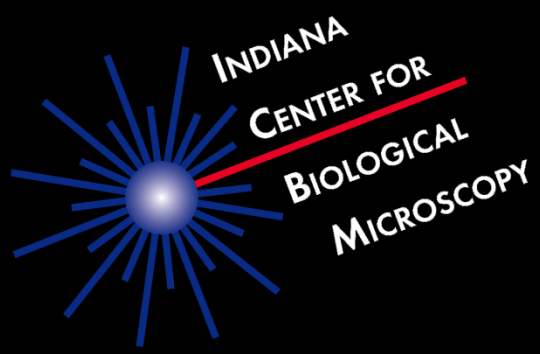
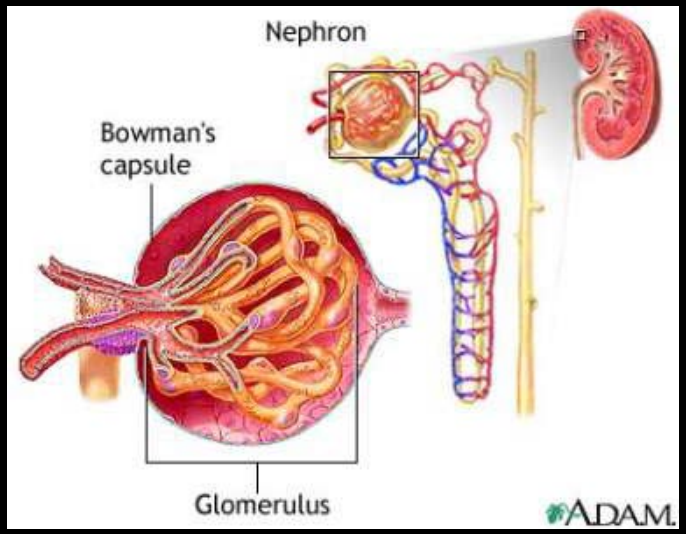
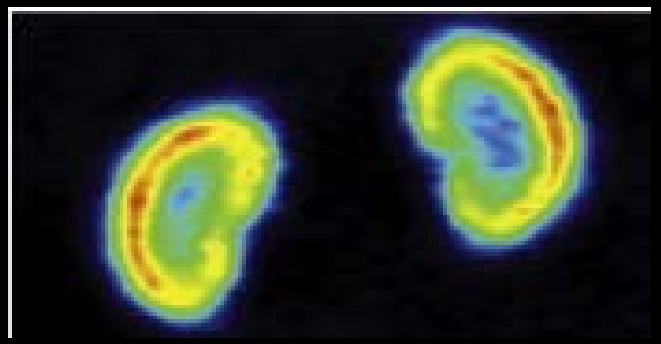


Intravital multiphoton microscopy – Principles and challenges

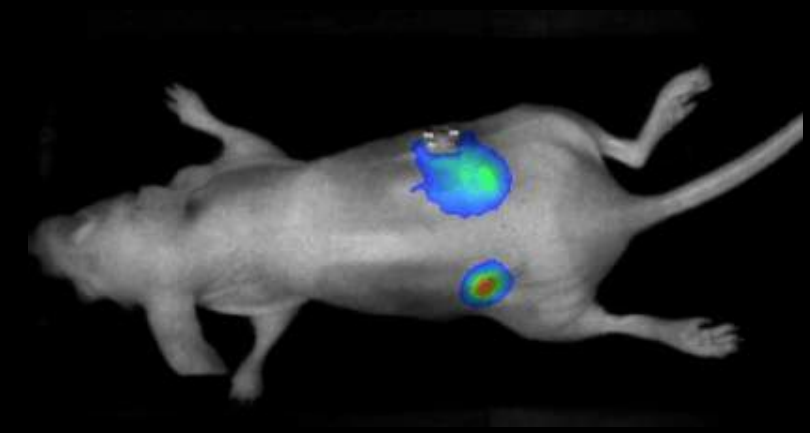
Ken Dunn, PhD
Scientific Director
Indiana Center for Biological Microscopy
Indiana University Medical Center

In vivo imaging of the kidney

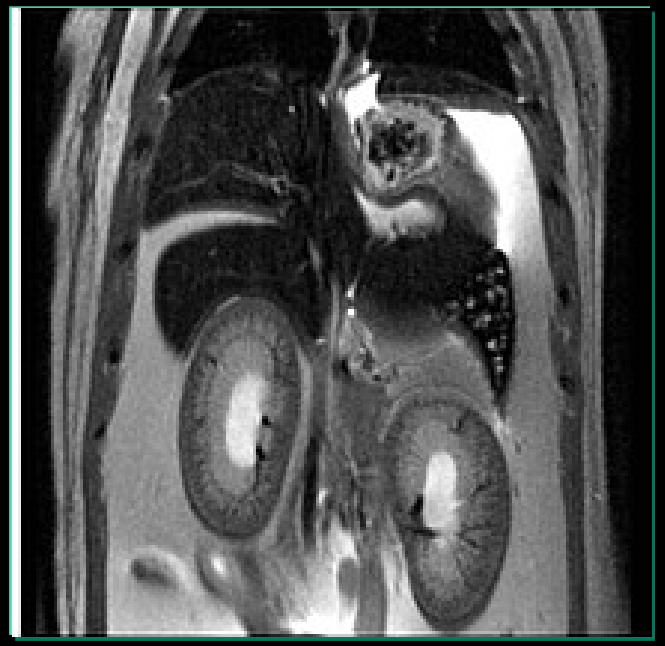




PET
Ceccarini et al., 2009 Cell Metab.

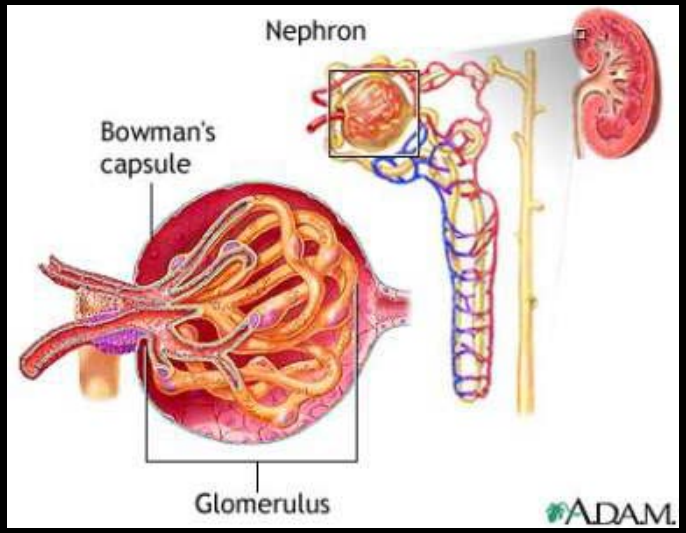


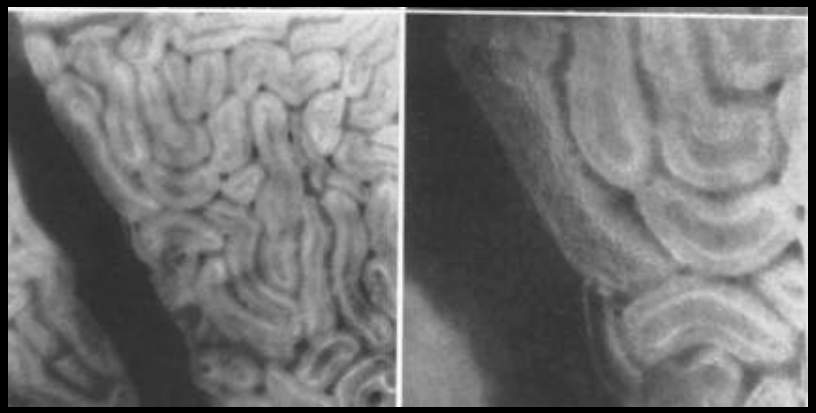
Bioluminescence
Que, Kajizel and Löwik, LUMC, Netherlands



MRI
Bruker Biospin

Intravital microscopy of the kidney





Wide-field microscopy
Steinhausen et al., 1963.

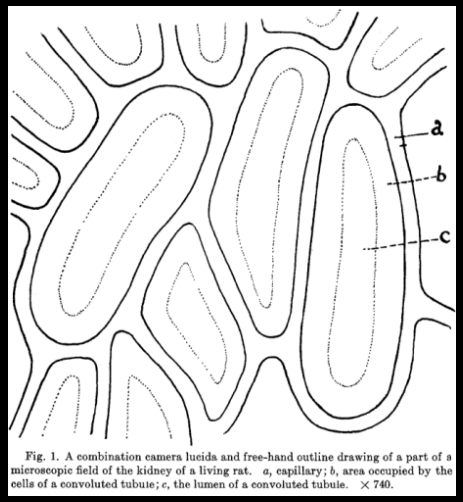
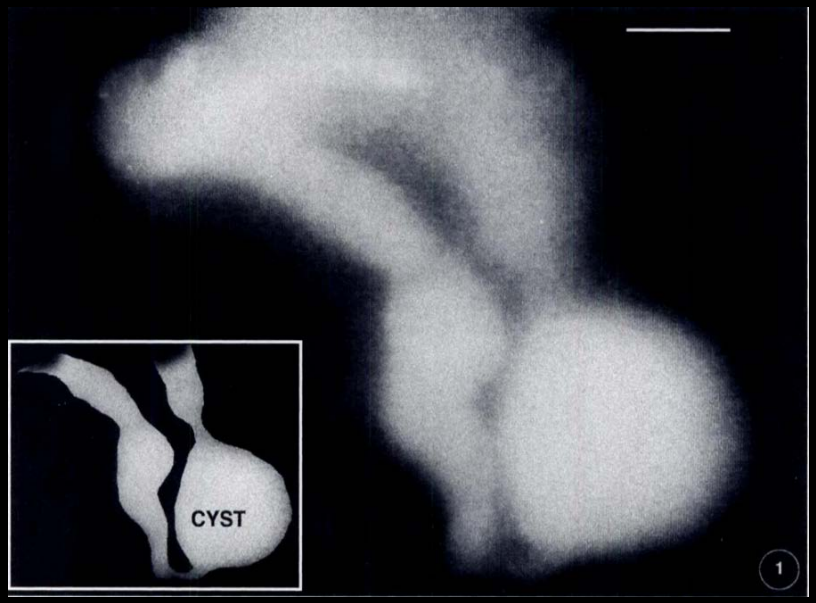


Fig. 1. A combination camera lucida and free-hand outline drawing of a part of a microscopic field of the kidney of a living rat. *a*, capillary; *b*, area occupied by the cells of a convoluted tubule; *c*, the lumen of a convoluted tubule. × 740.

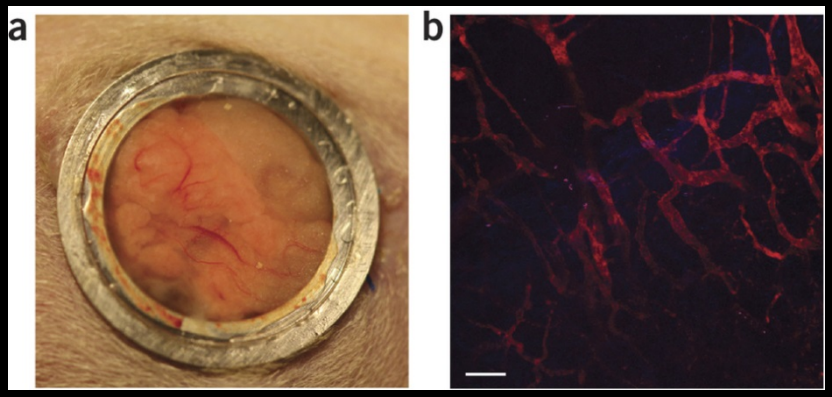
Wide field microscopy
Edwards and Marshall, 1924



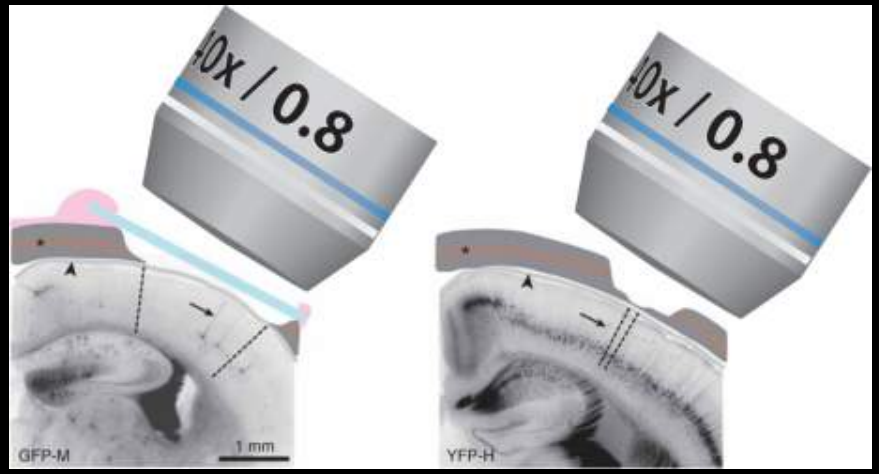
Fluorescence microscopy
Tanner et al., 1997, JASN

Optical access to tissues in vivo

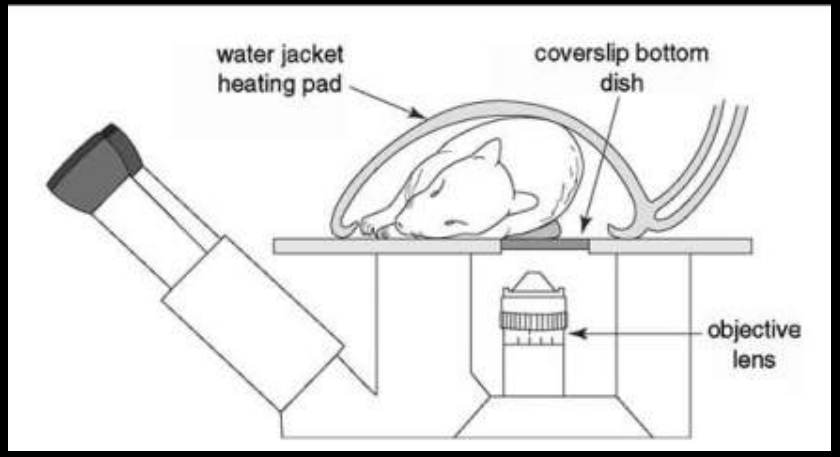
Oslo University Hospital



Ritsma et al., Nature Protocols 2013

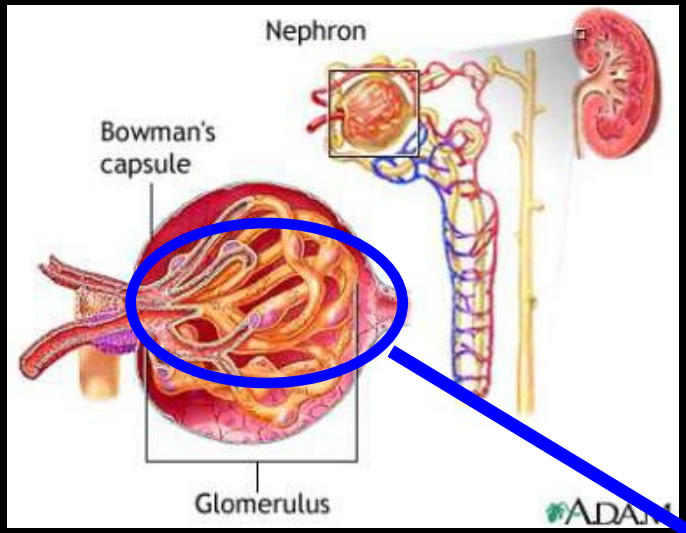


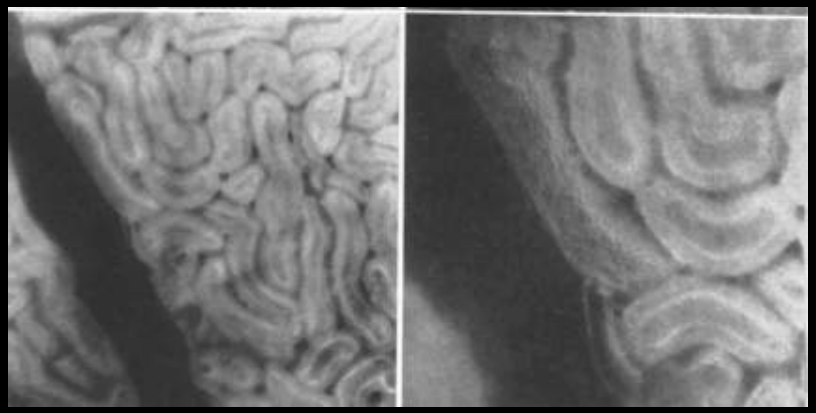
Holtmaat et al. Nature Protocols, 2009



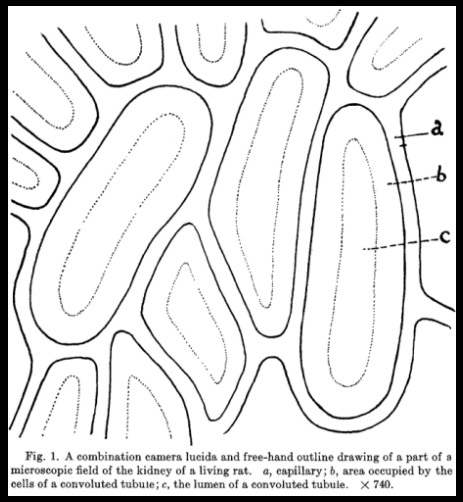
Dunn, Sutton and Sandoval. 2007. Curr. Protocols Cytometry.

Intravital microscopy of the kidney





Wide-field microscopy
Steinhausen et al., 1963.



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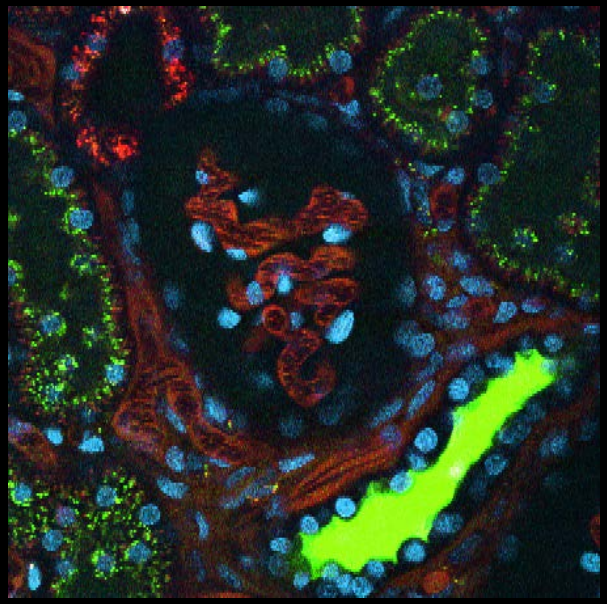
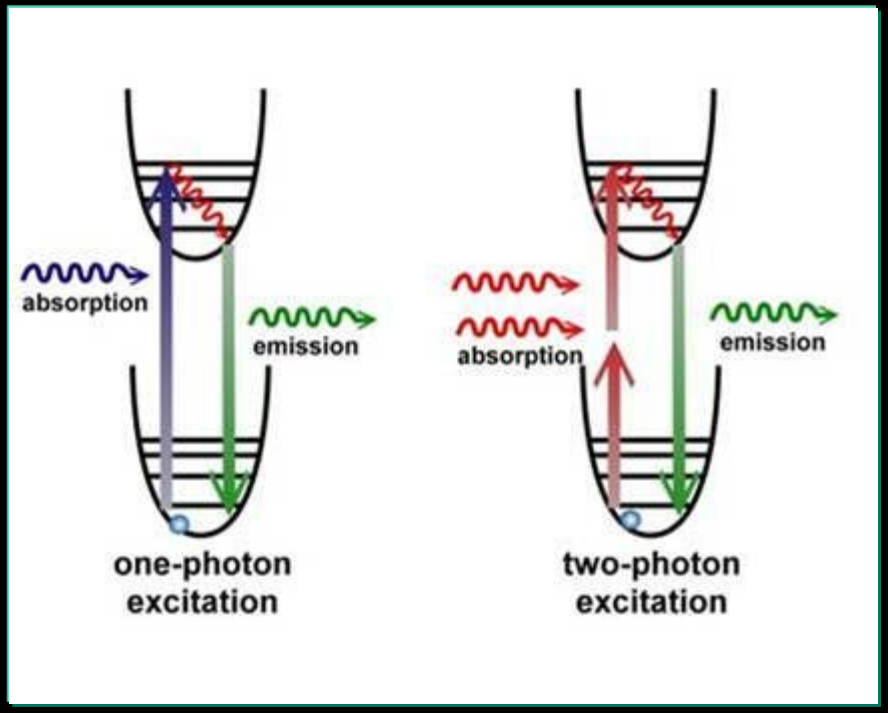


Dunn, Sandoval and Molitoris. 2003. Intravital imaging of the kidney using multiparameter multiphoton microscopy Nephron. 94:e7-11

Multiphoton fluorescence excitation



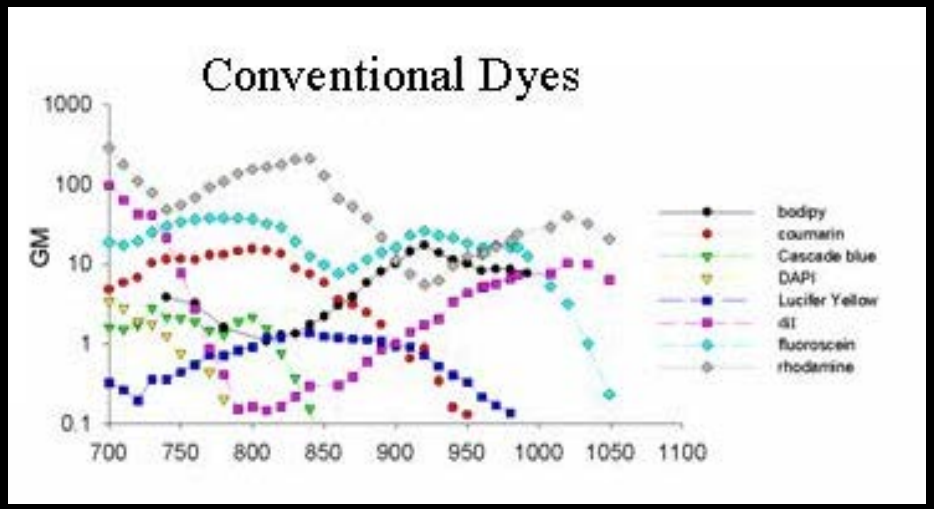
Biophotonics Imaging Lab, Univ. Illinois Urbana-Champaign

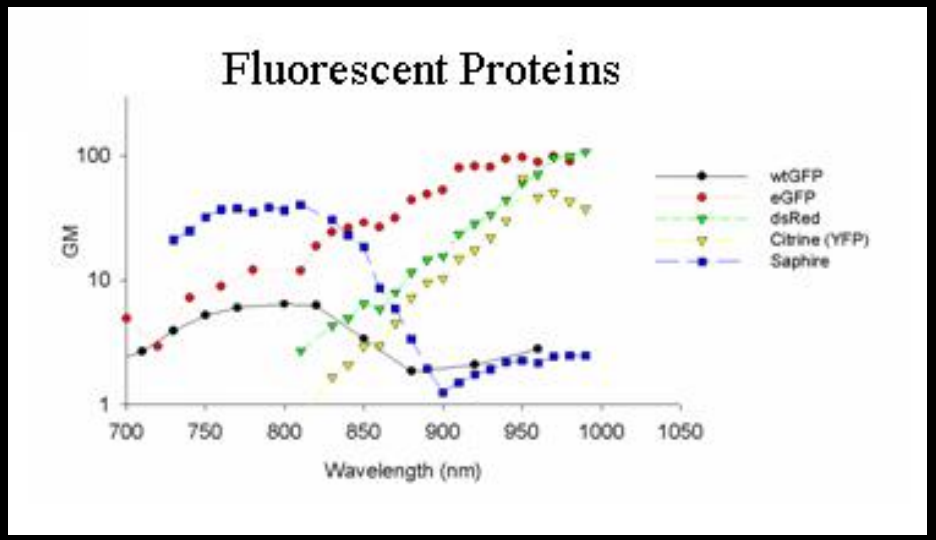
Fluorescence can be stimulated by the absorption of one photon of a particular energy level.

