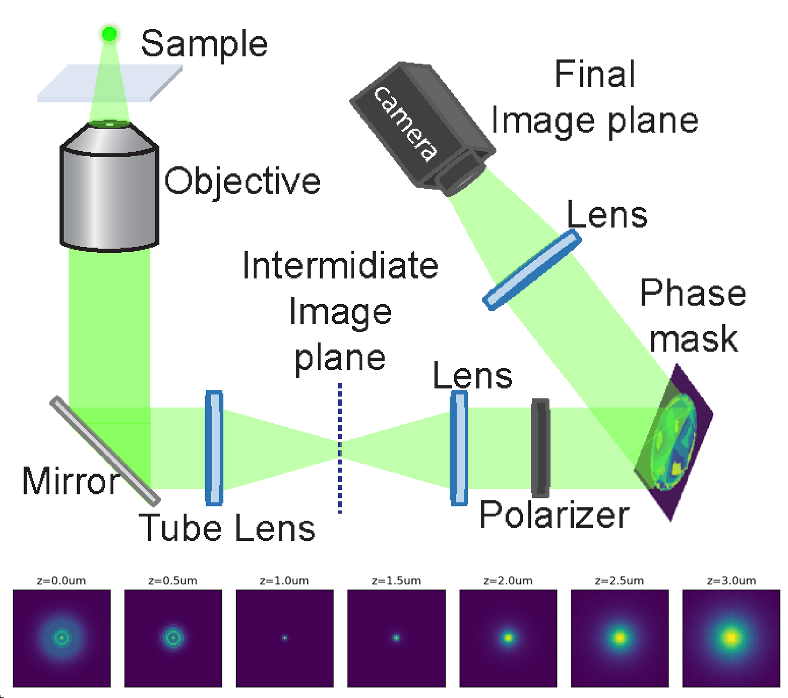
# Learning optimal multicolor PSF design for 3D pairwise distance estimation

Ofri Goldenberg[1], Boris Ferdman[1,2], Elias Nehme[1,3], Yael Shalev Ezra[1], Yoav Shechtman[1,2]

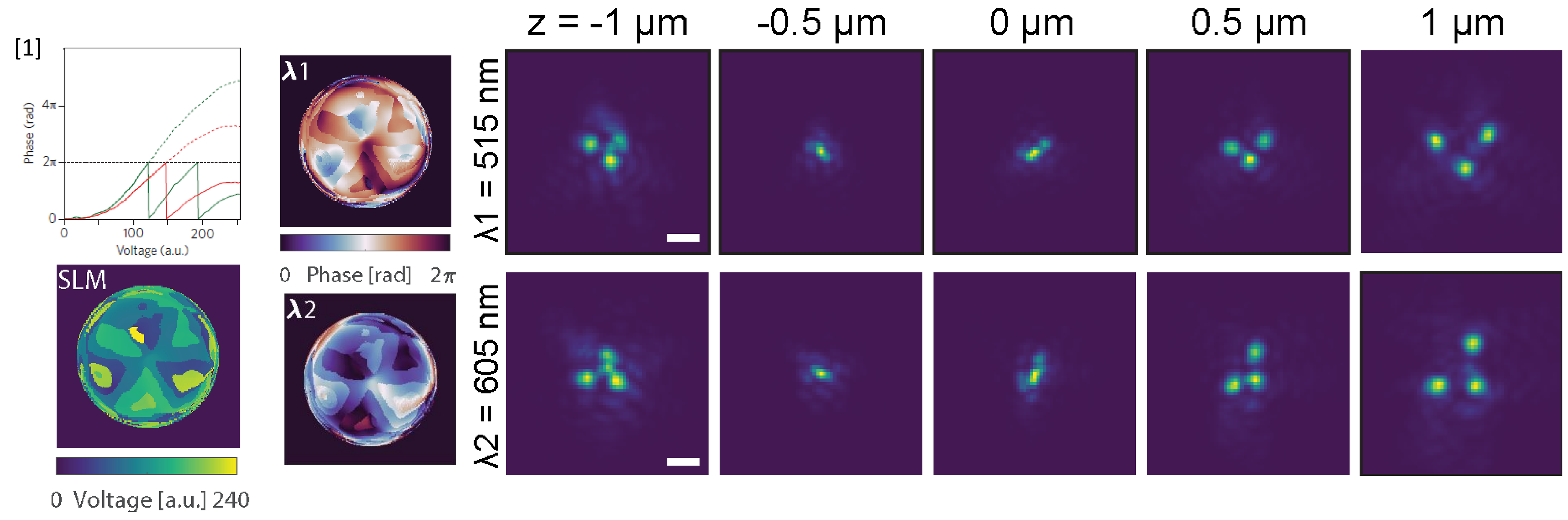
[1]Department of Biomedical Engineering and Lorry I. Lokey Interdisciplinary Center for Life Sciences and Engineering , Technion IIT,
[2]Russell Berrie Nanotechnology Institute, Technion IIT, [3]Viterby Faculty of Electrical and Computer Engineering, Technion IIT

