Bi/BE 227 Winter 2016

Assignment #4

Live imaging: tools for neuro science

Schedule:

Feb 3: Assignment

Feb 5: Work on assignment

Feb 17: Assignment due

A. Imaging Live brain.

We will image the central nerve system (brain + VNC) of the larval fly brain. This fly expresses GCaMP6S (calcium indicator) under a pan-neuronal driver.

1. We will image dissected brains to see the development of the neuro-blast. What's the reason we can't use live animals?

2. GCaMP is a GFP based calcium indicator. Set the system to image GFP. Find the right condition (laser intensity, dwell time, frame rate, etc) to observe activity of the cells. Can you see spontaneous cellular activity? Can you detect action potentials with GCaMP imaging?

See reference: http://www.ncbi.nlm.nih.gov/pubmed/23868258

3. Set a time-lapse image so that you can observe cell division. What are the things you need to consider when you decide the frame rate for this experiment?

4. How fast do the cells divide?

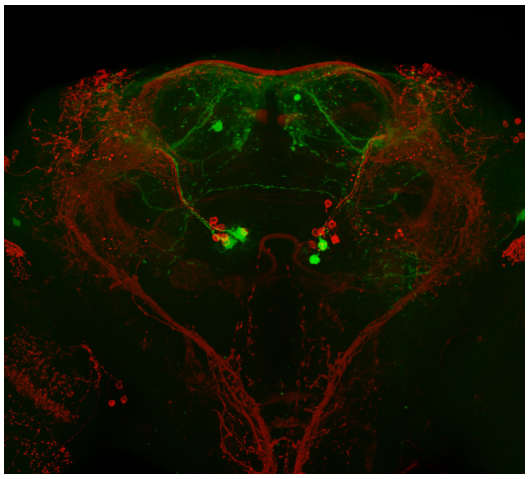
B. Immunohistochemistry

Immunohistochemistry (**IHC**) refers to the process of detecting antigens (e.g. proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. For example one might use “goat driven anti-tubulin” primary antibody with “anti-goat-alexa 488” secondary antibody to visualize non-fluorescent tubulin molecules.

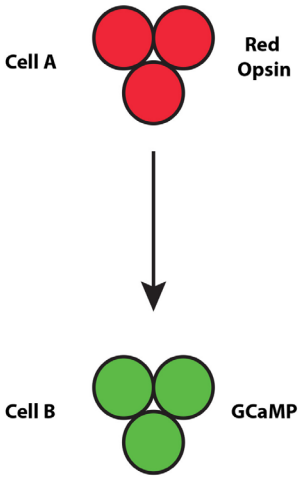
1. IHC was initially aimed to visualize non-fluorescent molecules, but many people use anti-GFP or anti-RFP as primary antibodies to increase signal to noise ratio. What are the other advantages of using IHC over native fluorescent imaging?

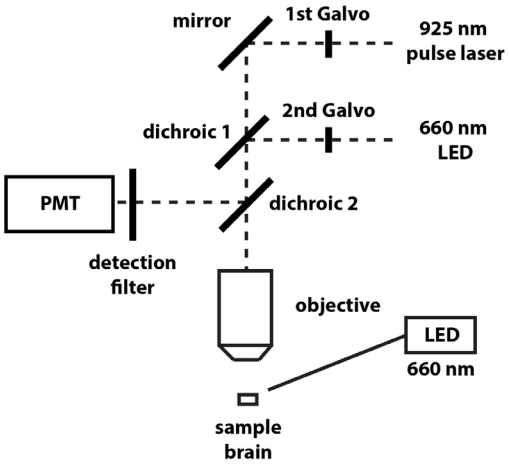
2. What are other ways to increase signal to noise ratio besides IHC?

3. Below is an image of a fly brain that expresses GFP in one population of cells and RF in another populations of cells. Please choose the primary and secondary antibody combinations used to take this two-color image.



C. All optical neuro-physiology





You want to know whether cell population A is functionally connected to cell population B. In order to do answer this question, you expressed channel-rhodopsin that will activate neuronal activity with 660nm illumination in cell A, and GCaMP in cell B.

1. During your preliminary experiment, you want to activate Cell A using 660 nm LED coupled with optical fiber that is located closed to your sample while imaging Cell B with 925 nm pulse laser. Choose the right filter for dichroic 1, and dichroic 2.

2. For the detection filter, you have 525nm/50nm (center, width) BP and 520nm/20nm BP. Both options have pros and cons in this experiment. What are the pro and con for each filter, and which one do you want to choose?

3. From your preliminary experiment, you discovered Cell B is functionally downstream of Cell A. Now you want to know whether each cell in cluster A is connected to different cells in cluster B. To study this, you want to image Cell B while activating individual cells in cluster A by targeting 660nm activation laser to cells in cluster A. Choose the right filters for dichroic 1 and 2.