

# Visualizing chromatin organization with single molecule localization microscopy

Clayton W. Seitz

November 12, 2023

# Outline

## Single molecule localization microscopy

The time resolution of *d*STORM

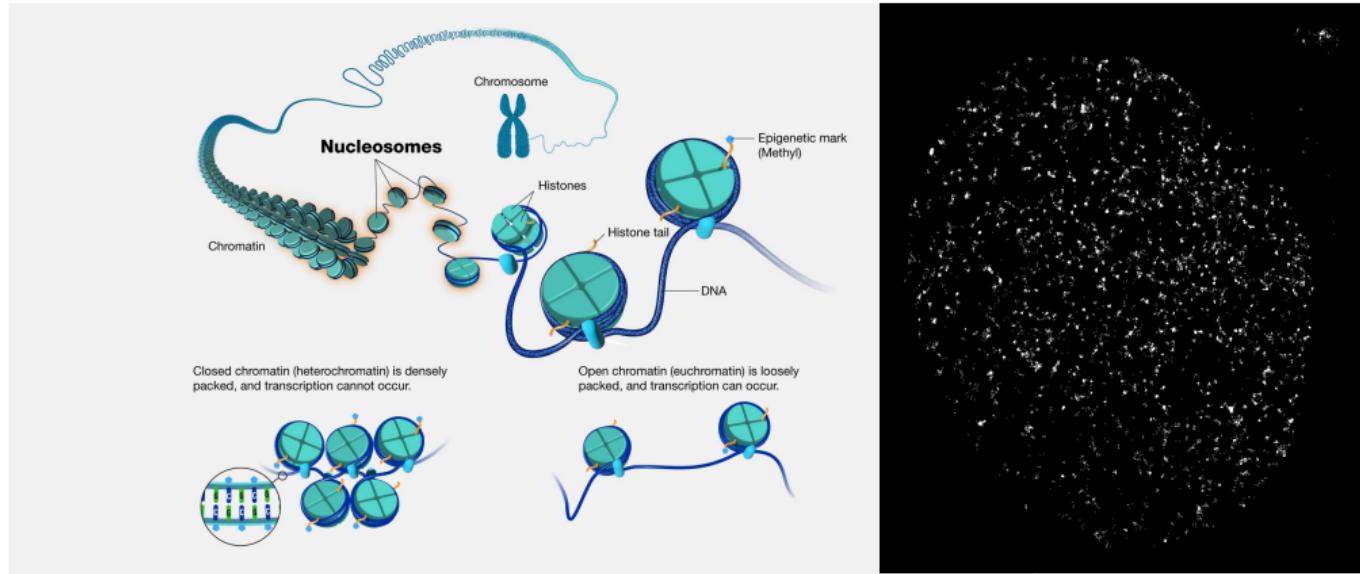
Dense localization with deep learning

Dense localization by fluorescence antibunching

## Phase separation of chromatin

## Single molecule localization microscopy

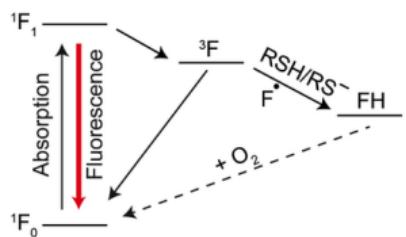
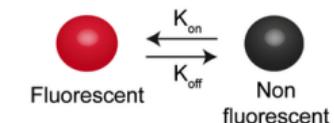
# Genome organization and single molecule localization microscopy



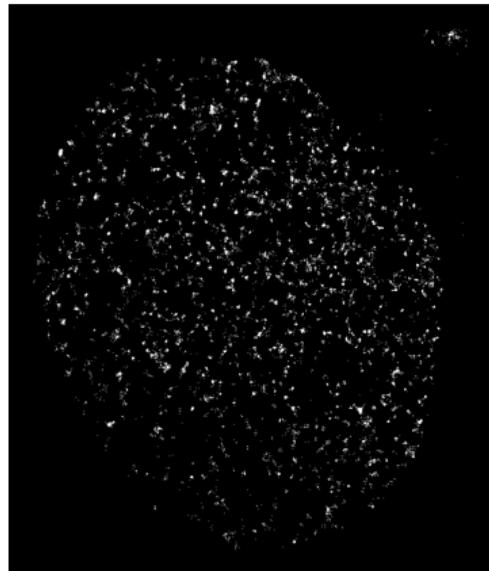
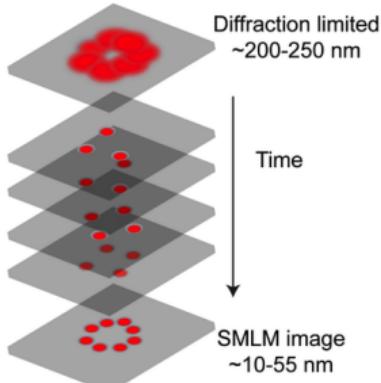
- ▶ Genome has a hierarchical structure, fundamental unit is the nucleosome
- ▶ We study chromatin organization by localizing fluorescently tagged nucleosomes

