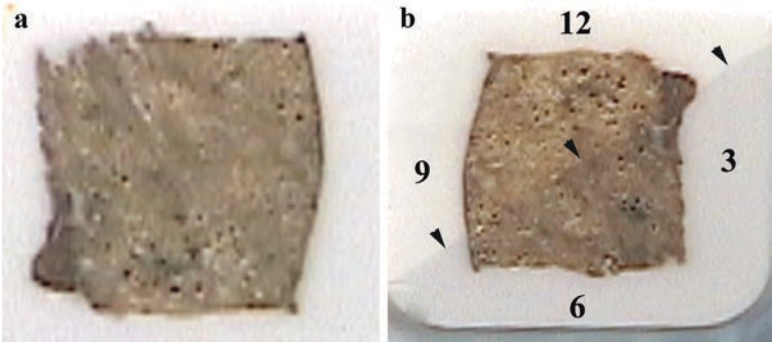

Fig. 4.10 Rotating the block. A block containing lung tissue trimmed before and after 90 degree rotation in a cryostat that is in poor *x–y* orientation. (**a**) A trimmed frozen section block of lung tissue. (**b**) The block trimmed again after 90 degree rotation. The lower right side of the block, now out of orientation has been shaved away before reaching the upper left. The block must be readjusted by **slightly** tilting the upper left corner forward along the axis defined by the three arrows

degree. Only if the block has been prepared so that the block face is parallel to the chuck face and the chuck face is in perfect *x–y* orientation with the blade, will the block rotate without affecting the *x–y* orientation. Figure 4.9 shows a block which has been trimmed, rotated 180 degrees and retrimmed. It is obvious how dramatically the orientation changed with this rotation. **Always take the block backward before starting to trim again.** Approach the block slowly and see where the tissue begins to shave the block. If the section begins to cut away from center specimen, *x–y* orientation will need to be adjusted before beginning to trim again or pay the price of wasted tissue. This is particularly critical when cutting very thin specimens. Figure 4.10b shows that the lower right corner has been shaved away similar to Fig. 4.9. To re orient this block, the upper left corner of the block at 9 and 12:00 will have to tilt forward along the axis denoted by the arrow heads (Fig. 4.10).

4.2.6.4 Removing and Returning the Block to the Chuck Holder

When removing the block for any reason, it is a good idea to make a small mark at 12:00. When the block is returned to the chuck holder for additional sectioning, it will be easy to achieve the same position and avoid orientation change and unnecessary tissue wastage. Always, first move the block backward and approach it gently to assess any orientation change before aggressive trimming.

4.2.6.5 Trimming Minute Specimens

More frequently than we would like, we are called upon to examine the minute biopsies or the thin core biopsies with diameters of a less than mm. In such cases it is imperative to embed the tissues in our flattest plane and to begin with a well oriented block.

Whenever possible concentrate the specimen centrally in the block to minimize the effects of malorientation. If tissue is visible on the surface and not covered in embedding medium, begin with a layer of plastering. Next trim the block gently until the tissue becomes visible. At the point that the tissue appears very close to the surface of the block, look carefully at each cut section until the early signs that the tissue is becoming uncovered. From this point pick up a section on a slide and look to see if the tissue is present. If tissue is present, this is your first level to stain. With single turns of the wheel, observe the increasing amount of tissue available at the surface and take the sections until the complete tissue face has been reached. By slowly approaching the tissue and carefully examining the section both on the cryostat stage and on a slide, we can recognize the moment the tissue is reached, take only what we need and preserve the tissue for paraffin sections (Fig. 4.11).

4.2.7 Cutting the Final Sections

Now that our block is trimmed to the correct level we will take our final sections for interpretation.

