Institut-Hôpital neurologique de Montréal

Montreal Neurological Institute-Hospital

McGill

**EDDU Protocols**

# Cryosectioning of fixed and frozen tissue

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# 1 Introduction

## 1.1 Objectives

This protocol will provide a standardized procedure on methods developed for cryosectioning of fixed or fresh frozen tissue in Optimal Cutting Temperature (OCT) using a motorized cryostat. This SOP also outlines minimum assessment criteria that should be in place to evaluate the quality and integrity of frozen tissue sections and troubleshooting steps when issues with sectioning arise.

## 1.2 Reference to Other SOP or Documents

| Type of Document | Organization | Document Title |
|---|---|---|
| SOP 08.03.006 e2.0 | CTRNet | Sectioning of Tissue – Paraffin and Optimal Cutting Temperature (OCT) Embedded Tissue |
| Operator Guide | Thermo Scientific | Thermo Scientific CryoStar NX70 / NX50 Operator Guide 387928 |
| SOP TTP-P-0001 | Tissue Translational Platform of the Neuro | Cryosectioning |

## 1.3 Protocol overview

This protocol is for users interested in cryosectioning fixed and live tissue for research purposes only. This is optimized for the cryostat within the group, and when working with another cryostat model, the methods outlined will need to be optimized.

## 1.4 Technical and safety considerations

The following information should be read before starting:

This procedure is intended to ensure that tissue samples preserved for research studies ONLY, are sectioned in a safe and consistent manner, while eliminating the risks of contamination, loss of structural integrity and block orientation. Consistency in procedure is important for obtaining comparable and reliable test results from section to section, and from sample to sample.
This procedure is adapted from the Neuro's TTP SOP (TTP-P-0001, Cryosectioning) and CTRNet SOP 08.03.006 e2.0 (consult CTRNet SOP List to access this SOP).

- **Note:** The sooner the blocks are cryosectioned after fixation, the higher the quality of the sections. Optimal results are obtained when the block is still fresh.
- **Note:** should you need to store blocks prior to sectioning, be sure to place them at -80°C freezer or in a moisture proof freezer to keep the blocks well preserved.

- **Note:** Sectioning tissue can be dangerous and carries a biohazard risk. Personnel sectioning tissue should receive adequate training from both McGill University Environment Health and Safety department (or equivalent from a different institute) and EDDU personnel in charge of the equipment.
- Due to the nature of the work, EHS training on biosafety and WHMIS are required to be passed.

- **Note:** Sectioning involves working with sharp blades and knives. It is critical you use the blade guard when your hand is inside the chamber. In the event of a cut or injury with the equipment, please communicate with your supervisor or your immediate superior as soon as possible. You also need to let the EDDU personnel in charge know what happened so proper steps can be taken in cleaning and decontaminating of the instrument and the area.
- Any injury regardless of size or severity has to be reported to your local Environmental Health and Safety Department. For users at McGill University, an incident report form that can be found at: https://www.mcgill.ca/ehs/forms/forms/accident-and-incident-report must be completed.
- If you believe you need medical assistance you need to go seek attention at your nearest medical facility.

- **Note:** This procedure focuses on manual sectioning. Should you be interested in automatic sectioning please consult the instrument's instructions manual.

# 2 Materials

Refer to the product datasheet from the supplier for further details on storage and preparation instructions.

