

Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

Clayton W. Seitz

August 4, 2023

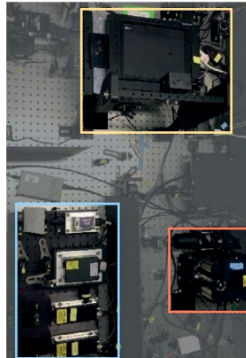
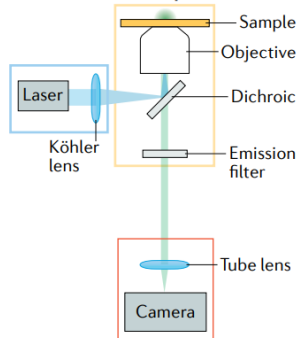
Introduction

Summary

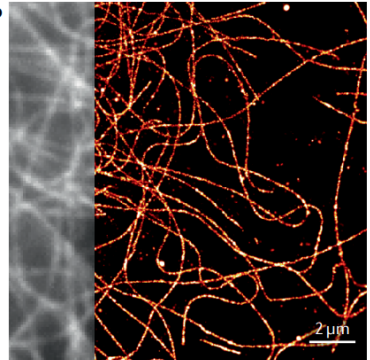
- ▶ We study the organization of nucleosomes in living cells, and I am interested in the impact of BRD4 phase separation (fusion/fission) on chromatin packing, as previous work has shown chromatin nanodomains are highly dynamic structures (Barth 2020)
- ▶ We study chromatin structure using SMLM, which 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

Direct stochastic optical reconstruction microscopy

a Basic SMLM set-up



b



Maximum likelihood localization of an isolated fluorescent emitter

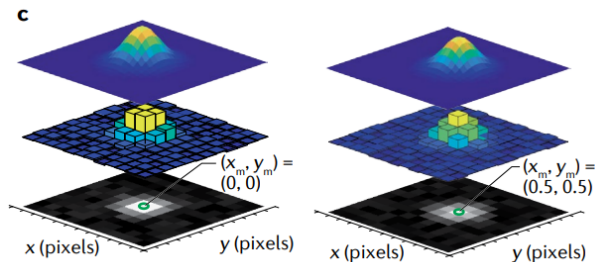
Localization: $\theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$

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

η – quantum efficiency

N_0 – emission rate

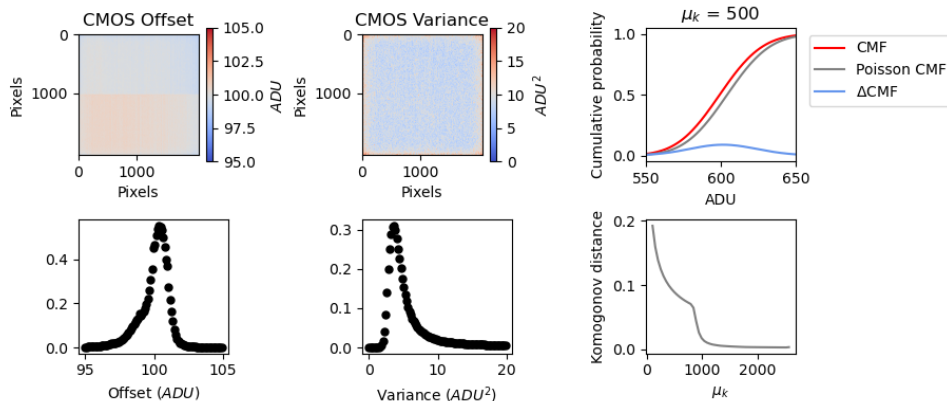
Δ – exposure time



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

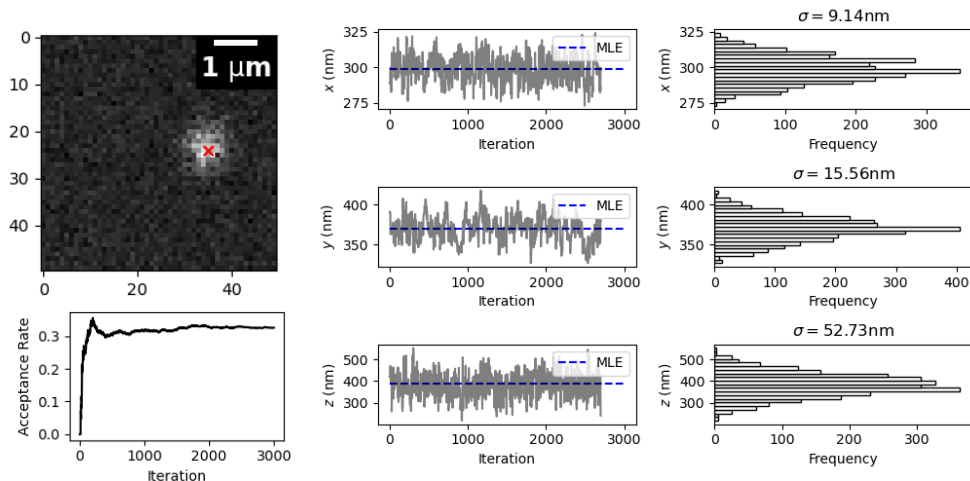
$P(H_k|\theta)$ can be approximated as Poisson at high signal-to-noise (SNR)

