

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

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Outline

Introduction

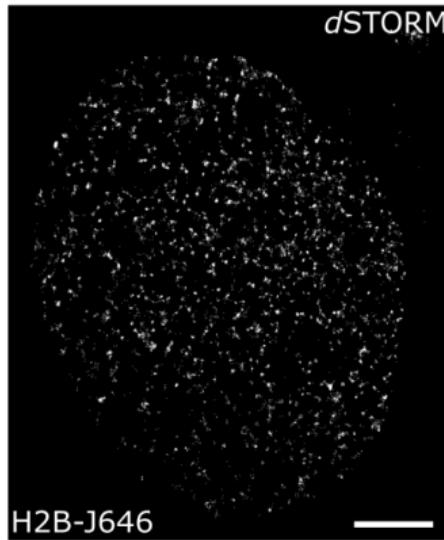
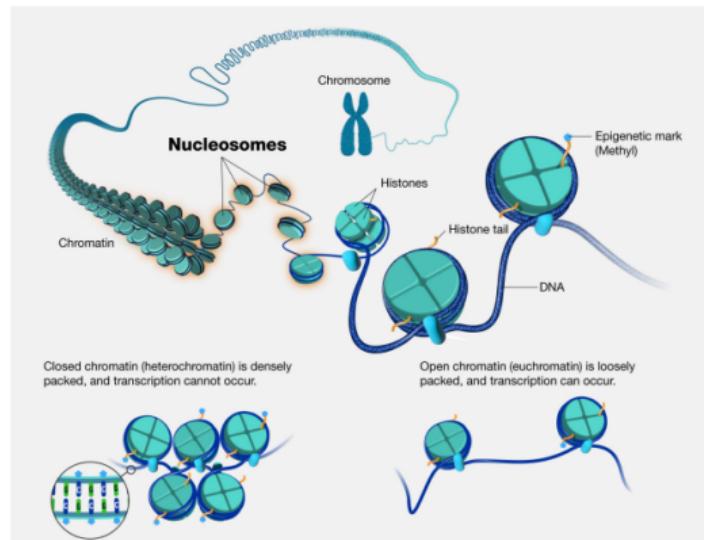
Novel theory for SMLM

Enhanced SMLM with photon statistics

Super resolution of chromatin nanodomains

Introduction

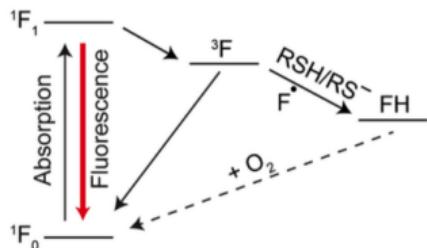
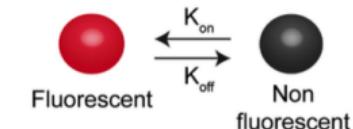
Chromatin organization and super resolution imaging



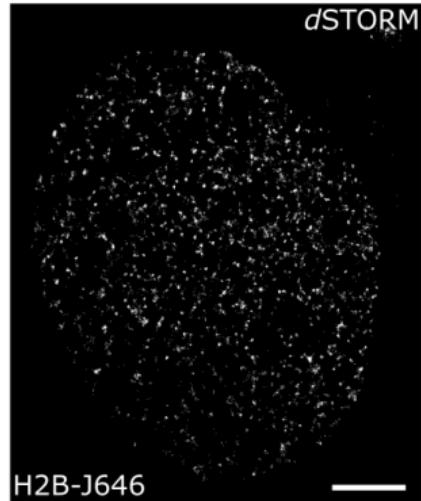
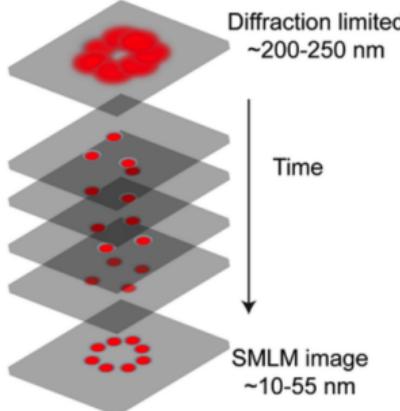
- ▶ Chromatin has a hierarchical structure, fundamental unit is the nucleosome
- ▶ We study chromatin organization with SMLM

