

# Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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# Outline

Single molecule localization microscopy

The time resolution of *d*STORM

Dense localization with deep learning

The nucleosome as a Brownian harmonic oscillator

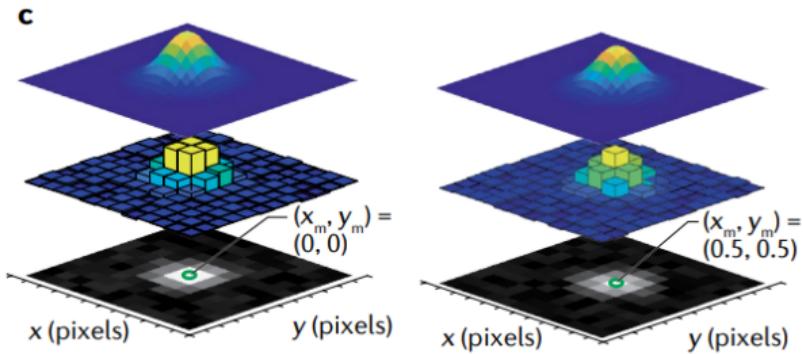
Phase separation of chromatin

# Single molecule localization microscopy

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

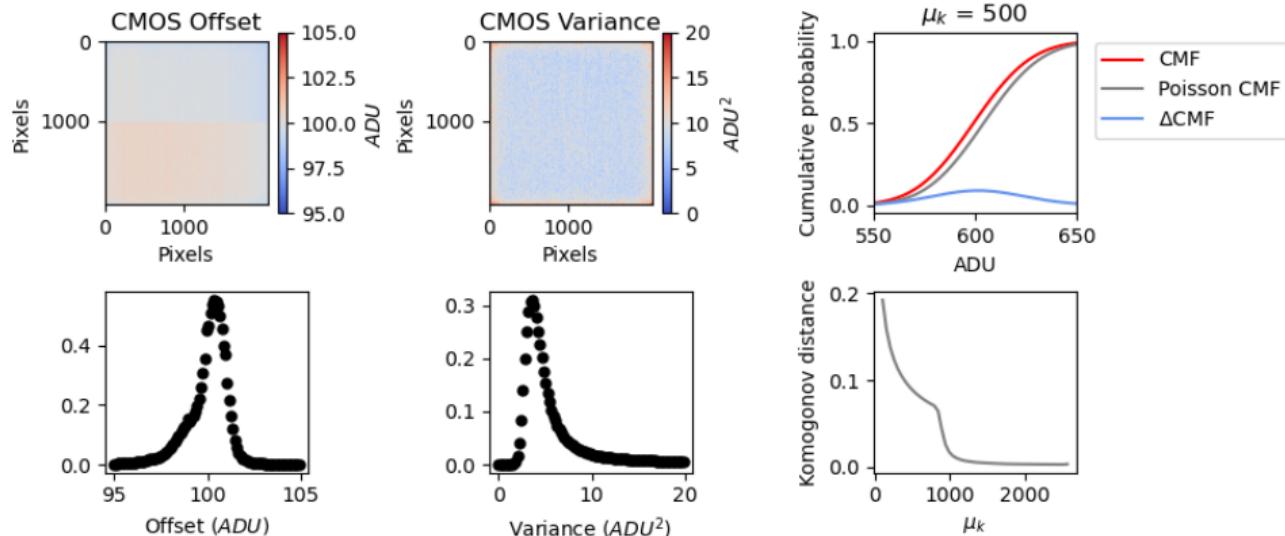
$$\mu_k = g_k \eta N_0 \Delta \int_{\text{pixel}} G(x, y) dA$$

$\eta$  – quantum efficiency  
 $N_0$  – photon count  
 $\Delta$  – exposure time



