PUSHING THE RESOLUTION OF PARALLELIZED RESOLFT LIVE-CELL IMAGING WITH PHOTOSWITCHABLE FLUORESCENT PROTEIN DREIKLANG

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Today, point-scanning approaches in superresolution microscopy like STED and RESOLFT are very popular. STED nanoscopy has been used for more than 10 years and demonstrated impressive resolution up to 20 nm in biological specimen and wide applicability including living organism. The first demonstration of RESOLFT was done in 2005 \cite{1}, but only recently with development of new reversibly switchable fluorescent proteins (RSFPs) \cite{2,3} it has revealed its full potential for low light level long-term super-resolution imaging.

Fundamental improvements in imaging speed has been realized by parallelization of RESOLFT \cite{4} and more recently by STED with incoherently crossed standing wave (ICS) pattern. The switching process in STED is very fast, albeit it inherently requires high irradiances (MW/cm\textsuperscript{2}) since stimulated emission depletion beam has to act on a very short-lived (ns) excited state of a fluorophore. Limited by the available power levels of STED lasers, current implementations of parallelized STED support 2000 donuts at a time. The genuine advantage of RESOLFT is the requirement of only very low light levels (W/cm\textsuperscript{2}) because it uses states with much higher lifetimes (ms-s). This allows to stretch off-switching photons in time and makes implementations of more than 100,000 donuts at a time possible with RESOLFT.

Yet the resolution of RESOLFT lags behind STED, since the number of detectable photons per cycle was limited for utilized RSFPs. Here, we applied to parallel RESOLFT microscopy the novel protein Dreiklang \cite{3}, that belongs to a new class of RSFPs, where switching is decoupled from read-out. Thus, the total number of photons per cycle is significantly improved. Consequently the resolution of Dreiklang RESOLFT images was improved to better than 60nm in the parallel scheme. Furthermore the parallel scheme enables a lower requirement of switching cycles since the light pattern in ICS requires less imaging steps compared to scanning a single beam. This favors the use of Dreiklang with comparably low number of switching cycles of ~200 in the parallel scheme.