

# Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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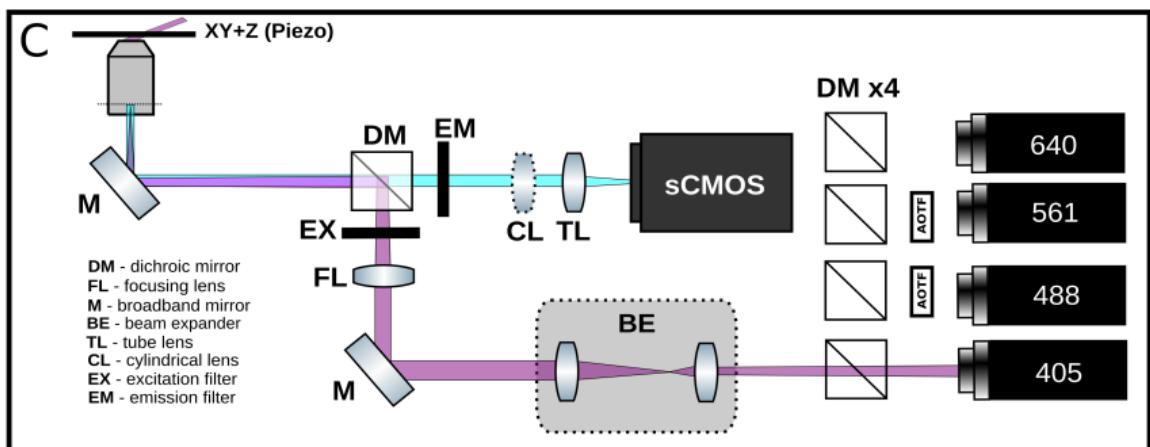
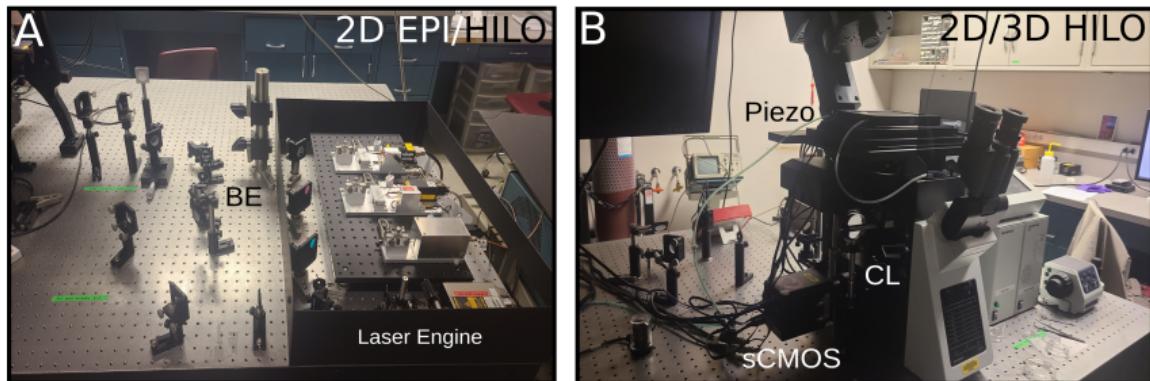
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# Introduction

