

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

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May 21, 2024

Outline

Single molecule localization microscopy

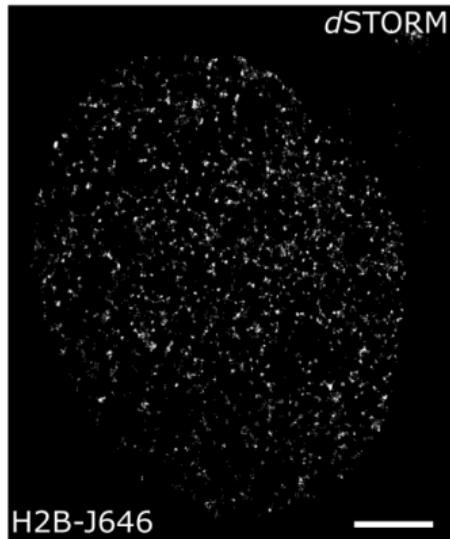
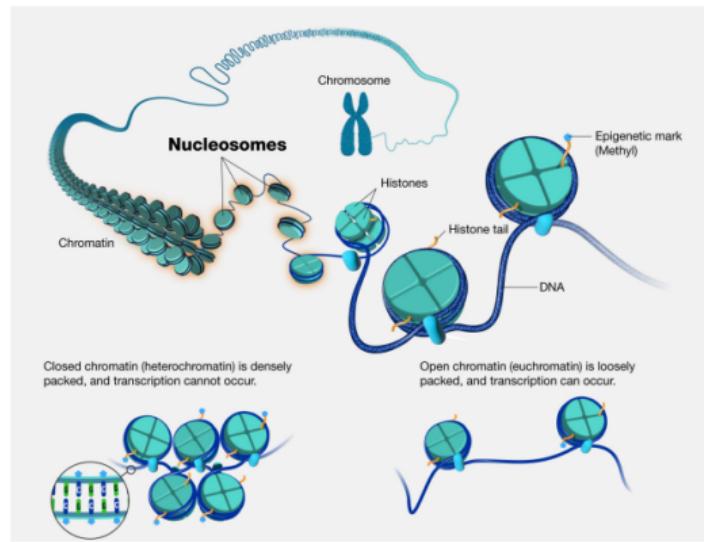
Super-resolution of nucleosome nanodomains *in-vivo*

Enhanced SMLM with deep generative models

Enhanced SMLM with photon statistics

Single molecule localization microscopy

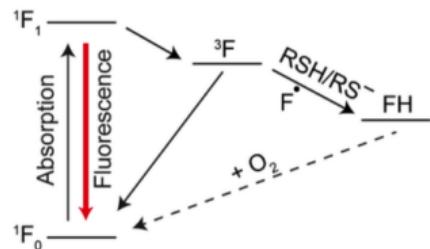
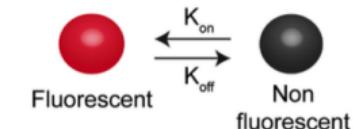
Chromatin organization and super resolution imaging



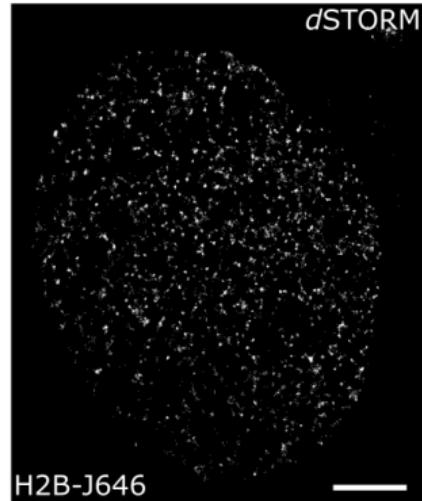
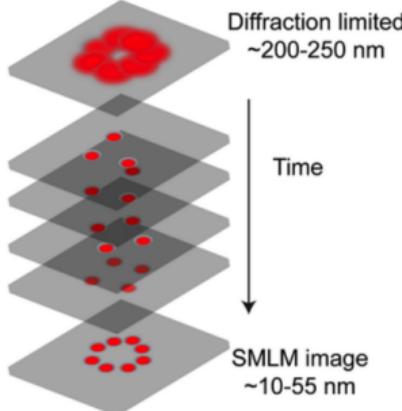
- ▶ Chromatin has a hierarchical structure, fundamental unit is the nucleosome
- ▶ We study chromatin organization with SMLM

