

Ratiometric photon assignment to improve resolution in pulse STED microscopy

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1. INTRODUCTION

Lower-peak-intensity depletion STED beam with longer pulse duration (~600ps) has been recently applied to reduce the photobleaching in pulse-STED implementation, and the resulting decreased resolution can be recovered by time-gated detection[1]. Ratiometric photon assignment based on lifetime in our STED implementation can keep the long lifetime photons emitted from fluorophores located in the focal point and abandon short lifetime photons in the periphery, achieving the resolution improvement without significantly decreasing the SNR.

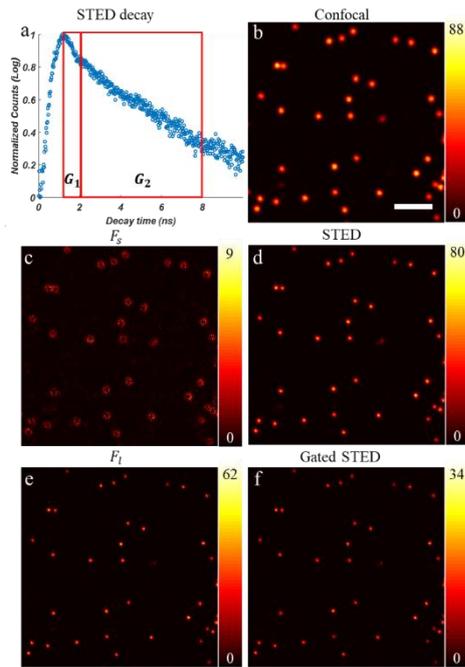


Fig.1 Ratiometric photon assignment to improve resolution. Scale bar, 2 μ m.

2. METHODS AND RESULTS

In Fig.1(a), Photons detected from two gates (G_1 and G_2) can be regarded as the linear combination of the photons with long lifetime (emitted after depletion window, F_l) and short lifetime (emitted during depletion window, F_s),

$$\begin{bmatrix} G_1 \\ G_2 \end{bmatrix} = \mathbf{M} \begin{bmatrix} F_l \\ F_s \end{bmatrix} = \begin{bmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{bmatrix} \begin{bmatrix} F_l \\ F_s \end{bmatrix}. \quad (1)$$

Once the fraction matrix \mathbf{M} is determined, detected photons can be assigned to F_l and F_s through the inverse operation of Eq. 1. Therefore, photons with short lifetime in the periphery can be rejected (Fig.1(c)), achieving higher resolution (shown in Fig.1(e)) compared to normal STED image (Fig.1(d)) and higher SNR compared to traditional time-gated STED image (Fig.1(f)). Meanwhile, compared with pSTED-SPLIT[2], our proposed method is less complexity in system implementation and data processing as there is no need of lifetime measurement. Only a fanout buffer is needed to copy the detected signals so that photons in G_1 and G_2 can be collected simultaneously to achieve resolution improvement in pulse STED microscopy.

[1] M. Castello, G. Tortarolo, et al. "Gated-STED microscopy with subnanosecond pulsed fiber laser for reducing photobleaching," *Microsc. Res. Tech.*, **79**, 785–791(2016).

[2] G. Tortarolo, et al. "Photon-separation to enhance the spatial resolution of pulsed STED microscopy." *Nanoscale*, **11.4**, 1754-1761(2019).