

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

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December 19, 2023

# Outline

Introduction

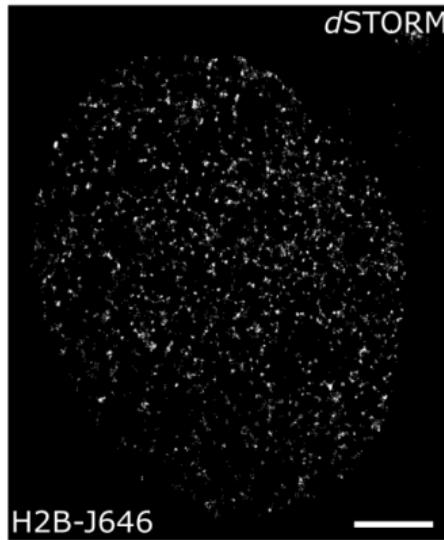
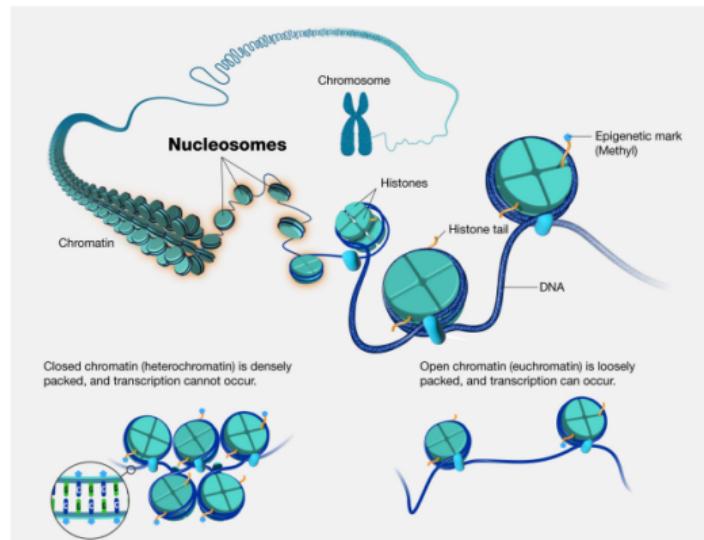
Novel theory for SMLM

Enhanced SMLM with photon statistics

Super resolution of chromatin nanodomains

# Introduction

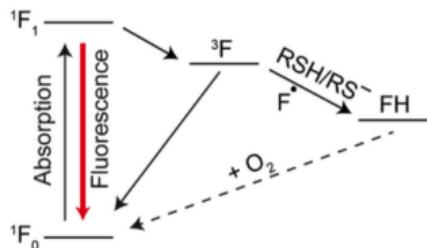
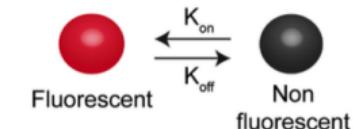
# Chromatin organization and super resolution imaging



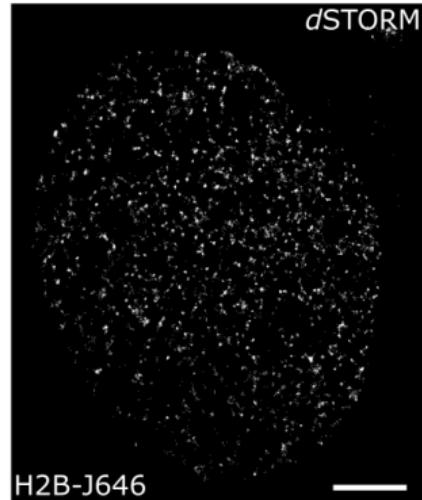
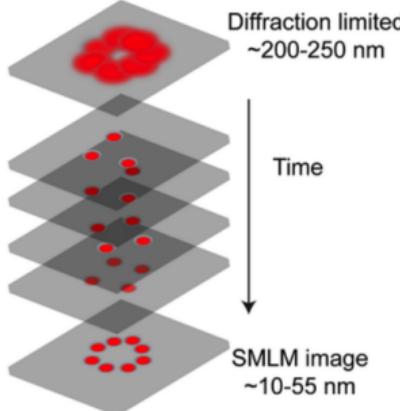
- ▶ Chromatin has a hierarchical structure, fundamental unit is the nucleosome
- ▶ We study chromatin organization with SMLM

