# About Confocal Microscopy

This page gives a brief overview of confocal microscopy and the advantages of using confocal microscopy. If you would like a more detailed explanation of confocal microscopy, please view this page.

### What is Confocal Microscopy?

Confocal microscopy is a powerful tool in which we are able to obtain high resolution images of real-life objects as they were meant to be seen -- in 3 dimensions -- rather than in 2 dimensions. Through the use of confocal microscopes we are able to obtain high resolution images with the use of lasers, filters, pinholes, and electronic detectors. Confocal microscopy allows us to see a re-made 3D image representation of the real object.

### Why do we need Confocal Microscopy?

We need confocal microscopy because, in our research, we need to be able to microscopically analyze thick tissue specimens in order to gain quantitative information about the molecular and structural properties of cells in our specimens. This type of analysis is not possible with the use of conventional microscopy. Conventional microscopy gives us a projected and out-of-focus 2-dimensional (2D) image of a thick 3-dimensional (3D) object.

### How does a conventional microscope work?

A conventional microscope works well for thin specimens (i.e. less than 4µm). This is because the specimen is approximately 2-dimensional and thus all of it lies in the same focal plane. However, when dealing with a thick specimen, this resolution is lost in conventional microscopy. This is because only one thin slice through the specimen can be in-focus at any given time. The rest of the specimen (most of it) is out-of-focus resulting in an image that is mainly out-of-focus.

### How does a Confocal Microscope work?

Confocal microscopy is different because only the in-focus light is detected, while the out-of-focus light is blocked out. This is done with the use of a pinhole. A pinhole is put at the image plane and an electronic light detector is put behind pinhole. But by doing this, it only allows one point in the specimen to be focused upon at a time. We can construct an image of the entire specimen by having a laser beam scan over the entire focal plane and can mechanically move the specimen to change the depth of the optical plane through the specimen. Thus we are able to get a 3D representation of the specimen with all planes in focus in the final, reconstructed image.

## Detailed Explanation of Confocal Microscopy

This page will (hopefully) help explain confocal microscopy in further detail. This page will thus assume that the reader has some understanding of microscopy and optics.

### Fluorescence

When fluorescent molecules are exposed to a light source of one color, through a microscope a user will usually see a light of a different color being emitted (Figure 1). This is the basic principle of fluorescence. Molecules at **ground** state will absorb a high energy light photon which increases the energy state of the molecules. This is called the **excited** state. Some of the energy from the light is lost internally within the molecule. The molecule will then quickly move back to its **ground** state. For this to occur, energy must leave the molecule, and this happens in the form of an **emitted** light. This lesser energy photon being emitted from the molecules will thus be a different color of light. The excitation light and the emission light are both dependent upon the molecules being examined. The advantage of this is that you can attach a fluorescent dye molecule to specific molecules in your sample/cell, so that only those molecules of interest in the sample/cell will be seen.

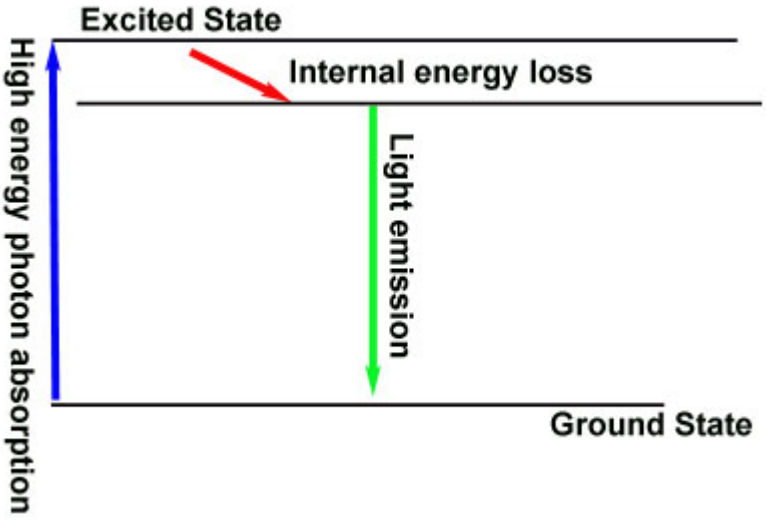


Figure 1

### Fluorescence Microscopy

The unique element of a fluorescence microscope is the **dichoric mirror** (also called a **dichromatic mirror**). This mirror is used because its special properties allow it to reflect light that emits below a certain wavelength while allowing light emitted above that wavelength to pass through the mirror. You can see the necessity of this when using fluorescence microscopy (Figure 2) because it stops any of the excitation light from entering the eyepiece, giving the user a false image. The dichroic mirror reflects

the excitation light that is reflected back down the path it came from.

