

# Modern approaches to live cell fluorescence microscopy

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# About Me

## Education

- ▶ PhD @ Purdue University, MS @ University of Chicago

## Research interests

- ▶ Bioimaging, Machine learning methods for live cell imaging
- ▶ Quantitative single molecule localization microscopy
- ▶ Generative models, statistical physics, theory of deep learning

# Outline of the talk

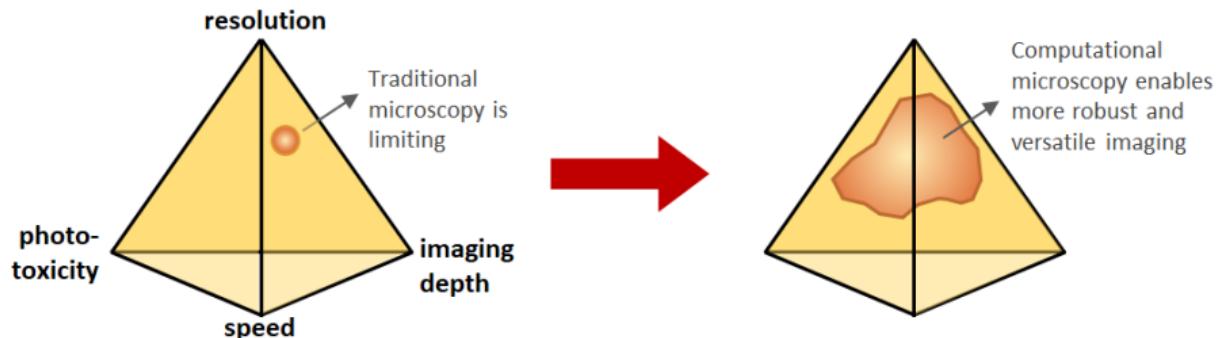
Live cell super resolution microscopy

Deep learning models for modern microscopy

## Live cell super resolution microscopy

# A live cell fluorescence imaging zoo

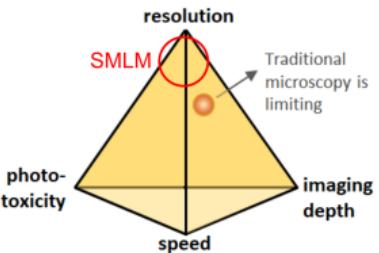
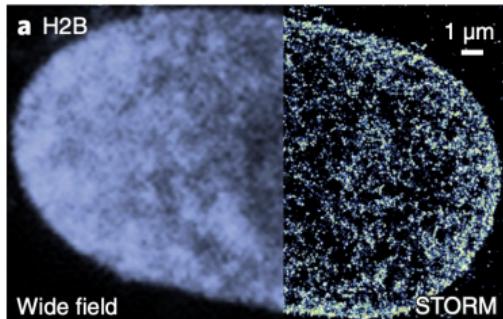
# The inherent tradeoffs of fluorescence microscopy



Courtesy of Chowdhury lab at UT Austin

- ▶ Ex. increase in resolution is often accompanied by loss of imaging speed
- ▶ Computational methods such as AI/ML algorithms can help find a superior balance

# Single molecule localization microscopy (SMLM)

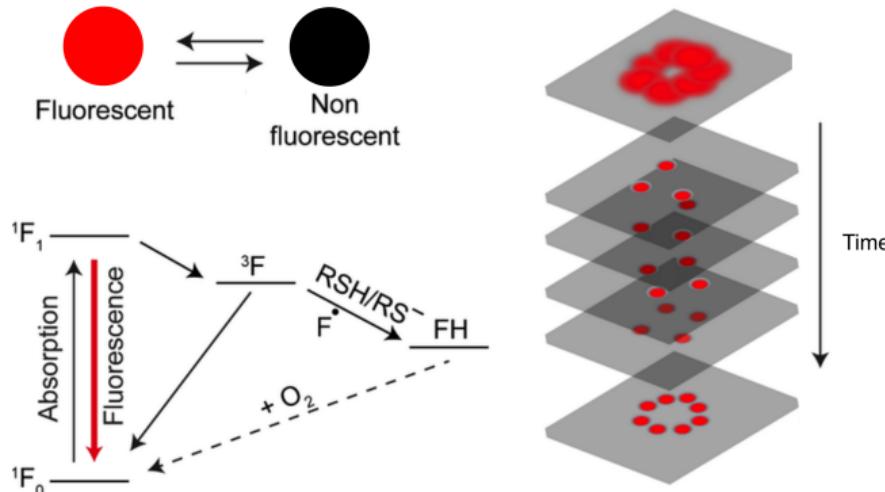


- ▶ SMLM techniques can achieve 10nm resolutions (20x higher than diffraction limit)

