

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

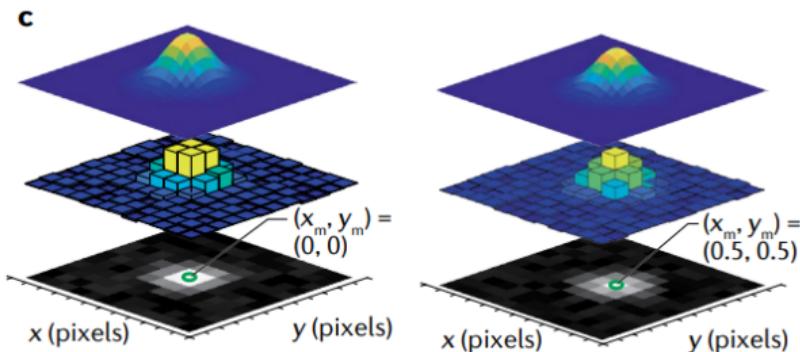
$$\text{Localization: } \theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$$

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

η – quantum efficiency

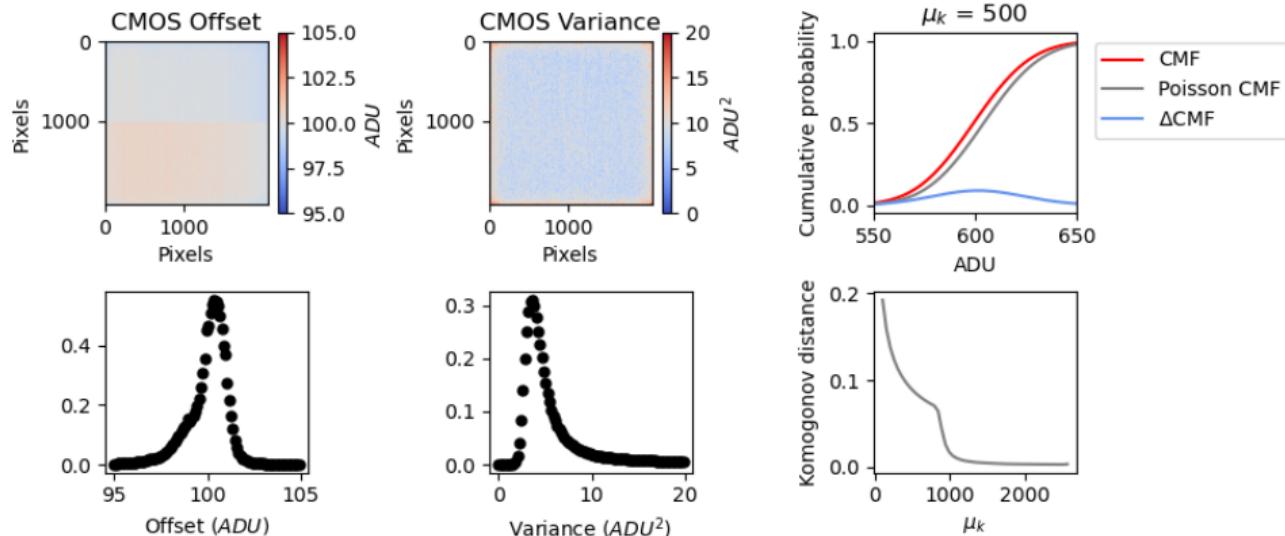
N_0 – photon count

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)

