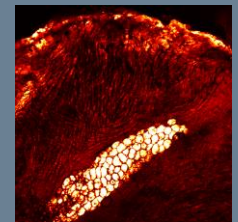
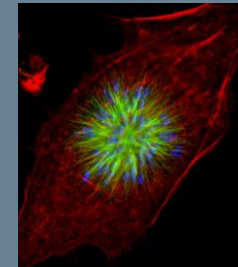
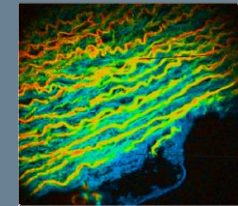
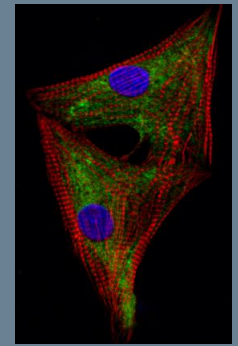
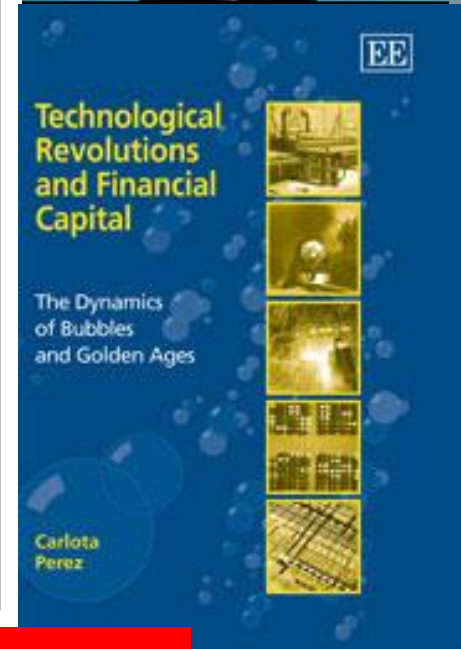
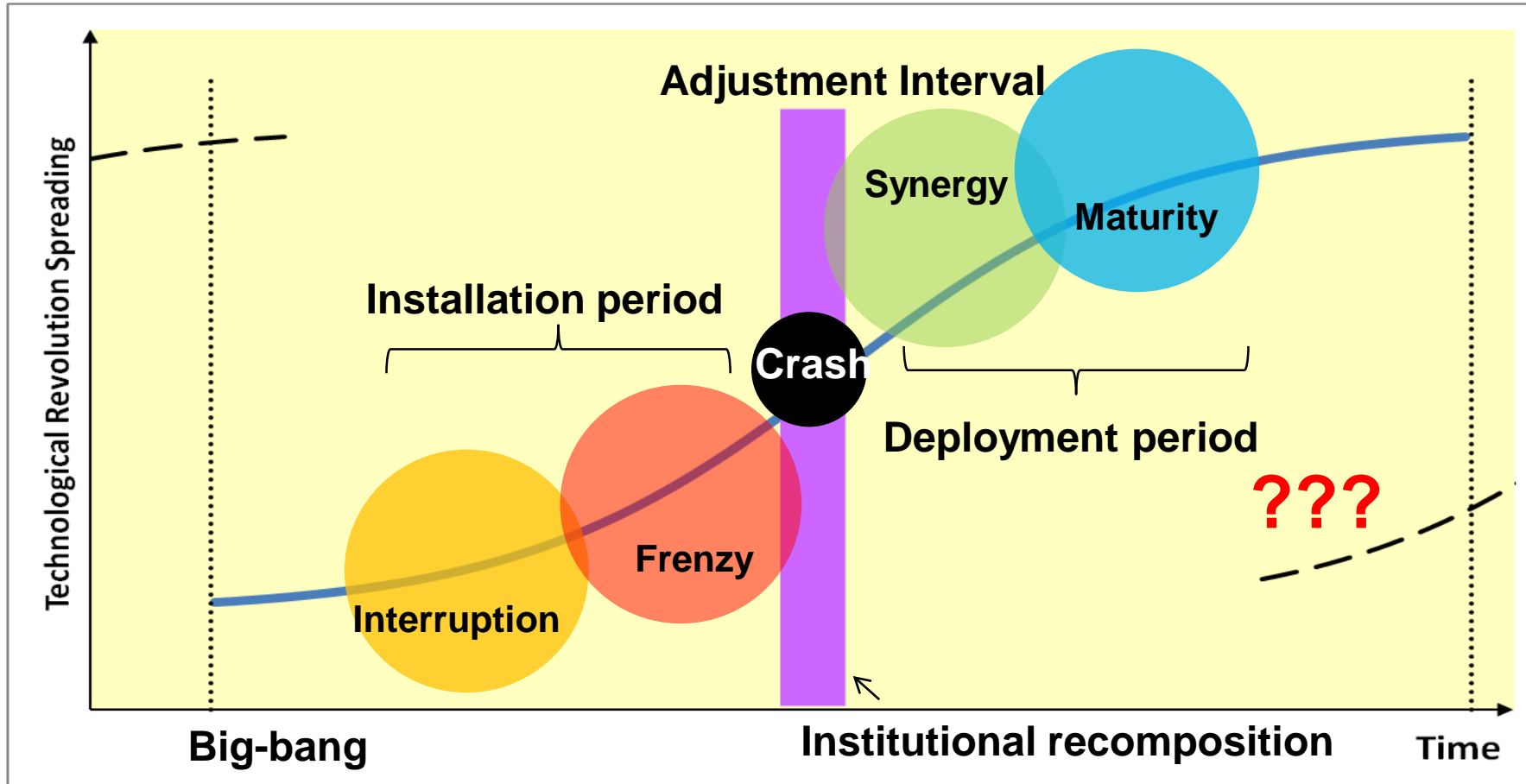


Multimodal Photonic Platform to Understand Biological Processes



Carlota Perez: Technological Revolutions and Financial Capital



- 1
- 2
- 3
- 4
- 5

Trillion dollars question: Next Revolution???

Our bet: control of biology at cell/molecular level

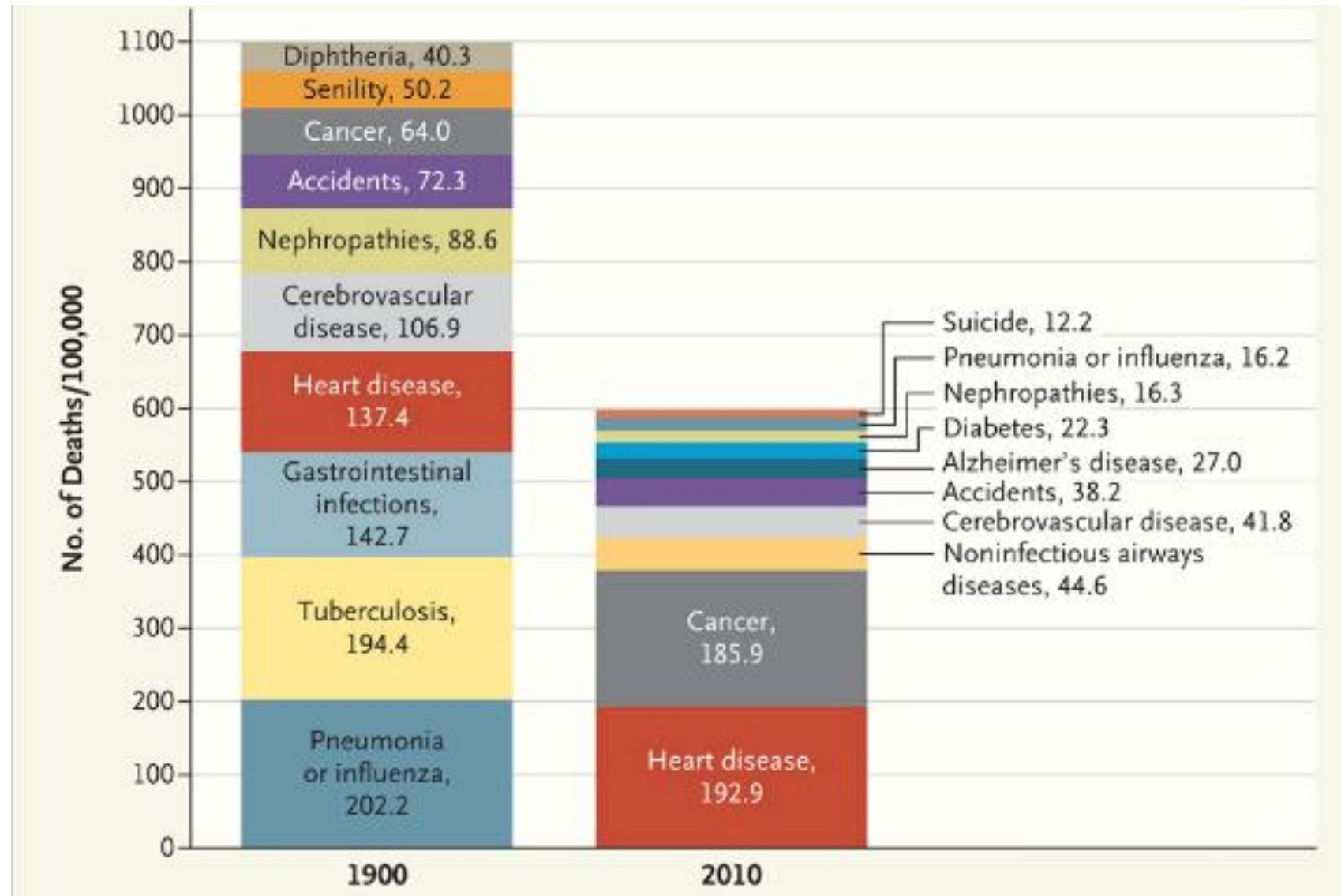
BIOECONOMY

6. Next revolution???

le

Death causes USA 1900 – 2010

<http://www.nejm.org/doi/full/10.1056/NEJMp1113569>





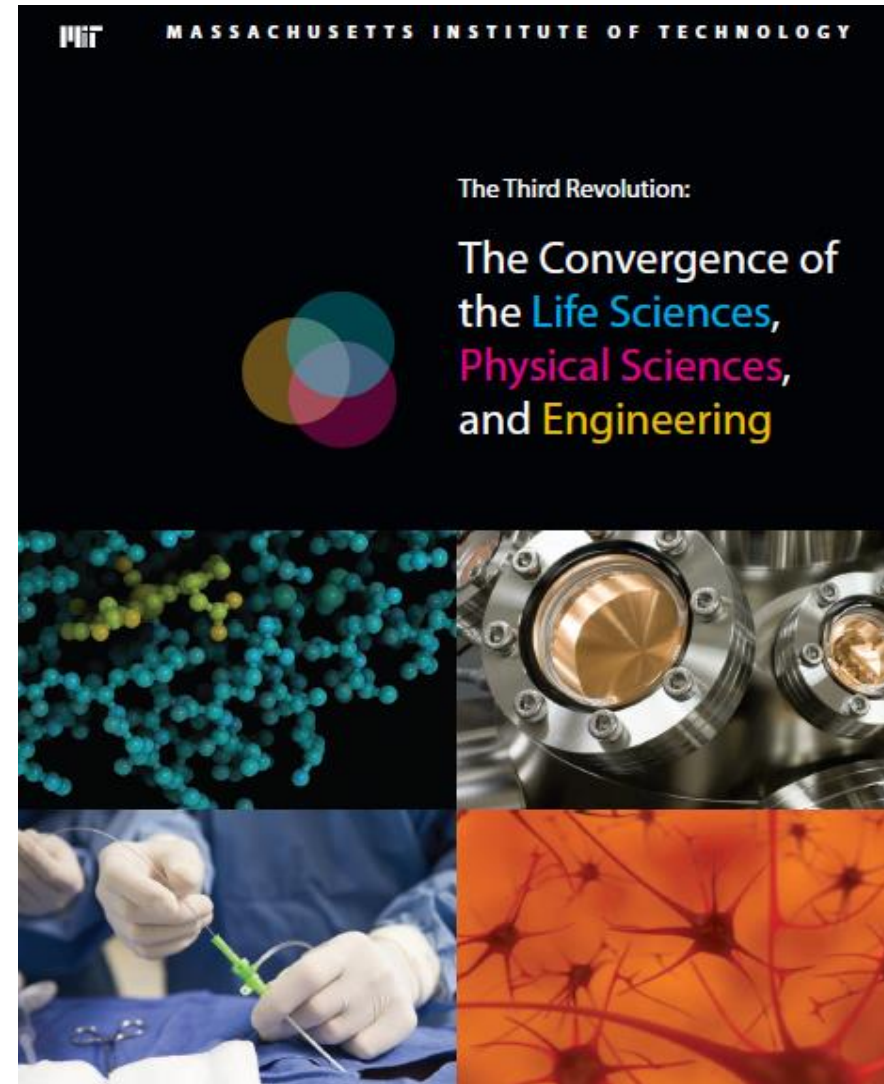
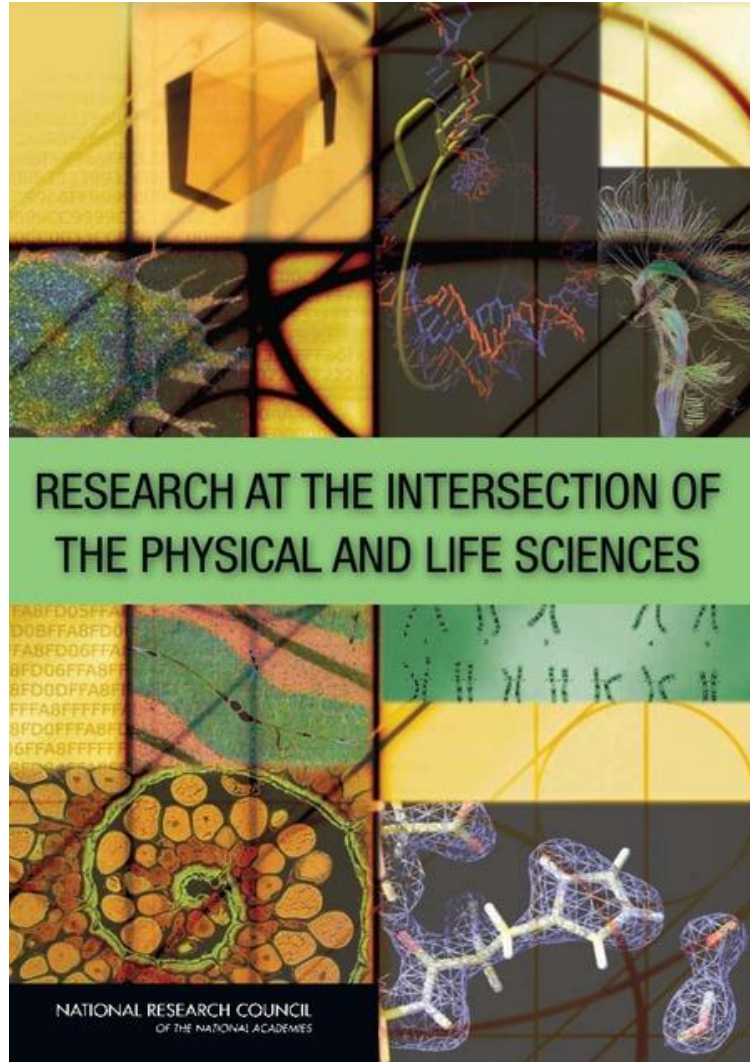
Tom Knight – engineer – pioneer of arpanet
Biochemistry classroom at 40 yo
father of synthetic biology, biobricks, iGEM

Synthetic biology is the technology of the century. This is going to change how we build things. Biology is fundamentally a manufacturing technology, and we're on the verge of figuring out how to control that. It's impossible to predict and estimate the impact of that, but it's going to be massive.

