EMS Microscopy Academy

Endless Possibilities ...

Kirsch notes

• How to Thin Section TEM Samples

How to Thin Section TEM Samples

The slicing of ultra thin sections for transmission electron microscopy (TEM) observation is challenging, requiring good instrumentation, proper environment, patience, technical skill and sometimes luck. Here, we describe how to thin section using RMC and Leica ultramicrotomes.

EMS Catalog Supplies

Perfect Scale 70617-10
45° Diatome Ultra 30-US
Eyelash Manipulator 71182
Perfect Loop (handle + loop) 70944
200 mesh Cu grids EMS200-Cu
Tweezers
- Straight Style 3, 120 mm 78518-3
- Style 3 Self Closing, 120 mm 78318-3x
- Style 3 Self Closing, 120 mm, extra fine tip 78319-4x
- Style 7 curved, 115 mm 78522-7

Grid Storage Box, 100 capacity 71146-02
Uranyless, Post Stain 22405

Directions

Specimen in microtome chuck.

1. Place trimmed block securely in microtome chuck.
2. Place clean diamond knife in microtome holder, secure, and set clearance angle 4°-6°
3. Turn on microtome overhead and bottom lighting.

Diamond knife in microtome holder.

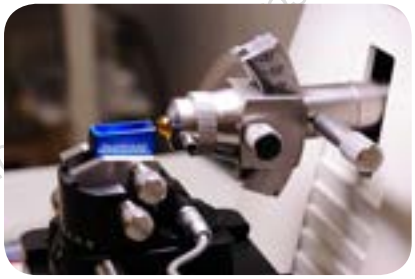
LEICA Lighting

RMC Lighting

4. Secure the specimen in microtome arm with the arc adjustment vertical and scale on the right.
5. Reset specimen advance.

LEICA Ultramicrotome

RMC Ultramicrotome



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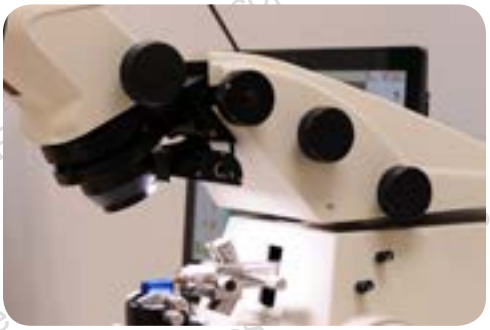
LEICA Ultramicrotome



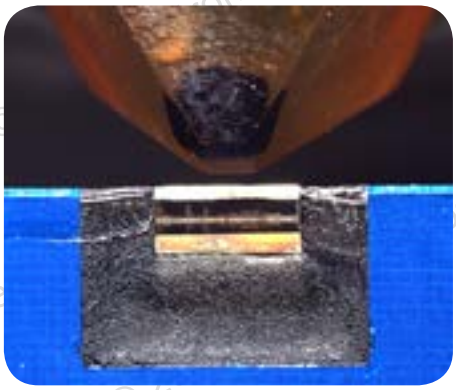
Step 7

RMC Ultramicrotome

Step 8

6. Slide the knife holder into the microtome and carefully advance it forward close (2 mm) to the block and secure in place.
7. Adjust microtome optics, lateral, tilt, magnification, and focus to get a clear view of the block face and knife edge.
8. Adjust the lateral position of the knife
9. Rotate the sample to get the leading edge of the block parallel to the knife edge.
10. If the knife arc is visibly rotated from the plane of the block face, rotate the knife to the approximately correct position and reassess the specimen rotation.

This is what you should see after making the adjustments in steps 7 & 8.



Specimen TILTED.

Specimen PARALLEL.

Knife edge

Adjusting specimen rotation.

