

# Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

Clayton W. Seitz

August 3, 2023

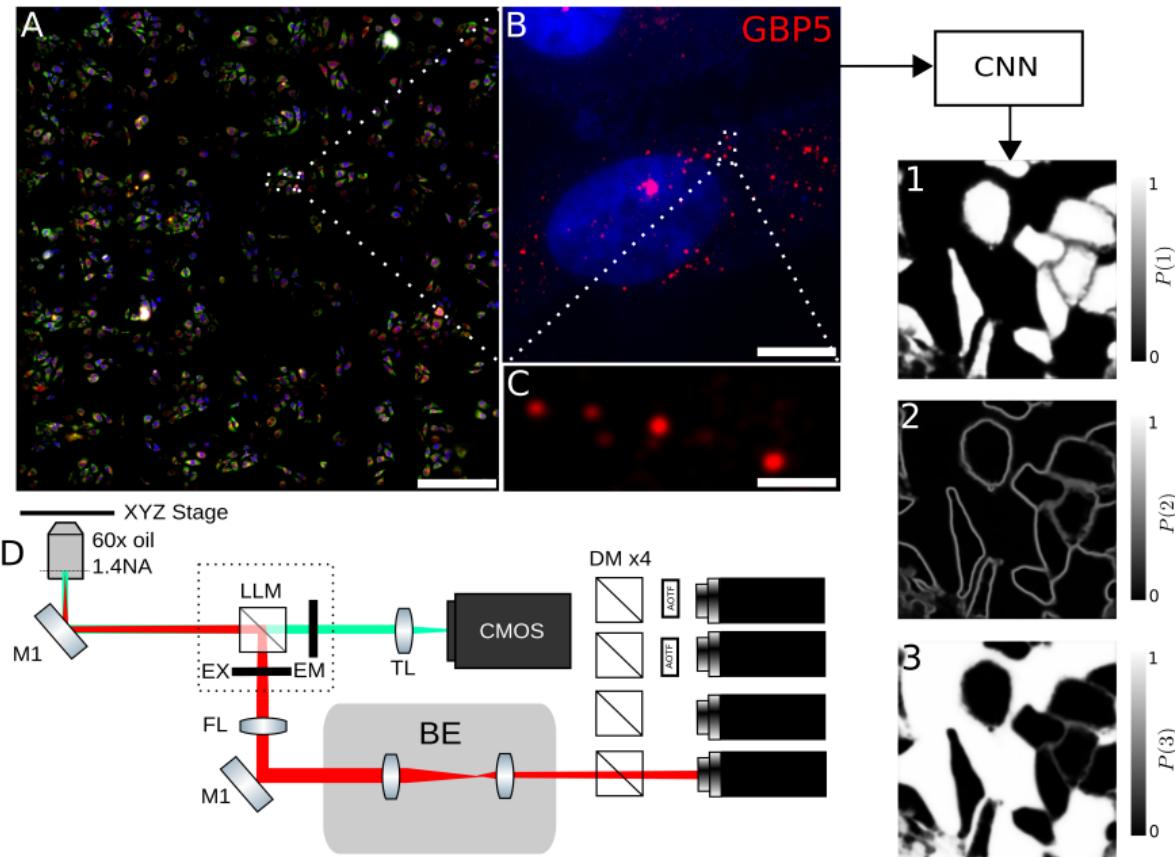
# Introduction

## Summary

- ▶ We study the organization of nucleosomes in living cells, and I am interested in the role of BRD4 protein on chromatin packing
- ▶ We study chromatin structure using SMLM, which can increase lateral resolution by one order of magnitude in living cells. This technique permits 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 determines the resolution limit (Cramer-Rao lower bound). Deep learning based localization methods can approach this bound in the limit, faster than maximum likelihood estimation
- ▶ SMLM achieves the highest resolution of SR methods, but requires sparse labeling of nuclear proteins, which in turn reduces the overall throughput and time resolution
- ▶ Therefore, we look to other alternatives for resolution enhancement which combine the power of deep methods e.g., ANNA-PALM, with the physical constraints of image autocorrelation based techniques e.g., SOFI/SACDj

