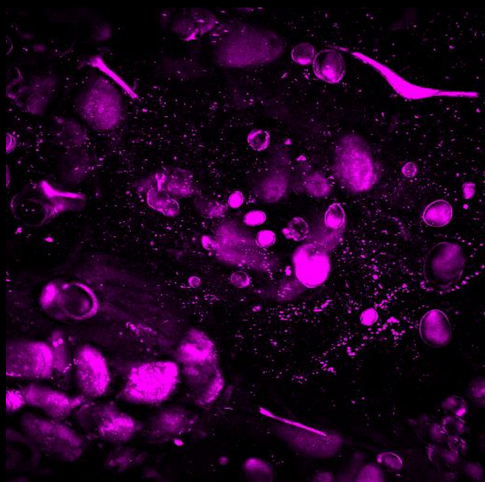
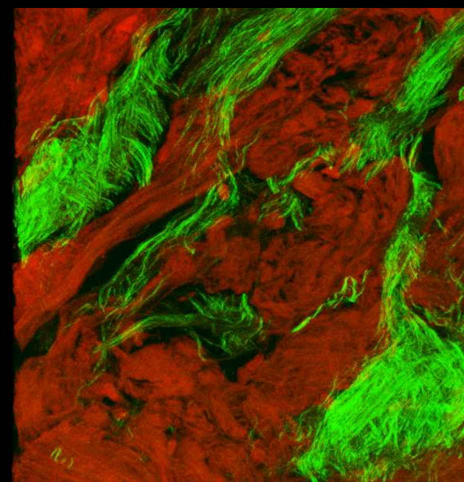
Biophotonics in the 6th scientific technological revolution

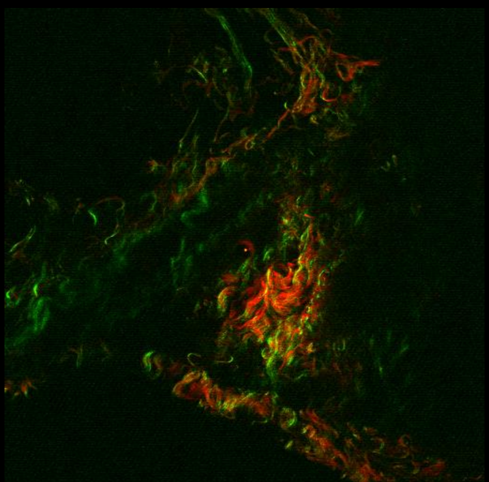
Carlos Lenz Cesar – Instituto de Física Gleb Wataghin - UNICAMP



THG potato

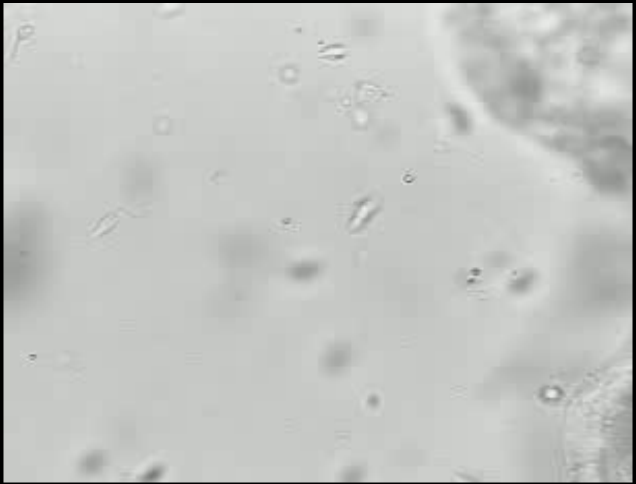


TPEF+SHG : heart fibroma



TPEF+SHG : Ovarium

spermatozoid



Trypanosoma Cruzi

Leishmania amozenensis

Midia´s future is always dark: if it bleeds, it leads

planet of the apes

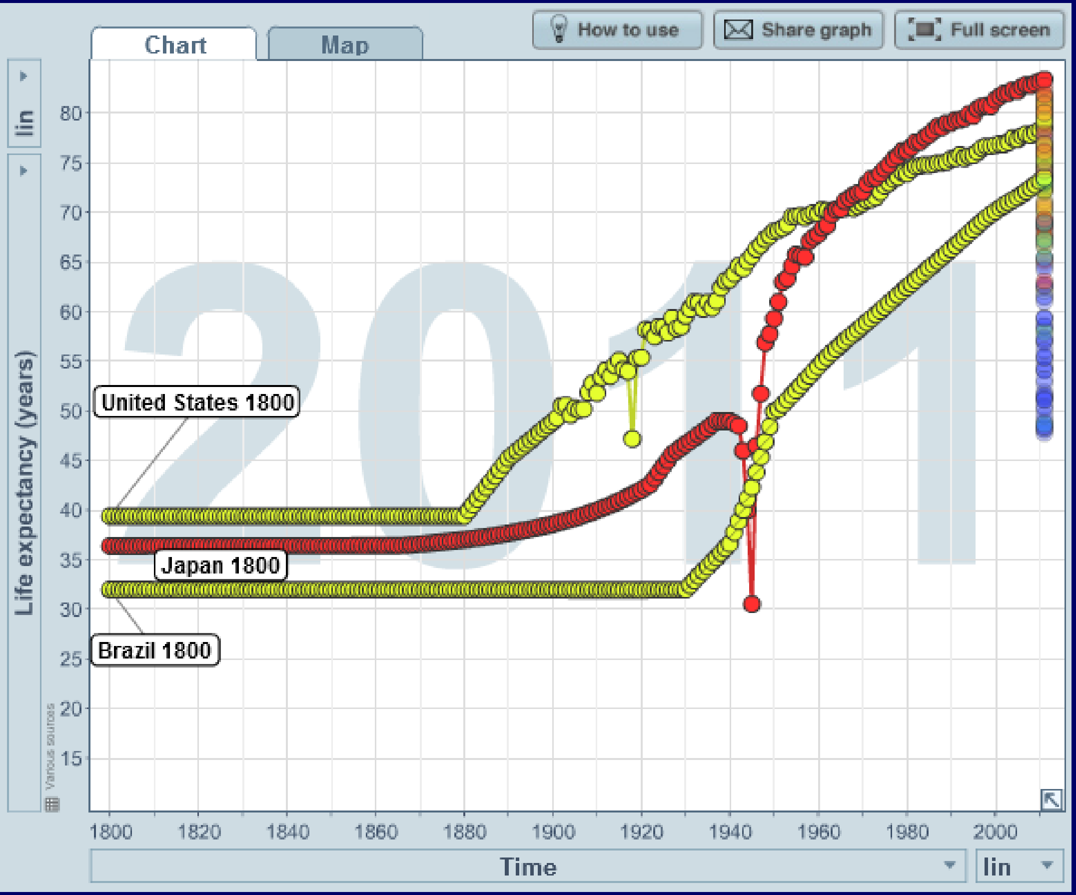
Mad Max

Water world

NY subway

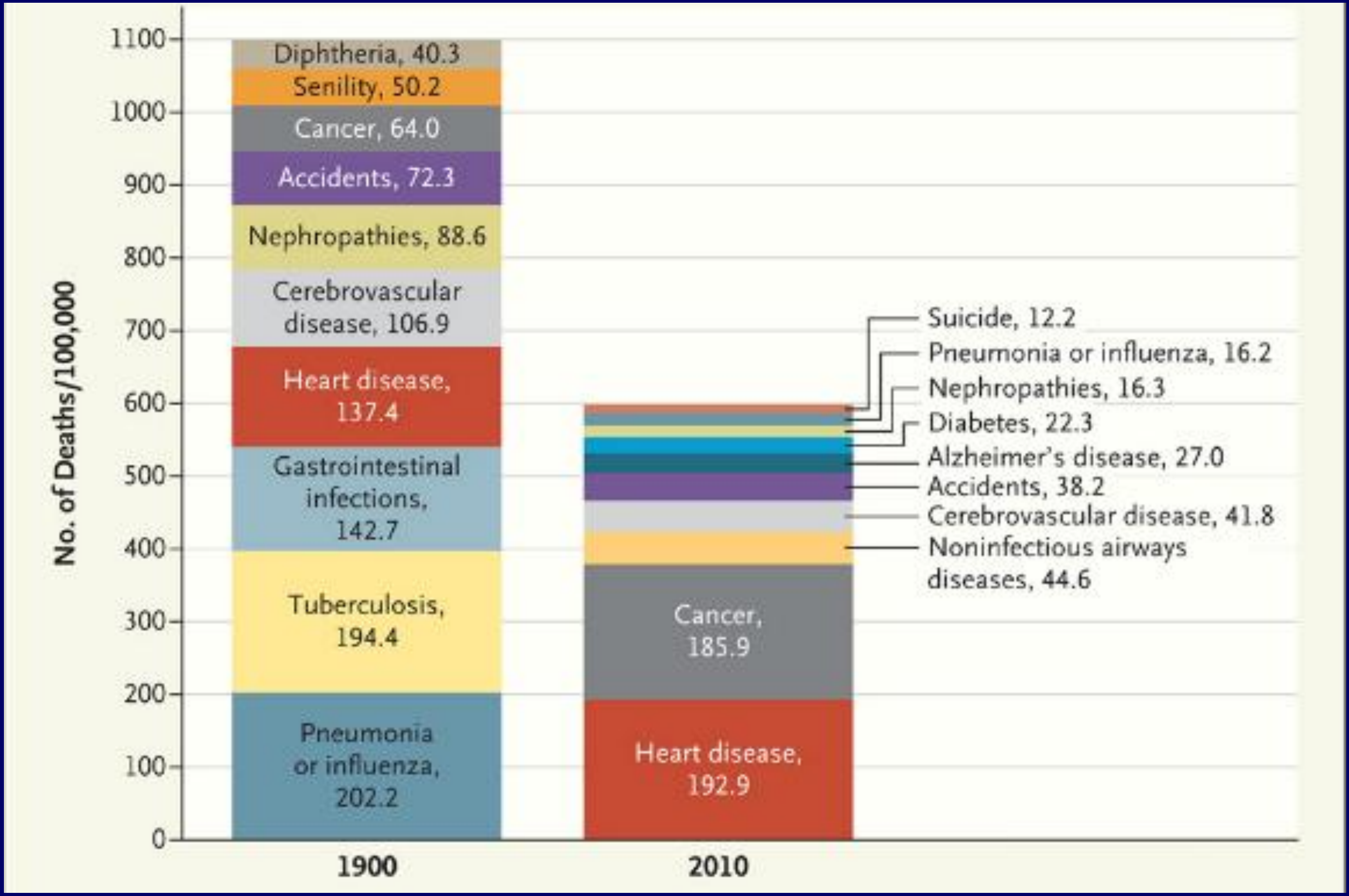
But the future can be the best ever seeing

Life expectancy from 1800 to 2010
www.gapminder.com



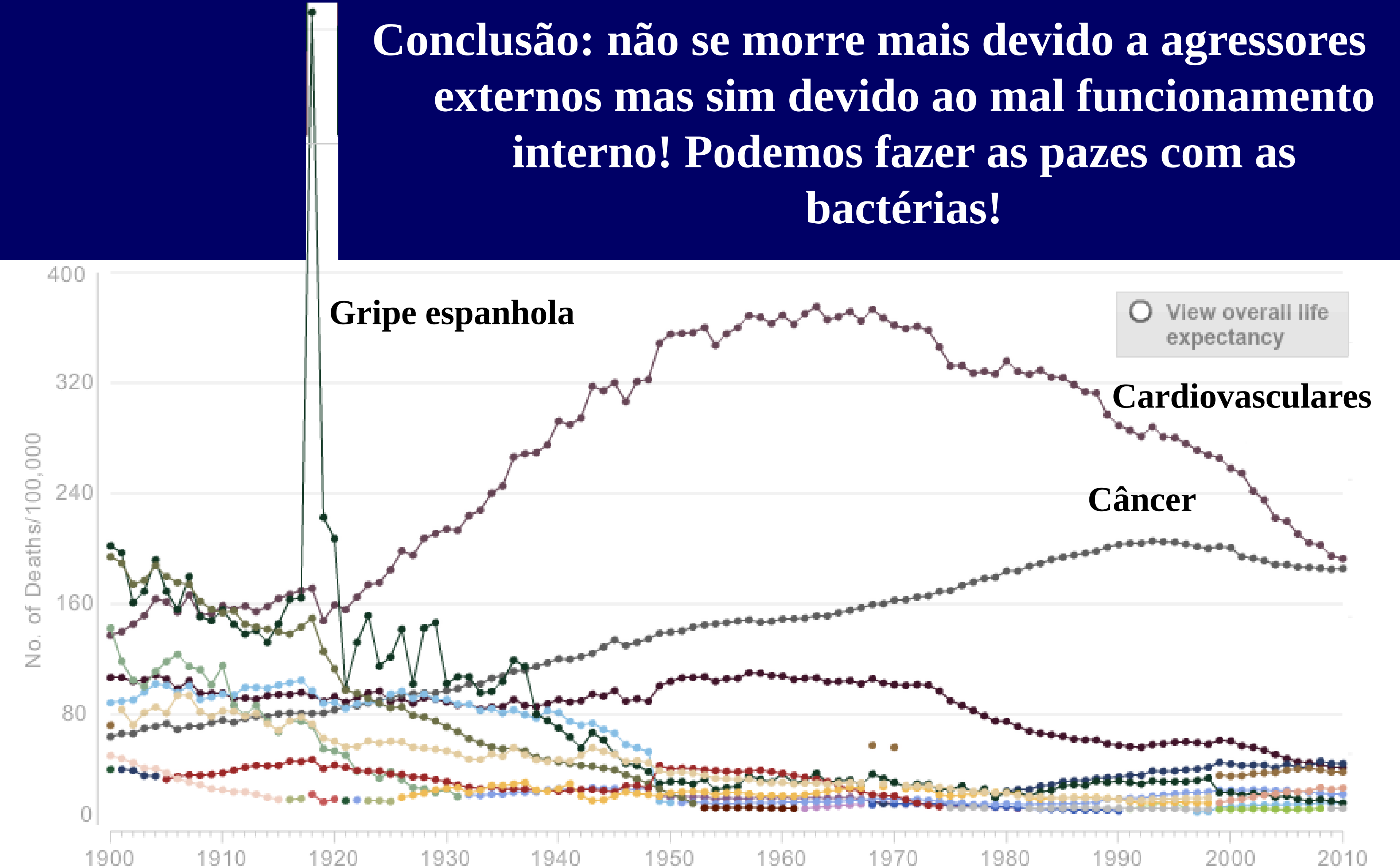
Causas de mortalidade nos EUA de 1900 – 2010

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

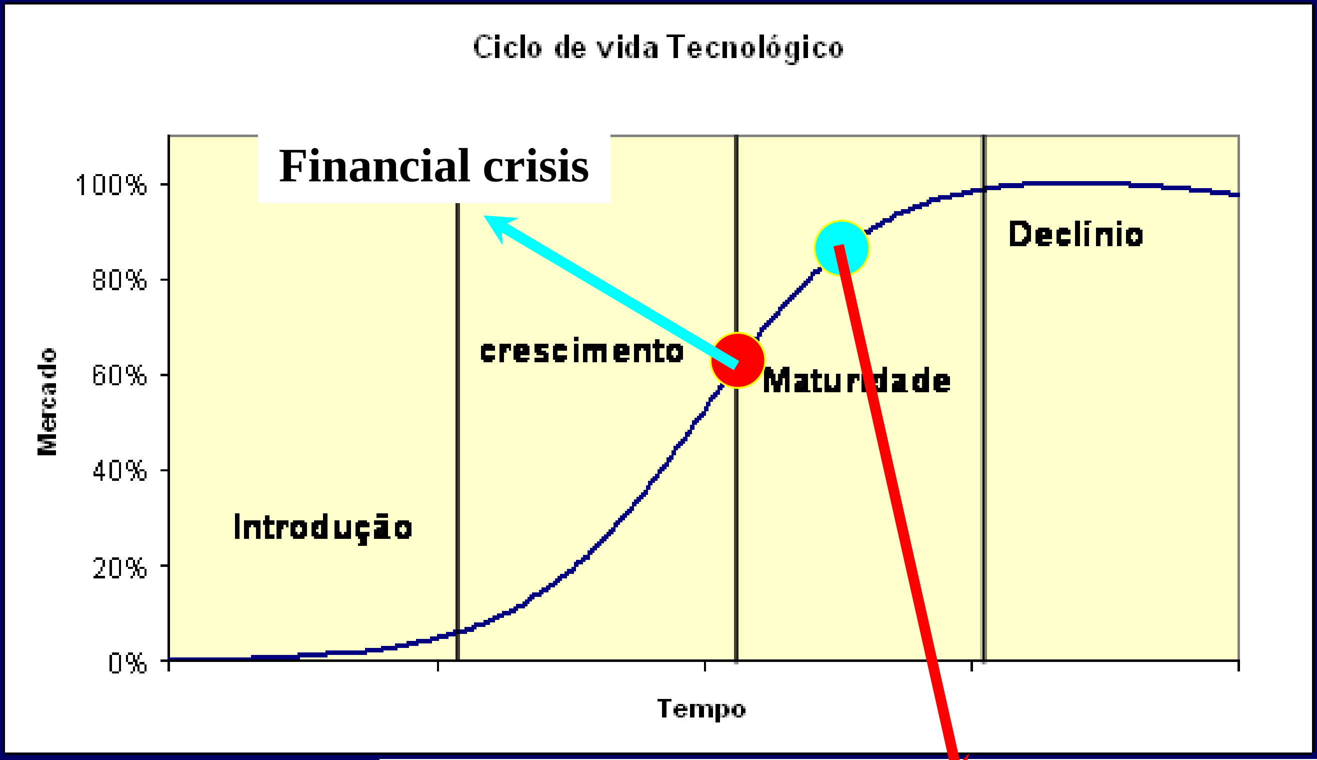


Mortalidade nos EUA de 1900 a 2010
Cancer já está acima das cardiovasculares

Conclusão: não se morre mais devido a agressores externos mas sim devido ao mal funcionamento interno! Podemos fazer as pazes com as bactérias!



Carlota Perez: Technological Revolutions and Financial Capital



Periphery deployment
No funds for other techwave up to this point

5 Revolutions - ~ 60 years total cycle

1. **Industrial Rev. – England – 1771**
2. **Steam and rail-road – England – 1829**
3. **Steel and eletricity – England+USA+Germany – 1875**
4. **Oil, cars and mass production – USA – 1908**
5. **Information and comunications – USA – 1971**

President Obama
2009 annual meeting of the National Academy of Sciences:

As you know, scientific discovery … requires the support of a nation. But it holds promise like no other area of human endeavor.

Surf wisdom: catch the wave before it breaks

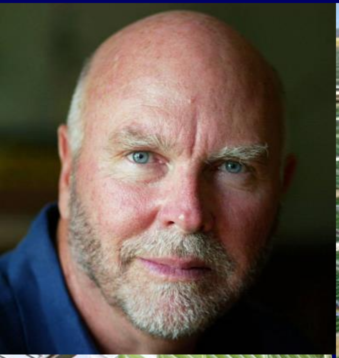
Maybe this time Brazil can catch the wave earlier

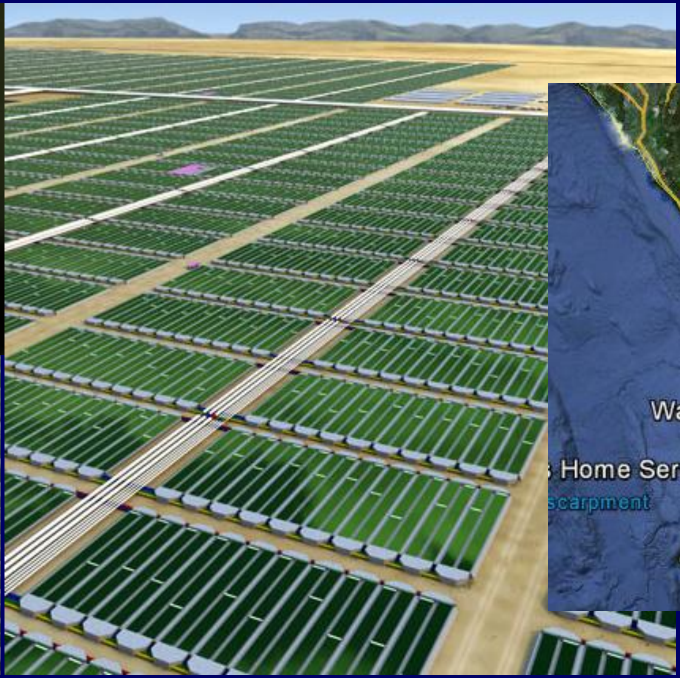
It is coming faster than we think:

Craig Venter and Synthetic Genomics – San Diego

http://www.syntheticgenomics.com/

Scientists Build First Man-Made Genome; Synthetic Life Comes Next

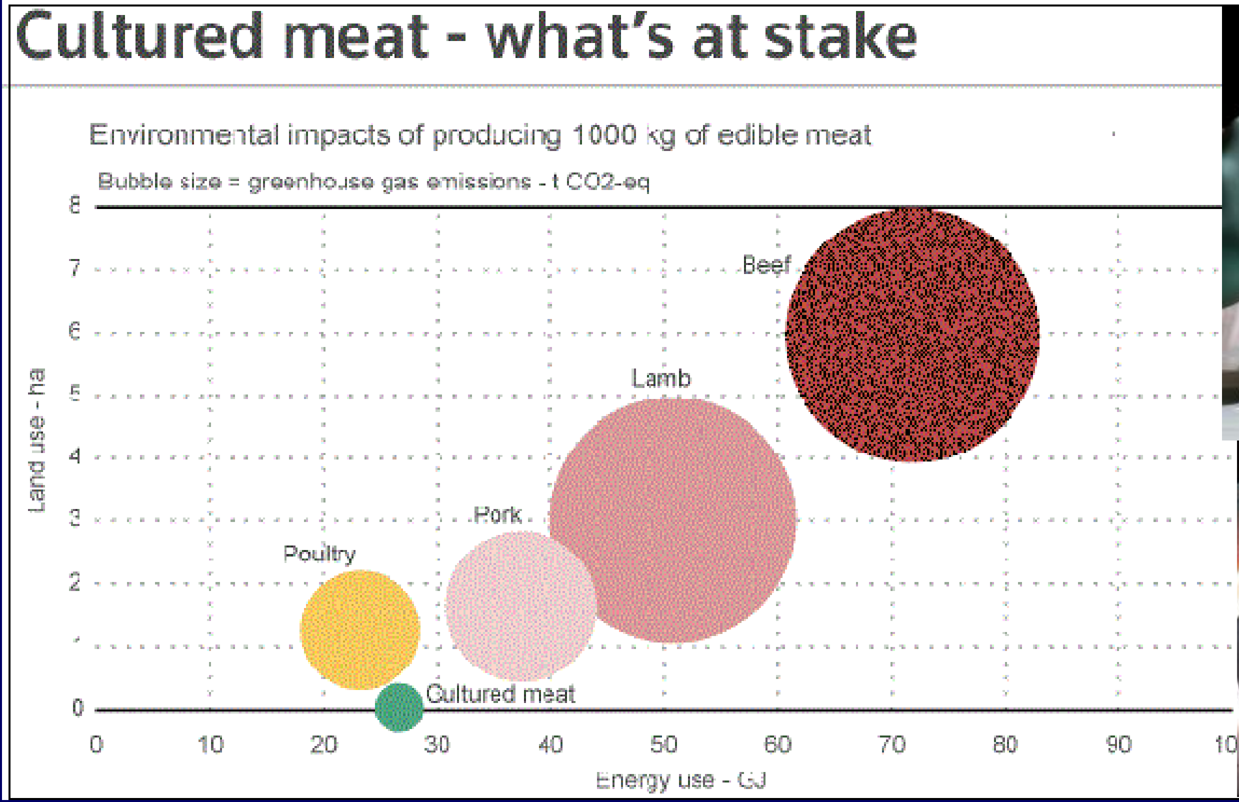


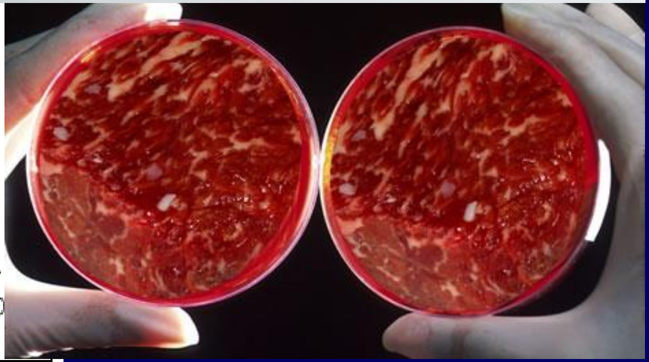


With the new ability to sequence a genome, scientists can begin to custom-design organisms, essentially creating biological robots that can produce from scratch chemicals humans can use. Biofuels like ethanol, for example.

Winston Churchill in 1932
Essay in Popular Mechanics : “50 years hence” (1982)

We shall escape the absurdity of growing a whole chicken in order to eat the breast or wing, by growing these parts separately under a suitable medium. Synthetic food will, of course, also be used in the future.



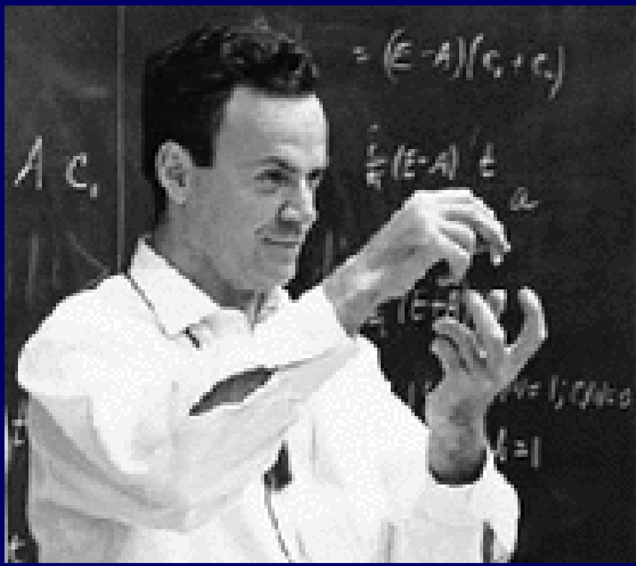


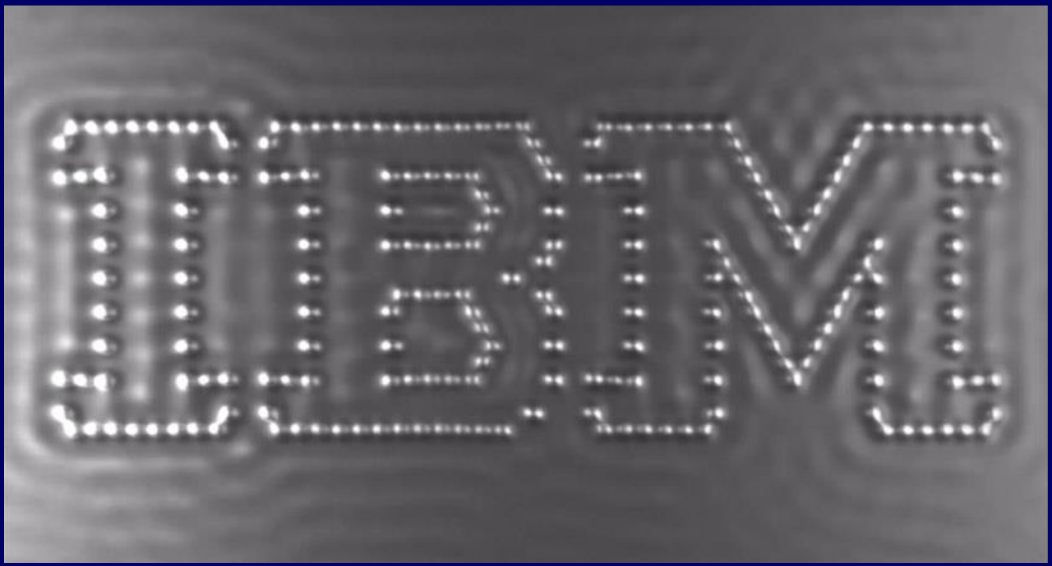
http://io9.com/5458425/is-vat+grown-meat-kosher-we-asked-a-rabbi?tag=cultured-meat

http://www.marksdailyapple.com/in-vitro-meat/#axzz29WgjfJ2o

There's plenty of room at the bottom

1953: Feynman proposed manipulation of individual atoms

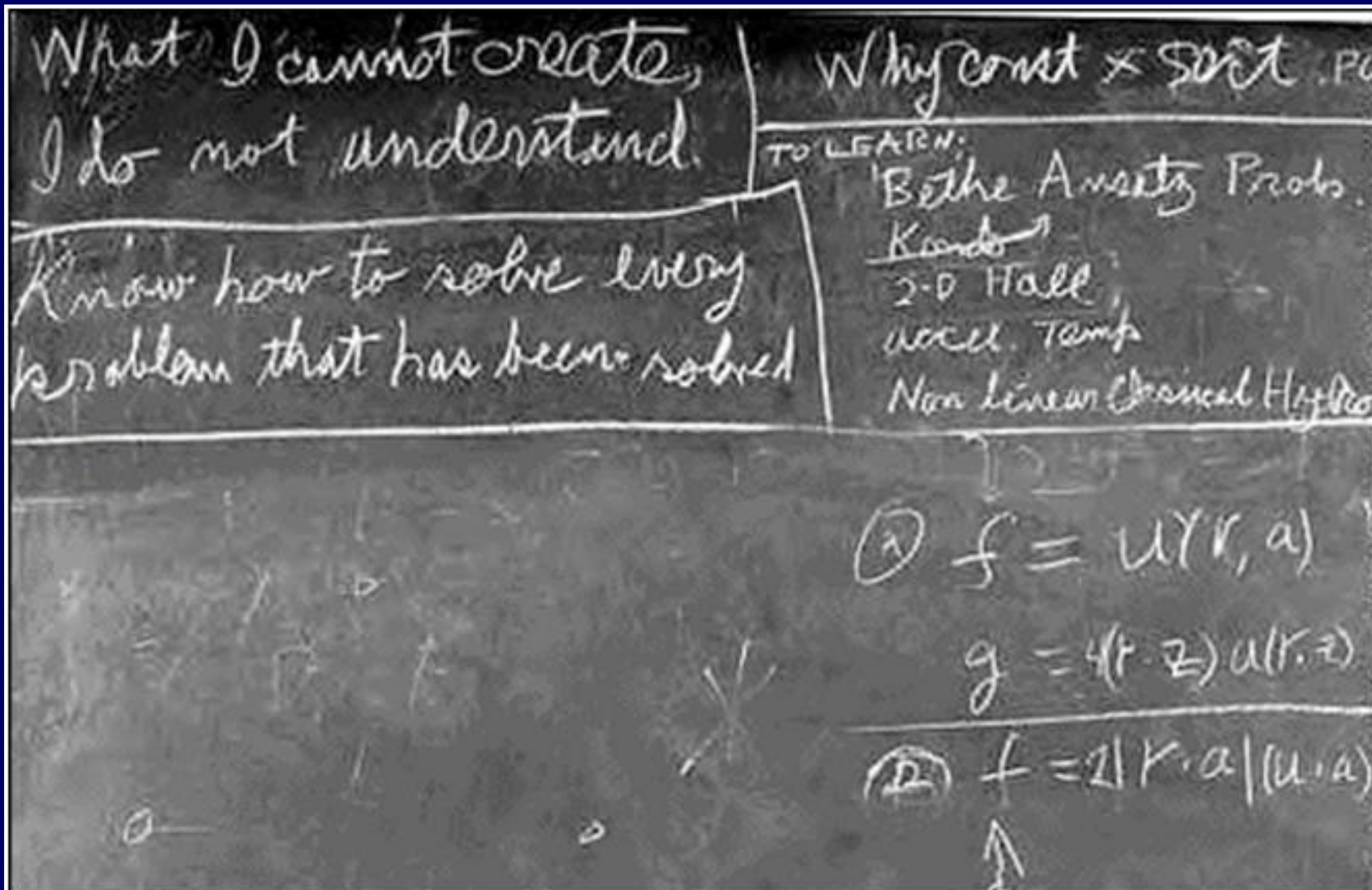




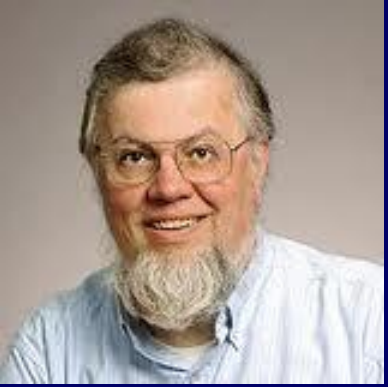
Talk that inspired the nanotechnology!
Information: Britannica on the head of a pin

Nobody implemented this experiment but:
Biological enzymes (ribosomes) function chemically close to Feynman's vision

Feynman´s last blackboard before his death



What I cannot create, I do not understand!



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.

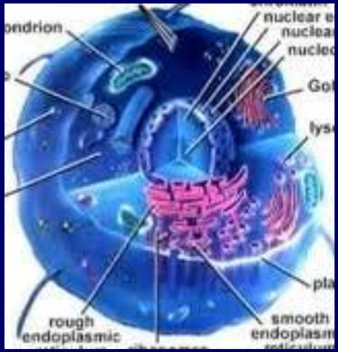
Be aware that we always overestimate what will happen in five years and always underestimate what will happen in ten.

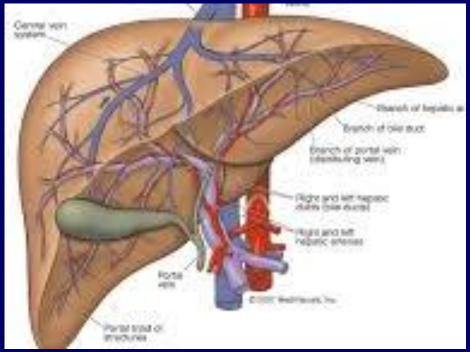
Simple versus complex!
Validity of reductionism?

A complex system that works is invariably found to have evolved from a simple system that works!
John Gaule [1603?-1687]

Inverse Problem

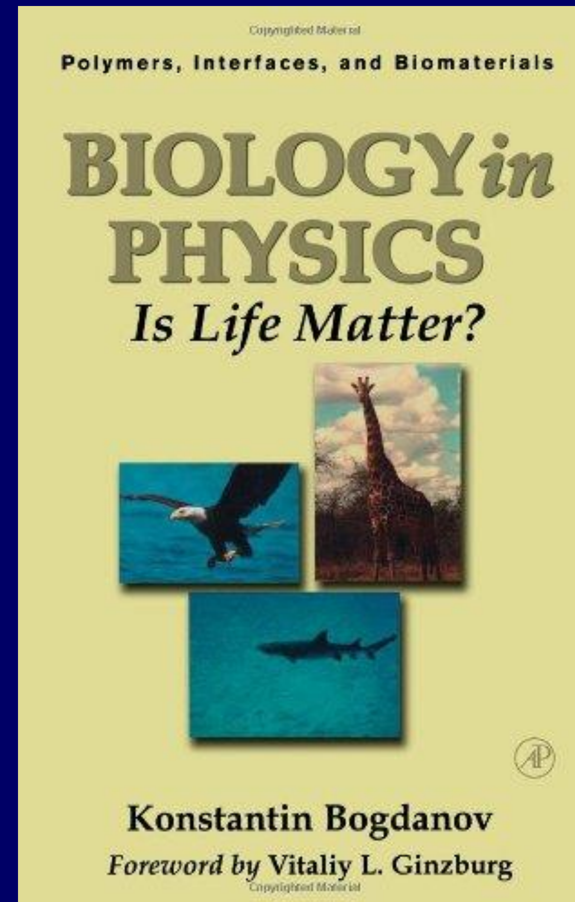
Complex





Simple

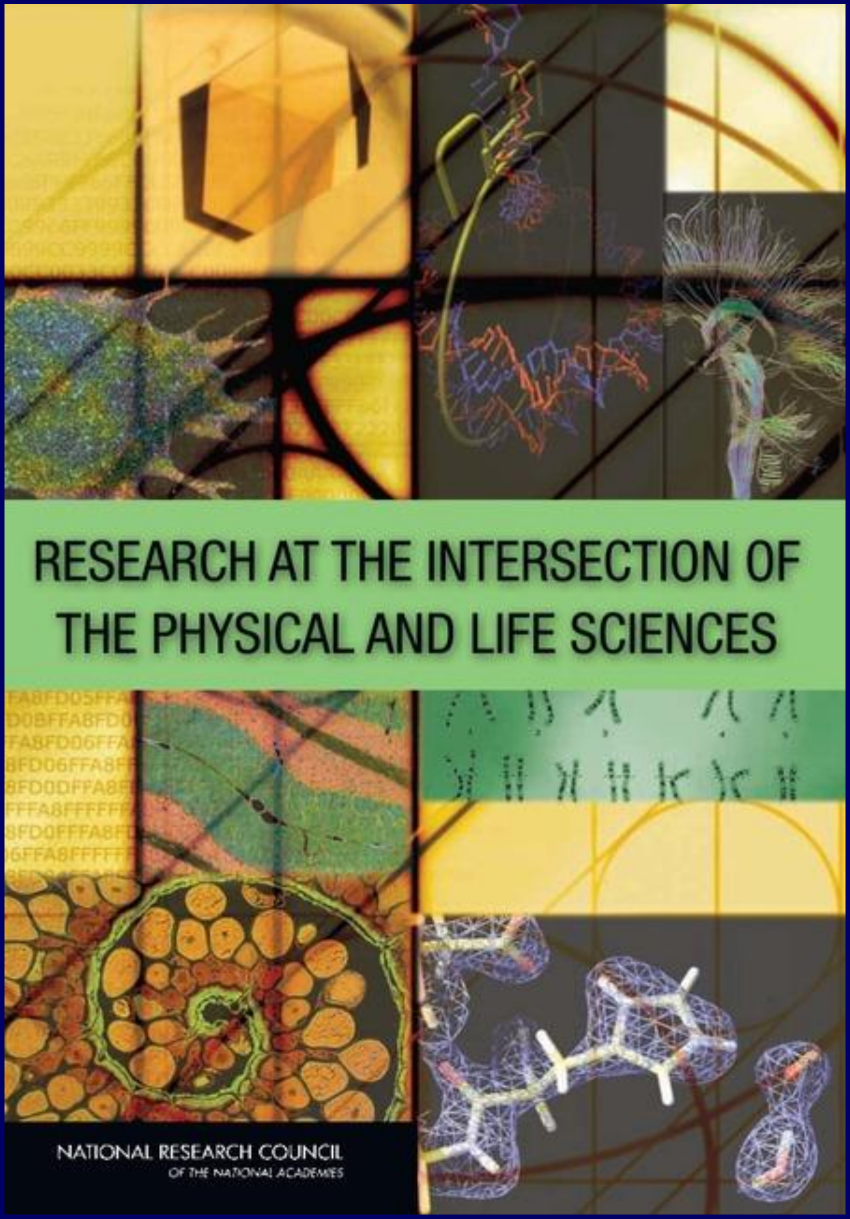
21st: leaded by biology?
Highest interest of human society!

