

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

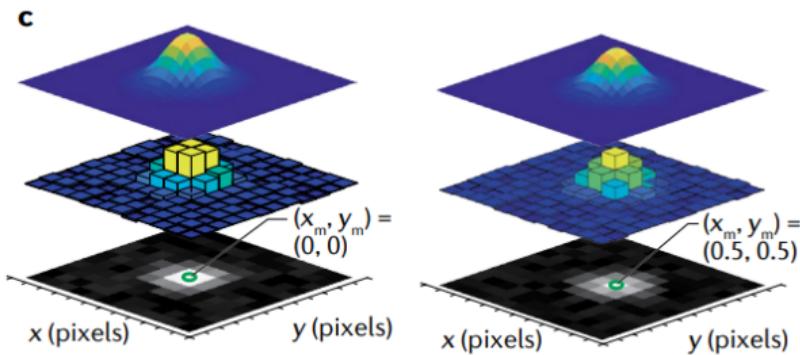
$$\text{Localization: } \theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$$

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

η – quantum efficiency

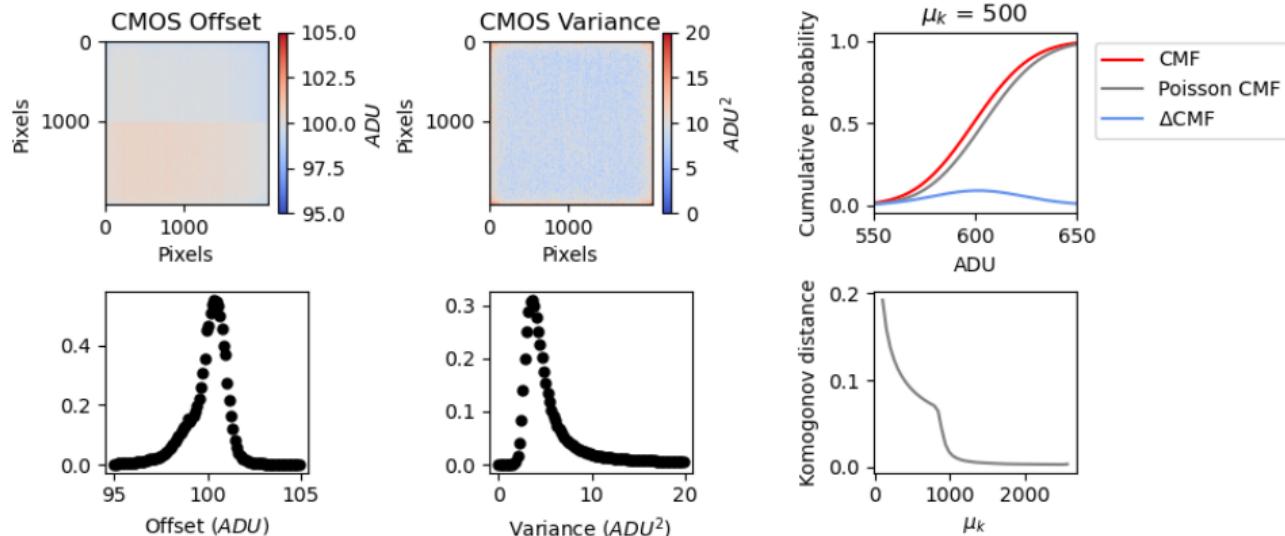
N_0 – photon count

Δ – exposure time



- ▶ SMLM techniques are diffraction unlimited
- ▶ SMLM techniques are suitable for **super-resolution** (SR) and **single molecule tracking** (SMT)