## 2.1 Labware

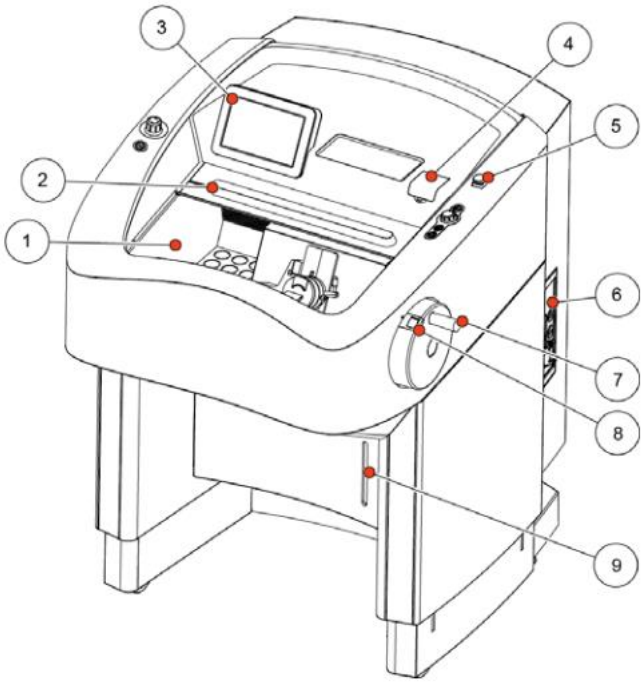
| Item | Supplier | Catalogue # |
|---|---|---|
| Solvent resistant markers, pencils or labels | | |
| Microtome blades MX35 Ultra | ThermoScientific | 3053835 |
| Fine tipped paint brush | | |
| Glass slides (electrostatically charged) | FisherScientific | 12-550-15 |
| Tray to hold slides | | |
| Slide storage boxes | | |
| Optimal Cutting Temperature Compound (OCT) | Fisher Healthcare | 23-730-571 |
| 99-100% Ethanol | MNI Store or any other supplier | |

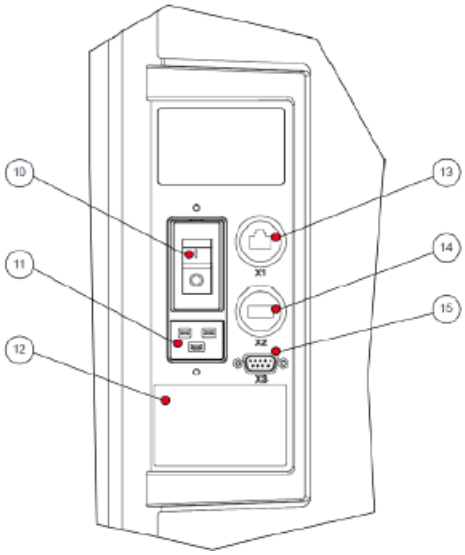
## 2.2 Equipment

| Item | Supplier | Catalogue # |
|---|---|---|
| Cryostat Cryostar NX70 | Thermo Scientific | 957000 |
| Microscope Stemi 508 | Zeiss | Zeiss Stemi 508 |

The following information is taken from "*Thermo Scientific CryoStar NX70 / NX50 Operator Guide 387928*" which is present in the group and all methods correspond to this specific model. Other Cryostats might have slightly different layouts and set up for usage. Please consult the manual made for the model of cryostat you are using for more detailed and accurate information.

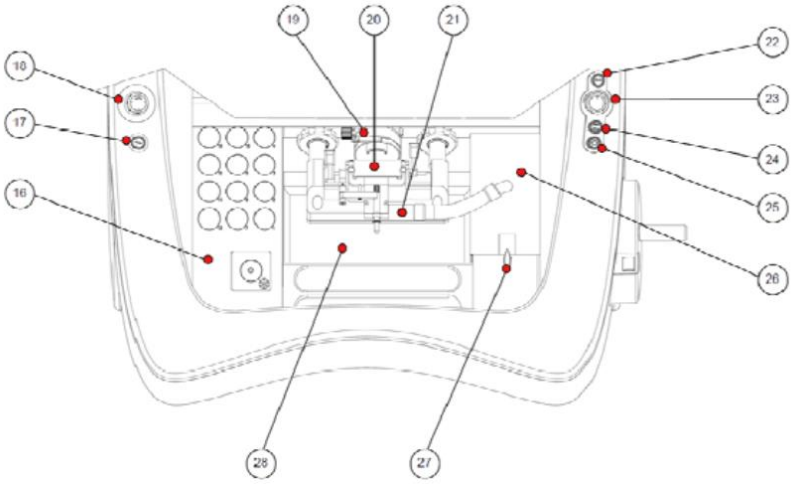
### 2.2.1 Cryostat Parts: Overview and Connection Panel



