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

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Outline

Introduction to fluorescence nanoscopy

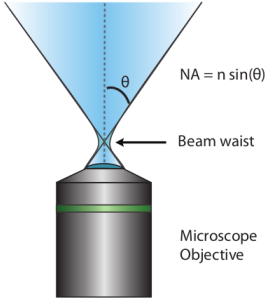
Enhanced nanoscopy with deep generative models

Super-resolution of nucleosome nanodomains *in-vivo*

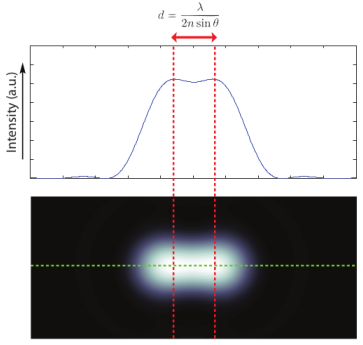
Introduction to fluorescence nanoscopy

Fluorescence microscopy and the diffraction limit

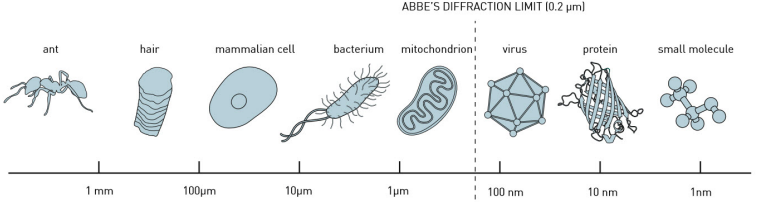
Minimal resolvable distance $d \sim \lambda$



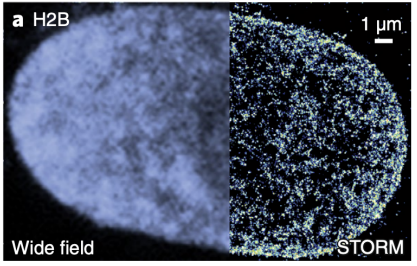
(a)

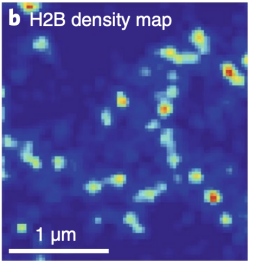


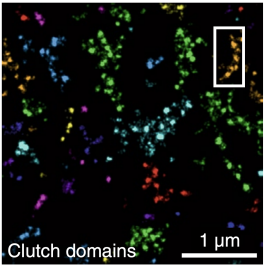
(b)

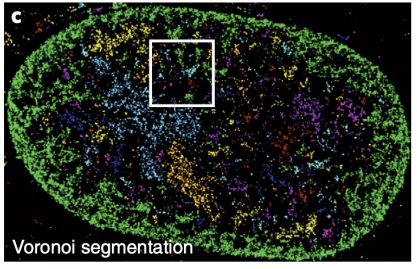


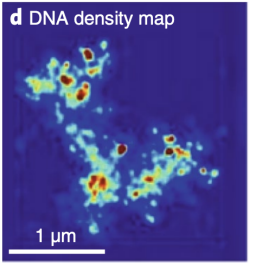
Stochastic optical reconstruction microscopy (STORM)

