Coherent anti-Stokes Raman scattering (CARS) microscopy near boundaries

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Coherent anti-Stokes Raman scattering basics (1)

CARS important features:
1. Resonant process (fluorescence)
2. Coherent process (SHG, THG)
Coherent anti-Stokes Raman scattering basics (2)

Two contributions to CARS generation

Resonant contribution ($\chi_R^{(3)}$)

- Vibrational origin

Nonresonant contribution ($\chi_{NR}^{(3)}$)

- Electronic response of the medium

Presence of molecules with oscillating vibrational mode $\Omega_R$

$\Rightarrow$ Enhancement of the signal at angular frequency $\omega_{as}$
CARS microscopy: a stain free microscopy

Advantages:

1. Fluorescent staining useless.
2. Chemical selectivity of the contrast.
3. Intrinsic 3D imaging.
CARS imaging of interfaces: motivations (1)

From the literature...


Polystyrene beads in agarose


Melanin beads in water


Yeast cells in water

Interference or refractive effects ???
CARS imaging of interfaces: motivations (2)

CARS as a third-order nonlinear process:

\[ \overline{P^{(3)}}(\vec{r}, -\omega_{as}) = \chi^{(3)}(\omega_{as}) : \overline{E_p}(\vec{r}, \omega_p) : \overline{E_p}(\vec{r}, \omega_p) : \overline{E_s}(\vec{r}, \omega_s) \]

Spring dephasing

Normalized resonance detuning

Object

Surrounding

Excitation volume

Excitation volume

Scanning direction
Spectral behavior of CARS signal (1)

\[ \chi^{(3)} (\omega) = \chi^{(3)}_R + \chi^{(3)}_{NR} \]

For an isolated Raman line:

\[ \chi^{(3)}_R = \frac{1}{\Delta \omega_R} \left( \omega_p - \omega_s - \Omega_R \right) + i \Gamma \]

Electronic response spectrally independent: \( \chi^{(3)}_{NR} \) is real & constant

\[ I_{CARS} \propto \left| \chi^{(3)}_R + \chi^{(3)}_{NR} \right|^2 \]

\[ I_{CARS} \propto \left| \chi^{(3)}_R \right|^2 + \left| \chi^{(3)}_{NR} \right|^2 + 2 \text{Re} \left( \chi^{(3)}_R \cdot \chi^{(3)}_{NR}^* \right) \]

Homodyne terms
Heterodyne term

CARS resonance lineshape

Potma et al., *J. Raman Spectrosc.* 34, 642 (2003)
Spectral behavior of CARS signal (2)

\[ \chi^{(3)}(\zeta,\eta) = \rho(\zeta,\eta) \cdot \exp[i\phi(\zeta,\eta)] \]

with

\[ \rho(\zeta,\eta) = \chi_{NR}^{(3)} \cdot \left[ 1 + 4 \frac{1/\eta - \zeta}{\eta(\zeta^2 + 1)} \right]^{1/2} \]

\[ \tan[\phi(\zeta,\eta)] = \frac{2}{\eta(\zeta^2 + 1) - 2\zeta} \]

where

\[ \delta \omega = \omega_p - \omega_s \] \hspace{1cm} \text{Laser detuning}

\[ \zeta = (\delta \omega - \Omega_R) / \Gamma \] \hspace{1cm} \text{Normalized Raman resonance detuning}

\[ \eta = -2\Gamma \cdot \chi_{NR}^{(3)} / a \] \hspace{1cm} \text{Nonresonant part relative strength}
Spectral behavior of CARS signal (3)

Nonresonant part relative strength

\[ \eta = -2\Gamma \cdot \frac{\chi^{(3)}_{NR}}{\alpha} \]

Representation of the resonance in the complex plane
CARS imaging of interfaces: 1D model (1)

\[ I_{CARS}(x) = \begin{cases} 
\rho_o^2 & \text{if } x < \frac{\lambda}{2} \\
\left[\rho_o^2 + \rho_s^2 - 2\rho_o\rho_s \cdot \cos(\phi_o)\right] \left(\frac{x}{\lambda}\right)^2 & \text{if } \left|x\right| \leq \frac{\lambda}{2} \\
\left(\rho_o^2 - \rho_s^2\right) \cdot \frac{x}{\lambda} + \frac{1}{4} \left[\rho_o^2 + \rho_s^2 + 2\rho_o\rho_s \cdot \cos(\phi_o)\right] & \text{if } \left|x\right| > \frac{\lambda}{2} \\
\rho_s^2 & \end{cases} \]

\( I_{CARS} \) admits a minimum on the \([-\lambda/2; \lambda/2]\) range only if \( \cos(\Phi_o) < \min(\rho_o/\rho_s; \rho_s/\rho_o) \)
CARS microscopy near boundaries

9th French-Israeli Symposium on Non-linear & Quantum Optics

CARS imaging of interfaces: 1D model (2)

\[ I_{CARS}(x) = \left| \begin{array}{c|c}
\text{Sample} & \text{Excitation field} \\
\hline
\text{Object} & 1 \\
\text{Surrounding} & 0
\end{array} \right| \times x \]

\[ x = \frac{\rho_o - \phi_o}{\rho_s \lambda} \]
CARS microscopy near boundaries

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CARS imaging of interfaces: 3D model

Considered set-up

Forward detected signal (F-CARS)

Collection

NA=0.5

ωas

Scanning directions

x

y

z

Sample

Backward detected signal (E-CARS)

E_s(ω_s)

E_p(ω_p)

NA=1.2 in water

Glass slide

6µm

Oil

Theoretical 1D scans (3D model)

Normalized CARS intensity

Scan position (µm)

Bead

Oil

Interface

Resonance peak

Dip

Off resonance

Phase maximum

Normalized CARS intensity

Scan position (µm)

Interface

Bead

Oil

Phase maximum

Off resonance

Collection NA=0.5

Scanning direction
Experimental set-up

- **λ_p = 730 nm**
- **P_p = 500 μW**
- **Pulse rate = 3.8 MHz**
- **Pulse width ~ 3 ps**

- **λ_s ~ 787 nm**
- **P_s = 500 μW**
- **Pulse rate = 3.8 MHz**
- **Pulse width ~ 3 ps**

- **BC: beam combiner**
- **BS: beam splitter**
- **C: condenser (NA=0.5)**
- **F: filter**
- **L: lens**
Experimental evidence (1)

CARS microscopy near boundaries

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n=1.556@25°C, phenyl free

Immersion oil

Polystyrene bead (Φ=6 µm)

Glass slide

n~1.6@25°C, phenyl group

CARS resonance

Bead 2D scans

Off-resonance

CARS intensity (kcps)

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

Phase (°)

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

\( \chi_{NR}^{(3)} \)

Immersion oil

Polystyrene bead

Raman shift (cm\(^{-1}\))

Source: http://www.aist.go.jp
Experimental evidence (2)

Bead experimental 1D scans
Experimental evidence (3)

Scans features:

1. Influence of the bead/oil refraction index mismatch…

2. …but a spectral effect can be seen, conveying the interference variation between the bead and the oil.

Bead experimental 1D scans (selected scans)
Discussion (1)

Potma et al., J. Raman Spectr. 34, 642 (2003)

CARS spatial coherence

Discussion (2)

A fruitful analogy: the Michelson interferometer

<table>
<thead>
<tr>
<th>Path 1</th>
<th>Path 2</th>
<th>Dephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giant</td>
<td>Unilamellar</td>
<td>Variation of distance between the GUV and the slide</td>
</tr>
<tr>
<td>Vesicle</td>
<td>Resonant</td>
<td>Dephasing of the incident pulse spectral components</td>
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<tr>
<td>Non-resonant</td>
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<td></td>
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<tr>
<td>contribution</td>
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<tr>
<td>Object</td>
<td>Surrounding</td>
<td>Raman resonance detuning</td>
</tr>
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Experiment by Potma et al.  \(\rightarrow\) spatial interferences
Experiment by Oron et al.  \(\rightarrow\) spectral interferences
Our experiment  \(\rightarrow\) combined effect
Conclusion & perspectives

1. Theoretical & experimental study of CARS imaging of interfaces.

2. Strong analogy with previous works on spatial & spectral interferences in CARS → combined effect.

3. Experiment suitable for multiplex CARS microscopy (one shot experiment, phase-retrieval procedures in congested fingerprint region).

4. On small objects (biological membranes), the procedure seems interesting to improve image contrast (high non-resonant surrounding & reduced refraction-index mismatch)