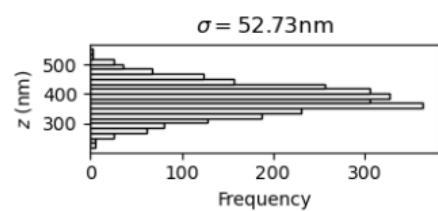
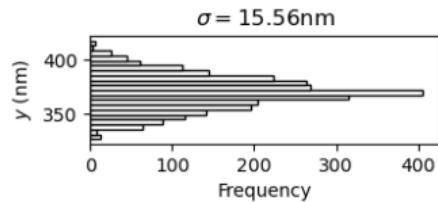
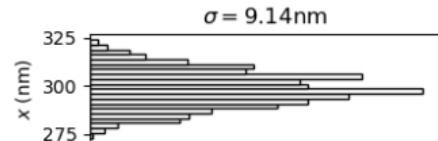
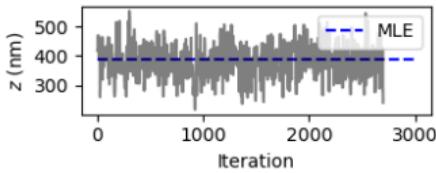
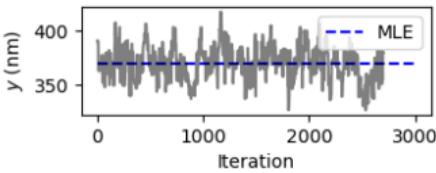
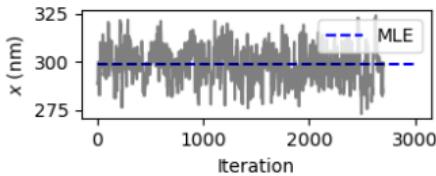
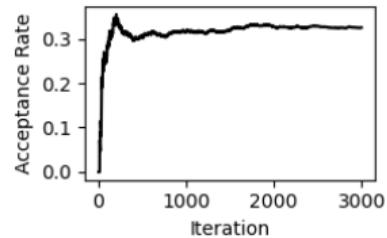
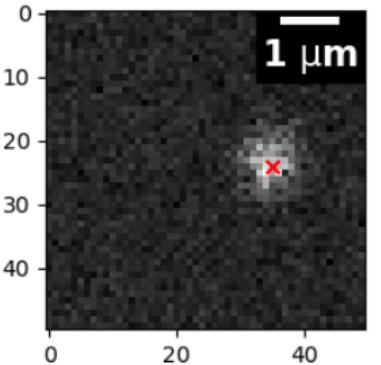
A Poisson approximation at moderate SNR simplifies SMLM



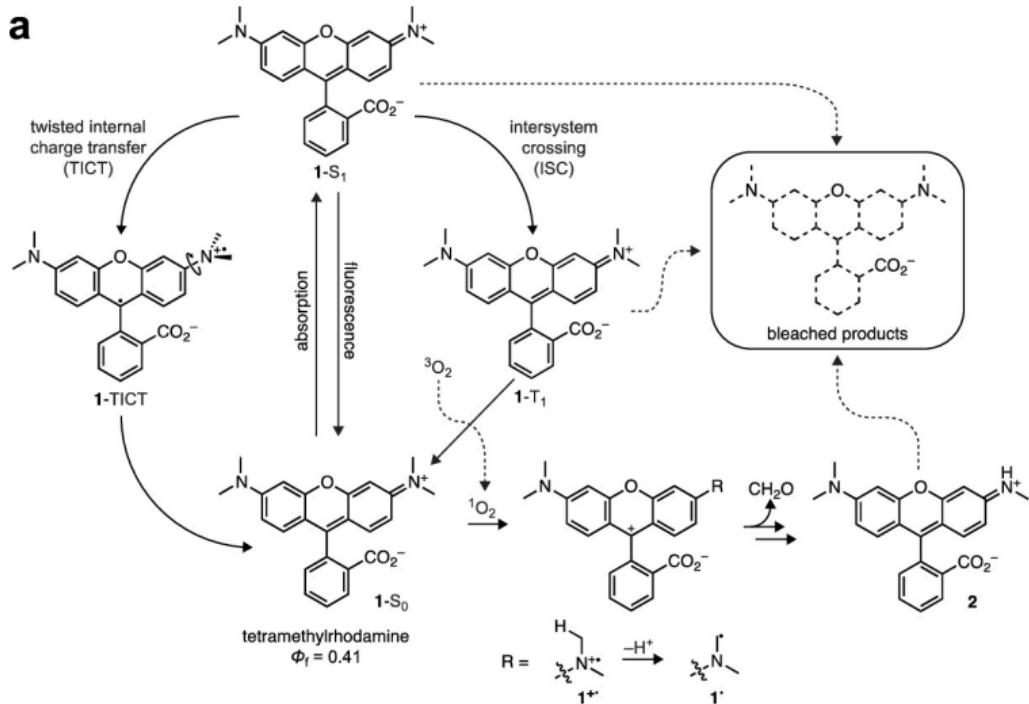
$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi\sigma_k^2}} e^{-\frac{(H_k - g_k q - \sigma_k)^2}{2\sigma_k^2}}$$

