c. Tape the inserts to the sides of the cardboard box.

CRITICAL To prevent coverslips from slipping below the inserts and stacking on top of one another, the inserts have to be taped in a manner to ensure there is no allowable movement up or down.

3.3 Fresh Frozen Tissue Sectioning

Fresh-frozen tissue sections are mounted directly onto poly-L-Lysine-coated coverslips. Appropriate preparation and storage of tissue sections are critical to ensure sample integrity. The instructions provided in this manual are limited to what is specific to the CODEX® workflow, and they are not intended to be a comprehensive guide on how to process and cut tissue sections. Further guidance on tissue processing of Fresh-Frozen samples can be found in our Technical note: Tissue processing – Best practices

Guidelines

Tissue Sections

- Tissue sections adhered to Poly-L-Lysine-coated coverslips can be stored at -80°C for up to 6 months before staining.
- It is critical not to exceed a tissue thickness of 10 µm because it can affect the autofocusing capabilities of the microscope.
- For best results, tissue sections should be devoid of folds and tears.
- To ensure that tissue sections are not adversely affected, it is critical that the tissue coverslips are not stacked on top of one another.

Pre-Experiment Preparation

Materials NOT Included in Kits:

- Poly-L-Lysine-coated coverslips prepared in section 3.1.
- Cryo/Freezer tissue storage box with tube inserts prepared in section 3.2.
- Fresh Frozen Tissue Block of interest
- Aerosol Spray
- Dry Ice
- Polystyrene container for Dry Ice
- Blade for Tissue Sectioning (we recommend 63069-LP Low Profile Microtome Feather® Blade by Electron Microscopy Sciences)

Prepare Cryostat Chamber

Standard cryostats with temperature control are recommended for tissue sectioning. Most tissues are sectioned in temperature ranges of -15°C to -25°C. The exact temperature is unique to each tissue and needs to be selected according to standard slicing procedures.

Fresh Frozen Tissues - Sectioning Instructions

a. Set the cryostat chamber to tissue-specific temperature range.
b. Place the prepared storage box in the cryostat chamber to equilibrate at the selected cryostat temperature.
c. Once the cryostat has reached the selected temperature, transfer the tissue from the -80°C freezer to the cryostat. Use a container filled with dry ice for transporting the tissue block.
d. Use an aerosol spray to clean coverslips from dust and lint before use.
e. Place the previously prepared Poly-L-Lysine-coated coverslips prepared in a cryostat chamber to equilibrate for approximately 20-30 seconds.
f. Slice the tissue between 5-10 µm thick.

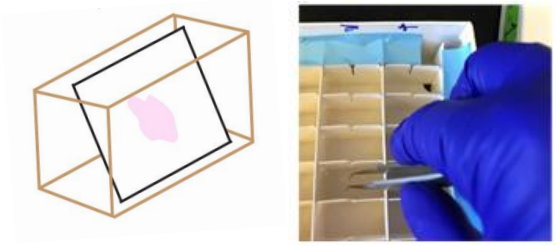
CRITICAL	Do not exceed 10 µm because it can affect the autofocusing capabilities of the microscope. Avoid folds and tears because they will affect image quality and data analysis.

g. Gently place the tissue section in the center of the coverslip
h. Adhere the tissue section to the coverslip by placing a gloved finger on the underside of the coverslip just below the tissue for 1-2 seconds.

CRITICAL	Do not keep your finger on the coverslip for more than the minimum time necessary to quickly melt OCT.

NOTE	The directed heat transfer should effectively melt the OCT, thereby ensuring tissue adherence. Chemical fixation of the tissue will take place during the staining protocol.

i. Place the mounted coverslip in a single slot of the prepared tissue storage box.



j. Repeat steps f - i for each tissue section.
k. Once complete, cover the tissue storage box with the lid.
l. Place the box of mounted coverslips on dry ice and transport it to a -80°C freezer.

