

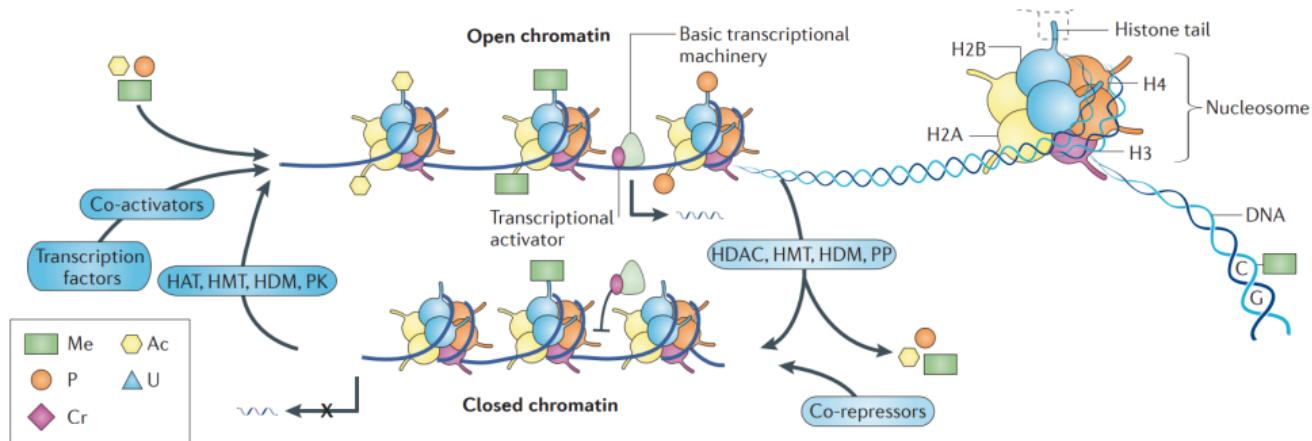
Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

Clayton W. Seitz

August 22, 2023

- ▶ Overview of biological system and some literature review
- ▶ Open questions in that system
- ▶ A novel method to study that system
- ▶ Major results so far
- ▶ Future goals and perspectives

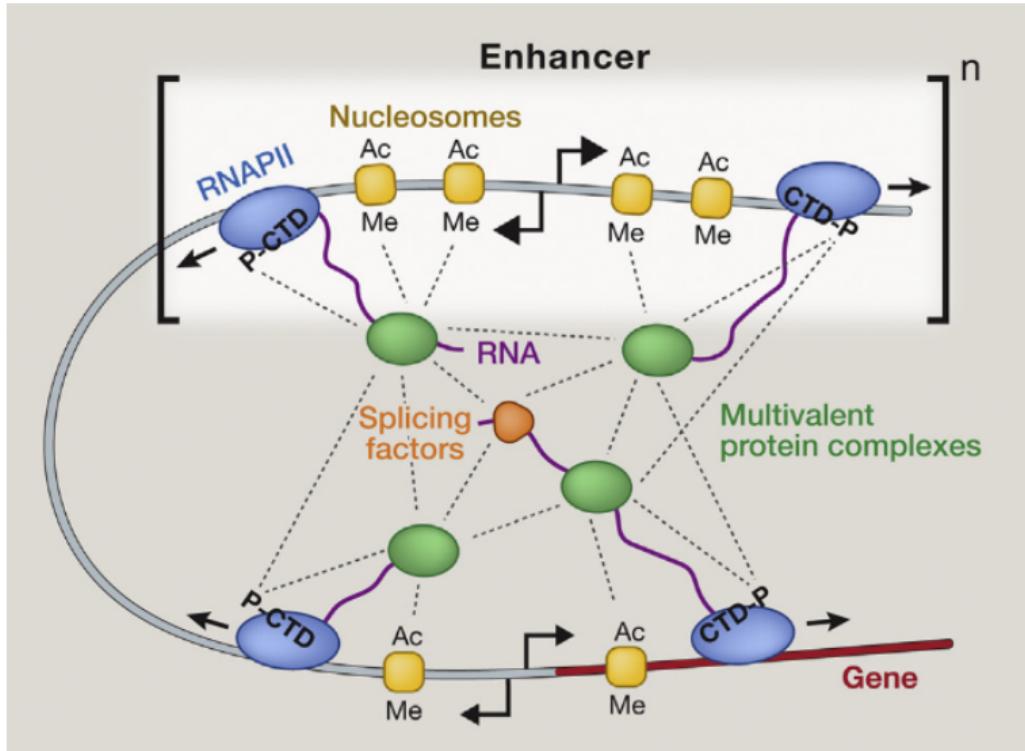
The textbook view of histone acetylation



Graff et al. Histone acetylation: molecular mnemonics on the chromatin

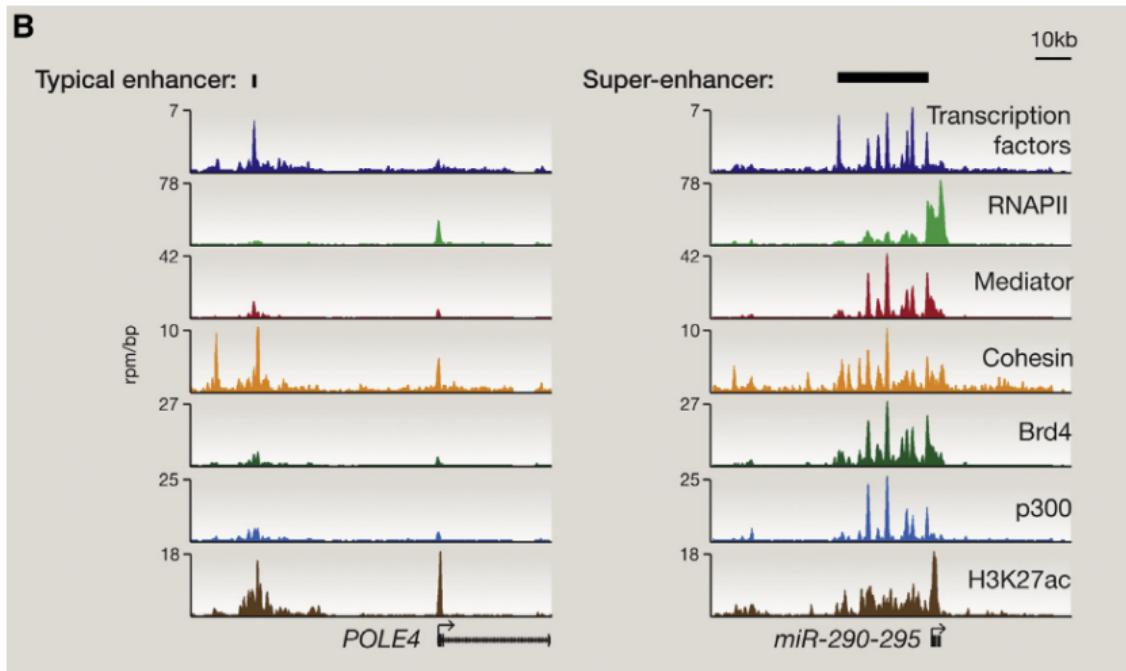
- ▶ I am interested in the impact of BRD4 protein on chromatin structure
- ▶ Previous work has shown BRD4 is associated with histone acetylation
- ▶ Live cell super-resolution imaging is a useful tool