Direct stochastic optical reconstruction microscopy (dSTORM)

a Photoswitching

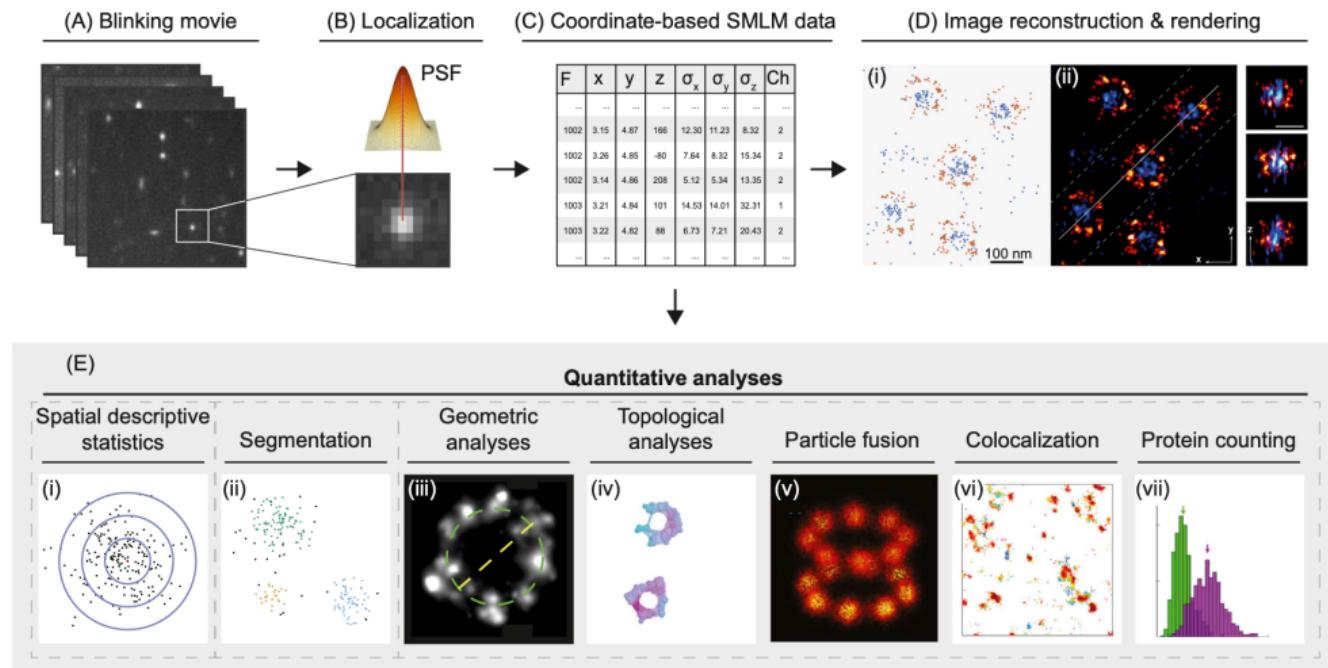


b Temporal separation



- ▶ 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.