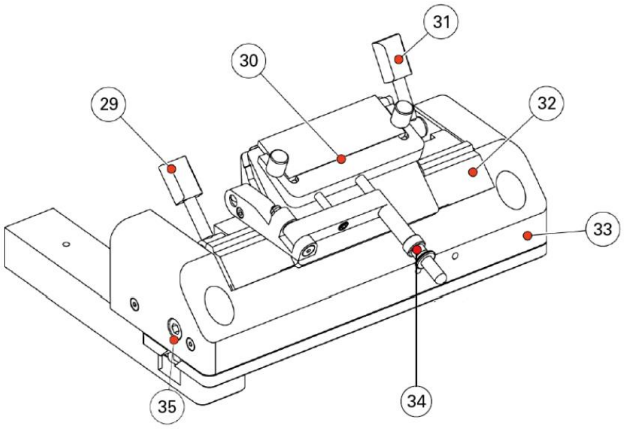

| | | | |
|---|---|---|---|
| 1 | Cryochamber | 9 | Water Drain Reservoir Cover |
| 2 | Cryochamber Window | 10 | Power Switch |
| 3 | Touch Screen | 11 | Power Socket |
| 4 | Cold D Reservoir Cover | 12 | Rating and Serial Number |
| 5 | Emergency Stop Button | 13 | X1 –RJ45 port (for service purpose only) |
| 6 | Connections Pane | 14 | X2- USB port |
| 7 | Handwheel | 15 | X3- Foot Pedal Connector |
| 8 | Handwheel Mechanical Brake | | |

### 2.2.2 Cryostat Parts: Cryochamber



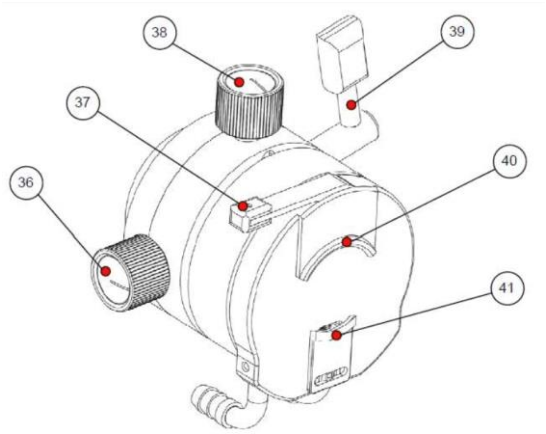

| | | | |
|---|---|---|---|
| 16 | Cryobar and Cooled Storage Area | 23 | Cutting Speed Control |
| 17 | Trim Button | 24 | Motorized Cutting Start/Stop Button |
| 18 | Joystick | 25 | Handwheel Electronic Brake Button |
| 19 | Specimen Head | 26 | Vacutome filter Cover |
| 20 | Blade Holder with Anti-Roll Plate | 27 | Vacutome Filter Cover Release Knob |
| 21 | Vacutome | 28 | Section Waste Tray |
| 22 | Mode Selection Button | | |

### 2.2.3 Cryostat Parts: Blade Holder




| | | | |
|---|---|---|---|
| 29 | Lateral Adjustment Lever | 33 | Blade Holder Base |
| 30 | Anti-Roll Plate | 34 | Anti-Roll Plate Adjustment |
| 31 | Blade Clamping Lever | 35 | Clearance Angle Adjusting Screw |
| 32 | Blade Holder Base | | |

### 2.2.4 Cryostat Parts: Specimen Head




| | | | |
|---|---|---|---|
| 36 | Y-Axis Fine adjustment Knob | 39 | Specimen Head Clamping Lever |
| 37 | Specimen Chuck Release Lever | 40 | Upper Specimen Chuck Jaw (Static) |
| 38 | X-Axis Fine Adjustment Knob | 41 | Lower Specimen Chuck Jaw (Moving) |

# 3 Protocol

## 3.1 Block Mounting

a) Set cryostat temperature according to the optimal cutting temperature of your sample. This may need to be optimized for sectioning new tissues, examples of temperatures used for specific tissue types already optimized are shown below in **Table 1**.