# Methods

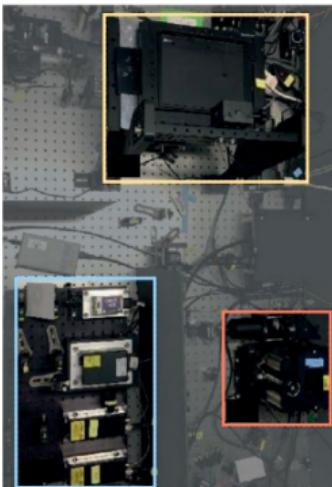
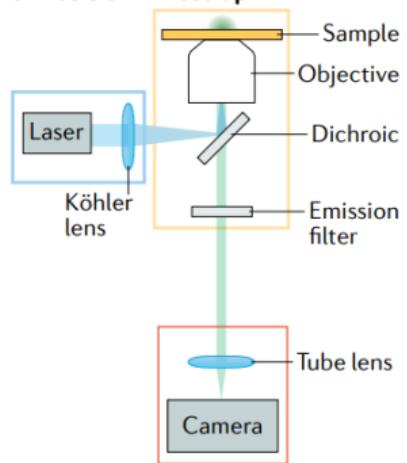
# High throughput widefield microscopy



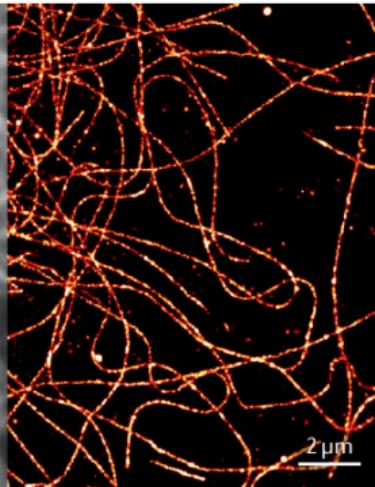
# Direct stochastic optical reconstruction microscopy

# Direct stochastic optical reconstruction microscopy

a Basic SMLM set-up



b



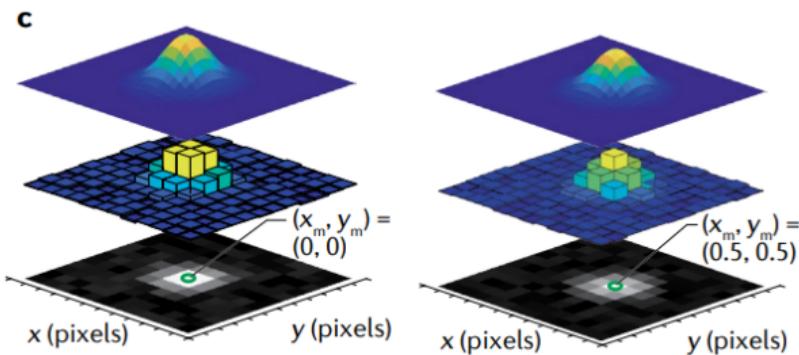
# Direct stochastic optical reconstruction microscopy

# Maximum likelihood localization of an isolated fluorescent emitter

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

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

- $\eta$  – quantum efficiency
- $N_0$  – emission rate
- $\Delta$  – exposure time

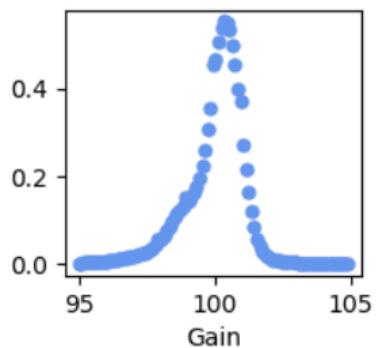
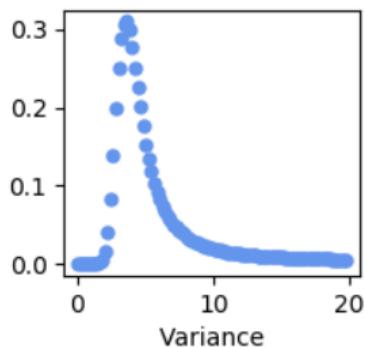
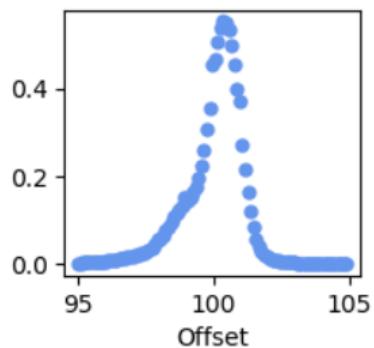
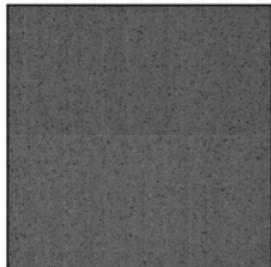


