

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

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# Outline

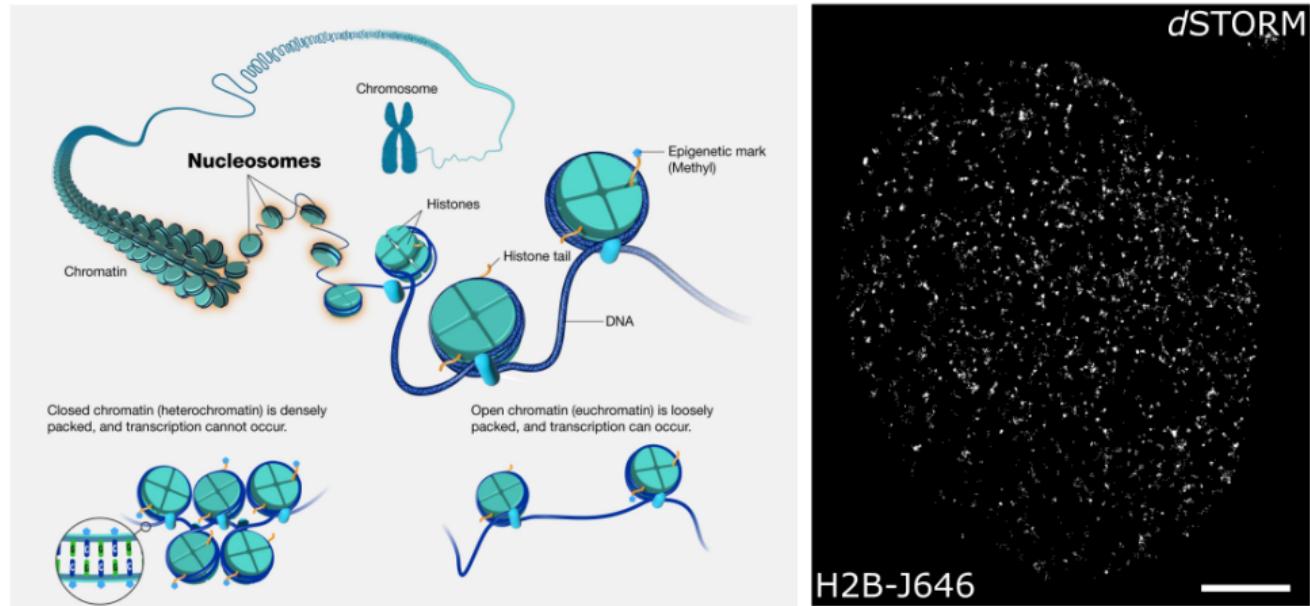
Single molecule localization microscopy (SMLM)

A novel approach to dense localization microscopy

Probing chromatin structure with SMLM

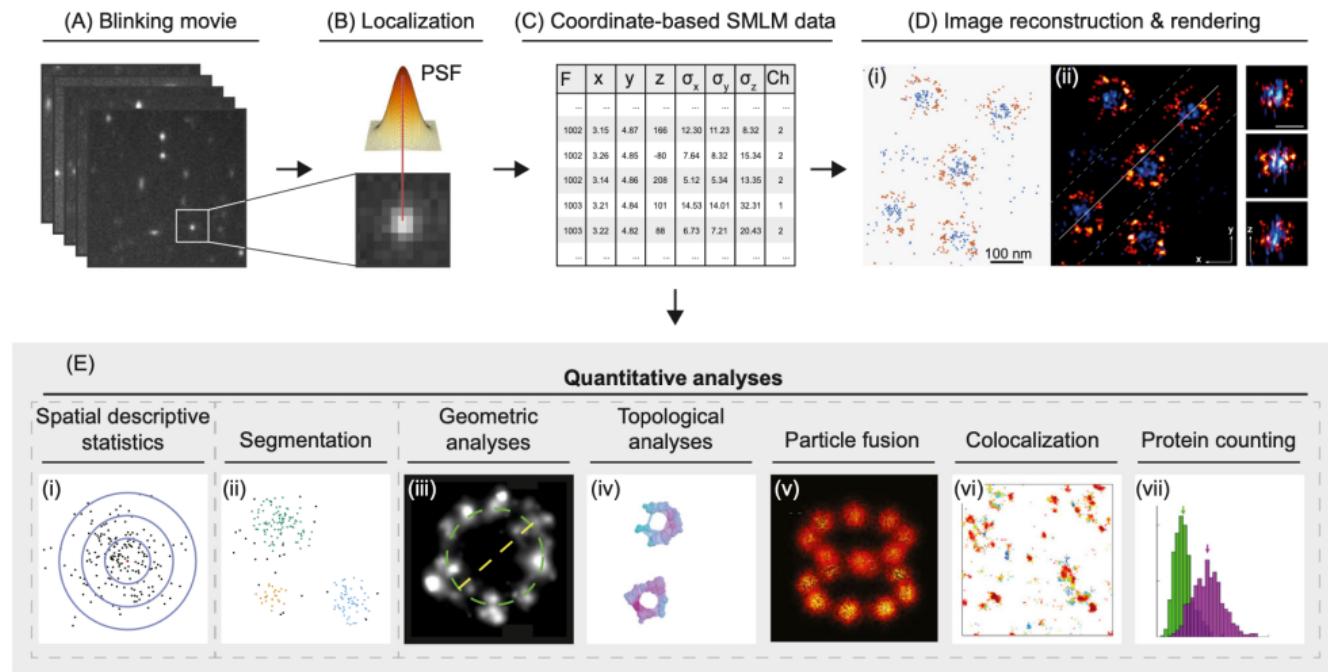
## Single molecule localization microscopy (SMLM)