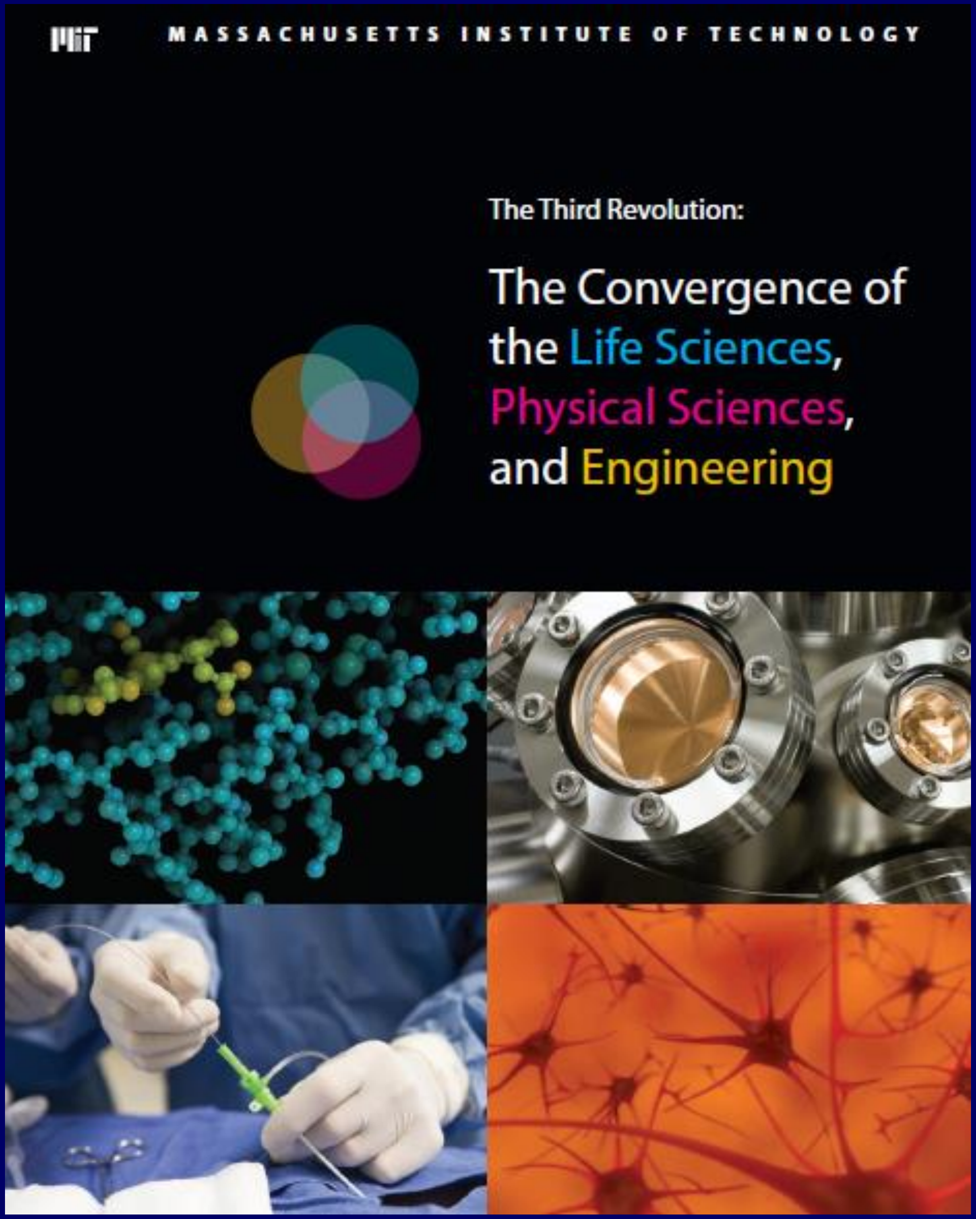


Reductionism:
Can physics explain biology?
Can physics explain living organisms' processes.
Can molecular physics explain transformation of complex molecules to simplest organisms that can self-regenerate, the step between life and nonlife?

National Research Council Report

MIT white paper





Molecular/cell biology are in the center of next wave

Grand Challenges/Recommendations

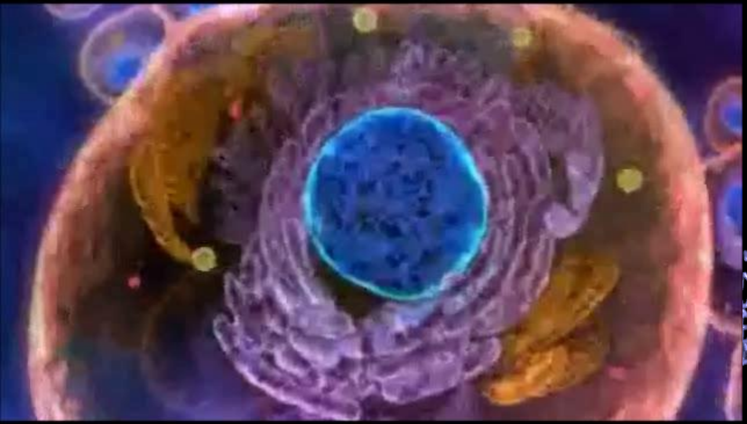
Synthesizing Lifelike Systems/Understanding Brain/Predicting Organisms' Characteristics from DNA Sequence.

Funding of integrated research of life sciences with physics and engineering.

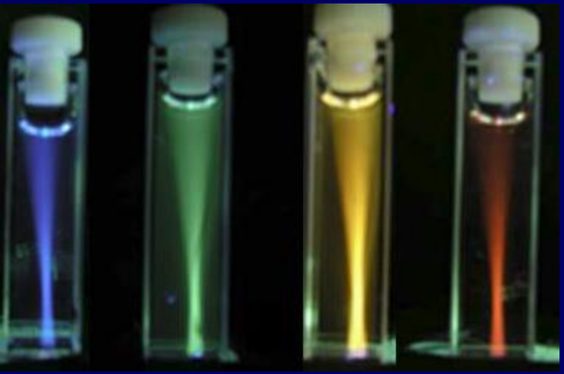
Special multidisciplinar collaborative programs: Suggested 10 years of investments.

More multidisciplinar education of next generation

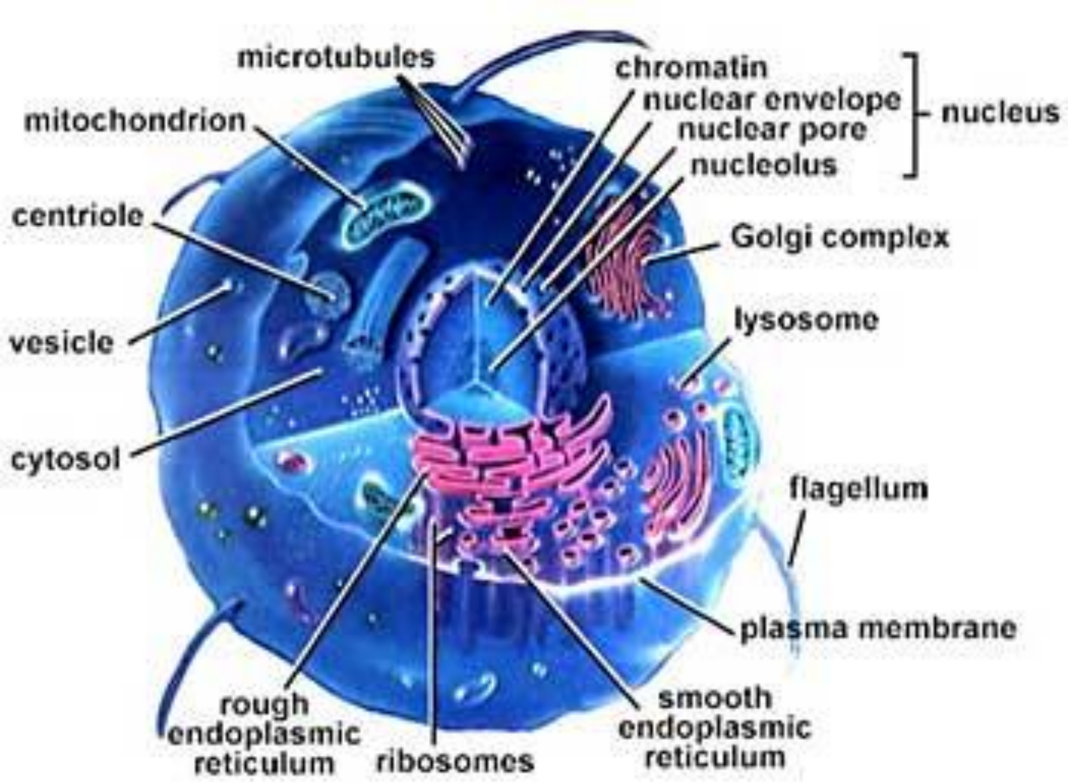
In Singulo Biochemistry

Trends in Biology: single cell → single molecule



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



Cells are made equal
but cells are not identical
Neither in space nor in time

Spatial organization –
external /internal signalling

Best thing for us:

CONTROLLABLE

TECHNOLOGY FEATURE

THE DEEPEST DIFFERENCES

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The NIH gets singular

To understand biological heterogeneity, researchers are learning how to profile the molecular contents of individual cells.

The challenges of single-cell analysis have caught the attention of the US National Institutes of Health (NIH). The agency has launched a programme to fund advances in single-cell research, with a budget of around US$90 million over five years from the NIH Common Fund, which backs science that crosses disciplines. Grant applications are due early next year, and the NIH expects to make the first awards by September 2012, says Andrea Beckel-Mitchener, a programme officer at the NIH campus in Bethesda, Maryland. The programme will fund new techniques in areas ranging from microscopy to biochemistry, and foster their commercialization. The NIH also sees a big need for tools to examine cells in their natural environment.

Many of the techniques need an extra push. "It's still really difficult for individual labs to move into that area; the group of researchers who work on this is still highly specialized," says Beckel-Mitchener. "If you want to reach the next level you really have to push the envelope." C.S.

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CAREERS

SINGLE-CELL ANALYSIS

Imaging is everything

Advances in single-cell imaging bring opportunities for physicists, biologists and chemists alike.

Researchers should apply to universities at which there are already regular collaborations between imaging centres and medical schools or cell-biology departments.

Lessons for the physicits:

We learn about Hydrogen atom – very hand-waving about multielectron atoms – jump to solid state crystals. Enough for information revolution.

But where are the molecules??
Biology is made out of molecules – soft matter – alive.

Protein presents tons of different conformations
Biochemical reactions happens in some of them
We can observe single molecules now:
What about mechanical statistics of 1 molecule?
Thermodynamic equilibrium?

Without observations theory will be lost in this area

The need of photonics to observe molecular/cell processes

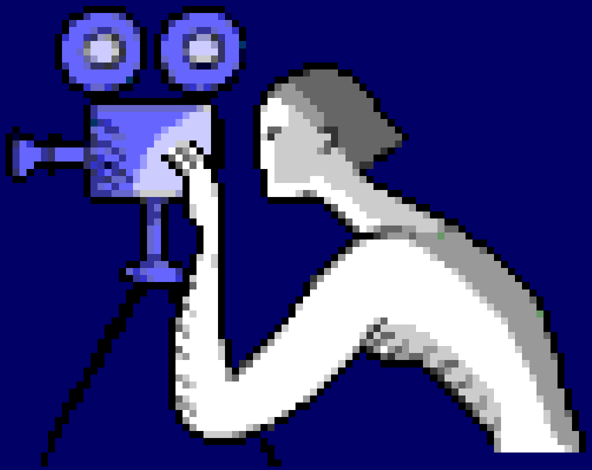
Microscope is not only a visualization tool:

Analytical tool with space, spectral and time resolution.

Single molecule sensitivity

Any optical characterization could be performed in a microscope.

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!



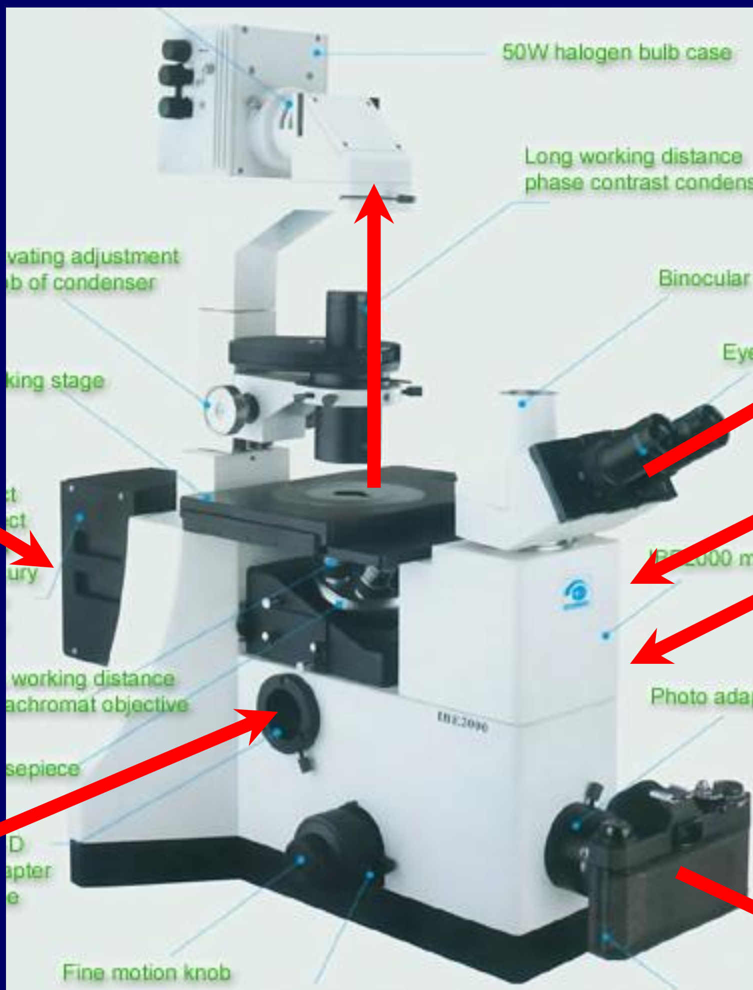
LABEL FREE

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

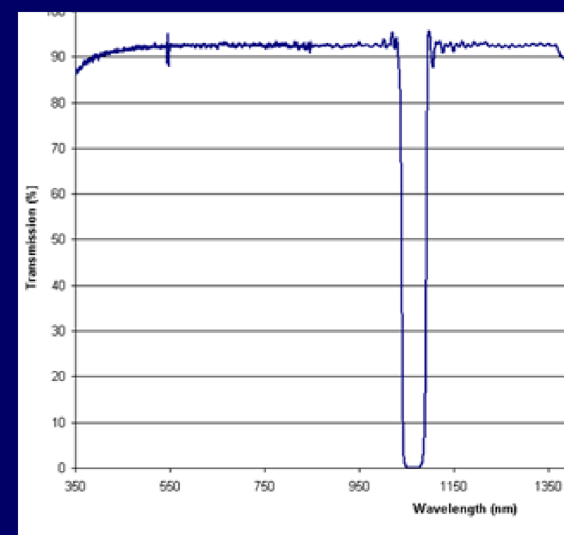
Questions to be answered: where, when and what happened

Resolved in time, space and spectrally

Optics allows Multimodality in the same platform

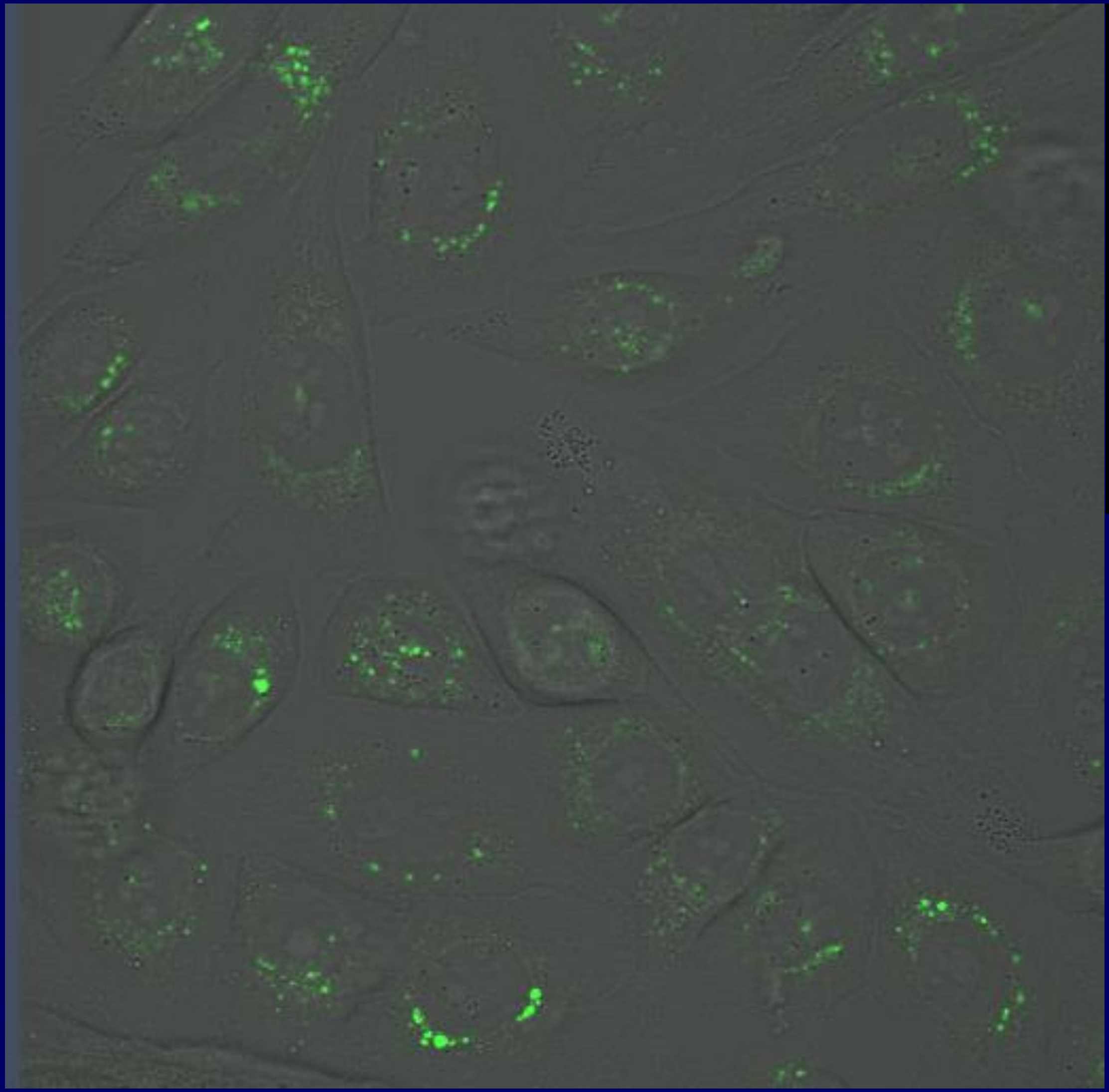


Optical beams do not collide!



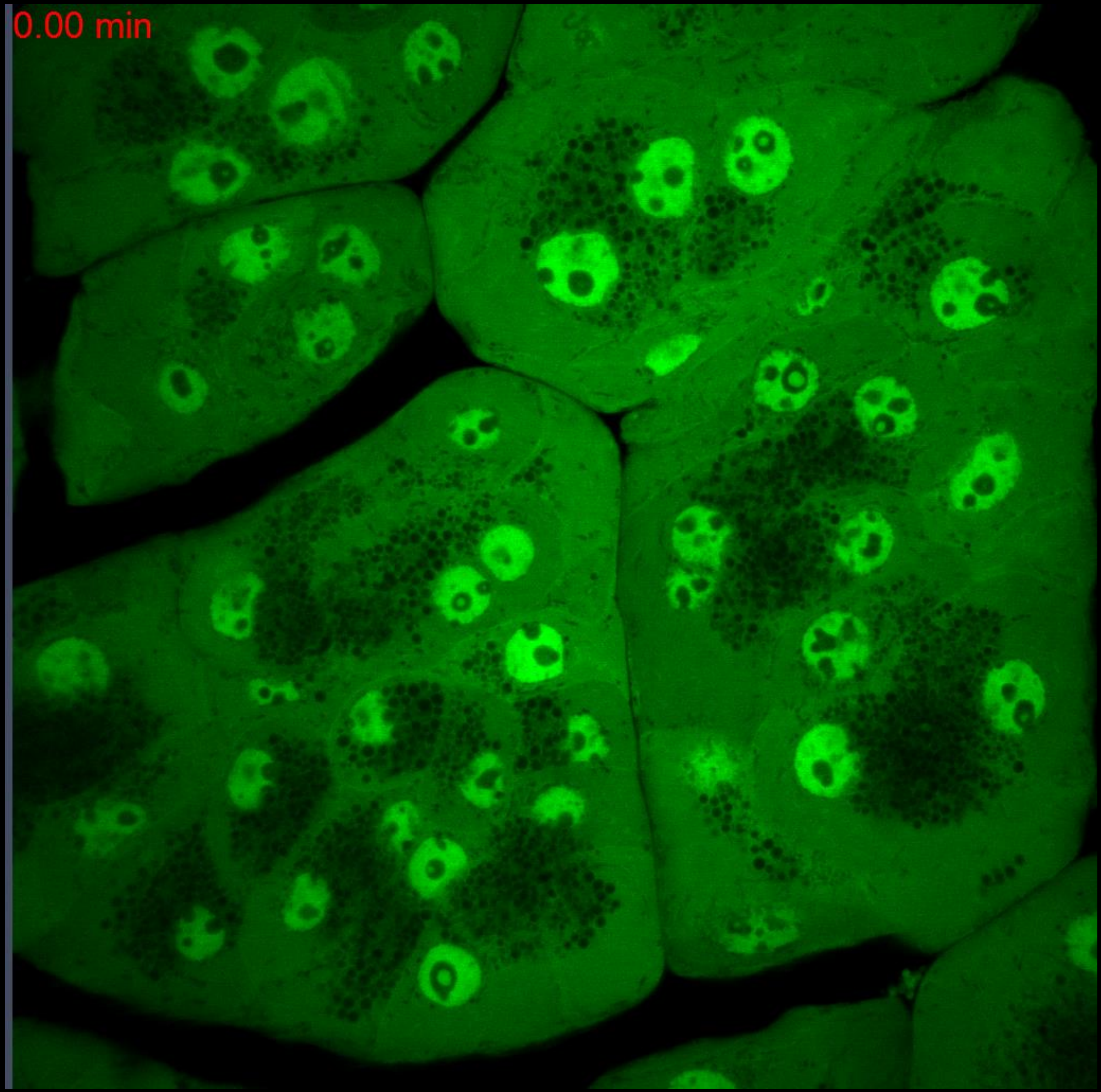
Dichroics filters

Time lapse movie: 14 h/5min
Prostate endothelial cell with Clusterin with Hernandes Carvalho

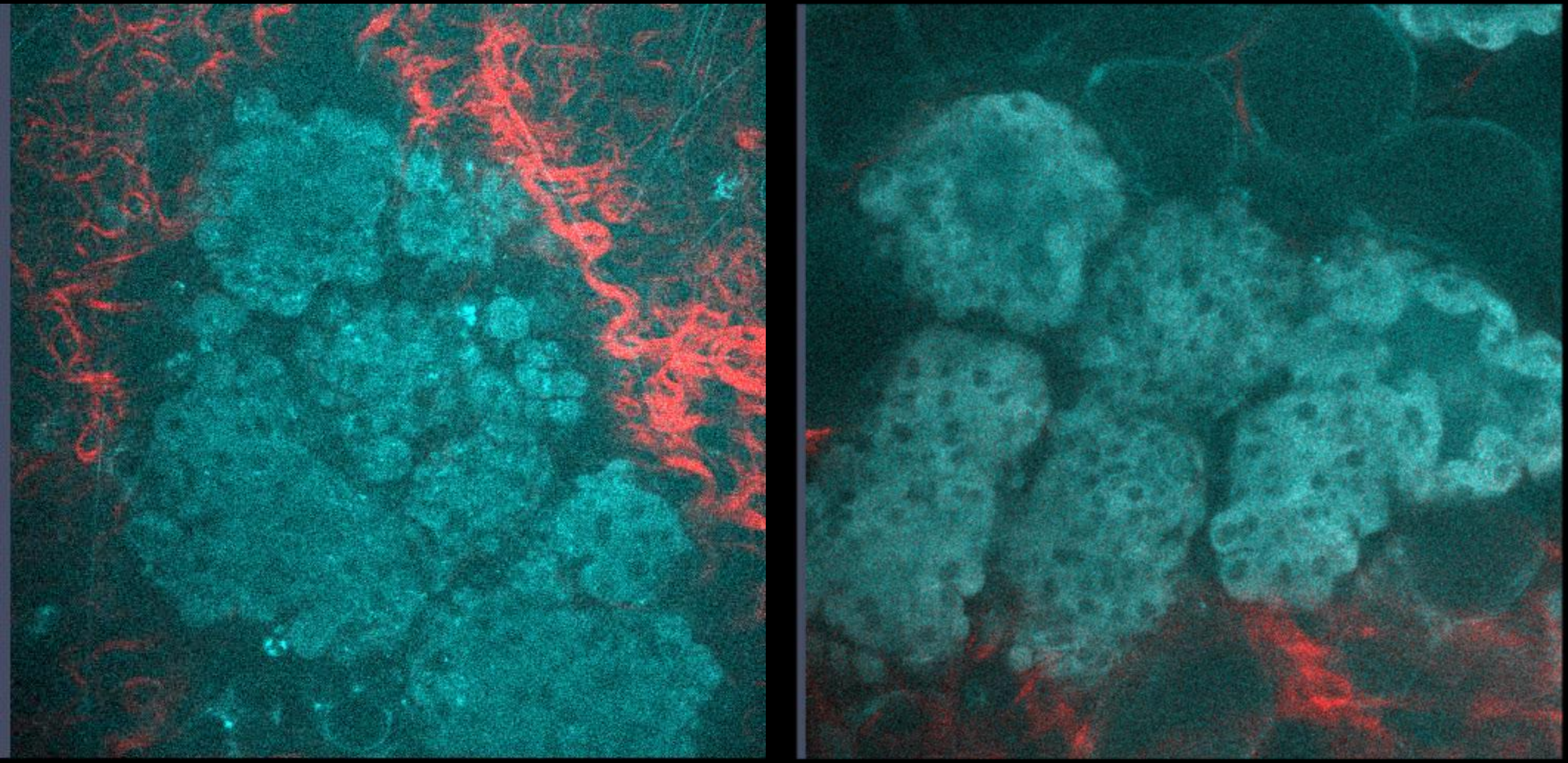


2-photon 880 nm
Goat spermatozoid
Nunes UECE

In vivo microscopy: Mice pancreas



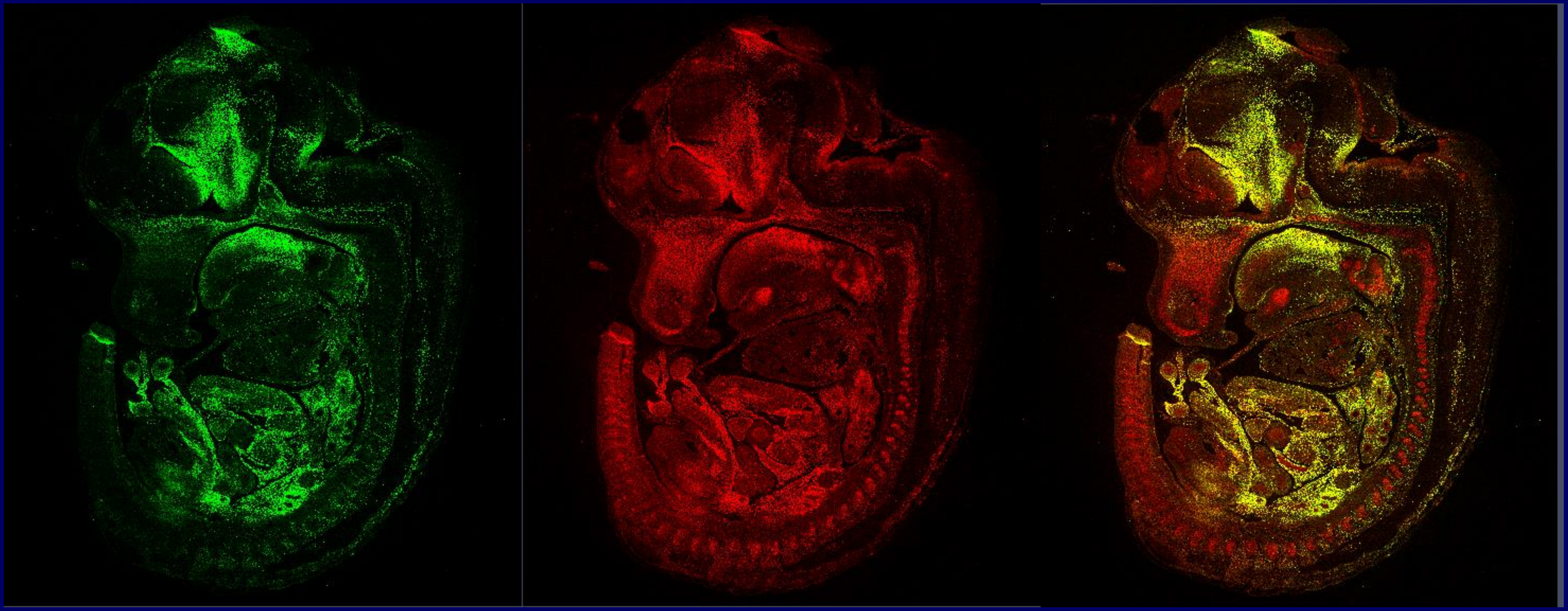
In vivo microscopy:
Mice mammary glands - milk secretion



EMBRYO´s Development

Strategical studies to understand stem cell differentiation

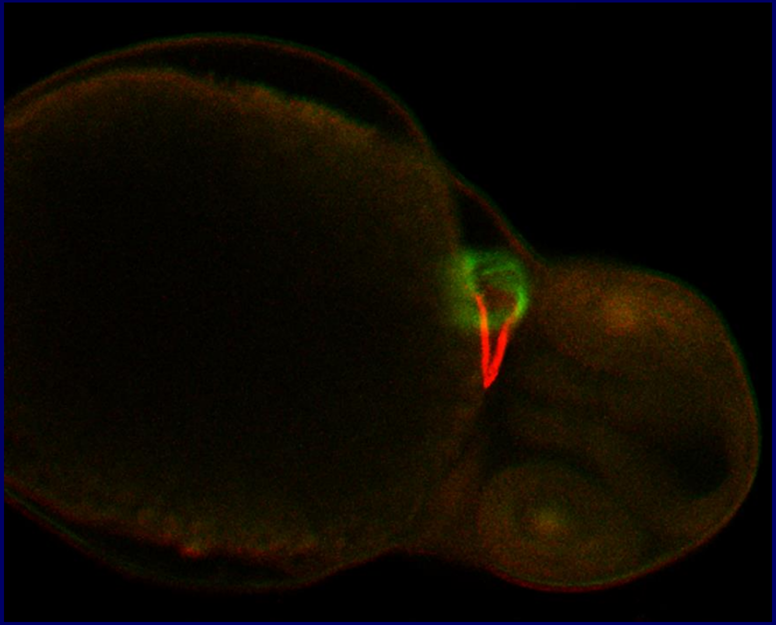
Several disease cures and cancer understanding

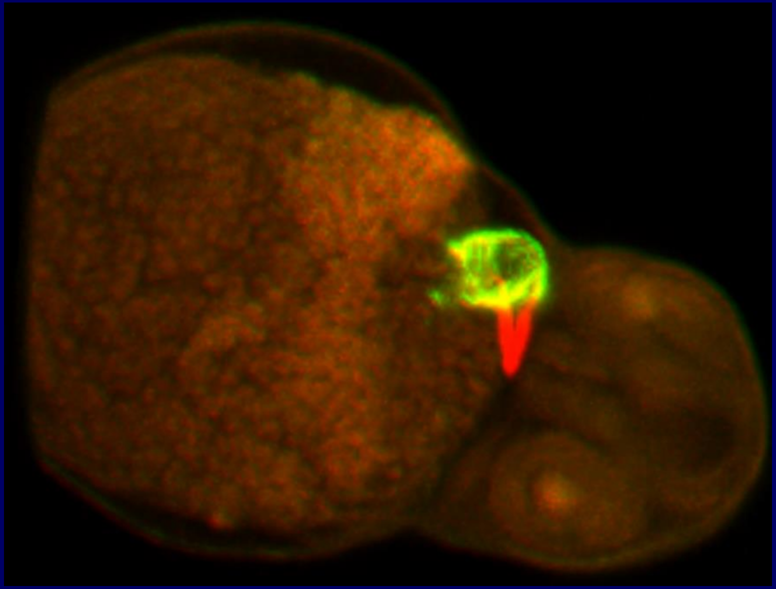


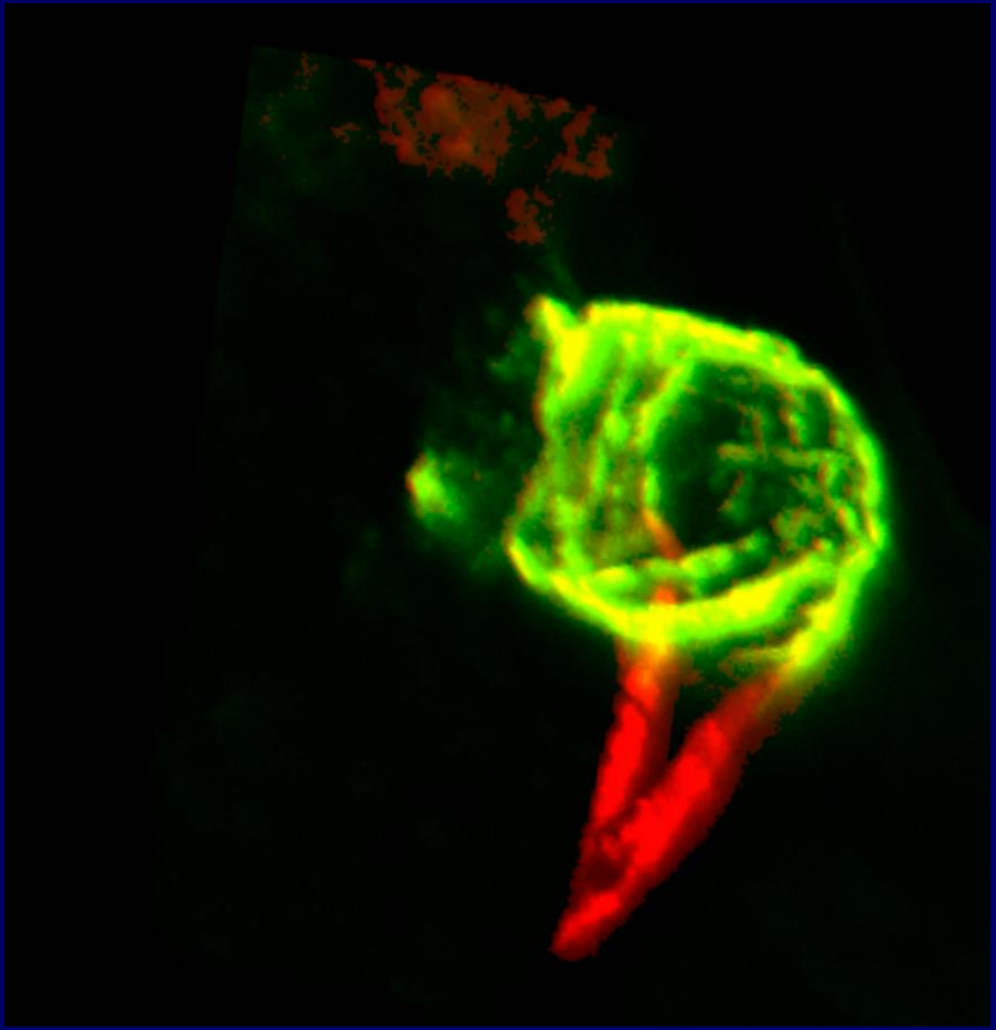
Mouse embryo – 8 mm x 5 mm

MOSAIC technique – 6 hours for the whole image

Zebra fish Heart [paulistinha]







