

Visualizing chromatin organization with time resolved 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

Single molecule localization microscopy

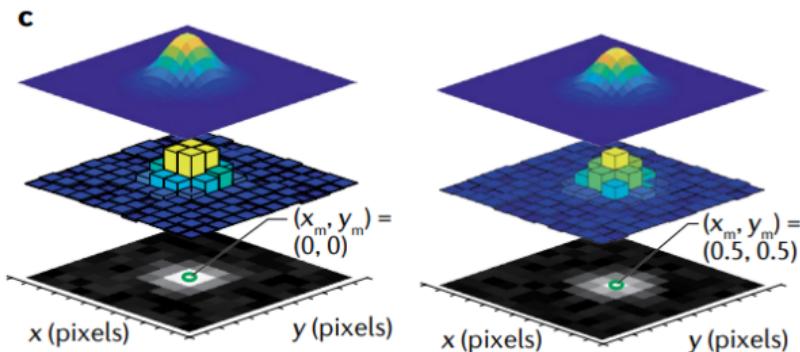
$$\text{Localization: } \theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$$

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

η – quantum efficiency

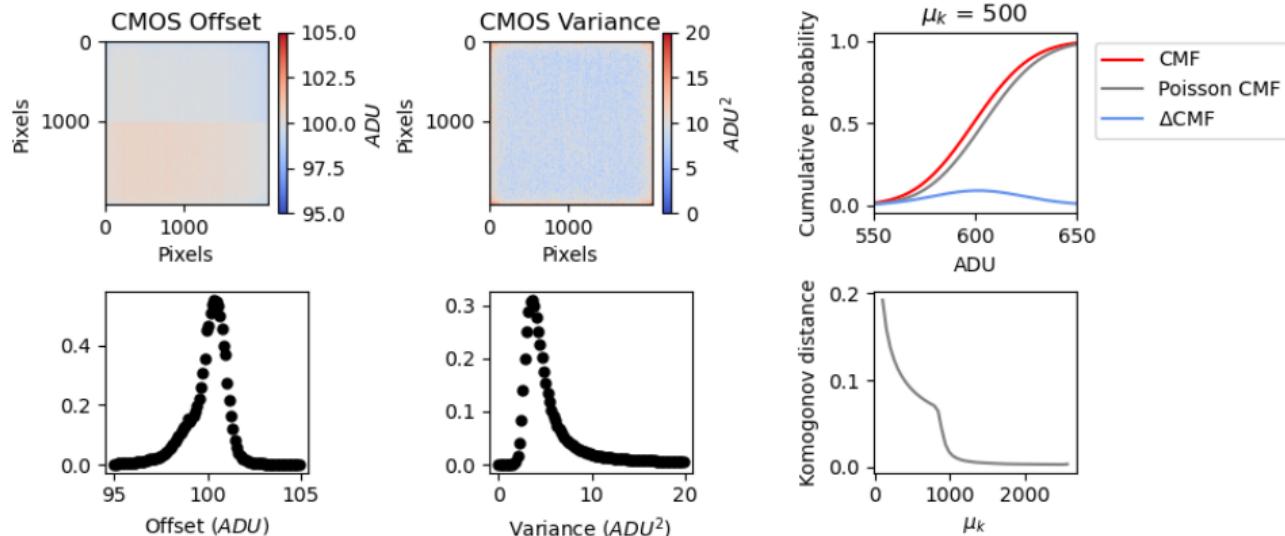
N_0 – photon count

Δ – exposure time



