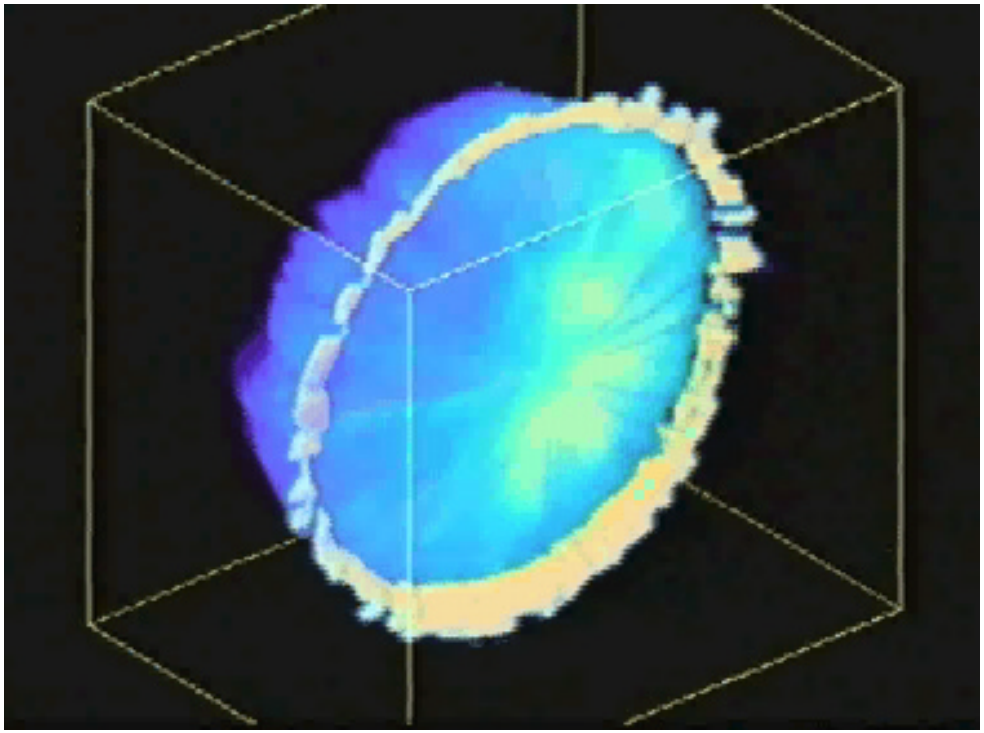
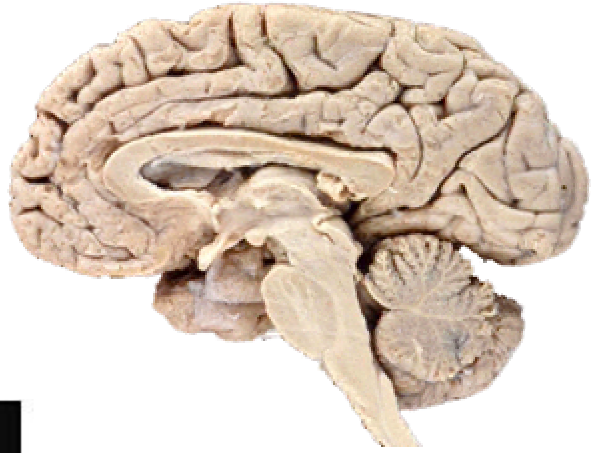
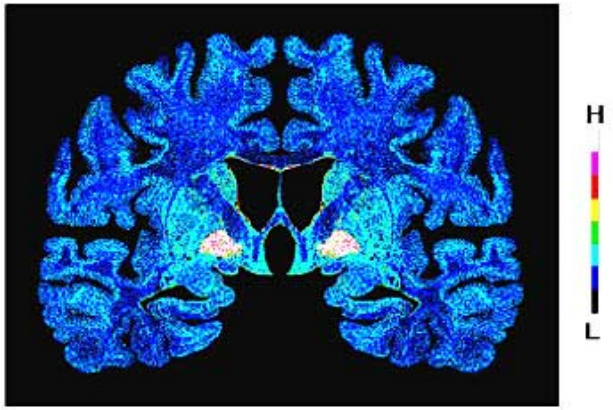
3D Microscopy: Confocal Imaging

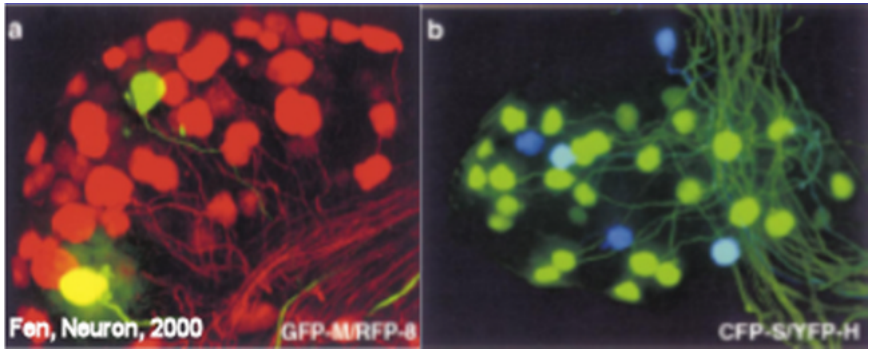


The Need For 3D Resolved Imaging

Biological systems are inherently 3D!

