3D OPTICAL NANOSCOPY

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Far-field fluorescence microscopy would be almost ideal for non-invasively investigating the three-dimensional (3D) interior of transparent objects in biology and material sciences if it could discern details that are substantially smaller than half the wavelength of light. However, its resolution stagnated at this limit - imposed by diffraction - for a century.

In recent years, the use of the joint aperture of two opposing objective lenses provided nearly isotropic 3D imaging by improving the axial resolution by 3- to 7-fold, and the switching of the fluorescence of adjacent markers enabled the diffraction barrier to be overcome [1].

Here we report on the progress to push the 3D-resolution isotropically below 50 nm using opposing lenses, specifically by the method of isoSTED [2], which has non-invasively visualized 3D nanostructures of polymers [3] and organelles in the interior of cells [4] that have so far required electron microscopy.

Figure 1: (a) Confocal microscopy overview of a mammalian (PtK2) cell outlining the mitochondrial network (white) and the nucleus (blue). (b) Sketch of an isoSTED nanoscope optically dissecting the interior of a mitochondrion with a spherical effective PSF. (c) isoSTED imaging at the mitochondrial equatorial plane reveals immunolabelled F1F0ATPase proteins of the inner mitochondrial membrane and the cristae as essential structural elements of this organelle. Panels from refs. 4,5.