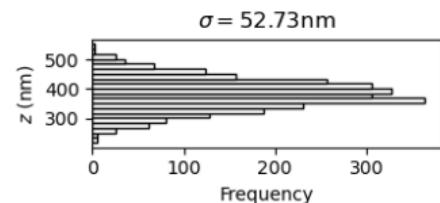
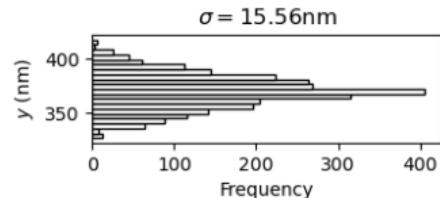
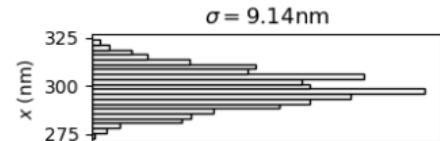
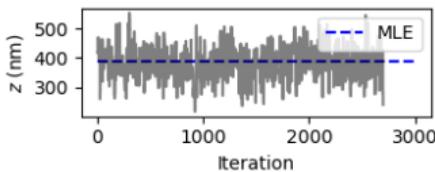
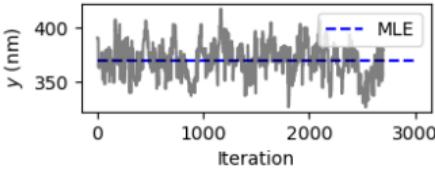
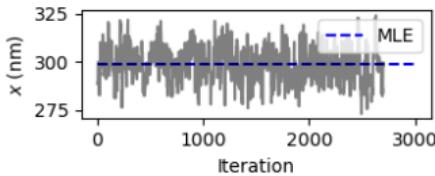
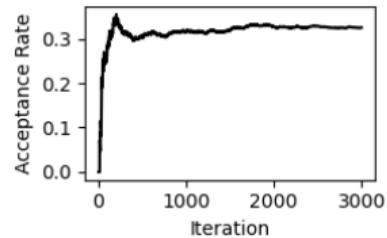
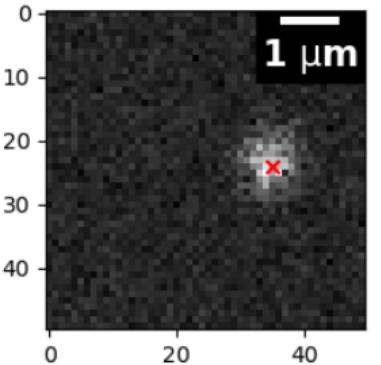
A Poisson approximation at moderate SNR simplifies SMLM



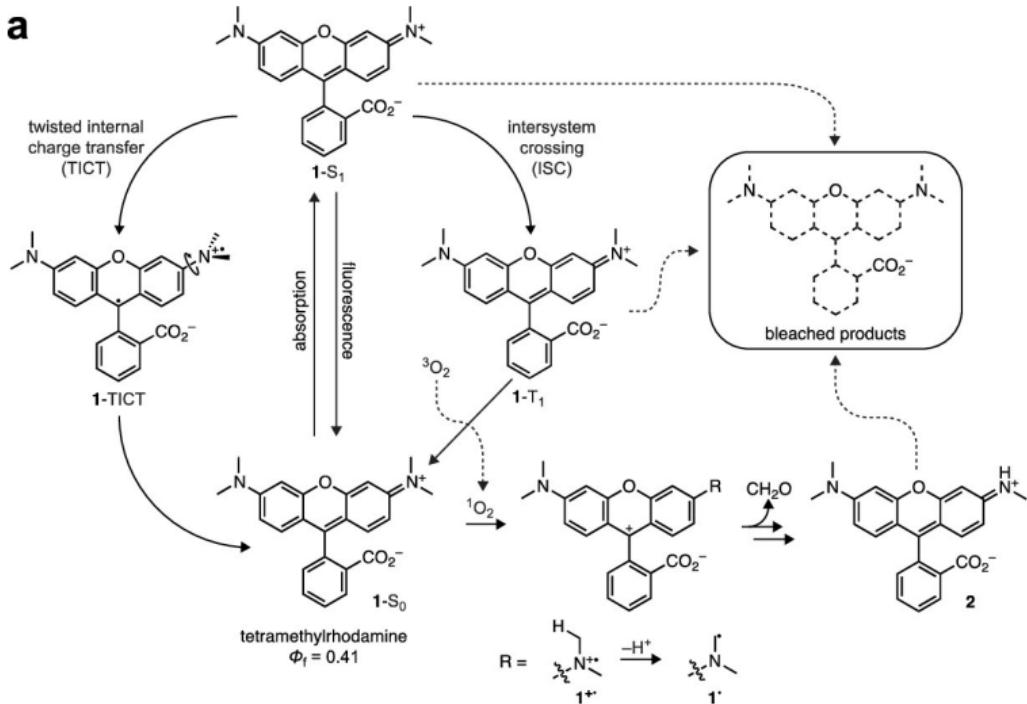
$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi\sigma_k^2}} e^{-\frac{(H_k - g_k q - \sigma_k)^2}{2\sigma_k^2}}$$

