Technical Note

Cryosectioning of heat stabilized tissue samples

Introduction

Cryo sectioning of heat stabilized samples can be challenging and may require special considerations. A number of key conditions have been identified that improve section quality from heat stabilized material. A list of preferred techniques, equipment and steps related to cryo sectioning of heat stabilized tissue have been collected below into two tiers. A first tier with steps not requiring special equipment and that should be easy to implement. These steps will be routine in most labs and should not present much of a problem. Most tissues will section OK using these steps. If sufficient sections quality cannot be obtained using the first tier steps, it may be necessary to implement some of the second tier steps. In the second tier other, more demanding, tips have been collected. These steps may require special equipment or other preparations that may not be standard in most labs. Using the right combinations of first and second tier steps it is generally possible to get good quality sections of different tissue types suitable for MALDI imaging and other analytical workflows requiring cryosectioning although optimization of sectioning may be needed.

First tier steps: General advice easy to implement without special equipment

In this tier of steps, more general advice concerning the actual heat stabilization procedure, freezing and the transfer of sections is covered. Implement these steps first and depending on results advance to 2nd tier steps:

If possible, heat stabilize tissue intended for cryosectioning directly from a fresh state. Heat stabilization from a frozen state will notably reduce quality of the final sections.

Always use Structure Preserve method setting on the Stabilizor™ instrument to reduce deformation of tissue.

Figure 1. Method selection screen of the Stabilizor

If normal cavity vacuum deform the gross morpholgy too much see application note ”Manual control of ..” for tips on how to reduce deformation.

After heat stabilization, immediately open the cavity of the Maintainor® Tissue card to avoid condensation of steam which may cause deformation of tissue as the cavity collapses.

When freezing heat stabilized tissues, do so rapidly, e.g. using dry ice cooled organic solution e.g. isopentane kept at -20 °C to -30°C, or floating in an alu foil “boat” in the vapor phase of liquid nitrogen. This is to prevent formation of large ice crystals, which may form if the sample is frozen too slowly, e.g. at -20°C or with dry ice, or micro cracks, which may form if the sample is frozen too rapidly, e.g. using liquid nitrogen. Both fenomena will interfere with the fine structure of the sections.

If possible, section shortly after sample collection and if there is a need to store heat stabilized tissue make sure to store it in air tight containers at -80°C. Freeze drying of heat-stabilized tissue will adversely affect quality of sections.

If the downstream application is flexible with regards to section thickness, thicker sections tend to be of higher quality than thinner sections.

Use a thin artist brush to transfer the section to the pre-chilled slide rather than placing the cold or room temperature slide over the section and lifting it by touching the slide to the section. Sections from heat stabilized tissue are more fragile than sections from non-stabilized tissue and brush transfer tends to be gentler.

Figure 2. Artist brush for section transfer

When thaw mounting the section onto the frozen slide, start from one side and progress under the section to the other side of the section. This will minimize wrinkling and trapped air under the section and improve section quality.

Second tier steps: Motorized sectioning, embedding in supportive medium and tape assisted transfer systems

In this next tier of steps, more advanced advice requiring specialized equipment is covered.

Use motorized sectioning, set at the lowest possible speed. More advanced cryostats, e.g. Leica CM 3050-S, are equipped with a sectioning motor which moves the specimen at a constant speed across the blade and can do so much slower and more consistent than what is possible by hand.

For some tissue really thin sections, 5 µm, have worked well in combination with slow motorized sectioning. This sections are however very difficult to transfer intact to the slide.

Embed the heat-stabilized tissue in supportive medium e.g. 2.5% CMC (Carboxy methyl cellulose, Sigma #C4888) using a cryomold. The embedding will support and reduce strain on the fragile heat stabilized tissue during sectioning to preserve morphology. CMC, in contrast to OCT, is generally assumed to be “invisible” in the mass spectrometer and does not need to be washed away prior to matrix application. However, there are potential concerns that it may reduce signal. If this becomes a problem, 2.5 % CMC could potentially be substituted with 10% gelatin or water as a supportive medium. Water will not be as easy to work with but should have less impact on downstream analysis.

Special considerations must be taken when making and using the 2.5% CMC gel. When added to water, the dry CMC powder tends to form inclusion bodies with dry powder inside that take a long time to dissolve. To avoid this: Add the powder slowly while stirring the water to make sure all the powder has been wetted. This can be done using a magnetic stirrer; however do not continue to stir the gel once all powder is wetted as this may change the viscosity of the final gel. Once wetted, the CMC will dissolve into a smooth gel in a few hours without further stirring. Refrigerate the gel in a sealed container until needed, it will keep for at least a week.

