

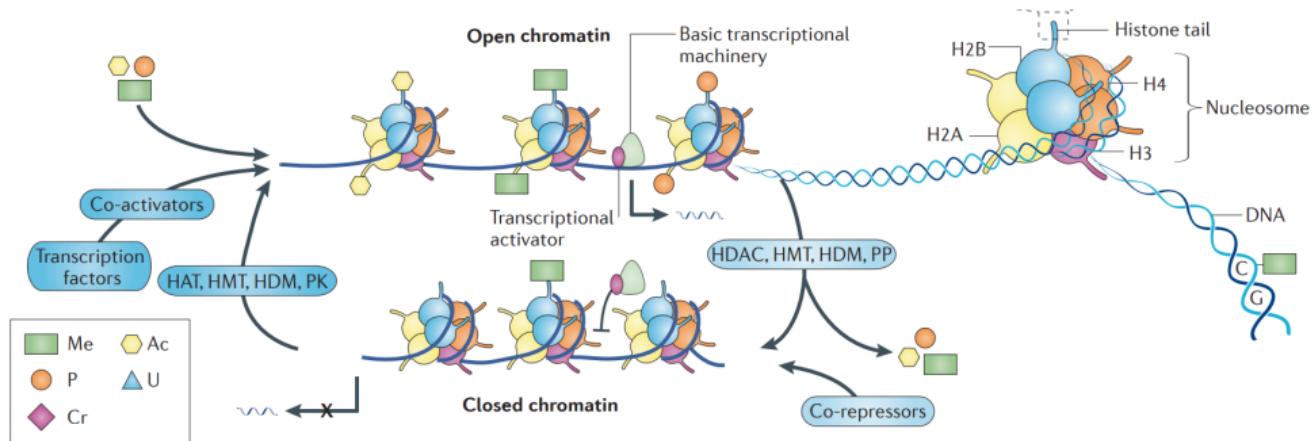
Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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Introduction

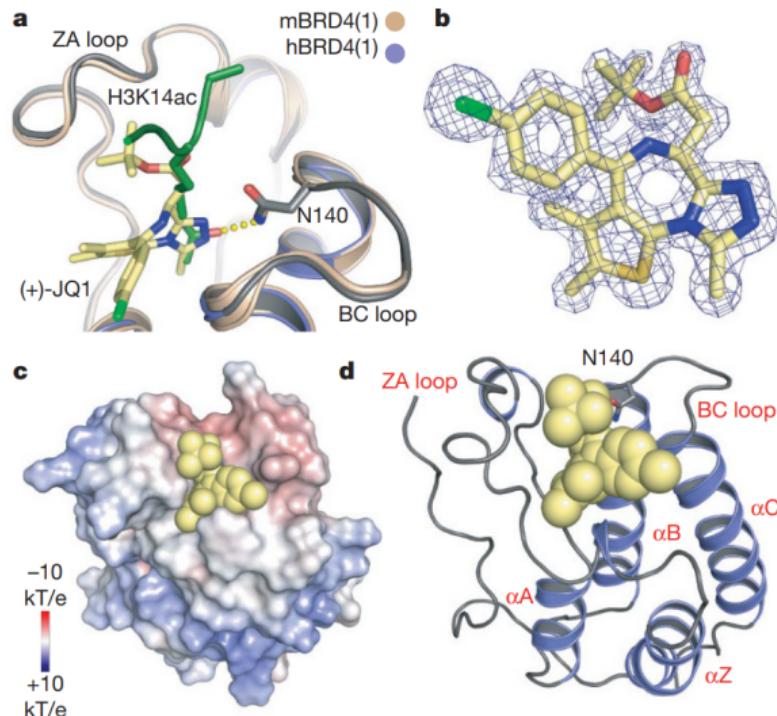
The textbook view of histone acetylation



