

Diffusion based generative models for modern fluorescence microscopy

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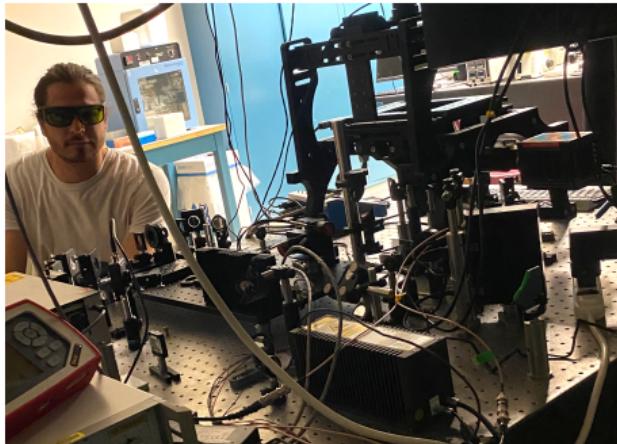
February 4, 2025

Outline of the talk

Live cell super resolution microscopy

Deep learning models for modern microscopy

Research Interests

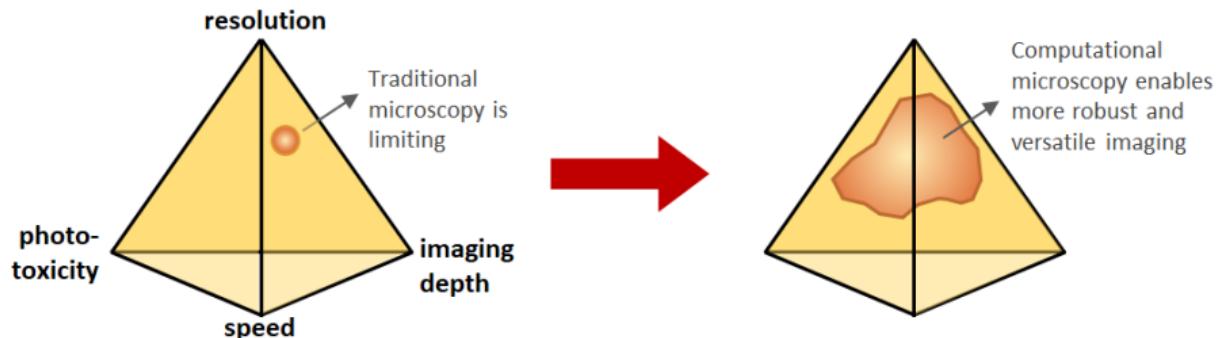


- ▶ Machine learning methods for enhanced light microscopy
- ▶ Quantitative single molecule localization microscopy
- ▶ Generative models, theory of deep learning

Live cell super resolution microscopy

A live cell fluorescence imaging zoo

The inherent tradeoffs of fluorescence microscopy



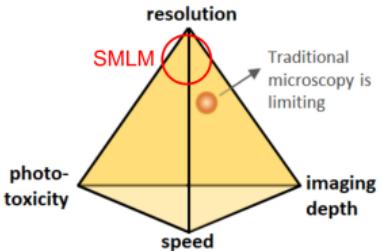
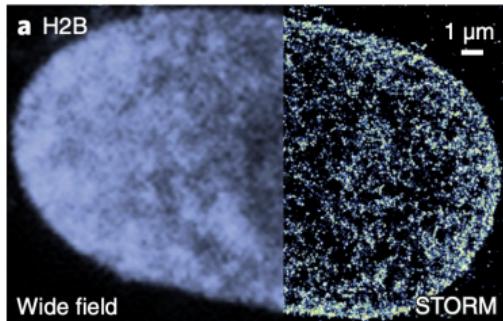
Courtesy of Chowdhury lab at UT Austin

- ▶ 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)

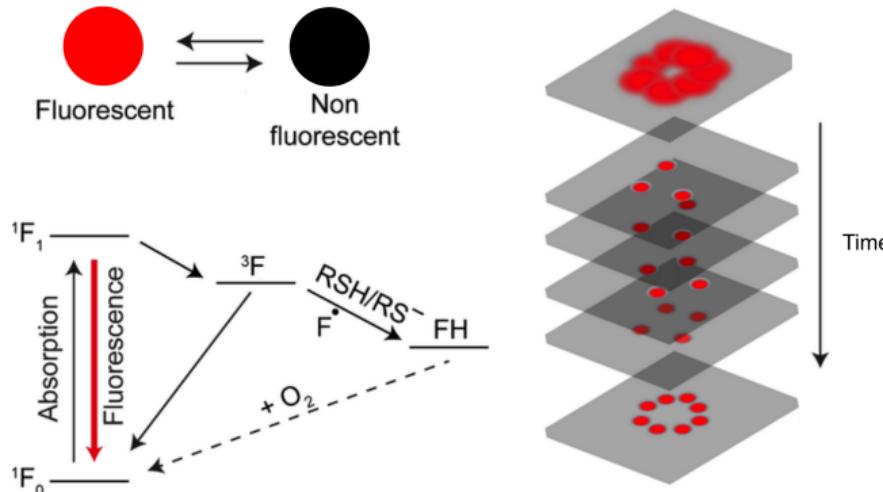
SMLM is all about *control* of the fluorescence

