

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

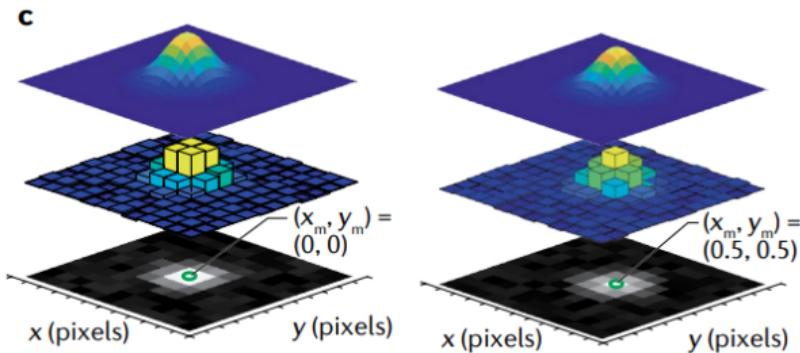
$$\text{Localization: } \theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$$

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

η – quantum efficiency

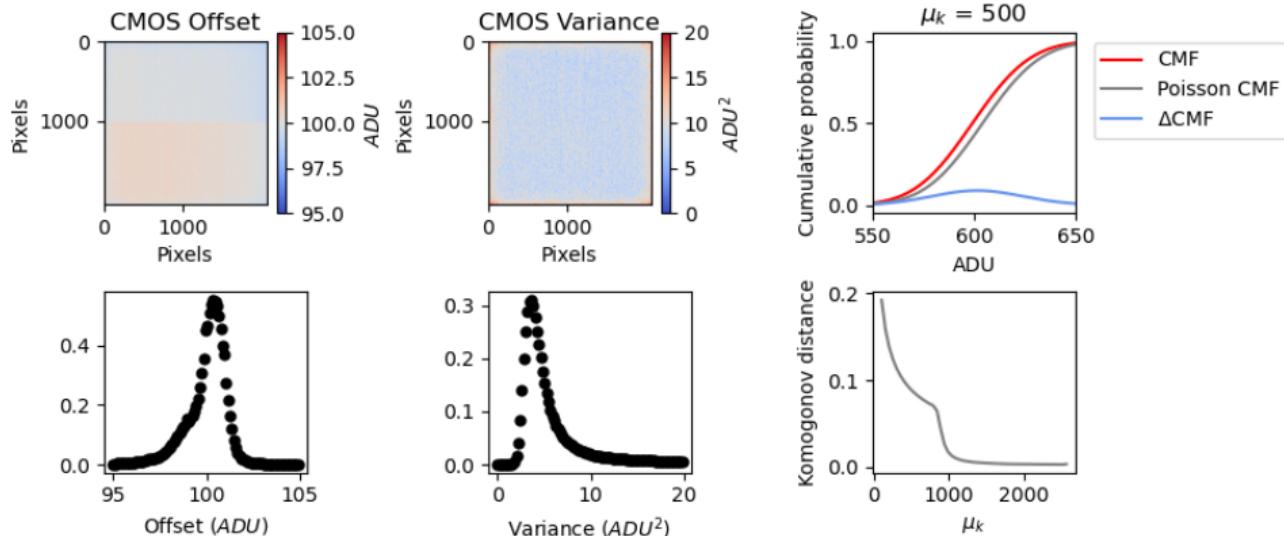
N_0 – photon count

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)

