

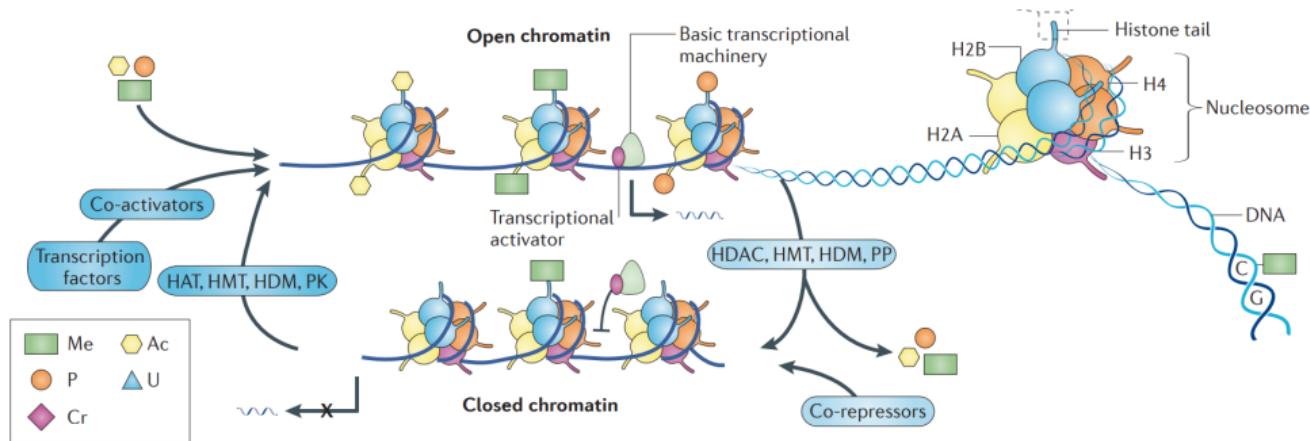
# Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

Clayton W. Seitz

August 21, 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

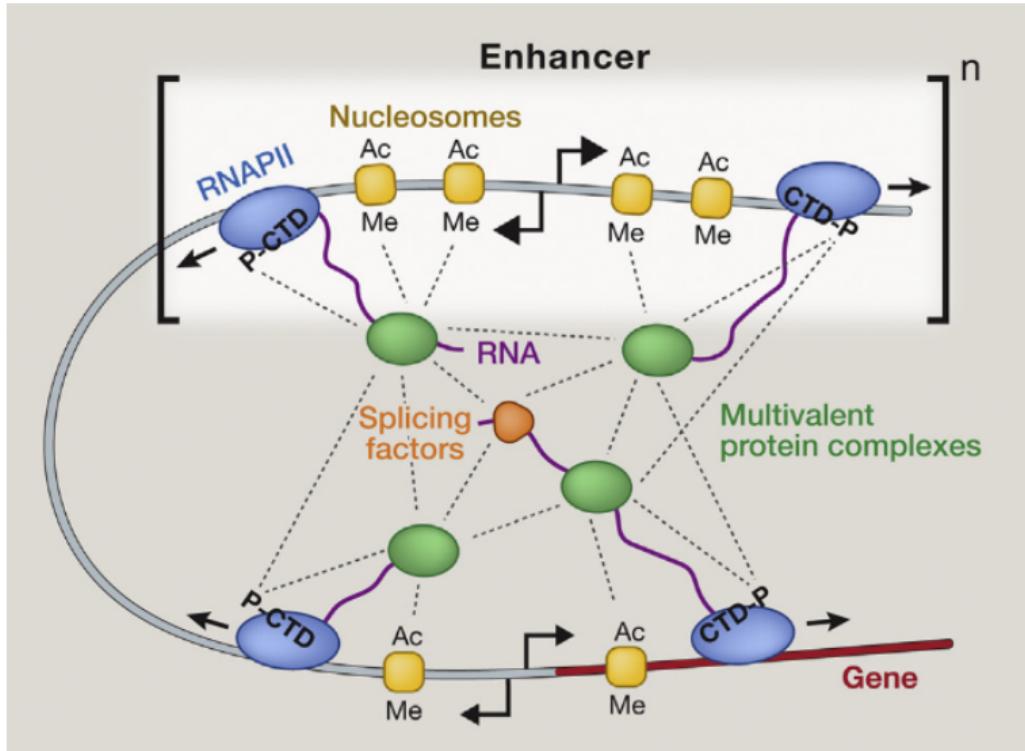
# The textbook view of histone acetylation



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

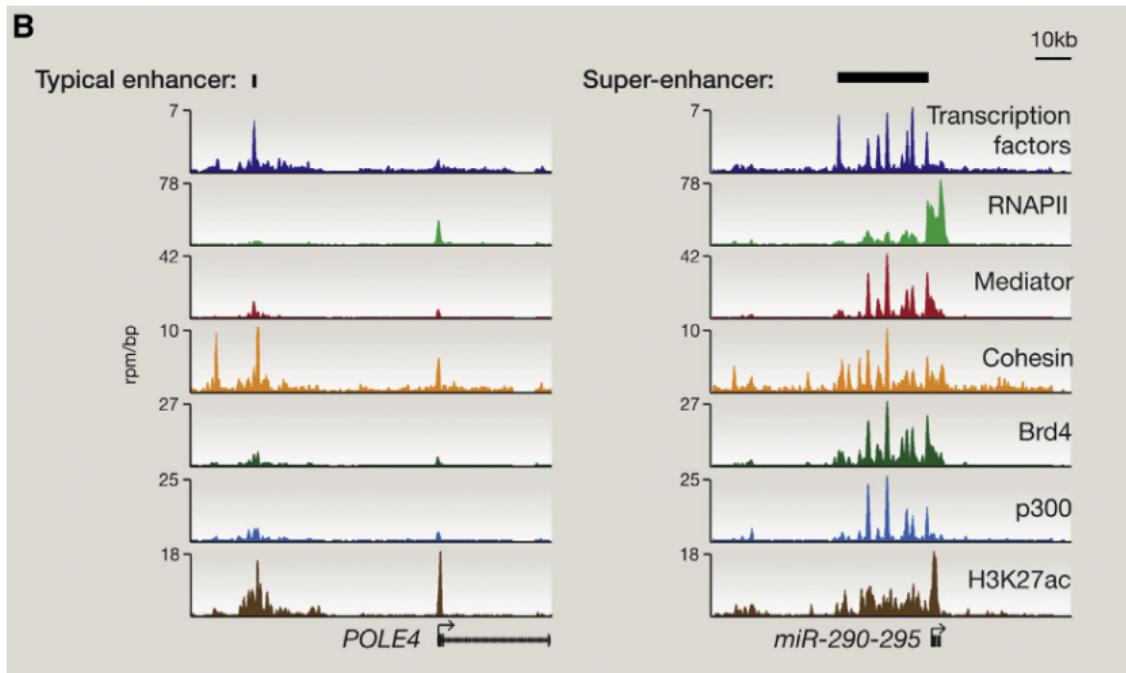
- ▶ I am interested in the impact of BRD4 protein on chromatin structure
- ▶ Previous work has shown BRD4 is associated with histone acetylation
- ▶ Live cell super-resolution imaging is a useful tool

