

CSSI NEUROSCIENCE SUMMER 2011

SEEING NEURONS I: DYES

TECHNIQUES

- Worm Farming
- Dissecting Microscopy
- Behavioral observations

GOALS

- Label chemosensory neurons with the neuronal tracer DiI
- Mount worms for compound microscopy
- Observe *C. elegans* using fluorescence microscopy

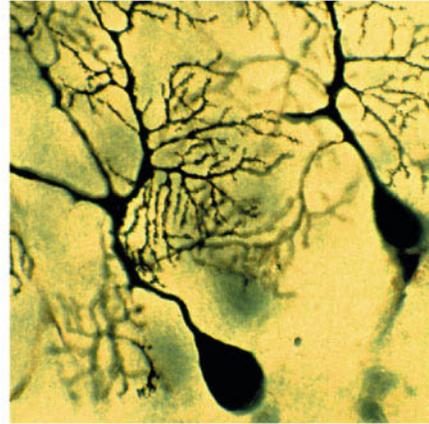
INTRODUCTION

It's hard to study what you cannot see

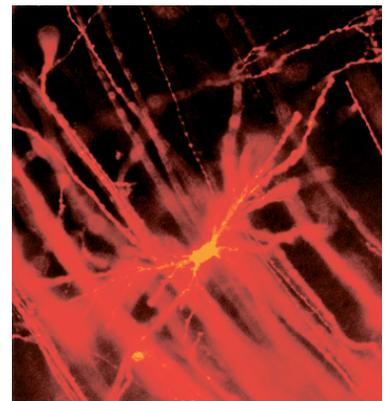
Neuroscientists who want to study the structure of the nervous system are faced with a special problem: neurons are packed and tangled tightly together, especially in the brain and spinal cord, making it nearly impossible to see the delicate structure of each individual neuron. One of the first people to solve this problem was Camillo Golgi, who in 1873 developed a method that used silver nitrate to stain individual neurons so that they could be viewed under the microscope. Golgi's method (cleverly called Golgi staining) revolutionized neurobiology. Spanish neuroscientist Santiago Ramon y Cajal was one of the first scientists to use Golgi staining to study the structure of neurons in detail. Ramon y Cajal ultimately proposed that the nervous system is composed of discrete nerve cells that communicate with each other through specialized contacts. We now know these contacts as synapses. Both Golgi and Cajal were awarded the Nobel Prize in Physiology or Medicine for their pioneering contributions to neuroscience.

Fluorescent labels

Modern neuroscientists have a wide variety of neuronal labeling techniques in their toolkit. Many of these techniques involve fluorescent dyes and proteins that allow them to observe neurons in living organisms. Today, we will focus on one of these techniques, the use of the fluorescent dye DiI (pronounced "dye-eye") to label individual neurons or groups of neurons. DiI is readily taken up by cells, particularly those that have openings to the external environment, such as sensory neurons. DiI emits red-orange fluorescence when excited by a specific wavelength of light. Thus, neurons that have taken up DiI will glow under a microscope used to view fluorescent samples.



Purkinje neurons in the cerebellum stained using the Golgi Method (Source: Purves Neuroscience)

