Document Number: **SASoM/METHOD/144.v1**

Title: **Sectioning Formalin fixed paraffin embedded (FFPE) blocks using the Leica RM2255 or Shandon Finesse microtome.**

Version: **v1**

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SOP History		
Number	Date	Reason for Change
v1	01/11/2021	Original

1.0 Purpose –

This SOP describes the current procedures for performing Sectioning FFPE (Formalin fixed paraffin embedded) blocks using a microtome in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff performing Sectioning FFPE (Formalin fixed paraffin embedded) blocks using a microtome in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff performing Sectioning FFPE (Formalin fixed paraffin embedded) blocks using a microtome in this manner are responsible for ensuring that the methods are followed in accordance with this SOP. All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

Lab 248 currently uses two Leica RM2255 microtomes and one Thermo Finesse microtome (Figure 1). Users should refer to the appropriate instruction manual prior to use. These are bench-mounted instruments and are adapted for use with disposable metal or glass blades. These devices operate with a staged rotary action such that the actual cutting is part of the rotary motion. In a rotary microtome, the knife is typically fixed in a horizontal position.

Figure 1 Microtomes available in 248 lab

After use, the excess wax trimmings and sections should be brushed from the microtome and put in the bin. Always brush upwards near the blade and do not brush the cutting edge of the blade. The blade should be removed before cleaning for safety and **ENSURE FINGERS DO NOT COME INTO CONTACT WITH THE CUTTING SURFACE OF THE BLADE**. The microtome should then be properly cleaned with histoclear or Lotoxane.

4.1 Ice plates

Blocks should be placed on an ice plate to cool before sectioning. Plastic containers filled with water and stored frozen in the freezer are used for this. After use these ice trays should be wiped clean, refilled and placed back into the freezer.

4.2 Water baths

The water bath is filled with tap water. Check the temperature dial is at correct setting (it should be 40-45°C). After use, the water is poured out and the inside dried with paper towel.

4.3 Disposable blades

Used blades may be kept for use with block trimming (see below) or placed into the bottom of the blade container that forms part of the blade dispenser or into a SHARPs bin awaiting disposal. **ALWAYS ENSURE FINGERS ARE KEPT AWAY FROM THE SHARP CUTTING SURFACE OF THE BLADE.** When

starting a new blade, start sectioning from one end of the blade and as each part becomes blunt work along the length of the blade.

4.4 Block trimming

• Before sections can be taken from a block, excess wax has to be trimmed from the surface of the cutting face. This process is termed “trimming” or “facing-in” and is performed on a microtome. This is for newly processed and embedded tissue samples.
• Make sure the safety catch is on and the knife guard is in place.
• Place the block into the microtome chuck (cassette holder). Position the face of the block close behind the blade edge. Do this by either moving the blade holder unit back to the block or advancing the block holder to the blade.
• Align the block face with the blade edge using the adjusters. **KEEP FINGERS AWAY FROM BLADE EDGE.**
• Tighten all clamps.
• Set microtome section thickness to 10µm or use the microtome advance mechanism.
• Continue rotating the microtome handle until the surface wax has been removed and the full face of the tissue can be seen.

4.5 Sectioning (Figure 2)

• Once the block has been trimmed, place it back onto the ice plate for 20 – 60 minutes to cool before sectioning.
• Apply the microtome safety catch.
• Ensure that a new piece of sharp disposable blade is used to cut sections. • Place the block back into the microtome holder and realign the block with the blade edge.
• Make sure all clamps have been tightened.
• Set the microtome cutting thickness to 2-6 µm.
• Release the safety catch.
• Rotate the microtome handle until a full face is achieved.
• Dispose of unwanted sections using forceps (or a brush).
• Rotate handle in a steady continuous motion to cut a section.
• Using forceps (or brushes) remove the section(s) carefully from the blade avoiding contact with the blade edge.
• Float the section(s) using a dragging motion into a clean water bath set at 40-45°C. The heated water will cause the section to expand and flatten.
• Inspect the section and compare with the block to make sure there is a full face. If a full face is not achieved initially then the block should be trimmed further.
• Small folds in the tissue may be gently teased out using a probe.
• Sections containing large folds or tears should be discarded.