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

Estimator precision in localization microscopy

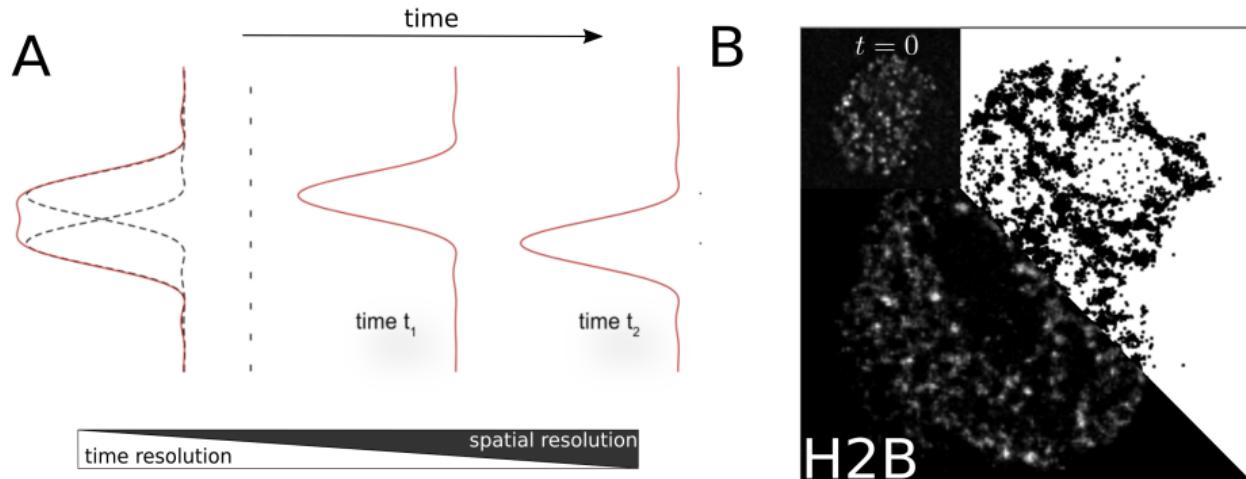


Super resolution with photoswitching of rhodamines



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

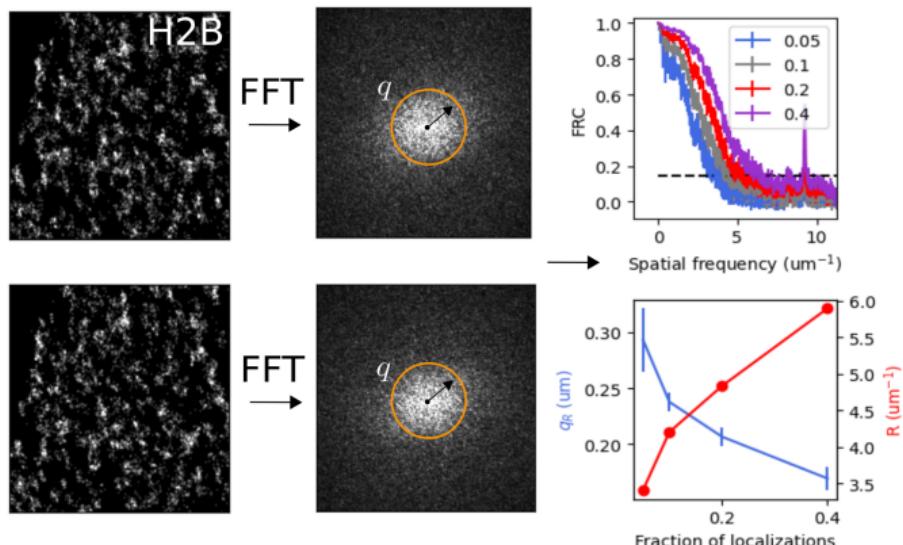
Direct stochastic optical reconstruction microscopy



