

# Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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# Outline

Super resolution imaging with *d*STORM

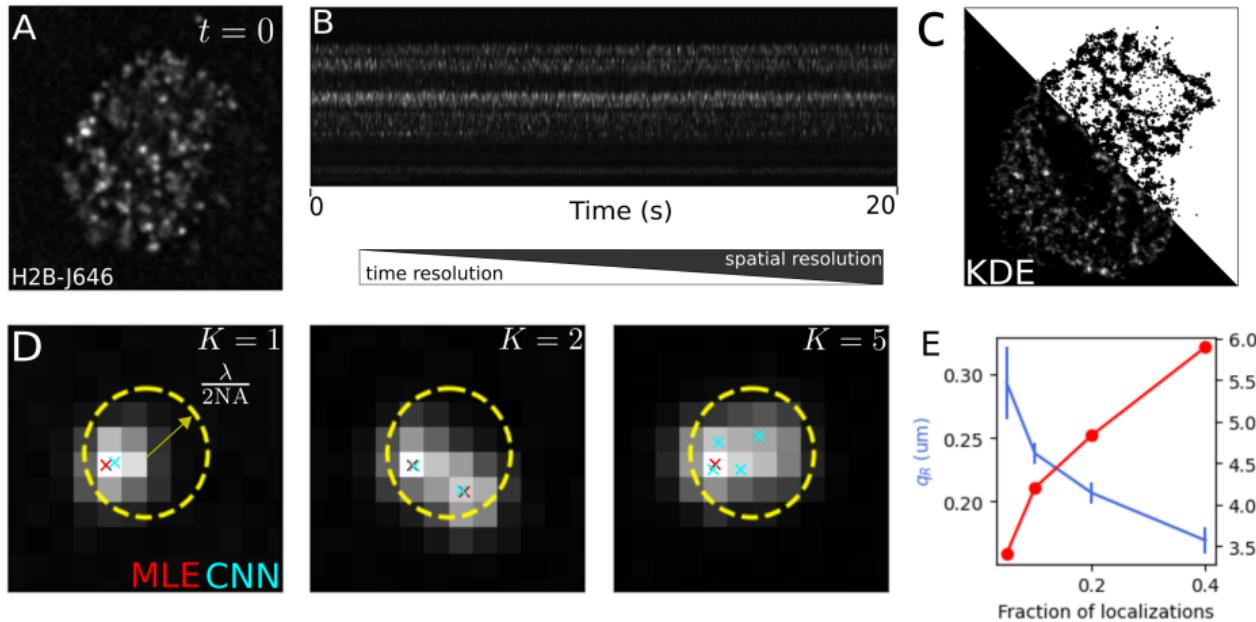
The time resolution of *d*STORM

Dense localization with deep learning

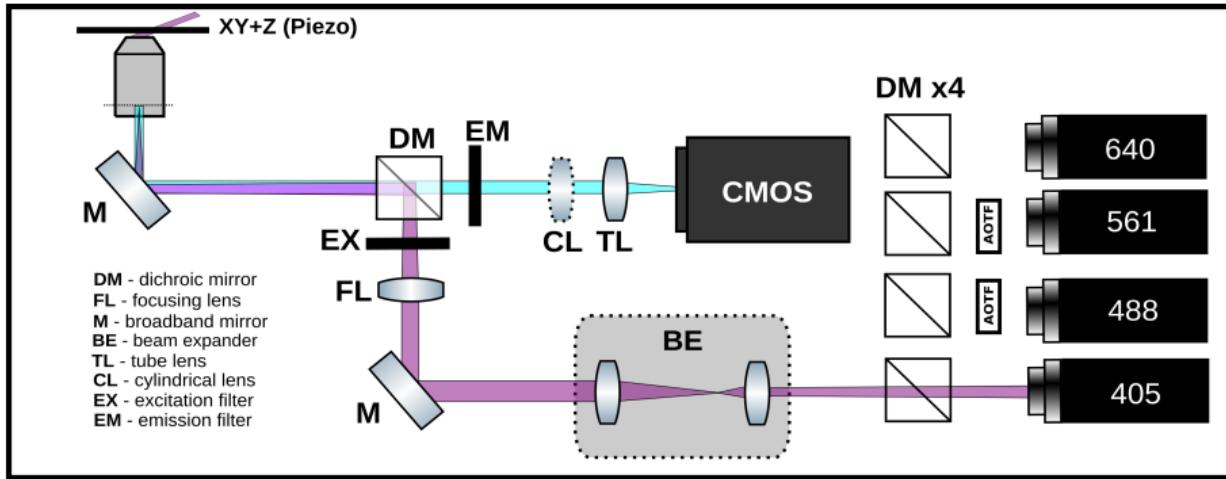
The nucleosome as a Brownian harmonic oscillator