## Single molecule localization microscopy (SMLM)

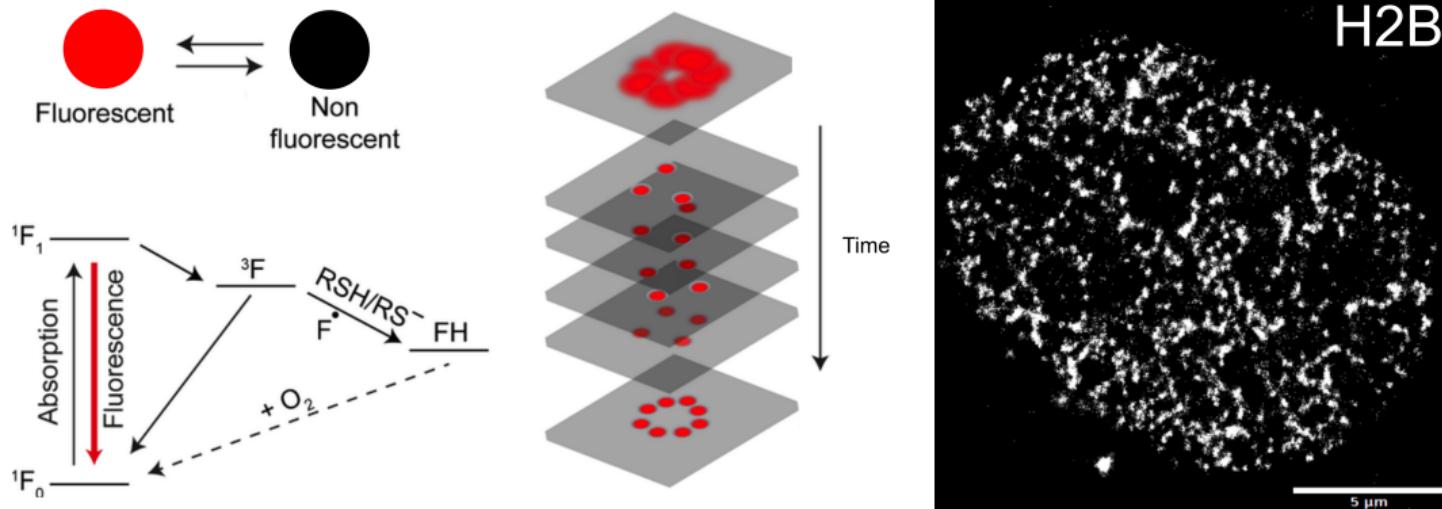
SMLM is all about *control* of the fluorescence

# Single molecule localization microscopy



- ▶ STORM and similar nanoscopy techniques are limited by localization precision
- ▶ Higher lateral/axial resolution than other methods (e.g., SIM, STED, Confocal)
- ▶ Poor time resolution

# Stochastic optical reconstruction microscopy (STORM)



- ▶ STORM and similar nanoscopy techniques are limited by localization precision
- ▶ Higher lateral/axial resolution than other methods (e.g., SIM, STED, Confocal)
- ▶ Poor time resolution