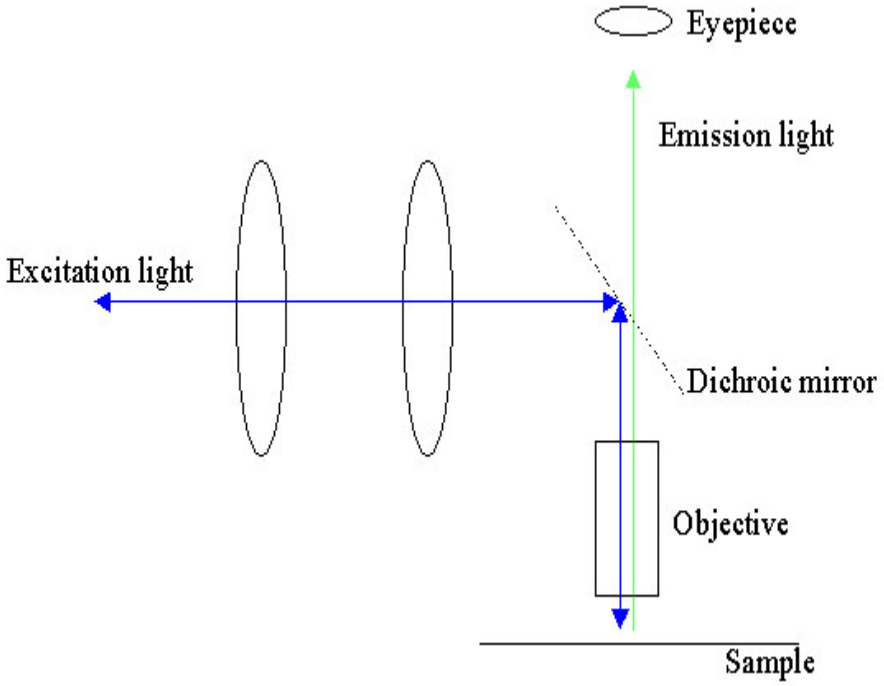


Figure 2

**Confocal Microscopy**

Confocal microscopy takes these properties of fluorescence and fluorescence microscopy, and goes a step further. Confocal microscopy adds a pinhole and laser scanning in order to produce high resolution images. Instead of the emitted light from the fluorescent molecule being emitted back through an eyepiece (as this could be very dangerous), the light is instead sent through to a detector that is placed behind the pinhole (Figure 3). The detector is used to detect the light that is able to make it through the pinhole, thus producing an image from only the emitted light from one point in the specimen. This point is where the laser is focused so it is an "in-focus" point. In other words, the detector is very efficient at being able to reject out-of-focus emitted light originating from out-of-focus points before and after the in-focus point. Through the use of scanning mirrors, the laser can scan different points of the specimen until an entire area, or plane, is scanned. Thus, this allows the user to see a thin section of the entire specimen with a small depth of field. If many of these thin section images are taken at different depth of fields within the sample, we are able to reconstruct a 3D image of the entire specimen. The inventor of the confocal microscope, Marvin Minsky, has posted his memoirs on the web. If you have any questions or comments about the topics introduced on this page, please feel free to e-mail the staff at the Confocal Microscopy Lab at the National Cancer Institute at Frederick, and we'll be happy to be of help.

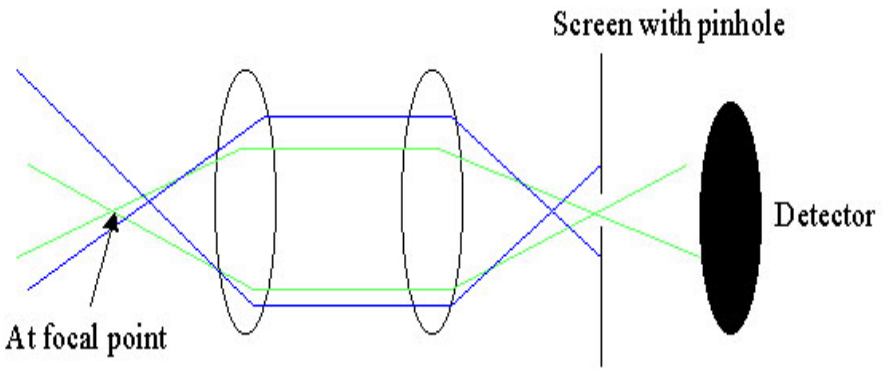


Figure 3

**Confocal Laser Scanning Microscope Schematic**

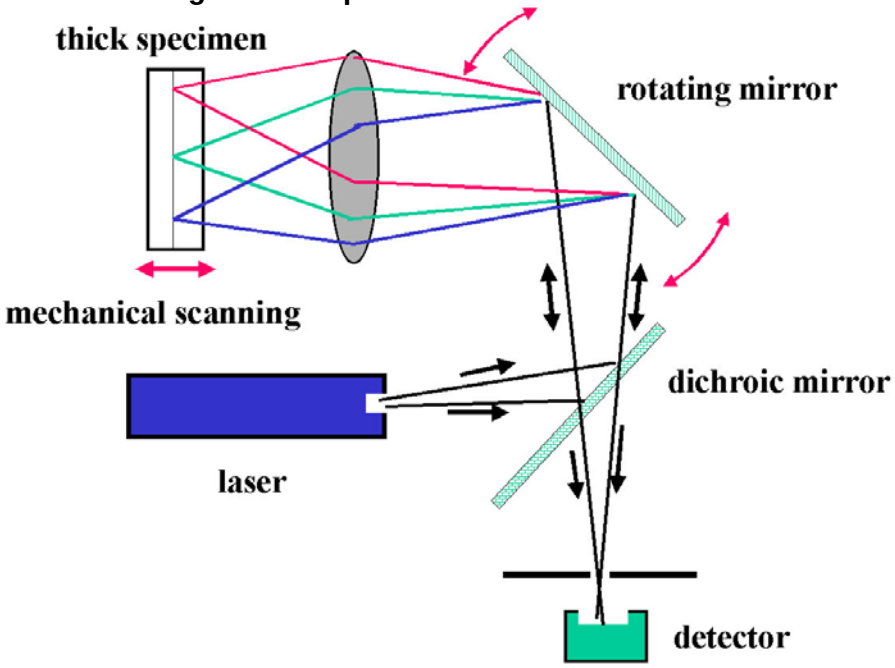


Figure 4