Phase separation of chromatin

# Direct stochastic optical reconstruction microscopy

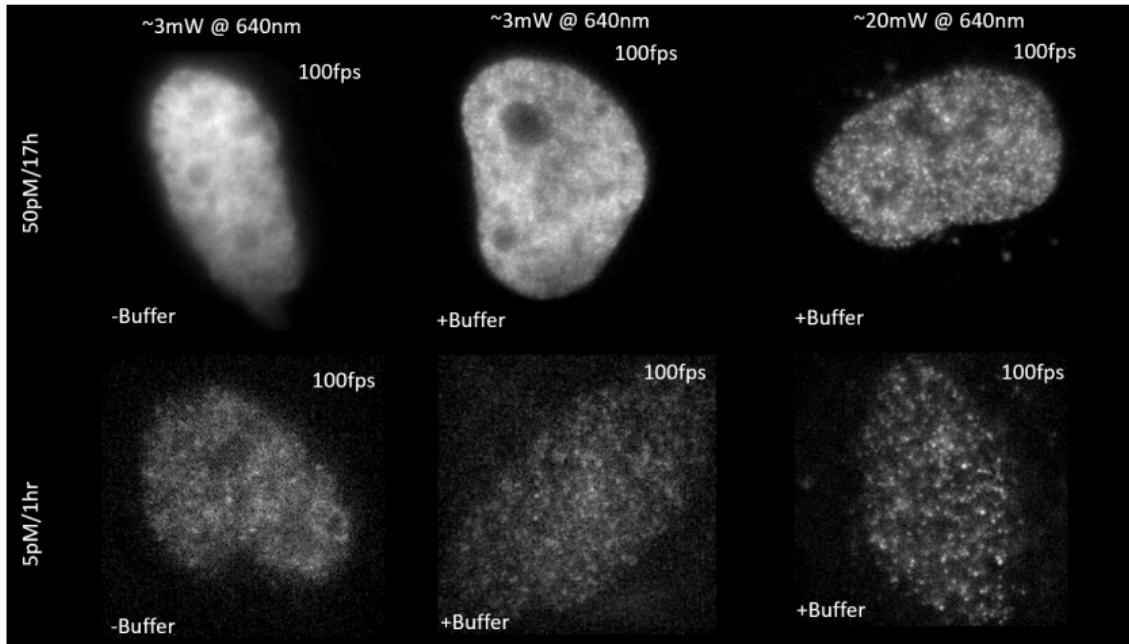


# Instrumentation for single molecule localization microscopy



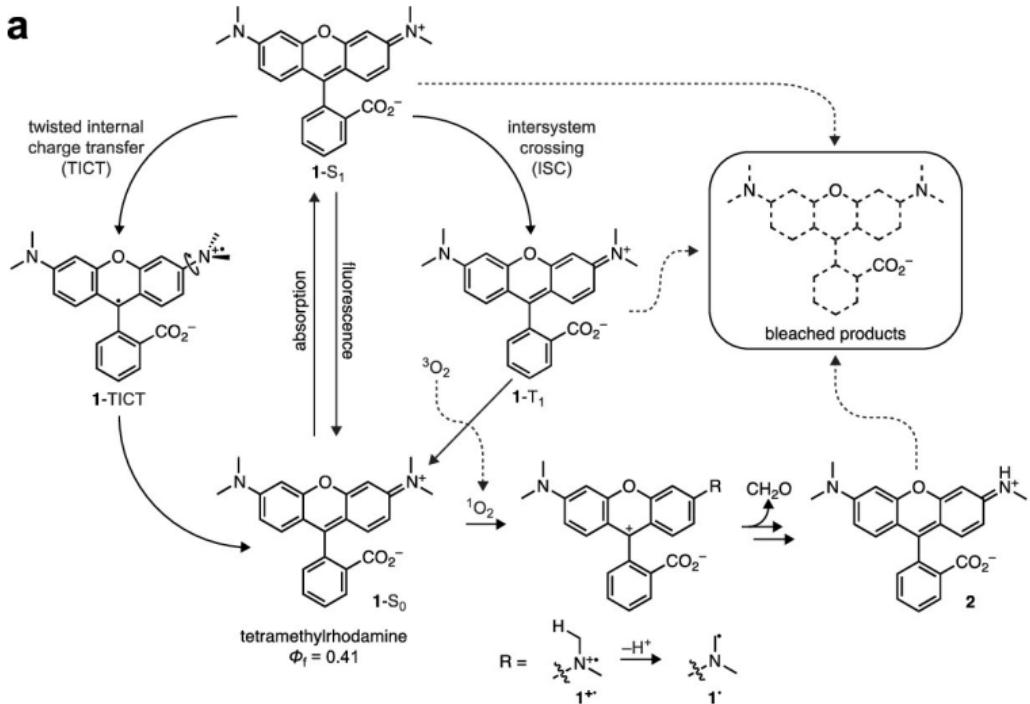
- ▶ Selectable widefield and oblique illumination
- ▶ Widefield useful for high throughput
- ▶ Oblique method illuminates a thin section of nuclei

# Dense labeling of histone H2B in fixed cells at RT



- ▶ Dense labeling of H2B-Halotag w/ fluorescent ligand JF646
- ▶ Reducing buffer is usually a primary thiol like cysteamine (MEA)
- ▶ Photoswitching of JF646 allows us to beat the diffraction limit

# Direct STORM: The photophysics of rhodamines



- ▶ Reduction of the T<sub>1</sub> state yields a dark, long-lived, and stable radical state

