

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

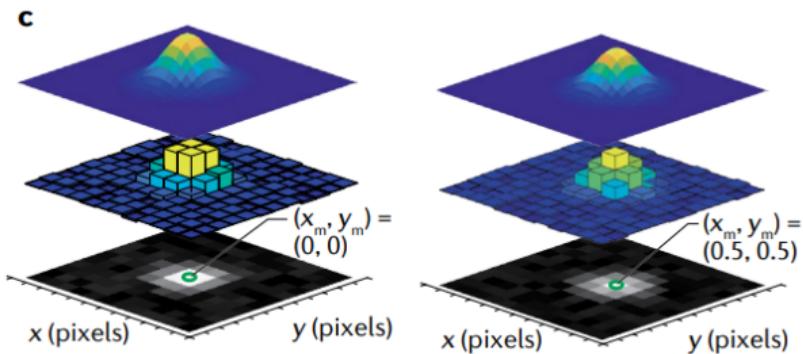
Phase separation of chromatin

Single molecule localization microscopy

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

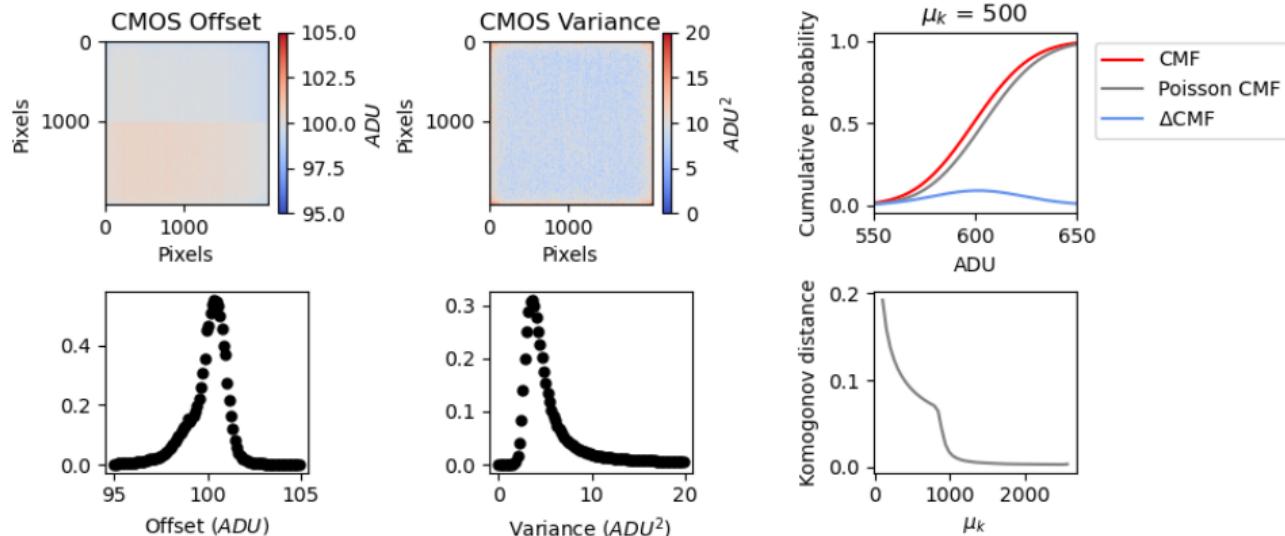
$$\mu_k = g_k \eta N_0 \Delta \int_{\text{pixel}} G(x, y) dA$$

