Supplementary Information

Statistical Deconvolution for Super-Resolution Fluorescence Microscopy

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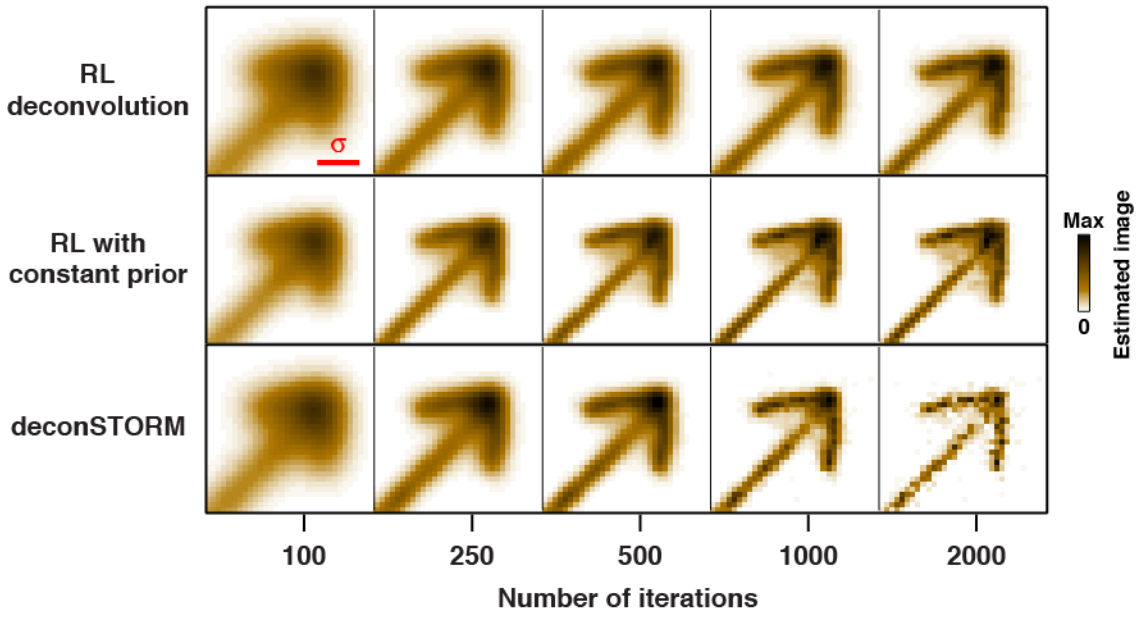

FIGURE S1

Effect of the number of iterations on deconvolution performance. σ is the width parameter of the Gaussian PSF. See detailed explanation of simulation methods in caption of Fig. 2.

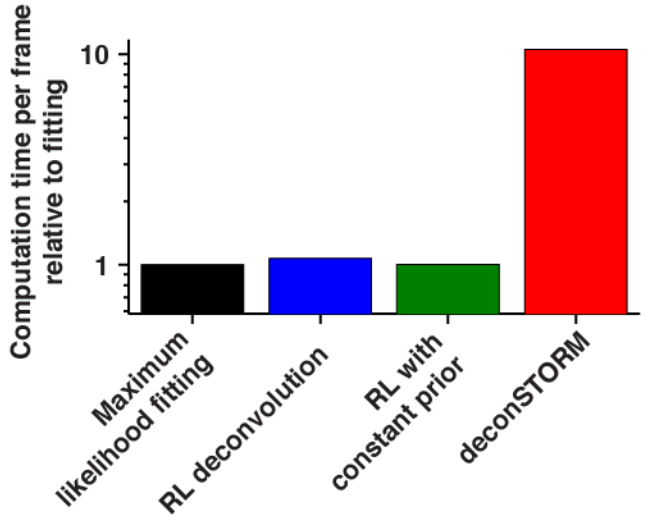

FIGURE S2

Computation time per movie frame for each algorithm, normalized to the computation time for the single-emitter maximum likelihood fitting method. All analyses were implemented in MATLAB and performed on a MacBook Pro with a 2.5 GHz Intel Core 2 Duo processor.

Supplementary Methods

Analysis of STORM images of cellular samples. To test the image analysis methods on cellular samples, we recorded STORM images of microtubules in cells immunohistochemically labeled with the Alexa-647 dye. BS-C-1 cells were plated in 8-well chambered coverglasses (LabTek-II, Nalgene Nunc) and allowed to grow to near confluence. They were fixed with 3% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffered saline (PBS) for 10 min, followed by a 7 min treatment with freshly prepared 0.1% sodium borohydride. Fixed samples were then washed thoroughly with PBS, and blocked with blocking buffer (BB, 3% w/v BSA, 0.1% v/v Triton X-100 in PBS) for 15 min. Samples were first labeled with mouse monoclonal anti-beta-tubulin primary antibody (T5201, Sigma) in BB, washed with PBS and then labeled with Donkey anti-mouse secondary antibody (715-005-150, Jackson Labs) conjugated with Alexa 405 and Alexa 647. Imaging was performed on a STORM microscope constructed as described previously (23). The amount of spatial overlap between adjacent activated fluorescent molecules was adjusted with the 405 nm activation laser.

Microtubule data shown in Fig. 4 consisted of 5000 fluorescence frames. For computational efficiency, we divided the data set into 8 segments with 625 frames each and analyzed each segment independently. Results from the 8 independent segments were combined by averaging the final estimates. The STORM analysis results use the single-emitter localization procedures that were described previously (23). This single-emitter localization analysis determined the following average parameters for all well-isolated emitters within the field of view: background fluorescence intensity, b = 102 photons/pixel/frame; peak width parameter, σ = 173 nm = 1.04 pixels; the average number of consecutive frames in which a given emitter remains active, $1/\alpha$ = 2.2; density of activated single emitters (excluding overlapping emitters), ρ = 0.34/μm^2/frame, which corresponds to activation rate $\beta = 6.5 \times 10^{-5}$. We used these parameters for the deconvolution analysis. For the gain parameters, we tested four values for γ and g and selected values (γ = 1/16 and g = 1/256) that, to our eyes, best balanced sharp detail and continuity of image features after n = 1000 iterations. We found that the results were robust in that the final image was not substantially altered when the gain parameters varied between 50-200% of the selected values.

Supplementary Software

A MATLAB toolbox for implementing the deconSTORM algorithm is available at http://zhuang.harvard.edu/software.html. Documentation is also provided at the same address.