

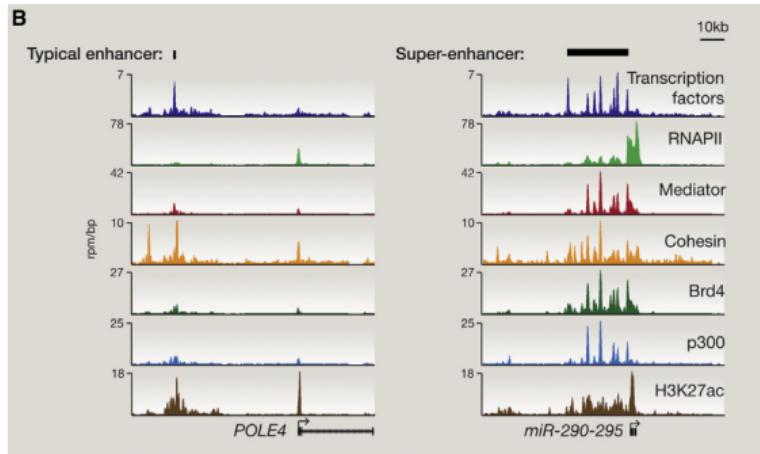
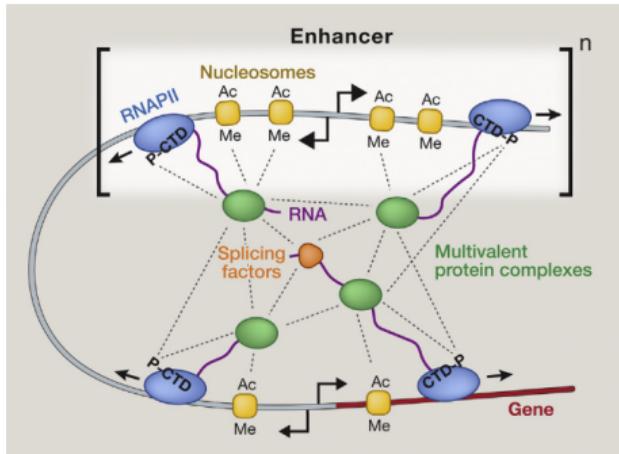
Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

Clayton W. Seitz

August 28, 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

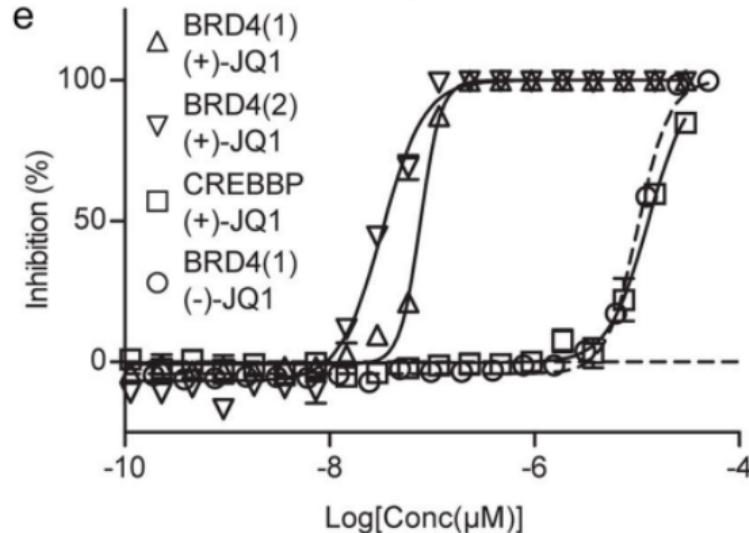
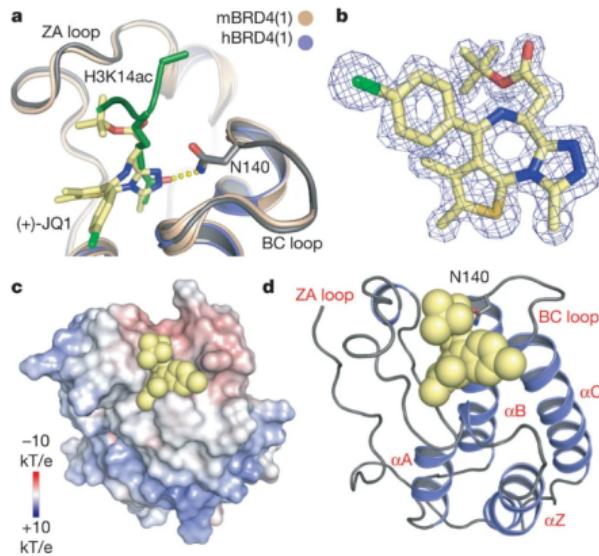
A phase separation model for transcriptional control



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

- ▶ Super-enhanced genes are regulated by large molecular assemblies
- ▶ BRD4 is an interesting target since specific and non-specific inhibitors exist
- ▶ Nucleosome organization is poorly characterized at super-enhancers

