

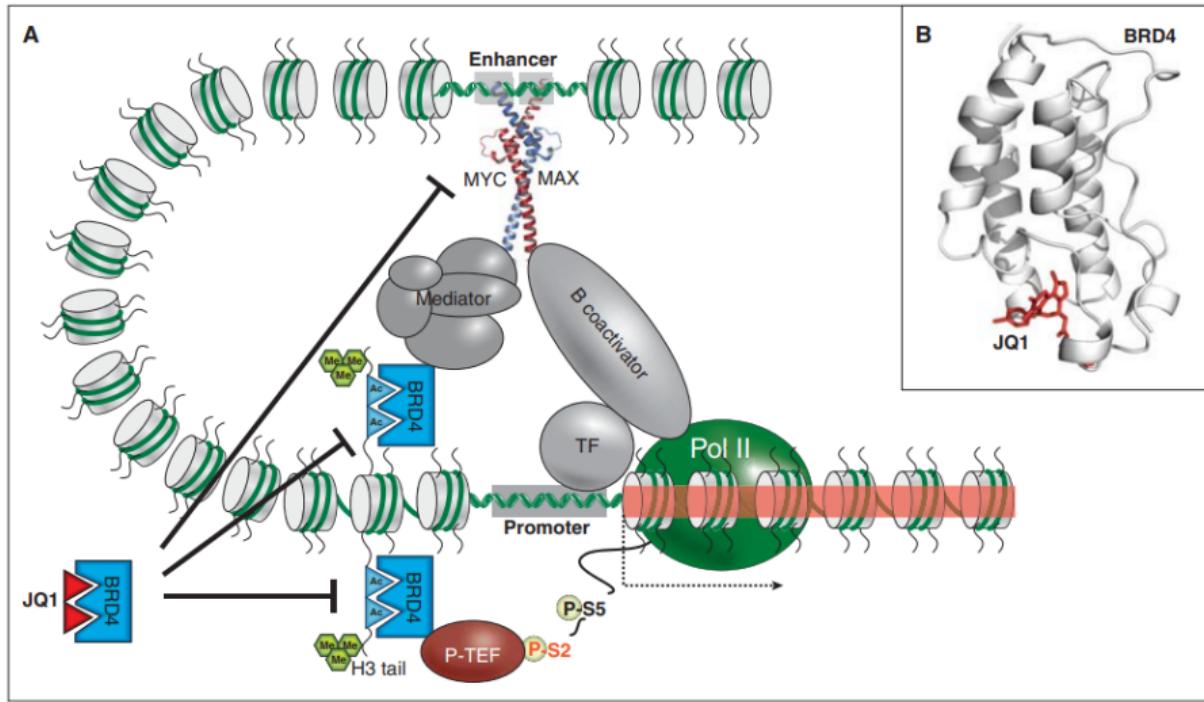
Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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August 1, 2023

