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Cutting FFPE Sections for Imaging Mass Cytometry

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We use this protocol and it's working

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Abstract

FFPE block are cut into thin sections.

Materials

MATERIALS

- FFPE block
- Rotary Microtome **Thermofisher Catalog #HM355S**
- FFPE Block Cooling Unit **Catalog #TES99.430**
- Stretching table
- Deionized Water
- Microtome Blade
- Brushes
- Glass Slides Superfrost 25×75×1.0mm **Thermo Fisher Scientific Catalog #10.0344.01**

Troubleshooting

Safety warnings

- ! The microtome blade is very sharp, care must be taken to avoid injury.

Cooling the FFPE Block

- 1 **1.1 Cool down the FFPE block**
 - a. Switch on the cooling device and set it to 4°C
 - b. Put the FFPE block into the cooling device.

Switching on the microtome

- 2 **1.2 Prepare the microtome**
 - a. Connect the cooling unit in the back with the cable and switch it on at the front.
 - b. Switch on the microtome using the button at the back of the machine.
 - c. Install the water bath with the filter.
 - d. Add deionised water to the water bath and lower the compartment until the grid is slightly covered with water.
 - e. Place a fresh blade in the blade holder.
 - f. Check the water flow. If water is not flowing rinse the tubing with a syringe filled with water.
 - g. Set the water flow to 3 or 4.
 - h. Fix the precooled FFPE block to the specimen clamp holder.
 - i. Adjust the angle between blade and FFPE block by unlocking the clamping lever and then adjusting the orienting screws accordingly.
 - j. Set the trimming (Trim) and cutting (Feed) thickness by pressing the top knob once or twice, respectively.
 - k. Unlock the wheel with the mechanical hand wheel brake on the right-hand side underneath the wheel.
- 1.3 Switch on the stretching table**
 - a. Switch it on and set it to 37°C.

Cutting

- 3 **1.4 Trimming**
 - a. Always use a new sharp blade.
 - b. Trim carefully and make sure that your cutting angle is fine, if needed readjust.
 - c. As soon as you think you are cutting tissue, take up a cut and have a look under the microscope.
 - d. Exchange the water bath before starting cutting sections that you intend to collect.
- 1.5 Cutting**
 - a. Cut in a uniform and gentle rotation.
 - b. Let the cut slide down into the water bath.
 - c. Wait until the cut is flattened before taking it up on a glass slide.



- d. Wipe the glass slide dry on the bottom side before placing it on top of the stretcher.
- e. Exchange water on a regular basis if sample produces a lot of paraffin debris and between samples.

Switching off

4 1.6 Switching off the microtome

- a. Block the wheel to avoid any injuries as soon as you are done cutting
- b. Dispose of the blade
- c. Stop the water flow by turning the wheel down to 0.
- d. Discard the water, clean the water bath, filter and grid, place them to dry.
- e. Clean the water slide.
- f. Clean the microtome from paraffin debris.
- g. Switch off the microtome at the back and disconnect the cooling block.
- h. Switch off the FFPE Block cooling device.

Section drying and storage

5 1.7 Drying sections

- a. Dry the sections overnight or for **1-2 hours** at 37°C. Do not extend the drying for too long.

1.8 Storage of FFPE sections

- a. Store all FFPE fresh cut and dried sections at -80°C or stain them within a week.