“Introdução á Óptica Não Linear”

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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 biology at cell/molecular level
Our contribution: Multimodal Platform based on a laser scanning spectral confocal microscopy!

Descanned Array Detectors APDs 10nm

Scan horizontal

Scan vertical
Why laser scanning Confocal Microscopy?

Confocal patent: 1957

Minsky scanned the sample – images were not that great!


Laser scanning made all the difference!

Marvin Minsky
Mathematician

Artificial Intelligence
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

$S_0 \rightarrow S_1 \rightarrow S_{In}$

Fluorescence

Non Linear Optical processes

Virtual

$S_0 \rightarrow S_1 \rightarrow S_{In}$

TPE Fluorescence

Virtual

SHG

THG

Hyper Raman

CARS

Energy transfer

FRET

$\omega_{phonon}$
Inelastic processes: heat dissipation

Fluorescence

$S_0 \rightarrow S_1 \rightarrow S_{In}$

Energy transfer

FRET

donor molecule

acceptor molecule

Raman

virtual

$\omega_{phonon}$

TPE

Fluorescence

$S_0 \rightarrow S_{In} \rightarrow S_1$

virtual

$\omega_{phonon}$

Hyper Raman
Elastic processes

SHG

THG

CARS

$\omega_{\text{phonon}}$
Understanding Optical Processes
Light is an Electromagnetic Field

Dipole $P = q \cdot x$
Light Irradiation: Molecular vibration

infrared \sim 10 \, \mu \text{m}

Dipole \quad P = q \times

Radio wave emission

molecule light emission
Forced spring-mass system: resonance

\[ \omega = \sqrt{\frac{K}{m}} \]

Oscilador

Amplitude de oscilação

A \sim \frac{F}{K}

A \sim \frac{1}{\omega^2}

\omega_{\text{ressonância}}

Freqüência

electron mass \ll 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

$A \sim \frac{F}{K}$

$A \sim \frac{1}{\omega^2}$
Hooke's law is an approximation

\[ F = -k \, x \]

\[ F = -k \, x - \beta x^2 - \gamma x^3 - \delta x^4 \ldots \]

**Inversion symmetry:**

Force is antisymmetric: if \( x \rightarrow -x \) then \( F \rightarrow -F \)

\[ k \, x + \beta x^2 + \gamma x^3 + \delta x^4 \ldots = k \, x - \beta x^2 + \gamma x^3 - \delta x^4 \ldots \]
Molecular symmetry

Symmetric Molecule
Unnoticed change $x$ by $-x$

\[ F = -k x - \gamma x^3 \cdots \]

Non-symmetric molecule
 Noticed change $x \rightarrow -x$

\[ F = -k x - \beta x^2 - \gamma x^3 - \delta x^4 \cdots \]
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

\[ P[x_n, x_e] = P_o + a x_e + b x_n + c x_e^2 + d x_e x_n + e x_n^2 + f x_e^3 + g x_n^3 + \cdots + h x_e^4 x_n + \cdots \]

Electronic Absorption
Rayleigh absorption

Infrared absorption

SHG/SFG

DFG

\( \chi^{(2)} \)

Raman

\( \chi^{(1)} \)

Overtone

NIR

\( \chi^{(3)} \)

THG

\( \chi^{(3)} \)

CARS

NLO vocabulary – powers of E – only electron counts
Trygonometry

\[
\cos(a + b) = \cos a \cos b - \sin a \sin b \\
\cos(a - b) = \cos a \cos b + \sin a \sin b \\
\cos a \cos b = \frac{1}{2} \cos(a + b) + \frac{1}{2} \cos(a - b)
\]

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

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

\[
\cos^3(\omega t) = \frac{3 \cos(3\omega t) + \cos(3\omega t)}{4}
\]
Infrared [IR] absorption

\[ x_n \cos(\omega_n t) \]

IR absorption

\[ x_n^2 \cos^2(\omega_n t) \]

1st overtone IR absorption

\[ x_n^3 \cos^3(\omega_n t) \]

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

\[ \alpha E x_n \cos[(\omega - \omega_n)t] \]
Raman Stokes
Inelastic - heating

\[ \alpha E x_n \cos[(\omega + \omega_n)t] \]
Raman AntiStokes
Inelastic - refrigeration

\[ P[x_n, x_e] = \cdots + x_e + \cdots + x_e x_n + \cdots \]
Second order elastic processes

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

Sum Frequency Generation

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

Second Harmonic Generation

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

Difference Frequency Generation

\[
P[x_n, x_e] = \cdots + \chi_e^2 + \cdots
\]
Second order inelastic processes

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

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

\[ \alpha^3 E^3 \cos(3\omega t) \]

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

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

**Third Harmonic Generation**

**Coherent AntiStokes Raman Scattering (CARS)**

**Coherent Stokes Raman Scattering (CSRS)**

\[ P[x_n, x_e] = \cdots + x_e^3 + \cdots + x_e^2 x_n + \cdots \]
Optical Circuits
Excitation

- cw lasers
- optical fiber coupling
- NLO pulsed lasers 690 – 2200 nm
- avoid optical fibers
- Optical Tweezers lasers 1064 nm
NLO & cw Laser combining dichroics

Fluorescence

cw lasers

NLO lasers
Zeiss Spectral Confocal

NLO lasers

cw lasers

PTC Laser Ports

TwinGate Beam Splitter
NLO lasers combination

OPO$_1$

OPO$_2$

HWP

HWP

HWP

PBS

attenuator

Ti:S
Make room to add an Optical Tweezers and laser cutting

red blood cell

Trypanosoma cruzi

Laser cutting

“Light Sucks”
NLO microscopy properties
Linear Optics

Rayleigh scattering
elastic

Raman Stokes
Inelastic - heating

Raman AntiStokes
Inelastic - refrigeration

\[ \alpha E \cos(\omega t) \]

\[ \alpha E x_n \cos[(\omega - \omega_n) t] \]

\[ \alpha E x_n \cos[(\omega + \omega_n) t] \]

\[ P[x_n, x_e] = \cdots + x_e + \cdots + x_e x_n + \cdots \]
Rayleigh Scattering:
Electron follows the laser - nucleus do not
Espalhamento Rayleigh \( \propto \frac{1}{\lambda^4} \)

Penetração aumenta na direção do infravermelho

\[ 2^4 = 16 \text{ mais penetração} \]
Deep penetration: > 1600 µm

Chris Xu
Cornell
Raman: Electron follows the laser, nucleus don't.
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

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

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

- Heart fibroma
- Mammary duct
- muscle

Descanned Array Detectors APDs

Scan horizontal
Scan vertical

BP filter

Squid pen

Mouse aorta
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
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]

Pump: 532 nm

Signal: 690 nm – 990 nm

Idler: 1150 nm – 2300 nm

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

Coherent AntiStokes Raman Scattering (CARS)

Coherent Stokes Raman Scattering (CSRS)

\[ P[x_n, x_e] = \cdots + x_e^3 + \cdots + x_e^3 x_n + \cdots \]
THG of homogeneous sample - Gouy phase shift

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

THG of homogeneous sample tends to ZERO

Sensitivity to interfaces

\( \chi^3 \) contrast can be orders of magnitude higher than \( \chi^1 \)

THG: lipid membranes and droplets
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