Supplemental Document

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Design of experiments for the optimization of SOFI super-resolution microscopy imaging: supplement

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1. EXPERIMENTAL OBSERVATIONS

A. Material and methods

The samples were obtained from Thomas Schlichthaerle (Jungmann lab). The measurement was performed using a DNA-paint style labeling on U2OS cells. For this we used mitochondrial targetting anti-TOMM20 primary antibody (Santa Cruz: sc-17764), donkey anti-mouse IgG as secondary antibody (Jackson immuno research: 715-005-151) coupled to the P3 docking strand and as dye Cy3b coupled to the P3 imager strand. Specifics of coupling and the oligo's can be found in [1, 2].

Dye was added to 200 ml PPT (DNA-paint imaging buffer), to obtain final concentrations of 4.65 nM, 10 nM, 21.5 nM, 46.5 nM and 100 nM. The measurements for the different dye concentrations were made sequentially: between each measurement the sample was washed with dye-free buffer solution and then exposed to a new dye-imager solution. For each measurement up to 6250 frames were taken, with an exposure time of 20 ms, using a 561 nm laser.

The experiments were done using an Olympus IX83 inverted microscope with cellTIRF module and 150 mW 561 nm laser. The objective used was an Olympus UPlanFL N 100X/1.30 ∞ /0.17/FN26.5.

B. Results

In DNA-PAINT the t_{on} is defined as the average binding time between the two oligonucleotide strands, while t_{off} is the average time passing between two successful bindings. While the t_{on} depends uniquely on the affinity between two strands, the t_{off} can be easily manipulated by the operator by modifying the concentration of dye in solution (and therefore of free strands available for the binding). In fact, the concentration of the dye in the imager solution is inversely correlated to the t_{off}: a higher dye concentration will correspond to a lower mean t_{off} for each binding site on the structure [1].

Figure S1 shows SOFI images that are obtained in different experimental conditions: in particular, 5 different dye concentrations were used during the measurement, and 5 different number of frames were used to generate SOFI images. If looking from left to right, it possible to see the quality of the SOFI image increasing with a higher number of frames used. It can also be seen that the quality of the image improves when higher concentration of the dye is used, with images measured at higher dye concentration consistently better that images with same number of frames and a lower concentration. This is true excluding the highest amount of dye used (*i.e.* the line **i**), where the high amount of stray light due to the excessive dye concentration lead to impoverished measurements. This aspect, though, was not accounted for in the simulations conducted in this work. Therefore, especially in real case studies, any operator should always tailor the optimization design to the specific operative conditions considered.

Figure S2 presents the SNR distribution relative to the DNA-PAINT dataset. The SNR has been obtained by using consecutive measurement to obtain several replicate SOFI images. As for the simulated datasets, the per-pixel SNRs were then calculated as the ratios of the average intensity and standard deviation seen for that particular pixel in the replicate images. The overall SNR was then calculated by averaging the per-pixel SNRs.

The SNR is plot in function of measurement time and dye concentration. Since the dye concentration is inversely proportional to the average t_{off}, these observations seems to indicate that lower t_{off} and higher measurement time will result in higher SNR. This is in accord with the conclusion obtained from the simulated data.