# 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 and its applications

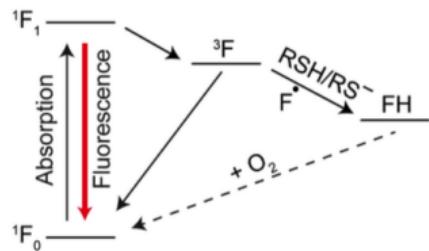
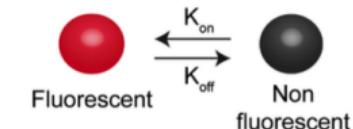


Trends in Cell Biology

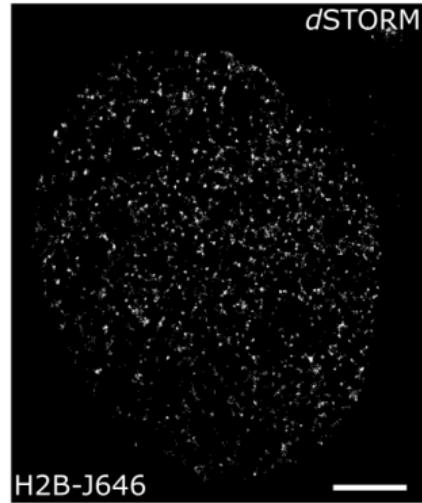
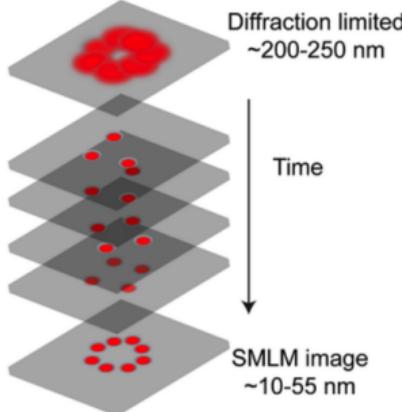
Wu et al. Quantitative Data Analysis in Single-Molecule Localization Microscopy.

# Direct stochastic optical reconstruction microscopy (dSTORM)

## 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

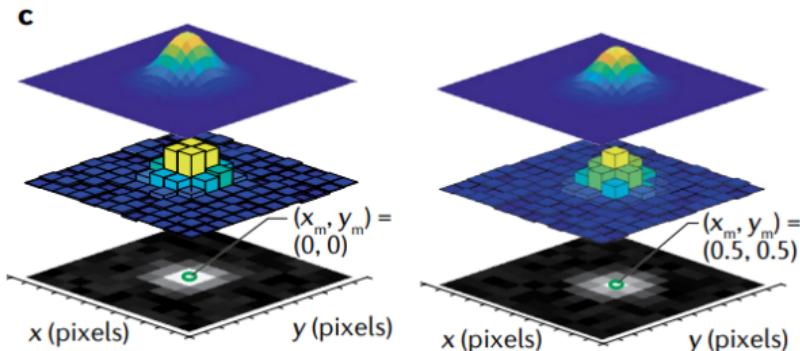
$$\mu_k = i_0 \int_{\mathbf{k}} h_{\theta}(x_0, y_0) dx dy$$