Figure 2 Example of Sectioning

4.6 Picking up sections (Figure 3)

• Once a satisfactory section has been obtained it must be “picked up” onto the appropriately labelled slide.
• To pick the section up, hold the slide at the top and immerse the slide in the water close to the section.
• Slope the slide away from the section and advance the slide gently until it encounters the section.
• Keeping the slide sloped away from the section and lift the slide slowly out of the water. The section will adhere to the slide as it is lifted from the water bath. Make sure that the section is picked up on the correct side of the slide and is placed on the lower 2/3rds of the slide. Avoid being placed on the very edges by leaving a 3mm margin on each side (indicated in blue). By placing sections on the lower 2/3 rds of the slide it reduces the possibility of sections drying out close to the upper edge of the cover tile where the solutions are dispensed if immunodetection is performed using Bond robots.
• Once the section has been picked, up tap off any excess water on a tissue and place the slide into a staining rack.
• Put slide rack into a 65°C oven and bake overnight.

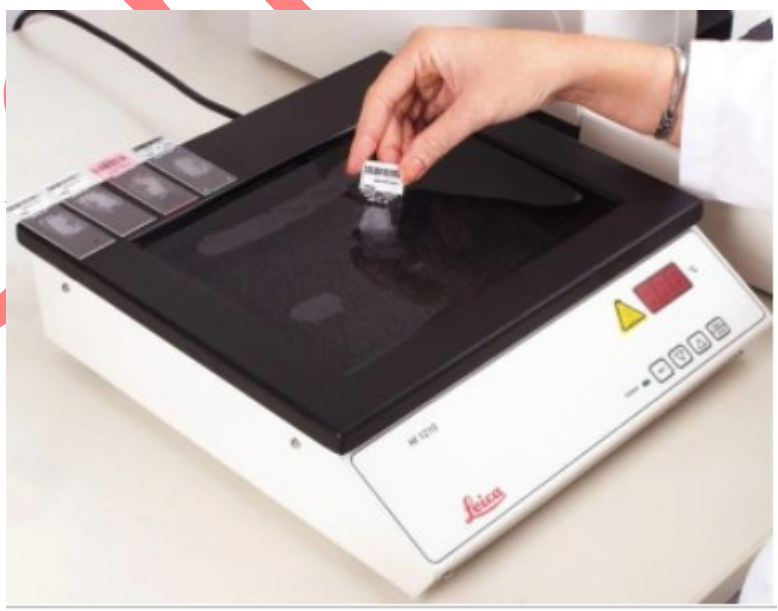

Figure 3 Picking up sections

4.7 Surface decalcification

• Occasionally, blocks are received which have small areas of calcification that will not section.
• To enable a section to be taken from these blocks they should be placed in a jar of surface decalcifying agent for a period of half an hour or more depending on the amount of calcium present. This will decalcify a small amount of calcium close to the cutting surface of the block.
• Do not leave blocks in surface decalcifying agent for long periods of time as this can cause tissue damage.

4.8 Tissue softener

• Some tissue blocks are composed of hard dense material that does not section well.
• If this problem is encountered then the block can be placed into tissue softening solution (Von Ebners) for a few hours.
• Blocks should not be left in this solution for long periods of time as this can cause tissue damage.

4.9 Embedding problems

• If a block is found to be too thin, or if when sectioning the block becomes loose, or the wax cracks, the block should be taken for re-embedding before a section is cut.

4.10 Microtome cleaning

• Brush up excess wax and clean with histoclear or lotoxane.

5.0 Personal protection –

A Howie coat must be worn at all times.

6.0 Spillages –

Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard. Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training –

All staff should undergo training in this technique before performing the procedure.

8.0 Related documents –

- Risk Assessment: CHARM_20176_Serial Sectioning of FFPE blocks.
- SOP: SASoM/EQUIP/108_Leica RM2255 Fully Automated Rotary Microtome.
- Manual for the instrument (available online).

9.0 Approval and sign off –

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Name: Peter Mullen

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Signature:  Date: 03/11/2021

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Signature:  Date:03/11/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE