Creating adaptable multi-focal arrays (MFAs) for pRESOLFT with a spatial light modulator (SLM)

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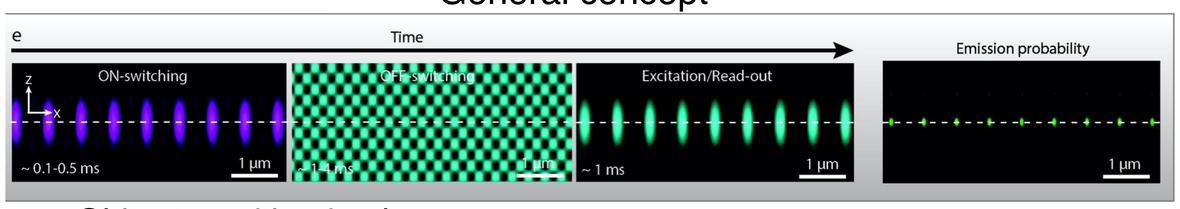
Abstract

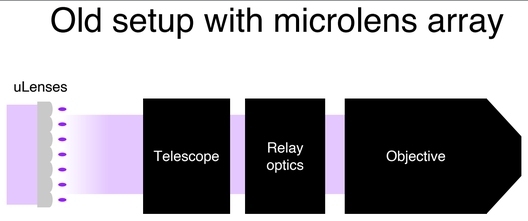
A common goal in super-resolution microscopy techniques is to increase the number of cells imaged per unit time (i.e., throughput). Multi-focal arrays (MFA) are used to speed up and provide optical sectioning in techniques that are based on a point-scanning process, such as conventional confocal and RESOLFT microscopy. Typically, the MFAs are created with fixed optics, such as a microlens array. However, these approaches are limited to a fixed periodicity and number of foci. Being able to adjust the MFA periodicity is crucial for adapting the imaging technique to various biological samples that require different scanning speeds, depths, and axial resolution. For example, a large periodicity is beneficial for reducing crosstalk and out of focus light in thick samples, while a small periodicity substantially reduces the acquisition time by minimizing the amount of scanning steps.
In this work, we use a spatial light modulator (SLM) in the Fourier plane of a lens to create MFAs with varying periodicities and number of spots and apply it to super-resolution imaging. The MFAs are created by displaying a computer-generated hologram (CGH) on the SLM. Adjustments to location and intensity of individual foci are made by changing the displayed CGH without having to adjust any additional optics. The CGHs are created through the weighted Gershberg-Saxton algorithm[2] and adaptively corrected to compensate for nonuniformity in intensity[3], which increases the usable field of view and thus the throughput. We show how the setup can be easily adjusted to optimize for different samples by altering the CGH that produces the MFA.

Setup for MFA illumination

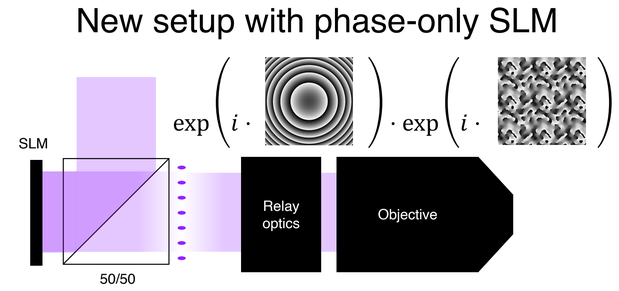
General concept[1]



