

Instructions for Cryostat Slicing Leica Model CM 3050S

Set-up:

1. Make sure cryostat is on and cold (-15 to -20 degrees C). Place tissue to be sliced in chamber to bring it to temperature (tissue stored at -80 should be in the chamber for at least 30 minutes prior to slicing). Also place brushes, forceps, slide cases, etc. in the chamber. If you are not using a disposable blade, also put the microtome knife in place. Specimen disks (chucks) should be left at room temperature until you are ready to mount your tissue. Also leave slides at room temperature (at least until you have mounted and dried all of the desired sections on them).
2. Mount your tissue. First, place the specimen disk inside the chamber and apply a generous circle of O.C.T. (or other freezing mounting medium). When medium *begins* to freeze (starts to turn white), place tissue specimen in medium and freeze completely. You may add additional mounting medium around tissue for added security.
3. Once specimen is securely embedded in frozen medium, place the specimen disk in the specimen head, orient your tissue as desired, and tighten the knob (upper left) to secure. To adjust the plane of your specimen, release the lever on the right and use the knobs behind the head (to the left) to make the needed adjustments.

Note: For your safety, keep the microtome knife guarded at all times while you are working in the chamber! For disposable blades, use the anti-roll plate as your guard. For the reusable knife, use the knife guard.

Trimming:

1. Begin by putting the specimen head in "home" position (all the way back). Use the stage forward button to bring your specimen very close to the knife.
2. When you are ready to begin trimming your tissue, press the trim/section button. The trim function is on when the green light is lit. Use the + and - buttons to change your trimming thickness (5-150 um).
3. Use any of the motorized sectioning buttons and footswitch, or the manual handwheel to trim your tissue until you near the place where you will begin to collect tissue sections. As you are trimming, you may need to make further adjustments to the plane of your tissue.
4. When you are near the place you will collect tissue sections, TURN THE TRIM FUNCTION OFF by pressing the trim function button once (Note: the trim function will automatically turn off each time you lock the handwheel).

Note: if you are using an automatic slice setting (i.e. the footswitch), be sure the handle is in the center of the handwheel!

Collecting Tissue Specimens:

1. First, set your sectioning window. Use the handwheel to place the bottom of your specimen about 3 mm above the blade and press the Sectioning Window key (see

- Key Functions). Next, turning the wheel clockwise, place the top of your specimen about 3 mm below the blade and press the sectioning window key again.
2. Before collecting tissue sections, put the anti-roll plate in place and practice a few sections so that you can make the proper adjustments for smooth tissue sections. If sections are curling, turn the knob of the anti-roll plate clockwise. If sections are shredding, turn the knob counter-clockwise. Usually, only minute adjustments to the anti-roll plate are necessary to make a difference in tissue section quality. (For more tips on section quality, see “Troubleshooting”).
 3. When you have made all of the necessary adjustments and are at the place where you wish to begin collecting tissue sections: slice a section, carefully lift the anti-roll plate away, use a brush to position your section, and carefully collect the section on a room temperature slide.
 4. If you are refreezing your tissue sections, be sure to allow all sections to dry at room temperature before placing the slides in the cryostat chamber.

Clean-up:

1. When you are finished slicing, remove the remaining tissue specimen from the specimen head. If you are planning to discard the remainder of the specimen, put the specimen outside of the chamber until it begins to thaw, at which time you can easily remove it from the specimen disk. If you plan to reuse your specimen it can be left on the specimen disk, covered, and stored at -20 degrees C. However, because of the limited number of specimen disks, please do not use them for long-term storage (check the sign-out sheet to see if someone will be using the equipment after you). **DO NOT** leave specimens in the cryostat chamber overnight, as the cryostat will automatically defrost every 24 hours!
2. Use the large brush to sweep most of the discarded tissue sections into the drop pan below the head and empty the pan (use biohazard waste).
3. Use diluted detergent (e.g. Liquinox) and dH_2O to wash and rinse the drop pan, brushes, specimen disks and other instruments. Once dry, place them back in the chamber for the next person's use.
4. Use 100% EtOH to wipe out the cryostat chamber. **DO NOT** use water, as it will cause the chamber to become frosty.
5. Before you leave be sure the handwheel is locked, the light is turned off, the chamber is clean, and the surrounding area is clean.

