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

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Outline of the talk

Introduction to fluorescence nanoscopy

Novel methods: Probabilistic modeling approaches to fluorescence nanoscopy

- Approach I: Enhance nanoscopy with deep generative models
- Approach II: Integrated single photon counting and widefield single molecule imaging

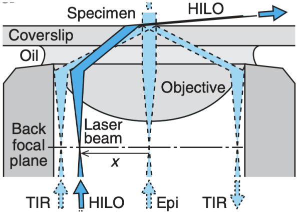
Super-resolution of nucleosome nanodomains *in-vivo*

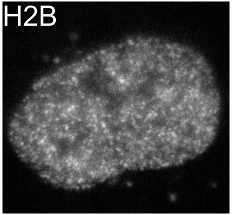
- Interaction of transcriptional condensates with nucleosome nanodomains

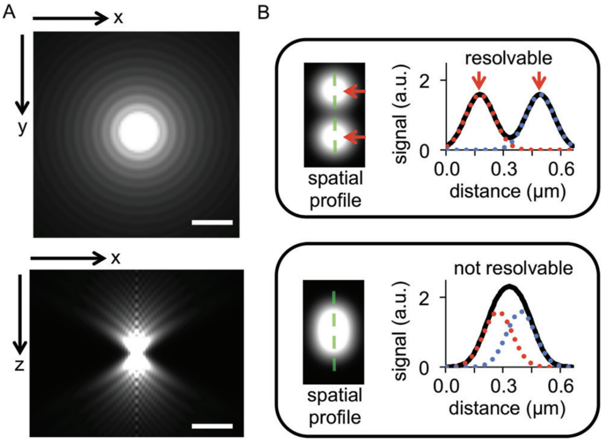
Introduction to fluorescence nanoscopy

Fluorescence microscopy and the diffraction limit

- Minimal resolvable distance $d \sim \lambda/2$

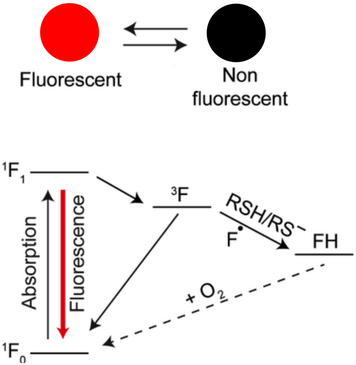


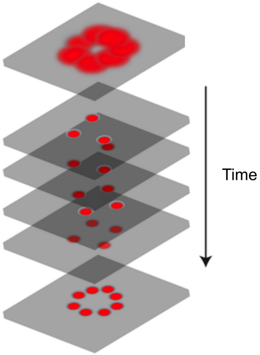


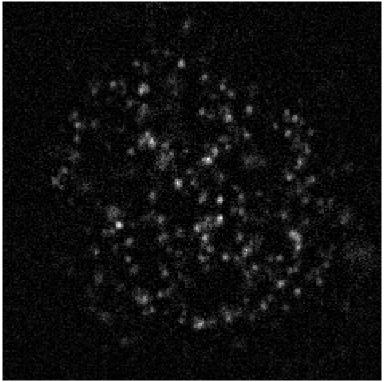


Herbert et al. Microscopy and Microanalysis.

Stochastic optical reconstruction microscopy (STORM)

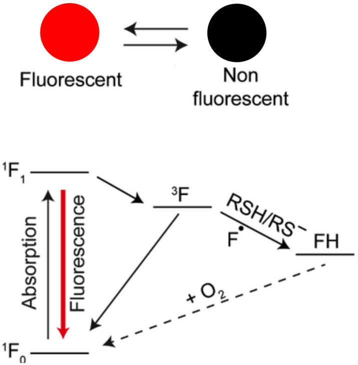


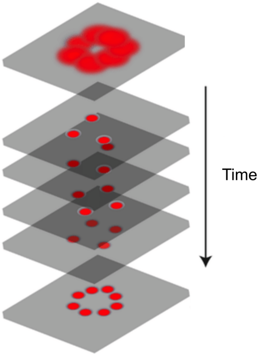


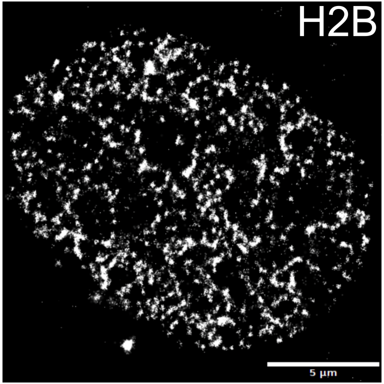


- STORM and similar nanoscopy techniques are limited by localization precision
- Higher lateral/axial resolution than other methods (e.g., SIM,STED,Confocal)
- Poor time resolution

Stochastic optical reconstruction microscopy (STORM)







- STORM and similar nanoscopy techniques are limited by localization precision
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Nanoscopy by localizing isolated fluorescent emitters

