Histology

By Vivian Hernandez, Sarah Lorenzen, Nick Bush, and Shoai Hattori
Updated 1/10/25 by Claire Cho

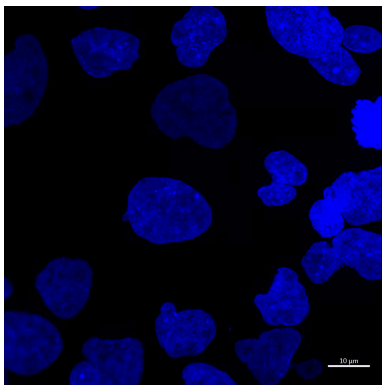
Objectives

1. Understand how histology is used in neuroscience research
2. Understand the different ways to image neurons and how these methods can be used to visualize parts of a neuron, distinguish neurons from other cells in the brain, and see how neurons change in disease

Background Information

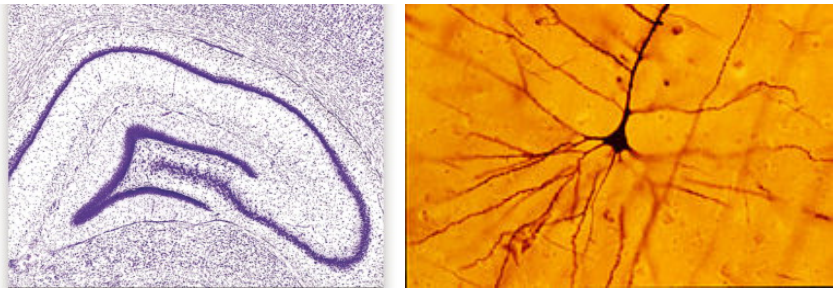
Neuroscientists study how the brain works, and to do this they need to understand the structure and function of the cells (i.e. neurons) that make up the brain. One way to understand how these neurons work is to observe them. However, it's hard to see individual neurons and their structure by simply looking at brain tissue. This is why we use histological techniques to prepare and stain brains.

One way we stain neurons today is with fluorescent proteins or dyes. These proteins glow, or fluoresce, when you shine a different color light on them. The most famous fluorescent protein is called green fluorescent protein and was originally discovered in jellyfish. Today, we have engineered many fluorescent proteins that come in many colors like red, green, blue, and cyan, and they can be used to image many different aspects of the neuron. For example, DAPI is a blue fluorescent stain that binds to DNA, effectively allowing scientists to image neuron nuclei.



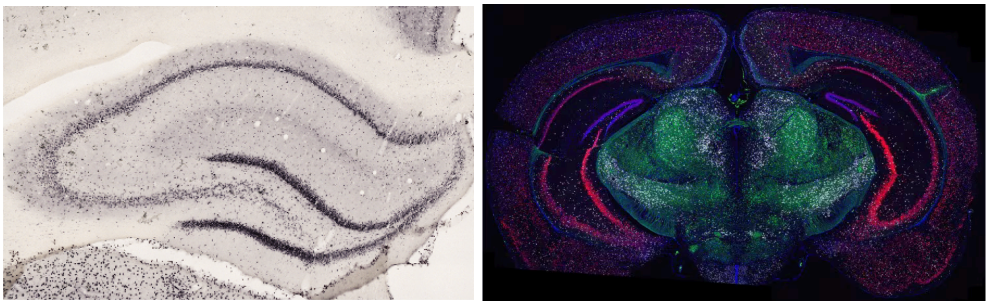
DAPI stain showing DNA in cell culture.

Not all staining has to use fluorescence. Another type of stain is the Nissl stain which uses purple dyes like cresyl violet or thionin. These dyes bind to Nissl bodies, which are aggregates of the rough endoplasmic reticulum located in the neuronal cell body. It is useful for studying neuronal structure and cell density as well as identifying brain regions based on neuronal arrangement. Another non-fluorescent stain is the Golgi stain. This uses silver to turn the neurons black. Though it only stains a very few number of neurons, it stains the entire in great detail, allowing scientists to observe the finer details.