η – quantum efficiency
 N_0 – photon count
 Δ – exposure time



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

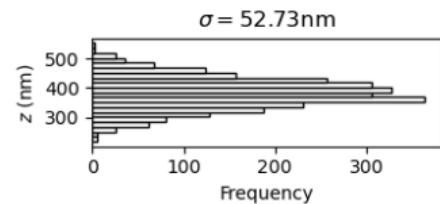
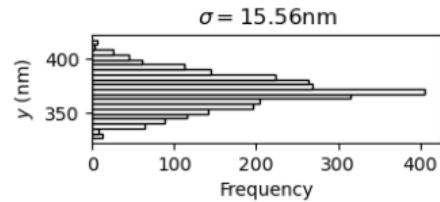
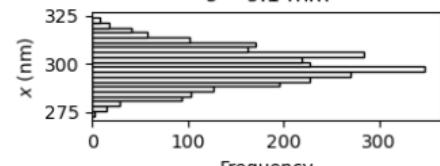
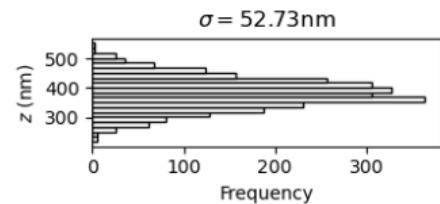
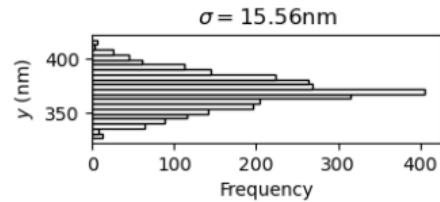
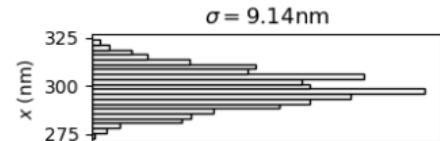
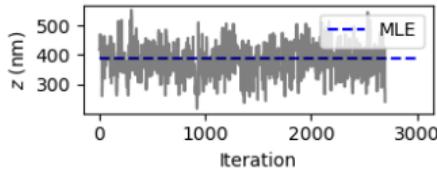
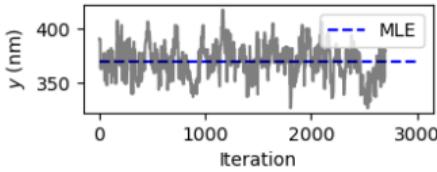
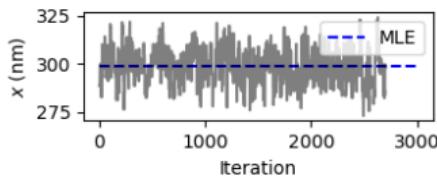
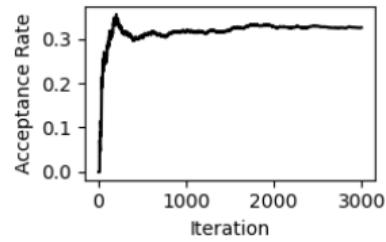
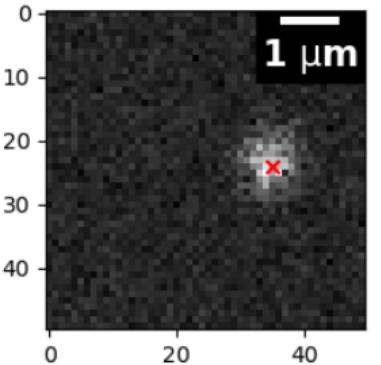
A Poisson approximation at moderate SNR simplifies SMLM



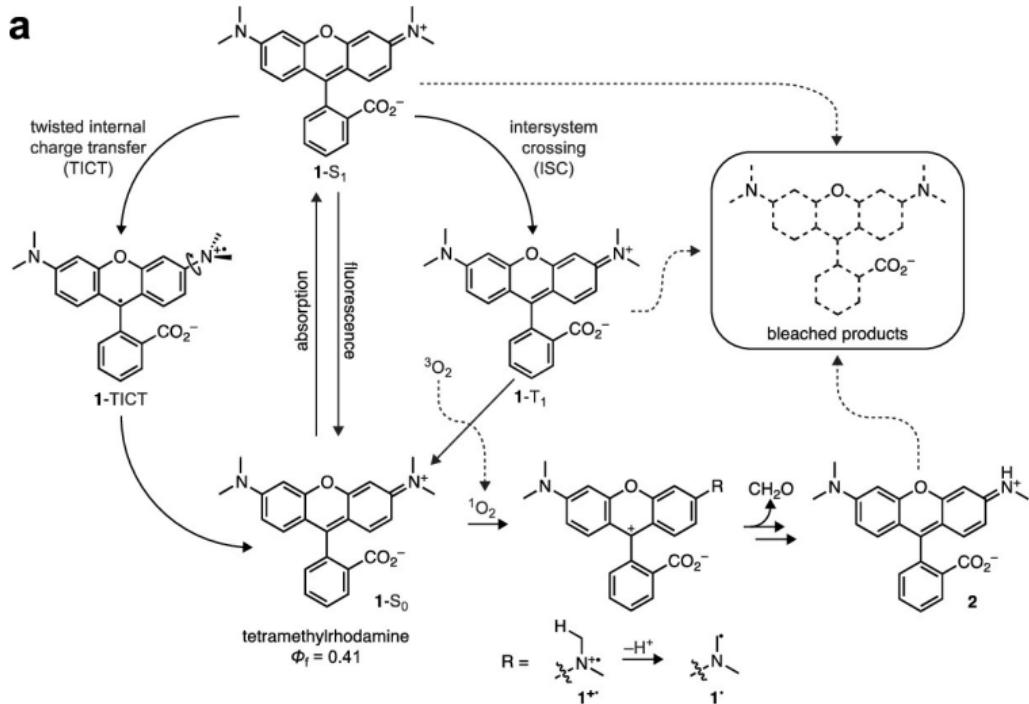
$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi\sigma_k^2}} e^{-\frac{(H_k - g_k q - \sigma_k)^2}{2\sigma_k^2}}$$