Old setup with microlens array

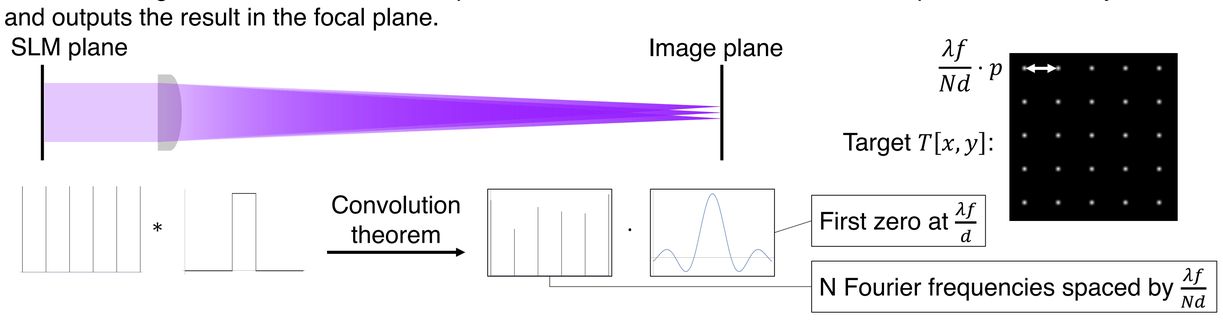


New setup with phase-only SLM



Generating holograms for a phase-only SLM

We are making use of the fact that a lens performs the Fourier transform on the complex electric field just before it and outputs the result in the focal plane.



For an $N\times N$ array of square SLM pixels, there will be an $N\times N$ array of diffraction-limited spots formed in the focal plane, with each spot corresponding to a Fourier frequency.

Weighted Gershberg-Saxton (WGS) algorithm[2]

We want to find the set of SLM pixel phases that maximizes the coefficients of the discrete Fourier transform frequencies that we have selected for our MFA.

$$maximize \rightarrow \sum_m w_m|V_m|$$

V_m: amplitude of the electric field at the m^{th} focus at $(\vec{x}_m+\vec{y}_m)$ w_m : weight to improve uniformity
k: iteration number ψ_m: m^{th} focus phase T_m: target amplitude for m^{th} focus
ϕ_j: phase of the j^{th} SLM pixel at $(\vec{x}_j+\vec{y}_j)$

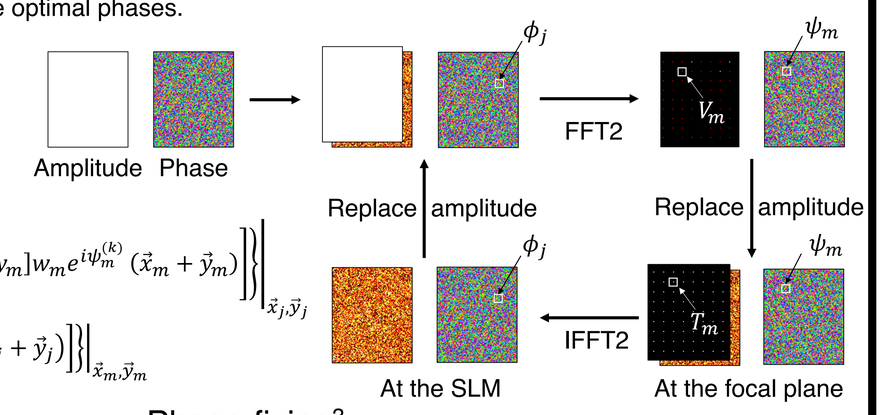
The WGS algorithm works by starting with a random initial guess for each ϕ_j and applying the maximization condition to take a step towards the optimal phases.

$$0th\ step:\ w_m^0=1, \phi_j^{(0)}=random$$

$$kth\ step: w_m^{(k)}=w_m^{(k-1)}\frac{\left\langle V_m^{(k-1)}\right\rangle}{\left|V_m^{(k-1)}\right|},$$

$$\phi_j^{(k)}=\arg\left\{FFT2^{-1}\left[\sum_m T[x_m,y_m]w_m e^{i\psi_m^{(k)}}(\vec{x}_m+\vec{y}_m)\right]\right\}\Big|_{\vec{x}_j,\vec{y}_j}$$

