Reversibly photoswitchable fluorescent proteins for RESOLFT live-cell nanoscopy

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All super-resolution microscopy (nanoscopy) concepts that fundamentally overcome the diffraction barrier in far-field optical microscopy utilize fluorophore transitions between two states, typically a fluorescent 'on'- and a non-fluorescent 'off'-state. In RESOLFT (reversible saturable optical fluorescence transition) and the related STED (stimulated emission depletion) microscopy concepts, a doughnut or a pattern is scanned across the sample, determining at any point in time the nanosized coordinate range where the fluorophores are in the 'on'-state.
RESOLFT super-resolution microscopy utilizes reversibly switchable fluorescent proteins (RSFPs) that can be repeatedly photoswitched between fluorescent and non-fluorescent states by irradiation with distinct light wavelengths to overcome the diffraction barrier. RESOLFT nanoscopy is particularly suited for live cell imaging because it requires relatively low light levels to overcome the diffraction barrier. Early RSFPs allowed only a limited number of switching cycles before photodestruction. We have generated a number of new and improved RSFPs with different photophysical properties that exhibit strong resistance against photobleaching and switching fatigue. These very photostable RSFPs allow live cell imaging of tissues and of cells in intact animals such as *Drosophila melanogaster* larvae. Recent progress in live-cell RESOLFT nanoscopy will be discussed.

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