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

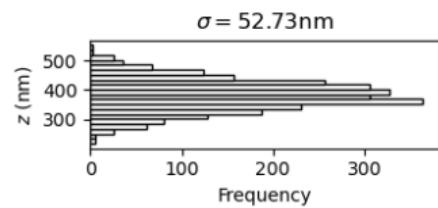
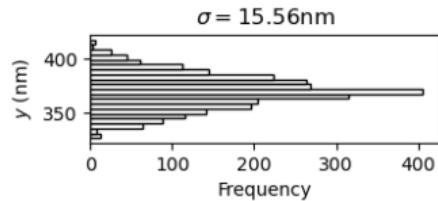
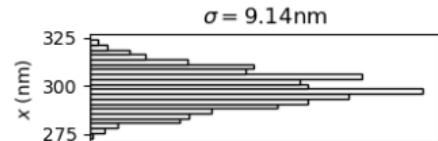
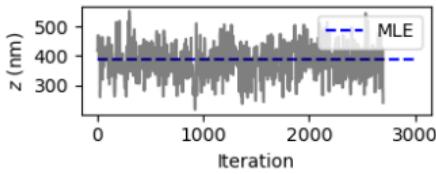
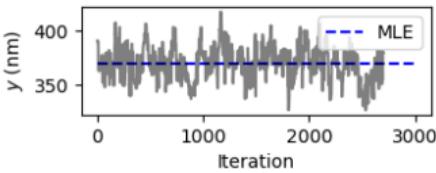
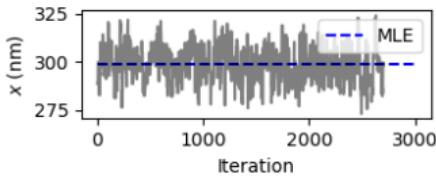
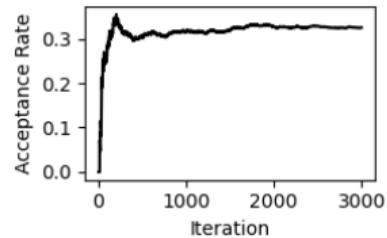
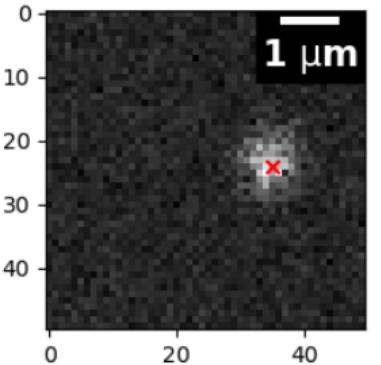
A Poisson approximation at moderate SNR simplifies SMLM



