Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy
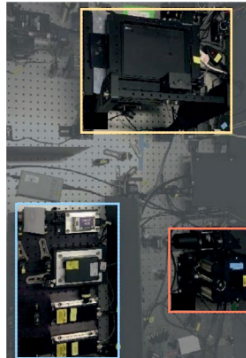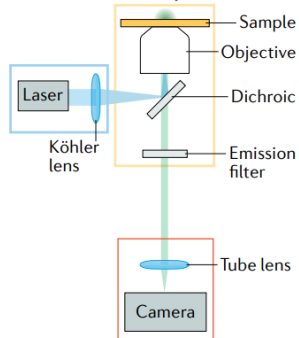
Clayton W. Seitz

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Introduction

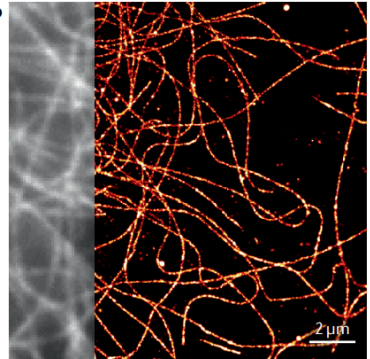# Visualizing nucleosome cluster dynamics

Methods

Direct stochastic optical reconstruction microscopy

Direct stochastic optical reconstruction microscopy

a **Basic SMLM set-up**

Sample
Objective
Dichroic
Laser
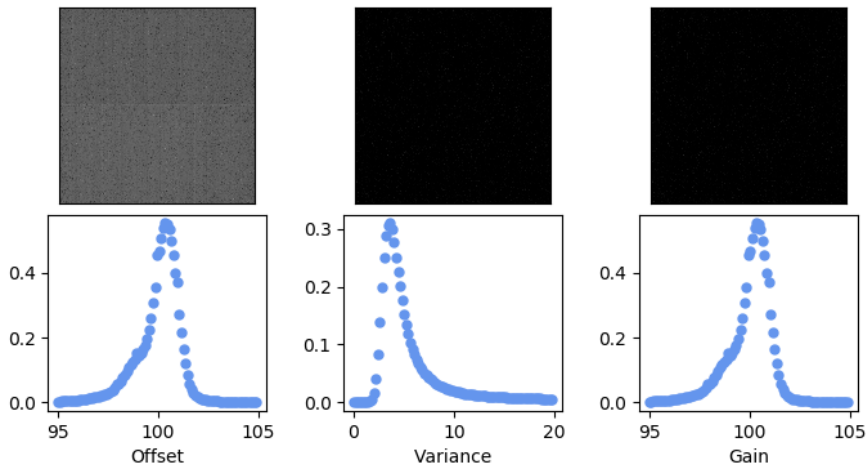Köhler lens
Emission filter
Tube lens
Camera

b

2 μm

Direct stochastic optical reconstruction microscopy

Readout noise of sCMOS cameras

Hamamatsu ORCA v3 CMOS, air cooled to -10C

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

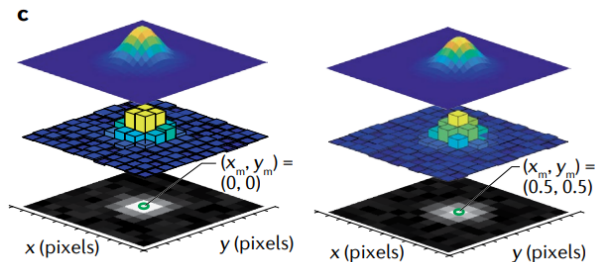# Maximum likelihood localization of an isolated fluorescent emitter

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

c

$(x_m, y_m) = (0,0)$

$(x_m, y_m) = (0.5, 0.5)$
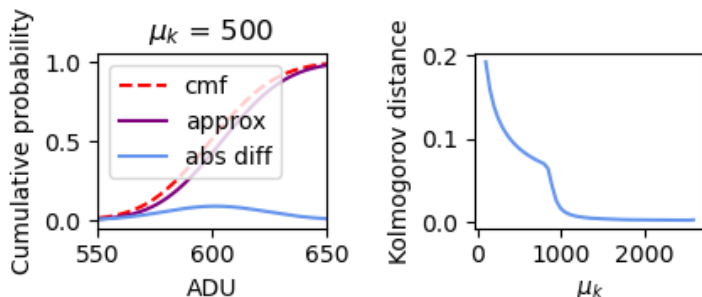
x (pixels)   y (pixels)

x (pixels)   y (pixels)

