Visualizing chromatin organization with single molecule localization microscopy

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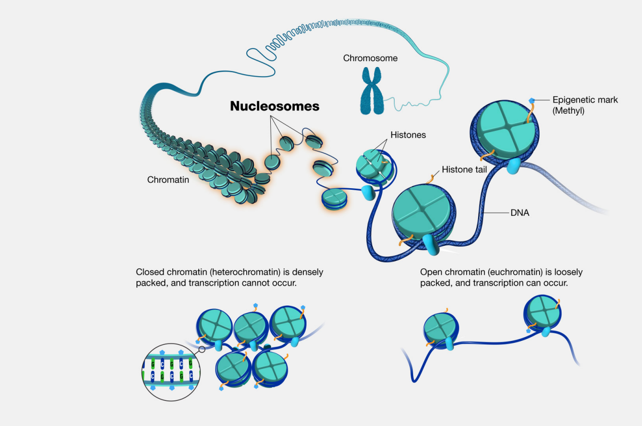
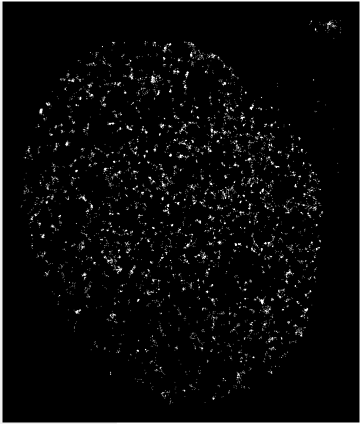
Outline

- Single molecule localization microscopy
  - The time resolution of *d*STORM
  - Dense localization with deep learning
  - Dense localization by fluorescence antibunching
- Phase separation of chromatin

Single molecule localization microscopy

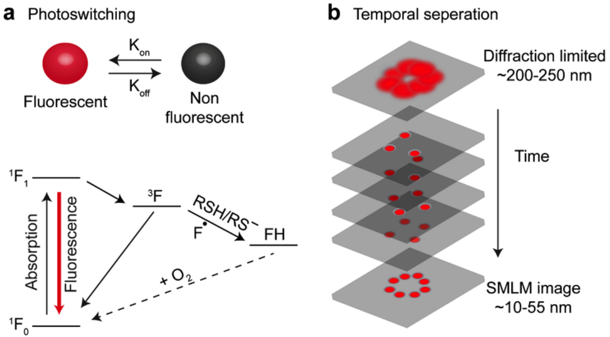
Genome organization and single molecule localization microscopy

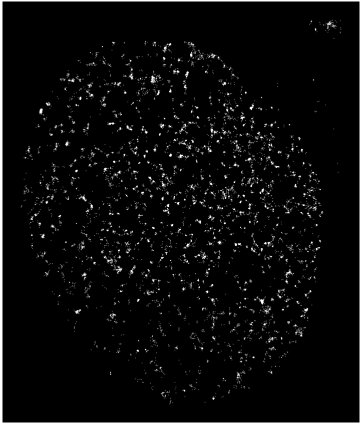




- Genome has a hierarchical structure, fundamental unit is the nucleosome
- We study chromatin organization by localizing fluorescently tagged nucleosomes

Single molecule localization microscopy





- SMLM techniques are diffraction-unlimited
- Photoswitching enables resolution of emitters in time rather than space

Single molecule localization microscopy

Modeling the point spread function permits sub-pixel localization

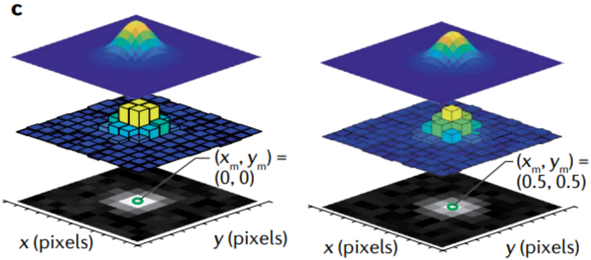
$$\mu_k = i_0 \int_{\mathrm{k}} h_\theta(x_0, y_0)\,dxdy$$