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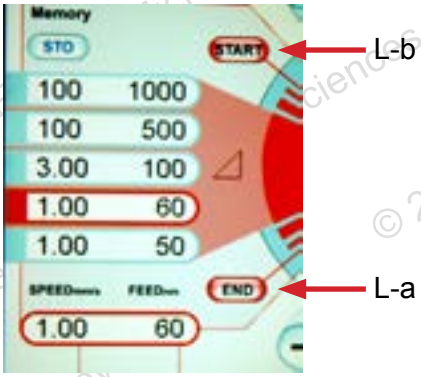
Steps 11.1 & 11.2



LEICA Ultramicrotome

RMC Ultramicrotome

11.1 Set the cutting window by moving the specimen below the knife edge. *(See photos above)*

For Leica: *Press* "END" (L-a)

For RMC: with monitor – *Click* "Adjust Lower" (RMC-a)
no monitor – *Press* LOWER "Cutting Window" button (RMC-aa)

11.2 Continue the cutting stroke clockwise until the block comes down to the position where you want the cutting speed to begin.

For Leica: *Press* "START" (L-b)

For RMC: with monitor – *Click* "Adjust Upper" (RMC-b)
no monitor – *Press* UPPER "Cutting Window" button (RMC-bb)

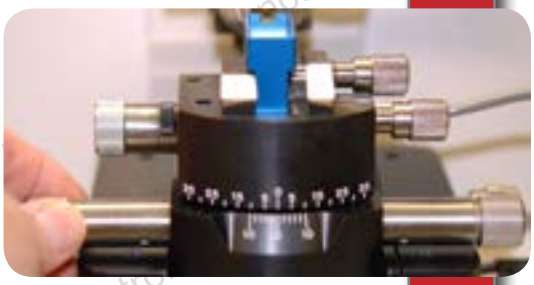
Special Notes

- When the specimen is rotated to the bottom of the cutting stroke, the arm retracts, so it will not hit the knife on its upward return stroke. Be sure to complete the cutting stroke so when the specimen wheel is being turned clockwise the arm is moving down.
- In making knife or block arc adjustments always back the knife off some to avoid running the block into the knife edge.

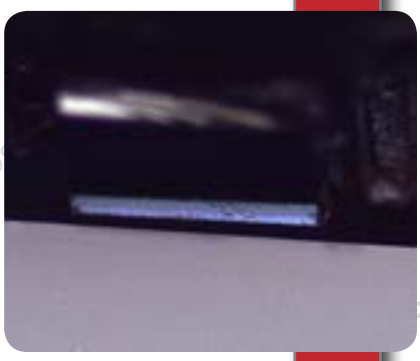
12. With the bottom edge of the block at the same level as the knife edge, Increase the magnification and, using the manual knife control, advance closer to the block until a bright reflection of the knife edge is visible on the block face.
13. This reflection represents the proximity of the knife to the block and will be used to align each arc so sections are generated across entire block face and from top to bottom.
14. Leaving the sample stationary observe the reflection it should be even in "thickness" from side to side.
15. If not back the knife up, make an adjustment by rotating the knife in the proper arc and re-approach the block.

Steps 13 - 15

Reflection TILTED.



Adjusting knife arc.



Reflection PARALLEL.

