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Multi-focus coherent anti-Stokes Raman scattering microscopy

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Recently, new nonlinear microscopy using coherent anti-Stokes Raman scattering (CARS) spectroscopy has been developed, and the non-staining imaging with three-dimensional resolution has been demonstrated[1-3]. Figure 1 shows the CARS process. In CARS spectroscopy, two laser beams with different wavelengths are used for excitation. When the frequency difference $\omega_1 - \omega_2$ is equal to the frequency of the Raman active molecular vibration Ω, CARS is generated as the coherent emission at $2\omega_1 - \omega_2$. The advantages of CARS spectroscopy over conventional Raman (spontaneous Raman) spectroscopy are the high intensity emission and the separation from undesired fluorescence signal because CARS is the anti-Stokes side emission.

We have developed a multi-focus CARS microscope system for fast multi-spectral imaging. Figure 2 and 3 show the schematic layout of the developed system and the principle of multi-focus CARS microscopy, respectively. The system consists of a picosecond tunable laser based on an optical parametric amplifier excited by a regeneration amplifier, a rotating micro-lens array disk, and a transmission microscope with an intensified CCD camera. A few tens of points are excited simultaneously by the micro-lens array disk, and these excited spots are scanned by rotating the array disk. Therefore, a CARS image is obtained by the intensified CCD at once.

Figure 4 shows CARS images of the mixture of polystyrene (diameter = 4.5 μm) and glass beads (diameter = 3~5 μm) observed (a) at 990 cm^{-1} that is near the peak of the phenyl breath mode of polystyrene and (b) at 1050 cm^{-1} apart form the band peak. One can recognize that some beads with larger diameters in Fig. 4 (a) shows brighter image than those in Fig. 4 (b), though the intensity of the beads with smaller diameters was not changed appreciably. Figure 5 shows resultant CARS spectra of beads positioned at the cross point of α and at β in Fig.4. The spectral distinction of these beads was clearly detected, and therefore, the distribution of analytical sample can be identified by the multi-spectral CARS images. We have also developed the CARS microscopy system with two synchronized ps Ti:Sapphire lasers have narrower spectral band widths. The results of the new system will be also shown.

Reference

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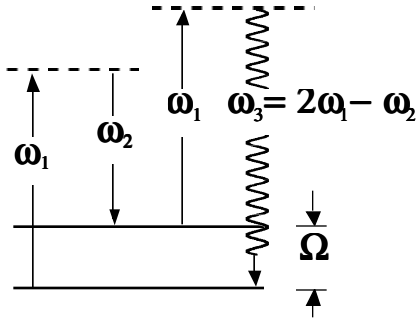

Figure 1. CARS process

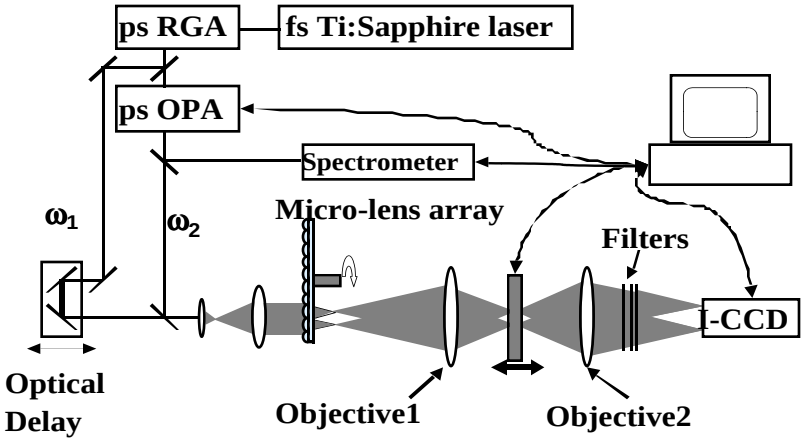

Figure 2. Schematic layout of multi-focus CARS microscopy system

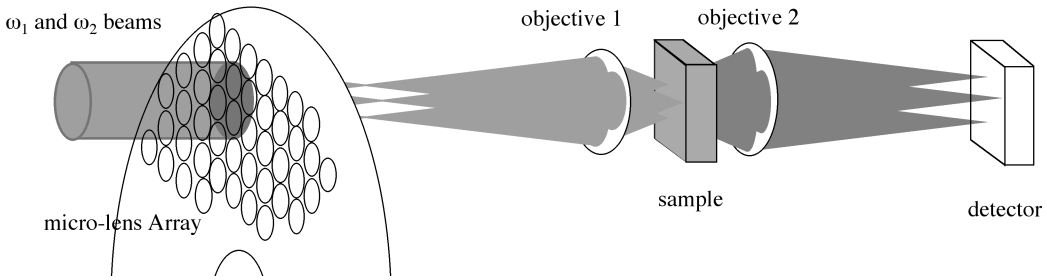

Figure 3. Principle of multi-focus CARS microscopy

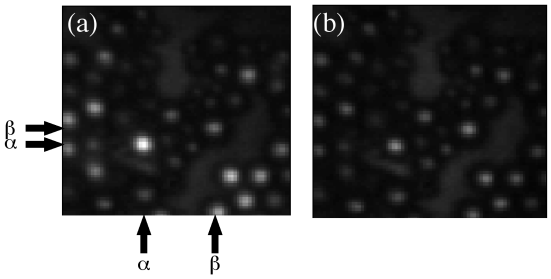

Figure 4: CARS image of the mixture of polystyrene at 990 cm^{-1} (a) and 1050 cm^{-1}(b)

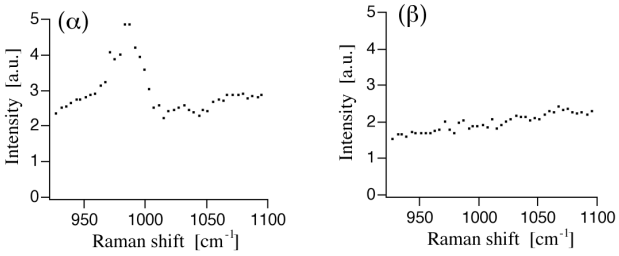

Figure 5: CARS spectra at (α) and (β) in Fig. 4

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