BRC-Imaging Overview

Rebecca Williams,
Director, BRC-Imaging

Imaging facility staff



Rebecca Williams
Director



Carol Bayles
Microscopy manager



Johanna Dela Cruz
Vet Imaging Manager



Mark Riccio
CT Director



Fred von Stein
CT Manager



Bob Doran
Vet Research Support

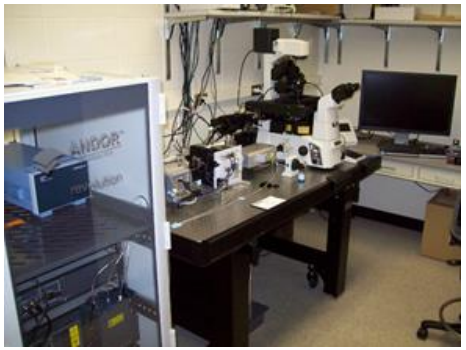
Optical microscopy and image analysis
B46 Weill Hall Imaging Corridor

IMAGING FACILITY

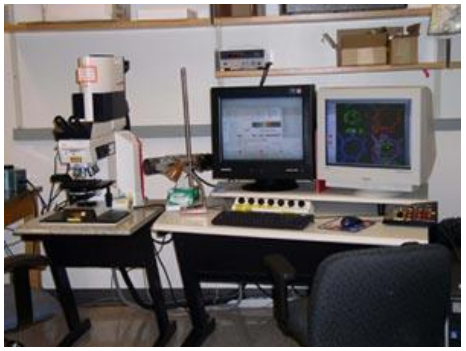
Instruments:



Zeiss LSM710 (B46 Weill) and LSM510 Meta (T5008A VRT)



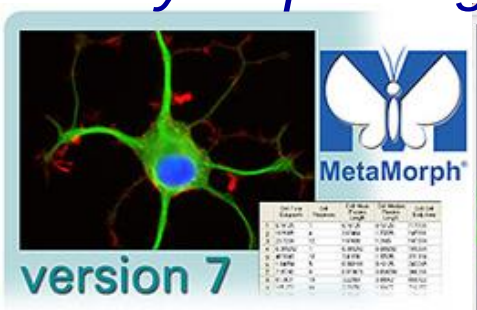
Andor Spinning Disk (B46 Weill)

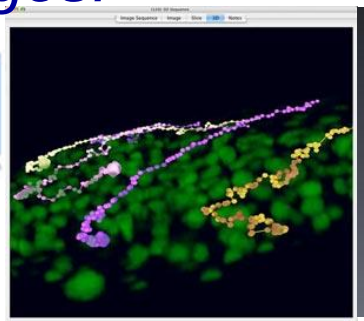


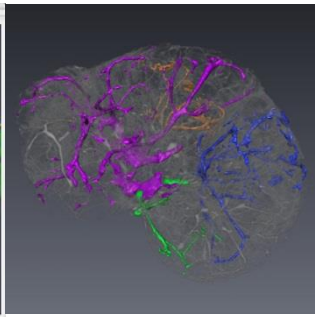
Leica TCS SP2 (B46 Weill)

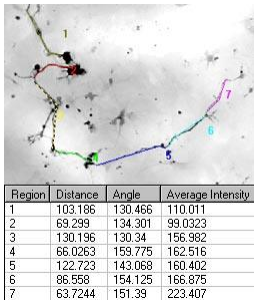
…and others

Analysis packages:







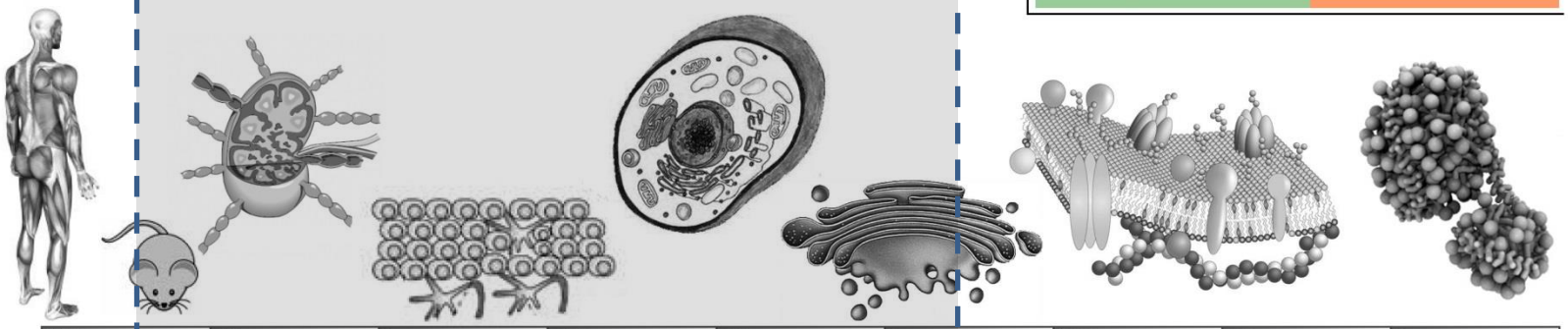


Region	Distance	Angle	Average Intensity
1	103.186	130.466	110.011
2	69.299	134.301	99.0323
3	130.196	130.34	156.982
4	66.0263	159.775	162.516
5	122.723	143.068	160.402
6	86.558	154.125	166.875
7	63.7244	151.39	223.407

Volocity, MetaMorph, Avizo, et al.


BRC
Imaging

BRC Imaging


Standard
Research
Live
Fixed
100
10
1
100
10
1
100
10
1
Millimeters->
Microns->
Nanometers->
X-Ray Computed tomography
Micro CT
3D X-Ray microscopy
Magnetic Resonance Imaging
Micro MRI
SPECT/PET
Research SPECT/PET
Ultrasound
Research ultrasound
Light/Laser Microscopy
Super Resolution
Electron Microscopy

Imaging for Biomedical Research

Physical concepts

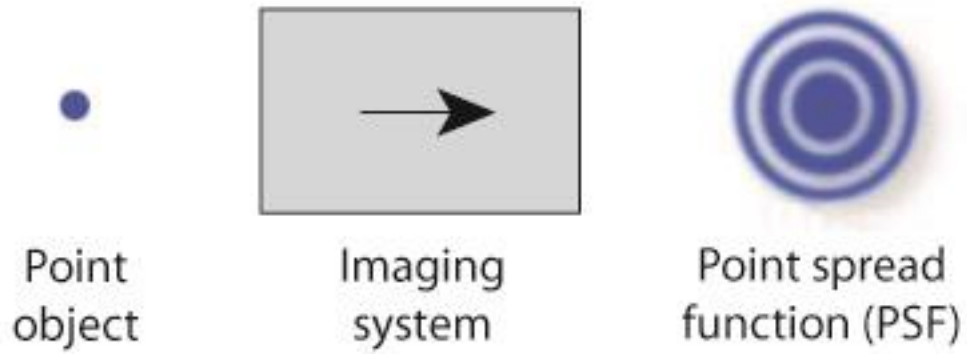
Optical microscopy and related tools

Optical macroscopy (molecular imaging)

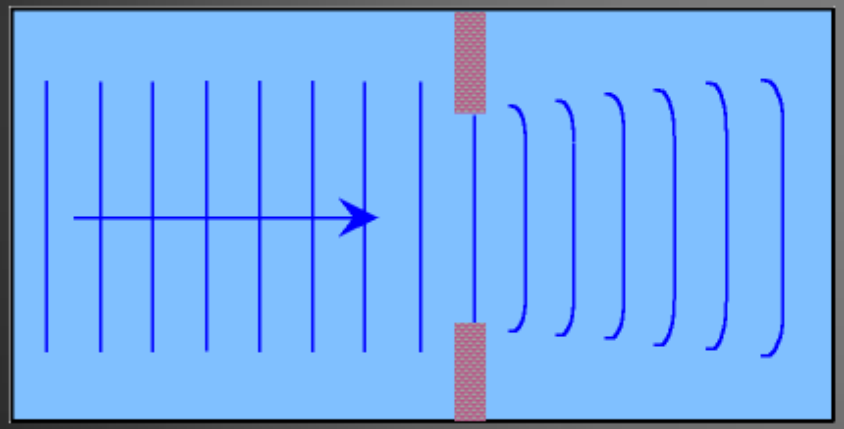
High resolution ultrasound

High resolution X-ray CT

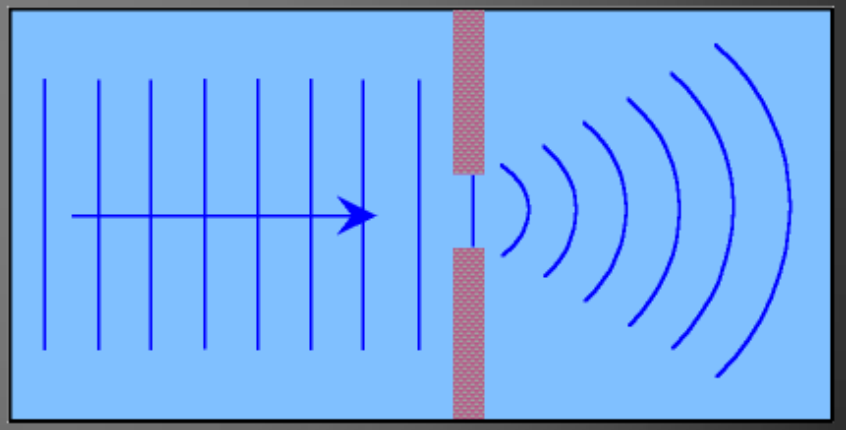
Image Resolution (and the PSF)



Resolution is determined by diffraction in a perfect optical system



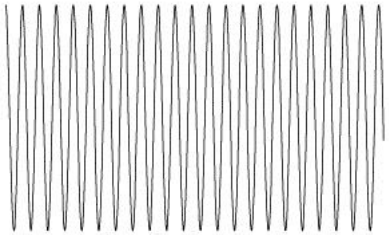
Big object



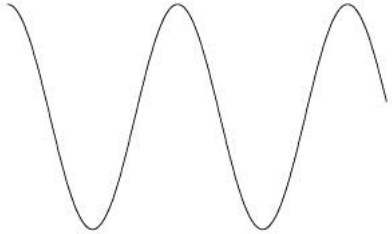
Small object

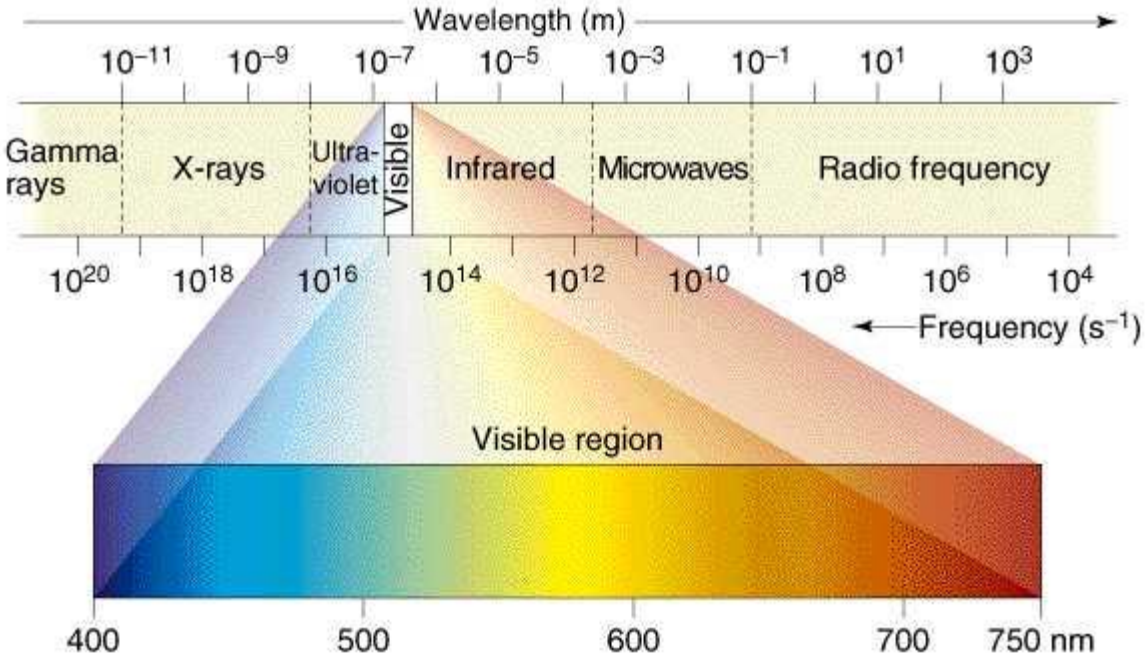
EM Spectrum

Short wavelength
High frequency
High energy

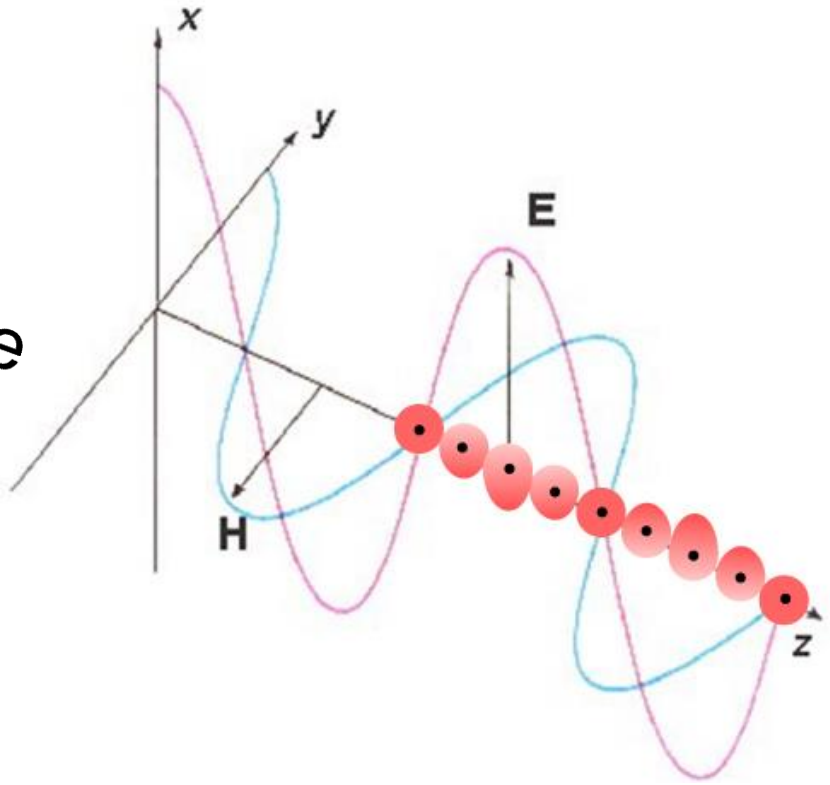


Long wavelength
Low frequency
Low energy

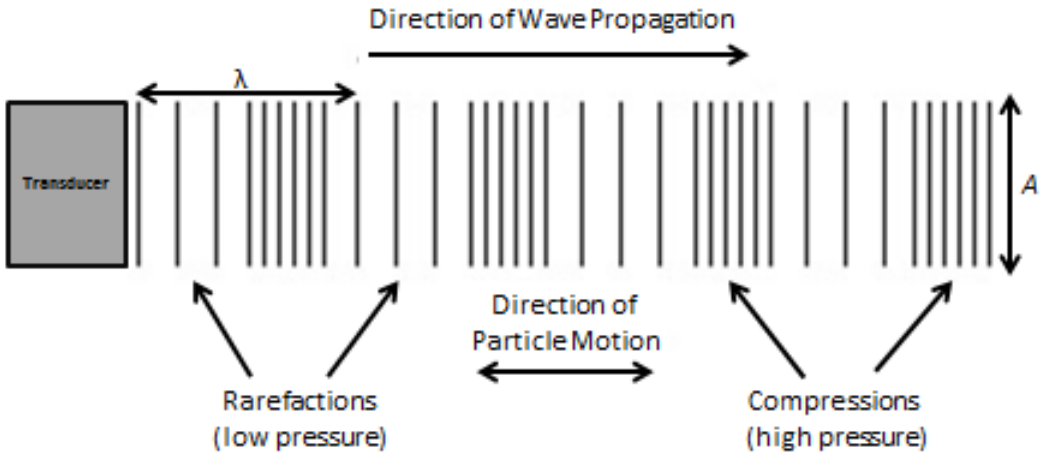




Electromagnetic wave
(Light)

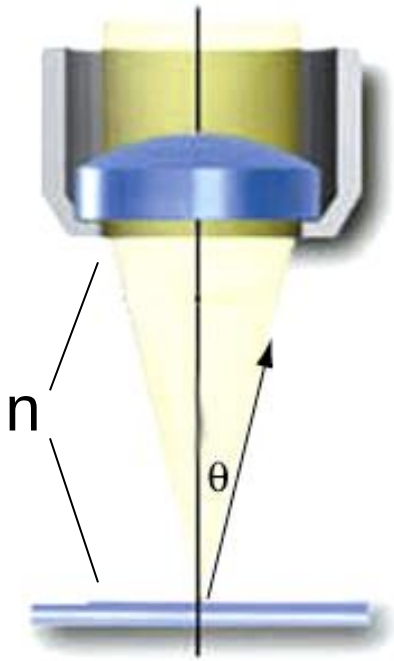


Pressure wave
(Sound)



Resolution determined by Numerical Aperture (NA)

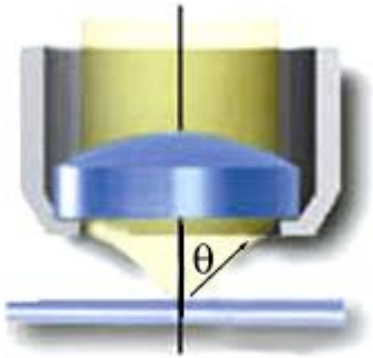
$$NA = n \sin(\theta)$$



NA = 0.12

θ ~ 7°

Long working distances



NA = 0.90

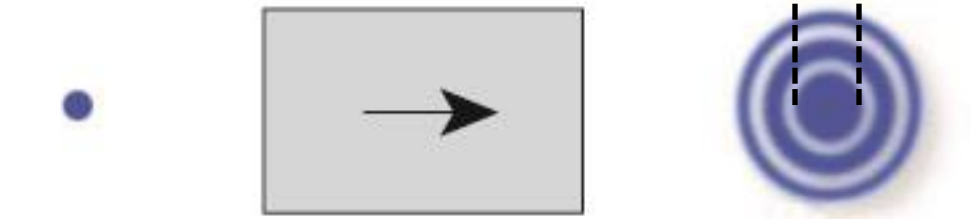
θ ~ 64°

Short working distances

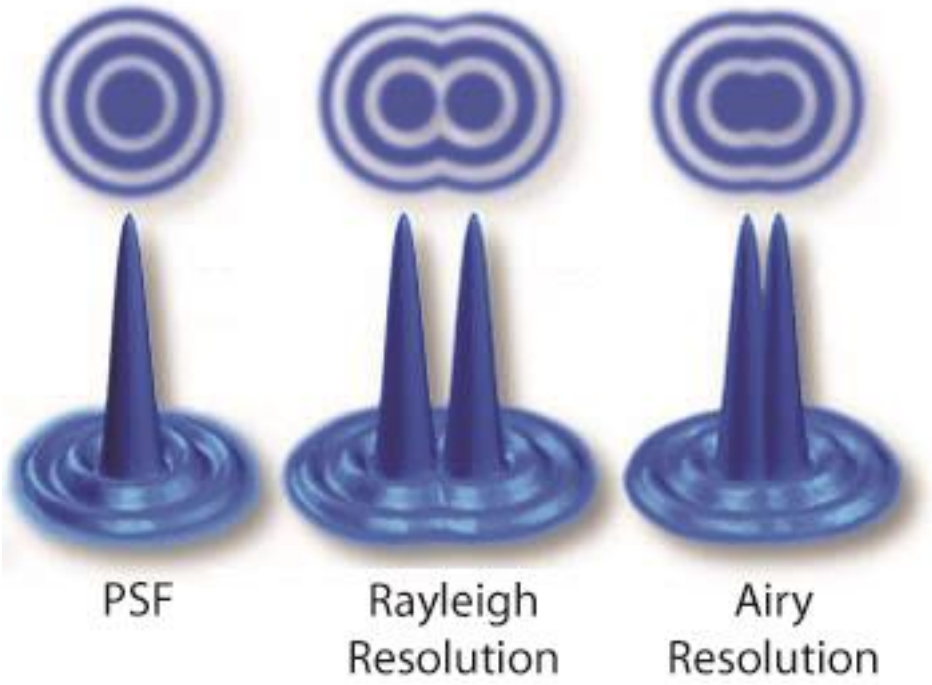


Image Resolution

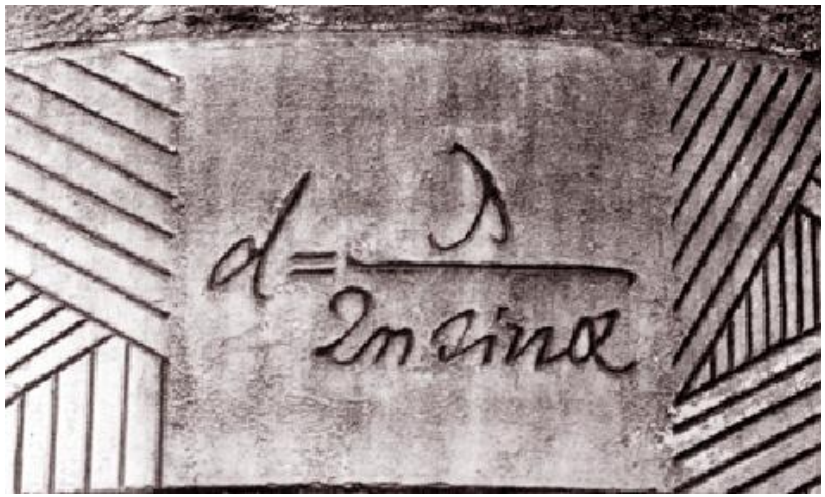
$\lambda/2*NA$



Point object | Imaging system | Point spread function (PSF)

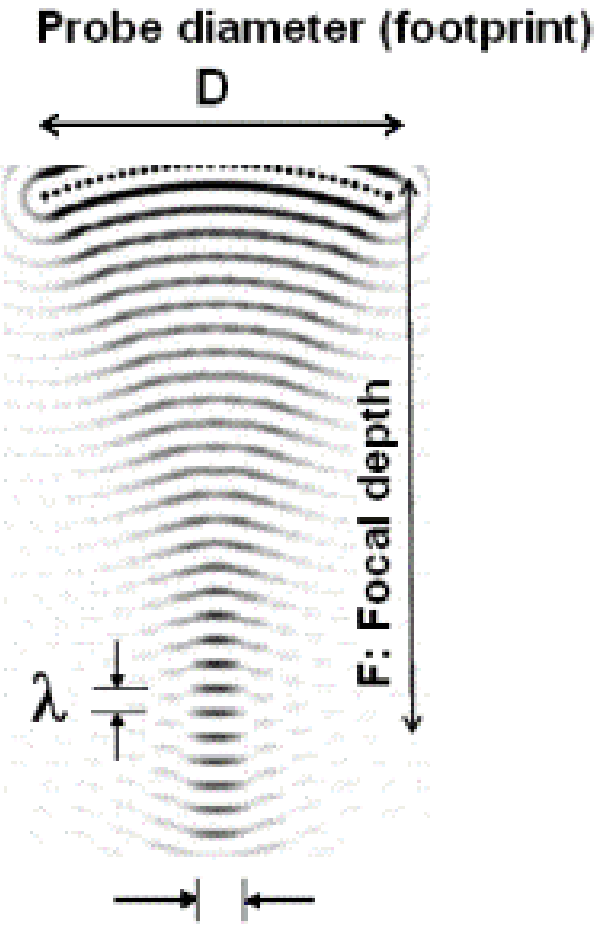


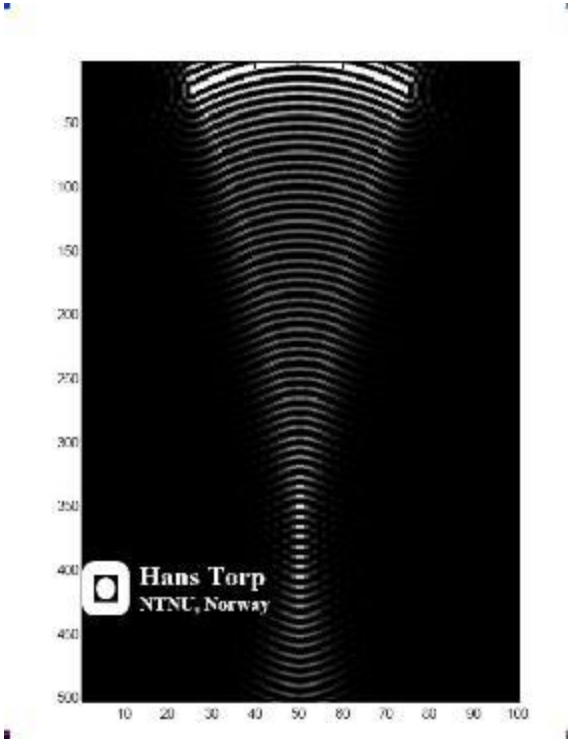
PSF | Rayleigh Resolution | Airy Resolution