Maria Goppert-Mayer, 1931 –

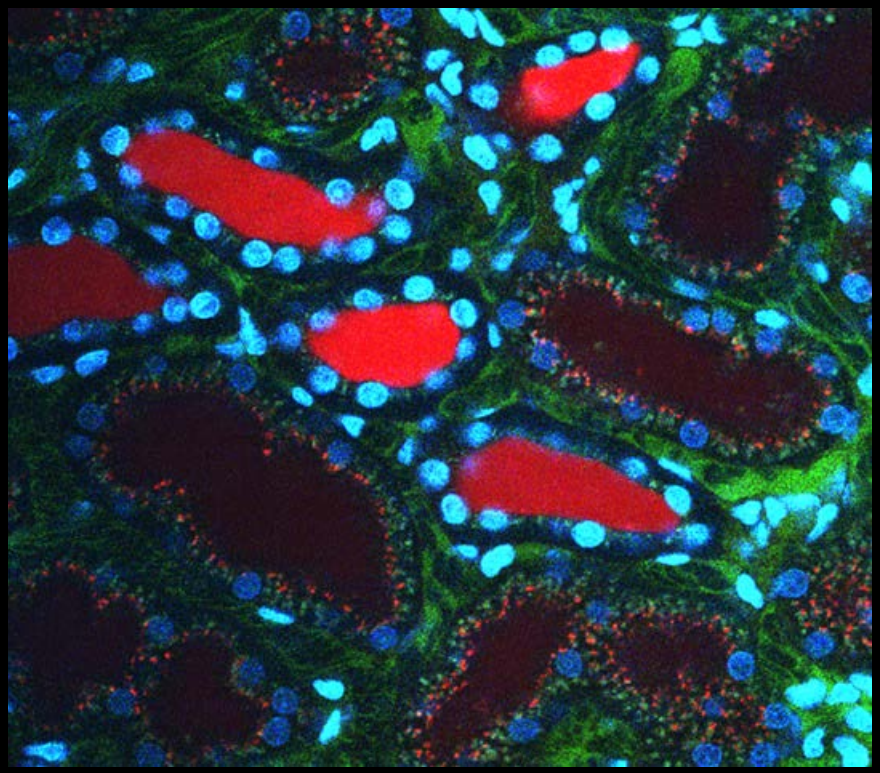
Fluorescence can also be stimulated by the simultaneous absorption of multiple, low-energy photons

Two-photon action cross sections





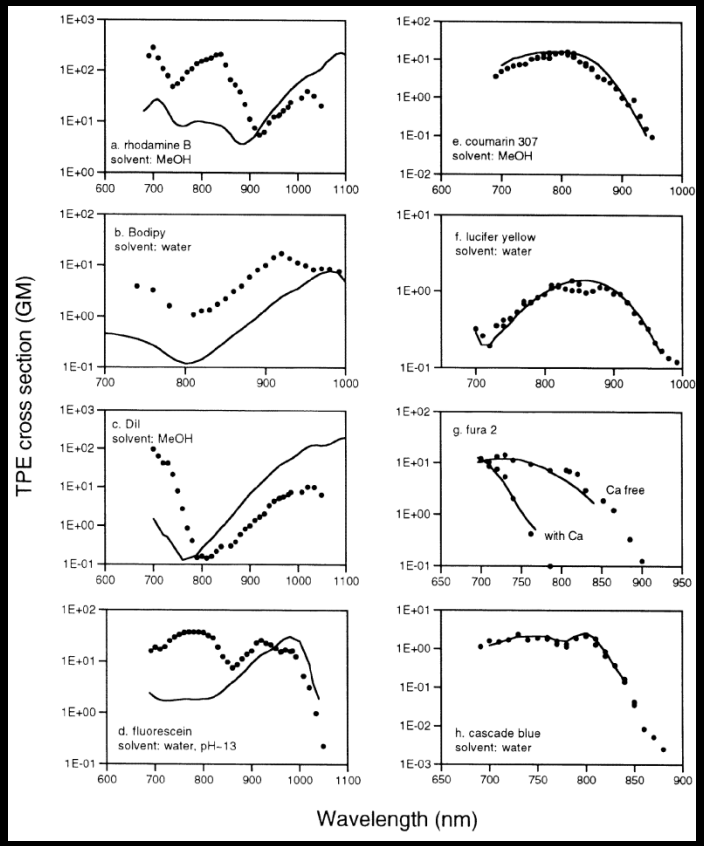
Developmental Resource for Biophysical Imaging Opto-electronics, Cornell
(Watt Webb and Warren Zipfel)



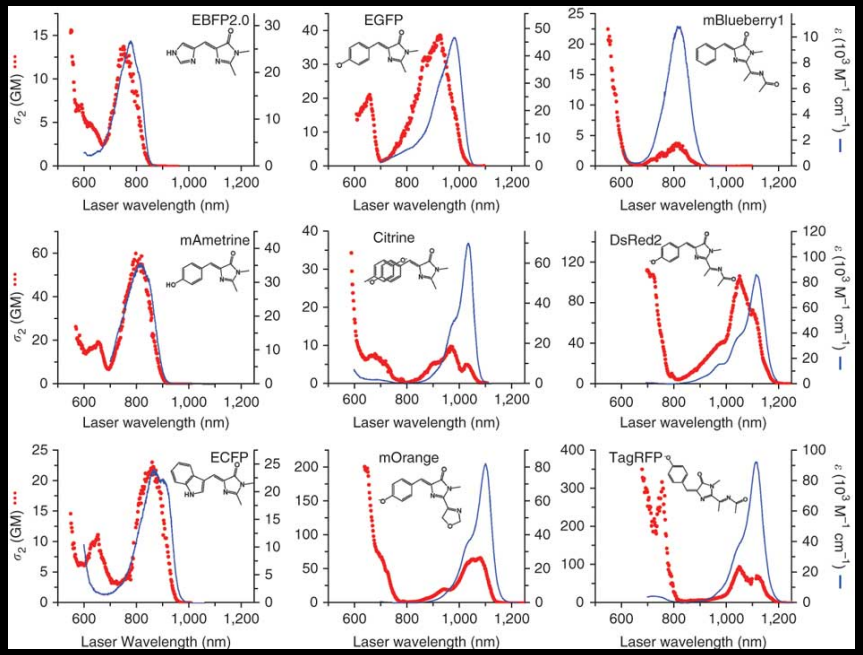
10 kD Rhodamine Dextran,
500 kD Fluorescein Dextrn
Hoechst 33342

Dunn et al., 2002. AJP Cell. 283:C905-C916

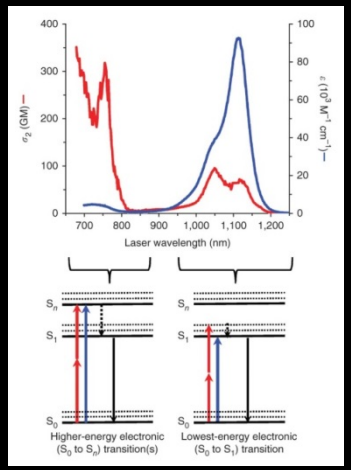
Relation between one and two photon absorption
Not always 2X



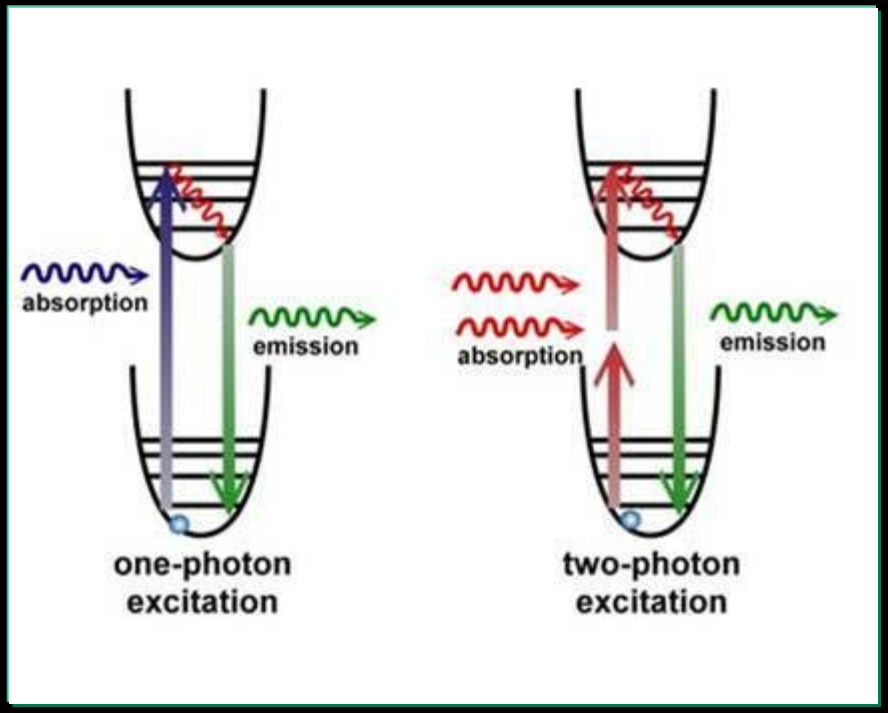
Xu et al., 1996. Bioimaging



Drobizhev et al., 2011.
Nature Methods



Multiphoton fluorescence excitation Requires an enormous density of photons



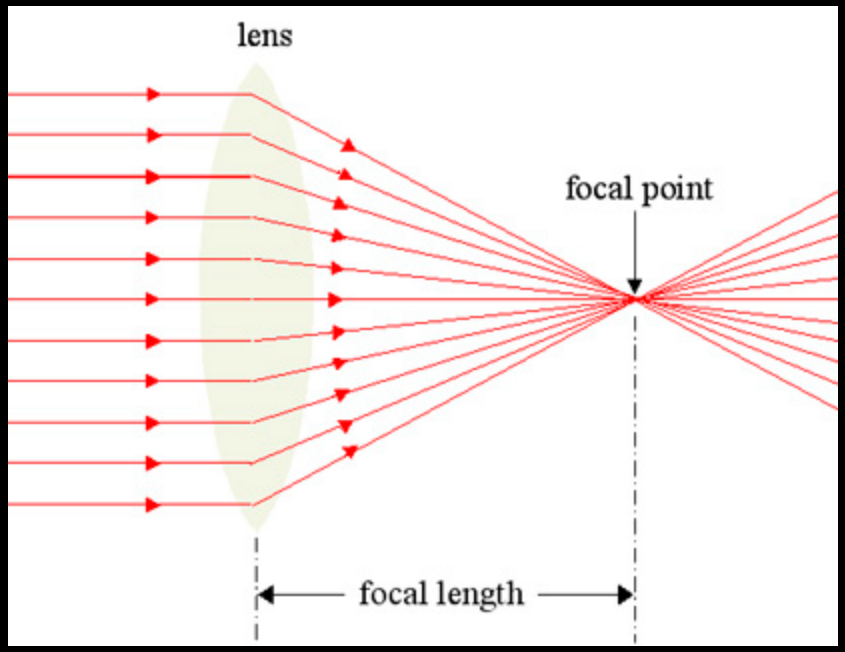
Biophotonics Imaging Lab, Univ. Illinois Urbana-Champaign

"Simultaneous absorption of two photons" – within ~ 10 attoseconds (10^{-17} seconds)

Detectible two-photon fluorescence excitation requires peak power on the order of 200 GW/cm^2
~ 300,000x the surface of the sun

Two photon fluorescence excitation first demonstrated with development of the ruby laser – Kaiser and Garrett, 1961

Multiphoton fluorescence excitation Requires an enormous density of photons



DiracDelta Science

Detectible two-photon fluorescence excitation requires peak power on the order of 200 GW/cm^2
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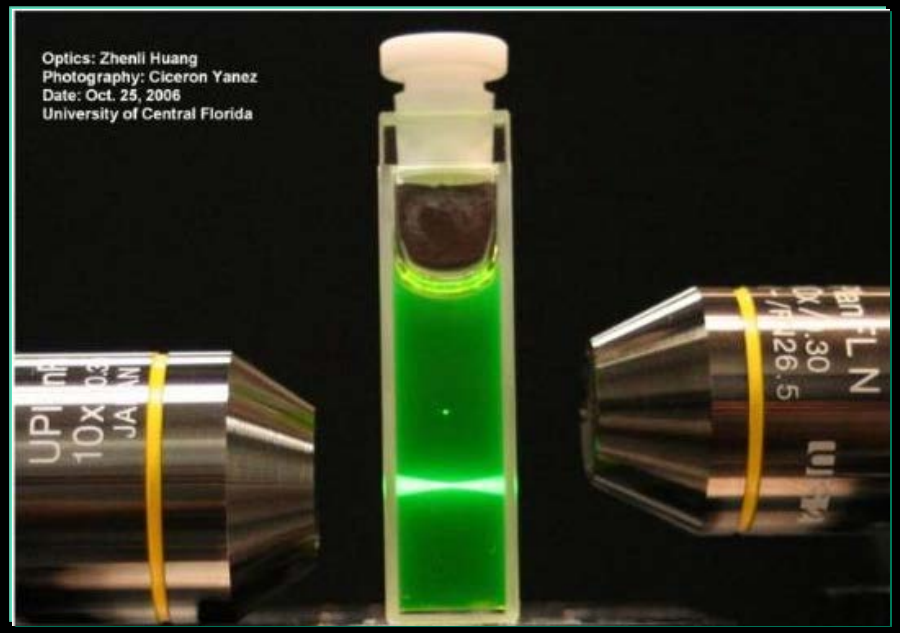
For multiphoton microscopy, this density is provided by focusing laser illumination through high NA objective lenses.

Photon density decreases with the 4th power of distance from the lens focus

Multiphoton fluorescence excitation Requires an enormous density of photons



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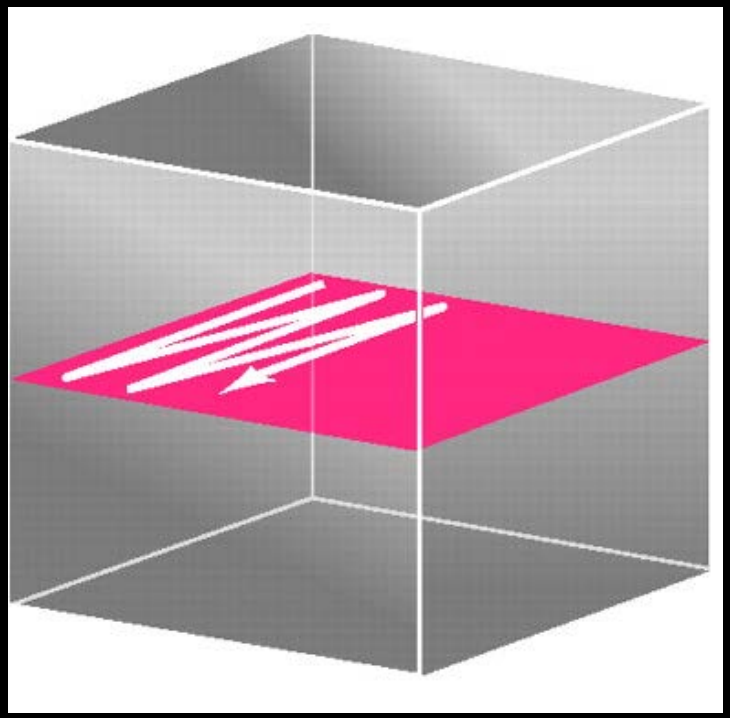
For multiphoton microscopy, this density is provided by focusing laser illumination through high NA objective lenses.

Photon density decreases with the 4th power of distance from the lens focus

Two-photon absorption occurs ONLY in the sub-femtoliter volume at the focus

Multiphoton excited fluorescence is mapped to form an image

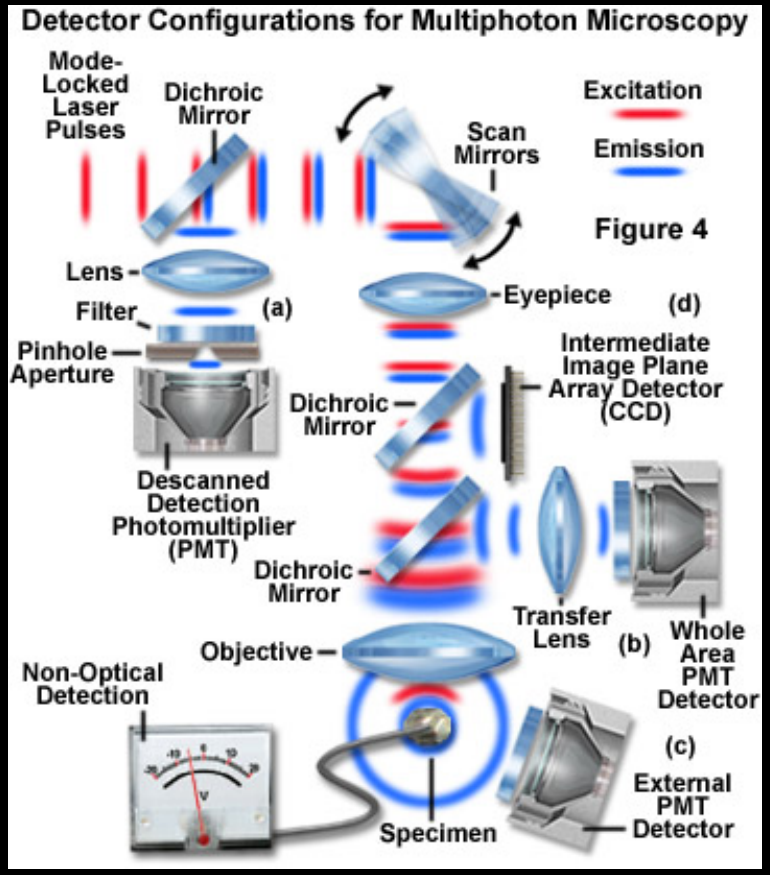


Göbel and Helmchen 2007

Build an image from single points

- A 2D image is formed by raster scanning a laser over the sample
- A 3D image is formed by collecting a set of 2D images at different depths

Multiphoton excited fluorescence is mapped to form an image



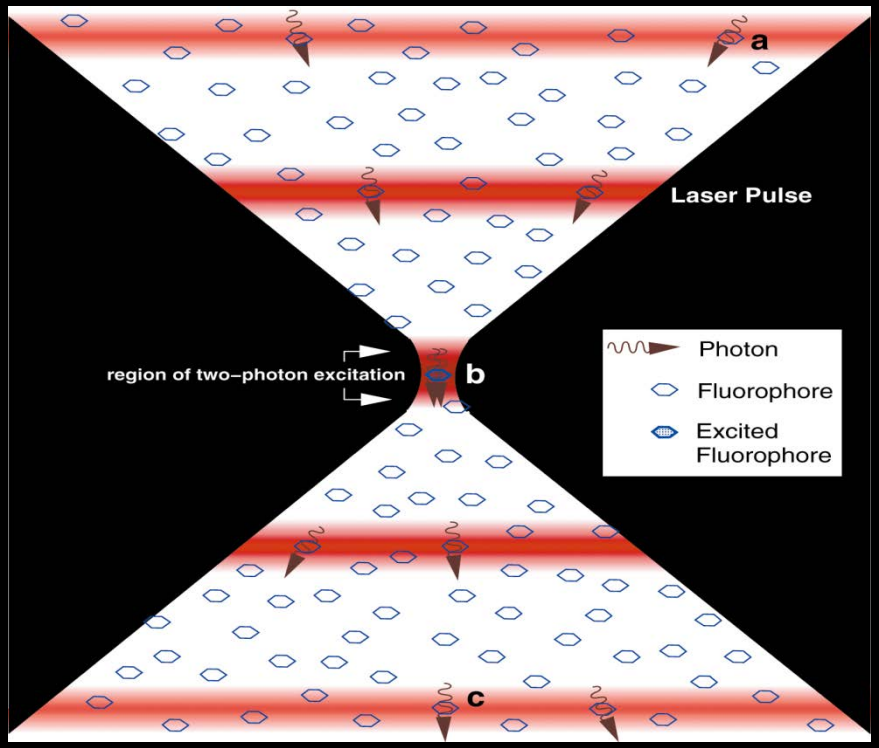
Zipfel et al., 2003

Build an image from single points

- A 2D image is formed by raster scanning a laser over the sample
- A 3D image is formed by collecting a set of 2D images at different depths
- Image is formed by collecting, rather than imaging photons

Warren Zipfel
Optimizing Microscope Design for Multiphoton Excitation Studies
Wednesday at 9:20 am

But 300,000 times brighter than the surface of the sun????



Dave Piston

Denk, Strickler and Webb, 1990

- Use a pulsed laser ~100 femtosecond pulses at a rate of 80 MHz

Peak power sufficient for multiphoton excitation, but average power low enough to minimize damage.

Laser is mostly off –

- Emitting only 0.001% of the time
- Duty cycle equivalent to 1 sec pulse/day

Note that pulses do not REALLY limit the volume of excitation

- Pulses ~ 45μ long and ~ 4 meters apart

Multiphoton microscope designs

Gregor Lab, Princeton

Roberto Weigert
Building your own 2P microscope system
Thursday, 9:20 am

Leica SP8 MP

Ken Dunn
Writing a Shared Instrumentation Grant
Thursday, 11:10 am

Fun facts about multiphoton microscopy

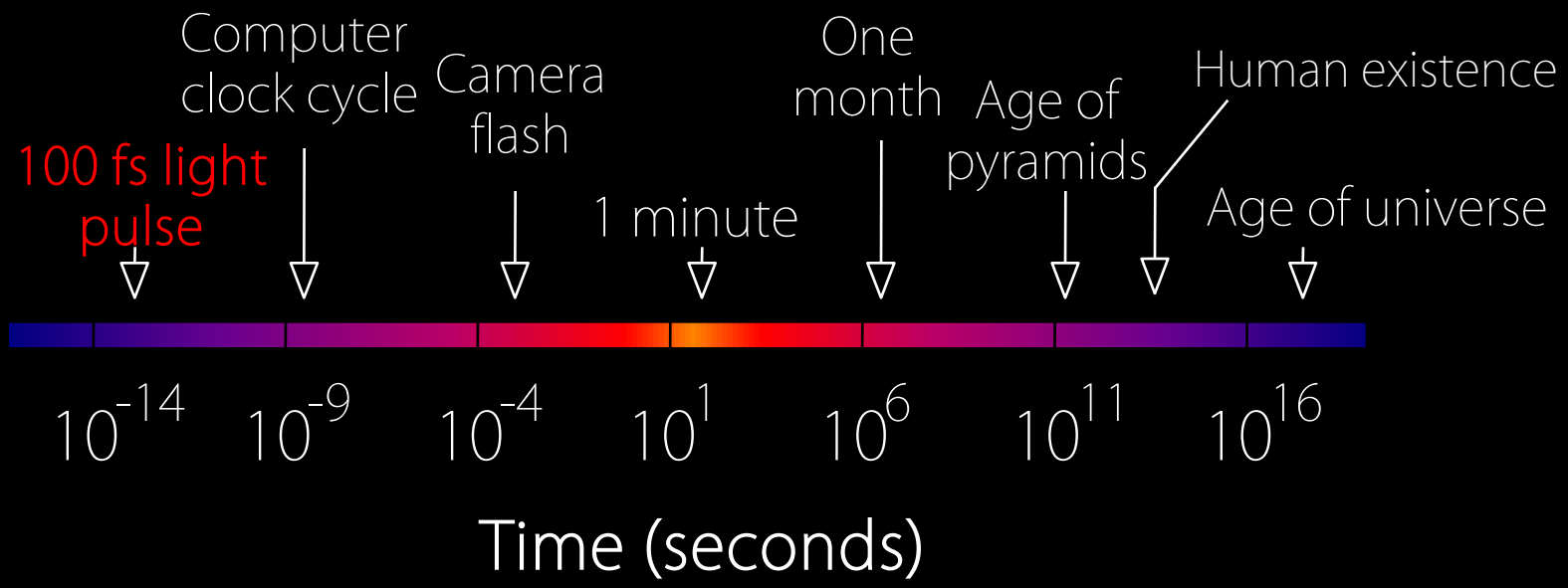
How frequently does multiphoton fluorescence excitation occur outside of a microscope?

Calculations made by Winfried Denk indicate that a molecule of rhodamine B exposed to direct sunlight will experience:

- A one-photon absorption around once per second.
- A two photon absorption once every 10,000 years.
- A three-photon absorption . . . never in the history of the universe.

Fun facts about multiphoton microscopy

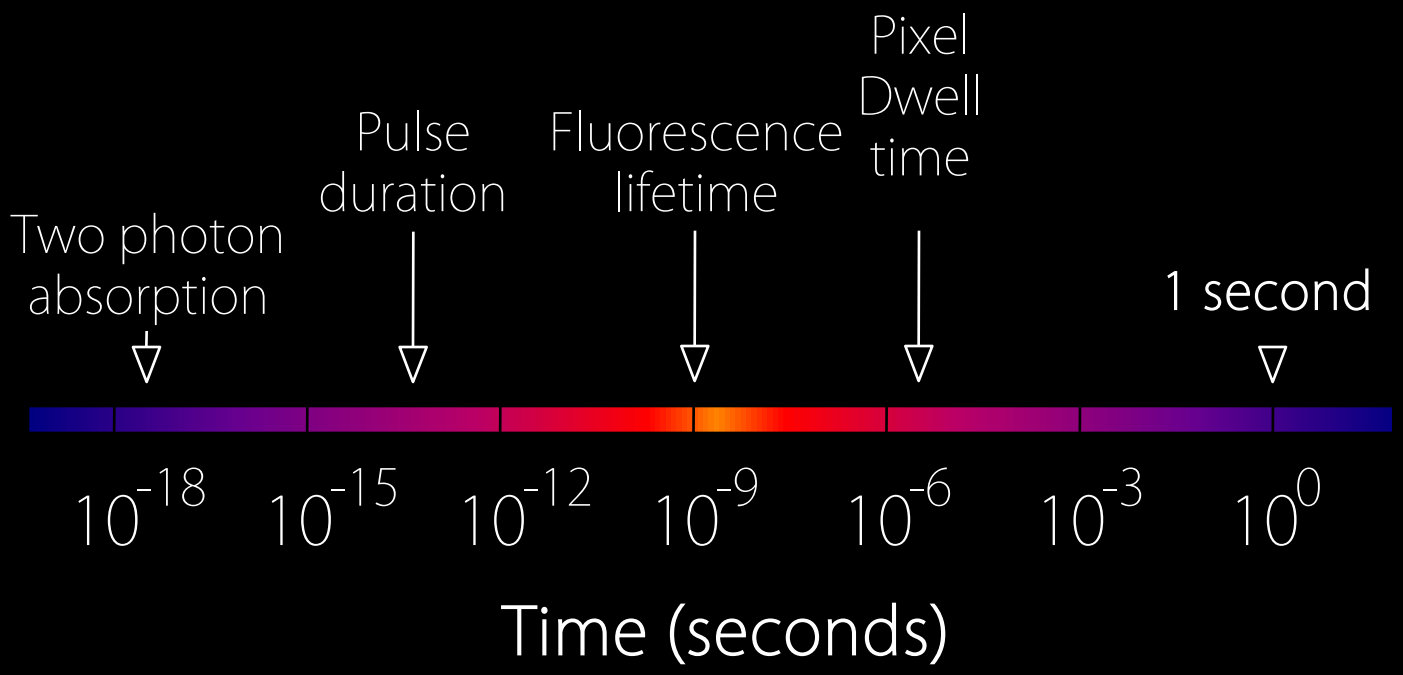
Ridiculous time scales



100 fs is to 10 minutes as 10 minutes is to the age of the universe.

