

Diffusion based generative models for modern 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

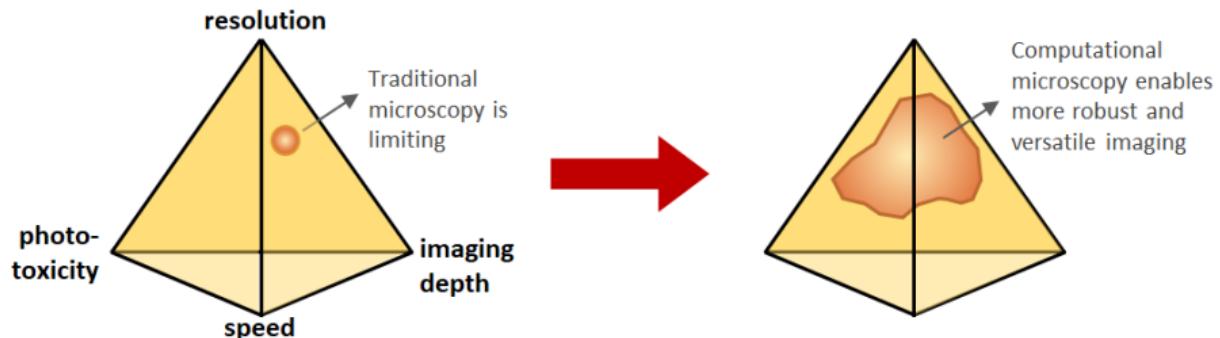
Live cell super resolution microscopy

Deep learning models for modern microscopy

Live cell super resolution microscopy

A live cell fluorescence imaging zoo

The inherent tradeoffs of fluorescence microscopy



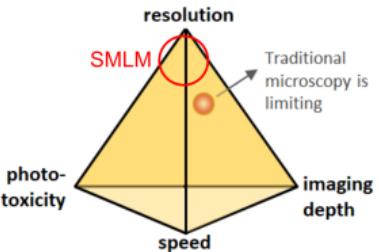
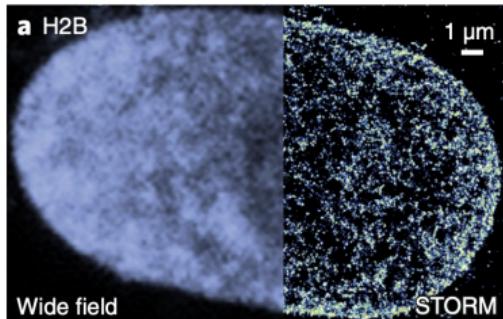
Courtesy of Chowdhury lab at UT Austin

- ▶ 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)

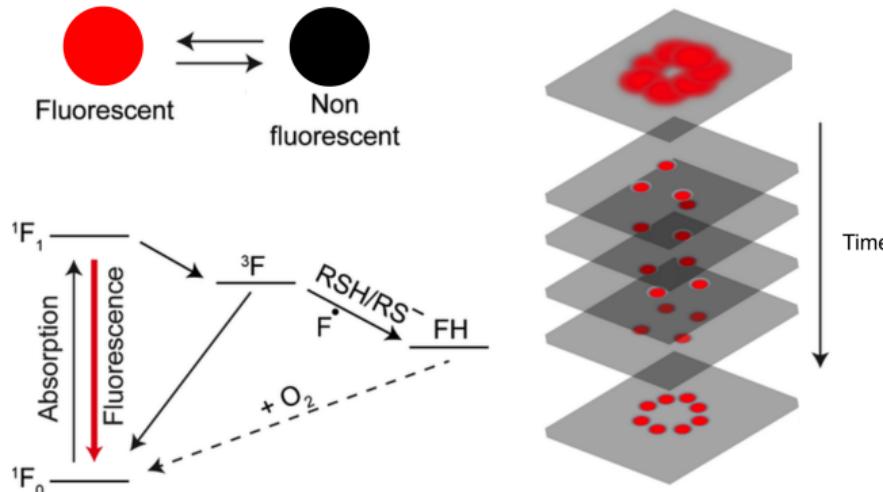
SMLM is all about *control* of the fluorescence

Single molecule localization microscopy (SMLM)