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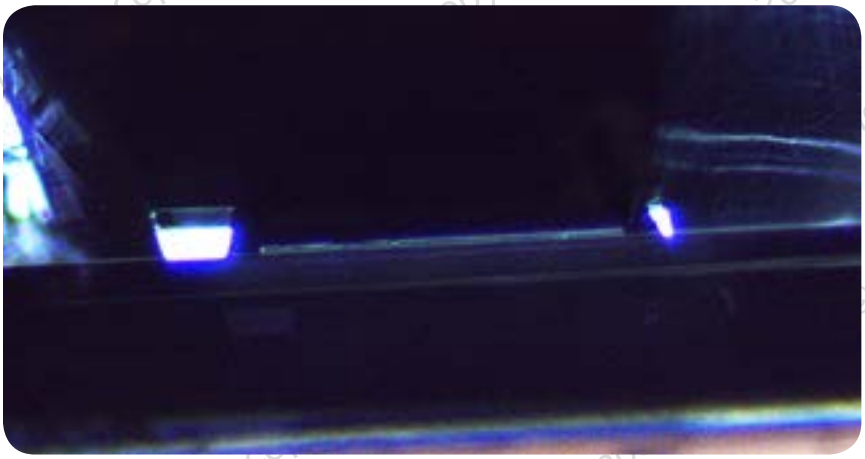
16. Repeat this as necessary until, at high magnification and very close proximity to the block, the reflection is a very thin, even strip.
17. Back the knife up until the reflection is in the middle of the block and, using the manual specimen movement wheel, lower and raise the block relative to the knife edge while observing the reflection.
18. Ideally the reflection should remain the same “thickness”.
19. If the reflection gets thinner as the block moves down, the face is “closed” and needs to be rotated down to “open” its position. The opposite is the case if the reflection gets thicker.
20. Repeat this as necessary until, at high magnification and very close proximity to the block, the reflection is the same “thickness” as the block face is lowered and raised.
21. At this point magnification should be high to see the knife very, very close to the block face with an even sliver of reflection across the face, remaining exactly the same when the block is lowered and raised.

22. Using the fine knife advance or the specimen “step advance”, bring the knife so close that the reflection just disappears.
23. On the way there (close to the block), any final adjustments can be made to the specimen and or knife arc by backing up slightly, making the change and re-advancing.



Steps 16 & 21

Reflection even: top-to-bottom and side-to-side.

Step 22

Fine knife advancement.

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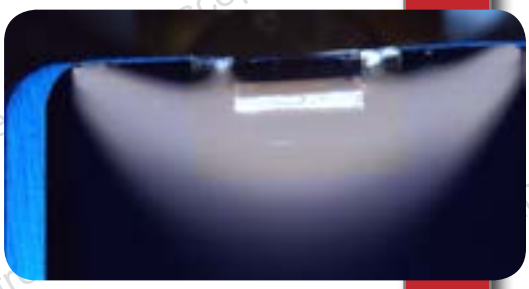
Too much water.

24. Leave the specimen arm in its lowered position and fill the knife boat with water so it forms a meniscus and, when looking at the knife, it can be clearly seen through the water.
25. Using a pipette or syringe with a needle remove the water until it forms a bright mirror like reflection at the knife edge. Check to make sure there is still water at the knife edge.

Correct level of water.



LEICA speed and thickness (feed)

RMC digital monitor and manual speed and thickness

26. Select the section thickness (40 – 100 nm.) and cutting speed (0.5 – 3 mm/sec) and select the automatic “CUT”.
27. As the automatic cut cycle continues through full cutting strokes, make very fine manual knife or specimen “step advances” until the specimen starts to cut.

