



Microtomy and Paraffin Section Preparation

Scientia

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Introduction

The preparation of high-quality sections for histopathology requires skill and experience – but we all have to start somewhere. This booklet has been prepared as a training aid for newcomers to microtomy and paraffin section preparation and as a refresher course for more experienced histologists. It covers the essential elements for set-up and safe operation of a rotary microtome to prepare paraffin sections. Some of the more common faults seen in sections are illustrated and troubleshooting suggestions are provided.

The microtome pictured throughout this booklet is a Leica RM2235 manual microtome, but the suggestions and comments provided can be applied to most modern rotary microtomes. A description of the mechanical and safety features of other microtome models and instructions for their use can be found in the particular instrument's instruction manual.

Scientia

Knowledge. Science. Skill.

The Scientia education series from Leica Microsystems is part of our commitment to improving the theory and practice of histology through education, training and scientific discourse.

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1. Fix Specimens Properly

Fixation is the most important step in performing histologic specimen preparation techniques. No matter how much care is taken in processing and sectioning tissue specimens, essential morphologic detail will only be demonstrated if the tissue is promptly and adequately fixed.

- Poorly fixed specimens are almost always more difficult to section than those that are well fixed.
- Poorly fixed tissue will always produce inferior morphology even if optimally processed and carefully sectioned.

2. Process Tissue Properly

A paraffin block will be difficult to section unless a well-fixed specimen is properly processed using an appropriate schedule

- Specimens may be under-processed (specimen too large, schedule too short) or over-processed (schedule too long for size and nature of specimen). In both cases they may be difficult or impossible to cut.
- There are a number of techniques that can be used to help obtain sections from difficult blocks.
- If the block is difficult to section because the tissue is hard or friable, the exposed surface can be soaked in cold water, or a softening agent such as weak detergent solution, fabric softener or Mollifex™.
- For specimens that contain calcium, the use of a surface decalcifying agent applied to the exposed tissue for 10 minutes or more, may allow several sections to be obtained. Rinse blocks well before mounting into the microtome specimen holder and recutting, as traces of the decalcifying agent will damage the pressure plate of the blade holder.
- The Leica Paraffin Tape-Transfer System™ is recommended for obtaining sections from difficult blocks where fixation, processing or infiltration may be faulty and normal sectioning methods are unsuccessful.
- Where it is impossible to obtain sections from a block because the specimen has been under-processed in the first place, it may be possible to reprocess it.



This block of pancreas had insufficient fixation and was processed using a protocol that was far too short. It shows the typical effects of under-processing that has resulted in considerable shrinkage of the specimen within the surrounding wax. The tissue was soft and mushy and was impossible to section. It required reprocessing.

3. Embed Specimens Carefully

Embedding is an important step that requires a thoughtful approach. Careless embedding can make microtomy much more difficult.

- Avoid under-filling the cassette as this can allow unstable clamping in the microtome and lead to cutting “thick then thin” sections and other problems.
- Avoid over-filling cassettes as this can interfere with the correct alignment of the block face for sectioning.
- Any excess wax on the outside of a cassette should be removed before clamping to ensure the block is firmly held during sectioning.
- Specimen orientation is very important (see 8. on page 11).



Examples of under-filled (left), over-filled (right) and correctly filled cassettes (centre). Under-filled and over-filled cassettes can cause problems during microtomy

4. Locate Microtome Appropriately

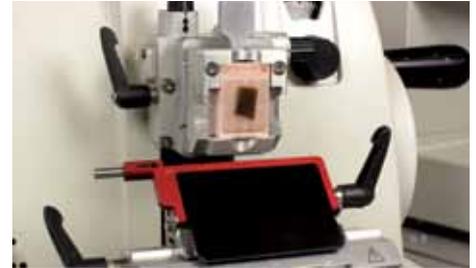
The location of the microtome in the laboratory is important.

- Position the microtome on a stable bench, away from air drafts, doorways and passing staff. Any air movement from air conditioners or other causes can make section handling very difficult.
- A height-adjustable bench and ergonomic chair are preferred.
- It is very important that staff are not distracted when using the microtome because of the risks of injury from extremely sharp blades. The potential for interaction with other staff members should be considered when positioning microtomes in a laboratory.
- It is preferable to have non-slip flooring in the vicinity of microtomes because, inevitably, wax fragments will find their way onto the floor where they can produce a slippery surface. Many laboratories use non-slip matting to make the environment safer.

5. Utilize Safety Features Properly

You must be familiar with the safety features of the microtome you are using and observe some basic rules when cutting sections.