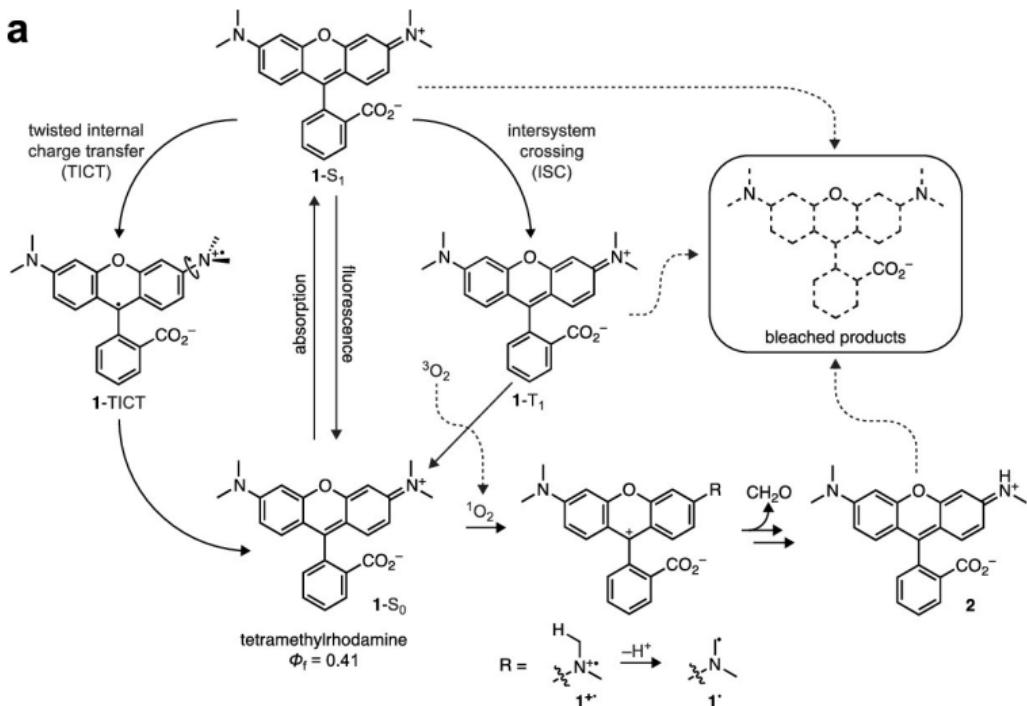
## Summary

- ▶ 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

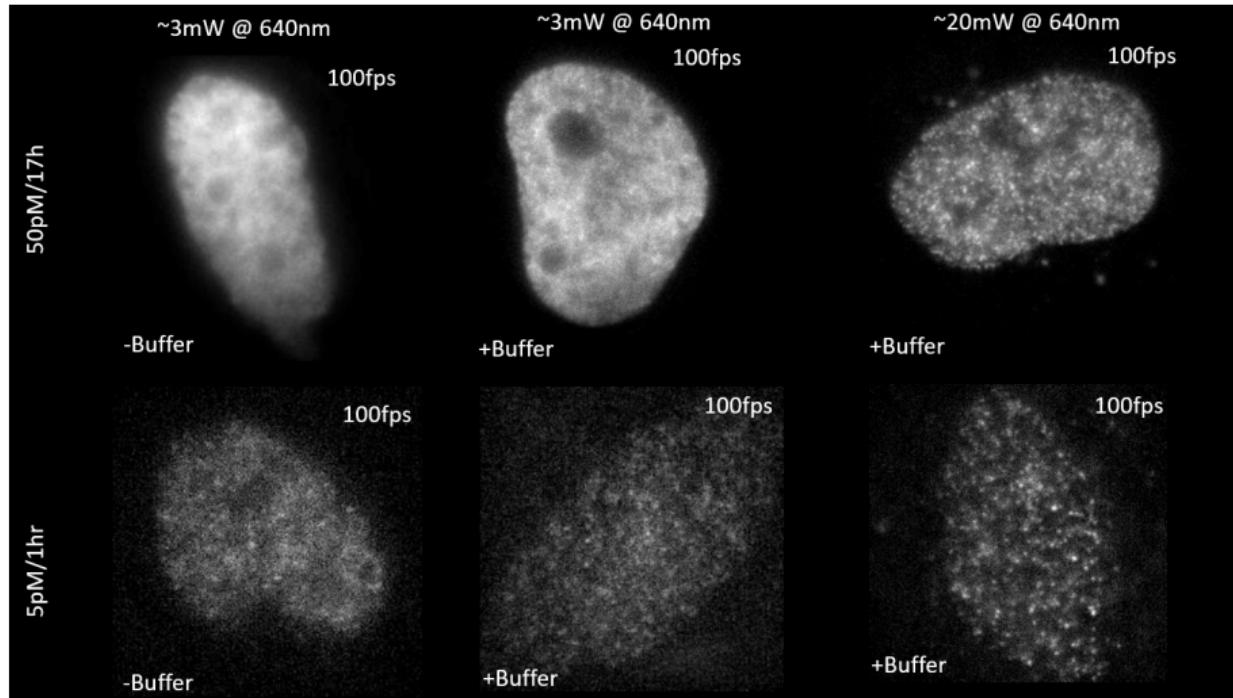


# The photophysics of rhodamines



Grimm et al. A general method to improve fluorophores using deuterated auxochromes. ACS 2021

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



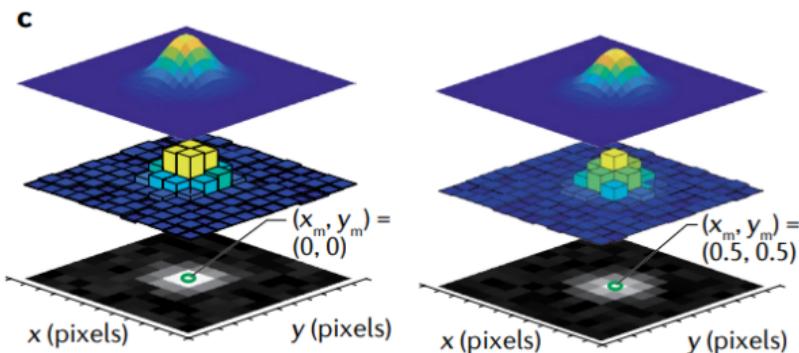