## Introduction

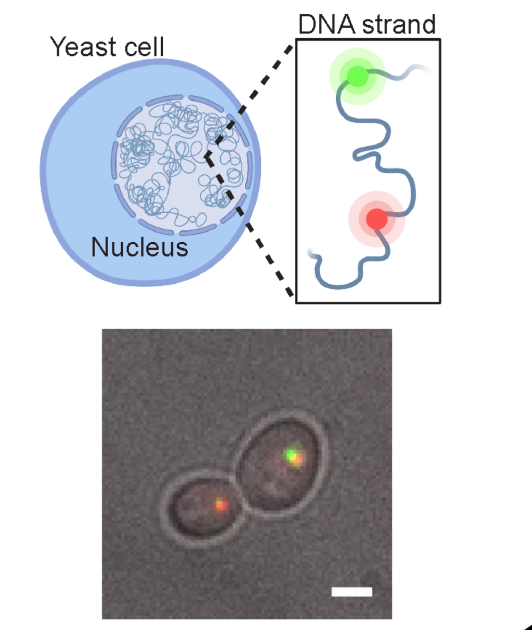
**4f optical setup**




**Multicolor PSF engineering**

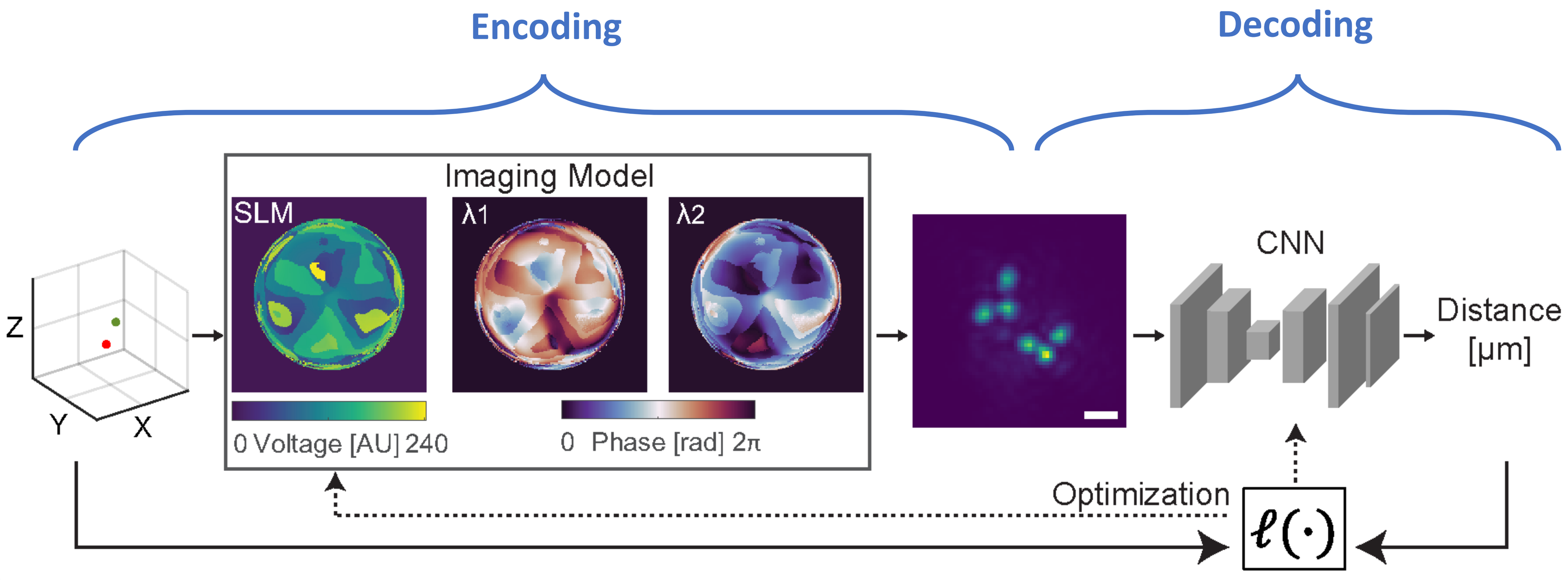



**3D reconfiguration of chromosomes in yeast cells**

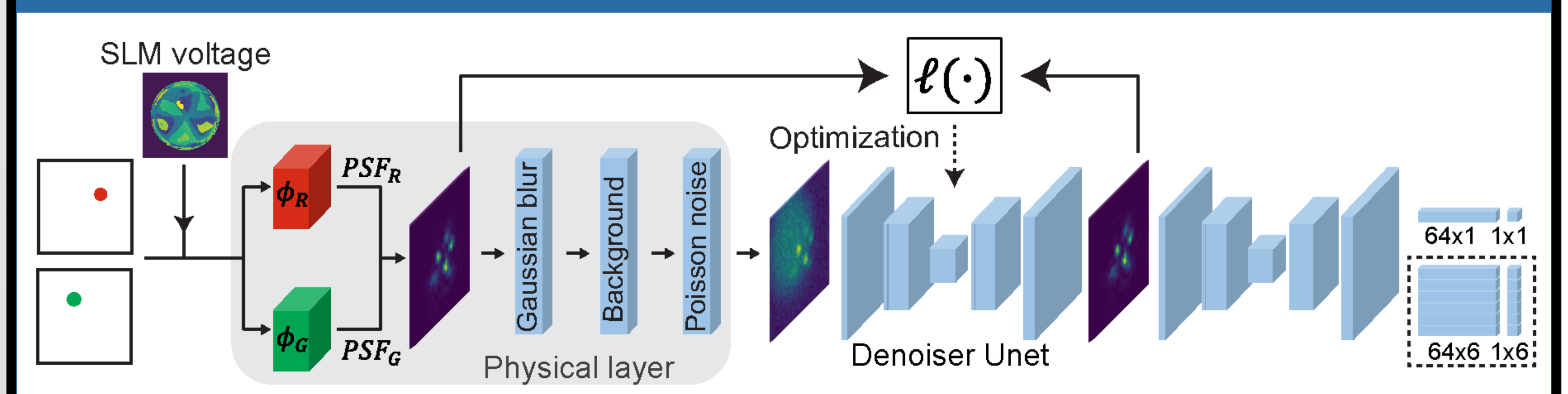



## Project Goal

Design an optical system that images microscopic samples and directly produces the **scalar 3D distance** between two emitters, labeled with different fluorescent labels. We use multicolor PSF engineering, and design a neural network to jointly learn both the encoding – the **optimal phase mask** for the task, and the decoding – neural network that computes the absolute distance given the modified images.