- Microtome knives and disposable blades are extremely sharp and can inflict serious injuries unless appropriate care is taken when working with them. Accidents occur when a microtome operator is distracted and not concentrating fully.
- Use forceps or brush instead of your fingers to pick up sections or wax fragments from blade or block face.
- Leica rotary microtomes are equipped with a safety guard (knife guard or finger guard), a handwheel lock and a handwheel brake to enable safe operation.
- The safety guard can be positioned to cover the whole length of the cutting edge.
- The handwheel lock will lock the object head at the top of the cutting stroke and must be used when changing blocks.
- The guard must be in place and the handwheel lock engaged when a block is being placed into or removed from the cassette clamp, or when any manipulation of the block is being undertaken while the knife or blade is in place. The guard must also be used when the microtome is left unattended.
- The handwheel brake will lock the microtome when the handle is in any position and is used when realigning a block face or adjusting the coarse feed.

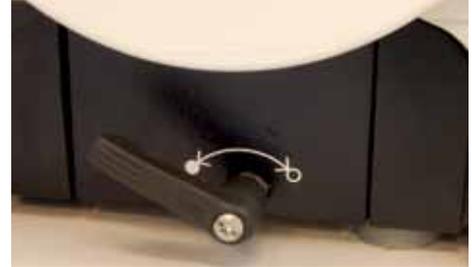


The red safety guard on the Leica RM2235 Microtome has been flipped up into place in this illustration. It completely covers the cutting edge of the blade.



The handwheel lock is shown here with the handwheel in the 12 o'clock position and the lock engaged. This lock will only engage in this position at the top of the cutting stroke.

- The knife or blade should be removed from the microtome when the instrument is left unattended or when cleaning the instrument. This is best done by unclamping the blade, then using the blade ejector on the left side of the guard to start moving the blade laterally out of the clamp. It can then be grasped with forceps (not fingers) or picked up with the magnet at the end of the Leica brush and safely removed. Used blades should be disposed of appropriately in a “sharps” container or into the “used blades” slot in the base of the blade dispenser.
- Never place a knife or blade on the bench or in a box with the cutting edge facing up. If you happen to drop a blade, let it fall. On no account try to catch it (a natural reflex action that you must guard against).

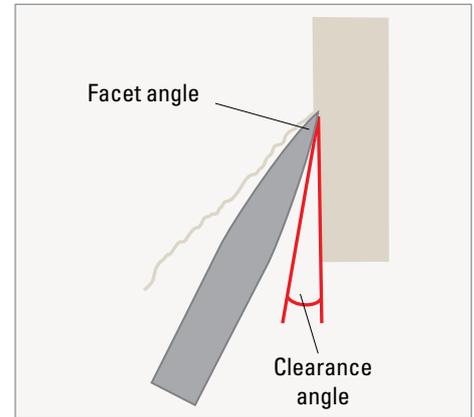


The handwheel brake of the Leica RM2235 is shown here in the locked position. The brake can be activated with the handwheel in any position. Either the handwheel lock or the handwheel brake can be used to immobilize the microtome.

6. Set Blade Clearance Angle Optimally

Blade clearance angle is adjustable and must be set for optimum performance.

- The clearance angle prevents contact between the knife facet and the face of the block.
- The facet angle is the angle between the two facets that form the cutting edge. For routine use knives and disposable blades are made with a facet angle of approximately 35° , but this angle can vary with the blade type and from manufacturer to manufacturer.
- Therefore for each blade type the clearance angle must be optimally set.
- Follow the microtome manufacturer's guidelines for the recommended angle setting. For Leica knife and blade holders a setting of between 1° and 5° is recommended¹.



7. Maximize Blade Life

There are some simple strategies for getting the maximum life from each blade.

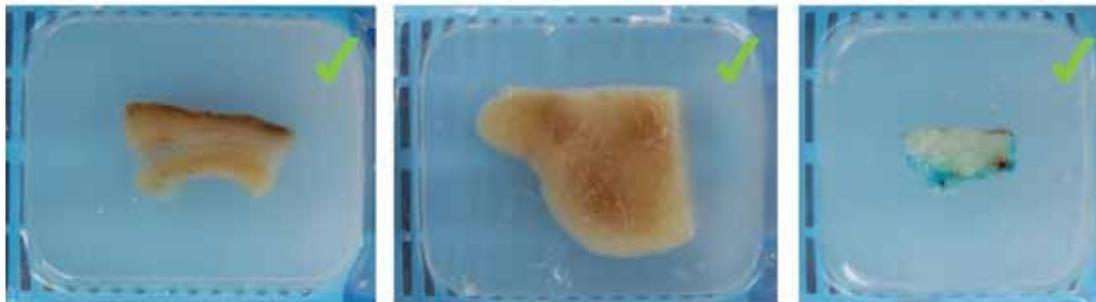
- When cleaning the blade avoid dragging anything along the cutting edge. Even cellulose fibres can cause damage to the blade.
- Avoid touching the edge with any hard objects such as forceps or brush.
- Use the blade systematically, working from one end to the other. This will give you maximum life from every part of the blade.
- Use one part of the blade for trimming and another new part for final sectioning, or use separate blades for these two procedures.
- A retracting microtome extends blade life by moving the specimen away from the blade on the upstroke and preventing the build-up of debris on the back of the blade.