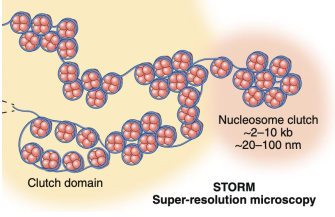






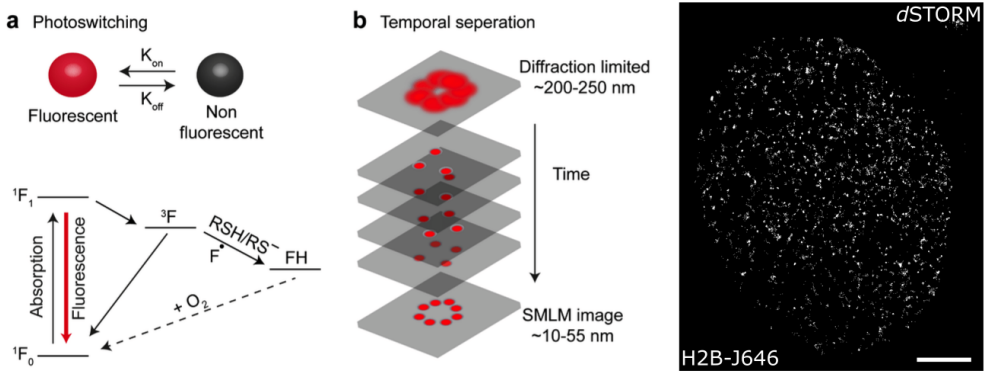






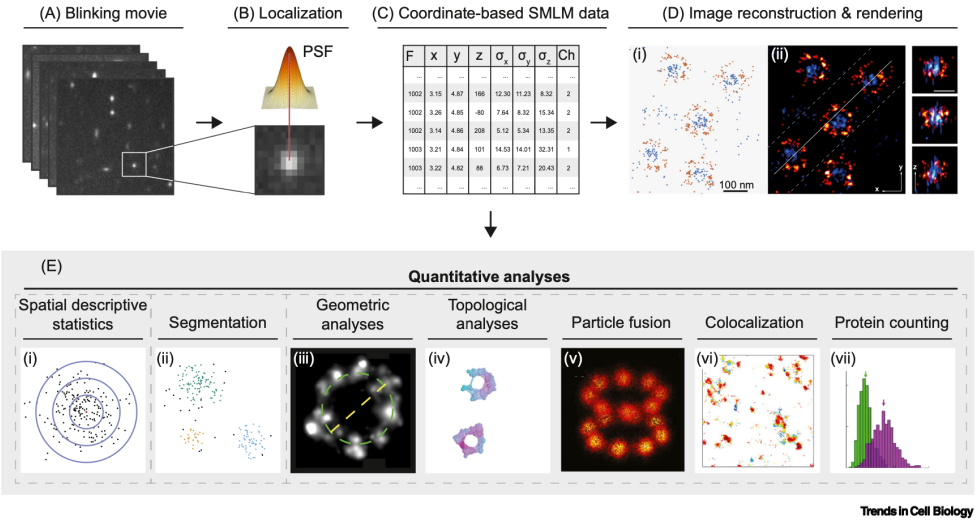
Lakadamyali, M. et al. Nature Methods **17**, (2020).

Stochastic optical reconstruction microscopy (STORM)



- Single molecule localization microscopy (SMLM) techniques are diffraction-unlimited
- Photoswitching enables resolution of emitters below the diffraction limit

Applications of single molecule localization microscopy



Wu et al. Trends in Cell Biology. **30** (2020)

Enhanced nanoscopy with deep generative models

Localization of isolated fluorescent emitters

Modeling the point spread function permits sub-pixel localization

$$\mu_k = i_0 \int\int O(u,v)dudv$$

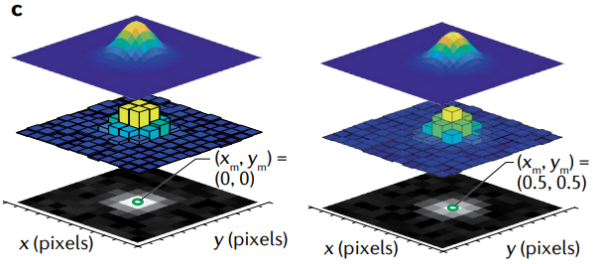
$$i_0 = g_k \eta N_0 \Delta$$

g_k – pixel gain

η – quantum efficiency

N_0 – photon emission rate

Δ – exposure time

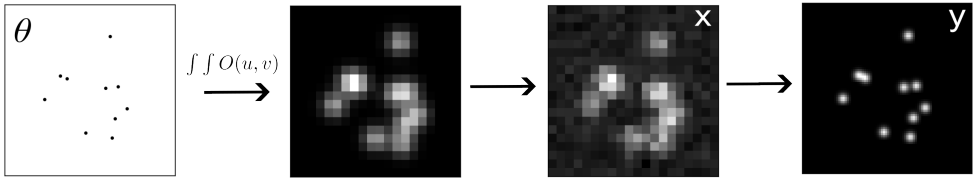


Assume N_0 is constant over Δ (homogeneous Poisson)

$$\theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(\mathbf{x}_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(\mathbf{x}_k|\theta)$$

How to pack more localizations in a single frame?