Introduction

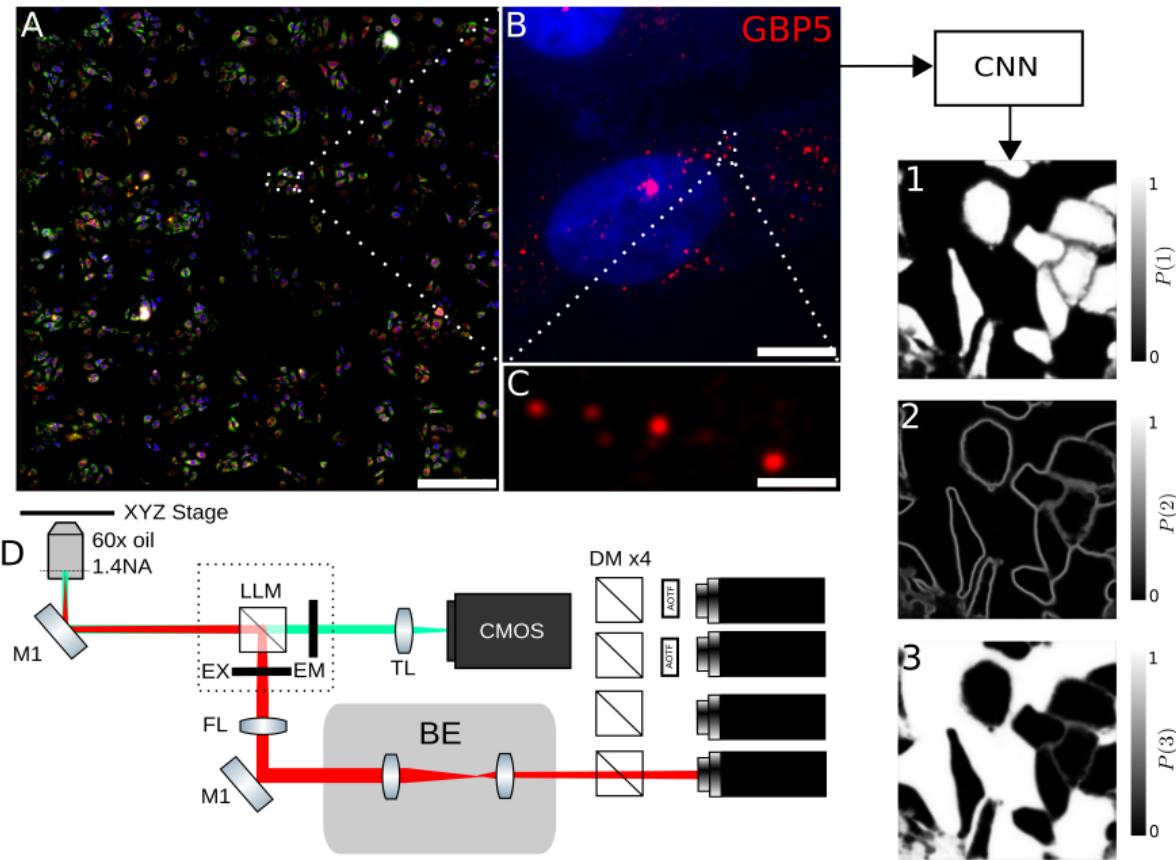
Visualizing nucleosome cluster dynamics



Jun Qi. Bromodomain and Extraterminal Domain Inhibitors for Cancer Therapy

Methods

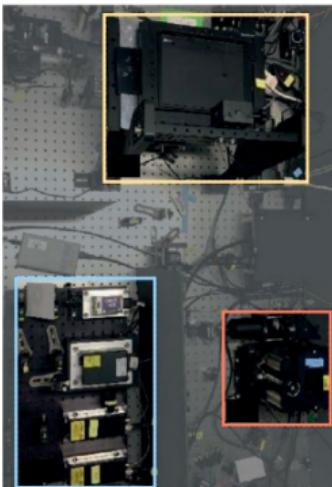
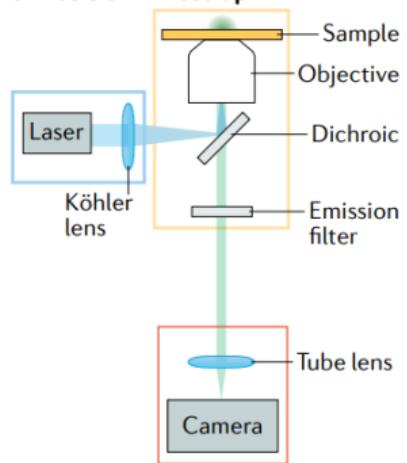
High throughput widefield microscopy