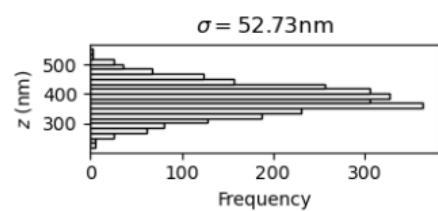
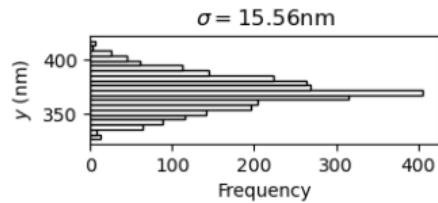
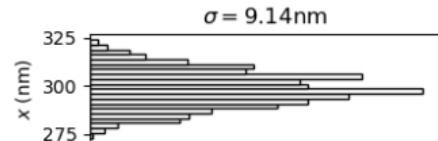
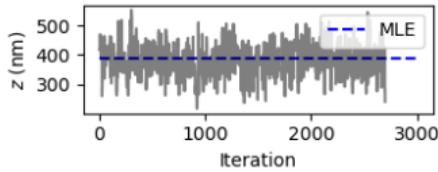
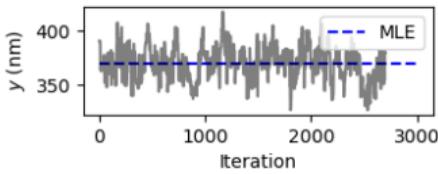
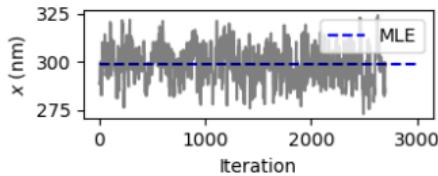
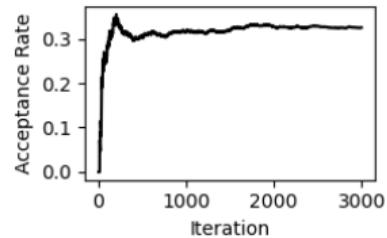
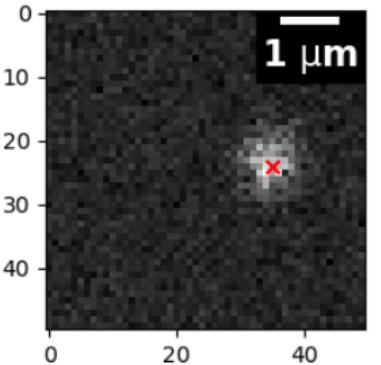
Classical emission statistics of fluorescent markers



