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Optical Microscopy for Biomedical Applications

Welcome to the Olympus Microscopy Resource Center, designed to provide an Internet-based educational forum on all aspects of optical microscopy, photomicrography, and digital imaging. Current advances in microscopy are being propelled by new techniques such as laser scanning confocal, multi-photon fluorescence, and wide-field deconvolution microscopy. Although these techniques were once considered exotic methods, available only to a few research microscopists, they are now frequently utilized in laboratories around the world for analysis of cellular structure and molecular dynamics.

We invite you to explore the exciting world of optical microscopy with our extensive microscopy essays that are supported by interactive Java and Flash tutorials. Together with scientists and programmers at [Molecular Expressions](#), Olympus microscopists and optical engineers have constructed a web-based instructional format that starts with the basics and proceeds through the most current research techniques in microscopy.

[Olympus BioScapes™ Digital Imaging Competition](#) - Olympus America Inc. is sponsoring an exciting new photo competition that will honor extraordinary microscope images of life science subjects. Entry deadline is September 30, 2009, and first prize will be Olympus microscope or camera equipment valued at \$5,000. Nine additional winners will also receive valuable prizes from Olympus. The Olympus BioScapes Digital Imaging Competition will recognize the finest images of life science specimens captured through light microscopes, using any magnification and any brand of equipment. Each person entering can submit up to five movies, images, or image sequences. Entries will include information on the importance or "story" behind the images. Winners will be notified in late September, and publicly announced at an event in October.

[NEW! - Fluorescent Protein Fluorophore Maturation Mechanisms](#) - Autocatalytic formation of the fluorophore (also referred to as a **chromophore**) within the shielded environment of the polypeptide backbone during fluorescent protein maturation follows a surprisingly unified mechanism, especially considering the diverse natural origins of these useful biological probes. Shortly after synthesis, most fluorescent proteins slowly mature through a multi-step process that consists of folding, initial fluorophore ring cyclization, and subsequent modifications of the fluorophore. The spectral properties of fluorescent proteins are dependent upon the structure of the fluorophore as well as the localized interactions of amino acid residues in the immediate vicinity, and in some cases, residues far removed from the fluorophore. The interactive tutorials in this section explore fluorophore formation in a wide variety of spectrally diverse fluorescent proteins deduced from crystallographic studies.

[Rat Brain Tissue Sections](#) - The rat brain has served as an excellent model for elucidating the complex anatomy and physiological mechanisms of the human brain. As a result, a significant amount of information on brain diseases, such as dementia and Parkinson's disease, has been determined from investigations using rat brains. Brain tissue has been mapped into dozens of major and hundreds of minor regions that are anatomically and functionally distinct. Individual brain cells segregate into specialized areas by expressing a wide spectrum of specific housekeeping proteins, enzymes, transporters, and receptors. This digital image gallery explores many regions of the rat brain as observed with immunofluorescence in coronal, horizontal, and sagittal thick sections using laser scanning confocal microscopy.

[Plant Tissue Autofluorescence Gallery](#) - Autofluorescence in plant tissues is a common and useful phenomenon arising from a variety of endogenous biomolecules that absorb light in many regions of the near-ultraviolet and visible light spectrum. One of the primary contributors of plant autofluorescence is chlorophyll, but lignins, carotenes, and xanthophylls also produce a significant level of fluorescence emission when stimulated with the proper wavelengths. This digital image gallery examines natural autofluorescence in plant tissue thin sections using multiple excitation wavelengths with laser scanning confocal microscopy.

[Colocalization of Fluorophores in Confocal Microscopy \(Interactive Java Tutorial\)](#) - Two or more fluorescence emission signals can often overlap in digital images recorded by confocal microscopy due to their close proximity within the specimen. This effect is known as **colocalization** and usually occurs when fluorescently labeled molecules bind to targets that lie in very close or identical spatial positions. This

interactive tutorial explores the quantitative analysis of colocalization in a wide spectrum of specimens that were specifically designed either to demonstrate the phenomenon, or to alternatively provide examples of fluorophore targets that lack any significant degree of colocalization.