$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi\sigma_k^2}} e^{-\frac{(H_k - g_k q - o_k)^2}{2\sigma_k^2}}$$

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

# Readout noise of sCMOS cameras

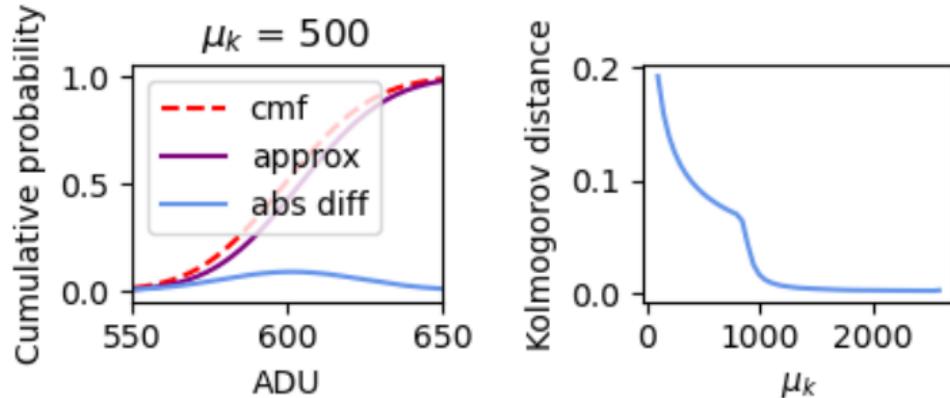
Hamamatsu ORCA v3 CMOS, air cooled to -10C



