Lab 2 - Part 1

Microscope Skills & Mitosis

Using the Compound Light Microscope :

We use a light microscope to view histology slides for many of the Anatomy & Physiology labs. It is very important to get good at using the microscope yourself and to be able to troubleshoot common problems. First, let's review the names and basic functions of the many parts of the compound light microscope you will use in lab.

□ **Ocular Lenses**
— Two of them, each with a magnification of **10X**.
— One of them usually has an arrow you can position.
— attached to the head, and can swivel left & right.
— the distance between the ocular lenses is also adjustable to fit the distance between your eyes.

□ **Objective Lenses**
— **RED** Objective Lens has a magnification of **4X**.
- also called the "scanning" objective.
- best objective to start getting a slide in focus.
- "safest" objective to use the coarse focus knob.
- field diameter is about **4,700 µm**.

— **YELLOW** Objective Lens has a magnification of **10X**.
- also called the "low" power objective.
- "still ok" to use the coarse focus knob, can't hit slide.
- field diameter is about **1,800 µm**.

— **BLUE** Objective Lens has a magnification of **40X**.
- also called the "high & dry" power objective.
- ONLY use the fine focus knob.
- field diameter is about **470 µm**.
- MOST COMMON objective lens used to view slides.

— **WHITE** Objective Lens has a magnification of **100X**.
- also called the "oil immersion" objective.
- ONLY use the fine focus knob.
- field diameter is about **180 µm**.

□ **Rotating Nosepiece**
— rotates the 4 objective lenses attached to it.

□ **Mechanical Stage**
— The metal clips that hold your slide on the Stage.

□ **Stage**
— The black platform you put your slide on.

□ **Condenser**
— has a light concentrating lens that is usually kept close to the the slide on the stage.

□ **Condenser Knob**
— regulates the height of the condenser.

□ **Iris Diaphragm Lever**
— adjusts the *"amount of light"* passing through the condenser.
— contrast tends to improve as you close it.
— open it if the field of view is too dark.
— you will use the diaphragm lever more than you think!

□ **Arm** — One the Two places to hold the microscope when carrying it.

□ **Base** — One the Two places to hold the microscope when carrying it.

□ **Substage Light & Light Control** — adjusts the *"brightness (intensity) of the light"* being used.
— you will need a higher light intensity at higher magnifications.

□ **Coarse Focus Knob** — "safest" to use with the "scanning" RED objective lens (4X).
— "still ok" to use with the "low" power objective lens (10X).
— NEVER USE with the 40X and 100X objective lenses.
You will hit the microscope slide and potentially break it!

□ **Fine Focus Knob** — Safe to use with any objective lens.
— It is the smaller knob set inside the Coarse Focus Knob.

Other Microscope Use & Care Tips:

□ Use two hands to transport the microscope. One hand on the Arm, the other on the Base.

□ Use Lens Paper to clean the lenses and slides before viewing. Fingerprints, skin oils, immersion oil and dust can make it impossible to get things in focus at higher magnifications.

□ Always start focusing using the RED objective lens (4X). Then move up to the YELLOW objective lens (10X), and finally the BLUE objective lens (40X). We only use the White oil immersion objective lens (100X) for looking at blood slides in BIOL 221.

□ Return the microscope back to the counter on the side of the lab with the RED objective lens in place and the cord wrapped around the base.

□ Don't take the microscope apart. If there is a mechanical problem, just let your professor know.

□ Return any slides back to the proper box. The slides are numbers to assist you in this task.

□ "PARKING" slides is like parking a car… only one per spot. Don't cram the slides into a full spot and don't "park" them at angles. Keep the slides strait. This simple request keeps the slides nice for everyone and a nice slide is always nice to study from and for the lab exams.

□ I draw a "smily face" on slide labels that are just really, really good. Unfortunately, not all slide are *awesome*… you may need to look at a few to good at recognizing stuff on some of them. If you are struggling finding stuff on one slide, just put it back and try another one.

Microscope Usage Terms:

□ Working Distance

— the amount of space between the objective lens and the microscope slide.
— The working distance gets less and less as you increase the magnification.
— With the BLUE objective lens (40X) and the White objective lens (100X) the working distance is so small, you can easily collide the objective lens with the slide and break it if you accidentally use the coarse focus knob.

□ Total Magnification (TM)

— The Total magnification is what you will see noted somewhere with each histology or cell microphotograph.
— Ocular Magnification X Objective Magnification = **TM**
— Your microscope in lab has only 4 Total Magnifications:
- 10X X 4X = **40X**
- 10X X 10X = **100X**
- 10X X 40X = **400X**
- 10X X 100X = **1000X**

— 400X is the most common Total Magnification view you will spend time studying for most slides.
— The "X" is the unit for magnification. Don't forget to write your "X" after the number when writing Total Magnifications!

