Use of the Microscope and Cytology

Introduction:

A true study of anatomy not only considers the large, visible structures of an organism, but also the small structures that provide the organism its form and function. To view these small structures, we require a tool to magnify the near invisible details. Our microscopes are compound, binocular, bright-field microscopes. These microscopes utilize a combination of lenses to magnify and clarify small objects of interest. The specimen is illuminated with light and will appear as a darker or stained image on a light background. Our microscopes are designed to be parfocal- meaning that when an object is in focus with one power, it should be in focus on another power (or very close to being focused).

Parts of the Microscope:

1. ocular (magnifies 10X)
2. body tube
3. arm
4. nosepiece
5. objectives
   a. scanning objective (magnifies 4X)
   b. low power objective (magnifies 10X)
   c. high power objective (magnifies 40X)
6. stage platform with stage clips
7. mechanical stage controls
8. coarse adjustment knob
9. fine adjustment knob
10. condenser
11. iris (disc) diaphragm
12. light source/power source
13. base

Click for a Numbered Diagram of the Microscope

Calculating Total Magnification:

The microscopes used in the labs are compound microscopes, meaning that both ocular and objective lenses are used during observation. The use of both lenses provides a higher degree of magnification than using a single lens. Total magnification can be calculated for the microscope by using the following formula:

\[
\text{Total Magnification} = \text{Power of Objective lens} \times \text{Power of the Oculars}
\]

Ex. High Power = 40X, Oculars= 10X

<table>
<thead>
<tr>
<th>Power of Lens</th>
<th>Total Magnification when in use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning:</td>
<td></td>
</tr>
<tr>
<td>Low:</td>
<td></td>
</tr>
<tr>
<td>High:</td>
<td></td>
</tr>
</tbody>
</table>

40X \times 10X = 400X (Total magnification with high power lens in place)
Proper use of the microscope:

1. Carefully place the microscope on the tabletop in a position comfortable for viewing. Make sure to grasp the arm of the microscope when moving the microscope. Do not attempt to move the microscope by grasping the body tube.

2. Check to be sure that the power switch is in the off position and that the light intensity on the base is at the lowest setting. Plug the microscope into the nearest outlet.

3. Using lens paper and cleaning solution, clean the oculars and the objectives of the microscope to remove dust and oil. Be sure to use only the lens paper provided. Normal paper towel is too abrasive.

4. Turn the nosepiece until the scanning power objective (red ring) is in place.

5. Move stage down using the course adjustment to put slides in place. Slides should be placed between the stage clips, not underneath them.

6. Use the course adjustment knob to move the stage up and down while on scanning power to achieve focus of the specimen. It is usually helpful to bring the stage to its highest position while on scanning power and focus the specimen away from the objective. This cuts out a lot of needless adjustment and makes finding the specimen quicker and easier.

7. When focus is achieved on scanning power, the nosepiece can be turned to bring the low power objective into place.

8. Using the fine adjustment knob, SLOWLY adjust the position of the stage until the image is in focus. Since the microscopes are parfocal, this should be a small adjustment.

9. Repeat the previous step to view the specimen at high power. You may have to adjust the position of the slide on the stage using the stage control knobs.

10. When you have completed viewing the slide, move the nosepiece to scanning power and lower the stage using the course adjustment. Be careful not to attempt this with the microscope at the high power setting as you could drive the lens through the slide, damaging both.

11. When you complete the viewing of all slides, return the slides to the proper trays, clean the microscope lenses again, and return the microscope to the proper cabinet with the dust cover in place.

Inversion:

1. Obtain a prepared slide of the letter “e.” Mount the slide on the stage of the microscope. Center the “e” under the objective.

2. While on scanning power, move the stage to the highest position. Careful! You must be on scanning power only. Any other objective could damage the slide.

3. Once at the highest position, use the course adjustment to bring the “e” into focus. Slow careful movements will ensure that you do not over focus.

4. Notice that the image on the slide is different when view through the microscope than when viewed with the unaided eye. The image is upside down and appears backwards.

This effect is referred to as inversion and is caused as the light passing through the specimen is passed through lenses. Students need to remember to account for inversion when moving the slide using the mechanical stage controls. Since the image is inverted, movements of the stage will likewise be reversed.
Field of View:

1. After noting the effects of inversion, move the nosepiece so that the low power objective is in place. Since the microscopes are parfocal, only a small amount of focusing with the fine adjustment knob is necessary to refocus the image.