# A phase separation model for transcriptional control



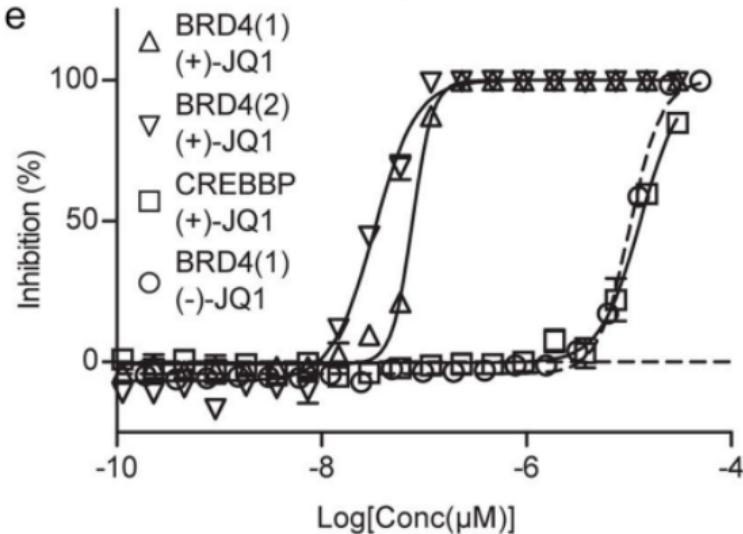
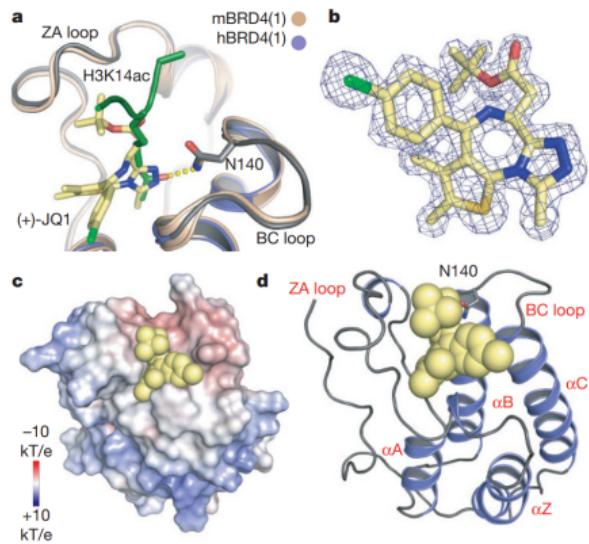
Hnisz et al. A phase separation model of transcriptional control. Cell 2017

# A phase separation model for transcriptional control



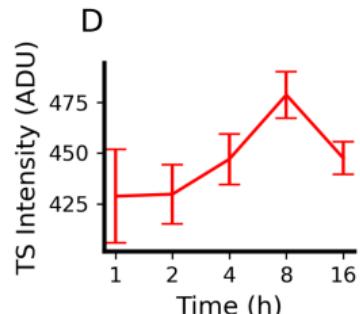
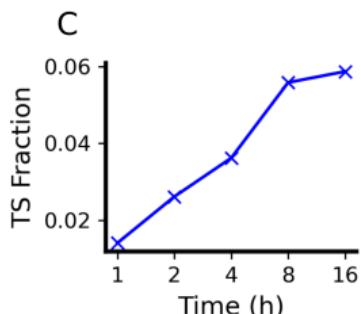
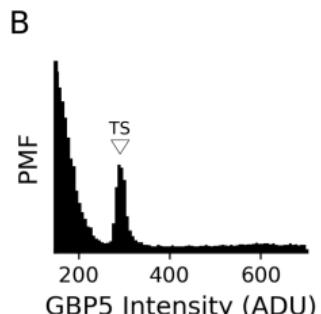
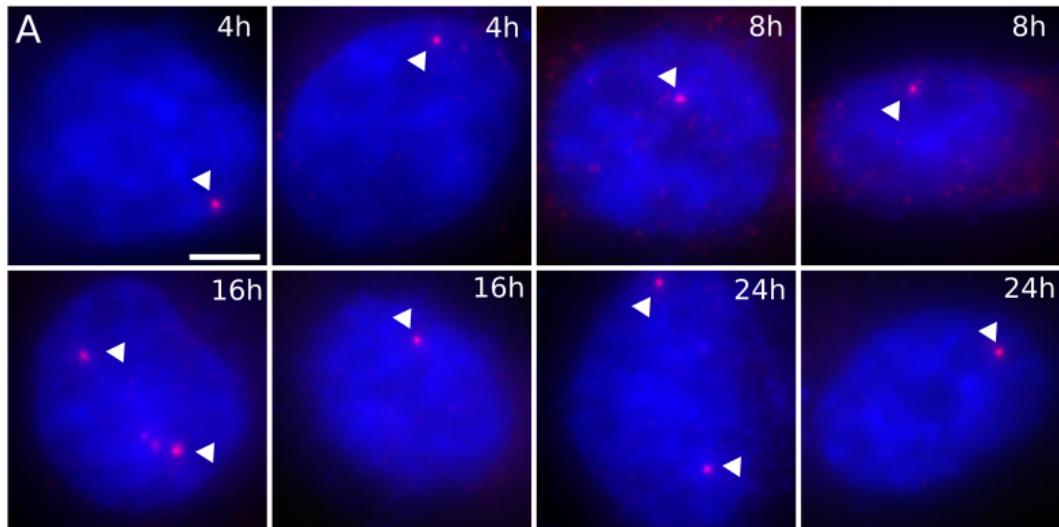
*Hnisz et al. A phase separation model of transcriptional control. Cell 2017*

