

Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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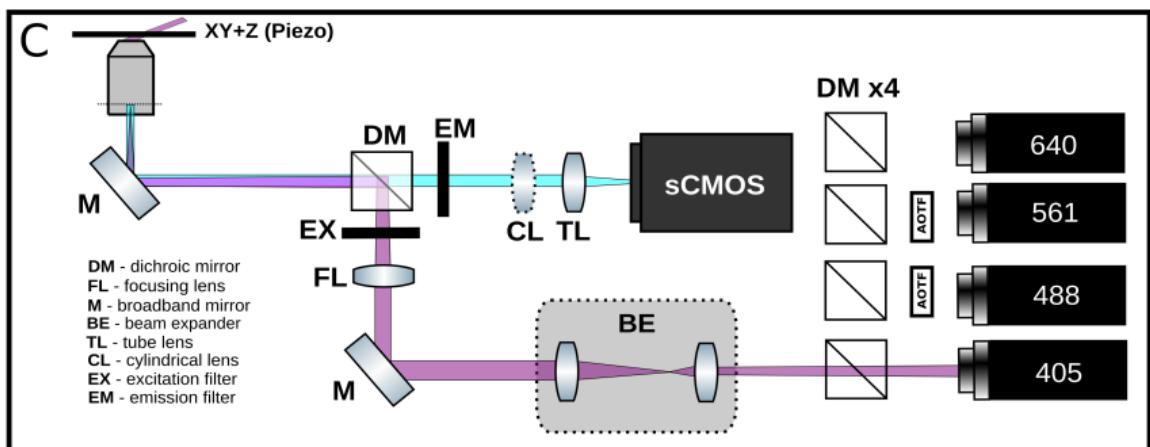
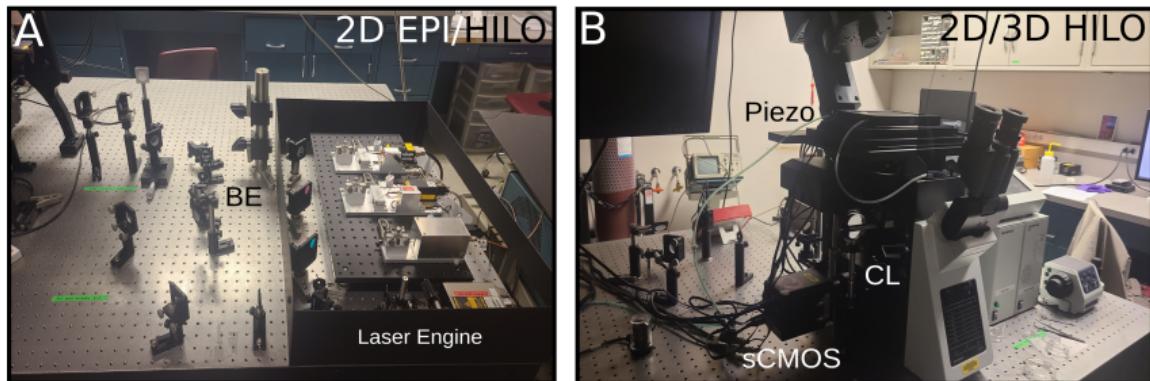
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Introduction