- **Table 1**

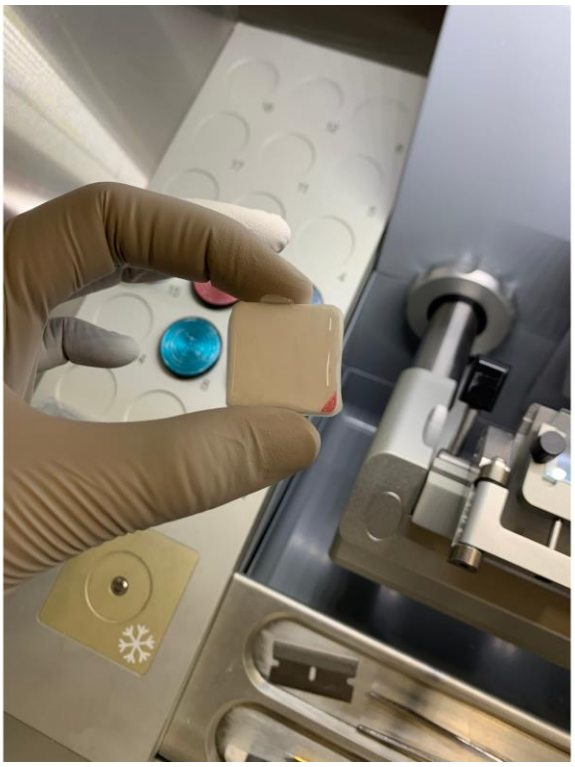
| Tissue type | Cutting temperature range |
|---|---|
| Fixed brain organoid | -9ºC to -13ºC* |
| Fresh frozen human brain | -21ºC to -18ºC* |

* This is an indicative range can vary depending on the cryostat model and brand, as the thermostat can be different.

b) Place block in the cryostat for ~30 minutes prior to sectioning to equilibrate the temperature of the block within the cryostat.

c) Remove block from the mold, always keeping track of orientation. Marking the bottom side of the block by cutting an edge prior to removal helps in this regard. Record the orientation of the mark in your lab notebook for further reference when analyzing the sections.

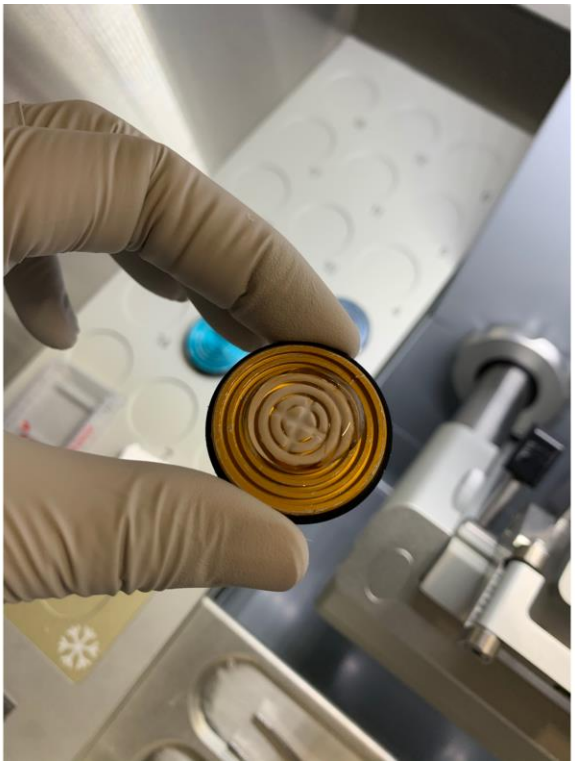
d) Evenly cover the surface of the block that will be in contact with the blade with a thin layer of OCT.



e) Press it down on the cooled storage area to make it flat, remove it when the OCT has solidified.

