

Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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Outline

Super resolution imaging with *d*STORM

The time resolution of *d*STORM

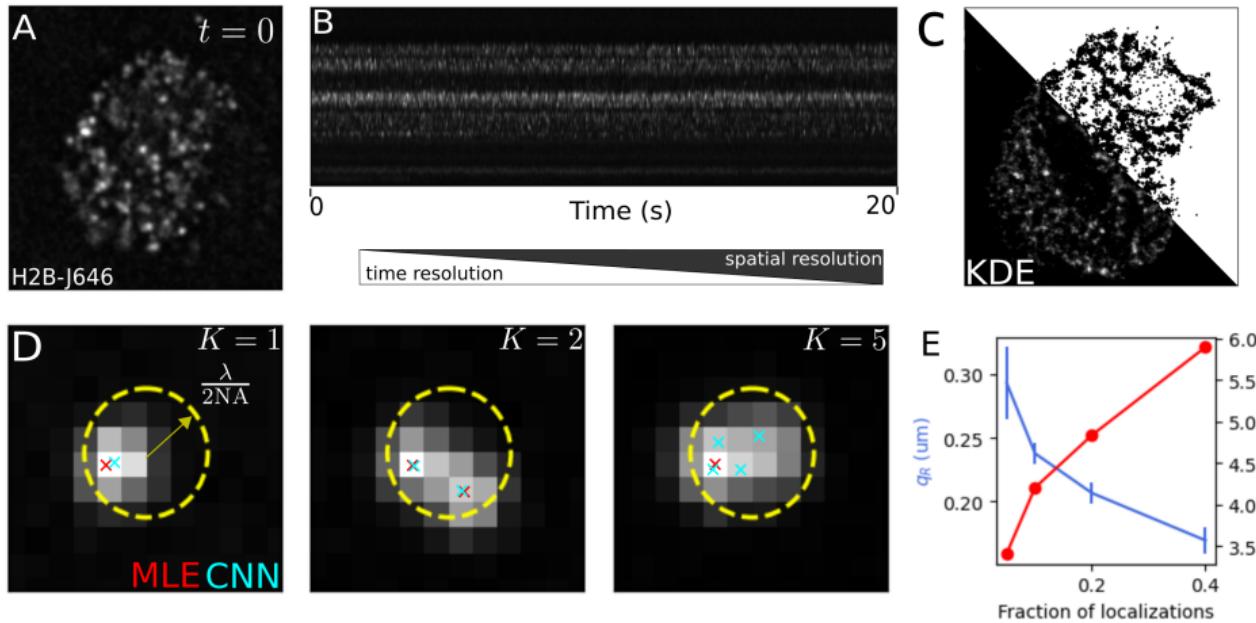
Dense localization with deep learning

The nucleosome as a Brownian harmonic oscillator

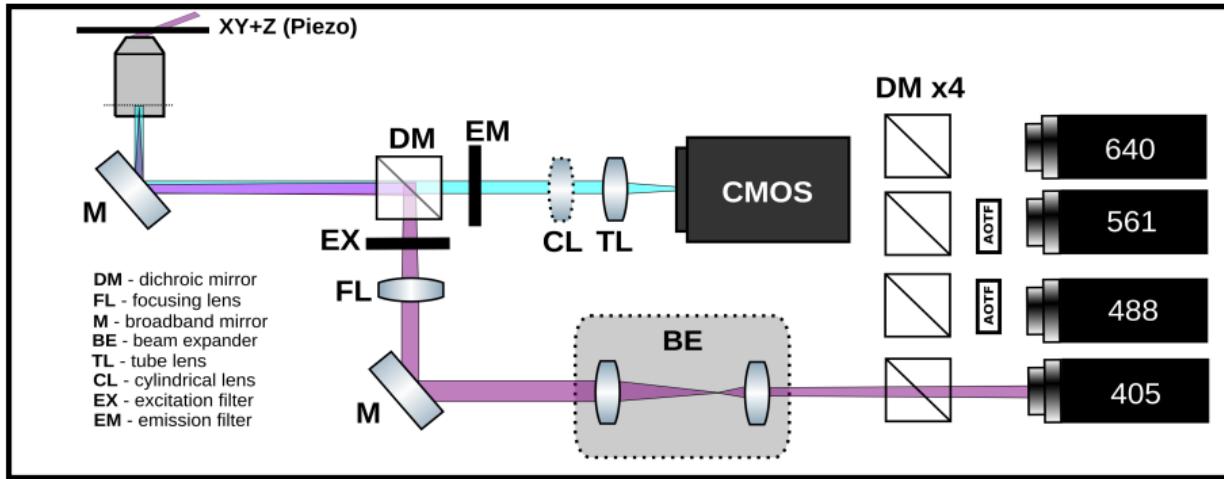
Results and future aims

