Breaking the Diffraction Barrier Using Fluorescence Emission Difference Microscopy

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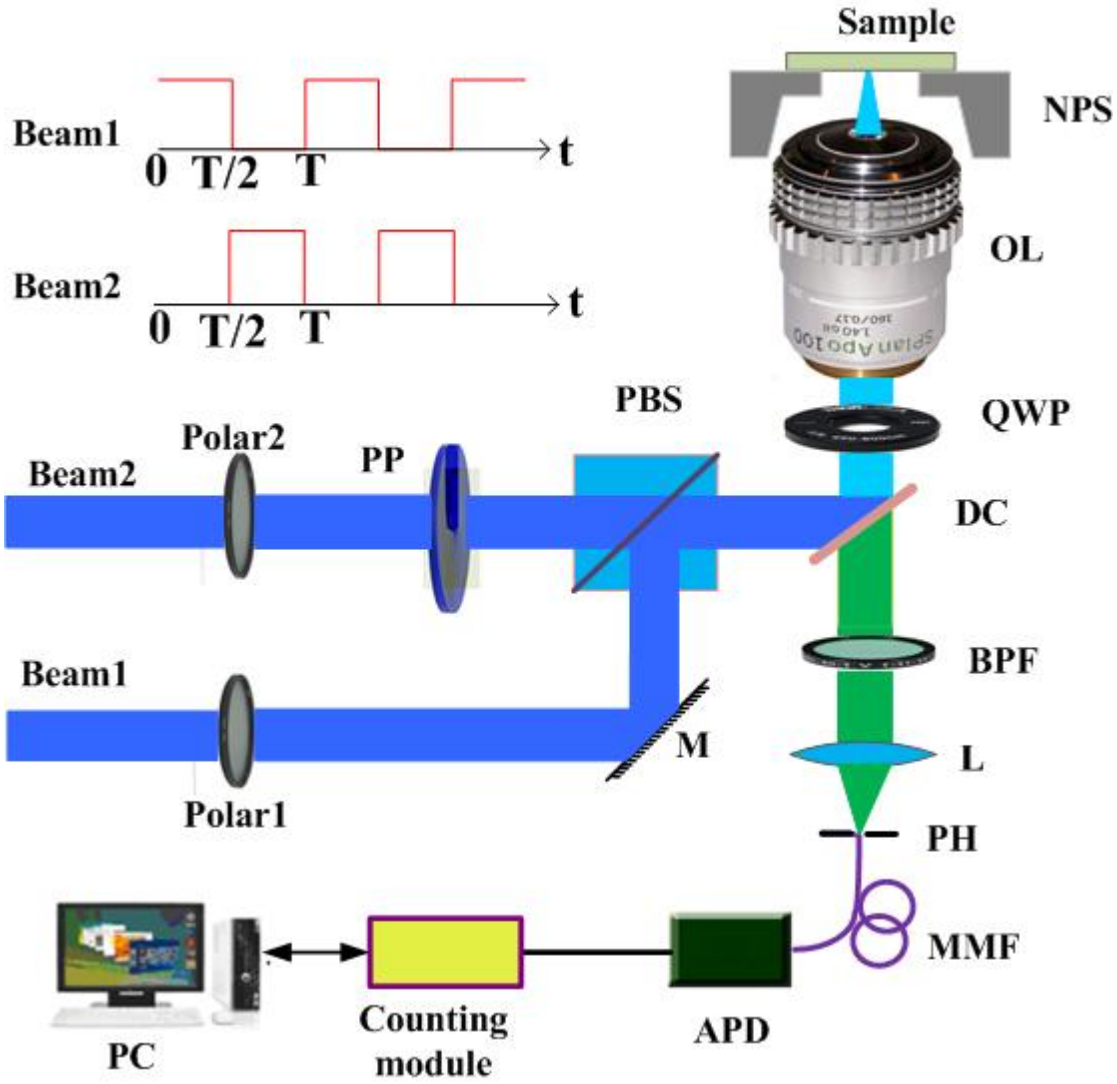
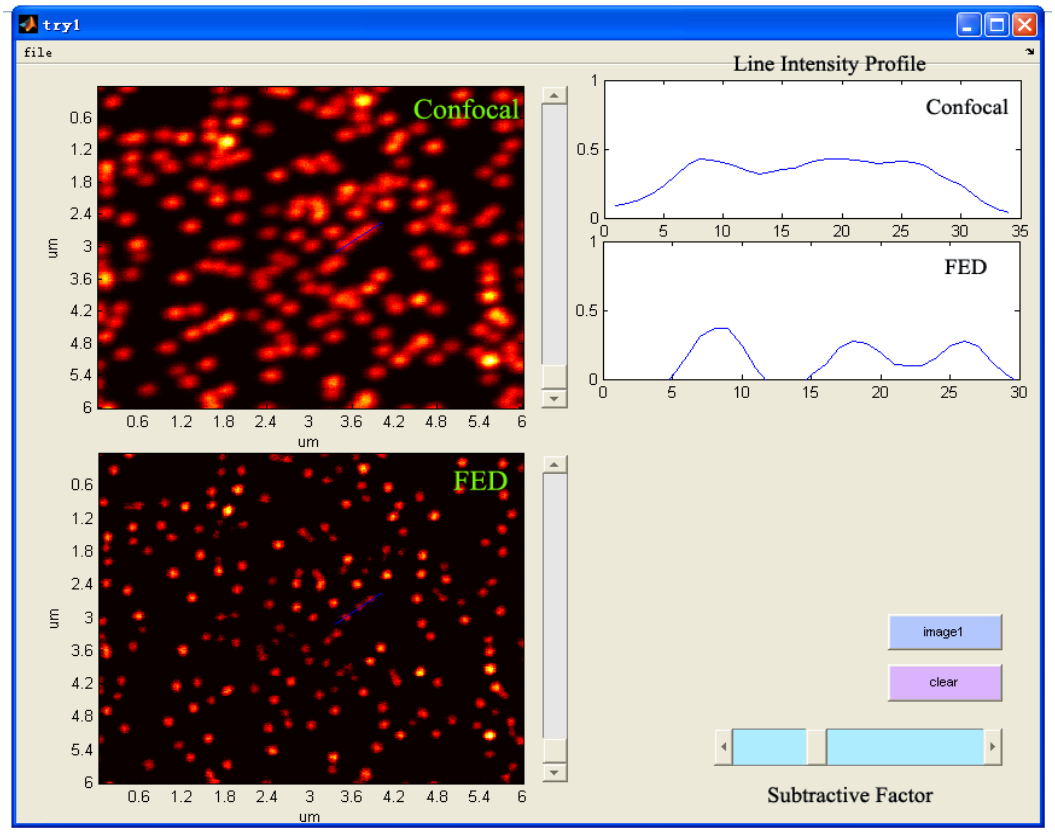
Supplementary Table S1. Resolution attainable by FED under different conditions (in units of the illumination beam wavelength).