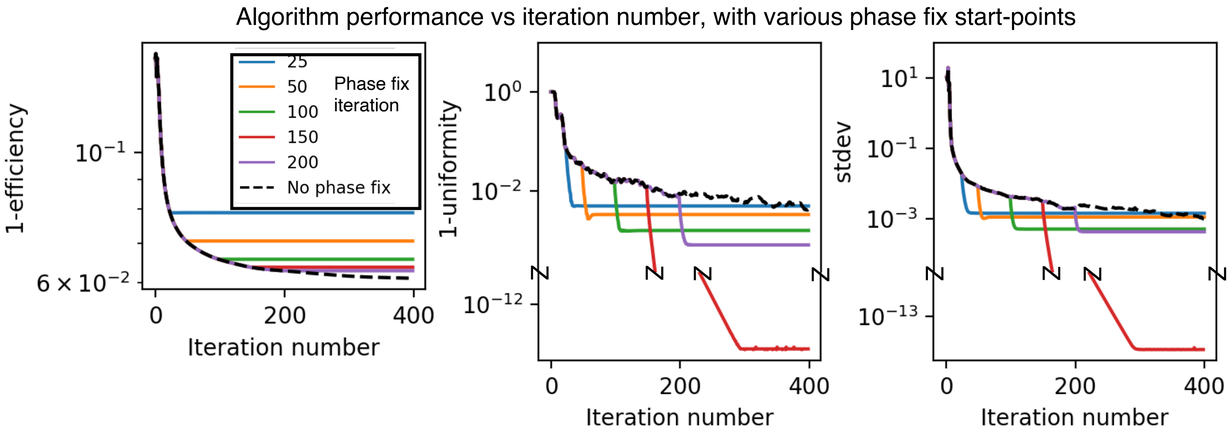
$$\psi_m^{(k)}=\arg\left\{FFT2\left[\sum_j e^{i\phi_j^{(k-1)}}(\vec{x}_j+\vec{y}_j)\right]\right\}\Big|_{\vec{x}_m,\vec{y}_m}$$



Phase-fixing[3]

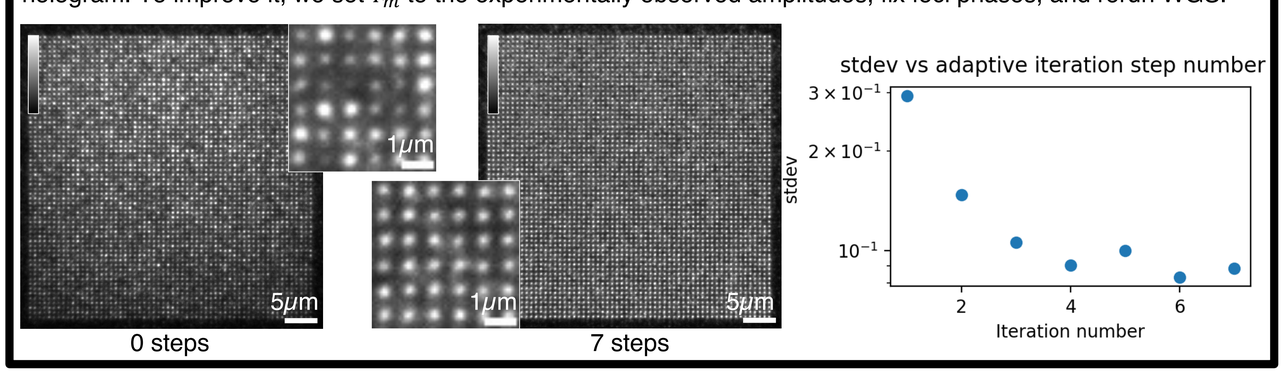
Fixing the foci phases (ψ_m) after a certain number of iterations quickly improves the uniformity and stdev by about two orders of magnitude. Sometimes, there is a massive leap in the theoretical performance for certain choices. The best choice of iteration number is unclear.