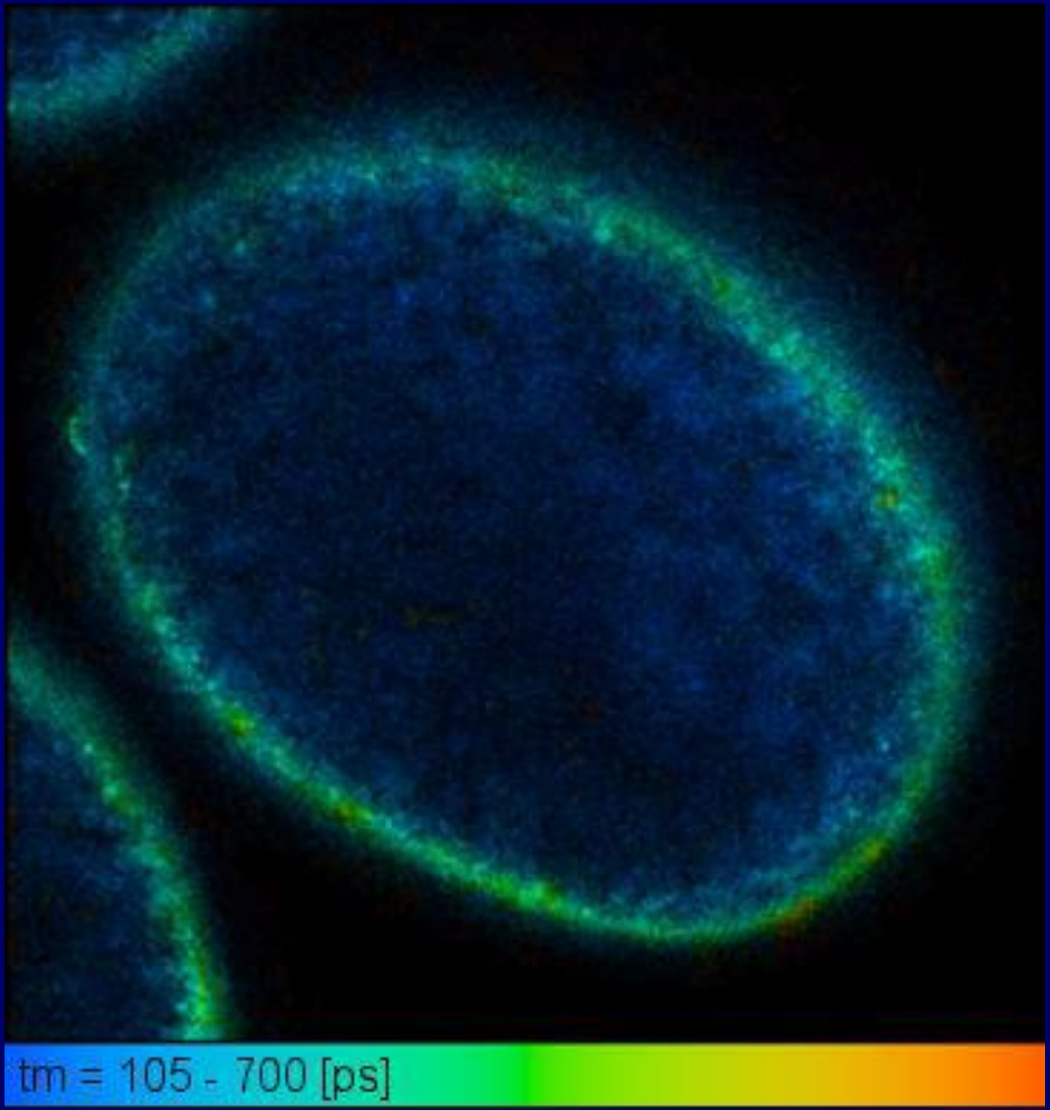
Parhyale hawaiensis embryo

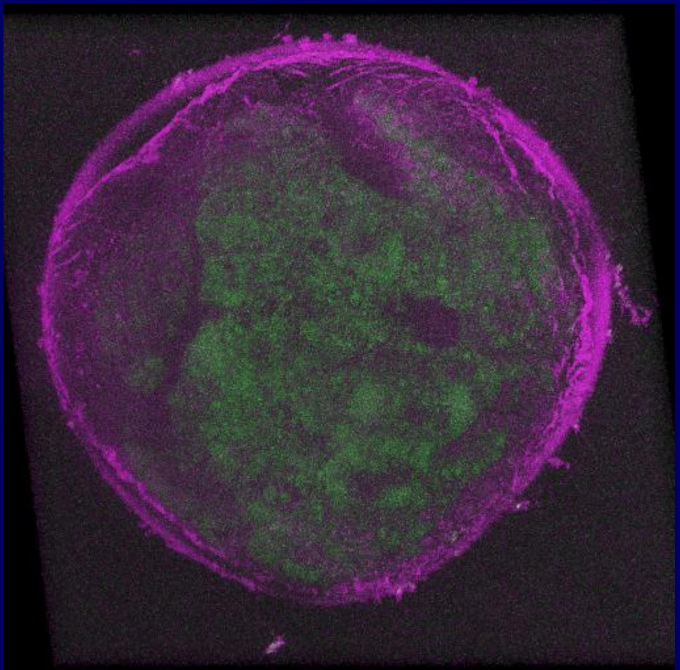


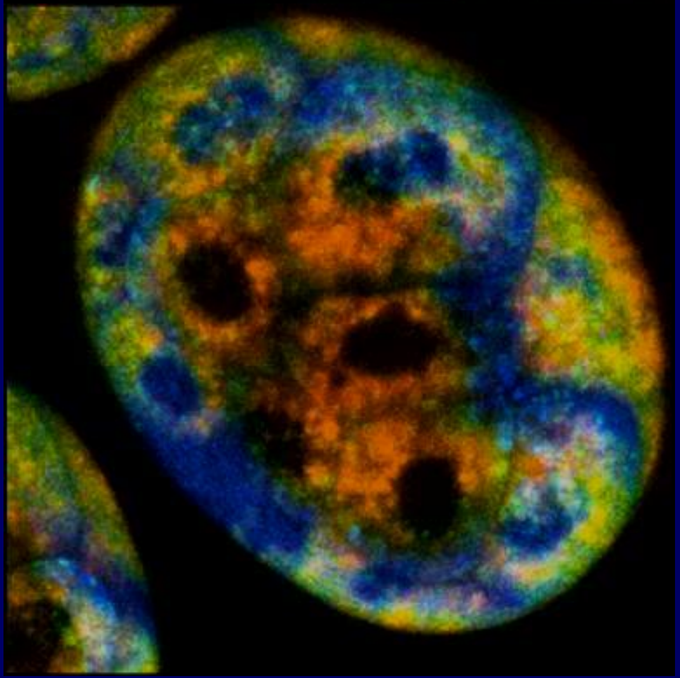


Size of the eggs: 200 μm

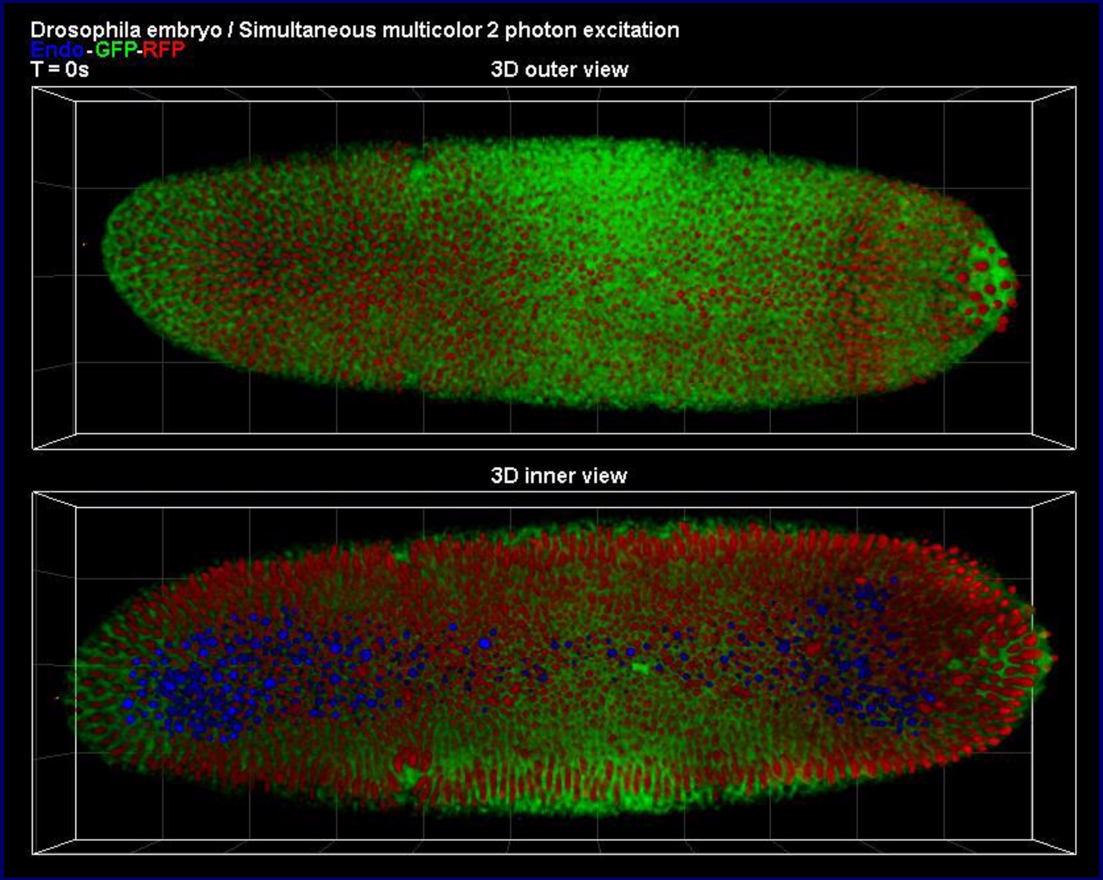
Parhyale hawaiensis embryo

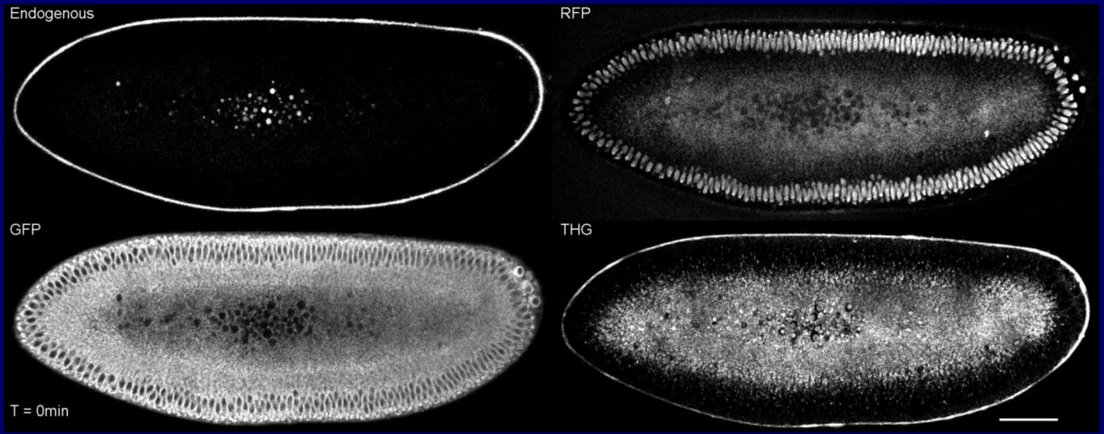






Embryology INSERM – France Results

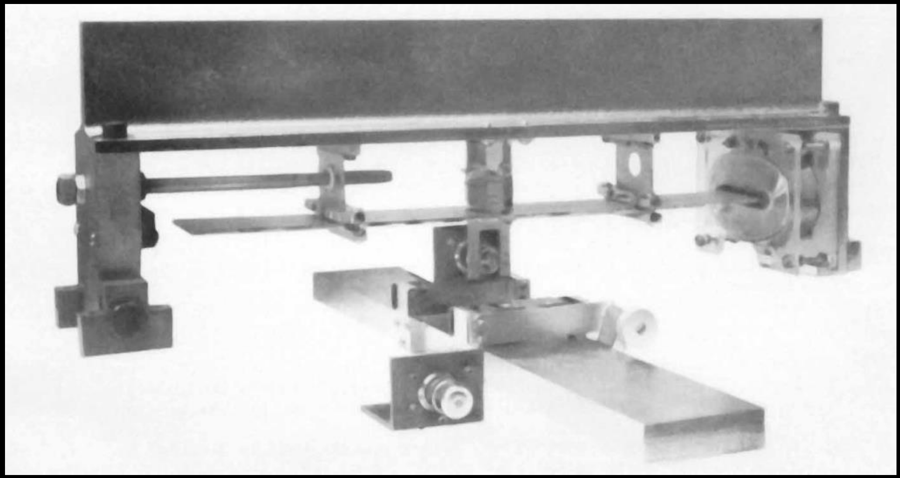


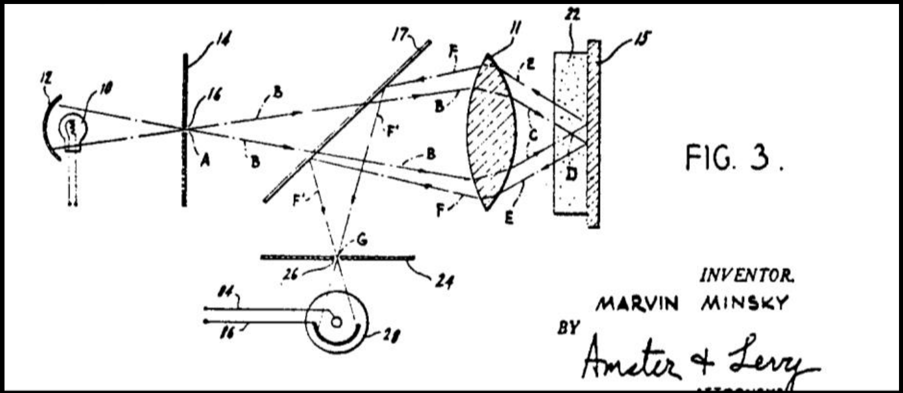


Confocal Microscopy

Marvin Minsky
Mathematician

Artificial Inteligence



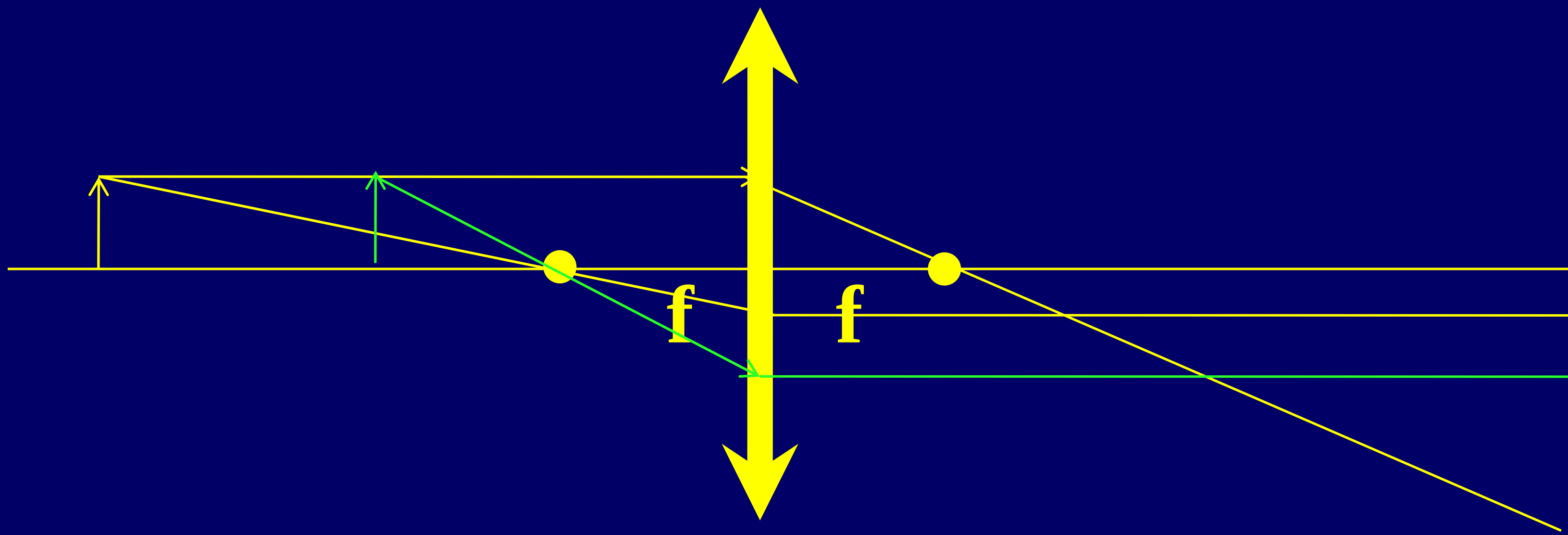


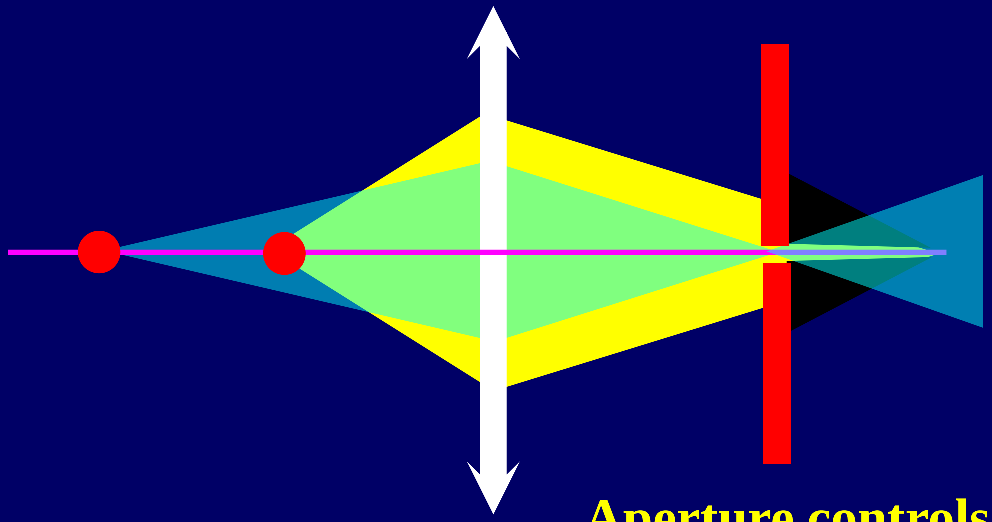
Confocal patent: 1957

Laser scanning 1987 Amos&White

Commercial - BioRad 1988

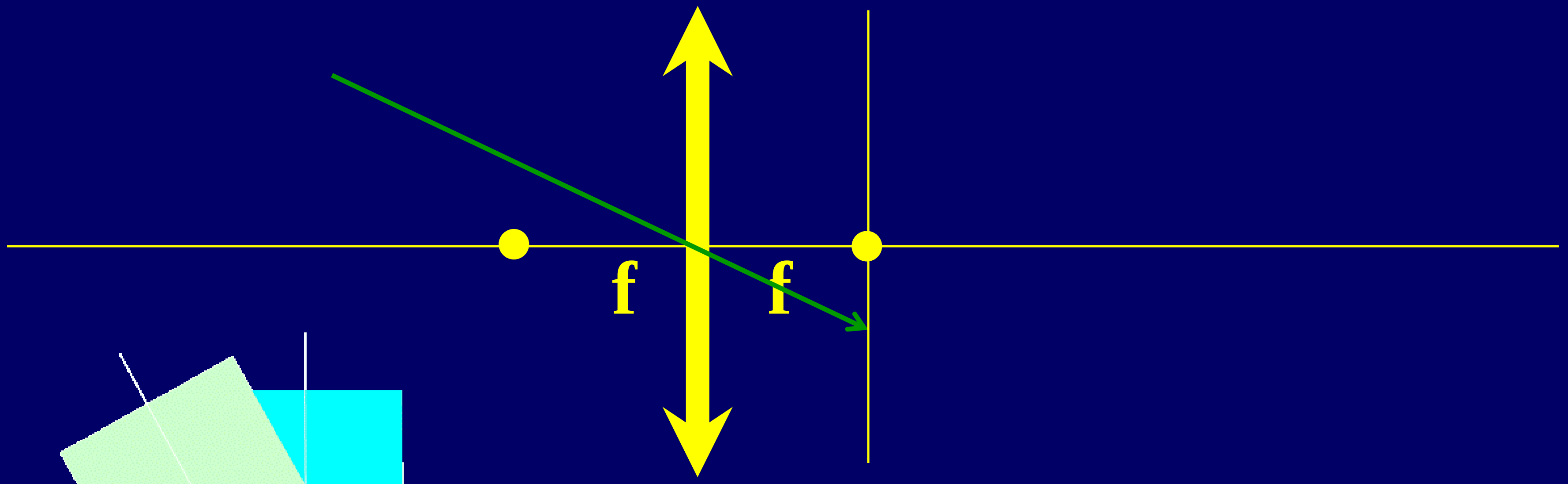
Lentes: raio paralelo passa no foco; passa no foco sai paralelo

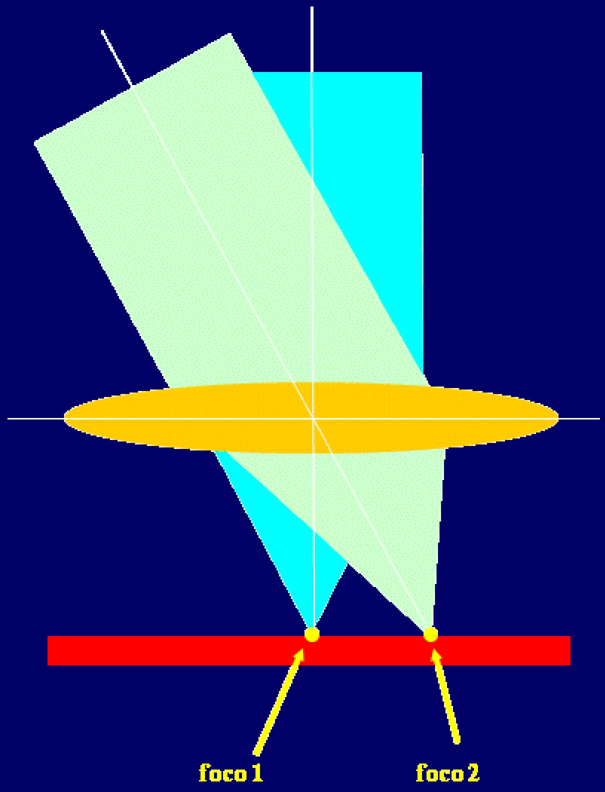


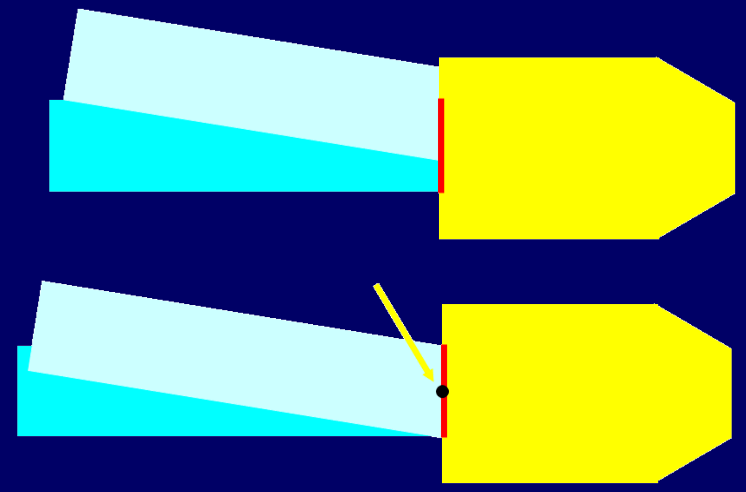


Aperture controls
Vertical resolution

Varredura - raio que passa no centro da lente não é desviado







Pivotando

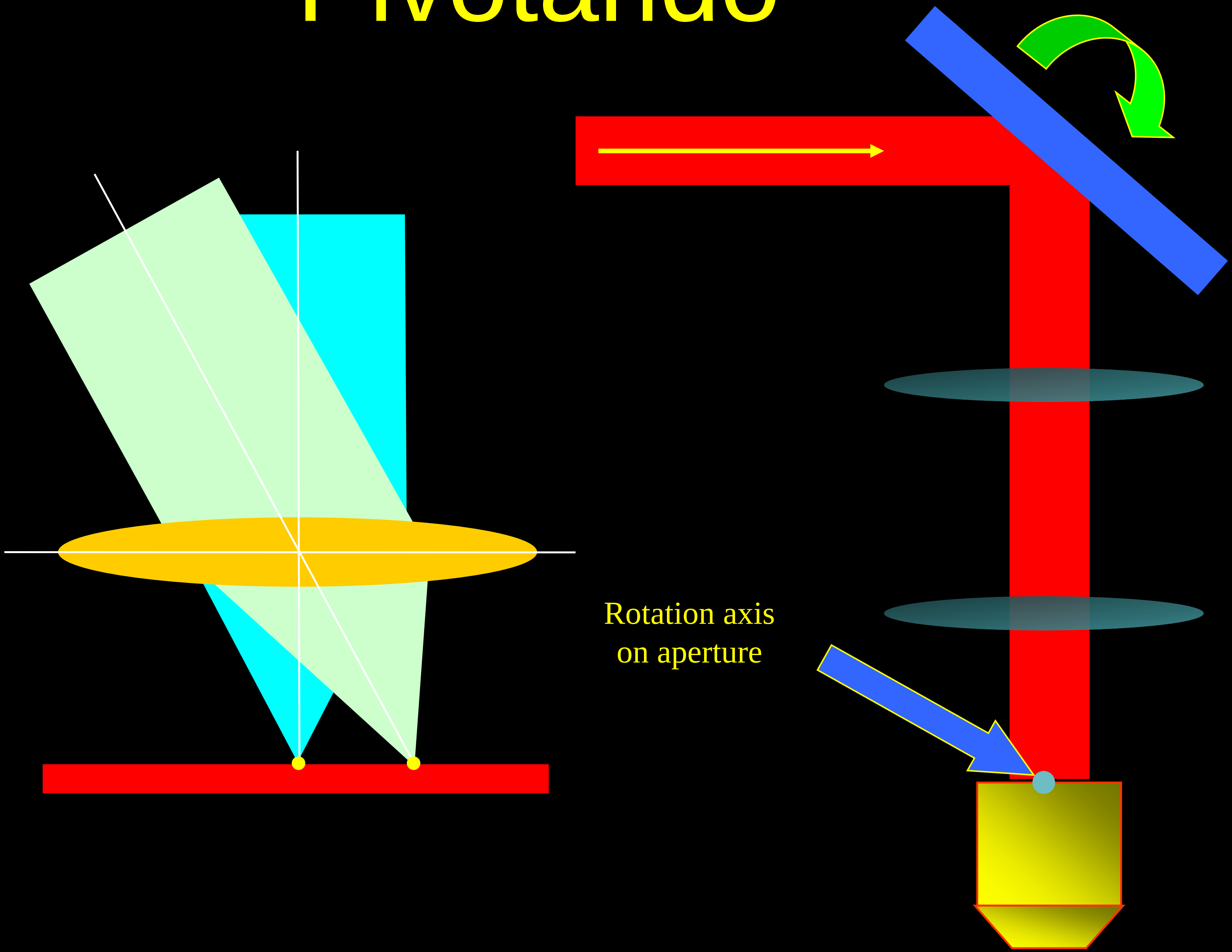
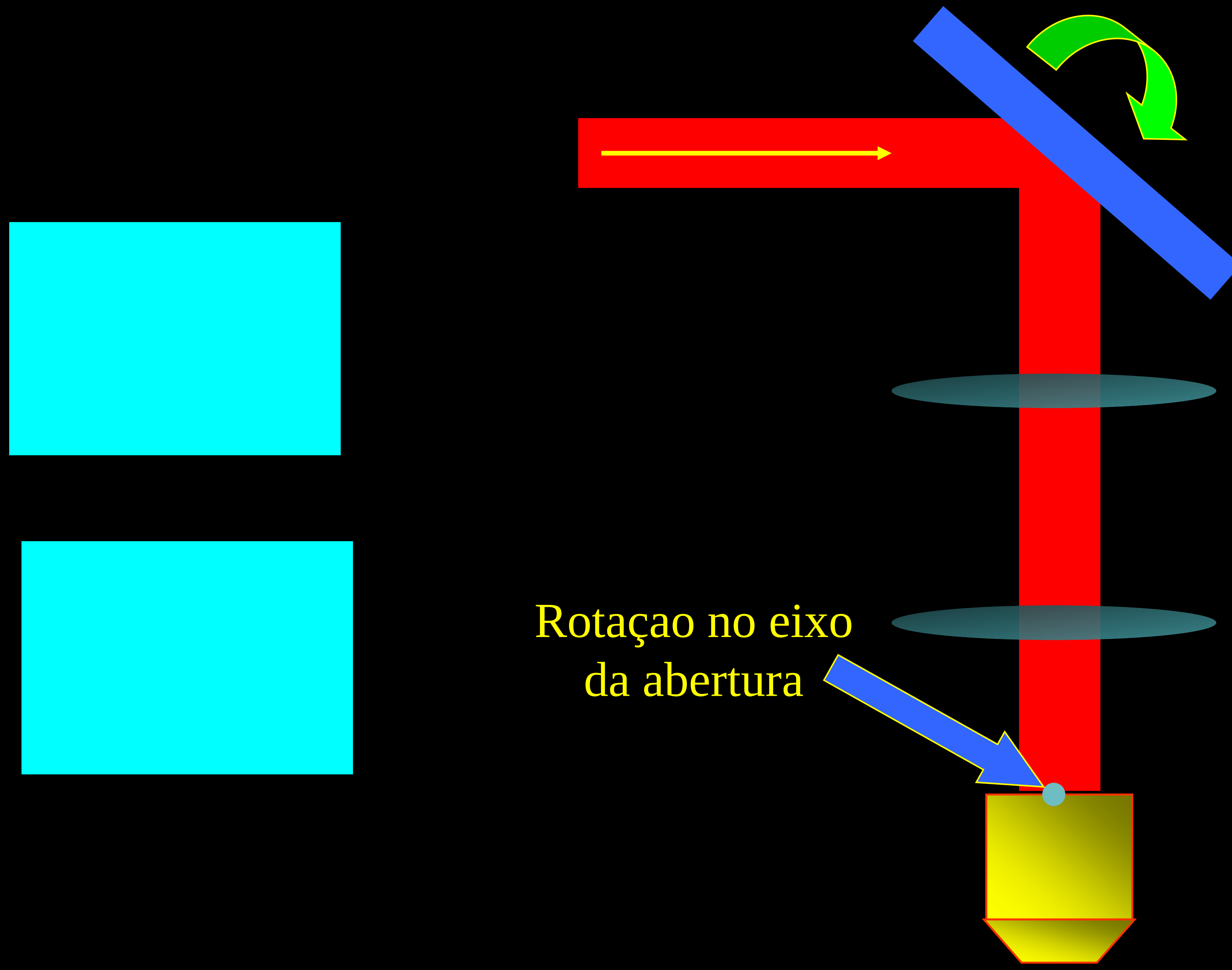
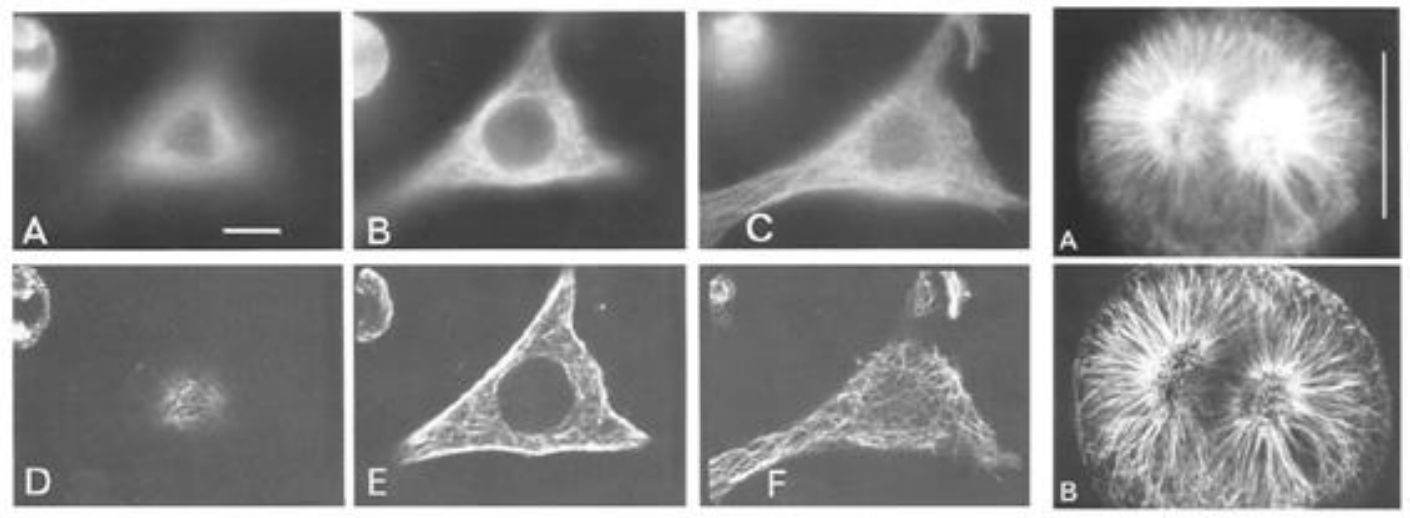
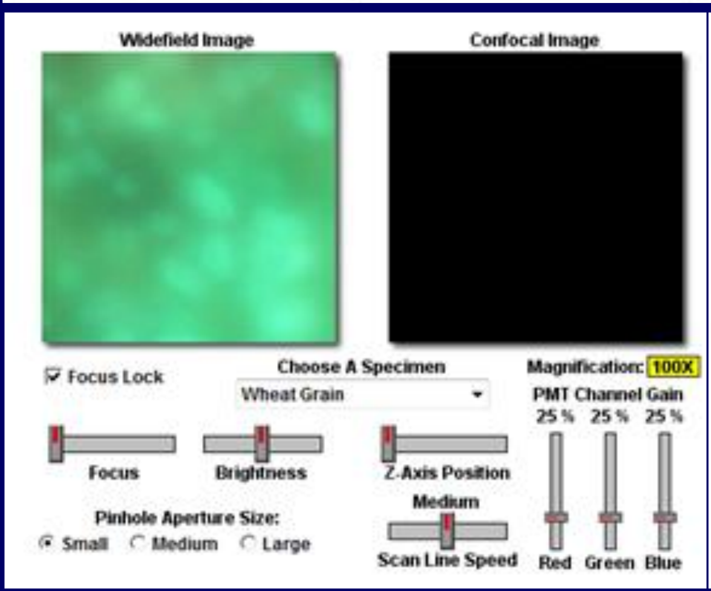


