

Compact and fast 4Pi beam scanning microscope for imaging fixed and live cells

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Confocal microscopy is the method of choice in biological 3D-imaging, but in commonly accessible systems its axial resolution hardly exceeds 500 nm. During the last decade it has been demonstrated that the axial resolution can be substantially improved with 4Pi – microscopy.

We now report a 4Pi microscope for biological applications. This novel 4Pi-microscope has been realized as a fast beam scanning system featuring a 4Pi opto-mechanical module linked to a state-of-the-art confocal microscope. As a result, the advantages of a state-of-the-art-confocal system are retained in scanning, detection and imaging speed. Sensitive multicolor detection is accomplished through a acousto-optic beam splitting filter.

The 4Pi microscope is designed both for 1- photon and 2-photon picosecond excitation, and also enables joint coherent illumination and detection (i.e. 4Pi of type C). The superior PSF and OTF of the system enables 80 nm axial resolution in cells mounted in aqueous media. We present the optical design solution and demonstrate an up to 7-fold optical sectioning in live cells.