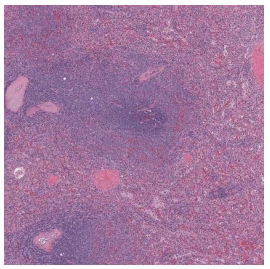
Dec 13, 2019 Version 2

HuBMAP Tissue Sectioning for FFPE Specimens V.2



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Protocol status: Working
We use this protocol in our group and it is working.

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Abstract

This method details our microtomy (sectioning) process for reasearch specimens involved with HuBMAP.

This process follows the microtomy process that follows after receiving, processing, and embedding of research specimens into formalin fixed, paraffin embedded tissue blocks.

Guidelines

- Managers and supervisors - are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.

- Laboratory personnel - are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs.

Materials

MATERIALS

- Water
- KimWipes **Fischer Scientific**
- Shandon™ Cartilage Curved Thumb Forceps, Curved, Fine Point, standard, 5 in. (12.7cm) **Thermo Fisher Catalog #**1631TS
- Flotation Bath **Fisher Scientific**
- Microtome Blades (Thermo Scientific Ultra) **Thermo Scientific Catalog #**3053835
- Gauze 4×4 Non-Sterile Squares **Fisher Scientific Catalog #**MSD-1400249
- Microtomy Brush **Cancer Diagnostics Catalog #**SLK1000
- Microtome **Diagnostic Pathology Catalog #**HM 315 / HM 325
- Fisherbrand™ Superfrost™ Plus Stain Slides **Fisher Scientific Catalog #**22-034979
- EZ-QUIK SLIDE STAINING RACK **Fisher Scientific Catalog #**NC0103846
- Tissue-Tek® Cold Plate **VWR International (Avantor) Catalog #**25608-942
- Moist Mark Plus Slide/Cassette Marker **Cancer Diagnostics Catalog #**SKU: MP2100

Safety warnings

- Use extreme care when working with microtome blades. They are extremely sharp.
- Use physical safety precautions when working with sharps (disposable blades).
- As these specimens are fixed in formaldehyde, gloves and other PPE are optional / personal preference.

Before start

- Ensure you have proper slides, blades, forceps, and your personal preference of gauzes/wipes

Microtome Preparation

1 Locate your tissue flotation bath / water bath and fill it completely with de-ionized or purified water. 5m

2 Turn the flotation bath on, and set the water temperature to 42°C. 1m 30s

42 °C Flotation Bath

Note

At this step, also inspect the microtome you are planning to use; Ensure it is clean, well maintained, and set at the correct angle.

Tissue Block Preparation

3 5m

- The best practice for most sectioning is to place a few paper towels or Wypall on top of the tray and dampen it with water.

Cold Plate Example: Sakura Tissue Tek Poly Cold Plate

Note

- Ensure the tissue blocks you have chosen to section have been correctly embedded, and all wax has been scraped/cleaned off the sides of the block.
- Ensure the tissue blocks have been correctly labeled with proper identification (case #, type, etc)

Tissue Block Facing

15m

4

2m

- After locating the appropriate blades to use for your microtome, carefully select a blade from its' container, and place it inside the microtome's knife holder area. Be sure to secure it in place with the knife clamp/lock spanner.

Microtome blade dispensing from storage box.