8. Orientate Specimen Appropriately

The orientation of the specimen to the blade during the cutting stroke can affect the ease with which a ribbon can be obtained and directly influence section quality.

- In most laboratories all cassettes are placed in the object clamp with the same orientation (eg. label to the left for east-west orientation or label uppermost for north-south orientation). This is done to facilitate roughing of multiple blocks prior to preparing the high-quality sections and to allow deeper cuts or re-cuts without excessive loss of tissue. The orientation of the specimen to the blade must therefore be considered **at the embedding stage. This requirement is often overlooked.**
- The illustration on page 12 shows the preferred orientation of some typical specimens. Opinions will differ as to what orientation is best for particular specimen types, but orientation is important and must be considered.
- Example A. Intestine: blade passes through the mucosa last
- Example B. Cervix: it is better to present a point of dense tissue to the blade rather than a straight edge.
- Example C. Skin: blade passes through the epidermis last.
- Microtomes such as the Leica RM2235 also have a precision orientation device with calibrated controls that make it easy to find a zero position or a measurable variable on the x/y axis. Having this capability is very useful when re-cutting blocks prepared in other laboratories or on other microtomes where precise alignment is required to prevent unnecessary loss of tissue. For routine use when cutting

multiple blocks, it is important that this holder is set to a zero position for both axes before commencing to section. This is easily achieved by using the indicators (red), notch points (click stops) and indicator markings.



A. Intestine

B. Cervix

C. Skin



9. Learn About the Design Features of Your Microtome and How to Use Them

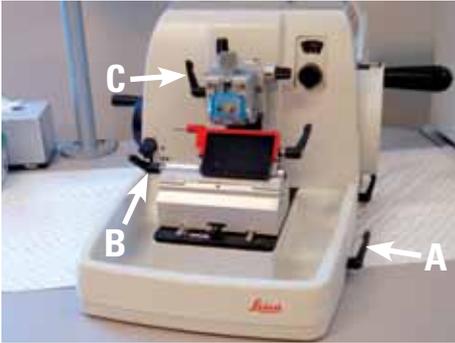
Retracting microtomes are designed so that the specimen retracts away from the blade on the up stroke. It is important to know whether your microtome has this capability.

- Retraction is a design feature that provides distinct advantages during sectioning and prolongs blade life.
- When using a microtome that incorporates retraction, alignment of the block face to the knife edge, must be carried out with the block on the down stroke (in the forward position – not retracted).
- If a block is aligned close to the knife edge while the specimen arm is in retraction, on the next full revolution of the handwheel the block will advance by the retraction value plus the selected section thickness. This will result in a thick slice being cut that could damage both the specimen and blade.
- Microtomes can also be manual, semi-automated or fully automated.
- Automated instruments reduce repetitive movements that can contribute to musculo-skeletal disorders.

10. Take Care When “Trimming”, “Facing”, or “Roughing”

This stage in microtomy requires great care as tissue of diagnostic importance can easily be lost or the block surface damaged.

- Before you commence trimming always make certain that all of the clamping mechanisms are tightened securely.
- The goal of properly trimming a block is to conservatively expose the tissue down to a level where a representative section can be obtained.
- Trimming is usually done at thicknesses between 10 and 30 μm .
- Rapid coarse trimming of brittle tissue risks damaging the specimen surface². Take particular care using the mechanical trimming device at 30 μm .
- As a final step, polish the block face by gently cutting a few thin sections. This will avoid the problem illustrated in 18B on page 24.



The knife holder base clamp A, the lateral displacement clamp B, and the x-y orientation clamp C should be firmly locked before trimming your block. Failure to do so may result in damage to both block and blade.



The mechanical trimming device should be used carefully when roughing, particularly the lower (..) setting that produces an advance of 30 μm when activated.

