ENHANCED PHOTON COLLECTION ENABLES FOUR-DIMENSIONAL FLUORESCENCE NANOSCOPY OF LIVING SYSTEMS

Francesca Pennacchietti[1], Andreas Boden[1], Luciano Masullo[1], Ilaria Testa[1]

[1] KTH Royal Institute of Technology/Science for Life Laboratory, Tomtebodavägen 23A, 17165 Stockholm, Sweden

E-mail: francesca.pennacchietti@scilifelab.se

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The observation of the dynamics of the organelles as well as the interaction of specific macromolecular complexes inside living cells and tissues requires the continuous development of minimally invasive optical systems able to achieve high spatio-temporal resolution. Although, the theoretically unlimited spatial resolution of fluorescence nanoscopy often comes at the expense of time, contrast and increased dose of energy for recording.

We developed a gentle fluorescent nanoscope based on the RESOLFT (REversible Saturable-Switchable OpticaL Fluorescent Transition) concept, capable of imaging structures at a scale of 45–65 nm within the entire cell volume at low light intensities (W–kW/cm^2). Our approach, named MoNaLISA, for Molecular Nanoscale Live Imaging with Sectioning Ability, is based on thousands of focal spots which switch and read-out the fluorescence signal emitted by reversible switchable fluorescent proteins simultaneously. The three-step imaging scheme is achieved by three distinctly modulated illumination patterns crafted and combined in an optimized way to gain fluorescence ON-OFF switching cycles and image contrast. By maximizing the detected photon flux, MoNaLISA enables prolonged (40–50 frames) and large (50 x 50 μm^2) recordings at 0.3–1.3 Hz with enhanced optical sectioning ability. We demonstrated the general use of our approach by 4D imaging of organelles and fine structures in epithelial human cells, colonies of mouse embryonic stem cells, brain cells, and organotypic tissues.