- We use rhodamine derivatives JF549 and JF646 HaloTag ligands to label H2B protein in transiently transfected H2B-HaloTag HeLa cells

# Maximum likelihood localization of an isolated fluorescent emitter

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

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

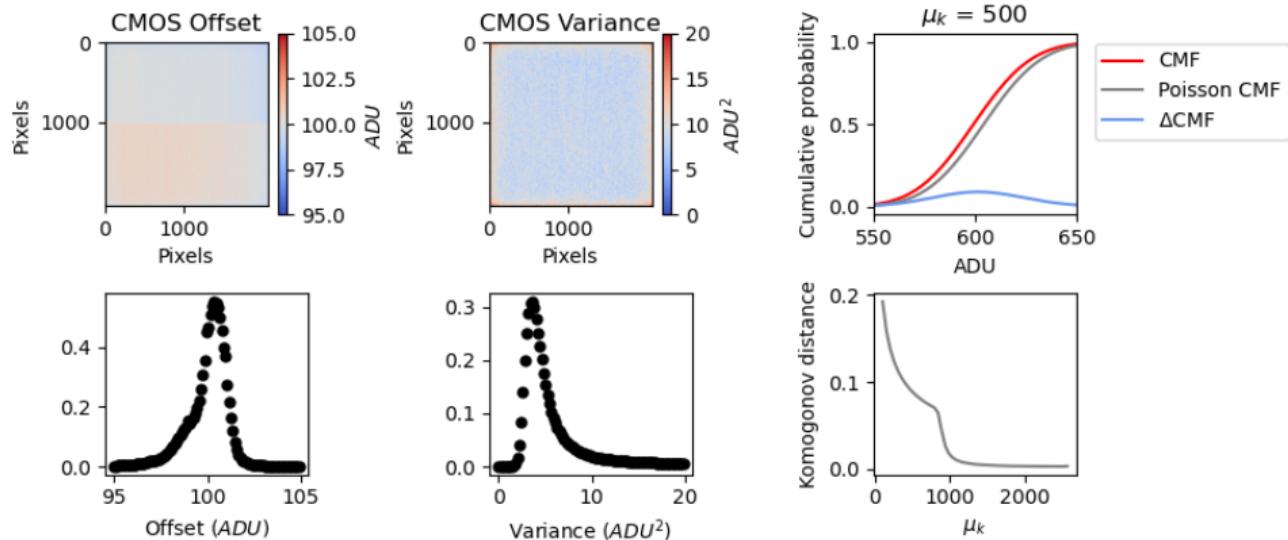
- $\eta$  – quantum efficiency
- $N_0$  – emission rate
- $\Delta$  – exposure time



