3D ISOTROPIC SUPER RESOLUTION IN PARALLELIZED RESOLFT MICROSCOPY

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In recent years, the development of parallelized super resolution microscopy techniques have shown impressive results combining diffraction unlimited lateral spatial resolution (~60-70 nm) with increasingly fast acquisition speeds (~ 1 second)[1]. However, the diffraction unlimited resolution improvement in these systems are always limited in one way or another to the lateral dimension. Here we present a novel design of illumination patterns that allows for 3-dimensional isotropic diffraction unlimited resolution while maintaining the high imaging speeds of a parallelized imaging system. Much like in the original MoNaLISA [1] the system is based on the RESOLFT principle of controlling the spatial distribution of ON-OFF states in a sample labelled with reversibly switchable fluorescent proteins (rsFPs). Using rsFPs as labels allows for high resolution imaging at very low light doses and is thus very suitable for live cell imaging. Designing illumination patterns that are modulated in all three spatial dimensions and combining these with the saturation of rsFP emission states lets us create emission patterns with theoretically unlimited frequency content along all three spatial dimensions. The extended frequency content in the emission pattern manifests itself as isotropic diffraction unlimited resolution in the final images.

The spatial modulation in all 3 dimensions is made possible by the incoherent superposition of several independent illumination patterns, each pattern highly modulated in one spatial direction. Proper co-alignment of these patters results in sharply confined zero intensity volumes that, together with saturation of the fluorophore OFF-state, can create diffraction unlimited emission volumes. Quantification and reassignment of the emission from these volumes allows for reconstruction of the final isotropic super resolution images.

[1] Masullo, L; Bodén, A; Pennacchietti, F: Coceano, G; Ratz, M; Testa, I. “Enhanced photon collection enables four dimensional fluorescence nanoscopy of living systems” *Nat. Com.* **Vol 9**, Article number: 3281 (2018)