# Maximum likelihood localization of an isolated fluorescent emitter

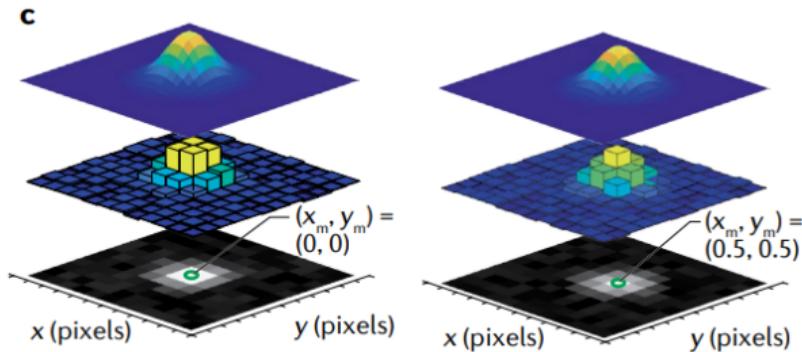
$$\text{Localization: } \theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$$

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

$\eta$  – quantum efficiency

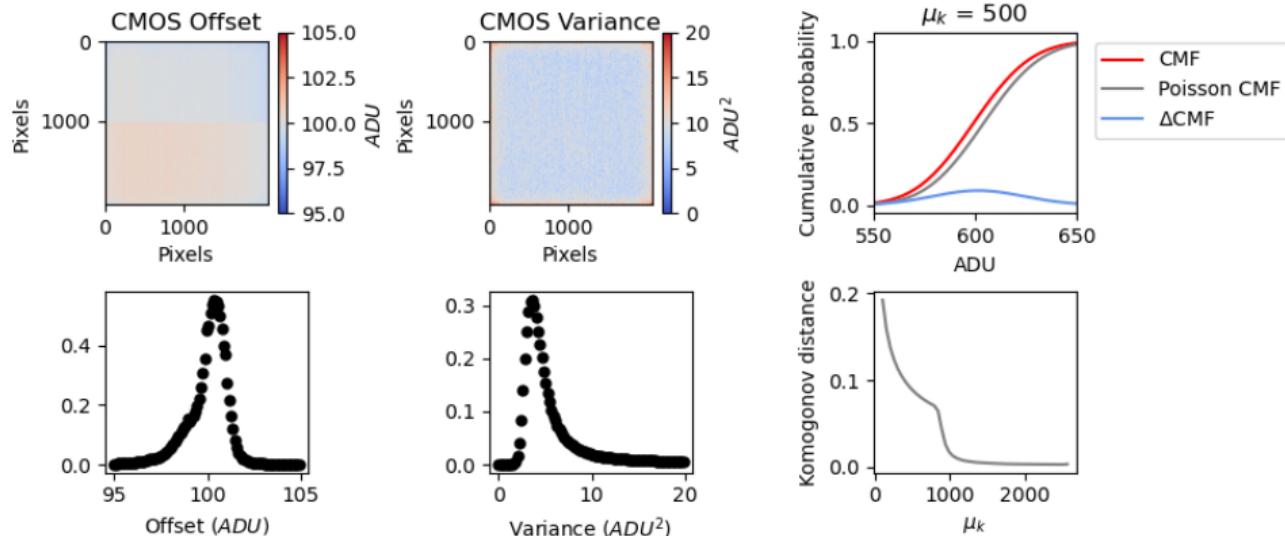
$N_0$  – photon count

$\Delta$  – exposure time



- Likelihood function can be directly optimized with gradient descent

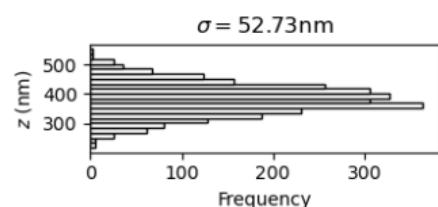
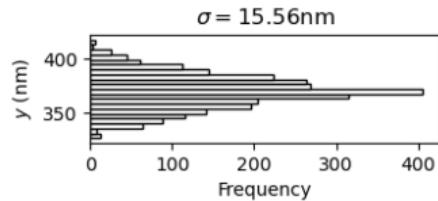
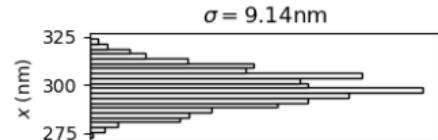
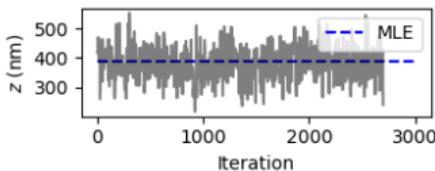
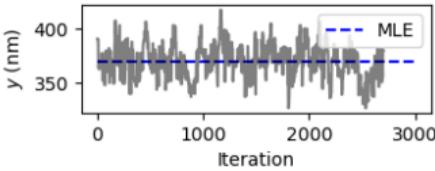
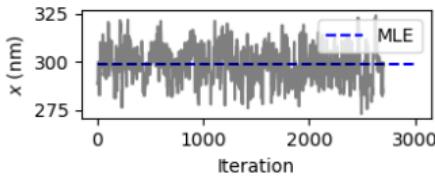
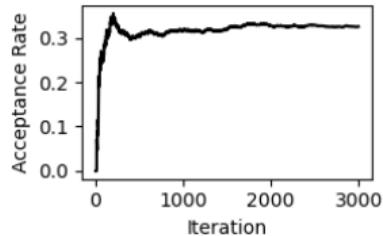
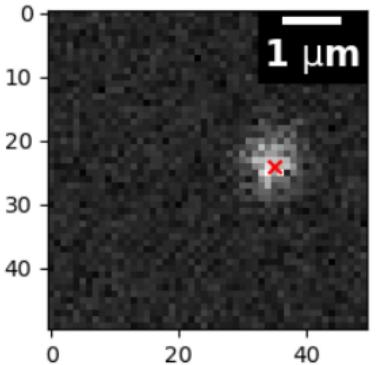
# A Poisson approximation at moderate SNR simplifies SMLM



