

Visualizing chromatin organization with single molecule localization microscopy

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Outline

Single molecule localization microscopy

The time resolution of *d*STORM

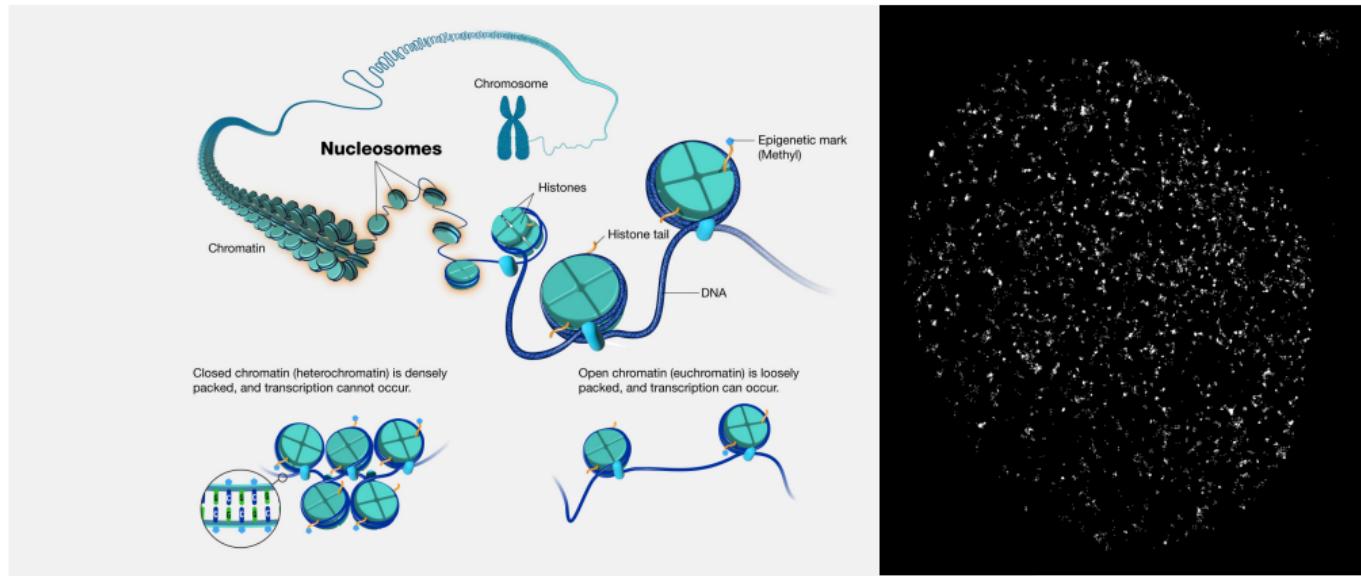
A novel approach to dense localization microscopy

Dense localization by fluorescence antibunching

Phase separation of chromatin

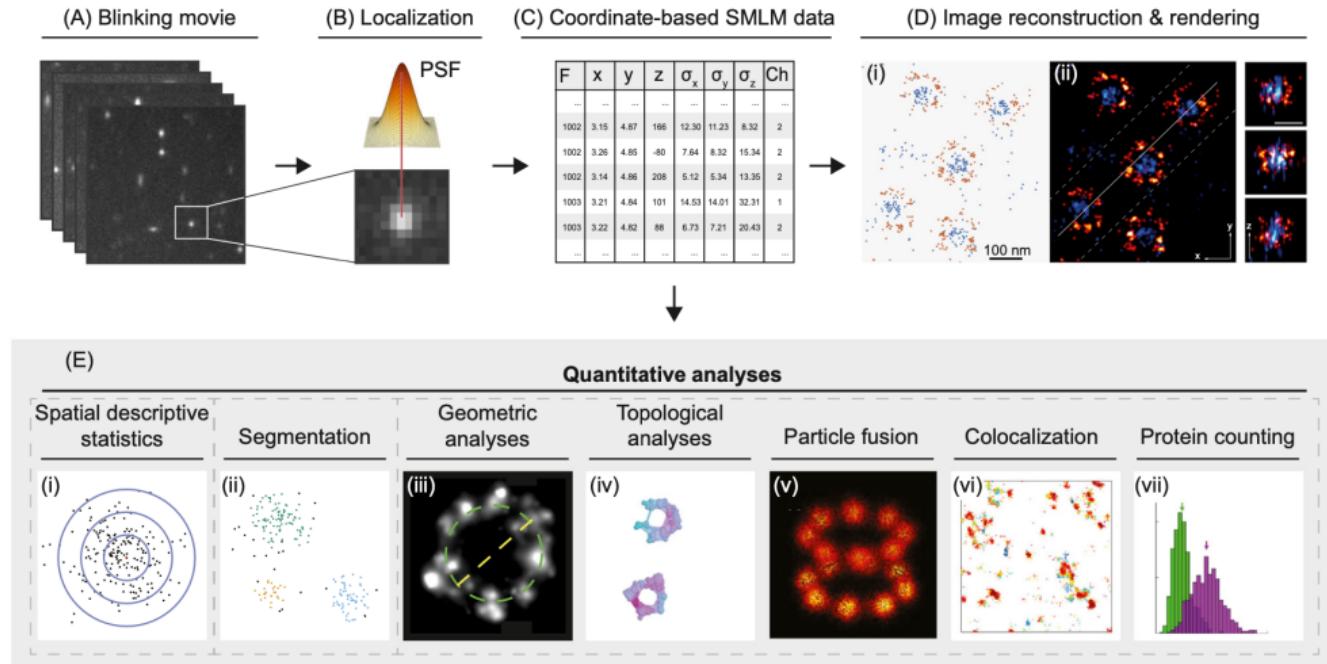
Single molecule localization microscopy

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

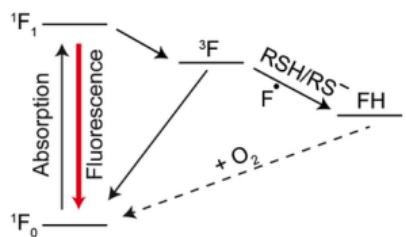
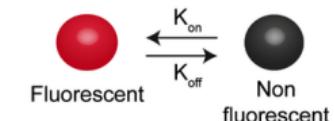
Single molecule localization microscopy



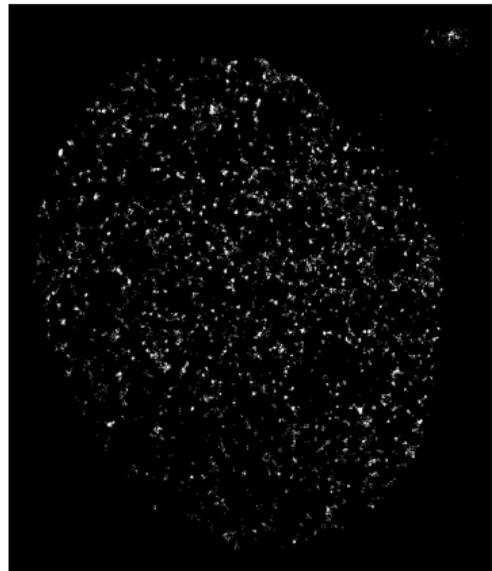
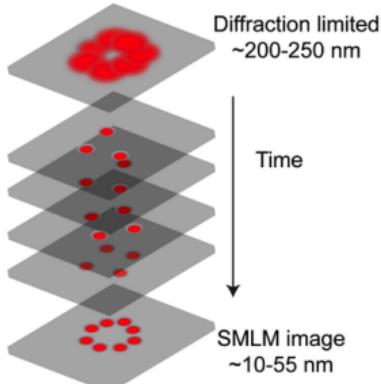
- Chromatin structure is dynamic and undergoes **phase separation** → We are pushing the limits of SMLM for time-resolution

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

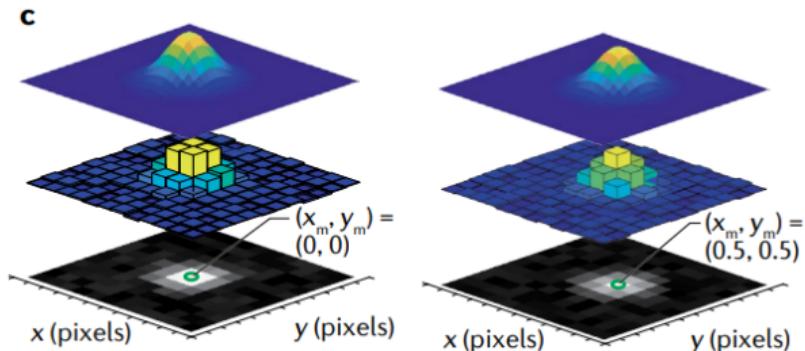
$$\mu_k = i_0 \int_{\mathbf{k}} h_\theta(x_0, y_0) dx dy$$

