

Operating Procedure for Leica UCT Ultramicrotome (room temperature / conventional sectioning)

Ultramicrotomy

An ultramicrotome is used to produce ultra-thin sections (60-200 nm thick) for the purposes of imaging/analysis in the transmission electron microscope. Conceptually, the process is similar to a deli-meat slicer in that you pass a large block of material against a knife, incrementing the block slightly with each progressive cycle. Conventional room temperature sectioning is considered 'wet', sections are floated atop a reservoir of water prior to transfer onto a TEM grid. Whereas cryo-sectioning is 'dry', carefully transferring the section ribbon onto the grid and adhering with electrostatics.

Generally, the process of room temperature ultramicrotomy can be broken down into the following steps:

- Dehydration/embedding sample into a resin block
- Trimming the resin block to form a pyramid for cutting
- Cutting ultra-thin sections
- Transfer sections to a TEM grid

DIRECTIONS FOR USE

NOTE: instructions here assume you have your sample already embedded in a polymerized resin block

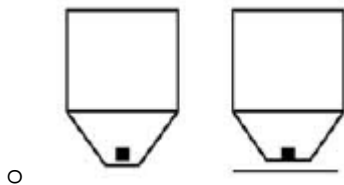


Start-up:

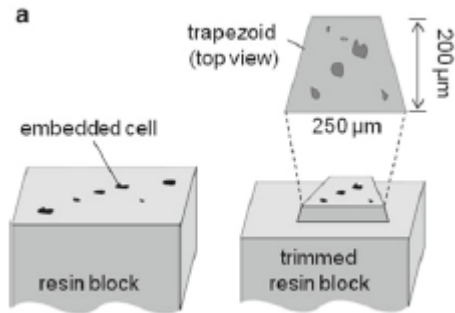
- Log into the ultramicrotome through the login computer
- Turn on switch in back of the controller unit
- Activate the above and below illumination lights

Trimming the resin block:

- Resin containing the sample of interest must be trimmed away (either with a straight-edge razor blade by hand or in a semi-automated fashion with a diamond trimming knife) to produce a pyramid-shaped block with a flat surface area for specimen sectioning.
 - Bulk resin removal: Cut the surface down, removing resin, until the specimen is reached in the resin block.



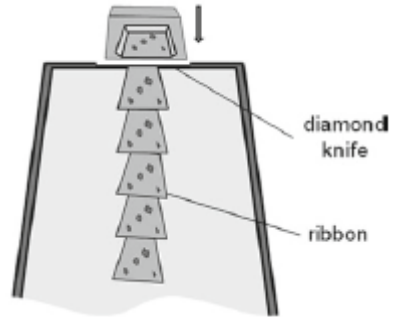
- Mount sample on the stage (so that the newly exposed specimen in the block can be seen through the binoculars).
- Using a razor, trim the exposed resin block to form a pyramid.



- Accomplish this by cutting resin slowly, moving from the outside of the block inwards. Final dimensions of any given side should be less than 0.5mm. The smaller the cross-sectional area cut per section, the better the sections will cut.

Cutting ultrathin sections:

- Mount the trimmed resin block onto the ultramicrotome arm. Mount either a glass knife or diamond trimming knife
 - Face-off: using the glass or diamond trimming knife, carefully remove the very top surface of the pyramid. This will remove the roughness generated from previous trimming steps and exposes a perpendicularly-oriented surface for sectioning (Cutting speed can be fairly quick for this step, 10 mm/s. Removing 100-200 nm per cut)
- Switch to a diamond knife (or well-cut glass knife with a water reservoir attachment).
- Carefully fill the reservoir with clean, freshly filtered DI water. NOTE: be careful not to splatter any droplets of water onto the resin block as this will cause problems with sectioning later.
 - Adjust water level appropriate for resin type. Some resins cut better with the water level flat with the knife edge. Others require the assistance of gravity, so require the water level to be slightly lower, but ensure that the water touches the knife edge.
- Cut ultrathin sections (section thickness ranging from 55 – 85 nm thick depending on application. 65 or 70 nm is “standard” thickness).
 - Make sure your cutting window is set properly (using Cut-start and cut-stop selections). The beginning of the cut should be just before the resin block makes contact with your knife edge. The stop point should be after the block has passed completely through the block.
 - Confirm that the cut speed during the window of sectioning is 1 mm/s or slower. This ensures that the movement is slow enough for a good section. The slower, the better. However, the slower, the longer the sectioning session will take.
 - Cut such that the wider part of your trapezoidal shaped pyramid makes contact with the diamond knife first. This also enables better/smooth cutting.



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- Sections will come off the knife edge and if the top and bottom of your trapezoid were trimmed well (parallel to each other), the sections should stick to the previous one, forming a ribbon that floats out onto the water reservoir.
- If the sections don't seem to come off, or stick to the back-side of the knife edge, it is likely that you have a water droplet on the surface of your resin block. Remove both the knife and sample block, remove water from the reservoir and air-dry the surface of the pyramid before remounting and trying again.

Transfer of sections to TEM grid:

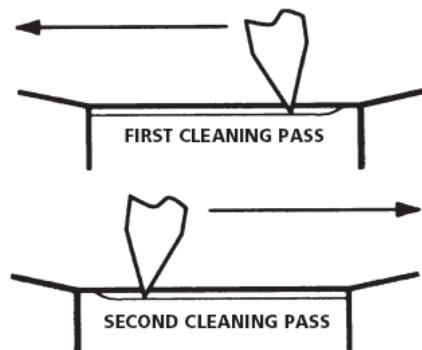
- Sections float on the water (preferably as a ribbon) due to surface tension. These sections/ribbons can be manipulated to different regions of the reservoir with an eyelash manipulator (eyelash or fine hair of some sort stuck onto a wooden stick with nail polish).
- Prepare grids by placing them on 2 sheets of filter paper with the support film side facing up.
- Pick up sections with a loop and transfer them to the TEM grid.



- Excess liquid should be wicked away by the filter paper and the grid will initially stick to the loop. Patiently continue to carefully blot the grid and wait until enough liquid evaporates that the grid falls off the loop. Allow grid to air dry further.

Cleaning the diamond knife:

- If you are using a diamond knife for your sectioning, proper care must be taken to clean it. Otherwise dried-on sections will quickly degrade the cutting capacity of the knife.
- Pour out water from the reservoir.
- Using a squirt bottle with DI in it, thoroughly rinse both sides of the knife (this can be done over the trash can)
- Using a squirt bottle with ethanol in it, thoroughly rinse both sides of the knife (this can be done over the trash can)
- Take the diamond knife polystyrene cleaning rod and cut a new brush edge (use a clean razor to do this as you do not want resin particulates embedded in your cleaning rod)
- Dip the brush edge into 100% ethanol and run orthogonally along the knife edge (as seen below)



- Allow to air dry.

Shut-down:

- Clean diamond knife
- Clean workspace area
- Shut down ultramicrotome controller unit
- Log out

Remember:

Sign the logbook and LOGOUT from the Login system computer.

*** Please contact Paul Shao (8-3851. pshao@princeton.edu) or Nan Yao (8-6394. nyao@princeton.edu, 120 Bowen Hall) if you have any questions.**

Emergency Information:

Medical Emergencies: **Contact 911 and Public Safety (609) 258-1000**

Room / facility emergencies: **Contact Public Safety (609) 258-1000**

Issues related to the instrument:

1. **Contact IAC Staff.**
2. **If unsure, leave system as is.**
3. **Try to power down the system.**

Audible/Siren Emergency Alerts:

Follow previous steps 2 & 3 and leave the building.

Emergency Contact Information:

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