# Direct stochastic optical reconstruction microscopy (dSTORM)

## a Photoswitching

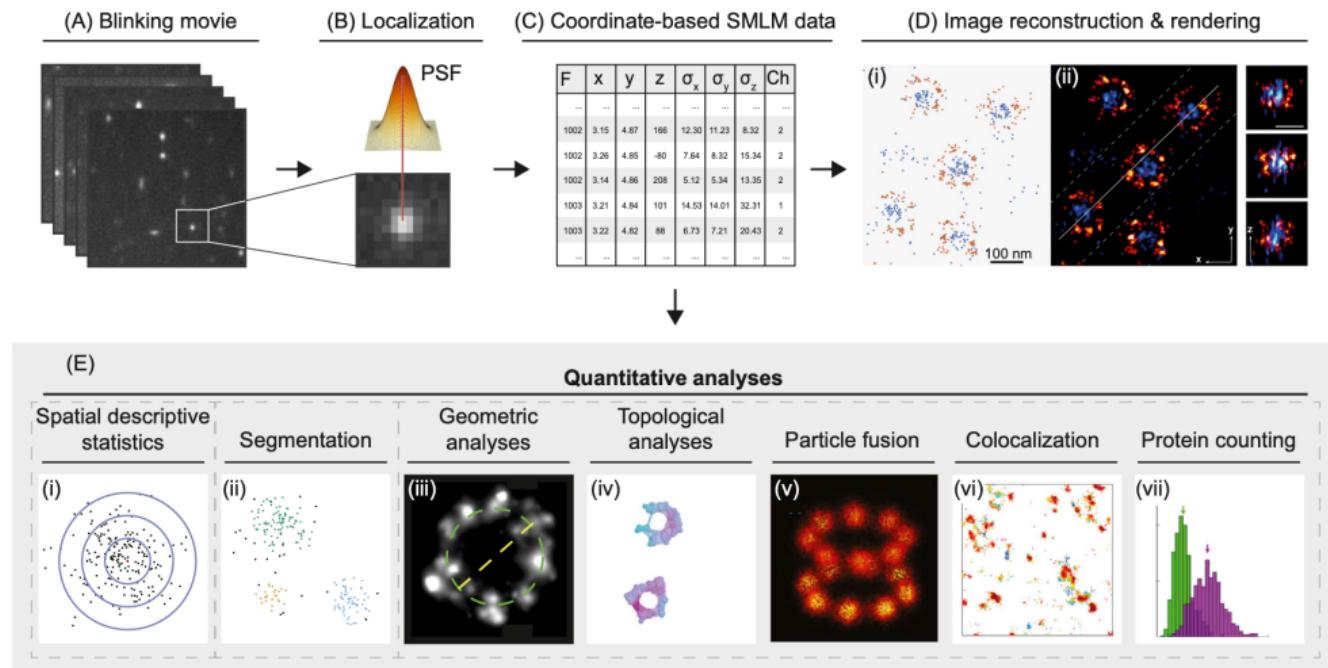


## b Temporal separation



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

# Single molecule localization microscopy and its applications



Trends in Cell Biology

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

## Novel theory for SMLM

# A first pass at SMLM for super-resolution

Modeling the point spread function permits sub-pixel localization

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

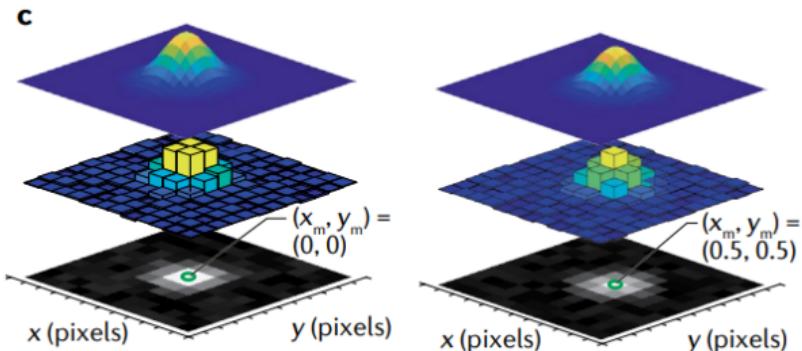
$$i_0 = g_k \eta N_0 \Delta$$

$g_k$  – pixel gain

$\eta$  – quantum efficiency

$N_0$  – photon emission rate

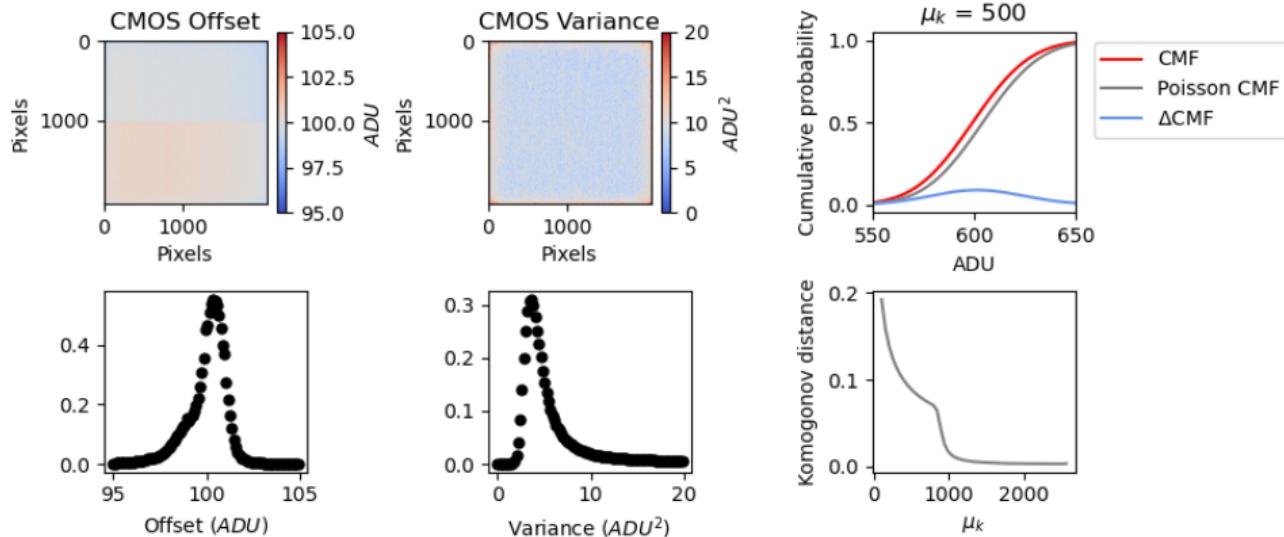
$\Delta$  – exposure time



Assume  $N_0$  is constant over  $\Delta$  (homogeneous Poisson)