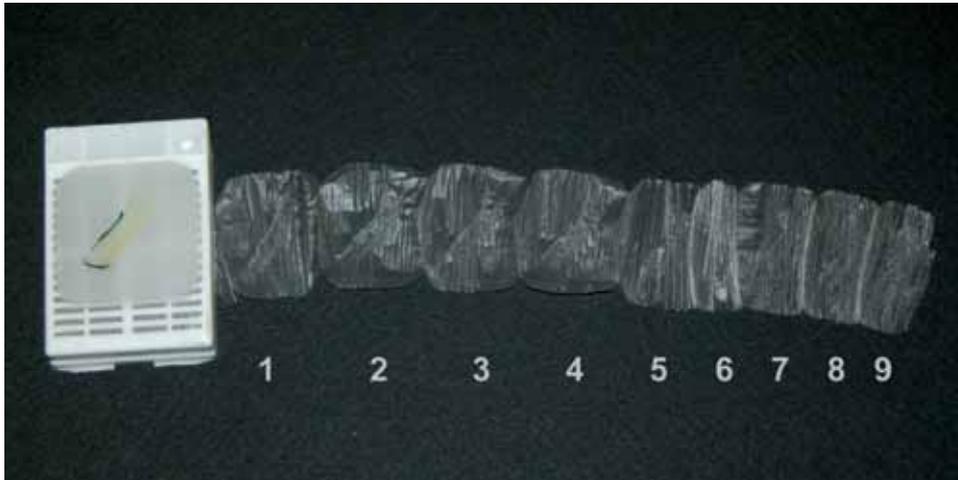


The coarse feed wheel needs to be used carefully when roughing to avoid cutting unintentional thick slices from the specimen surface and causing damage.

11. Consider Factors Affecting Section Thickness

Set the microtome at the desired setting but note that there are a number of factors that determine the actual section thickness.

- A cohesive section of 4 μm may provide more information than a severely disrupted section of 2 μm .
- The actual thickness of the first couple of sections in a ribbon may be thicker than indicated because of thermal expansion^{3,4} when cutting a cold block (as seen in sections 1, 2 & 3 below).
- Other factors such as speed of rotation, clearance angle setting and the condition of the cutting edge can influence the actual thickness achieved.



12. Ensure Blocks are Cold

Sectioning is generally improved when the specimen and the wax are well matched in hardness. It is for this reason that most paraffin blocks must be cold when sections are cut. The actual method used to chill the block is important.

- Cold wax provides better support for the harder elements in a specimen allowing thinner sections to be obtained.
- Place the blocks on a cold plate or a cold **wet** surface for a few minutes (such as the surface of melting ice).
- Water penetrates a small distance into the block face, swelling tissues and making them more amenable to cutting. This is particularly important to over-dehydrated, dry or crumbly tissues.
- Placing blocks in a freezer can cause surface cracking, where tissue separates from the surrounding wax. This can make it more difficult to obtain cohesive sections.

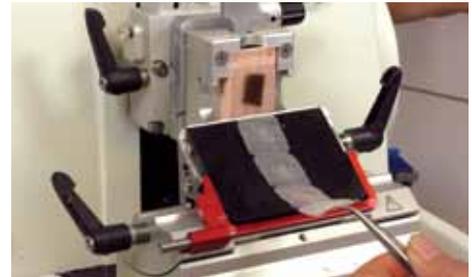


The sections on the left were cut from a relatively warm block without chilling. The sections on the right were cut from the same block after chilling on the surface of melting ice.

13. Learn the Technique for Cutting Consistent, High-quality, Thin Sections

There is no substitute for experience but there are some fundamental steps that will make the task easier.

- Use a section of blade that has not been used for rough trimming.
- When using the coarse feed, avoid cutting unintentional thick sections as this will damage your knife and possibly the block face.
- Re-chilling of the block may be required if the block face becomes warm or if deeper levels are required.
- Generally a slow, uniform cutting stroke produces the best results and the least compression.
- Do not stop and restart during a cutting stroke as this will produce bands of different thickness across the section.
- The practice of gently breathing on the face of a chilled block immediately before cutting each section, is common practice in some laboratories. The application of warm, moist breath tends to make sections more cohesive, but it also causes thermal expansion thus making the section thicker³.
- Debris adhering to upper or lower edges of the block, or the back of the blade, can make it difficult to obtain cohesive ribbons and cause the ribbon to lift off the blade on the upstroke. If debris is present clear it away, re-chill your block and start again.



This ribbon has been cut with a slow and steady stroke from a well processed, thoroughly chilled block. The sections show very little compression even before flotation.