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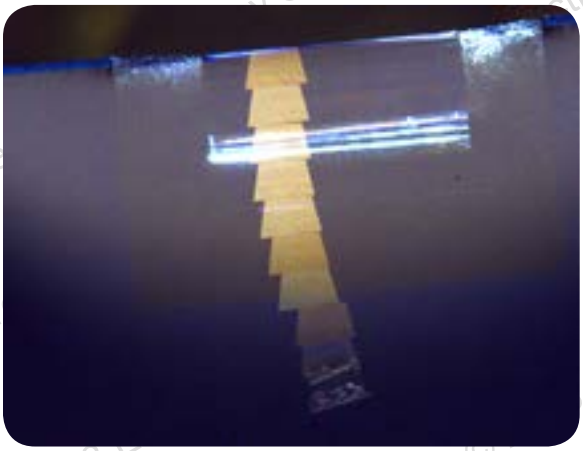
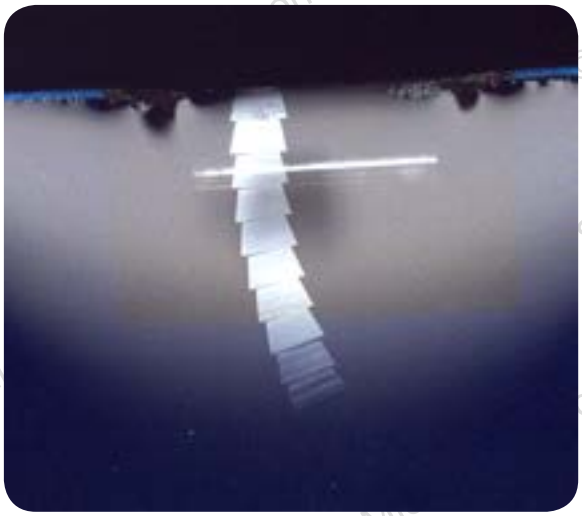
28. Remove any sectioned material from the knife edge with an eyelash manipulator
29. Observe the cutting action, the sections should form a straight ribbon, with the same interference color, and lacking any chatter bands or knife marks within each section.
30. After a ribbon (~ 10 sections) is formed stop cutting, leaving the specimen arm down.





Three Techniques for Retrieving Thin Sections

There are 3 basic techniques used to get thin sections on grids for TEM observation: Top Down, Scoop, Perfect Loop. Each technique has its advantages and disadvantages.

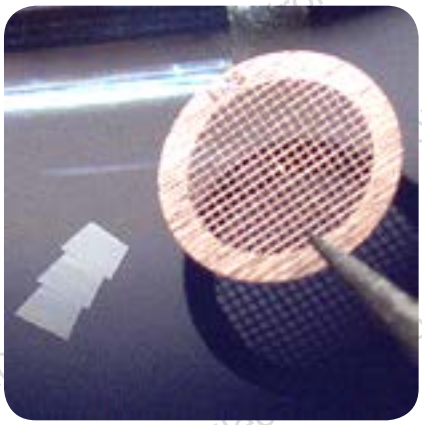
Either side of the grid can be used for picking up but be consistent with which side you use.

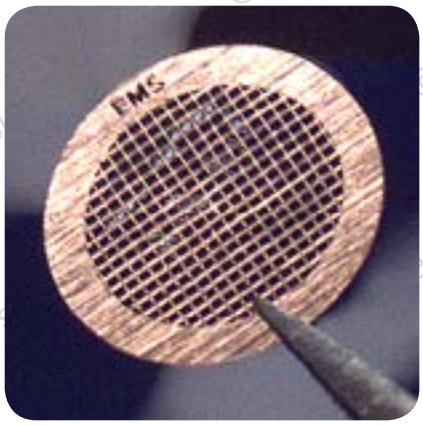
Top Down

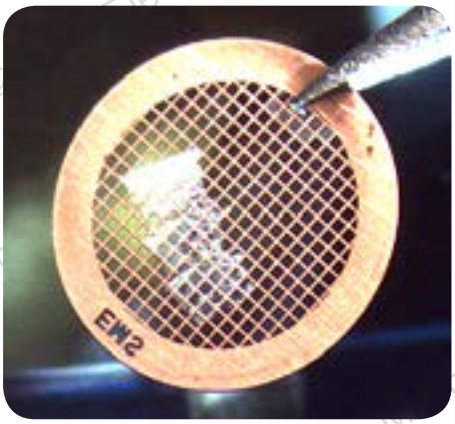
1. From the ribbon, using the eyelash, carefully dislodge about 3-4 sections from the back (end away from the knife edge) of the ribbon and float to the middle of the boat.
2. Take a grid in the tweezer and touch it down onto the floating sections and lift the grid up from the surface of the water.
3. Place back of grid down on a piece of filter paper to dry then place in grid storage box.

Advantage: Easy, with little technique required.
Disadvantage: Section placement on grid is random and may cause wrinkles or folds.