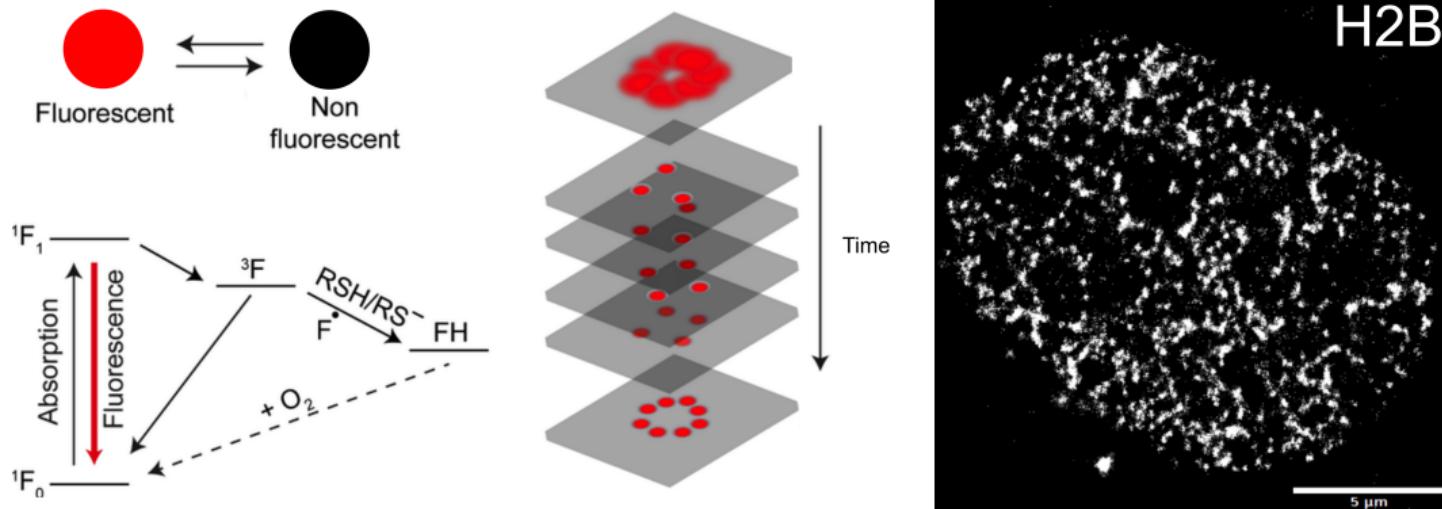
- ▶ SMLM techniques can achieve 10nm resolutions (20x higher than diffraction limit)

