

Implementation of High-Speed Laser Technology based Microscopy Systems for Various Biological Applications

Presenter – Dr. Lance Davidson



University of Virginia

W.M. Keck Center for Cellular Imaging

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(A University Imaging Center)

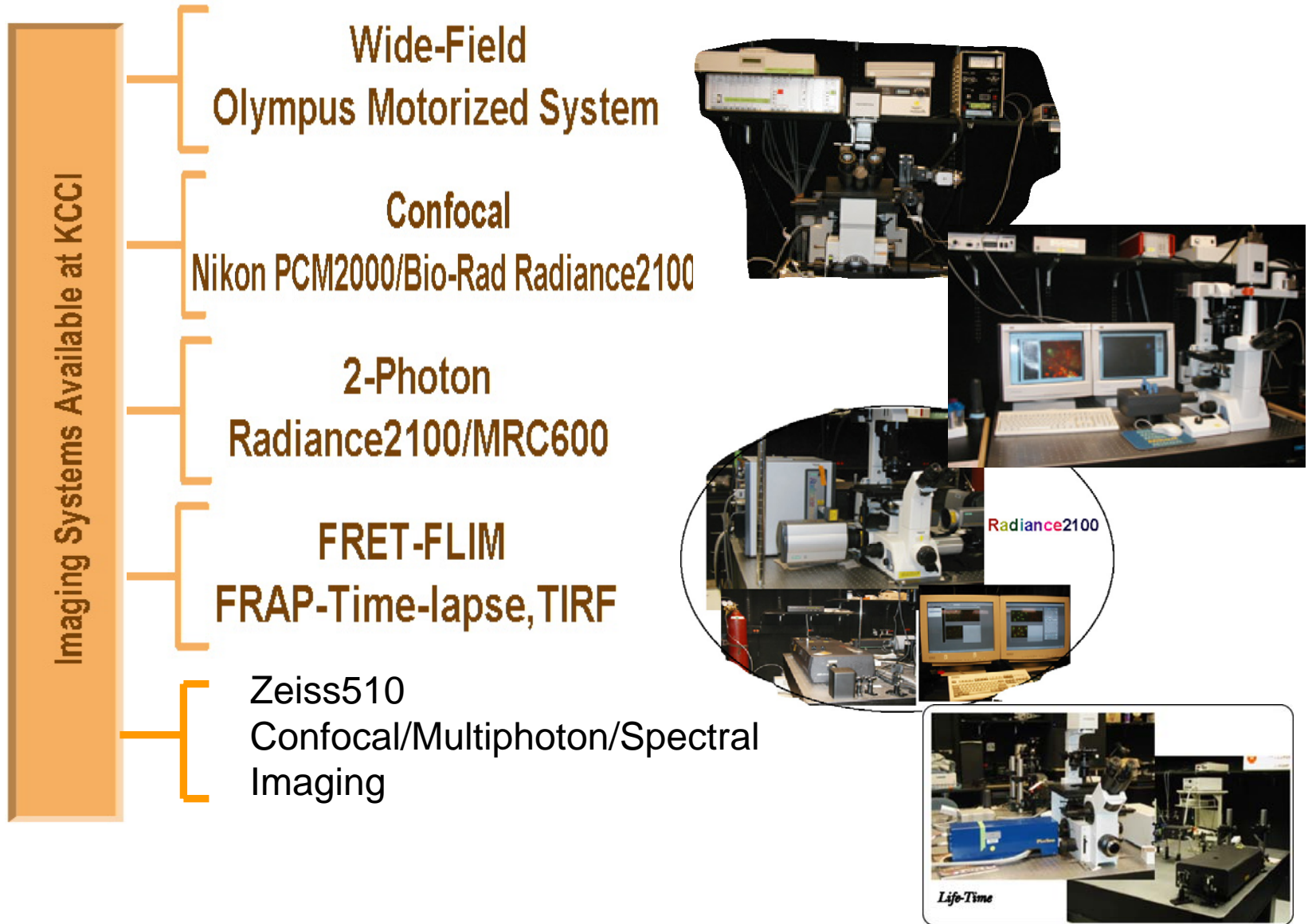
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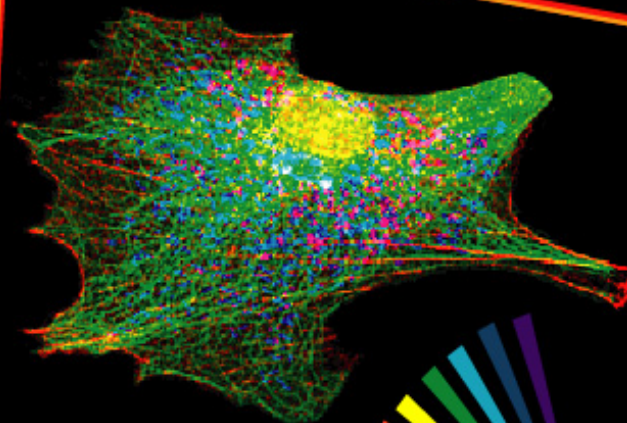


Methods in Cellular Imaging

edited by

Ammasi Periasamy

Published by Oxford University Press



Other Topics:

Fluorophores
Detectors
Confocal
Multiphoton
Spectral Imaging
TIRM
AFM
Laser Tweezer
Bioluminescence

FRET-FLIM Chapters by:

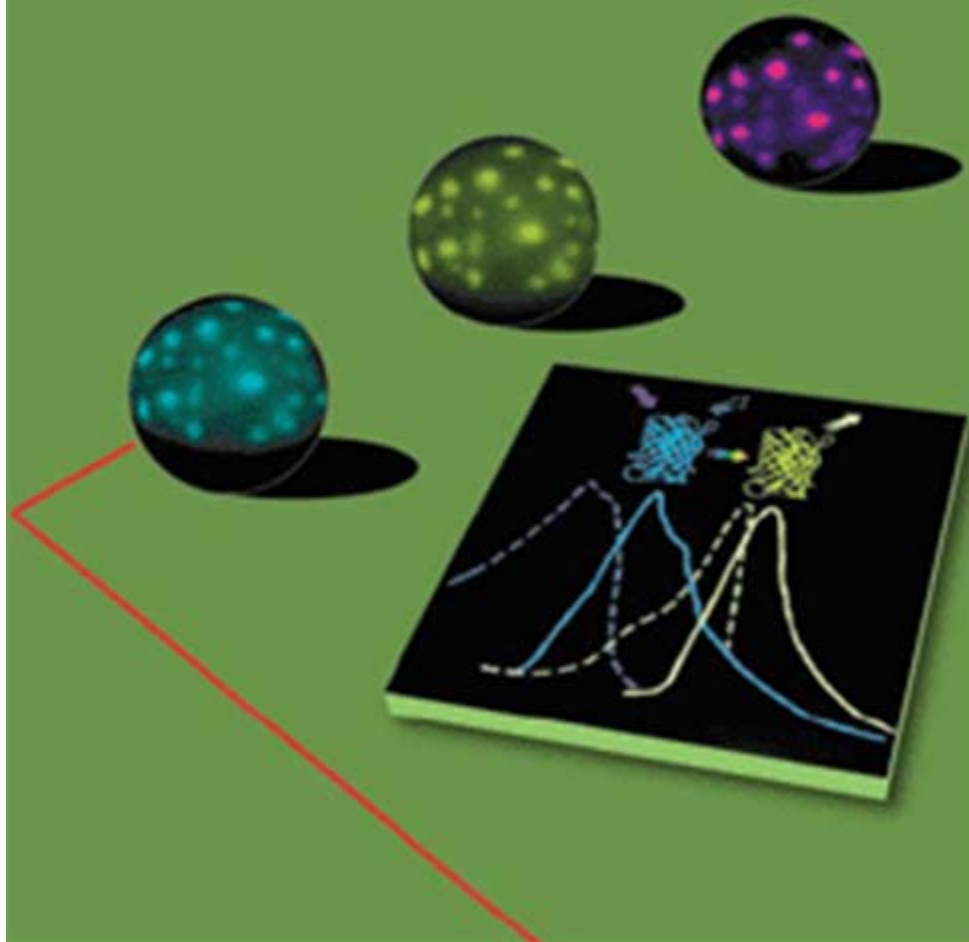
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MOLECULAR IMAGING