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

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

Estimator precision in localization microscopy

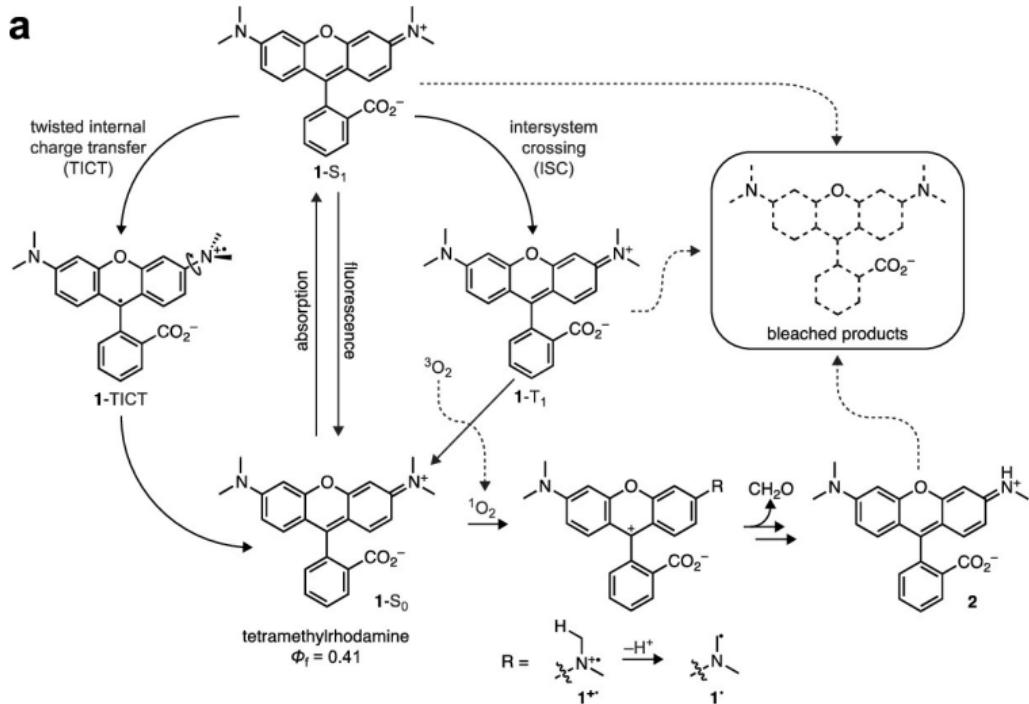


$\sigma = 9.14 \text{ nm}$

$\sigma = 15.56 \text{ nm}$

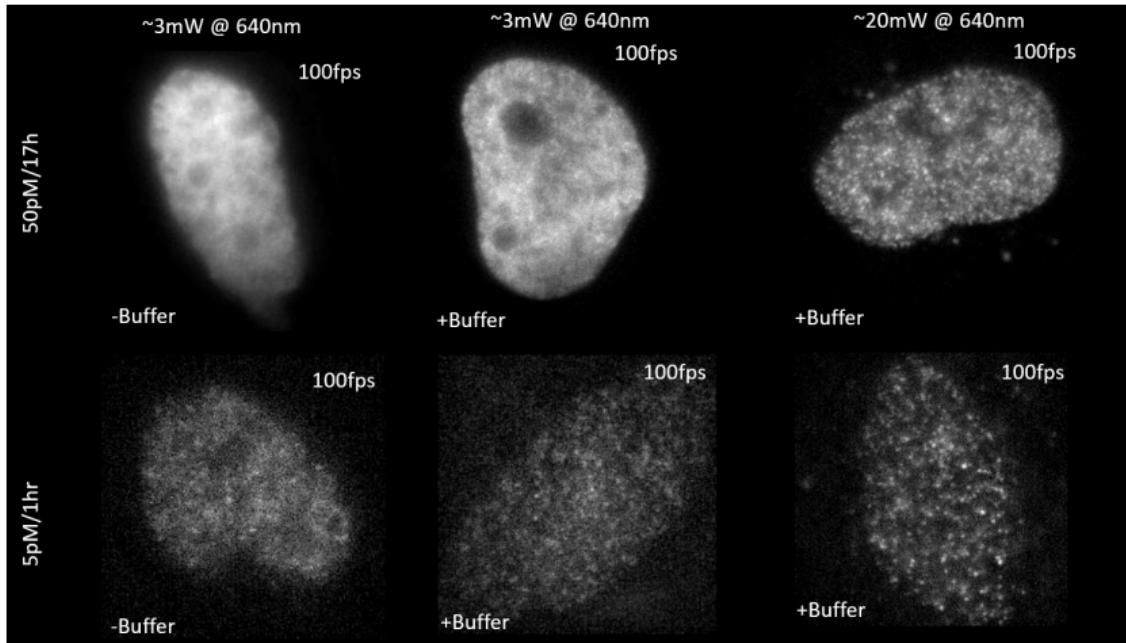
$\sigma = 52.73 \text{ nm}$