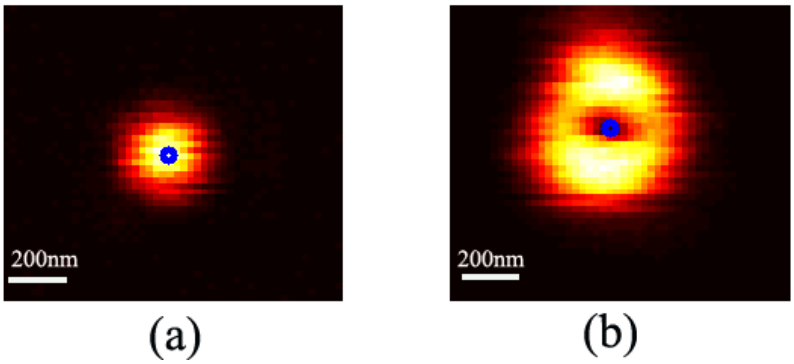
Subtractive factor r / Saturation factor ζ	0.7	0.85	1
1	0.256	0.242	0.226
2	0.192	0.18	0.172
3	0.148	0.138	0.134
4	0.124	0.118	0.112
5	0.112	0.104	0.102
6	0.098	0.094	0.092
7	0.092	0.086	0.082
8	0.086	0.082	0.076
9	0.08	0.076	0.07
10	0.076	0.07	0.068
11	0.07	0.068	0.066
12	0.068	0.066	0.064
13	0.066	0.064	0.062
14	0.064	0.062	0.06
15	0.062	0.06	0.058



Supplementary Figure S1. FED system setup. (Inset) Modulated intensities of beam1 and beam2. Polar, polarizer; PP, vortex 0–2π phase plate; M, mirror; PBS, polarization beam splitter; DC, dichroic mirror; QWP, quarter-wave plate; OL, objective lens; NPS, nano-positioning stage; BPF, bandpass filter; L, lens; PH, pinhole; MMF, multimode optical fiber; APD, avalanche photodiode.



Supplementary Figure S2. To process and analyse the detected data, we developed data processing software based on MATLAB. The input parameters required in this software are the detected intensity values of the corresponding confocal and negative confocal images, which should be saved in.dat files. Because the subtractive factor r is tunable in the software, we can easily generate FED images under different conditions. Line intensity profiles of the confocal and FED images can be plotted automatically simply by drawing the line to be analysed, which permits a quick comparison of the resolving power of the two images.



(a) (b)

Supplementary Figure S3. Images taken to calibrate the FED system. (a) Confocal image of a single fluorescent nanoparticle. (b) Corresponding negative confocal image. Blue circles denote the central positions of the two illumination patterns.