FRET Microscopy and Spectroscopy



Edited by

Ammasi Periasamy • Richard N. Day

W.M. Keck
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Cellular Imaging

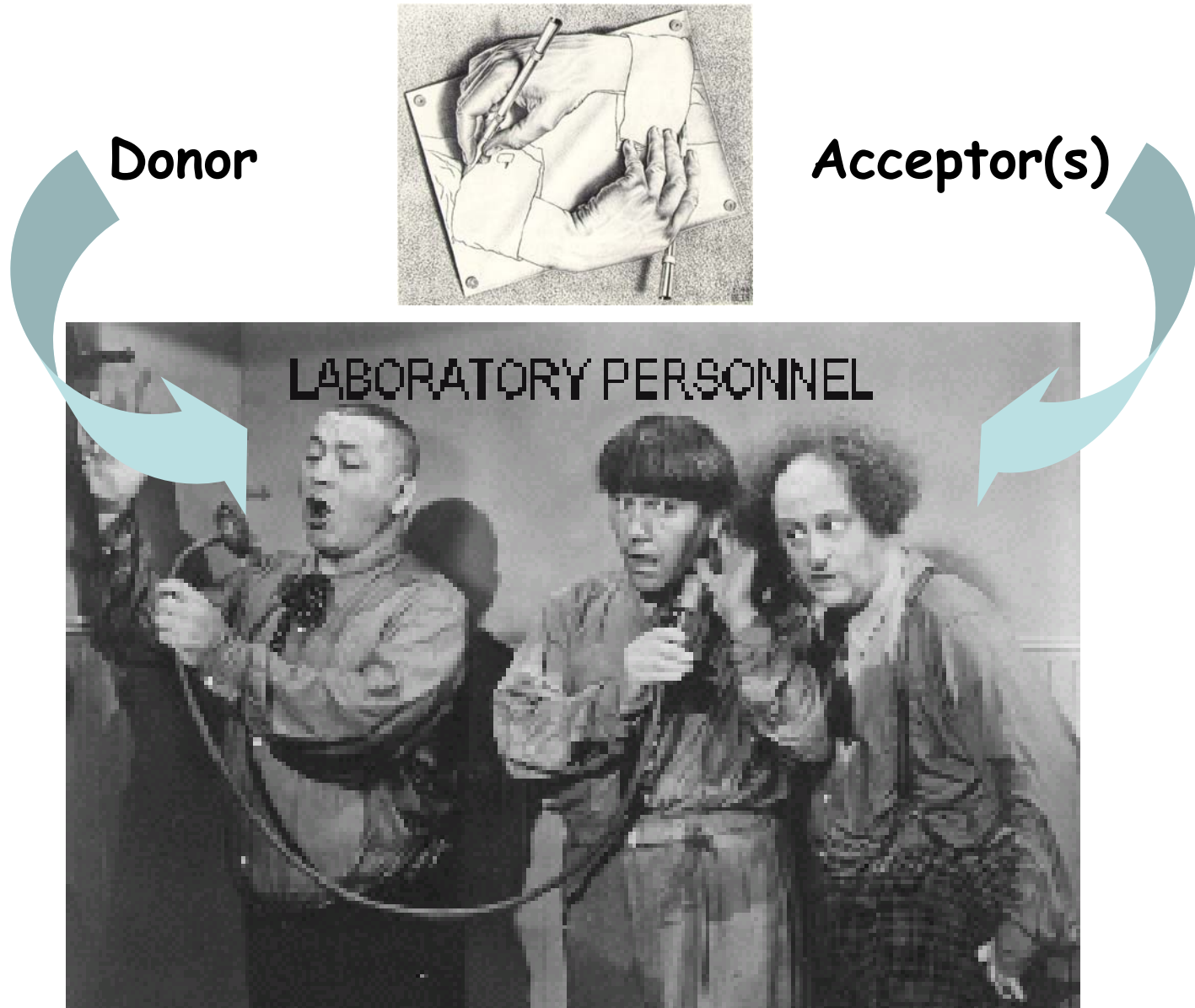


5th Annual
FRET Microscopy
Workshop

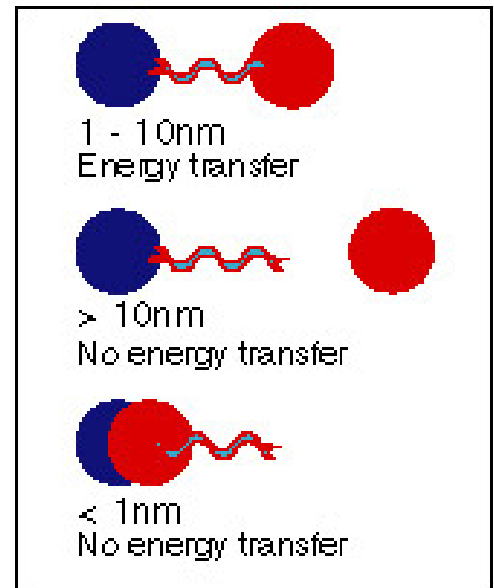
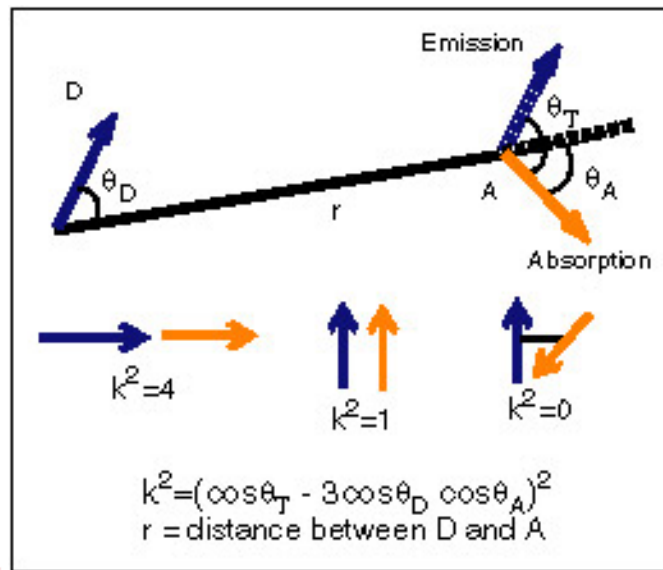
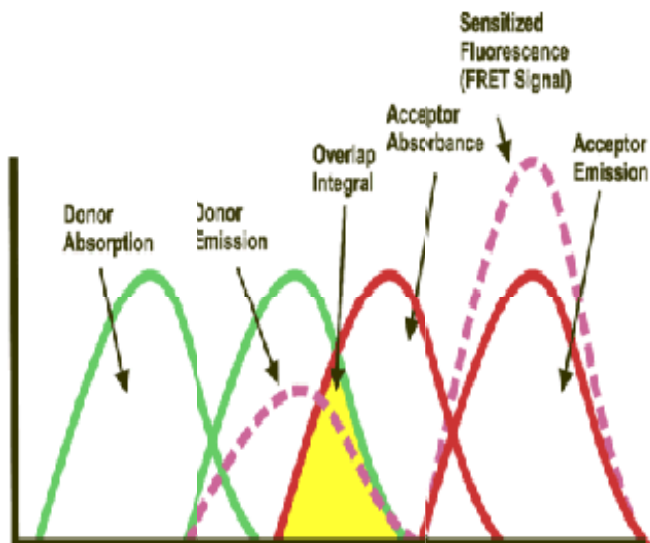
March 7-11, 2006

