STUDYING THE FAST NANOSCALE DYNAMICS OF CELL ADHESIONS USING RESOLFT NANOSCOPY

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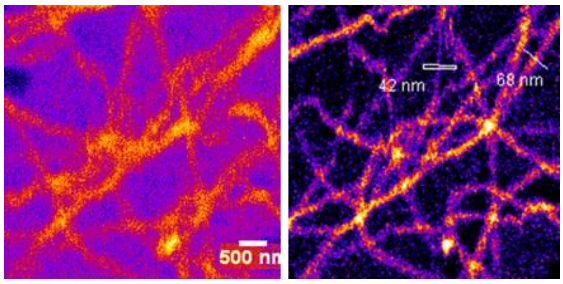
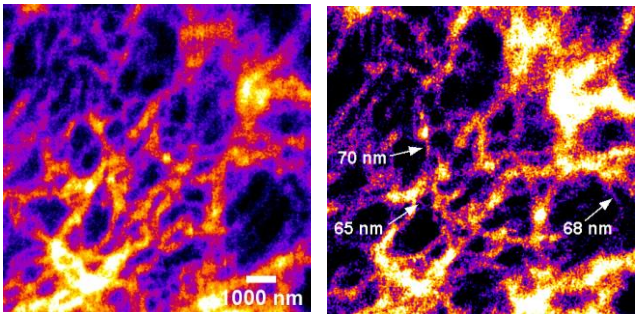
Cells adjust their adhesive and cytoskeletal organizations according to changes in the biochemical and physical nature of their surroundings. Certain critical cellular processes involved in cell shaping and migration, proceeds through cycles lasting from seconds to minutes. Super resolution imaging techniques can spatially resolve these sub-cellular structures, but they must be live cell compatible and fast enough to follow their dynamics.

RESOLFT (REversible Saturable Optical Linear Fluorescent Transitions) nanoscopy is a unique super resolution technique, as it impart only very little photon dosage (up to 4 orders of magnitude less compared to other techniques) on cells for constructing a super-resolution image [1]. This makes it suitable for long term live cell imaging. We have implemented a zero-dimensional RESOLFT which is capable of studying protein dynamics in the order of minutes.



Confocal and RESOLFT images of microtubules with tubulin rsEGFP2 using zero-dimensional RESOLFT



Widefield and RESOLFT images of U2OS cells expressing Vimentin rsEGFP2 using parallelized RESOLFT

To study faster dynamics occurring in live cells, we developed a parallelized RESOLFT. Large 2D parallelization for STED [2] and RESOLFT [3] has already been reported with optical lattices. The optical lattice for depletion was realized by interference of four beams generated by a spatial light modulator. By using a complimentary lattice for activation, we minimized the effect of photobleaching. With the parallelized scheme, we can achieve long term live cell imaging (~50 frames) at a rate of 0.4 Hz. This speed will enable us to study the fast dynamic reorganization of adhesive and cytoskeletal structures in cells.

[1] T Grotjohan, I Testa, M Reuss, T Brakemann, C Eggeling, SW Hell, S Jakobs: "*rsEGFP2 enables fast RESOLFT nanoscopy of live cells*" elife1: e00248 (2012)

[2] Y Bin, F Przybilla, M Mestre, J.B Trebbia, B Lounis: "*Large parallelization of STED nanoscopy using optical lattices*"; Optics Express, vol. 22, issue 5, p. 5581

[3] A Chmyrov, J Keller, T Grotjohann, M Ratz, E d'Este, S Jakobs, C Eggeling & S.W Hell: "*Nanoscopy with more than 100,000 'doughnuts" Nature* Methods **10**, 737–740 (2013)