



Document Number: SuRF-HIS-005.02
Title: Microtome Use and Cleaning
Version 2.0
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Reviewed annually	

SOP History		
Number:	Date:	Reason for Change:
01	01/06/2013	Original
02	19/02/2014	Procedure update

1.0 Purpose:

The purpose of this Standard Operating Procedure (SOP) is to describe the current procedure for the microtomy of paraffin embedded tissues.

2.0 Scope:

This SOP applies to all Histology staff, students, visitors and any other supervised/trained individuals involved in this procedure within the Shared University Research Facilities (SuRF), based in the Queen’s Medical Research Institute (QMRI), Edinburgh.

3.0 Responsibilities:

This document is a guide only – on site training is essential before use

- 3.1 All staff involved in this procedure are responsible for ensuring that methods are followed in accordance with this SOP after suitable training.
- 3.2 All staff must have read and signed any relevant risk assessment document(s) relating to this SOP before performing this procedure.
- 3.3 All staff are strongly advised to have an up-to-date Hepatitis B vaccination.

4.0 Procedure:

4.1 Safety considerations

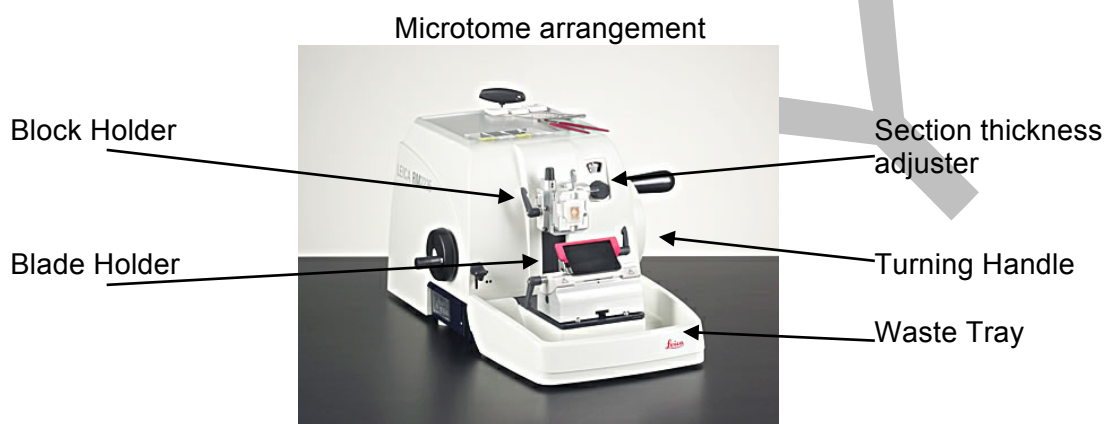
- Gloves must be worn at all times.

- Remember that the knife is very sharp and is therefore a potential hazard.

High-risk samples are not permitted in the Histology laboratory

4.2 Equipment: Microtome

The centre currently uses two Leica RM2135 microtomes, one Reichert-Jung 2035 and one Thermo Finesse microtome. Users should refer to appropriate instruction manual prior to use. These are bench-mounted instruments and are adapted for use with disposable metal or glass blades; non-disposable diamond knives are also available. 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.



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.

4.3 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.

4.4 Water baths

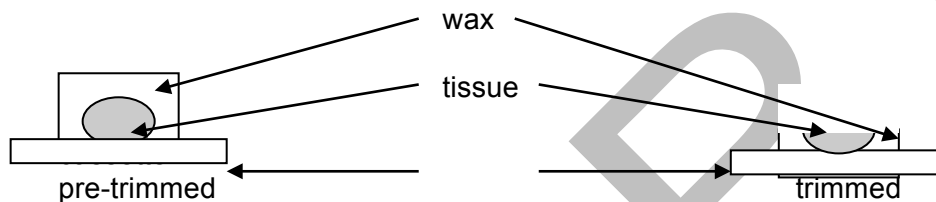
The water bath is filled with tap water. Check the temperature dial is at correct setting (it should be 40-45°C). After use, the water is poured out and the inside dried with paper towel.

4.5 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.

4.6 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.
- 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 20 μ m, 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.



4.7 Sectioning

- 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 3, 4 or 5 μ m.
- 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.

4.8 Picking up sections

- 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.