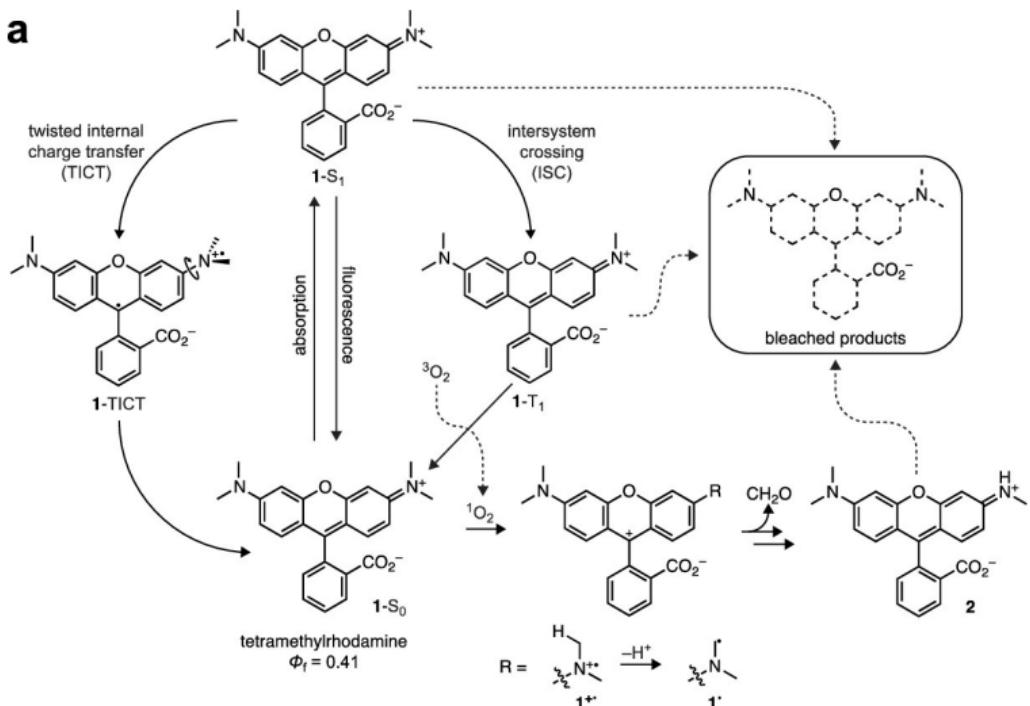
Summary

- ▶ We study the organization of nucleosomes in living cells, and I am interested in the impact of BRD4 phase separation (fusion/fission) on chromatin packing, as previous work has shown chromatin nanodomains are highly dynamic structures (Barth 2020)
- ▶ We study chromatin structure using SMLM, which can increase lateral resolution by one order of magnitude in living cells compared to standard widefield microscopy
- ▶ SMLM enables simultaneous super-resolution of chromatin structure and single molecule tracking to probe the physical properties of chromatin nanodomains
- ▶ In general, the uncertainty of a statistical estimator in SMLM determines the resolution limit (Cramer-Rao lower bound).
- ▶ Deep learning can generalize SMLM to three dimensions, particularly in sparsely labeled regimes
- ▶ SMLM achieves the highest resolution of SR methods; however, there is a fundamental tradeoff between spatial and temporal resolution (Shroff et al)
- ▶ Therefore, we look to other alternatives for resolution enhancement which combine the power of deep image translation methods e.g., ANNA-PALM

