Introduction to Microscopy and Cell Structure

For the cell biologist, the microscope is a basic necessity; without it, our subjects of study would be unobservable. Original simple microscopes consisted of a single lens (objective) that increased the resolution of objects viewed with it. However, these lenses had to be held close to the eye in order for the image to fall on the retina; the greater the resolution, the closer to the eye they had to be. With the invention of the compound microscope, this real image produced by the objective lens could be viewed with a telescope, the ocular or eyepiece, and this lens could be used to produce a virtual image with enhanced magnification. This virtual image is projected onto the retina of the eye. Typical compound microscopes today have a series of objectives with varying resolution/magnification; the oculars/eyepieces are usually 10 X. Total magnification of the object under study is the product of the magnification printed on the objective lens and the ocular magnification. Although magnification can help to visualize small objects, the critical feature of microscopes in their ability to enhance resolution. This increases clarity, not just size, of the image. Resolution is reflected in a value called the numerical aperture (N.A.) which is dependent on the radius of curvature of the lens. This determines the angle of the cone of light that is accepted by the lens. The best image is produced when the light cone impinging on the lens is the same size as the maximum that can be accepted. Matching the light source to the lens is the purpose of the substage iris diaphragm, attached to the substage condenser which projects an image of the light source onto the object, making it, in effect, self-luminous. In order to match the size of the light source to the substage condenser, some microscopes also have a field iris diaphragm. In order to optimize resolution, these iris diaphragms must be adjusted for the objective lens being used and all the components in the light path must be aligned. The process of Koehler illumination accomplishes this.

Resolution is also dependent on the wavelength of light, with shorter wavelengths giving greater resolution. In practice, we utilize an incandescent bulb as a light source, thereby having a mix of wavelengths in the visible spectrum. Each of these wavelengths will be diffracted by the lenses to a different extent, leading to a concentric set of images of different colors. This chromatic aberration is partially corrected in the lenses of most microscopes, although it is not eliminated completely. Lenses are also corrected to varying degrees, depending on their specific design and quality, for two other problems inherent in their shape. One of these is spherical aberration and the other is curvature of field; spherical aberration produces fuzzy, curved images and curvature of field means that the entire field is not in focus at a particular setting. An achromat lens has partial correction of chromatic and spherical aberration; plan objectives produce a flat field of view.

Objective lenses also vary in three other characteristics that are of practical significance. Each has a characteristic working distance (between the lens and the object being viewed), depth of field (the depth into the object that will be in focus at any given time), and diameter of field. In general, these all become smaller as N.A. (resolution/magnification) increases, although some higher N.A. objectives can be manufactured to have greater working distances, for instance.
The highest N.A. objectives exceed the N.A. (1.0) of air. In practice, the material with the smallest N.A. through which the light passes will provide the limit of resolution. Therefore, objectives with an N.A. greater than one must be used with immersion oil (higher N.A.) replacing the air in the light path. **Immersion oil should be used sparingly and always cleaned off the components before the microscope is put away.**

This exercise will familiarize you with the theory and practice of operating the compound optical microscope, utilizing it to compare the characteristics of various plant and animal cells. You will observe the effects of staining specimens on increasing their apparent resolution.

**Procedures**

1. The instructor will discuss the history and theory of optical microscopy and then demonstrate the proper procedure for setting up Koehler illumination.

   To obtain Koehler illumination:
   1. Place a slide on the microscope stage and obtain an in-focus image.
   2. Close the field iris completely; focus the image of the field iris with the substage condenser.
   3. Open the field iris until it nearly fills the field of view, making sure that the image is centered using the substage condenser.
   4. **CAREFULLY (!) remove one of the oculars.** Looking down the barrel of the microscope, open the substage iris until it fills about 80% of the back of the objective lens. Replace the ocular.

   **This procedure must be repeated every time you change the objective lens that you are using.**

2. Students will practice setting up microscopes at each of the available magnifications until they can demonstrate proficiency to the instructor. They will also learn to utilize ocular and stage micrometers to measure specimens.

   **Calibrating an ocular micrometer:**
   1. The micrometer inserted into the ocular of a microscope is a unit-less scale. In order for it to measure real distances, it must be calibrated for each objective with which it will be used. This is done with a stage micrometer which resembles a microscope slide; begin by placing a stage micrometer onto the stage and focusing on the image.
   2. Stage micrometers have scales etched onto them; these scales are divided into units that are indicated on the label. For example, 2 mm might be divided into 0.1 mm or 10 micrometer parts. Be sure you know what the scale is on your micrometer.
   3. Superimpose the lines at one end of the ocular micrometer and stage micrometer scales (you might have to rotate the ocular); move your eye down the ocular scale until you find another pair of lines that are superimposed. Count the number of
divisions on the ocular micrometer and the number of divisions on the stage micrometer. Knowing the real distance subtended on the stage micrometer, divide this by the number of divisions on the ocular micrometer to give the measurement for each one. You now can measure specimens on a slide using the ocular micrometer and then applying the conversion factor calculated to convert those measurements into real distances.

4. With a slide of tissue on the stage, measure the size of some component (e.g., a nucleus.) Repeat this measurement ten times; calculate an average and note how much variation occurs among the measurements.

3. Students will observe both pre-prepared slides and make wet mounts of fresh tissues and other materials available; examine fresh tissues both with and without stain to determine how stains increase apparent resolution by enhancing contrast. Data should be recorded as detailed drawings with labels. (You might think you’ll recall what this stuff was later, but memory can be a fragile thing!) Be sure to note which objective lens is being used.

Points to Ponder (as you write your lab report.)

1. What is the purpose of the iris diaphragms in a microscope?

2. Why should you not use the substage iris to vary the brightness of the light source?

3. Why must Koehler illumination be set up again each time you change objectives?

4. What are the ways in which one could make an image with the optical microscope have more contrast? How does contrast relate to resolution?

5. What organelles are visible with the light microscope; why does a cell have specialized organelles to perform cellular functions?