Rick Trebino, University of Georgia

Fun facts about multiphoton microscopy
Relative time scales

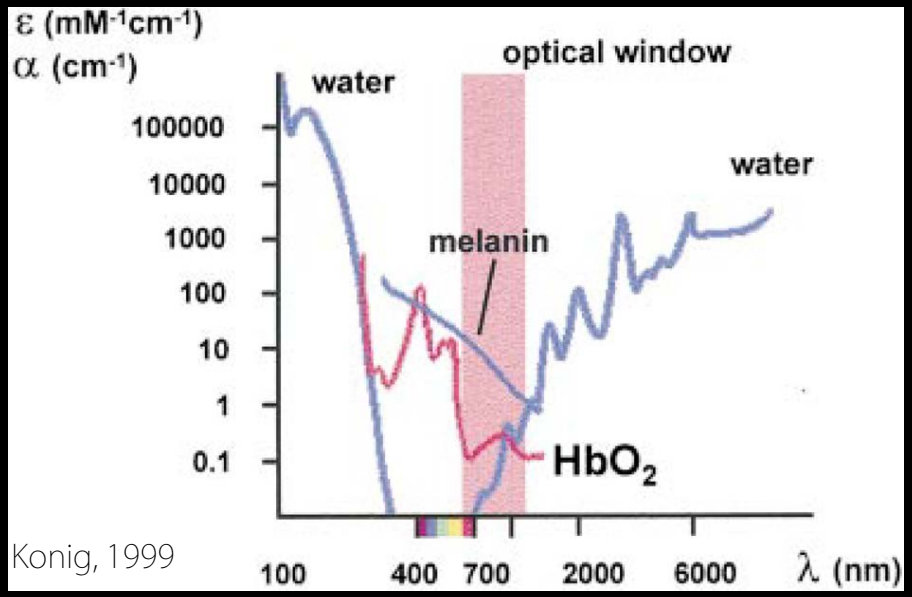


- Fluorescence lifetimes are ~ 1 billion times the length of the virtual intermediate state
- At 80 MHz, the laser pulses are spaced ~ 3 fluorescence lifetimes apart (serendipitous)
- A fluorophore will be illuminated ~320 times during a 4 microsecond pixel dwell time

Why multiphoton microscopy?

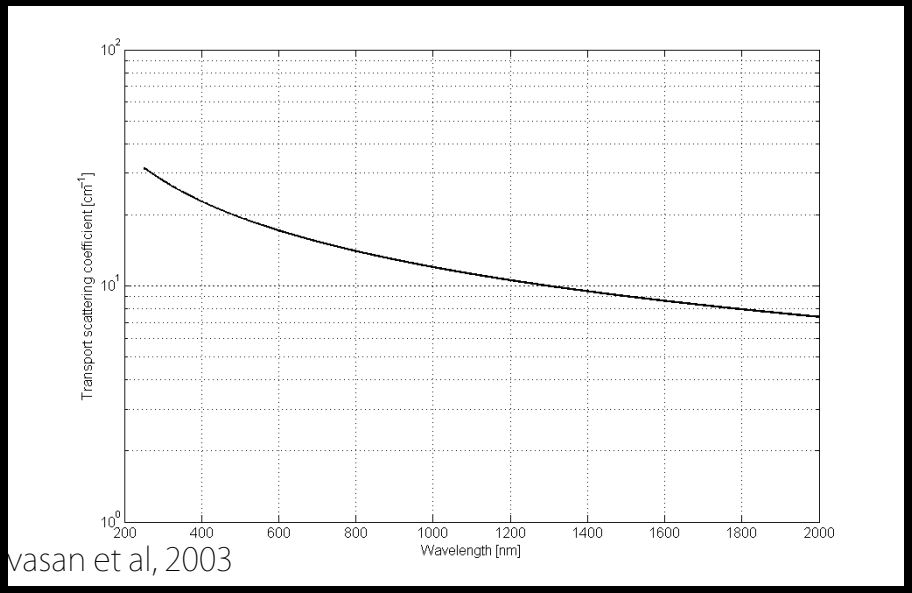
Complex
Expensive
Poorer resolution

Why multiphoton microscopy?



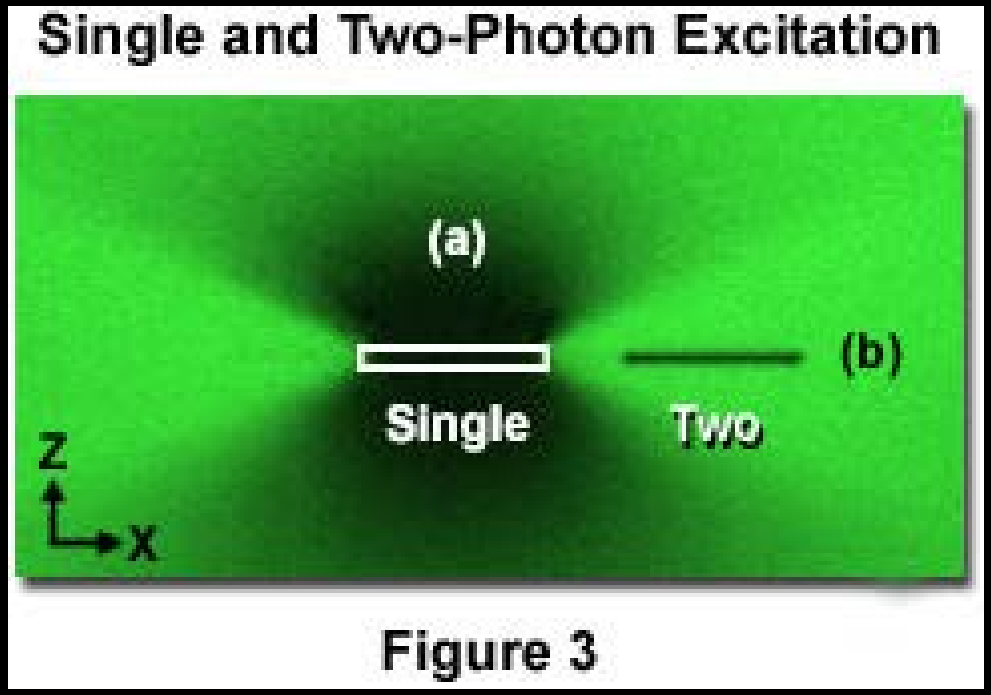
Konig, 1999

- IR light penetrates deeper, with less damage



vasan et al, 2003

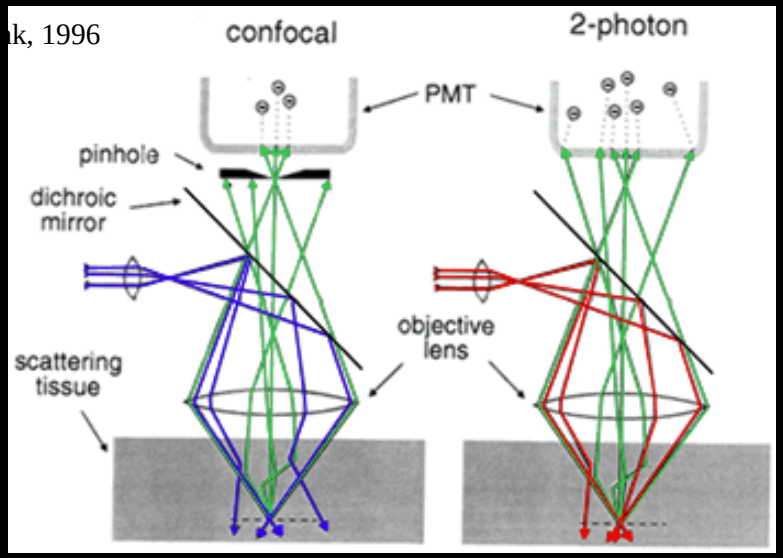
Why multiphoton microscopy?

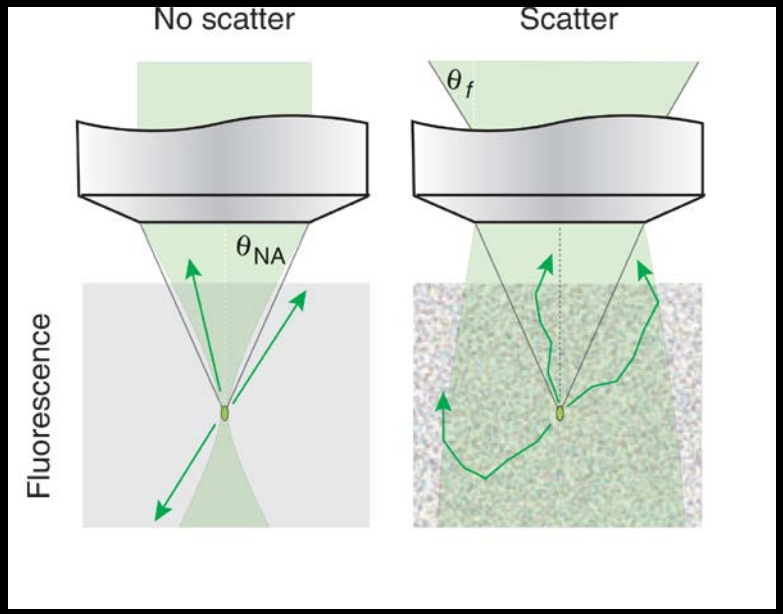


Nikon MicroscopyU

- IR light penetrates deeper, with less damage
- Photobleaching only in the focal plane (but more of it)

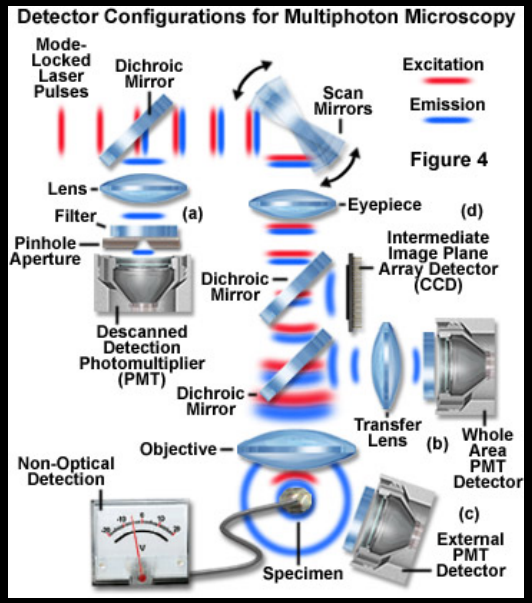
Why multiphoton microscopy?

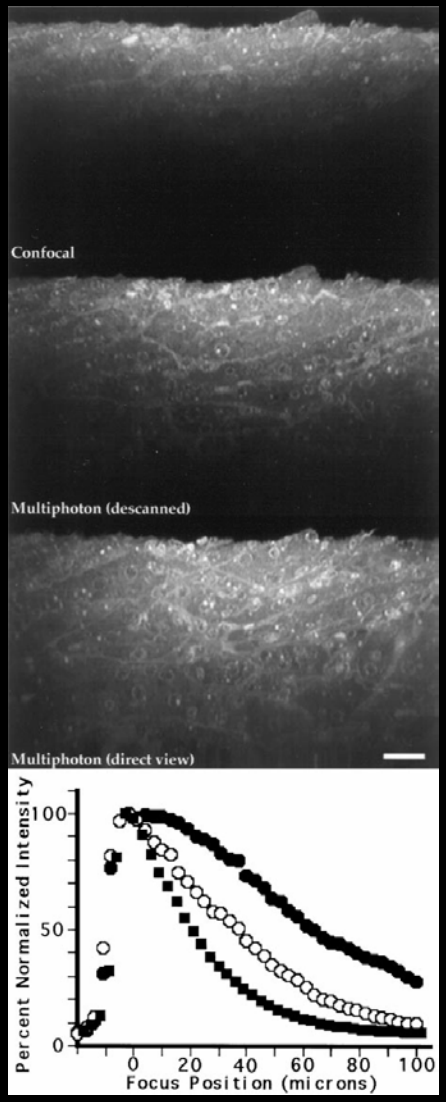




- IR light penetrates deeper, with less damage
- Photobleaching only in the focal plane (but more of it)
- Optical sectioning without an emission aperture - less loss to scattering

Why multiphoton microscopy?





Centonze and White, 1998

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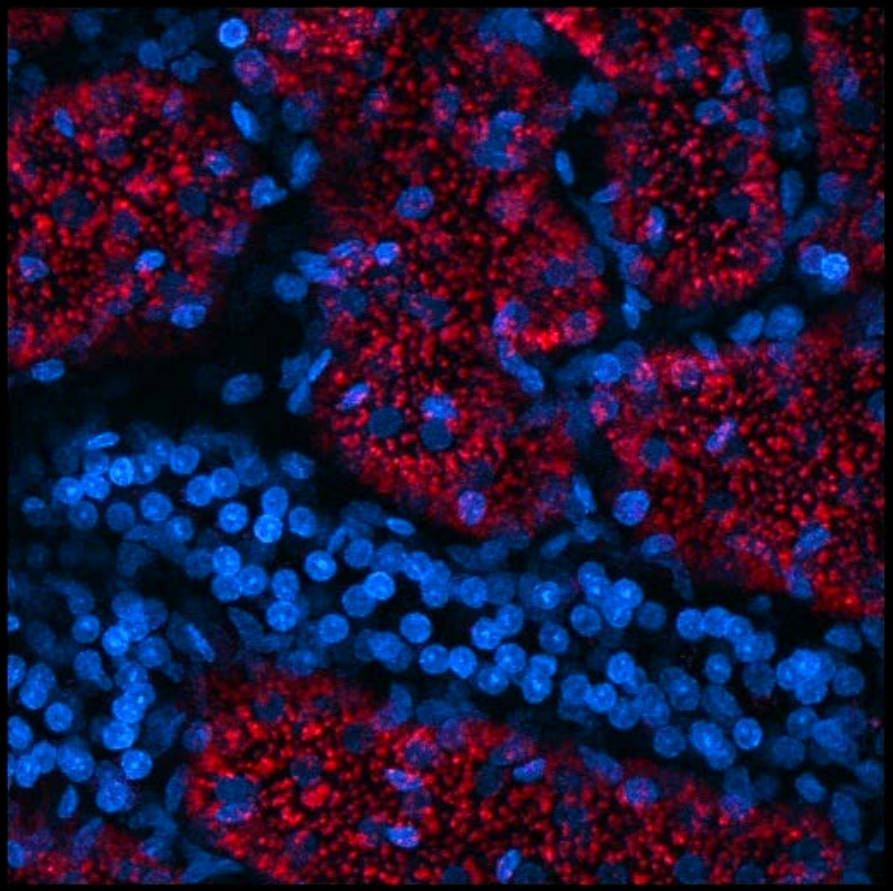
Why multi-photon microscopy? Intravital microscopy



- Intravital microscopy provides submicron resolution and nanomolar sensitivity in under a second.
- Multiphoton microscopy has extended the reach of intravital microscopy to the scale of tissues and the functional components of organs.

Applications of intravital multiphoton microscopy in biomedical research

Brain function and pathology
- Neural development and activity - Winfried Denk, Karel Svoboda
- Alzheimer's disease - Brian Bacskai, Brad Hyman
- Astrocyte-neuron signaling - Jan Nedergard
- Vascular function in the brain - David Kleinfeld

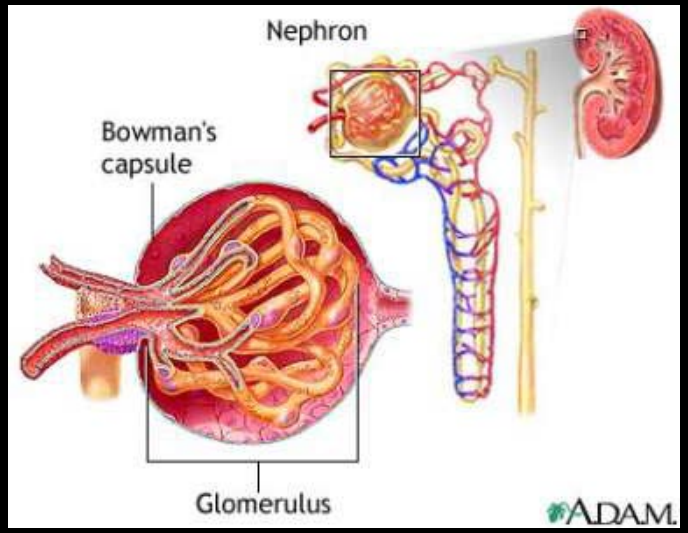
Tumor biology
- Tumor cell dynamics and metastasis - John Condelis, John Segal
- Angiogenesis/vascular function, gene expression - Rakesh Jain

Immunology
- T-cell interactions and dynamics- Michael Dustin, Ullrich Von Andrian, Michael Cahalan
- Immune surveillance in the brain - Fritjof Helmchen, W.B. Gan

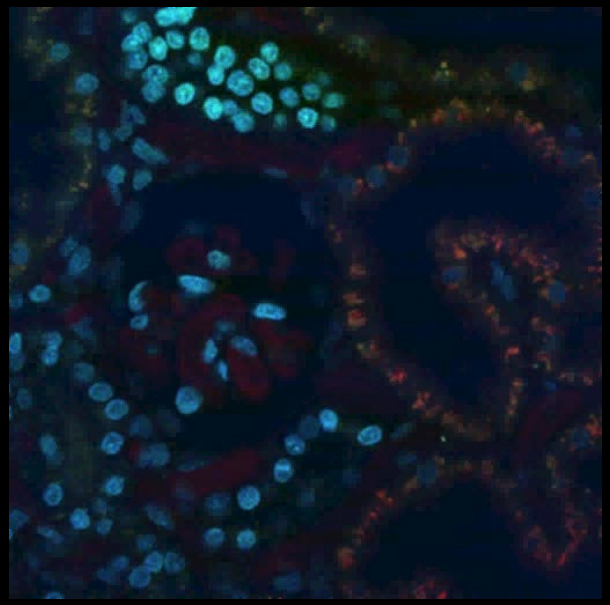
Liver function
- Mitochondria and liver injury - John Lemasters
- In vivo analysis of hepatobiliary transport – Ken Dunn

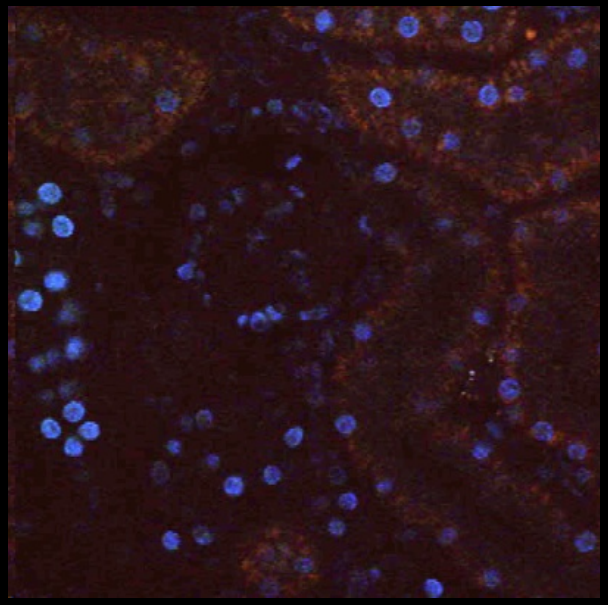
Kidney function
- Pathology and treatment of renal ischemia - Bruce Molitoris
- Pathobiology of AKI - Katherine Kelly
- Renal septic injury - Pierre Dagher
- Microvascular function in renal injury - Tim Sutton
- Glomerular function - Janos Peti-Peterdi

Roberto Weigert
Membrane Remodeling Through Membrane Trafficking in Live Animals
Tuesday, 5pm



Dynamic intravital microscopy of the kidney



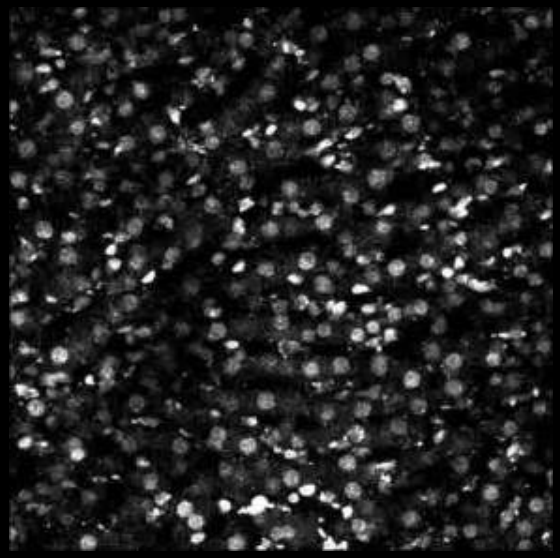


Ruben Sandoval and Bruce Molitoris

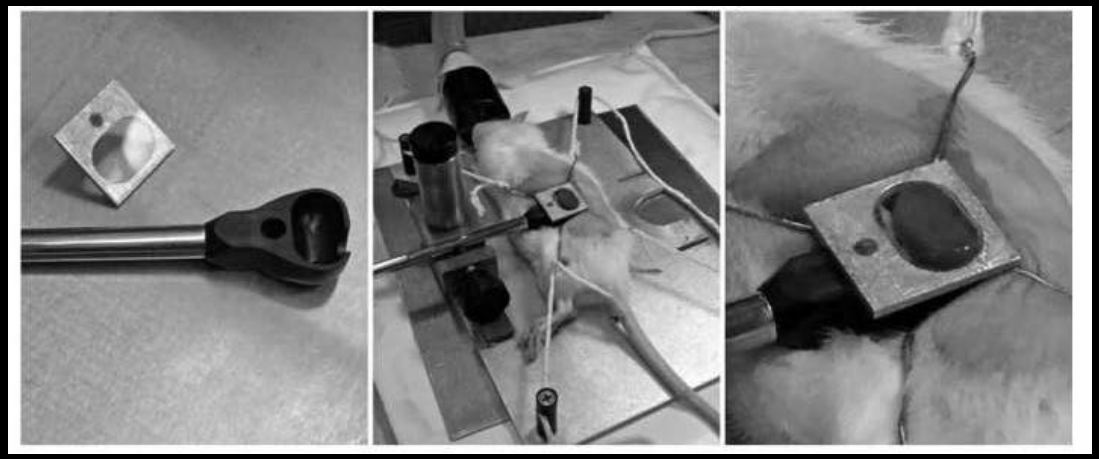
Challenges of subcellular imaging in living animals

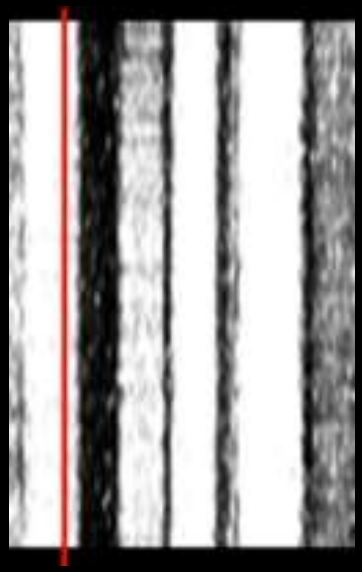
- Motion artifacts from respiration and heartbeat

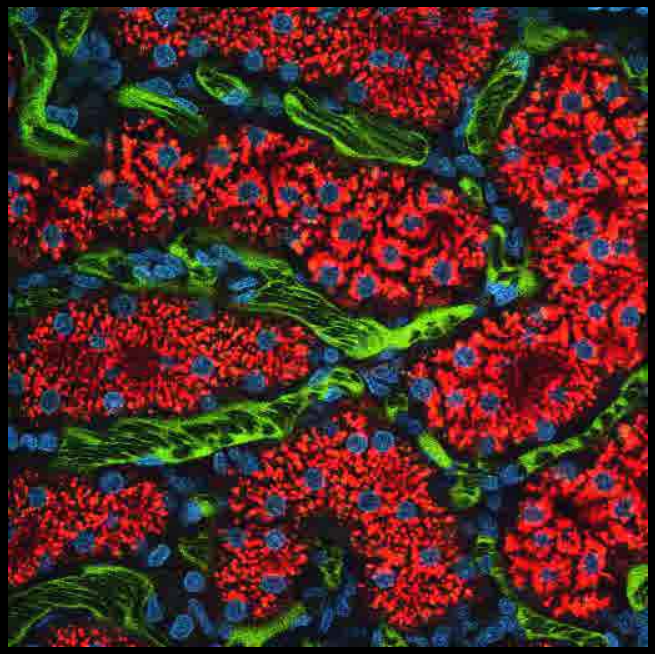
Reducing motion artifacts in intravital microscopy - Immobilize the organ



