

# Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

Clayton W. Seitz

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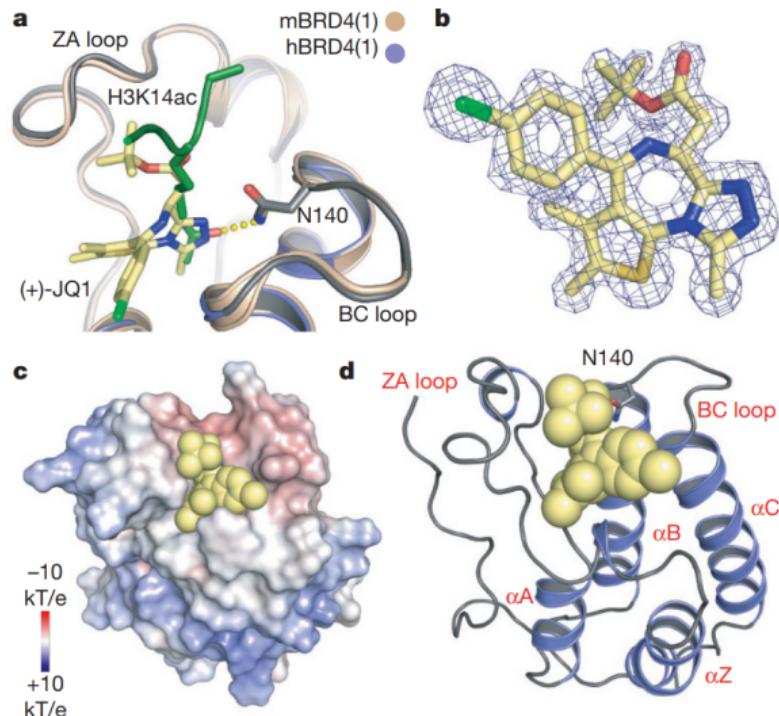
# Introduction

## 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

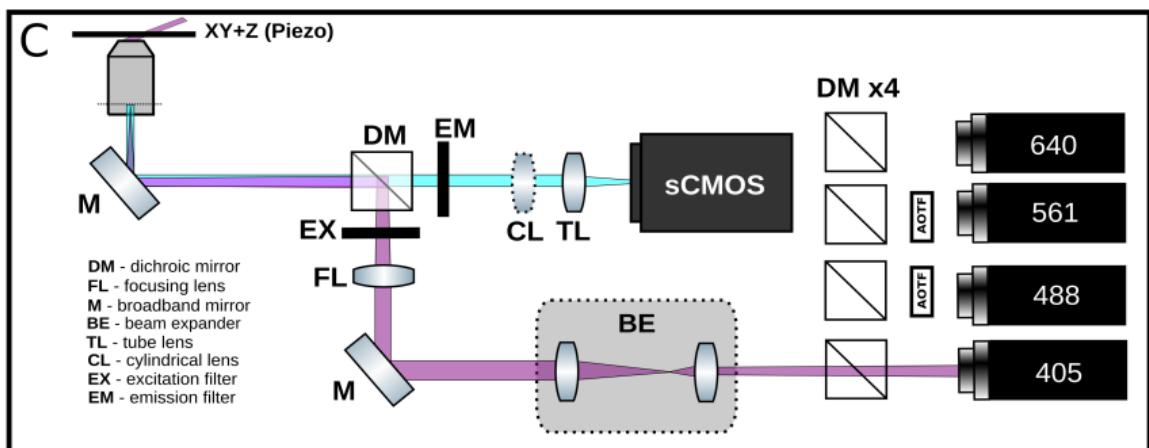
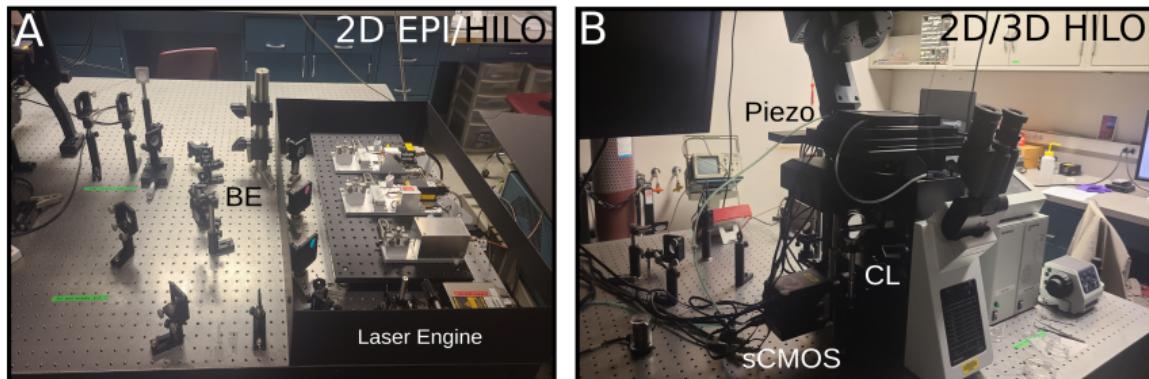
## The textbook view of histone modifications