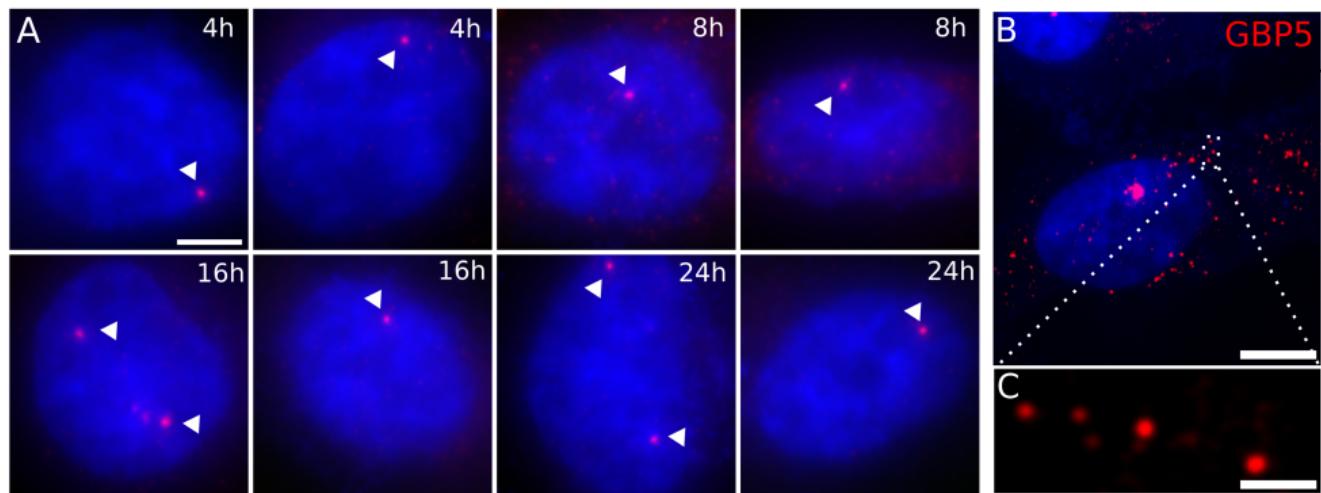
(+)-JQ1 in complex with BRD4 protein



Filippakopoulos. Selective inhibition of BET bromodomains. *Nature*

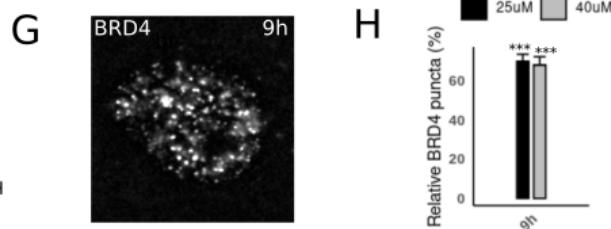
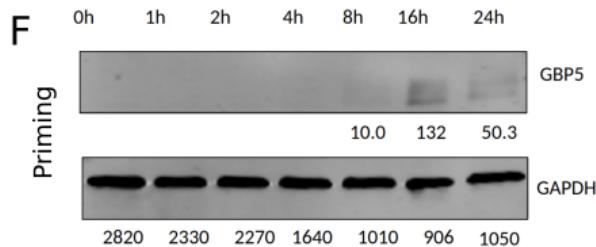
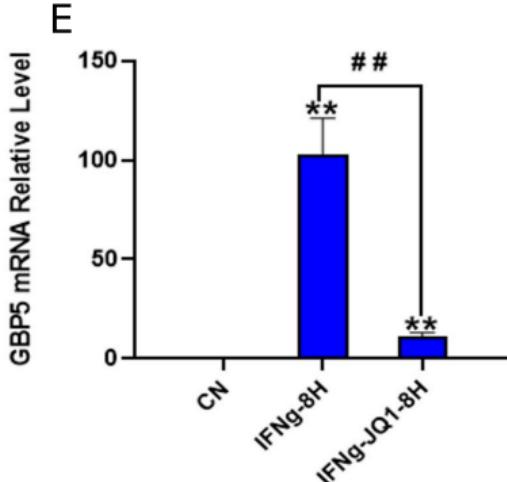
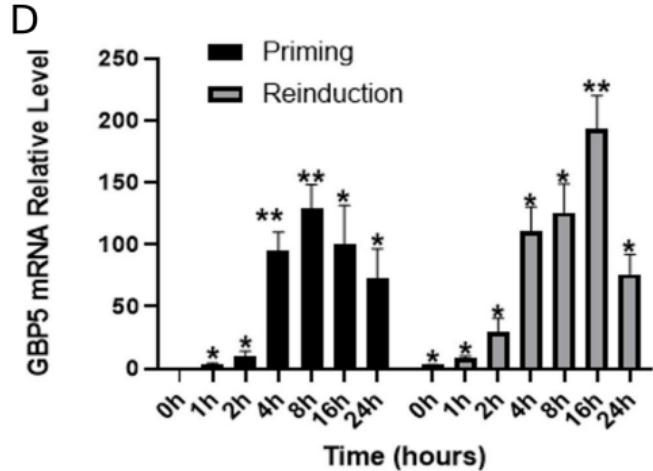
- BRD4 has disordered domains which are thought to promote phase separation
- Non-specific phase separation inhibitors exist

