Life Sciences Outreach Fall Faculty Speaker Series
Teacher Professional Development Activity
Microscopy Activity

THE DEREK BOK CENTER FOR TEACHING AND LEARNING
HARVARD

Table of Contents

Activity Authors

Doug Richardson, PhD
Director of Imaging at the Harvard Center for Biological Imaging
Lecturer on Molecular and Cellular Biology

Emily Rivard
PhD Candidate in Molecular and Cellular Biology

Tarun Kumar, PhD
Research Specialist in Organismic and Evolutionary Biology

Overview

This activity was designed for an audience of classroom high school biology teachers as part of a professional development program to further their knowledge in a research field. It has not been formally formatted or tested for a high school student audience because we believe that teachers are the best interpreters of content for their students. Therefore, we welcome teachers to adapt this activity for their own classroom needs.

Objective

In this activity, participants will be introduced to the design of several fluorescence microscopes and observe how they are used to image living organisms. By the end of the session, participants will be able to:

1. Describe the advantages of fluorescence microscopy over traditional transmitted white light microscopy
2. Understand the basic lens arrangement of a light microscope
3. Understand the principle of optical sectioning and why it is needed to image thick, fluorescent samples

Activity

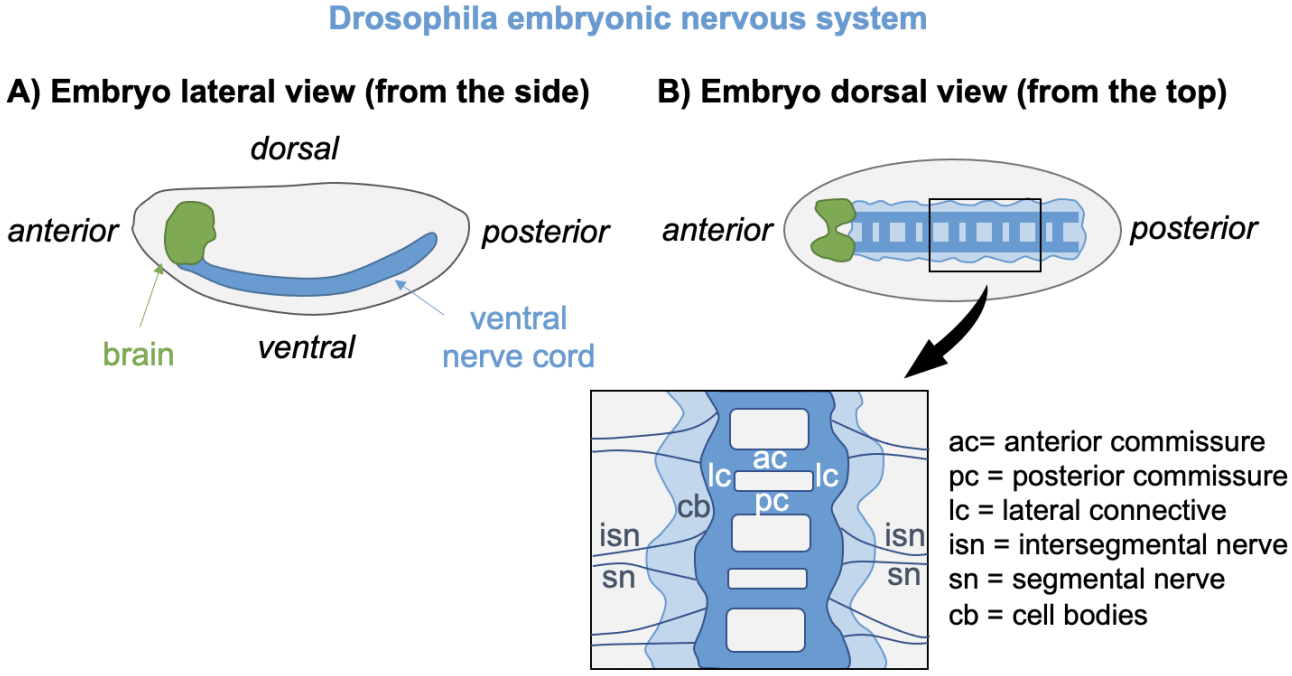
About the tissues

Today we are going to be using different microscopes to visualize tissue samples from the embryos of the model organism, the fruit fly (*Drosophila melanogaster*).

