COURSE: 322-HISTOLOGICAL TECHNIQUE

PRACTICAL -1

INTRODUCTION and DEMONSTRATION

The safety protocols:

1-Wear gloves and a lab coat with closed toed shoes, long pants, mask and goggle when selection of tissue blocks and when preparing fixatives.

2-Avoid eating inside the lab or any drinks…

3-Wash hands properly after practical.

Definitions:

Histopathology, the microscopic study of diseased tissue, it is an important tool in anatomical pathology.

Histology is the scientific study of the fine details of biological cells and tissues using microscopes to look at specimens of tissues that have been carefully prepared using special processes called “Hiatological technique” or Histotome.

Microtome is a sectioning instrument that allows for the cutting of extremely thin slices of material, known as sections. Microtome are an important device in microscopy preparation.

Microtome knives use steel, glass, or diamond blades depending upon the specimen being sliced and the desired thickness of
the sections being cut.

1-**Steel blades**
2- **Glass knives**
3- **Industrial grade diamond knives.**
4- **Gem quality diamond knives.**

- **Demonstration:**

1. Setting the Microtome
2. Section cutting
3. Picking section.
4. Mounting section
5. Draining & drying section

1. Setting the Microtome

Microtome:
An instrument that is used to cut a specimen, as of organic tissue, into thin sections for microscopic examination. The exposed ends of the knife or blade must at all time be protected by magnetic or clip-on knife guards.

- (Semi-automatic Rotary microtome)

(Routine Rotary microtome)

Section cutting

a. Trimming of the blocks:
Before trimming the block, put the block into cold water or ice trays. This has the advantage of cooling both the tissue and the wax given them a similar consistency.
The trimming may be done by either setting the thickness 15-10um adjuster or by advancing the block using course feed mechanism. All screws must be firmly tightened.

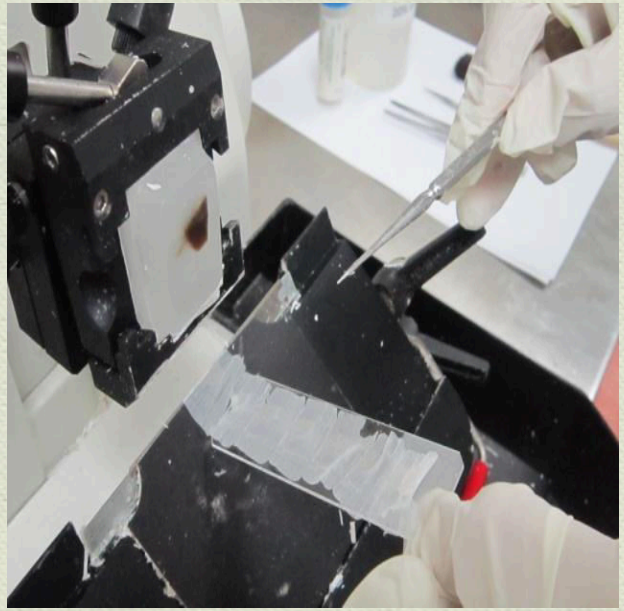
*Goal of trimming:
To expose a suitable area for sectioning.

b. Cutting sections:

After trimming the blocks to expose a suitable area for sectioning, adjust the cutting thickness up to 3-5um to the optimal thickness.

Ribbon of sections..

When a ribbon of six to eight sections has been cut, the first section is held by forceps or needle and the last section eased from the knife edge by a small brush which itself will affect the last section.

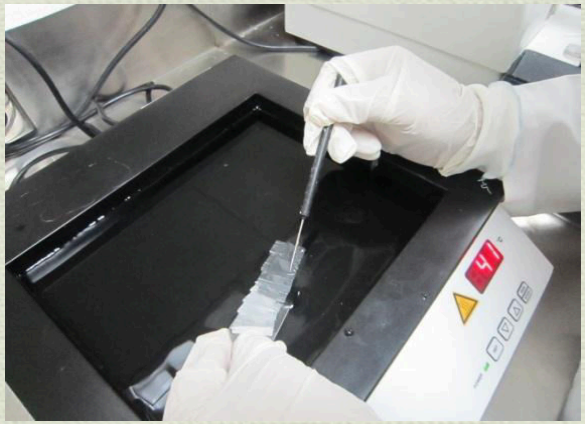


Ribbon of section of 6 to 8 sections

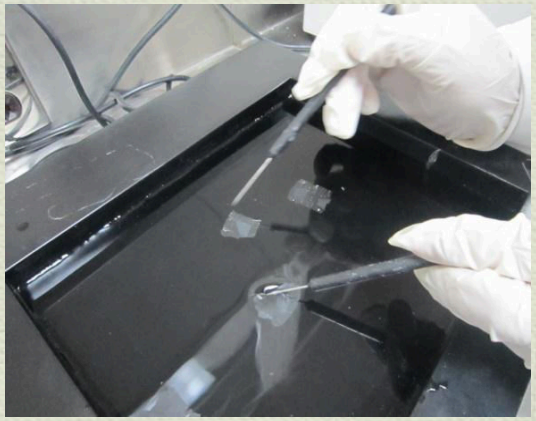
3. Floating out sections:

After cutting apply 20% alcohol (why?)

