

# Advancing super resolution microscopy for quantitative in-vivo imaging of chromatin nanodomains

Clayton W. Seitz

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# Outline

Single molecule localization microscopy

The time resolution of *d*STORM

A novel approach to dense localization microscopy

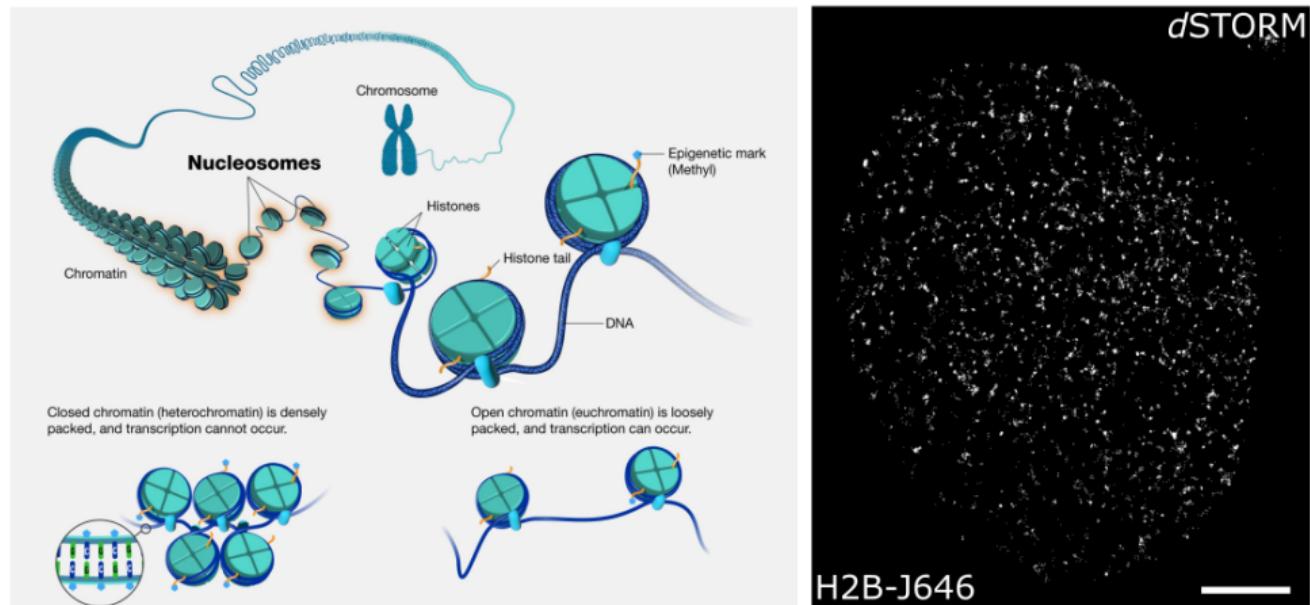
Dense localization by fluorescence antibunching

Phase separation of chromatin

Future Aims

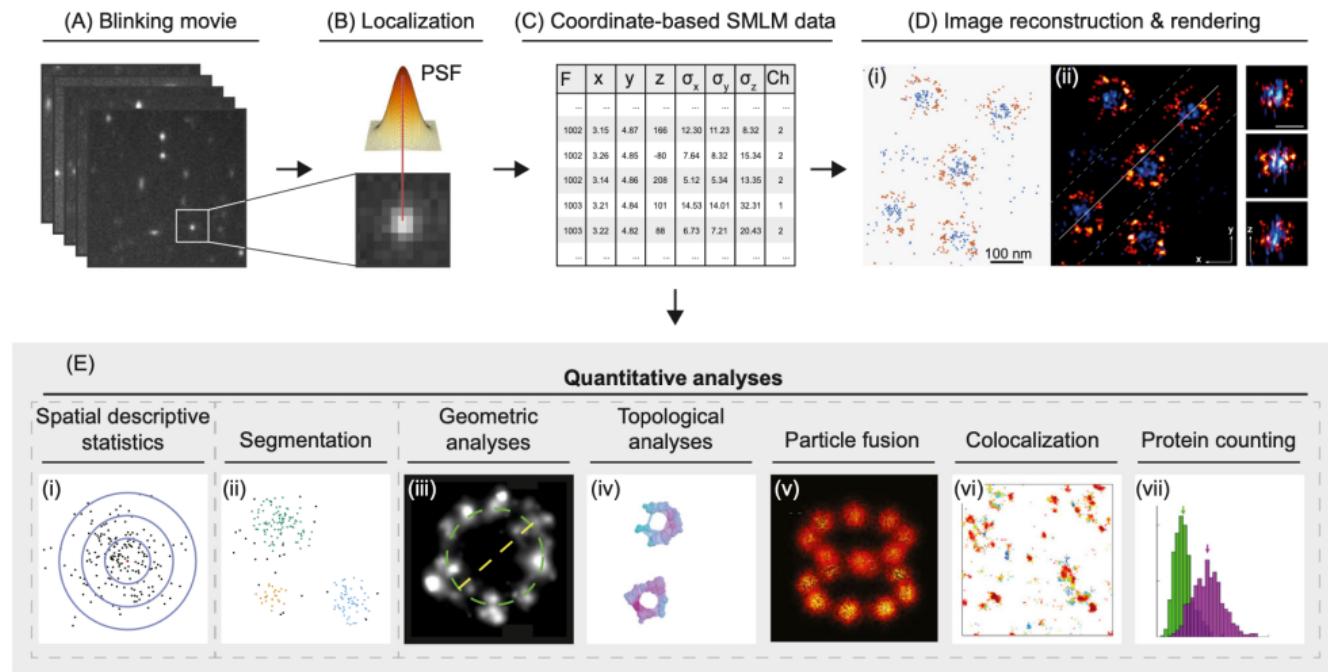
## Single molecule localization microscopy