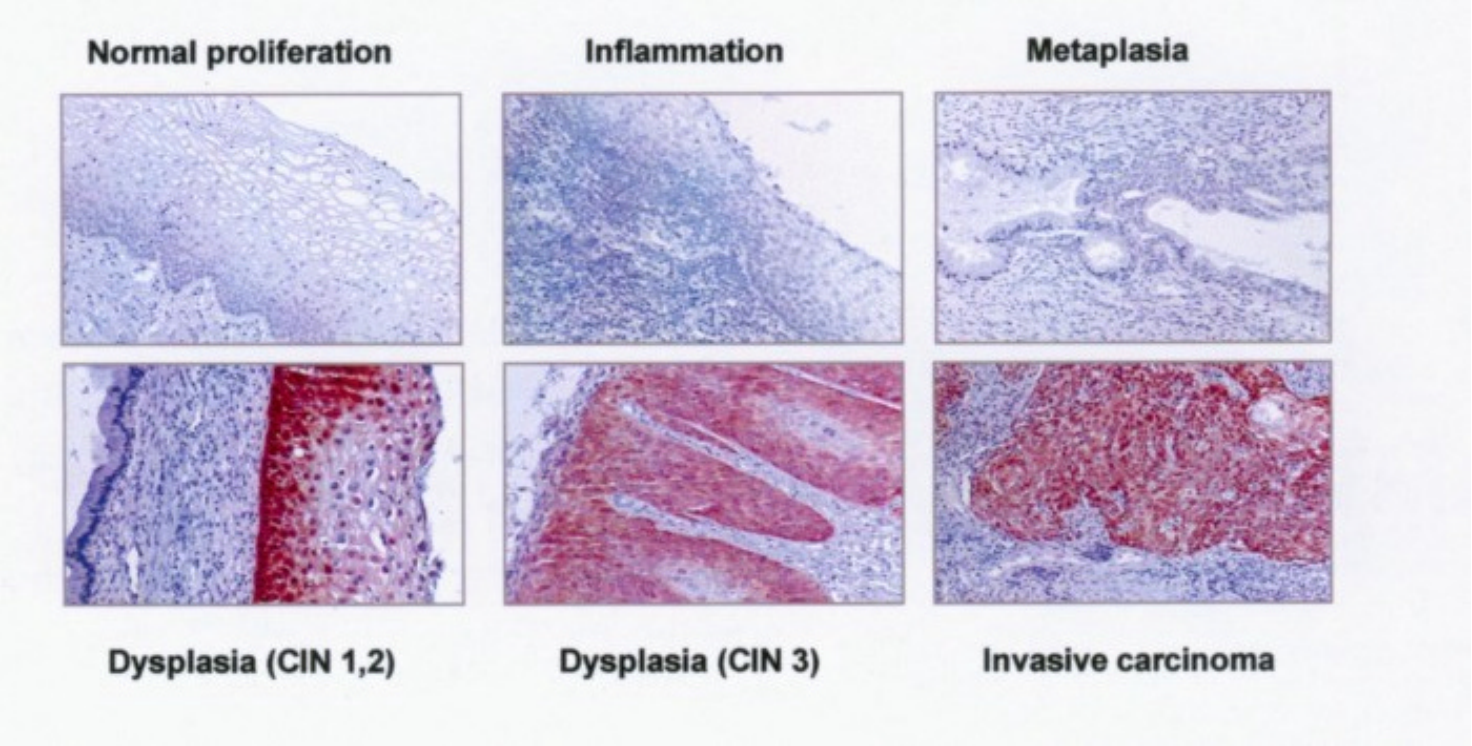






Biological processes also occur on multiple length scale

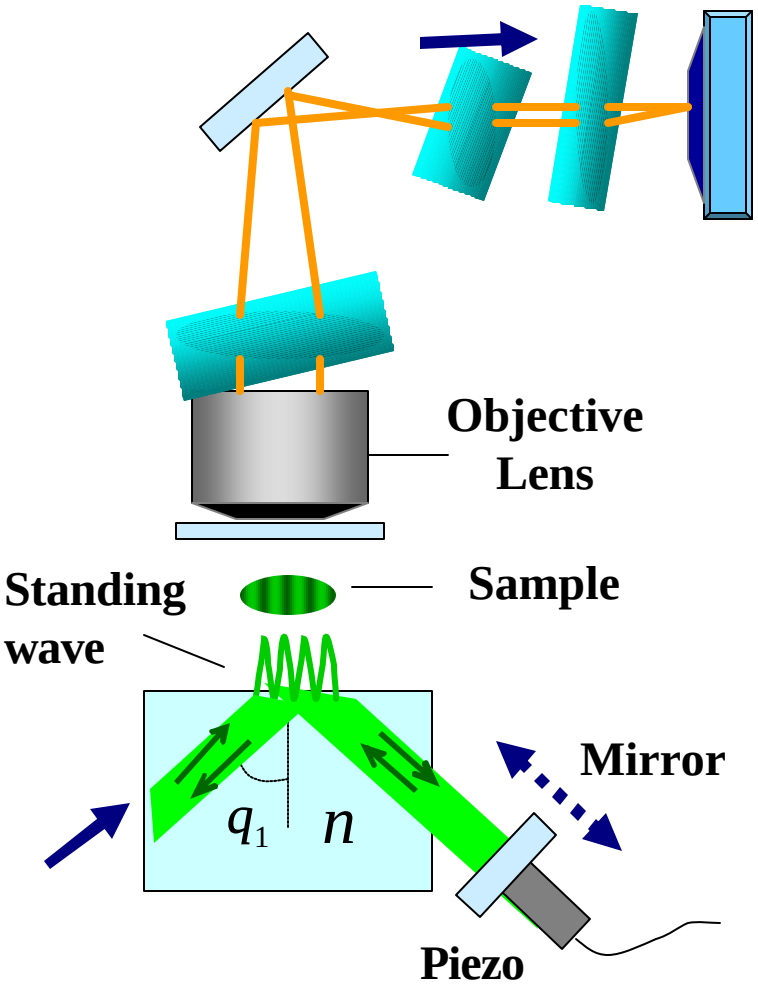
Histopathology



Solution: mechanical sectioning of specimen

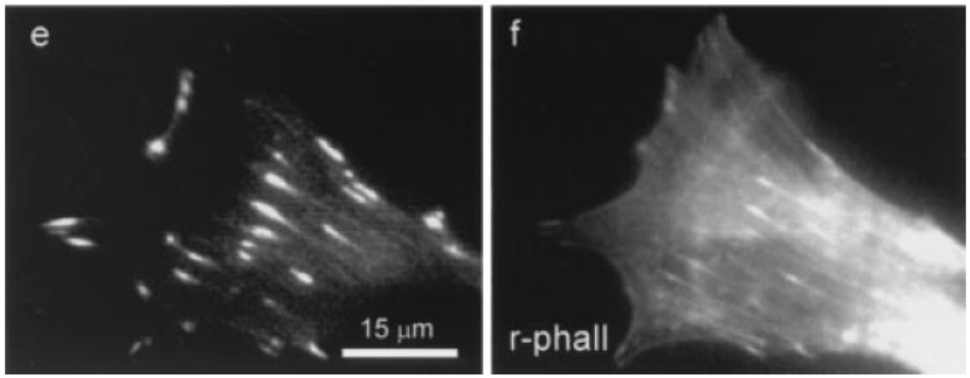
Comment: (1) Clinical standard (2) Simple technology
(3) Sectioning artifacts (4) Not in vivo

Total Internal Reflection Microscopy



Solution: Evanescence wave at interfaces

Comments: (1) only basal surface structure (2) high z resolution, 50 nm



TIRF Wide Field

Sund & Alexrod, Biophys J 2000

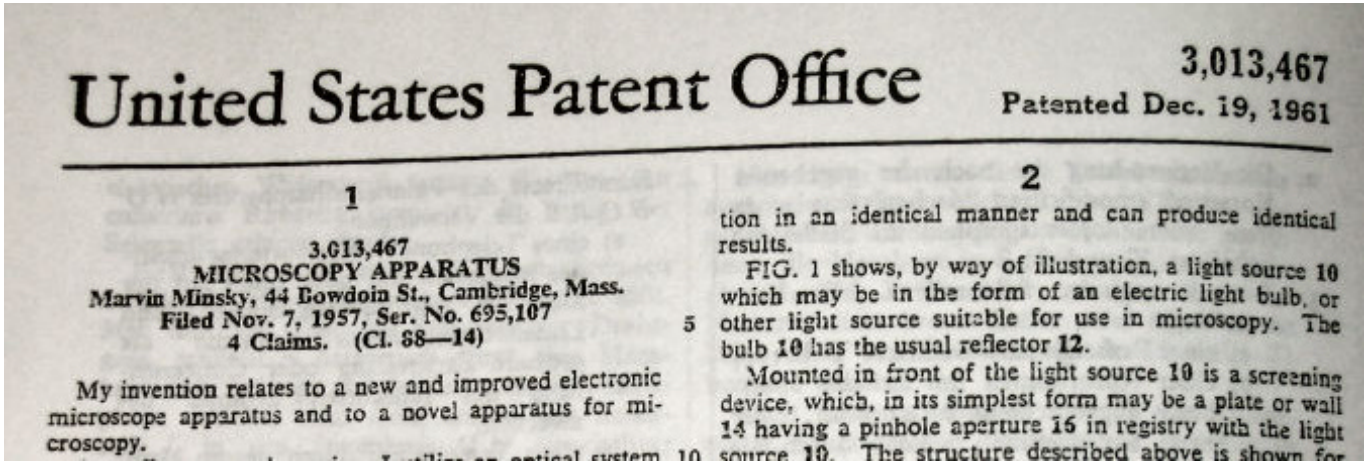
True 3D Microscopy

Confocal Microscopy: Minsky, US Patent, 1961

Two-Photon Microscopy: Sheppard et al., IEEE J of QE, 1977
Denk et al., Science, 1990

The Invention of Confocal Microscopy

Confocal microscopy is invented by Prof. Melvin Minsky of MIT in about 1950s.



United States Patent Office

3,013,467
Patented Dec. 19, 1961

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3,013,467
MICROSCOPY APPARATUS
Marvin Minsky, 44 Bowdoin St., Cambridge, Mass.
Filed Nov. 7, 1957, Ser. No. 695,107
4 Claims. (Cl. 88—14)

My invention relates to a new and improved electronic microscope apparatus and to a novel apparatus for microscopy.

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tion in an identical manner and can produce identical results.

FIG. 1 shows, by way of illustration, a light source 10 which may be in the form of an electric light bulb, or other light source suitable for use in microscopy. The bulb 10 has the usual reflector 12.

Mounted in front of the light source 10 is a screening device, which, in its simplest form may be a plate or wall 14 having a pinhole aperture 16 in registry with the light source 10. The structure described above is shown for