- Modeling the point spread function permits sub-pixel localization

$$\mu_k = i_0 \int \int O(u,v) du dv + \lambda$$

$$i_0 = g_k \eta \zeta \Delta$$

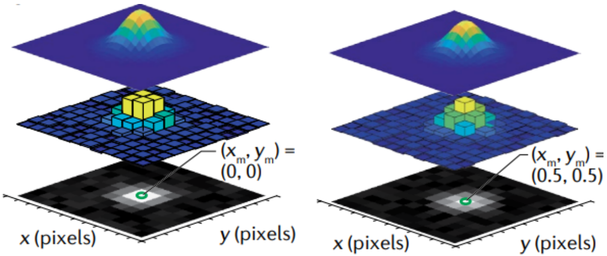
g_k – pixel gain

η – quantum efficiency

ζ – photon emission rate

Δ – exposure time

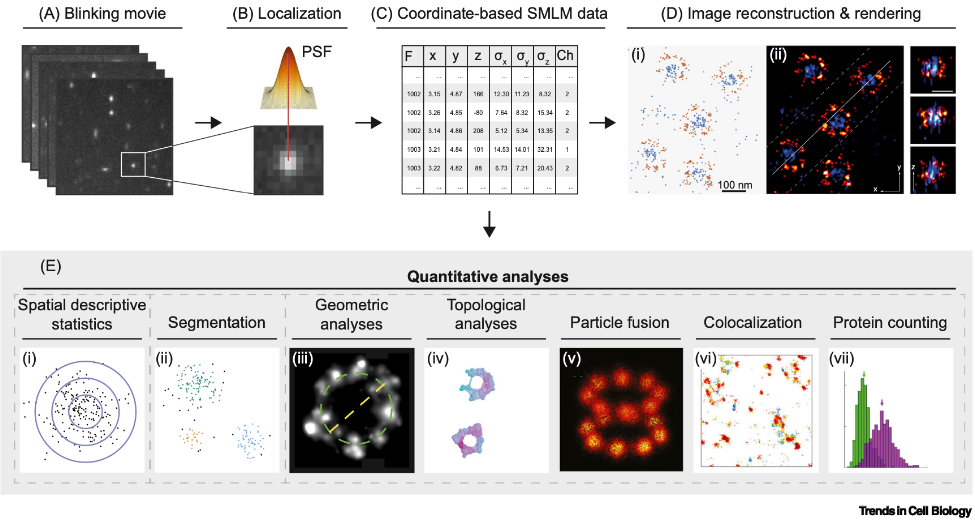
λ – background rate



Maximum likelihood localization:

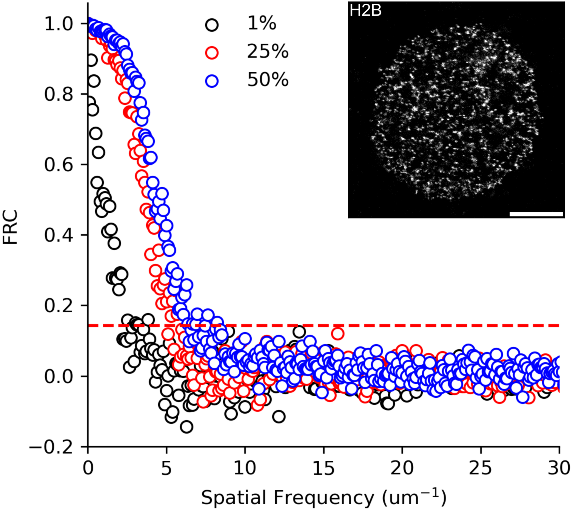
$$\theta^* = \underset{\theta}{\mathrm{argmax}} \prod_k p(\mathbf{x}_k|\theta) = \underset{\theta}{\mathrm{argmin}} - \sum_k \log p(\mathbf{x}_k|\theta)$$

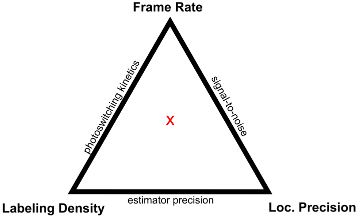
Applications of single molecule localization microscopy



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

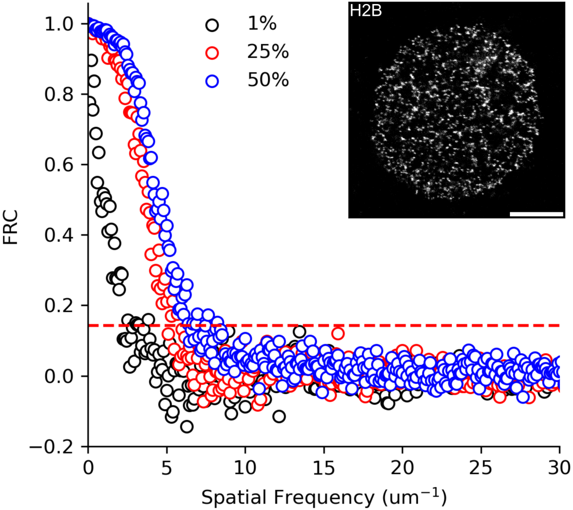
Maximization of density, minimization of error