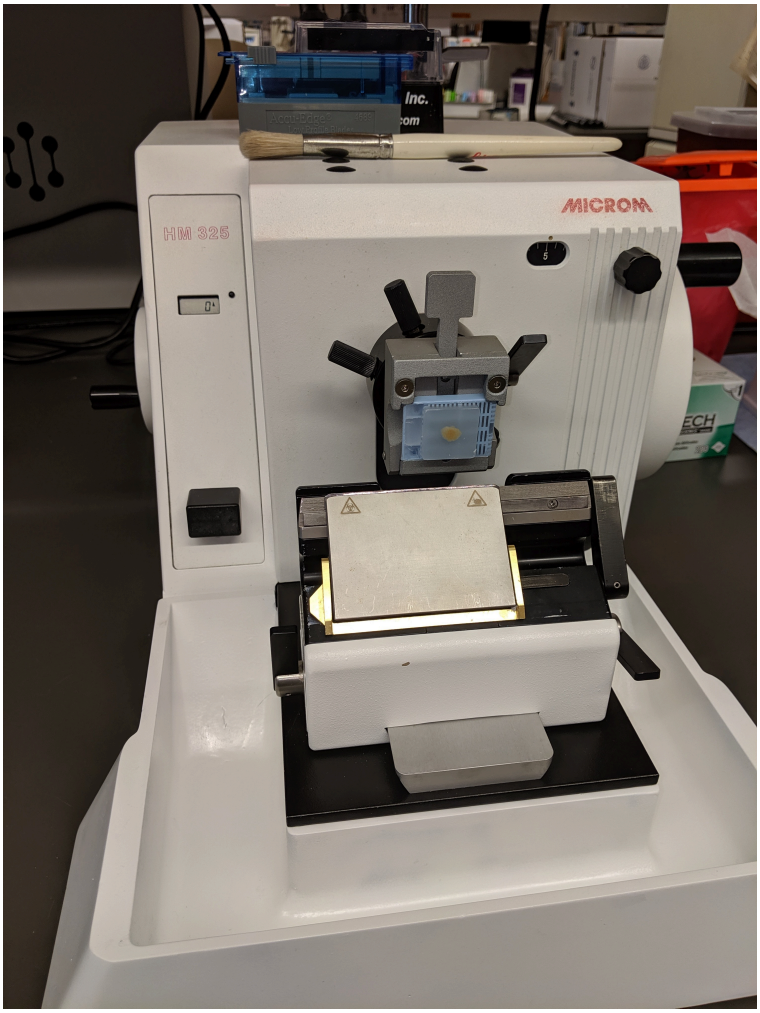
Note

Before putting a microtome blade into the knife holder, BE SURE to utilize the locking lever under the flywheel.

5

After securing your knife and double checking your microtome settings one final time, retrieve a block from your ice tray and secure it in the microtome's chuck (block holder).

3m



A set up microtome, with a secured knife in the knife holder and tissue block in the microtome chuck.

6 After the block is secured, use the coarse advance wheel on the left side of the microtome to carefully, approach the block with the blade and cut a few thin sections to ensure the positioning is correct. Adjust if necessary.

7 Trim gently into the block to expose the tissue surface to a level where a complete representative section can be cut.

Note

Trimming is normally done at a thickness of 10-30 µm. This can be performed with the advance flywheel alone, or a combination of both the advance and coarse advance wheels using the rocking method.

8 After each tissue block has been trimmed to expose a representative surface of the specimen, place each block back on your ice tray. Let each block chill for approximately 10-15 minutes.

Note

Cold wax allows thinner sections to be obtained by providing support for harder elements within the tissue specimen. The small amount of moisture that penetrates the block from the melting ice pieces or ice water will also make the tissue easier to cut.

Slide Preparation

30m

9 5m

- Using a pencil or specialized marker for microscope slides, label each slide you plan to use ahead of time with it's relevant information. (case #, type, test, etc)

Note

- Be sure to locate the glass charged microscope slides that are appropriate for the tissue type or project you are about to perform microtomy for.
- Wear gloves for this step to keep the slides clean of debris/prints, and to prevent ink/graphite from smearing.

Performing Microtomy

2h 30m

10 1m After your tissue blocks have chilled on the ice tray and are very cold to the touch, they should now be suitable for precision sectioning.

Remove your first block from the ice tray and secure it into the microtome's block chuck.

11 1m Using your coarse adjustment wheel on the left, adjust the block holder to be as close as possible to the edge of the knife, but not touching it.

Note

A helpful tip: When you adjust your chuck holder towards the blade and begin to see small water droplets from the condensation from the frozen block accumulate near the knife holder, stop using the coarse advance wheel.

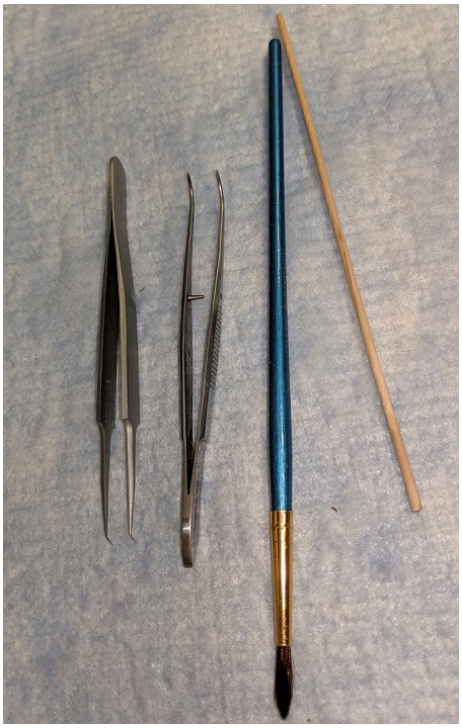
12 Very carefully, unlock and rotate the hand wheel on the right side of the microtome so the block holder is moving up and down in a steady, even manner.
A ribbon of wax and tissue should begin to form down the front of the metal plate covering the knife holder.