We have very little ability to put atoms exactly where we want them. Semiconductor engineers don't get to put atoms where we want them. Biology puts every atom in the place it wants with precise control. We can use that as a very powerful manufacturing technology.

National Research Council Report

MIT white paper



Combining molecular/cell Biology with physics & engineering.

Movement towards biological physics:

Nobel prize in 2014 for biophotonics microscopy -

NSF funded 9 Physics Frontier Centers with 2 [22%] in the biological physics area:

Center for the Physics of Living Cells – Univ of Illinois

Center for Theoretical Biological Physics - UC San Diego/Rice Univ.

JILA and Kavli also develop research on this area

Department of physics of Harvard ~25-27% of the faculty dedicated to biophysics

We need to catch the wave earlier

SURF WISDOM: Catch the wave before it breaks

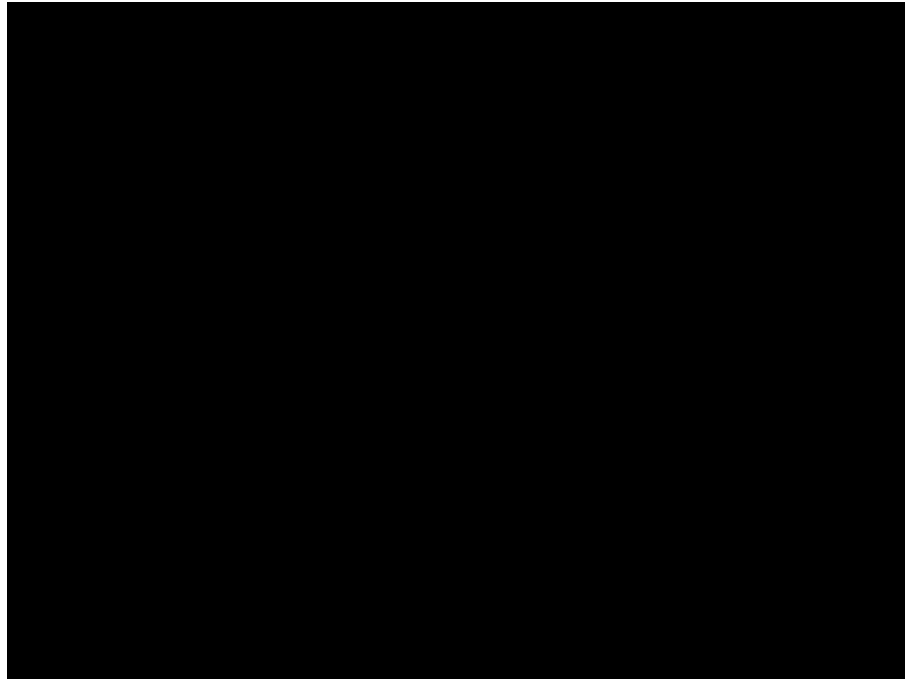


Does biology need physics?

- 1. Developing the tools to observe, manipulate and understand biology – down to molecular level. Photonics, x-rays, synchrotron, etc!**
- 2. To understand and manipulate biological molecular machinery – in singulo biochemistry – synthetic biology**
- 3. To understand biological interactions and control**
- 4. Complex systems of molecules, cells, organisms, social organizations of living beings**

Control of Biology at Sub/Celular Level

Synthetic Biology: If biology does it, we can make it too!!!

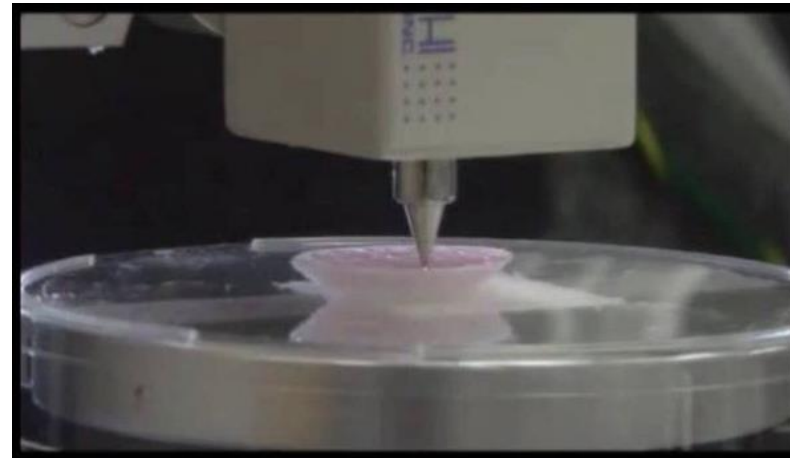


Animation produced by Harvard



Cultured Meat

<http://io9.com/5458425/is-vat+grown-meat-kosher-we-asked-a-rabbi?tag=cultured-meat>

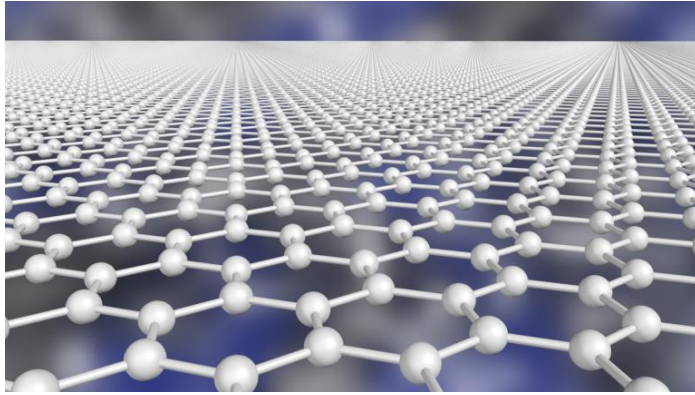


A. Atala
Wake
Forest
Institute

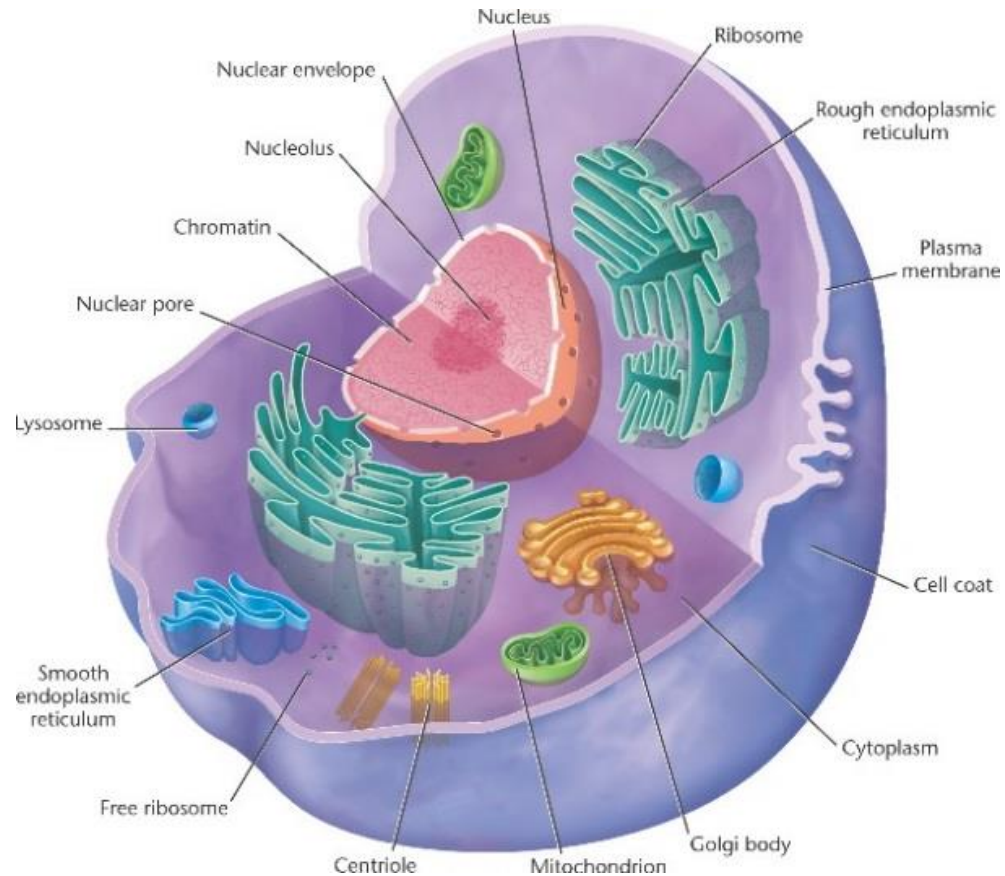


D. Taylor
U. of
Minnesota

Cells are heterogenous!



Physics + Chemistry: 10^{20} identical molecules:
Perfect statistics – strong accumulated signal
Averaging in space and time



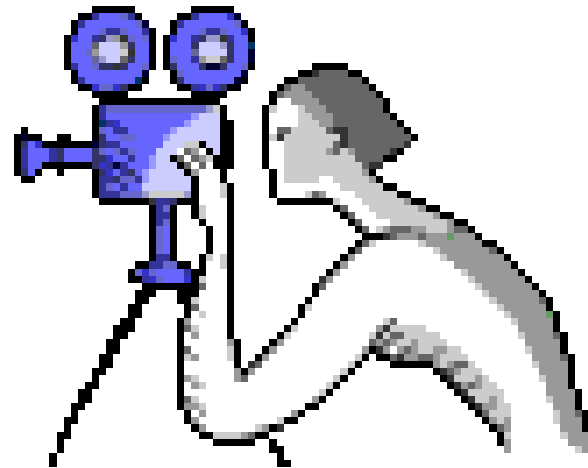
Cells are identical
But they are performing
different functions at
different times

PROCESS is a sequence of events in time.

Time evolution is crucial.

Tool needed: capable of real time observations.

No more pictures – we need movies!



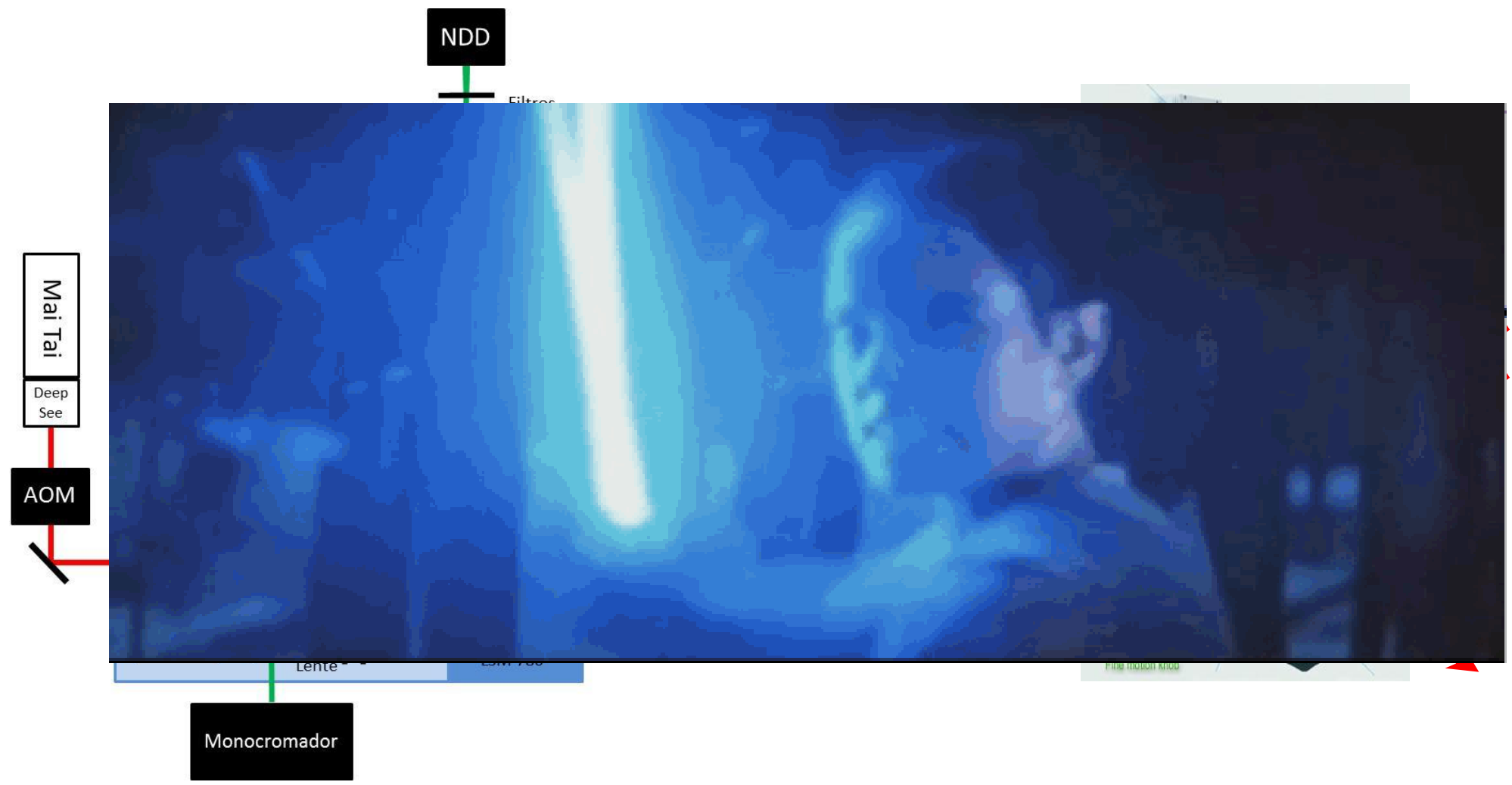
LABEL FREE

Non destructive – remote – capable to bring biochemical & biomechanical information – spatial resolution sub-cellular level [ideal molecular level] – 3D image reconstruction.