Phase separation of chromatin

Direct stochastic optical reconstruction microscopy

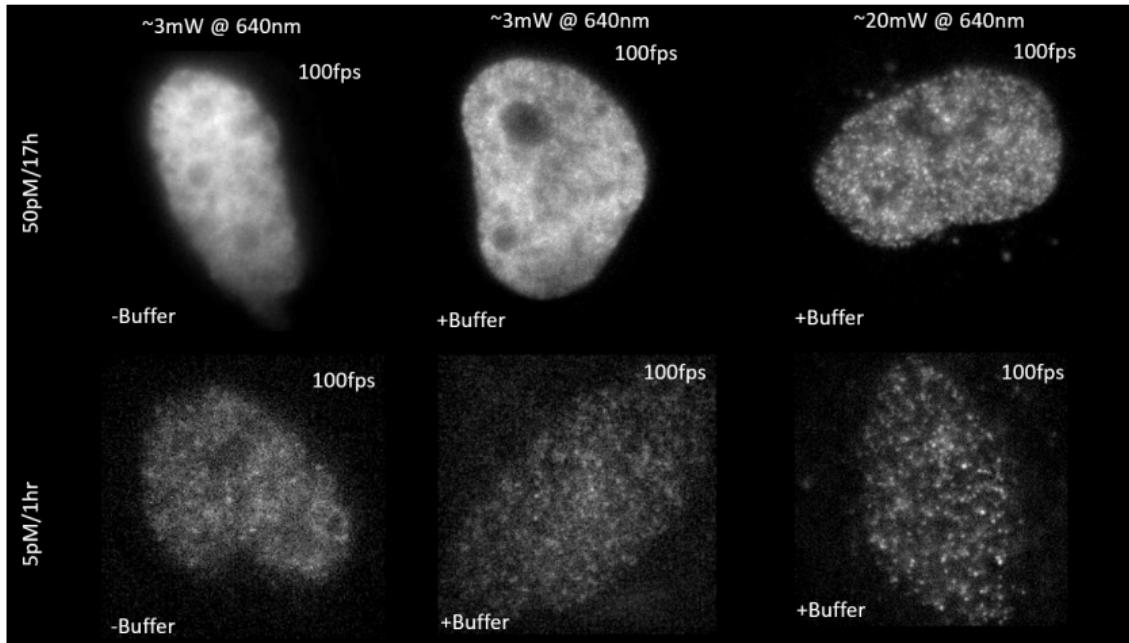


Instrumentation for single molecule localization microscopy



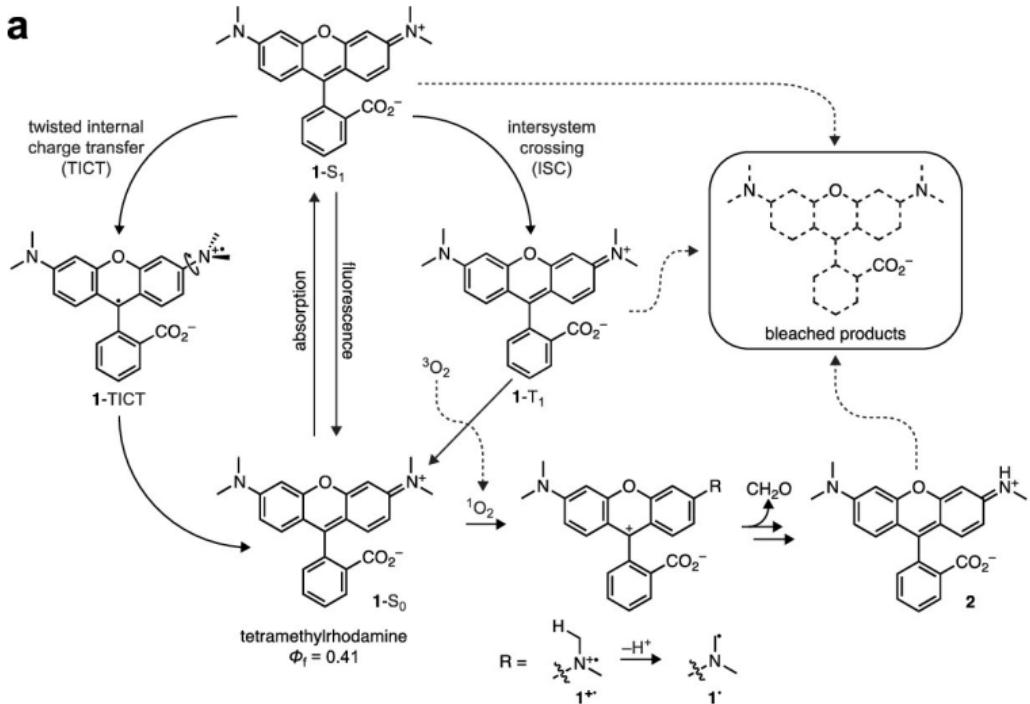
- ▶ Selectable widefield and oblique illumination
- ▶ Widefield useful for high throughput
- ▶ Oblique method illuminates a thin section of nuclei