Instrumentation for super-resolution and high throughput microscopy

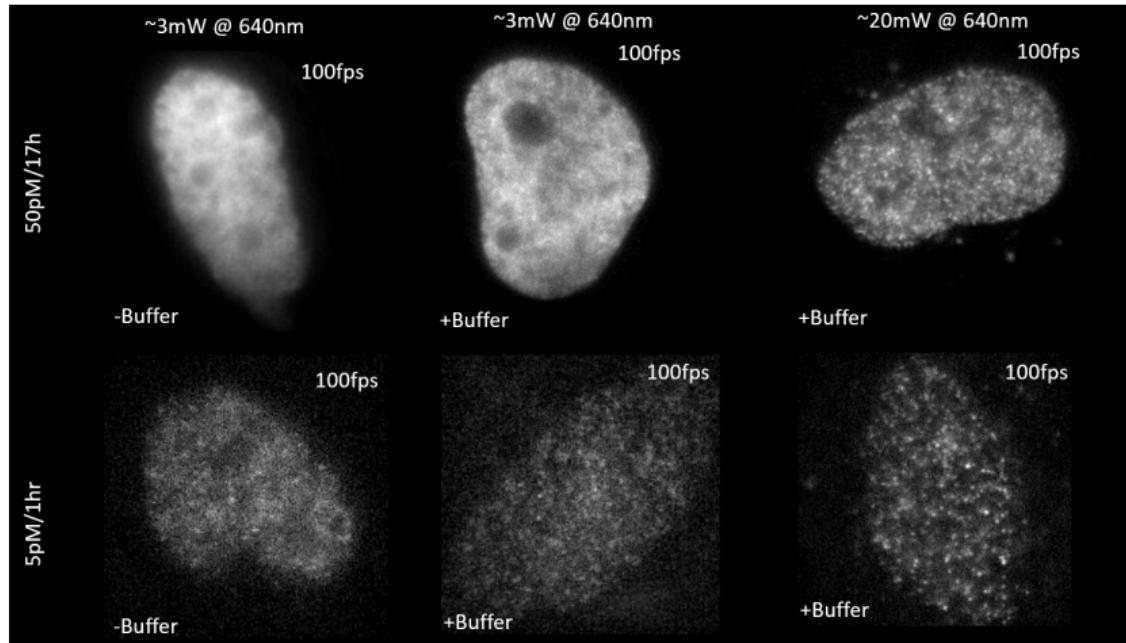


The photophysics of rhodamines



- ▶ 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



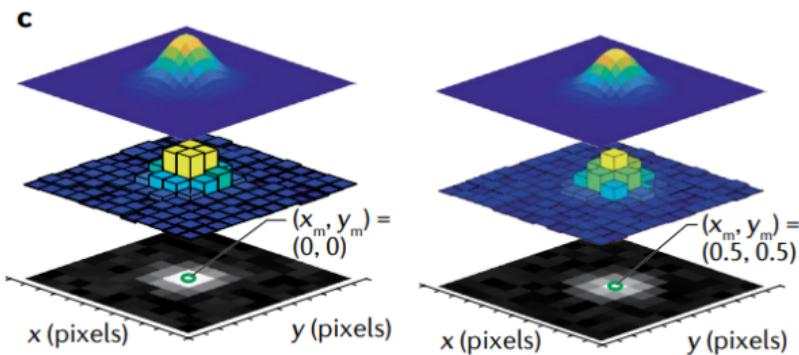
- ▶ SMLM is desirable for SR due to very high res and no scanning (STED)
- ▶ Less control over photophysical state, but high throughput
- ▶ High power compensates for dense labeling

Maximum likelihood localization of an isolated fluorescent emitter

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

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

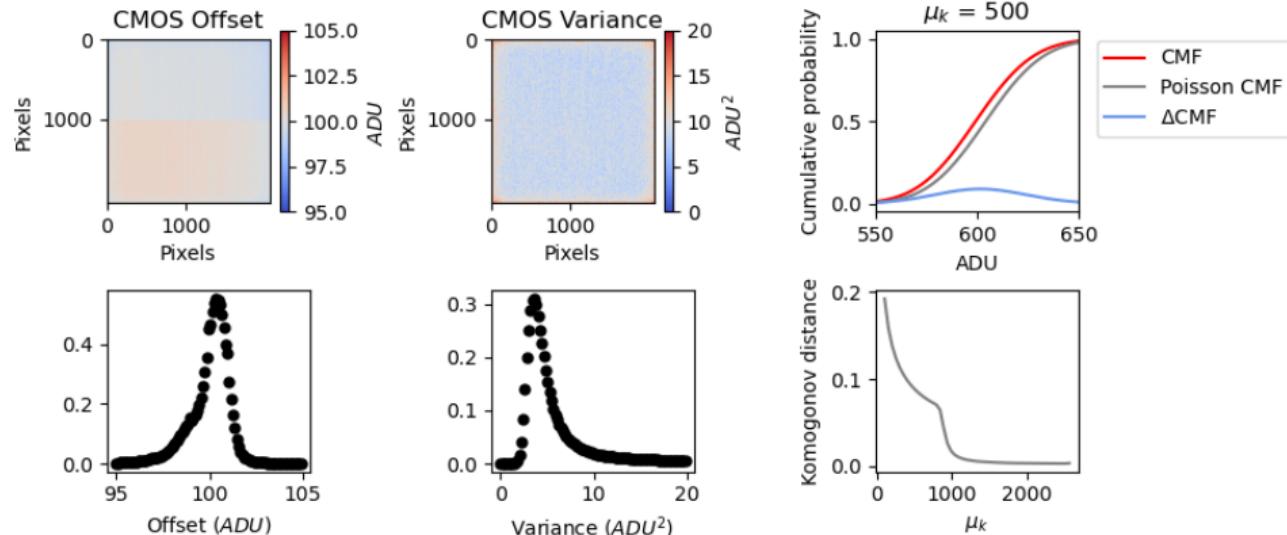
- η – quantum efficiency
- N_0 – emission rate
- Δ – exposure time