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

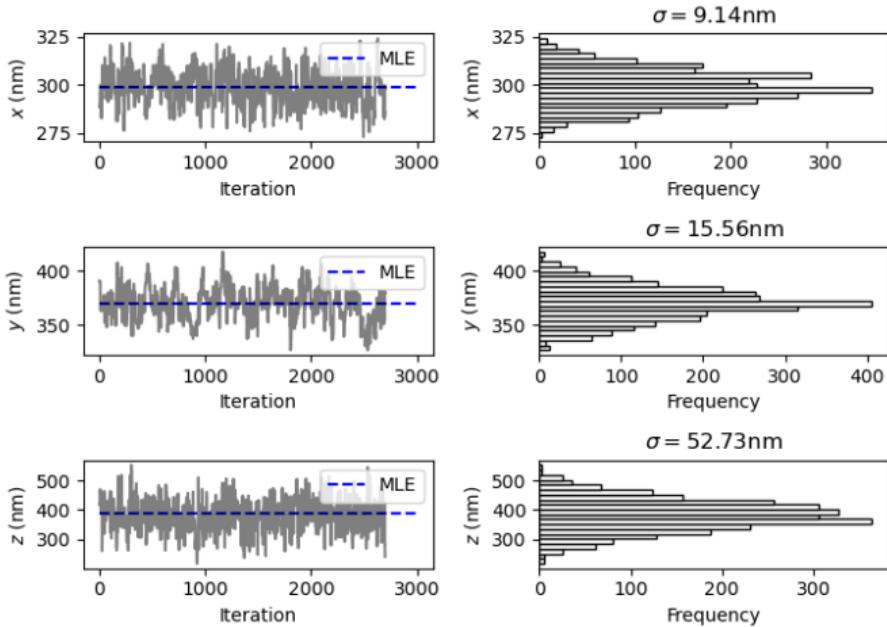
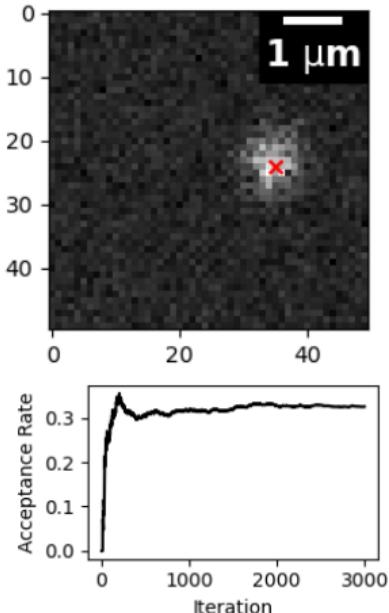
$P(H_k|\theta)$  can be approximated as Poisson at high signal-to-noise (SNR)

