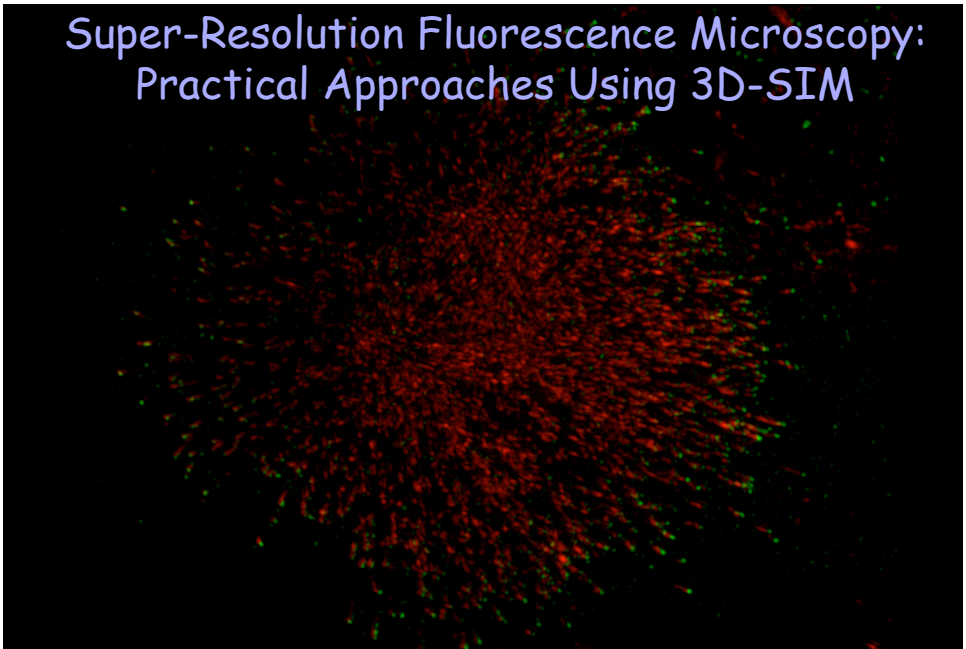
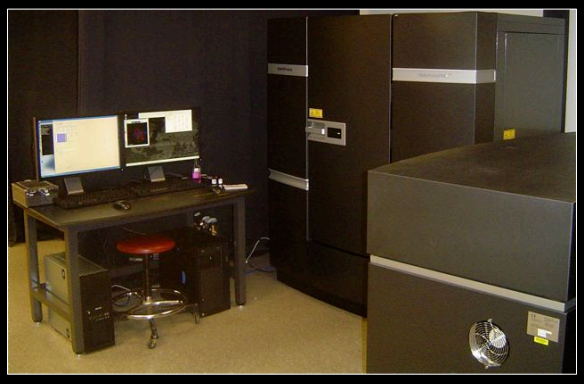
Super-Resolution Fluorescence Microscopy: Practical Approaches Using 3D-SIM

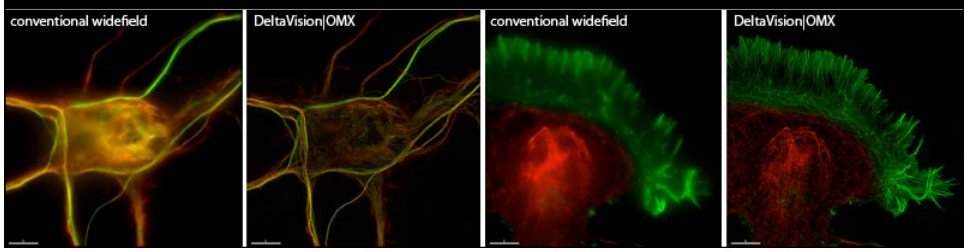


Claire Walczak
IUB Light Microscopy Imaging Center

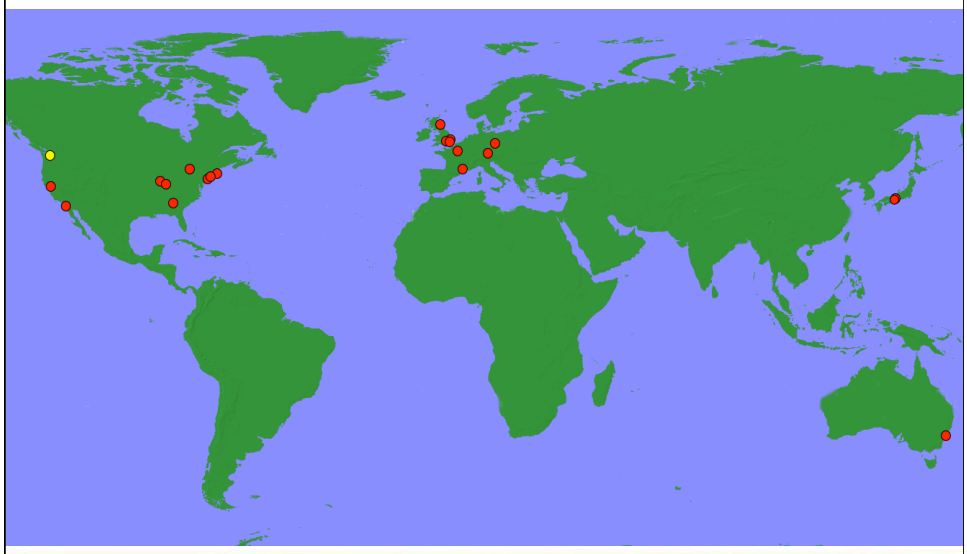
Super-Resolution Fluorescence Microscopy Using Structured Illumination