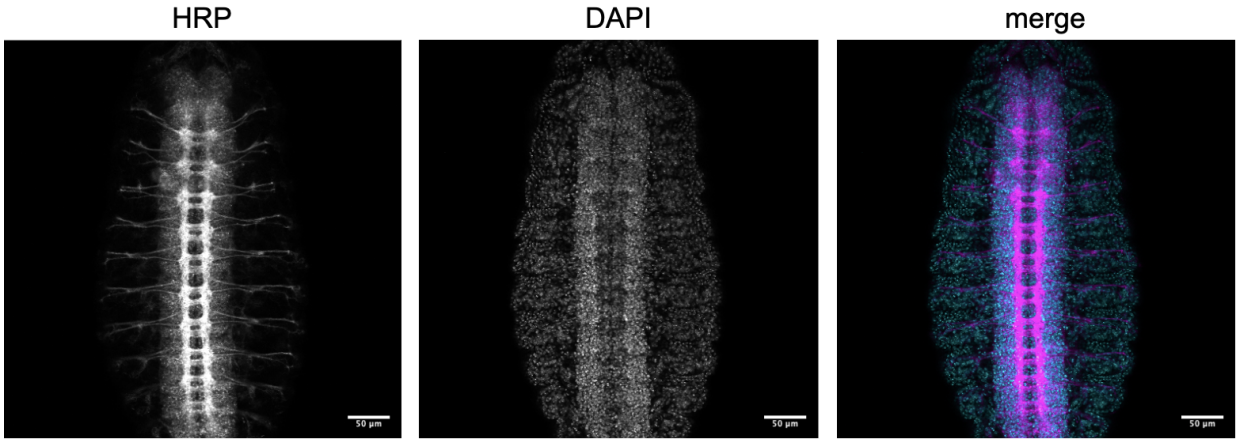
Drosophila melanogaster (Wikipedia)

Embryonic nervous system

One tissue we are going to study is the *Drosophila* embryonic nervous system. The central nervous system (CNS) of *Drosophila* has two parts, a **brain** and a **ventral nerve cord** (the functional equivalent of a spinal cord in vertebrates). In the ventral nerve cord, the axons of the neurons assemble in a ladder-like pattern. Axons arrange into two **longitudinal connectives** that run along the anterior-posterior axis of the embryo. Neurons also extend axons across the middle of the embryo along two paths called the **anterior** and **posterior commissures**. Motorneurons extend axons out of the CNS and sensory neurons extend axons into the CNS via **intersegmental** and **segmental nerves**.



We will be observing embryos that are fixed and stained with antibodies to observe the embryonic nervous system. We are using an antibody to horseradish peroxidase (anti-HRP), which binds to neuronal membranes in flies and therefore serves as an effective marker of different kinds of neurons. We also are using DAPI, a stain that binds to the nuclei of all of the cells in the embryo.

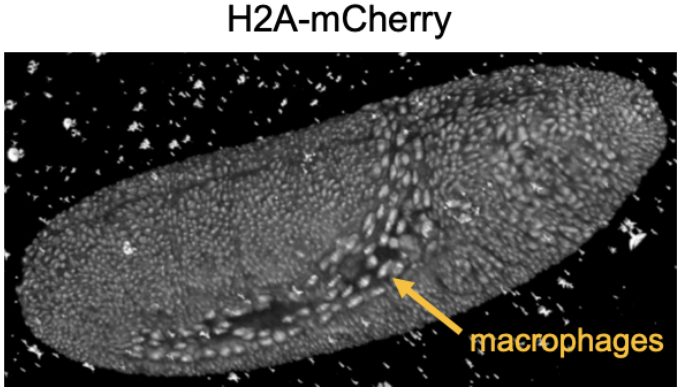


Drosophila embryos labeled with the neural marker, HRP (magenta), and nuclear marker, DAPI (cyan).