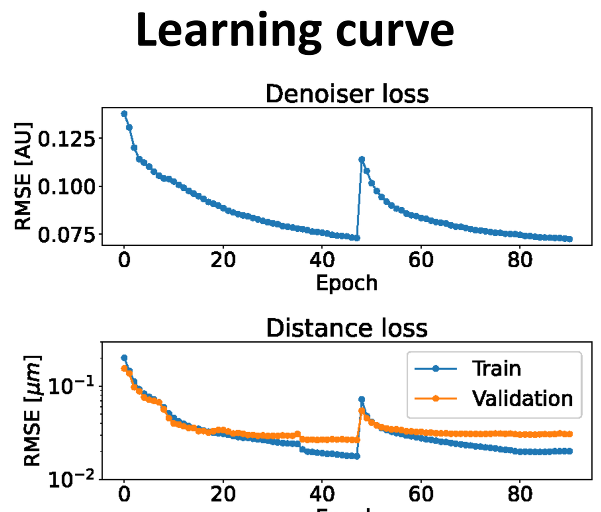

## Methods



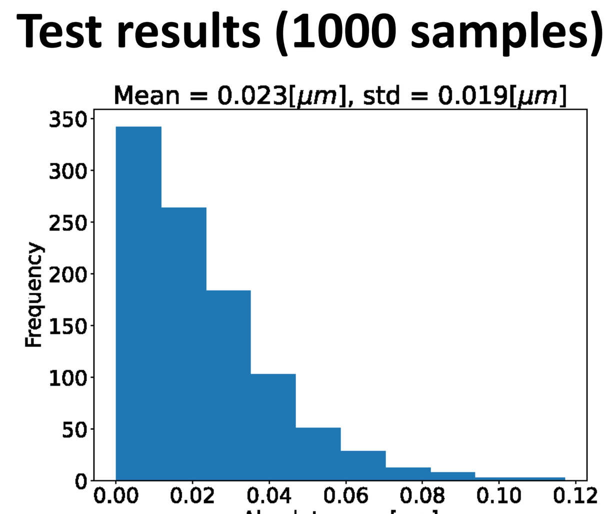

- **Differentiable physical layer** for image generation, implementation and optimization of the voltage mask.
- **Reconstruction module** based on U-net [2] architecture for denoising and multiscale feature extraction, estimating scalar 3D distance.
- **Training and validation** sets consists of randomly sampled 3D coordinates of the two emitters, and random photon count for each emitter. Training set: 60000 samples, validation set: 5000 samples, test set: 1000 samples.
- Two steps training in each epoch: first only the denoiser was optimized, while the PSF is unchanged, with the denoiser loss only- MSE between the intermediate image (the first U-net output) and the simulated image of the PSFs before the added noise. Then the voltage mask was trained together with the rest of the network weights to optimize the MSE loss between the output and the true distance.
- The network was implemented in Pytorch and trained on a single Titan RTX GPU for ~8 hours.