Direct stochastic optical reconstruction microscopy (dSTORM)

a Photoswitching

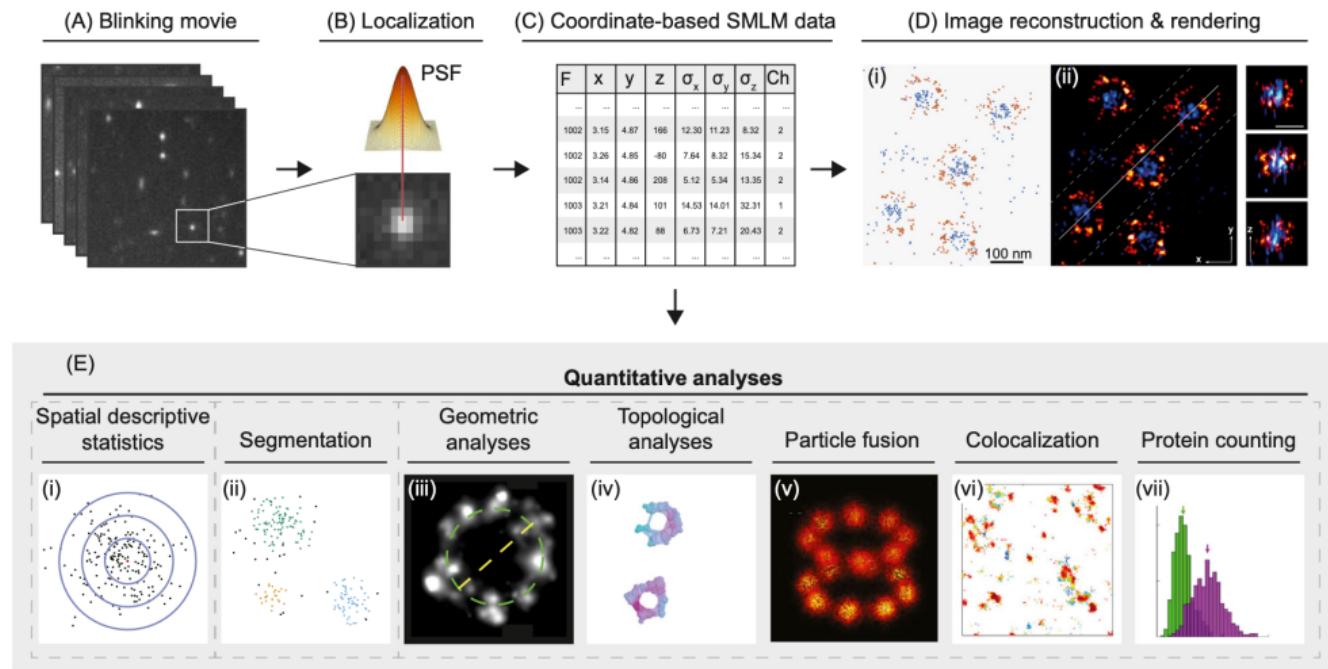


b Temporal separation



- ▶ 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

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

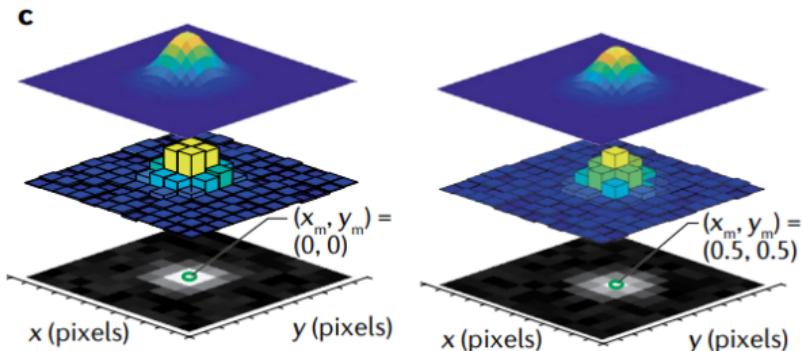
$$i_0 = g_k \eta N_0 \Delta$$

g_k – pixel gain