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

$$\mu_k = i_0 \int \int O(u, v) du dv + \lambda$$

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

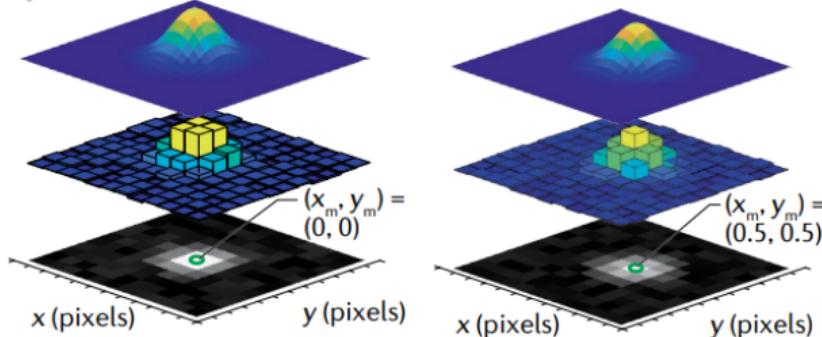
g_k – pixel gain

η – quantum efficiency

ζ – photon emission rate

Δ – exposure time

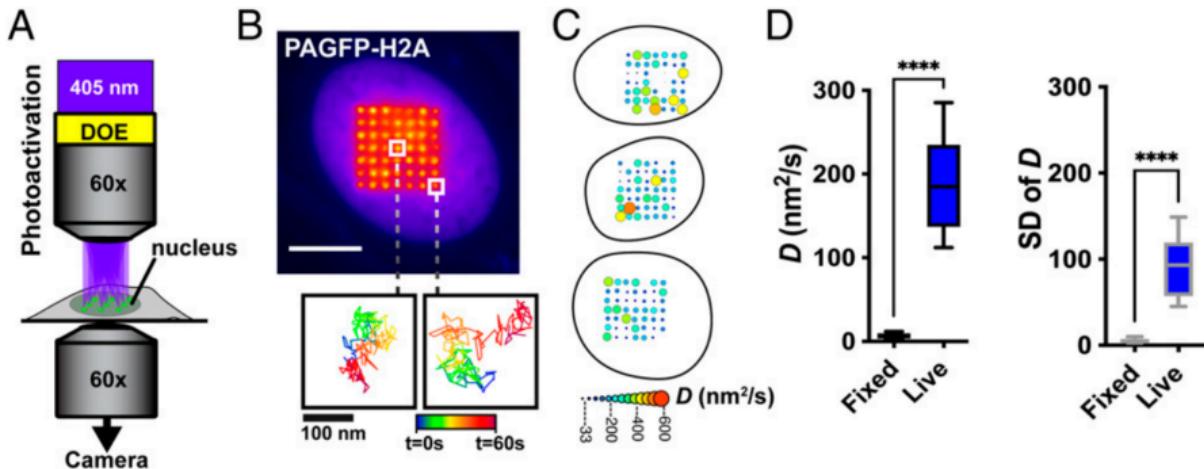
λ – background rate



Maximum likelihood localization:

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

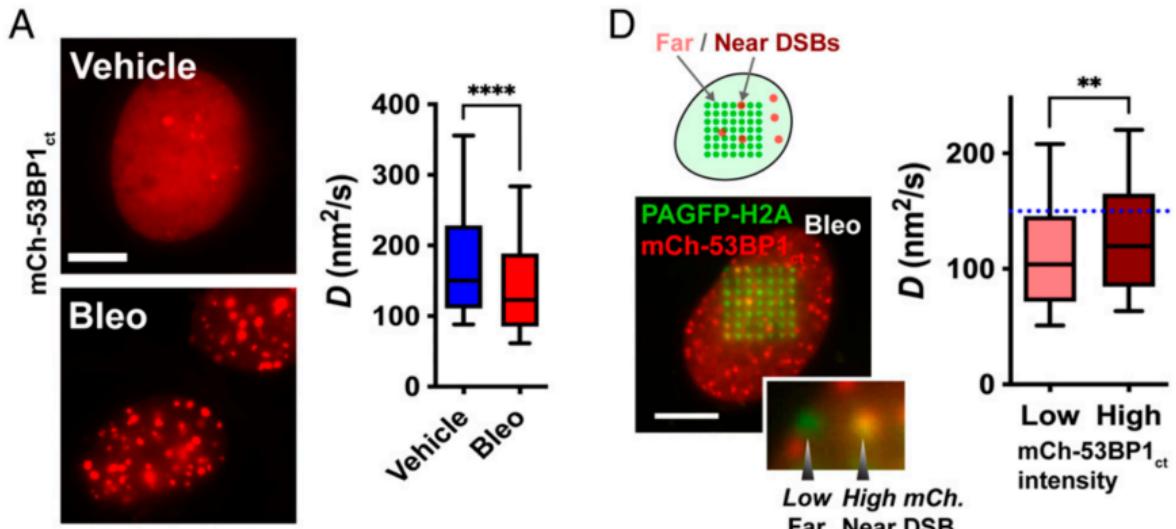
Tracking chromatin loci with photoactivated localization microscopy