Questions to be answered: where, when and what happened

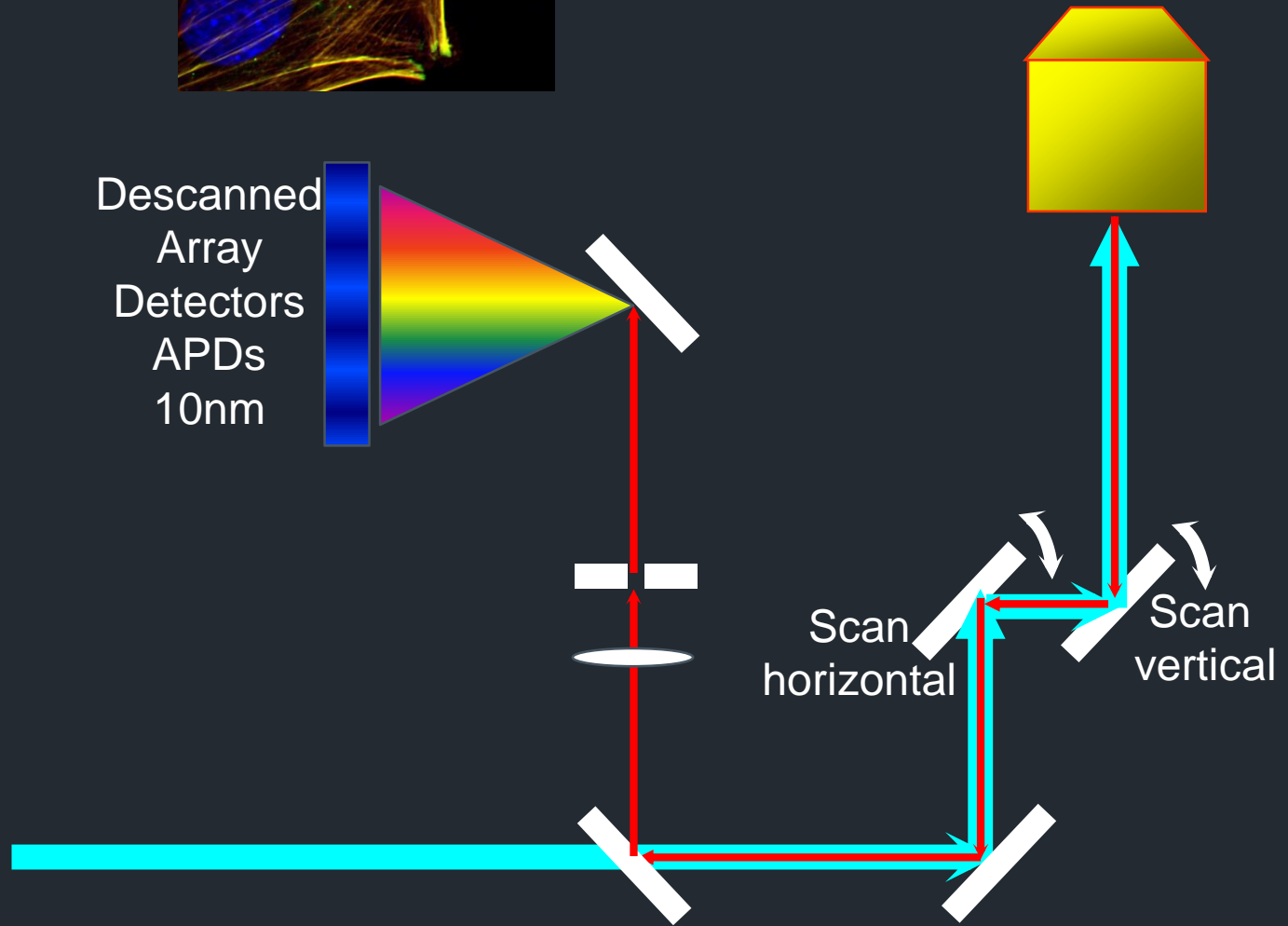
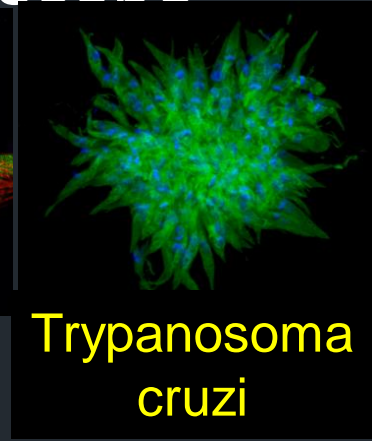
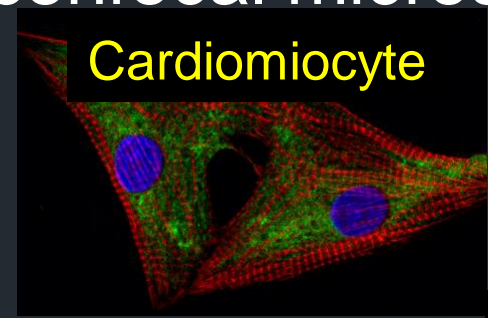
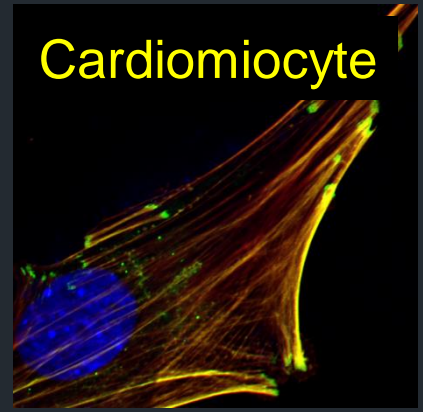
Resolved in time, space and spectrally

Multimodal Biophotonics Platform

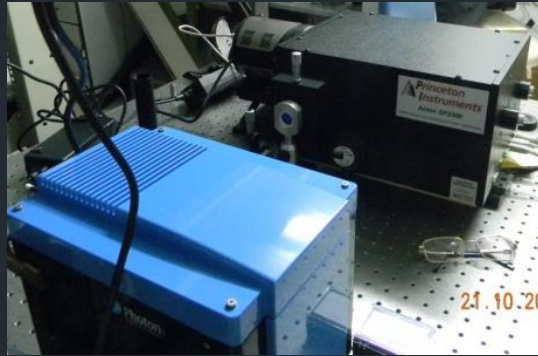


Integration: Step by step

Starting with a spectral confocal microscope



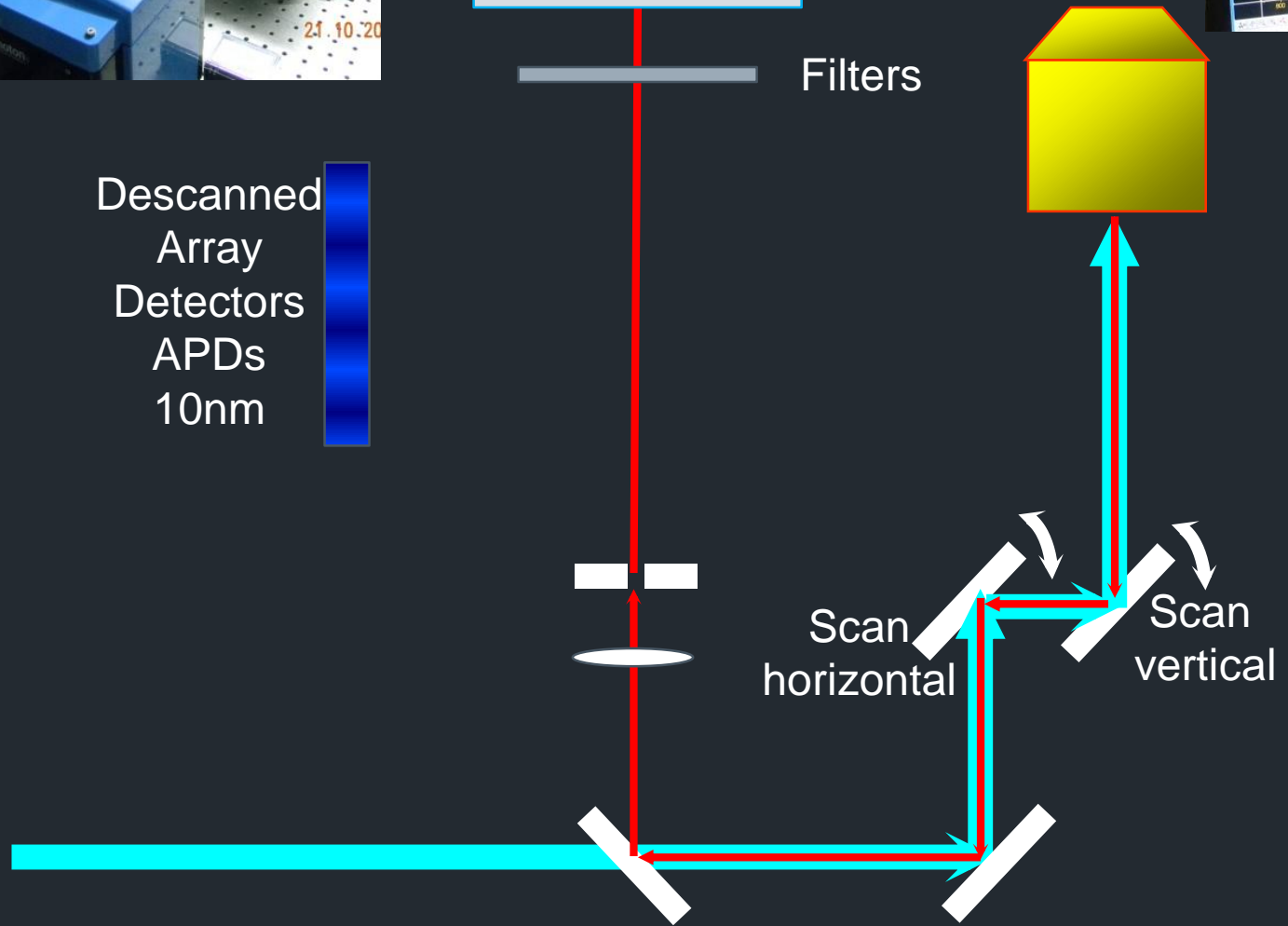
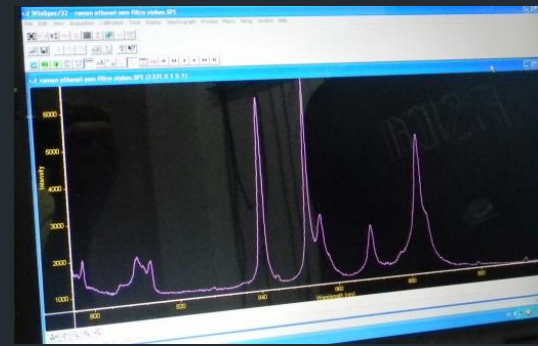
Starting with a spectral confocal microscope



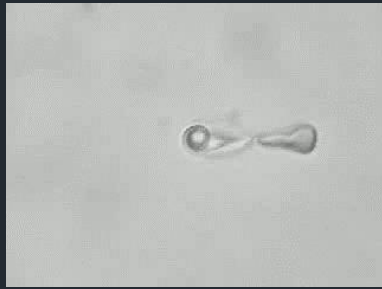
Descanned
Array
Detectors
APDs
10nm

Monocromator

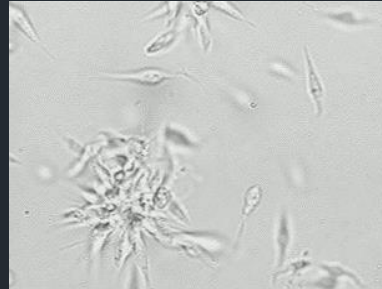
Filters



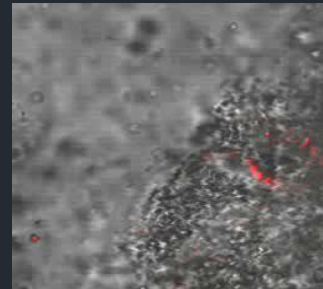
Make room to add an Optical Tweezers and laser cutting



