ANAT 2160/BIOL 3430 Introduction to the histology laboratory

Laboratory rules

1) No eating or drinking in the laboratory at any time. The laboratory benches are shared with classes in neuroanatomy in which preserved human brains are sometimes used on the open benches. Although the benches are cleaned after every laboratory session, there may be occasional traces of contamination left on the bench surfaces. Do not bring food or drink into the laboratory.

2) Do not remove any teaching materials, microscopes or specimens from the laboratory.

3) Return microscopes and slide sets to the correct locker after use. The correct locker number is indicated on all microscopes and slide sets.

A) Cytology overview: cellular features important for histology.

Objectives: The summary and exercises below are meant to demonstrate some of the features of cells that can be used to help identify histological specimens, and to familiarize you with using the microscope.

Cytology: Cells are the basic building blocks of tissues, organs and ultimately the whole body. Many of the features of individual cells are submicroscopic (can't be seen with a light microscope), but some features are obvious and can be used to identify different cell types. The following cellular components, and in particular the way these components may differ in different cell types, will be important clues as you learn to examine microscope slides of body tissues.

- Plasma membrane
- Cytoplasm
  - cytosol
  - organelles
    - mitochondria
    - endoplasmic reticulum
    - Golgi apparatus
    - vesicles
  - cytoskeleton
    - microtubules
    - microfilaments
    - intermediate filaments
  - inclusions
    - nutrients
    - pigments
- Nucleus
  - membrane
  - chromatin
  - nucleolus

B) Introduction to the microscope.

The correct use of the compound microscope is key to visualizing the tissues you will be learning about in this course. The brief description below is intended to familiarize you with basic microscope operation, and gives you some useful tips when looking at the slides. Parts of the microsope are labelled on the accompanying drawing.

Light source. All microscopes require a source of light to properly illuminate the tissue you are viewing. Some of the microscopes in this laboratory have internal light sources (you can tell this by the electrical cord attached to the bottom of the microscope), but most microscopes will require an external light source (a lamp on the bench) that you will need to direct toward the mirror under the stage. Place the lamp about 10 cm away from the mirror, turn on the light, aim the lamp directly at the mirror and adjust the mirror so that light is directed into the bottom of the condenser under the stage.

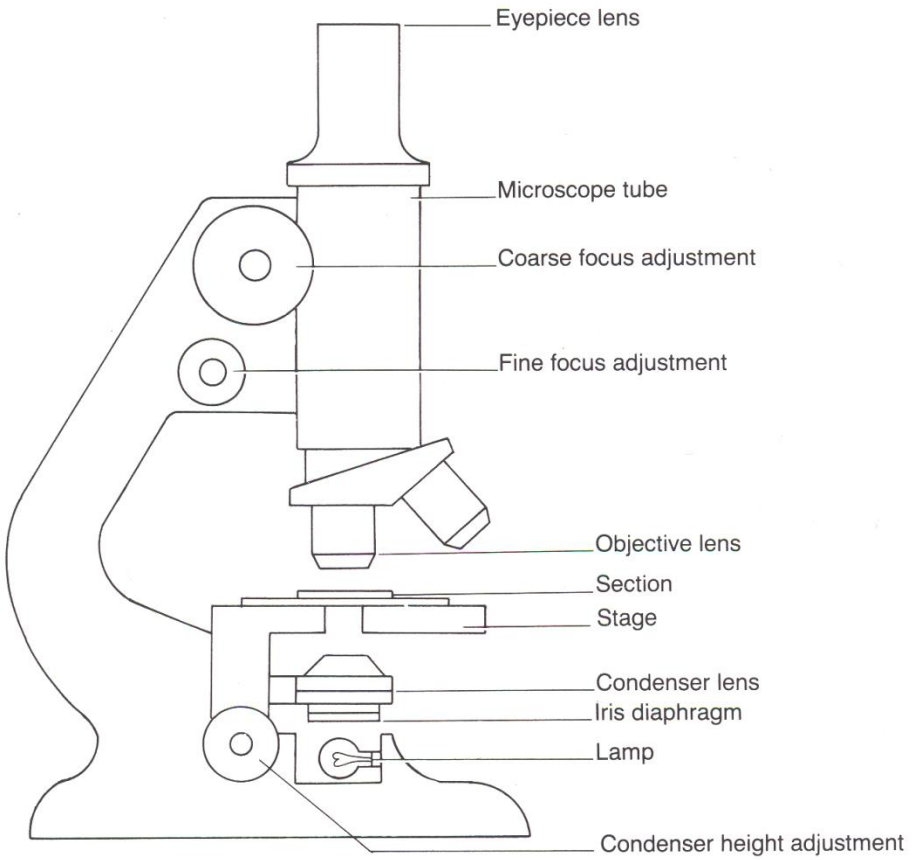
Initial setup. Once the lamp and mirror are aimed correctly (or the internal light is turned on), set up the rest of the microscope starting from the base and working upward. Open the iris diaphragm in the condenser body to its maximum, move the small top lens on the condenser (if there is one) out of the light path, and raise the condenser to the top limit of its travel. Place a slide on the stage so that some tissue is illuminated (there should be a bright spot on the slide from the light coming through the condenser). Turn the objective turret so that the shortest (lowest magnification) objective lens is above the slide, and turn the coarse focus knob to bring the lens toward the slide (but do not touch the slide with the lens). Looking through the eyepiece, adjust the coarse focus knob until an image forms. Use the fine focus knob to get the best focus possible.