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

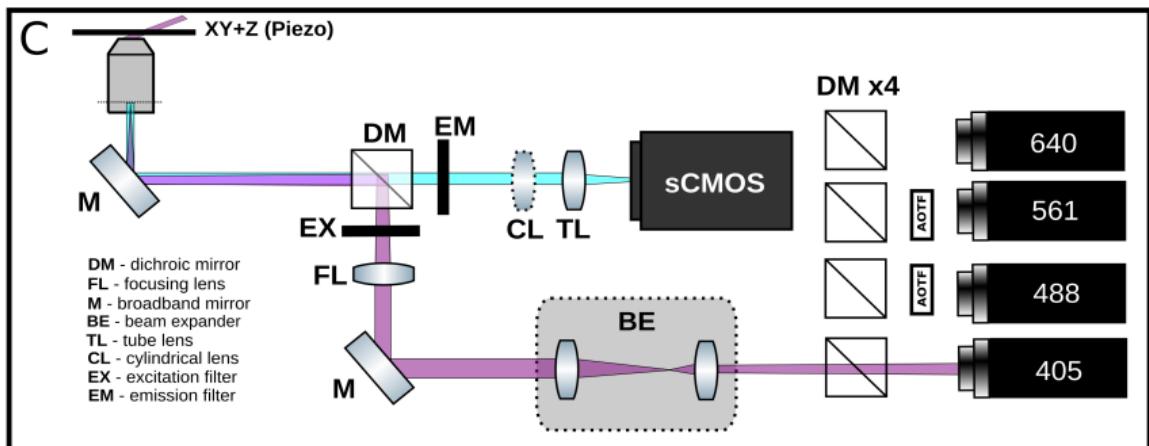
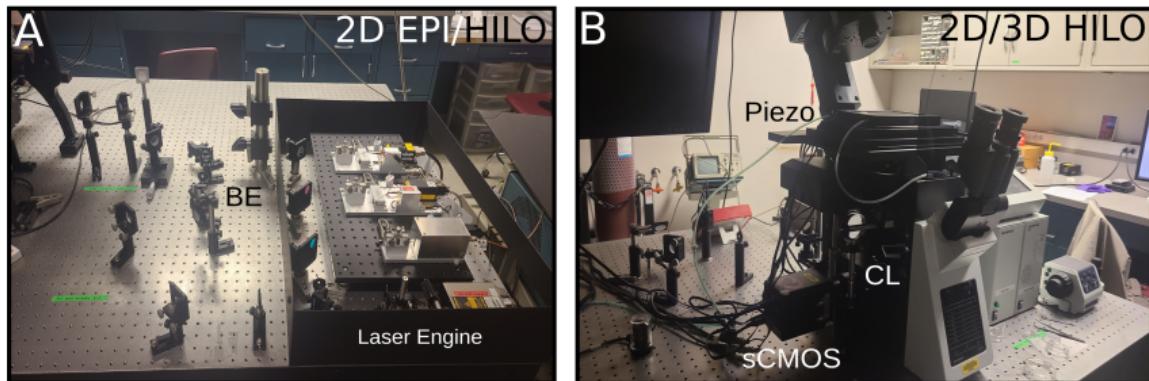
- ▶ I am interested in the impact of BRD4 protein on chromatin structure
- ▶ Previous work has shown BRD4 is associated with histone acetylation
- ▶ Live cell super-resolution imaging is a useful tool