Dislodge sections from ribbon ... place grid over sections and push down onto sections ... lift out of the water ...



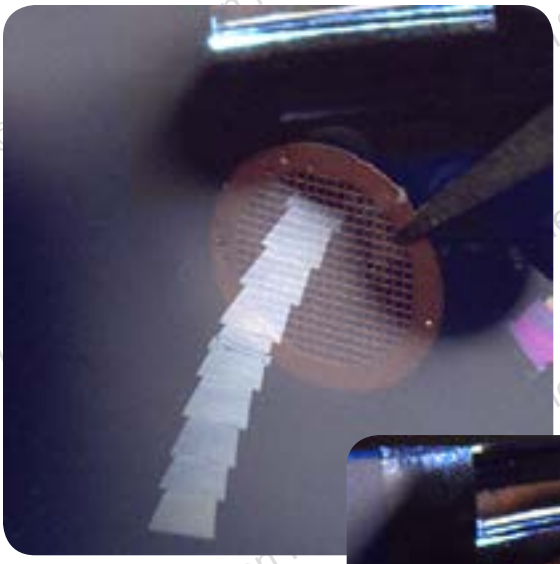




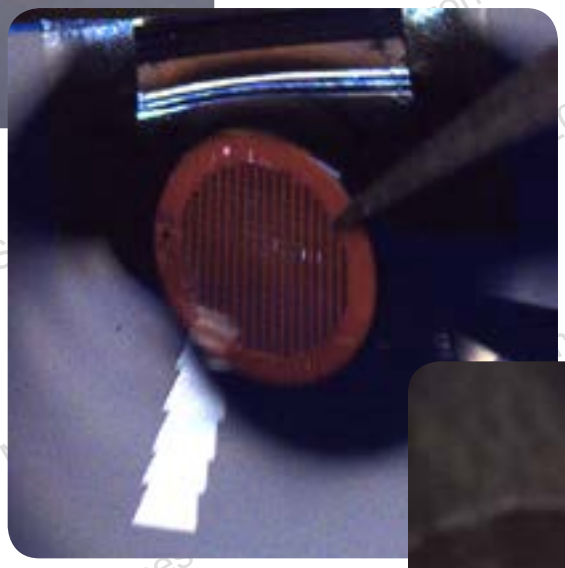
Scoop

1. Dislodge about 3-4 sections from the end of ribbon farthest from the knife edge.
2. Taking a grid in the tweezer immerse it vertically into the middle of the boat.
3. Nudge the sections over to the immersed grid with eyelash manipulator..
4. Keeping the grid almost vertical raise the grid catching a small portion of the first section on the edge of the grid.
5. Lift out and placing a small wedge of filter paper next to the grid at the tip of the tweezer release it onto a piece of filter paper or directly into grid storage box.

Advantage: You have more control as to where the sections will be on the grid; fewer wrinkles than the Top Down method.
Disadvantage: Needs a very steady hand and practice for good technique.

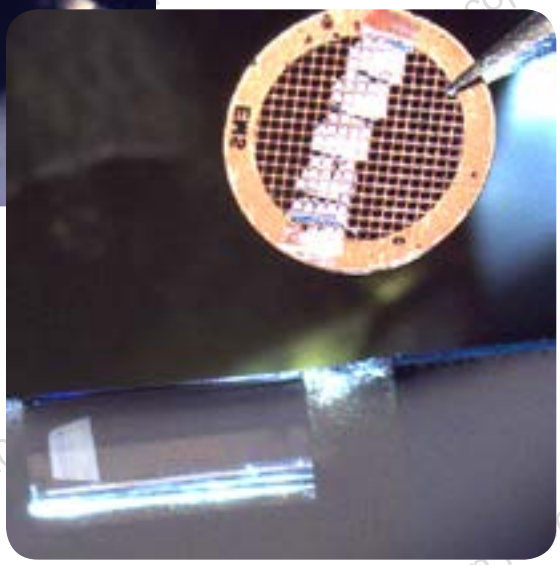


Immerse tweezer vertically in water ...