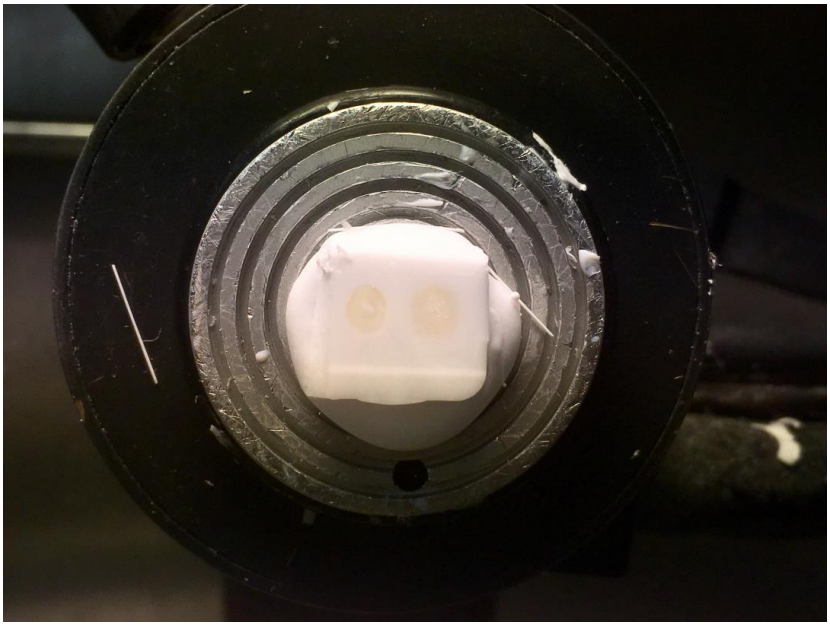

f) Trim with a razor blade to have straight edges. Should the orientation of the block matter, be sure to keep track of it at this stage.

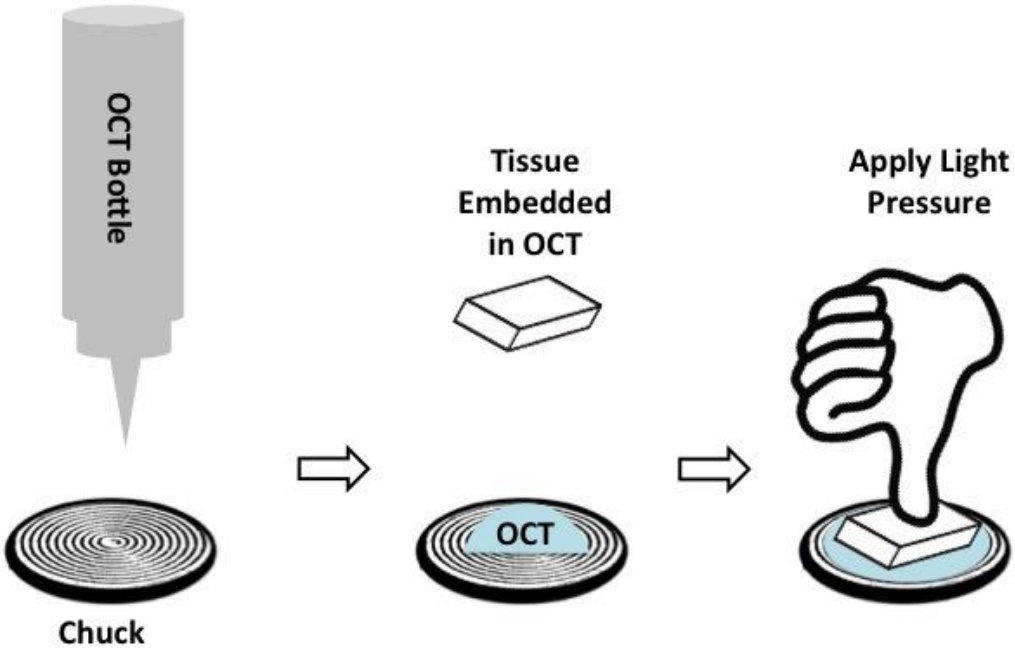

g) Apply an amount of OCT to the chuck (sample holder) sufficient to make the base a little larger than the block area.



h) Place block on top of the OCT base immediately after and “stamp” it using the heat extractor to ensure the block becomes as horizontal as possible.

