Fluorescence Microscopy

microtutor

Course Syllabus

Chapter 1

The first chapter introduces light, fluorophores and fluorescence, helping to set the stage for understanding the design of the fluorescence microscope.

Fluorescence: Introduces the electromagnetic spectrum, and the relationship between energy, wavelength, and color of light. The molecular basis of fluorescence is illustrated using Jablonski diagrams.

Properties of Fluorophores: Reviews critical fluorophore properties, including brightness and rate of photobleaching, and how to use spectra to assess fluorophore excitation and emission

Biological Fluorescence Microscopy: Introduces fluorescent proteins and small molecule fluorophores, as well as some of the most common methods used to conjugate fluorophores to biological targets

Chapter 2

Next, we explore the design of the fluorescence microscope. The purpose of the major parts and pieces are covered, with a focus on the fluorescence filter sets used to image fluorophores.

Filter Sets: Introduces how filters are used to excite and collect fluorescence

Filters in the Microscope: Places filter sets in the context of the microscope and introduces the design of the epifluorescence light path

Choosing Filters: Reviews guidelines for choosing appropriate filter sets, then put your skills to the test in a filter choice activity using spectraviewers from FPbase.org

The Fluorescence Microscope: Covers the anatomy of the fluorescence microscope, including critical hardware such as light sources and the field diaphragm

Chapter 3

The final chapter of this course focuses on issues that come up when performing imaging experiments with biological specimens. The most common errors introduced by the fluorescence microscope that, if ignored, may compromise the accuracy of imaging data. For each, controls that can be used to reveal and measure the impact of the error on imaging data are reviewed, as well as a selection of strategies that can be used to minimize the impact.

Imaging Multiple Fluorophores: Introduces sequential acquisition of multiple fluorophores, setting us up for the next few sections

Bleedthrough: Introduces the concept of bleedthrough, a major source of error in multi-fluorophore experiments. You'll use a spectraviewer-based activity to reinforce your understanding, and then learn some strategies to detect and deal with bleedthrough.

Chromatic Shifts: Introduces sources of lateral and axial registration shifts between images of different fluorophores, and provides recommendations for measuring and correcting these shifts

Autofluorescence: Discusses the potential for endogenous fluorescence in biological materials, and suggests some methods to decrease its influence on your results

Acquisition Speed: Deals with the potential for artifacts in multi-channel experiments when imaging dynamic specimens