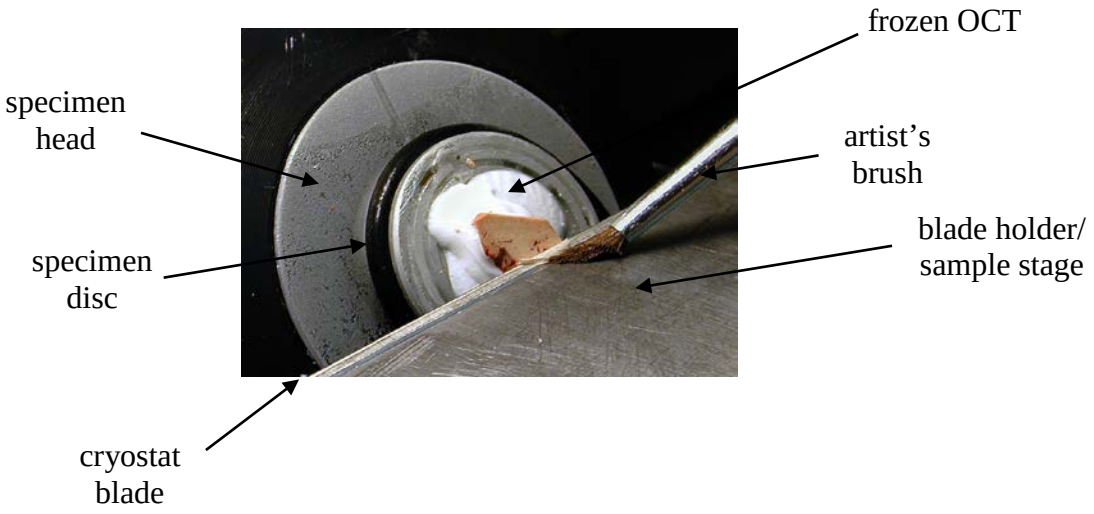
Leica CM 3050S and Leica CM 1900 Cryostats: Cutting Tissue



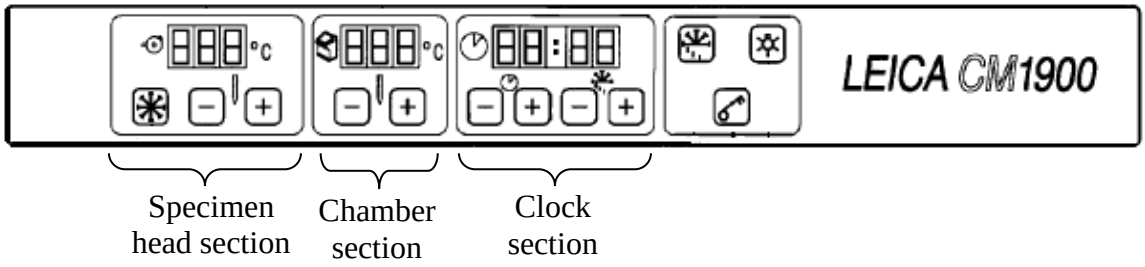
Equipment and Materials:

- CM 3050S and/or CM 1900 cryostat by Leica
- Ethanol (EtOH), 100%, as disinfectant
- Embedding media: e.g., OCT, optimal cutting temperature polymer, Leica #0201 08926, or Fisherbrand embedding media #SH75-125D
- Cryostat blades, disposable, high profile: e.g., Tissue Tek Accu-Edge (Fisher #NC9527669)
- Sample holders (specimen discs): e.g., Leica 30 mm (#0370 08587)
- Artist's paintbrushes: various, available at Vanderbilt bookstore
- Glass slides: e.g., Superfrost plus slides (Fisher #12-550-15)

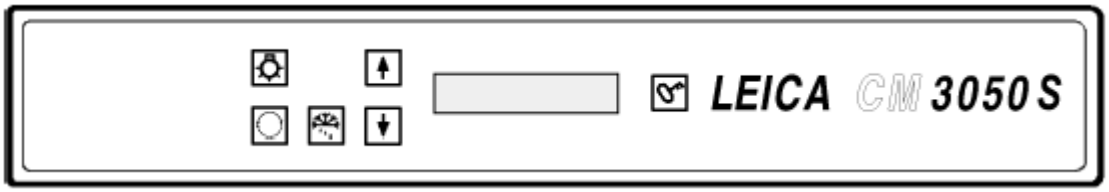
Procedure:

