CARS Microscopy for Imaging

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***Abstract* — Optical microscopy grows in its importance with the development of modern nanotechnology, biotechnology, methods of diagnostics and treatment of most dangerous diseases for mankind [1-3]. There are several important goals of optical microscopy for biomedical studies among which the next three may be distinguished: fast imaging with high lateral spatial resolution, 3-D sectioning capability and high contrast for chemical selectivity. To meet these specific requirements, various types of both linear and nonlinear optical microscopy were elaborated [4,5].**

***Index Terms* — Raman spectroscopy, microscopy, CARS imaging, biomedical applications.**

I. INTRODUCTION

Raman spectroscopy, as a vibrational "fingerprint" of a molecule, is an adequate analytical technique used for cells and tissues studies due to its non-destructive nature without external markers. However, Raman scattering normally exhibits a very weak signal which results in long integration process. One of the most promising imaging tool in optical microscopy is based on the nonlinear variant of Raman scattering – Coherent Anti-Stokes Raman Scattering (CARS) [6-8].

CARS can be considered as one of the powerful imaging tool in general, and for biomedical applications in particular, as it provides remarkable signal enhancement due to the coherent nature of process [9]. An important point is that CARS imaging speeds up data processing from hours to seconds.

II. SETUP AND FIRST RESULTS

Recently at the Joint Institute for Nuclear Research (JINR) in Dubna, a multimodal optical platform based on Raman and CARS microspectroscopy and microscopy was established as a complimentary tool to the neutron scattering methods used for condensed matter studies and traditionally implemented at JINR. This multimodal platform was developed and manufactured at the "SOLAR TII" company (at present "SOL Instruments Ltd") in Minsk, Belarus (Fig.1).

Fig.1 The general view of the CARS microscope at JINR

The details of the optical platform can be found in [10]. A part of a diode-pump Nd:YVO4 picosecond laser (7 ps, 5 W, 85 MHz) is used as a source of the Stokes wave. Intracavity optical parametric oscillator (OPO) provides generation of pulses tunable from 690 nm to 990 nm, with pulse duration of 6 ps and typical output power of 40-130 mW. Two picosecond beams recombined and overlapped in space and time, through a computer-controlled galvano-scanner are directed to the inverted microscope assembled on the NIKON 2000S mount, with wide aperture (60x, NA-1.2) water-immersion lens (Olimpus) installed on the Z piezo-scanner with a minimal step of 100 nm and a scanning range of 80 microns.

The CARS signals generated on the sample both forward (F-CARS) and backward (E-CARS) are collected with the collimators, optically filtered of the initiating beams, and are directed to the corresponding registration channels for further digital processing and imaging.

We have tested Raman and CARS spectroscopy and imaging for a number of specimens: liquids, solids and powders. As an example Figure 2 shows Raman spectra of polystyrene beads with a diameter of 2.8 μ.

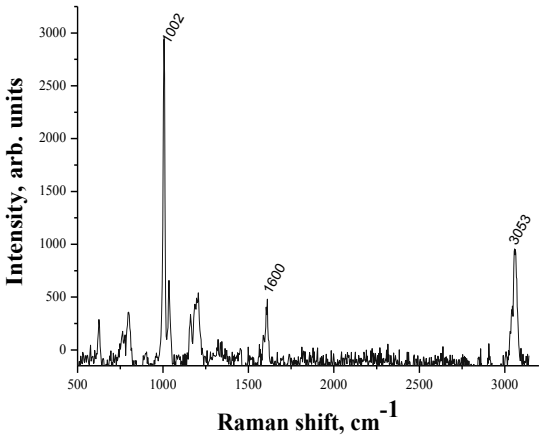

Fig.2 Raman spectra of polystyrene beads

Corresponding CARS image of these polystyrene beads is given in the Figure 3.

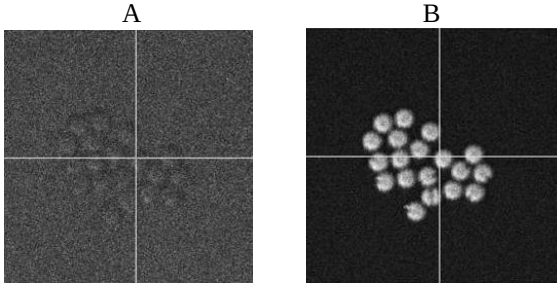

Fig. 3 CARS images of polystyrene beads:
(A) – at 3140 cm^{-1} (off-resonance condition),
(B) – at 3053 cm^{-1} (at the resonance).

It is worth noticing that since the CARS signal is generated only from the focal volume, this provides a natural way of 3-D sectioning without the need for confocal geometry.

The JINR CARS microscope data-acquisition software has an operational control of the main system parameters, such as spectral splitting of the signal, the scanning region,
enlarging the image (Zoom) with selection of the scanning zone, accumulation of the signal according to the scan series, simultaneous visualization of up to 4 images that correspond to different registration channels, construction of 3-D images with registration of still-frame images at automatic stepwise shifts of the scanning plane with the controlled scanning step and the subsequent emulation of 3-D images, recording of the images in the electronic form, etc.

III. CONCLUSION

At present the JINR multimodal optical platform for Raman and CARS microspectroscopy and microscopy has passed its testing period and is ready for full-scale operation, including prospects for CARS imaging of biosamples.

IV. ACKNOWLEDGMENTS

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