Bi 1X, Spring 2012

Week 1

- **Session 2: Microscopy and Powers of Ten**

Introduction: The Relative Sizes of Things in Biology

IBM's short film "Powers of Ten" uses orders of magnitude to take a new look at physics, from the tiny subatomic particles in a proton to the huge, sweeping arms of distant galaxies. Many advances have been made that now take us well beyond the picture painted in 1977 and concern exotic states of matter at very small and very large scales.

Many other advances, in biology and medicine, fit comfortably below 1 meter. In a perfect world, we could use microscopes to resolve all of these scales in the ponds, but diffraction, the way light scatters and spreads when it meets small objects, prevents us from seeing it all. What is the resolution limit of the microscope you are using? You may find this site useful:
http://www.microscopyu.com/articles/formulas/formulasresolution.html

The scales of different organisms dictates in part how they interact with their environment: a frog swims just fine by kicking, but *E. coli* and other microbes would have trouble moving by a similar motion due to an decrease in the influence of inertia. To get around that, they have found other ways of moving with flagella. For a great explanation of the importance of size in motion, see "Life at low Reynolds number" by EM Purcell (check out the "Readings" section on the Bi1x website).

Goal:
Use prepared slides to take images of a graticule, fluorescently labeled slides and pond water. Do so with the 10x, 40x, and 100x objectives. After the images have been taken use Matlab to convert pixels to microns on the graticules for each magnification and add scale bars to each picture. Concatenate images taken at different fluorescent channels using Matlab with a caption on the false colors and scale bars.

Protocol:
Throughout the course, you will acquaint yourself with modes of light microscopy: bright-field (BF) and phase contrast (PC). Additionally, certain samples are either naturally fluorescent or they have been modified chemically or genetically to have specific fluorescent markers. You will examine these samples using fluorescence microscopy – a mainstay of modern biological microscopy.

1. Go over the Micro-Manger Manual attached at the end of this handout with your TA.

2. Understand the basic mechanisms and light paths of light and fluorescent microscopy, know the location of: eyepieces, objectives, stage, focus adjustments, condenser aperture diaphragm, field stop, phase ring, lumen, fluorescent filter block turret (i.e. dichroic), and intensity filters (excitation and emission).
3. Be able to setup Köhler Illumination in bright-field.

4. Familiarize yourself with the basics of objectives: magnification, depth-of-field, working distance, numerical aperture (objective and condenser), and the effect of imaging medium (air vs. oil) on resolution.
5. Have a working understanding of how to capture images with a CCD camera, including how contrast, saturation, binning, exposure time, frame rate and image format are all related to the quality of video or still images.
6. Spatially calibrate your microscope at three different magnifications: use a "graticule" to directly correlate pixel size with spatial dimension, estimate the error in your calibration.
7. Take images on the following samples and save for future analysis. Make sure to write down the magnification, exposure time, and fluorescent channels used in your notebook!
    a. Prefixed cells with organelles fluorescently labeled
    b. Pond water. Your TA will show you how to mount the specimens.
    c. Optional: Diatoms – unicellular organisms with a cell wall made of silica called frustule, which can be a way to test the resolving power of a microscope.

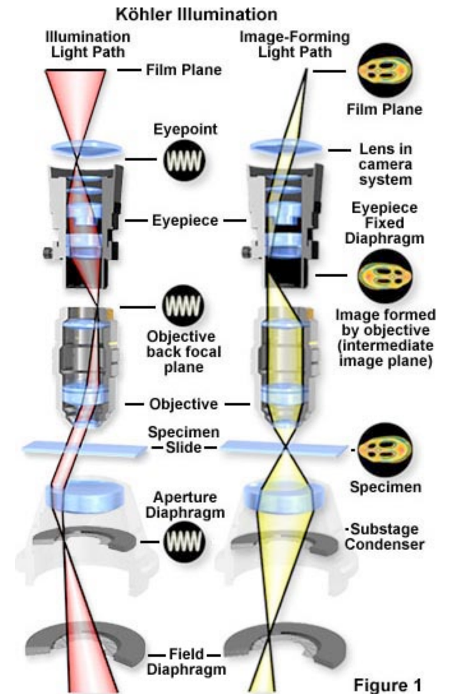

Figure 1