Instrument panels

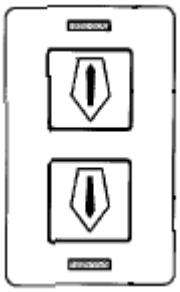
CM 1900 – control panel 1 (front)



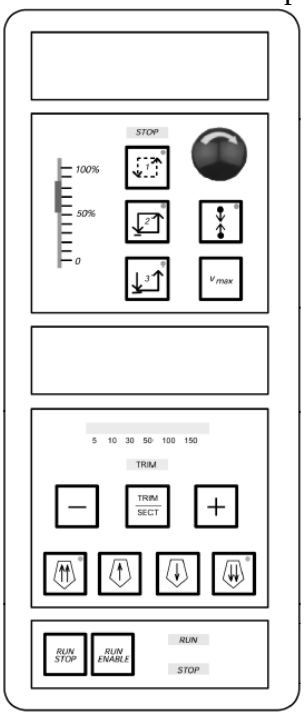
CM 3050S – control panel 1 (front)



CM 1900 – control panel 2 (left side)



CM 3050S – control panel 2 (left side)



Section A: These controls refer to the motorized sectioning options available on the 3050S instrument, but will not be discussed in this protocol.

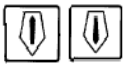
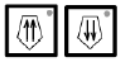
Section B: These controls allow the user to select trim mode, adjust trim thickness, and move the specimen head forward or backwards (towards or away from the knife, respectively).

Section C: These controls refer to the motorized sectioning options available on the 3050S instrument but will not be discussed in this protocol.

Overview of symbols:

Both Lock key: Locks/unlocks instrument panel

Both Light key: Turns light in cryochamber on/off

	CM 3050S	Menu key: Used to select menu items for adjusting parameters
	CM 3050S	Selection keys: Used to adjust set values and display current values
	Both	Selection keys: Used to adjust set values
	CM 3050S	Trim key: Activates trim mode
	Both	Coarse feed keys (slow): Used to move specimen head forward or back
	CM 3050S	Coarse feed keys (fast): Used to move specimen head forward or back

Preparing the cryostat and samples for sectioning

Make sure the cryostat is on and cool.

1. Wipe down the cryochamber, brushes, and forceps with EtOH.

   Note: The order of the next 7 steps is not important, but for safety, the blade should be inserted just before use.

2. Place frozen tissues in the cryochamber to equilibrate (specimens stored at -80°C need to warm up to ~-20°C for optimal sectioning; the time required for this varies with the size of the sample).

3. Unlock the chamber by pressing the key button and holding it down for ~5 sec.

   CM 1900: Display is locked when LED's between the hour and minute numbers on the time panel are OFF. Unlocking the display turns the LED's on.

   CM 3050S: Display is locked when the background of the display panel is DARK. The display background lights up when unlocked.

4. Set the chamber temperature to -20°C +/- 5°C. The final temperature required will depend on many factors (including the type of sample being analyzed, the room temperature, etc.)

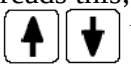
   CM 1900: Adjust the chamber temperature by using the  keys on the chamber section of the front control panel.

   CM 3050S: Press the menu key  to select the chamber temperature menu (the display will read "Set temp CT") and then use the arrow keys  to adjust the temperature.

5. Turn on and set the specimen head temperature (object temperature) to -20°C +/- 5°C. The final temperature required will depend primarily on the type of tissue being sectioned – fattier tissues typically require colder temperatures. The object temperature should generally be slightly warmer than the chamber temperature, to prevent frost buildup and to ensure the sections don't stick/melt to the sample stage.

CM 1900: Depress either selection key  on the specimen head section of the front control panel (to show the current set temperature). WHILE THE SET TEMPERATURE IS DISPLAYED, depress the lock key  . The display will show the temperature is turned on by turning off the blinking red LED's in the specimen head display panel.