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

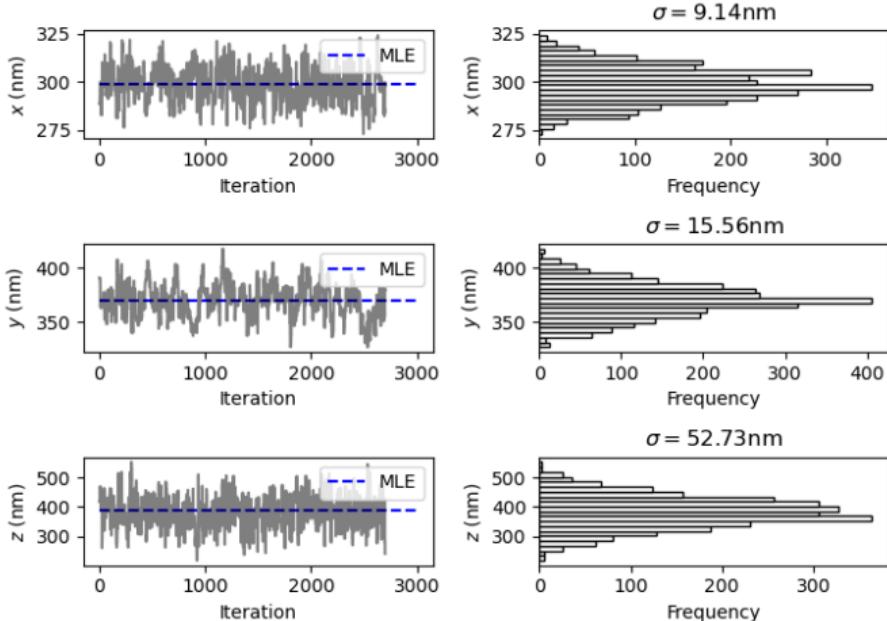
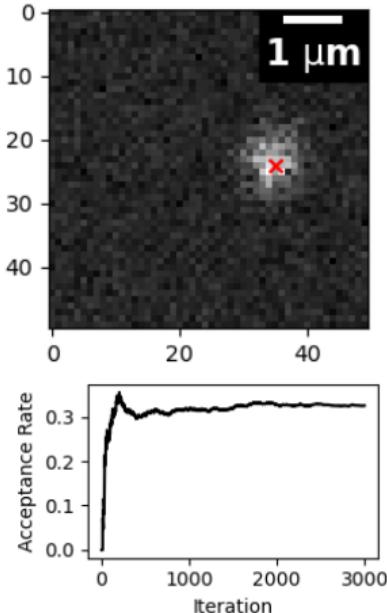
$P(H_k|\theta)$ can be approximated as Poisson at high signal-to-noise (SNR)

A Poisson approximation at moderate SNR simplifies SMLM



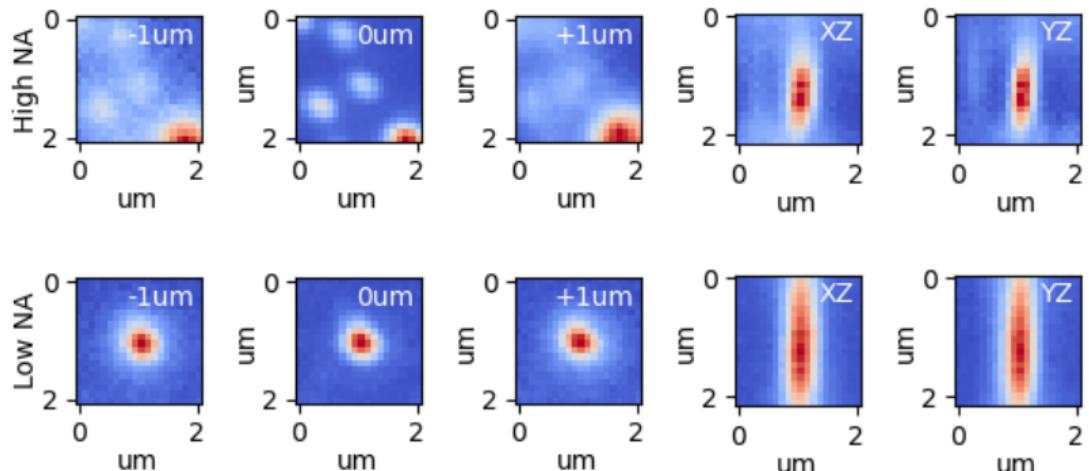
- ▶ $P(H_k - o_k | \theta) = \text{Poisson}(\mu_k + \sigma_k^2 | \theta)$ for pixel offset o_k noise variance σ_k^2
- ▶ Fisher information and Cramer-Rao lower bound (CRLB) can be computed analytically for Poisson log-likelihood ℓ (Smith 2010, Huang 2013)