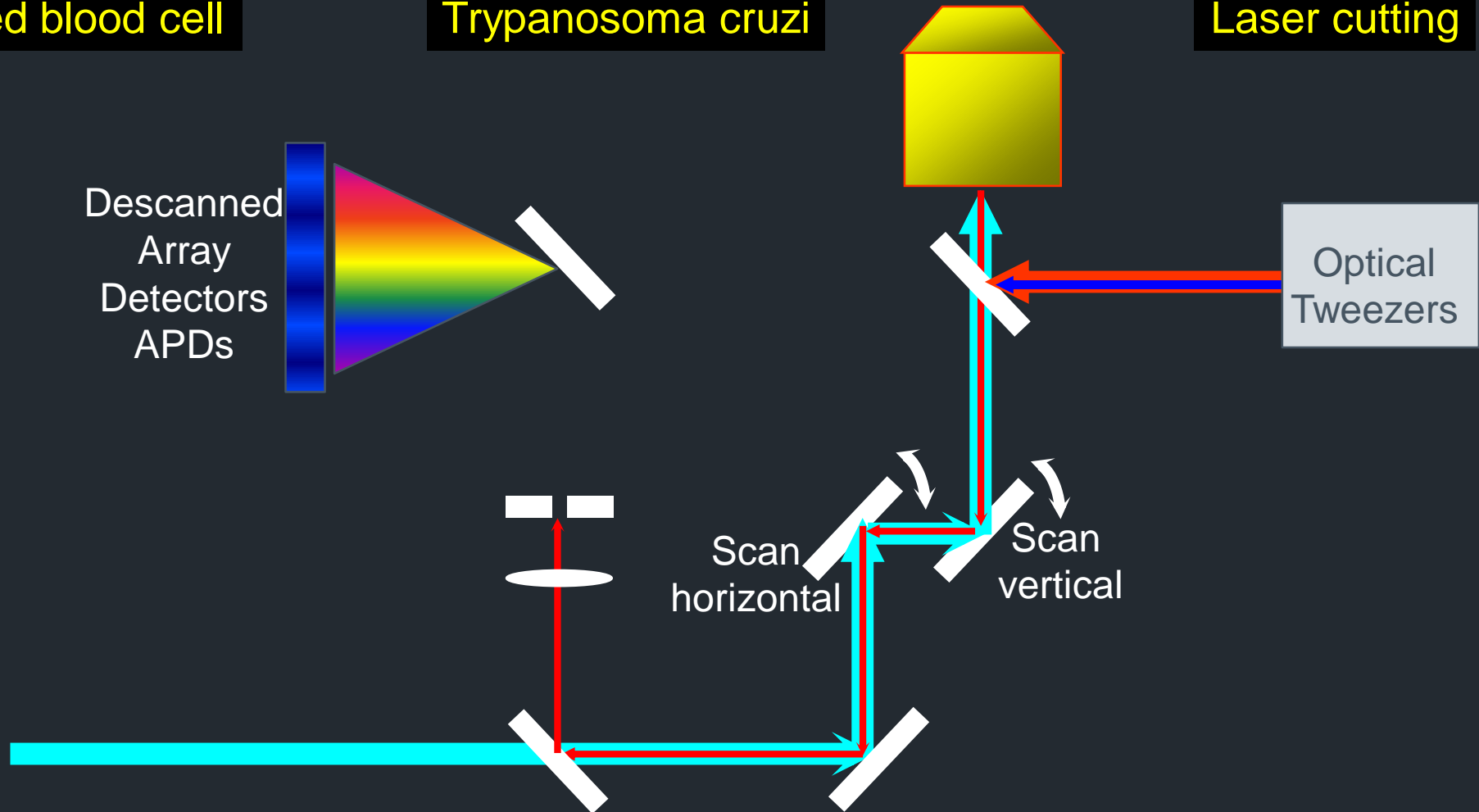
red blood cell



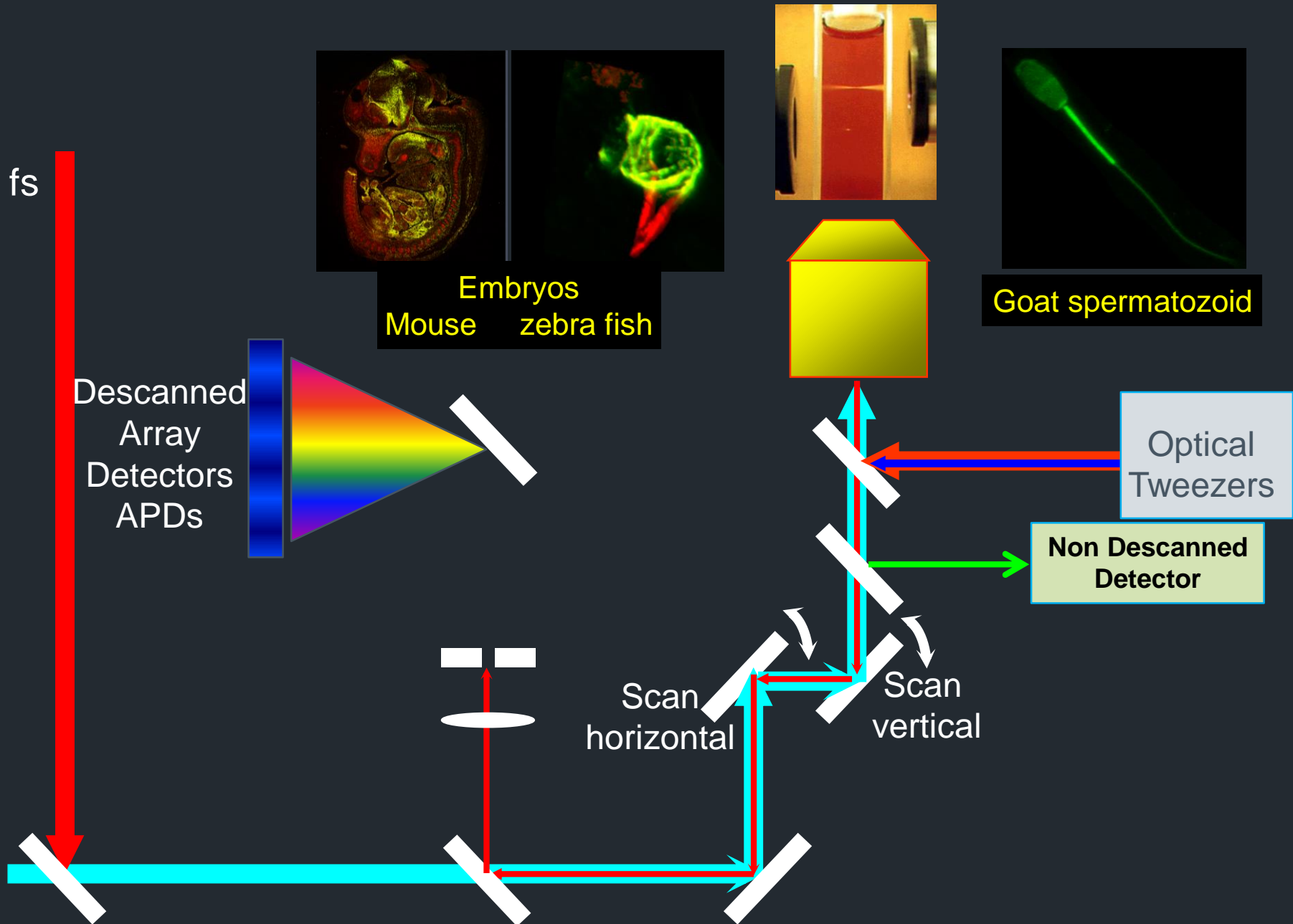
Trypanosoma cruzi



Laser cutting

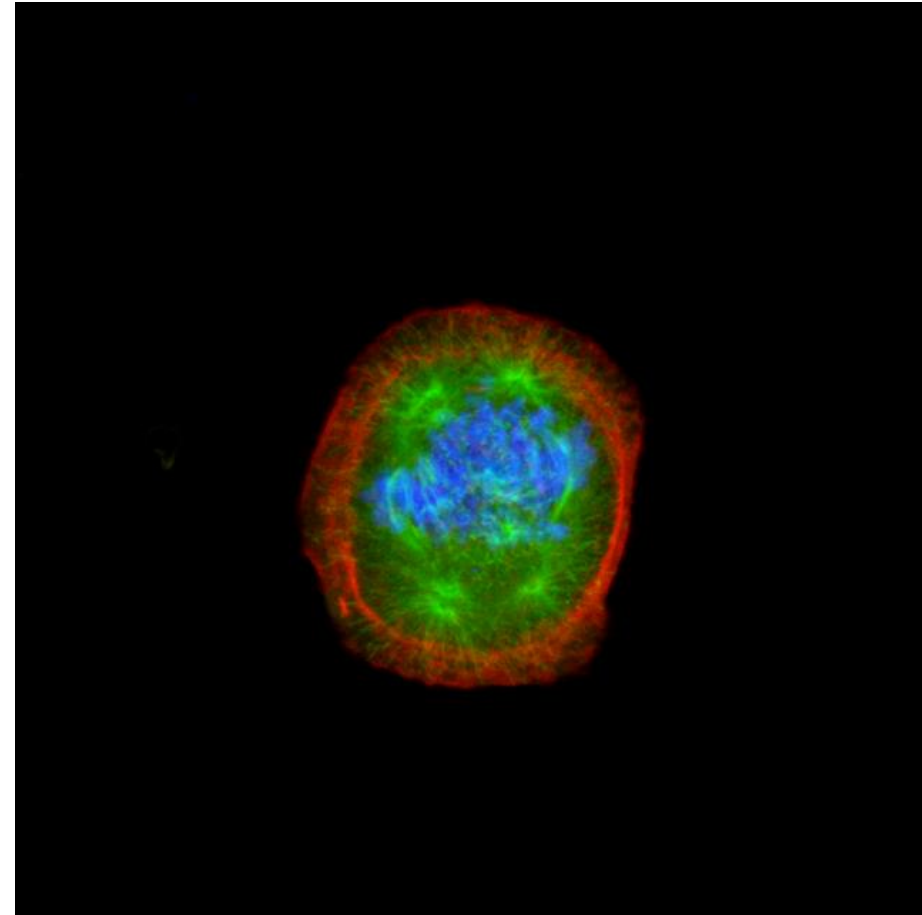
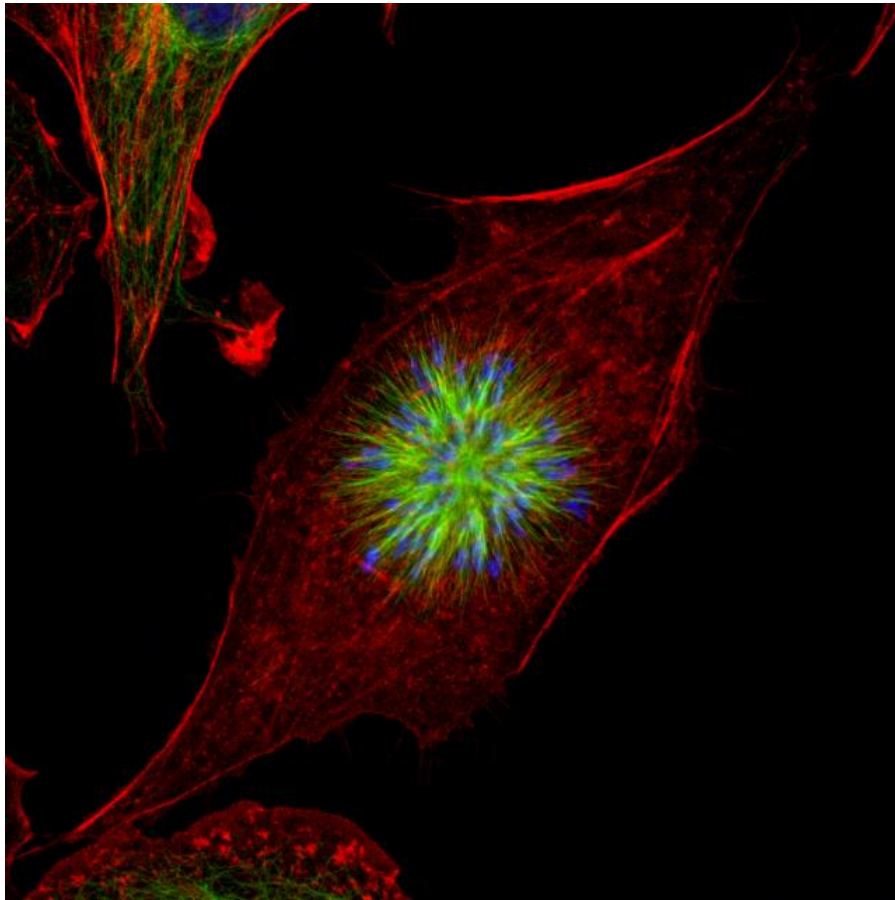


