ADVANCES IN STIMULATED EMISSION DEPLETION MICROSCOPY

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Stimulated Emission Depletion (STED) Microscopy overcomes the diffraction limit of light microscopy by spatially targeted quenching of fluorescence emission, usually in a ring pattern centered on the excitation focus of a laser scanning microscope. Since the first introduction of the principle by Stefan W. Hell\textsuperscript{[1]}, the technique has seen remarkable progress and now routinely generates biological images at tens of nanometers resolution\textsuperscript{[2]}.

We will present our latest results in the development and application of STED microscopy achieved at Yale. Specifically, a recently introduced novel far-red fluorescent protein\textsuperscript{[3]} suitable for live cell STED microscopy using \textasciitilde750 nm depletion wavelength and advances in live-cell super-resolution imaging with a commercial 2-color Leica TCS STED microscope will be discussed. Furthermore, we will present our latest progress in the construction of a custom-built STED microscope (Fig. 1d-f).

Figure 1: (a-c) A mammalian cell expressing EB3-TagRFP657 which labels microtubules was imaged with a Leica STED microscope. (b) shows the area denoted by the white box in (a) in a magnified view. (c) shows a profile across the tubule as indicated by the white box in (b) and demonstrates that sub-100 nm resolution is achievable with TagRFP657. (d-f) 20-nm crimson beads imaged in confocal (d) and STED (e) mode with our custom-built setup. (f) shows the lateral profile through a bead demonstrating approx. 26 nm resolution.