Embryonic immune system

We also will be looking at a group of immune cells in the *Drosophila* embryo called plasmatocytes, which are functionally equivalent to **macrophages** in vertebrates. Macrophages are the most abundant blood cell type in *Drosophila.* These cells play roles in numerous biological processes. They are important for *Drosophila* immunity as they engulf bacterial and viral invaders. They also are necessary for proper development since they degrade superfluous, apoptotic cells during embryogenesis. *Drosophila* macrophages rapidly move around the embryo along specific routes during development. These cells serve as a popular model for investigating the mechanisms of cell migration.

We will be able to see macrophages moving in live embryos. We have generated embryos in which the nucleus of every cell is marked because these flies are expressing a red fluorescent-tagged copy of a histone protein (H2A-mCherry). The cells with large nuclei you will see moving around the middle of the embryo are the macrophages.

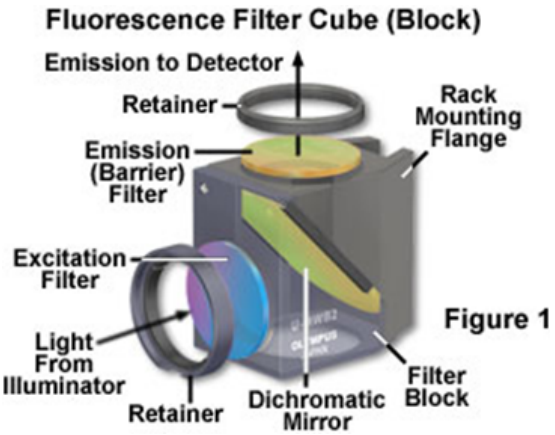


About the microscopes

Today we are going to be using different microscopes to visualize our fruit fly tissue samples. We will begin with a microscope called the “AxioZoom.” This is a multifunctional microscope similar in design to a stereo microscope that you may have in your classroom.

Carl Zeiss AxioZoom V16

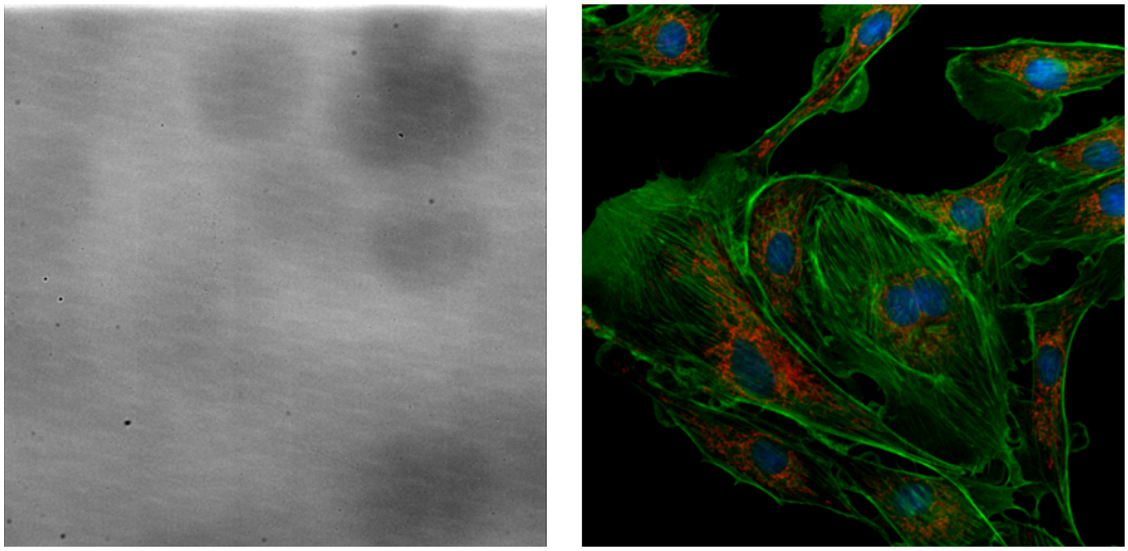
We can use transmitted light imaging to view the overall anatomy of the fruit fly; however, this does not give us any information about how the individual molecular components (proteins, DNA, RNA, etc.) are arranged relative to one another. To achieve that level of detail we will use fluorescence microscopy. To build a fluorescence microscope, we must install a **filter cube** behind the objective. A filter cube has three components. The **excitation filter** selects the correct wavelengths of light needed to excite a specific fluorescent dye (for example blue light around 480 nm) and reflects all other wavelengths of light. The excitation light enters the filter cube and is reflected toward the objective by the **dichroic mirror**. The dichroic mirror has a special metal coating on it that reflects lower wavelengths of light (such as the 480 nm excitation light) but allows longer wavelengths to pass through it. Therefore, the fluorescence light (for example, green 525 nm light) will be collected by the objective, enter the filter cube and pass through the dichroic mirror. Beyond the dichroic mirror lies the **emission filter** which performs a final clean up of the light before it goes to the detector to ensure the 525 nm fluorescence light does not contain excitation or autofluorescent light.



