Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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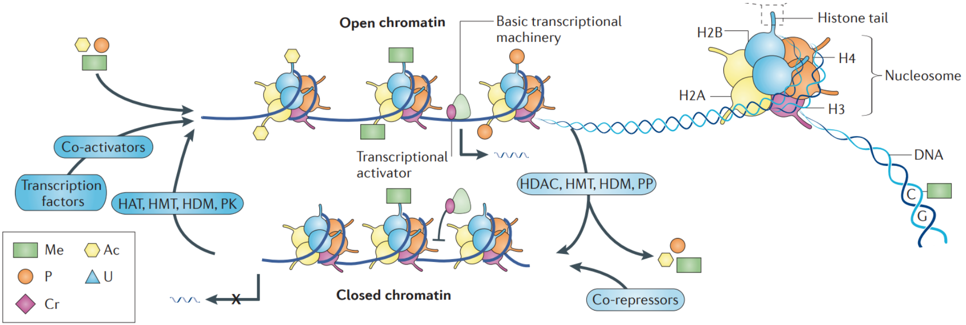
Introduction

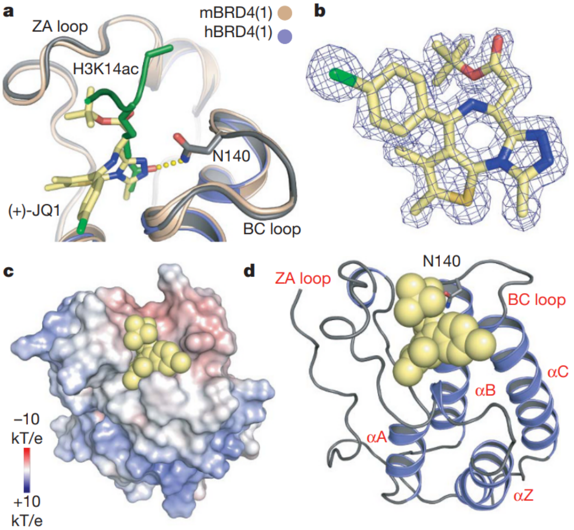
The textbook view of histone acetylation



Graff et al. Histone acetylation: molecular mnemonics on the chromatin

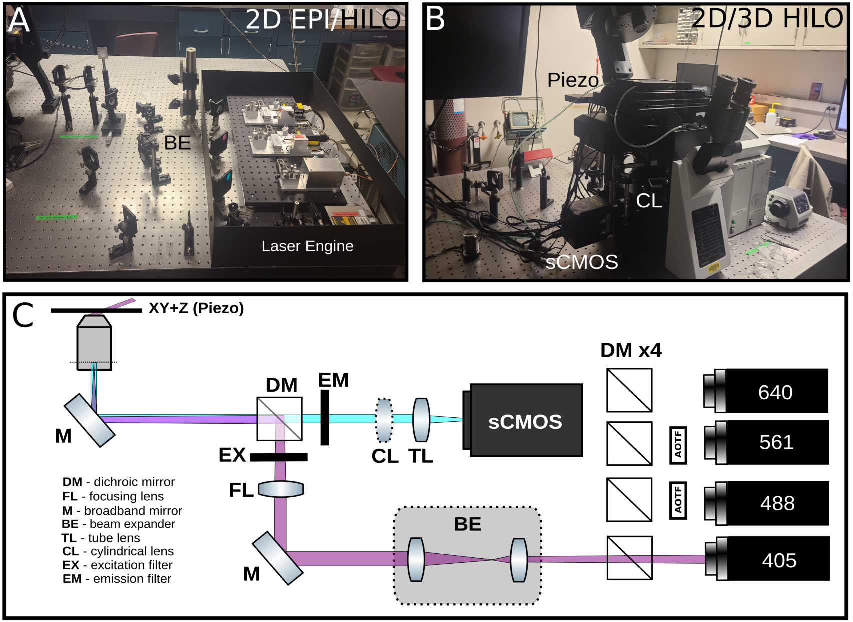
- I am interested in the impact of BRD4 protein on chromatin structure
- Previous work has shown BRD4 is associated with histone acetylation
- Live cell super-resolution imaging is a useful tool