Inhibition of a super-enhanced gene with JQ1



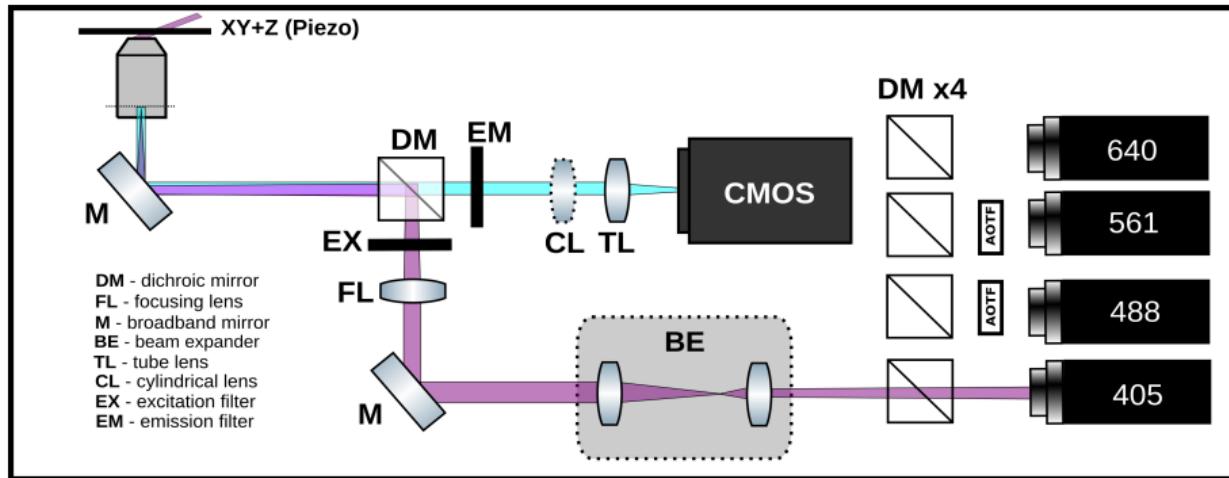
- ▶ Blue - DAPI (binds DNA minor groove)
- ▶ White arrows highlight putative GBP5 transcription sites