Add a femtosecond laser: multiphoton microscopy

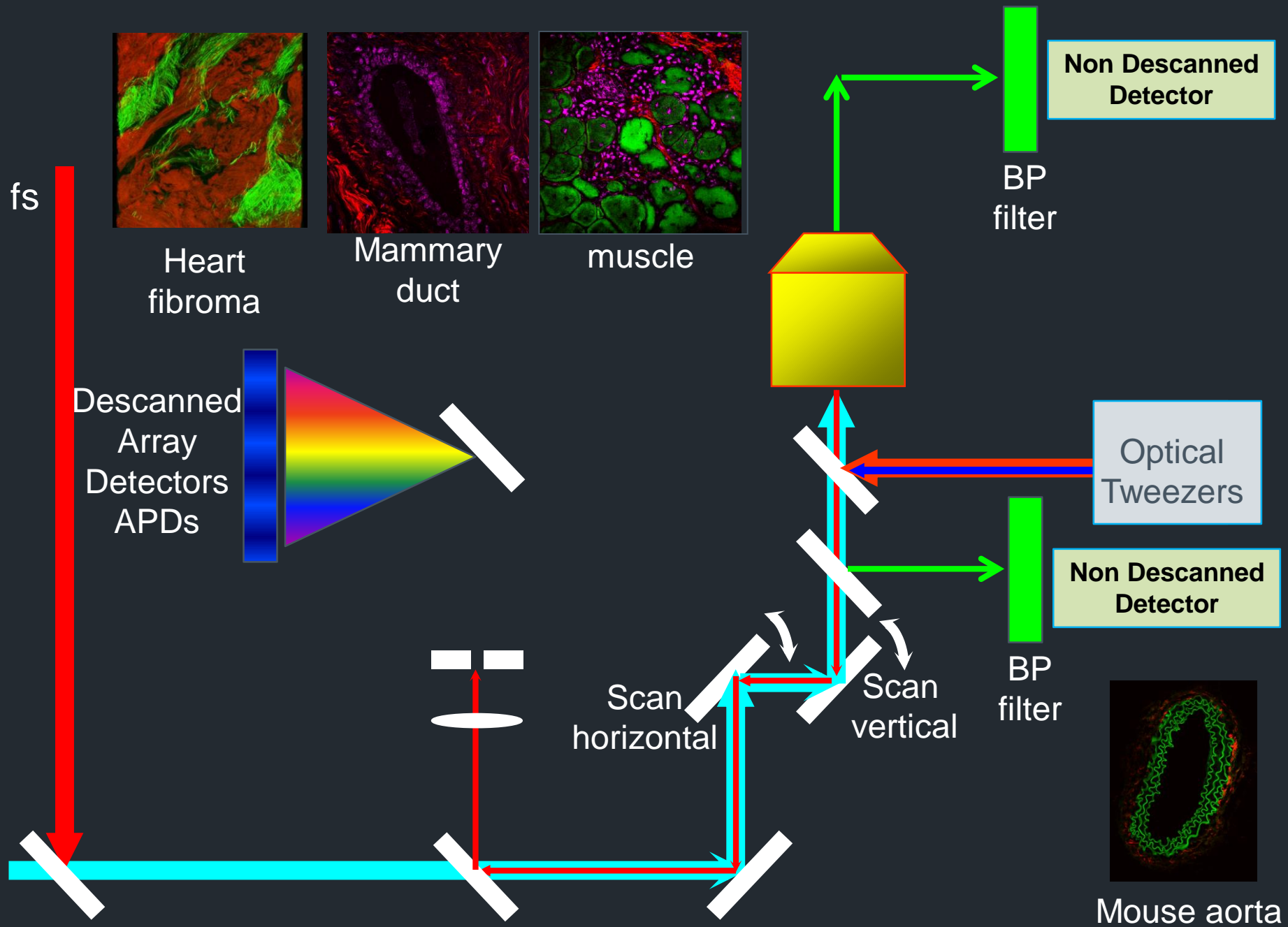


Cellular Division

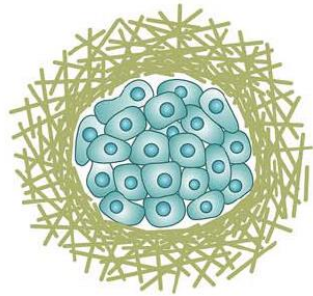
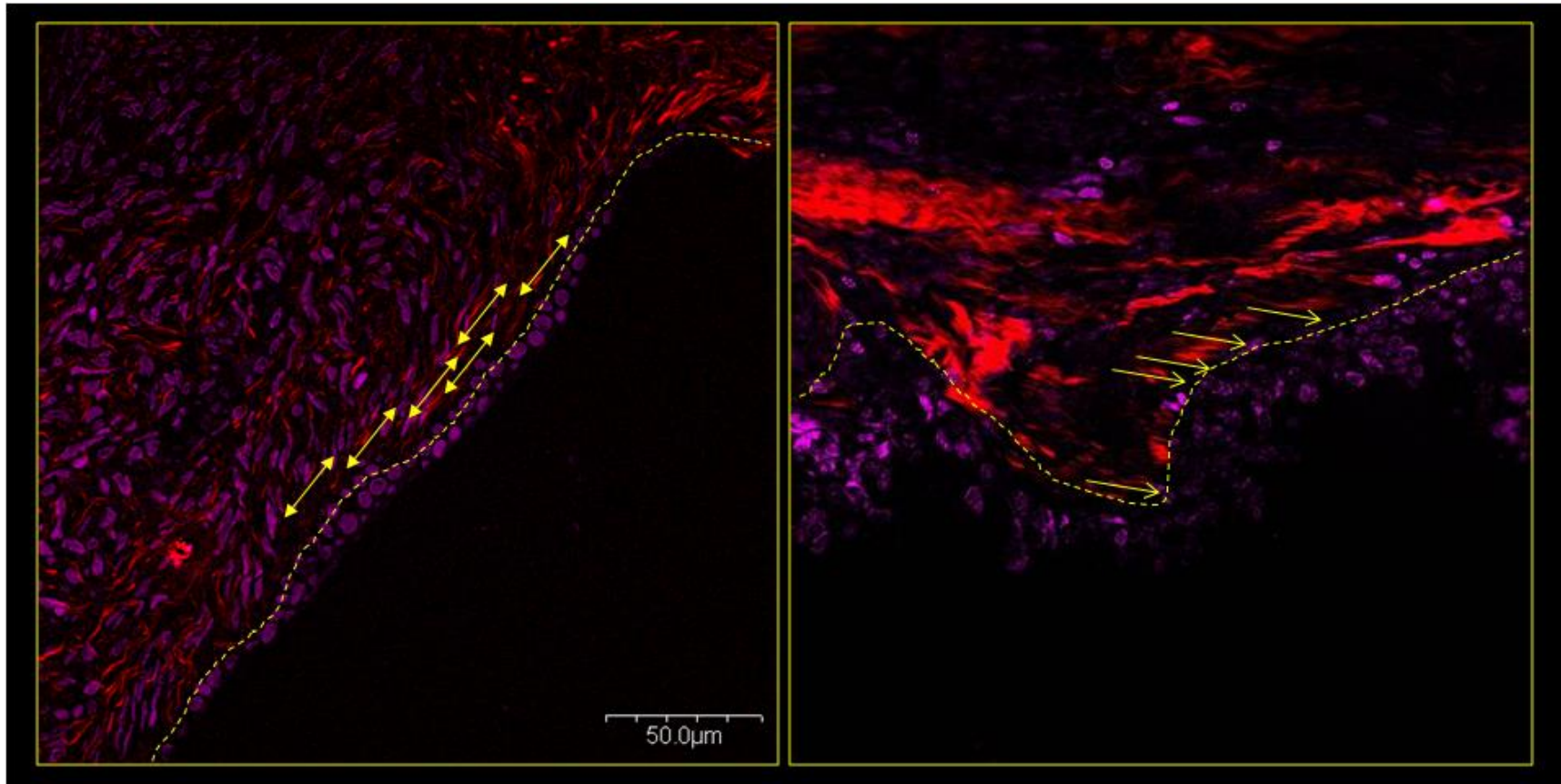
Actin, Tubulin and Nucleus



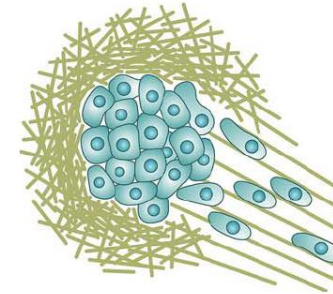
Second/Third Harmonic Generation comes for free



SHG + THG Ovarian Comparison normal vs adenocarcinoma



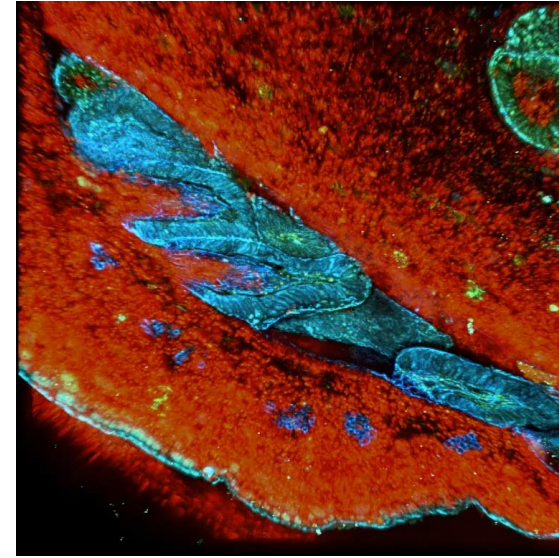
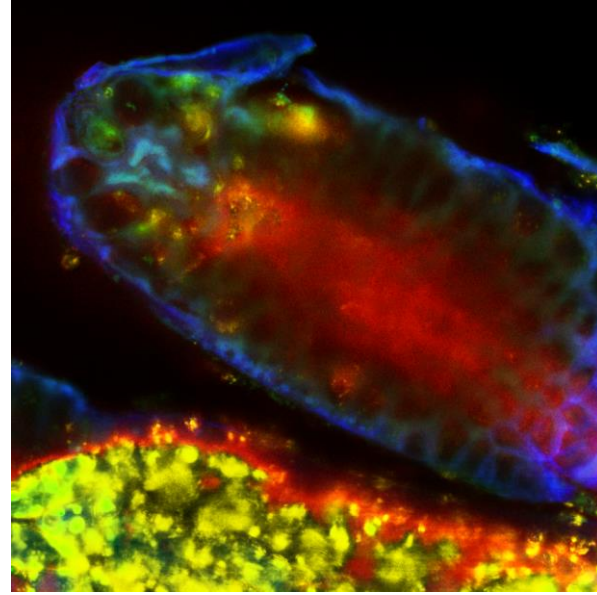
**TACS-2, collagen
tangential fibers**



**TACS-3, radial
collagen fibers**

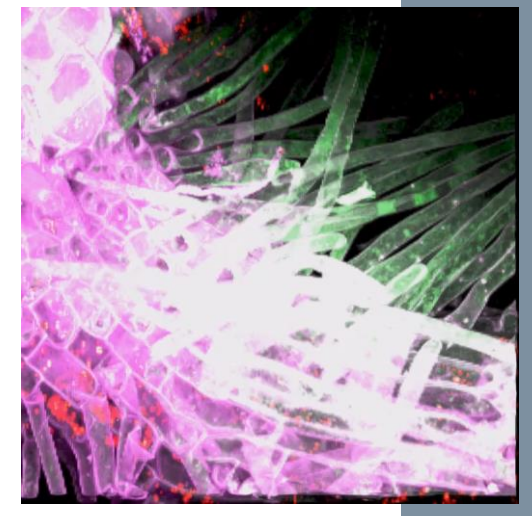
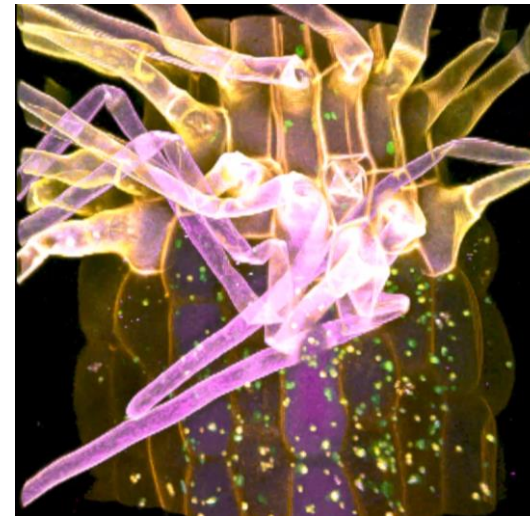
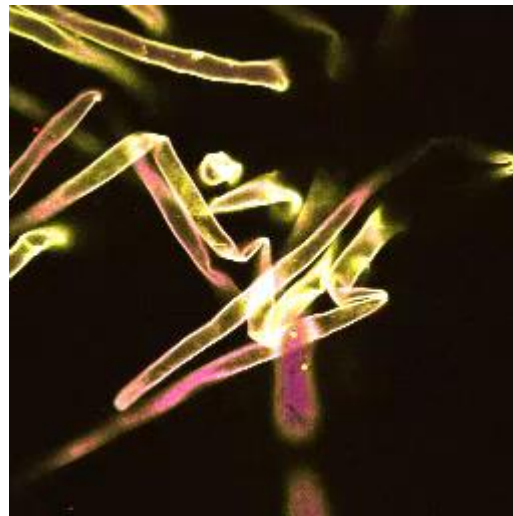
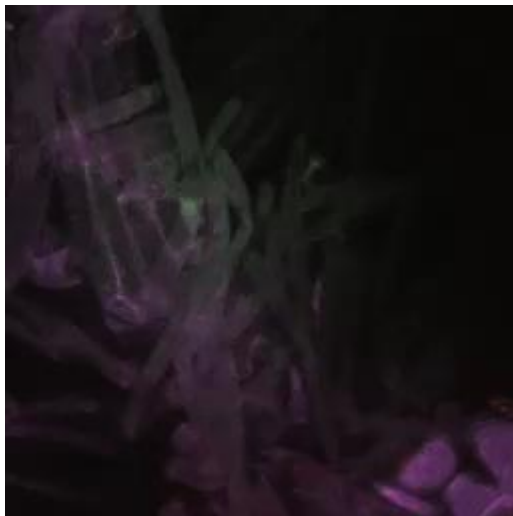
Plants: Coffee flower button Coleter TPEF + SHG

Mazzafera
Biol

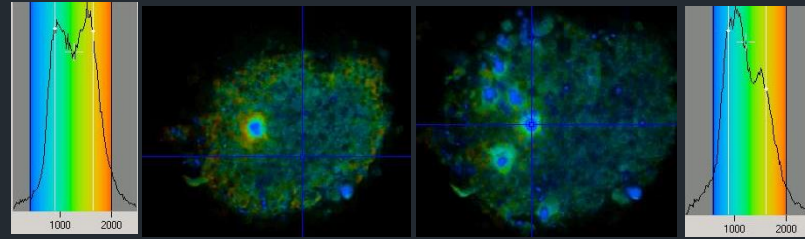
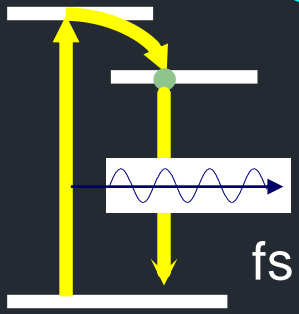


