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A Multi-mode Fluorescent Microscope

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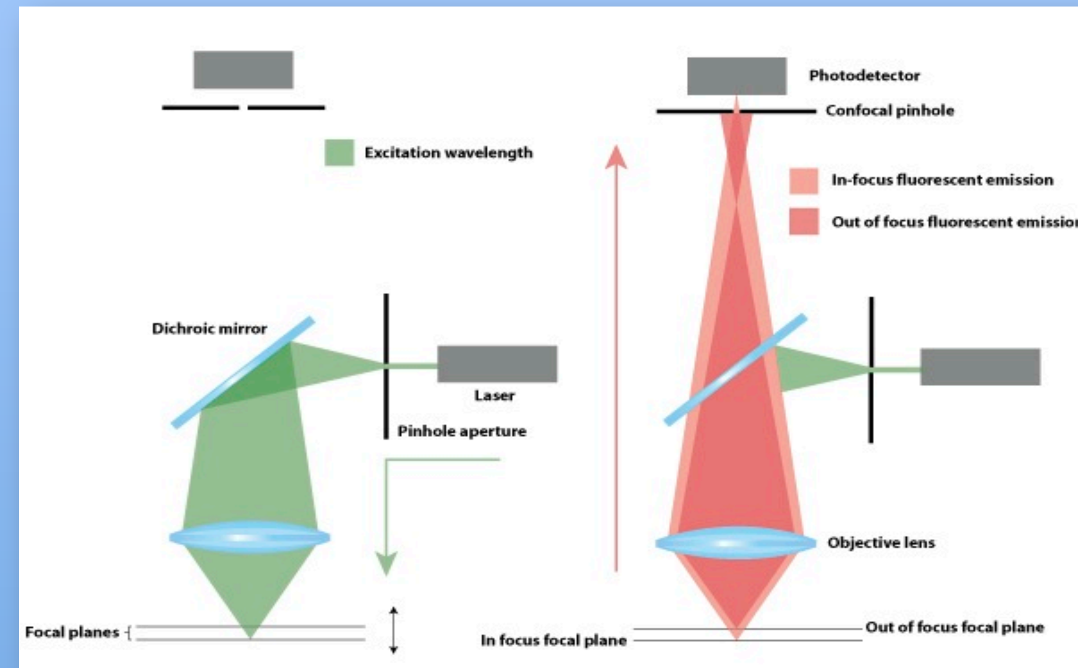
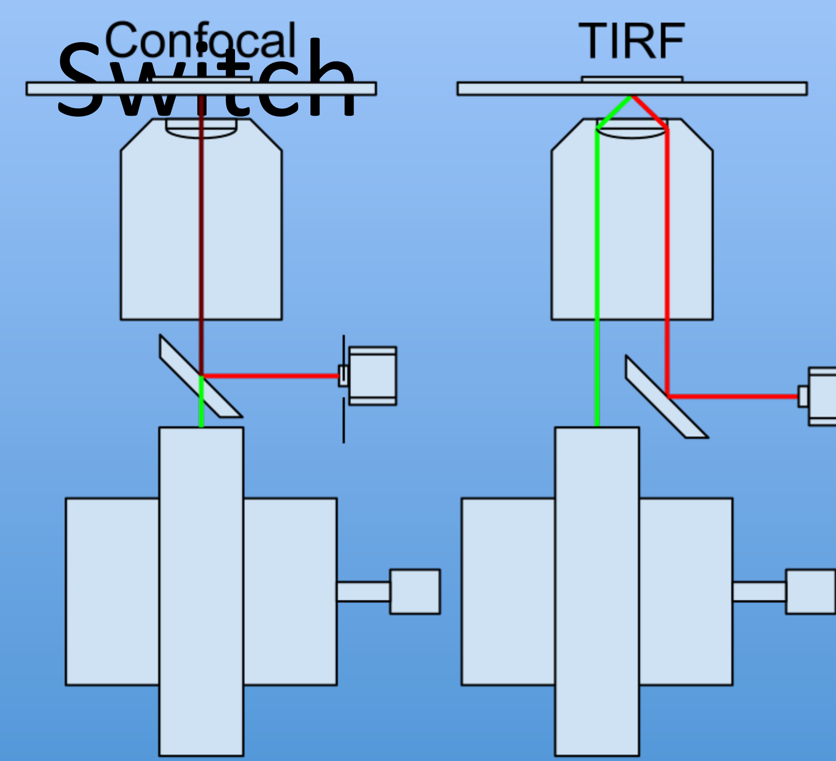
Confocal Microscopy

In a conventional wide-field microscope, thick specimens will produce an image that represents the sum of sharp image details from the in-focus region, combined with blurred images from all the out-of-focus regions. As a result, a specimen having a thickness greater than three to five microns will produce an image in which most of the light is contributed by the regions that are not in exact focus.



