General Sectioning Protocol

This is a general guideline of how to use a microtome with samples embedded in resin, settings may vary depending on the microtome.

- 1. Switch on microtome and set up for sectioning.
- 2. Remove block from capsule using a razor and metal block.
- 3. Place the resin block into the sample holder and the holder into the trimming block.
- 4. Trim the resin block to roughly 1mm around the sample in a trapezium shape. Do the initial trim with a single edged razor and a second trim with a double edged razor to ensure clean straight edges.
- 5. Place the sample holder into the specimen arm and position, making sure the angle is correct.
- Place the knife into the knife holder and the holder onto the stage. Ensure everything is screwed tightly into place and the knife holder is as far back as it will go.
- 7. Ensure the angle of the knife is angled at 6° and fill the boat with enough water to reach the knife edge (a brush can be used to push the water toward the knife edge).
- 8. Position knife to specimen block, use the finer adjustment (often on the left with newer microtomes but possibly at the front with older models) to get the knife and the block as close as possible without damaging either the block or knife. Line up the knife so the bottom edge is parallel with the block. Also check the angle of the knife is straight.
- 9. If available, set the cutting window and the trimming size/cut width.
- 10. Check everything is lined up correctly by taking a few slow hand sections.
- 11. Begin sectioning, resetting the specimen arm every so often, keeping the water topped up and clearing off sections as they come through.
- 12. Once you have reached the sample test slides can be made to see if the sections are good. Place a drop of water onto the slide, and carefully lift the section out of

- the water boat onto the drop using a paintbrush. Place the slide onto a hotplate to dry and then stain to see your section.
- 13. When happy with the sections, final ones can be taken using the same method for light microscopy. TEM sections need to be stretched using chloroform and collected on TEM grids.
- 14. Once sectioning is finished remove capsule from holder and knife from knife block (if glass knife put into glass waste) switch off microtome, cover and clean up area.