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

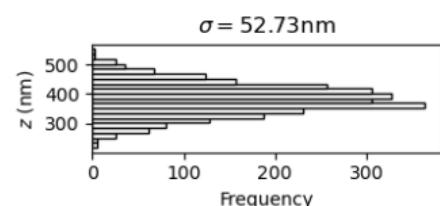
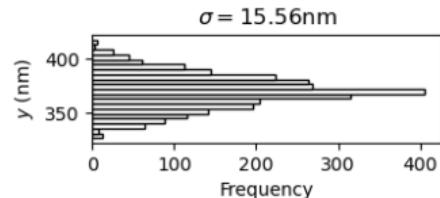
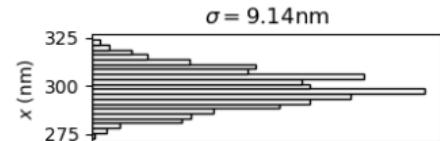
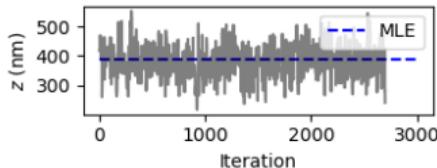
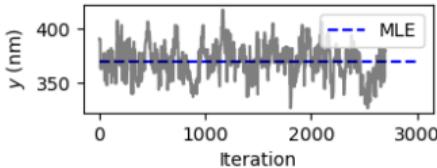
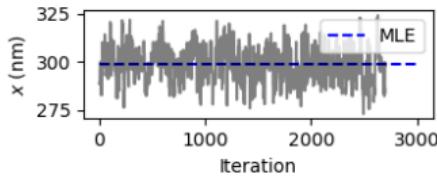
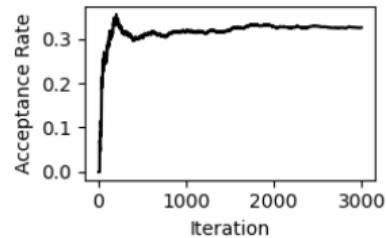
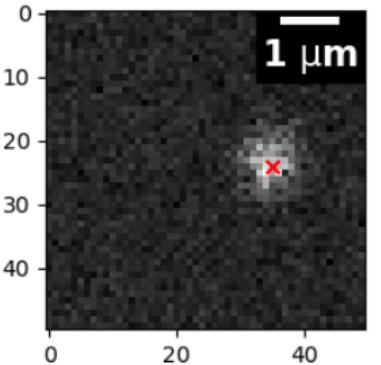
# A Poisson approximation at moderate SNR simplifies SMLM



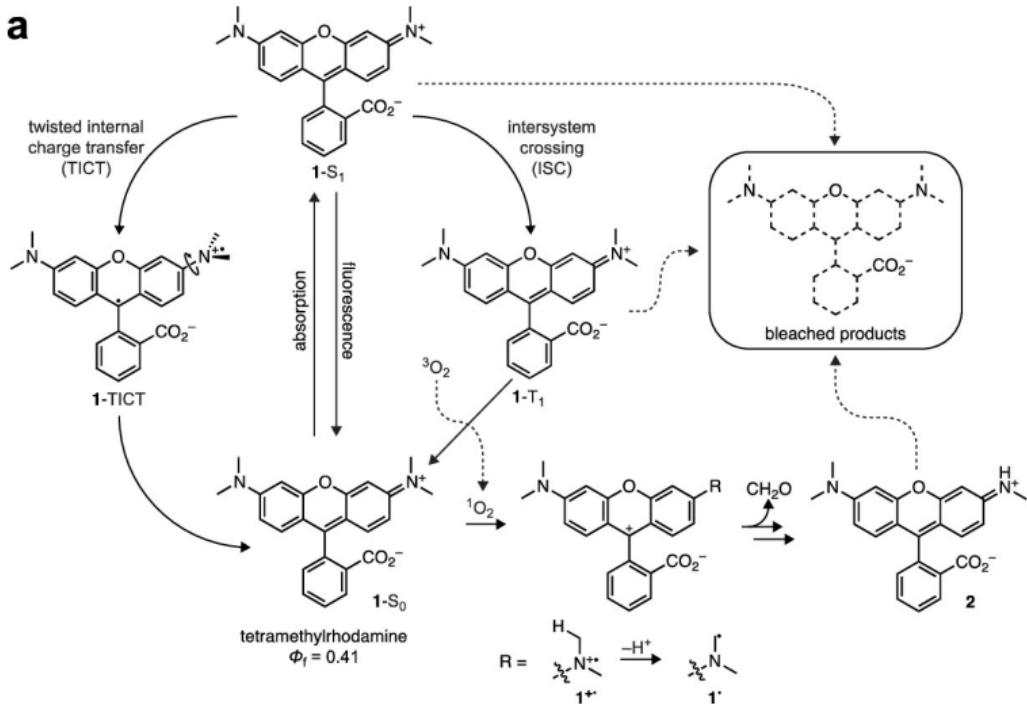
$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi\sigma_k^2}} e^{-\frac{(H_k - g_k q - \sigma_k)^2}{2\sigma_k^2}}$$

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

# Estimator precision in localization microscopy

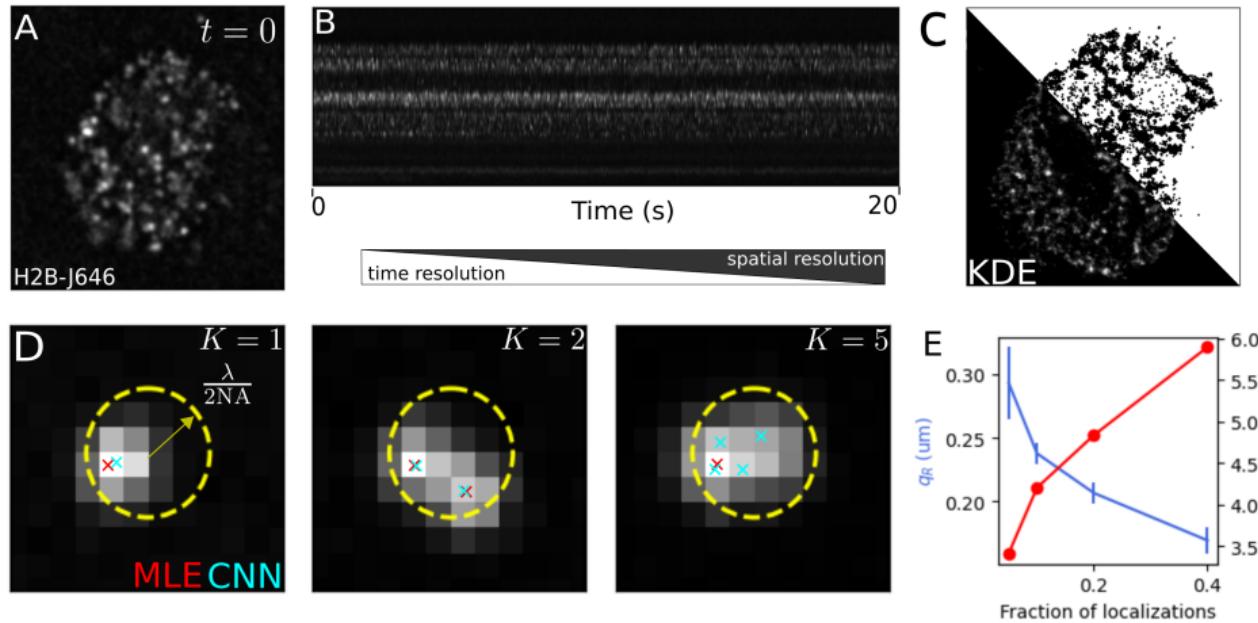


# Super resolution with photoswitching of rhodamines



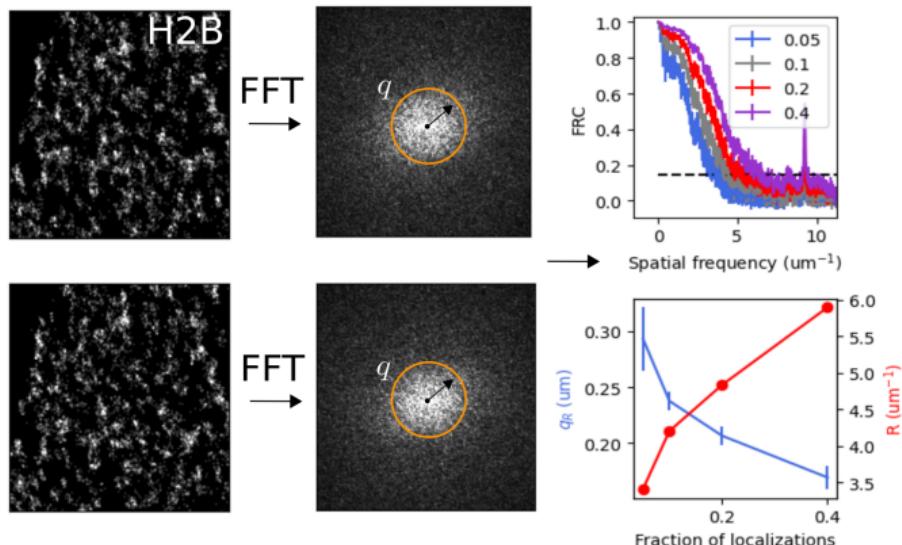
- ▶ Reduction of the T<sub>1</sub> state yields a dark, long-lived, and stable radical state

