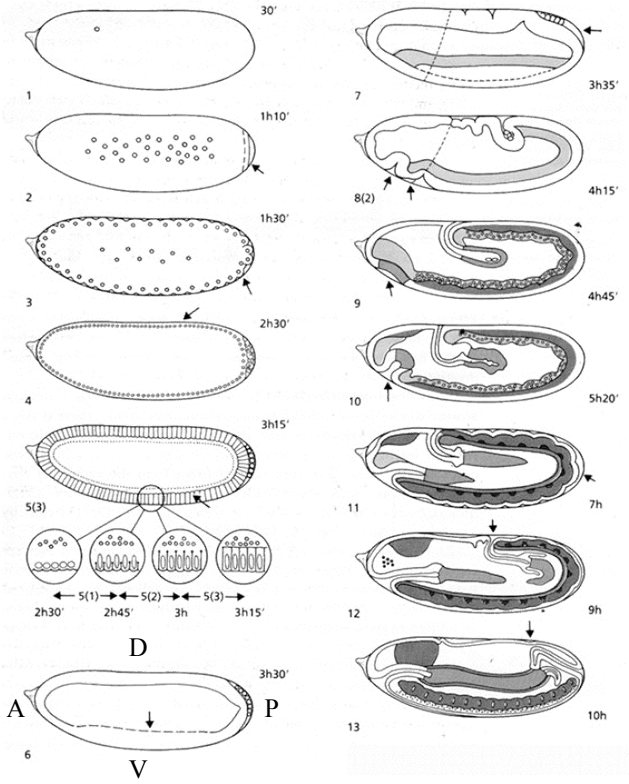
Bi227 Assignment #6 **Fall 2007**

Goals for this week:

1) Understand the challenges of sample collection and mounting
2) *in-vivo* time-lapse imaging of *Drosophila* embryos with either:
   - H2A - Cherry (Cherry fluorescent protein fused to histones found in all nuclei)
   - Dorsal - GFP (Green fluorescent protein fused to the transcription factor Dorsal found in a nuclear gradient with highest amounts in ventral regions)

***Drosophila melanogaster* imaging**



We will be imaging 0-5 hour embryos. This includes the first 10 stages of embryogenesis.

You will collect embryos by changing the apple-juice plate on the collection bottle where the parents are mating. Age the plate for the appropriate number of hours to see the stage you want to image. Note: these stages are based on development at 25°C, so your embryo's development may vary depending on the temperature of the room, stage, or heating due to imaging.

Prepare a slide by placing a drop of heptane glue in the middle of the slide. Put double-stick tape (two pieces thick) on either side of the heptane glue area. This will provide the supports of a cover slip "bridge" for the embryos to lie under.

Mounting is the same for both the Zeiss LSM 310 and Zeiss LSM 410

The TA will show you how to dechorinate the embryos using double stick tape and a sharp probe. After the chorion (protective outer casing of the embryo) has been removed, carefully transfer the embryo(s) to the heptane glue-coated slide using a paintbrush or the probe. Be sure to orient the embryos with the area of interest facing up, as you will not be able to image through the slide and the glue. Drop a large drop of halocarbon oil on top of your embryos. Gently place a coverslip over your embryos. You may use any objective you choose between 20x-63x, but please be exceptionally careful working with the oil objective as the working distance is very small. Due to its short size and a limitation in the software, the 100x objective is not suitable for automatic z-stack acquisitions.

Imaging Suggestions:

1) Find an embryo with either "green" or "cherry" fluorescent proteins
These are new constructs and have not been homozygosed yet, meaning that the transgene is only on one of the chromosomes. Not all of the animals you will be imaging will have the fluorescent protein that you want to see. These proteins are also maternal which means that only one half of the progeny will have either the green or cherry fluorophores. Make sure you dechorinate enough embryos (more than 4) to try to bias the statistics in your favor.

2) 3D *in vivo* imaging
Acquire z-stacks of the entire embryo. How deep is it possible to image before the signal dies out? Try different frame scan times and determine how long it takes to acquire one volume. Collect several z-stacks testing out the different parameters (pinhole size, attenuation).

3) Time-lapse imaging of 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 time and the sample survives).
NOTE: Always choose intervals that are longer than the time it takes to acquire one z-stack.

4) Feel free to experiment
Pick any of the exercises we have discussed throughout the term. Have fun!

Stage-specific imaging:
- Stages 1- 2 (age for 0 - 30 minutes) Image the mitotic divisions using H2A-cherry. Divisions occur approximately every 15 minutes at 25°C.
- Stages 2 – 5 (age for 1 hour) Capture the dynamics of the Dorsal protein by imaging Dorsal - GFP. This protein shuttles in and out of the nucleus with each mitotic division. You will have to image more frequently than once a division to capture the dynamics.
- Stages 1.5-5.5 (age for 30 minutes - 1 hour) Image the formation of the Dorsal gradient.
- Stages 3.5 (age for 3 hours) Image mesoderm migration. Image the ventral side of the embryo to see the invaginated cells move up the sides of the embryos

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

Remember to include in your figures: scalebars, timestamps, the name of microscope, objective used (brand, type, magnification, NA, immersion medium), sample age, flurophores used, excitation wavelength and emission filters wavelength range, and pinhole size.