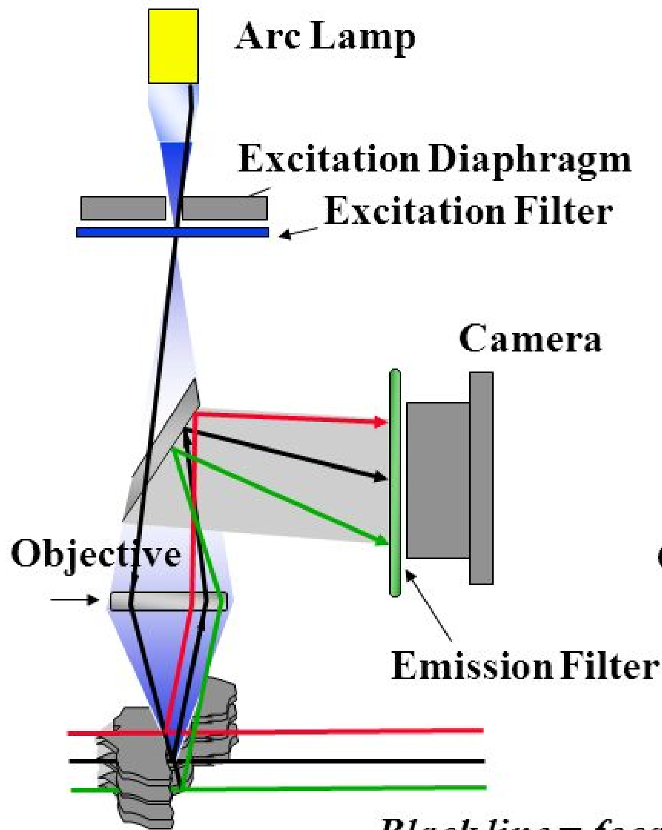
Confocal microscopy

Tanvi Gupte

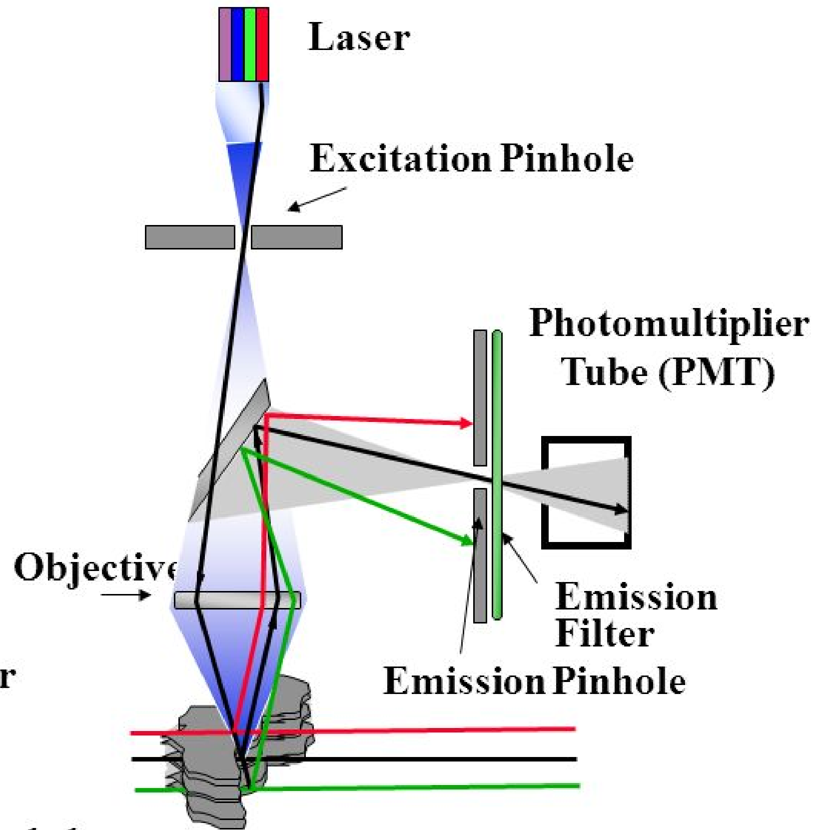
Introduction and history

- Confocal microscopy is an optical imaging technique for generating high-resolution images of specimen stained with fluorescent probes.
- Multiple two-dimensional images are captured at different depths in a sample which enables the 3-D reconstruction of a specimen. This process is known as **optical sectioning**.
- The principle of confocal imaging was patented in 1957 by Marvin Minsky, who made the first stage scanning confocal microscope.
- The key feature of confocal microscopy is its ability to produce in-focus images and eliminating out of focus light.
- Images have better contrast and are less hazy.

Wide-field fluorescent Microscope



Confocal Microscope



Black line = focal plane
Red line = above focal plane
Green line = below focal plane

Principle

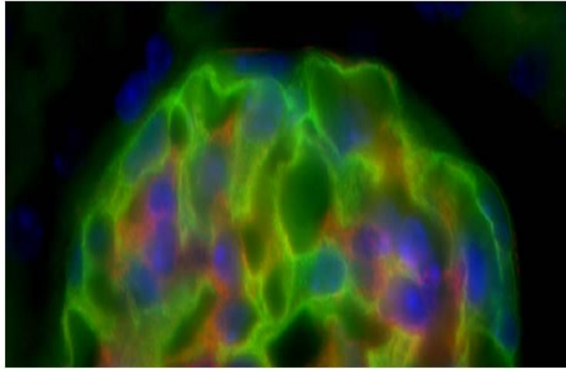
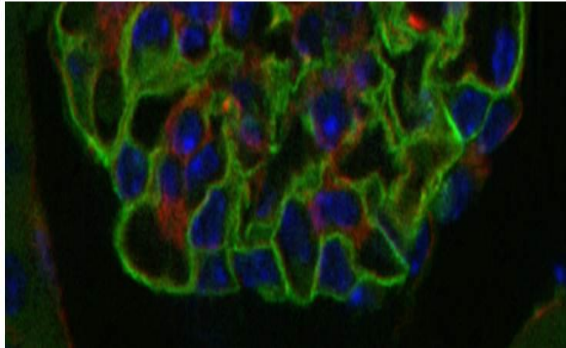
In confocal microscopy two pinholes are typically used:

- A pinhole is placed in front of the illumination source to allow transmission only through a small area. The focusing helps to limit the excitation of fluorophores above and below the plane of focus.
- Thus only a point on or in the specimen is illuminated at a time, termed as **point illumination**.