Gravestone, Sir George Airy (1801–92)

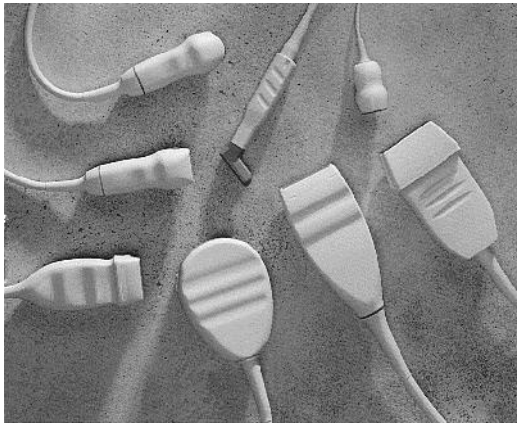
Ultrasound focusing





$$\text{Lateral resolution} = \frac{F * \lambda}{D} \quad \sim\lambda/2*NA$$

Ultrasound probes
(analogous to optical objective lenses)

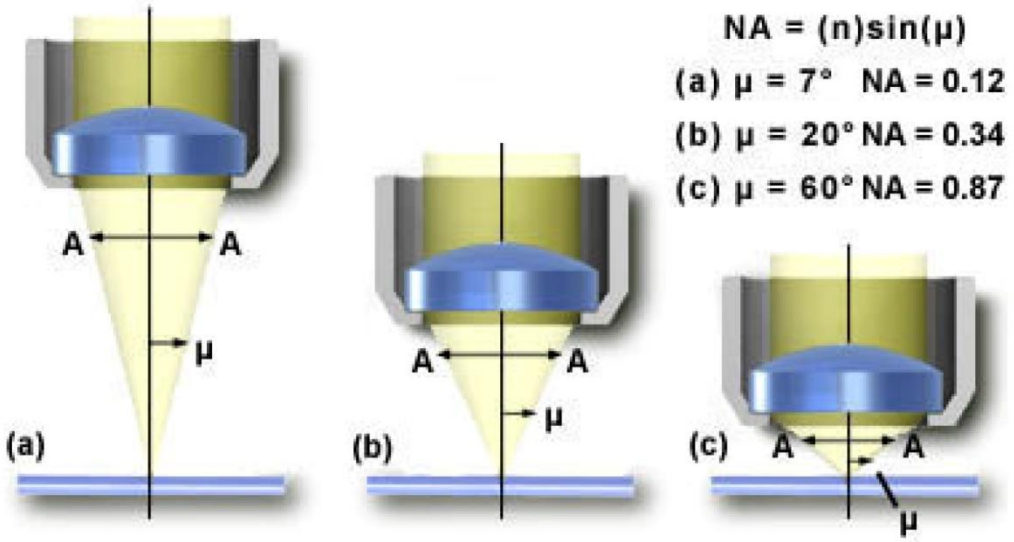


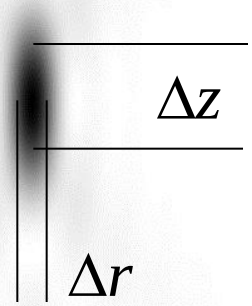
Probe	Application	Frequency (MHz)	F#	Focal length (mm)	Lateral resolution (um)	Axial resolution (um)
RMV 704	High resolution near	40	2.0	6.0	~75	40
RMV 706	Mouse general near	40	2.4	6.0	~90	40
RMV 707B	Mouse cardiology	30	2.1	12.7	~80	55

Resolution determined by Numerical Aperture (NA)

$$\Delta z(FWHM) = \frac{0.88\lambda}{\mathrm{n} - \sqrt{\mathrm{n}^2 - \mathrm{NA}^2}}$$

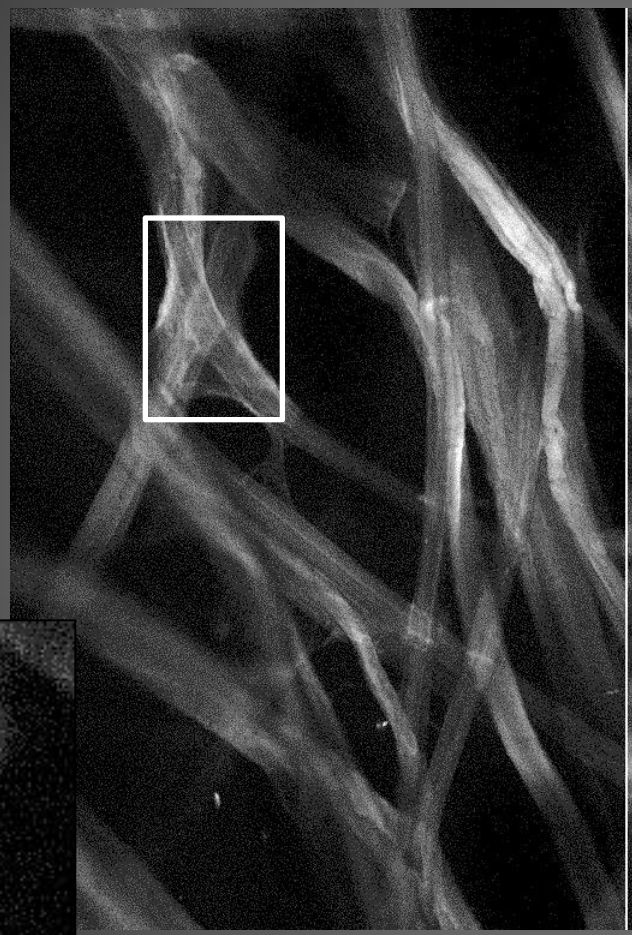
$$\Delta r(FWHM) = \frac{0.53\lambda}{\mathrm{NA}}$$



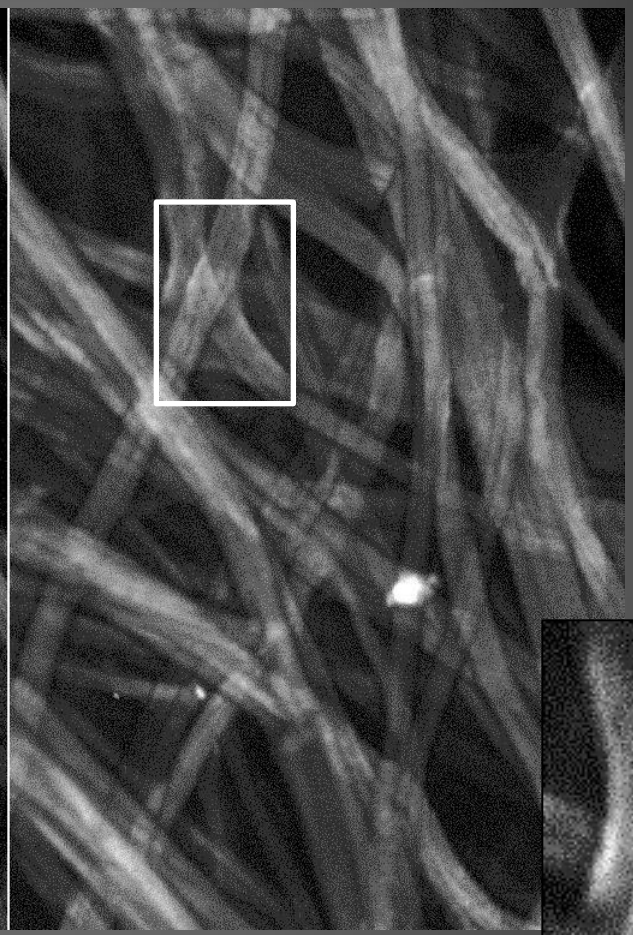




High vs Low NA images





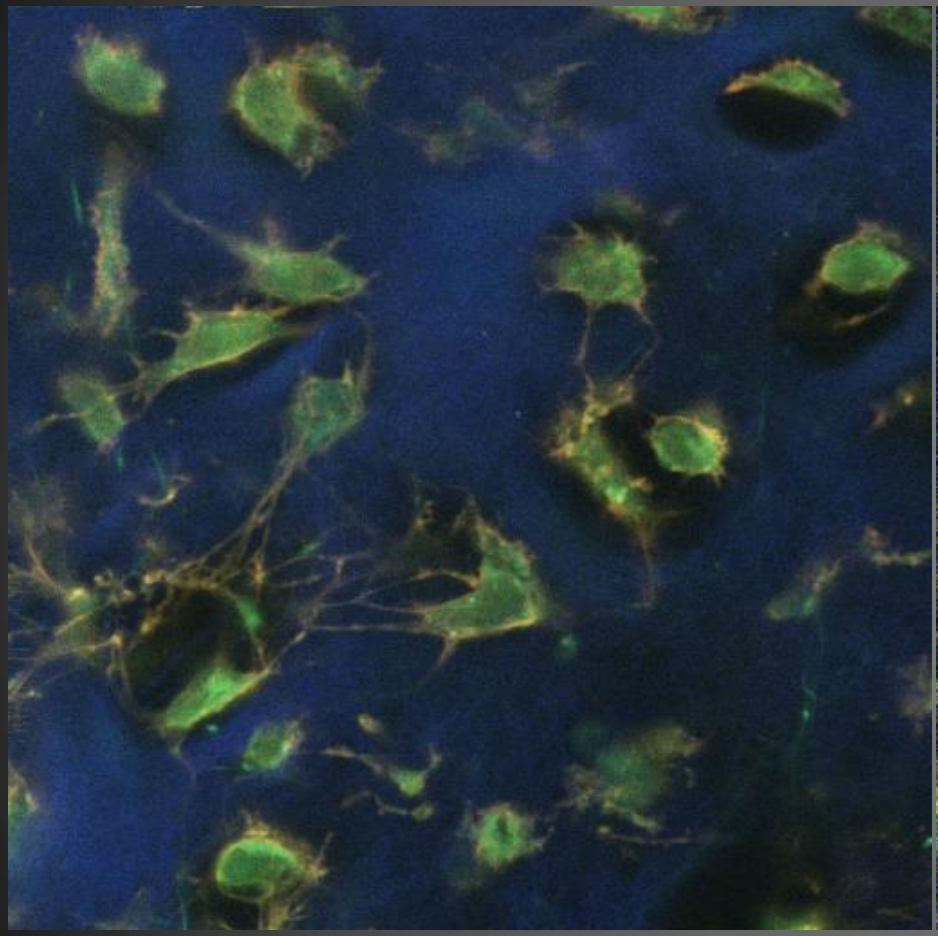


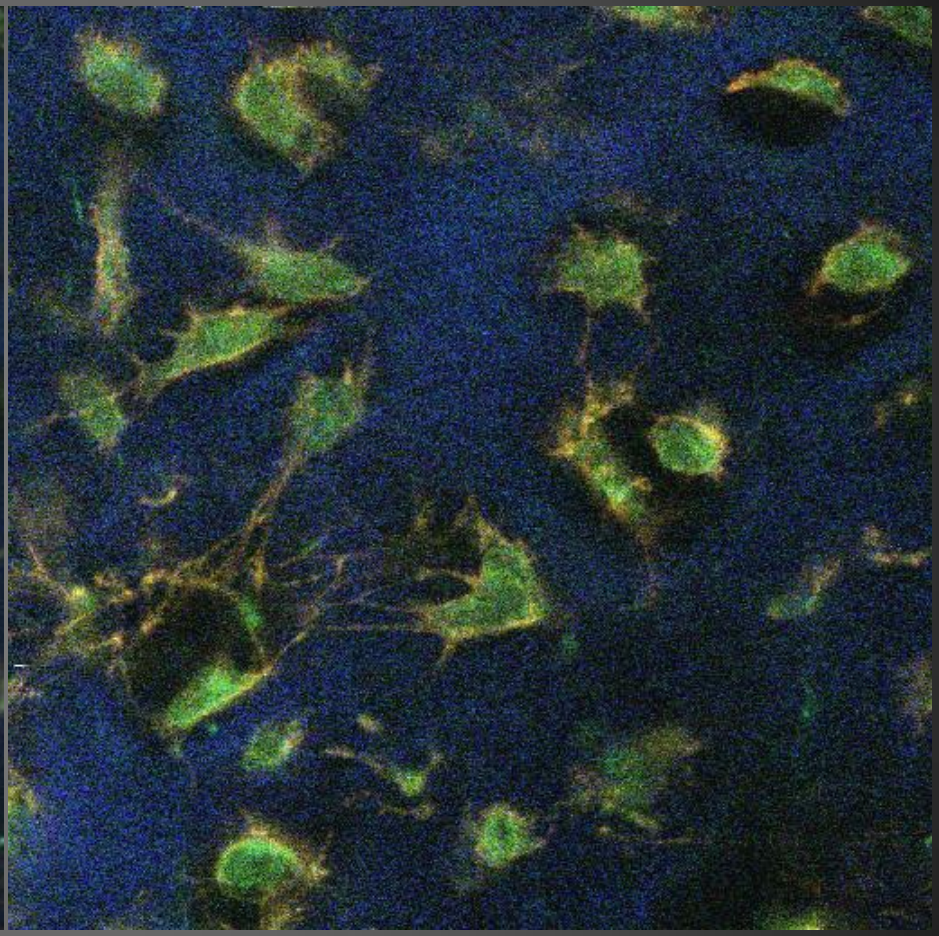


High NA

Low NA

Image “Brightness”





Many photons/Low gain

Few photons / High gain

(Same mean pixel value)

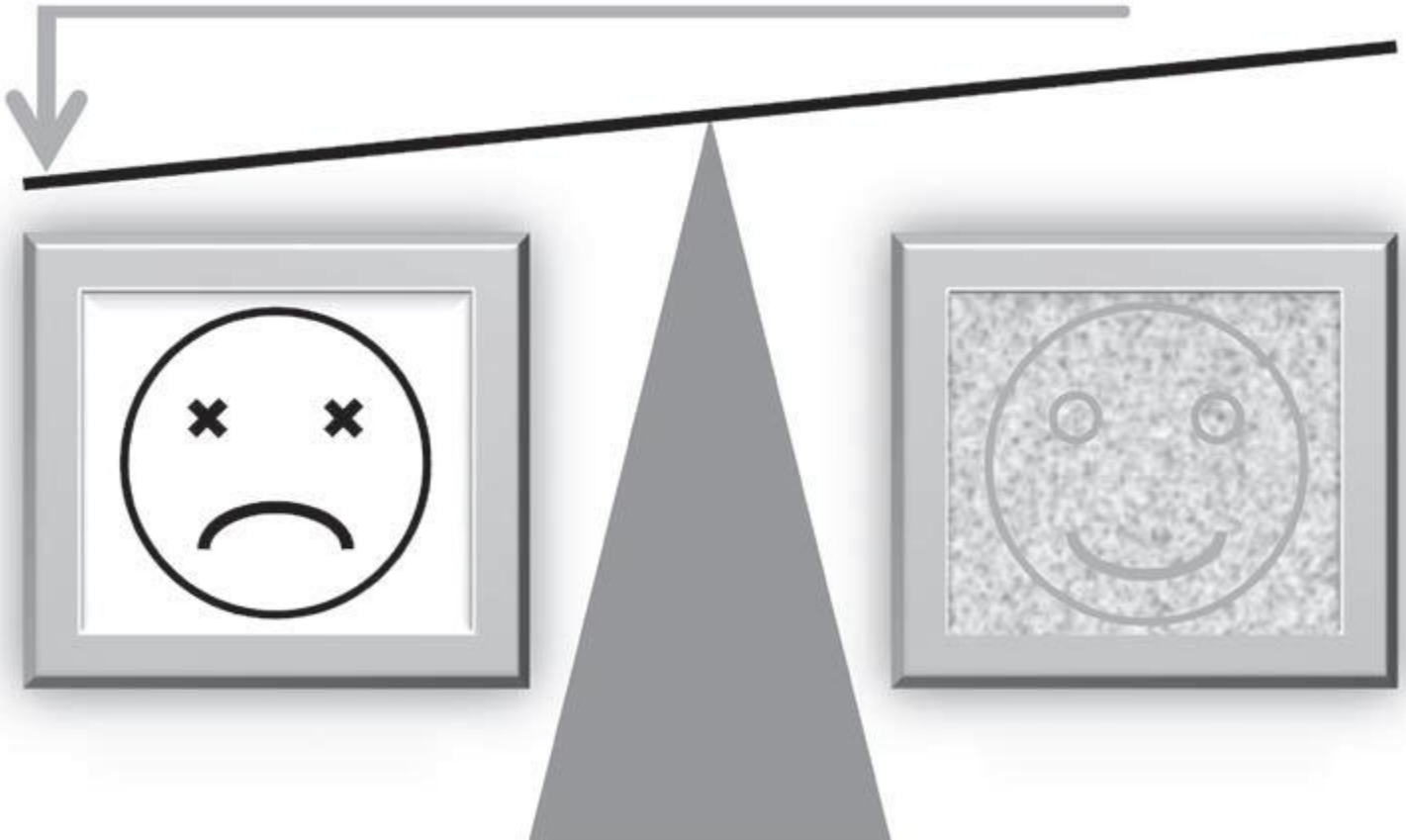
Intravital imaging is often a balancing act

More illumination:

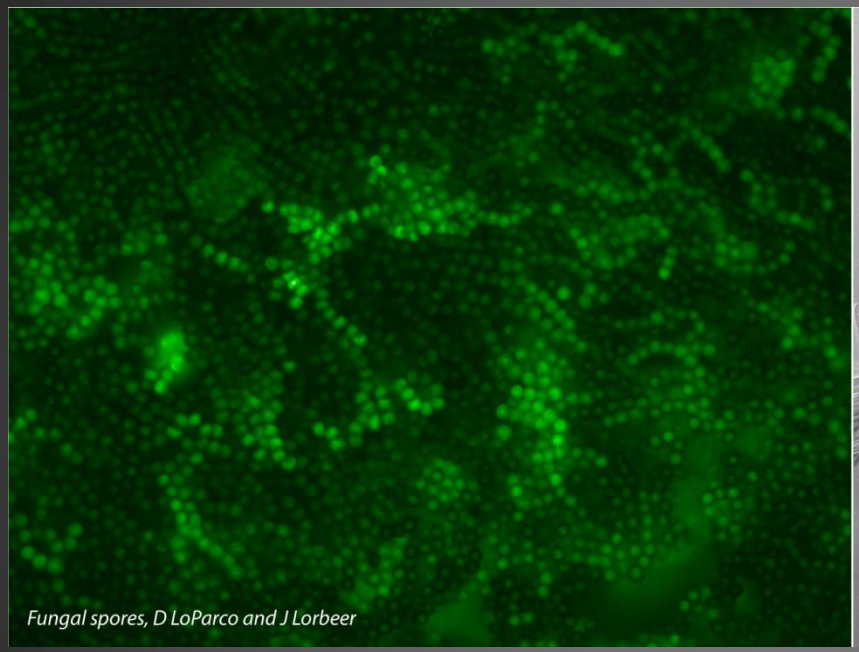
Better images

Less illumination:

Less phototoxicity
Less photobleaching



Basic microscopes, stereoscopes and spectroscopic tools



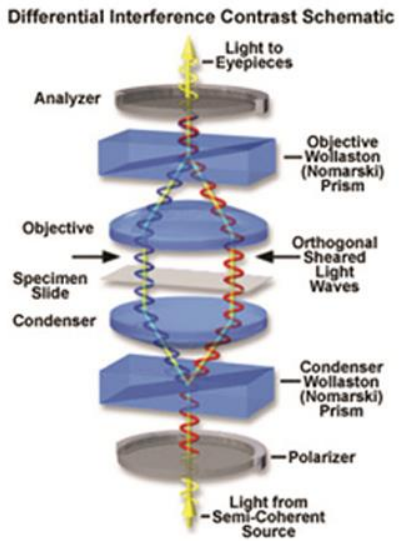


Olympus BX-50 (B46 Weill)
Olympus OV-10 0 (VRT 5013B)
PTI Spectrofluorometer

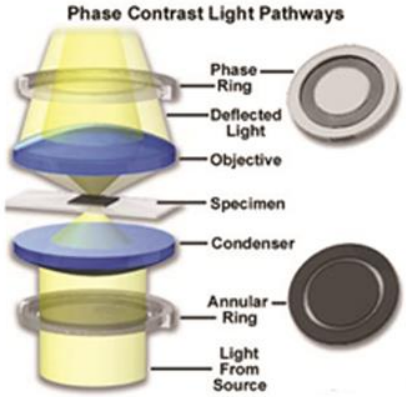
Standard fluorescence packs
Stereo microscopes
Polarization optics
DIC and phase optics