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

η – quantum efficiency

N_0 – photon count

Δ – exposure time

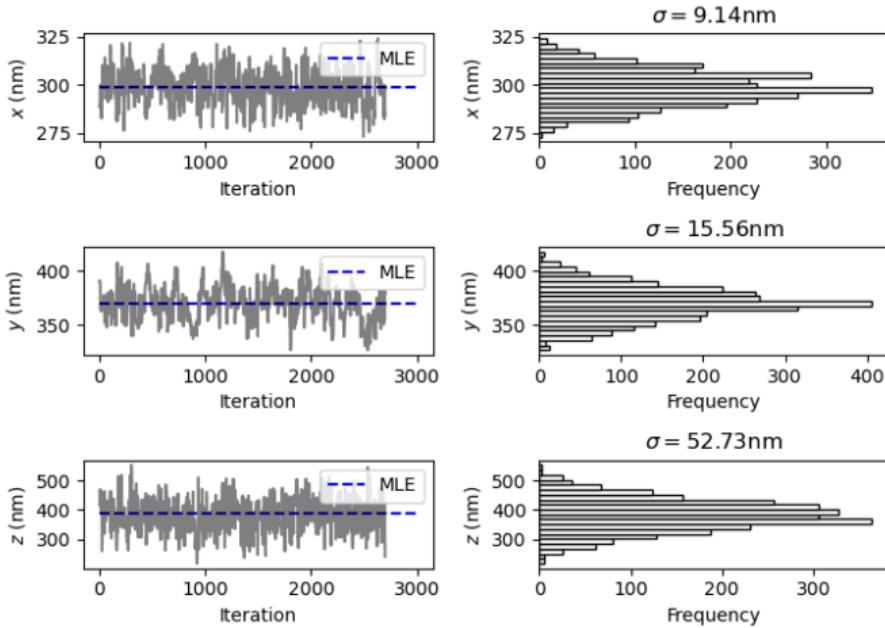
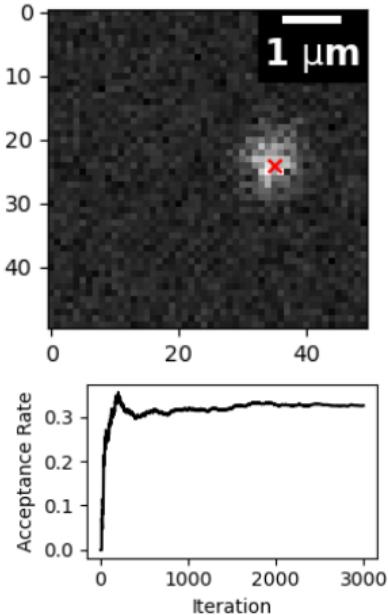


Long $\Delta \rightarrow$ pixels are iid:

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

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

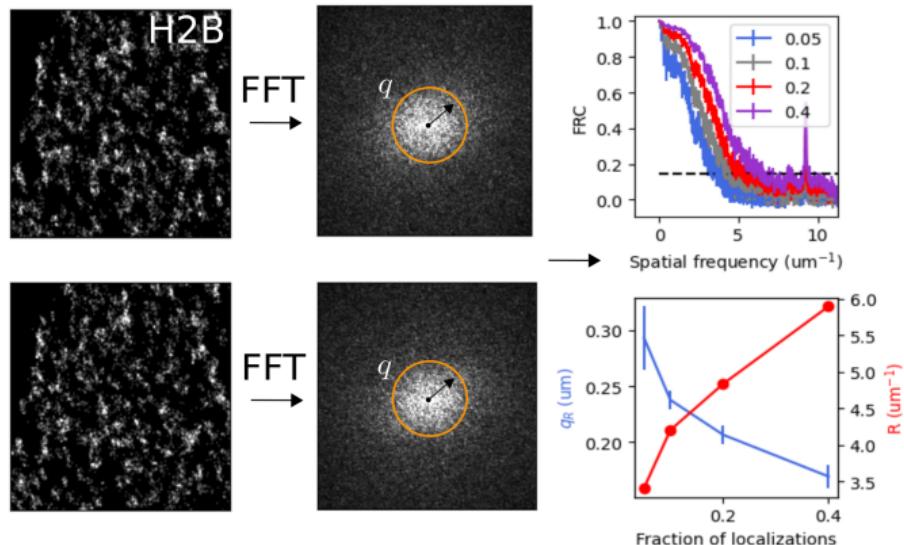
Estimator precision determines resolution in localization microscopy



- One can derive a lower bound on the variance of a statistical estimator of the coordinates θ