# 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

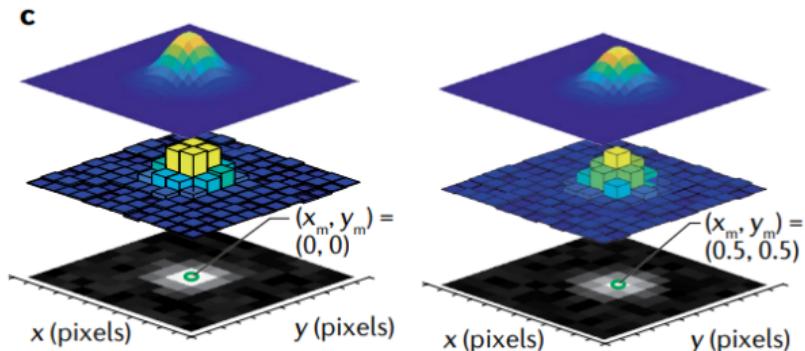
$$\mu_k = i_0 \int_{\mathbb{K}} h_\theta(x_0, y_0) dx dy$$

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

$\eta$  – quantum efficiency

$N_0$  – photon count

$\Delta$  – exposure time



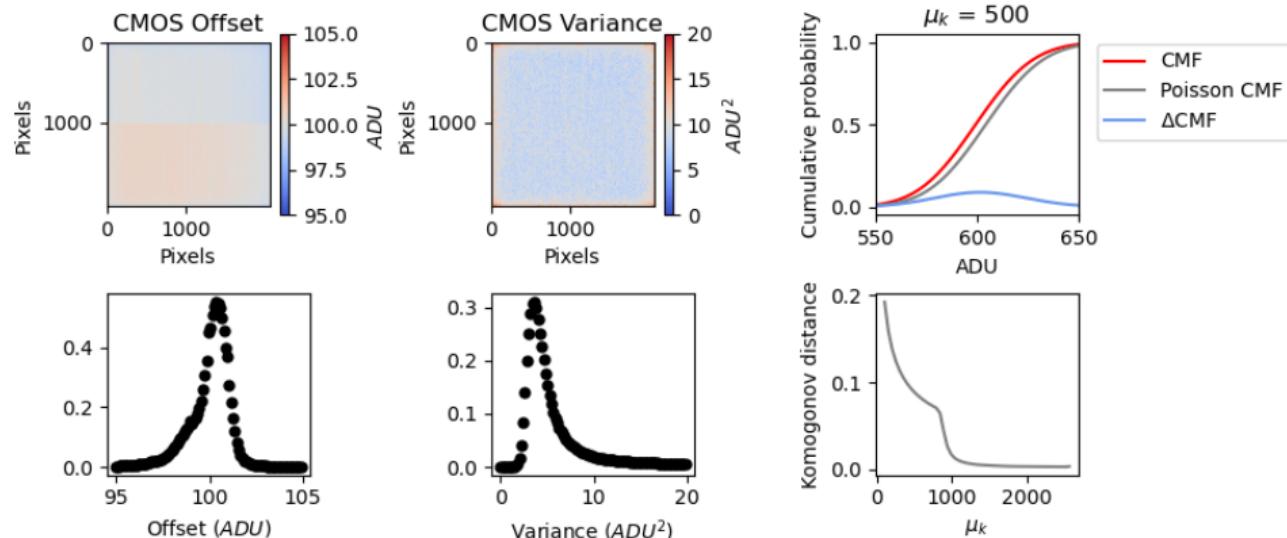
Long  $\Delta \rightarrow$  pixels are iid:

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

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

# Classical emission statistics of fluorescent markers

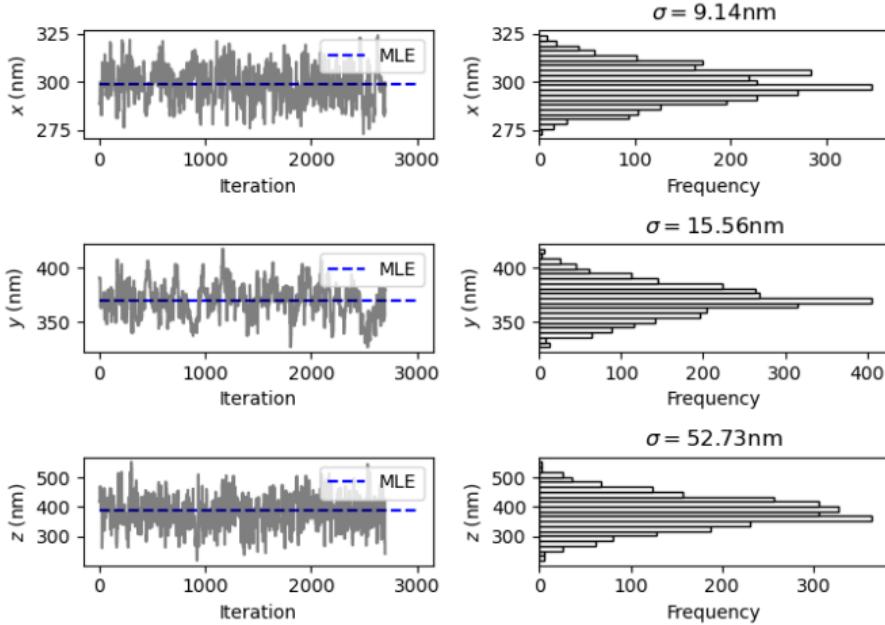
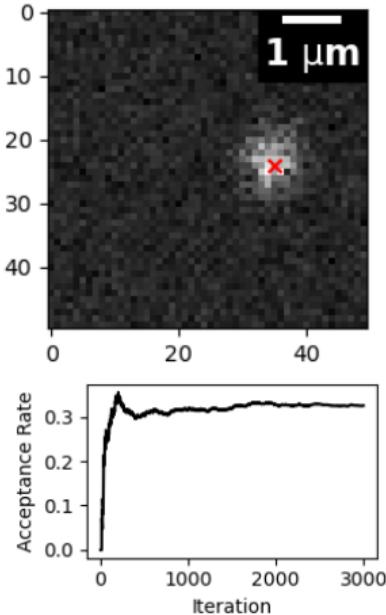
Long integration times  $\Delta \rightarrow$  intensity fluctuations are Poisson



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

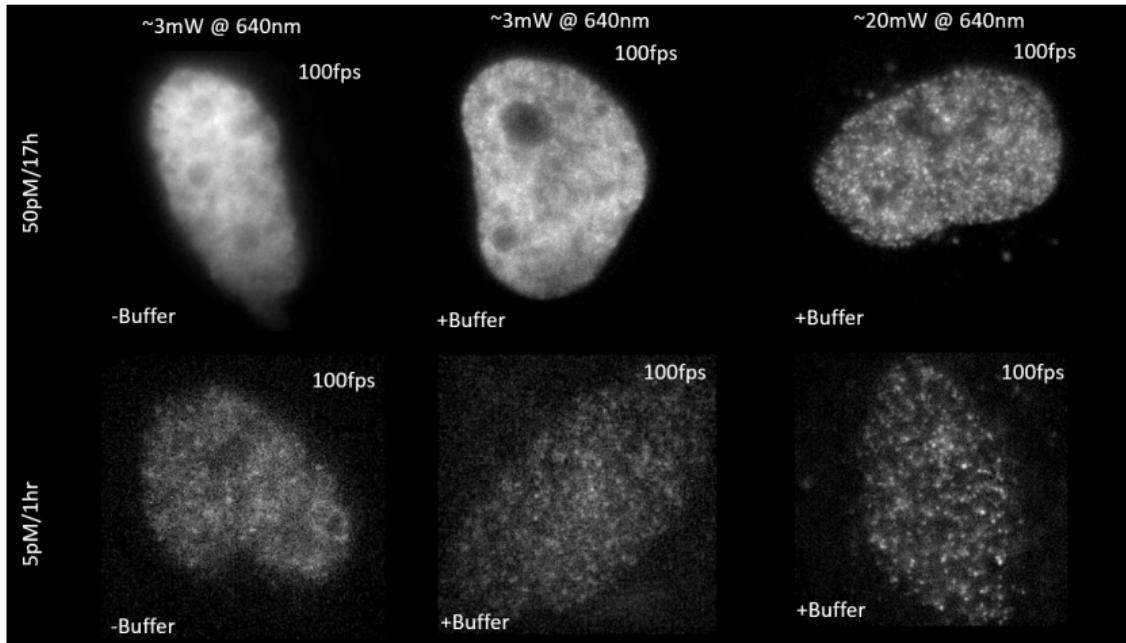
$P(H_k|\theta)$  can be approximated as Poisson at high signal-to-noise (SNR)