Phase Enhancement

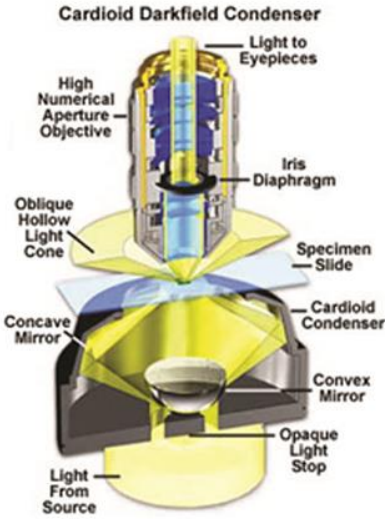
DIC

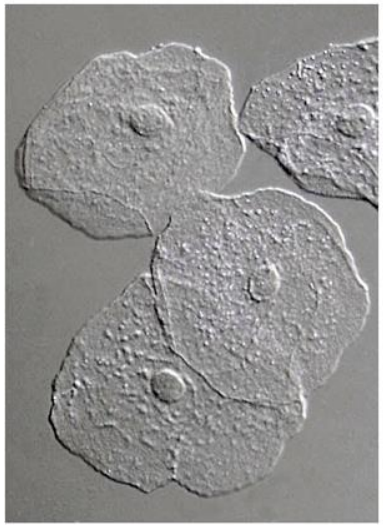


Phase

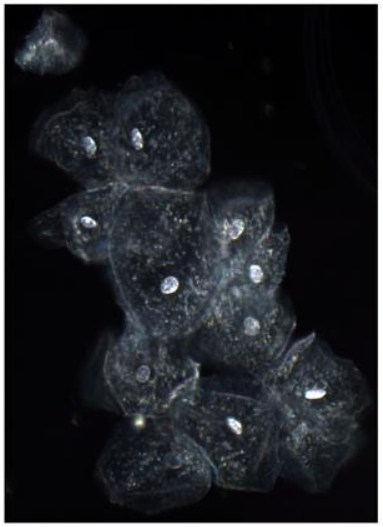


Darkfield

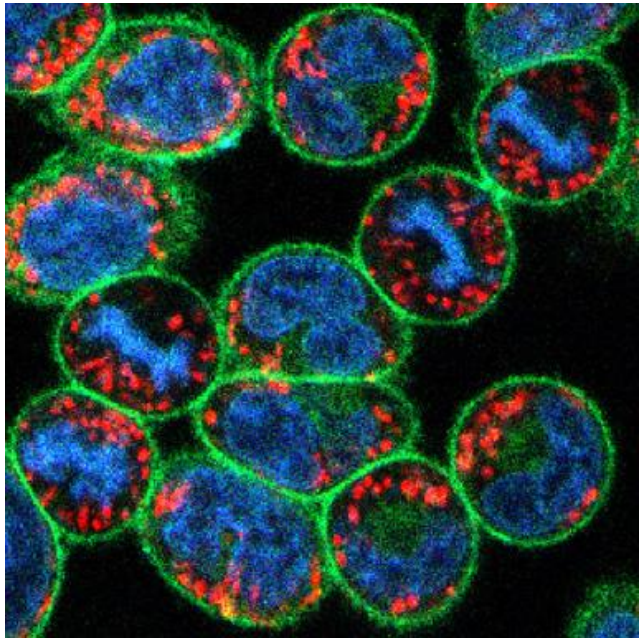








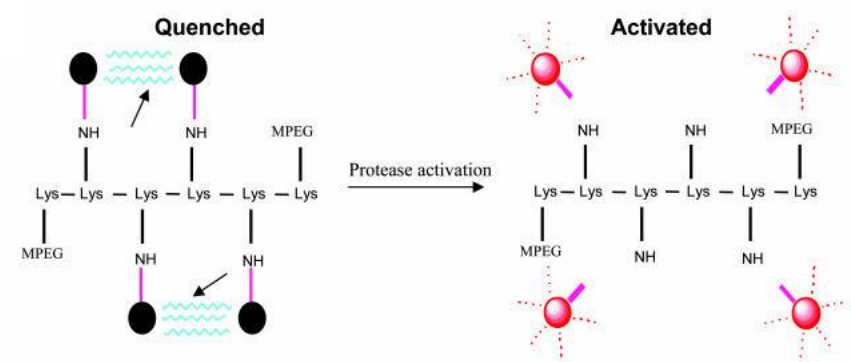
Fluorescence indicators



Organelle-specific fluorophores



Genetically-encoded fluorophores

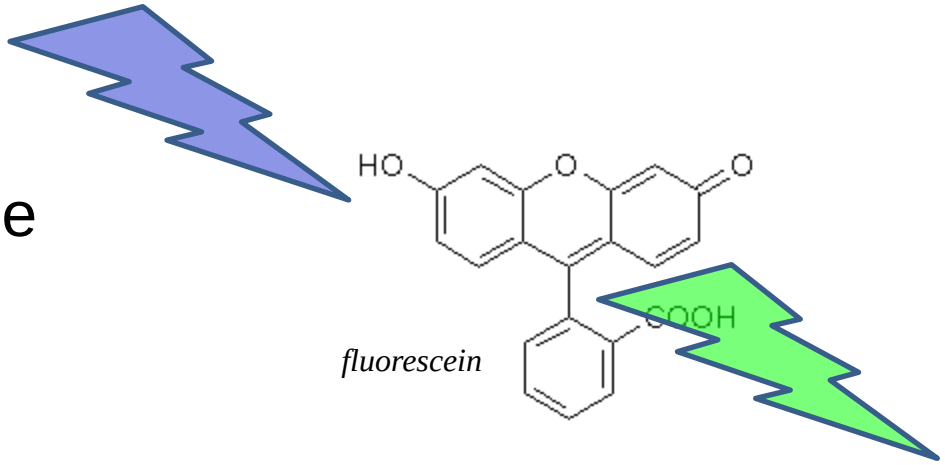


Enzymatic and ion indicators

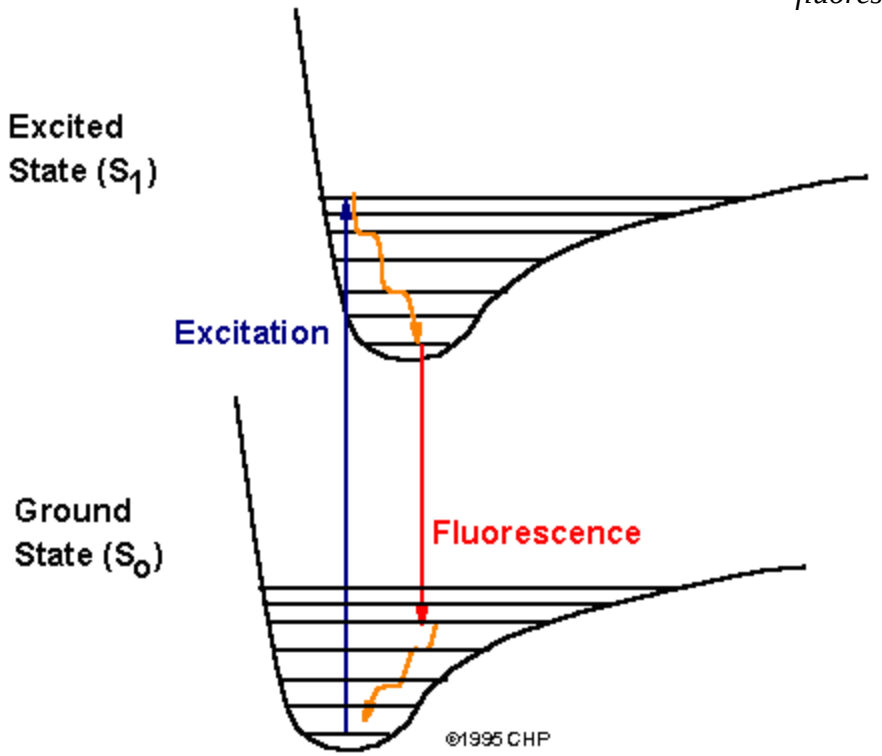


Photo-convertible fluorophores

Fluorescence



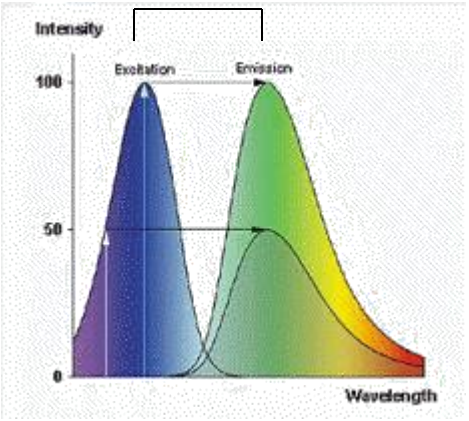
fluorescein



Energy →

Distance from nuclei →

Stokes shift



http://chemistry.rutgers.edu/grad/chem585/lecture2.html

For proteins – ANS, dansyl chloride rhodamine; fluorescein

For nucleic acids – acridine orange, ethidium bromide.

Most fluorophores in the visible and NIR have 2 or more “benzene ring” structures in them. Large delocalized pi orbital systems and many double (C=C) bonds make good fluorophores.

Check out:

Molecular Probes www.probes.com

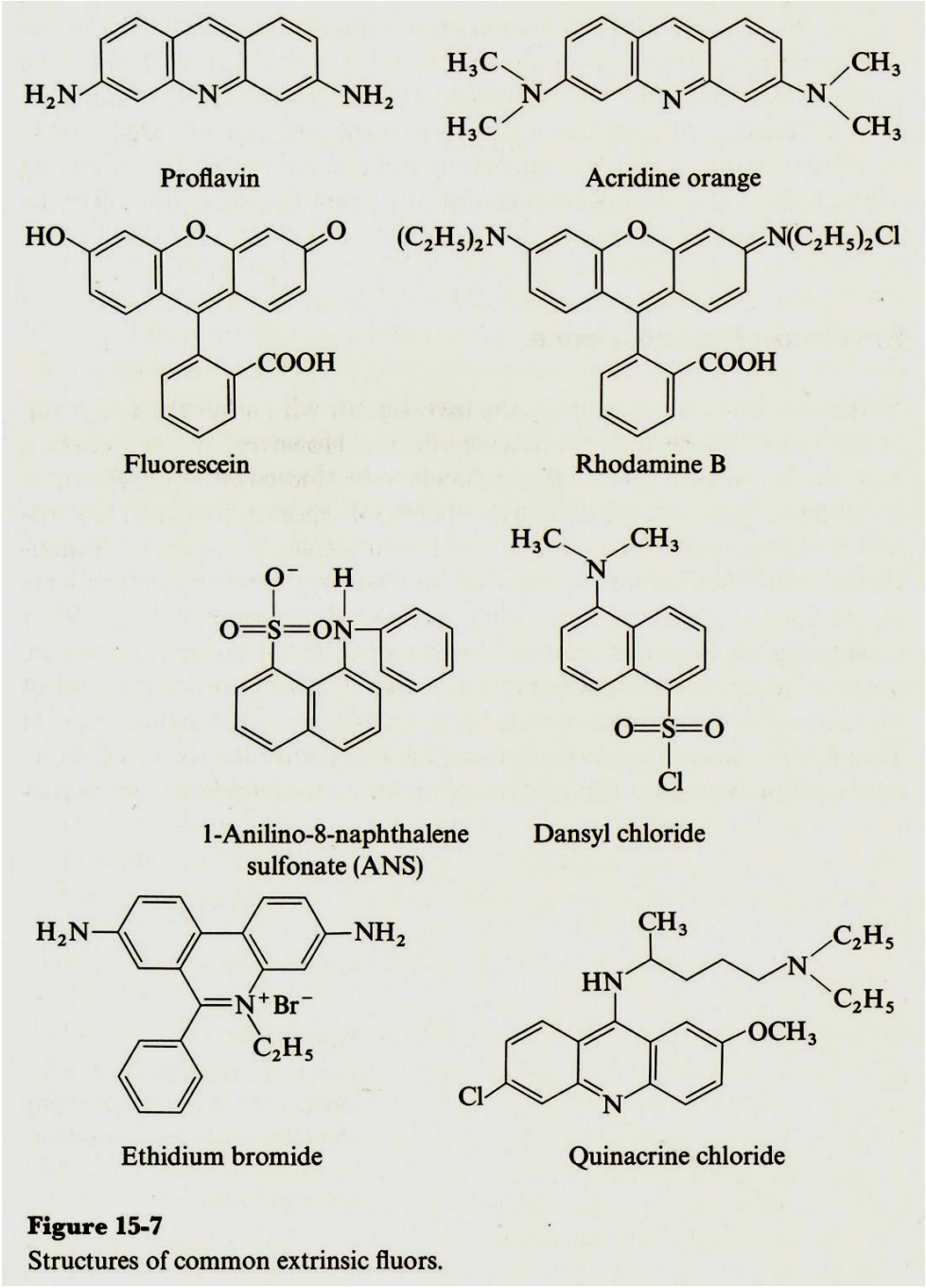
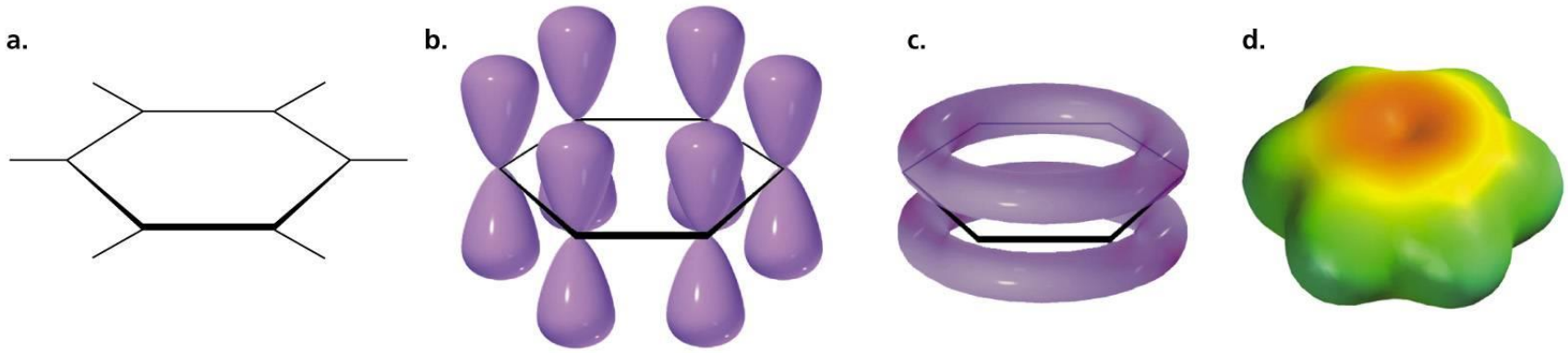
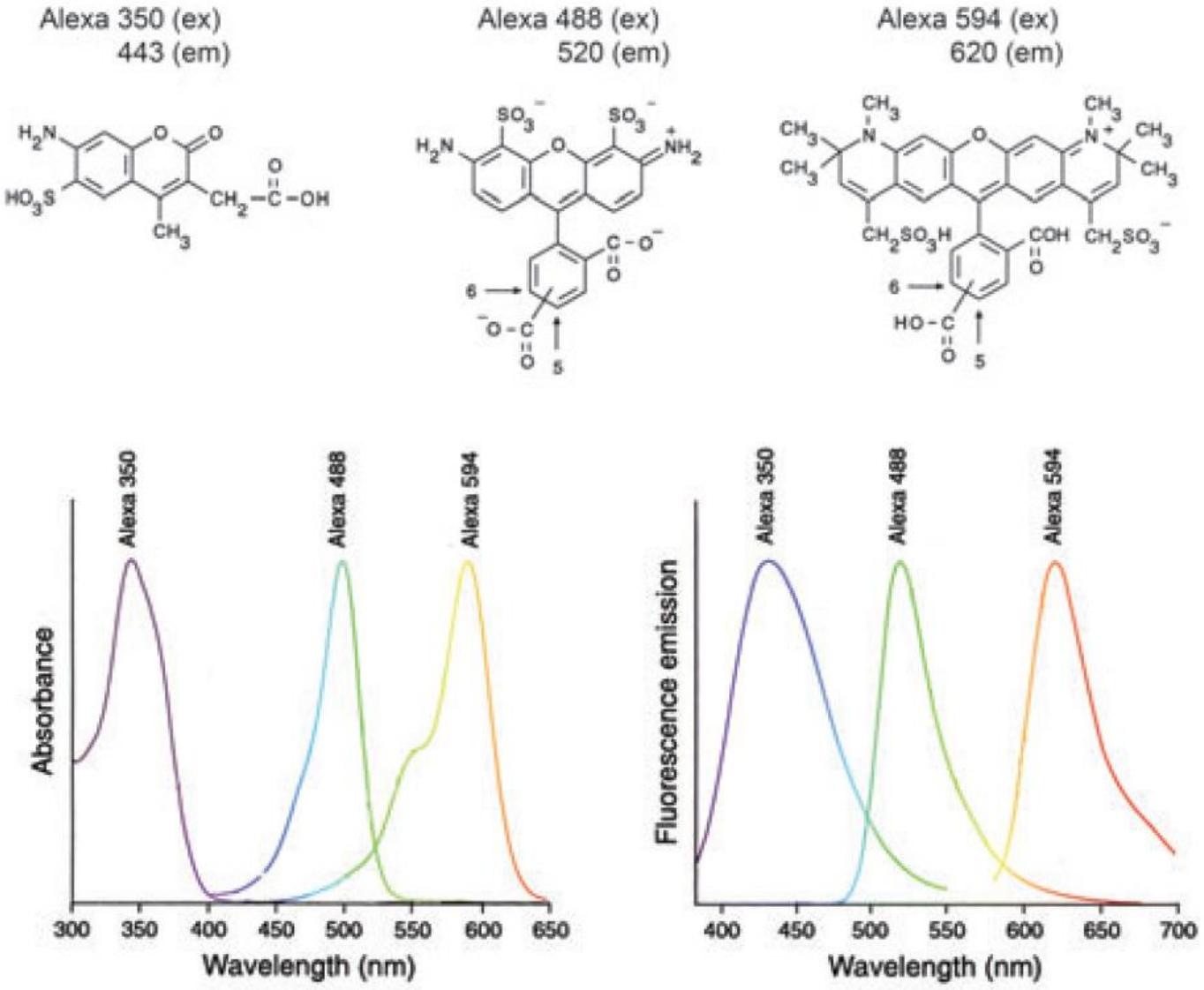


Figure 15-7
Structures of common extrinsic fluors.

delocalized pi-bonding system (in benzene)

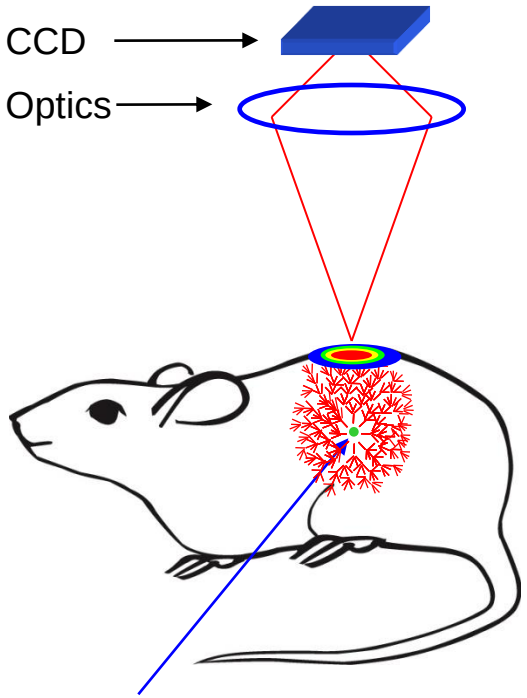


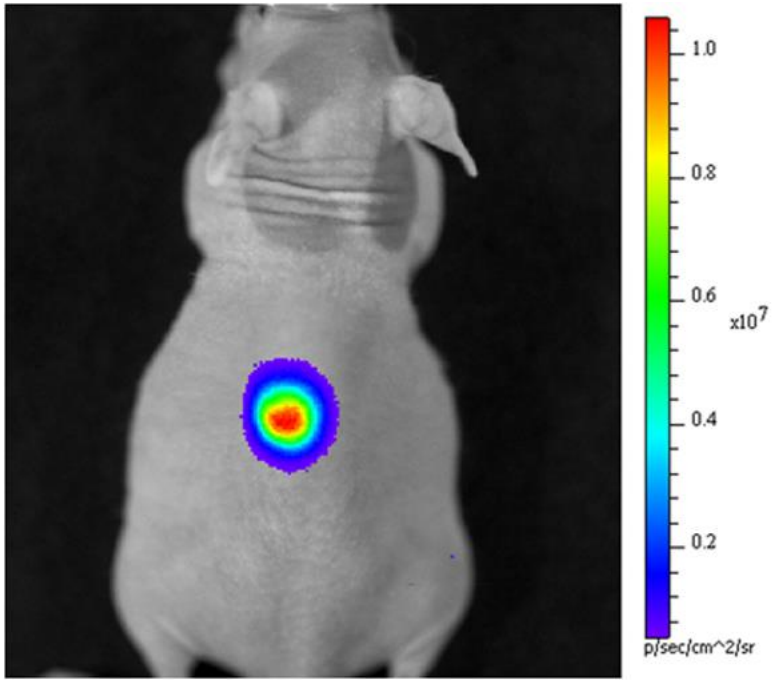
Fluorophore size



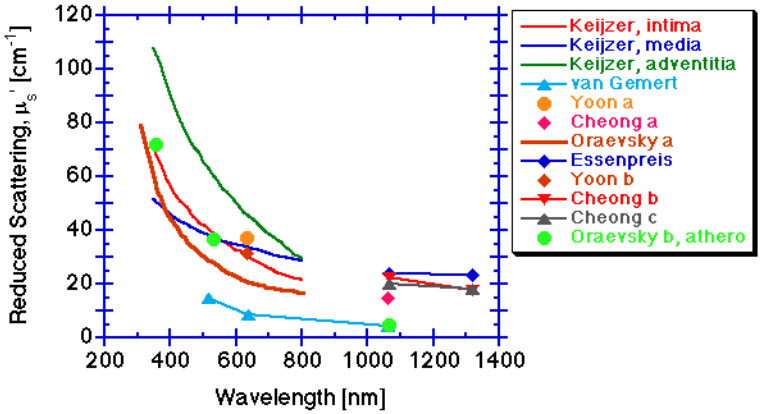
Lichtman, J.W., and J.A. Conchello (2005) Fluorescence microscopy. Nat Methods 2(12):910-919.

Whole mouse molecular imaging

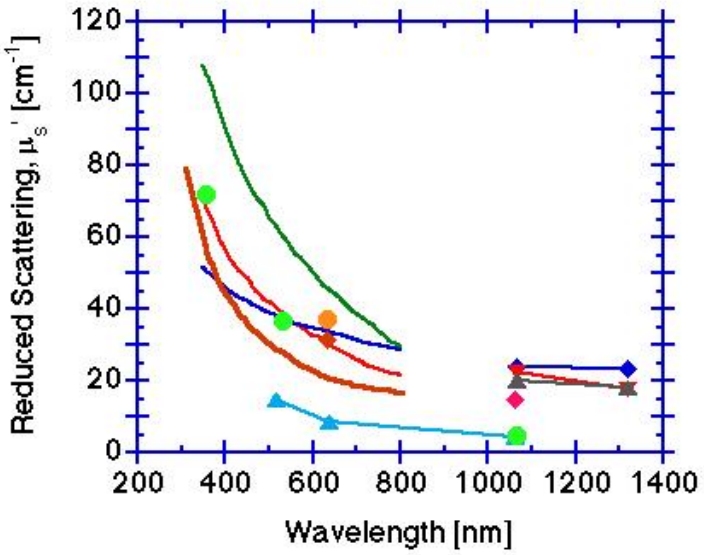




Tissue scattering in Aorta
http://omlc.ogi.edu/spectra/aorta/index.html

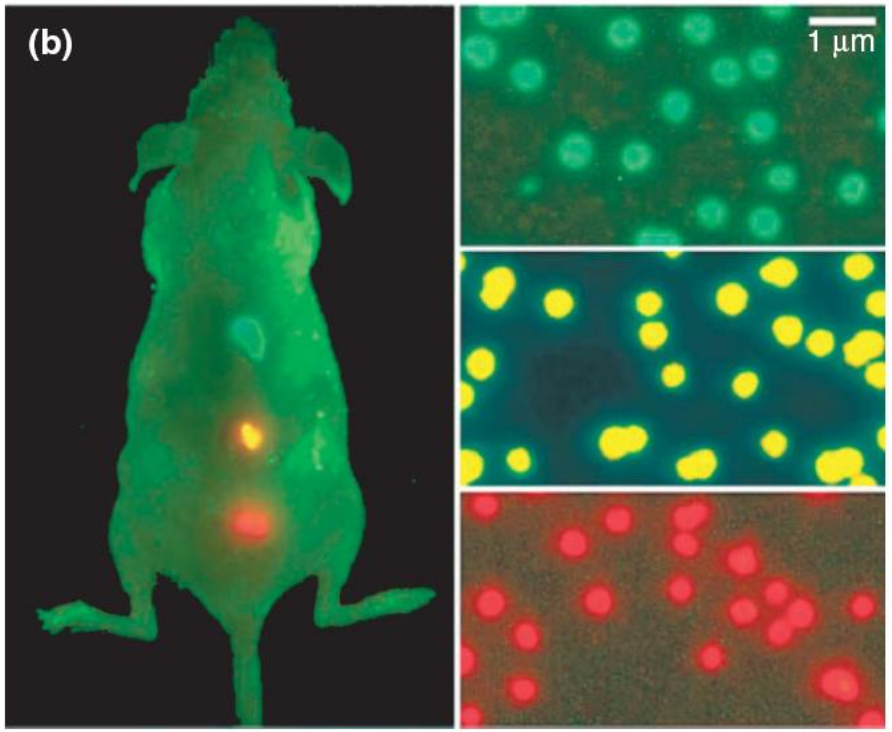


Trend towards IR probe development



Tissue scattering is lessened

(*Aorta, http://omlc.ogi.edu/spectra/aorta/index.html)*

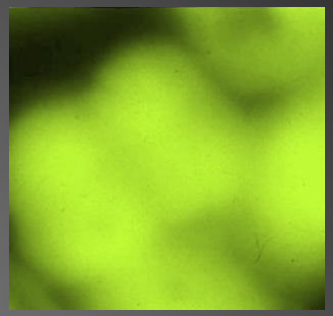


Autofluorescence less of a problem

*Nanomed Nanobiotechnol 2011 3:*11–32

Optical Sectioning in Biological Microscopy

Conventional light microscopy doesn't work well on thick (> few microns) specimens