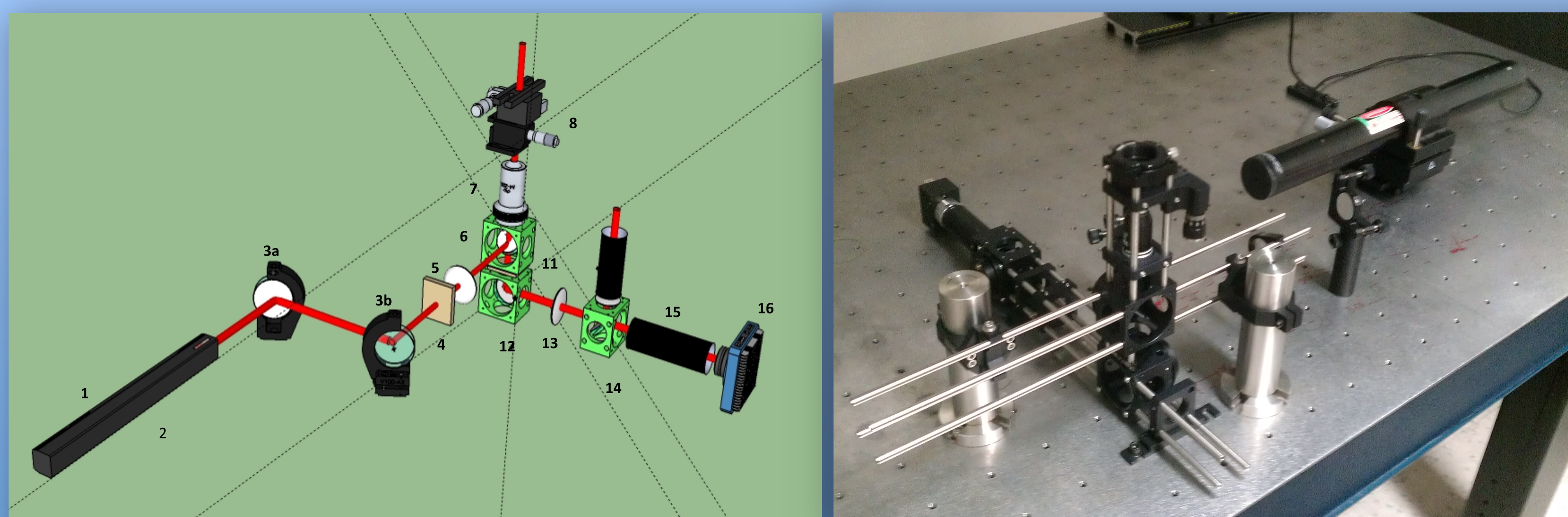
In confocal microscopy, a point-light light source (laser) is focused by an objective onto a sample. The spatial extension of the focus spot on the sample is determined by the wavelength the numerical aperture of the lens, and the quality of the image formation. The image spot is then focused through the same lens onto a pinhole and onto a detector. This pinhole is situated at a plane where the light from the in-focus part of the image converges to a point. Light from object planes above or below that of the focused images do not converge at the pinhole and hence is mostly blocked by it. Paras N. Prasad, Introduction to Biophotonics

Mode Switch



The design of this microscope enables the user to switch in between confocal and total internal reflection (TIR) mode. When centered, the green laser from 532 nm He-Ne laser overlaps with the optical axis of the objective lens. As a result, the laser is delivered orthogonal to the sample stage. After absorption, the light emitted by the fluorophore goes through a pinhole and forms a confocal image on the CCD camera. When placed towards the left, the laser deviates from the optical axis of the objective lens. When the laser goes through the lens, it is bent towards the center of the sample stage, and hits the sample with an angle larger than the critical angle. As a result, only the evanescent wave is able to reach the sample. The emitted light is then reflected by a mirror and is registered onto the CCD camera.