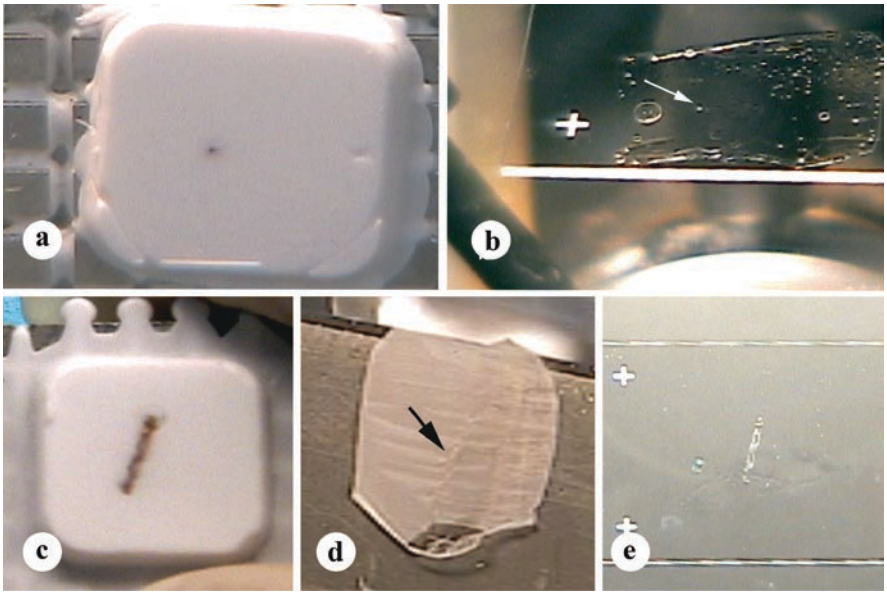

Fig. 4.11 Cutting minute specimens. (**a**) A frozen section block with a minute sample just as it is reached at the surface. (**b**) A glass slide on which a section has been picked up. The arrow points to a minute speck of tissue on the slide. (**c**) Frozen section block trimmed with a small core biopsy specimen at the surface. (**d**) A cut section of the block in picture (**c**) on the cryostat stage. The arrow points to the tissue visible in the section. (**e**) A glass slide upon which the section from picture (**d**) has been retrieved. A complete section of the tissue is visible

4.2.7.1 Turning the Wheel

Our goal is to simply turn the wheel or crank of the cryostat in a continuous uniform motion without hesitation. In fact, we will be trying to imitate the function of an automated cryostat. With practice, by holding the brush as I described, the operator is capable of catching and guiding the section while the block continues in motion.

4.2.7.2 Movement of the Brush

The technique will be described in four simple motions. Figure 4.12a shows that the starting position of the brush is in the center at the bottom two millimeters of the block. This is *home plate***.**

Ride the Block

As the block begins its descent toward the knife, the brush moves downward keeping pace with the block. The brush can gently rest on home plate and ride the block to the blade. It is the downward movement of the brush that allows you to maintain continuous motion as you grab hold of the section and continue on (Fig. 4.12a).

Gently Lift Up While Crossing the Blade

As the brush meets the blade, the section will begin to form under the brush. The brush gently lifts up as it reaches the blade while holding onto the edge of the newly forming section (Fig. 4.12b).

Hold Onto the Curl

As the first few millimeters of the section passes the knife, there will be some degree of curling of the section. As the curl begins, the moving brush is in position on top of this curl of the tissue and is holding onto it. With the curl in hand, the brush changes to a horizontal motion toward you. The path of the brush is much like a child coming down a slide in an “elbow” shape down and then toward you in a continuous motion (Fig. 4.12b)

Glide the Section Across the Stage

The motion continues horizontally as the forming section is drawn across the stage. Figure 4.12c illustrates that by being extremely delicate with the brush; one can