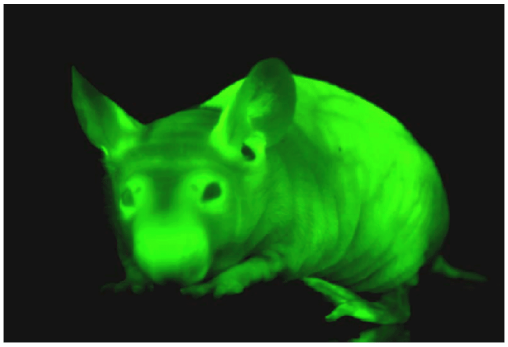
Nissl stain (left) showing Nissl bodies in the rat hippocampus. Golgi stain (right) showing cortical pyramidal neuron.

Some staining methods, like immunostaining and in situ hybridization, allow us to look at protein expression levels throughout the tissue. These techniques can help researchers understand how proteins are distributed and expressed in cells. Immunostaining uses antibodies tagged with fluorescent or non-fluorescent dyes to bind to a specific protein of interest. Your body makes antibodies when you are sick so that it can fight off invading viruses or bacteria; the antibodies made are very specific to the invaders, binding to those and nothing else. In immunostaining, the antibodies are very specific to a protein of interest in the cell. These antibodies are tagged with dyes so that you can easily see where in the tissue they are binding to. Similarly, in situ hybridization uses a DNA or RNA probe that is tagged with fluorescent or non-fluorescent dyes. Instead of binding to a protein, like in immunostaining, it binds to a specific DNA or RNA sequence. It can tell you useful information like where or when a gene is expressed or what DNA looks like when a cell is dividing.



Non-fluorescent immunostain (left) showing pCREB protein in the rat hippocampus. Fluorescent in situ hybridization (right) showing Vglut1 (red), Vglut2 (green), and Vgat (white) RNA expression in mouse brain.

The previously discussed methods are all done in extracted tissue samples. However, we are able to label cells in an animal throughout its lifetime, even before it is born. Scientists can insert a piece of DNA that codes a fluorescent protein into an animal's DNA to create a transgenic animal. You can restrict the fluorescence to a specific cell type of interest by controlling its expression using a promoter. The promoter is in front of the inserted DNA and acts as its "boss", telling it where and when it can be expressed. Based on what kind of promoter you have, you can restrict the expression of a fluorescent protein to a specific cell type, like neurons versus glia.



Transgenic mouse expressing green fluorescent protein in all cell types

Materials

Item	**Quantity**	**Notes**
Light microscope	2-4	Borrowed from science teaching labs
Slide of unstained brain slice	2	Borrowed from Northwestern University lab to show how visualizing neurons is difficult without staining
Histology slides	As many as possible	Borrowed from various labs throughout Northwestern University. Ideally have slides of different stains (i.e. Nissl, Golgi) obtained from distinct areas of the brain (i.e. hippocampus, striatum) and from various species (i.e. mouse, rabbit, monkey)
Images of fluorescent staining	As many as possible	Print pictures of fluorescent images, insert in sheet protectors, and put in a binder. Can also make a poster

In the Class

If you have access to light microscopes and slides:

1. Place slides in a microscope stage. Each microscope should be used to visualize only one slide to avoid having to refocus every time a slide is changed.
2. Have students rotate between microscopes and make observations about what the staining reveals.

If you do not have access to light microscopes and slides:

1. Create a poster/binder/powerpoint with images and examples of histology. Direct students to relevant images as they are discussed and explain what details are stained.

Image Credits (in order of appearance)

1. https://www.rndsystems.com/products/dapi_5748

2. Watson, C., Kirkcaldie, M., & Paxinos, G. (2010). Techniques for studying the brain. In C. Watson, M. Kirkcaldie, & G. Paxinos (Eds.), *The Brain* (pp. 153-165). Academic Press. https://doi.org/10.1016/B978-0-12-373889-9.50011-5
3. https://en.wikipedia.org/wiki/Pyramidal_cell
4. Zhang, X. O., et al. (2024). Enhancing associative learning in rats with a computationally designed training protocol. *Biological Psychiatry Global Open Science 4(1)*. 165-181. https://doi.org/10.1016/j.bpsgos.2023.07.006
5. https://acdbio.com/science/applications/research-areas/neuroscience
6. Hoffman, R. M. (2008). Imaging in mice with fluorescent proteins: From macro to subcellular. *Sensors 8(2)*. 1157-1173. https://doi.org/10.3390/s8021157