Adjust the specimen head temperature by using the  keys on the specimen head section of the front control panel.

CM 3050S: Press the menu key  to select the object temperature menu (the display will read "Set temp OT"). While the display reads this, briefly press the lock key  to turn on the refrigeration. Use the arrow keys  to adjust the temperature.

6. Turn on the chamber light by pressing the light key on the control panel. 

7. For greater ease of cleanup, place Kim-wipes on the waste tray to collect waste sections.

8. Place sample collection media (e.g., MALDI plates, glass slides) into the cryochamber to cool down.

9. Verify that the handwheel is locked. Insert a disposable blade into the knife holder. NOTE: These blades are coated with an oil to make them slide out of their dispenser easily. This should be wiped off with EtOH prior to insertion into the knife holder. USE CAUTION AS KNIVES ARE SHARP!! Lock blade in place by turning the black handle on the right side of the specimen stage clockwise ~3/4 turn.

10. Mounting a sample on the specimen disc

11. Place a small amount of OCT on a room temperature specimen disc. For most tissues, use enough to cover about a dime-sized area of the center of the disc; use more or less as required for larger or smaller tissues, respectively. For tissues to be analyzed by MALDI, take care not to get OCT on the bulk of the tissue being sectioned, as it will cause ion suppression.

12. Place a frozen specimen firmly on the OCT-covered disc, and place it into one of the freezing stations to freeze.

13. Once frozen (the OCT turns from shiny and clear to matte and white), place the specimen disc into the specimen head. Rotate the disc until the desired surface is facing the blade and tighten the screw.

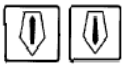
14. The angle of the sample on the specimen head may be adjusted to obtain the desired sectioning plane.

    CM 1900: Turn the clamping lever on the specimen head counterclockwise to loosen the specimen head. Adjust the head to the desired angle, and retighten the clamping lever.

    CM 3050S: Turn the clamping lever on the specimen head counterclockwise (up) to loosen the specimen head. Turn the two adjustment screws (one for left-right and one for top-

bottom adjustment) as desired until specimen is at the desired angle, and retighten the clamping lever (push it down).

Trimming the tissue

1. Unlock the handwheel and slowly lower the specimen head to evaluate how close/far away the edge of the specimen is to the blade.
2. If there is no space between them and the tissue overhangs the blade, either move the blade holder (sample stage) away from the specimen head and/or move the specimen head back away from the blade. [The specimen head should be kept in the home position (farthest away from the blade) when the cryostat is not in use.]
3. If the distance is roughly >1 cm, move the blade holder closer to the specimen head and continue with 5.4.2.
4. If the distance is <1 cm, adjust the position of the specimen head so that it is almost in contact with the blade.
5. CM 1900: Use the  keys on the left control panel to move the specimen
6. Head back or forward, respectively. Note, pushing the back key  will move the
7. Specimen head continuously back. Push the key again to stop it. For forward movement, the specimen head will move as long as the forward key is depressed and it will stop automatically when the key is released.
8. CM 3050S: There are two speeds for moving the specimen head on this instrument. The
9. Fast coarse feed backward key  will move the specimen head continuously back to its home position. The slow coarse feed keys act the same as on the CM 1900.
10. Set the desired trimming thickness (µm).
11. CM 1900: Use the black knob in the cryostat chamber to set the desired thickness. 
12. CM 3050S: Depress the trim key  on the left instrument panel, and then use the 
13. Keys to select section thickness. Thickness will be displayed on the LED display on the left control panel.
14. Trim the tissue by turning the handwheel to precisely move the specimen head forward so that the tissue contacts the blade. Continue trimming until the desired cutting plane has been reached. Use the artist's brushes to sweep discarded sections on to the waste tray and off of the tissue block.
15. Set the desired section thickness (µm). Typical values are ~5-20 µm, depending on tissue and application.
16. CM 1900: Use the black knob in the cryostat chamber to set the desired thickness. 

