Laser Confocal Microscopy

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Technology

Laser Confocal microscopy uses laser light to control the depth of field, and a pin hole to eliminate out of focus light, thus allowing visualisation of an image in a single horizontal plane.

If you consider a normal microscopic image to be like looking through a transparent book, where you can see something of the images on all the top pages, then confocal microscopy allows you to visualize each page separately (see diagram). This allows exact location of cellular structures, and objects such as infecting bacteria. Taking multiple slices allows rendering of the image into three-dimensional display.

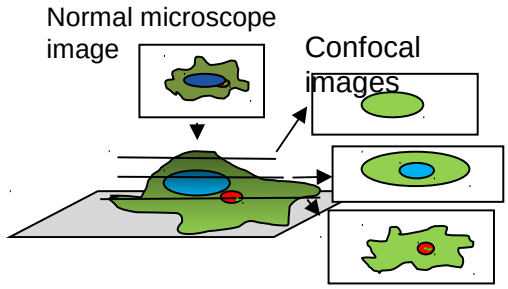
Images must be stained with **fluorescent dyes**, **fluorescently bound antibodies** or be expressing **natural fluorescence**. The dyes are excited by laser beam (at the appropriate wavelength), they then emit light at a lower wavelength which is detected.

Cells can be grown on microscope cover-slips or petri dishes with cover-slip bases, thus imaging can be done on cells *in situ* without disturbing fine cell structures, and cell-to-cell interactions can be studied.

Images

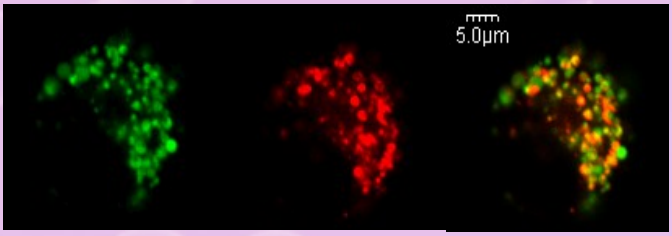
Careful setting of the laser intensity is required to ensure that only light from the relevant horizontal plane is viewed. When this is achieved, spatial correlation of a labeled target is possible and dual fluorescence proves co-localisation (**Image 1**).

Multiple images taken over a range of tissue depths allow 3D imaging of whole cells which can then be viewed as a series of images, as a movie or a 3D structure (**Image 2**). Images can be viewed from different perspectives (**Image 3**).



Pictorial showing the difference between a normal microscope view and confocal image

Image 1. An intracellular protein vaccination system (green) co-localised in acid vacuoles (red)



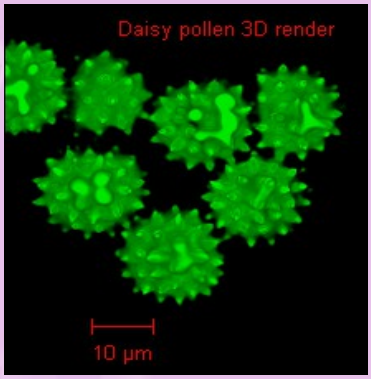


Image 2. naturally fluorescent pollen viewed in 3D

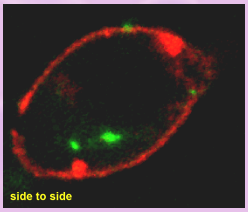


Image 3. Intracellular bacteria viewed from different perspectives