# Estimator precision determines resolution in localization microscopy



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

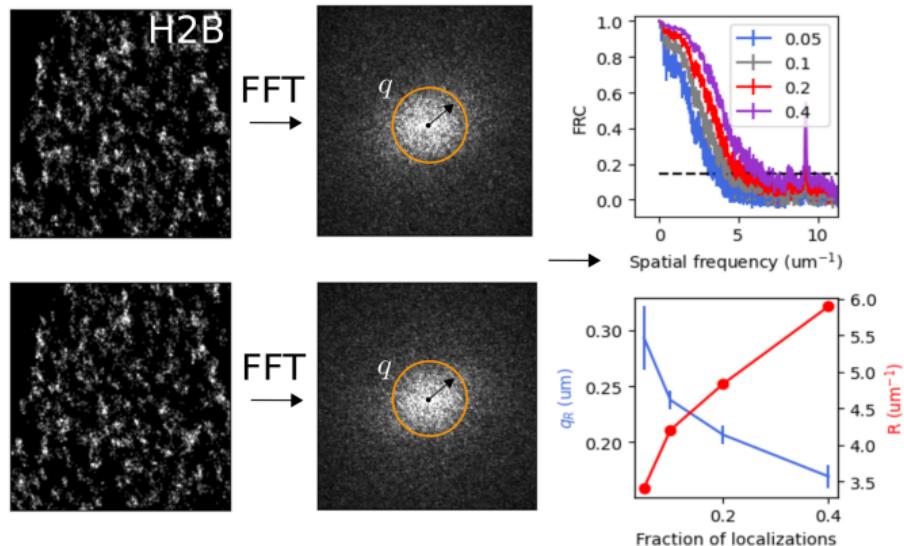
# Dense labeling of histone H2B in fixed cells at RT



- ▶ 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

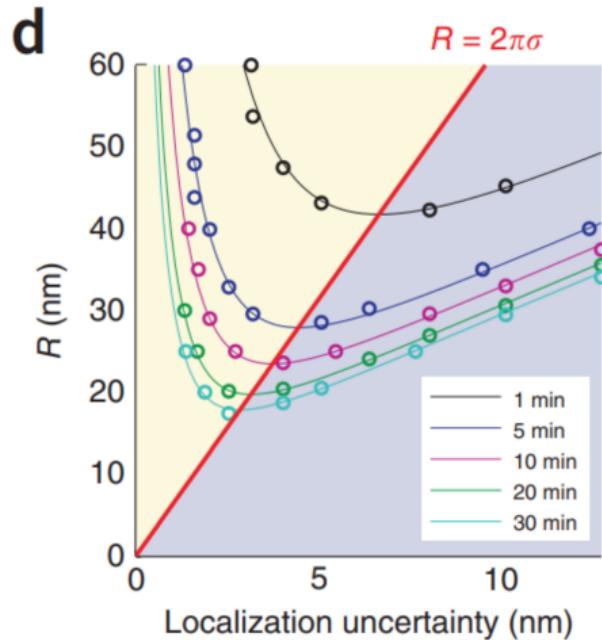
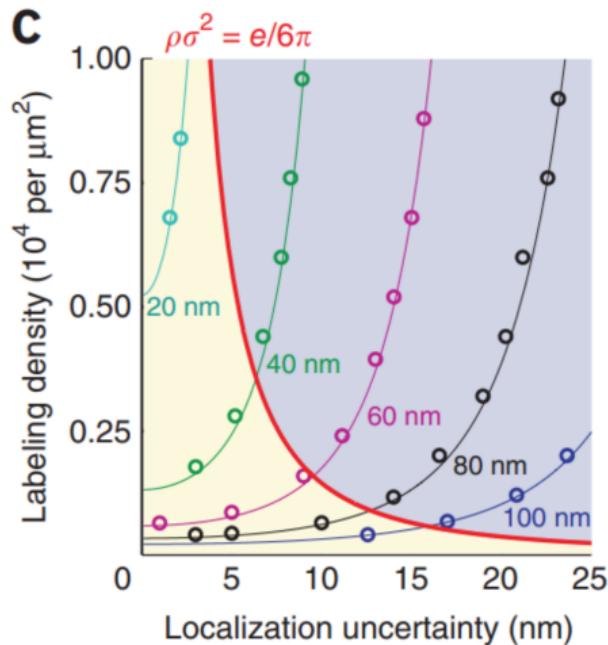
# Dense localization increases time resolution