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

$\eta$  – quantum efficiency

$N_0$  – photon count

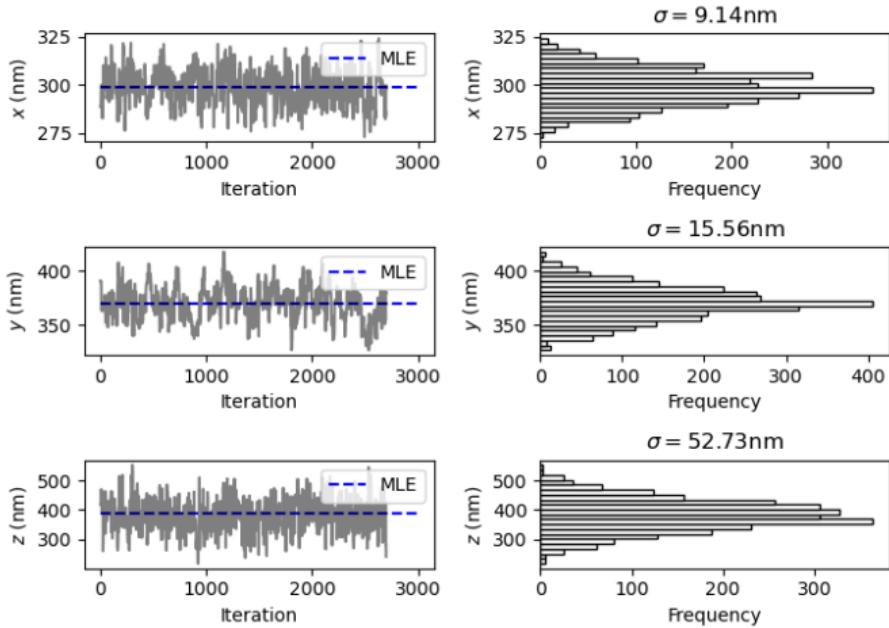
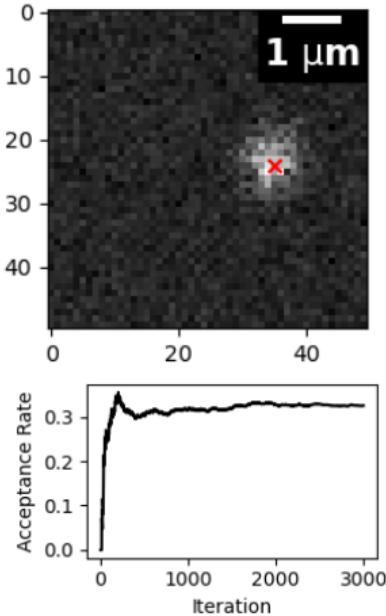
$\Delta$  – exposure time



Assume  $\mu_k$  is constant over  $\Delta$  (homogeneous Poisson)

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

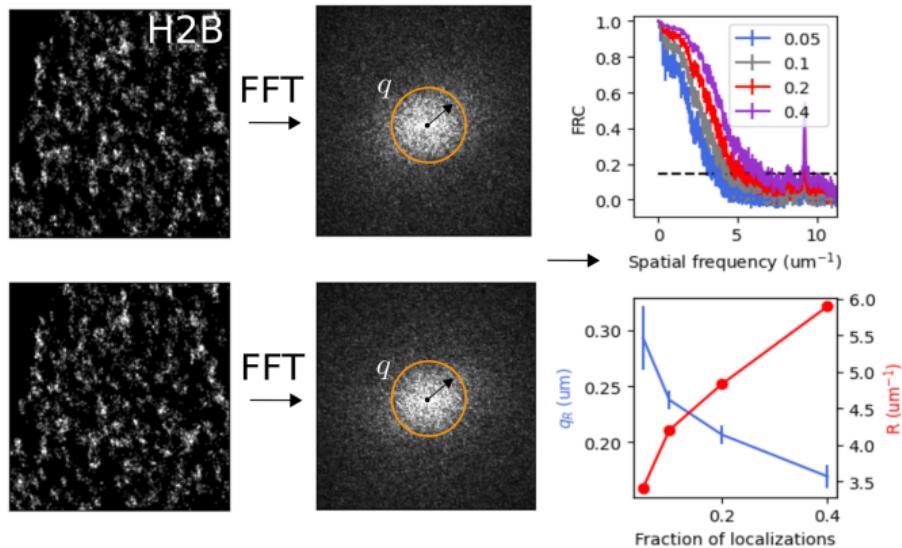
# The definition of resolution in SMLM



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

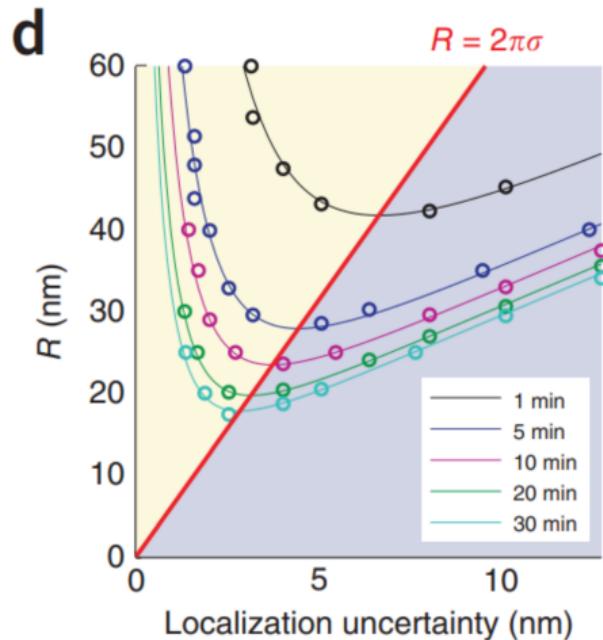
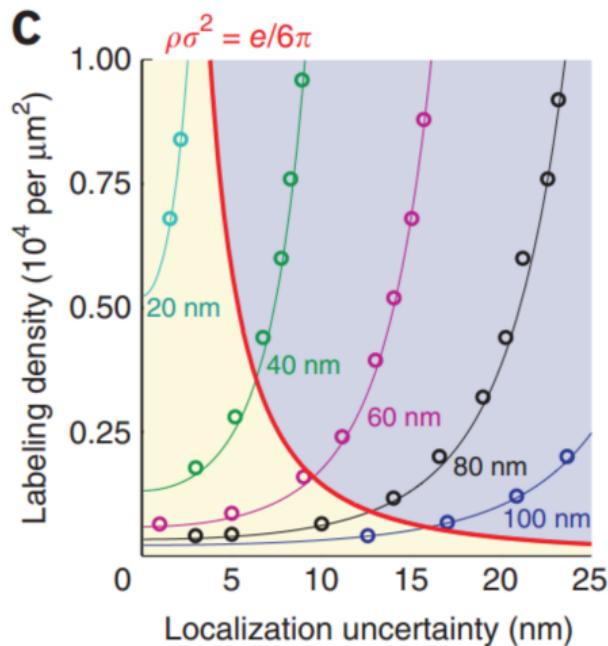
# Dense localization increases time resolution

