Pathology Partners

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Leica MICROSYSTEMS

Maintain the Best View: Take Care of Your Microscope

By Doug Giszczynski, *Leica Senior Marketing Manager*

Excellent tools . . . one of the keys to success in the surgical pathology suite. Accurate diagnosis demands the best information available, and a properly maintained microscope delivers optimal performance. Latex glove powder residue combines with blood, tissue, stains, buffers, and solvents to leave behind a sticky mess that diminishes a microscope's optical performance and gums up focus and stage gears. Microscope care is simple, but must be done regularly. Perform the the following steps daily at a minimum; and ideally, after each use to insure the highest quality images.

- Thoroughly wipe the exposed surfaces of the microscope with a 70% ethanol solution to clean and decontaminate. Pay special attention to the stage surface and controls, binoculars, eyetubes, and focus knobs
- Use commercial glass cleaner and lens tissue to clean the lens surfaces of the objective lens, eyepieces, and condenser lens. If oil objectives are used, remove immersion oil from the lens surface with cotton swabs and glass cleaner. Swabbing is essential for 63x objectives, as lens tissue is unlikely to reach the concave surface of the lens.
- Once a week run the focus knobs, stage controls, and condenser focus rack though a full course of travel. In addition to helping maintain even distribution of lubricant, this will ensure that the components function properly. *Note: adjustment of the condenser will necessitate re-focusing for proper Koehler illumination* (*see page 2 for instructions*).
- Report any microscope problems immediately and have them repaired by manufacturer-certified technicians.

CAP inspectors require annual maintenance and instrument performance certification. However, the special challenges of the surgical pathology suite make it a good idea to inspect twice a year.

Welcome to *Pathology Partners*, the Leica-sponsored e-newsletter designed to provide balanced reporting on pathology and clinical/anatomical topics as a practical resource for you.

Play It Safe: Cryostat Disinfection Techniques

By Jan Minshew, HT, HTL (ASCP), *Leica Marketing Manager*

Properly frozen tissue specimens can be sectioned in a cryostat because, like formalin-fixed paraffin embedded specimens, cellular structures are well supported and can withstand the cutting force without distortion. The trick, of course, is freezing at an extremely rapid rate to prevent the formation of damaging ice crystals and sectioning at the appropriate temperature so the tissue is neither brittle (temperature too low) nor soft (temperature too high). *Note: This topic will be discussed in a future issue of Pathology Partners.*

Frozen sectioning techniques are most often performed on fresh specimens to obtain rapid diagnosis for intraoperative consultation, although frozen sectioning is used for other procedures as well. Since fresh specimens are not exposed to chemical fixatives, processing solutions or heat (like formalin-fixed paraffin embedded specimens), many enzymes, diffusible compounds, and antigens are well preserved within the specimen. This makes the technique ideal for enzyme histochemistry, immunohistochemistry, and the demonstration of various neurological elements and lipids.

For the histotechnician, there are special concerns when working with fresh, frozen tissue. Pathogens also remain well preserved within a frozen specimen and are quite capable of infecting the cryostat operator, service personnel, and other individuals in the working area.

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Make the Most of Your Microscope

10 Easy Steps to Achieve Perfect Koehler Illumination

By Doug Giszczynski, *Leica Senior Marketing Manager*

Without light, there is no vision. For microscopes, the quality of the light is critical to achieving a crisp, consistent image. Even the best microscopes yield "soft" or poor images when the illumination system is not properly adjusted. Most microscopes use Koehler (named after its 1893 inventor, Augustus Koehler) illumination to produce bright, even, glare-free illumination. All clinical microscopes should be capable of Koehler illumination.

There are several mechanical requirements for a microscope to achieve Koehler Illumination:

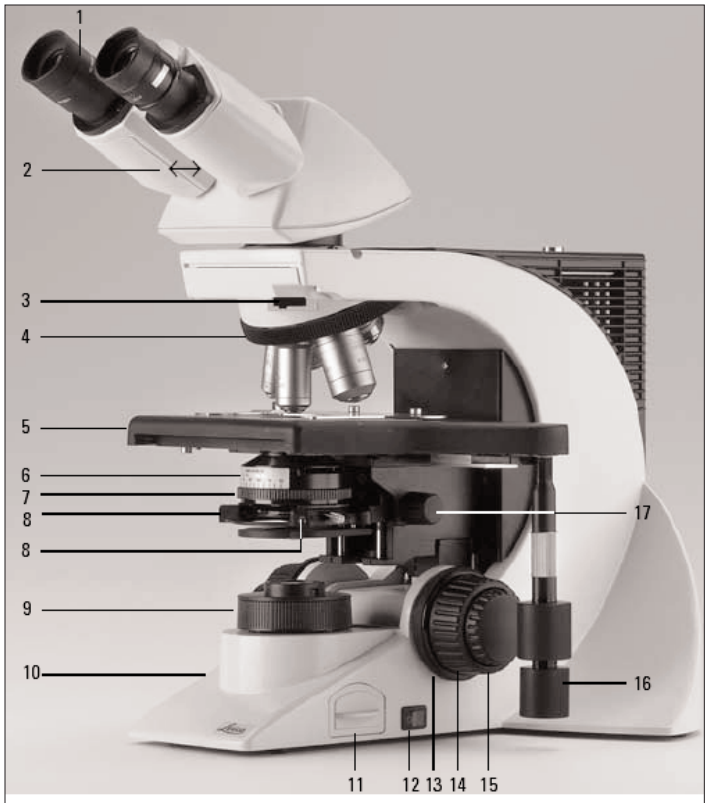
- Focusable sub-stage condenser (centerable or factory pre-centered)
- Adjustable aperture diaphragm (in the condenser)
- Field diaphragm near the light source (adjustable or factory pre-set)
- Light source lamp (centerable or factory pre-centered)