- 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

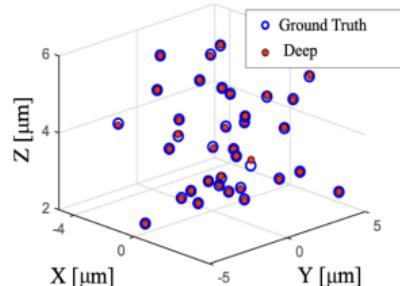
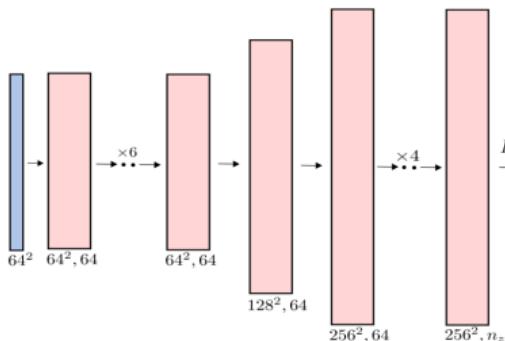
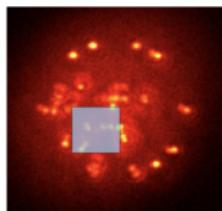


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

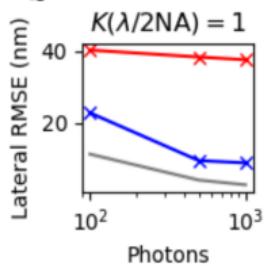
- ▶ Increased localization uncertainty requires higher density for same resolution
- ▶ Longer acquisitions have higher resolution

# Dense localization with deep learning

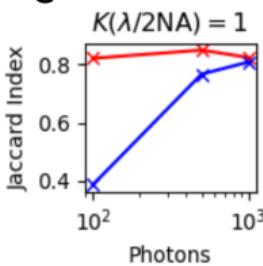
a



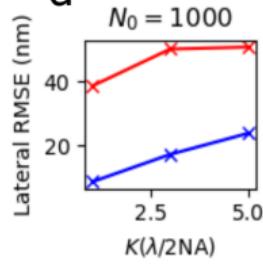
b



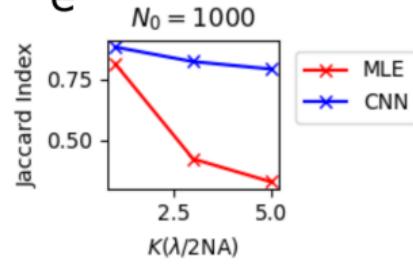
c



d

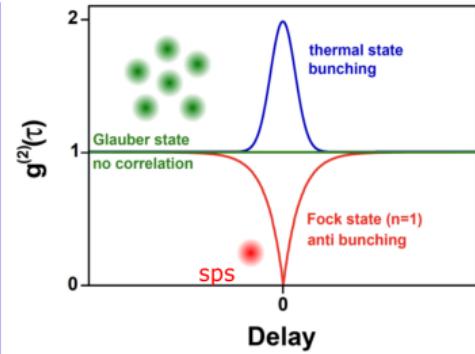
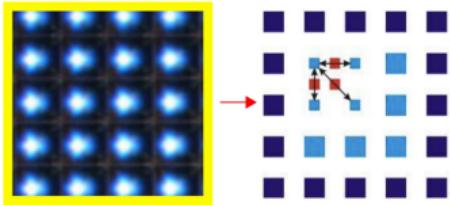
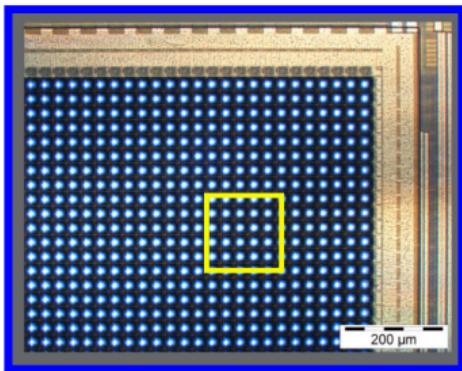
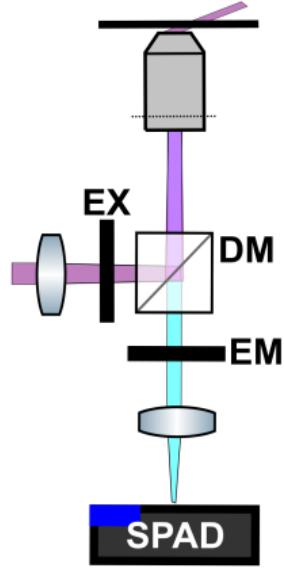


e



- We can model  $P_\Psi(Z)$  with a convolutional neural network  $\Psi$

# Dense localization with a photon counting camera



0101010010...  
0101011101...