- 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

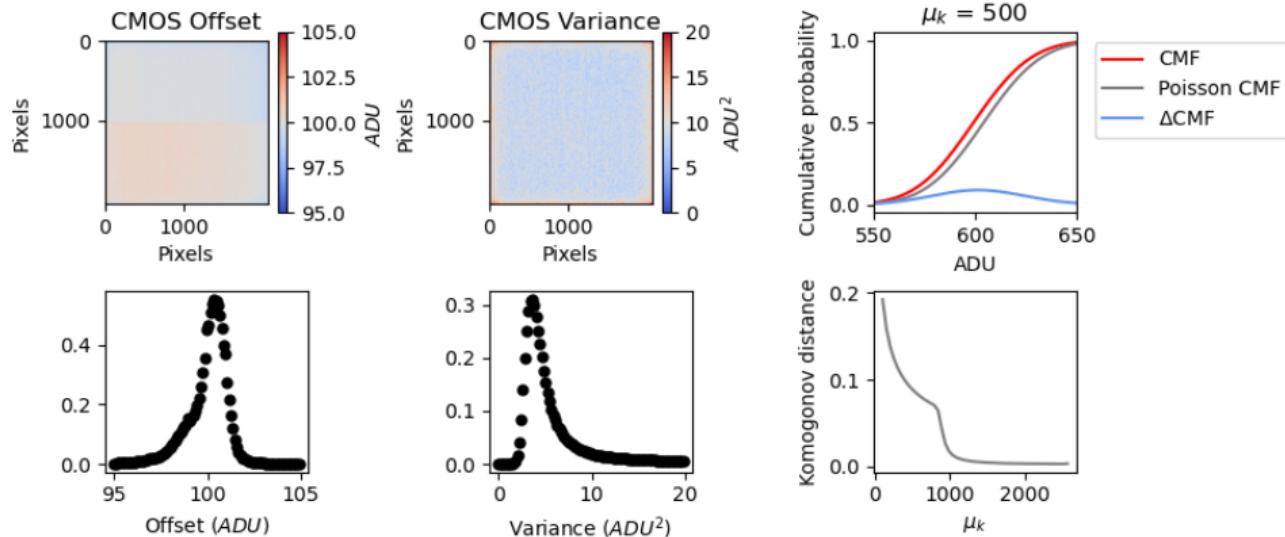


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

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

## A novel approach to dense localization microscopy

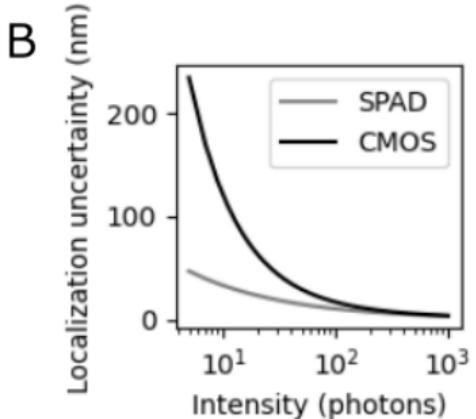
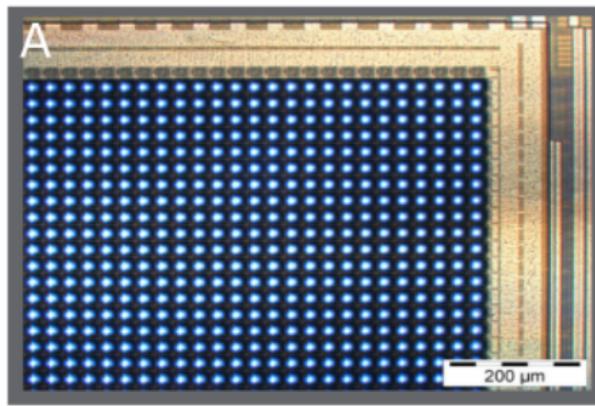
# CMOS readout noise limits SMLM