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

Estimator precision in localization microscopy

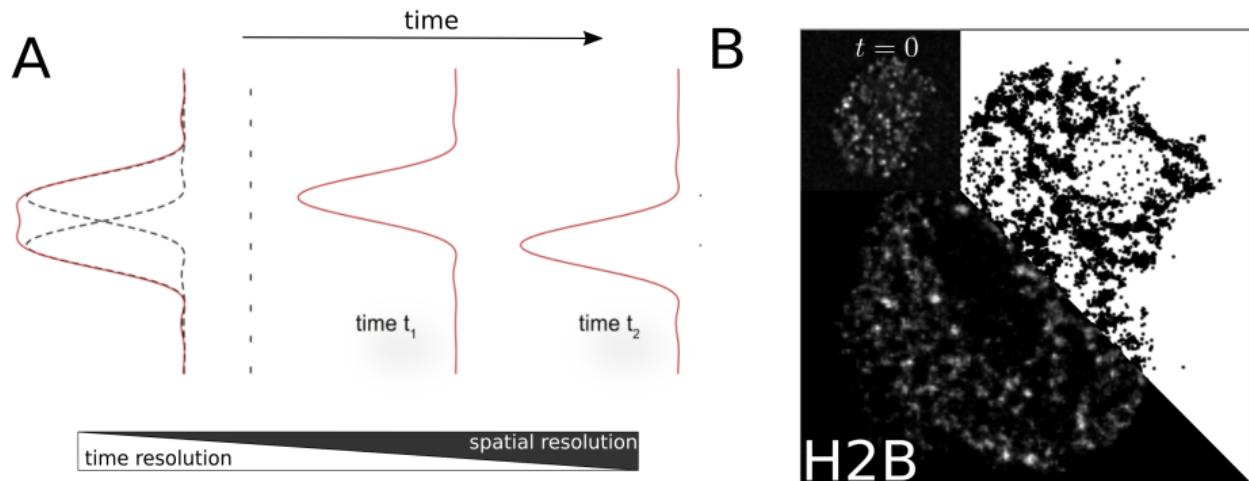


Super resolution with photoswitching of rhodamines



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

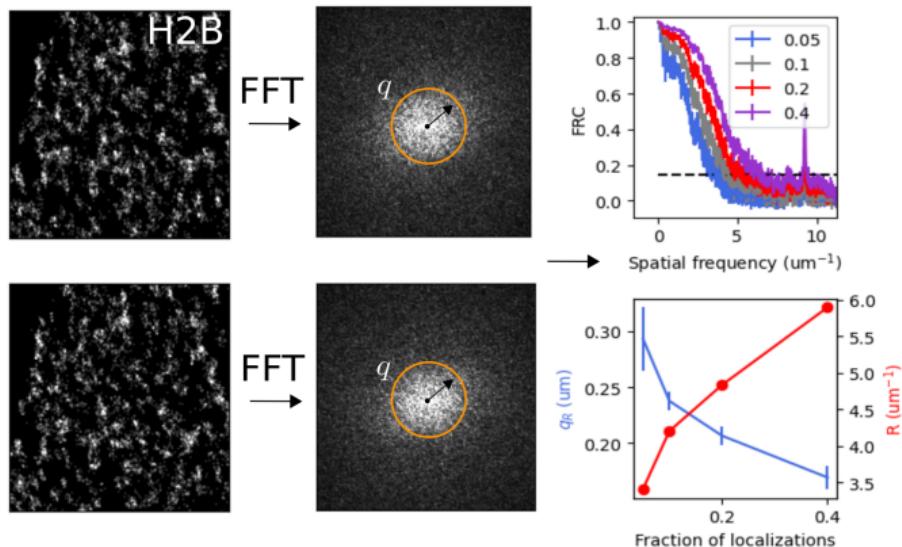
Direct stochastic optical reconstruction microscopy