# (+)-JQ1 in complex with BRD4 protein

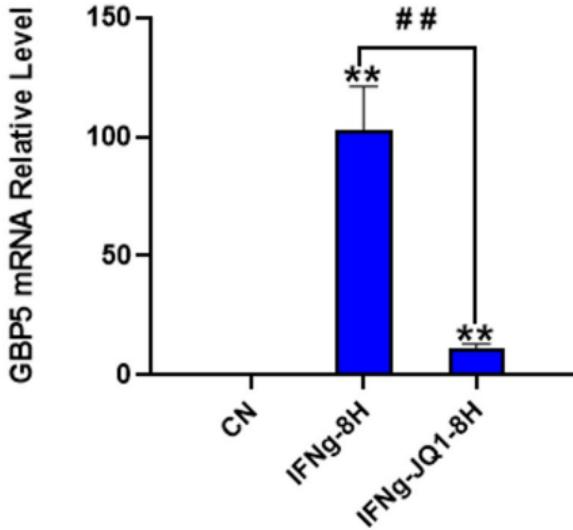
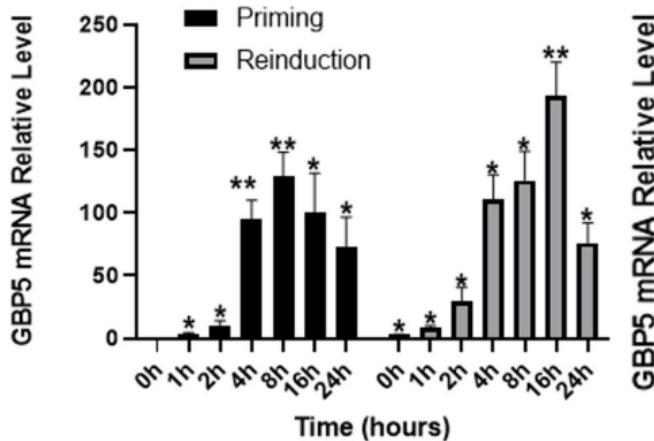


Filippakopoulos. Selective inhibition of BET bromodomains. *Nature Communications*

# Induction of a BRD4-controlled gene with cytokine treatment

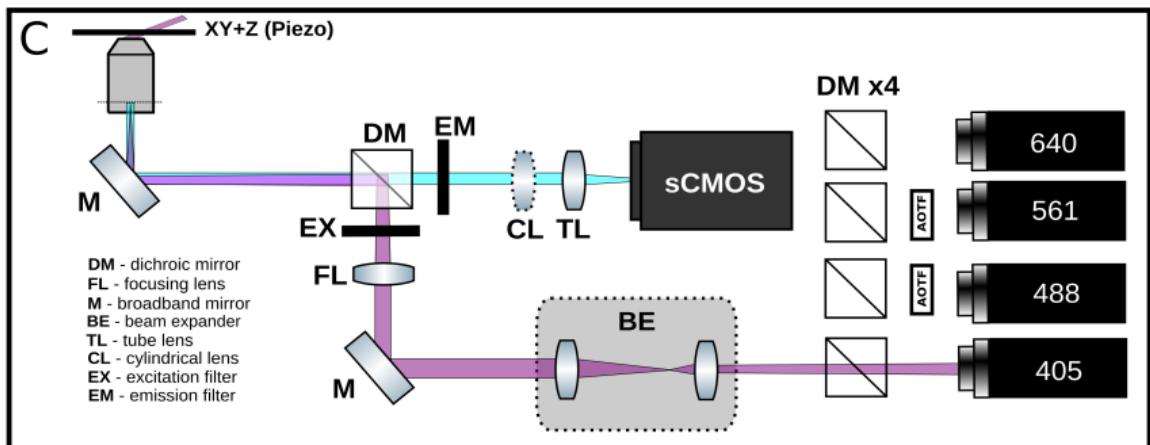
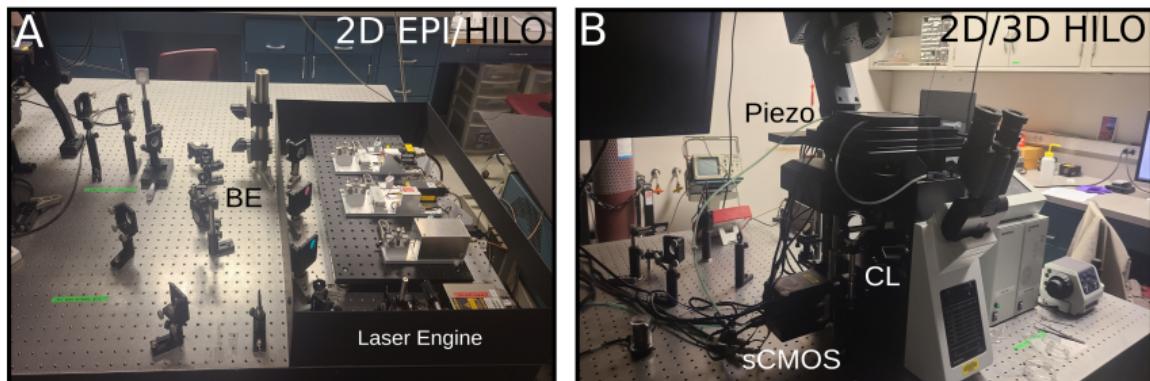