14. Choose Slide and Adhesive Correctly

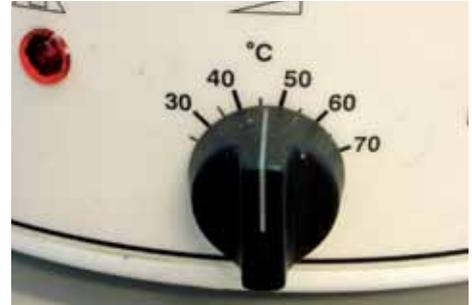
The choice of slide and adhesive will be influenced by the staining methods to be subsequently applied.

- Slides must always be grease and dust free and stored and handled correctly.
- If staining is to include antigen retrieval (IHC), enzyme pretreatment (ISH), or prolonged incubation steps, charged slides or an adhesive such as aminoalkylsilane (AAS) must be used. Some special stains, particularly those that employ alkaline reagents, can also cause sections to lift.
- Slides must always be accurately and appropriately labelled in a manner compliant with local regulations.

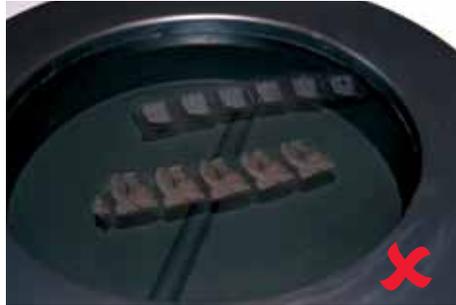
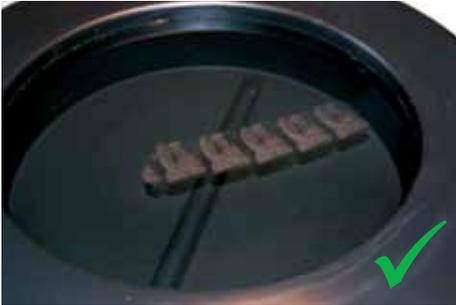
15. Float Out Sections Carefully

Flotation should expand the section to its original dimensions and ensure it is completely flat.

- Monitor the temperature carefully. The temperature will need to be 5 - 9 °C below the melting point of the wax.
- Make sure the water is clean and free of bubbles and section waste (to avoid cross-contamination).
- Place sections with the smooth (shiny) side down.
- Place the sections onto the water surface with a gentle sweeping action.
- Sections are very easily damaged when dislodging wrinkles or bubbles with brush or forceps.
- Examine each section as it floats on the water surface as imperfections can be readily seen.
- Leave the section on the water surface just long enough for it to flatten. Over-expansion can spoil the morphology in susceptible sections.
- To promote efficient drainage and to prevent the section slipping down the slide, remove slides vertically from the water.



- Before placing slides in a slide drier or oven drain them vertically for a brief time to remove excess water.
- Skim the water surface with lint-free tissue between blocks to avoid the possibility of cross-contamination.
- To avoid any chance of a mix-up float out sections from one block at a time.



Sections from two different blocks must not be floated out simultaneously. Even though the specimens may be of a different type, there is a possibility of cross-contamination and confusion leading to incorrect identification. This procedure should therefore be avoided at all times.

16. Dry Slides Adequately

Proper drying ensures that sections are completely dehydrated, free of heat damage, flat and unlikely to lift during staining.

- Drain excess water from beneath the section before drying. This is vital if slides are dried flat on a hot plate⁵.
- Slides can be stored in racks in an upright position, then dried in an oven.
- Generally drying temperatures should not exceed 65 °C.
- Excessive heat can cause droplets of water underneath a section to boil and this will cause damage.
- Dry sections for between 10 and 30 minutes.
- Some delicate specimens will produce best results when dried at 37 °C for a longer time (several hours to overnight).

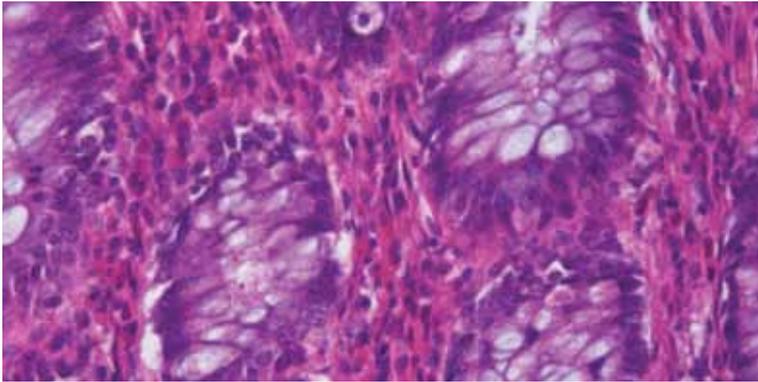
17. Clean and Maintain the Microtome Thoroughly

It is important to remove accumulated tissue debris and wax after use. Regular preventative maintenance is important.