Note

- - Double check the micron thickness setting prior to this step. Normal thickness for sections is 4-5 µm.
- - It is normal to have to scrap the first few sections that appear on your ribbon due to holes or other cutting artifacts.

13 After your ribbon has begun to form on the front of the knife holder plate, use a pair of forceps (or whatever your preference is for transferring ribbons from the microtome to the water bath) to gently grip the bottom of the ribbon and another pair of forceps to gently grip the top of the ribbon nearest the blade.

Gently pull the ribbon up and away from the microtome and towards the water bath.



A selection of tools ideal to use for securing and floating tissue ribbons during microtomy.

Lifting a ribbon of complete sections away from the knife holder plate.

Small clip of sectioning a spleen by MJ @ University of Florida

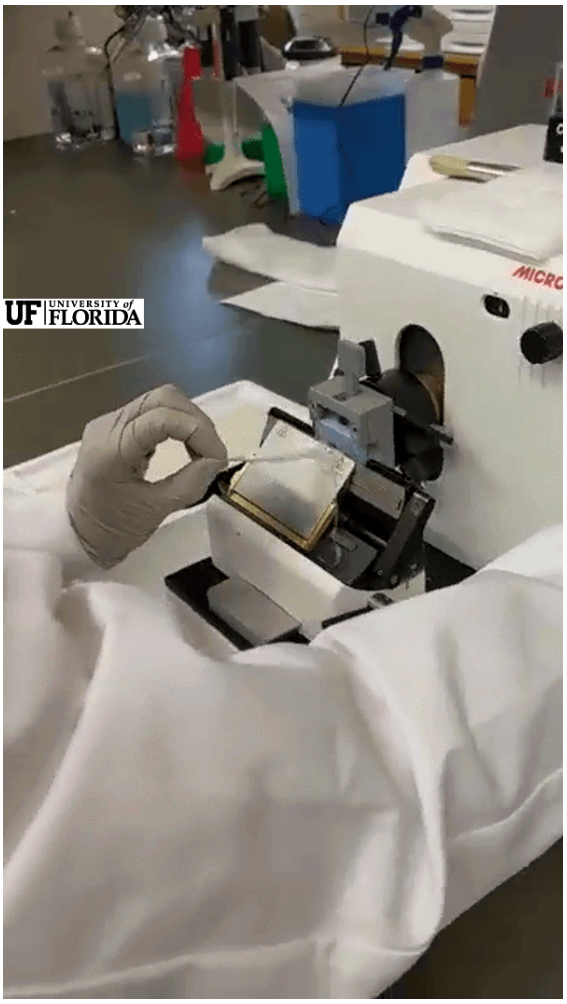
14

- As carefully as possible, shift the ribbon of tissue sections toward your waterbath, and then quickly but gently lay the tissue out on the bath in a "dragging" type motion (either gently towards you, or gently away from you).

- Wait a few moments while the tissue sections sit on the waterbath, as the heated water will help expand any compression and remove some of the wrinkling or folding in the section.

Preparing to place a paraffin ribbon of tissue sections across a water bath prior to creating a slide.

Small ribbon of tissue sections afloat on a water bath - note they are very nice, with no wrinkling, folding, or knife lines.