A phase separation model for transcriptional control



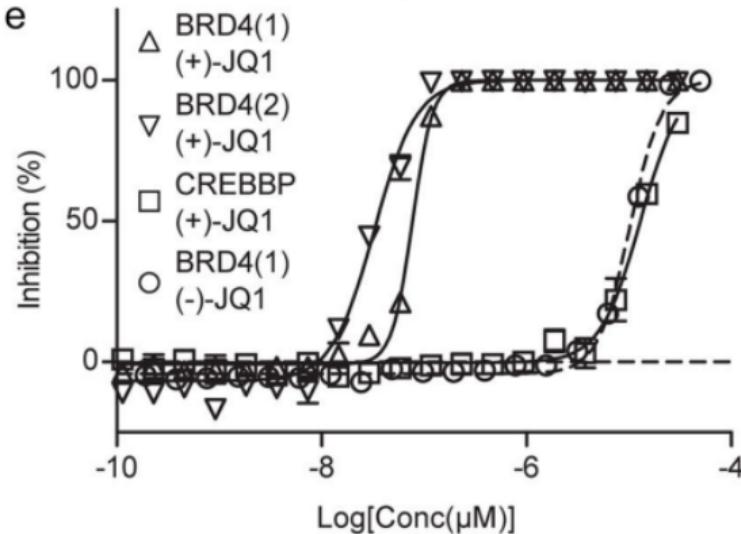
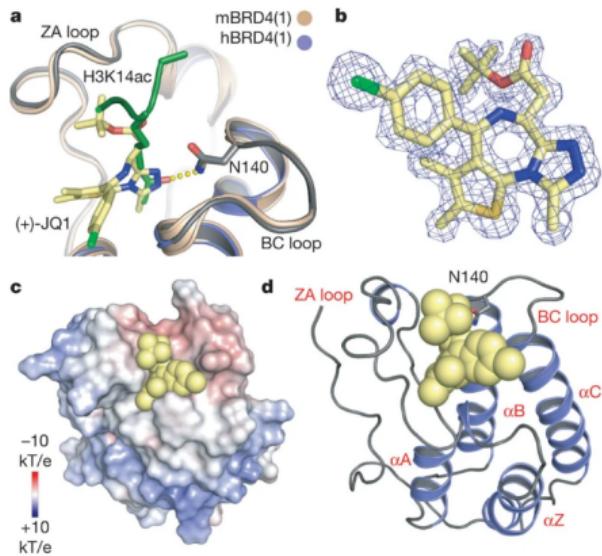
Hnisz et al. A phase separation model of transcriptional control. Cell 2017

A phase separation model for transcriptional control



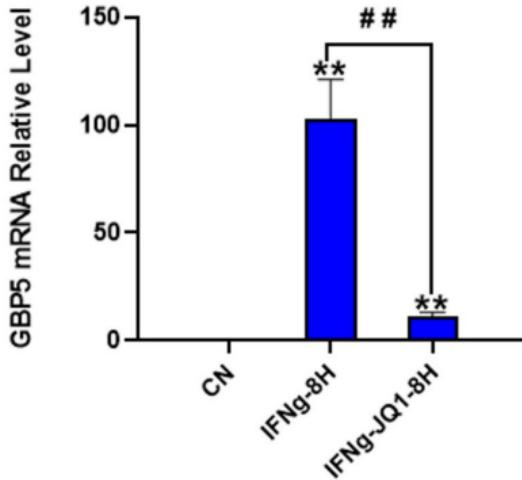
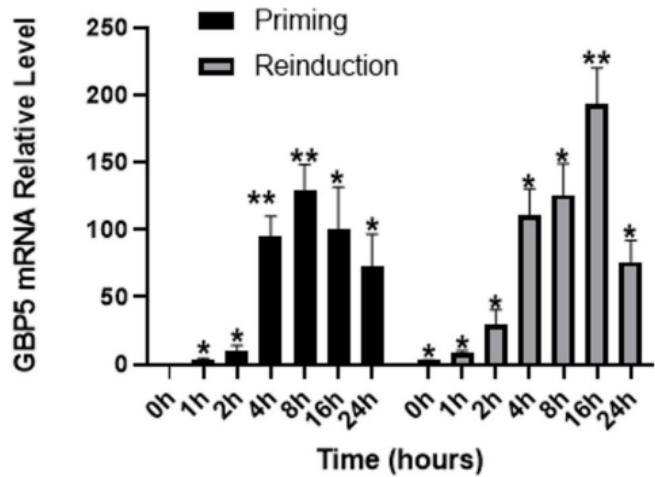
Hnisz et al. A phase separation model of transcriptional control. Cell 2017

(+)-JQ1 in complex with BRD4 protein



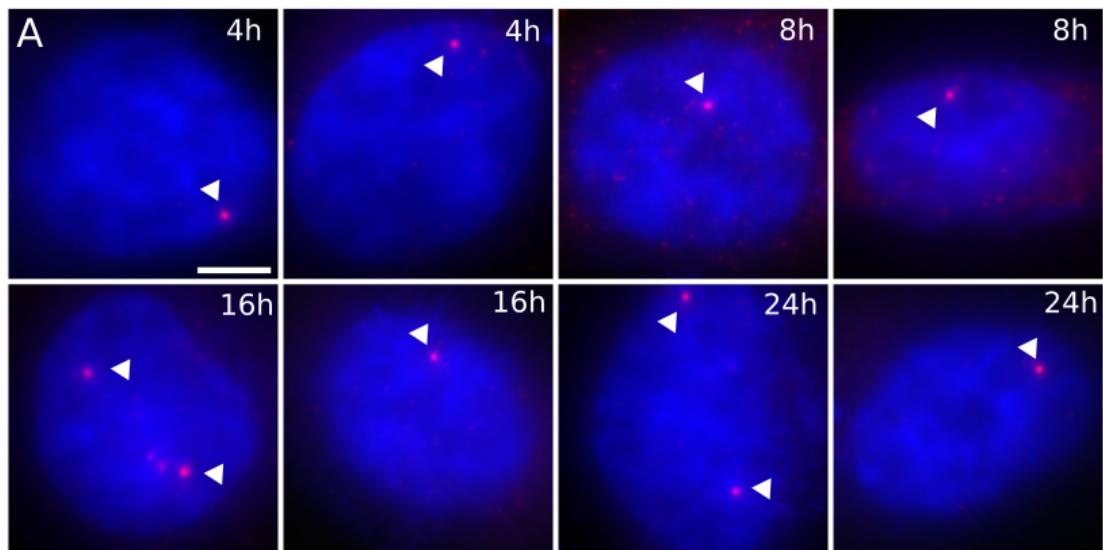
Filippakopoulos. Selective inhibition of BET bromodomains. *Nature Communications*