# (+)-JQ1 in complex with BRD4 protein

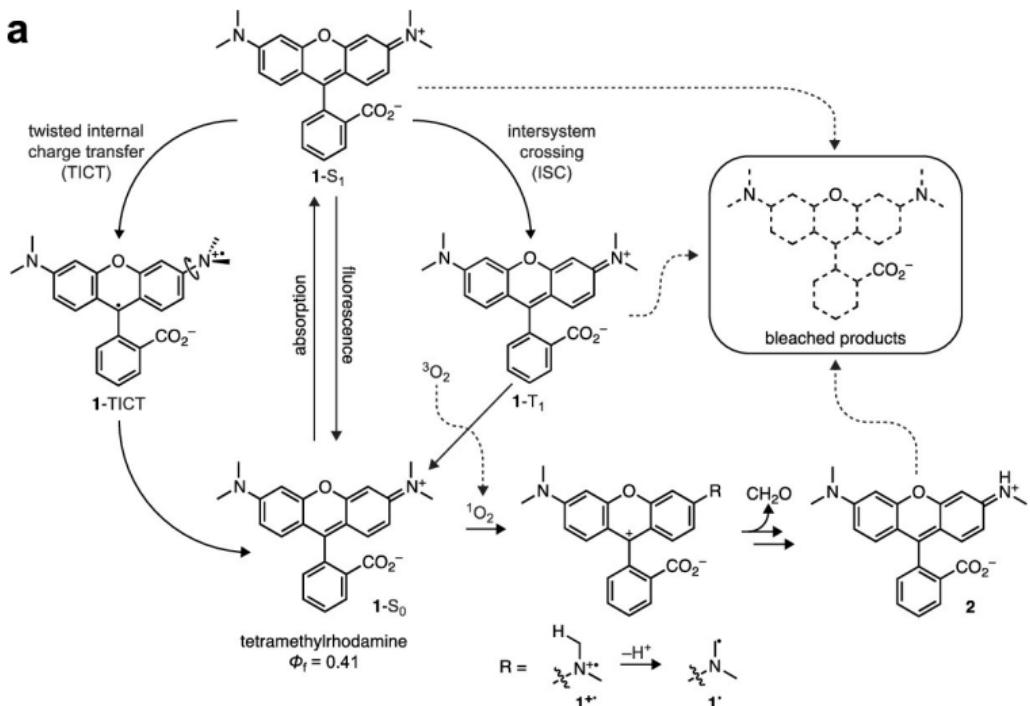


Filippakopoulos. Selective inhibition of BET bromodomains. *Nature Communications*

