

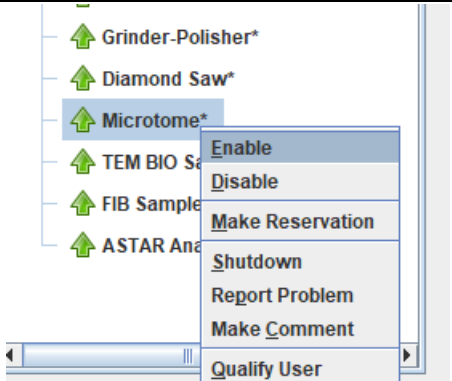

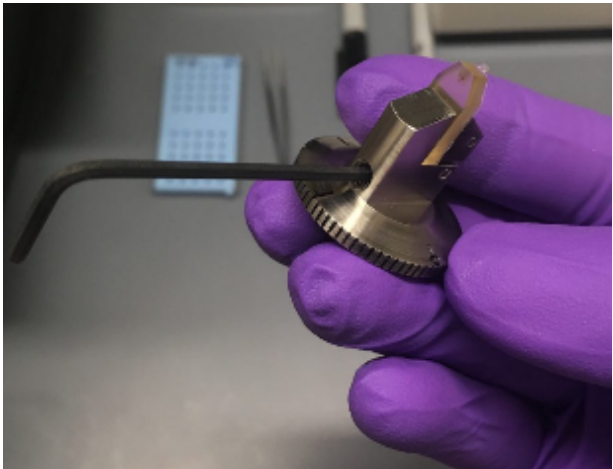
Ultramicrotome – (Sorvall MT-2B) Standard Operating Procedure





These instructions are intended for reference only, and will *not* replace the thorough training required for proper system operation. Contact a clean room staff member with questions or to report a system problem.

Written by Dr. Amir Zangiabadi



<p>1. Enable the tool in BADGER</p>	
<p>2. With a microtome, you are able to slice relatively soft materials, like polymers: plastics, epoxy, etc. This picture shows some biological samples embedded in Spurr resin.</p> <p>This mold size is 15mm x 6mm x 3mm</p> <p>Very large samples don't fit in the mold for slicing. Very small samples can be embedded in a resin.</p>	
<p>3. There are two types of sample holders; square molds and cylinder molds (next image).</p> <p>Use the L-wrench to fasten the sample. Tight enough to prevent sample movement during slicing. Not too strong</p>	<p align="center">Flat sample holder</p> 

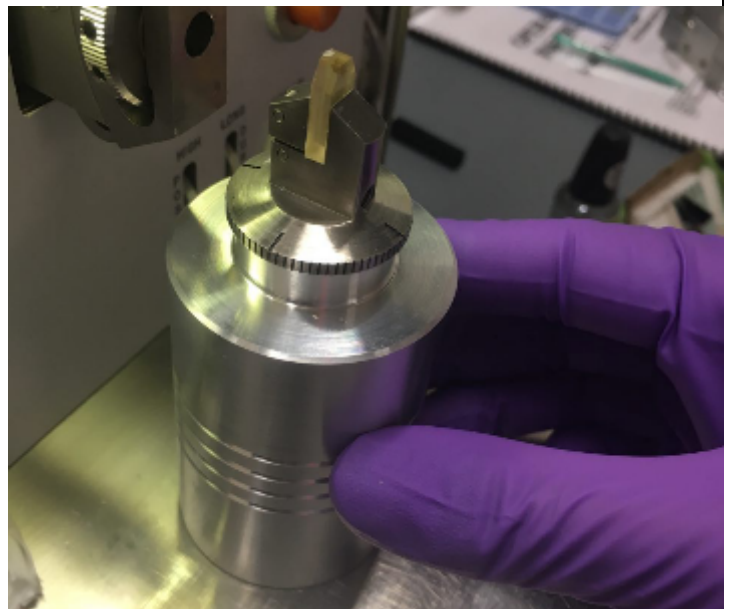
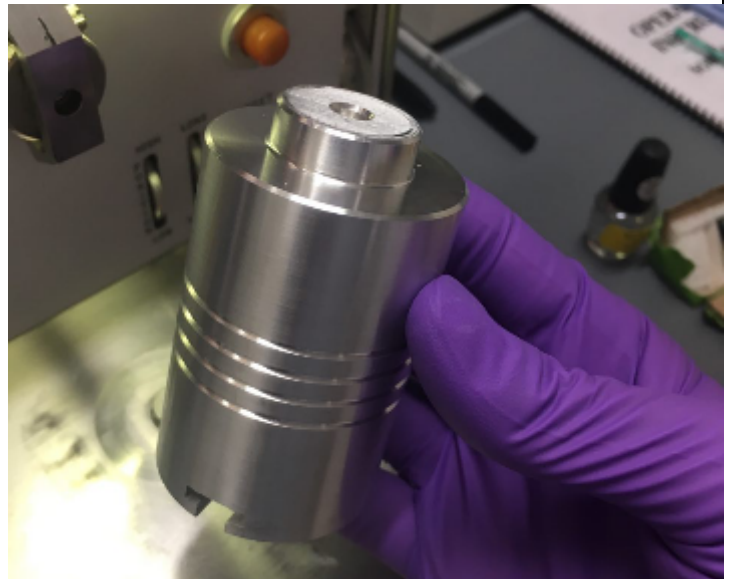


<p>to avoid damaging the polymer. For the cylinder sample holder, you just need to twist the top part relative to the bottom base to tighten it.</p>	<p>Cylindrical sample holder</p> 
<p>4. Prior to sectioning, the specimen must be trimmed to a proper shape and size.</p> <p>1-Remove the stage, by twisting the handle bar shown in the image CCW, and put the stage on the table</p> <p>After loading the sample on the specimen holder (step 3), load the specimen holder on the trimming fixture (shown below).</p>	<p>Knife stage</p> 



Put the trimming fixture on the front microtome plate, first by twisting the stage handle bar to raise the pin, then slide the fixture forward on the pin. Then release the twisting handle bar to tighten the fixture position on the plate.