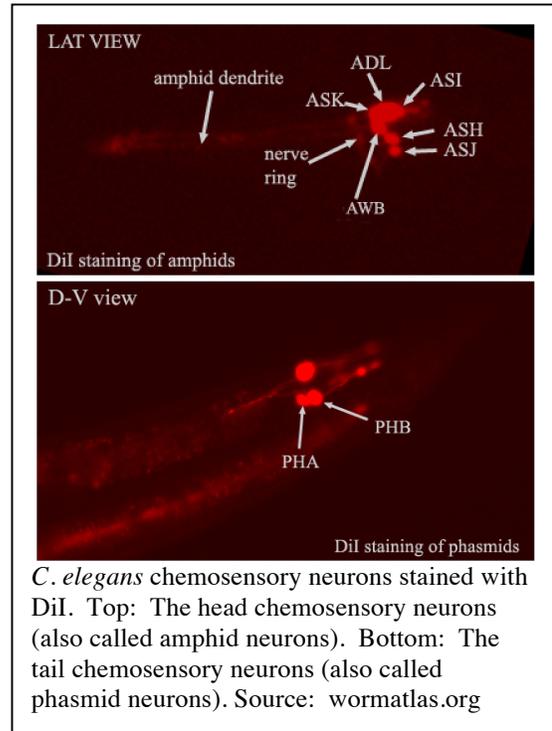


Neurons in the rat thalamus labeled with DiI.
<http://www.jneurosci.org/content/vol22/issue19/cover.shtml>

Chemosensory Neurons in *C. elegans*

The *C. elegans* nervous system is specialized to allow worms to find food, avoid danger, and to find other worms, including appropriate mating partners. To do this, *C. elegans* samples its environment using chemosensory neurons located in its head and its tail. (Yes, a worm can “smell” with its backside.) These neurons are exposed to the environment through openings in the worm’s outer covering, the cuticle. This allows these neurons to directly sense and respond to chemicals hence the name “chemosensory” neurons.

One of the behaviors controlled by chemosensory neurons is **chemotaxis**, or movement in a direction controlled by a gradient of a diffusible chemical. Worms chemotax toward substances that attract them (sometimes called chemoattraction—think “yum!”) and away from chemicals that repel them (sometimes called chemorepulsion—think “yuck”). Your guided research project will focus on chemotaxis behavior, and we will discuss it in more detail tomorrow.



Labeling *C. elegans* chemosensory neurons with DiI

C. elegans chemosensory neurons are exposed directly or indirectly to the external environment, allowing them to sense chemical cues. This exposure to the external environment also allows some of these chemosensory neurons to take up neuronal tracers like DiI. In hermaphrodites exposed to DiI by soaking, 8 pairs of neurons have been shown to stain (or “fill”) with DiI. These are the head sensory neurons ASK, ADL, AWB, ASH, ASJ, and ASI, and the tail sensory neurons PHA and PHB (See figure above).

Compound and Epifluorescence Microscopy

The compound microscopes at your benches and in the microscope room will allow you to see your *C. elegans* samples magnified between 40 and 400 times. You will be able to see far more detail in your worms using the compound scopes than you could using your dissecting scope. In order to see and photograph worms on the compound scope, they will be anesthetized so that they will not move or crawl away. As you view your worms on your compound microscope, look for the structures shown in the diagram in yesterday’s handout, including the pharynx (grinding organ), intestine, gonad, and fertilized in embryos in the uterus.

Epifluorescence microscopy allows the visualization of fluorescent molecules by using filters to direct light of specific wavelength onto the sample. The fluorescent molecules in the sample (DiI, for example) are excited by this light, and in turn emit light of a different wavelength. It is

this emitted light that you will see when you look through the compound fluorescence microscope.

TODAY'S EXPERIMENT

You will work with two different worm strains today:

1. Wild-type *C. elegans*. This is the same strain that you observed in class yesterday.
2. *che-2* mutant *C. elegans*. This strain has a mutation in a gene called *che-2* (for chemosensation-2)

Your goal is to use compound and epifluorescence microscopy to compare DiI staining in wild-type and *che-2* mutant worms. At the end of the lab, we will discuss the differences (if any) that you observe between these strains.

Behavioral Observations

Use your dissecting scope to compare the behavior of wild-type and *che-2* mutant worms. Do you notice any differences between these two strains?