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

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

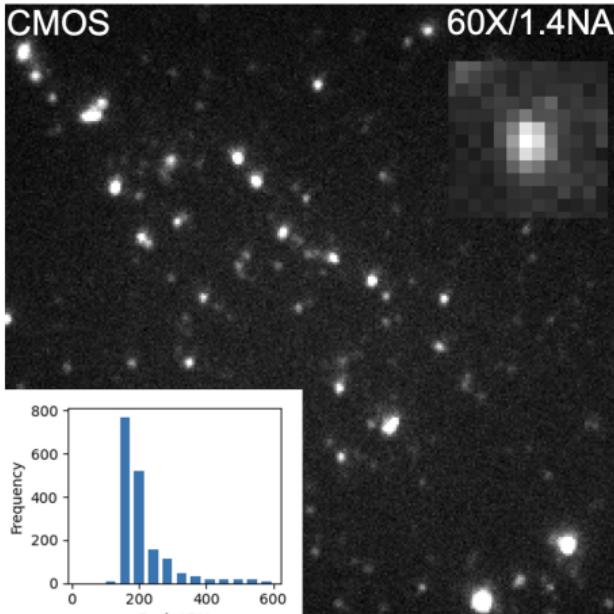
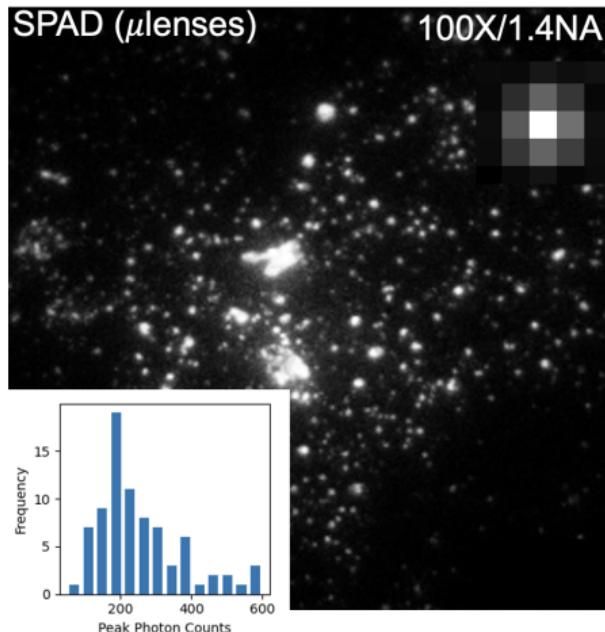
# Single photon avalanche diode (SPAD) cameras



(A) Courtesy of Pi imaging technologies

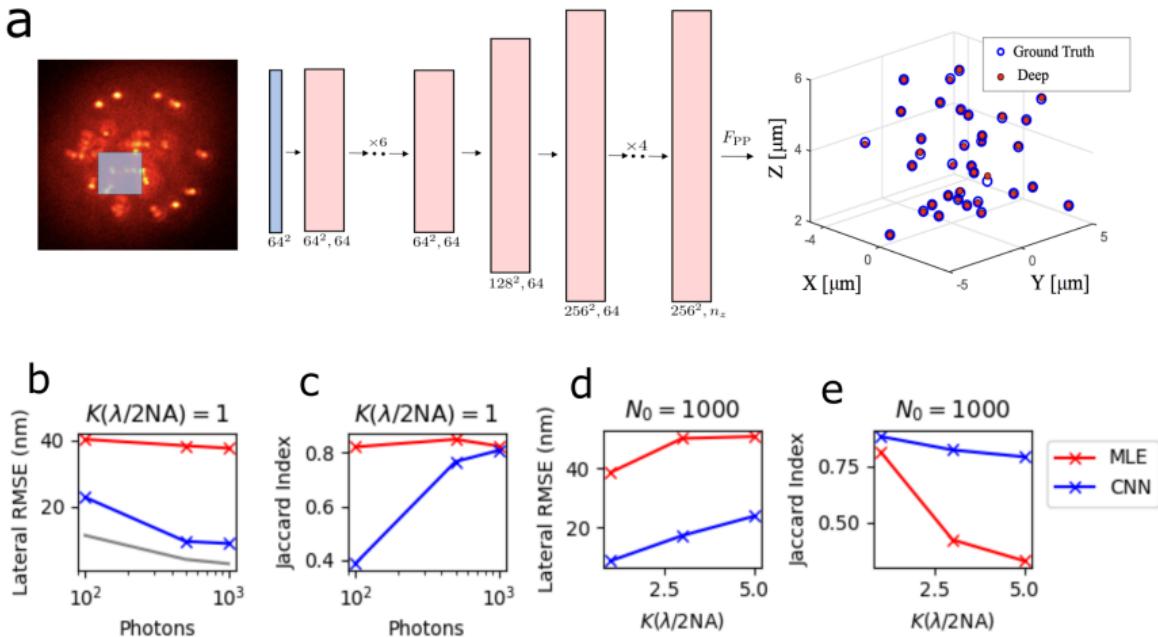
- ▶ Poisson statistics at any signal level (zero readout noise)
- ▶ Reduced quantum efficiency ( $\eta \approx 0.5$ ), but frame rates up to 1MHz

