

IMAGE FORMATION IN IMAGE SCANNING MICROSCOPY

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Image scanning microscopy (ISM) is a variation of confocal microscopy in which the confocal pinhole is replaced by a detector array.^{1,2} The simplest way to reconstruct the image is using the principle of pixel reassignment, where the signal from each detector element is reassigned to an effective imaging point, different from the illumination point.^{1,3} After summation over the detector array, the result is that the signal level is equal to that of a confocal microscope with a pinhole of the same size as the detector array, but with substantially improved resolution. In practice, a small array is sufficient to achieve good overall imaging performance.⁴

Image formation in ISM is reviewed.^{1,3,5} Both ISM and confocal microscopy with finite-sized arrays/pinhole can be regarded as exhibiting fundamentally different imaging properties from a conventional imaging system. The limiting resolution behaviour of both modalities for a small array/pinhole size is the same as true confocal imaging, and the limiting behaviour of confocal microscopy with a large array is the same as scanning non-confocal imaging. On the other hand, the limiting behaviour of ISM with a large array depends on the reassignment factor.

ISM acquires a 4D image from a 2D object,⁶ which can be represented in terms of a 4D OTF. Then images for conventional, confocal, or ISM can be calculated from the 4D spatial frequency content. The 4D image can be regarded as a cross-correlation between the illuminated and detected points. Then the 4D spatial frequency response is like the spectral correlation function, related to the ambiguity function of phase-space optics.⁷

ISM can be performed in a two-photon excitation fluorescence mode, substantially improving the resolution, optical sectioning and axial resolution of two-photon fluorescence microscopy, while retaining good signal collection efficiency.

The effect of using Bessel beam illumination in ISM and confocal microscopy is also analyzed.

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