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

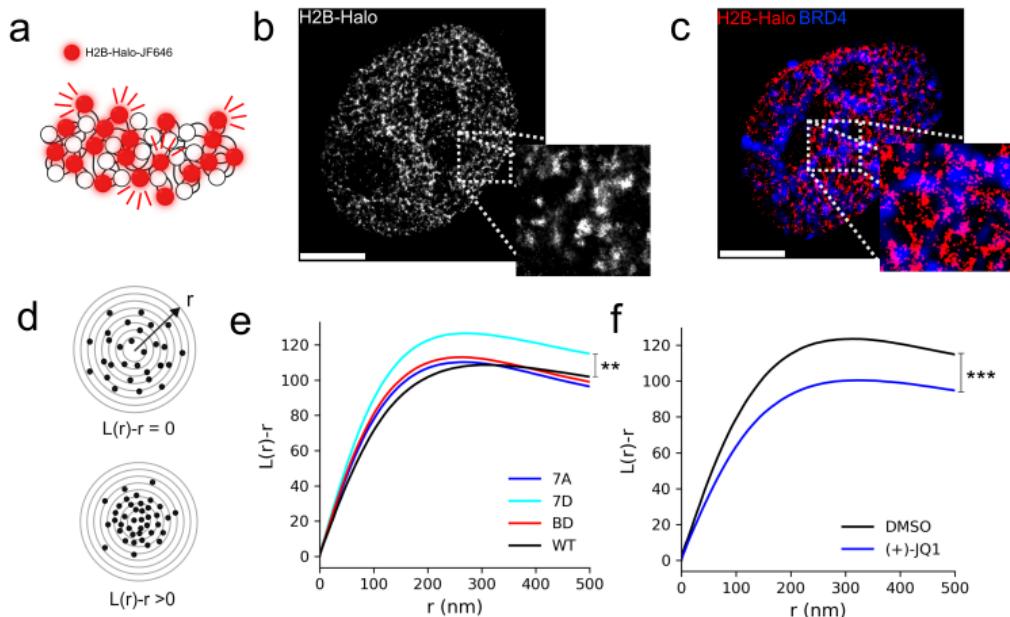
- ▶ A diffractive optical element (DOE) is used to photoactivate chromatin microdomains
- ▶ Used for understanding the spatial correlations of chromatin diffusion

Tracking chromatin loci with photoactivated localization microscopy



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

Analyzing the structure of nucleosome nanodomains with SMLM



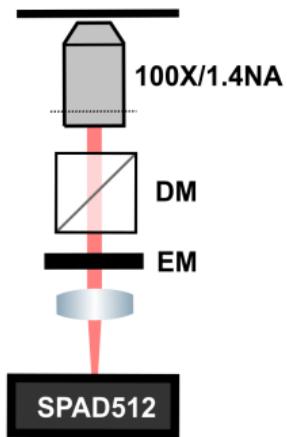
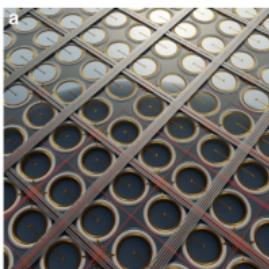
Seitz et al. bioRxiv, Cells, In Review (2025)

- ▶ H2B is densely labeled for super-resolution imaging

Fast single photon sensitive cameras for localization microscopy

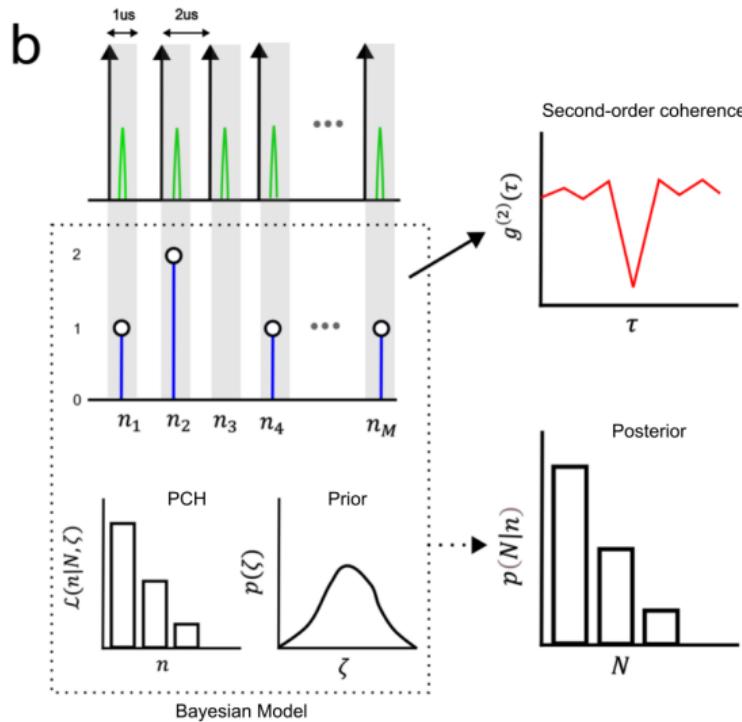
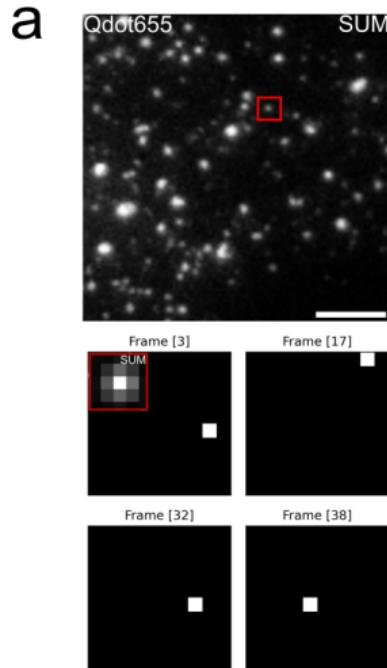