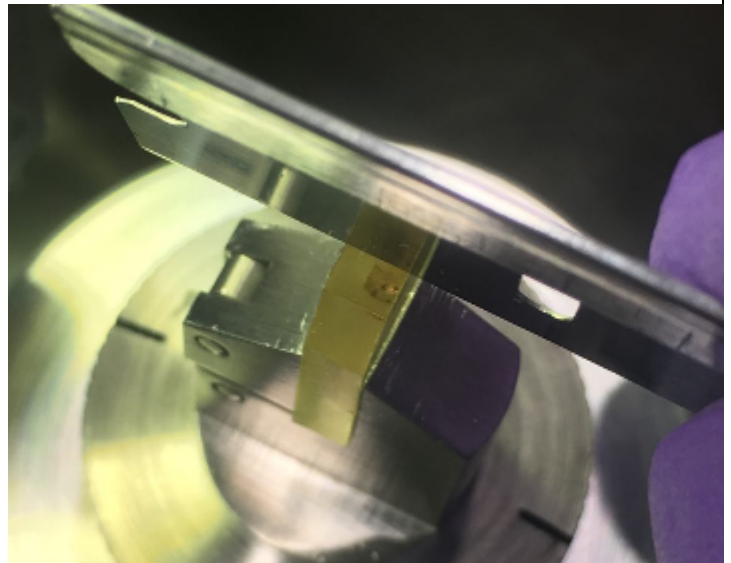
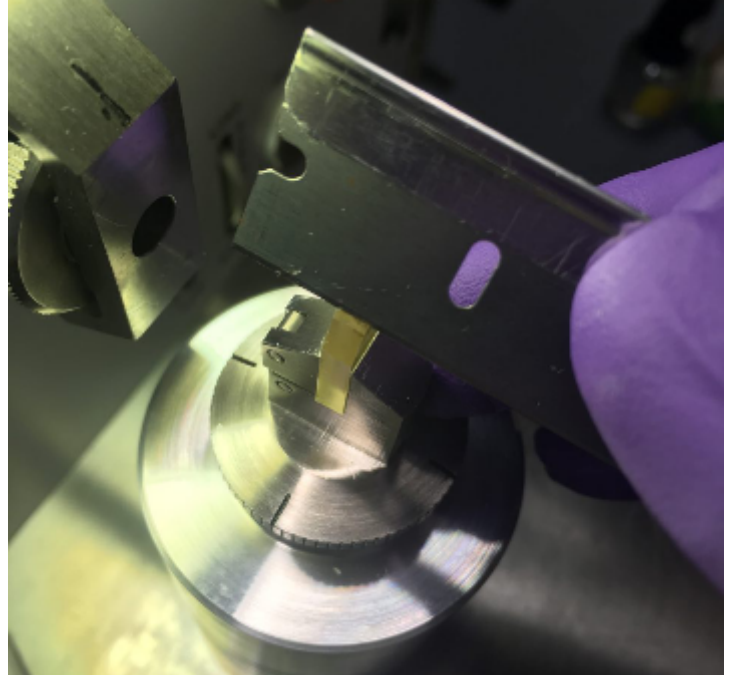
Trimming fixture:

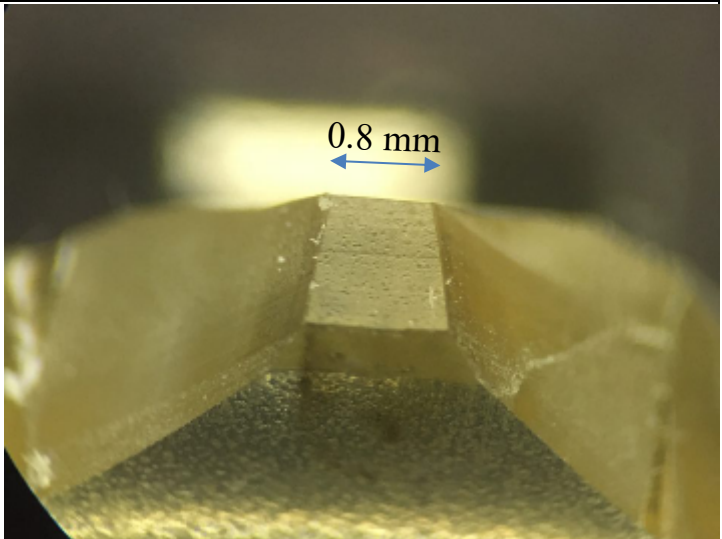
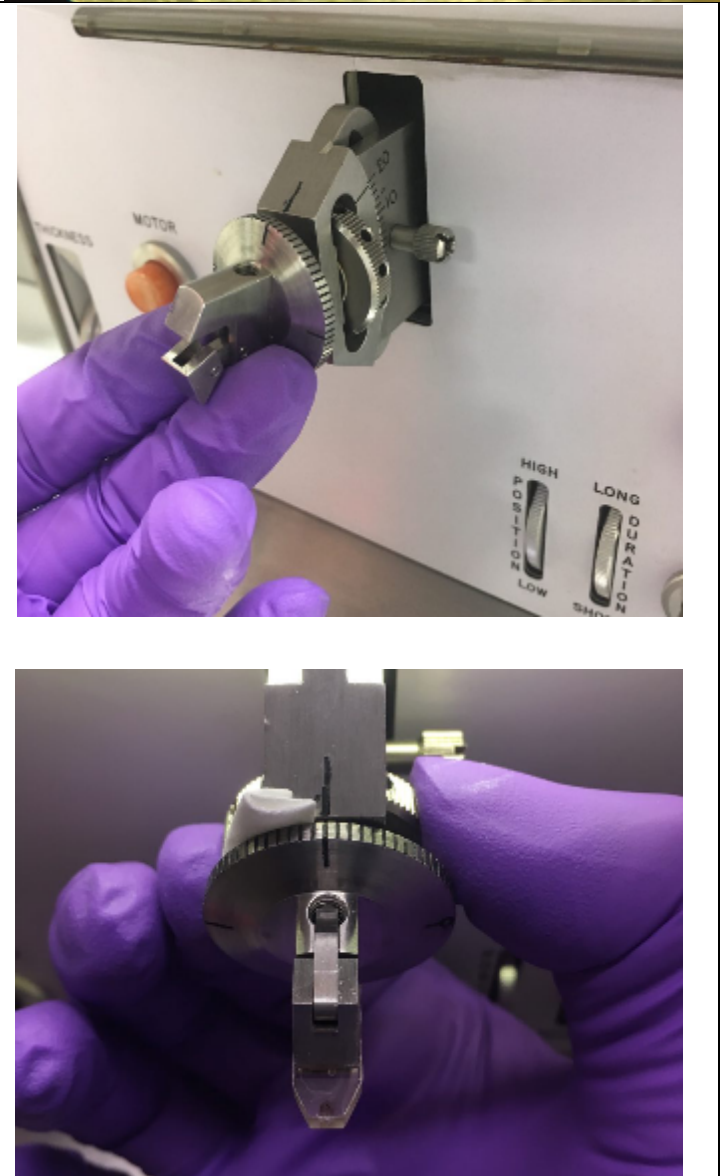


5. Since the sample surface is large, it needs to be trimmed by a razor. There are two stages to the trimming procedure: rough trimming and precision trimming.