A filter cube. Credit: Molecular Expressions (https://micro.magnet.fsu.edu)

Fluorescence microscopy is essential to modern biology as it provides:

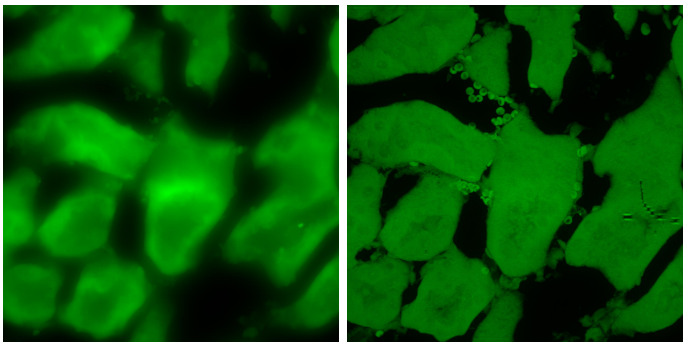
1) High contrast
2) Molecular specificity (each molecule of interest can be labelled with a different color)



Left: Transmitted light image of the cells lining a cow's blood vessel (bovine endothelial cells). They are too thin to be seen. Right: The same field of view imaged by fluorescence. The blue dye stains DNA in the nucleus, the green dye stains actin and the red dye stains mitochondria.

Confocal microscopy

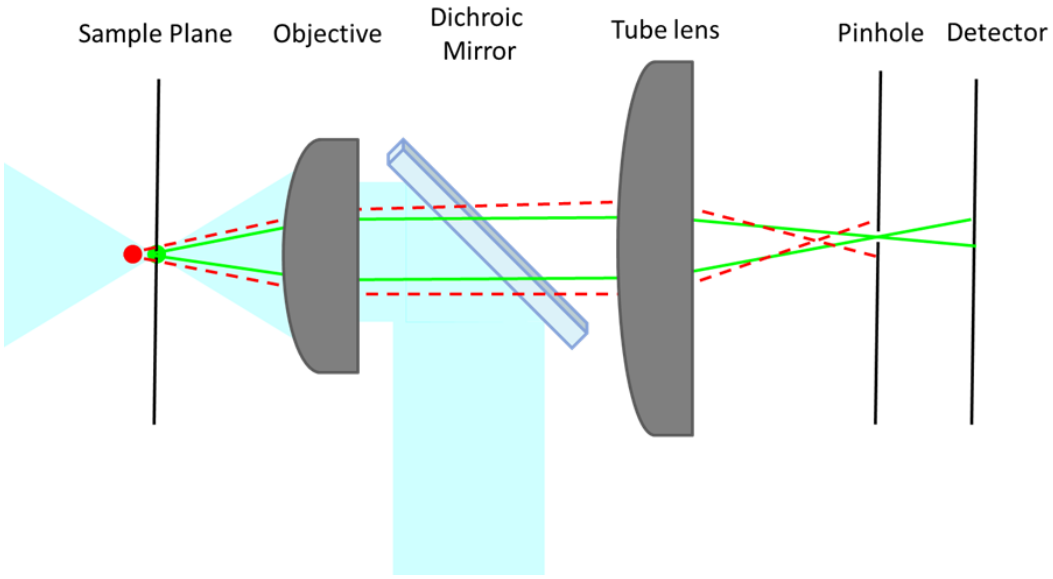
A major limit to transmitted light and fluorescence microscopy is that as the sample becomes thicker, the image becomes blurrier.