Validation of JQ1 efficacy for BRD4 inhibition in HeLa cells



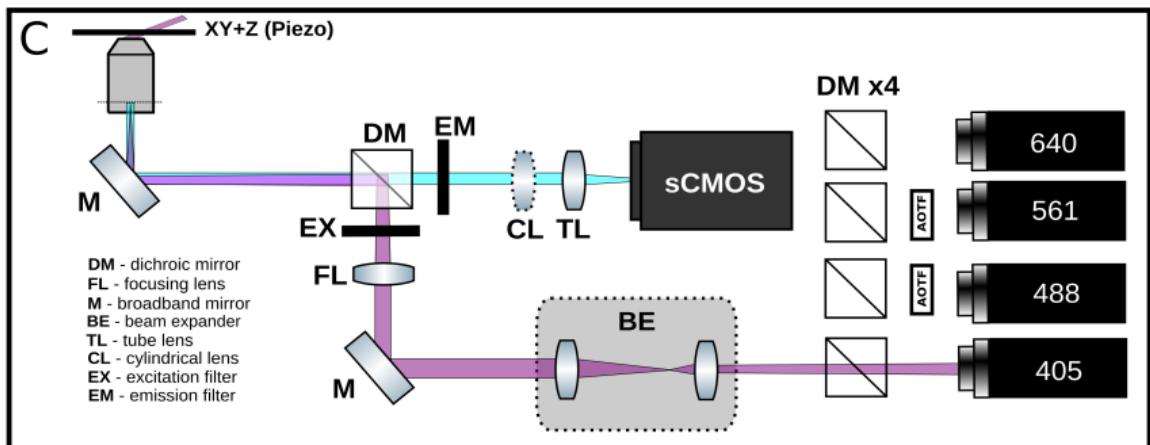
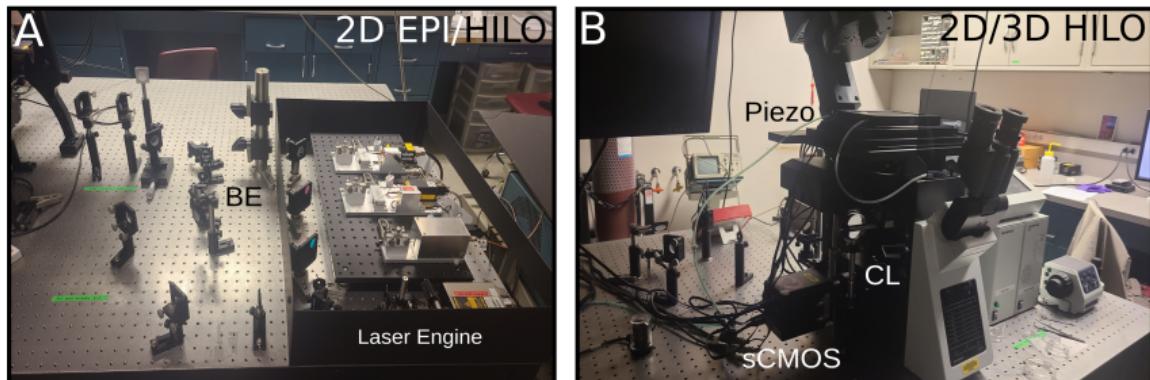
- RT-qPCR quantified using $2^{-\Delta\Delta C_t}$ method using GAPDH as a reference gene
- *: $P \leq 0.1$, **: $P \leq 0.01$

Induction of a BRD4-controlled gene with cytokine treatment

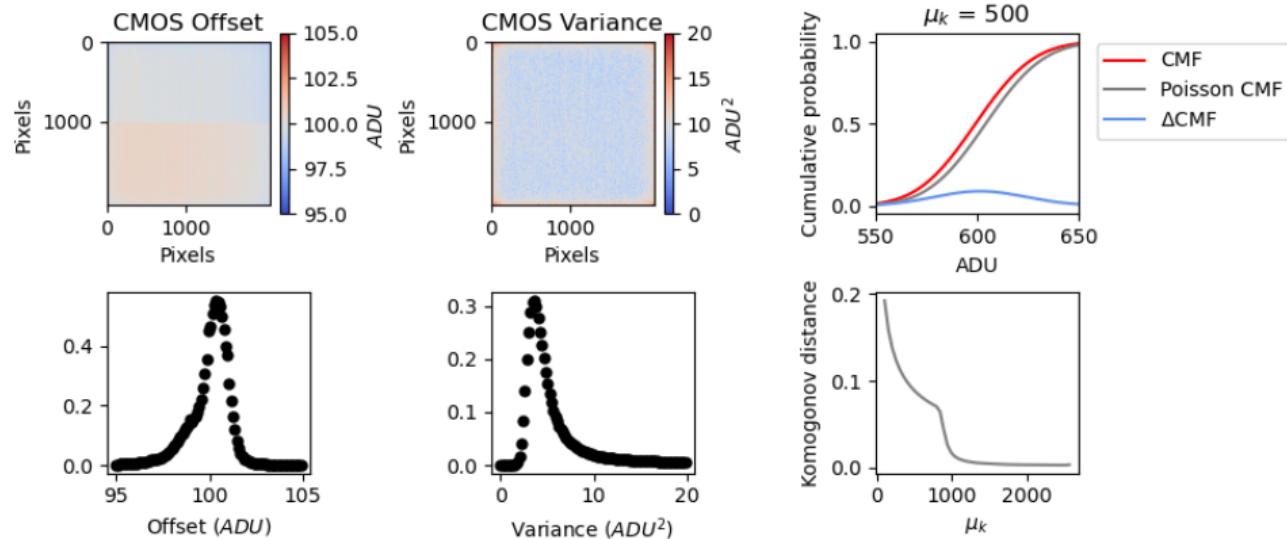


- ▶ GBP5 transcription is regulated by BRD4 (Lin 2022)

