



Center for Toxicology, Southwest Environmental Health Sciences Center (an NIEHS Center)

Experimental Pathology Service Core

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http://www.pharmacy.arizona.edu/exp_path.html

Histology lab personnel news:

- The Cell Biology & Anatomy Histology Service Lab is pleased to announce the hiring of Andrea Grantham as the new full-time Sr. Research Specialist in the lab. She will be the primary point of contact for users of the lab. She comes to the lab with over 20 years of clinical histology experience, ASCP certification and she's even the president of the Arizona Society for Histotechnology this year.
- Katie Propst is the other new face in the Histology lab. Katie will be working for the lab on an on-call basis. Katie is also ASCP certified and she has 20+ years of experience in clinical histology labs.
- The CBA Histology Service lab is located in the College of Medicine, room 4212. The lab can be reached by calling 626-4415 or sending an email to <histo@u.arizona.edu>. Information about the lab, as well as a current price list is available on line at: <http://www.cba.arizona.edu/histology-lab.html>

Choosing appropriate dye combinations for the Leica confocal microscope:

- There are many different commercially available fluorescent dyes that are suitable for confocal microscopy. A problem can occur when a dye is selected that does not match the capabilities of the microscope. Please consider the following information when selecting fluorescent dyes for your confocal experiments.
- Fluorescent dyes absorb light of one wavelength (*excitation*) and emit light of a longer wavelength (*emission*). The first two unfilled curves in the adjacent spectral graph (*Molecular Probes Handbook, 7th ed.*) are for the well known dye fluorescein (*the gray curve is the emission curve for rhodamine red*). The excitation/emission maxima for fluorescein are 494/520 nm. The distance between the maxima is referred to as the "Stokes shift".
- The graph illustrates a common problem with using two or more dyes. Fluorescein and rhodamine red excite with separate wavelengths, but the emission spectrum of fluorescein is so broad that it is almost impossible to create an optical filter that can fully separate the emitted light of the two dyes when they are excited at the same time. This is especially a problem if the fluorescein emissions are very bright in comparison to that of rhodamine red, the weaker rhodamine red signal can become almost completely obscured. With the confocal microscope users can acquire images by exciting the dyes separately and combining the images. This alleviates the problem of overlap, but is not a recommended practice if the user is trying to demonstrate co-localization of the two dyes.
- Occasionally users will select two dyes that have overlapping excitation spectra (*e.g., fluorescein and propidium iodide*). This causes a different problem in that there is no longer a way to adjust the confocal so that one dye can have more or less excitation than the other.
- Some users prefer to use the dark red fluorescent dye CY-5 because it is bright, it rarely overlaps with other dyes and it is far removed from the wavelengths associated with tissue autofluorescence (*usually similar to that of fluorescein*). The disadvantage of CY-5 is that the human eye cannot easily "see" the 670 nm emission maxima, which makes it difficult to quality control your staining. CY-5 does not easily fade in the confocal, however, it can be particular about which anti-fade mounting media is used.
- When you are evaluating fluorescent dyes for your experiments, here is some important information about the Leica confocal. It has three wavelengths (488, 568 and 647 nm) for exciting fluorescent dyes. The available filters allow us to capture fluorescent emissions in the ranges of: 515-545 nm, 585-615 nm and greater than 665 nm (*the ranges are for the bandpass filters, we have longpass filters also*). There are currently no confocal microscopes on campus that are capable of exciting dyes that require UV wavelengths (*there is one at ASU*).
- The Doug Cromey, Dr. Lantz or Dr. Payne would be happy to assist you in selecting dyes that are appropriate for the Leica confocal.