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

Estimator precision in localization microscopy

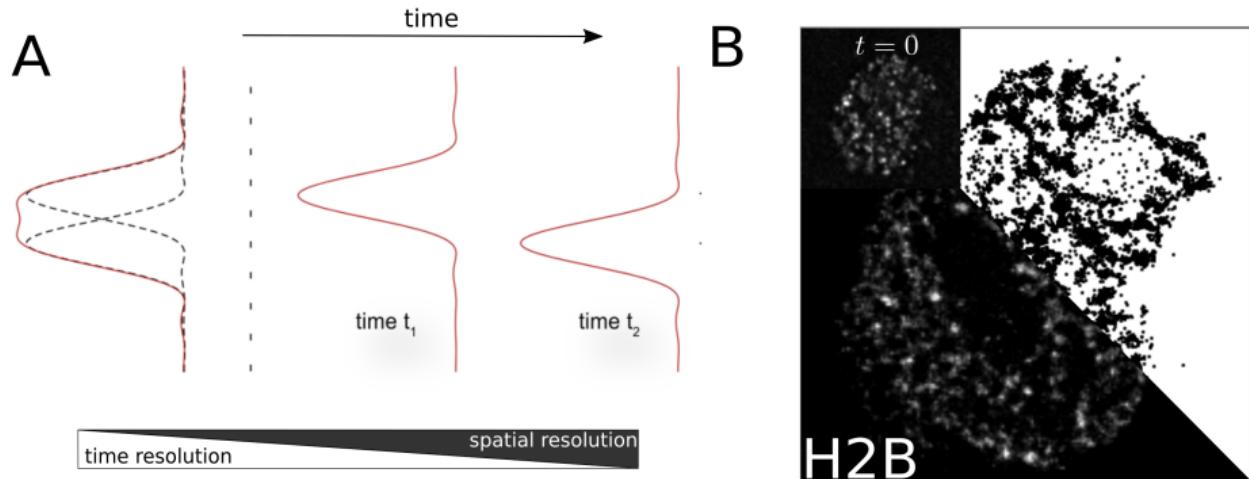


Super resolution with photoswitching of rhodamines



- ▶ Reduction of the T₁ state yields a dark, long-lived, and stable radical state

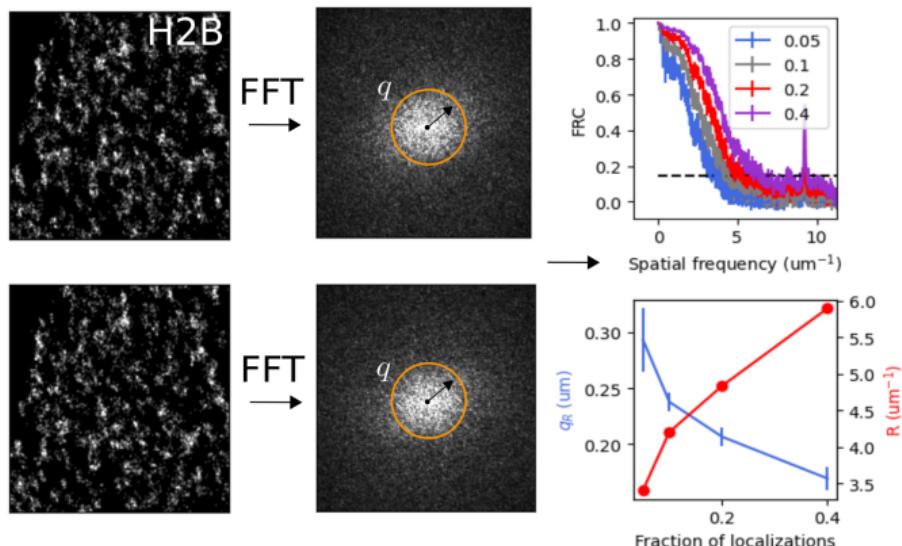
Direct stochastic optical reconstruction microscopy