# Direct stochastic optical reconstruction microscopy



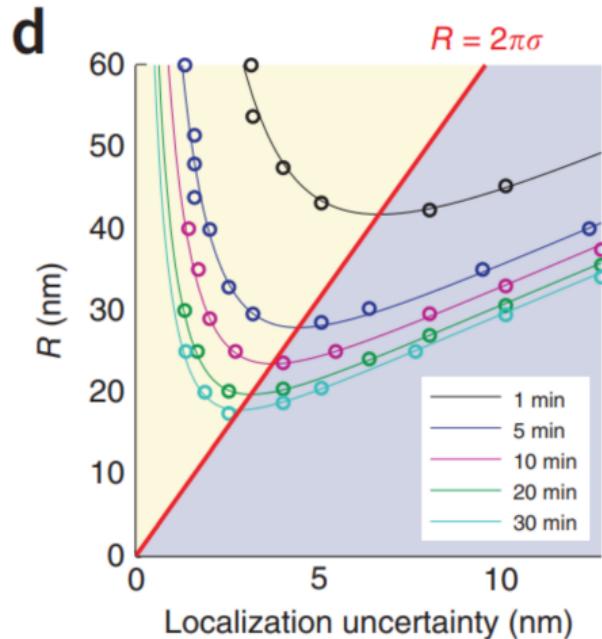
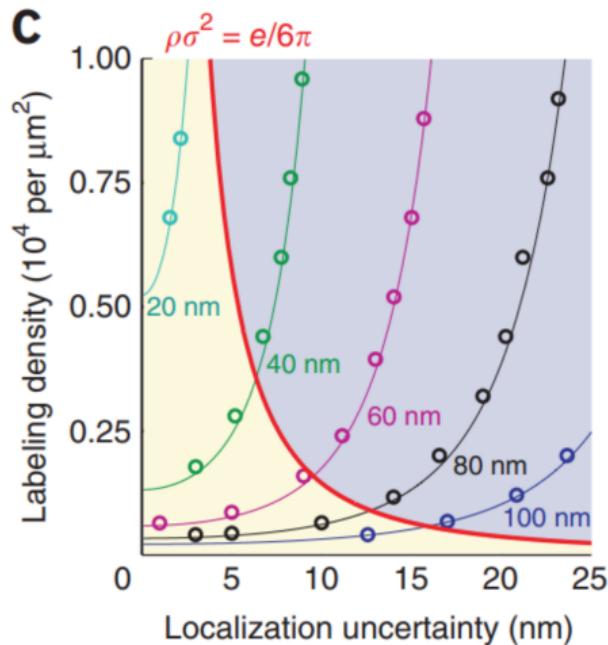
# Fourier ring correlation links spatial and temporal resolution