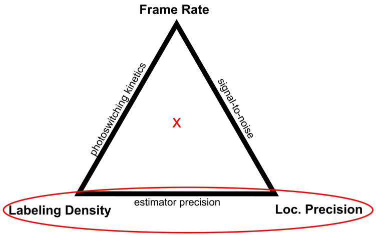




- Maximize density and in turn spatial/temporal resolution
- Minimize localization errors

Maximization of density, minimization of error





- Maximize density and in turn spatial/temporal resolution
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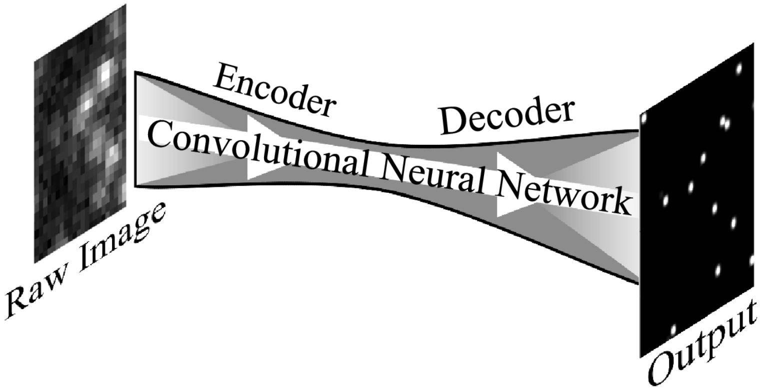
Novel methods: Probabilistic modeling approaches to fluorescence nanoscopy

Resolution enhancement by deep learning

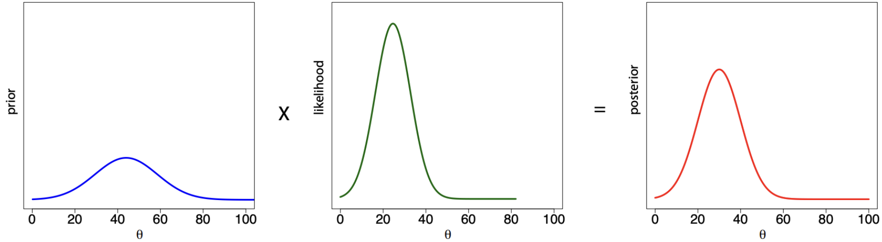


Nehme E. et al. Optica **5**, 458-464 (2018)

- Prediction of high resolution images from low resolution ones
- Cannot report uncertainty, leading to overconfident results

The Bayesian calculation

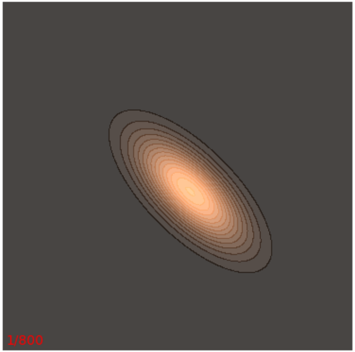
Bayes Rule: $p(\theta|\boldsymbol{x}) = \frac{p(\boldsymbol{x}|\theta)p(\theta)}{\int p(\mathbf{x},\theta)d\theta} \propto p(\boldsymbol{x}|\theta)p(\theta)$



- The posterior $p(\theta|\boldsymbol{x})$ is can be hard to obtain
- We can instead try to sample from $p(\theta|\boldsymbol{x})$
- Can naturally represent uncertainty during inference

Approach I: Resolution enhancement with a diffusion model

- Can sample from $p(\theta|\boldsymbol{x})$ using a stochastic process called Langevin dynamics



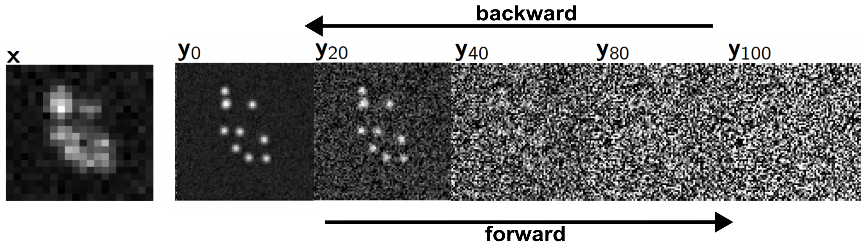
Drift and diffusion: $\theta_t = \overbrace{\theta_{t-1} - \frac{\beta}{2}\nabla f(\theta)}^{\mu} + \sqrt{\beta}\xi \quad \xi \sim \mathcal{N}(0, I)$

Figure credit: Roy Friedman

Approach I: Resolution enhancement with a diffusion model

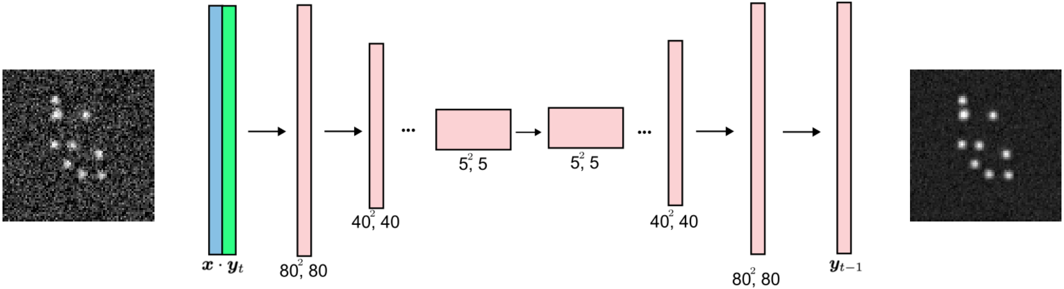
- Task: infer a high resolution image $\mathbf{y}_0$ from low resolution $\mathbf{x}$
- Drift is not available for image data, but can be learned from pairs $(\mathbf{x}, \mathbf{y}_0)$