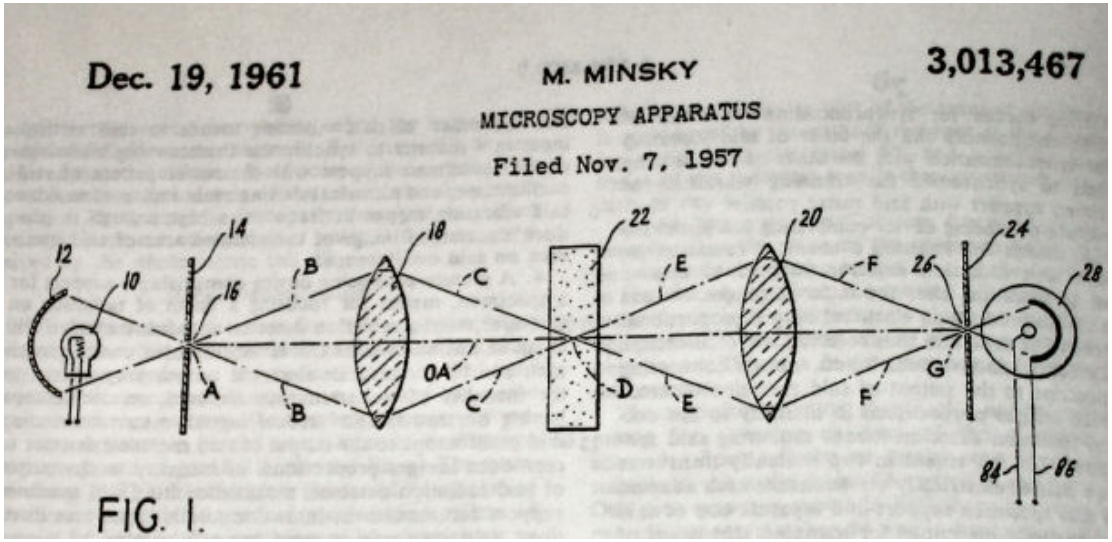
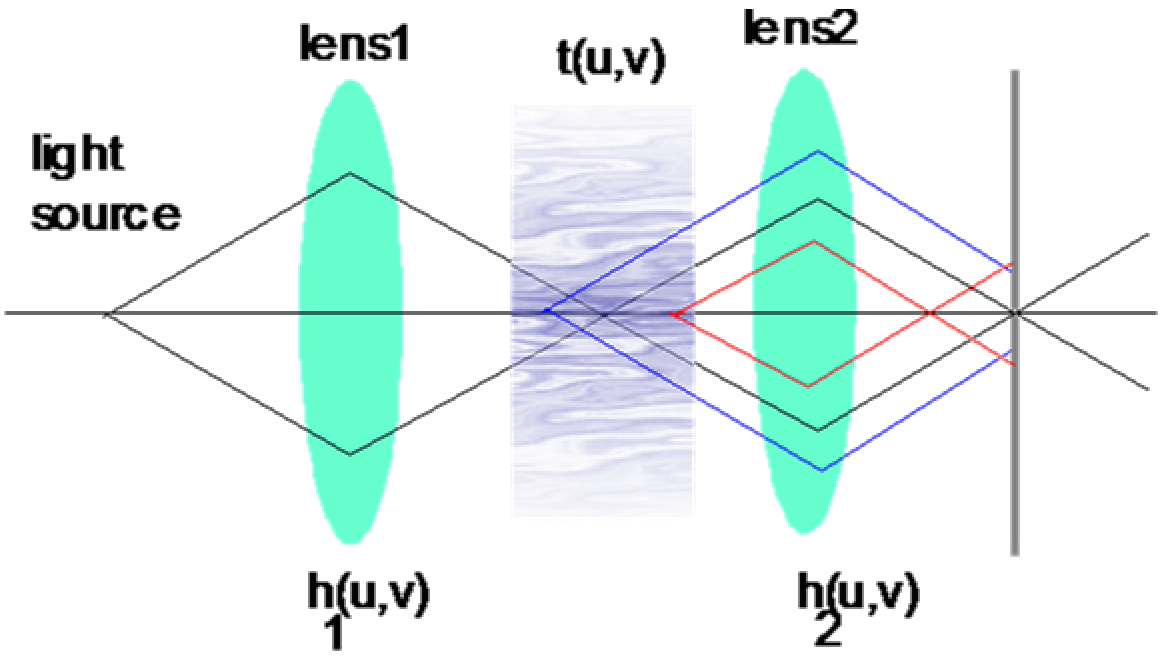


FIG. 1.

Principle of Confocal Microscopy



Point Spread Function of Confocal Microscopy

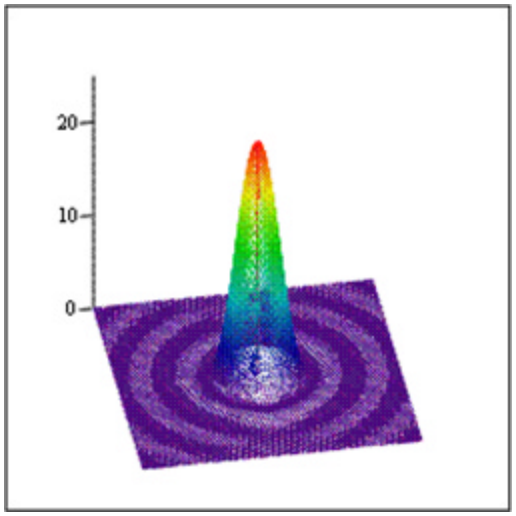
Lateral Dimension: Airy function

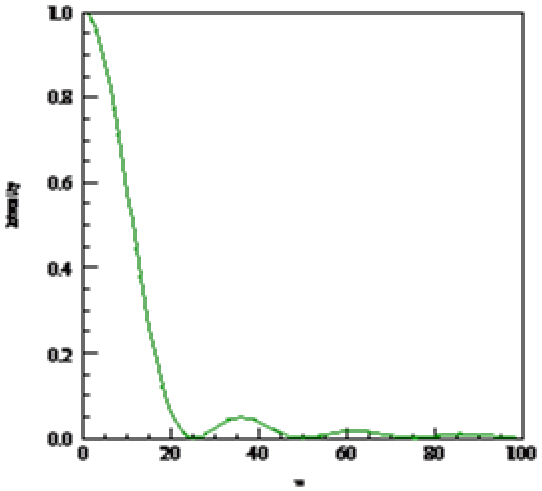
$$PSF_{confocal}(kr) \propto [\frac{2J_1(kr)}{kr}]^4$$