Estimator precision sets the resolution limit in localization microscopy

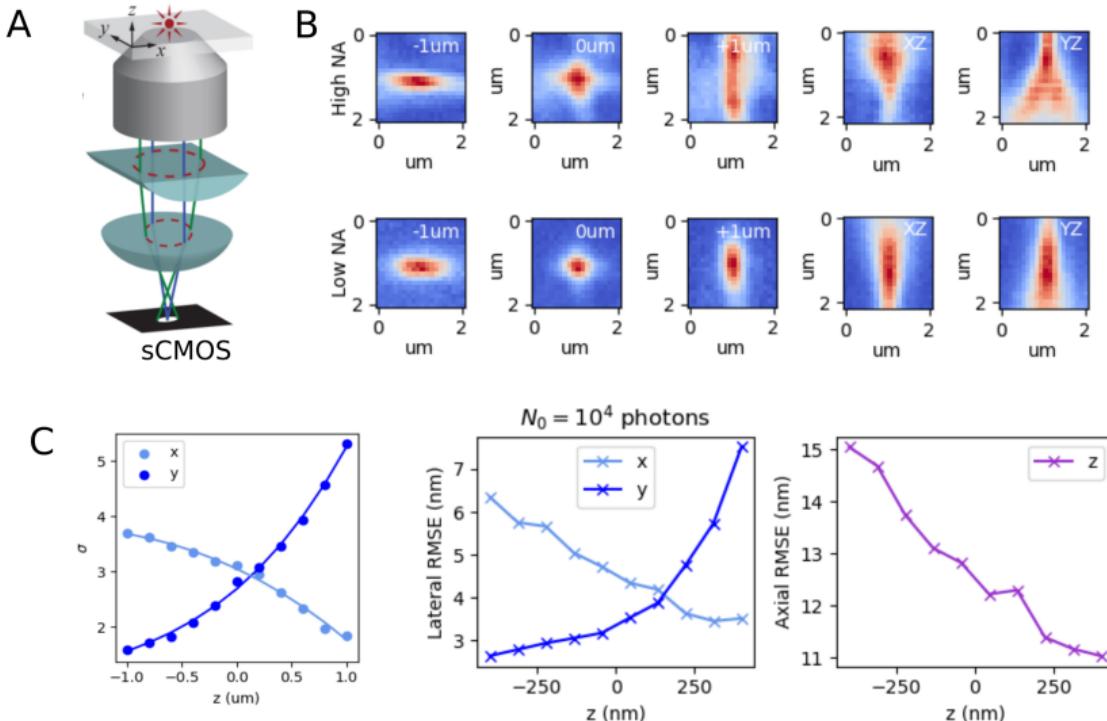


- ▶ Variance of the posterior $P(\theta|\vec{H})$ is a useful particle filter
- ▶ We assume uniform priors on coordinates

