

# Visualizing chromatin organization with single molecule localization microscopy

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# Outline

Single molecule localization microscopy

The time resolution of *d*STORM

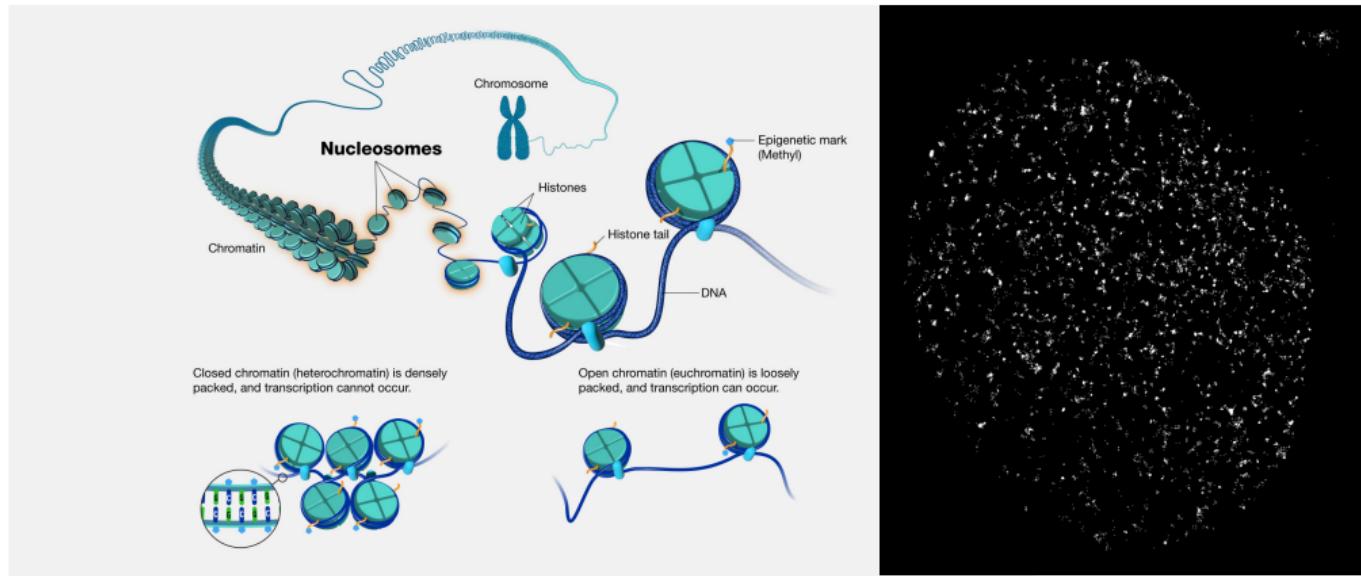
A novel approach to dense localization microscopy

Dense localization by fluorescence antibunching

Phase separation of chromatin

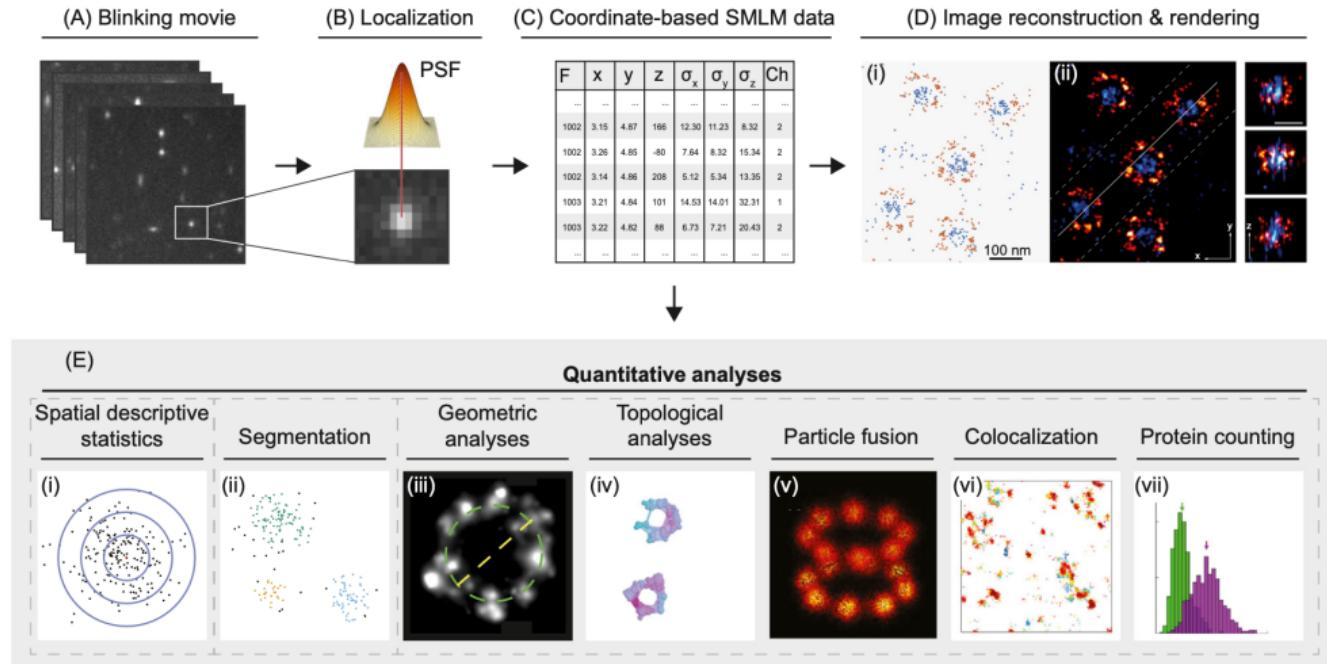
## Single molecule localization microscopy

