

# Correcting for photodestruction in super-resolution optical fluctuation imaging - Supplementary information

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## 1 Effect of uncorrected photodestruction on third order SOFI imaging

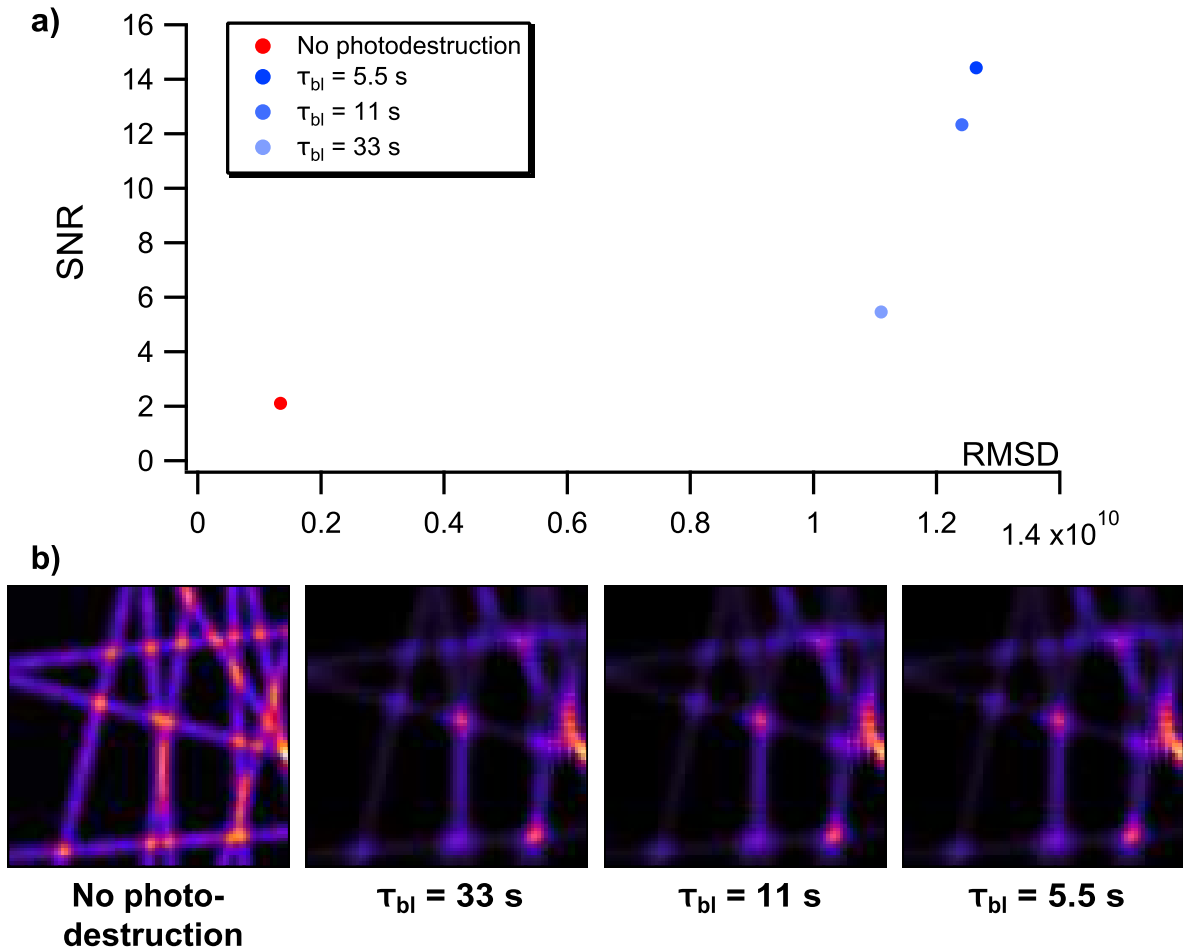


Figure S1: Effect of photodestruction on 3<sup>rd</sup> order SOFI calculations. (a) 2D plot of SNR and RMSD values of data with varying degrees of photodestruction. Photodestruction was simulated by defining a characteristic time constant of the photodestruction ( $\tau_{bl}$ ). The average SOFI image of 100 simulations of 20,000 frames without photodestruction was used as a reference to determine RMSD values. (b-e) Average 3<sup>rd</sup> order SOFI images with varying degrees of photodestruction (lower  $\tau_{bl}$  values correspond to more photodestruction). SOFI images of 100 simulations were averaged to yield these images.

## 2 Correcting photodestruction in second order SOFI simulations

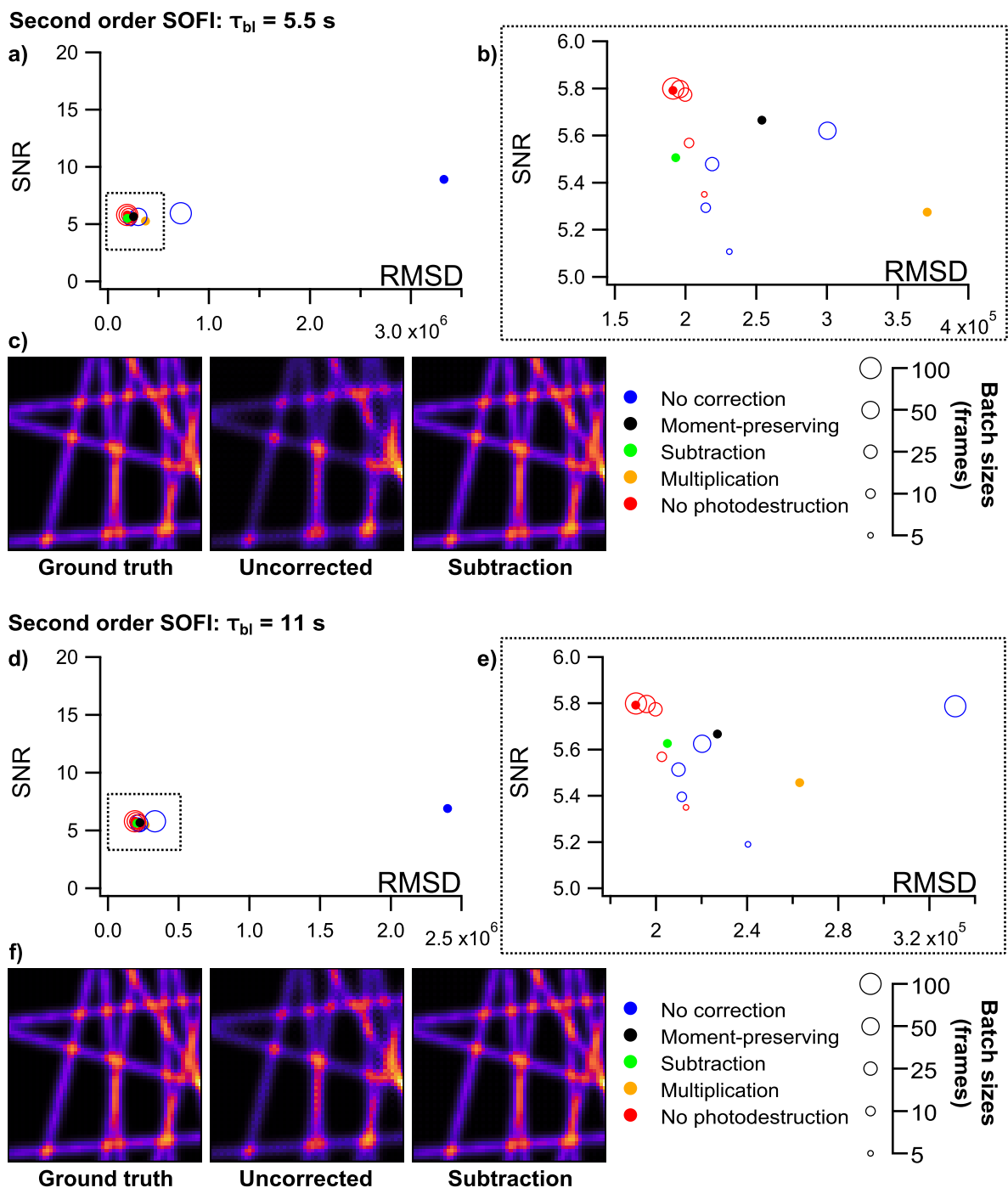


Figure S2: (a) Evaluation of different photodestruction-correcting methods on simulated data with fluorophores assigned a  $\tau_{bl}$  value of 5.5 s for 2<sup>nd</sup> order SOFI calculations. (b) Close-up of region in dotted box in (a). (c) Images of certain datasets to serve as a visual reference for RMSD values. These images are averaged SOFI images of 100 repetitions. (d-e-f) correspond to (a-b-c) respectively for simulations with a  $\tau_{bl}$  value of 11 s. Batch sizes of 100, 50, 25, 10, and 5 frames were examined and are visualized by different marker sizes.

