Refractive effects in Coherent Anti-Stokes Raman Scattering (CARS) Microscopy

Nadia Djaker, Pierre-Francois Lenne, Herve Rigneault
Institut Fresnel UMR CNRS 6133, Ecole Generaliste d Ingenieurs de Marseille (EGIM), Universite Aix-Marseille, Domaine Universitaire Saint Jerome, 13397 Marseille cedex 20, France
Mail : nadia.djaker@fresnel.fr, herve.rigneault@fresnel.fr

Key words: Four-wave mixing, Coherent anti-Stokes Raman scattering (CARS) Microscopy, chemical imaging

Coherent anti-Stokes Raman scattering microscopy (CARS) is a new approach for chemical imaging of molecular systems, with high sensitivity, high spatial resolution, and three dimensional sectioning capability, without using fluorophores that are prone to photobleaching. This technique permits to map selectively molecular species, by using vibrational properties of their chemical bounds. CARS is described by a four wave-mixing process, where the signal intensity depends nonlinearly on the incident intensities: $I_{CARS} \sim I_P{}^2 I_S$, and generated in a direction determined by the phase-matching condition. The CARS signal can be detected in both forward and backward directions [1]. The two signals provide complementary information about a sample: forward-detected CARS (F-CARS) microscopy is suitable for imaging objects of a size comparable to or larger than the excitation wavelength. Epi-detected CARS (E-CARS) microscopy provides a sensitive means of imaging objects having an axial length much smaller than the excitation wavelength because it avoids the large backgrounds from the solvent [2, 3, 4]. The E-CARS and F-CARS intensities ratio depends on the shape, the size of the sample, as well as the index of the solvent. The back-reflection of the F-CARS at an index-mismatched interface contributes to the contrast in the epi-detected CARS image [2], although that was theoretically predicted, not any systematic characterization was reported.
In this presentation, we show the first CARS studies of the refractive effect of the sample, comparing the E-CARS and F-CARS signals for different diameters of polystyrene beads, in different refractive index solvents. This effect is dominant in all cases where the refraction index of the solvent is different than the refractive index of the sample.
In addition, we present several simulations based on geometrical optics, comparing forward-detected and backward-detected signals in different sized polystyrene beads, embedded in different index solvents, and we show that, the backward-reflected F-CARS dominates the experimentally epi-detected signals (except when the solvent have the same refractive index of the sample). Furthermore, we demonstrate experimentally and theoretically that the maxima of forward and epi-detected signals are generated at different positions along the Z axis in the sample.

References

[1] A. Volkmer, J-X. Cheng, X.S. Xie, *phys. Rev. Lett.* **87**, 23901 (2001).
[2] J-X. Cheng, X.S. Xie, *J. Phys. Chem B* **108**, 827 (2004).
[3] J-X. Cheng, A. Volkmer, X.S. Xie, *J. Opt. Soc Am. B* **19**, 1363 (2002).
[4] N. Djaker, PF. Lenne, H. Rigneault, *Proc. SPIE*. **5463**, 133 (2004).