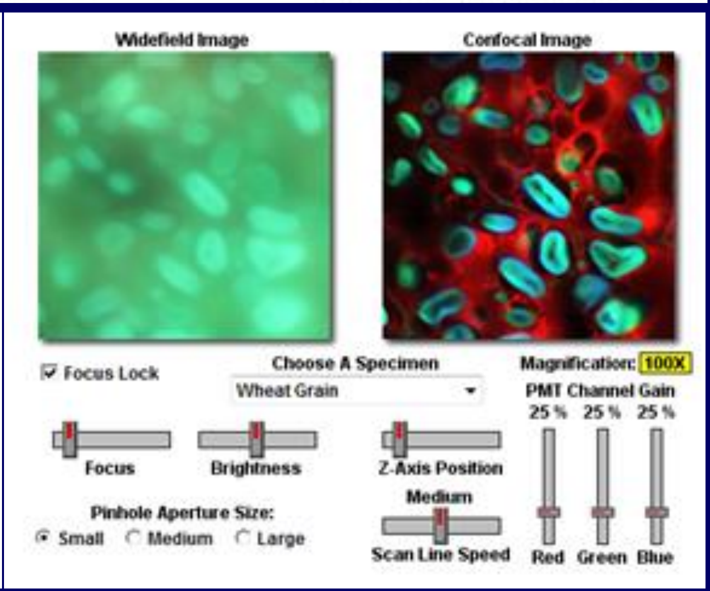
Imagem por Varredura a LASER



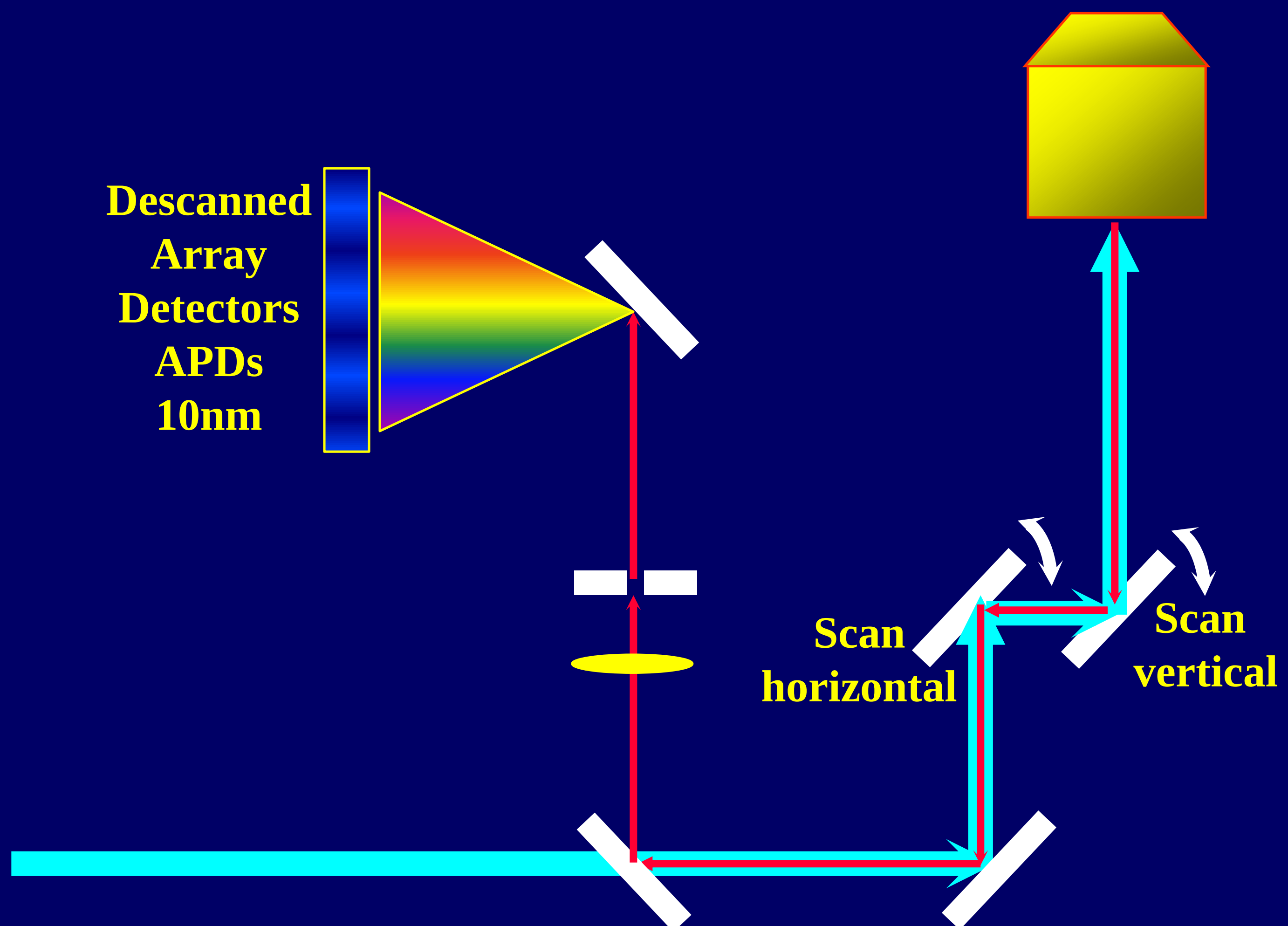
Sectioning capability



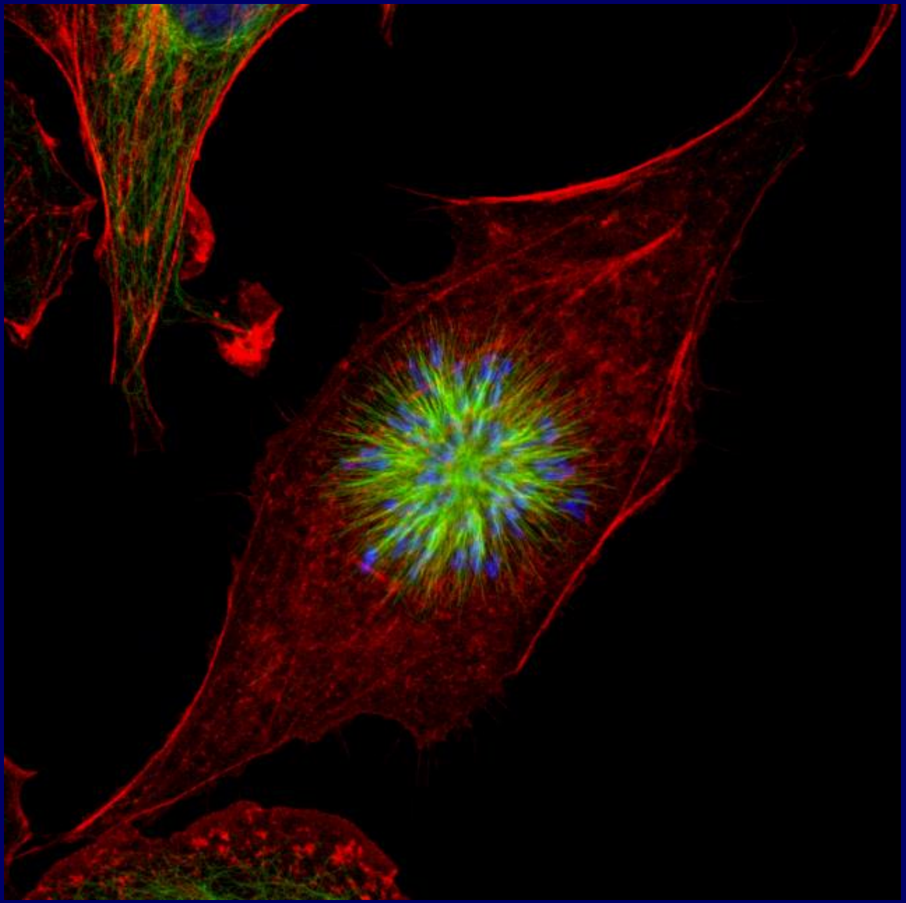


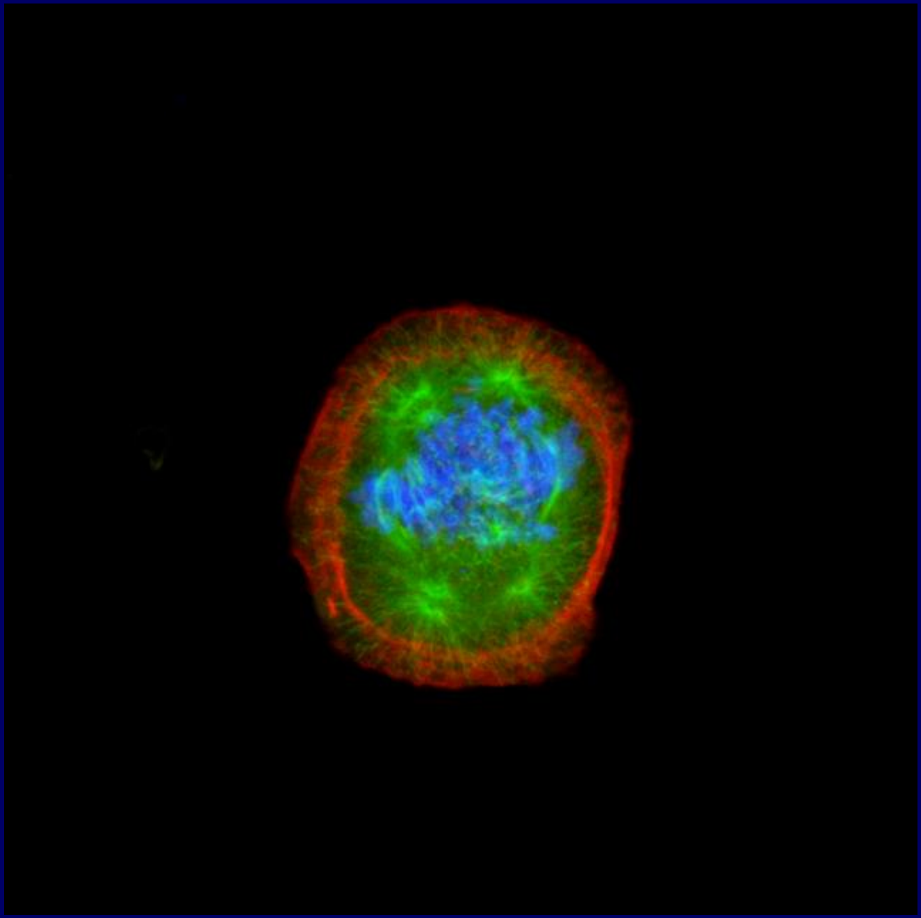


Starting with a spectral confocal microscope



Cell division phases – tubulin, actin and nuclei





Multiphoton Microscopy

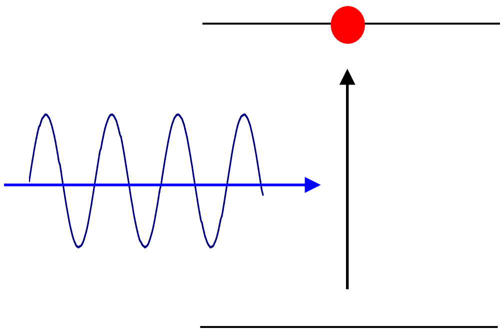
Watt W. Webb – WWW
Physicist – Cornell

http://www.drbio.cornell.edu/personnel/webb.html

More than one photon processes

Linear Optics

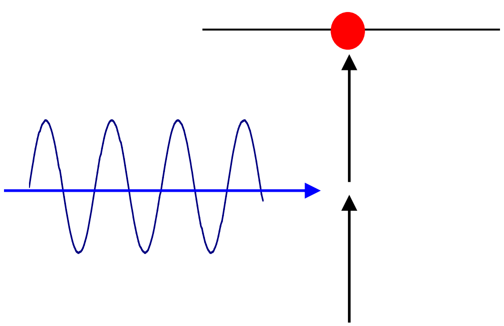
$$p \rightarrow e \qquad V \propto [p] = I$$



$$I = \frac{power}{area}$$

Non Linear Optics

$$p + p + \cdots + p \rightarrow e$$



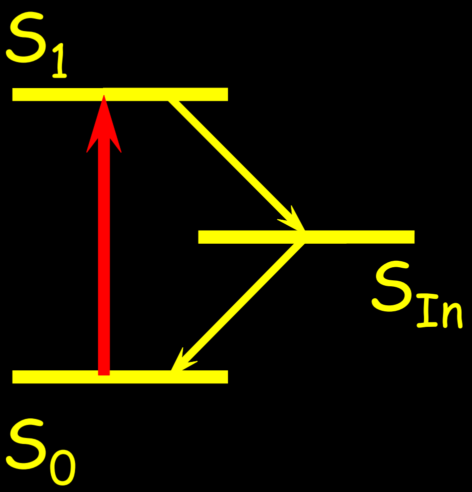
$$V \propto [p]^n = I^n$$

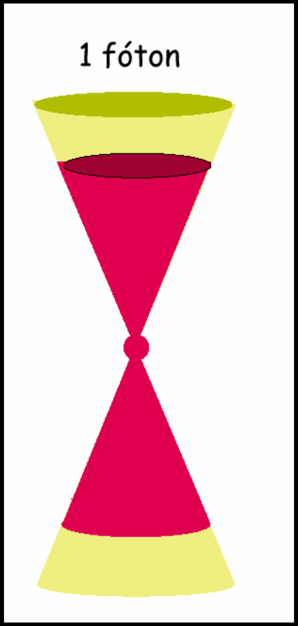
Confocality of NLO Non linear Optics

$$N_{events} \propto I \times area = \frac{power}{area} \times area$$

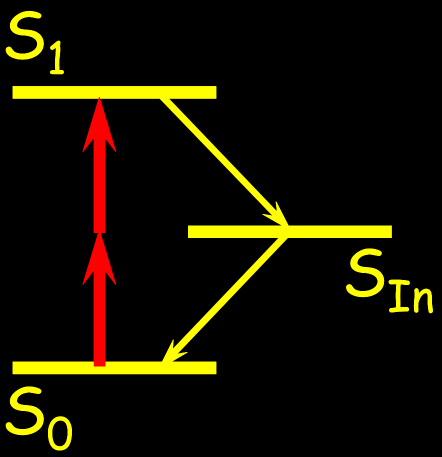
$$N_{eventos} \propto I^2 \times area = \frac{power^2}{area}$$

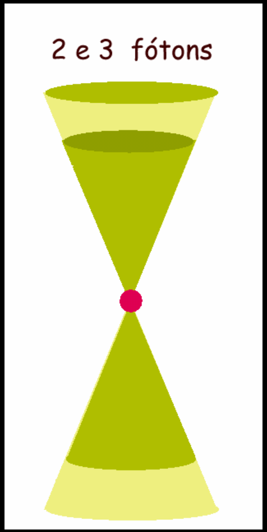
1 fóton (I)



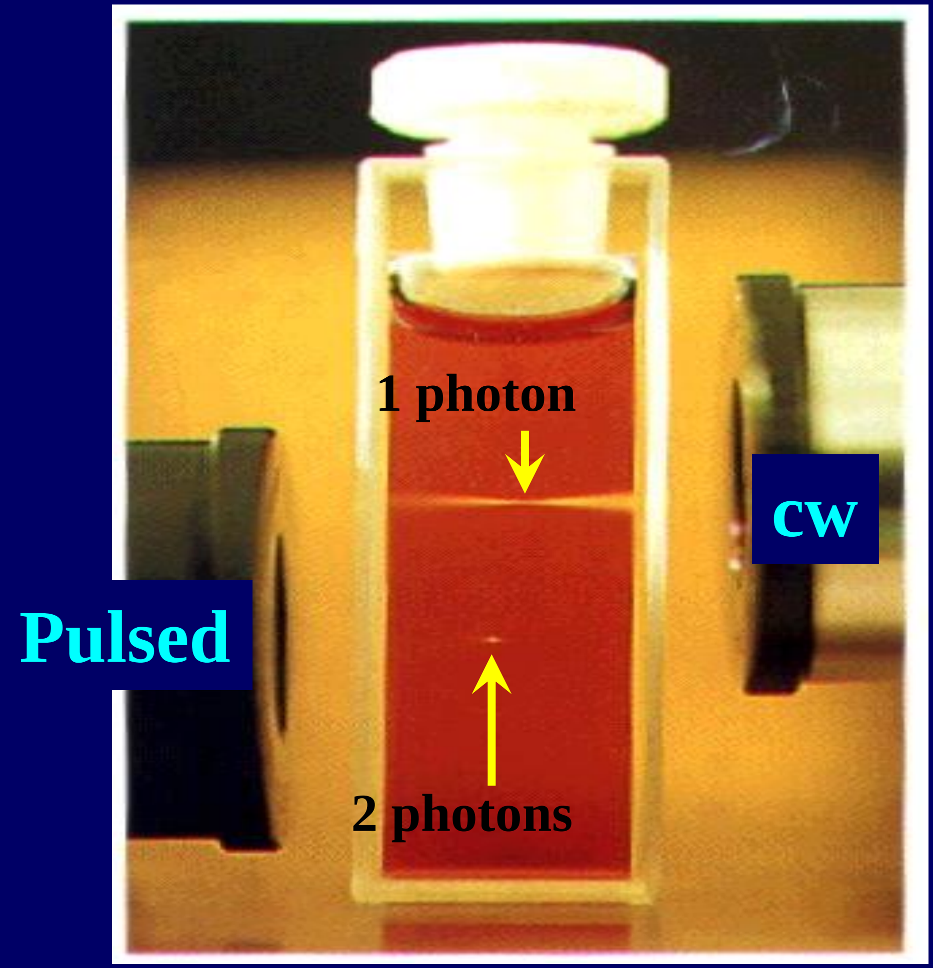


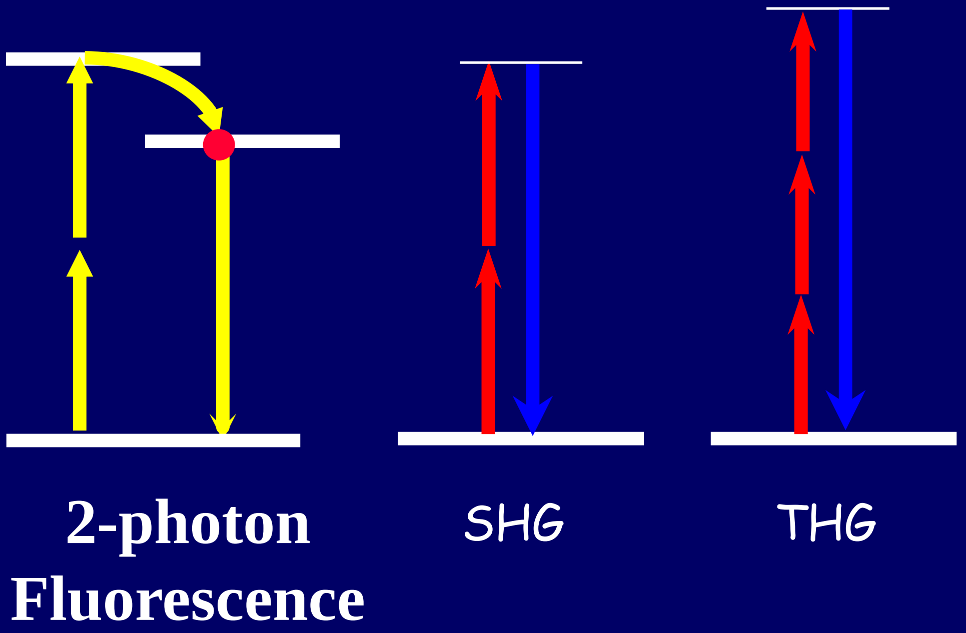
2 fótons (I^2)

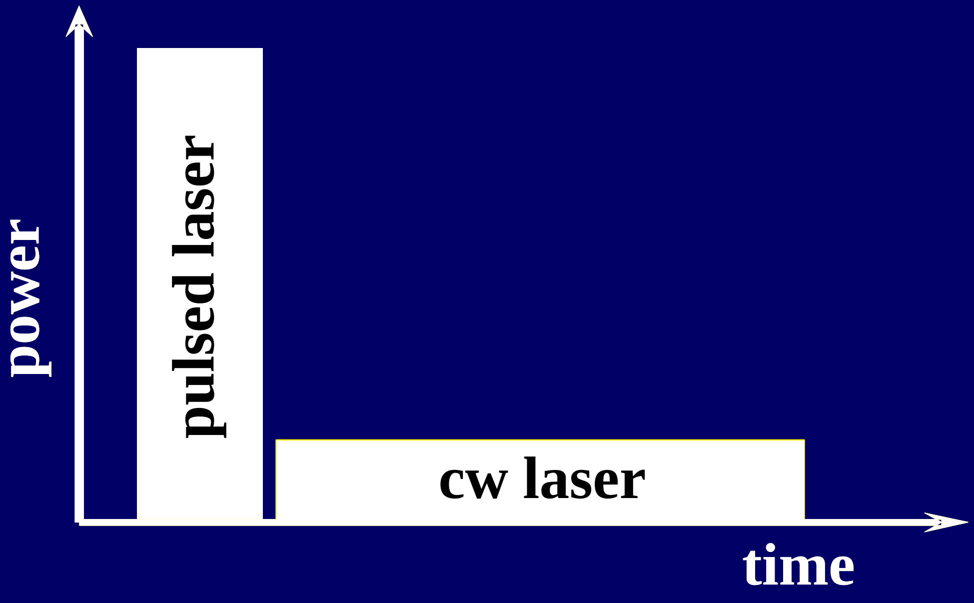




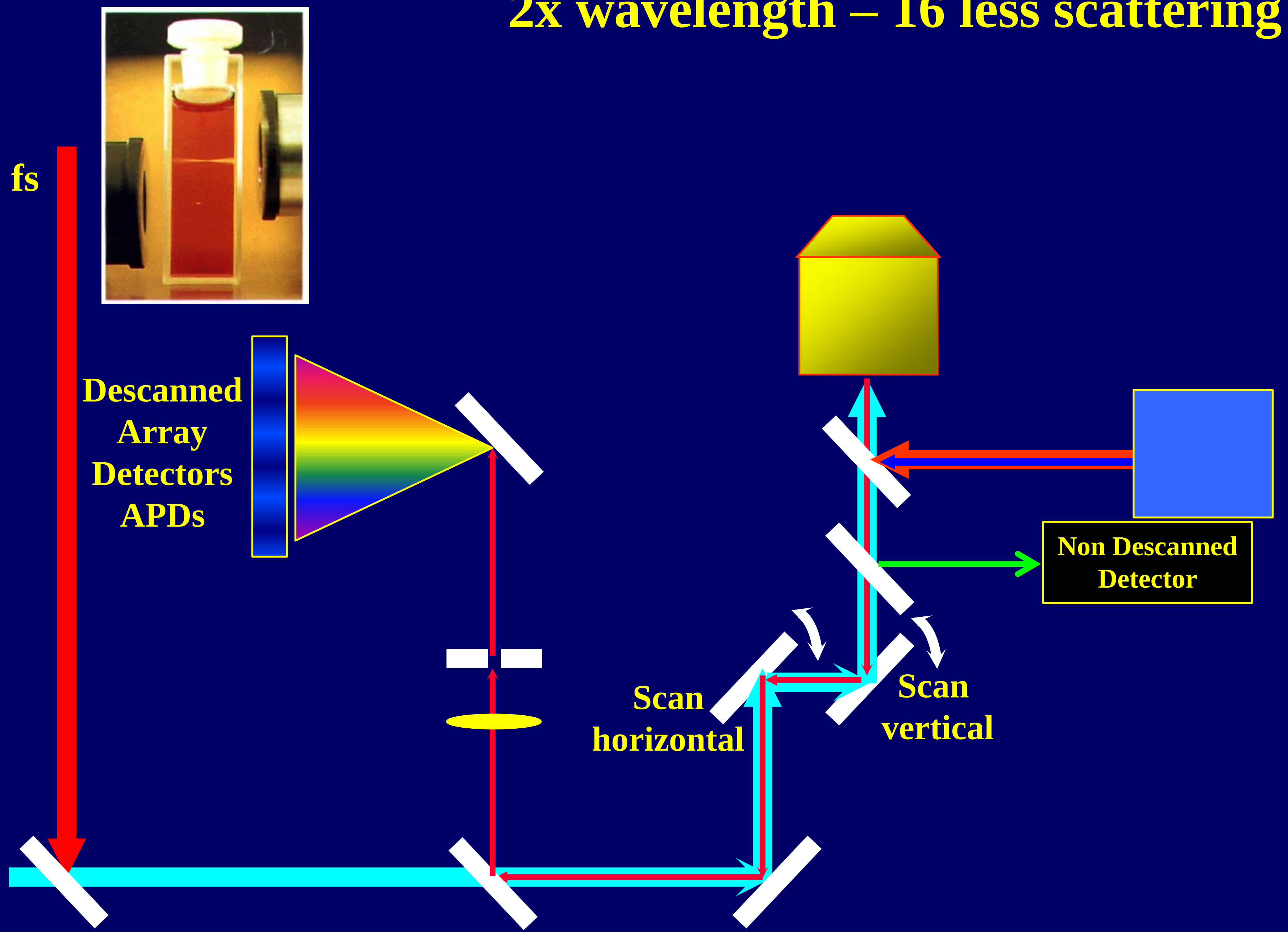
Multiphoton – spatial localization



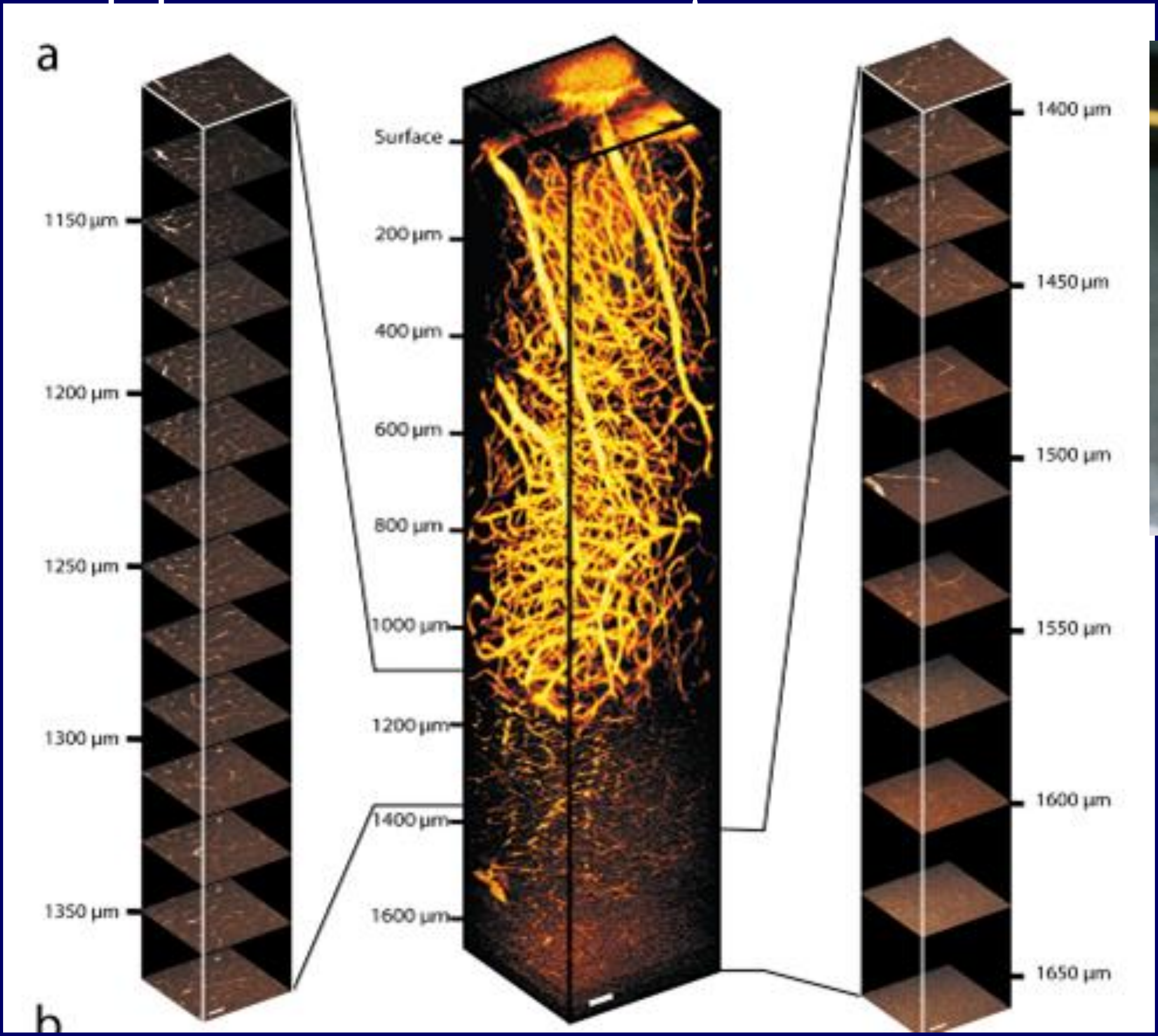




Add a femtosecond laser: multiphoton microscopy
2x wavelength – 16 less scattering



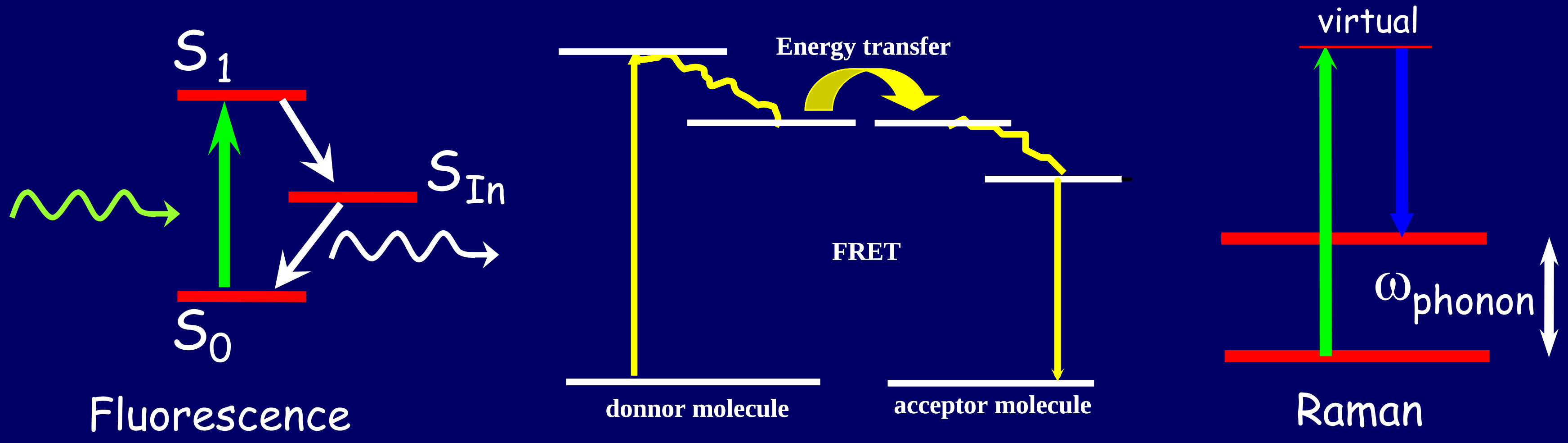
Deep penetration: > 1600 μm



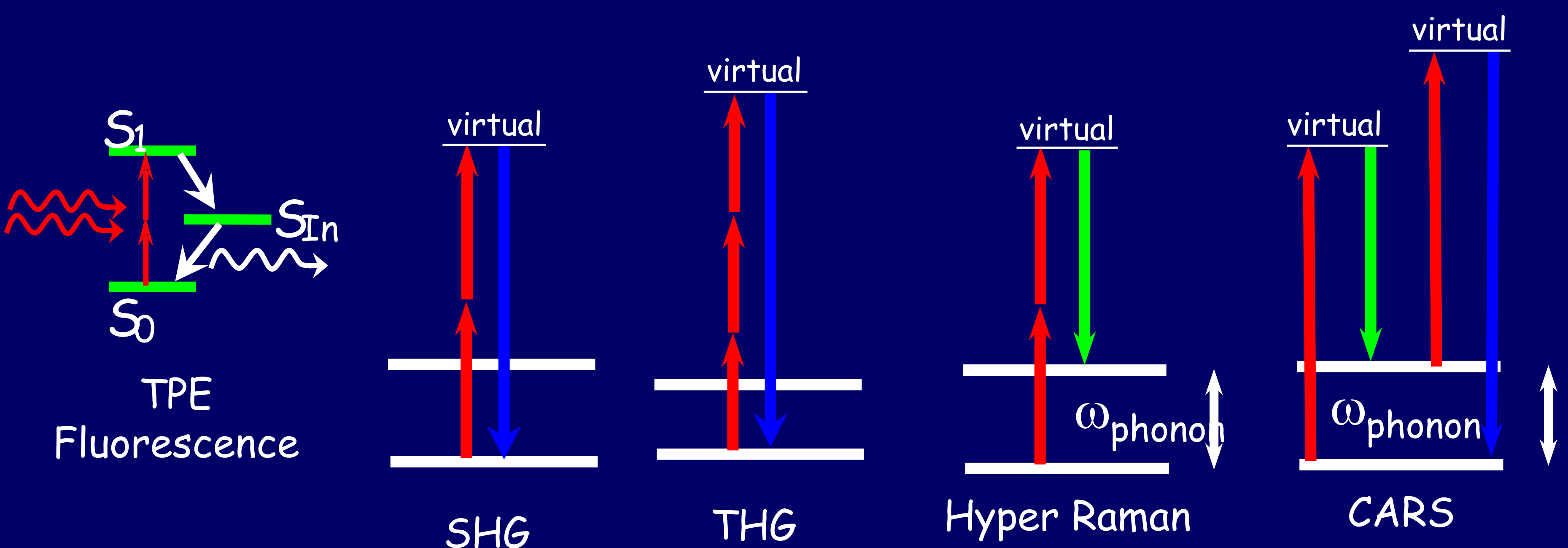
Chris Xu

Cornell

Linear Optical processes

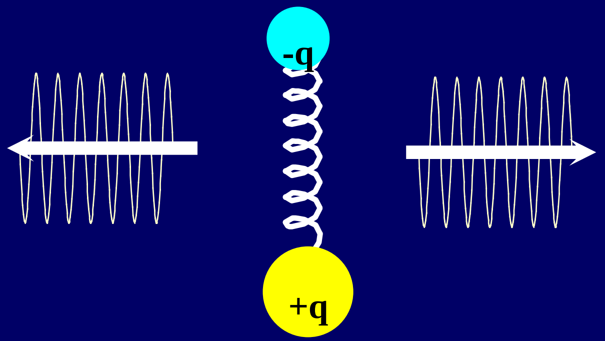


Non Linear Optical processes

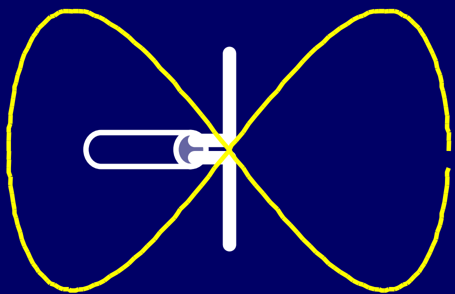


Como gerar luz? Irradiação de dipolo

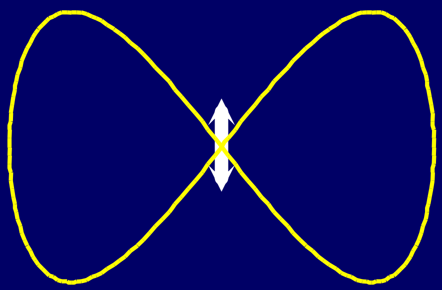
Dipolo   P = q x



antena

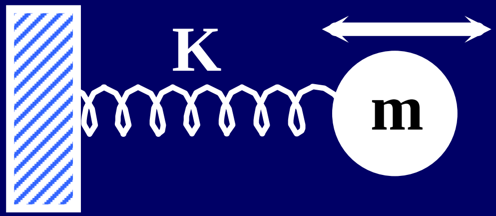


Emissão de onda de radio

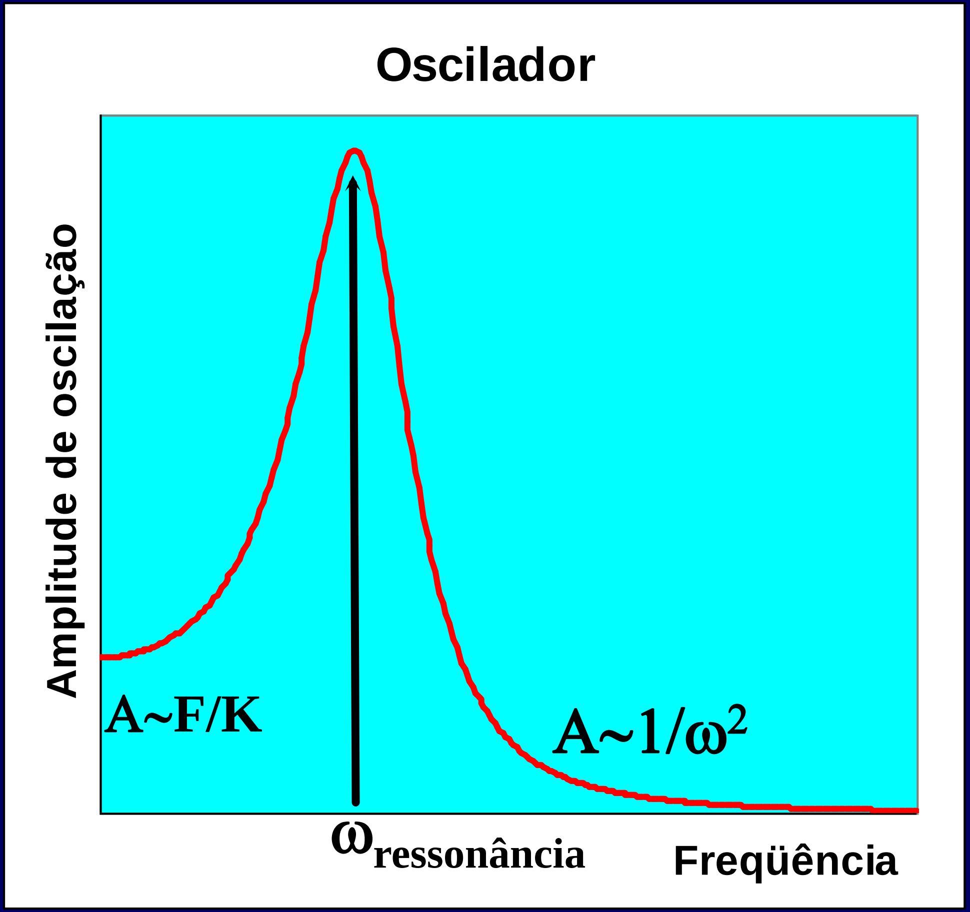


Emissão de luz por moléculas

Forced spring-mass system: resonance

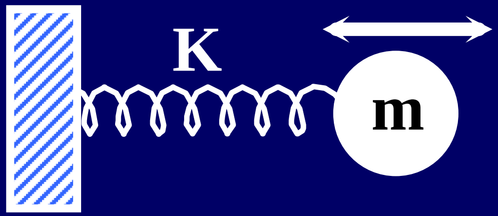


$$\omega = \sqrt{\frac{K}{m}}$$



electron mass << nucleus mass
electron follows light frequency
nucleus do not follows light

Hooke´s law is an approximation



$$F = -k\,x$$

$$F = -k\,x - \beta x^2 - \gamma x^3 - \delta x^4 \cdots$$

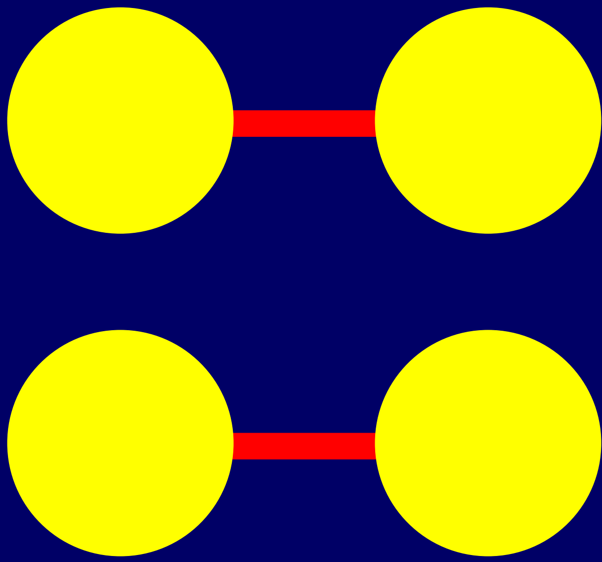
Inversion symmetry:

Force is antisymmetric: if x → - x then F → - F

$$k\,x + \beta x^2 + \gamma x^3 + \delta x^4 \cdots = k\,x - \beta x^2 + \gamma x^3 - \delta x^4 \cdots$$

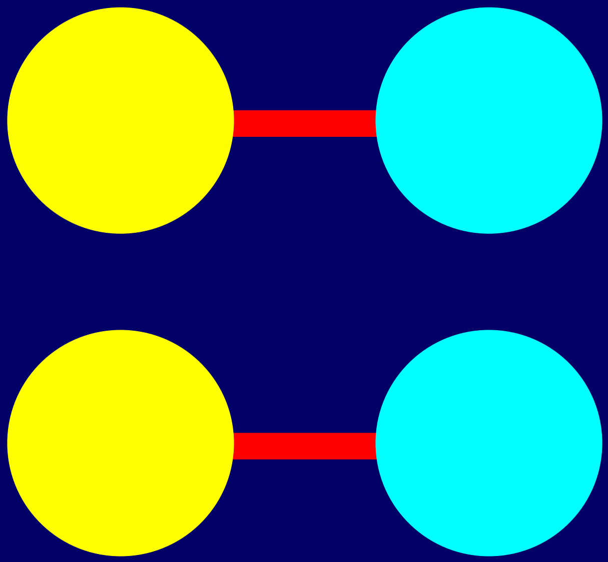
Molecular symmetry

Symmetric Molecule
Unnoticed change x by –x



$$F = -k\,x - \gamma x^{3} \cdots$$

Non-symmetric molecule
Noticed change x → -x



$$F = -k\,x - \beta x^{2} - \gamma x^{3} - \delta x^{4} \cdots$$

Electron follows optical frequency

Molecule polarization

$$P[x_n, x_e] = P_o + a\,x_e + b\,x_n + c\,x_e^2 + d\,x_e x_n + f\,x_n^2 + g\,x_e^3 + \cdots + g\,x_e^3 x_n$$

- Electronic Absorption, Rayleigh scattering ($a\,x_e$): $\chi^{(1)}$
- Infrared absorption ($b\,x_n$)
- SHG/SFG, DFG ($c\,x_e^2$): $\chi^{(2)}$
- Raman ($d\,x_e x_n$): $\chi^{(1)}$
- Overtone NIR ($f\,x_n^2$)
- THG ($g\,x_e^3$): $\chi^{(3)}$
- CARS ($g\,x_e^3 x_n$): $\chi^{(3)}$

NLO vocabulary – only electron counts

Raman $P[x_n, x_e] = \cdots + d\, x_e x_n + \cdots$

$$\cos a \cos b = \frac{1}{2}\cos(a+b) + \frac{1}{2}\cos(a-b)$$

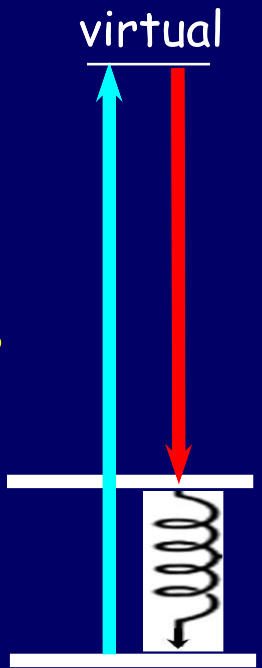
$$a = \omega_e t \quad b = \omega_n t$$

Stokes **AntiStokes**

$$\cos(\omega_e t)\cos(\omega_n t) = \frac{1}{2}\cos\left[(\omega_e - \omega_n)t\right] + \frac{1}{2}\cos\left[(\omega_e + \omega_n)t\right]$$

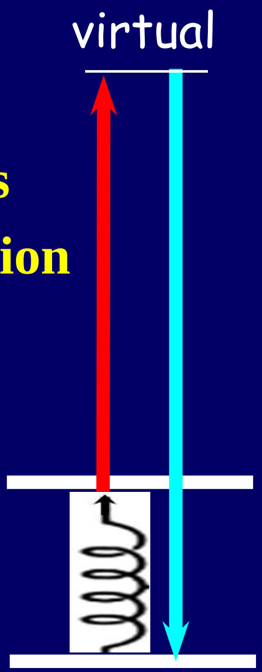
Raman Stokes

Inelastic - heating

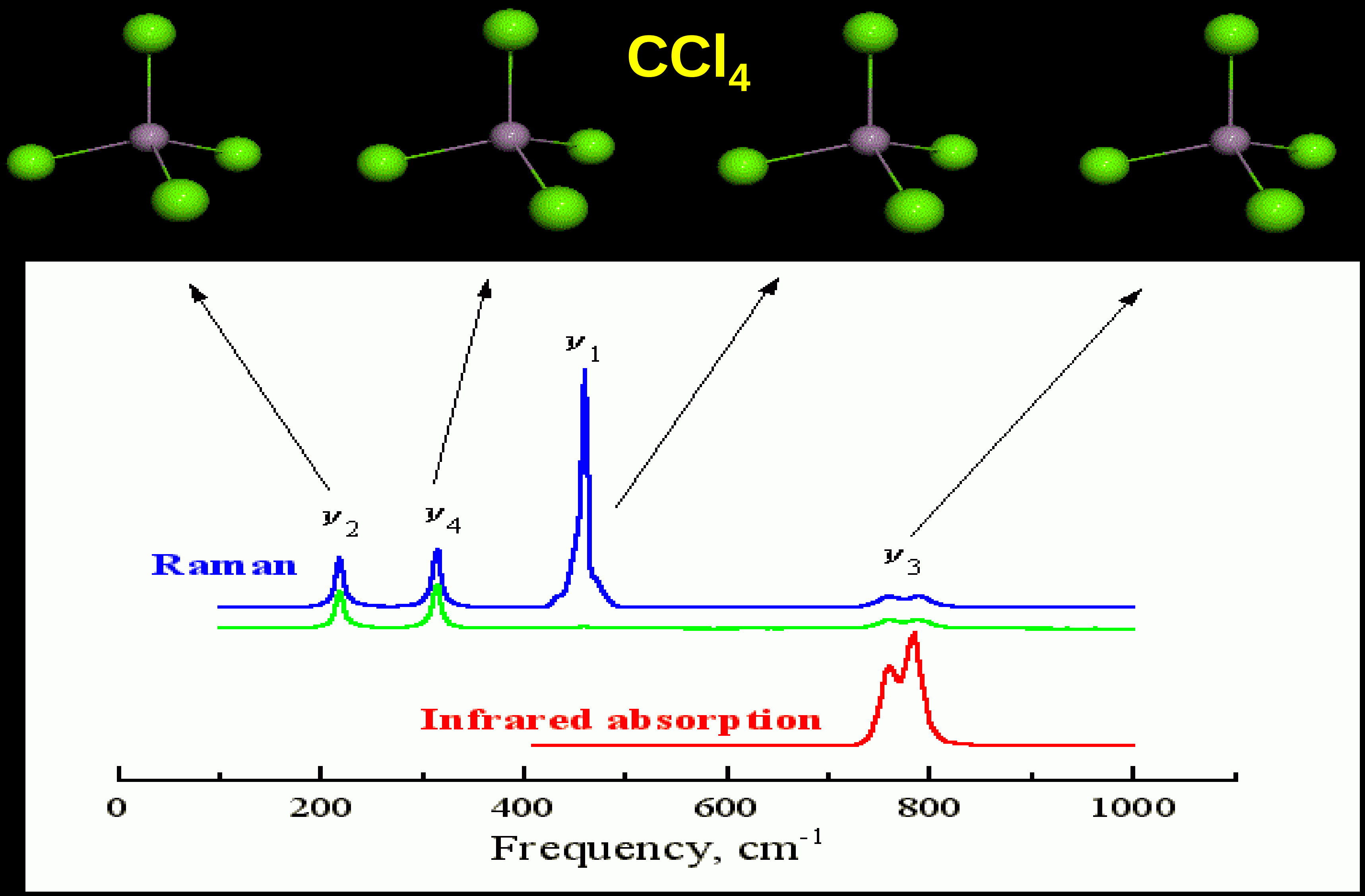


Raman AntiStokes

Inelastic - refrigeration

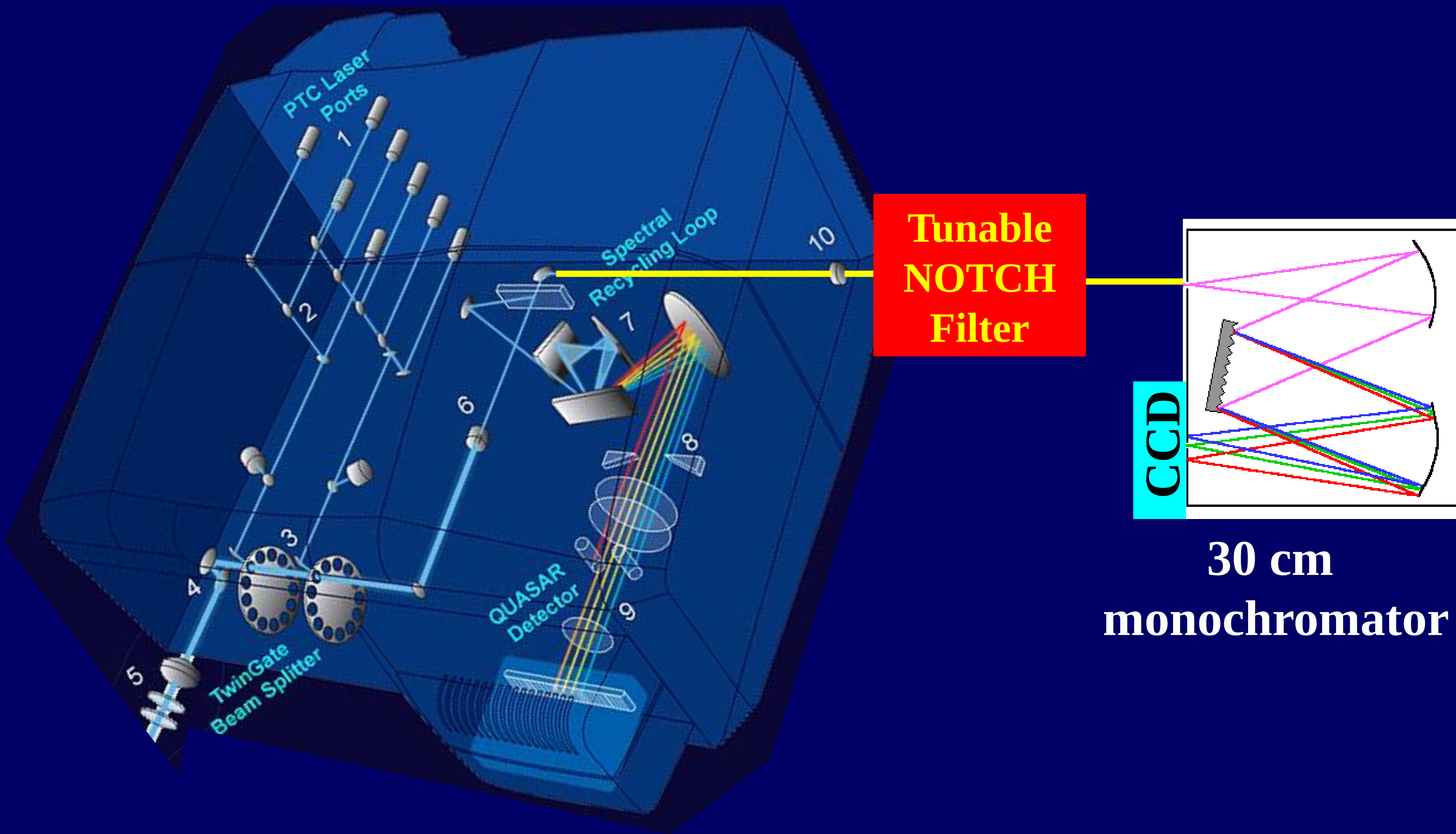


Biochemical information of molecular vibrations

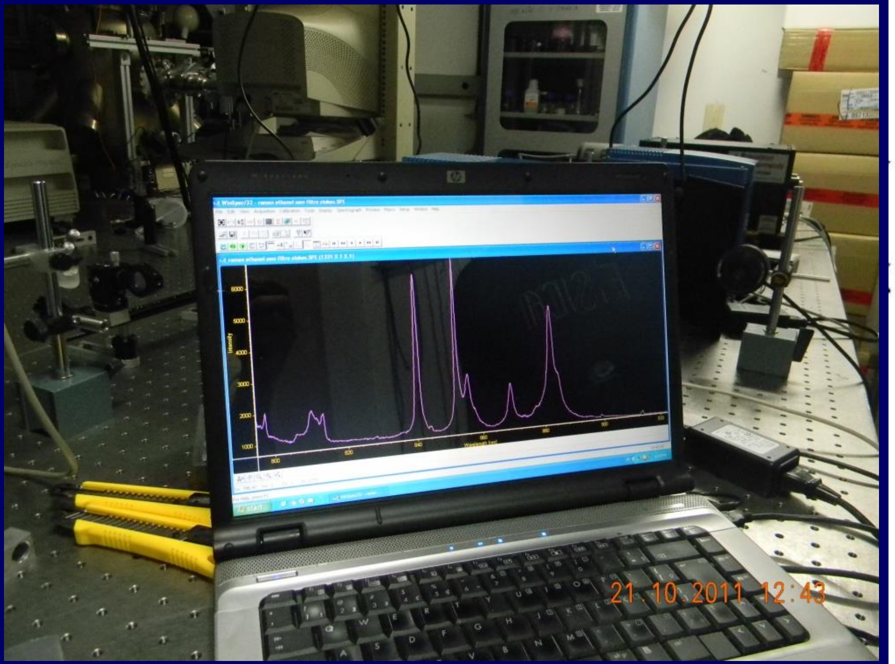


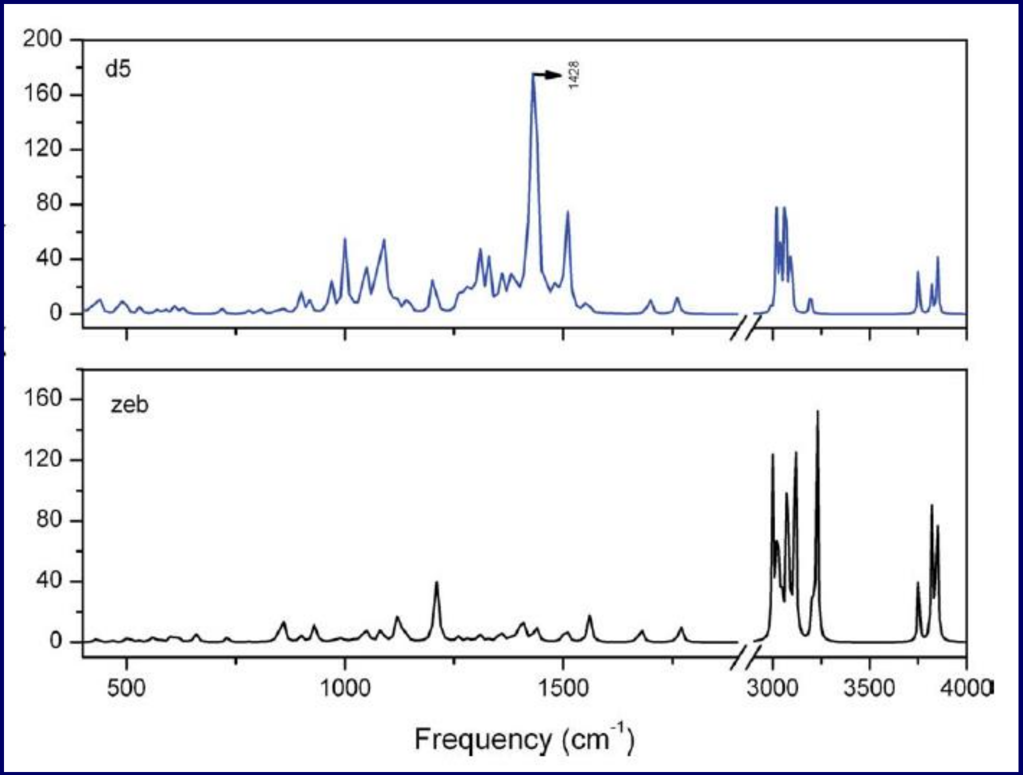
http://fy.chalmers.se/~brodin/MolecularMotions/CCl4molecule.html

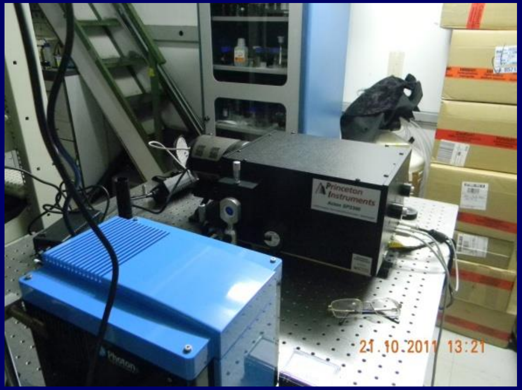
Add a confocal Raman Spectroscopy



Raman - Methylated vs non-methylated DNA

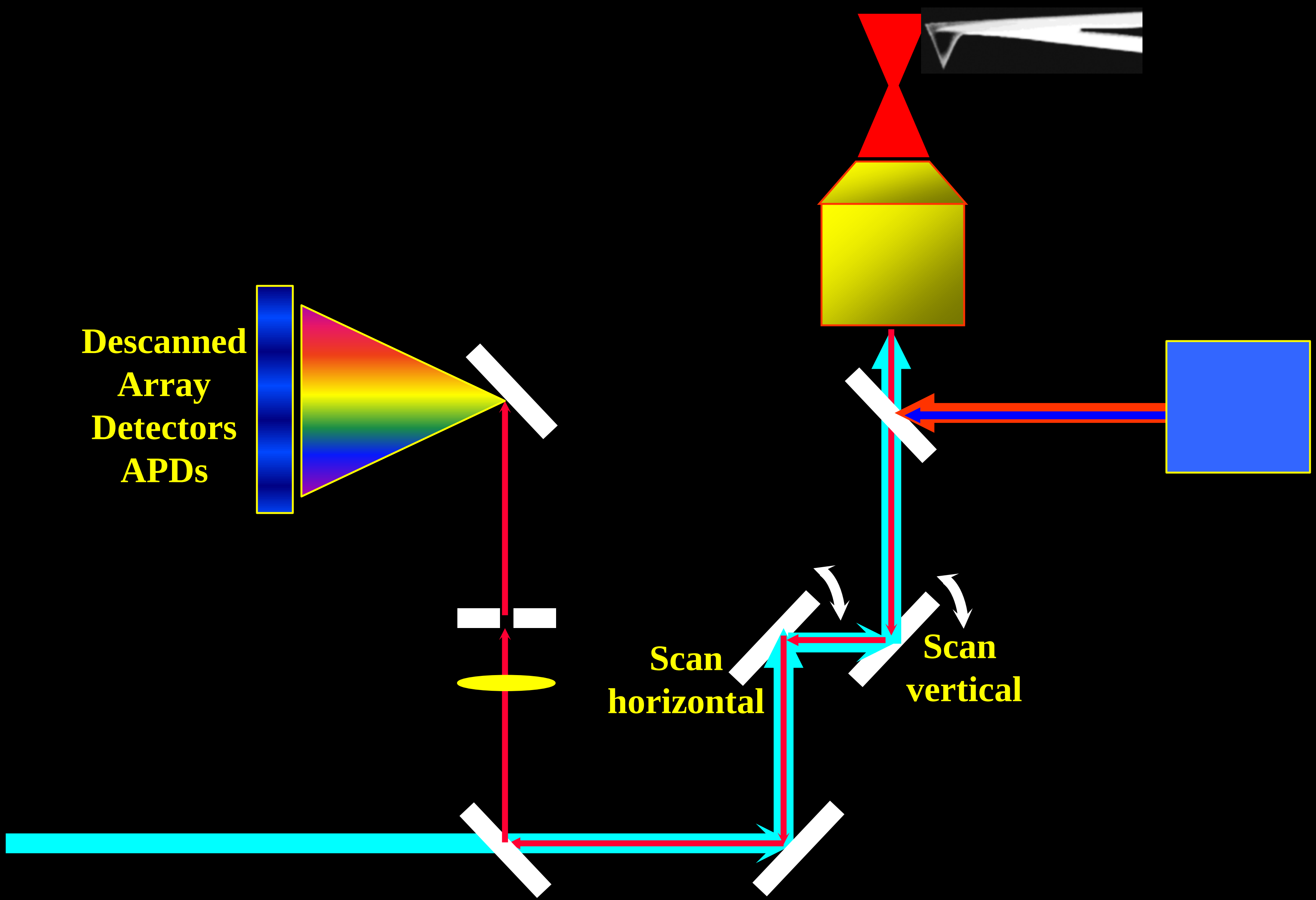




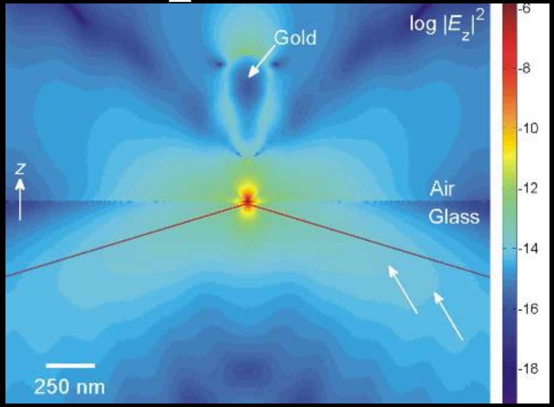


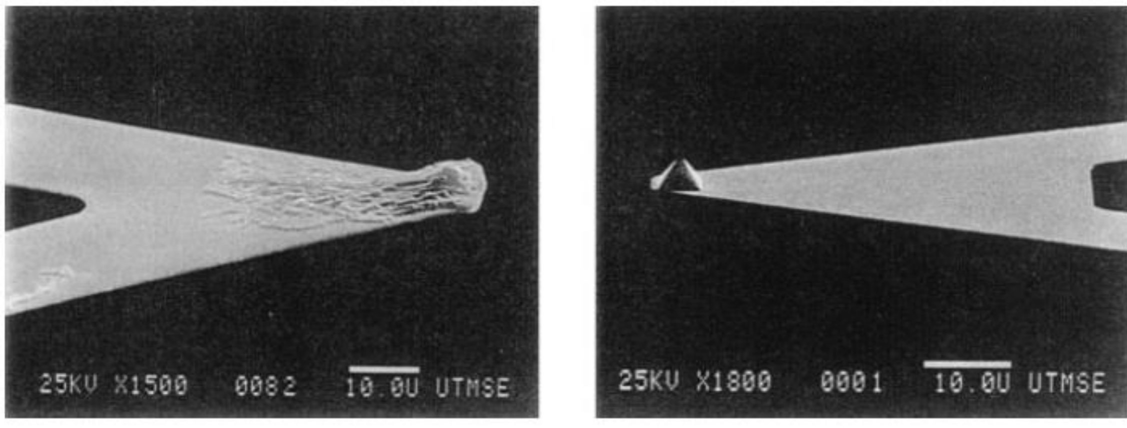
AFM &
Tip-enhancement near field microscopy

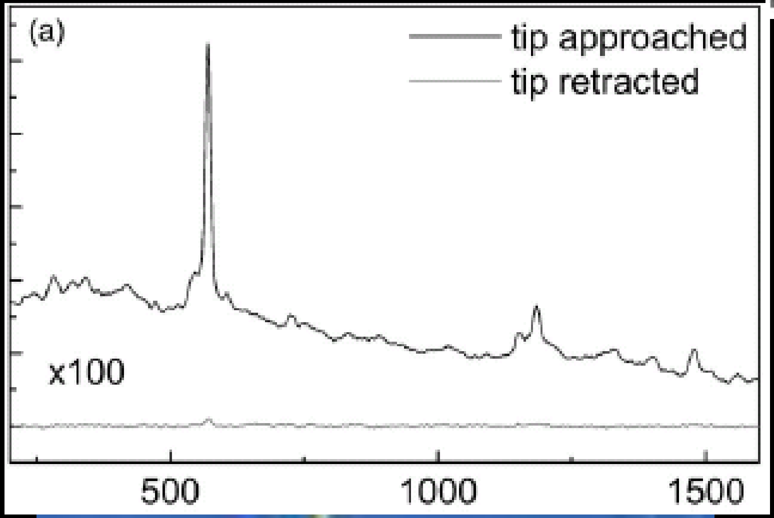
Add an AFM/Tip-enhancement system on top

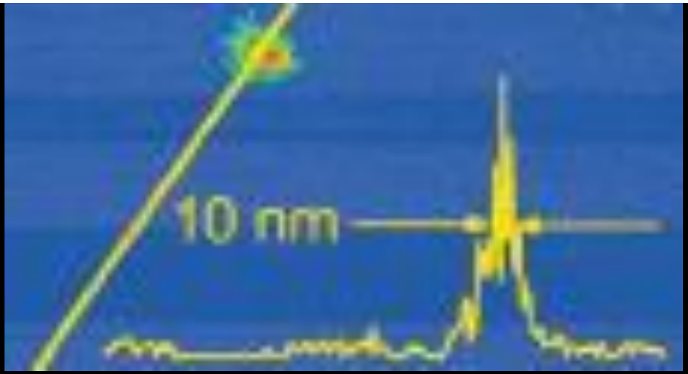


Tip-Enhanced microscopy & spectroscopy



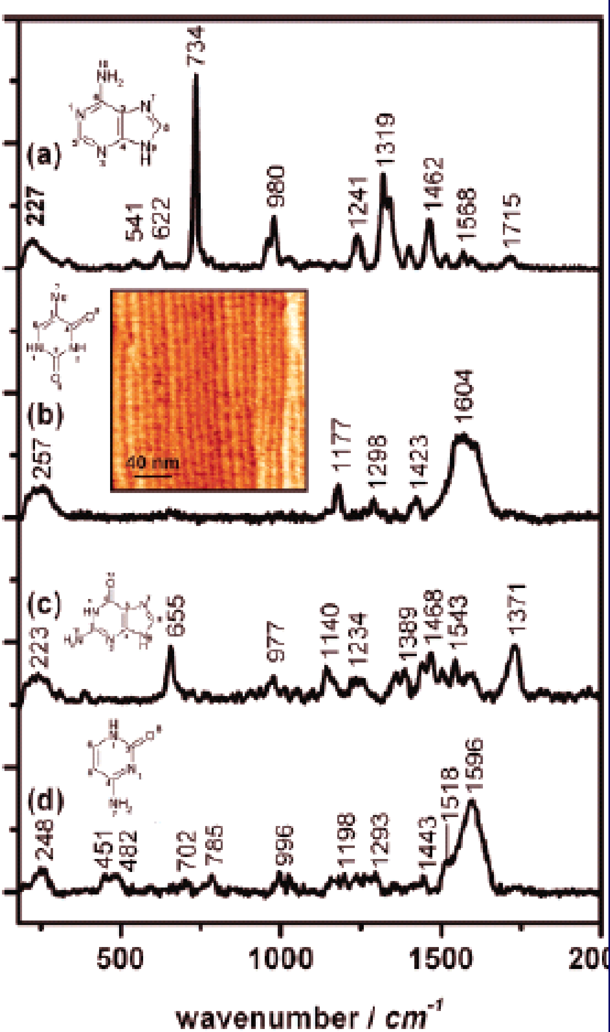






Single molecule photo-biochemistry & sequencing

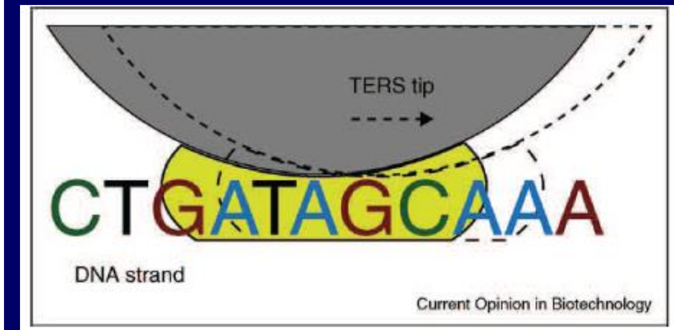
TERS

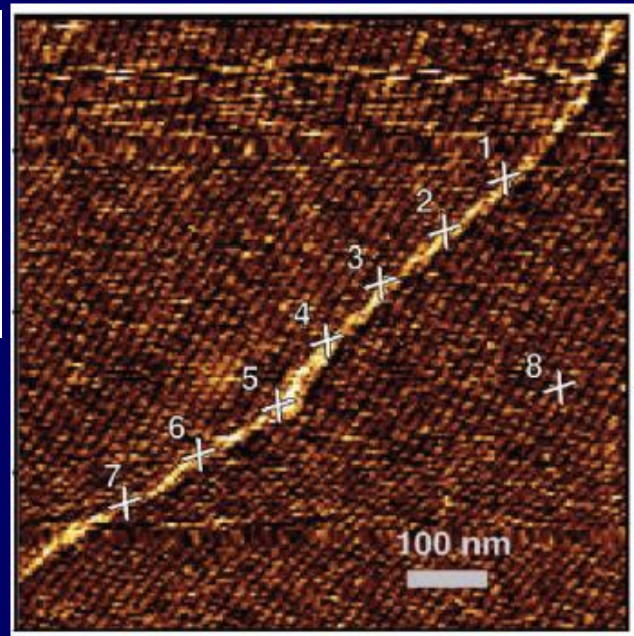


Recent advances in single-molecule sequencing

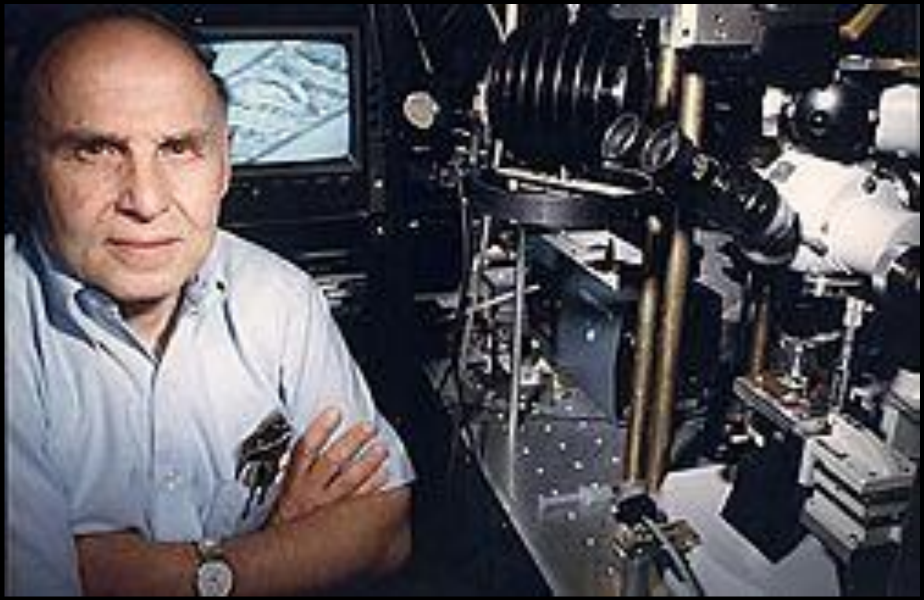
Regina Treffer[1] and Volker Deckert[1,2]

Current Opinion in Biotechnology 2010, 21:4–11



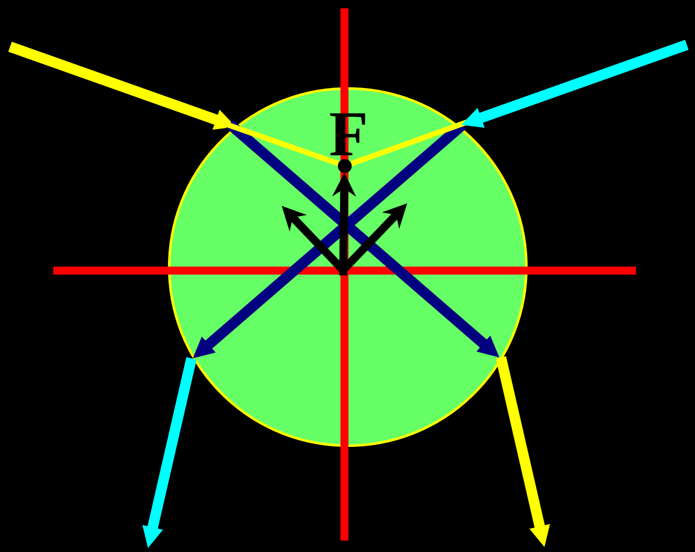


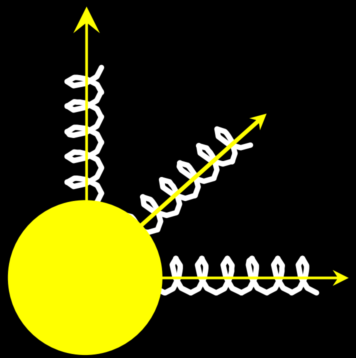
Optical Tweezers

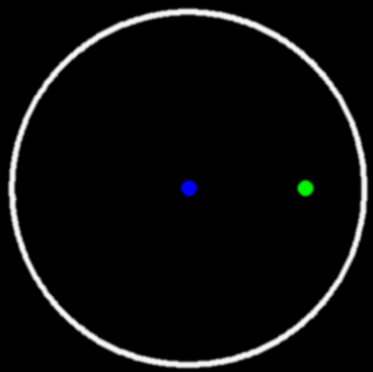


Arthur Ashkin - 1986
Physicist – AT&T Bell Laboratories

Stable trap: Single Beam Optical Tweezers

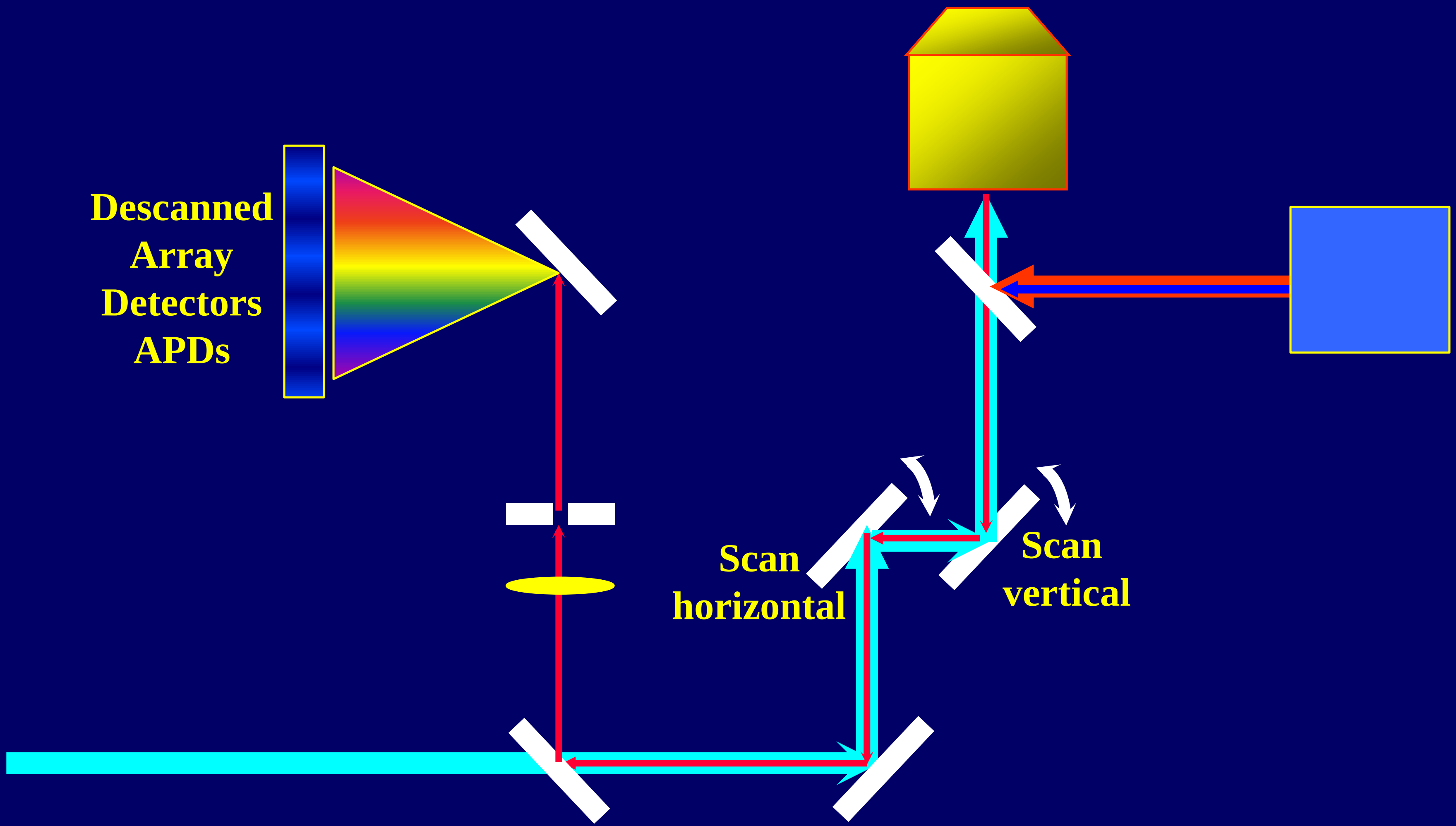




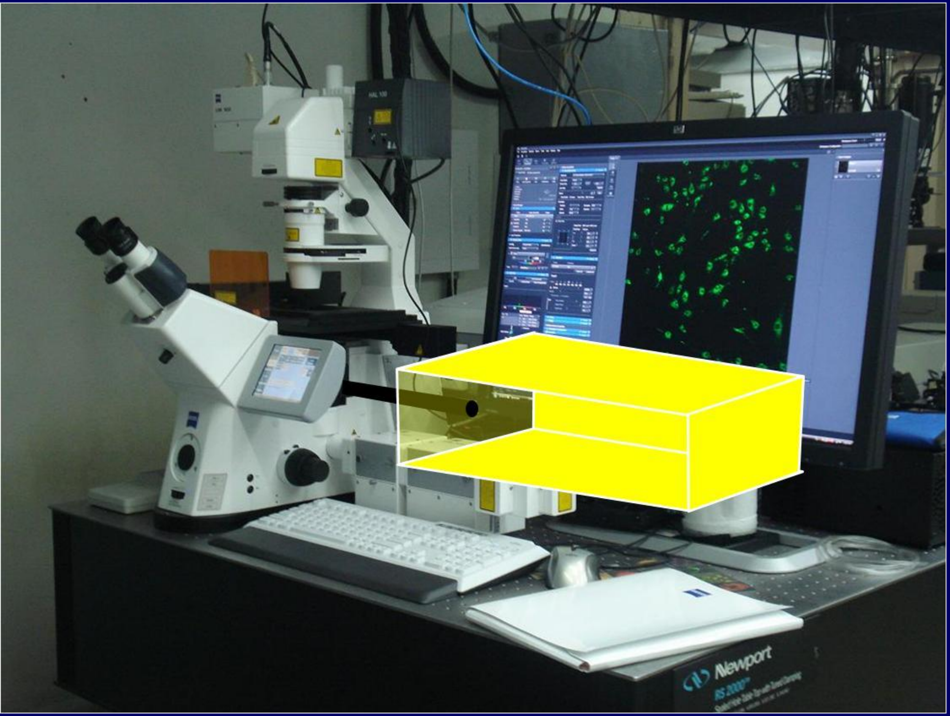


Restorative force: always tending to bring the center to the focus

Make room to add an Optical Tweezers and laser cutting

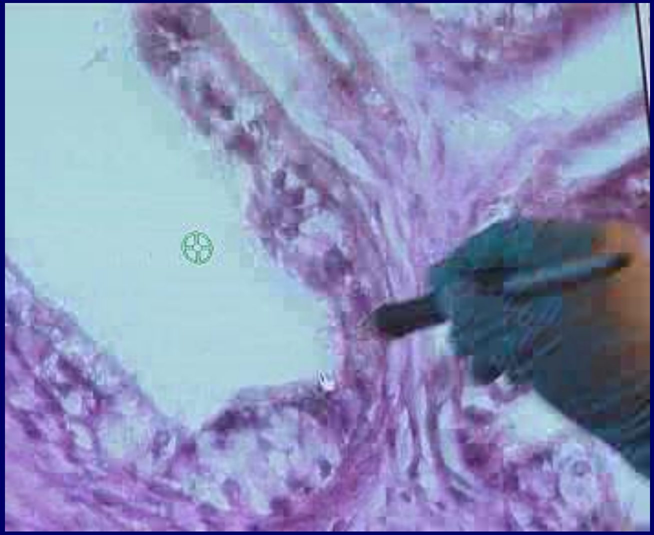


Optical Tweezers & Laser Cutting



Biomechanics F~500 pN
Cell rheology; manipulation;
Zeta potential ...