**Second order SOFI:  $\tau_{bl} = 33$  s**

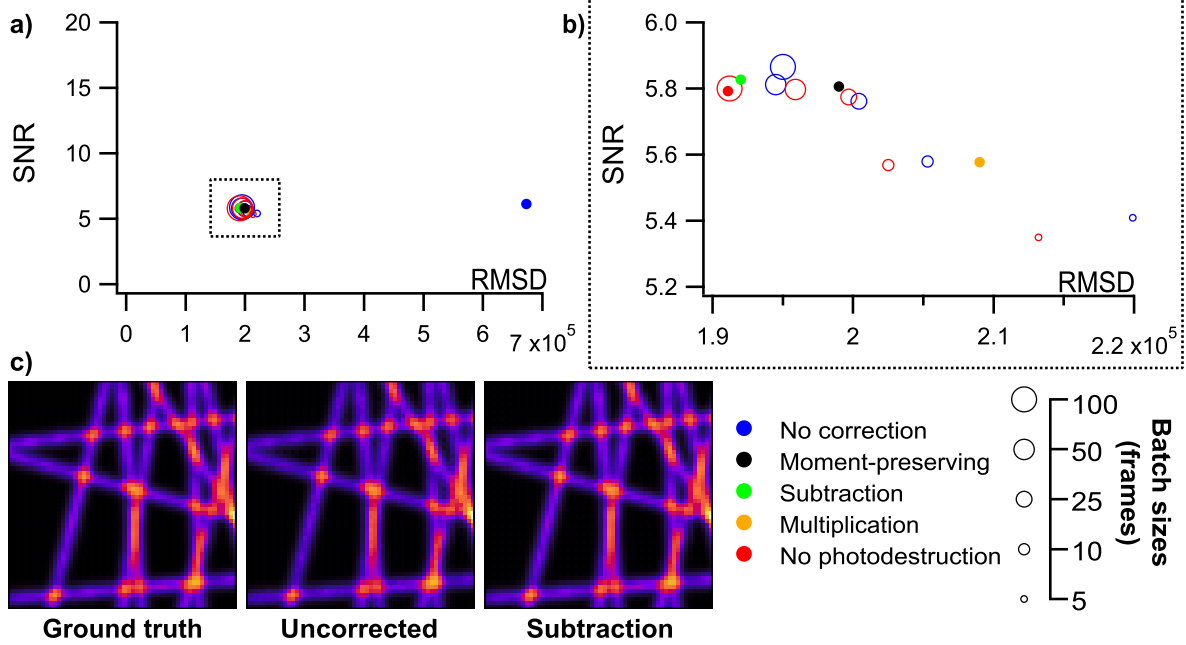


Figure S3: (a) Evaluation of different photodestruction-correcting methods on simulated data with fluorophores assigned a  $\tau_{bl}$  value of 33 s for  $2^{nd}$  order SOFI calculations. Batch sizes of 100, 50, 25, 10, and 5 frames were examined and are visualized by different marker sizes. (b) Close-up of region in dotted box in (a). (c) Images are shown for certain datasets to serve as a visual reference for RMSD values. These images are averaged SOFI images of 100 repetitions.