## Simulation results

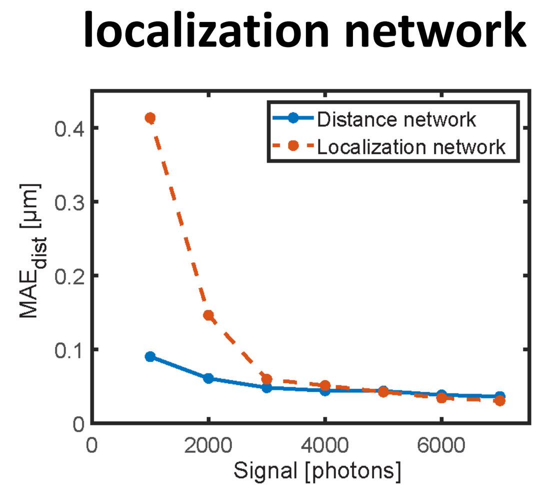
**Learning curve**




**Test results (1000 samples)**

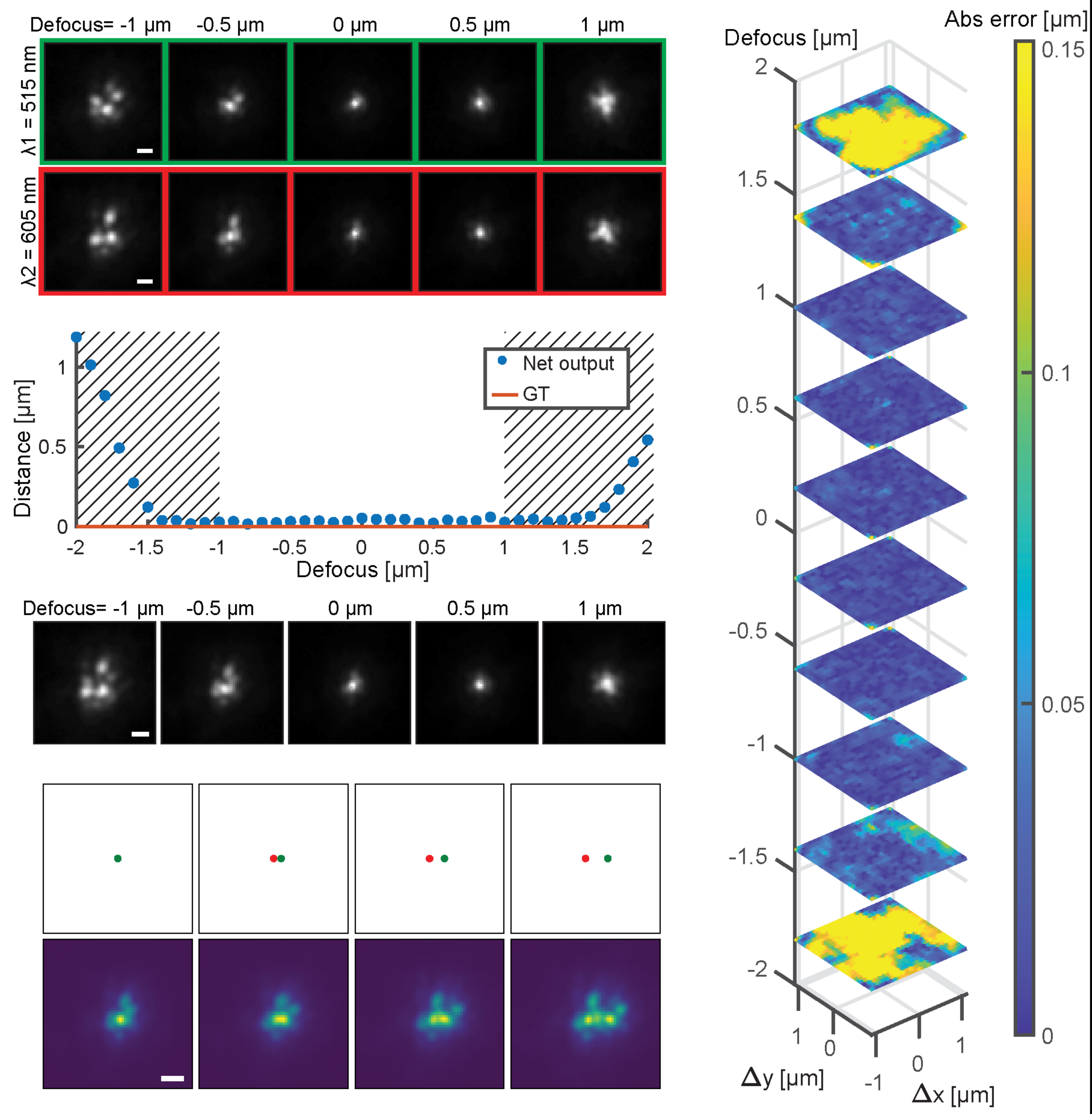



**Comparison to localization network**

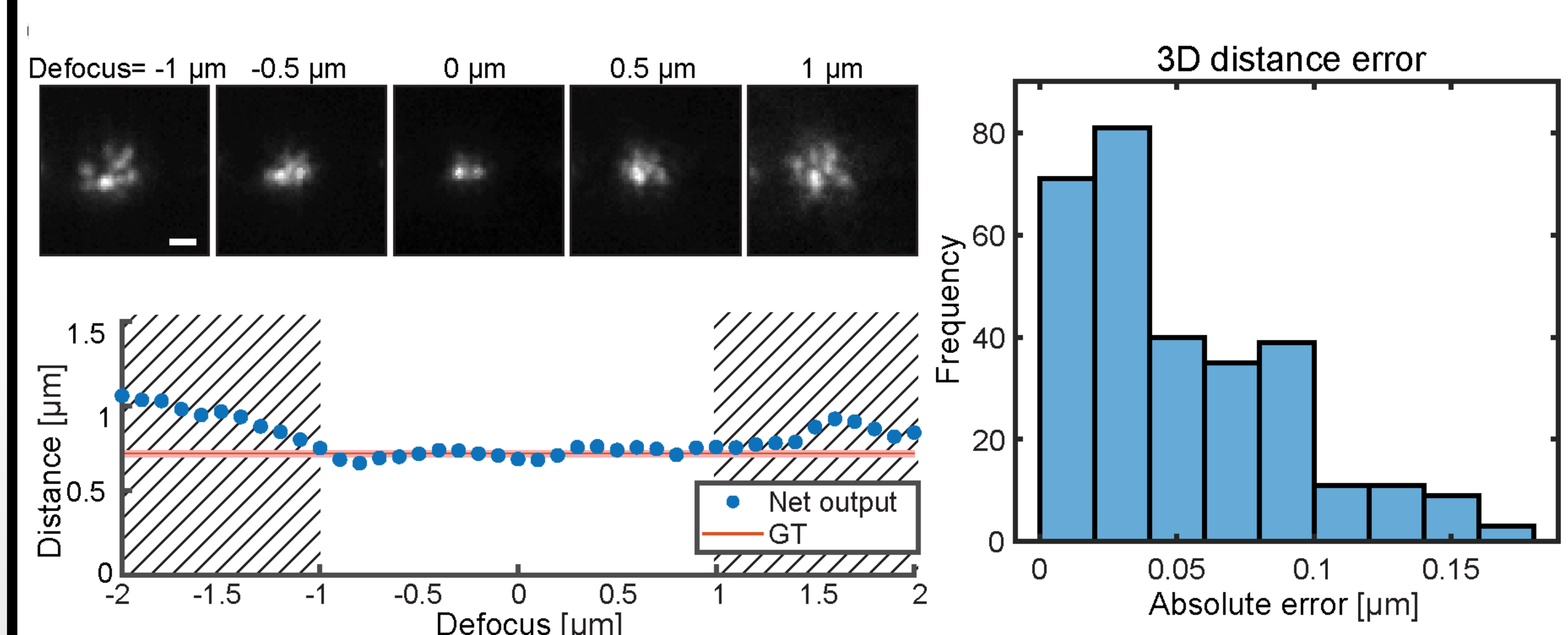



## Experimental Results

**1) Hybrid experiment**

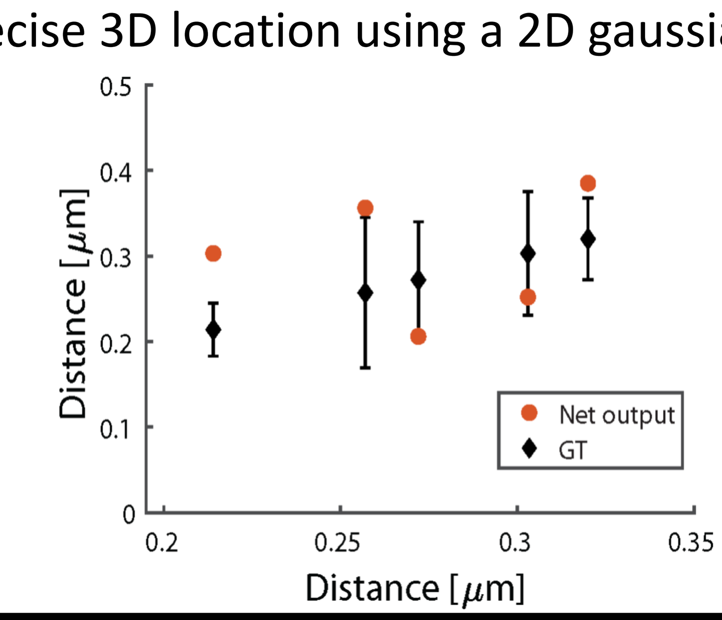


