

# Visualizing chromatin organization with single molecule localization microscopy

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# Outline

Single molecule localization microscopy

The time resolution of *d*STORM

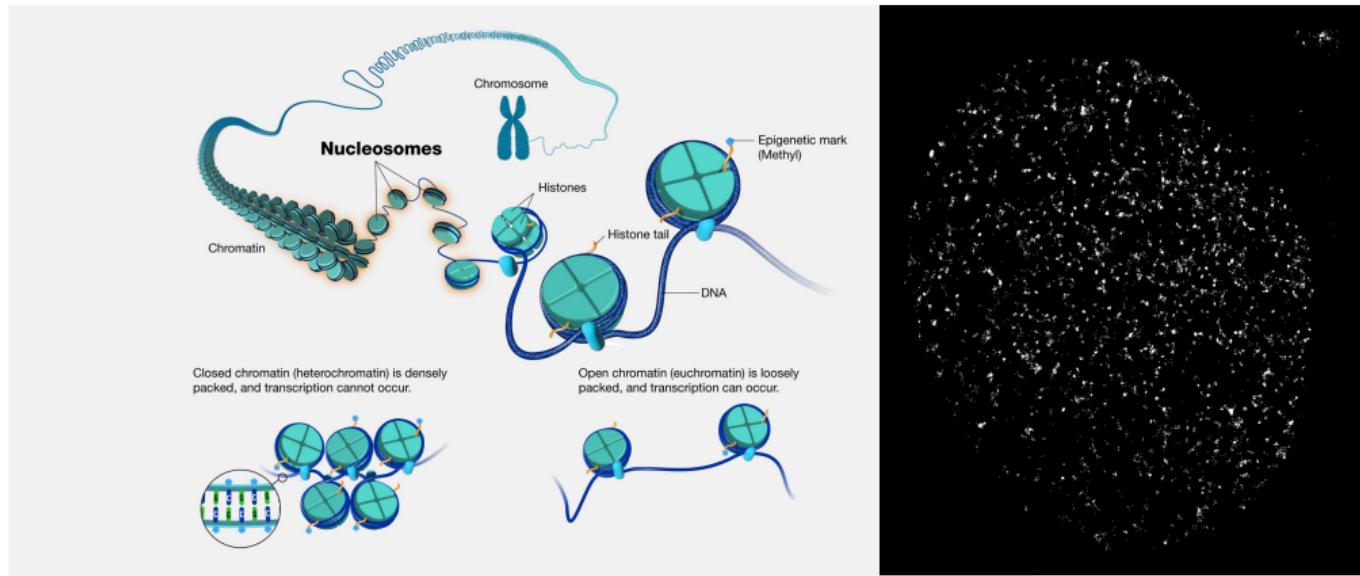
Dense localization with deep learning

Dense localization by fluorescence antibunching

The nucleosome: lost in phase space

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

# Single molecule localization microscopy

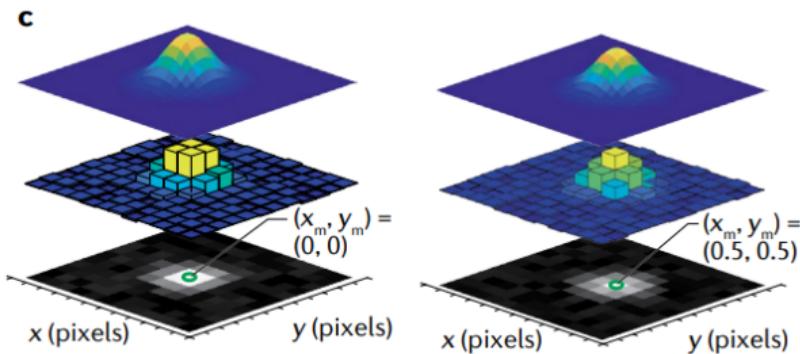
$$\text{Localization: } \theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$$