(+)-JQ1 in complex with BRD4 protein

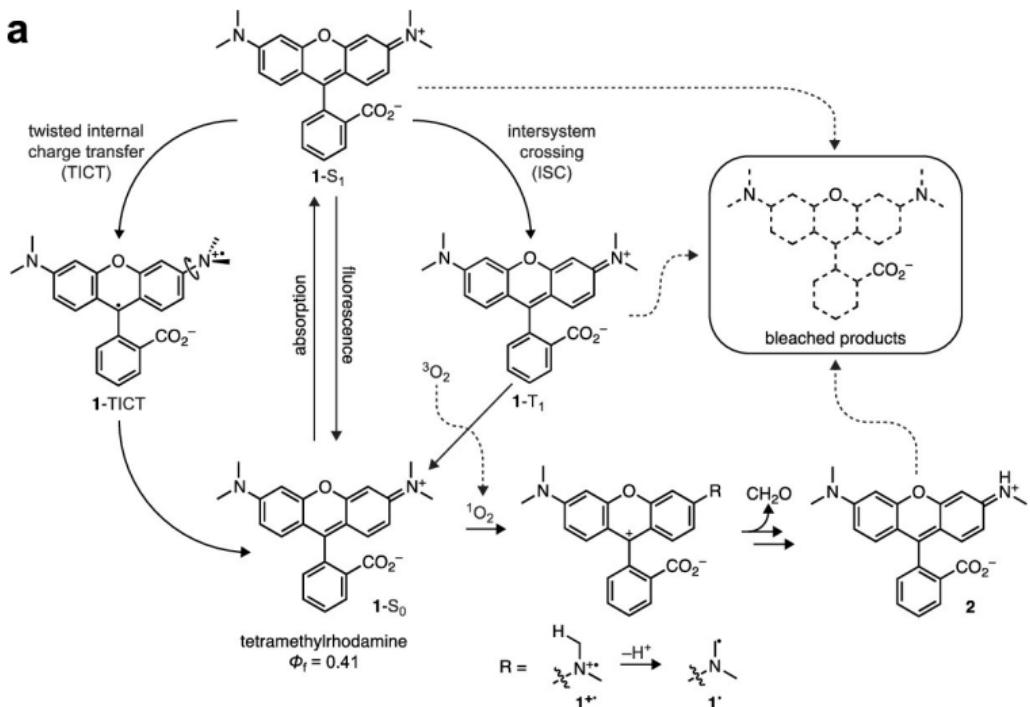


Filippakopoulos. Selective inhibition of BET bromodomains. *Nature Communications*

Instrumentation for super-resolution and high throughput microscopy

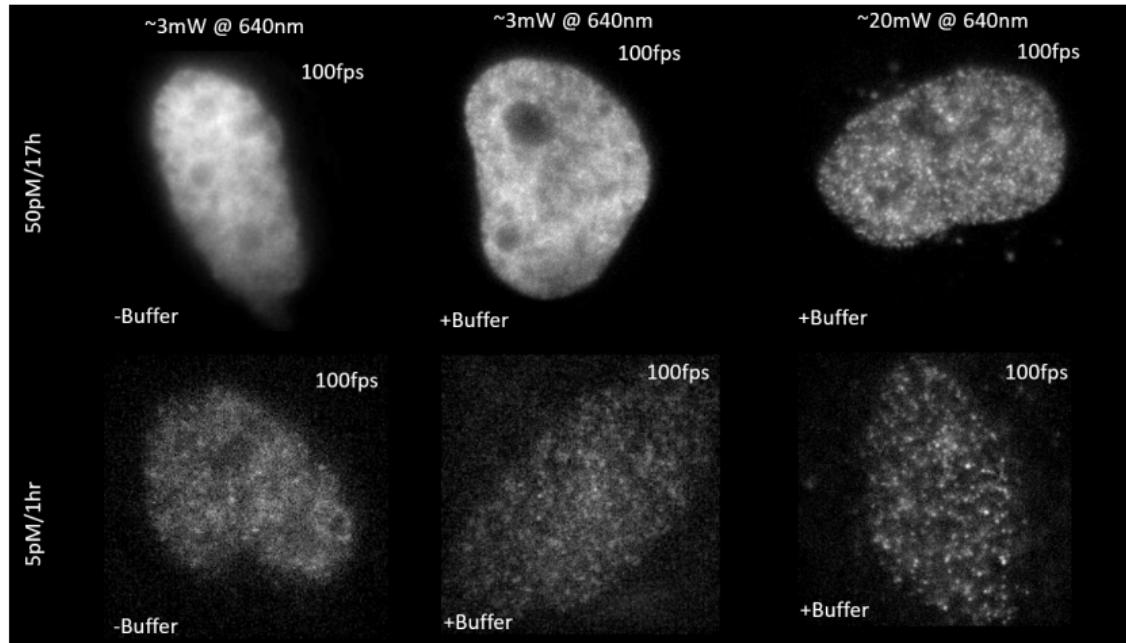


The photophysics of rhodamines



- ▶ 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



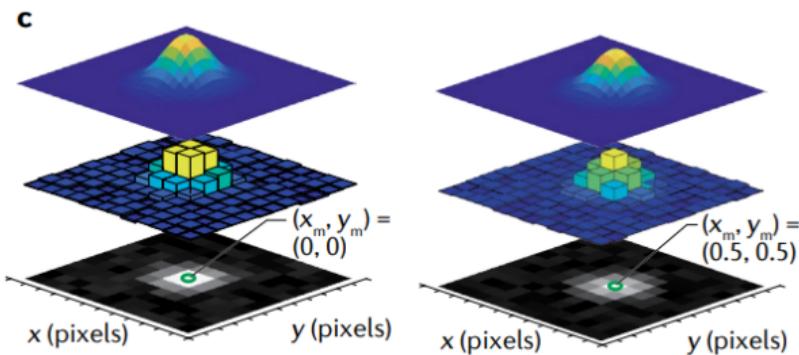
- ▶ High power maintains the OFF state, potentially by promoting triplet state formation

