BI 227 Assignment #5 **Fall 2007**

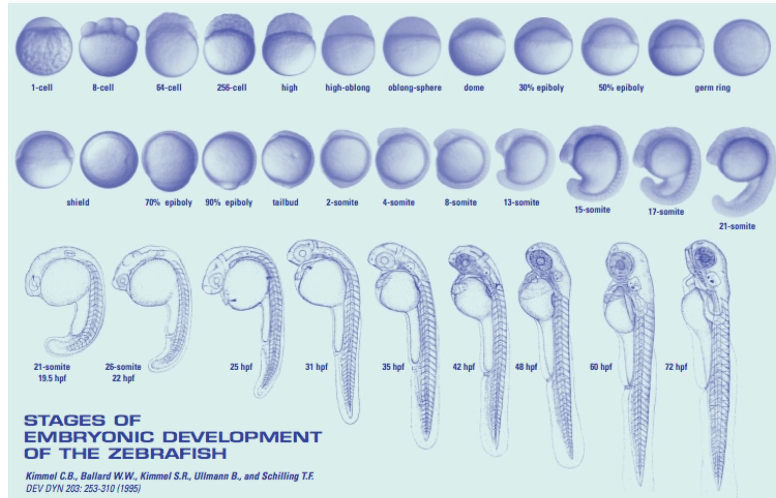
Goal for this week:

Understand the challenges of sample mounting
in vivo time-lapse imaging of zebrafish embryos

Note: You should have signed-up for scope time and set a time to meet with the TA to get the embryos prior to your imaging session.

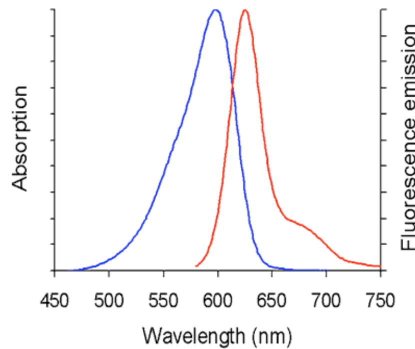
Zebrafish

We will image 10 to 96 hours post fertilization (hpf) zebrafish embryos that are either transgenic or have been soaked in a bright green or red fluorescent dye.

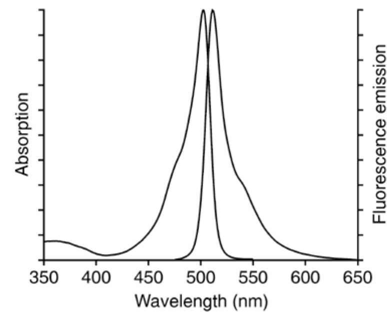


Eggs will have already been collected for you from mating pairs after they are laid in the morning. They will have been cleaned and unfertilized eggs discarded. Embryos are incubated at 28°C in artificial pond water. In order to prevent formation of pigments, the embryos are transferred to a solution of 0.003% 1-phenyl-2-thiourea (PTU) in artificial pond water after around 10-20 hpf.

Embryos are soaked overnight in 0.001% Bodipy FL C_5 Ceramide (Green, targets membranes) or 30 minutes in 2% CellTrace BODIPY TR methyl ester (Red, targets cytoplasm). The fish are then washed several times in artificial pond water to remove excess stain and are transferred to a new dish. 15 minutes before mounting, embryos are anesthetized in a 0.016% tricaine in artificial pond water solution.



