



Endless Possibilities ...

Kirsch
notes

Thick Sectioning
for TEM
Using the Leica UC7
Ultramicrotome

Thick Sectioning Using the Leica UC7 Ultramicrotome

The following directions will address the topic of thick sectioning samples for later viewing in the TEM using the Leica UC7 Ultramicrotome.

The thick section or the survey section has a thickness of between 0.5 - 2 μm . It is collected, stained and imaged on the Optical Light Microscope (OLM) to determine if the tissue:

- a. Contains the desired Region of Interest (ROI)
- b. Has been fixed and embedded properly
- c. And has proper trimming orientation of the block face for thin sectioning

Thick sectioning also serves to smooth the block face in order to prepare it for subsequent thin sectioning.

Supplies Needed

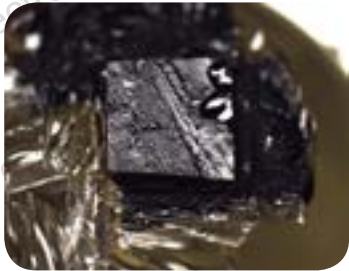
- Trimmed block
- Diatome 6 mm Histo knife or glass knife with boat
- Clean glass slide
- Pipette and DI water
- 70°C hot plate
- Epoxy tissue stain
- Eyelash manipulator
- Perfect loop
- Perfect Scale
- Glass slides w/ etched circle
- Gold Seal 22 mm cover glass

EMS Catalog Supplies

Diatome 6 mm Histo knife – #60-HIS
Glass slide – #71879-10
Epoxy tissue stain – #14950
Eyelash manipulator – #71182
Perfect loop (handle and loop) – #70944
Perfect Scale – #70617-10
Glass slides w/ etched circle – #71879-10
Gold Seal 22 mm cover glass – #63757-01

Directions for Thick Sectioning Using the Leica UC7 Ultramicrotome

1. Insert trimmed block into specimen holder and set arc to 0° and secure into specimen arm.



Razor blade trimmed sample.



Diamond knife trimmed sample.

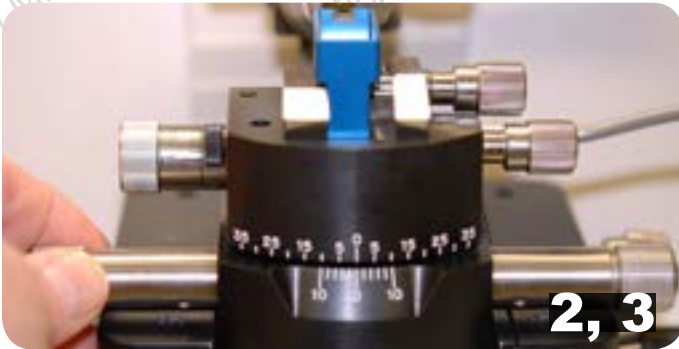


Specimen holder (left)
Securing holder (above)

2. Place facing off or sectioning knife into holder and secure tightly.

3. Set knife arc to 0° and clearance angle to 6°

4. Turn on overhead and back lighting.



2, 3



4



3



5

5. Slide knife holder/knife into base and carefully push forward until knife is ~ 2-3 mm from block face.

2

6. Observing the block and knife edge adjust specimen cutting stroke wheel so bottom or leading edge of block is parallel to knife edge.
7. Turn specimen cutting stroke wheel CW until the specimen is descending and stop when the leading edge of the block is ~2-3 mm above the knife edge and set Upper mark for the cutting window.



6



6



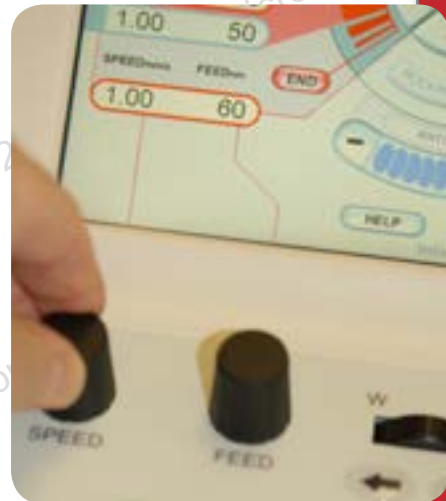
7



7

8. Continue rotating the wheel, lowering the block face to slightly below the knife edge and set the Lower mark of the cutting window.

8

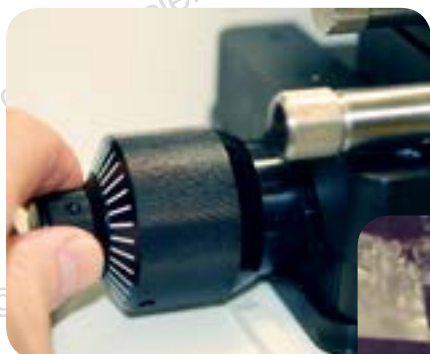


9. Select the desired cutting speed (2-5 mm/sec) and thickness (0.5-2 μm)

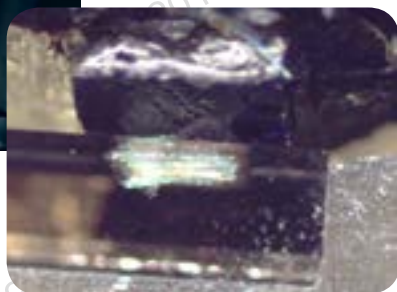


10. Adjust the knife horizontal / lateral so the desired area of the knife is under the block face.

11. While observing at higher magnification, use the coarse knife advance control to move the knife very close to the specimen while rotating the specimen wheel through full cutting strokes.