THE KEY FUNCTIONS

| | |
|---|---|
|  | ON/OFF key for cryochamber illumination |
|  | To select the menu items for setting/changing instrument parameters (CT=chamber temp, OT=object temp) |
|  | In the status indication, pressing the arrow keys displays current parameters. In the menu, pressing the arrow keys changes the set values. New values are automatically stored once displayed. |
|  | Activates the manual defrost cycle (cryochamber). Key is functional when "SET TEMP..." is selected first. |
|  | Lock/unlock key: locks display to avoid accidental change of parameters; activates/deactivates a number of functions. |
|  | Sectioning mode 1 – Intermittent stroke: specimen head travels up and down as long as footswitch is depressed. |
|  | Sectioning mode 2 – Continuous stroke: specimen head travels up and down until it is stopped by depressing footswitch. |
|  | Sectioning mode 3 – Single stroke: specimen head carries out one sectioning stroke and then stops in the lower limit position. |
|  | Sectioning window key: use to set sectioning window as described in the slicing instructions. |
|  | Vmax key: while depressed, sectioning motion is carried out at maximum speed (210mm/s); sectioning window is inactive. |
|  | Emergency stop: sectioning is interrupted at once. |

Trimming/sectioning key: activate or deactivate trim function; use “+” and “-“ keys to select trimming/sectioning thickness.

Course feed fast backward: specimen head travels away from knife to the rear limit (“home”) position.

Coarse feed slow backward: specimen key travels backward as long as the key is held.

Course feed fast forward: specimen head travels forward as long as key is held (1000 $\mu\text{m/s}$)

Course feed slow forward: specimen head travels forward as long as key is held (500 $\mu\text{m/s}$)

RUN STOP/RUN ENABLE keys: pressing these keys together is the same as depressing the footswitch.

MAINTENANCE

1. The cryochamber must be cleaned after each use (swept out and wiped with 100% EtOH).
2. The black intake vents on each side of the cryostat must be vacuumed to remove dust when needed (task should be assigned to a specific person).
3. The cryostat should be thoroughly cleaned and dried approximately every 6 months (task should be assigned to a specific person). Consult instruction manual for details (p. 53).
4. Replacement fluorescent bulbs (13W) can be found at Home Depot or elsewhere. Consult the instruction manual (p. 56) for details.
5. The specimen head and levers should be lubricated (blue Leica oil) every 1-2 weeks (task should be assigned to a specific person, however, if you find the head needs oil, you may add 1 or 2 drops).

SPECIAL FEATURES

Some of the special features of the Leica CM3050S you may want to know about are:

1. **Hydraulic lift:** The cryostat may be moved up or down for more comfortable body posture. Use the up/down switch located on the right side of the cryostat toward the rear.
2. **Interchangeable knife holders:** You may use either disposable microtome knives or a regular knife. To change the knife holder, consult the knife holder instruction manual.
3. **Object Fast Freeze:** You have the option of fast freezing a specimen by using the 90° prism (see instruction manual p. 40)
4. **Heat extractor:** The stationary heat extractor, located on the quick-freeze shelf, can be used to speed the freezing process of a specimen (for details see instruction manual p. 39)
5. **Motorized sectioning:** You can use the footswitch (located to the right of the cryostat) for motorized sectioning of tissue. Refer to slicing instruction and key functions for details.

TIPS AND TROUBLESHOOTING

Error messages:

- If an error message is displayed (e.g., power failure, service, coarse feed malfunction, or temperature indication, consult manual p. 46 and call for technical service).
- If a red “STOP” appears in control panel 2, the emergency stop button has been accidentally activated. Unlock by using lock/unlock key.
- If the footswitch is not properly connected, cryostat will not run. Check the connection and connect properly if necessary.

