Tissue Sectioning Guidelines (Fresh Frozen)- CODEX/Phenocycler-Fusion

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We use this protocol and it's working

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ABSTRACT

The purpose of this SOP is to outline the techniques for sectioning and storage of fresh-frozen tissue samples for PhenoCycler-Fusion experiments

GUIDELINES

Fresh-frozen tissue sections are mounted directly onto slides. Appropriate preparation and storage
of tissue sections are critical to ensure sample integrity.

- Tissue sections adhered directly onto slides can be stored at -80°C for up to 6 months before

staining.

- Tissue thickness must not exceed 10 µm as this can affect the autofocusing capabilities of the

microscope.

- For best results, tissue sections should be completely adhered to the slide without folds or tears.
- To ensure that tissue sections are not damaged, it is critical that the tissue slides are not

stacked on top of one another

MATERIALS

1. Frozen tissue block of interest
2. Dry ice
3. 1” x 3” positively charged glass microscope slides. NOTE: Please do not use extended frost/ extended label versions of the slides. These slides will interfere with proper adhesion of the flow cell or result in flow cell breakage
4. Tissue sectioning blade - 63069-LP Low Profile Microtome Feather® Blade by Electron Microscopy Sciences
5. Cryostat

PREPARE CRYOSTAT CHAMBER

1 Standard cryostats with temperature control are recommended for tissue sectioning. Most tissues are sectioned in temperatures ranging from -15°C to -25°C. The exact temperature is unique to each tissue type and should be determined according to standard sectioning procedures.

FRESH-FROZEN TISSUES - SECTIONING PROCEDURE

2 Set the cryostat chamber to tissue-specific temperature range.

3 Place the slide storage box in the cryostat chamber to equilibrate to the cryostat temperature.

4 Once the cryostat reaches the programmed temperature, transfer the tissue from the - 80°C freezer to the cryostat using a container filled with dry ice.

5 Use compressed air to remove dust and lint from the slides before use.

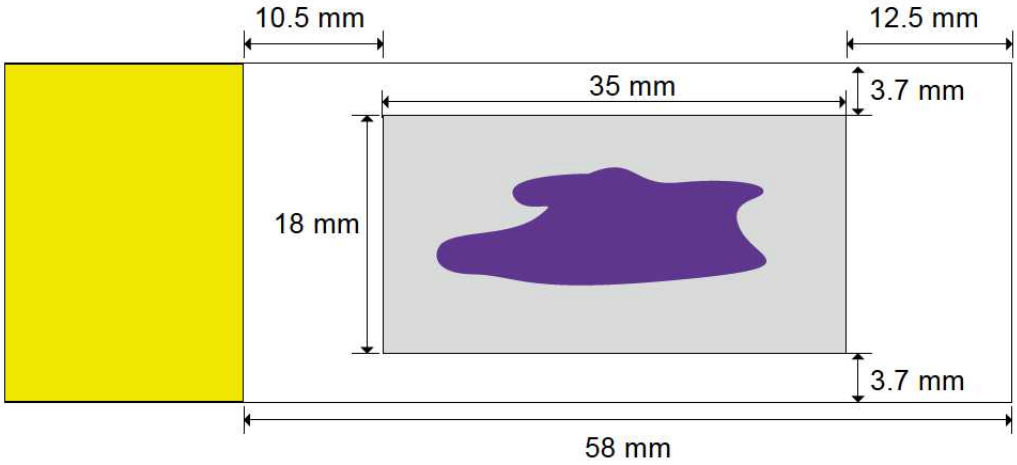
6 Place the prepared slides in the cryostat chamber to equilibrate temperature for 20-30 seconds.

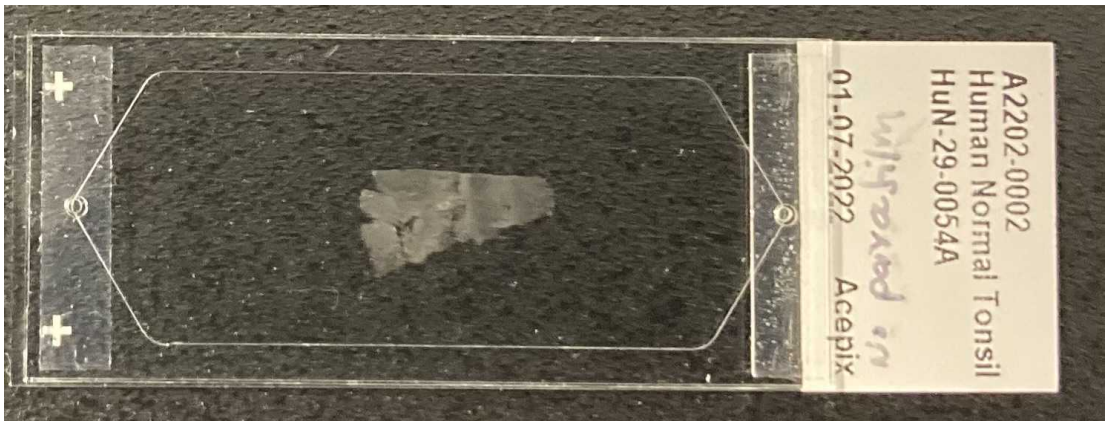
7 Section the tissue at a **thickness of 5-10 µm.**

Note

Do not exceed 10 µm as this will affect the autofocusing capabilities of the microscope. **Avoid folds and tears in the tissue**, as these artifacts will affect image quality and data analysis.

8 Gently place the tissue section in the center of the slide within the imageable area as shown in **grey rectangle** below. Multiple tissues can be placed within this region as long as there is no overlap of tissue-on-tissue or OCT-on-tissue.





Example of good tissue placement

9 Adhere the tissue section to the slide by placing a gloved finger underneath the slide for 1- 2 seconds

> **Note**
>
> Do not keep your finger on the slide for longer than the minimum time necessary to melt the OCT (Optimal Cutting Temperature compound). The directed heat transfer should melt the OCT, thereby ensuring tissue adherence. Chemical fixation of the tissue will take place during the staining protocol.

10 Place the mounted slide in a single slot of a microscope slide box. Once complete, cover the slide

storage box with the lid. Place the box of mounted slides on dry ice for transport to a -80°C freezer.

Store slides at -80°C for up to 6 months