# Nanoscopy by localizing isolated fluorescent emitters

- Modeling the point spread function permits sub-pixel localization

$$\mu_k = i_0 \int \int O(x, y) dx dy + \lambda$$

$$i_0 = g_k \eta \zeta \Delta$$

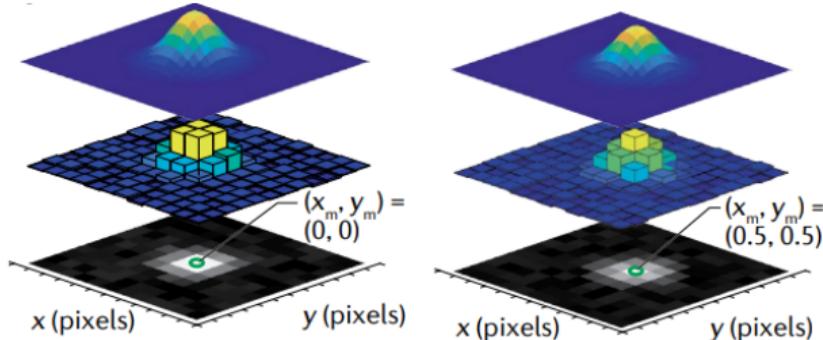
$g_k$  – pixel gain

$\eta$  – quantum efficiency

$\zeta$  – photon emission rate

$\Delta$  – exposure time

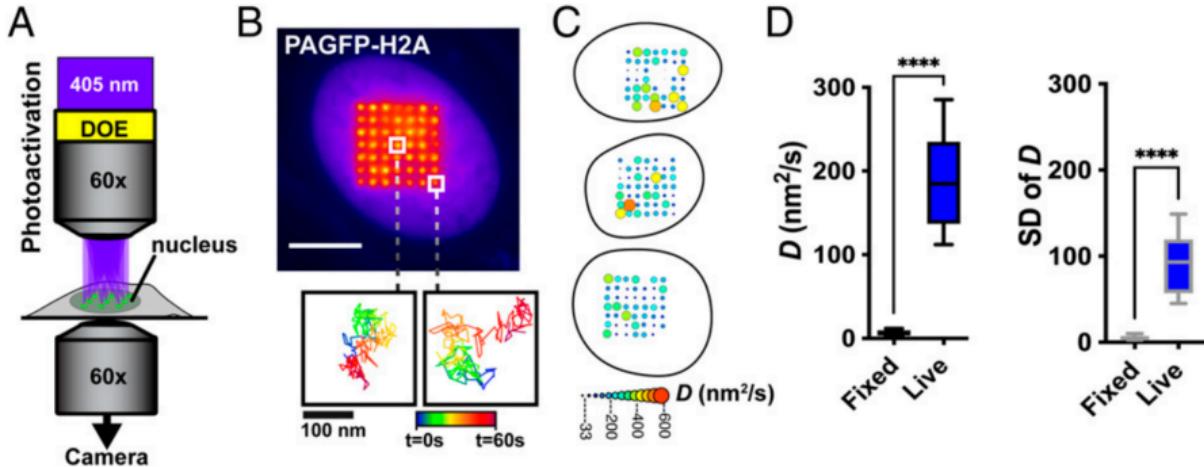
$\lambda$  – background rate



Maximum likelihood localization:

$$\theta^* = \operatorname{argmax}_{\theta} \prod_k p(\mathbf{x}_k | \theta) = \operatorname{argmin}_{\theta} - \sum_k \log p(\mathbf{x}_k | \theta)$$

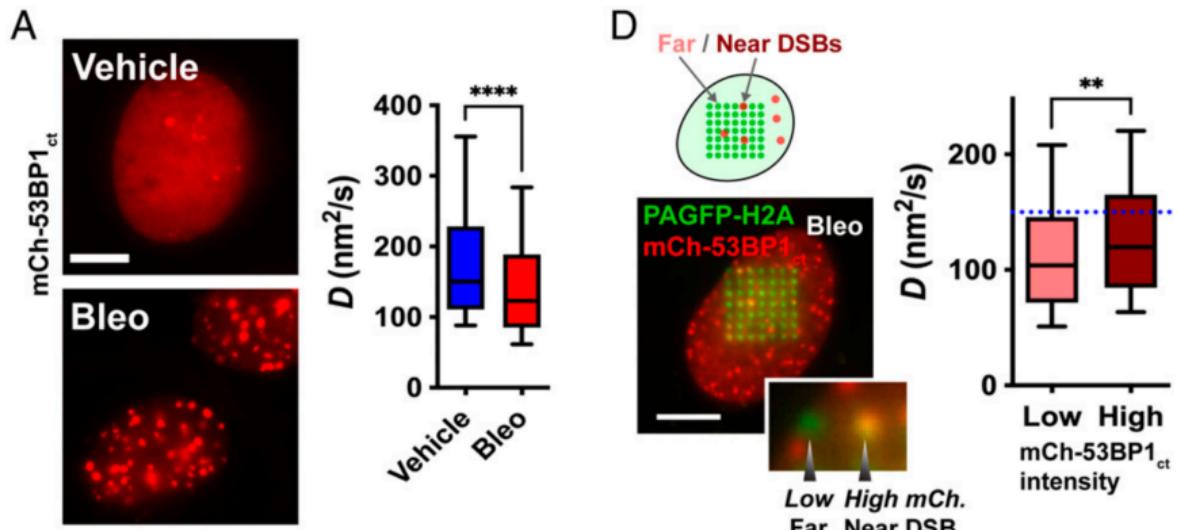
# Tracking chromatin loci with photoactivated localization microscopy



Locatelli, Seitz et al. PNAS **29** (2022)