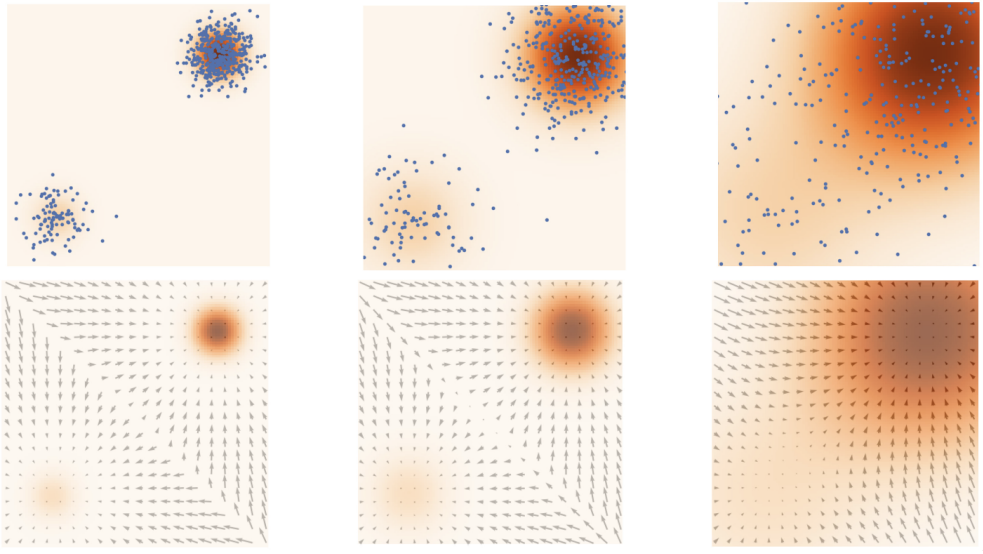
Cast localization as *image restoration*



Seitz et al. Under Review. (2024)

- Would like to estimate a high-resolution **y** from low-resolution **x**, but it is many to one
- Must then model a *distribution* over **y** i.e., $p(\mathbf{y}|\mathbf{x})$
- How to model a distribution over images?

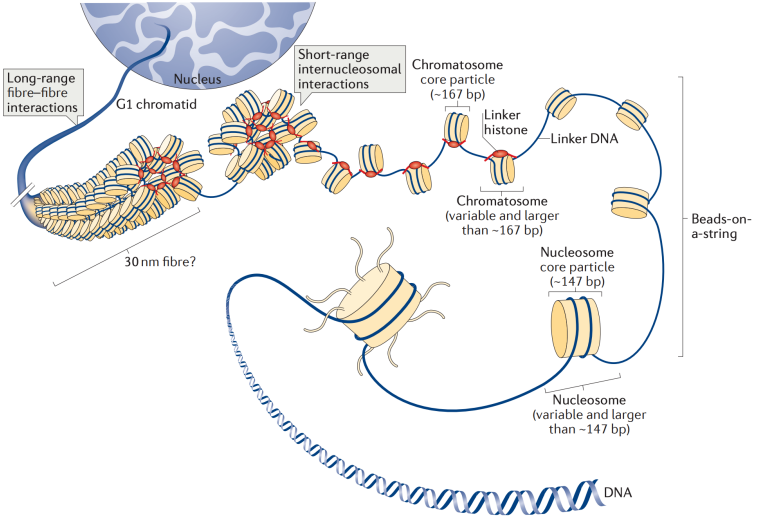
Bayesian image restoration with diffusion models



$$
\begin{aligned}
-\log p(\mathbf{y}_0) &\leq -\mathbb{E}_{q(\mathbf{y}_{1:T}|\mathbf{y}_0)} \log \left(\frac{p_\psi(\mathbf{y}_{1:T}, \mathbf{y}_0)}{q(\mathbf{y}_{1:T}|\mathbf{y}_0)} \right) && (1) \\
&= D_{KL}(q(\mathbf{y}_T|\mathbf{y}_0)||p(\mathbf{y}_T)) + \mathbb{E}_{q(\mathbf{y}_1|\mathbf{y}_0)} \log p(\mathbf{y}_0|\mathbf{y}_1) + \mathcal{L}_\psi && (2)
\end{aligned}
$$

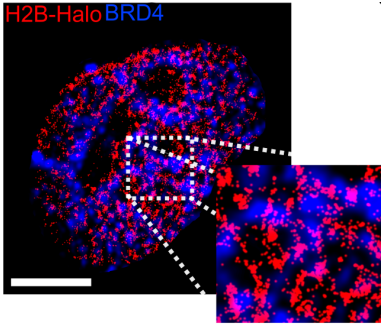
Super-resolution of nucleosome nanodomains *in-vivo*

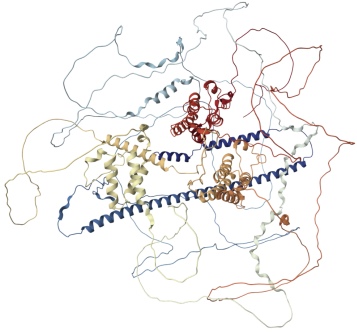
Hierarchical structure of chromatin

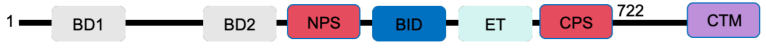


Fyodorov, D. et al. Nat Rev Mol Cell Biol **19**, (2018).