Single molecule localization microscopy (SMLM)



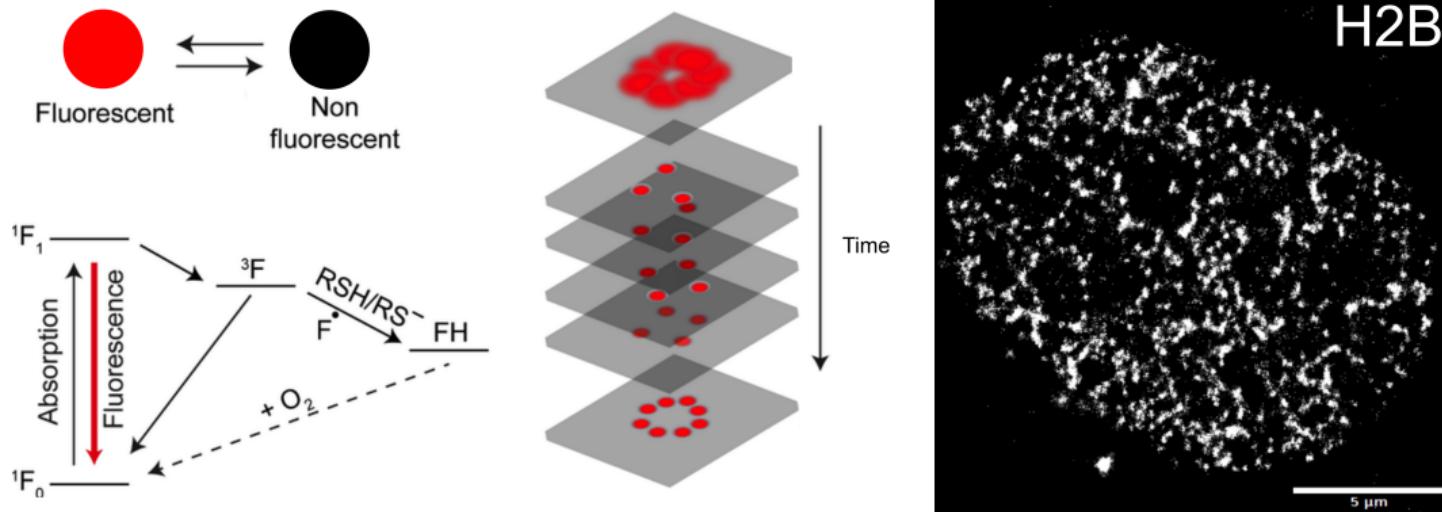
- ▶ SMLM techniques can achieve 10nm resolutions (20x higher than diffraction limit)