SPAD512



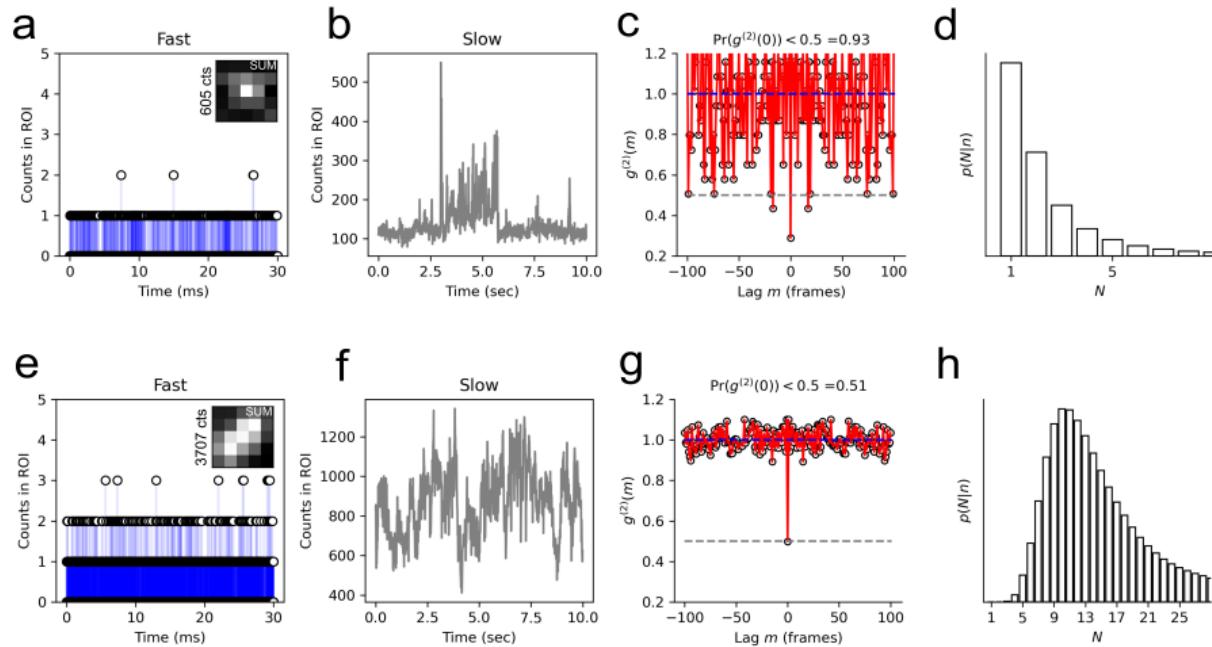
- ▶ Single photon avalanche diode (SPAD) array
- ▶ Frame rates up to 100kHz enable generating images photon by photon

Quantitative SMLM by measuring the photon counting histogram



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

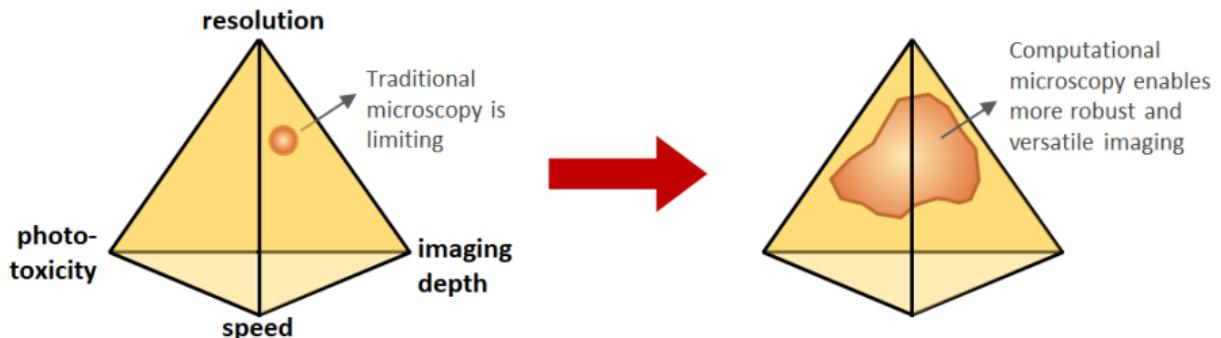
Quantitative SMLM by measuring the photon counting histogram