# Instrumentation for super-resolution and high throughput microscopy

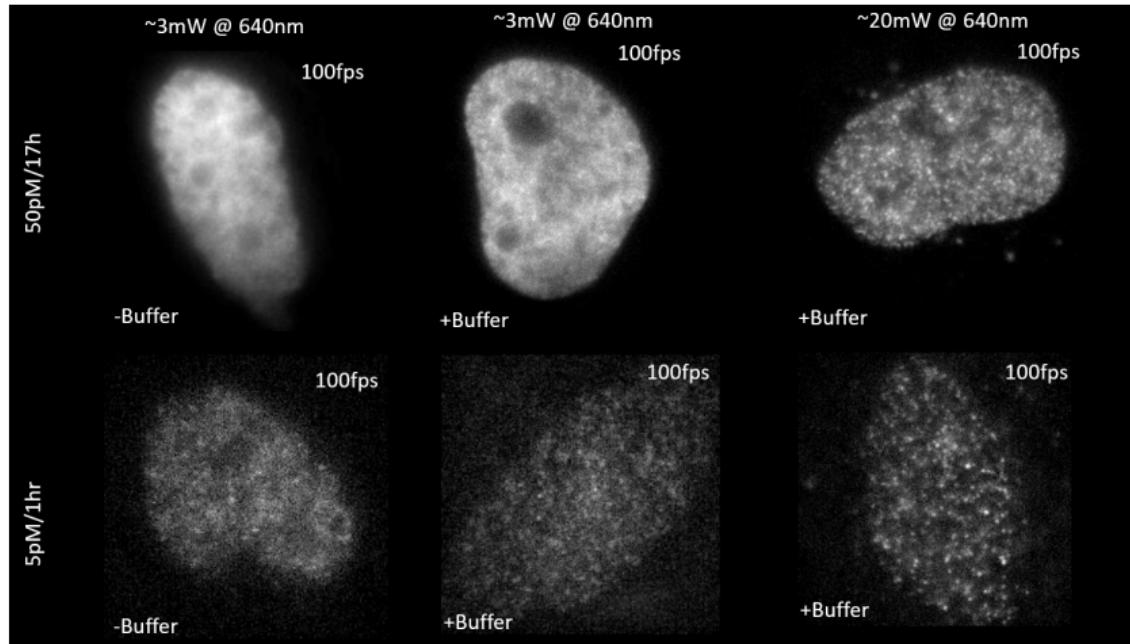


# The photophysics of rhodamines



- ▶ Reduction of the T<sub>1</sub> 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



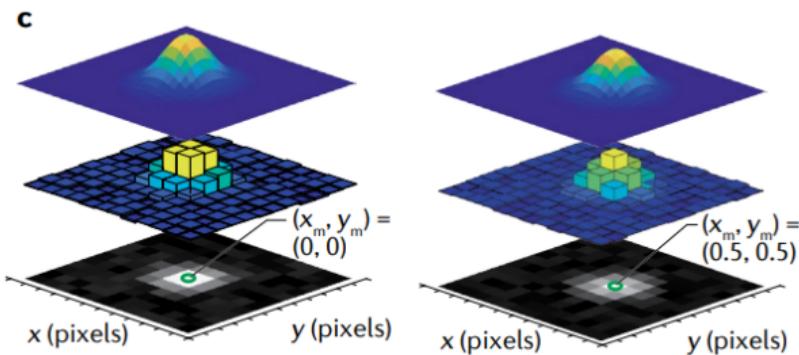
- ▶ SMLM is desirable for SR due to very high res and no scanning (STED)
- ▶ Less control over photophysical state, but high throughput
- ▶ High power compensates for dense labeling

# Maximum likelihood localization of an isolated fluorescent emitter

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

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

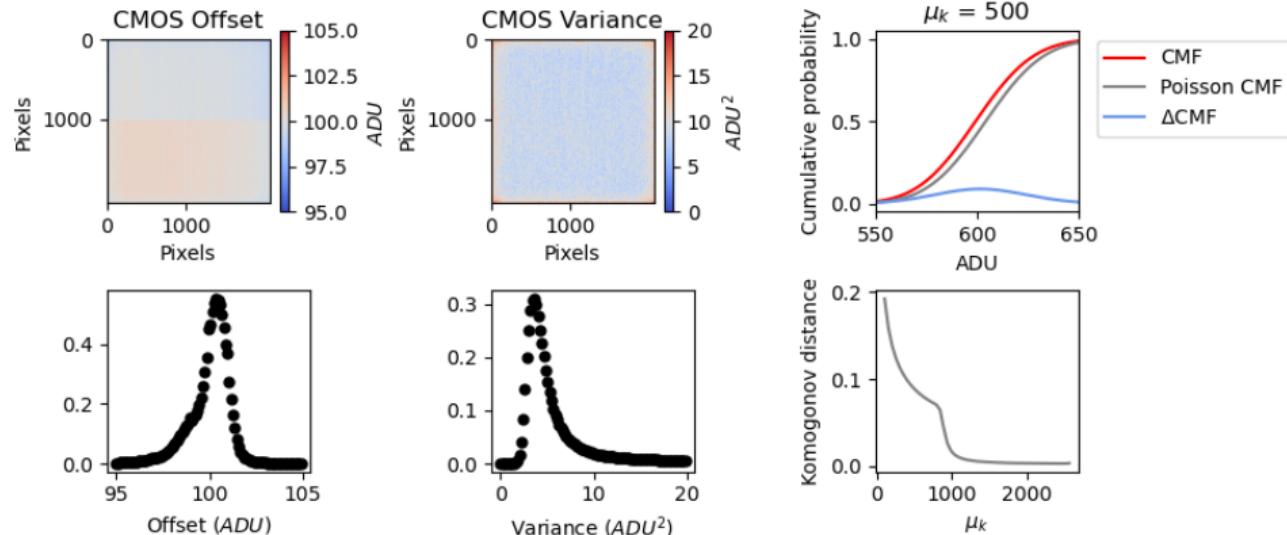
- $\eta$  – quantum efficiency
- $N_0$  – emission rate
- $\Delta$  – exposure time