University of Virginia

FRET = molecular communication



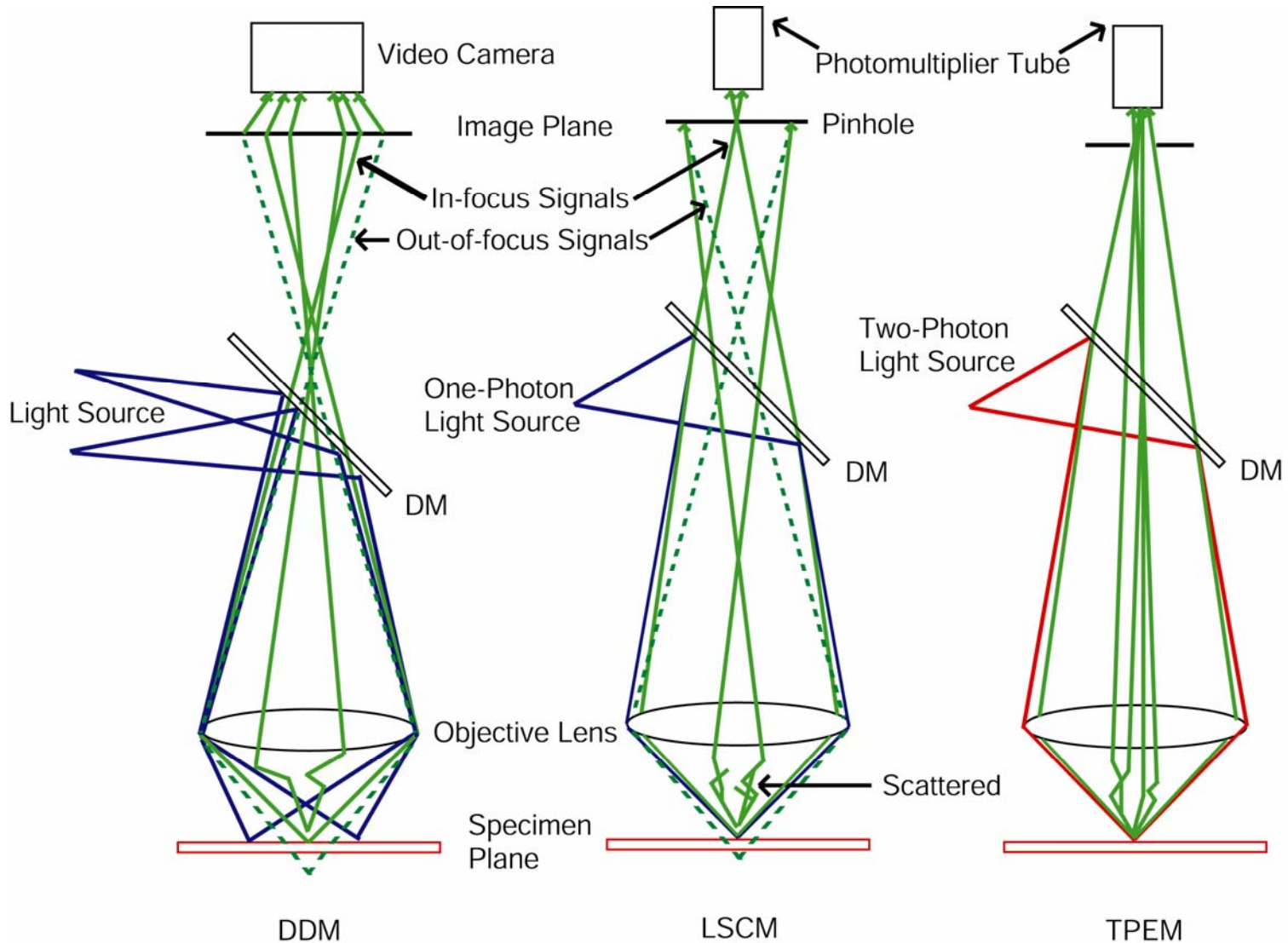
Conditions for FRET to Occur



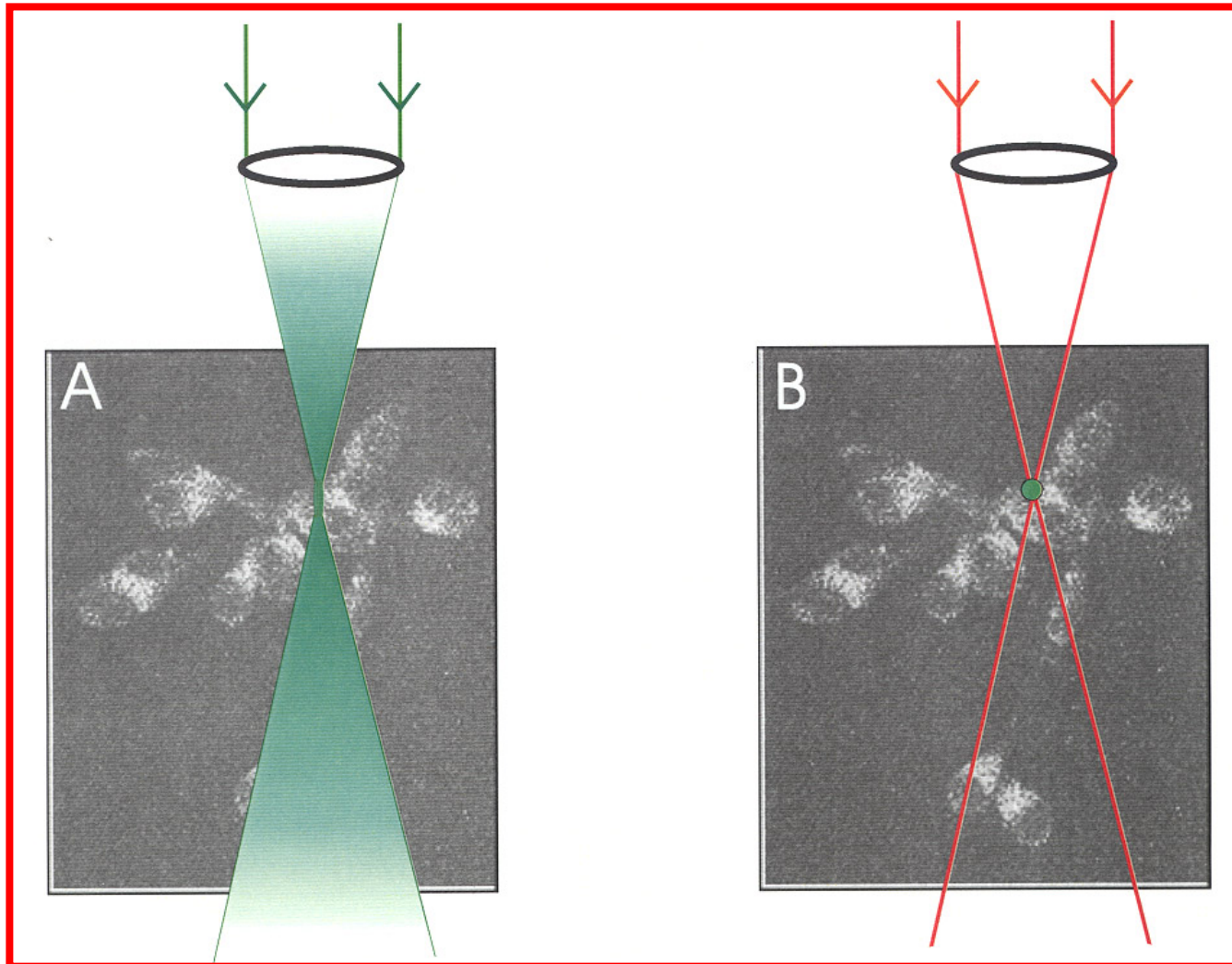
What are the FRET microscopy techniques?

- **Wide-field or digitized video FRET (*WF-FRET*)**
- **Laser Scanning Confocal FRET (*C-FRET*)**
- **Two-photon excitation FRET (*2p-FRET*)**
- **Lifetime Imaging FRET (*FLIM-FRET*)**
- **Spectral Imaging FRET**
- **Photobleaching FRET**
- **Correlation Spectroscopy**