- Applied Precision OMX system
- 4 EM-CCD Cameras
- 4 laser excitation





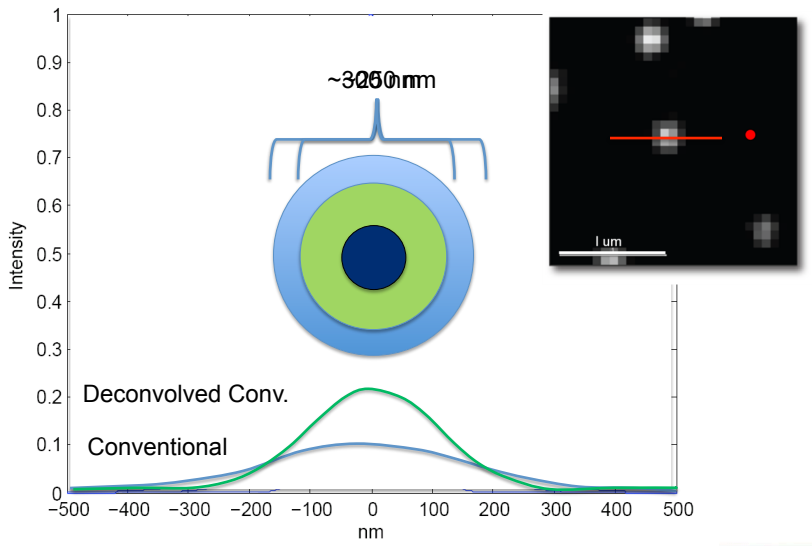
DeltaVision OMX® System Installations



Imaging Beyond the Resolution Limit

- Problem…
  - Light Microscopes are diffraction limited.
  - You can't break the Laws of Physics, but you can use them creatively to answer important questions.
- What that means…
  - Even if you image an object you know to be smaller than the diffraction limit of your microscope, you cannot create an image of that object that represents its true size or shape.

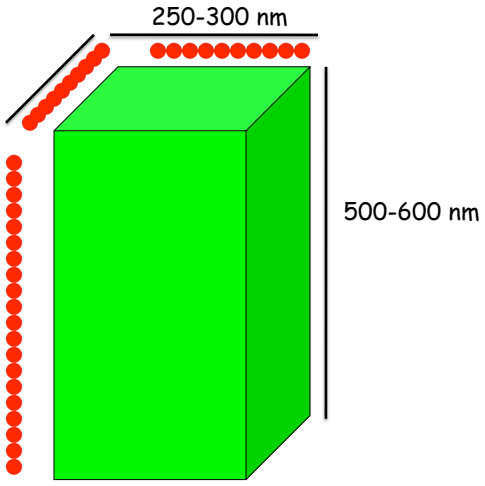
Practical Issues Regarding Object Size



Resolution of Microscope Systems

Diffraction limited volume
- Lateral ~250-300 nm
- Axial 500-600 nm

Approximate size of secondary antibody



What Limits Resolution?

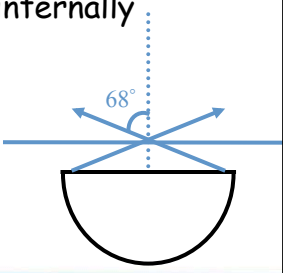
$$D = \frac{0.61 \times \lambda}{NA}$$

Rayleigh Resolution Limit
λ = Wavelength
NA = Numerical Aperture

Improve resolution by:
- Shortening the wavelength of light
- Increasing numerical aperture of lens

….And why that doesn't work

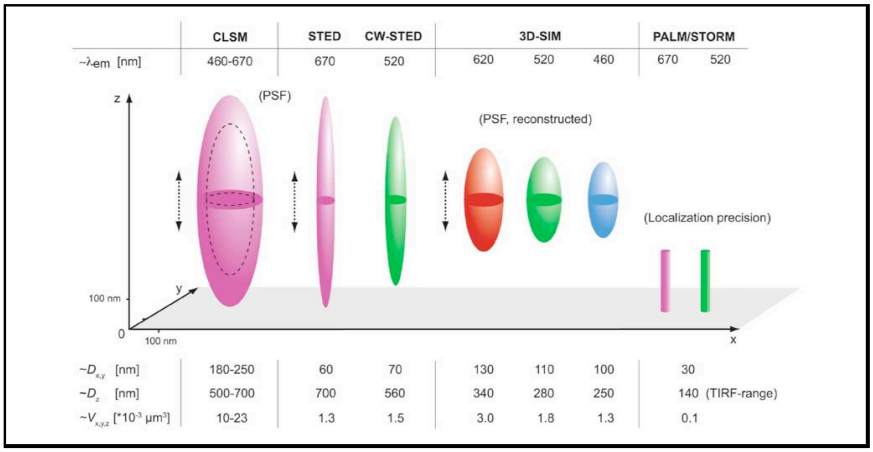
- Making optics to pass light below 250 nm is very expensive and difficult.
- Increasing NA requires increasing the acceptance angle of the lens
- However you can only bend light so much before you run into problems at the interfaces between different materials and light becomes internally reflected



Practically: How can you Increase Resolution?

- Localization Microscopy
  - Determining location of individual fluorophores by selectively turning them on or off
    - PAL-M, STORM
- Illumination Manipulation
  - Modifying the point spread function or illumination system of microscope
    - (SIM, STED)

Super Resolution Comparisons



J Cell Biol. 2010 Jul 26;190(2):165-75.
A guide to super-resolution fluorescence microscopy, Schermelleh L, Heintzmann R, Leonhardt H.

3D-Structured Illumination Microscopy

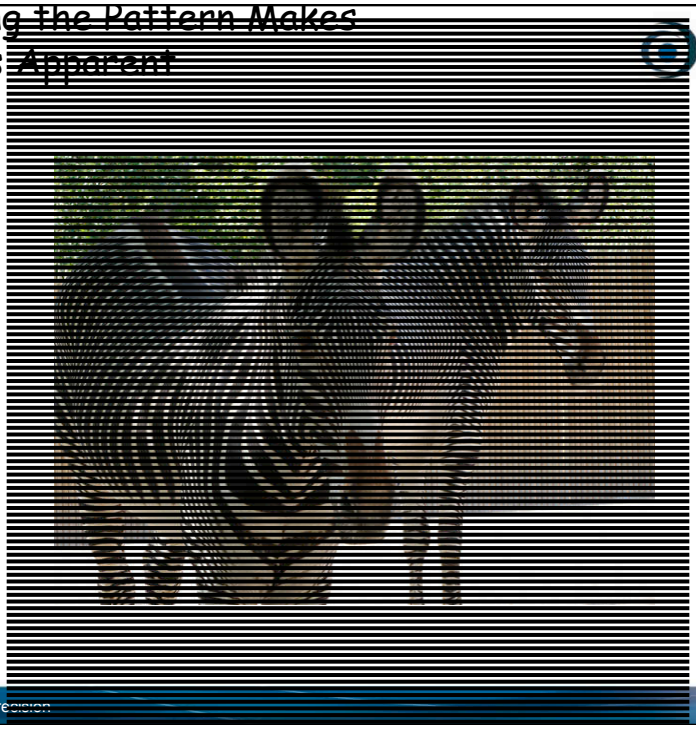
- Utilizes a 3D structured light pattern to extract higher resolution information from sample
- Works with standard dyes and fluorescent proteins
- XY Resolution 80-130 nm
- Z Resolution 250-350 nm
- Widefield imaging technology (not confocal)