(+)-JQ1 in complex with BRD4 protein

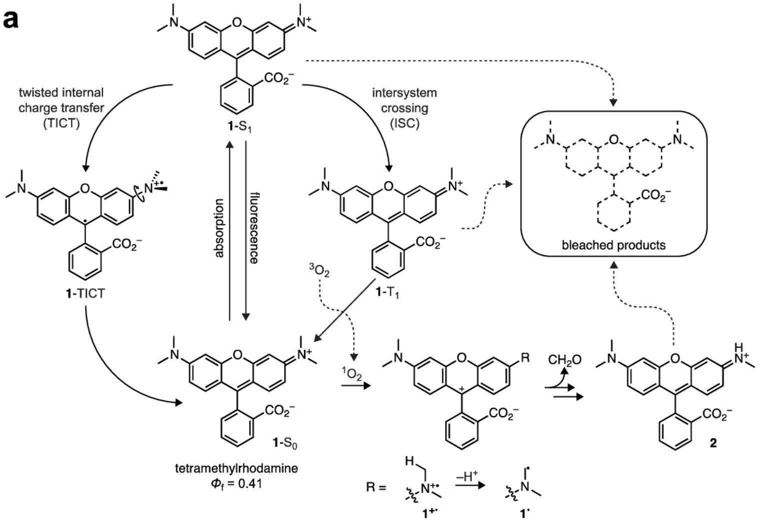


Filippakopoulos. Selective inhibition of BET bromodomains. Nature Communications

Instrumentation for super-resolution and high throughput microscopy

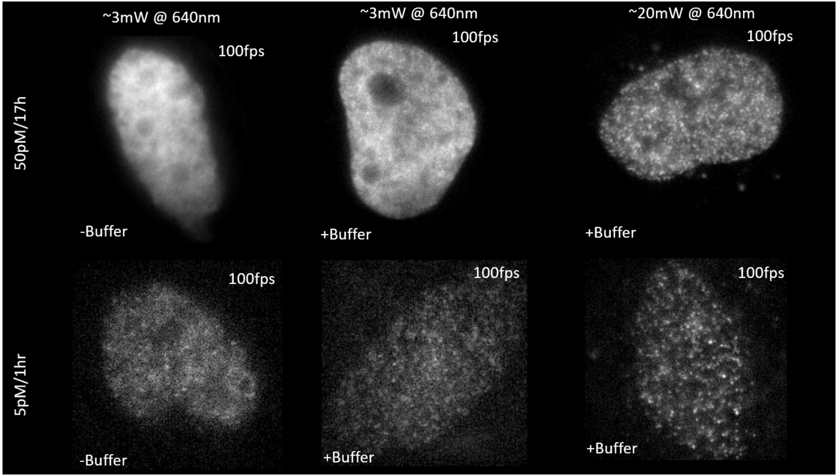


The photophysics of rhodamines



- Reduction of the T1 state yields a dark, long-lived, and stable radical state
- The reducing agent is usually a primary thiol like cysteamine (MEA)

The OFF state of JF646 can be maintained with high laser power



- High power maintains the OFF state, potentially by promoting triplet state formation

Maximum likelihood localization of an isolated fluorescent emitter

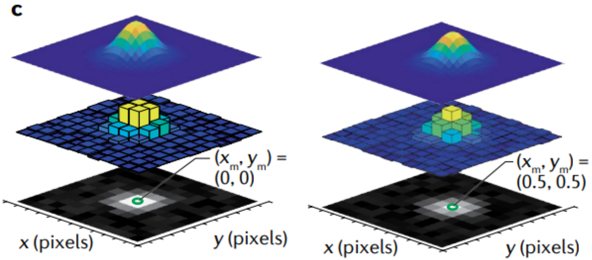
Localization: $\theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$