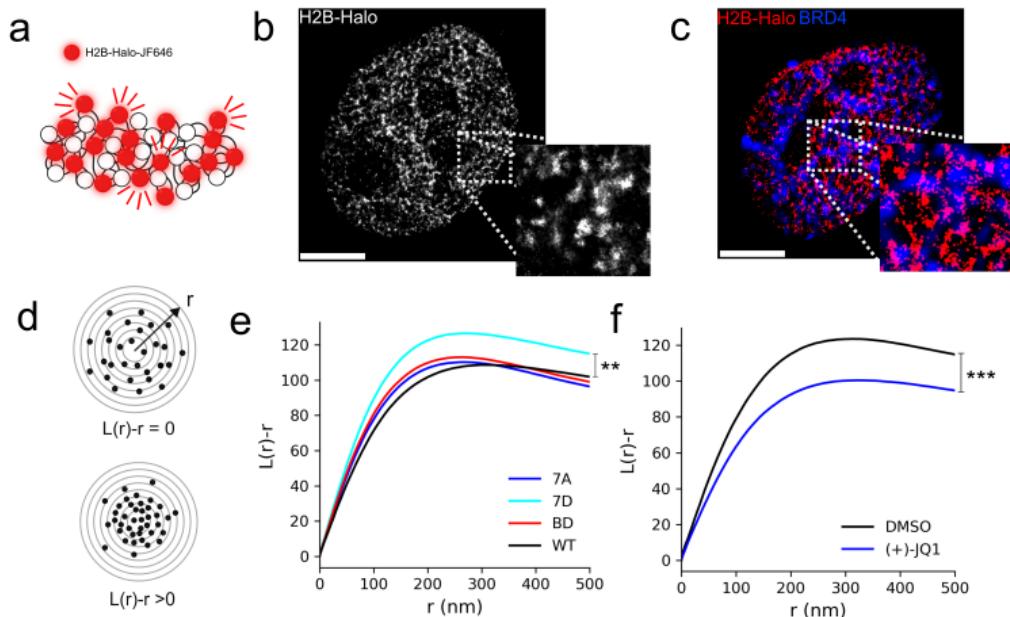
- ▶ A diffractive optical element (DOE) is used to photoactivate chromatin microdomains
- ▶ Used for understanding the spatial correlations of chromatin diffusion

# Tracking chromatin loci with photoactivated localization microscopy



Locatelli, Seitz et al. PNAS **29** (2022)

# Analyzing the structure of nucleosome nanodomains with SMLM



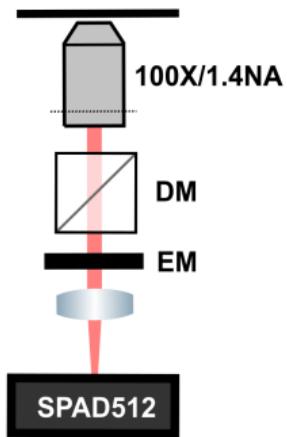
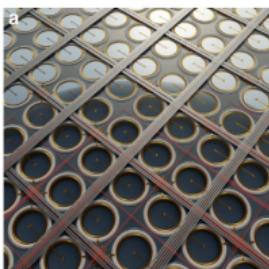
Seitz et al. bioRxiv, Cells, In Review (2025)

- ▶ H2B is densely labeled for super-resolution imaging

# Fast single photon sensitive cameras for localization microscopy

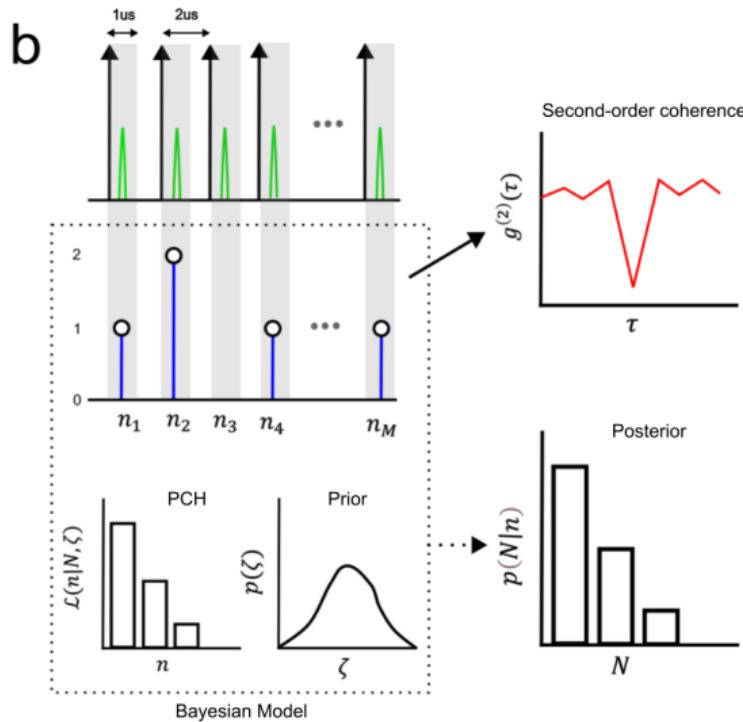
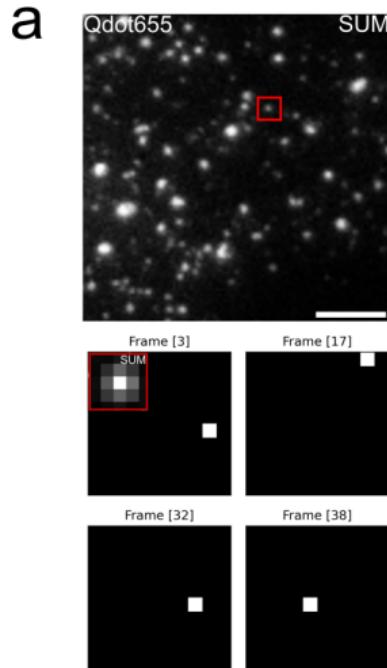