Structured Illumination Microscopy

Courtsey Stephen Cody Ph.D, Monash Micro Imaging

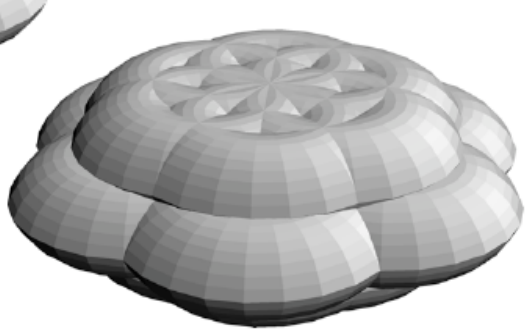
Interference Patterns Known as Moire Fringes

Rotating the Pattern Makes Fringes Apparent



3D SIM Image Allows Larger Frequency Range to Be Covered

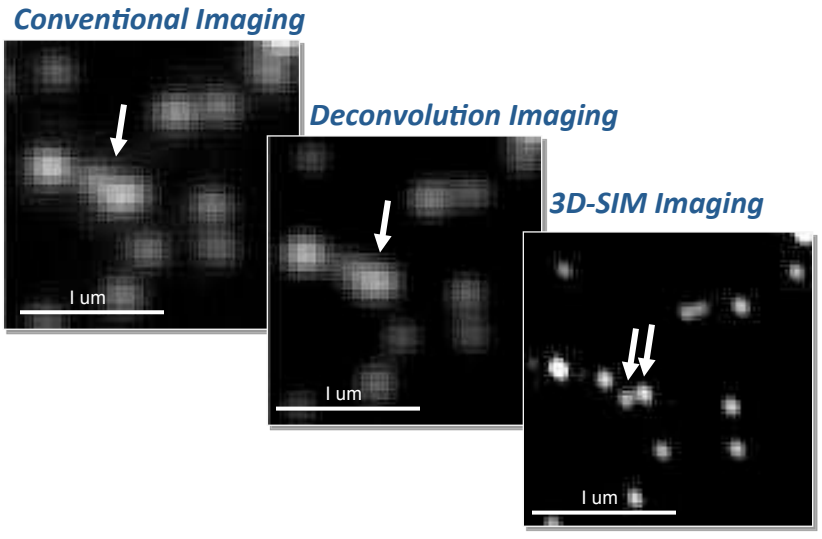
Single PSF Fourier (OTF)



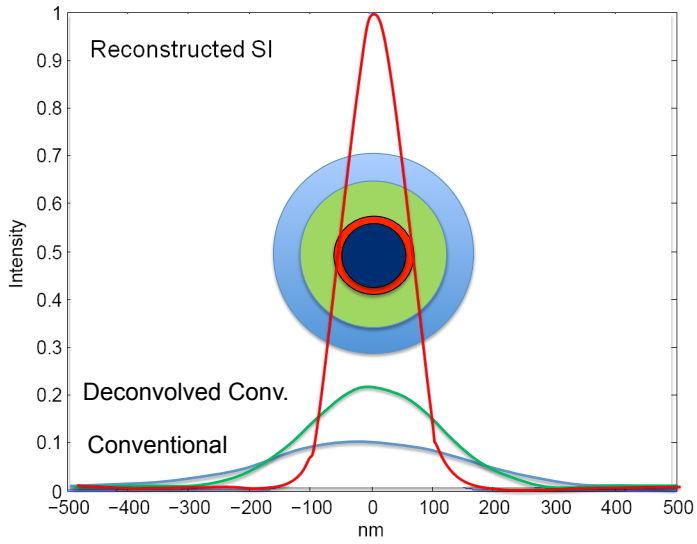
Overlapping OTFs Eliminates Missing Cone Information

Result- improved resolution in X, Y, and Z

Improvements in Resolution

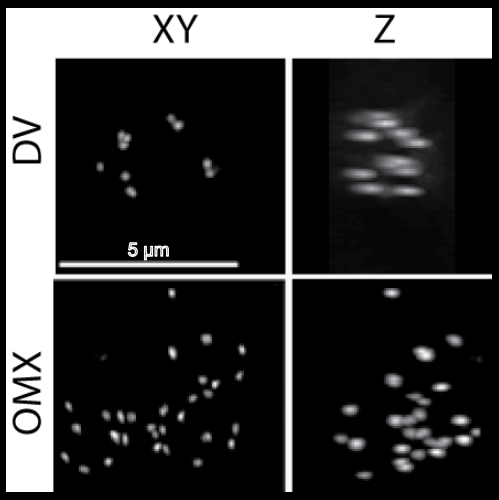


SIM Resolution Relative to Conventional and Deconvolution Approaches



Practical Aspects of Improved Resolution

GFP-centrin in Toxoplasma Gondi



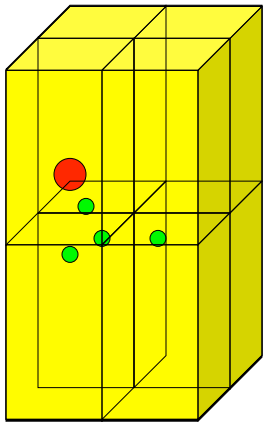
Ke Hu- Indiana University

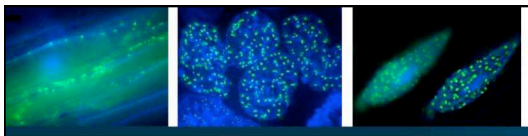
What Does This Get You?