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

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

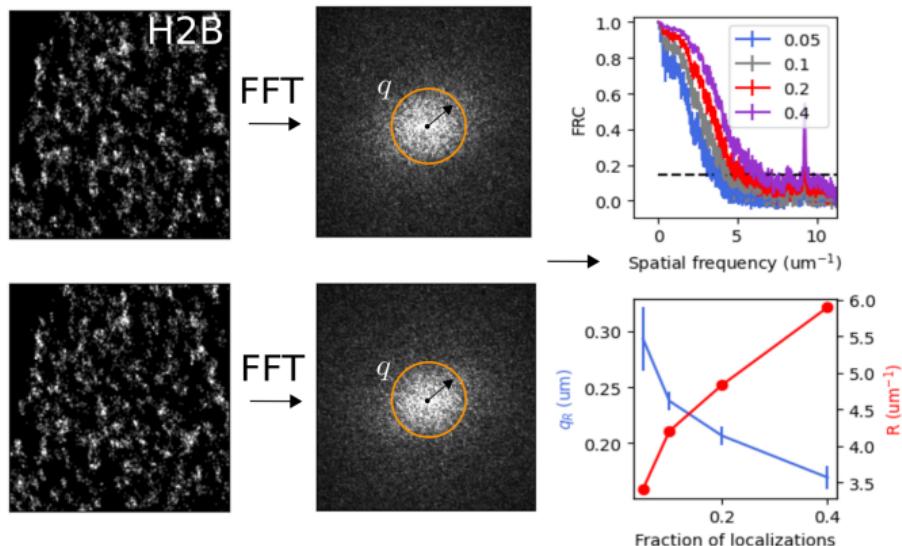
# Estimator precision in localization microscopy



- ▶ SMLM techniques are diffraction unlimited
- ▶ This makes them desirable for super-resolution, but they are slow

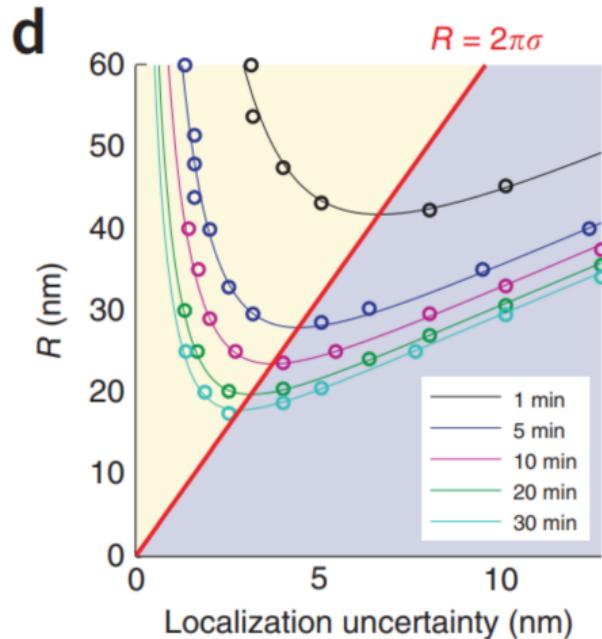
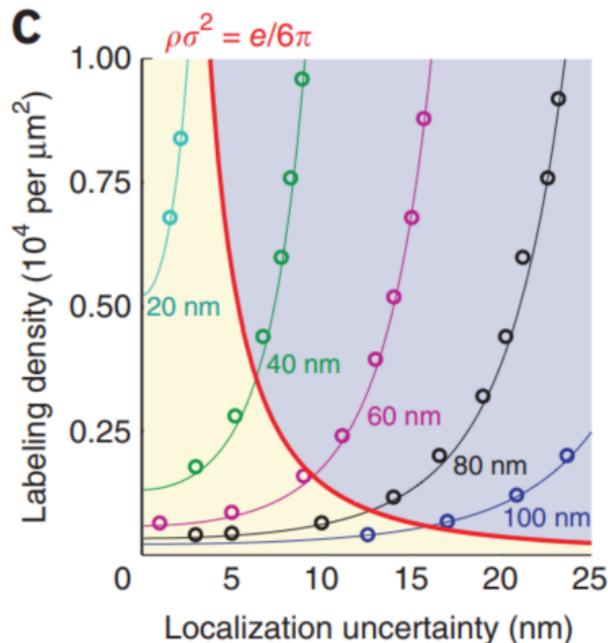
# Fourier ring correlation links spatial and temporal resolution