The goal is to make the top surface of the sample block as a **small trapezoid** in the range of 1 mm x 1 mm or smaller (as shown in image below).

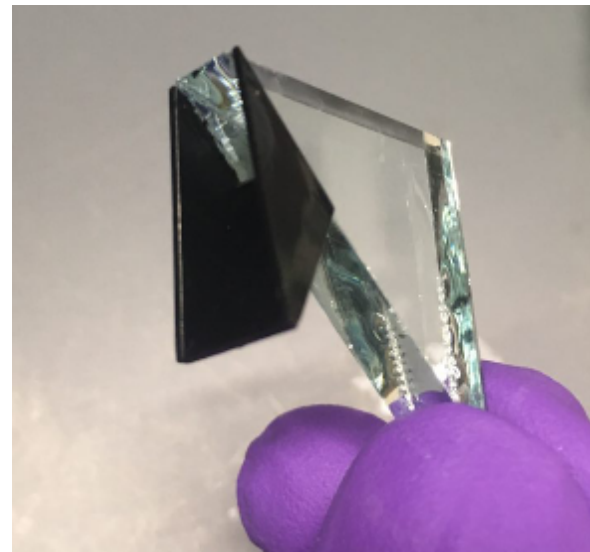
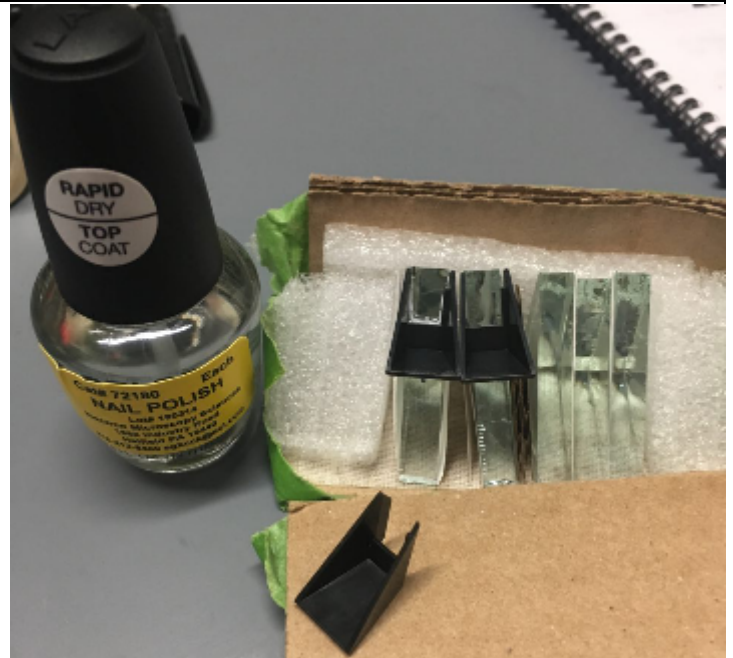
For precision trimming use the stereomicroscope overhead to cut the edges of the epoxy to make the surface as a flat trapezoid, that only contains a small part of the sample required for imaging (shown below).



	
<p>6. Remove the sample holder and trimming fixture from the microtome front plate and install the sample holder on the front face of the microtome.</p> <p>Twist the sample holder and the free wheel on the back to make the sample holder completely fixed.</p> <p>It is recommended to put a small piece of paper between the flat sample holder and the back plate to make it fully tight.</p> <p>The sample should be placed like the image on the right, so the wide side of the epoxy is horizontal.</p>	



7. There are two types of knives. Glass knife and diamond knife.
- Glass knife is used first for 10 μ m-thick sections on the top surface to make it more flat and reach the desired area of interest.
- Diamond knife is used later for nanometer thick sections.
- The glass knives come in a triangle shape. A small plastic boat is attached to top part near the sharp edge to make sure the tip is being wet during sectioning. The plastic boat is attached and sealed to the glass knife by using nail polish.



8. Place the stage back to the front plate of the microtome and make sure the 0 degree is aligned to the indicated line.

Twist the advancement knob CCW to make sure the stage is completely retracted (as shown in the image). Don't twist more than it is shown here, since the knob will come out.

P.S. On the advancement knob it is inscribed 1 μm for the smallest unit, but it is actually 10 μm . One full rotation is 1 mm advancement.

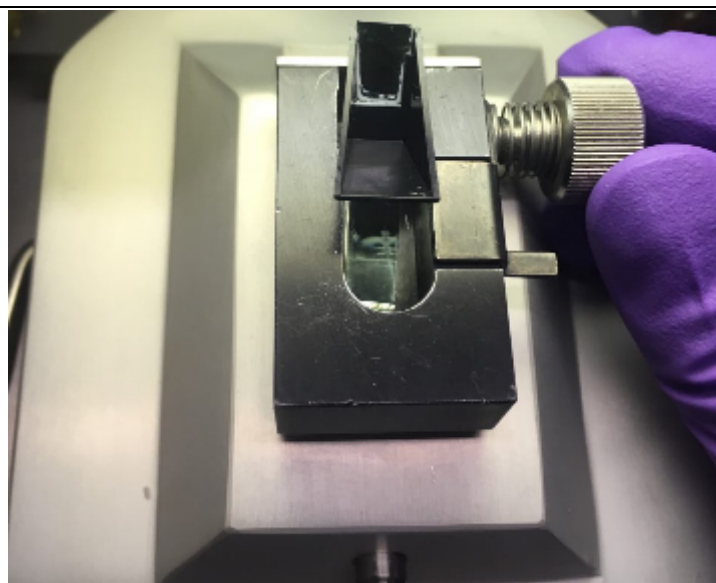


Advancement knob



9. Put the glass knife on the knife holder. Make sure the front face of the glass is completely attached to the front plane, then twist the screw on the right to make the glass tight (as shown).

