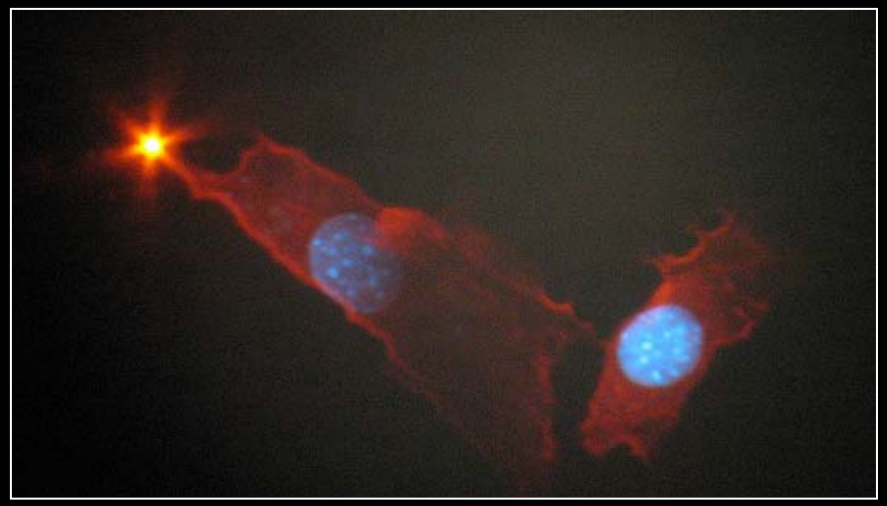
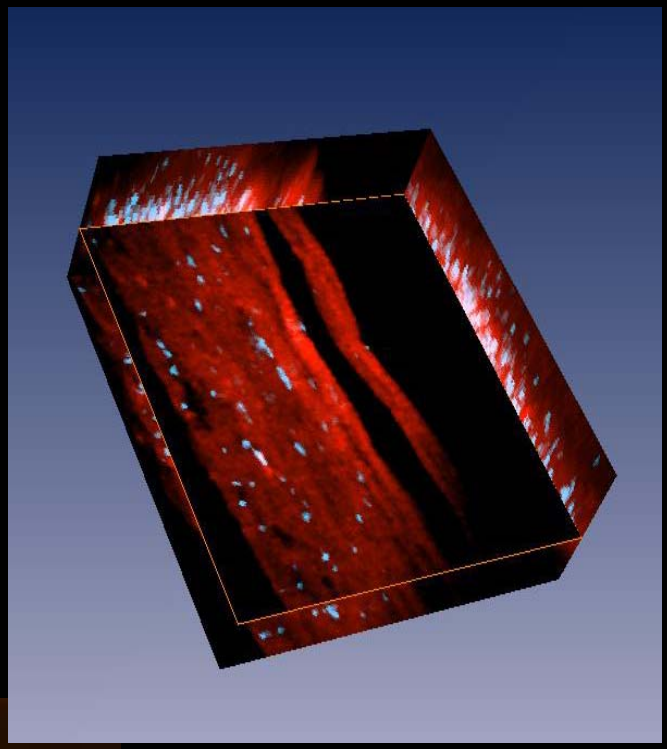
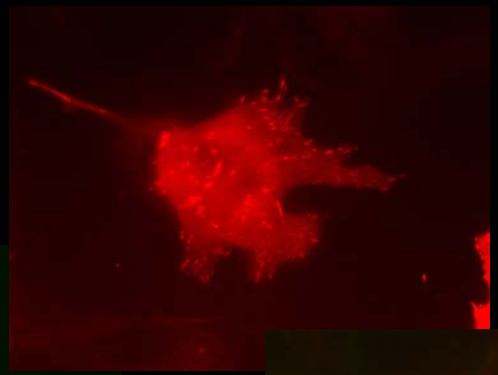
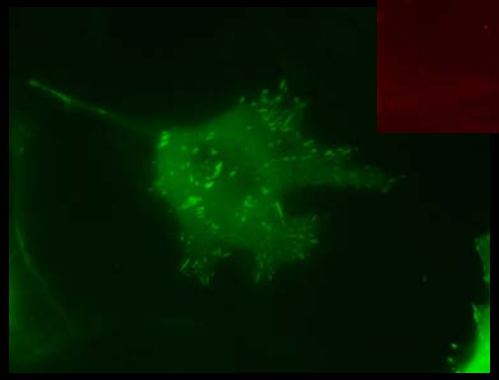
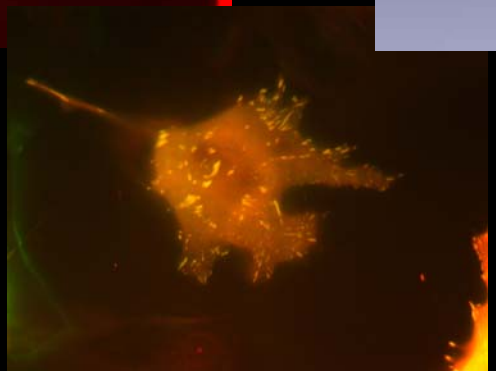
3D Microscopy: Deconvolution, Confocal, Multiphoton



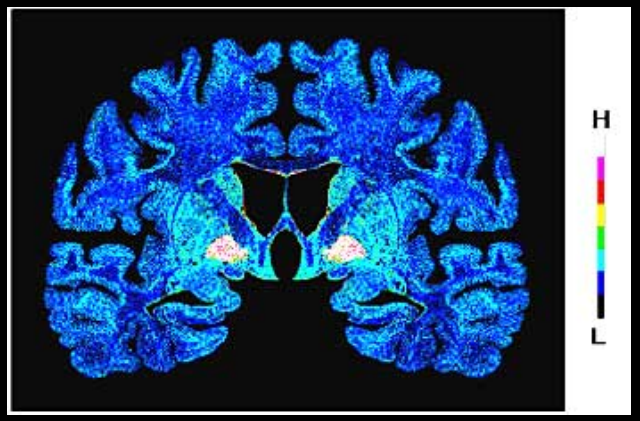


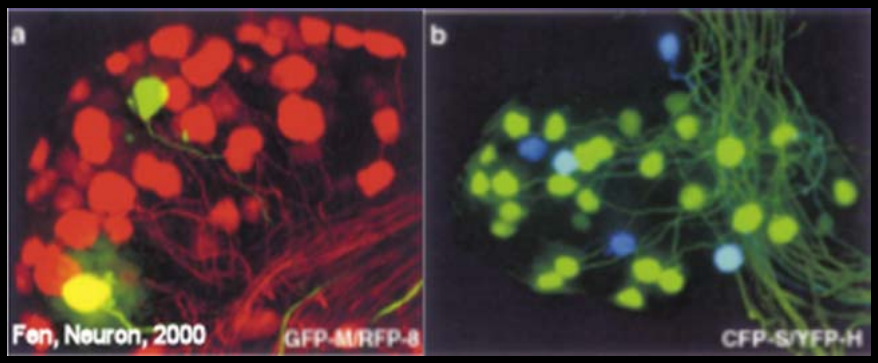






Biological systems are inherently 3D!





Biological processes also occur on multiple length scale

3D Microscopy

Deconvolution:

Hiraoka, Science, 1987

McNally, Methods, 1999

Confocal Microscopy:

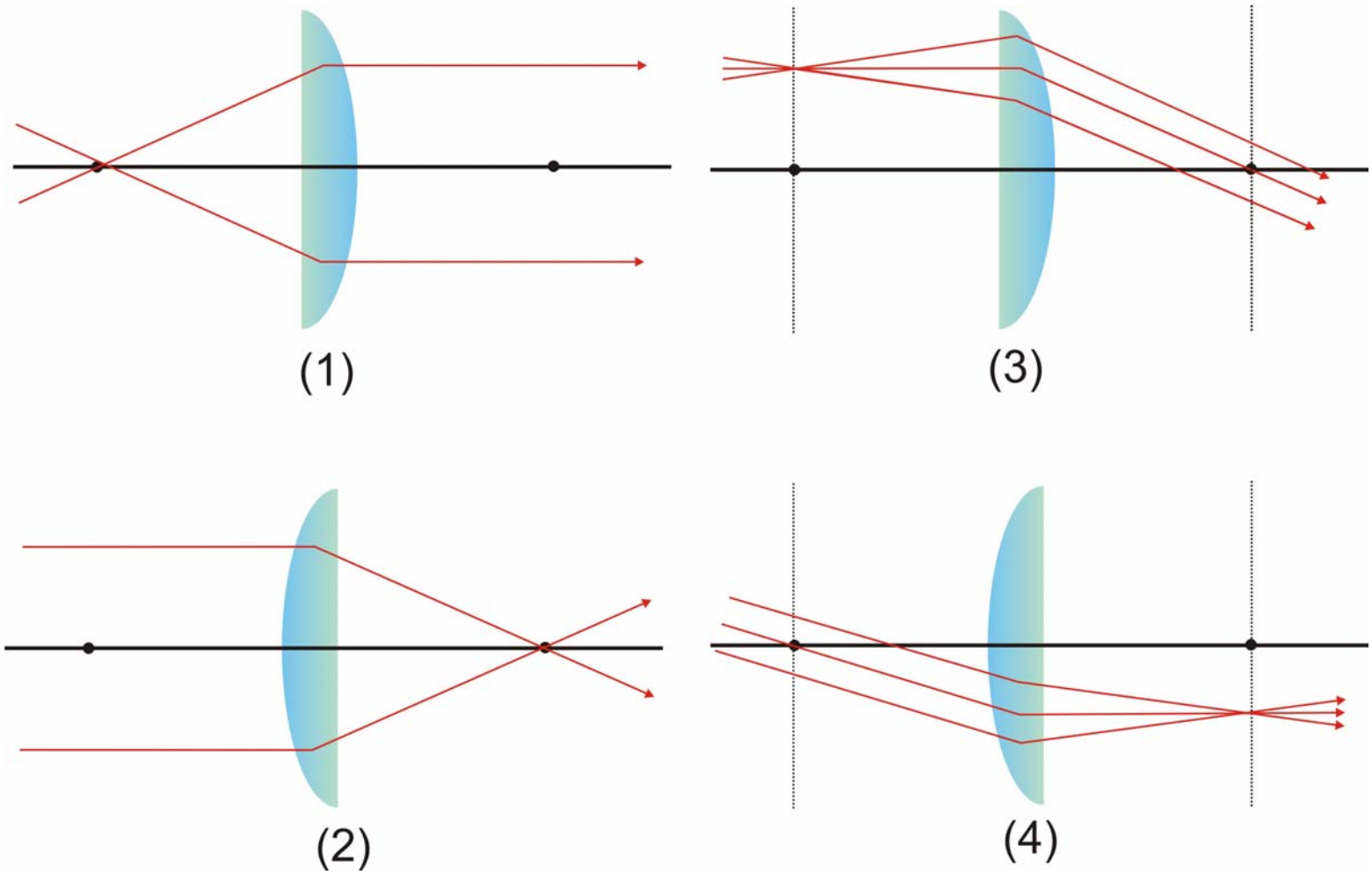
Minsky, US Patent, 1961

Two-Photon Microscopy:

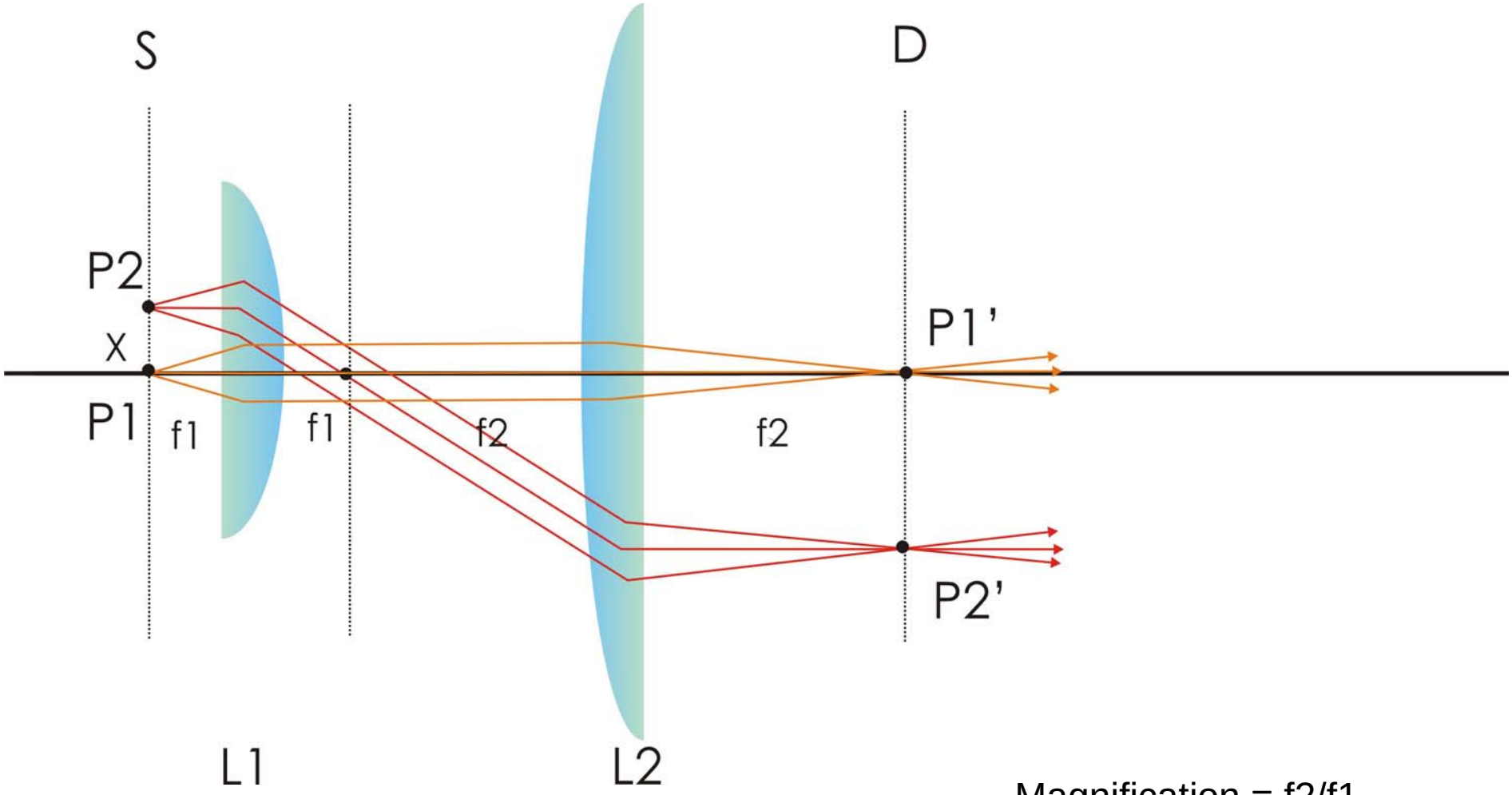
Sheppard et al., IEEE J of QE, 1978

Denk et al., Science, 1990

Understanding Optics: 4 simple rules of tracing light rays



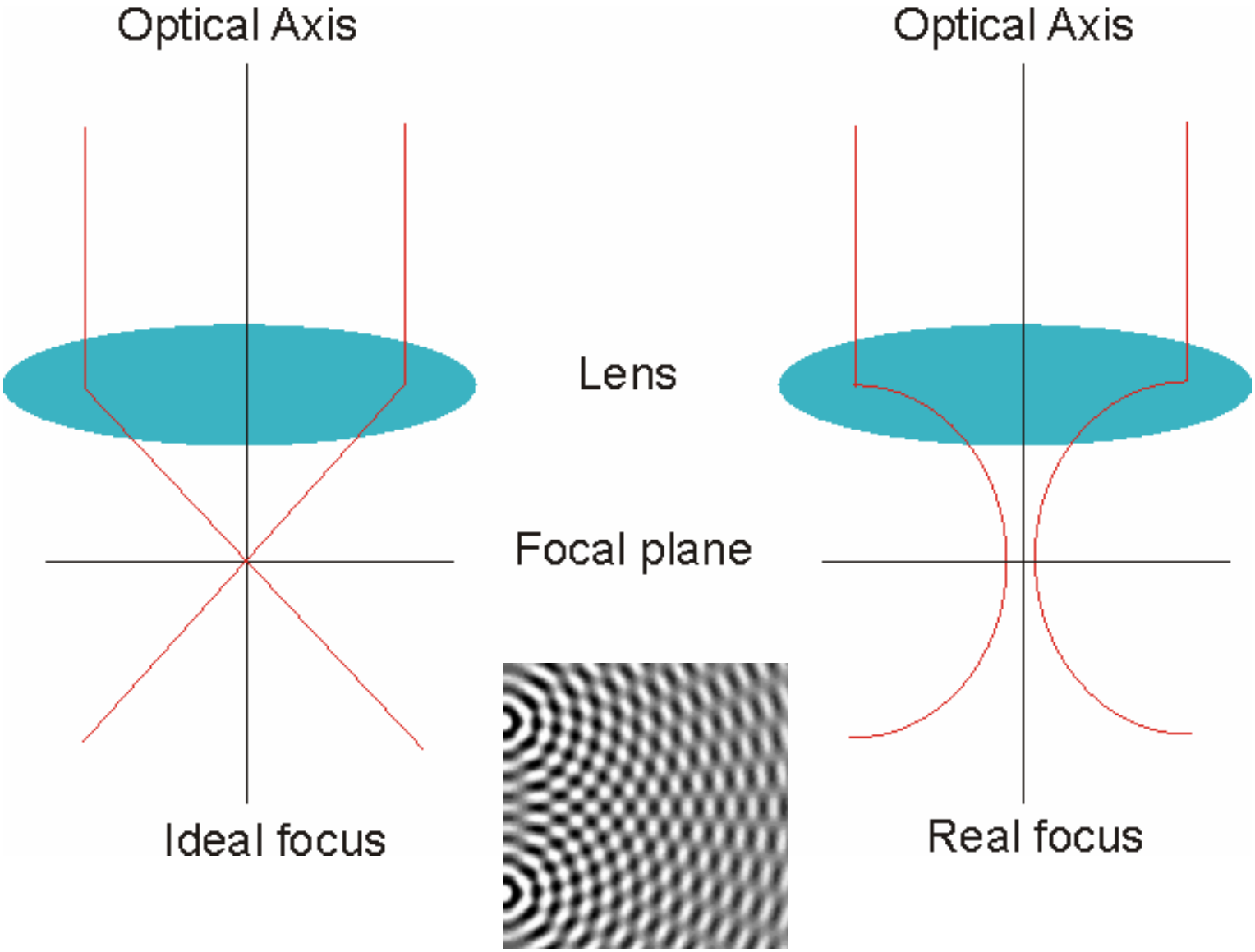
What is a microscope?



Magnification = f2/f1

This is a wide field microscopy

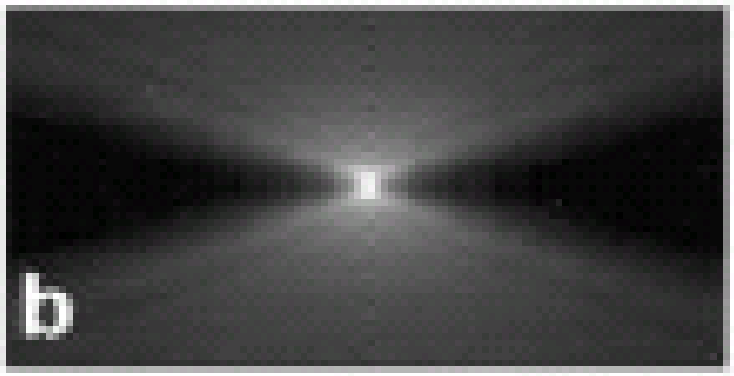
How light focus by a microscopy objective?



Interference & Diffraction Effects are Important at the Focus

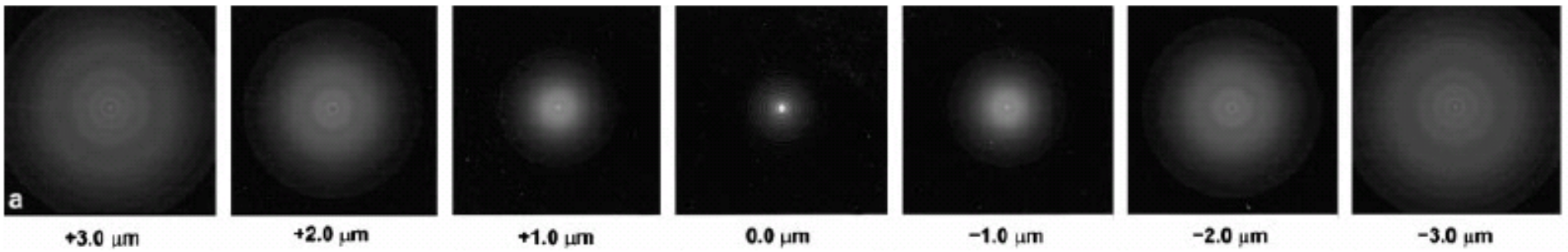
Experimentally Measuring the Light Distribution at Focus

What we observe?



(1)Radial resolution
--the lateral dimension is NOT infinitely small

(2) Axial resolution
--light is generated above & below the focal plane



McNally, Methods, 1999

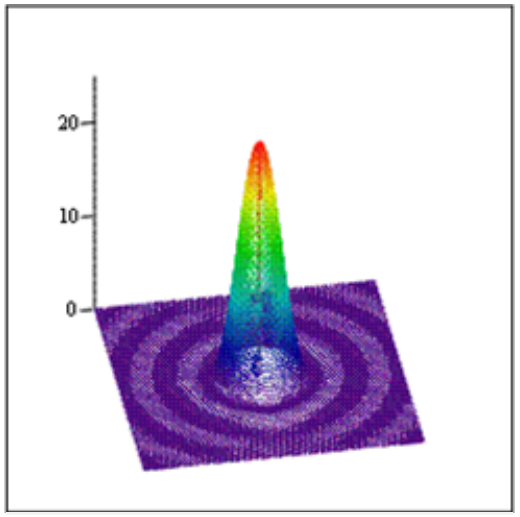
Point Spread Function – Image of an Ideal Point

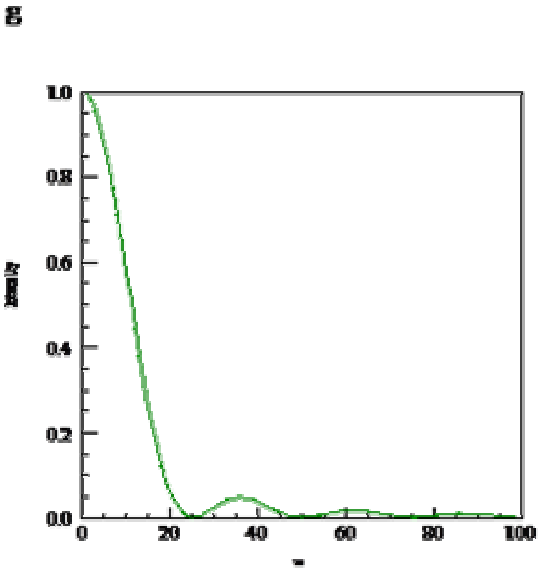
Lateral Dimension: Airy function

$$PSF(kr) \propto [\frac{2J_1(kr)}{kr}]^2$$

$k = \frac{2\pi}{\lambda}$ is the wave number

$$\text{FWHM} \approx \frac{\lambda}{2}$$ Resolution



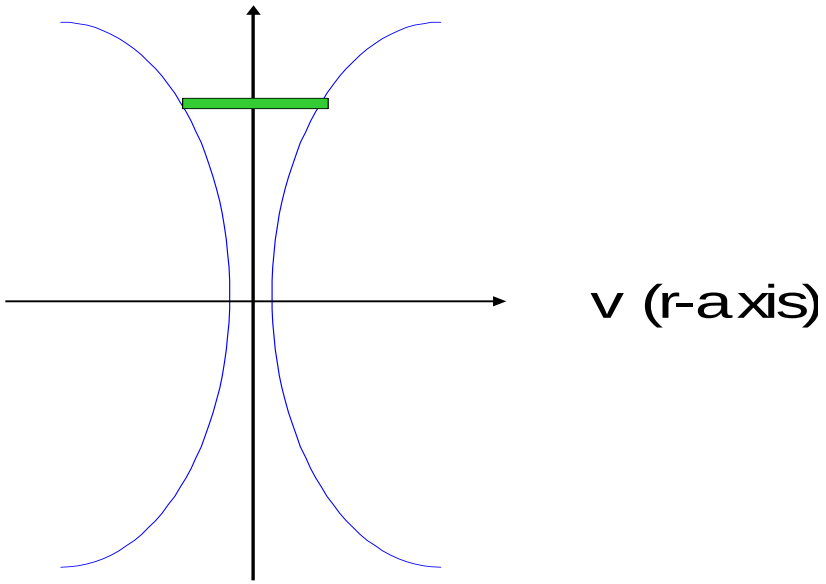


Axial Dimension : Sinc function

$$PSF(kz) \propto [\frac{\sin(kz)}{(kz)}]^2$$

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.