Super resolution with photoswitching of rhodamines



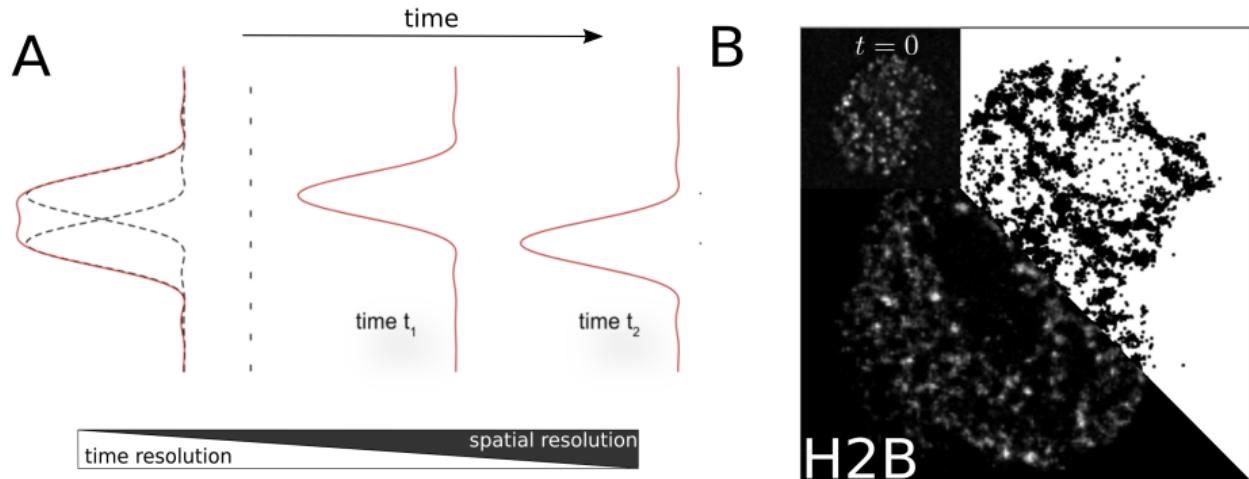
- ▶ Reduction of the T₁ state yields a dark, long-lived, and stable radical state

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

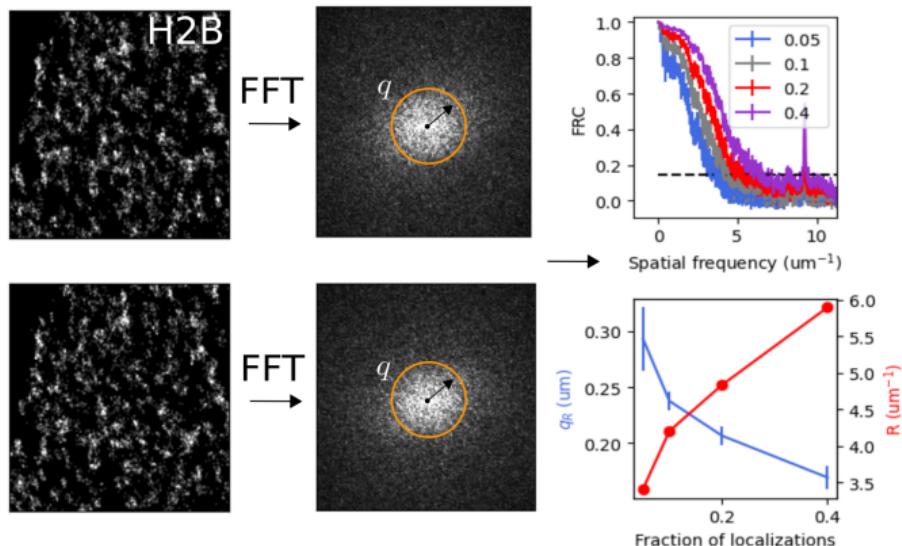
Direct stochastic optical reconstruction microscopy