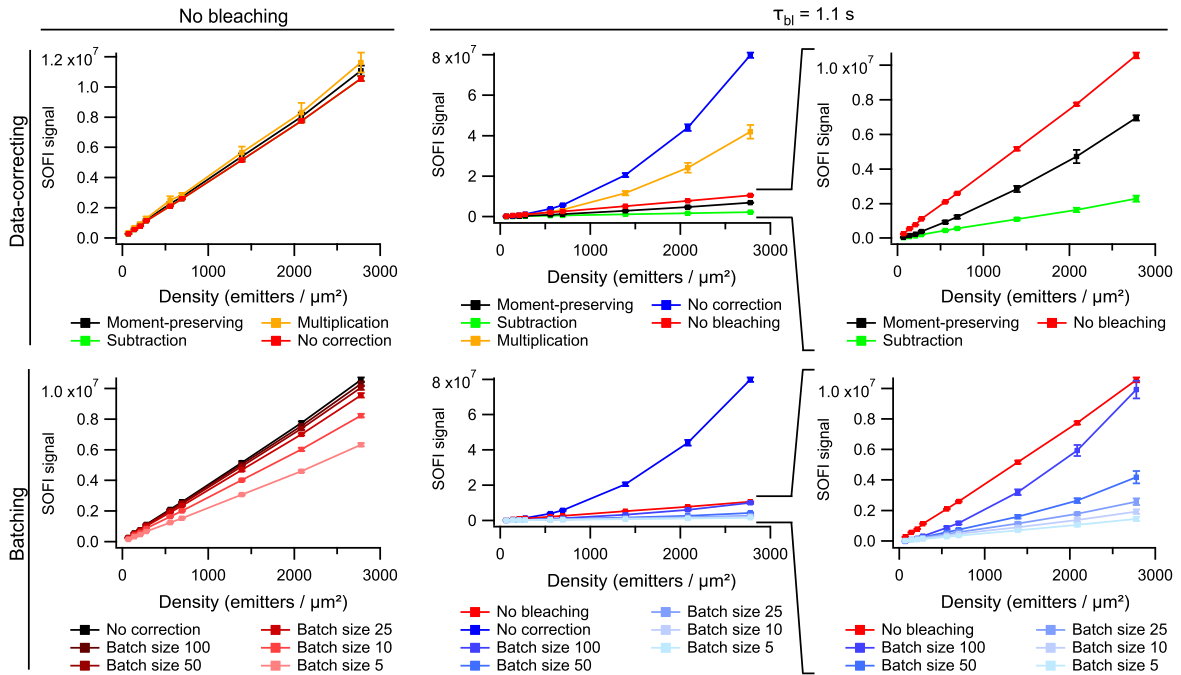


Figure S4: Analysis of photodestruction effects and correction methods on  $2^{nd}$  order SOFI signal with various emitter density. Each simulation contained 500 frames and was repeated 10-fold. Average SOFI signal is shown with standard deviation.