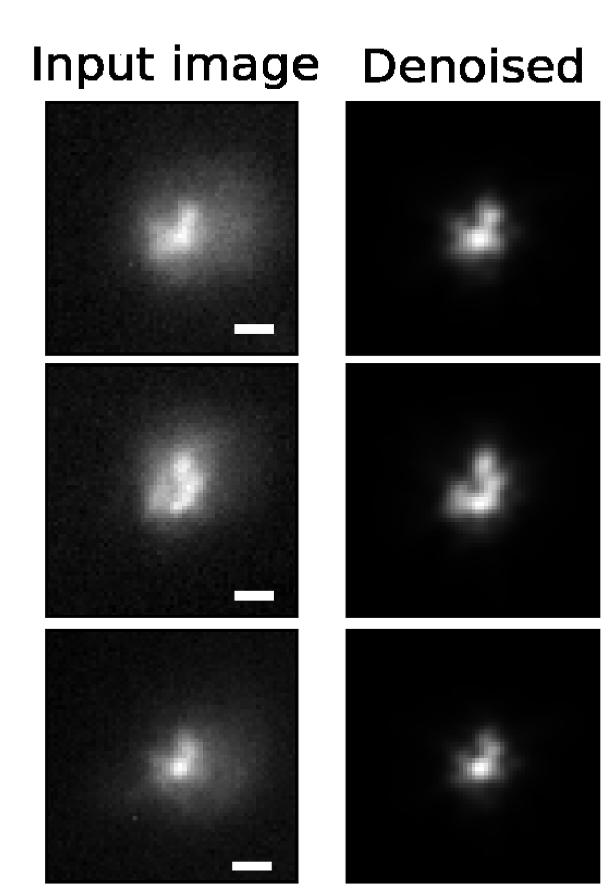

**2) Multicolored bead-pairs experiment**




**3) Yeast cells experiment**

Ground truth distance was estimated by imaging each of the colors separately by a z-stack, without the mask, to find their precise 3D location using a 2D gaussian fit.




[1] Shechtman, Y., Weiss, L. E., Backer, A. S., Lee, M. Y. & Moerner, W. E. Multicolour localization microscopy by point-spread-function engineering. Nat. Photonics 10, 590–594 (2016); [2] Ronneberger, O., Fischer, P. & Brox, T. U-net: Convolutional networks for biomedical image segmentation