Tissue Processing – Best Practices

Tissue dissection, processing, and embedding are very critical for the preservation of tissue morphology and the quality of the staining. We highly recommend that these procedures are performed by trained users.

The purpose of this document is to highlight some tips for avoiding commonly observed artifacts in Fresh-Frozen and FFPE tissue sections, and it is not intended as a comprehensive manual for tissue processing and sectioning. Detailed technical guide and some useful links on processing Fresh-Frozen [1-3] and FFPE [4,5] samples are indicated at the end of this document.

Fresh-Frozen Tissues

Tips for Tissue Sectioning

- Place the tissue block in a container full of dry ice when transferring the tissue block from the -80 °C freezer to the cryostat.
- Make sure that the cryostat has reached the selected temperature before transferring the tissue.
- Before starting tissue sectioning, make sure to equilibrate the tissue at the selected cryostat temperature for at least 20 minutes.
- We recommend using 63069-LP Low Profile Microtome Feather® Blade by Electron Microscopy Sciences. Please consider that each sectioning session may require a new blade to avoid tears.
- Fatty tissues, such as lymph nodes and breast samples, require the blade to be wiped with ethanol to remove residual fat every few sections.

A comprehensive report on artifacts of fresh-frozen tissues can be found in reference [1] below, which also contains pictures related to freezing artifacts.

Artifacts, Causes, and Tips

1. Tissue disruption when sectioning

Causes: Nicked blade. Composite samples as fatty tissues (e.g., lymph nodes, breast, skin) might be difficult to cut due to the presence of fat.

Tips:
- Use a new sectioning blade in each sectioning session.
- Choose the temperature of the cryostat ideal for the specific tissue of interest.
- Wait longer for the tissue to equilibrate at the cryostat temperature if it is too firm for sectioning.

2. Holes

Causes: Autolysis*, the formation of ice crystals (refer to point 3), over- or under-freezing.

*Autolysis is the process of cell self-digestion (and consequent destruction) by the action of the endogenous enzymes. It is caused by the delay between the time when the tissue becomes anoxic and when it is fully frozen.

Tips:

- Minimize the time between tissue dissection and freezing. After dissection, quickly freeze the tissue.
- Piling dry ice over the tissue before dissecting it from the animal can also help.
- For *over-freezing*: polish the block with a couple of extra turns of the blade to create friction and warm up block by pressing on it with your gloved finger (5 - 10 seconds).
- For *under-freezing*: Make sure the cryostat reached the selected temperature before transferring the tissue, in some cases adding a heat sink to block can help.

3. Ice crystals and Swiss Cheese Appearance

Causes: The tissue freezing is too slow and gives water the time to crystallize. Large ice crystals can rupture cell membranes, causing the formation of holes.

Tips:

- Freeze tissues faster (3 or 4 seconds until solid) to prevent water crystallization (water will turn into a vitreous form instead). This might require immersion in -80 °C or colder fluid, or complete embedding in powdered CO_2. Cold (-80 C) isopentane is recommended for full tissue immersion.
- Do not freeze fatty tissue surrounding the tissue of interest.
- Work with smaller tissues - dimensions should be smaller or equal to 0.5 x 0.5 x 0.3 cm.
- Do not use tissues larger than the diameter of the chuck.
- Dry the surface of the tissue by pressing with gauze before making the block.
- Once frozen, put a small amount of refrigerated (4 °C) OCT on a refrigerated pedestal, then drop the tissue on it and dip the pedestal to freeze the OCT quickly.
- After freezing, do not store tissue at -20 °C for more than an hour.

4. Air Bubbles

Causes: Air bubbles can be trapped under coverslips can cause the tissue to dry out.

Tips:

- Keep the coverslips in the cryostat.
- Gently move air bubbles off the slide with finger or tweezers.
- Some users find it helpful to place the section directly on the zone of the coverslip while the finger is placed on the back. This difference in temperature may allow a good spread of the section
- Moving the coverslip up and down can also help to spread the tissue.

FFPE Tissues

Artifacts, Causes, and Tips

1. Tissue does not attach to the paraffin block or bounces out of paraffin block while on the microtome for sectioning

Cause: Insufficient dehydration and paraffin infiltration resulting from water left in the tissue.

Tip: Use fresh reagents for FFPE tissue processing.

2. The tissue separates rapidly when the ribbon is placed in the water bath

Causes:

- If the temperature of the water bath is between 45-50° C, then the tissue is under-processed.
- Tissue may have too thick for processing conditions.
- Tissue may have been processed on a program that was too short for that tissue type.
- Processing reagents may be saturated with water.
- Paraffin may be saturated with xylene or isopropanol.

Tip: Change reagents and reprocess tissue on correct processing protocol.

3. The tissue does not adhere to slide or falls off easily

Causes:

- Tissue slides are placed in the oven prior to deparaffinization in xylene.
- Tissue is under-processed.
- Reagents saturated with water or contaminated with the preceding reagent.

Tip: Change reagents and paraffin and reprocess tissue on proper processing protocol.

4. The tissue feels soft or mushy during embedding

Causes:

- Tissue may have too thick for processing conditions.
- Tissue may have been processed on a program that was too short for that tissue type.
- Processing reagents may be saturated with water.
- Paraffin may be saturated with xylene or isopropanol.

Tips: Change reagents and paraffin and reduce tissue thickness and reprocess tissue on proper protocol.

5. Shattering, cracked or folded sections from large blocks processed through paraffin wax

Cause: Over-dehydration. Too much moisture was removed from the block during processing.

Tip: Place a soaked cotton gauze over the block face for 5 to 10 seconds before cutting. This allows enough moisture to penetrate 15 to 20 µm allowing a quality section to be cut.

Resources

[1] S.R. Peters (ed.), *A Practical Guide to Frozen Section Technique*, DOI 10.1007/978-1-4419-1234-3_2, © Springer Science + Business Media, LLC 2010

[2] https://www.feinberg.northwestern.edu/research/docs/cores/mhpl/tissuefreezing.pdf

[3] http://www.ihcworld.com/_protocols/histology/frozen_section_technique_1.htm

[4] https://www.agilent.com/cs/library/technicaloverviews/public/08002_ihc_staining_methods.pdf

[5] F.L Carson, C.H. Cappellano, *Histotechnology: A Self-Instructional Text*, 4th Edition, ISBN: 9780891896319, ASCP 2015