Estimator precision sets the resolution limit in 2D

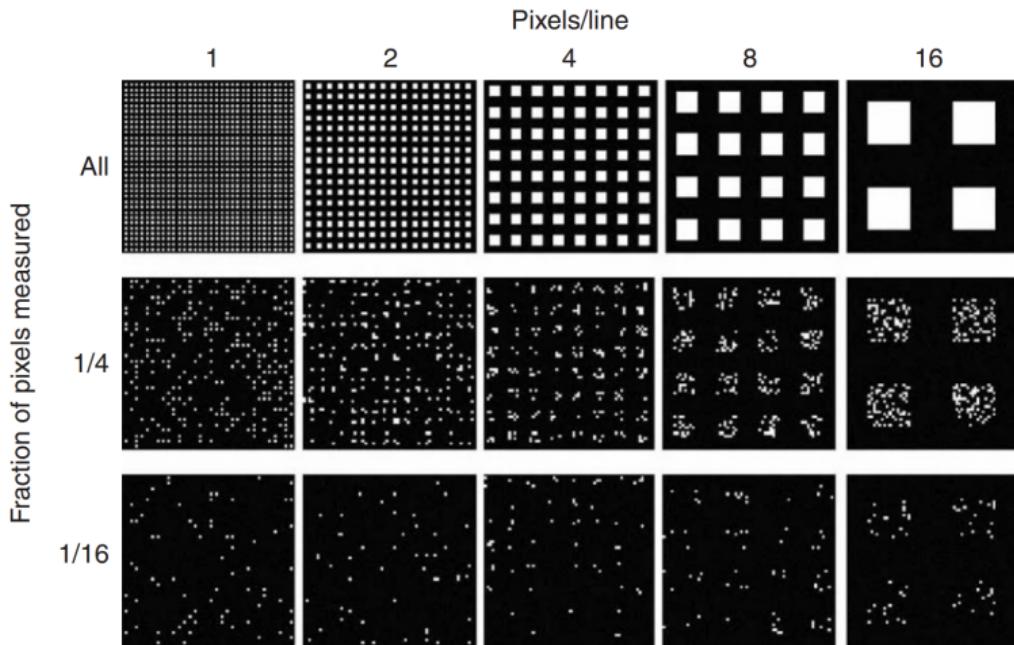


Estimator precision sets the resolution limit in 3D



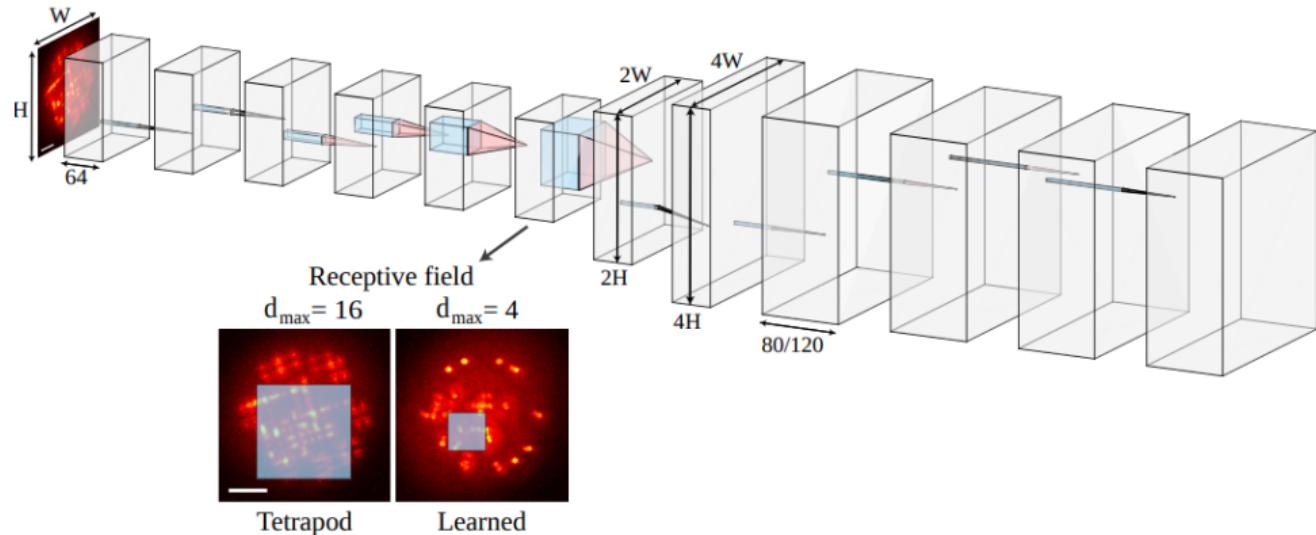
- A weak ($f = 10\text{m}$) cylindrical lens breaks the axial symmetry of the PSF

