Name:	Leica RM2235 Microtome Procedure	**Created:**	September 9, 2025
Number:	Histo-4	**Revised:**	September 12, 2025
Category:	Instrument Operation	**Author(s):**	Anna Chernatynskaya

1.0 Purpose

This document outlines the standard procedure for the safe and correct operation, cleaning, and basic maintenance of the Leica RM2235 Rotary Microtome. Following this procedure ensures consistent and high-quality sectioning of tissue blocks, maximizes user safety, and preserves the integrity of the equipment, based on the manufacturer's instruction manual.

2.0 Policy

2.1 The use of all histology equipment in the histology core lab at Missouri S&T is currently managed by the Center for Biomedical Research (CBR) staff.

2.2 All personnel working in the histology core are required to complete general laboratory safety and BSL-2 training through EHS.

- 2.2.1 More training may be required in the future. Please check with CBR staff before beginning work to ensure all required training is complete.

2.3 Eating or drinking is not permitted in the lab.

2.4 PPE is required for all users. This includes, at minimum, gloves and a lab coat. A mask and/or goggles should also be worn if working with noxious chemicals (i.e. xylene) or chemicals with the potential to splash.

2.5 Bertelsmeyer 220 is a shared lab space, therefore all users must be familiar with the supplies and equipment available to them before keycard access to the lab will be granted.

2.6 All samples should be labeled with your name, date, and sample identification. **Any samples not labeled will be thrown out.**

2.7 Each user is required to sign in and out of each equipment logbook while working in the histology core.

- 2.7.1 Please also schedule all equipment use through the online Outlook calendar for the Histology Users – MST Outlook group.
  - 2.7.1.1 Please view the Outlook calendar instructions pdf sent with the Outlook group invite for further information.

2.8 Each person working in the lab is responsible for cleaning work surfaces, such as benches, and any used equipment before leaving.

- 2.8.1 Cleaning tasks must be documented daily on the provided checklist.

2.9 Each person leaving the lab, including temporary visitors, is required to wash their hands before leaving.

2.10 No user fee is currently being charged, however, a list of supplies to be provided by users is outlined in the Supplies section below.

2.11 The operator is responsible for carefully following all steps outlined in this SOP, performing the required cleaning after each use, and immediately reporting any equipment malfunctions, damage, or safety concerns to the lab supervisor.

2.12 Please contact Anna Chernatynskaya or Katie Tooley if you have any questions.

3.0 Safety Precautions:

3.1 **Blade Hazard:** The microtome blade is extremely sharp. Always handle the blade and blade holder with extreme caution. **Always use the blade guard when not in use.** Never place your fingers near the blade's cutting edge.

3.2 Never place a blade anywhere with the cutting edge facing upwards and never try to catch a falling blade.

3.3 **PPE:** Always wear appropriate personal protective equipment (PPE), including gloves, when handling tissue blocks and blades to protect against physical injury and biological hazards.

3.4 **Securing the Specimen:** Ensure the specimen head and blade holder are securely locked before beginning sectioning. The handwheel lock must be engaged before mounting or removing the specimen to prevent any unintended movement of the handwheel.

3.5 **Never Leave Unattended:** Do not leave the microtome unattended with an exposed blade. After use, the blade must be removed and the blade guard engaged.

4.0 Procedure

4.1 Preparation and Setup

4.1.1 **Surface Cleaning:** Using a lint-free cloth and a cleaning agent such as 70% ethanol, thoroughly clean the microtome's work surfaces, especially the area around the blade holder and specimen clamp. Ensure there is no dust or paraffin debris.

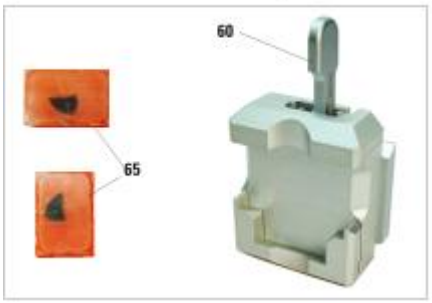
4.1.2 **Blade Holder Check:** Verify that the blade holder is clean and free of any residual tissue or debris. Check that the blade clamping lever functions smoothly.

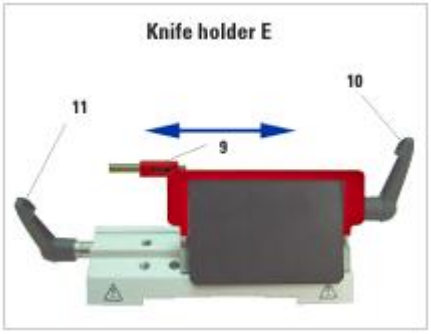
4.1.3 **Handwheel Brake:** Engage the handwheel brake by pulling the handwheel lock lever towards you. This will lock the handwheel in place and prevent accidental movement during specimen and blade setup.

4.2 Blade and Specimen Setup

4.2.1 **Tissue Block Preparation**: Retrieve a prepared paraffin-embedded tissue block. Ensure the block is well-chilled on an ice bath (or cold plate to a temperature of approximately 0°C to -10°C) for optimal sectioning.

6.2.5 Universal cassette clamp





4.2.2 **Specimen Mounting**: Unlock the specimen head clamping lever by releasing the lever to the left. Insert the tissue block into the specimen head, ensuring it is centered, and the top surface of the block is parallel to the blade holder's edge. And then move the lever to secure the cassette. The block should not move or wobble when gently pushed.

4.2.3 **Blade Insertion:** Fold blade guard (9) downward. To insert the blade, flap the right clamping lever (10) forward and down. Carefully slide a new, disposable blade into the blade holder's slot. Make sure that the blade is clamped parallel to the upper edge of the pressure plate. To clamp the blade, rotate clamping lever (10) back upwards.

Note: The blade holder has a lateral movement, so that the entire width of the blade can be used. The clamping levers on the blade holder are not interchangeable. The two clamping levers (10, 11) must remain in the position shown at all times, as otherwise isolated malfunctions of the blade holder can occur. Clamping lever for the blade (10) at the right, clamping lever for the lateral displacement (11) at the left.

4.3 **Sectioning. Trimming:**

4.3.1 Set the section thickness control to a coarse trimming thickness of ~10 µm – 30 µm.

4.3.2 Unlock the handwheel brake.

4.3.3 Turn the handwheel in a slow, continuous motion until the tissue is fully exposed and the block face is flat.

4.3.4 Lock the handwheel brake after trimming to prepare for fine sectioning.

4.4 **Fine Sectioning:**

4.4.1 Set the section thickness control to the desired thickness for your application (e.g., 3-5 µm).

4.4.2 Always use a different area of the cutting edge for trimming and cutting. Use lateral movement, it is sufficient to move the blade holder sideways.

4.4.3 It is recommended to chill blocks on a cold wet surface and are always cold when cut (the surface of melting ice is excellent).

4.4.4 Unlock the handwheel brake.

4.4.5 Turn the handwheel smoothly and continuously to create consistent sections. Avoid jerky movements, as this can cause chatter marks or compression. The final sections from each block are cut gently with a uniform, slow rotation.

4.4.6 Use the fine feed control to advance the specimen towards the blade incrementally if needed to maintain cutting.

4.4.7 Use a fine brush or forceps to gently remove the sections from the blade and place them on a warm water bath to flatten them. You can use intermediate a cold (room temperature) water bath that helps separate each tissue slices with forceps because forceps will not sick to the paraffin.

- The water bath temperature should be 4-5°C below the melting point of the wax. Sections should be readily flattened, but the wax should not melt. If sections left in hot water bath for more than 15 seconds, the wax melts. If the wrinkles remain in the sections, the bath may be too cold. Avoid formation of the bubbles in the flotation bath. Any visible bubbles need to be removed before the sections are laid on the water.
- Use charged glass slides (frosted).
- Sections needed to be drained briefly before being placed in the slide dryer or onto the hotplate.
- Dry slides on the hotplate. The temperature of the hot plate should not be very hot. The excessive heat can produce hot spot and sections and cause uneven staining (nuclear meltdown usually at the perimeter of specimens. The drying times vary considerably.

