Name:	Leica CM1860 Cryostat Procedure	**Created:**	September 17, 2025
Number:	Histo-7	**Revised:**	September 22, 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 Cryostat CM1860. 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 **Never leave samples in the cryochamber!** The instrument is not suitable for storing frozen specimens as the refrigeration dehydrates the specimens!

2.8 All components removed from the cold cryostat **must be carefully dried before returning them** to the cryochamber to prevent ice buildup!

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

2.9.1 Please also schedule all equipment use through the online Outlook calendar for the Histology Users – MST Outlook group.

2.9.1.1 Please view the Outlook calendar instructions pdf sent with the Outlook group invite for further information.

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

2.10.1 Cleaning tasks must be documented daily on the provided checklist.

2.10.2 **Remove section waste after EVERY change of specimen!** Do not start any chemical disinfection until you have done this! Each new specimen is a potential source of contamination.

2.10.3 **Remove section waste after EVERY sectioning operation and BEFORE changing specimens**. Remove the section waste by using a paper towel soaked in an alcohol-based disinfectant. **Use only alcohol-based disinfectants for disinfecting the cryochamber.** Do not use any solvents (xylene, acetone, etc.) for cleaning or disinfection.

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

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

2.13 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.

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

3.0 Safety Precautions:

3.1 **Blade Hazard:** The cryostat 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. **Always lock the handwheel prior to manipulating the knife and specimen, changing the specimen or taking a break.**

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 micro's work surfaces, especially the area around the blade holder and specimen clamp. Ensure there is no dust or paraffin debris.

4.1.2 **Temperature check**: Set the chamber temperature according to the tissue type to be sectioned (→ P. 59 –7.5 Temperature selection chart (in minus °C)).

4.1.3 **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.

4.1.4 **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.

4.1.5 Install all necessary parts, whether standard or optional (e.g. panels, waste and brush tray and the selected knife or blade holder). Make sure that they are mounted and cooled.

4.2 Blade and Specimen Setup

4.2.1 **Specimen Mounting:** Freeze specimen onto a specimen disc (→P. 41 – 7.3 Specimen discs) and mount it into the specimen head (→ P. 41 – 7.3.1) inserting the specimen discs into the specimen head).

4.2.2 **Blade Insertion:**

4.2.3 **Premium blade holder (Fig 29) and Blade Holder CE (Fig 30).**

Make sure that the knife or blade is inserted in the knife or blade holder, see (→ P. 45 –7.4.4 Blade holder CE) or (→P. 52 – 7.4.6 Knife holder CN).

Fig. 29

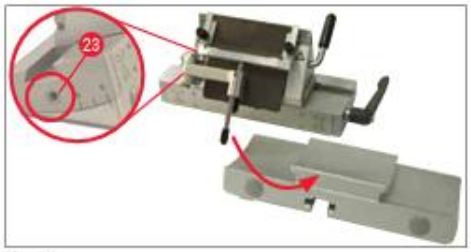

Fig. 30

Fig. 31

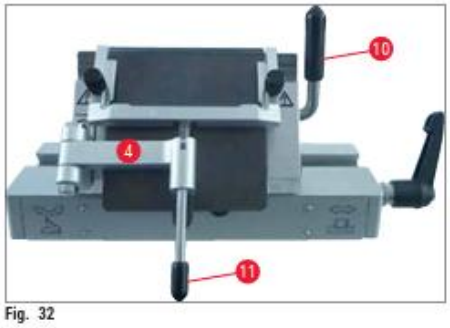

Fig. 32

4.2.3.1 **Inserting the blade holder CE :** Push the upper part of the blade holder onto the base. Clamp the blade holder into place on the left side using a Hexagon key (No. 4) (→Fig. 30-23).