SPAD512



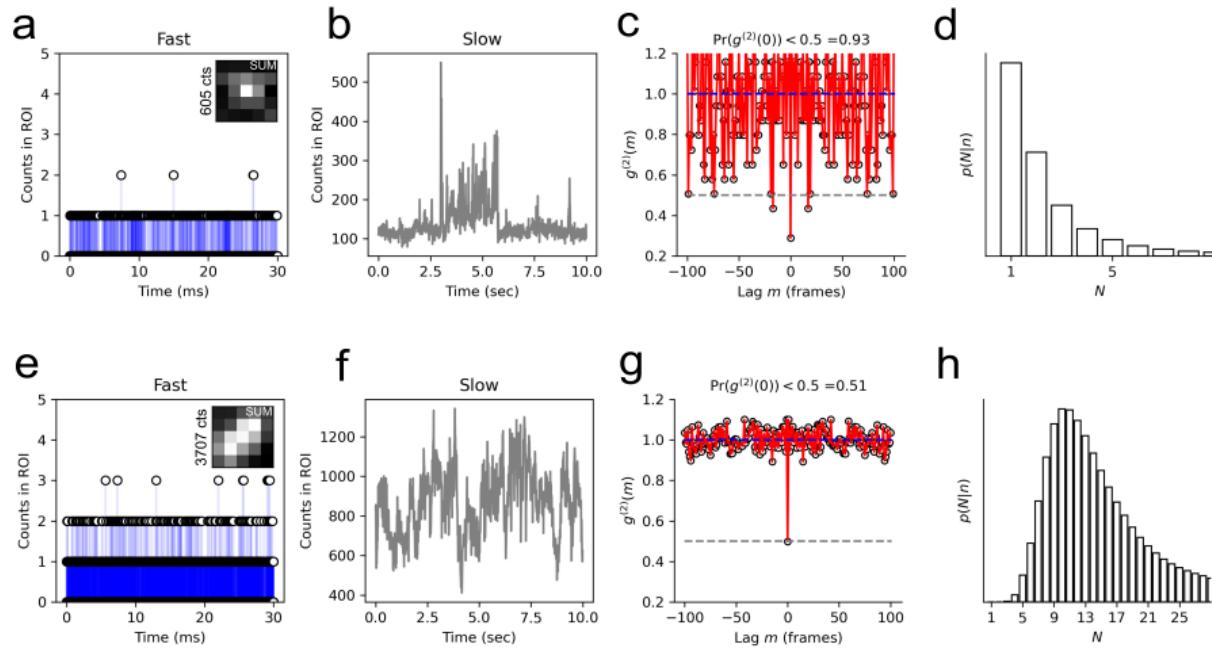
- ▶ Single photon avalanche diode (SPAD) array
- ▶ Frame rates up to 100kHz enable generating images photon by photon

# Quantitative SMLM by measuring the photon counting histogram



Seitz et al. Communications Physics, In Review (2025)

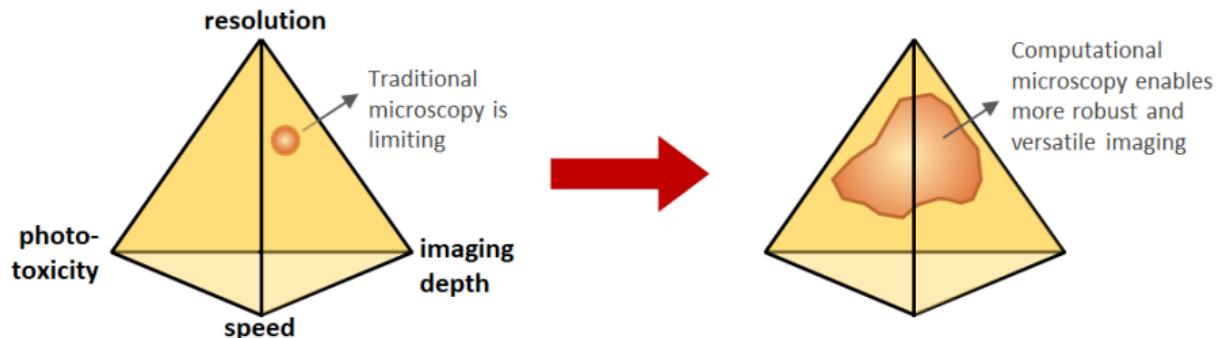
# Quantitative SMLM by measuring the photon counting histogram



Seitz et al. Communications Physics, In Review (2025)