STEP 1

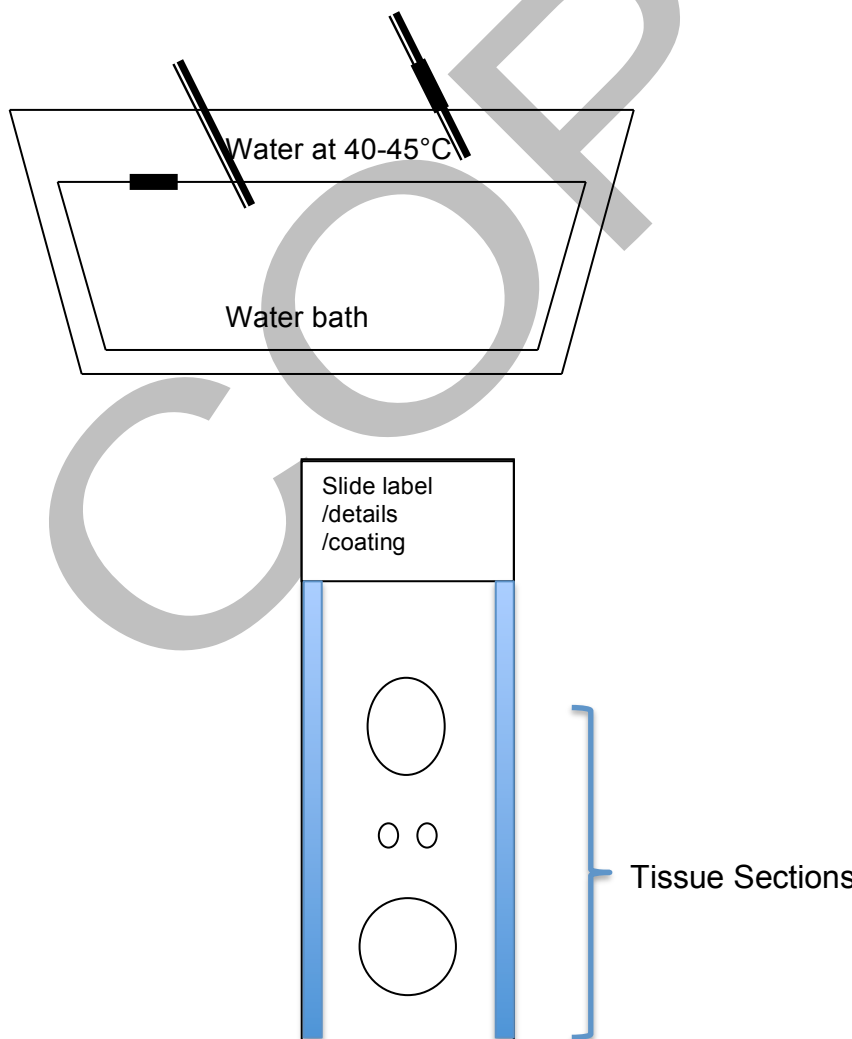
Advance Slide

Slide

Section

STEP 2

Lift Slide





- 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 55°C oven and bake overnight. If slides are for immuno staining the oven should only be at 37°C.

4.9 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.

4.10 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.

4.11 Blood

- Some blocks contain a large proportion of blood clot or blood cells, which tends to fragment and disintegrate on sectioning.
- To remedy this situation the surface of the block can be placed on a tissue soaked with acetone.
- The acetone prevents the section from disintegrating when it leaves the blade edge.

4.12 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.

4.13 Post treatment

- Stored at 40°C or 55°C overnight or until required.
- Block can be returned to client, sectioned or stained for light microscopy or immunohistochemistry as required.
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4.14 Microtome cleaning

- Brush up excess wax and clean with histoclear.



5.0 Related documents /references:

- RA1: SuRF-RA1-005: Microtome Use and Cleaning
- COSHH: SuRF-COSHH-005: Microtome Use and Cleaning
- Manufacturer's manual / instructions
- The University of Edinburgh Health & Safety Policy / Codes of Practice (available on University's Health and Safety Department website)
- College of Medicine and Veterinary Medicine Health and Safety Manual (available on University's Health and Safety Department website)
- Bancroft J.D., and Gamble M. Theory and Practice of Histological Techniques 6th Edition (2008) Churchill Livingstone, Elsevier Limited

6.0 Approval and sign off:

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