75 images collected over 30 min

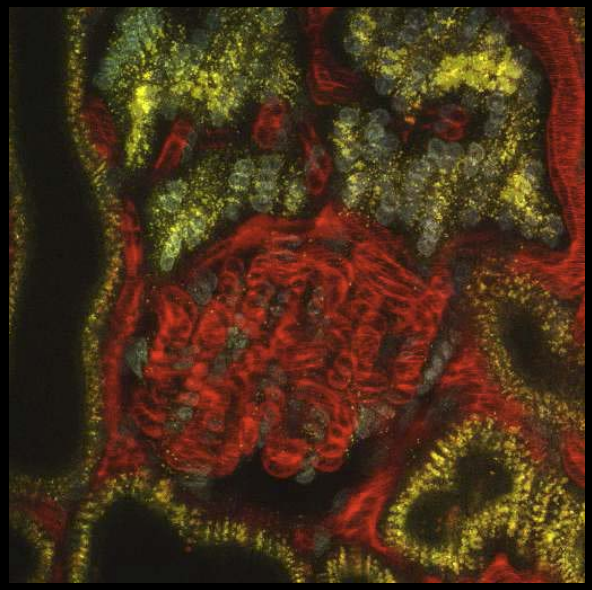




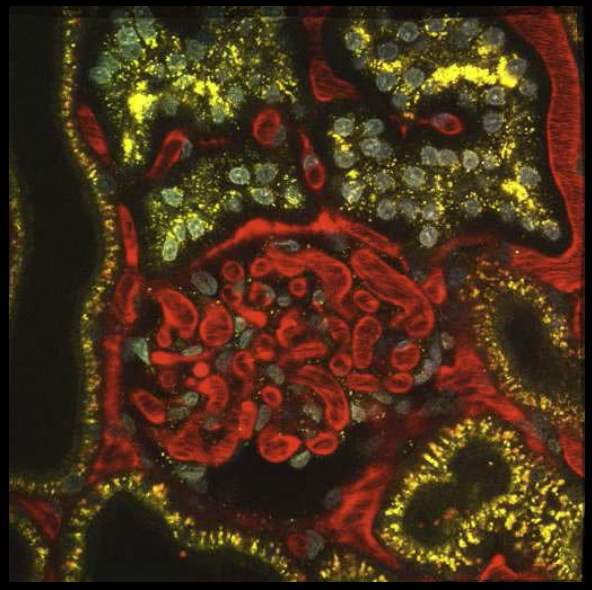


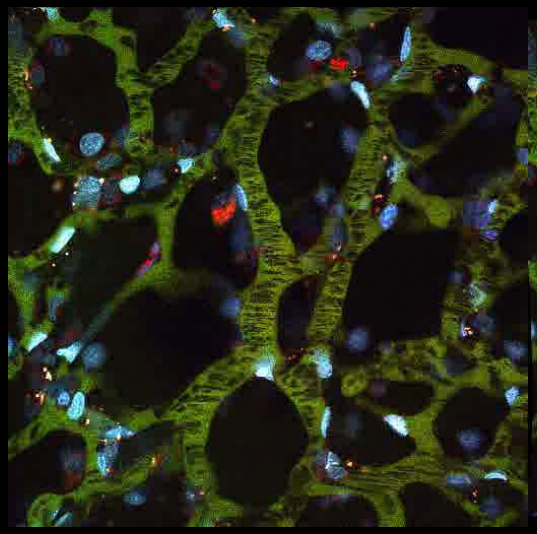
Reducing motion artifacts in intravital microscopy – Digital image registration

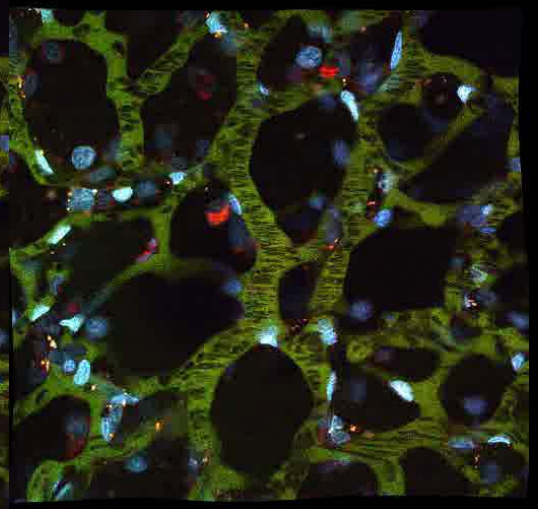
Before



After





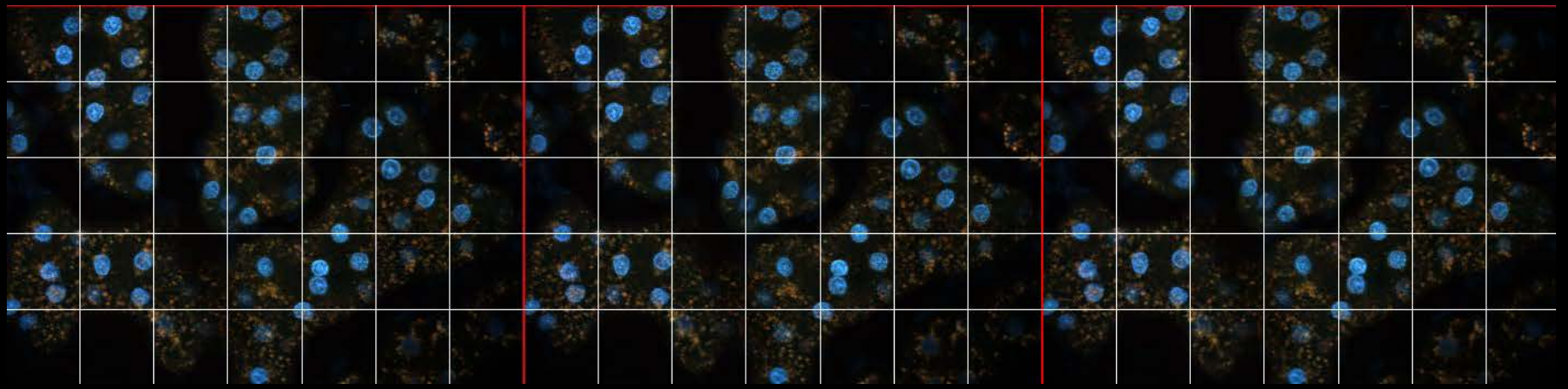


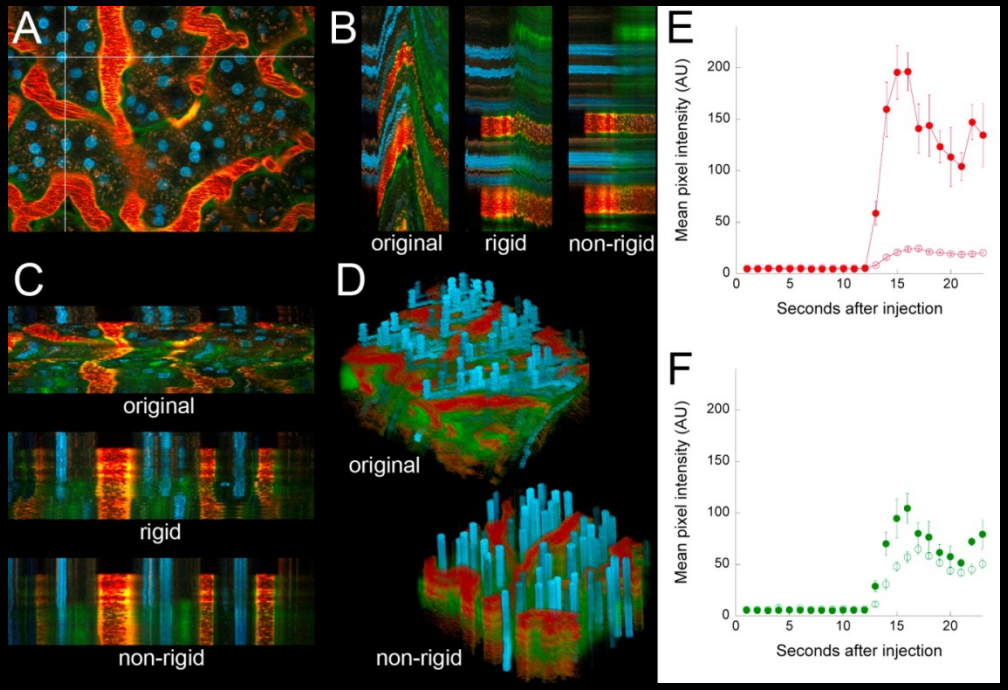
Before *After*

Presson, R., Brown, M., Sandoval, R., **Dunn, K.**, Lorenz, K., Delp, E., Salama, P., Molitoris, B. and Petrache, I. 2011. Two-photon imaging within the murine thorax without respiratory and cardiac motion artifact. Am. J. Path. 179:75-82

Lorenz, K.S., Salama, P., **Dunn, K.W.** and E.J. Delp. 2011. Non-rigid registration of multiphoton microscopy images using B-splines. Progress in Biomedical Imaging SPIE proceedings. 7962.

Reducing motion artifacts in intravital microscopy – Digital image registration





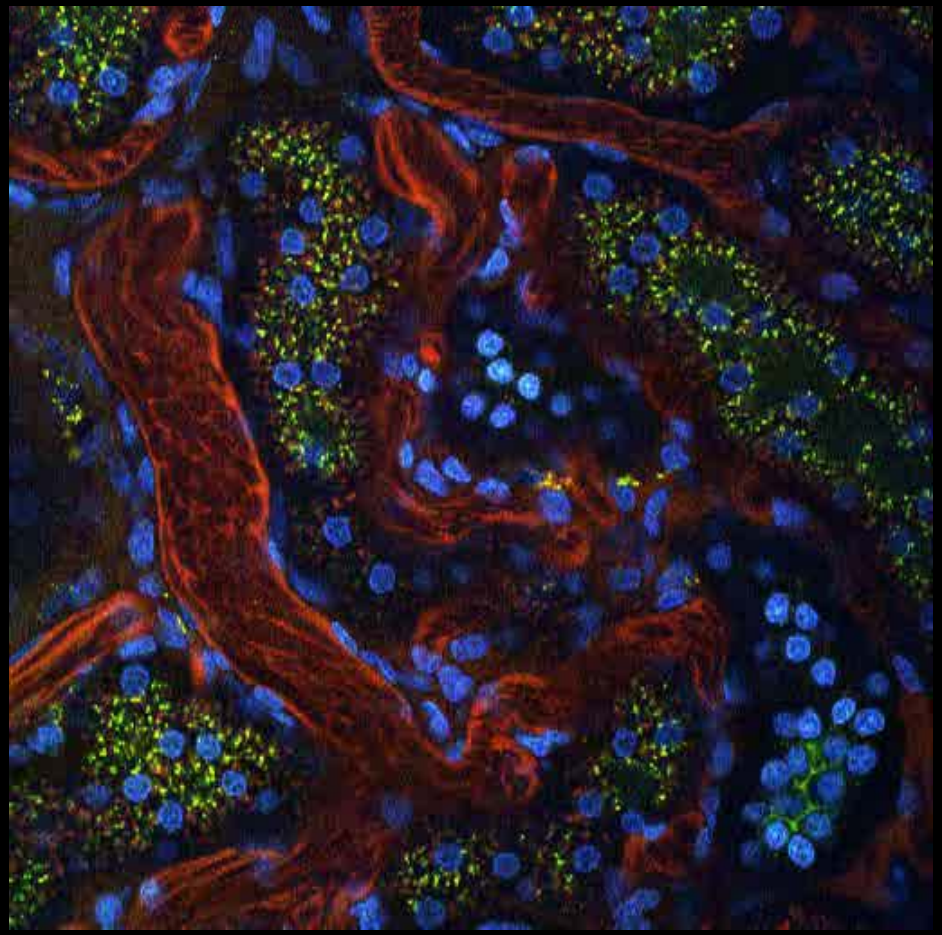
Dunn, Lorenz, Salama, and Delp. 2014. IMART software for correction of motion artifacts in images collected in intravital microscopy. Intravital. 3:e28210-1-10

Edward Delp
Use of Digital Image Analysis for Studies of Renal Physiology
Tuesday at 9:20 am

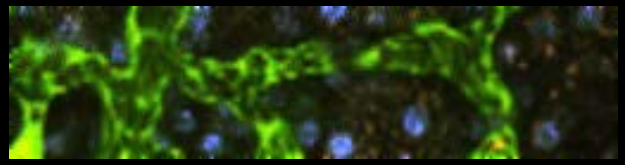
Challenges of subcellular imaging in living animals

- Motion artifacts from respiration and heartbeat
- Slow image capture rate

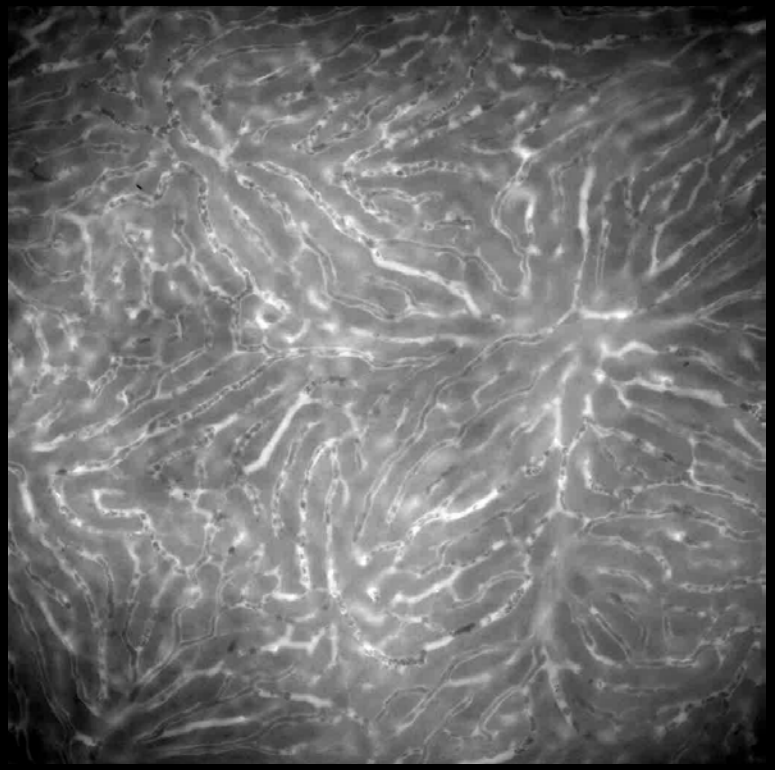
Single point scanning with a galvanometer
512 lines per second



One 512x512 frame per second

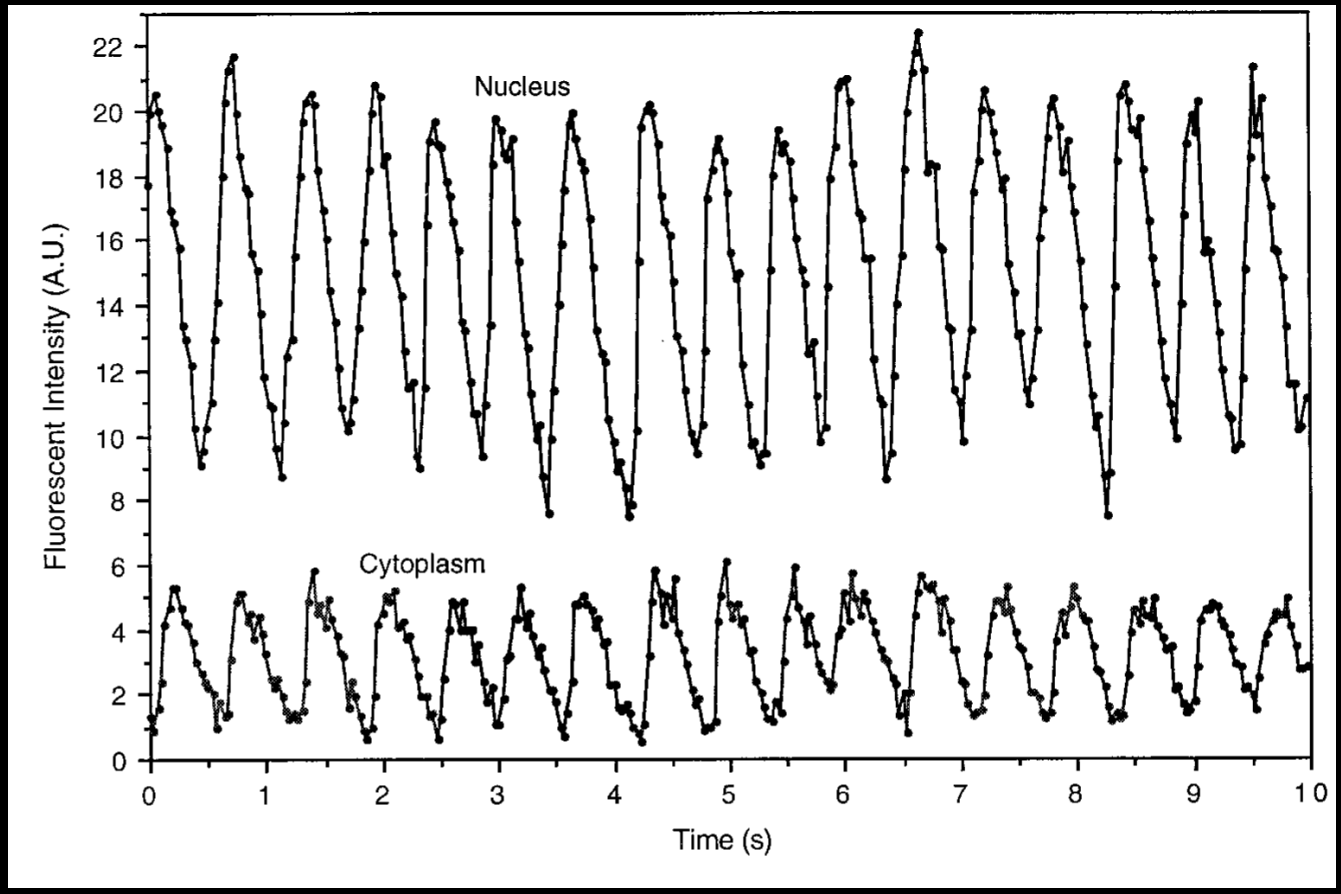


Four 512x128 frames per second



100 fps - widefield epifluorescence microscopy
1 minute, playing at 1/3rd speed

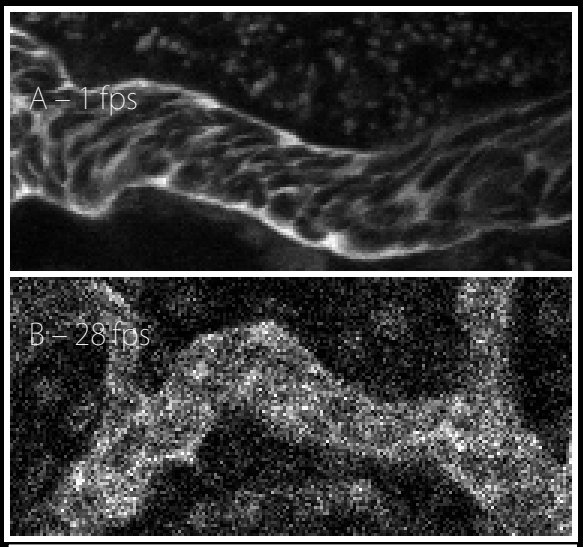
Single point scanning with a resonant scanner 15000 lines per second (30 fps)



Fan et al., 1999. Biophys. Journal 76:2412

Speed limits for multi-photon microscopy

30-fold higher frame rates require 30-fold shorter pixel dwell times which then requires 30 fold better signal. For most applications increasing the signal requires increasing the excitation.



Images of renal capillary of a living rat collected at 1 frame per second (A) or 28 fps (B).

- In many applications of multiphoton microscopy, power is already limiting – you may not be able to extract more signal with more power - 30 fold increase in signal requires 5.5 fold more power

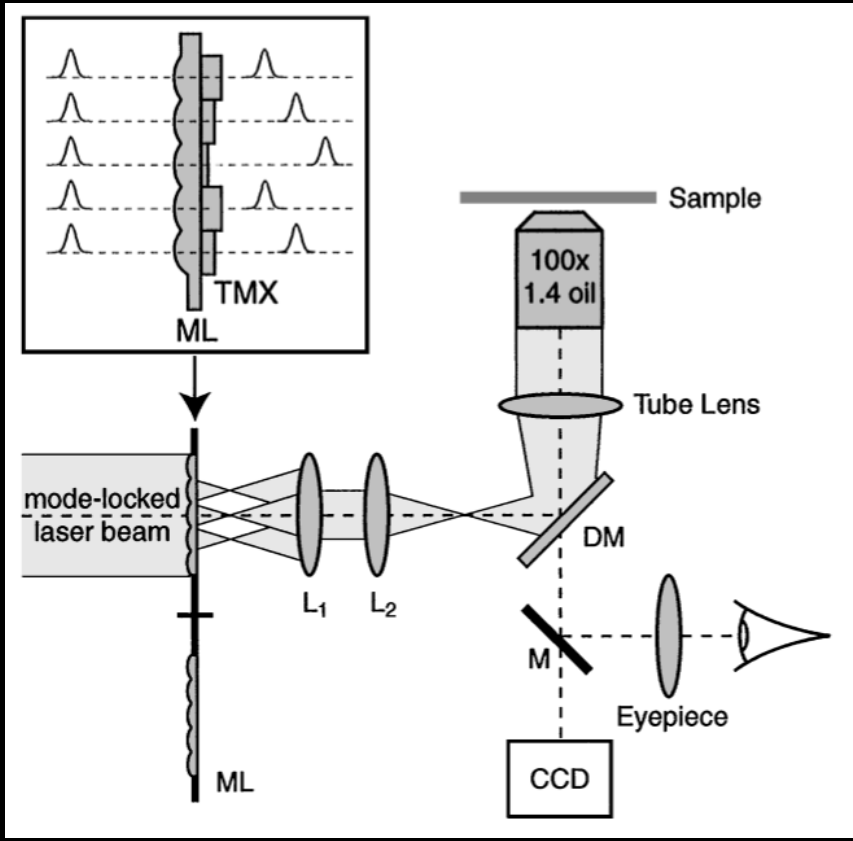
- In many applications, multiphoton imaging is done at power levels very close to fluorophore saturation – you may not get proportional increases in signal

- Photobleaching increases at greater than the cube of power – disproportionately more photobleaching than signal with more power

But, for experimental systems with sufficient fluorescence, resonant scanners are capable of capturing dynamics in vivo

Try the Leica SP8 MP system

Multi-point scanning multiphoton microscopy



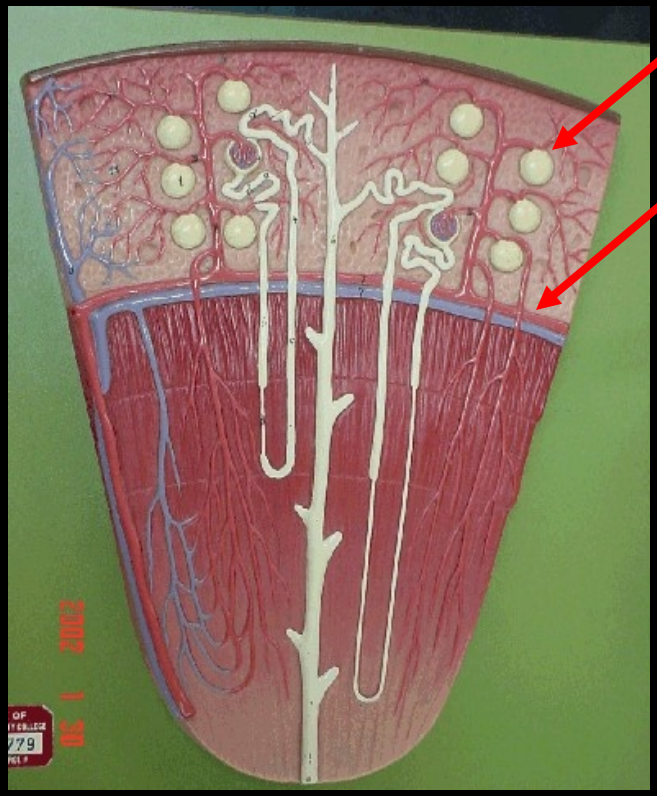
Andresen et al., 2001. Optics Letters 26:75

- Multi-focal systems speed image acquisition by parallel scanning
- Power is divided among multiple excitation points – laser power limits multiplexing
- Imaging onto a CCD results in scattered emissions being attributed to the wrong position in the image, increasing background and reducing resolution
- The system would thus be of limited utility for imaging deep into tissues, the conditions that justify multiphoton microscopy in the first place

Challenges of subcellular imaging in living animals

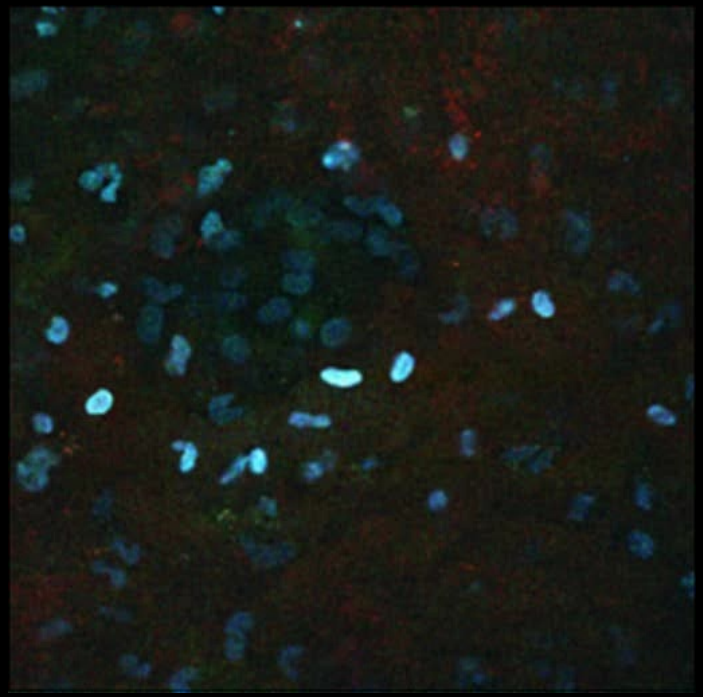
- Motion artifacts from respiration and heartbeat
- Slow image capture rate
- Limited reach/signal attenuation with depth