17. CM 3050S: Turn trimming off by pressing the trim key 

Press the  keys to adjust section thickness. Thickness will be displayed on the front instrument panel display.

18. Cut and discard ~5 sections at the desired section thickness (and anytime after changing the desired thickness) to ensure a consistent section thickness is obtained.

Sectioning the tissue

1. Place the anti-roll plate in position over the blade. There should be minimal space visible between the glass plate and the blade.

2. Cut a section. It should smoothly slide between the anti-roll plate and the blade. If not, the anti-roll plate may need to be adjusted. Use the screw on the front of the anti-roll plate to move the plate up or back as necessary. Once adjusted, the anti-roll plate should not need major adjustments for subsequent sections.

   Troubleshooting: Besides placement of the anti-roll plate, many other factors can affect the quality of the tissue sections. The troubleshooting section of the Leica manual accompanies this protocol (section 8.0) and should be referred to when sections are not optimal.

   A few additional tips:

   A. It may be useful to optimize the anti-roll plate on frozen embedding media (OCT), especially when the tissue sample is extremely small and/or valuable.

   B. If one suspects that the tissue is too cold, the tissue can be quickly warmed up by placing a gloved finger on the tissue block for a few seconds and taking a few new sections. If the sections look better, adjust the object temperature to warm the tissue up and continue sectioning.

3. Fold back the anti-roll plate away from the blade. The section should remain on the plate behind the blade. It may need to be gently dislodged from the edge of the blade with an artist's brush.

Mounting the tissue on a plate or slide

1. Move a pre-cooled plate or slide to the sectioning plate.

2. Using an artist's brush, gently guide the section on top of the plate. This may be done by "pushing" the section onto the plate, placing the brush underneath the section and carrying it to the plate, or by "sticking" the brush gently to the top of the tissue section and carrying it to the plate.

3. Care should be taken to MINIMALLY manipulate the tissue section at this point. Excessive flattening of the tissue or adjusting of its position can lead to tissue tearing or warming so that it becomes sticky and its shape is deformed.

4. Once the tissue is on top of the cooled plate and in the desired position, place a finger or two directly underneath the tissue on the underside of the plate to thaw-mount the

tissue section onto the plate. Keep warming the section until it is entirely dry (~30 sec-1 min).

5. Note: Sections taken for staining on glass slides should be warmed just until they have thawed onto the glass slide – overdrying may disturb the cells for a pathological review of the stained section.

6. Return the plate to the cryochamber to re-cool so it is ready for the next section. Place the plate vertically in a slide box or on several sheets of cheesecloth for added protection against freezer burn (i.e., don't place the plate directly in contact with the cold metal of the cryochamber.)

7. The tissue block may be removed from the specimen disc when finished by removing the disc from the specimen head and using a razor blade to dislodge the tissue and OCT from the specimen disc.

8. Carefully wipe the blade with EtOH and/or move the cutting surface to a new location to prevent cross contamination between tissues.

9. Repeat sections 5.3-5.6 for each tissue block that requires sectioning.

Clean-up/shutdown procedures

1. Lock the handwheel.
2. Remove the disposable blade and discard in sharps container.
    a. Note: The order of the next 8 steps is not important.
    b. Home the specimen head.
    c. CM 1900: Press the  key on the left control panel once. The specimen head will travel to its home position, and the light above the key will stay lit when home. Press the  key until the light turns off.
    d. CM 3050S: Press the  key on the left control panel once. The specimen head will travel to its home position, and the light above the key will start flashing. Press the  key until the light stops flashing (and turns off).
3. Clean out tissue waste from the cryochamber and dispose of it in a biohazard bag.
4. Wipe down the cryochamber and all accessories (brushes, forceps, specimen discs) with EtOH.
5. Wipe down the handwheel and the control panels with EtOH.
6. Turn the chamber light off by pushing the light key.
7. Turn the specimen head (object) temperature off.
    a. CM 1900: Depress either  key on the specimen head section of the front control panel (to show the current set temperature). WHILE THE SET

TEMPERATURE IS DISPLAYED, depress the lock key. The display will show the temperature is off by blinking red LED's in the specimen head display panel.

b. CM 3050S: Press the menu key  to select the object temperature menu (the display will read "Set temp OT"). While the display reads this, briefly press the lock key  to turn off the refrigeration. The display will not show a temperature next to OT when it has been turned off.