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

Ans: Photon number at each z-section is the same (little absorption) →

The amount of light generated at each z-section is the same!

$$F_{z-\sec}(u) = \text{ Constant}$$

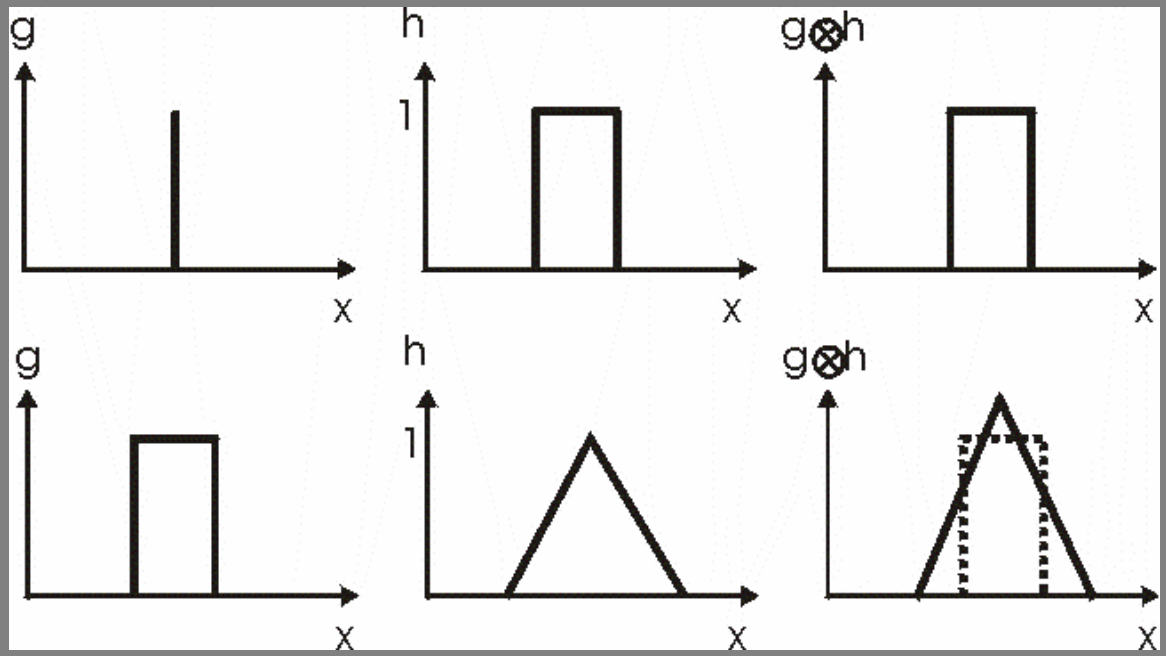
There is no depth discrimination!!!

What is Convolution?

Recall the definition of convolution:

$$g(t) \otimes h(t) = \int_{-\infty}^{\infty} g(\tau) h(t-\tau) d\tau$$

Graphical explanation of convolution:

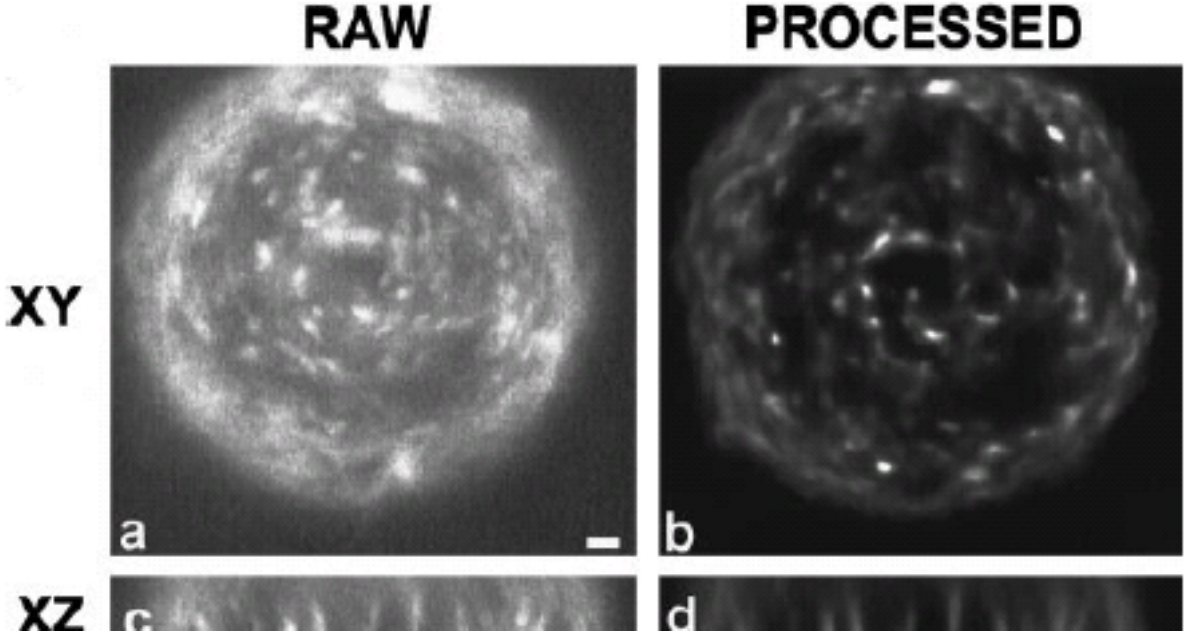


Convolution is a smearing operation

What is the effect of finite side PSF on imaging?

$$I(\vec{r}) = O(\vec{r}) \otimes PSF(\vec{r})$$

The finite size point spread function implies that images are “blurred” in 3D!!!



McNally, Methods, 1999

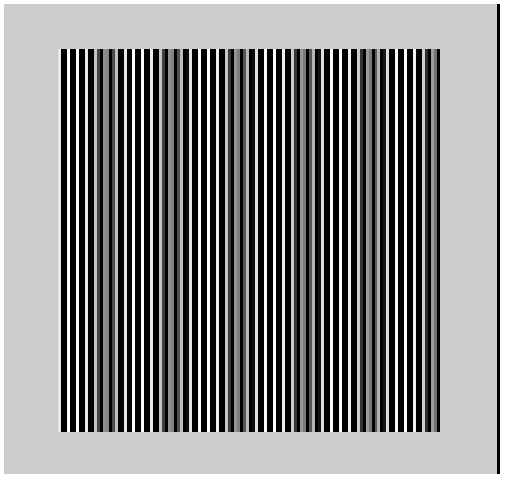
A View of Resolution and Depth Discrimination
In terms of Spatial Frequency

2D Fourier Transform

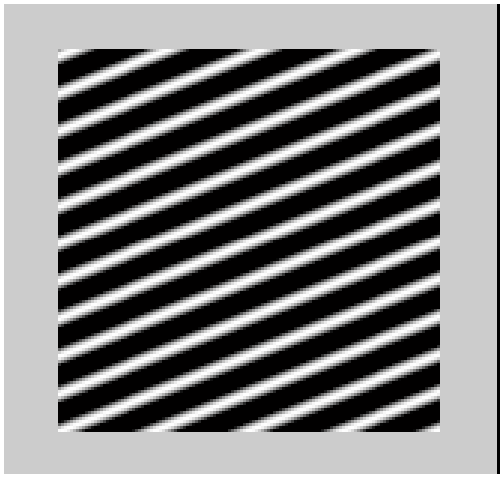
$$\tilde{I}(\vec{k}) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} I(x,y,z)\exp[-2\pi i(k_x x + k_y y + k_z z)]dxdydz$$

Power Spectrum $\tilde{P}(\vec{k}) = \left|\tilde{I}(\vec{k})\right|^2$

Two dimensional examples



High frequency



Low frequency

Convolution Theorem

$$\Im(g \otimes h)(f) = \tilde{g}(f)\tilde{h}(f)$$

Proof in 1-D

$$\int_{-\infty}^{\infty} g \otimes h(t) e^{-i2\pi ft} dt = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} g(\tau)h(t-\tau)d\tau e^{-i2\pi ft} dt$$

$$= \int_{-\infty}^{\infty} d\tau g(\tau) e^{-i2\pi f\tau} \left(\int_{-\infty}^{\infty} dt h(t-\tau) e^{-i2\pi f(t-\tau)} \right)$$

$$= \int_{-\infty}^{\infty} d\tau g(\tau) e^{-i2\pi f\tau} \left(\int_{-\infty}^{\infty} dt' h(t') e^{-i2\pi f(t')} \right)$$

$$= \tilde{g}(f)\tilde{h}(f)$$

where $t' = t - \tau \quad dt' = dt$

Fourier transform of the convolution of two functions is the product of the Fourier transforms of two functions

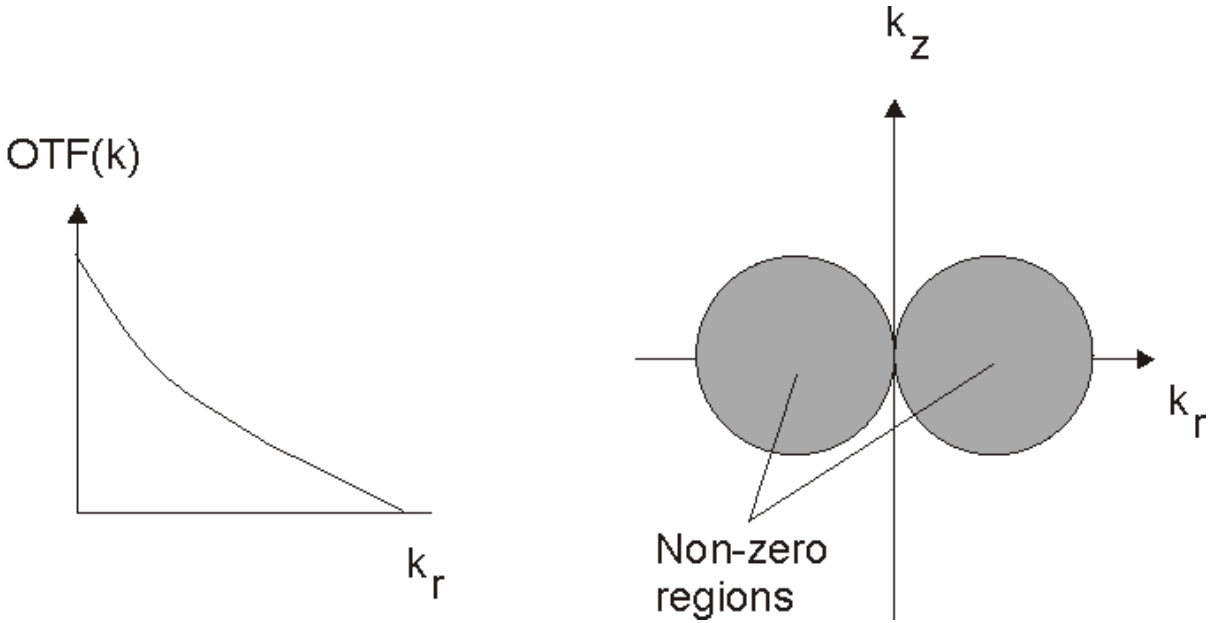
Resolution and Discrimination in Frequency Domain

$$I(\vec{r}) = O(\vec{r}) \otimes PSF(\vec{r})$$

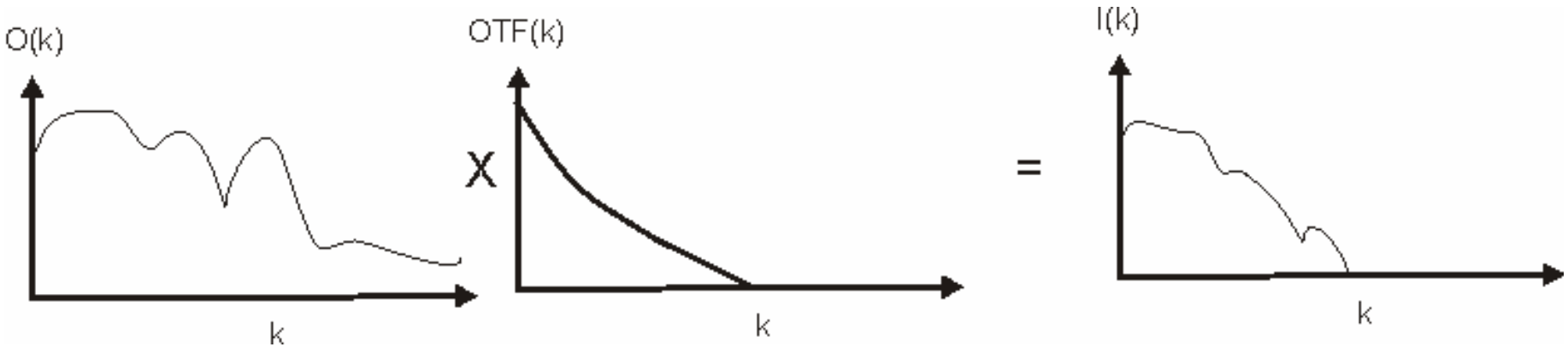
$$\tilde{I}(\vec{k}) = \tilde{O}(\vec{k}) \cdot OTF(\vec{k})$$

Goes from convolution
To simple multiplication

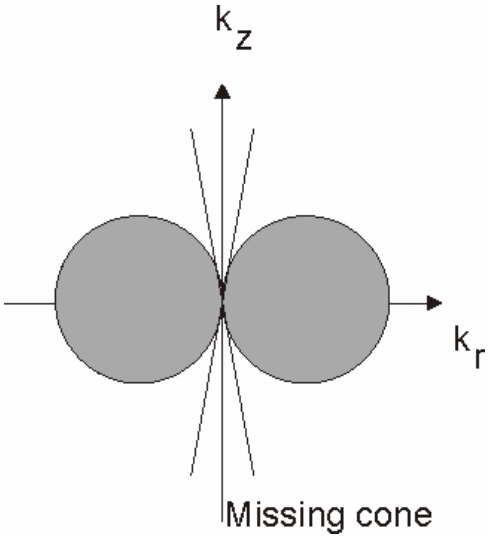
Optical transfer function, OTF, is the Fourier transform of PSF.
How does it looks like?