Microscope Schematic



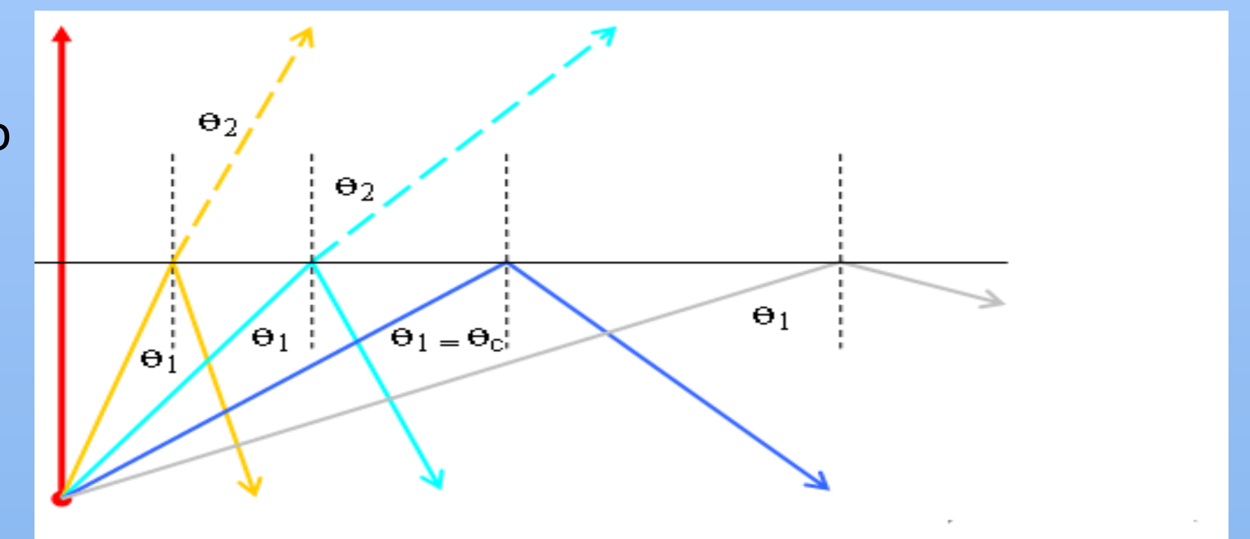
1) 15mW He-Ne Laser
2) Translation Stage under laser
3) 100% Aluminum Mirror
4) Neutral Density Filter
5) 100mm Convex Lens
6) Dichroic Beamsplitter
7) Objective Lens
8) XYZ Translation Stage
9) Optical Fiber*
10) Lamp*
11) Barrier Filter
12) 100% Aluminum Mirror
13) 175 mm Convex Lens
14) 50/50 Aluminum Mirror
15) 100mm Convex Lens/Eyepiece
16) CCD Camera w/ Computer

Research Directions

Photoluminescence blinking—random switching between states of high (ON) and low (OFF) emissivities—is a universal property of molecular emitters found in dyes, polymers, biological molecules and artificial nanostructures such as nanocrystal quantum dots, carbon nanotubes and nanowires. For the past 15 years, colloidal nanocrystals have been used as a model system to study this phenomenon. However, this model was recently challenged in several reports (Galland C., Ghosh Y., et al, 2011). This microscope is designed to further investigate the quantum dot blinking phenomenon.

Total Internal Reflection Microscopy

When a beam of light propagates from one medium to another, at the interface, refraction would occur at a small incidence angle. But when the angle of incidence exceeds a value θ_c,



the *critical angle*, the light beam is reflected from the interface, a process called *total internal reflection* (TIR). The critical angle θ_c is given by the equation.

$$\theta_c = \sin^{-1}\left(\frac{n_2}{n_1}\right)$$

Even under the condition of TIR, a portion of the incident energy penetrates the prism surface. This penetrating light is termed an *evanescent wave*, whose electromagnetic field decays rapidly. This decay is described as

$$E_z = E_0 \exp\left(\frac{-z}{d_p}\right)$$

where E_z is the electromagnetic field amplitude, E_0 is the electromagnetic field at the interface, z is the distance into the sample, parameter d_p is the penetrating depth constant, which is given as

$$d_p = \lambda / \left\{2\pi n_1\left[\sin^2\theta - \left(\frac{n_2}{n_1}\right)\right]^{\frac{1}{2}}\right\}$$

The rapid decaying nature of the evanescent light ensures that only the near-surface specimen generates fluorescence. This also limits any light-induced damage to the cell viability. Paras N. Prasad, Introduction to Biophotonics