$$\mu_k = g_k \eta N_0 \Delta \int_{\text{pixel}} G(x, y) dA$$

η – quantum efficiency

N_0 – photon count

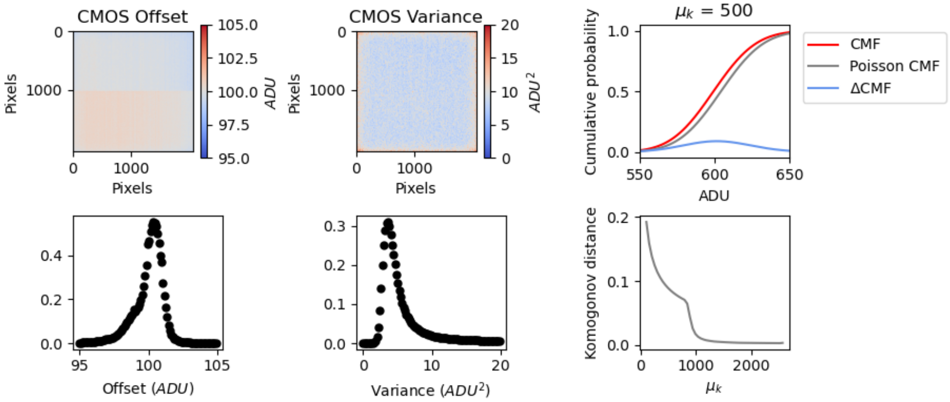
Δ – exposure time



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

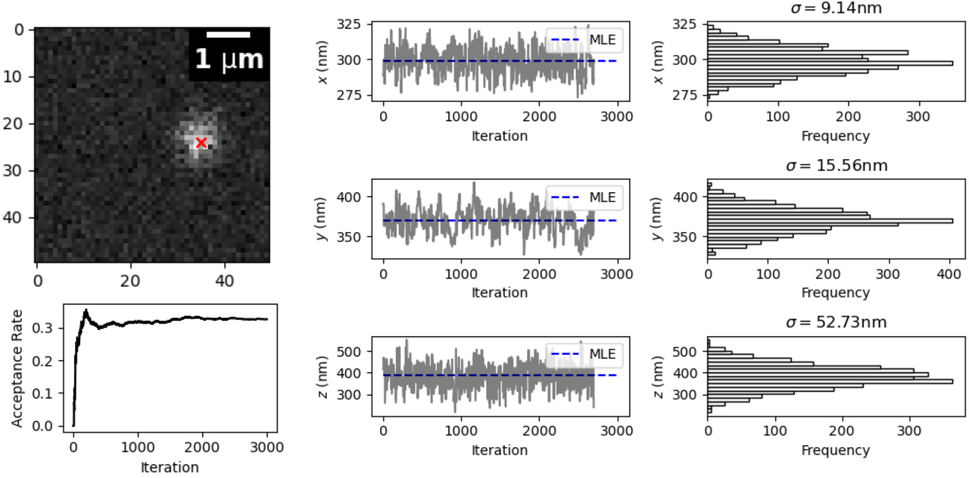
$P(H_k|\theta)$ can be approximated as Poisson at high signal-to-noise (SNR)

A Poisson approximation at moderate SNR simplifies SMLM



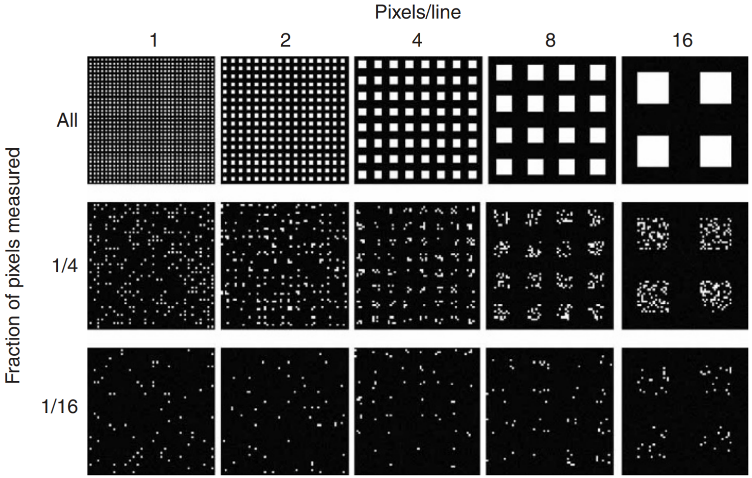
- $P(H_k - o_k|\theta) = \mathrm{Poisson}(\mu_k + \sigma_k^2|\theta)$ for pixel offset o_k noise variance σ_k^2
- Fisher information and Cramer-Rao lower bound (CRLB) can be computed analytically for Poisson log-likelihood ℓ (Smith 2010, Huang 2013)

Estimator precision sets the resolution limit in localization microscopy



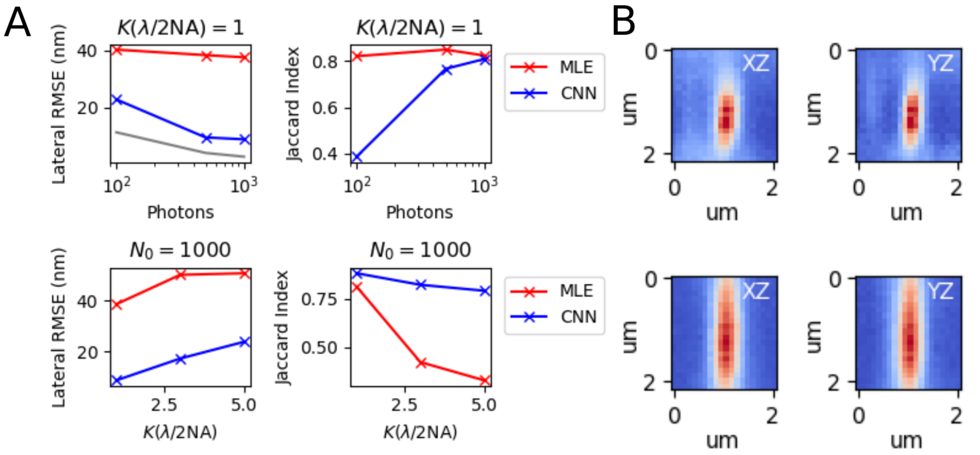
- Variance of the posterior $P(\theta|\vec{H})$ is a useful particle filter
- We assume uniform priors on coordinates