CellTrace BODIPY TR



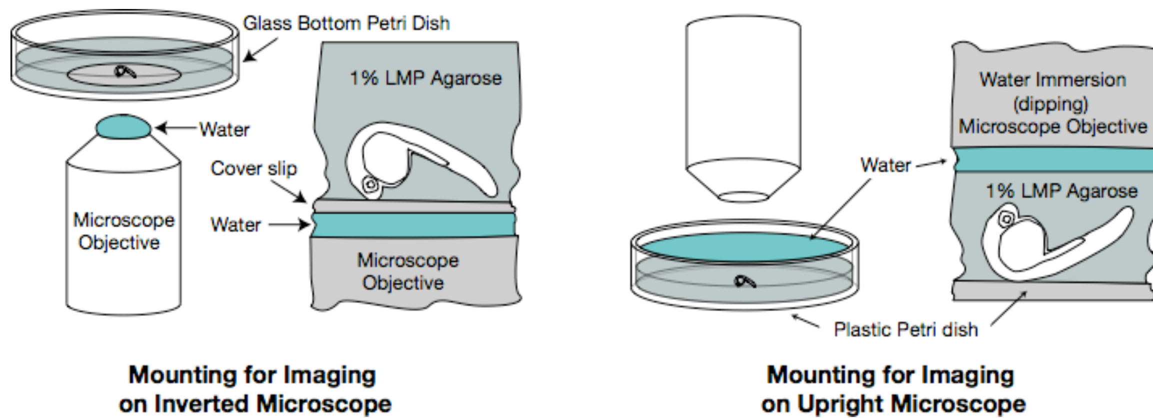
Bodipy FL C_5 Ceramide

Mounting for Imaging on Inverted Microscope (Zeiss LSM 410)

Mount the fish in a custom glass bottom dish. Pour a few milliliters of liquid agarose at 37°C onto the dish and add one or several embryos with a transfer pipette (with as little water as possible). Orient the embryos with pipette tips or needles under the dissecting scope. The region of interest should be facing *down* (inverted microscope). Because of the limited microscope objective working distance, it is essential to mount the embryos as closely as possible to the bottom of the dish. Let the agarose polymerize for 15-30 minutes.

On the LSM410, image with *air* or *water* objectives *only* (and never use oil objectives, their working distance is too shallow). Consult with the TA as to which microscope objective you should use depending on the age of your sample. Be very careful to not touch (and break) the bottom coverslip with the objective. Should that nevertheless happen and you spill water on the objective, remove the Petri dish, soak excessive water with lens paper and seek advice from the TAs *immediately*.

Mounting for Imaging on an Upright Microscope (Zeiss LSM 310)
On the LSM 310, image with the water immersion objectives *only.* Mount the fish in a standard (no glass-bottom) Petri dish. Pour a few milliliters of liquid agarose at 37°C onto the dish and add one or several embryos with a transfer pipette (with as little water as possible). Orient the embryos with pipette tips or needles under the dissecting scope. The region of interest should be facing *up* (upright microscope). Let the agarose polymerize for 15-30 minutes. Once the agarose has set, add some artificial pond water (with tricaine) to the Petri dish.



Mounting for Imaging on Inverted Microscope

Mounting for Imaging on Upright Microscope

Assignments

1) 3D *in vivo* Imaging
Acquire z-stacks in several different regions of the fish. How deep is it possible to image before the signal dyes off? Try out different frame scan times and count how long it takes to acquire one volume. Collect several z-stacks testing out the different parameters (pinhole size, attenuation).

2) Experimenting with Photobleaching
a) Adjust the setting as to obtain a nice 2D image, save it and write down your settings. Reduce the laser attenuation (i.e. more power) and scan the image for an extended period of time at a low scan speed (either launch a continuous scan for approximately 1 minute or launch an Average scan >16)
b) Move the stage approximately half of the field of view and take another image with the initial settings. c) If you have succeeded, you should be able to see the bleached section with decreased signal on one half of the new image and the other should look unbleached. If you haven't bleached enough to see it, try again with even less laser attenuation and/or longer scanning time.

3) Time-Lapse Imaging of a Thick Sample
Choose a region for time-lapse imaging and set up a 3D + time time-lapse experiment.
For your time-lapse try to image every few minutes (take less z slices if necessary) for at least one hour (longer is better if you have the time and the sample survives).
NOTE: Always choose intervals that are longer than the time it takes to acquire one z-stack.

What are the considerations for time-series collection in live samples (laser attenuation, pinhole setting, frame scan speed, photobleaching, frame averaging)?

Don't forget to add scalebars, timestamps, the name of microscope, the objective (brand, type, magnification, NA, immersion medium), the sample age and dye, the excitation wavelength and emission filters wavelength range, and pinhole size to your figure.