IMAGE SCANNING MICROSCOPY USING SMALL DETECTOR ARRAYS

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Image scanning microscopy is a technique of confocal microscopy in which the confocal pinhole is replaced by a detector array, and the image reconstructed, usually by the process of pixel reassignment [1-6]. The detector array collects most of the fluorescent light collected from the sample, so the signal-to-noise ratio is much improved compared with confocal microscopy with a small pinhole, while at the same time the resolution is improved compared with conventional microscopy. In previous studies it has usually been assumed that, neglecting Stokes shift, pixels should be reassigned to a point midway between the illumination and detection spots. Here it is shown that the peak intensity of the effective point spread function can be further increased by 5% by appropriate choice of the pixel reassignment factor. Further, it is shown that image scanning microscopy exhibits a superior axial resolution than a confocal microscope with a pinhole the same size as the detector array.

It has been shown previously, that Bessel beam illumination further improves the resolution of confocal microscopy [7], while for image scanning microscopy with a large array, imaging performance is degraded [5]. We therefore analyze image scanning microscopy with Bessel beam illumination together with a small array, and show that an improvement in transverse resolution by a factor of 1.78 compared with a conventional fluorescence microscope can be obtained. The peak of the point spread function can be doubled compared with that in a conventional microscope.

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