2. Using the stage controls, center the “e” in the center of the field of view. Notice that not only is the “e” magnified, therefore larger, but that you cannot see as much of the “e” or the surrounding background.

   As magnification of a specimen increases, the amount of the specimen, or the **field of view**, decreases. This means that more of the specimen is visible at the lower powers. Good microscope use begins with the lower powers. Students often have a difficult time locating structures with a microscope because they try to start with the highest magnification. This is poor technique because so little of the slide is visible at the higher powers.

Depth of Focus (Depth of Field):

1. Properly mount the slide named “Colored Threads” on the stage.

2. Find the area of the slide where the three threads cross with scanning power.

3. Move to low power and adjust the focus with the fine adjustment knob.

4. Try to focus the microscope so that all three threads are in perfect focus.

   Is it possible to focus all three threads? The answer is no. While the threads are small and thin, they still have a dimensional height. Since the threads are stacked atop each other, they are in different planes. This means trying to focus all the strings at the same time is not possible. This demonstrates the concept of **depth of focus (field)**.

Notes:

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Cytology: The Study of the Cell

Introduction:

All living things are composed of cells and all cells arise from preexisting cells. This idea is the **cell theory**, which is the one of the unifying principles of biology. Cells are the smallest unit of life. Some organisms exist as single cells, while other organisms are made up of trillions of cells. In complex organisms, cells group together with similar cells to form functional units called tissues. Tissues provide many various functions and can further group together to create organs. In advanced organisms, organs form systems that allow for complex functions to be carried out by the organism.

Cells have specific regions and structures that carry out the basic functions necessary for life. Many of these structures are tiny membrane-bound structures called **organelles** (“small organ”). Like the organs of the body, they perform the biological processes necessary to maintain life in the cell.
Cell Structures and Their Functions:

<table>
<thead>
<tr>
<th>Structure</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell (plasma) membrane</td>
<td>Acts as a protective barrier for the cell; plays a role in the movement of substances into and out of the cell</td>
</tr>
<tr>
<td>Cytoplasm (cytosol)</td>
<td>Fluid filling the internal spaces of the cell and suspending the organelles within in the cell</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Contains the genetic information of the cell; guides cell functions through the expression of genes</td>
</tr>
<tr>
<td>Nucleolus</td>
<td>Assembly site for ribosome particles</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Oxidizes food material to produce cellular energy in the form of ATP (cellular respiration)</td>
</tr>
<tr>
<td>Ribosomes</td>
<td>Produce proteins</td>
</tr>
<tr>
<td>Rough Endoplasmic Reticulum</td>
<td>Attached ribosomes function in protein synthesis; acts to store proteins and allows for modification of proteins and transport of proteins to other cell areas</td>
</tr>
<tr>
<td>Smooth Endoplasmic Reticulum</td>
<td>Site of lipid synthesis, lipid metabolism, and drug detoxification.</td>
</tr>
<tr>
<td>Golgi apparatus</td>
<td>Packages proteins and other substances for export out of the cell or for inclusion into the cell membrane</td>
</tr>
<tr>
<td>Centrioles</td>
<td>Help to form the spindle apparatus during cell division; form the basis of cilia and flagella</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>Contain digestive enzymes used to break down substances brought into the cell, old organelles, and even the entire cell if necessary.</td>
</tr>
<tr>
<td>Peroxisomes</td>
<td>Helps to detoxify alcohols, hydrogen peroxide, and other harmful substances that enter the cell.</td>
</tr>
</tbody>
</table>

Click the following links for larger pictures: [ONE](#)  [TWO](#)

Observation of Human Cells:

Like all living things, humans are composed of cells. An easy way to view human cells is to perform a check cell smear and observe with a microscope. Use the following direction to prepare a slide to observe.

1. Obtain a clean slide, cover slip, applicator stick, and methylene blue stain bottle from the tables in the center of the room.
2. Gently scrap the inside of your mouth with the applicator stick to remove cells.
3. Rub the stick on the center of the slide, using a circular, rolling motion to transfer the cells.
4. Add a drop of methylene blue to the top of the smear. This will add the need contrast to the cells to make them easier to see.
5. Place the cover slip atop the smear, being careful to avoid a lot of air bubbles.
6. Observe the stained smear at all powers from scanning to high power. Use caution when going to high power. If the lens will not freely rotate into place, do not force it, as you will likely damage your slide/microscope.