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

$\eta$  – quantum efficiency

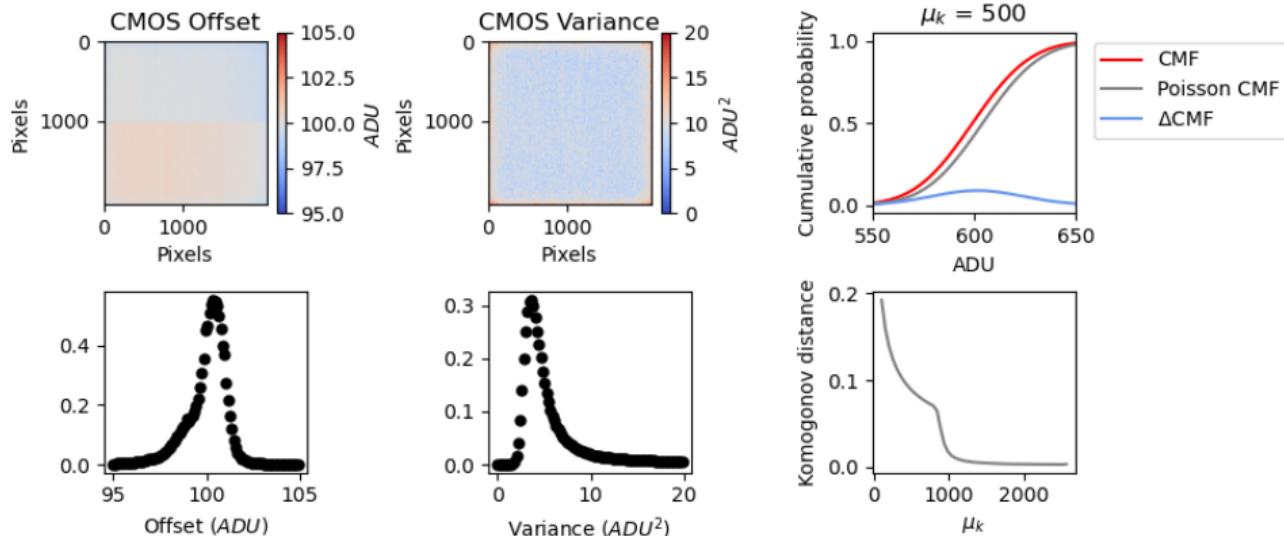
$N_0$  – photon count

$\Delta$  – exposure time



- ▶ SMLM techniques are diffraction unlimited
- ▶ Exposures are typically ten to hundreds of ms
- ▶ SMLM techniques are suitable for **super-resolution** (SR) and **single molecule tracking** (SMT)

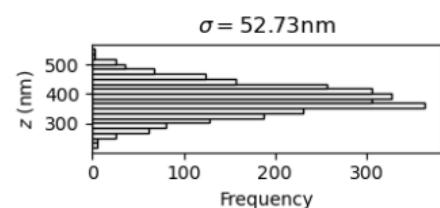
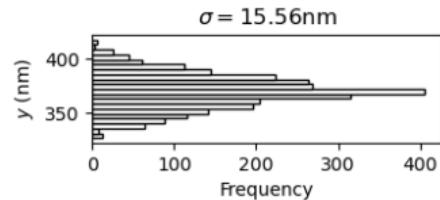
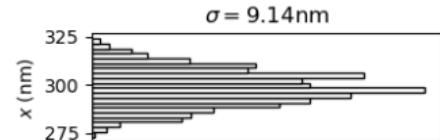
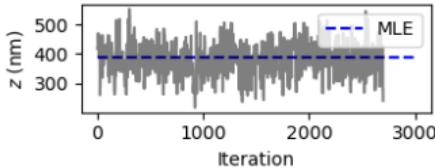
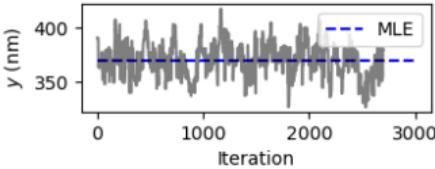
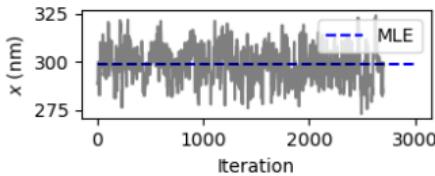
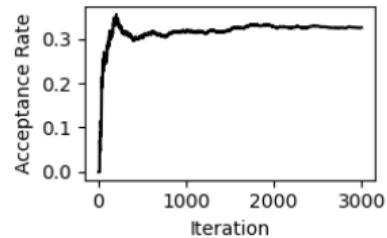
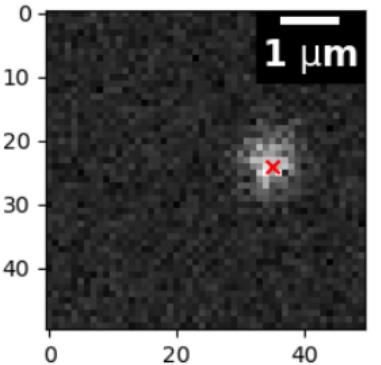
# Classical emission statistics of fluorescent markers



