

# Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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# Introduction

# TO DO

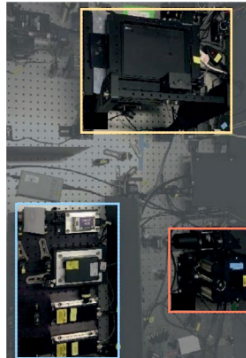
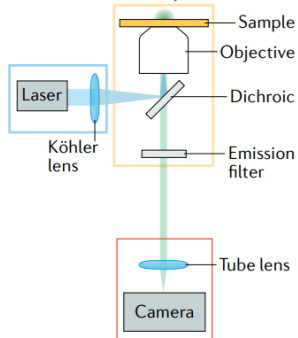
- ▶ Figure 1b:  $k_1, k_2$  inference (Monday)
- ▶ Figure 1c,d: Performance comparison of CNN with LoG algorithm (Tuesday-Thursday)
- ▶ Implement the Dataset object - a universal data object for simulated and experimental data. It can have an arbitrary shape, with axes for different conditions, for example. Ground truth information is attached to the object
- ▶ Hardware autofocus (focus drifts after 5-10 seconds)
- ▶ Precise exposure control (using an Arduino)
- ▶ Optimizing two-color labeling
- ▶ Setting up CO<sub>2</sub>
- ▶ Later: Caged diffusion dynamics (Singh 2018, Ashwin 2019)

# Methods

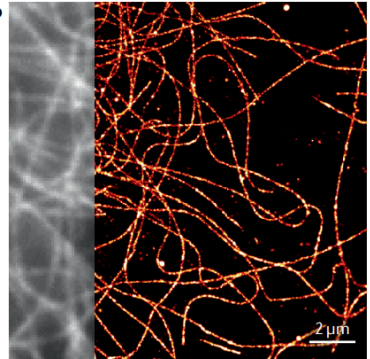
# Direct stochastic optical reconstruction microscopy

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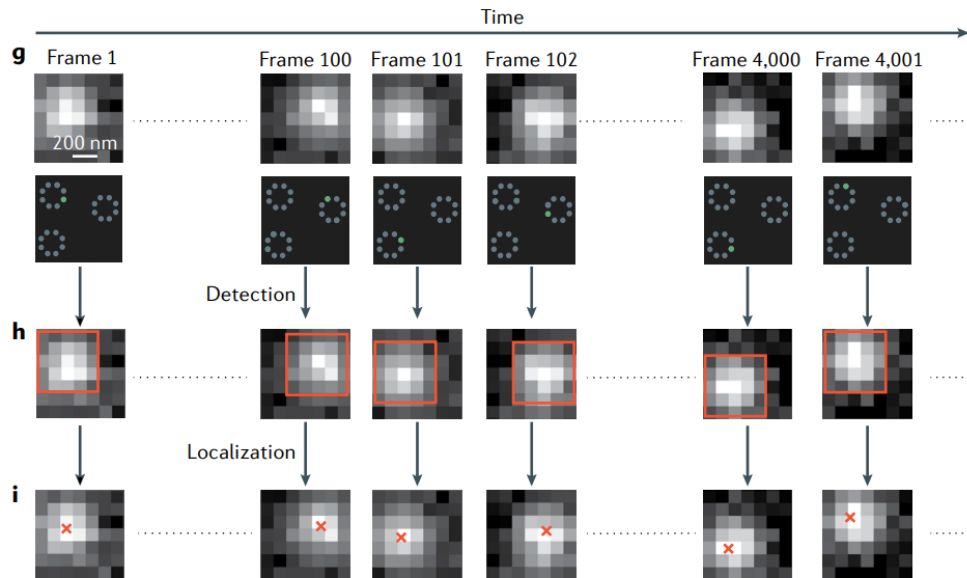
**a Basic SMLM set-up**