$$p_\psi(\mathbf{y}_{t-1}|\mathbf{y}_t, \mathbf{x}) = \mathcal{N}\left(\mu_\psi, \beta_t I\right)$$



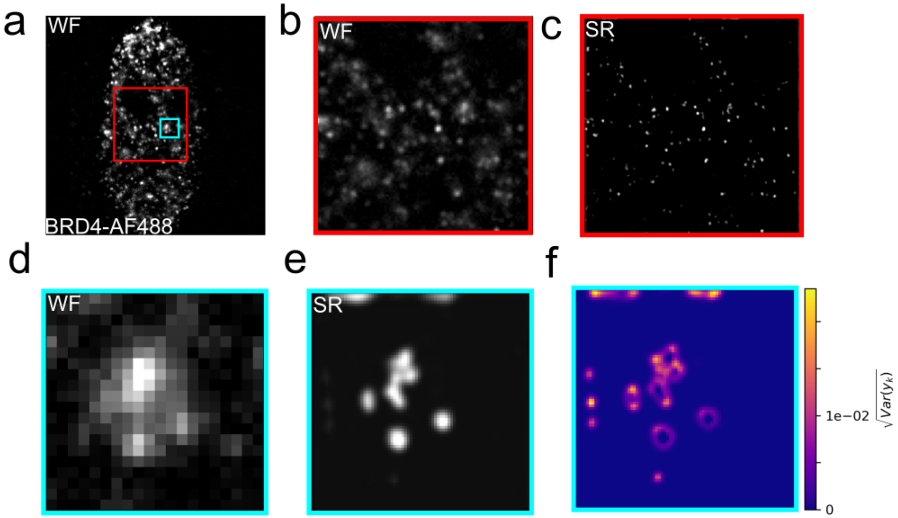
$$q(\mathbf{y}_t|\mathbf{y}_{t-1}) = \mathcal{N}\left(\sqrt{1-\beta_t}\mathbf{y}_{t-1}, \beta_t I\right)$$

Approach I: Resolution enhancement with a diffusion model



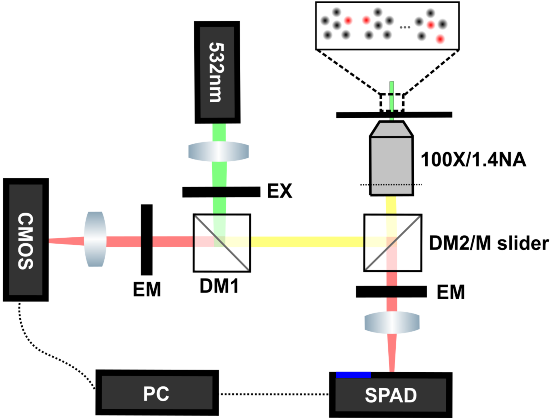
- A convolutional neural network ψ estimates the drift μ_ψ
- Denoising step: $y_{t-1} \sim p_\psi(\mathbf{y}_{t-1}|\mathbf{y}_t, \mathbf{x}) = \mathcal{N}(\mu_\psi, \beta_t I)$

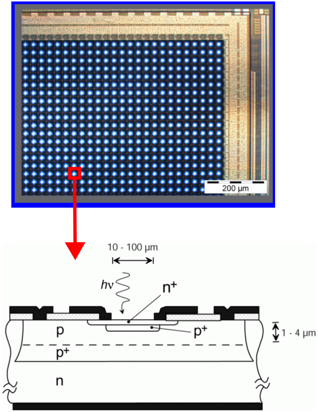
Super resolution of BRD4 protein in a HeLa cell



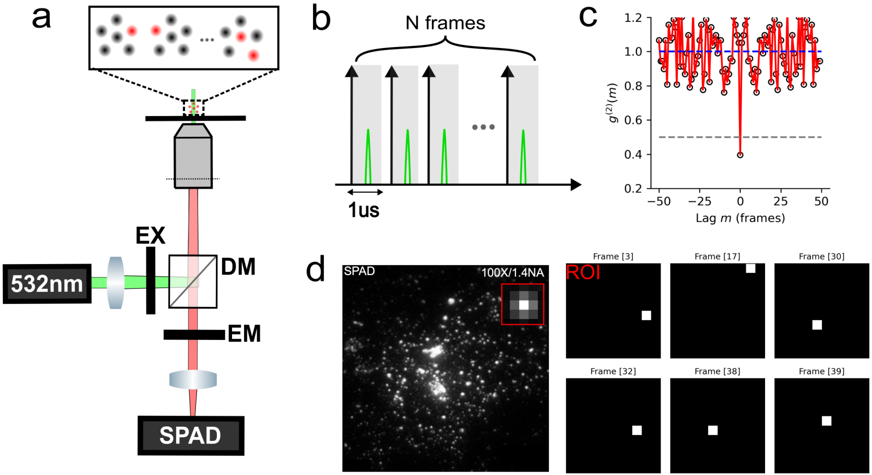
Approach II: Integrated single photon counting and widefield single molecule imaging