Inhibition of a super-enhanced gene with JQ1

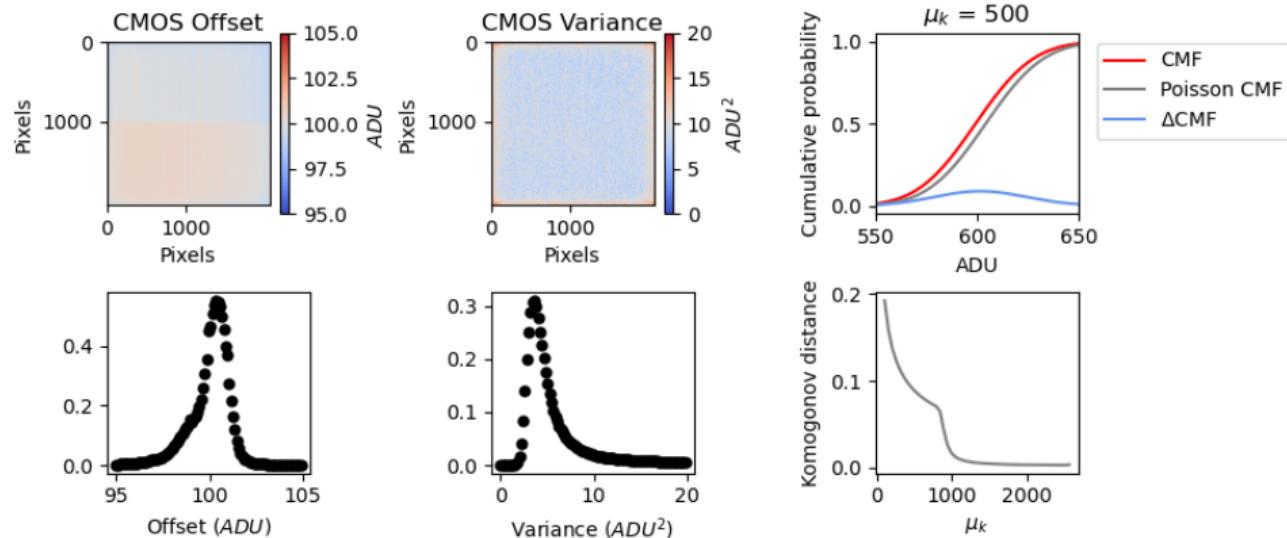


► *: $P \leq 0.1$, **: $P \leq 0.01$

Instrumentation for single molecule localization microscopy



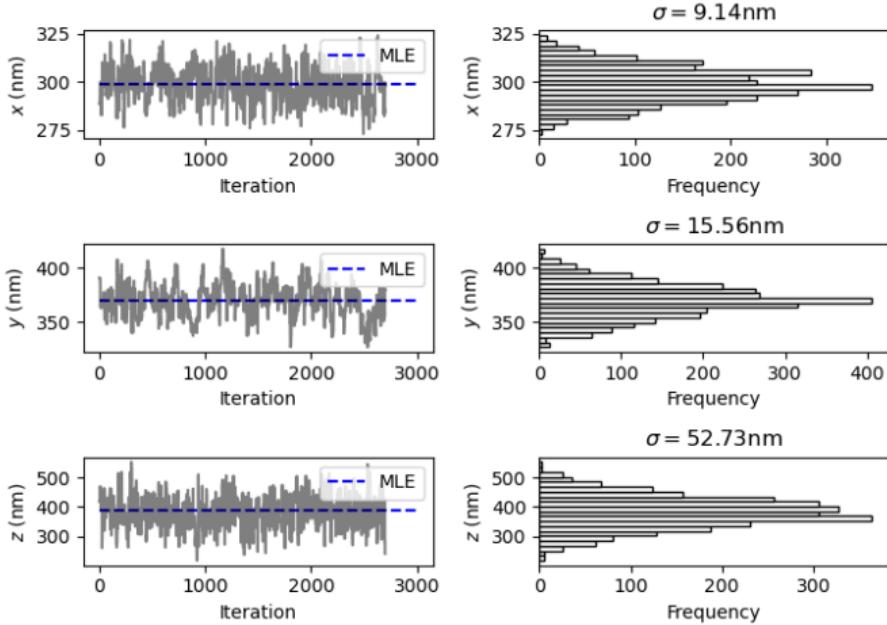
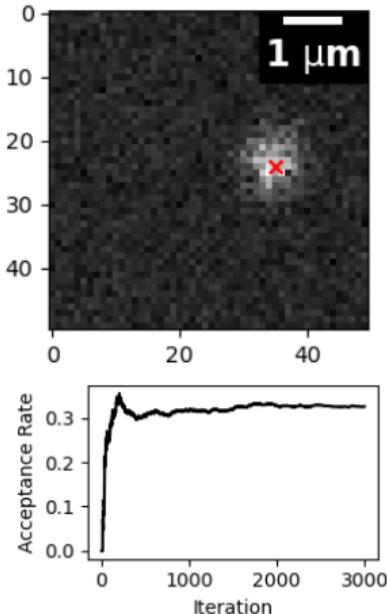
A Poisson approximation at moderate SNR simplifies SMLM



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

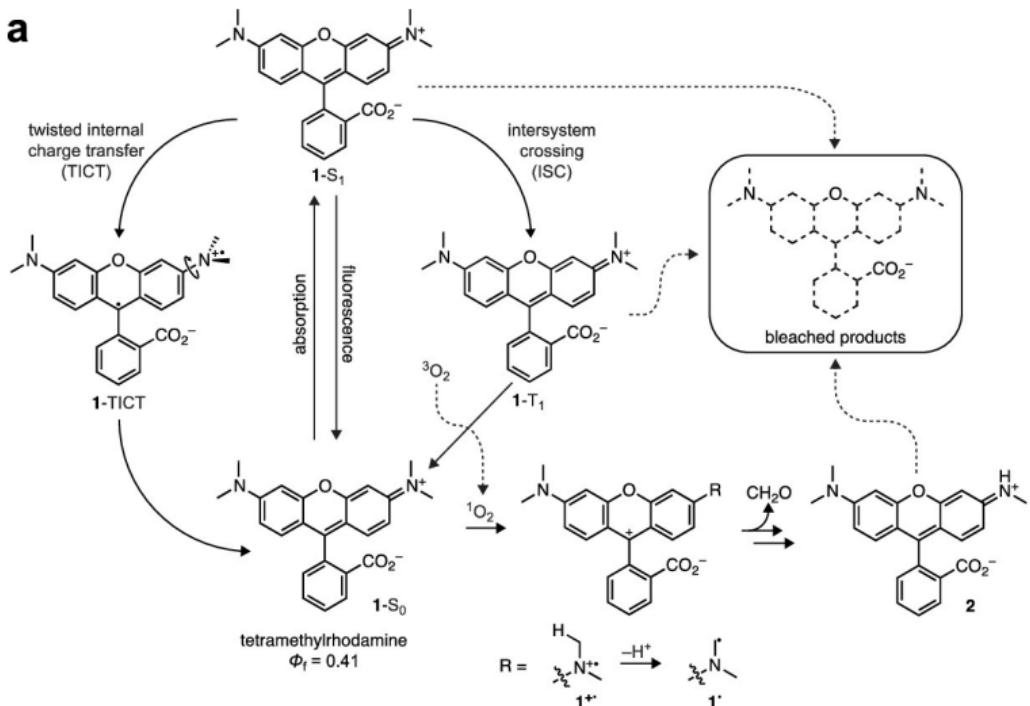
$P(H_k|\theta)$ can be approximated as Poisson at high signal-to-noise (SNR)