k is the wave number

Axial Dimension : Sinz function

$$PSF_{confocal}(kz) \propto [\frac{\sin(kz)}{(kz)}]^4$$





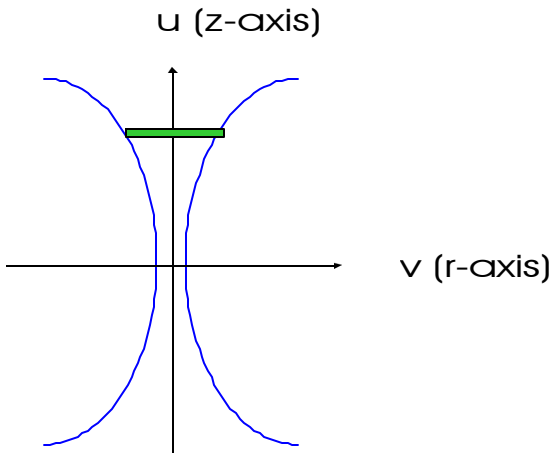
Resolution:

Lateral $\propto NA$

Axial $\propto NA^2$

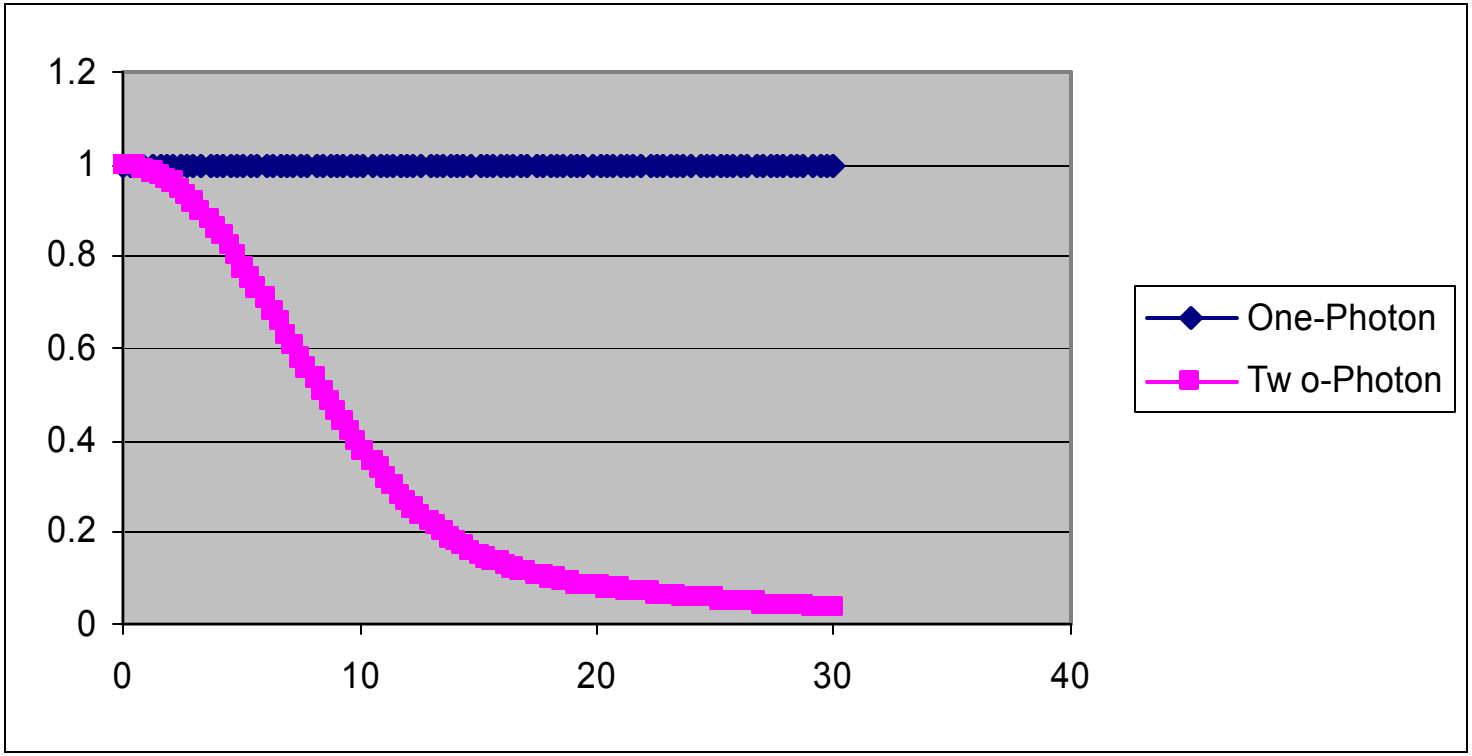
Depth discrimination

For a uniform specimen, we can ask how much fluorescence is generated at each z-section above and below the focal plane
assuming that negligible amount of light is absorbed throughout.