Use a tape transfer system to support the sections. Tape transfer of fragile and difficult to cut sections is standard practice in many pathology labs. The cryo compatible film transfer system that have worked best in our hands are the CryoJane tape transfer system (Leica Microsystems, Wetzlar, Germany). The Cryo Jane system is compatible with imaging mass spectrometry when self-coated ITO slides are used and the MALDI matrix is applied by spraying. In Japan the Kawamoto film method (Section lab, Japan) is also available and have been reported to work.

The CryoJane tape transfer system consists of glass slides coated with a UV activated polymer and a UV light source. The UV light source fits inside the cryostat and the procedure is done in the cryostat while the sections remain frozen. The CryoJane system is used with embedded tissue, e.g. in CMC. The tape is attached to the surface to be cut and a section is cut. The section remains attached to the tape which is then placed, with the sample side down, on the coated slide. The slide-section assembly is exposed to UV light which activates the polymer on the slide that attaches the section to the slide. The tape is then removed. The pre-coated CryoJane slides are not compatible with mass spectrometers requiring a conductive sample surface, so slides must be coated by the user using two solutions supplied by Leica (Slide Pre-Treatment Solution, Catalog #: 39475270 and Polymer solution, Catalog #39475271).

Figure 3. CryoJane tape transfer system (Leica Micro systems, Germany)

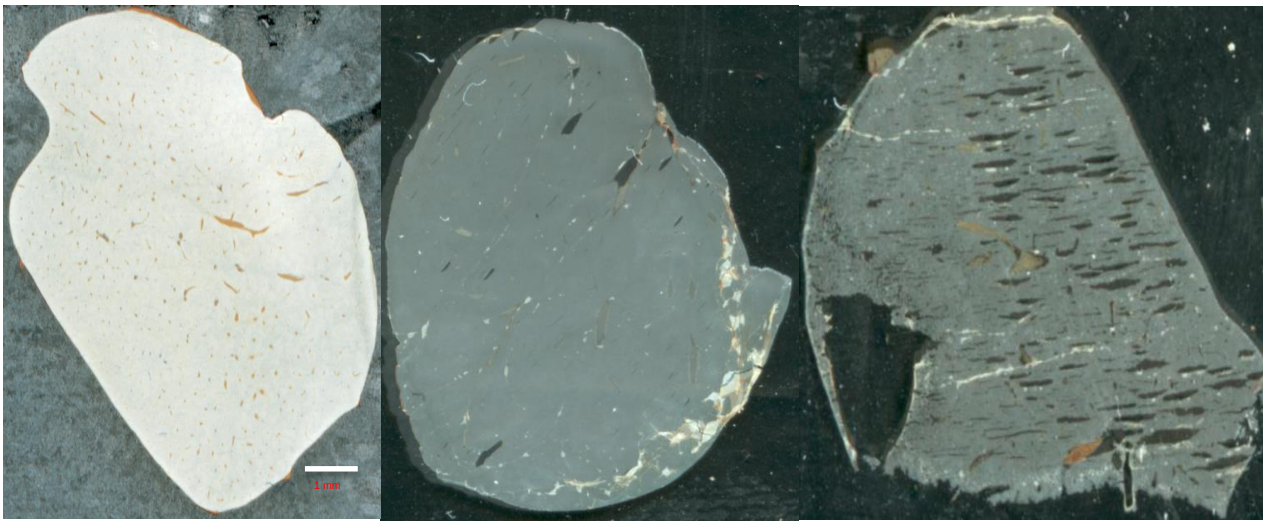

Figure 4. Cryotome sections on ITO slides of mouse liver stabilized using structure preserve from a fresh state and frozen in isopentane cold with dry ice, obtained using a microtome sectioning at -15°C and different combinations of tier 1 and 2 steps. Left panel: 12 µm CMC embedding using Cryo Jane, Center panel: Slowest motorized sectioning at 5 µm without embedding, note the lower edge folding over due to difficulty of transfering the thin section, Left panel: 12 µm manual section without embedding.

After sections are cut further downstream analysis can usually be done according to established practice without further adaptation. However, H&E or other histological staining procedures can be harsh and for some tissues result in partial loss of sections. Pre-treating slides with Biobond (BB-20. BioCell, Cardiff, UK) has been shown to lessen this problem.

The advice above contains the current best practice of cryosectioning of heat stabilized tissue. The working hypothesis on why sectioning of heat stabilized tissue is different from non-heat stabilized tissue is that protein-protein interactions are weaker due to denatured proteins in heat stabilized tissue. This will make the sections more fragile and more prone to holes and cracks. Brain tissue tends to be easier to section after heat stabilization compared to other tissues, presumably due to the high fat content that helps keeping the section together. Different tissues will present unique challenges and solutions to enable cryo sectioning after heat stabilization.

Acknowledgement

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Further reading for MALDI imaging with heat stabilized tissue

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Blatherwick, E. Q., et al. "Localisation of adenine nucleotides in heat-stabilised mouse brains using ion mobility enabled MALDI imaging", International Journal of Mass Spectrometry, 2013