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

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

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

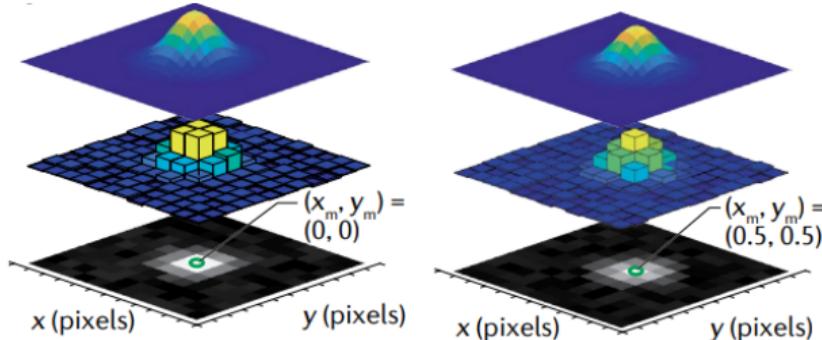
g_k – pixel gain

η – quantum efficiency

ζ – photon emission rate

Δ – exposure time

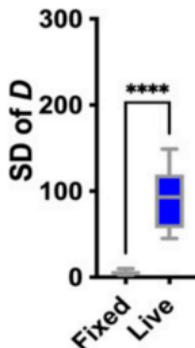
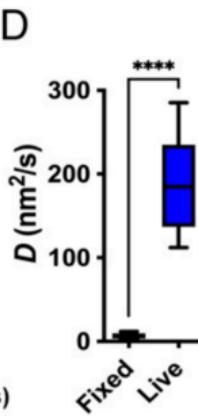
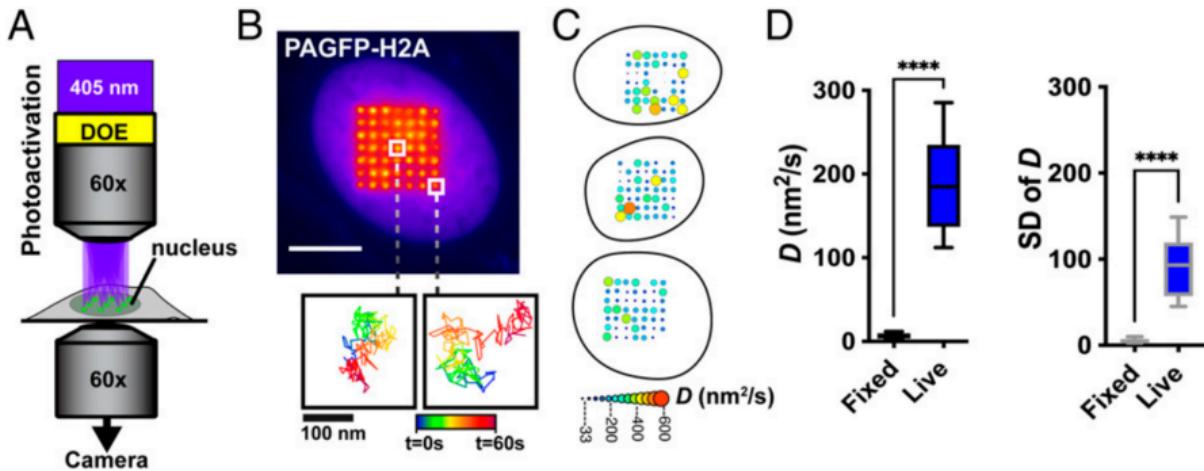
λ – background rate



Maximum likelihood localization:

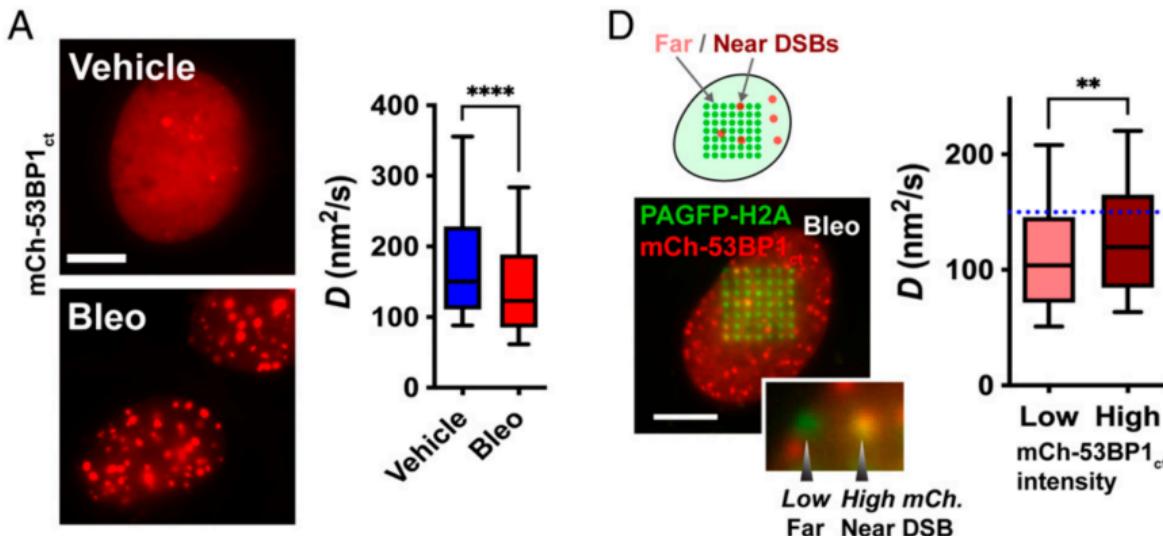
$$\theta^* = \operatorname{argmax}_{\theta} \prod_k p(\mathbf{x}_k | \theta) = \operatorname{argmin}_{\theta} - \sum_k \log p(\mathbf{x}_k | \theta)$$

