Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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Introduction

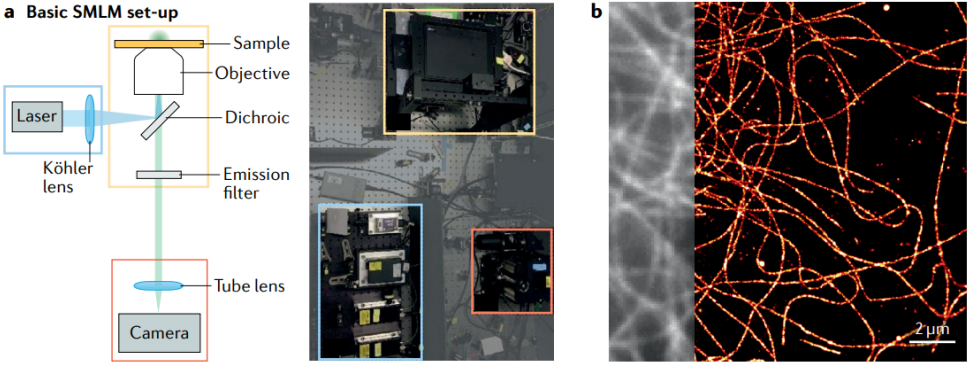
TO DO

- U2OS two-color test (if JF549 looks good, take long movies)
- Hela long movie test for 10pM, 20pM, 50pM JF646 and 1pM and 5pM JF549
- Clustering analysis - assess point accumulation with time
- Performance comparison of CNN with LoG algorithm
- Later: Caged diffusion dynamics (Singh 2018, Ashwin 2019)