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

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

Estimator precision in localization microscopy

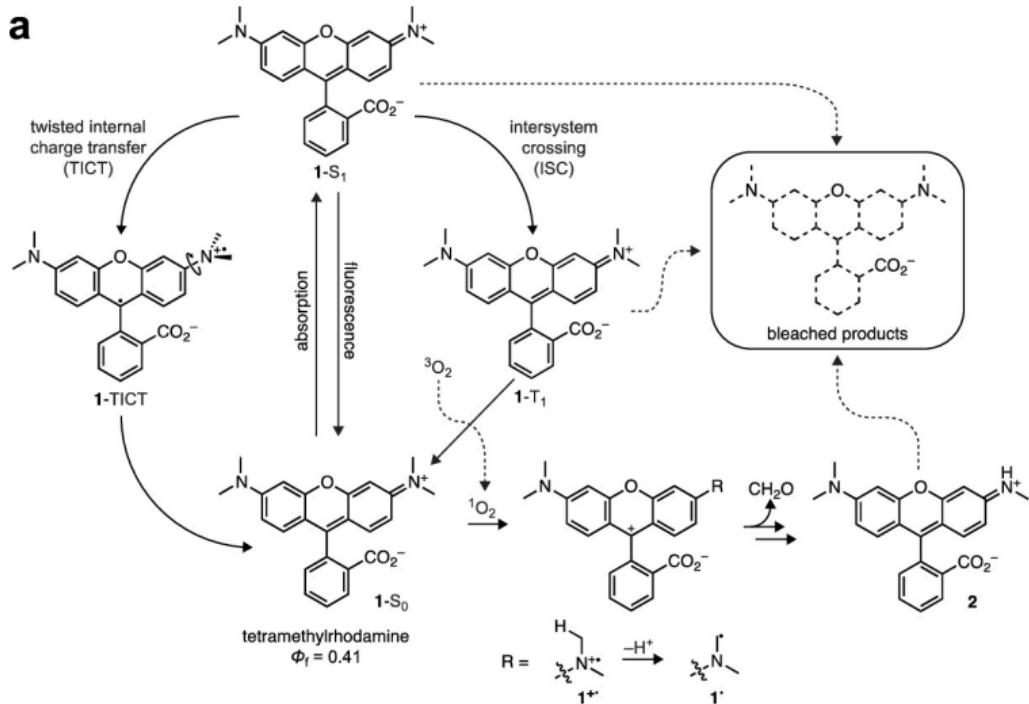


$\sigma = 9.14 \text{ nm}$

$\sigma = 15.56 \text{ nm}$

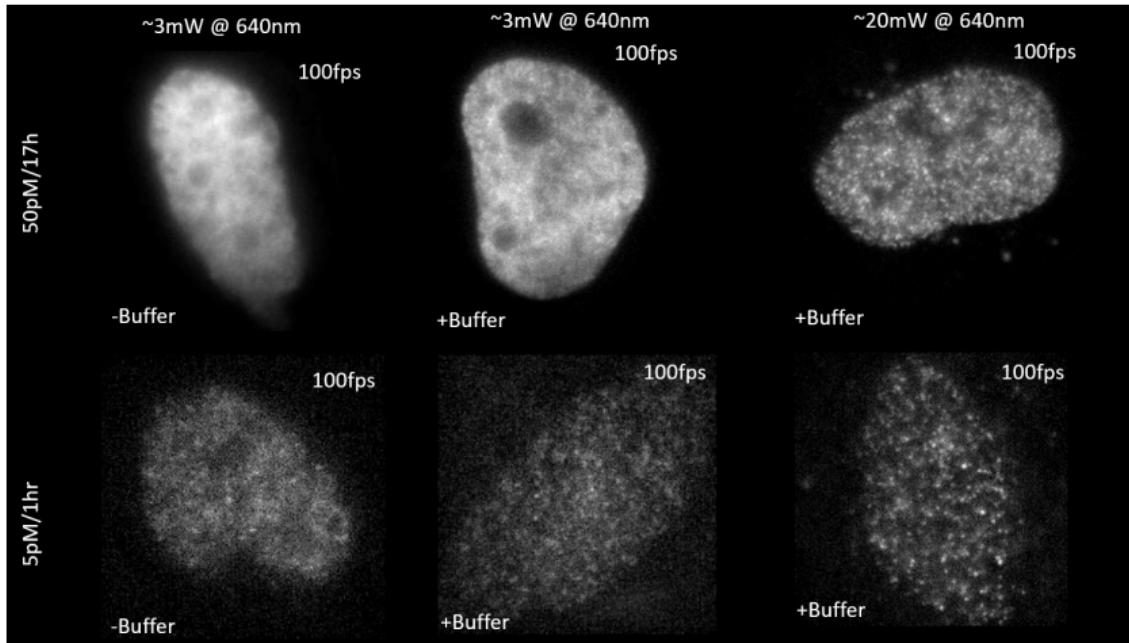
$\sigma = 52.73 \text{ nm}$