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

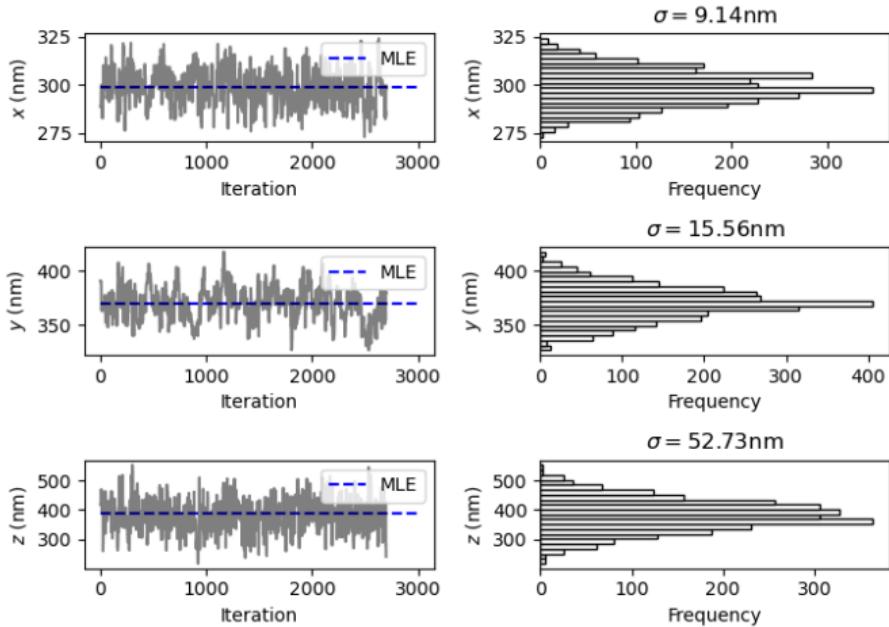
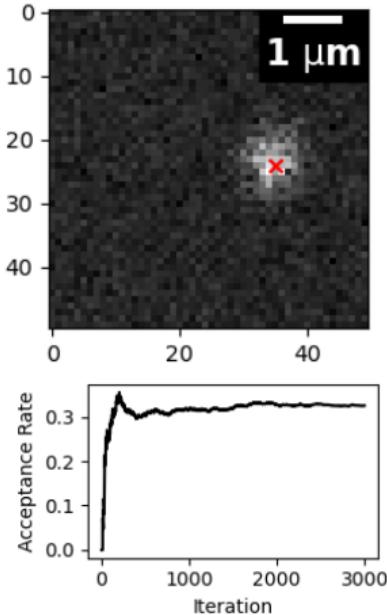
# CMOS readout noise limits SMLM