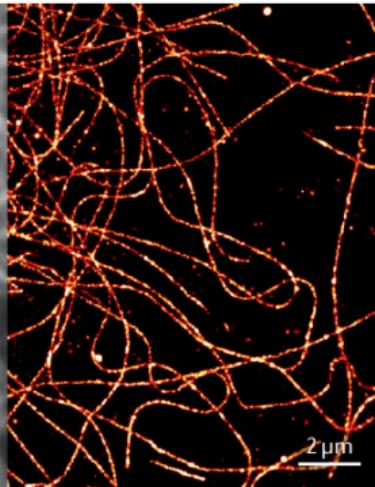
Direct stochastic optical reconstruction microscopy

Direct stochastic optical reconstruction microscopy

a Basic SMLM set-up



b



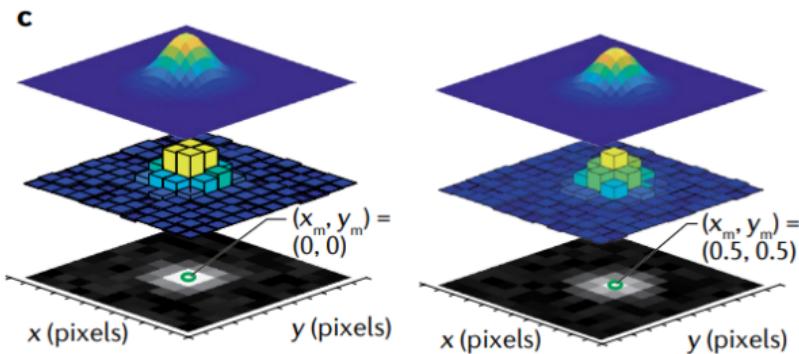
Direct stochastic optical reconstruction microscopy

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

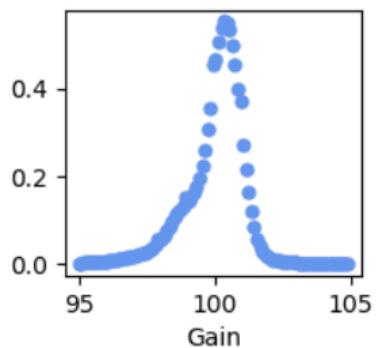
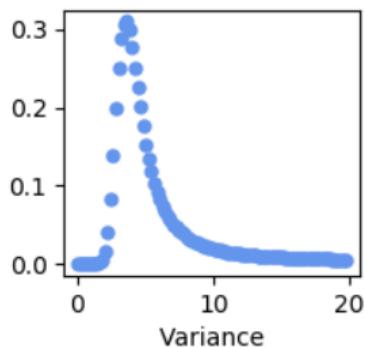
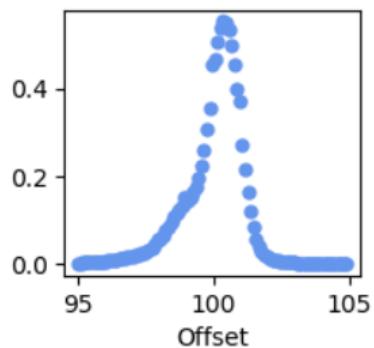
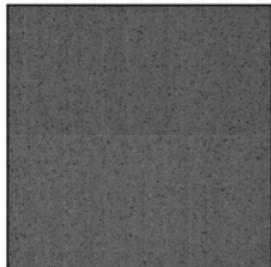


$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi\sigma_k^2}} e^{-\frac{(H_k - g_k q - o_k)^2}{2\sigma_k^2}}$$

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

Readout noise of sCMOS cameras

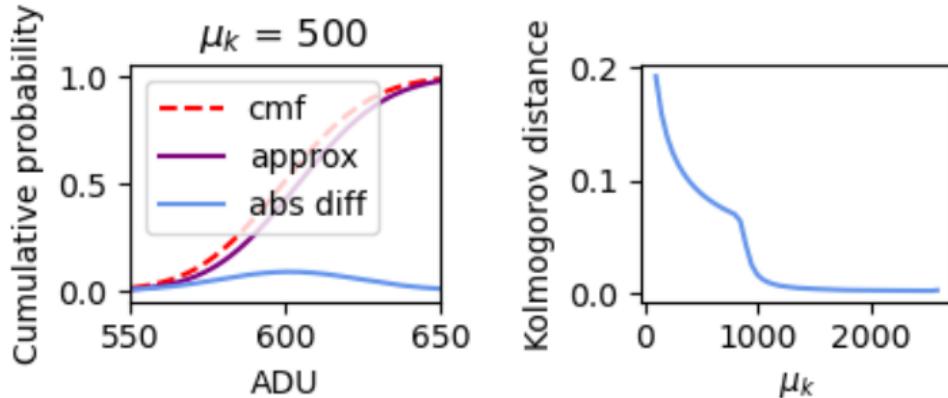
Hamamatsu ORCA v3 CMOS, air cooled to -10C



Measured signal: $H_k = S_k + \xi_k$, $S_k \sim \text{Poisson}(\mu_k)$, $\xi_k \sim \mathcal{N}(o_k, \sigma_k^2)$

Quality of the Poisson approximation depends on SNR

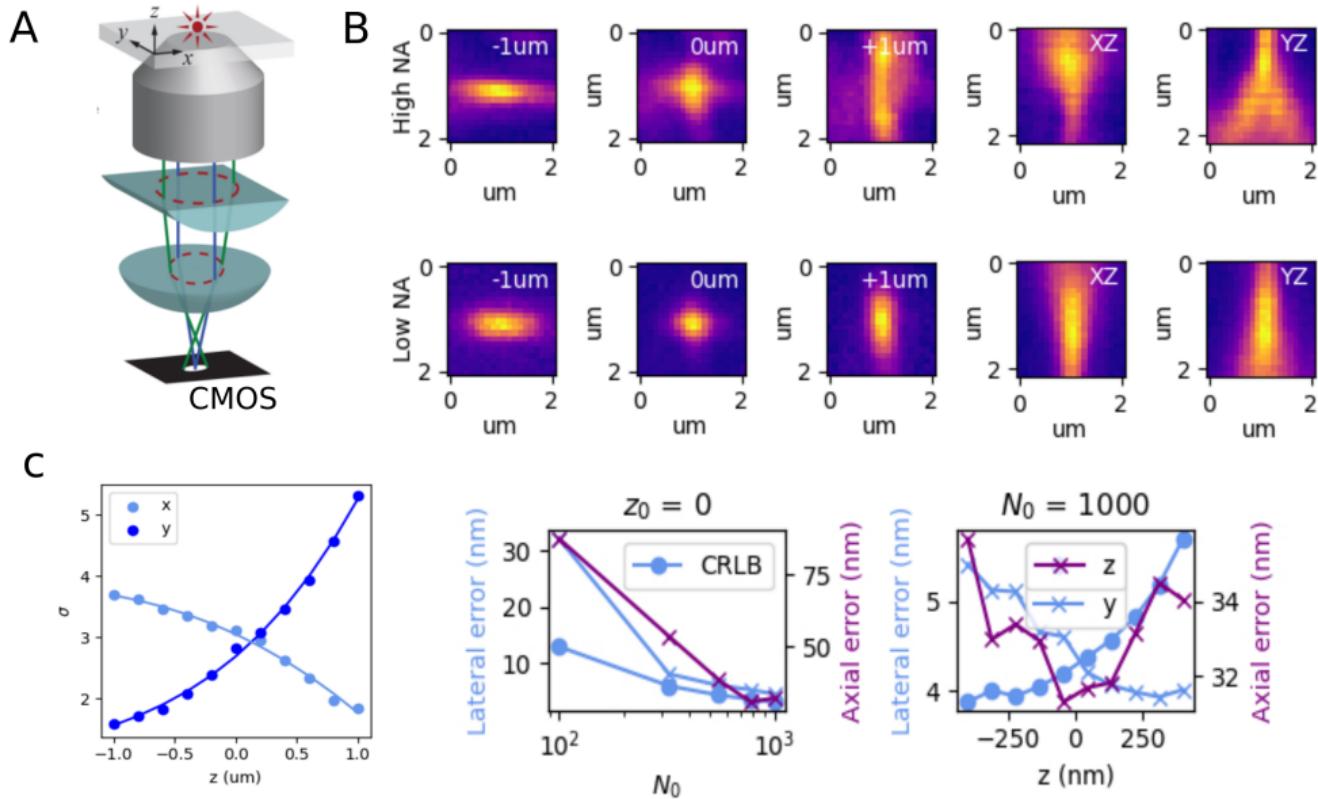
$P(H_k|\theta) \approx \text{Poisson}(\mu_k + \sigma_k^2)$ for $N_0 > 500$ assuming $\Delta = 100\text{ms}$



