Sample Sectioning Tips and Tricks

For CosMx™ SMI and GeoMx® DSP Experiments

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NanoString Technologies® CosMx SMI and GeoMx DSP platforms assist researchers in exploring subtle differences in the structure and function of tissues. For studies to reflect the true biology of the samples, quality sample preparation is critical. In this guide, we've compiled suggestions for recognizing and correcting common mistakes in frozen and formalin-fixed paraffin embedded (FFPE) histology, as it relates to the CosMx SMI and GeoMx DSP workflows. This guide does not provide comprehensive training on sample sectioning but aims to supplement formal training to help reduce frustration in the development and execution of your spatial biology experiments.

Contents

Before Sectioning

Before sectioning, plan the placement of the tissue in the center of the slide's scan area, indicated in green in Figures 1 and 2 for the CosMx SMI and GeoMx DSP platforms, respectively.

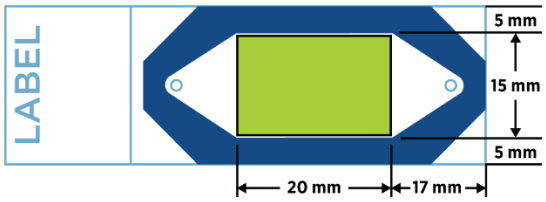

Figure 1. CosMx SMI tissue scan area

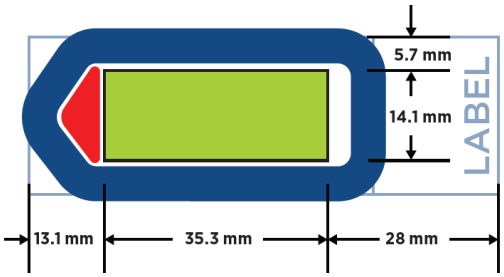

Figure 2. GeoMx DSP tissue scan area

Cryosectioning Frozen Specimens

Successful completion of downstream assays depends on good technique during sectioning and slide preparation. The following walkthrough provides a strategy for cryosectioning that will produce useful slides. For a comprehensive guide, consult A Method for Preparation of Frozen Sections[1].

Trimming

After securing and aligning the OCT block on the cryostat specimen head, the first task is to expose the tissue by slicing away any OCT that covers it. **Figure 3** demonstrates how difficult that may be to judge just by looking at the block face. These pictures show the same block before (left) and after (right) ~200 µm of sectioning. Though the tissue is visible in the 'before' picture, there is still a hard-to-see film of OCT covering it. Cutting at this stage will produce only sections of OCT. Ensure your block is trimmed all the way into the tissue by scrutinizing the sections you produce. A distinct difference between **tissue and OCT should be visible on a mounted slide, by eye.**

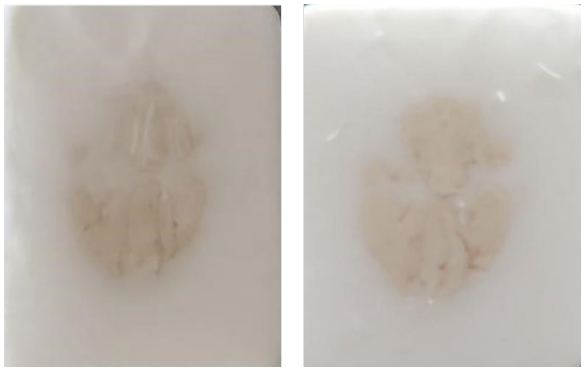

Figure 3. Frozen OCT block before and after trimming

3-Step Sectioning

For new users, it is helpful to visualize the block face as having two distinct regions, as shown in **Figure 4**. The orange-shaded areas contain only OCT. The knife can dwell here during sectioning without any harm or deformation to the tissue. The green-shaded area contains tissue. The knife must move steadily through this region to produce artifact-free sections of precise thickness. Where the blade lingers, the block melts faster, resulting in varying thickness across the section. In fresh (unfixed) frozen tissue, this may even cause zones of inconsistent RNA degradation. Therefore, it is important to cut across the tissue with a smooth, consistent turn of the hand wheel.

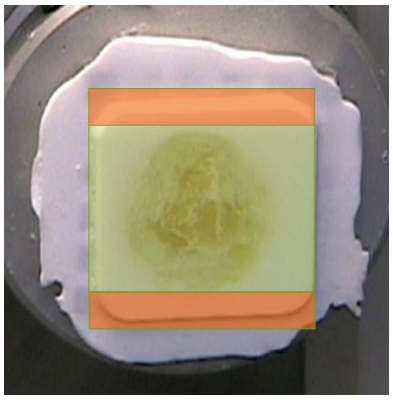

Figure 4. Visual guide to the block face (image adapted from A Method for Preparation of Frozen Sections[1])

Until you are comfortable quickly and smoothly sectioning from the block, cut your sections in three steps (described below): #1 create a 'handle' of OCT media, #2 slice through the tissue section, and #3 mount the tissue section.