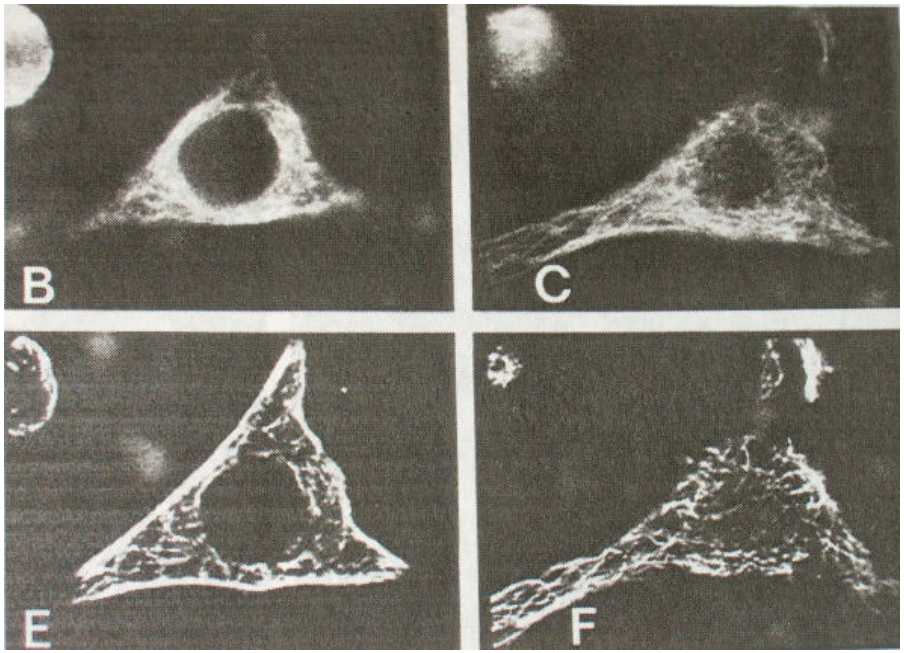


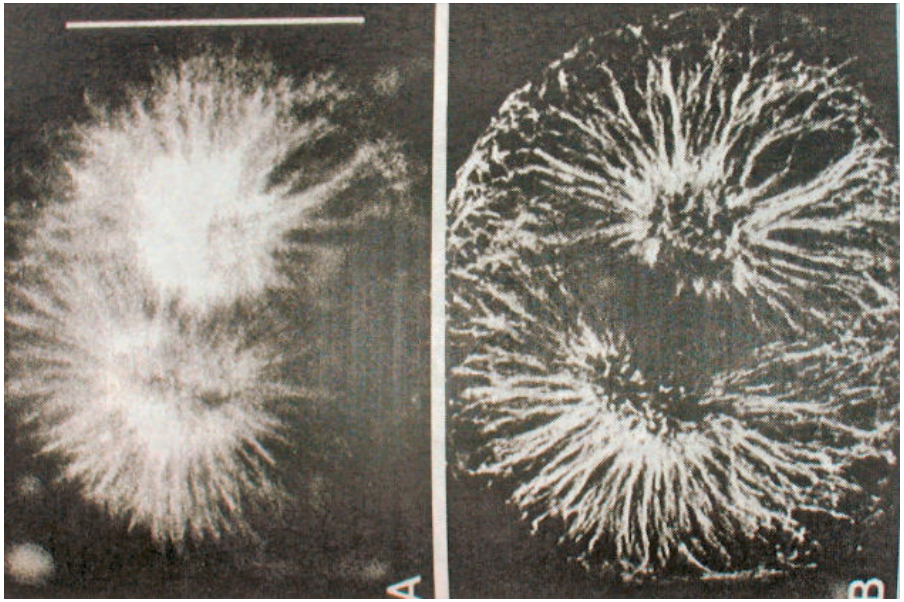
$$F_{z-\sec}(u) \equiv 2p \int_0^\infty F(u,v) v dv$$

Depth discrimination



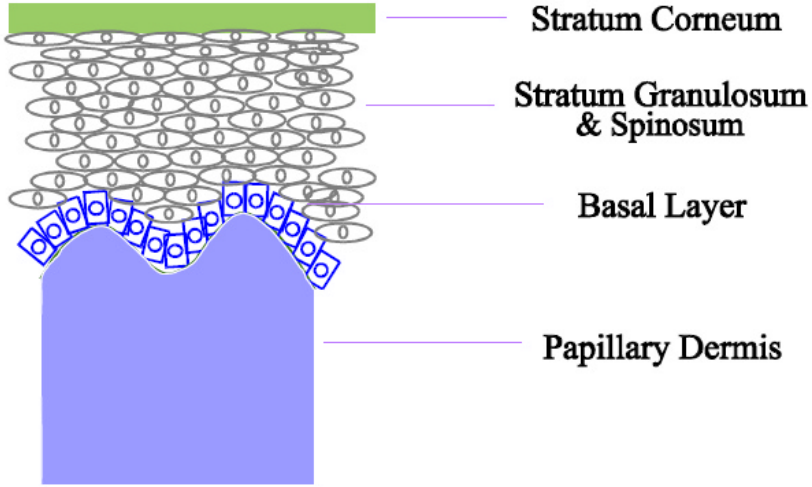
Early Demonstration of Confocal Microscopy in Biological Imaging