Tracking chromatin loci photoactivated localization microscopy



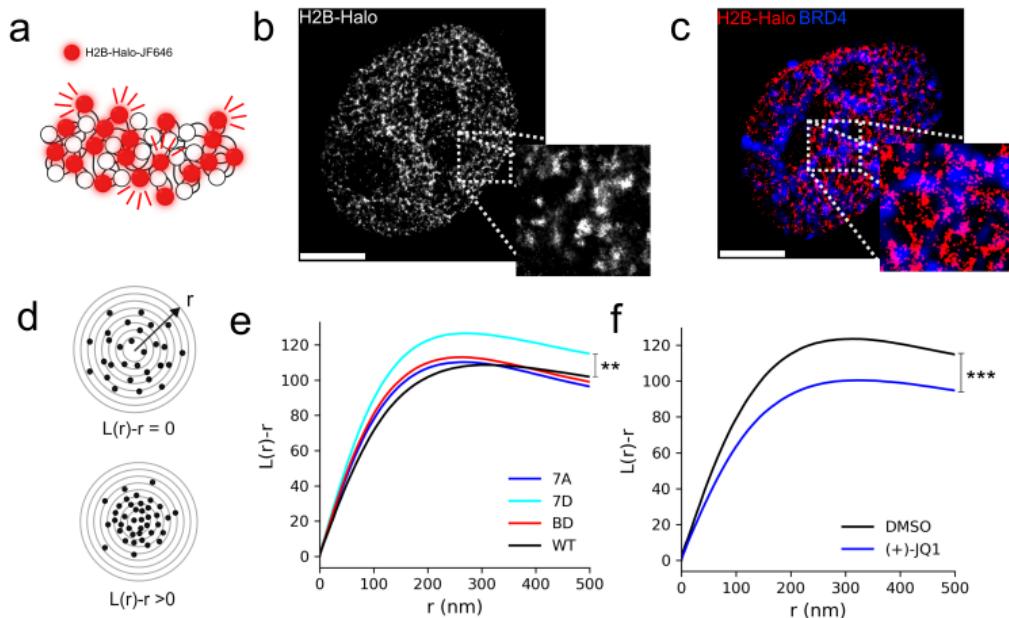
Locatelli, Seitz et al. PNAS **29** (2022)

Tracking chromatin loci photoactivated localization microscopy



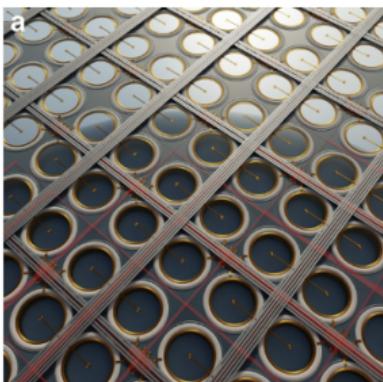
Locatelli, Seitz et al. PNAS **29** (2022)

Analyzing the structure nucleosome nanodomains with SMLM



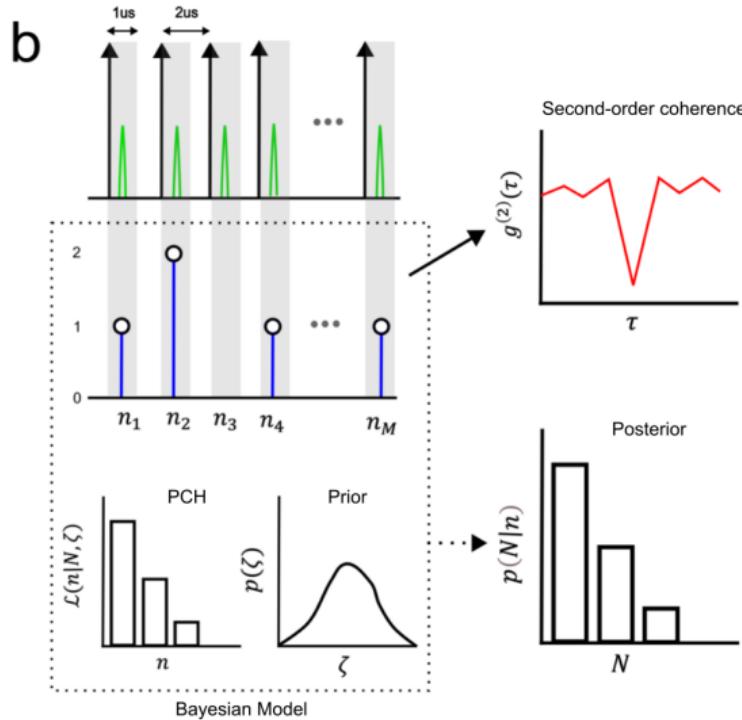
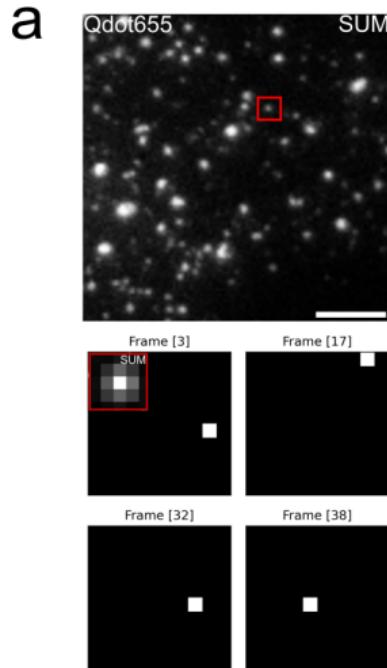
- ▶ Seitz et al. bioRxiv, Cells, In Review (2025)
- ▶ H2B is densely labeled for super-resolution imaging
- ▶ BRD4 chromatin binding activity controls nanodomain density