# Genome organization and super resolution imaging



- ▶ Genome has a hierarchical structure, fundamental unit is the nucleosome
- ▶ We study chromatin organization by localizing fluorescently tagged nucleosomes

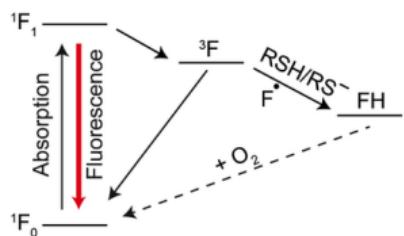
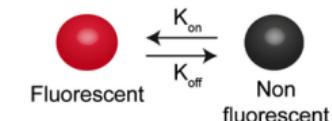
# Single molecule localization microscopy



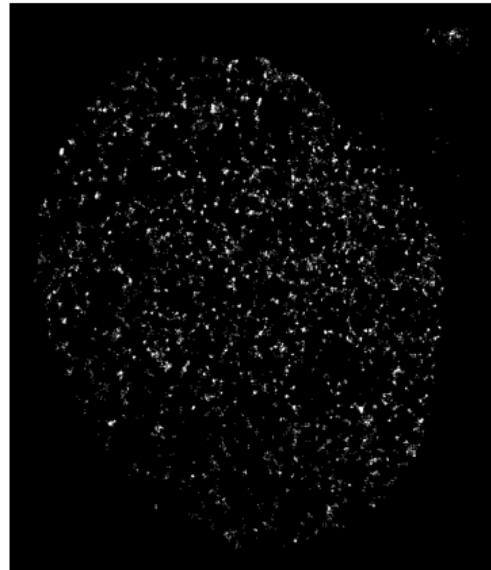
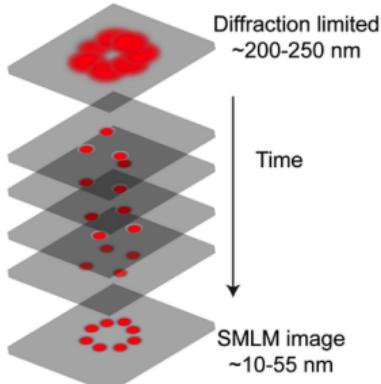
- Chromatin structure is dynamic and undergoes **phase separation** → We are pushing the limits of SMLM for time-resolution

# Single molecule localization microscopy

## a Photoswitching



## b Temporal separation



- ▶ SMLM techniques are diffraction-unlimited
- ▶ Photoswitching enables resolution of emitters in time rather than space

# Single molecule localization microscopy

Modeling the point spread function permits sub-pixel localization

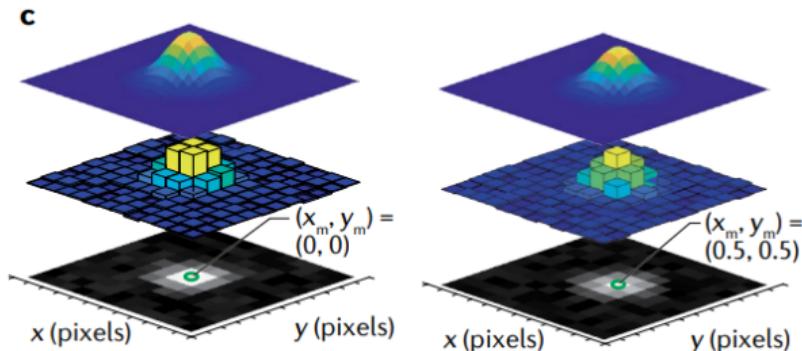
$$\mu_k = i_0 \int_{\mathbb{K}} h_\theta(x_0, y_0) dx dy$$