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 STORM: The photophysics of rhodamines



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

Maximum likelihood localization of an isolated fluorescent emitter

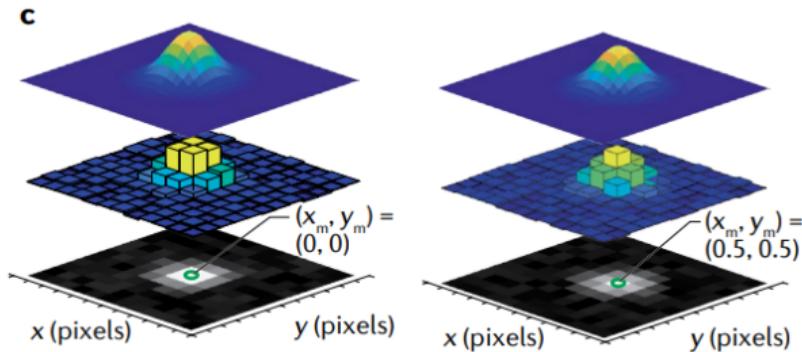
$$\text{Localization: } \theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$$

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

η – quantum efficiency

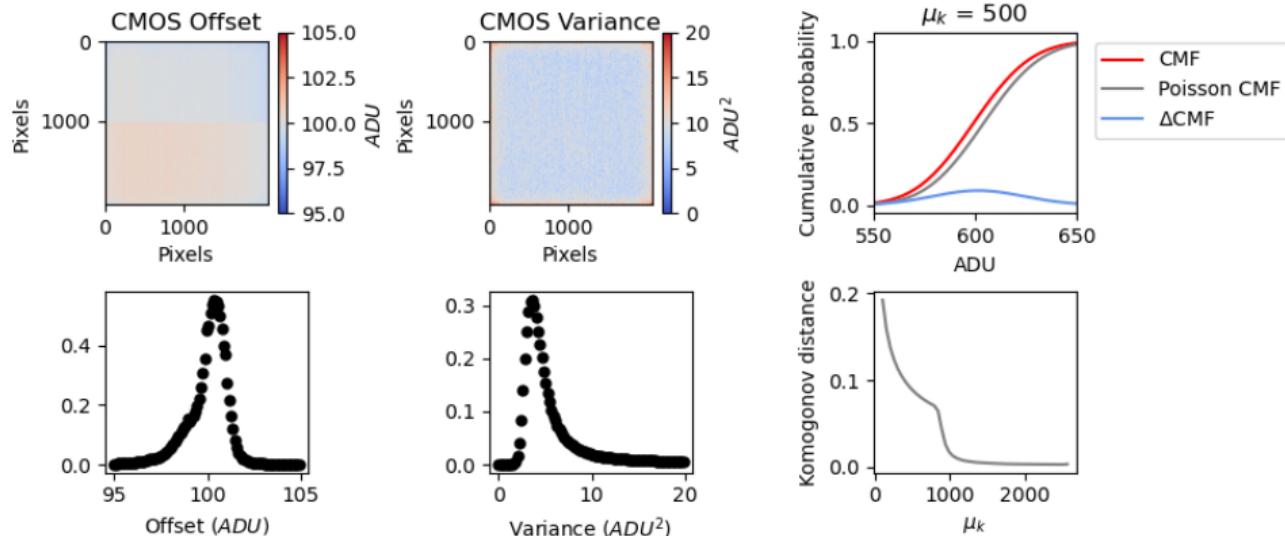
N_0 – photon count

Δ – exposure time



- Likelihood function can be directly optimized with gradient descent