Algorithm performance vs iteration number, with various phase fix start-points



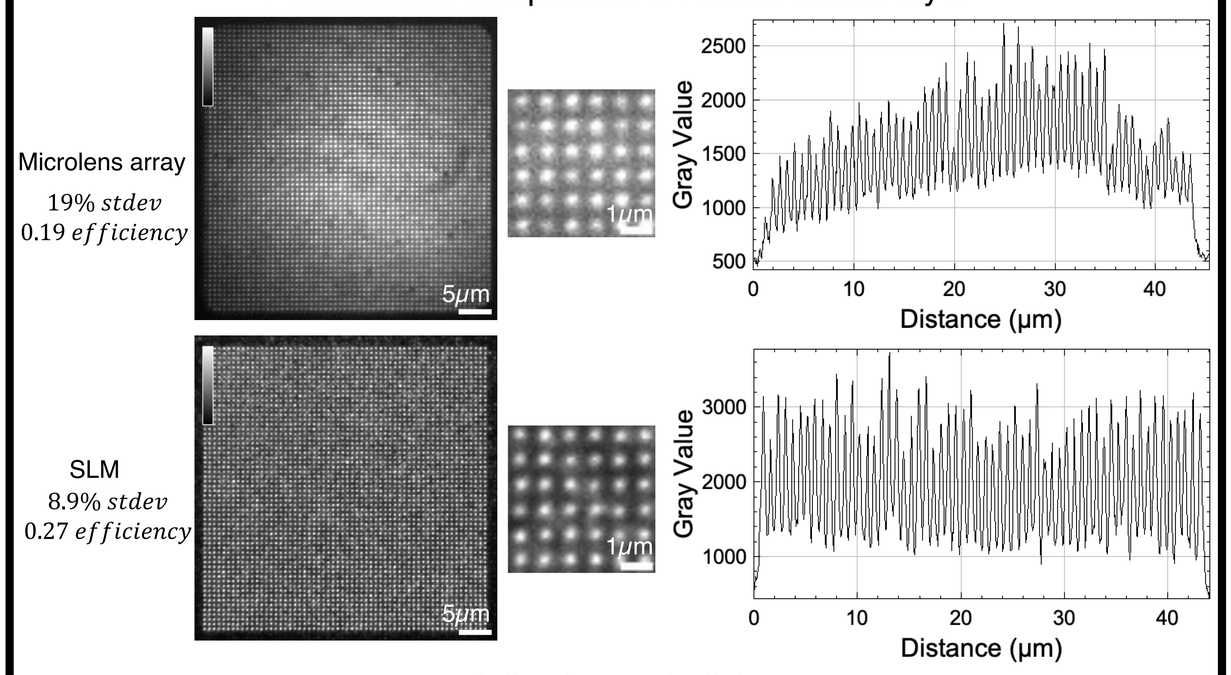
Adaptive correction[3]

In practice, the stdev is two orders of magnitude worse than the ideal values calculated when generating the hologram. To improve it, we set T_m to the experimentally observed amplitudes, fix foci phases, and rerun WGS.