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

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

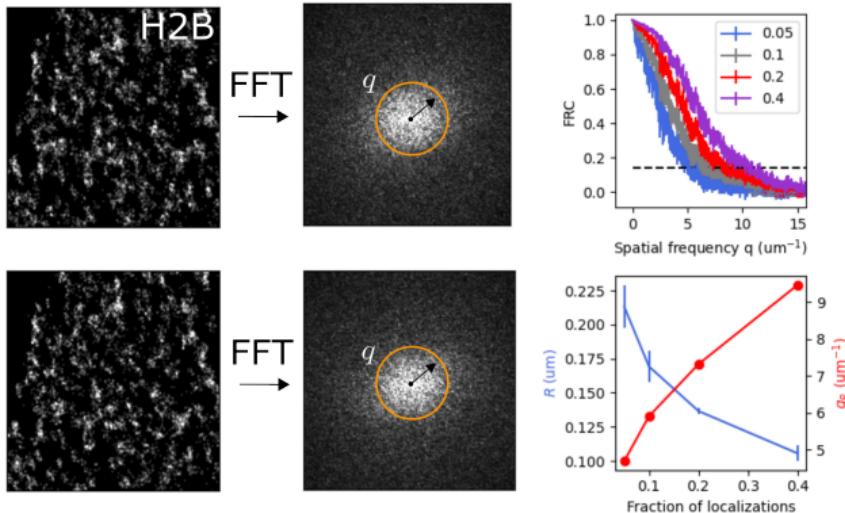
# Localization uncertainty in SMLM



- ▶ Sampling the coordinates  $\theta$  gives uncertainty estimates

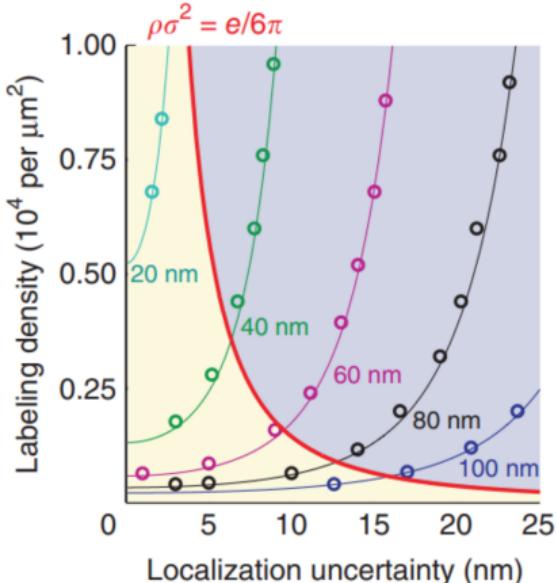
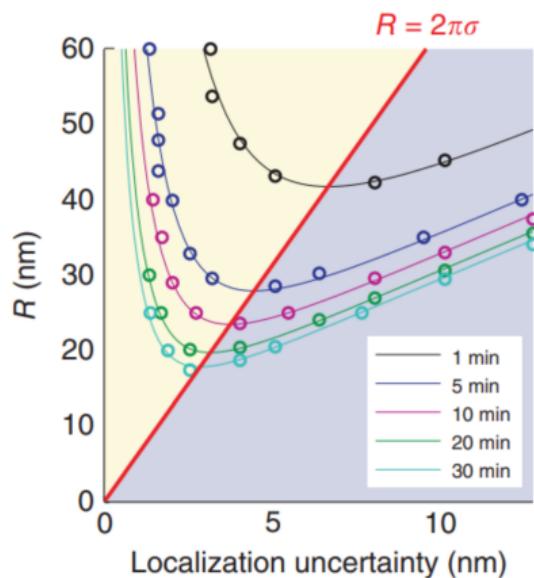
# The definition of resolution in SMLM

- We can view dSTORM as sampling from a density



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

# The definition of resolution in SMLM

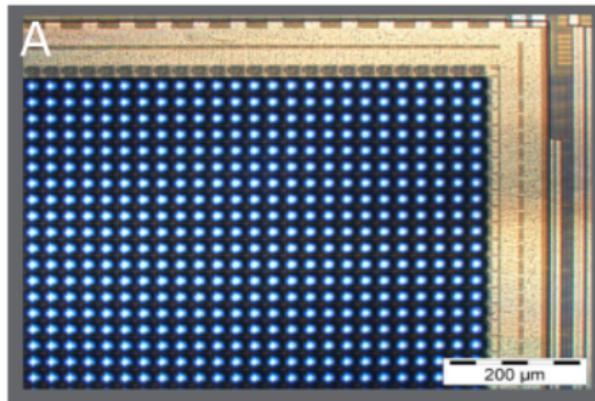


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

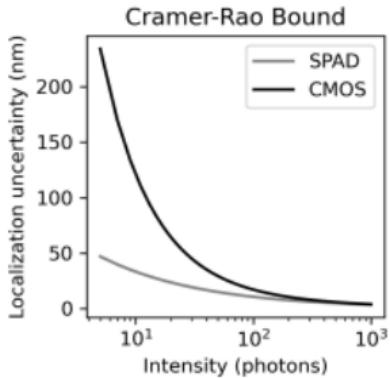
- ▶ Uncertainty-limited resolution (blue), Density-limited resolution (yellow)
- ▶ Labeling density  $\rho$  scales with  $\sigma$  to maintain constant resolution