Conventional microscope
250 x 250 x 600 nm

Vs

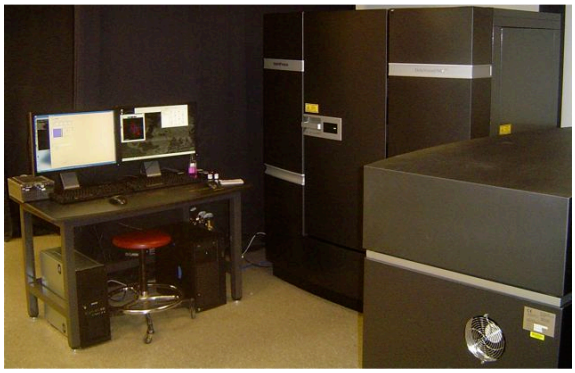
3D-SIM
100 x 100 x 300
=
8 fold volume resolution improvement





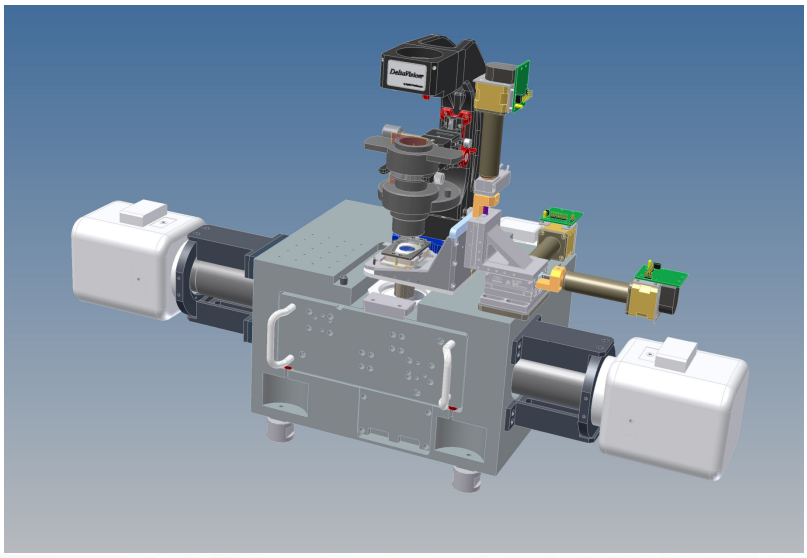
Applied Precision OMX System at IUB-LMIC

- 4 EM-CCD Cameras
- 4 laser excitation (405, 488, 561, 642) for imaging DAPI, green, red and far red fluorophores.
- Environmental Control

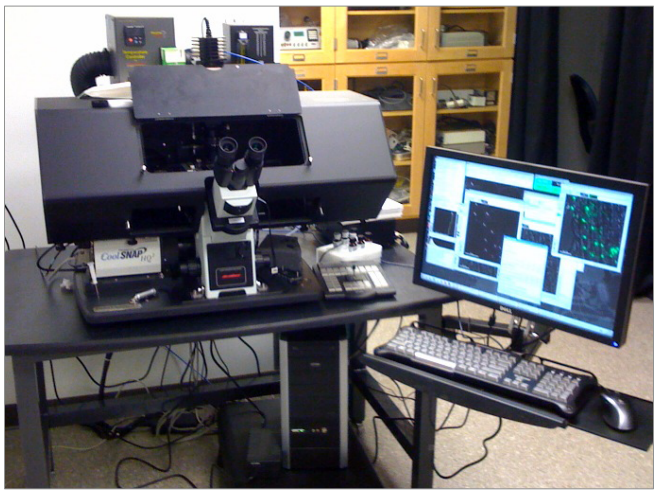


Based on exclusive license from UCSF (Agard, Sedat, Gustafsson)

DeltaVision OMX® System

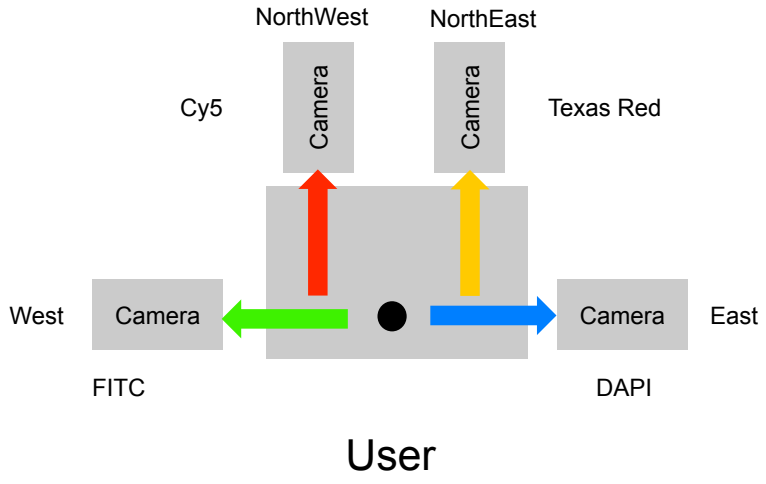


Conventional DeltaVision System Included

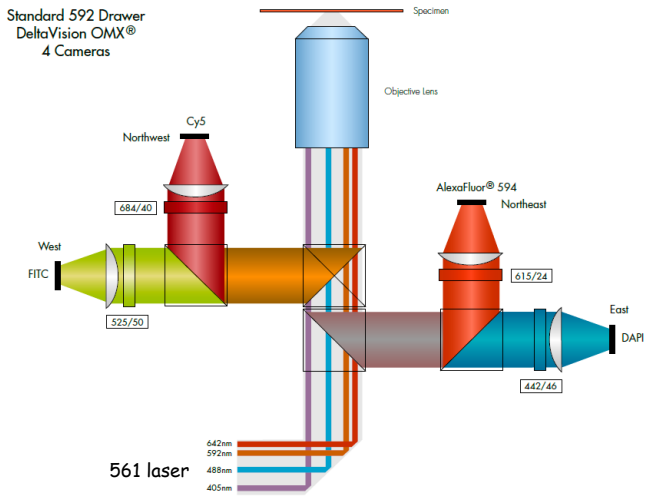


Precision-matched Stage
Serves as sample spotting microscope
Also- stand alone DV

DeltaVision OMX® Plan View



System is Equipped with Standard Laser Drawer



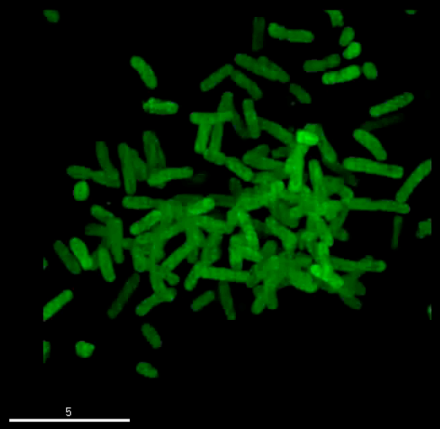
DeltaVision OMX® Capabilities

- Unique optical block designed for:
  - High photon efficiency
  - Multichannel simultaneous imaging
- Multiple imaging modalities
  - 3D Structured Illumination Microscopy
  - *TIRF/Localization Microscopy*
  - Widefield fluorescence imaging

