IMAGE SCANNING MICROSCOPY WITH A DOUGHNUT BEAM

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In confocal microscopy, the pinhole throws away valuable signal, limiting how small the pinhole can be, and therefore the attainable spatial resolution. In image scanning microscopy (ISM), most of the available light is detected by a detector array, and reassigned in the image to its most likely origin in the sample [1-7]. The point spread function (PSF) for a particular detector pixel is given by the product of the illumination and detection PSFs, and can be interpreted as the probability that a detected photon emanates from different sample positions. The most likely position is defined by the peak of the PSF [7]. After pixel reassignment to the peak, and summation over the pixels, we achieve the maximum possible total signal, i.e. it can be considered as a maximum likelihood restoration, performed noniteratively.

In the presence of aberrations, the signal is reassigned independently for each individual detector pixel. An extreme example of an aberration is a vortex beam, which produces a doughnut focus. The central detector pixel therefore detects no energy, but off-axis pixels do detect a significant signal. After reassignment and summation around a ring of detector pixels, the PSF is circularly symmetric, and for a wide range of ring radii approximates a Gaussian remarkably well. For a disk-shaped detector array with size of 2 Airy units (AU), the width of the PSF is 0.69 times that in a conventional microscope, even better than for a confocal microscope with a point detector (giving 0.72 times). In our model, we assume the total energy in the doughnut focus is equal to that in the conventional Airy spot: nevertheless the intensity at the peak of the PSF after reassignment and summation over 2 AU is actually 1.4 times higher than for a conventional image. Axial resolution reaches an optimum value at 1.3 AU, when the axial width of the PSF is 0.78 times that of a conventional microscope. Surprisingly, the optical sectioning strength, defined as the distance that the image intensity of a fluorescent sheet drops to one half its peak value is actually better than in ISM with Airy disk illumination. Use of a doughnut illumination also spreads out the detected signal more uniformly over the detector array, so that dynamic range is improved.

Experimental results broadly confirm our theoretical predictions. The measured resolution of doughnut ISM is improved over Airy disk ISM by a factor 1.18, probably because of the improved noise performance.

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