Maximum likelihood localization of an isolated fluorescent emitter

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

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

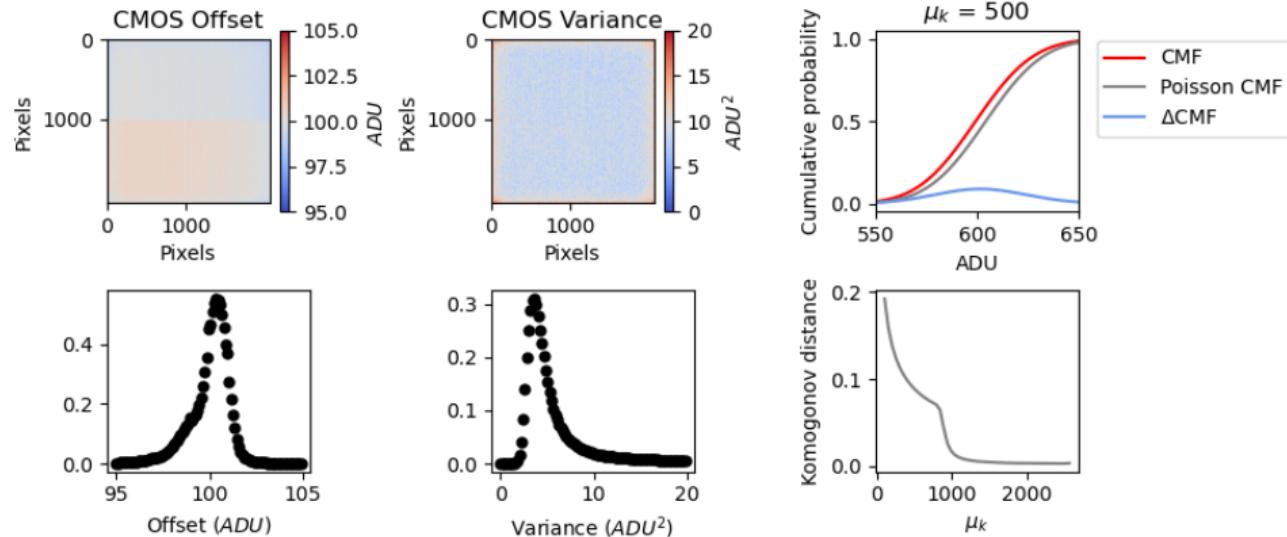
- η – quantum efficiency
 N_0 – photon count
 Δ – exposure time



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

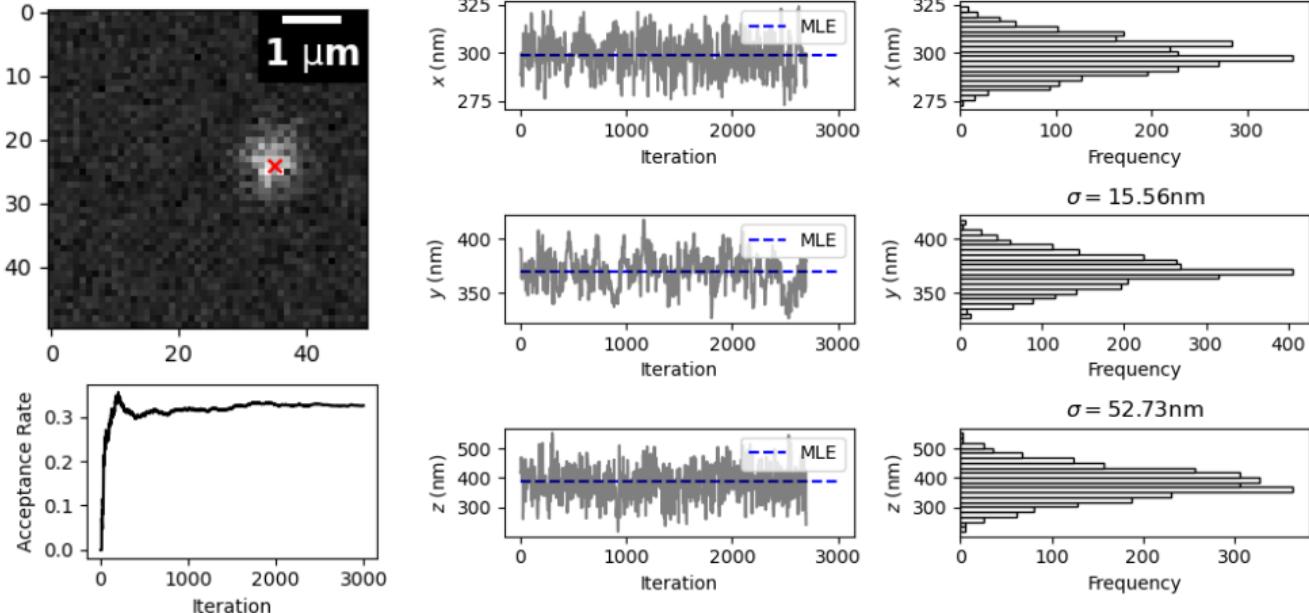
$P(H_k|\theta)$ can be approximated as Poisson at high signal-to-noise (SNR)