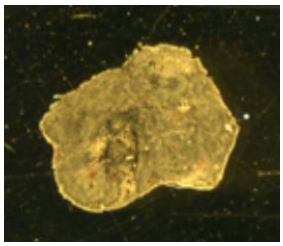
8. Leave the chamber temperature at -20°C when finished so it is ready for the next user.

CM 1900: Replace the white plastic cover over the freezing stations to prevent frost buildup.

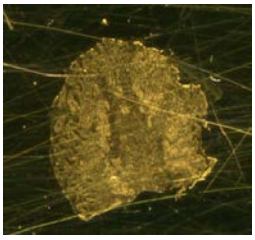
Shut the top cover.

Expected Outcome:

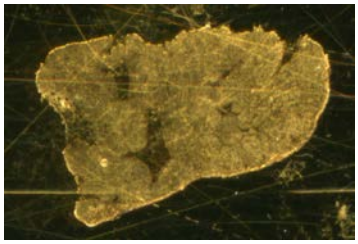
High-quality sections should show no obvious deformations or tears, and should accurately reflect the shape of the bulk tissue.



mouse tumor tissue



human gastric tumor



human heart tissue

References:

Instrument manuals.

Schwartz, S. A., Reyzer, M. L., and Caprioli, R. M., “Direct tissue analysis using matrix-assisted laser desorption/ionization mass spectrometry: practical aspects of sample preparation”, *J. Mass Spectrom.*, **2003**, *38*, 699-708.

Troubleshooting (copied from Leica instrument manual)

6.2 Potential problems - causes and remedies

Problem	Causes	Remedies
Frost on chamber walls and microtome	- Cryostat is exposed to draft (open windows, doors, air-conditioning). - Sliding window has been open for a long period of time at a very low chamber temperature. - Frost build-up caused by breathing into the cryochamber.	- Eliminate draft sources or change place of installation of the cryostat. - Close sliding window. - Consider wearing a mask.
Sections smear	- Specimen not cold enough. - Knife and/or anti-roll plate not yet cold enough - sections melt.	- Select lower temperature. - Wait until knife and/or anti-roll plate have reached chamber temperature.
Sections splinter Sections not properly flattened	- Specimen too cold. - Static electricity / draft. - Specimen not cold enough. - Large surface specimen. - Anti-roll plate poorly adjusted. - Anti-roll plate poorly aligned relative to knife edge. - Wrong clearance angle selected. - Knife blunt or damaged.	- Select higher temperature. - Eliminate cause. - Select lower temperature. - Trim specimen parallel; increase section thickness. - Readjust anti-roll plate. - Align correctly. - Set correct clearance angle. - Use different part of knife or replace knife.
Sections not properly flattened despite correct temperature and correctly aligned anti-roll plate	- Knife and/or anti-roll plate dirty. - Edge of anti-roll plate damaged. - Blunt knife.	- Clean with dry cloth or brush. - Replace anti-roll plate. - Use different part of knife or replace knife.

6. Troubleshooting, applications tips

Problem	Causes	Remedies
Sections curl on anti-roll plate.	- Anti-roll plate does not protrude far enough beyond the knife edge.	- Readjust correctly.
Scraping noise during sectioning stroke and specimen return stroke.	- Anti-roll plate protrudes too far beyond the knife edge and is scraping against the specimen.	- Readjust correctly.
Ondulated sections.	- Knife damaged. - Edge of anti-roll plate damaged.	- Use different part of the knife or replace knife. - Replace anti-roll plate.
Chatter marks form during sectioning.	- Specimen insufficiently frozen onto specimen disc. - Specimen disc not clamped tightly enough. - Specimen holder ball joint not clamped. - Knife not clamped tightly enough. - Selected section thickness too thick - specimen has come off the specimen disc. - Specimen very hard and inhomogeneous. - Blunt knife. - Knife profile inappropriate for the type of specimen to be sectioned. - Wrong clearance angle.	- Refreeze specimen onto disc. - Check disc clamping. - Check ball joint clamping. - Check knife clamping. - Refreeze specimen onto disc. - Increase section thickness; if possible, reduce specimen surface. - Use different part of knife or replace knife. - Use knife with a different profile. - Readjust clearance angle setting.
Condensation forms on anti-roll plate and knife during cleaning.	- Brush, forceps, cloth or other cleaning item too warm.	- Accessories and cleaning material to be used pre-cooled only. - Store on storage shelves inside the cryochamber.