- See the Image and the schematic below as an example.




## 3.2 Sample Aligning

a) Move the blade away from the specimen head using the joystick

b) Insert chuck on specimen head and rotate it so that the block edges are aligned to the head.

c) Bring blade closer to block and adjust vertical-horizontal alignment to the blade:

   I. Approach block until one part of it touches the blade (move up and down to check).

   II. If any part of it touches the blade, release the head lock lever and adjust alignment so that the block no longer touches the blade.

   III. Approach the block further until it touches the blade again and repeat steps i and ii

   IV. When the only part that touches the blade is the center of the block or it there is no position that no part of the block touches the blade, alignment is complete.

d) Retract blade for the next step.

## 3.3 Block Trimming

a) Use cryostat trim function or set cutting thickness to a larger value (40-60 µm) to shave off excessive OCT from refacing and mounting.

b) When samples start to become visible in the surface of the block but before they are actually exposed, set cutting thickness to the desired value and continue trimming until samples begin to be cut. A testing slide may be used to confirm whether samples are being cut or not.

c) If the antiroll plate will be used, it is ideal to use step b (previous step) to set its positioning and to equilibrate its temperature to block and blade.

## 3.4 Sectioning

a) Using either the antiroll plate or brush methods, cut sections while preventing their rolling.

- **Note:** If using a slide labeller, the slides must be pre-labelled. If labelled manually, slides can be labelled before or after the sections are made using a pencil or solvent resistant markers.

b) Use a slide kept at room temperature to pick up section using the thaw-mounting method.

- **Note:** Make sure slide are clean and don't show any scratches before use.

c) Repeat this step until enough slides have been produced or the sample ends.

d) Let sections air dry at room temperature between 30 minutes to 2 hours.

e) Use slides for desired purpose or store them at -80 ºC. Ice buildup can be prevented by adding a bag of desiccant to the box used for storage.

f) Write down information about the sections (Block, slide number, section thickness, box location) in a cryo blocks logbook and/or in your lab book.

## 3.5 Clean-up

a) Carefully remove blade from microtome. If the blade is damaged, discard it. If it is still sharp and undamaged, it may be reused.

b) Remove chuck and sample from microtome head and place outside for a minute to allow the sample to warm up, making it easier to separate from the chuck.

c) Wipe all OCT shavings into collection tray and discard shavings in a trashcan. Temporarily remove the brush holding tray and wipe the waste from underneath it.

d) Use 99-100% ethanol to wipe all the internal walls of the cryo chamber.

# 4 Quality Control/ Quality Assurance

At a minimum, assessment must consist of morphologic review of tissue sections under the microscope.

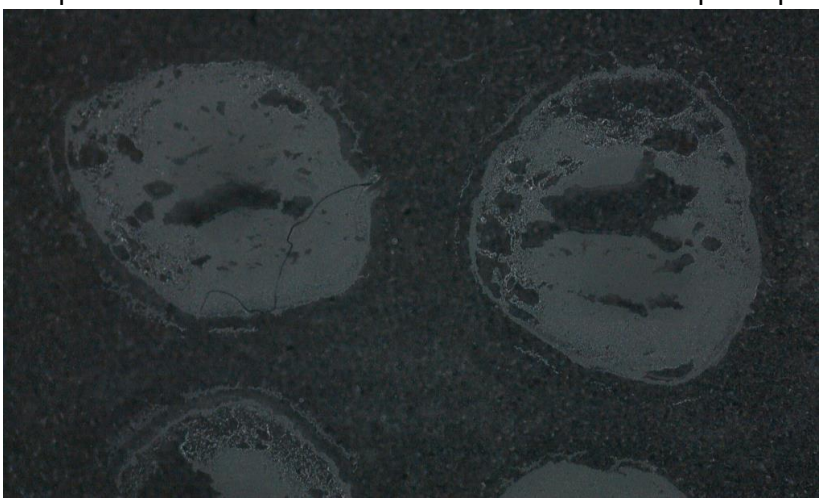
- **Note:** when looking under the microscope, check to see if all of the tissue of interest is visible in the section. Signs for cracks and uneven thickness across the section should also be examined. See **section 5.2.2 and 5.2.3** for example images.