The tradeoff between spatial and temporal resolution in SMLM



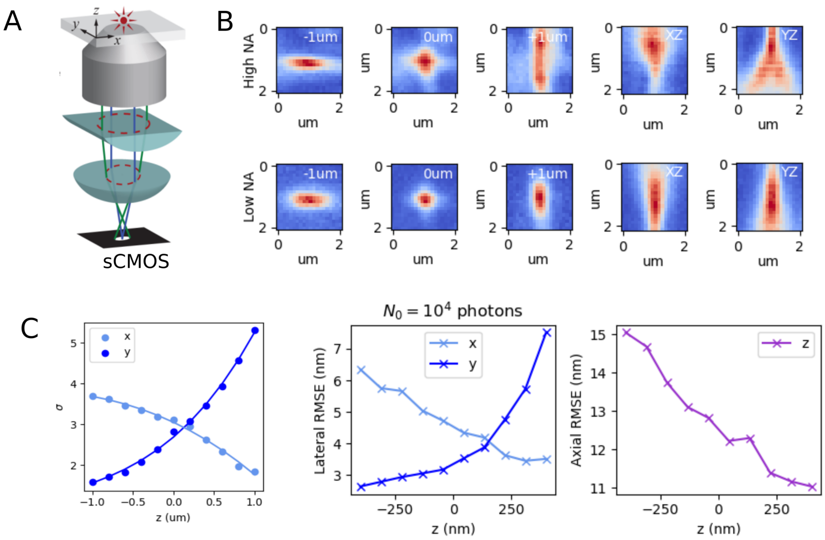
- SMLM is desirable for SR due to very high res and no scanning (e.g., STED)
- Less control over photophysical state, but high throughput

Deep learning enables dense localization in two-dimensions



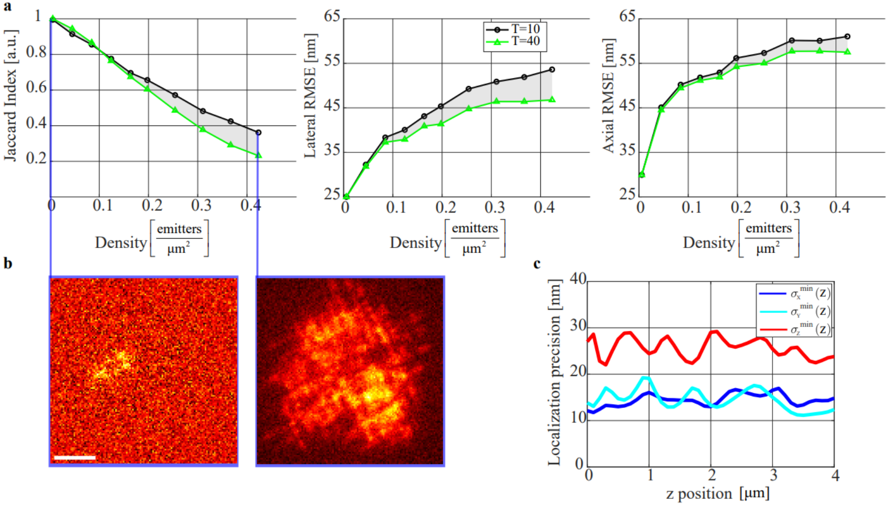
- $K(\lambda/2\mathrm{NA})$ is Ripley's K function at the diffraction limit
- Convolutional neural networks (CNNs) approach the CRLB (gray) at high photon counts and generalize to the dense regime

Astigmatism based three dimensional imaging



- A weak ($f = 10\text{m}$) cylindrical lens breaks the axial symmetry of the PSF