η – quantum efficiency

N_0 – photon emission rate

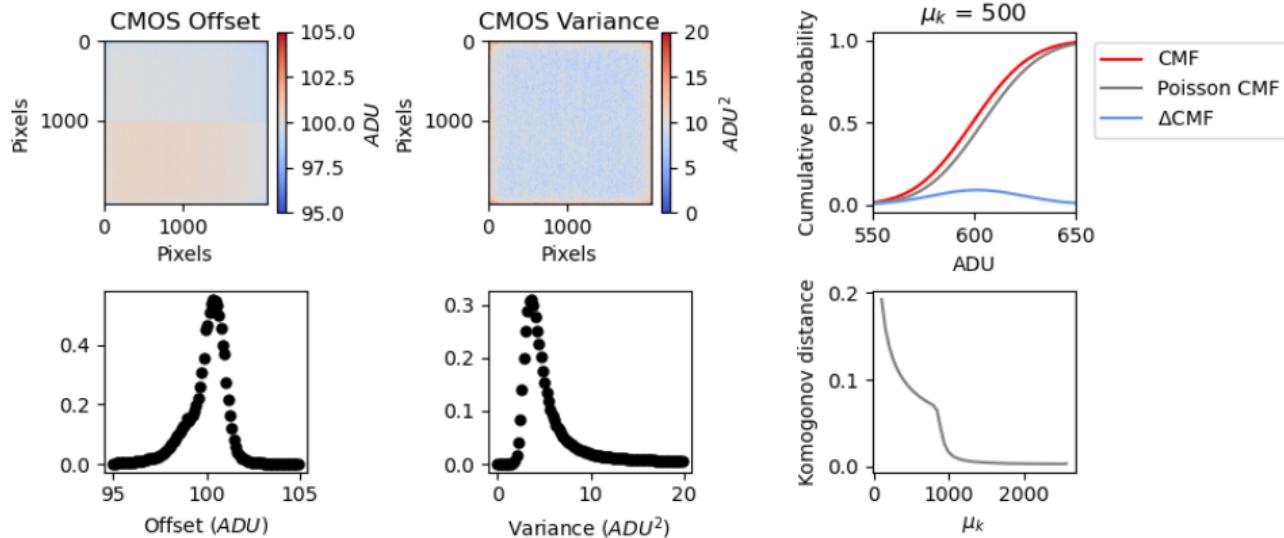
Δ – exposure time



Assume N_0 is constant over Δ (homogeneous Poisson)

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

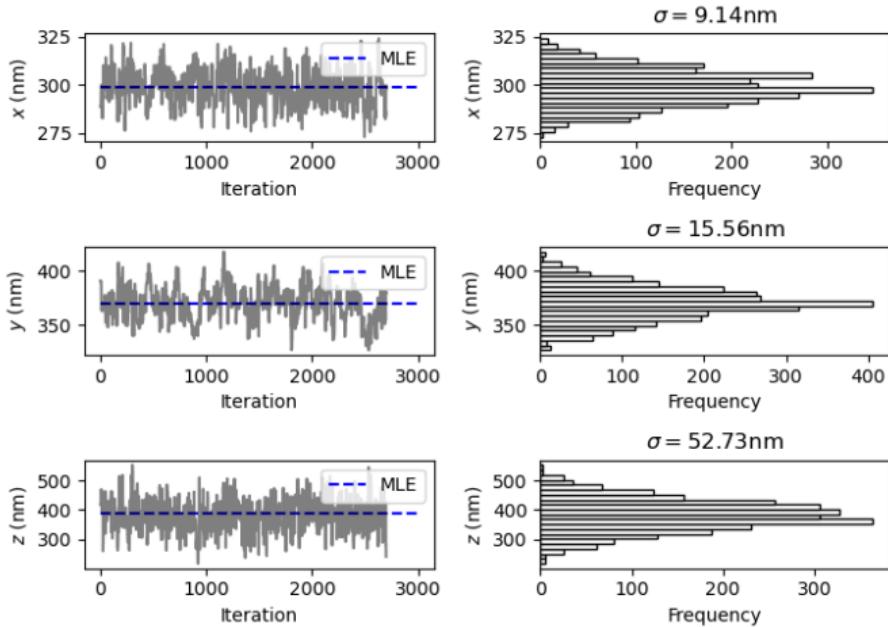
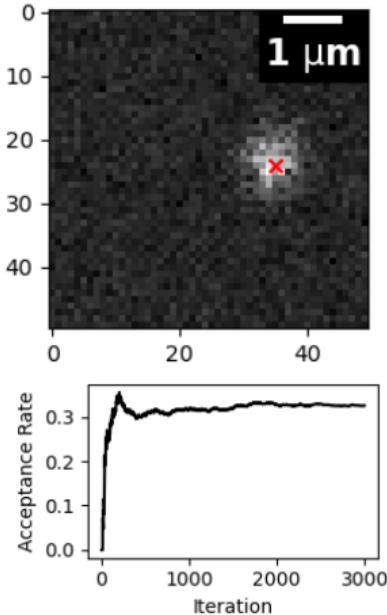
CMOS readout noise limits SMLM