A Poisson approximation at moderate SNR simplifies SMLM



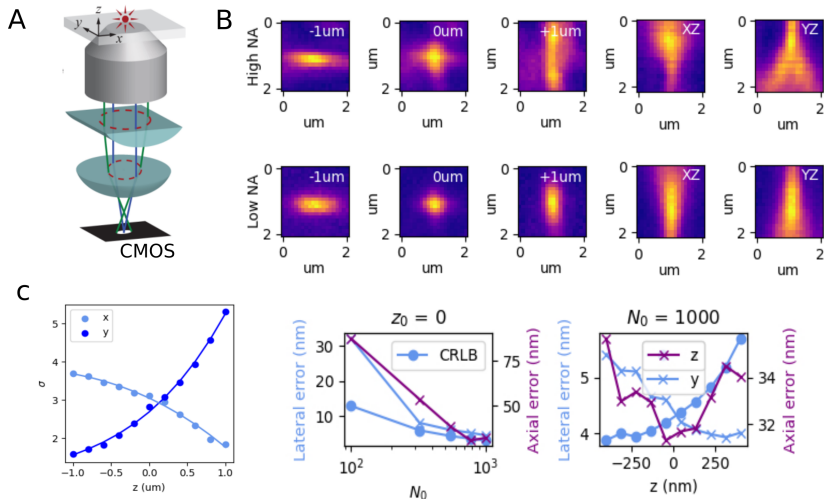
$$\ell(\vec{H}|\theta) = -\log \prod_k \frac{e^{-(\mu'_k)} (\mu'_k)^{n_k}}{n_k!} = \sum_k \log n_k! + \mu'_k - n_k \log (\mu'_k)$$

Estimator precision sets the resolution limit in localization microscopy



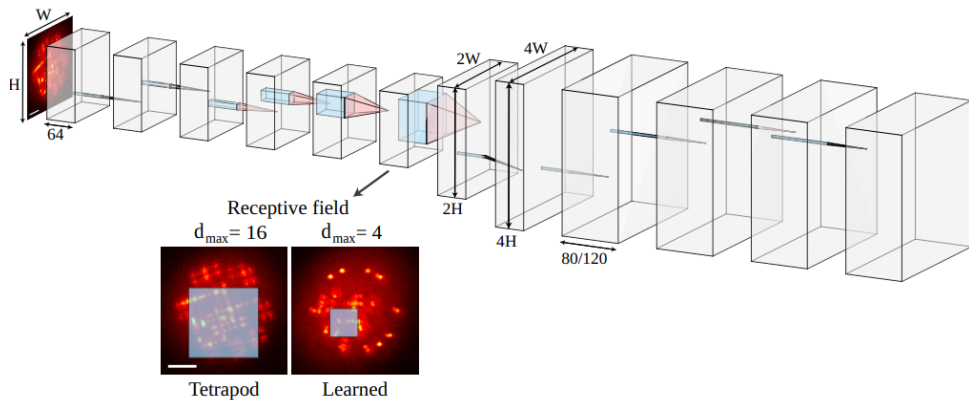
- ▶ Localization uncertainty can be quantified with Metropolis-Hastings MCMC
- ▶ MCMC is asymptotically exact, but slow

