

# University of Toledo's Leica CM1950 Cryostat

For a good overview of using the instrument:

[youtube.com/watch?v=ImHYzGO6V5c&ab\\_channel=ManchesterBioimaging](https://www.youtube.com/watch?v=ImHYzGO6V5c&ab_channel=ManchesterBioimaging)

Note – our instrument does NOT come equipped with vacuum or automatic trimming/sectioning as seen in the video; all other functionality is the same

## Operation

### General notes

- Always wear gloves
- Labs should be providing their own blades and removing them when done
- Labs should be providing their own OCT
- ALWAYS clean up thoroughly, following instructions
- Ensure the handwheel is locked at the apex unless actively sectioning or trimming
  - o The handwheel moves the object head up and down against the back plate
    - If it's not locked, the object head wants to move down b/c gravity and you would need to put fingers too close to the blade while mounting the sample
- Dry all parts that are taken out of chamber when putting them back in (use 200 proof EtOH)
- **If you see a glowing red symbol next to the clock, please notify ICenter staff.**

### Cooling

- Cryochamber can take quite a long time to cool down, especially relative to the object head which cools quickly
- Do not turn on unless the cryochamber is completely dry, otherwise you will have frost formation
- User manual provides temperatures to use for a variety of common tissue samples
- Peltier shelf is used for fast cooling; cannot adjust temperature
- Snowflake button on object head = set to minimum temperature

### Manual defrost

- If frost is coating cryochamber walls, Peltier/cooling shelf, or object head, should defrost
- If large chunks of ice are present, let ICenter staff know, longer defrosts may need to be performed
- Press melting snowflake button (no need to hold down), you will hear the instrument beep
- Click on a button (either + or - ) for the section you want to defrost, other section temperature reading will go dark
- 12 minute cycle
- Wipe away melted water gently with gauze or kimwipe and then wipe with 200 proof EtOH to prevent re-freezing of defrosted area(s)
- Key button on control panel – likely will not need to use
  - o Hold down for 5 seconds to lock entire keyboard (you'll see the LEDs in the clock go out)

- Hold down briefly, then “-“ key in specimen head control panel field, switches OFF the specimen head; hold briefly then “+“ key in specimen head control panel fields switches the specimen head back ON

### **Automatic defrosting**

- 1x/day – set by using the +/- buttons under the melting snowflake, under the clock
  - The timing is based on what the clock shows
- Defrosts the top of the chamber, not the whole chamber

### **Changing blade**

- Pull up on handle next to blade (on right side) to prop used blade loose
- Use blade ejector button on left side (pushes in towards blade and blade should slide out); can also use magnetic brush
- Slide new blade in and then pull handle other way to lock in place
  - secure = handle pointing away from user
  - loose = handle pointing towards user
- New blades should be degreased with acetone or alcohol before use
- Blades should be pre-cooled before using for sectioning/trimming

### **Sample Preparation**

- Trim front of block using razor
- Squeeze OCT onto pre-cooled specimen disc inside cryostat
  - Specimen disk is also sometimes called a “chuck”
  - OCT = optimal cutting temperature compound – used to embed tissue samples prior to frozen sectioning on a microtome-cryostat; mounts slices (sections) of a sample onto slides for analysis
- Place specimen block in OCT
  - When dealing with really small specimens, you can place a piece of cork soaked in water on top of the OCT to lift it up away from the aluminum specimen head, then put some more OCT on top of that and then mount the sample on top (helps protect the aluminum head AND the razor from damaging)
  - Ideally specimen should be snap frozen
- Can use heat sink to help with rapid specimen freezing
- Let OCT freeze before proceeding by sitting it in the Peltier position on the freeze shelf
  - Wait until the specimen is completely frozen; be aware that if they may become too cold from the Peltier freezing and can split apart during sectioning, so time is needed for specimens to acclimate

### **Cutting specimens**

- Double arrows = moves continuously (rough adjustment)
- Single arrows = moves only when pressed (adjusts in microns)
- trim = trimming; sect = sectioning
- Change thickness in microns using +/- buttons
  - Routinely can use 10 microns sectioning, 30 microns trimming

- Flip open blade guard and the anti-roll plate (glass plate sitting on top of cold plate)
- Position sample so that bottom of block is just above the blade
  - o Allows you to gauge distance between block and blade, then advance the object mount
- To pass sample over the blade is one complete turn of the handwheel
- Then adjust the position of the object head
  - o You may want to trim off a bit of the outer layer of specimen before taking the sections that you'll use for analysis
- Replace the anti-roll plate – if the object head is too far forward, it can push it upwards
  - o In a cutting (down) stroke, this can damage the block and the anti-roll plate
- To adjust the anti-roll plate, there's screw on the back of the plate (closest to the user)
  - o Check distance on an up-stroke (less likely to damage instrument)
  - o Need the anti-roll plate far enough back so that the specimen is cut by the blade, but far enough forward that the sample is cut off in a flat section down the plate

### **Mounting sample on slide**

- Use a paint brush to brush the slice of sample flat
  - o Safest to do this towards the bottom of the cold plate
- Quickly pick it up with slide by placing slide face down on top of sample and then picking up again
- Brush off frost print on cold plate
- Alternate method – if specimen is difficult to pick up, freeze the slide at the bottom of the cold plate, then use the brushes to pull/slide the sample on top of the slide, then pick up the slide and put your finger behind the sample so it unfreezes and drops into place on the slide

### **Safely detaching specimen**

- Use a cold sink placed behind the specimen disk
- Once the sample is warm enough, use a blunt edge to pick off the sample
  - o If any force at all is needed, then it's not warm enough
- Place unmounted sample upside down on cold plate for it to re-freeze

### **Cleaning up**

- Spray and wipe down outside touch points
  - o Only used alcohol based cleaners or those recommended in the manual, no organic solvents or anything super harsh
- Start UVC disinfection cycle – must be performed for samples containing diseases
  - o When pressed you see the lamp turn on (blueish color/UV)
  - o Only can operate with sliding door closed – will shut off if door is opened

### **Misc. Notes**

- Good monolayer = 5 micrometers
- If platform is shaking, there's an shaft that needs to be cleaned