Laser Scanning Confocal Microscope Simulator - Perhaps the most significant advance in optical microscopy during the past decade has been the refinement of mainstream laser scanning confocal microscope (**LSCM**) techniques using improved synthetic fluorescent probes and genetically engineered proteins, a wider spectrum of laser light sources coupled to highly accurate acousto-optic tunable filter control, and the combination of more advanced software packages with modern high-performance computers. This interactive tutorial explores multi-laser fluorescence and differential interference contrast (**DIC**) confocal imaging using the Olympus FluoView FV1000 confocal microscope software interface as a model.

Optical Highlighter Fluorescent Proteins - Protein chromophores that can be activated to initiate fluorescence emission from a quiescent state (a process known as **photoactivation**), or are capable of being optically converted from one fluorescence emission bandwidth to another (**photoconversion**), represent perhaps the most promising approach to the *in vivo* investigation of protein lifetimes, transport, and turnover rates. Appropriately termed **molecular** or **optical highlighters**, photoactivated fluorescent proteins generally display little or no initial fluorescence under excitation at the imaging wavelength, but dramatically increase their fluorescence intensity after activation by irradiation at a different (usually lower) wavelength. Photoconversion optical highlighters, on the other hand, undergo a change in the fluorescence emission bandwidth profile upon optically-induced changes to the chromophore. These effects result in the direct and controlled highlighting of distinct molecular pools within the cell.

Co-Localization of Fluorophores in Confocal Microscopy - During the examination and digital recording of multiply labeled fluorescent specimens, two or more of the emission signals can often overlap in the final image due to their close proximity within the microscopic structure. This effect is known as **co-localization** and usually occurs when fluorescently labeled molecules bind to targets that lie in very close or identical spatial positions. The application of highly specific modern synthetic fluorophores and classical immunofluorescence techniques, coupled with the precision optical sections and digital image processing horsepower afforded by confocal and multiphoton microscopy, has dramatically improved the ability to detect co-localization in biological specimens.

Choosing Fluorophore Combinations for Confocal Microscopy - In planning multiple label fluorescence staining protocols for widefield and laser scanning confocal fluorescence microscopy experiments, the judicious choice of probes is paramount in obtaining the best target signal while simultaneously minimizing bleed-through artifacts. This interactive tutorial is designed to explore the matching of dual fluorophores with efficient laser excitation lines, calculation of emission spectral overlap values, and determination of the approximate bleed-through level that can be expected as a function of the detection window wavelength profiles.

Matching Fluorescence Filter Blocks with Fluorescent Probes - The essential feature of any fluorescence microscope is to provide a mechanism for excitation of the specimen with selectively filtered illumination followed by isolation of the much weaker fluorescence emission using a second filter to enable image formation on a dark background with maximum sensitivity. These conditions are satisfied in modern fluorescence instruments by a combination of filters that coordinate excitation and emission requirements based on the action and properties of the dichromatic beamsplitter. This interactive tutorial enables visitors to determine the optimum fluorophore characteristics necessary to maximize the efficiency of excitation and emission in conjunction with current Olympus fluorescence filter blocks.

Olympus FluoView Laser Scanning Confocal Microscopy - The new Olympus FluoView™ FV1000 is the latest in point-scanning, point-detection, confocal laser scanning microscopes designed for today's intensive and demanding biological research investigations. Excellent resolution, bright and crisp optics, and high efficiency of excitation, coupled to an intuitive user interface and affordability are key characteristics of this state-of-the-art optical microscopy system.

Fluorescence Microscopy of Cells in Culture - Serious attempts at the culture of whole tissues and isolated cells were first undertaken in the early 1900s as a technique for investigating the behavior of animal cells in an isolated and highly controlled environment. The term **tissue culture** arose because most of the early cells were derived from primary tissue explants, a technique that dominated the field for over 50 years. As established cell lines emerged, the application of well-defined normal and transformed cells in biomedical investigations has become an important staple in the development of cellular and molecular biology. This fluorescence image gallery explores over 30 of the most common cell lines, labeled with a variety of fluorophores using both traditional staining methods as well as immunofluorescence techniques.