Notice that all the organelles covered earlier are not visible even at the high power (400X). These organelles are too small to be able to resolve with our light microscopes. Typically, you will be able to see the cell membrane, the nucleus, and the cytoplasm. It is also likely that you will find food debris and bacteria on these smears as well.
Cell Physiology - Tonicity

Cells interact and respond to the environment around them. The solutions inside the cell and outside in the environment can have an effect on the cell's shape. The ability of a solution to change the shape of a cell is referred to as **tonicity**. These changes are caused by the movement of molecules through the membrane, altering the contents of the cell. Molecules typically move from areas of higher concentration to areas of lower concentration, a process referred to as **diffusion**. Water behaves in the same way, moving from high concentration to low concentration, a process called **osmosis**. A solution that contains the same concentrations of solutes (dissolved substances) as the cell is termed **isotonic** and causes no change to the cell's shape. If a solution contains more solutes than the cell, it is termed **hypertonic**, and will cause the cell to shrink in size due to the movement of water out of the cell. This shrinking is referred to as **crenation**. If a solution contains less solute than the cell, it is termed a **hypotonic** solution and it will cause the cell to expand due to the movement of water to the inside of the cell. The expansion of animal cells can lead the cell to **lyse**, or rupture. Your instructor will demonstrate tonicity to you using blood cells and different types of solutions.

Cell Division:

An organism must be able to produce new cells in order to survive. These cells are necessary to grow, repair damage, replace aging cells, and to reproduce. Cell division can be thought of in two distinct sequences; nuclear division and splitting of the cytoplasm (**cytokinesis**). Let us consider mitosis, a specific type of cell division that generates 2 identical daughter cells from one parent cell. This type of division allows for growth, repair, and replacement of cells in humans. Before we consider mitosis, let us first consider take a look at the cell cycle. All living cells move through a cyclic series of events culminating with mitosis.

In the diagram, you can see the stages of the cell cycle. The G₁, S, and G₂ collectively are referred to as interphase, a stage in the cell's life when it is not actively dividing. These phases include important processes necessary for successful cell division. As the stages are completed, the cell can undergo mitosis and create daughter cells for the purposes mentioned above.

<table>
<thead>
<tr>
<th>Cell Cycle Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁ (Gap 1)</td>
<td>Period of vigorous growth and protein synthesis</td>
</tr>
<tr>
<td>S (Synthesis)</td>
<td>DNA is replicated</td>
</tr>
<tr>
<td>G₂ (Gap 2)</td>
<td>Enzymes and other proteins needed for division are synthesized</td>
</tr>
<tr>
<td>M (Mitosis)</td>
<td>Nuclear division occurs (cytokinesis also takes place in most cells as well at this time)</td>
</tr>
</tbody>
</table>
After the S phase, the cell contains a double amount of DNA needed for normal function. These two copies will be separated to create the new cells at the end of mitosis. To make the DNA more manageable to move about, it is wound up tight into a structure called a chromosome. The diagram below shows the structure of a chromosome. The separation of the duplicated chromosome into sister chromatids is the key to ensure that each cell gets the proper amount of genetic material.

Notes:

Phases of Mitosis:

Mitosis consists of four stages, each recognized by the changes occurring to the nucleus of the cell during the process. Recognizing the different stages of mitosis requires an understanding of what is occurring to the nucleus during each phase. The diagram shows cells in the different phases of mitosis. The table below summarizes the events of each phase.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophase</td>
<td>Chromosomes condense and become visible, nuclear envelope starts to breakdown</td>
</tr>
<tr>
<td>Metaphase</td>
<td>Chromosomes align across cell’s equator</td>
</tr>
<tr>
<td>Anaphase</td>
<td>Sister chromatids are separated and pulled towards the cell’s poles, cleavage furrow forms due to contractile ring</td>
</tr>
<tr>
<td>Telophase</td>
<td>Nucleus starts to reorganize, chromosomes relax, cytokinesis completed.</td>
</tr>
</tbody>
</table>

Obtain a slide labeled Animal Mitosis (or Whitefish Blastula) and mount it on your microscope. On this slide are several clumps of cells called blastulas. Within these blastulas are examples of each phase of mitosis. Using high power, examine the cells and determine their specific phase.

Notes: