Vibrational imaging and microspectroscopies based on coherent anti-Stokes Raman scattering (CARS)

by

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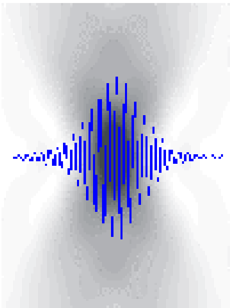

AG Volkmer
(Coherent microscopy &
single-molecule spectroscopy)

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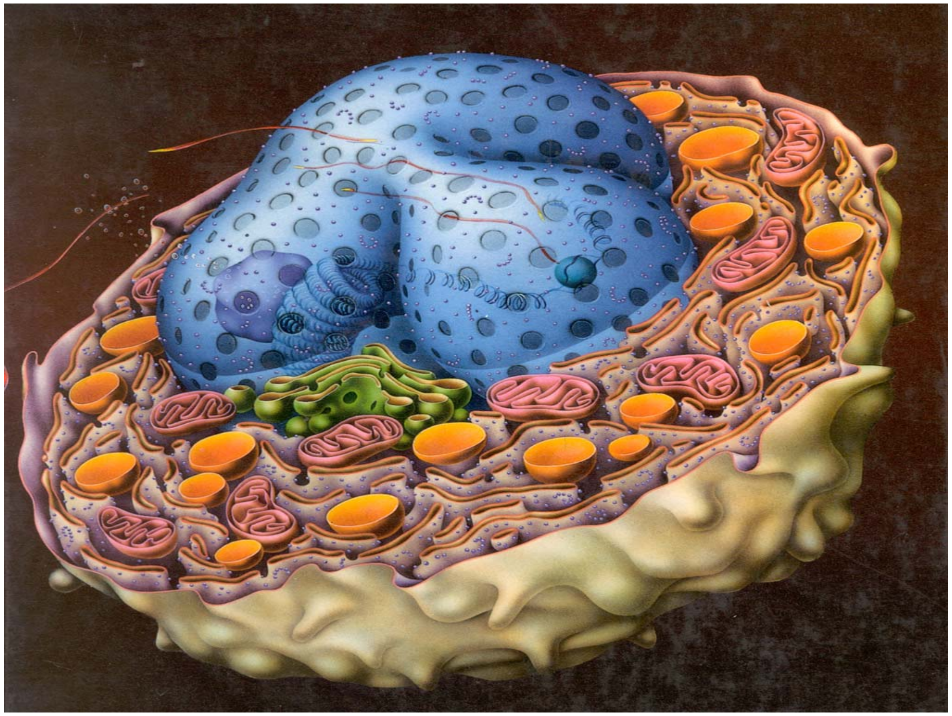
FRISNO-8, Ein Bokek, 20-25 February 2005

Ultimate goal in Optical Microscopy

Noninvasive three-dimensional characterization of mesoscopic objects within complex heterogeneous systems

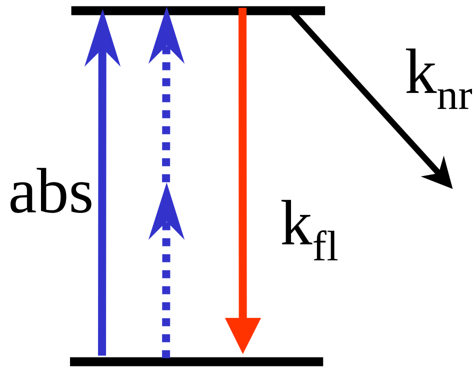
- **with high spatial resolution,**
- **with high spectral resolution,**
- **with high temporal resolution,**
- **and with high sensitivity.**

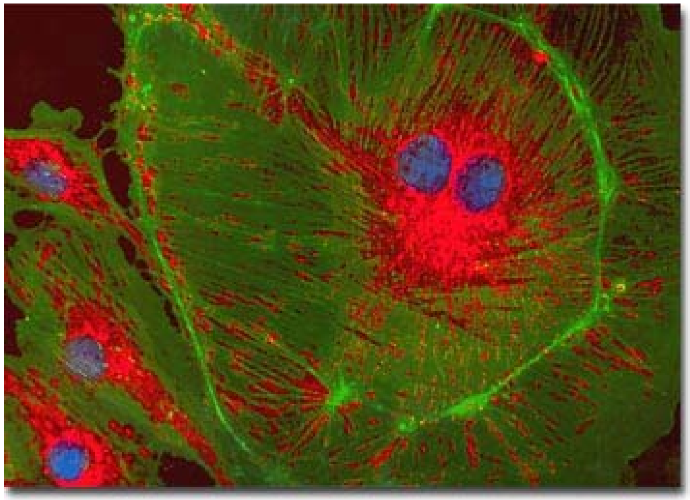


Fluorescence-based microscopy

→ Confocal fluorescence laser scanning microscopy

→ Two-photon induced fluorescence laser scanning microscopy



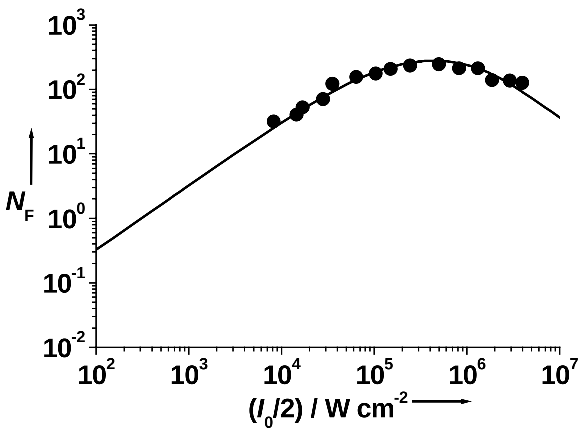


! Limitations of fluorescence-based spectroscopic studies:

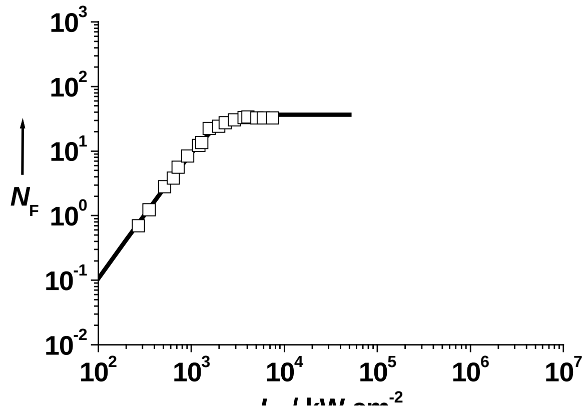
- dye labeling required (photo-toxicity)
- perturbation of structure and dynamics by fluorophore
- photo-stability (# emitted photons)

Fluorescence photobleaching of Rhodamine 6G / water

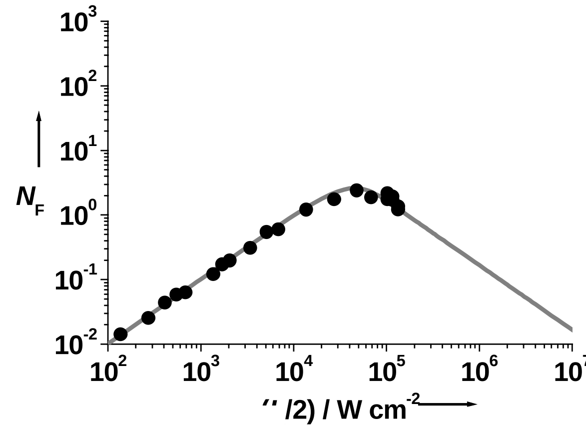
CW (one-photon) excitation at 514 nm



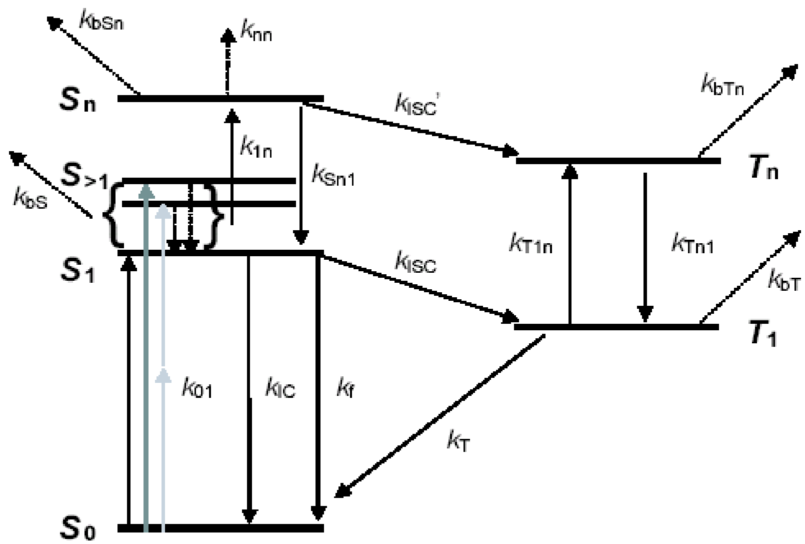
Pulsed fs (two-photon) excitation at 800 nm



Pulsed fs (one-photon) excitation at 350 nm



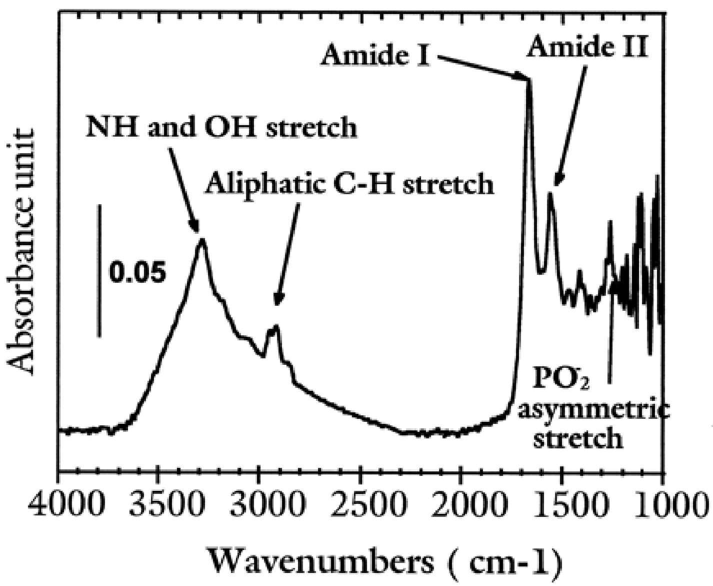
Excited-state photolysis model:



Eggeling, Volkmer, Seidel, *Chem. Phys. Chem.* (2005) submitted.

Intrinsic chemical contrast mechanism

Chemical contrast mechanism based on molecular vibrations, which is intrinsic to the samples: NO requirement of natural or artificial fluorescent probes!



[N. Jamin et al., *PNAS* **95** (1998) 4837-4840]

species	**Raman bands / cm^{-1}**
DNA backbone, C-O stretching	**~ 1000**
Polypeptide backbone, C=O stretching (Amide I)	**1500-1700**
Lipids, C-H stretching	**2900-3000**

<u>Infrared</u> microscopy:

- low spatial resolution (~2-3 µm)
- low S/B ratio
- water absorption

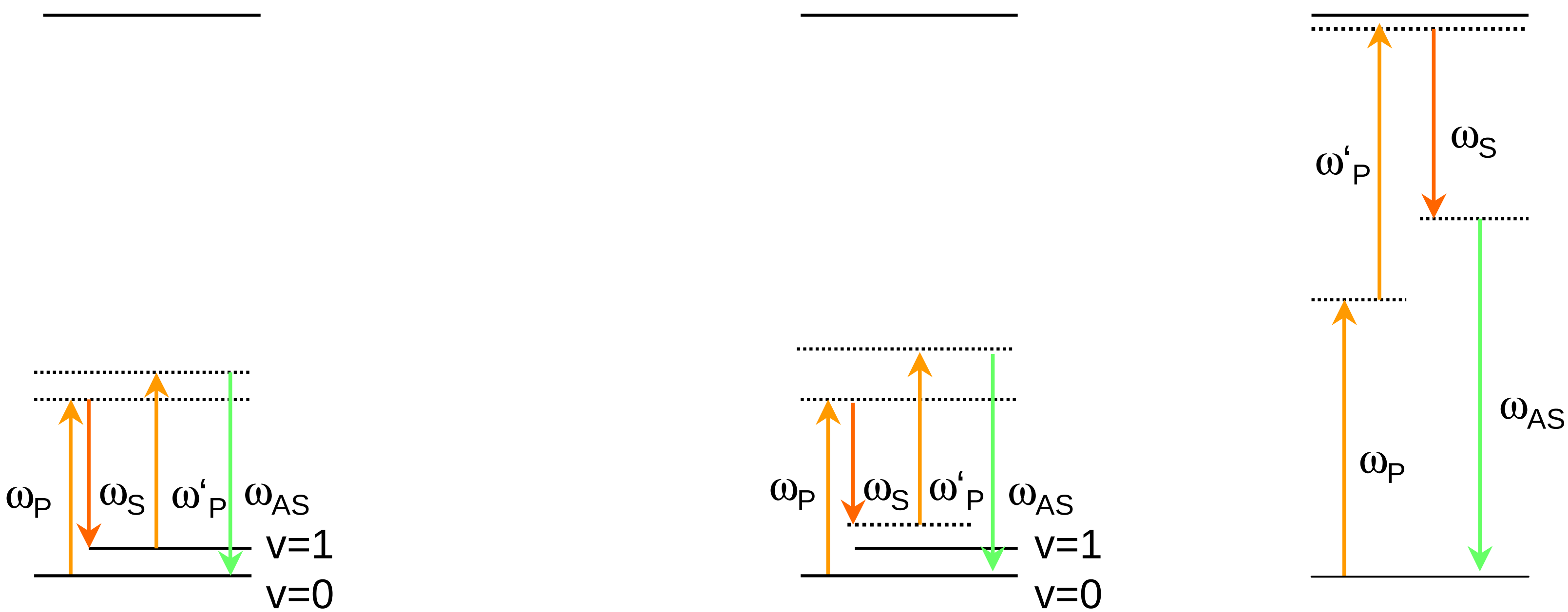
<u>Spontaneous Raman</u> microscopy:

- weak signal
  => requirement for high excitation power
- fluorescence background

CARS fundamentals

induced third-order polarization: $P_{AS}^{(3)}(\omega_{AS}) = \left[\chi_r^{(3)}(\omega_{AS}) + \chi_{nr}^{(3)}\right] E_P^2(\omega_P) E_S^*(\omega_S)$

CARS signal: $I_{CARS} \propto \left| P_{AS}^{(3)}(\omega_{AS}) \right|^2 \qquad (\omega_{AS} = 2\omega_P - \omega_S)$



Resonant CARS

Non-resonant CARS

Two-photon enhanced non-resonant CARS

$$\chi_r^{(3)} = \frac{1}{\left[\Omega - (\omega_{p1} - \omega_s)\right] + i\Gamma}$$

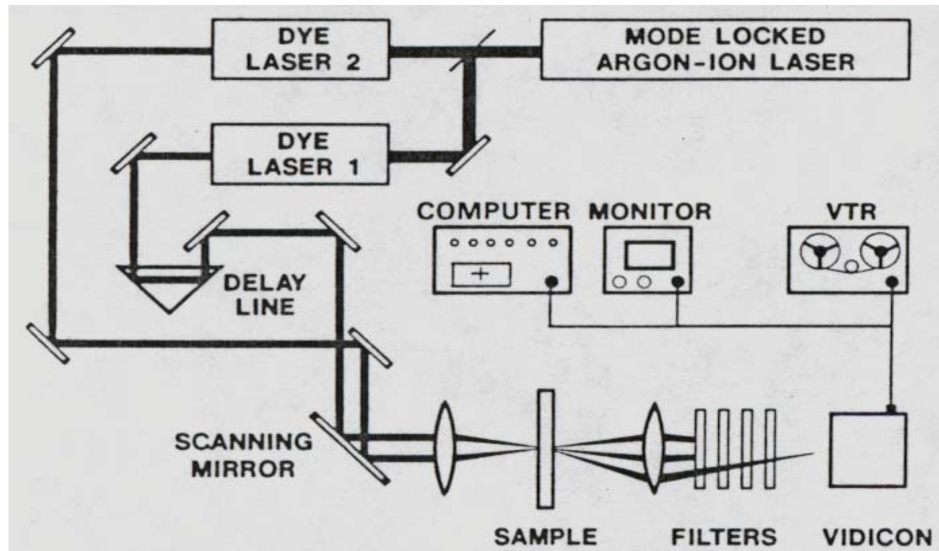
$$\chi_{nr}^{(3)} = const.$$

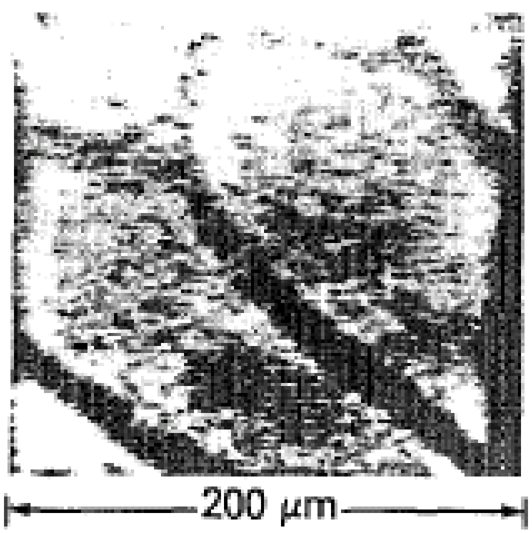
→ **No vibrational contrast !**

Development of CARS Microscopy

1982 - Duncan, Reintjes, Manuccia, *Optics Lett.* 7, 350

Picosecond visible laser,
Noncollinear geometry



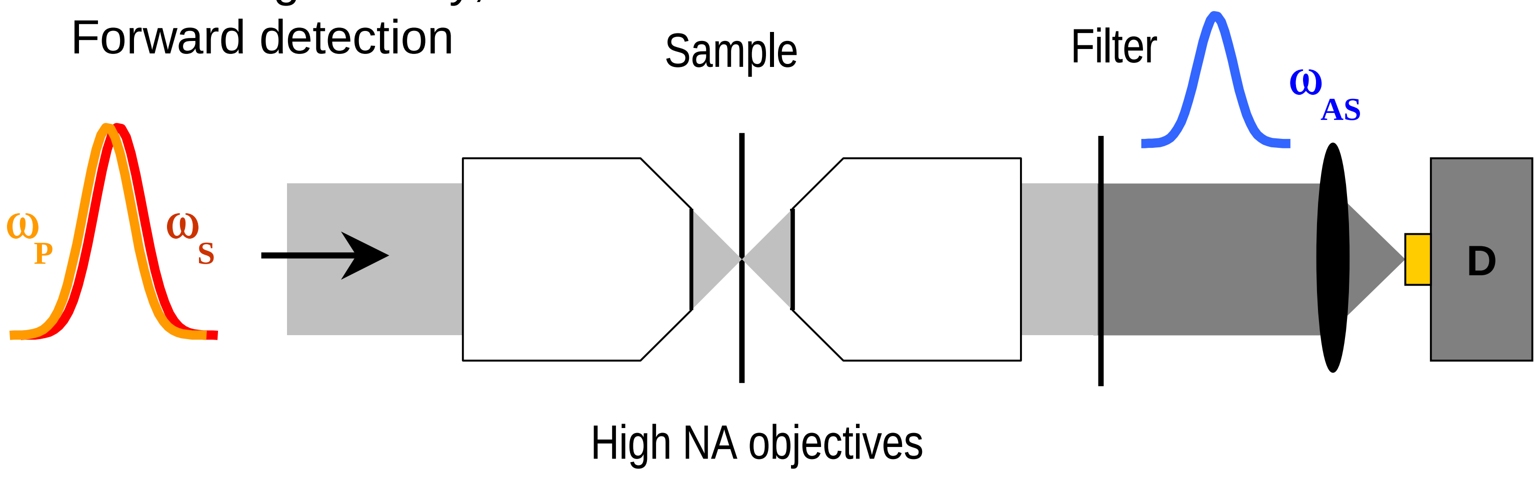


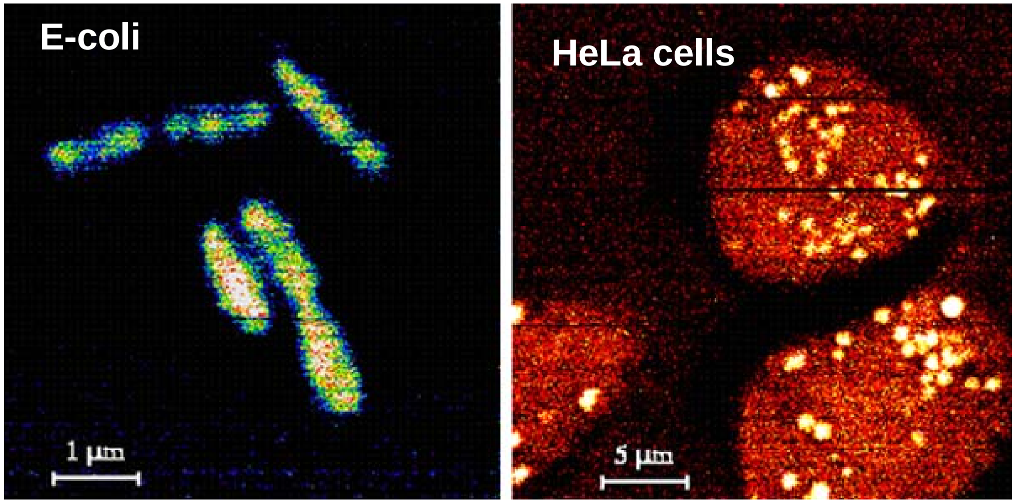
Onion-skin cells, soaked in D_2O

(CARS image on the 2450-cm^{-1} band of D_20)

1999 - Zumbusch, Holtom, Xie, *Phys. Rev. Lett.* 82, 4142

Femtosecond near-IR laser,
Collinear geometry,
Forward detection





853 nm (100 µW)

1135 nm (100 µW)

⇒ CARS signal at 675 nm

(Raman-shift of 2913 cm^{-1}, on resonance with C-H vibrations)

Advantages of CARS-microscopy

- Intrinsic sensitivity to specific chemical bonds

  => No dye labeling

- Coherent signal enhanced by orders of magnitudes

  => Less laser power required compared to conventional Raman

  compared to spontaneous Raman signal microscopy

- No population of higher electronic states

  => No photobleaching

- Confinement of nonlinear excitation to confocal volume

  => Inherent 3D spatial sectioning capability

Theory of collinear CARS microscopy

Distinct features:

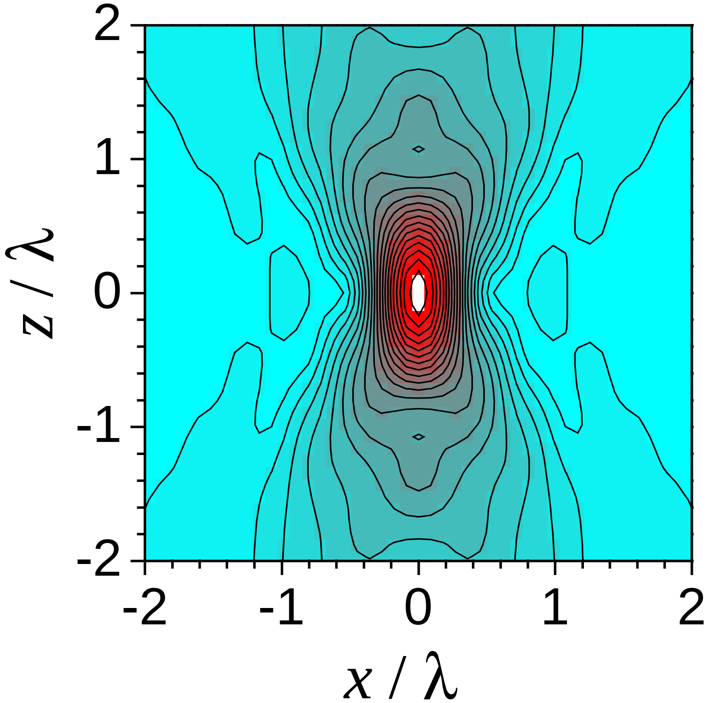
(i) Under tight focusing conditions -> breakdown of paraxial approximation

(ii) Actual extent of wave-vector mismatch is controlled by geometry for propagation directions of both incident beams and the CARS radiation

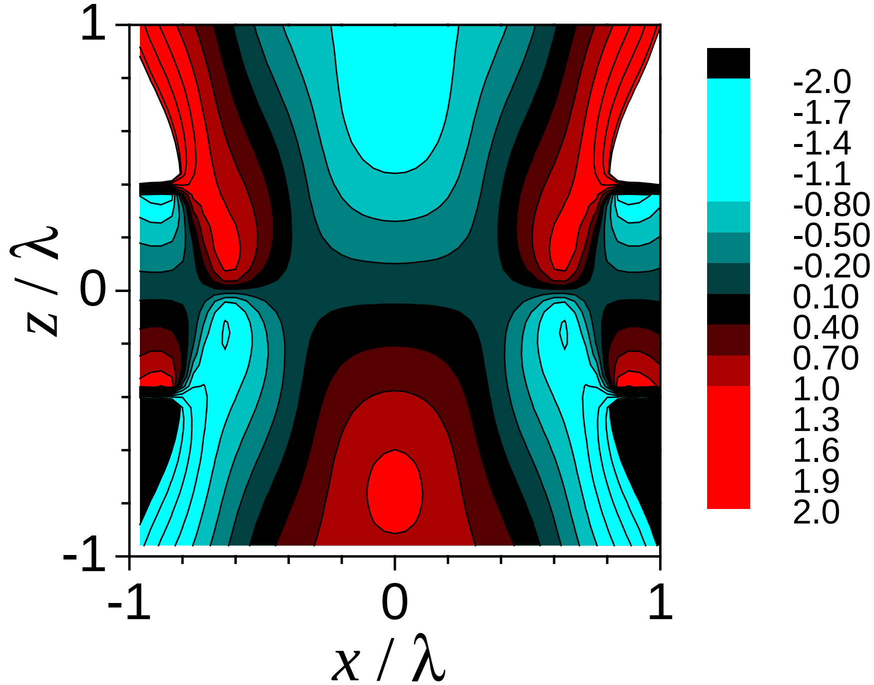
(iii) Heterogeneous sample of Raman scatterers of arbitrary shape and size embedded in nonlinear medium

(i) Description of a tightly focused Gaussian field

Amplitude distribution



Phase distribution (ϕ-*kz*)



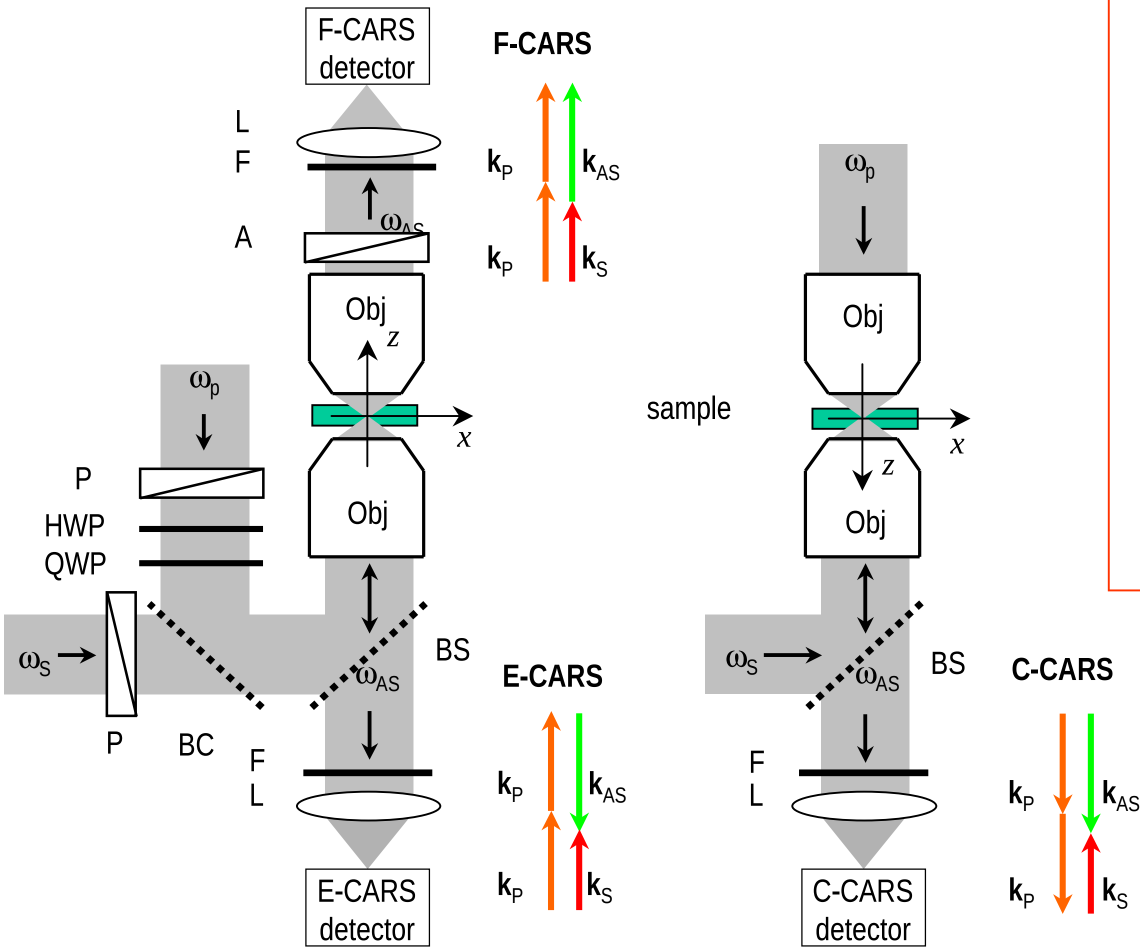
Cheng, Volkmer, Book, Xie, *JOSA B*, **19** (2002) 1363

(ii) Wave-vector mismatch in collinear CARS microscopy

Wave-vector mismatch in collinear beam geometry: $\Delta \mathbf{k} = \mathbf{k}_{AS} - (2\mathbf{k}_P - \mathbf{k}_S)$

phase matching condition: $D << \pi / |\Delta \mathbf{k}|$

(interaction length << coherence length)



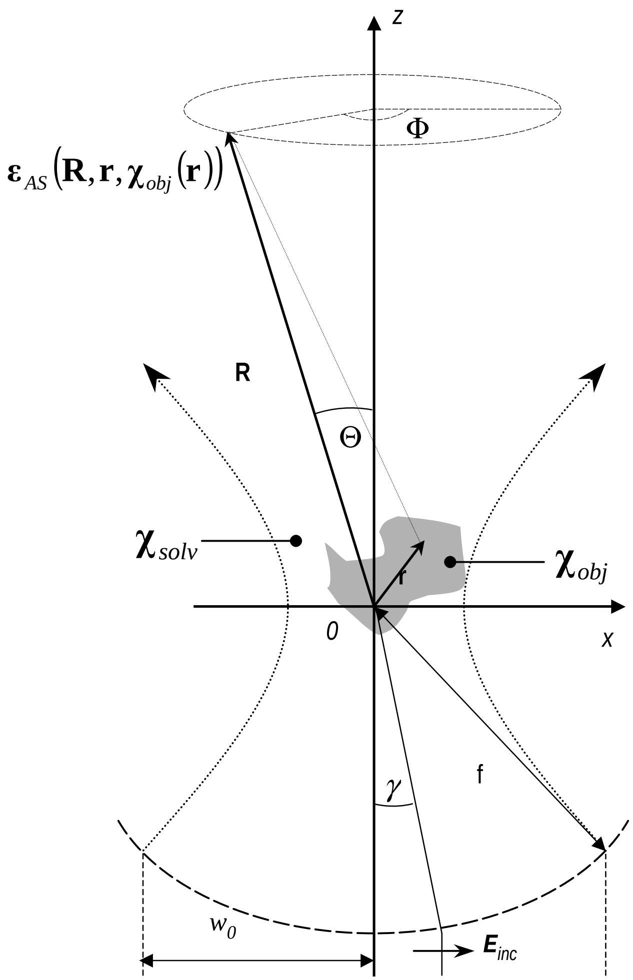
F-CARS (forward-detected) $|\Delta \mathbf{k}| = 0$

E-CARS (epi-detected) $|\Delta \mathbf{k}| = 4\pi n / \lambda_{as}$

C-CARS (counter-propagating) $|\Delta \mathbf{k}| = 4\pi n / \lambda_s$

Cheng, Volkmer, Book, Xie, *JOSA B*, **19** (2002) 1363

(iii) CARS signal generation for microscopic scatterer

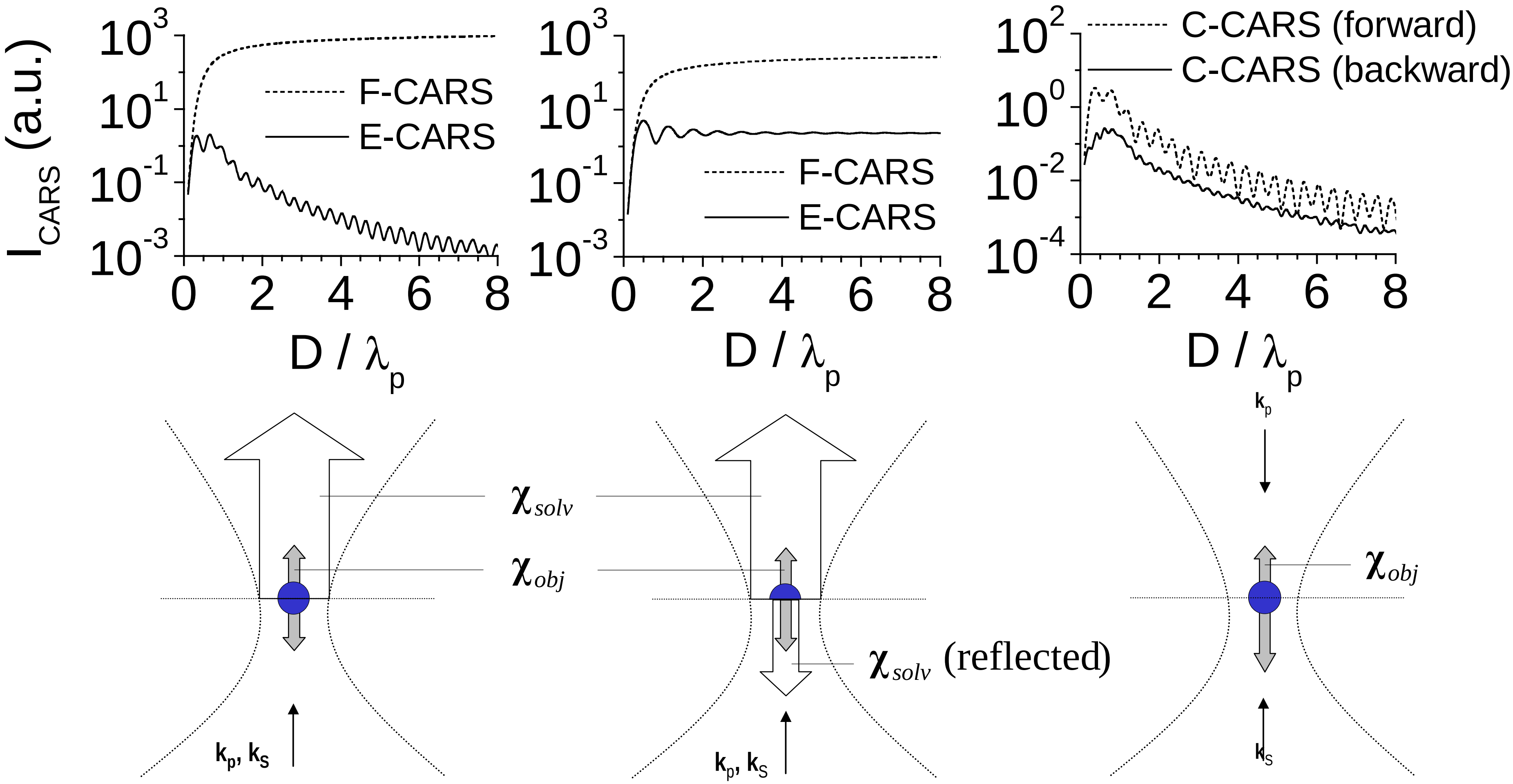


Assuming:

- tightly focused incident Gaussian fields
- Incident fields are polarized along the *x* axis
- refractive index mismatch between sample and solvent is negligible

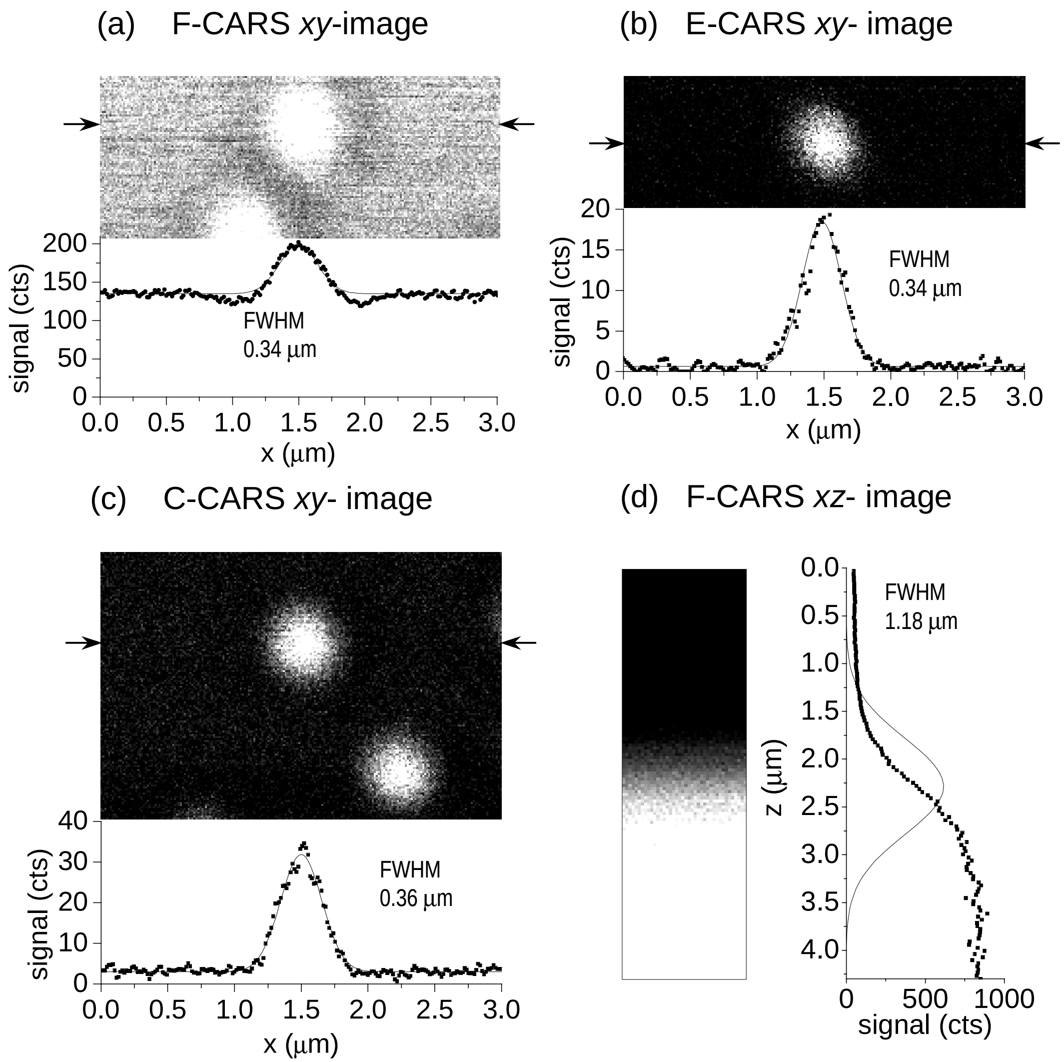
Volkmer, Cheng, Xie, *Phys. Rev. Lett.* **87,** 023901 (2001).

Simulated size dependence of CARS signals



Volkmer, *J. Phys. D : Appl. Phys.* **38** (2005) R59

Experimental characterization of CARS microscopy for a single 500-nm polystyrene bead in water (Raman shift ~1600 cm^{-1})

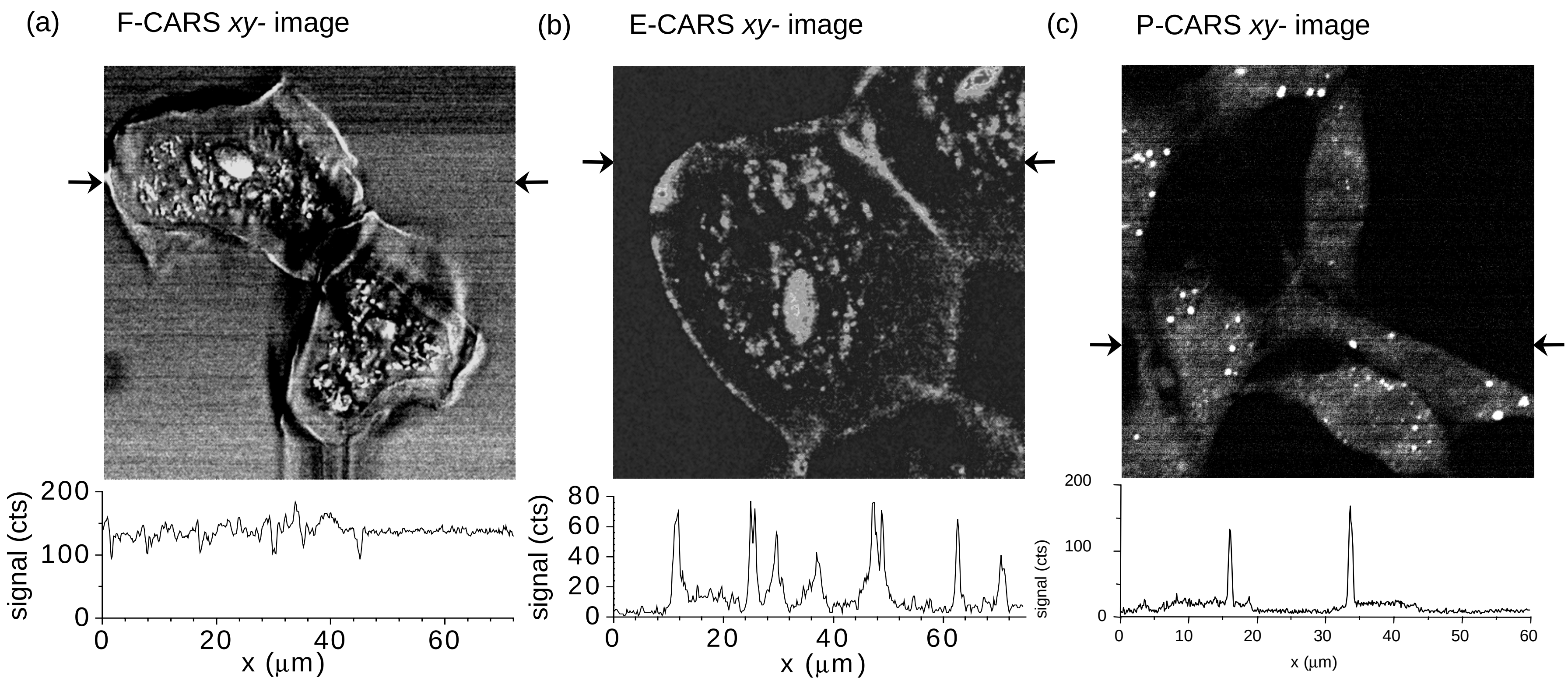


Volkmer, *J. Phys. D : Appl. Phys.* **38** (2005) R59

Picosecond CARS imaging of a live unstained cell

Epithelial cells
@ Raman shift ~1570 cm^{-1}
(amide I)

NIH3T3 cells
@ Raman shift ~2860 cm^{-1}
(C-H strectch)



Simulation of CARS spectra as a function of pulse widths

$$\chi^{(3)} = \frac{A}{\Omega - (\omega_p - \omega_s) - i\Gamma} + \chi_{nr}^{(3)}$$

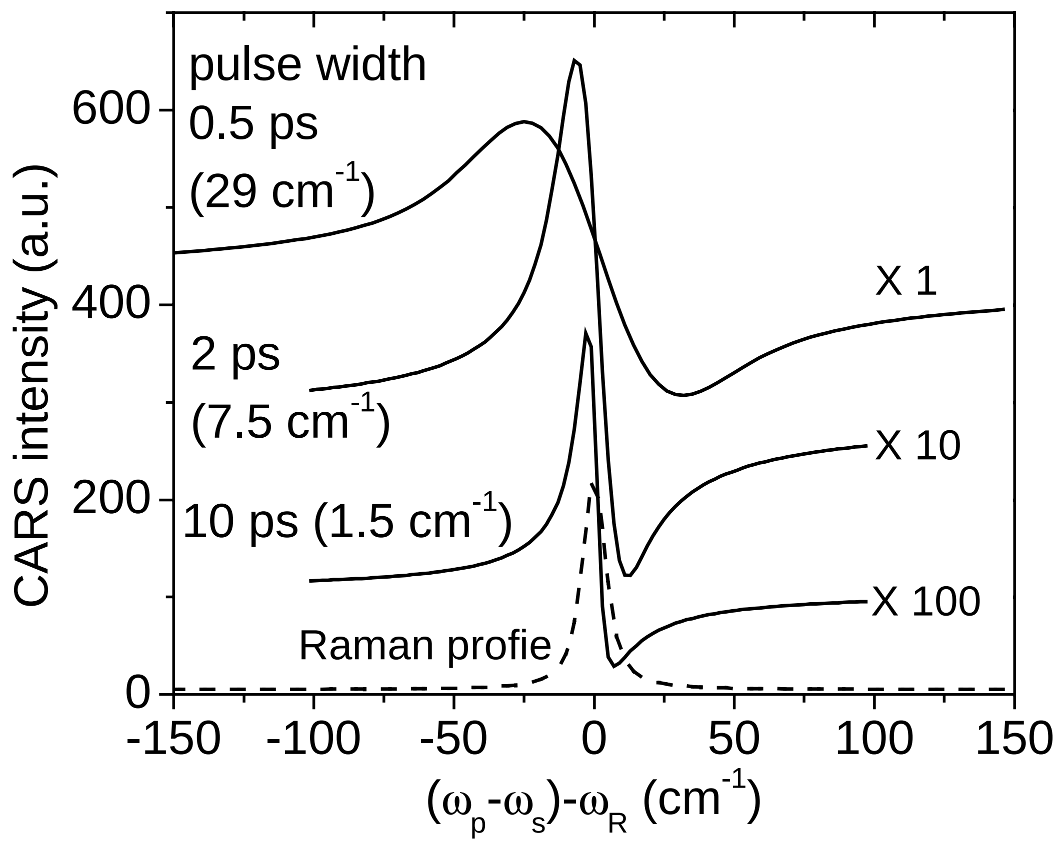
$2\Gamma = 10\ \mathrm{cm}^{-1}$ … line width

Ω … vibration frequency

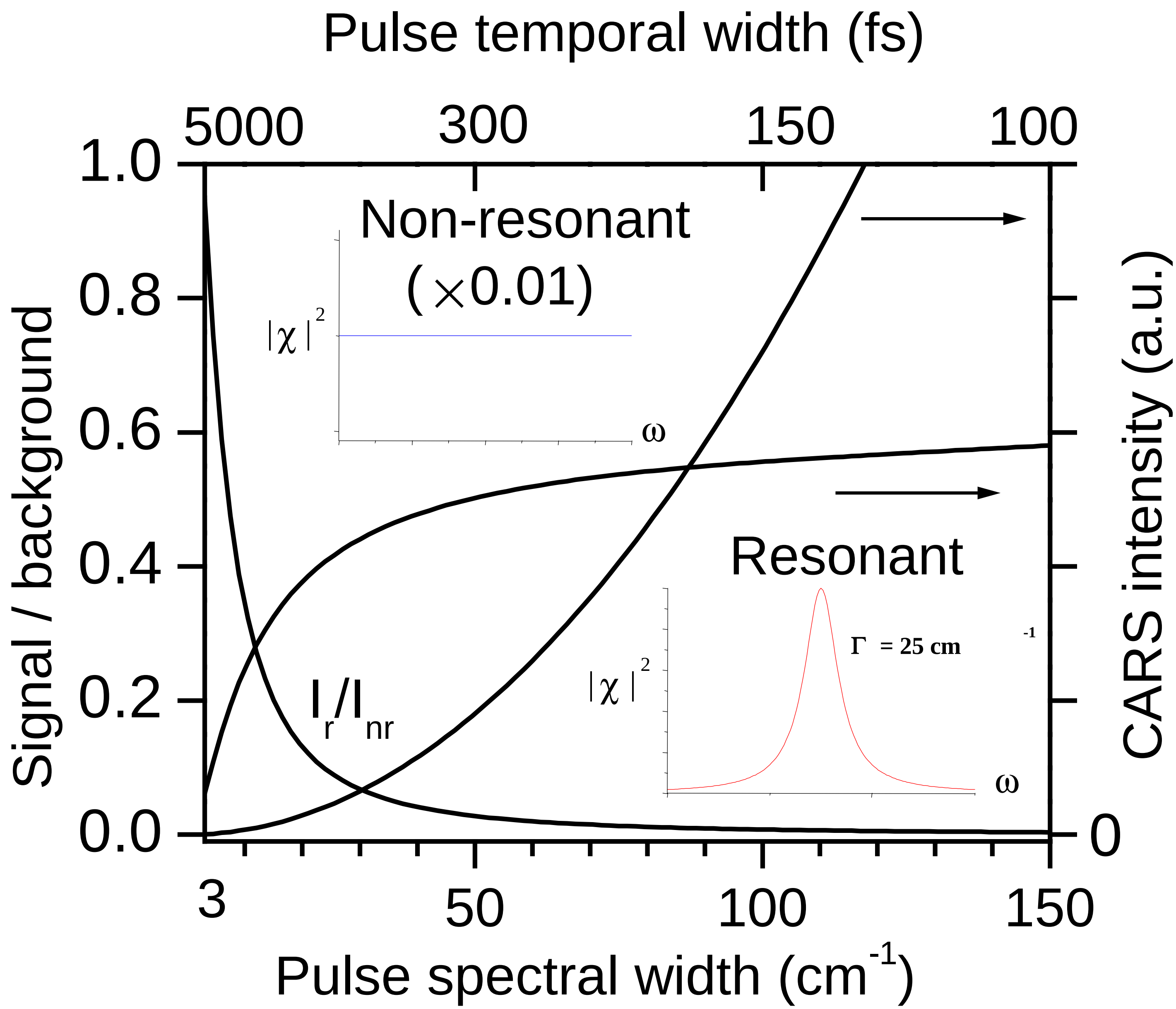
$\chi_{nr}/A = 0.2$

The CARS intensity is:

$$I_{CARS} = \int_{-\infty}^{+\infty} \left| P^{(3)}(\omega_{as}) \right|^2 d\omega_{as}$$

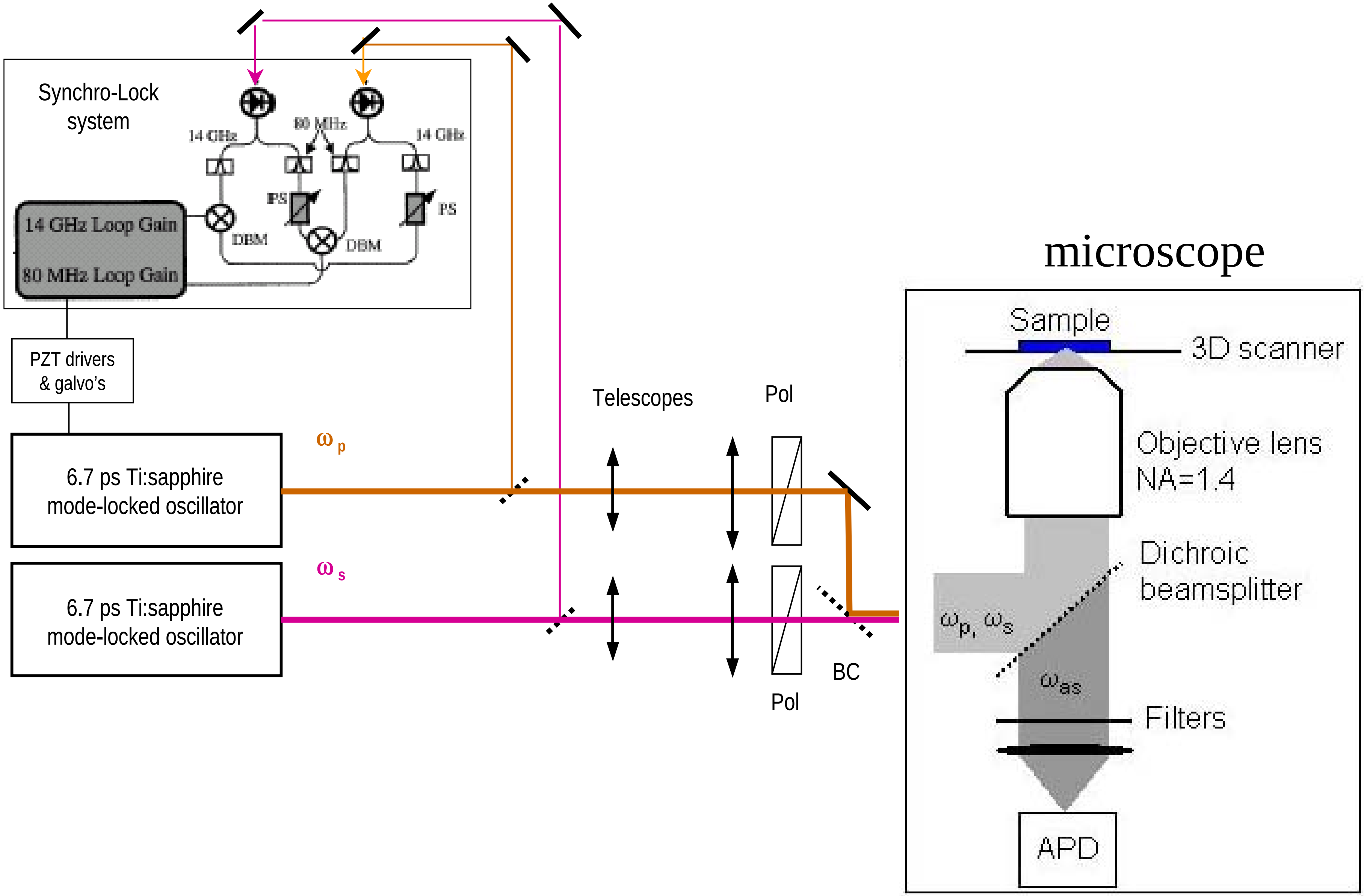


CARS intensity *vs.* excitation pulse spectral width



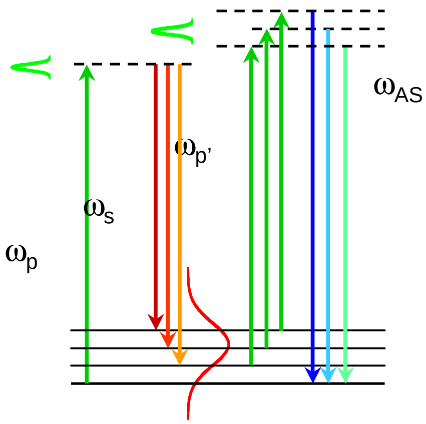
Cheng, Volkmer, Book, Xie, *J. Phys. Chem. B* **105,** 1277 (2001).

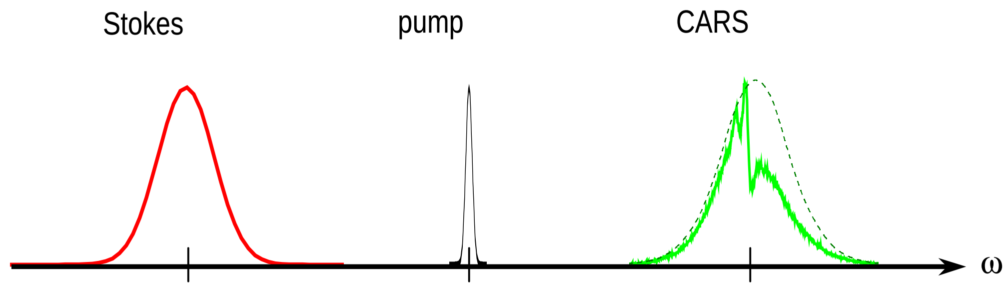
The CARS microscope

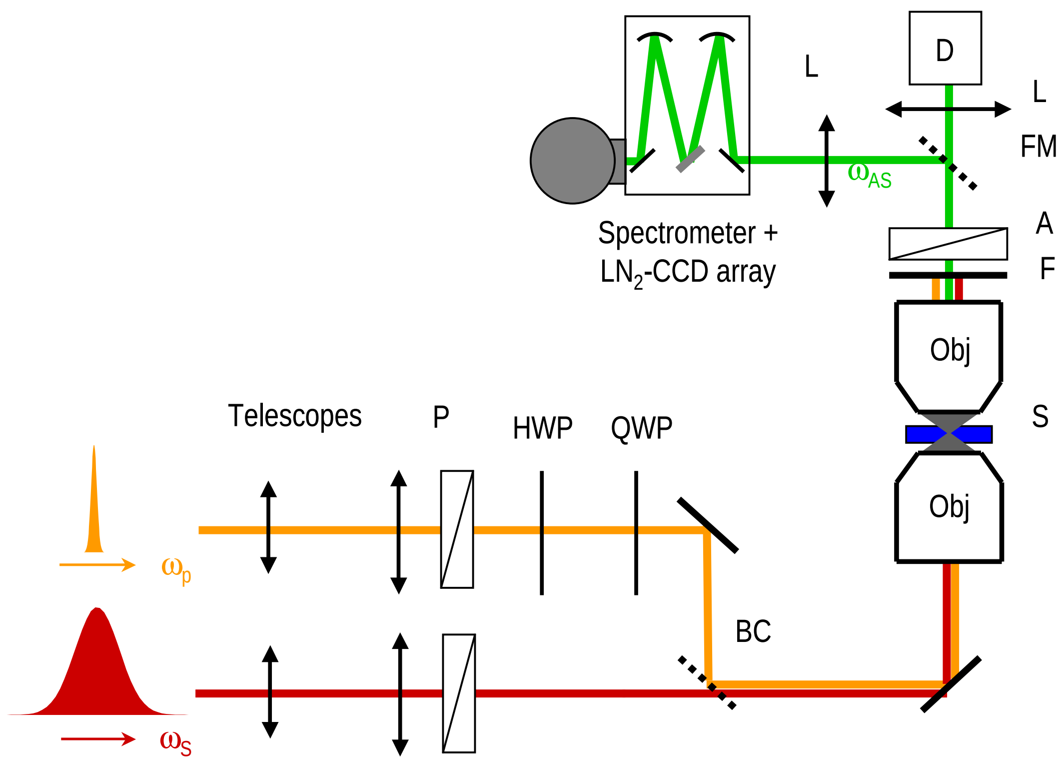


Multiplex-CARS Microspectroscopy in the Frequency-Domain

➔ **acquisition of CARS spectrum in one"shot"!**

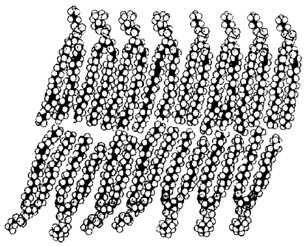






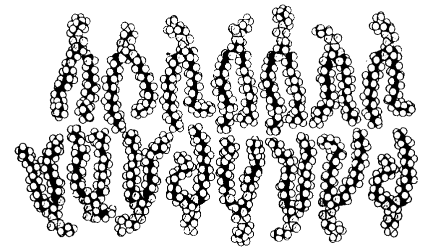
Example: Monitoring the thermodynamic state of phospholipid membranes in the C-H stretch region

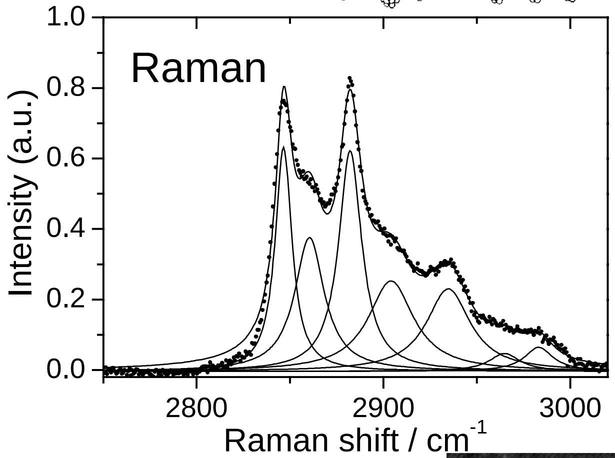
DSPC
T_g=55°C

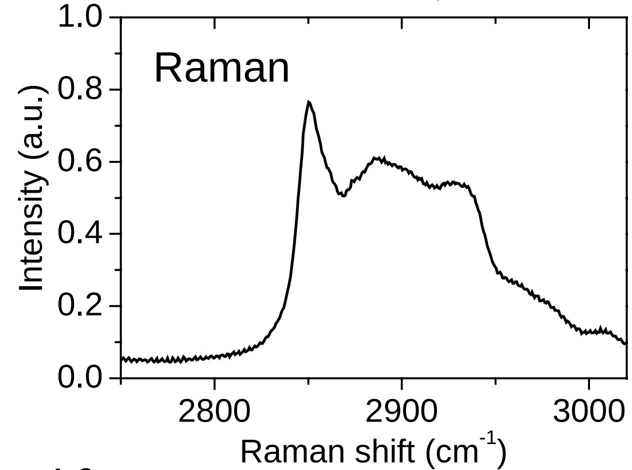


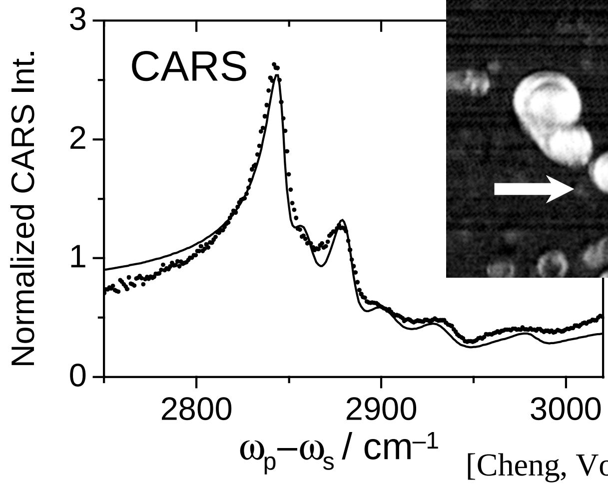
entropy

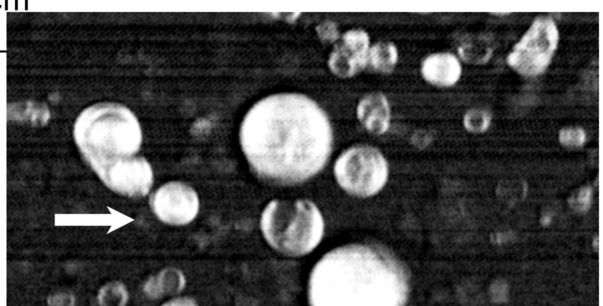
DOPC
T_g=-20°C

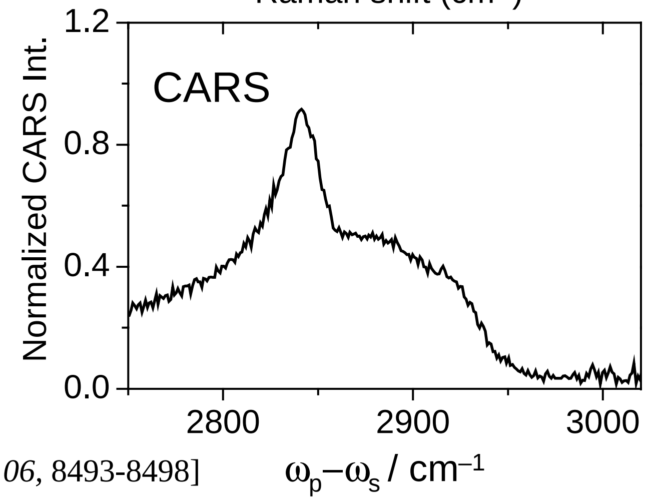






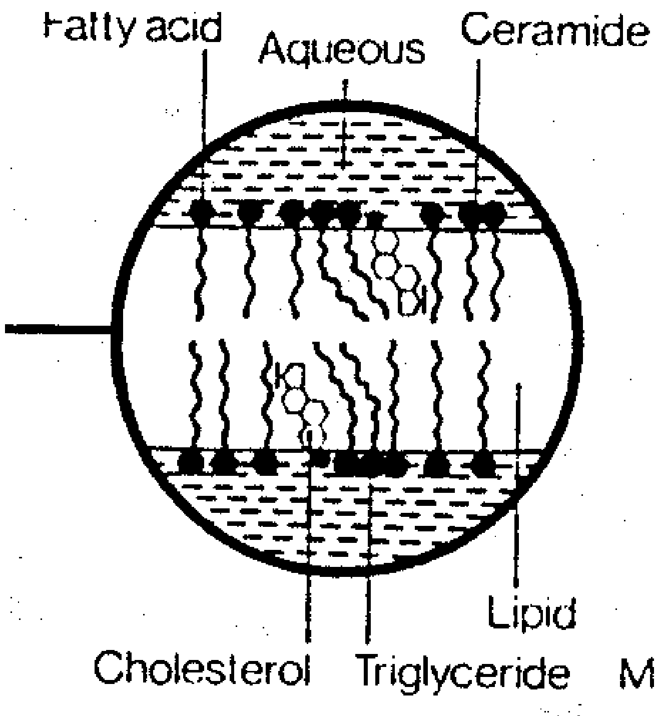




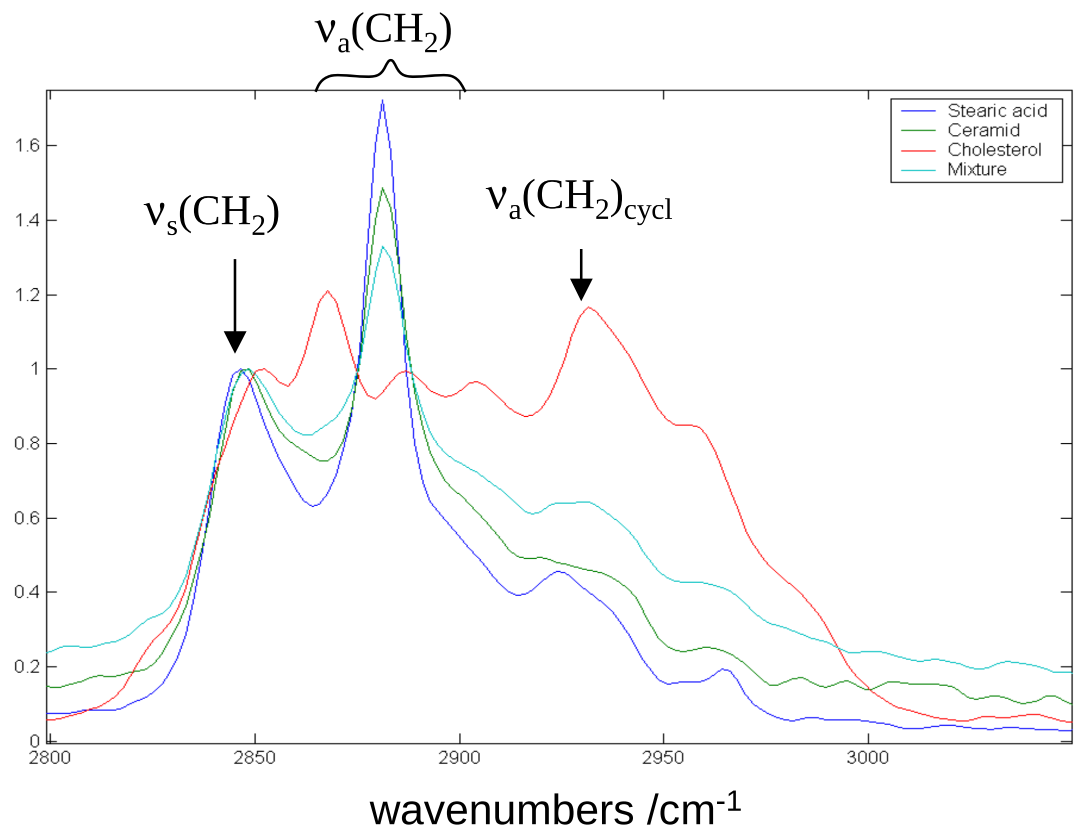


[Cheng, Volkmer, Book, Xie, *J. Phys. Chem. B* **2002,** *106*, 8493-8498]

Model system for Stratum Corneum lipids



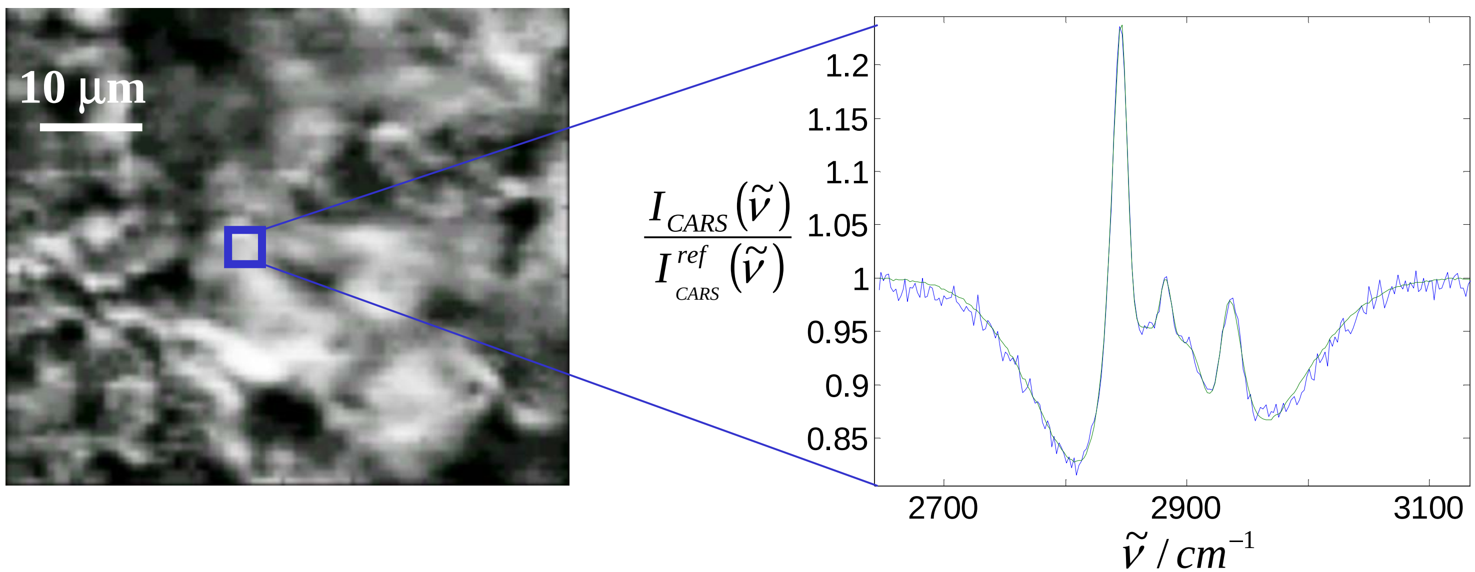
Raman spectra in CH-stretching mode region



Hyper-spectral CARS imaging of a Stratum Corneum

Spectrally integrated CARS image section

Extracted CARS ratio spectra for each image pixel

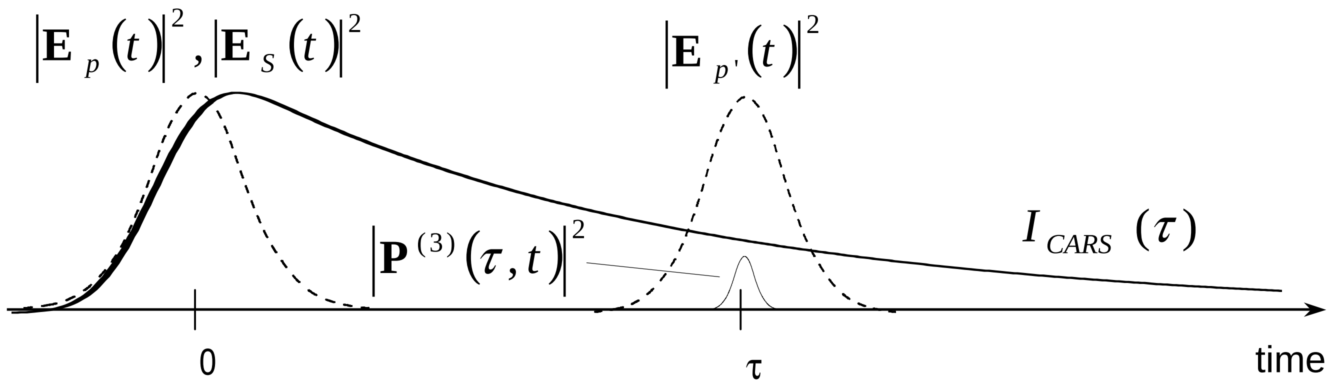


➔ **Existence of cholesterol-enriched micro-domains**

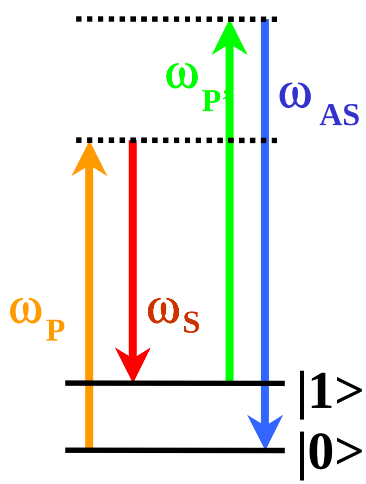
(see Poster by Nandakumar *et al* : Mo-4)

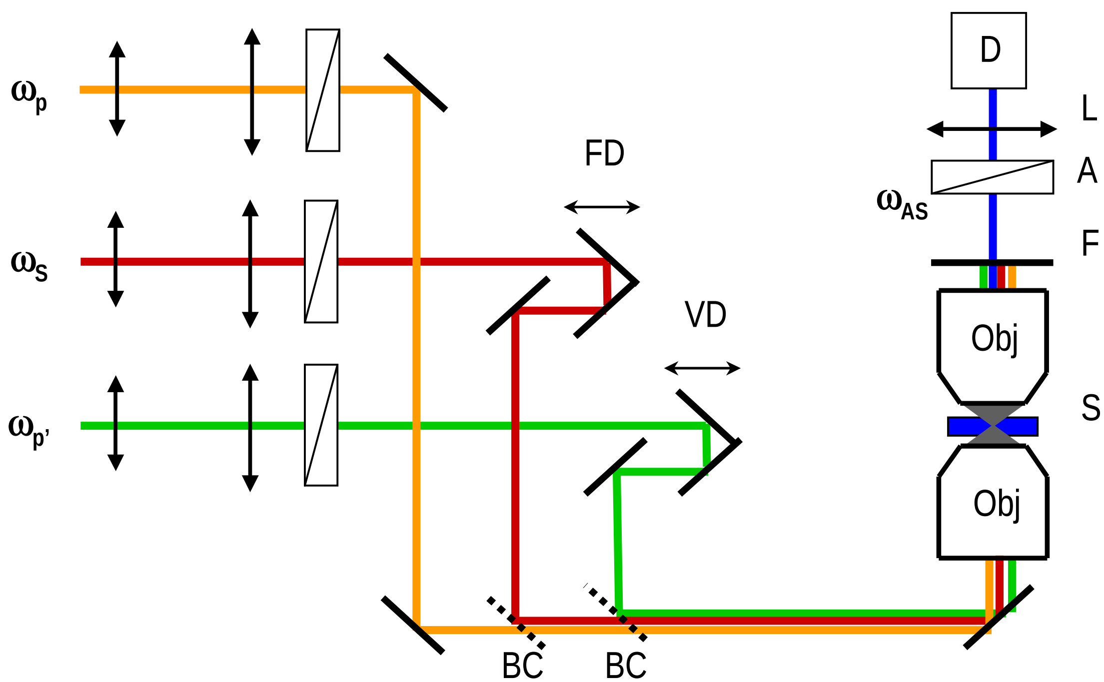
CARS microspectroscopy in the time-domain

Raman Free Induction Decay (RFID):