$$i_0 = g_k \eta N_0 \Delta$$

$\eta$  – quantum efficiency

$N_0$  – photon count

$\Delta$  – exposure time



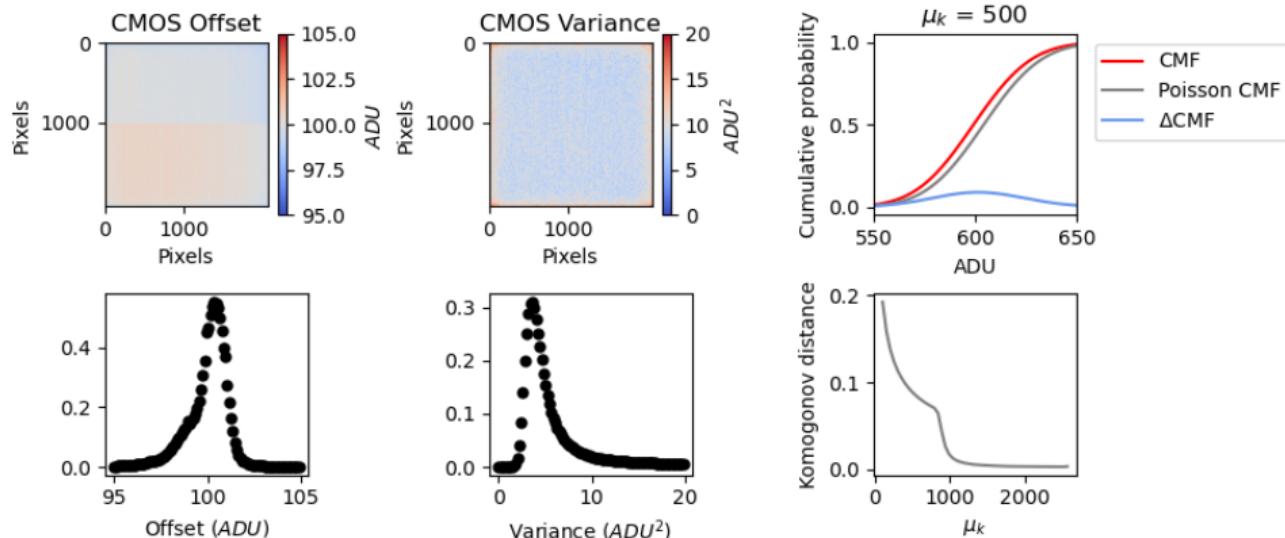
Long  $\Delta \rightarrow$  pixels are iid:

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

What is  $P(H_k | \theta)$ ?

# Photon statistics of fluorescent markers

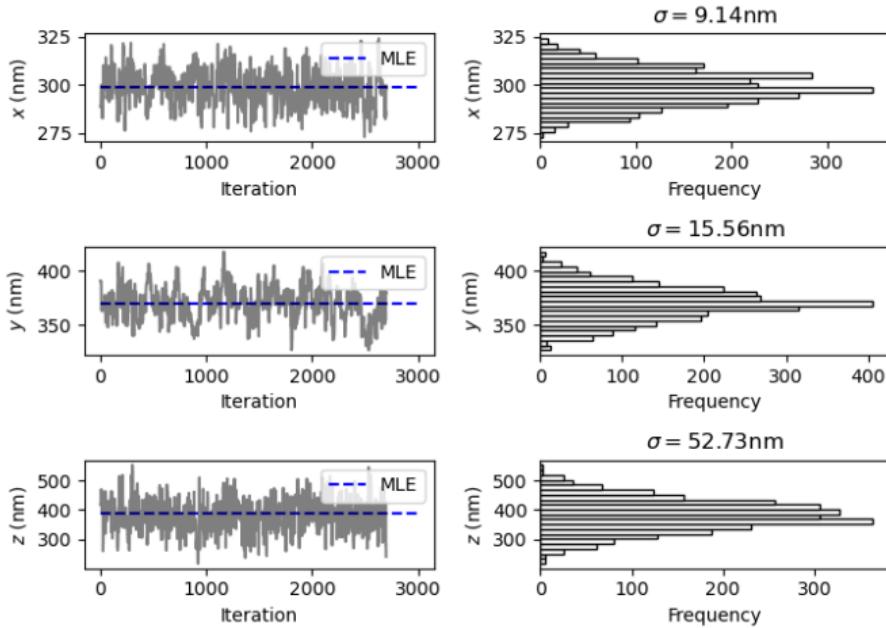
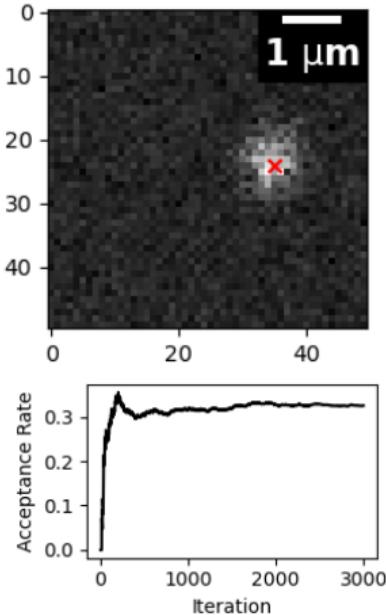
Long integration times  $\Delta \rightarrow$  intensity fluctuations are Poisson