Super resolution with photoswitching of rhodamines



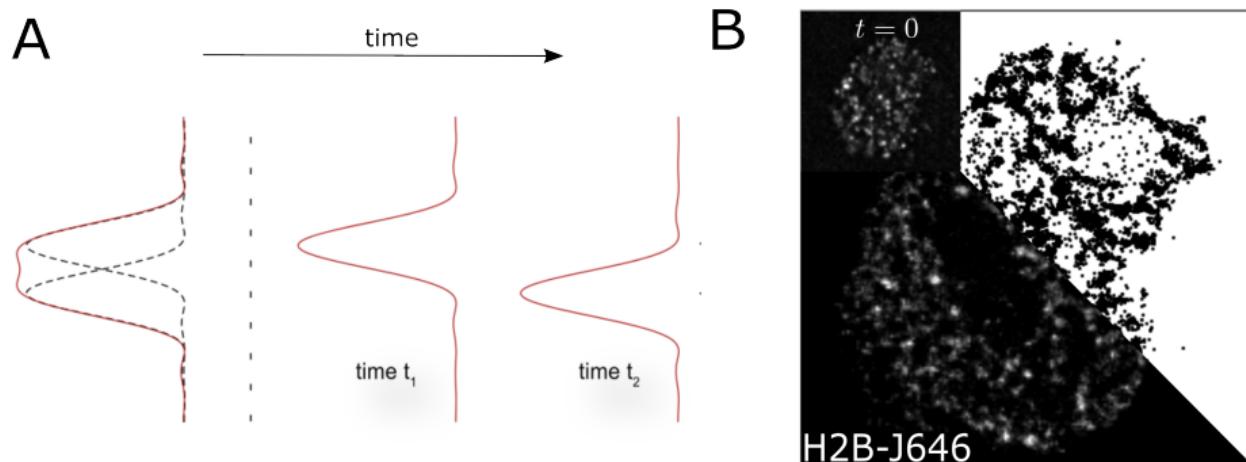
- ▶ Reduction of the T₁ state yields a dark, long-lived, and stable radical state

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

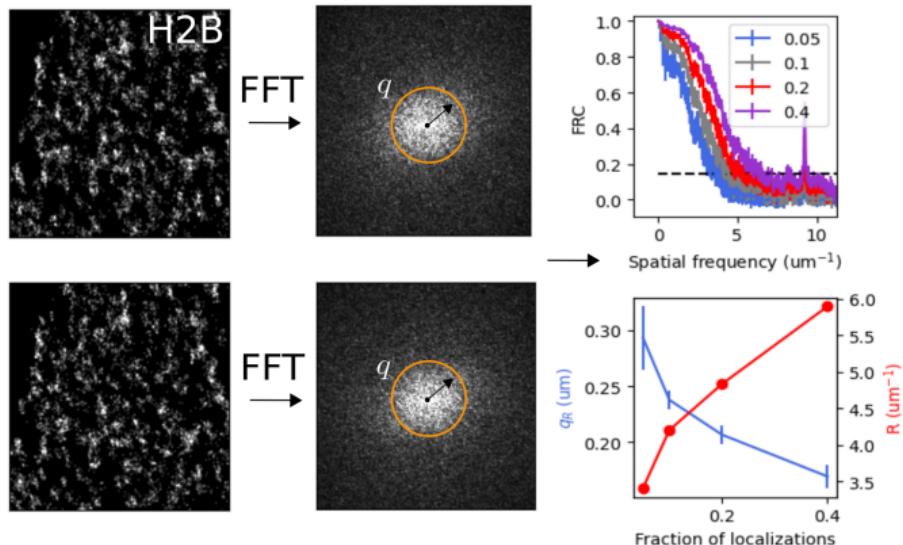
Direct stochastic optical reconstruction microscopy