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

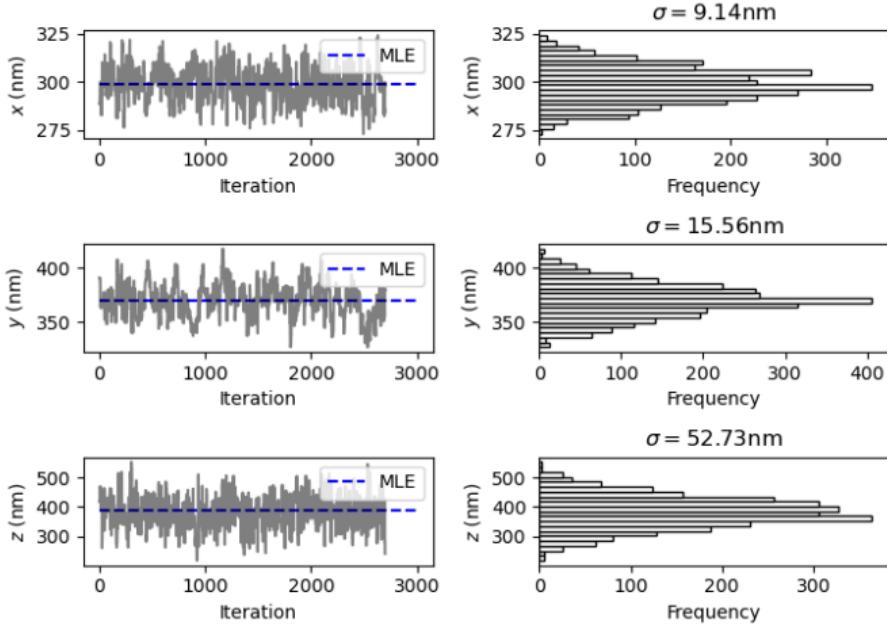
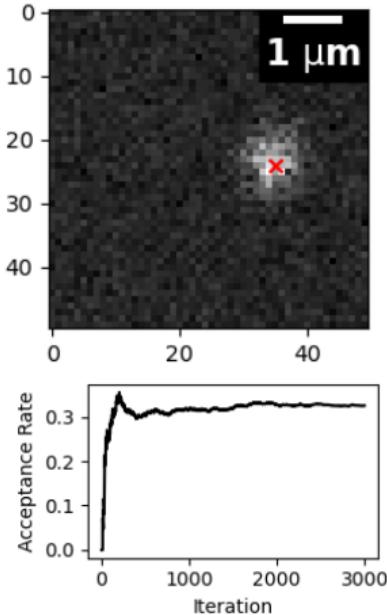
$P(H_k|\theta)$  can be approximated as Poisson at high signal-to-noise (SNR)