# Genome organization and super resolution imaging



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

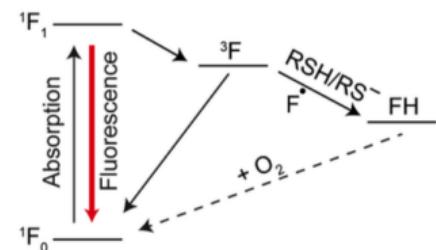
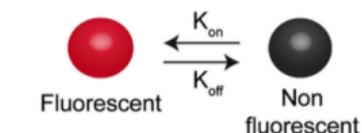
# Single molecule localization microscopy



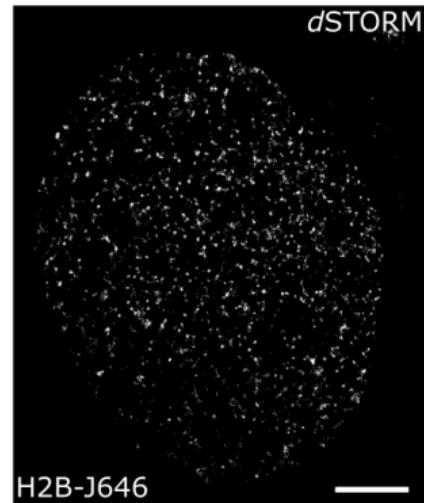
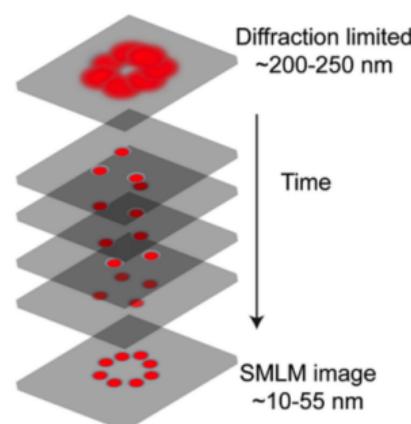
*Wu et al. Quantitative Data Analysis in Single-Molecule Localization Microscopy.*

# Single molecule localization microscopy

## a Photoswitching



## b Temporal separation



- ▶ SMLM techniques are diffraction-unlimited
- ▶ Photoswitching enables resolution of emitters below the diffraction limit

# Single molecule localization microscopy

Modeling the point spread function permits sub-pixel localization

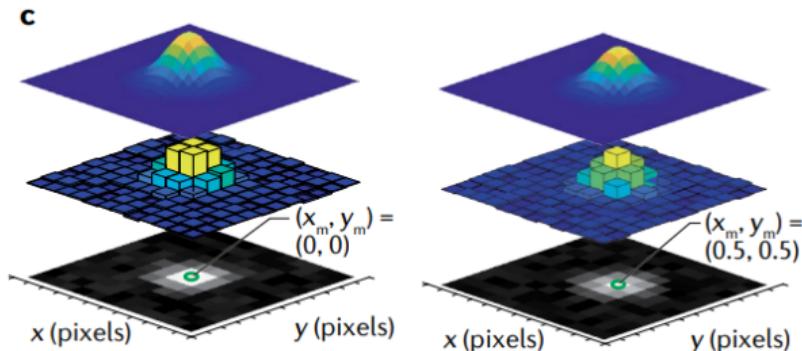
$$\mu_k = i_0 \int_{\mathbb{K}} h_\theta(x_0, y_0) dx dy$$