[Olympus FluoroScapes Screen Saver for Windows](#) - Explore the amazing world of fluorescence microscopy and review the performance of Olympus widefield fluorescence and confocal microscopes. This unique screen saver features 36 fluorescence digital images captured with either the BX51 or FluoView FV300 Laser Scanning Confocal Microscope. Specimens include cells in culture from 19 individual established lines (hosts: human, mouse, rat, monkey, mink, tahr, dog, pig, deer, and rat kangaroo), as well as human, mouse, rat, and sheep thin tissue sections. Each specimen was double or triple labeled with a combination of synthetic fluorophores using standard protocols and immunofluorescence techniques. The screen saver software also contains 15 user-configurable display modules that allow an almost infinite number of image display transition motifs.

Latest Articles, Interactive Tutorials, and Galleries

[Microscope: Basics and Beyond \(50 pages in PDF format; 20.7 Mbytes\)](#) - Download the latest edition of Mortimer Abramowitz's renowned introduction to optical microscopy in full color. The volume covers all of the important basic concepts, ranging from simple magnifiers to complex compound microscopes, including illumination, objectives, eyepieces, condensers, aberration, Köhler illumination, resolution, numerical aperture, and depth of field. Numerous appendices review focusing of the microscope and oil immersion, and contain useful numbers, formulas, and a short bibliography.

[Purchase Educational Microscopy Books](#) - This series, written by Mortimer Abramowitz, Fellow, New York Microscopical Society, contains six volumes (including **Microscope: Basics and Beyond**), which cover many aspects of microscopy including contrast methods, reflected light, fluorescence, photomicrography, and basic optics. Mr. Abramowitz was the recipient of the New York Microscopical Society Ernst Abbe Memorial Award in November 2002 for his outstanding contributions to microscopy as a photomicrographer, educator, and author.

[Olympus DP70 Digital Camera System](#) - The latest generation of digital cameras designed for wide-ranging applications in optical microscopy combine excellent resolution, high sensitivity, and rapid data transfer to a host computer. The Olympus DP70 is a 12.5 million-pixel cooled digital color camera system that incorporates the latest innovations in imaging technology to enable the capture of superb images in the most demanding current microscopy applications, including differential interference contrast (DIC), darkfield, phase contrast, polarized light, and most widefield fluorescence techniques.

[Differential Interference Contrast Digital Image Gallery](#) - Thin unstained, transparent specimens are excellent candidates for imaging with classical differential interference (DIC) microscopy techniques over a relatively narrow range (plus or minus one-quarter wavelength) of bias retardation. The digital images presented in this gallery represent a wide spectrum of specimens, which vary from unstained cells, tissues, and whole organisms to both lightly and heavily stained thin and thick sections. In addition, several specimens exhibiting birefringent character are included to demonstrate the kaleidoscopic display of color that arises when anisotropic substances are imaged with this technique. All of the images presented in this gallery were captured with an Olympus DP70 camera system operating on a BX60 transmitted microscope.

[The Olympus MIC-D Digital Microscope](#) - Olympus has thrown the doors open to a new era in optical microscopy education with the introduction of the MIC-D inverted digital microscope. Designed specifically for a wide spectrum of applications ranging from basic classroom instruction to more advanced laboratory analysis, this versatile microscope features a palette of contrast enhancing techniques that rival many research-level instruments.

[MIC-D Digital Microscope Quick Start Guide \(PDF Format; 5.2 Mb\)](#) - Download the official Olympus MIC-D Quick Start Guide, which provides a brief explanation of the microscope hardware and software, as well as hints on specimen choice, illumination techniques, and image processing. In the laboratory or in the field, the Olympus MIC-D portable digital microscope captures still and moving images instantly. The guide will introduce beginners to the microscope's innovative design and spectacular imaging capabilities, enabling them to collect images on the microscope with a minimum of effort.

[Digital Imaging in Optical Microscopy](#) - Digitization of a video or electronic image captured through an optical microscope results in a dramatic increase in the ability to enhance features, extract information, or modify the image. When compared to the traditional mechanism of image capture, photomicrography on film, digital imaging and post-acquisition processing enables a reversible, essentially noise-free modification of the image as an ordered matrix of integers rather than a series of analog variations in color and intensity. This section addresses a variety of current topics in image acquisition and processing for optical microscopy.

