

Holographic and Coherent Anti-Stokes Raman Scattering Microscopy

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Abstract. The objective of the research is to combine holographic and super resolved coherent anti-Stokes Raman scattering (CARS) microscopy with diode lasers for 3D visualization of biological objects at cellular and sub-cellular levels. Experimental results for parallel holographic recording of fixed cells with detection of the CARS signals from different groups at molecular level - (S-H), (C-H) and (=CH) in them are obtained [1]. Intensity of the detected CARS signals from different groups is in 5 till 6 orders of magnitudes higher than the expected one [1]. This phenomenon is similar to the published results for the so called “surface enhanced CARS”, explained with the additional population of the first excited vibrational level, due to the introduced clusters of silver or gold nanoparticles into the objects [2]. In our case the results are obtained without nanoparticles into the objects that is essential for investigation of living cells in their natural environments. The goals of the next stage of the study is to record 2D images from the CARS signals, to obtain transfer function - point spread function (PSF) of optical system, and to create super resolved 3D images of the objects, similarly to the approach in fluorescent microscopy (Nobel price for 2014) [3-5]. The advantages of CARS microscopy are in the markers free labeling and in illumination with the safe for living cells wave lengths in the red and NIR spectral regions. Phase information obtained by parallel holographic recording of the same objects will be used as an additional degree of freedom, and to improve axial resolution, which is 2 – 3 times less than the lateral one in fluorescent microscopy. Super resolved 3D imaging of living cells could be expected. As the intensities of the CARS signals are about hundred nanowatts, for recording of 2D images of living cells new generation “single photon sensitivity” electron multiplying camera (EM CCD) has to be used (Princeton Instruments or Oxford Instruments).

References

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