IMAGE SCANNING MICROSCOPY WITH SMALL DETECTOR ARRAYS

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Image scanning microscopy (ISM) is a technique of confocal microscopy, in which the confocal pinhole is replaced by a detector array [1-7]. The image is reconstructed by pixel reassignment, i.e. by reassigning the pixel value to the appropriate object coordinates, which vary for different points of the array. Alternatively, the data set with images from different detector pixels can be deconvolved using the known theoretical point spread function as a priori information. The advantage of ISM over conventional confocal microscopy is that an improvement in spatial resolution is achieved, but with greatly improved signal collection efficiency.

It is found that if the detector array is very large, the optical sectioning effect of confocal imaging disappears, and so in practice the size is limited to the central region of the focused spot in the detector plane. We explore the effects of using small number of detector pixels: an odd number 5, 9, 13 or 21, or an even number 4, 12, 16, 24. An approximate model based on assuming the pixel is a delta-function times its area is found to be useful, improving in accuracy as the number of pixels increases.

The spatial sampling rate is also an important issue. Neglecting Stokes’ shift, the reassignment is to the point midway between the illumination and detection points. If the sampling in the detector plane is equal to the sampling of the object illumination, the reconstructed image will exhibit double the sampling rate, which is appropriate for the increased spatial frequency bandwidth. So sampling of the illumination can be at conventional Nyquist rate, rather than at confocal Nyquist rate, giving a corresponding speed advantage. A similar behaviour occurs even in the presence of Stokes’ shift.

1. C. J. R. Sheppard, Optik 80, 53-54 (1988). (available on ResearchGate)