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

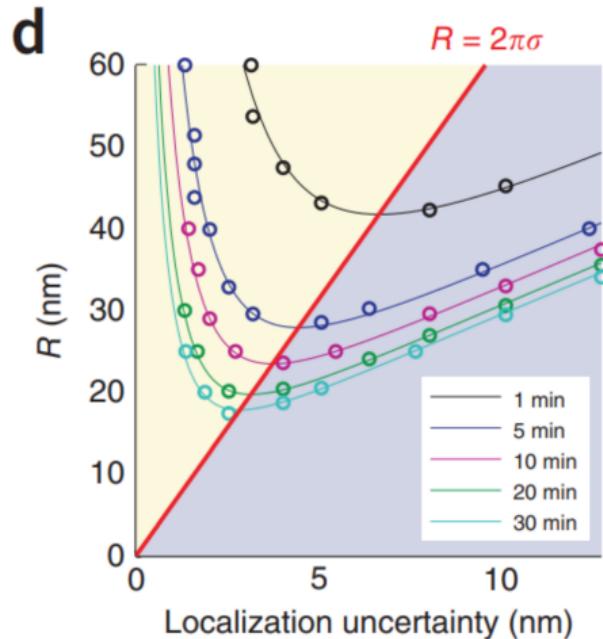
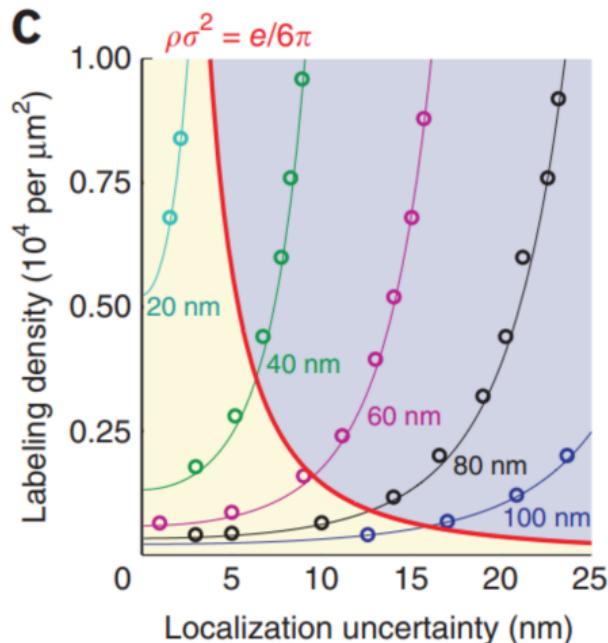
Fourier ring correlation links spatial and temporal resolution

- 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

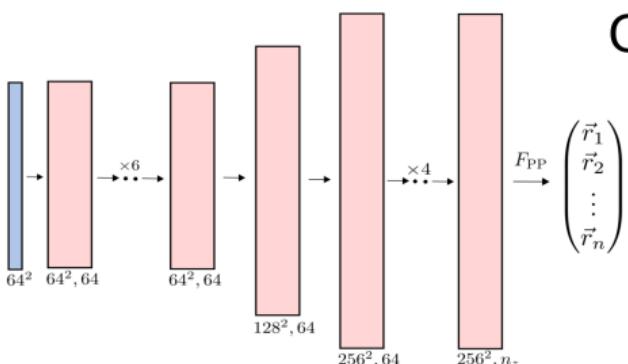


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

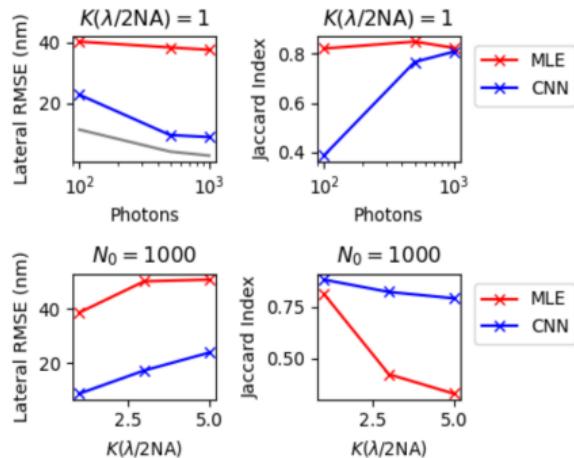
- ▶ Increased localization uncertainty requires higher density for same resolution
- ▶ Longer acquisitions have higher resolution

