Supplemental Information

Resolution improvement in STED super resolution microscopy at low power using a phasor plot approach

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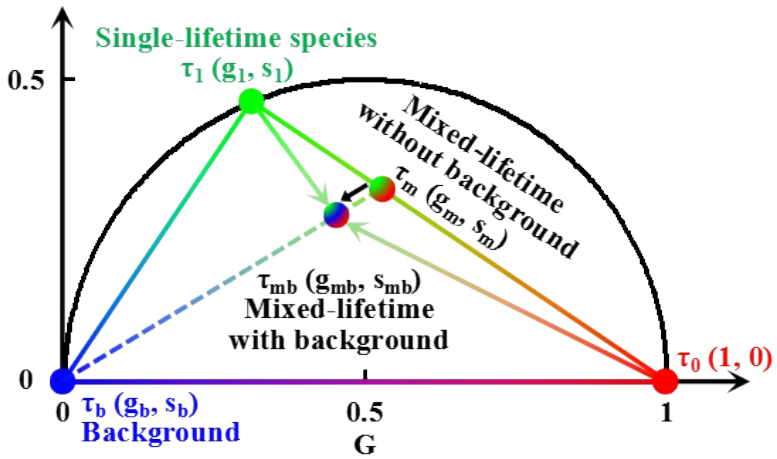

Figure S1. The mixed-lifetime in the phasor plot. Five points in the phasor plot denote different lifetime: τ_0 is zero lifetime with the coordinates of (1, 0); τ_1 is the lifetime of single species with the coordinates of (g_1, s_1) at the scale of ns; τ_b is the lifetime of background with the coordinates of (g_b, s_b); τ_m is the mixed-lifetime of τ_0 and τ_1 with the coordinates of (g_m, s_m); τ_{mb} is the mixed-lifetime of τ_0, τ_1 and τ_b with the coordinates of (g_{mb}, s_{mb}). Theoretically, the average lifetime in STED mode should locate on the line connecting the single-lifetime species and τ_0, and approaching to the point (1, 0) when depletion power increases indefinitely. However, background signal with the lifetime several orders higher than τ_1 (almost close to point (0, 0)) makes the lifetime deviate from the original line. Therefore, the mixed-lifetime (τ_{mb}) locates inside the triangle connecting the three points, resulting in a lifetime with the coordinates (g_{mb}, s_{mb}) deviated from the coordinates (g_m, s_m).

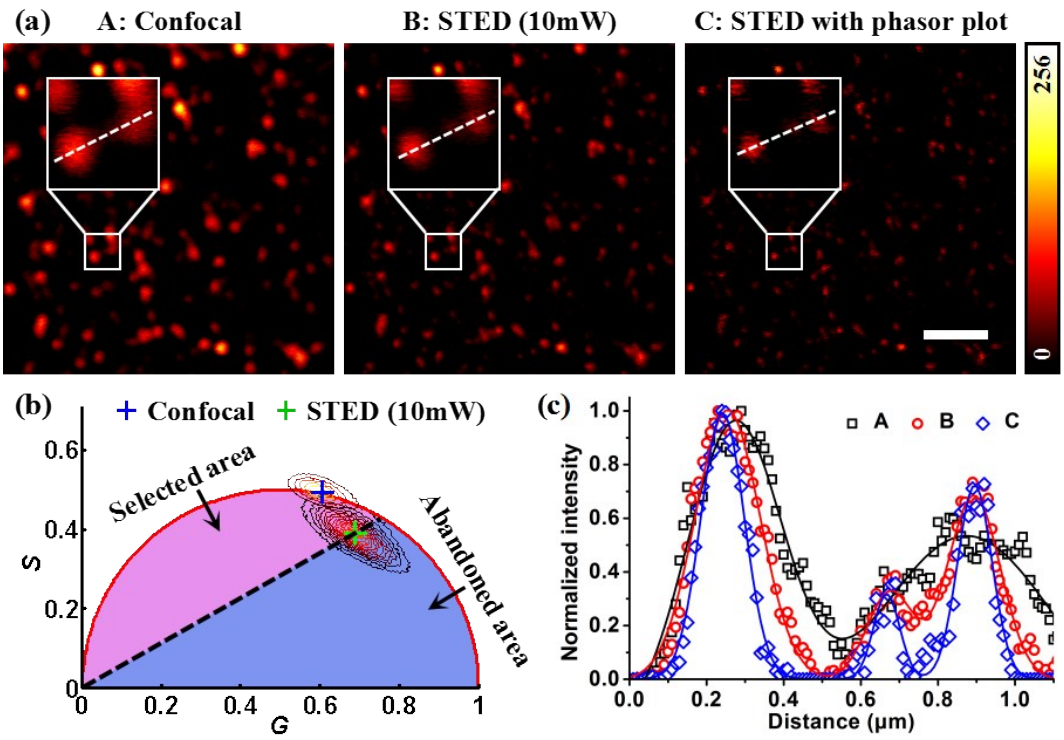

Figure S2. Resolution improvement of 40 nm fluorescent microspheres at the depletion power of 10 mW. (a) Time-resolved images (the lifetime in confocal mode: 1.8 ns; the lifetime in STED mode: 1.1 ns). Scale bar, 2 μm. (b) Phasor plots and the principle of resolution improvement. (c) The normalized intensity profiles are given by the dotted lines in (a).

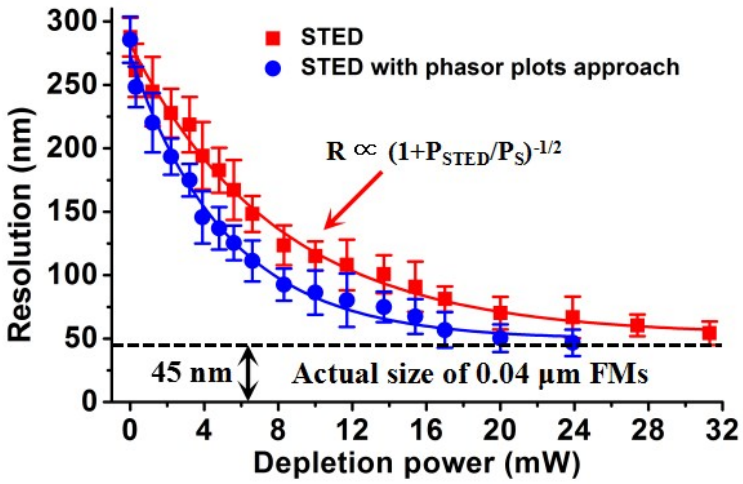

Figure S3. Resolution improvement of 40 nm fluorescent microspheres (actual size: 45 nm) at different depletion power by using phasor plot analysis approach.