Step 1. Slowly cut into the first section of the **OCT-only** portion of the block. Stop before the blade meets tissue, as in **Figure 5.** Using a fine brush, gently contact a small piece of the cut OCT. This **handle** will help guide the section as it is cut, ensuring that it comes out flat.

Figure 5. Creating a handle of OCT media

Step 2. When you are ready, begin smoothly turning the handwheel. Maintain gentle tension on the section using the brush on the handle. Guide the section down the knife stage, matching the speed it is cut from the block. Pulling too swiftly or too hard will tear the tissue. Pulling too gently or too slowly will result in wrinkling and curling. Once the blade has passed the tissue, be sure to stop cutting before fully severing the section from the block, as in **Figure 6**. Keeping the section attached to the block gives you a chance to flatten out any visible wrinkles by gently applying tension using the brush. When satisfied, hold the section taut and sever from the block, as shown in **Figure 7**. The section is now ready for mounting.

Figure 6. Slicing entirely through the block's tissue portion, leaving the section attached by the OCT-only zone

Step 3. Ensure there is enough room on the cryostat stage to maneuver the slide as much as needed. Once ready, gently press the slide onto the section.

Figure 7. Flattening and severing the section

Ideally, sections are flat. If so, room temperature slides work well to immediately melt a section in place on a slide.

If the sections are not flat, they are unlikely to mount well. The sections in **Figure 8** contain folds, wrinkles, and tears, gathering on one side due to a dull blade. These artifacts are very likely to be transferred to the slide (or worsened) when mounted. Take caution to flatten sections as much as possible before mounting.

Imperfect sections that wrinkle, fold, tear or gather on one side may still be salvaged by using a slide chilled in the cryostat. By using a chilled slide, you'll have more time to maneuver and flatten the tissue before it begins to melt and adhere.

Figure 8. Consequences of mounting imperfect sections

If it seems impossible to generate a section that isn't wrinkled or torn, it is worth trying with a fresh blade, especially if lines appear throughout the sections. If that doesn't solve the problem, changes to the cryostat setup may be needed. If the tissue consistently tears, the temperature of the specimen head is too cold. Conversely, if wrinkles are too hard to remove, a colder temperature may be required. Use **Table 1** to confirm the tissue is at the correct temperature for cutting.

Tissue Type	Working Temperature
Brain	-12°C
Liver	-14°C
Lymph Node	-14°C
Kidney	-16°C
Spleen	-16°C
Muscle	-20°C
Thyroid	-20°C
Skin	-25°C
Breast	-25°C
Breast with Fat	-30°C or below
Adipose Tissue	-30°C or below
Fixed Tissue	-12°C to -17°C

Table 1. Recommended temperatures for cutting unfixed frozen tissues (adapted from ihcworld.com[2])

Microtomy for FFPE specimens

As in cryosectioning, there are numerous potential artifacts in paraffin sectioning. For a comprehensive guide, consult Leica Biosystems Introduction to Microtomy: Preparing & Sectioning Paraffin Embedded Tissue[3].

For the purposes of CosMx SMI and GeoMx DSP experiments, section **adhesion** and **consistency** of thickness have the most impact on downstream success.

Section Adhesion

There is an increased chance of section detachment with cell pellet arrays and more delicate tissue types (such as breast tissue). Take these steps to reduce the risk of section detachment:

- Allow sections to fully expand in the water bath – fully expanded sections should have a glass-like surface, with a matte effect. Shining a light from above will allow you to see any remaining wrinkles more clearly as light will bounce off them. Failure to allow sections to fully expand in the water bath increases the chances the section will detach.
- Immediately after sectioning, to maintain tissue adhesion throughout the protocol, dry or bake the slides following the guidance in the CosMx SMI or GeoMx DSP Slide Prep User Manuals. Adjustments and optimization may be needed for your own experimental conditions. Place slides at an angle to allow excess paraffin to flow off the slide.
- Try more adhesive slides – in our experience, Leica BOND Plus slides are more adhesive than Apex BOND slides, which are more adhesive than VWR SuperFrost Plus slides. For semi-automated and automated workflows, we recommend Leica BOND Plus slides. Although not validated by NanoString, products like Epredia™ Tissue Section Adhesive (Fisher Scientific, Catalog No. 86014) or TOMO® Adhesion Microscope Slides (VWR, Catalog No. 10000-038) may also work well.