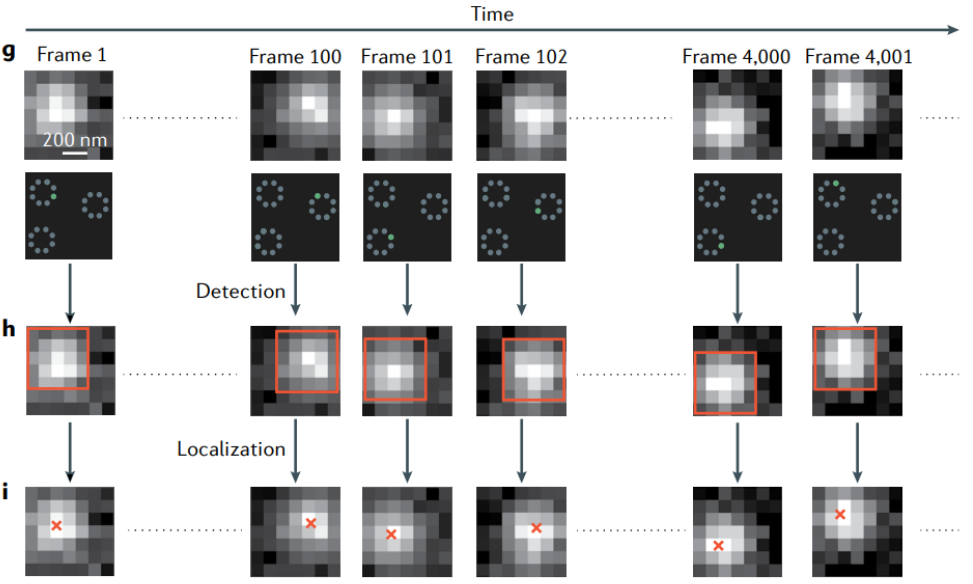
Methods

Direct stochastic optical reconstruction microscopy

Direct stochastic optical reconstruction microscopy

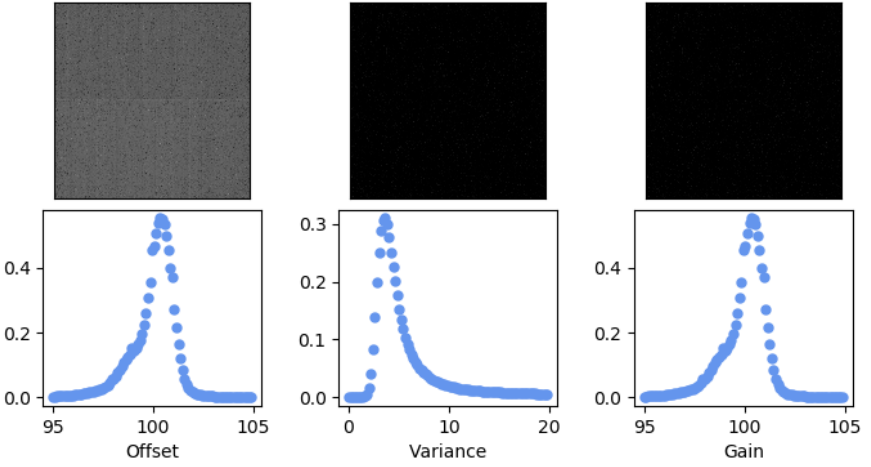


Direct stochastic optical reconstruction microscopy



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

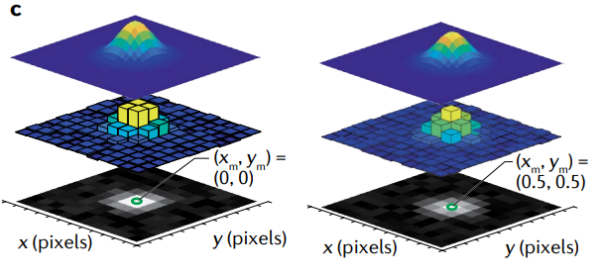
Localization: $\theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$

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

η – quantum efficiency

N_0 – emission rate

Δ – exposure time

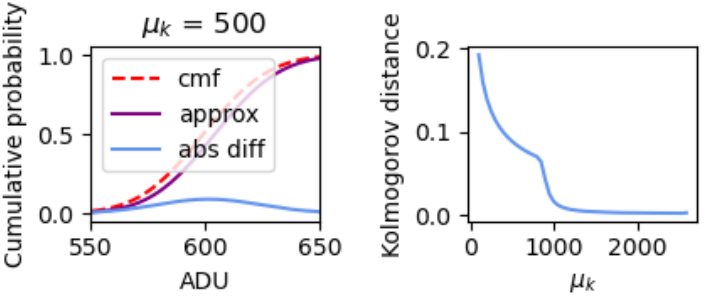


$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi}\sigma_k} e^{-\frac{(H_k - g_k q - o_k)}{2\sigma_k^2}}$$

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

Quality of the Poisson approximation depends on SNR

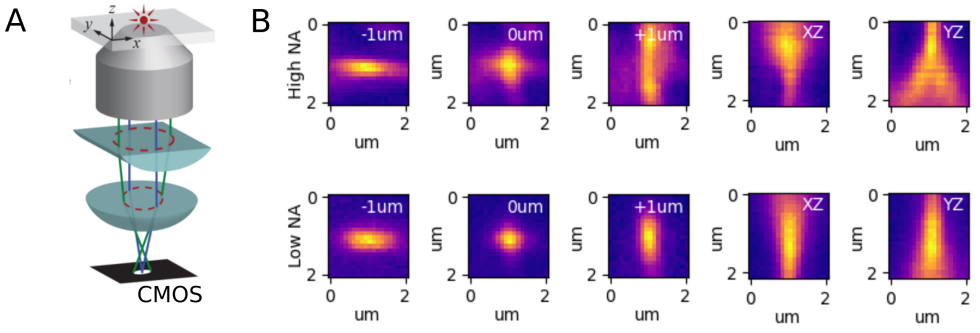
$P(H_k|\theta) \approx \mathrm{Poisson}(\mu_k + \sigma_k^2)$ for $N_0 > 500$ asssuming $\Delta = 100$ms