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

Deep learning models for modern microscopy

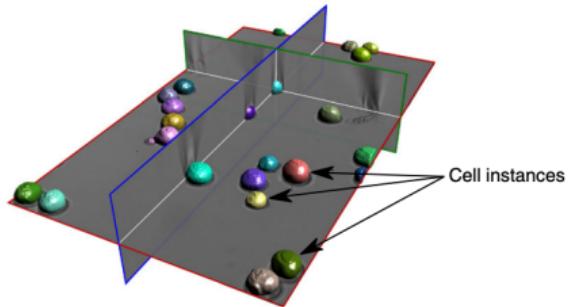
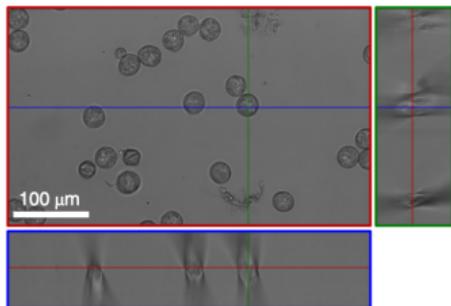
The inherent tradeoffs of fluorescence microscopy



Courtesy of Chowdhury lab at UT Austin

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

Deep learning based 3D reconstruction

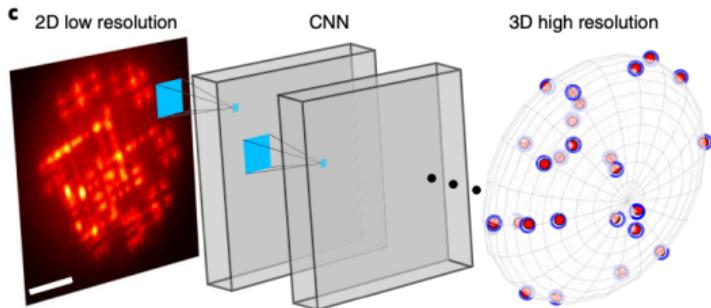


Falk et al. Nat Methods **16** 2019

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

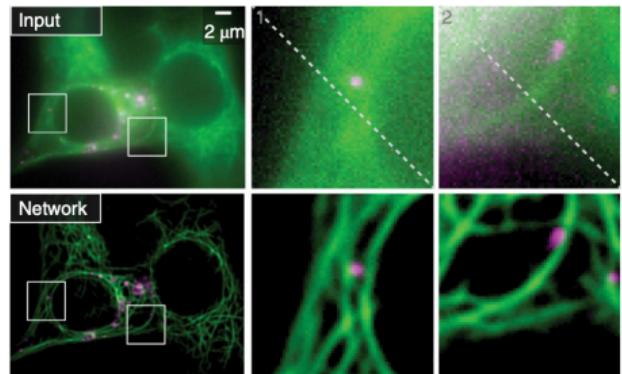
Robust 3D localization microscopy using deep learning

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 θ is some latent of interest and we make a measurement \mathbf{x}

