Biophysical Journal, Volume *120*

Supplemental information

Chromatin investigation in the nucleus using a phasor approach to structured illumination microscopy

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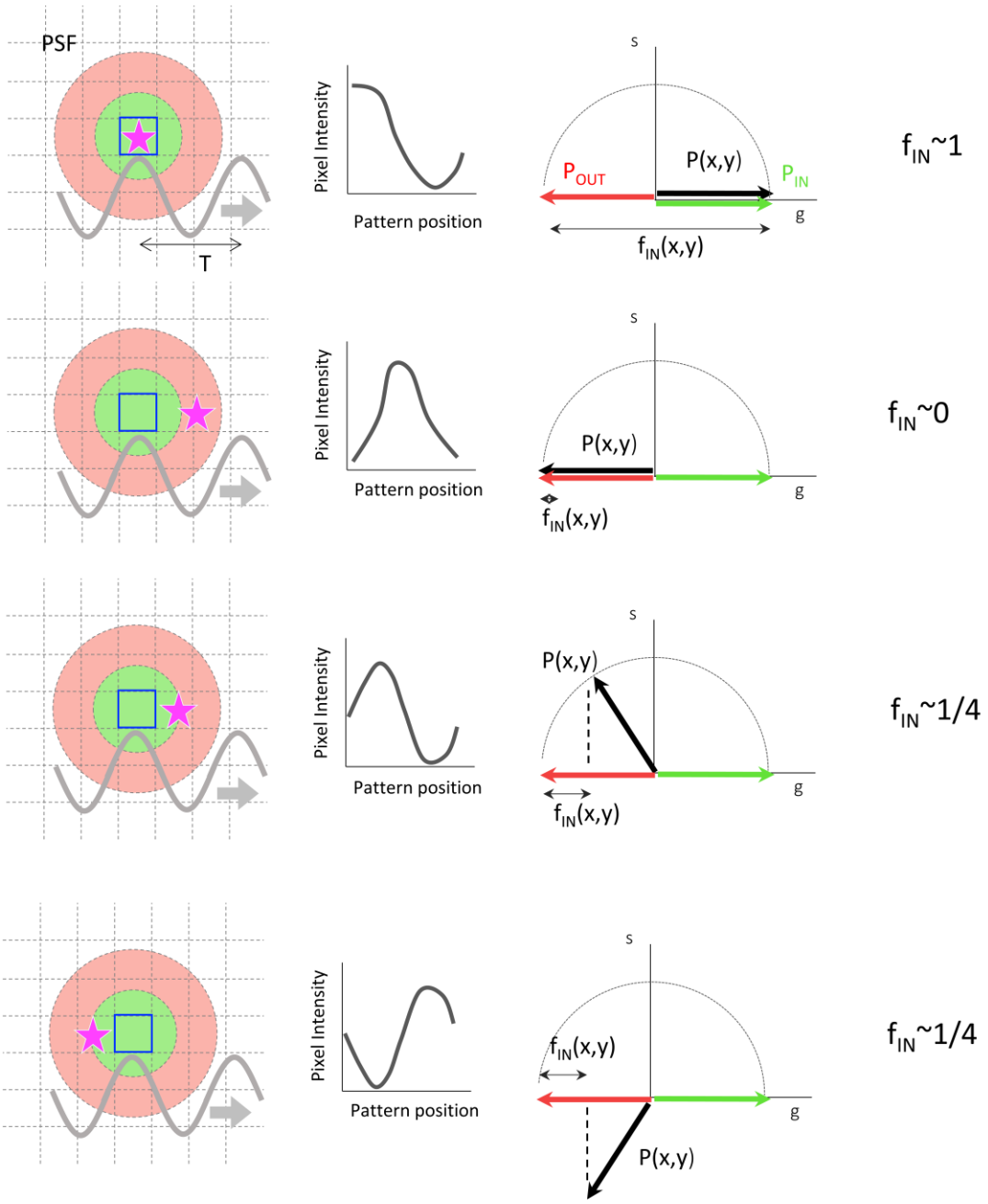

Figure S1 | Phasors of single fluorophores. a) Schematic of the SPLIT-SIM processing for the case of a single fluorophore located at different position with respect to the center of the pixel. Shown are (from left to right) the PSF and an illumination pattern of period T translating in the direction of the arrow, the profile of the pixel intensity as a function of the pattern position, the decomposition of the corresponding phasor $\mathbf{P}(x,y)$ in the components $\mathbf{P}_{in}$ and $\mathbf{P}_{out}$. Different positions of the fluorophore (indicated by a star) correspond to different phasors and to different values of the calculated fraction $f_{IN}(x,y)$.