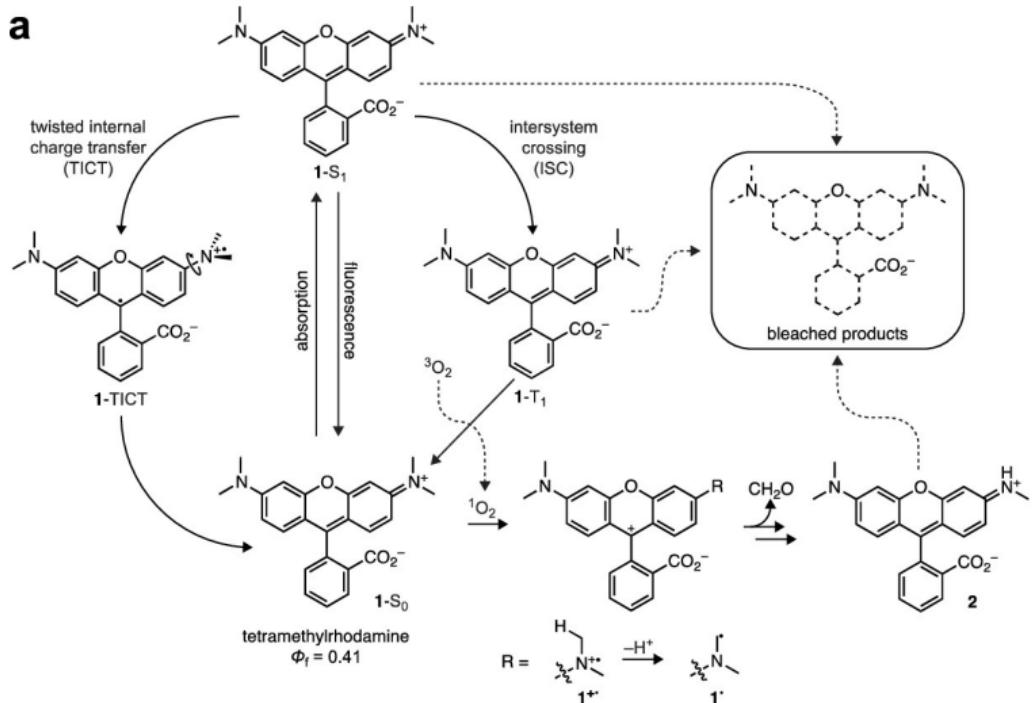
$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi\sigma_k^2}} e^{-\frac{(H_k - g_k q - \sigma_k)^2}{2\sigma_k^2}}$$

