MULTICOLOR SUPER-RESOLUTION IMAGING OF SUBCELLULAR STRUCTURES BY MEANS OF A DIFFRACTION-LIMITED IMAGING SYSTEM

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Radially polarized ring shaped laser beams (RPRBs) produce a needle-like field distribution in the focus of a 1.4 NA lens, i.e. the RPRBs depth of focus is about 2 µm, while the transversal FWHM range is between 80 and 120 nm. These laser sources have been studied in detail [1]. In this work, we show the huge potential that RPRBs have in imaging of biological structures. After installing an RPRB in a standard confocal setup, multicolor images can be recorded with a super lateral resolution ranging between 80 and 130 nm. For any excitation wavelength, the image is formed by one scanned picture, does not require pinhole size reduction, any deconvolution or image post processing tool. Figure 1 compares images taken by means of a standard 633 nm Linearly Polarized Gaussian Beam (LPGB, see Figure 1 d-f) and by a 633 nm RPRB (see Figure 1 a-c). A cell was labelled for Nup153, a component of the nuclear pore complex (NPC). The single NPCs shown in Figure 1 (b,c) and in Figure 1 (e,f) are the ones isolated by the rectangles drawn in Figure 1 (a,d). These, as well as many others, show the dramatic optical resolution improvement offered by the RPRBs with respect to LPGBs. Moreover, the NPCs shown in Figure 1 (a) can be resolved with at least 130 nm accuracy (measured analyzing the FWHM of small spots underneath the line depicted in Figure 1 a). Since confocal imaging with RPDBs does not present any challenge, we believe that this new kind of diffraction-limited super-resolution imaging will have huge impact and provide a great improvement in many bio-imaging assays, especially in live imaging experiments.

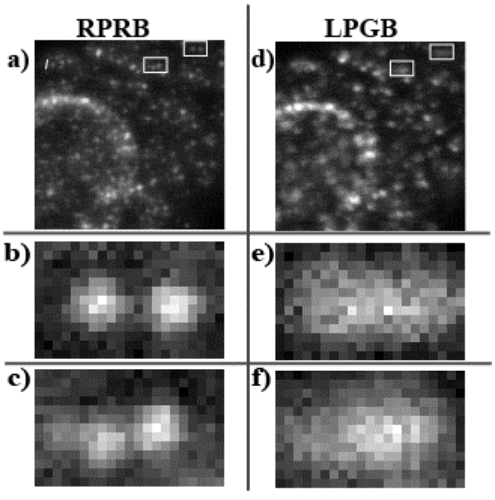

Figure 1: Images of Nup153 (immuno-labelled and stained with AlexaFluor647) taken by using a 633 nm RPRB (a-c) and by using a 633 nm LPGB (d-f).

[1] H. F. Wang, L. P. Shi, B. Lukyanchuk, C. Sheppard, and C. T. Chong, "Creation of a needle of longitudinally polarized light in vacuum using binary optics," *Nat. Photonics* **2**, 501–505 (2008).