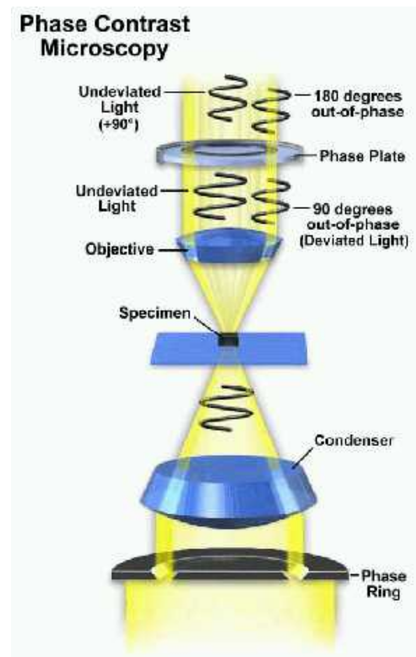
APh 162 –Week 2 Session 1 and 2

Session 1 objectives:

1. Movie of *E. coli* growth
2. Movie of *S. cerevisiae* growth
3. Movie of *C. reinhardtii* cilia beating
4. Cell-cell competition
5. Photobleaching curve

Phase microscopy:



The problem is that these biological samples don't create enough amplitude differences in light for our cameras to detect, nor do they create any sort of color that our eyes would detect (the cameras still wouldn't, since there are no colour filters on the ccd chip). Thus, we need a method of creating contrast in the specimens. One approach is to add dye molecules to the samples so that they can be seen under fluorescence, another is to encode fluorescent proteins, such as GFP (green fluorescent protein) in the target of interest. Yet another way is to externally modulate the light so the heretofore transparent specimens become opaque (see diagram on the right). In this laboratory, the type of brightfield microscopy used to create contrast is called "phase contrast". In brief, a $\pi/2$ phase shift is introduced into the zero-order components of the Fourier transform of the object through the use of a spatial filter at the back focal plane of the objective (Fourier plane). What we first notice from the diagram is that the *phase ring*, since it is a new source of light, is conjugate to the *phase plate*. Light that passes through the specimen that does not diffract is therefore focused onto the *annular ring* in the phase plate, and hence encounters a $\pi/2$ phase shift. The annular ring also absorbs some light. Light that the specimen does diffract misses the ring and is spread over the entire back focal plane. Thus the zeroth order light is reduced in intensity, and the diffracted light and zero-order light are out-of-phase by half a wave. These two combine to yield an image of higher contrast.

Growth movies protocol (required for E. coli or S. cerevisiae):

1. Inoculate an overnight culture of cells at 1/100 dilution factor into minimal media plus carbon source of choice 2-3 hours before microscopy session.
2. Turn on microscope. If using fluorescence, make sure the light source has been on for at least 30 minutes before use. This ensures that the illumination has stabilized as the bulb has reached

thermal steady state. Check to see that the microscope sample chamber is at the correct temperature.

3. Prepare 1.5% w/v agarose pads (YPD for yeast) with carbon source of choice (either LB media or minimal media+ carbon).
4. Pipette 2 uL of cells onto pads and let dry for 5 minutes at 37 C.
5. Place pad+cells onto Matek dish and focus. Check that the Koehler illumination and phase rings are aligned correctly.
6. Take a movie, making sure all the exposure times, number of frames, etc., makes sense.

C. Reinhardtii imaging (not required):

1. Wash microscope coverslips vigorously by boiling in a 1% alconox bath for 30 minutes. Let air dry.
2. Construct flow chamber from a slide and the cleaned coverslip using double sided tape (see TA).
3. Pipette into the chamber 0.05% high molecular weight poly-L-lysine (roughly 10 uL depending on flow chamber size). Let sit for 30 minutes.
4. Rinse vigorously with water (at least 5 chamber volumes).
5. Set on hot plate and boil off water. Store flow chambers at 4 C.
6. Flow into chamber chlamydomonas and image.
7. Aim a red laser at the cells and see if their cilia beating pattern changes.

Photobleaching of fluorescent E. coli or S. cerevisiae (required):

See http://micro.magnet.fsu.edu/primer/java/fluorescence/photobleaching/

1. Follow the growth movies protocol, except using minimal media + carbon source to avoid autofluorescence.
2. When imaging, add a fluorescence channel. You might want to leave the fluorescence (lumen) shutter open, depending on your individual preferences.

Cell competition assay (not required):

1. Follow the protocols above for imaging *E. coli*, except have two variants of the cells growing.
2. When the time comes (exponential phase of growth, an OD of approximately 0.2), mix the two together in an Eppendorf at equivalent volume.
3. Using a Q-tip, dab a sterile razor blade with the cells.

4. Apply to agarose pad (be careful not to puncture the agar) and image. Note that the cells will expand outwards fairly quickly, so prepare to take images (of both colours!) that are far from the original source of cells.

Homework:

1. Based on your movies, how fast does E. coli/yeast double (depending on the organism you chose)? What temperature did you use? What was the carbon source?
2. Post a movie with a scale bar and time bar.
3. Write a Matlab program to automatically find cells and measure their fluorescence intensity as a function of time, for the photobleaching assay. How did you calculate the fluorescence of a cell? Fit a function to the decay of fluorescence. Each data point should have an error bar. Specify what type or error bar you used (you can read up on error bars in Cumming et al. posted on the course website).
4. Any other experiments you do, make sure that it is posted!