+ Laser cutting:
Controlled transfection;
Cell surgery;
Material collection; ...



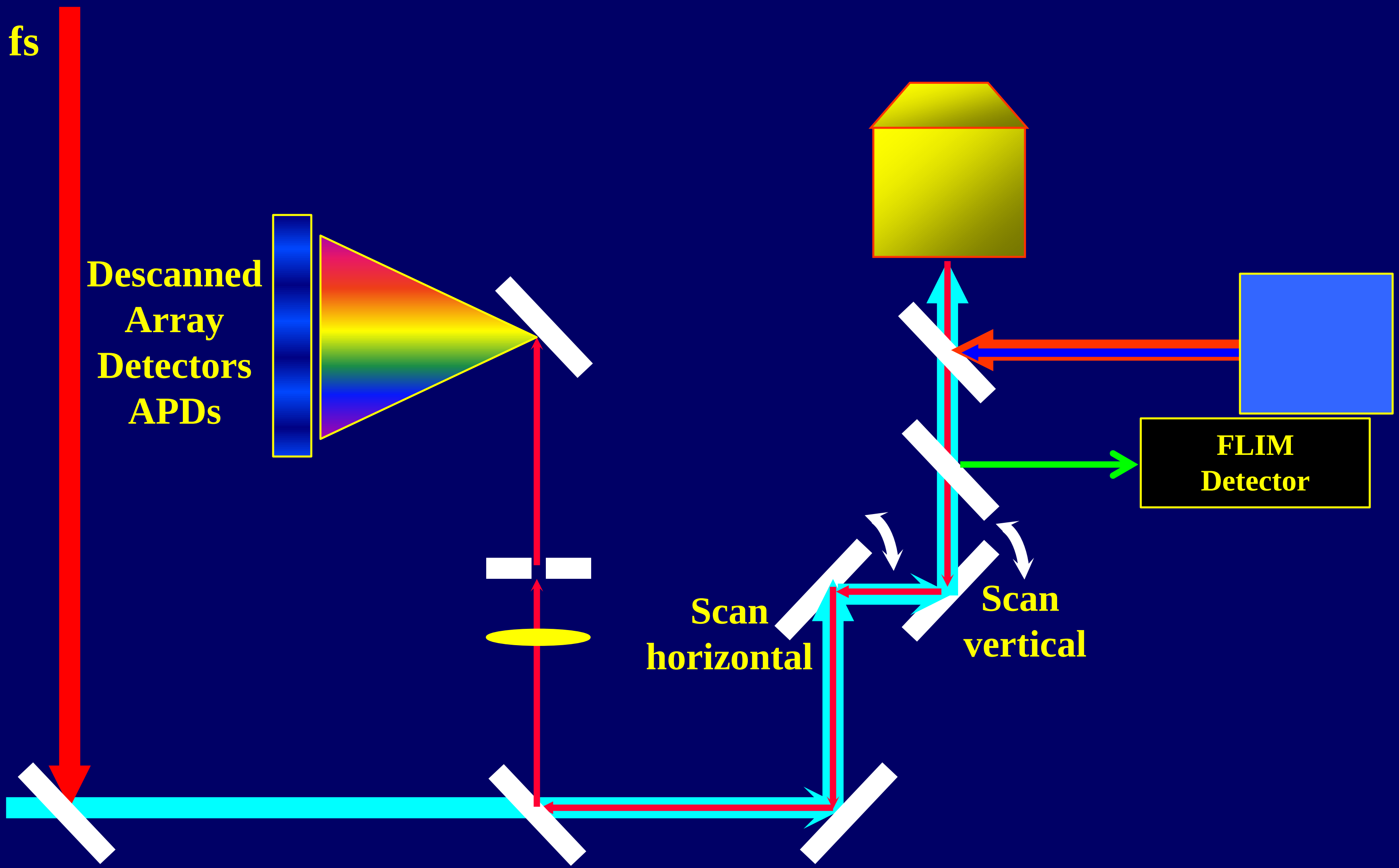
Optical Tweezers

"Light Sucks"

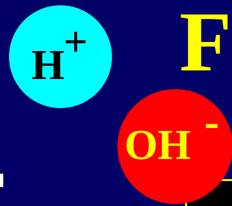
Biomechanics F~200 pN
Cell rheology
Cell manipulation
12 papers with hemocenter

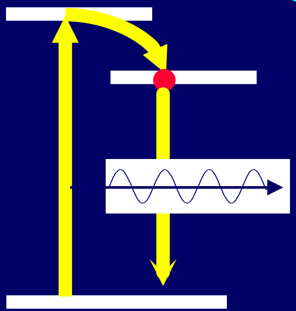
+ Laser microdissection:
Controlled transfection;
Cell surgery;
Material collection;

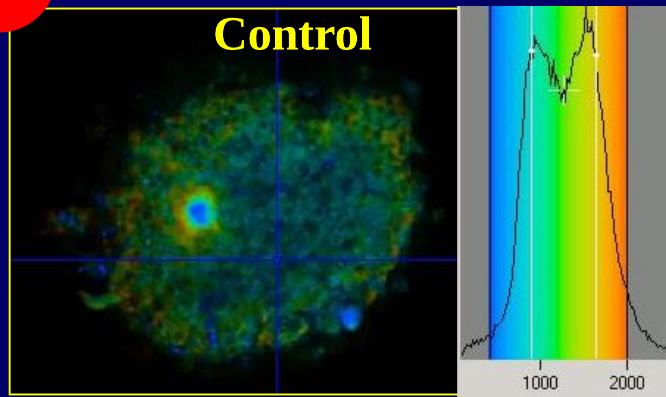
Add a cronometer – get the arrival time of each photon FLIM – fluorescence lifetime imaging

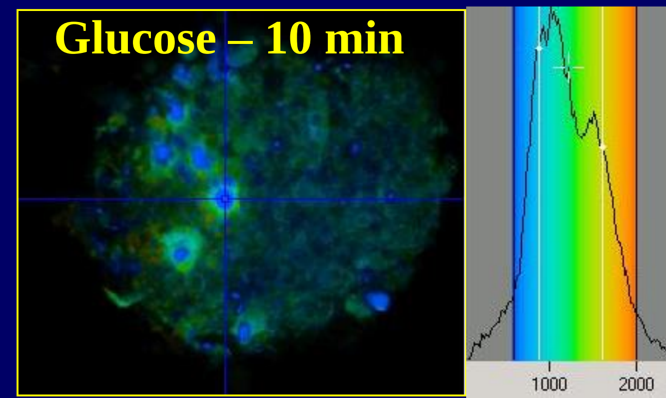


FLIM : Fluorescence Lifetime Imaging

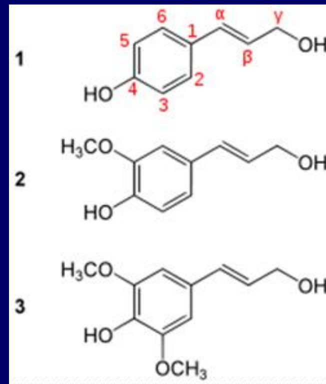


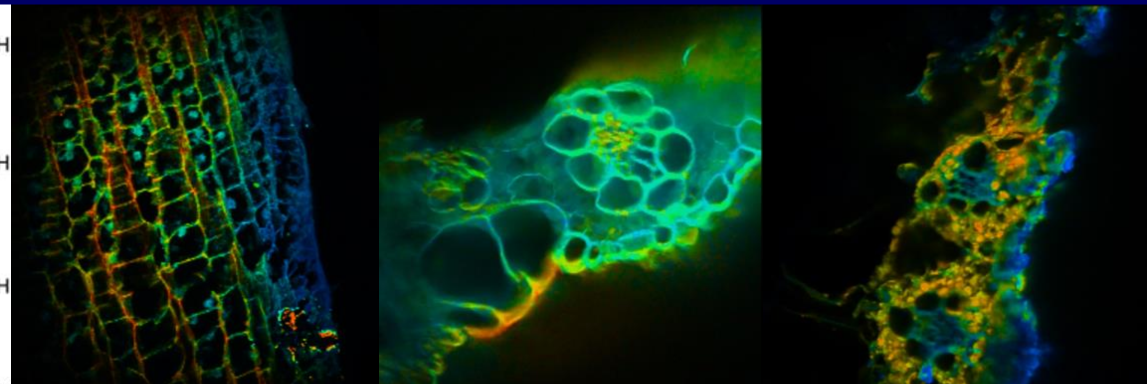




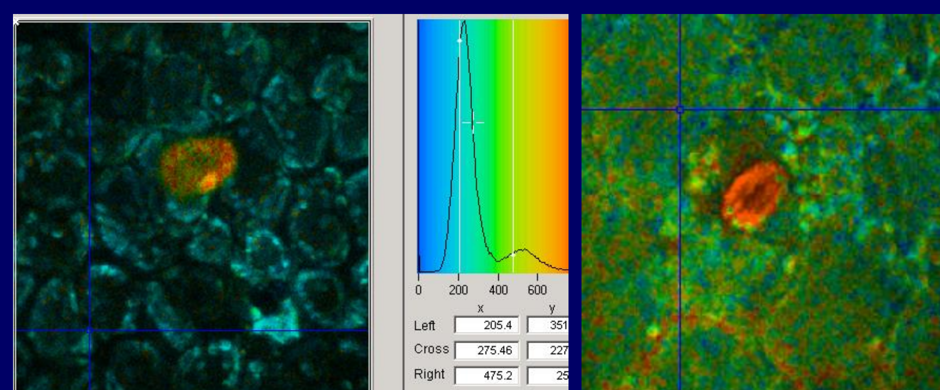


Langerhan´s Island
Everardo Biol



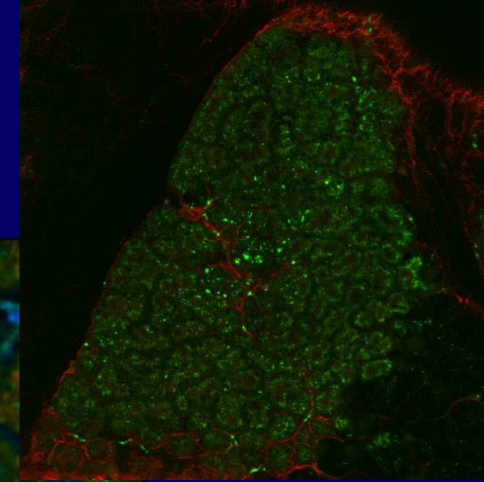


Sugar cane leaf, stem and root lignin autofluorescence
Mazzafera Biol

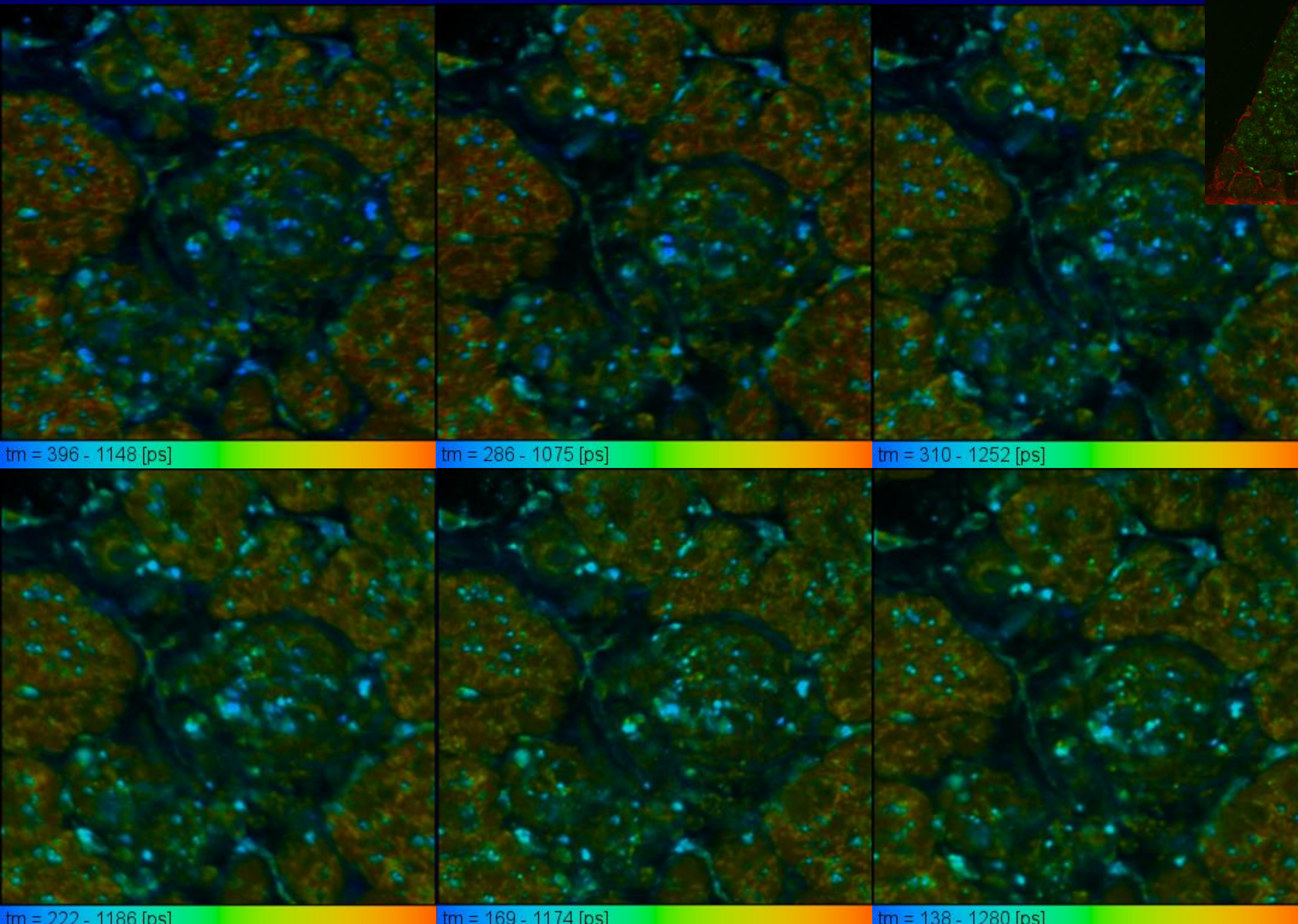


Oregano leaf
stomata and chloroplasts

In vivo microscopy – mice pancreas

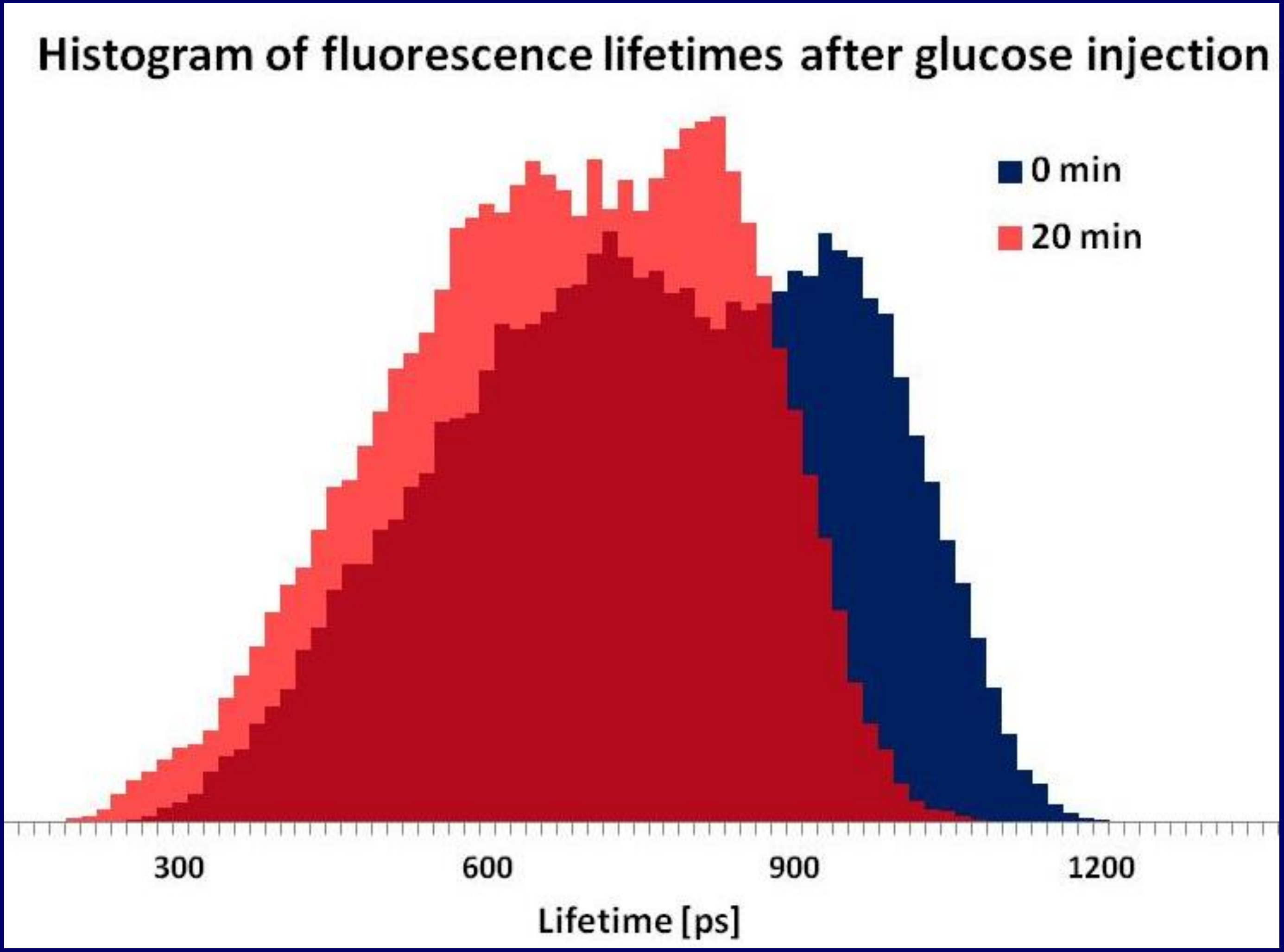


0 min 5 min 10 min



15 min 20 min 40 min

In vivo microscopy: mice pancreas lifetime histogram after glucose injection



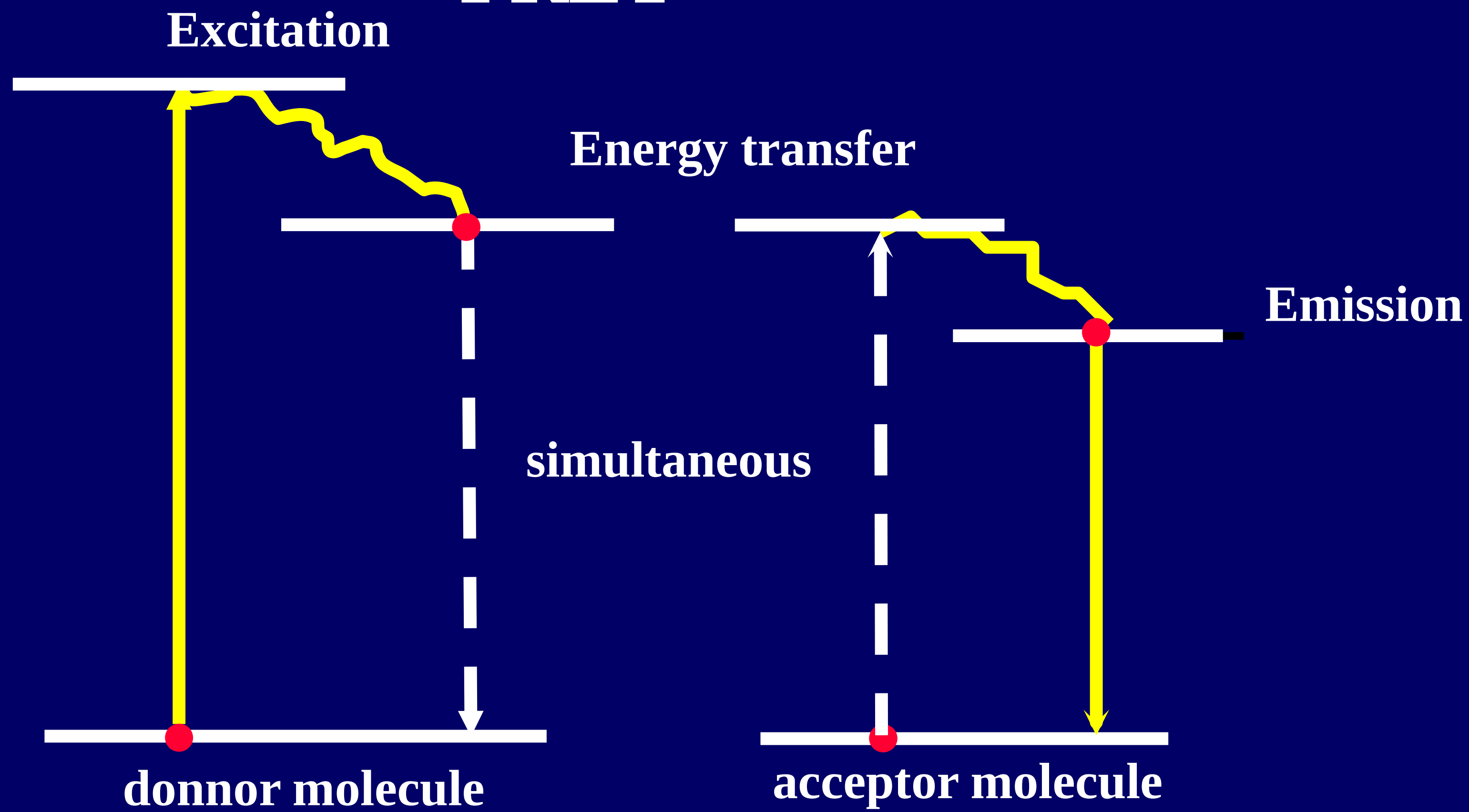
FRET
Förster Resonant Energy Transfer

“Guilt by association”

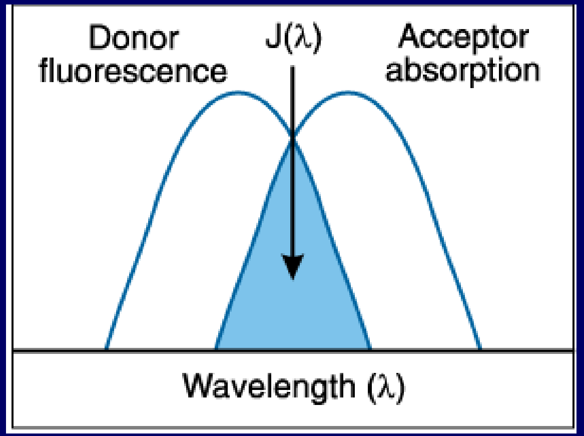
ou

“Dize-me com quem andas e te direi quem és”

FRET



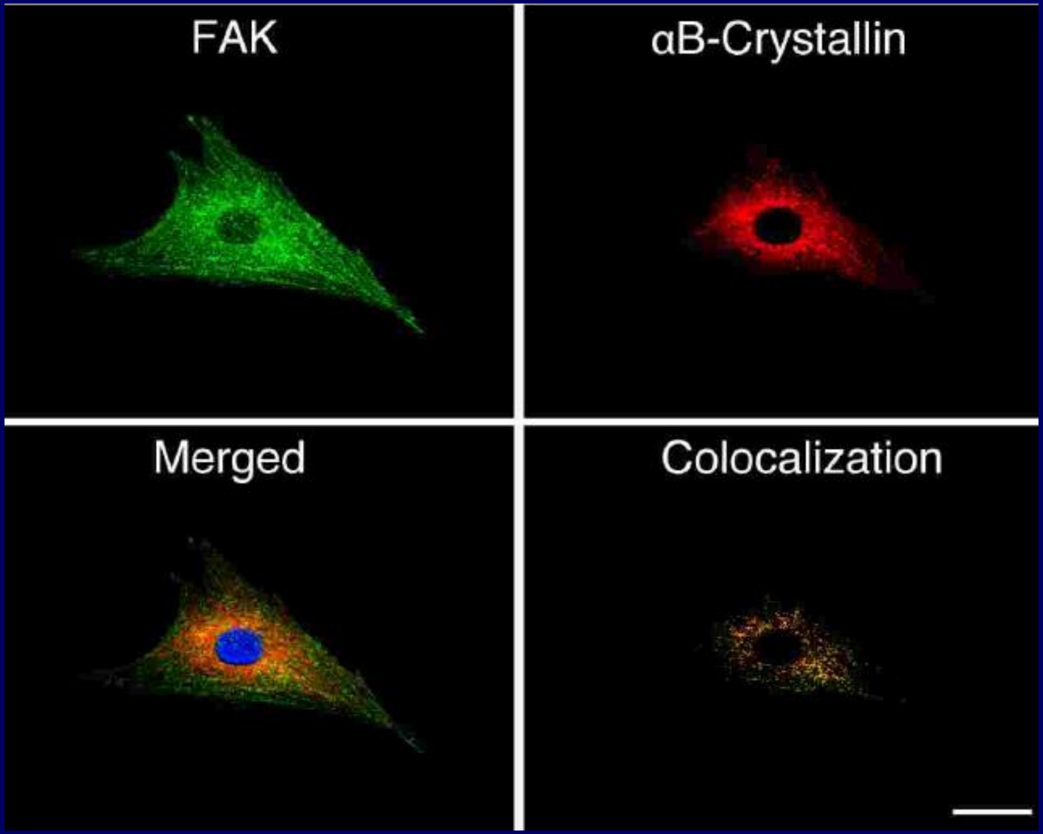
FRET only happens if molecules are very close ~ 5-10 nm: probing of intermolecular proximity

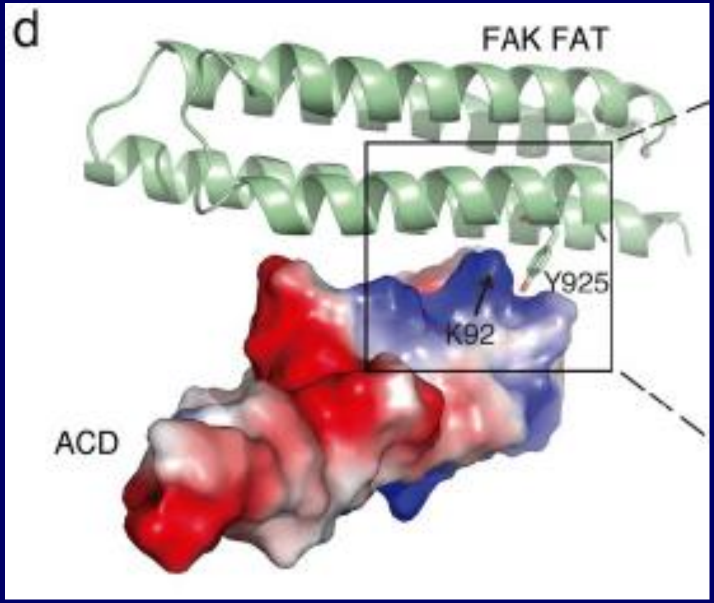


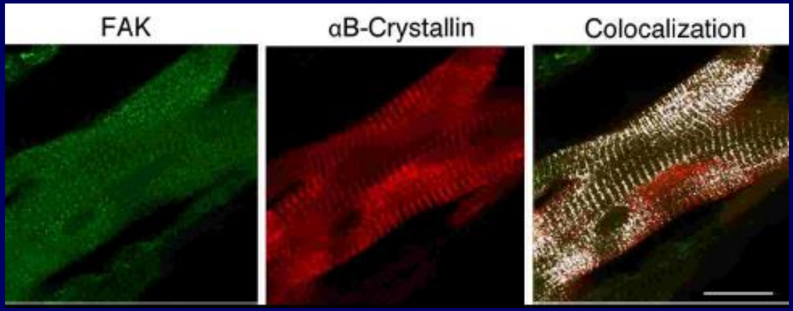
αB-Crystallin recruits FAK to promote the survival of cardiomyocytes upon induction of mechanical stress

M. B. M. Pereira, A. M. Santos, D. Gonçalves, A. C. Cardoso, S. Consonni, F. C. Gozzo, P. S. Oliveira, A. R. Figueiredo, A. O. Tiroli, C. Ramos, A. A. de Thomaz, C. L. Cesar and K. Franchini.