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

$\eta$  – quantum efficiency

$N_0$  – photon count

$\Delta$  – exposure time



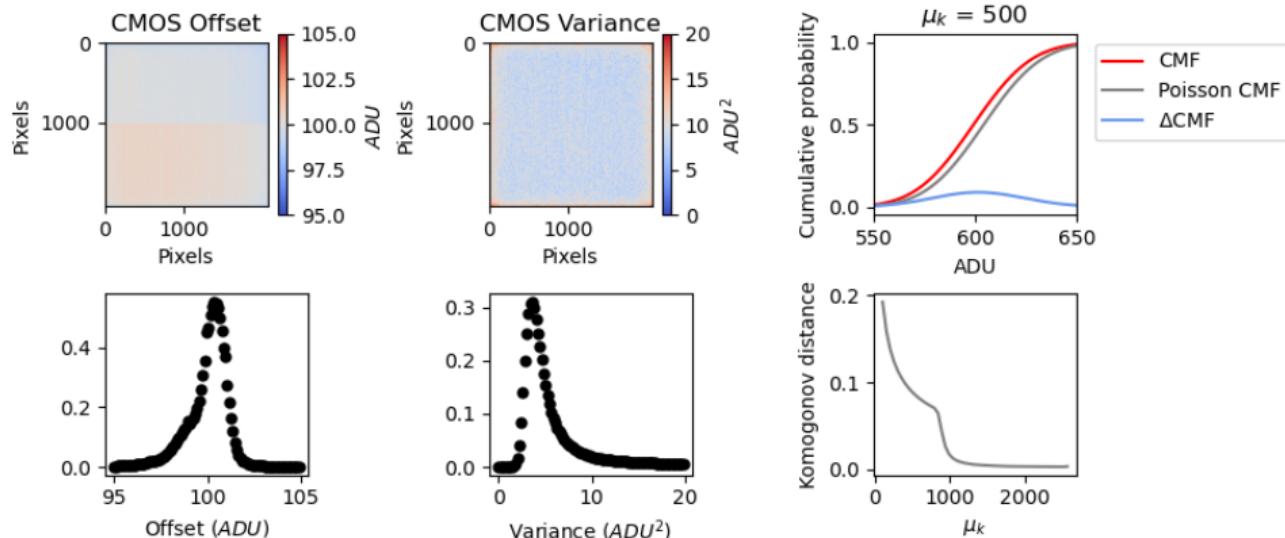
Long  $\Delta \rightarrow$  pixels are iid:

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

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

# Photon statistics of fluorescent markers

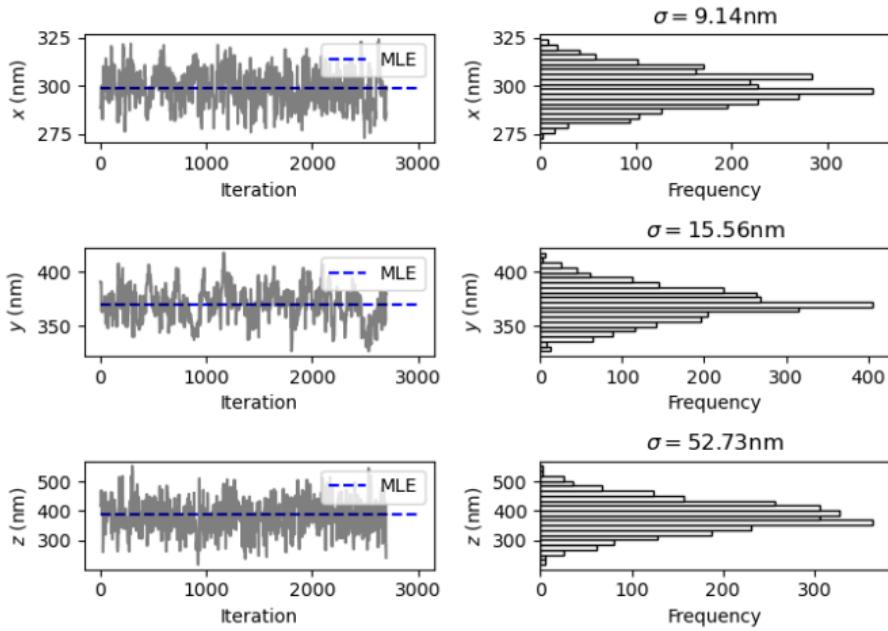
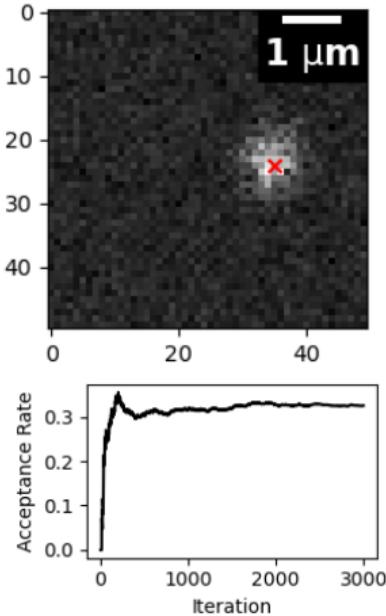
Long integration times  $\Delta \rightarrow$  intensity fluctuations are Poisson



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

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

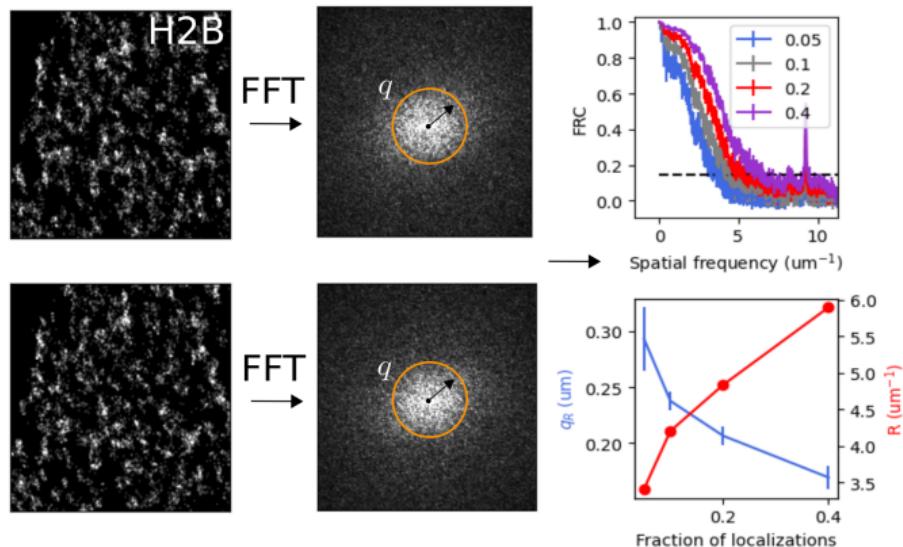
# The definition of resolution in SMLM



- One can derive a lower bound on the variance of a statistical estimator of the coordinates  $\theta$