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

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

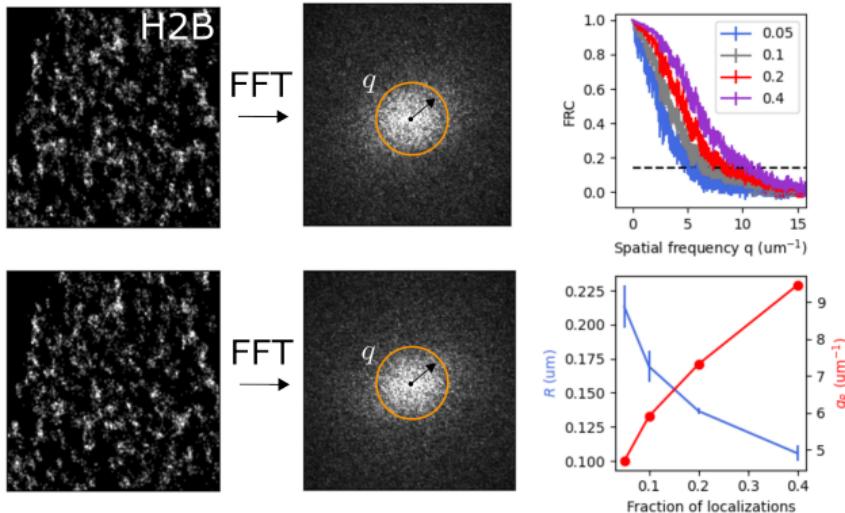
Localization uncertainty in SMLM



- ▶ Sampling the coordinates θ gives uncertainty estimates

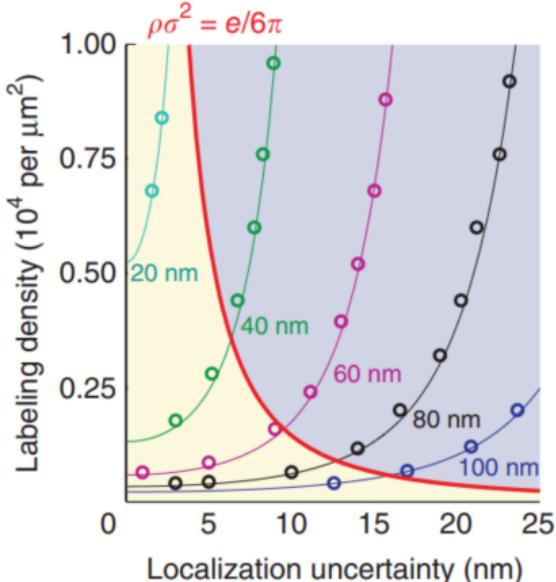
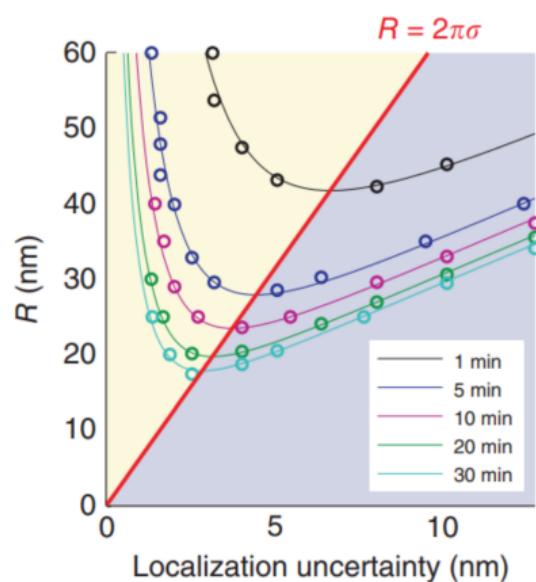
The definition of resolution in SMLM

- 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

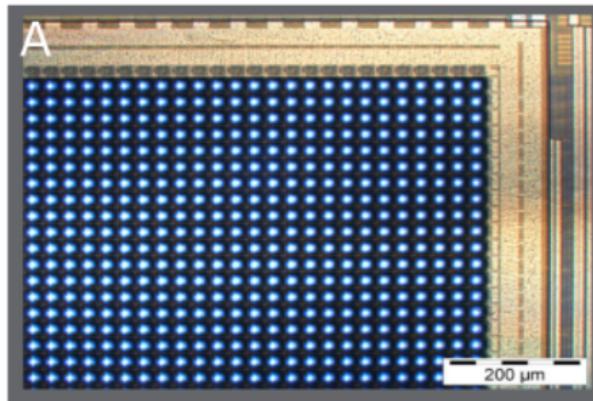


Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

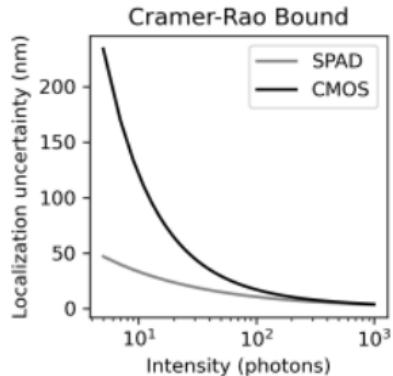
- ▶ Uncertainty-limited resolution (blue), Density-limited resolution (yellow)
- ▶ Labeling density ρ scales with σ to maintain constant resolution