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

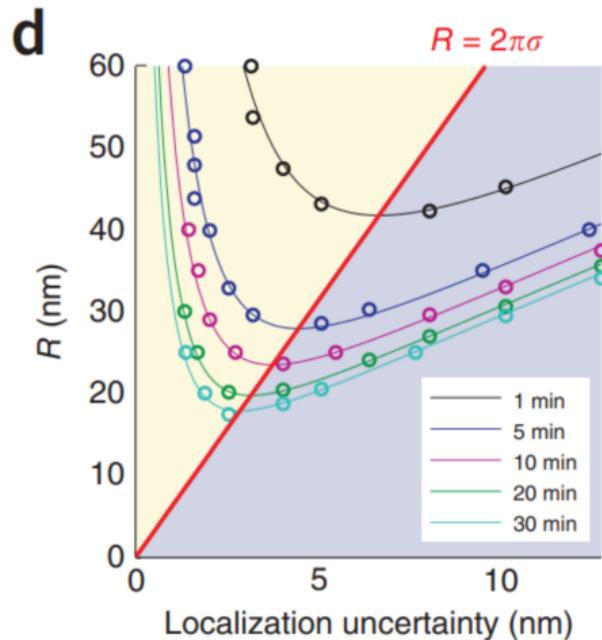
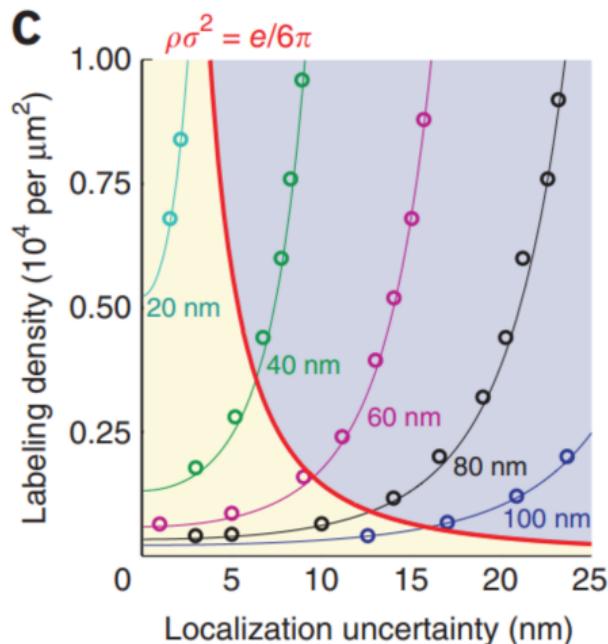
Fourier ring correlation links spatial and temporal resolution