Estimator precision sets the resolution limit in localization microscopy

B

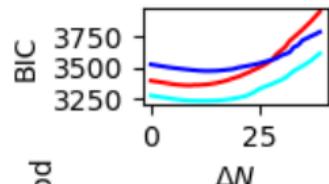
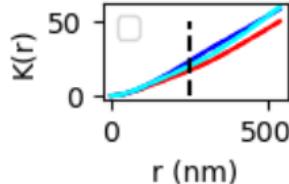
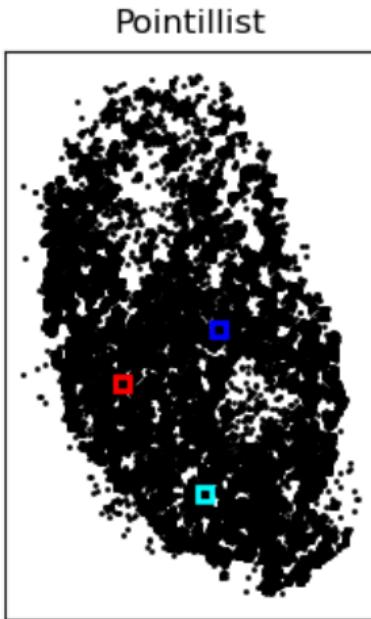
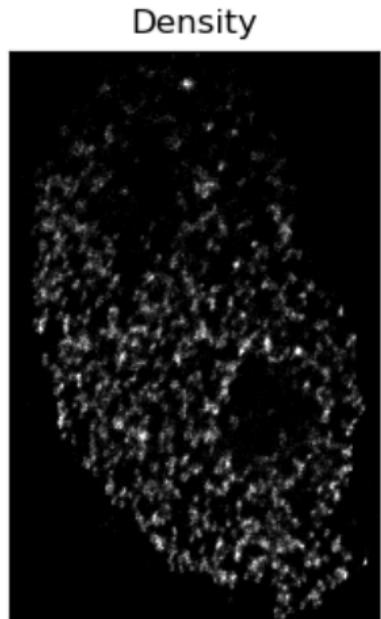


C

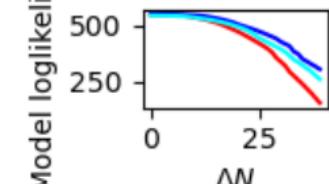


- ▶ $K(\lambda/2NA)$ is Ripley's K function at the diffraction limit ($\lambda = 640\text{nm}$)
- ▶ Convolutional neural networks (CNNs) approach the Cramer-Rao lower bound (gray)

Chromatin nanodomains in a living Hela cell nucleus



Model loglikelihood



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

Dense localization by fluorescence antibunching

If the emission from a single molecule represents a sub-Poisson process, then the detection process is also sub-Poisson, with a reduced rate.

Consider point processes $n_i(t)$ and $n_j(t)$. The second order coherence function reads

$$g_{ij}^2(\tau) = \frac{\langle n_i(t + \tau) n_j(t) \rangle}{\langle n_i(t) \rangle \langle n_j(t) \rangle}$$

$n_i(t)$ and $n_j(t)$ are parameterized by θ . Suppose they are Poisson processes, with rates that can be computed from θ e.g., $\mu_i = f(\theta)$ and $\mu_j = g(\theta)$. Then,

$$g_{ij}^2(0) = \frac{\langle n_i(t) n_j(t) \rangle}{\mu_i \mu_j} = 1$$

However, when they are sub-Poisson processes, then

$\langle n_i(t) n_j(t) \rangle \leq \langle n_i(t) \rangle \langle n_j(t) \rangle$. Why?

The presence of multiple emitters in the local region makes photons appear more “bunched” w.r.t these two detector elements i and j , and therefore $g_{ij}^2(0) > 0$. I expect we can use $g_{ij}^2(0)$ over 3x3 neighborhoods of pixels where i the center pixel.