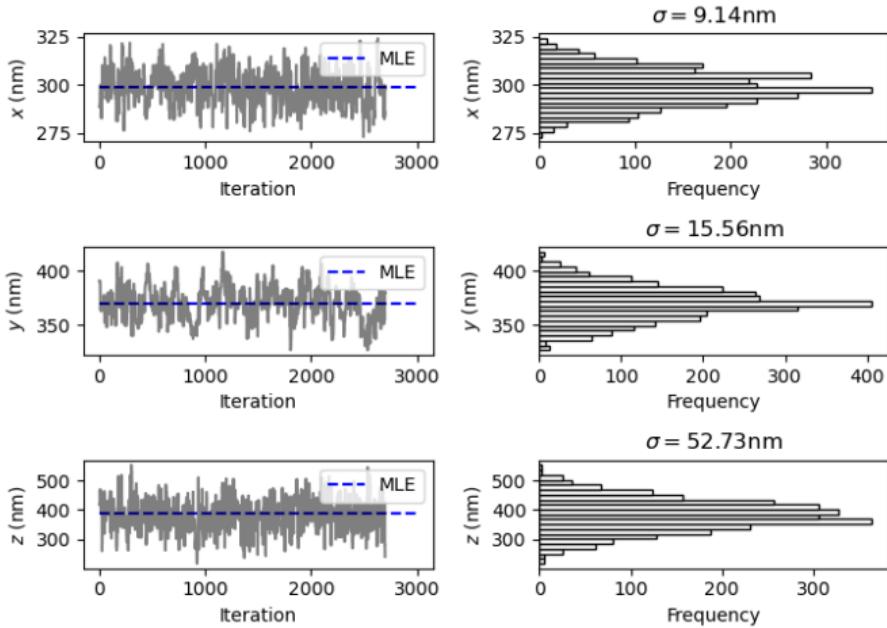
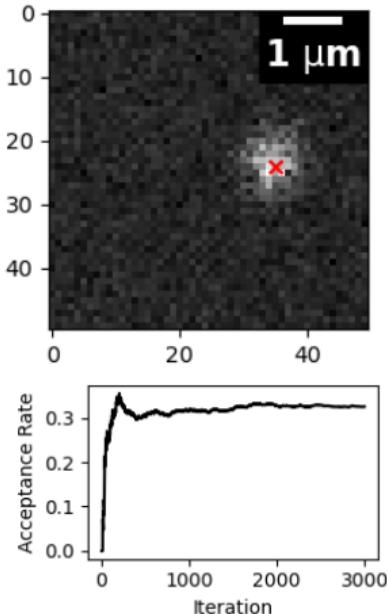
Using the approximation we can write

$$\ell(\vec{H}|\theta) = -\log \prod_k \frac{e^{-(\mu'_k)} (\mu'_k)^{n_k}}{n_k!} = \sum_k \log n_k! + \mu'_k - n_k \log (\mu'_k)$$