- 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

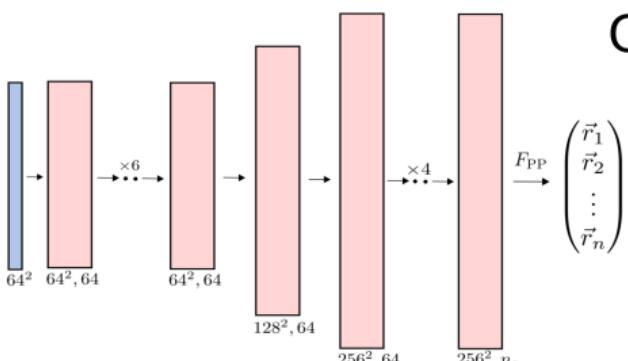


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

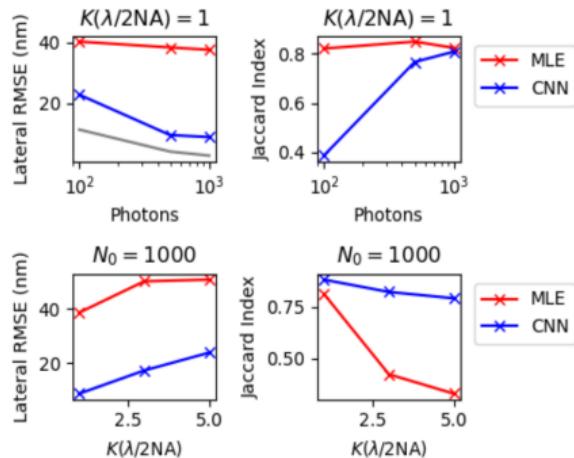
- ▶ Increased localization uncertainty requires higher density for same resolution
- ▶ Longer acquisitions have higher resolution

Estimator precision sets the resolution limit in localization microscopy

B

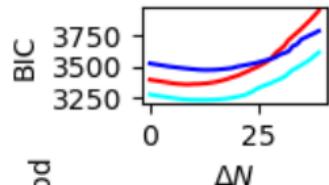
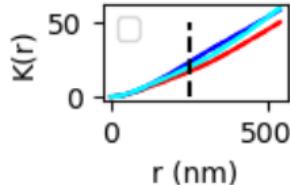
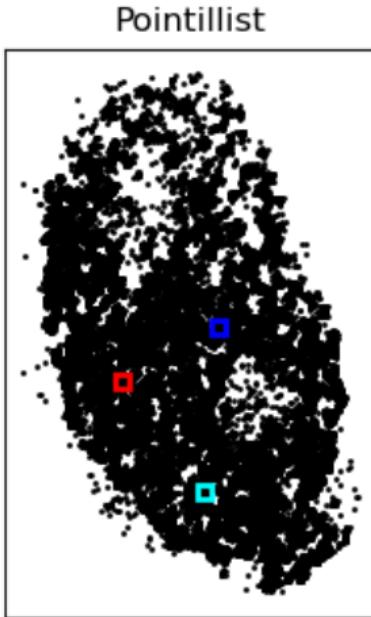
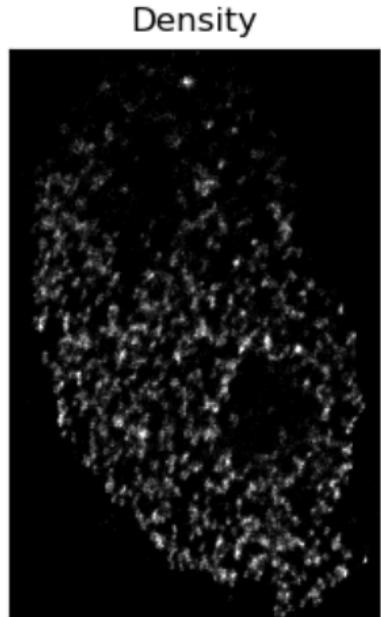


C

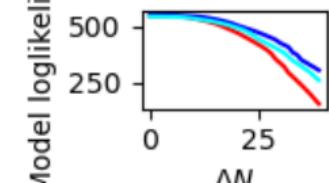


- ▶ $K(\lambda/2NA)$ is Ripley's K function at the diffraction limit ($\lambda = 640\text{nm}$)
- ▶ Convolutional neural networks (CNNs) approach the Cramer-Rao lower bound (gray)