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

# Estimator precision in localization microscopy

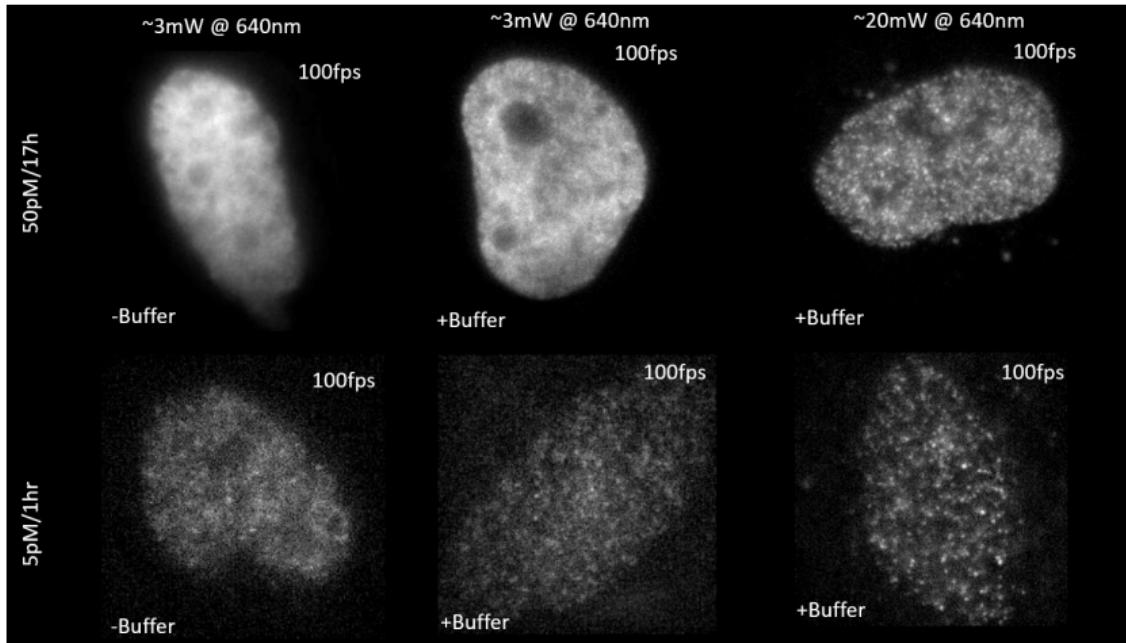


# Super resolution with photoswitching of rhodamines



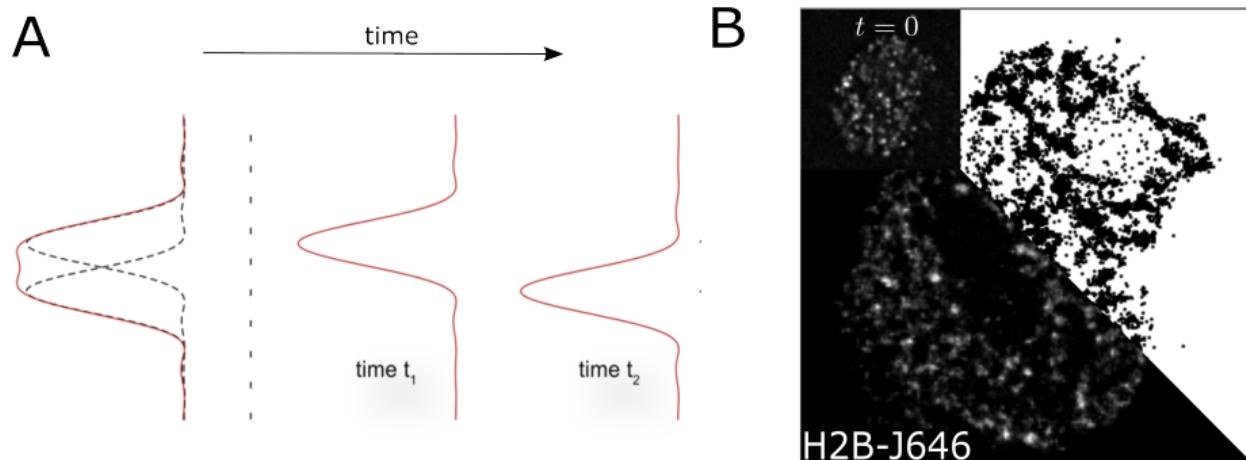
- ▶ Reduction of the T<sub>1</sub> state yields a dark, long-lived, and stable radical state