Left: blurry "widefield" image. Right: sharp "confocal" image.

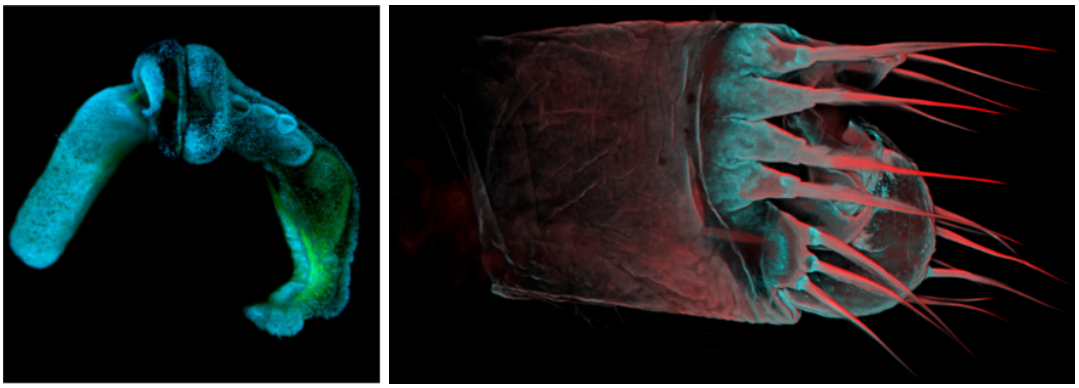
In fluorescence microscopy, the blurriness is the result of light from out of focus planes reaching the detector along with the infocus light. The out of focus light eventually overwhelms the finer details of the image. Therefore, we use a technique called "optical sectioning" for imaging thick (> 50 um) fluorescent tissues. Similar to an MRI, we can image thin (< 1 um) sections of a sample without actually slicing it up with a knife. One of the most commonly used tools for this is a confocal microscope. The confocal microscope uses two optical "tricks" to create an optical section. First, instead of bathing the entire field of view in excitation light, the microscope objective is used to focus the light to a very small (~250 nm) point. This point is scanned across

the sample and the image is built up one pixel at a time. Secondly, the collected fluorescent light is forced to pass through a pinhole. The pinhole is specifically placed where any light originating from the focal plane will be tightly focused and pass through. Light coming from above or below the focal plane is not focused and is excluded by the pinhole. This generates a thin optical section.



Lightpath of a confocal microscope: Excitation light (cyan) is focused to a point in the sample plane. It excites both in focus (green dot) and out of focus (red dot) fluorophores. The out of focus light (red dashed lines) is excluded by the pinhole whereas the in focus light (green dashed lines) passes through to the detector.

By moving the objective of the microscope vertically and collecting many 2D optical sections, a 3D image of the sample can be composed.



Left: Acorn worm from Cape Cod. Nuclei stained in blue, actin stained in green. Right: Spider appendage (autofluorescence)