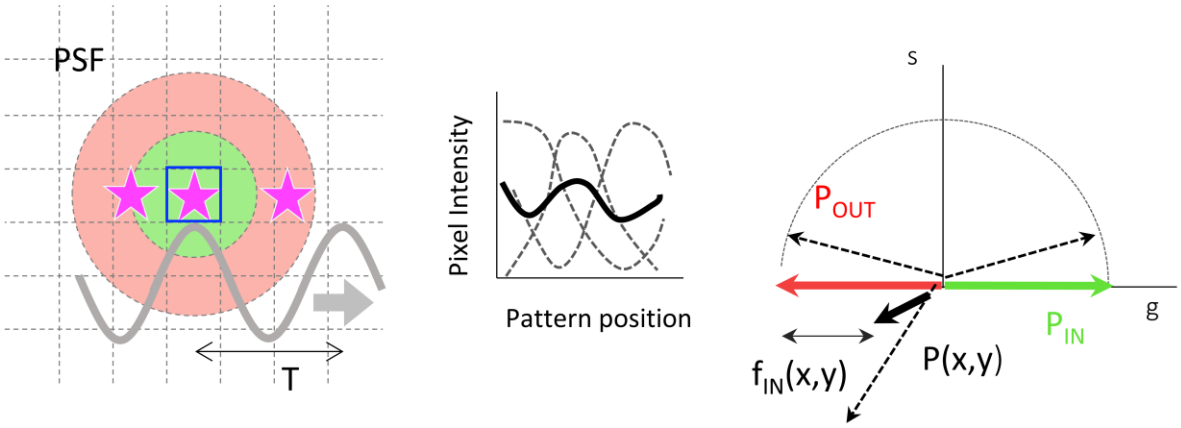

Figure S2 | Phasors of multiple fluorophores inside the PSF. a) Schematic of the SPLIT-SIM processing for the case of a multiple fluorophores (indicated by stars) inside the PSF. Shown are (from left to right) the PSF and an illumination pattern of period T translating in the direction of the arrow, the profile of the pixel intensity as a function of the pattern position, the decomposition of the corresponding phasor $\mathbf{P}(x,y)$ in the components $\mathbf{P}_{in}$ and $\mathbf{P}_{out}$. The phasor $\mathbf{P}(x,y)$ is the linear combination of the phasors of the single fluorophores (indicated by dashed arrows).