Instrumentation for single molecule localization microscopy



A Poisson approximation at moderate SNR simplifies SMLM



$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi}\sigma_k} e^{-\frac{(H_k - g_k q - o_k)^2}{2\sigma_k^2}}$$

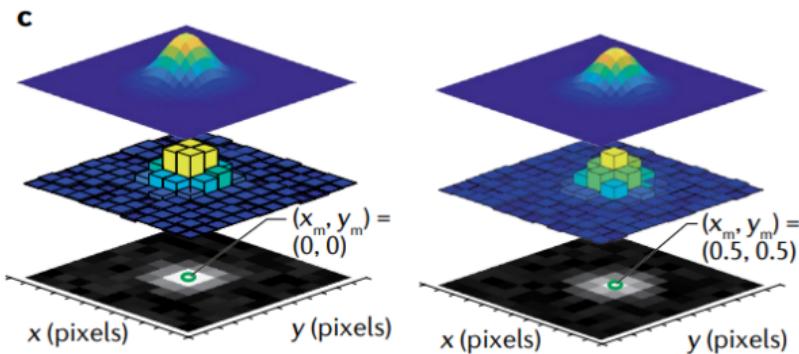
$P(H_k|\theta)$ can be approximated as Poisson at high signal-to-noise (SNR)

Maximum likelihood localization of an isolated fluorescent emitter

$$\text{Localization: } \theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$$

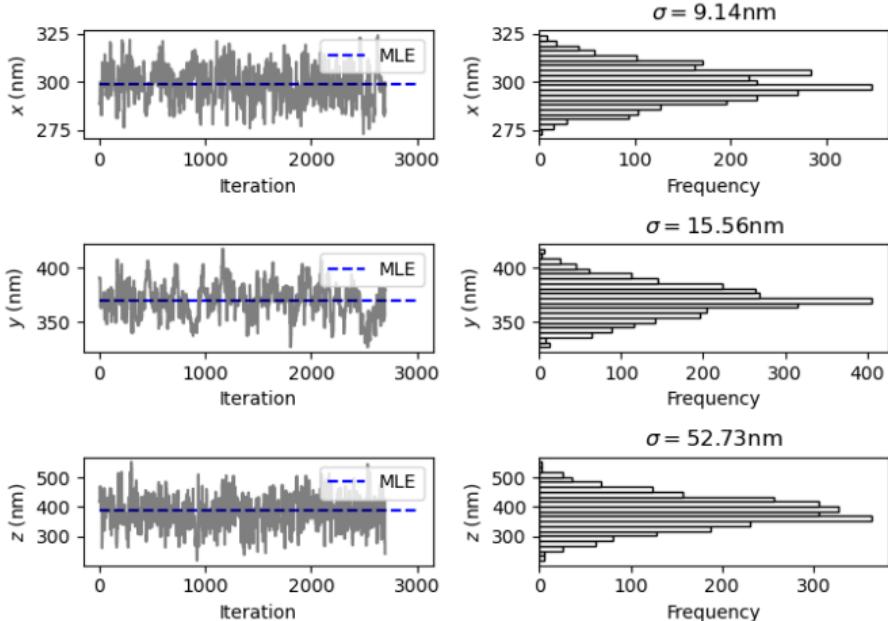
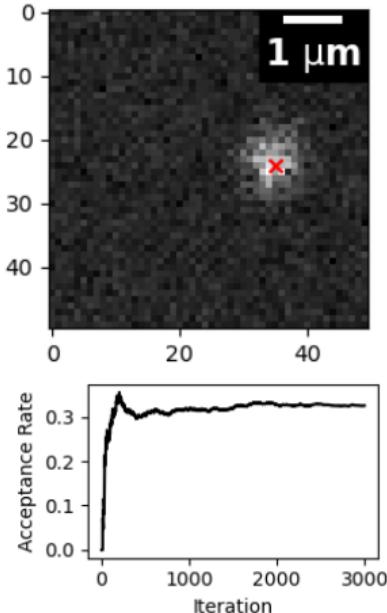
$$\mu_k = g_k \eta N_0 \Delta \int_{\text{pixel}} G(x, y) dA$$

η – quantum efficiency
 N_0 – photon count
 Δ – exposure time



- ▶ Fisher information and Cramer-Rao lower bound (CRLB) can be computed analytically for Poisson log-likelihood ℓ (Smith 2010, Huang 2013)

Estimator precision sets the resolution limit in localization microscopy



- ▶ Variance of the posterior $P(\theta|\vec{H})$ is a useful particle filter
- ▶ We assume uniform priors on coordinates

Computing the CRLB for static errors in two dimensions

Fisher information (separable case):

$$I_{ij}(\theta) = \mathbb{E}_{\theta} \left(\frac{\partial \ell}{\partial \theta_i} \frac{\partial \ell}{\partial \theta_j} \right) \quad (1)$$

Let $\mu'_k = \mu_k + \sigma_k^2$. For an arbitrary parameter,

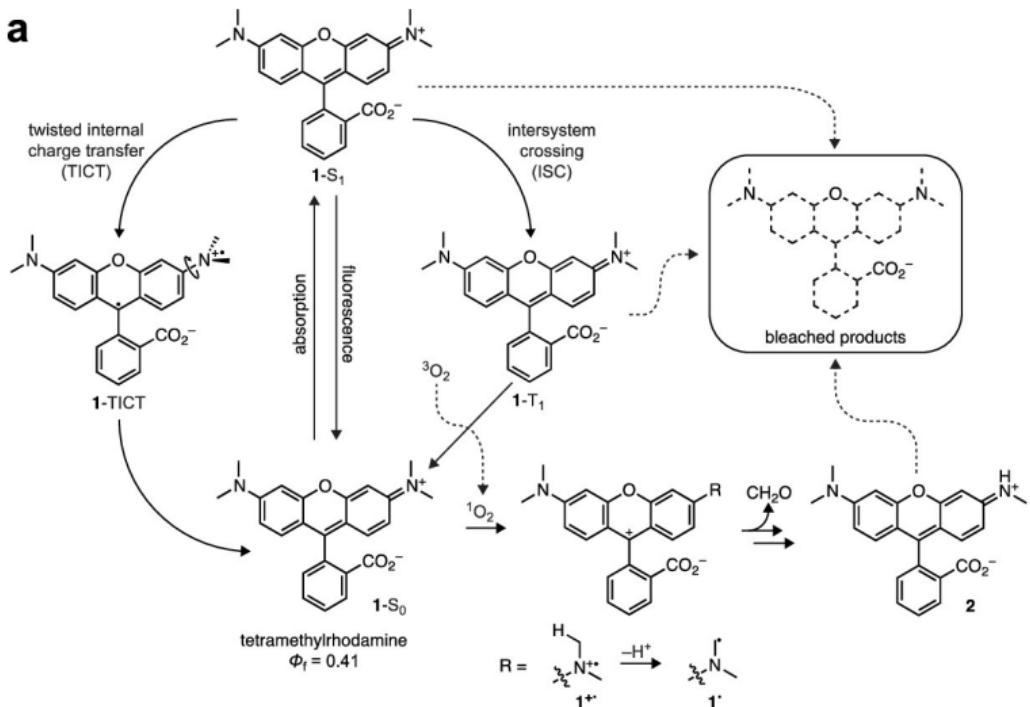
$$\frac{\partial \ell}{\partial \theta_i} = \frac{\partial}{\partial \theta_i} \sum_k x_k \log x_k + \mu'_k - x_k \log (\mu'_k)$$