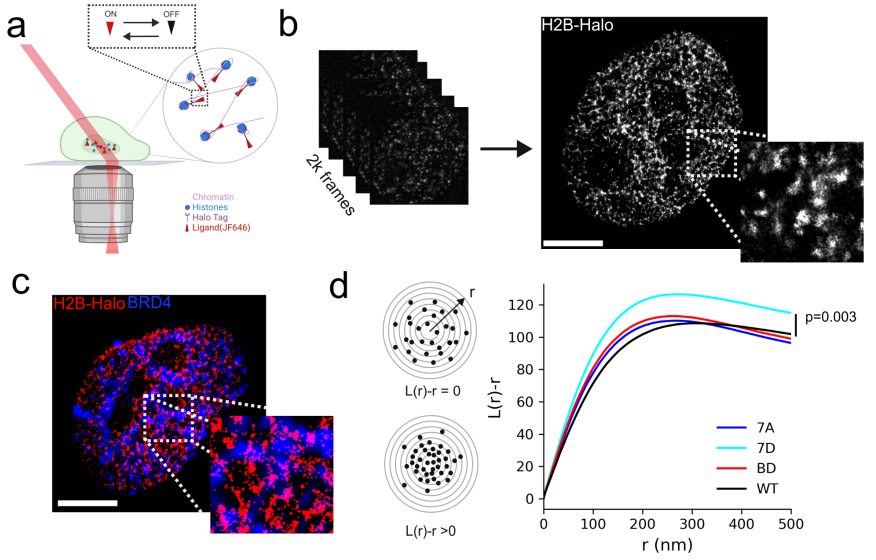
Bromodomain protein 4 (BRD4) binds acetylated chromatin



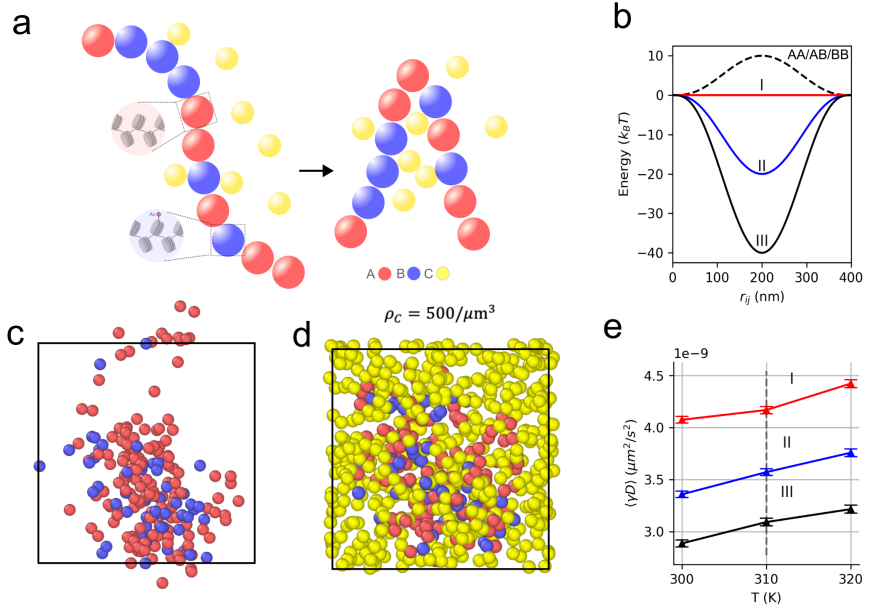




BD	140-A-141	433-A-434	484-SSSDSSSDSSSDSDSSTDDS-503
7A	140-N-141	433-N-434	484-ASSDASSDASADSDAATDDA-503
7D	140-N-141	433-N-434	484-DSSDDSSDDSDDSDDDTDDD-503



Seitz et al. Under Review. (2020)



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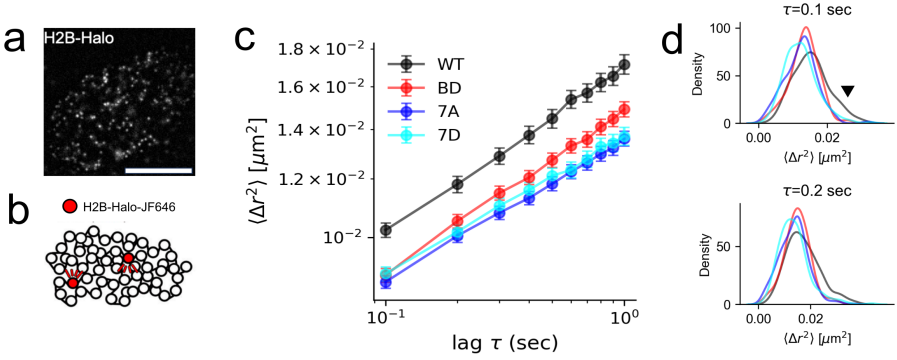

Seitz et al. Under Review. (2024)

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

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Thank you!