5.0 Cleanup and waste disposal

5.1 **Engage Brake:** Engage the handwheel brake to lock the handwheel.

5.2 **Blade Removal:** Carefully remove the blade from the blade holder using the ejector and immediately dispose of it in a designated sharps container. Never leave a used blade on the microtome.

5.3 **Specimen Removal:** Remove the tissue block from the specimen clamp and return it to its designated storage location.

5.4 **Thorough Cleaning:** Clean the blade holder, specimen clamp, and all work surfaces with a lint-free cloth and cleaning agent. Do not use xylene or acetone, they will damage the finished surfaces. Remove all residual paraffin and tissue fragments with a dry brush. To remove paraffin residue, please use mineral oil or a plastic scraper.

5.5 **Biohazardous materials:** If samples are biohazardous, place all solid waste in the designated container with a biohazardous tag.

6.0 Maintenance

6.1 **Daily:** Clean the microtome and surrounding area after each use. Check for any loose parts or obvious damage.

6.2 **Monthly:** Lubricate moving parts according to the manufacturer's manual to ensure smooth operation.

6.3 **As needed:** If a malfunction occurs, refer to the manual's troubleshooting section. If the issue persists after attempting the recommended fixes, contact the lab supervisor for professional service.

7.0 Supplies provided by users:

7.1 **Blades for microtome and cryostat**: Epredia HP35 Ultra microtome blades (please stick with this exact product, our microtome is set up for easy change-outs of these specific blades) - $289.09 for a pack of 50, Catalog #31-537-35, https://www.fishersci.com/shop/products/thermo-scientific-ultra-disposable-microtome-blades-2/3153735?searchHijack=true&searchTerm=thermo-scientific-ultra-disposable-microtome-blades-2&searchType=Rapid&matchedCatNo=3153735

7.2 **Camel hair brushes, small** (links include what we use, but feel free to shop around, must be camel hair) - $70.75 for a pack of 12, Catalog #1910, https://www.fishersci.com/shop/products/cryotome-cryostat-accessories-camel-hair-brush/1910#?keyword=1910%20brush

7.3 **Camel hair brushes, large** - $26.28 each, Catalog #03-661, https://www.fishersci.com/shop/products/fisherbrand-long-handled-camel-s-hair-brush/03661#?keyword=03661

7.4 **1 gallon of Fisher histological grade ethanol** (only needed if you'll be processing more than 50 samples) - $113.10, Catalog #A405F-1GAL, https://www.fishersci.com/shop/products/ethanol-anhydrous-histological-fisher-chemical-3/A405F1GAL?searchHijack=true&searchTerm=ethanol-anhydrous-histological-fisher-chemical-3&searchType=Rapid&matchedCatNo=A405F1GAL

7.5 **Glass microscope slides** (charged slides are best for tissue retention during staining) – Fisherbrand Superfrost Plus Microscope Slides, $47.66 for pack of 144 slides, Catalog # 22-034979, https://www.fishersci.com/shop/products/fisherbrand-superfrost-plus-stain-slides/22034979?searchHijack=true&searchTerm=fisherbrand-superfrost-plus-stain-slides&searchType=Rapid&matchedCatNo=22034979

7.6 **Glass cover slips** (personal preference, but this is what we use) - Epredia Signature Series Cover Glass, $83.55 for a pack of 10 boxes, Catalog #22-050-232, https://www.fishersci.com/shop/products/signature-series-cover-glass-24-x-50mm/22050232#?keyword=22050232

7.7 **Microscope slide box** (feel free to shop around, item linked is an example) – Fisherbrand Microscope Slide Box, 100 slots, $9.58, Catalog #03-446, https://www.fishersci.com/shop/products/fisherbrand-microscope-slide-boxes-numbered-slots-3/03446#?keyword=03-446

8.0 References

8.1 Microtome: Leica RM2235 https://rankinbiomed.com/wp-content/uploads/Leica-2235-Operators-manual.pdf

8.2 Google search with key words (histology procedures, steps to process tissues, etc.)

SOP REVISION HISTORY

VERSION #	APPROVED	DETAILS
1	9/9/25	Created
2	9/12/25	Added additional information to sections 2 & 7
3		