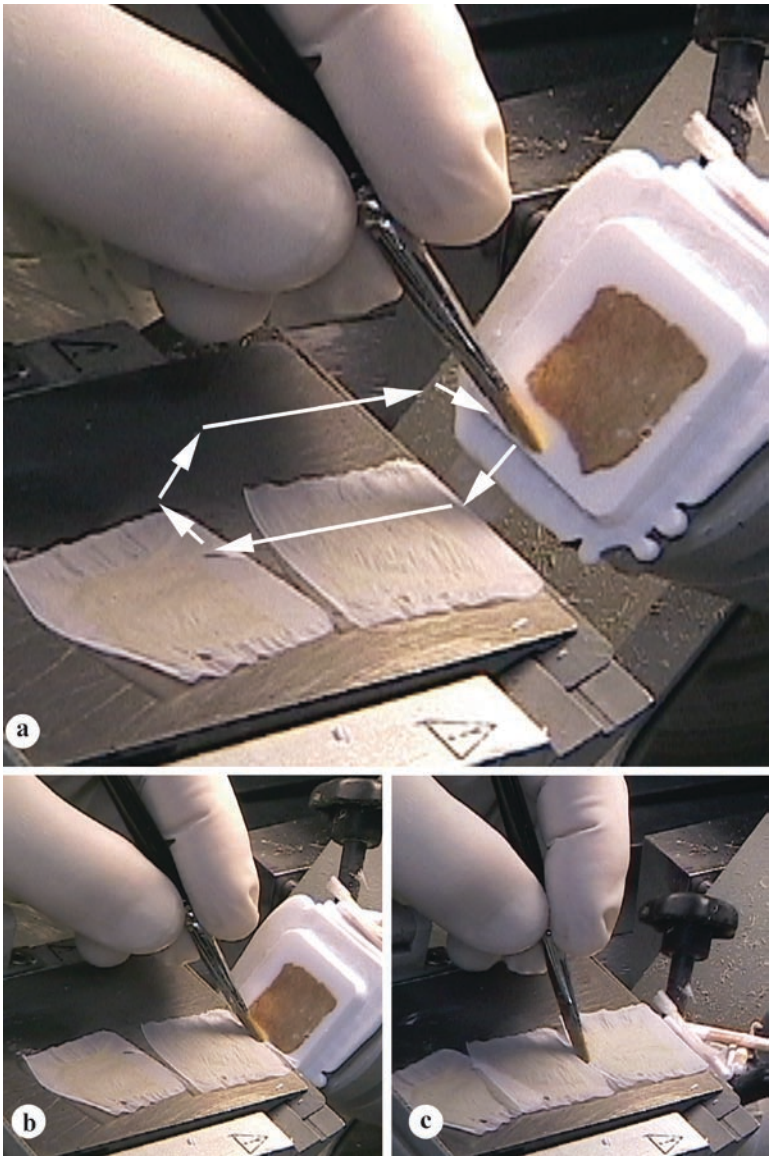

Fig. 4.12 Continuous motion brush technique. Figure (**a**) shows the frozen section brush at the starting position, *home plate*, gently resting on edge of the block in its up position. The white arrows show the elliptical path that the brush will travel in continuous motion. From this point the brush will ride the block to the blade. (**b**) The block and brush have now descended to the blade. Upon reaching the blade, the brush gently lifts up as it crosses the blade while holding onto the edge of the curl of the forming section. The brush now changes to a horizontal path to carry the section across the stage. (**c**) The brush gently glides the section across the stage without pressing the tissue to the stage. The brush follows the path of the arrows in continuous motion to return to the block in the up position

hold onto the edge of the curl and glide the section across the stage. *Avoid pressing tissue to the cryostat stage* which can result in adhesion of the tissue to the stage, especially in fatty tissues. This will result in a smeared section and a need to clean the stage.

If the section we have cut meets our standards, we will stop to retrieve the tissue on a slide. If not, the brush returns to take the next section completing a cycle. As the brush returns to take the next section, it completes a continuous elliptical motion. By repeating this process in a uniform fashion, the movement of the brush continually traces the path of this ellipse as shown by the arrow in Fig. 4.12a. The continuous repeated sectioning of a block is like turning the pedals of a bicycle. Both hands are circling in synchrony. As I said earlier, when taking our final section, it usually takes at least three cycles in continuous motion until the cutting process reaches equilibrium and the best quality sections begin to appear.

4.2.7.3 Using the Handle

As I said at the end of the last chapter, there are many advantages to preparing blocks with a *handle* of embedding medium completely surrounding the tissue. The block in Fig. 4.12 has an ample handle of white embedding medium surrounding the brown tissue. The handle provides margin of error when both cutting and retrieving the tissue. When cutting the block, we can grasp this handle with the brush without having to engage the tissue. In sections having a tendency to form holes such as fatty and necrotic tissues, the handle will hold the section together and resist crumpling and tearing. It acts like a frame giving strength to the section. Blocks made with a handle of embedding medium will have fewer tendencies to smear if this handle is inadvertently pressed to the stage.