## Validation of JQ1 efficacy for BRD4 inhibition in HeLa cells

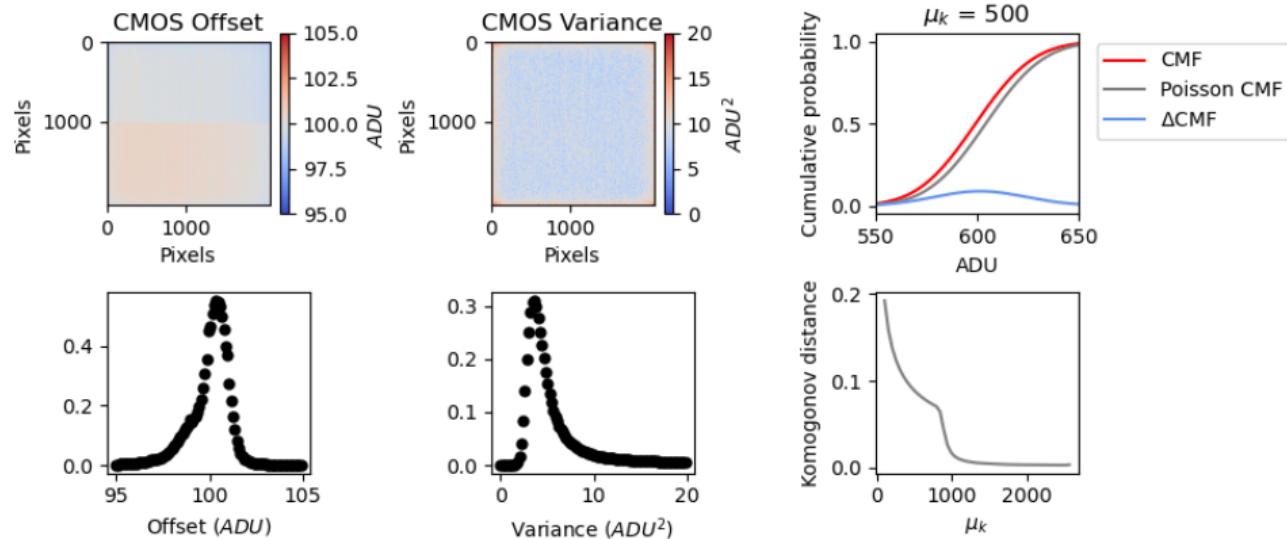


- RT-qPCR quantified using  $2^{-\Delta\Delta C_t}$  method using GAPDH as a reference gene
- \*: $P \leq 0.1$ , \*\*: $P \leq 0.01$

# Instrumentation for single molecule localization microscopy



# A Poisson approximation at moderate SNR simplifies SMLM



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

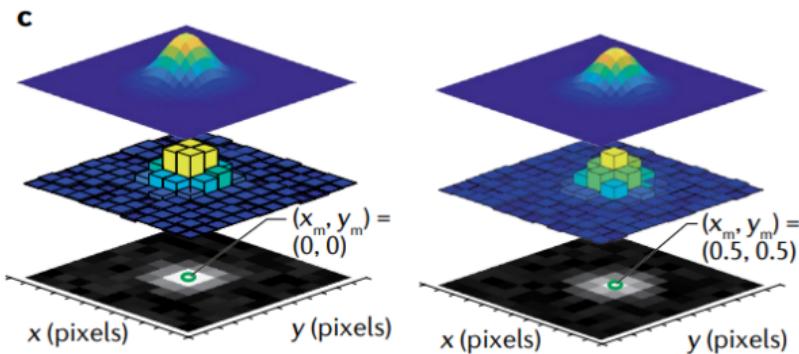
$P(H_k|\theta)$  can be approximated as Poisson at high signal-to-noise (SNR)

# Maximum likelihood localization of an isolated fluorescent emitter

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

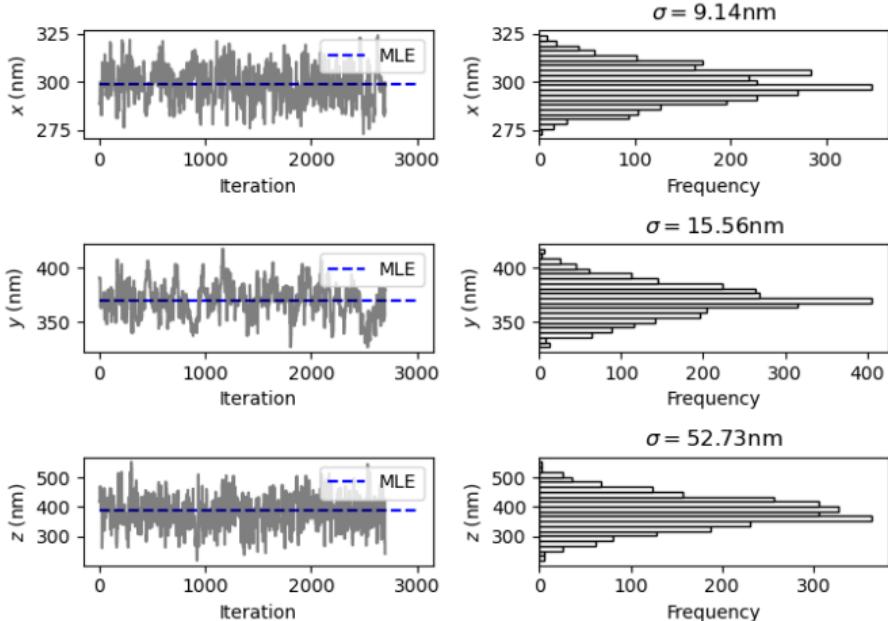
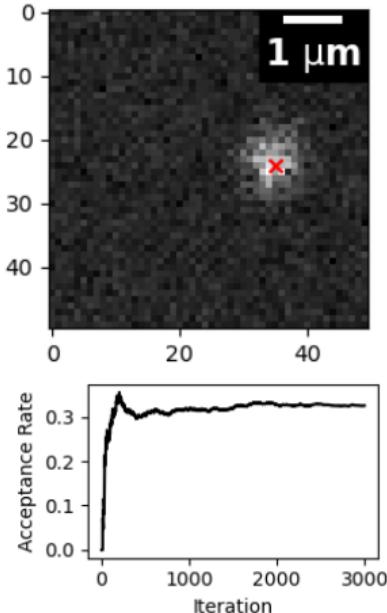
$$\mu_k = g_k \eta N_0 \Delta \int_{\text{pixel}} G(x, y) dA$$

$\eta$  – quantum efficiency  
 $N_0$  – photon count  
 $\Delta$  – exposure time



- ▶ Fisher information and Cramer-Rao lower bound (CRLB) can be computed analytically for Poisson log-likelihood  $\ell$  (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,