Multiphoton microscopy
Attenuation of signal with depth in the kidney



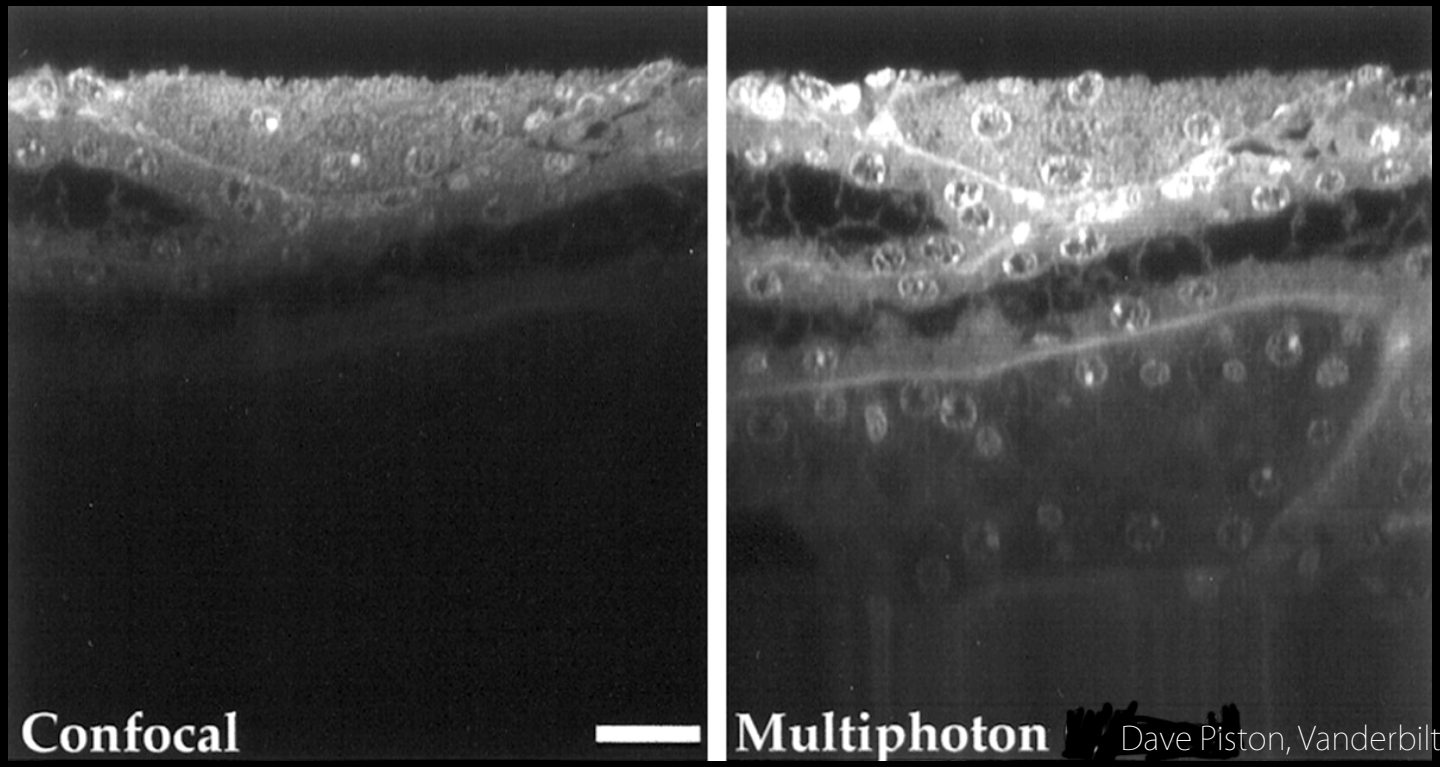
Glomeruli of most rats, mice are around 400 microns from the surface of the kidney

Cortico-medullary boundary is around 2 mm from the surface of the kidney



With a 60X NA 1.2 water immersion objective, signal attenuation prevents imaging deeper than around 100 microns in kidney of living rats

Multiphoton microscopy
Sources of signal attenuation of signal with depth



- Light absorption
- Light scattering
- Spherical aberration

Multiphoton microscopy
Light absorption as a source of signal attenuation with depth

Model light extinction as - $I_z = I_0 e^{-az}$

Where

I_z = intensity of light at depth z

a = the absorption coefficient

For fluorescence excitation

Near infrared light, $a = .05$ to $2\ cm^{-1}$ in biological tissues

So transmission attenuated by 50% in 0.35 - 14 cm

But excitation is proportional to I^2,

Excitation is attenuated by 75% in 0.35 - 14 cm

- Excitation reduced 75% in ~ 3500 to 140,000 microns - not a big deal

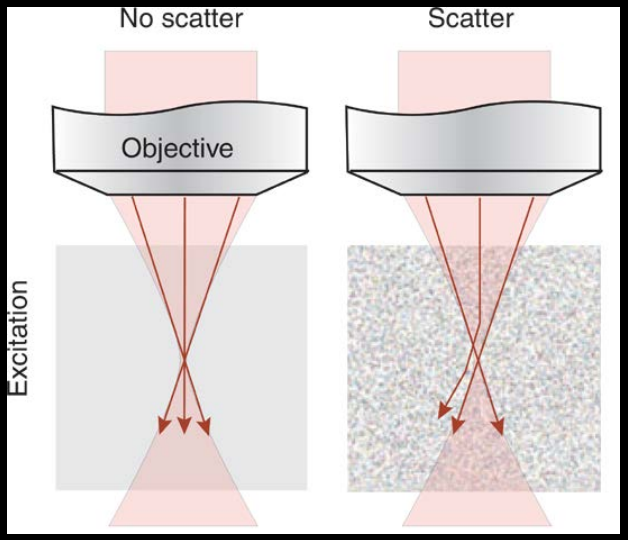
For collection of fluorescence emissions

For 550 nm light, $a = 4$ to $20\ cm^{-1}$ in biological tissues

- Transmission reduced 50% in 350-1730 microns – not a real big deal

Most investigators agree, absorption is seldom significant in biological tissues,
except in skin (melanin) and under blood vessels (hemoglobin)

Multiphoton microscopy
Light scattering as a source of signal attenuation with depth



Model light extinction as - $I_z = I_0 e^{-sz}$

Where

I_z = intensity of light at depth z

s = the scattering coefficient

For fluorescence excitation

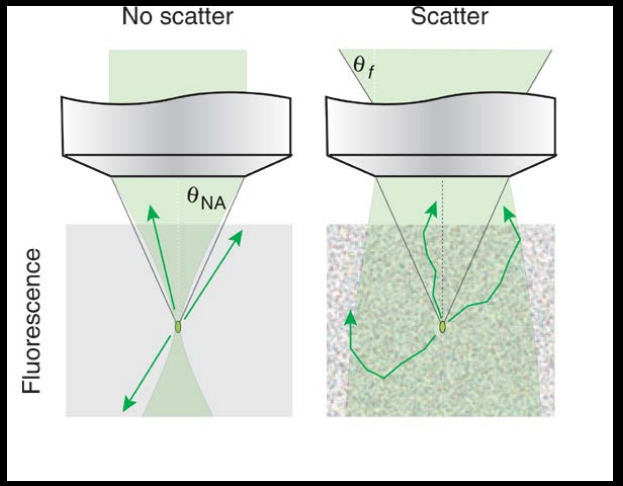
Near infrared light, s = 5 - 100 cm^{-1} in biological tissues

So transmission attenuated by 50% in 70 to 1400 microns

But excitation is proportional to I^2,

Excitation attenuated by 75% in 70 to 1400 microns

Multiphoton microscopy
Light scattering as a source of signal attenuation with depth



Model light extinction as - $I_z = I_0 e^{-sz}$

Where

I_z = intensity of light at depth z
s = the scattering coefficient

For fluorescence excitation

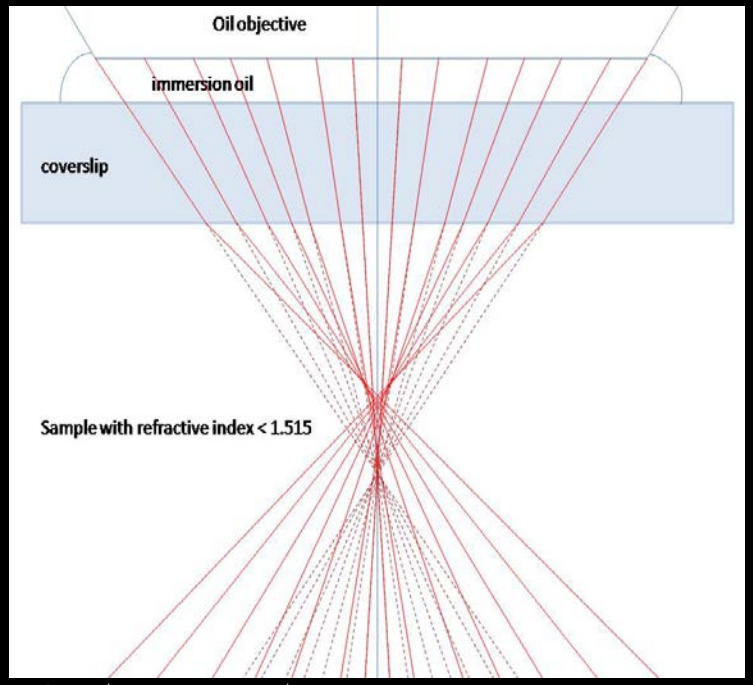
Near infrared light, s = 5 - 100 cm^{-1} in biological tissues
So transmission attenuated by 50% in 70 to 1400 microns
But excitation is proportional to I^2,
Excitation attenuated by 75% in 70 to 1400 microns

For collection of fluorescence emissions

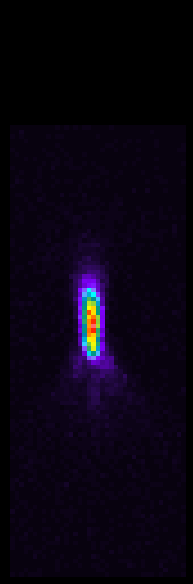
For 550 nm light, s = 100 – 500 cm^{-1} in biological tissues
So transmission attenuated by 50% in 15 - 70 microns

Multiphoton microscopy
Spherical aberration as a source of signal attenuation

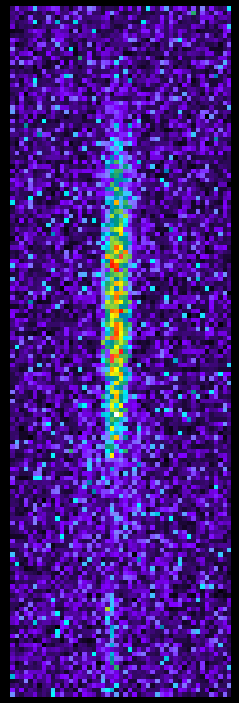
Two photon microscopy of fluorescent beads mounted in different media
using an oil-immersion objective



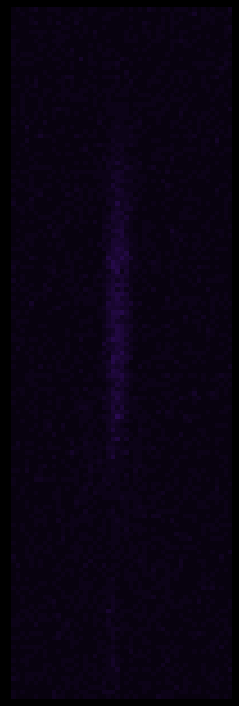
An oil-immersion objective imaging into an aqueous medium



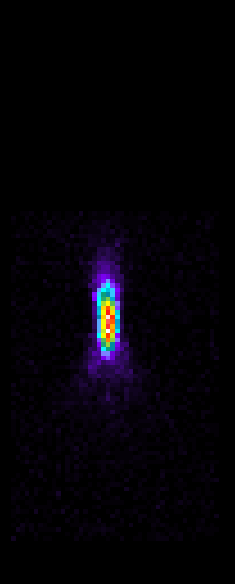
Surface
Aqueous



100um
Aqueous



100um
Aqueous
(w/out stretch)

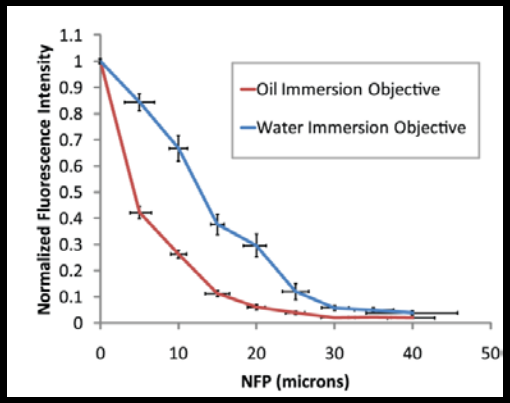


75um
1.5 RI medium

Since the average refractive index of the kidney is ~1.4, we incur spherical aberration with either water immersion or oil immersion objectives

Limited optical reach in the living kidney
Potential solutions

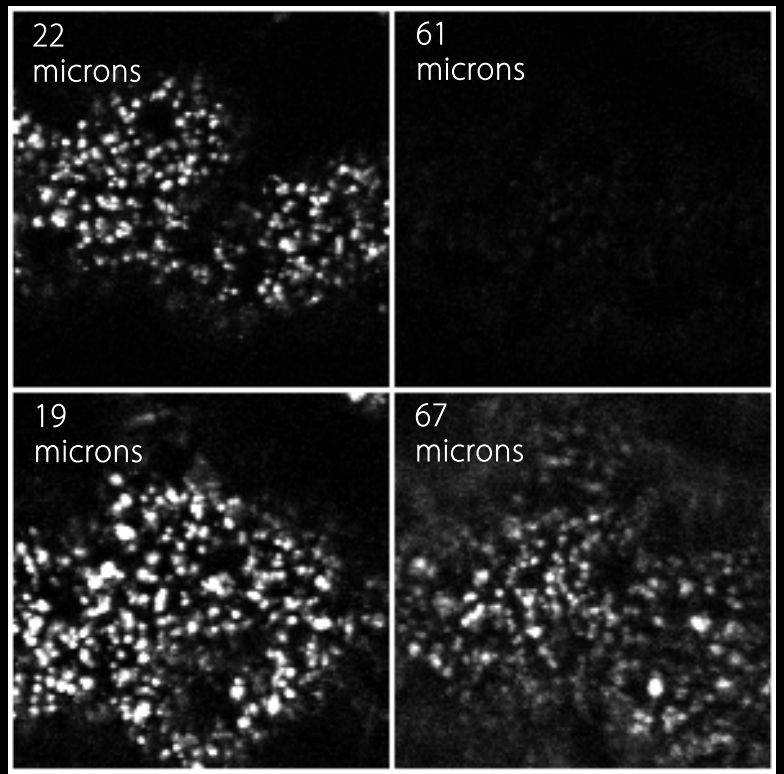
- Reduce optical aberrations



Young, Clendenon, Byars, Decca and Dunn. 2011. J. Microscopy

Olympus 25X, NA 1.05
Water immersion
objective
RI 1.33

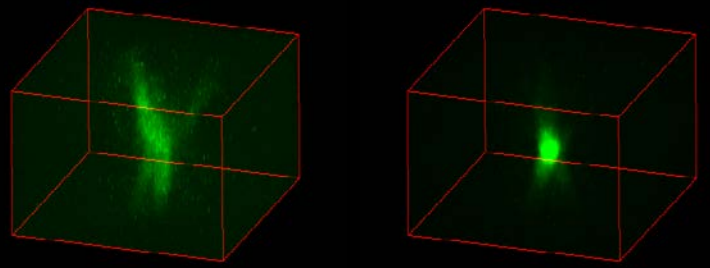
Olympus 30X, NA 1.05
Silicone oil immersion
objective
RI 1.4



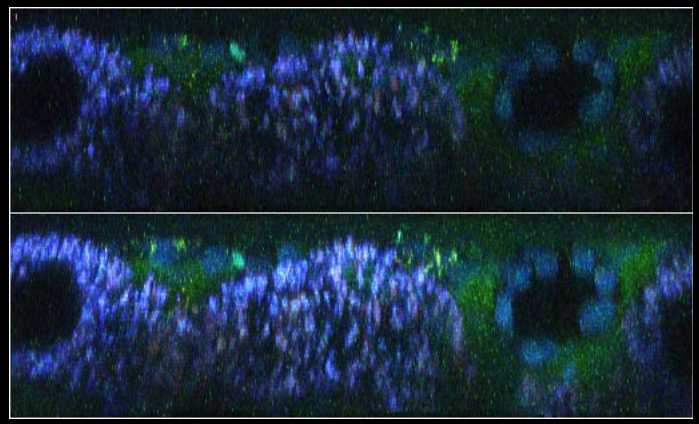
Limited optical reach in the living kidney
Potential solutions

- Reduce optical aberrations

Adaptive optics



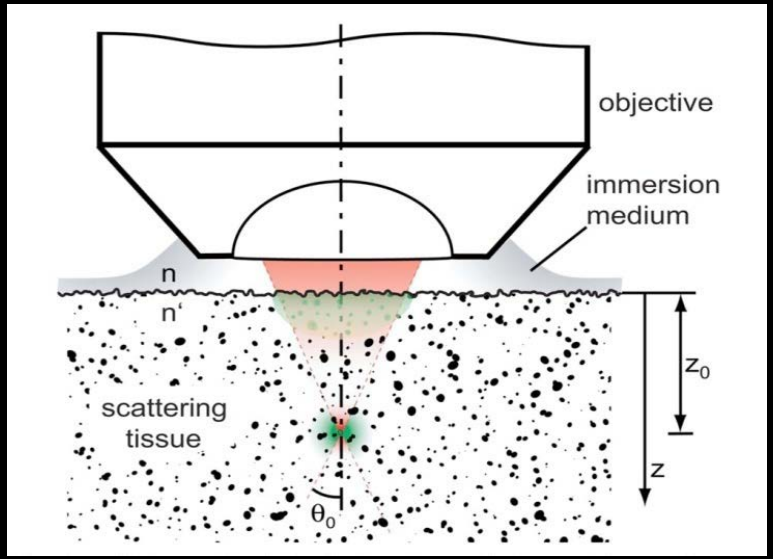
Confocal microscope images of 1 micron fluorescent spheres located below 100 microns of kidney tissue before (A) and after (B) adaptive optics correction – Joel Kubby, UCSC



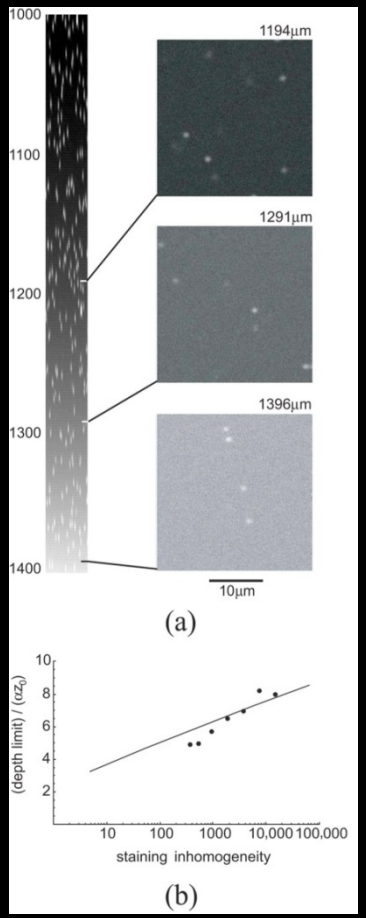
Seth Winfree and Ruben Sandoval

Limited optical reach in the living kidney
Potential solutions

- Reduce optical aberrations
- Jack up the laser



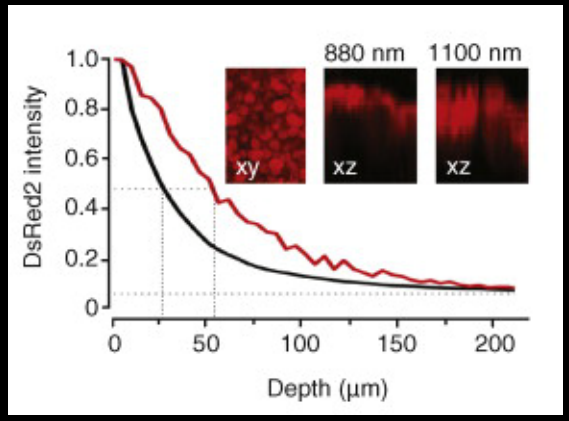
Imaging depth is ultimately limited by excitation of near-surface fluorescence
- signal-to-background ratio goes to 1
- Theer and Denk, 2006



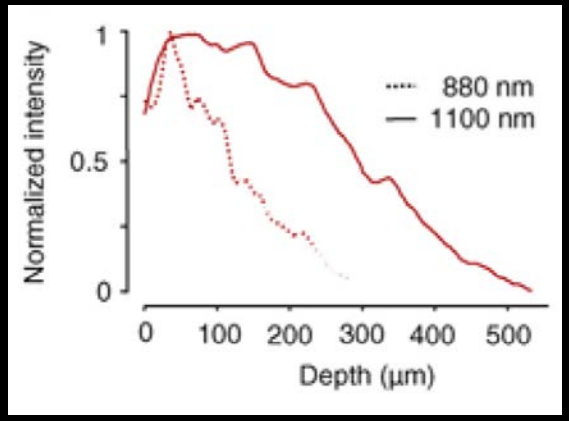
Limited optical reach in the living kidney
Potential solutions

- Reduce optical aberrations
- Jack up the laser
- Longer wavelengths of light

Benefit of long-wavelength excitation



Tissue spheroid



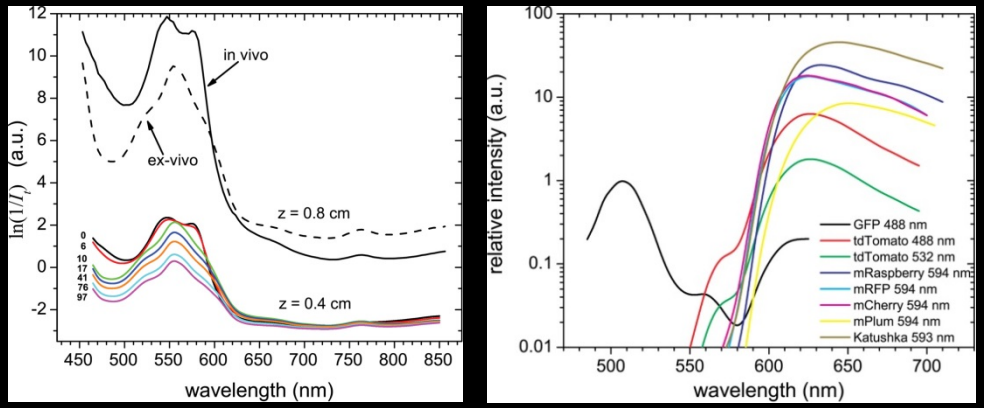
Tumor xenograft

Andresen et al., 2009. Infrared multiphoton microscopy: subcellular-resolved deep tissue imaging. Curr. Opin. Biotech.

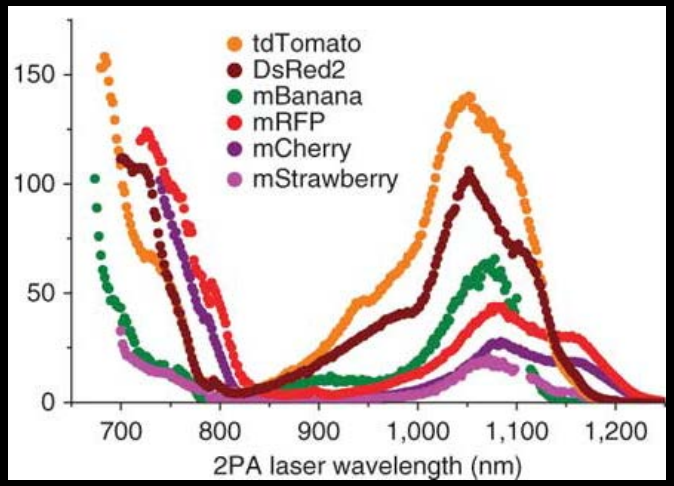
Limited optical reach in the living kidney
Potential solutions

- Reduce optical aberrations
- Jack up the laser
- Longer wavelengths of light

Benefit of long-wavelength emissions



Deliolanis et al., 2008. J. Biomed. Opt.