Nudge sections towards submerged grid ...

Raise grid vertically out of water.



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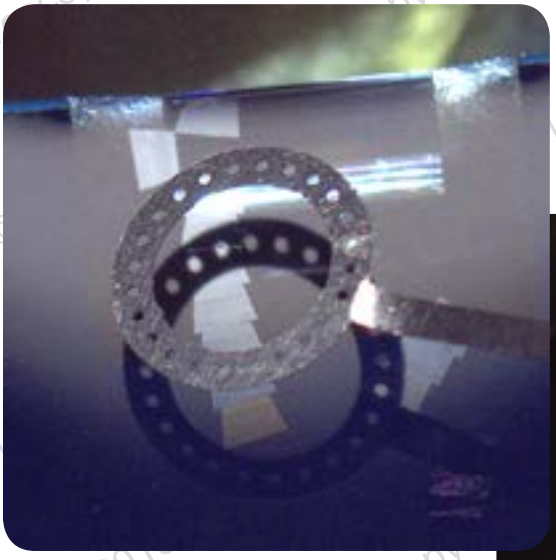
Perfect Loop

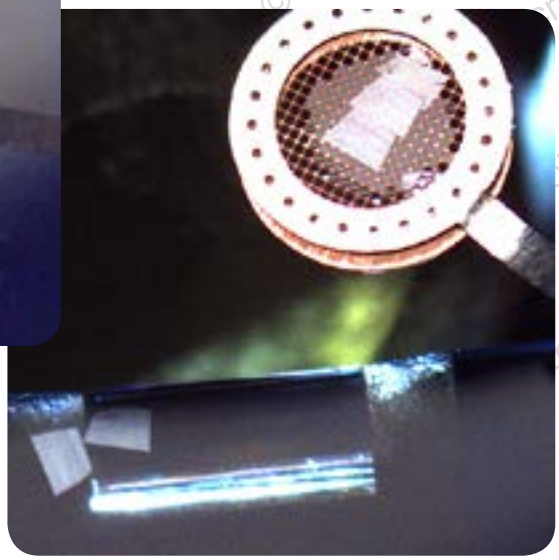
1. Dislodge about 3-4 sections from the end of ribbon farthest from the knife edge.
2. Center the Loop over the sections and lower it over the sections and touch the water.
3. The sections are now in the droplet of water in the loop.
4. Lower onto a grid sitting on filter paper. The filter paper absorbs the water and the sections adhere to the grid.
5. The grid may stick to the Loop, grab the grid with the tweezer and place in the grid storage box.

Advantage: Easy to do; fewer wrinkles.
Disadvantage: Position on grid variable.

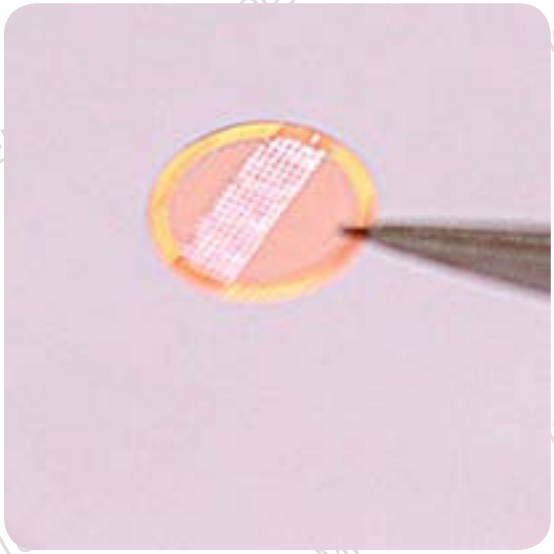


Center loop over sections ... lower over sections and touch the water ...

... lift out of water .. place over grid and touch ...



... let dry on filter paper ...



...store ...

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