- Single photon avalanche diode (SPAD) arrays give us new modeling approaches



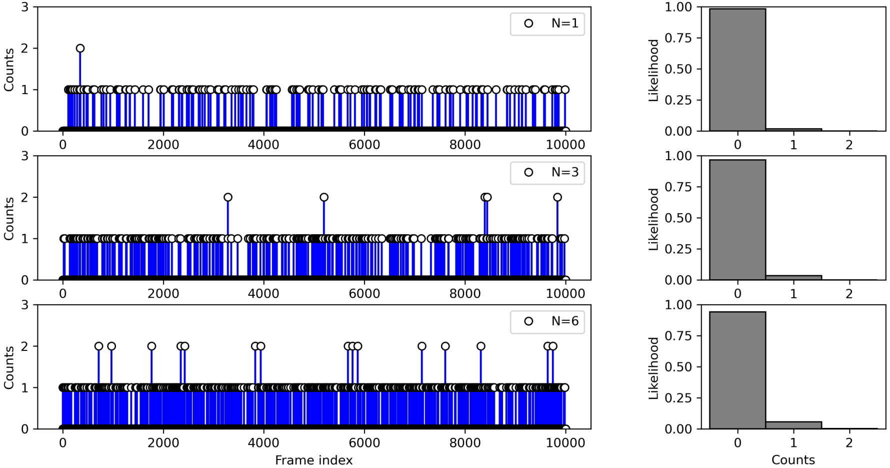


Imaging Qdot655 photon by photon



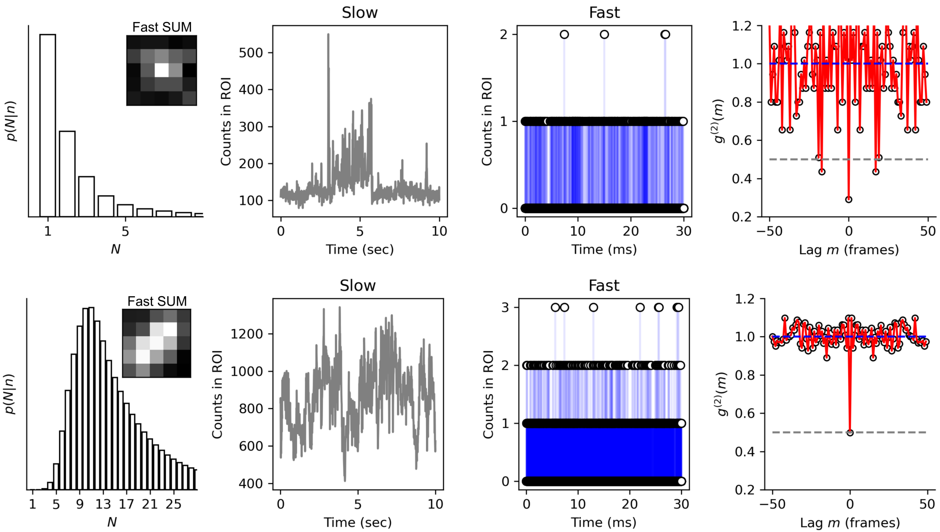
- Second order coherence $g^{(2)}(m)$ measures degree of photon antibunching

Poisson-Binomial photon count data (simulation)



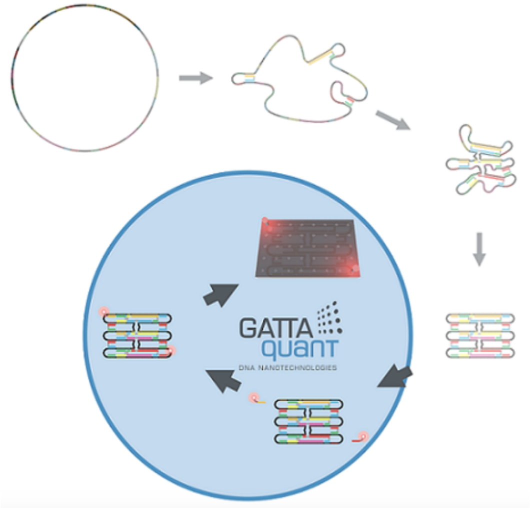
- SPAD arrays permit modeling of time-series of photon counts n_t
- Likelihood on counts: $\mathcal{L}(n_t|N,\zeta,\lambda) = \mathrm{Poisson}(\lambda) * \mathrm{Binomial}(N,\zeta)$