Quantitative SMLM by measuring the photon counting histogram



SPAD512

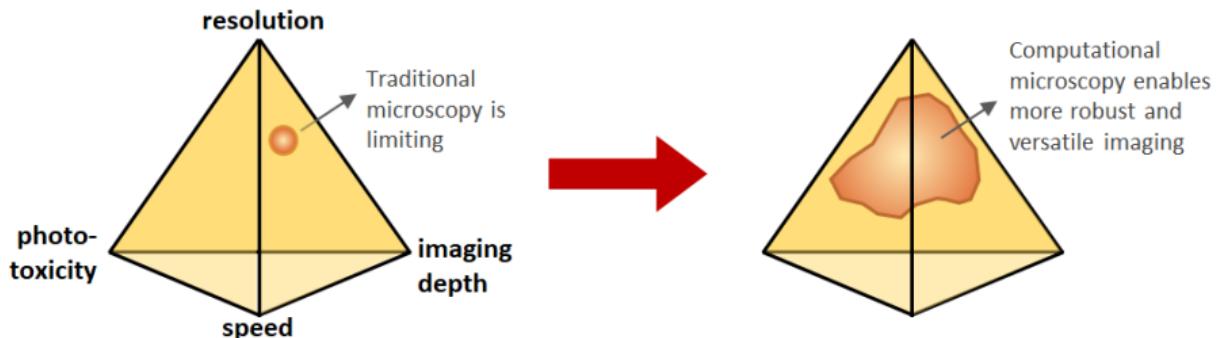
Quantitative SMLM by measuring the photon counting histogram



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

Deep learning models for modern microscopy

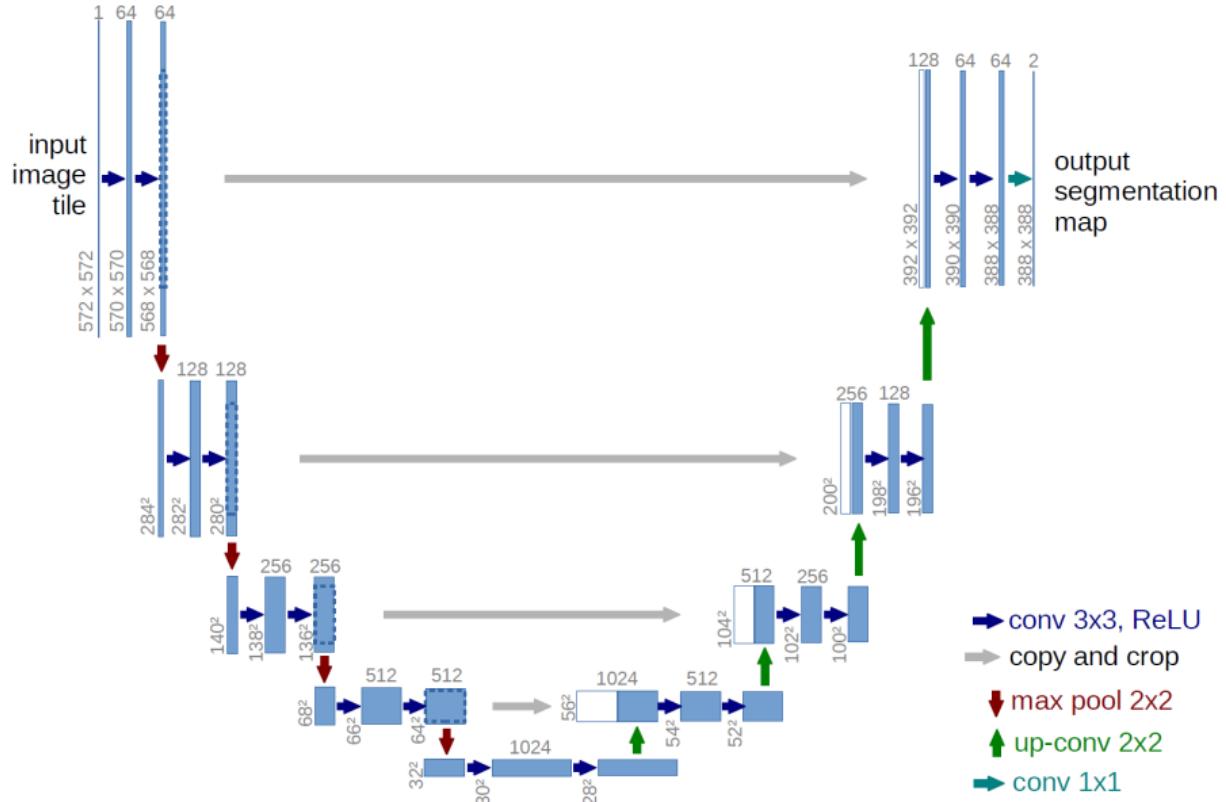
The inherent tradeoffs of fluorescence microscopy