Estimator precision sets the resolution limit in localization microscopy



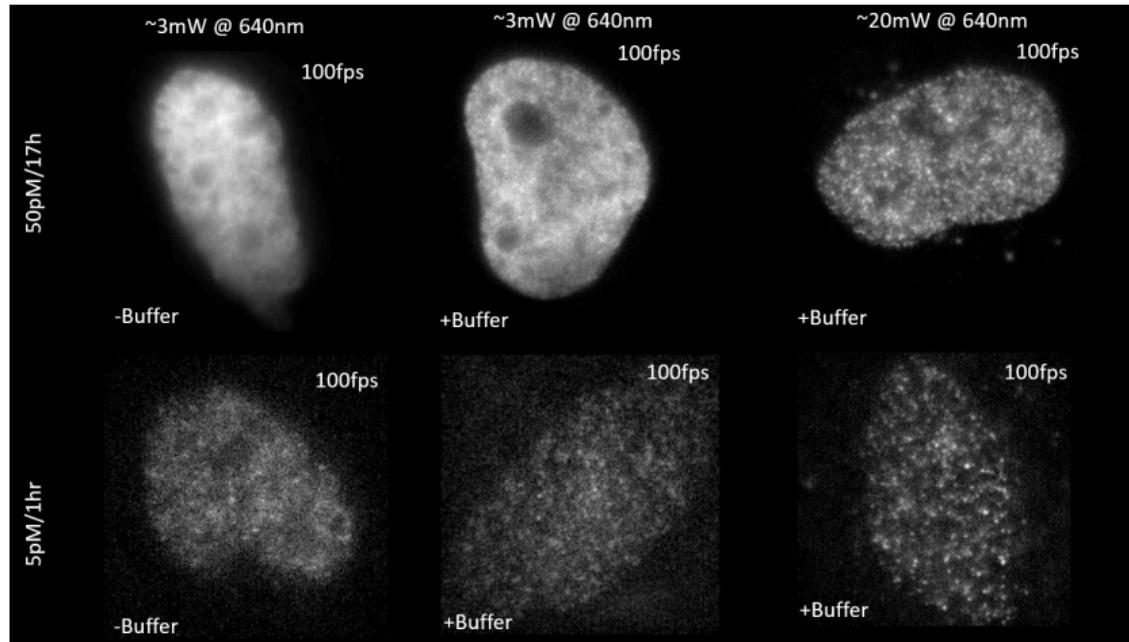
- ▶ Variance of the posterior $P(\theta|\vec{H})$ is a useful particle filter
- ▶ We assume uniform priors on coordinates

Direct STORM: The photophysics of rhodamines



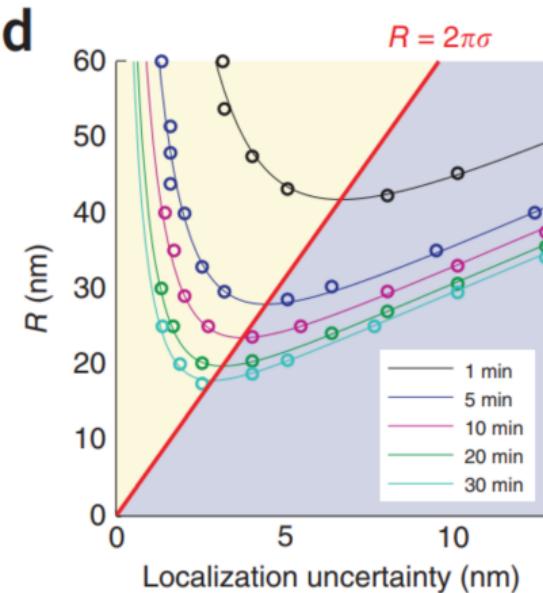
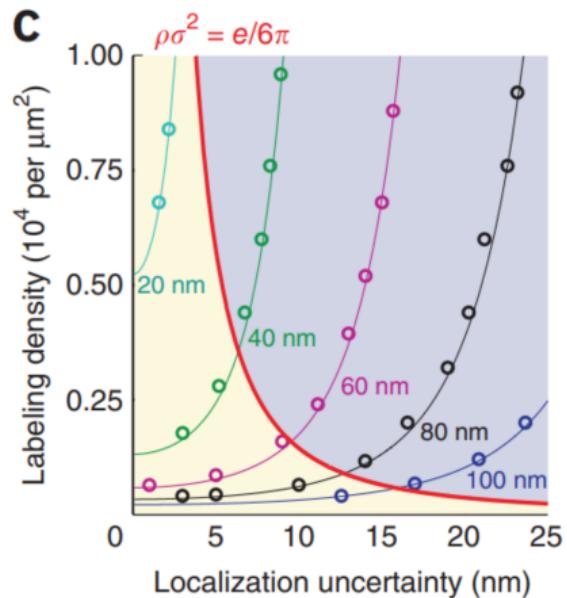
- ▶ Reduction of the T₁ state yields a dark, long-lived, and stable radical state
- ▶ The reducing agent is usually a primary thiol like cysteamine (MEA)

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



- ▶ Laser power controls the OFF state lifetime
- ▶ Dense labeling is possible for a stable OFF state