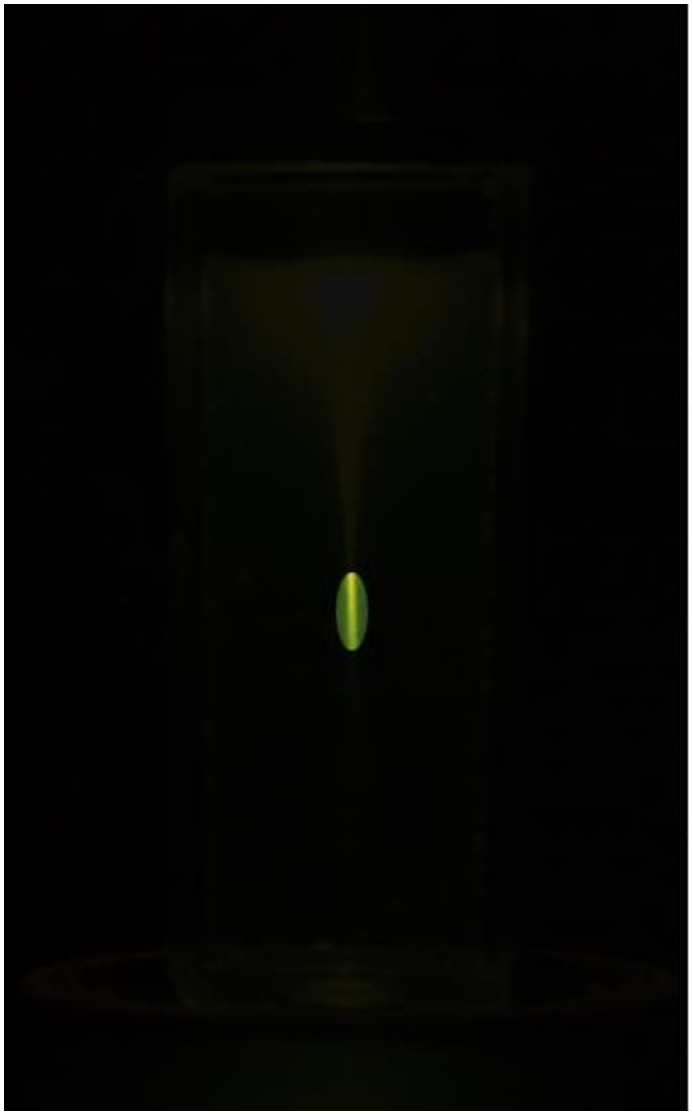


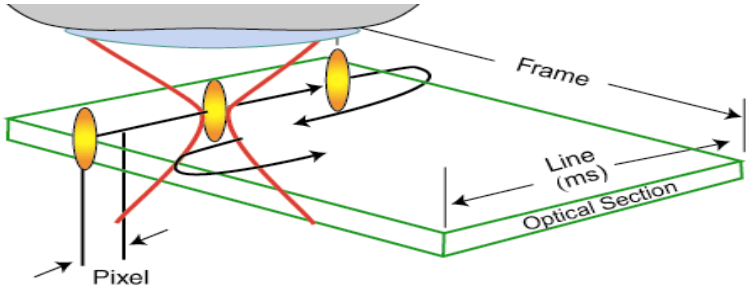
Live specimens

- Widefield Fluorescence → Fixation and Physical Sectioning
- Widefield Fluorescence → Deconvolution Methods
- Widefield Fluorescence → Selective Plane Illumination
- Widefield Fluorescence → Structured Illumination
- Confocal Microscopy → Confocal Aperture
- Multiphoton Microscopy → Nonlinear Optical Processes

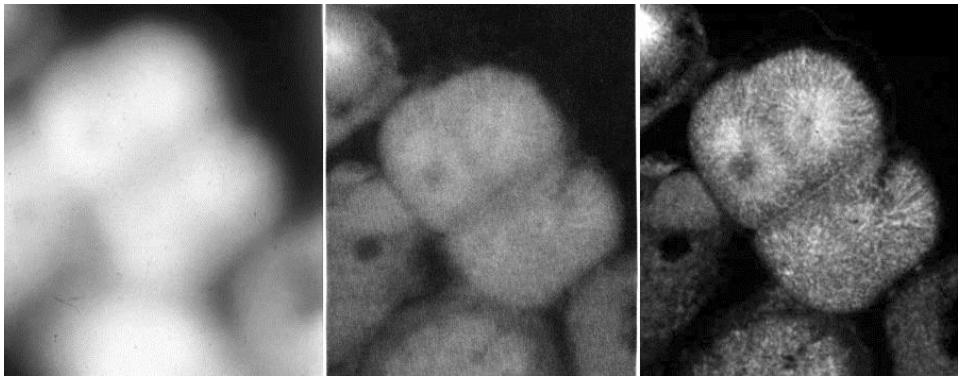


Confocal microscopy



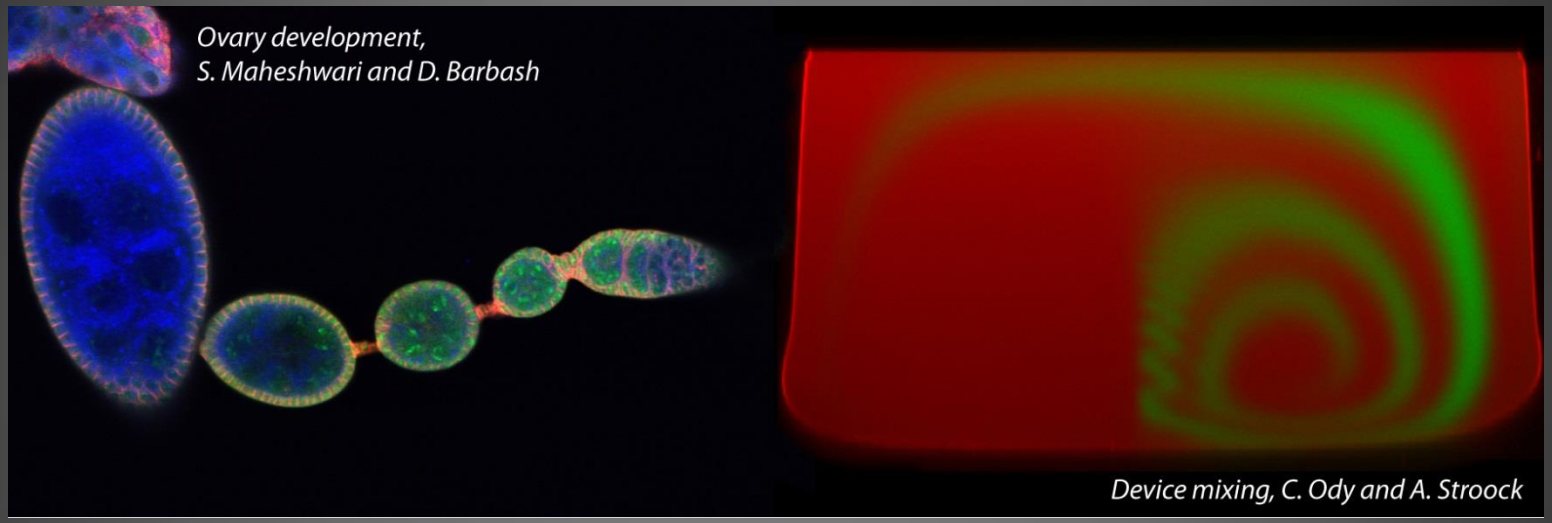


Widefield 23 ou 3.3 ou



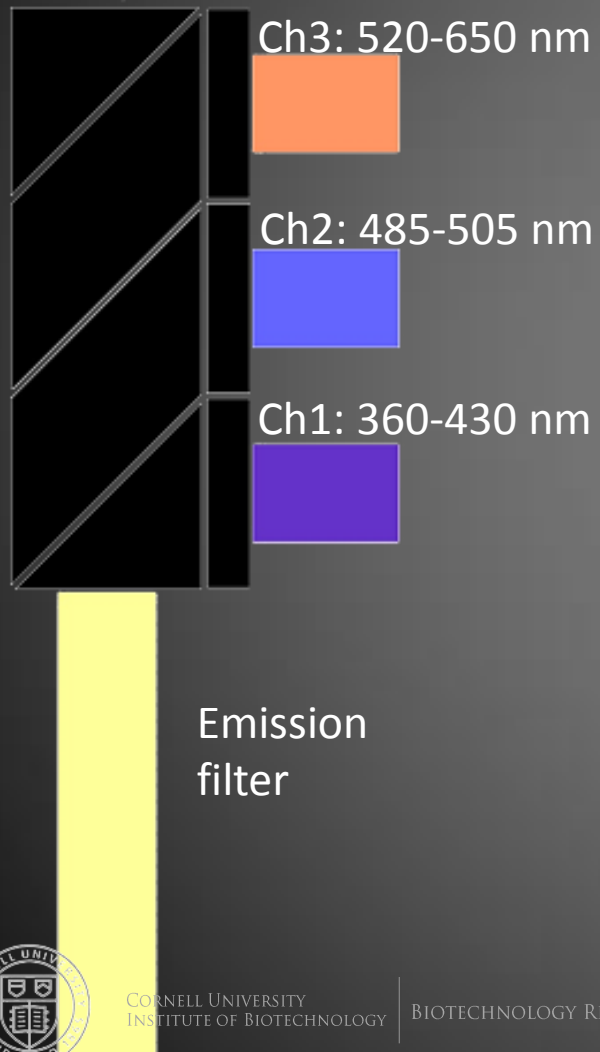
Confocal aperture

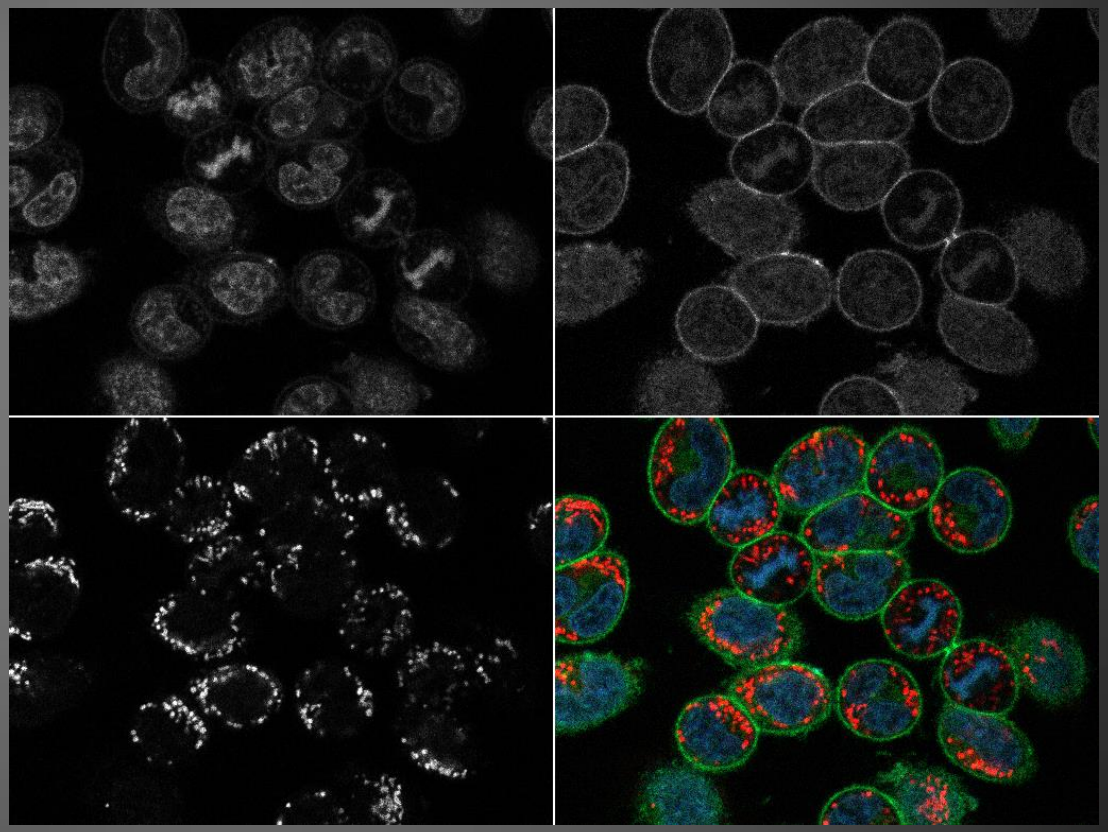
Confocal microscopy



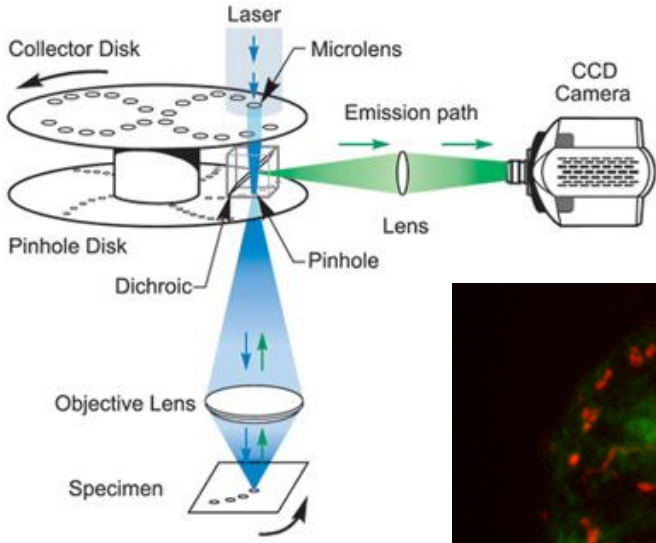
Zeiss LSM710 (B46 Weill)
Zeiss LSM510 (VRT 5008A)
Leica SP2 (B46 Weill)
Andor Revolution Spinning Disk (B46 Weill)

RGB *merged* image comes from 3 separate channels of data merged into Red, Green and Blue channels



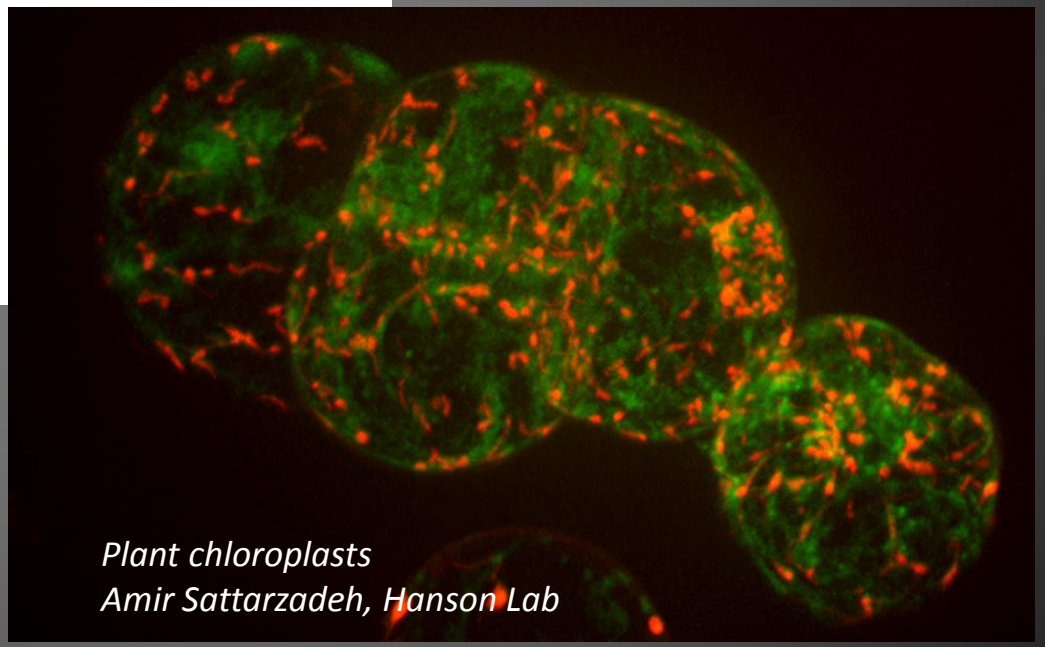


Spinning disk confocal (Andor Revolution)



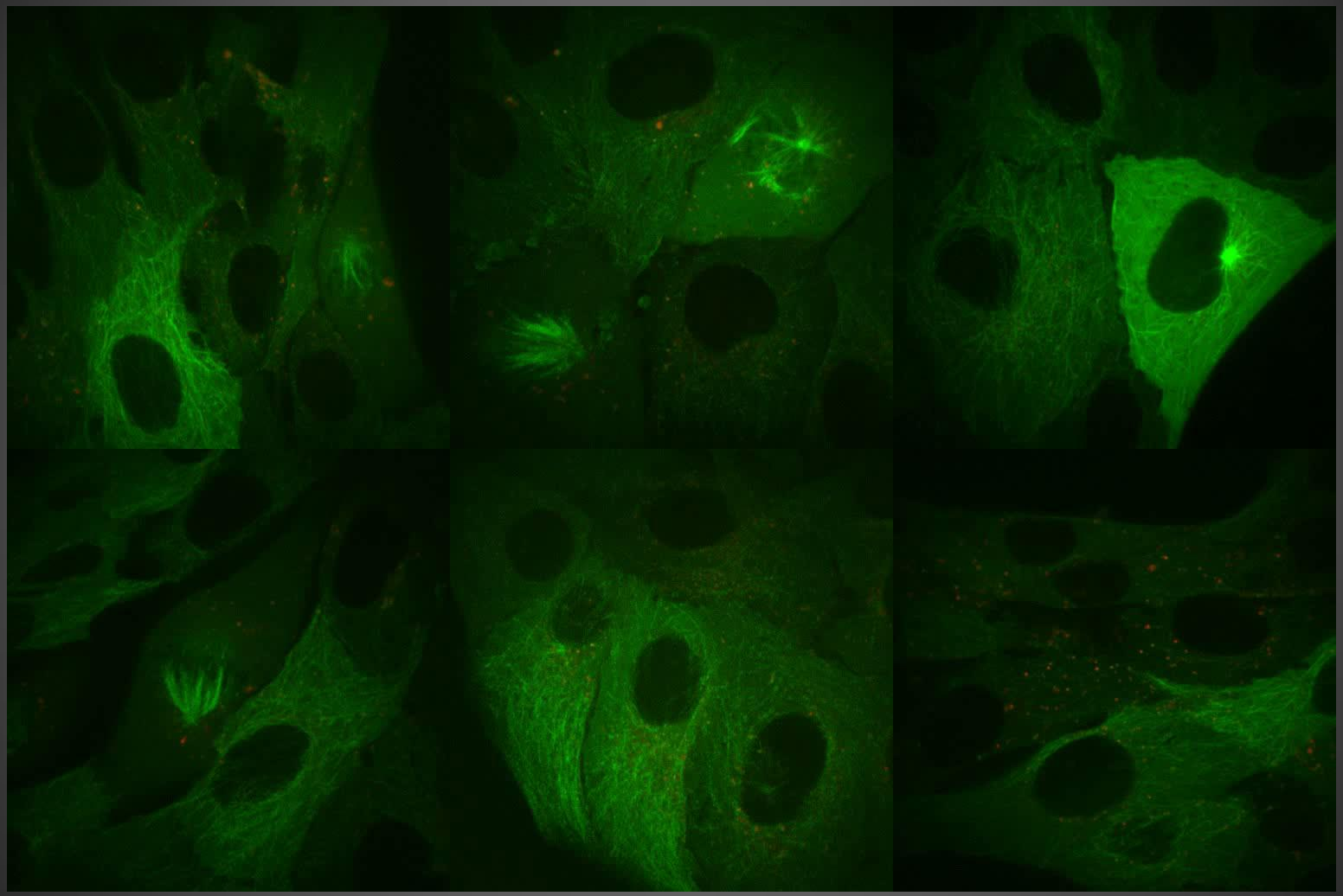
New!

- *~100 images/sec*
- *Fast piezo-electric focusing*
- *Automated stage*
- *Environmental chamber*
- *Targeted photo-activation*



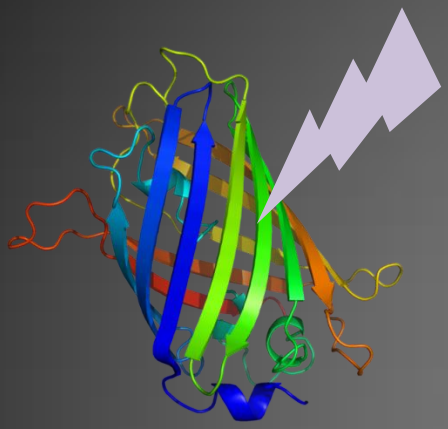
Plant chloroplasts
Amir Sattarzadeh, Hanson Lab

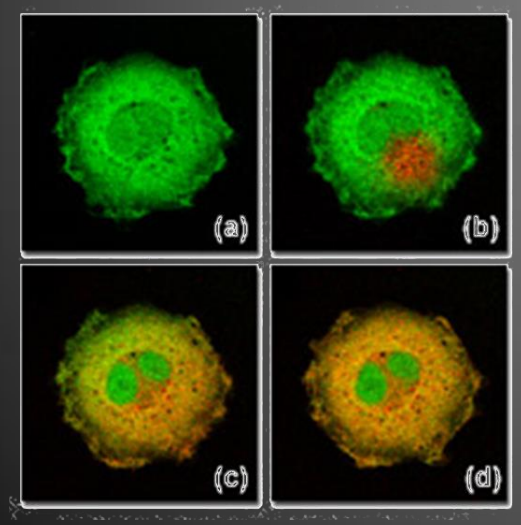
Simultaneous fields-of-view timelapse. . . .

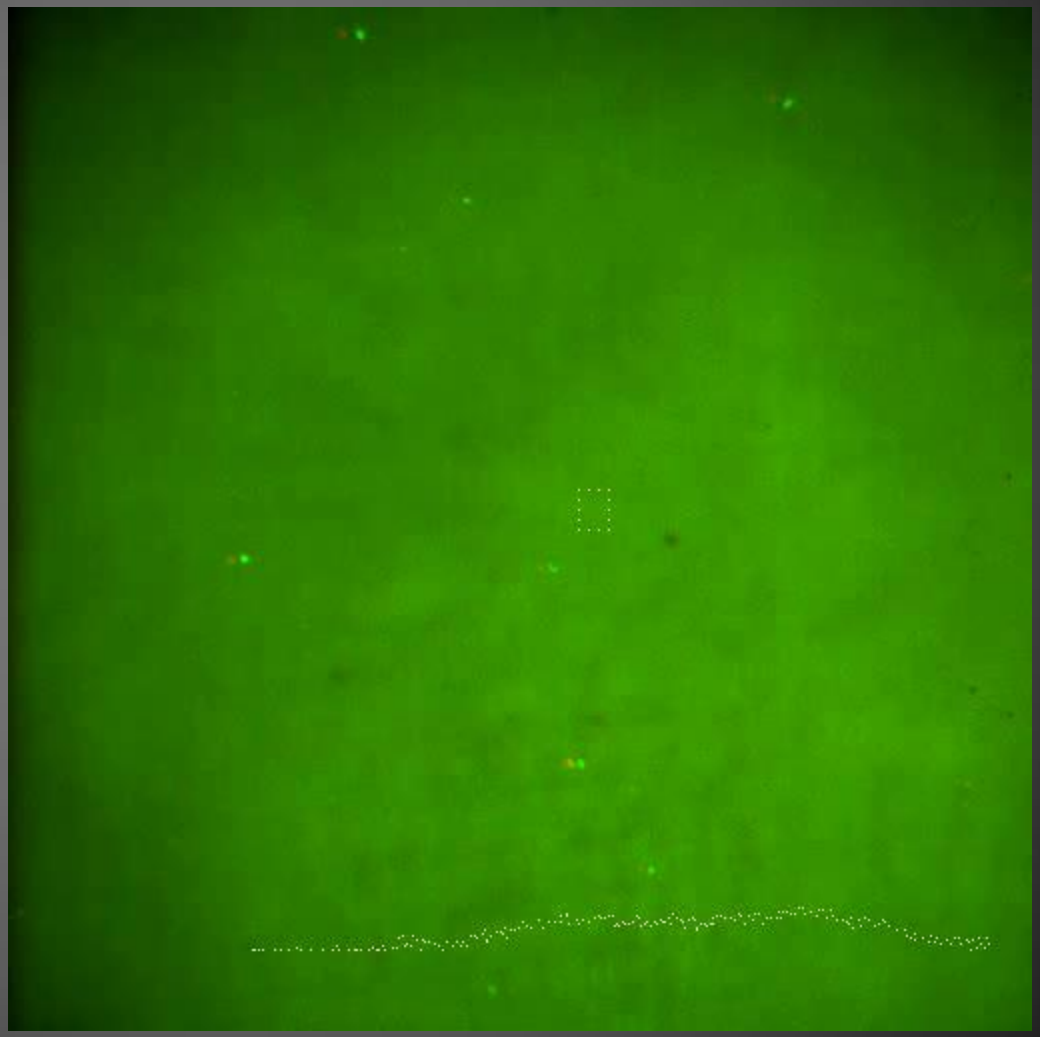


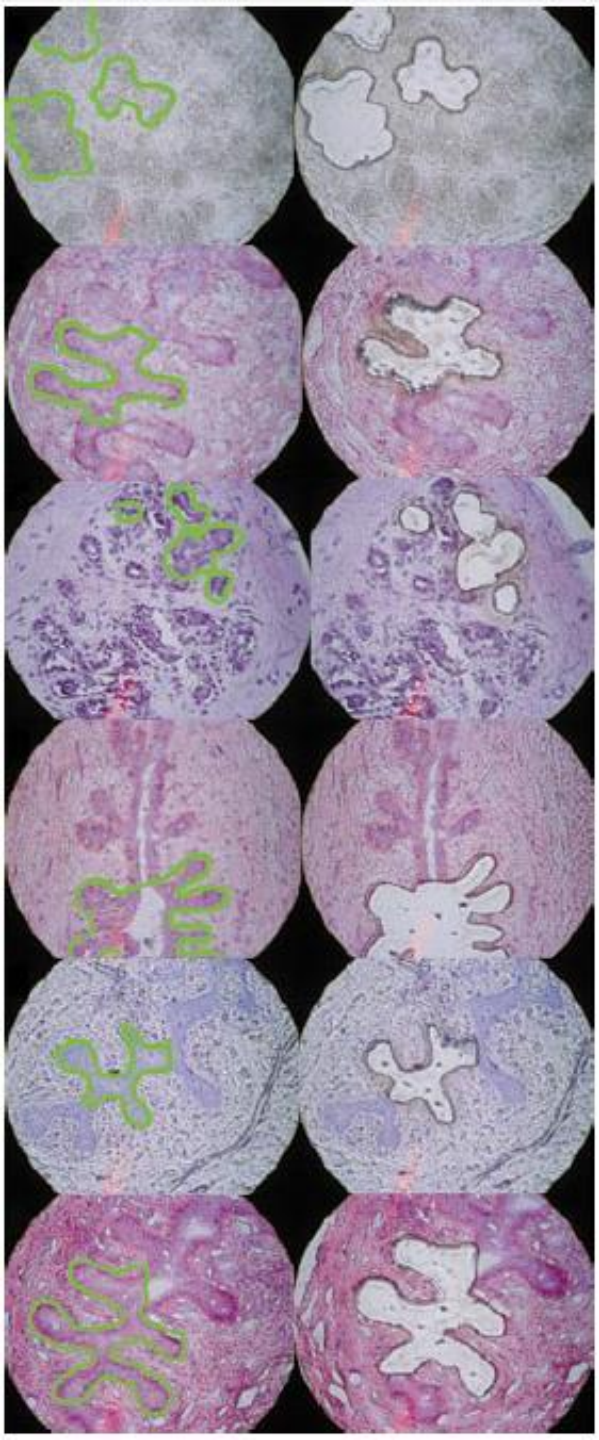
Fast mobility measurements (mEOS)