Compare 1p-2p FRET Microscopy

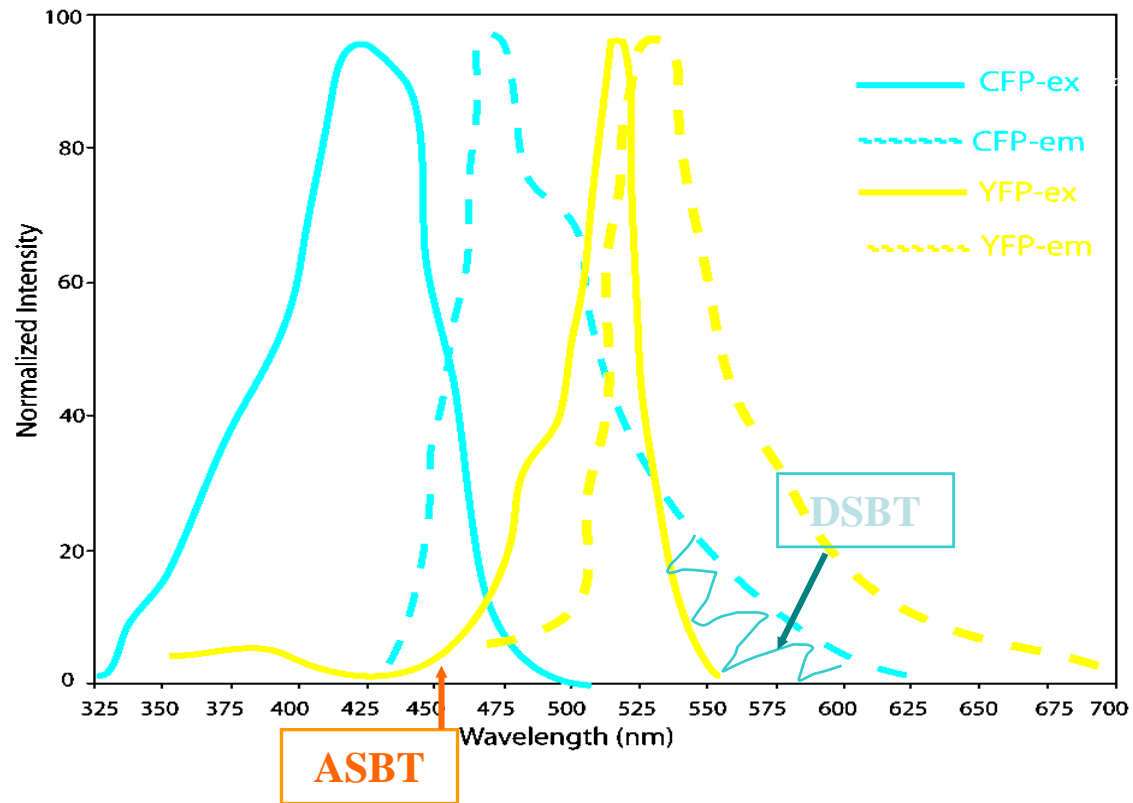


Illumination of Excitation Light in One- and Two-photon Microscopy

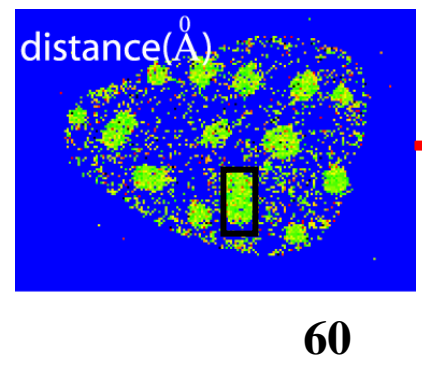
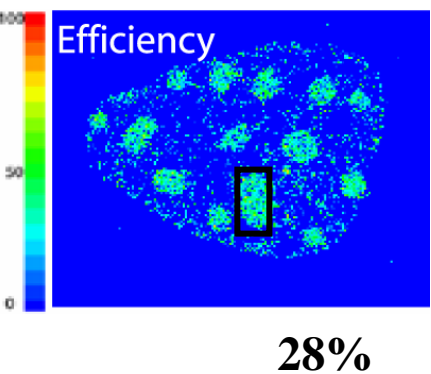
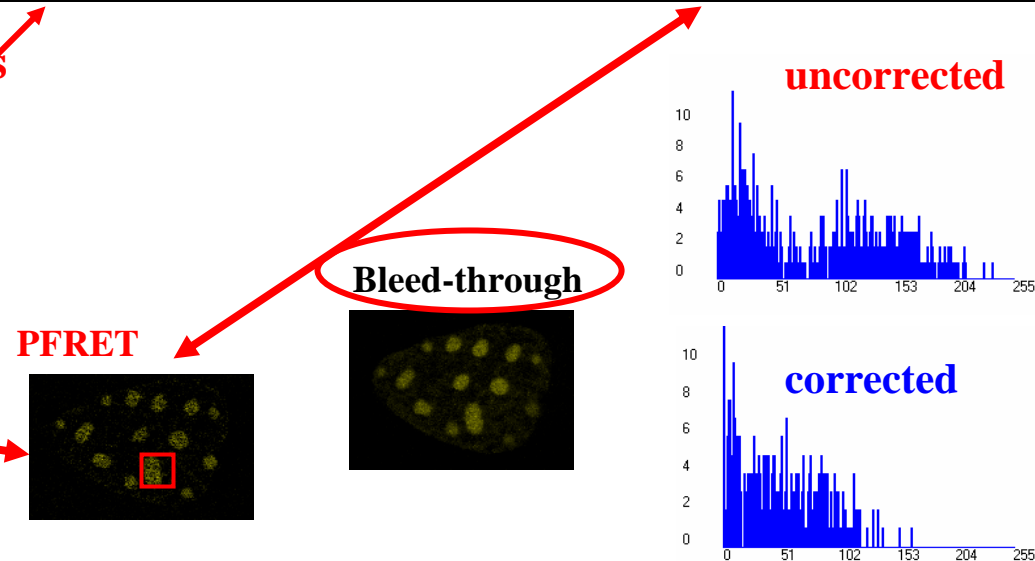
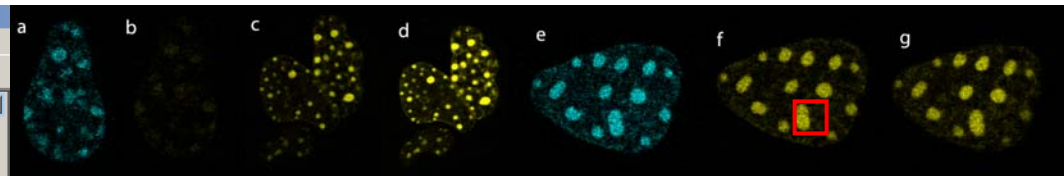
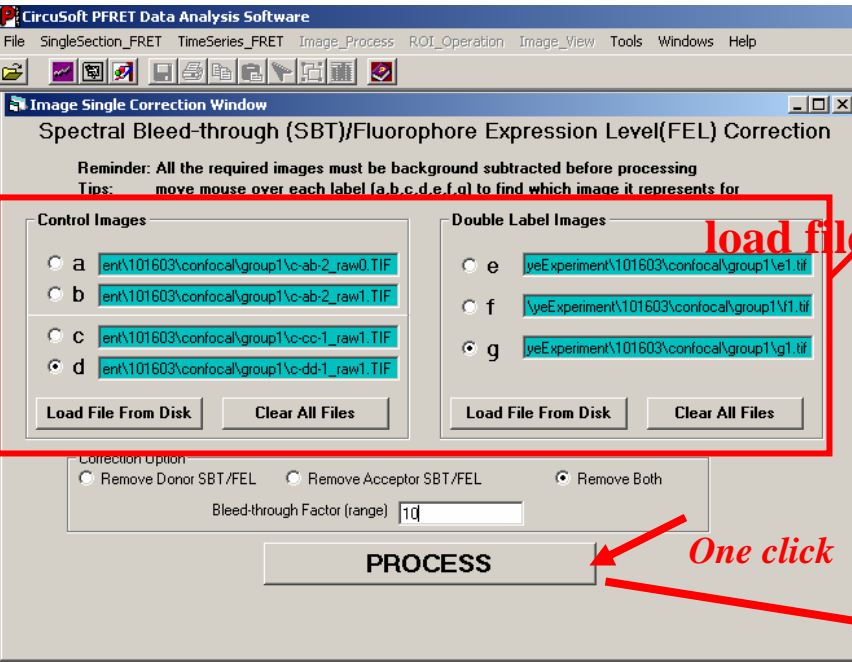


Contamination in the FRET Image

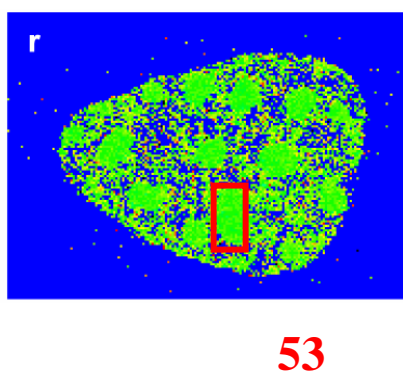
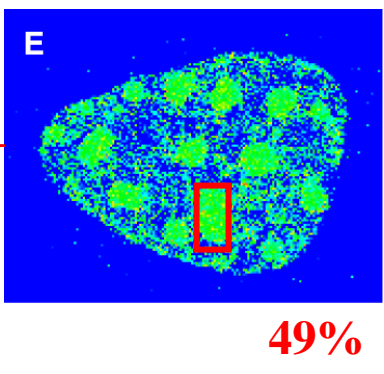
- **Background Signal**
- **Donor spectral bleedthrough (DSBT)** - donor excitation causes donor emission into the acceptor channel
- **Acceptor spectral bleed-through (ASBT)** - donor excitation wavelength also excites acceptor molecules which cause more signal detected in the acceptor emission.