A Poisson approximation at moderate SNR simplifies SMLM



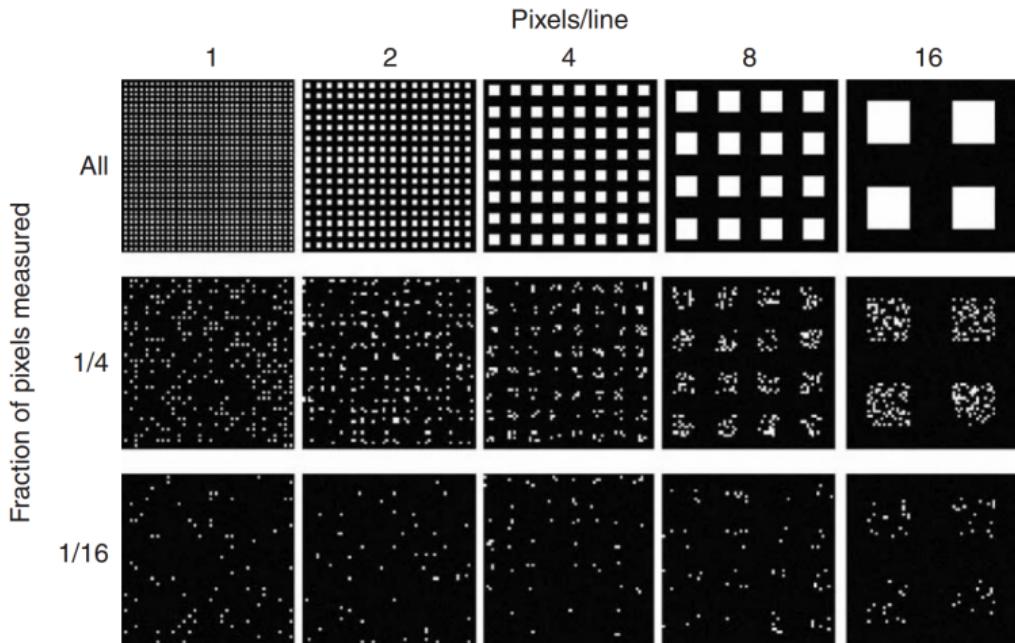
- ▶ $P(H_k - o_k | \theta) = \text{Poisson}(\mu_k + \sigma_k^2 | \theta)$ for pixel offset o_k noise variance σ_k^2
- ▶ Cramer-Rao lower bound (CRLB) in 2D can be computed analytically for Poisson log-likelihood ℓ (Smith 2010, Huang 2013)