# A Poisson approximation at moderate SNR simplifies SMLM



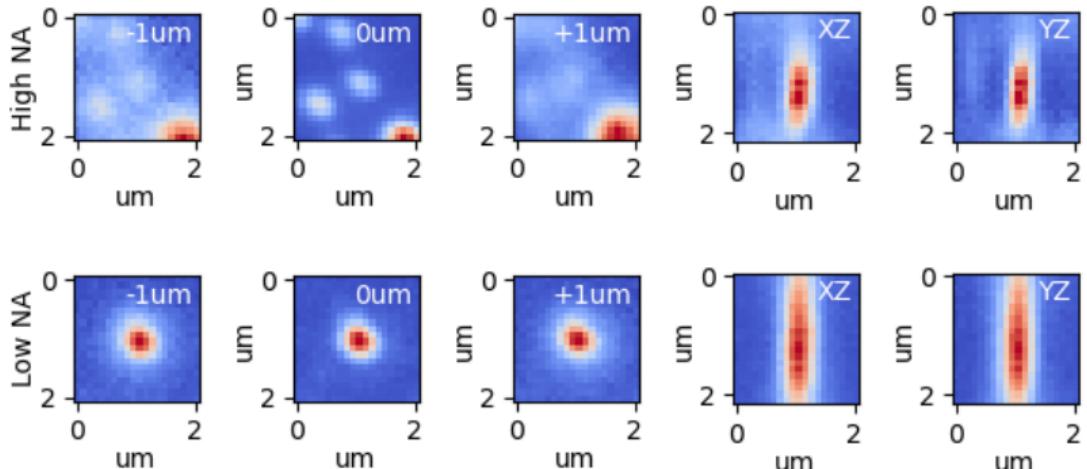
- ▶  $P(H_k - o_k | \theta) = \text{Poisson}(\mu_k + \sigma_k^2 | \theta)$  for pixel offset  $o_k$  noise variance  $\sigma_k^2$
- ▶ Fisher information and Cramer-Rao lower bound (CRLB) can be computed analytically for Poisson log-likelihood  $\ell$  (Smith 2010, Huang 2013)

# Estimator precision sets the resolution limit in localization microscopy

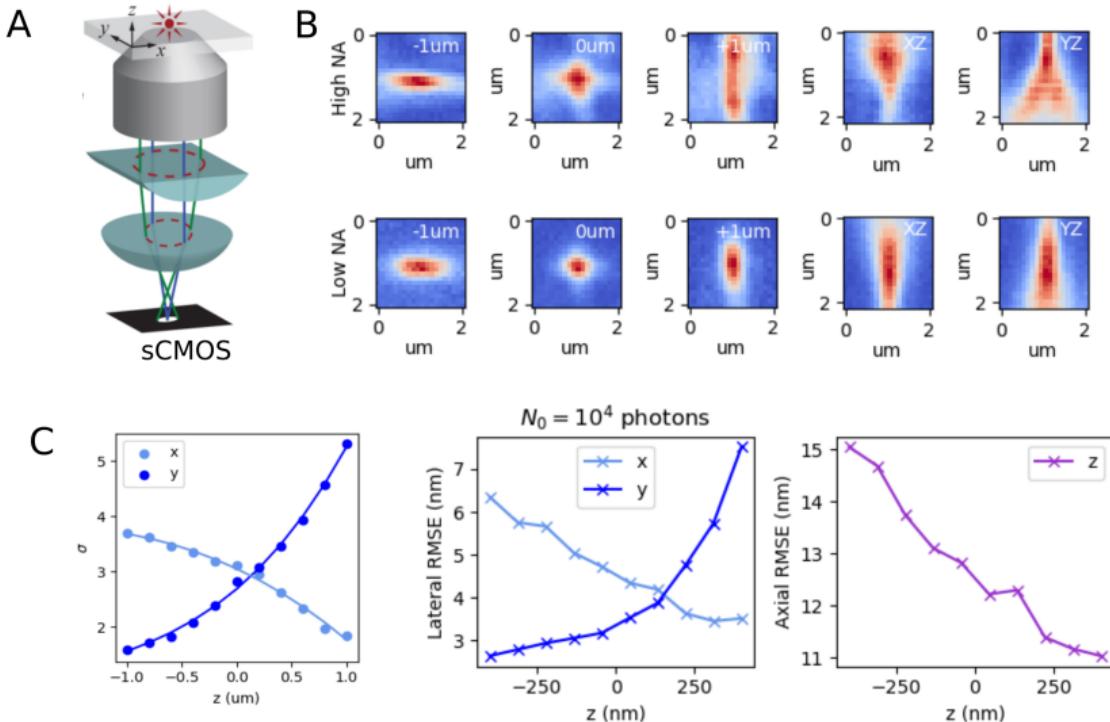


- ▶ Variance of the posterior  $P(\theta|\vec{H})$  is a useful particle filter
- ▶ We assume uniform priors on coordinates