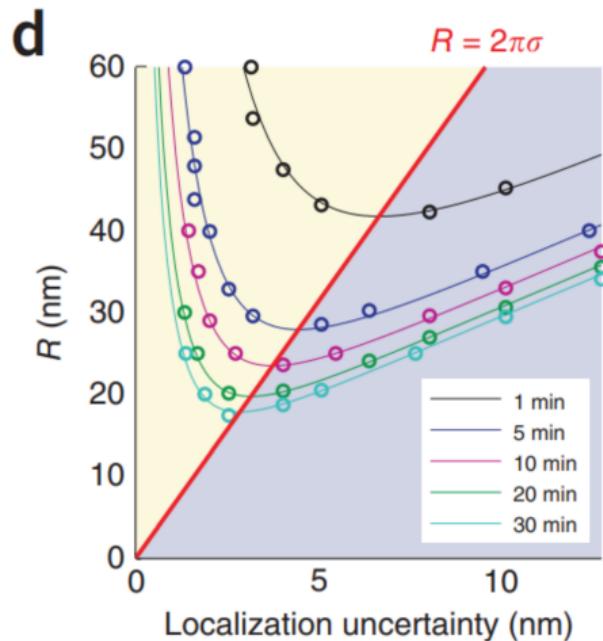
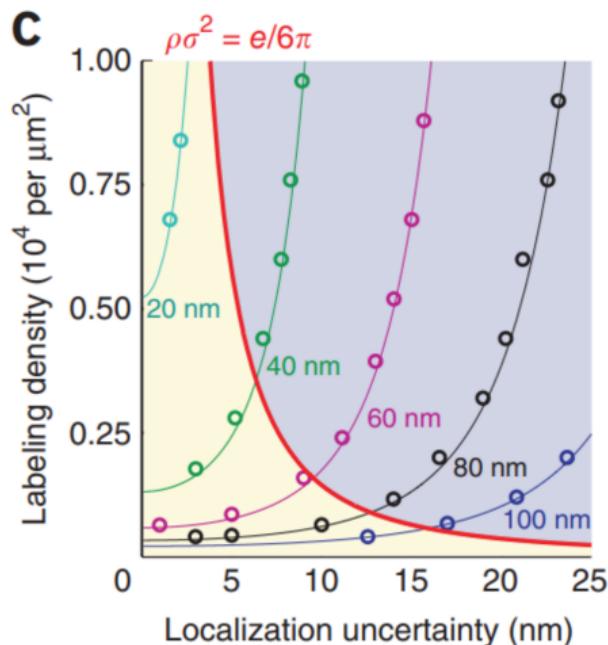
# Dense localization increases time resolution