Effect of OTF on Image – Loss of Frequency Content



Effects: (1) lower amplitude at high frequency
(2) completely loss of information at high frequency



Missing all info along kz axis.
"Missing cone" is the origin of no depth discrimination

Deconvolution Microscopy

What is Deconvolution Microscopy?

$$\tilde{I}(\vec{k}) = \tilde{O}(\vec{k}) \cdot OTF(\vec{k})$$ Convolution

$$\tilde{O}(\vec{k}) = \tilde{I}(\vec{k}) \cdot OTF(\vec{k})^{-1}$$

$$O(\vec{r}) = \mathrm{F}^{-1}[\tilde{O}(\vec{k})]$$ Deconvolution

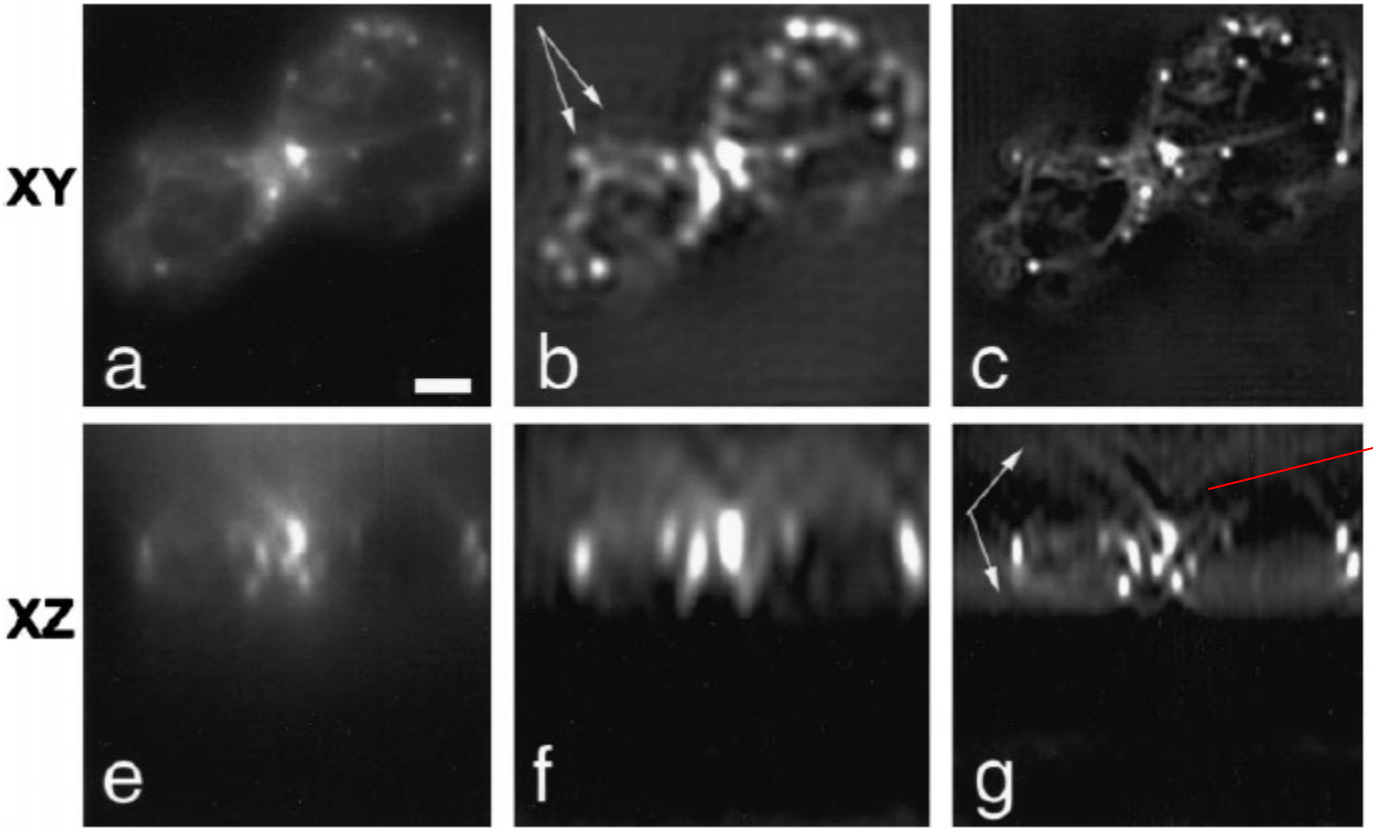
What is the problem of this procedure?

OTF is zero at high frequency…. Divide by 0???

There are many possible “O” given “I” and “OTF”
This belongs to a class of “ill posted problem”

The “art” of deconvolution is to find constrains that allow the best estimate of “O”. An example of these constraints is positivity

Application of Deconvolution I



Artifacts?

Improves resolution and 3D slicing

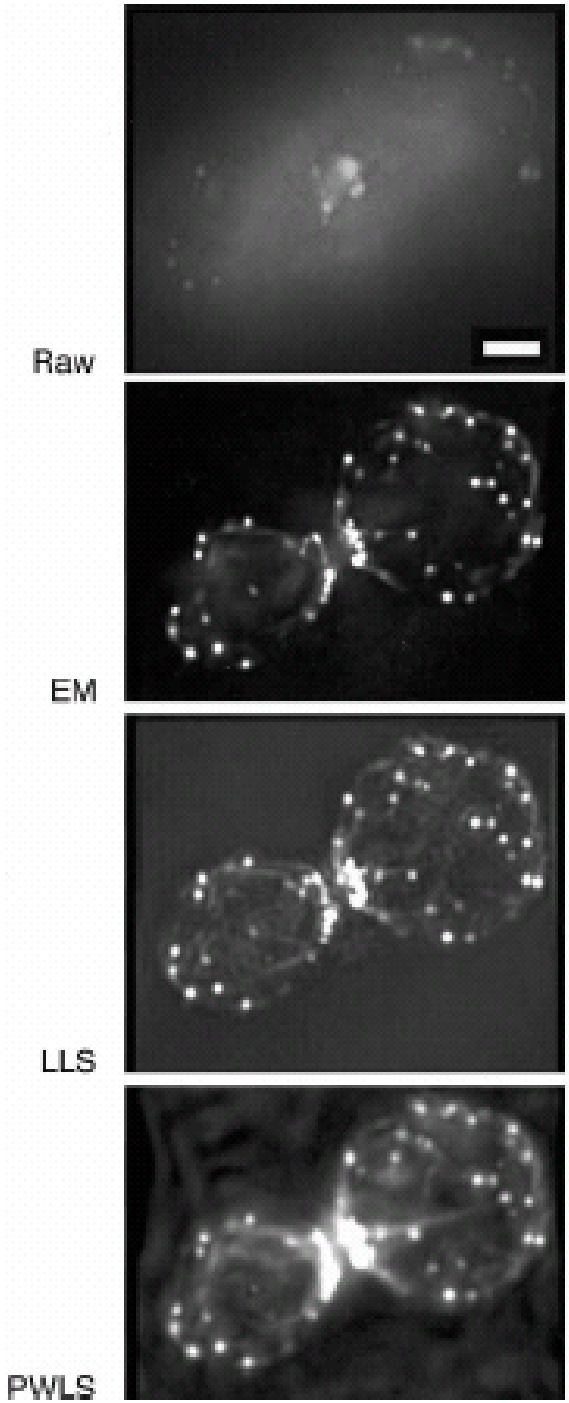
McNally, Methods, 1999

Application of Deconvolution II

Raw images deconvoluted by 3 different methods

Depending on deconvolution algorithm chosen different “features” and “artifacts” are seen

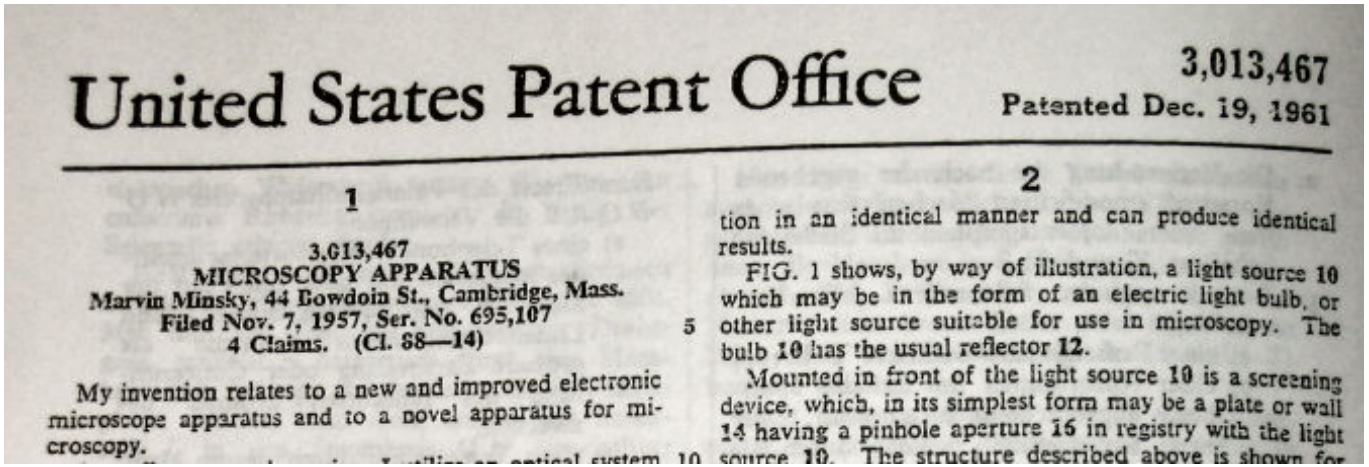
McNally, Methods, 1999



Confocal Microscopy

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

1

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.

2

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

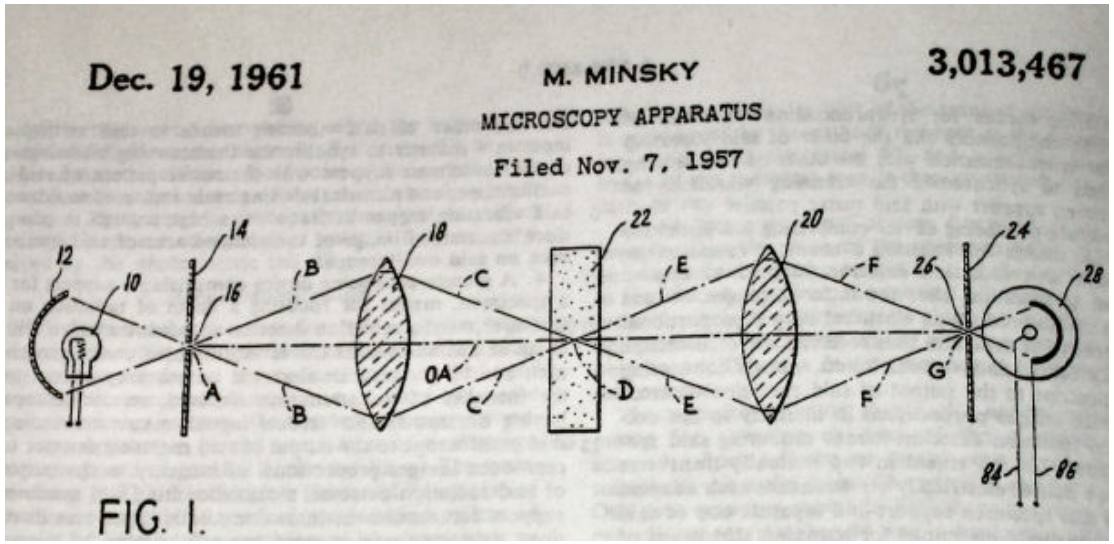
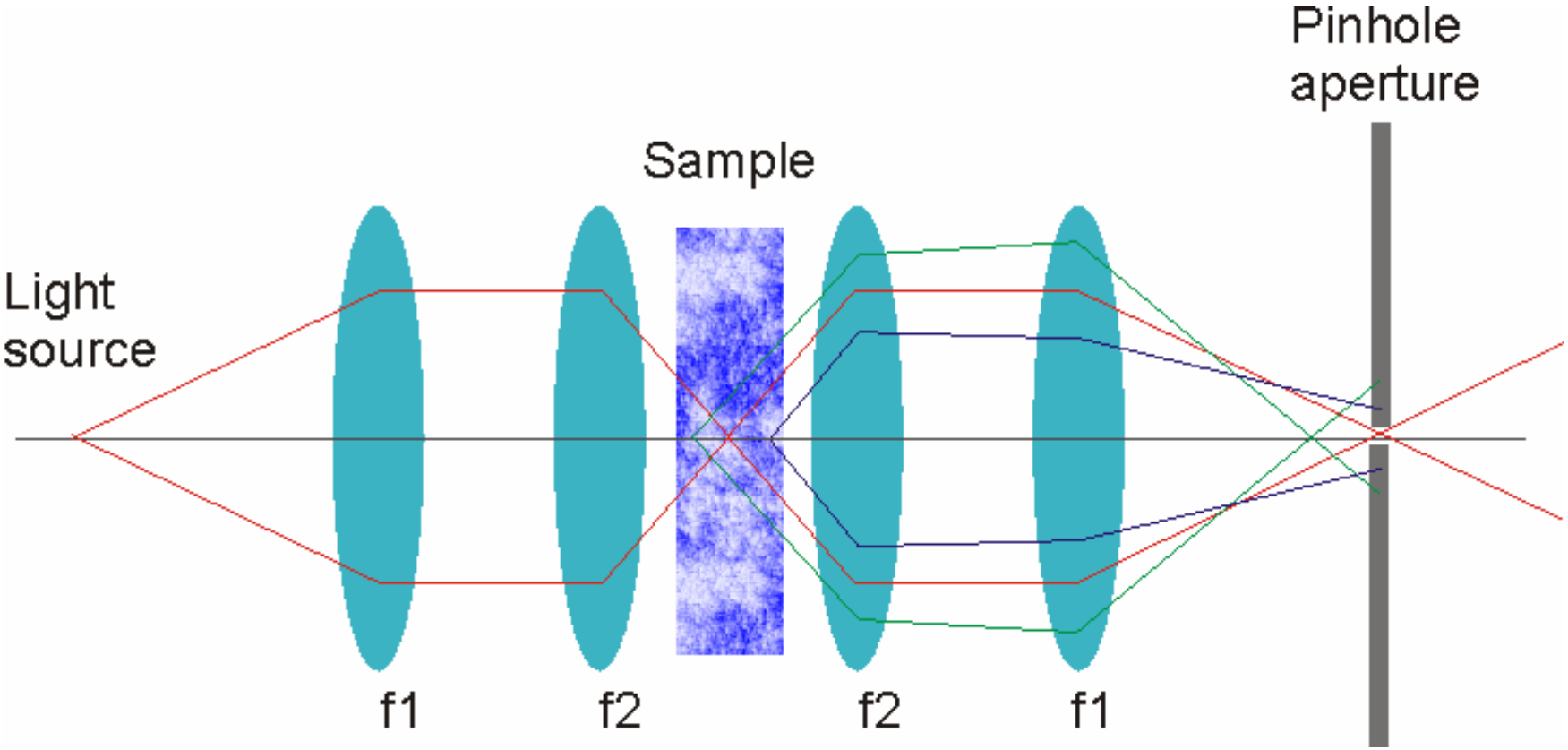