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

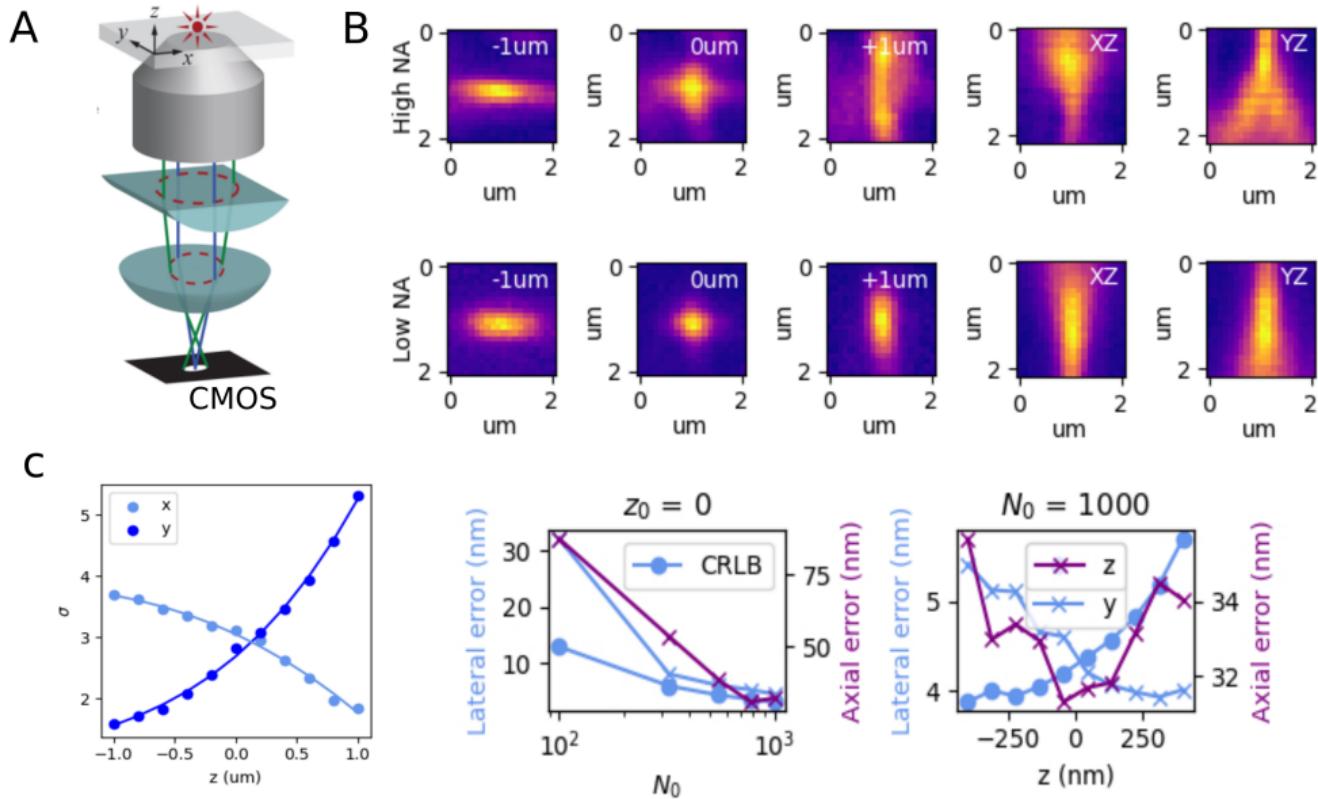
$P(H_k|\theta) \approx \text{Poisson}(\mu_k + \sigma_k^2)$  for  $N_0 > 500$  assuming  $\Delta = 100\text{ms}$



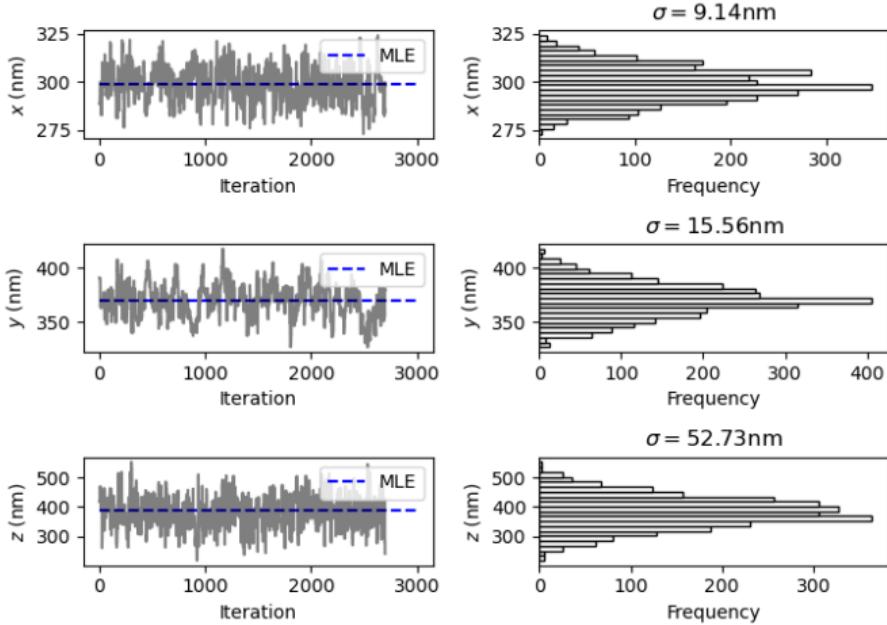
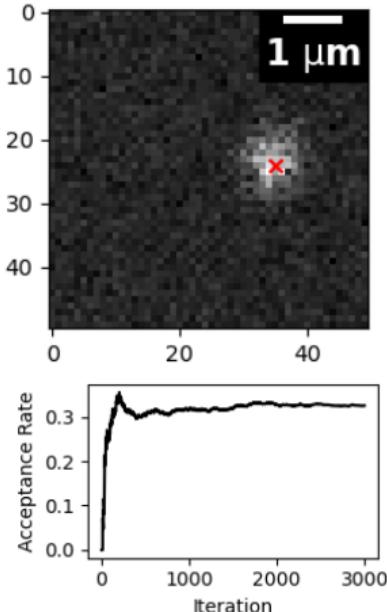
Using the approximation we can write