Chromatin nanodomains in a living Hela cell nucleus



Model loglikelihood



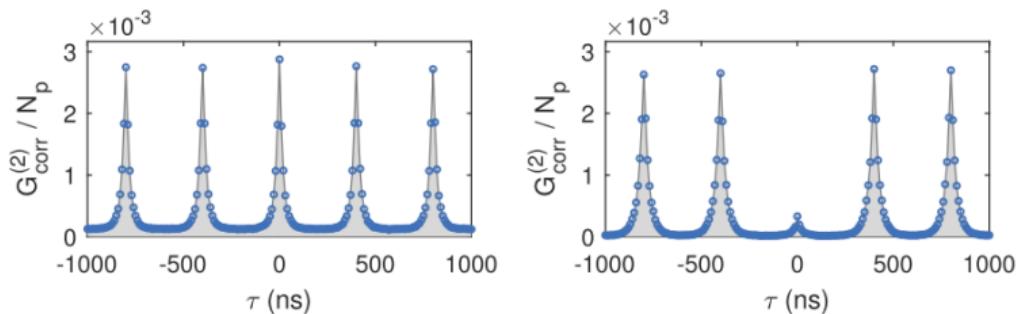
- ▶ Histone DE using 30x30nm bins
- ▶ Likelihood is computed under a Gaussian Mixture Model (GMM)

Next generation SMLM with photon counting cameras

SOFI techniques conventionally compute autocorrelation functions $G_{ii}^2(\mathbf{r}, \tau)$

SOFI images are usually $G_{ii}^2(\mathbf{r}, 0)$ for each pixel, for long integration times

It is reasonable that $G_{ij}^2(\mathbf{r}_i, \mathbf{r}_j, \tau)$ contains information about molecular positions



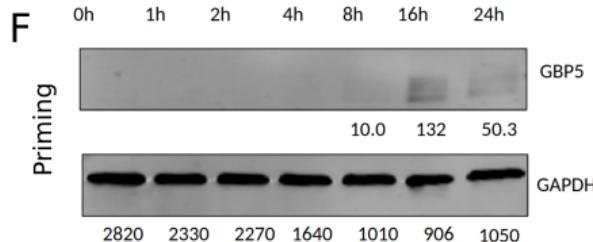
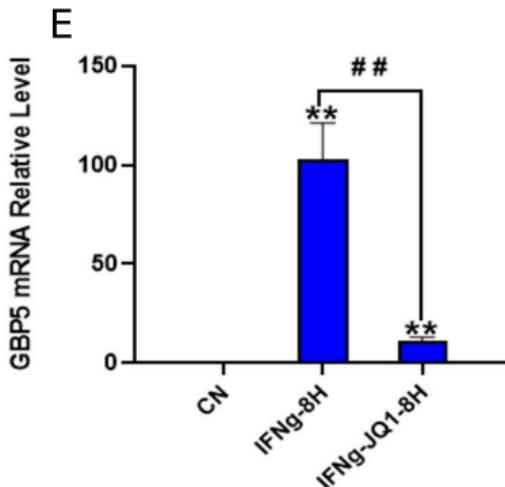
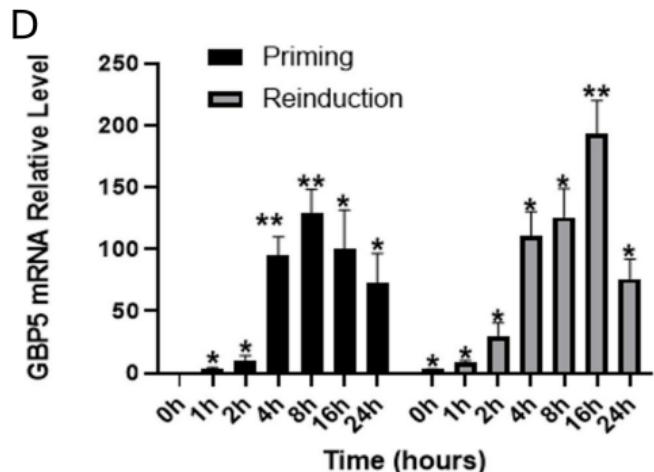
For single photon counts, I don't think $G_{ij}^2(\mathbf{r}_i, \mathbf{r}_j, 0)$ contains this information (peaks in G_{ij}^2 at nonzero lag is evidence for position) Cross-cumulants may be more appropriate (Girsault 2016), which comes with virtual pixels May also need pulsed excitation synced with the SPAD camera (can guarantee single photon per molecule per frame) Higher order correlations might give stronger localization results, but could be intractable to compute (RNNs?)