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

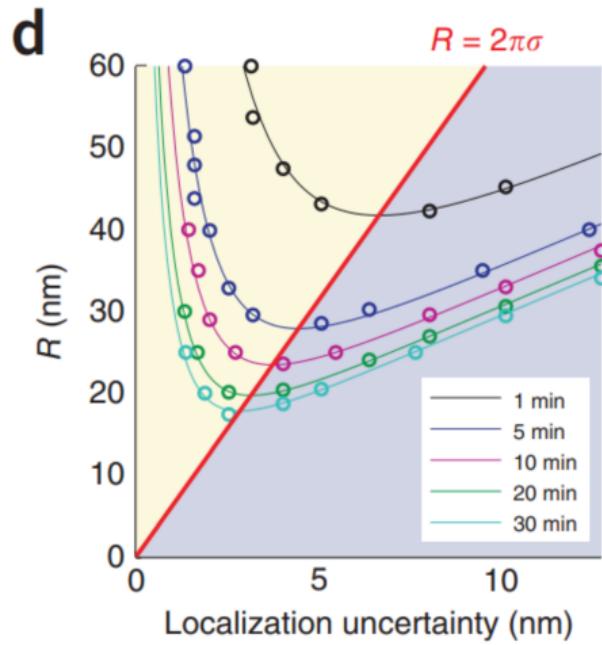
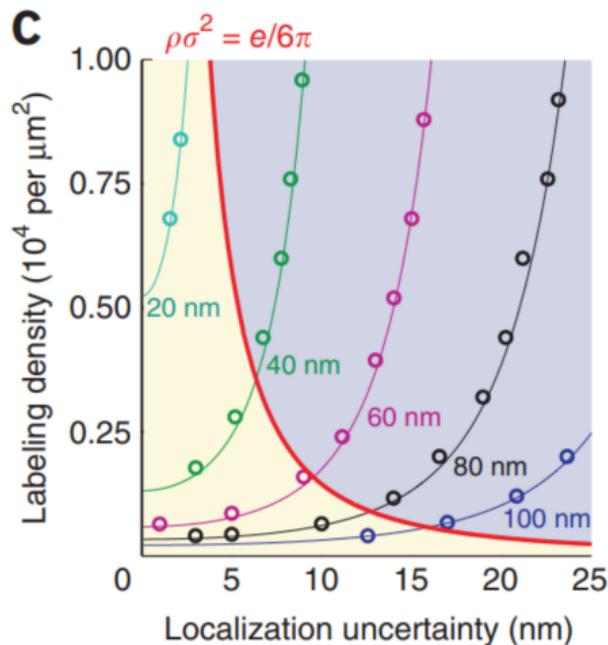
Fourier ring correlation links spatial and temporal resolution

- 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

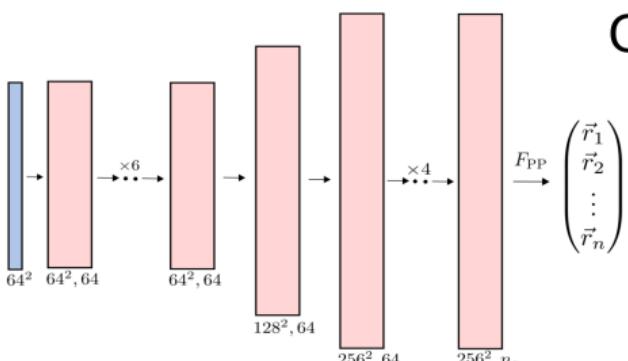


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

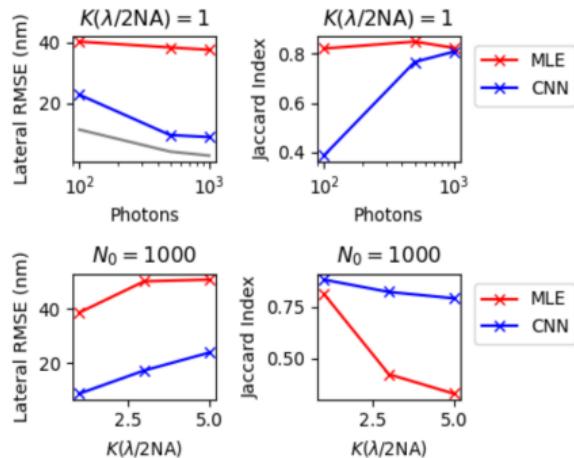
- ▶ Increased localization uncertainty requires higher density for same resolution
- ▶ Longer acquisitions have higher resolution

Estimator precision sets the resolution limit in localization microscopy

B

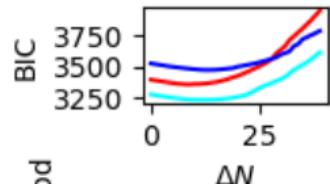
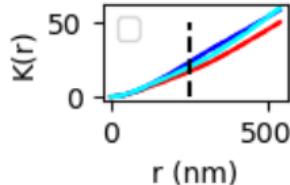
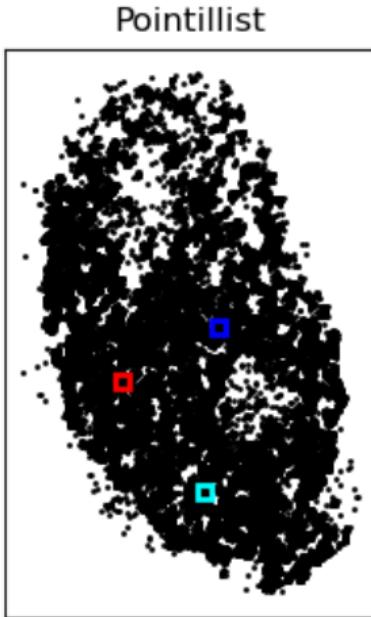
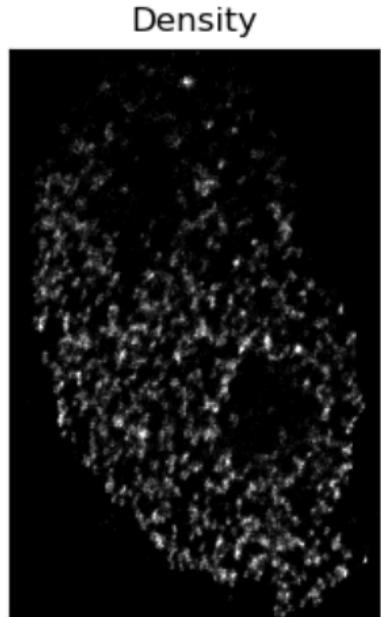


C

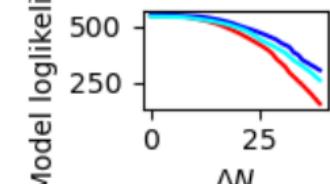


- ▶ $K(\lambda/2NA)$ is Ripley's K function at the diffraction limit ($\lambda = 640\text{nm}$)
- ▶ Convolutional neural networks (CNNs) approach the Cramer-Rao lower bound (gray)

Chromatin nanodomains in a living Hela cell nucleus



Model loglikelihood



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

Dense localization with fluorescence antibunching

- ▶ Pixels represent independent categorical variables, where the category is the number of photons collected
- ▶ Define tensors $\Omega, \Theta, \Upsilon, \Phi$ as horizontal adjacent, vertical adjacent, right diagonal and left diagonal pixel pairs

Suppose pixel i collects n_i photons and pixel j collects n_j photons. Call this pair n . We have,

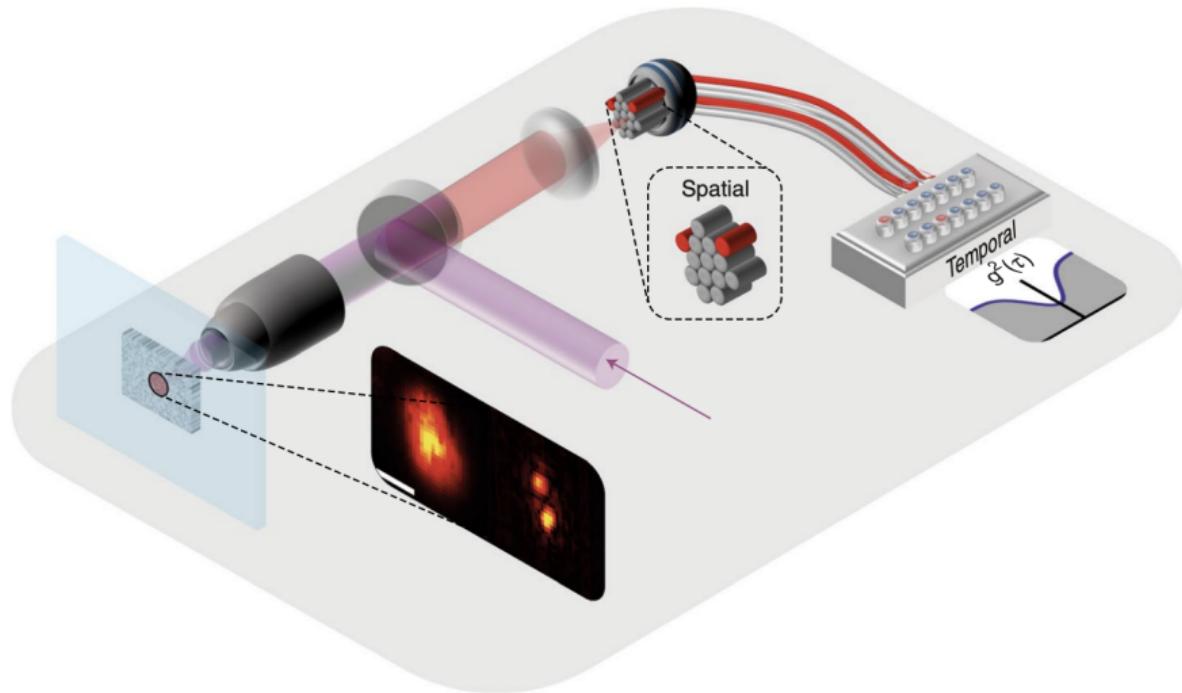
$$\Omega_n = P(X_i = n_i, X_j = n_j) = \left[\sum_{\alpha} \left(\prod_n p_i^{\alpha_n} (1 - p_i)^{\alpha_n} \right) \right] \left[\sum_{\beta} \left(\prod_n p_i^{\beta_n} (1 - p_i)^{\beta_n} \right) \right]$$