**b**

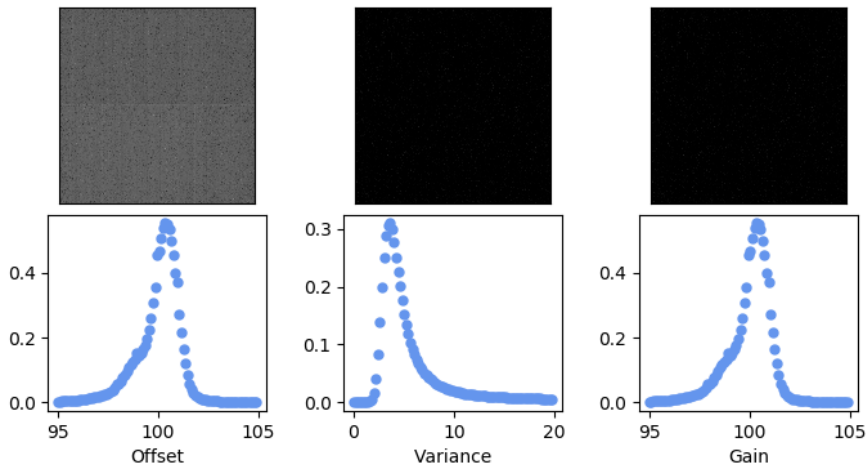


# Direct stochastic optical reconstruction microscopy



# Readout noise of sCMOS cameras

Hamamatsu ORCA v3 CMOS, air cooled to -10C



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



# Maximum likelihood localization of an isolated fluorescent emitter

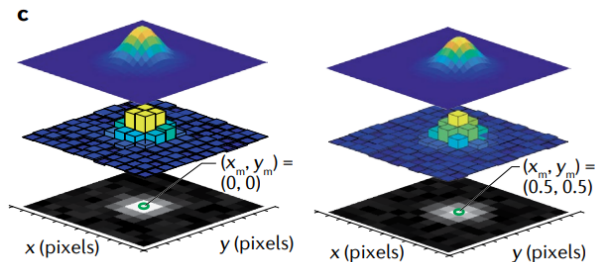
Localization:  $\theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$

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

$\eta$  – quantum efficiency

$N_0$  – emission rate

$\Delta$  – exposure time

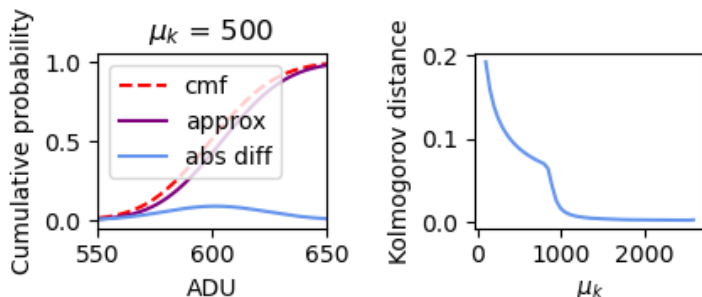


$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi\sigma_k}} e^{-\frac{(H_k - g_k q - o_k)^2}{2\sigma_k^2}}$$

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

## Quality of the Poisson approximation depends on SNR

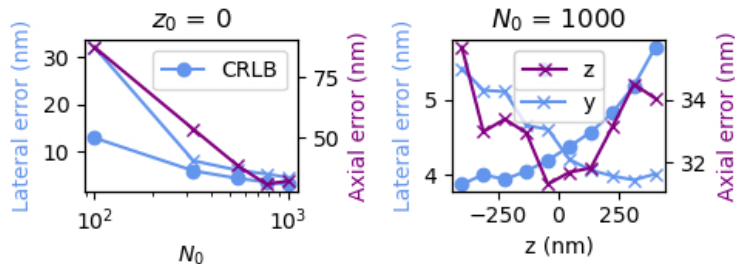
$P(H_k|\theta) \approx \text{Poisson}(\mu_k + \sigma_k^2)$  for  $N_0 > 500$  assuming  $\Delta = 100\text{ms}$



Using the approximation we can write

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

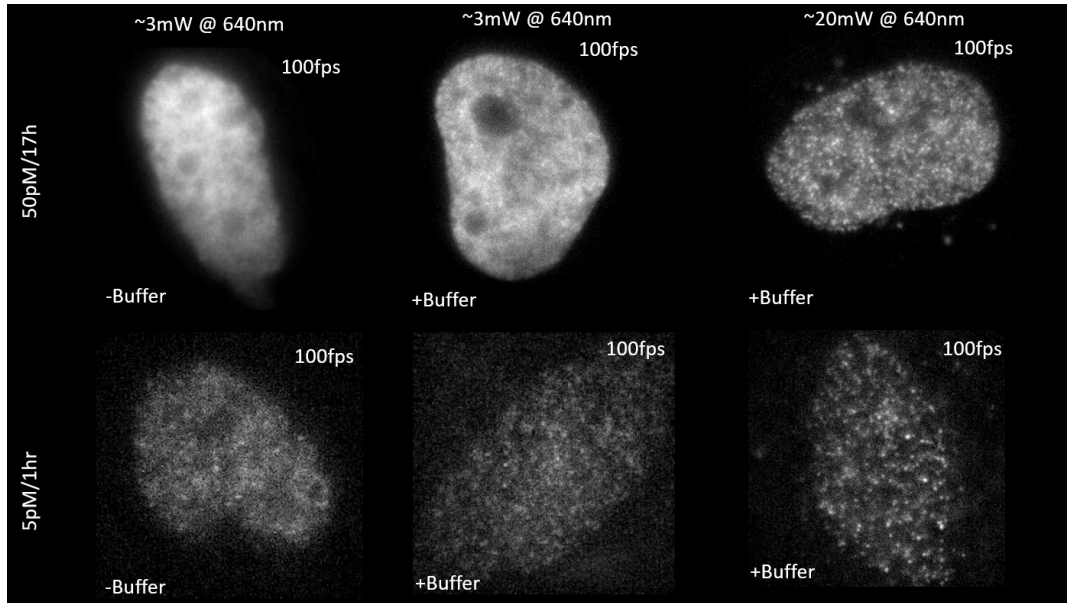
# Estimator precision sets the resolution limit in localization microscopy