Please see **Figure 1** for location of microscope components.

How to properly adjust a microscope for Koehler illumination:

1. Power on the microscope and place and adjust the eyepiece diopters to neutral.
2. Fully open both the aperture and field diaphragms.
3. Lower the stage using the microscope coarse focus knob and place a specimen (i.e., stained microscope slide) on the stage. Rotate the objective turret to move the 10x objective into working position.
4. Use the coarse focus knob to raise the stage until it reaches the upper stop of its travel range; then close your left eye and use the fine focus knob to bring the specimen into focus.
5. Close your right eye, and use the left eyepiece diopter to bring the specimen into focus.
6. While viewing through the microscope, close the field diaphragm until you see the iris leaves in the field of view.
7. Turn the condenser focus knob to bring the leaves of the field diaphragm into sharp focus.
8. Center the image of the field diaphragm by turning the condenser centering screws simultaneously. Next open the field diaphragm until the leaves just barely disappear from view.
9. Remove the right eyepiece and view through the eyetube. Close the aperture (condenser) diaphragm completely, then reopen it until the leaves obscure approximately 20% of the outer field of view (**Figure 2**). Finally, replace the eyepiece.

You are now ready to view your specimen under Koehler illumination.



1. Eyetube containing eyepiece with adjustable diopter
2. Interpupillary distance adjustment
3. Objective prism slider fixture
4. Objective turret with objectives
5. Stage with specimen holder
6. Condenser
7. Aperture diaphragm
8. Condenser centering screws
9. Field diaphragm
10. Brightness adjustment
11. Built-in illumination
12. On/off switch
13. Focus threshold setting
14. Coarse focus
15. Fine focus
16. Coaxial drive for x/y stage movement
17. Condenser height adjustment

Figure 1

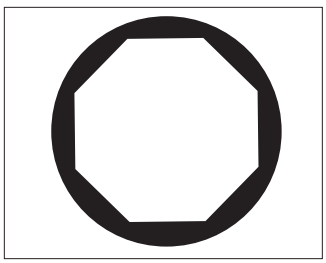


Figure 2 *(at right)*

A Day in the Life . . .

One Pathologist's Approach to Teaching Frozen Sectioning

By Dr. Stephen Peters

Anyone who has visited Dr. Peter's website tutorial on frozen section techniques knows that he is a true believer in the brush technique. Let's learn how he teaches this valuable skill to his residents:

The first step is to find a comfortable hand position to maximize fine motor ability for this very delicate maneuver. For this, I have my students hold the brush like a pen and gently rest a finger on the anti-roll plate or at the edge of the cryostat stage. Next, I tell them to try to write their names on the edge of the block with the brush to gain a sense of the fine movements they will use in the sectioning process.

We then turn the cryostat wheel continuously, without any hesitation, and catch the sections with the brush. This precise, delicate motion can be compared to catching a falling snowflake with the brush. In order to do this, the brush must continue to move in an elliptical motion, which in conjunction with the right hand turning the wheel is like turning the pedals of a bicycle. I have found that the best way to teach this skill is to hold hands.

Once a student is holding the brush in the correct position, I take hold of the student's hands and ask them to let their hands to go totally limp. Standing next to the student I move the right hand (holding the brush) while moving the left hand (holding the wheel handle). We cut together in continuous motion for a minute or two to give the student the muscle memory of what this motion feels like. Next, the student uses the brush alone with the left hand, while I continuously turn the wheel with my right. After a minute of this, I will again take both hands and get started in continuous motion. After another 30 seconds of turning both hands together I let go. If there is hesitation, I nudge the student along until he or she is cutting continuously on their own. After a few minutes of practice, most students will now be cutting continuously. If there is still hesitation, we repeat the steps as necessary.

My next goal is to make sure that students are pulling the sections along the stage like pulling the covers over yourself in bed. I emphasize not to press the brush and tissue to the stage so that the tissue will not stick to the stage, a problem particularly common while cutting fatty tissues. To foster the correct motion across the stage I sharpen a point on an applicator stick and have the students catch a section with the pointed stick rather than the brush. Pressing the section to the stage with the pointed stick results in tearing, and it teaches them to grab just the edge of the section and pull in gently across the stage.