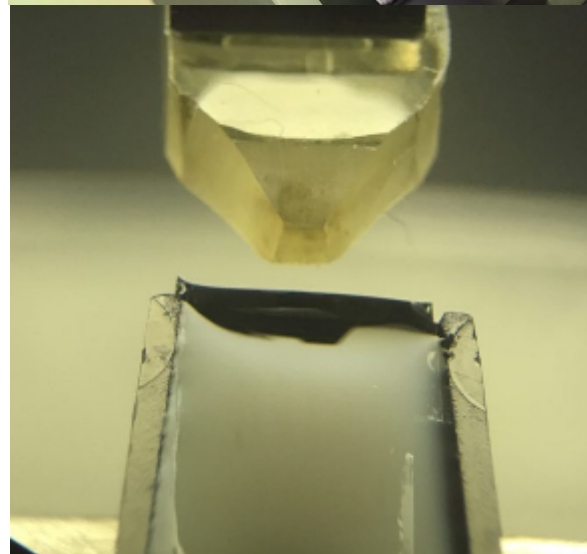
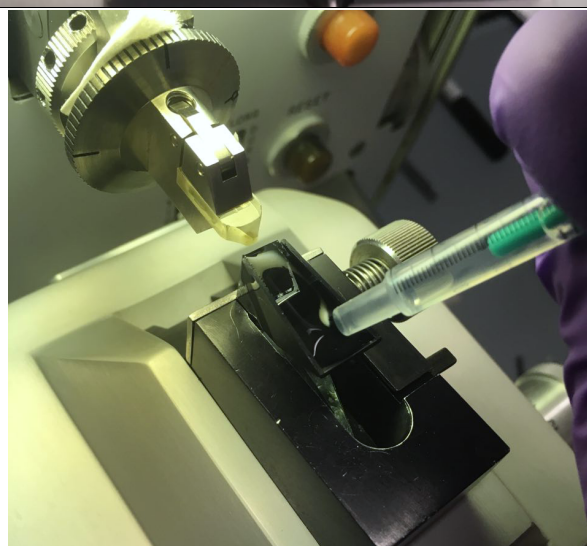
The glass edge **should not** touch the sample while tightening it.



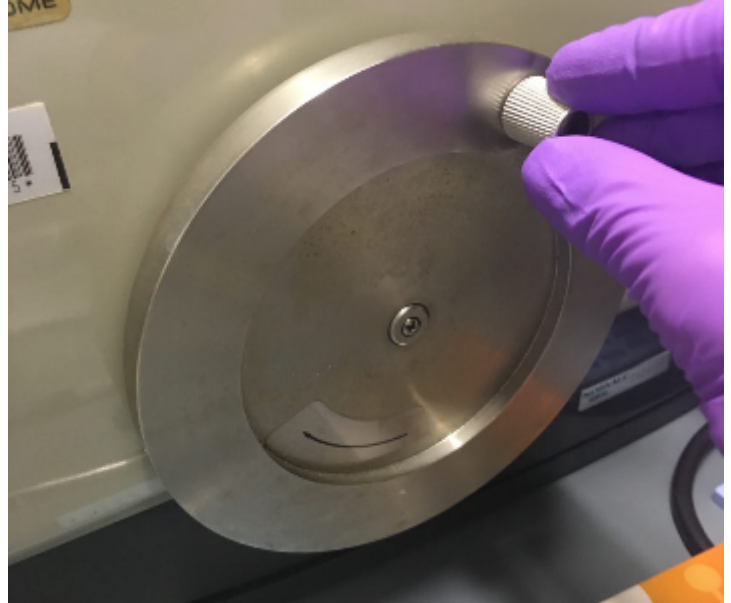
10. Use a container and fill it with the DI water faucet located on top of the sink.

Use a 1 mL syringe and fill in the plastic boat.

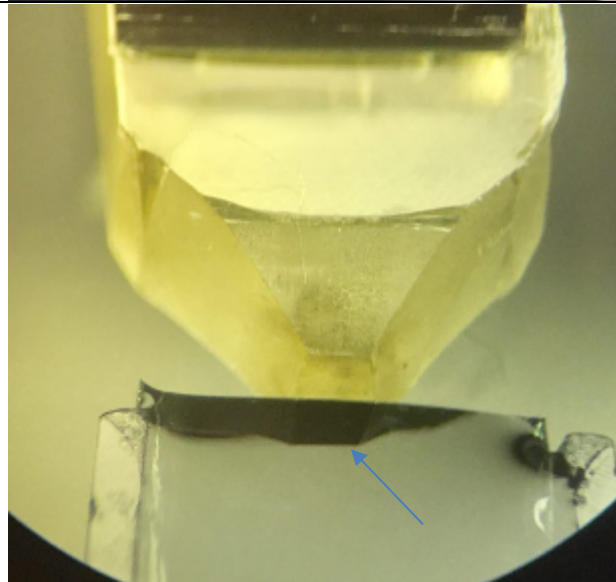
First fully fill with the water till the water is bulging and touching the glass edge, then remove the excess water gently till the water surface is almost flat and the knife edge is still wet.



- 11.** Rotate the handwheel clockwise to make sure the sample face is at the same height as the glass knife edge.
- Then, use the advancement knob and bring the knife closer to the trapezoid surface (don't let it touch it).



- 12.** When the knife edge is getting very close to the sample surface, you can see the reflection of the sample surface in the water.

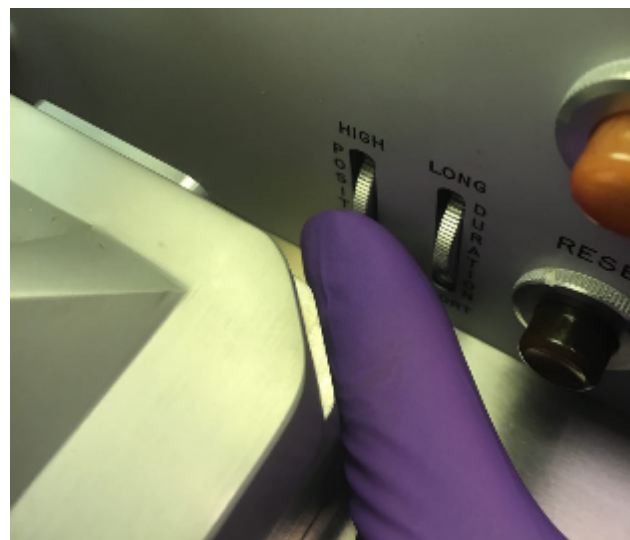
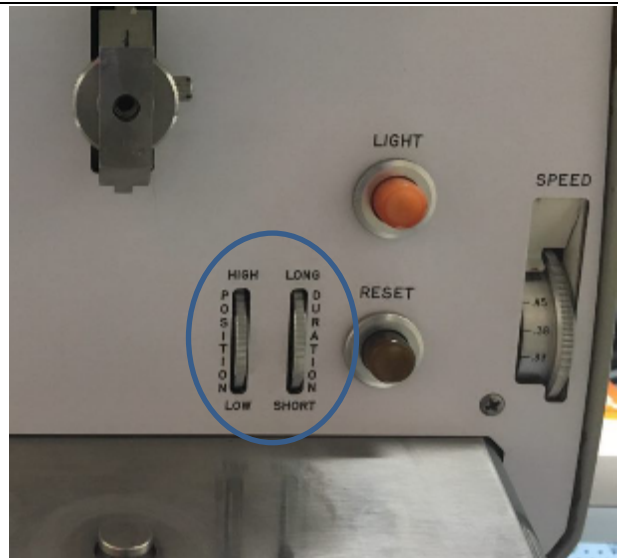


13. The slicing is done by moving the sample down while the knife is fixed. In order to save time and have a clean cut, the microtome has a slow movement during the cutting and then moves faster between cutting cycles. This is called **cutting window**.