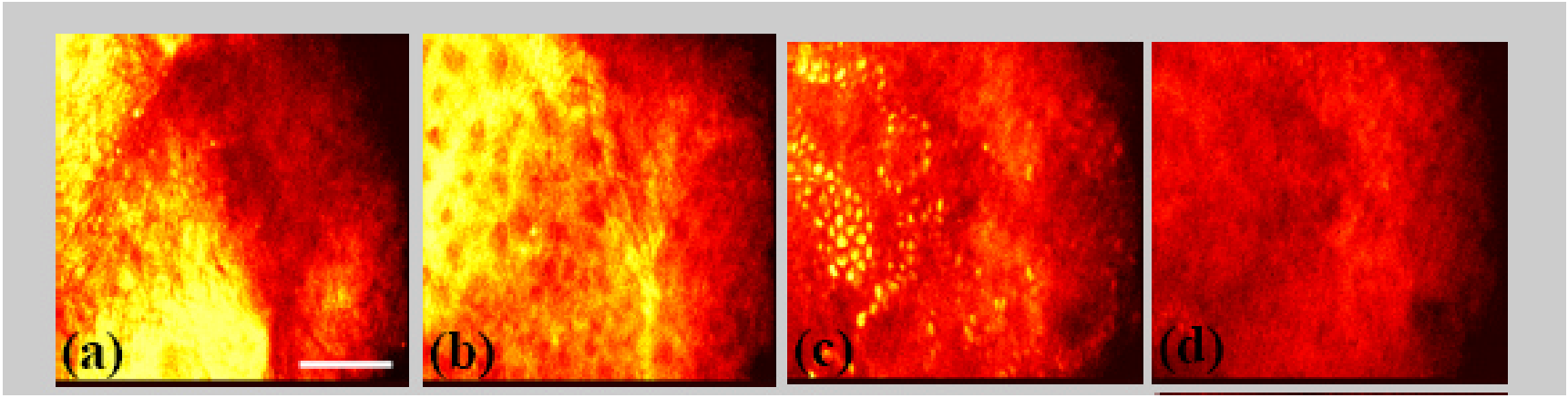




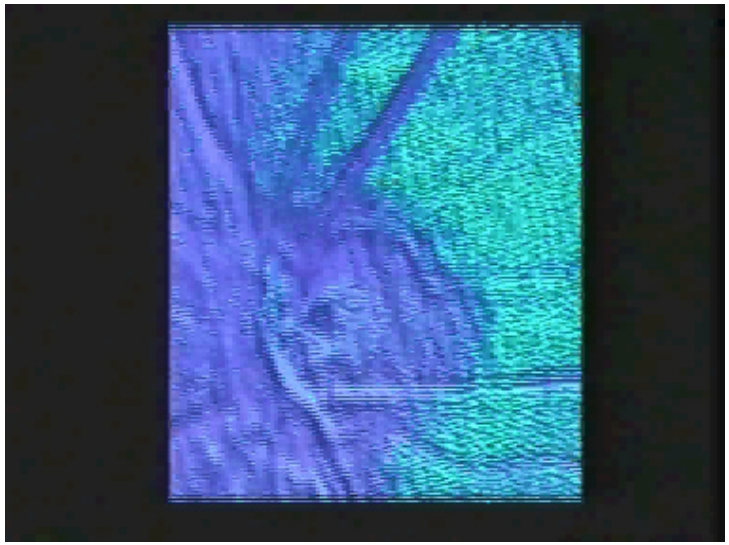
White et al., JCB 1987

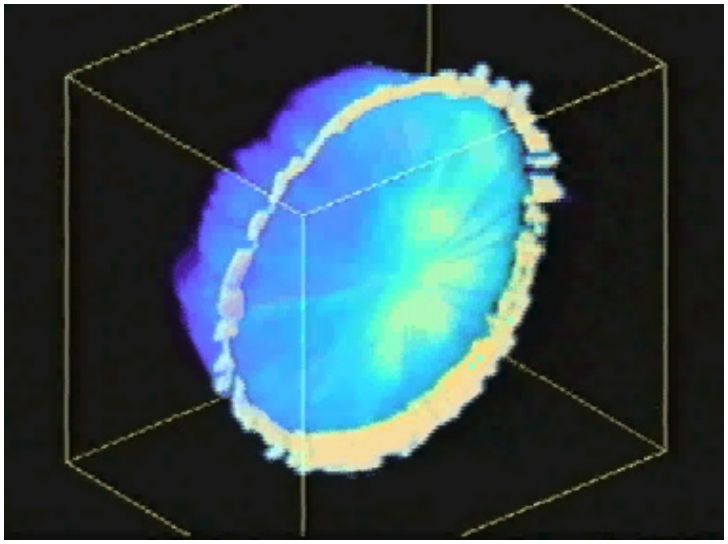
Some Recent Application of Confocal Tissue Imaging