My last goal is for students to learn all of the information in my cryosectioning tutorial. The tutorial covers such things as adjusting the block temperature so that sections will cut optimally; proper embedding and sectioning of tissues relative to the knife blade; and most importantly, how to recognize various thickness variations and artifacts inherent in cryosectioning to assess the quality of the result while sections are taken, not later when the slide is under the microscope.

My advice: Know how your cryostat works, learn simple maintenance and troubleshooting, and above all use a sharp blade! To learn more about this pathologist's approach to cryosectioning visit Dr. Peter's tutorial at:
http://pathologyinnovations.com/frozen_section_technique.htm.

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Ask Mari Ann . . .

Dear Mari Ann,

I have been cutting the same specimen for one hour and all of a sudden I am getting thick and thin sections. What could be the cause?

There are several things that can cause thick and thin sections.

1. The knife may have to be changed.
2. Check the knife holder and make sure everything is tight.
3. The temperature of the cryostat may have warmed up a bit. It would be good to stop cutting, close the door, and let temperature equilibrate for about 10 minutes.
4. The next thing to check is the specimen chuck. Make sure the specimen is still firmly attached to the chuck.

Thank you for your question.
Please send your questions to Mari Ann at:
pathologypartners@leica-microsystems.com
Mari Ann Mailhiot, BA, HT(ASCP), product application specialist with over 37 years of experience in the field of histology, will choose one question to answer each issue.

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Play It Safe

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Cryostat disinfection techniques are extremely important. The CAP Laboratory Accreditation Program Inspection Checklist requires a documented procedure for routine decontamination of a cryostat at defined intervals, and also requires evidence of decontamination records.

The following disinfection guidelines can be used to maintain compliance with CAP inspection criteria and improve laboratory safety:

Wear Personal Protective Equipment (PPE)

Personal Protective Equipment, such as gowns, puncture and penetration resistant gloves, and eye protection must be worn when performing cryostat disinfection procedures.

Cryostat Preparation

Remove used blades/knives from their holder. Although not a requirement, steel mesh gloves should be worn when changing knife blades. Dispose of blades according to the regulations of your institution or disinfect knives before reusing by soaking in disinfecting solution. Remove ALL debris and utensils (pencils, forceps, brushes, gauze, etc.) from the chamber. Debris must be removed because organic material (blood and proteins) may contain high concentrations of microorganisms and could inactivate the chemical disinfectant or prevent access to contaminated surfaces. Debris should be treated as a biohazard and disposed of according to the policies and procedures of your institution. Utensils must be disinfected before reusing. 70% ethyl or reagent alcohol can be used to clean the cryostat and partially disinfect the cryostat. The germicidal activity of ethyl alcohol is most effective in the 70% range because it can penetrate tubercle bacteria and has an advantage over isopropyl alcohol by its ability to kill hydrophilic viruses.

Chemical Disinfection

To disinfect a cryostat using a chemical disinfectant, the instrument MUST be at room temperature before the process is started. Do not create an airborne mist by spraying disinfectant (or anything else) in an open cryostat chamber. Instead, pour disinfectants onto surfaces or absorbent disposable towels and allow them to remain in contact with contaminated surfaces for the length of time specified in the instructions of the individual agents. Properly dispose of paper towels. Use a tuberculocidal disinfectant that is non-corrosive. The EPA posts a list of Antimicrobial Chemical/Registration Number Indexes (**http://www.epa.gov/oppad001/chemregindex.htm**) and updates it regularly. From this link you can find agents effective against bloodborne pathogens such as *mycobacterium tuberculosis*, human HIV-1 virus, and Hepatitis B or Hepatitis C virus. **Critical:** NONE of the listed solutions have been tested at low temperatures and can ONLY be used at room temperature.

Following Chemical Disinfection

After the disinfection procedure is complete, the cryostat must be thoroughly dried and lubricated before being put back into service at cold temperatures. Only use lubricants that are recommended by the cryostat manufacturer and only in the recommended amounts. For optimum sectioning, allow at least two and a half hours for the metal microtome parts to reach the cold temperature setting.

Built-in Disinfection

There are several cryostats manufactured with built-in disinfection systems, including Leica's CM1850 UV and the CM1900 UV. Prior to production of these instruments, an independent laboratory, Ecoscope, Laboratory for Microbiology and Ecotoxicology in Amtzell, Germany, performed tests in various positions in the chambers to validate the efficiency of the UVC surface disinfection. As a result of those tests, the Amtzell Laboratory established a certificate attesting to the UVC's efficiency and the recommended irradiation times to inactivate all kinds of bacteria, spores, fungi, and viruses, including the Avian Influenza A (H5N1).

Although regularly scheduled chemical disinfection is still suggested, UVC disinfection is an excellent means of rapidly reducing the exposure to dangerous pathogens without warming or defrosting the cryostat. Please feel free to contact your Leica representative or authorized dealer for information regarding the cryostats or the certificates.

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Note: We are interested in your comments and thoughts about the newsletter. Please feel free to email your comments to:
pathologypartners@leica-microsystems.com