$$= \sum_k \frac{\partial \mu'_k}{\partial \theta_i} \left(\frac{\mu'_k - x_k}{\mu'_k} \right)$$

$$I_{ij}(\theta) = \mathbb{E}_{\theta} \left(\sum_k \frac{\partial \mu'_k}{\partial \theta_i} \frac{\partial \mu'_k}{\partial \theta_j} \left(\frac{\mu'_k - x_k}{\mu'_k} \right)^2 \right) = \sum_k \frac{1}{\mu'_k} \frac{\partial \mu'_k}{\partial \theta_i} \frac{\partial \mu'_k}{\partial \theta_j}$$

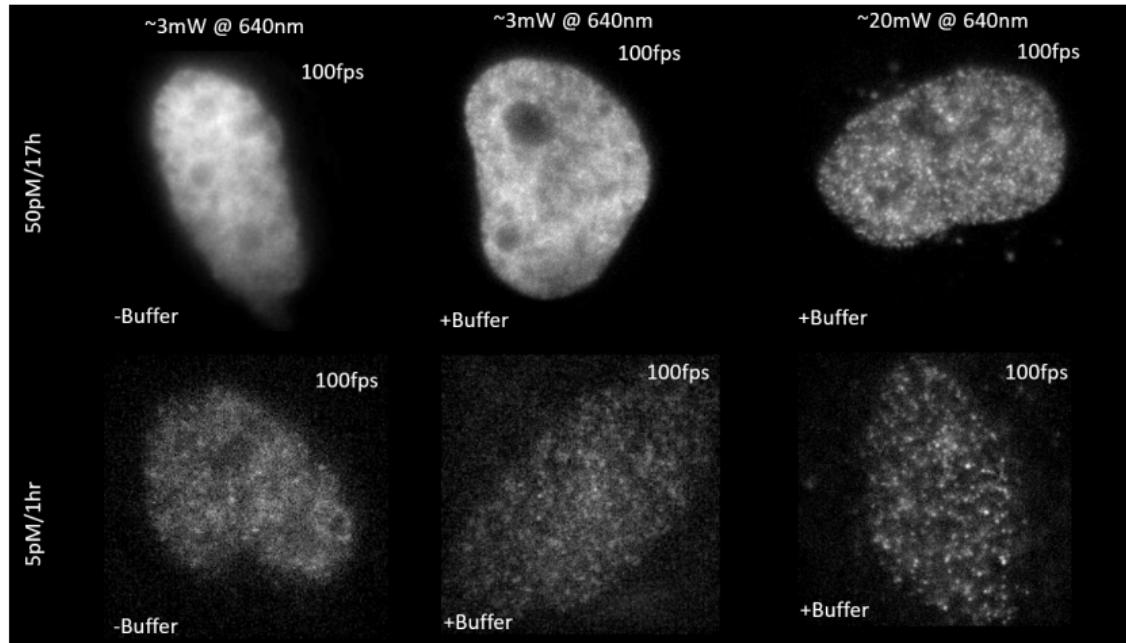
The CRLB is a frequentist

Direct STORM: The photophysics of rhodamines



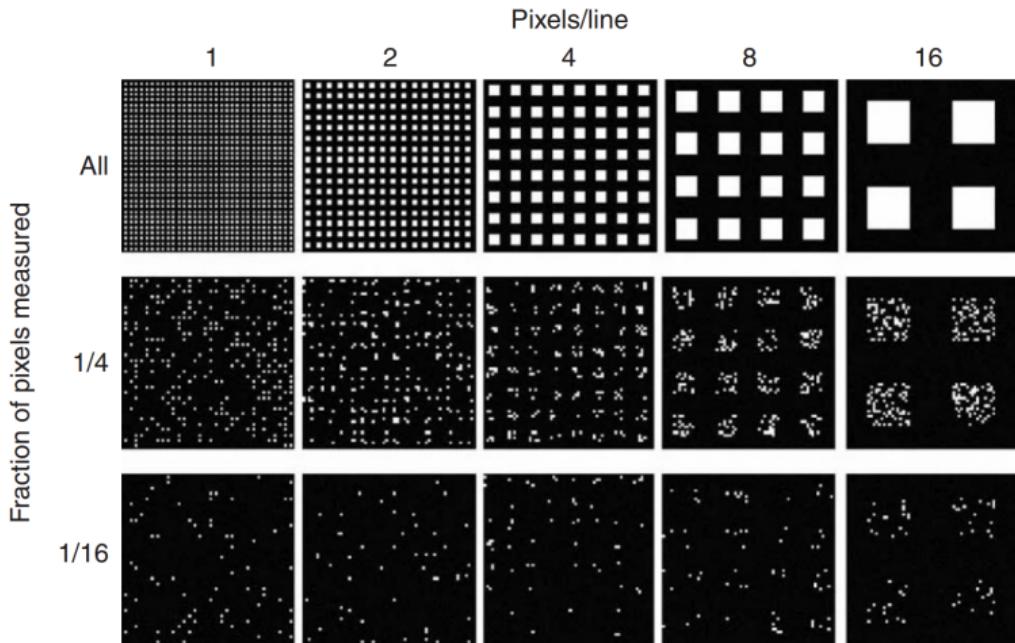
- ▶ Reduction of the T₁ state yields a dark, long-lived, and stable radical state
- ▶ The reducing agent is usually a primary thiol like cysteamine (MEA)

