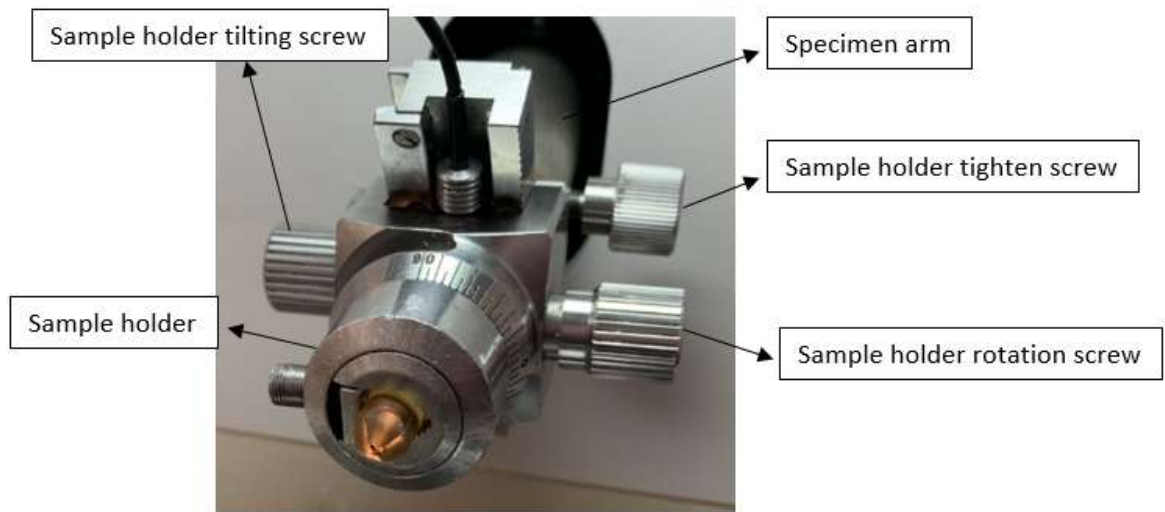
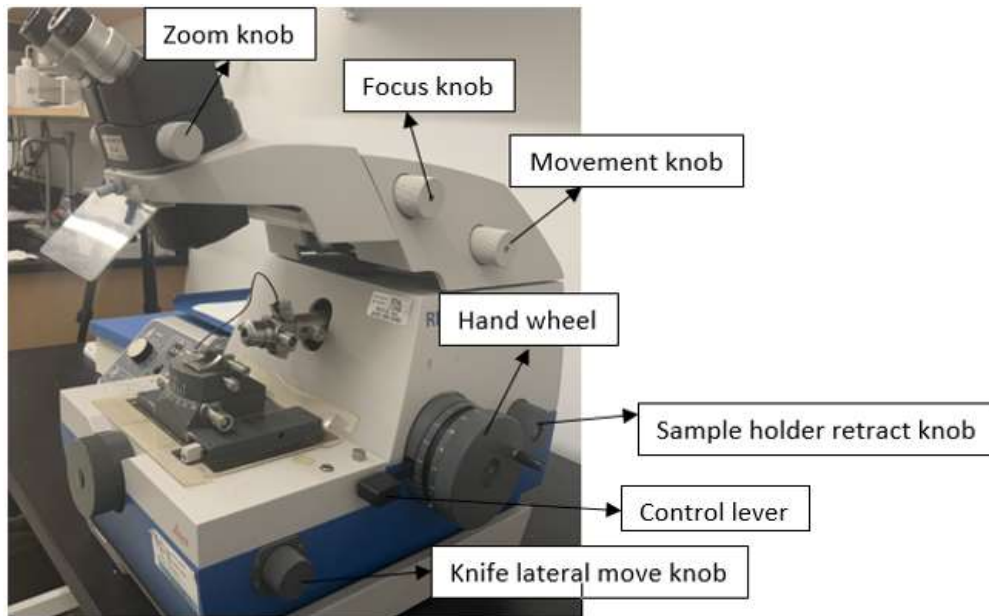