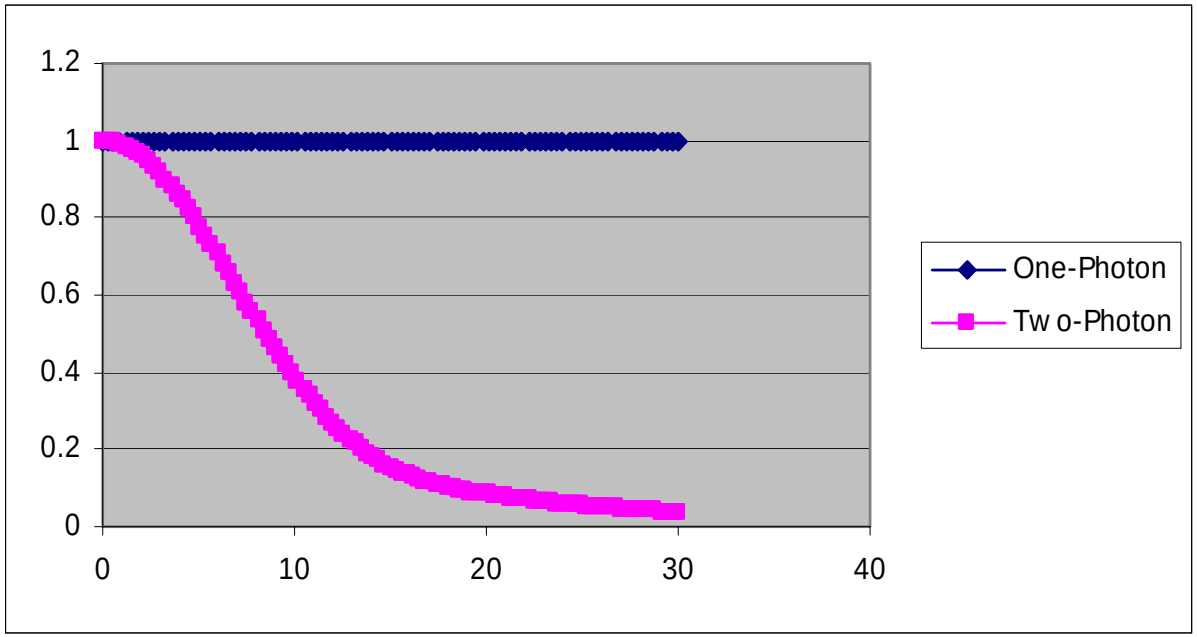
FIG. 1.

Principle of Confocal Microscopy

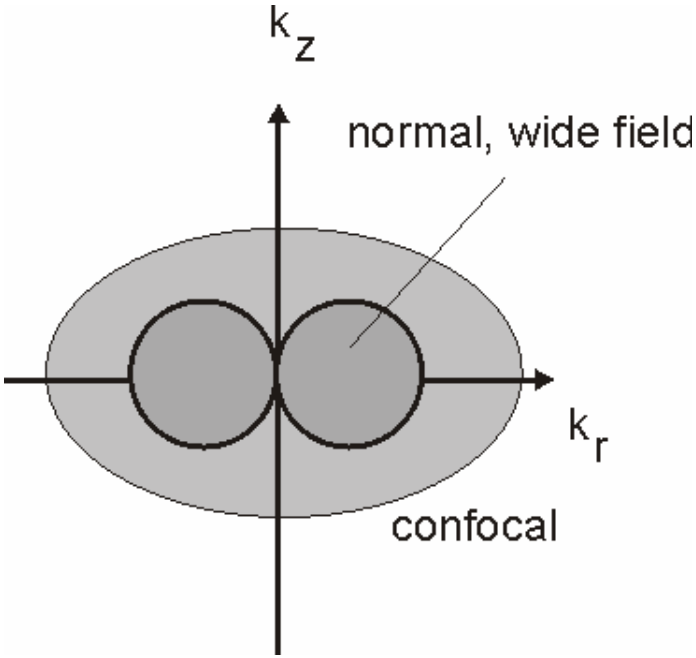


Information comes from only a single point. Needs to move the light or move the sample!

Depth discrimination



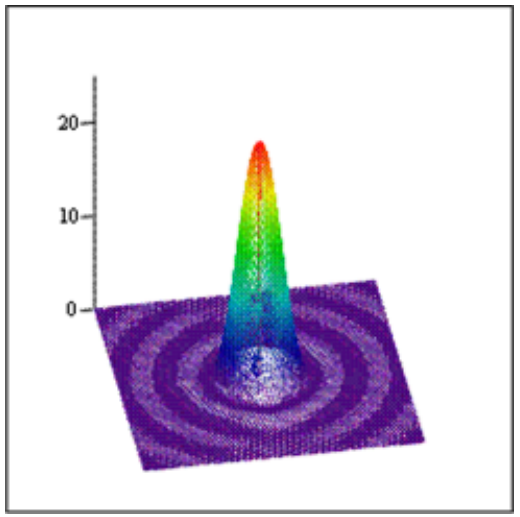
OTF



Point Spread Function – Image of an Ideal Point

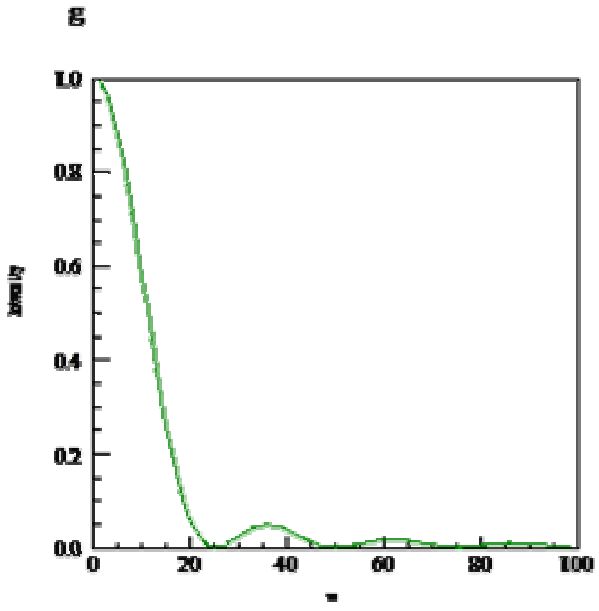
Lateral Dimension: Airy function

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



Axial Dimension : Sinc function

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

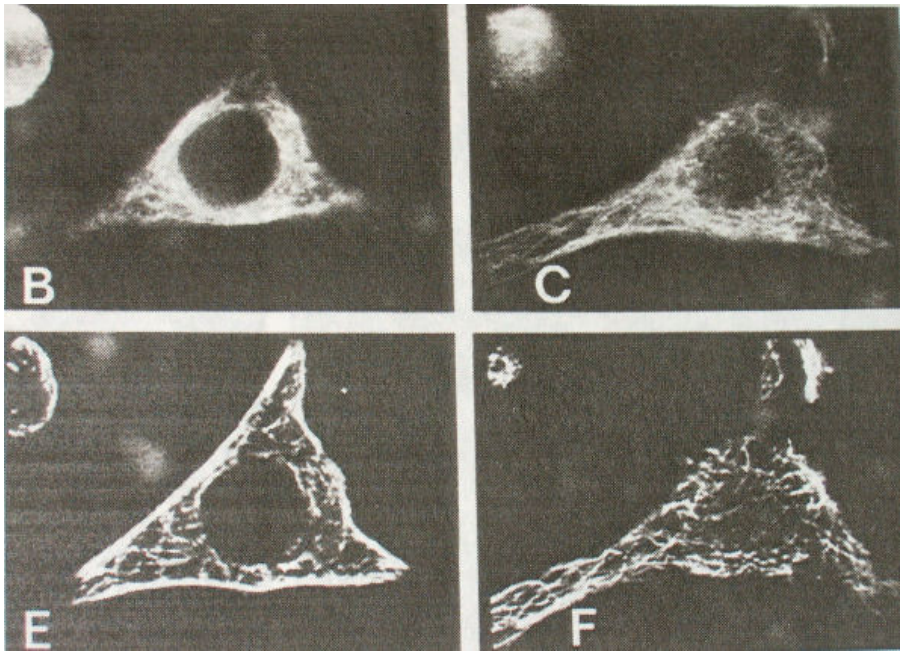


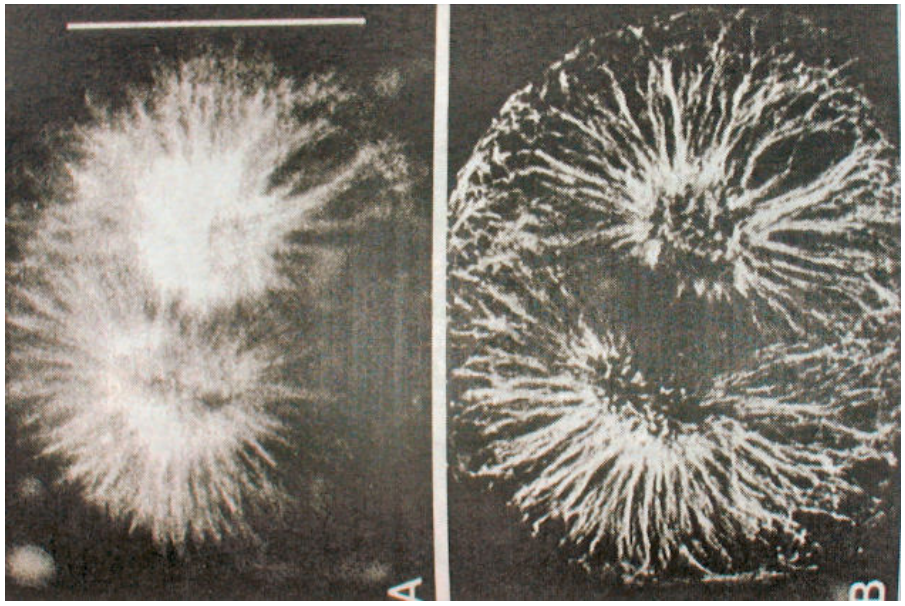
The PSF of confocal is the square of the PSF of wide field microscopy