- We can view dSTORM as sampling from a density



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

## Dense localization increases time resolution

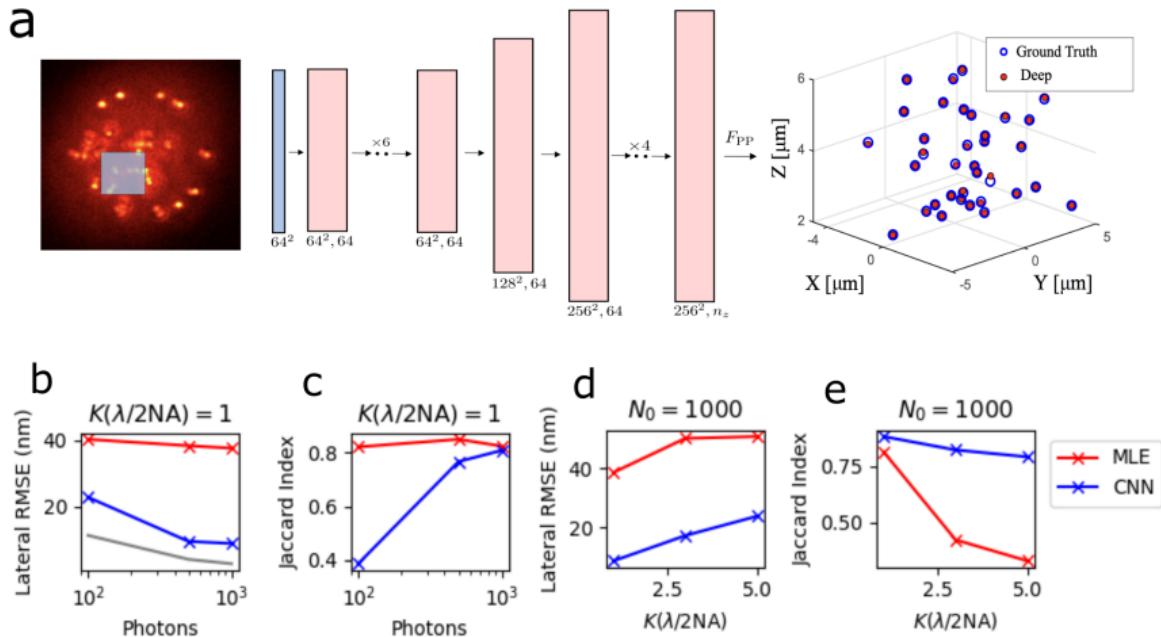


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

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

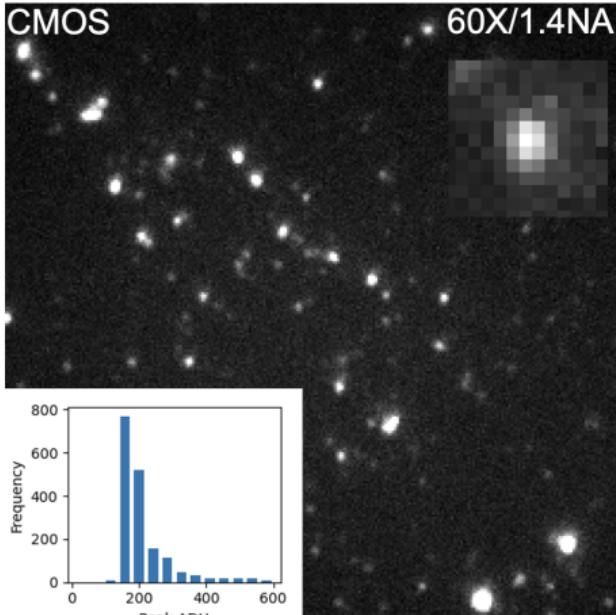
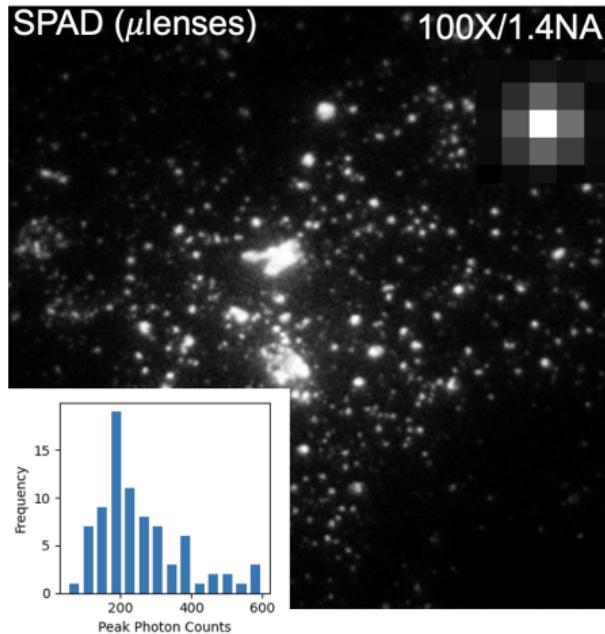
## A novel approach to dense localization microscopy

# An early approach to dense SMLM with deep learning



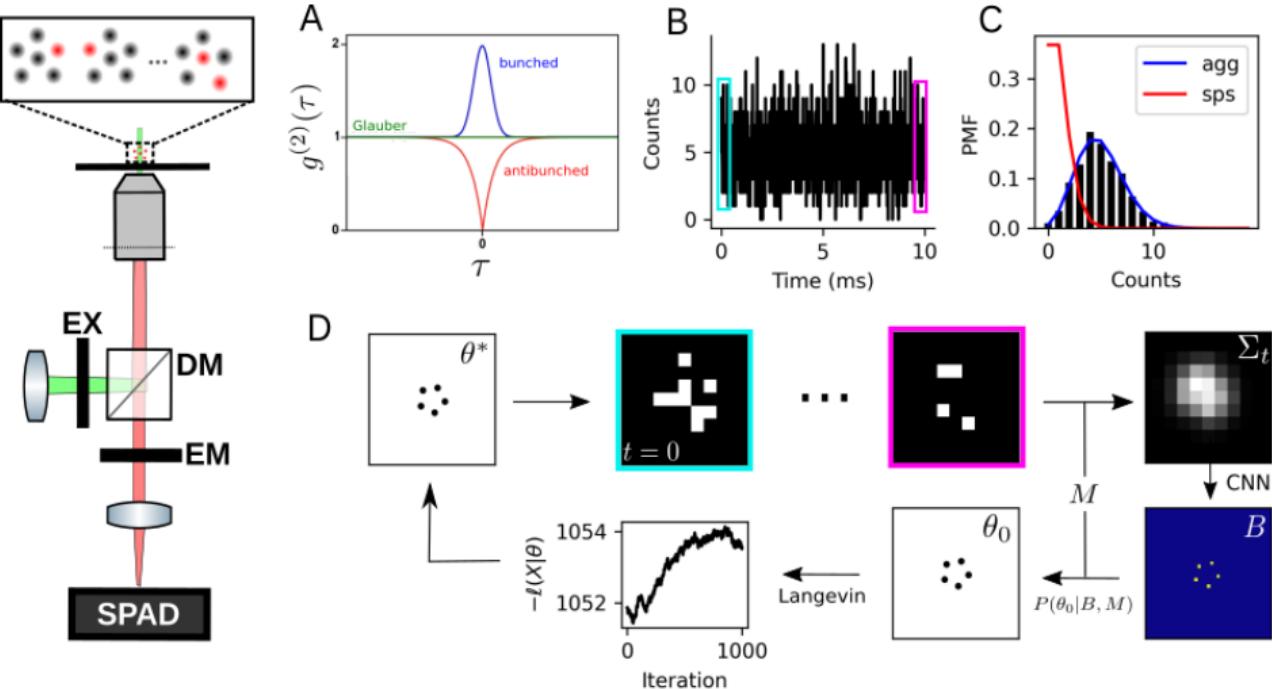
- ▶ MLE in high dimensional spaces can quickly become intractable
- ▶ We can model  $P_\Psi(\theta_0)$  with a convolutional neural network  $\Psi$