Depending on the height of the knife or sample thickness, you need to adjust the cutting window by its **position** (height) and **duration** (how thick).

Rotate the handwheel and see the sample surface and observe when the movement slows down and then speeds up again. Use the **position** and **duration** knobs to place the cutting window based on knife edge height and sample thickness.

This step requires several rotation cycles to make sure the cutting window is correctly adjusted and overlaps the sample thickness.



14. Rotate the handwheel continuously and in each cycle, rotate the advancement knob only 2 small units (μm) in each cutting cycle until eventually the sample reaches the glass knife.

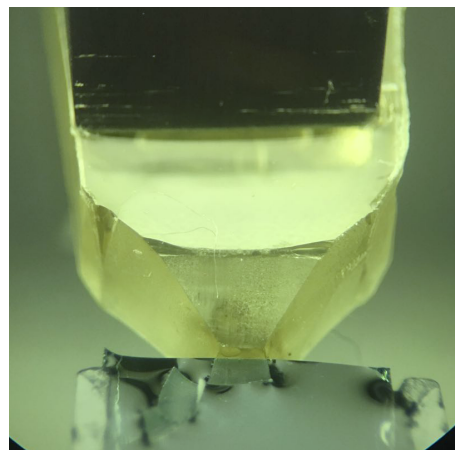
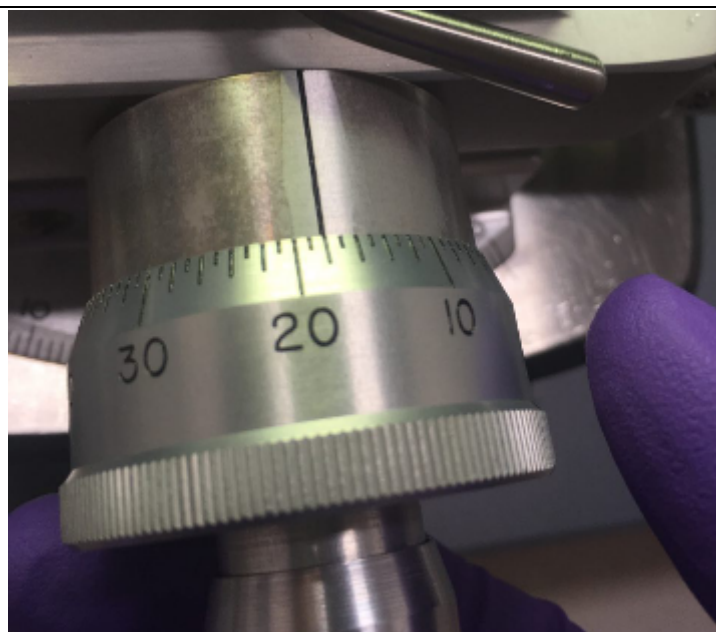
Then click on the **Motor button** on the front panel to make the cutting cycle automatic.

In each cycle, you can advance 1-2 unit. Don't exceed this limit, since the sample or the glass knife will break.

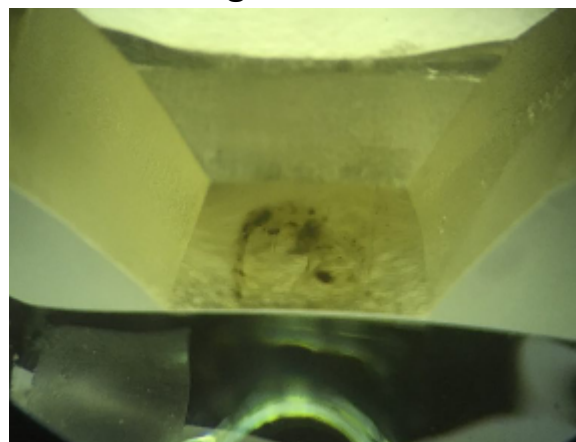
Make several slices till you get a complete section from the area of interest.

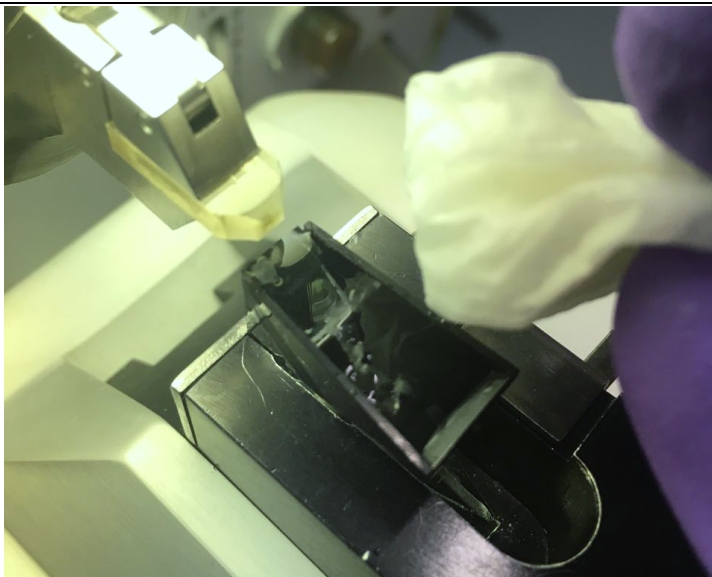
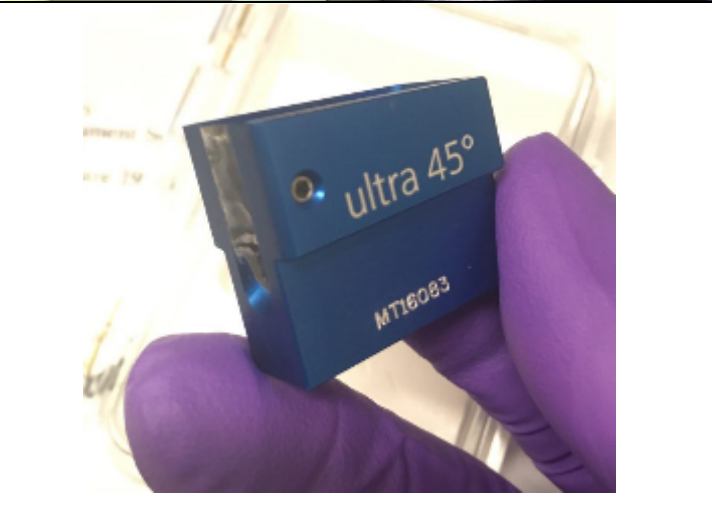
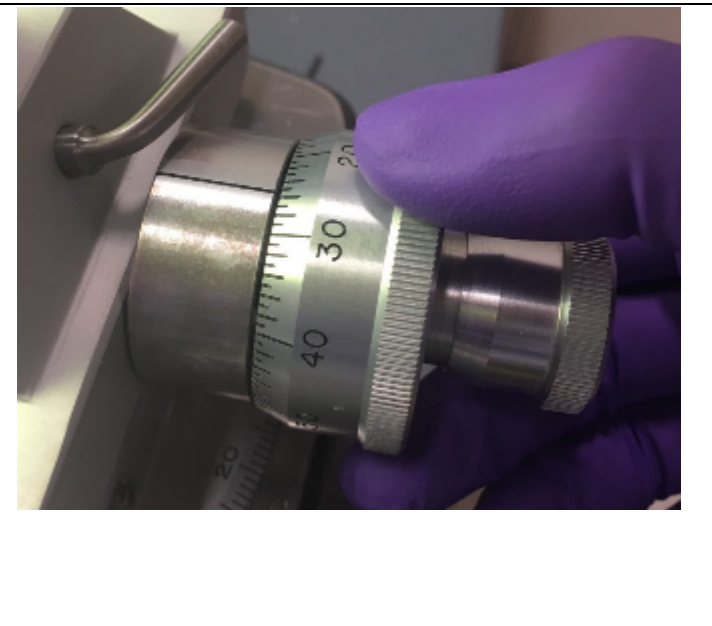
When you don't advance manually, the machine advances forward in nanometer scale.

