

SATURATED STRUCTURED SUBTRACTION MICROSCOPY (S³M) WITH THEORETICALLY UNLIMITED RESOLUTION

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Keywords: Point Spread Function Engineering, Fluorescence, Subtraction Microscopy

Recent developments (confocal, theta, SPIM, 4Pi, patterned excitation microscopy) have permitted to overcome the conventional resolution limits. The better resolution is obtained by improving the illumination or the detection process or both, but fundamentally, these instruments are still diffraction limited. In order to further improve the resolution, non-linear phenomena have to be involved. With fluorescence saturation combined with wide-field structured illumination, particles as small as 50 nm have been imaged [1].

We propose an alternate method, which combines subtraction microscopy [2] with a structured excitation from an azimuthally polarized excitation beam, and permits, in the linear regime, to already double the resolution compared to a wide field microscope. The advantage with respect to wide field structured illumination [3] is that only two images are required.

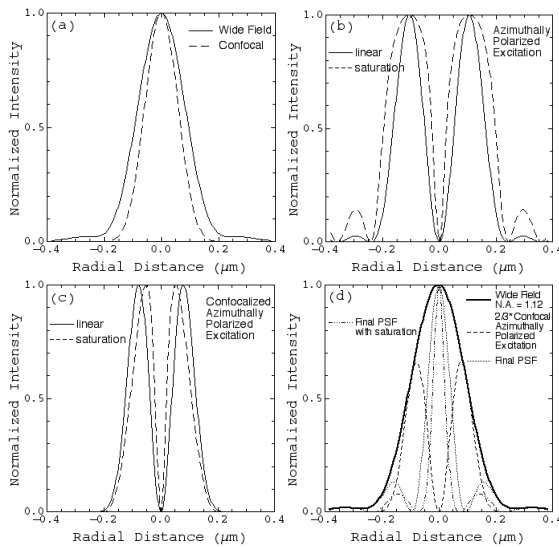


Fig. 1: (a): wide-field PSF_{wf} and confocal PSF for randomly polarized illumination (b): excitation PSF_{ill-az} for azimuthally polarized illumination. (c): confocal hollow $PSF_{conf-az}$. (d): subtraction microscopy PSF.

Figure 1 displays PSF_{wf} for wide field microscopy, PSF_{conf} for confocal microscopy, the illumination PSF_{ill-az} with an azimuthally polarized laser beam, the confocal $PSF_{conf-az}$, obtained by multiplying the former by the latter, and the final subtraction microscopy PSF_{sub-az} . For these computations, we consider a NA=1.4 oil immersion objective, an excitation performed at 400nm, and a detection at 450nm. The final PSF_{sub-az} has a FWHM of 90nm only, to be compared with the theoretical lateral resolution of 190nm for the wide field microscope (this value is larger than the usual $R_{Abbe}=\lambda/(2NA)=161nm$, which is derived from a scalar diffraction model, and overestimates the resolution). For the ideal usual confocal microscope, a 130nm resolution is predicted.

When combined with fluorescence saturation [4], this saturation structured subtraction microscopy (S³M) is characterized by a theoretically unlimited resolution, as for example STED [5] or nonlinear wide-field structured-illumination microscopy [1,4]. Saturation reduces the width of the central hole of the illumination $PSF_{exc-az-sat}$ as well as that of the structured confocal $PSF_{conf-az}$ (Fig. 1(b,c)), which is subtracted from the wide field PSF_{wf} , leaving a narrower peak for the final $PSF_{sub-az-sat}$ (Fig. 1(d)). In that case, a resolution of 45nm is predicted with a saturation level of 5/6 as in Ref. [4]. The advantage would be that no fast instrumentation is needed, and still only two images are required.

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