# High speed imaging for enhanced SMLM



- ▶ SPAD frame is sum of  $10^4$  1us exposures
- ▶ This inspires counting molecules in widefield images for enhanced SMLM

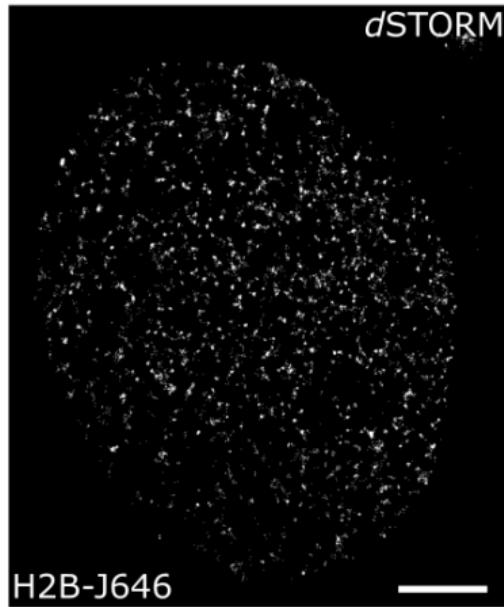
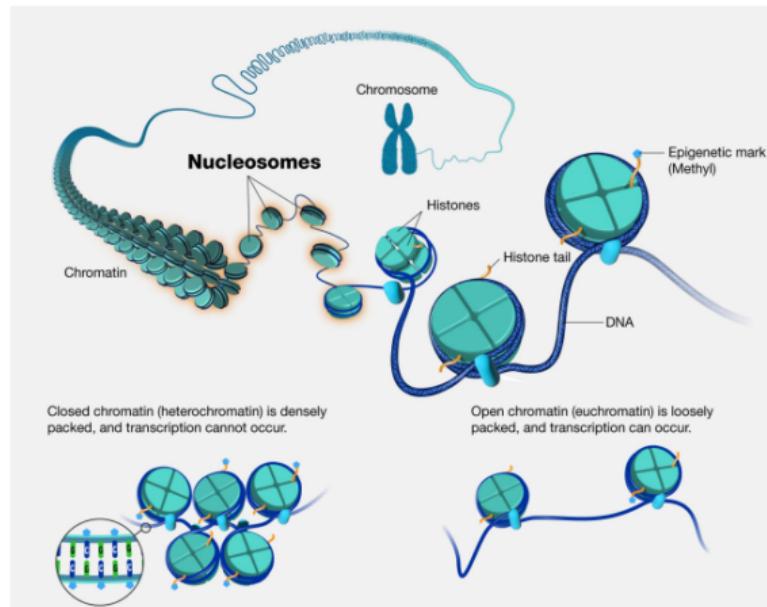
# Single photon counting for enhanced SMLM



- At higher time resolution we can sample from this Poisson PMF 100-1000s of times in a single 10ms exposure