## Deep learning models for modern microscopy

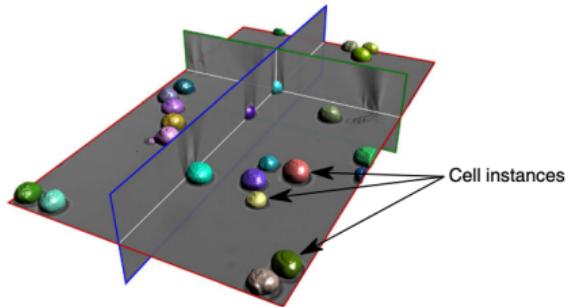
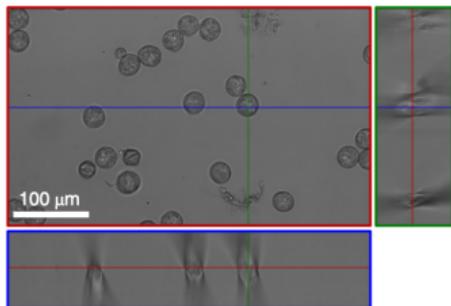
# The inherent tradeoffs of fluorescence microscopy



Courtesy of Chowdhury lab at UT Austin

- ▶ Ex. increase in resolution is often accompanied by loss of imaging speed

## Deep learning based 3D reconstruction

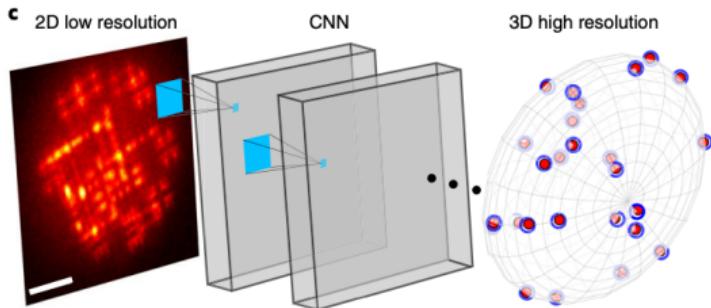


Falk et al. Nat Methods **16** 2019

- ▶ A deep neural network UNet is trained to predict cell volumes
- ▶ Can predict more axial slices than measured

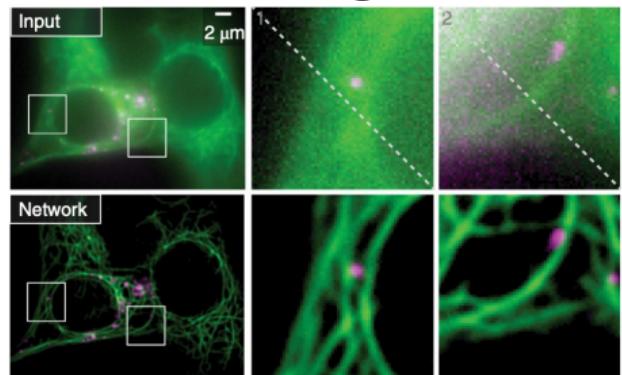
# Robust 3D localization microscopy using deep learning

## DeepSTORM3D



Nehme et al. Nat Methods **17** 2020

## Content-aware image restoration



Weiger et al. Nat Methods **15** 2018

- ▶ DeepSTORM3D localizes fluorescent molecules in 3D
- ▶ Content aware image restoration - image denoising for rapid imaging

## Inverse problems in microscopy are often ill-posed

- ▶ Inverse problems deal with the task of finding parameters of interest from observations
- ▶ Inverse problems are usually *ill-posed*
- ▶ “Ill-posed” means that observations underdetermine the system

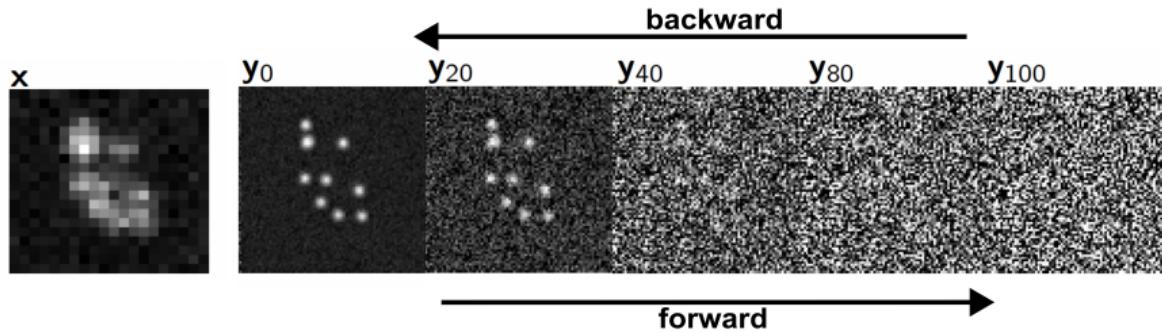
Suppose  $\theta$  is some latent of interest and we make a measurement  $\mathbf{x}$