- We can view dSTORM as sampling from a density



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

# Fourier ring correlation links spatial and temporal resolution

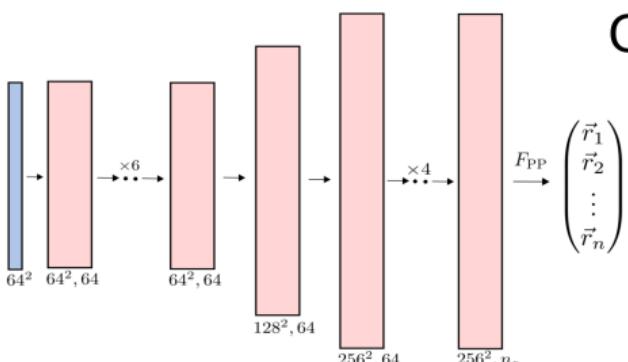


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

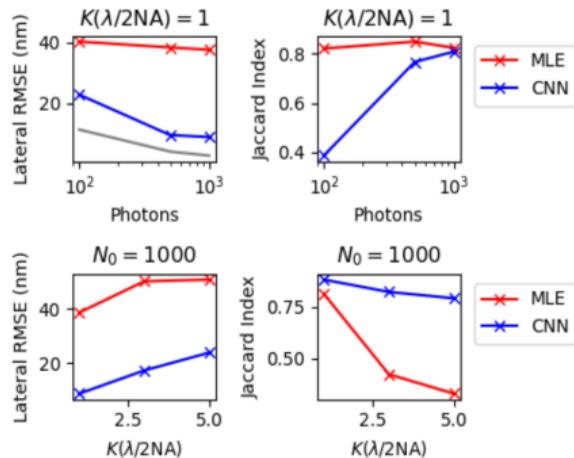
- ▶ Increased localization uncertainty requires higher density for same resolution
- ▶ Longer acquisitions have higher resolution

# Estimator precision sets the resolution limit in localization microscopy

B

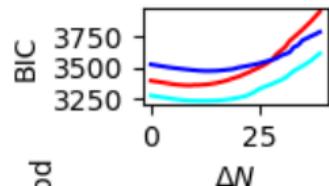
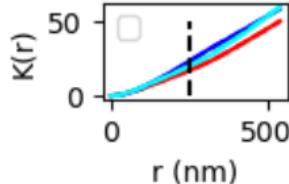
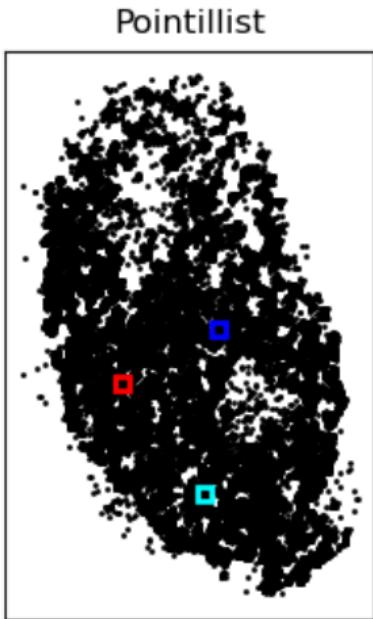
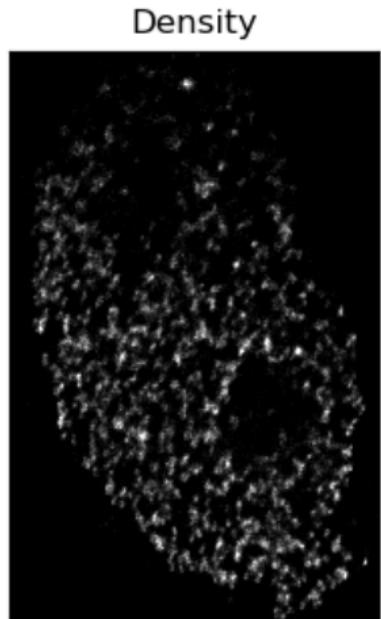


C

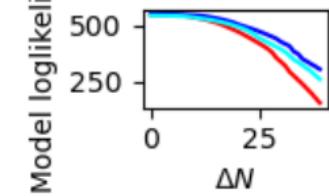


- ▶  $K(\lambda/2NA)$  is Ripley's K function at the diffraction limit ( $\lambda = 640\text{nm}$ )
- ▶ Convolutional neural networks (CNNs) approach the Cramer-Rao lower bound (gray)