DiI labeling of *C. elegans*

These steps were done before lab:

1. Prepare a stock solution of DiI (Invitrogen Molecular Probes). Stock solution is 2 mg/ml in dimethylformamide, stored at -20° in a foil wrapped tube.
2. Dilute the stock 1:500 in phosphate buffered saline (PBS) (8 g NaCl, 0.24 g KH₂PO₄, 1.44 g Na₂HPO₄, 0.2g KCl in 1L). Some dye will precipitate when you do this; don't worry about it.
3. Wash worms from plates into a 15 ml centrifuge tube using PBS buffer. Pellet worms by spinning gently (2 minutes at about 1.5K RPM) in a clinical centrifuge. Remove supernatant carefully, using a pipette. Be careful not to disturb the worm pellet; leave about 1 ml of buffer above the worms.
4. Using a glass Pasteur pipette, transfer worms to a 1.5 ml microfuge tube. Fill tube with PBS, and centrifuge worms at 3000 RPM for 2 minutes. Carefully remove most of the supernatant, being careful not to disturb the worms.
5. Resuspend worms in about 250 μ l DiI staining solution. Incubate for 2 hours at room temperature with gentle agitation.

You will do these steps during lab:

****We will demonstrate the techniques for you before you do them****

6. Wash worms 3X in PBS to destain. (For each wash, pellet worms in the microfuge as described above, remove supernatant, and fill tube with PBS.)
7. Prepare a slide by placing 3 ul of pelleted worms + 3 ul of TRI/TET anesthetic on an agarose pad and covering carefully with a coverslip.
8. Observe your worms using the compound microscope at your bench. What structures can you see? Draw a diagram of your worm and as many internal structures as possible in the space below (use yesterday's handout as a guide to the names of some of the structures).

9. When it is your turn, observe and photograph your worms on one of the compound fluorescence microscopes. Observe your worms under white and fluorescent light, and take digital pictures of your samples (we will show you how).
10. When it is your turn, observe your worms on the fluorescence dissecting microscope at the back of the lab. What are the advantages of this type of microscope over the compound fluorescence microscope? What are the disadvantages?

Summary Questions

1. How many head neurons are stained with DiI in your wild-type worms?

In your *che-2* mutant worms?

How many tail neurons are stained with DiI in each strain?

2. Consider your behavioral and microscopic comparisons of wild-type and *che-2* mutant worms. What are some possible functions for of the *che-2* gene in *C. elegans*?

CSSI Neuro 2011

Seeing neurons II: Green fluorescent protein reporter transgenes

TECHNIQUES

- Green fluorescent protein (GFP) reporters
- DIC and Fluorescence microscopy
- Wormbase.org

GOALS

- Observe and identify neurons in *C. elegans* with green fluorescent protein reporter transgenes
- Use wormbase.org to research *C. elegans* gene information

GFP reporters

Reporter transgenes are valuable tools for labeling cells in developing organisms. The gene encoding the green fluorescent protein (GFP) from jellyfish is a commonly used reporter gene. Cells that express GFP glow green when excited by a specific wavelength of light. The development of cells expressing GFP can be observed in living specimens in real time. (Pretty amazing stuff—amazing enough for a Nobel Prize for Martin Chalfie and Roger Tsien.)

The transgenic *C. elegans* strains that you will observe today have been genetically engineered so that the expression of GFP is controlled by cell-specific regulatory elements (enhancers and promoters). For example, one strain might contain a transgene in which the gene that encodes GFP has been placed under the control of the regulatory elements for the *unc-119* (*uncoordinated-119*) gene, which is expressed in the nervous system. This transgene was introduced to *C. elegans* by injection into the gonad and passed through the germline to progeny to create a transgenic strain in which the GFP gene is transcribed and translated into protein in neurons (Figure 1). (See attached handout for more information on GFP.)

