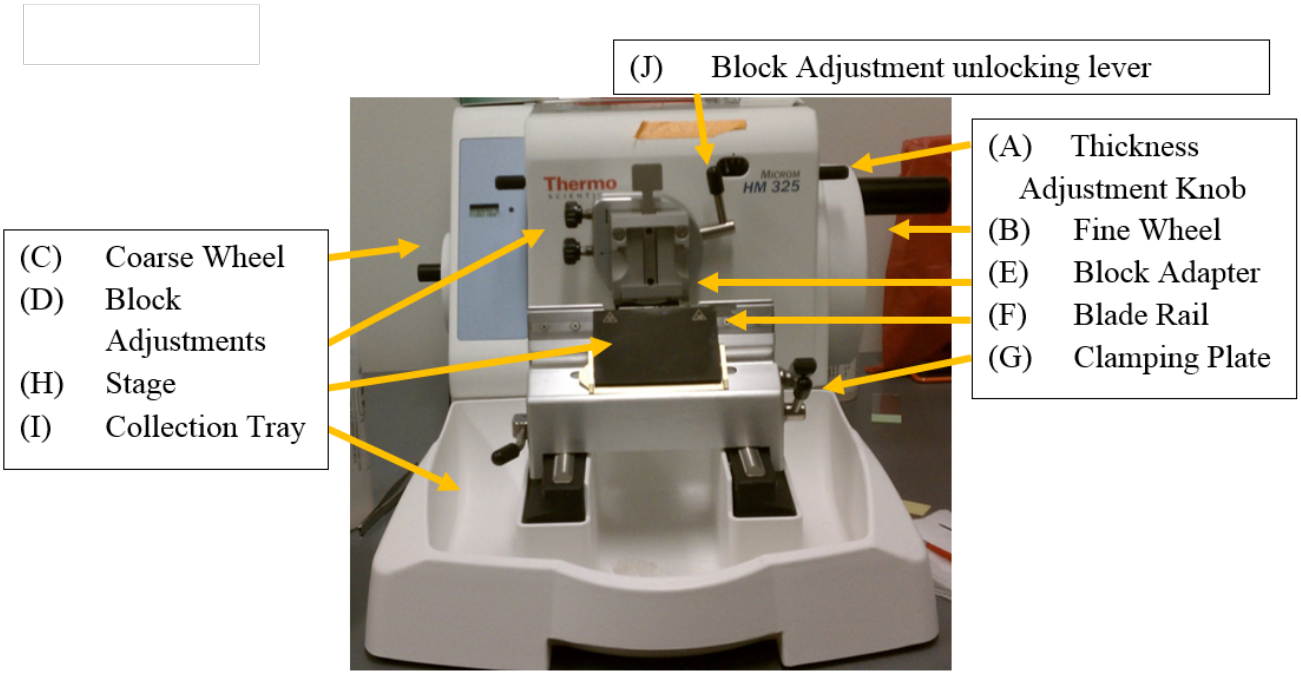
Sectioning Embedded Tissues with the Microtome

Equipment and Materials:

Microtome, Thermo Microtome HM 325
Microtome Blades
Dissection probe

100% Ethanol
ITO Sample Slides, Delta Technologies Cat# CG-81IN-S115
Glass Histology Slide, FisherBrand ColorFrost Plus, Cat# 12-550-18
Razor blade
KimWipe
Room Temperature Milli-Q Waterbath
45-50°C Milli-Q Waterbath
Heating Plate
ParaGard Paraffin removing solution Thermo, Cat# 350170



Procedure:

Pre-cutting preparation:

1. Place the FFPE block in a beaker filled with ice slurry for 1 hour.
2. Set up a water bath using Milli-Q water at room temperature.
3. Set a second water bath water to 45°C using Milli-Q, as indicated by a black mark on the hot plate's temperature knob. Set the stir bar to slowly stir the warm water bath.

Setting up the Microtome:

1. Adjust the thickness setting to your desired thickness using knob A. Most applications use 6 µm.

2. Clean a razor blade, and dissection probe, with EtOH and set aside. (This will be used to separate successive slices).
3. Move the Block Holder to the furthest point back using the coarse adjustment wheel, C, turning clockwise.
4. Remove the Microtome blade, from holder and clean it with EtOH.
5. Place the blade into the rail, F, on the microtome with the knick facing you. Then lock the blade into place with the lever on the right, G, pushing back until tight. Note: Over-tightening will deform the blade.