$$F_{z-\sec}(u) = 2\pi \int_0^\infty PSF_c(u,v) v dv$$

$$= 2\pi \int_0^\infty PSF^2(u,v) v dv \neq \text{constant}$$

Early Demonstration of Confocal Microscopy in Biological Imaging

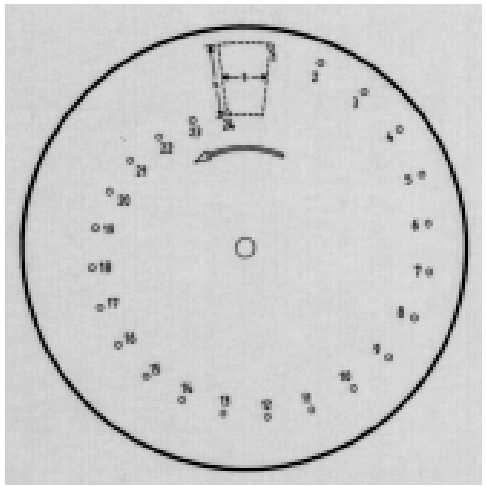




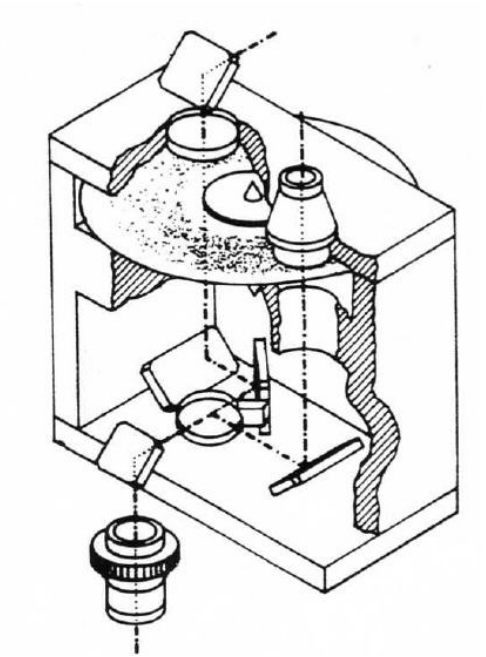
White et al., JCB 1987

Tandem Scanning Confocal Microscope

Utilizes a Nipkow Disk

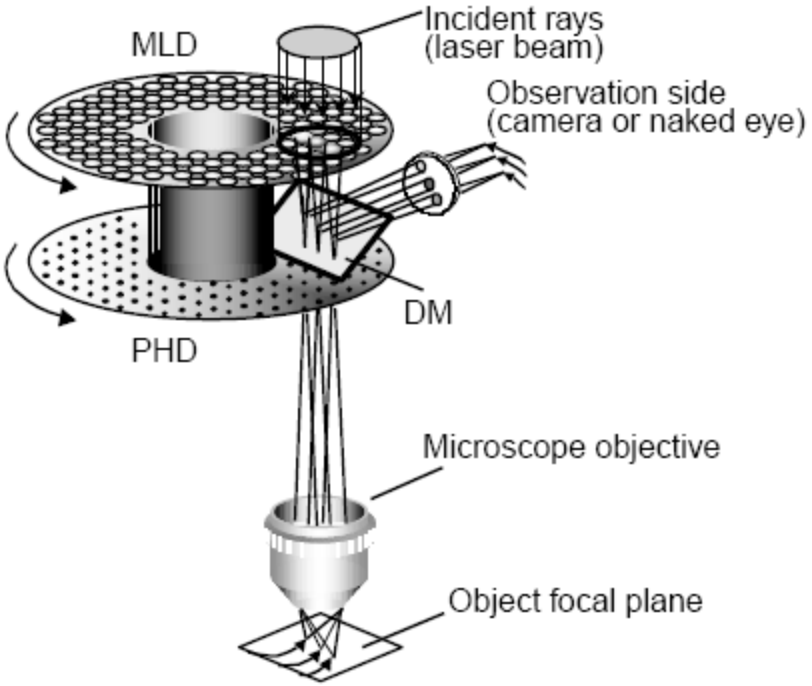


Holes organize in an Archimedes spiral

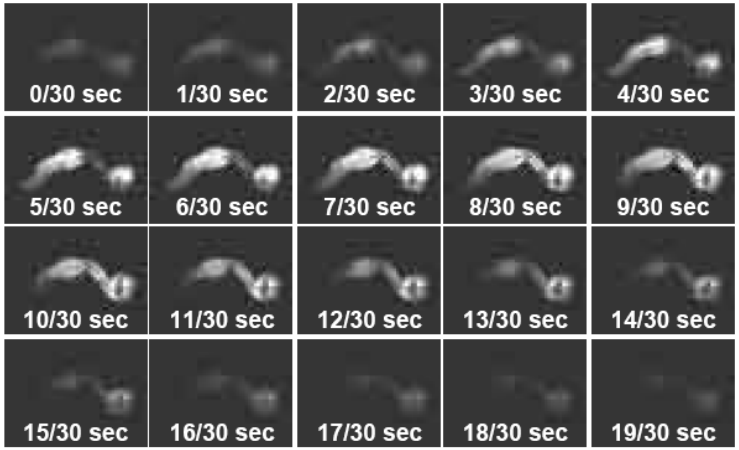


Petran's System

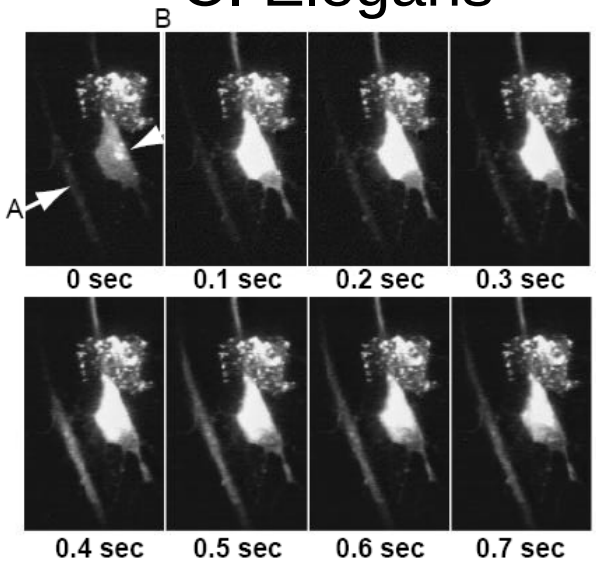
A Model Tandem Confocal Microscope Utilizing Yokogawa Scan Head



