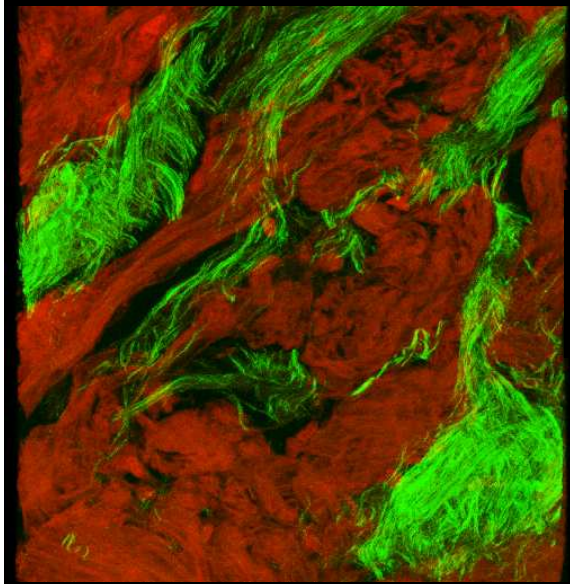
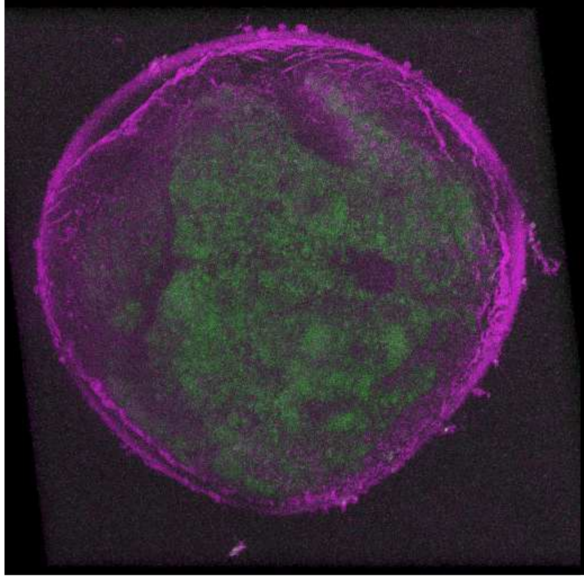
“Introdução á Óptica Não Linear”

Carlos Lenz Cesar: IFGW - UNICAMP



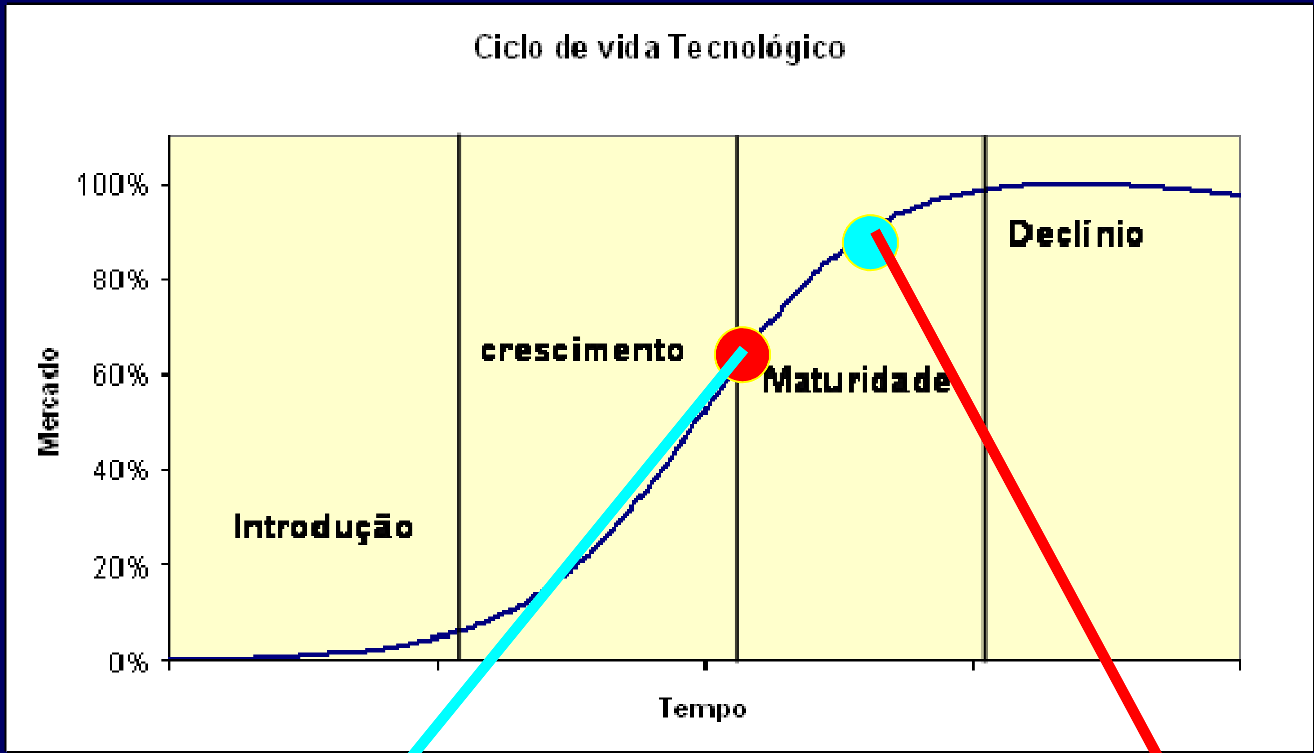
TPEF + SHG :
heart fibroma



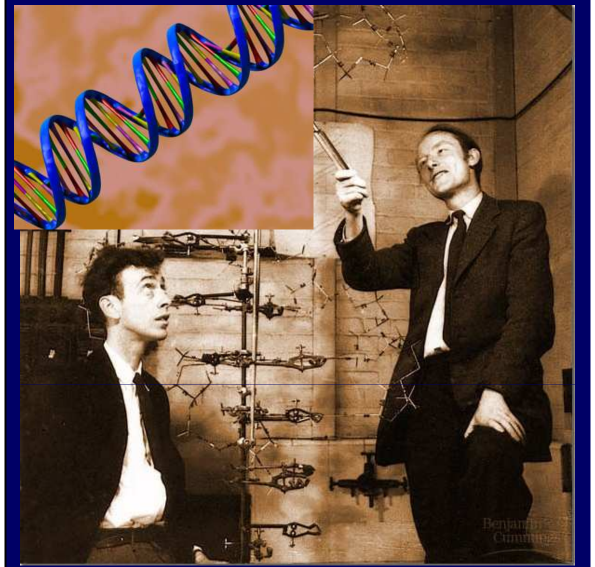
THG
embryo

CARS + SFG
Mice myelin sheet

Signs of New Era: World is ready to launch the next scientific-technological revolution



Watson & Crick 1953
~ 60 years old knowledge



Signs:

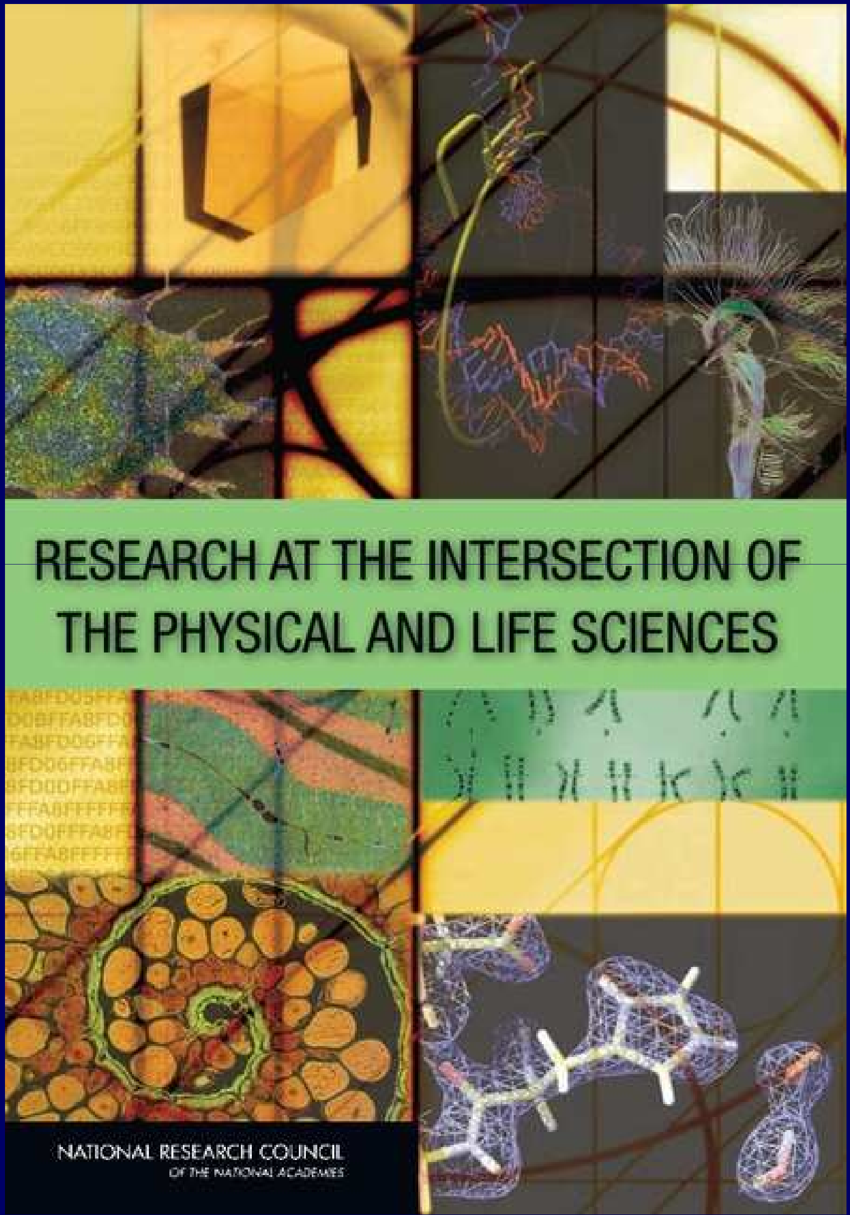
The crisis already appeared **The Information era evolution is here**

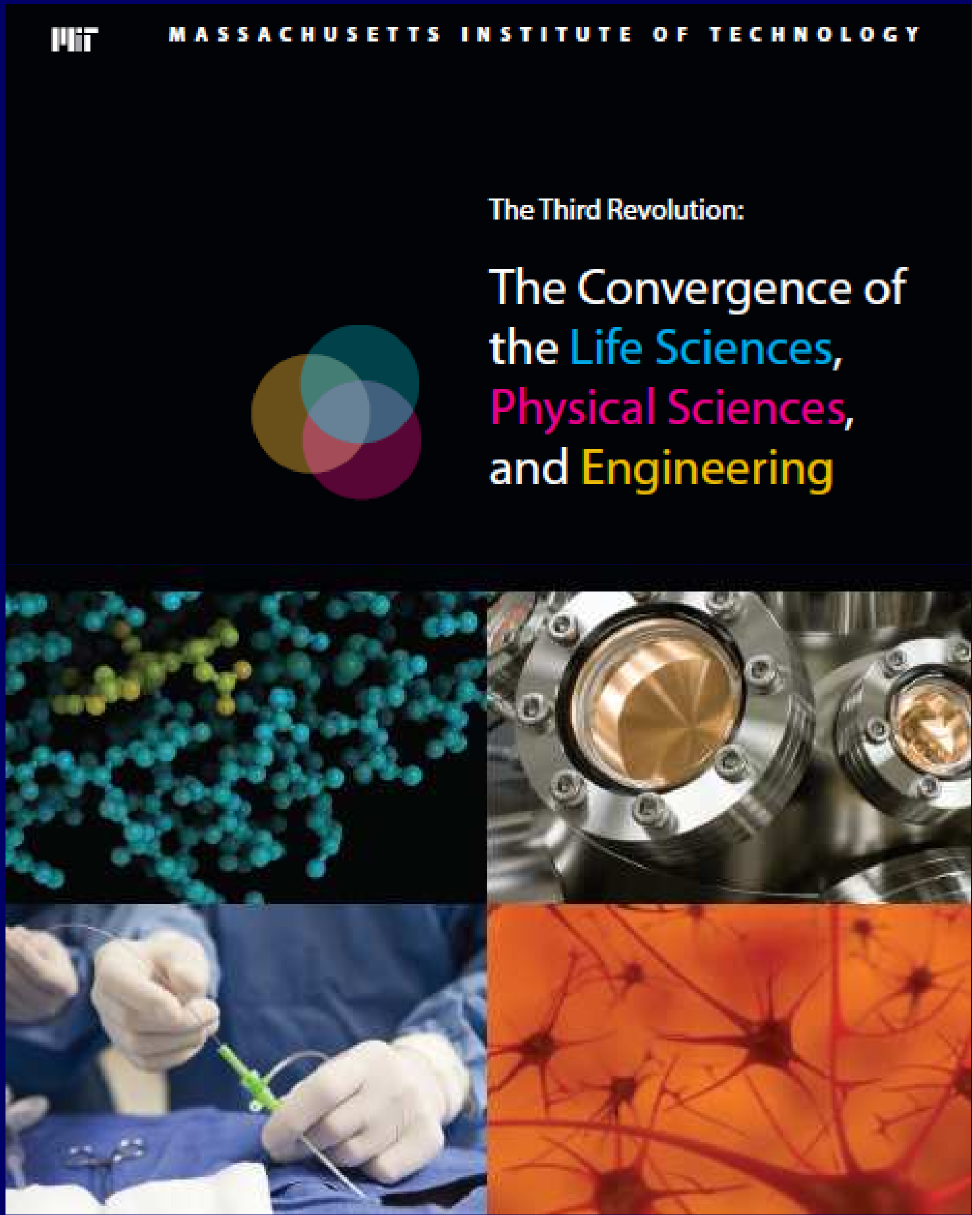
Next Revolution???

Our bet: control of biologyy at cell/molecular level

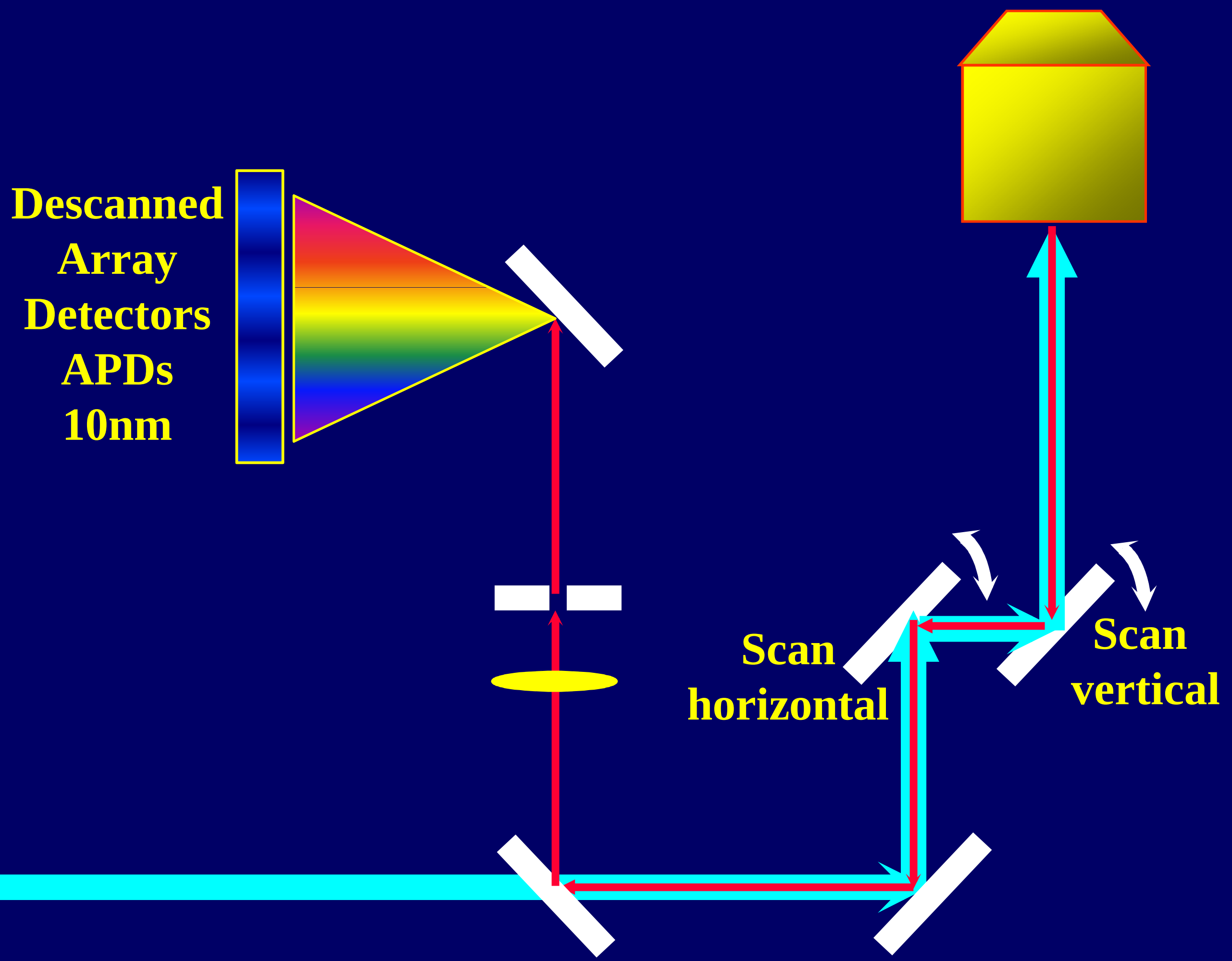
National Research Council Report

MIT white paper





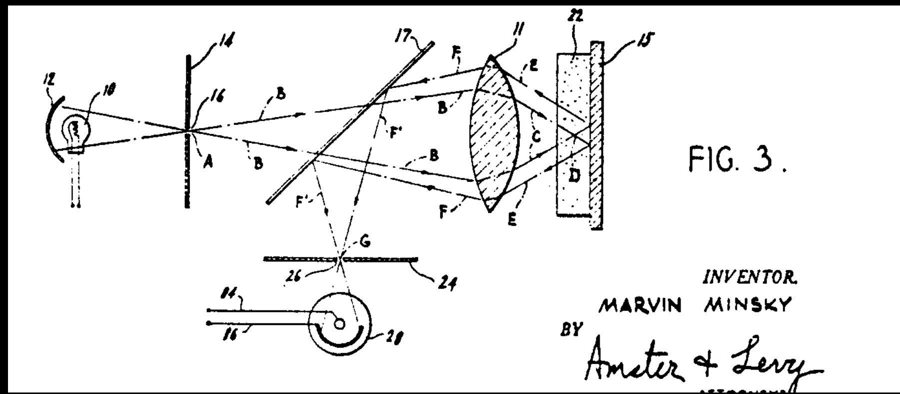
Our contribution:
Multimodal Platform based on a laser scanning spectral confocal microscopy!



Why laser scanning Confocal Microscopy?

Marvin Minsky
Mathematician

Artificial Inteligence



Confocal patent: 1957

Minsky scanned the sample – images were not that great!

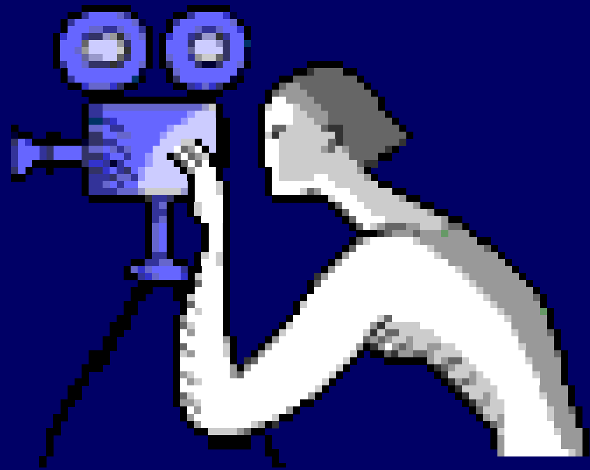
First commercial confocal appeared in 1988 – Amos&White (Cambridge UK). BioRad.

Laser scanning made all the difference!

Why Multimodal Platform?

To understand cell processes!

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

Which Platform?

Multimodal platform.

Laser scanning + 3D + time-lapse capabilities

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

SHG + THG

Raman + CARS

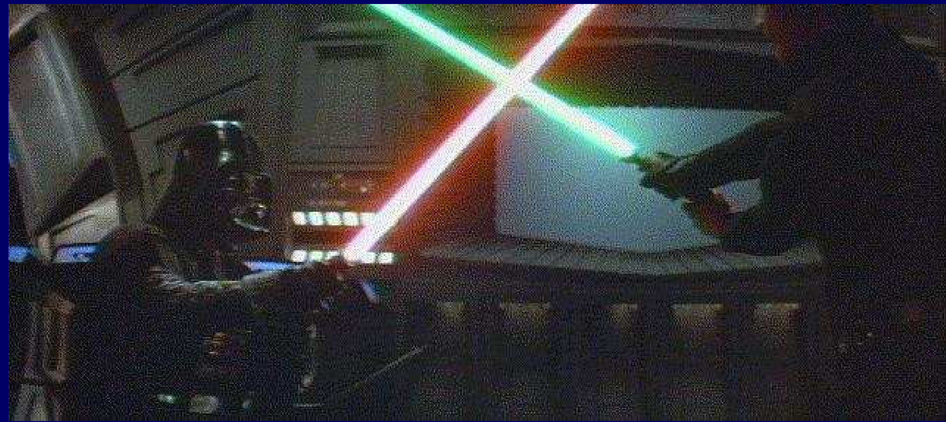
Tip-enhancement + conventional AFM

Optical Tweezers + laser cutting

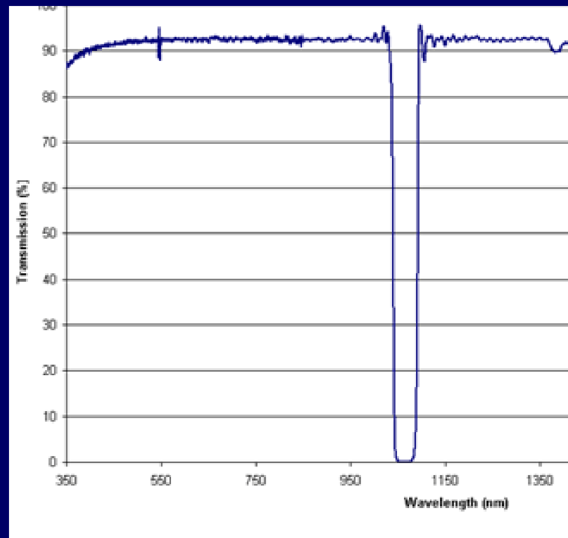
Physiological controlled cell – temperature + atmosphere

Is it possible?

Optics allows Multimodality in the same platform



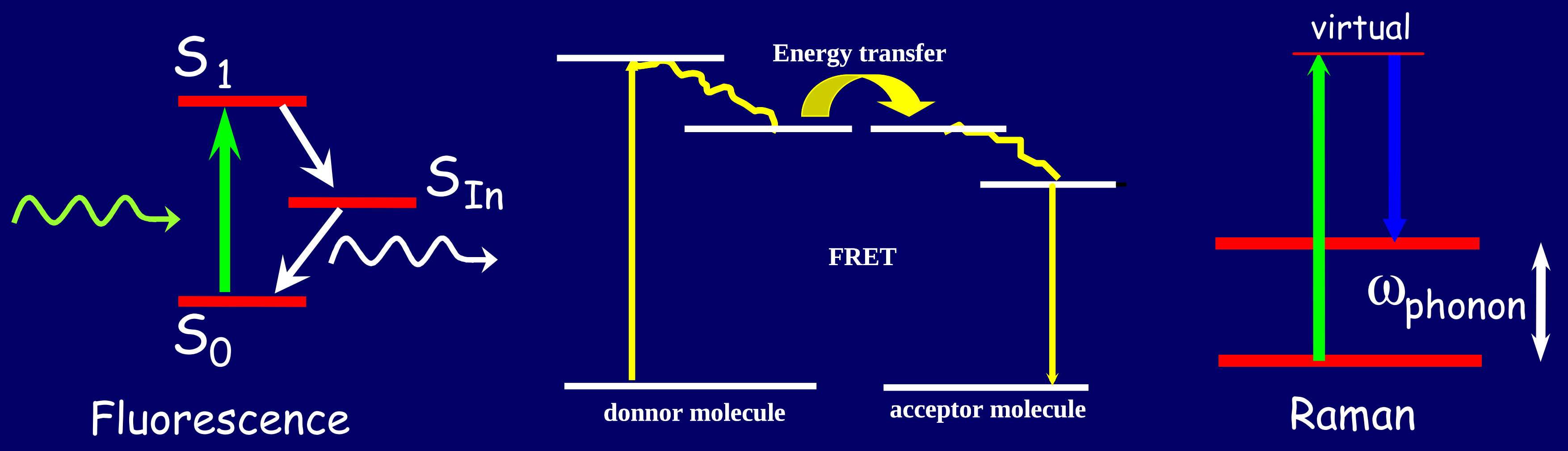
Optical beams do not collide!