4.2.8 Retrieving the Section

Retrieving the section refers to the process of picking up the cut frozen section onto a glass microscope slide. Tissue can be retrieved from the cryostat stage or from the block face. When retrieving tissue from the stage, the direction from which you approach the stage will be dictated by the design of the cryostat. The most ergonomic approach given the anatomy of our wrist is to retrieve the tissue from the side of the stage. Unfortunately, some cryostats are designed with various levers, knobs and antiroll devices obstructing the lateral approaches to the stage. In this case, the operator is forced to approach the stage from the front which is a bit more awkward due the need to hyperextend and rotate the wrist. *Tissue can also be picked up from the back of the stage but the operator must be acutely aware of the blade at all times.* While our slide will be crossing over the blade to retrieve the tissue, our hands will be getting dangerously close to the blade.

This process in which tissue is picked up onto a slide happens in a moment's time as tissue is drawn to the slide by static attraction. During that time the tissue can develop folds or be stretched or torn by erratic motion. The operator must appreciate the delicacy of the tissue section and the kinetics of this brief process to avoid artifacts. It is an advantage to have a "handle" of embedding medium surrounding the tissue allowing a margin of error for curling or flipping at both ends of the section before it involves the tissue.

High levels of static electricity can become a problem in some laboratories particularly in dry climates and during cold weather heating season when air becomes dry. This can manifest in the form of freshly cut frozen sections jumping as much as an inch land on our slide in a most haphazard way. In this setting, retrieving the section from the stage can become an Olympic event and is easier accomplished by retrieving from the block. Suggestions for dealing with static are described in Chap. 1.

4.2.8.1 Retrieving from the Stage

When the section is complete, the tissue can be up picked from the stage up by holding the slide just above the section and angle the slide down to touch a portion of the tissue as in figure a. Static attraction will draw the section to adhere to and quickly melt on to the warm slide. One can use a fingertip as in figure b to stabilize the front edge of the slide while levering the slide down to meet the tissue in a very well controlled way (Fig. 4.13).

If the section is in a position on the stage which is awkward to retrieve, we can will simply drop the slide onto the tissue from a height of about a quarter inch above the tissue. Tissue sticking to the brush can also create problems and will be minimized by keeping brushes clean so there is less sticking and by having several brushes available. If the sections are at ideal temperature and lying flat, they can be

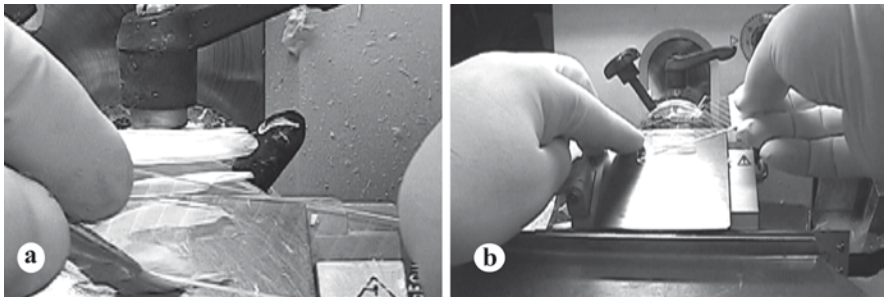

Fig. 4.13 Retrieving from the stage. Figure (**a**) shows the slide being levered down on the edge of the stage to meet the tissue. Figure (**b**) shows the finger tip being used as a hinge to lever down the slide onto the tissue. Both techniques allow fine control of the slide as it comes in contact with the tissue

moved with the brush into whatever position is easiest to pick up on the slide. We can also use two brushes to stretch, uncurl, or orient the sections and to move them into position to retrieve. Our tissue will be the easiest to retrieve and position when it is cut at the ideal temperature so the sections lie flat on the stage. See Chap. 5.