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



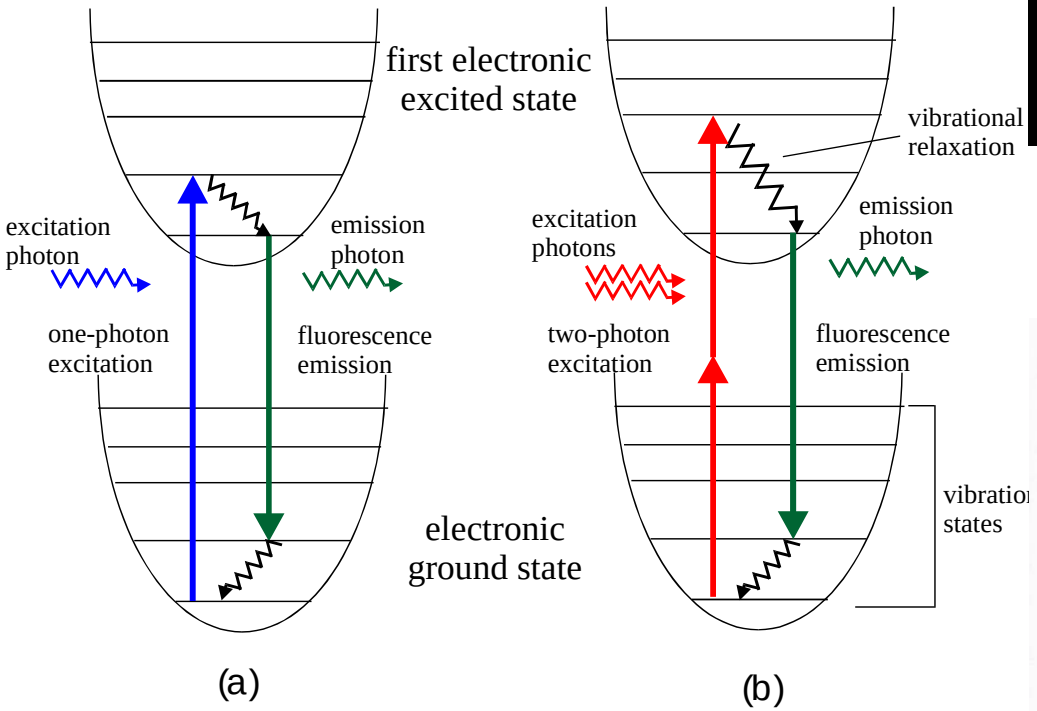
C. Elegans

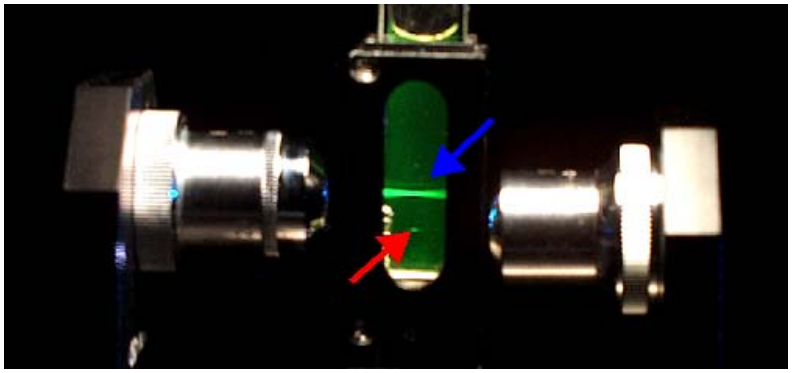


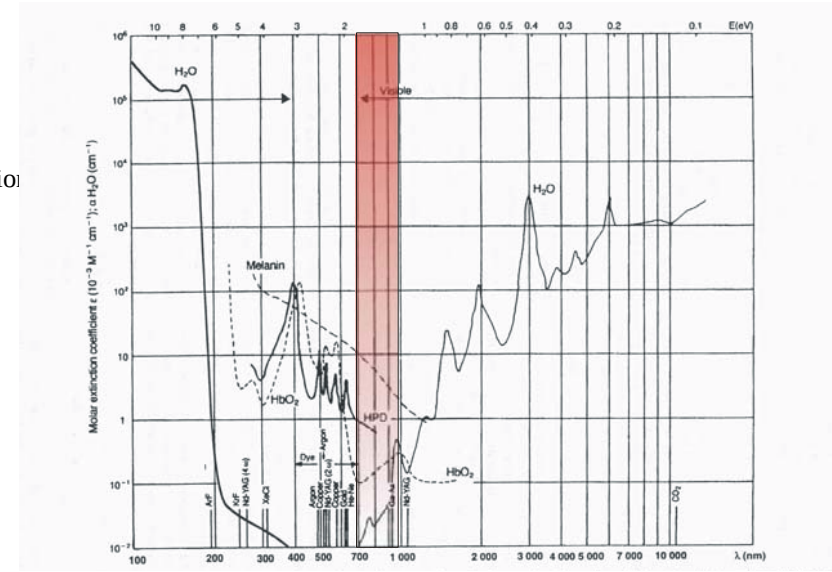
Calcium events in nerve fiber

Multiphoton Microscopy

Two-Photon Excitation Microscopy



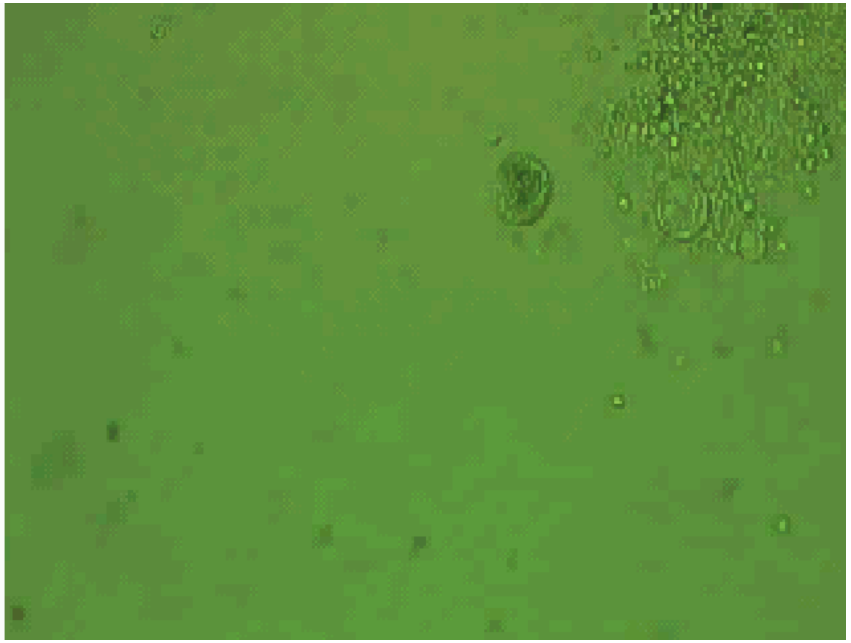


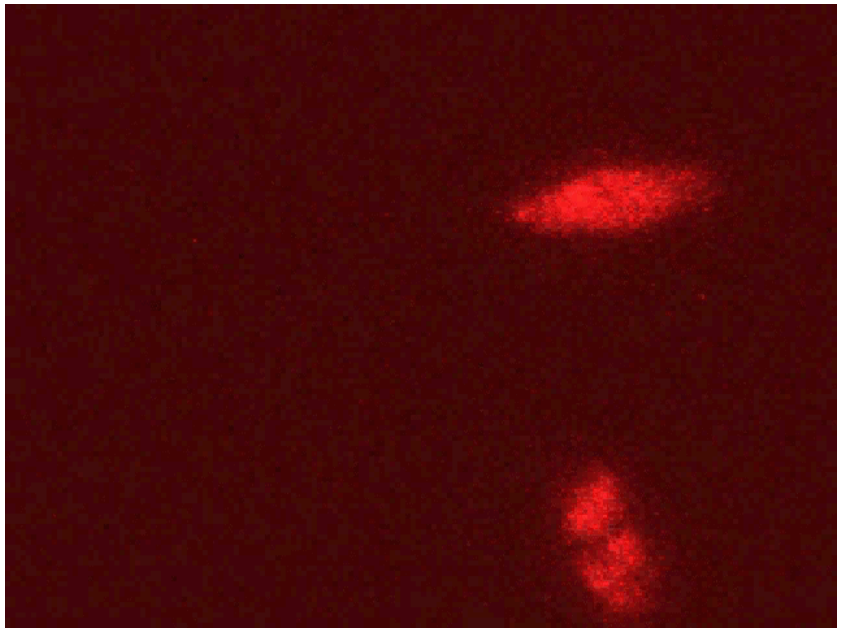


A comparison of two-photon and confocal microscopes

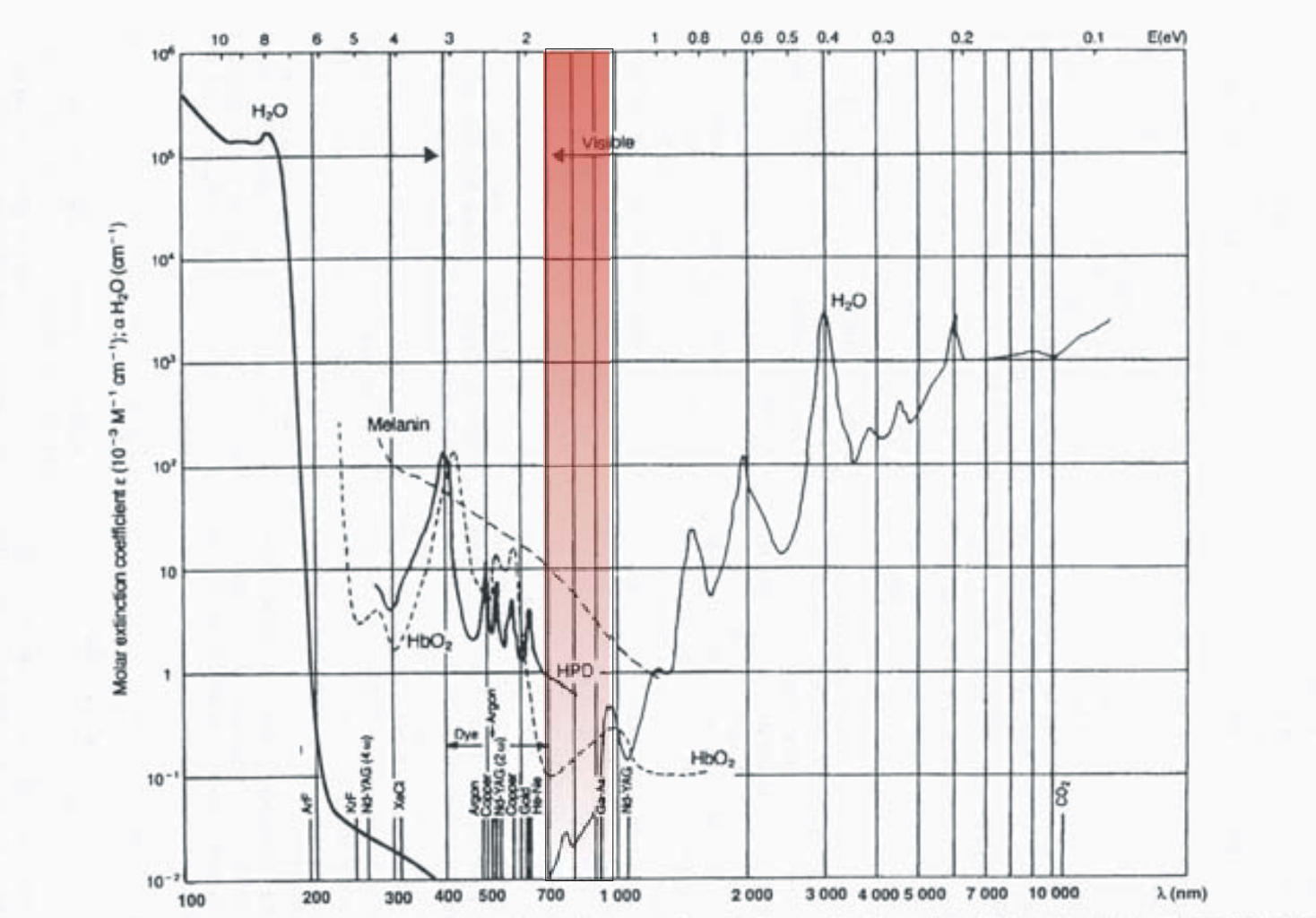
(1) Confocal microscopes have better resolution than two-photon microscopes without confocal detection.

(2) Two-photon microscope results in less photodamage in biological specimens. The seminal work by the White group in U. Wisconsin on the development of *c. elegans* and hamsters provides some of the best demonstration. After embryos have been continuously imaged for over hours, live specimens are born after implantation.

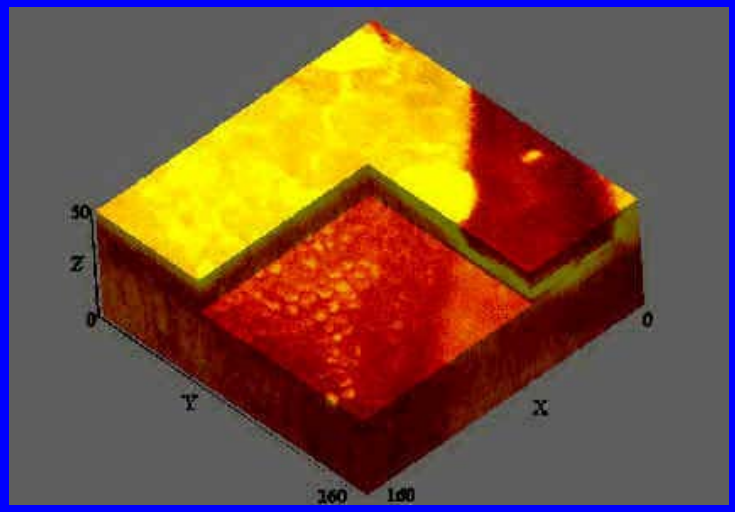


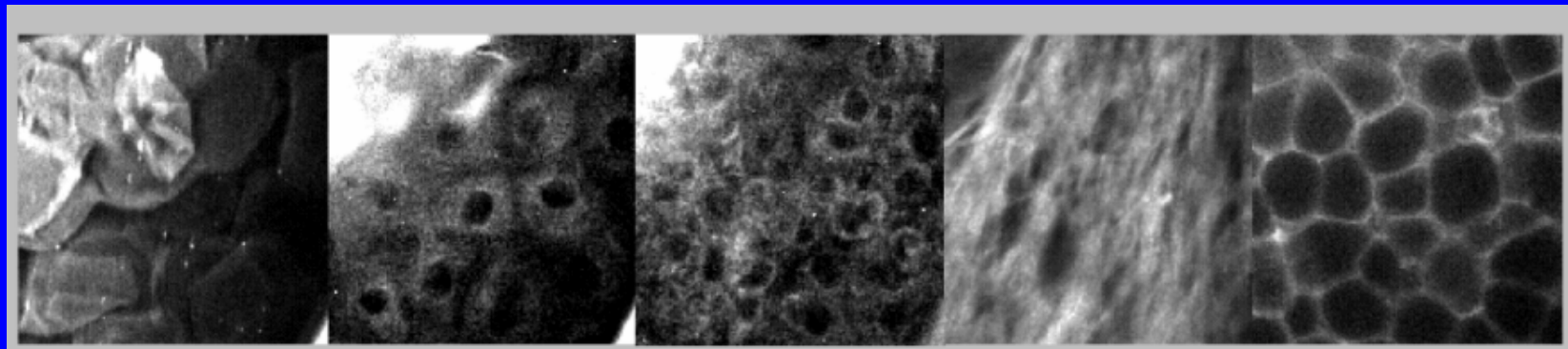


(3) Two-photon microscope provides better penetration into highly scattering tissue specimen. Infrared light has lower absorption and lower scattering in turbid media.



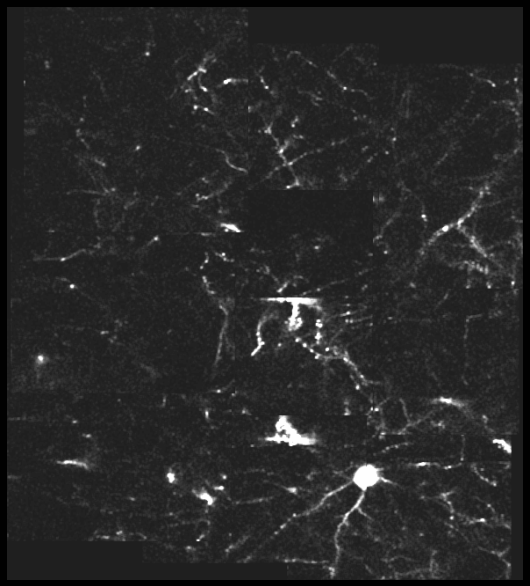
A 3D Reconstructed Movie Of Skin Structures From A Mouse Ear Tissue Punch