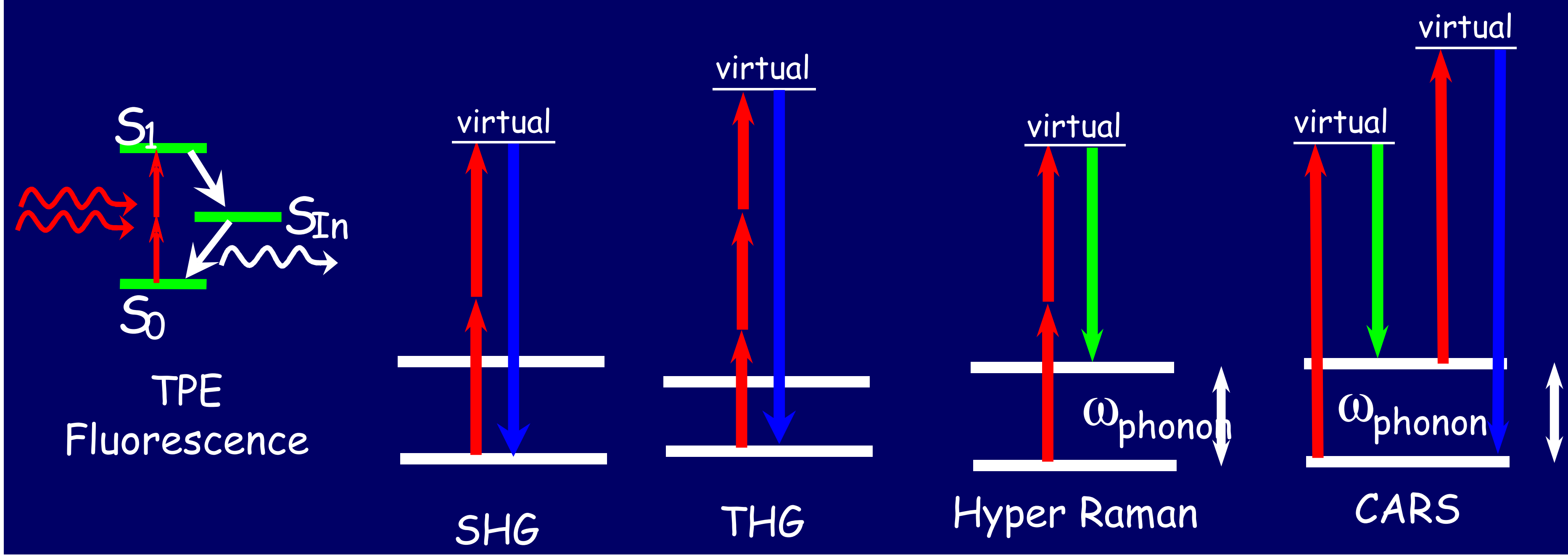
Dichroics filters

Platform Optical Processes

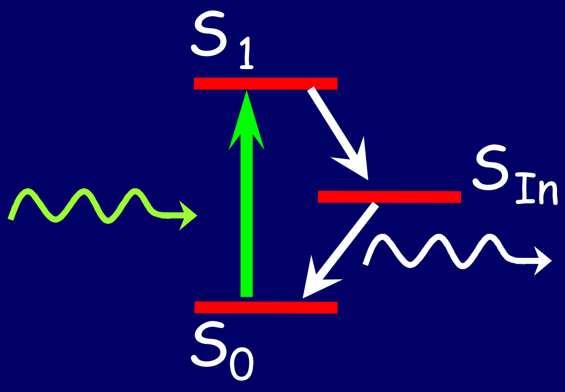
Linear Optical processes



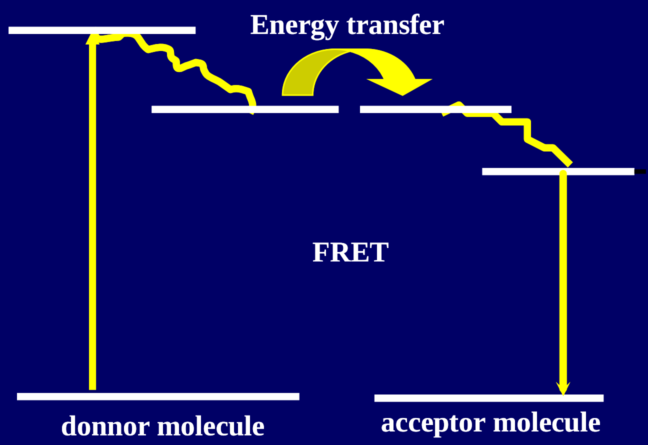
Non Linear Optical processes

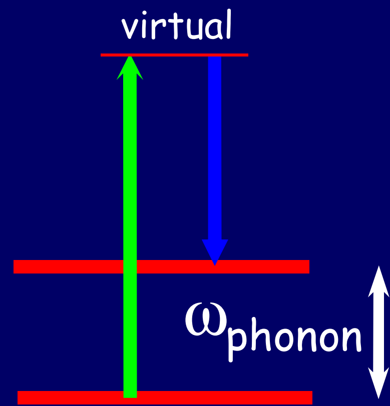


Inelastic processes: heat dissipation

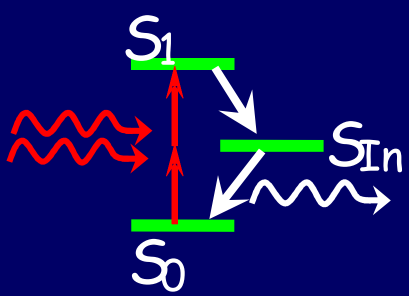


Fluorescence

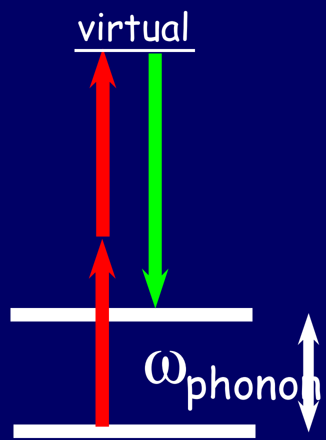




Raman

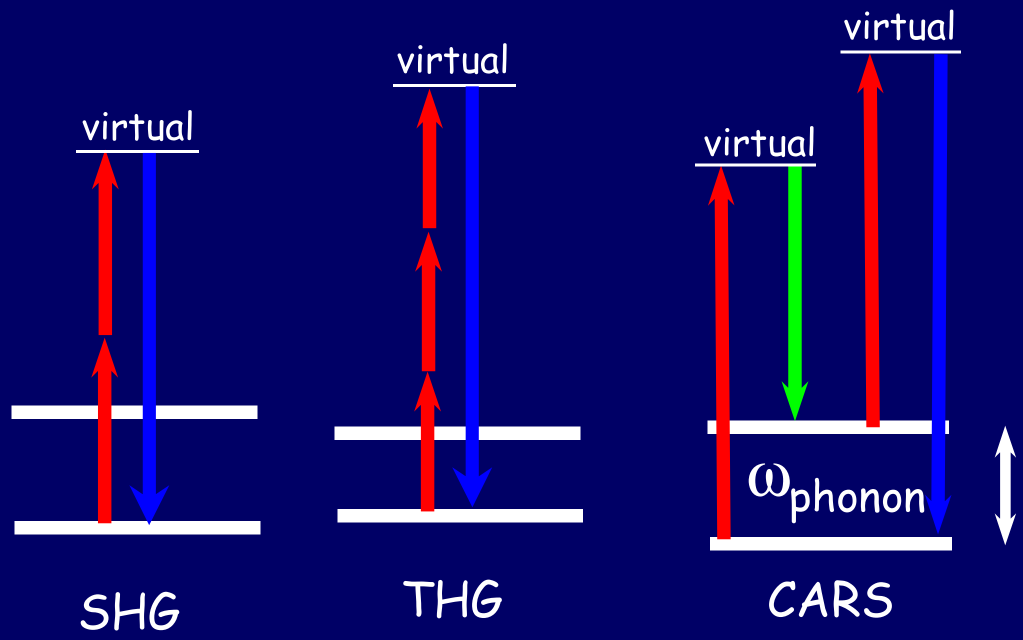


TPE
Fluorescence



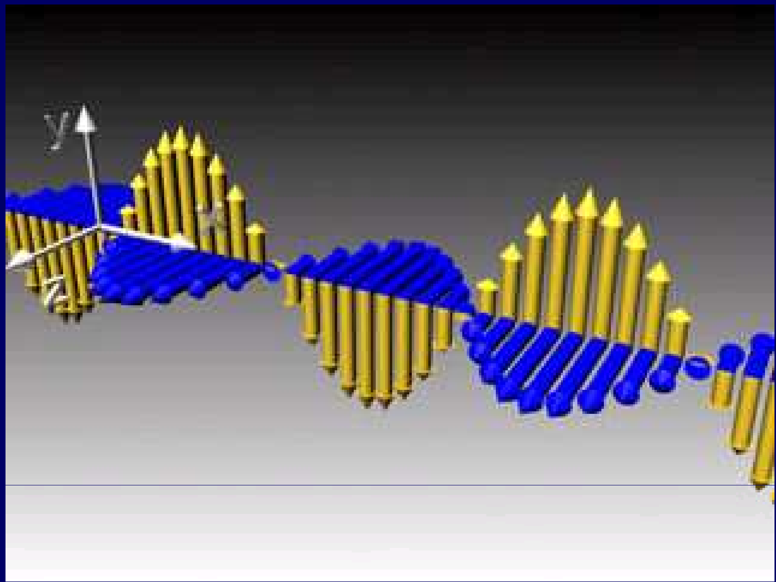
Hyper Raman

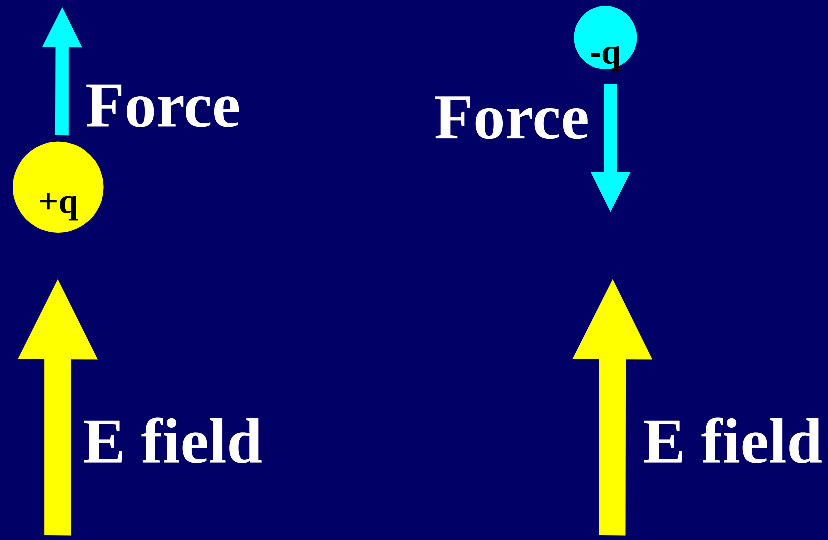
Elastic processes



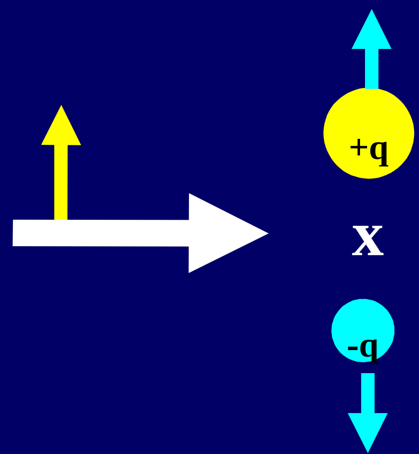
Understanding Optical Processes

Light is an Electromagnetic Field





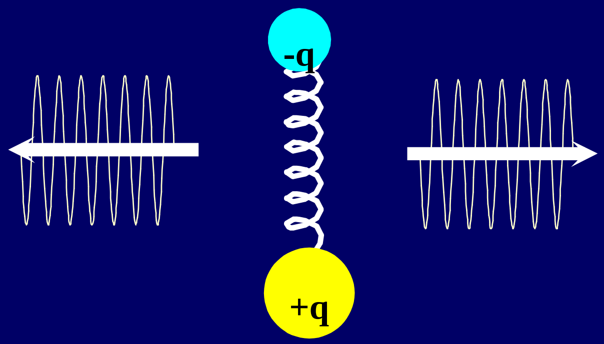
Dipole $P = q\,x$

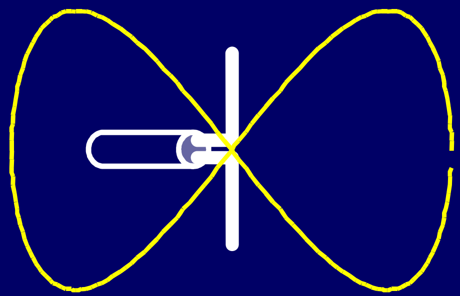


Light Irradiation: Molecular vibration

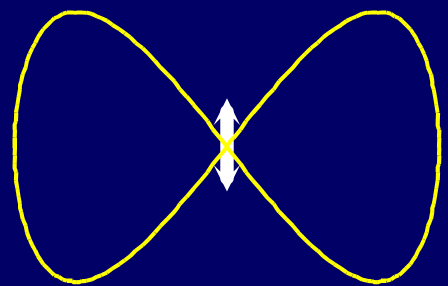
infrared ~ 10 μm

Dipole   P = q x



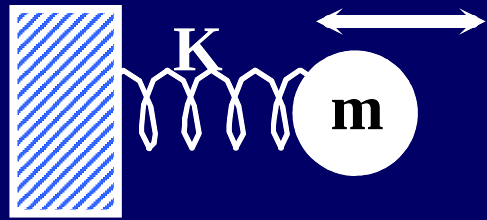


Radio wave emission

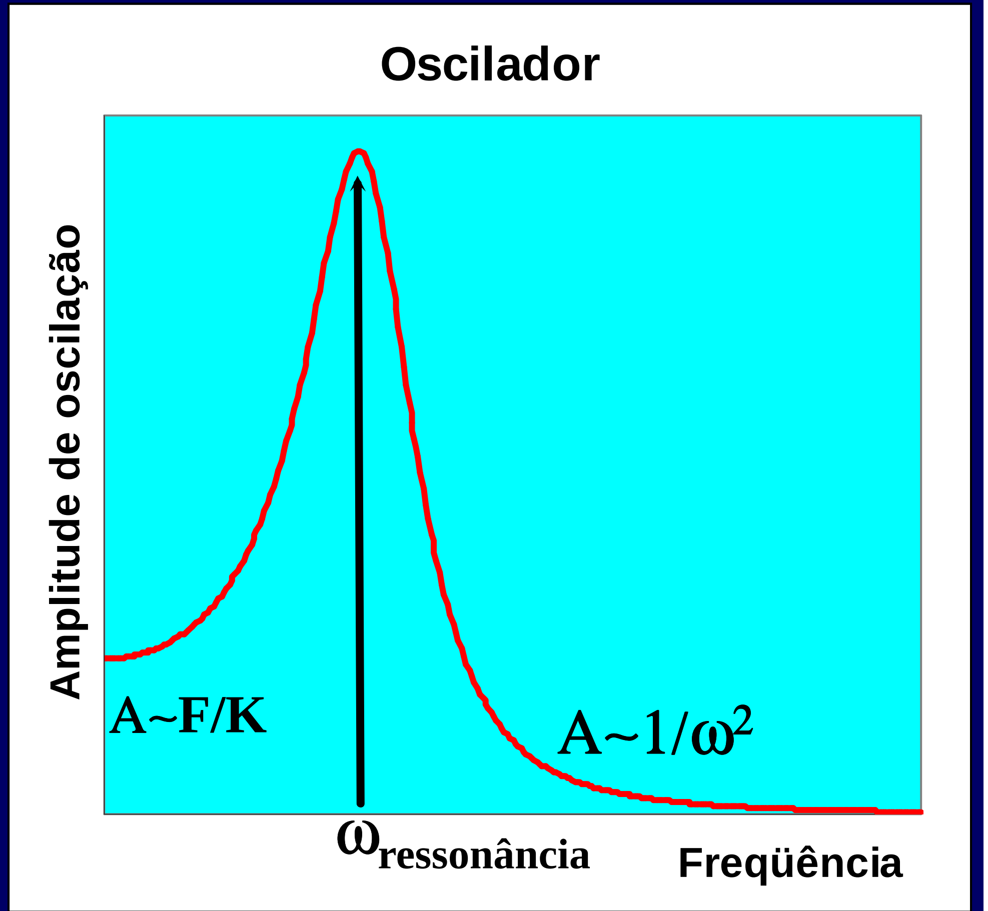


molecule light emission

Forced spring-mass system: resonance



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



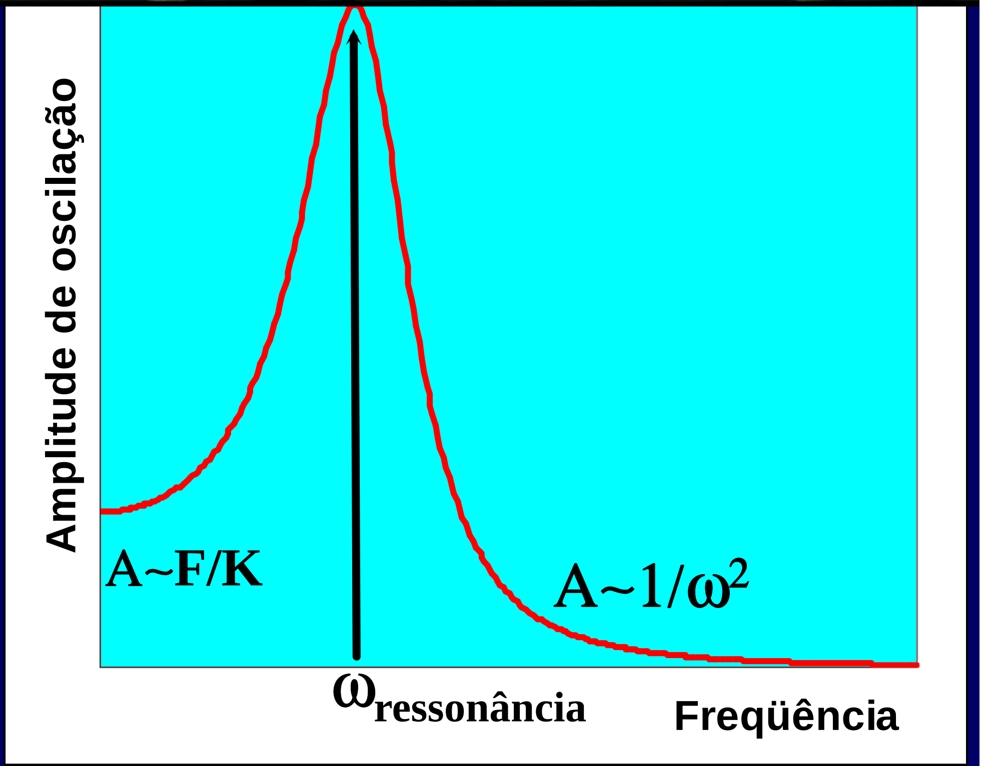
electron mass << nucleus mass
electron follows light frequency

nucleus do not follows light

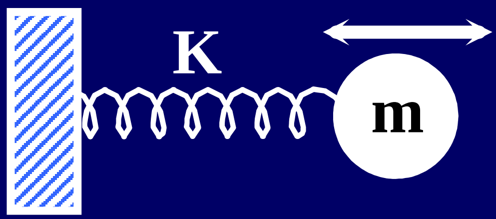
Forced spring-mass system: resonance

electron mass << nucleus mass
electron follows light frequency

nucleus do not follows light



Hooke´s law is an approximation



$$F = -k\,x$$

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

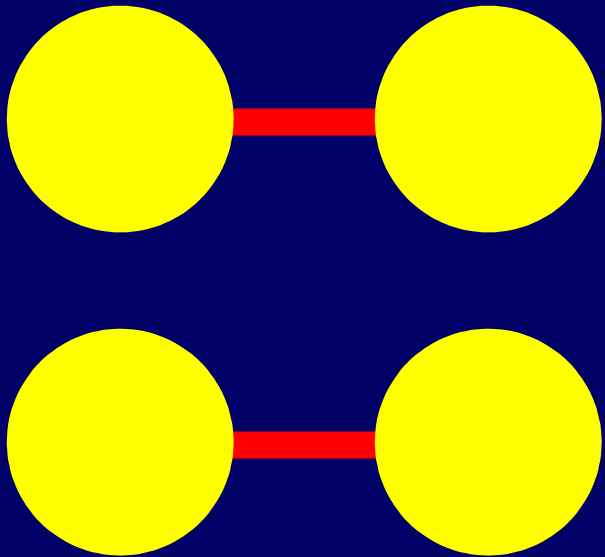
Inversion symmetry:

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

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

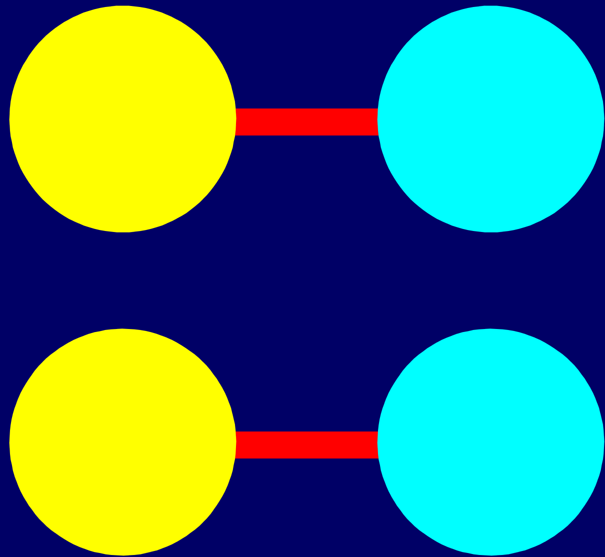
Molecular symmetry

Symmetric Molecule
Unnoticed change x by –x



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

Non-symmetric molecule
Noticed change x → -x



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

Electrons follow optical frequency: $x_e = \alpha E_o \cos(\omega t)$

Nuclei don't: $x_n = x_o \cos(\omega_n t)$

Molecule polarization

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

Electronic Absorption Rayleigh scattering $\chi^{(1)}$

Infrared absorption

SHG/SFG DFG $\chi^{(2)}$

Raman $\chi^{(1)}$

Overtone NIR

THG $\chi^{(3)}$

CARS $\chi^{(3)}$

NLO vocabulary – powers of E – only electron counts

Trygonometry

$$\cos(a+b) = \cos a \cos b - \sin a \sin b$$

$$\cos(a-b) = \cos a \cos b + \sin a \sin b$$

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

$$\cos(\omega_1 t)\cos(\omega_2 t) = \frac{1}{2}\cos\left[(\omega_2 + \omega_2)t\right] + \frac{1}{2}\cos\left[(\omega_1 - \omega_2)t\right]$$

SFG **DFG**

$$\cos^2(\omega t) = \frac{1+\cos(2\omega t)}{2}$$

SHG

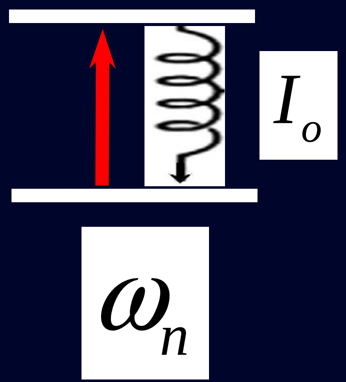
$$\cos^3(\omega t) = \frac{3\cos(3\omega t) + \cos(3\omega t)}{4}$$

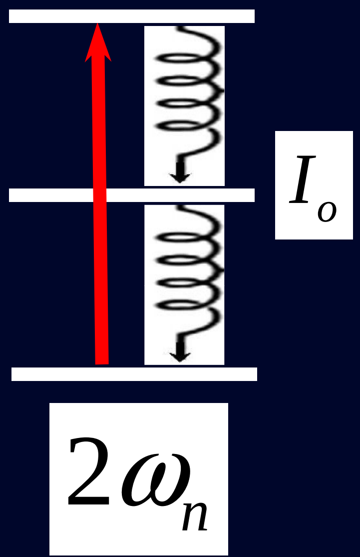
THG

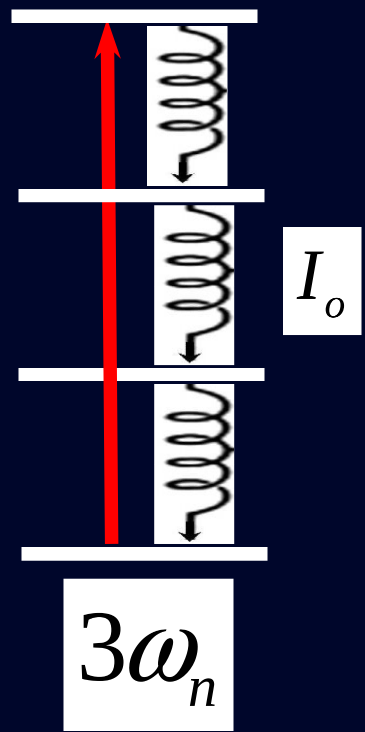
Infrared [IR] absorption

$x_n \cos(\omega_n t)$ $x_n^2 \cos^2(\omega_n t)$ $x_n^3 \cos^3(\omega_n t)$

IR absorption **1st overtone IR absorption** **2nd overtone IR absorption**







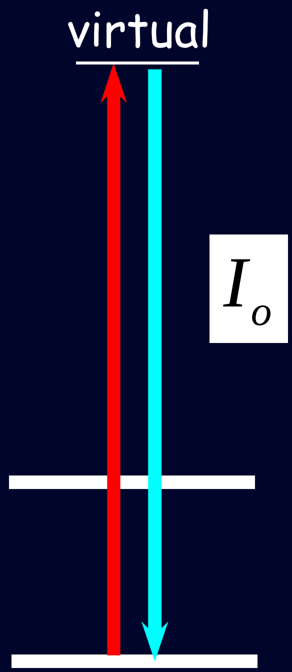
$$P[x_n, x_e] = \cdots + x_n + \cdots + x_n^2 + x_n^3 + \cdots$$

Linear Optics

$$\alpha E \cos(\omega t)$$

Rayleigh scattering

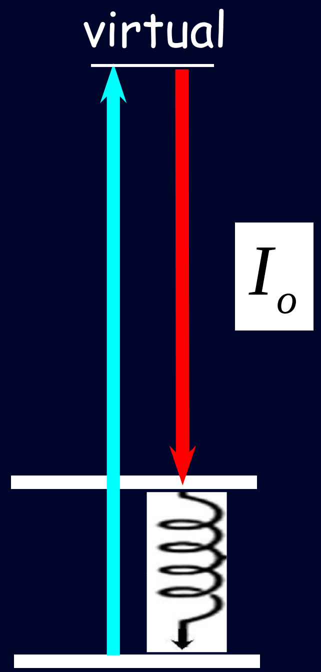
elastic



$$\alpha E\, x_n \cos\left[(\omega - \omega_n)t\right]$$

Raman Stokes

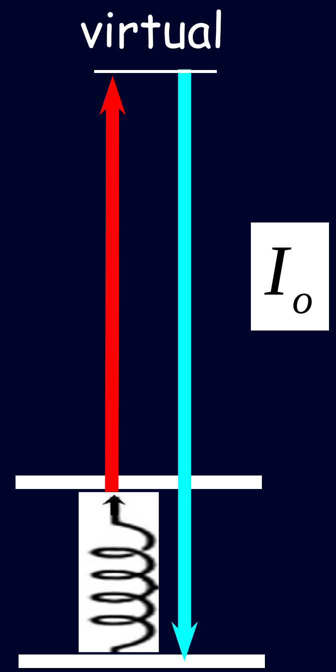
Inelastic - heating



$$\alpha E\, x_n \cos\left[(\omega + \omega_n)t\right]$$

Raman AntiStokes

Inelastic - refrigeration



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

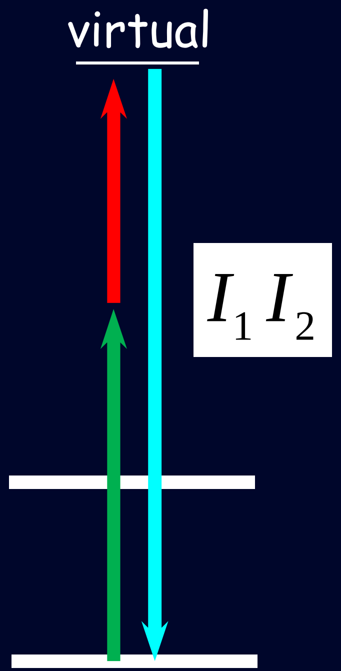
Second order elastic processes

$$\alpha^2 E_j E_k \cos\left[\left(\omega_j + \omega_k\right)t\right]$$

$$\alpha^2 E^2 \cos\left[2\omega t\right]$$

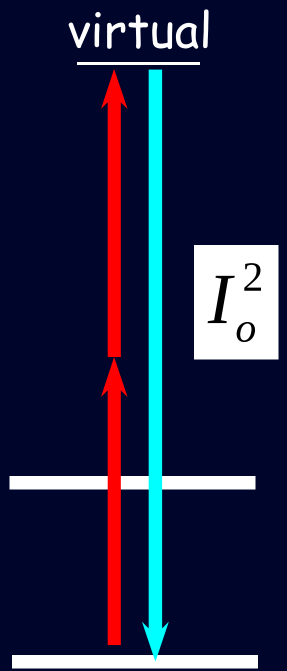
$$\alpha^2 E_j E_k \cos\left[\left(\omega_j - \omega_k\right)t\right]$$

Sum Frequency Generation

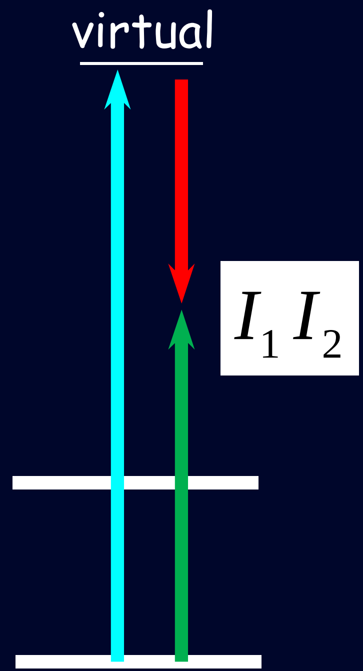


Second Harmonic Generation

$\omega_j = \omega_k$



Difference Frequency Generation



$$P[x_n, x_e] = \cdots + x_e^2 + \cdots$$

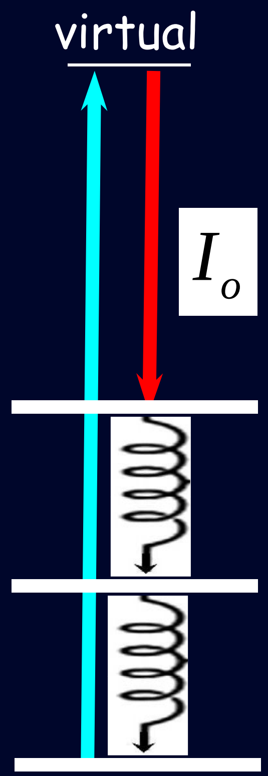
Second order inelastic processes

$$x_n^2 \alpha E \cos\left[\left(\omega \pm 2\omega_n\right)t\right]$$

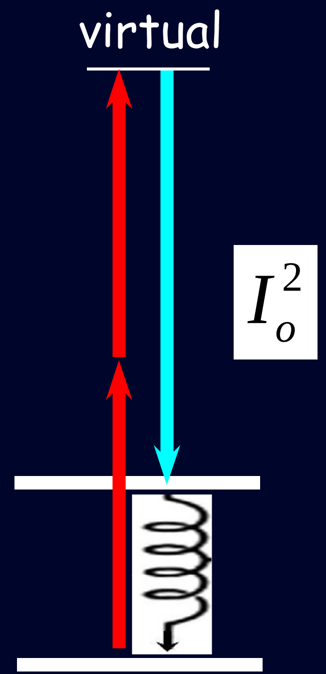
$$x_n \alpha^2 E^2 \cos\left[\left(2\omega - \omega_n\right)t\right]$$

$$x_n \alpha^2 E^2 \cos\left[\left(2\omega + \omega_n\right)t\right]$$

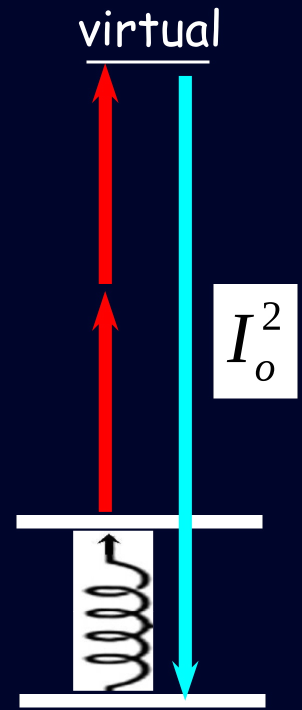
Raman 1st overtone



Hyper Raman Stokes



Hyper Raman AntiStokes



$$P[x_n, x_e] = \cdots + x_e x_n^2 + \cdots + x_e^2 x_n + \cdots$$

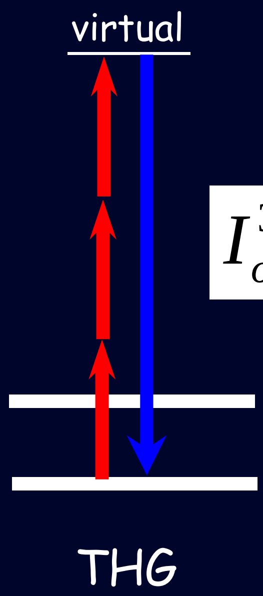
Third order elastic processes

$$\alpha^3 E^3 \cos(3\omega t)$$

$$x_n \alpha^3 E_j^2 E_k \cos\left[\left(\omega_j + \omega_n\right)t\right]$$

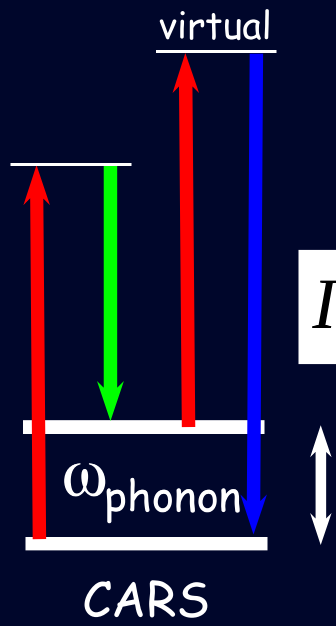
$$x_n \alpha^3 E_j^2 E_k \cos\left[\left(\omega_j - \omega_n\right)t\right]$$

Third Harmonic Generation



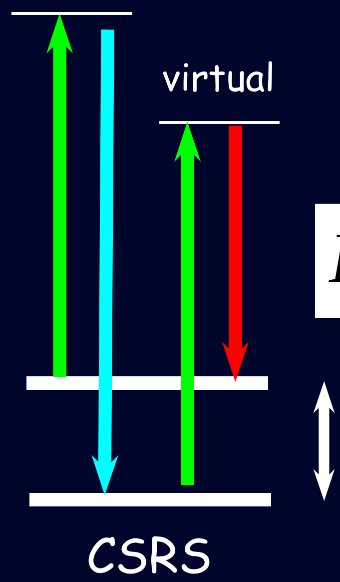
$$I_o^3$$

Coherent AntiStokes Raman Scatterinng CARS



$$I_1 I_2^2$$

Coherent Stokes Raman Scatterinng CSRS

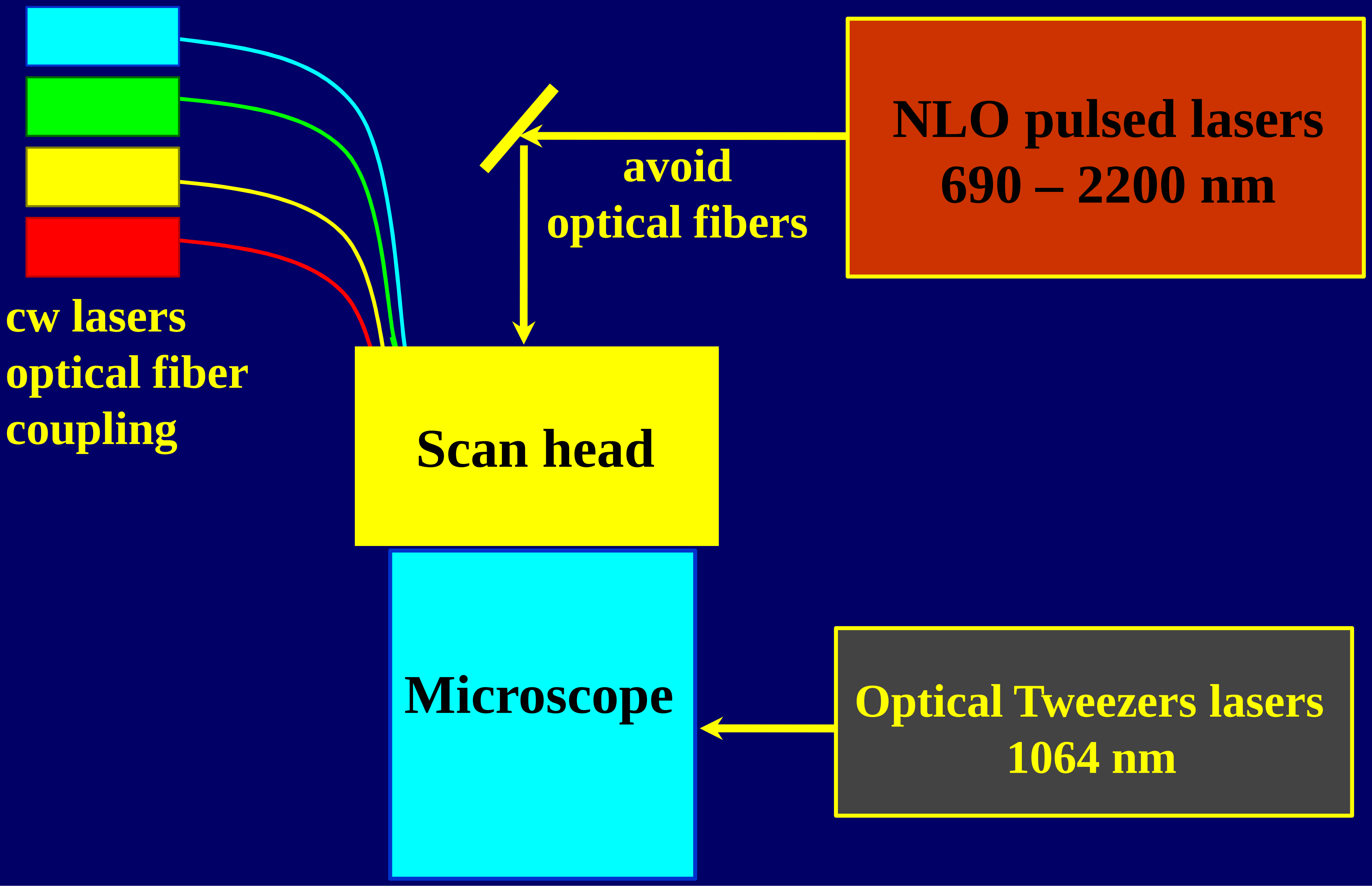


$$I_1^2 I_2$$

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

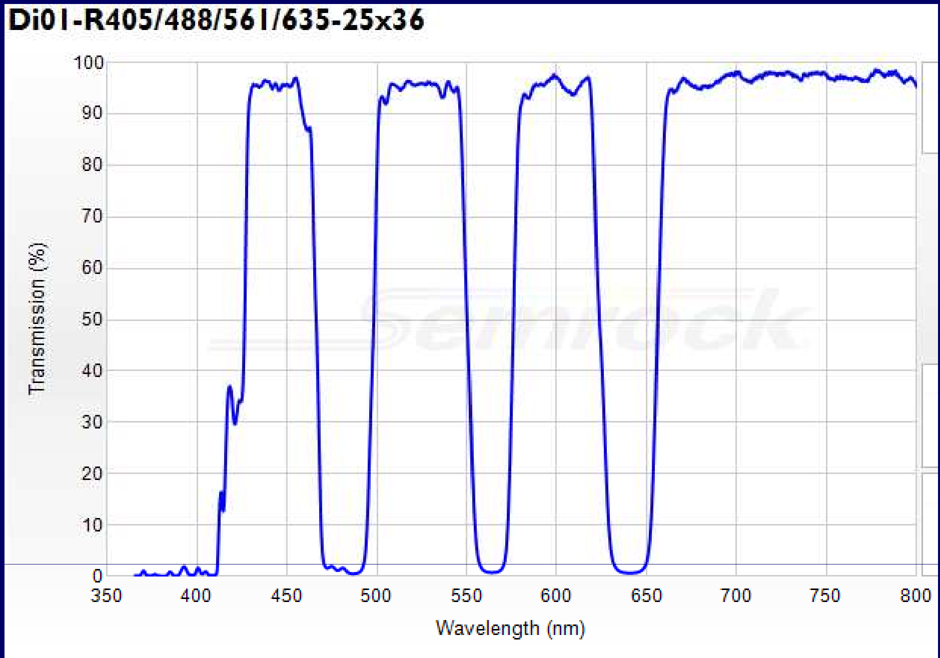
Optical Circuits

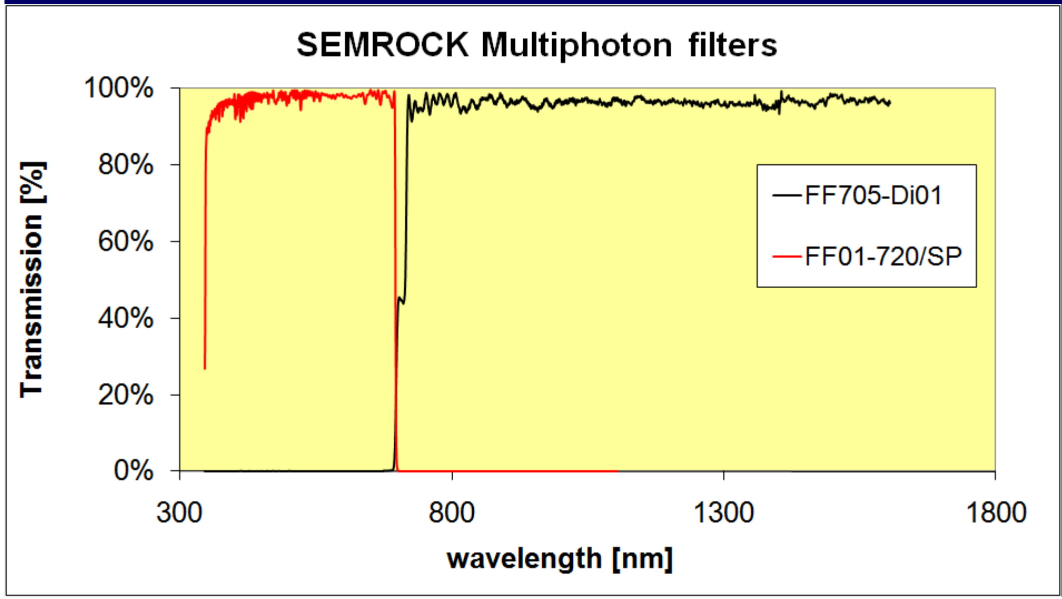
Excitation

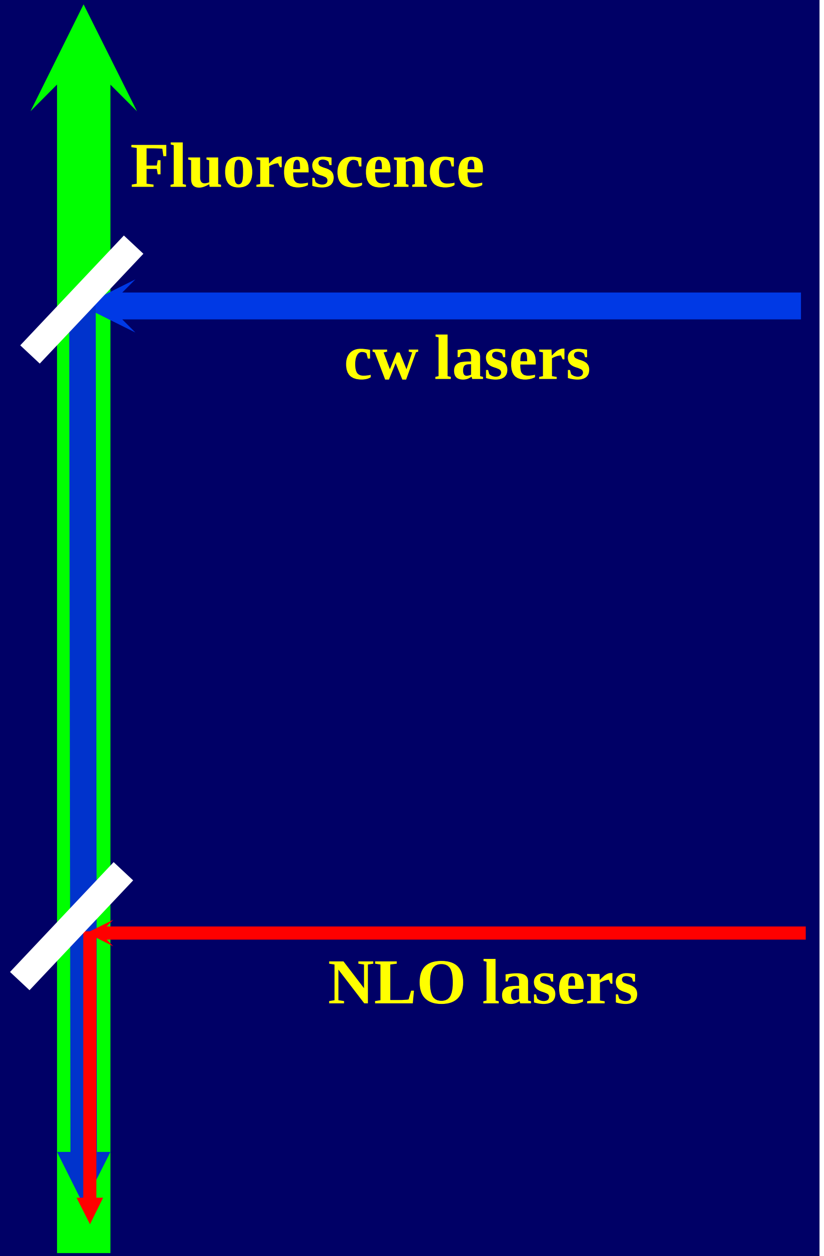


NLO pulsed lasers
690 – 2200 nm
avoid
optical fibers
cw lasers
optical fiber
coupling
Scan head
Microscope
Optical Tweezers lasers
1064 nm

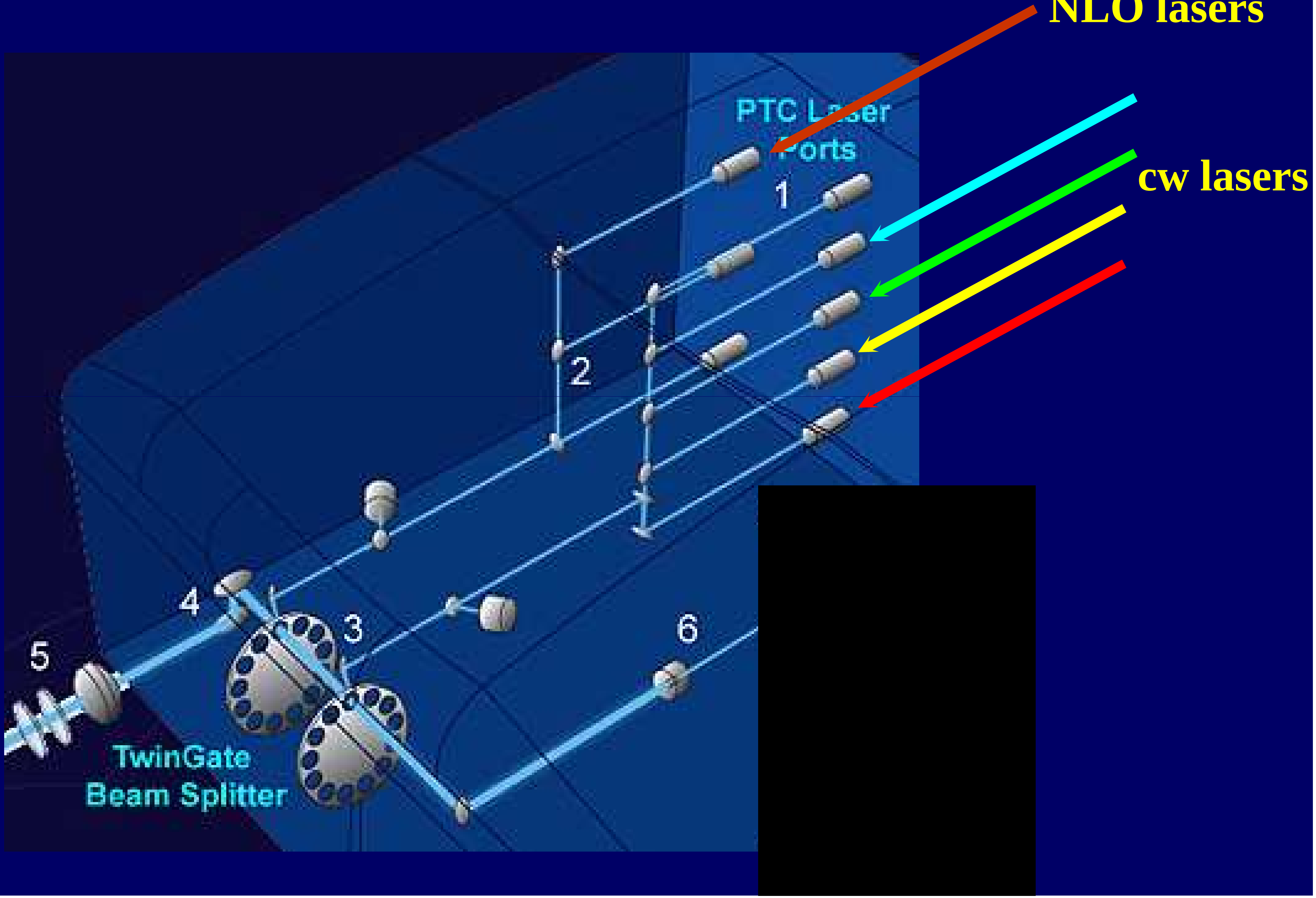
NLO & cw Laser combining dichroics



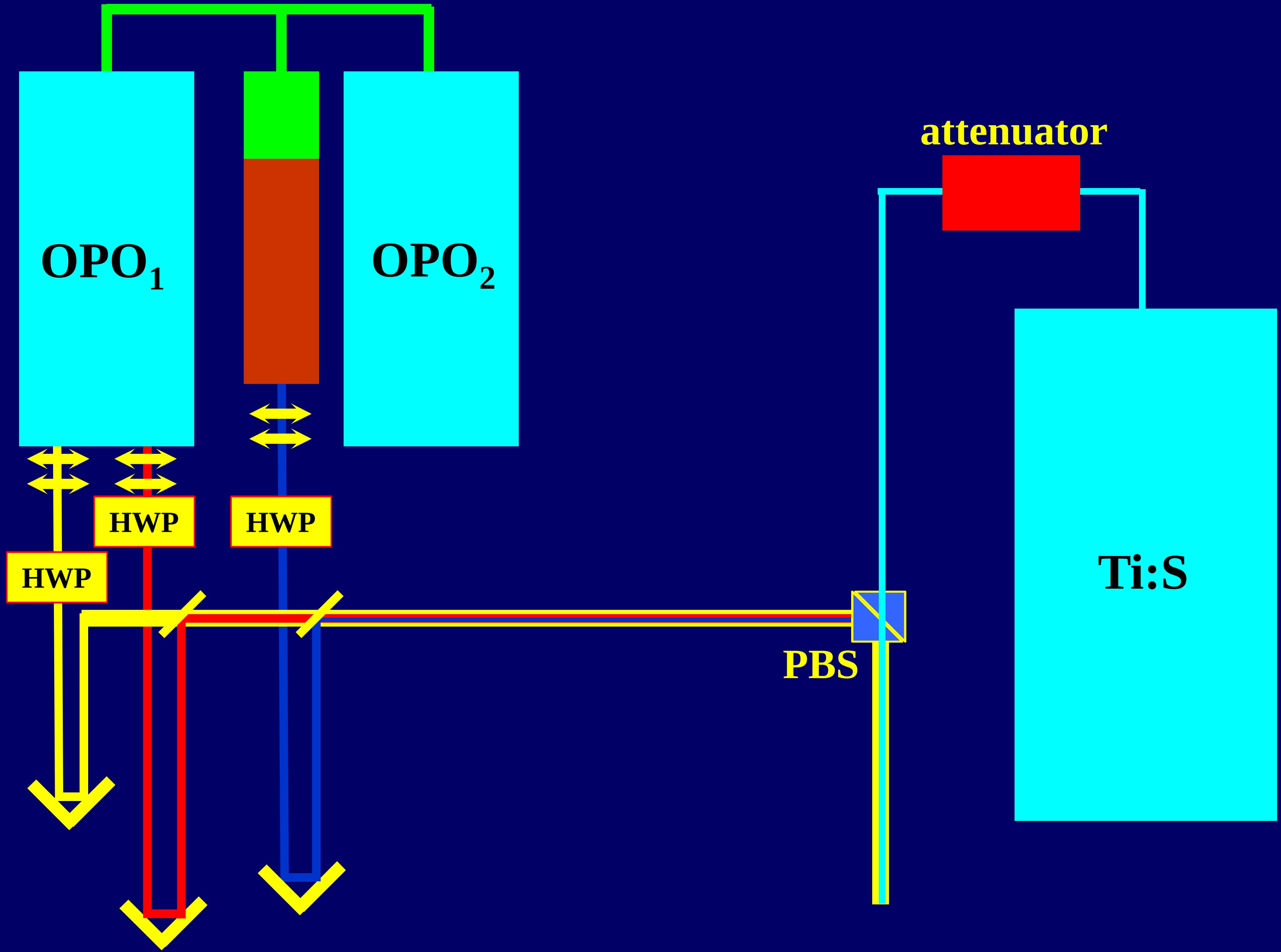


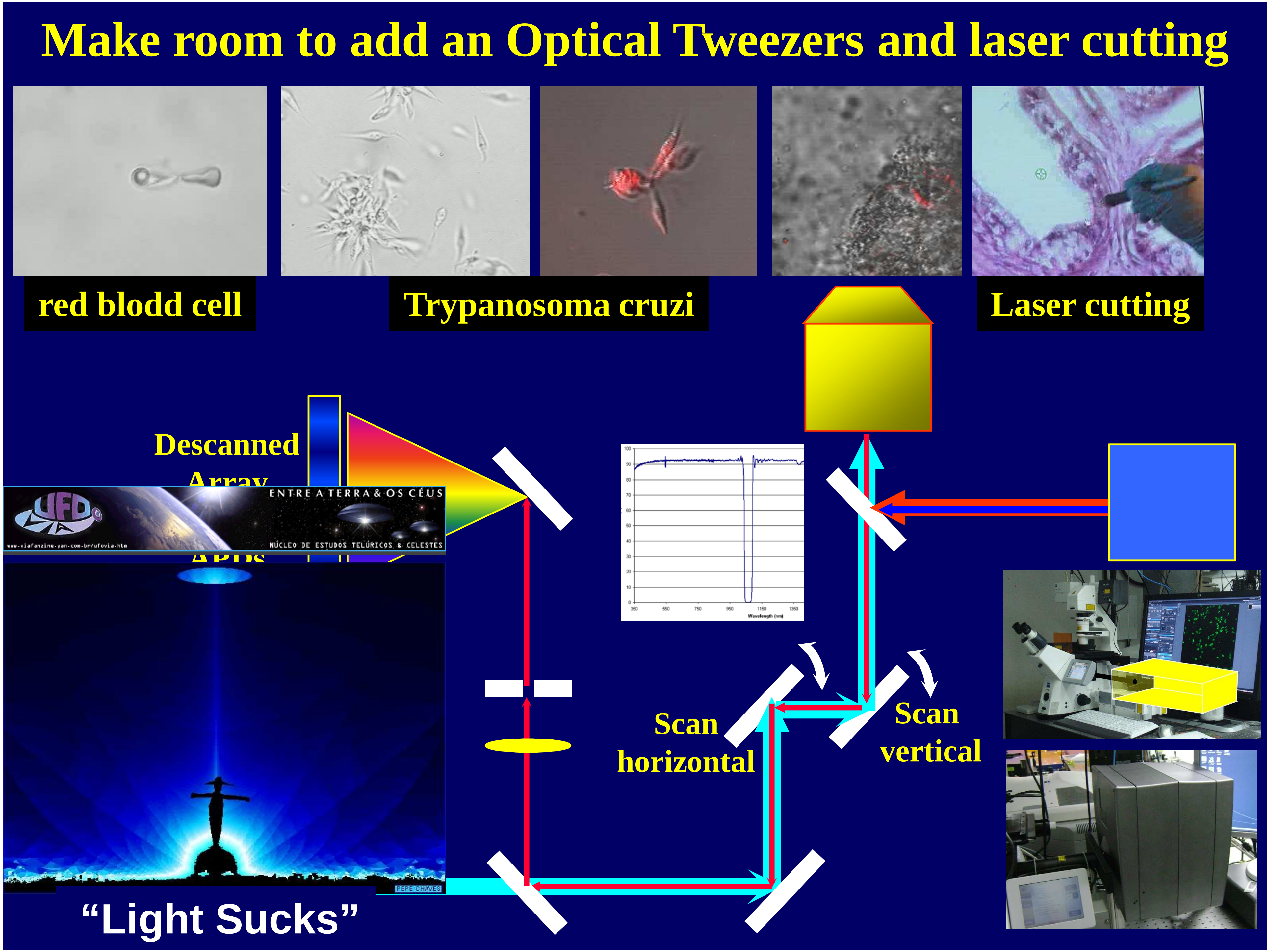


Zeiss Spectral Confocal



NLO lasers combination




Make room to add an Optical Tweezers and laser cutting
red blodd cell
Trypanosoma cruzi
Laser cutting
Descanned
Array
ENTRE A TERRA & OS CÉUS
NÚCLEO DE ESTUDOS TELÚRICOS & CELESTES
Scan horizontal
Scan vertical
"Light Sucks"
PEPE CHAVES

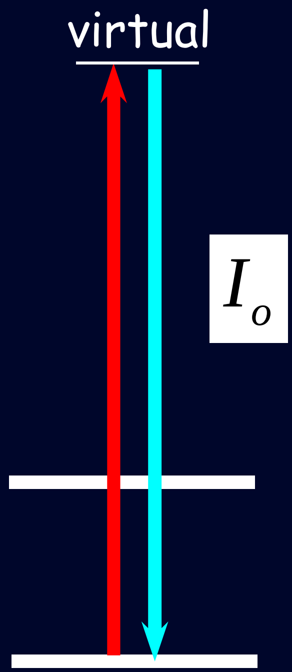
NLO microscopy properties

Linear Optics

$$\alpha E \cos(\omega t)$$

Rayleigh scattering

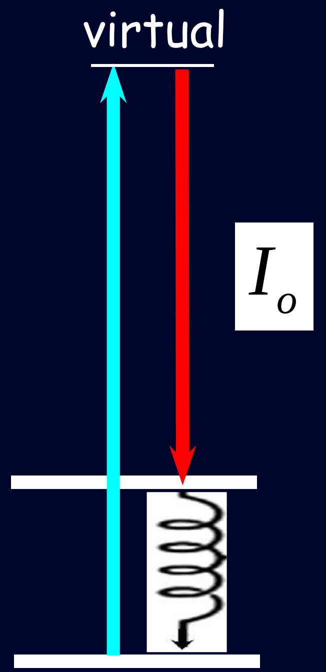
elastic



$$\alpha E\, x_n \cos\left[(\omega - \omega_n)t\right]$$

Raman Stokes

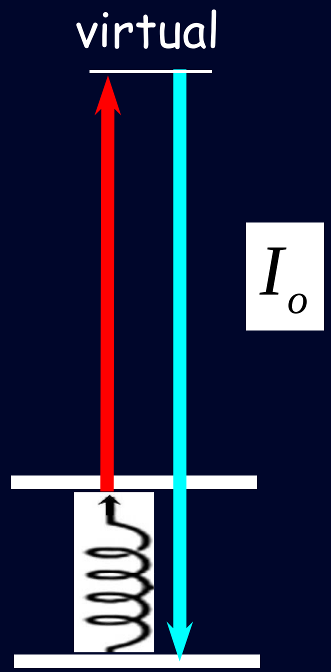
Inelastic - heating



$$\alpha E\, x_n \cos\left[(\omega + \omega_n)t\right]$$

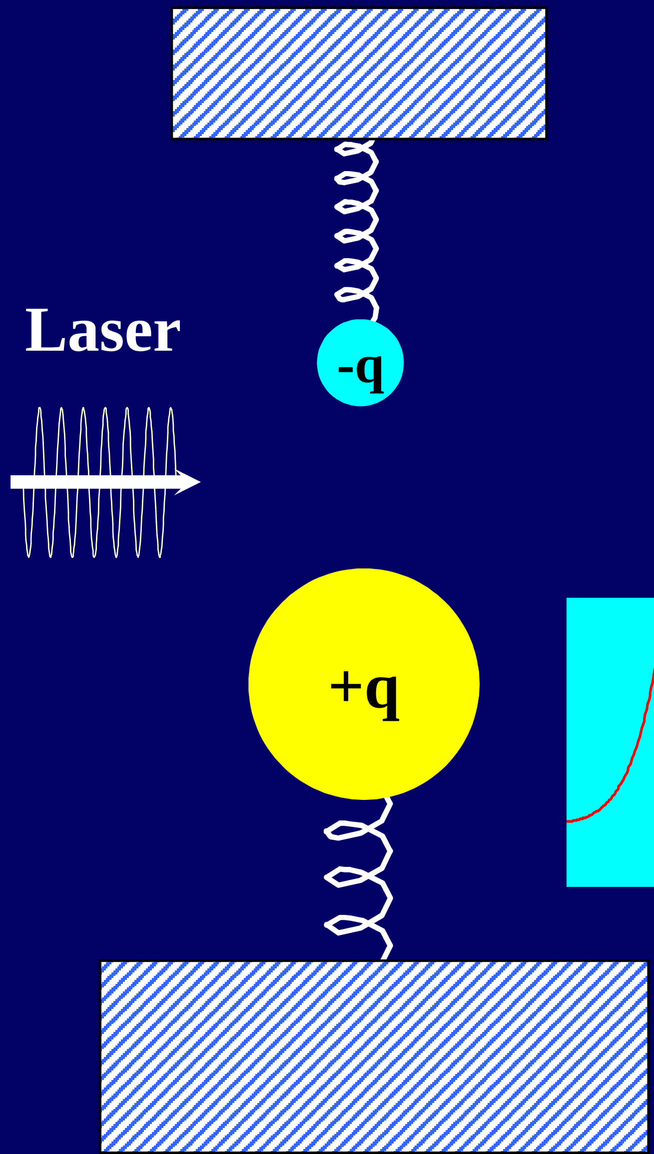
Raman AntiStokes

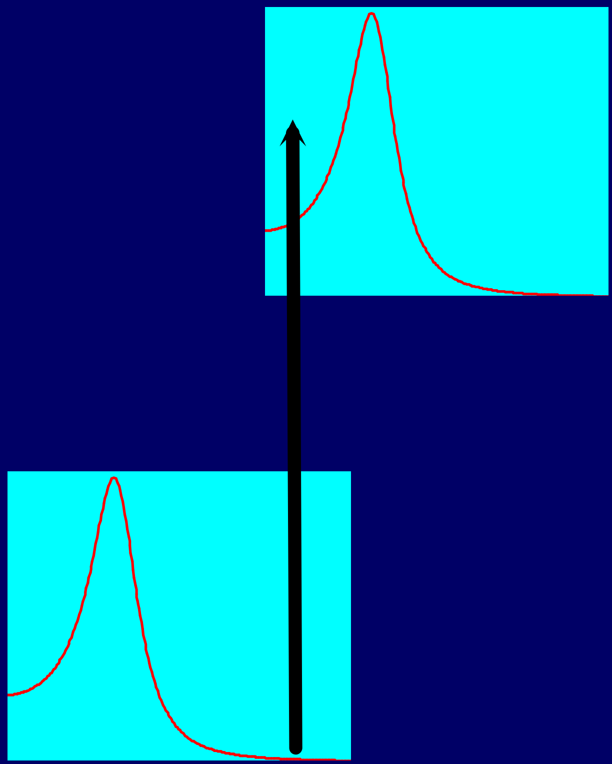
Inelastic - refrigeration



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

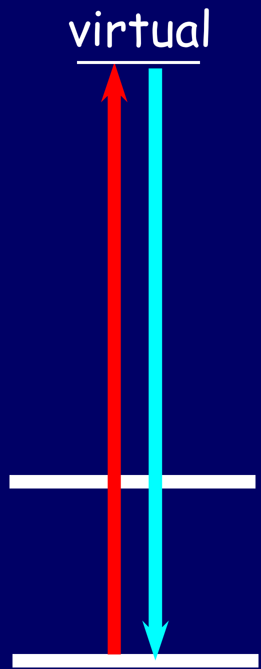
Rayleigh Scattering:
Electron follows the laser - nucleus do not





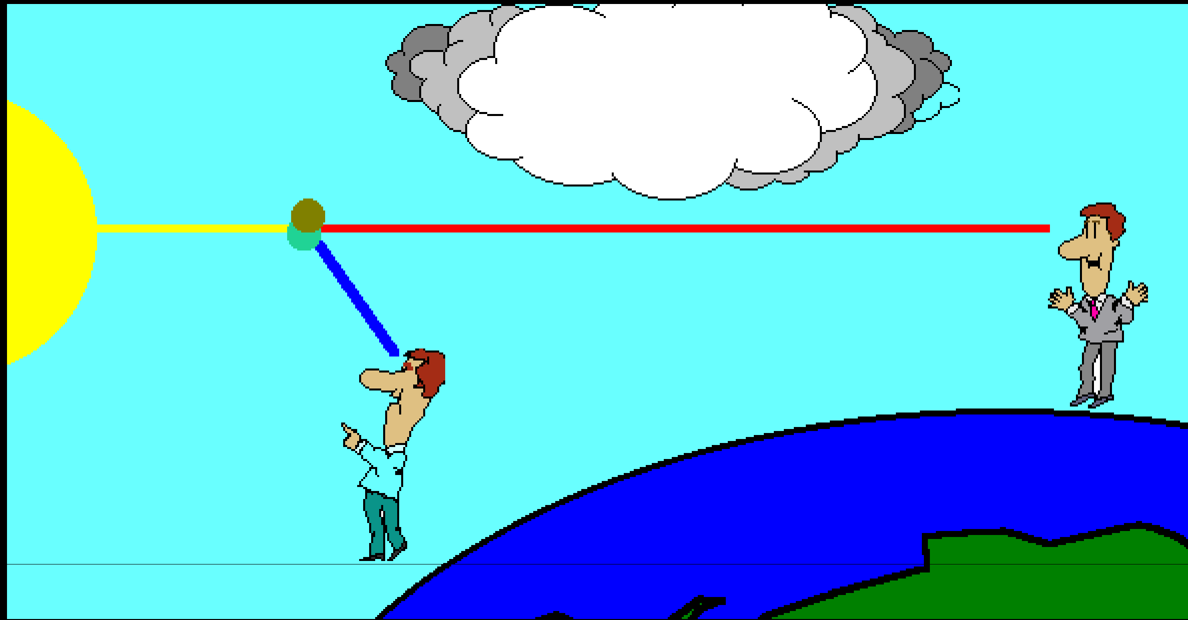
Rayleigh scattering

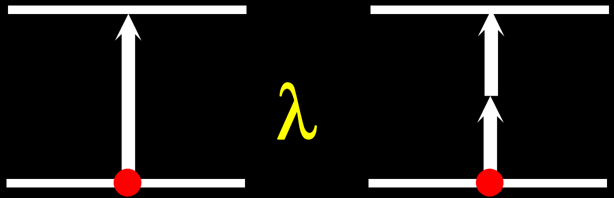
elastic



$$I_{Rayleigh} \propto \frac{1}{\lambda^4}$$

Espalhamento Rayleigh α $1/\lambda^4$



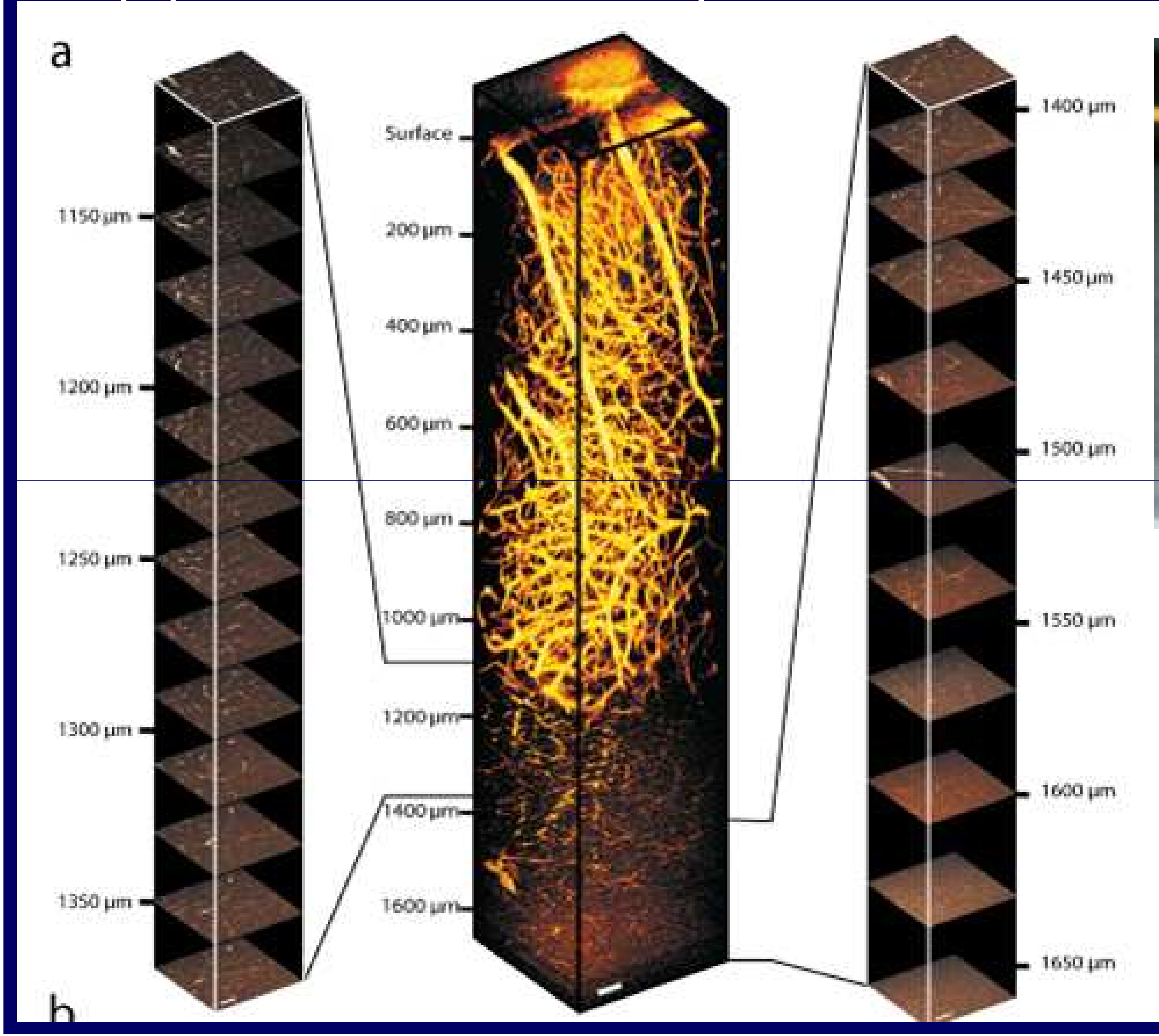


λ 2λ

2^4 = 16 mais penetração

Penetração aumenta na direção do infravermelho

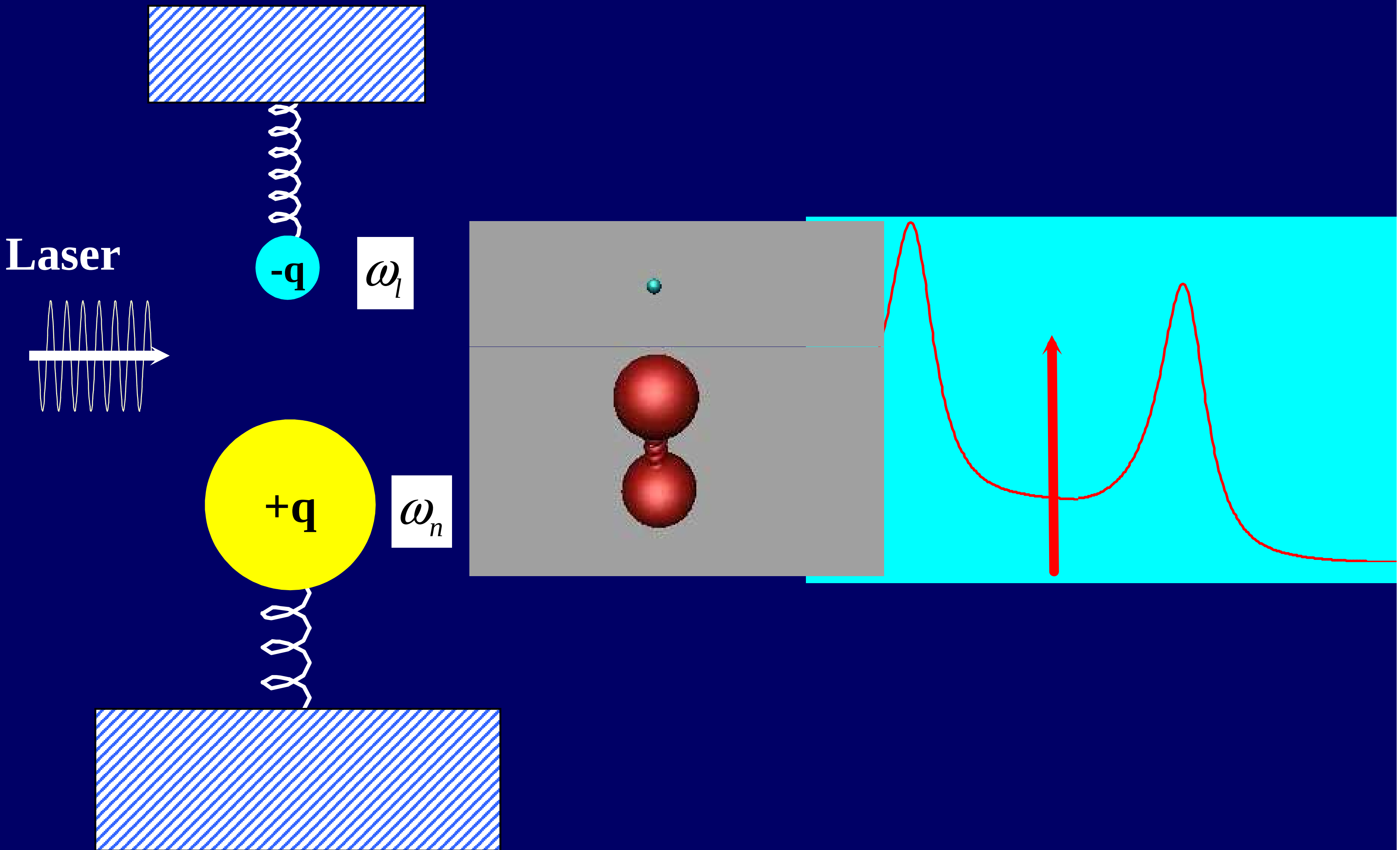
Deep penetration: > 1600 μm



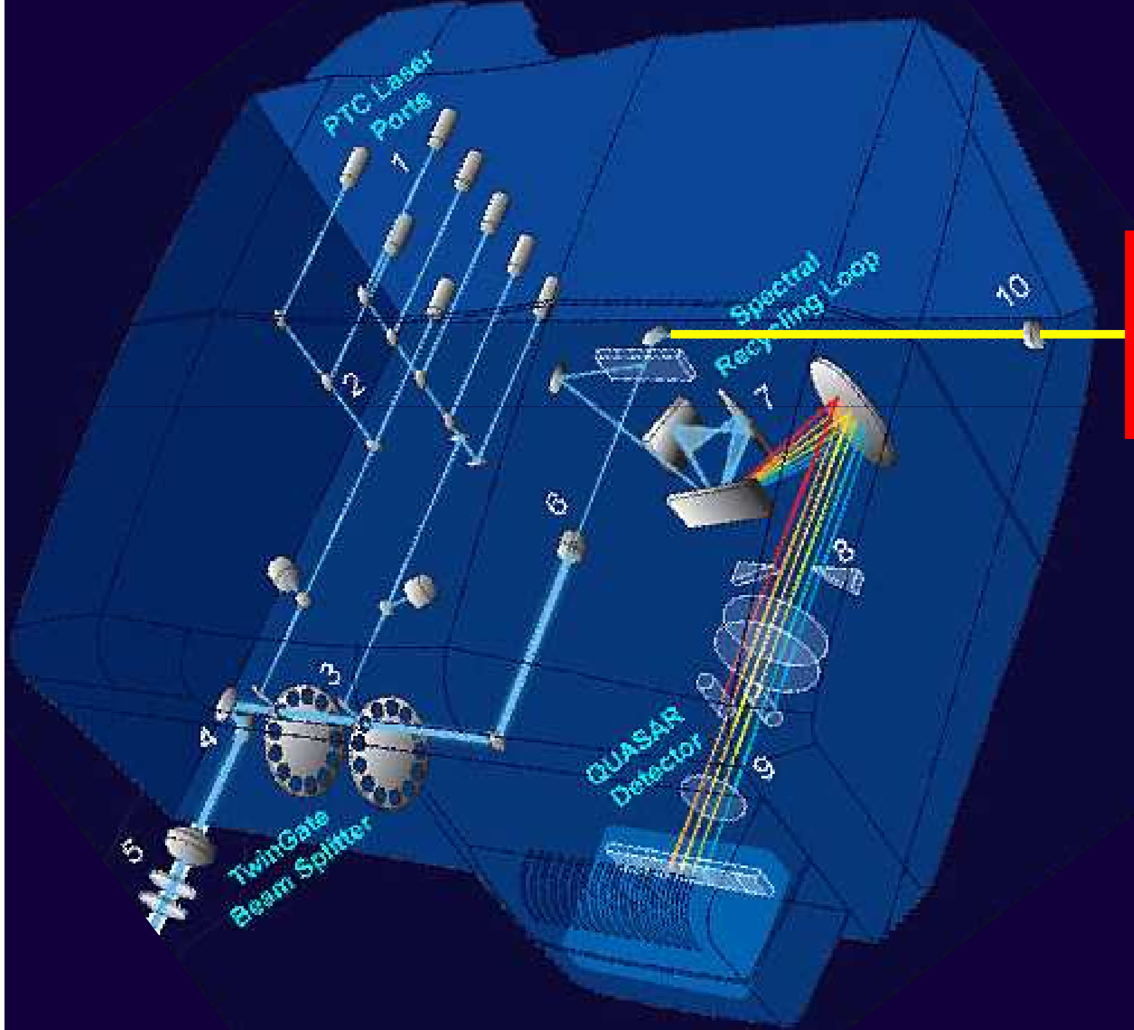
Chris Xu

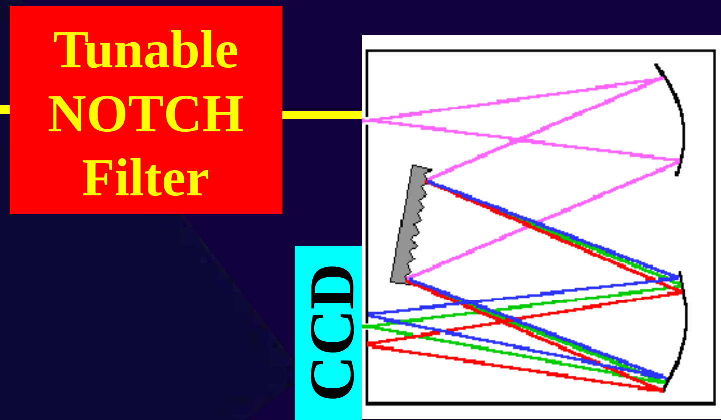
Cornell

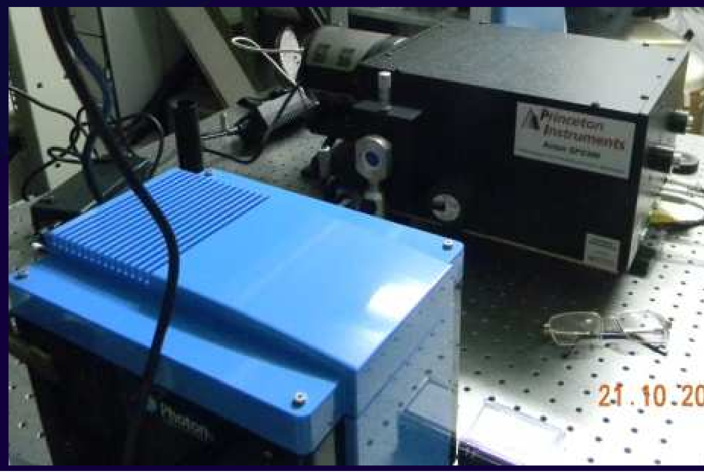
Raman: Electron follows the laser, nucleus dont



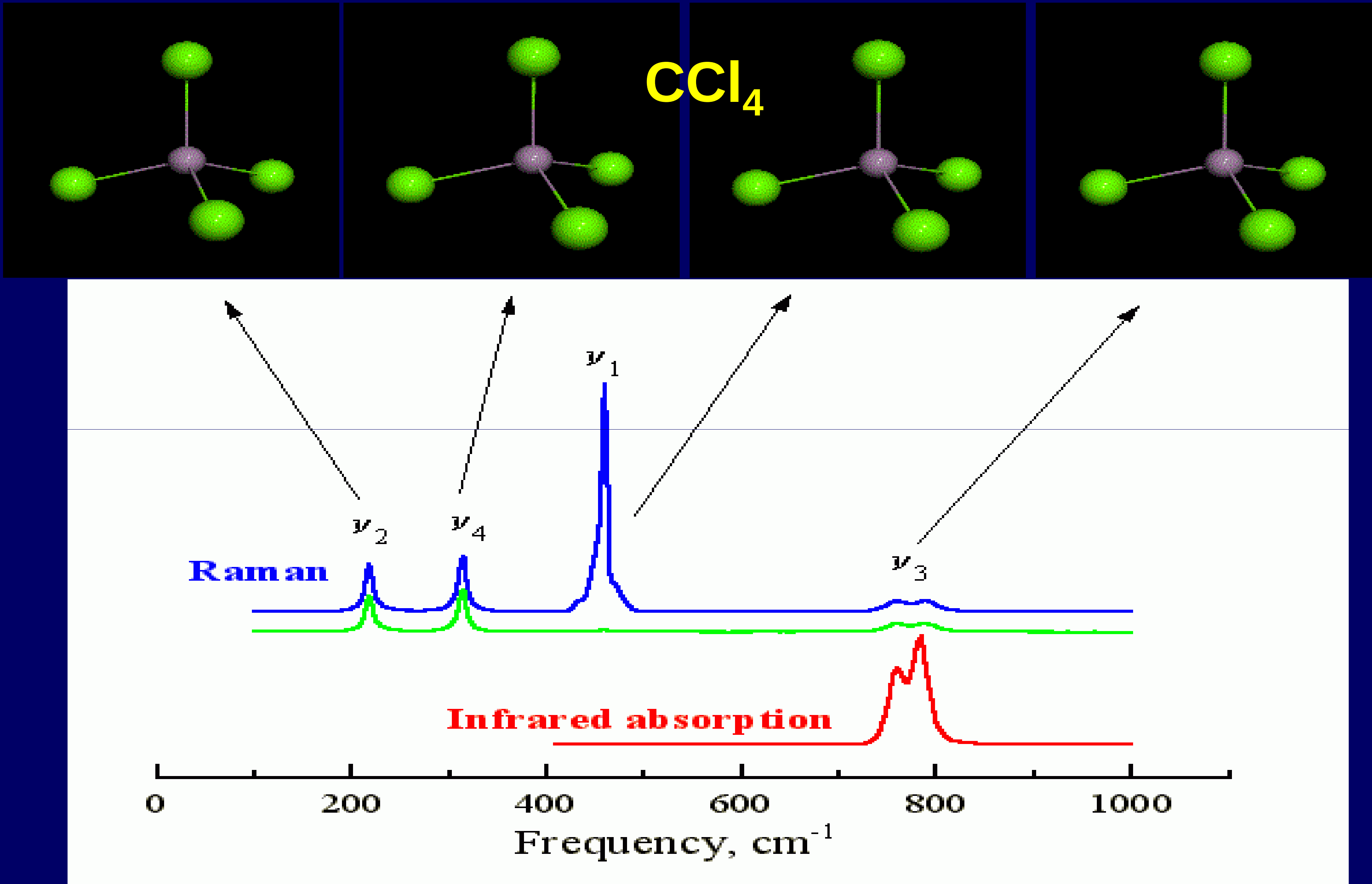
Add a confocal Raman Spectroscopy to get: Raman + Hyper Raman



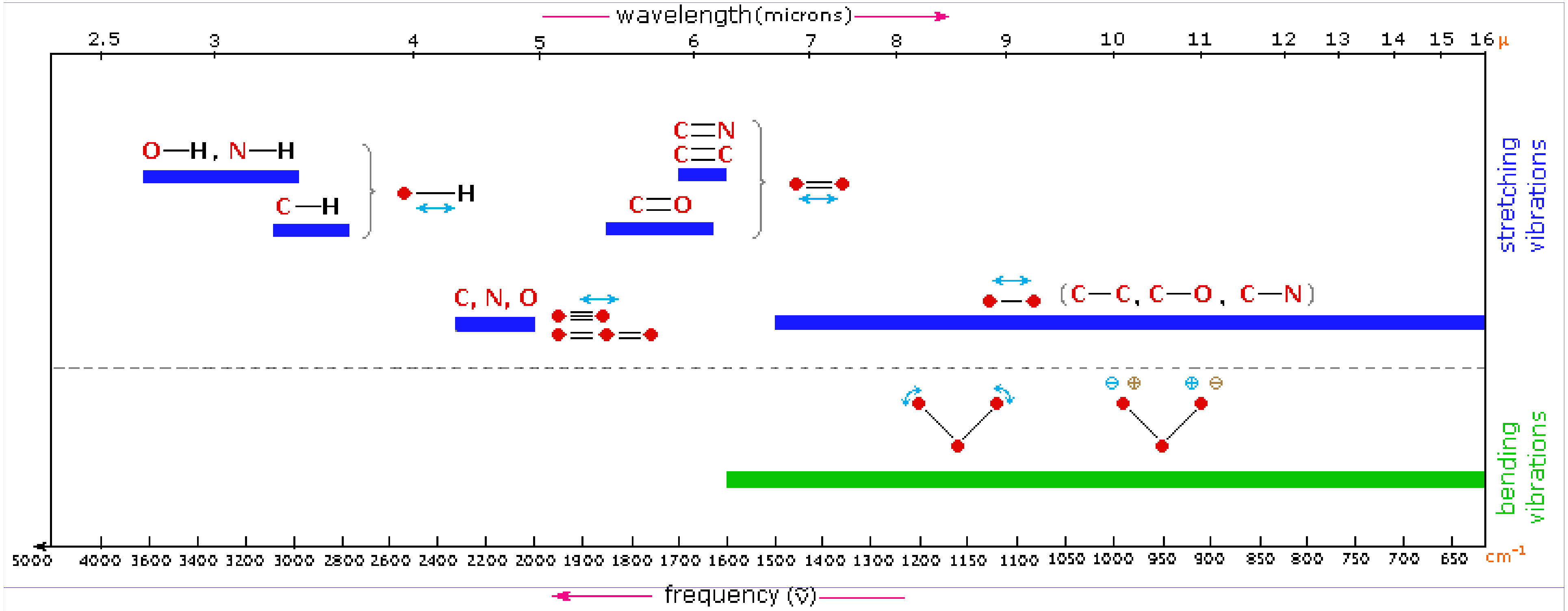




Biochemical information of molecular vibrations



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



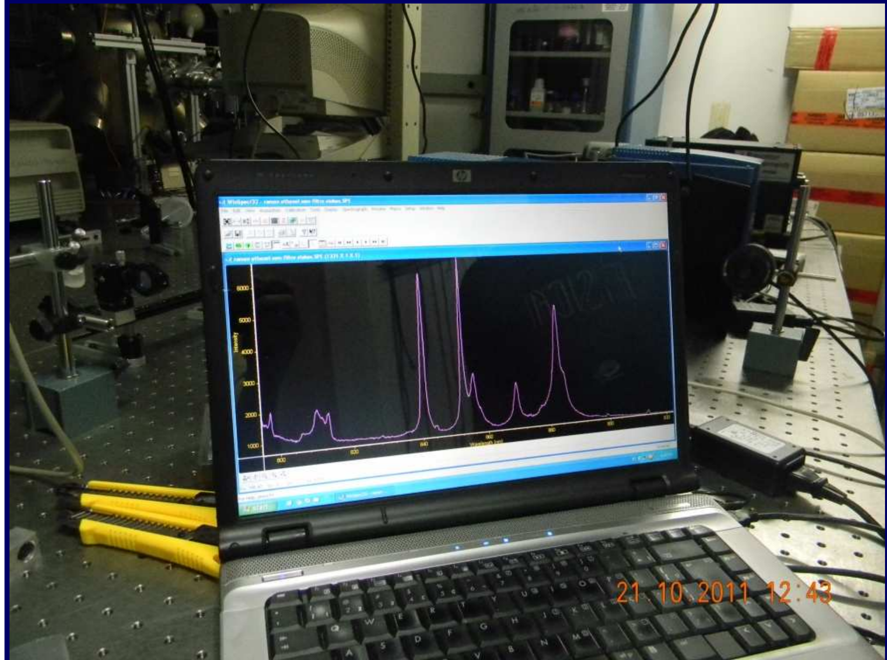
General Trends:

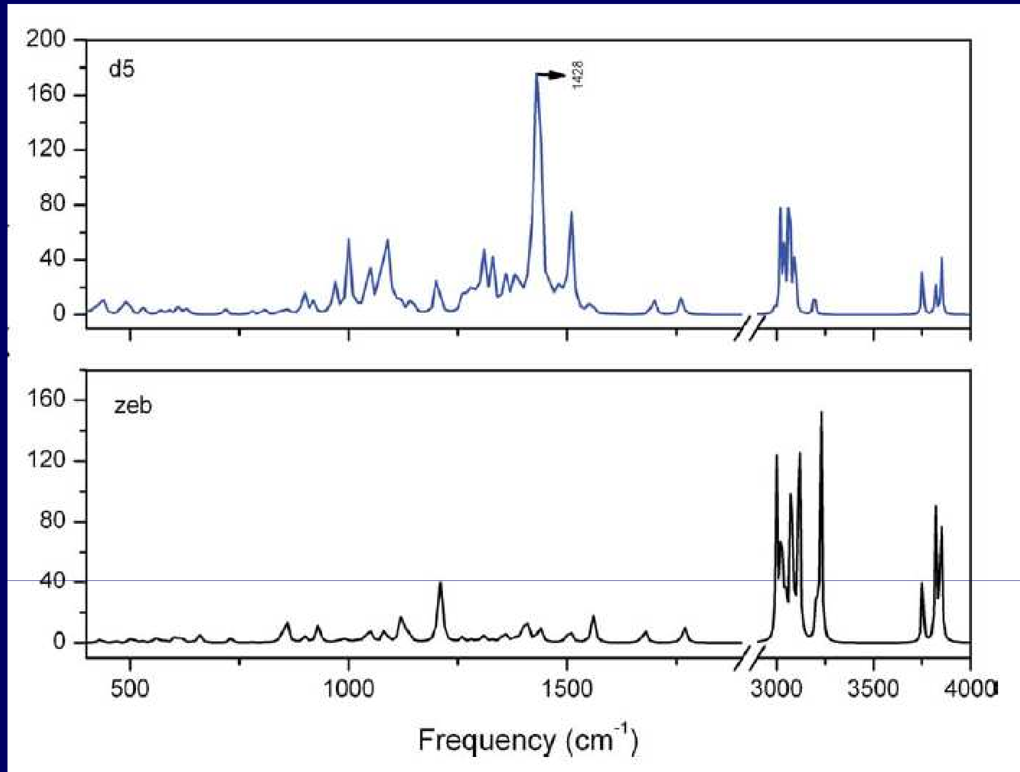
i) Stretching frequencies are higher than corresponding bending frequencies.

ii) Bonds to hydrogen $\omega = \sqrt{\frac{K}{m}}$ have higher stretching frequencies.

iii) Triple bonds stretching frequencies > double bonds > single bonds.

Raman - Methylated vs non-methylated DNA





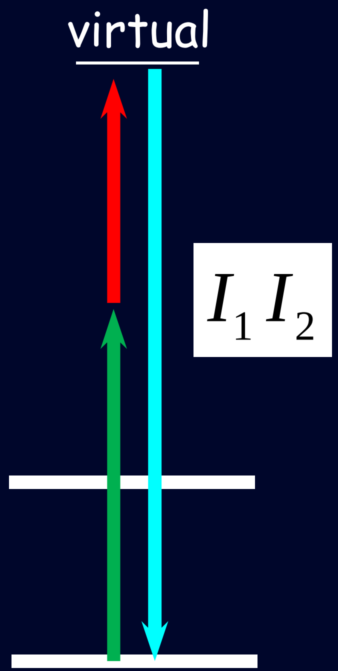
Second order elastic processes

$$\alpha^2 E_j E_k \cos\left[\left(\omega_j + \omega_k\right)t\right]$$

$$\alpha^2 E^2 \cos[2\omega t]$$

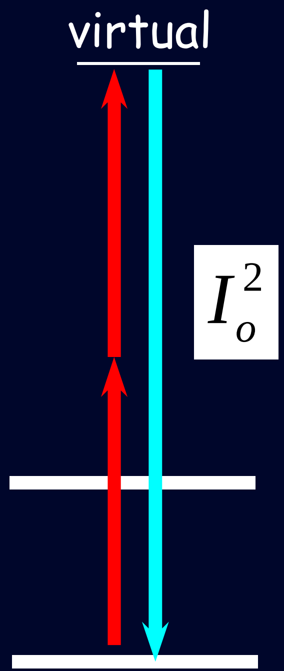
$$\alpha^2 E_j E_k \cos\left[\left(\omega_j - \omega_k\right)t\right]$$

Sum Frequency Generation

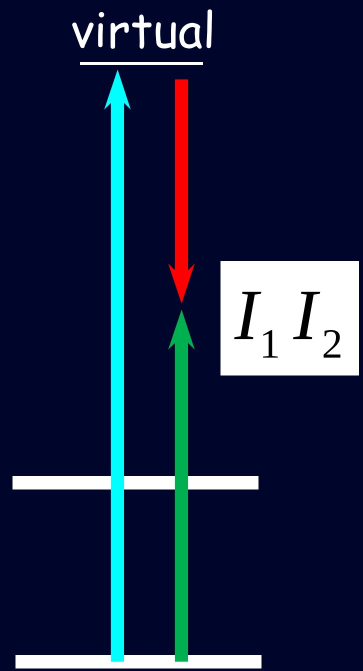


Second Harmonic Generation

$\omega_j = \omega_k$

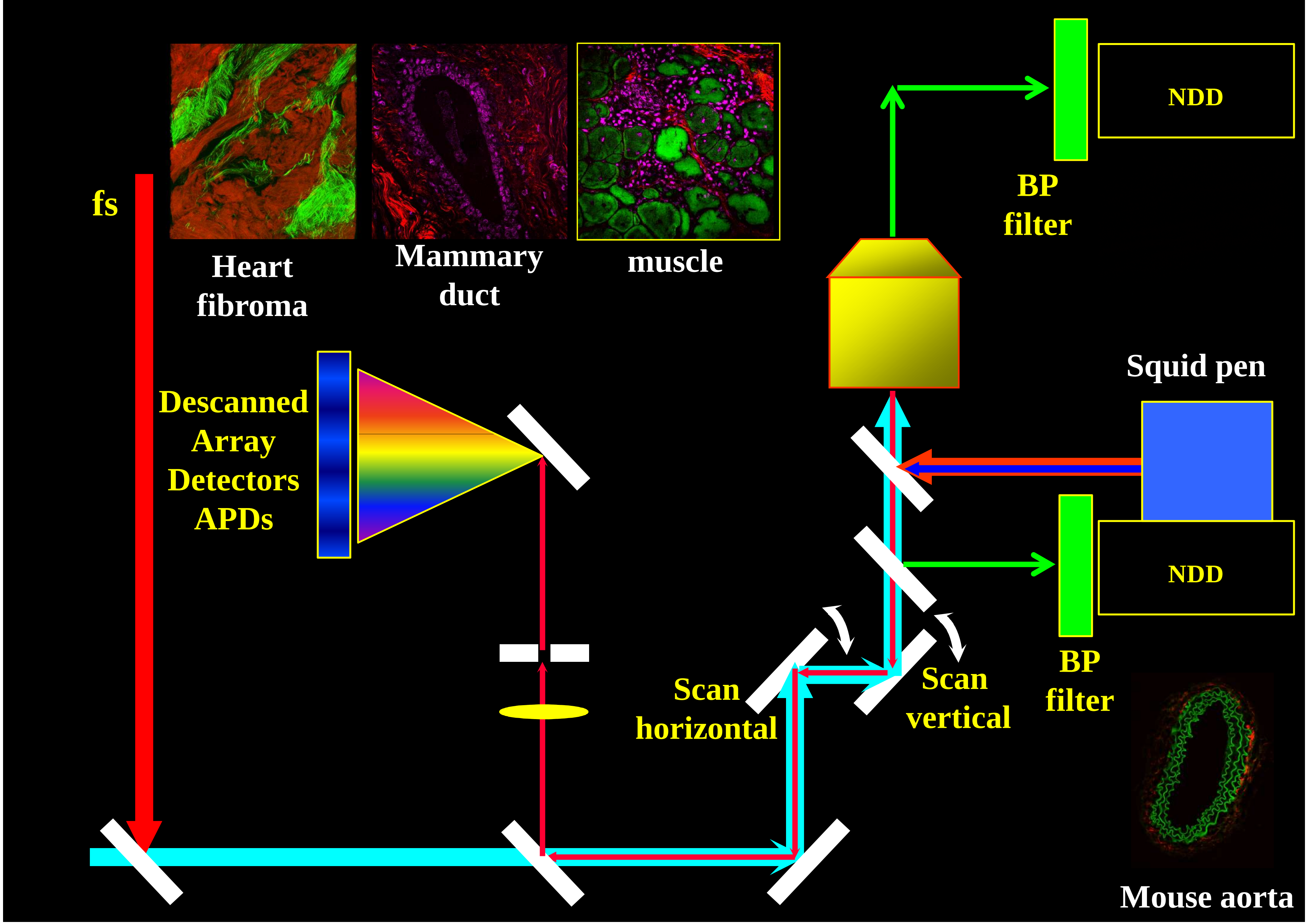


Difference Frequency Generation



$$P[x_n, x_e] = \cdots + x_e^2 + \cdots$$

Second/Third Harmonic Generation comes for free



SHG Microscopy

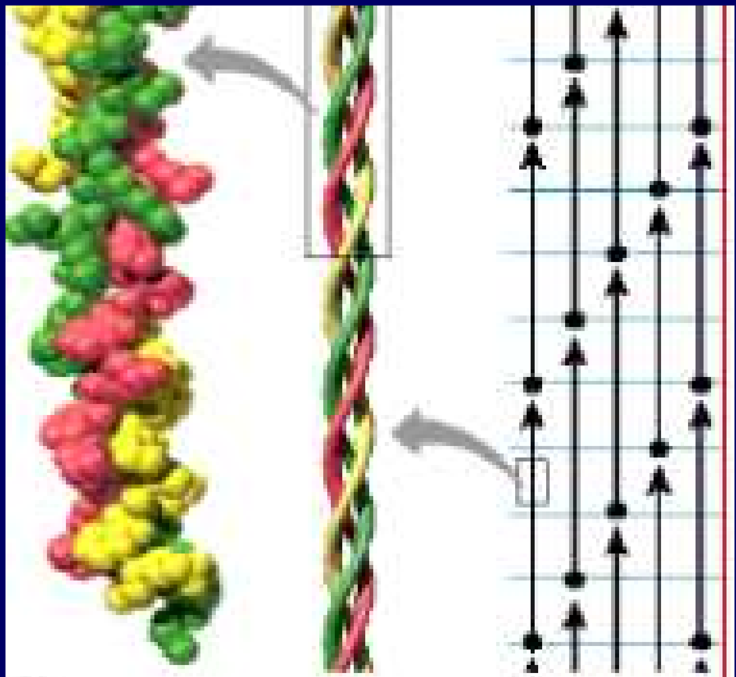
Non-linear optics – 3D reconstruction

No stains – endogenous signal

Instantaneous Elastic process – no thermal damage

SHG - noncenter symmetric molecules

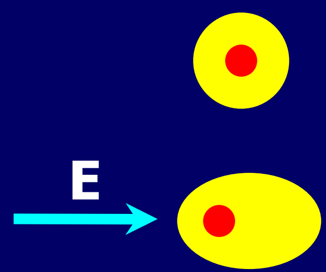
SHG: Collagen Triple Helix



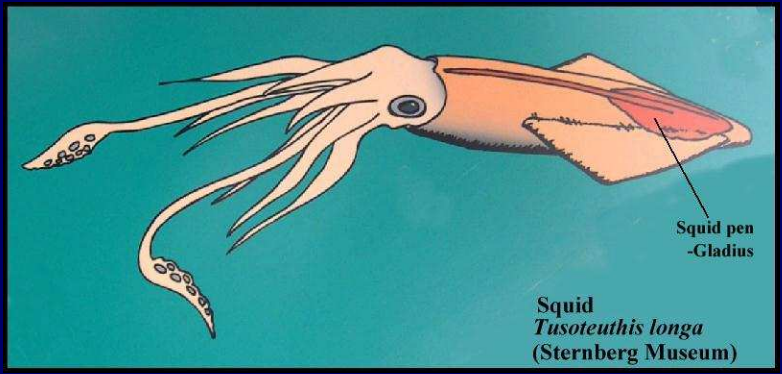
Electric fields & interfaces break symmetry

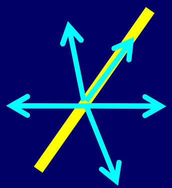
O=C=O

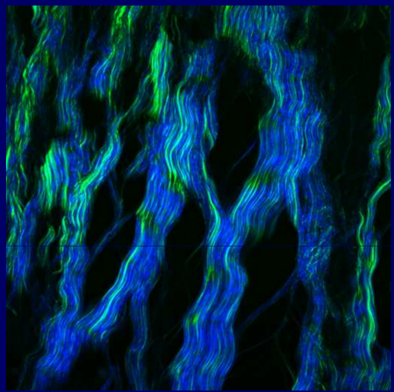
O = C=O



Forward/Backward and polarization signals have information



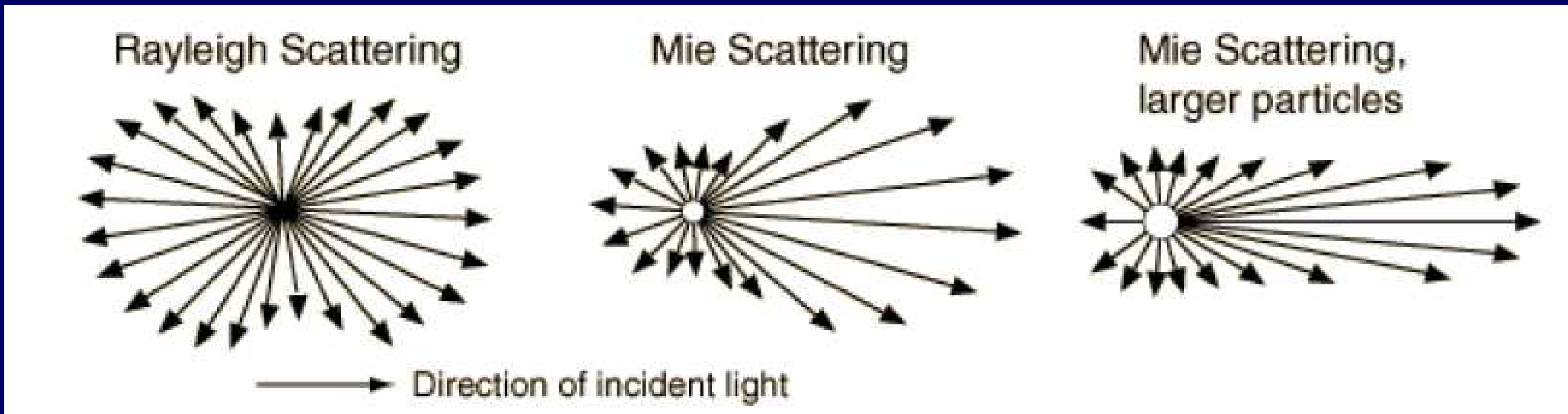




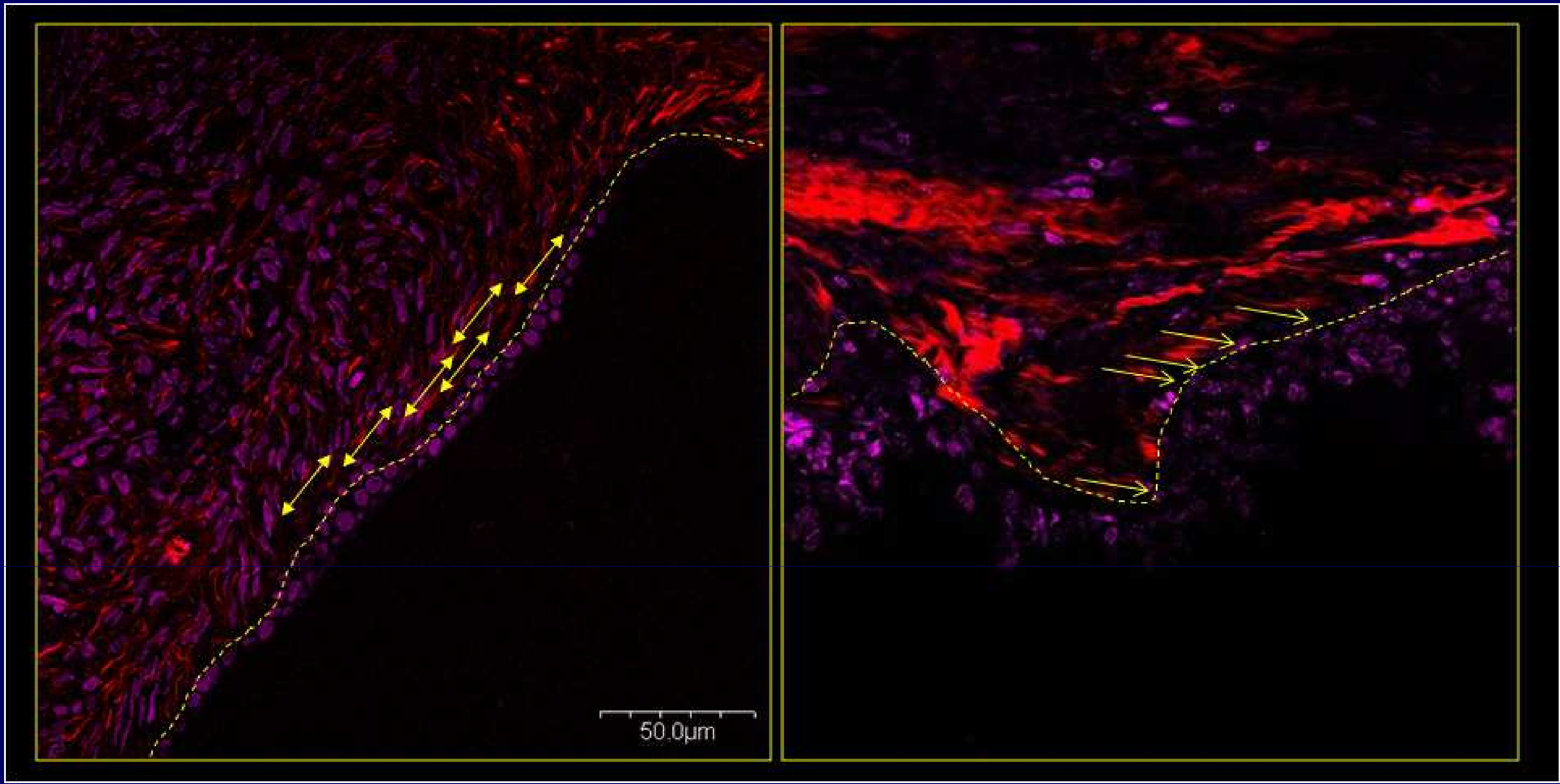
tendom

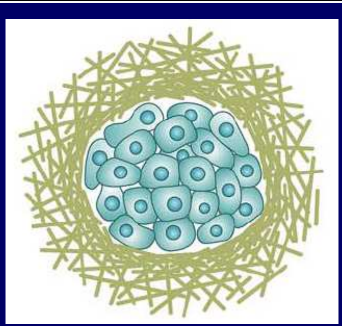
Squid

pen

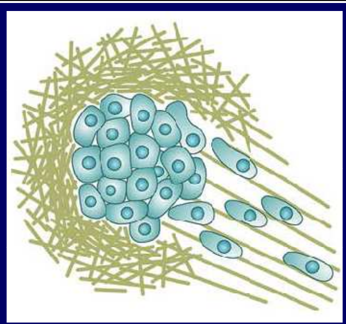


SHG + THG Ovarian Comparison normal vs adenocarcinoma





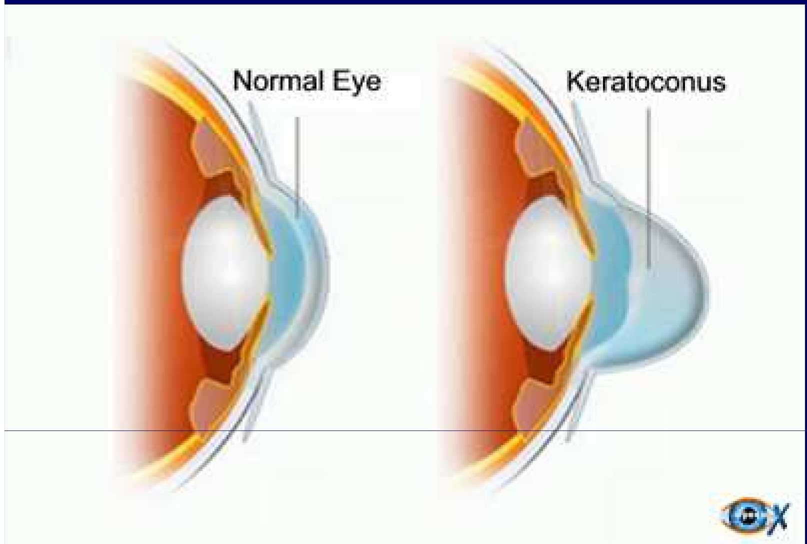
TACS-2, collagen tangential fibers

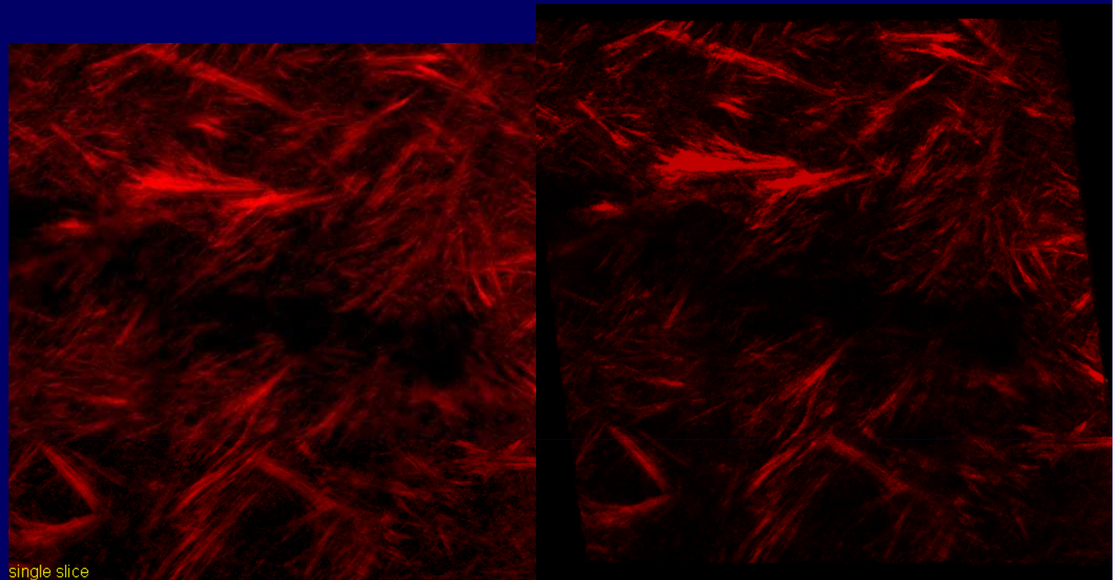


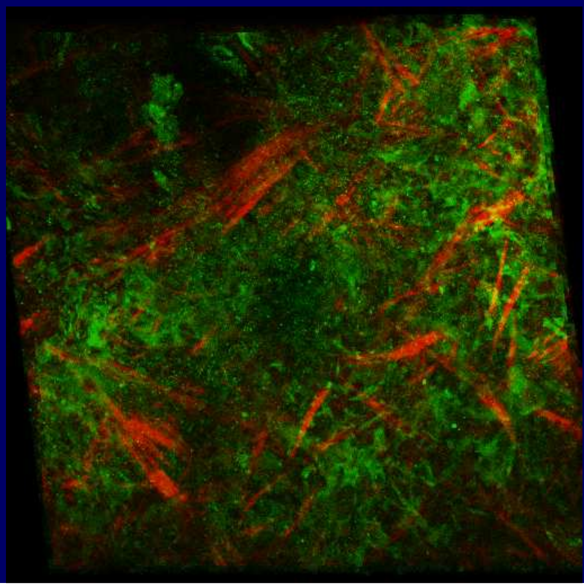
TACS-3, radial collagen fibers

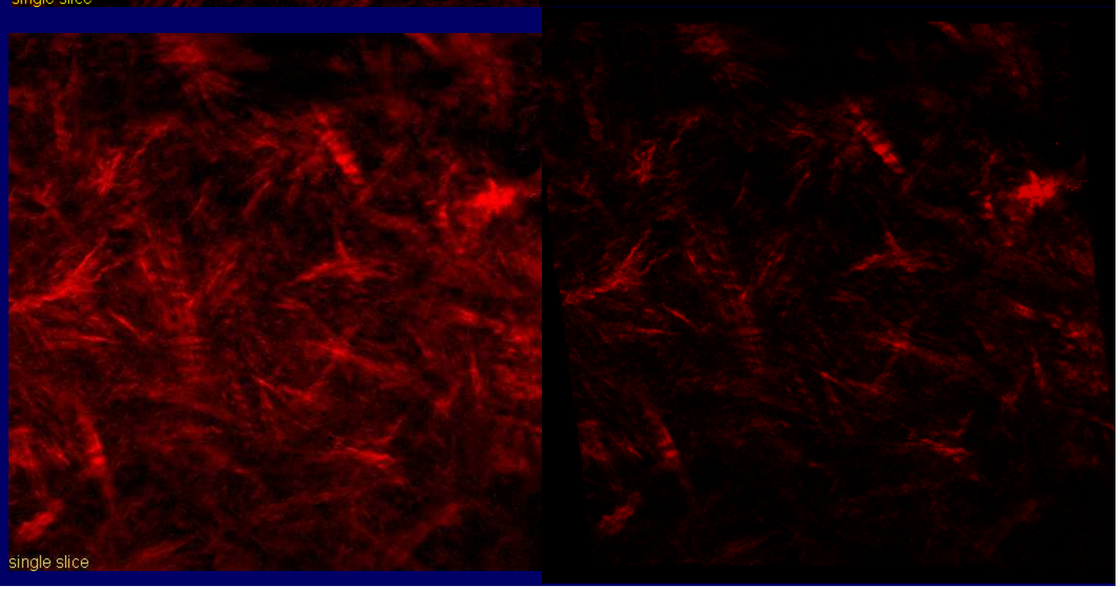
Cornea Keratoconus – UNIFESP + IPEN collaboration

UV + Riboflavin collagen croslinking

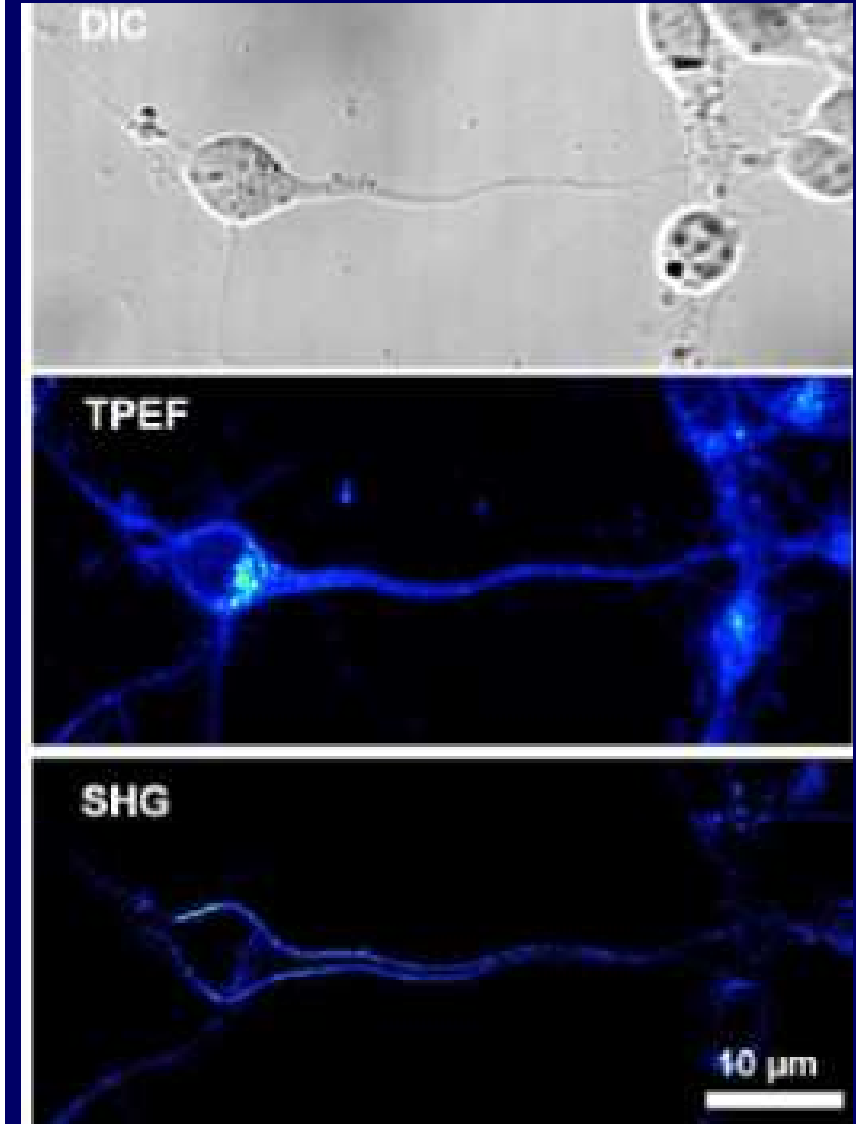


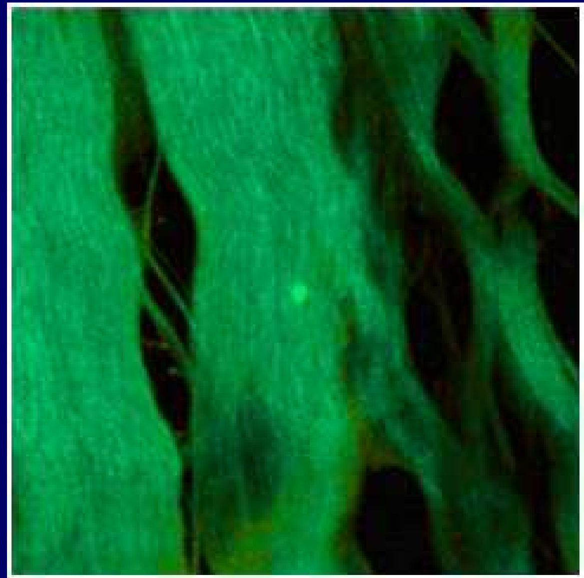


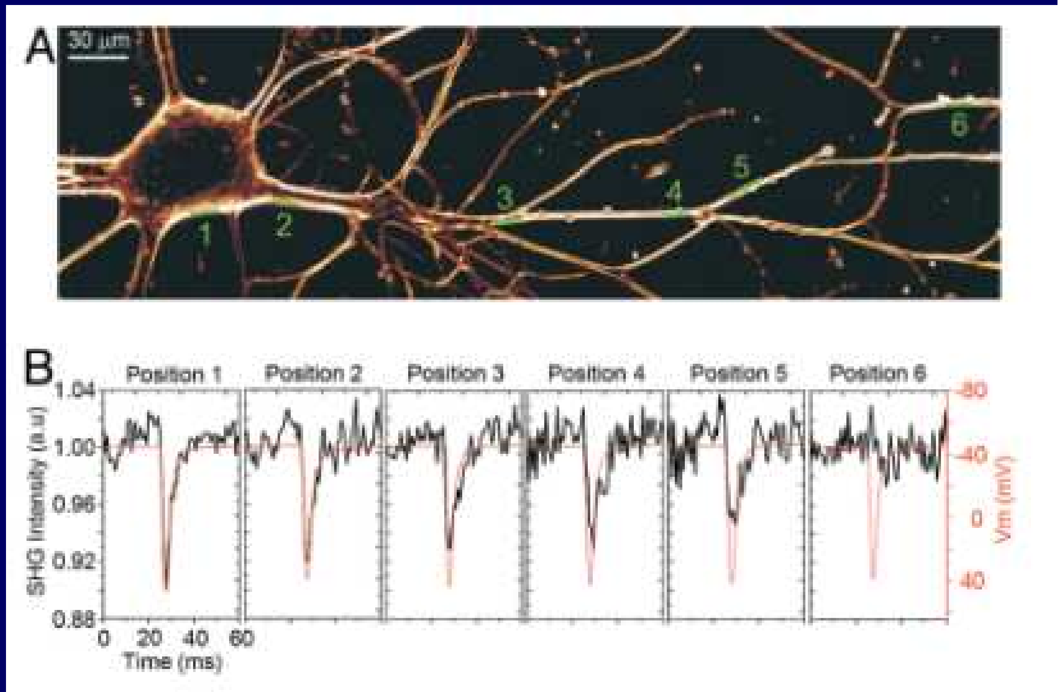




Instantaneous, symmetry breaking

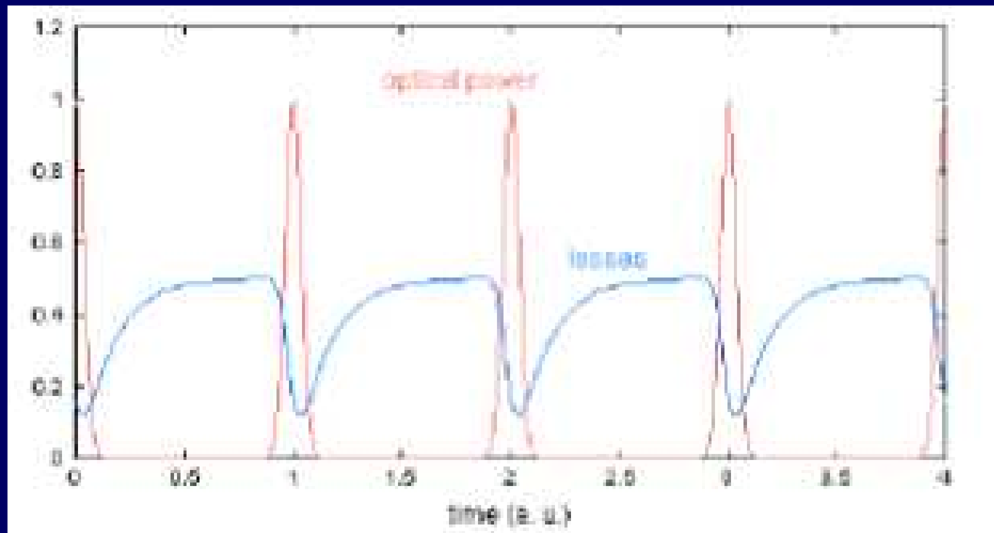


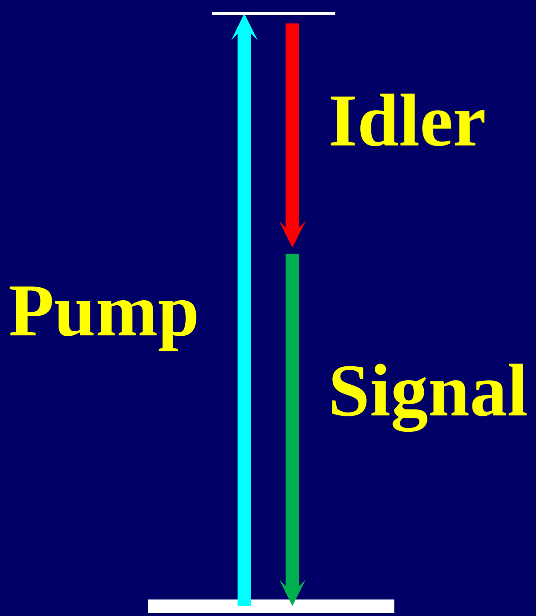


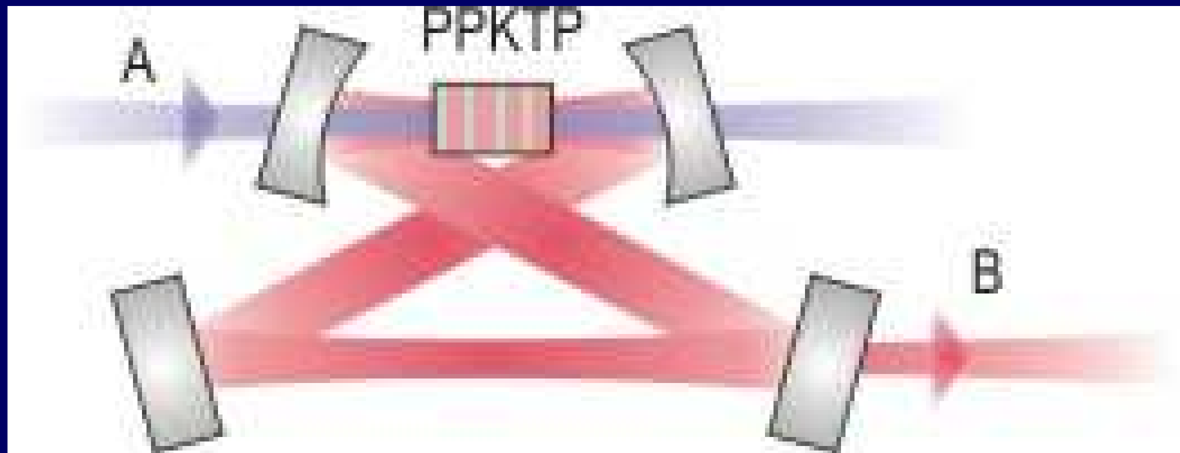


Inverse Process

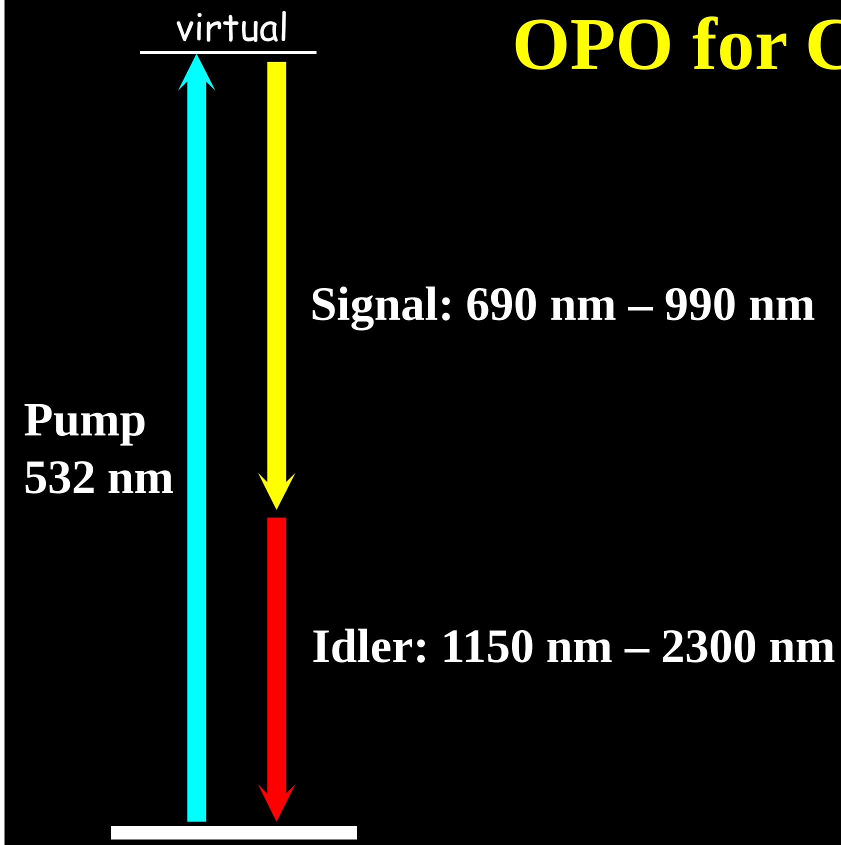
OPO = Optical Parametric Oscillator
OPA = Optical Parametric Amplifier

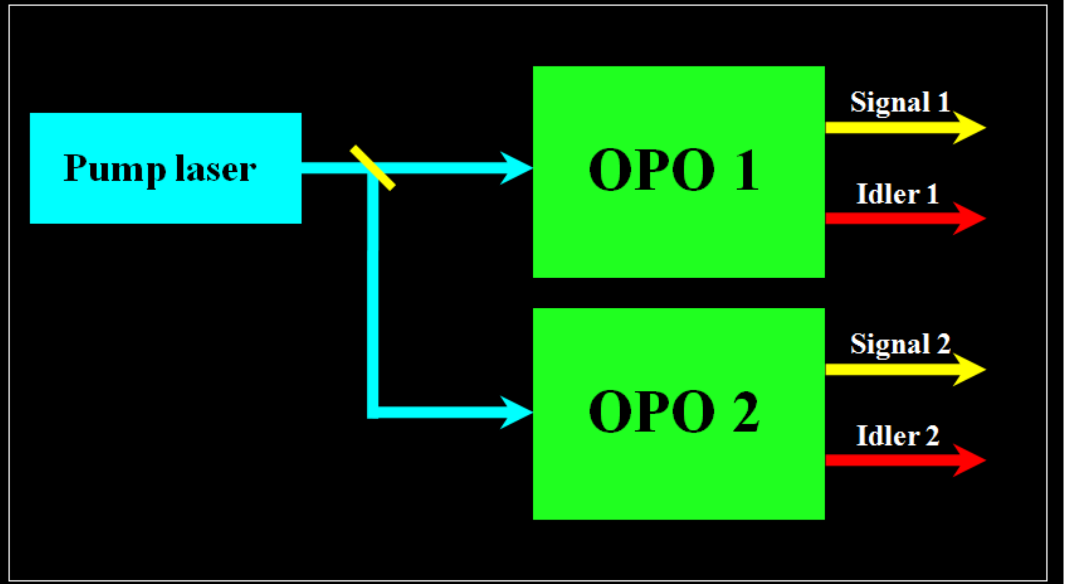






OPO for CARS [5 ps pulses]





Laser lines combinations

Fundamental: 1064 nm

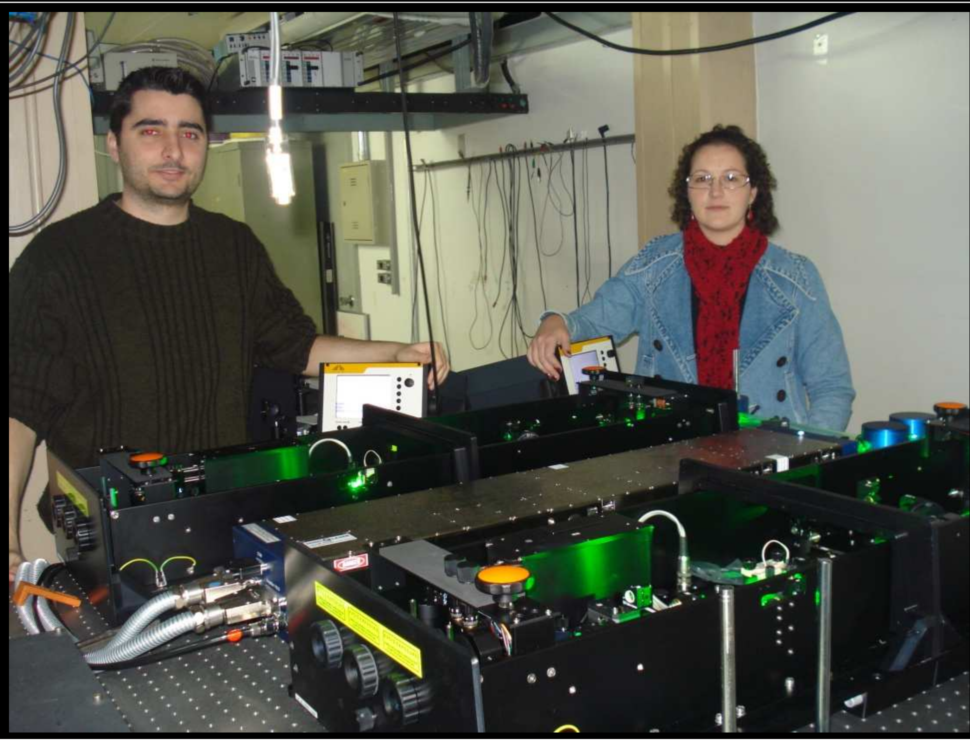
S1: 690 – 990 nm + 1064 nm [700 – 5000 cm^{-1}]

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}]



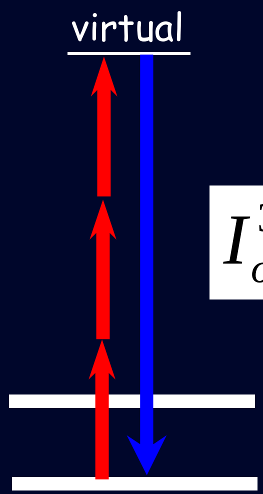
Third order elastic processes

$$\alpha^3 E^3 \cos(3\omega t)$$

$$x_n \alpha^3 E_j^2 E_k \cos\left[\left(\omega_j + \omega_n\right)t\right]$$

$$x_n \alpha^3 E_j^2 E_k \cos\left[\left(\omega_j - \omega_n\right)t\right]$$

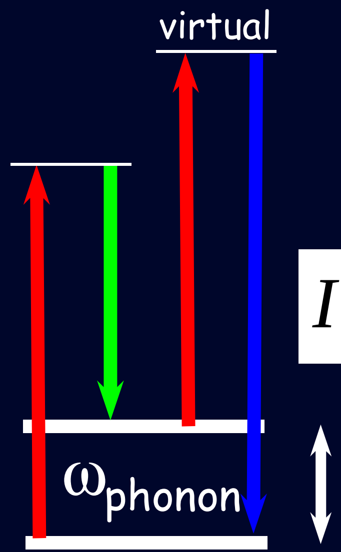
Third Harmonic Generation



$$I_o^3$$

THG

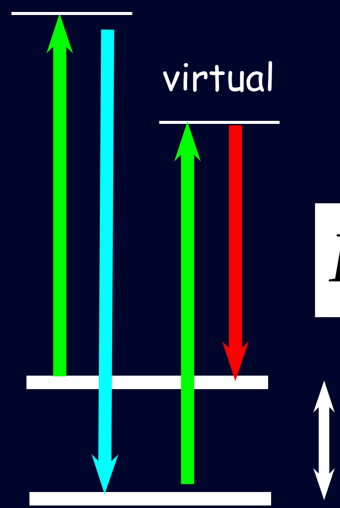
Coherent AntiStokes Raman Scatterinng CARS



$$I_1 I_2^2$$

CARS

Coherent Stokes Raman Scatterinng CSRS



$$I_1^2 I_2$$

CSRS

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

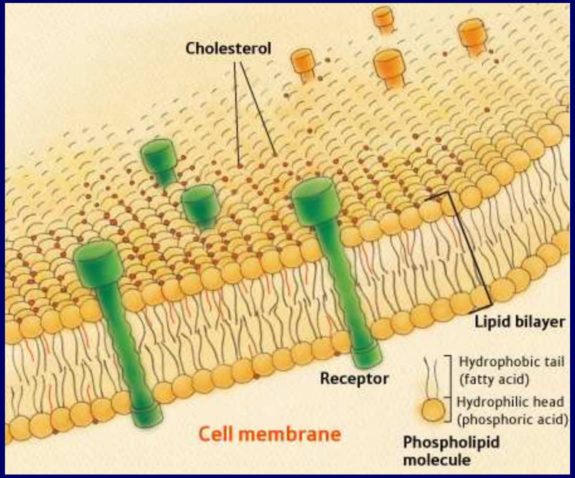
THG of homogeneous sample - Gouy phase shift

$$\vec{E}(x,y,z)=\vec{E}_0\,\frac{\omega_0}{\omega(z)}\,e^{-\frac{\rho^2}{\omega^2(z)}}\,e^{i\left[kz-\arctan\left(\frac{z}{z_o}\right)+\frac{k\rho^2}{2R(z)}\right]}$$

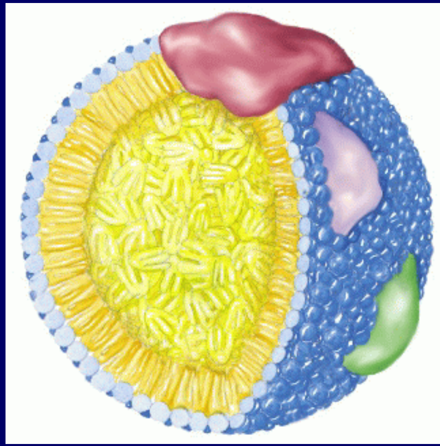
THG of homogeneous sample tends to ZERO

Sensitivity to interfaces

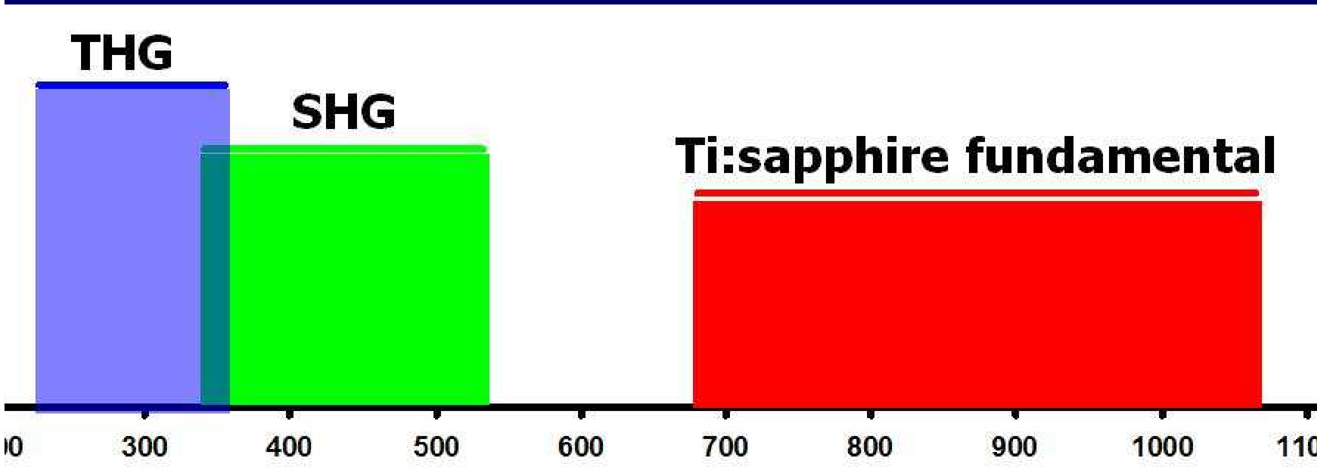
χ^3 contrast can be orders of magnitude higher than χ^1