Accepted in Nature Communications

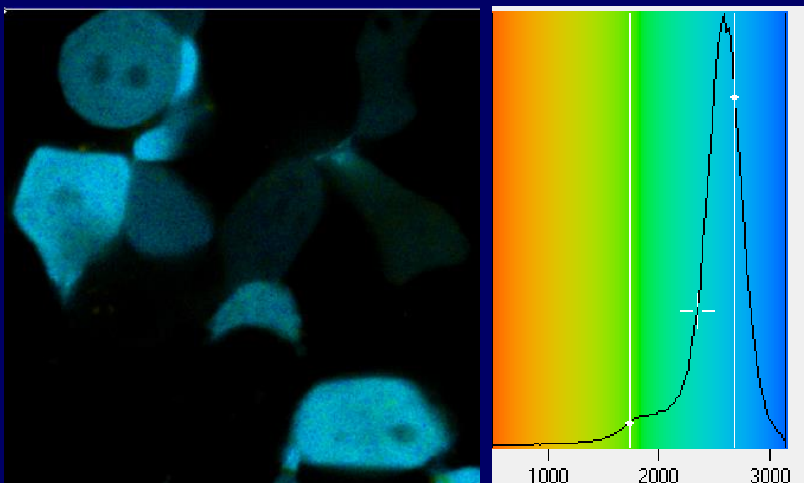


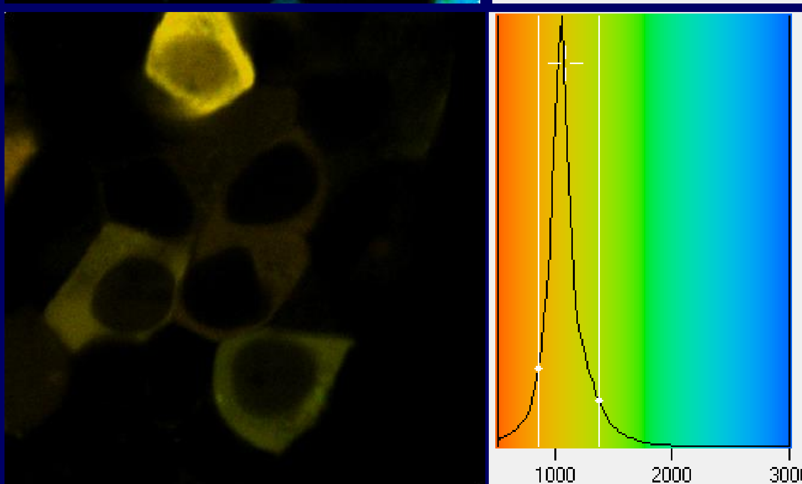




Our first FRET result

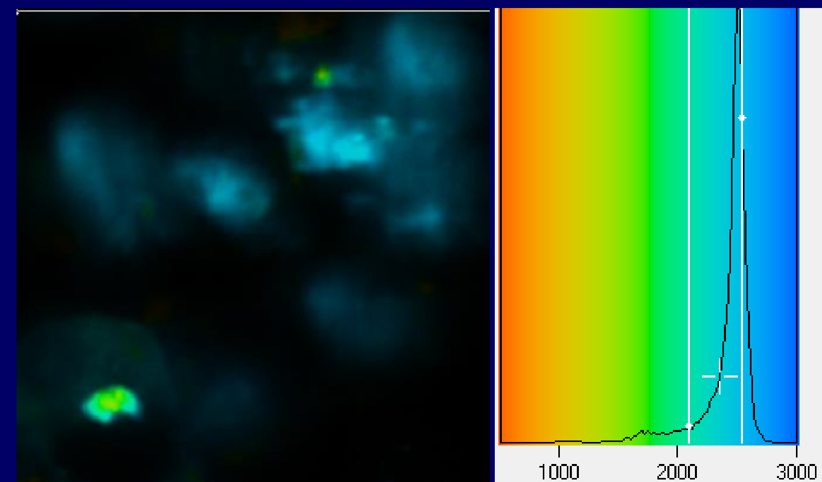
CFP + YFP:
Negative control

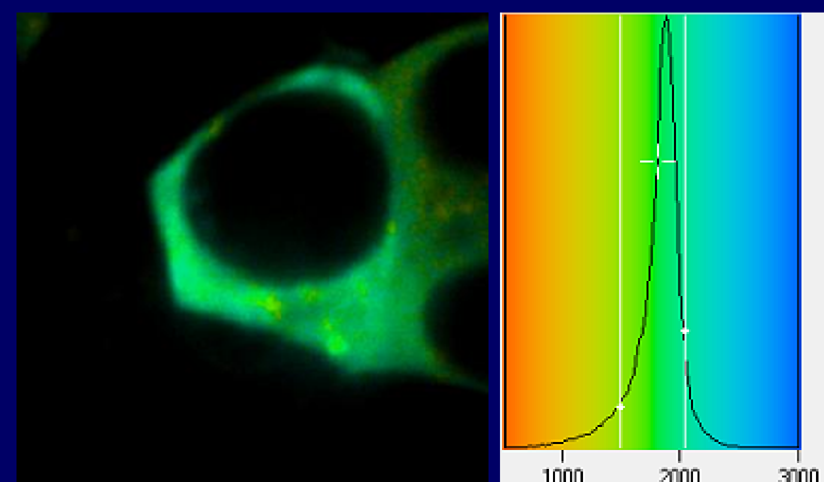




CFP - 15 AA - YFP

CFP-Cry-ab + YFP:
Negative Control

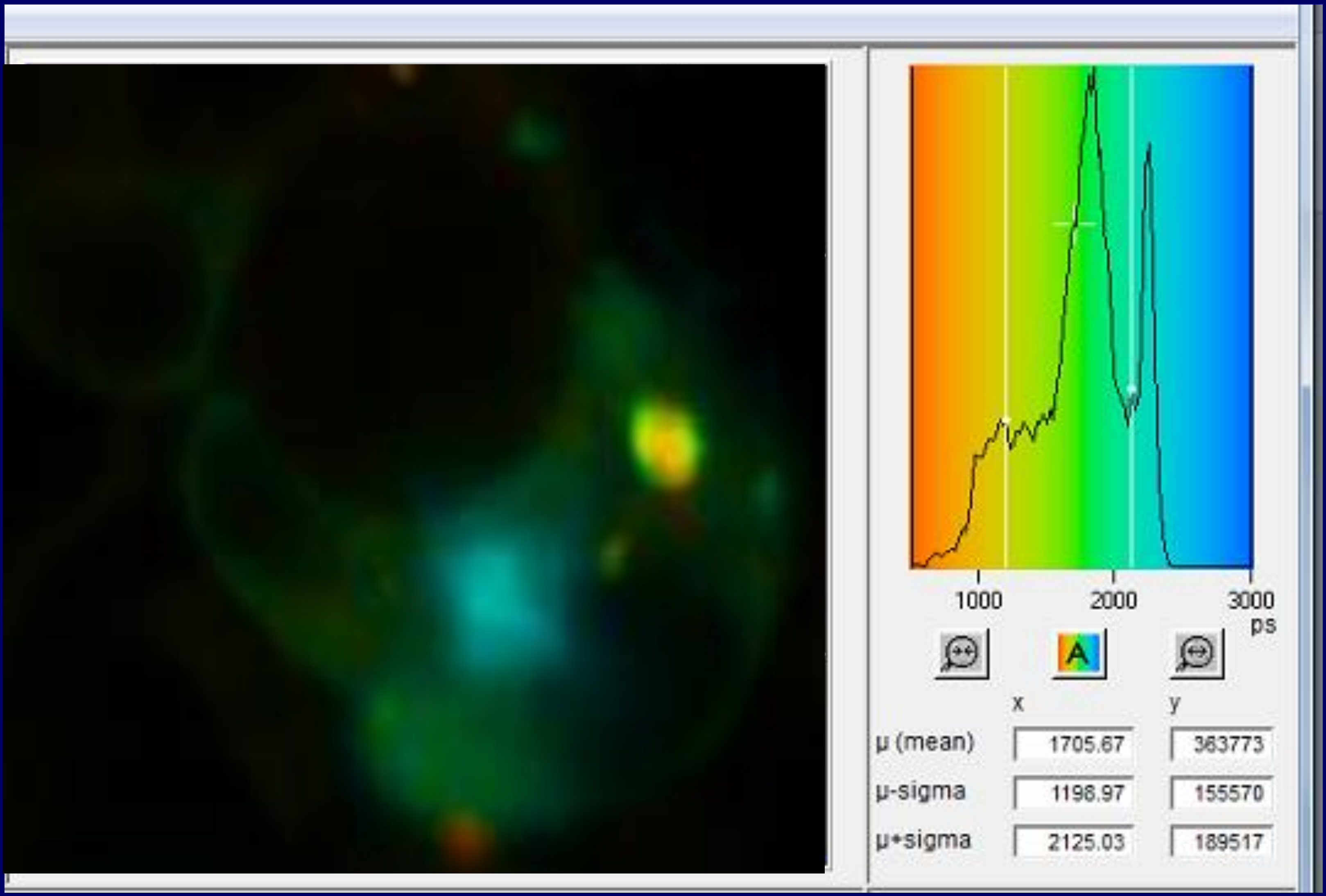




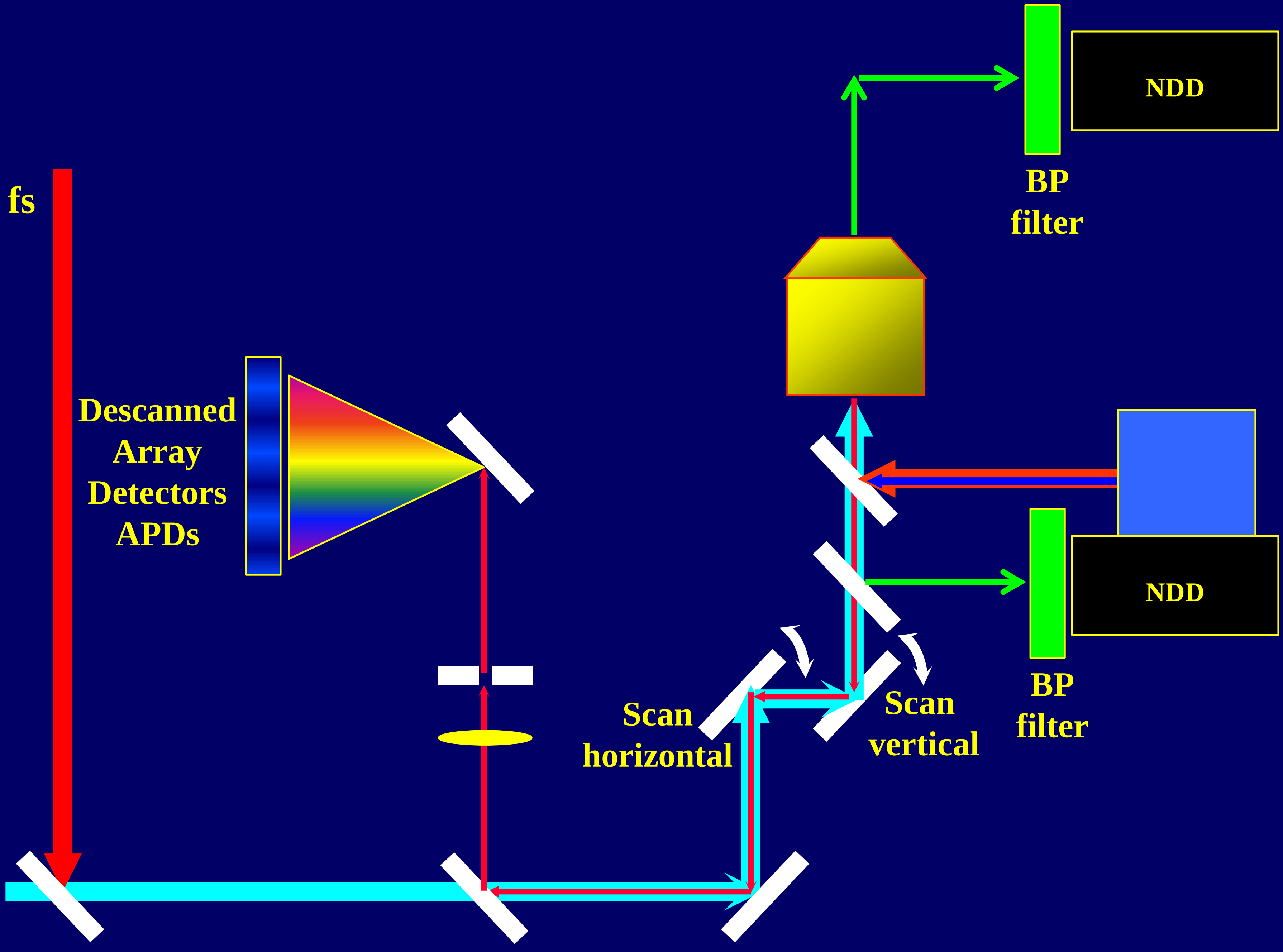
CFP-Cry-ab + YFP-FAK-CT

FRET everywhere

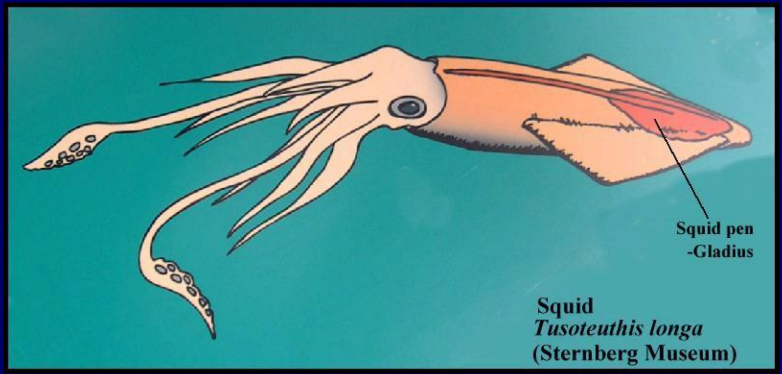
CFP-Cry-ab + YFP- FAK-CT

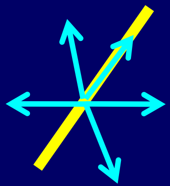


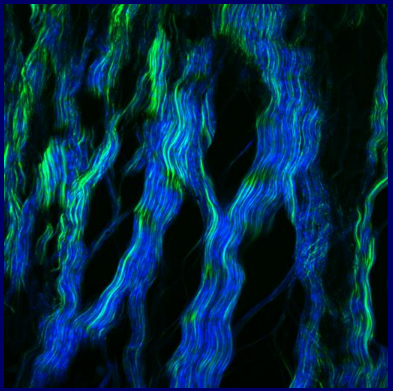
Second/Third Harmonic Generation comes for free



Forward/Backward and polarization signals have information



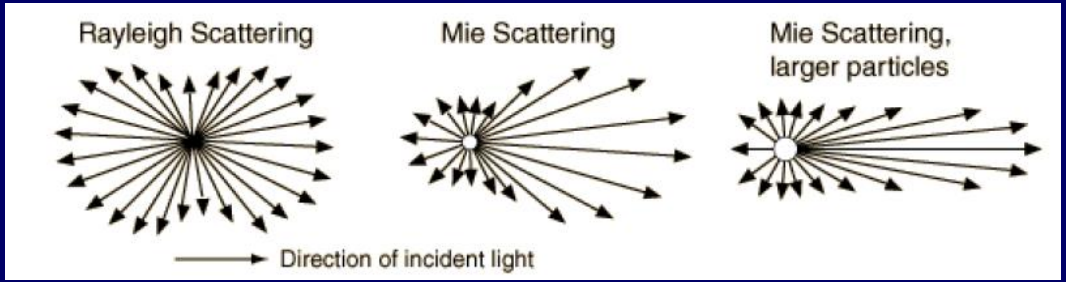




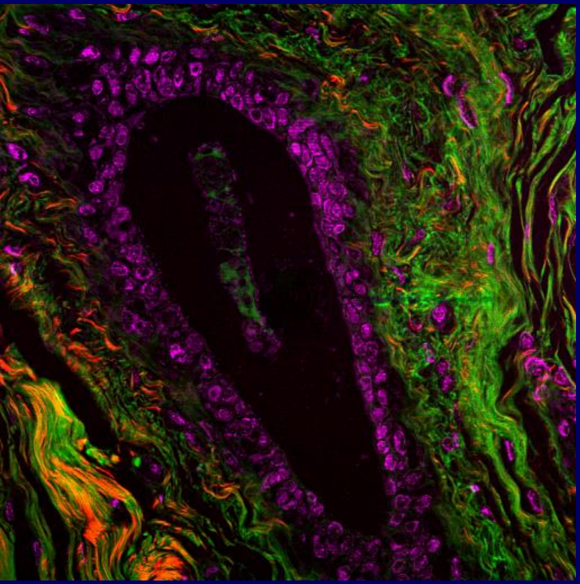
tendom

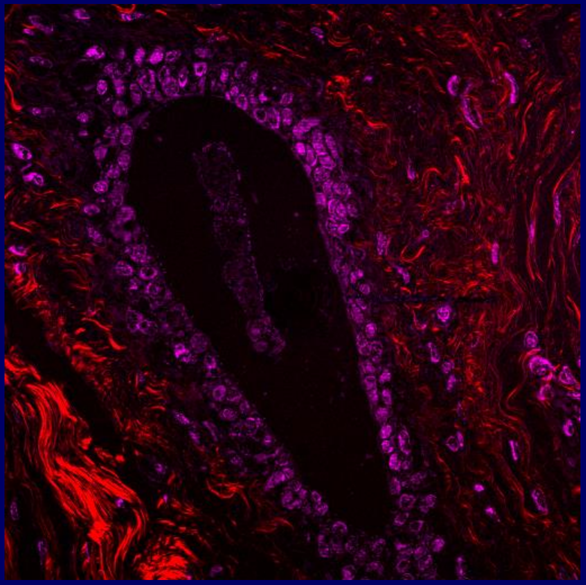
Squid

pen



TPFE + SHG + THG

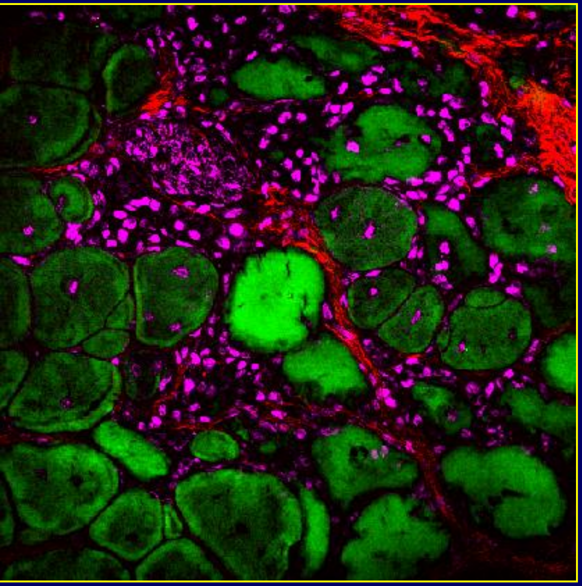


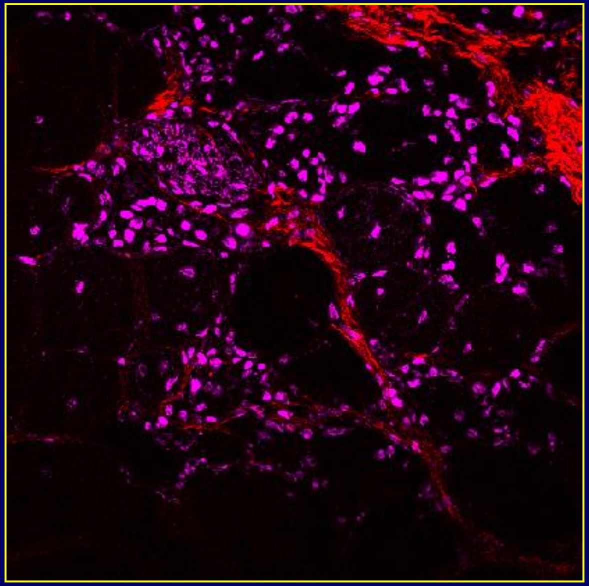


Normal breast tissue

Duct region

Fátima Böttcher
Liliana Andrade
CAISM - UNICAMP

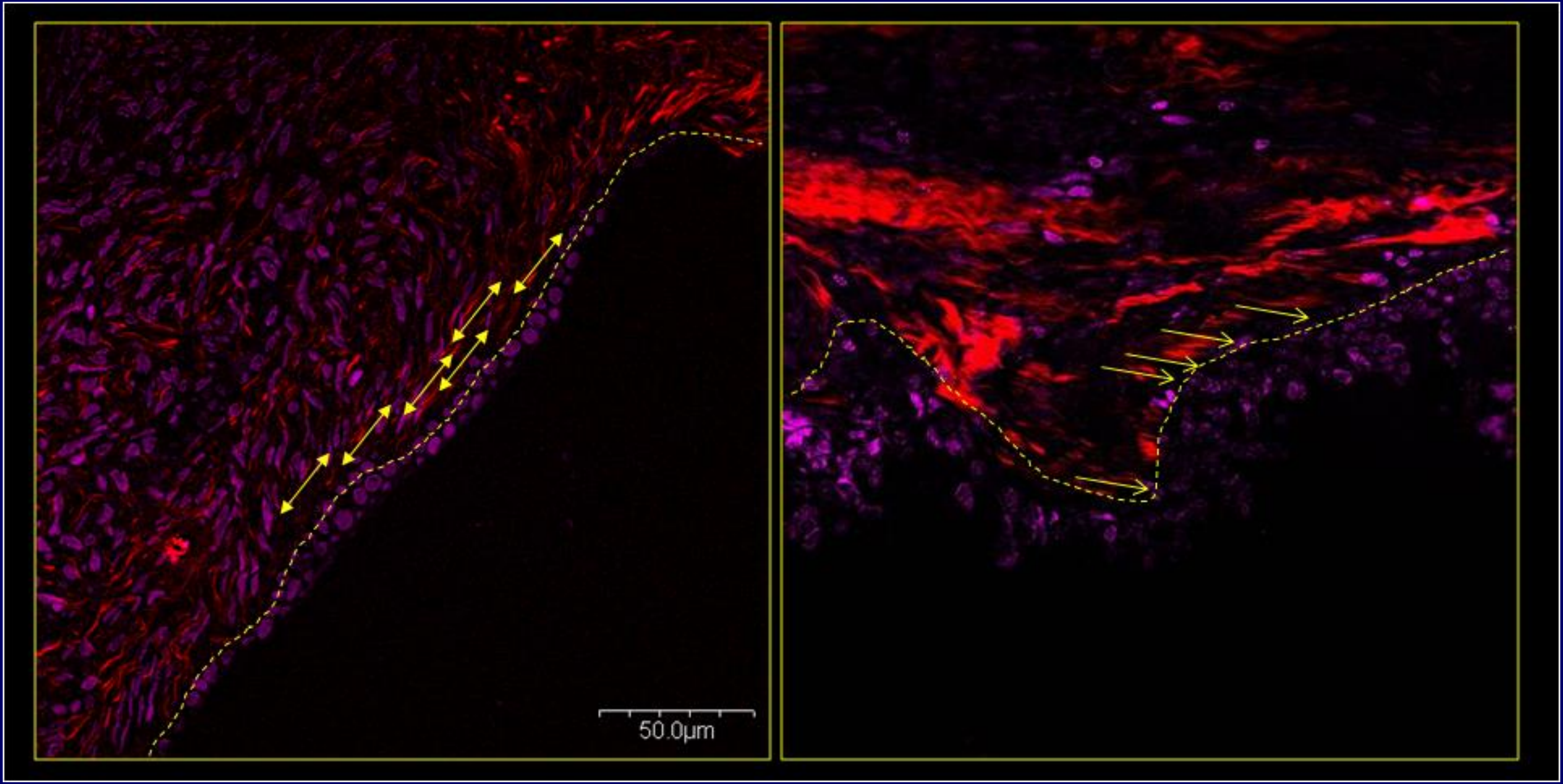


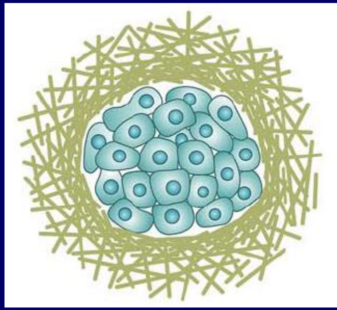


Muscle tissue

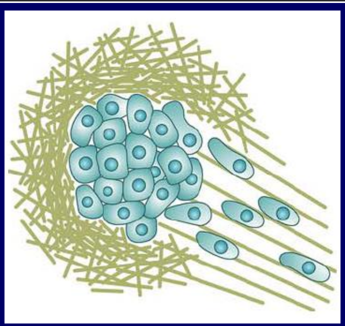
Mayana Zatz
Mariz Vainzof
IBC - USP

SHG + THG Ovarian Comparison normal vs adenocarcinoma





TACS-2, collagen tangential fibers

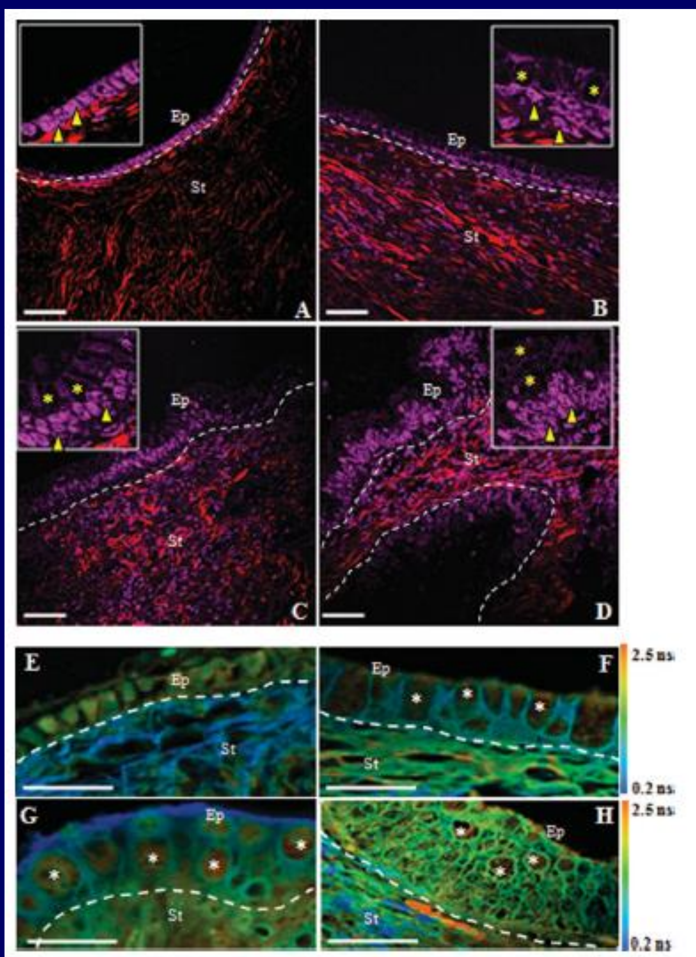


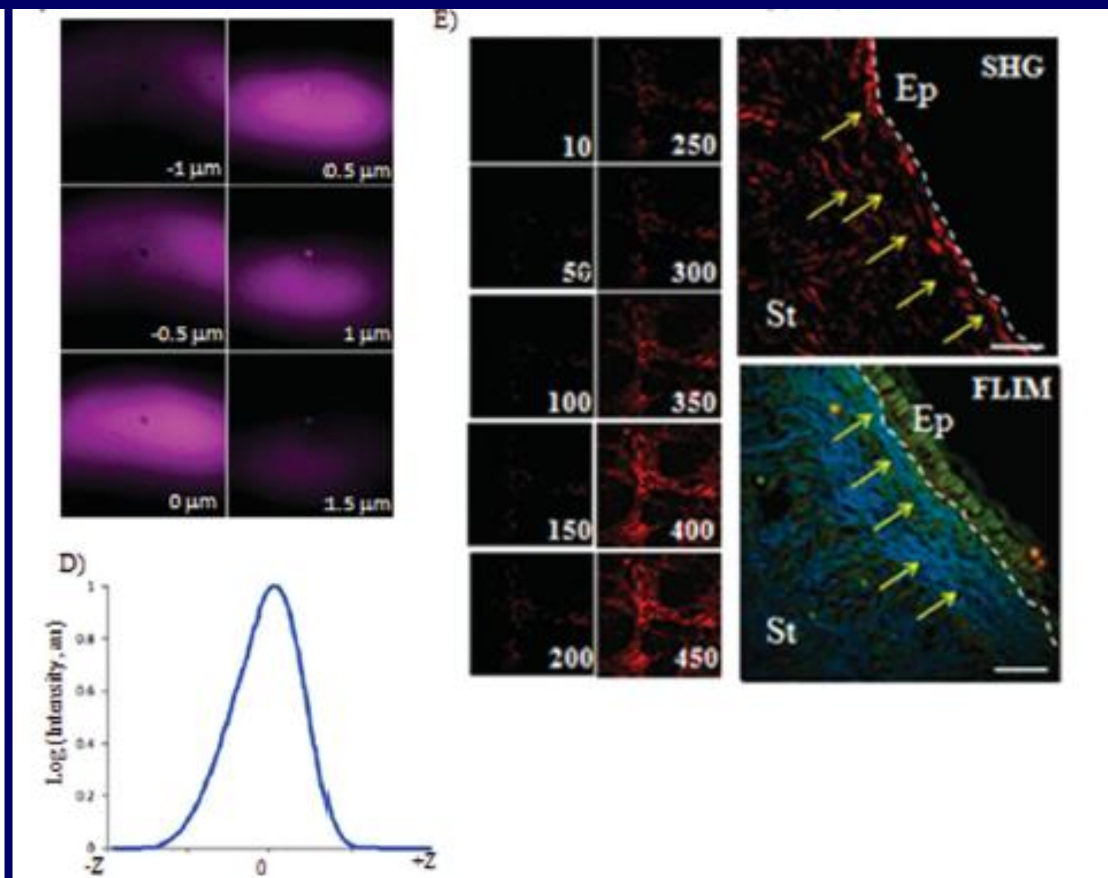
TACS-3, radial collagen fibers

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. CESAR[1]

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



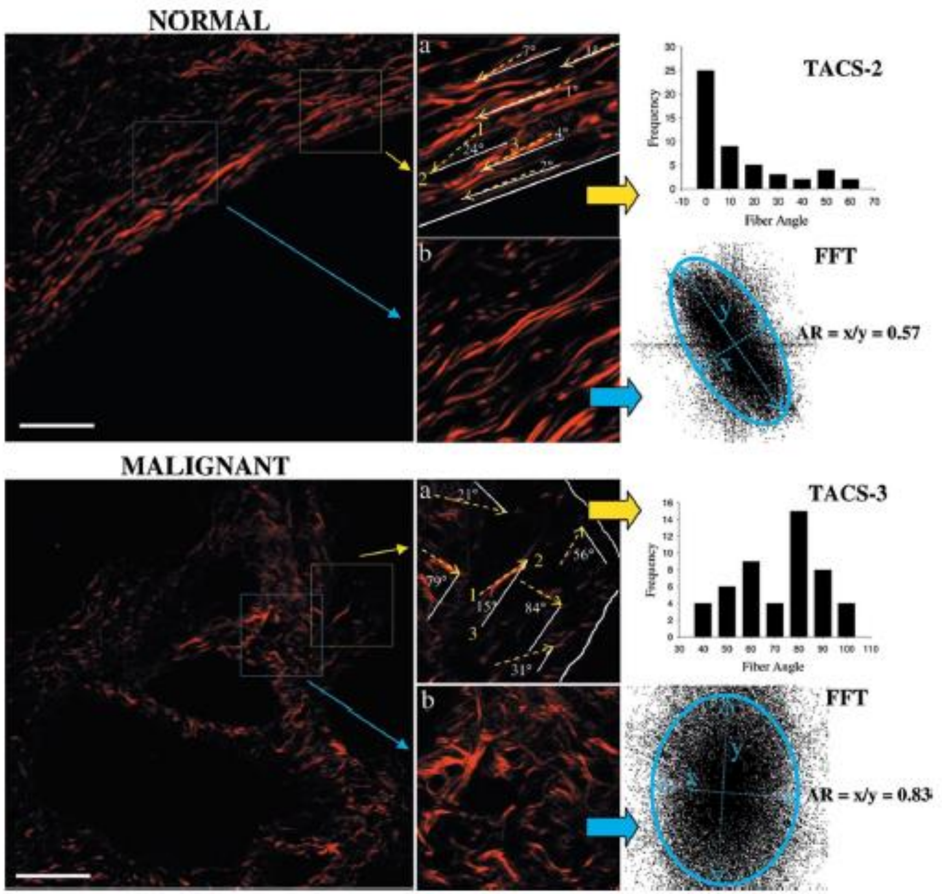


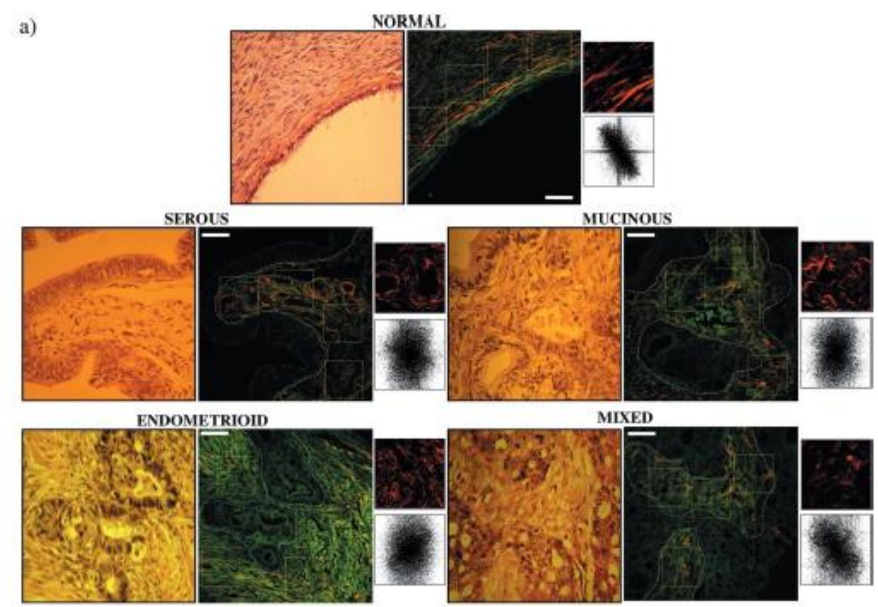
Second harmonic generation microscopy as a powerful diagnostic imaging modality for human ovarian cancer

Javier Adur[*,1,2], *Vitor B. Pelegati*[1], *Andre A. de Thomaz*[1], *Mariana O. Baratti*[3], *Liliana A. L. A. Andrade*[4], *Hernandes F. Carvalho*[3,5], *Fátima Bottcher-Luiz*[3,6], *and Carlos Lenz Cesar*[1,3]

Journal of BIOPHOTONICS

J. Biophotonics 1–13 (2012) / **DOI** 10.1002/jbio.201200108





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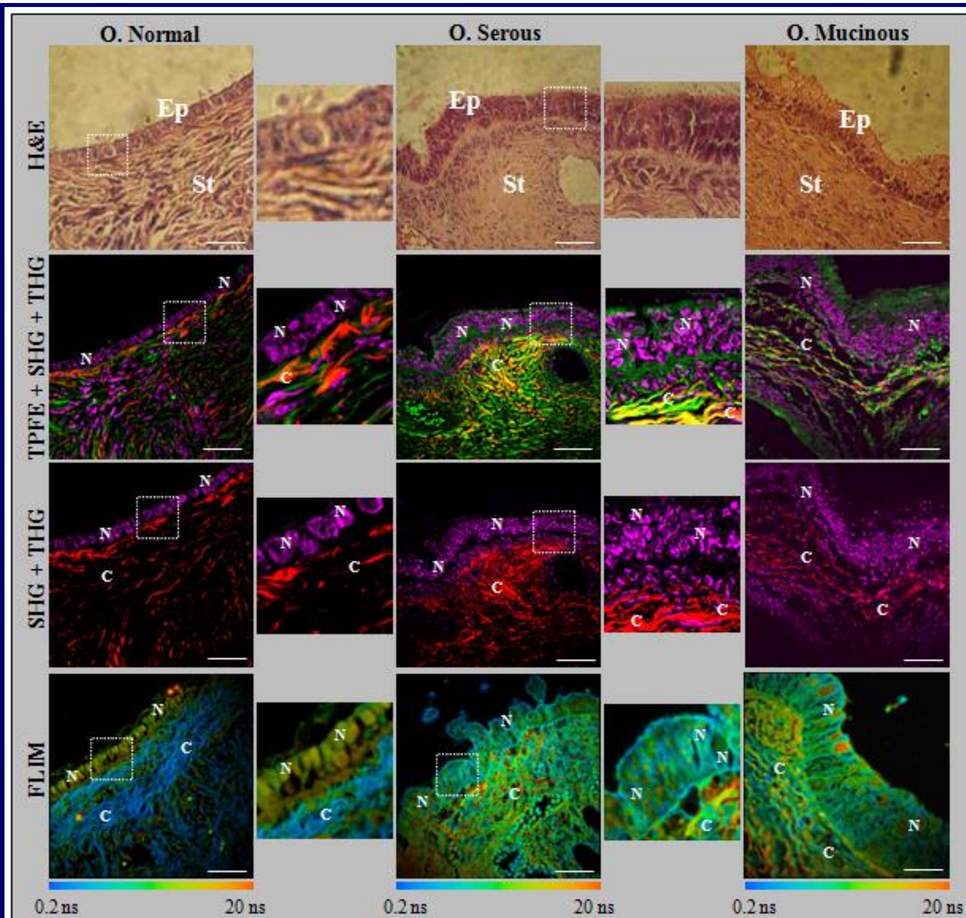
October 2012 | Volume 7 | Issue 10 | e47007
PLOS | ONE

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

Javier Adur[1,2]*, Vitor B. Pelegati[1], Andre A. de Thomaz[1], Mariana O. Baratti[6], Diogo B. Almeida[1], L. A. L. A. Andrade[3], Fátima Bottcher-Luiz[4], Hernandes F. Carvalho[5,6], Carlos L. Cesar[1,6]



H&E stained

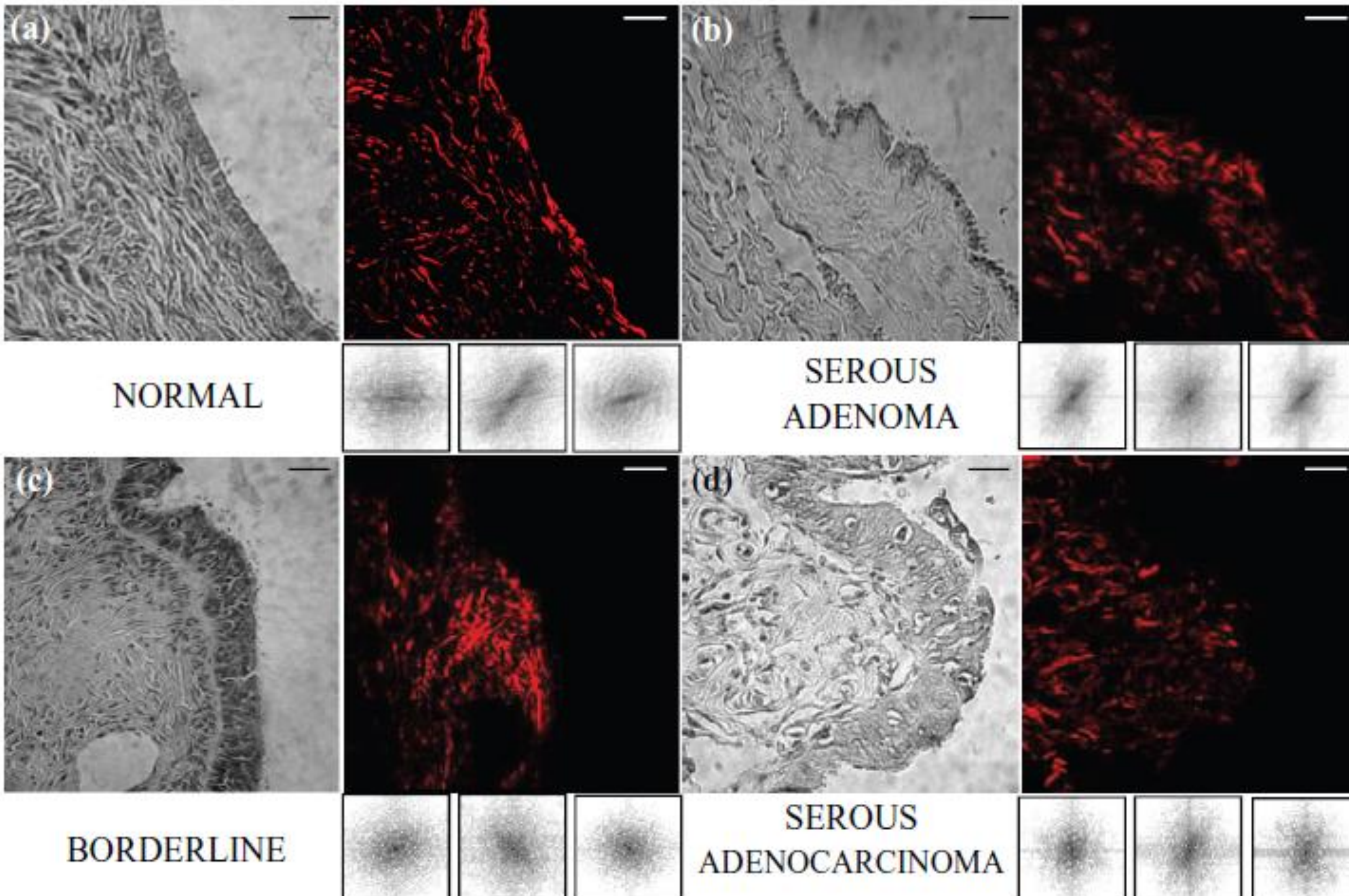
Two-photon +SHG+THG 940 nm

FLIM Non H&E only parafin 890 nm

Recognition of serous ovarian tumors in human samples by multimodal nonlinear optical microscopy

Journal of Biomedical Optics 16(9), 096017 (September 2011)

