

## Student instructions and data for Virtual Fluorescent Immunocytochemistry Lab Activity

This week's "lab" work:

1. Go to the JoVE Science Education access URL below.

<https://www.jove.com/science-education-library>

2. Click on Start your JoVE education free access, and set up your free account.

3. Go to the URL below for Jove Video on Immunocytochemistry and Immunohistochemistry. View the video and take notes on it. You may want to look at the modified questions for this data report in the document below the JoVE links to guide that note taking.

[Immunohistochemistry and Immunocytochemistry: Tissue Imaging via Light Microscopy](#)

4. Go to the URL below for Jove Video on Immunofluorescence Microscopy of tissue sections. View the video and take notes on it. You may want to look at the modified questions for this data report in the document below the JoVE links to guide that note taking.

[Immunofluorescence Microscopy: Immunofluorescence Staining of Paraffin-Embedded Tissue Sections](#)

5. Look at the Word document of immunocytochemistry results. These are the raw data you may use to create your data report, IF you did not already hand in two data reports earlier this semester. (this was a separate document posted on our learning management system).

### **Images and modified summary questions for immunocytochemistry virtual experiment lab report**

Please use these images to create your data report figures.

- Remember to combine them into multi-panel Figures and provide a Figure legend beneath them, as in the example Figures I handed out early this semester in class. You can copy/paste and resize them as you see fit. Creating a Table and pasting images into the Table is a convenient way to do this.
- Remember to provide text in the results section to describe what you see in each image.
- Remember to follow the instructions for preparing data reports from your lab manual.

source of cultured cells: C57Bl6/J mouse cerebellum, grown for 14 days in vitro before staining

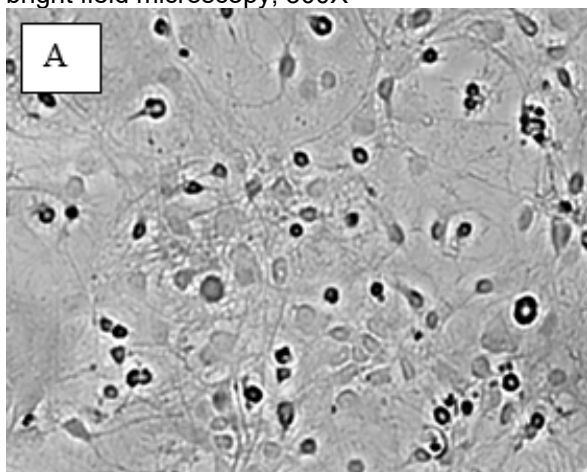
primary antibodies: rabbit anti-calbindin 1:1500 dilution

mouse anti-glial fibrillary acidic protein (GFAP) 1:200 dilution

secondary antibodies: goat anti-rabbit Cy3 (red) 1:300 dilution

goat anti-mouse Alexa 488 (green) 1:300 dilution

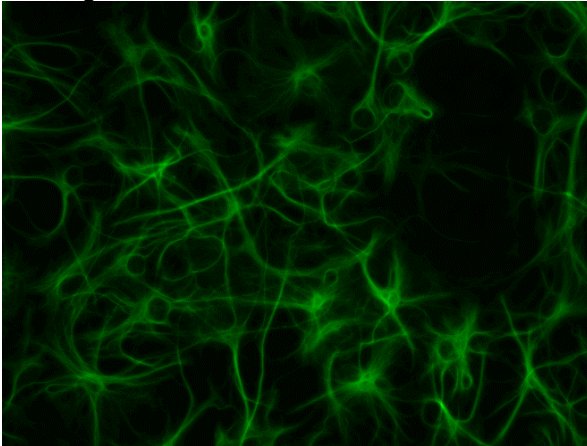
bright field microscopy, 300X



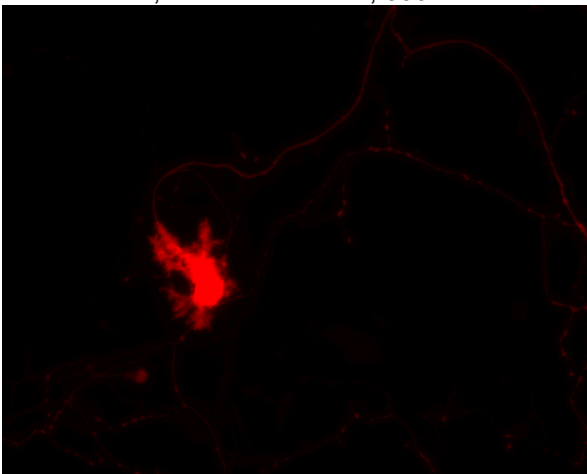
no primary antibodies/secondary antibody only control, fluorescent filters, 300X



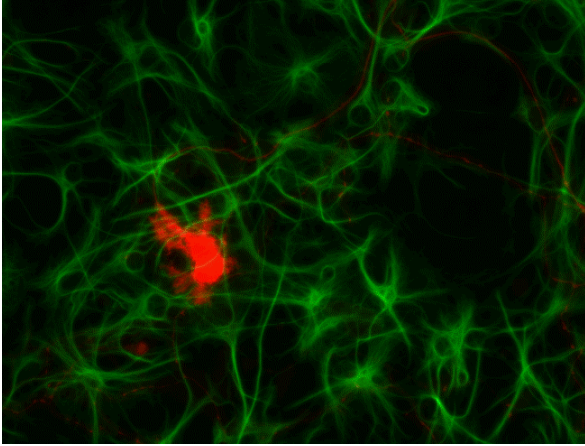
GFAP green, fluorescent filters, 300X



calbindin red, fluorescent filters, 300X



Calbindin Red, GFAP green, fluorescent filters, 300X



**Modified questions for immunocytochemistry data report:**

1. What is the function of the blocking step?
  2. What is the function of the washes?
  3. What is the function of the triton-X-100? Hint: it is a detergent.
  4. What is the function of the goat anti-rabbit Alexa 488-conjugated secondary antibody?
  5. What reagents and controls are needed for an indirect immunofluorescence assay?
  6. What does the green color indicate in this assay?
  7. What does the red color indicate in this assay?
  8. Compare and contrast the light paths for a regular light microscope and for a fluorescent microscope.
  9. What is the purpose of the barrier filters and dichroic mirror in the fluorescent microscope?
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