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

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

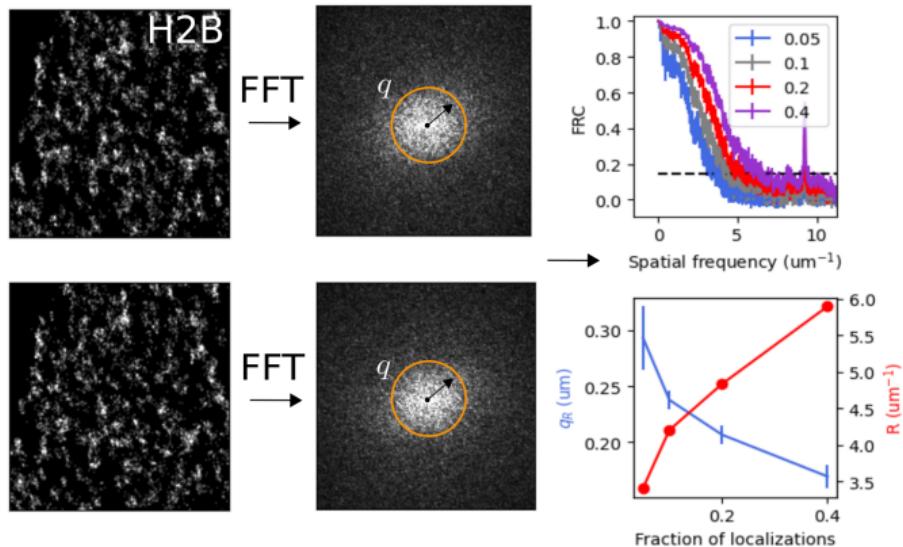
# Estimator precision determines resolution in localization microscopy



- One can derive a lower bound on the variance of a statistical estimator of the coordinates  $\theta$

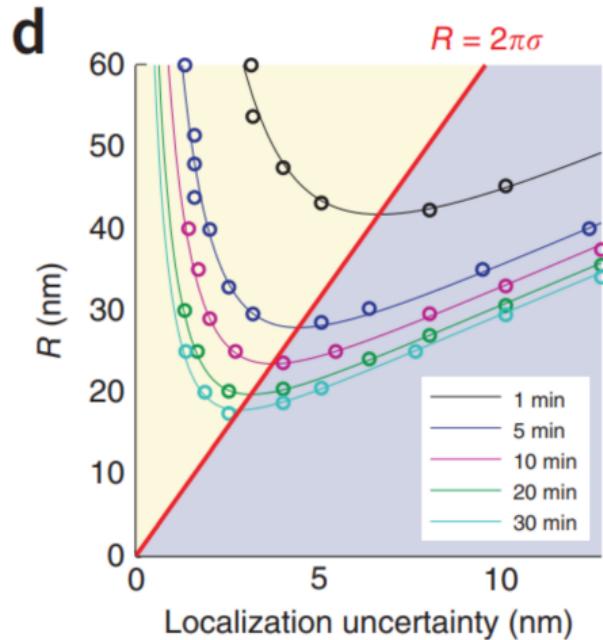
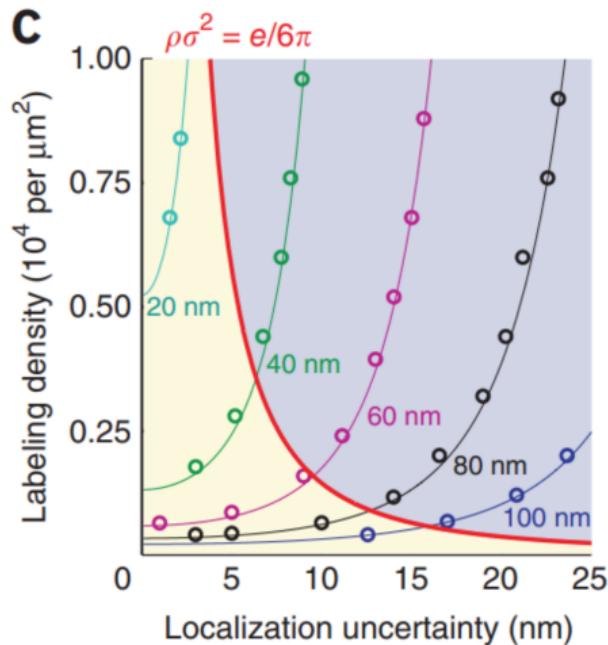
# Dense localization increases time resolution