Consistency of Thickness

One advantage of FFPE samples is that, once trained, it is easy to produce a ribbon of consistent, serial sections. For any study rooted in the comparison of specific tissues or anatomy, this is invaluable. For CosMx SMI and GeoMx DSP experiments, consistent thickness is desired as variation between sections will differentially affect the number of probes bound. No matter how skillfully sectioned, there will always be a measure of variation between sections in a ribbon. Remaining mindful of a few key points, however, will minimize the effect this variation has on the data.

The temperature of the FFPE block will influence section thickness and quality. Before sectioning, chill and hydrate the block on melting ice. As a room temperature blade meets a chilled block, the block face is slightly warmed, generating thicker sections. As more sections are sliced from the block, the temperature eventually stabilizes. Discard the first two sections of every ribbon

generated. This avoids both over-warmed, over-thick sections, as well as any effects of oxidation on the exposed block face.

When floating sections, pay attention to any obvious differences in transparency between sections (indicative of different thicknesses).

Sample Processing

Beginning with flat, well-mounted sections is critically important to maintaining tissue integrity. **Figure 9** shows the difference between a good and bad section. The section shown on the left has few flaws and performed well in the GeoMx DSP workflow. The section on the right contains many tears and wrinkles and performed poorly in the workflow. Throughout the assay, tears are likely to grow wider, and wrinkles are likely to become holes. Too many of either imperfection ultimately results in tissue detaching from the slide.

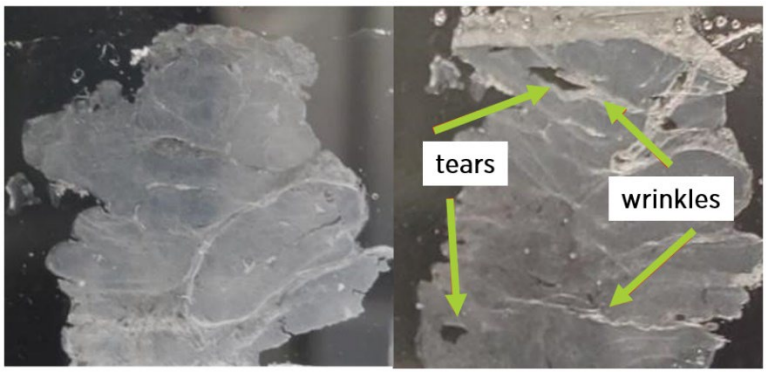

Figure 9 - Good (left) and bad (right) sections mounted on a slide

For both the CosMx SMI and GeoMx DSP workflows, adjustments to the high-temperature antigen or target retrieval step may help preserve tissue adhesion. Fat-rich tissues, such as breast cancer, may benefit from **shorter incubation times** to avoid complete loss of adipose tissue. Delicate tissues and cell pellets may benefit from **lowering target retrieval temperatures**. Consult the Slide Preparation User Manuals for recommended target retrieval conditions based on tissue type. Keep in mind that modifications to the validated protocols are performed at your own risk and NanoString is not responsible for any errors, failures, sample loss, or data loss resulting from your testing and development.

Conclusion

With proper technique and practice, many of the artifacts and frustrations discussed in this guide can be avoided. For additional support, refer to the resources listed below, then consult with your NanoString Application Scientist or histology/pathology experts at your institution or elsewhere.

References and Suggested Reading

1. Peters, S. (not dated). *A Method for Preparation of Frozen Sections.* University of Basel Department of Biomedicine. https://biomedizin.unibas.ch/fileadmin/user_upload/biomedizin/core_facilities/histology/Guides/Frozen_Section_Methods.pdf
2. IHC World. (not dated). *Methods and Techniques for Frozen Tissue Sections.* http://www.ihcworld.com/_protocols/histology/frozen_section.htm
3. Rolls, G. (not dated). *Introduction to Microtomy: Preparing & Sectioning Paraffin Embedded Tissue.* Leica Biosystems. https://www.leicabiosystems.com/knowledge-pathway/steps-to-better-microtomy-flotation-section-drying/

Bruker Spatial Biology, Inc.
530 Fairview Avenue North
Seattle, Washington 98109
T (888) 358-6266
F (206) 378-6288
customerservice@nanostring.com

Sales Contacts
United States: us.sales@nanostring.com
EMEA: europe.sales@nanostring.com
Asia Pacific & Japan: apac.sales@nanostring.com
Other Regions: customerservice@nanostring.com