## Estimator precision sets the resolution limit in 2D

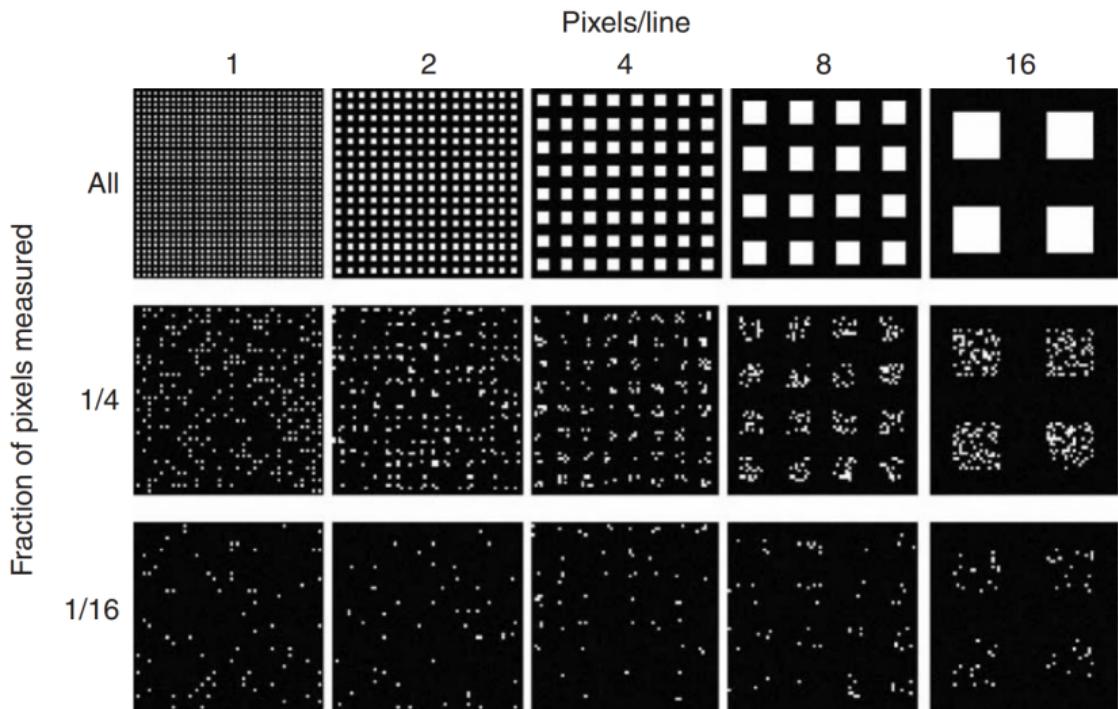


# Estimator precision sets the resolution limit in 3D

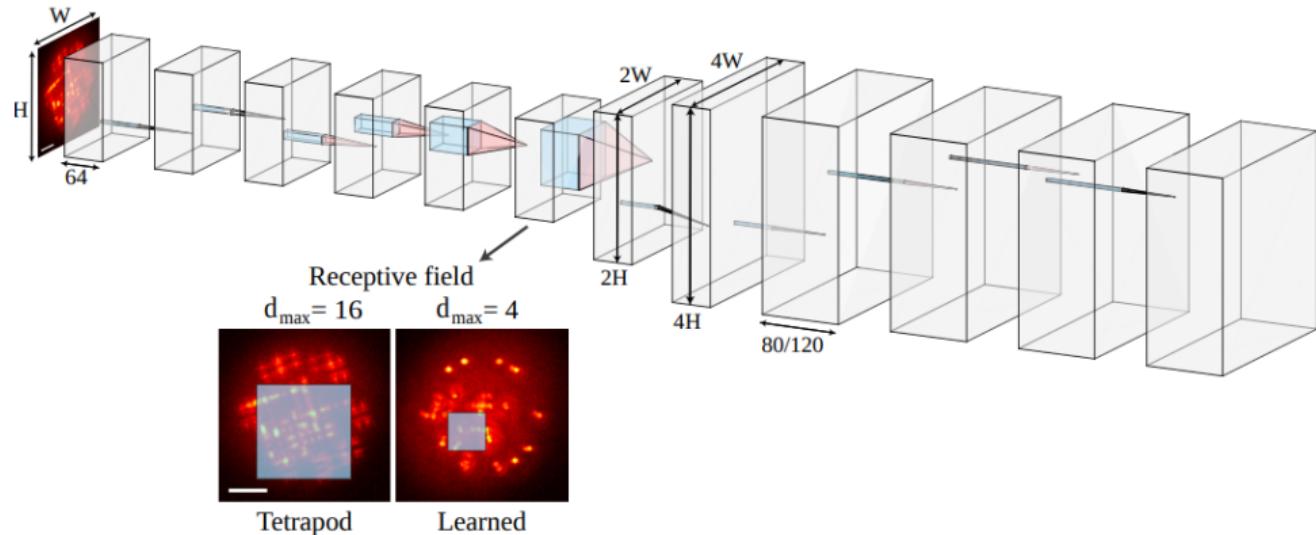


- A weak ( $f = 10\text{m}$ ) cylindrical lens breaks the axial symmetry of the PSF