where α, β are binary strings indicating which emitters contribute to the count. Ω_n has the dimension of the state space of (X_i, X_j) . Clearly,

$$\langle X_i X_j \rangle = \sum_{x_i, x_j} x_i x_j \Omega_n$$

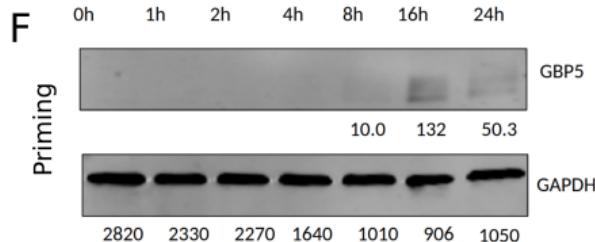
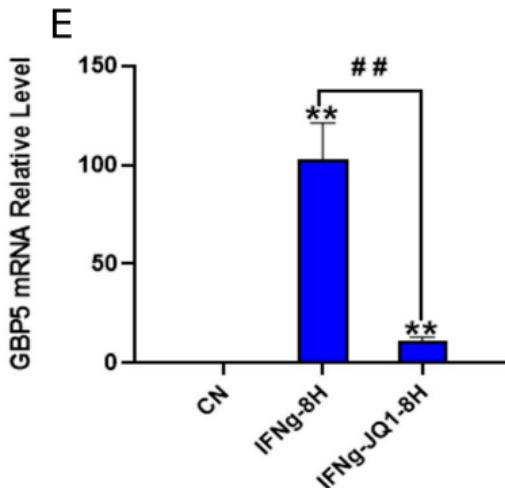
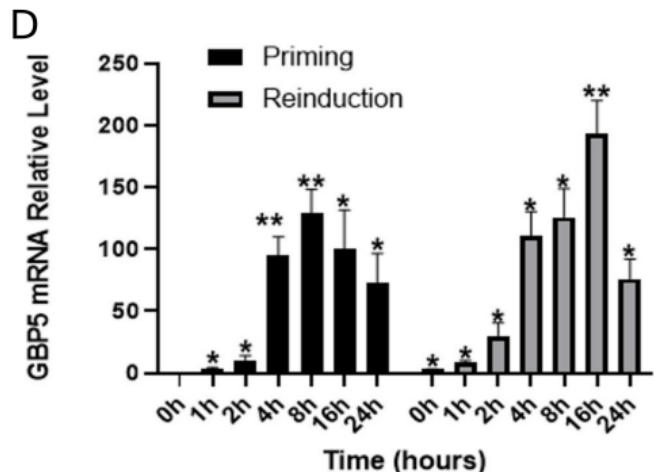
It still isn't clear how to map $n \rightarrow (i, j)$ to make this computationally efficient

Dense localization by fluorescence antibunching

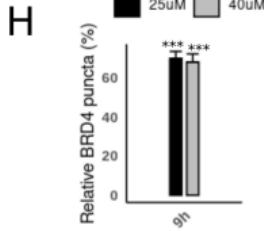
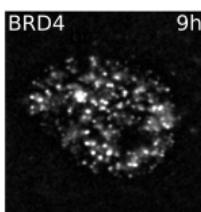


Andrew Forbes and Valeria Rodriguez-Fajardo. Super-resolution with quantum light. Nature Photonics 2019.