12. Advance slowly using the fine knife advance, until the first hints of cut resin start to appear.



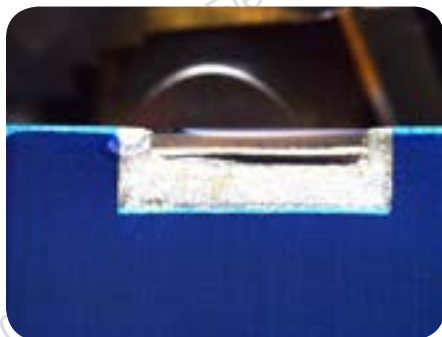
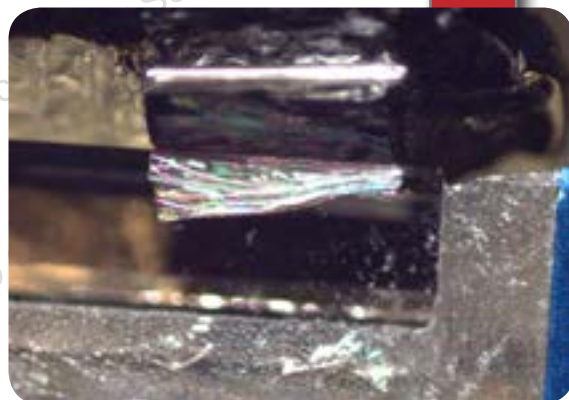
13. If you used a diamond knife / trim tool for trimming go to # 14, otherwise, if you used a razor blade continue to 13a

13a. To advance the knife slowly while moving the specimen through full cutting strokes, taking "fly wing" sections.

13b. Advancing and cutting until the entire block face is cut smooth and reflective.

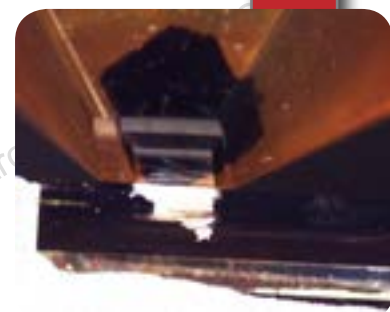
13c. Move to the good cutting area of the knife or change to your good sectioning knife.

13d. Repeat steps #10-12.



Too much water!

14. After the initial cut leave the specimen arm down and fill the boat with water to form a slightly convex surface. It should be transparent at this point and you can see the knife supporting resin clearly through it.

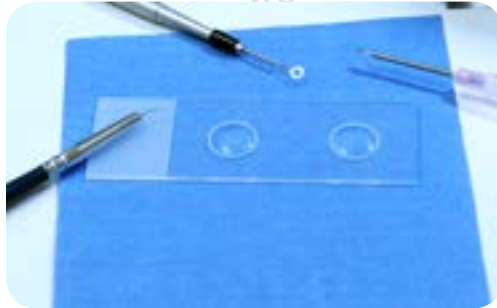
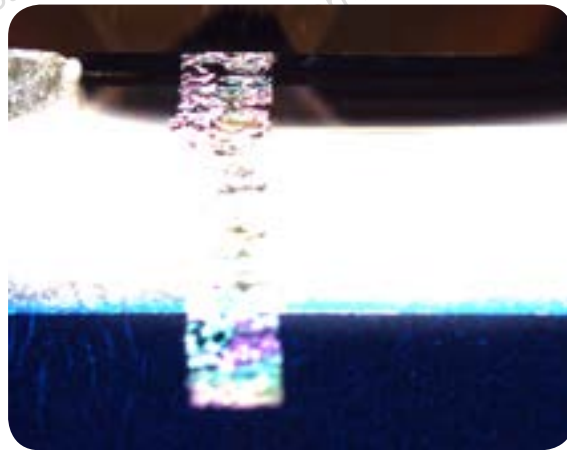


15. Water from the boat until a silver reflection forms at the knife edge almost obscuring your vision of the knife supporting resin.

NOTE: If the water does not stay at the knife edge, wipe your eyelash manipulator on your tongue, put it in the water close to the edge and draw the water along the edge with the eyelash.



16. Having already set your cutting window, thickness, and speed press the auto cut button and observe the cut sections.



17. Using your eyelash tool or Perfect Loop remove sections from the water and place them on the slide.

18. Collect several sections 3-4 on each inscribed circle and place it on the "warm" 70°C hot plate until dry.

19. After the sections have dried on the slide, add 2-3 drops of Epoxy stain to one circle and let sit for approximately 1 min. then rinse thoroughly and dry.

20. The sections are ready to observe under the OLM. If needed stain remaining circle after evaluating first circle.