slowed 2x for viewing



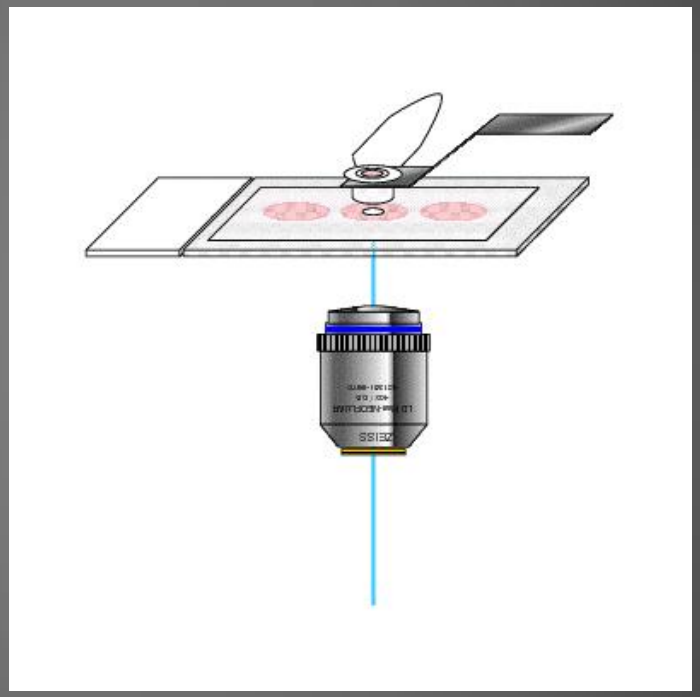






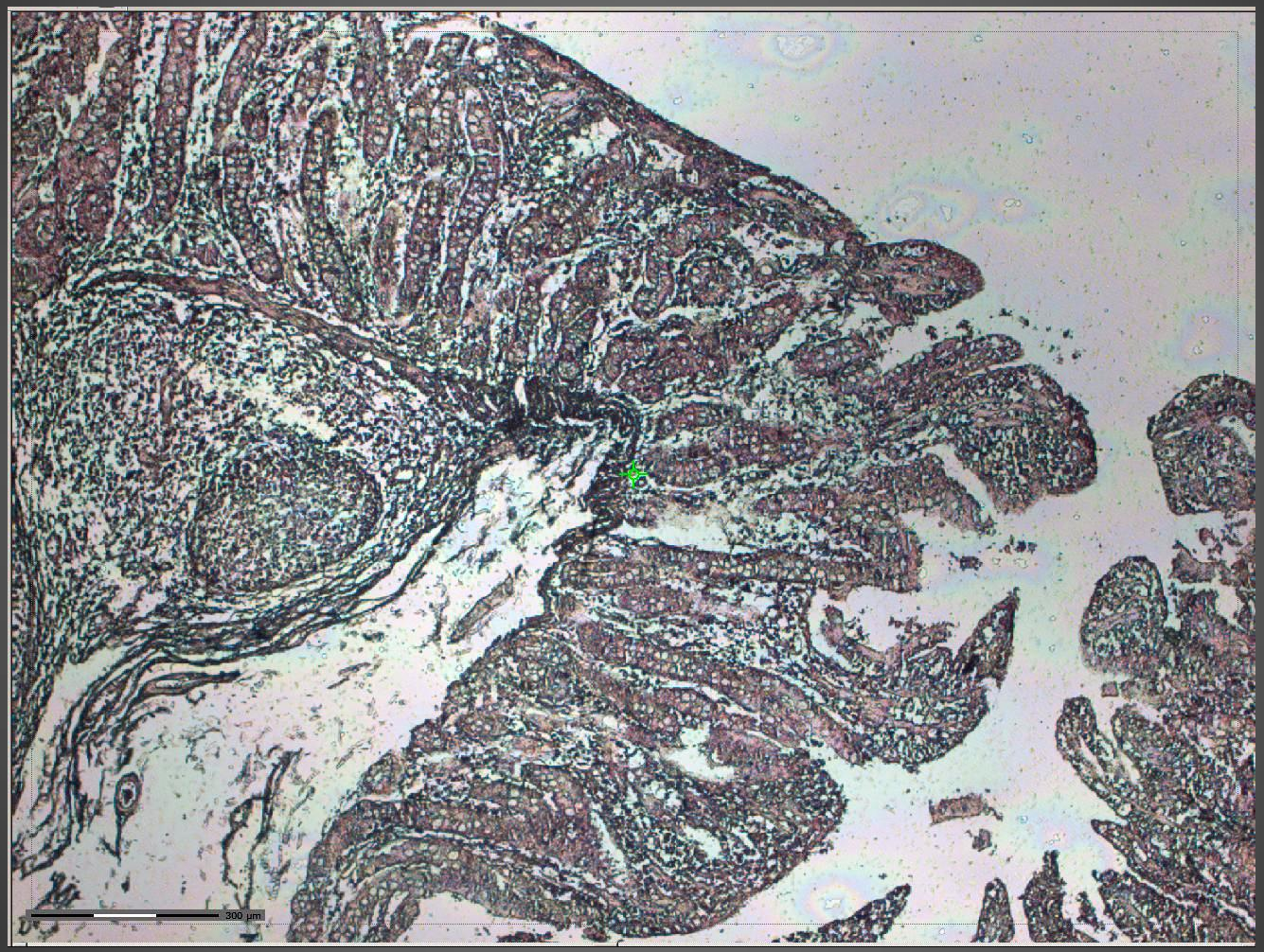
New!

Laser capture microdissection (Zeiss)

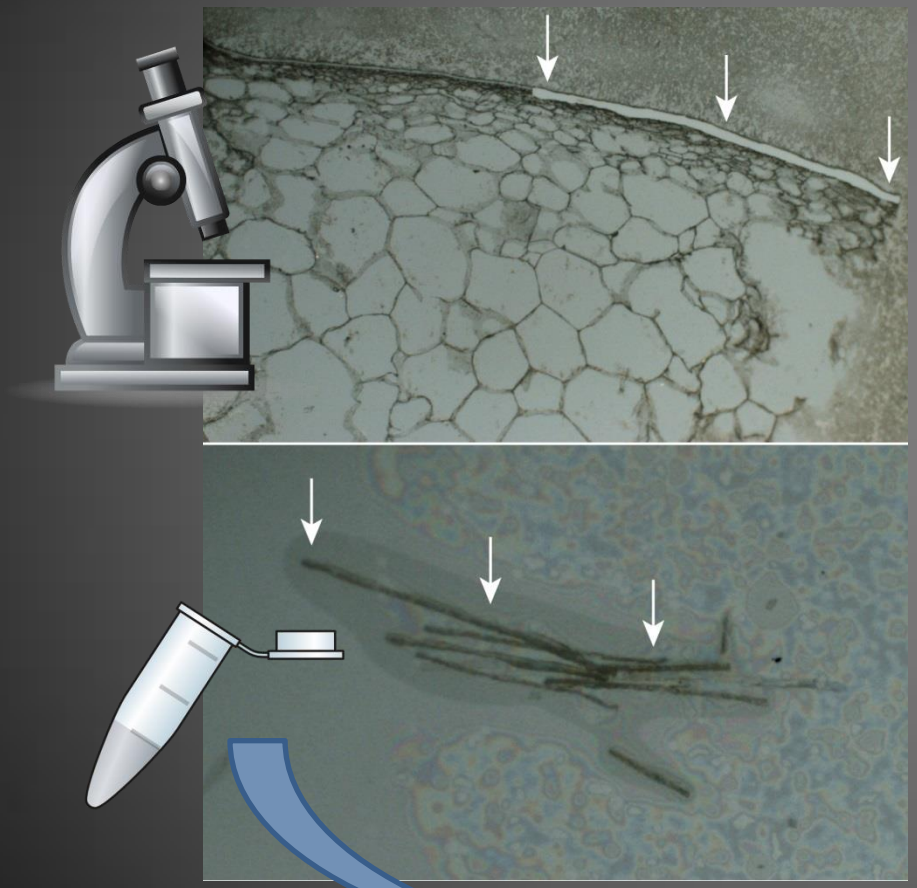


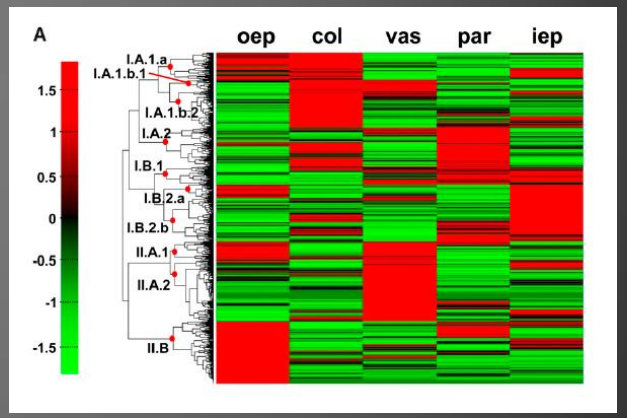
fresh frozen breast tissue
BioTechniques, Vol. 43, No. 1, July 2007, pp. 41–48

Laser capture process



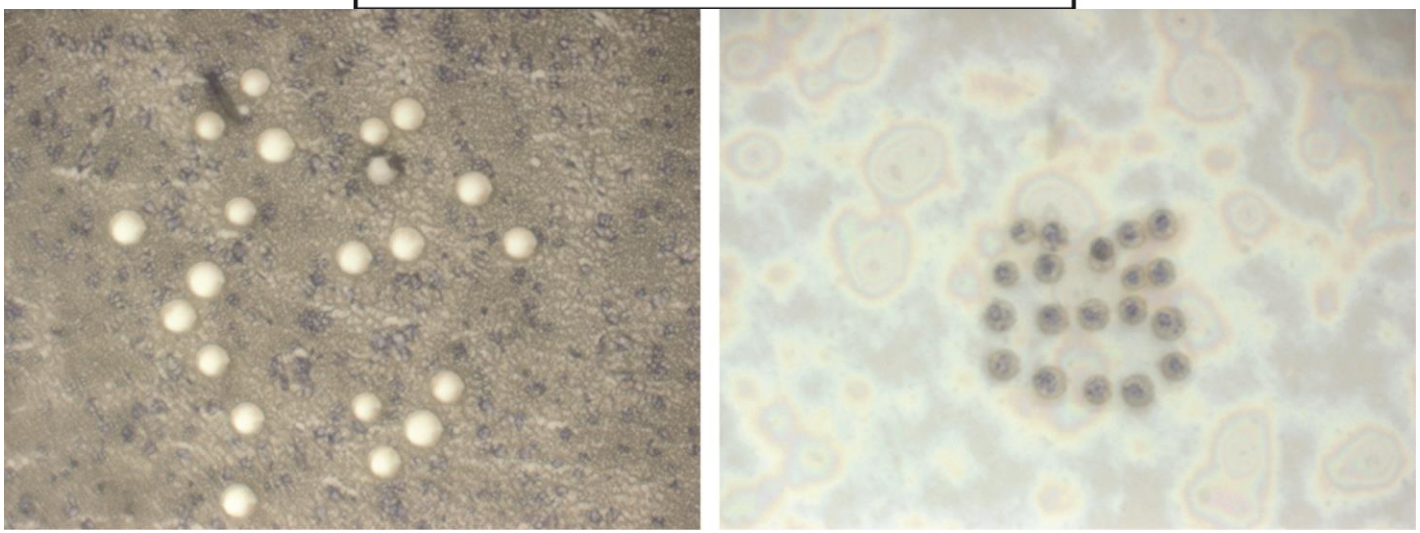
Tomato RNA analysis . . . Rose Lab





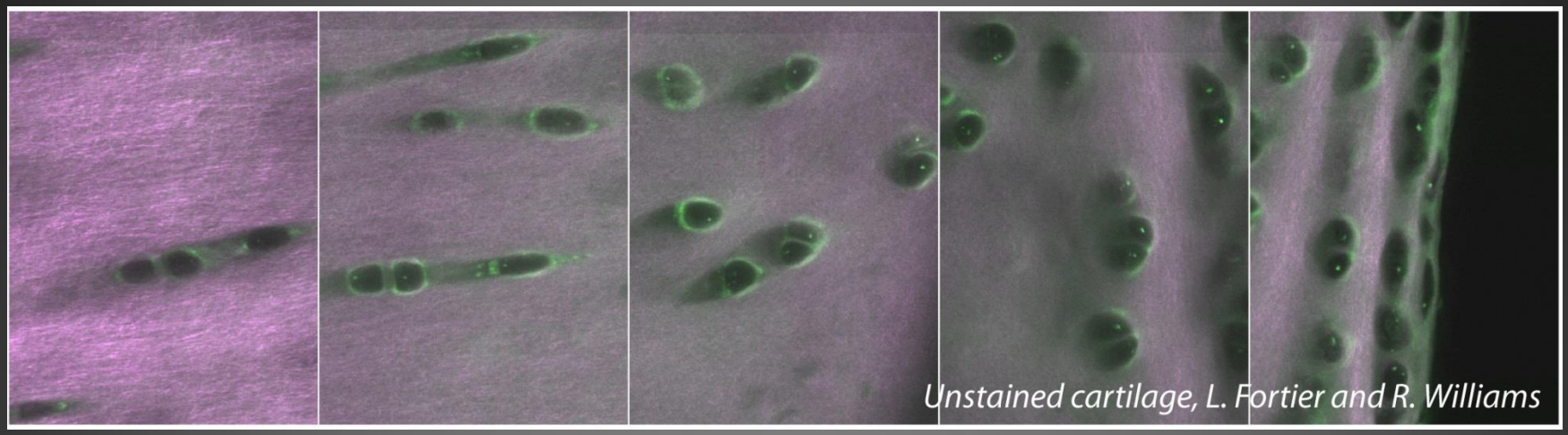
To Genomics Facility

Laser capture microdissection
(Zeiss microBeam LCMD)



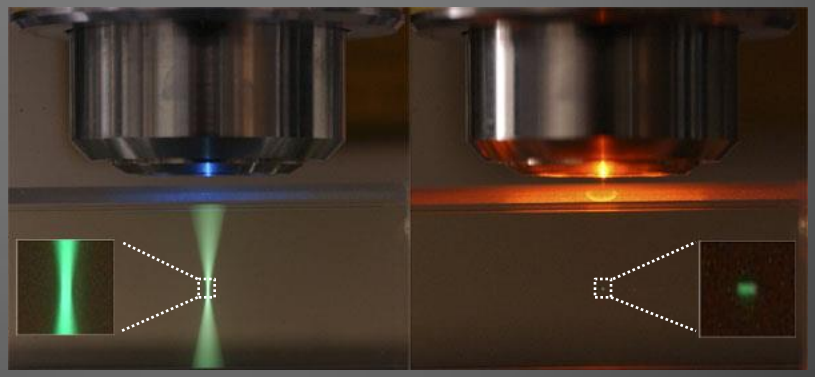
Single cells collected from brain slice

Multiphoton microscopy



Custom built (B46 Weill)

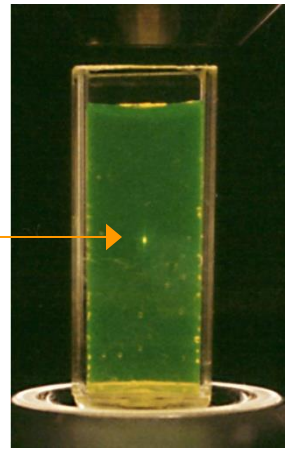
1P

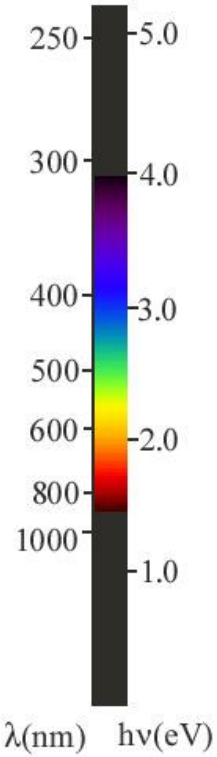


2P

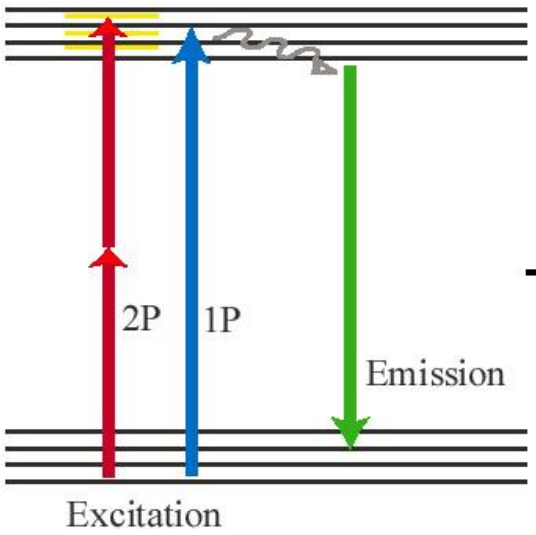
Multiphoton microscopy: Photophysical principles

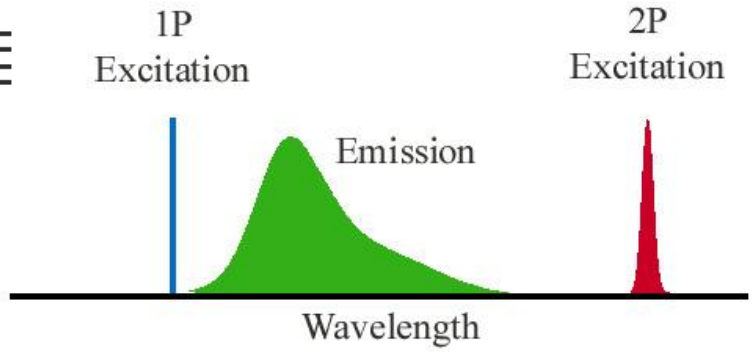
predicted in 1931 by Maria Göppert-Meyer in her PhD thesis



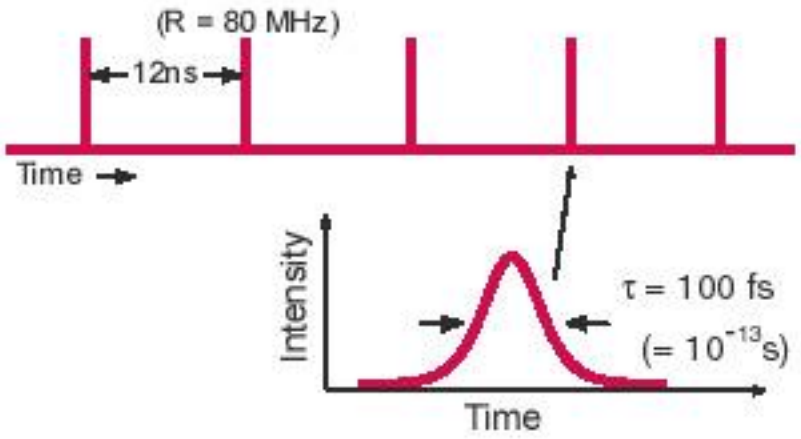


Simultaneous absorption of two lower energy photons produces an excitation equivalent to the absorption of a single, higher energy photon.

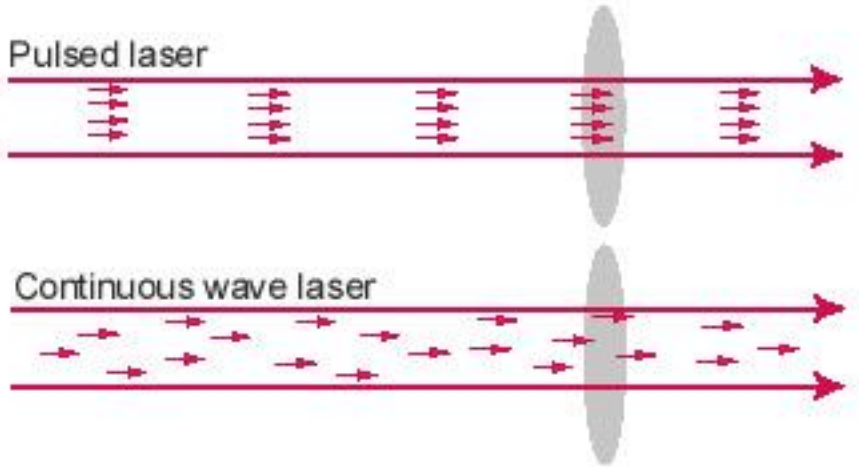




Multiphoton microscopy: Pulsed lasers



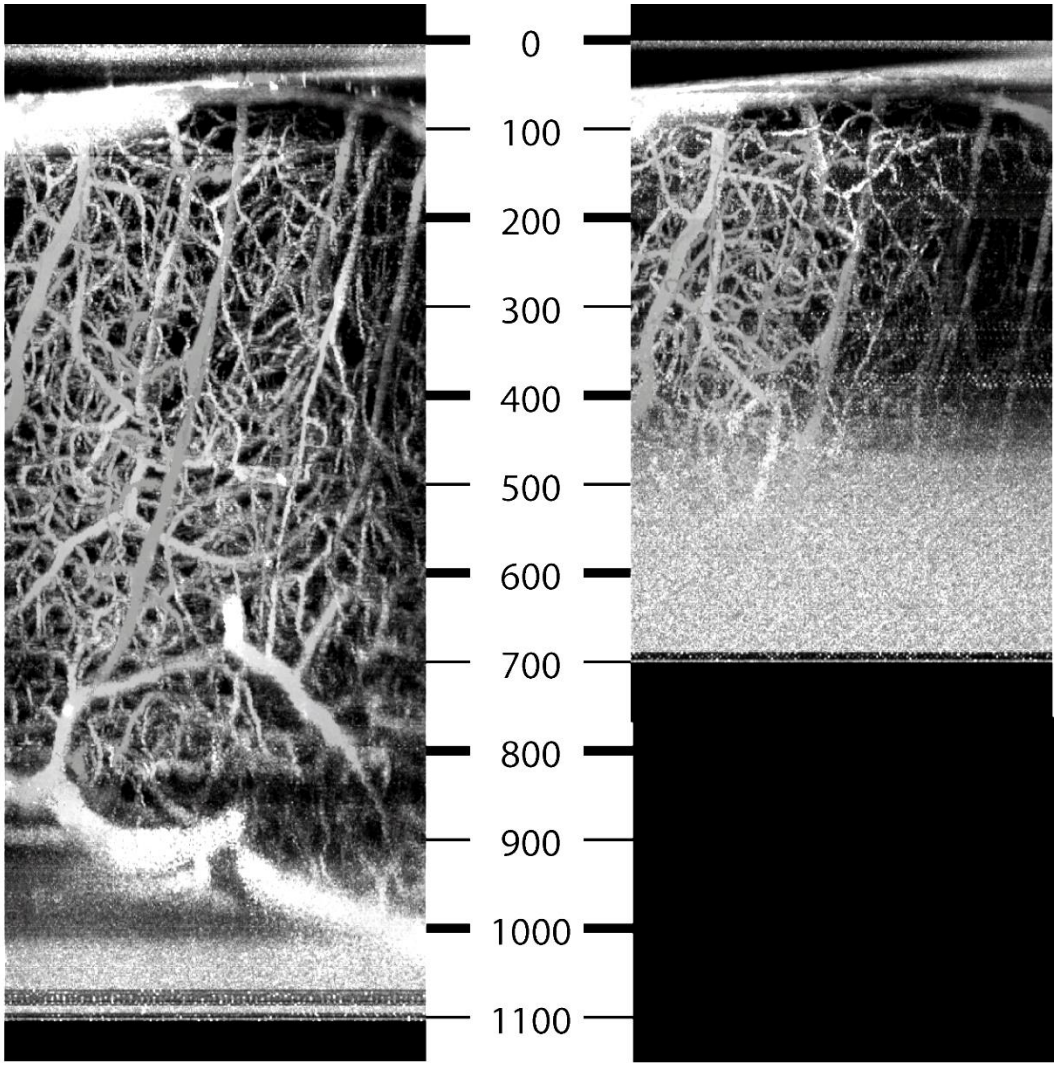
Pulsed lasers are used for optimal multiphoton excitation with minimal average power



The frequency of multiphoton excitation events is typically 10^5 times greater with the same ave power.

$$\frac{100\,\text{fsec}}{1\,\text{sec}} \sim \frac{1\,\text{sec}}{3000\,\text{years}}$$

MPM of brain vasculature (mouse)



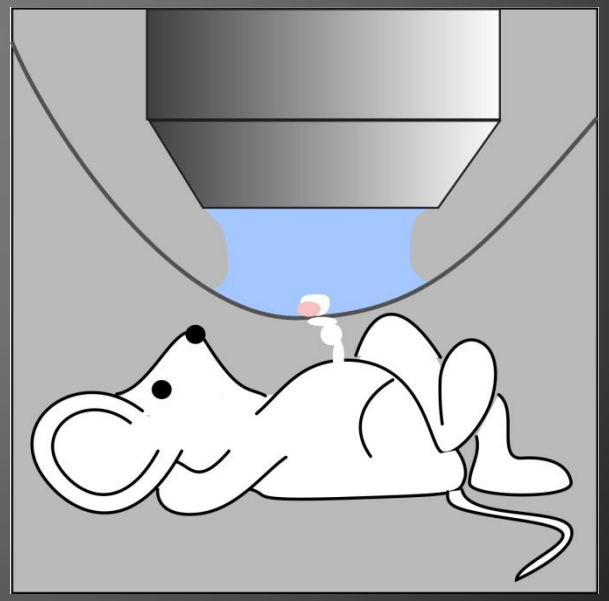
1280 nm: No observable damage with 100 mW at sample surface.