Data Analysis—PFRET Software



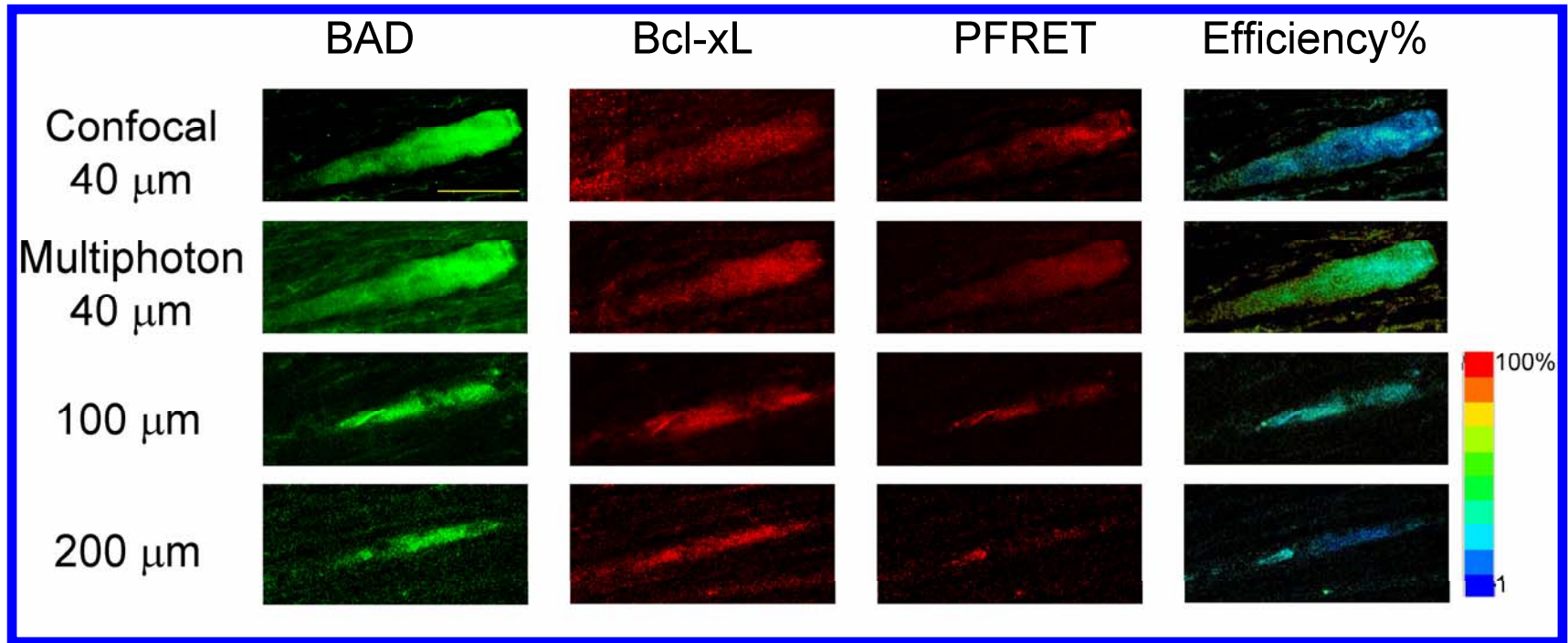
No correction



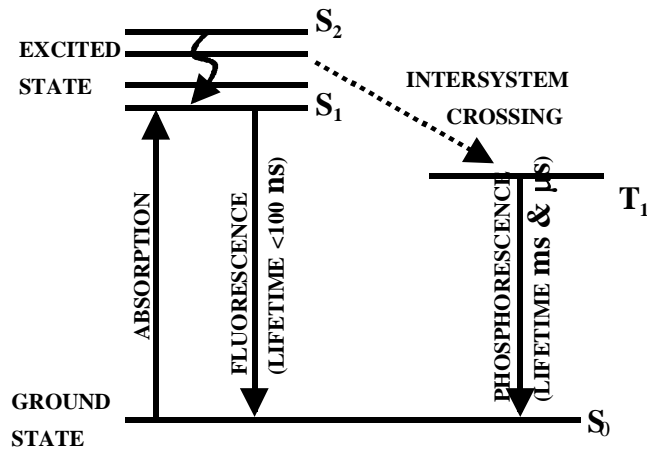
Tissue FRET-Multiphoton Microscopy

- For the first time FRET has been used to demonstrate protein interactions in whole brain tissue.
- We have shown that BAD and Bcl-xL form a heterodimer in axons following injury, indicating that the apoptotic cascade may be an important factor in secondary brain injury.

Deep Tissue FRET Imaging in Traumatic Axonal Injury – Multiphoton FRET Microscopy



Six hours post injury; tissue labeled with BAD/Alexa 488 (donor) and Bcl-xL/Alexa 555 (acceptor) demonstrates energy transfer consistent with BAD-Bcl-xL heterodimerization.



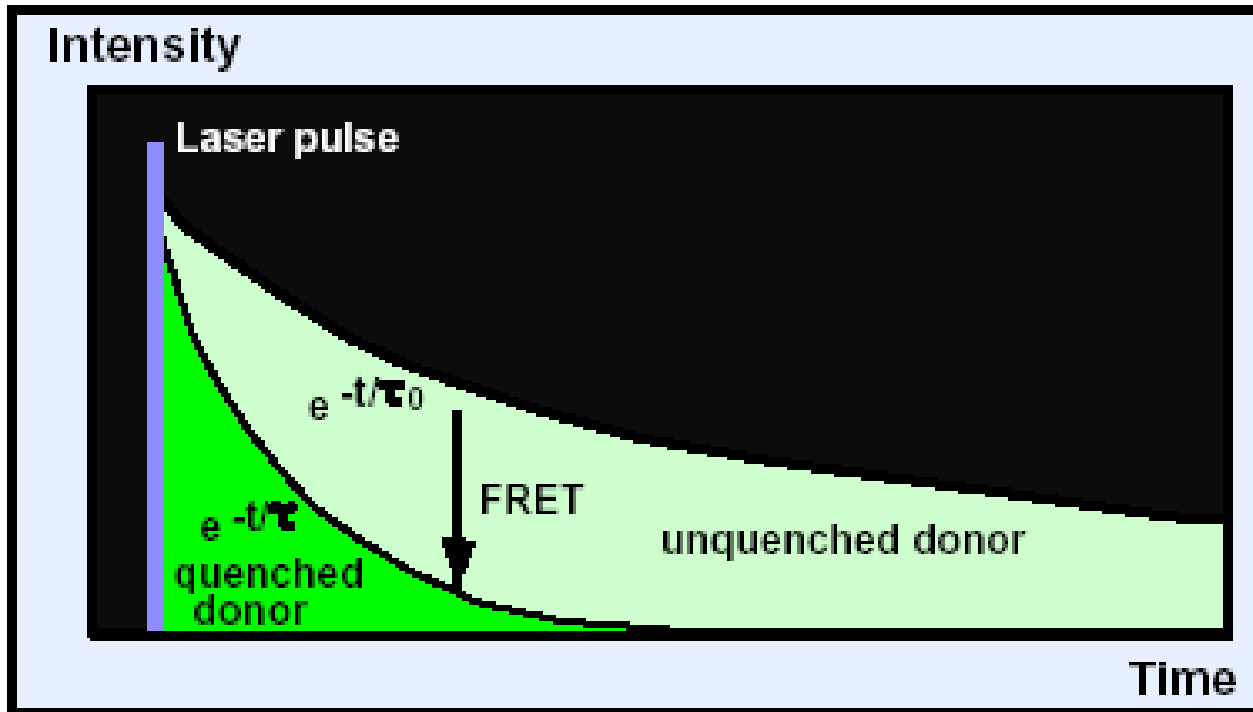
WHAT'S FLUORESCENCE LIFETIME?

The Fluorescence Lifetime is the average time that a molecule remains in the excited state prior to return to the ground state.

Lifetime generally falls in the range of 1 to 100 ns.

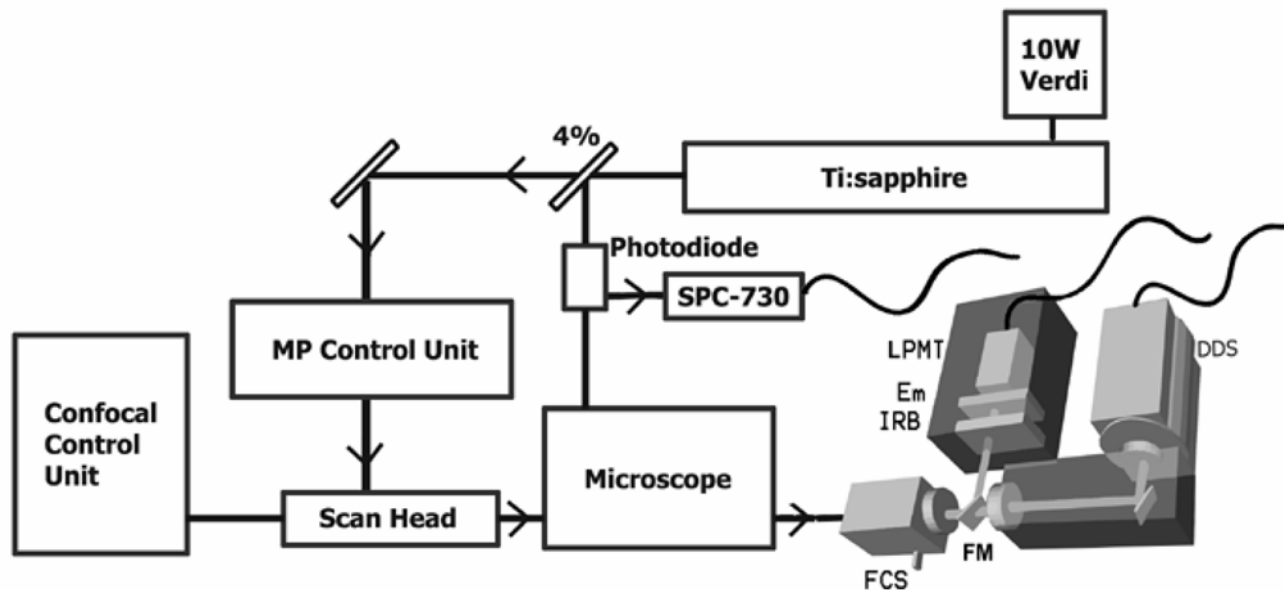
***Independent** of change in excitation light intensity, probe concentrations, and light scattering, but highly dependent on the local environment of the fluorophore.