Using Reeichert-Jung Ultracut E Ultramicrotome to cut semithin section and ultrathin section

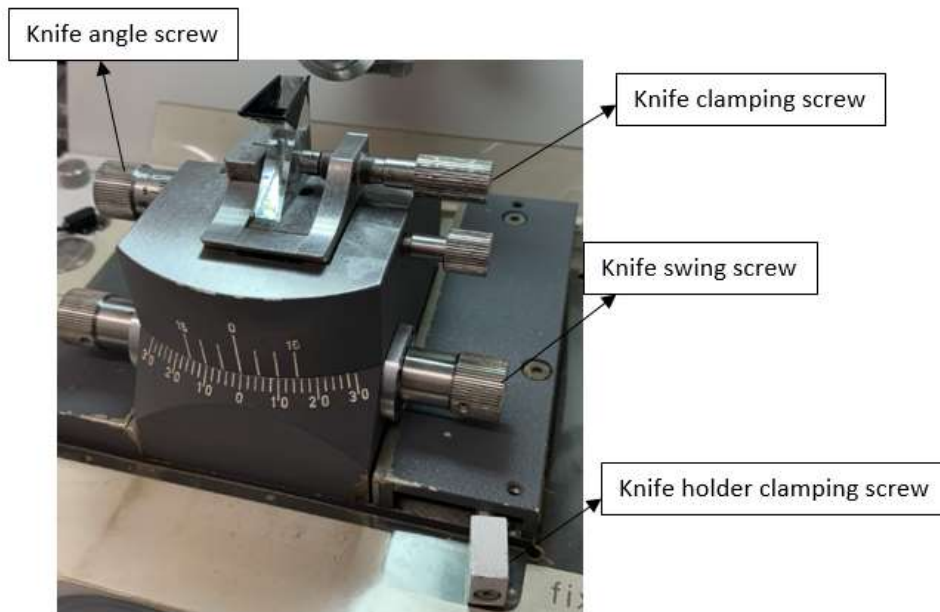
1. Put a trimmed block into the sample holder and clamp securely
2. Secure the sample holder in the specimen arm by tightening the sample holder tighten screw. Adjusting the sample holder tilting angle to 0° by sample holder tilting screw.



3. Turn on microtome by pushing the green button .
4. Turn the sample holder retract knob clockwise until it stops, then turn it counterclockwise one quarter of a turn.



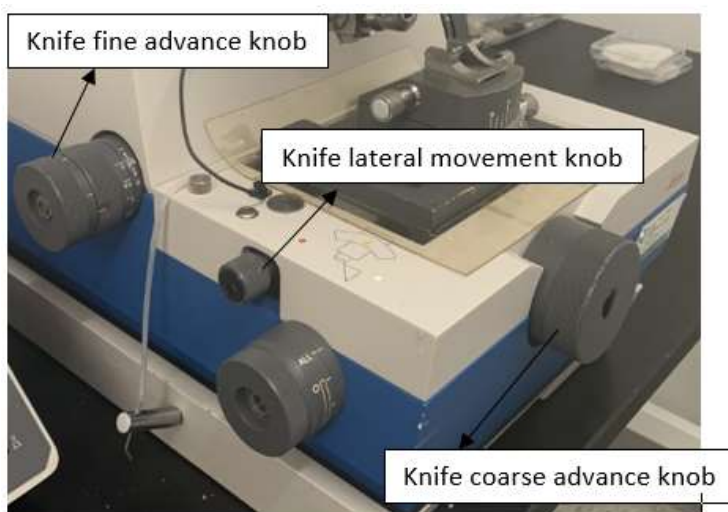
5. Put a glass or diamond knife on the knife holder and securely tighten the knife by knife clamping lever.
6. Make sure the knife angle is 6° . If not, adjust the knife angle to 6° by knife angle screw.



