Zoological Studies 34, Supplement I (1995)

Resolution beyond the Diffraction Limit: 4Pi-confocal, STED, and GSD

Stefan W. Hell[1, 2], Pekka E. Hänninen[1], Martin Schrader[1, 2], Tony Wilson[2] and Erkki Soini[1]

[1] *Department of Medical Physics and Biotechnology Center, University of Turku, P.O. Box 123, 20521 Turku, Finland*

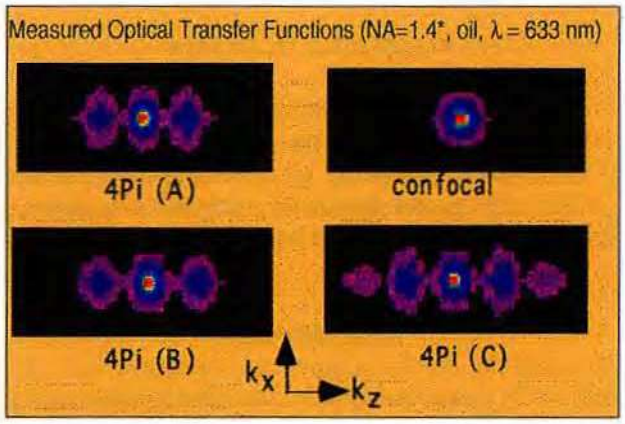
[2] *Department of Engineering Science, University of Oxford, Parks Road, Oxford OX1 3PJ, England*

In far-field light microscopy resolution is determined by diffraction. In a far-field light microscope such as the confocal scanning light microscope, the resolution is governed by the extent of the squared intensity distribution in the focal region. Precise measurements of the confocal PSF (Brakenhoff et al. 1979, Hell et al. Appl Phys Lett, 64, 1994, Schrader et al. 1995) have shown that the axial and lateral resolution of a confocal microscope (NA=1.4 oil, λ= 633 nm) is 520nm and 200nm (FWHM), respectively. At a wavelength of 375nm, this amounts to a resolution of 300 nm (axial) and 120 nm (lateral), obtainable with a confocal microscope of high aperture.

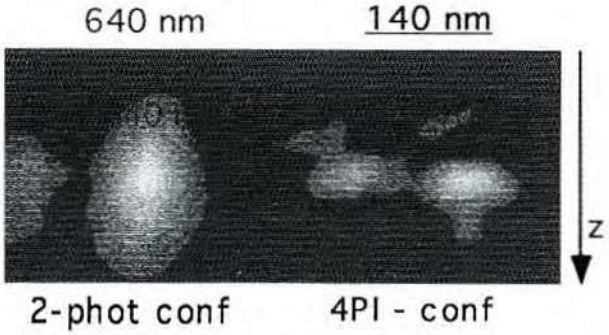
A 3-7 fold increase in axial resolution is achieved with a 4Pi-confocal microscope (Hell 1990, Hell et al. 1992). The 4Pi- confocal micro-scope uses two high numerical aperture objective lenses that are used coherently for illuminating or detecting the same point in the object space. The present paper deals with the latest develop-ments in the field of 4Pi-confocal microscopy. The Optical Transfer Functions (OTF) of 4Pi-con-focal microscopies of type A, B, and C as realised in Oxford are measured and compared with their standard confocal counterpart. The application of the three point deconvolution of Hänninen et al. (Appl Phys Lett, in press) demonstrates an axial resolution of 140 nm (4Pi type A,B and 2-photon excitation type A) of 75nm (4Pi type C) at a (com-paratively long!) wavelength of 633 nm. Furthermore we show that the increased axial bandwidth of the 4Pi-confocal OTF (Gu and Sheppard, JOSA A, 1994) can be used to determine the axial distance between two point objects with a high precision.

Furthermore we discuss STED-fluorescence microscopy which is a concept to overcome the diffraction resolution limit in lateral direction Hell and Wichmann (Opt Lett, 19, 1994). The resolution increase is based on the reduction of the lateral extent of the effective PSF in a scanning fluorescence microscope by preventing exited fluorescent molecules from undergoing spontaneous emission. This is achieved by depleting the excited molecules at the edge of the focus by stimulated emission. The physics of the lateral resolution increase as well as the depletion process is discussed and demonstrated.

An althernate but related concept of depleting the ground state of the fluorophore (Ground State Depletion Microscopy) is introduced.



140 nm axial resolution in chromosome sample at an excitation wavelength of 750 nm (!)



(Fig. Hänninen et al. Appl Phys Lett, in press)