# 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

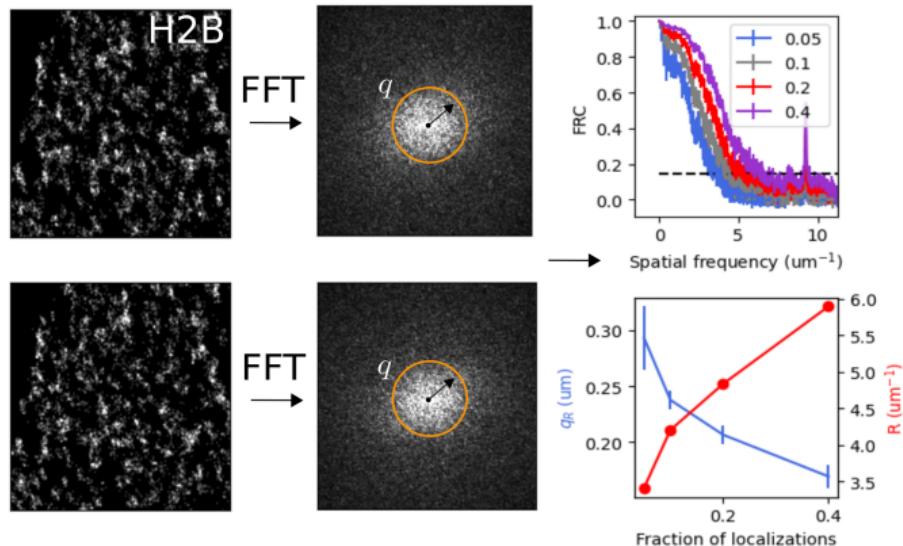
# Direct stochastic optical reconstruction microscopy



- ▶ Photoswitching enables resolution of emitters in time rather than space
- ▶ Presents a tradeoff between spatial and temporal resolution

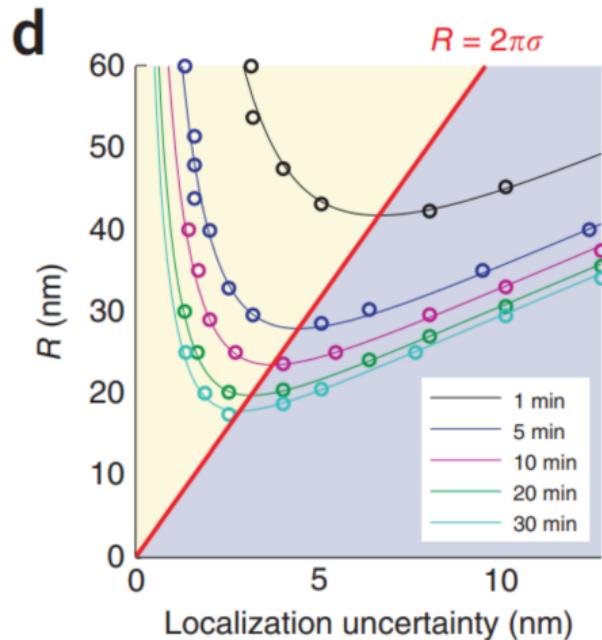
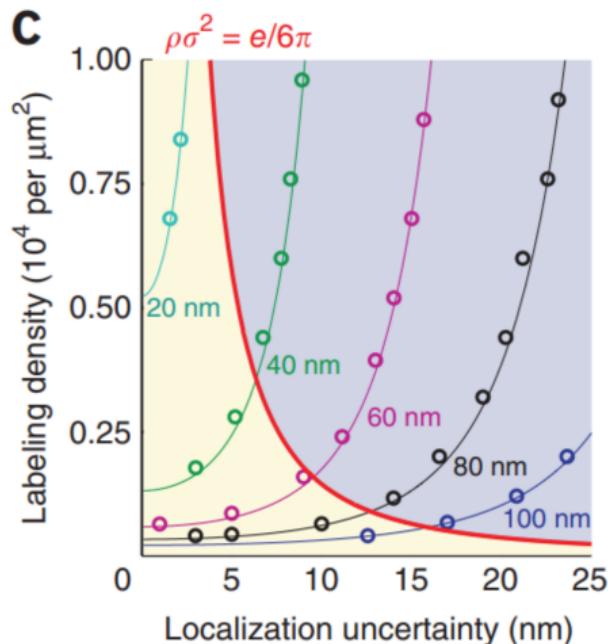
# Dense localization increases time resolution