Estimator precision sets the resolution limit in localization microscopy

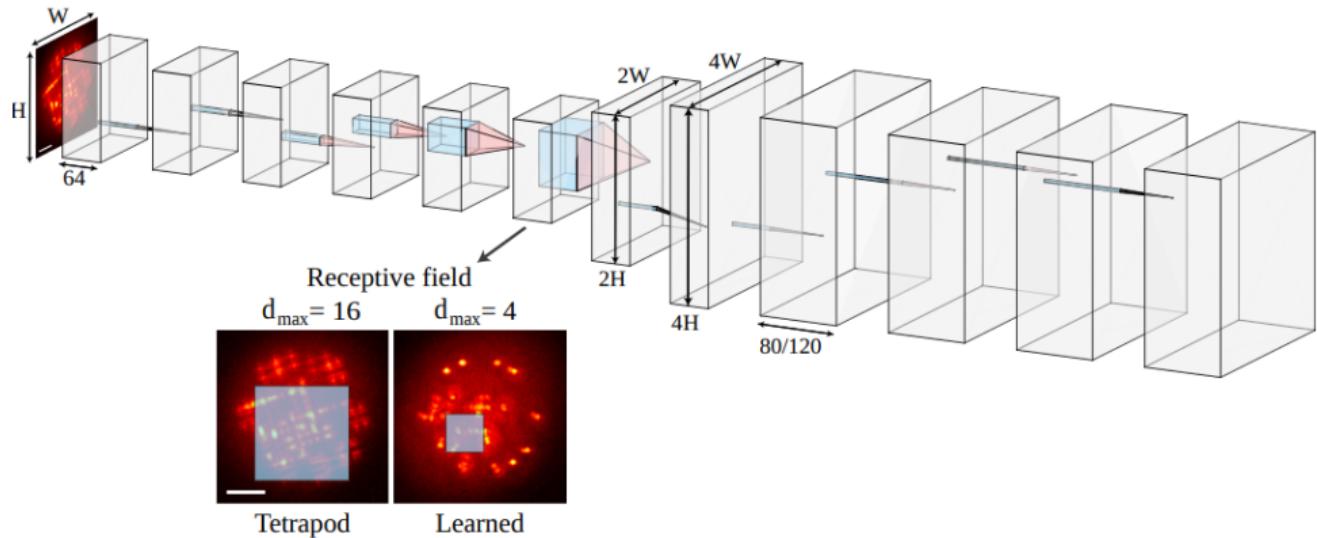


Estimator precision sets the resolution limit in localization microscopy



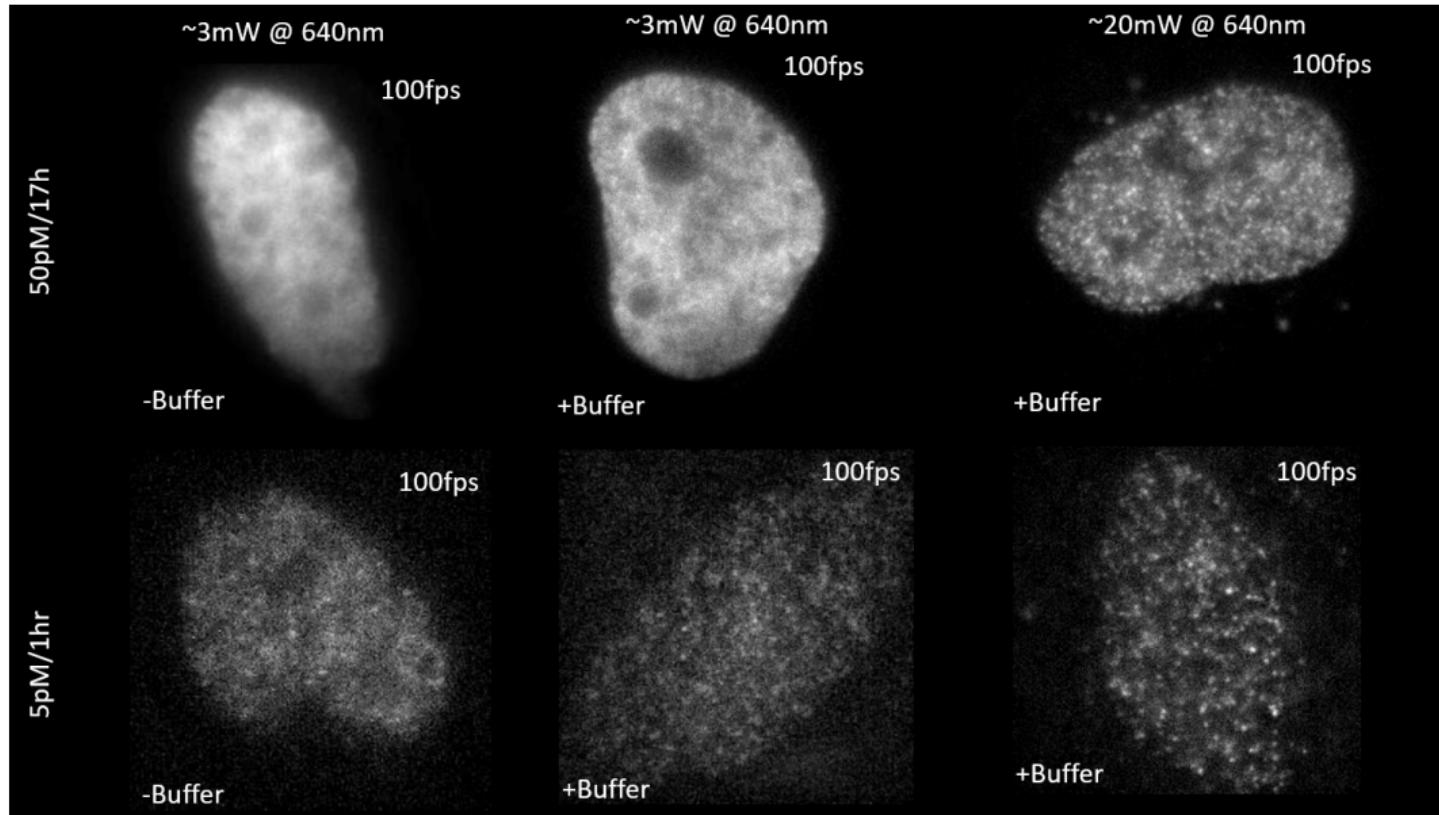
- ▶ Localization uncertainty can be quantified with Metropolis-Hastings MCMC