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

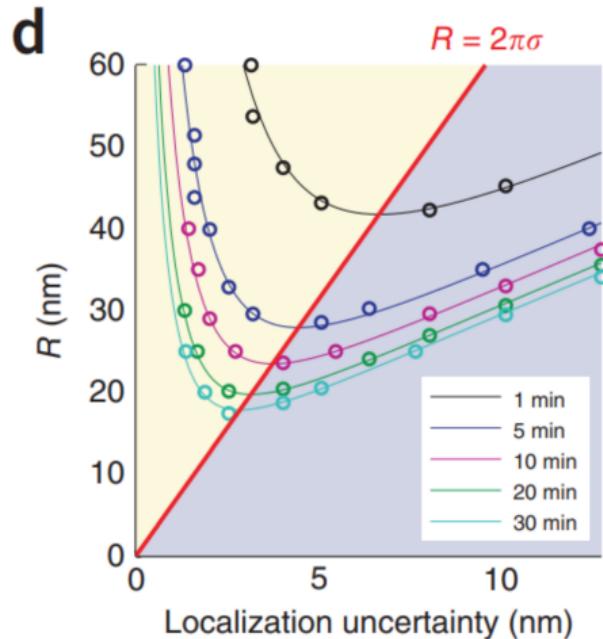
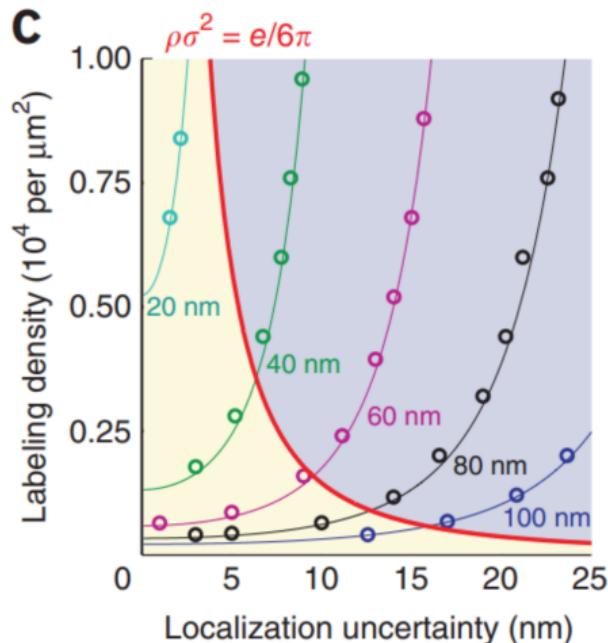
Fourier ring correlation links spatial and temporal resolution

