AXIAL TOMOGRAPHY IN SINGLE CELL FLUORESCENCE MICROSCOPY

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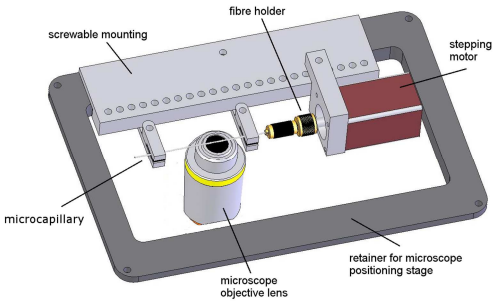
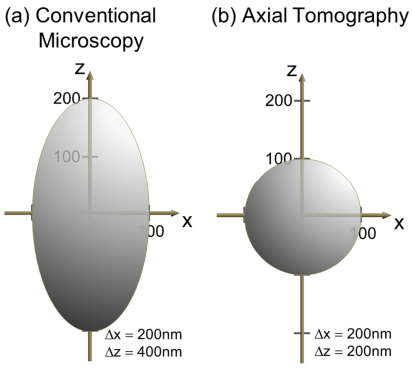
Axial tomography in combination with various techniques of 3D microscopy including confocal laser scanning microscopy, structured illumination microscopy or light sheet based fluorescence microscopy improves visualization of single living cells or small cell clusters in three dimensions.

After adaptation of an innovative device for sample rotation [1], single cells embedded in agarose and located within micro-capillaries are observed from different sides. Thus, z-stacks of 3D samples can be recorded in different directions, providing additional information, since cells or organelles which appear superimposed in one direction, may be well resolved in another one.
Furthermore, since lateral resolution is better than axial resolution by at least a factor 2 (see below: point spread functions), an improved effective 3D resolution is achieved upon sample rotation [2].
The method is tested and validated with single cells expressing a membrane or a mitochondrially associated Green Fluorescent Protein (GFP), or cells accumulating fluorescent quantum dots.
A further step towards super-resolution with non-phototoxic light doses is the combination of axial tomography with structured illumination microscopy, which at best results in doubling of resolution. Using a spatial light modulator to generate an interference pattern in the sample plane [3], live cell microscopy can be performed with a resolution slightly above 100 nm in the image plane.





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