Discrimination of single and multiple quantum dots

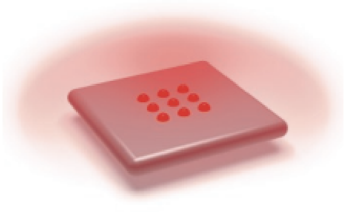


Blinking in quantum dots occurs due to non-radiative relaxation pathways

Upcoming work: counting ATTO532 dye bound to DNA origamis

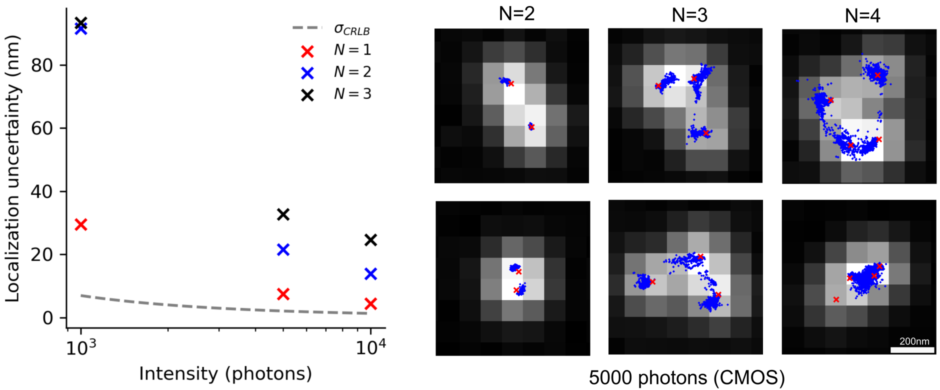


Courtesy of GATTAquant DNA Nanotech



- DNA origami 2um grid
- Each binds N ATTO532 dyes
- Verification of counting method

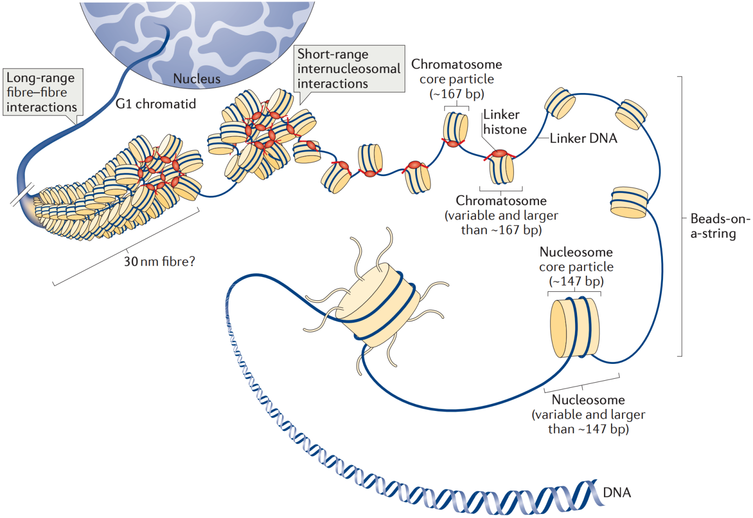
Constrained multi-emitter localization (simulation)



- Constrained multi-emitter localization when N is known
- Multiple emitters can be fit simultaneously using MCMC sampling

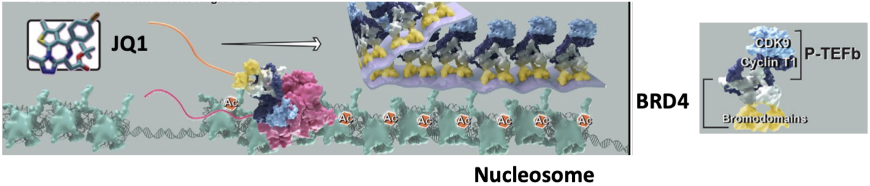
Super-resolution of nucleosome nanodomains *in-vivo*

Hierarchical structure of chromatin

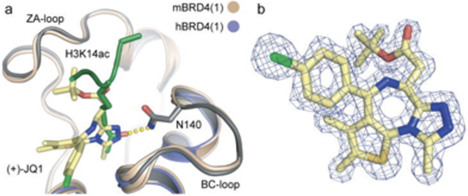


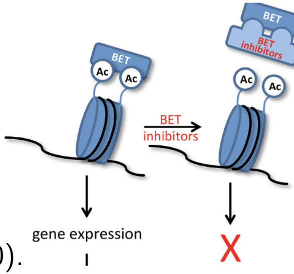
Fyodorov, D. et al. Nat Rev Mol Cell Biol **19**, (2018).

Bromodomain protein 4 (BRD4) binds acetylated chromatin



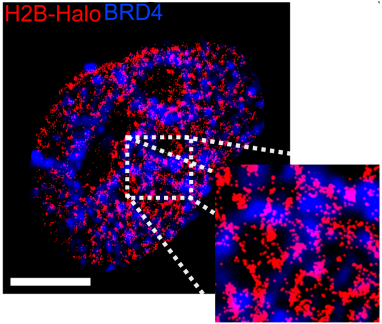
Zheng, B. et al. Molecular Cell **16**, (2023).

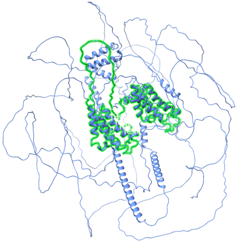




Fillapakoulos, P. et al. Nature **468**, (2010).