### 3 Correcting photodestruction in third order SOFI simulations

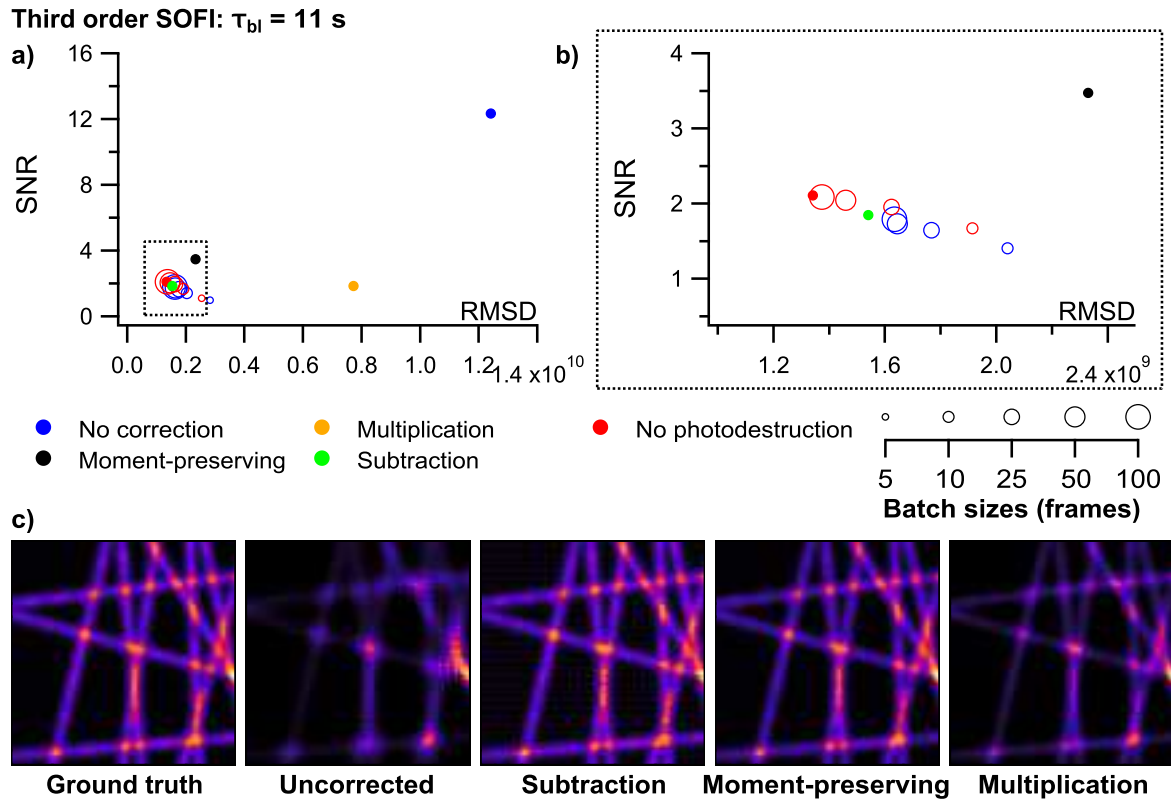


Figure S5: (a) Evaluation of different photodestruction-correcting methods on simulated data with fluorophores assigned a  $\tau_{bl}$  value of 11 s for 3<sup>rd</sup> order SOFI calculations. Batch sizes of 100, 50, 25, 10, and 5 frames were examined and are visualized by different marker sizes. (b) Close-up of region in dotted box in (a). (c) Images are shown for certain datasets to serve as a visual reference for RMSD values. These images are averaged SOFI images of 100 repetitions.

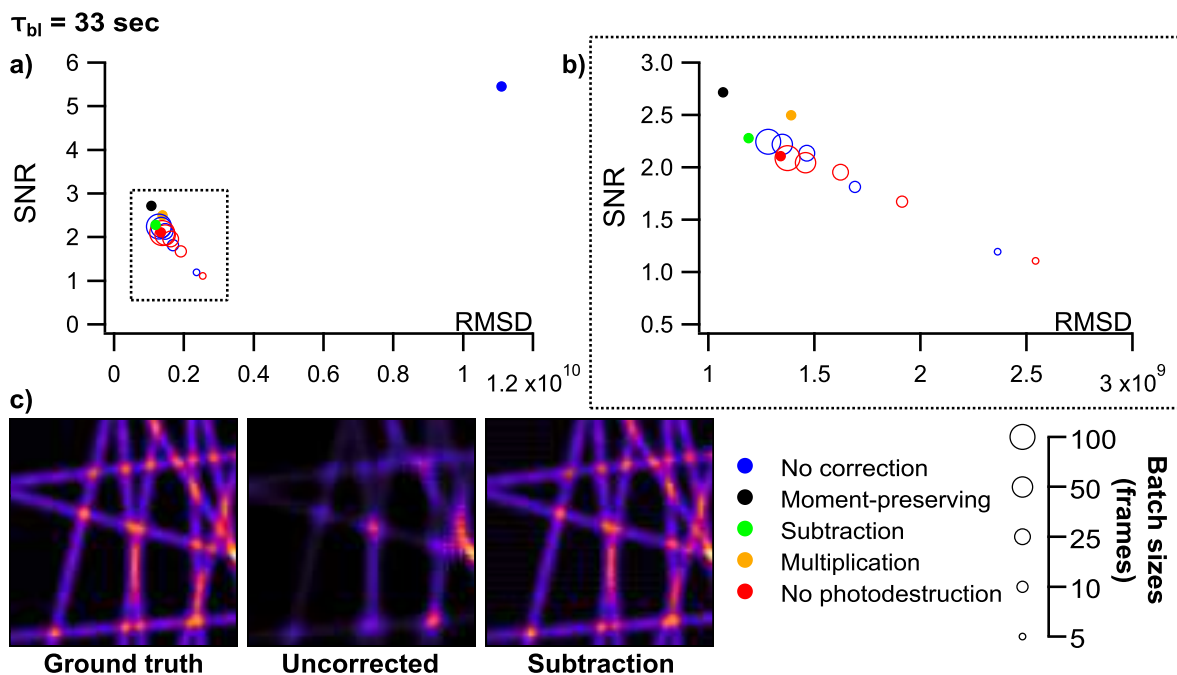


Figure S6: (a) Evaluation of different photodestruction-correcting methods on simulated data with fluorophores assigned a  $\tau_{bl}$  value of 33 s for  $3^{rd}$  order SOFI calculations. Batch sizes of 100, 50, 25, 10, and 5 frames were examined and are visualized by different marker sizes. (b) Close-up of region in dotted box in (a). (c) Images are shown for certain datasets to serve as a visual reference for RMSD values. These images are averaged SOFI images of 100 repetitions. Deviations are attributed to insufficient convergence of the simulations.