## Dense localization with fluorescence antibunching

We need to compute the joint distribution  $P(X_i, X_j)$ . We compute  $P(X_i = N_i, X_j = N_j)$  by considering now microstates  $\alpha_i, \alpha_j$ , which are binary vectors, s.t.  $\sum \alpha_i = N_i$  and  $\sum \alpha_j = N_j$  and have  $\alpha_i \text{ AND } \alpha_j = 0$

$$P(X_i = N_i, X_j = N_j) \propto \sum_{\alpha, \beta \in \mathcal{A} \otimes \mathcal{B}} \prod_n \mathbf{p}_i^\alpha \mathbf{p}_j^\beta$$

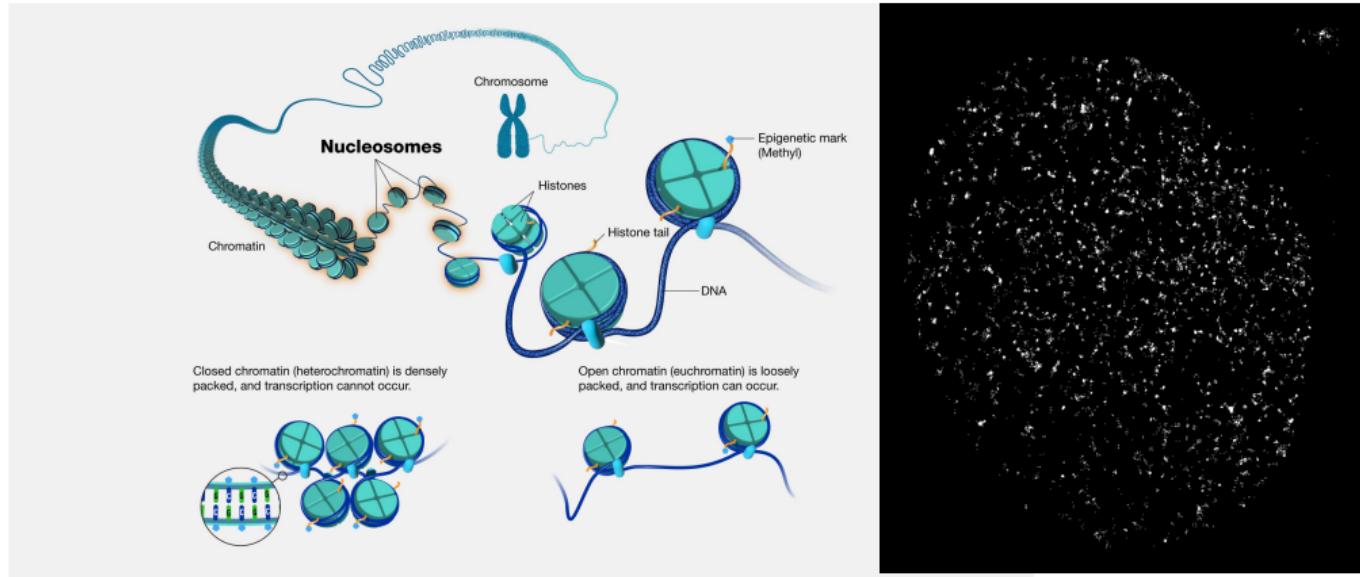
But now consider

$$\langle X_i X_j \rangle = \sum_{(N_i, N_j)} N_i N_j P(X_i = N_i, X_j = N_j)$$

Antibunching now becomes apparent. If only a single emitter exists (and we have designed  $\alpha$ 's correctly) then this expectation must be zero for all  $(i, j)$ .