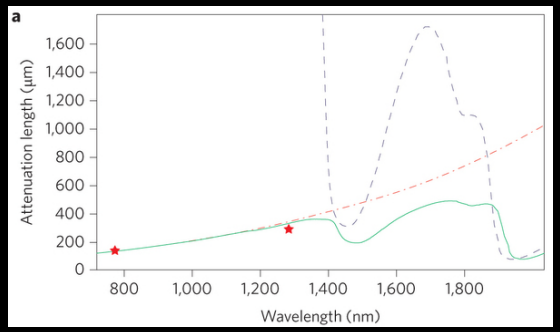


Drobizhev et al., 2011. Nature Methods

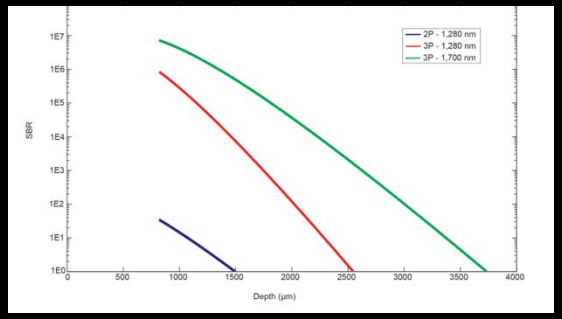
Limited optical reach in the living kidney
Three-photon fluorescence excitation

- Reduce optical aberrations
- Jack up the laser
- Longer wavelengths of light

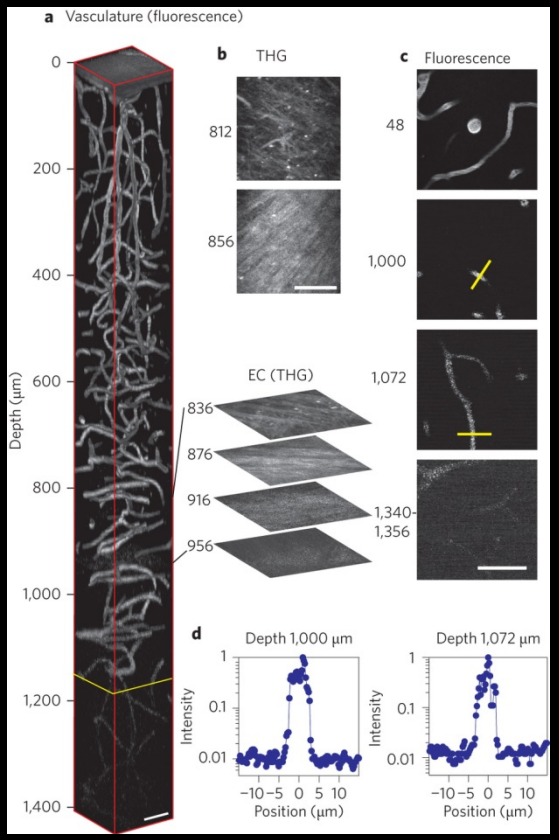
Benefits of 3-photon excitation at 1700 nm



Mie scattering and water absorption as a function of wavelength



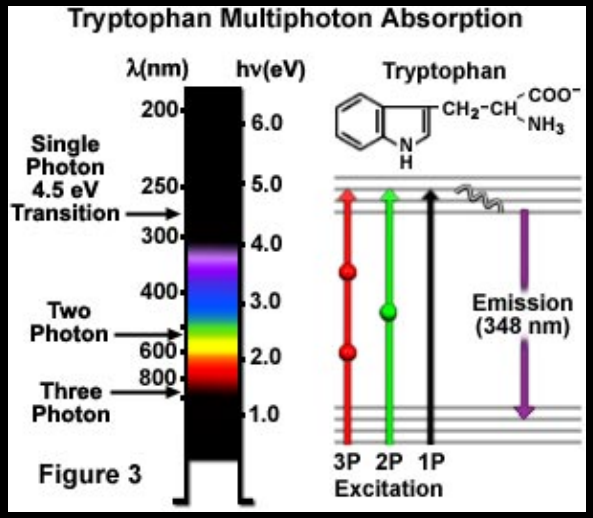
Signal-to-background ratio for 2P and 3P excitation



Horton et al., 2013. Nature Photonics

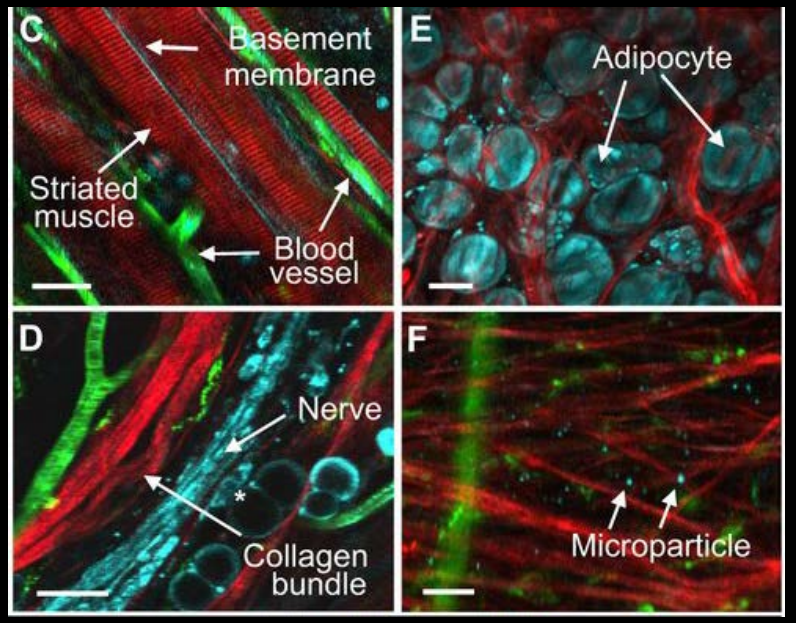
Additional benefits of three-photon excitation

Deep-UV excitation



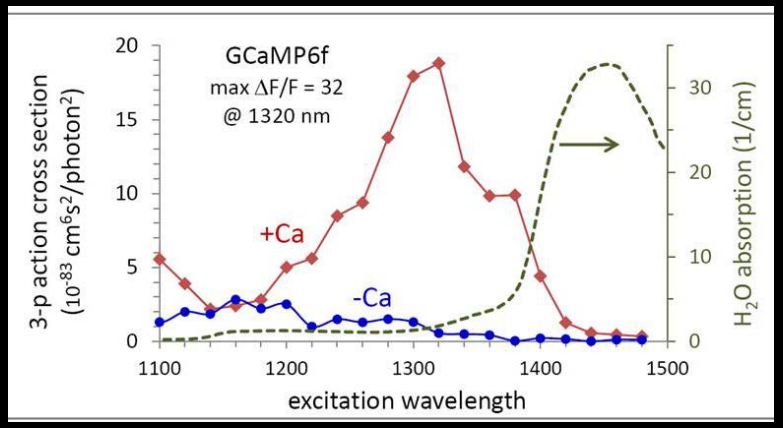
Molecular Expressions

Third harmonic imaging



Weigelin et al., 2016. J. Cell Science

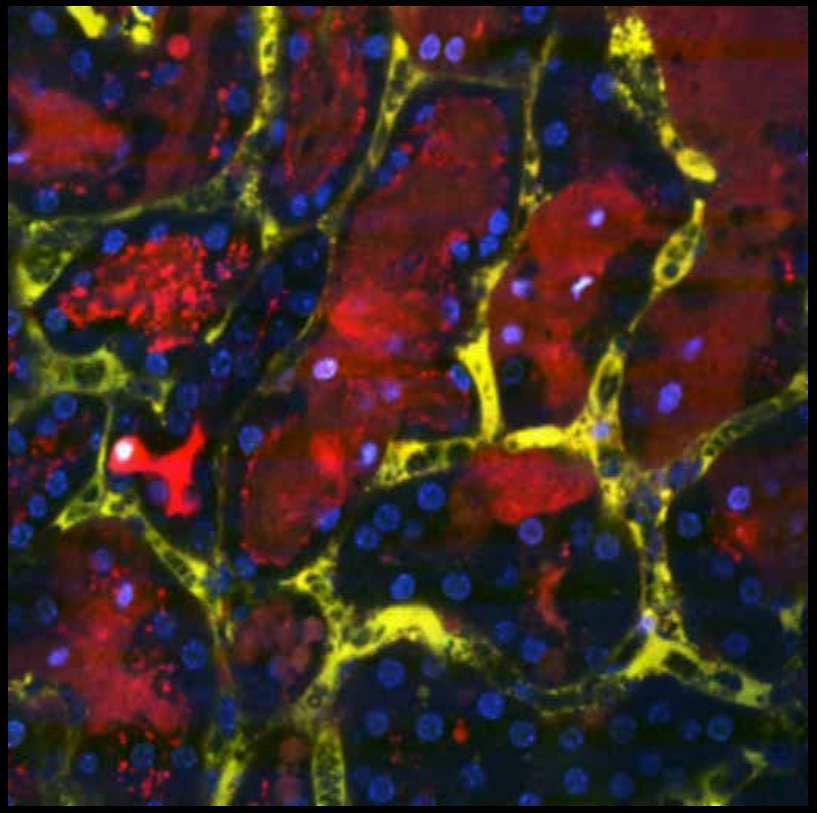
Deep tissue imaging of conventional probes



Tim Harris Lab, Janelia Farms

Limited optical reach in the living kidney
Potential solutions

- Reduce optical aberrations
- Jack up the laser
- Longer wavelengths of light
- Invade the kidney - "cut down", GRIN lens, micro-prisms



Kidney “cut-down” or “parenchymal window”

Limited optical reach in the living kidney
Potential solutions

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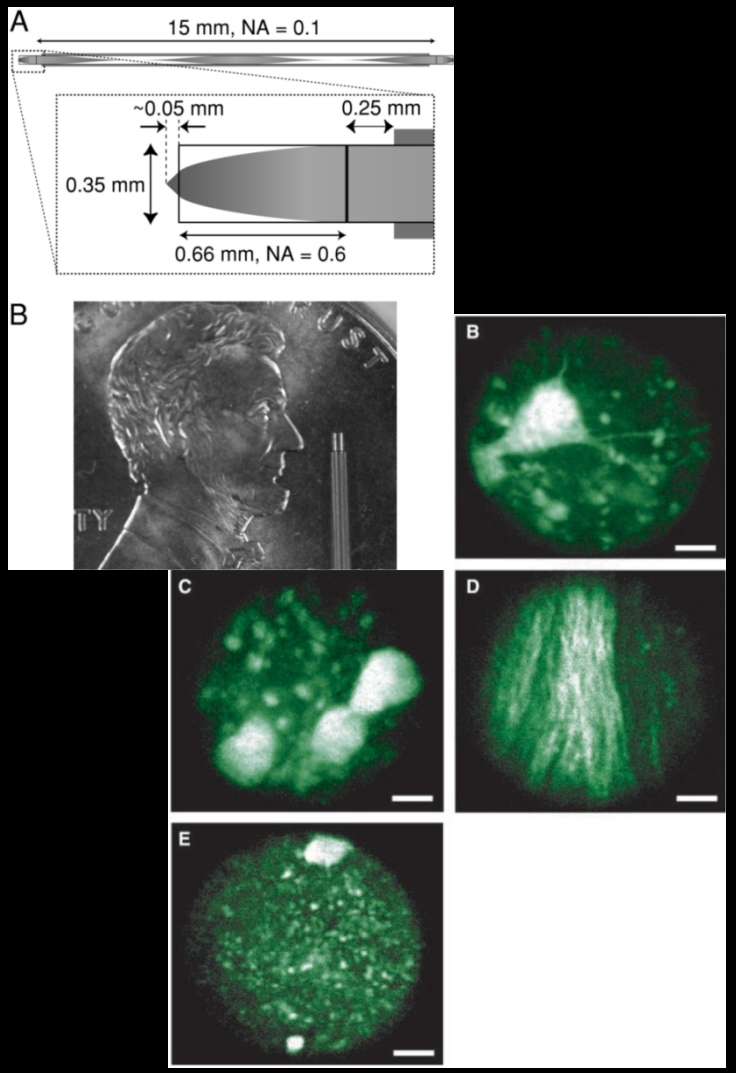
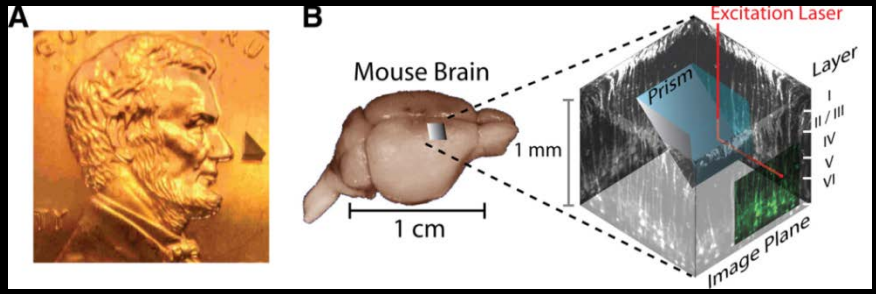
- Jack up the laser
- Longer wavelengths of light
- Invade the kidney - "cut down", GRIN lens, micro-prisms

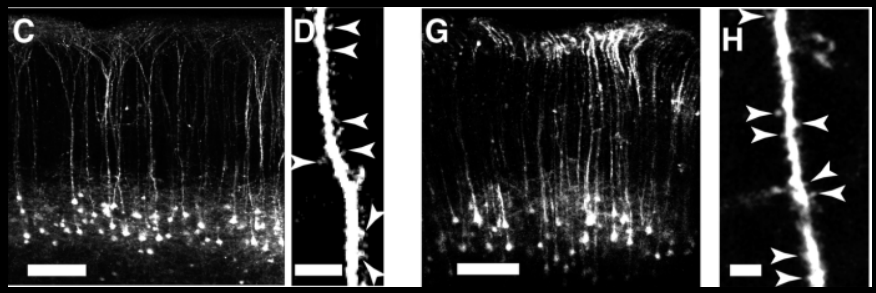


Gradient Index (GRIN) lens
Levene et al., 2004, J. Neurophysiol

Limited optical reach in the living kidney
Potential solutions

- Reduce optical aberrations
- Jack up the laser
- Longer wavelengths of light
- Invade the kidney - "cut down", GRIN lens, micro-prisms





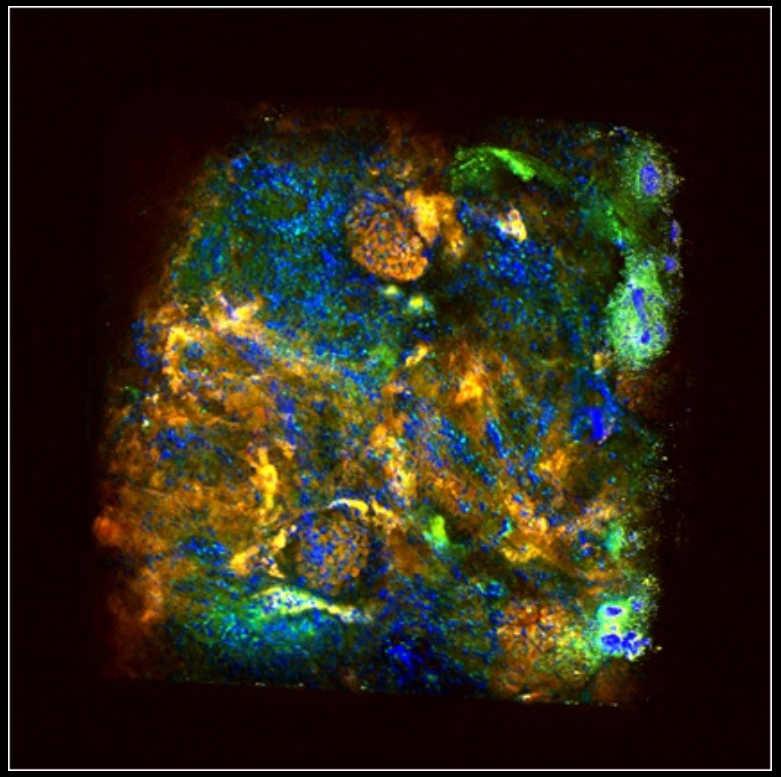
Conventional XY image in brain slice

XZ image in vivo with prism

Microprisms
Chia and Levene. 2009.

Limited optical reach in the living kidney
Potential solutions

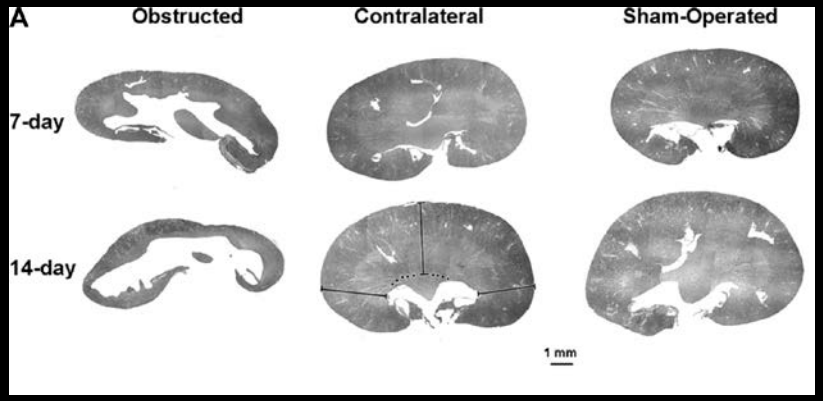
- Reduce optical aberrations
- Jack up the laser
- Longer wavelengths of light
- Invade the kidney - "cut down", GRIN lens, micro-prisms



Microprisms
Gosia Kamocka and George Rhodes

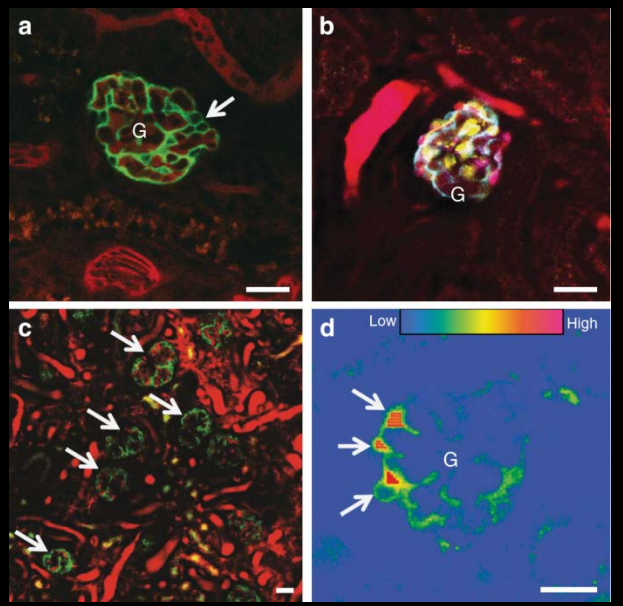
Limited optical reach in the living kidney
Potential solutions

- Reduce optical aberrations
- Jack up the laser
- Longer wavelengths of light
- Invade the kidney - "cut down", GRIN lens, micro-prisms
- Bring the structures to the surface



Forbes et al., 2012. Fight-or-flight: murine unilateral ureteral obstruction causes extensive proximal tubular degeneration, collecting duct dilatation and minimal fibrosis. AJP Renal 303 F120-129

Peti-Peterdi et al., 2015

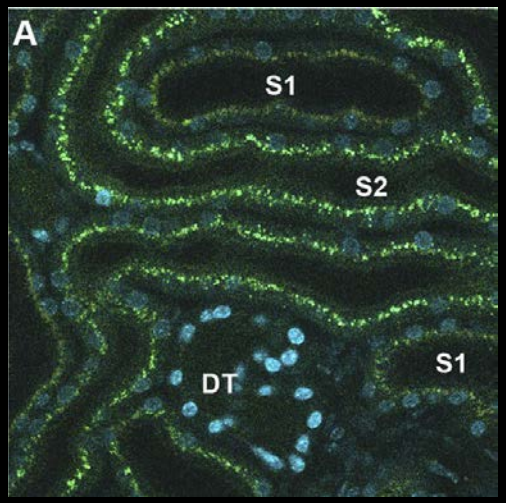


Challenges of subcellular imaging in living animals

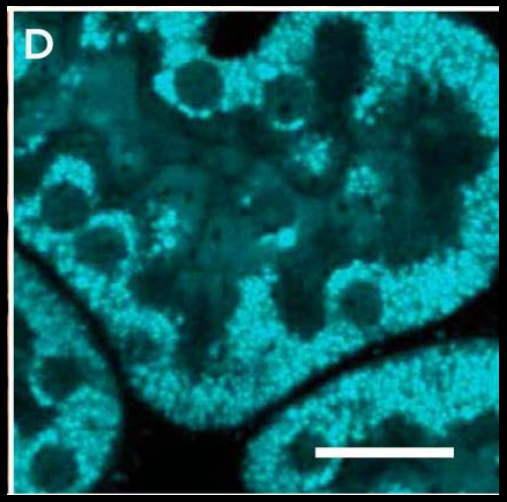
- Motion artifacts from respiration and heartbeat
- Slow image capture rate
- Limited reach/signal attenuation with depth
- *In vivo* labeling

Sources of contrast in intravital multiphoton microscopy

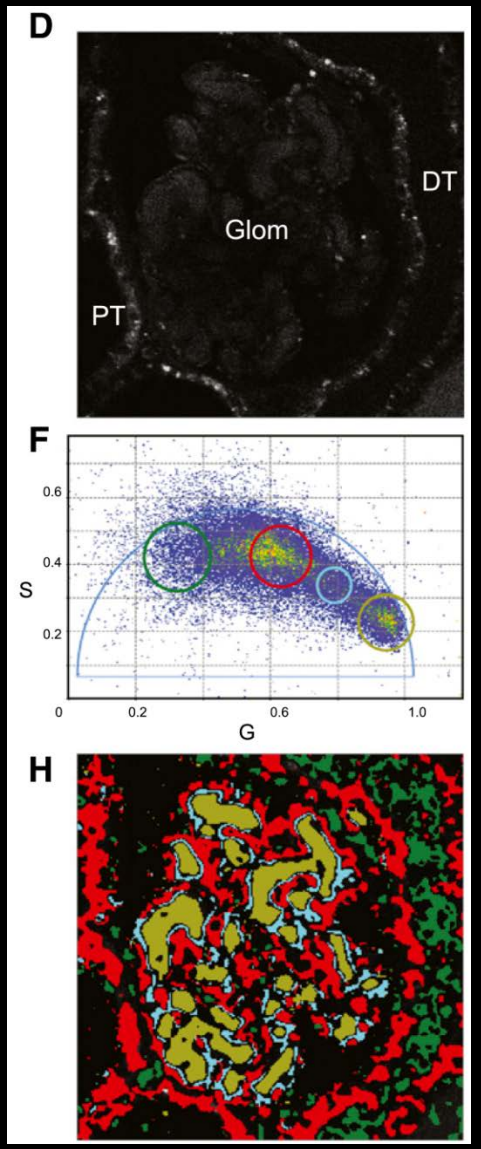
- Autofluorescence



Hato, Ashkar and Dagher, 2013. AJP Renal



Hall and Molitoris, 2014. AJP

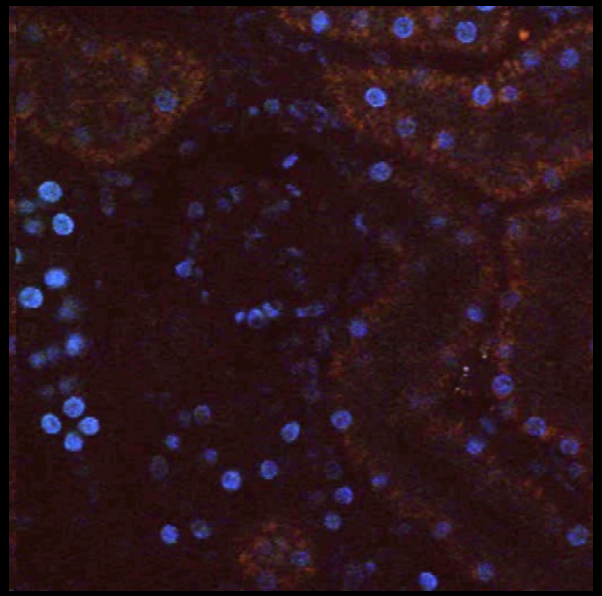


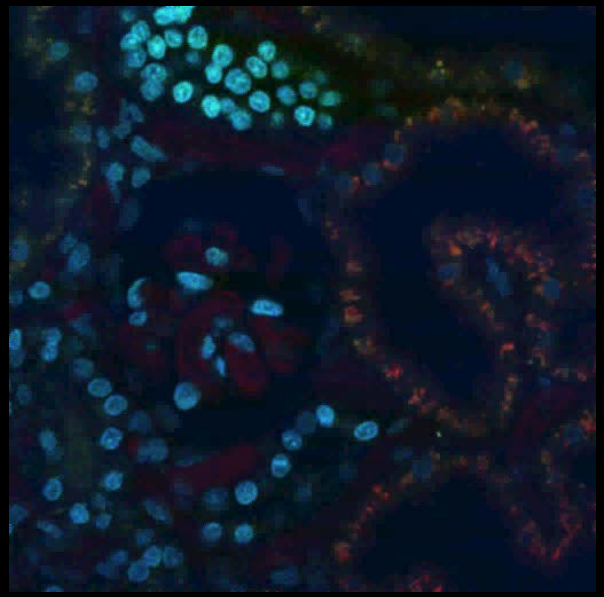
Hato et al., 2017, JASN

Takashi Hato
Applications of micropuncture techniques and fluorescence lifetime imaging for intravital studies
Wednesday at 12:10 am