Arabidopsis root: TPEF + SHG

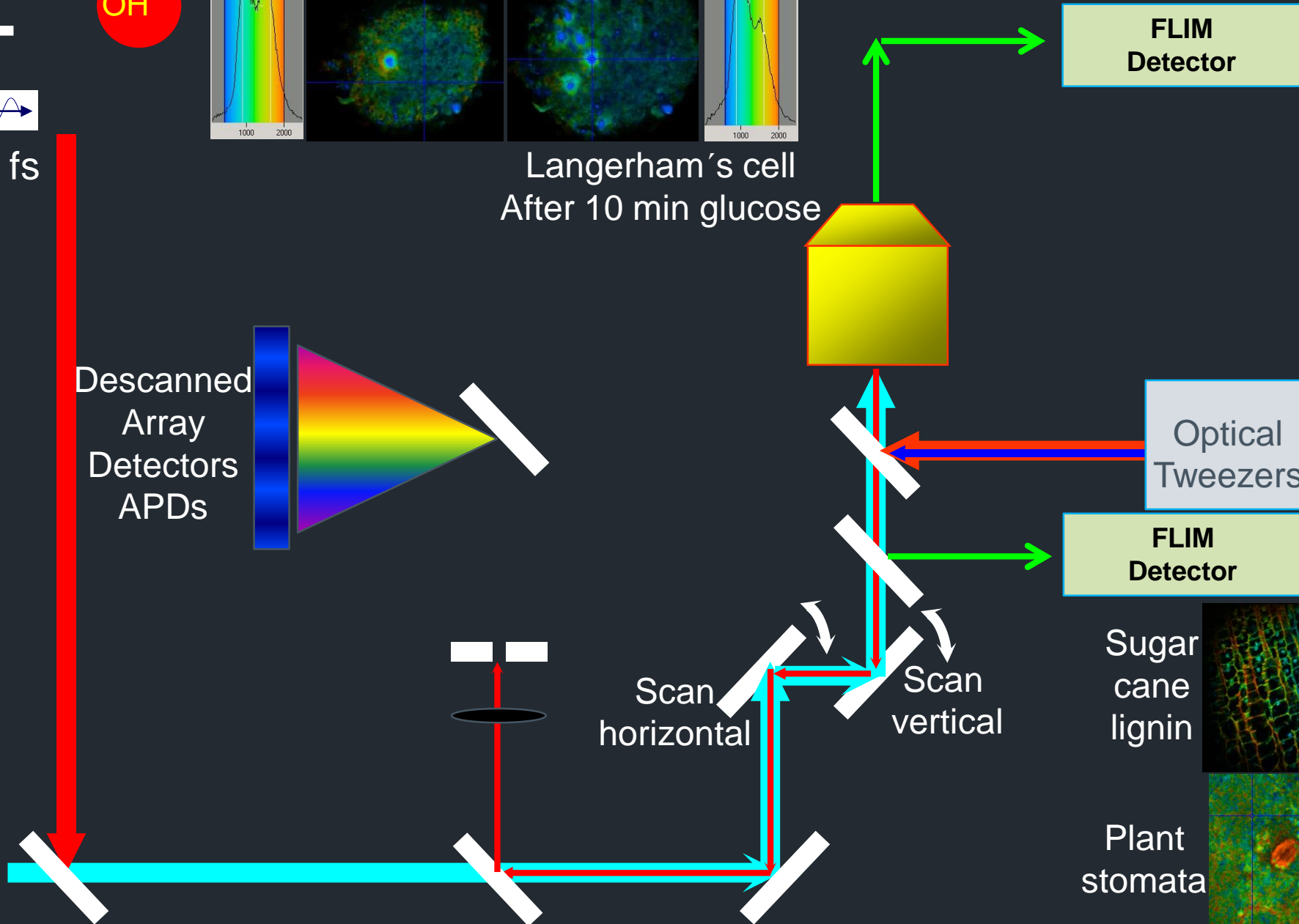
Salgado
Biol



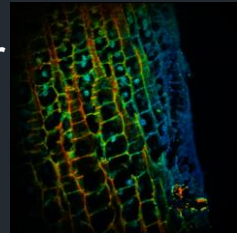
FLIM – fluorescence lifetime imaging



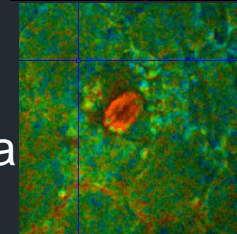
Langerham's cell
After 10 min glucose



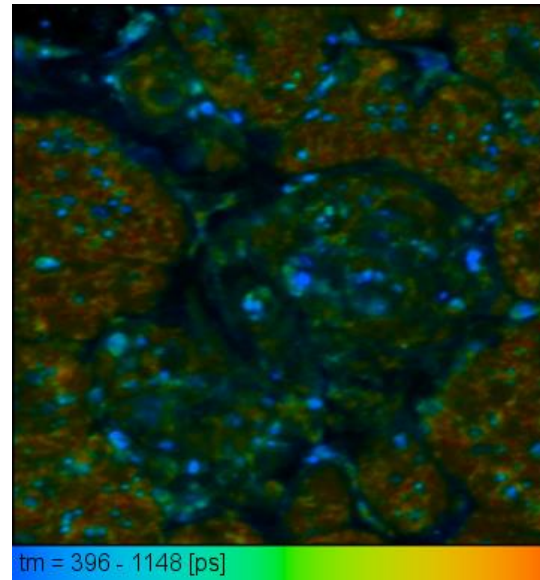
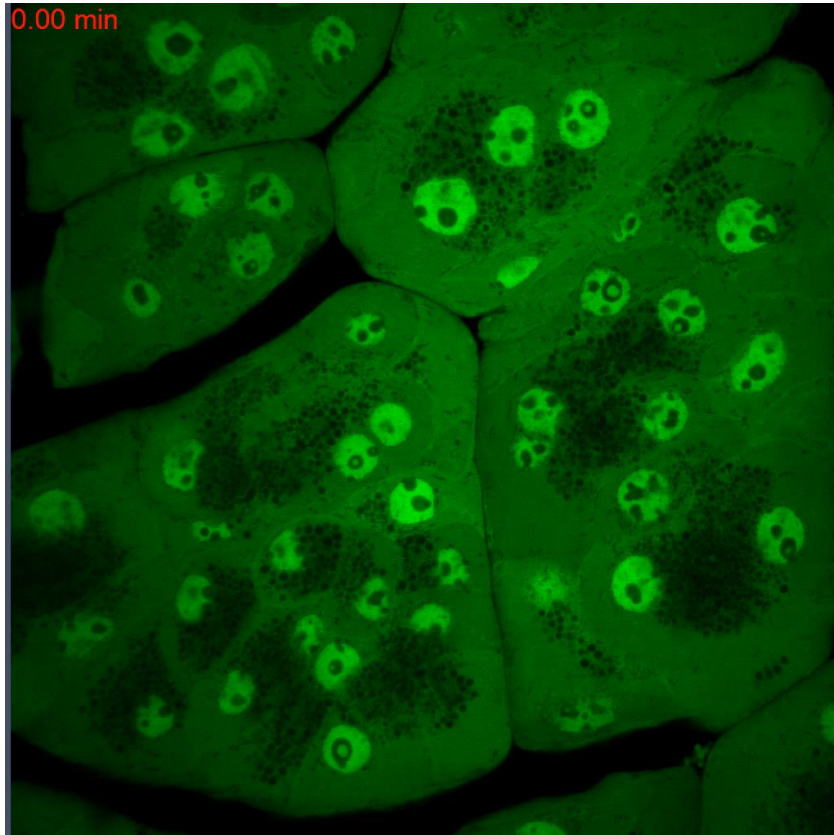
Sugar cane lignin



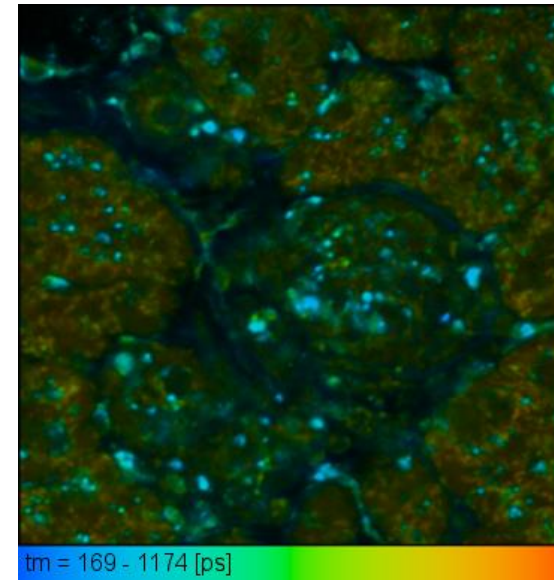
Plant stomata



Example 1: In vivo mice pancreas



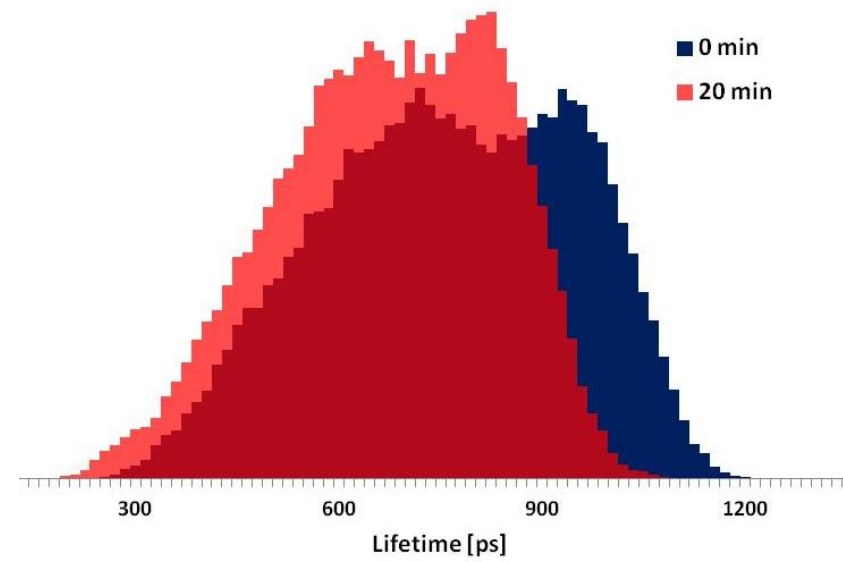
0 min



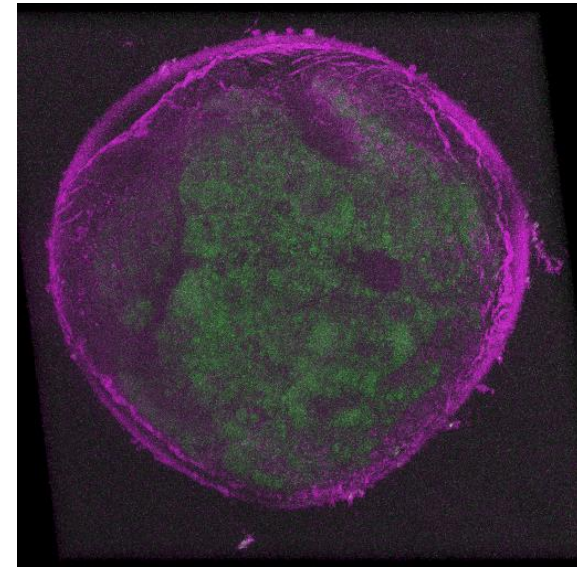
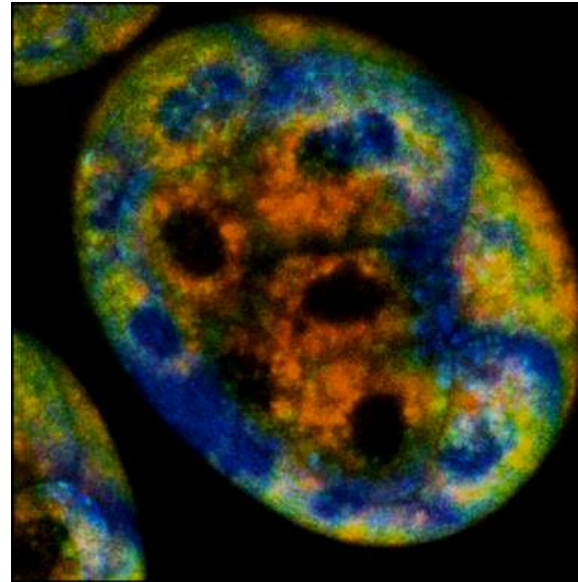
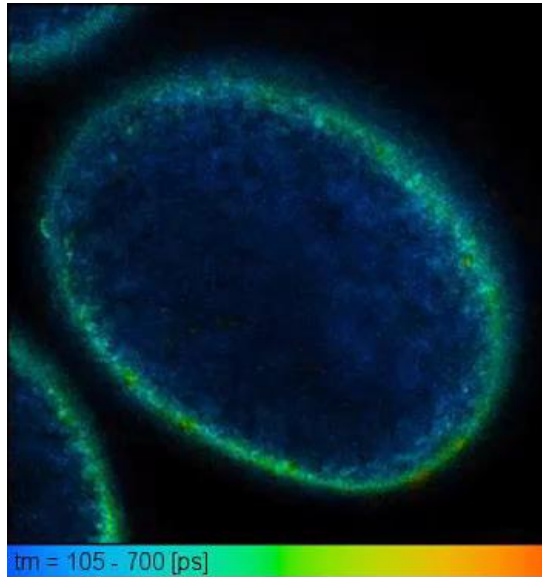
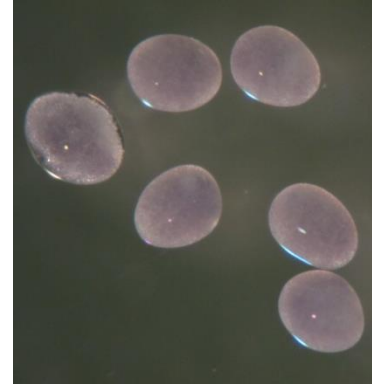
20 min

FLIM after glucose injection

Histogram of fluorescence lifetimes after glucose injection



Example 2: Parhyale hawaiiensis embryo development



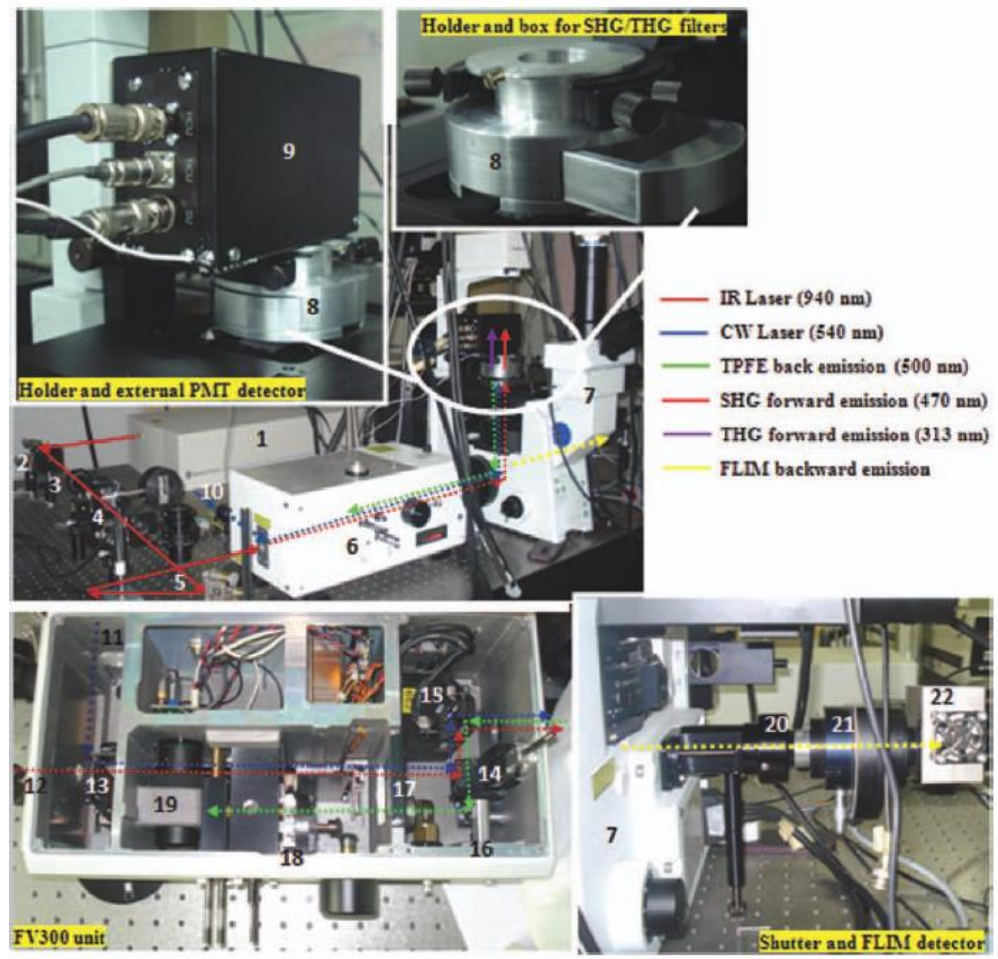
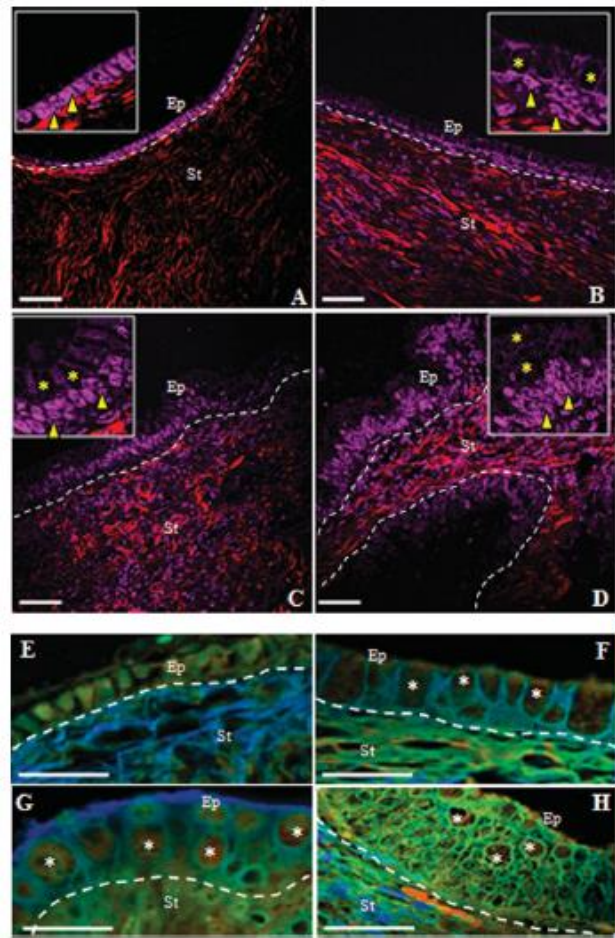
EMBRYO's Development

