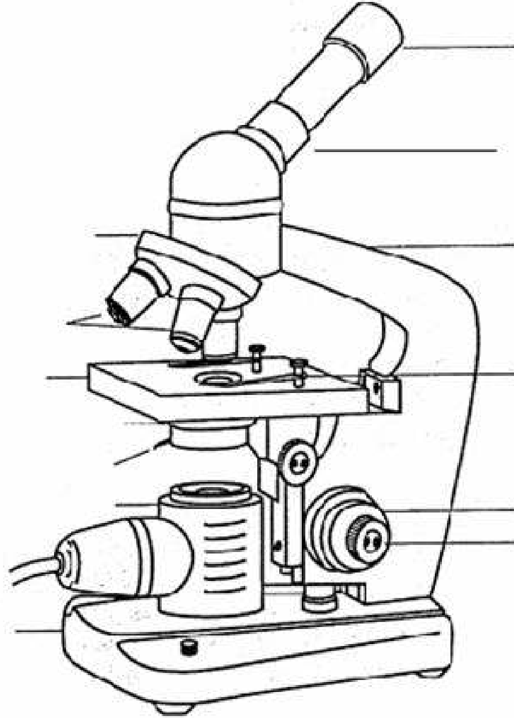
INS 2007-08 Pre-lab I Questions

Note: You will benefit the most by reading through the laboratory handouts first before attempting to answer these questions without the help of the handouts. After you answer the questions, go back to the handouts to find the answers and correct your work. Use your pre-lab questions as a mock quiz! Open-ended questions are not meant to have one specific answer.

1) Label the parts of the compound light microscope in the diagram below (13 parts in total).



2) What is the total magnification of your specimen with the 40X objective in place?

3) What are the lowest and highest magnification capabilities of your microscope?

4) What can you say about the relationship between magnification and field of view?

5) Why is acridine orange mutagenic?

6) What is the purpose of mitosis?

7) How does mitosis differ from cytokinesis?

8) Design a feasible experiment to estimate the proportion of time that cells in actively dividing tissues, such as the root tip, actually spend in M-phase, cytokinesis, and Interphase.

Introduction to Natural Science
Biology Lab 1 - Microscopy and Introduction to Mitosis

I. Lab Orientation

It is very important to know your way around the laboratory and be able to find any equipment that you may need. During the first half hour we will help orient you to the lab.

II. Microscopy

Part A – Learning how to use a compound microscope

The discovery of the cell and its organelles was enabled by the development and refinement of the microscope. There are two basic types of microscopes: light microscopes and electron microscopes. The light microscope uses glass lenses and visible light to form a magnified image of an object. An electron microscope uses magnets to focus an electron beam. Since we cannot see electrons, the electron microscope directs them at a fluorescent screen or photographic film to create a visible image.

The light microscope is a standard instrument in the biology laboratory. The images that we see can be described graphically (by drawing and photography), qualitatively (describing shape, color, and movement), and quantitatively (measuring the number and dimensions of individual structures). Throughout the program we will emphasize the importance of being quantitative when making observations. In this lab, as you change the magnification of the image, you should pay particular attention to scale (characterizing an object as being “small” and “large” is relative, and not very informative in absolute terms).

Goals

1) Identify the parts of the compound microscope and be proficient in their correct use.
2) Determine the diameter of the field of view and use it to estimate the size of the specimen
3) Understand relative sizes of different cell types
4) Familiarize yourself with the location and function of the optical and mechanical parts of the compound and microscope
5) Learn the proper technique for preparing a wet-mount slide

The Compound Light Microscope

The compound microscope is used to magnify and resolve fine detail within a transparent specimen (one through which light can pass). It has two separate lens systems (hence the term compound). The objective lens is located near the specimen and magnifies the image to a certain degree (4X, 10X, 40X, 100X). The ocular lens is located in the eyepiece and further magnifies the image formed by the objective lens system (10X). The total magnification of the image seen by the observer is the product of the magnification of the two lens systems (objective X ocular).

The resolving power of the microscope is equally as important as its ability to magnify. Resolving power is the minimum distance two points can be separated and still be distinguished as two separate points. The resolution of a light microscope is limited by the wavelength of visible light. The maximum possible resolution of the light microscope is 0.2 μm; the highest magnification in a light microscope with maximum resolution is approximately 1000X.

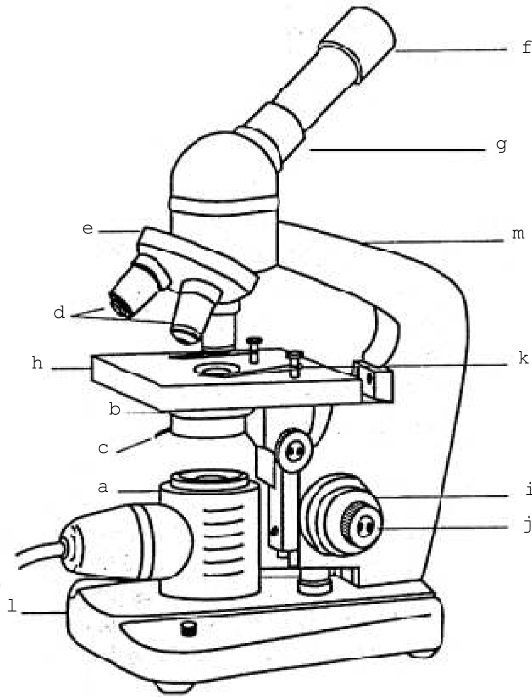
Observe the following precautions when using the microscope:

Always carry the microscope in an upright position. Use one hand to grasp the arm (m in diagram below) of the microscope; use the other to support the base (l in diagram below). The eyepiece (ocular lens) slides into the body tube and could fall out if the microscope is tilted.
Never place the microscope close to the edge of the lab table or counter. Be sure to place the electrical cord out of the way and not in a position where it could catch and drag the microscope to the floor.

Use only lens paper or a Q-tip soaked with lens cleaner for cleaning the lenses. Using tissue paper, other materials, or a dry Q-tip could damage the lenses.

Never put a microscope away with the high power objective in the viewing position or with a slide on the stage. When you are finished with your observations, remove the slide, turn off the illuminator, clean the lens, rotate the low-power objective into viewing position and make sure the stage is clean.

Parts of the microscope



a. Light source. The light source is built into the base with a lens that focuses light onto the lower condenser lens and is covered by an adjustable field diaphragm.

b. Condenser. The condenser contains a system of lenses that focuses light on the specimen.

c. Iris diaphragm. The iris diaphragm is used to adjust the amount of light striking the object. It can be opened or closed using the lever on the side of the condenser.

d. Objective lenses. The objectives are mounted on a revolving nosepiece or turret (e) and each contains a complex lens system. Most new microscopes are parfocal. (Once you have focused on an object using one objective lens -usually the low power- you can then switch to high power without completely losing focus.)

e. The nosepiece holds the following objectives (the magnification is indicated on the side of the objective): **4X** magnification (low power objective), **10X** magnification (low power objective), **40X** magnification (high power objective)**, 100X** magnification (oil immersion objective)

f. Ocular Lens or Eyepiece. The ocular lens is the lens that you look through. It will usually magnify the image 10X. Focus each ocular and use both eyes to look at your specimen. One of the oculars contains a scale within the eyepiece (ocular micrometer) that you will use to make measurements. Some models are fitted with a movable body tube or head piece (g) which can be rotated.

h. Stage. The specimen slide sits on the stage and is held in place by the mechanical stage (k). Adjustment knobs connected to the mechanical stage allow you to move the slide left and right and forward and backward. The stage can be moved vertically by turning the coarse adjustment knob (i) or the fine adjustment knob (j). These will be located in different places in different types of microscopes, and are usually nested. As the stage is moved up and down the object will come in and out of focus. The coarse adjustment is used for initial focusing at low power. Fine adjustment makes very slight changes and is used for precision focusing at higher power.

Using the Microscope

To look at a fixed slide:

1. Move the stage down all the way.
2. Place a slide on the stage and move the specimen into position.
3. Move the lowest power objective into position
4. Looking at the stage, move the objective down all the way, toward the slide with the coarse focus adjustment.
5. Then, looking through the oculars, focus away from the specimen (moving the objective away from the slide with the coarse focus adjustment) until it is in focus.

Adjustments:

1. Adjust interocular distance so that you are using both eyes.
2. Focus each ocular.
3. Adjust light level to comfortable level.

Pointers:

Always begin with the lowest magnification to locate your specimen, and then increase magnification as necessary. Because depth of field (thickness of specimen that may be seen in focus at one time) will decrease as you increase magnification, bigger is not always better. If you have trouble finding the proper focal plane, it may be helpful to first focus on the edge of the coverslip, then move back to the center, and then slowly focus down on your sample.

Be sure you have your specimen in good focus and location (centered in the field of view) before attempting to use the oil immersion lens (see Appendix I for Koehler Illumination). Once you have added oil to the coverslip it is almost impossible to return to lower magnifications. If something goes wrong, you will have to clean the oil from the slide and start over; with wet mounts this may mean making a new slide.

After you have completed your observations remove the slide from the stage. Thoroughly remove all oil from the objective lens as instructed. If you have spilled oil anywhere on the microscope (the stage etc.) clean that up as well.

Part B - Making measurements with the microscope

The size of objects viewed with the compound microscope can be estimated by first determining the diameter of the field of view for a particular microscope objective and then estimating the size of the specimen by comparing it with the total diameter of the field of view.

1) Place a transparent ruler across the field of view under scanning power (lowest power objective) and record the diameter in millimeters (mm) __________. What is this diameter in micrometers (µm)?

2) The diameter of the field of view using the scanning objective (A) can be used to calculate the diameter of the field of view using any other objective (B)

Remember: total magnification = objective magnification X ocular magnification

$$\frac{\text{Total magnification A}}{\text{Total magnification B}} \text{ X diameter A } (\mu\text{m}) = \text{diameter B } (\mu\text{m})$$

3) In your lab notebook, calculate the diameters of the fields of view using each of the objectives on your microscope. Create a table in your notebook similar to the one shown here.

objective	diameter field of view (µm)
4 x	
10 x	
40 x	
100 x	

4) If you have a **stage** **micrometer**, you can measure the diameter of the field of view and compare it to the calculated value.

Note: the scale of an **ocular** **micrometer** changes with total magnification, and thus has no absolute value. Therefore, you need to use a stage micrometer or the calculation above to determine the relative size of the ocular micrometer at each magnification.

5) Obtain a prepared slide of a eukaryotic organism and estimate the length of one cell in micrometers. Make a sketch in your notebook including the estimated size of the cell. Be sure to indicate what specimen you are looking at and any other pertinent details that would help someone to repeat your work.

6) For the sake of size comparison, look at a slide of bacterial cells that we have provided. Provide an estimate of their size.

Part C - Basic wet-mount technique

1) Place a drop of water/saline in the center of a clean microscope slide. Add the specimen (mixed culture, tissue etc.) to the drop. (In some cases you may place the specimen directly into a drop of stain.)

2) Place one edge of a coverslip at the edge of the water drop and gently lower it so that the liquid containing the specimen completely spreads out under the coverslip (Figure 5). Take care not to trap air bubbles under or around the specimen. Do not press down on the coverslip. If there is too much water, draw off the excess by touching the corner of a paper towel, Kimwipe or blotting paper to the edge of the coverslip.

3) Examine your wet mount under low power and then under high power.

4) After you have finished with your slide, remove the coverslip and discard it in the glass disposal bin; wash and dry the slide. Clean, dried slides should be returned the slide storage box.

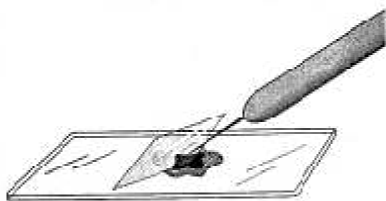


Figure 5. Preparation of a wet mount. Place a drop of water and your specimen on the slide. Place a coverslip at a 45° angle with the slide, then slowly lower a coverslip onto the slide using a dissecting needle or your fingers. Try to avoid trapping air bubbles under the slide.

Freshwater aquatic plant cells

1) To prepare a wet-mount of an aquatic plant leaf, choose one of the smaller leaves and tear it in half. Do not cut it. When you are examining the specimen you will be able to see more clearly if you look near the torn edge of the leaf. Why is this?

2) What is the approximate cell size? Record your observations in your notebook.

Note: Your observations should include drawings and labels as well as descriptive text. Remember these are your notes and they are all you will have when you are trying to recall this lab (or any lab)!

Human epithelial cells

The epithelial cells that line your cheek are thin flat cells that you can remove easily from you cheek with a toothpick or a swab. Prepare a wet mount of your own epithelial cells.

1) Use a clean toothpick to gently scrape the inside of your cheek several times.

2) Roll the end of the toothpick though a drop of water that you have placed on a clean microscope slide. Add a small drop of methylene blue and a coverslip.

3) Observe your cheek epithelial cells under the microscope (begin with low power). Try to find some cells that are not folded over and study them in detail at a higher power. Can you identify the cell membrane, nucleus and cytoplasm?

How are the animal cells different from the plant cells? Record your observations

III. Introduction to Mitosis

Part A - Examining and measuring mitotically dividing chromosomes

It was not until the early 1900s that chromosomes were suspected of carrying the genetic material of the organism. It is generally difficult to observe chromosome structure in G1, S, and G2 phases of the cell cycle (referred to as Interphase), as chromosomes exist as long, thin strands. In M-phase of the cell cycle chromosomes undergo condensation in preparation for mitosis or meiosis, and are thus easier to observe.

Onion root tips are often used to demonstrate the stages of mitosis because cell division is especially rapid in the growing root tip. We have treated some root tips overnight in Carnoy's fixative (a mixture of acetic acid and methanol). You will further prepare the root tips by digesting with hydrochloric acid, then staining with acridine orange dye. The acridine orange will bind to chromosomal DNA, staining the DNA orange (be careful - acridine orange is a mutagen!)

Prepare a slide of a stained onion root tip as follows:

1) Remove 2 root tips from the Carnoy's fixative tube and immerse them in a new tube filled with 1 mL of 1N HCl. Incubate for 12 minutes at 60C.

2) Remove the HCl with a Pasteur pipet and discard in the drain with running cold tap water.

3) Add 0.5 mL Acridine Orange and incubate at room temperature for about 10 minutes or until the root tip appears dark orange.

4) Place one drop of 45% acetic acid on a clean microscope slide.

5) Place a root tip into the slide in the drop of acetic acid. With a scalpel or razor blade, remove all but the darkly stained very tip of the root.

6) Add a cover slip and place the slide on a sheet of white paper on your bench.

7) Tap gently and straight down with the eraser end of a pencil until the stained tip is spread out to a faint monolayer. Do not smear the coverslip sideways – this will shear the chromosomes!

8) Examine your spread using the low power objective to make sure you have spread the cells in a thin monolayer. If the cells are still in thick layers, tap with the pencil eraser some more

9) Once you have a nice monolayer (hopefully one cell thick) switch to your oil immersion objective and identify the various stages of M-phase (prophase, metaphase, anaphase, and telophase). Make a sketch of

each of these in your laboratory notebook and properly label them with a title and a description of what is happening in each stage. Cells that are not in mitosis are in Interphase of the cell cycle and will not have condensed chromosomes; thus, you will not be able to see the chromosomes in these cells.

10) Calculate the length of 5 different onion tip cells and chromosomes in micrometers (µm). Collect data from two other students in the laboratory and compare the results. Were there any noticeable differences in the measurements? If so, what could account for this?

Part B – Experimentation
Conduct the experiment you designed in your pre-lab to estimate the proportion of time that cells in actively dividing tissues, such as the root tip, actually spend in M-phase, cytokinesis, and Interphase.

Appendix I: Setting up Koehler Illumination
1. Place a slide on the stage and rotate in the 10X objective.

2. Adjust the inter-ocular distance if you are using a binocular scope.

3. Focus on the specimen with the coarse focus.

4. Adjust the eyepieces if you are using a binocular scope.
--close the eye which uses the adjustable eyepiece
--focus on some detail of the specimen with the coarse focus
--open your closed eye and close the other eye. Use the **eyepiece** to focus on the same detail with the second eye

5. Completely open the condenser iris diaphragm.

6. Partially close the field iris diaphragm - until its image appears in the field of view

7. Focus the condenser until the image of the field diaphragm is clear (if you have an Abby condenser, this will be the point between the orange/blue switch)

8. Center the field iris diaphragm with the condenser screws. This is easiest if the iris diaphragm is opened until the image nearly fills the field of view.

9. Open the field iris diaphragm until the field of view is completely illuminated.

10. Remove eyepiece and look at the back focal plane of the objective. Close the condenser iris diaphragm until its image just encroaches on the illuminated area of the back focal plane of the objective (7/10)

Note: *When you change to a higher objective you should adjust the aperture of the condenser iris diaphragm in order to provide a sufficient amount of light for adequate resolution of detail.* (Repeat steps 9 & 10 above.)