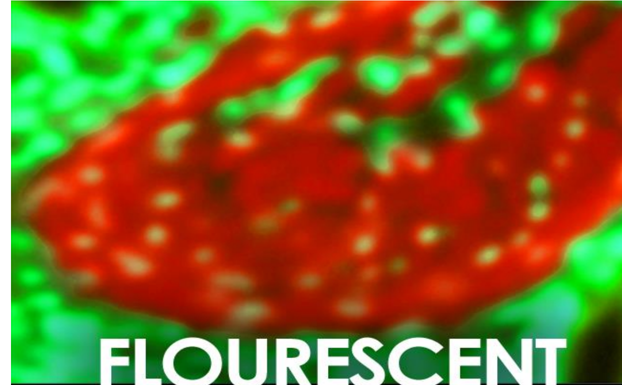
- Fluorescence generated at the focal plane is imaged onto a confocal pinhole placed in front of the detector.
- The specimen is scanned by moving the stage in the vertical & horizontal directions and optics are kept stationary.
- Scanning of successive small sections is done. A series of thin slices of the specimen are assembled to generate a 3- dimensional image.

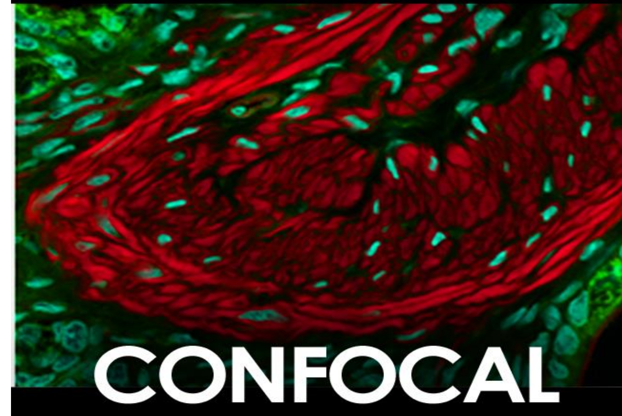
Advantages

- Better resolution and much sharper images by reducing the image-degrading effects of out-of-focus light.
- Serial optical sections can be collected without physical sectioning.
- Analysis of fluorescent labelled thick specimens.
- Ability to serially produce thin (0.5 to 1.5 micrometer) optical sections - fluorescent specimens.
- Thickness ranging up to 50 micrometers or more.
- Multidimensional analysis of living cells and tissues.
- Several combination of lasers can be coupled in order to increase the number of excitation wavelengths.
- Imaging of two or more fluorescent stains.

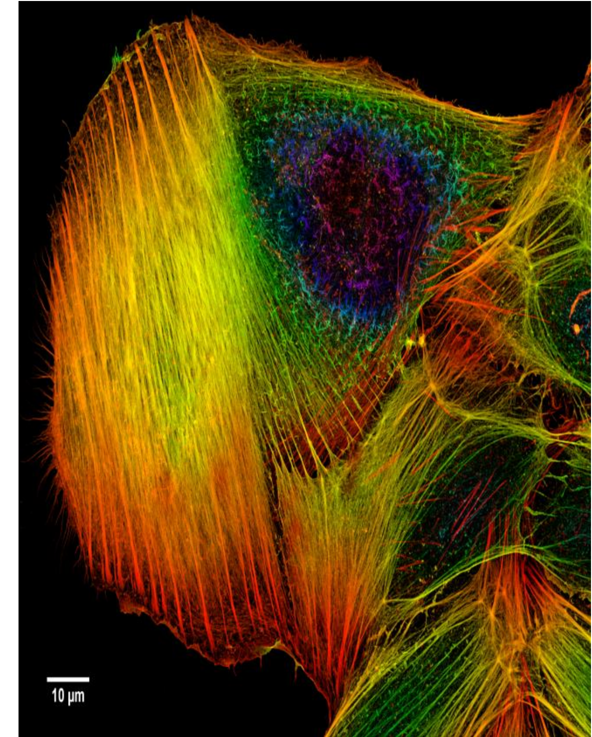



Kidney cells
(fluorescence vs Confocal
microscope





An Intestine Section



Colour coded image of actin
filaments in a cancer cell

Pawley JB (editor) (2006). *Handbook of Biological Confocal Microscopy* (3rd ed.). Berlin: Springer. ISBN 0-387-25921-X

LIMITATIONS

- **Pin hole size** : Strength of optical sectioning depends on the size of the pinhole.
- **Fluorophores :**
  - Fluorophores should be sensitive enough for the given excitation wave length.
  - They should have narrow spectral profiles of excitation and emission and whose peak values are distinctly separate both from each other.
  - It should not significantly alter the dynamics of the organism in the living specimen.
- **Photobleaching**
  - It involves the interaction of fluorophore & oxygen which destroys fluorescence and yields free radicals, that cause death of live cells and tissues.

Thank you!