$$i_0 = g_k \eta N_0 \Delta$$

η – quantum efficiency

N_0 – photon count

Δ – exposure time



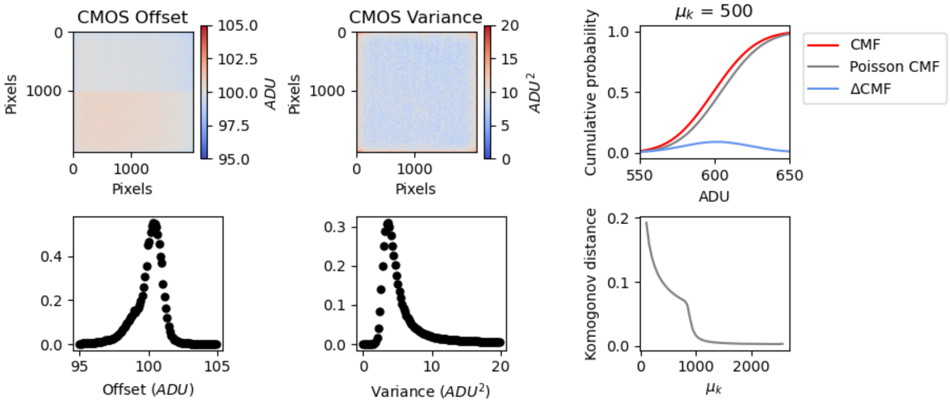
Long $\Delta \rightarrow$ pixels are iid:

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

What is $P(H_k|\theta)$?

Classical emission statistics of fluorescent markers

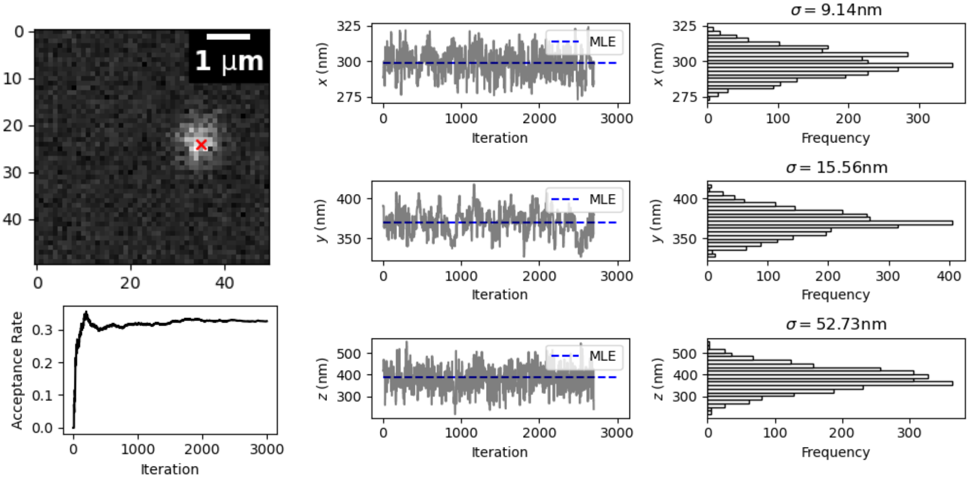
Long integration times $\Delta \rightarrow$ intensity fluctuations are Poisson



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

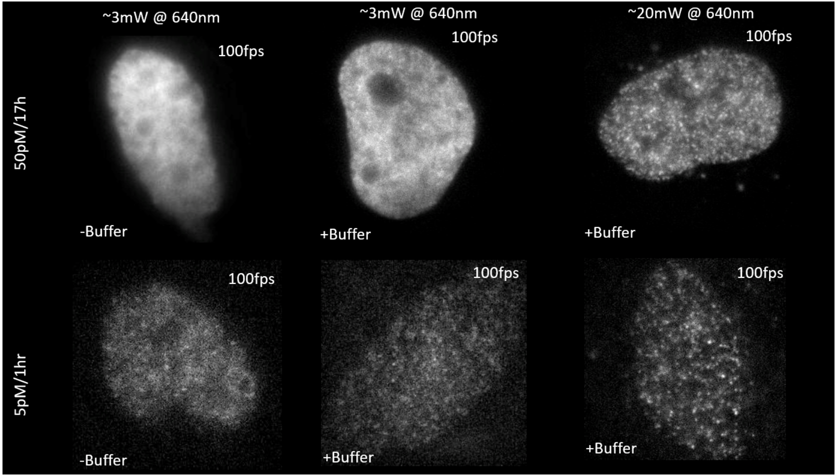
$P(H_k|\theta)$ can be approximated as Poisson at high signal-to-noise (SNR)

Estimator precision determines resolution in localization microscopy



- One can derive a lower bound on the variance of a statistical estimator of the coordinates θ

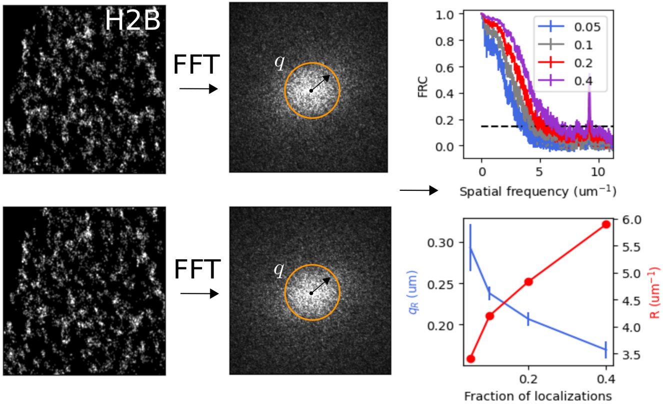
Dense labeling of histone H2B in fixed cells at RT