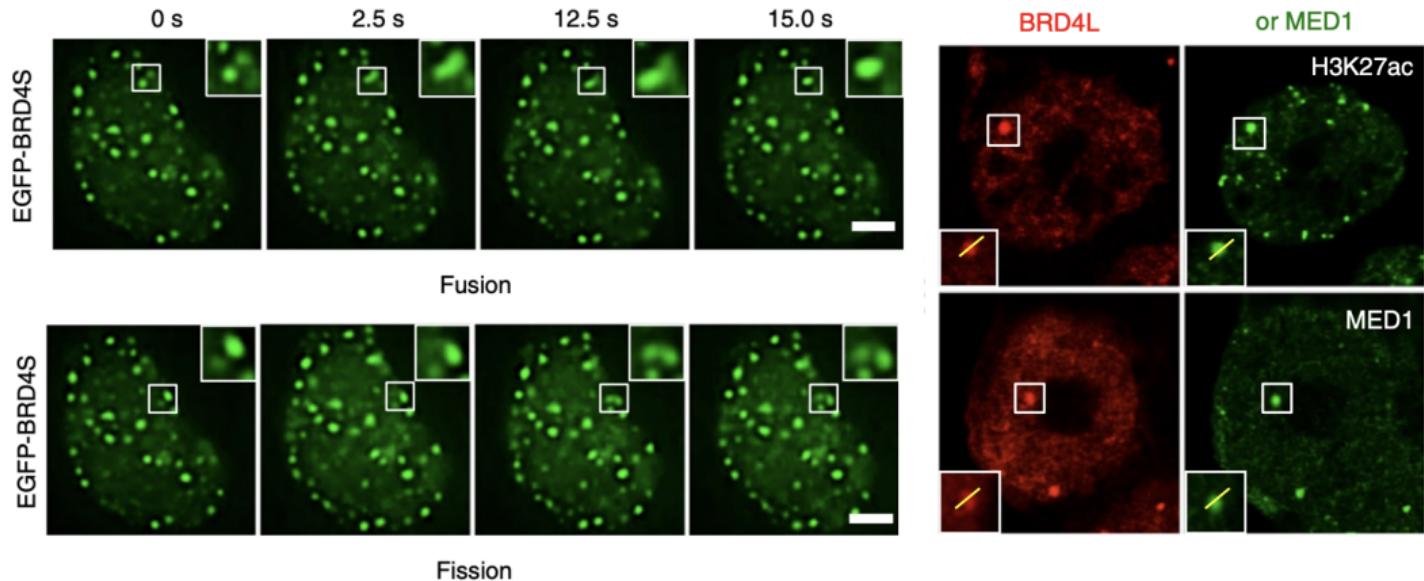
## Phase separation of chromatin

# Genome organization and single molecule localization microscopy



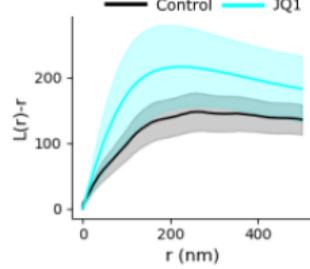
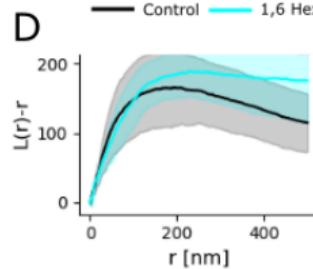
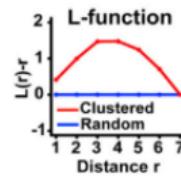
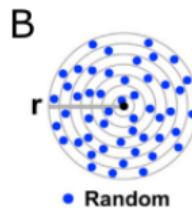
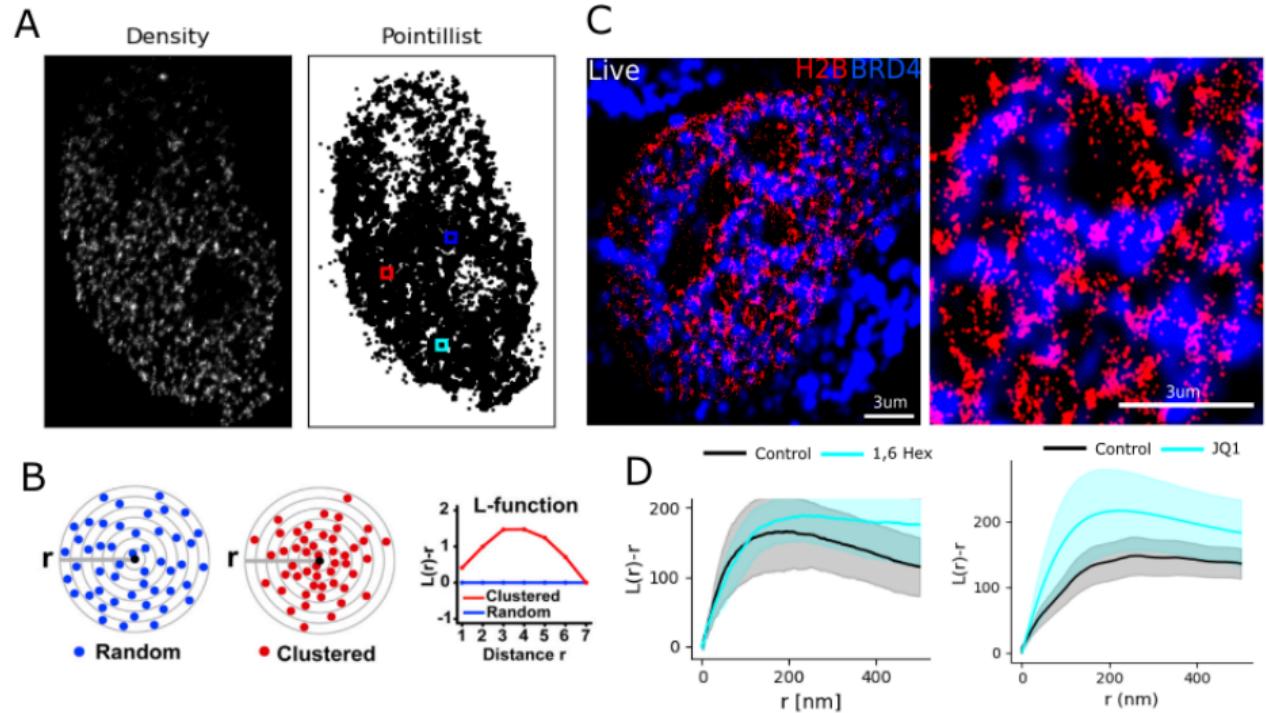
- ▶ Genome has a hierarchical structure, fundamental unit is the nucleosome
- ▶ We study chromatin organization by localizing fluorescently tagged nucleosomes