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

# Estimator precision sets the resolution limit in localization microscopy

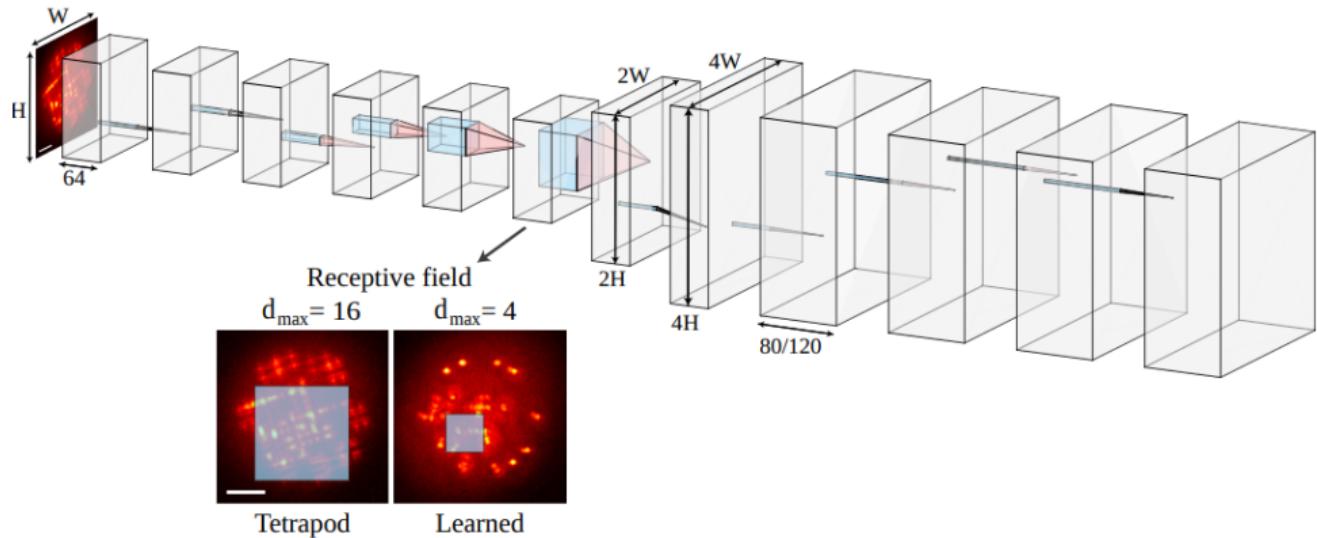


# Estimator precision sets the resolution limit in localization microscopy



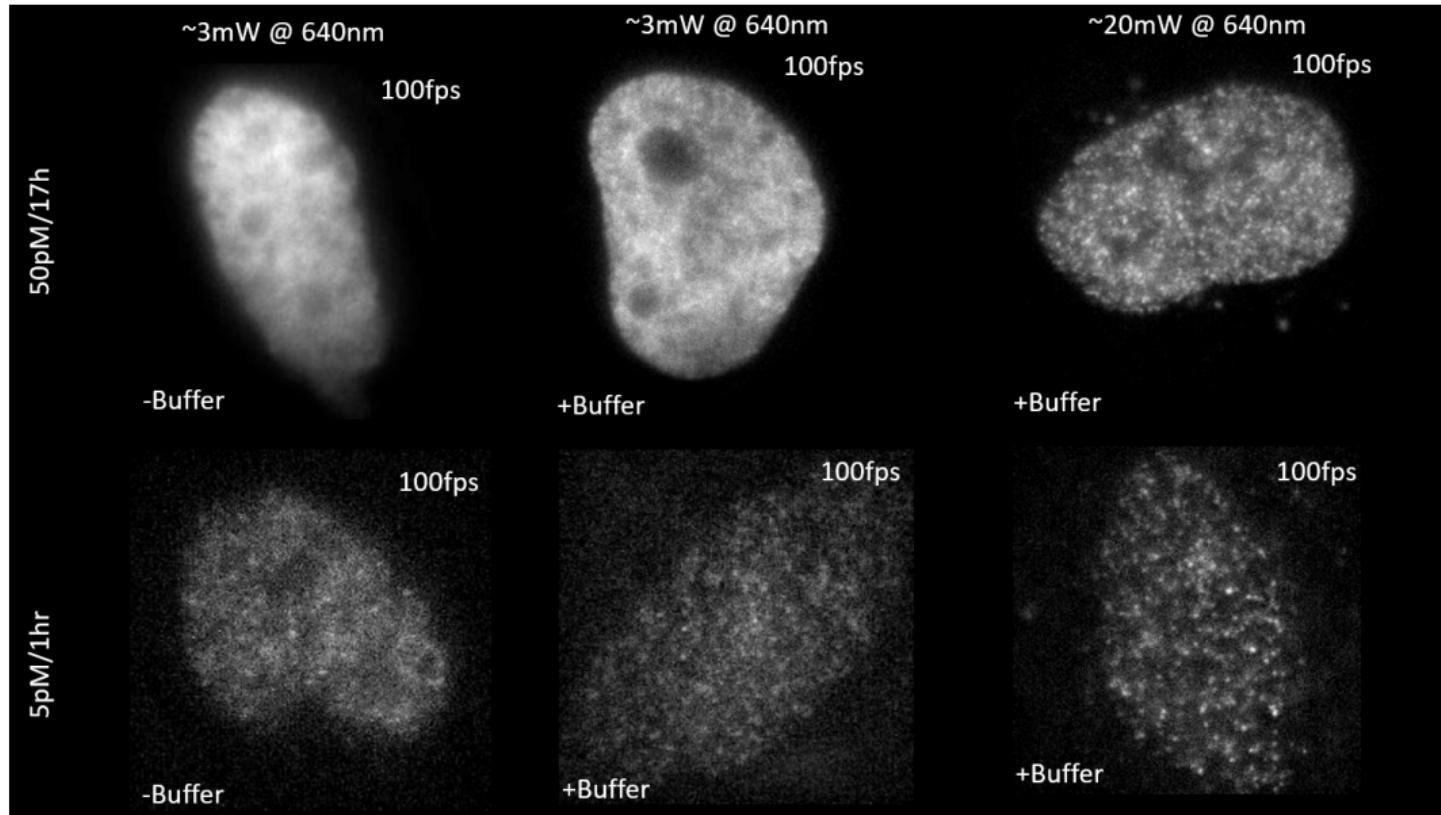
- ▶ Localization uncertainty can be quantified with Metropolis-Hastings MCMC
