An imaging system such as a microscope can be constructed using either an imaging detector, or by scanning using a focused illumination spot. Either approach results in the same resolution, except that for fluorescence imaging if there is a Stokes shift (typically 10%) the scanning method gives a resolution correspondingly better. Various techniques have been proposed that image using both the illuminating light and the detected light. The result is a spatial frequency bandwidth equal to the sum of those for illumination and detection. With no Stokes shift the bandwidth is doubled. If there is a Stokes shift, compared with a conventional imaging detector, the bandwidth is more than doubled.

These techniques include the confocal microscope, spinning disk microscope, structured illumination, subtractive imaging, programmable array microscope and image scanning microscopy [1, 2]. Sometimes similar techniques are known by alternative names, some other terms used being pixel reassignment [3], photon reassignment, virtually structured detection, scanning patterned detection, and scanning patterned illumination.

Structured illumination has been demonstrated to give a factor of two improvement in resolution compared with conventional microscopy. However, as structured illumination requires a demodulation step, this is usually combined with image restoration, and should therefore really be compared with a deconvolved conventional image. Confocal microscopy requires no subsequent image processing, but has the disadvantage of weak collection efficiency, as a result of the physical confocal pinhole. Pseudo random illumination and detector arrays can benefit from Feligett’s multiplex advantage. Although illumination and detection are equivalent in imaging as a result of the principle of reciprocity, they are very different from the point of view of signal level for a given light exposure. Spinning disk and structured illumination can be seen to be in principle similar to each other.

Different designs of image scanning microscopy include optical implementations and multifocal illumination, to increase imaging speed. The approach can also be applied to multiphoton microscopy.

References