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

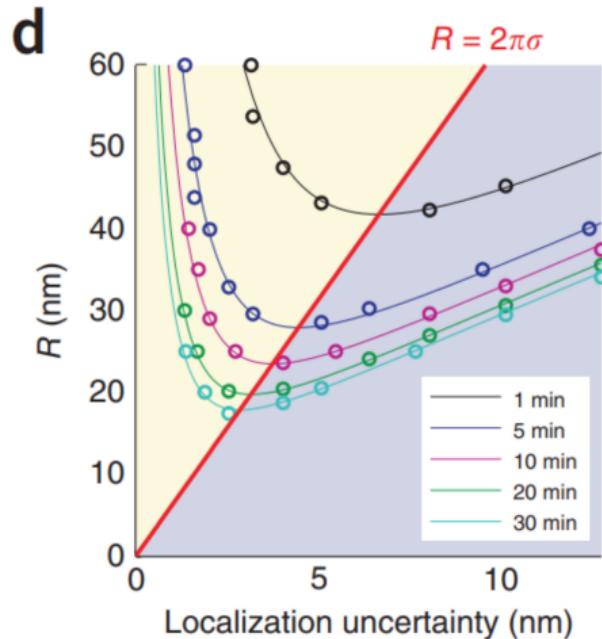
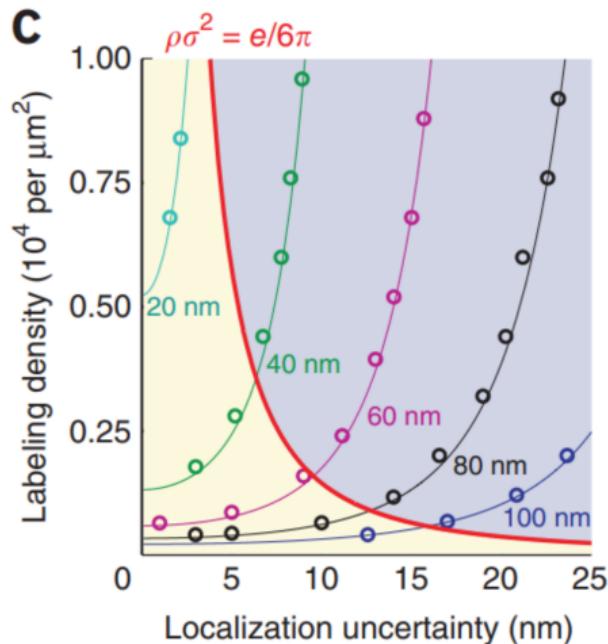
Fourier ring correlation links spatial and temporal resolution

- 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

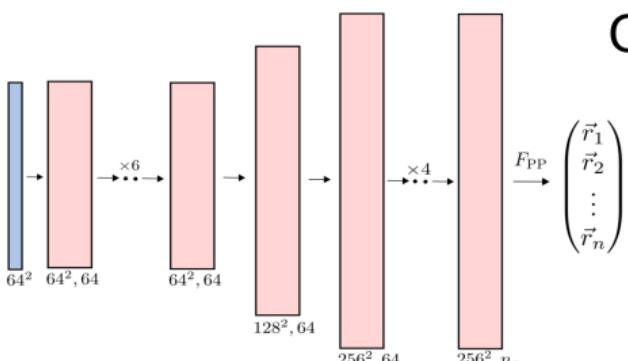


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

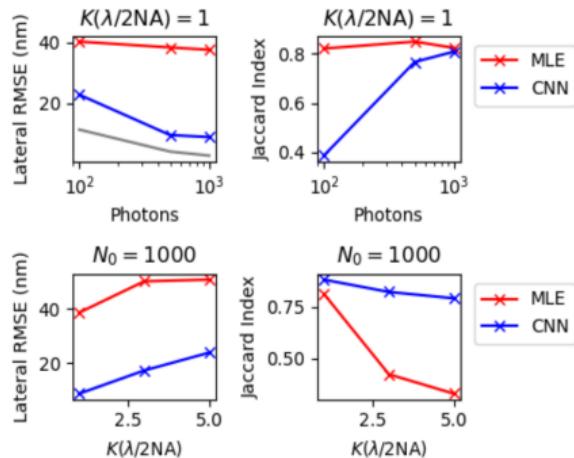
- ▶ Increased localization uncertainty requires higher density for same resolution
- ▶ Longer acquisitions have higher resolution