A first pass at SMLM for super-resolution

Modeling the point spread function permits sub-pixel localization

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

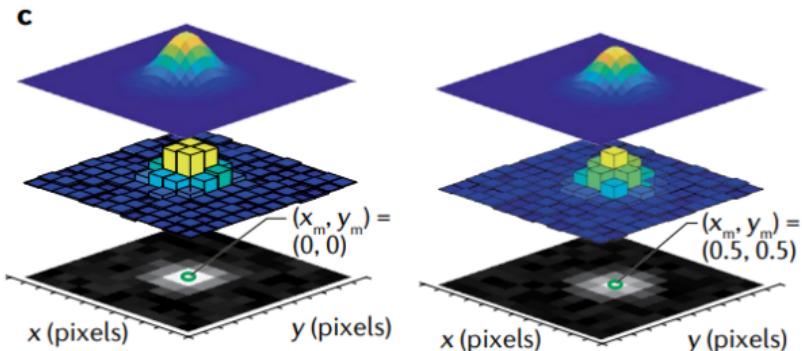
$$i_0 = g_k \eta N_0 \Delta$$

g_k – pixel gain

η – quantum efficiency

N_0 – photon emission rate

Δ – exposure time

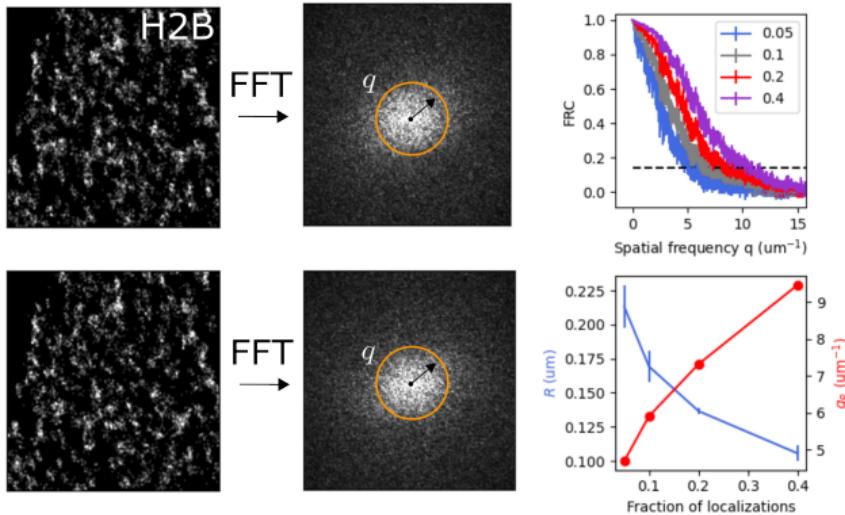


Assume N_0 is constant over Δ (homogeneous Poisson)

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

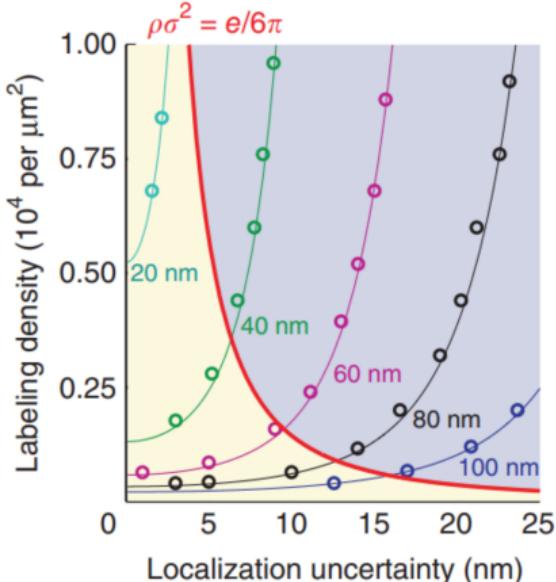
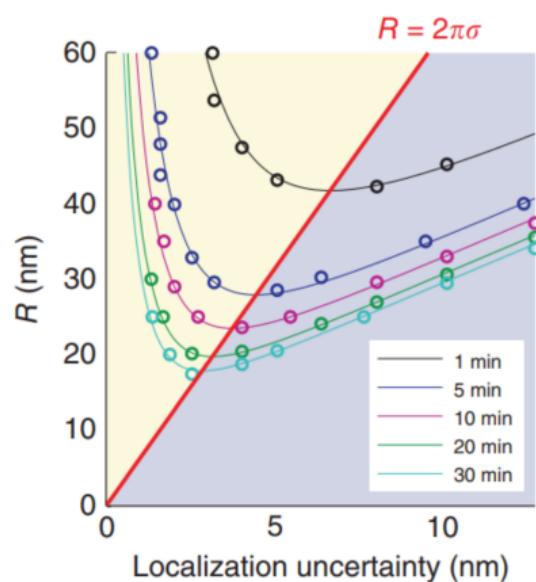
The definition of resolution in SMLM

- 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.

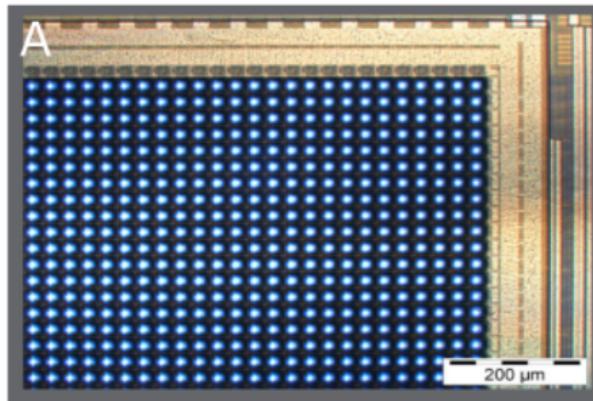
- ▶ Uncertainty-limited resolution (blue), Density-limited resolution (yellow)
- ▶ Labeling density ρ scales with σ to maintain constant resolution

Super-resolution of nucleosome nanodomains *in-vivo*

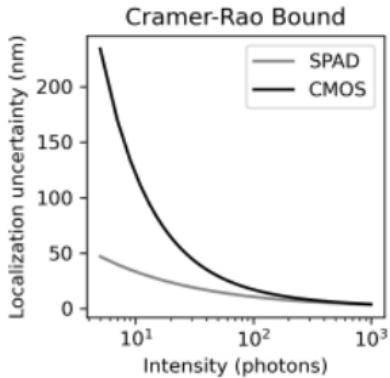
Enhanced SMLM with deep generative models

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

Recent Publications

- ▶ 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!