# The tradeoff between spatial and temporal resolution in SMLM

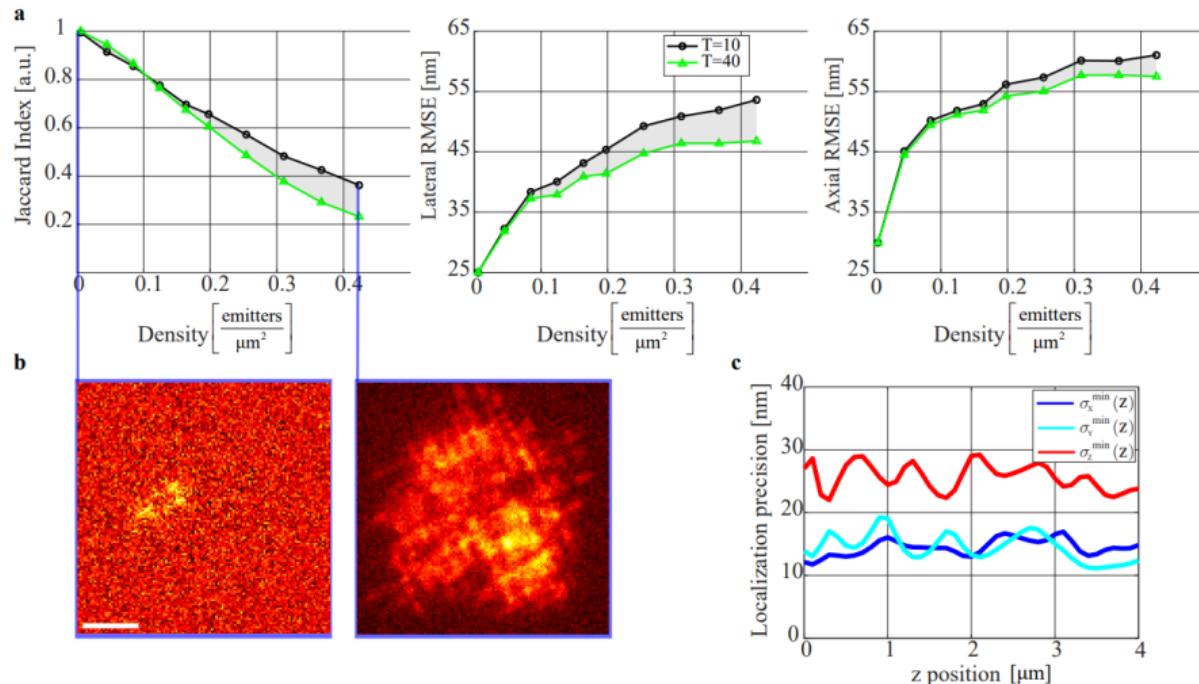


# The tradeoff between spatial and temporal resolution in SMLM



- ▶ 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

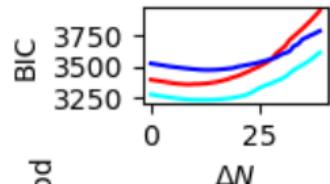
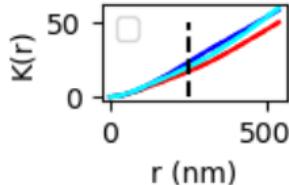
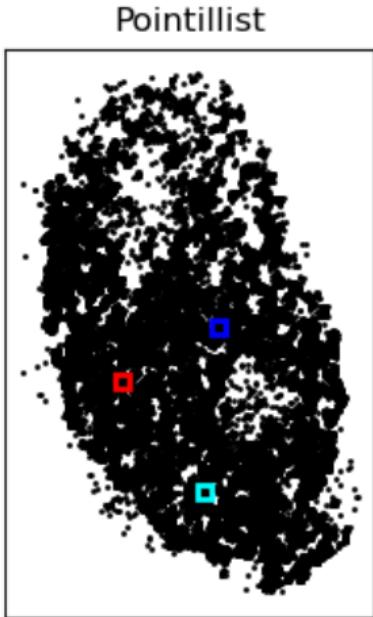
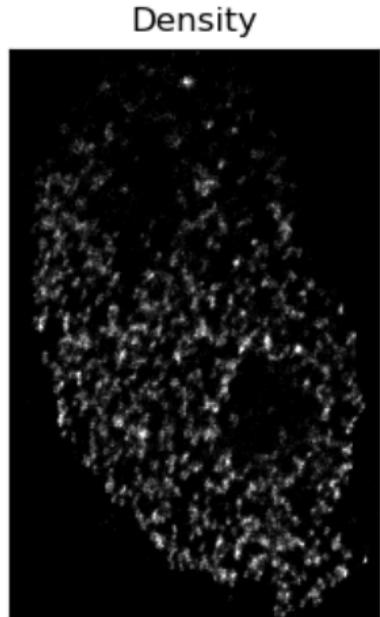
# Deep learning can generalize precise SMLM to three dimensions