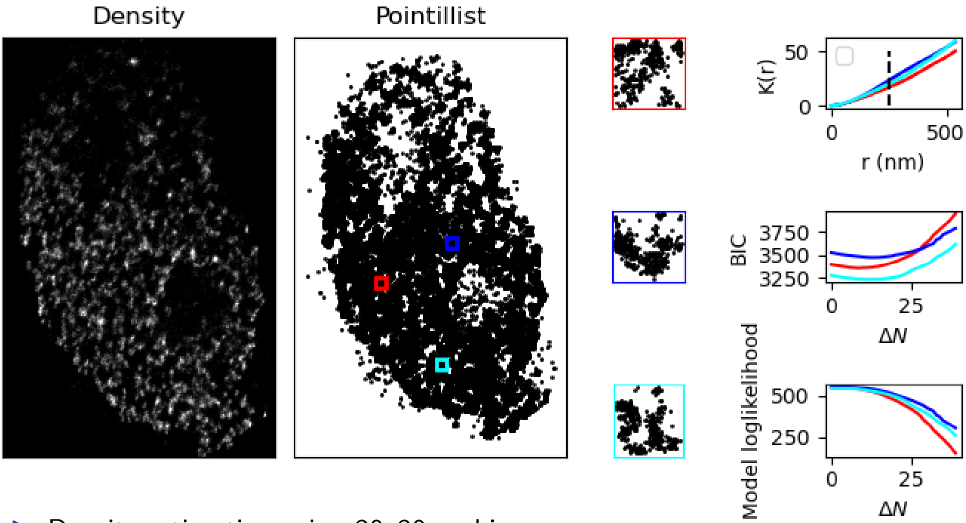
Deep learning can generalize precise SMLM to three dimensions



Several methods have been designed to break the time-resolution barrier

- Density estimation using 30x30nm bins
- Bayesian information criterion (BIC) used to reduce the effect of multiple blinking, assuming 10nm lateral uncertainty

Chromatin nanodomains in a living Hela cell nucleus at 37C



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Diffusion increases localization uncertainty in live-cell SMLM

Nucleosome diffusion has been modeled in various potentials:

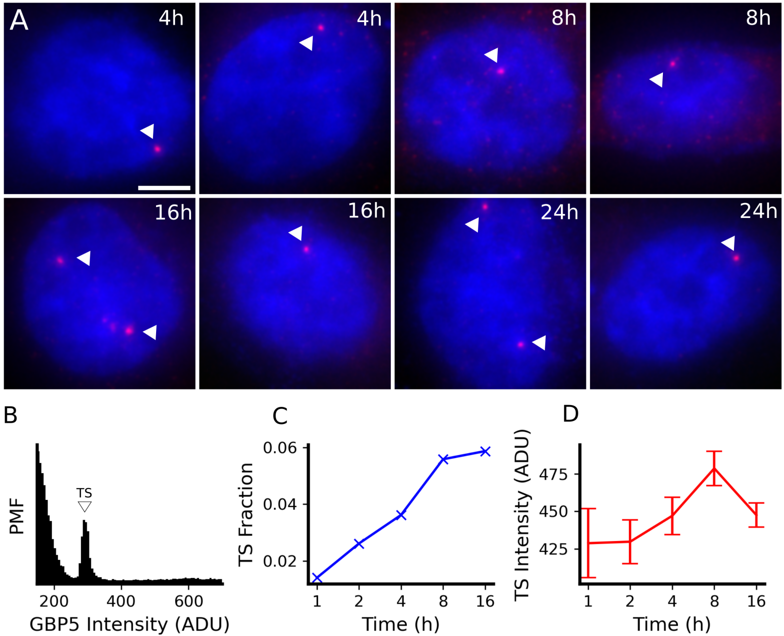
- Bead model: $V(r_{ij}) = \epsilon_0 (r_0/r_{ij})^{12} - \epsilon_{ij}(r_0/r_{ij})^6$ (Ashwin 2019)
- Harmonic: $V(\vec{\Delta r}) = \frac{1}{2}k|\vec{\Delta r}|^2$ (XXX)

The latter is attractive because the stationary distribution of Brownian motion in a harmonic potential is known:

$$\partial_t P(r) = \hat{\mathcal{L}}_{FP} P(r); \hat{\mathcal{L}}_{FP} = \hat{\mathcal{L}}_{FP} = \left(-\frac{\partial}{\partial x} M^{(1)}(t) + \frac{1}{2}\frac{\partial^2}{\partial x^2} M^{(2)}(t) \right)$$

$$x', t' | x, t = \mathcal{N}(\mu, \Sigma)$$

Validaton of JQ1 efficacy for BRD4 inhibition in Hela cells



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