- ▶ MCMC is asymptotically exact, but slow

# Deep learning beats MLE at 2D and 3D localization

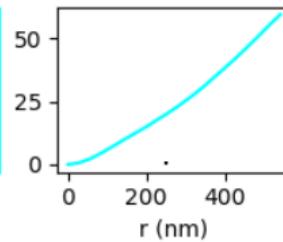
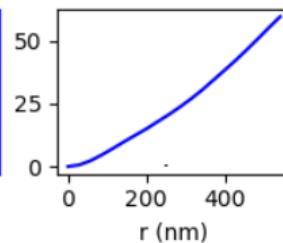
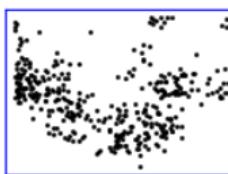
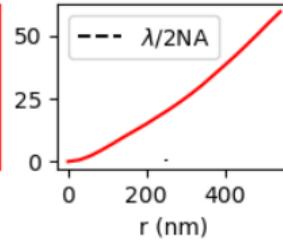
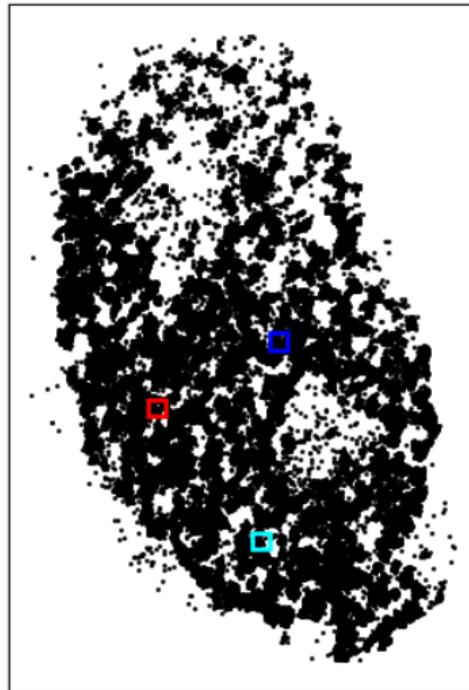