775 nm: Blood vessel damage with 60 mW at sample surface.

Xu and Schaffer labs

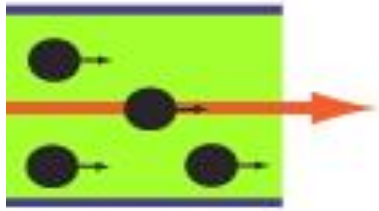
Multiphoton microscopy of Bloodflow in mouse ovary

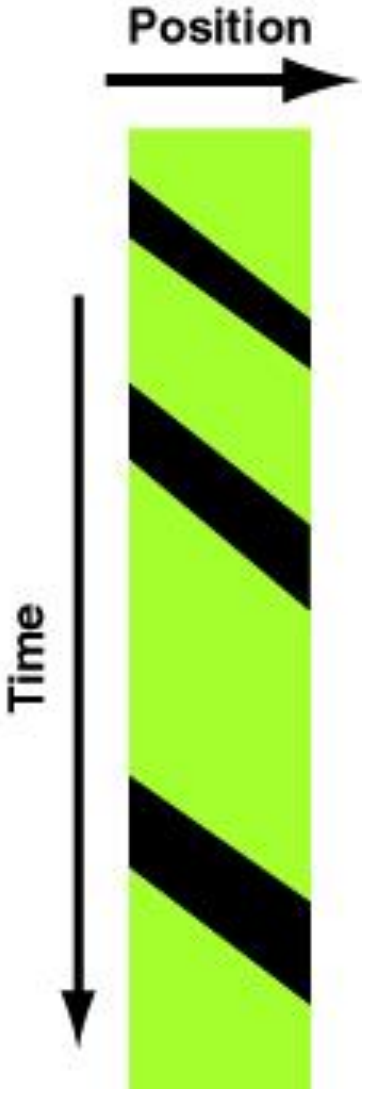
Bob Cowan & Fernando Migone,
Quirk laboratory

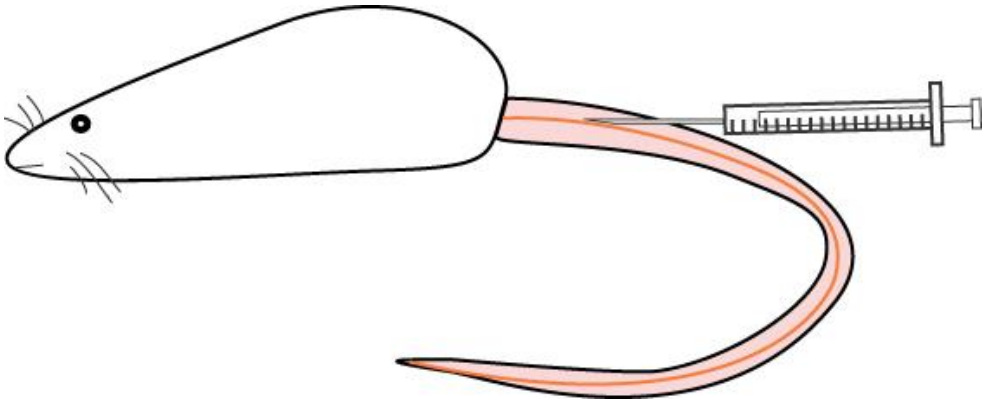


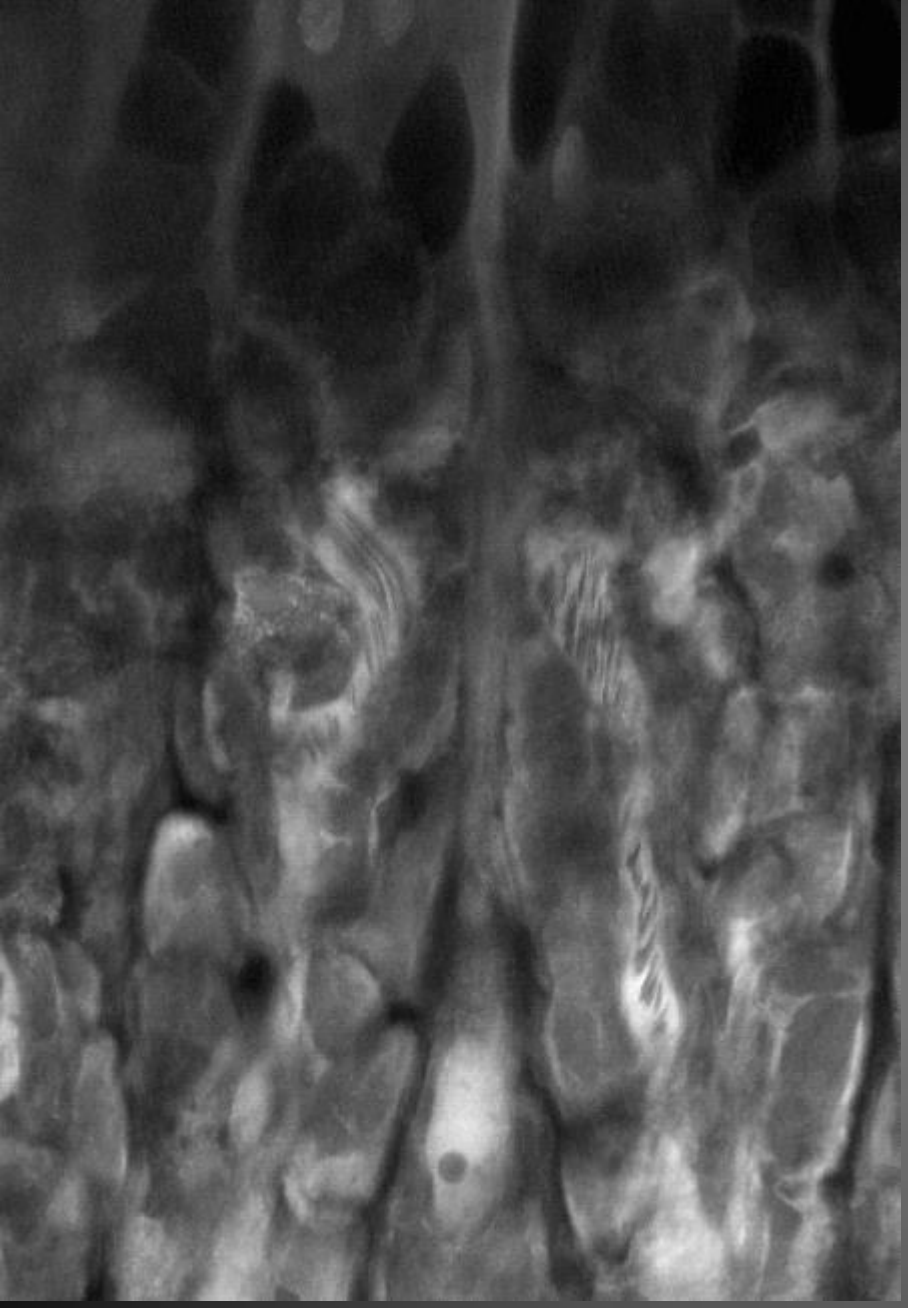
Measuring blood velocity with multiphoton fluorescence angiography

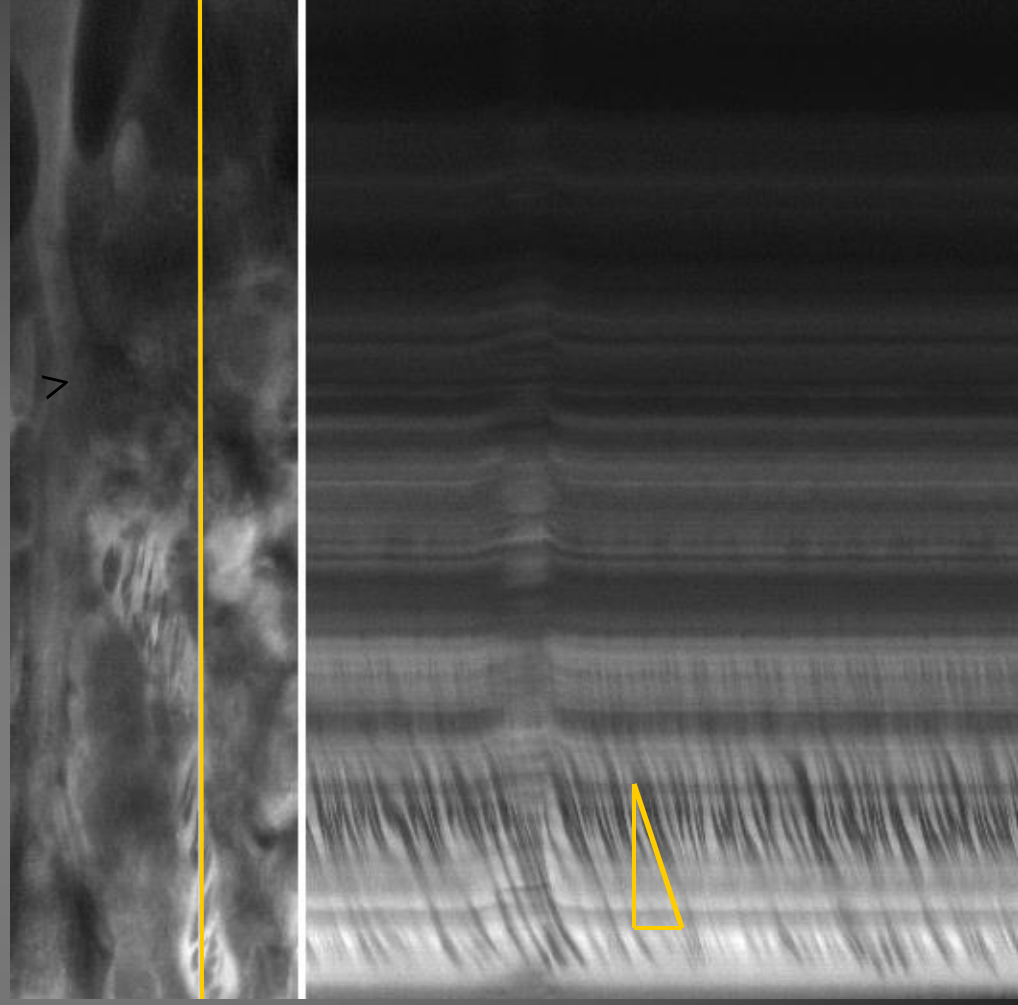
Injection of water-soluble tracers produces capillary images in which red blood cells appear as shadows within the tracer-rich plasma. The laser is repeatedly scanned in a single line at ~2 ms/line along a capillary. The blood velocity is measured by analyzing the slope of the shadows.

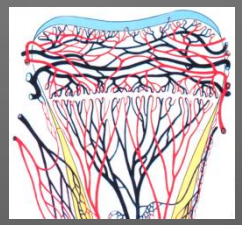










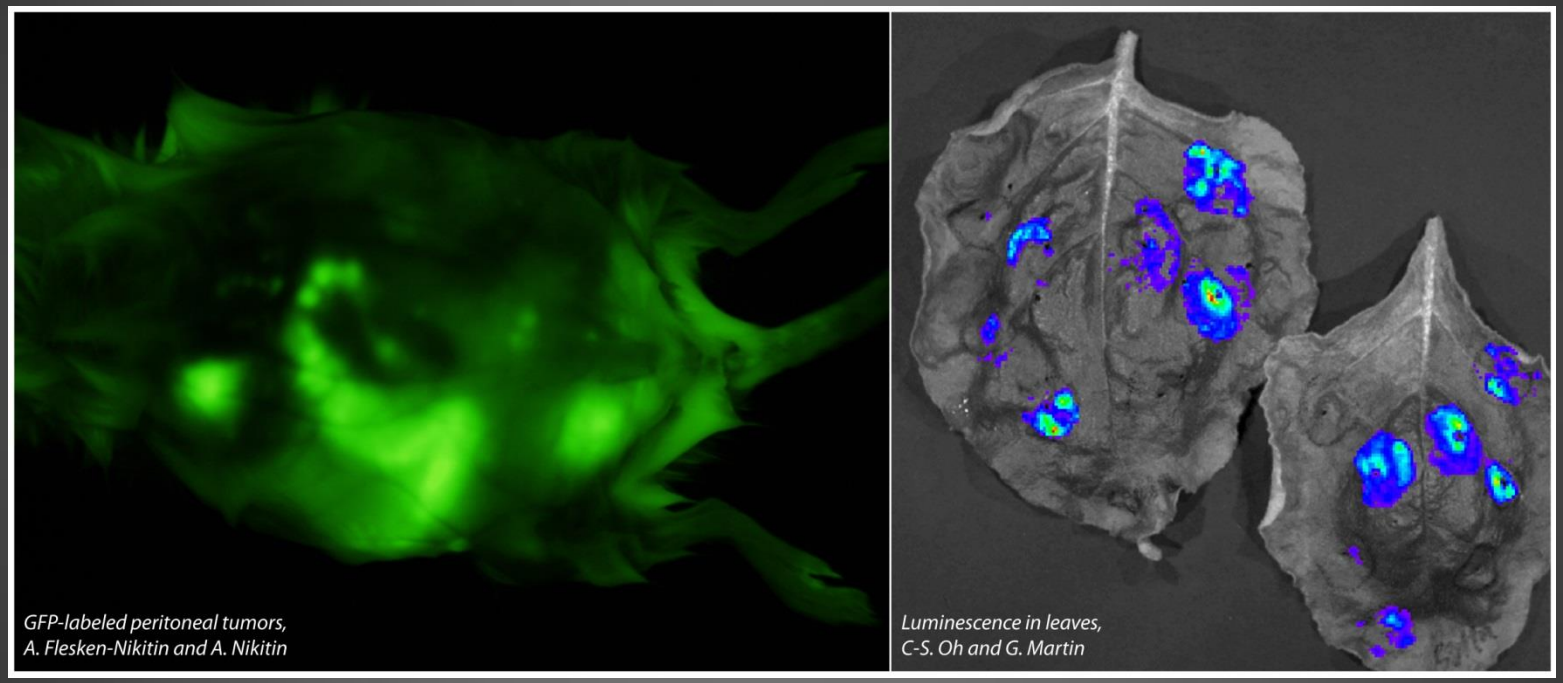


$\Delta x/\Delta t$ = 660 um /sec

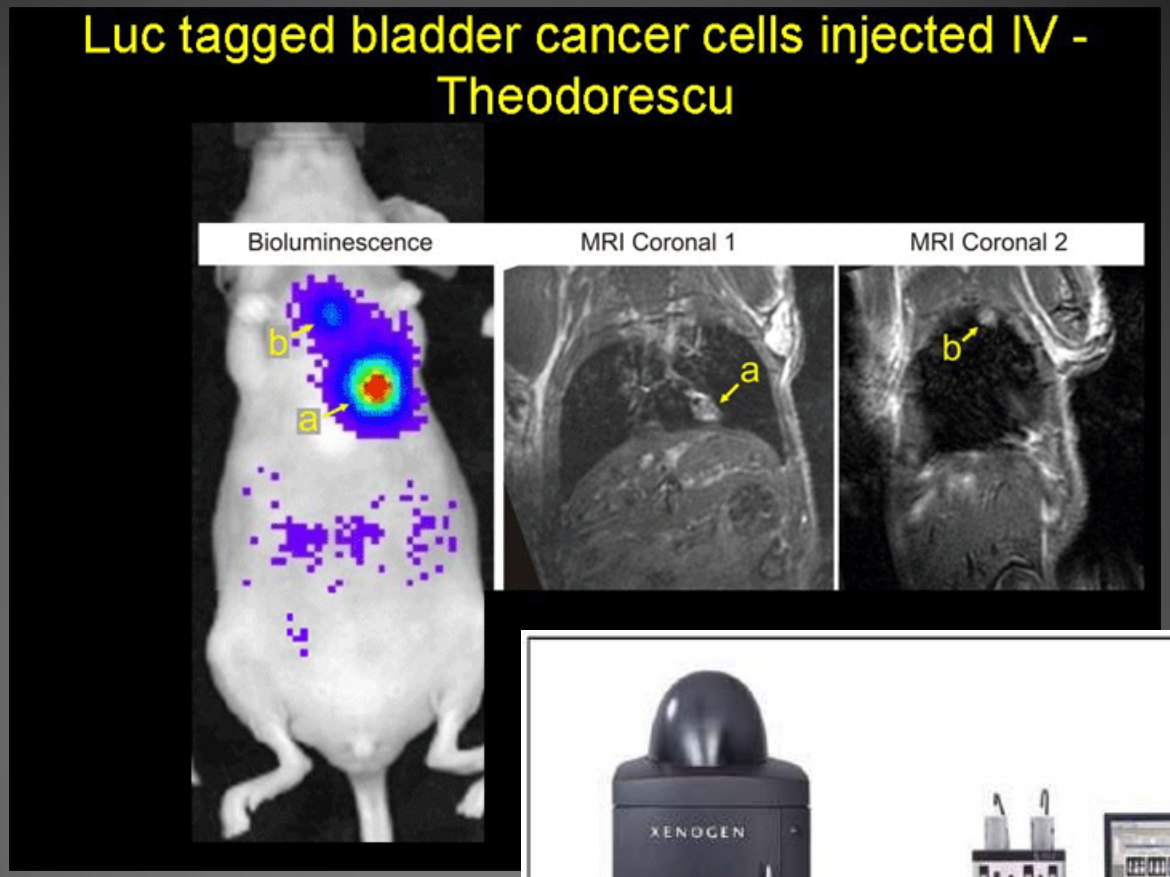
Blood flow peripheral to growth plate with Farnum lab

40 x 3.8 sec intervals

Macro level luminescence and fluorescence imaging



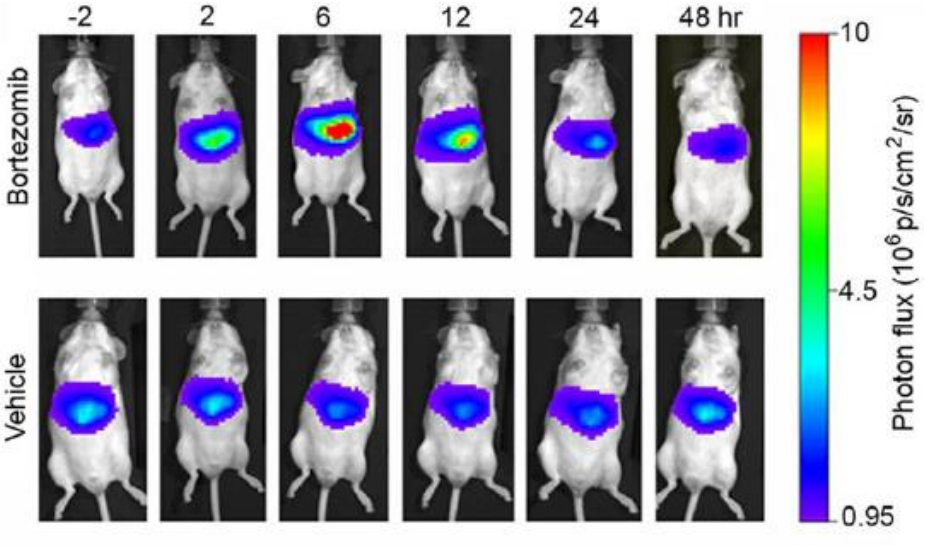
Olympus OV-100 (VRT 5013B)
*Xenogen IVIS-200 (*C1-005A TMCF)
Stereo microscopes

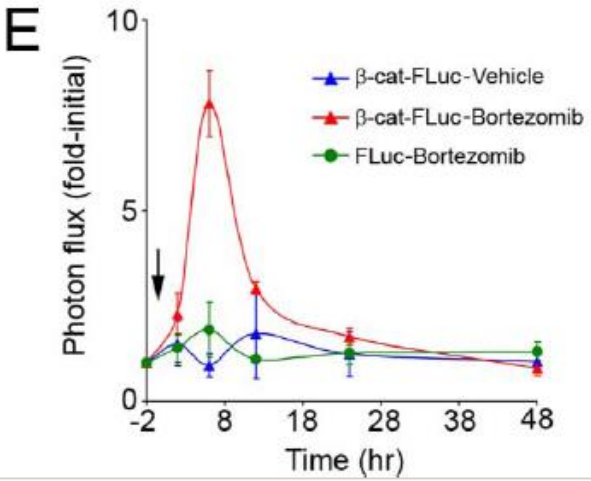




Whole mouse luminescence imaging
(Molecular Imaging)
Xenogen IVIS-200

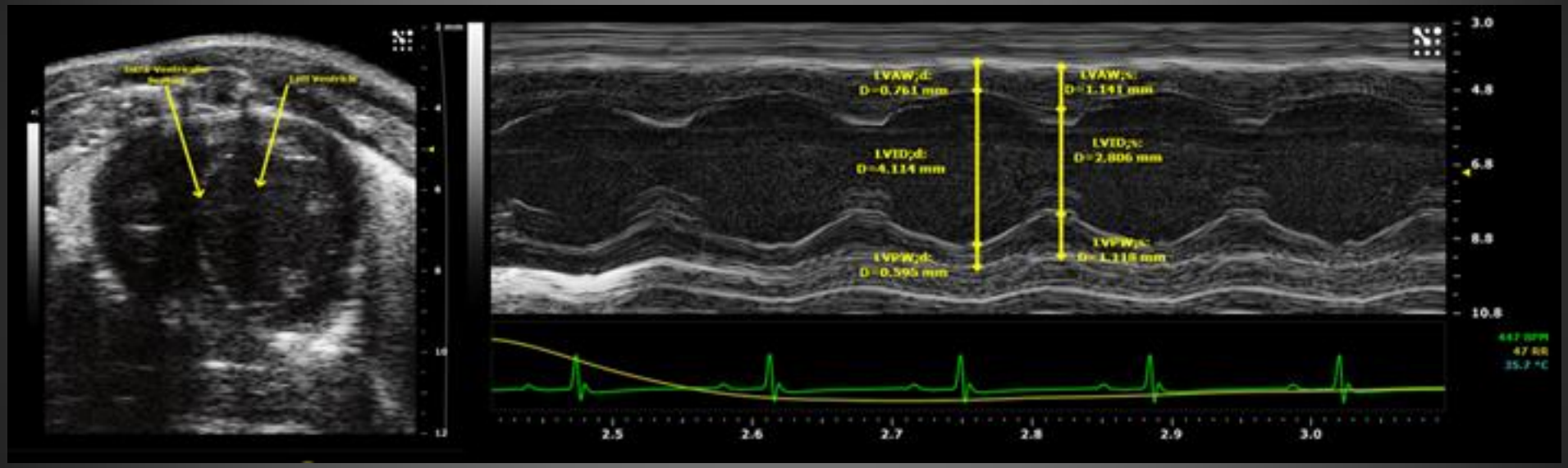
Example: Wnt pathway signaling in vivo





PNAS (2009) 104:44: 17465–17470

High-resolution ultrasound



*Mouse cardiac measurements, VisualSonics Vevo-770 (*C1-011 TMCF)