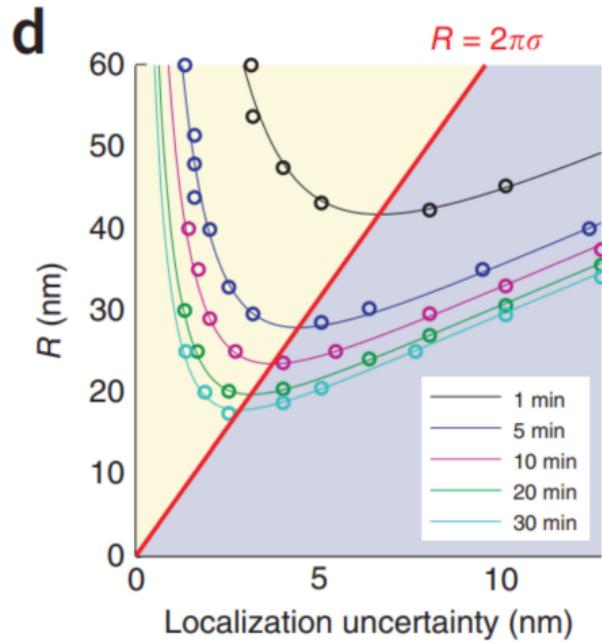
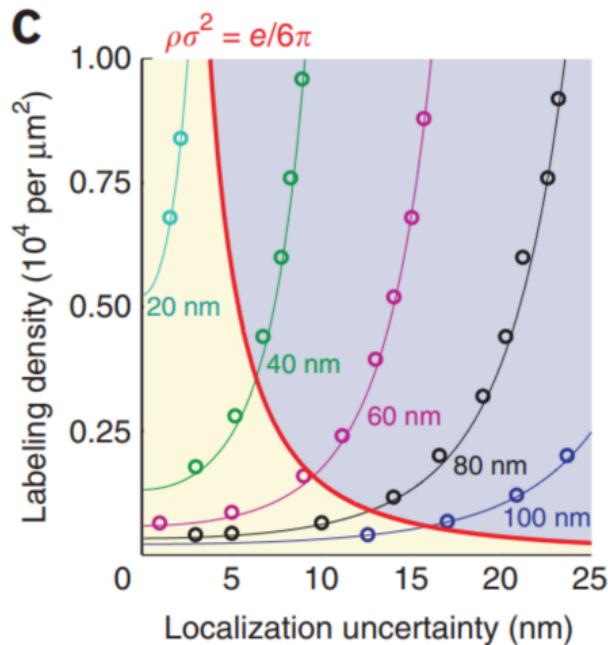
Dense localization increases time resolution