P.S. On the advancement knob it is inscribed $1\mu\text{m}$ for the smallest unit, but it is actually $10\mu\text{m}$.



A flat region of interest:



<p>15. After making several slices, the water is filled with thick epoxy slices. Use a piece of tissue and touch the water and slices to remove some of them, then refill the water boat and continue slicing (if needed). You can repeat this to remove most of the slices.</p>	
<p>16. When you are satisfied with the sample surface and you find it flat, it is time to make nanometer thick slices with the diamond knife.</p> <p>Diamond knife is inside a box. While handling the knife, never touch the diamond edge.</p>	
<p>17. Retract the knife stage from the sample by rotating the advancement knob CCW.</p> <p>Remove the glass knife, and place the diamond knife in the knife holder.</p> <p>Repeat similar steps for loading and adjusting the diamond knife:</p>	



1. Fill the boat with the DI water till the water is making a bulging surface and makes the diamond edge wet. Remove the excess water till the water surface is flat (Like the image).

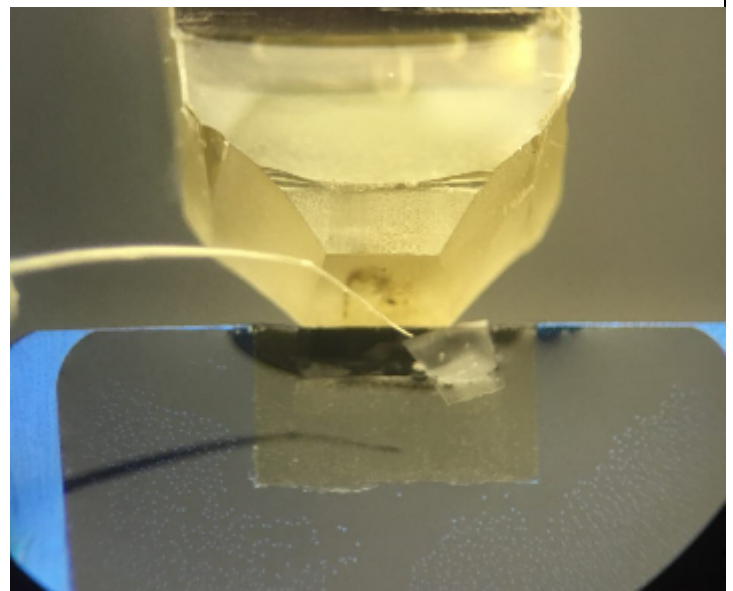
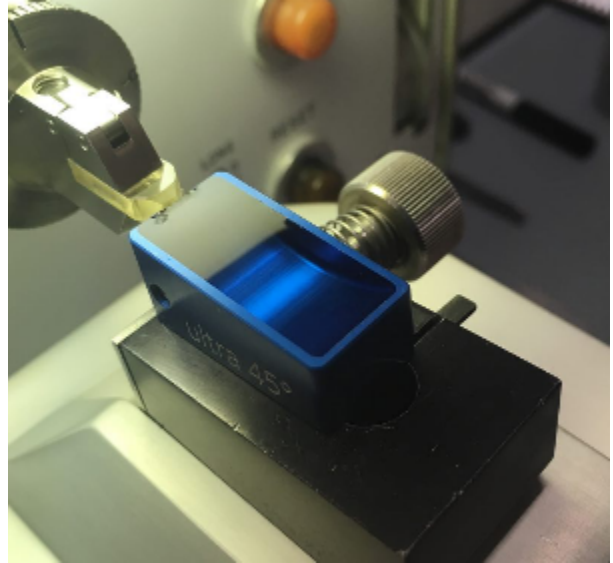
2. Rotate the handwheel till the sample surface is roughly at the same height of the knife edge.


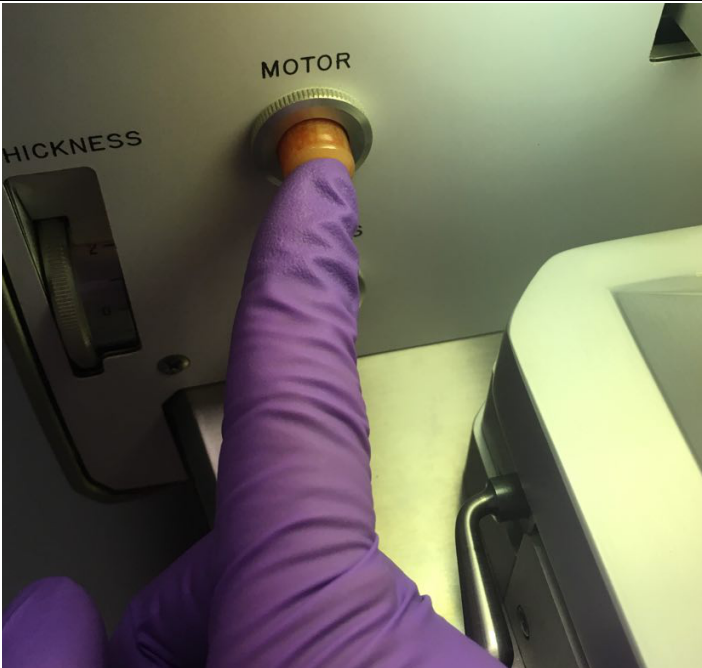
3. Rotate the advancement knob with 1-2 μ m steps, till the knife edge is **close** to the sample surface and you start seeing part of the reflection and still there is a gap.