# A Poisson approximation at moderate SNR simplifies SMLM



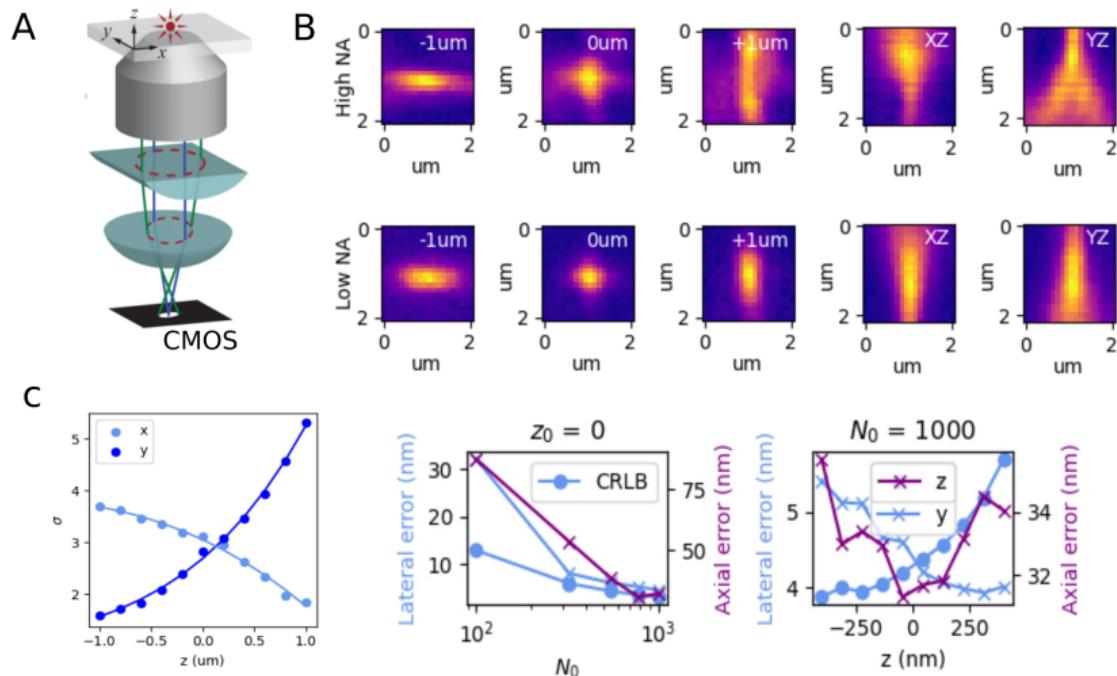
$$\ell(\vec{H}|\theta) = -\log \prod_k \frac{e^{-(\mu'_k)} (\mu'_k)^{n_k}}{n_k!} = \sum_k \log n_k! + \mu'_k - n_k \log (\mu'_k)$$

# Estimator precision sets the resolution limit in localization microscopy



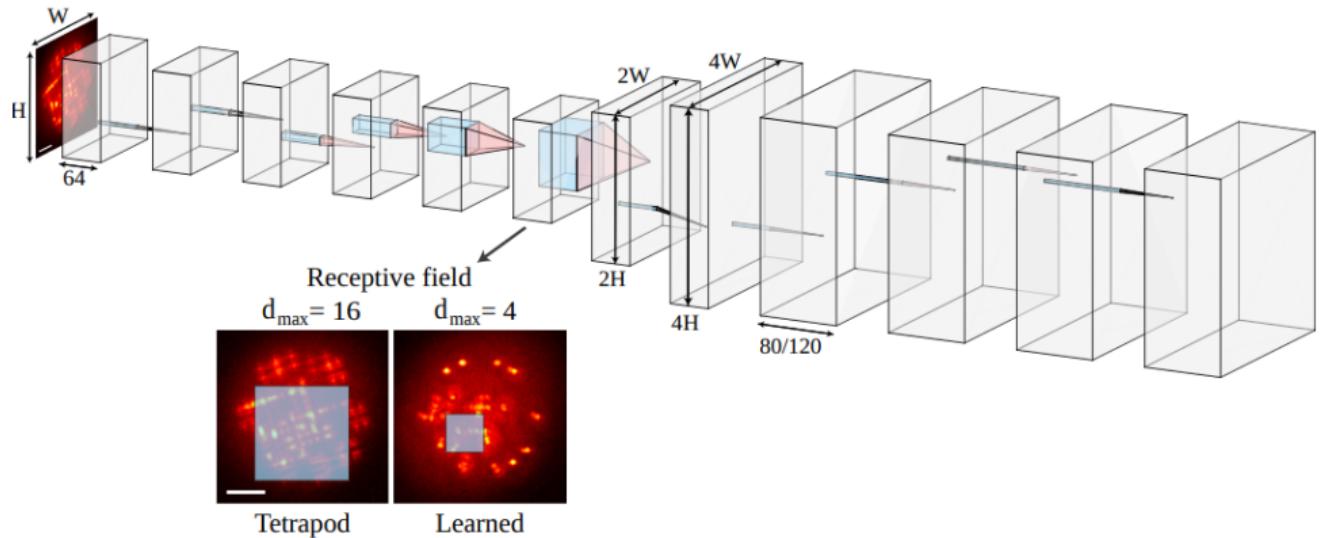
- ▶ Localization uncertainty can be quantified with Metropolis-Hastings MCMC
- ▶ MCMC is asymptotically exact, but slow