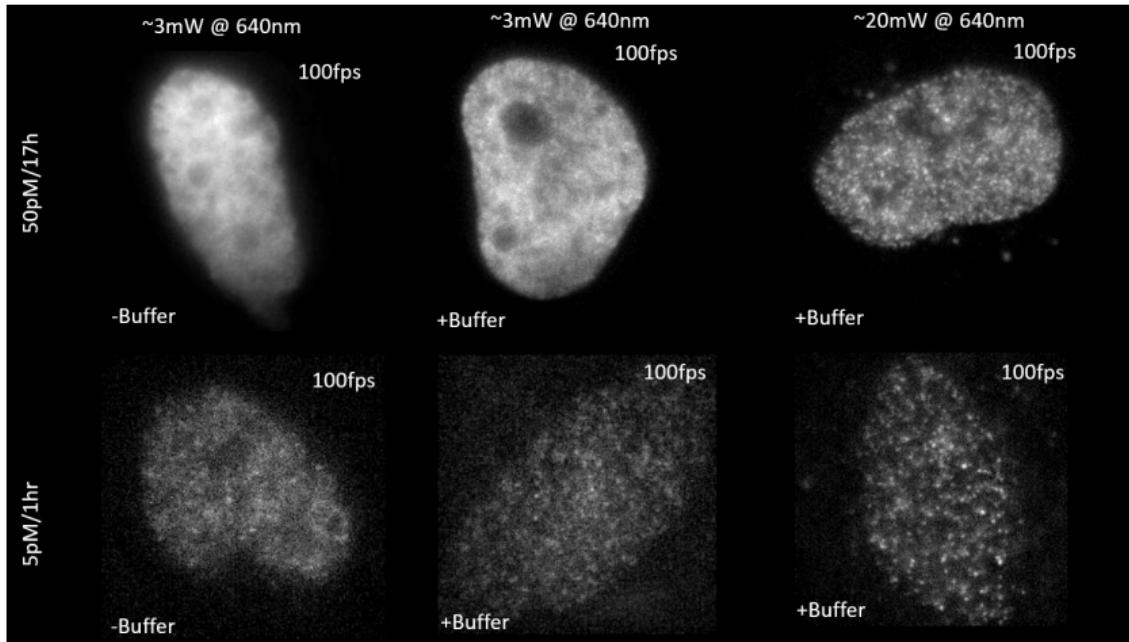
## Phase separation of chromatin

# Genome organization and single molecule localization microscopy



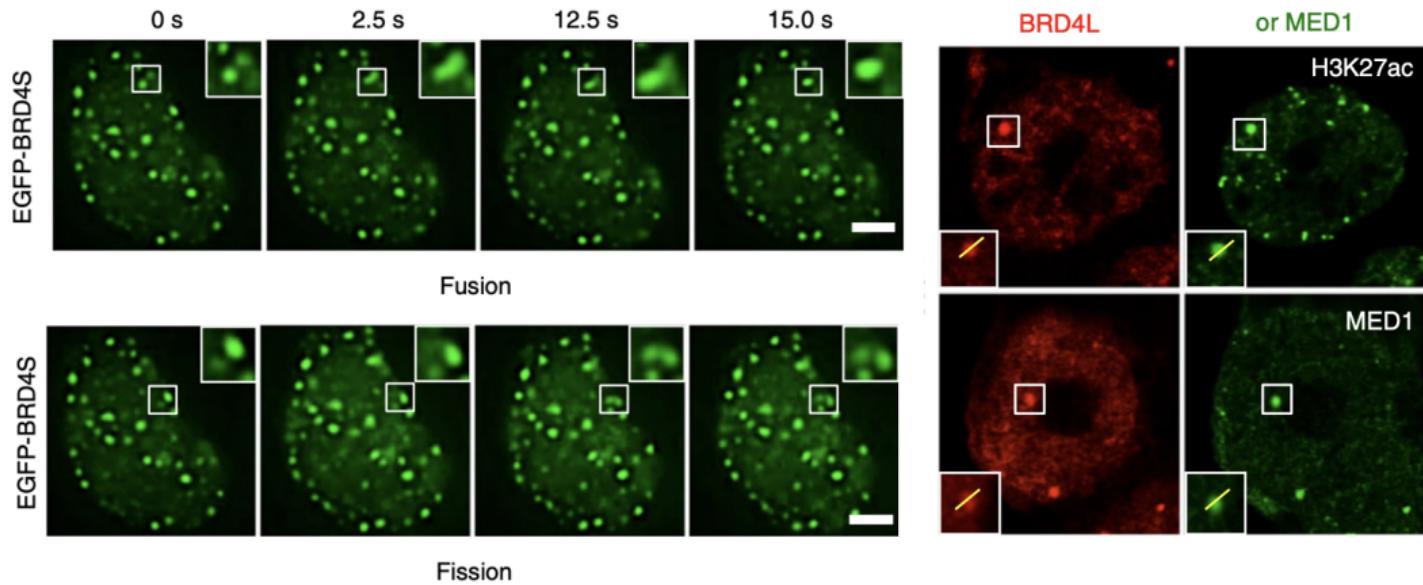
- ▶ Genome has a hierarchical structure, fundamental unit is the nucleosome
- ▶ We study chromatin organization by localizing fluorescently tagged nucleosomes