Estimator precision sets the resolution limit in localization microscopy

B

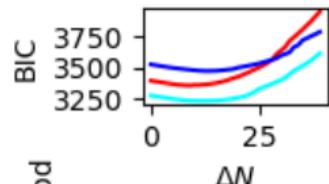
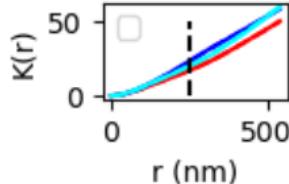
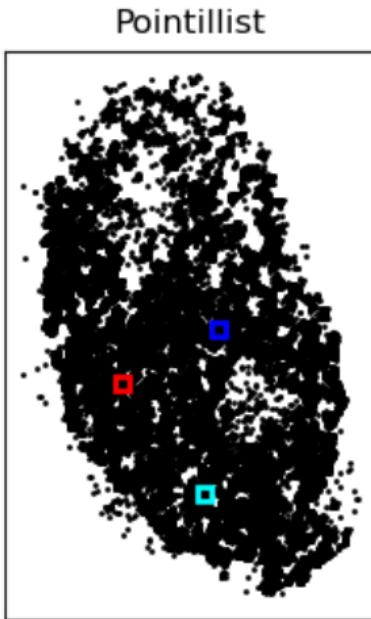
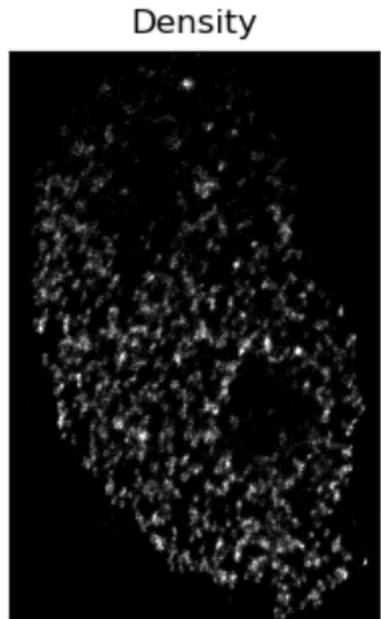


C

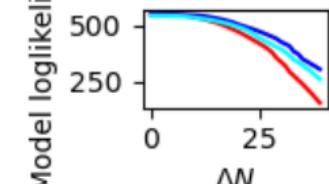


- ▶ $K(\lambda/2NA)$ is Ripley's K function at the diffraction limit ($\lambda = 640\text{nm}$)
- ▶ Convolutional neural networks (CNNs) approach the Cramer-Rao lower bound (gray)

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

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 α_i, α_j , which are binary vectors, s.t. $\sum \alpha_i = N_i$ and $\sum \alpha_j = N_j$ and have $\alpha_i \text{ AND } \alpha_j = 0$