Enhanced SMLM with photon statistics

Single photon avalanche diode (SPAD) cameras



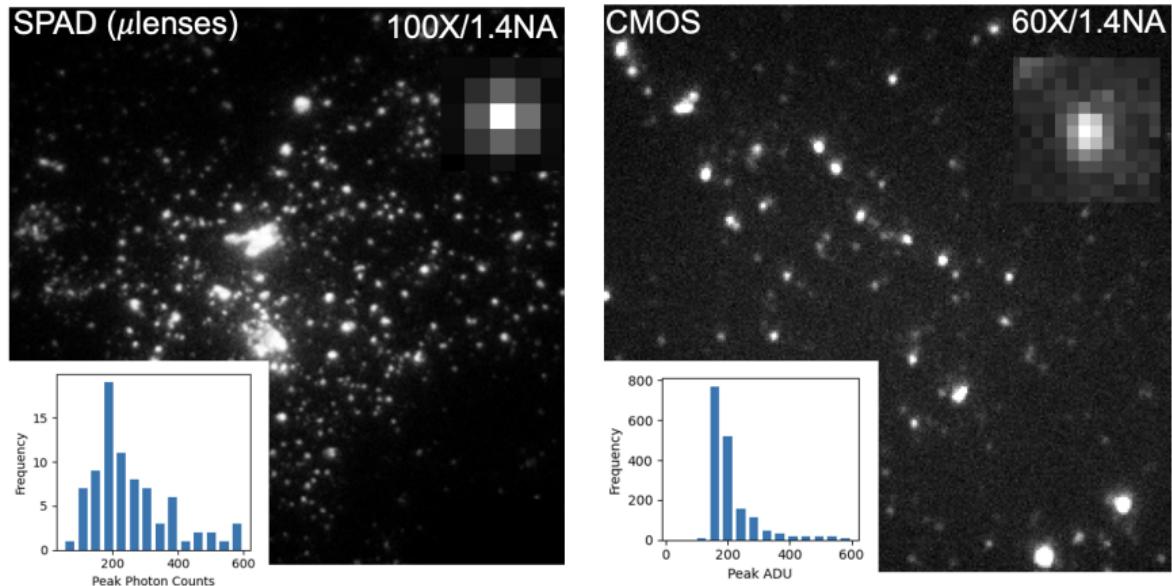
B



(A) Courtesy of Pi imaging technologies

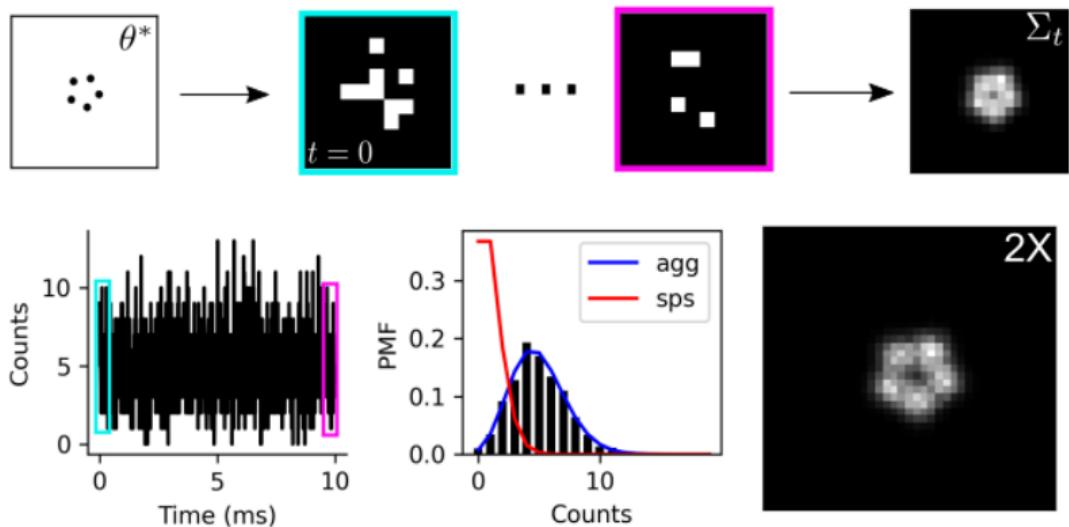
- ▶ Imaging in low light conditions (near zero readout noise)
- ▶ Reduced quantum efficiency ($\eta \approx 0.5$), but frame rates up to 1MHz

High speed imaging for enhanced SMLM



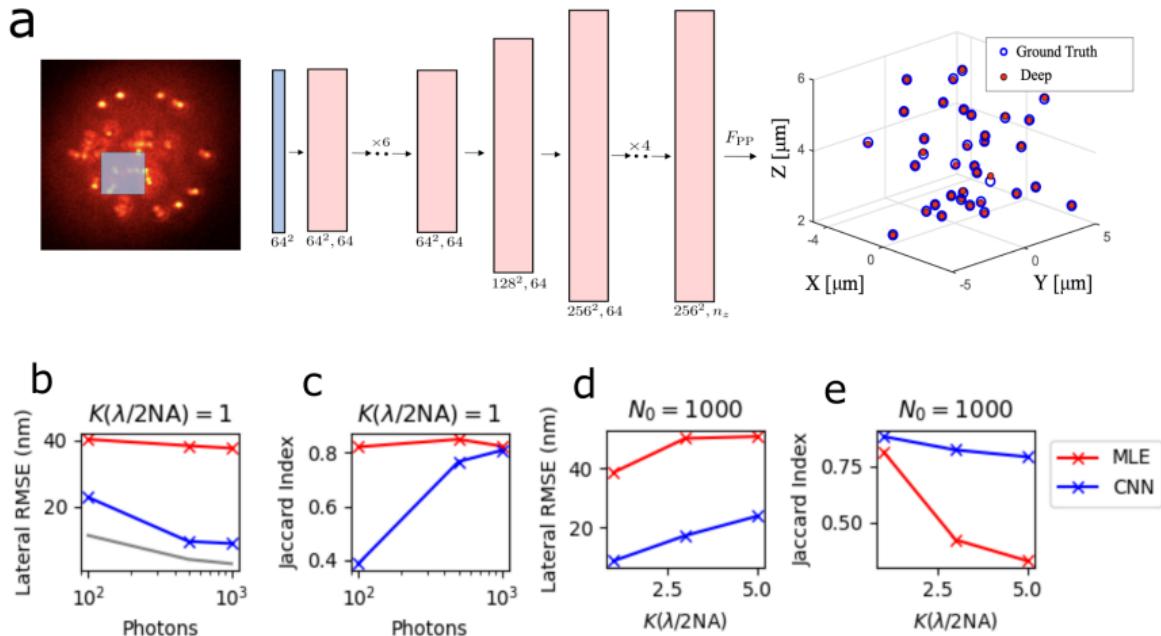
- ▶ SPAD frame is sum of 10^4 1us exposures
- ▶ This inspires counting molecules in widefield images for enhanced SMLM