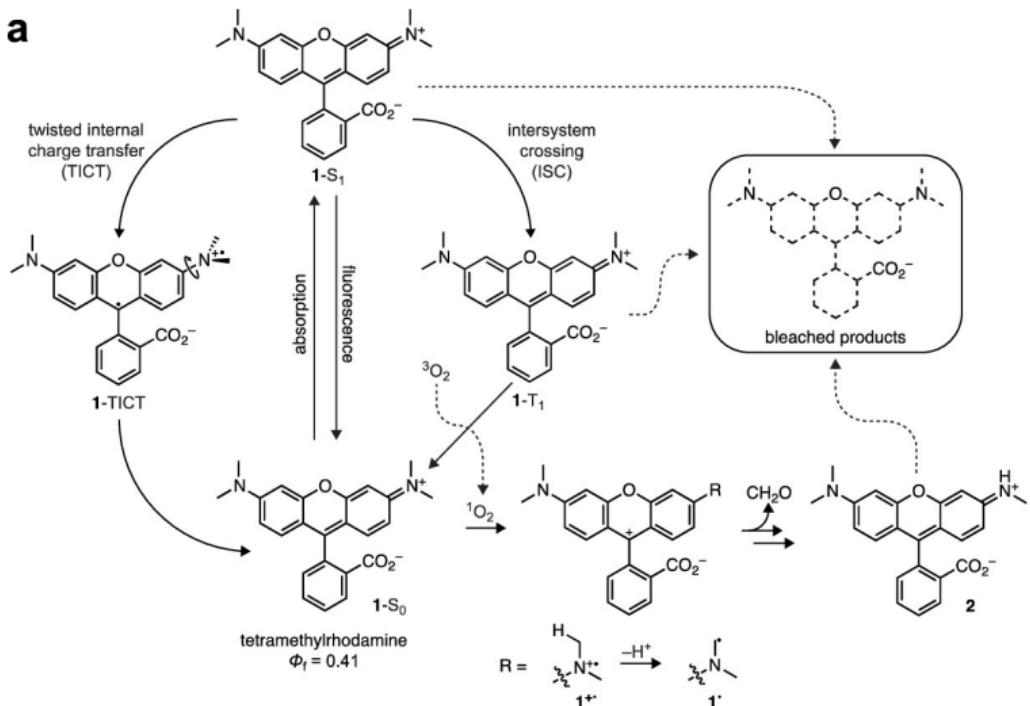
$$\frac{\partial \ell}{\partial \theta_i} = \frac{\partial}{\partial \theta_i} \sum_k x_k \log x_k + \mu'_k - x_k \log (\mu'_k)$$

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

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

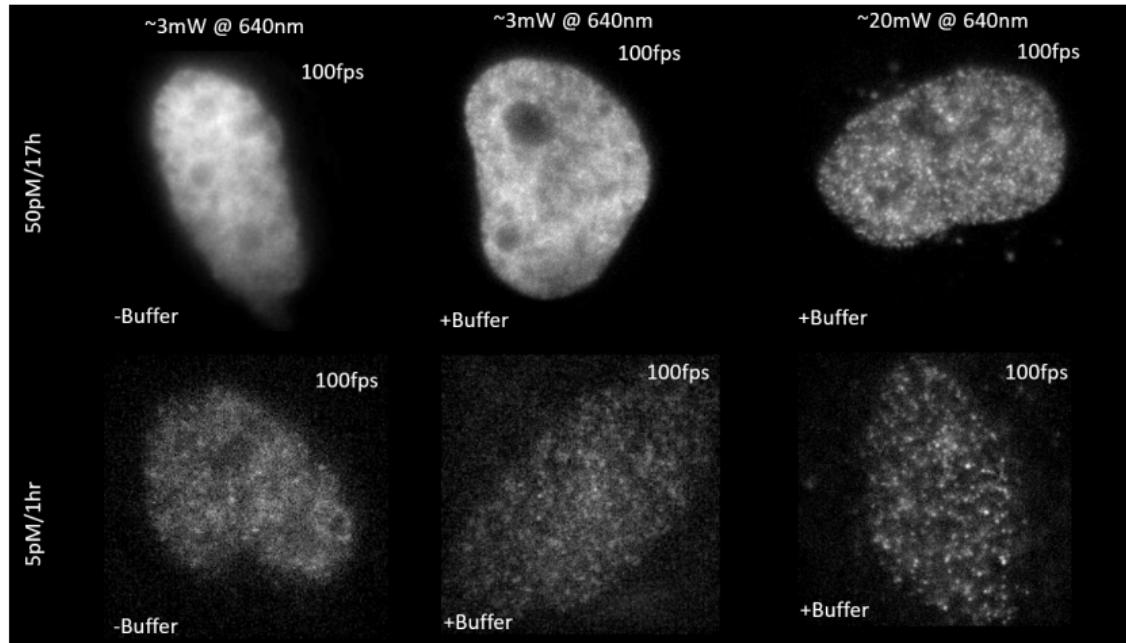
The CRLB is a frequentist

# Direct STORM: The photophysics of rhodamines



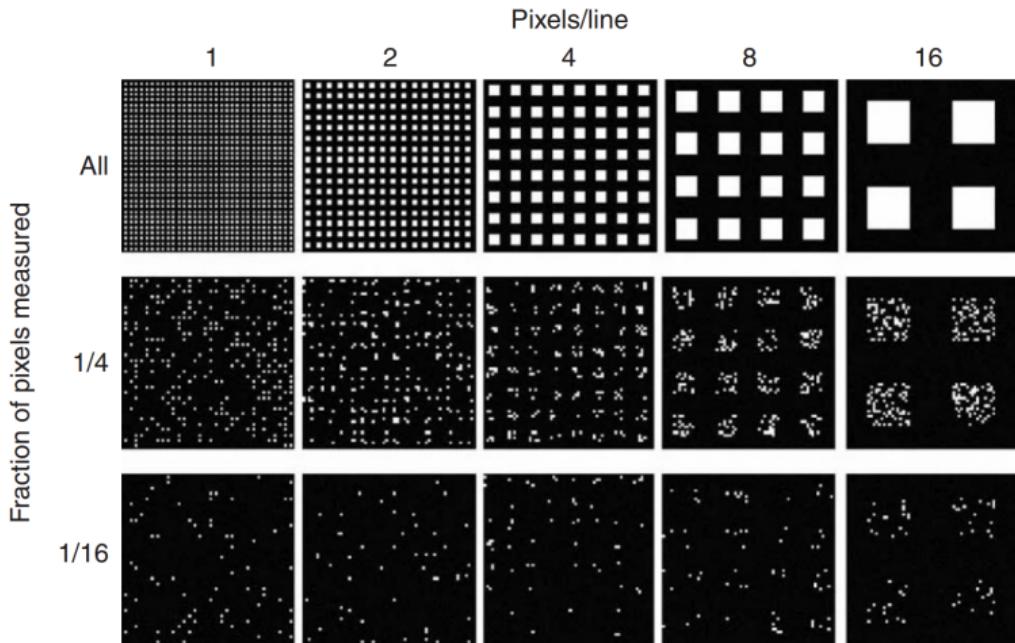
- ▶ Reduction of the T<sub>1</sub> state yields a dark, long-lived, and stable radical state
- ▶ The reducing agent is usually a primary thiol like cysteamine (MEA)