If your microscope has an external lamp, at this point you may have to adjust the angle of the mirror slightly to get even illumination of the whole field of view. For both types of microscope, remove the eyepiece and look down the barrel of the microscope at the bright spot of illumination. Slowly adjust the iris diaphragm so that light fills about 3/4 of the field of view. Replace the objective and check that the slide is still in focus.

Viewing slides. The above procedure sets the microscope up for using the lowest magnification lens. When you change slides or turn the objective turret to the lens of the next higher magnification, you should look at the tip of the lens from the side as you are swinging it into position to make sure that it does not bump the slide. If it comes close to the slide, raise the lens using the coarse focus knob, then once the lens is in position bring the tissue into focus by slowly adjusting the focus knob as you look through the eyepiece. You may have to move the condenser slightly to get a bright and high-contrast image after changing objective lenses.

Tips:
Before putting a slide on the microscope stage, take a look at the tissue on the slide with your eyes against a white background. This is useful to see where on the slide the tissue is located, or to determine which part of a large tissue slice you might want to look at under the microscope.

Always start examining a slide using the lowest magnification objective to get an overview of the tissue before switching to higher magnification objectives.



Parts and simplified optical path of the light microscope.

C) Examples of cellular components

The slides listed by number in these exercises are found in the slide boxes that are common to each two adjacent positions at your benches. Slides numbered 1-100 are found in one box, slides with higher numbers are located in the other box (may be labeled "Extra slides"). Remove the slides indicated below from the boxes, and place them on a sheet of white paper or other light background. In this exercise you will look at examples of cellular features that are visible at the level of the light microscope.

Note: you will encounter all of these slides in more detail later in the course when you examine the specific organs and systems from which these specimens are taken.

Caution: do not open a slide box upside down. Place the box flat on the benchtop with the label facing upward. If you open a box of slides upside down, most of the slides within will come out, and you will have to sort them back into their slots. At worst some will fall on the floor, break and will need to be replaced. Please be careful with these boxes!

69, liver. The liver contains a very large number of cells, called hepatocytes, that are shaped roughly like cubes and are packed tightly into functional units. Look at this slide first with your eye against a light background, select a part of the reddish-stained tissue section somewhere in the centre of the section, and place the slide on the microscope stage so that this part of the section is in the illuminated field of view. At low magnification you will be able to make out many very small dark dots throughout the field of view; these are the dark-staining nuclei of individual cells. When you increase the magnification by changing the objective lens the nuclei will become more visible and you will begin to see more cellular detail, including some cell-to-cell borders. At the highest magnification nuclei and some cellular borders are clear. You will not be able to see the plasma membranes of individual cells, but you can see thin, dark red lines marking places where there is extracellular material between some cells. Note that there are nuclei stained to different degrees of darkness, and that their size varies. Since there is usually one nucleus per cell in the human body, the number and size of nuclei visible in a field of view is a good indication of how cellular the tissue is (the more cellular the tissue, the more nuclei are present); nuclei of different sizes may also indicate that there are different types of cell present. The cytoplasm of hepatocytes holds a considerable amount of glycogen (one of the nutritive inclusions in cell cytoplasm) but this cannot be seen without special staining.

66, parotid gland. This slide shows a section of a gland associated with the mouth, at the sides of the jaws, that secretes saliva. The cells in this gland have very extensive and active endoplasmic reticulum and Golgi apparatus, although these cannot be seen at the light microscopy level. However, the cytoplasm of many of these cells has vesicles (surrounded by a membrane very similar to the plasma membrane) containing secretory products, mostly enzymes, that are stained red and look granular. These secretory vesicles will merge with the plasma membrane and empty their products into ducts which in turn empty into the mouth.

86, skeletal muscle. More than 90 % of the volume of skeletal muscle cells (myocytes) is filled with the contractile proteins actin and myosin, part of the cytoskeleton. The presence of these proteins in each cell is evident in the strong cross-banding of these muscle cells. The banding shows up at medium and high magnification. You will not see many nuclei in these cells. The dark red, fibrous material on this slide is collagen in the form of tough fibres that join the muscle cells to bone.