The tradeoff between spatial and temporal resolution in SMLM



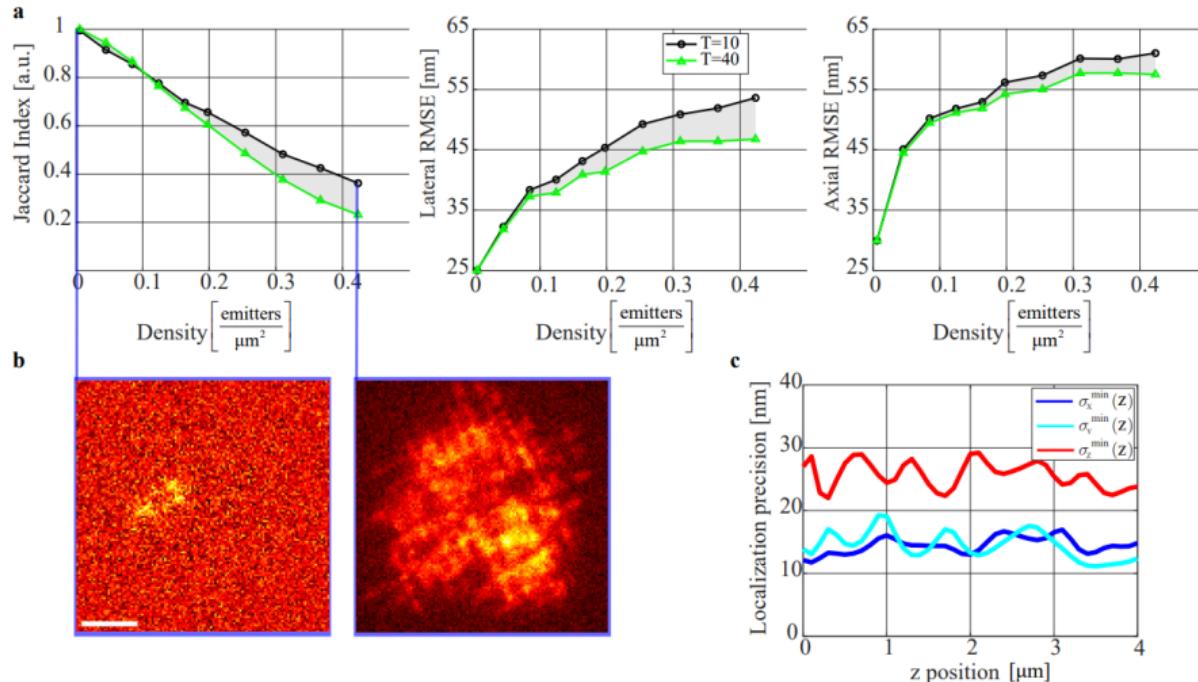
Shroff et al. Live-cell photoactivated localization microscopy of nanoscale adhesion dynamics. Nature Methods.

The tradeoff between spatial and temporal resolution in SMLM



- ▶ ANNA-PALM (Ouyang 2018) use prior information to generate SR images from fewer localizations
- ▶ DECODE (Speiser 2021) and DeepSTORM3D (Nehme 2020) instead process dense images to increase localizations per unit time

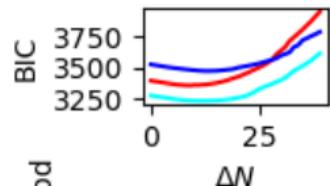
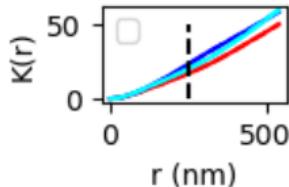
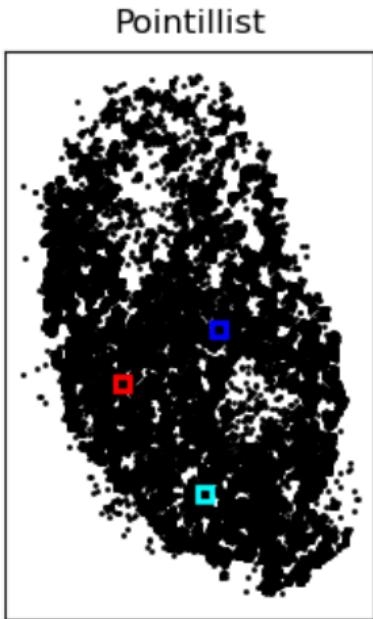
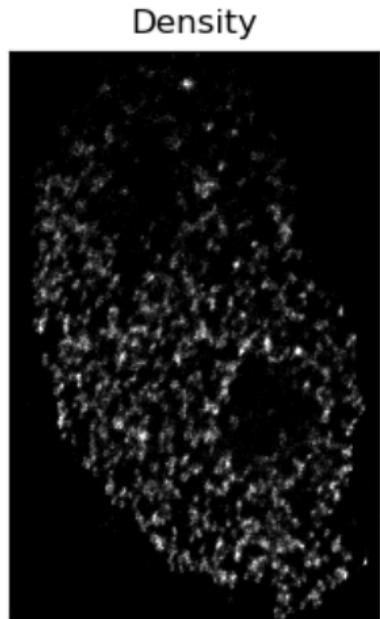
Deep learning can generalize precise SMLM to three dimensions



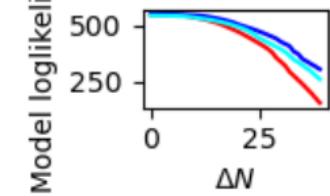
Several methods have been designed to break the time-resolution barrier

- ▶ 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:

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

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

Resolution is dependent on photoswitching kinetics

A molecule is considered "detected" in principle if the measured ADU signal satisfies $\tilde{s} = \mu\tau \geq \delta$ where δ is a number of photons which satisfy a criterion on localization accuracy.