Localization precision sets the resolution limit in localization mic



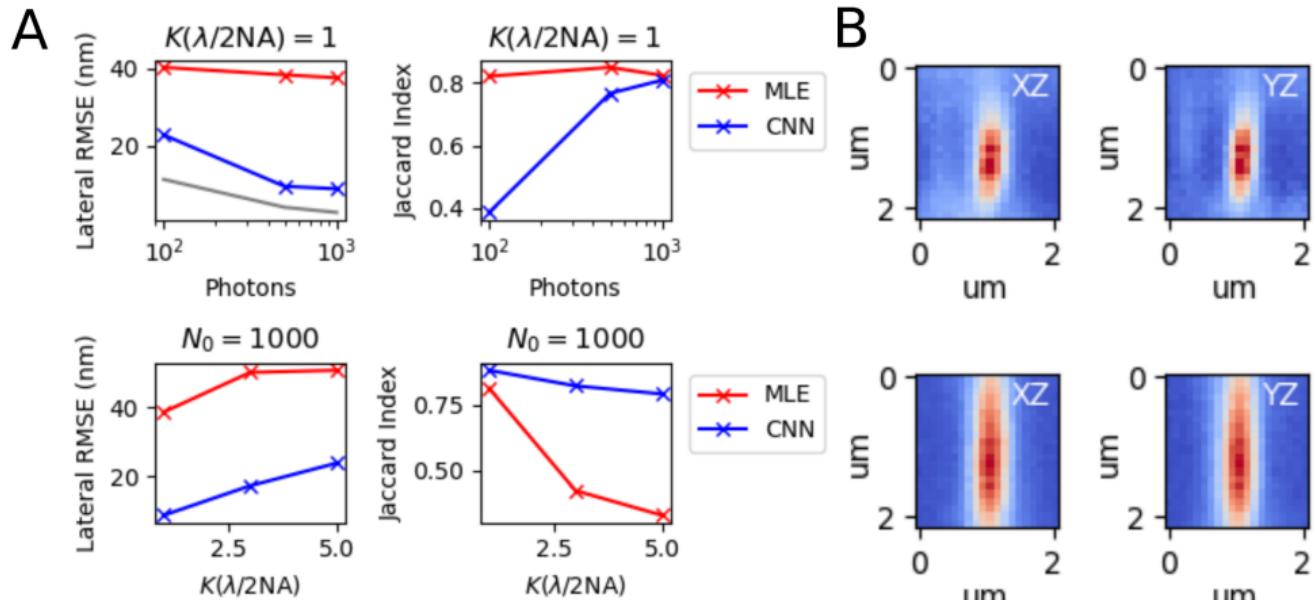
- ▶ Variance of the posterior $P(\theta|\vec{H})$ is a useful particle filter
- ▶ We assume uniform priors on coordinates

The tradeoff between spatial and temporal resolution in SMLM



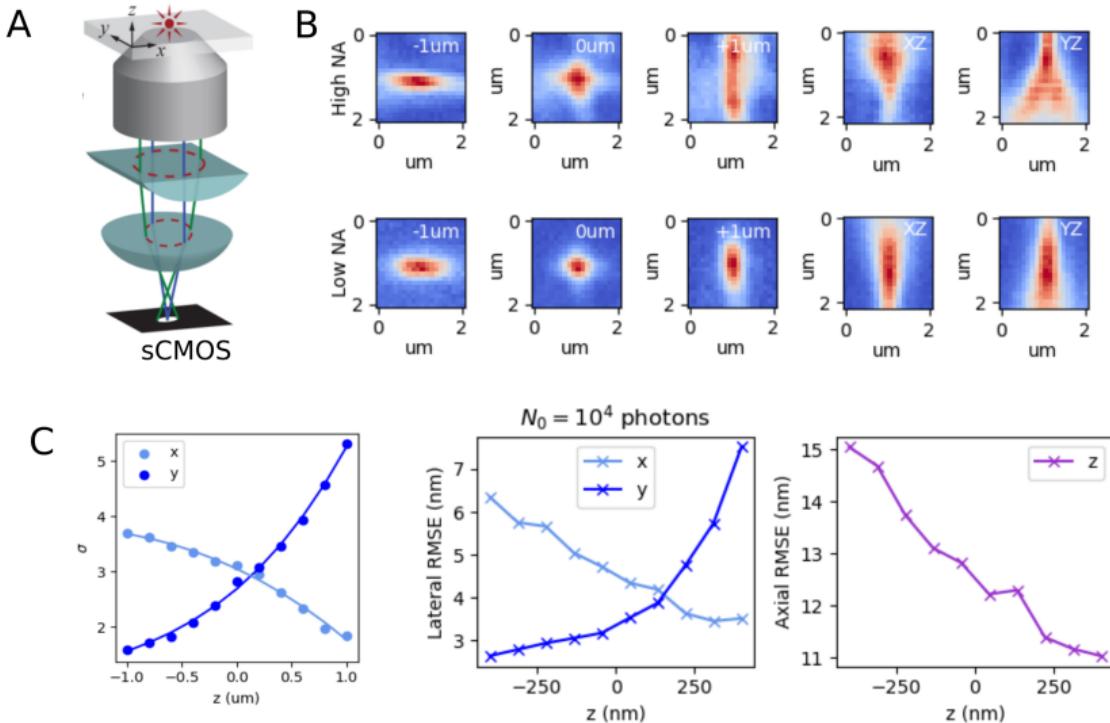
- ▶ SMLM is desirable for SR due to very high res and no scanning (e.g., STED)
- ▶ Less control over photophysical state, but high throughput