The floating out bath should be filled with the fluid of choice and raise to the appropriate temperature . Section must be laid shining side down on the floating out bath. If folds have occurred, they may be removed by gently teasing with forceps.

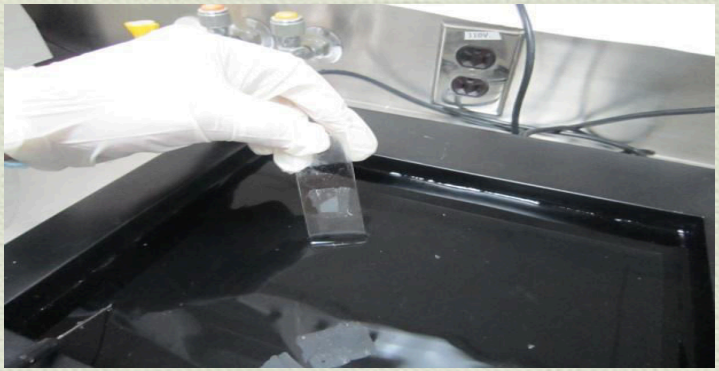


Floating out section



Separate the section using the needle

4. **Picking section:**



5. **Drying sections:**

The sections will allow further flattening to occur when heat is applied to dry the sections.

The temperature should be at the melting point of the wax.

Instruments needed in section cutting:

1.Microtome
2.Water bath
3.Hot plate
4.Prepared blocks
5.Cold water or ice trays
6.20% alcohol Gelatin
7. Forceps
8. Needles
9. Brush
10. Blade/knife
11. Glass slides
12. Gelatin

Types of Microscopes:

- **Compound microscopes** -can be found in most biology and science classrooms. They are electrically operated and use light to enhance the image of a cell. They will have multiple lenses for viewing.

- **Dissecting microscopes** are also known as stereo microscopes. They have low magnification and are also light powered. These microscopes can view objects larger than what a compound microscope is able to handle, in three dimensions.

- **SEM- Scanning Electron Microscope** uses electrons instead of light to create an image. These microscopes produce three-dimensional images with high resolution and magnification. They also have a larger depth of focus.

- **TEM-Transmission Electron Microscopes** use electrons instead of light to create an image. The material prepared must be very thin. The beams of electrons that pass through it give the viewer high magnification and resolution. These give two-dimensional images.

Care and Handling:

Transporting:	When you pick up the microscope and walk with it, grab the arm with one hand and place your other hand on the bottom of the base. DON'T SWING THE MICROSCOPE!
Handling & Cleaning:	Never touch the lenses with your fingers. Your body produces oil that smudges the glass. This oil can even etch the glass if left on too long. Use only LENS PAPER to clean the glass. TOILET PAPER, KLEENEX, AND PAPER TOWELS HAVE FIBERS THAT CAN SCRATCH THE LENSES.
Storage:	When you are finished with your "scope" assignment, rotate the nosepiece so that it's on the low power objective, roll the nosepiece so that it's all the way down to the stage, then replace the dust cover. DON'T FORGET TO USE PROPER TRANSPORTING TECHNIQUES!

Types of Microscopes:

	Compound	Scanning Electron Microscope (SEM)	Transmission Electron Microscope (TEM)
description	Compound microscopes are light illuminated. The image seen is two dimensional. This microscope is the most commonly used. You can view individual cells, even living ones. It has high magnification,and low resolution.	SEM use electron illumination. The image is seen in 3-D. It has high magnification and high resolution. The pictures are in black and white.	TEM is electron illuminated. This gives a 2-D view. It has high magnification and high resolution.
source of radiation	visible light	electrons	electrons
nature of lenses	glass	one electrostatic lens with a few electromagnetic lenses	one electrostatic lens and a few electromagnetic lenses
Focusing	mechanical	electrical	Electrical i.e. current of the objective lens coil is changed.
Providing specimen Contrast	Light Absorption	electron scattering	electron scattering