- We can view dSTORM as sampling from a density



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

## Dense localization increases time resolution

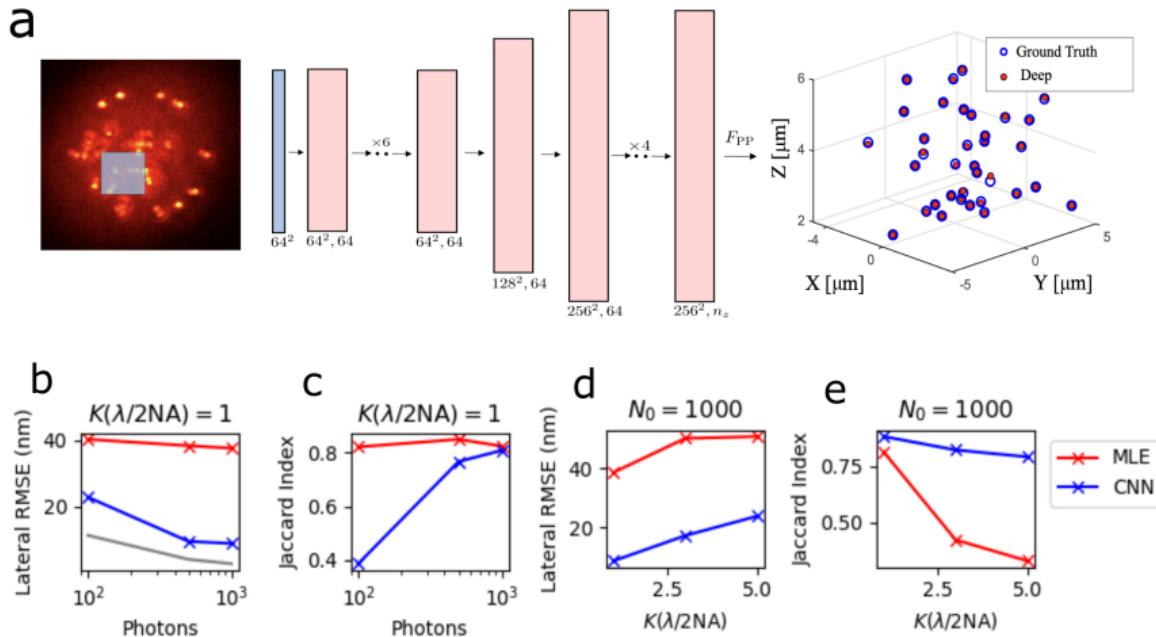


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

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

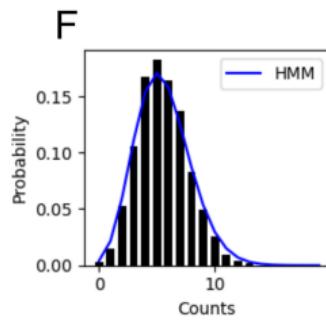
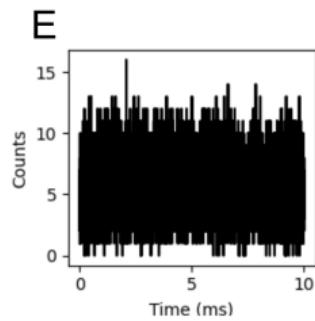
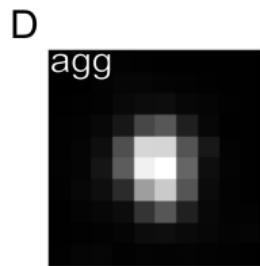
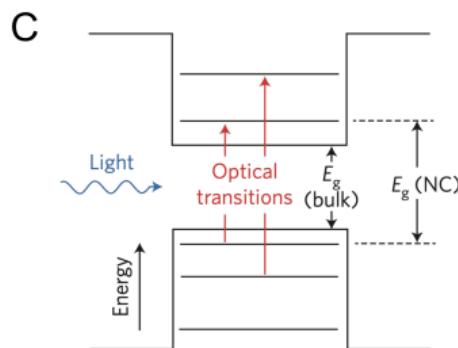
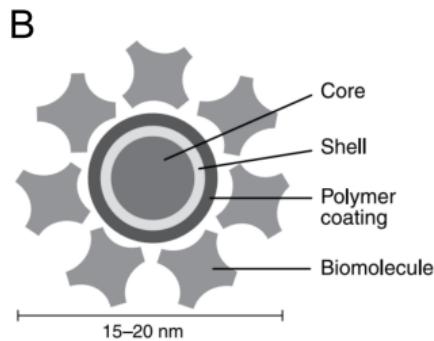
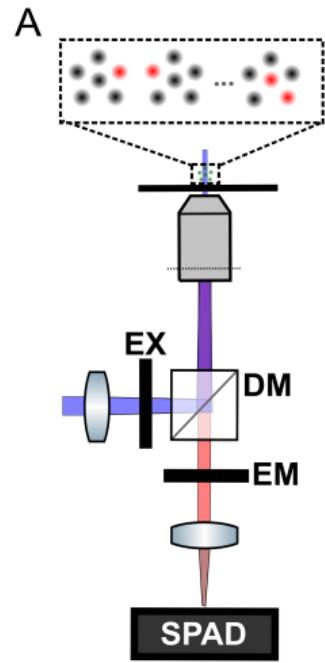
## A novel approach to dense localization microscopy

# DeepSTORM: An early approach to dense SMLM with deep learning



- ▶ MLE in high dimensional spaces can quickly become intractable
- ▶ We can model  $P_\Psi(Z)$  with a convolutional neural network  $\Psi$

# Counting fluorescent molecules with a photon counting camera



Suppose we have a model  $\mathcal{M} = (S, T)$  with a spatial part  $S$  and temporal part  $T$ . We want to compare different models, based on the ratio  $P(\mathcal{M}_1|D)/P(\mathcal{M}_2|D)$ .

$$\frac{P(\mathcal{M}_1|D)}{P(\mathcal{M}_2|D)} = \frac{P(D|\mathcal{M}_1)P(\mathcal{M}_1)}{P(D|\mathcal{M}_2)P(\mathcal{M}_2)}$$