Common tissue quality problems:

| Problem | Possible cause(s) |
|---|---|
| Sections smear | Specimen, knife, or anti-roll plate not cold enough |
| Sections splinter Sections not properly flattened | Specimen too cold; static electricity or draft; specimen not cold enough; large surface specimen; anti-roll plate poorly adjusted or poorly aligned with knife; wrong clearance angle selected; knife blunt or damaged. |
| Sections not properly flattened despite correct temperature and correctly aligned anti-roll plate | Knife or anti-roll plate dirty; edge of anti-roll plate damaged; blunt knife |
| Sections curl on anti-roll plate | Anti-roll plate does not protrude far enough beyond the knife edge |
| Scraping noise during sectioning | Anti-roll plate protrudes too far |
| Chatter marks form during sectioning | Specimen insufficiently frozen to disk; specimen disk not clamped tightly enough; specimen holder ball joint not clamped; knife not clamped tightly; selected section thickness too thick; specimen hard and inhomogeneous; blunt knife; wrong knife for specimen type; wrong clearance angle |
| Tissue sticks to anti-roll plate | Plate too warm or incorrectly adjusted; static electricity; fat on corner/edge of plate; knife rusty |
| Sections curl up as anti-roll plate is lifted | Static electricity or draft; anti-roll plate is too warm |
| Sections tear | Temperature too low; knife blunt/dirty; upper edge of anti-roll plate damaged; hard particles in the tissue; rear knife face dirty |

For other technical problems and causes not listed here, refer to pp. 47-50 of the instruction manual.

TIPS AND TROUBLESHOOTING CONT'D

Other hints and tips:

- If you find your new disposable blade is too sharp (i.e., tissue is shredding despite all other appropriate adjustments), you may use a cork to dull the blade.
- If you have problems with static (curling tissues), run a static sheet or pouch across platform and anti-roll plate.
- If the blade becomes dull, you can move the blade head to either side to use a different part of the blade for slicing.
- Although somewhat variable, the typical clearance angle to use with the disposable blades is 4-6°. The angle for the steel knife will vary depending on the condition of the knife.

Table of Contents

| | |
|-------------------------------|----|
| Special Features..... | 1 |
| Instrument Properties..... | 2 |
| Key Functions..... | 4 |
| Safety Information..... | 6 |
| Instructions for Slicing..... | 7 |
| Tips and Troubleshooting..... | 9 |
| Maintenance..... | 11 |

Cryostat Slicing for Dummies

*A Quick Reference to Slicing on
the Leica CM3050S*

Safety Information
(MUST READ BEFORE OPERATING)

Safety Devices:

- Handwheel lock
- Handwheel grip centering (for motorized sectioning)
- Emergency stop function
- Knife holder equipped with knife guard

To lock the handwheel:

- Rotate handwheel until grip is in upper or lower position (upper locking position is marked by a black dot).
- To lock, press pin to the right into position.

Note: the sectioning motor is now blocked and “LOCKED” appears on the screen.

To center the handwheel grip:

- Pull outwards and pivot into center of handwheel.
- When released, the grip locks into position.

Note: the grip should always be in the center position while using motorized sectioning.

Activating and deactivating the emergency stop function:

- To activate, press emergency stop button or step on footswitch forcefully (“STOP” (red) lights up).
- To deactivate, rotate emergency stop button in direction of arrow.

Note: if emergency stop has been activated by the footswitch, deactivation is not necessary.

Always cover the cutting edge with the knife guard (anti-roll plate, in the case of disposable blades):

- Prior to doing any work on knife and /or specimen.
- Prior to exchanging specimens.
- During work breaks.

Handling microtome knives/blades:

- Cutting edges are extremely sharp! Handle with utmost care.
- Never leaves knives/blades in unprotected places.
- Never place knife, no matter where, with cutting edge facing upwards.
- Never try to catch a falling knife.
- Always insert specimen before inserting the knife.

During motorized sectioning:

- Never interrupt sectioning by setting the sliding potentiometer to zero speed! This will not switch the sectioning function off, so if speed is moved again, sectioning will resume immediately.