PROTOCOL FOR SERIAL PARAFFIN SECTIONING

1. Fix specimens at least 24 hours in FAA. After 24 hours, they may either be transferred to 70% ETOH for storage or kept in FAA. If specimens fail to sink when placed in the fixative, they should be placed under vacuum in a vacuum chamber for 15 minutes to remove all air.

2. Transfer specimens from fixing solution to TBA 1 (note: TBA = tertiary butyl alcohol); after 1 hour in TBA 1 (=40% ETOH, 10% TBA, 50% dH_2O), begin dehydration through the graded TBA series, with a minimum of 1 hour in each of the solutions through TBA 5. [Note: TBA solutions are as follows: TBA 2 = 50% ETOH, 20% TBA, 30% dH_20; TBA 3 = 50% ETOH, 35% TBA, 15% dH_20; TBA 4 = 40% ETOH, 55% TBA, 5% dH_2O; TBA 5 =25% absolute ETOH, 75% TBA; TBA 6 = 100% TBA.] The specimens may be left overnight in either TBA 2, TBA 5 or TBA 6. TBA 5 has a few grains of eosin Y stain in it to stain the specimens for orientation. At all stages of the dehydration, the vials should be tightly capped to prevent evaporation of the alcohols.

3. Specimens should remain in TBA 6 overnight, on top of a slide warming tray. Absolute TBA solidifies at about 20 ° C, so it must be kept above this temperature to remain liquid.

4. The next morning, replace the TBA in the vial with new TBA 6 to half-full. Then add paraffin chips to fill the vial, cap it, and place it back on the slide warming tray for 24 hours. The paraffin will begin dissolving into the TBA under these conditions, slowly infiltrating the tissues with a mixture of paraffin and alcohol.

5. The next day, uncap the vial and place it in a 56° C oven. The paraffin will melt and the TBA will slowly evaporate overnight. Leave undisturbed for 24 hours.

6. The next day, make two paraffin exchanges, one in early morning and the second about 3 hours later. To make a paraffin exchange, remove the vial from the oven and carefully, but quickly, decant the paraffin off into a disposal can next to the oven. Refill the vial with fresh melted paraffin from the oven. The specimens will be ready to embed by late afternoon of this day.

7. To embed, decant melted paraffin to about half-fill an embedding boat. Flame a spatula and move it across the surface of the paraffin in the boat to keep the surface melted, while the paraffin begins to solidify at the bottom of the boat. Once the bottom fourth of the paraffin has solidified, quickly decant the vial, with specimen, into the boat. Use a pair of flamed needles to orient the specimen into the proper plane for sectioning. Sections will be prepared from the bottom of the boat upwards, so plan your orientation accordingly. After the paraffin has solidified around the specimen, float the boat in a dish of ice water to complete the solidification process.

8. The paraffin embedded specimens are now ready to be mounted onto sectioning stubs or wooden blocks, trimmed, and sectioned with a rotary microtome following standard procedures.