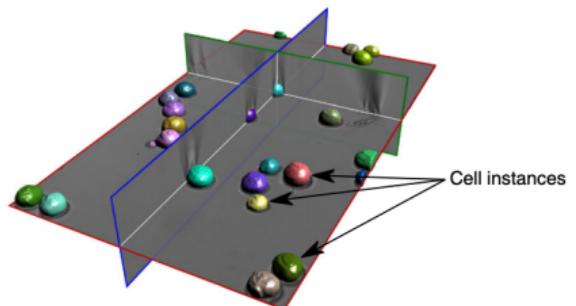
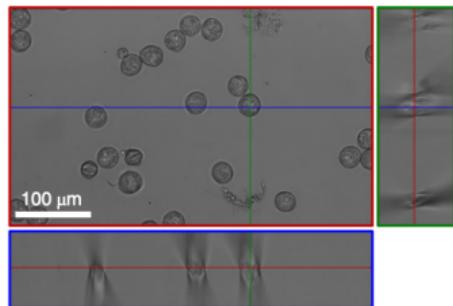
Courtesy of Chowdhury lab at UT Austin

- ▶ Ex. increase in resolution is often accompanied by loss of imaging speed
- ▶ Computational methods such as AI/ML algorithms can help find a superior balance
- ▶ Fluorescence microscopy is often not very quantitative (more on this later)

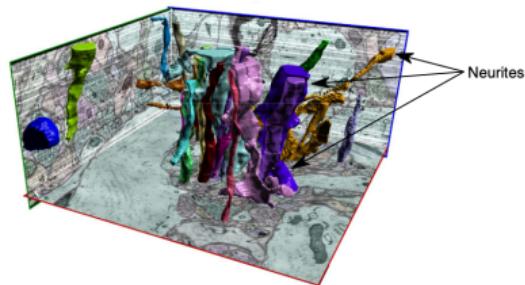
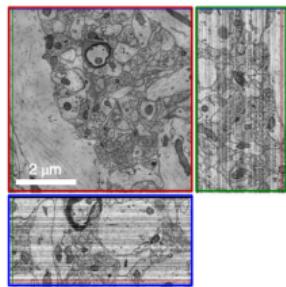
Deep learning based 3D reconstruction



Deep learning based 3D reconstruction



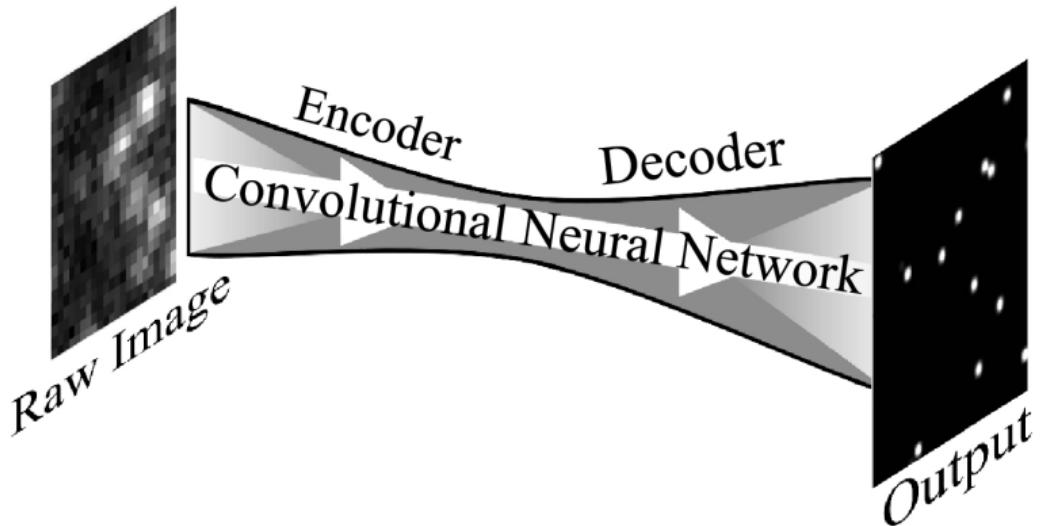
Deep learning based 3D reconstruction



Three-dimensional localization microscopy using deep learning

Deep learning based quantitative phase imaging

Resolution enhancement by deep learning



Nehme E. et al. Optica 5, 458-464 (2018)

