gates. Since this factor applies for all locations, it will not affect the spatial extent of the effective PSF of the microscope. The $I(r)$ terms in both the prefactor and the exponent, on the other hand, will improve the spatial resolution.

Figure 4b illustrates the effect of time-gating with a CW STED beam on the effective PSF of the microscope. When a CW STED beam is applied without time-gating, the width of the effective PSF drops; however, the peak amplitude of the PSF—a measure of the probability of detecting emitted photons—remains constant. Thus, the brightness of a point-emitter will be unchanged. However, when a time-gate is applied, both the width and the maximum amplitude of the effective PSF decrease; thus, a point emitter will appear both sharper and dimmer. To better illustrate the decrease in the spatial extent of the PSF, Fig. 4c plots these same PSFs but normalized to the maximum amplitude. Figures 4d and 4e quantify the decrease in the FWHM with different CW-STED parameters and reveal that increasing either the STED strength or the ratio of the time-gate to the fluorophore lifetime increases resolution.

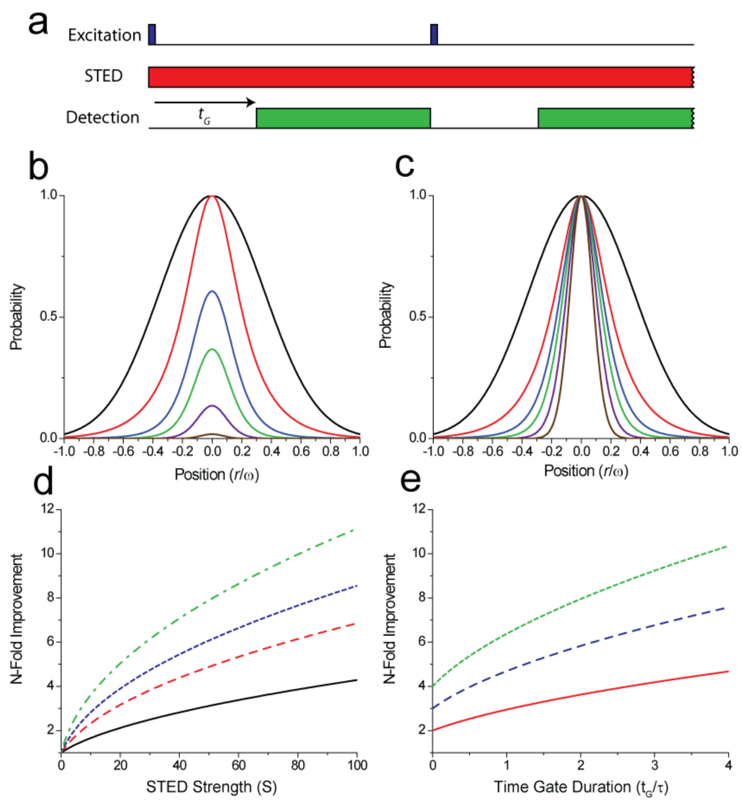

Fig. 4. CW T-STED. (a) Timing diagram for time-gating with a CW STED beam. (b) The effective PSF for different CW T-STED parameters. Plotted are the confocal PSF (black); the effective PSF with a CW STED beam with STED power sufficient to reduce the FWHM to 1/2 that of the confocal PSF (red; $S = \sigma\gamma\omega^2\tau = 16.5$); the same STED beam with $t_G = 0.5\tau$ (blue); $t_G = \tau$ (green); $t_G = 2\tau$ (purple); and $t_G = 4\tau$ (brown). (c) The effective PSF corrected for the decrease in image brightness. The color scheme is the same as in panel (b). (d) The N-fold improvement in the FWHM of the effective PSF with respect to the confocal PSF versus strength of the STED beam. $t_G = 0,1,2,4\tau$ are plotted as solid black, dashed red, dashed blue, and dashed green respectively. (e) The N-fold improvement in the FWHM due to CW T-STED as compared to the confocal PSF versus time-gate duration for different STED beam strengths. Red, blue, and green correspond to STED strengths that decrease the FWHM by 2, 3, and 4-fold in the absence of a time-gate. All distances are measured in units of the 1/e waist of the electric field of the excitation beam. FWHM values were calculated numerically from Eqs. (11) and (23).

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As above, we complement the numerical results in Fig. 4 with an analytical estimate for the effective waist of a CW T-STED PSF. We Taylor expand the natural logarithm of the product of the CW T-STED depletion factor, Eq. (23), with the confocal PSF and use the second order coefficient to derive the effective waist

$$\omega_{eff}/\omega = \left(4 + \sigma\gamma\omega^2\tau\left(1 + t_G/\tau\right)\right)^{-1/2} = \left(4 + S'\left(1 + t_G/\tau\right)\right)^{-1/2}. \qquad (24)$$

where we slightly modify the STED strength by replacing the pulse duration, undefined for a CW STED beam, with the unstimulated fluorophore lifetime, $S' = \sigma\gamma\omega^2\tau$.