Practical Applications of OMX at IU

Practical Applications of OMX at IU

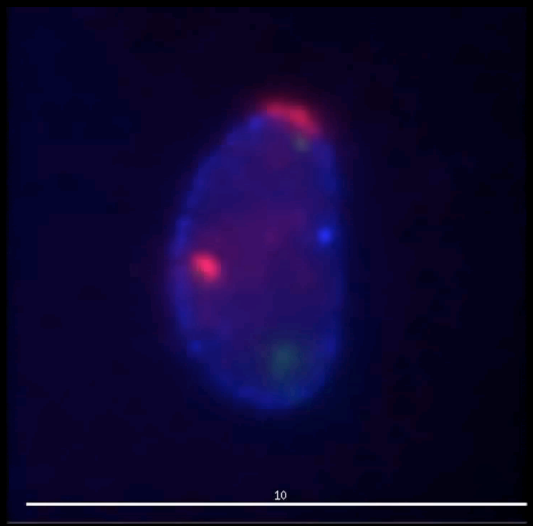
Biofilm Formation

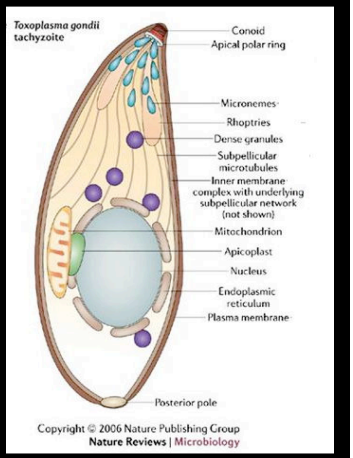


Clay Fuqua- IUB

Practical Applications of OMX at IU

Toxoplasm Gondi

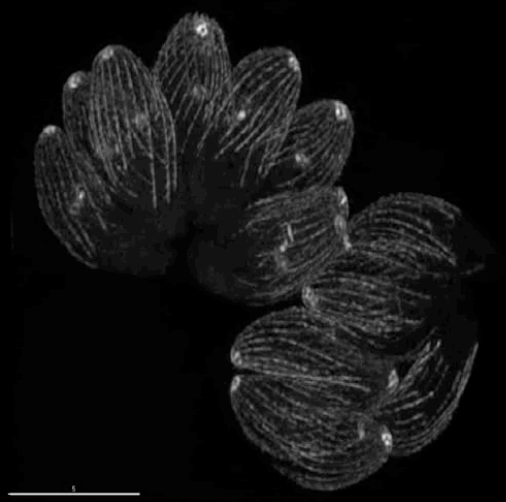


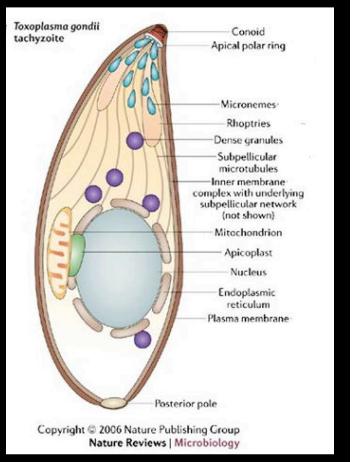


Ke Hu- IUB

Practical Applications of OMX at IU