- ▶ Prediction of high resolution images from low resolution ones
- ▶ Cannot report uncertainty, leading to overconfident results

Inverse problems in microscopy are 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

Resolution enhancement with a diffusion model

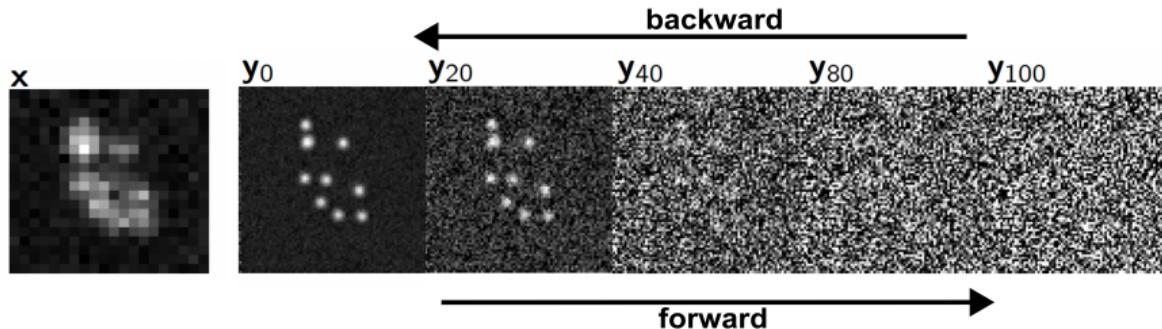
- ▶ Can sample from $p(\theta|x)$ using a stochastic process called Langevin dynamics

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

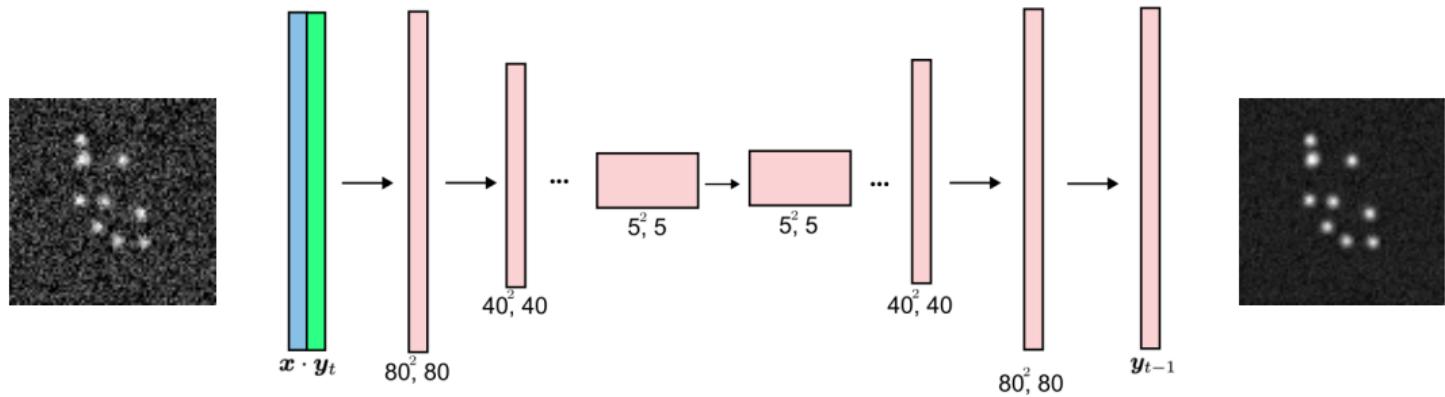
- ▶ Task: infer a high resolution image \mathbf{y}_0 from low resolution \mathbf{x}
- ▶ Drift is not available for image data, but can be learned from pairs $(\mathbf{x}, \mathbf{y}_0)$

