MICROTOMY & CRYOTOMY

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Microtomy

- Final aim of most procedures in histology = good quality, stained section on a glass slide
- Microtomy is how we produce these sections
- AKA “Cutting” or “Sectioning”
The Rotary Microtome

- Most popular in routine histology
- Designed to cut paraffin embedded tissue
- Rolling advancement mechanism
- Advances block to knife by present amount (µM)
The Rotary Microtome
Safety

- Microtomes are very dangerous!
- Be alert – most accidents occur due to lapses in concentration
- Microtome blades are very sharp!
Safety

- Wheel **MUST** be locked when your hand is not in control of it
- Knife guard **MUST** be up when:
  - Inserting or removing block
  - Any manipulation when blade in place
  - Microtome is unattended
Safety

- Do **NOT** use your fingers to pick up sections from the blade
- Remove blades when cleaning and leaving the microtome
- Dispose of blades appropriately
Microtomy “How To” - Preparation

1. Locate blocks & slides
2. Check cold plate & waterbath
3. Ensure the wheel is LOCKED
4. Cover blade with knife guard
5. Insert and secure your blade
Microtomy “How To” - Trimming

1. Clamp block in holder
2. Lower guard, unlock wheel
3. Move block until it just clears knife
4. Chill block when at full face
5. Trim the block Until it is “full face”
Microtomy “How To” - Sectioning

1. Ensure knife is clean and sharp
2. Clamp block and adjust to correct position
3. Turn the wheel using smooth, even strokes
4. Dip slide into water behind section and lift vertically
5. Grasp end of ribbon and transfer to water bath
6. Slide is dried and submitted for staining
Factors Affecting Section Quality

- Clearance Angle
- The Block
- Fixation, Processing, & Embedding
- The microtome
- The Knife
- Mounting & Drying Technique
- Temperature
Cutting Tips

- Clean work area
- Trimming blade - decent
- Cutting blade – clean and sharp
- Cold blocks
- Blood rich tissues – moisture
- Brain – chill face up, cut at 7μM
Contamination

- Sources:
  - Wax trimmings
  - Section debris on and around microtome
  - Surplus sections and debris on waterbath

- How to avoid it ...

CLEANLINESS!
Common Microtomy Faults

- Section Too Thick
- Holes
- Moth Eaten
Common Microtomy Faults

Knife Lines

Disruption
Common Microtomy Faults

Micro Chatter

Coarse Chatter
Common Microtomy Faults

Folds

Compression
Common Microtomy Faults

- Bubble Under Section
- Over Expansion
Cryotomy – Frozen Sections
What are Frozen Sections and Why do we do them?

- Tissue sections cut from fresh, snap frozen tissue

- Indications:
  - Rapid intra-operative diagnosis
  - Immediate patient management
  - To determine further testing
  - Demonstration of lipids
  - Staining
Snap Freezing Tissue

- Several different methods
- Specimen frozen onto cryostat chuck in OCT (Optimal Cutting Temperature) Medium

- Note: Unlike paraffin embedding, the TOP surface will be cut
Ice Crystal Artefact

- Human tissues full of water
- $\text{H}_2\text{O}$ (liquid) $\rightarrow$ Ice = Expansion
- Occurs in frozen sections when:
  - Freeze too slowly
  - Too much OCT
  - Specimen too large/dense
Ice Crystal Artefact

- Ice crystals begin to form at 0°C and 1 atmosphere

- Slower Freezing – Hexagonal Crystals
- Rapid Freezing – Cubic Crystals
- Very Rapid Freezing – Vitreous Ice Formation

Cell Damage & Loss of Morphology
Freezing Methods

- **Cold Contact/Rapid Freeze Bar** (-30°C - -40°C)
  - Slower
  - Cooling/freezing from below
  - Best with a pre-chilled chuck
  - May be assisted by aerosol spray
Freezing Methods

- **Dry Ice/Solvent Mixture**
  - Rapid Freezing
  - Isopentane packed in dry ice — must be allowed to stabilise (-80°C)
  - Tissue and OCT lowered into isopentane until frozen (stops bubbling)
Freezing Methods

- **Liquid Nitrogen/Isopentane**
  - Very Rapid Freezing
  - -150°C
  - Unforgiving, may crack tissue
Freezing Methods

- **Aerosol Spray**
  - Fast evaporating liquids
  - Reduce surface temperature
  - Approx \(-50^\circ C\)
  - Not ideal as stand alone method
The Cryostat

- Instrument used to cut frozen sections, “Cryotomy”
- Cold chamber with microtome mechanism inside
- -20°C - -30°C
The Cryostat
Safety

- Fresh Tissue = Potentially Infectious
- PPE – gloves, gown, goggles
- Same safety precautions as microtome
  - Blades are just as sharp when they are cold!
- Bare skin – Freeze burns
Cryotomy – Cutting a Frozen Section

- Same as microtomy but in a cold chamber
- Apply PPE
- Mechanism secure, wheel locked, knife guard
- Secure chuck in holder- tighten the screw
- Use coarse advance to trim top layer of OCT
- Fine trim until almost full face
Cryotomy – Cutting a Frozen Section

- Have slides and fixative ready
- Frozen sections cut at 5-6µM
- Use small brush to coax section off knife
- Drop/lower slide onto section
- Place in fixative
- Stain with a rapid H&E and cover slip
Limitations

- Time
- Freezing Artefacts
- Nature of the tissue
Frozen Section

Paraffin Section

Ice Crystals

Nuclear Chromatin Changes
Important Factors

- Temperature – chamber, knife, plate, and OCT
- Knife – angle, quality of edge
- Cutting technique – speed, specimen size, orientation of block
- Mounting and Fixation
- Equipment
References

- http://library.med.utah.edu/WebPath/HISTHTML/HISTO.html#1
- The Royal Children’s Hospital – Anatomical Pathology
- The Royal Women’s Hospital – Anatomical Pathology
- RMIT University – Laboratory Medicine (Histopathology)
Any Questions?