High resolution ultrasound imaging (20-40MHz)
Visual Sonics Vevo770



E13.5 mouse

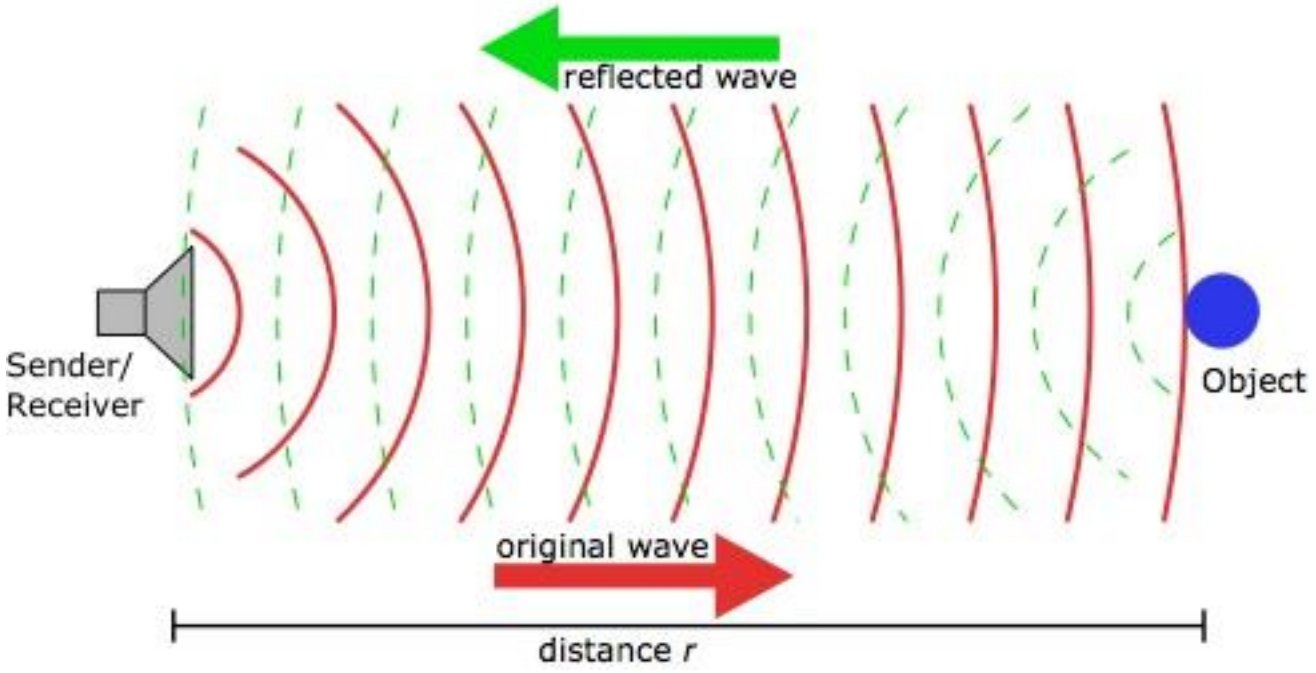


Available probes:

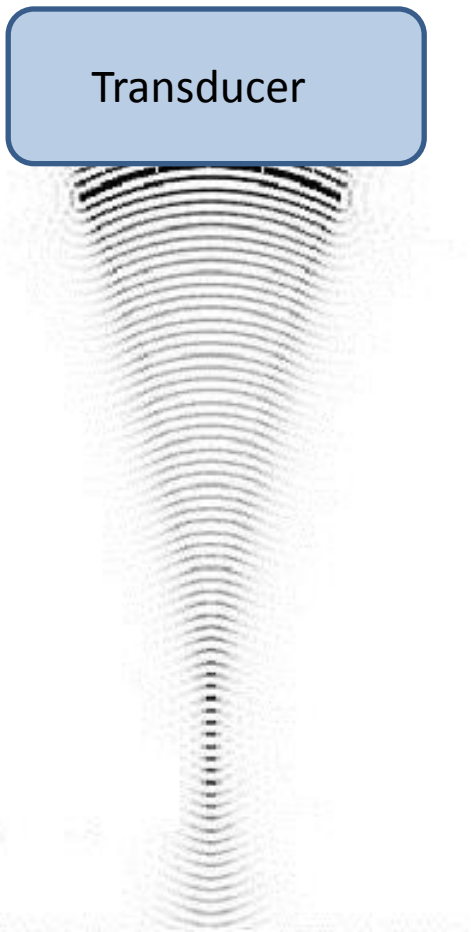
Probe	Application	Frequency (MHz)	F#	Focal length (mm)	Lateral resolution (um)	Axial resolution (um)
RMV 704	High resolution near	40	2.0	6.0	~75	40
RMV 706	Mouse general near	40	2.4	6.0	~90	40
RMV 707B	Mouse cardiology	30	2.1	12.7	~80	55

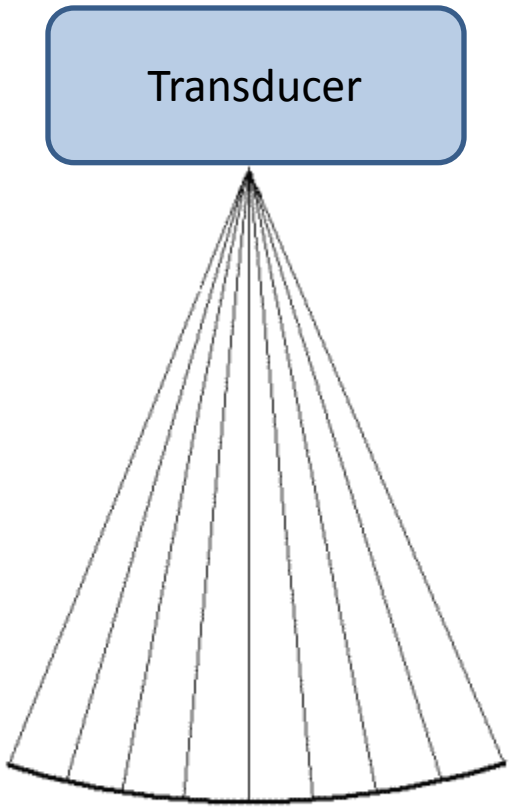
Screening
Blood flow analysis
Image-guided injections
3D visualization and measurement

Depth information is time-coded (assuming v = 1540 m/sec)



“B-mode imaging”





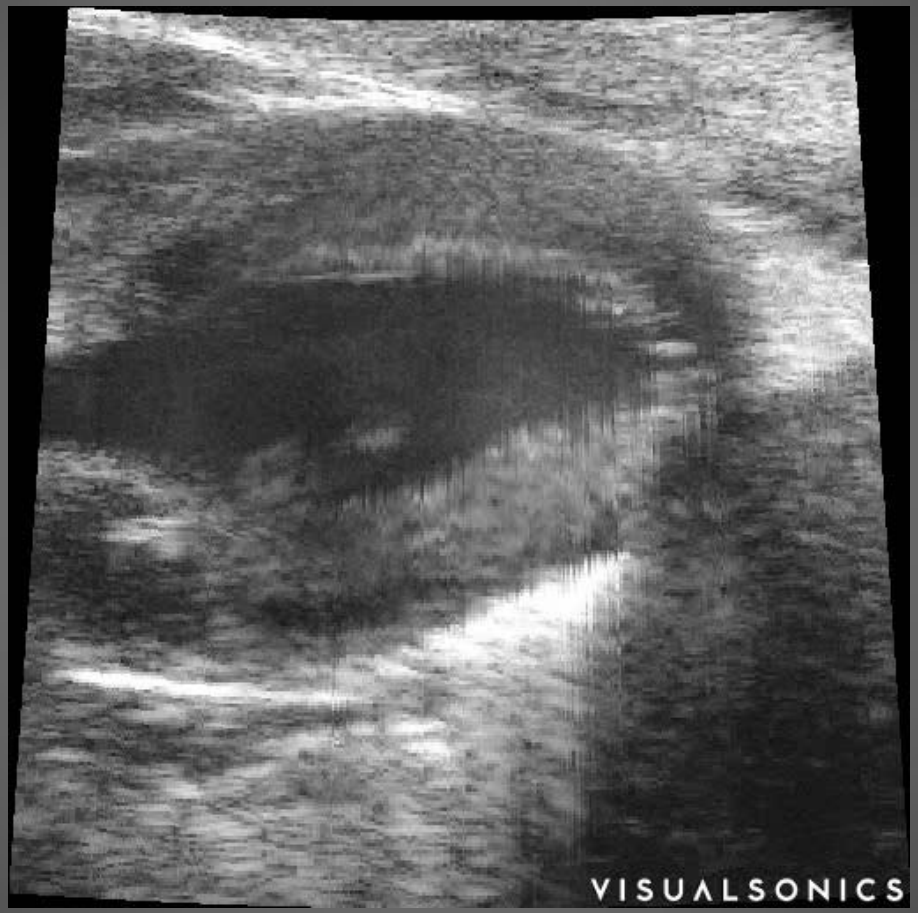


B-mode vs M-mode

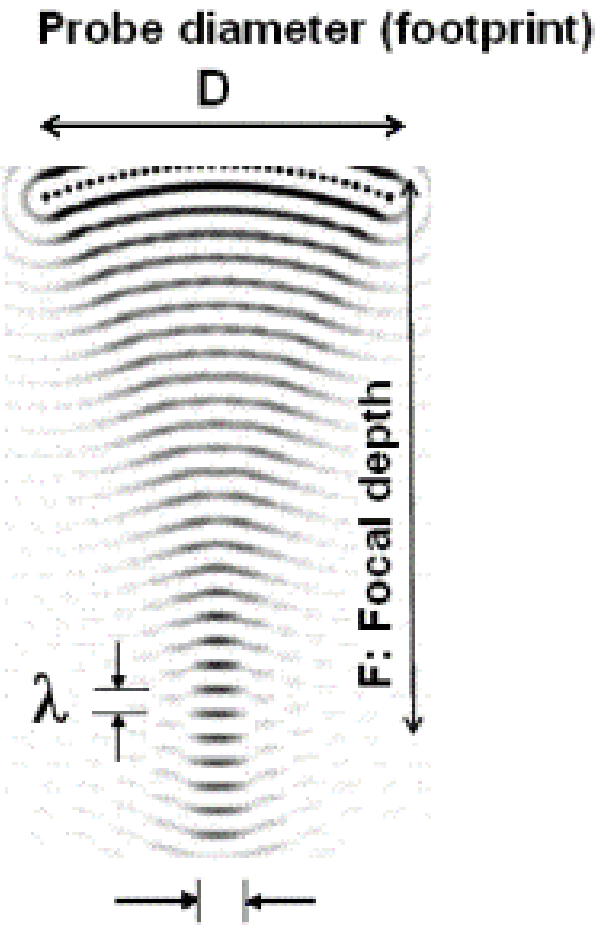


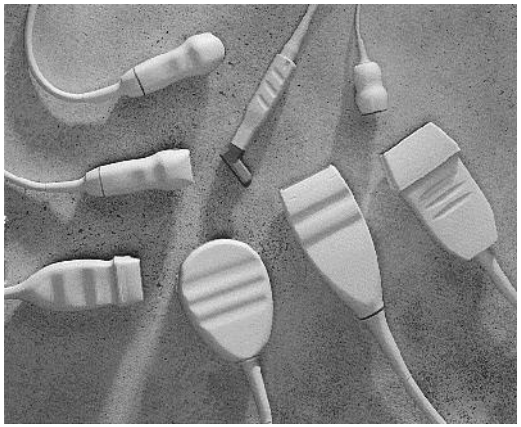
Parasternal Short Axis view in Adult Mouse

Mouse Heart beat (10 msec)



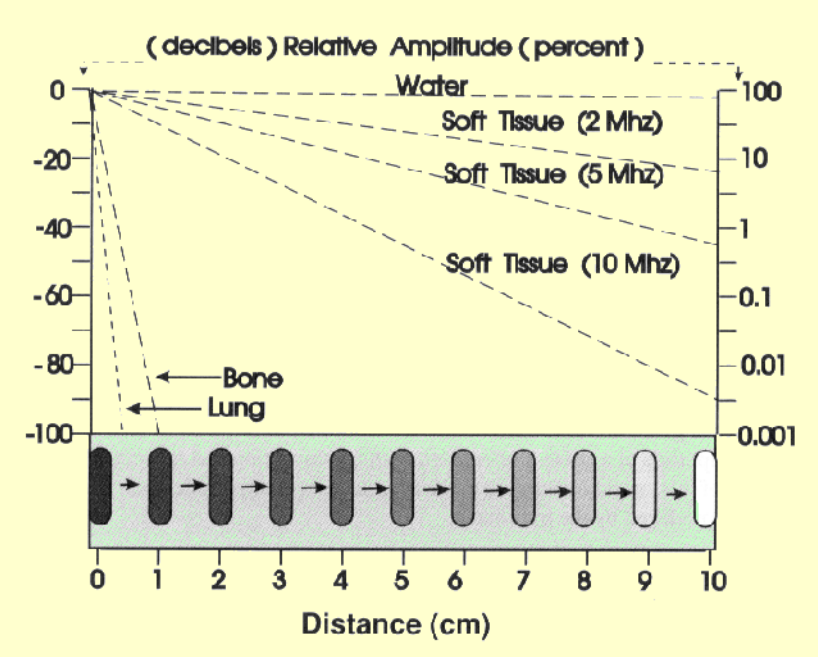
Lateral resolution



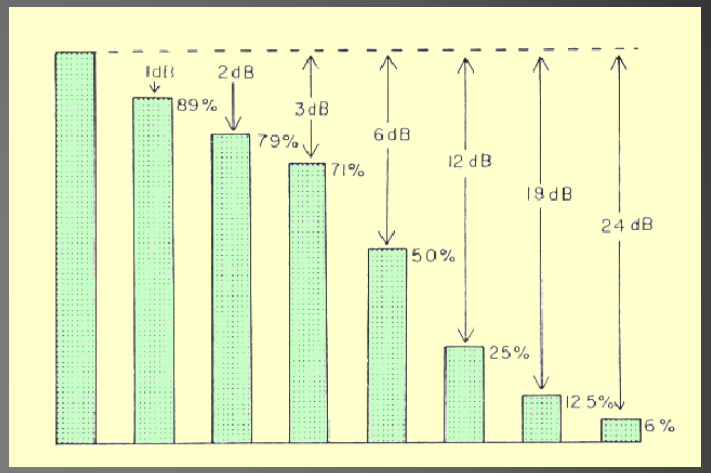


$$\text{Lateral resolution} = \frac{F * \lambda}{D} \quad \sim\lambda/2*NA$$

Ultrasound attenuation

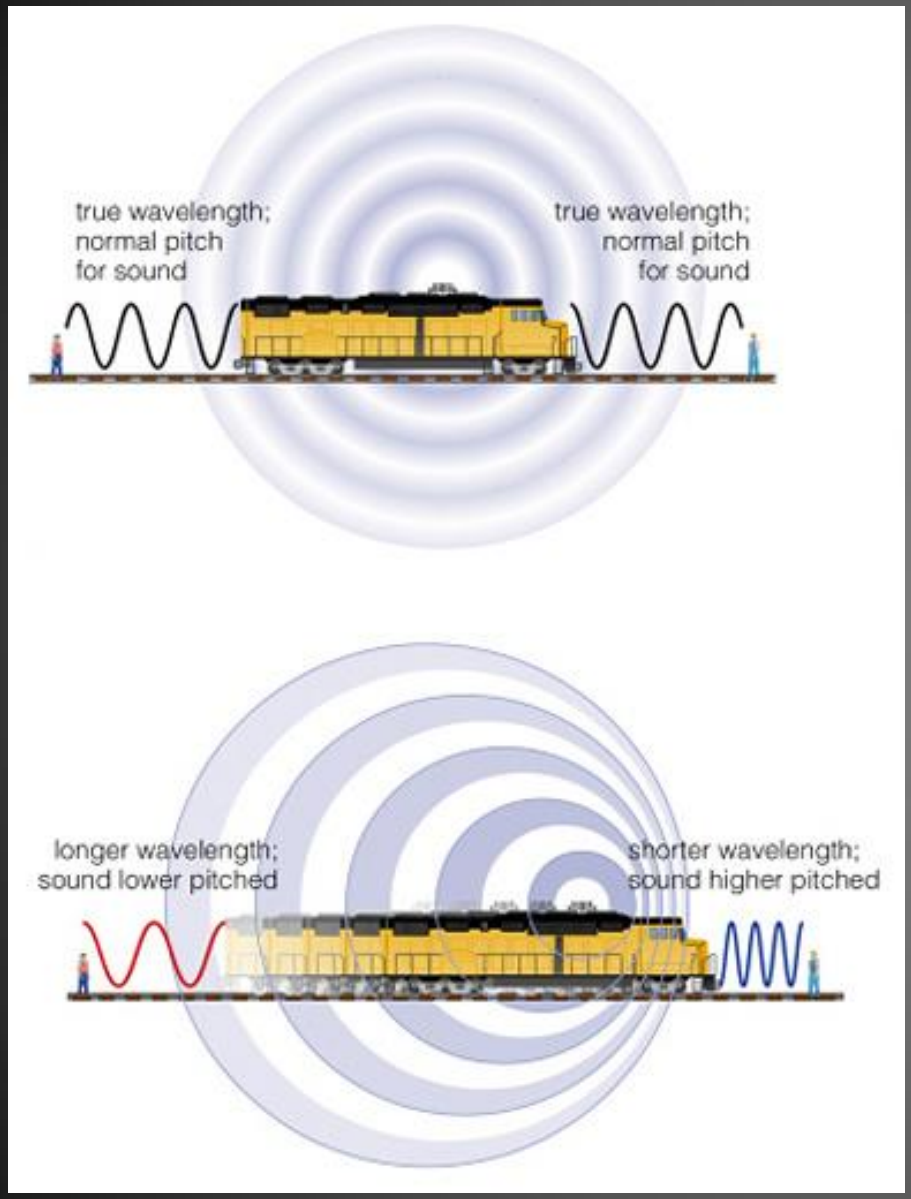


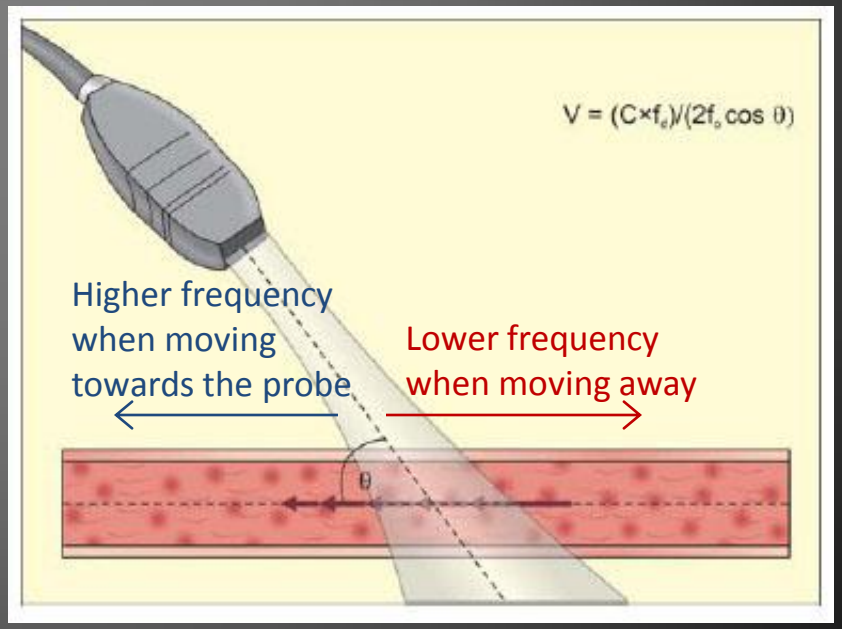
http://www.sprawls.org/ppmi2/USPRO/#Transducer%20Focusing



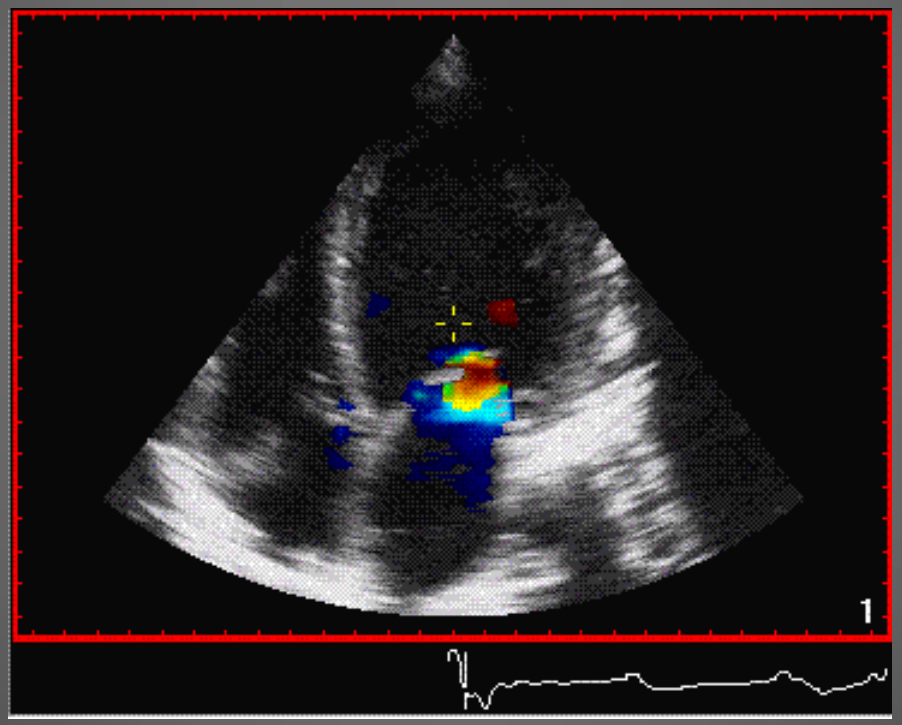
Material	*Coefficient (dB/cm MHz)*
Water	0.002
Fat	0.66
Soft tissue (average)	0.9
Muscle (average)	2.0
Air	12.0
Bone	20.0
Lung	40.0