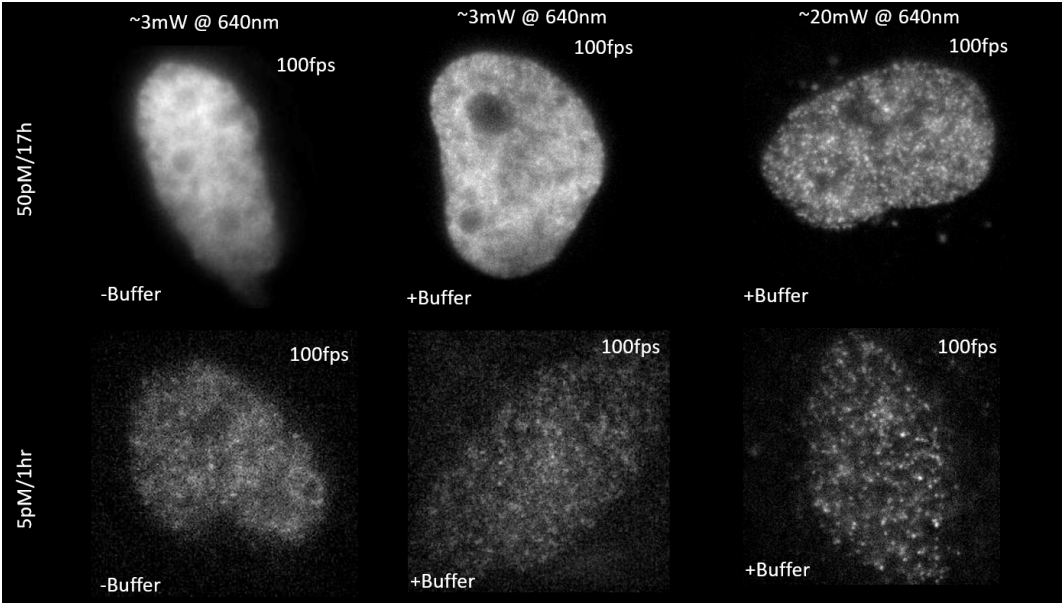
Using the approximation we can write

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

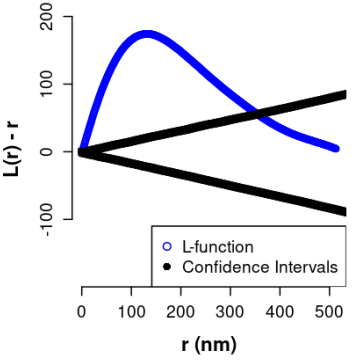
Estimator precision sets the resolution limit in localization microscopy

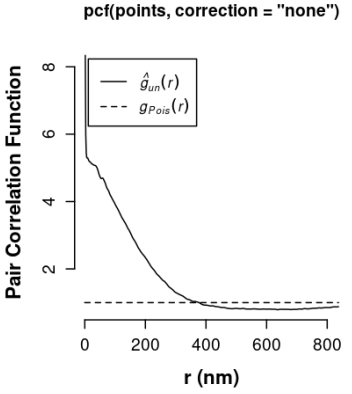


The metastable OFF state can be maintained with high laser power



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.