BRD4 mutations to probe effects on chromatin structure

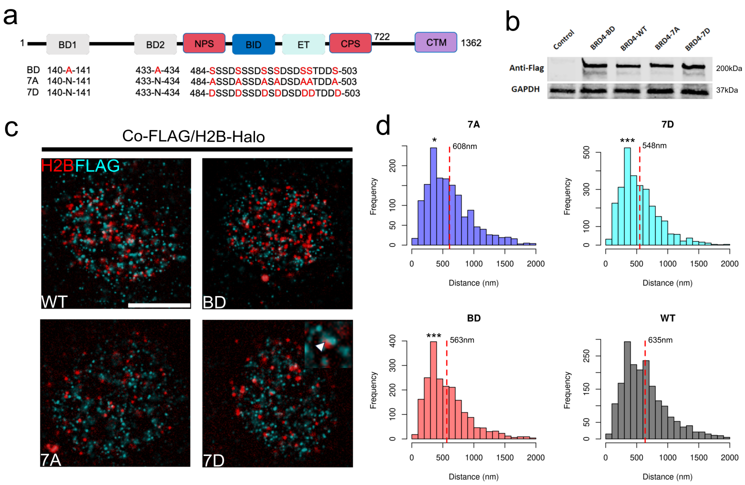




AlphaFold BRD4 1-1362aa

- 7A mutant - Constitutively unphosphorylated
- 7D mutant - Constitutively phosphorylated
- BD mutant - Bromodomain deactivated

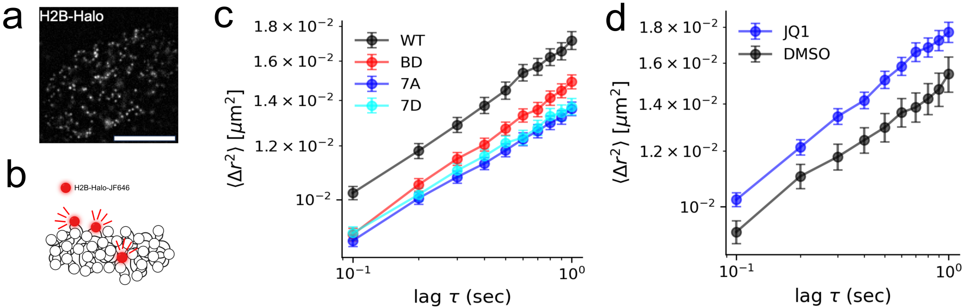
BRD4 recruitment to nucleosome nanodomains



- Phosphorylation enhances BRD4 binding to acetylated chromatin
- Hyperphosphorylation - resistance mechanism to BD loss (Shu 2016)

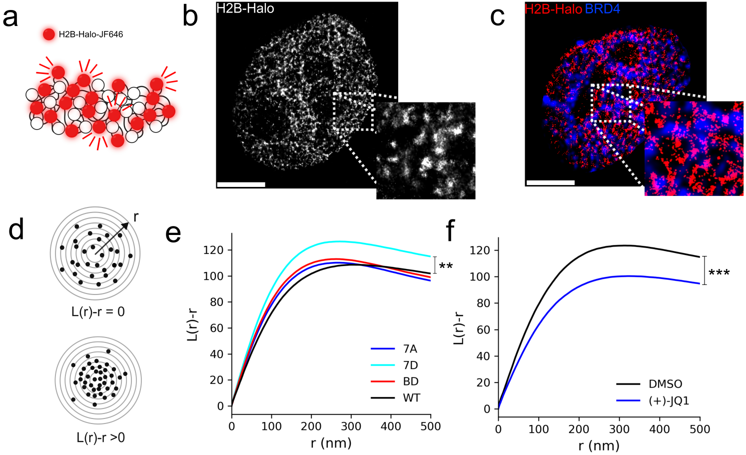
Seitz C. et al. 2024 (In Review) Phys Rev Lett.

BRD4 chromatin binding controls chromatin mobility



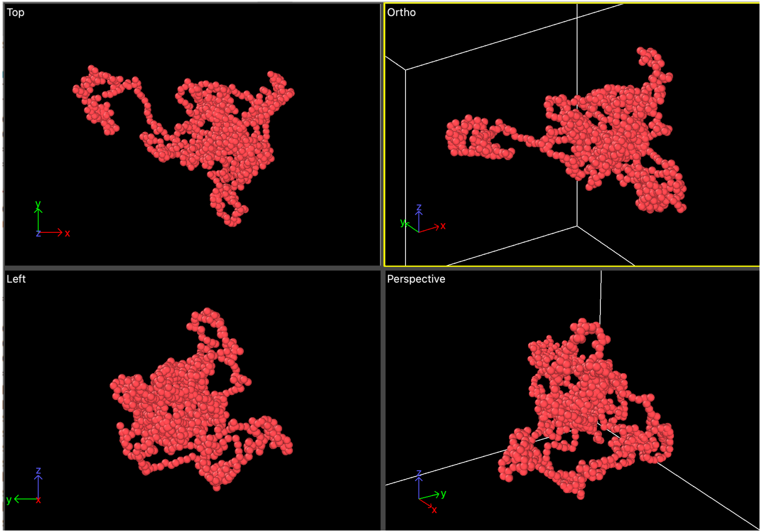
- H2B is sparsely labeled for particle tracking
- Reduced diffusion coefficient D in BRD4 mutants
- Increased D in cells exposed to (+)-JQ1