NEVER advance the knife till the knife edge hits the sample, you may break it (\$4000).

4. Click on the Motor button while there is a gap between the knife edge and the sample and observe the **cutting window**.

And adjust the cutting window by position and duration.

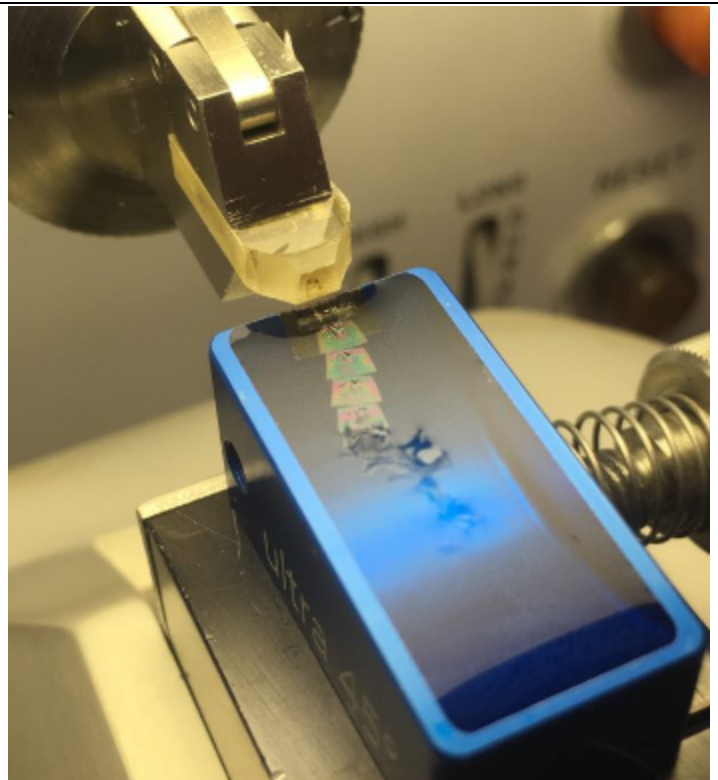


<p>5. While the motor is on, at the end of the cutting window advance the knife only 1-2 units (μm) at each cycle till the knife reaches the sample and starts cutting. Never exceed this limit, otherwise the knife or the sample will get damaged. For harder sample advance 1 unit (μm) at each cycle.</p> <p>6. When the knife reaches the sample reduce the advancement to a half unit (μm), and continue till a full slice is being cut from the sample surface.</p>	
<p>18. For cutting nanometer thick slices, turn on the motor, then adjust the auto-advancement knob located on the left side of the panel to 18. If you multiply this number by $\sim 40\text{nm}$, you roughly get the slice thickness ($\sim 720\text{nm}$).</p> <p>Then make few slices (as shown). Reduce the knob to 16, then 14, and continue to reach number 2.</p>	 <p style="text-align: center;">Thickness calculation</p>



At number 2, you should get slices with a thickness of around 100 nm, which should be good enough for Transmission electron microscopy (TEM).

Then turn off the Motor, by pushing the button.



19. Thickness calculation:
 The upper thickness knob is showing numbers in nanometer (right now it is set to 50nm). We normally don't change it.
 The Thickness dial number is set from 0-20.
 To get the **slice thickness** multiply the Thickness dial number (2) to the upper thickness knob (50nm).

Thickness dial


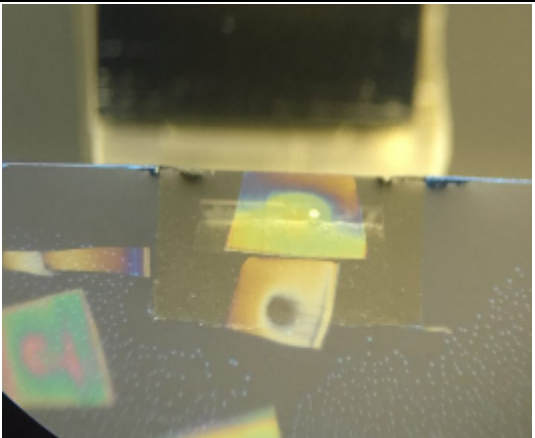


X Upper Thickness knob



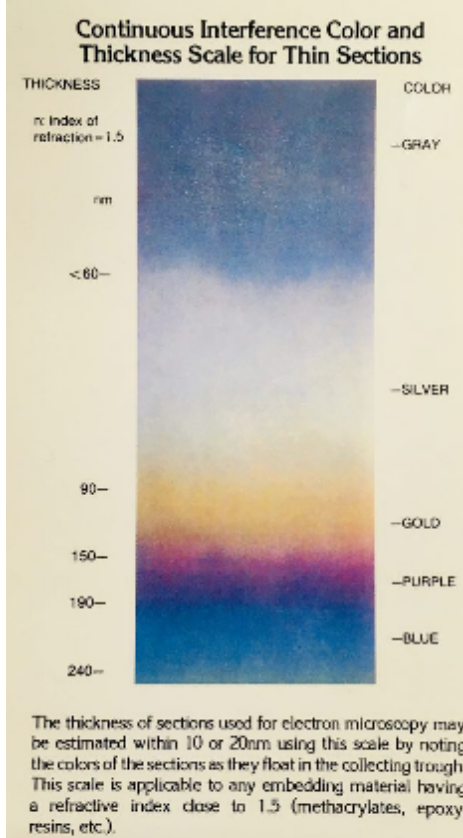
$$2 \times 50 \text{ nm} = 100 \text{ nm slice thickness}$$



<p>20. To get a thick slice: Hold the By-Pass button for several cycles (say 5), then release it. The obtained slice thickness will be the number of bypassed cycles multiply by the thickness of one slice ($5 \times 100\text{nm} = 500\text{nm}$).</p>	
<p>21. Another indicator of slice thickness is the reflection color from the slices.</p> <p>If slices are in the color range of gold or silver, they are thin enough for TEM (gold color is preferred; silver slices are thinner but more fragile to rupture.)</p>	