Sources of contrast in intravital multiphoton microscopy

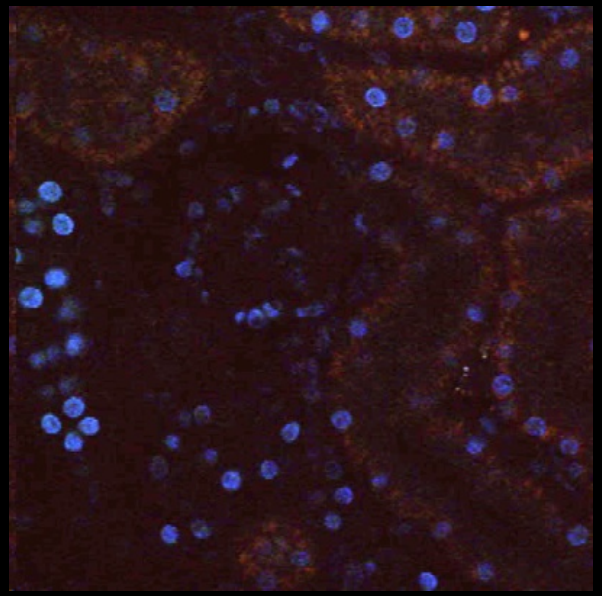
- Autofluorescence
- Hoechst 33342

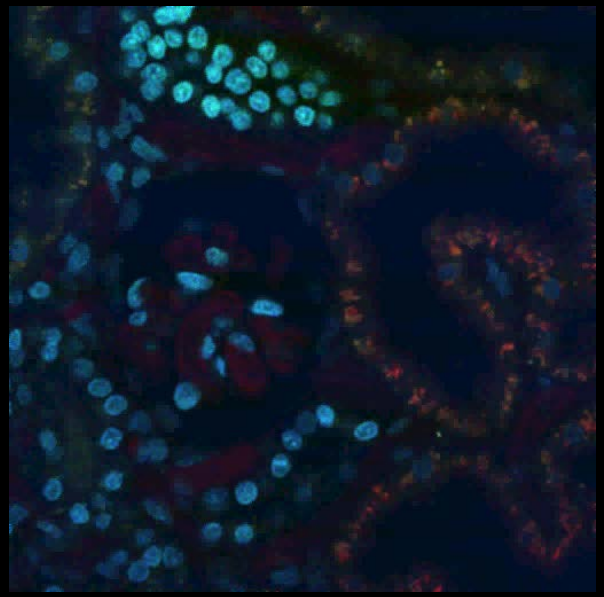




Sources of contrast in intravital multiphoton microscopy

- Autofluorescence
- Hoechst 33342
- Intravenous bulk probes

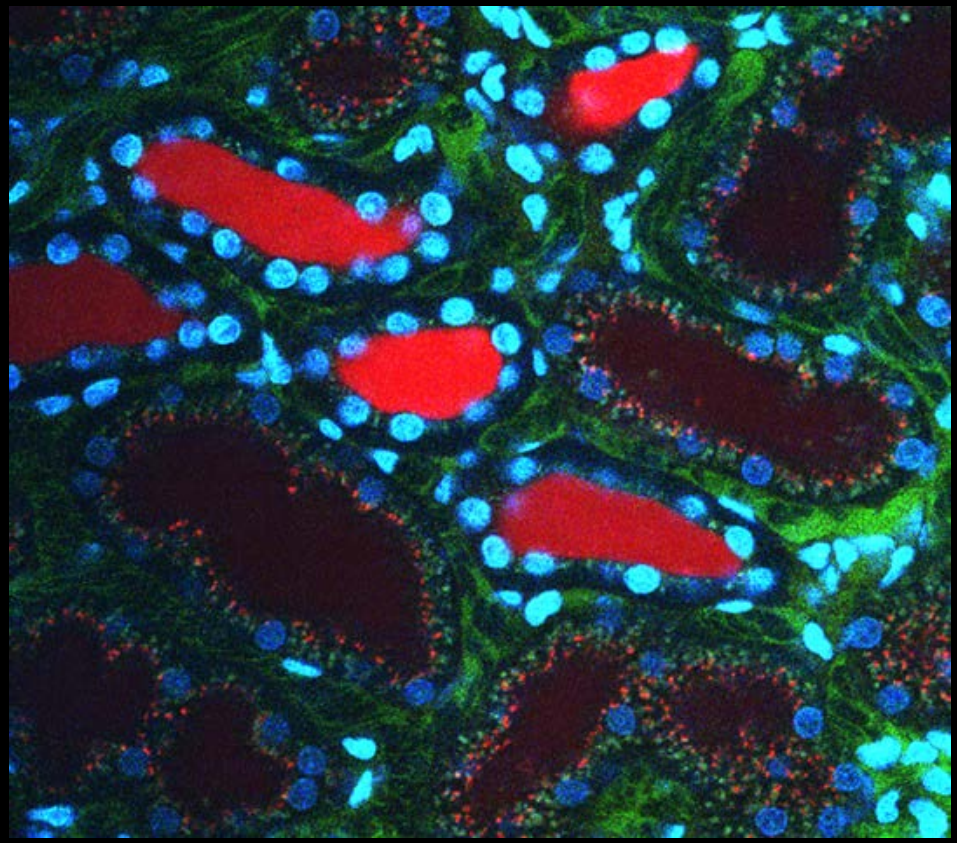




Intravital microscopy of multiple kidney functions via intravenous injection of fluorescent probes

Simultaneous imaging of a rat injected with fluorescent dextrans and Hoechst 33342 reveals multiple processes

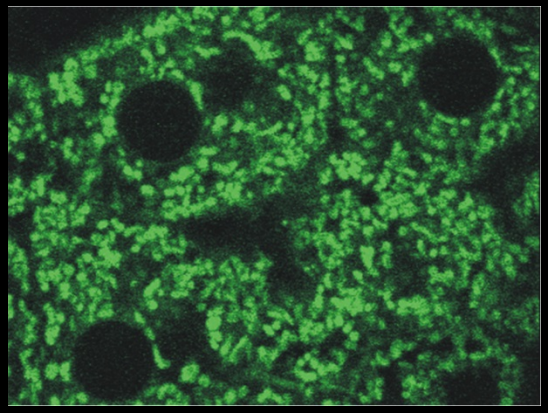
- glomerular filtration
- proximal tubule endocytosis
- tubular solute concentration
- tubular flow
- capillary blood flow
- vascular permeability
- apoptosis
- tubular sloughing



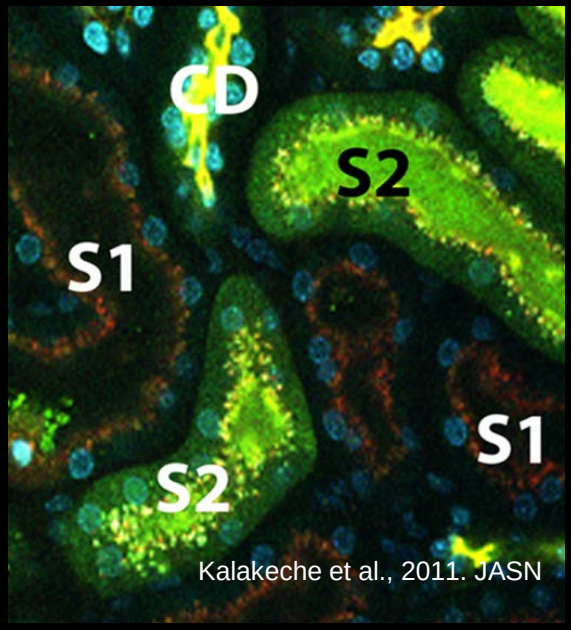
Dunn et al., 2002. AJP Cell. 283:C905-C916

Sources of contrast in intravital multiphoton microscopy

- Autofluorescence
- Hoechst 33342
- Intravenous bulk probes
- Permeant bio-indicators



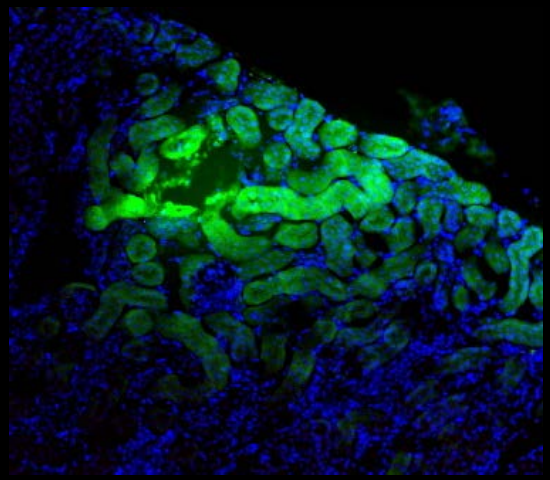
Rhodamine 123 in liver mitochondria –
John Lemasters



DCFDA labeling S2 cells in oxidative stress
Pierre Dagher

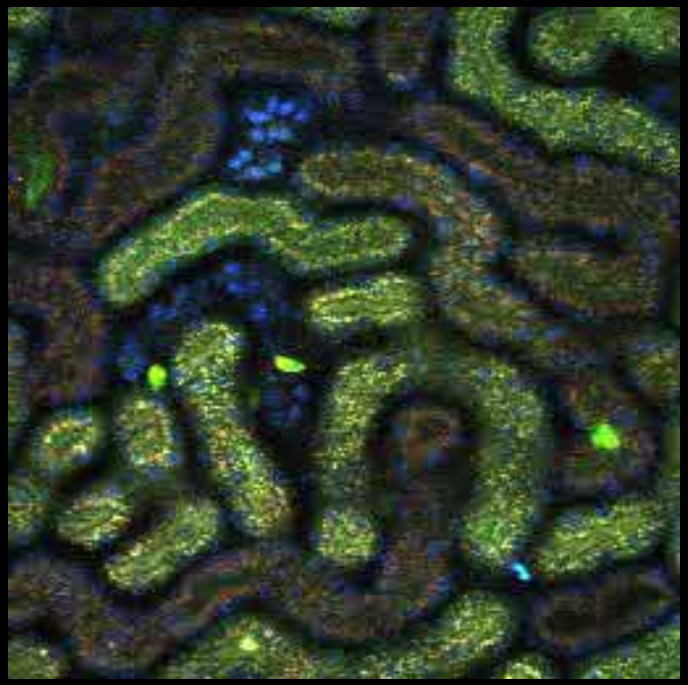
Pierre Dagher
Fluorescent probes for studying AKI
Tuesday at 11:10 am

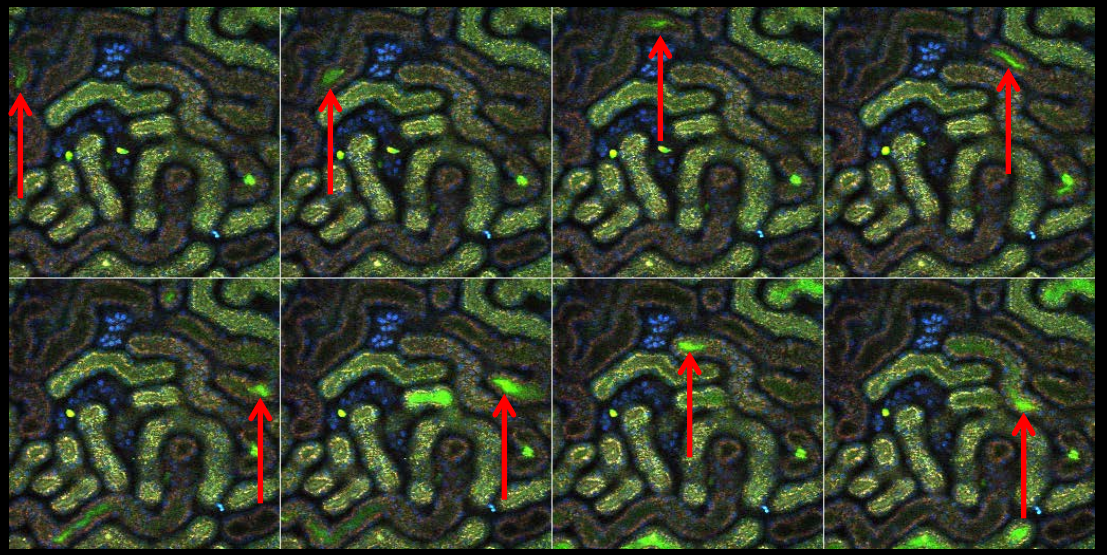
Tim Sutton
Using fluorescent probes to study tubule metabolism
Thursday at 8:30 am

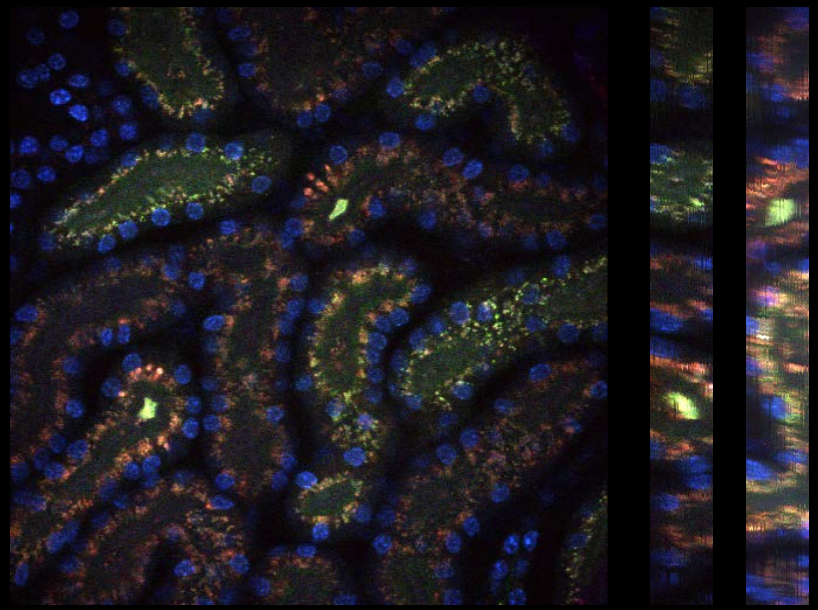


CMFDA delivered to cytosol by
subcapsular injection
Rudy Juliano, UNC

In vivo labeling of renal macrophages





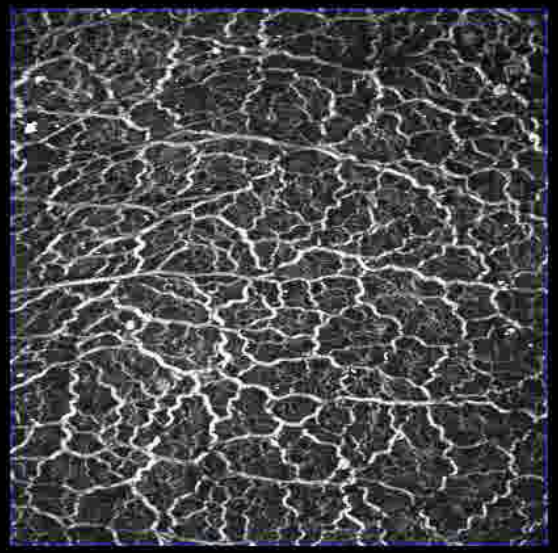


Calcein loaded into macrophages by phagocytosis of pH-sensitive liposomes containing calcein
Takashi Hato and Pierre Dagher
(30x)

Calcein-loaded pH-sensitive liposomes provided by Rudy Juliano, UNC

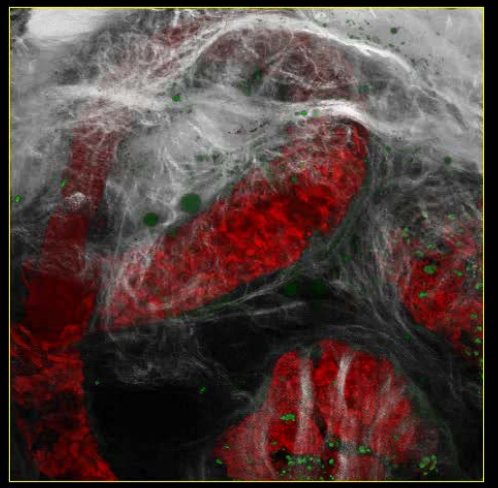
Sources of contrast in intravital multiphoton microscopy

- Autofluorescence
- Hoechst 33342
- Intravenous bulk probes
- Permeant bio-indicators
- Second harmonic imaging



Liver of living mouse
Gosia Kamocka and Amy Zollman

Fibrotic mouse kidney, Vuillemin et al., 2016. Scientific Reports

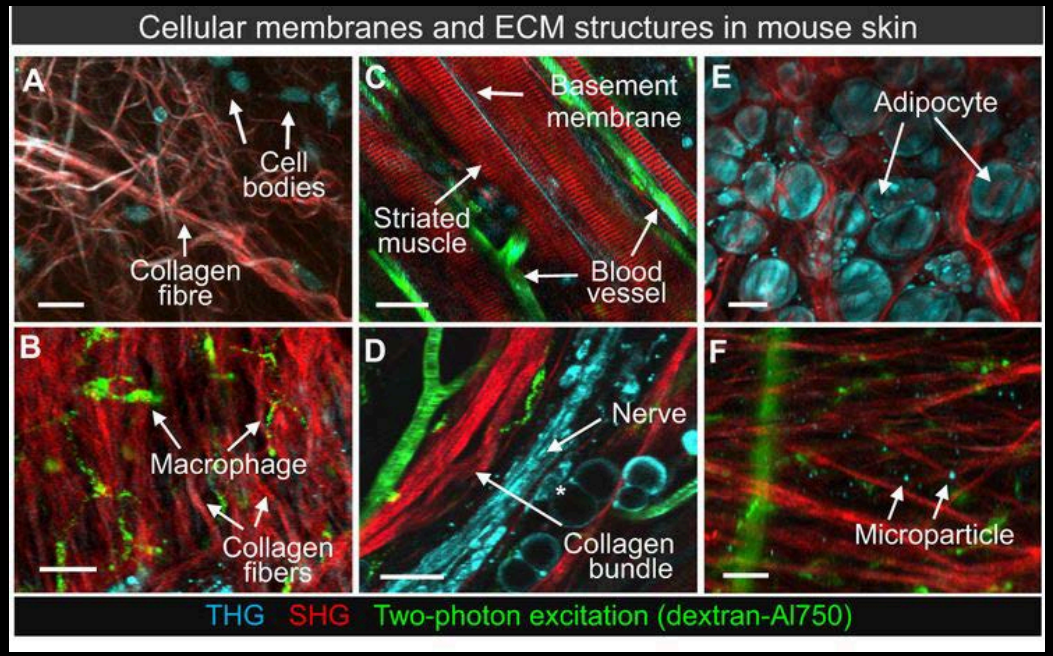


Ex vivo human sweat gland
Mary Beth Brown

Sources of contrast in intravital multiphoton microscopy

- Autofluorescence
- Hoechst 33342
- Intravenous bulk probes
- Permeant bio-indicators
- Second harmonic imaging
- Third harmonic imaging

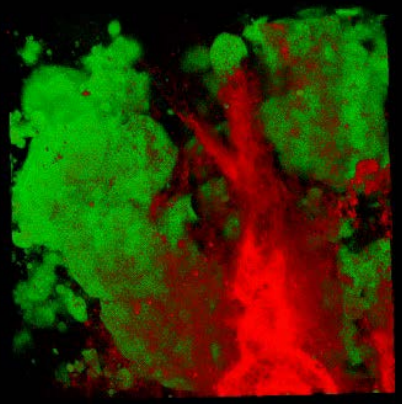
Fluorescence, SHG and THG excitation at 1180 nm



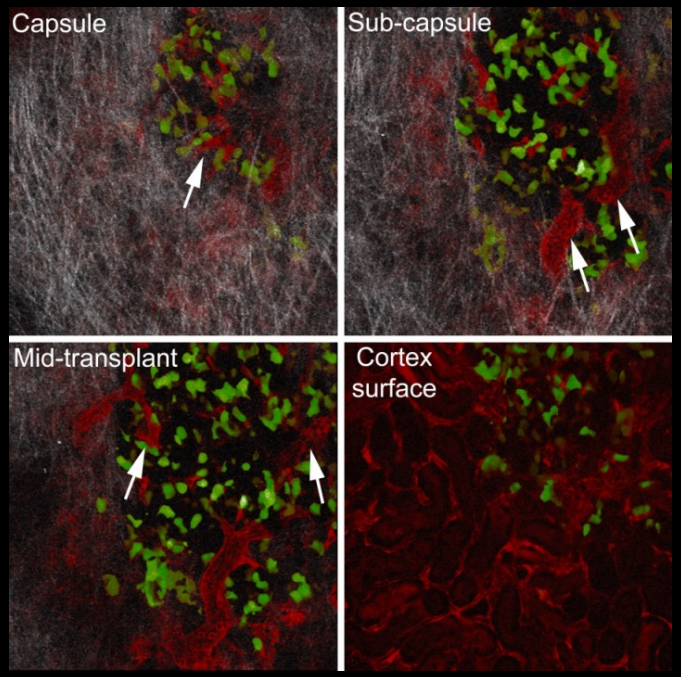
Weigelin et al., 2016. J. Cell Science

Sources of contrast in intravital multiphoton microscopy

- Autofluorescence
- Hoechst 33342
- Intravenous bulk probes
- Permeant bio-indicators
- Second harmonic imaging
- Third harmonic imaging
- Adoptive transfer



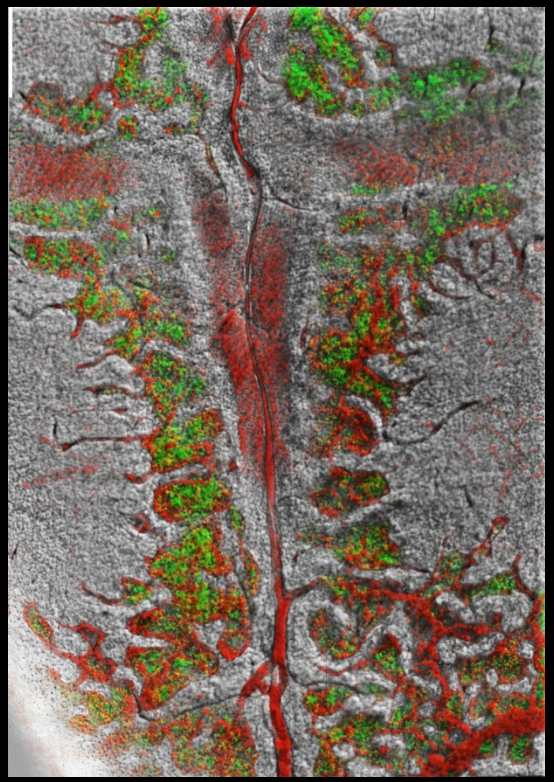
Prostate tumor
Tom Gardner



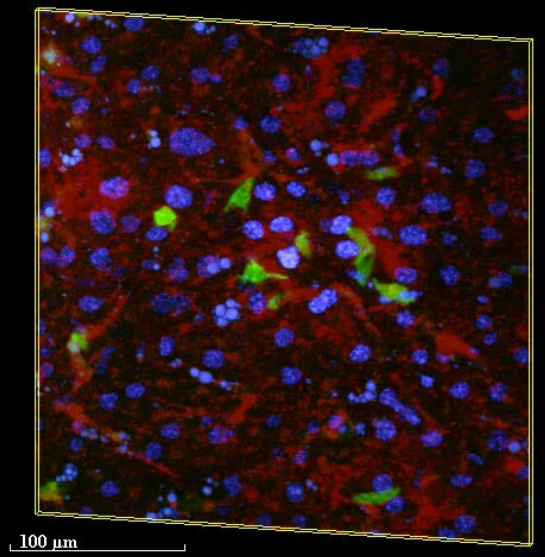
Pancreatic islets transplanted into mouse kidney
Jennifer Ryan and Natalie Stull

Sources of contrast in intravital multiphoton microscopy

- Autofluorescence
- Hoechst 33342
- Intravenous bulk probes
- Permeant bio-indicators
- Second harmonic imaging
- Third harmonic imaging
- Adoptive transfer
- Transgenic animals



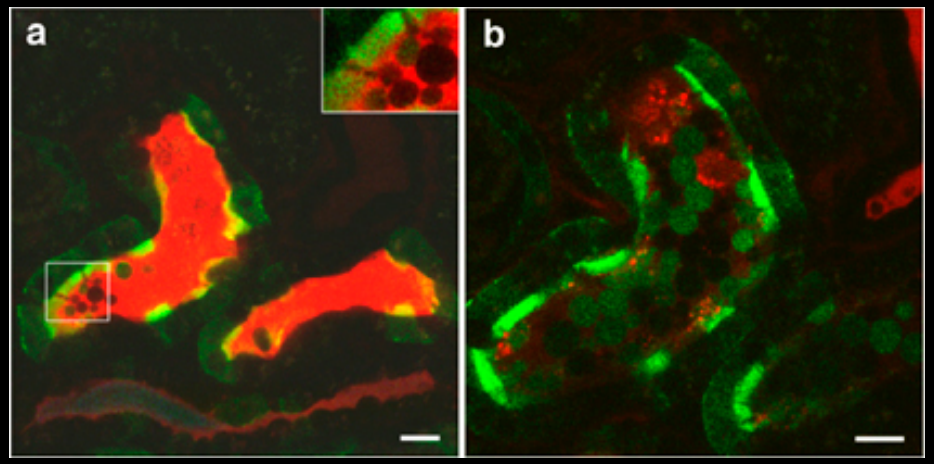
GFP-expressing hematopoetic stem cells in mouse calvarium
Gosia Kamocka, Amy Zollman, Nadia Carlesso