# Chromatin nanodomains in a living Hela cell nucleus



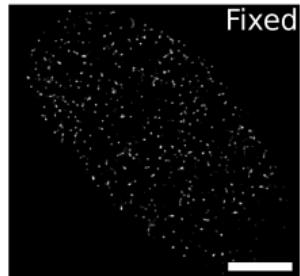
Model loglikelihood



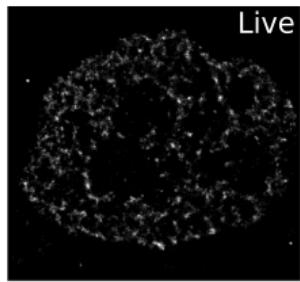
- ▶ Histone DE using 30x30nm bins
- ▶ Likelihood is computed under a Gaussian Mixture Model (GMM)

# Chemical fixation and LLPS inhibitors change the appearance of nucleosome clustering

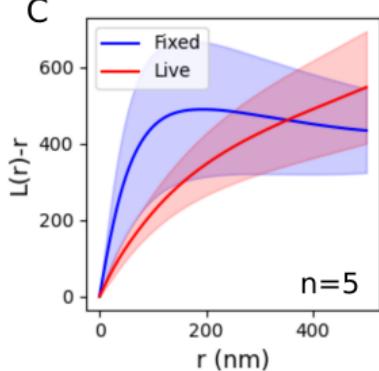
A



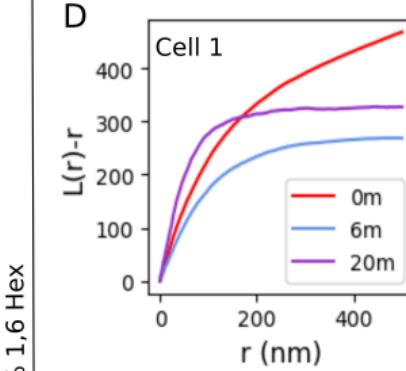
B



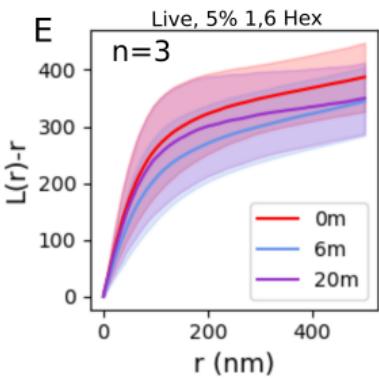
C



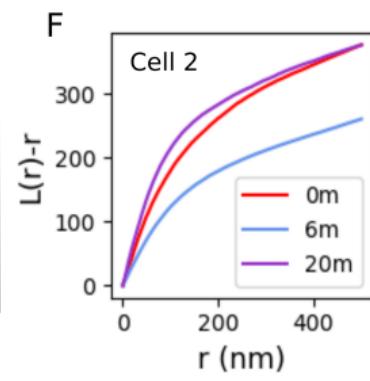
D



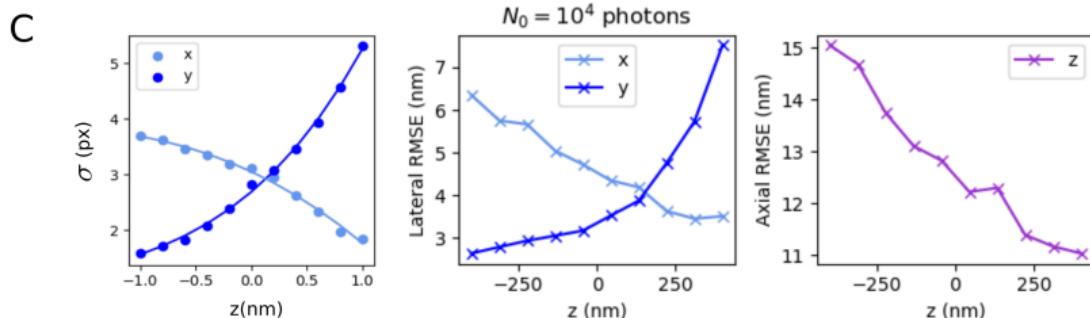
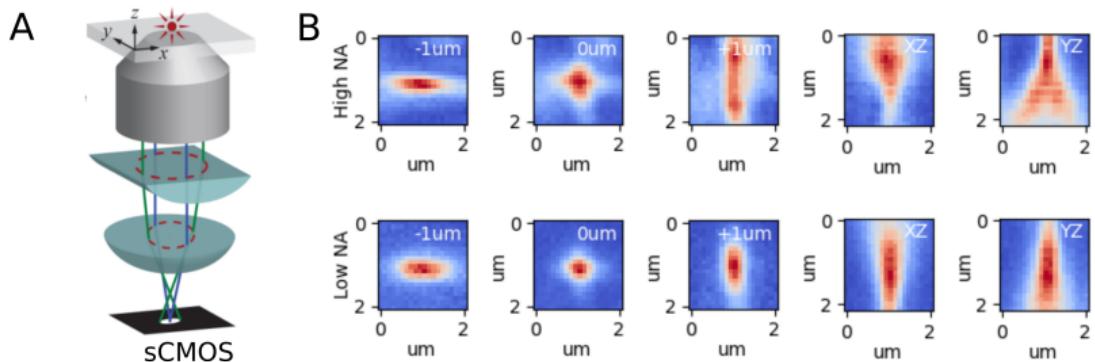
E