- 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

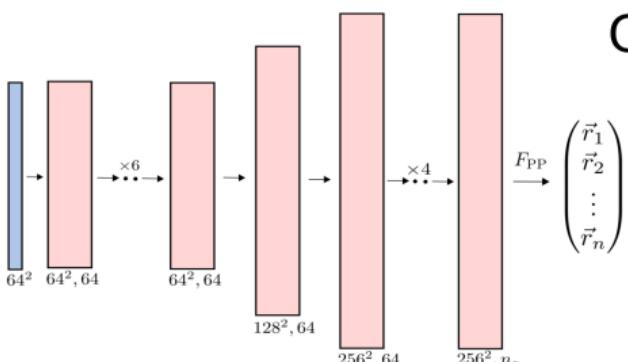


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

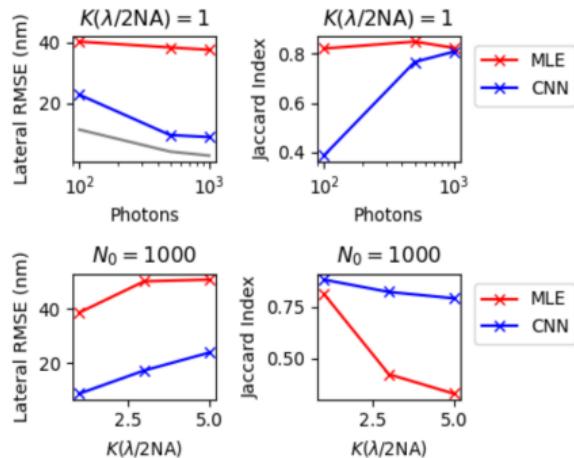
- ▶ Increased localization uncertainty requires higher density for same resolution
- ▶ Longer acquisitions have higher resolution

# Estimator precision sets the resolution limit in localization microscopy

B

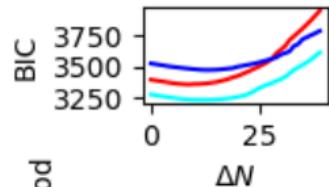
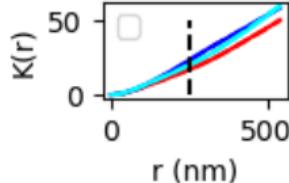
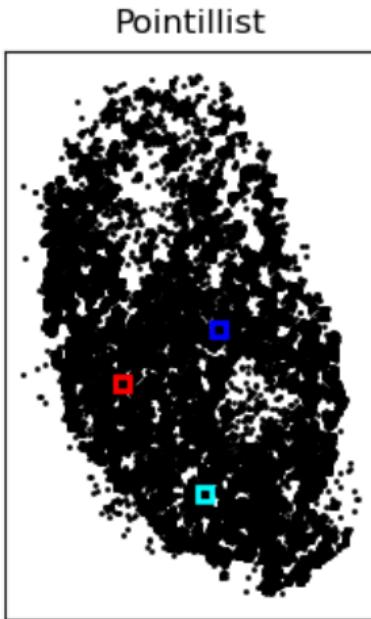
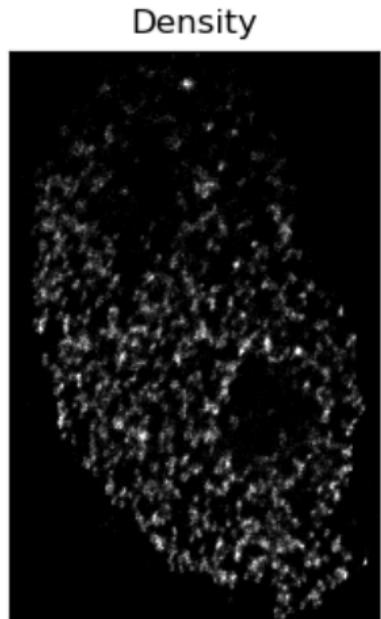


C

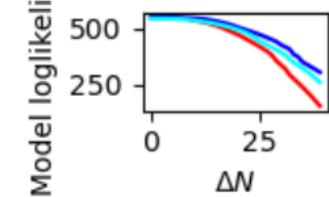


- ▶  $K(\lambda/2NA)$  is Ripley's K function at the diffraction limit ( $\lambda = 640\text{nm}$ )
- ▶ Convolutional neural networks (CNNs) approach the Cramer-Rao lower bound (gray)

# Chromatin nanodomains in a living Hela cell nucleus



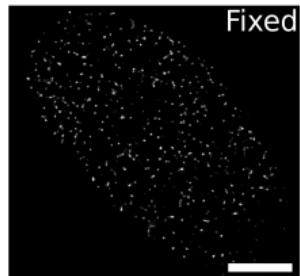
Model loglikelihood



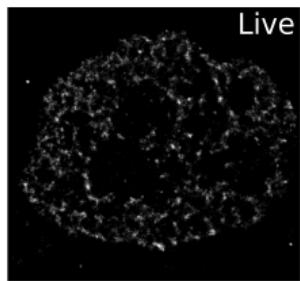
- ▶ Histone DE using 30x30nm bins
- ▶ Likelihood is computed under a Gaussian Mixture Model (GMM)