Counting molecules and enhancing resolution with a SPAD camera



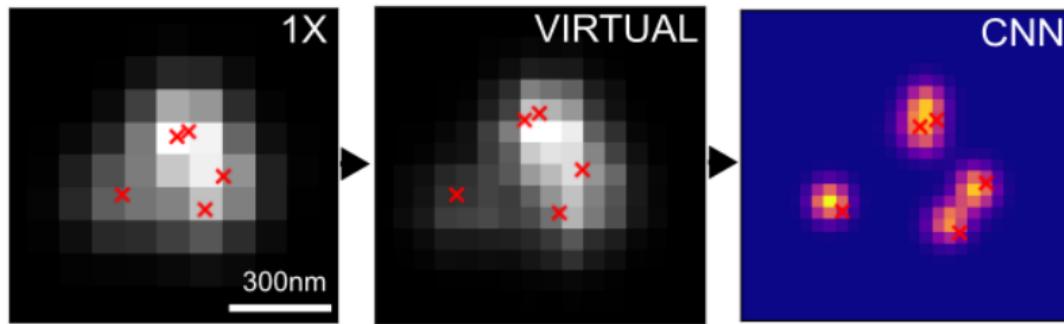
- ▶ Molecular counting for constrained multi-emitter localization
- ▶ Pixel doubling using correlation functions

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 Ψ

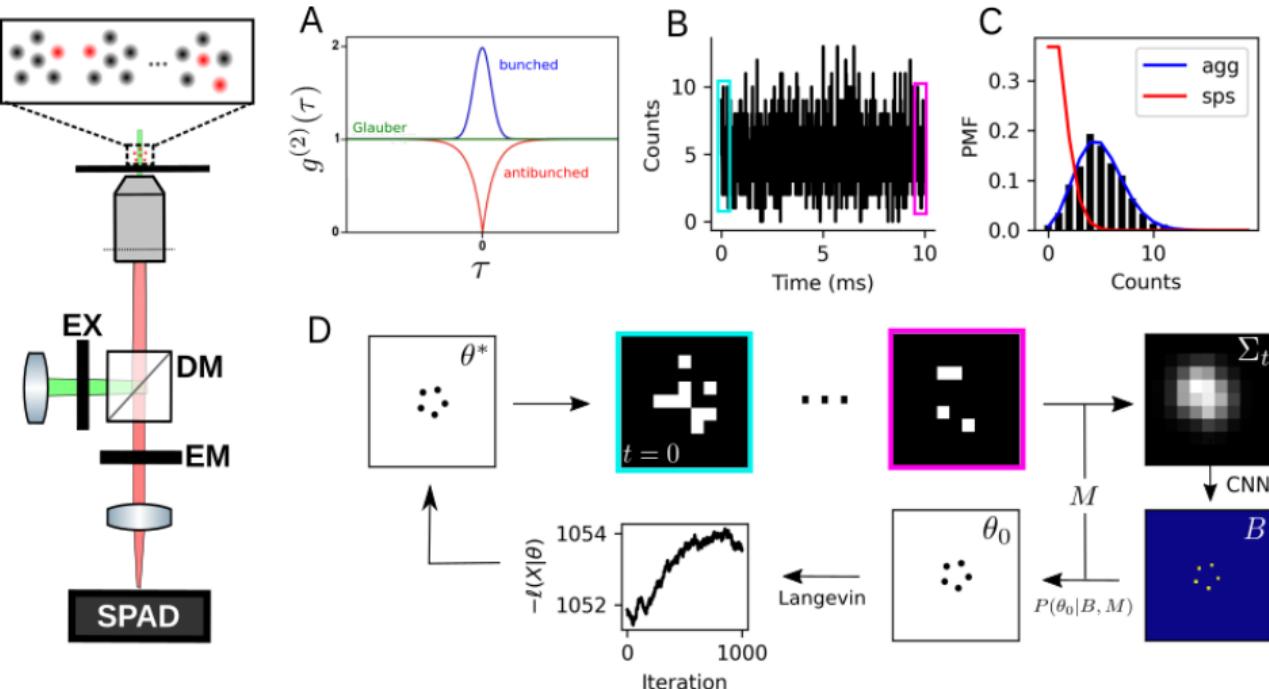
Counting molecules and enhancing resolution with a SPAD camera