STOPPING POINT	Samples can be stored at -80°C for up to six months prior to staining with care not to tip the container. Make sure the container stays upright.
NOTE	Tissue processing and sectioning are critical processes and need to be performed by trained users. Resources for best practice procedures and recommendations for avoiding artifacts can be found in Technical note: Tissue processing – Best practices

3.4 FFPE Tissue Sectioning

FFPE tissue sections are mounted directly onto poly-L-Lysine-coated coverslips. Appropriate preparation and storage of tissue sections are critical to ensure sample integrity. Given instructions are limited to what is specific to the CODEX® workflow, and they are not intended to be a comprehensive guide on how to process and cut tissue sections. Further guidance on tissue processing for FFPE samples can be found in the Technical note: Tissue processing – Best practices

Guidelines

Tissues

- FFPE tissues sectioned onto poly-L-Lysine-coated coverslips can be stored at 4° C for up to six months prior to staining.
- It is critical not to exceed a thickness of 10 µm because it can disrupt the autofocusing capabilities of the microscope.
- For best results, the tissue should be devoid of folds and tears.
- To ensure that tissue sections are not adversely affected, it is critical that the tissue coverslips are not stacked on top of one another.

Pre-Experiment Preparation

Materials NOT Included in Kits

- Poly-L-Lysine-coated coverslips prepared in section 3.1 of the User Manual.
- Cardboard freezer box with tube inserts prepared in section 3.2 of the User Manual.
- FFPE tissue block
- Blade for Tissue Sectioning (we recommend using 63069-LP Low Profile Microtome Feather® Blade by Electron Microscopy Sciences)
- Aluminum Foil
- Aerosol Spray
- 40°C water bath
- Clean Surface

Prepare Microtome

Prepare the Microtome of choice for use at RT following the standard procedures of the instrument.

FFPE Tissues - Sectioning Instructions

a. Prepare a water bath at 40°C and place it next to the Microtome.
b. Prepare a clean, dry surface for placing the coated coverslips next to the Microtome.
c. Use an aerosol spray to clean coverslips from dust and lint prior to use.
d. Place the poly-L-Lysine-coated coverslips next to the Microtome.
e. Insert a new blade for sectioning each new block
f. Section the tissue between 5-10 µm thick.

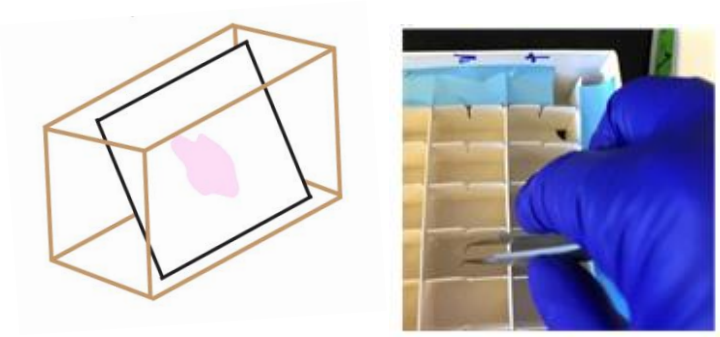
CRITICAL Do not exceed 10 µm because it can disrupt the autofocusing capabilities of the microscope. Avoid folds and tears because they will affect image quality and data analysis.

g. Place the sectioned tissue in the water bath for a few seconds and observe it expanding.
h. Once the tissue is expanded enough and is devoid of folds and wrinkles, using the forceps, quickly place a Poly-L-Lysine-coated coverslip in the water bath and gently move it towards the tissue. Doing so, the tissue will lay on the coverslip as it is moved out from the water bath.

NOTE Make sure that the tissue section is placed in the center of the coverslip.

i. Put the coverslip on a clean surface with the tissue-side facing up and let it air-dry overnight.
j. Repeat steps f - i for each tissue section
k. When the sections are dry, place each tissue coverslip in a single slot of the storage box, and cover the storage box with the lid.

NOTE The box of mounted coverslips can be kept at 4°C for up to 6 months.



STOPPING POINT	Samples can be stored at 4° C for up to six months prior to staining with care not to tip container. Make sure the container stays upright.

NOTE Tissue processing and sectioning are critical processes and need to be performed by trained users. Resources for best practice procedures and recommendations for avoiding artifacts can be found in Technical note: Tissue processing – Best practices