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

Resolution enhancement with a diffusion model

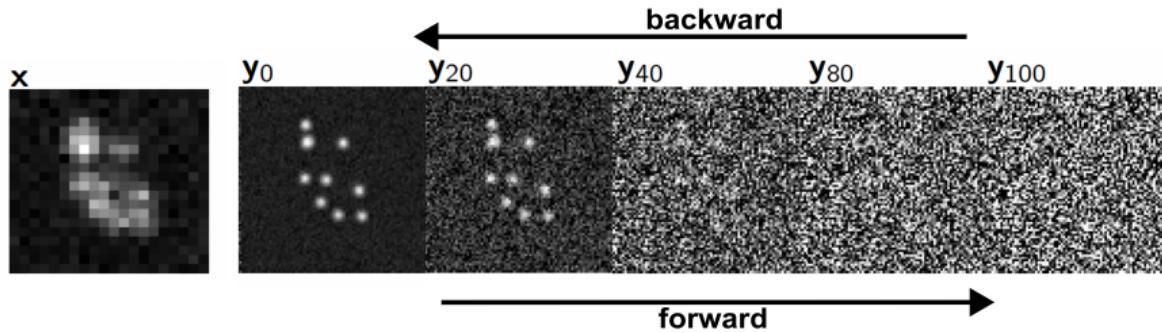
- ▶ Can sample from $p(\theta|x)$ using a stochastic process called Langevin dynamics

Drift and diffusion: $\theta_t = \overbrace{\theta_{t-1} - \frac{\beta}{2} \nabla f(\theta)}^{\mu} + \sqrt{\beta} \xi \quad \xi \sim \mathcal{N}(0, I)$

Resolution enhancement with a diffusion model

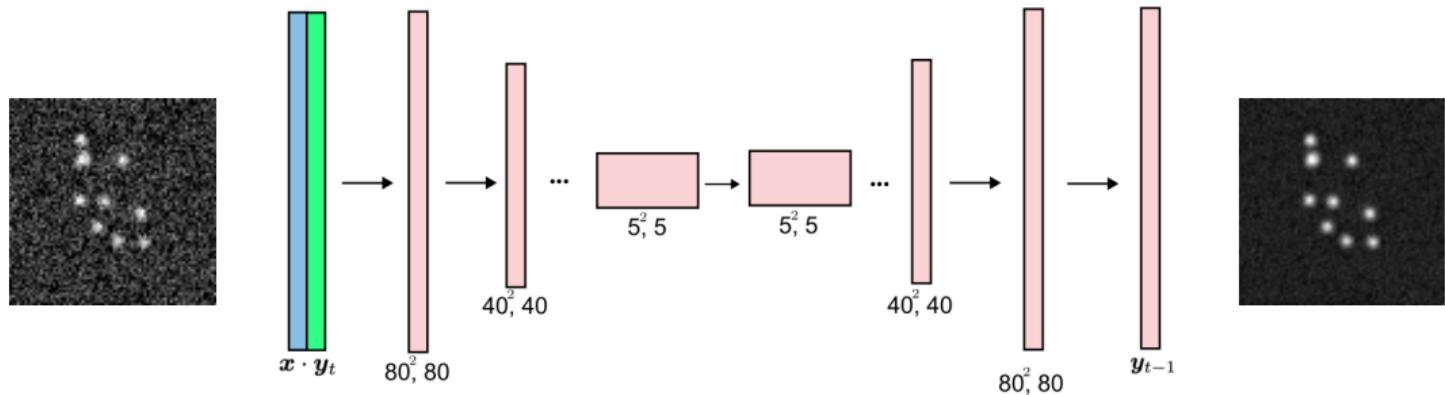
- ▶ Task: infer a high resolution image \mathbf{y}_0 from low resolution \mathbf{x}
- ▶ Drift is not available for image data, but can be learned from pairs $(\mathbf{x}, \mathbf{y}_0)$