4.2.8.2 Retrieving from the Block

Occasionally when faced with a difficulty retrieving from the stage, I may have more success retrieving the section from the block. First cut through the tissue stopping when the handle of medium on the far side of the tissue has been reached and leaving the last 1–2 mm or of the section attached to the block. By rotating the cryostat wheel backward, the block is drawn back up away from the blade with the tissue still attached to the block. The fixed edge of the section can now be gently stretched downward with the brush while the slide is placed over the block picking up the section. I have found this useful in problems arising from curling, tissue sticking to the brush, fat sticking to the stage, and in high static conditions. Some operators prefer this technique for the majority of their sections or are forced to use this technique when cryostat design leaves little access to the stage (Fig. 4.14).

4.2.9 Teaching Continuous Motion

Teaching a newcomer continuous motion technique is surprisingly easy once you have comfortably mastered the technique yourself. I recommend the following exercise which takes about 15–20 min in most cases. It is easiest to start with a

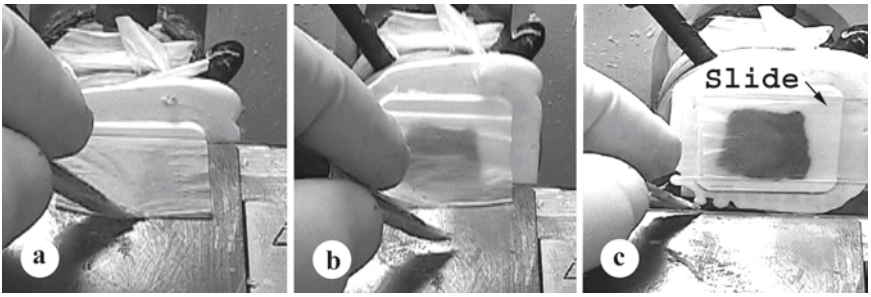

Fig. 4.14 Retrieving from the block. Figure (**a**) shows the section which has been cut and stopped in the last two mm. before completely cutting across the entire block. Figure (**b**) shows the block with attached section being brought backward and up so the section now rests on the block face. Figure (**c**) shows the slide (*arrow*) retrieving the section off the face of the block while the section is gently held stretched in position by the brush.

block made only of embedding medium, frozen to optimal temperature. Have the student sit in proper position holding the brush as described above making certain the brush will be meeting the tissue flat like a broom.

Steps One – Ask them to write their name across the bottom of the block using the brush in the left hand so they can appreciate the fine motor movements they will be using to maneuver the brush, while performing a task familiar to them.

Step Two – With their hands in position and the teacher standing on the right beside them, asks the student to go totally limp so there is no resistance; the teacher, while grasping their hands, begins the process of cutting using their hands. Carry their hands through multiple cycles of the cutting process for about a minute. I believe this step lets the student experience what it is like to cut without any hesitation as they are developing muscle memory for this movement.

Step Three – Give the student the brush in their left hand alone. The teacher now turns the wheel in a fairly slow continuous motion. The student stumbles a bit at first but soon finds they are keeping pace with the microtome. Correct any deviations in technique and position of hands and brush. After a minute or two, most are ready to go on. A few may need another minute at step two.

Step Four – Next start the student cutting again holding and guiding both hands in continuous motion and then let go to let the student use both hands alone. If they begin to hesitate, give a gentle touch to the wheel hand to keep them going until they are cutting without hesitation.

Step Five – Sharpen a point on an applicator stick and have the student use this pointed object in place of the brush. This will enlighten them to the even finer touch of the stick and teach them to precisely catch the edge of the section and guide it gently across the stage without pressing it to the stage. If they try to press down, the stick will tear the section. At this juncture, they will need to practice on their own.

If you are learning continuous motion on your own, try having a colleague turn the wheel continuously at a slow uniform pace for you while you learn to *catch the falling snowflake.* Figure 4.15 When turning the wheel for someone else holding the brush it will help them to sing in a waltz tempo at the speed you are turning the wheel.

Fig. 4.15 Illustrates teaching continuous motion technique. Sitting comfortably, the resident is placed in correct hand position and told to let his hands go completely limp. The aging teacher moves the resident's hands while cutting in continuous motion for a minute or more. This exercise passes along a strong physical sense for the continuous movement