Inhibition of a super-enhanced gene with JQ1

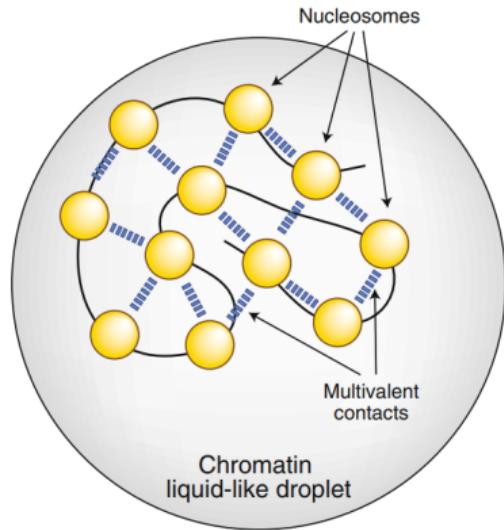


G



► *: $P \leq 0.1$, **: $P \leq 0.01$

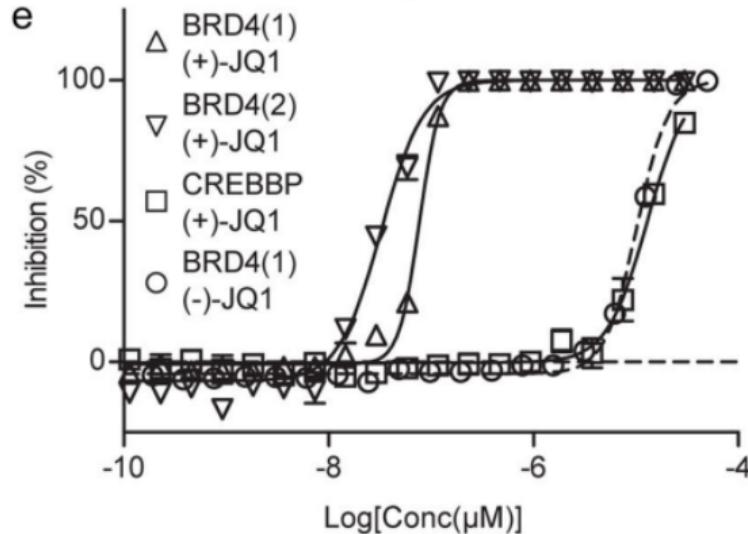
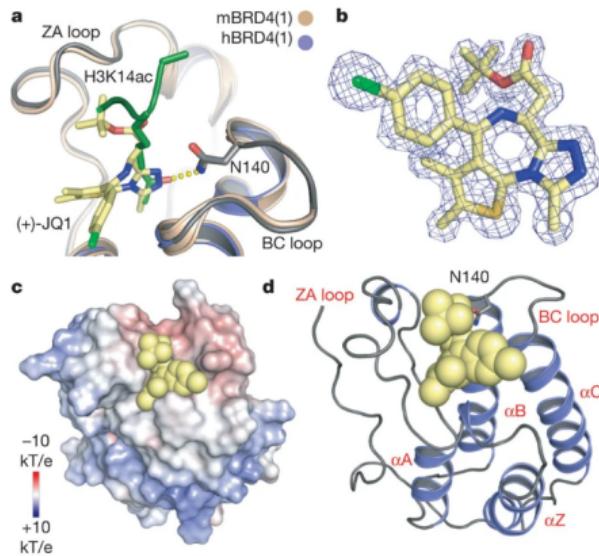
Chromatin has an intrinsic ability to undergo phase separation



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