Deep learning beats MLE at 2D and 3D localization

# The metastable OFF state can be maintained with high laser power



## Resolution is dependent on photoswitching kinetics



## 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  $\delta$  is a number of photons which satisfy a criterion on localization accuracy.

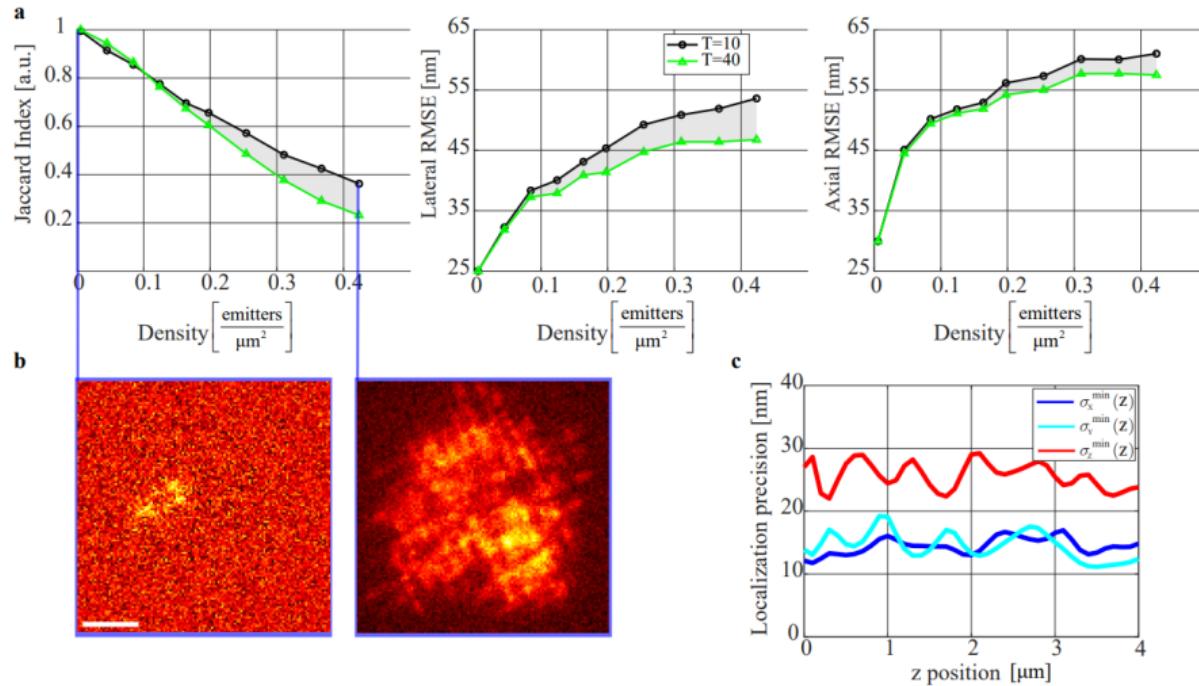
$$\alpha = \int_{\delta}^{\Delta} \left( \sum_{n=0}^{\infty} Q(N=n) \psi(\tau|n; \vec{k}) \right) d\tau \approx \mathbb{E}_{\tau \sim P(\tau)} (\mathbb{I}[\tau > \delta])$$

$P(\tau)$  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