The OFF state of JF646 can be maintained with high laser power



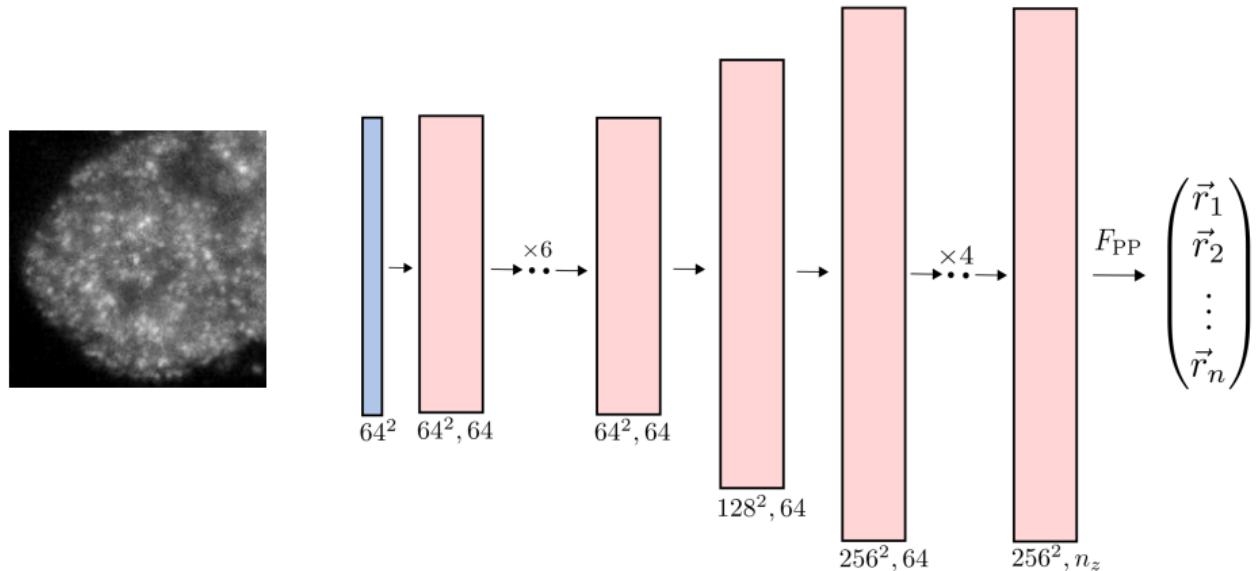
- ▶ High power maintains the OFF state, potentially by promoting triplet state formation

The tradeoff between spatial and temporal resolution in SMLM



- ▶ SMLM is desirable for SR due to very high res and no scanning (e.g., STED)
- ▶ Less control over photophysical state, but high throughput

Deep learning enables dense localization in two-dimensions

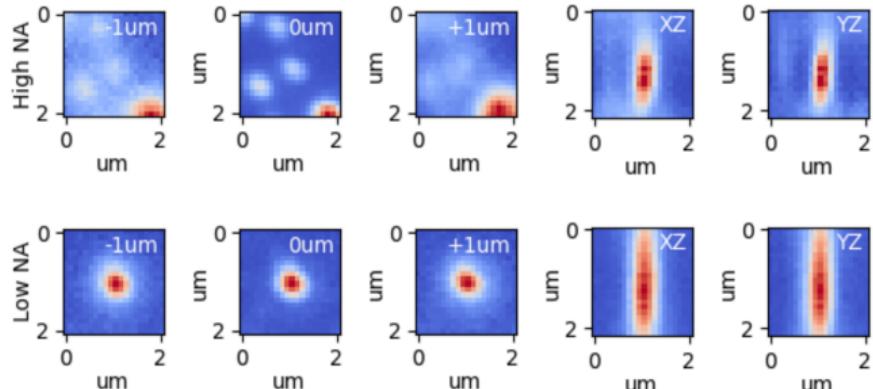


Localization is cast as semantic segmentation of the high resolution tensor:

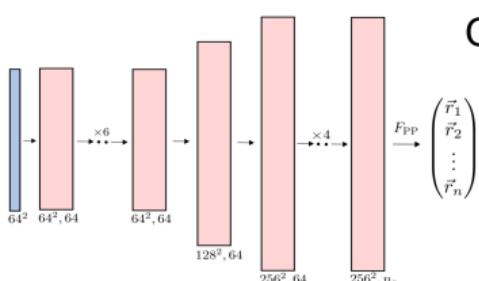
$$\mathcal{L} = \sum_{i,j} \log p_{ij}(\tilde{x}) = \sum_{i,j} \log \frac{\exp(-s_{ij}(\tilde{x}))}{\sum_{x \in \chi} \exp(-s_{ij}(\tilde{x}))}$$