Equation (24) bears a striking similarity to the improvement in spatial resolution derived previously [18] and above, in the limit of an infinitesimally short pulse, Eq. (20). The only distinction is that the effective STED strength is increased by the factor $\left(1 + t_G/\tau\right)$ in Eq. (24). Remarkably, the effective STED strength scales linearly with the time-gate duration for a CW T-STED measurement. Since this duration is bounded only by the repetition period of the excitation beam, which can be made as large as desired, the STED strength can be increased arbitrarily simply by increasing the time-gate, without increasing the power of the stimulating beam. In other words, the resolution of a time-gated STED measurement with a CW STED beam is theoretically unbounded for finite laser power. Of course this increase in resolution comes at a price. The prefactor $\exp\left(-kt_G\right)$ implies that images collected with time-gates of increasing duration will be exponentially dimmer.

This result is in sharp contrast to the results derived for a pulsed STED situation, in which time-gating cannot increase the resolution beyond the resolution achieved with an infinitesimally short pulse with the same total energy. The discrepancy between the performance of a pulsed measurement and a CW measurement highlights the physical origin of the increase in resolution: the effective energy of the STED beam. In a pulsed measurement, this energy is fixed by the duration of the pulse, time-gating can only use this energy more effectively, not increase it; thus, resolution is bounded by the resolution achieved with an infinitesimally short pulse. However, in a CW measurement the effective STED energy can be increased simply by integrating the STED beam over larger time-gates, producing increased depletion and increased spatial resolution.

3. Discussion and conclusions

In this Letter, we solve a simple kinetic model for the temporal dynamics of excited fluorophores in the presence of a spatially varying stimulating beam of variable duration and demonstrate that spatial information is contained in the arrival times of the spontaneous emission. To extract this extra information, we propose that the fluorescence signal should be time-gated, and we derive the increase in spatial resolution provided by time-gating for both pulsed and CW STED beams. In both cases, time-gating provides higher resolution but dimmer images.

When might T-STED be a useful alternative to a STED measurement? The lifetimes of the fluorophores useful for STED measurements range from 100s of ps to a few ns. STED pulse durations, on the other hand, range from 10s of ps [19] to ~1 ns [20]. Thus, for pulsed STED measurements, the range of α values is large, ~0.01 - 10. For measurements, with small α values, <0.5, the resolution enhancement provided by time-gating will likely be negligible. In fact, the improvement may be so modest that it is not measurable—an observation that may explain why a change in STED depletion as a function of pulse duration was not observed in a previous study [21]. However, for larger α values, the improvement in resolution is significant, and these measurements will likely benefit from time-gating.

Interestingly, the benefits of time-gating in a pulsed STED measurement are not limited to improved resolution. First, as has been widely recognized, time-gating can remove stray scattered light from the STED beam not removed by filters, decreasing background noise significantly in some situations. However, beyond this concern, we have now revealed that

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pulse durations can be increased, with no loss in resolution, when time-gating is applied. The ability to spread the pulse power over longer durations, decreasing the instantaneous intensity of the pulse, may allow experimentalists to avoid known issues such as inefficient depletion during vibrational relaxation [17] and STED intensity-dependent photobleaching [21]. STED measurements are often limited by the photostability of current dyes; thus, the ability to reduce photobleaching through longer pulses with no loss in resolution is an important additional benefit of T-STED.

The major benefit of T-STED with a CW laser source will likely be the ability to increase resolution significantly without the use of additional laser power. However, it is important to note that while, in theory, the resolution of a CW TSTED measurement is unbounded. In practice, there will likely be a maximum resolution given the specific STED laser, the fluorophore lifetime and photostability, and the background noise of the measurement. Image resolution increases slowly with the time-gate duration—as the square-root—while the brightness decreases exponentially. Thus, there will be a maximum time-gate beyond which the image will not be bright enough to be distinguished from background noise, and this maximum time-gate will set the maximum image resolution. To surpass this practical limit additional laser power will be needed. Alternatively, our analysis reveals that an increase in the resolution can also be produced by using fluorophores with longer lifetimes. This observation makes color centers in diamond all the more exciting as STED probes since these fluorophores have particularly long lifetimes, >10 ns [13,22]. Thus, through the simple addition of a time-gate, it may be possible to significantly increase the 6 nm resolution recently reported with CW-STED imaging of color centers [13].

While our discussion here has focused on a measurement in which all fluorescence before a certain period is discarded, practical implementations of T-STED will likely collect all fluorescence and record arrival times using time correlated photon counting hardware. In fact, such a time-resolved STED instrument has already been created and used to perform lifetime imaging in a pulsed STED setting. Initial observations [23] support the increase in resolution with time-gating we describe here. The advantage of measuring the arrival time of all photons, particularly for CW T-STED, is that time-gating can be applied in offline analysis, allowing the experimentalist to dynamically adjust the balance between resolution and image brightness to meet the demands of the specific application.

It was long thought that the resolution of far-field optics was fundamentally limited by diffraction, but it is now clear that a variety of techniques can embed additional spatial information in the optical measurement and leverage this additional information to image spatial features well below the diffraction limit. Here we show that minor differences in fluorescence lifetimes, created by differences in the local intensity of the STED beam, can be amplified into measureable differences in arrival times, revealing that the last photons to arrive carry the most spatial information. The construction of microscopes to extract this extra information should help push the practical limits of far-field imaging even further beyond the diffraction limit.

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