- ▶ MCMC is asymptotically exact, but slow

Deep learning beats MLE at 2D and 3D localization

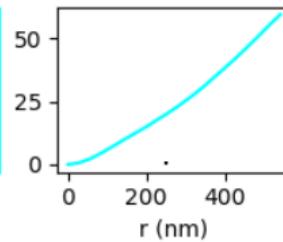
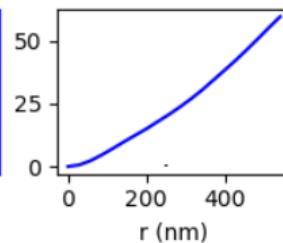
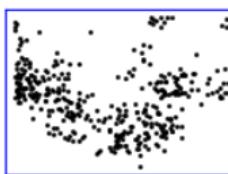
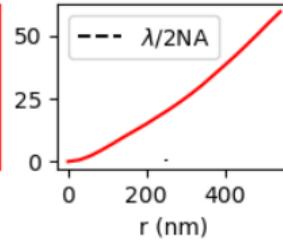
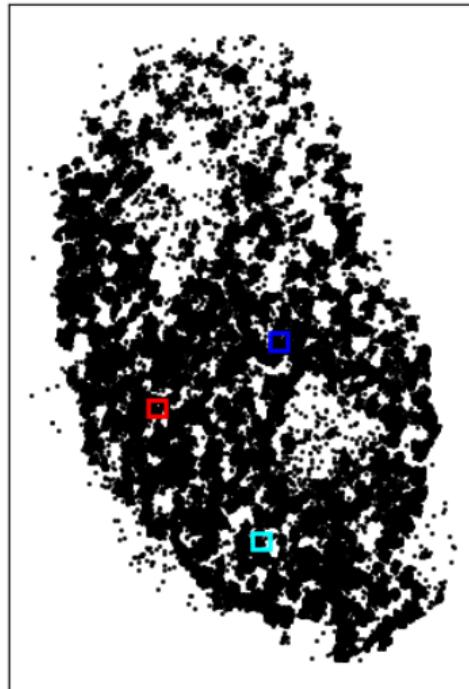


