# Fast Scanning RESOLFT microscopy for the investigation of live cellular processes

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The field of super resolution microscopy has lead the way for many great discoveries in the last decade. However many of the techniques requires high laser powers or other harsh conditions that make live-cell and time-lapse imaging difficult to perform. RESOLFT microscopy is a super resolution technique that requires only a fraction of the light of for instance STED microscopy, and it does not have any requirement of chemicals that could be toxic to the cells. RESOLFT is based on reversible switchable fluorescence proteins (rsFP), and a three phase pulsing scheme ensure the super resolution: (i) switching the rsFP into a ON, potentially fluorescent state; (ii) switching the rsFP in a dark OFF state with a light pattern featuring a "zero" or multiple "zeros" of intensities (donut or line shaped); (iii) probing by fluorescence the residual rsFPs still in the ON state with a third light pattern[1].

RESOLFT offers many advantages for investigation of molecular process in living cells and tissue [2], however one caveat, especially in the point-scanning mode, is the slow acquisition speed due to the three sequential steps required, with often the off switching being the time delimiting steps. Recent years development in the rsFP, have resulted in fairly fast switching kinetics, and for instance rsEGFP2 and DronpaM159T [3] have off switching in the µs range, however even a faster switcher would require a recording time of about a minute to acquire a 10 by 10 µm field of view.

In this work we present a new and faster scan method that decrease the overall frame time of several factors. In a nutshell, instead of scanning the entire frame, we only scan structures of interest inside the frame. The illumination beam spends a very short time to sample the pixel before it is actually recorded. During this time a quick decision is taken to "record and RESOLFT" or not the pixel. This means that only the fluorescence structures are being RESOLFT imaged and not the dark background. Typically in fluorescence microscopy most of the sample are sparse in space, namely many pixels do not contain information. As a matter of fact, this technique can be used to greatly improve the temporal resolution of point-scanning RESOLFT microscopy.

[1] Rego EH, Shao L, Macklin JJ, Winoto L, Johansson GA, Kamps-Hughes N, Davidson MW, Gustafsson MG, "Nonlinear structured-illumination microscopy with a photoswitchable protein reveals cellular structures at 50-nm resolution", *Proc Natl Acad Sci*. 2012 Jan 17;109(3)
[2] I. Testa; N. T. Urban; S. Jakobs; C. Eggeling; K. I. Willig; S. W. Hell: "Nanoscopy of Living Brain Slices with Low Light Levels" *Neuron* 75, 992 – 1000 (2012).
[3] T. Grotjohann, I. Testa, M. Reuss, T. Brakemann, C. Eggeling, S. W. Hell, and S. Jakobs, "rsEGFP2 enables fast RESOLFT nanoscopy of living cells." *eLife* 1, e00248 (1 (2012).