- 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

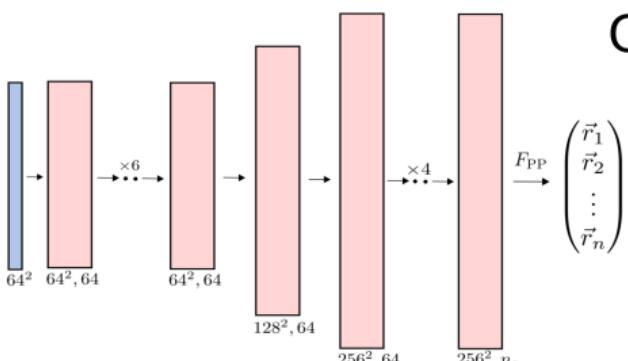


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

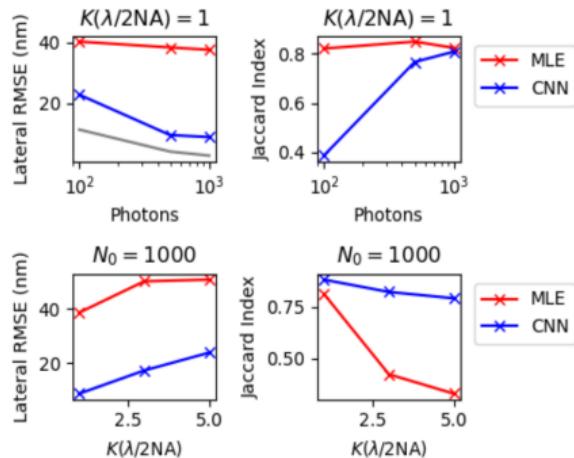
- ▶ Increased localization uncertainty requires higher density for same resolution
- ▶ Longer acquisitions have higher resolution

Estimator precision sets the resolution limit in localization microscopy

B

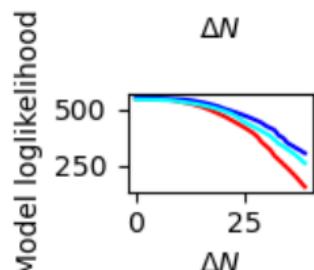
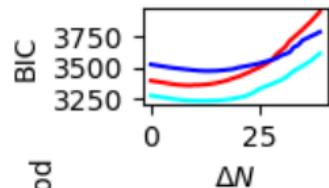
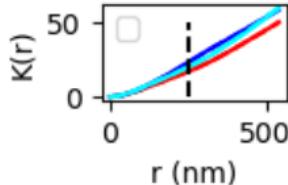
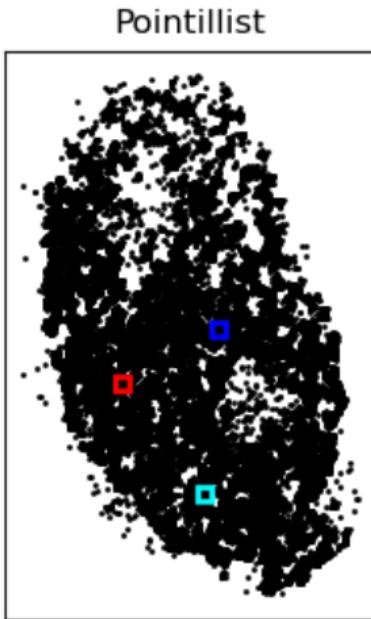
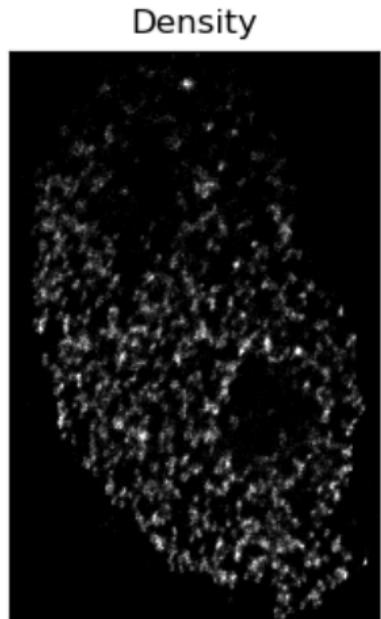


C



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

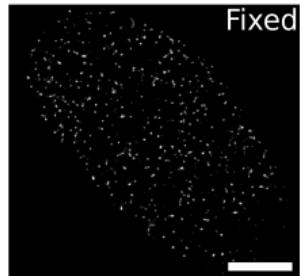
Chromatin nanodomains in a living Hela cell nucleus



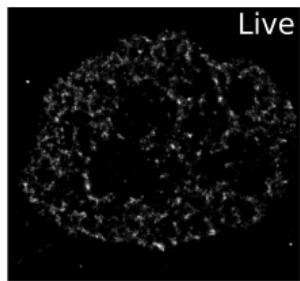
- ▶ Histone DE using 30x30nm bins
- ▶ Likelihood is computed under a Gaussian Mixture Model (GMM)

Chemical fixation and LLPS inhibitors change the appearance of nucleosome clustering

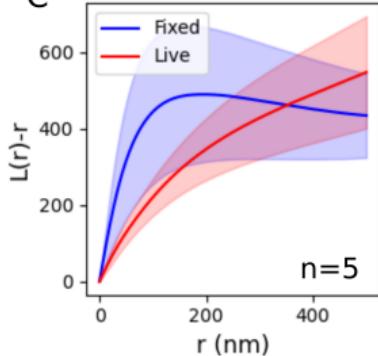
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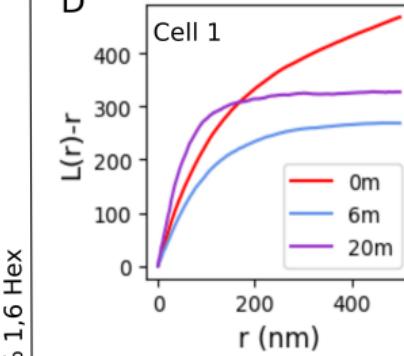
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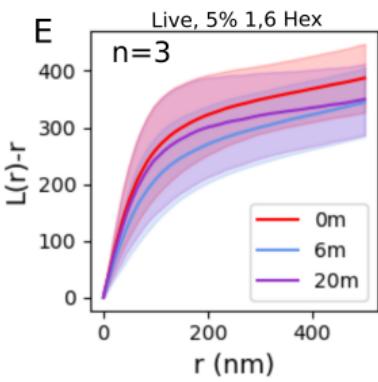
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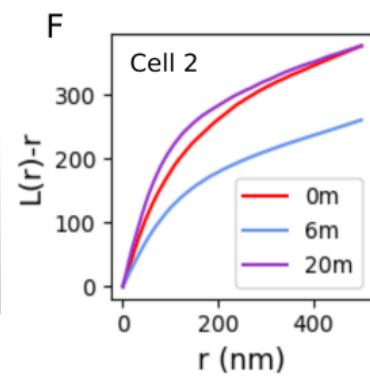
D



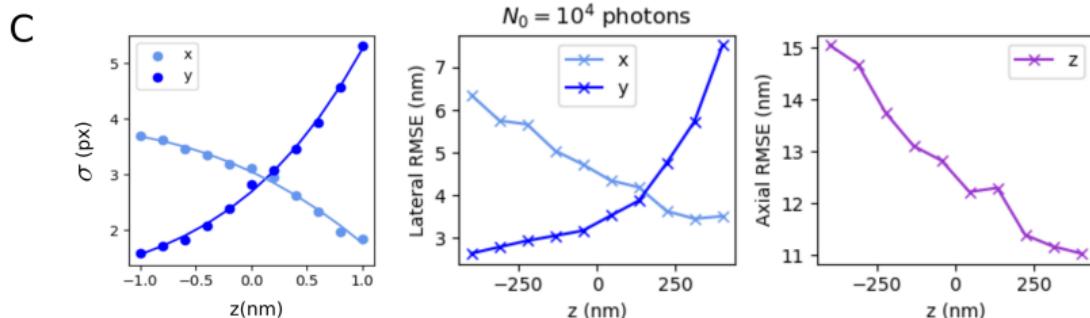
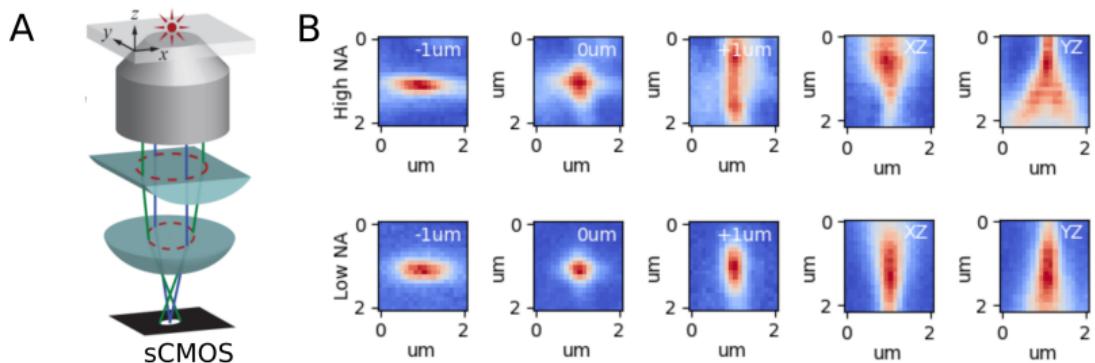
E