Many  $\theta$  may be consistent with the measurement  $\mathbf{x}$

## Resolution enhancement with a diffusion model

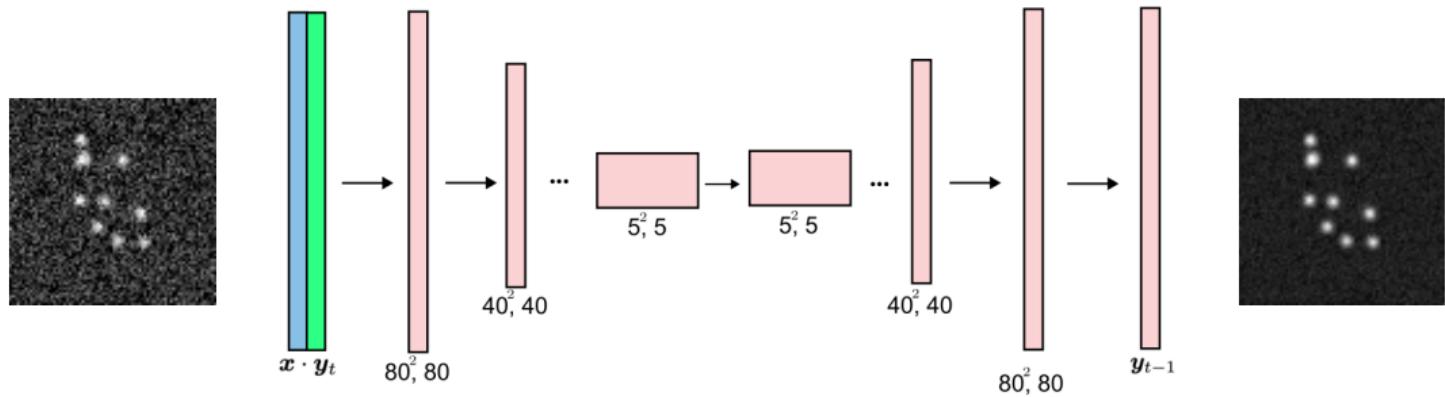
- ▶ Task: infer a high resolution image  $\mathbf{y}_0$  from low resolution  $\mathbf{x}$
- ▶ Drift is not available for image data, but can be learned from pairs  $(\mathbf{x}, \mathbf{y}_0)$

$$p_{\psi}(\mathbf{y}_{t-1} | \mathbf{y}_t, \mathbf{x}) = \mathcal{N}(\mu_{\psi}, \beta_t I)$$



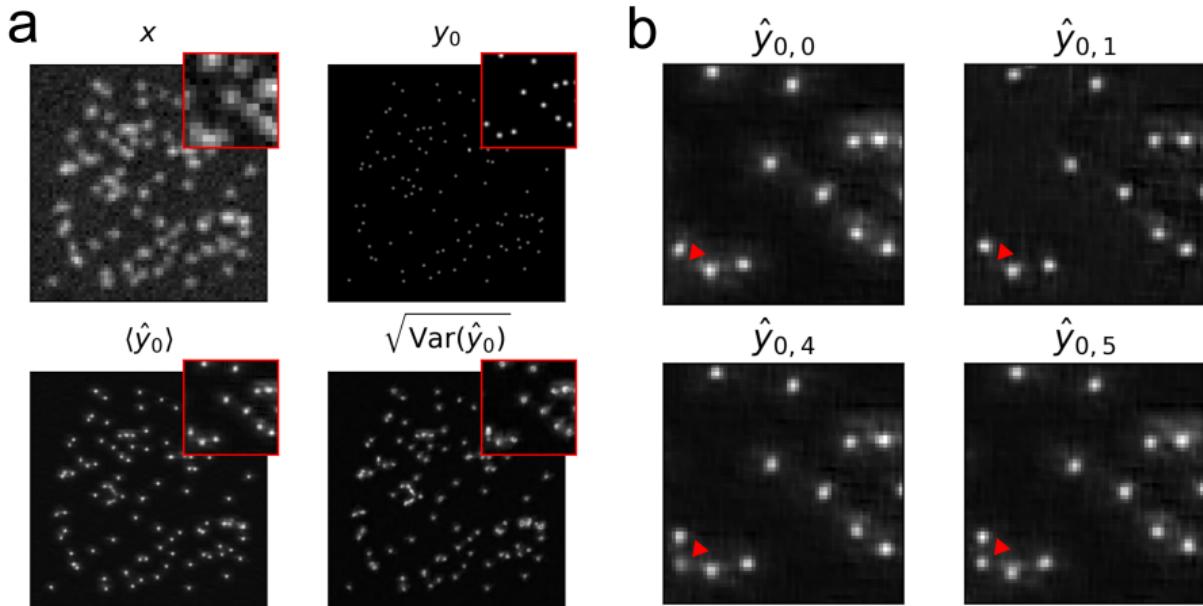
$$q(\mathbf{y}_t | \mathbf{y}_{t-1}) = \mathcal{N}\left(\sqrt{1 - \beta_t} \mathbf{y}_{t-1}, \beta_t I\right)$$