Sectioning:

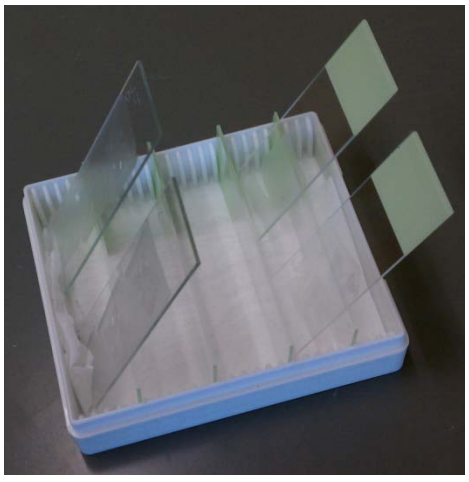
1. Place a piece of gauze into the ice containing the tissue block.
2. Remove the FFPE block from the ice and dry it using a KimWipe
3. Place the FFPE block into adapter, E.
4. Using the levers to the left of the adapter, D, make the front face of the FFPE block level and flat so you have an even face for sectioning. In order to adjust these levers, you must first unlock them using the lever to the right of the adapter, J.
5. Move the FFPE block forward using the small knob on the left, C, until the tissue block is next to the blade.
6. Start rotating the large knob, B, to the right of the instrument to get the tissue block to move up and down (sectioning).

7. Commence cutting until there is a ribbon of sections which have the desired tissue in them. Dispose of this ribbon into the collection tray, I.
8. Start cutting again until you have a ribbon of tissues 5 to 6 sections in length.
9. Carefully transfer the ribbon to the room temperature bath using either your fingers or a clean brush.
10. Turn off the stir bar in the warm water bath.
11. Separate the sections in the ribbon using either the razor or Dissection probe. One recommended collection is 1 for H&E and as many as you need for analysis. This depends on your application.
12. Using a glass slide, transfer the needed sections from the room temp bath to the warm bath.
13. Now, using either an ITO slide or a (+) labeled histology slide (depending on your need), pick up the desired number of sections onto the slide. Do this by dipping the slide into the water bath and approaching the section. When the section is lined up with the slide, pull the slide out at a slight angle bringing the tissue section with it.
14. Store the plates vertically so water can drip from them.
15. Continue this process until you have the needed number of sections. Every 30 sections or so, rinse the blade and stage with EtOH to prevent sticking. Also, at this time use the gauze which has been on ice to cool the FFPE block in order to aid the cutting process.

After Cutting:

Place the samples in the oven at 37°C overnight in the vertical position. This position can be achieved by using a slide box and placing blank glass slides at a spacing of about 5 slides and standing your samples against them. After which point, the samples will be ready for paraffin removal and staining or antigen retrieval.

Alternatively, you can dry for 1 hour at 55°C, this is less desirable but it will depend on the application.



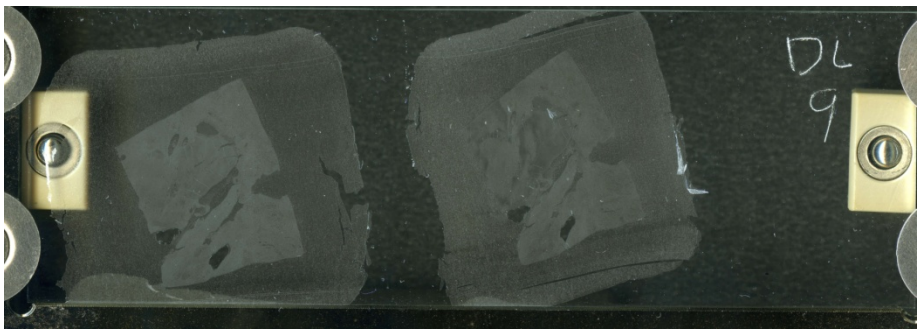
Cleaning the Microtome.

1. Remove the blade from the rail and properly dispose of it.
2. Dispose of all remnants of the sectioning process in the biohazard waste.

3. Rinse the surface of the stage as well as the collection chamber with EtOH. Any part of the microtome which may have come into contact with the tissue must be cleaned. Also any tools which you used must be cleaned.
4. If there is too much paraffin build up, use ParaGard to clean the stage and surrounding areas. When finished, wipe with EtOH.

Expected Outcome:

Once the sectioning is finished and placed on an ITO slide, this is what you will see.



References:

Microtome Manual

Prophet, E. B.; Mills, B.; Arrington, J. B.; Sobin, L. H. Laboratory Methods in Histotechnology; American Registry of Pathology: Washington, D.C., 1994.