BRD4 binding is necessary for maintenance of nucleosome nanodomains



- H2B is densely labeled for super-resolution imaging
- BRD4 chromatin binding activity controls nanodomain density

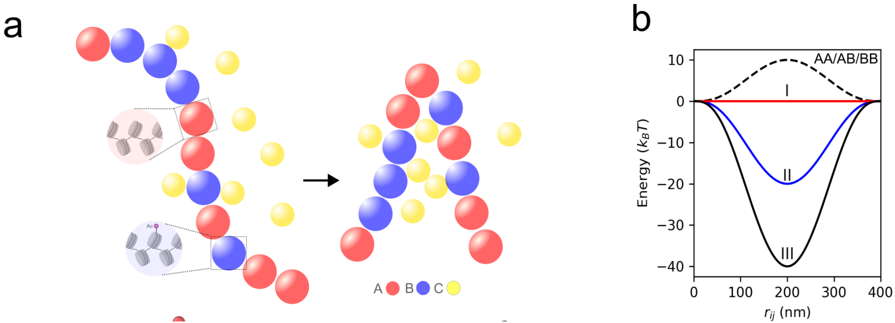
Coarse grained molecular dynamics of chromatin at 310K



- 100kb chromatin beads connected by harmonic bonds (Rouse model)

Seitz C. et al. 2024 (In Review) Phys Rev Lett.

Coarse grained molecular dynamics of chromatin binders at 310K

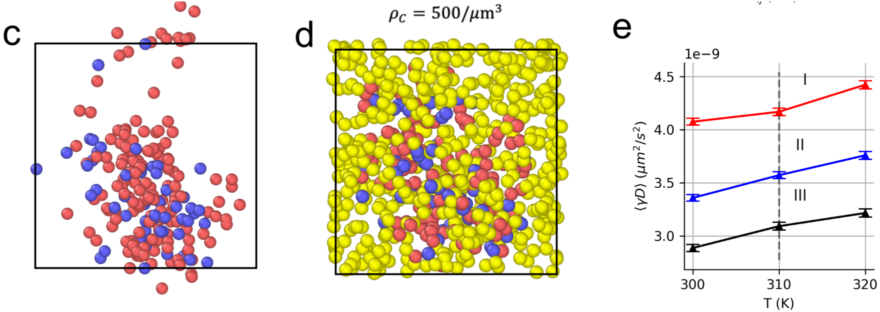


Chromatin chains interact with binders via the potential

$$U_{ij} = \epsilon \left(1 - \left(\frac{|r_{ij}|}{R_0}\right)^2\right)^3$$

- A (B) type particles represent unacetylated (acetylated) chromatin beads
- BRD4-like C particles bind B type particles with variable energies

Multivalent chromatin binding reduces chromatin mobility



Integrate Brownian dynamics: $\dot{r} = \gamma^{-1}\nabla U + \sqrt{2k_B T}\gamma^{-1/2}\xi \quad \gamma = 10^{-6}$

Stochastic forcing is a delta-correlated noise $\xi \sim \mathcal{N}(0,1), \langle \xi(t)\xi(t+\tau)\rangle = \delta(\tau)$

Summary of contributions

- First generative model of super-resolution microscopy images from low-resolution inputs
- Applied SPAD array for counting fluorescent molecules in widefield microscopy
- First data supporting control of chromatin architecture by transcriptional condensates

Selected Publications

- **C. Seitz**, D. Fu, M. Liu, H. Ma, and J. Liu. *BRD4 phosphorylation regulates the structure of chromatin nanodomains*. In Review. Phys Rev Lett. 2024
- **C. Seitz** and J. Liu. *Uncertainty-aware localization microscopy by variational diffusion*. In Progress. 2024
- **C. Seitz** and J. Liu. *Quantum enhanced localization microscopy with a single photon avalanche diode array*. In Progress. 2024
- M. Locatelli†, J. Lawrimore†, H. Lin†, S. Sanaullah, **C. Seitz**, D. Segall, P. Kefer, S. Moreno Naike, B. Lietz, R. Anderson, J. Holmes, C. Yuan, G. Holzwarth, B. Kerry, J. Liu, K. Bonin, P. Vidi. *DNA damage reduces heterogeneity and coherence of chromatin motions*. PNAS 12 July 2022; 119 (29): 1-11
- M. Zhang, **C. Seitz**, G. Chang, F. Iqbal, H. Lin, and J. Liu *A guide for single-particle chromatin tracking in live cell nuclei*. Cell Biology International 15 January 2022; 46 (5): 683-700
- S. Roy, **C. Seitz**, M. Samaddar, J. Liu *Bayesian Inference of Transcriptional Kinetics of GBP5 by Single-Molecule Imaging*. In Progress. 2024

Acknowledgements

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

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Thank you!