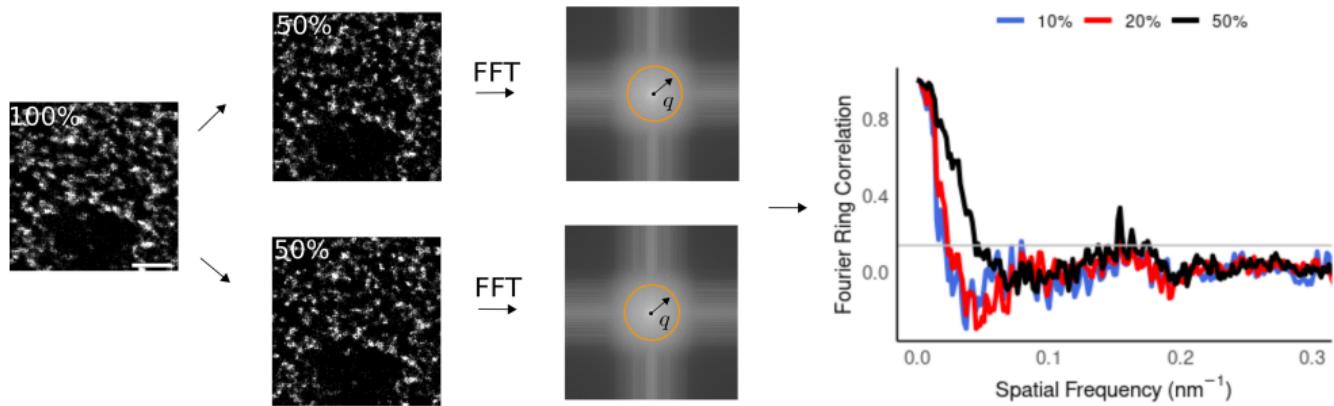
Fourier ring correlation links spatial and temporal resolution



Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy. *Nature Methods* 2013

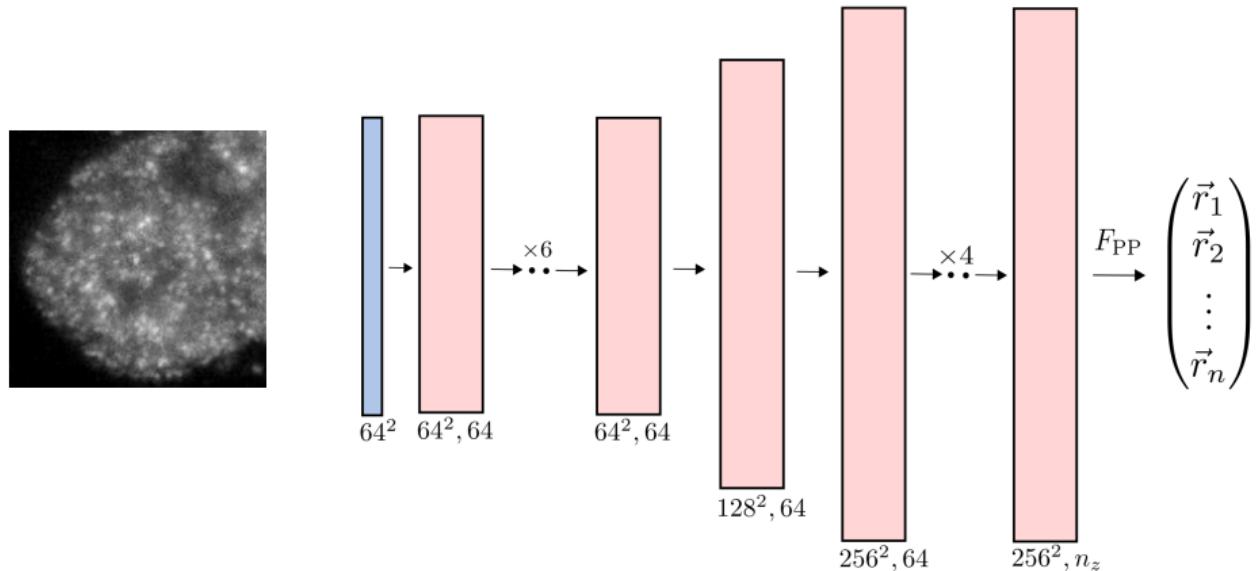
Fourier ring correlation links spatial and temporal resolution

- We can view dSTORM as sampling from a density
- This is why we use Gaussian kernel density estimation (KDE)



$$\text{FRC}(q) = \frac{\sum_{\vec{q} \in \text{circle}} \tilde{f}_1(\vec{q}) \tilde{f}_2(\vec{q})^*}{\sqrt{\sum_{\vec{q} \in \text{circle}} |f_1(\vec{q})|^2} \sqrt{\sum_{\vec{q} \in \text{circle}} |f_2(\vec{q})|^2}}$$

Deep learning enables dense localization in two-dimensions

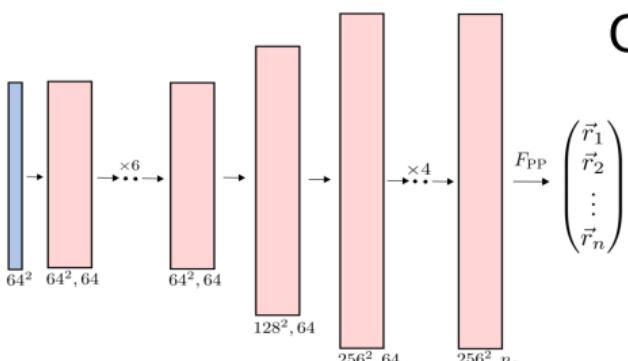


Localization is cast as semantic segmentation of the high resolution tensor:

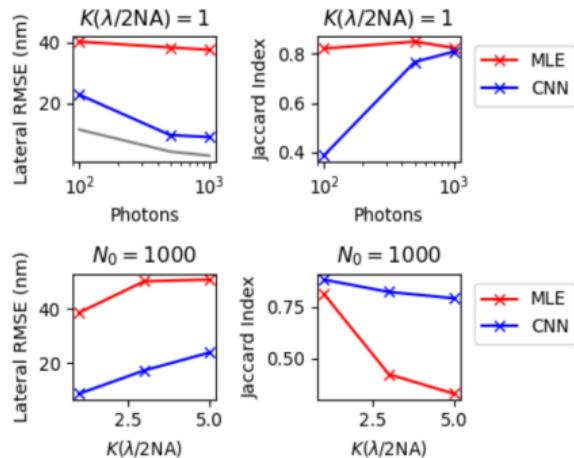
$$\mathcal{L} = \sum_{i,j} \log p_{ij}(\tilde{x}) = \sum_{i,j} \log \frac{\exp(-s_{ij}(\tilde{x}))}{\sum_{x \in \chi} \exp(-s_{ij}(\tilde{x}))}$$

Estimator precision sets the resolution limit in localization microscopy

B

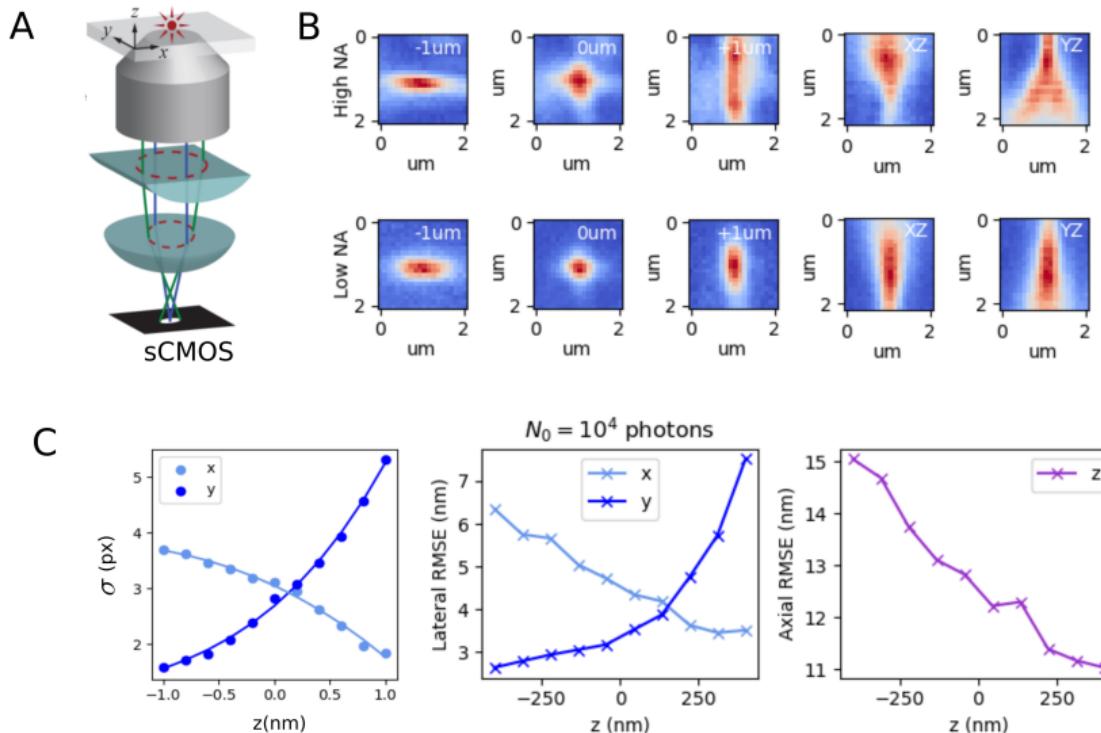


C



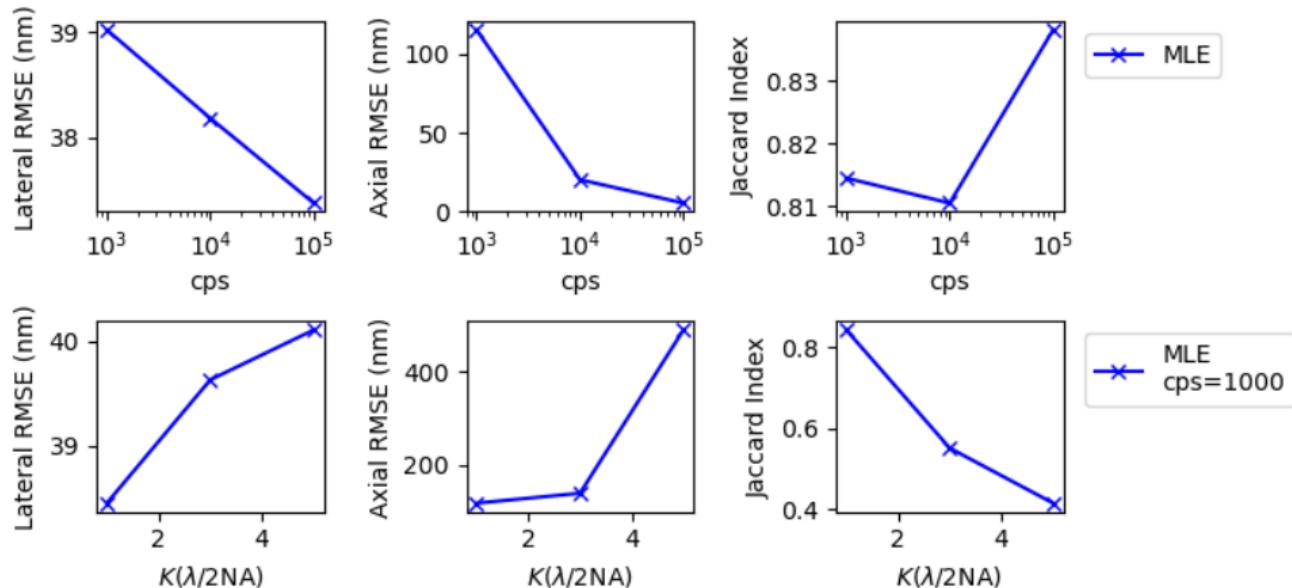
- ▶ $K(\lambda/2NA)$ is Ripley's K function at the diffraction limit ($\lambda = 640\text{nm}$)
- ▶ Convolutional neural networks (CNNs) approach the Cramer-Rao lower bound (gray)