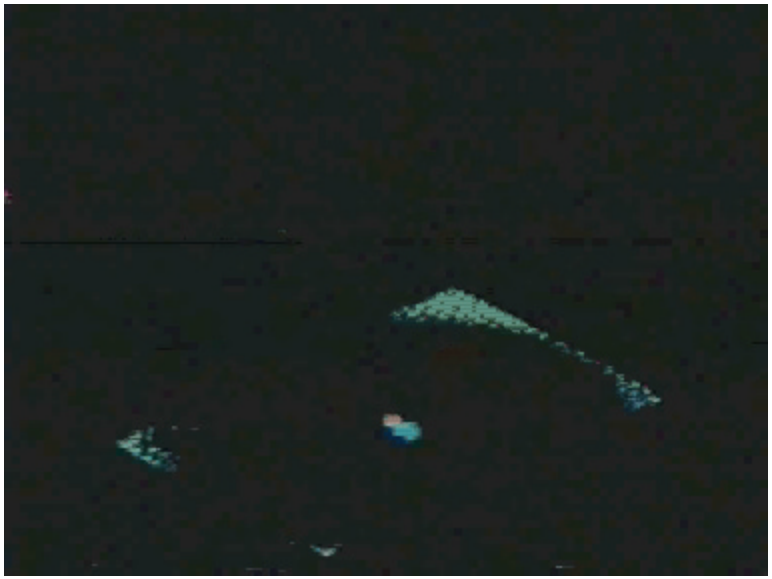




Confocal Tissue Imaging



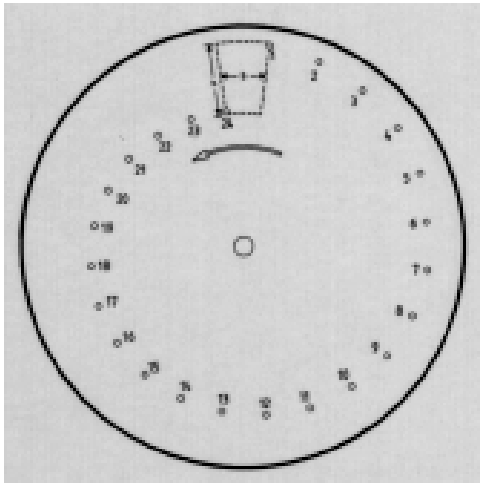




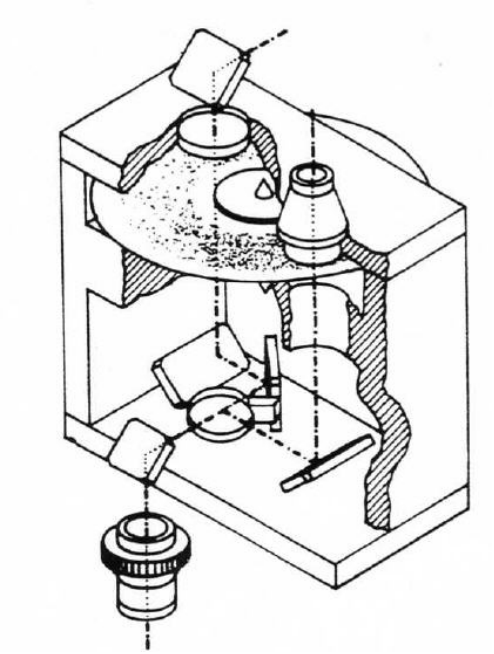
Masters, Opt Lett, 1999

Tandem Scanning Confocal Microscope

Utilizes a Nipkow Disk

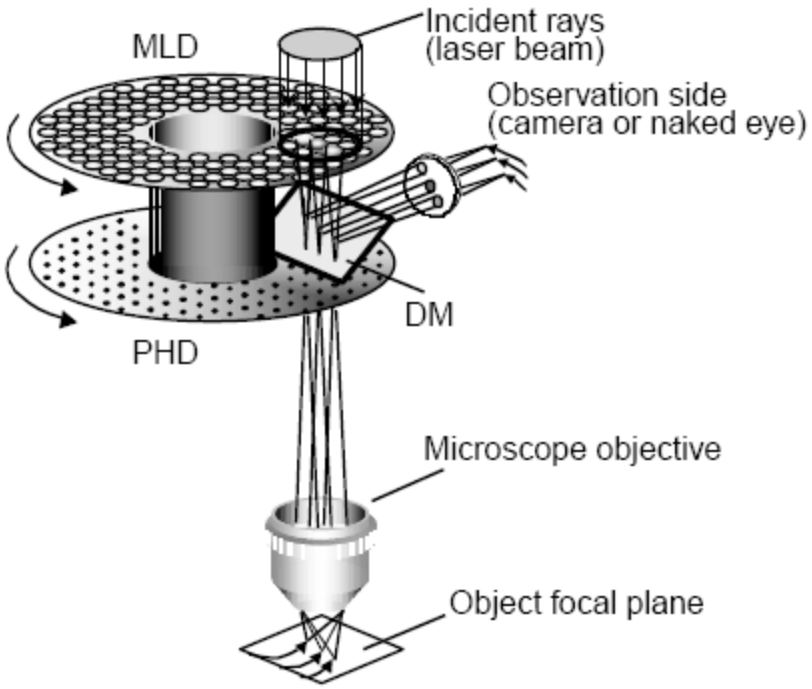


Holes organize in an Archimedes spiral

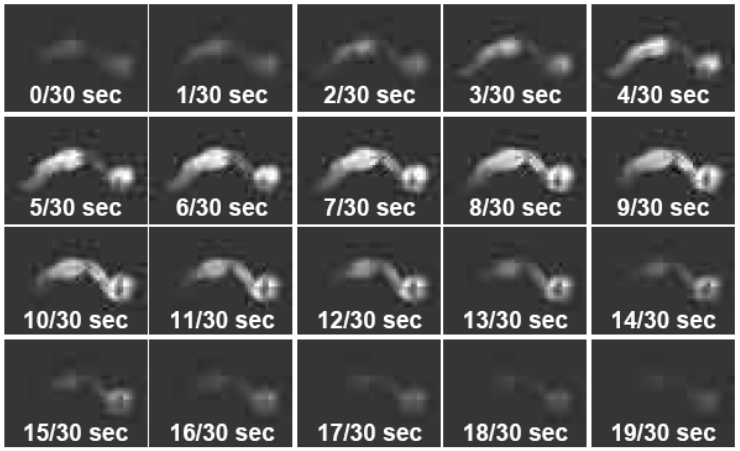


Petran's System

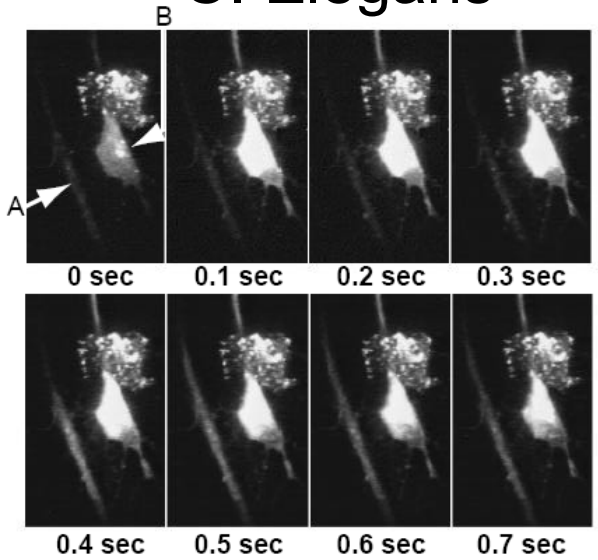
A Model Tandem Confocal Microscope Utilizing Yokogawa Scan Head



Eliminate light throughput Issue by spinning both a plate of lenslets and nother plate of pinholes



C. Elegans



Calcium events in nerve fiber