## Enhanced SMLM with photon statistics

# Single photon avalanche diode (SPAD) cameras



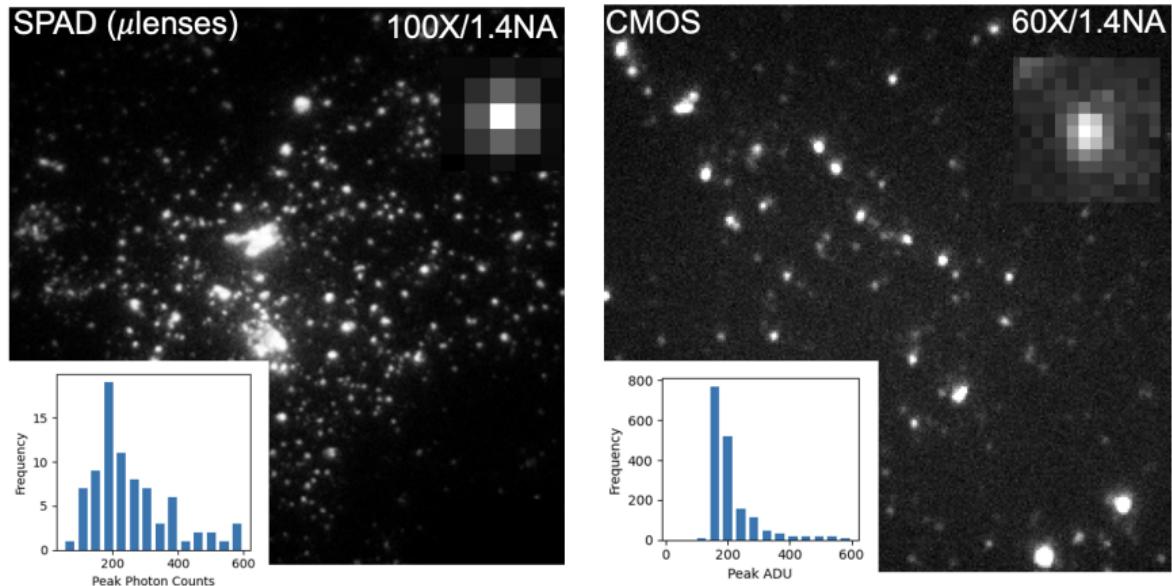
B



(A) Courtesy of Pi imaging technologies

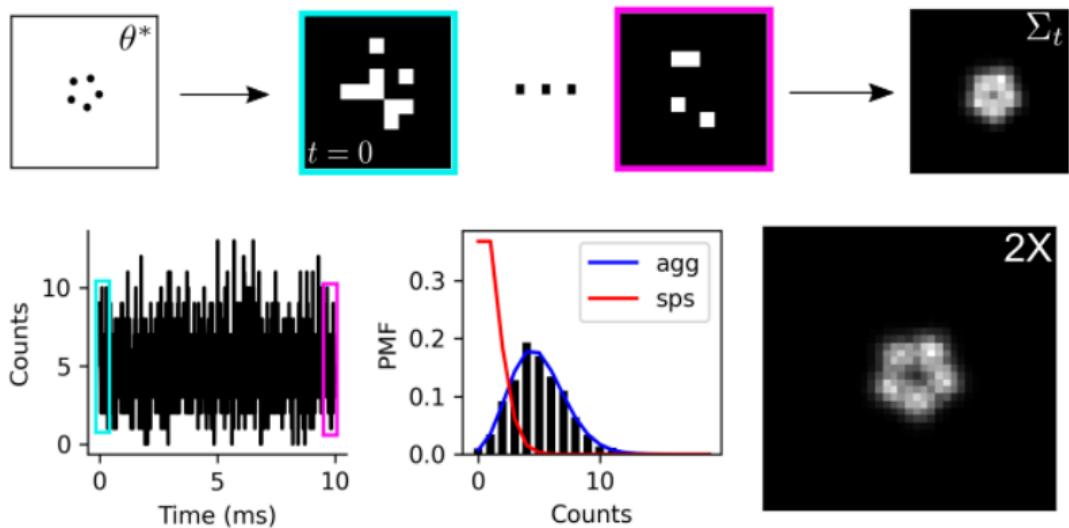
- ▶ Imaging in low light conditions (near zero readout noise)
- ▶ Reduced quantum efficiency ( $\eta \approx 0.5$ ), but frame rates up to 1MHz

# 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

# Counting molecules and enhancing resolution with a SPAD camera



- ▶ Molecular counting for constrained multi-emitter localization
- ▶ Pixel doubling using correlation functions

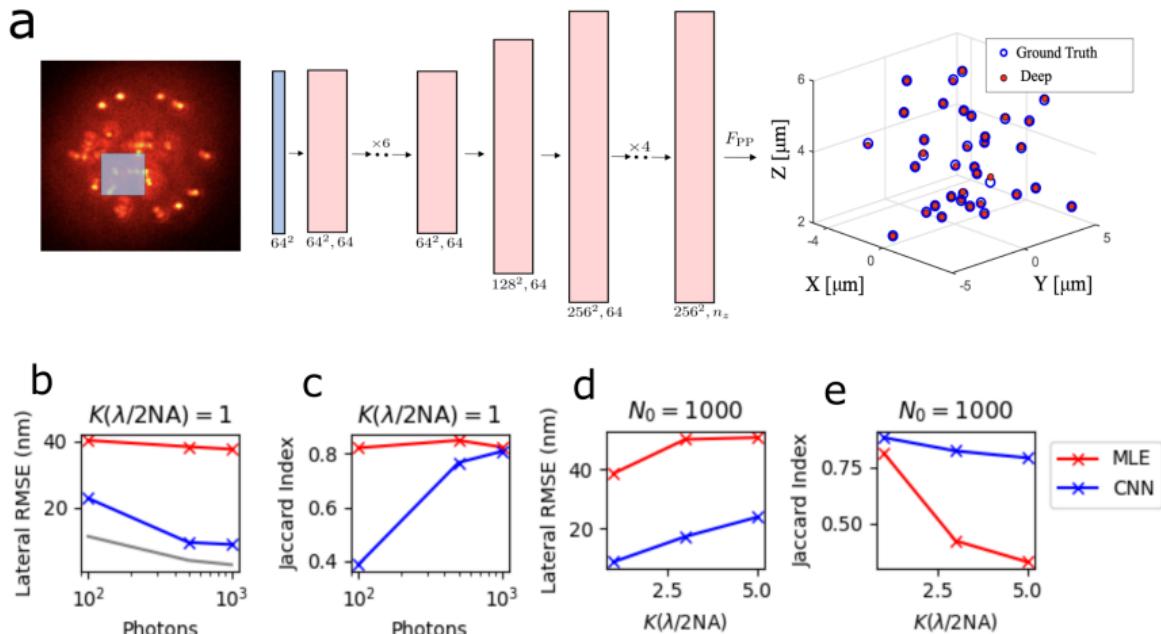
## Theory

$$g_{ij}^{(2)}(0) = \frac{\text{Tr}(a^\dagger a^\dagger a a \rho)}{\text{Tr}(a^\dagger a \rho)^2}$$