- 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

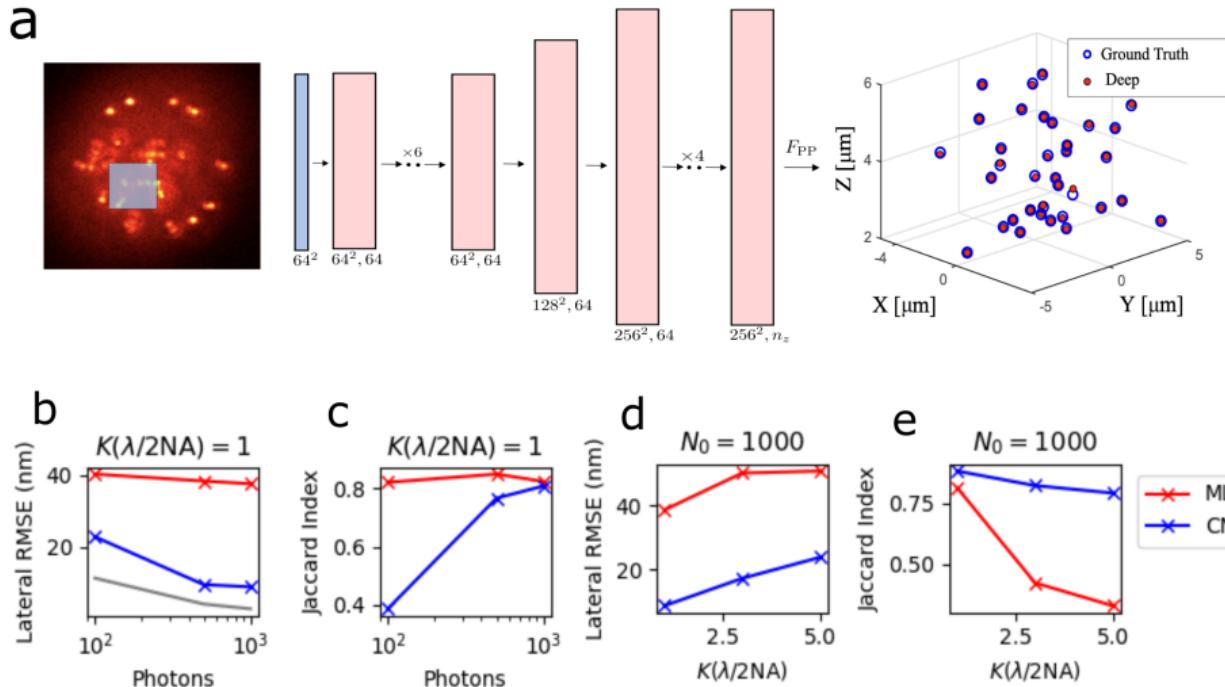


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

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

A novel approach to dense localization microscopy

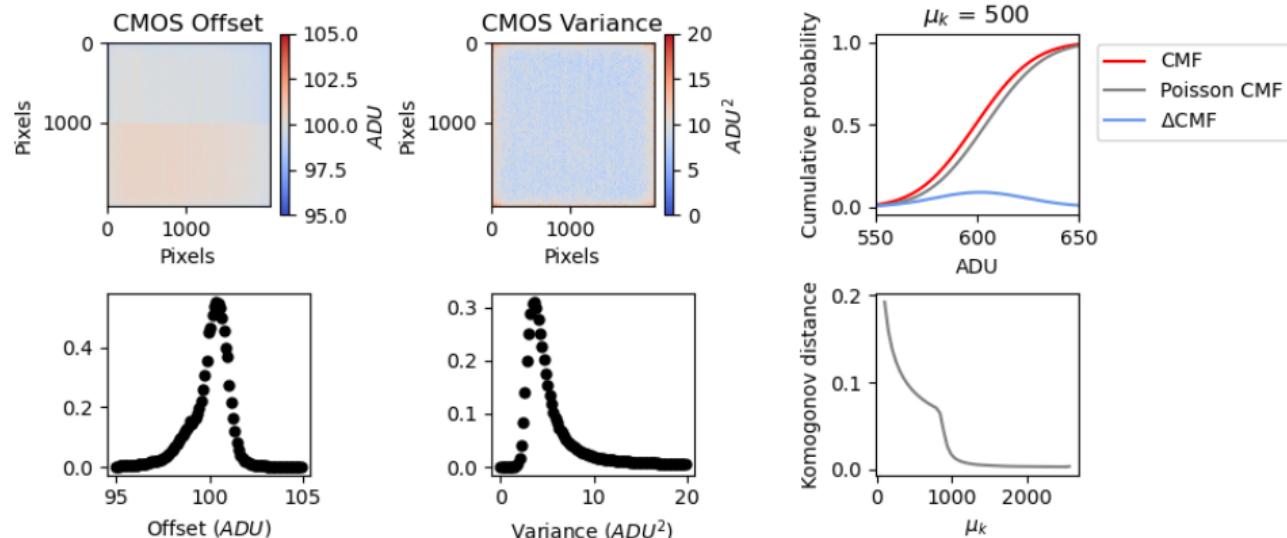
DeepSTORM: An early approach to dense SMLM with deep learning



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

Classical emission statistics of fluorescent markers

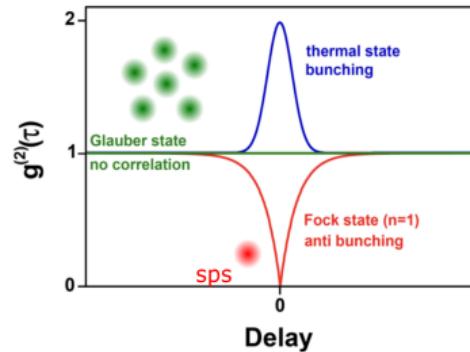
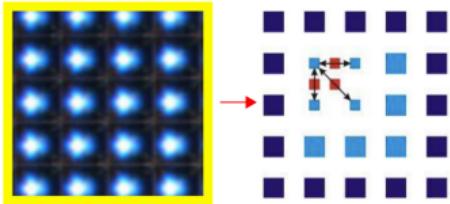
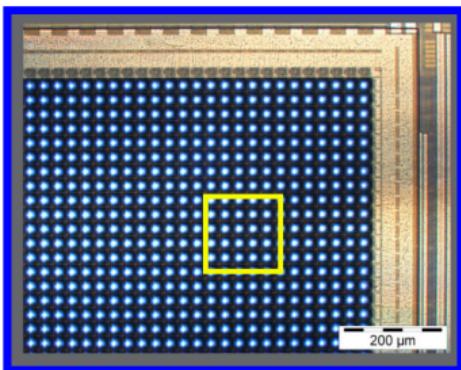
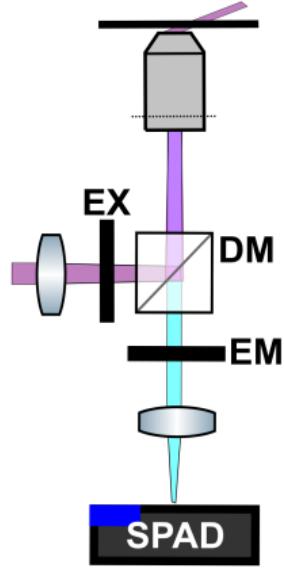
Long integration times $\Delta \rightarrow$ intensity fluctuations are Poisson



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

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

Counting fluorescent molecules with a photon counting camera



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0101011101...

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 θ with MCMC:

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

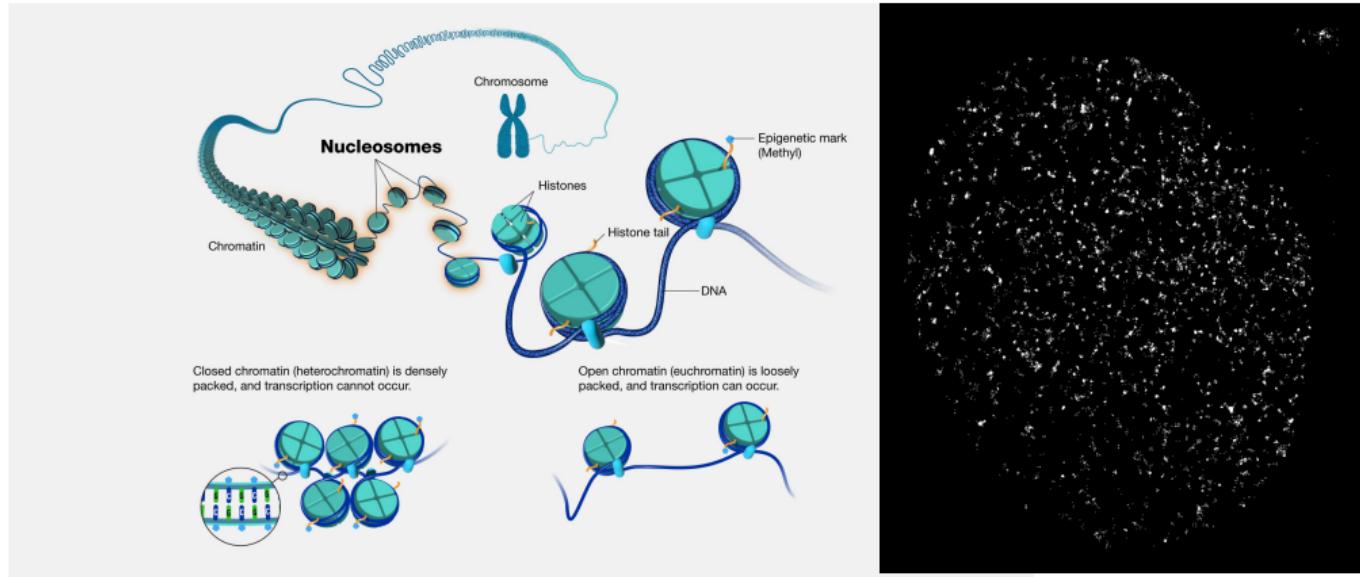
Then we use a Langevin Monte Carlo (LMC) sampler for the spatial model

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

Both of these sampling methods also provide the MAP estimates of the parameters θ, ϕ .