# Estimator precision sets the resolution limit in localization microscopy

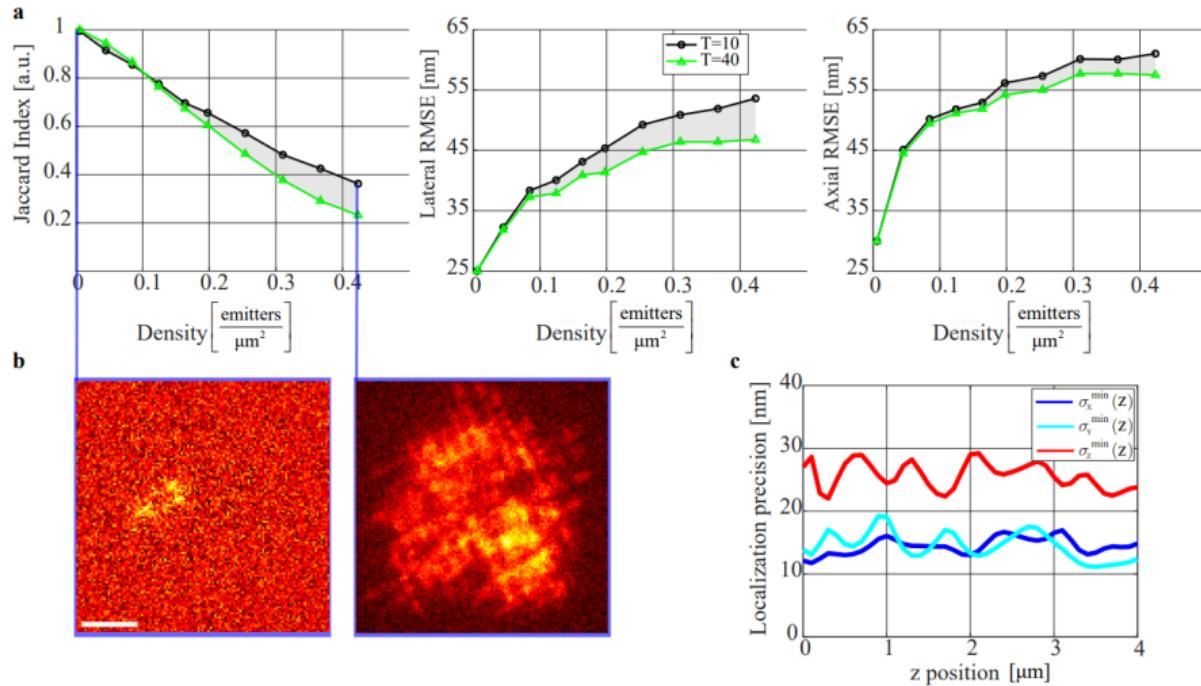


- ▶ A weak cylindrical lens breaks the axial symmetry of the PSF

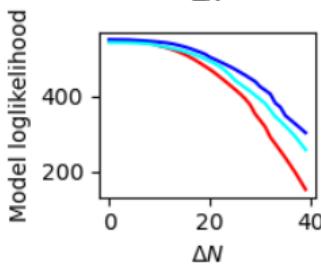
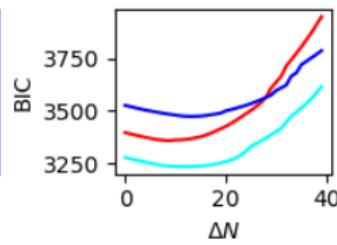