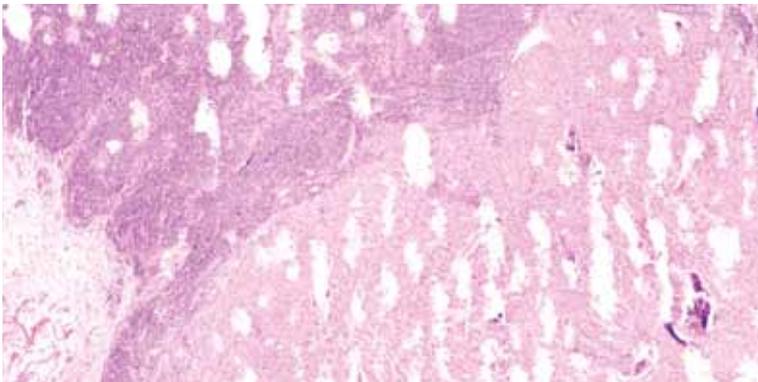
- Clean the instrument daily.
- Always remove the knife or blade before cleaning.
- The knife holder can easily be removed to facilitate access for cleaning.
- Section waste is best removed with a dry paintbrush.
- Do not clean the outer surfaces with alcohol or xylene as they are not resistant to these solvents and exposure to xylene should be avoided. Paraffin remover, mild commercial household cleaners or soap and water are recommended.
- No fluid must enter the inside of the instrument during cleaning.
- Have the instrument inspected at least once a year by a qualified service technician.
- Follow the lubrication instructions provided in your instruction manual using recommended lubricants.

18. Learn to Recognize and Correct Common Faults

Some of the most common faults seen in paraffin sections are:



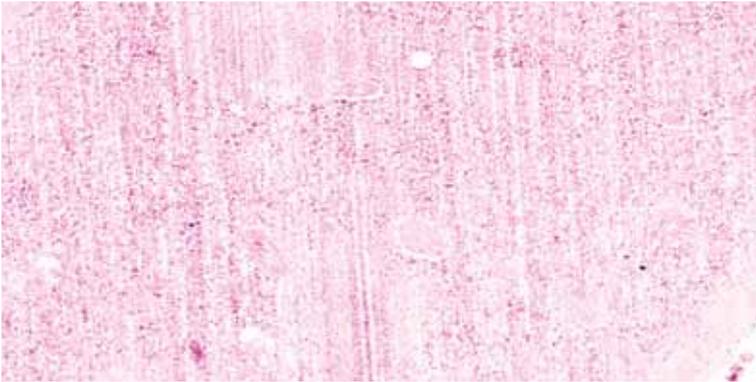
A. Section too thick



B. Holes from rough trimming

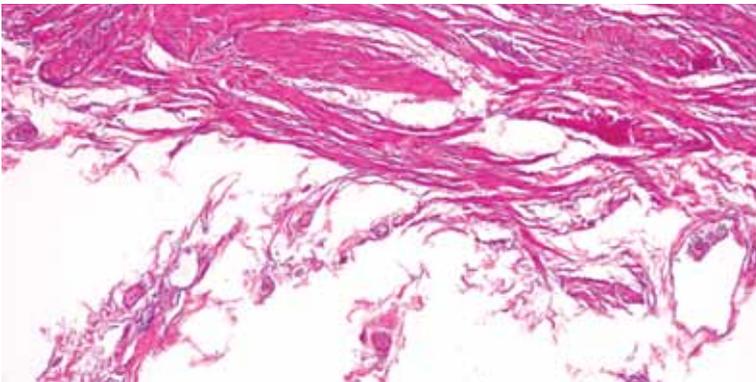
- Wrong micrometer setting
- Warm breath applied to cold block to facilitate sectioning
- First section in ribbon chosen
- Sectioning at too great a speed
- Poor processing
- Microtome needs recalibration

- Block trimmed too quickly
- Block surface not polished by cutting some thin sections after roughing
- Inappropriate section thickness used when trimming
- Block brittle (over-processed?) or too cold when trimmed



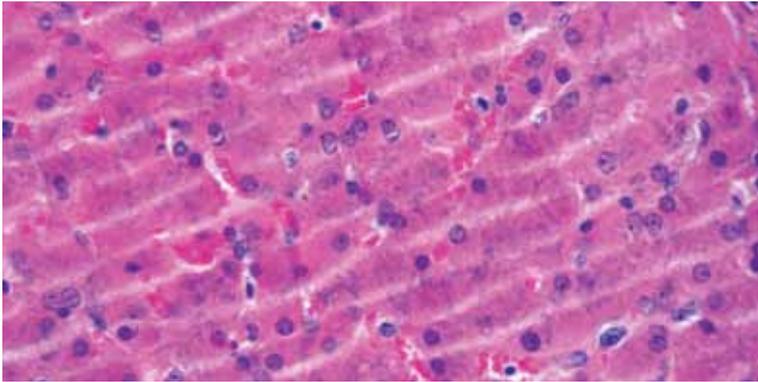
C. Knife lines (vertical striations in section)