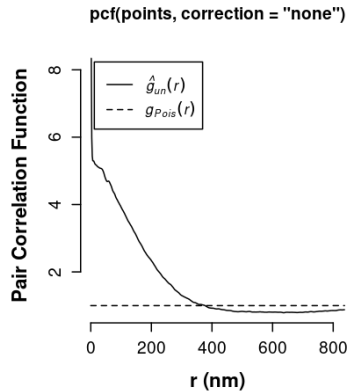
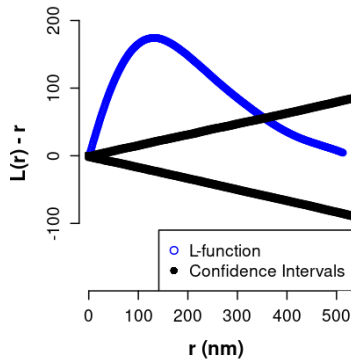
- MLE can approach the CRLB on simulated isolated emitter data



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

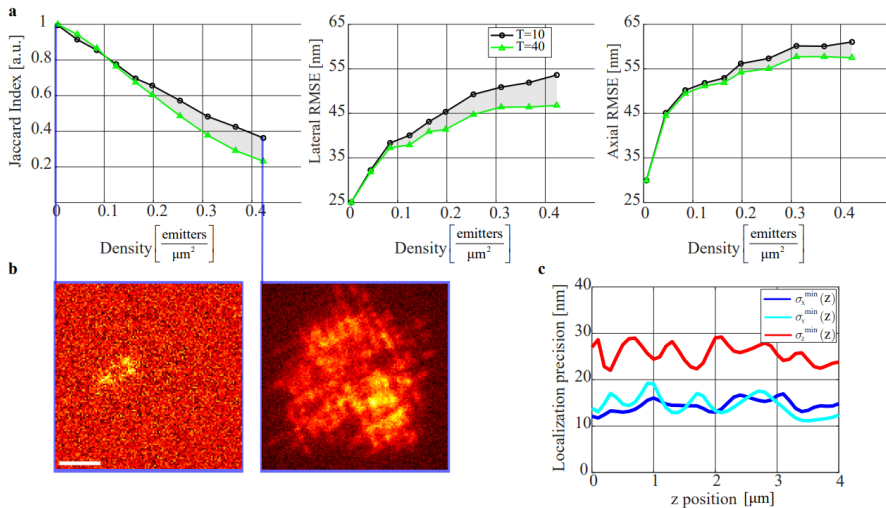
$$\alpha = \int_{\delta}^{\Delta} \left( \sum_{n=0}^{\infty} Q(N=n) \psi(\tau|n; \vec{k}) \right) d\tau \approx \mathbb{E}_{\tau \sim P(\tau)} (\mathbb{I}[\tau > \delta])$$

$P(\tau)$  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

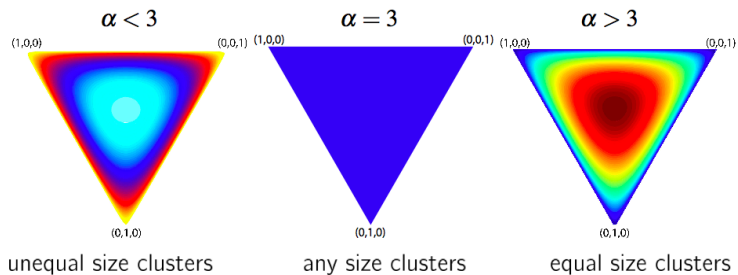
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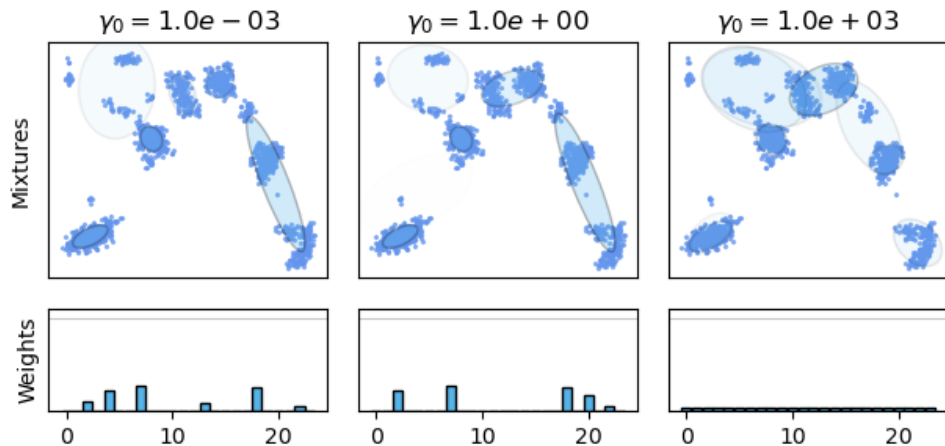


Deep learning enables accurate 3D localization and single molecule tracking

# Dirichlet process Gaussian mixture model (DPGMM)



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## Results

# GMM cluster analysis of H2B

Number of clusters is unknown apriori - Bayesian nonparametrics

# Mesoscale nucleosome organization and dynamics

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BRD4 associates with the small clusters

Besag's L-Function



# Besag's L-Function and 3D diffusion