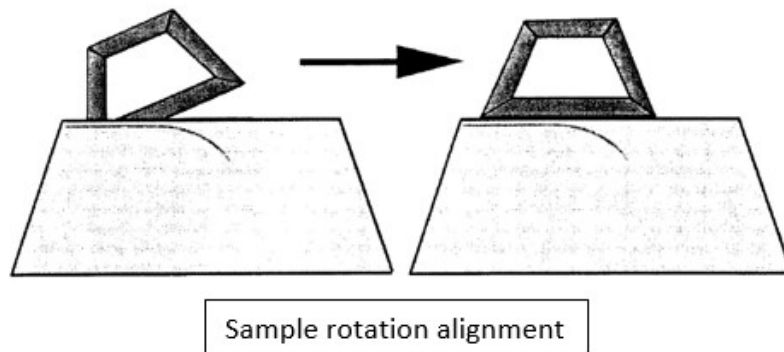
7. Bring the knife holder stage to within several millimeter of the block field in the specimen arm by looking from the side of the ultramicrotome.
8. Look through the microtome microscope and inject water into the knife boat, adjust the water level until a mirror like area on the water surface can be seen behind the knife edge and the knife edge is completely wet.
9. Use the hand wheel (on the right side) to bring the specimen to the same level as the knife edge.
10. Carefully move the knife forward until it is within about 1mm of the specimen block and lock the knife securely in place by the knife holder clamping screw.

11. Align the sample and knife:

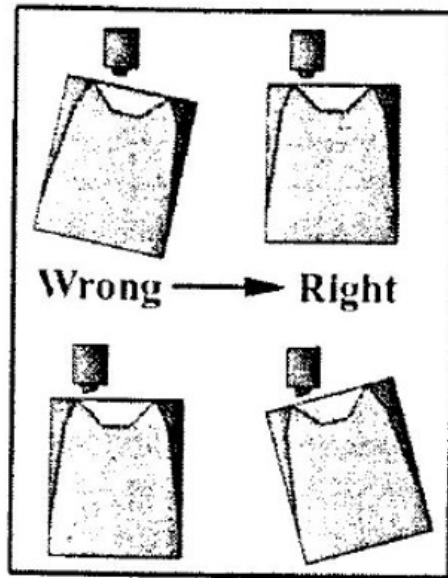
- 1) Look through the microscope, use the knife coarse advance knob to carefully move knife forward until a **reflection** of the knife can be seen on the sample block face. Use knife lateral movement knob to move the knife left or right to choose the portion of the knife, which will section the sample block face.



- 2) Rotate the sample block by sample holder rotating screw until the bottom of the block face parallel to the knife edge.

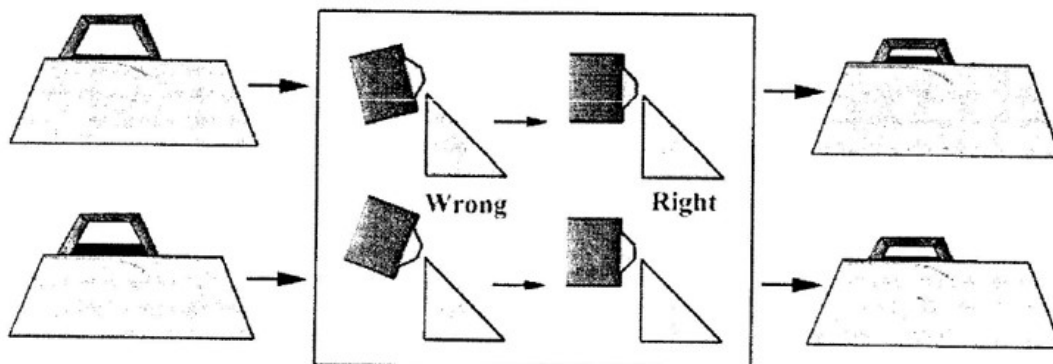


- 3) Rocking the sample up and down by the hand wheel to observe the knife edge reflection on the block face. Swing knife left or right by the knife swing screw to make the knife edge parallel to the block face (back off knife a little if needed before adjusting to avoid pump the sample block face).