# High speed imaging for enhanced SMLM



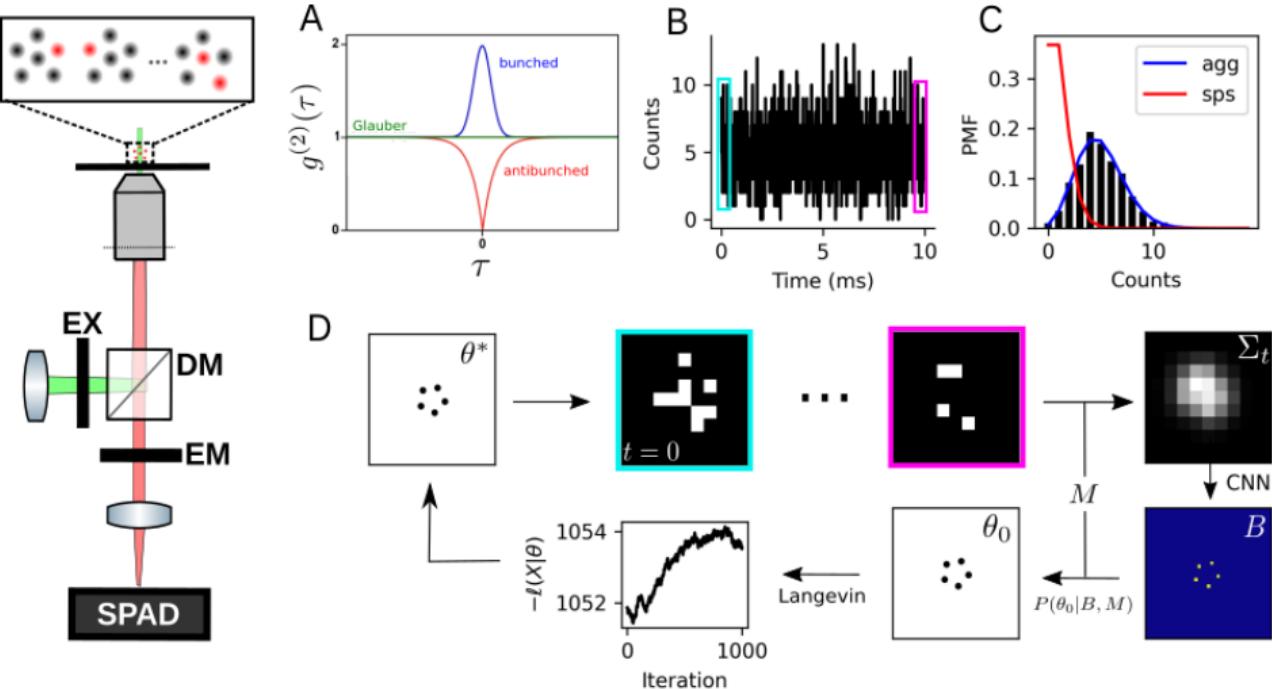
- ▶ SPAD frame is sum of  $10^4$  1us exposures
- ▶ This inspires counting molecules in widefield images for enhanced SMLM

# 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  $\Psi$

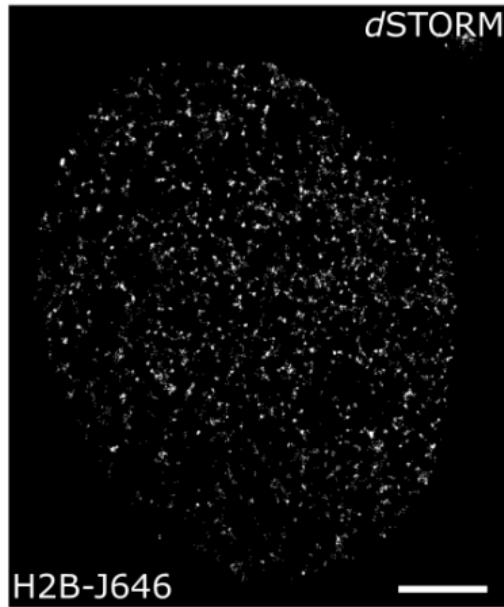
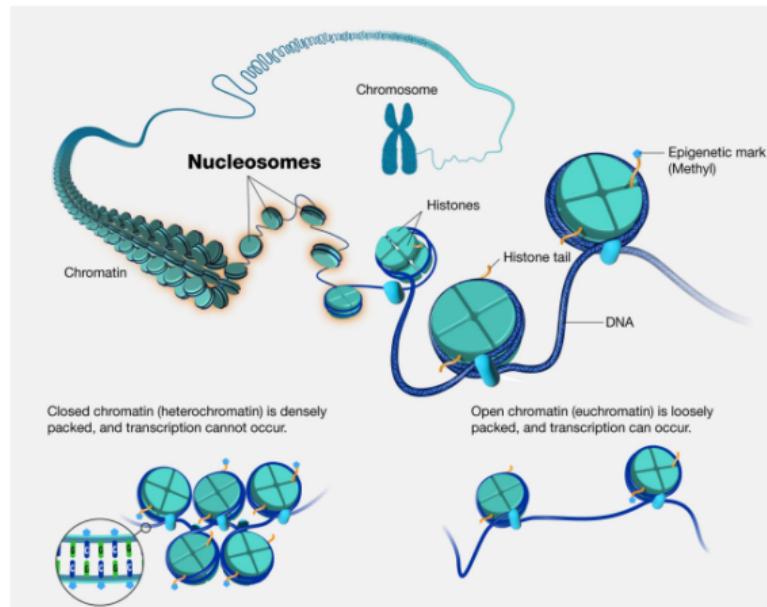
# Single photon counting for enhanced SMLM



