BI227 ASSIGNMENT #3 **FALL 2007**

Goal for this week: Imaging live samples.

General Notes:
Live cell imaging raises several issued that should be considered when determining imaging conditions:

a. **Temperature**. Consider the physiological temperature of the organism or cells. *Caenorhabditis elegans* are comfortable between 15-25˚C so no temperature changes are necessary.
b. **Movement**. We are going to be imaging embryos (that will not move) to avoid this problem temporarily.
c. **Phototoxicity**. Light is toxic to living cells. Prolonged exposure to laser light during imaging or when trying to find your samples could cause cells to die.
d. **Photobleaching**. Like fixed samples, fluorophores in live samples can be bleached.

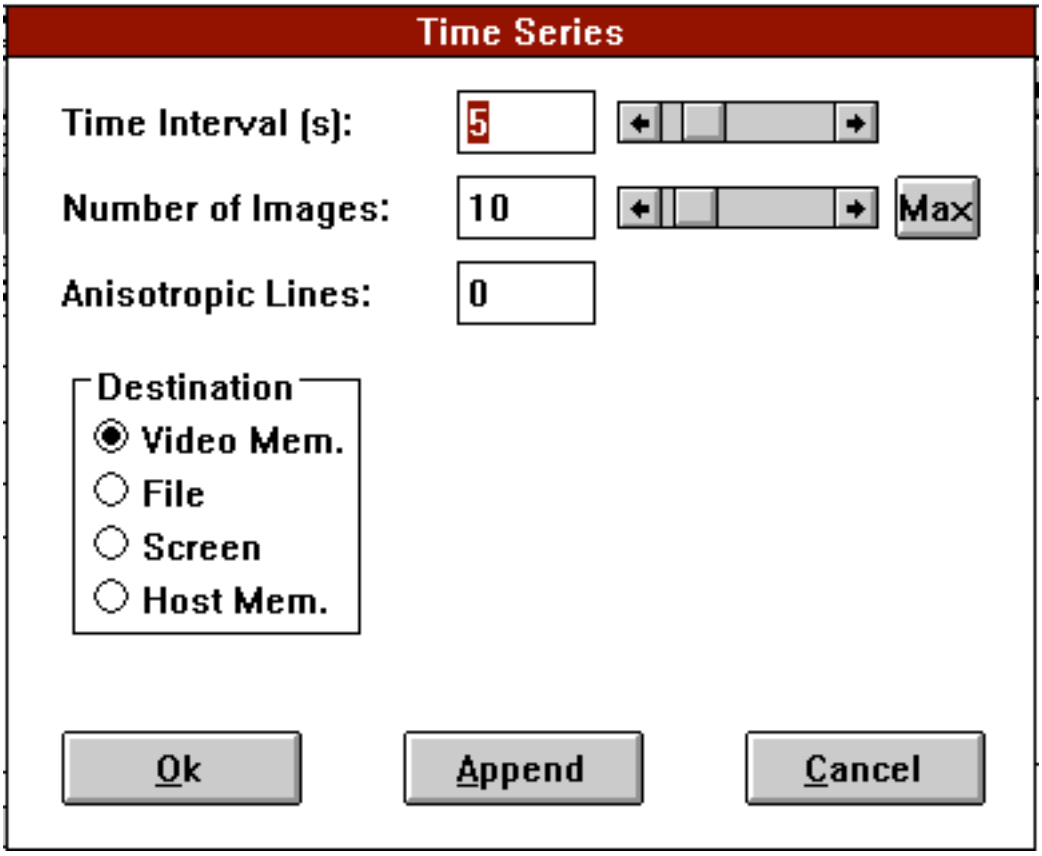
Samples:
C. elegans expressing ajm1::GFP which labels intercellular junctions between epithelial cells. For some really nice movies on these animals check out this website:
http://genetics.uchc.edu/MohlerLab/AJM-1GFPstereo-4D.html (You will need QuickTime)

Assignment for Friday: Prepare movies of time series image sequences.
1. Take a time series of the *C. elegans* embryos. Use a 100X objective or use the 63X and a zoom of 2-4X. Experiment with different scan times and scan intervals (eg. 1 second scan every 30 seconds, 2 second scan every minute…)
NOTE: Always choose intervals that are longer than the frame scan time.
Collect several time series testing out the different parameters. For each time series collection, try to image at least 10 minutes (longer is better if you have the time). Once you have found conditions you are satisfied with, set up a movie. It is fine to leave the movie for an hour or more and come back to check on it. This will allow you to image a longer period of development if you wish.

Before starting a time-lapse, always check what the size of the resulting data will be, and that it will fit on the computer:
Size (in MBytes) = N_x x N_y x N_z x N_c x N_t x N_b / (1024 x 1024), where N_x, N_y, and N_z are the number of pixels in the image in the X, Y and Z direction, N_c the number of channels, N_t the number of time points, and N_b is the number of bits per sample (bit-depth).
Example: if the images are 512 by 512 pixel, single z (N_z=1), single channel (N_c=1), 5 minutes (300 seconds) timelapse with images every 15 sec (N_t= 300/15 = 20) and 8-bit = 1byte images: (512 x 512 x 1 x 1 x 20 x 1)/(1024x1024) = 5 MB.

Things to think about:
What are the considerations for T-series collection settings?
->Laser attenuation? Pinhole setting? Frame scan speed? Photobleaching?
Is Frame averaging a good idea with live samples?

Time Series



Time Interval: User defined length of time in seconds

Number of Images: aka number of time points

Destination: ALWAYS SAVE TO FILE!