Deep learning beats MLE at 2D and 3D localization

The metastable OFF state can be maintained with high laser power



Resolution is dependent on photoswitching kinetics



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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.

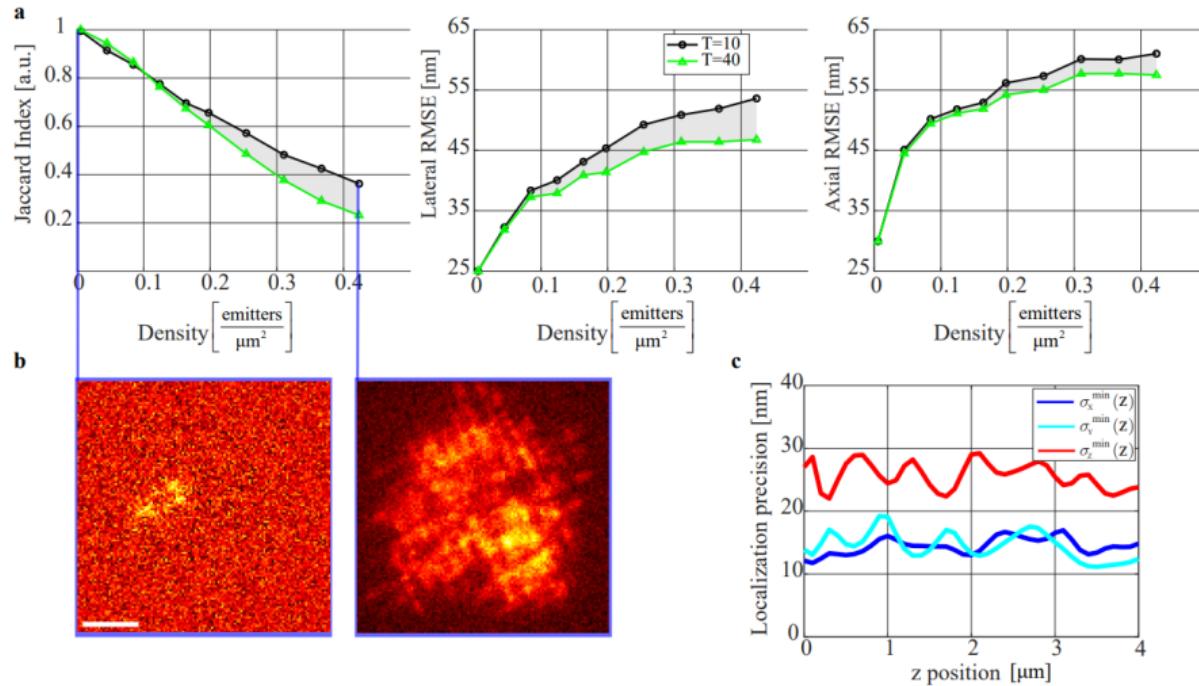
$$\alpha = \int_{\delta}^{\Delta} \left(\sum_{n=0}^{\infty} Q(N=n) \psi(\tau|n; \vec{k}) \right) d\tau \approx \mathbb{E}_{\tau \sim P(\tau)} (\mathbb{I}[\tau > \delta])$$

$P(\tau)$ 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

Resolution is dependent on photoswitching kinetics



Results

Validation of JQ1 efficacy for BRD4 inhibition in HeLa cells

