

Time-resolved fluorescence microscopy with sub-diffraction limited resolution via stimulated emission depletion (STED)

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Fluorescence microscopy has been widely applied in numerous disciplines, especially in biology due to its noninvasiveness, specificity, and sensitivity for *in vivo* imaging. However, the spatial resolution of the far-field light microscopy was limited by diffraction, as predicted by Abbe's theory over a century ago. In the laser scanning microscopy, the resolution is determined by the size of the diffraction limited focus spot. The proposed STED microscopy [1] utilizes the shaped point spread function (PSF) to scan the sample in the focal plane. The effectively shaped PSF can then overcome the diffraction barrier in far-field microscopy.

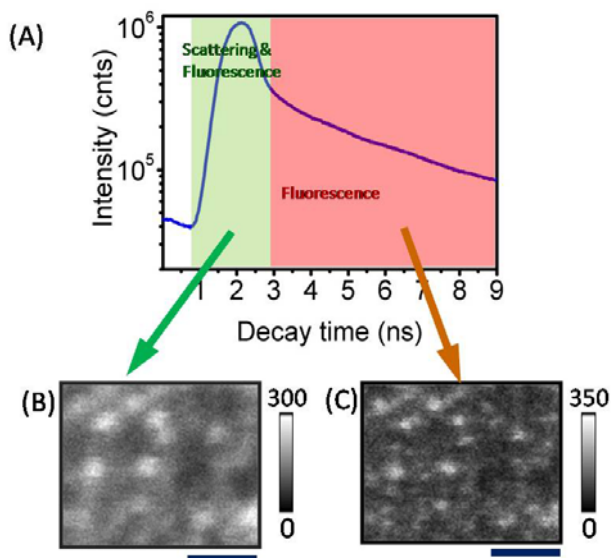


Figure 1: (A) fluorescence decay curve of crimson bead. Time-gated fluorescence image reconstructed from these photon in (B) IRF region and (C) arriving later. Scale bar: 1 μm .

respectively. In figure 1 (B), the image reconstructed from these photons in IRF region was similar to the confocal image. These photons delivered no super-resolution information may stem from the transition occurred before STED pulse arriving. The background photons will also destroy the resolution. Only the photons emitted later can contribute to super-resolution images, as shown in figure 1 (C). The resolution about 100 nm was demonstrated.

Reference:

[1]S. W. Hell and J. Wichmann, "Breaking the diffraction resolution limit by stimulated emission: stimulated emission depletion microscopy", *Opt. Lett.* 19, 780-782 (1994).

In this work, we are demonstrating time-resolved fluorescence microscopy with sub-diffraction limit resolution via stimulated emission depletion (STED). Through the use of time-correlated single photon counting (TCSPC) and high repetition rate pulsed laser, it becomes rather straightforward to integrate STED into fluorescence lifetime imaging microscopy, though pulsed irradiation is not a prerequisite in STED microscopy. As expected, high spatial resolution is achieved by shaping the point spread function of excitation with the STED beam. Figure 1 (A) shows the fluorescence decay curve under STED beam illumination. One can see that the fluorescence was quenched after IRF peak. The decay curve still retains the same lifetime with normal decay curve. Furthermore, these photons were divided into two groups, early and later arriving photons, and contributed to the images shown in figure 1 (B) and (C),