Strategical studies to understand stem cell differentiation

Harmonic Optical Microscopy and Fluorescence Lifetime Imaging Platform for Multimodal Imaging

VITOR B. PELEGATI,^{1,2} JAVIER ADUR,^{1,2*} ANDRÉ A. DE THOMAZ,¹ DIOGO B. ALMEIDA,¹
 MARIANA O. BARATTI,¹ LILIANA A. L. A. ANDRADE,³ FÁTIMA BOTTCHER-LUIZ,⁴
 AND CARLOS. L. CESAR¹

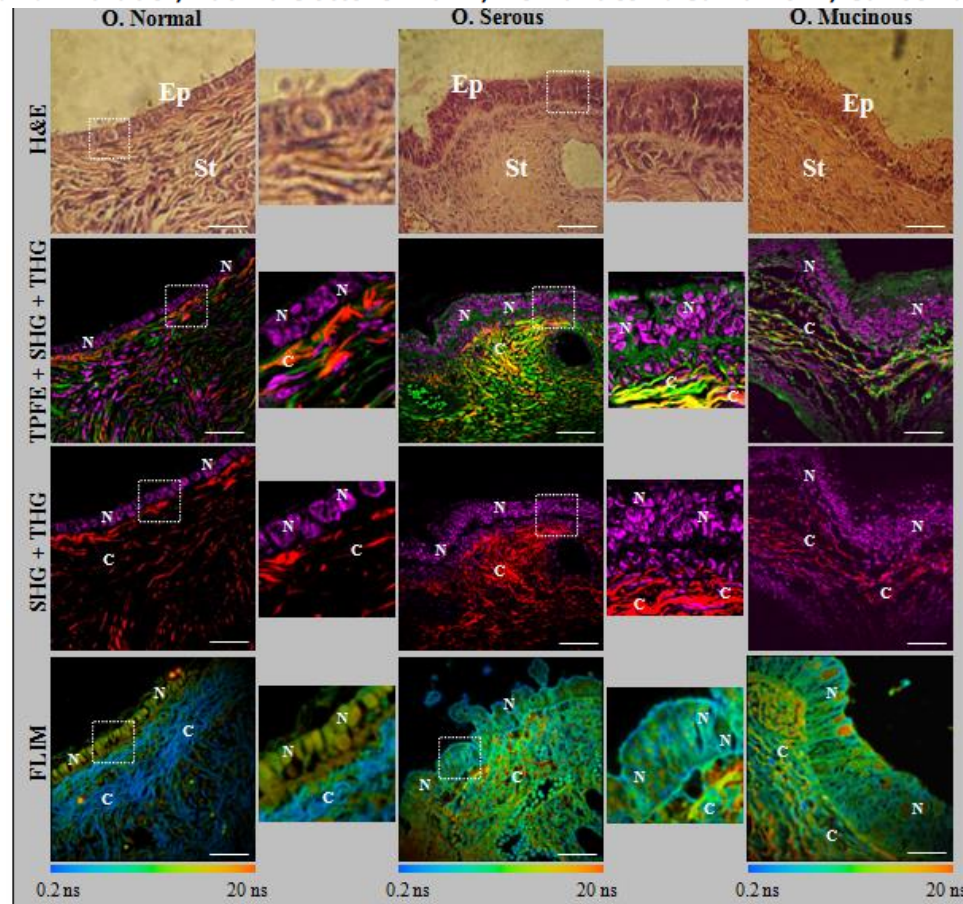
MICROSCOPY RESEARCH AND TECHNIQUE 75:1383–1394 (2012)



Several tools together

Optical Biomarkers of Serous and Mucinous Human Ovarian Tumor Assessed with Nonlinear Optics Microscopies

Javier Adur^{1,2*}, Vitor B. Pelegati¹, Andre A. de Thomaz¹, Mariana O. Baratti⁶, Diogo B. Almeida¹, L. A. L. A. Andrade³, Fátima Bottcher-Luiz⁴, Hernandes F. Carvalho^{5,6}, Carlos L. Cesar^{1,6}



H&E stained

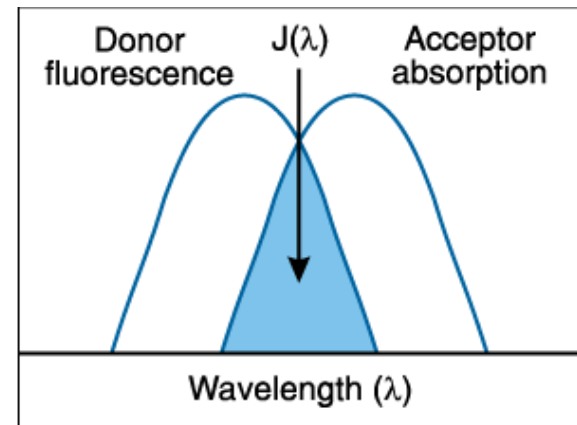
Two-photon
+SHG+THG
940 nm

FLIM
Non H&E
only parafin
890 nm

Förster Resonant Energy Transfer FRET

Use FLIM to measure distance donor-acceptor by **FRET**

FRET only happens if molecules are very close ~ 5-10 nm:
probing of intermolecular proximity



FRET: Förster Resonant Energy Transfer

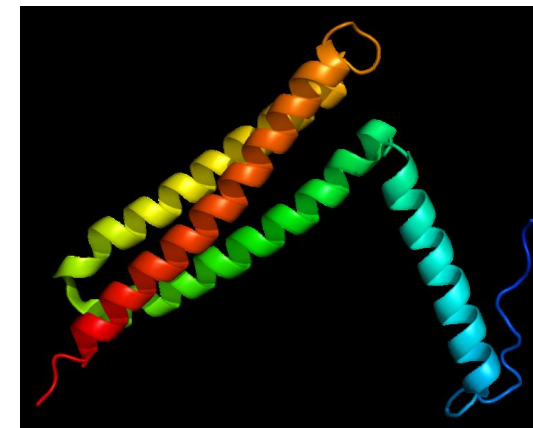


Nat. Comm. 5, art. 5159, october (2014)

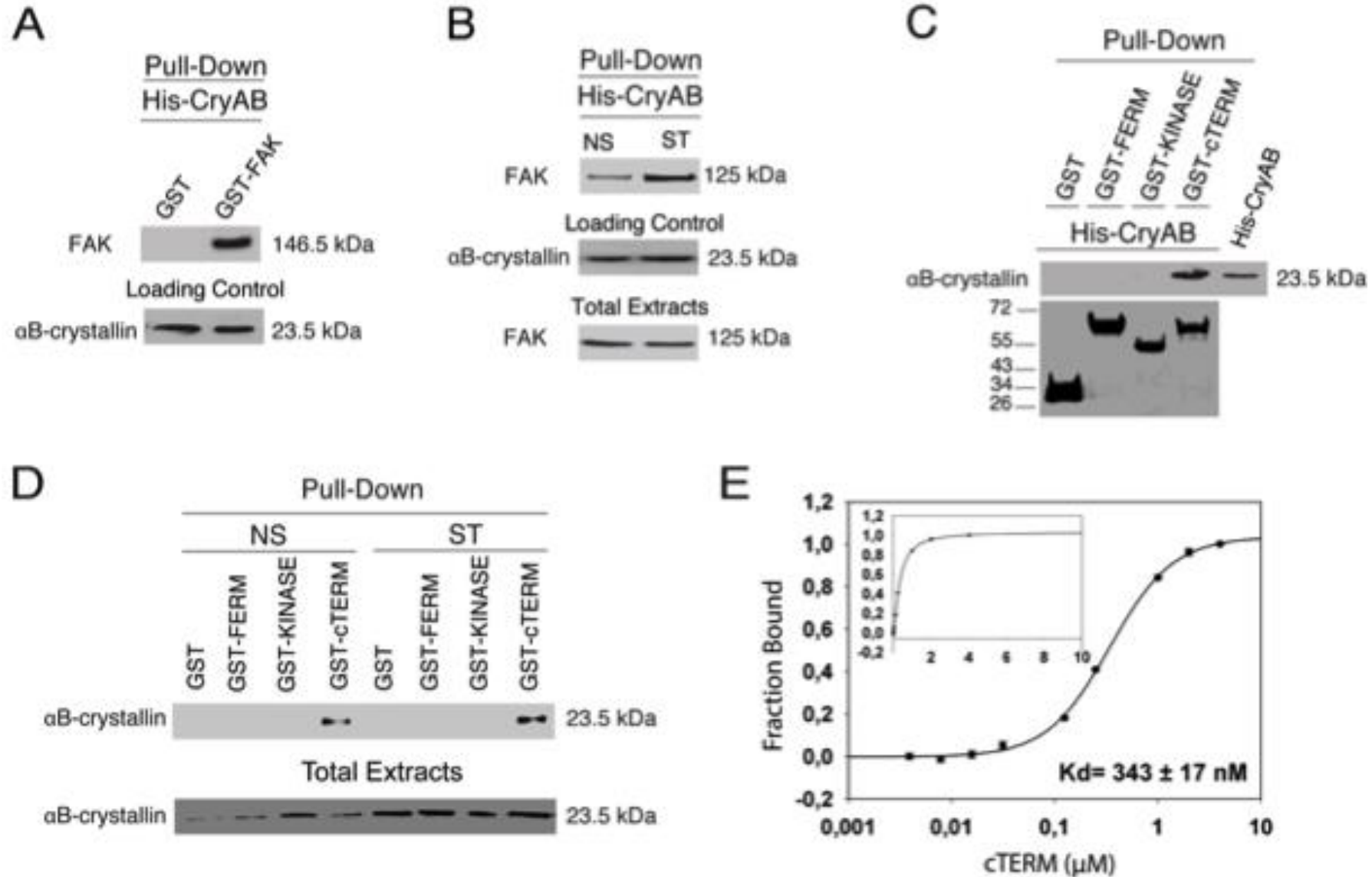
α B-crystallin interacts with and prevents stress-activated proteolysis of focal adhesion kinase by calpain in cardiomyocytes

Michelle B.M. Pereira^{1,*}, Aline M. Santos^{1,*}, Danieli C. Gonçalves¹, Alisson C. Cardoso¹, Silvio R. Consonni¹, Fabio C. Gozzo², Paulo S. Oliveira¹, Ana Helena M. Pereira¹, Alana R. Figueiredo², Ana O. Tiroli-Cepeda², Carlos H.I. Ramos², André A. de Thomaz³, Carlos L. Cesar³ & Kleber G. Franchini^{1,4}

Mechanical stress triggers interaction of α B-crystallin with FAK in cardiomyocytes

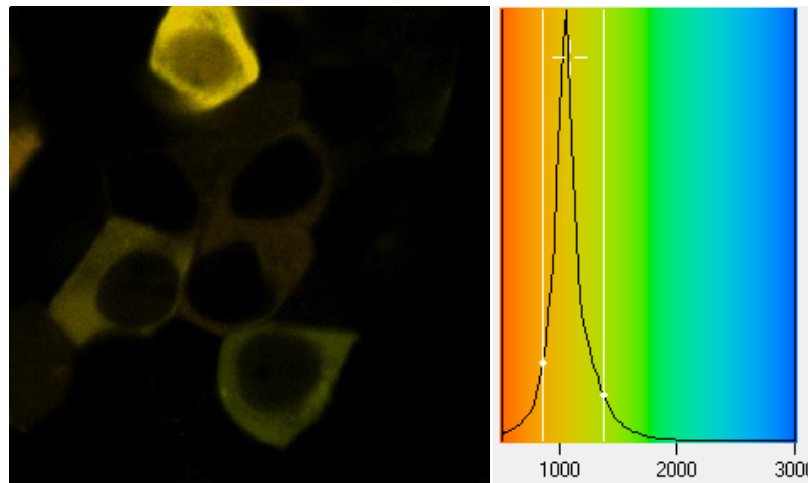
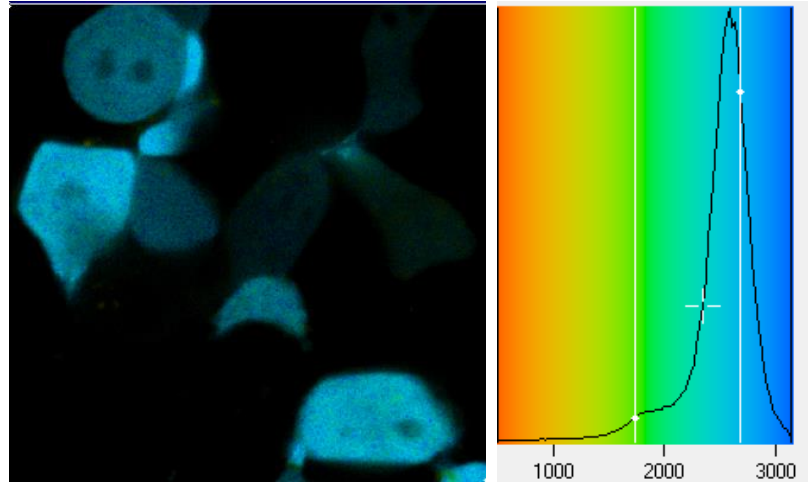