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

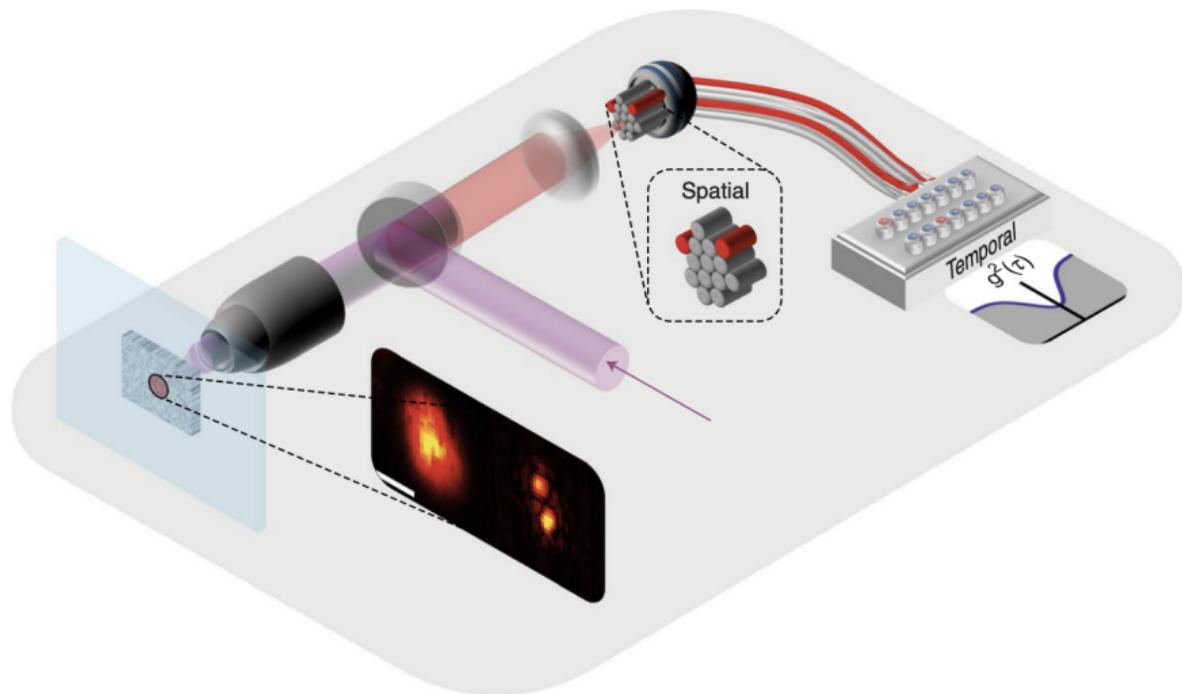
But now consider

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

Antibunching now becomes apparent. If only a single emitter exists (and we have designed α 's correctly) then this expectation must be zero for all (i, j) .

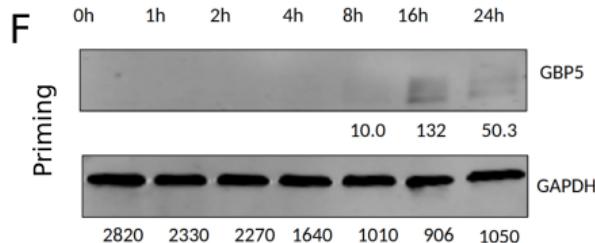
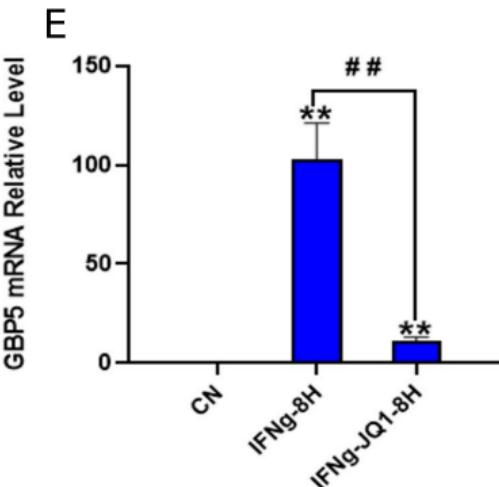
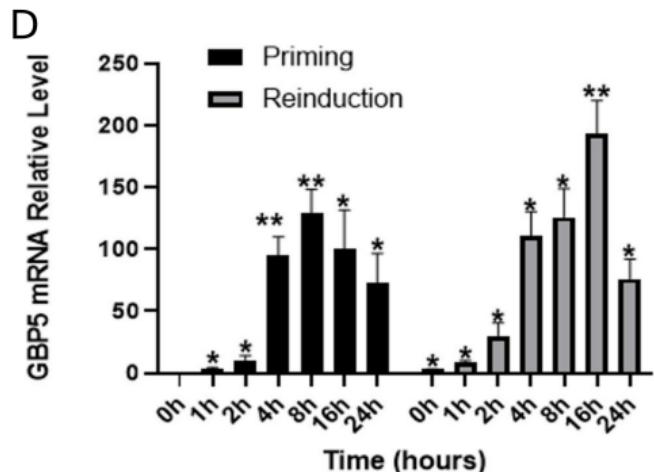
This time, you will have to iterate over the 2d state space (it is still pretty small), each time computing B which is a $2 \times M \times \binom{M}{N_i} \binom{M}{N_j}$ where M is the number of emitters. Then you can make some minor adjustments to the existing vectorized code to get the probability at each state space element. This gives you the probability over the state space for every possible pair, which can be used to compute $\langle X_i X_j \rangle$ for every pair.