Deep learning enables dense localization in two-dimensions



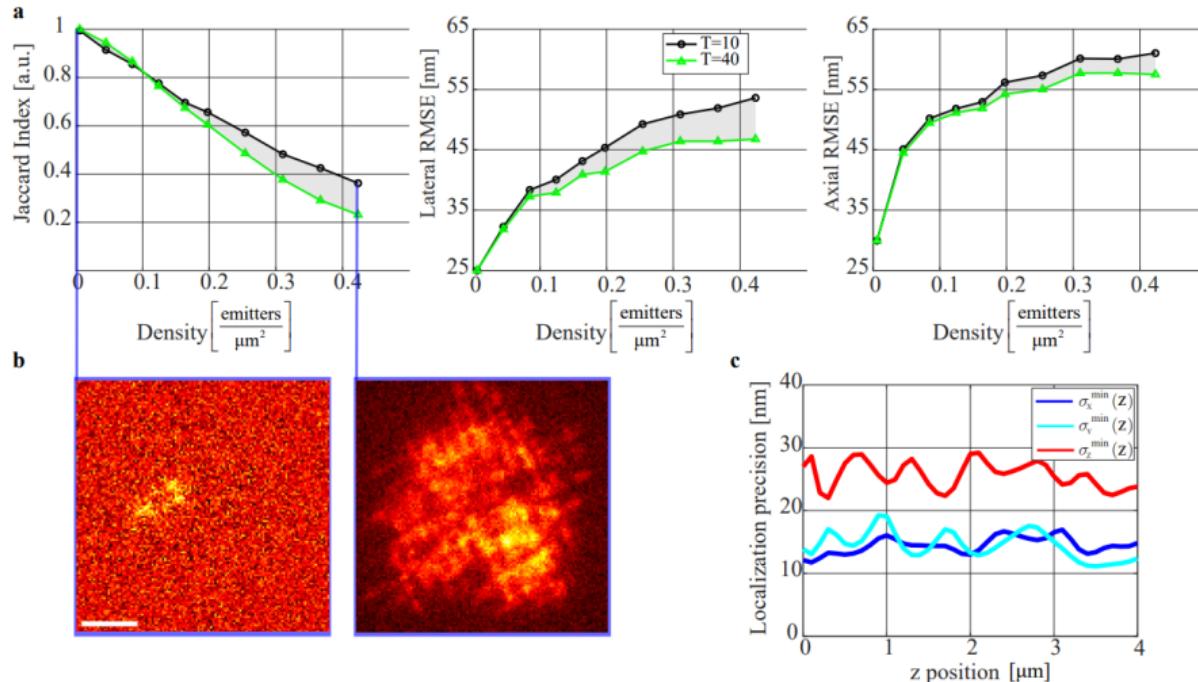
- ▶ $K(\lambda/2NA)$ is Ripley's K function at the diffraction limit
- ▶ Convolutional neural networks (CNNs) approach the CRLB (gray) at high photon counts and generalize to the dense regime

Astigmatism based three dimensional imaging



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

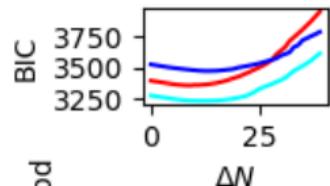
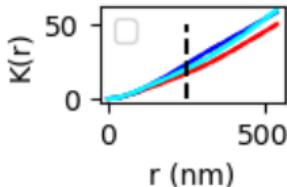
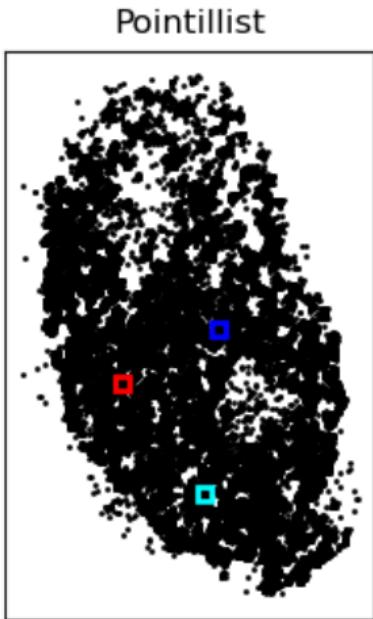
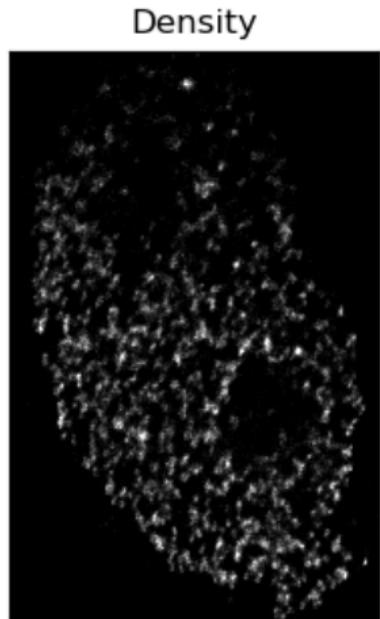
Deep learning can generalize precise SMLM to three dimensions