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

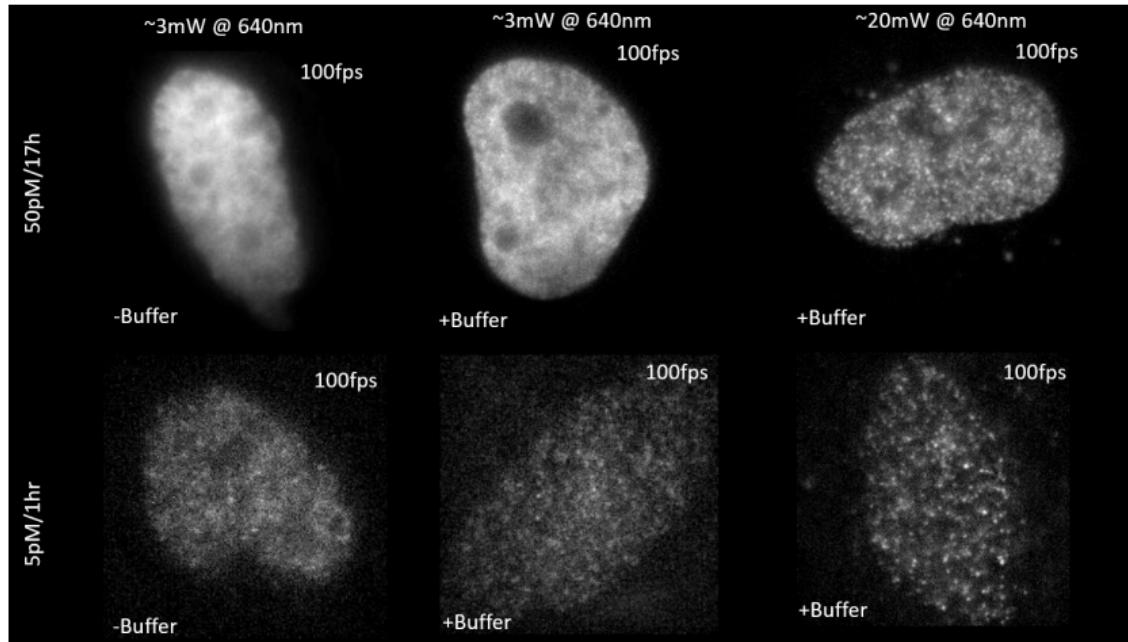
## Probing chromatin structure with SMLM

# Genome organization and single molecule localization microscopy



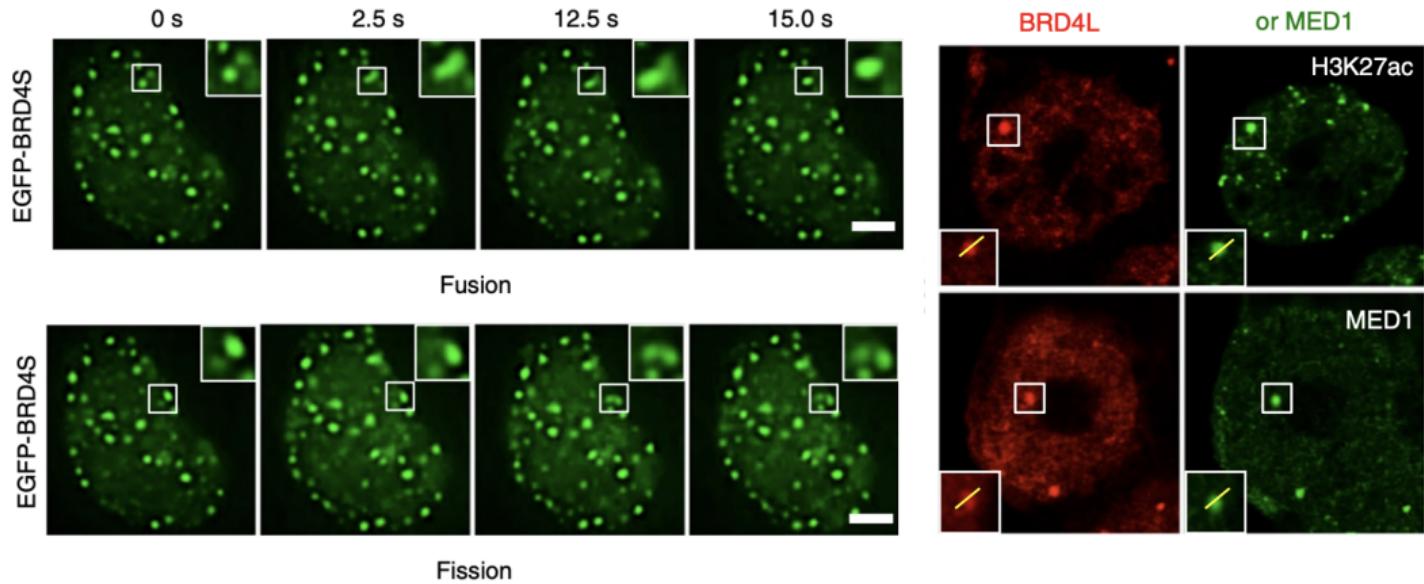
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# Dense labeling of histone H2B in fixed cells at RT



