Fluorescent Labeling in 3-D Live Cell Imaging

Sensitive Specimens Need Careful Microcopy

Preface

The advent of fluorescent labeling technologies along with the available light microscope techniques enable scientist to analyze critical changes in living cells and organisms as well as tracking dynamic movements in real time.

This workshop intended to cover two parts: specific methods such as fluorescent labeling, and delivery and detection of labeled molecules in cells; and experimental approaches ranging from the detection of single molecules to the study of dynamic processes in organelles.

The 3-D live-cell topic usually covers two general areas: Optics/Instrumentation and "Doing the experiment without killing the cells." These two areas are very different and many researchers start with the assumption that, really, only the second one is important. In fact, the two areas meet as soon as the experiment starts because only by doing the optics correctly can one get the best possible image with the least amount of damage to the specimen.

In fluorescence microscopy, doing the least possible damage implies using the lowest possible excitation power at the specimen. Unfortunately, good images require lots of photons, implying high excitation power or long exposure. The trick is to learn how to optimize the settings of your microscope to balance these two competing demands.

- Higher zoom magnification will increase the photodamage to the scanned area.
- Some dyes bleach more rapidly than others.
- Some dyes may be more prone to producing phototoxicity than others.
- Scan speed (or exposure time in wide field) may be a factor: but living cells move!
- Different cell types have different responses: plant leaves are better designed for a high photon-flux environment than mammalian embryos.
- Different objective lenses will vary in their ability to produce useful information from a given number of photons: Important variables include: NA (numerical aperture) and relative freedom from spherical aberration under the actual imaging conditions.
- The variations are almost endless.