## Resolution enhancement with a diffusion model



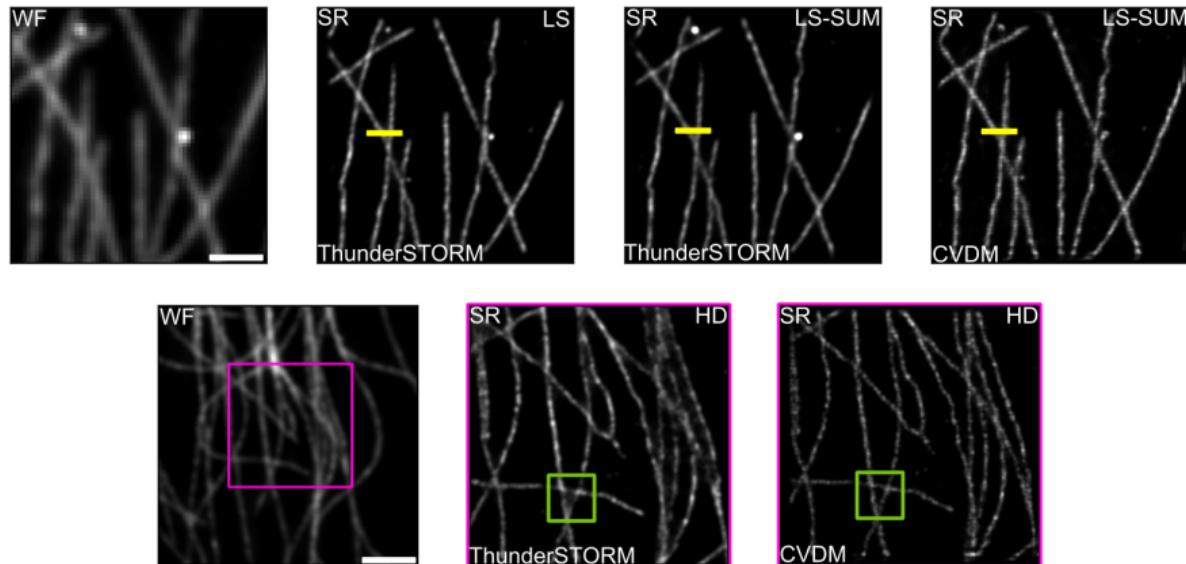
- ▶ A convolutional neural network  $\psi$  estimates the drift  $\mu_\psi$
- ▶ Denoising step:  $\mathbf{y}_{t-1} \sim p_\psi(\mathbf{y}_{t-1} | \mathbf{y}_t, \mathbf{x}) = \mathcal{N}(\mu_\psi, \beta_t I)$

## Super resolution with a diffusion model



- ▶ We can draw several samples  $\hat{\mathbf{y}}_0 \sim p(\mathbf{y}_0|\mathbf{x})$
- ▶ This allows computation of posterior statistics e.g.,  $\langle \hat{\mathbf{y}}_0, \sqrt{\text{Var}(\hat{\mathbf{y}}_0)} \rangle$

# Super resolution of microtubulues with a conditional diffusion model



- ▶ Strong agreement of diffusion model with existing algorithms, while modeling uncertainty

## Summary

## Selected Publications

- ▶ **C. Seitz**, D. Fu, M. Liu, H. Ma, and J. Liu. *BRD4 phosphorylation regulates the structure of chromatin nanodomains*. In Review. Phys Rev Lett. 2024
- ▶ **C. Seitz** and J. Liu. *Uncertainty-aware localization microscopy by variational diffusion*. In Progress. 2024
- ▶ **C. Seitz** and J. Liu. *Quantum enhanced localization microscopy with a single photon avalanche diode array*. In Progress. 2024
- ▶ M. Locatelli<sup>†</sup>, J. Lawrimore<sup>†</sup>, H. Lin<sup>†</sup>, S. Sanaullah, **C. Seitz**, D. Segall, P. Kefer, S. Moreno Naike, B. Lietz, R. Anderson, J. Holmes, C. Yuan, G. Holzwarth, B. Kerry, J. Liu, K. Bonin, P. Vidi. *DNA damage reduces heterogeneity and coherence of chromatin motions*. PNAS 12 July 2022; 119 (29): 1-11
- ▶ M. Zhang, **C. Seitz**, G. Chang, F. Iqbal, H. Lin, and J. Liu *A guide for single-particle chromatin tracking in live cell nuclei*. Cell Biology International 15 January 2022; 46 (5): 683-700

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