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



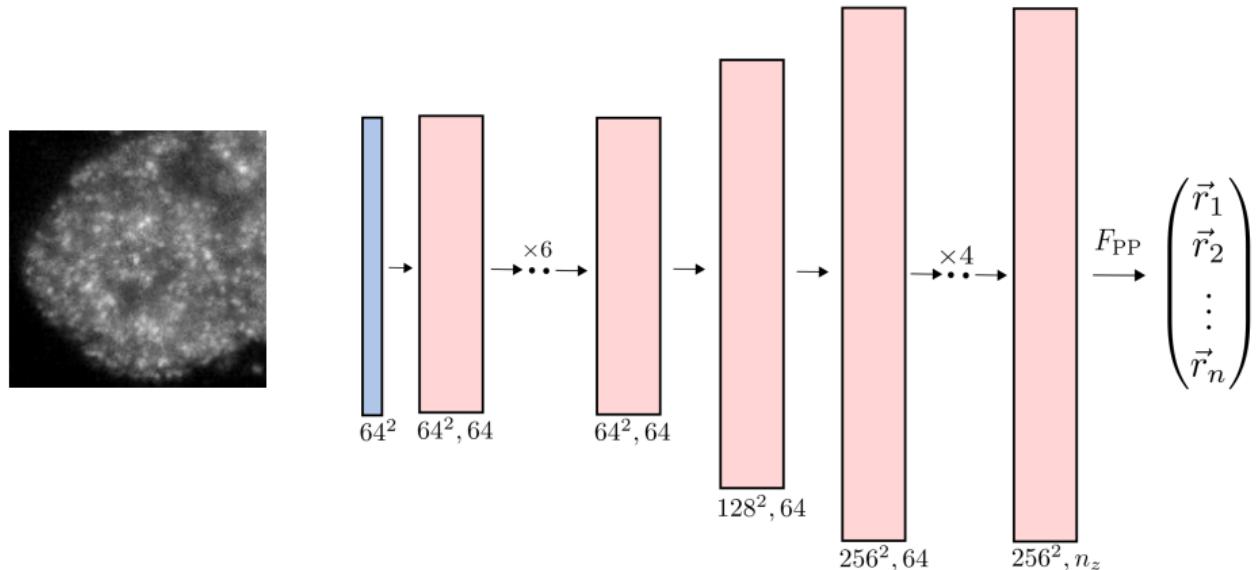
- ▶ High power maintains the OFF state, potentially by promoting triplet state formation

# 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

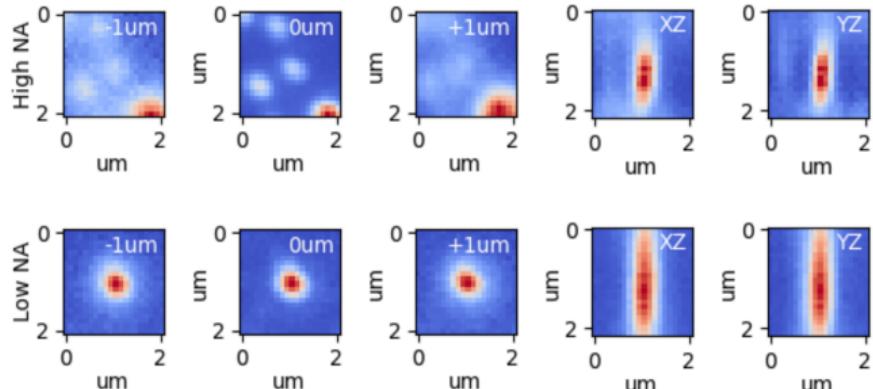


Localization is cast as semantic segmentation of the high resolution tensor:

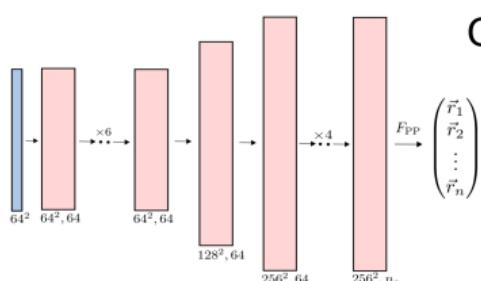
$$\mathcal{L} = \sum_{i,j} \log p_{ij}(\tilde{x}) = \sum_{i,j} \log \frac{\exp(-s_{ij}(\tilde{x}))}{\sum_{x \in \chi} \exp(-s_{ij}(\tilde{x}))}$$