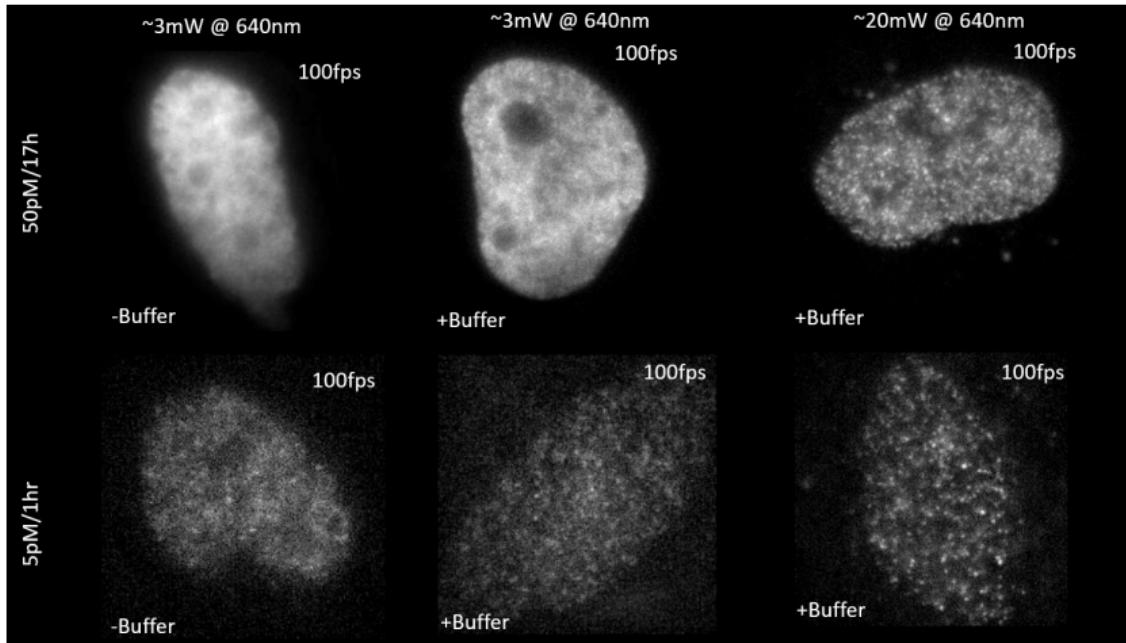
Phase separation of chromatin

Genome organization and single molecule localization microscopy



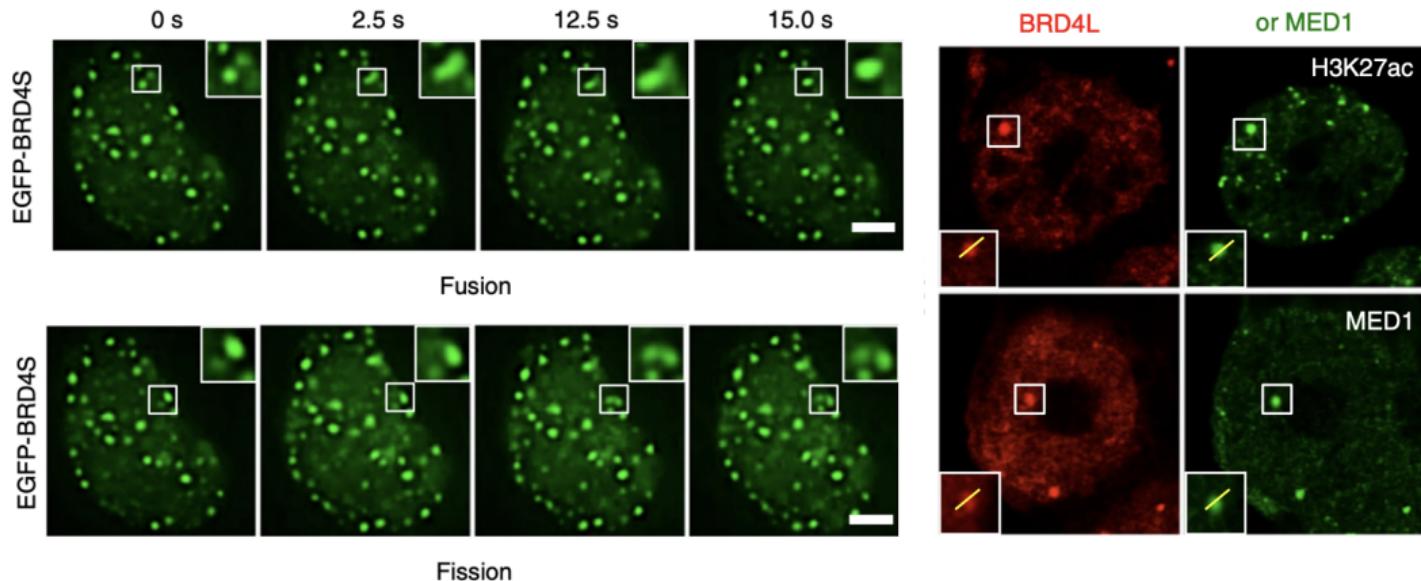
- ▶ Genome has a hierarchical structure, fundamental unit is the nucleosome
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Dense labeling of histone H2B in fixed cells at RT



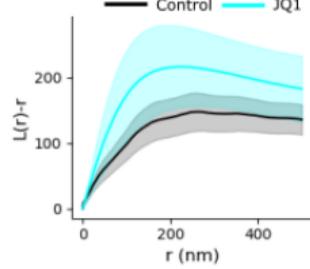
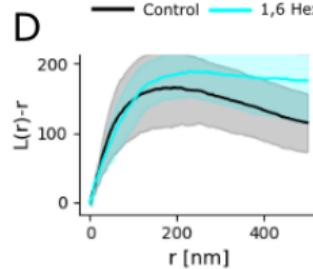
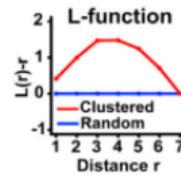
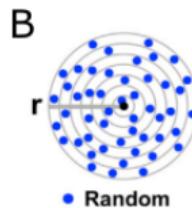
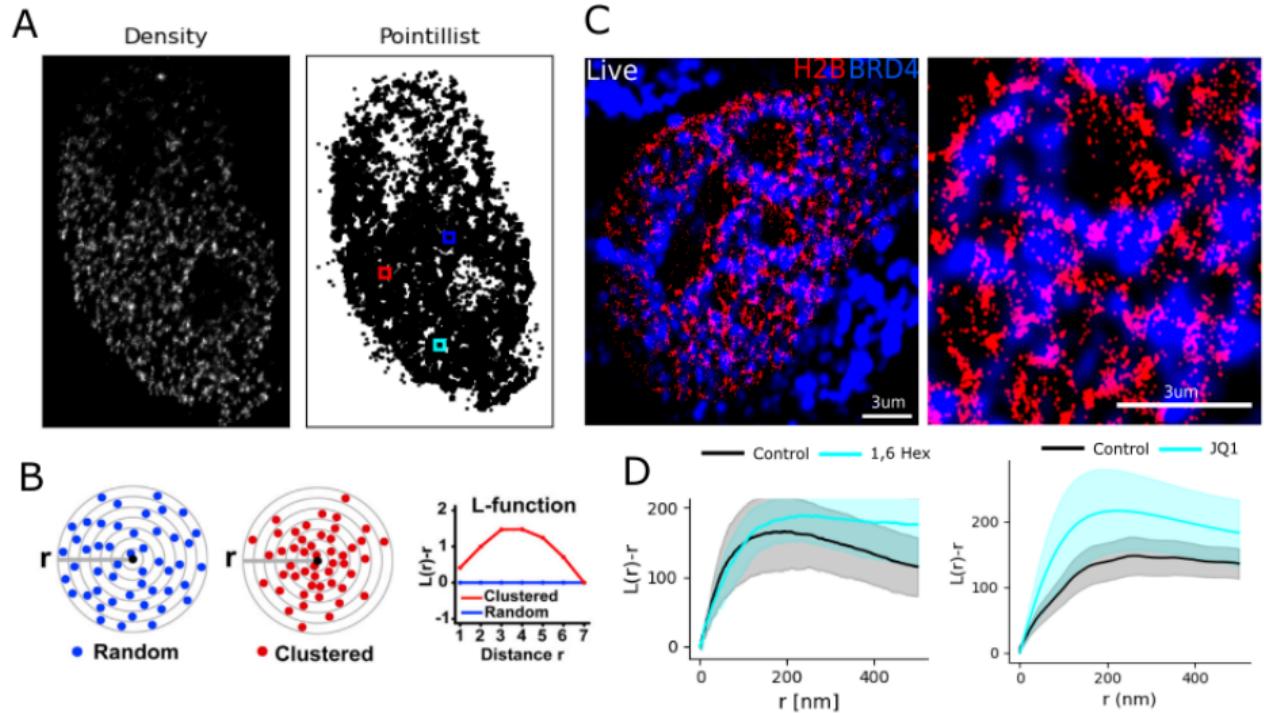
- ▶ Dense labeling of H2B-Halotag w/ fluorescent ligand JF646
- ▶ Reducing buffer is usually a primary thiol like cysteamine (MEA)
- ▶ Photoswitching of JF646 allows us to beat the diffraction limit