Toxoplasm Gondi

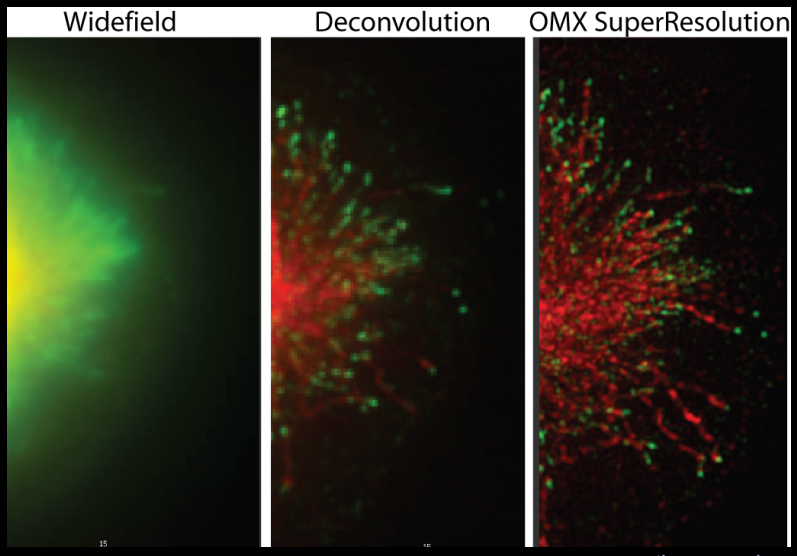




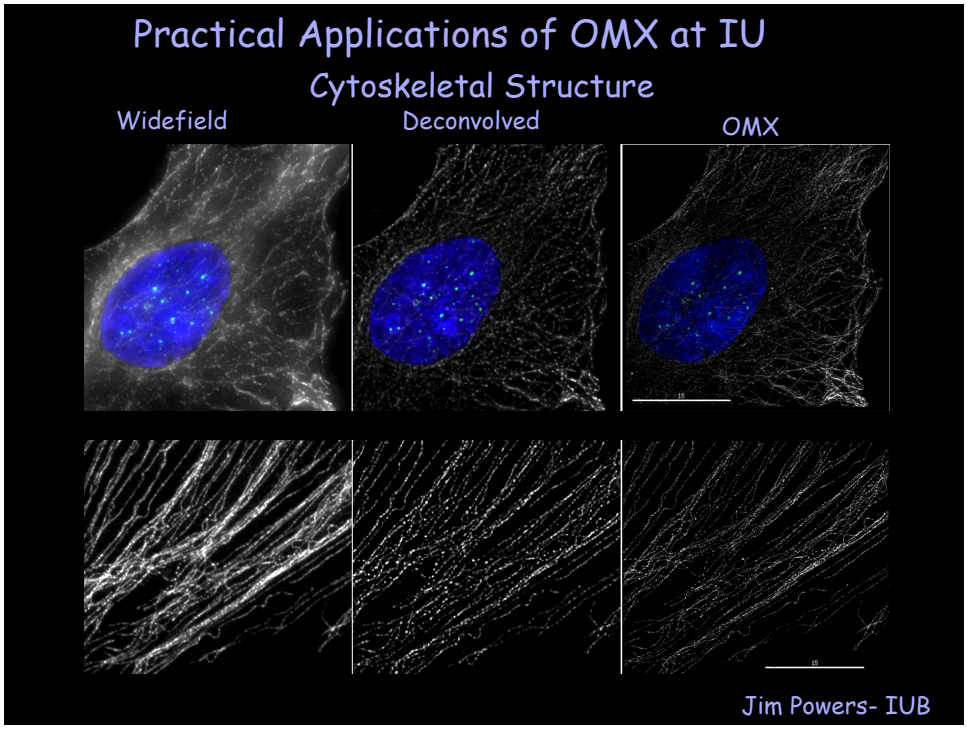
John Murray
Ke Hu- IUB

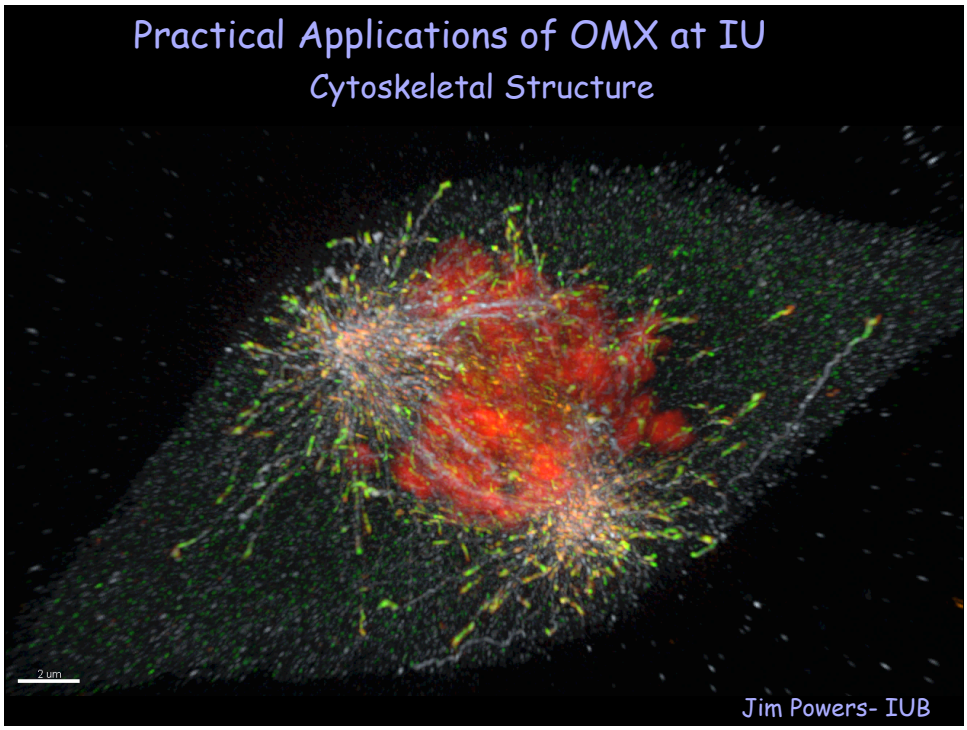
Practical Applications of OMX at IU

Cytoskeletal Structure



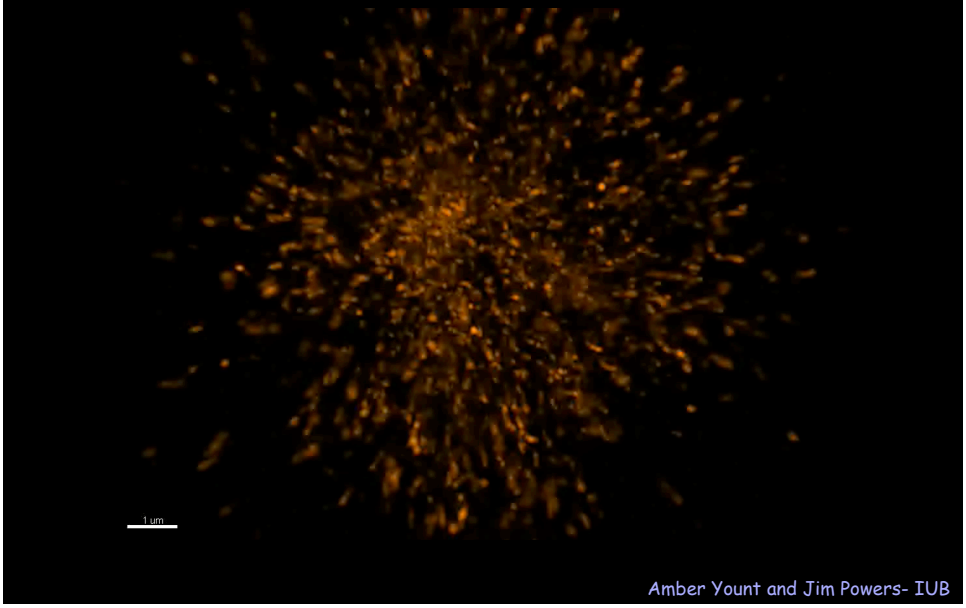
Claire Walczak- IUB


Practical Applications of OMX at IU
Cytoskeletal Structure
Widefield
Deconvolved
OMX
Jim Powers- IUB