Dense localization with fluorescence antibunching

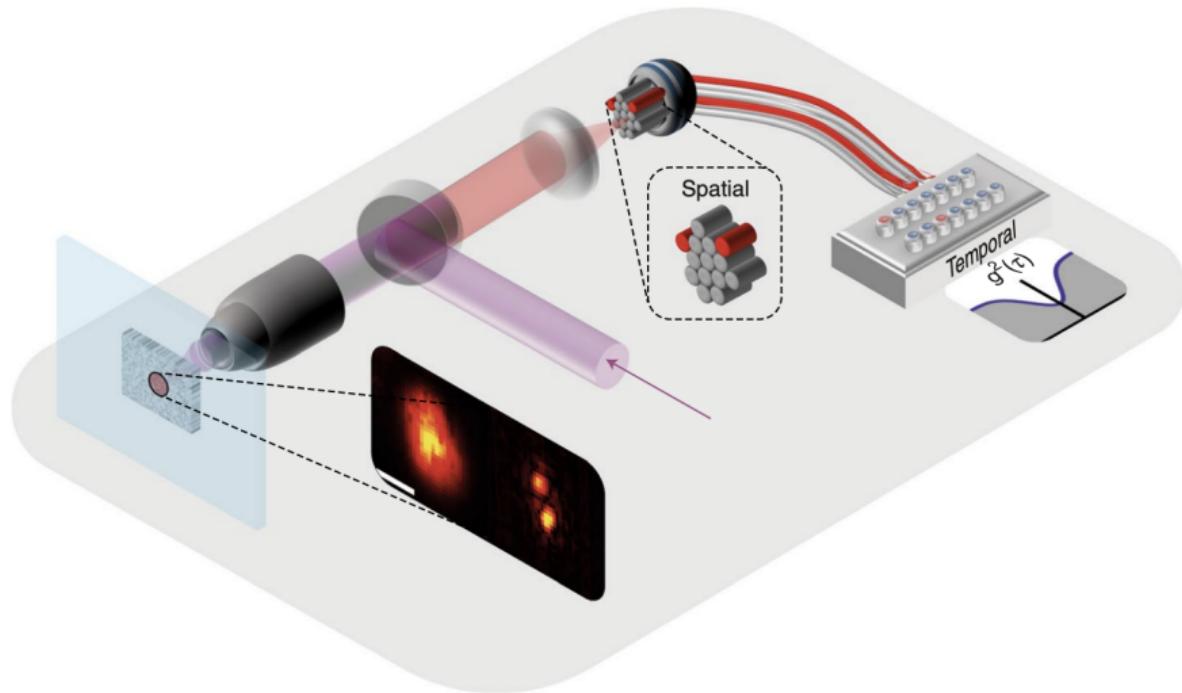
We require an estimate of emitter locations θ^* to approximate

- ▶ The number of photons collected in each pixel
- ▶ The second order coherence of photon counts in a pixel with its neighbors

Defining a likelihood on the entire time series is intractable. In intensity-based imaging, we would just analyze the sum of photon counts over time. However, the number of emitters is unknown *a priori*, which is part of the problem we are trying to solve. Instead we approximate the maximum likelihood estimate by a stochastic *online parameter estimation* procedure. I imagine emitters are added, if there is not reasonable probability that a photon was emitted by an existing one.

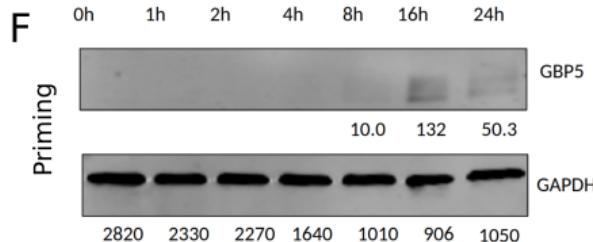
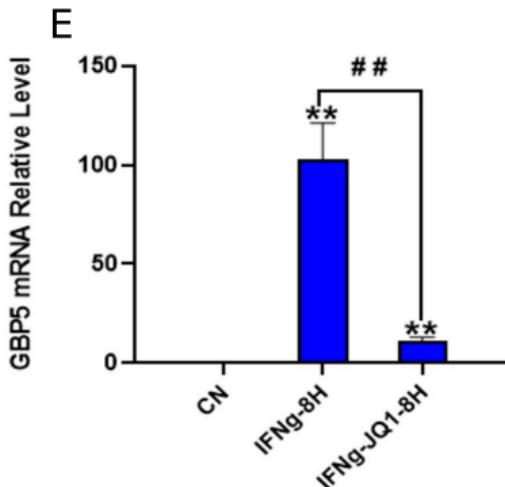
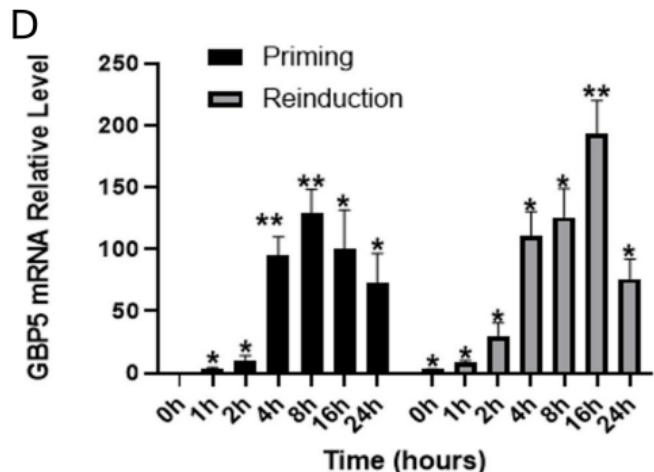
We are able to compute the likelihood of the time summed data and pairwise coherence calculations as the time series and optimization proceed.