BRD4 condensates exhibit LLPS properties

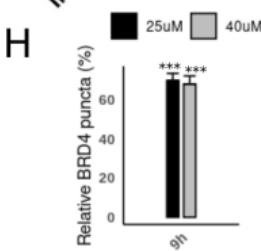
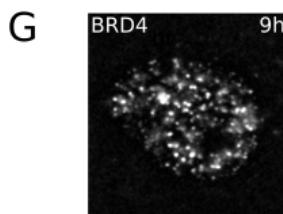
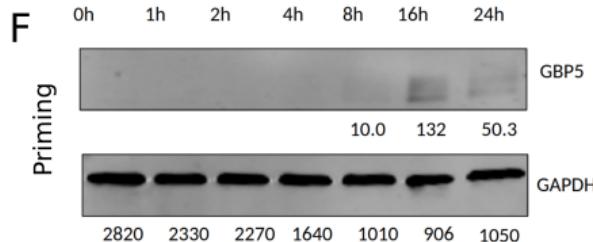
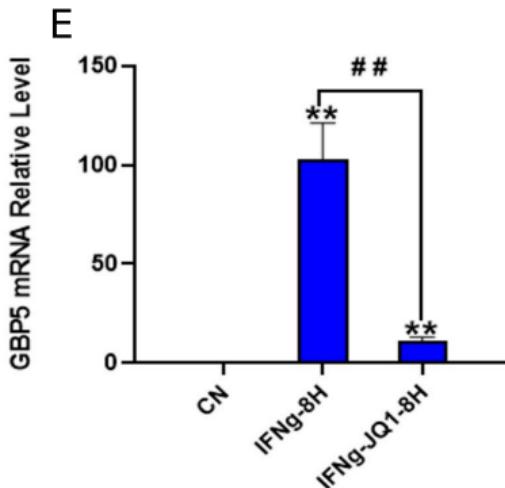
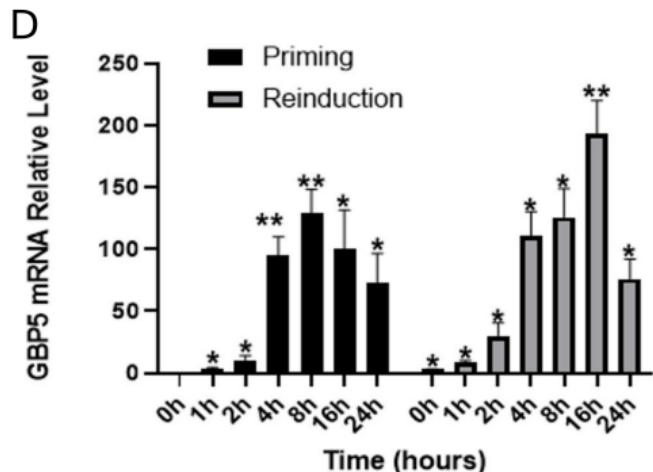


Han et al. Roles of the BRD4 short isoform in phase separation and active gene transcription. *Nature Structural and Molecular Biology*. 2020

BET inhibitors reduce nucleosome-BRD4 interactions in BRD4 condensates



Inhibition of a super-enhanced gene with JQ1



► *: P ≤ 0.1, **: P ≤ 0.01