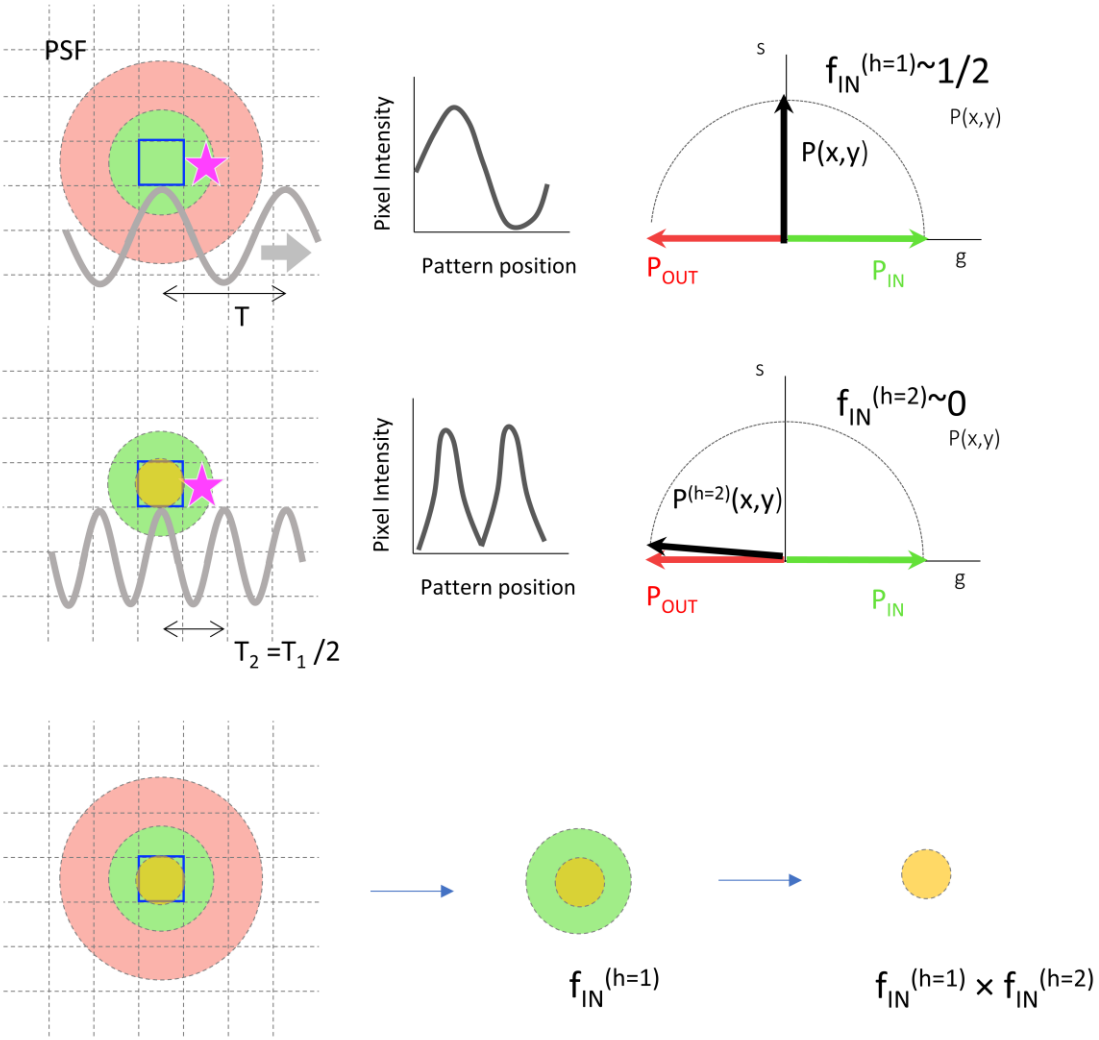

Figure S3 | Calculation of fraction from multiple harmonics. Schematic of the SPLIT-SIM processing using information from two harmonics. The top row shows the calculation of the fraction $f_{in}^{(h=1)}$ according to Eq.4 in the Main Text. The middle row shows the calculation of the fraction $f_{in}^{(h=2)}$ according to Eq.5 in the Main Text. The bottom row shows the calculation of the fraction $f_{in}=f_{in}^{(h=1)}f_{in}^{(h=2)}$ according to Eq.6 in the Main Text.