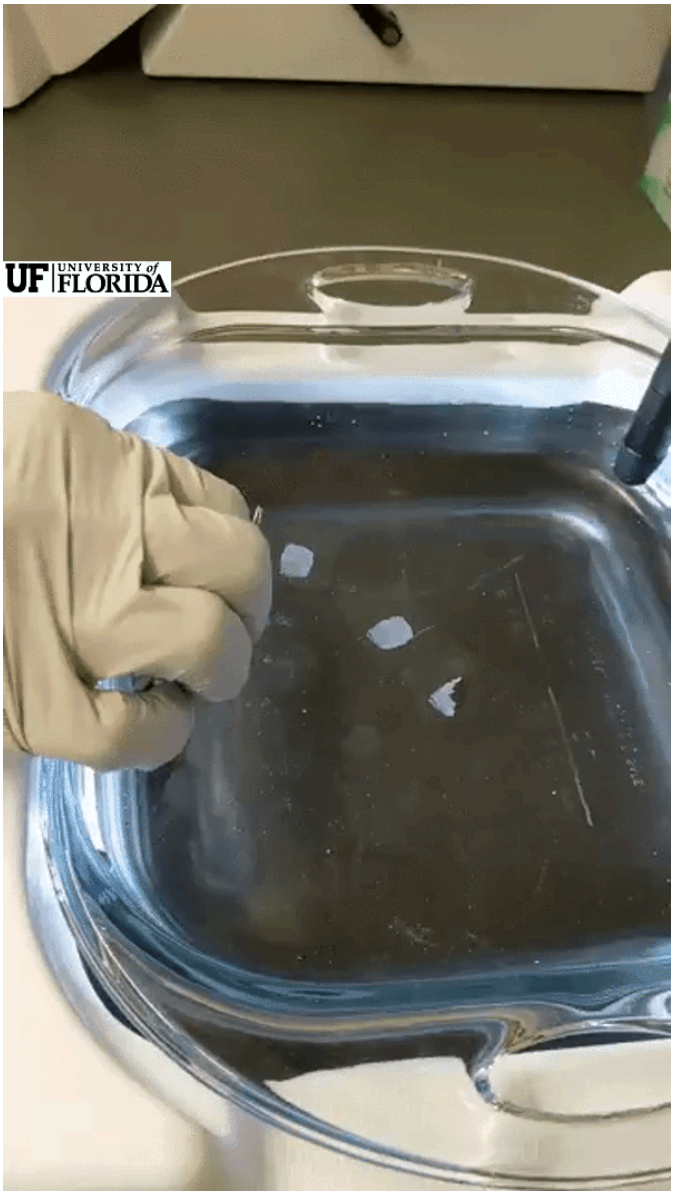
Clip of moving a paraffin tissue ribbon to water bath by MJ @ University of Florida

15 After your paraffin ribbon has floated on the water bath for approximately 20-45 seconds, use your forceps to select the section you wish to keep.

16 After selecting your section, find the appropriately pre-labeled microscope slide that matches the tissue block you sectioned.

16.1 Dip the slide into the waterbath, and place adjacent to and slightly under your chosen section.

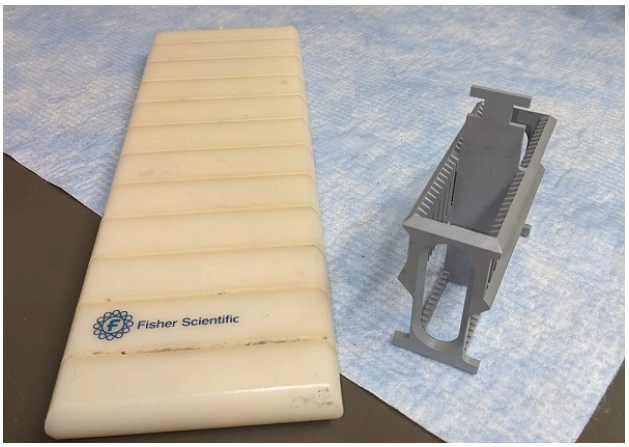
16.2 Adjust the section as desired on the slide with the forceps, and lift upwards out of the water bath, as shown in the clip below.



Clip of putting sections on a slide - MJ @ University of Florida

16.3 Place your newly made slide into a slide drying tray or into a slide staining rack, as pictured below. 1h

After completion, let your slides air dry overnight or dry in a 50°C oven for 1 hour.



A slide drying tray (left) and manual slide staining rack (right).

Cleanup Area

10m

17 2m

- After you have sectioned your last tissue block, unlock the microtome knife blade holder's lever/spanner.
- Using a magnet or forceps, carefully remove the microtome blade and place into the waste container on the blade's box, or in a sharps container.

Note

It is a good practice to clean your microtome if you are done for the day. This prevents paraffin gunk buildup on your microtome, water bath and floor area, in addition to being ideal for safety.

18 3m

Using a microtome brush, brush away all remnants of tissue and paraffin around your microtome, it's catch tray, chuck and behind the knife holder.

Note

A commercially prepared product known as "ParaGuard" can assist with the removal of wax and provide a more efficient microtome clean up. It should be lightly used and vigilantly wiped off with gauze after use.

Example of Paraguard

19 Carefully remove the glass dish from your water bath and dump the remaining water into your nearest sink. 1m

20 Clean the emptied water bath with warm water and dawn dish soap, then replace to the water bath's bench unit. 1m