22. This thickness color map is a good indicator.


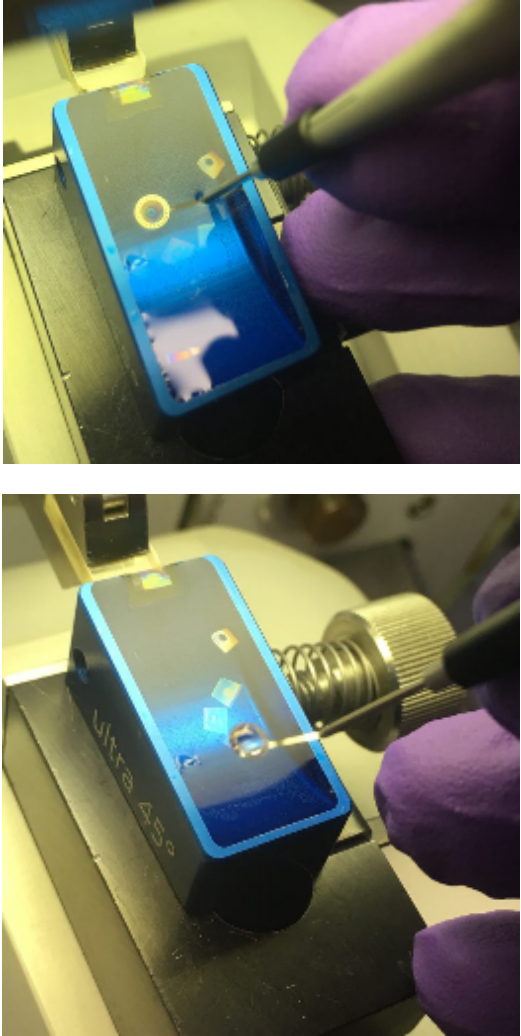


23. When you get a preferred slice thickness, bring in a filter paper, TEM grid, Pickup loop and the Eyelash with handle.



Eyelash and the Loop



	
<p>24. Use the Eyelash and move the slice to the middle of the water boat. Immerse the loop in the water boat. Then gently raise the loop to capture the sample in the middle of the loop.</p> <p>This will create a small water lens and the sample will be floating on top of it.</p>	




In an alternative method, you can lower the dry loop horizontally till it touches the water surface and the slice is in the middle of the loop, then lift it up, as shown in the schematic.

PERFECT LOOP (pat.)

HANDLING AND USE:
for easy section pickup and mounting on EM grids

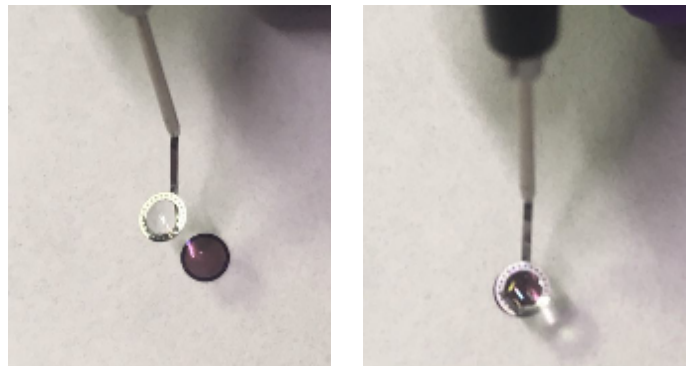
Have your cleaned grids sitting on clean filter paper in a Petri dish. Ensure that the ribbon of sections is short enough to fit inside the LOOP.



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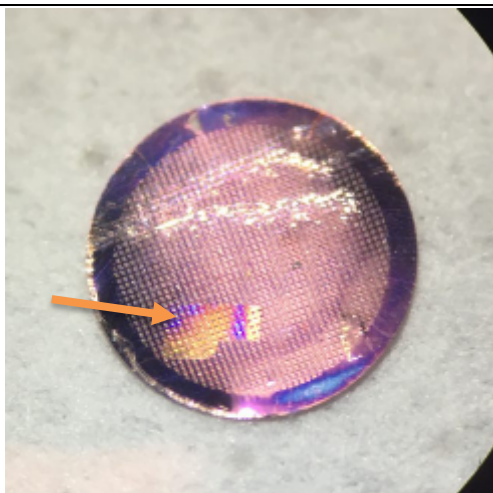
25. Place a TEM grid on a filter paper. If the grid is coated, place that layer on top (darker side).

Gently touch the loop with the grid, then use a piece of tissue to remove the excess water. Then by the eyelash or the tweezers remove the grid from the loop and wait till it gets dry.



26. Under the stereoscope, by tilting the sample, you should be able to see a reflection of your sample.

Now you can observe your sample with or without staining under the TEM (depending on your sample)

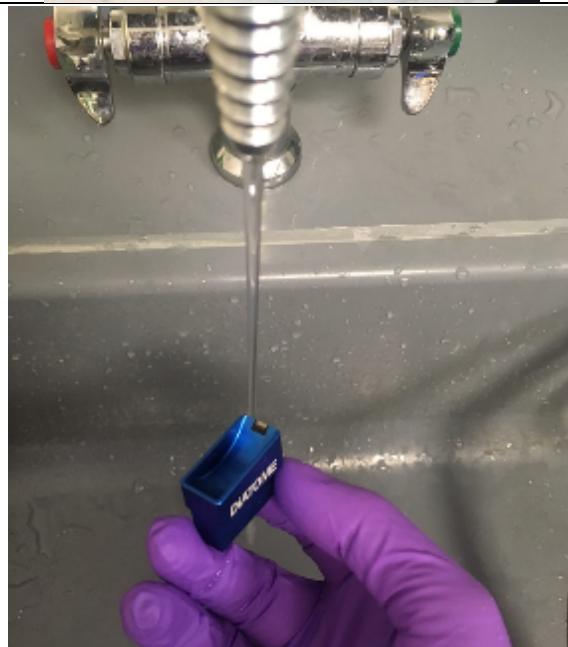




27. After being done with the microtome, retract the stage from the sample (by CCW rotation of the Advancement knob) and then remove it from the knife holder (by untwisting the grip, shown in Section 9).

Then put it under the running water.

Do not touch the diamond tip.

If there are tiny pieces of sample stuck to the knife edge, try to push them around with the eyelash, or very gently run a wet cloth on the edge once or twice. There is no need to clean it excessively. If you need assistance let the staff know.



<p>Do the same for the glass knife.</p> <p>Dry the knife metallic case and put it back in its box.</p>	
<p>28. Push the Reset button on the front panel.</p> <p>Remove your sample from the holder. Unscrew the holder from the microtome and place it in the drawer.</p> <p>Clean the loop and eyelash with water and place them in the drawer.</p> <p>Make sure the table is clean and dry for the next user.</p>	
<p>29. BADGER LOGOUT: Don't forget to disable the tool in badger after you're done.</p>	