Practical Applications of OMX at IU
Cytoskeletal Structure
2 um
Jim Powers- IUB

Practical Applications of OMX at IU

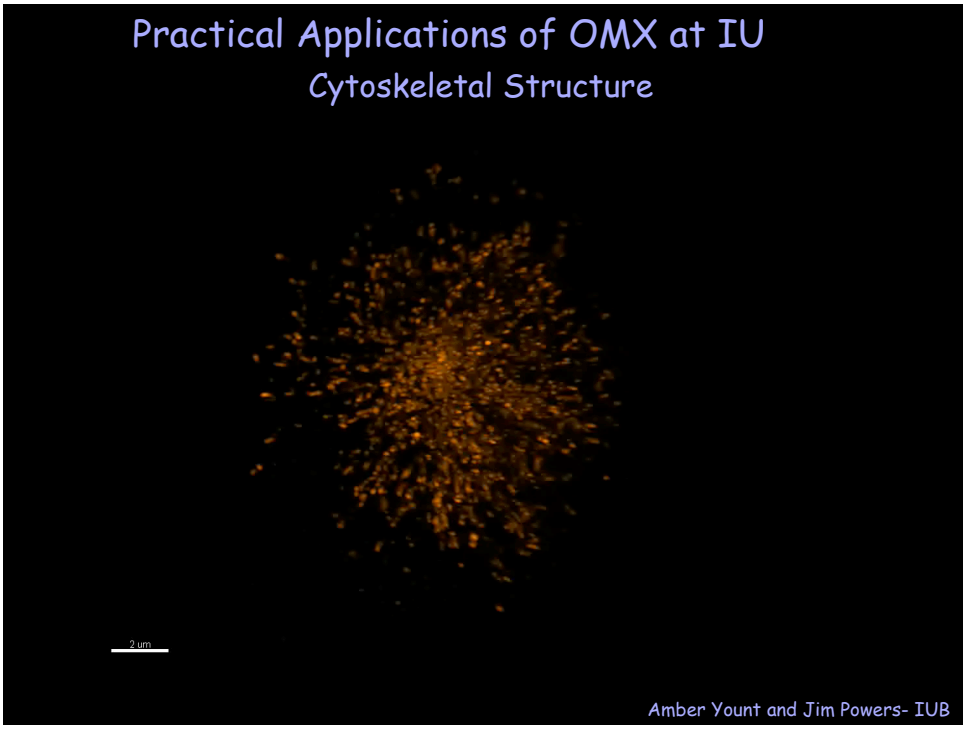
Cytoskeletal Structure



Amber Yount and Jim Powers- IUB

Practical Applications of OMX at IU

Cytoskeletal Structure



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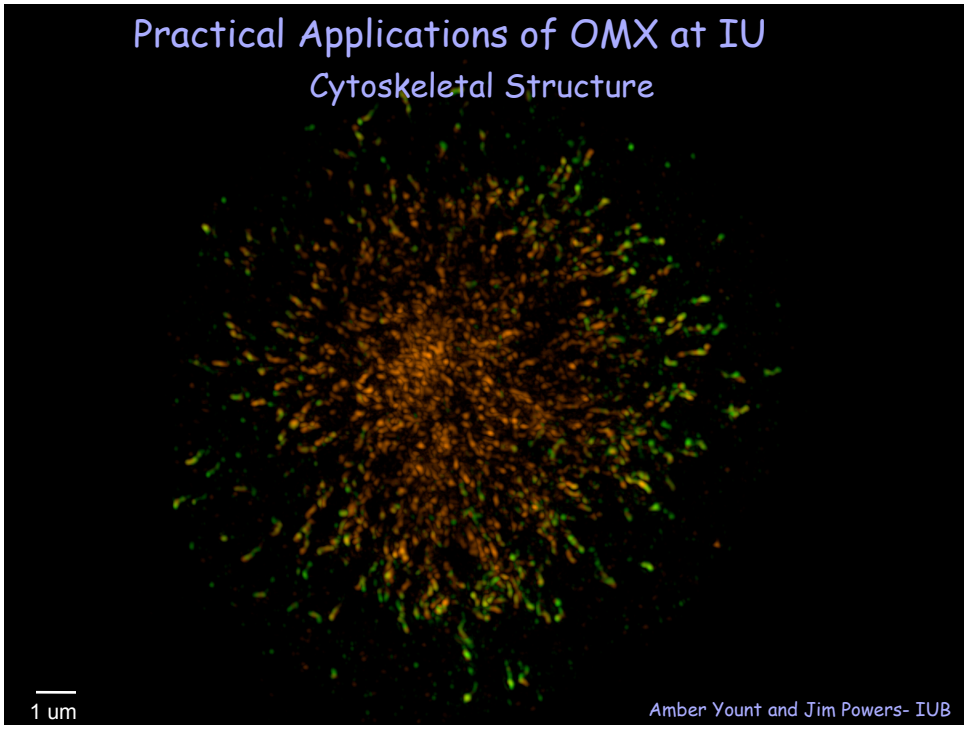
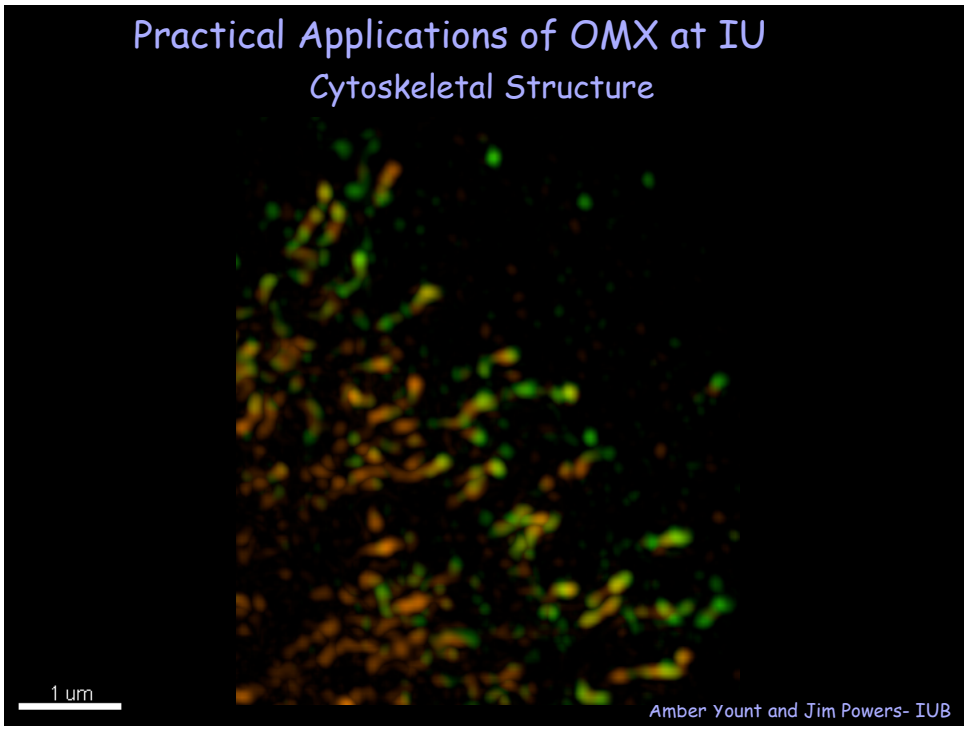
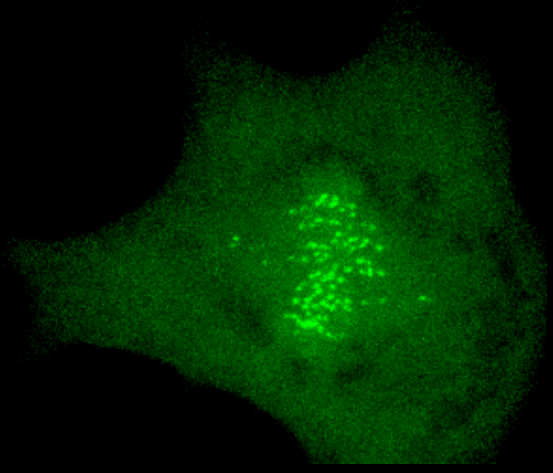