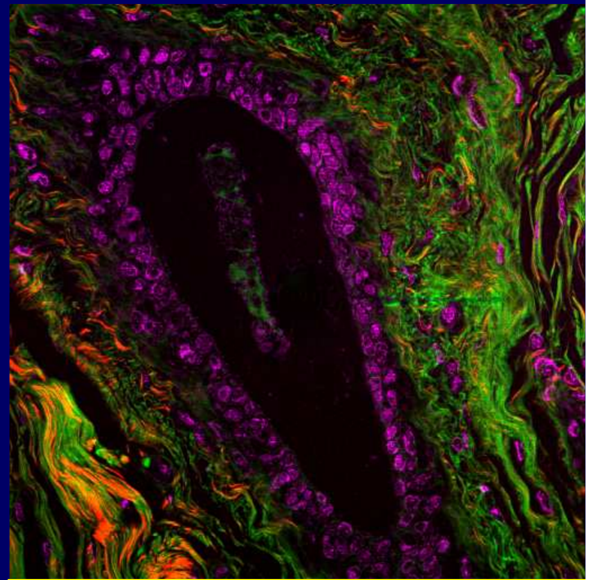


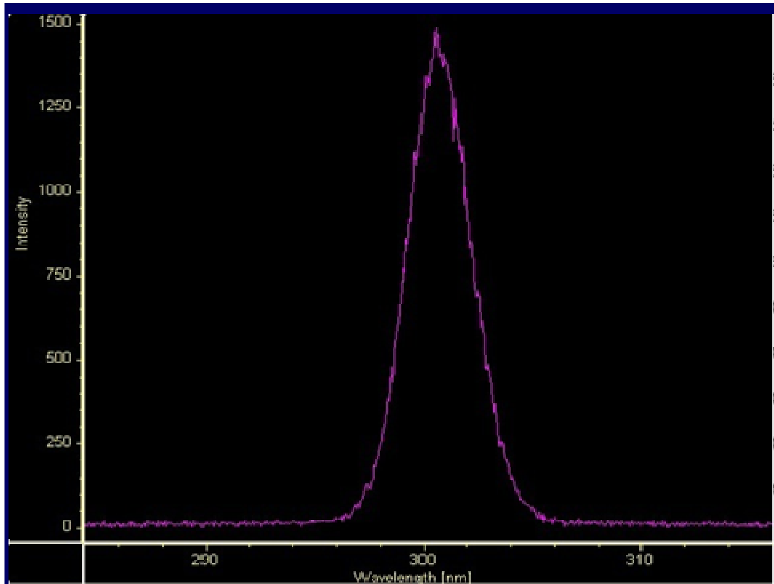
THG: lipid membranes and droplets

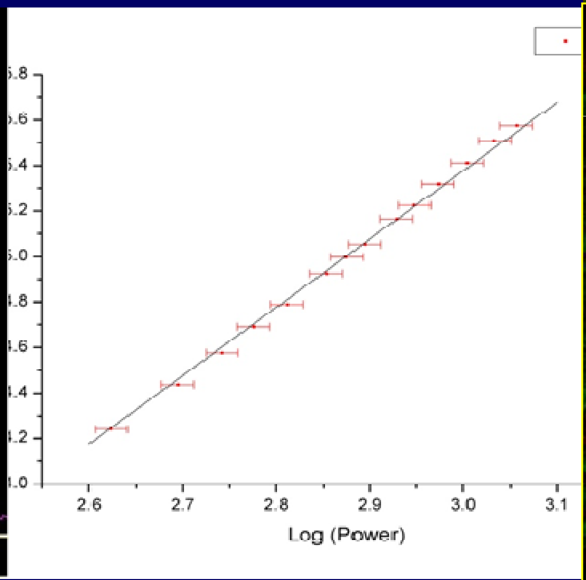


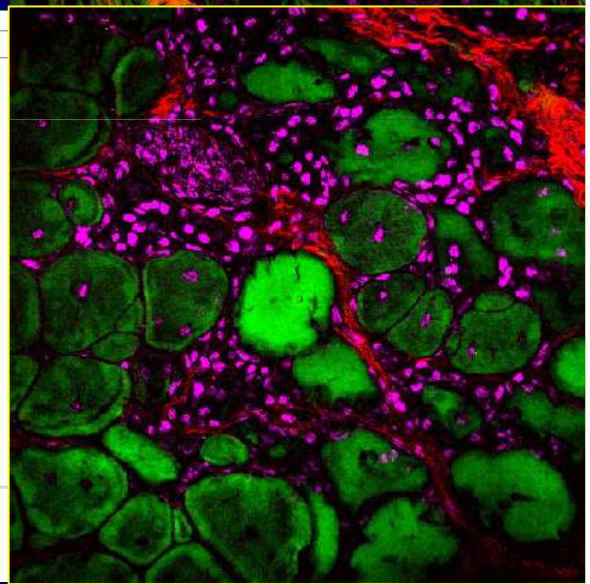
THG microscopy



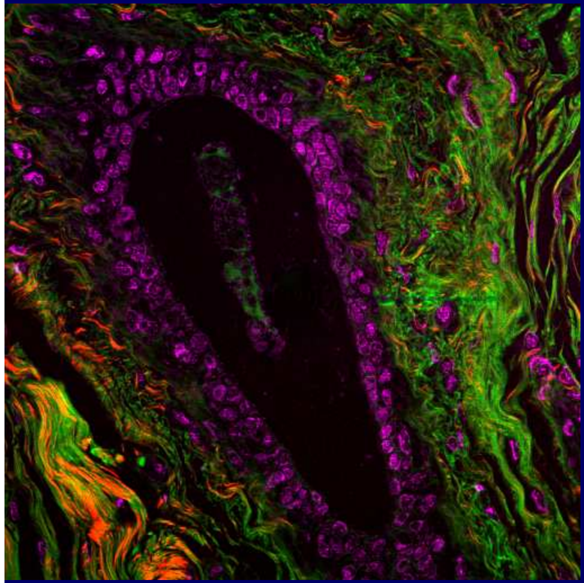


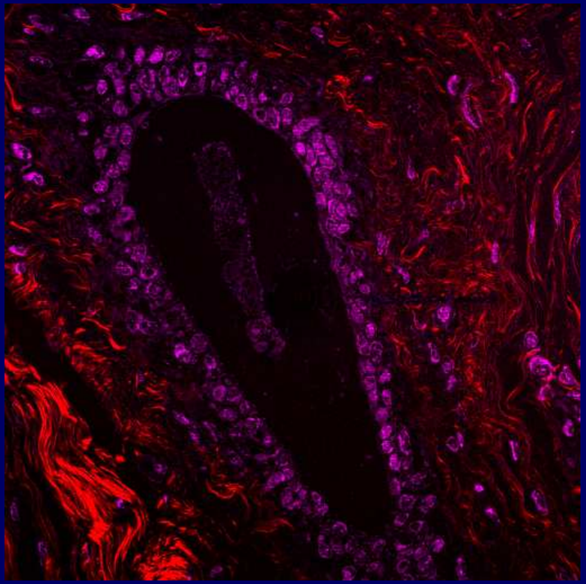






TPFE + SHG + THG

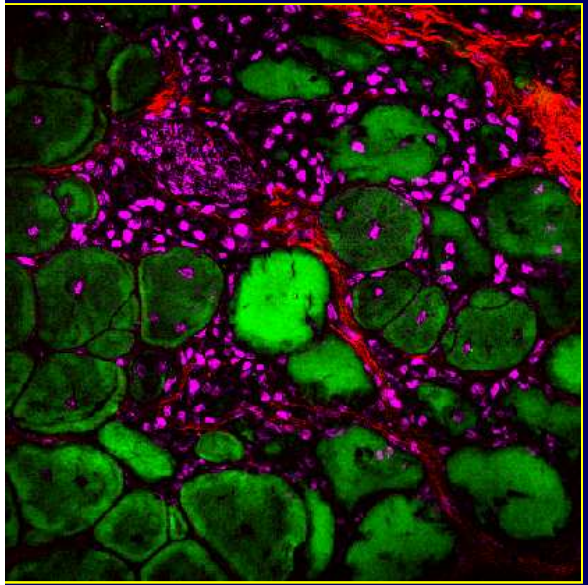


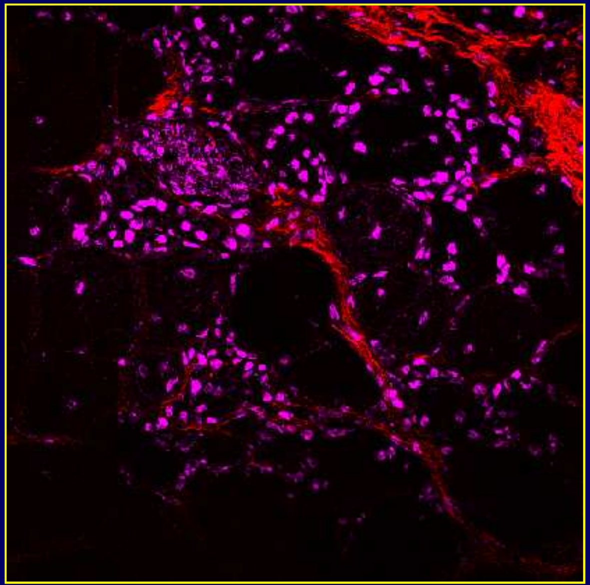


Normal breast tissue

Duct region

Fátima Böttcher
Liliana Andrade
CAISM - UNICAMP

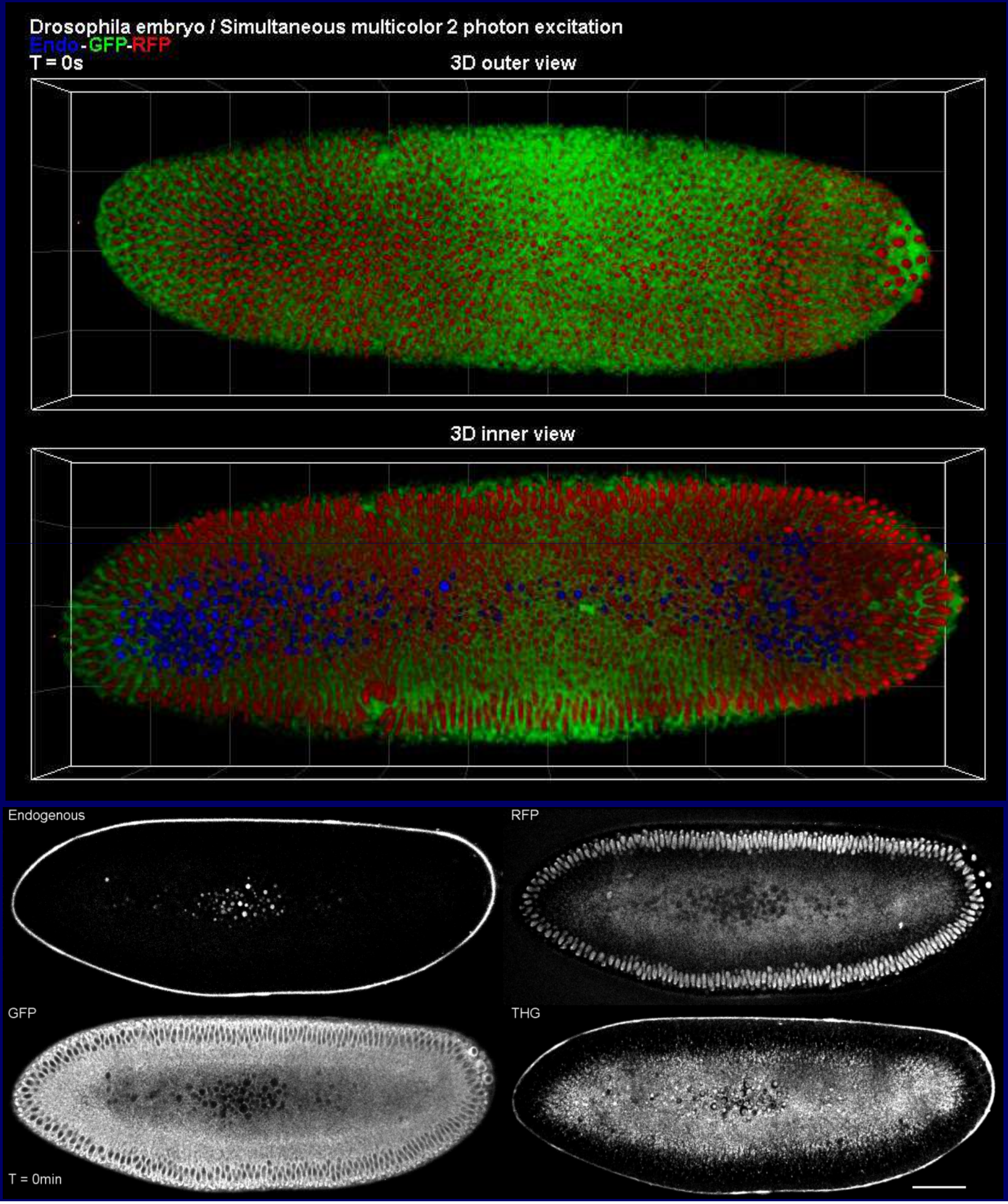




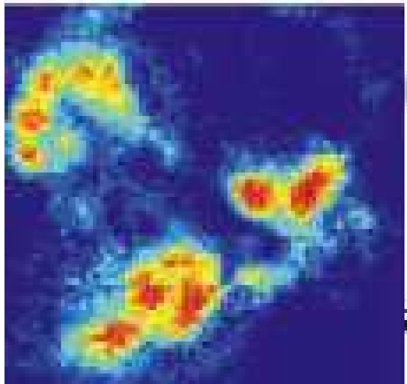
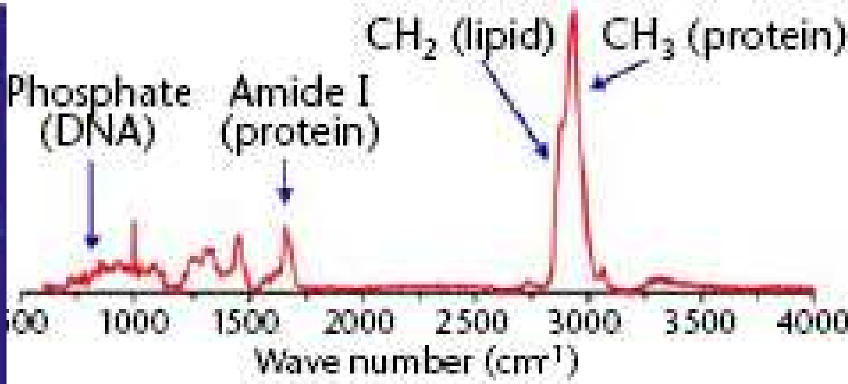
Muscle tissue

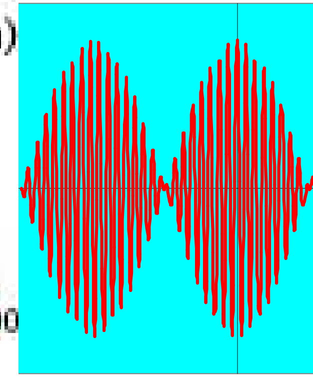
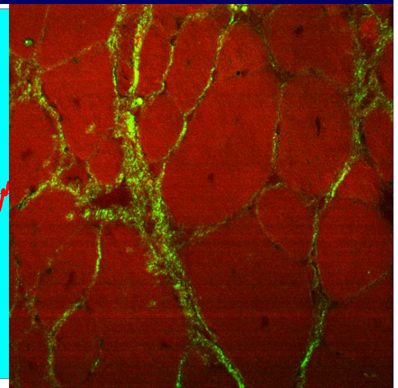
Mayana Zatz
Mariz Vainzof
IBC - USP

Embryology INSERM – France Results

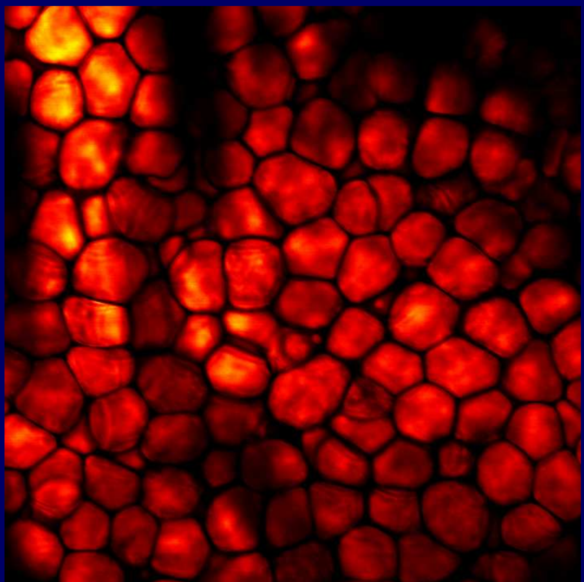


Coherent AntiStokes Raman Scattering CARS
Chemical specific imaging
The first CARS images of Brazil

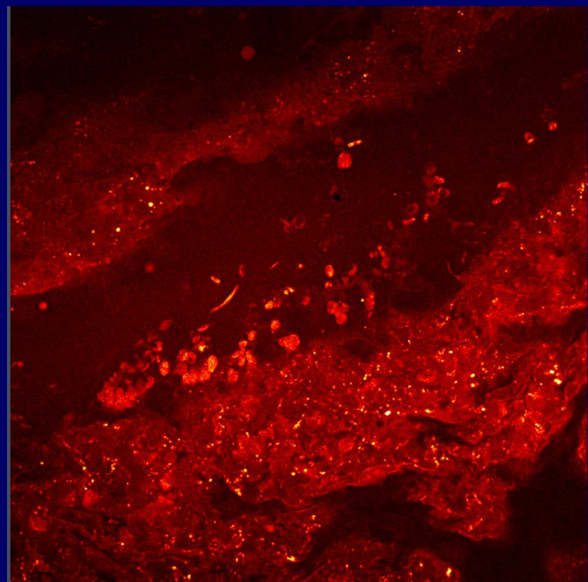



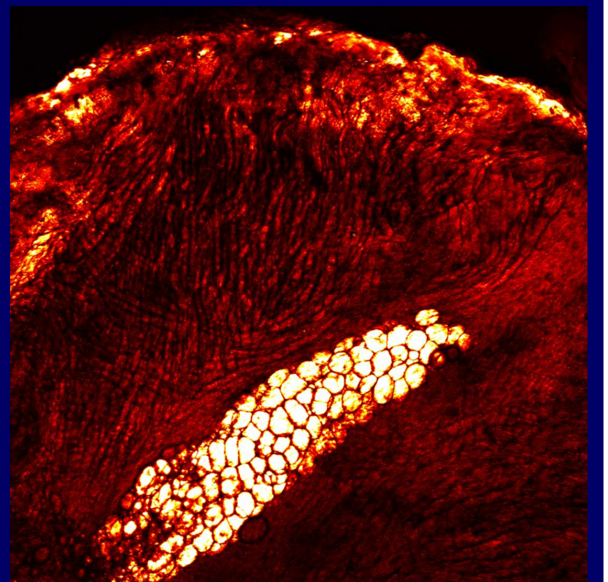
Raman image **CARS + SFG**



Mouse ear fat gland

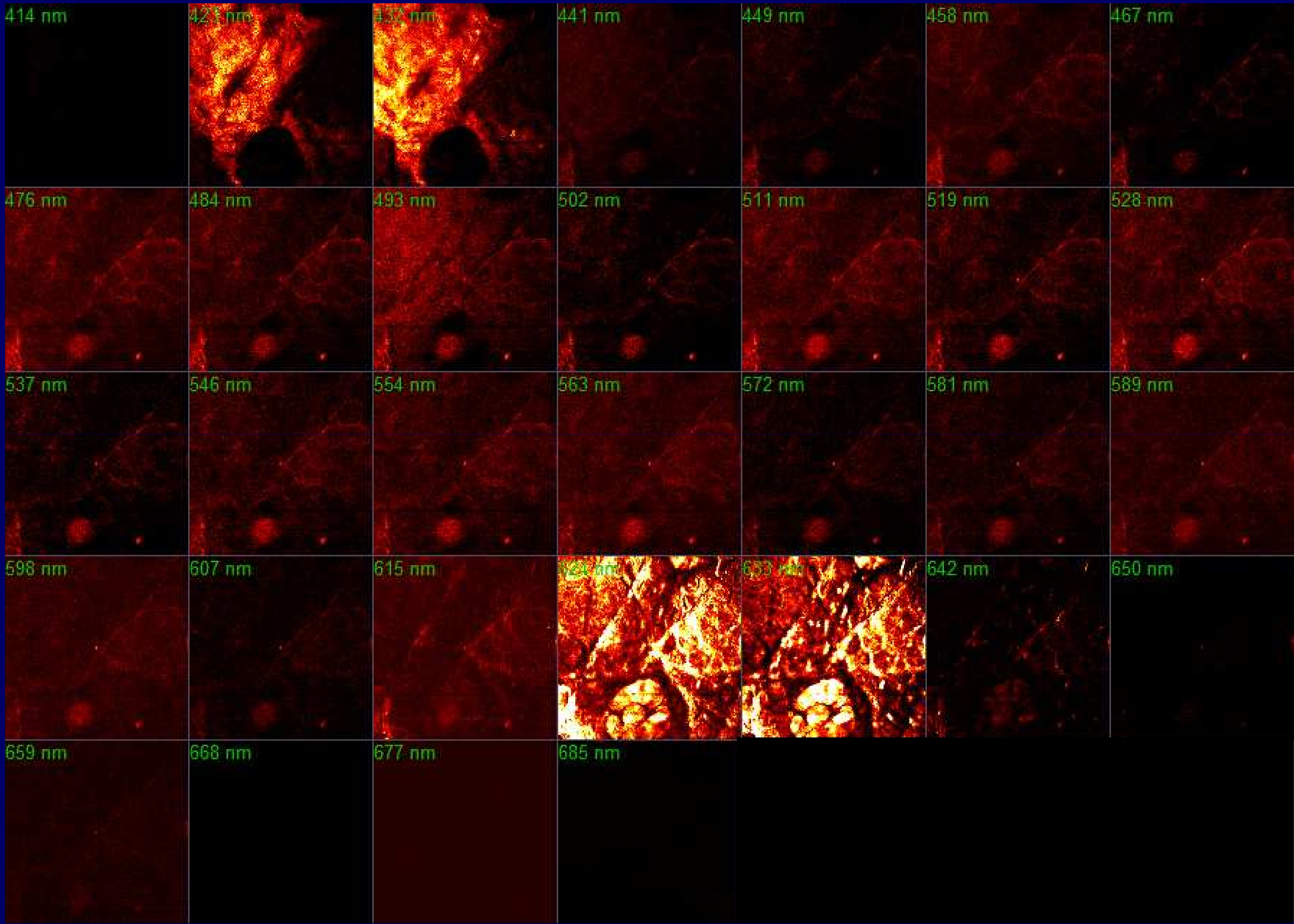


Lung artery

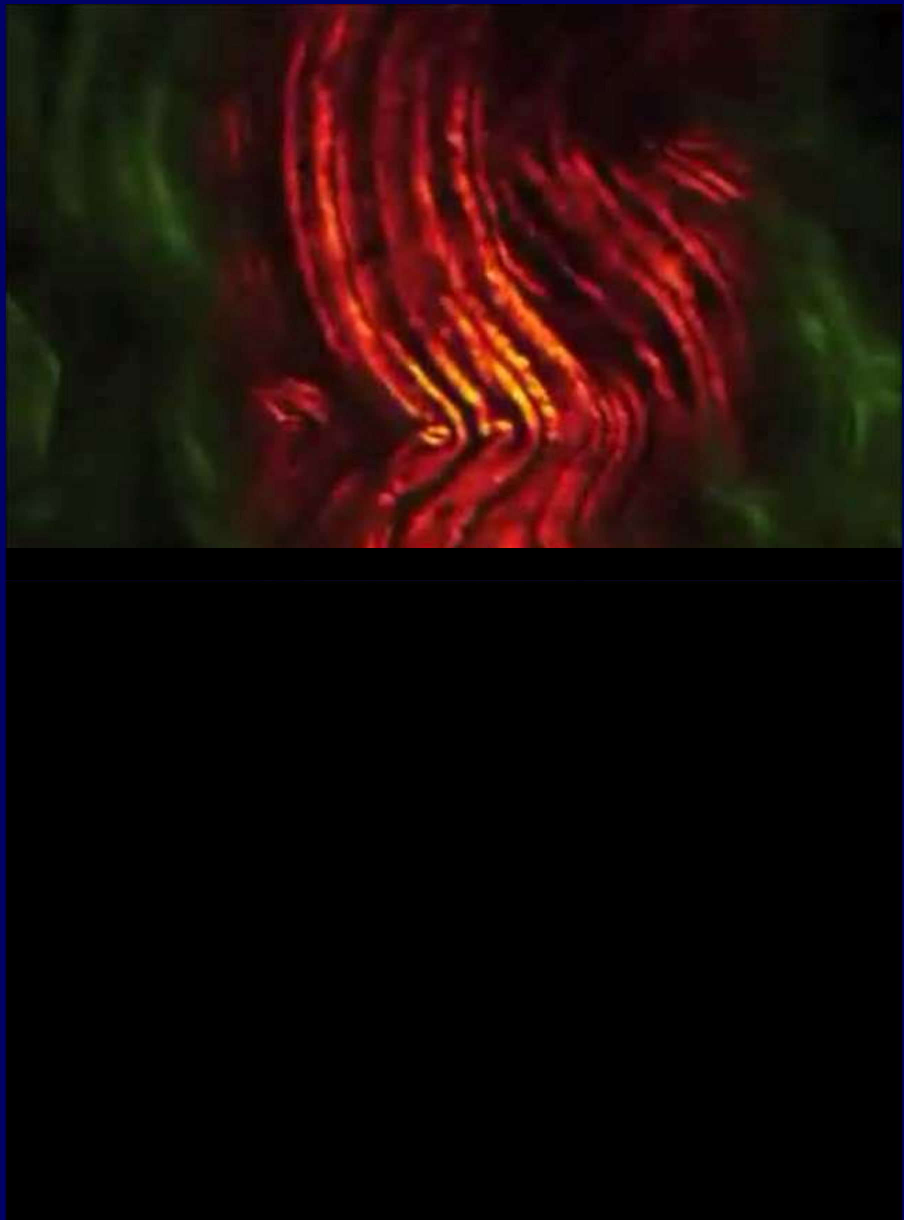


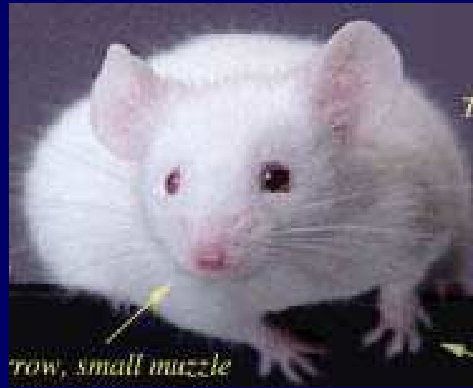
Heart

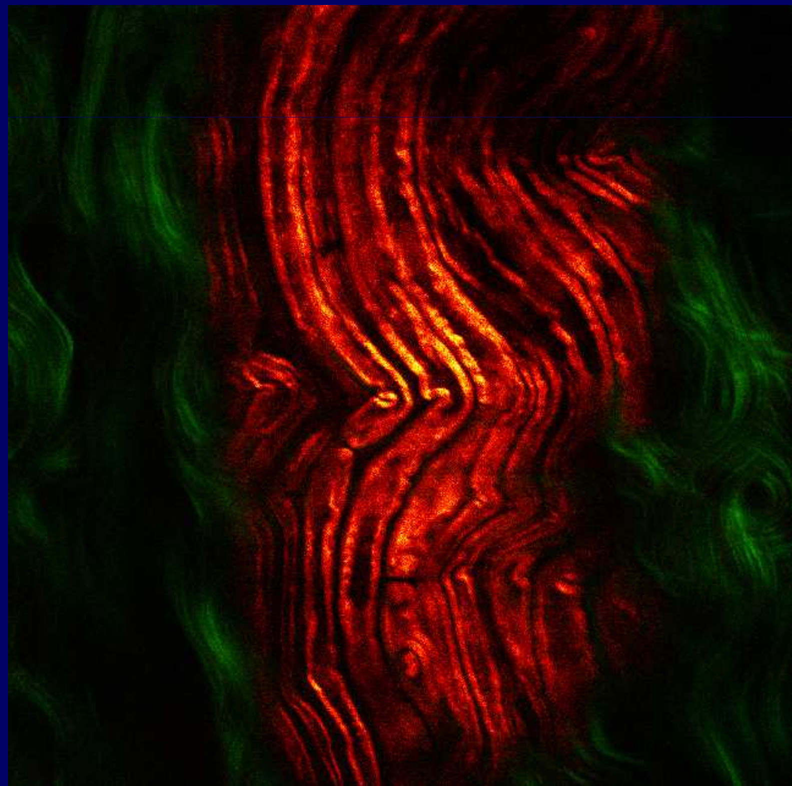
CARS + SFG Microscopy



CARS+SFG myelin sheath of mice sciatic nerve







Obrigado pela atenção!

Thanks for the attention