- Damaged knife or blade used
- Poor processing
- Hard material such as calcium in block
- Debris in unfiltered wax
- Buffer salts precipitated in specimens



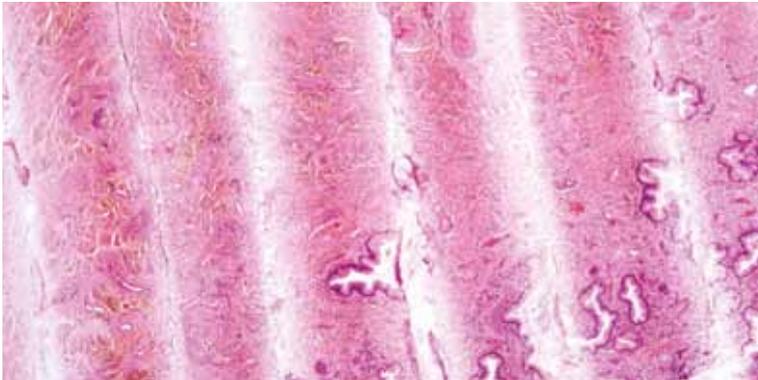
D. Disruption

- Rough handling of specimen during grossing
- Poor processing (incomplete dehydration, clearing or infiltration)
- Vigorous treatment to dislodge wrinkles during flotation
- Floating out for too long or using water that is too hot



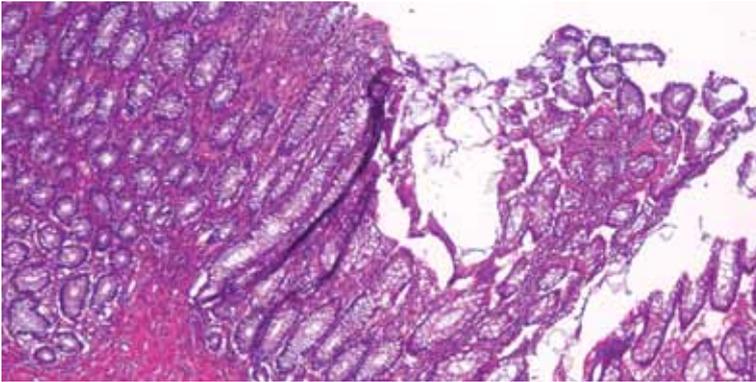
E. Fine cracks or micro-chatter

- Tissue over-processed
- Block too cold
- Cutting too fast
- Clamping mechanism not securely locked
- Clearance angle needs adjustment



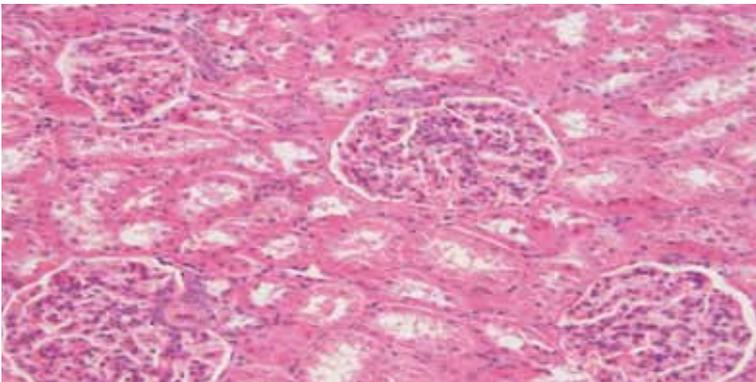
F. Coarse chatter

- Clamping mechanism not securely locked
- Very hard or large specimen
- Poor processing
- Insufficient clearance angle
- Sectioning too rapidly
- Worn microtome