Therefore, we need to find  $P(D|\mathcal{M}_n)$ . If we assume the two model components are independent

$$P(D|\mathcal{M}_n) = P(D|S_n)P(D|T_n)$$

We can use the HDP-HMM model to integrate out  $\theta$  with MCMC:

$$P(D|T_n) = \int P(D, \theta|T_n)d\theta$$

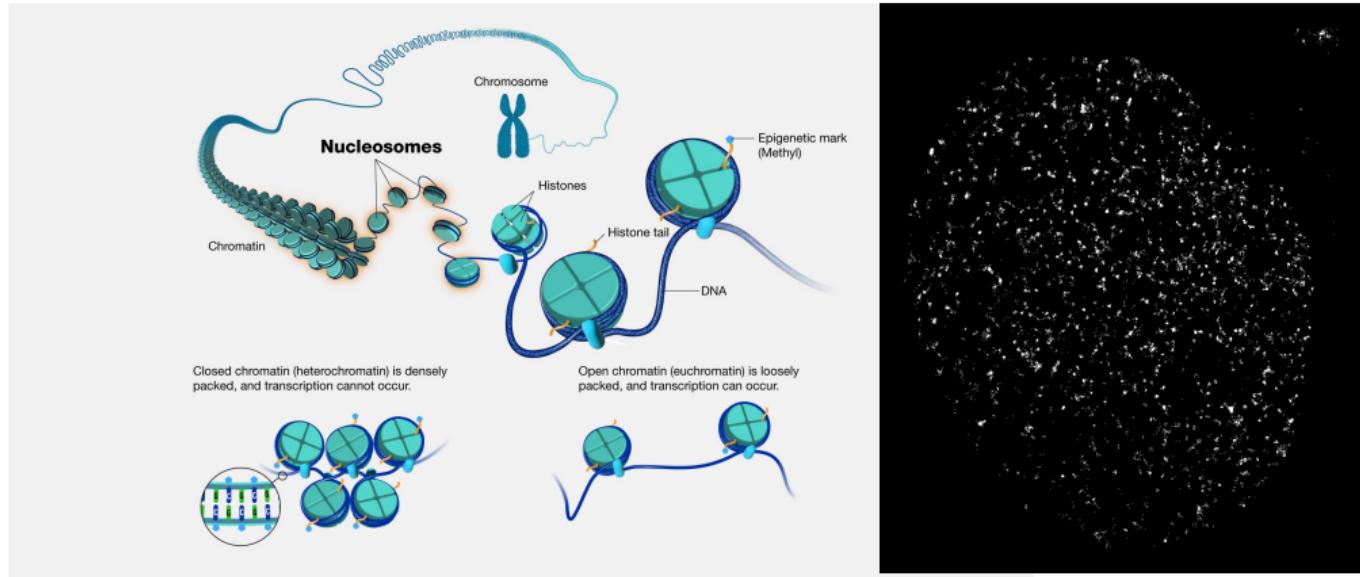
Then we use a Langevin Monte Carlo (LMC) sampler for the spatial model

$$P(D|S_n) = \int P(D, \phi|S_n)d\phi$$

Both of these sampling methods also provide the MAP estimates of the parameters  $\theta, \phi$ .