Dense localization by fluorescence antibunching

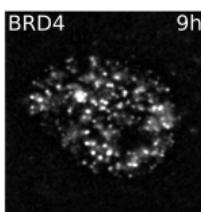


Andrew Forbes and Valeria Rodriguez-Fajardo. Super-resolution with quantum light. Nature Photonics 2019.

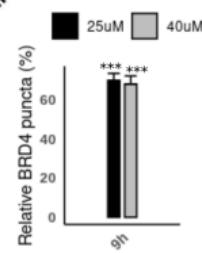
Inhibition of a super-enhanced gene with JQ1



G

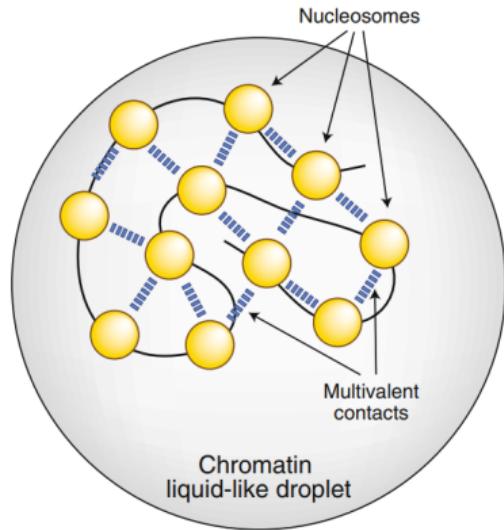


H



► *: $P \leq 0.1$, **: $P \leq 0.01$

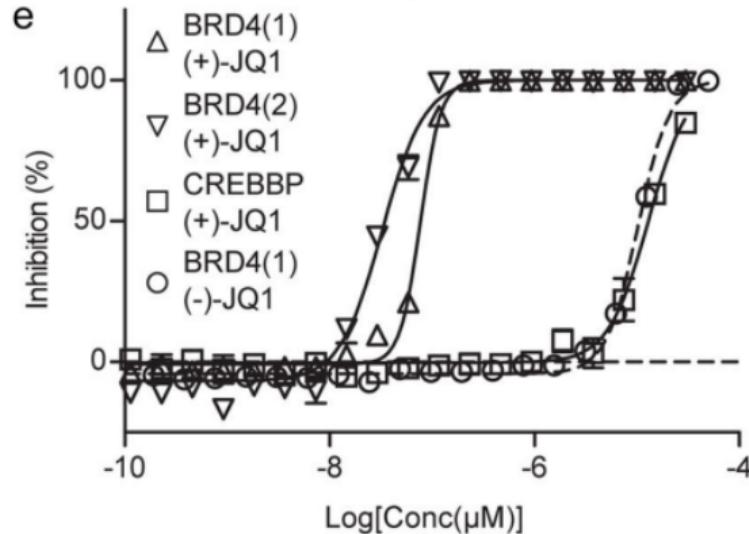
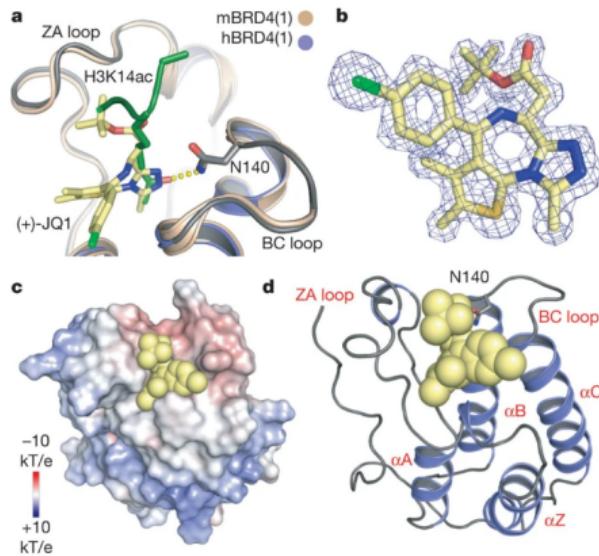
Chromatin has an intrinsic ability to undergo phase separation



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