$$\rho = \xi |\alpha\rangle\langle\alpha| + (1 - \xi) |0\rangle\langle 0|$$

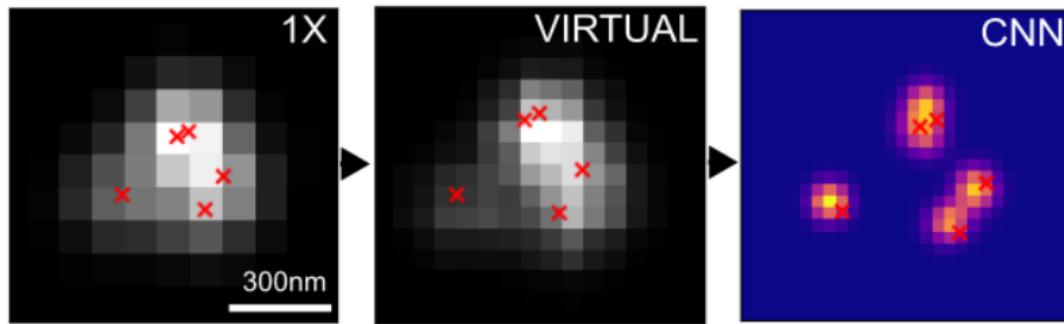
$$\text{Tr} \left( a^\dagger a^\dagger a a |n\rangle\langle m| \right) = \text{Tr} \left( n(n-1) |n\rangle\langle m| \right)$$

# DeepSTORM: 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  $\Psi$

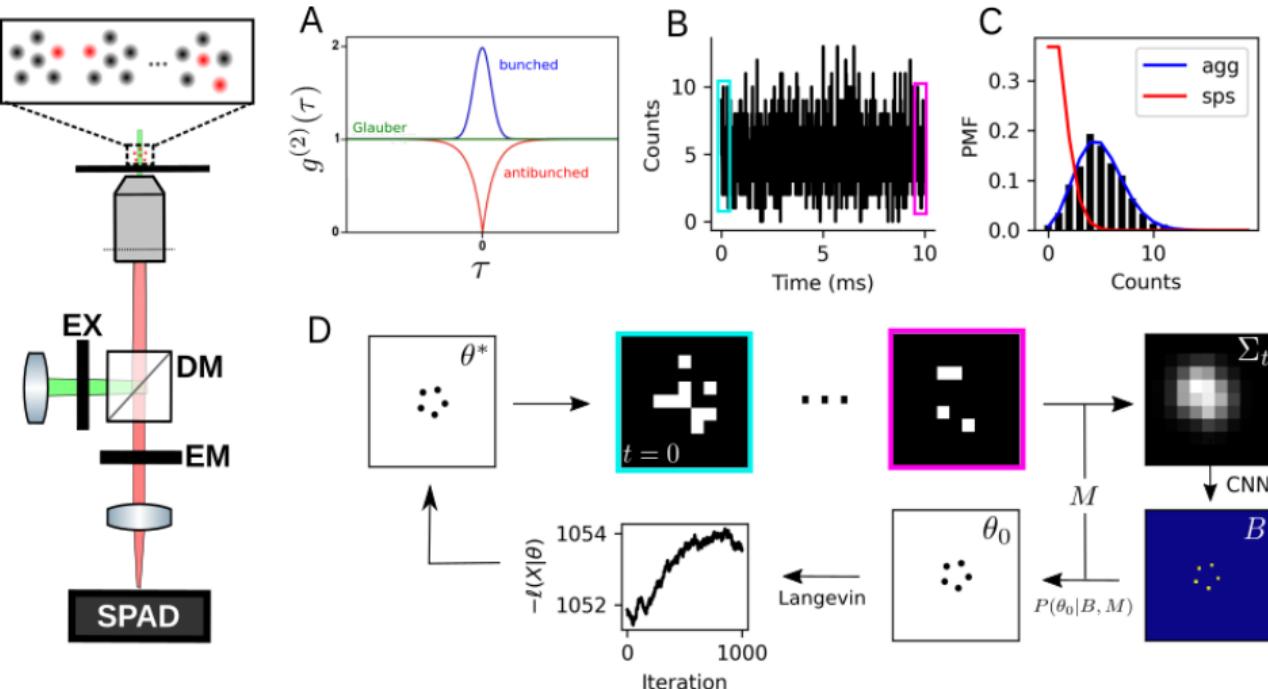
## Counting molecules and enhancing resolution with a SPAD camera



Simulated 5 blinking emitters, assigned virtual pixels  $\langle X_i(t)X_j(t)\rangle_t$  at or between existing pixels

- ▶ Relax assumption that  $N_0$  (emission rate) is constant
- ▶ For example, photoswitching ON or OFF
- ▶ Fluctuations in  $N_0$  may be **uncorrelated** between molecules