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



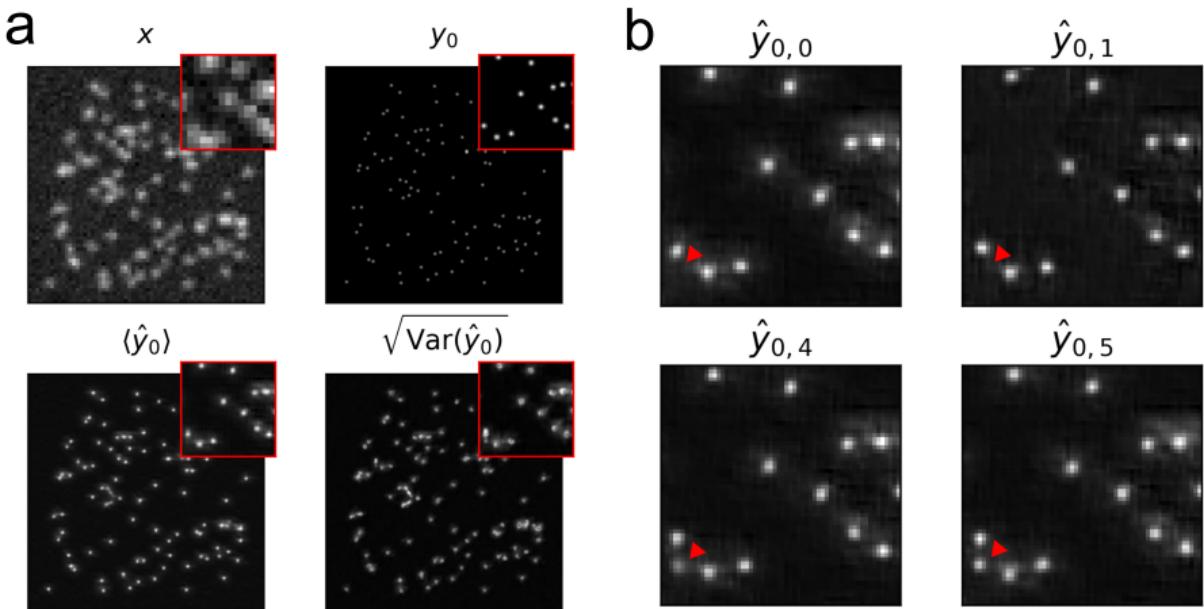
$$q(\mathbf{y}_t | \mathbf{y}_{t-1}) = \mathcal{N}\left(\sqrt{1 - \beta_t} \mathbf{y}_{t-1}, \beta_t I\right)$$

Resolution enhancement with a diffusion model

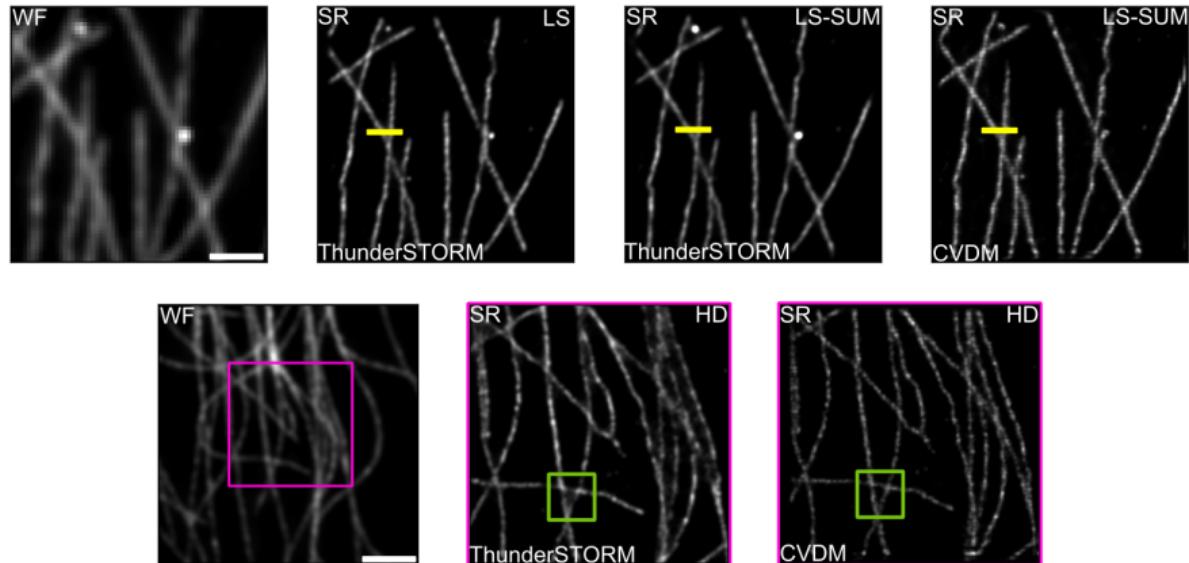


- ▶ A convolutional neural network ψ estimates the drift μ_ψ
- ▶ Denoising step: $\mathbf{y}_{t-1} \sim p_\psi(\mathbf{y}_{t-1} | \mathbf{y}_t, \mathbf{x}) = \mathcal{N}(\mu_\psi, \beta_t I)$

Super resolution with a diffusion model



Super resolution with a diffusion model



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