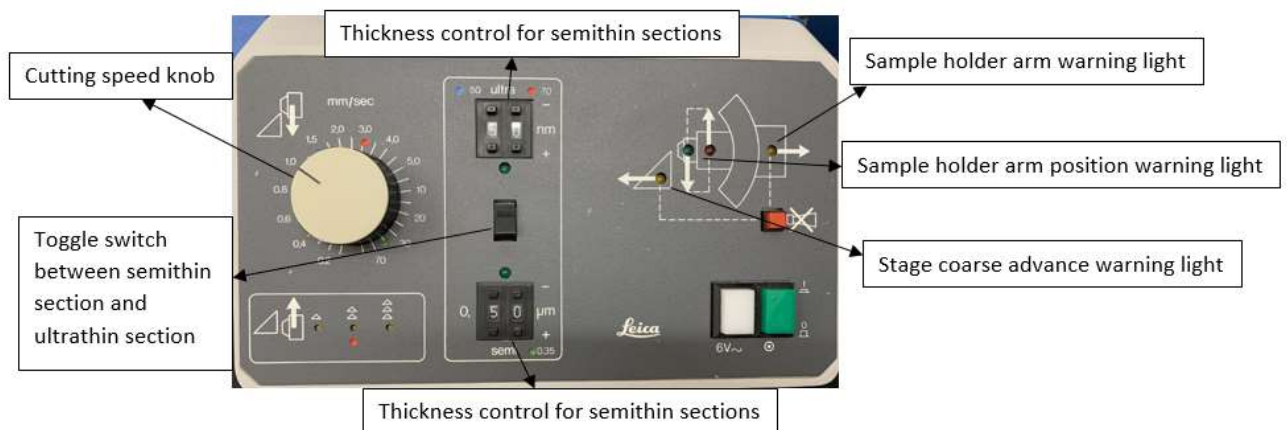
Knife tilt alignment

- 4) Tilt the sample block face up or down by the sample holder tilting screw to make the knife edge reflection on the top of the block face is equal to which on the bottom of the block face. After all the adjustments, the knife edge reflection is even side to side and stays the same width as the block is passed by the knife edge.



Sample tilt alignment

12. Slowly move forward the knife (using knife coarse advance knob) until the knife edge reflection just disappear.
13. Set cutting window: turn the hand wheel so the sample is on the forward cutting stroke. Lift the control lever (on the right side of the microtome). Turn the hand wheel until it stops. Then turn the tight feel hand wheel up or down until the top edge of the block face is just below the knife edge. Lower the control lever to its original position.
14. **To cut semithin sections**, set cutting speed at 2mm/sec by cutting speed knob, press down the toggle switch for semithin and ultrathin to select semithin section thickness control, select semithin section thickness (eg. 0.5 μ m).



15. Depress the control lever, microtome will automatically start to cut. When the first complete section is cut, continue cutting 4-10 sections.
16. **To begin cut ultrathin sections**, retract the knife stage away from the block face, then move the knife stage laterally to a fresh and sharp knife edge and reapproach the block face as in step 11. Make sure the water level in the knife is enough as described in step 8.

17. Reset the cutting window as in step 13.
18. To cut ultrathin sections, select set cutting speed at 1mm/sec by cutting speed knob, press up the toggle switch for semithin and ultrathin to select ultrathin section thickness control, select ultrathin section thickness (eg. 70nm).
19. Depress the control lever, microtome will automatically start to cut. When the first complete section is cut, continue cutting 4-10 sections. Lift the control lever to its original position, microtome will stop. Then use eyelash tool to move the last section away from the knife edge if needed and collect the sections.
20. When finished, loose the knife clamping screw, back off knife from the sample clock, take off knife to store or discard in the sharp container. Take the knife holder off the microtome. Take the sample block off the sample holder. Turn off the microtome (left side switch).