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Determination of spatial resolution of nonlinear laser scanning microscopy

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Microscope resolution is the shortest distance between two points on a sample that can be distinguished as separate entities. Due to the wave nature of light and the phenomenon of diffraction, it is fundamentally limited: even under theoretically ideal conditions and optical components, the microscope has a finite resolution.

In this paper, we determined lateral and axial resolution of a nonlinear laser scanning microscope by measuring its point spread function (PSF) in two ways: by imaging fluorescent beads using two-photon excited fluorescence (standard method), and by using monolayers of molybdenum disulfide – MoS_2 (non-standard method), obtained by chemical vapor deposition [1], which, due to the lack of central symmetry, efficiently generate second harmonic signal.

Parameters such as the numerical aperture of the objective and the excitation wavelength contribute to the resolution, so it changes depending on the current setting of the microscopic system. Measurements were performed for two different objectives and several standard excitation wavelengths, depending on the type of sample. As expected, the best resolution was obtained for the objective with the largest numerical aperture (40x 1.3) and the shortest excitation wavelength (730nm): $R_{lat} = 260nm$, $R_{ax} = 1648nm$. In addition, the values obtained by the non-standard method are closer to the theoretical values of the resolution, because the contributions of the out-of-focus signal are significantly smaller due to the two-dimensional nature of the layers. This implies that it is better to use this type of sample to determine the resolution of the microscope. The measured PSF can be further used to deconvolve the images obtained on this microscope.

Due to its properties such as large penetration depth of incident radiation and label-free imaging, as well as the possibility of obtaining 3D models, our microscope is widely used in examination of the samples of biological origin, such as: erythrocytes [2], chitinous structures [3], human colon tissue [4], collagen and dentin.

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