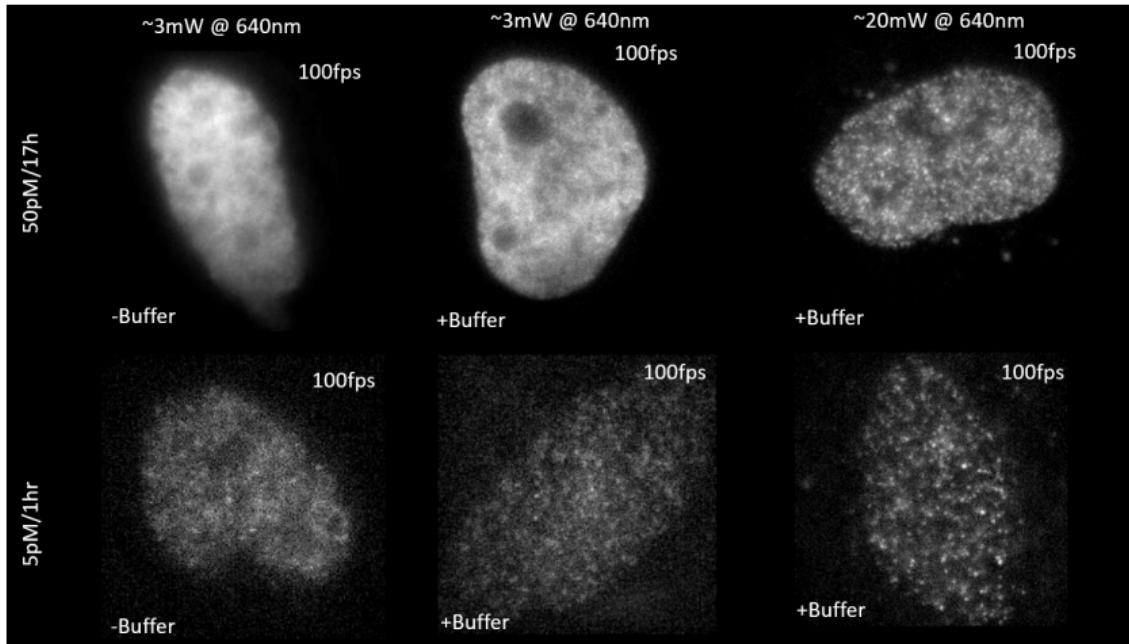
## Phase separation of chromatin

# Genome organization and single molecule localization microscopy



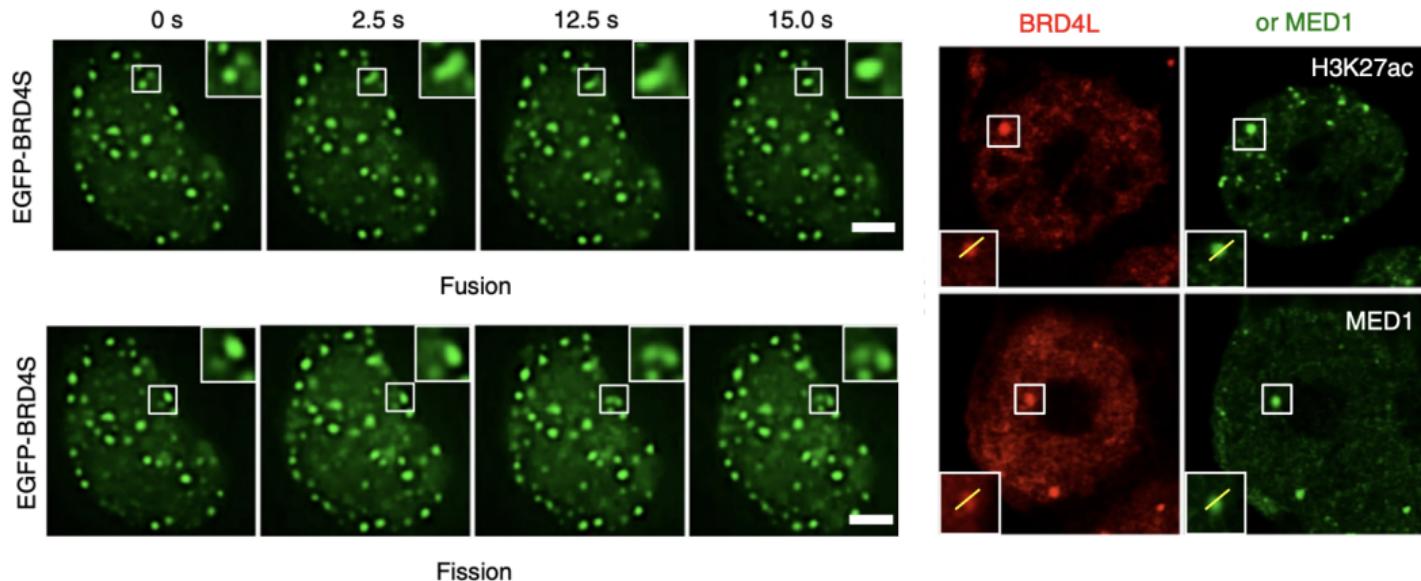
- ▶ Genome has a hierarchical structure, fundamental unit is the nucleosome
- ▶ We study chromatin organization by localizing fluorescently tagged nucleosomes

# Dense labeling of histone H2B in fixed cells at RT



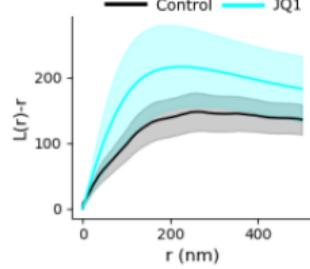
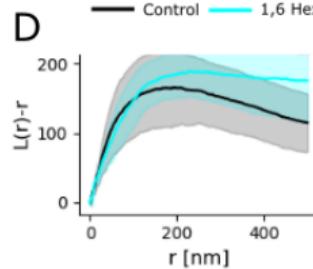
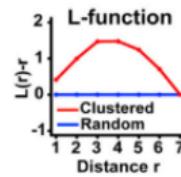
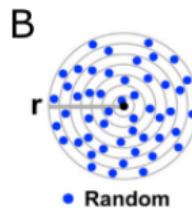
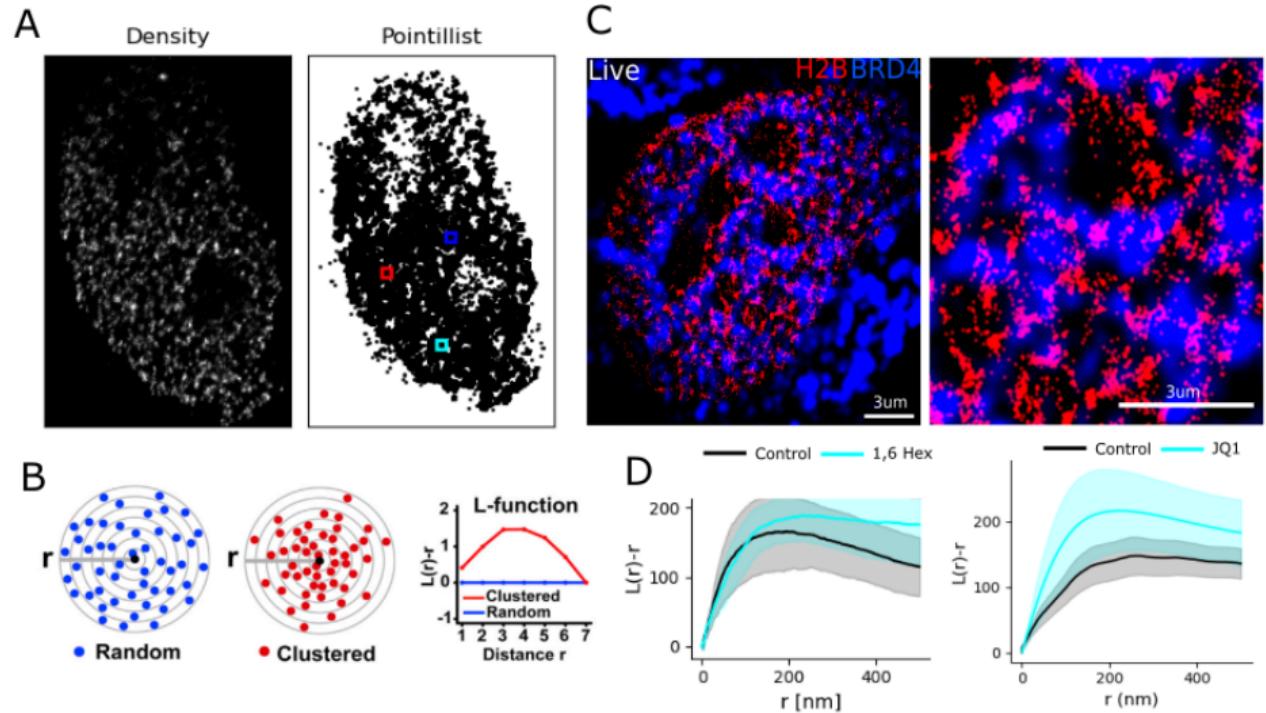
- ▶ Dense labeling of H2B-Halotag w/ fluorescent ligand JF646
- ▶ Reducing buffer is usually a primary thiol like cysteamine (MEA)
- ▶ Photoswitching of JF646 allows us to beat the diffraction limit