Estimator precision sets the resolution limit in localization microscopy

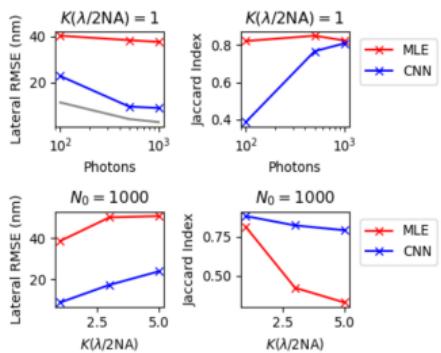
A



B

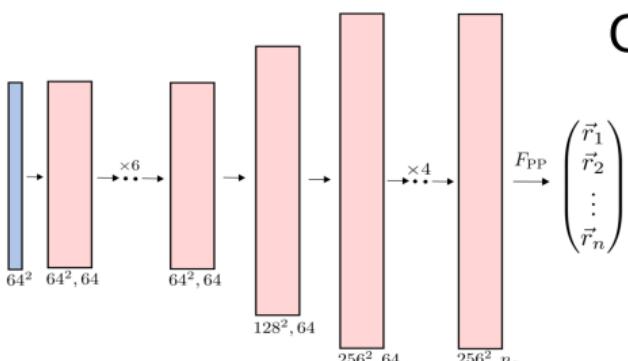


C

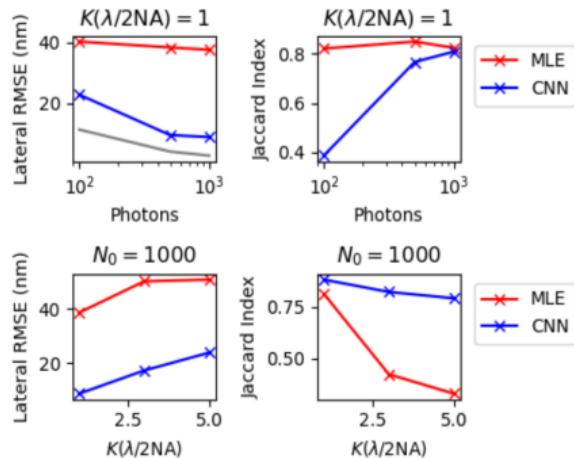


Estimator precision sets the resolution limit in localization microscopy

B

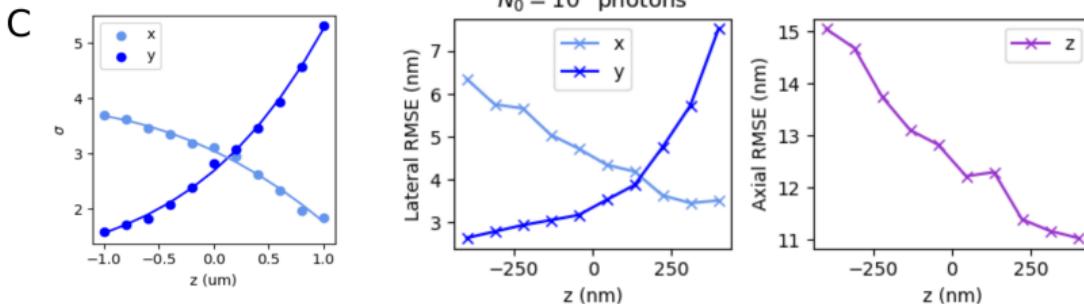
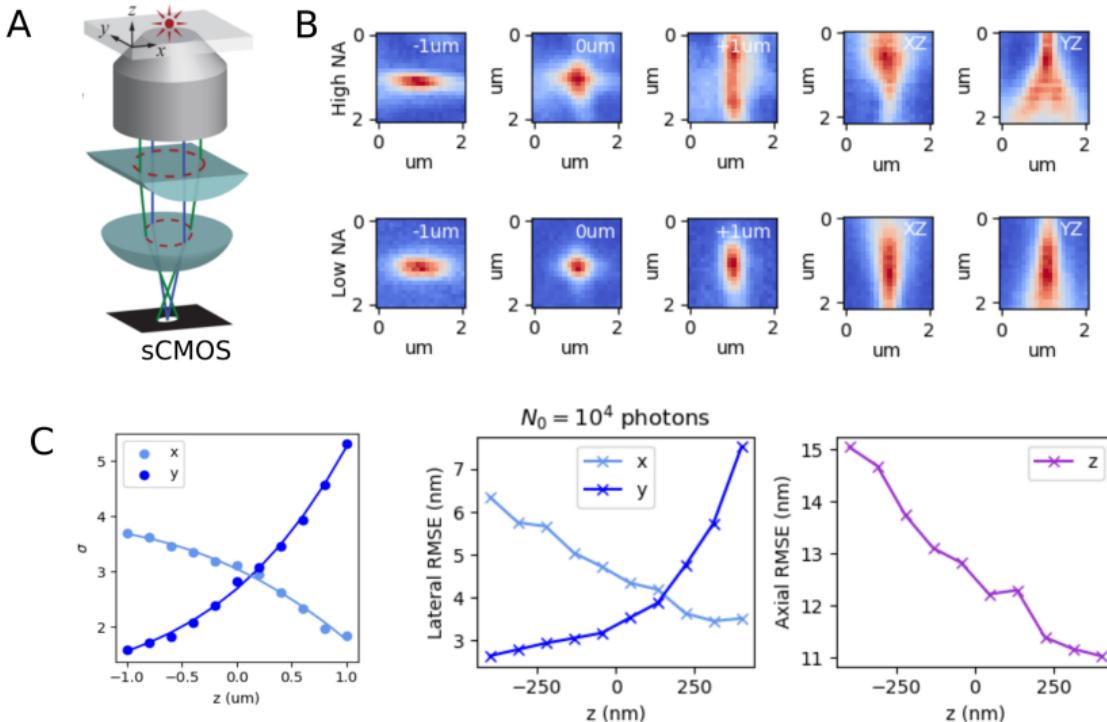


C



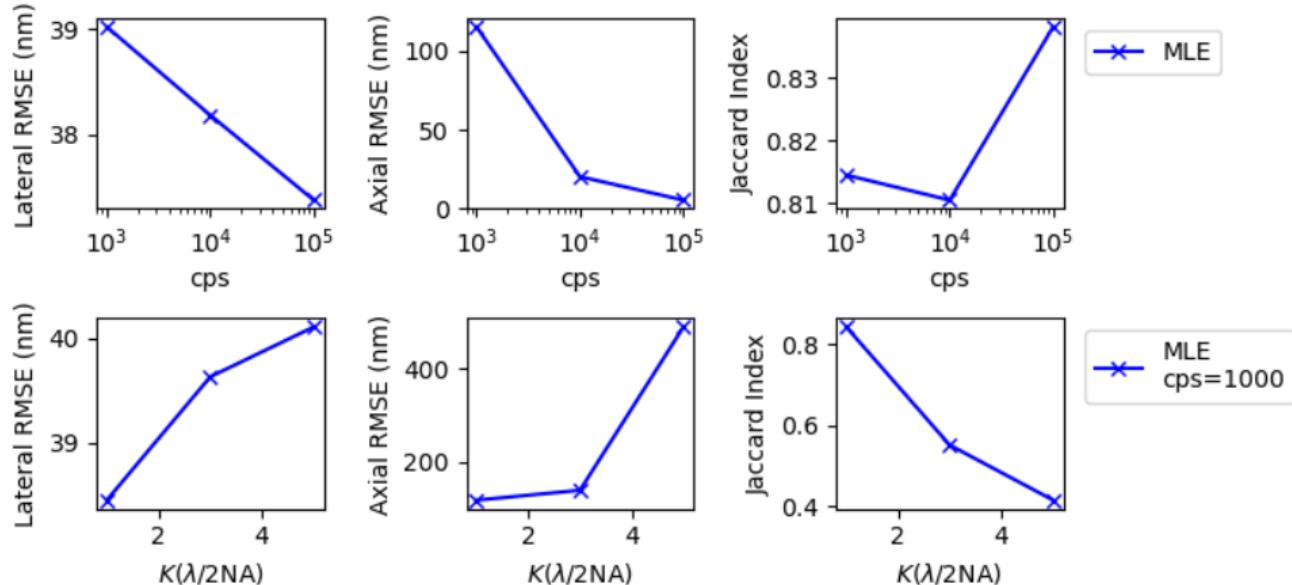
- ▶ $K(\lambda/2NA)$ is Ripley's K function at the diffraction limit ($\lambda = 640\text{nm}$)
- ▶ Convolutional neural networks (CNNs) approach the CRLB (gray) at high photon counts and generalize to the dense regime

Astigmatism based three dimensional imaging



- A weak ($f = 10\text{m}$) cylindrical lens breaks the axial symmetry of the PSF