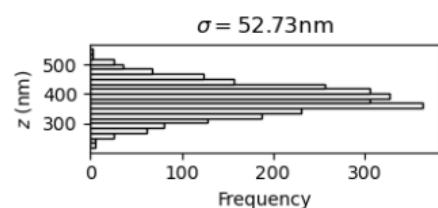
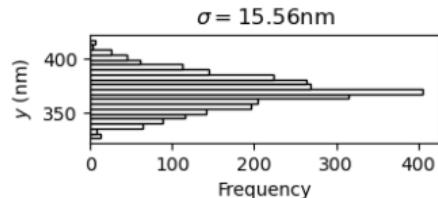
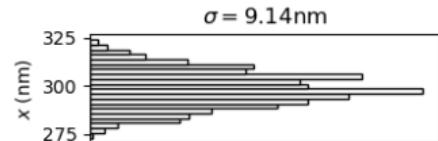
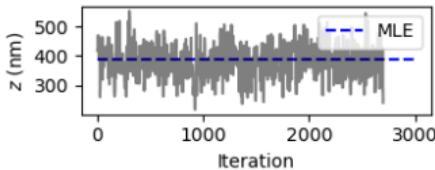
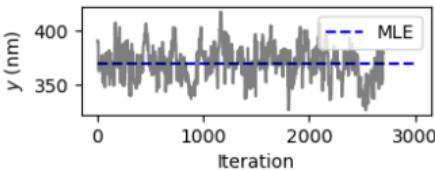
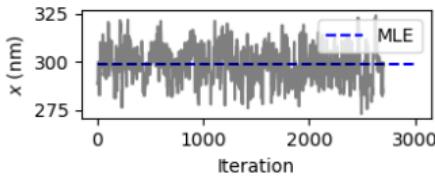
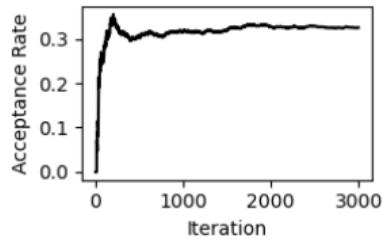
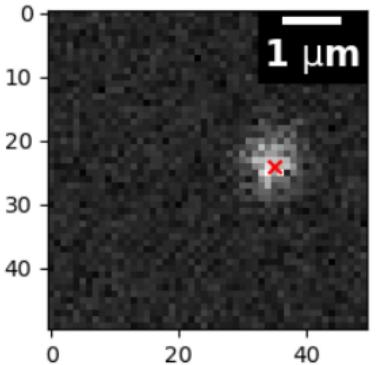
A Poisson approximation at moderate SNR simplifies SMLM



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

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

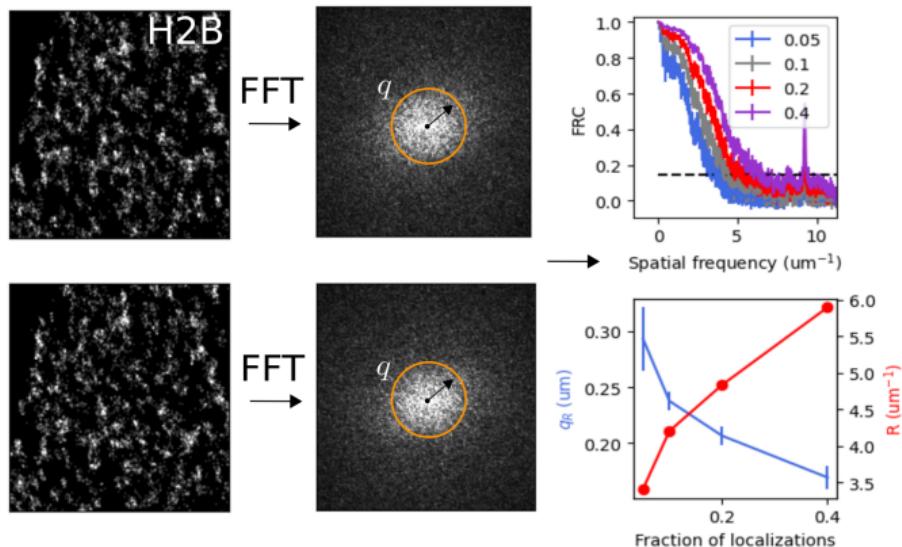
Estimator precision in localization microscopy



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

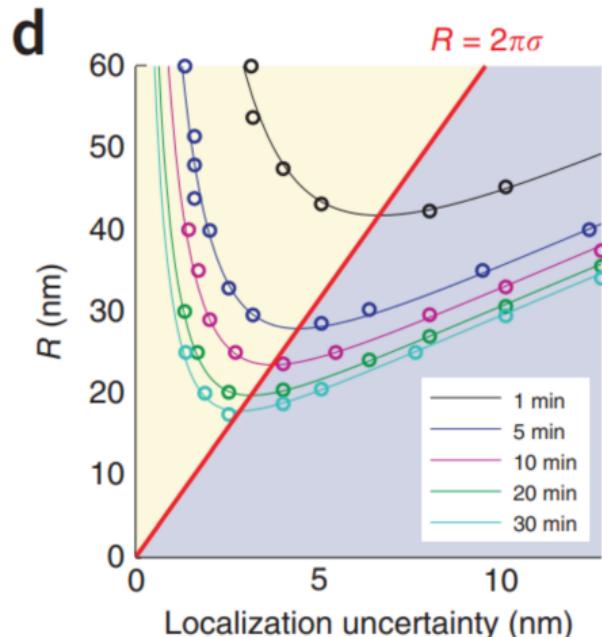
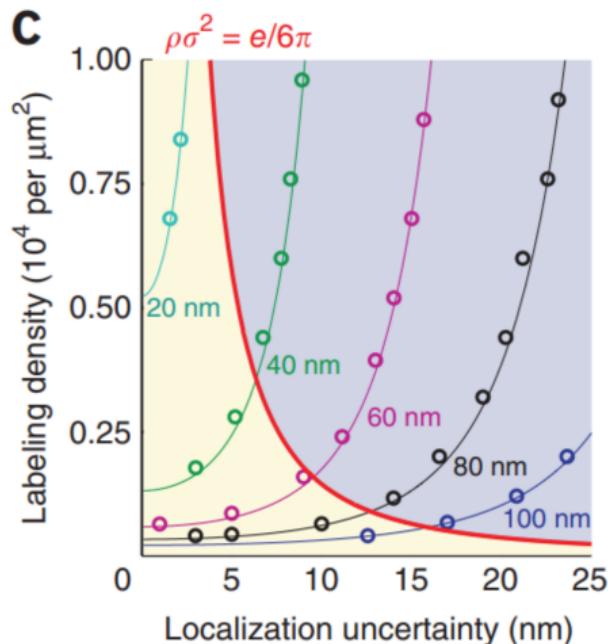
Fourier ring correlation links spatial and temporal resolution

- 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

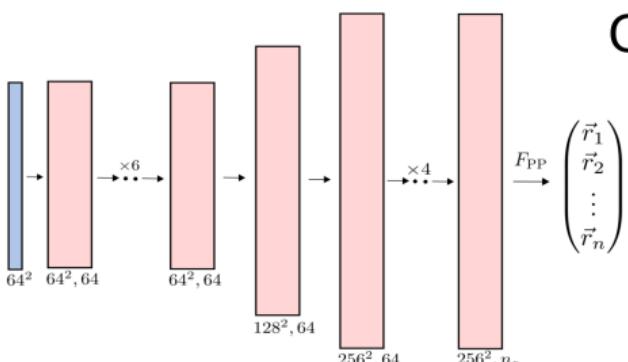


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

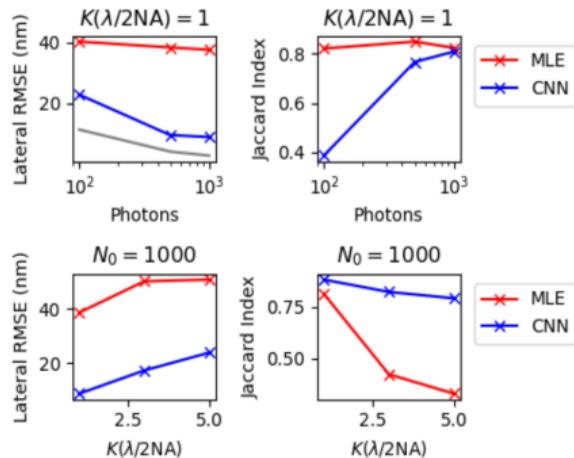
- ▶ Increased localization uncertainty requires higher density for same resolution
- ▶ Longer acquisitions have higher resolution

Estimator precision sets the resolution limit in localization microscopy

B



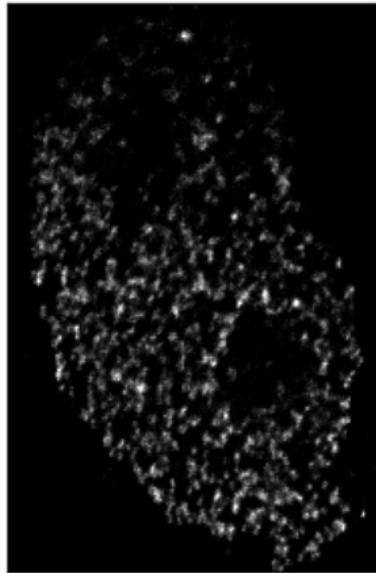
C



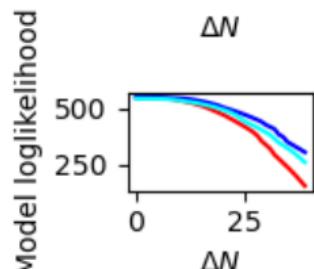
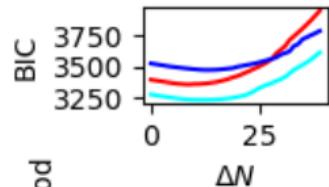
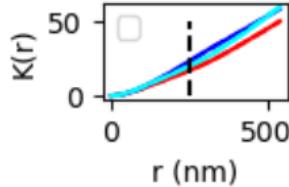
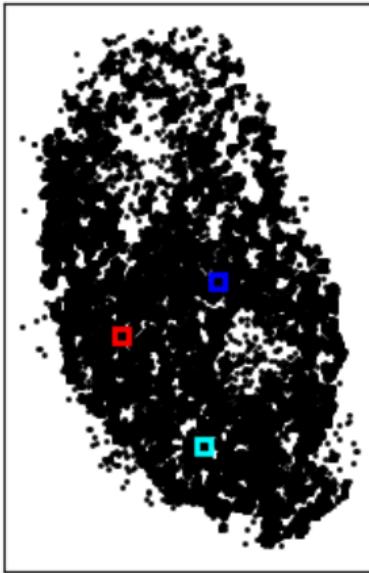
- ▶ $K(\lambda/2NA)$ is Ripley's K function at the diffraction limit ($\lambda = 640\text{nm}$)
- ▶ Convolutional neural networks (CNNs) approach the Cramer-Rao lower bound (gray)

Chromatin nanodomains in a living Hela cell nucleus

Density



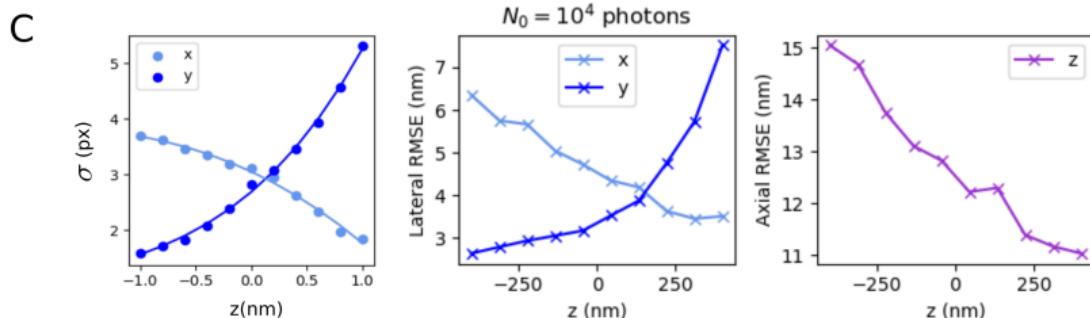
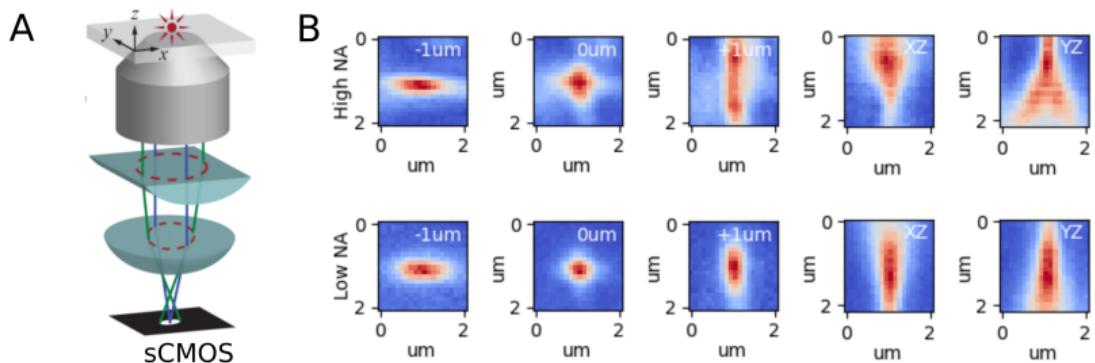
Pointillist



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

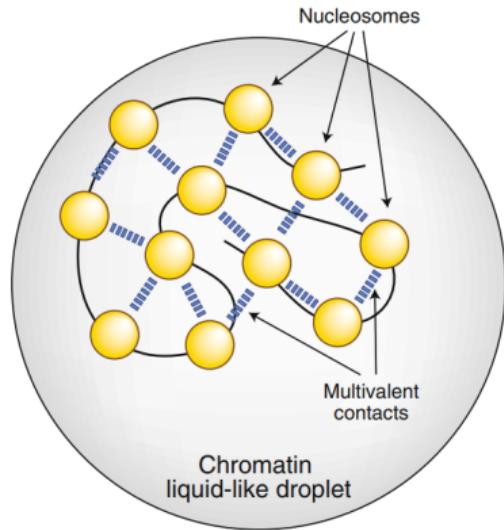
Chemical fixation changes nucleosome clustering

Astigmatism based three dimensional imaging



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

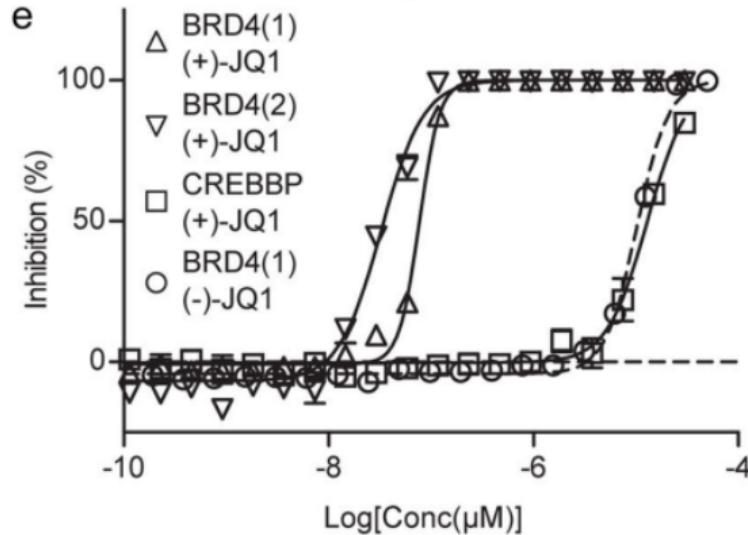
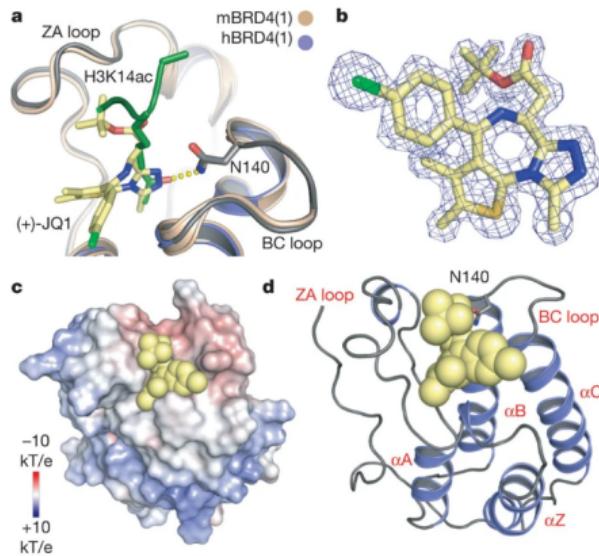
Chromatin has an intrinsic ability to undergo phase separation