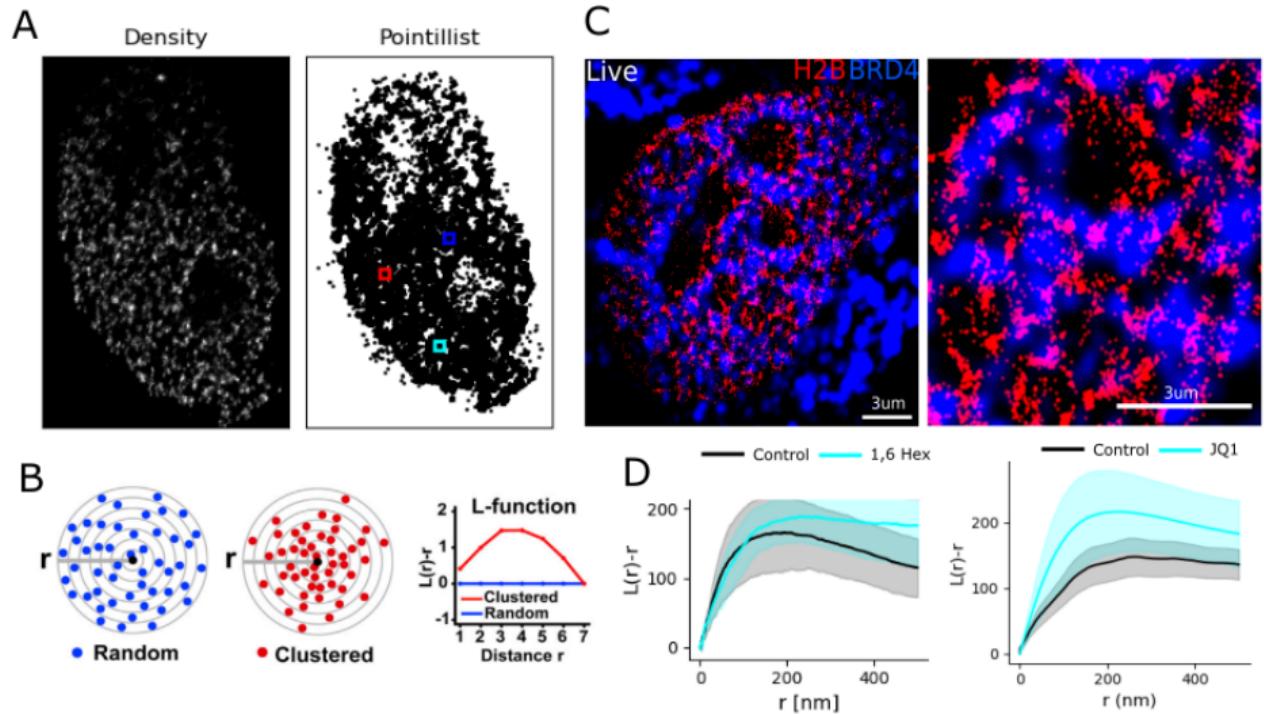
- ▶ Dense labeling of H2B-Halotag w/ fluorescent ligand JF646
- ▶ Reducing buffer is usually a primary thiol like cysteamine (MEA)

# 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



## Future Aims

## Recent Publications

- ▶ Seitz et al. *Super-resolution microscopy in-vivo reveals the bromodomain-dependent role of BRD4 in the maintenance of chromatin structure.* Unpublished
- ▶ Seitz et al. *Photon counting for enhanced single molecule localization microscopy.* Unpublished
- ▶ Maelle Locatelli<sup>†</sup>, Josh Lawrimore<sup>†</sup>, Hua Lin<sup>†</sup>, Sarvath Sanaullah, **Clayton Seitz**, ..., Pierre-Alexandre Vidi. *DNA damage reduces heterogeneity and coherence of chromatin motions.* PNAS. July 2022
- ▶ Mengdi Zhang, **Clayton Seitz**, Garrick Chang, Fadil Iqbal, Hua Lin, and Jing Liu *A guide for single-particle chromatin tracking in live cell nuclei.* Cell Biology International. January 2022.
- ▶ Wenting Wu, Farooq Syed, Edward Simpson, Chih-Chun Lee, Jing Liu, Garrick Chang, Chuanpeng Dong, **Clayton Seitz**, ..., Carmella Evans-Molina; *Impact of Proinflammatory Cytokines on Alternative Splicing Patterns in Human Islets.* Diabetes. January 2022

# Acknowledgements



(left to right) Charles Park, Garrick Chang, Jing Liu, David Buchanan, Mengyuan Liu, Hailan Ma



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Thank you!