Raman, CARS and SONICC spectroscopy and imaging of biomolecules.

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Introduction

A multiphoton microscopy is still a promising method to perform sophisticated studies on biological samples. Coherent anti-Stokes Raman Scattering (CARS) microscopy provides an advanced, minimally invasive (nondestructive) and label-free technique with high sensitivity and high lateral spatial resolution capable of selective chemical imaging of major types of macromolecules: proteins, lipids, nucleic acids, etc. Like spontaneous Raman, CARS probes vibrational modes in molecules and does not require exogenous dyes or markers, which is advantageous in imaging small molecules for which labeling may strongly affect their molecular properties.

Second Order Nonlinear Imaging of Chiral Crystals (SONICC) is an emerging technique for crystal imaging based on the second harmonic generation effect found in the majority of protein crystals.

The students involved in this project will get some experience in advanced methods of nonlinear optical imaging methods like CARS and SONICC realizing with high contrast and spatial resolution. Besides, they will have a good opportunity to learn and experimentally observe the advantages of nonlinear methods in comparison with spontaneous Raman spectroscopy and microscopy.

Experimental: ***Laser-scanning CARS microscope***

The multimodal optical platform (CARS microscope) for performing transmitted light, Raman, CARS and SONICC imaging is shown in Fig.1.

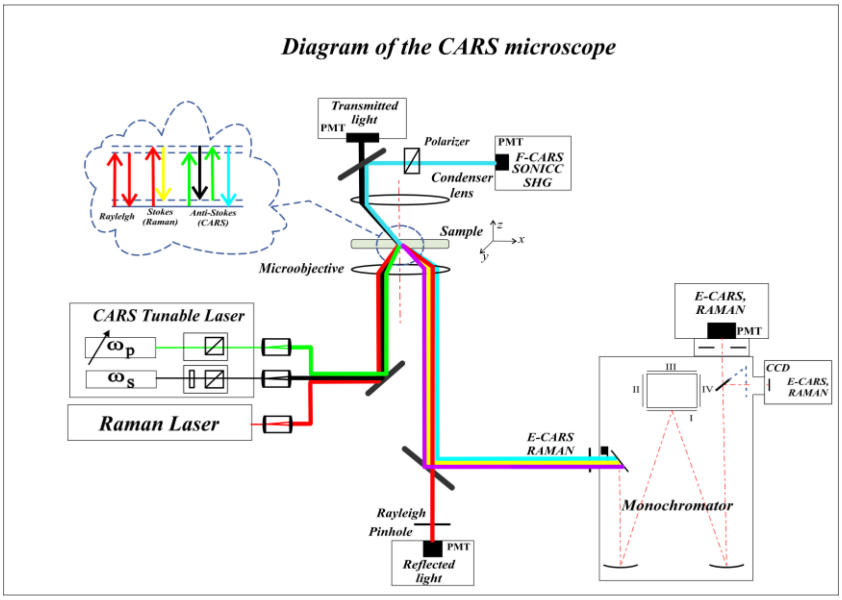

Fig. 1 The layout of the multimodal optical platform: “CARS” microscope

It’s well-known that the pulse duration of several picoseconds is a proper compromise between high intensity and narrow spectral bandwidth necessary for the CARS microscopy. Besides, its intensity is sufficient also for detection of other nonlinear processes, in particular second and sum harmonic generation. Thus, a picosecond Nd:YVO_4 tunable laser (EKSPLA, PT257-SOPO, Lithuania) with a pulse width of ~6 ps and a repetition rate of 85 MHz is used as the source of the

Stokes wave (ω_s) and is simultaneously used to synchronously pump an intracavity-doubled crystal optical parametric oscillator (SOPO). Thereby, the SOPO coherent device provides temporal synchronization with the Nd:YVO_4 and serves as a source of the pump beam (ω_p) tunable from 690nm to 990nm with a maximum output power of 300mW (Fig.2).

Fig.2 General view of the CARS microscope

Only a small portion of biologically tolerable laser power is used for CARS and SONICC imaging. The two picosecond laser beams are made coincident in time and in space utilizing an optical delay line and a series of dichroic mirrors. For CARS microscopy, we use an objective lens with a high numerical aperture to focus the beams tightly. With the tight foci, the phase-matching conditions are relaxed because of the large cone of wave vectors of the excitation beams and the short interaction length.

The measurement procedure

The potential participants (students) will be acquainted with the whole circle of the measurement procedure consisting of: spectra calibration, laser line alignment, choice of the optical filters and microobjective, sample preparation, scan and Raman mapping of the samples. An example of Raman and CARS imaging of bacteriorhodopcine (BR) crystal is shown in Fig.3.

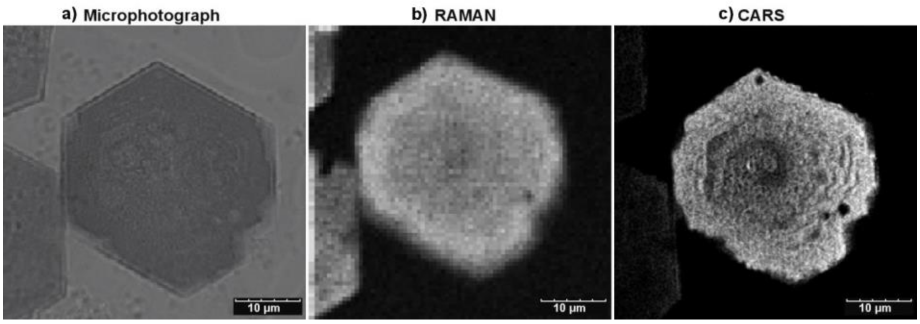

Fig.3 Visualization of bacteriorhodopcine (BR) crystals

Presentation of results

The results are supposed to be presented in the text format including introduction, obtained results, their description and discussion, as well as (desirable) in Power point format. The knowledge of ORIGIN and Power Point software packages for data processing and presentation is obligatory.

Proposed literature

J.-X. Cheng, Y. K. Jia, G. Zheng, X.S. Xie, “Laser-Scanning Coherent Anti-Stokes Raman Scattering Microscopy and Applications to Cell Biology”, Biophysical Journal **83,** 502-509 (2002).

Andreas Volkmer, “Vibrational imaging and microspectroscopies based on coherent anti-Stokes Raman scattering microscopy”, J. Phys. D: Appl. Phys. **38,** R59–R81 (2005).

L.G. Rodriguez, S.J. Lockett, G.R. Holtom, “Coherent Anti-Stokes Raman Scattering Microscopy: A Biological Review”, Cytometry Part A **69A,** 779–791 (2006).

P.J. Campagnola, and L.M. Loew, “Second-harmonic imaging microscopy for visualizing biomolecular arrays in cells, tissues and organisms”, Nat. Biotech. **21** 1356-1360 (2003).

M. Schmitt, Ch. Krafft, J. Popp, “Molekulares Imaging: Raman, CARS und TERS”, BIOspectrum **6,** 605-607 (2008).

Number of students: 1

The project supervisor

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