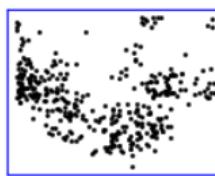
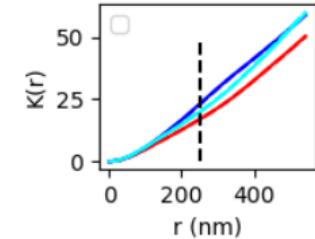
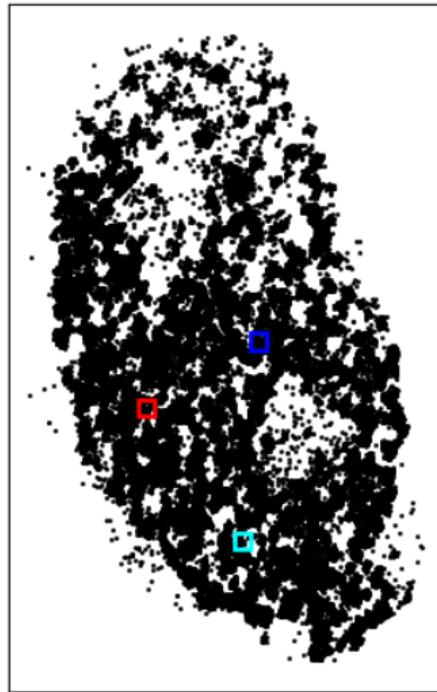
# Deep learning can generalize precise SMLM to three dimensions



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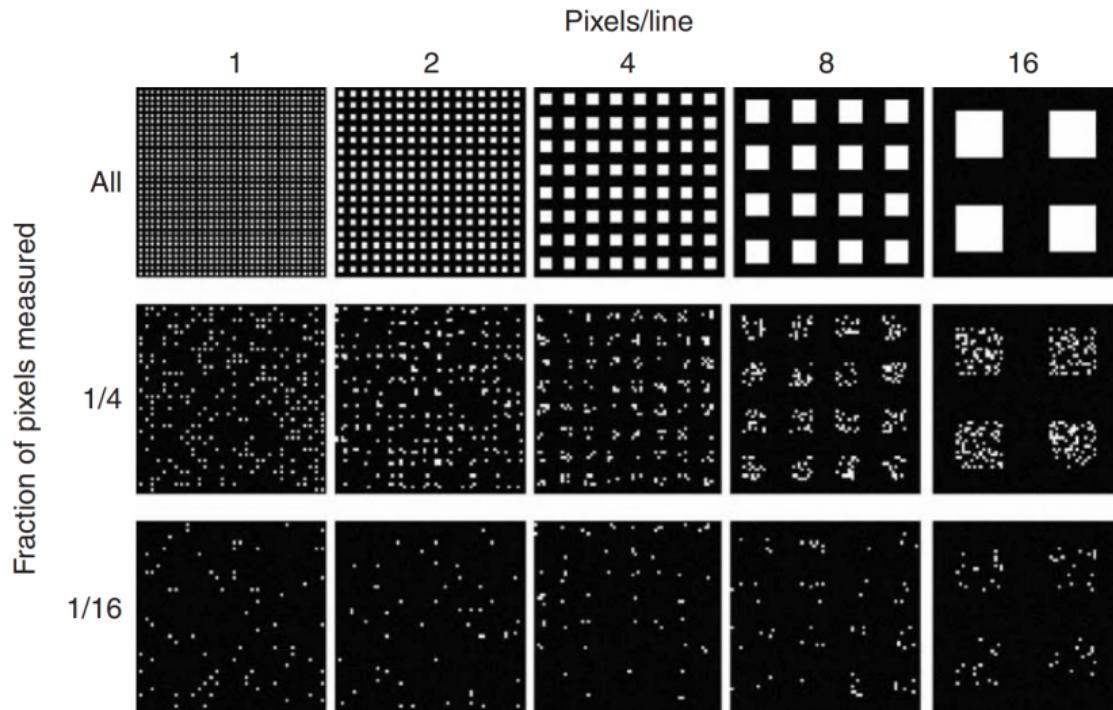