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

$P(H_k|\theta)$ can be approximated as Poisson at high signal-to-noise (SNR)
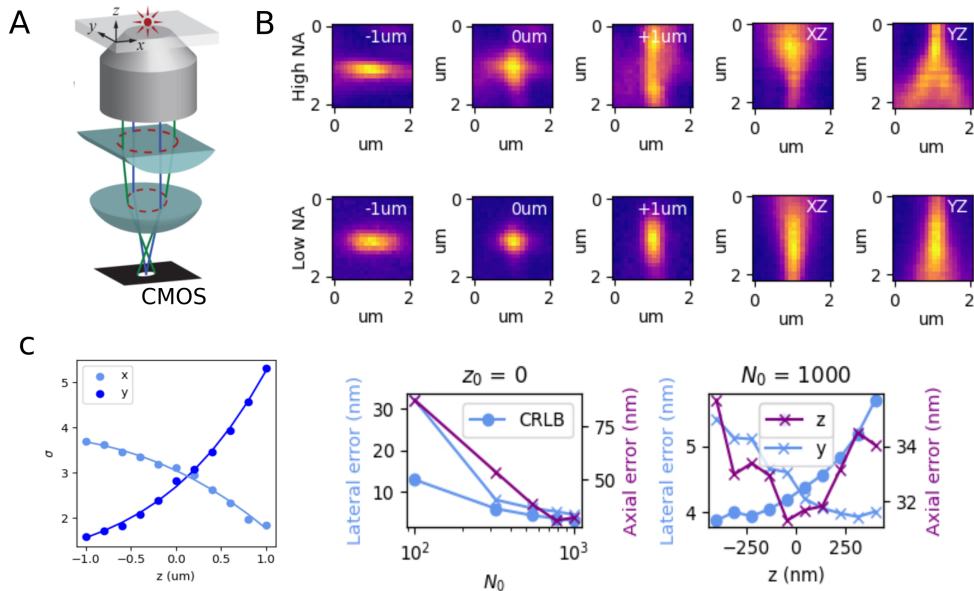
Quality of the Poisson approximation depends on SNR

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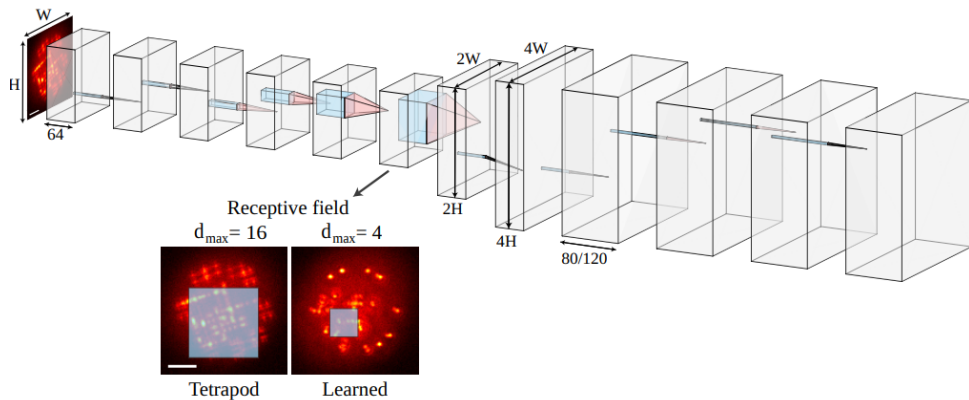