F

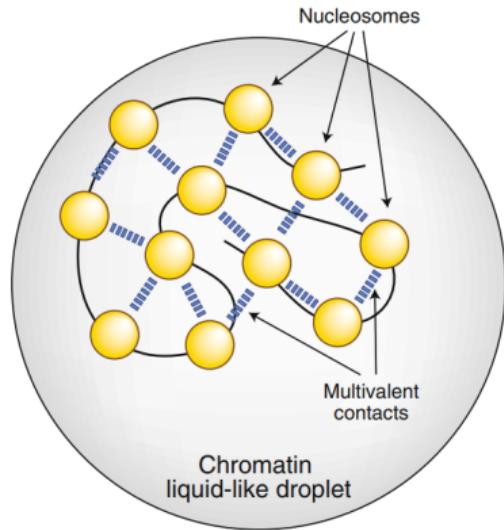


# Astigmatism based three dimensional imaging



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

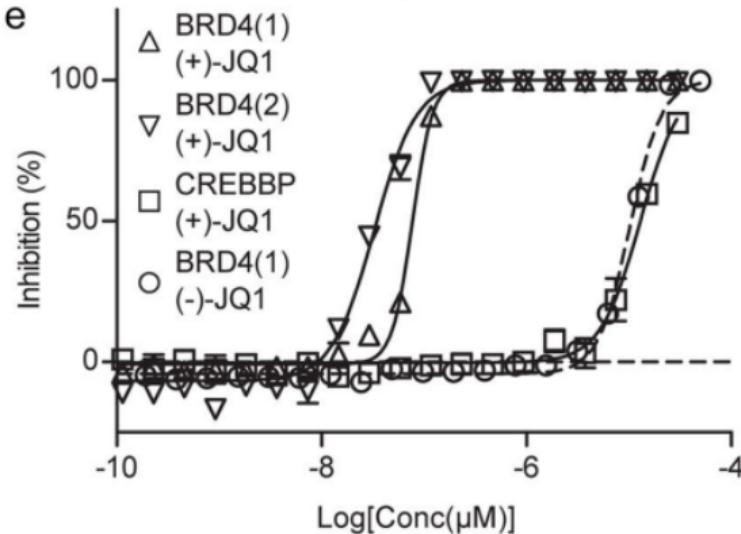
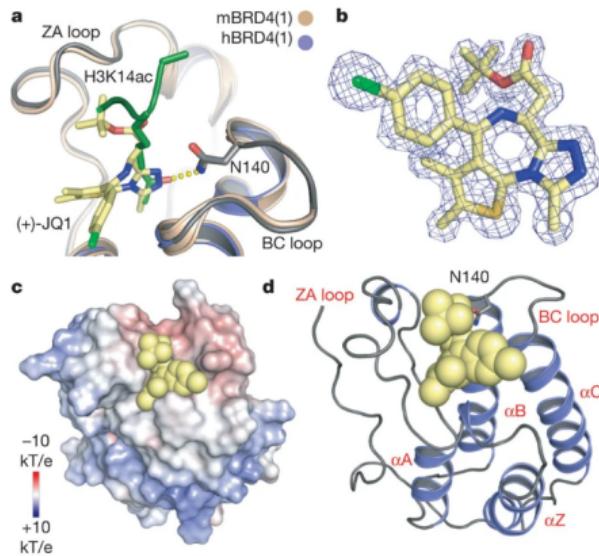
# Chromatin has an intrinsic ability to undergo phase separation



Regulatory factors of chromatin LLPS
Histone H1
DNA length between nucleosomes
Histone post-translational modifications
Nucleosome dynamics
Multivalent binding of proteins

- ▶ Super-enhanced genes are regulated by large molecular assemblies
- ▶ We study nucleosome clustering dynamics using super-resolution microscopy

