Balkan Physical Union

BPU11 CONGRESS

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Determination of spatial resolution of nonlinear laser scanning microscopy

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Introduction

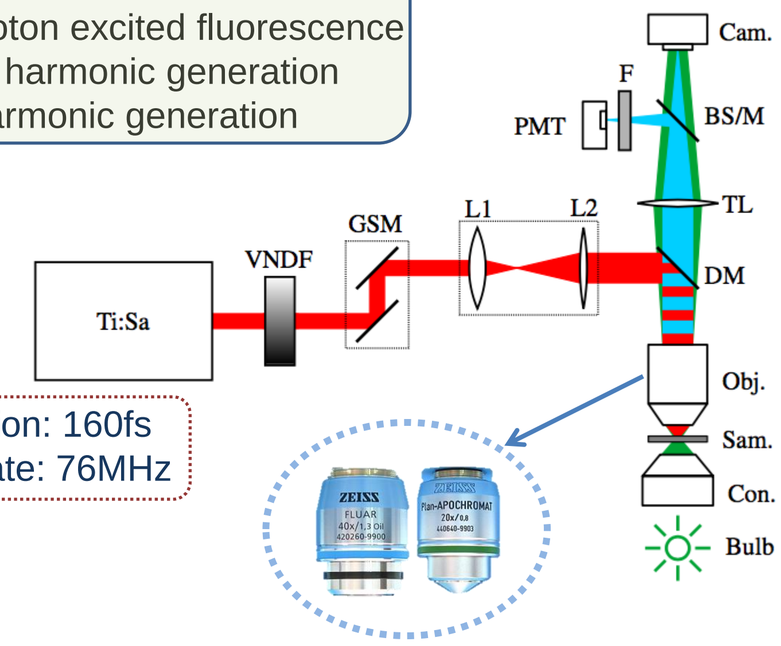
Microscope resolution is the shortest distance between two points on a sample that can be distinguished as separate entities. Due to the wave nature of light and the phenomenon of diffraction, it is fundamentally limited: even under theoretically ideal conditions and optical components, the microscope 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 (non-standard method), obtained by chemical vapor deposition, 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.

NLSM set-up

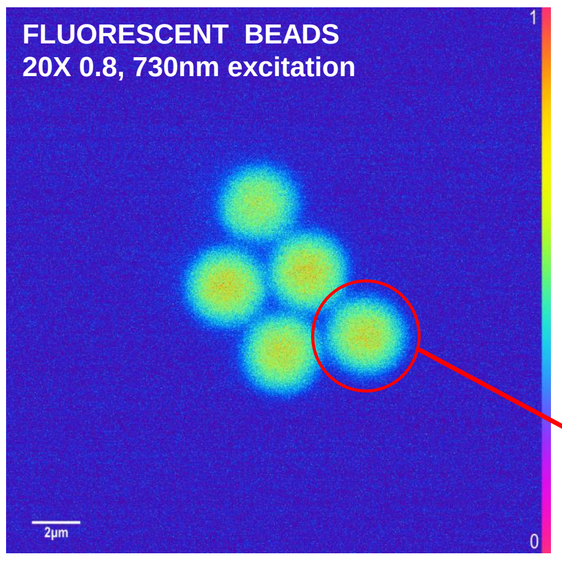
3 modalities:
- Two photon excited fluorescence
- Second harmonic generation
- Third harmonic generation

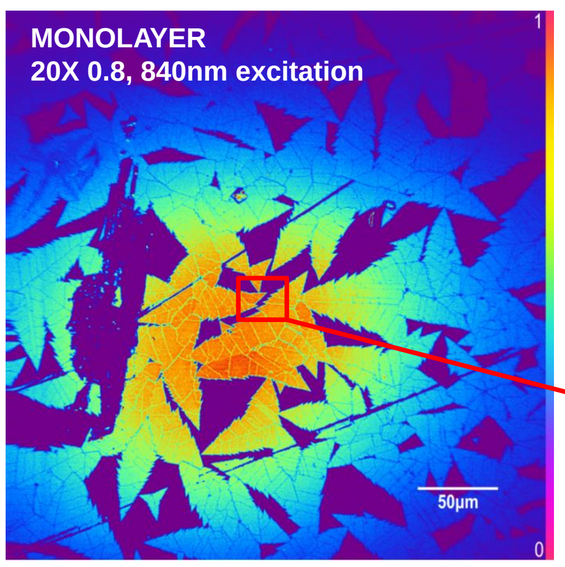


pusle duration: 160fs
repetition rate: 76MHz

VNDF - varial neutral density filter, GSM - galvo scanning mirrors, L1, L2 - 1:3.75 beam expanding lenses, DM - main dichroic mirror (shortpass), TL - tube lense, BS/M – beam splitter or mirror, F - bandpass filter, PMT - photomultiplier detector, Con - aspheric condenser lens, Cam, Sam, Obj - camera, sample, objective

