A Multimode 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)

**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) 100% Aluminum Mirror  
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**

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.

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)