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

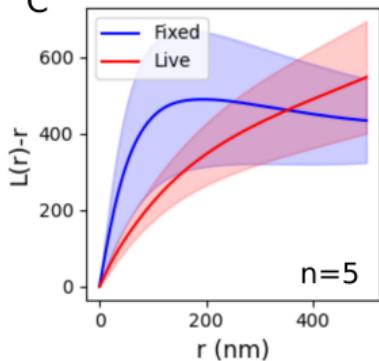
A



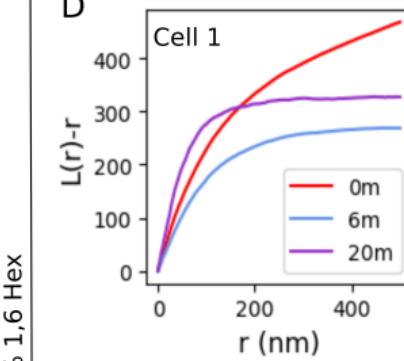
B



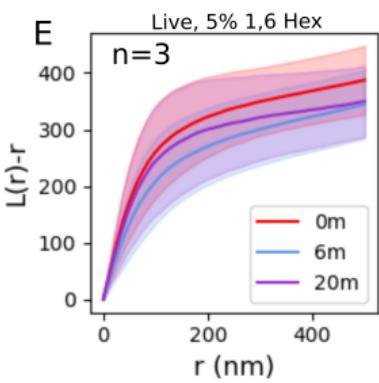
C



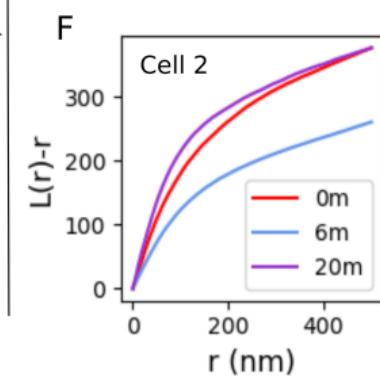
D



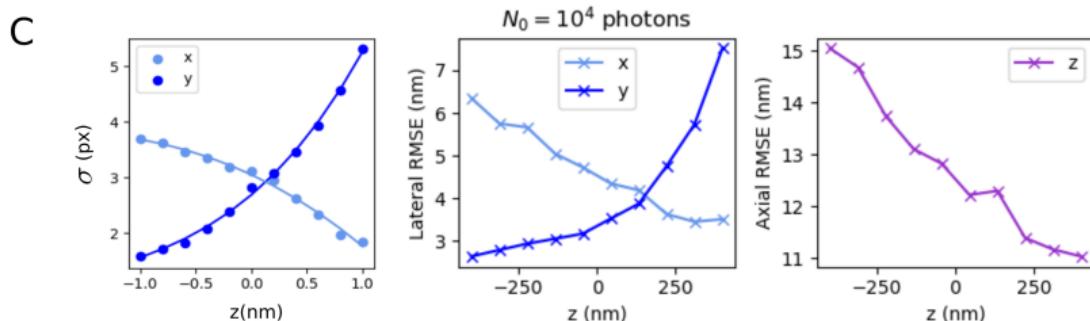
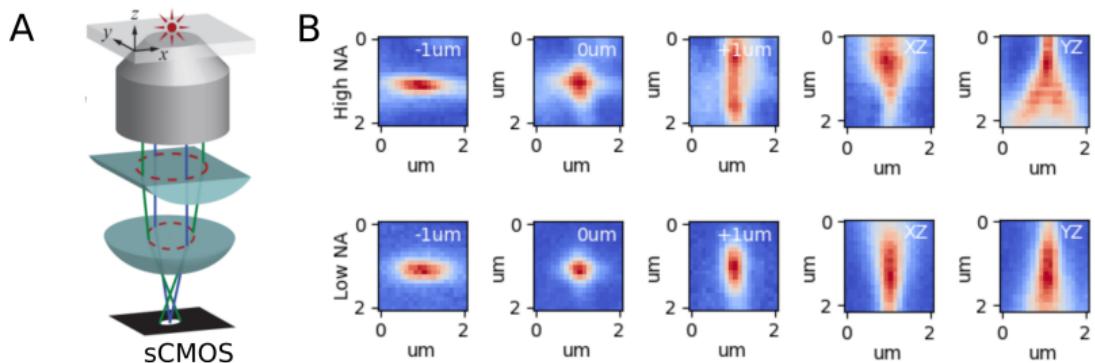
E