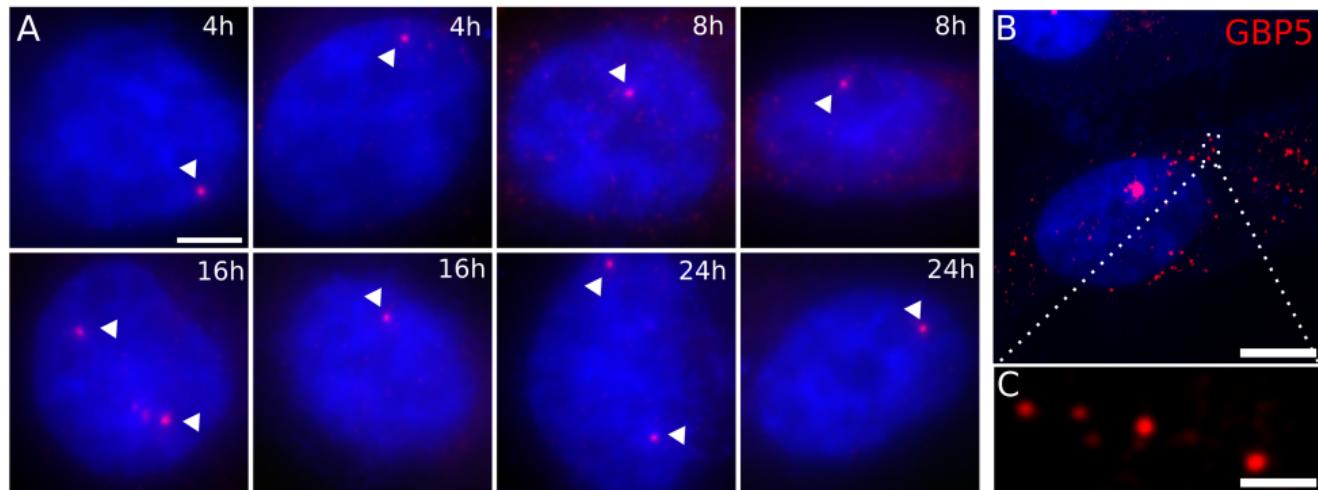
# (+)-JQ1 in complex with BRD4 protein



Filippakopoulos. Selective inhibition of BET bromodomains. *Nature*

- ▶ BRD4 is an interesting target since specific and non-specific inhibitors exist
- ▶ BET mimics including +JQ1 prevent binding of BRD4 to acetylated histones

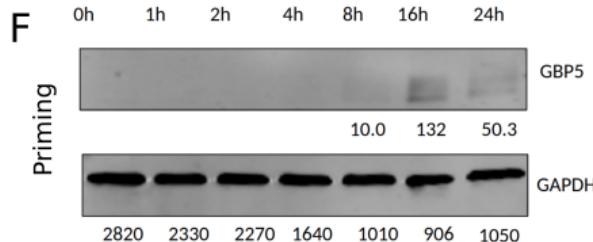
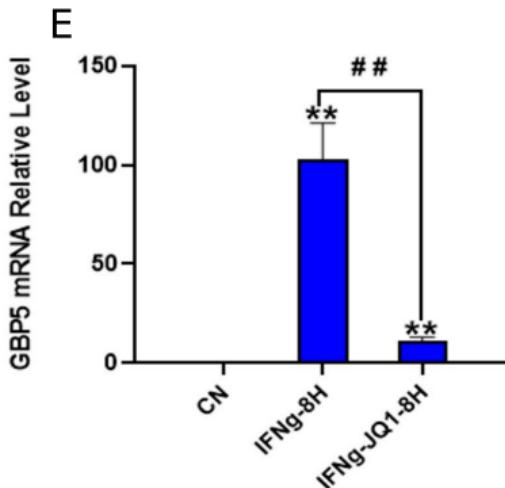
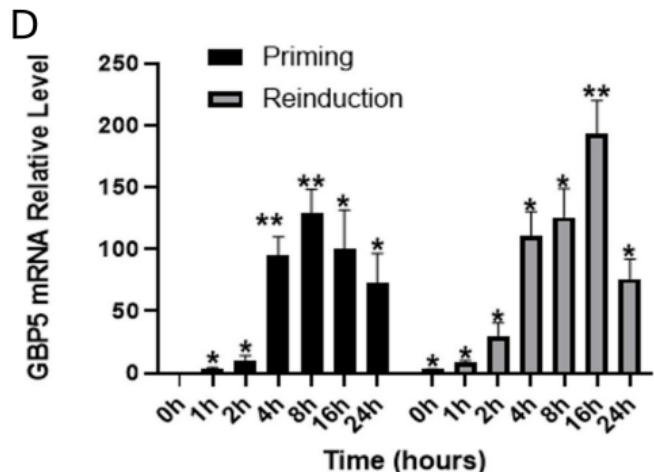
# Inhibition of a super-enhanced gene with JQ1



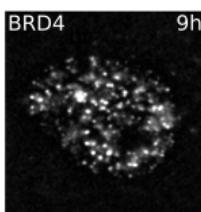
Blue - DAPI (binds DNA minor groove)

- ▶ Guanylate binding proteins (GBPs) are a family of GTPases induced by IFN- $\gamma$
- ▶ BRD4 is directly involved in GBP gene expression

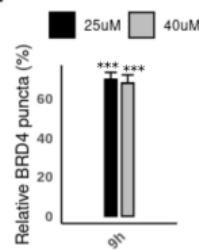
# Inhibition of a super-enhanced gene with JQ1



G



H



► \*: $P \leq 0.1$ , \*\*: $P \leq 0.01$

## Generative model for SPAD array data

$$F \sim \text{DP}(\alpha, F_0) \quad \theta | F \sim F \quad X | \theta \sim \mathcal{L}(X | \theta)$$

- ▶  $\text{DP}(\alpha, F_0)$  is a distribution over distributions
- ▶  $F$  is a prior on the model parameters and  $\theta$  are the parameters (coordinates of molecules)
- ▶ Likelihood  $\mathcal{L}$  can be chosen to use total photon counts or explicitly model the point process

$$\mathcal{L}(X | \theta) = \prod_n \left( \sum_{k=1}^K \mu_k(r_n | \theta_k) \right) \delta t^n$$

However, nearby molecules may emit at the same time, and this would be lost in the summation of total counts ( $g^2(0)$ ?)