Now I'm not so sure that the shape of G_{ij}^2 contains information on position. Translating a molecule along the axes changes the intensity of the individual processes, and I don't believe G_{ij}^2 is sensitive to the intensity of the processes. Imagine a molecule emits N photons. As the molecule approaches the neighboring pixel, more photons hit the neighboring pixel, but the delay between consecutive photons hitting pixel 2 and pixel 1 is unchanged.

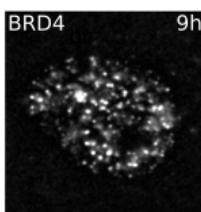
However, the intensity information and the anticorrelation of the processes are complementary. The degree of anticorrelated-ness of the various pairs determines how we can "group the pixels". Intensities then could then be used in a full generative model for the data.

A molecule at position θ will result in anticorrelated neighboring pixels. Perhaps $G_{ij}^2(\mathbf{r}_i, \mathbf{r}_j, 0)$ can be used to determine how anti-correlated the pixels are. Then, we use this value to create the "virtual pixels". Then define a likelihood function on the pixels+virtual pixels given the coordinates.

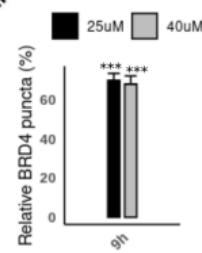
Inhibition of a super-enhanced gene with JQ1



G

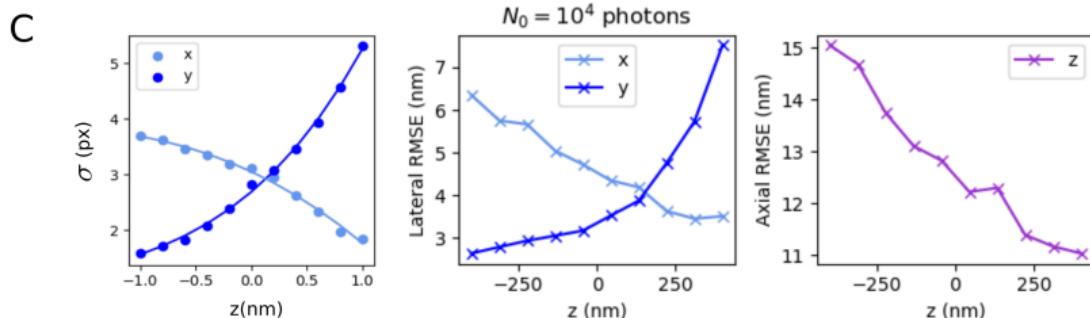
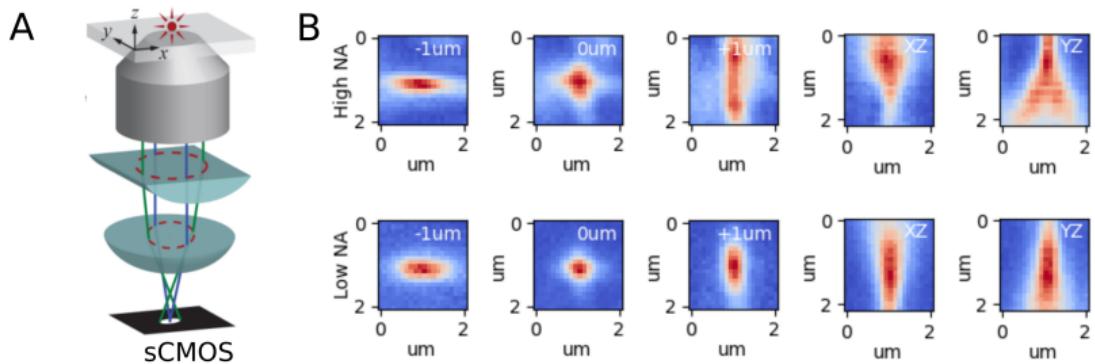