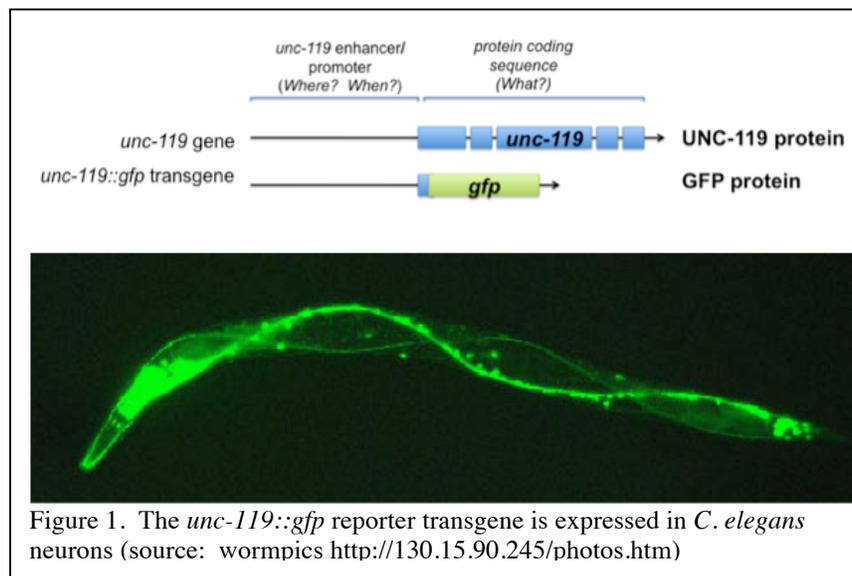


Figure 1. The *unc-119::gfp* reporter transgene is expressed in *C. elegans* neurons (source: wormpics <http://130.15.90.245/photos.htm>)

TODAY'S EXPERIMENT

You will be given a transgenic *C. elegans* strain containing a green fluorescent protein reporter. Your goal is to use DIC (differential interference contrast) and epifluorescence microscopy to characterize this reporter, and to draw some conclusions about where it is expressed.

ACTIVITIES

Observation of GFP lines

A. DIC and Epifluorescence Microscopy

Now that you're confident (or somewhat confident) in your worm bonking abilities, you should be able to mount your strain for DIC and epifluorescence microscopy. You should share your results with other groups so that everyone will have a chance to look at (or at least look at pictures of) all strains.

1. Mounting *C. elegans* for microscopy:
 - Prepare a 2% agarose pad on a microscope slide as demonstrated. Place 10-15 ul of 100 mM TRI/TET to anesthetize your worms.
 - Pick 5-6 adult worms into the drop of anesthetic on the slide. Try to be quick, so that the worms don't dry out. If worms do dry out while you're picking, add more Sodium Azide. Gently lay a coverslip over the worms.
2. Observe worms using DIC and epifluorescence microscopy. How many cells express GFP? Where are these cells found? If time permits, try to take a picture of GFP expression in your strain.

B. Observation GFP transgenic worms on the fluorescence dissecting scope

Observe your GFP transgenic strain(s) as they crawl around on their plates using the fluorescent dissecting microscopes in Jennifer's lab.

What advantages does a fluorescent dissecting scope have over a compound microscope? What disadvantages?

A NOTE ABOUT GENE NOMENCLATURE (NAMING) IN *C. ELEGANS*

In *C. elegans*, genes are given three-letter names that are determined in one of two ways:

- 1) If the gene was identified in a genetic screen, the name is based on the phenotype caused by mutation of the gene. For example, *unc-22* (*uncoordinated-22*) was the twenty-second gene identified in screens for worms with uncoordinated movement. *unc-22* encodes a muscle regulatory protein; *unc-22* mutant worms twitch uncontrollably.
- 2) If the gene was first identified on the basis of its homology (similarity) to a gene with a known function in another organism, the three-letter name is based on this homology. For example, *C. elegans tph-1* (*tryptophan hydroxylase-1*) encodes the enzyme tryptophan hydroxylase, which is essential for the production of the neurotransmitter serotonin.

In writing, *C. elegans* scientists use capitalization and italics to indicate whether they are describing a gene, the protein encoded by the gene, or the mutant phenotype as follows:

- 1) *unc-22* is a gene, DNA, or RNA.
- 2) UNC-22 is a protein.
- 3) Unc-22 (or simply Unc) is the phenotype caused when *unc-22* is mutated