F

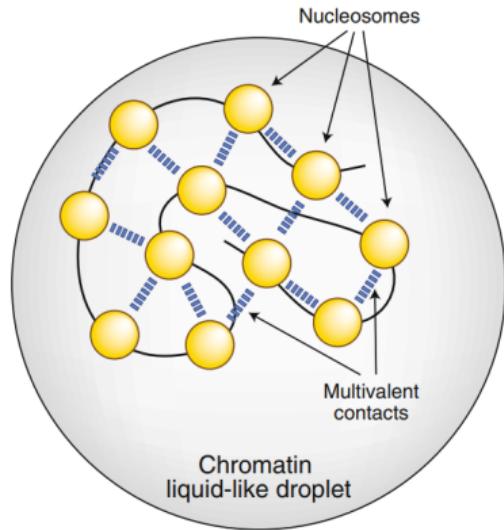


## Astigmatism based three dimensional imaging



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

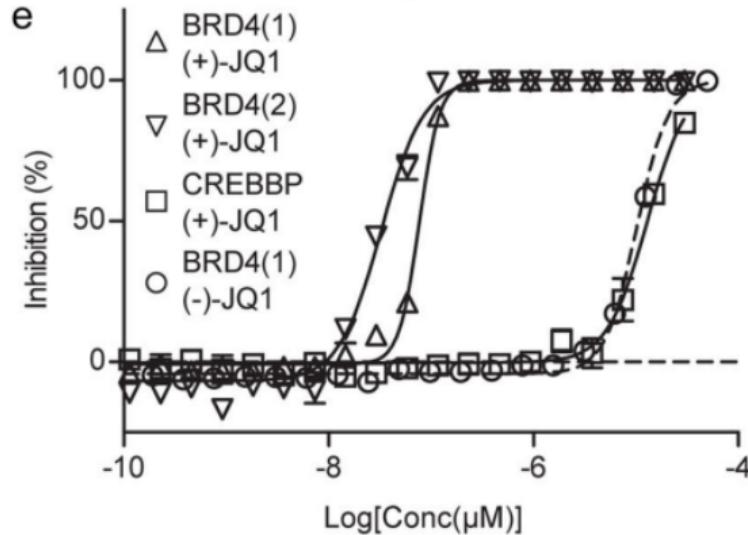
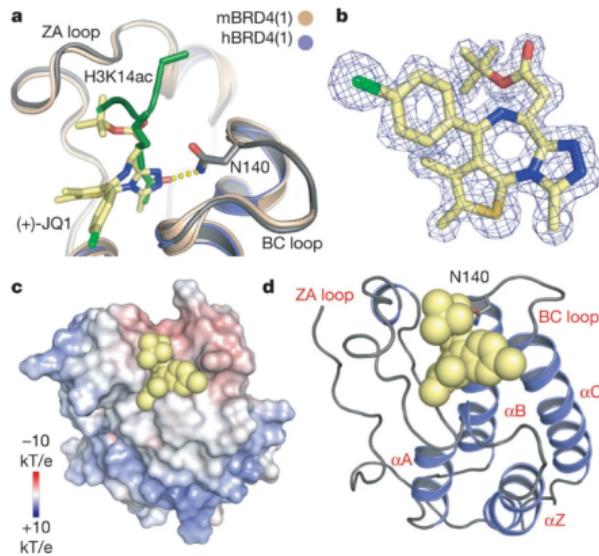
# Chromatin has an intrinsic ability to undergo phase separation