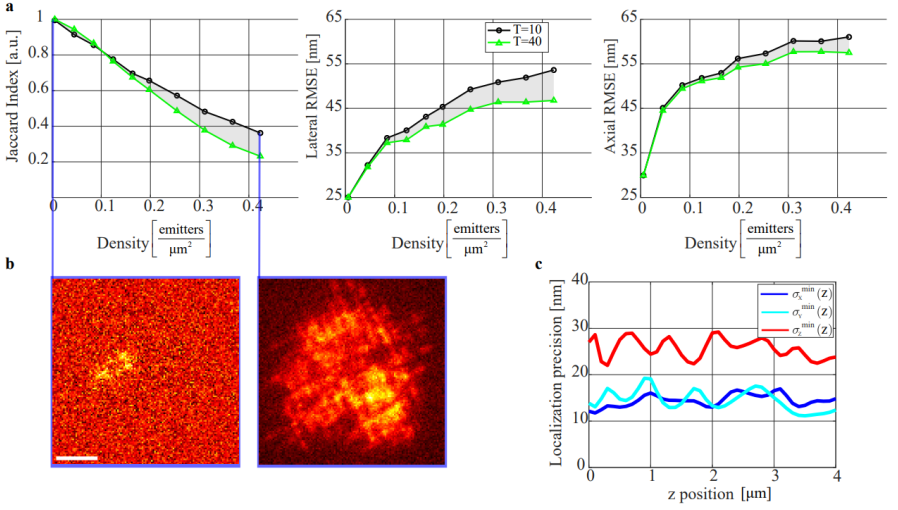
$$\alpha = \int_{\delta}^{\Delta} \left(\sum_{n=0}^{\infty} Q(N=n)\psi(\tau|n;\vec{k}) \right) d\tau \approx \mathop{\mathbb{E}}_{\tau \sim P(\tau)} (\mathbb{I}[\tau > \delta])$$

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

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

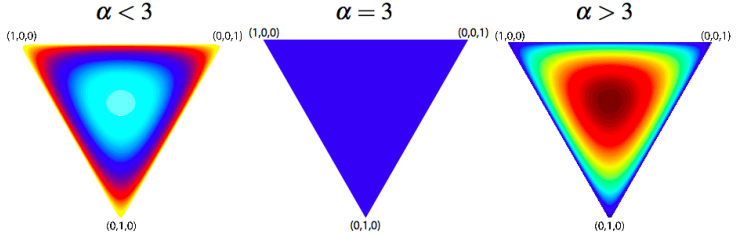
For actually inferring k_1, k_2, we need a measure of distance between $P(\tilde{s})$ and $P(s|k_1, k_2)$ for many k_1, k_2 pairs. Luckily we only need to compute $P(s|k_1, k_2)$ once, and we can then perform a grid search

Resolution is dependent on photoswitching kinetics



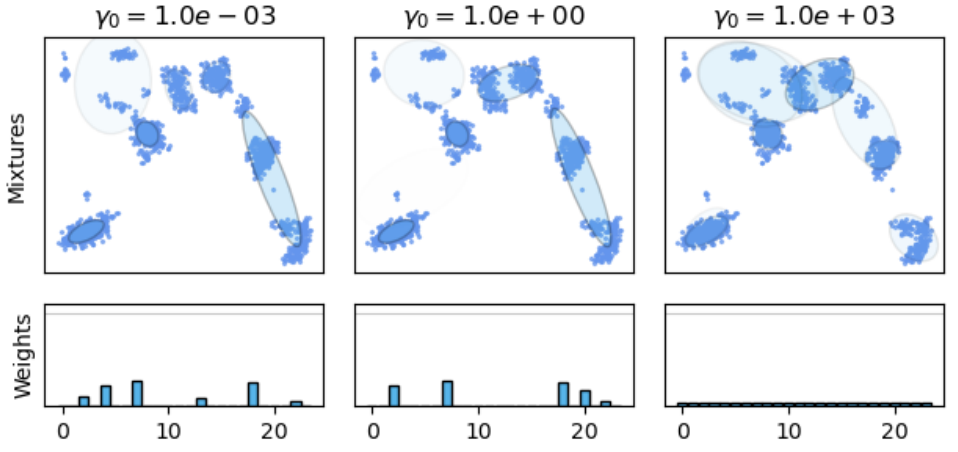
Deep learning enables accurate 3D localization and single molecule tracking

Dirichlet process Gaussian mixture model (DPGMM)



unequal size clusters | any size clusters | equal size clusters

Dirichlet process Gaussian mixture model (DPGMM)



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