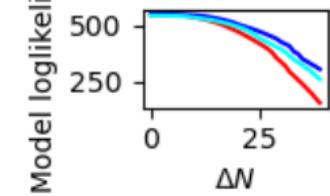
Several methods have been designed to break the time-resolution barrier

- ▶ 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



Model loglikelihood



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

Diffusion increases localization uncertainty in live-cell SMLM

Nucleosome diffusion has been modeled in various potentials:

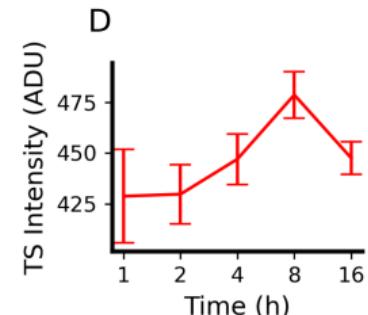
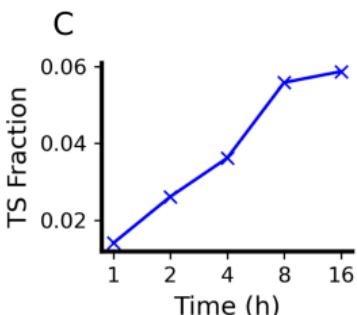
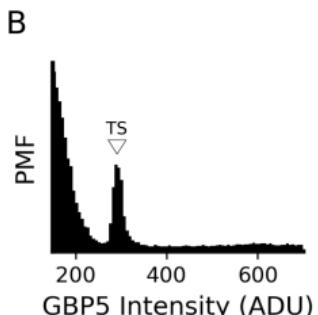
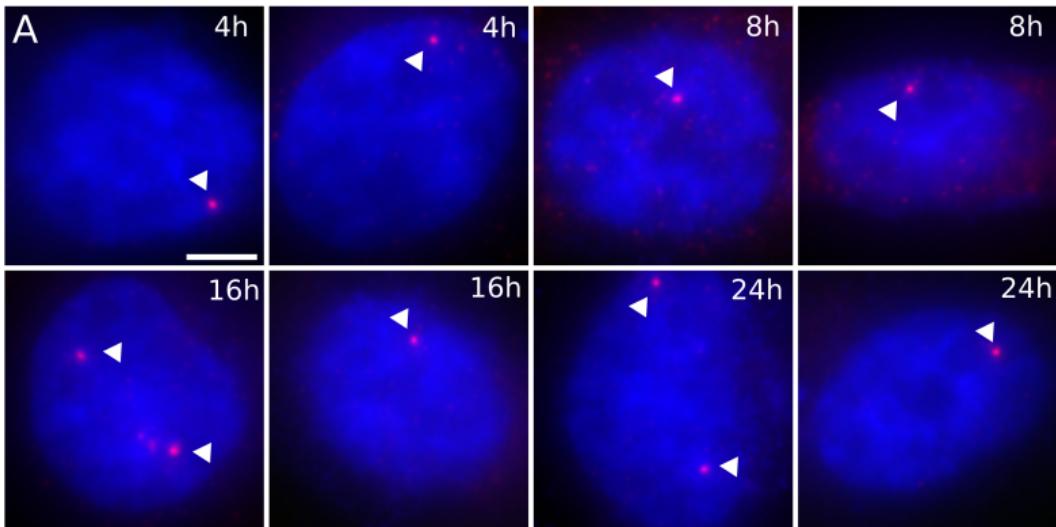
- ▶ Bead model: $V(r_{ij}) = \epsilon_0(r_0/r_{ij})^{12} - \epsilon_{ij}(r_0/r_{ij})^6$ (Ashwin 2019)
- ▶ Harmonic: $V(\vec{\Delta r}) = \frac{1}{2}k|\vec{\Delta r}|^2$ (XXX)

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

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

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

Validation of JQ1 efficacy for BRD4 inhibition in HeLa cells



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