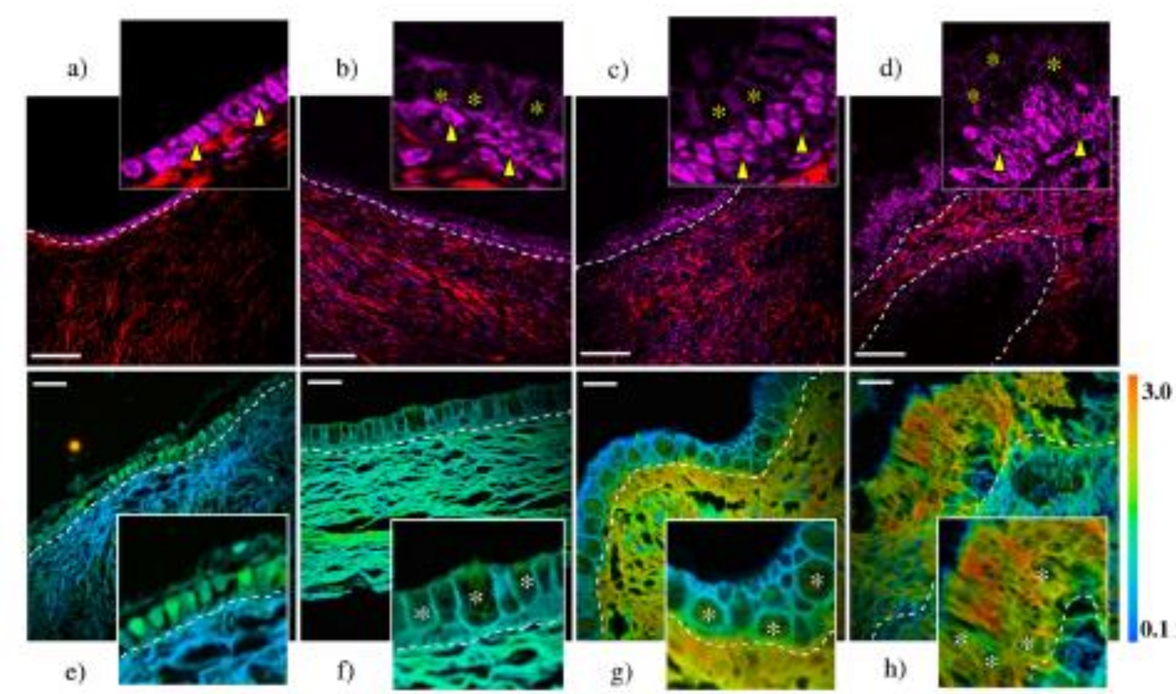
Javier Adur,[a,b] Vitor B. Pelegati,[a] Leverson F. L. Costa,[a] Luciana Pietro,[c] Andre A. de Thomaz,[a] Diogo B. Almeida,[a] Fatima Bottcher-Luiz,[d] Liliana A. L. A. Andrade,[c] and Carlos L. Cesar[a]

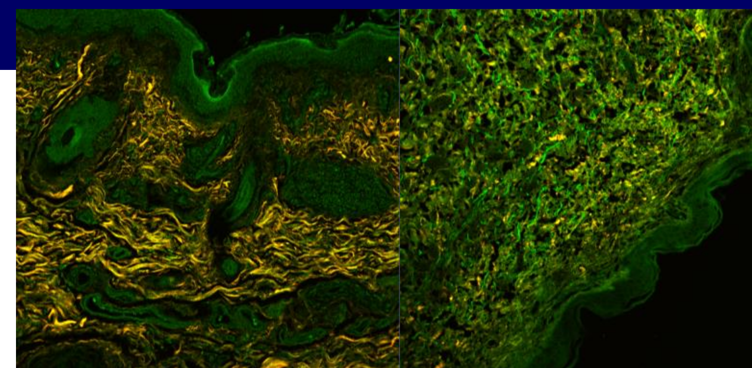


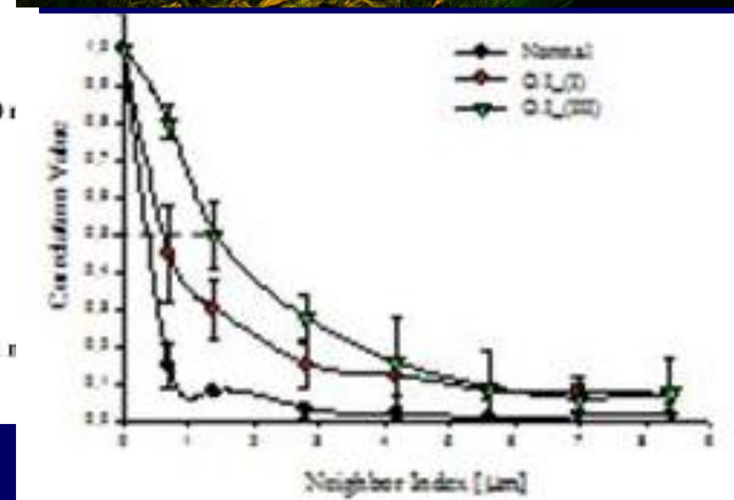
Quantitative changes in human epithelial cancers and osteogenesis imperfecta disease detected using nonlinear multicontrast microscopy

Journal of Biomedical Optics 17(8), 081407 (August 2012)

Javier Adur,[a,b] Vitor B. Pelegati,[a] Andre A. de Thomaz,[a] Lilia D'Souza-Li,[c] Maria do Carmo Assunção,[d] Fátima Bottcher-Luiz,[e] Liliana A. L. A. Andrade,[f] and Carlos L. Cesar[a]

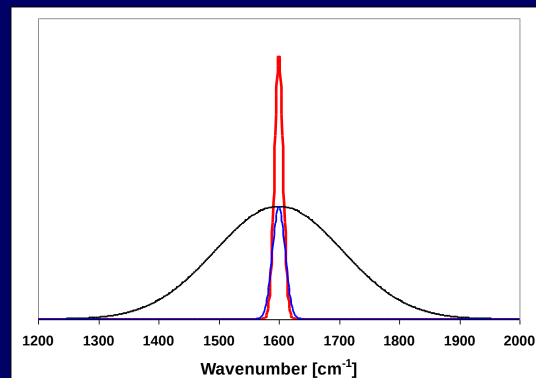






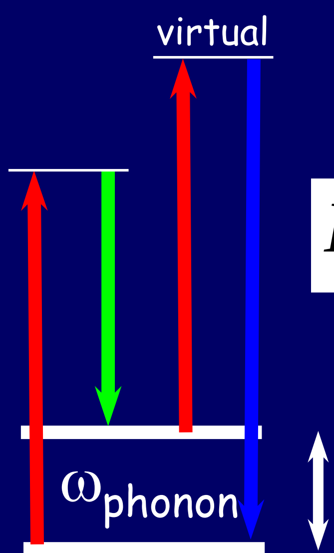
Third order elastic processes: CARS

$$P[x_n, x_e] = \cdots + g\, x_e^3 x_n + \cdots$$



10 ps pulse

Coherent AntiStokes Raman Scatterinng CARS

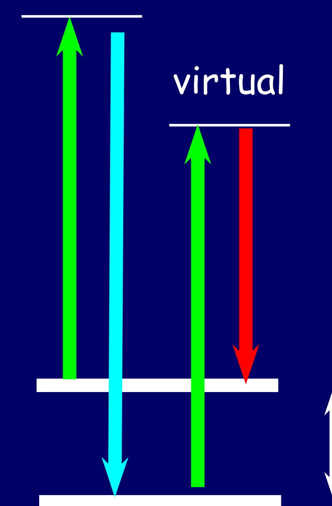


CARS

$$I \propto I_p^2 I_s$$

$$P = 2\omega_p - \omega_s + \omega_n$$

Coherent Stokes Raman Scatterinng CSRS



CSRS

$$I \propto I_p I_s^2$$

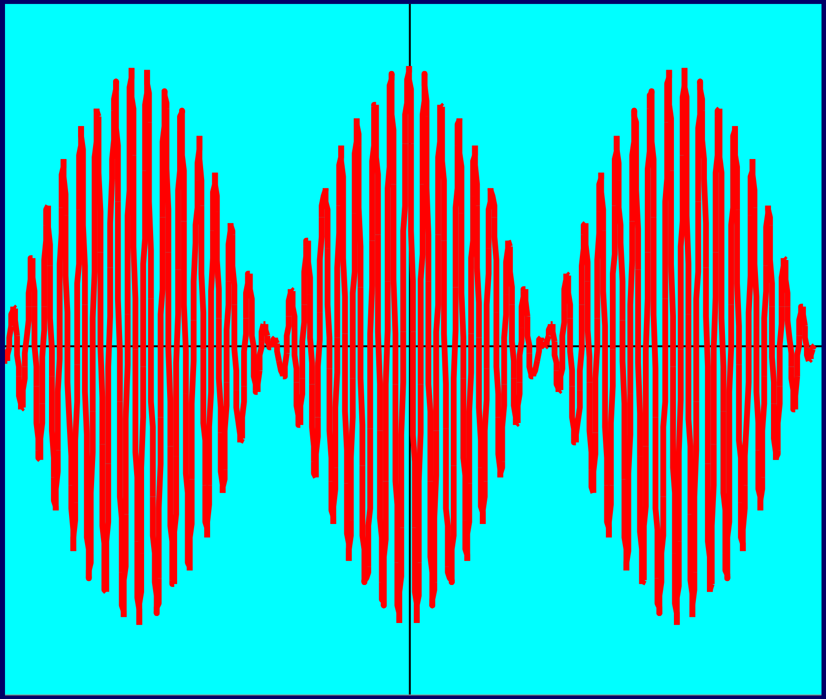
$$P = 2\omega_s - \omega_p - \omega_n$$

Beating

$$\cos(x)+\cos(y)=2\cos\left(\frac{x+y}{2}\right)\cos\left(\frac{x-y}{2}\right)$$

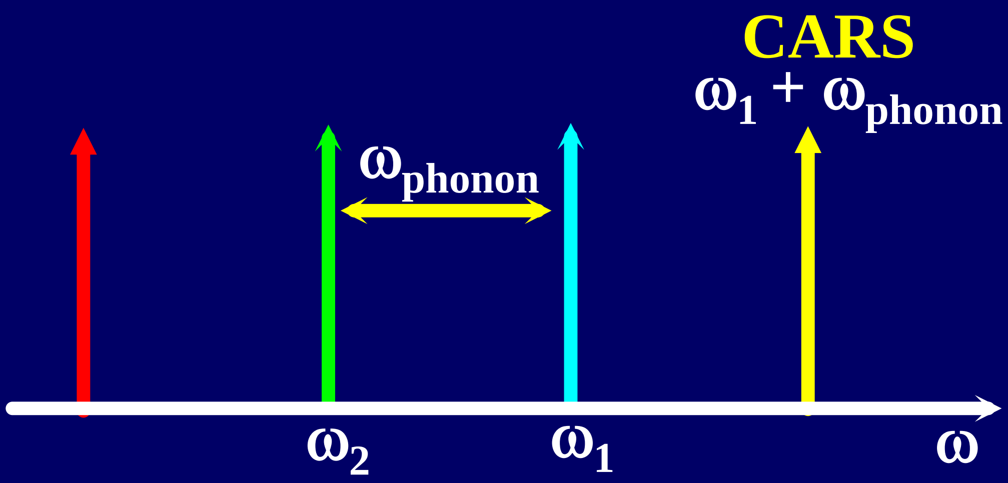
$$x=\omega_1 t \qquad y=\omega_2 t$$

$$\cos(\omega_1 t)+\cos(\omega_2 t)=2\cos\left(\frac{\omega_1+\omega_2}{2}t\right)\cos\left(\frac{\omega_1-\omega_2}{2}t\right)$$

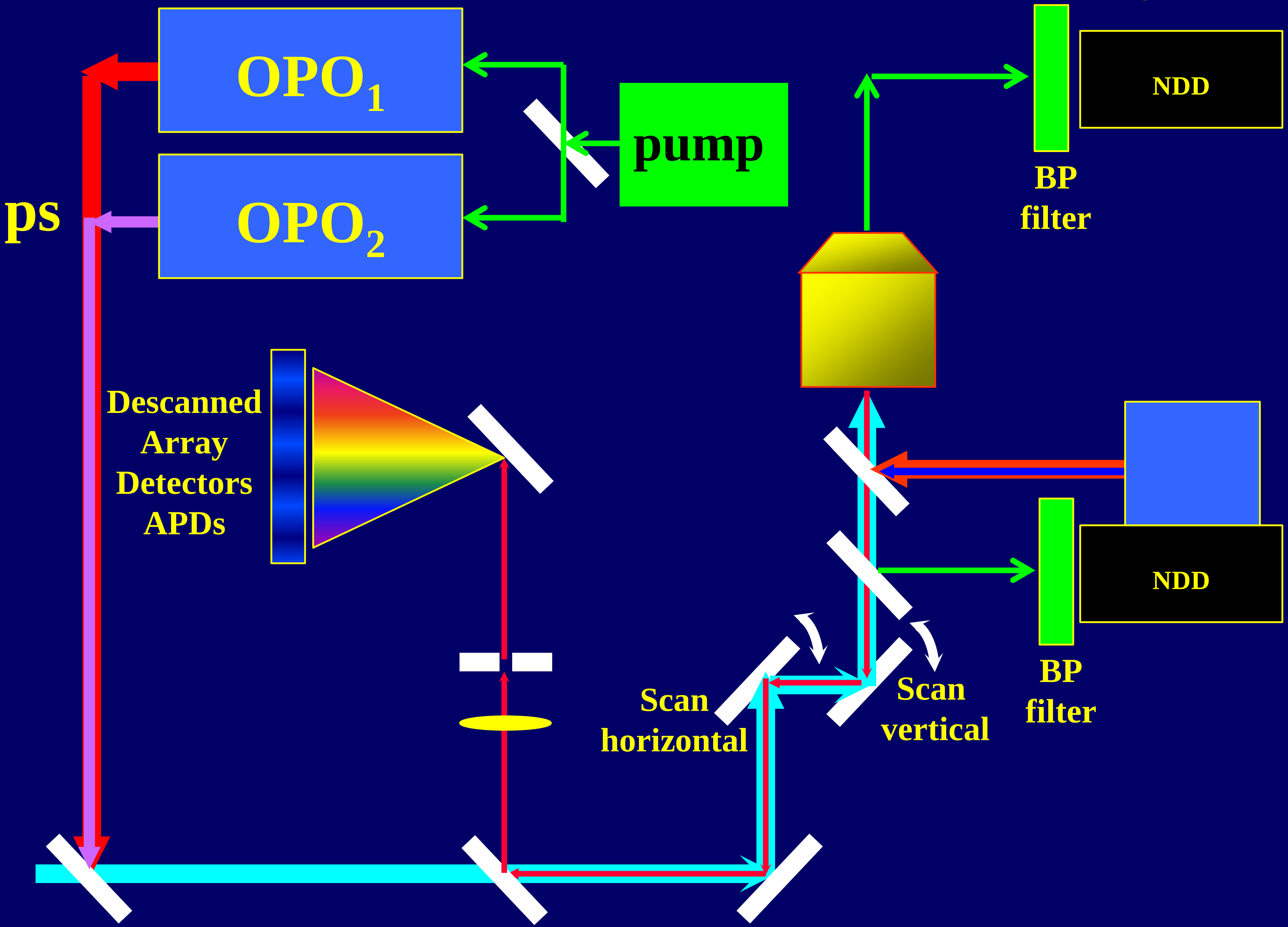


Resonant with nucleus vibrations if

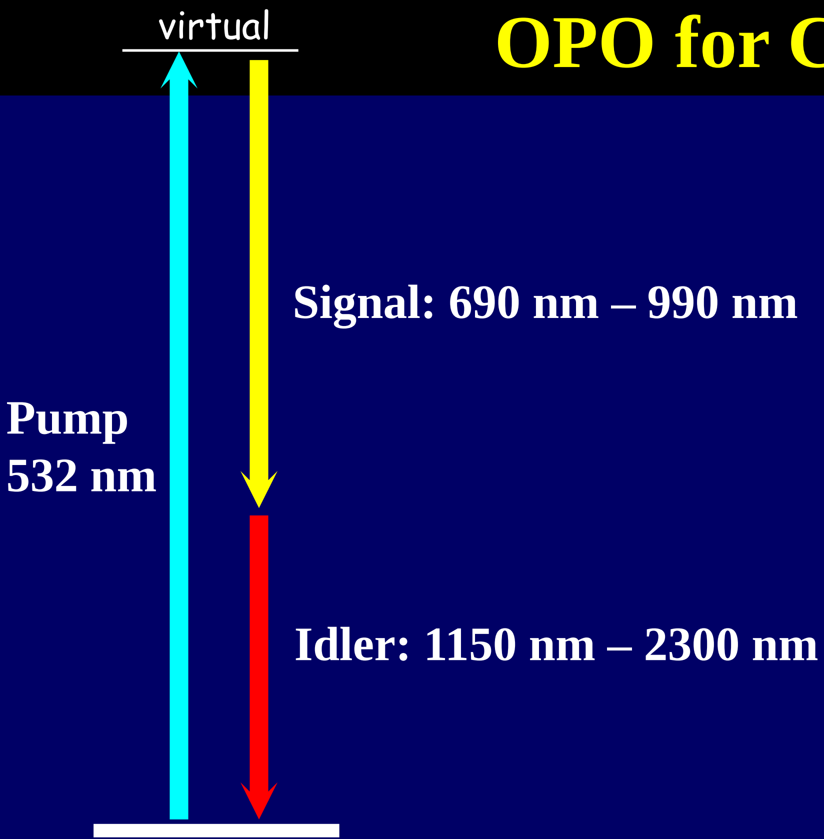
$$\omega_B = \omega_1 - \omega_2 = \omega_n$$

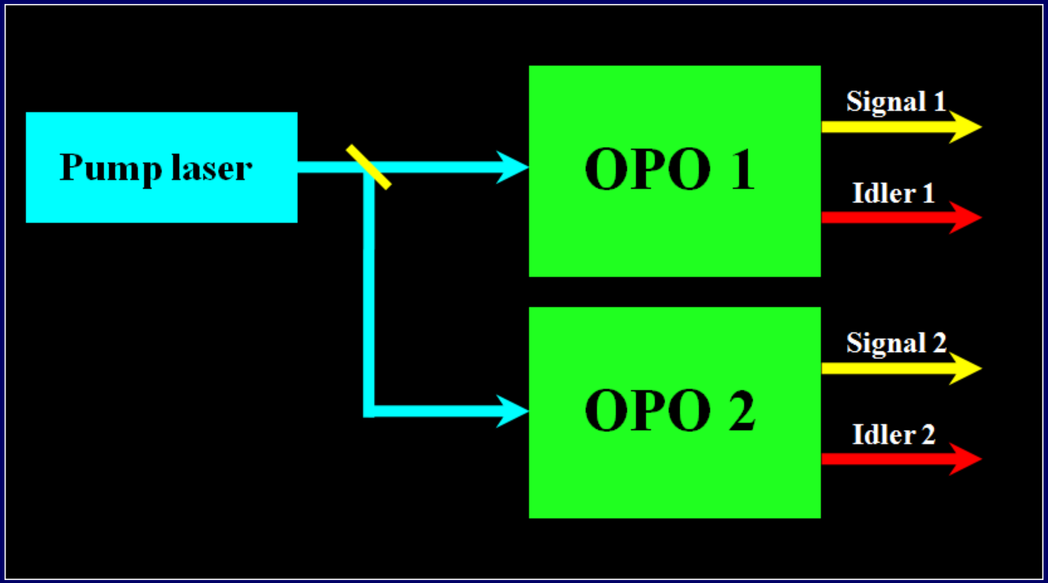


CARS: Coherent AntiStokes Raman Scattering



OPO for CARS [5 ps pulses]





Laser lines combinations

Fundamental: 1064 nm

S1: 690 – 990 nm + 1064 nm [700 – 5000 cm^{-1}]

S2: 690 – 990 nm + S1 [0 – 4400 cm^{-1}]

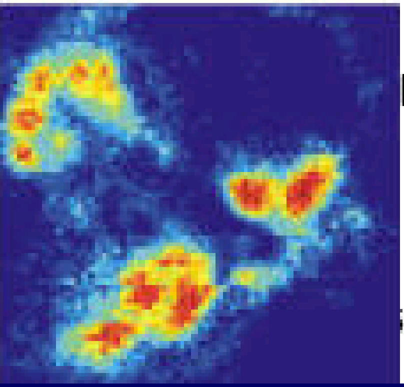
I1: 1150 – 2300 nm + 1064 nm [700 – 5000 cm^{-1}]

I2: 1150 – 2300 nm + I1 [0 – 4400 cm^{-1}]

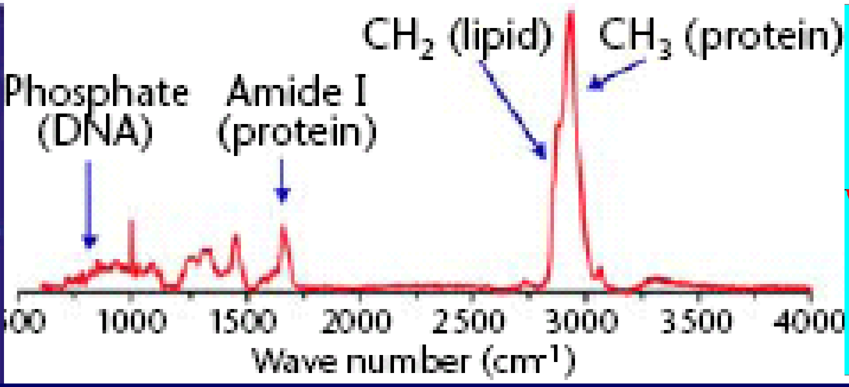
I2: 1150 – 2300 nm + S1 [5800 – 10000 cm^{-1}]

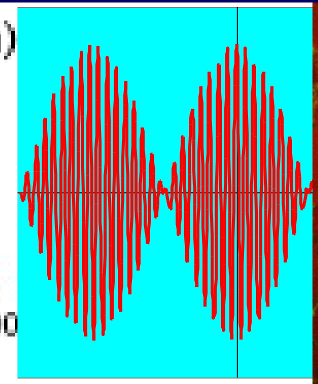
Coherent AntiStokes Raman Scattering CARS
Chemical specific imaging

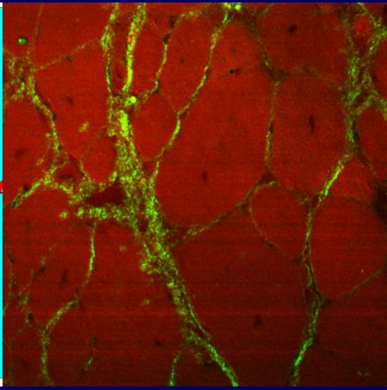
The first CARS images of Brazil



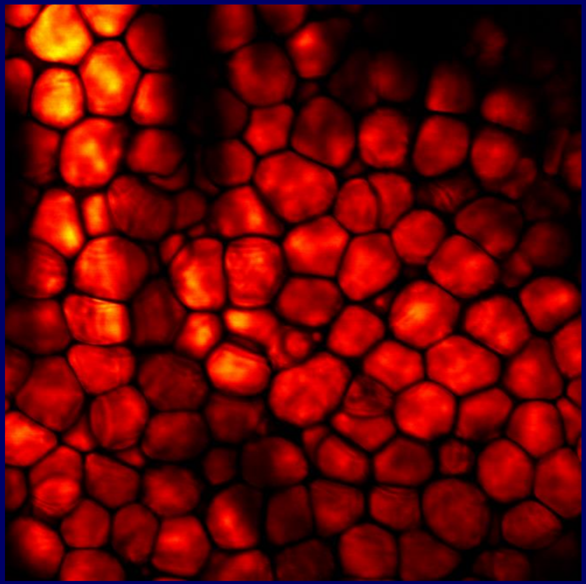
Raman image



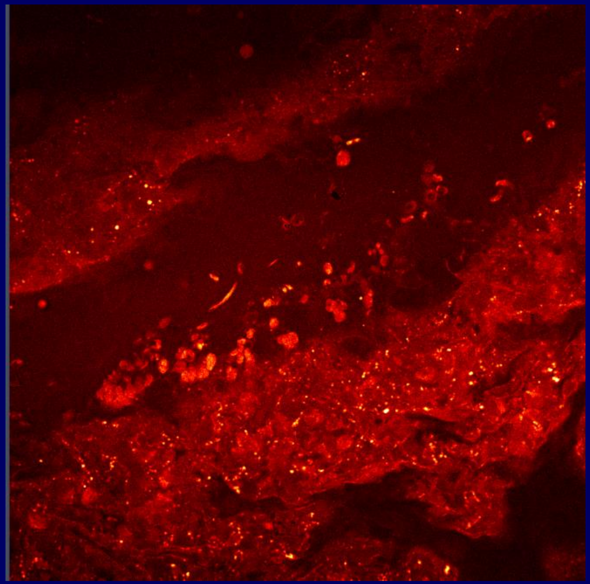




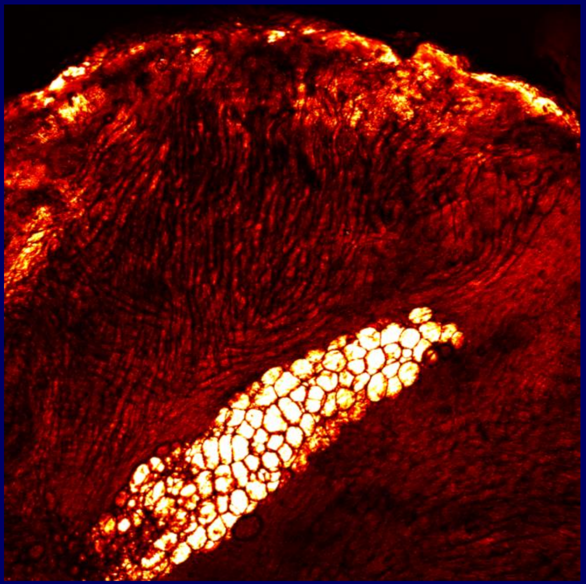
CARS + SFG



Mouse ear fat gland

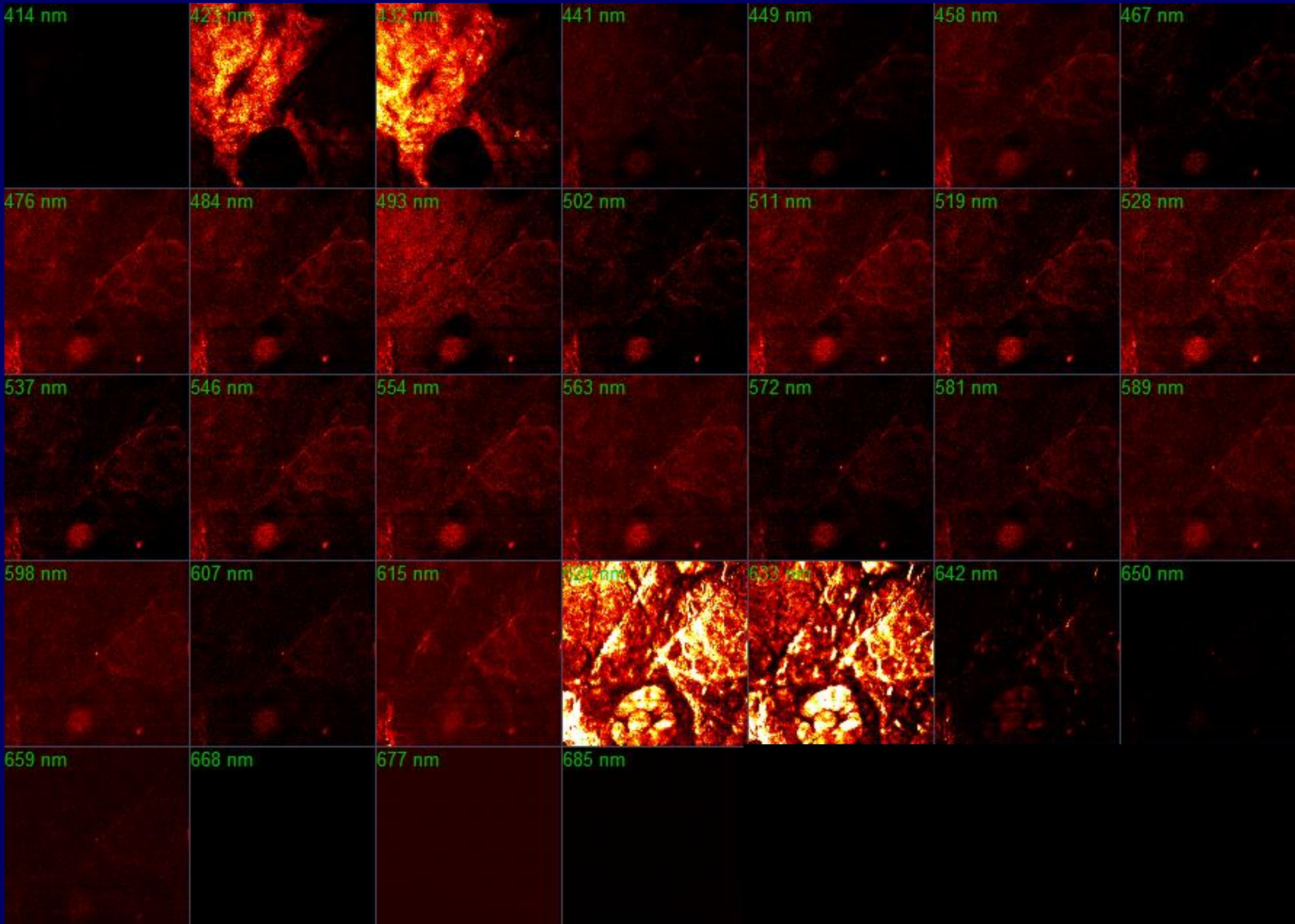


Lung artery

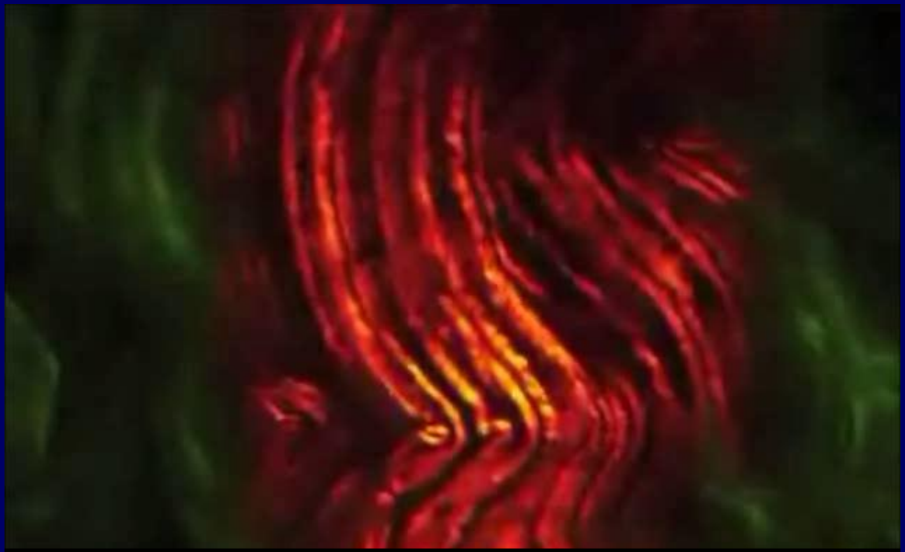


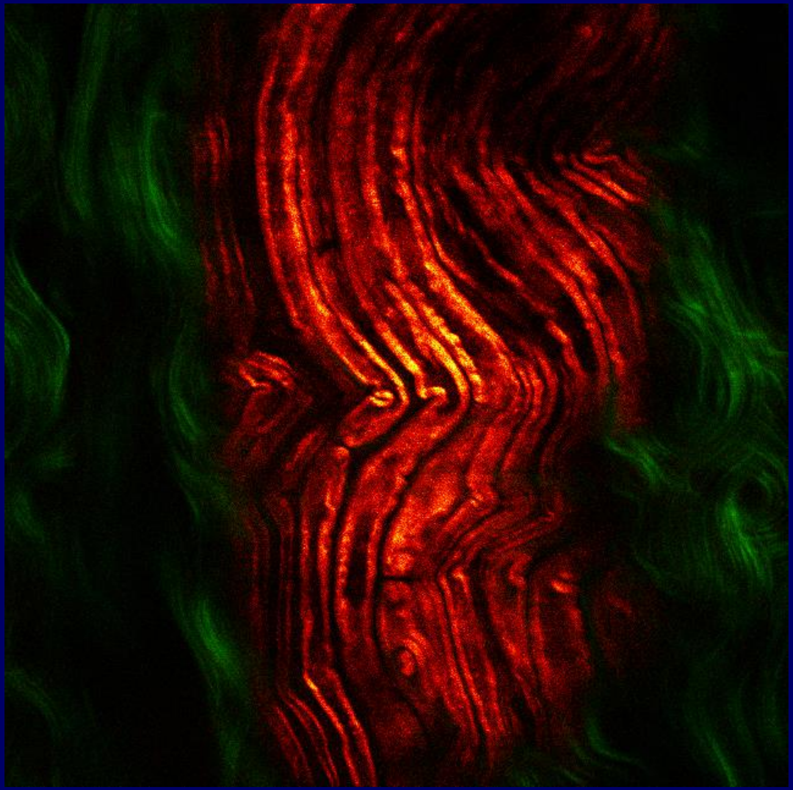
Heart

CARS + SFG Microscopy



CARS+SFG myelin sheath of mice sciatic nerve

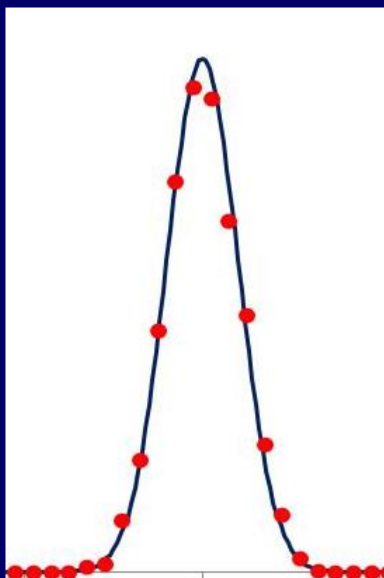
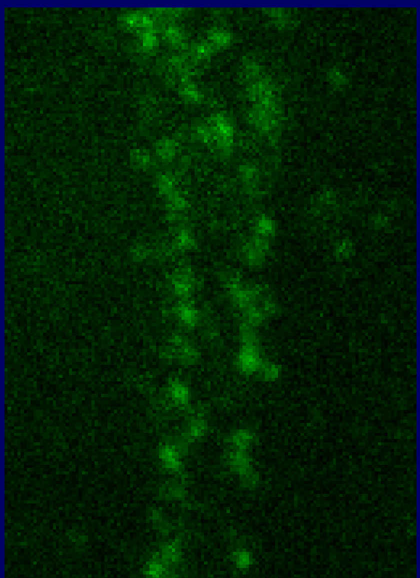
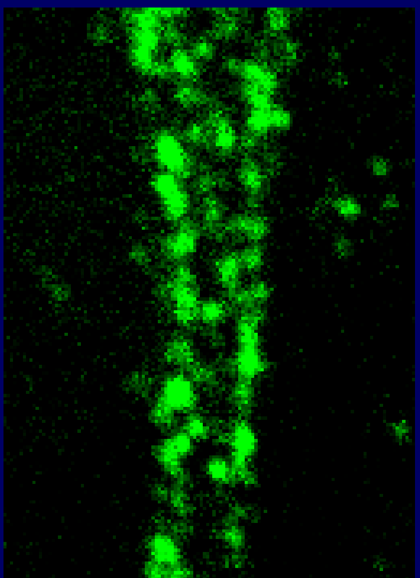
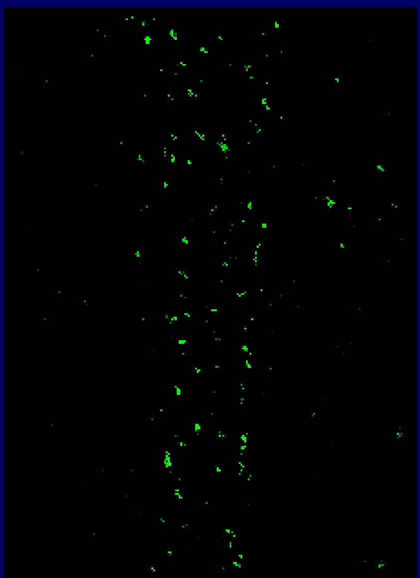
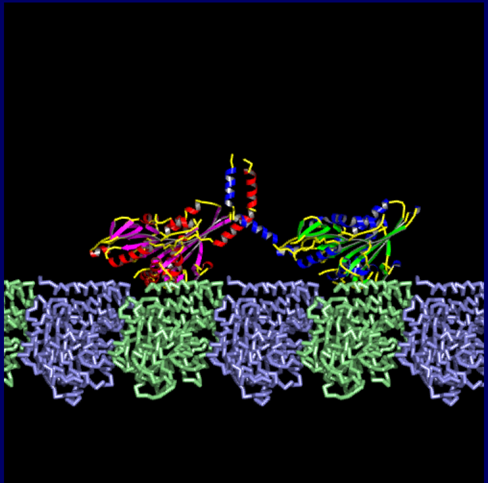
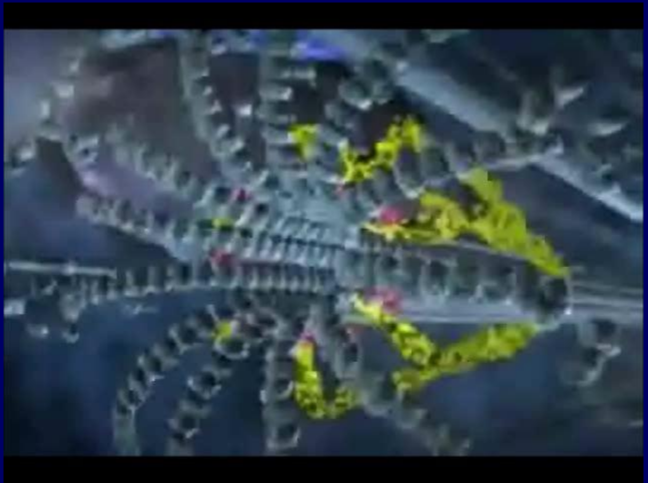




Super-resolution

Down to a molecule size 1-10 nm

Far field super resolution: localization
Present Limit < 10 nm single molecule
Proteins in tubulin ~ 30 nm

Integrated techniques into the same platform

3D + time-lapse capabilities

Single/multiphoton fluorescences: intensity spectral + FLIM + PLIM + FCS + FRET + F…

SHG + THG

Raman + CARS

Tip-enhancement + conventional AFM

Optical Tweezers + laser cutting

Physiological controlled cell – temperature + atmosphere

Obrigado pela atenção!

Thanks for the attention