# BRD4 condensates exhibit LLPS properties

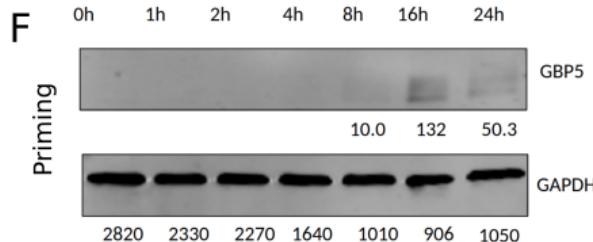
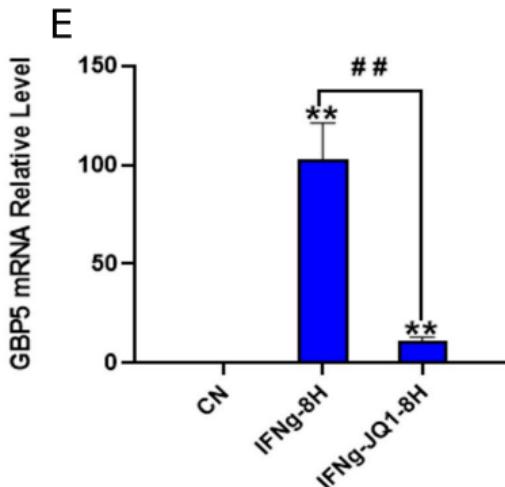
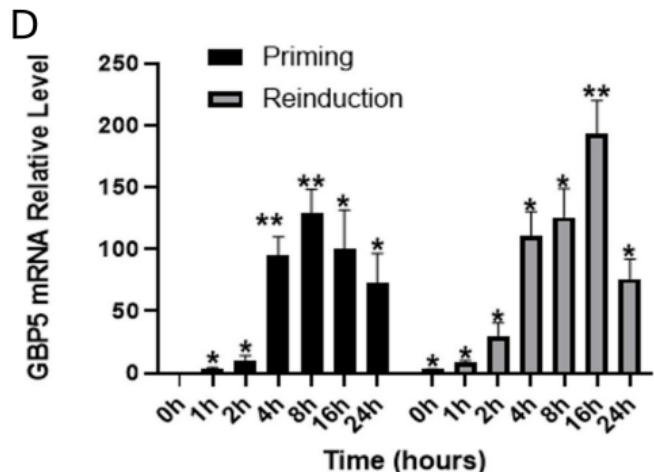


Han et al. Roles of the BRD4 short isoform in phase separation and active gene transcription. *Nature Structural and Molecular Biology*. 2020

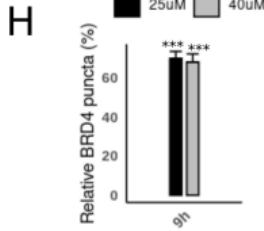
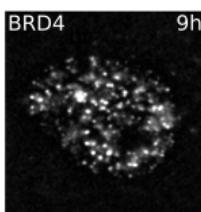
# BET inhibitors reduce nucleosome-BRD4 interactions in BRD4 condensates



# Inhibition of a super-enhanced gene with JQ1



G



► \*:P ≤ 0.1, \*\*:P ≤ 0.01