Practical Applications of OMX at IU
Cytoskeletal Structure
1 um
Amber Yount and Jim Powers- IUB


Practical Applications of OMX at IU
Cytoskeletal Structure
1 um
Amber Yount and Jim Powers- IUB

Practical Applications of OMX at IU

Superspeed Imaging



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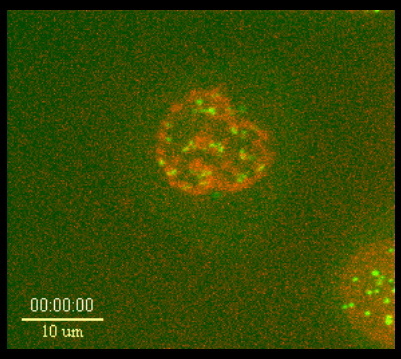
Practical Applications of OMX at IU

Superspeed Imaging



Two-channel simultaneous imaging

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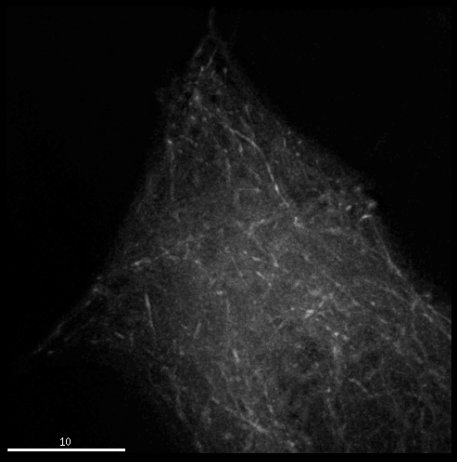
Practical Applications of OMX at IU

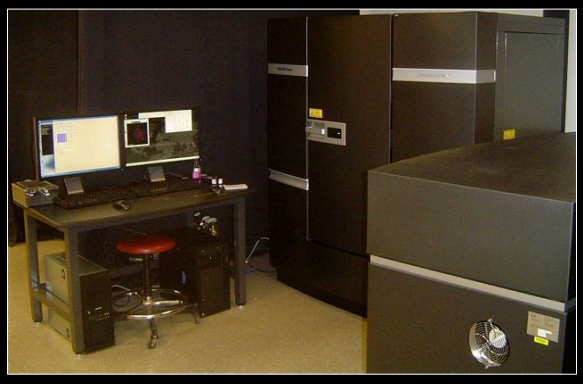
Superspeed Imaging



6- 0.2 μm z-steps
20 msec exposures
0.5 sec temporal resolution

Jim Powers- IUB

Practical Applications of OMX at IU



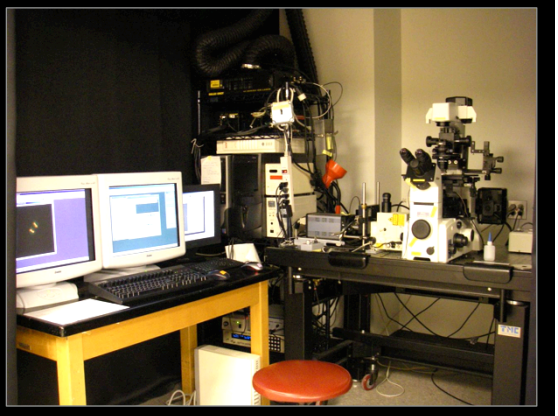
Super-Resolution or Super-Speed Imaging

Cannot do super-resolution of live specimens

Other Equipment at IU-LMIC

Spinning Disk Confocal Microscope

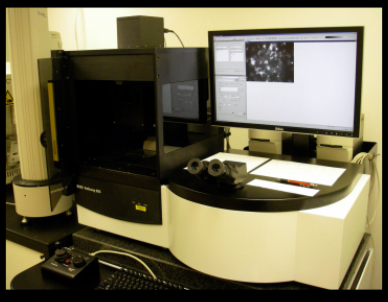
- Optimized for live-cell imaging. Inverted stand.
- Cascade-II EMCCD camera
- Yokogawa CSU-10 spinning disk confocal
- FRAP/Photoactivation using the Mosaic System (Photonics Inc.)
- Lasers for Green or Red fluorophores (ie. GFP. FITC, RFP, TRITC)
- Temperature control
- Metamorph imaging software



Other Equipment at IU-LMIC

BD Pathway High-Content Bioimager(s)

- Versatile system for high-throughput imaging of live or fixed samples.
- From single slides to multi-well plates.
- Multiple images per slide/well.
- Confocal fluorescence, widefield fluorescence, brightfield.
- Laser and image based autofocus.
- Environmental control. - Liquid handling.
- Robotics to handle loading/changing of multi-well plates.



ImageIN Microscopy Workshop Series

Super-Resolution Light Microscopy

Light Microscopy Imaging Center
Indiana University Bloomington
May 18, 2011

Speakers:

Hari Shroff, NIH
Paul Goodwin, Applied Precision Inc.
Richard Day, IUPUI
John Murray, University of Pennsylvania

- OMX Super-Resolution microscope demonstration
- Hands on scope time with your own specimens**

** additional hands on scope time May 19 - 20.

- The Applied Precision Mobile Imaging Lab on location

Free Registration - To register, please email: japowers@indiana.edu

More information available @ http://www.indiana.edu/~lmic/ImageINwebsite/Page1.html

Next Workshop:

Fluorescence Lifetime Imaging (FLIM) at IUPUI Sept, 2011

For information on the workshop series contact: japowers@indiana.edu

Thank You!!!

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IUB-COAS, Med Sciences, Optometry

NIH- NCRR