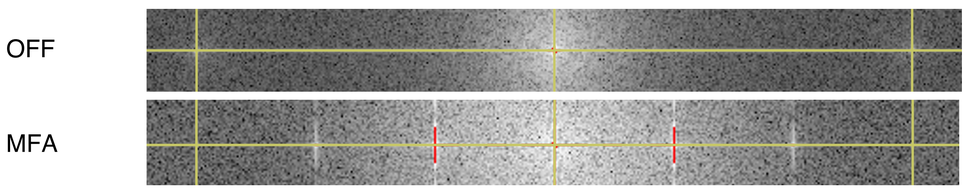
Comparing the SLM and microlens MFA

Performance comparison on a fluorescent layer

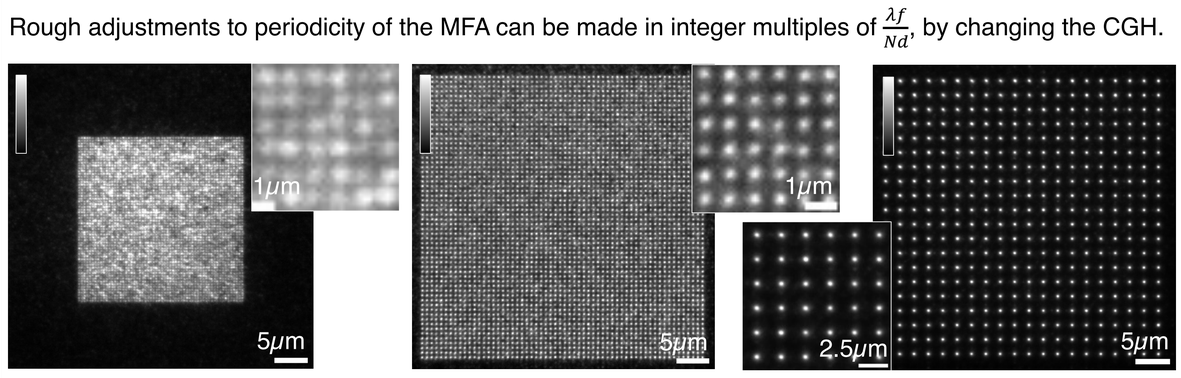


Adjusting periodicity

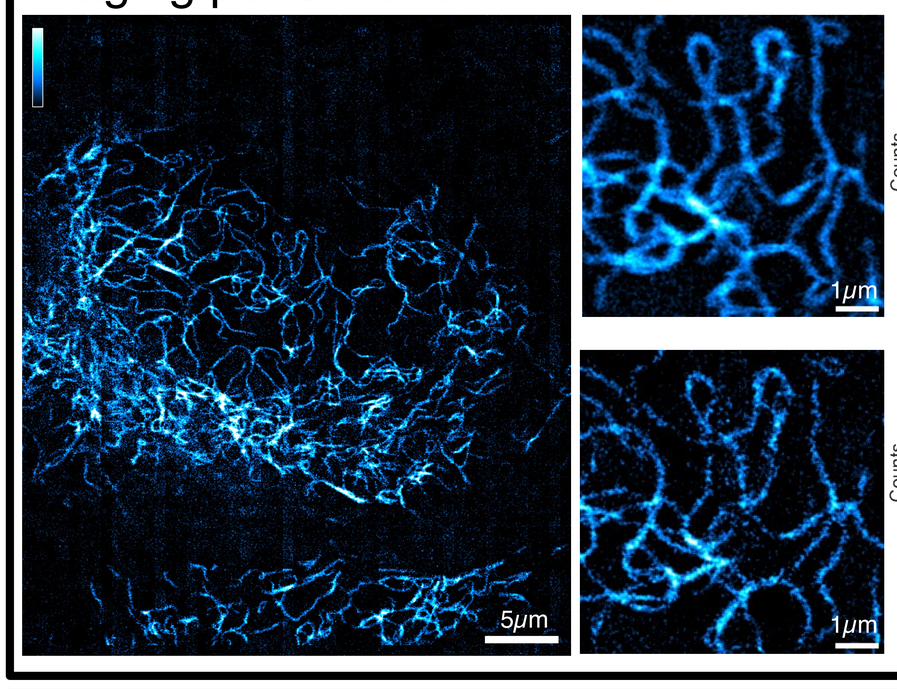
Fine adjustment of the periodicity of the MFA can be done without a telescope, just by adjusting the Fresnel lens focal length f. This was done to match the fundamental frequency of the SLM MFA to the 2nd harmonic of the off-pattern for the imaging in the following section.



Rough adjustments to periodicity of the MFA can be made in integer multiples of $\frac{\lambda f}{Nd}$, by changing the CGH.

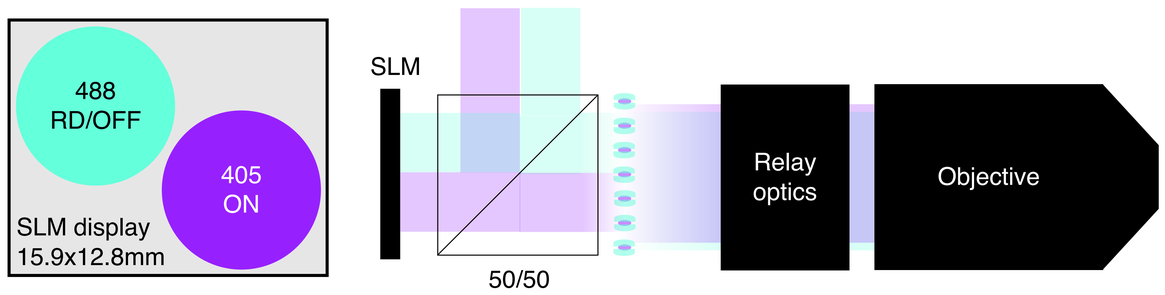


Imaging performance



Future steps

We plan to test the effect of pattern period on reconstructed image quality for different samples and imaging depths. We are also testing the possibility to address all three beam paths with the SLM, which would greatly increase the modularity of the setup.



[1] Bodén, A., Pennacchietti, F., Coceano, G. et al. Volumetric live cell imaging with three-dimensional parallelized RESOLFT microscopy. Nat Biotechnol 39, 609–618 (2021). https://doi.org/10.1038/s41587-020-00779-2

[2] Roberto Di Leonardo, Francesca Ianni, and Giancarlo Ruocco, "Computer generation of optimal holograms for optical trap arrays," Opt. Express 15, 1913-1922 (2007)

[3] Kim, Donggyu, et al. "Large-scale uniform optical focus array generation with a phase spatial light modulator." Optics letters 44.12 (2019): 3178-3181.

Acknowledgements

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