# Chromatin nanodomains in a living Hela cell nucleus



Caged nucleosomes are Brownian harmonic oscillators

# The tradeoff between spatial and temporal resolution in SMLM



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

## 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.

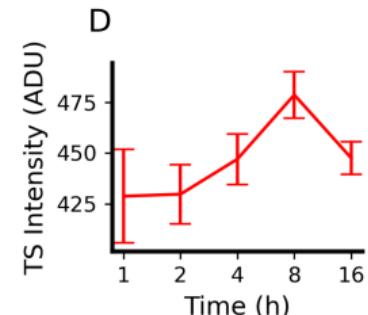
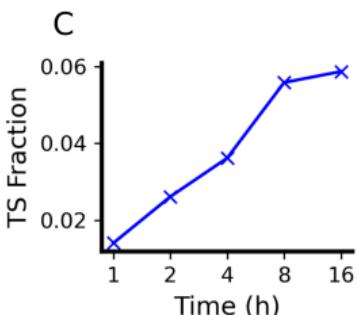
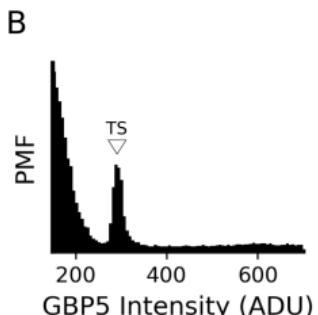
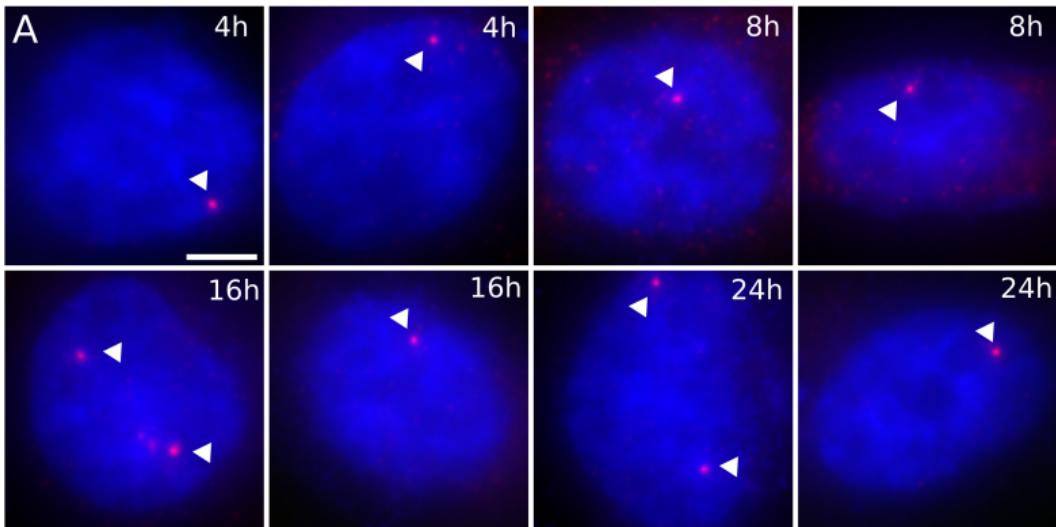
$$\alpha = \int_{\delta}^{\Delta} \left( \sum_{n=0}^{\infty} Q(N=n) \psi(\tau|n; \vec{k}) \right) d\tau \approx \mathbb{E}_{\tau \sim P(\tau)} (\mathbb{I}[\tau > \delta])$$

$P(\tau)$  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

# Validation of JQ1 efficacy for BRD4 inhibition in HeLa cells



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