Cryosectioning fresh frozen tissues for multimodal imaging

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We use this protocol and it's working

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ABSTRACT

This protocol describes sectioning for fresh frozen tissues for multimodal imaging including Hematoxylin & Eosin staining, DESI imaging mass spectrometry, SIMS imaging mass spectrometry and RNAscope imaging.

GUIDELINES

Upon finishing cryosectioning, remove the blade and dispose in sharps container before cleaning the cryostat. Discard tissue remnants in biohazard bag.

MATERIALS

Cryostat, Leica- CM 3050S
Accu-Edge Microtome Blades, Sakura- 4689
Anti roll glass, Leica 14047742497

1 Clean the cryostat surface, anti-roll blade and forceps with ethanol before sectioning.

2 Place the tissue block inside the cryostat at -20°C for 30 mins for equilibration. Also, place the slides and substrates inside the cryostat for cooling.

3 Set the chamber temperature of the cryostat to -20C and object temperature to -18C to -20C

4 Place the blade in place and lock it.

5 Trim the tissue block at 30-50µm thickness to obtain a flat surface.

6 Mount the tissue block on the chuck using water (no OCT) and adjust the angle of the chuck as needed.

7 Position the anti-roll bar appropriately so that it doesn't scrape the tissue (if placed too far) or roll off of the block (if placed too near).

8 Gently section the tissue at 8µm thickness using an anti-roll bar and place the consecutive sections on individual substrates. Adjust and flatten with a fine tipped brush if necessary. For H&E, DESI and RNAscope imaging, tissue sections are placed on regular or positively charged microscope slides and on gold coated 2.5cm^2 substrate for SIMS-imaging.

9 Thaw mount the sections on the target using heat from the finger under the slide.

10 Store at -80C until use.