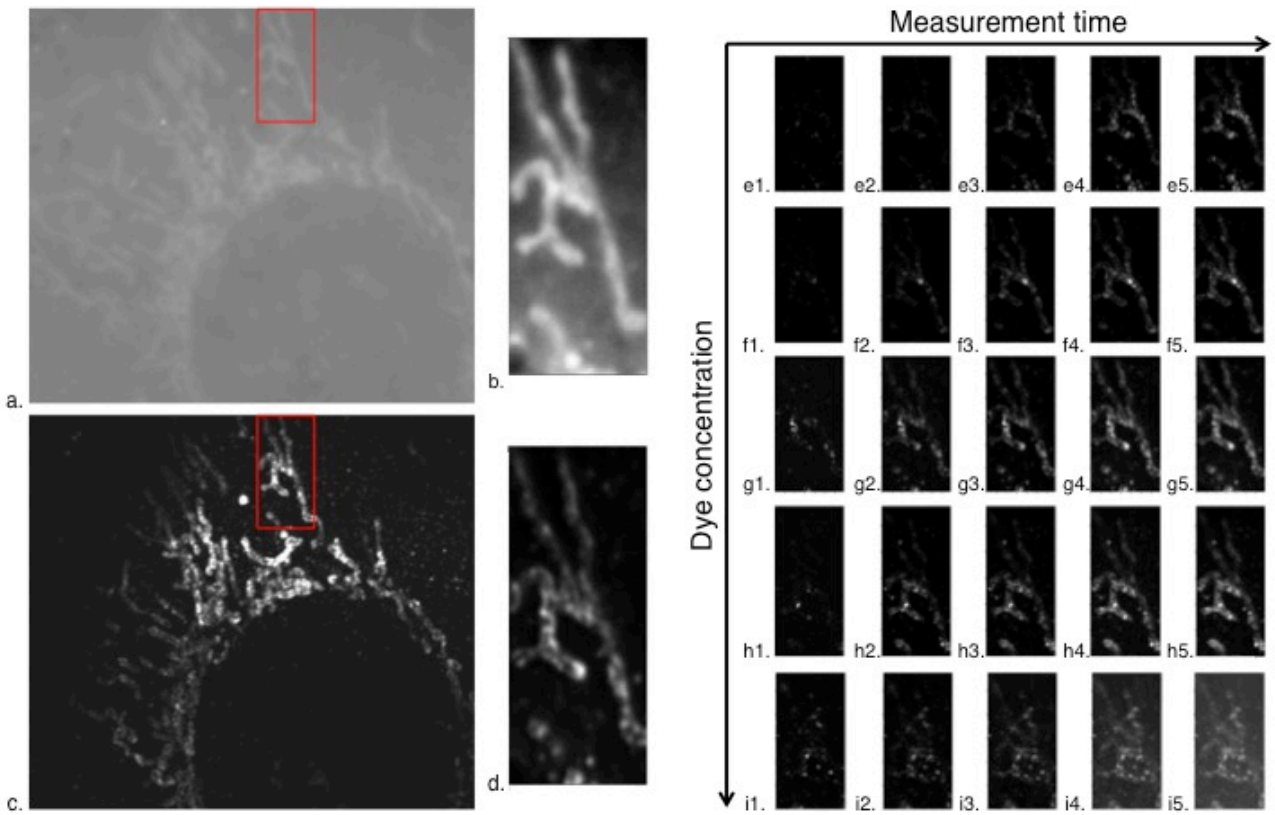

Fig. S1. Third-order SOFI images of mitochondria stained with DNA-Paint. a-b. Widefield (averaged) image, b. detail. c-d. Third-order SOFI image, 6250 frames, dye concentration 21.5 nM, d. detail. e-i. increasing dye concentration: e. 4.65 nM, f. 10 nM, g. 21.5 nM, h. 46.5 nM, i. 100 nM. 1-5. increasing measurement time: 1. 500 frames, 2. 2000 frames, 3. 3125 frames, 4. 5000 frames, 5. 6250 frames.

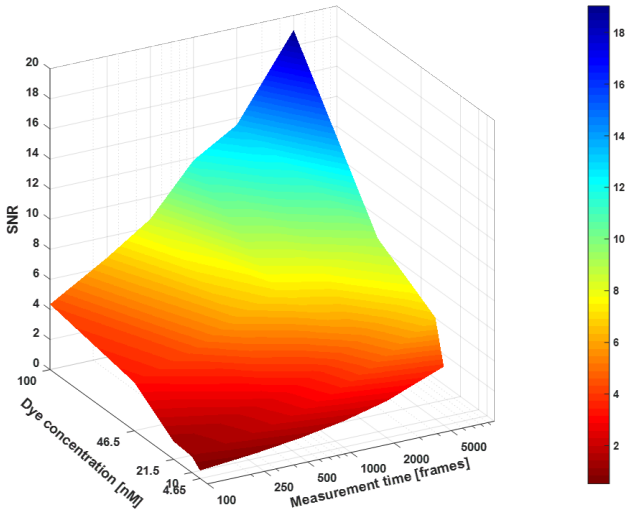

Fig. S2. SNR of the SOFI images obtained in the conditions reported in S1, plot in function of measurement time (500 frames, 2000 frames, 3125 frames, 5000 frames, 6250 frames) and dye concentration (4.65 nM, 10 nM, 21.5 nM, 46.5 nM, 100 nM).

2. COMPLETE MODELS

In equations S1 and S2 we present the full 21 terms model for second-order and third-order SOFI simulated images, respectively.

$$\begin{aligned} y = &+ 0.5126 + 0.1645\,T - 0.0400\,t_{on} - 0.2413\,t_{off} + 0.0090\,\epsilon^* - 0.0152\,b \\ &- 0.0044\,T\,t_{on}^* - 0.0427\,T\,t_{off} + 0.0043\,T\,\epsilon^* - 0.0173\,T\,b + 0.0734\,t_{on}\,t_{off} \\ &- 0.0101\,t_{on}\,\epsilon^* + 0.0717\,t_{on}\,b + 0.0151\,t_{off}\,\epsilon^* - 0.0140\,t_{off}\,b^* + 0.0123\,\epsilon\,b^* \\ &- 0.0468\,T^2 - 0.0035\,t_{on}^{2\,*} - 0.1487\,t_{off}^2 - 0.0139\,\epsilon^{2*} - 0.0053\,b^{2*} \end{aligned} \tag{S1}$$

$$\begin{aligned} y = &+ 0.4972 + 0.1094\,T - 0.1655\,t_{on} - 0.0227\,t_{off} + 0.0016\,\epsilon^* - 0.0035\,b^* \\ &- 0.0183\,T\,t_{on} - 0.0056\,T\,t_{off}^* - 0.0055\,T\,\epsilon^* - 0.0116\,T\,b^* + 0.1169\,t_{on}\,t_{off} \\ &- 0.0015\,t_{on}\,\epsilon^* + 0.0028\,t_{on}\,b^* + 0.0074\,t_{off}\,\epsilon^* + 0.0048\,t_{off}\,b^* + 0.0034\,\epsilon\,b^* \\ &- 0.0302\,T^{2*} + 0.0683\,t_{on}^2 - 0.0307\,t_{off}^{2\,*} - 0.0125\,\epsilon^{2*} - 0.0015\,b^{2*} \end{aligned} \tag{S2}$$

Terms marked with an asterisk have been found non-significant by the F-Test (p-value>0.05) and have been eliminated in the final model. The regression coefficients of the reduced model have to be recalculated after eliminating the non-significant terms.

REFERENCES

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