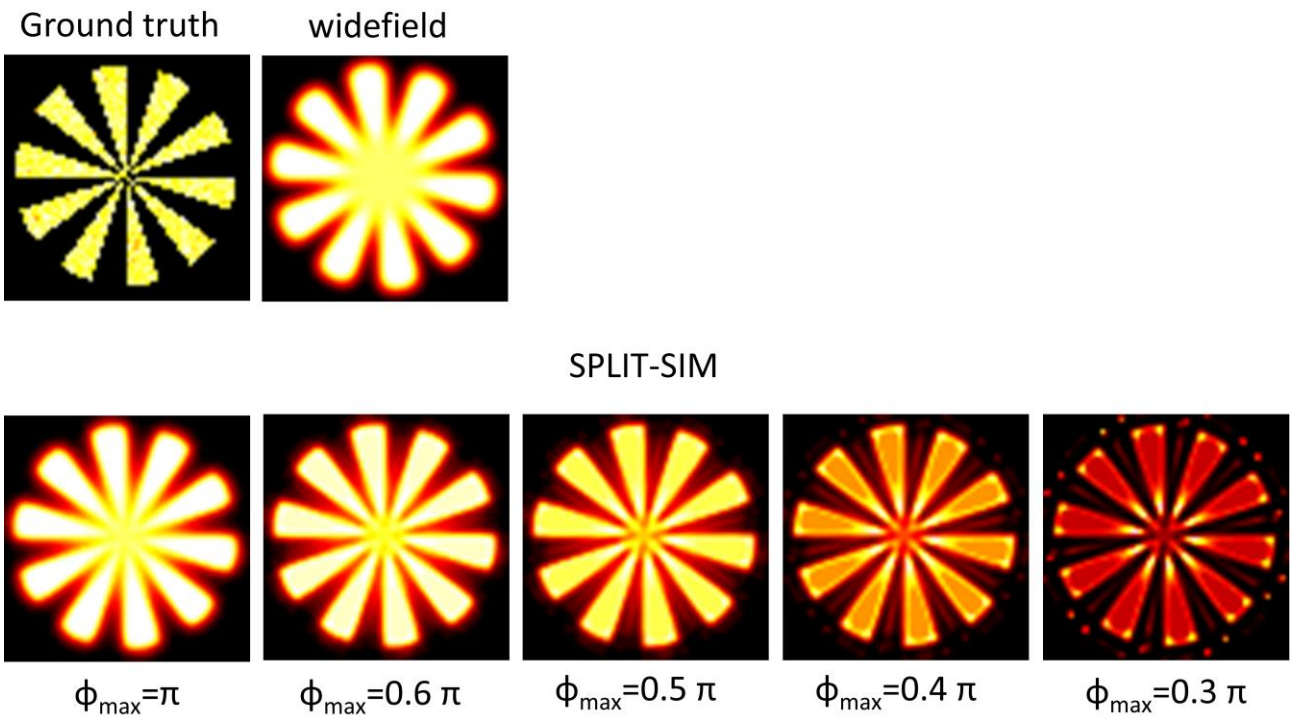

Figure S4 | SPLIT-SIM on Siemens star. SPLIT-SIM processing on a simulated SIM image of a Siemens star pattern as a function of the parameter ϕ_{max}. Shown is the Siemens star object used in the simulation (Ground truth), the widefield image (WF), the SPLIT-SIM images obtained for different values of ϕ_{max}.

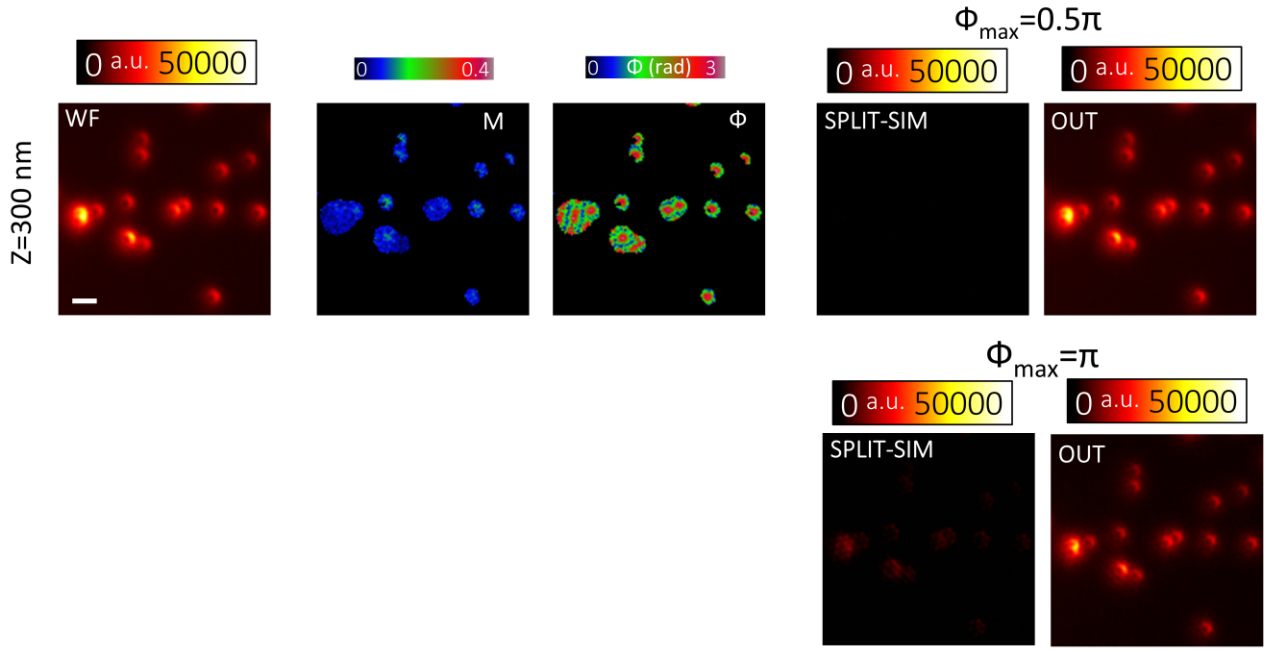

Figure S5 | SPLIT-SIM on out-of-focus signal. SPLIT-SIM is performed on a sample of 100-nm fluorescent spheres (same sample of Fig.3) at an out-of-focus distance z=300nm. Shown are the widefield image (WF), the phase and modulation images (first harmonic), the SPLIT-SIM images calculated with $\phi_{max}=0.5\pi$ or $\phi_{max}=\pi$, respectively, along with the corresponding residual components (OUT). This out-of-focus signal is correctly assigned to the OUT component, indicating optical sectioning effect. Scale bar 1μm.