3D parallelized RESOLFT microscopy for high resolution imaging of living cells

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Elucidating the volumetric architecture of organelles and molecules inside cells requires microscopy methods with a sufficiently high spatial resolution in all three dimensions. Current methods are limited by insufficient resolving power along the optical axis, long recording times and photobleaching when applied to live cell imaging. RESOLFT (Reversible Saturable Optical Fluorescence Transition) microscopy provides the possibility of imaging living cells at unprecedented levels of detail. From the initial point scanning implementations we have seen and participated in the development of parallelized systems for 2D and 3D resolution extension finally enabling fast volumetric studies of whole living cells (~40×40μm2) over time at sub-80 nm resolution in all spatial dimensions. The technical developments involve the creation of novel three-dimensional light patterns that together with the unique properties of reversible switchable fluorescent proteins allow for crafting of emission patterns modulated in all three dimensions. We also delve deep into the theoretical image formation model incorporating the unique behaviour of the switching fluorophores allowing for in-depth studies on the impact of different imaging and fluorophore parameters on the resulting image quality.