In Vitro Interaction is not enough



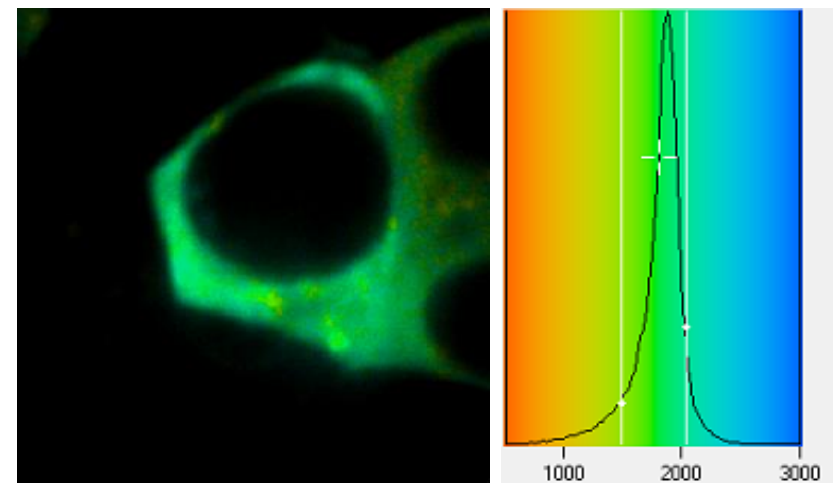
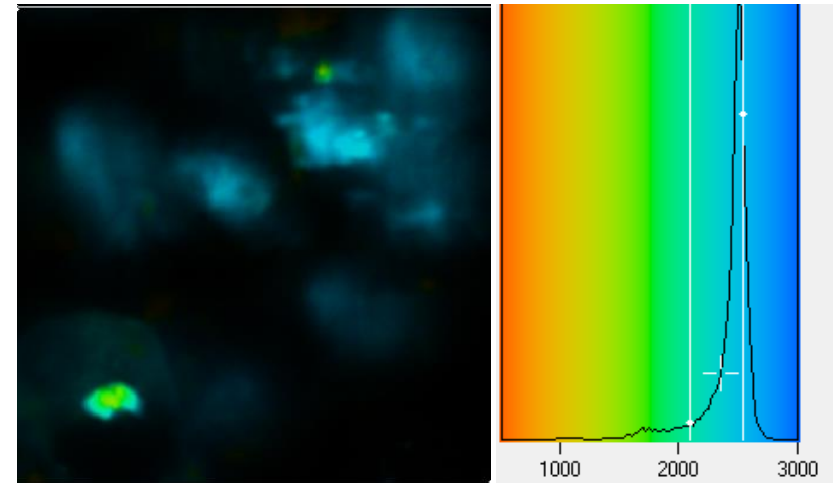
FRET

CFP + YFP:
Negative control



CFP - 15 AA - YFP

CFP-Cry-ab + YFP:
Negative Control



CFP-Cry-ab + YFP-FAK-CT

Integrated techniques into the same platform

3D + time-lapse capabilities

Single/multiphoton fluorescence: intensity

spectral + FLIM + PLIM + FRET + F...

SHG + THG

Raman

Optical Tweezers + laser cutting

Physiological controlled cell – temperature +

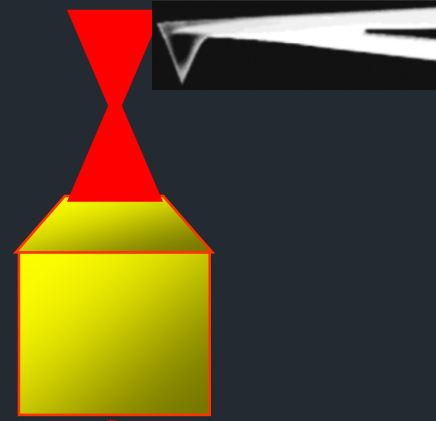
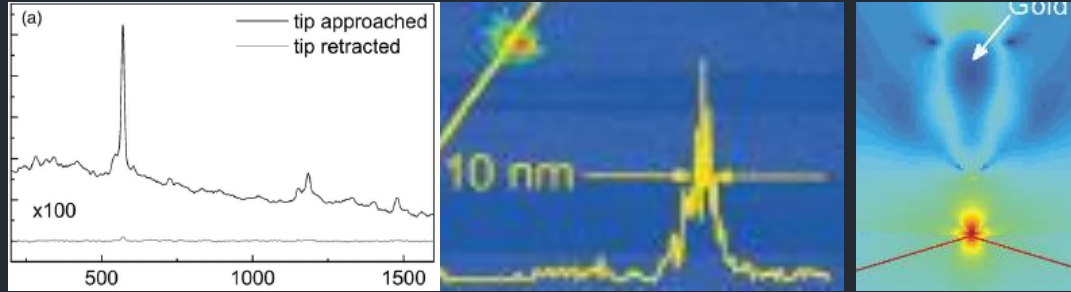
atmosphere

NEAR-FIELD

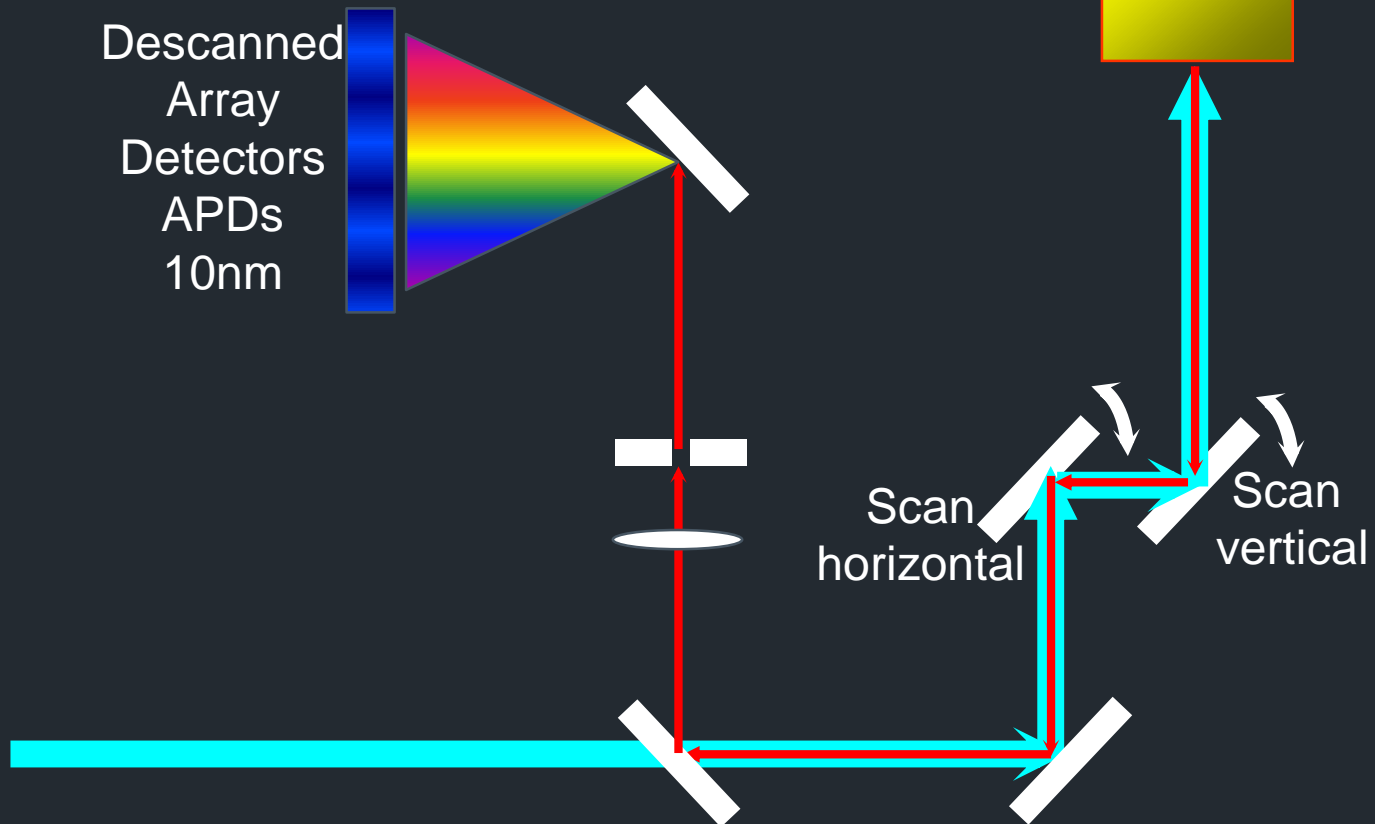
Super resolution

Tip-enhancement/AFM

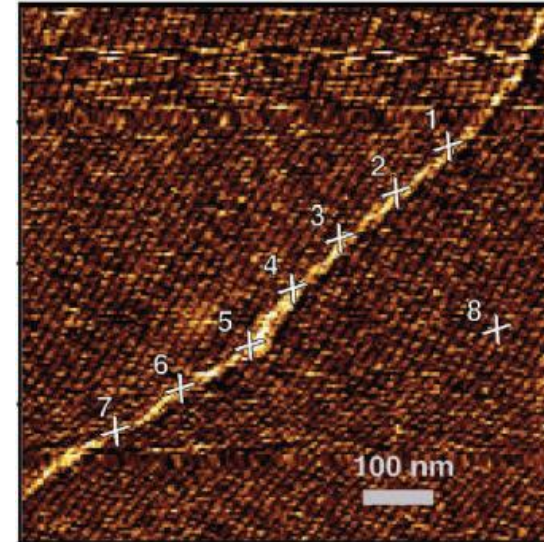
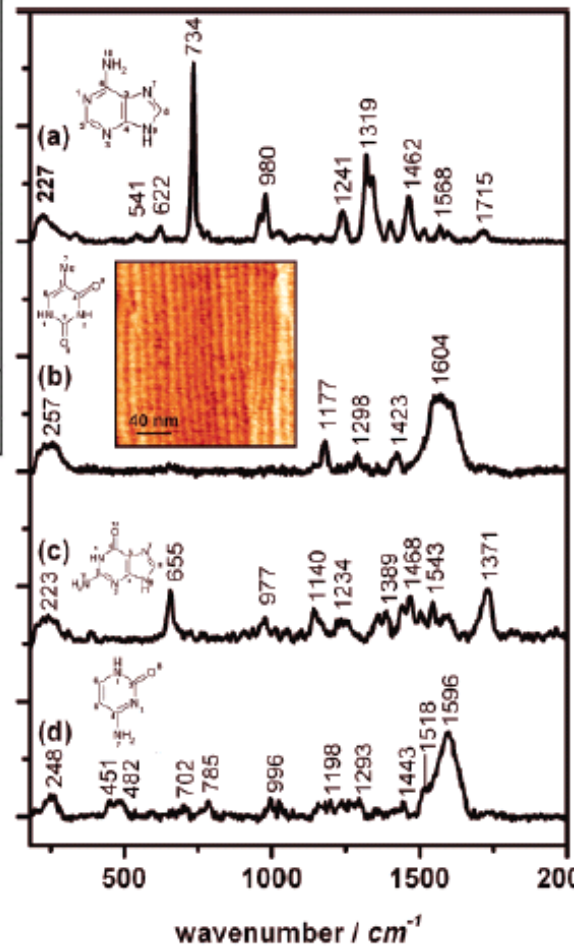
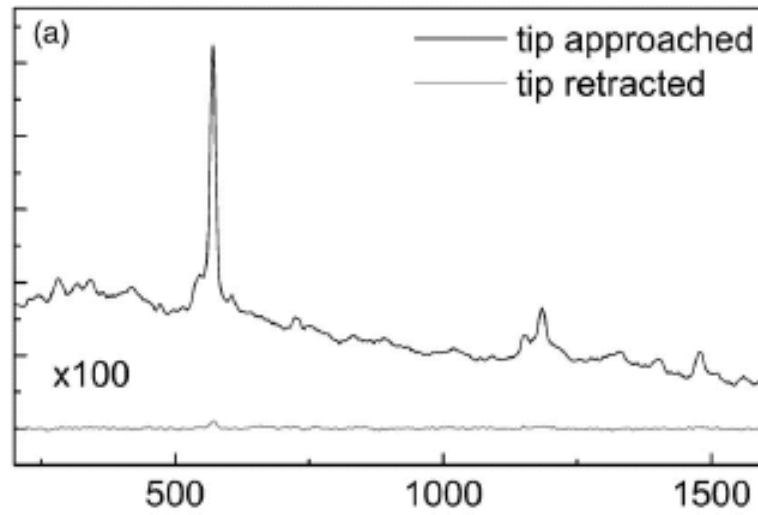
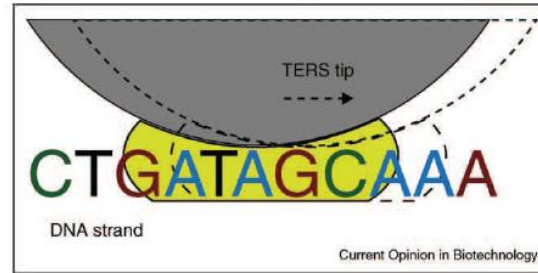
Add an AFM/Tip-enhancement system on top



Descanned
Array
Detectors
APDs
10nm



Tip-Enhanced microscopy & spectroscopy



TERS

INFABIC – Photonics in Cell Biology

NOSSA MISSÃO
Desenvolver e disponibilizar técnicas de última geração baseadas em óptica não linear para o estudo de materiais biológicos.

INFABIC

Home | Conheça o Instituto | Publicações | Pesquisas | Transferência de Conhecimento | Reserva de Equipamentos | Contato | Nomes Gerais INF-ABC | Imagens e Vídeos | Nova Proposta INCT - INF-ABC

ÚLTIMAS NOTÍCIAS
Acessar aqui o 4º Workshop INF-ABC, certificado, aulas, práticas e minicursos gratuitos. Para poder acessar todas as fotos do evento [clique aqui](#)
[saiba mais](#)

AGÊNCIAS FINANCIADORAS
FAPESP
CNPq
[saiba mais](#)

AGENDAR EQUIPAMENTOS
Microscópio Invertido LSM780, Microscópio Upright LSM780, Microscópio Spinning Disk Confocal, Microscópio PALM e AFM - JPK (sem microscópio)
[veja nossa agenda](#)

Galeria de Fotos
Galeria de Vídeos

	dom 16/11	lun 17/11	mar 18/11	mié 19/11	jue 20/11	vie 21/11	sáb 22/11
09:00		09:00-09:00 Viviane Rodrigues - Progo Herque Marques - Alexandre Oliveira - IB/Unicamp	09:00-09:00 Rodrigo - Prof. Alexandre Oliveira - IB/Unicamp	09:00-09:00 Viviane - Prof. Henrique Marques - IB/Unicamp	09:00-09:00 Ana Paula Konrad - FCM/Unicamp	09:00 - 18:00 FERIADO	09:00 - 18:00 Expediente e Suspenso
10:00							
11:00							
12:00							
13:00							
14:00							
15:00							
16:00							
17:00							
18:00							

People

Physics UNICAMP:

Wendel Moreira

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Obrigado pela atenção!



Thanks for the attention
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