- 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

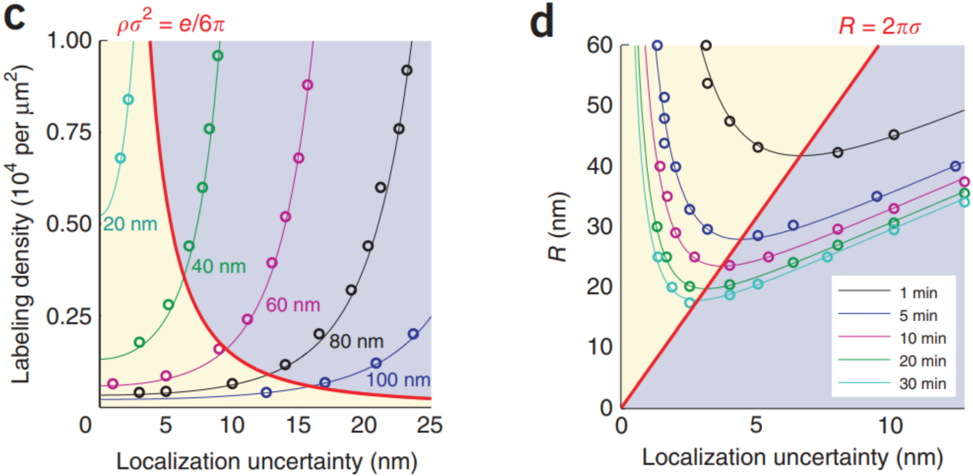
Dense localization increases time resolution

- We can view dSTORM as sampling from a density



$$\mathrm{FRC}(q) = \frac{\sum_{\vec{q}\in\mathrm{circle}} \tilde{f}_1(\vec{q})\tilde{f}_2(\vec{q})^*}{\sqrt{\sum_{\vec{q}\in\mathrm{circle}} |f_1(\vec{q})|^2}\sqrt{\sum_{\vec{q}\in\mathrm{circle}} |f_2(\vec{q})|^2}}$$

Dense localization increases time resolution

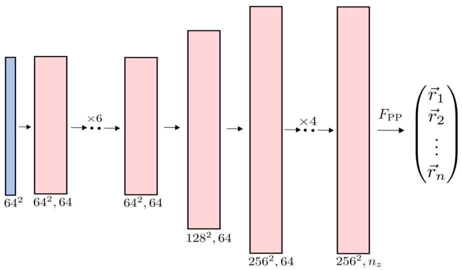


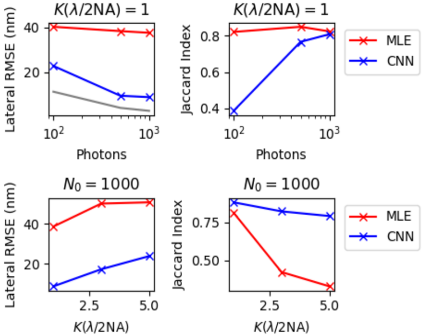
Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

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

Dense localization with deep learning

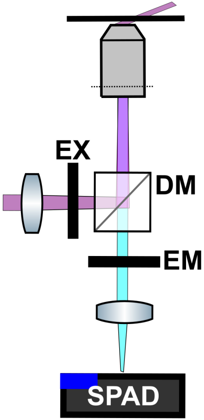
- Define Z as a high resolution binary image: $Z_k = 1$ if pixel k contains an emitter
- We can model $P_\Psi(Z)$ with a convolutional neural network Ψ

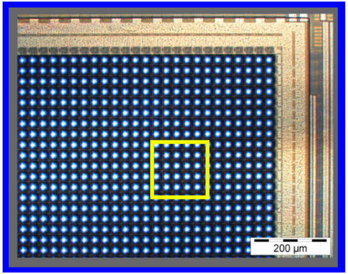


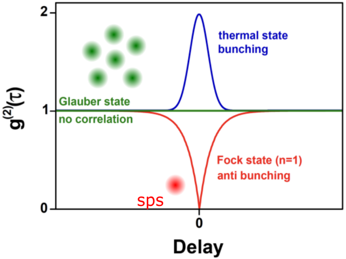


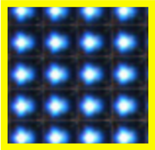
- Convolutional neural networks (CNNs) approach the Cramer-Rao lower bound (gray)