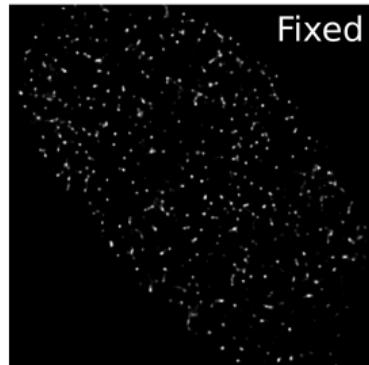
# An integrated method for dense SMLM



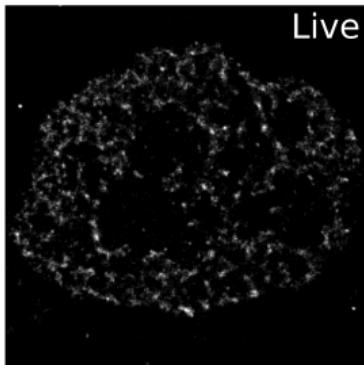
- At higher time resolution we can sample from this Poisson PMF 100-1000s of times in a single 10ms exposure

## Super resolution of chromatin nanodomains

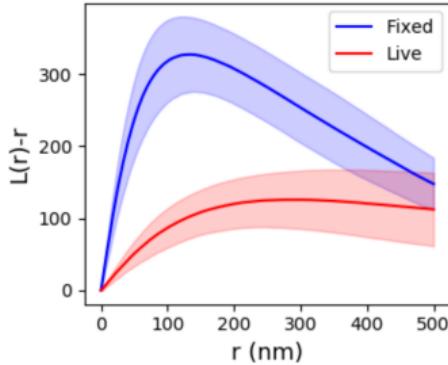
# Application of dSTORM in living cells



Fixed

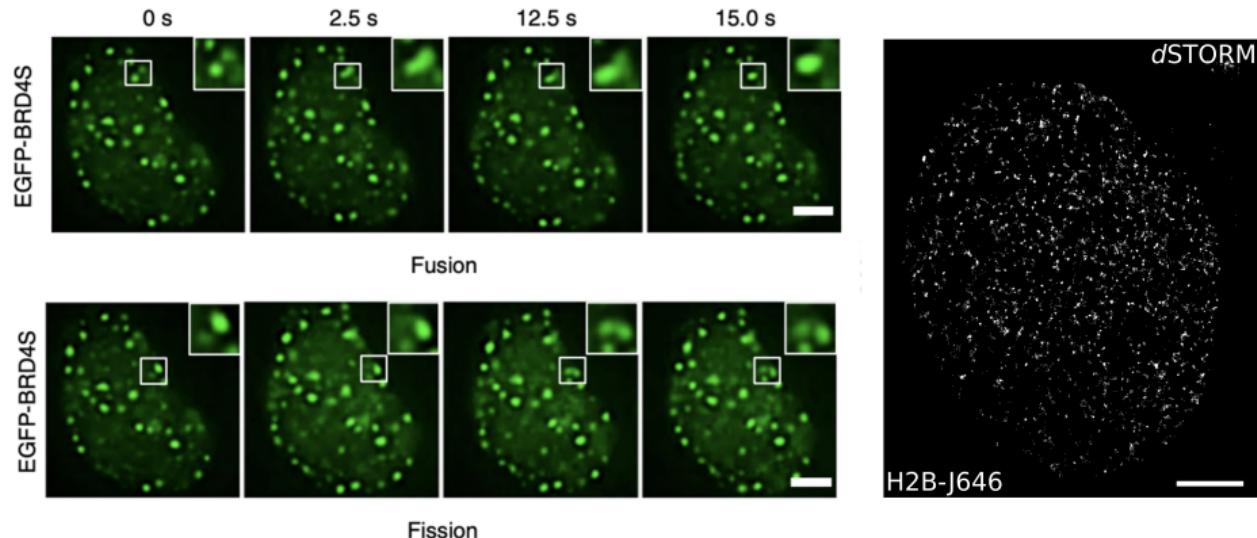


Live



- ▶ Fixation changes the appearance of nucleosome clustering
- ▶ Clusters are more dispersed in living cells
- ▶ Dispersion due to nucleosome diffusion and possibly affects of PFA on chromatin architecture