# BRD4 condensates exhibit LLPS properties

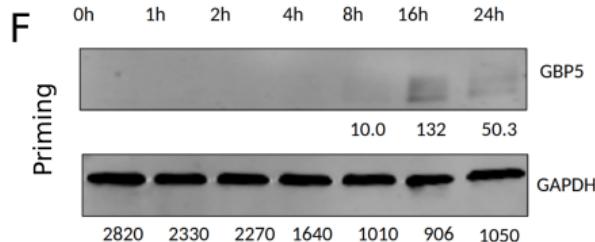
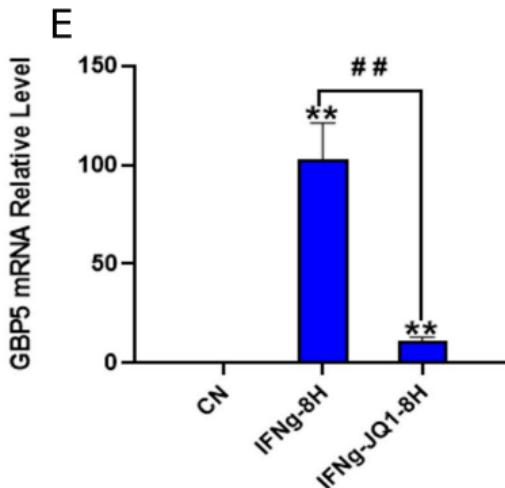
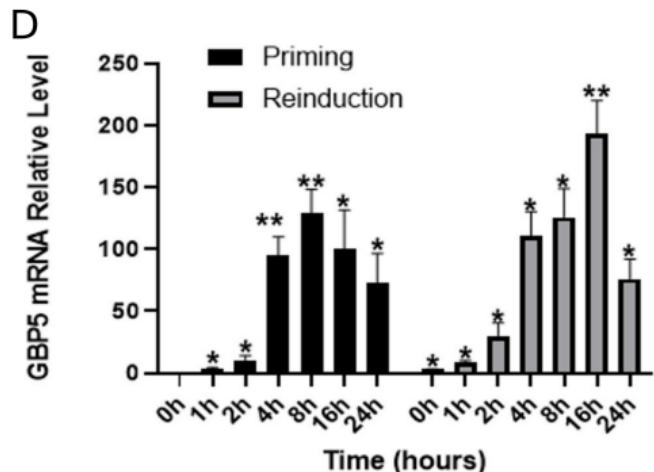


Han et al. Roles of the BRD4 short isoform in phase separation and active gene transcription. *Nature Structural and Molecular Biology*. 2020

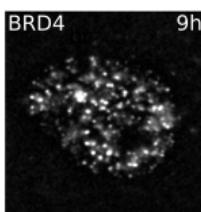
# BET inhibitors reduce nucleosome-BRD4 interactions in BRD4 condensates



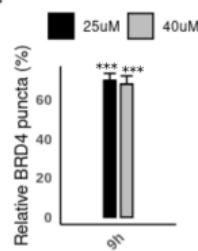
# Inhibition of a super-enhanced gene with JQ1



G



H



► \*: P ≤ 0.1, \*\*: P ≤ 0.01