

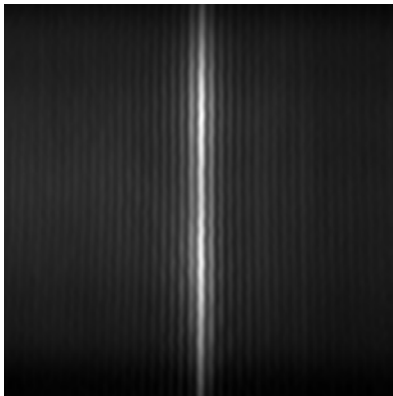
# CONFOCAL MICROSCOPY USING EXTENDED DEPTH-OF-FOCUS BEAMS

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Extended depth-of-focus beams, also known as Bessel or non-diffracting beams, have attracted considerable interest recently in a number of applications, including metrology, lithography and optical trapping. Their attraction for scanning microscopy stems from two of their properties: reduced size of the central peak which holds potential for higher lateral resolution and possibility to maintain this resolution over large depth of field due to non-diffractive property of the beam. However several problems hindered so far wider adoption of these beams in microscopy. Large sidelobes of the point spread function have detrimental effect on image quality and largely negate any improvement in resolution. More importantly, the lack of a light-efficient method of generating Bessel beams prevented their use in two-photon microscopy, where their properties could be used to most effect.

This work is further development of a technique developed in our lab [1], which uses diffractive optics to generate Bessel beams with unprecedented efficiency and improved uniformity of axial intensity distribution. The technique uses a diffractive phase mask followed by an annular aperture to form a beam with flattened axial profile and efficiency of the order of 50%. When such beam is used in conjunction with high NA microscope objective polarization properties become rather complex and careful consideration is required as to the selection of illuminating field. The best concentration of energy is achieved with the radially polarized input beam, but in this case the focused light is largely z-polarized, which may not be suitable for all imaging applications.



The improved efficiency of Bessel beam generation allowed us to achieve two-photon imaging with extended depth-of-focus within reasonable timescale. Using two-photon imaging also dramatically reduced the effect of sidelobes to the image quality. Additionally, by tilting the PSF and acquiring two images with different parallaxes we were able to produce two-photon fluorescence stereo images of thick samples without going through the process of acquiring a 3-D stack of images.

Fig.1 PSF of extended-depth-field microscope, x-z section, field of view 10x10  $\mu\text{m}$

[1] M.A.A.Neil, F.Massoumian, R.Juškaitis and T.Wilson, "Method for the generation of arbitrary complex vector wave fronts," *Opt. Lett.*, **27**, 1929-1931 (2002)