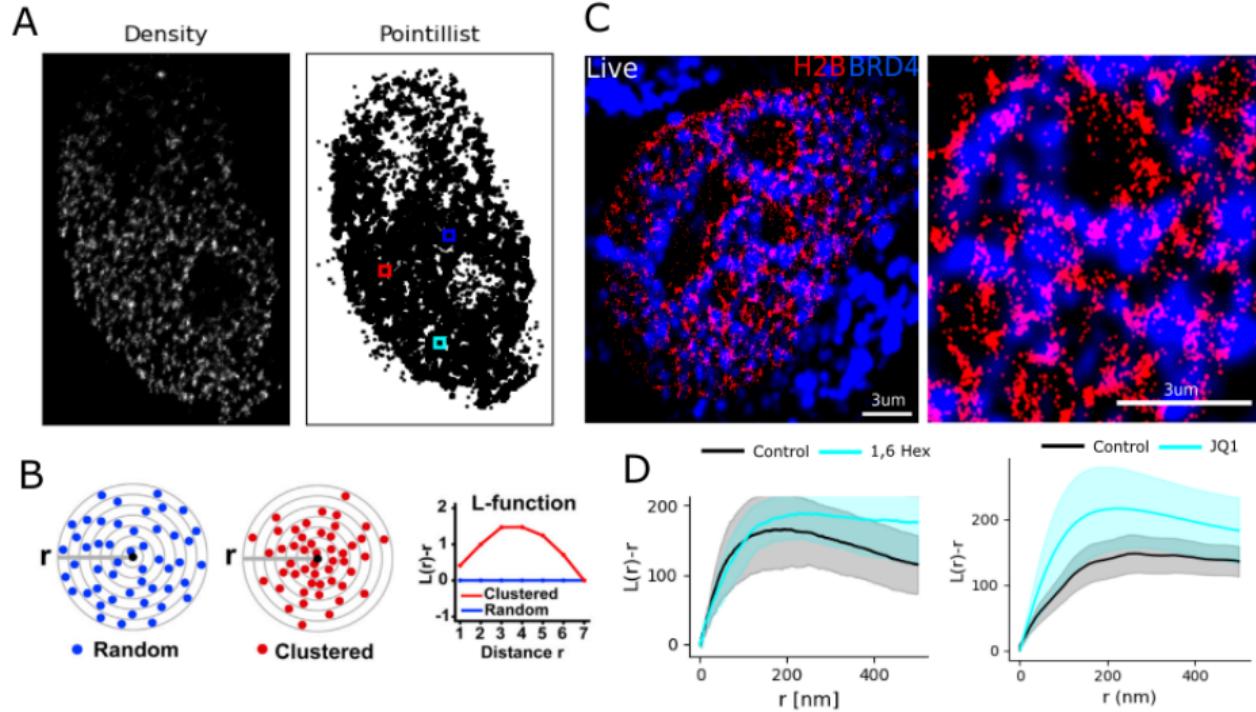
# BRD4 forms phase separated condensates in the nucleus



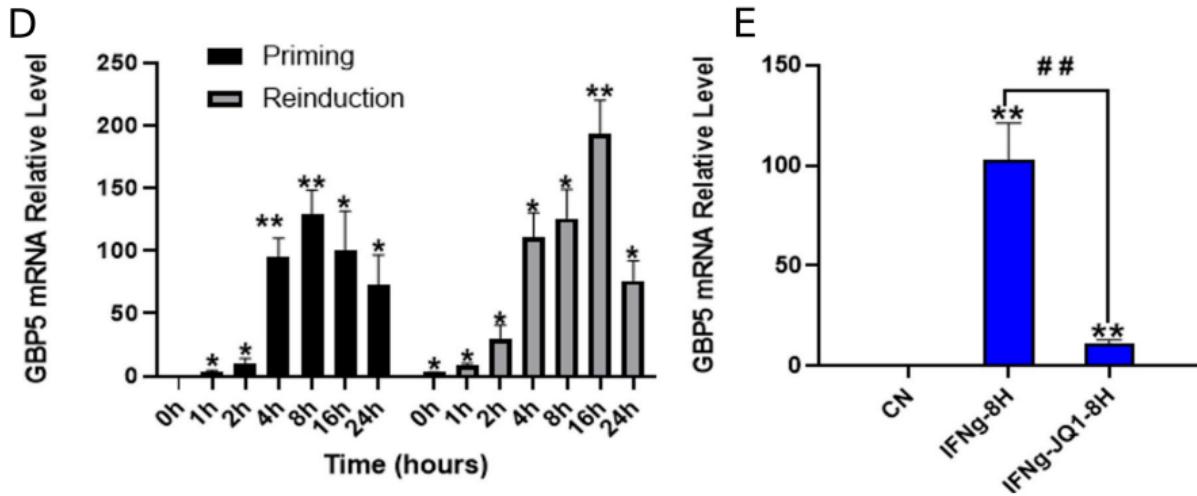
Han et al. Roles of BRD4 short isoform in phase separation and active gene transcription

- ▶ BRD4 interacts directly with the chromatin scaffold
- ▶ Super-resolution of BRD4 interactions with chromatin

# Super-resolution of nucleosome-BRD4 interactions in living cells



# Inhibition of a super-enhanced gene with JQ1



- \*: $P \leq 0.1$ , \*\*: $P \leq 0.01$
- JQ1 - a BRD4 inhibitor knocks down GBP5 expression

## Future Aims

### **Integrate deep models with counting algorithms for enhanced SMLM**

- ▶ Resolution enhancement and counting fluorescent emitters

### **Demonstrate that BRD4 phase separates with chromatin and cofactors**

- ▶ BRD4 mutation to inhibit (i) phase separation (ii) chromatin reading function
- ▶ Measure nucleosome diffusion after JQ1 exposure

## 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



Pancho



Donghong Fu



Norbert Scherer

Thank you!

## Selected References

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- [2] Nehme, E. et al. *DeepSTORM3D: dense 3D localization microscopy and PSF design by deep learning*. Nat Methods 17, 734–740 (2020).
- [3] Dertinger, T. et al. *Fast, background-free, 3D super-resolution optical fluctuation imaging (SOFI)*. PNAS
- [4] Nozaki, T. et al. *Dynamic Organization of Chromatin Domains Revealed by Super-Resolution Live-Cell Imaging*. Mol Cell 67, 282-293.e7 (2017).
- [5] Han, X. et al. *Roles of the BRD4 short isoform in phase separation and active gene transcription*. Nat Struct Mol Biol 27, 333–341 (2020).