# Estimator precision sets the resolution limit in localization microscopy

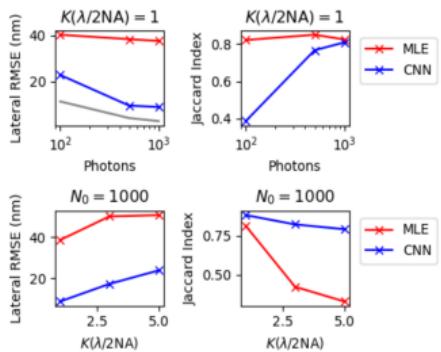
A



B

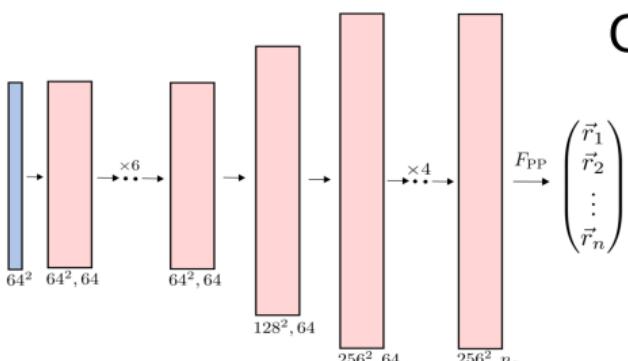


C

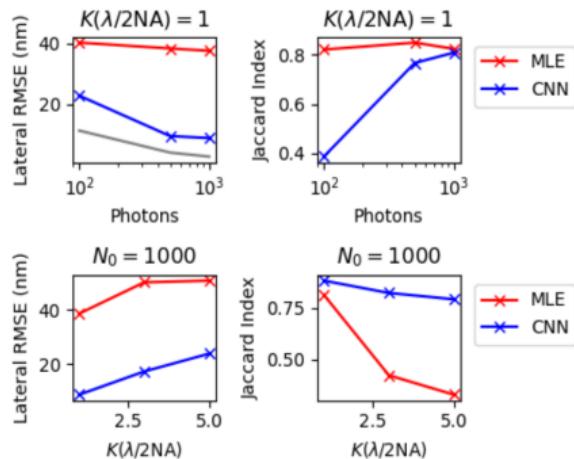


# Estimator precision sets the resolution limit in localization microscopy

B

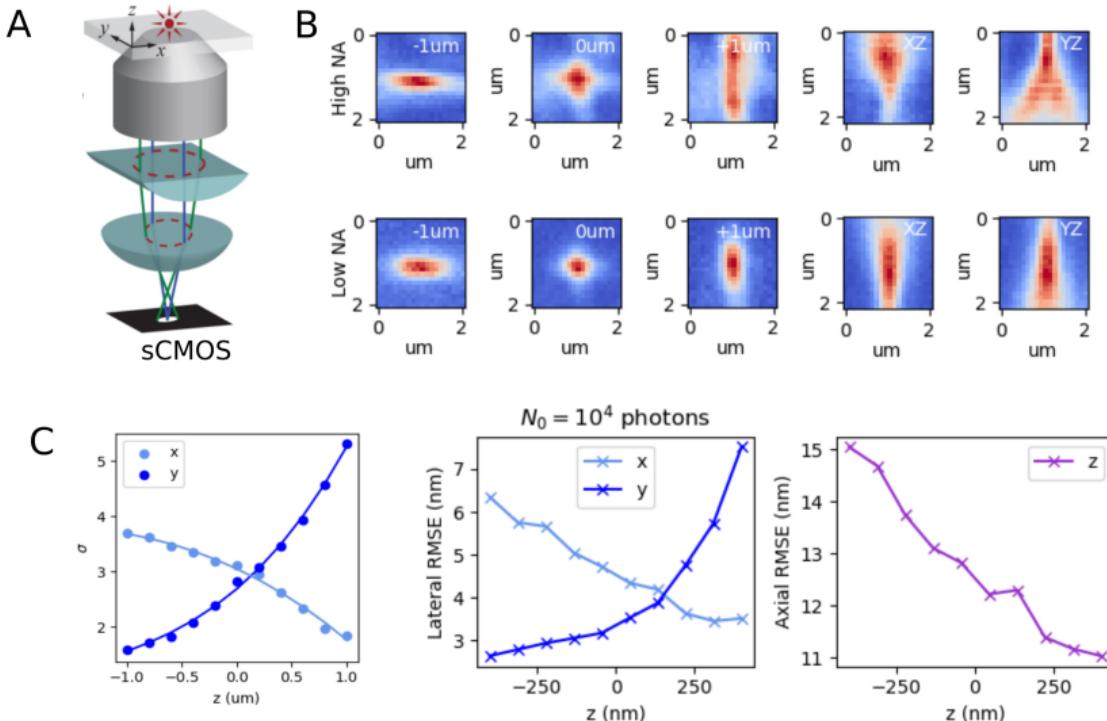


C



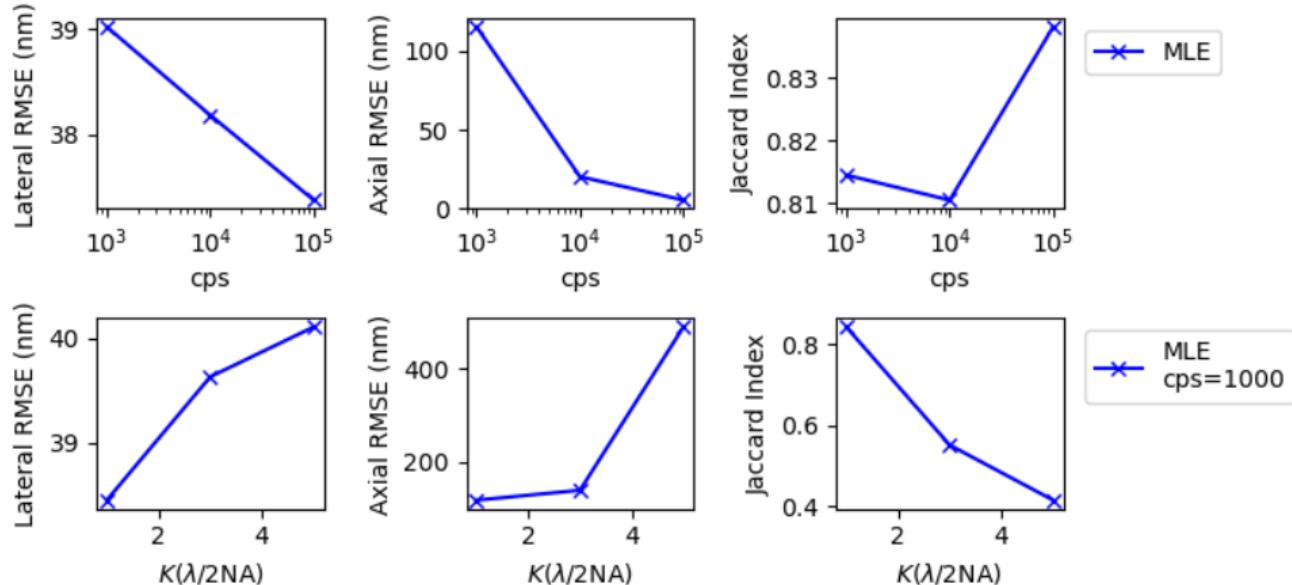
- ▶  $K(\lambda/2NA)$  is Ripley's K function at the diffraction limit ( $\lambda = 640\text{nm}$ )
- ▶ Convolutional neural networks (CNNs) approach the CRLB (gray) at high photon counts and generalize to the dense regime