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



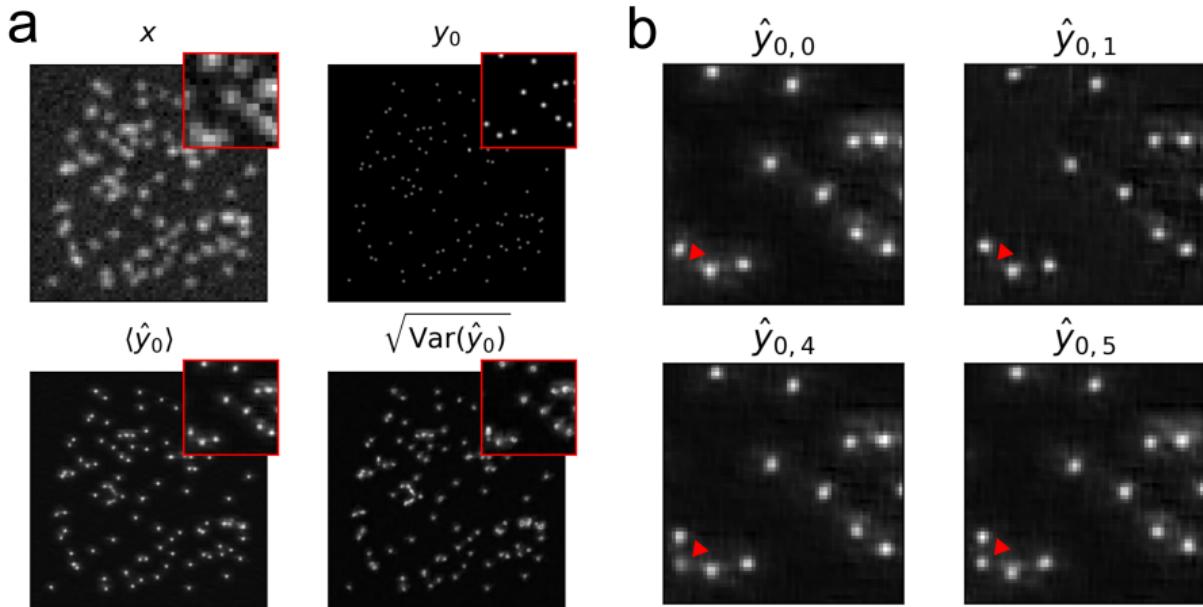
$$q(\mathbf{y}_t | \mathbf{y}_{t-1}) = \mathcal{N}\left(\sqrt{1 - \beta_t} \mathbf{y}_{t-1}, \beta_t I\right)$$

Resolution enhancement with a diffusion model



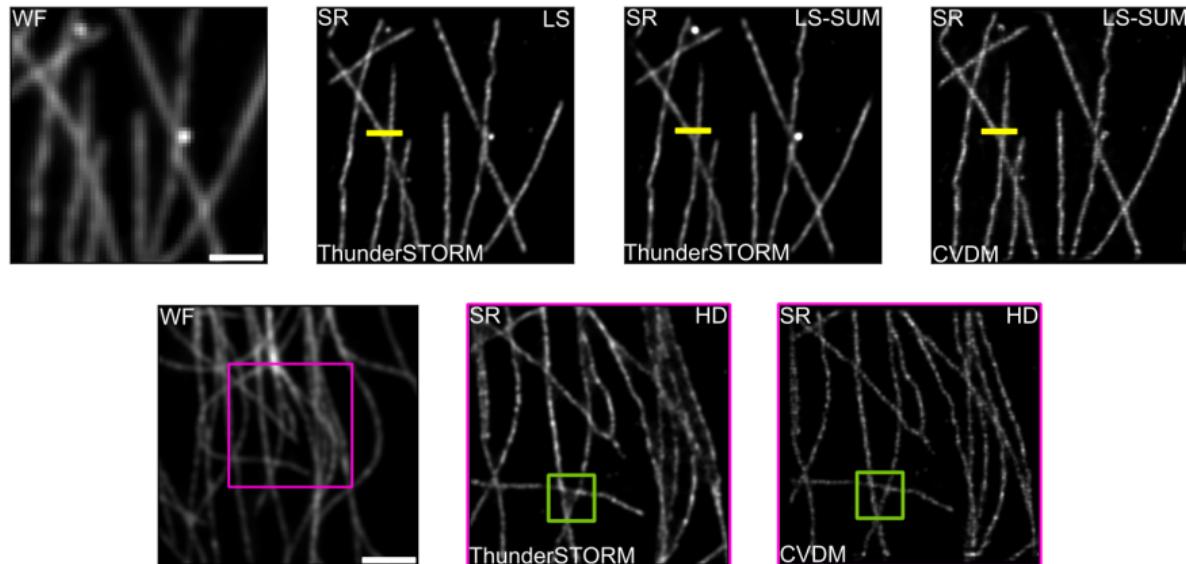
- ▶ A convolutional neural network ψ estimates the drift μ_ψ
- ▶ Denoising step: $\mathbf{y}_{t-1} \sim p_\psi(\mathbf{y}_{t-1} | \mathbf{y}_t, \mathbf{x}) = \mathcal{N}(\mu_\psi, \beta_t I)$

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[†], J. Lawrimore[†], H. Lin[†], 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