Time-domain



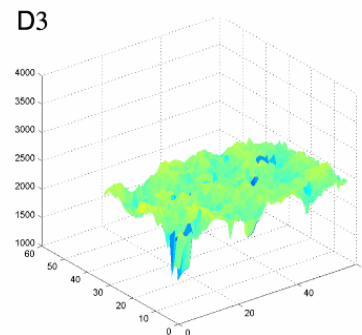
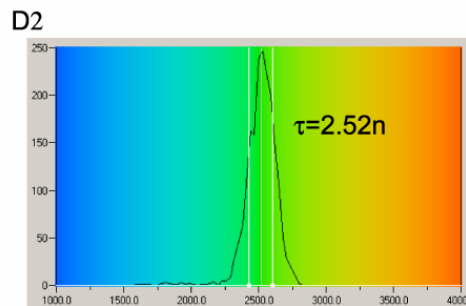
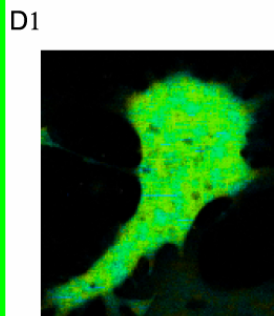
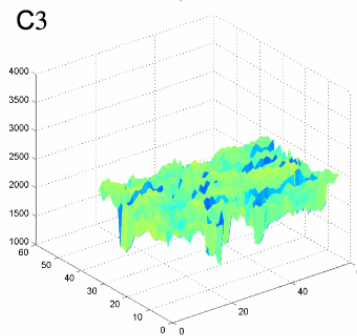
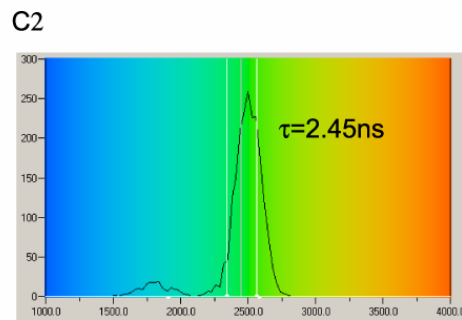
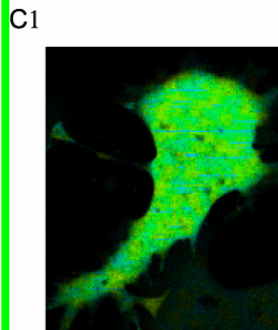
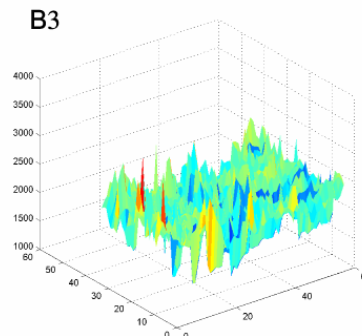
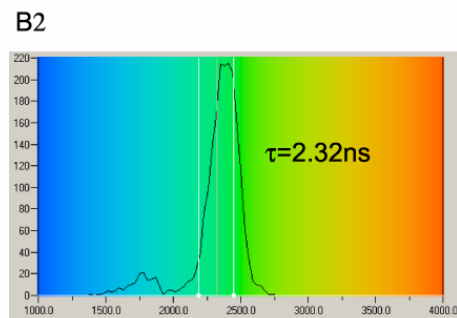
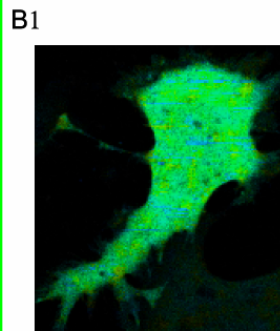
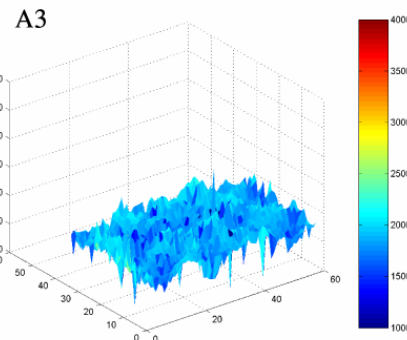
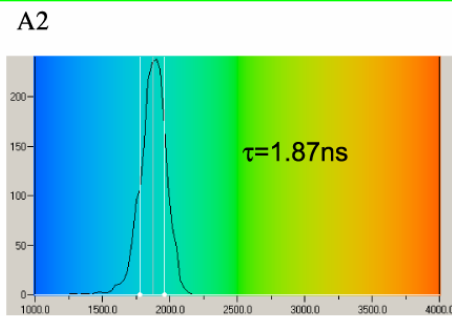
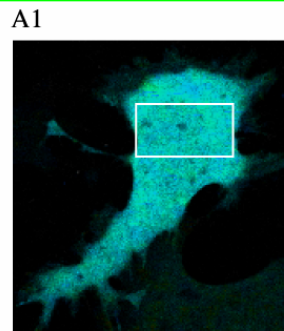
FRET results in dramatic decrease of donor lifetime. Now, the fluorescence decay function contains the fluorescence of quenched and of unquenched donor molecules, and is therefore double exponential.

Two-photon FLIM-FRET Microscopy



Chen and Periasamy, Molecular Imaging:FRET Microscopy and Spectroscopy, Chapter 13, Oxford University Press, 2005.

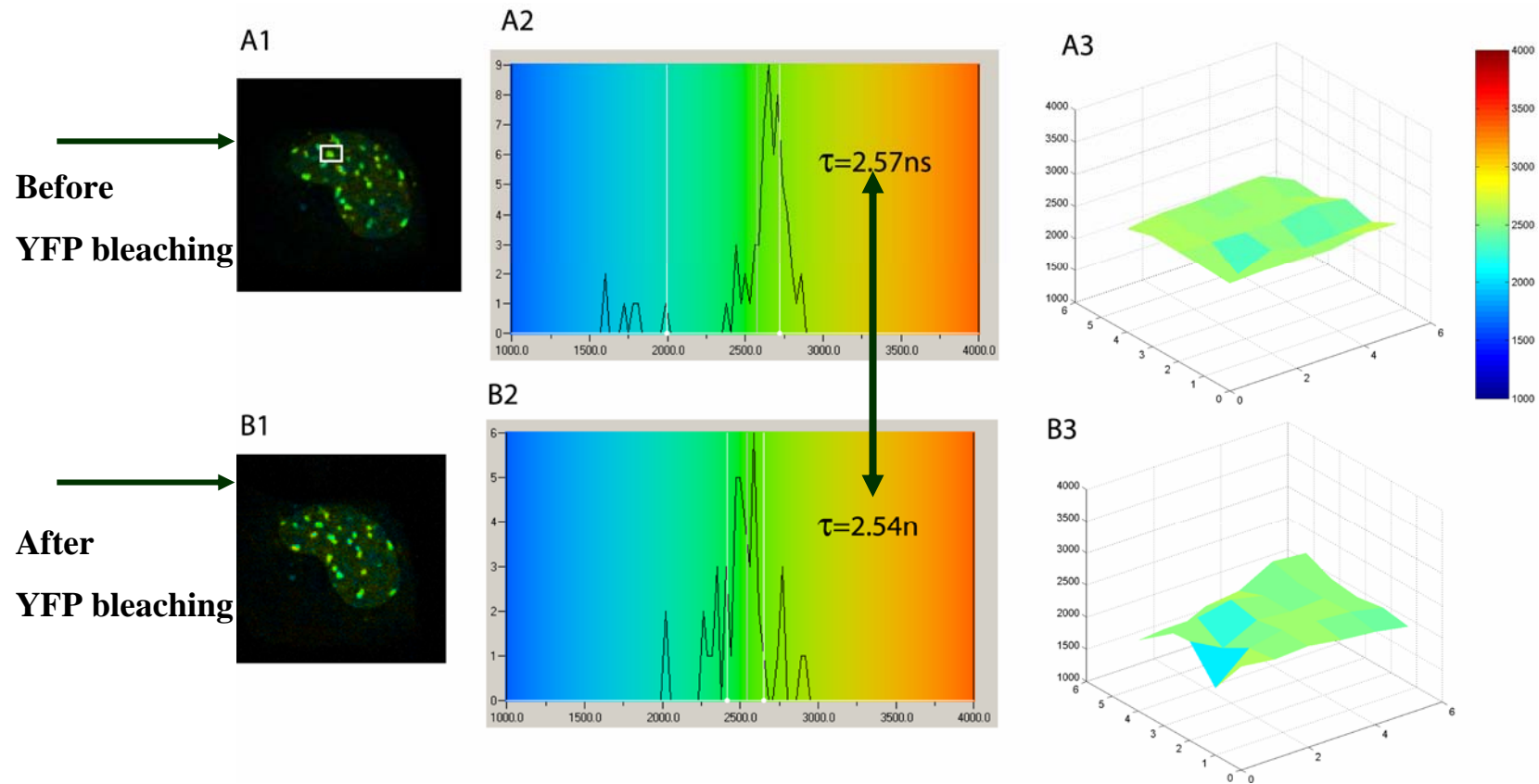
Photobleaching FLIM-FRET



Positive Control
CFP-15AA-YFP

Acceptor molecule
was photobleached
step-by-step

Acceptor photobleaching – Negative control



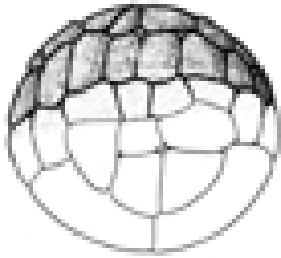
Cells co-expressing unlinked CFP and YFP, which were co-localized, but did not interact.