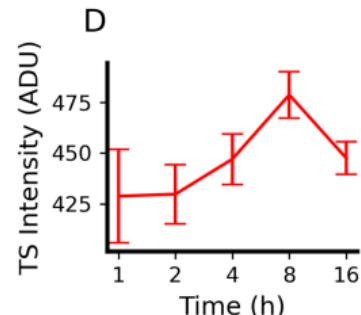
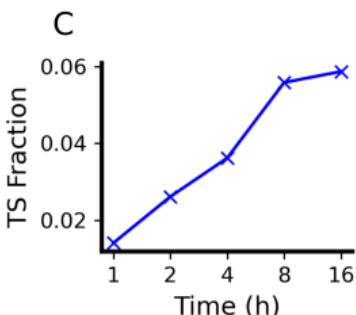
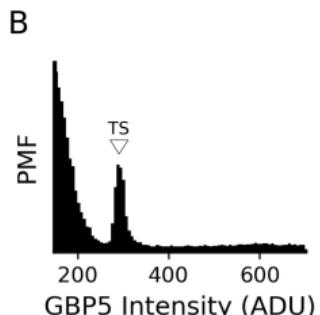
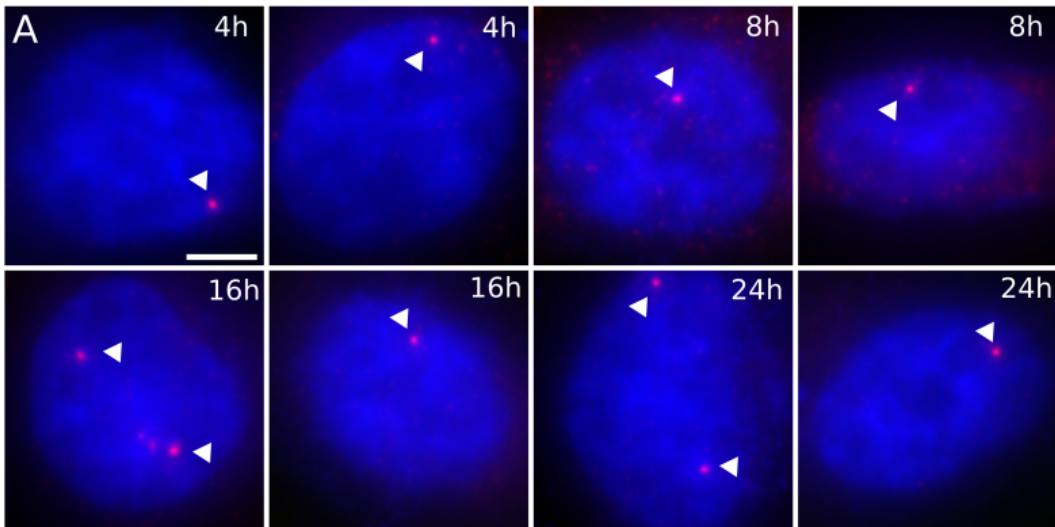
$$\alpha = \int_{\delta}^{\Delta} \left(\sum_{n=0}^{\infty} Q(N=n) \psi(\tau|n; \vec{k}) \right) d\tau \approx \mathbb{E}_{\tau \sim P(\tau)} (\mathbb{I}[\tau > \delta])$$

$P(\tau)$ is usually obtained by Monte Carlo simulation. This is useful for computing density measures and the total acquisition time:

$$D = \alpha K \left(\frac{\lambda}{2NA} \right) \quad T = \left(\Delta_{SR} + \frac{2N}{\log(1-\alpha)} \right)^2$$

For actually inferring k_1, k_2 , we need a measure of distance between $P(\tilde{s})$ and $P(s|k_1, k_2)$ for many k_1, k_2 pairs. Luckily we only need to compute $P(s|k_1, k_2)$ once, and we can then perform a grid search

Validation of JQ1 efficacy for BRD4 inhibition in HeLa cells



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