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

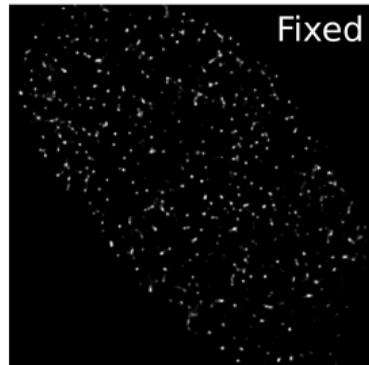
An integrated method for dense SMLM



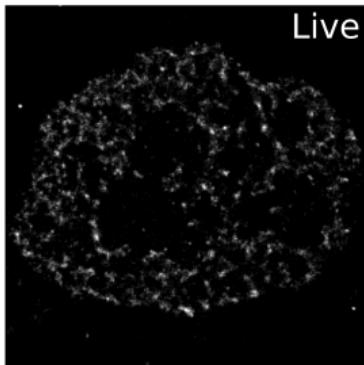
- At higher time resolution we can sample from this Poisson PMF 100-1000s of times in a single 10ms exposure

Super resolution of chromatin nanodomains

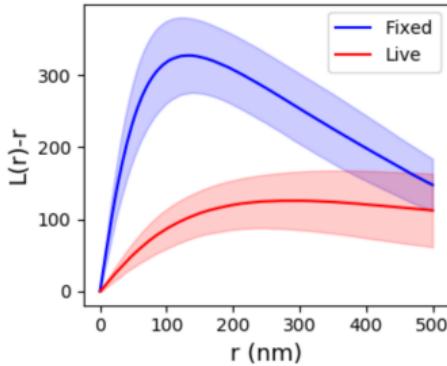
Application of dSTORM in living cells



Fixed

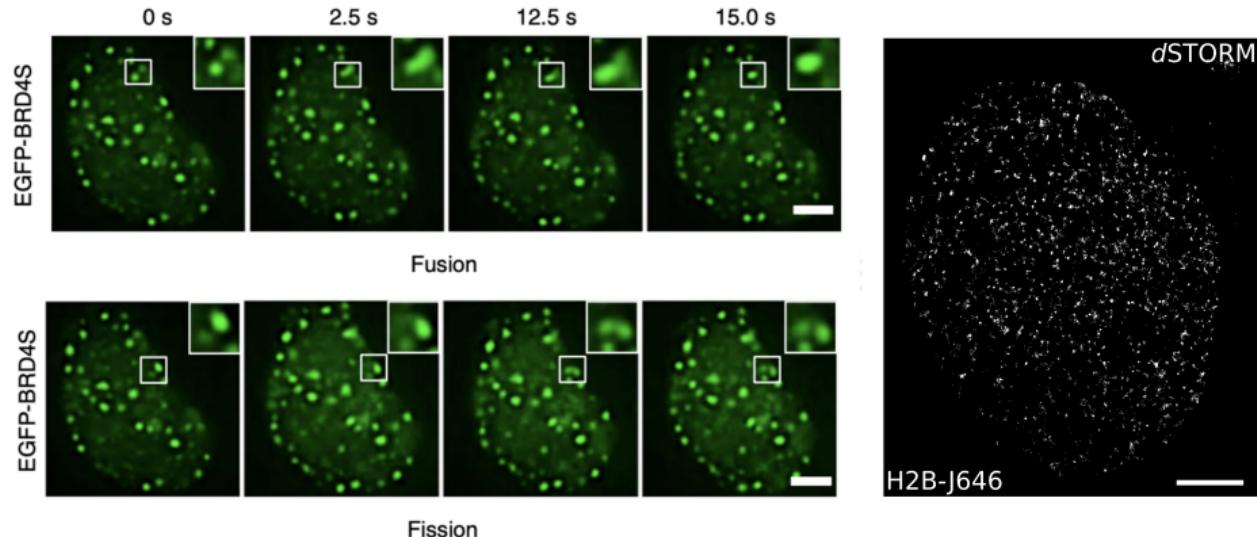


Live



- ▶ 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

