

Confocal microscopy

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Confocal Microscopy

Overview

Fluorescent microscopy uses fluorescent properties of an object to obtain a highly detailed, specific image of the desired structure with colours distinct to that of the light source.

Confocal Microscopy is an optical imaging technique developed in 1957. It uses fluorescence to increase resolution and contrast of an image. It involves the emission of light from a laser through a pinhole that is located on a confocal plane with a detector, where it also passes through a pinhole aperture. The focal point at the laser is the same as that of the detector, hence the name of the device. The light from the laser passes through a lens that focuses the rays onto a specific point, called the "scanning point". This is then reflected using a dichromatic mirror to a photomultiplier tube detector. As the light from the laser is simultaneously reflected and detected, fluorescence from the specimen is also reflected onto the photodetector.

A confocal microscope offers an enhanced, fully focused image of a small point on the desired object as opposed to a wide field fluorescence microscope, where the entire image is flooded with light and a largely unfocused. Confocal microscopy uses filters that remove glare and unfocused light and allows the user to control the depth of field and to also eliminate any background information. An important feature of confocal microscopy is that has the ability to produce 3D images from optical sections of thicker specimens, which is a reason as to why it has applications in a wide range of sciences.

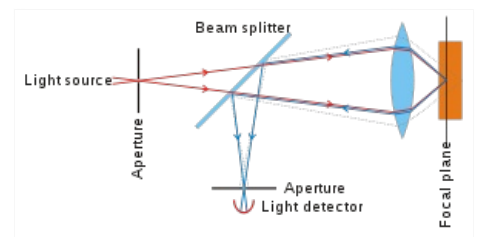


Figure 1 – The basic principle of confocal microscopy. Light source, specimen and detector are illustrated.

Relevance to Clinical Medicine

Confocal microscopy has the ability to image both fixed and living cells. An important clinical application of the confocal microscope is the imaging of the cornea and conjunctiva to study the corneal structure of the eye. Confocal microscopy is a non-invasive technique, thus is perfect for studying a patient's eye. It can detect a range of pathological conditions, such as infective keratitis ^[1]. However, the technique's major limitation when studying the eye is back-scattering of light, which has lead to specialised confocal microscopes being developed.

Types of Confocal Microscopes

There are 4 Main types of confocal microscopy:

- Confocal laser scanning microscopes
 - These use 2-3 mirrors to 'scan' the sample
- Spinning-disc confocal microscopy
 - These use moving pinholes on a spinning disc to illuminate specific points. As these devices use less energy than other types of confocal microscopes, the quality of image is better for imaging live tissues.
- Micro-lens enhanced/dual spinning disc confocal microscopy
 - Contain 2 spinning discs and micro-lenses behind the pinholes. This allows more light to be focused onto each focal point.
- Programmable array microscopes

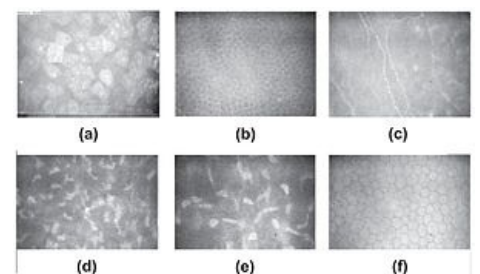


Figure 2: images from corneal layers taken from a confocal microscope [(a) - superficial epithelium (b) - Basal membrane (c) - Bowman's layer (d) - anterior stroma (e) - posterior stroma (f) - Endothelium.

- Uses a 'spatial light modulator', which contains moving pinholes and can be adjusted. A 'Charged Couple Device' then detects the resulting image.

Advantages and Limitations of Confocal Microscopy

Operating a confocal microscope is relatively easy, and images can be developed quickly. The technique produces high quality images of a specific area of a specimen. A limitation of confocal microscopy is that it can lose intensity due to light passing through small pinholes so long exposure is needed.

Despite only being invented in 1957, confocal microscopy has become an important technique across the life sciences and has been further developed into highly specialised techniques in the last 20-30 years. However, until recently many of these types (such as the spinning disc microscope) were not widely available for commercial use [2]. As technologies advance and the demand for non-invasive microscopic imaging of live cells in clinical practice increases, so has the production of confocal microscopes and its use around the world.

Links

References

1. Tavakoli M, Hossain P, Malik RA. Clinical applications of corneal confocal microscopy. (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2693976/>) Clinical ophthalmology (Auckland, NZ). 2008;2(2):435-445.
2. Cavanagh, H. D., J. V. Jester, and W. M. Petroll. Onlinelibrary.wiley.com. ([- 3. Memoir on Inventing the Confocal Scanning Microscope \(<http://web.media.mit.edu/~minsky/papers/ConfocalMemoir.html>\), Scanning 10 \(1988\), pp128-138.](http://onlinelibrary.wiley.com/store/10.1002/sca.4950160502/asset/4950160502ftp.pdf?v=1&t=ihpevkxm&s=424d52bf143e8af703c4c5bde58467e504f2213&systemMessage=Wiley+Online+Library+will+have+be+unavailable+on+Saturday+5th+December+from+10%3A00-14%3A00+GMT+%2F+05%3A00-09%3A00+EST+%2F+18%3A00-22%3A00+SGT+for+essential+maintenance.+Apologies+for+the+inconvenience.)

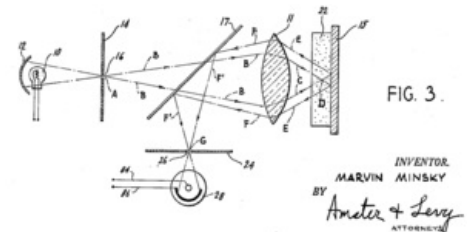


Figure 3: Minsky's original diagrams for his prototype confocal microscope [3]