- We can view dSTORM as sampling from a density



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

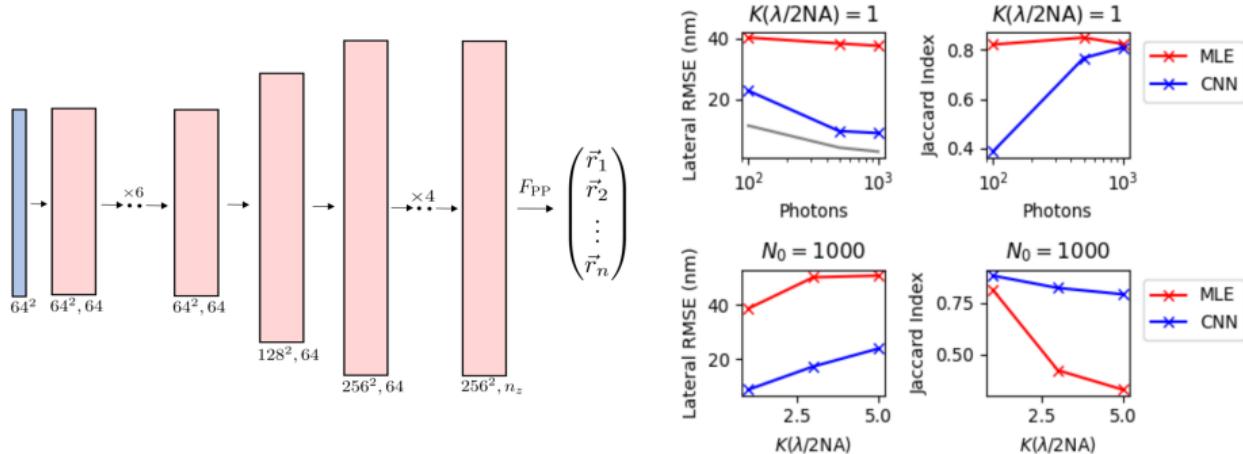
## Dense localization increases time resolution



Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

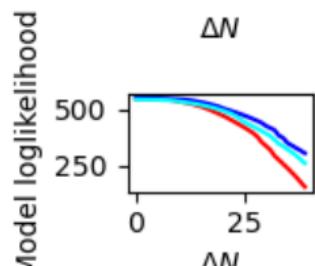
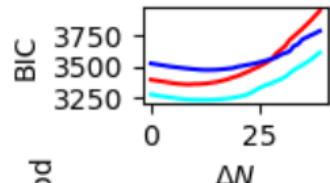
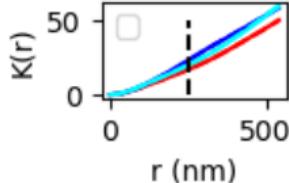
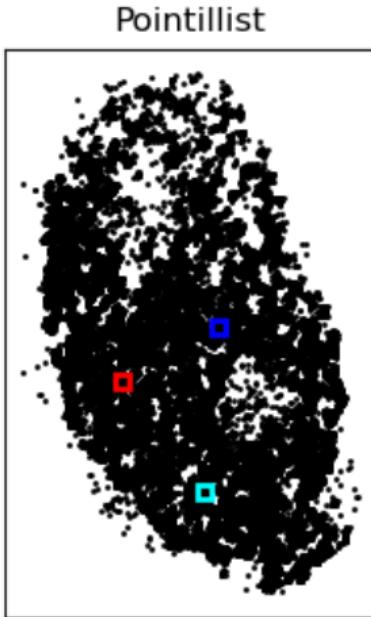
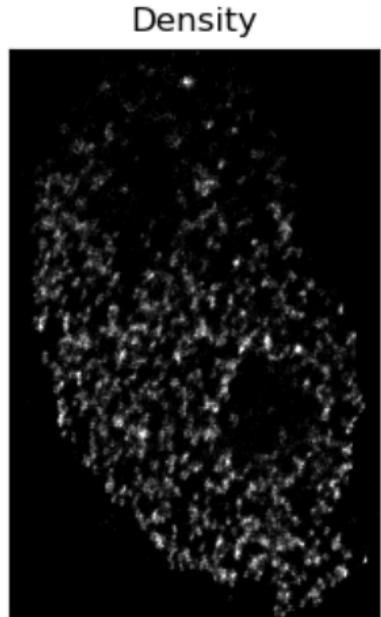
- ▶ Increased localization uncertainty requires higher density for same resolution
- ▶ Longer acquisitions have higher resolution