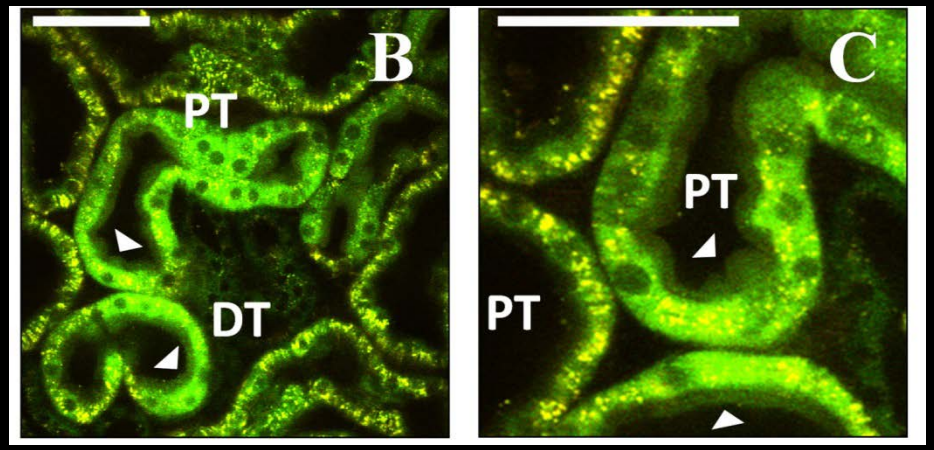
Fluorescent neutrophils in liver of GFP-Lys mouse
Cliff Babbey, Marwan Ghabril and Ken Dunn

Sources of contrast in intravital multiphoton microscopy

- Autofluorescence
- Hoechst 33342
- Intravenous bulk probes
- Permeant bio-indicators
- Second harmonic imaging
- Third harmonic imaging
- Adoptive transfer
- Transgenic animals
- In vivo gene transfer



Micropuncture injection of Adeno-GFP-actin
Ashworth et al., 2007

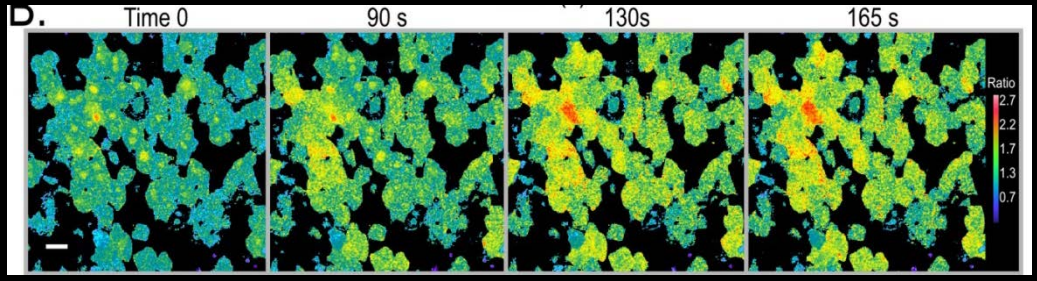


Hydrodynamic delivery ofAdeno-GFP-actin
Corridon et al., 2013

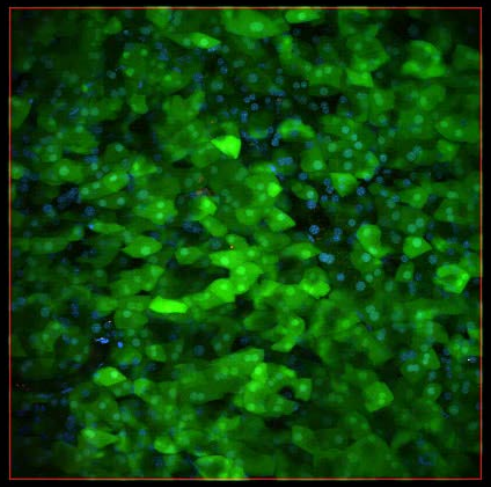
Sources of contrast in intravital multiphoton microscopy

- Autofluorescence
- Hoechst 33342
- Intravenous bulk probes
- Permeant bio-indicators
- Second harmonic imaging
- Third harmonic imaging
- Adoptive transfer
- Transgenic animals
- In vivo gene transfer

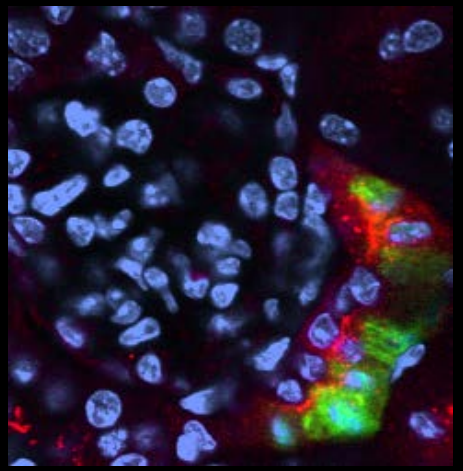
Intravenous injection of virus



Mouse liver Adeno-AKAR4.1



Mouse pancreas – AAV8-GFP



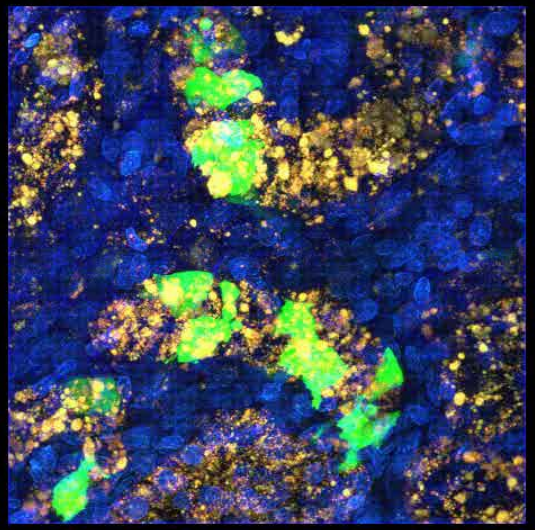
Mouse kidney
intravenous AAV9-GFP

Sources of contrast in intravital multiphoton microscopy

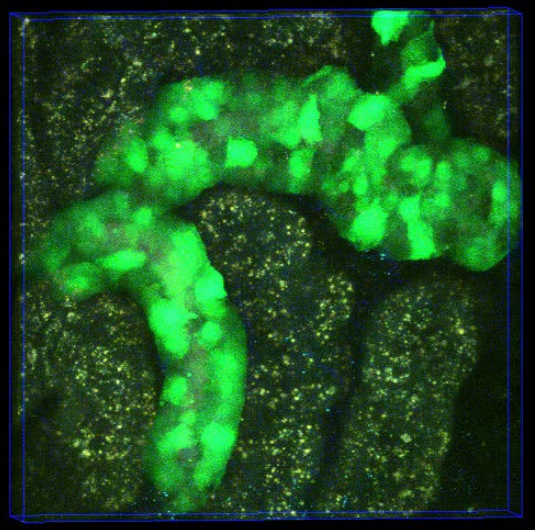
- Autofluorescence
- Hoechst 33342
- Intravenous bulk probes
- Permeant bio-indicators
- Second harmonic imaging
- Third harmonic imaging
- Adoptive transfer
- Transgenic animals
- In vivo gene transfer

Bob Bacallao
Expanding the toolbox for intravital imaging of the kidney using gene delivery techniques
Wednesday at 8:30 AM

Subcapsular injection of virus



Adeno-RGD-GFP

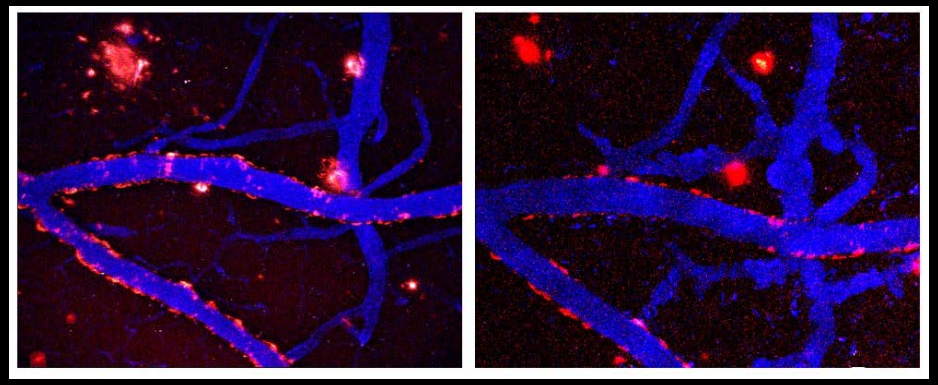


AAV9--GFP

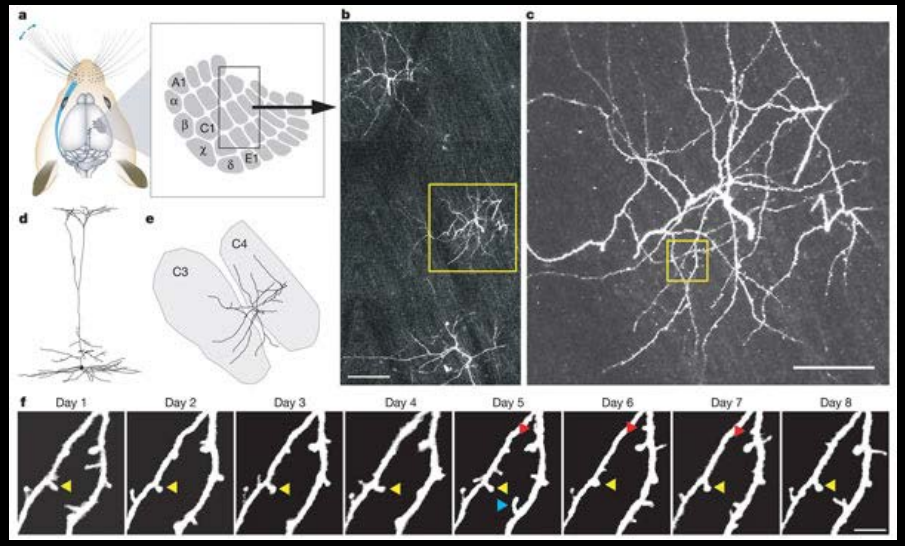
Challenges of subcellular imaging in living animals

- Motion artifacts from respiration and heartbeat
- Slow image capture rate
- Limited reach/signal attenuation with depth
- *In vivo* labeling
- Extracting quantitative data despite depth attenuation

Quantitative intravital microscopy circumventing the effects of depth on signal levels



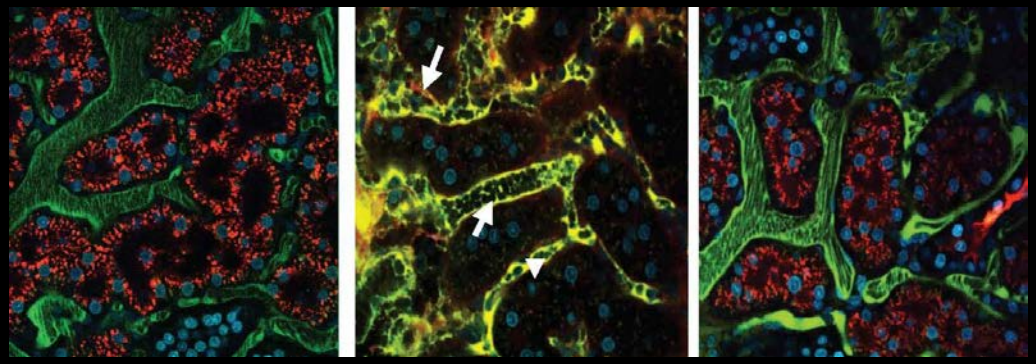
Amyloid plaque dynamics - Brian Bacskai, Harvard



Dendritic spine dynamics - Karel Svoboda, Janelia Farm

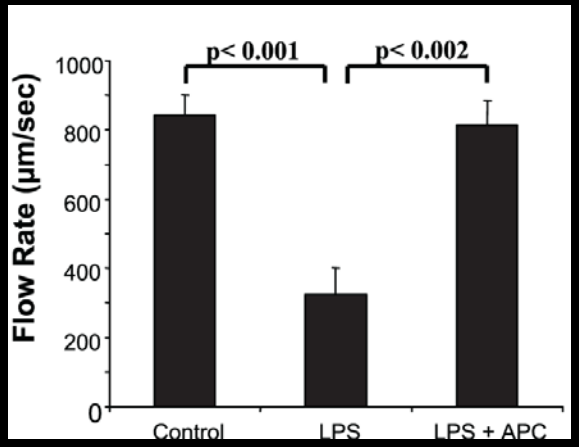
- Quantify without intensity
  –Structural changes

Quantitative intravital microscopy circumventing the effects of depth on signal levels



Sham LPS LPS + APC

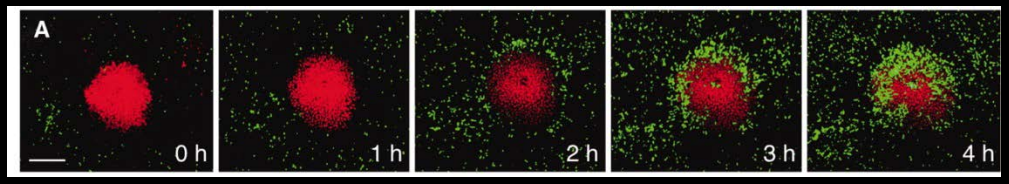
- Quantify without intensity
  - –Structural changes
  - –Motion

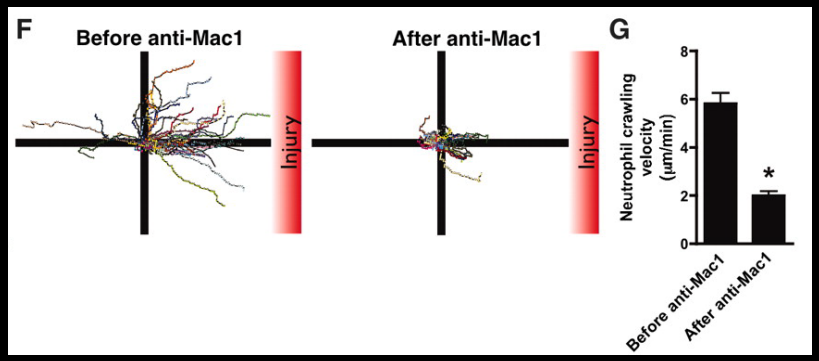


Microvascular flow

Sharfuddin Sandoval, Berg, McDougal, Campos, Phillips, Jones, Gupta, Grinnell and Molitoris. 2009. Soluble thrombomodulin protects ischemic kidneys. J Am Soc Nephrol:524-34.

Quantitative intravital microscopy circumventing the effects of depth on signal levels

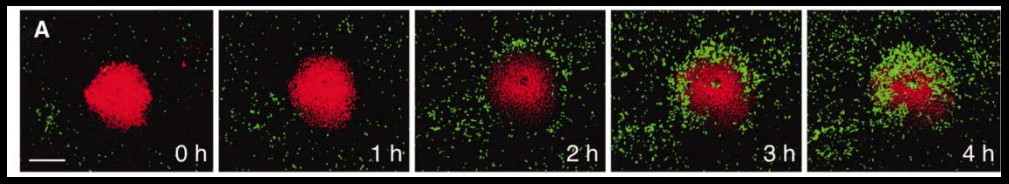


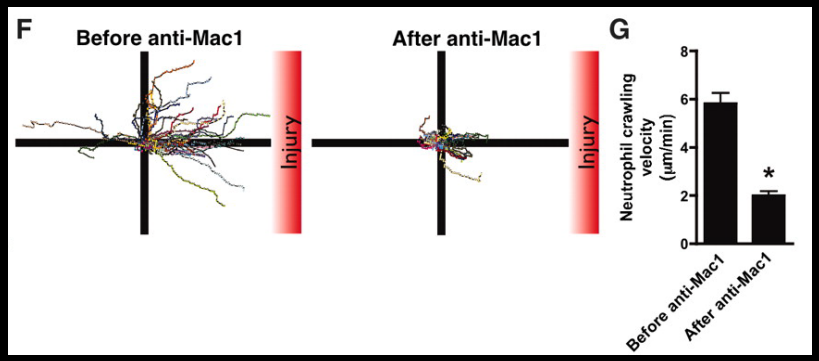


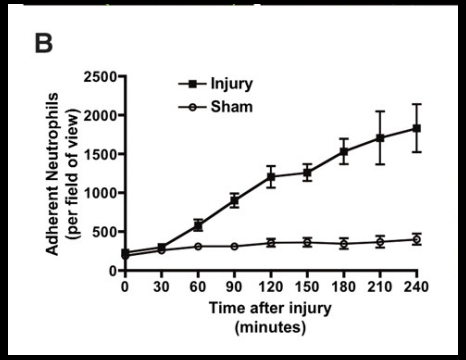
Neutrophil migration – Paul Kubes, Calgary

- Quantify without intensity
  - –Structural changes
  - –Motion

Quantitative intravital microscopy circumventing the effects of depth on signal levels



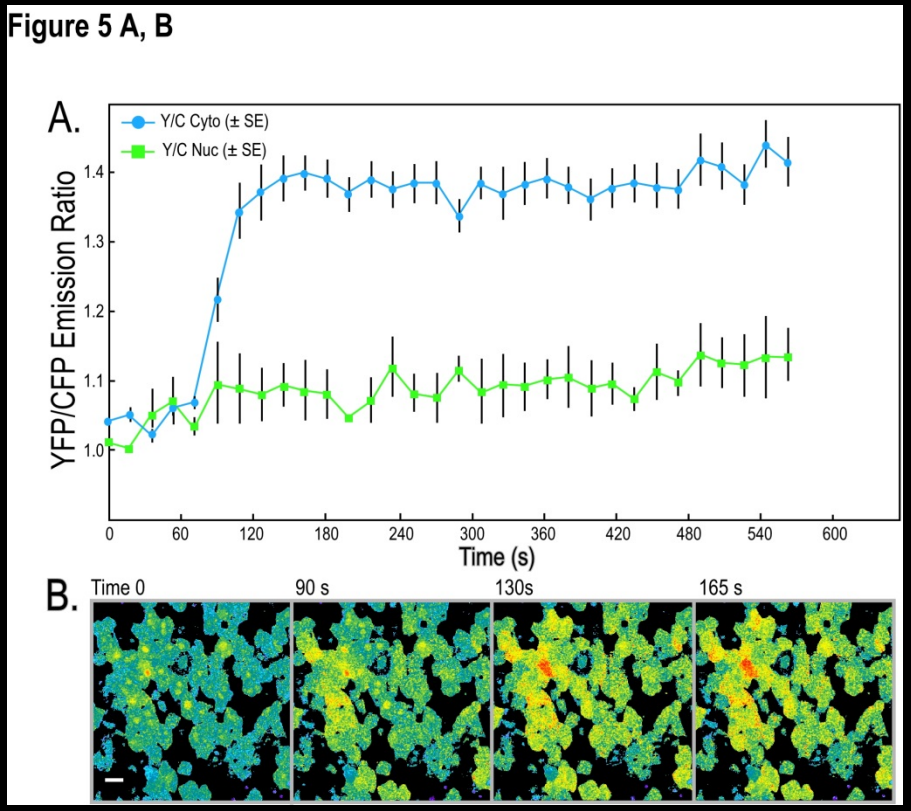




Neutrophil migration – Paul Kubes, Calgary

- Quantify without intensity
  - –Structural changes
  - –Motion
  - –Incidence

Quantitative intravital microscopy circumventing the effects of depth on signal levels



PKA activation in mouse liver– AKAR4.1 FRET sensor

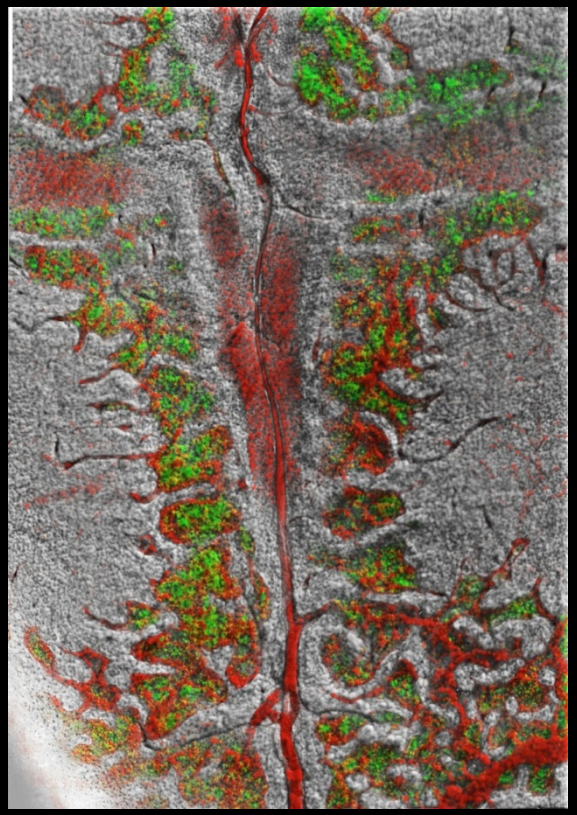
Practical method for monitoring FRET-base biosensor probe activities using two-photon excitation microscopy
Tao et al., in prep

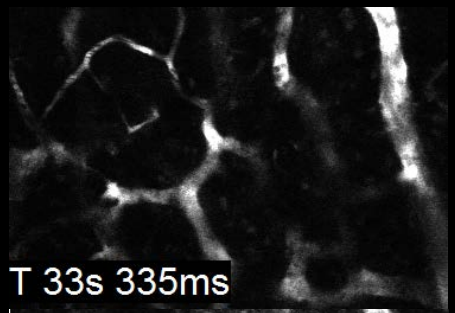
- Quantify without intensity
  - –Structural changes
  - –Motion
  - –Incidence
- Quantify relative intensities
  - –Ratiometric measures

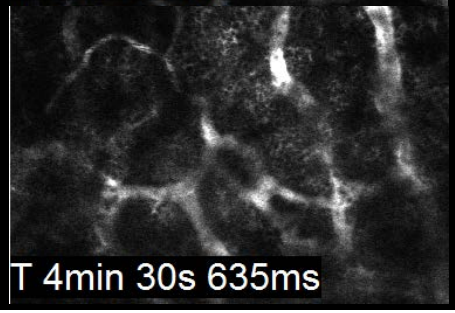
Richard Day
Fluorescent proteins and biosensors
Tuesday at 10:20 pm

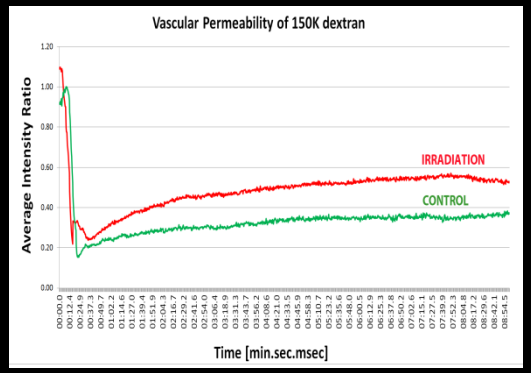
Forster Resonance Energy Transfer and Fluorescence Lifetime Microscopy
Wednesday at 11:10 am

Quantitative intravital microscopy circumventing the effects of depth on signal levels







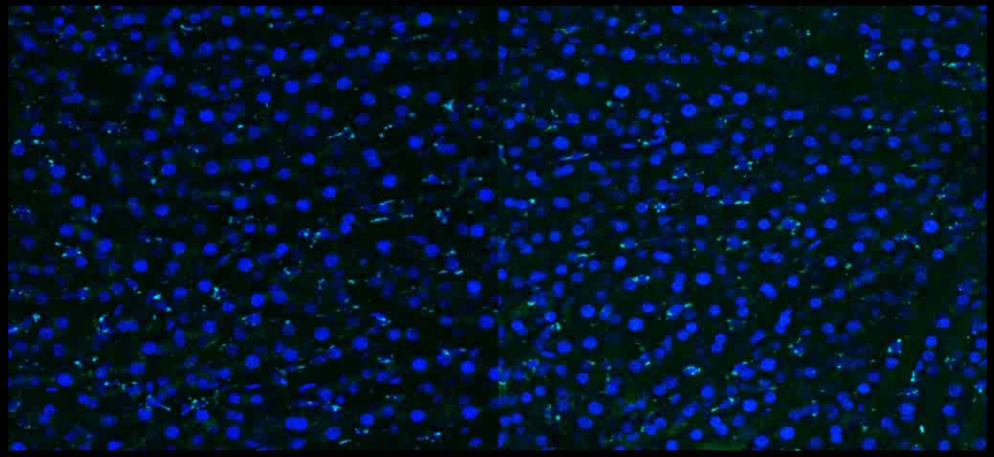


- Quantify without intensity
  - –Structural changes
  - –Motion
  - –Incidence
- Quantify relative intensities
  - –Ratiometric measures

Microvascular leakage in the bone marrow space of the mouse calvarium

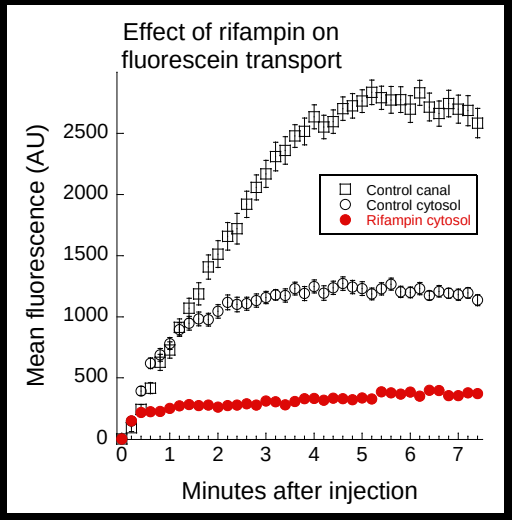
Malgorzata Kamocka, Amy Zollman and Nadia Carlesso - IUSM

Quantitative intravital microscopy circumventing the effects of depth on signal levels



Vehicle treated

Rifampin treated



- Quantify without intensity
  - –Structural changes
  - –Motion
  - –Incidence
- Quantify relative intensities
  - –Ratiometric measures
  - –Time-series measures

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Further information

Websites

http://www.drbio.cornell.edu/ - Watt Webb's laboratory
http://www.microscopyu.com/ - a great general microscopy education website
http://www.loci.wisc.edu - Laboratory for Optical and Computational Instrumentation

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