Using the approximation we can write

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

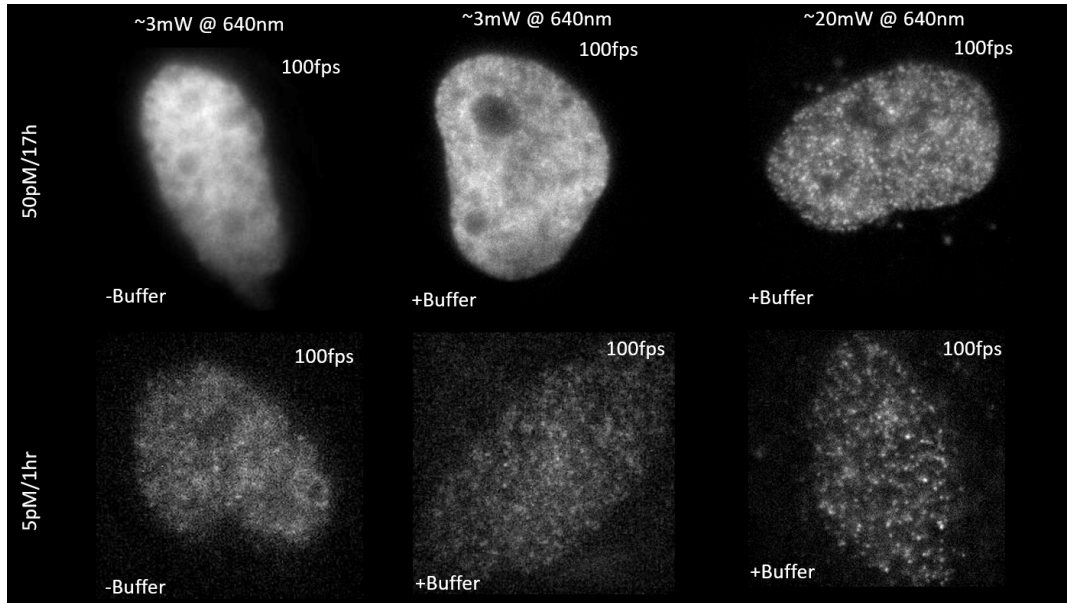# Estimator precision sets the resolution limit in localization microscopy

Deep learning beats MLE at 2D and 3D localization

Receptive field
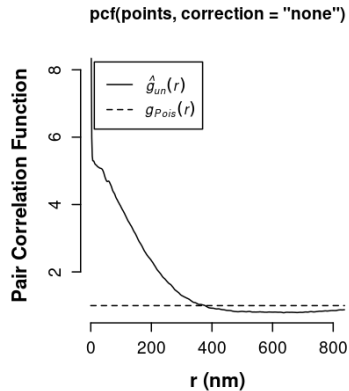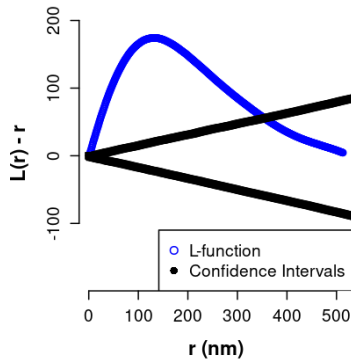$d_{max}= 16$ $d_{max}= 4$

Tetrapod   Learned

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

▶ MLE can approach the CRLB on simulated isolated emitter data

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.

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

$P(\tau)$ is usually obtained by Monte Carlo simulation. This is useful for computing density measures and the total acquisition time:

$$D = \alpha K \left(\frac{\lambda}{2\mathrm{NA}} \right) \quad T = \left(\Delta_{SR} + \frac{2N}{\log(1-\alpha)} \right)^2$$

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
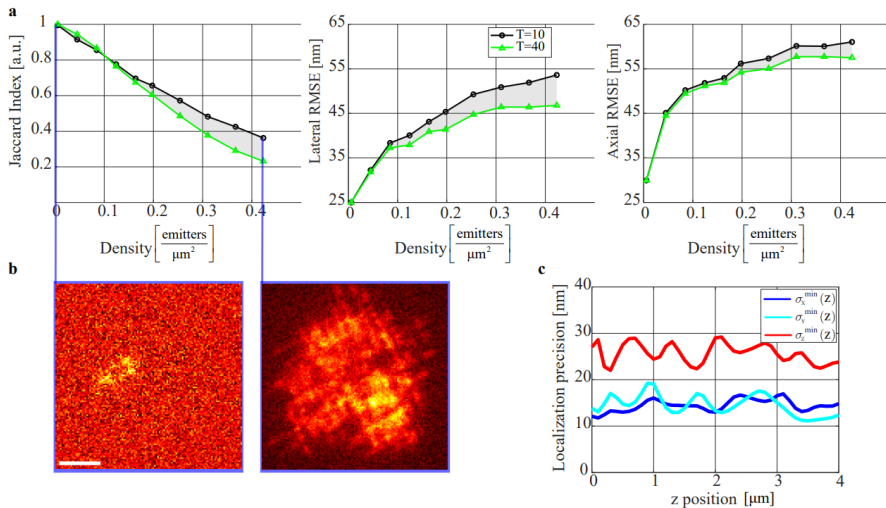
Results

Diffusion of H2B-JF549 depends on local nucleosome organization