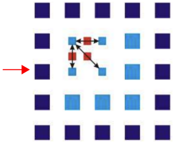
Dense localization with a photon counting camera











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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 $\alpha_i \text{ AND } \alpha_j = 0$

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

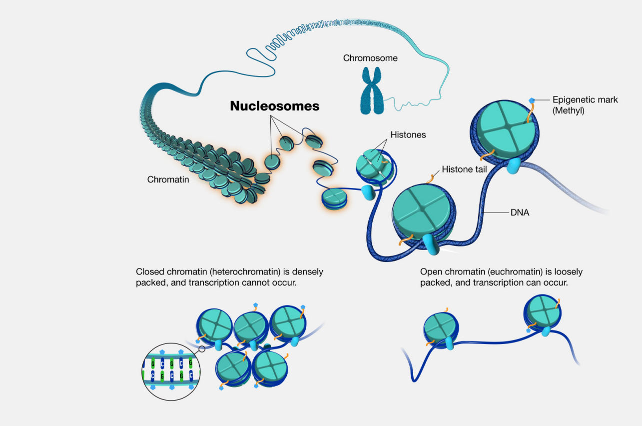
But now consider

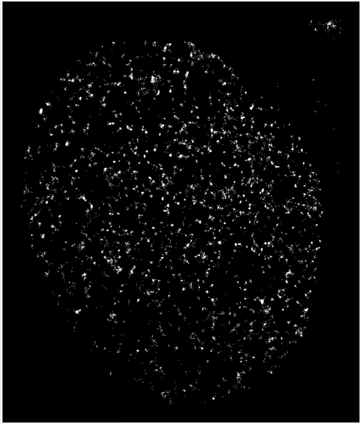
$$\langle X_i X_j \rangle = \sum_{(N_i, N_j)} N_i N_j P(X_i = N_i, X_j = N_j)$$

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).

Phase separation of chromatin

Genome organization and single molecule localization microscopy





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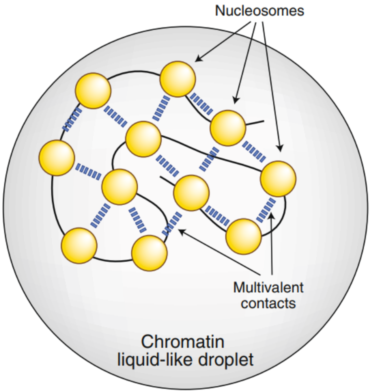
Chromatin has an intrinsic ability to undergo phase separation



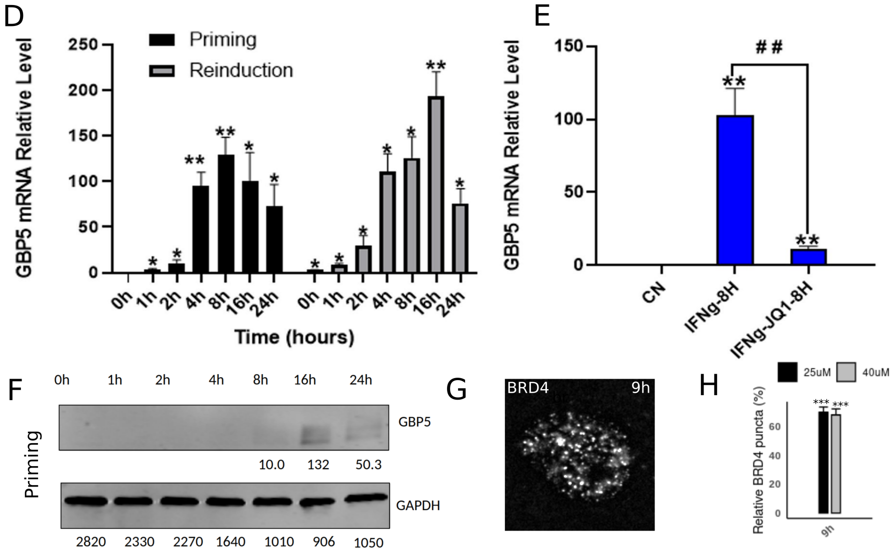
Regulatory factors of chromatin LLPS
Histone H1
DNA length between nucleosomes
Histone post-translational modifications
Nucleosome dynamics
Multivalent binding of proteins

- Super-enhanced genes are regulated by large molecular assemblies
- We study nucleosome clustering dynamics using super-resolution microscopy

BET inhibitors reduce nucleosome-BRD4 interactions in BRD4 condensates

BET inhibitors promote disordered BRD4 condensates

Inhibition of a super-enhanced gene with JQ1



- $*$: $P \leq 0.1$, $**$: $P \leq 0.01$