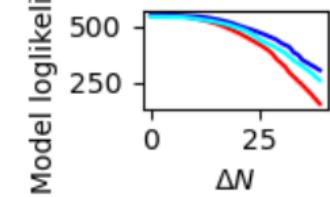
Several methods have been designed to break the time-resolution barrier

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

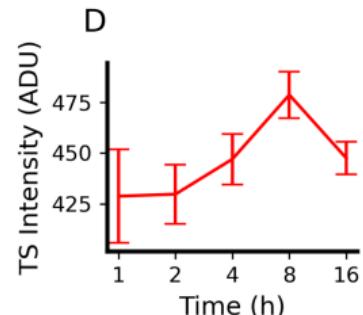
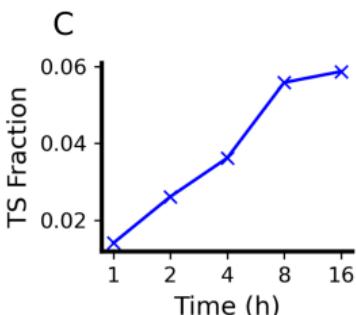
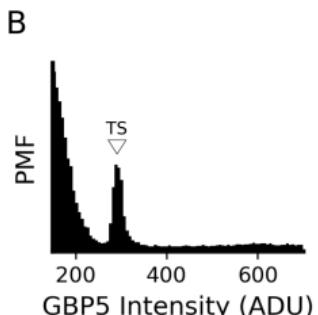
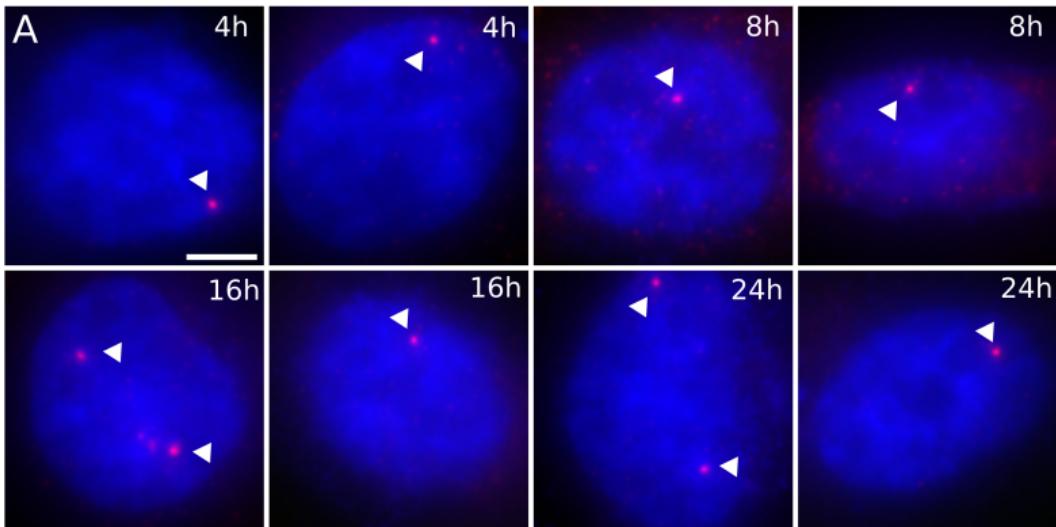
$$\alpha = \int_{\delta}^{\Delta} \left( \sum_{n=0}^{\infty} Q(N=n) \psi(\tau|n; \vec{k}) \right) d\tau \approx \mathbb{E}_{\tau \sim P(\tau)} (\mathbb{I}[\tau > \delta])$$

$P(\tau)$  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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