Estimator precision sets the resolution limit in localization microscopy

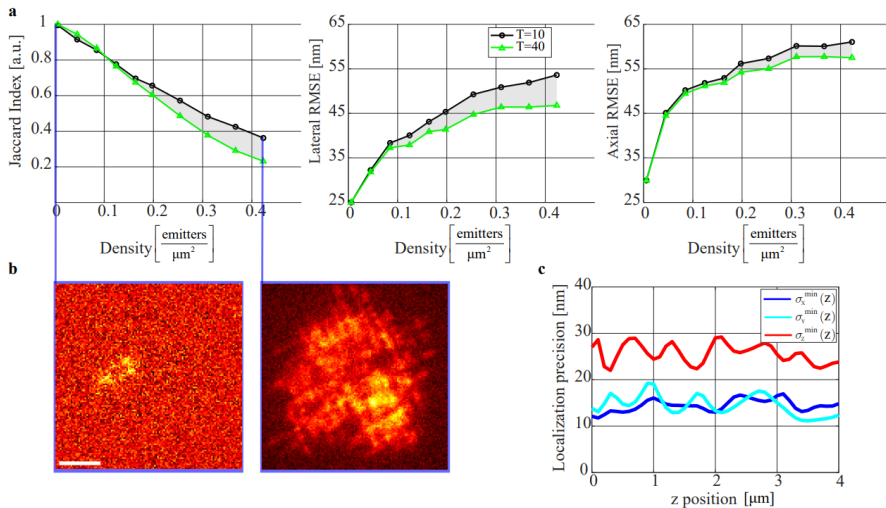


- A weak cylindrical lens breaks the axial symmetry of the PSF

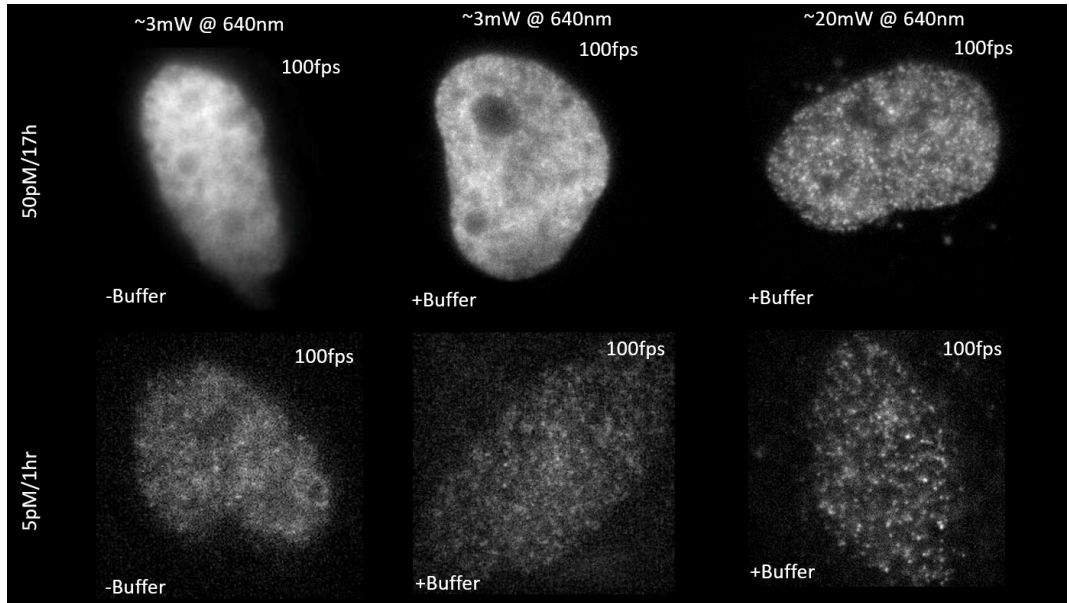
Deep learning can generalize precise SMLM to three dimensions



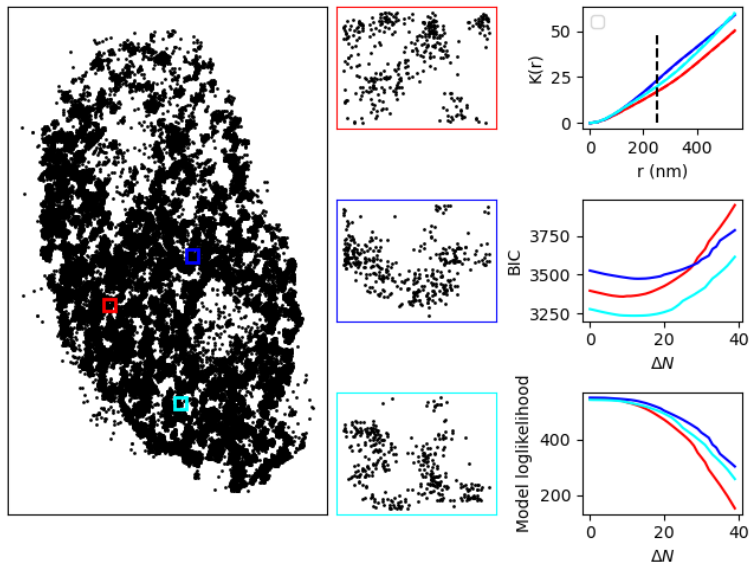
Deep learning can generalize precise SMLM to three dimensions



The metastable OFF state can be maintained with high laser power

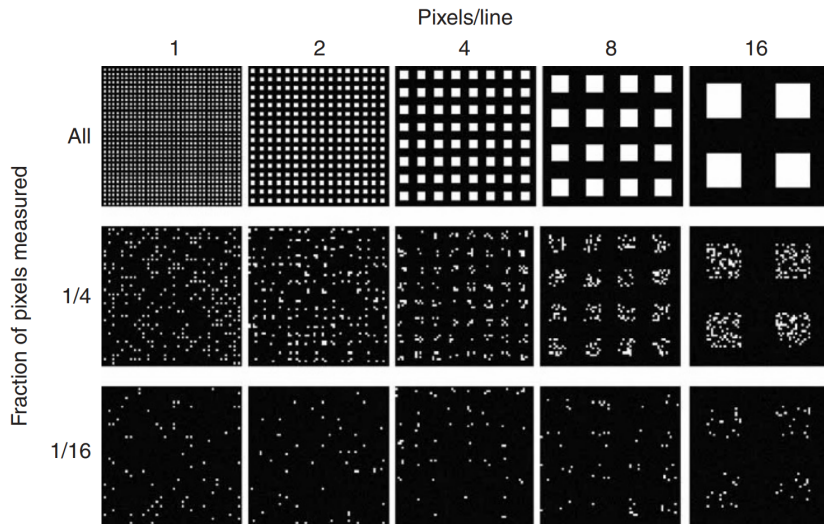


Chromatin nanodomains in a living Hela cell nucleus



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 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