[Near-Field Scanning Optical Microscopy](#) - For ultra-high optical resolution, near-field scanning optical microscopy (NSOM) is currently the photonic instrument of choice. Near-field imaging occurs when a sub-micron optical probe is positioned a very short distance from the sample and light is transmitted through a

small aperture at the tip of this probe. The near-field is defined as the region above a surface with dimensions less than a single wavelength of the light incident on the surface. Within the near-field region evanescent light is not diffraction limited and nanometer spatial resolution is possible. This phenomenon enables non-diffraction limited imaging and spectroscopy of a sample that is simply not possible with conventional optical imaging techniques.

Introduction to Lasers - Ordinary natural and artificial light is released by energy changes on the atomic and molecular level that occur without any outside intervention. A second type of light exists, however, and occurs when an atom or molecule retains its excess energy until **stimulated** to emit the energy in the form of light. Lasers are designed to produce and amplify this stimulated form of light into intense and focused beams. The word laser was coined as an acronym for **L**ight **A**mplification by the **S**timulated **E**mission of **R**adiation. The special nature of laser light has made laser technology a vital tool in nearly every aspect of everyday life including communications, entertainment, manufacturing, and medicine.

Laser Systems for Optical Microscopy - The lasers commonly employed in optical microscopy are high-intensity monochromatic light sources, which are useful as tools for a variety of techniques including optical trapping, lifetime imaging studies, photobleaching recovery, and total internal reflection fluorescence. In addition, lasers are also the most common light source for scanning confocal fluorescence microscopy, and have been utilized, although less frequently, in conventional widefield fluorescence investigations.

Multiphoton Excitation Microscopy - Multiphoton fluorescence microscopy is a powerful research tool that combines the advanced optical techniques of laser scanning microscopy with long wavelength multiphoton fluorescence excitation to capture high-resolution, three-dimensional images of specimens tagged with highly specific fluorophores.

Total Internal Reflection Fluorescence Microscopy - Total internal reflection fluorescence microscopy (TIRFM) is an elegant optical technique utilized to observe single molecule fluorescence at surfaces and interfaces. The technique is commonly employed to investigate the interaction of molecules with surfaces, an area which is of fundamental importance to a wide spectrum of disciplines in cell and molecular biology.

Prisms and Beamsplitters - Prisms and beamsplitters are essential components that bend, split, reflect, and fold light through the pathways of both simple and sophisticated optical systems. Cut and ground to specific tolerances and exact angles, prisms are polished blocks of glass or other transparent materials that can be employed to deflect or deviate a light beam, rotate or invert an image, separate polarization states, or disperse light into its component wavelengths. Many prism designs can perform more than one function, which often includes changing the line of sight and simultaneously shortening the optical path, thus reducing the size of optical instruments.

Physics of Light and Color - Visible light represents only a small portion of the entire electromagnetic spectrum of radiation that extends from high-frequency gamma rays through X-rays, ultraviolet light, infrared radiation and microwaves to very low frequency long-wavelength radio waves. The complex phenomenon of visible light is classically discussed in terms of rays and wavefronts. Starting with the nature of electromagnetic radiation, a wide variety of topics are covered in this section, including refraction, reflection, diffraction, interference, birefringence, polarization, primary colors, human vision, mirrors, prisms, beamsplitters, laser systems, geometrical optics, filtration, color temperature, and the speed of light.

Basic Concepts in Optical Microscopy - A thorough discussion of the elements that comprise modern microscopes and theories behind important concepts such as magnification, image formation, Köhler illumination, optical aberrations, immersion media, and light sources, among others.

Specialized Microscopy Techniques - More advanced topics in microscopy are covered in this section, including contrast enhancement, fluorescence microscopy, differential interference contrast, phase contrast and other optical techniques used in microscopy.

Use the links provided in our navigators to visit our photomicrography galleries featuring both film-based and digital images gathered from a wide variety of specimens imaged with the most popular and revealing illumination techniques. The **Olympus Microscopy Museum** is still under construction, but eventually will contain a rich illustrated history of Olympus microscopy. In addition the **Digital Video Galleries** are filled with time-lapse and real-time digital movies captured with Olympus microscopes.

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