F

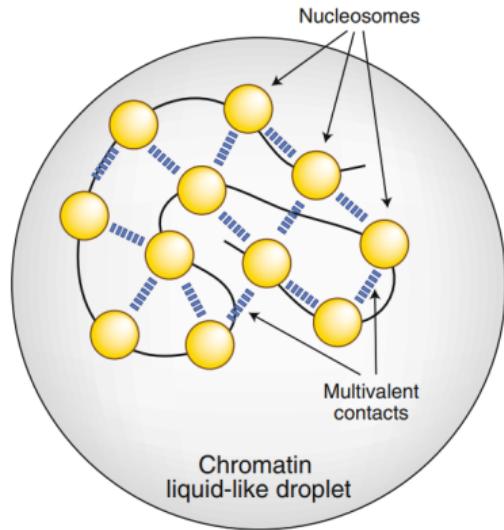


Astigmatism based three dimensional imaging



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

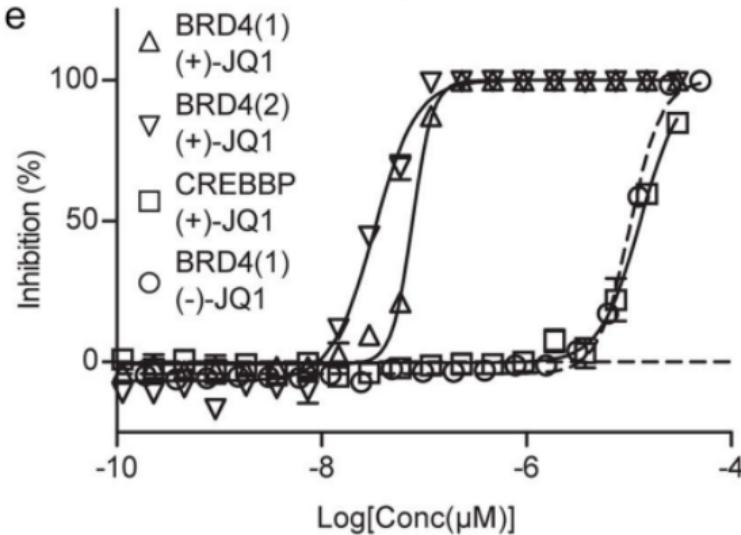
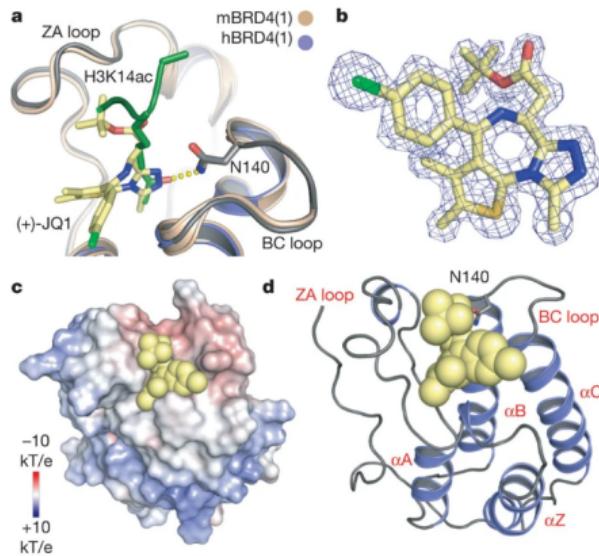
Chromatin has an intrinsic ability to undergo phase separation