***Xenopus laevis*: African Clawed Frog**

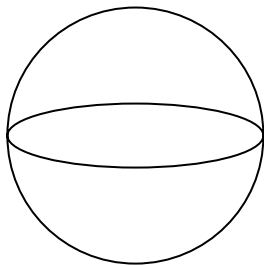
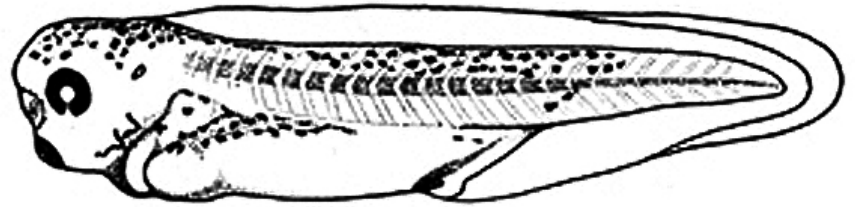


What is gastrulation?

blastula



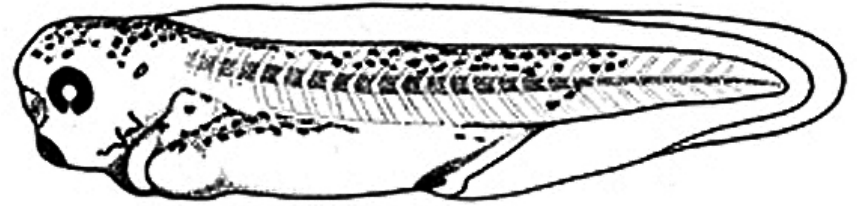
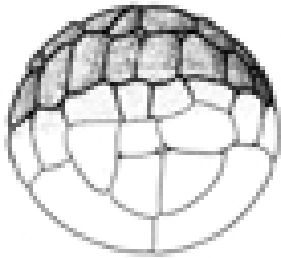
tadpole



ball



tube



mechanical molecules

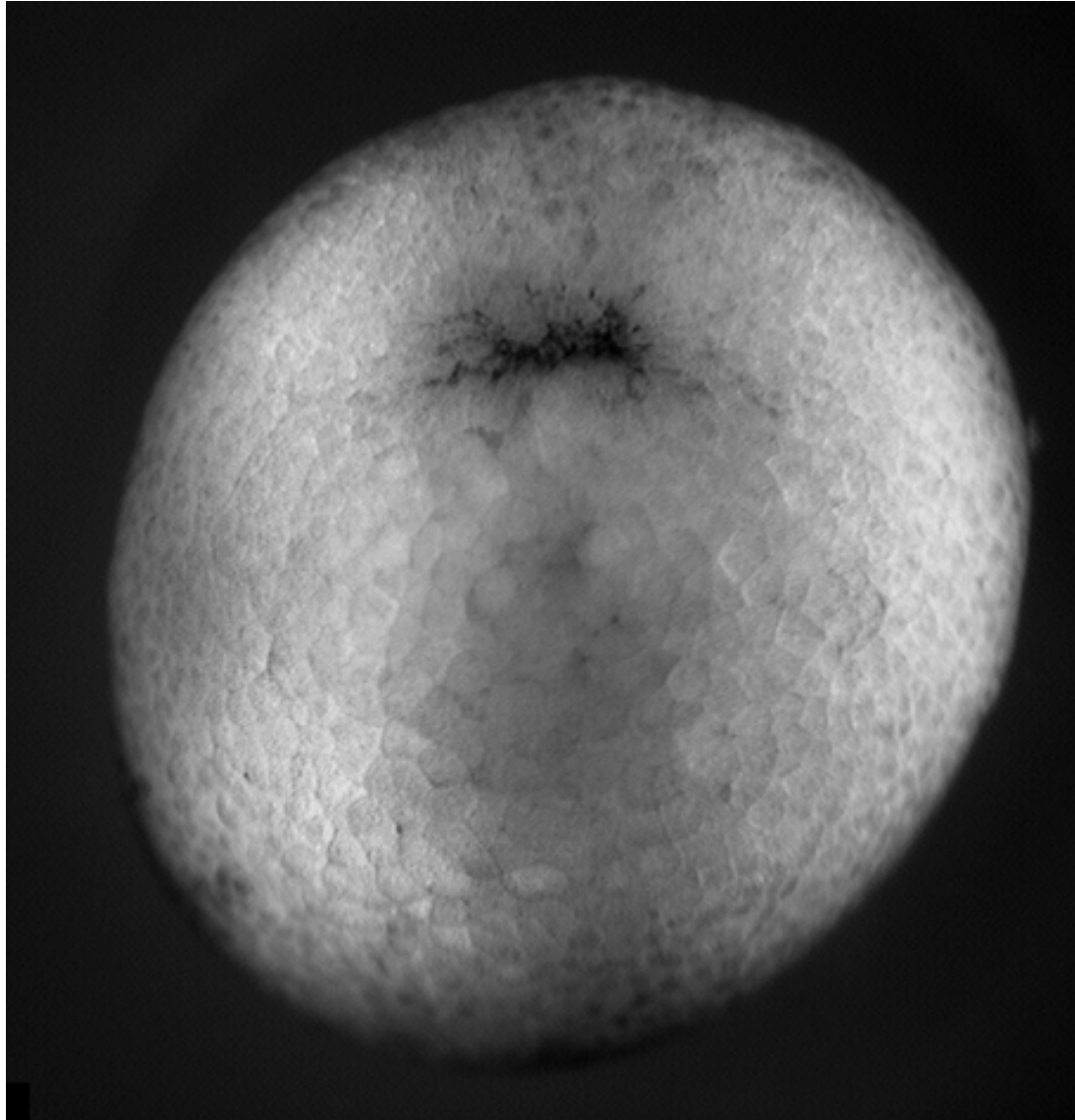
- motors
- cytoskeleton
- cell adhesion
- extracellular matrix

mechanical phenomena

- cell motility / shape change
- cell behaviors
- cellular environment
- force transmission
- tissue deformation