# Estimator precision sets the resolution limit in localization microscopy



- ▶  $K(\lambda/2NA)$  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



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

## 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  $\alpha_i \text{ AND } \alpha_j = 0$

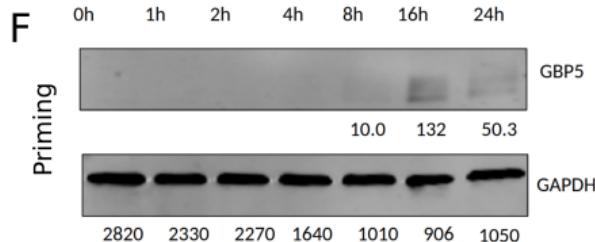
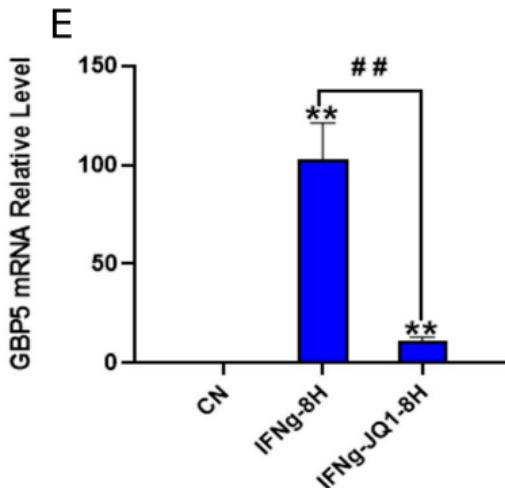
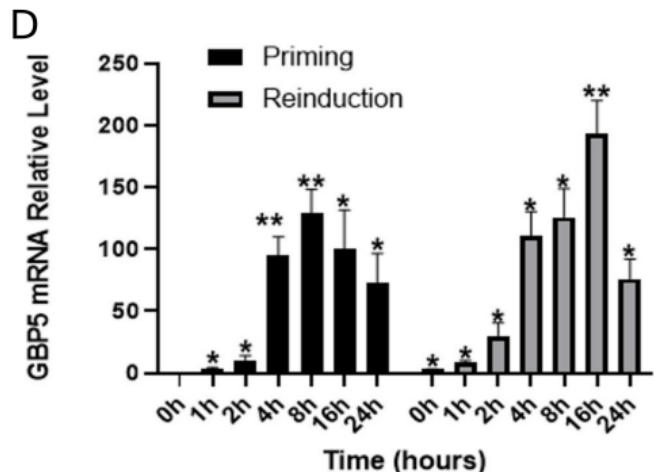
$$P(X_i = N_i, X_j = N_j) \propto \sum_{\alpha, \beta \in \mathcal{A} \otimes \mathcal{B}} \prod_n \mathbf{p}_i^\alpha \mathbf{p}_j^\beta$$

But now consider

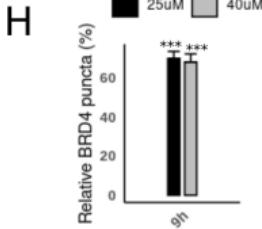
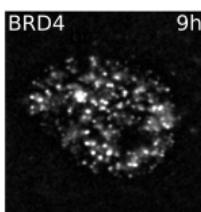
$$\langle X_i X_j \rangle = \sum_{(N_i, N_j)} N_i N_j P(X_i = N_i, X_j = N_j)$$

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  $(i, j)$ .