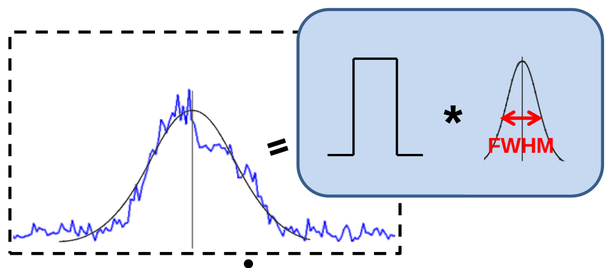
Analysis

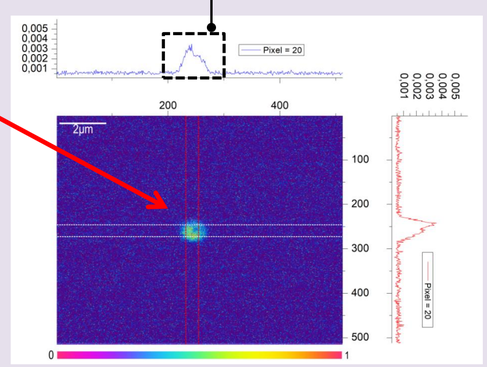




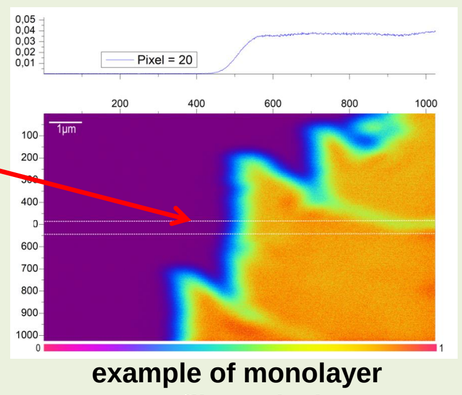
obtained profile is convolution of theoretical bead function and system's psf

Resolution = FWHM of psf





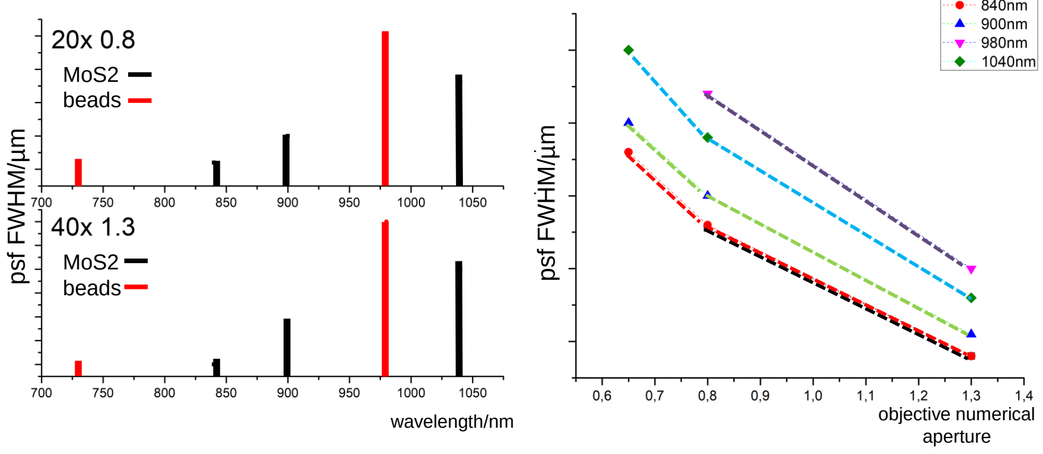
example of bead profile analysis with horizontal and vertical profiles



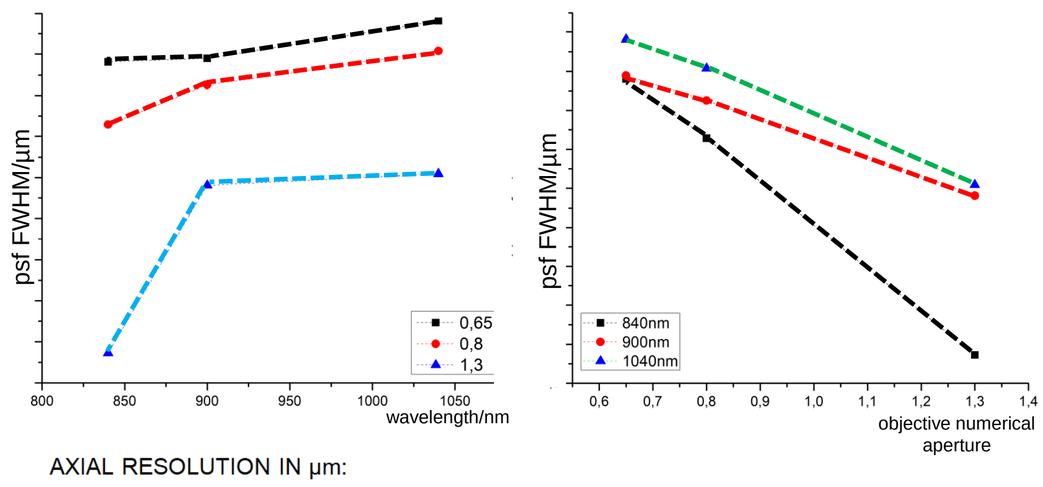
example of monolayer profile analysis

Results

LATERAL RESOLUTION



AXIAL RESOLUTION



AXIAL RESOLUTION IN μm:

	40x 0.6	20x 0.8	40x1.3
840nm	3.362	3.057	1.945
900nm	3.379	3.251	2.763
1040nm	3.562	3.415	2.818

LATERAL RESOLUTION IN μm – MONOLAYERS:

	40x 0.6	20x 0.8	40x1.3
840nm	0.560	0.460	0.280
900nm	0.600	0.500	0.310
1040nm	0.700	0.580	0.360

LATERAL RESOLUTION IN μm – BEADS:

	20x 0.8	40x1.3
730nm	0.450	0.250
980nm	0.630	0.400

Conclusions

- obtained resolution values are close to theoretical limits, which implies that our set-up is well-aligned
- the best resolution was, as expected, obtained for the objective with the largest numerical aperture and the shortest wavelength
- for the nonstandard method, the out-of-focus signal is significantly smaller due to the 2D nature of the monolayer, and there is no photobleaching when using SHG imaging
- measured PSF can be further used to deconvolve the obtained images

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