# Dense labeling of histone H2B in fixed cells at RT



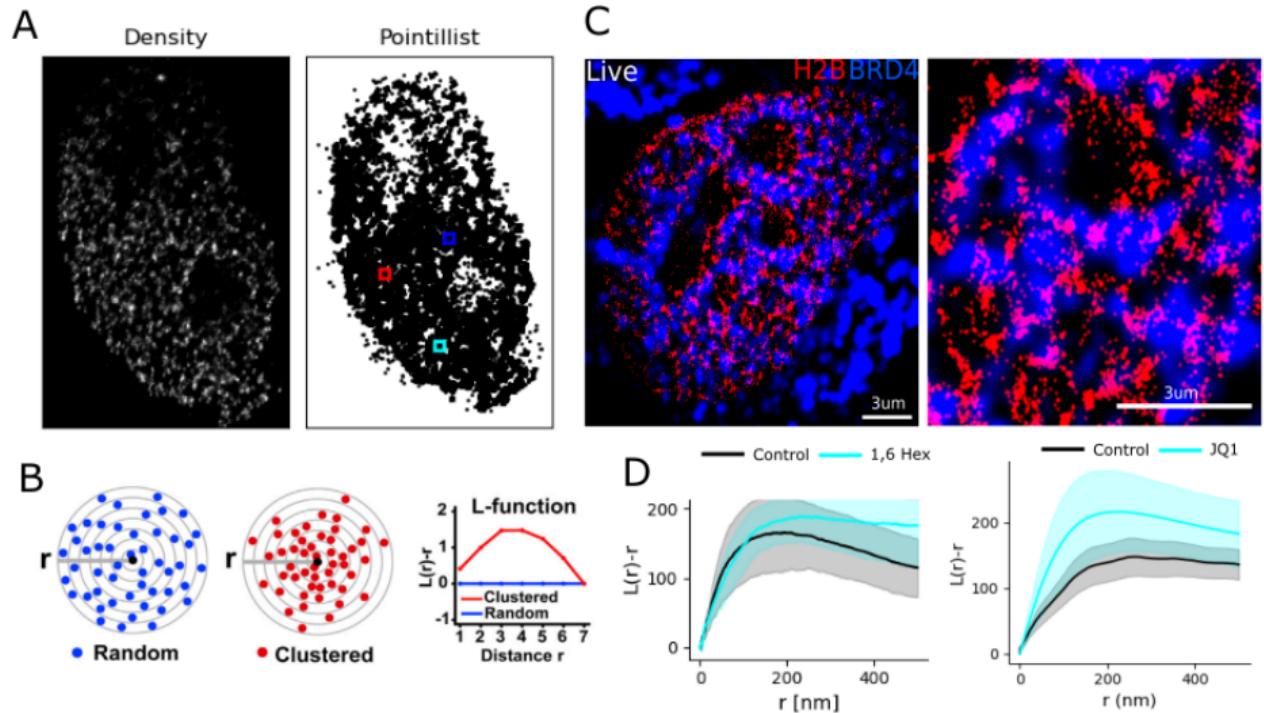
- ▶ Dense labeling of H2B-Halotag w/ fluorescent ligand JF646
- ▶ Reducing buffer is usually a primary thiol like cysteamine (MEA)

# BRD4 condensates exhibit LLPS properties

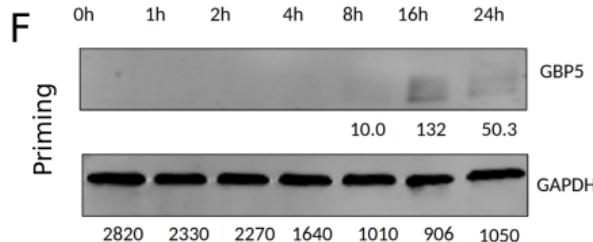
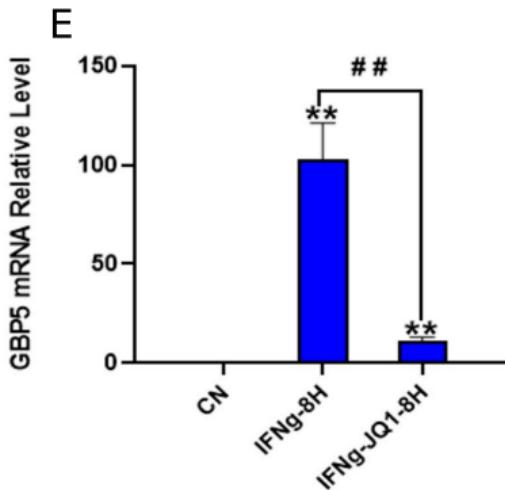
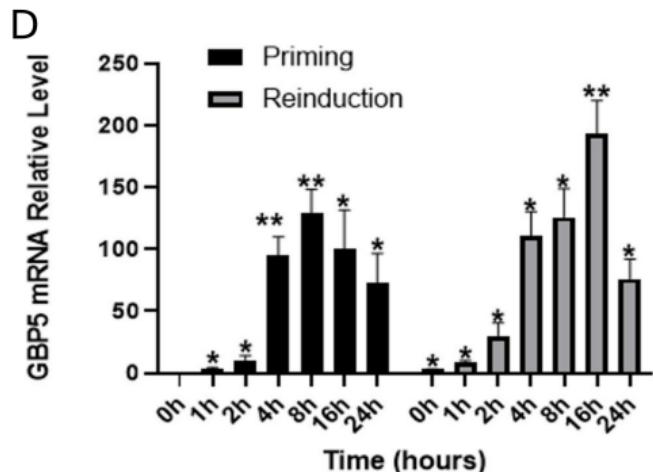


Han et al. Roles of the BRD4 short isoform in phase separation and active gene transcription. *Nature Structural and Molecular Biology*. 2020

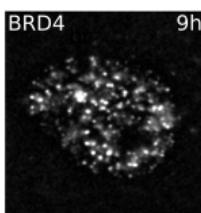
# BET inhibitors reduce nucleosome-BRD4 interactions in BRD4 condensates



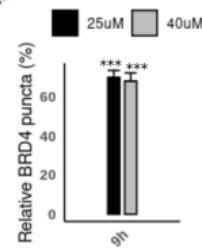
# Inhibition of a super-enhanced gene with JQ1



G



H



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

## Future Aims