Gastrulation

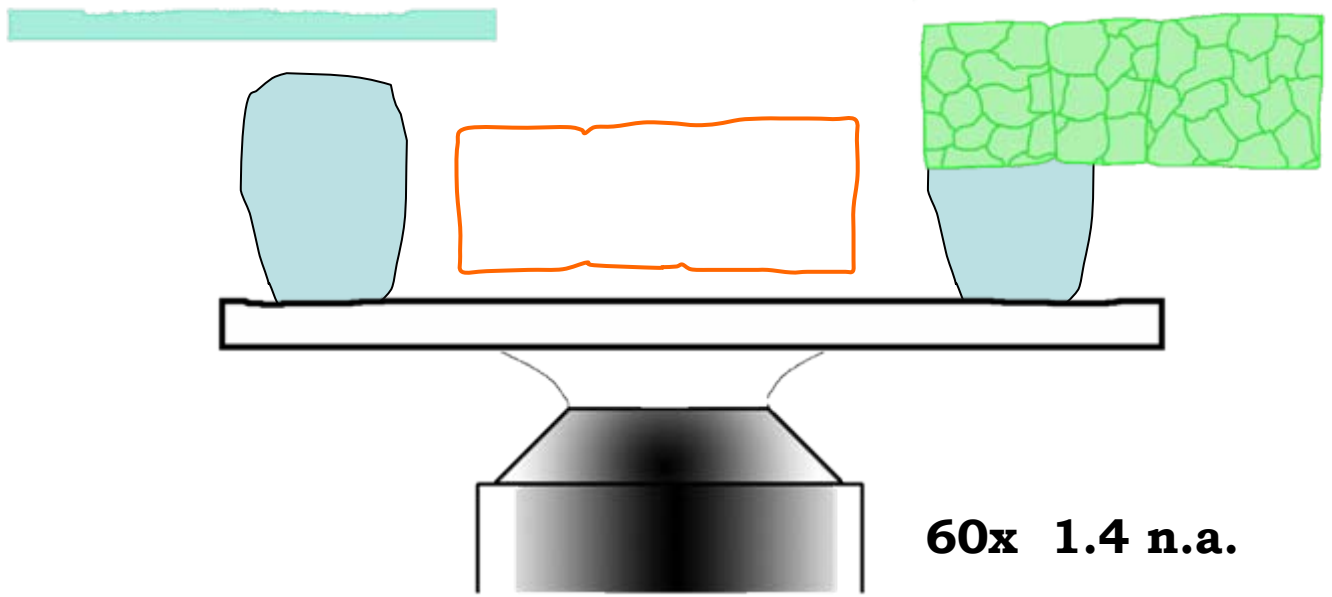
↑
1.2 mm
↓



14 hours elapsed time

 membrane-targeted GFP

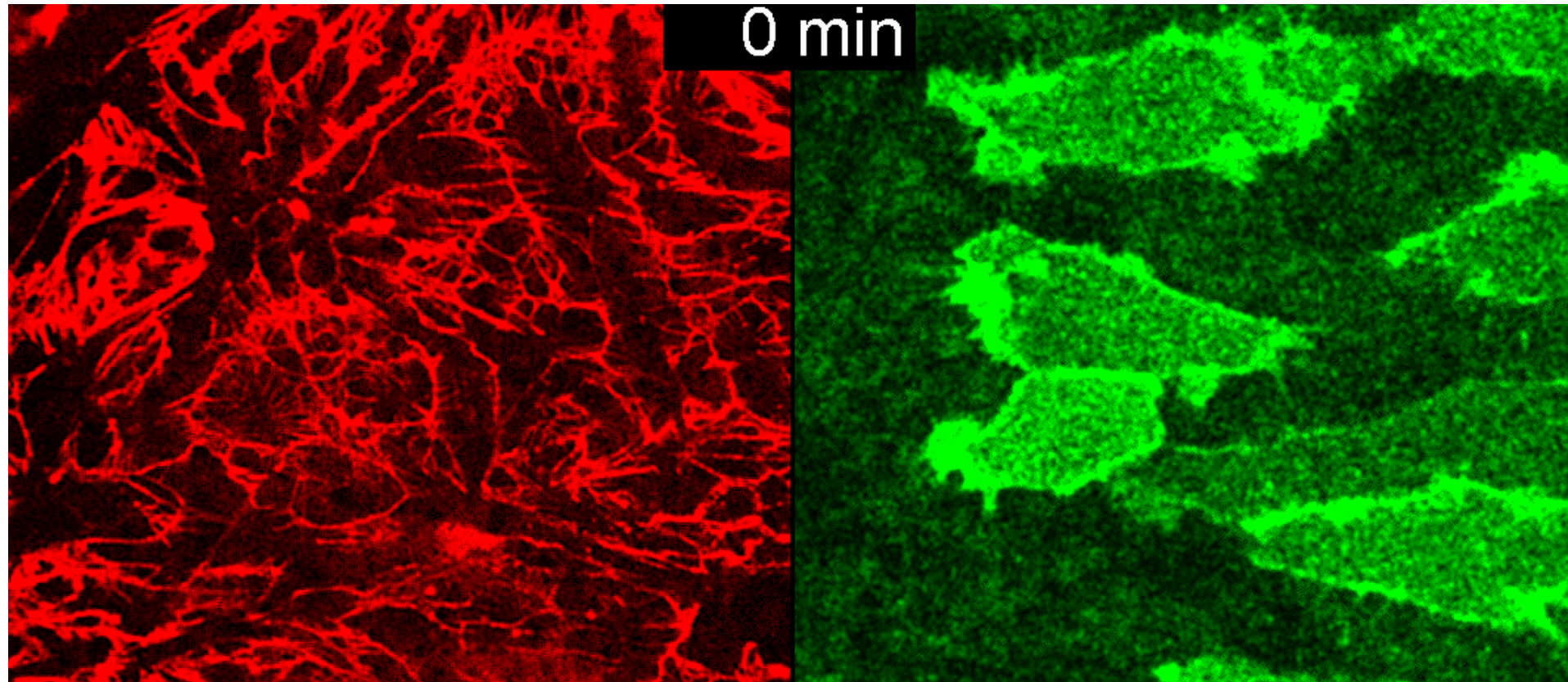
 Cy3-conjugated anti-Fibronectin mAb



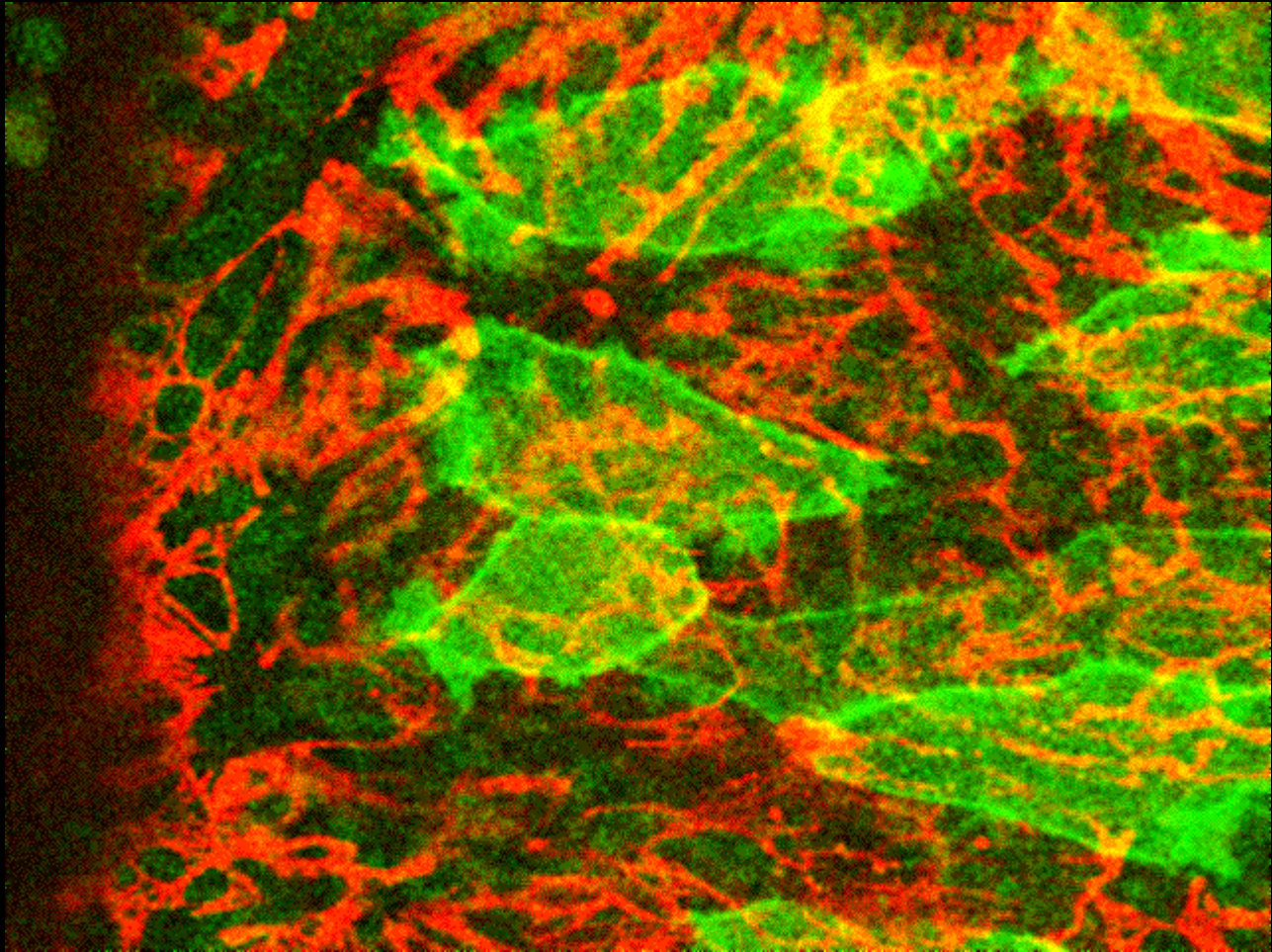
60x 1.4 n.a.

Fibronectin rich fibrils
labeled with Cy3-anti-FN

Scattered membrane-labeled
cells (with unlabeled background
cells) in same plane as fibrils



Live imaging of Fibronectin Fibrils and Cells



Biology advances when imaging technologies advance.

Key questions:

How to use the FEL to fundamentally advance imaging?

- Basic studies of tissue-spectroscopy.
- Using non-imaging methods to "interrogate" the cell and its environment for information during imaging.