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

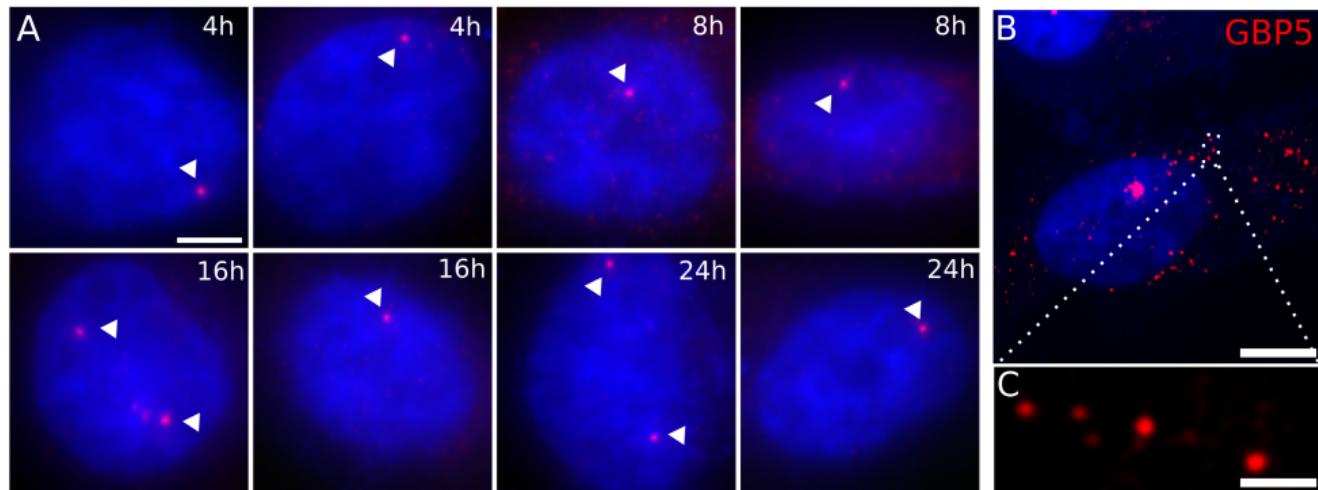
(+)-JQ1 in complex with BRD4 protein



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

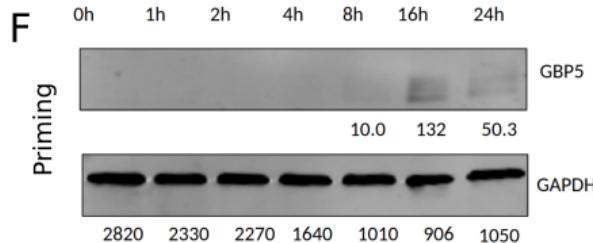
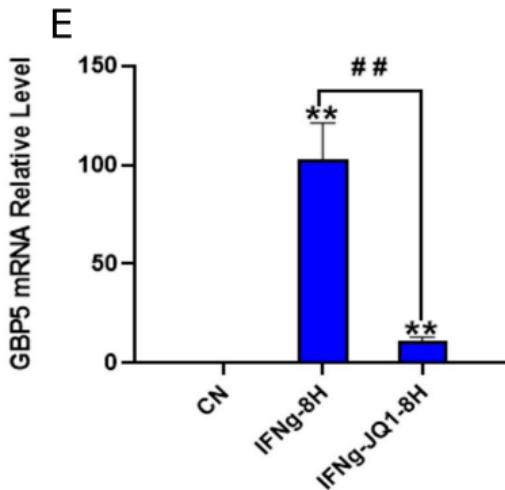
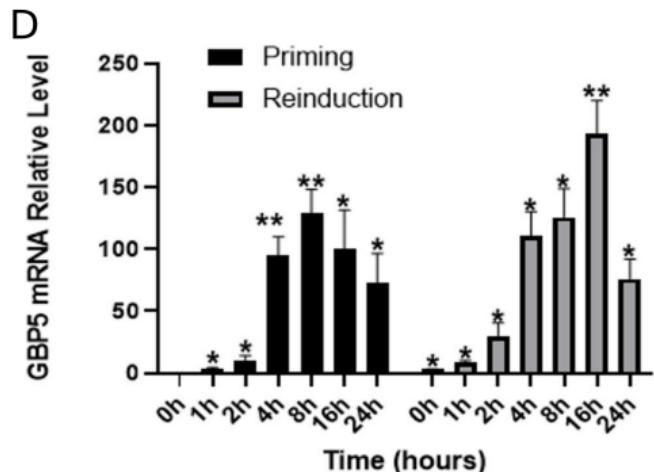
Inhibition of a super-enhanced gene with JQ1



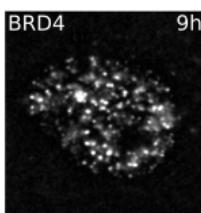
Blue - DAPI (binds DNA minor groove)

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

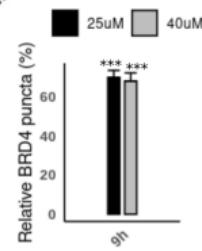
Inhibition of a super-enhanced gene with JQ1



G



H



► *: $P \leq 0.1$, **: $P \leq 0.01$