## 4.1 Images of sections

Below are two exemplary images that can help understanding block quality control better:

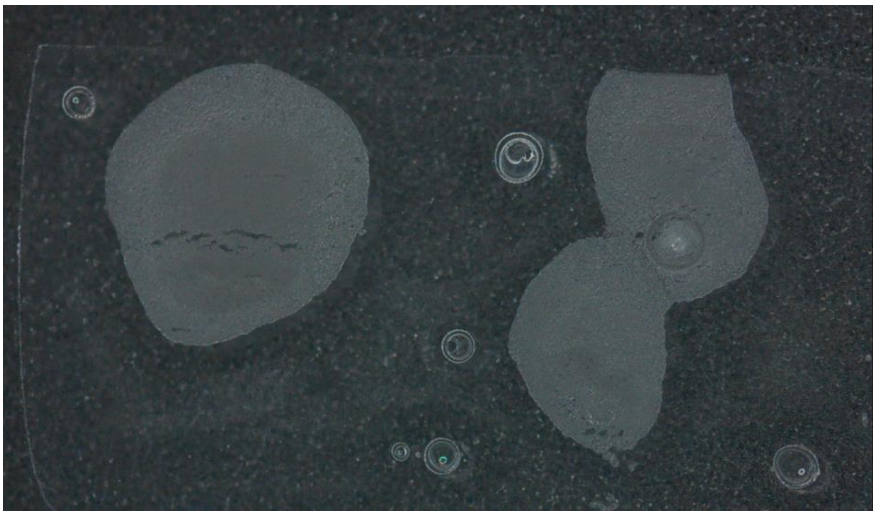
### 4.1.1 Quality Control Example I:

This is an example of quality control done on sections under the microscope (Zeiss Stemi 508). The entire section is not visible and there are many holes within the sections indicating thick and thin parts within each section. These sections did not pass quality control test.



### 4.1.2 Quality Control Example II:

The sections in this image show that the entire section is visible and the thickness across each section is roughly consistent. However, the section on the left has a crack, which is not ideal.



# 5 Annexes / Attachments

## 5.1 Troubleshooting:

The following information was taken from Thermo Scientific CryoStar NX70 / NX50 Operator Guide 387928:

| Problem | Cause | Remedies |
|---|---|---|
| Sections fold or crumple | 1. Specimen is too warm.<br>2. Knife holder too warm.<br>3. Anti-Roll plate too low.<br>4. Anti-Roll plate and/or clamp plate dirty. | Specimen is too warm<br>Knife holder too warm<br>*Tip – use freezer spray on clamp plate and blade to confirm.*<br>Anti-Roll plate too low<br>Clean with absolute alcohol & dry thoroughly. |
| Section rolls up under the Anti-Roll plat | 1. Specimen is too cold. | Raise specimen temperature. |
| Specimen curls after lifting Anti-Roll plat | 1. Anti-Roll plate & clamp plate too warm.<br>2. Static electricity in chamber<br>3. Blade blunt | Lower chamber temperature<br>*Tip – use freezer spray on clamp plate and blade to confirm*<br><br>Change blade |
| Sections tear or crack | 1. Specimen too cold.<br>2. Blade damaged or dirty<br>3. Specimen frozen too rapidly or specimen overly large. | Raise temperature<br>Change blade |
| Specimen and section chatter | 1.Knife holder not correctly clamped<br>2. Blade incorrectly clamped.<br>3. Specimen incorrectly clamped. | Check & tighten all stages of knife holder<br>Blade clamping force can be increased by tightening clamp screw at the rear of the top stage.<br>Check specimen is securely mounted and clamped in specimen head jaws. Check there is no debris or ice on the back of the chuck or on specimen head |
| Sections thick-thin | 1. Check knife holder and specimen correctly clamped.<br>2. Ensure specimen is securely attached to the Cryocassette/chuck.<br>3. Temperature of specimen incorrect. | <br>Raise or lower temperature<br>Change blade |

# 6 Appendix

NA