# Inhibition of a super-enhanced gene with JQ1

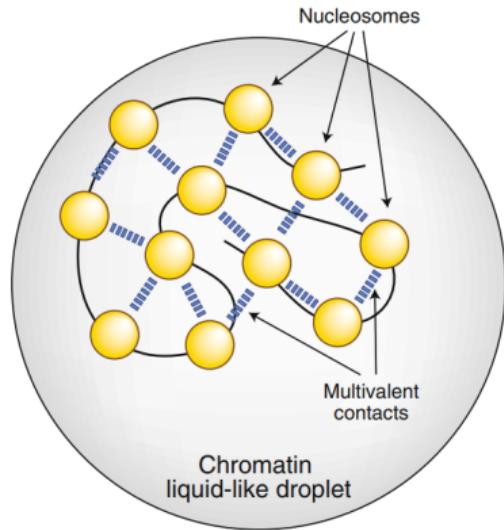


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► \* $P \leq 0.1$ , \*\* $P \leq 0.01$

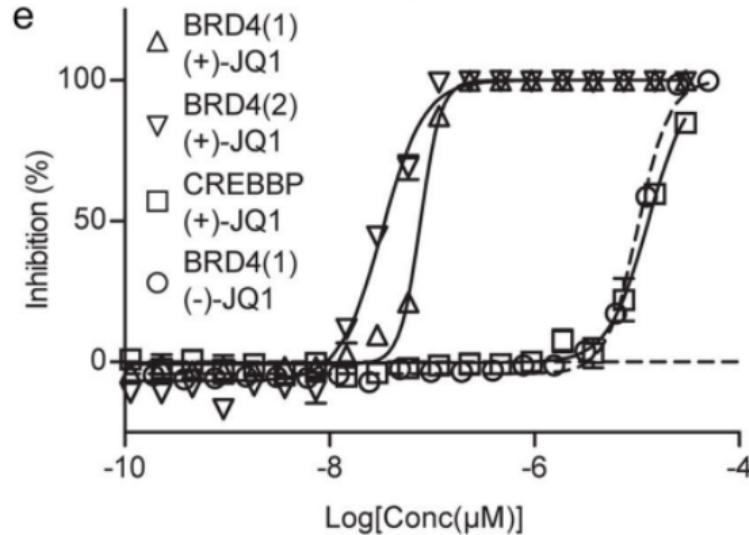
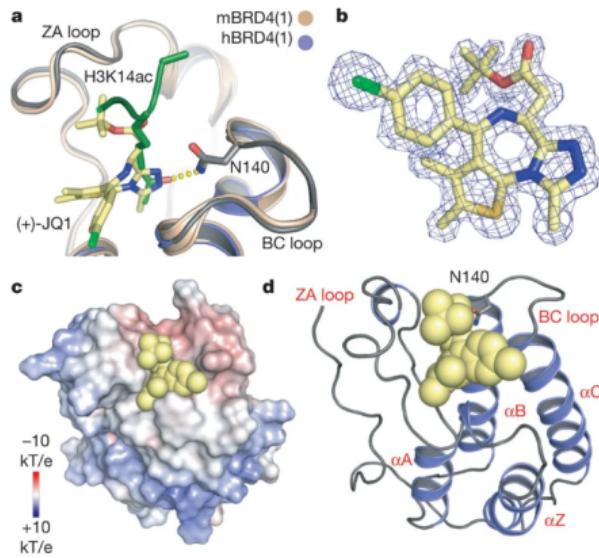
# Chromatin has an intrinsic ability to undergo phase separation



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

# BET inhibitors reduce nucleosome-BRD4 interactions in BRD4 condensates

## BET inhibitors promote disordered BRD4 condensates