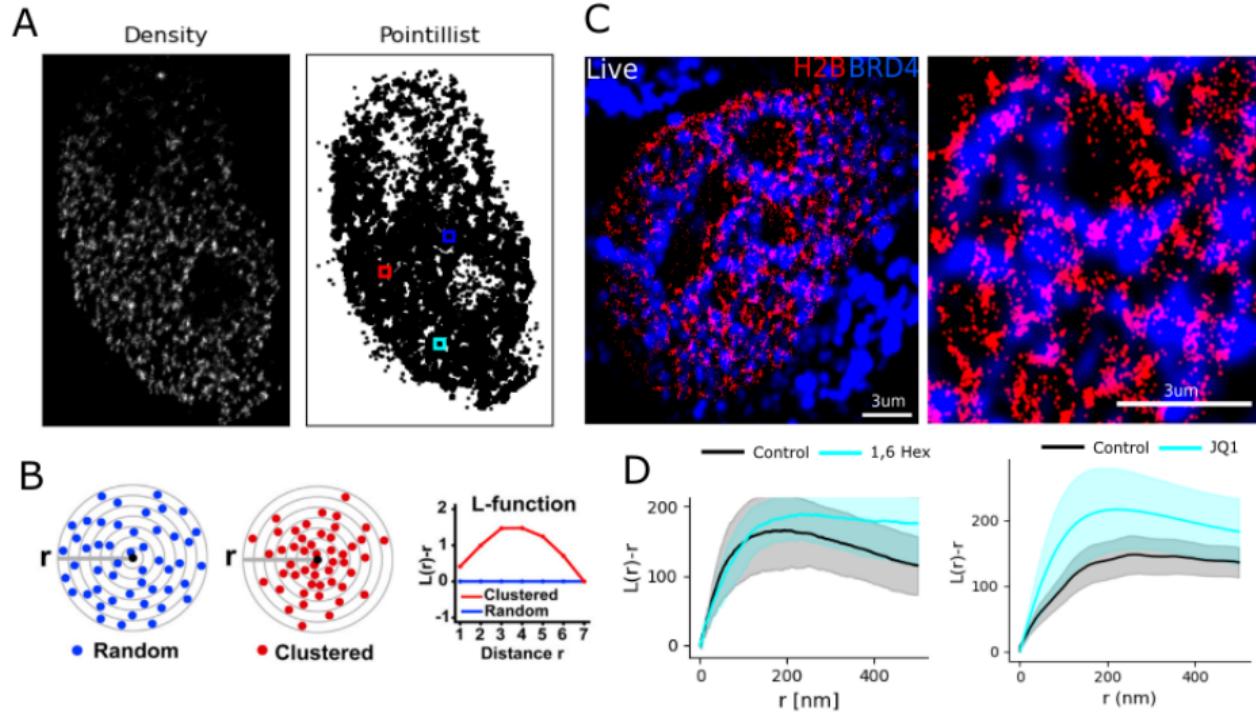
BRD4 forms phase separated condensates in the nucleus



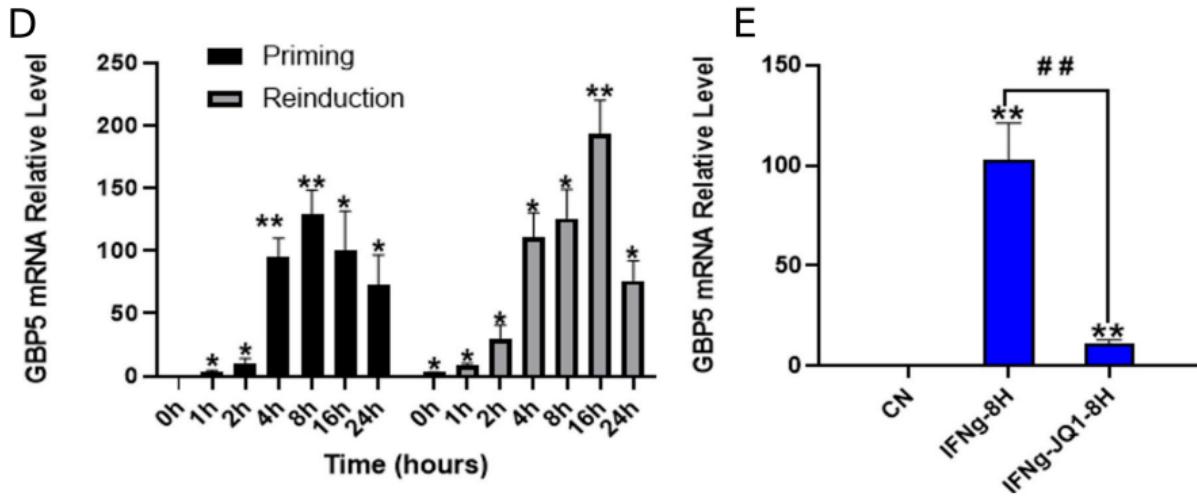
Han et al. Roles of BRD4 short isoform in phase separation and active gene transcription

- ▶ 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[†], Josh Lawrimore[†], Hua Lin[†], 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

- [1] Schermelleh, L. et al. *Super-resolution microscopy demystified*. Nature Cell Biology vol. 21 72–84 (2019).
- [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).