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

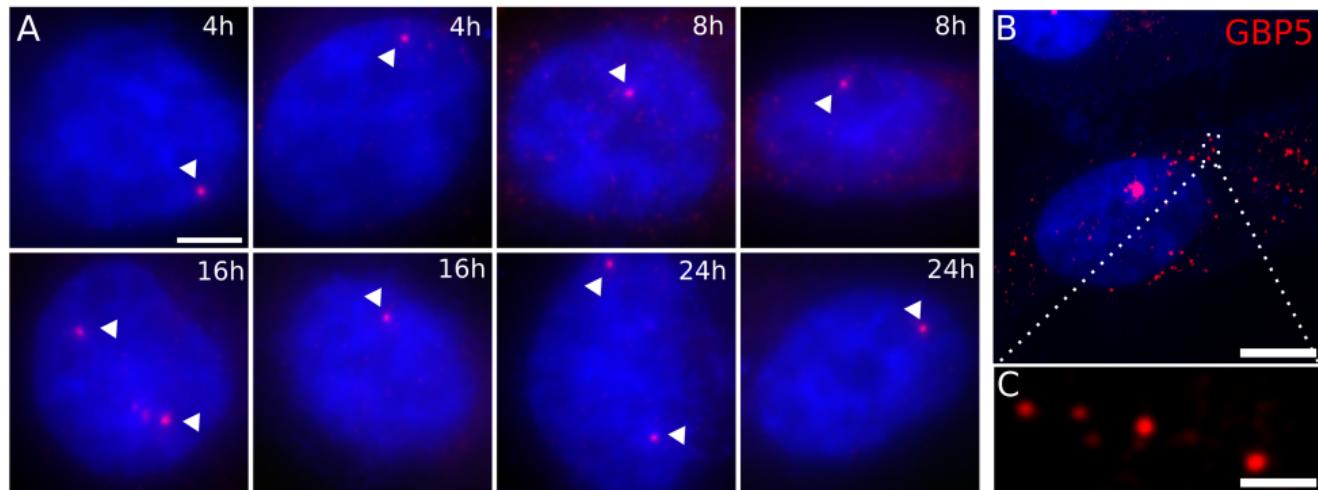
(+)-JQ1 in complex with BRD4 protein



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

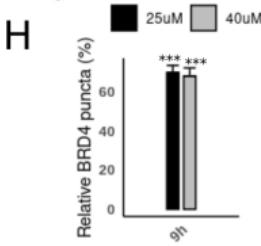
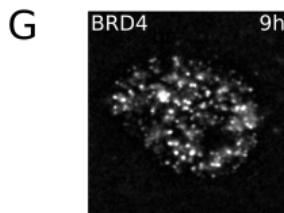
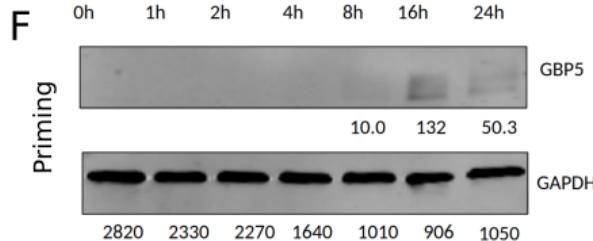
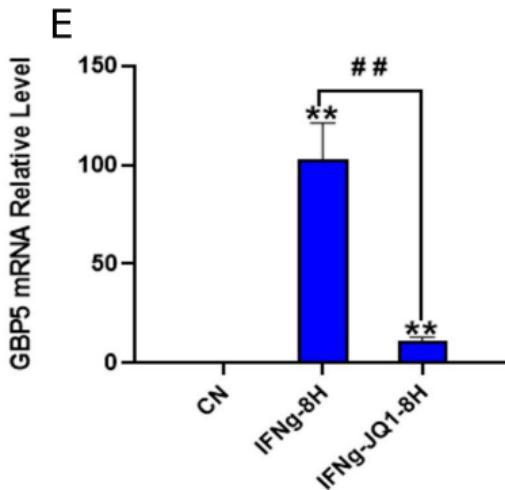
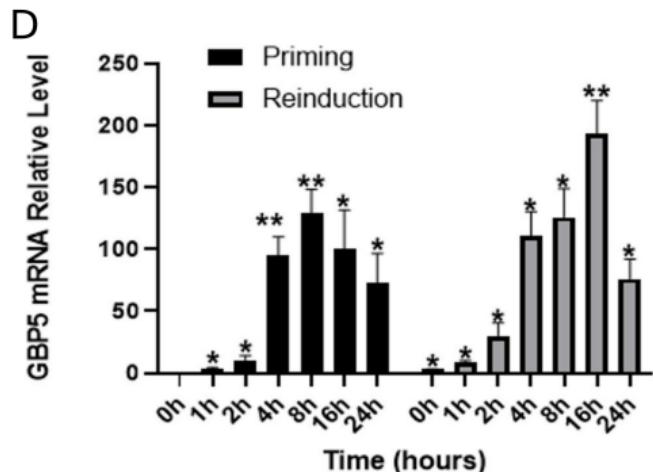
Inhibition of a super-enhanced gene with JQ1



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



► *: $P \leq 0.1$, **: $P \leq 0.01$