Doppler ultrasound



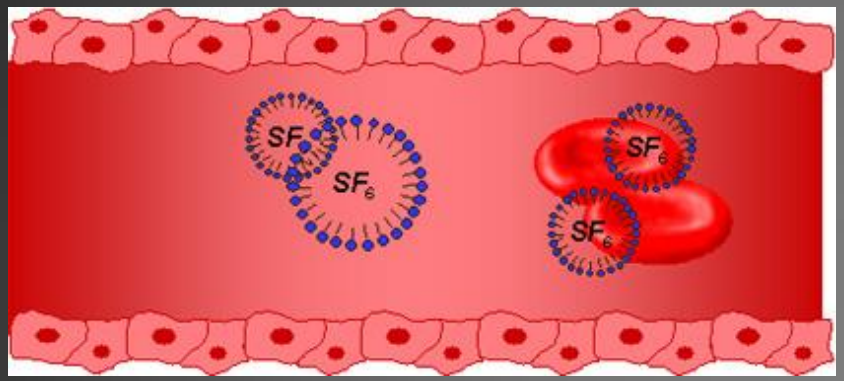


Color Doppler
Mitral valve regurgitation

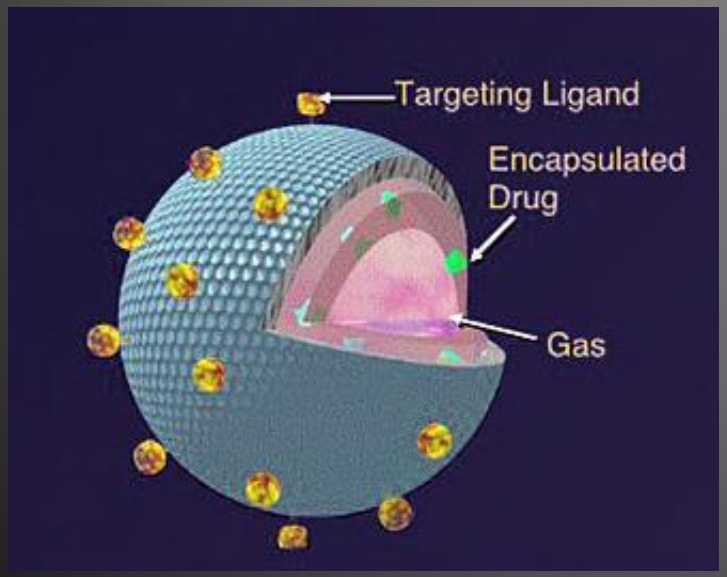


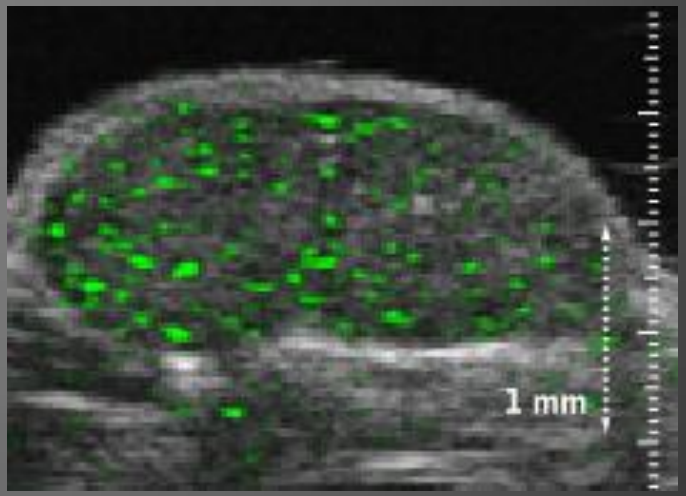
Molecular Imaging with ultrasound

Contrast agent = gas filled micro-bubbles



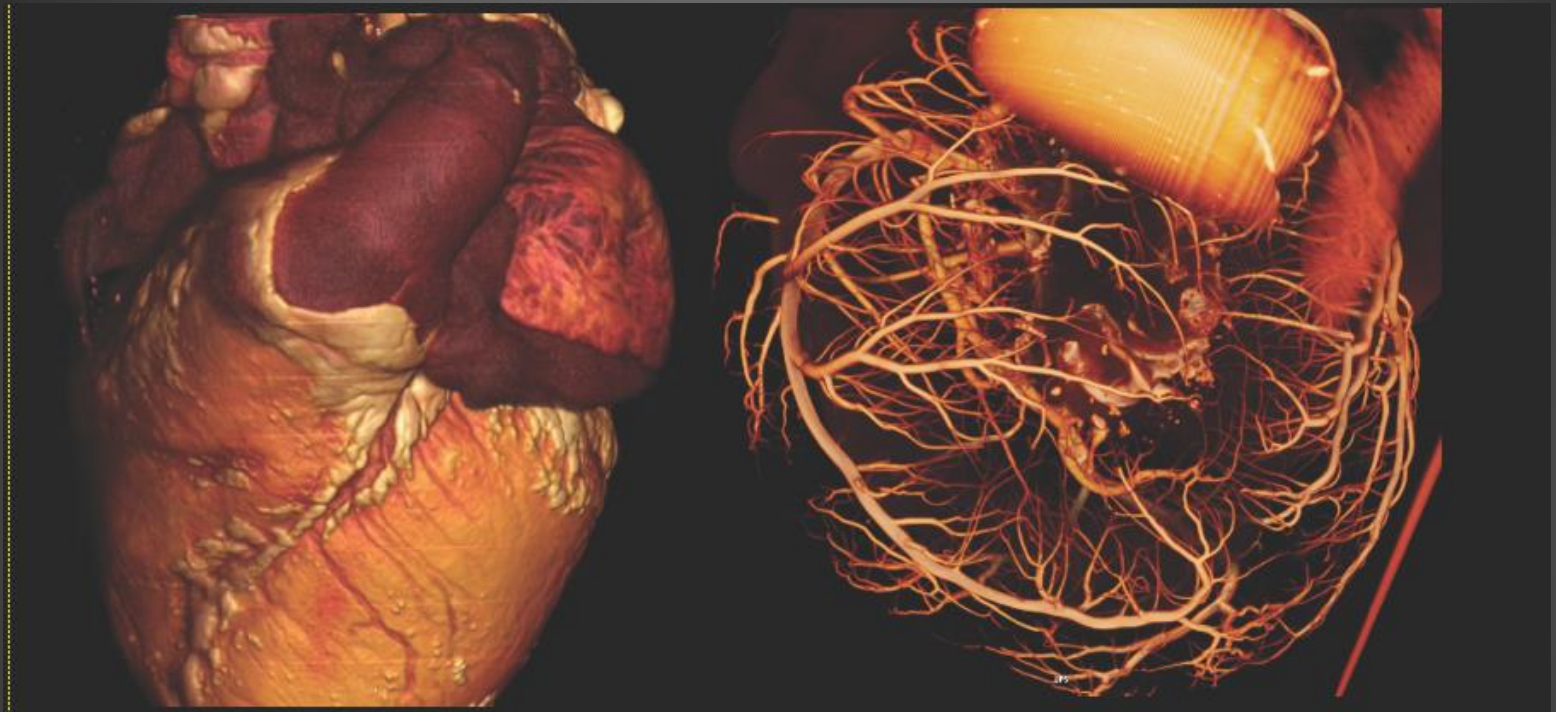
- molecular imaging
- targeted drug or gene delivery
- targeted tissue destruction





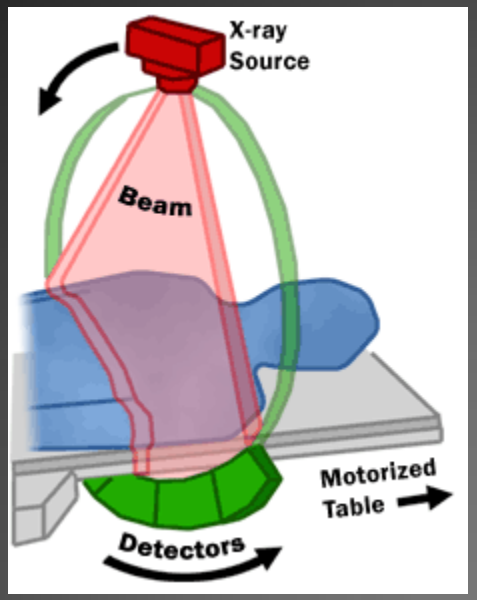
Mouse tumor (MeWo) showing targeted contrast agent (green) bound to VEGFR2, a biomarker that is expressed during angiogenesis.

High resolution X-ray CT



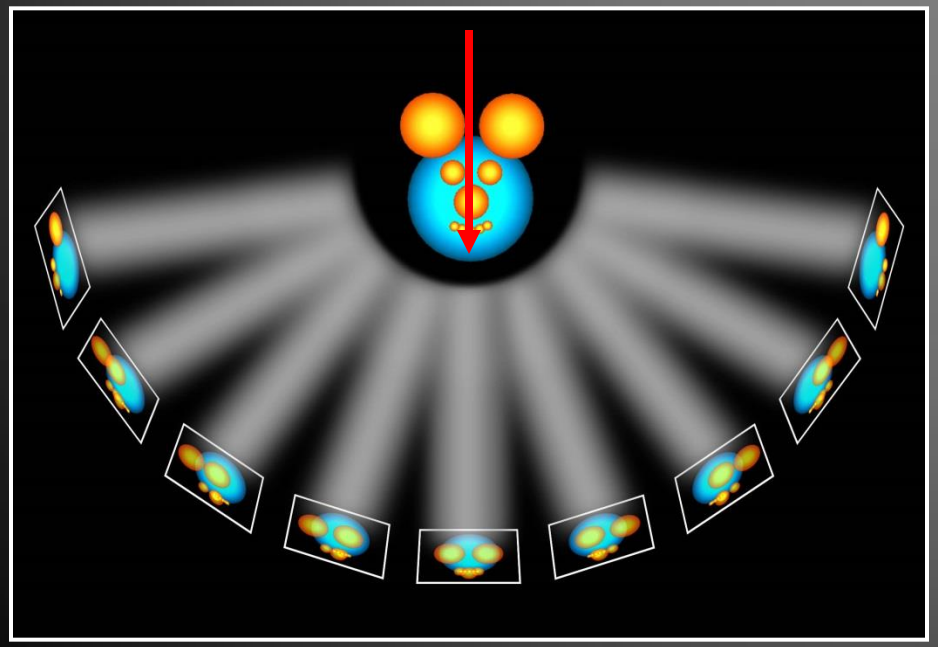
Cardiac structure and vasculature analysis are enhanced by using contrast agents
Geometrical data is imported into cardiac *in silico* models

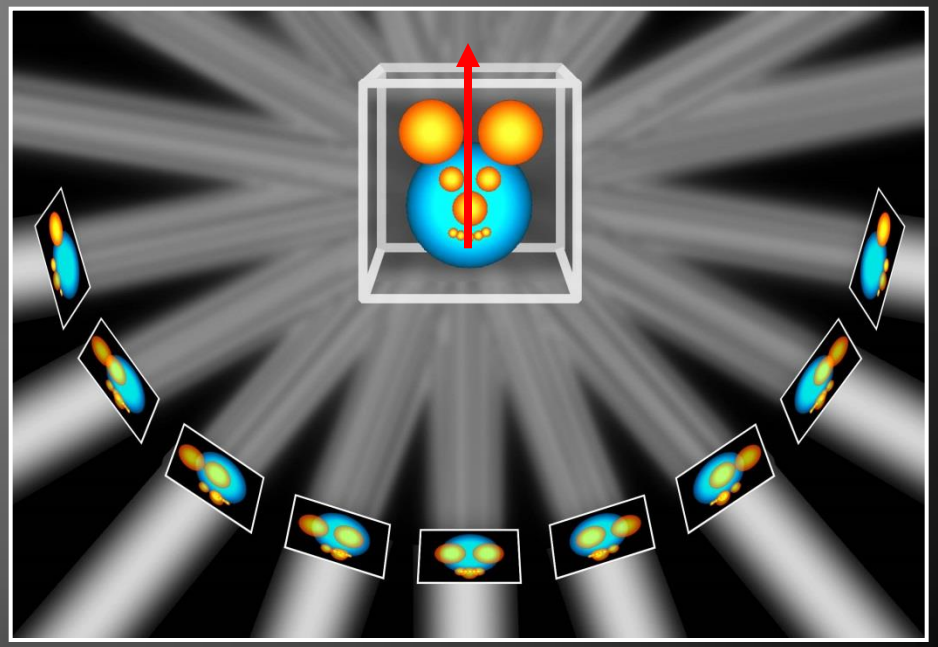
Xradia XRM-500 (B46 Weill)
GE CT-120 (B46 Weill)



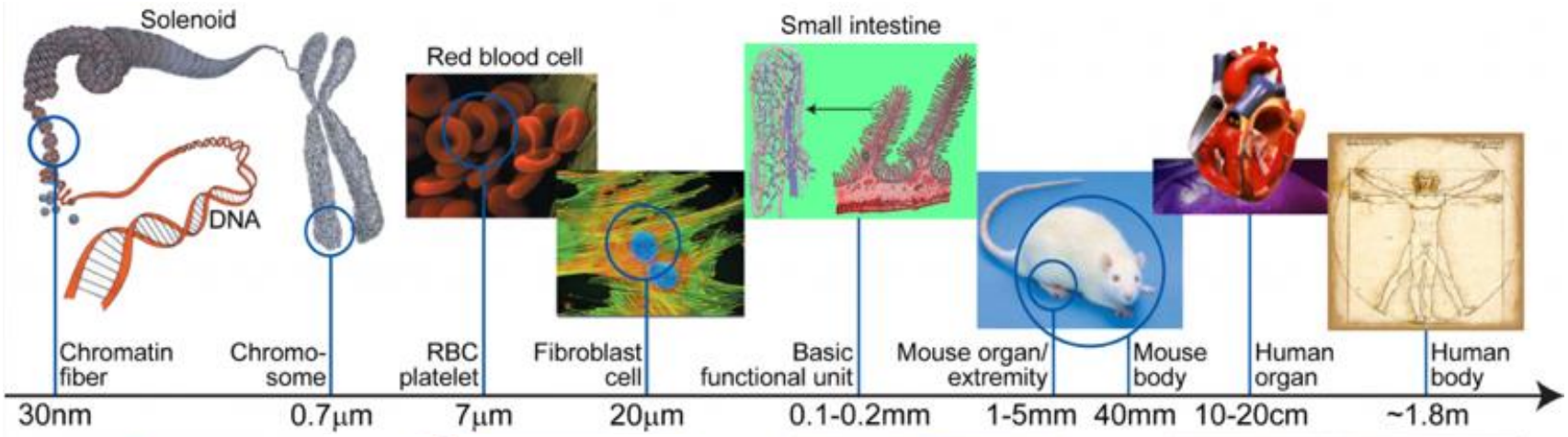
Computed tomography (CT)

3D object ⇨ set of 2D projections ⇨ 3D reconstruction





Multiscale CT Landscape





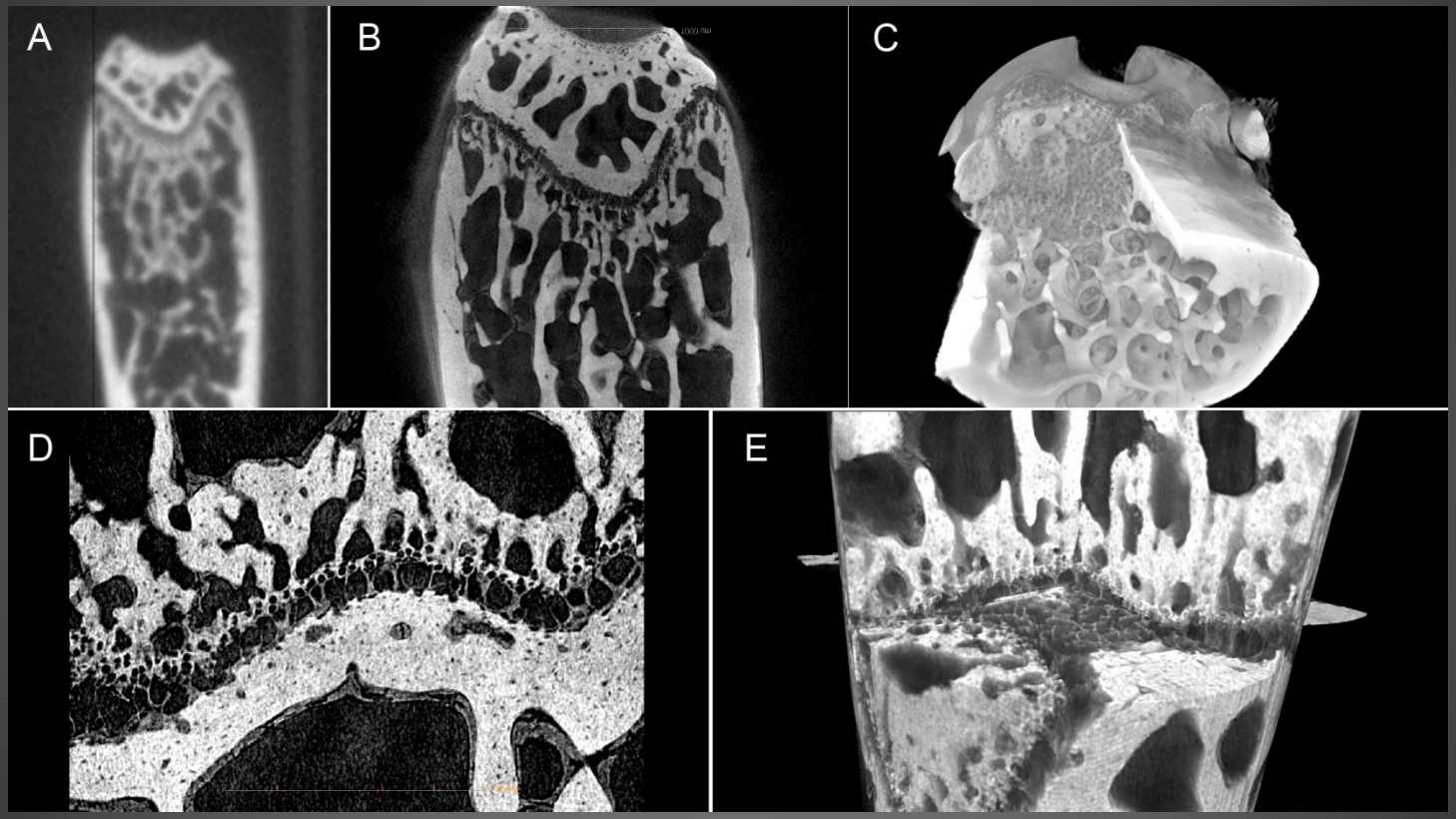






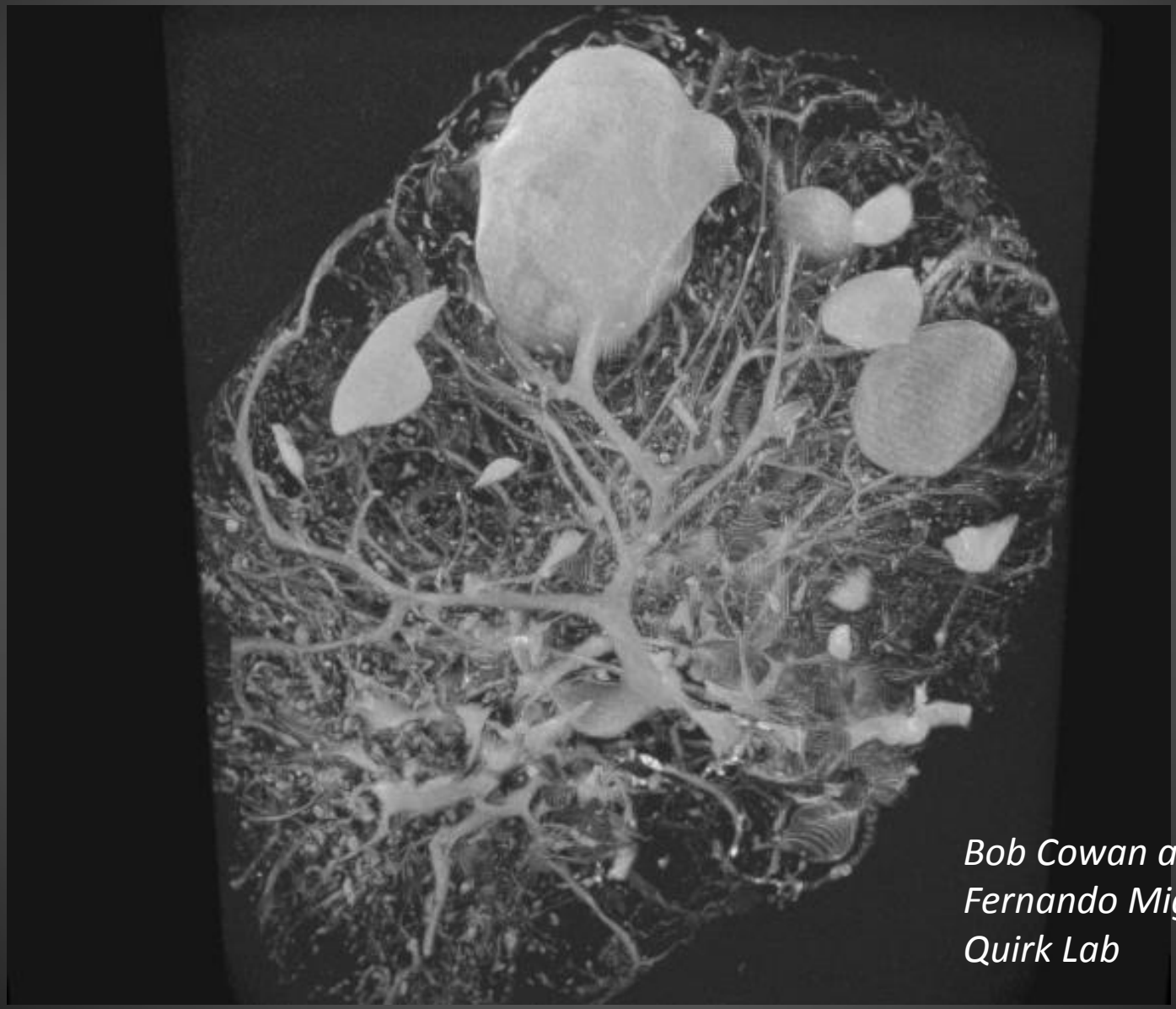
Ultra Nano CT	***Nano CT***	**Micro CT**	Clinical CT

Mouse tibia from the Xradia nano-CT (B-E)



Maria Serrat, Marshall Univ

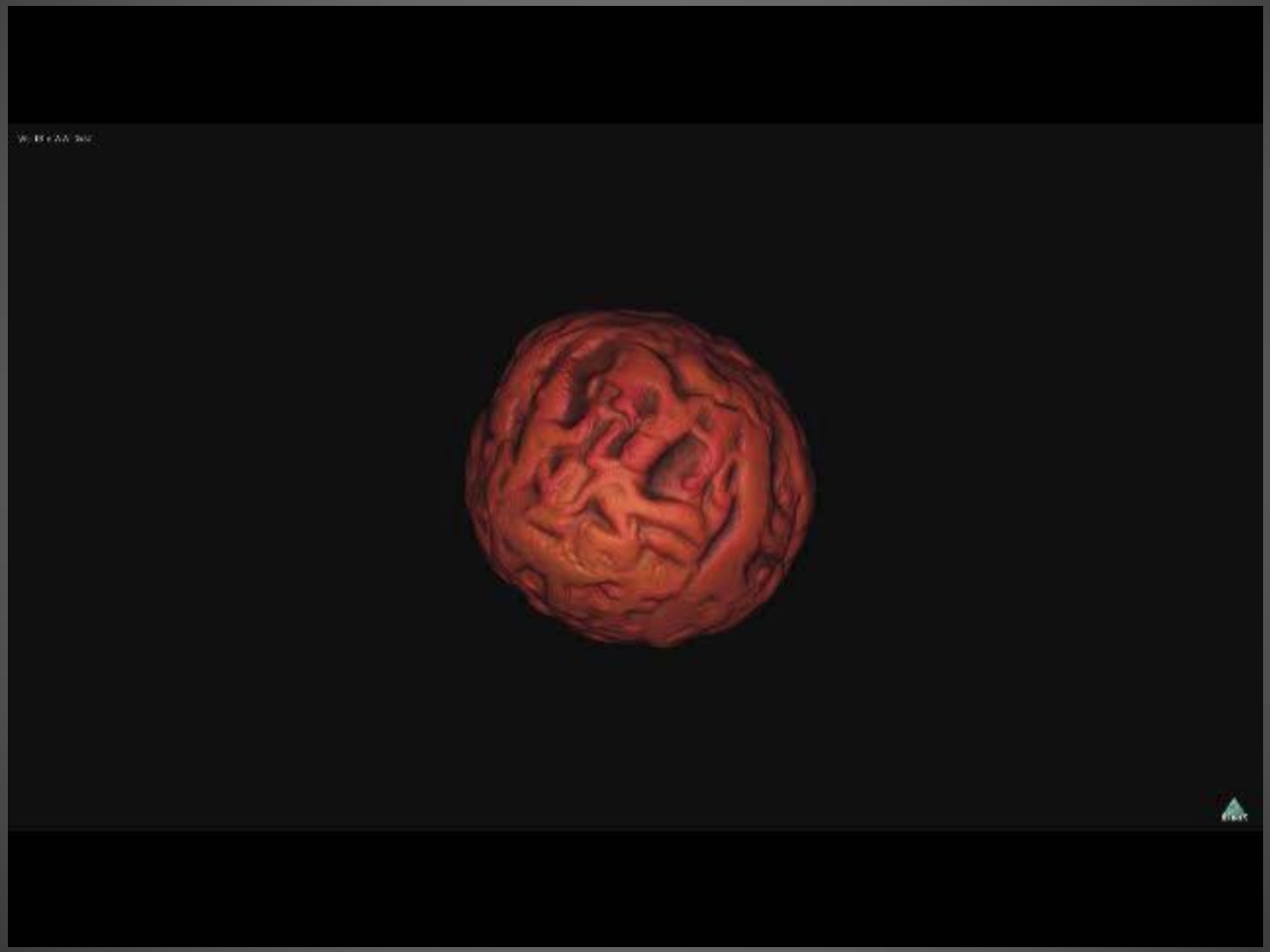
Mouse Ovarian Vasculature (Microfil labeled)



Bob Cowan and Fernando Migone, Quirk Lab

Early Sumerian “Tablet”

David Owen, Curator of Tablet Collections, Near Eastern Studies



BRC-Imaging

http://www.biotech.cornell.edu/brc/imaging