4.2.3.2 **Inserting a high-profile blade:**

1. Fold the anti-roll guide system (→ Fig. 31-4) towards the left – while doing so, hold the lever (→ Fig. 31-11) (NOT the adjusting screw of the anti-roll guide), so that the height of the anti-roll guide remains unchanged.
2. Open the clamping lever (→ Fig. 31-10) by turning it counterclockwise (→"Fig. 31").
3. Carefully insert the blade (→ Fig. 31-9) from above or from the side between the pressure plate and the blade rest. Make sure that the blade is inserted so that it is centered and lies evenly along the ledge (see red arrow in (→ "Fig. 31")).
4. Rotate clamping lever (→ Fig. 32-10) clockwise to clamp (→ "Fig. 32").
5. Fold the anti-roll guide system (→ Fig. 32-4) back to the right (towards the blade) using the lever (→Fig. 32-11).

4.3 **Orient the specimen** (→P. 42 –7.3.2 Specimen orientation).

4.4 **Lateral movement**

If the sectioning results are not satisfactory, the blade holder (on a base here (→ "Fig. 38")) can be shifted sideways in order to use another part of the blade, and to benefit from the entire length of the blade.

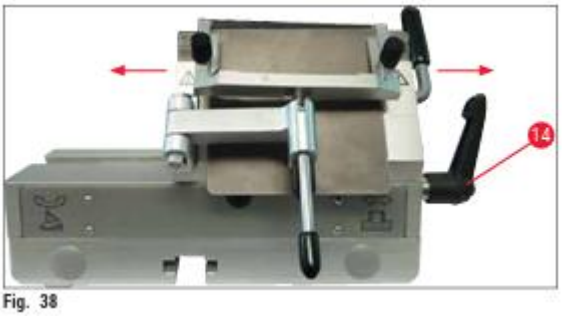

Fig. 38

1. Turn the clamping lever (→ Fig. 38-14) to the rear (counterclockwise) to release and then shift the blade holder sideways to the desired position.
2. To tighten, turn the clamping lever (→ Fig. 38-14) to the front (clockwise).

4.5 **Adjusting the anti-roll guide system:**

You can adjust the height of the anti-roll guide system using the knurled nut (→ Fig. 39-8):

- If you turn the nut counterclockwise, the anti-roll guide system moves toward the blade.
- If you turn the nut clockwise, the anti-roll guide system moves away from the blade.
- If the anti-roll guide system is in the wrong position relative to the blade, the following problems will result:

The section rolls over the glass insert of the anti-roll guide system (→Fig. 40-1). Error: Glass insert not high enough. Remedy: Turn the knurled nut counterclockwise until the section is pushed between the blade and anti-roll guide as shown in (→ Fig. 40-3).

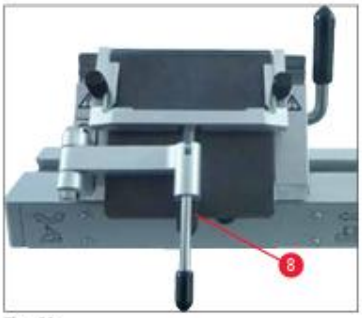

Fig. 39

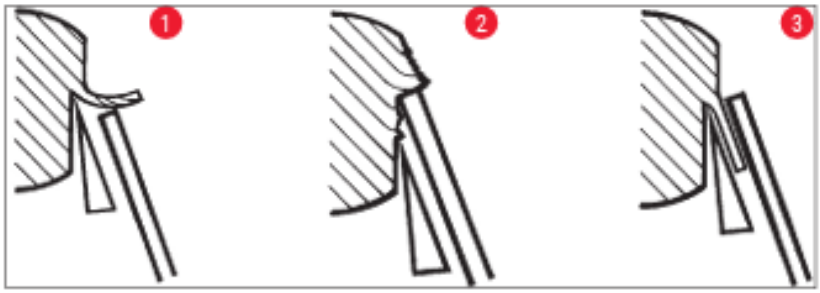

Fig. 40

Sections are compressed and block hits the glass insert (→ Fig. 40-2) after sectioning. Error: Anti-roll guide system is set too high. Remedy: Turn the knurled nut clockwise until the section is pushed between the blade and anti-roll guide as shown in (→Fig. 40-3).

