Sectioning

2011年12月12日
15:22

Ziru Li

Instructions for sectioning with an RMC microtome

Last modified 06/17/09

Thick sections for light microscopy

1. Turn on the microtome and the two lighting system on the microtome.
2. Insert the sample block into the sample holder, and then place the sample holder onto the trimming block. With a single edge razor blade, trim the sample block into a pyramid without removing any of the plant tissue. The only exception is at the top where the block should be trimmed until a thin slice of the plant tissue has been trimmed off.
3. Replace the trimming block with the sectioning block and place a small metal piece in the glass knife slot (if using glass knife). For glass knives, set clearance angle to 4 degrees. Take down the sample holder from the trimming block and put it on a bigger sample holder for sectioning.
4. Fill the glass knife boat with water until water is touching the edge of the knife blade. Make sure that water surface is not bulging at the same time as too much water can also cause problems.
5. Adjust the pedestal to left/right to choose the best part of the blade, and then adjust angles on the sample holder and the pedestal so that blade is aligned with the surface of the block that is about to be cut. What I mean by "aligned" is when the block moves up and down we are likely to see an entire, nice section to come off the block. Note though, the direction in which the block moves is not parallel to one side of the glass knife because of the clearance angle.
6. Set the cutting window and start cutting. Section thickness is usually set at 500nm and cutting speed is usually not a problem (as long as it is not too slow or too fast). The initial setting of 0.7mm/sec is good.
7. Advance forward slowly until it starts cutting. Obtain smooth, entire sections with a wooden rod/metal probe (take care not to touch the edge of the blade) and place them on a slide precoated with distilled water.
8. Put the slide on a hot plate preset at ~ 60-70C and let the water evaporate for 2-3 minutes. The sections will stretch out and stick to the slide once the water evaporates.
9. Stain the sample with an appropriate general dye such as toluidene blue or methylene blue for an appropriate length of time on the hot plate (toluidene blue for 15 seconds and methylene blue for 2 minutes). Rinse off the dye with distilled water and observe on a compound microscope.

Thin sections for electron microscopy

10. Trim the sample block further until the section area is ~ a tiny square with each side's length equivalent to 1.5x leaf thickness.
11. Repeat steps 3-6.
12. Set the cutting window and start cutting. Optimum section thickness is 70nm but unless you are extremely careful this is usually not attainable (the sections give off a silver color when floating on water due to interference). If sections are 90-120nm thick they are OK to be viewed under TEM (they have a golden appearance). If the sections look purplish that means they are >130nm thick and are not of the best quality, although (mediocre) images can still be retrieved from these sections. Play with the sectioning speed and section thickness to obtain the best sections. With regard to sectioning speed, anywhere from 0.4-1.3 could give a good result but there is no guarantee a single speed would give the best result. This is a trial-and-error process.
    - Note: If the sample block is tough, diamond knives would work much better than glass knives. For these blocks, obtain thick sections with glass knives and thin sections on diamond knives.

13. Section until enough sections has been obtained (usually this is when the boat is 1/4 – 1/2 full). Pull all the nice sections together with an eyelash (attached to a wooden applicator) and pick up the sections with copper grids. Legend has it that the brighter side of the copper grid is better at picking up and adhering to the sections. Dry the copper grids on a filter paper.