# Astigmatism based three dimensional imaging



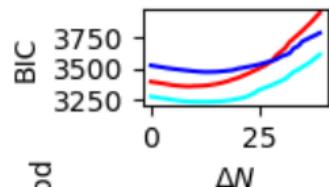
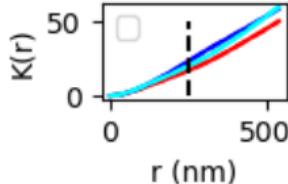
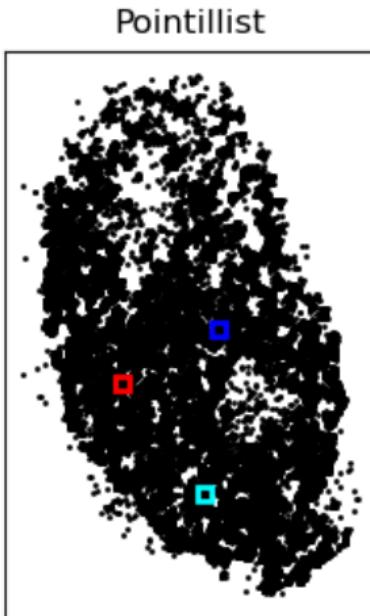
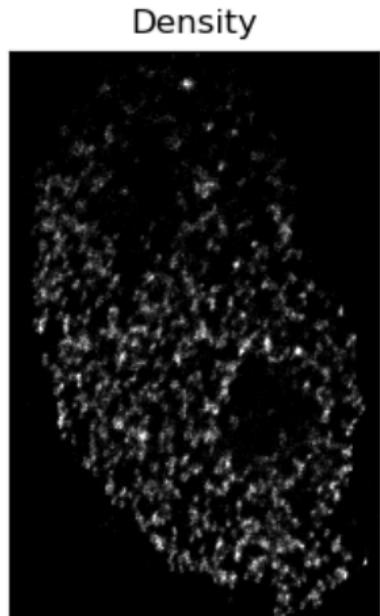
- A weak ( $f = 10\text{m}$ ) cylindrical lens breaks the axial symmetry of the PSF

# Astigmatism based three dimensional imaging

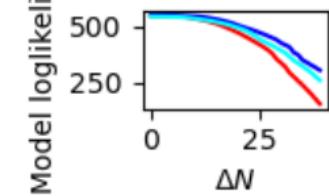


- ▶  $z_0 \sim U([-0.4, 0.4])$  um
- ▶ 3D imaging requires long exposure and sparse emitters for MLE
- ▶ Deep methods may be a suitable choice in future work

# Chromatin nanodomains in a living Hela cell nucleus at 37C



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)

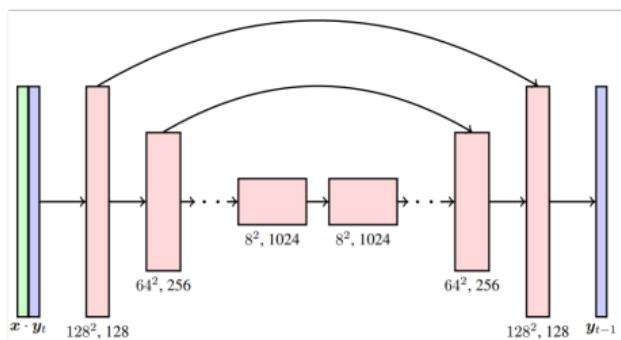
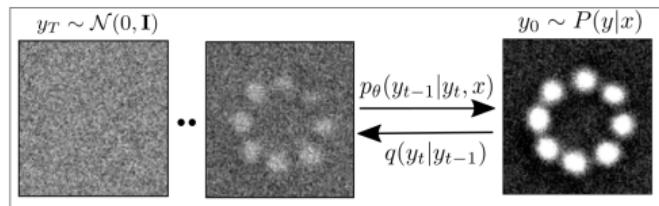
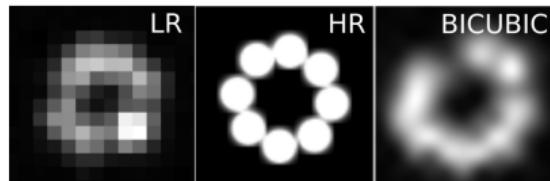
## Results and Future Aims

# Dissolving BRD4 condensates with 1,6 Hexanediol

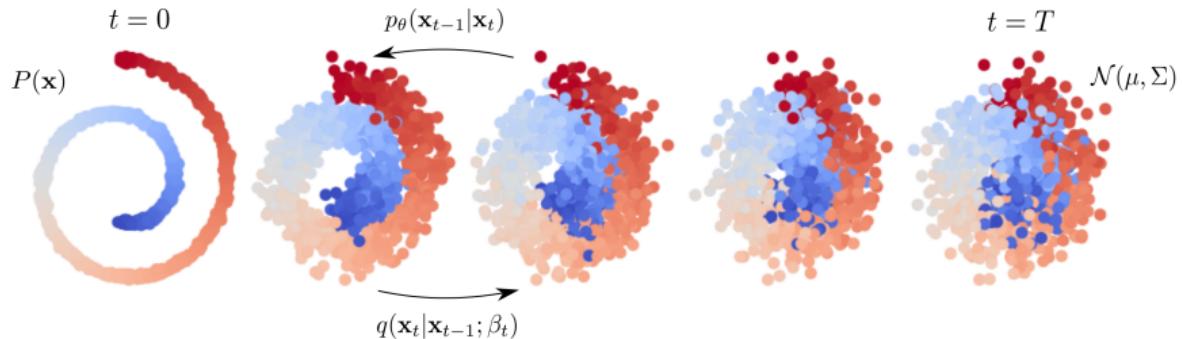
Fixed cell results

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"