□ Field of View

— the circular area that you see when looking in the microscope.
— the diameter of the field of view gets smaller and smaller as you increase the magnification.
— The approximate diameter of the field of view for each Total Magnification is measured in micrometers (μm):
- **40X — has a field of view of about 4,700μm**
- **100X — has a field of view of about 1,800μm**
- **400X — has a field of view of about 470μm**
- **1000X — has a field of view of about 180μm**

□ Parfocal

— modern microscopes are mechanically calibrated so that all you need is to do a little bit of fine focus knob adjustment to get things in focus as you advance in total magnification.

□ Resolution

— the ability to see two very very close objects as separate and distinct.
— without a microscope the human eye can resolve objects about 100 μm apart (0.1 millimeters). Any closer and the objects appear fused together.
— with a compound light microscope you can resolve objects about 0.2 μm apart under ideal conditions. Any closer and the objects appear fused together.
— increasing the intensity and amount of the light at higher magnifications will improve resolution.

□ Contrast

— most cells are colorless under natural conditions.
— contrast is improved by staining the cells.

Optional Microscope Skill Practice Slides:

*These slides are not tested on, but are good for learning the basics of getting microscope images in focus.

Slide 1-1-2 Newsprint / Letter "e":

□ Newsprint letters / letter "e"

— these lowercase letters are usually about 1 mm in diameter.
— 1 mm (millimeter) = 1000 µm (micrometers)
— slide is mostly used to help you understand size and the fact that the image you see in the microscope is flipped in two directions (upside-down & left-right backwards).

Slide 1-1-3 Stage Micrometer:

□ Stage Micrometer

— these slides have a 1 or 2 mm ruler on them.
— slide is for measuring the diameter of the field of view.

Slide 1-1-4 Crossed Fibers:

□ 3 Colored Threads

— good for practice focusing on objects at different depths.
— see if you can figure out what color is on top, in the middle and on the bottom.
— most histology slides are very, very thin and just one or two cells thick.

Notes:

Slide 1-1-1 Mitosis (Blastodisc of Fish Eggs):

□ **Interphase**
- — Nuclear Envelope: fully intact
- — DNA: in the "Chromatin" form
- — Nucleolus: usually 1 (or more), easily visible.
- — Centrosomes: present, usually not visible
- — Interphase is divided into 3 periods:
  - • G_1 New cell growth. Centrioles replicate.
  - • S DNA is replicated.
  - • G_2 More cell growth. Final division prep.

□ **Mitosis**
- — Divided into phases. Describes the division of the NUCLEUS.

□ **Early Prophase**
- — Nuclear Envelope: mostly (over 50%) *"intact"*.
- — DNA: begins condensing into chromosomes.
- — Nucleolus: begin to disappear.
- — Centrosomes: begins to migrate to opposite poles.

□ **Late Prophase**
- — Nuclear Envelope: mostly (over 50%) *"broken apart"*.
- — DNA: all chromosomes fully coiled up.
- — Nucleolus: none.
- — Centrosomes: at opposite poles.
- — Microtubules: kinetochore microtubules attach to the centromere on the chromosome.

□ **Metaphase**
- — Nuclear Envelope: none.
- — DNA: chromosomes lined up in the center of cell forming the "**Metaphase Plate**".
- — Nucleolus: none.
- — Centrosomes: at opposite poles.
- — Microtubules: kinetochore microtubules *"ready to pull"*.

□ **Anaphase**
- — Nuclear Envelope: none.
- — DNA: chromosomes appear in "**V-shape**" due to being pulled by the microtubules.
- — Nucleolus: none.
- — Centrosomes: at opposite poles.
- — Microtubules: kinetochore microtubules *shorten and begin "pulling"* on each daughter chromosome.

□ **Telophase**
- — Nuclear Envelope: begins to reform for both daughter cells.
- — DNA: chromosomes uncoil into chromatin.
- — Nucleolus: 1 or more appear in each nucleus.
- — Centrosomes: microtubules they formed disappear.

□ **Cytokinesis**
- — NOT a phase of Mitosis, but begins during Anaphase.
- — is the term describing the division of the cytoplasm and the rest of the other organelles into each new daughter cell.
- — a **contractile ring** of actin proteins at the **cleavage furrow** can usually be seen between the two small daughter cells.