H



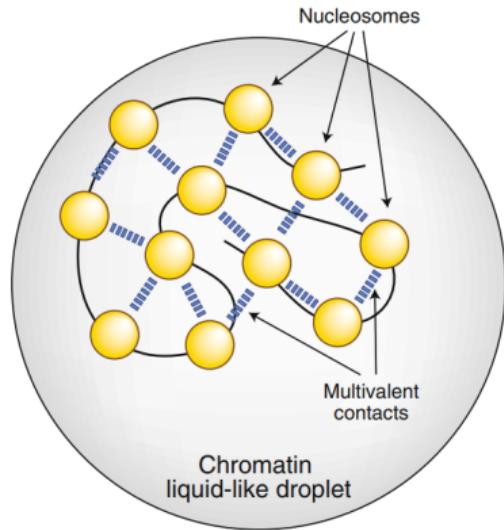
► *: $P \leq 0.1$, **: $P \leq 0.01$

Astigmatism based three dimensional imaging



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

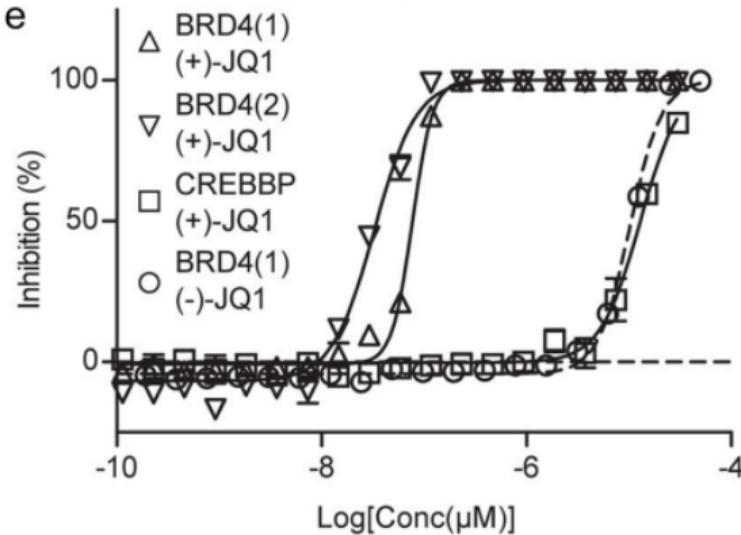
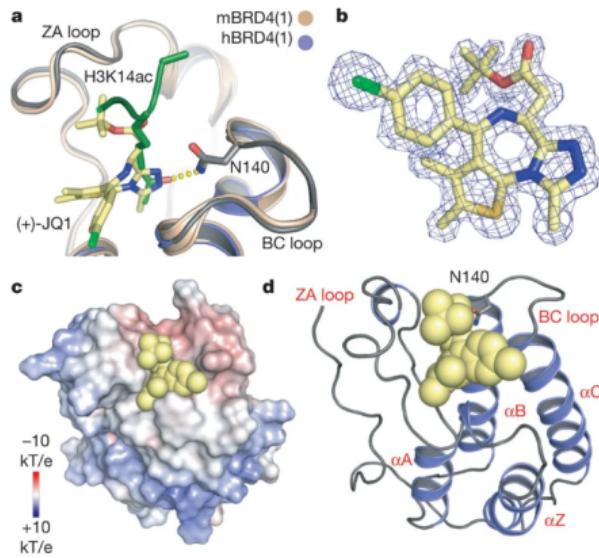
Chromatin has an intrinsic ability to undergo phase separation



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

BET inhibitors reduce nucleosome-BRD4 interactions in BRD4 condensates

BET inhibitors promote disordered BRD4 condensates

Langevin dynamics of BRD4 condensates