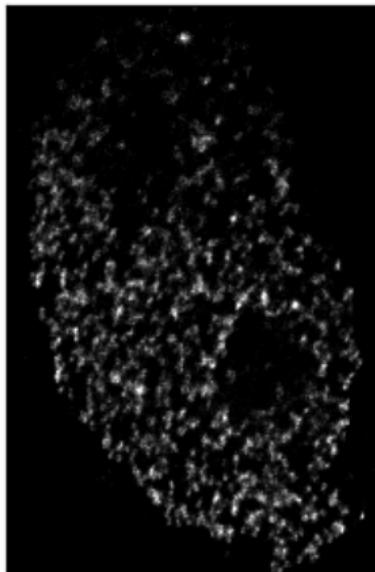
Astigmatism based three dimensional imaging



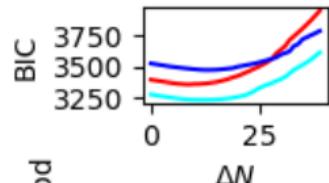
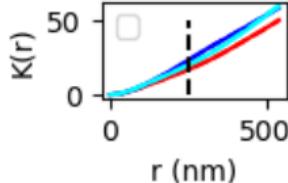
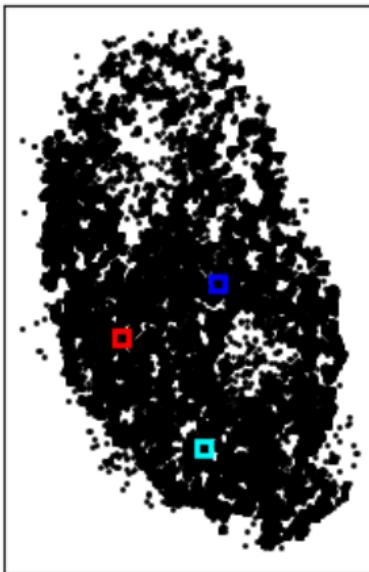
- ▶ $z_0 \sim U([-0.4, 0.4])$ um
- ▶ 3D imaging requires long exposure and sparse emitters for MLE
- ▶ Deep methods may be a suitable choice in future work

Chromatin nanodomains in a living Hela cell nucleus at 37C

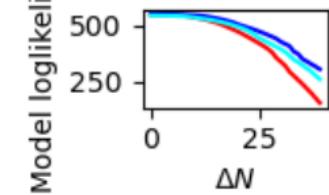
Density



Pointillist



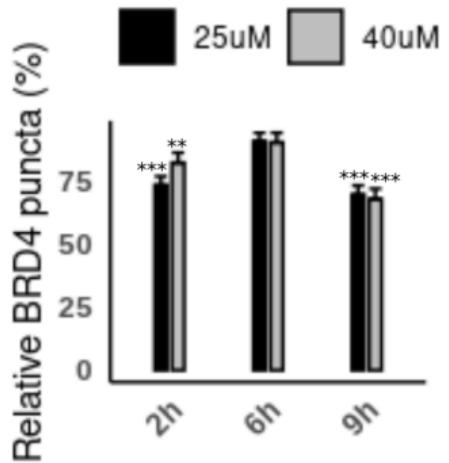
Model loglikelihood



- ▶ Density estimation using 30x30nm bins
- ▶ Closest pairs are merged one at a time, until we minimized the BIC
- ▶ Data likelihood is computed under a Gaussian Mixture Model (GMM)

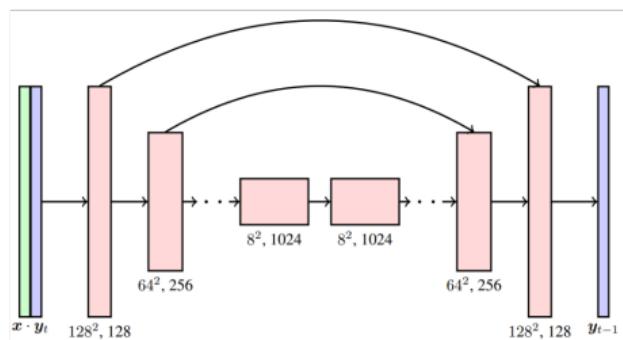
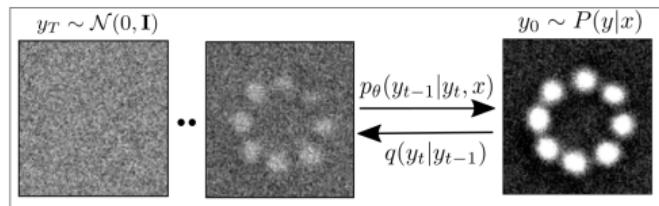
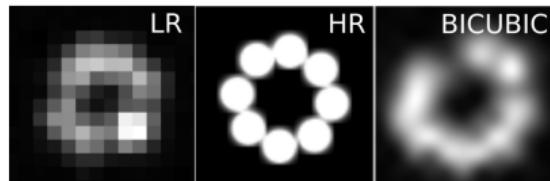
Results and Future Aims

Dissolving BRD4 condensates with JQ1 and 1,6 Hexanediol

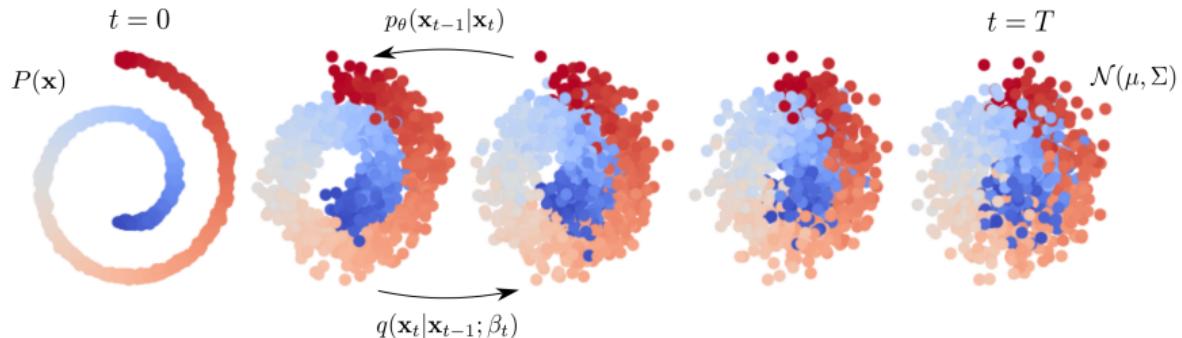


Proposed two and three color imaging experiments

Deep generative modeling with nonequilibrium thermodynamics



Deep generative modeling with nonequilibrium thermodynamics



- ▶ The data distribution is gradually converted into a analytically tractable distributon e.g., Gaussian
- ▶ Repeated application of a Markov transition kernel destroys data structure
- ▶ Use deep neural networks to learn to "reverse time"