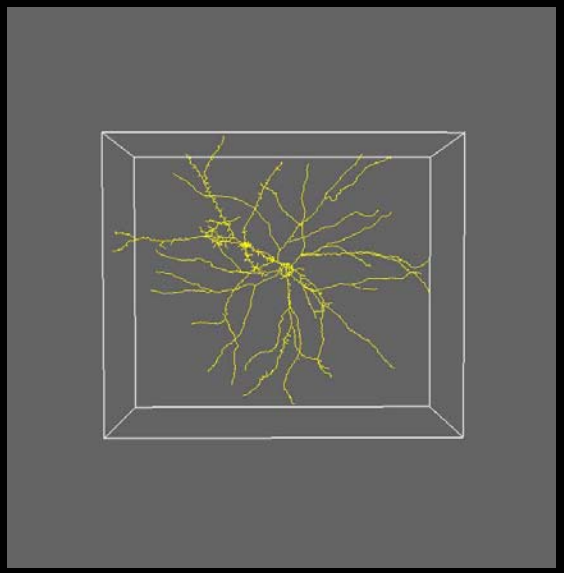


In collaboration with I. Kochevar, Wellman Labs, MGH and B. Masters

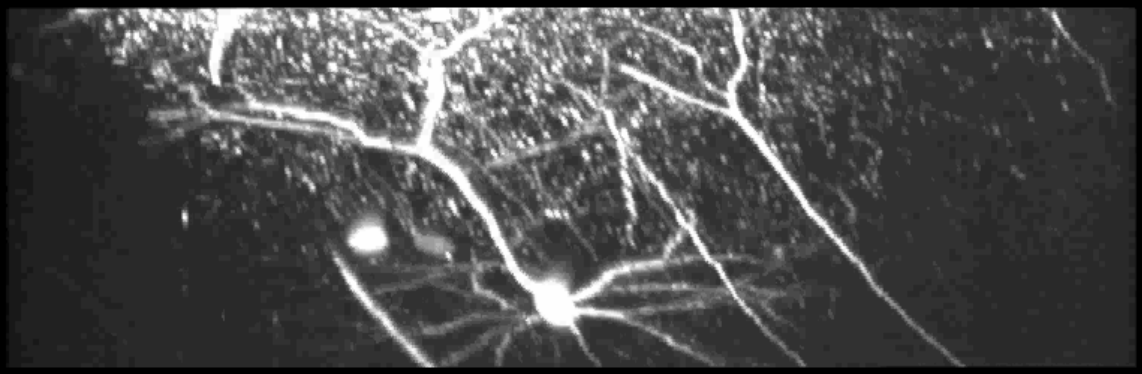
IN VIVO IMAGING OF NEURONAL DEVELOPMENT



Z-Stack, Individual Slices



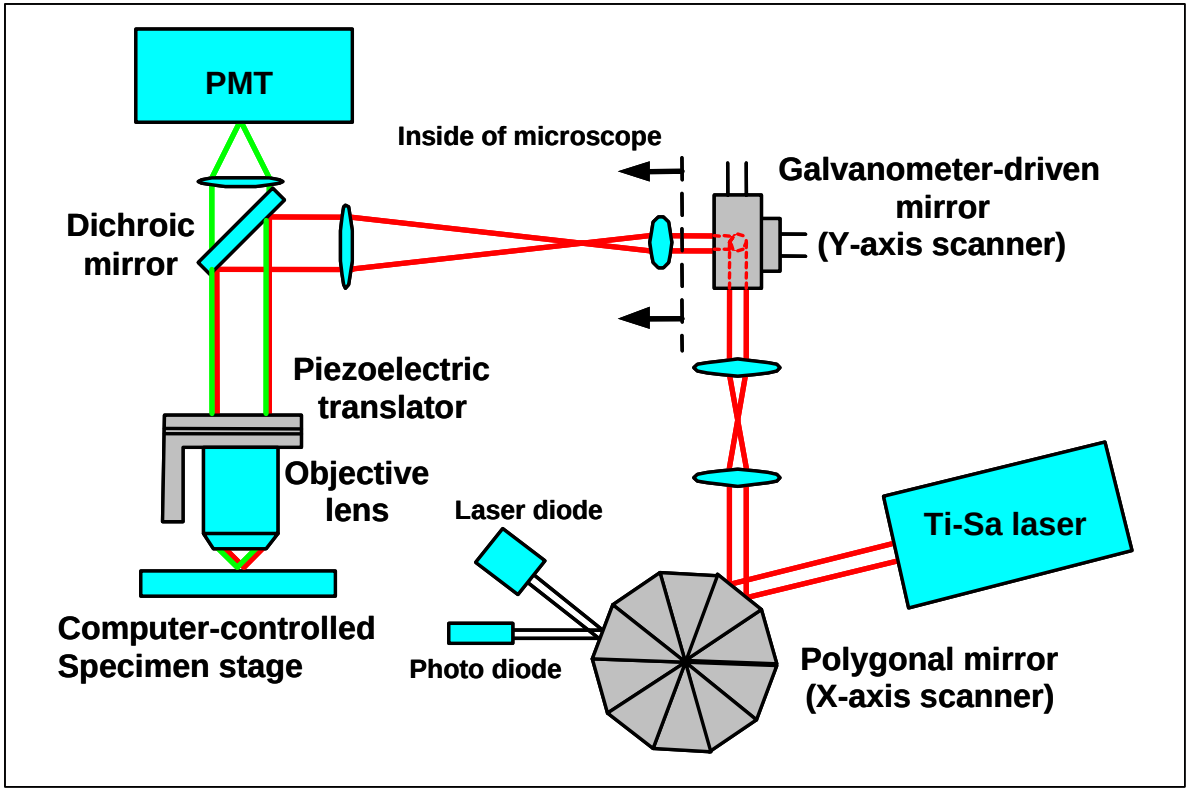
Computational Model of Dendrite Branches



Reconstructed 3-D View

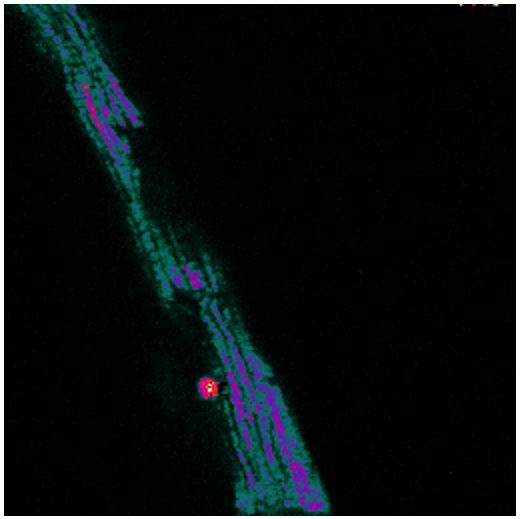
In collaboration with Wei Lee & Elle Nevidi, MIT

3D Multiple Particle Tracking with Video Rate Two-Photon Microscopy



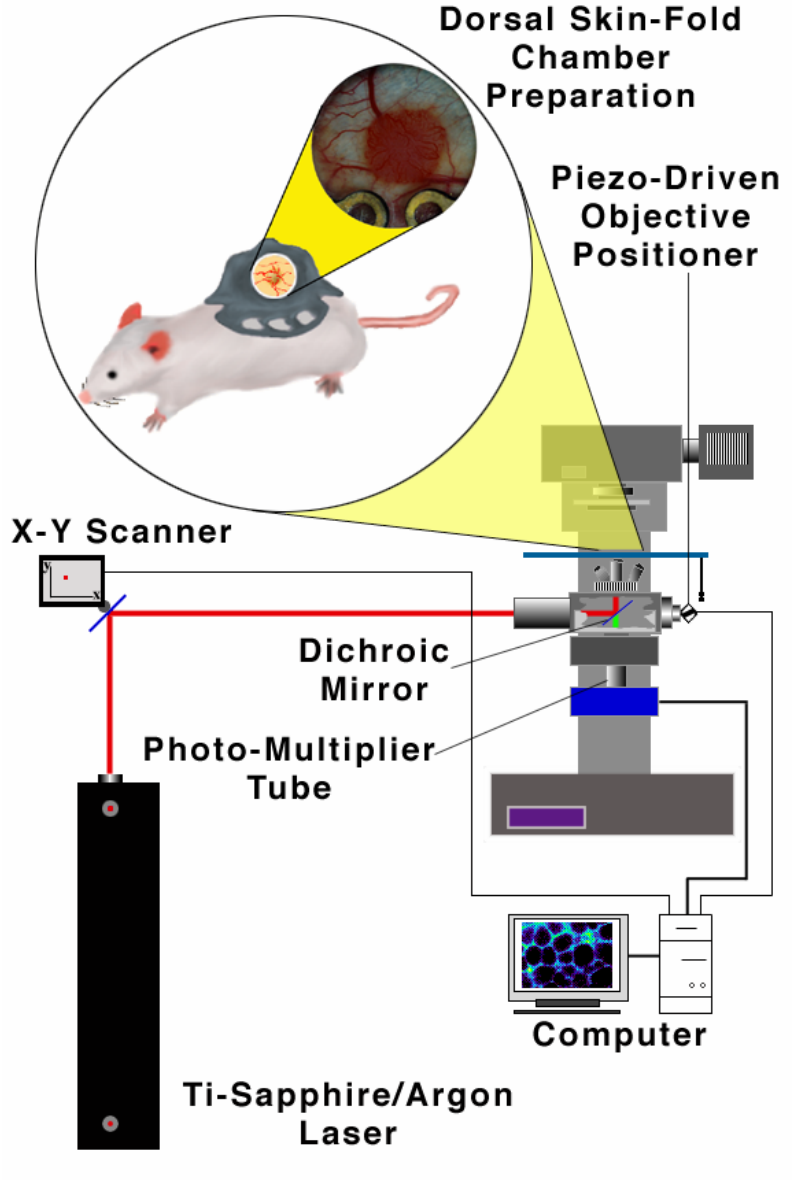
In collaboration with Ki Hean Kim (MIT)

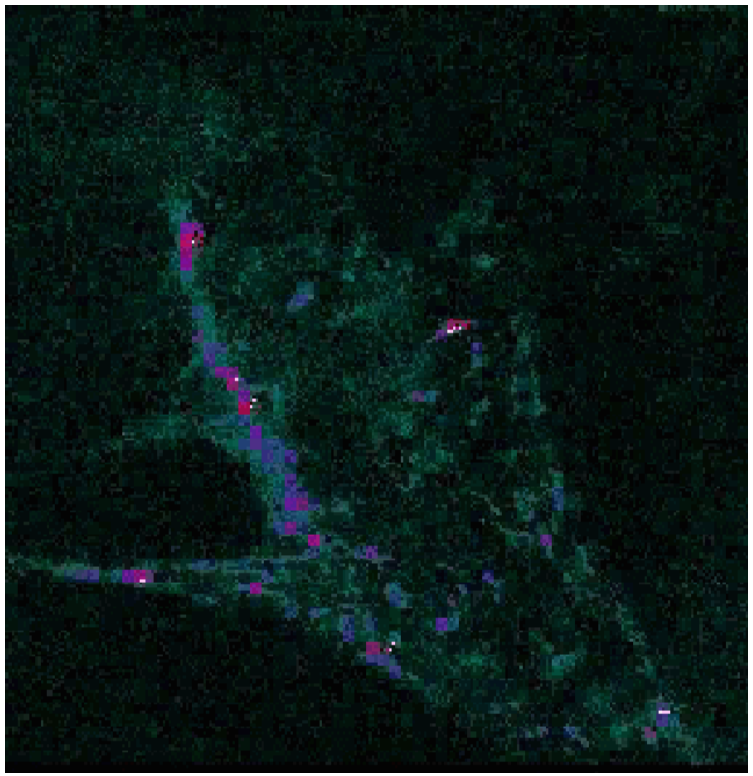
Imaging of myocyte contraction -- R6G labeled mitochondria



In collaboration with J. Lammerding, H. Huang, K. Kim, R. Kamm, R. Lee (MIT and Brigham & Women's Hospital)

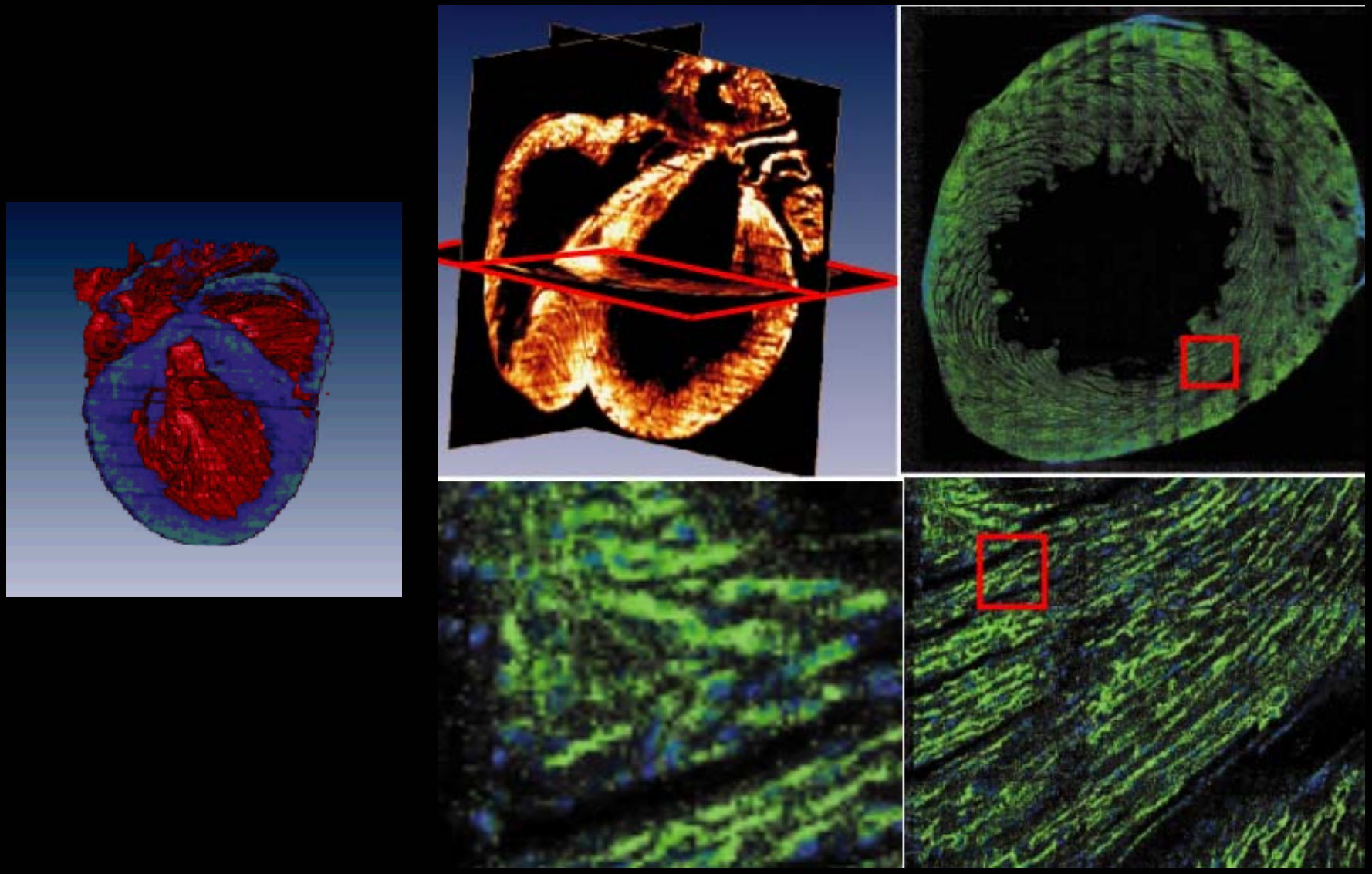
3D Quantification of Blood Flow in Solid Tumors





In collaboration with Rakesh Jain, MGH

QUANTIFYING AND UNDERSTANDING GENETICALLY INDUCED CARDIAC HYPERTROPY



Macroscopic View of Whole Mouse Heart with Microscopic Subcellular Image Resolution

A Comparison of The Three 3D Imaging Methods with Wide Field

	Wide field	Deconvolution	Confocal	Multiphoton
Resolution	NA	Better (depend on SNR)	Better	Similar
3D	No	Yes	Yes	Yes
Imaging depth	--	-	+	++
Uncertainty	+	--	+	+
Cost	$	$$	$$$$	$$$$$