Tag-free labeling of tubulin in live cells with fluorescent organic dyes

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T. Schvartz[I], N. Alush[II], E. Arbely[I], **N. Elia**[III]

[I]Ben Gurion University, Beer Sheva, Israel, [II]Ben Gurion Umiversity, Beer Sheva, Israel, [III]Ben Gurion University of the Negev, Beer Sheva, Israel

High-resolution fluorescence imaging, combined with labeling techniques available today have yet been able to provide the spatiotemporal resolution needed to record all cellular processes in live cells. Substituting the bulky fluorescent protein tags (such as GFP) currently used in live-cell applications with much smaller fluorescent dyes that possess superior photophysical characteristics can potentially improve both temporal and spatial resolutions in these advanced imaging techniques. Genetic code expansion and bioorthogonal labeling offer, for the first time, a non-invasive way to specifically and directly attach fluorescent dyes to proteins in live cells. Here we employ this strategy to directly tag α-tubulin in live mammalian cells with silicon-rhodamin (SiR). By screening different conditions we have optimized the system for quantitative high-resolution light microscopy studies of microtubules. We will present data demonstrating the feasibility and efficiency of the approach and will discuss the advantages and limitations of using genetic code expansion for quantitative high-resolution microscopy.