SMART RESOLFT nanoscopy to lower photo dosage and acquisition time

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The field of super resolution has lead the way for many great discoveries in the last few decades. However many of the techniques requires high laser powers or other harsh condition that make live cell and time-lapse imaging difficult to perform.

RESOLFT is a super resolution technique that requires only relatively low light dosage, and it does work without special medium that could be toxic to the cells. RESOLFT is based on reversible switchable fluorescence proteins (rsFP). In a typical point-scanning architecture (1) a first light pattern switches the rsFP into an ON (fluorescent state); (2) a second light pattern featuring a "zero" intensity point switches the rsFP in a dark OFF state, (3) a third light pattern probes by fluorescence the residual rsFPs still in the ON state [1] The iteration of this pulsing scheme for every pixel ensure the super resolution.

RESOLFT nanoscopy has been implemented in various optical schemes leading to super resolution images of living cells and tissues and even with multi-channels ability [2,3], however the 3 sequential steps have long been thought of as a caveat that limited RESOLFT speed of acquisition, as it results in a complete pixel/cycling times from 300μs to 10 ms.

In this work we are exploring the fluorescence emitted along the three sequential steps to great advantage in order to minimize the frame time and the light dosage. These novel features are made possible by a real time feedback loop based on FPGA and the inherent properties of the rsFPs. In short, each pixel/voxel is probed by a short light illumination, to detect the presence or absence of fluorescence protein/structures. After which a decision is made to either skip the pixel or fully measure it by going through the complete RESOLFT switching cycle consisting of OFF switching and readout illuminations. Through all of these steps the emitted photons are collected, stored and processed. This signal originated by a population of proteins in continuous evolution across time is combined into the final super resolved image, which results in a higher SNR and, importantly, acquired much faster than in conventional RESOLFT imaging.

We applied our smart RESOLFT nanoscopes to acquire super resolution imaging data of various biological samples, ranging from cell cultures, cultured neurons and hole organisms (C. *Elegans*). Demonstrating the robustness of the technique, its ability to perform deep inside tissue, and show a record breaking 4 Hz dynamic measurement for point scanning RESOLFT.

[1] Grotjohann, T.; Testa, I.; Leutenegger, M.; Bock, H.; Urban, N. T.; Lavoie-Cardinal, F.; Willig, K. I.; Eggeling, C.; Jakobs, S.; Hell, S. W. "Diffraction-unlimited all-optical imaging and writing with a photochromic GFP" *Nature,* **478**, 204−208 (2011)
[2] Testa; I., Urban; N. T., Jakobs; S., Eggeling; C., Willig; K. I., Hell, S. W. " Nanoscopy of living brain slices with low light levels", *Neuron*, **75**, 992 – 1000 (2012).
[3] Testa; I., D'Este, E., Urban; N. T., Balzarotti; S., Hell, S. W. " Dual Channel RESOLFT Nanoscopy by Using Fluorescent State Kinetics", *Nano letters*, **15**, 103-106 (2015)