Dense localization by fluorescence antibunching

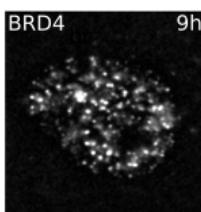


Andrew Forbes and Valeria Rodriguez-Fajardo. Super-resolution with quantum light. Nature Photonics 2019.

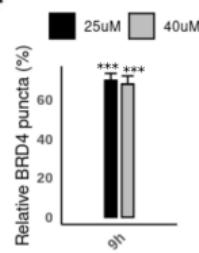
Inhibition of a super-enhanced gene with JQ1



G

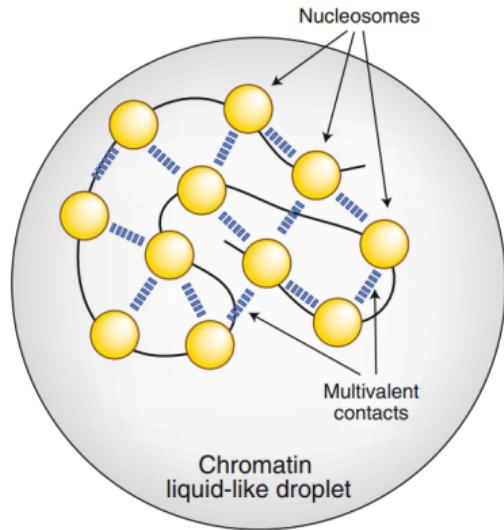


H



► *: $P \leq 0.1$, **: $P \leq 0.01$

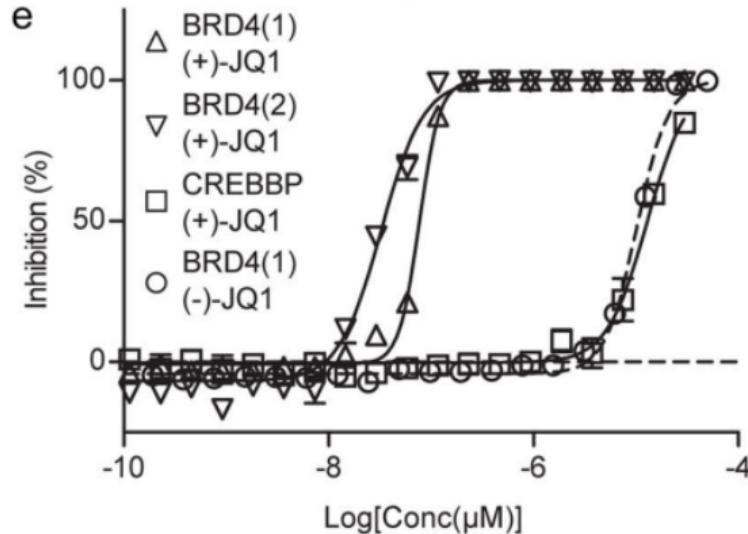
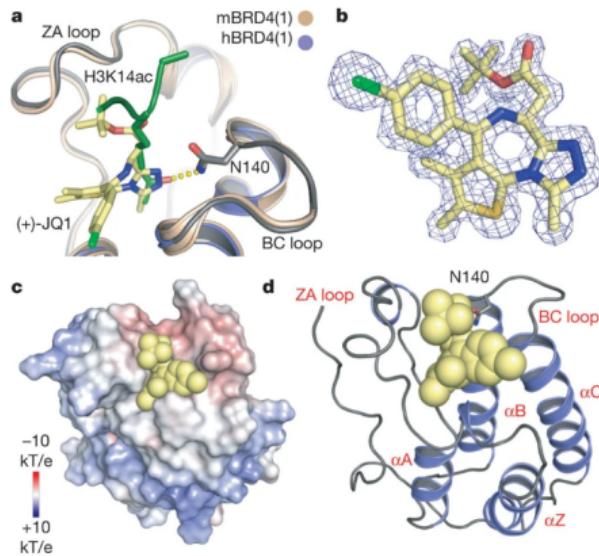
Chromatin has an intrinsic ability to undergo phase separation



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