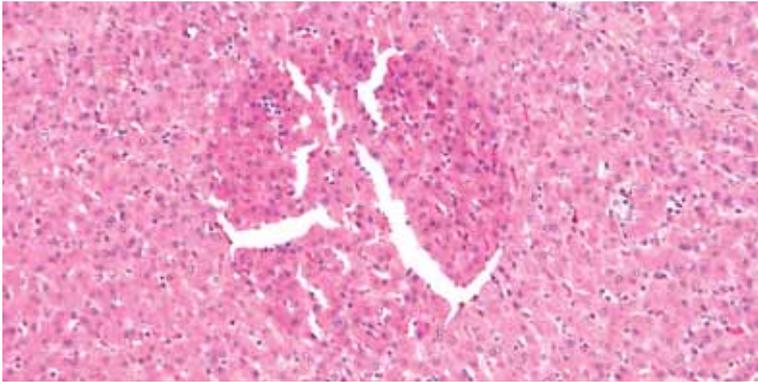
G. Folds

- Poor flotation technique
- Poor fixation and/or processing (insufficient support)
- Warm block
- Section too thin
- Clearance angle too great
- Water bath too hot



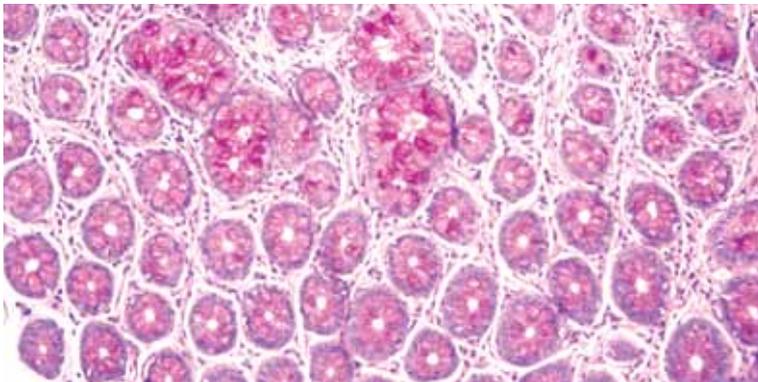
H. Excessive compression

- Poor processing (insufficient support)
- Warm block
- Cutting too fast
- Dull cutting edge
- Clearance angle too great
- Poor quality wax



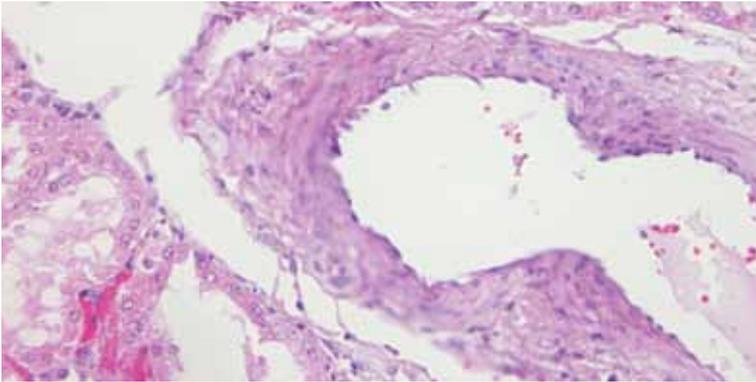
I. Bubbles under the section

- Bubbles adhering to base and sides of flotation bath
- Poor flotation technique trapping bubbles under section



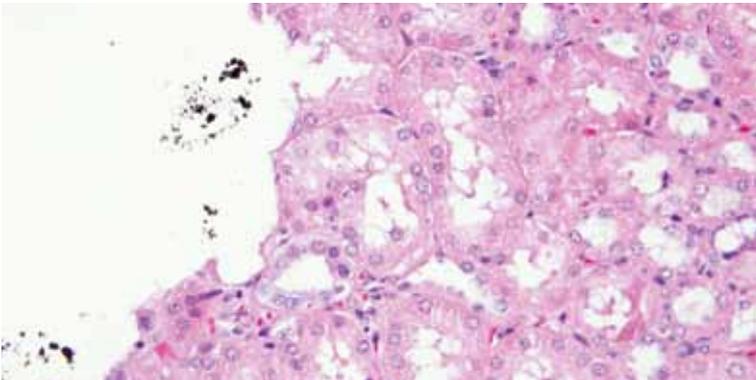
J. Over-expansion during flotation

- Temperature of bath too high
- Section left for too long on water
- Poor fixation and/or processing (residual solvent)



K. Section not flat (poor adherence)

- Poor quality section (wrinkles, bubbles)
- Flotation bath too cold
- Use of an uncoated slide
- Section not drained thoroughly after flotation
- Insufficient drying time
- Drying temperature too low



L. Dust present

- Dirty slide
- Flotation bath not skimmed or contaminated
- Slides drained, dried or stored in a dusty environment
- Fragments of pencil lead from labelling

References

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2. Rolls GO, Farmer NJ, Hall JB. Artifacts in Histological and Cytological Preparations. Melbourne: Leica Microsystems, 2008;106.
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