Astigmatism based three dimensional imaging



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

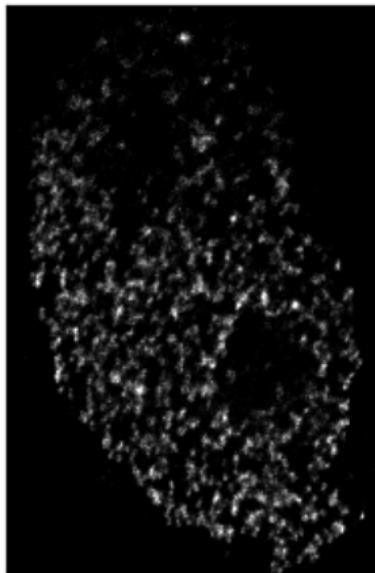
Astigmatism based three dimensional imaging



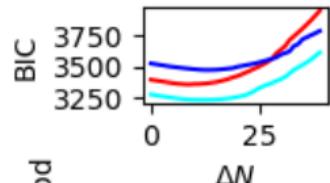
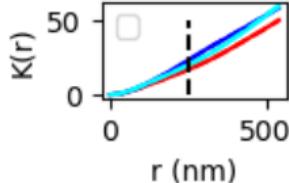
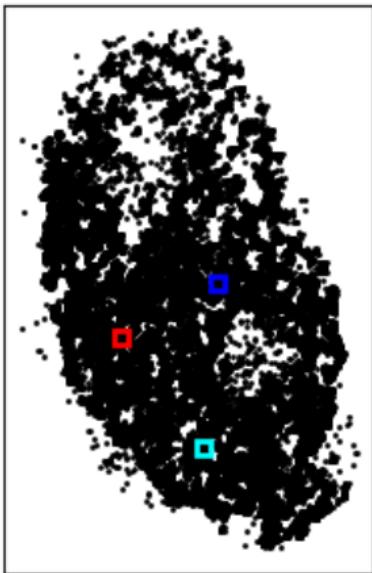
- ▶ $z_0 \sim U([-0.4, 0.4])$ um
- ▶ 3D imaging requires long exposure and sparse emitters for MLE
- ▶ Deep methods may be a suitable choice in future work

Chromatin nanodomains in a living Hela cell nucleus

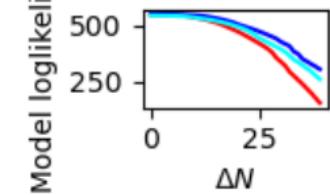
Density



Pointillist

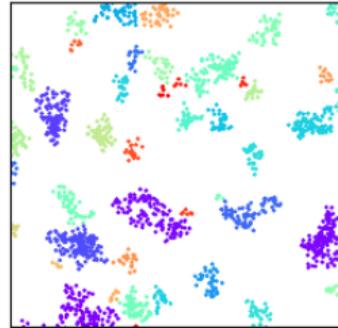
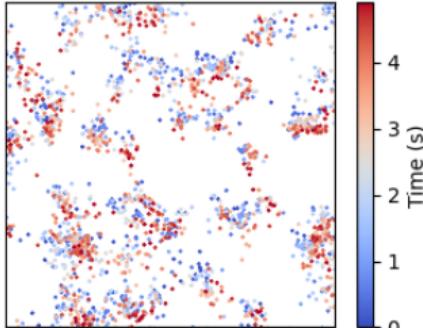
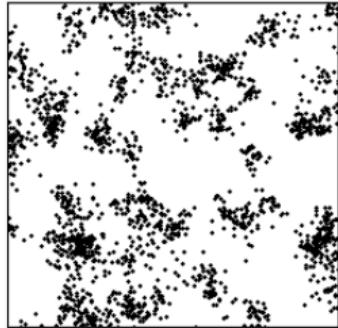


Model loglikelihood



- ▶ Isotropic Gaussian KDE using 30x30nm bins
- ▶ Closest pairs are fused one at a time, until we minimized the BIC
- ▶ Likelihood is computed under a Gaussian Mixture Model (GMM)

Chromatin nanodomains in a living Hela cell nucleus



Future Aims

- ▶ Integrate 2D SMLM and 2D/3D single molecule tracking (Brownian dynamics in a potential)
- ▶ Determine suitable metrics for measuring dynamics of the KDE
- ▶ Publish

