Fluorescence Microscopy in Cell Biology

This laboratory exercise has three parts that can be completed at any time. 
Please note that your written report is due by the date given in the class syllabus.

**Part 1.** The first part includes three web-based tutorials that will introduce you to the principles of fluorescence as it is applied to visualization of cellular structures.

**Objective.** To understand the basic theory and uses of fluorescence. Keep in mind that this material will not be covered in class but you are responsible for the material on the final exam.

**Begin.** Start the exercise at http://probes.invitrogen.com/resources/education/

You will find three tutorials, *Introduction, Spectra, and Light Filters and Sources*. As you review the tutorials, think about how using the appropriate techniques can enhance imaging quality and sensitivity. Pay attention to the images that accompany each tutorial as they will help you appreciate the uses of fluorescence.

When finished, you should be able to answer the following questions:

— Why use fluorescence to study cells?
— What is a fluorophore?
— How do fluorophores emit light?
— What is photobleaching?
— What is the importance of light spectrum and wavelength to fluorescence?
— What is the difference between a fluorescence excitation spectrum and a fluorescence emission spectrum?
— What variables affect the intensity of the emission?
— What is the significance of the Stokes shift?
— What are the two most common types of light sources? What are the properties of each?
— When would you use a filter for fluorescence microscopy?
— What is the difference between a longpass emission filter and a bandpass emission filter?

**Part 2.** You will gain firsthand experience with fluorescent microscopy by observing prepared fluorescent slides.

**Objective.** To see how fluorescence is used for cell biology research.

**Begin.** There is a sign-up sheet just inside the door of SL 430. Choose a time and day that is convenient for you and place your name on the list. Make a note of your time and date. (Please let your instructor know if you cannot make one of the scheduled times). On your scheduled date and time, please stop by SL 419A, for a 15-minute demonstration of fluorescent microscopy. Samples examined will vary from semester to semester but all represent current research topics.
Part 3. When you have completed the tutorials, you will examine images that were obtained using fluorescent dyes. One of these images will serve as an organizing ingredient for a written paper that summarizes an important aspect of cell biology.

Objective. To summarize your understanding of cellular structures and processes in a written report.

Begin. Go to http://probes.invitrogen.com/servlets/gallery/

Begin by looking at the Recent Additions section, which are represented by the thumbnail images that appear on the page. Click on any you like and just explore the images; they are truly astounding. Next, select a Gallery from the pulldown menu of the Browse heading. You will note that these are categorized by cellular locale or cellular process. Find an image/topic that is of interest to you and write a four- to five-page report on the image and its significance for understanding cells. You will need to use the figure legend to get started, and use your cell biology textbook, class notes (if appropriate), and research articles for additional information. When you are finished, you should have a complete, detailed summary of a cell biology topic. Include a color copy of the image with your report (the instructor can print this for you if you do not have access to a color printer).

You should provide references (other than your textbook) for the information you include. At least one of these references should be from a research article that covers a similar topic. Please double-space your report.

Grading Rubric

[20 points] **Introduction.** Provide sufficient background so that someone not familiar with cell biology can understand the significance of the topic under study. Be sure to provide citations for any information that is not common knowledge.

[20 points] **Techniques.** Provide any information you can about what fluorophore was used and how it was employed and detected. (Depending on the image, it may or may not be possible to go in to great detail here). Give the catalog cumber of the Invitrogen product and its units.

[40 points] **Discussion.** Describe the significance of the findings for cell biology. Here, your target audience is someone, like your instructor, who has a good knowledge of cells. What do the results indicate about the structure or function of cells? Are there any applications that you can think of? Provide citations for research studies that are related to this topic.