4.6 **Clearance angle adjustment:** If necessary, adjust the clearance angle (→ P. 44 – 7.4.2 Clearance angle adjustment).

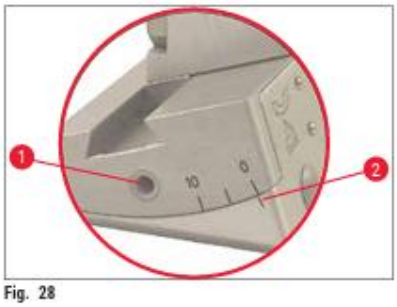

Fig. 28

- The clearance angle scale is located on the left side of the blade holder.
- Release the blade holder by turning the No. 4 Hexagon screw (→Fig. 28-1) counterclockwise. Select a clearance angle of 0°. To do so, align the number 0 with the index mark (→ Fig. 28-2) and tighten the Hexagon screw (→ Fig. 28-1). If the sectioning results are not satisfactory, increase the clearance angle in 1° increments until you achieve optimum results.

Notes:

- Settings of 2° - 5° (blade holder CE, CE-TC and premium blade holder) and 4°-6 °for the knife holder CN are well suited for most applications.

- The harder the specimen is, the larger the clearance angle must be chosen. However: The larger the clearance angle is, the larger the section buckling becomes.
- If the clearance angle is too small, sections that are too thick or too thin can result. Also consider that the position of the knife edge relative to the specimen can change when the clearance angle is adjusted. For this reason, **always place the specimen above the knife when adjusting the clearance angle**. Otherwise, the specimen could hit the knife while being raised. If necessary, move the specimen backwards to avoid collisions during further trimming.
- A clearance angle that is too steep or too shallow may lead to suboptimal sectioning results and could possibly damage the specimen. **We generally recommend using higher clearance angles for harder specimens and relatively lower clearance angles for softer ones.**

4.7 Check whether retraction is as wanted on or off (→ P. 38 – 6.3 Retraction).

4.8 **Sectioning and trimming:**

4.8.1 Select trimming thickness.

4.8.2 Adjust anti-roll device (→P. 49 – Adjusting the anti-roll guide system).

4.8.3 Trim using the handwheel (→ P. 58 – Trimming the specimen).

4.8.4 Select section thickness (→ P. 37 – 6.2.7 Setting the section thickness).

4.9 5. Carry out sectioning using the hand wheel, discard the first 2-3 sections.

4.10 6. Pick up the sections either by transferring onto a cold slide using a cold brush or a warm slide.

5.0 Cleanup and waste disposal

5.1 **Engage Brake:** 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.

5.3 **Specimen Removal:** Remove the specimen from the cryostat, e.g. fix it for later paraffin embedding.

5.4 **Thorough Cleaning:** Remove section waste using a cold brush. Empty the section waste tray. Clean the storage shelves and brush shelf. Alcohol-based disinfectants should be used for cleaning,

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

5.6 **Remove all specimen material from the cryostat.** Close the sliding window. Switch off the light. Do NOT switch off the instrument.

6.0 Maintenance

6.1 **Daily:** remove frozen section waste from the cryostat with a cold brush. Clean the cryostat and surrounding area after each use.

6.2 **Occasionally, or when required:** Lubricate moving parts according to the manufacturer's manual (plastic coupling, specimen cylinder, clamping piece, slot cover) to ensure smooth operation with a drop of cryo oil.

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 **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

7.5 **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

7.6 **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 chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.leicabiosystems.co

m/sites/default/files/media_product-download/2021-12/Leica_CM1860_eIFU_2v5S_US.pdf

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

8.3 Leica educational resources: https://www.leicabiosystems.com/us/knowledge-pathway/

8.4 Histological techniques: https://mmegias.webs.uvigo.es/02-english/6-tecnicas/1-introduccion.php

8.5 Cryosectioning techniques: https://pathology-innovations.squarespace.com/

SOP REVISION HISTORY

VERSION #	APPROVED	DETAILS
1	9/17/25	Created
2	9/22/25	Minor formatting edits
3		