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

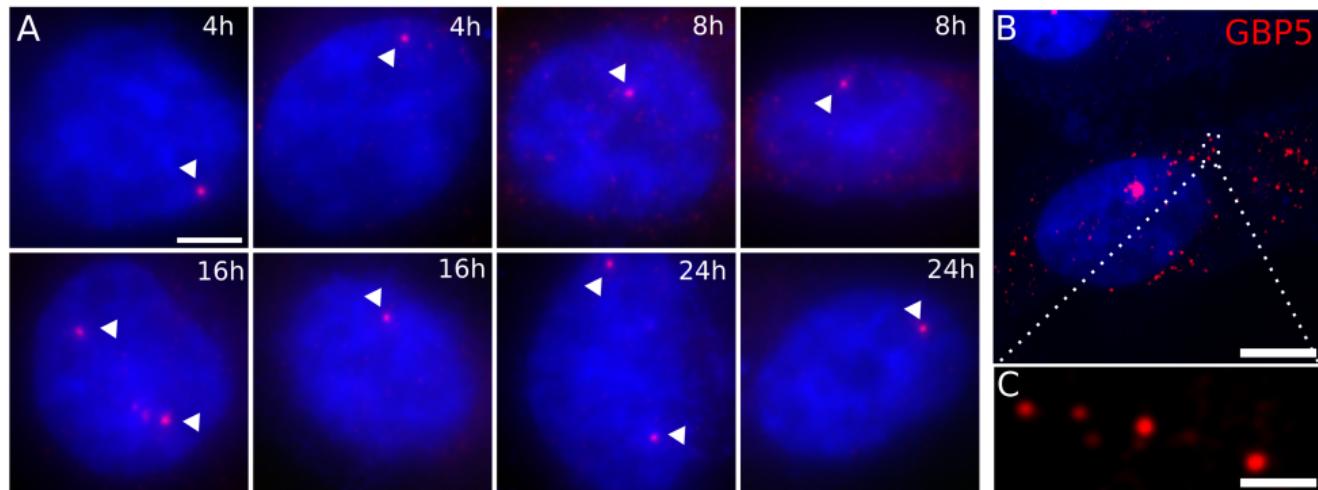
# (+)-JQ1 in complex with BRD4 protein



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

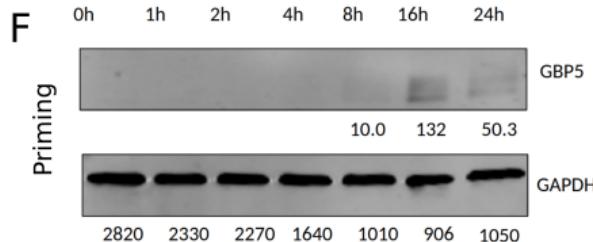
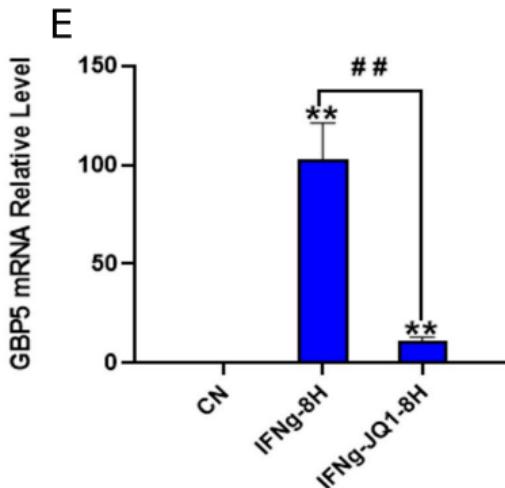
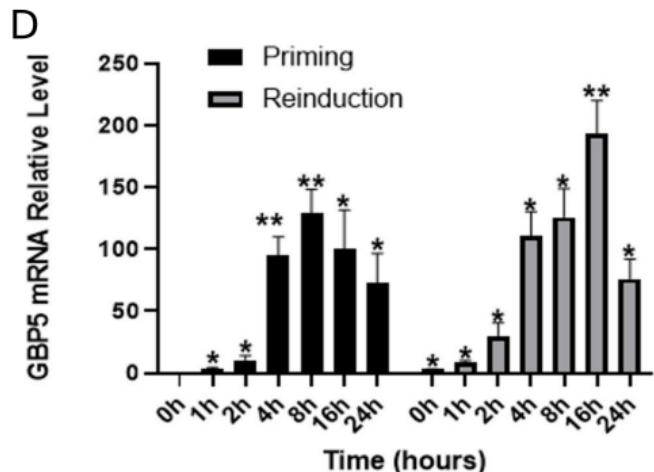
# Inhibition of a super-enhanced gene with JQ1



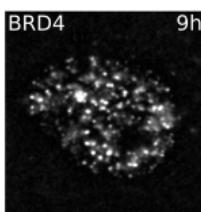
Blue - DAPI (binds DNA minor groove)

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

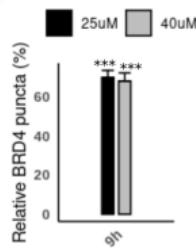
# Inhibition of a super-enhanced gene with JQ1



G



H



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