6. Troubleshooting, applications tips

Problem	Causes	Remedies
Anti-roll plate damaged after adjustment.	- Anti-roll plate protrudes too far beyond the knife edge. Adjustment was done in the direction of the knife.	- Replace anti-roll plate; in future, lift away from the knife when aligning. - Handle anti-roll plate more carefully.
Thick/thin sections.	- Wrong temperature for the tissue to be sectioned.	- Select correct temperature. - Wait until correct temperature has been reached.
	- Knife profile inappropriate for the specimen to be sectioned.	- Use knife with different profile (c or d) or possible switch to disposable blade system.
	- Ice build-up on the knife back.	- Remove ice.
	- Handwheel rotation not uniform or inappropriate rotation speed.	- Adapt speed.
	- Knife not clamped tightly enough.	- Check knife clamping.
	- Specimen disc not clamped correctly.	- Check disc clamping.
	- Embedding medium poured onto cold specimen disc; specimen comes off the disc once frozen.	- Place embedding medium on luke-warm specimen disc, inside the cryochamber place the specimen onto the disc and freeze in quick-freeze shelf.
	- Blunt knife.	- Use different part of knife or replace knife.
	- Incorrect clearance angle.	- Set correct clearance angle.
	- Specimen dried out.	- Prepare new specimen.
Tissue sticks to the anti-roll plate.	- Anti-roll plate too warm or incorrectly adjusted.	- Cool or readjust anti-roll plate.
	- Static electricity.	- Eliminate cause.
	- Fat on corner or edge of anti-roll plate.	- Remove fat (alcohol).
	- Knife rusty.	- Remove rust.
Flattened sections curl up as soon as anti-roll plate is lifted from the knife.	- Static electricity or draft.	- Eliminate cause.
	- Anti-roll plate too warm.	- Cool anti-roll plate.

6. Troubleshooting, applications tips

Problem	Causes	Remedies
Sections tear.	- Temperature too low for the tissue to be sectioned.	- Select higher temperature and wait for the new temperature to be reached.
	- Knife blunt, dirty, dusty, frosted or rusty.	- Eliminate cause (--> see details on individual problems).
	- Upper edge of anti-roll plate damaged.	- Exchange anti-roll plate.
	- Hard particles in the tissue.	- If application permits, section at lower plane.
	- Rear face of knife dirty.	- Clean.
Inconsistent or insufficient specimen feed.	- Microtome was not completely dry when refrigeration was switched on; ice build-up inside the micrometer mechanism.	- Remove microtome from chamber and dry - possibly with hairdryer (hairdryer only to be used outside the cryochamber!) or at ambient temperature.
	- Microtome defective.	- Call Technical Service.
Specimen disc cannot be removed.	- Specimen disc stuck to quick-freeze shelf or specimen head due to moisture on underside of specimen disc.	- Apply strong alcohol to contact point or heat the specimen head.
Cryostat inoperational.	- Mains plug not properly connected.	- Check mains plug connection.
	- Automatic cut-out has been triggered.	- Reconnect automatic cut-out (switch instrument back on).
Sectioning motor inoperational.	- Footswitch not connected.	- Connect footswitch.
	- Automatic cut-out of sectioning motor has been triggered.	- Reconnect automatic cut-out (push inwards until it locks in place).
	- Motor defective.	- Call Technical Service.
No or insufficient refrigerating performance.	- Stopper not placed properly into drain in bottom of cryochamber.	- Close drain with stopper.
	- Compressor defective.	- Call Technical Service.
	- Leak in cooling system.	- Call Technical Service.