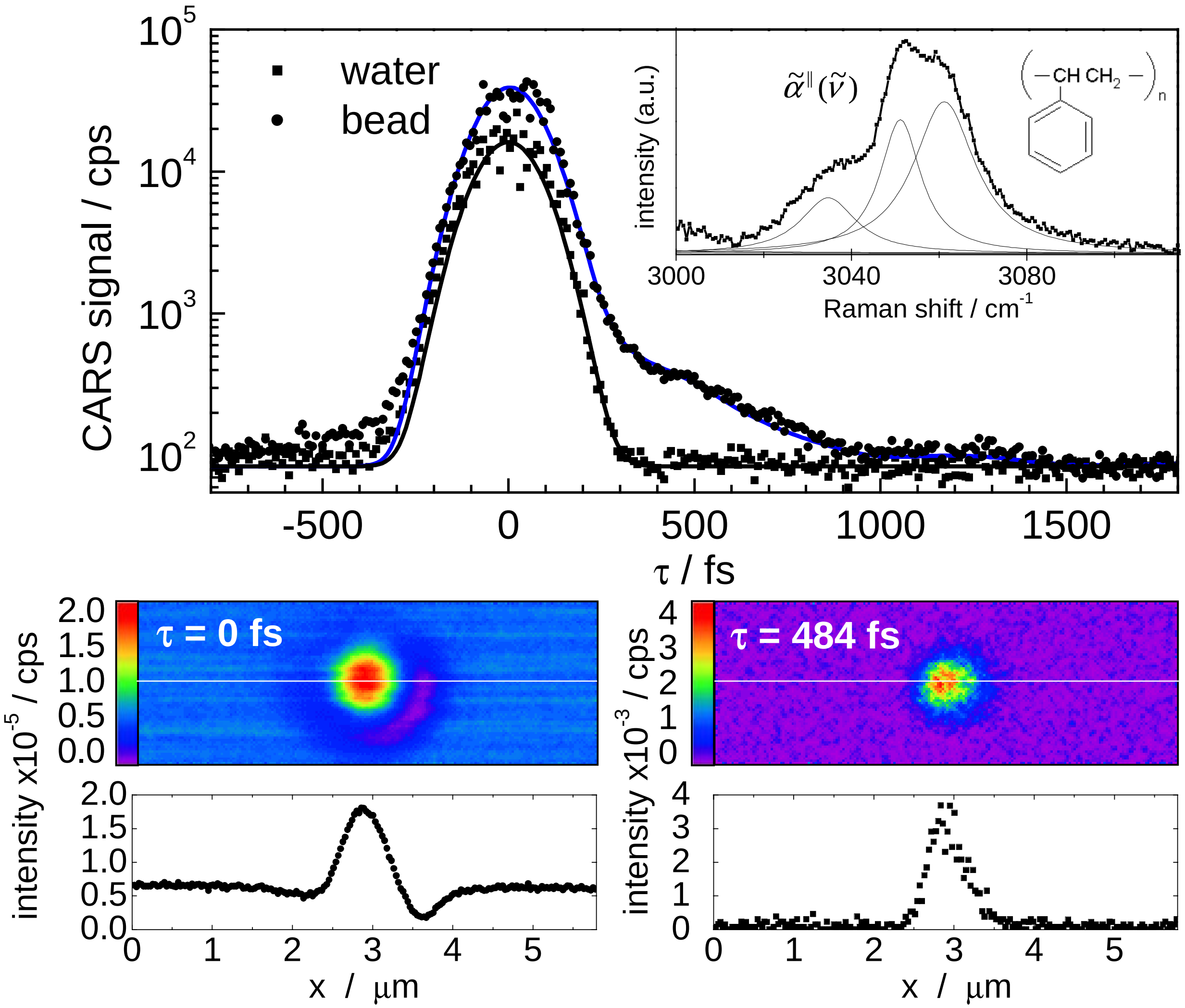


Three-color CARS set-up:





Example: RFID imaging of 1-μm polystyrene bead



λ_{P1} = 714.6 nm (~85 fs)

λ_{S} = 914.1 nm (~115 fs)

λ_{P2} = 798.1 nm (~185 fs)

→ **Quantum beat recurs at ~ 1280 fs (mode beating at difference frequencies of ~ 26 cm^{-1})**

S/B(τ=0) ≈ 3 **S/B (τ=484 fs) ≈ 35**

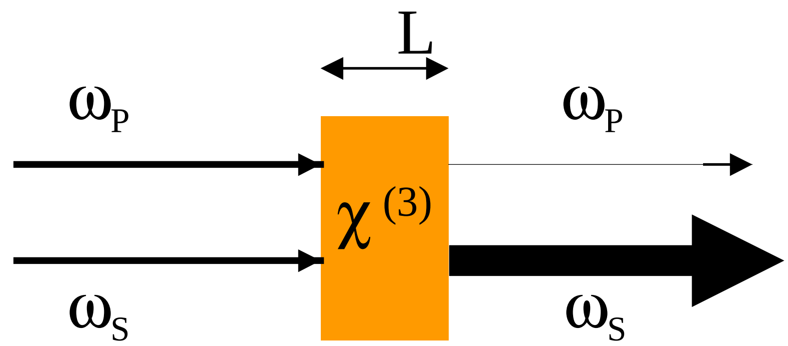
→ Complete removal of the non-resonant CARS contributions !

Volkmer, Book, Xie, *Appl. Phys. Lett.* **80** (2002) 1505c

Coherent Vibrational Imaging beyond CARS

Simplifying coherent Raman microscopy by use of a nonlinear optical imaging technique which maps only the **imaginary part of $\chi^{(3)}$**

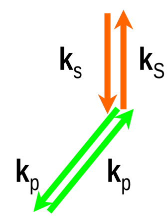
Stimulated Raman scattering (SRS) microscopy



$$\omega_S = \omega_S - \omega_p + \omega_p$$

$$k_S = k_S - k_P + k_p$$

Stimulated Raman gain for probe laser in the presence of strong pump laser, when frequency difference equals Raman frequency



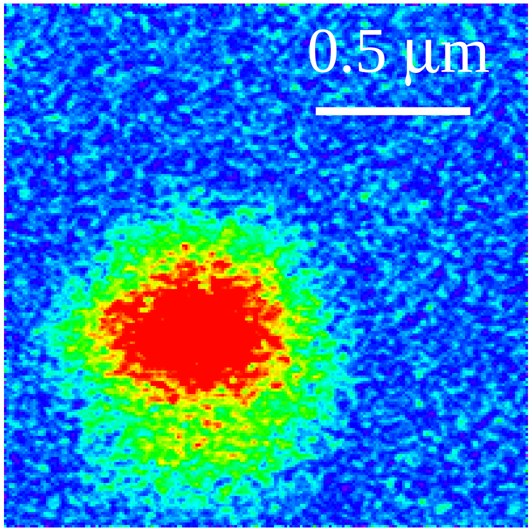
$$P(\omega_2) = \chi^{(3)}(-\omega_2; \omega_2, -\omega_1, \omega_1)\, E(\omega_2)\, |E(\omega_1)|^2$$

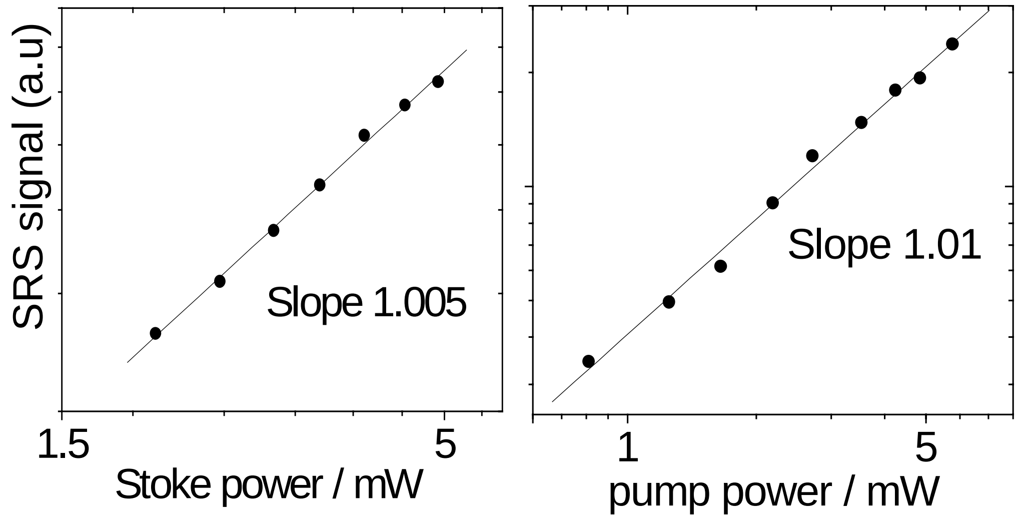
Advantages:

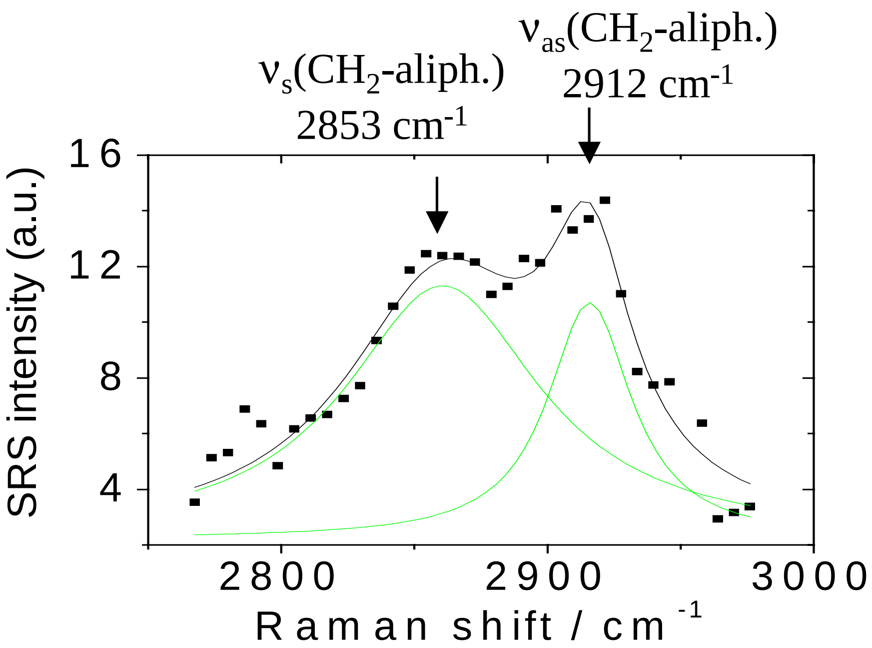
- ♦ Depends only on the Im $\chi^{(3)}$
- ♦ Linear on $\chi^{(3)}$
- ♦ Linear on number density
- ♦ Linear in pump and Stokes intensities
- ♦ Automatic Phase matching

Disadvantage: Tiny signal over huge background signal from the Stokes field!

SRS images of a polystyrene 1-μm bead in water







- Pixel intensity ∝ No. of C=C bonds
- No signal from surrounding water
- No interference effect in image contrast

Nandakumar, Kovalev, Volkmer, manuscript in preparation

Summary

- Under tight focusing conditions, size-selectivity in CARS signal generation is introduced by wave-vector mismatch geometries, e.g. epi-detected CARS (E-CARS) microscopy

  → allows efficient rejection of bulk solvent signal

  → E-CARS is easily implemented with a commonly used confocal epi-fluorescence microscope

- Combination of CARS microscopy with spectroscopic techniques provides wealth of chemical and physical structure information within a femto-liter volume in both the frequency-domain (multiplex CARS microspectroscopy) and time-domain (RFID imaging)

  → allows rejection of nonresonant background contributions by polarization-sensitive and time-delayed detection schemes

- Highly sensitive tool for the chemical mapping of unstained live cells in a spectral region for DNA, membranes and proteins.

[*J. Phys. D : Appl. Phys.* **38** (2005) R59 (Topical review)]

- First demonstration of Stimulated Raman Scattering (SRS) microscopy on model systems of polystyrene beads embedded in water

  → No interference effects with nonresonant contributions from both object and matrix

  → SRS spectra qualitatively reproduce the Raman spectra

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P. Nandakumar
A. Kovalev

Roswell Park Cancer Institute, Buffalo, NY:

A. Sen
M. Koehler

€€ **$$**

Emmy Noether Program

Faculty of Arts and Sciences of Harvard University