Standard operating procedure for using the Microm HM 525 CRYOSTAT

All researchers must be properly trained before using the cryostat. Any questions please ask Professor Iain McGregor.

Safety Device
- This cryostat is equipped with a locking hand wheel and blade guard.
- The wheel must be locked when changing a blade or specimen.
- When changing a specimen, the blade guard must also be in place.
- Both should be in place during breaks or when the cryostat is not in use.
- Lab coat, gloves and eye protection must be worn at all times.
- Liquids must never be placed inside the cryostat.
- The cryostat undergoes an automatic thaw cycle every night. Do not leave specimens inside the cryostat overnight.

Blades
- The cutting edge of the blade is extremely sharp and can cause severe injury.
- One should never leave blades lying around on the bench or in the cryostat.
- One should never clean the cryostat without the blade guard down.
- The blades used with this cryostat are disposable and should be placed in a sharps container immediately after being removed.
- Changing the blades:
  a. Lock the wheel
  b. Put the blade guard down
  c. Pull the lever on the left of the blade stage to release the blade.
  d. While doing this, be mindful that pulling this lever back also pulls the blade guard back as well – the blade becomes exposed and this can be a cutting hazard
  e. Using metal forceps, free the blade from the blade stage. Do not use your fingers!
  f. Using the forceps, place the used blade in the sharps container.
  g. Using the forceps, take the new blade and carefully insert it into the groove in the blade stage
  h. Using the forceps or the wooden handle of the brushes, slide the blade within the groove so that it is positioned properly under the specimen mount
  i. Push the lever on the left of the blade stage to lock the blade into position.
- Moving the position of the blade to place a sharper position under the sample:
  a. Lock the wheel
  b. Put the blade guard down
  c. Pull the lever on the left of the blade stage to release the blade.
d. While doing this, be mindful that pulling this lever back also pulls the blade guard back as well – the blade becomes exposed and this can be a cutting hazard

e. Using the forceps or the wooden handle of the brushes, slide the blade within the groove so that it is positioned properly under the specimen mount

f. Push the lever on the left of the blade stage to lock the blade into position.

**Tools**
- Only tools and brushes already placed in the cryostat may be used because they are cold.
- Using a tool or brush that is at room temperature will melt your tissue.
- Razor blades, scalpel blades or other sharps should never be stored in the cryostat.

**Procedure:**

**Freezing of Tissue**
1. Make sure the hand wheel is locked in place and the blade guard is in position before you start!!!!
2. Be sure the temperature of the cryostat is suitable for the work you are doing. Fixed brains may require a different temperature setting than fresh brains or stained brains (for example most fixed brains can be sliced at -21°C while golgi treated brains require the temperature to be -26°C). The temperature can be adjusted by using the up, down buttons.
3. Double click the “down” button to turn on the freezing platform. A “P” appears on the left hand side of the temperature display when the cycle is running. The freezing cycle lasts for approximately 10 min. Once it has finished its cycle the P on the display with disappear and you can double click the button to start it again.
4. Turn on the cryostat light by pressing the button with a light bulb on it.
5. Apply a layer of OCT (optimal cutting temperature) medium to a chuck. Try to be clean and not spread OCT medium all over the cryostat, it’s very difficult to clean up!
6. Place the chuck on the freezing platform. Within a few seconds it should begin to solidify and turn white.
7. Once the OCT medium has turned white place your specimen on top and cover with a layer of OCT medium. You may remove the chuck from the freezing platform to do this, just be sure to be quick! Place it back on the freezing platform. Freezing time will vary depending on the size of your sample!

**Slicing**
1. Ensure that the hand wheel is locked and the blade guard is in position.
2. Clamp the chuck into the specimen holder. You can change the angle of the specimen by adjusting the holder.
3. Select your desired slicing thickness using the arrows in the “section thickness” part of the display.
4. Move the blade stage as close to the specimen as possible. Be careful not to lift the blade guard.
5. Once in position, lift the anti-roll bar up and flip the blade guard back, gently place the anti-roll bar back into position. CAUTION: the blade is now exposed! Be EXTREMELY careful when working in the cryostat.
6. Unlock the hand wheel.
7. Rotate the wheel clockwise at a uniform speed to begin slicing.
8. When changing a specimen, be sure to put the blade guard down and lock the hand wheel.

Cleaning up
1. Lock the hand wheel
2. Position the blade guard
3. Remove the specimen from the holder.
4. Clean section waste from trays. All waste from the cryostat must be placed in the yellow clinical waste bin outside in the courtyard.
5. Wipe trays with 70% ethanol.
6. Carefully clean the stage and antiroll bar using a kimwipe and 70% ethanol.
7. Specimen chucks can be cleaned by running under warm water and scrubbing with a brush. Before placing back in the cryostat make sure they are completely dry!
8. Turn off the light.

If you spill something in the cryostat or experience other issues (e.g. a chipped or broken blade you aren’t sure how to remove) please inform Professor Iain McGregor immediately.