Bridging Mesoscale Nucleosome Organization and Dynamics with Super Resolution Microscopy

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Introduction

TO DO

- α-distribution, acquisition time scaling with α
- Deep learning approach for dense 2D and sparse 3D localization (see example figure)
- Tuning the GMM and cluster statistics
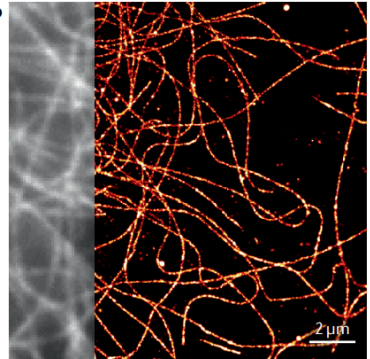- Caged diffusion dynamics (Singh 2018, Ashwin 2019)

Methods

Direct stochastic optical reconstruction microscopy

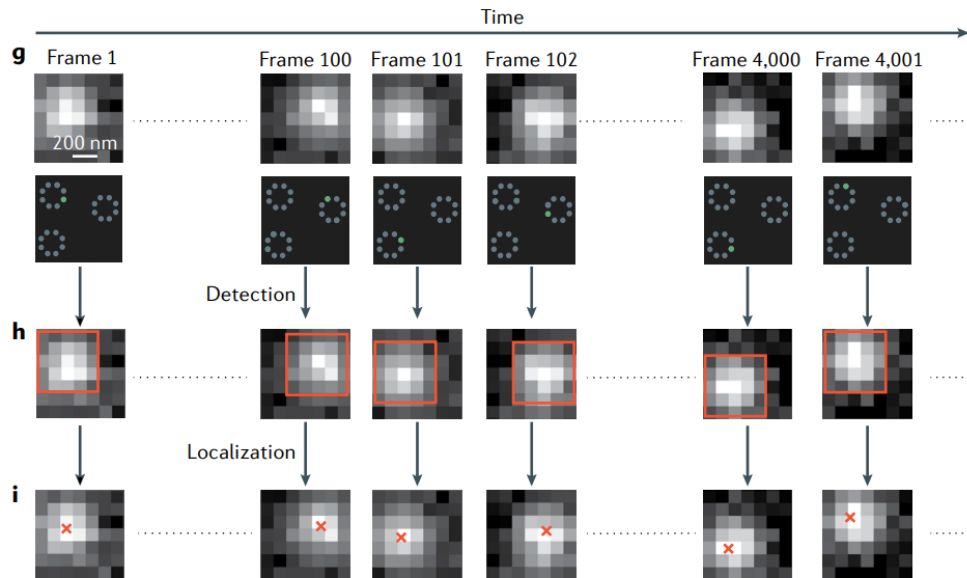# Direct stochastic optical reconstruction microscopy
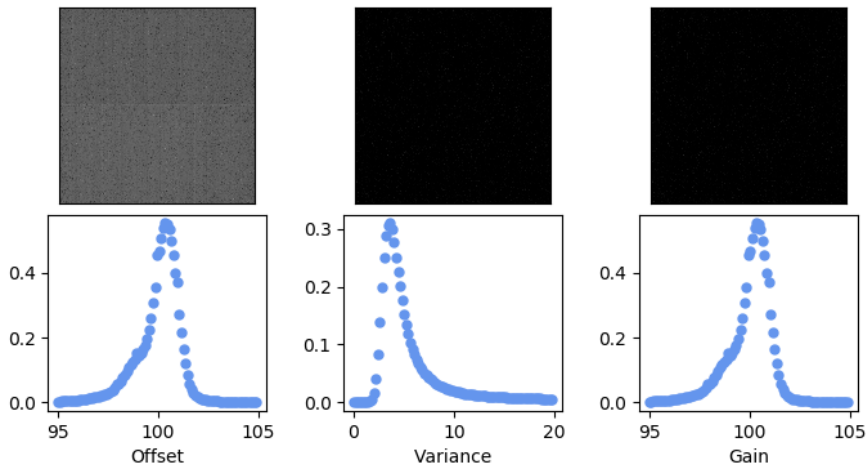

a Basic SMLM set-up
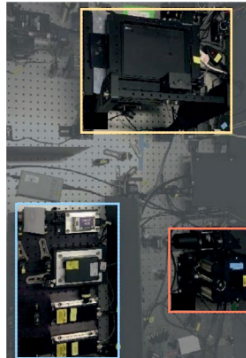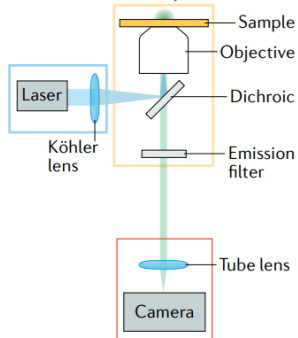
b

Direct stochastic optical reconstruction microscopy

Readout noise of sCMOS cameras

Hamamatsu ORCA v3 CMOS, air cooled to -10C

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

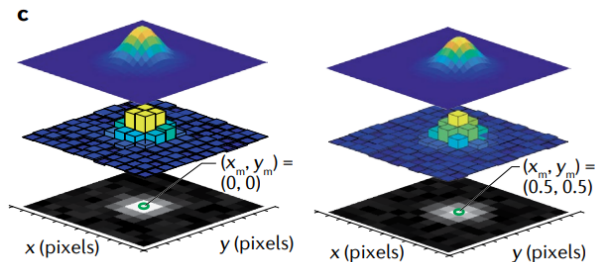# Maximum likelihood localization of an isolated fluorescent emitter

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
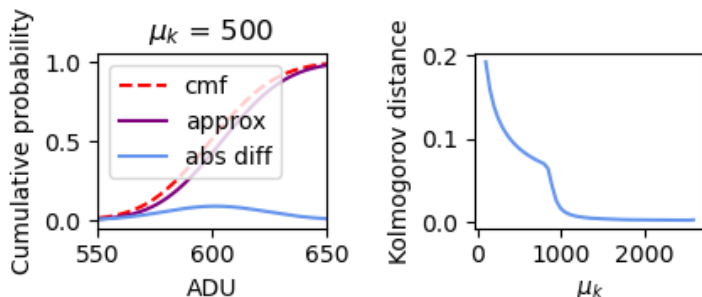
N_0 — emission rate

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)

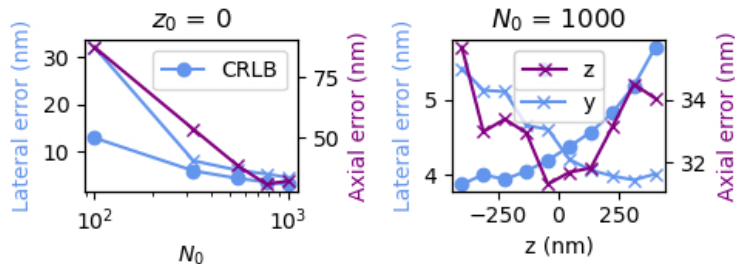# Quality of the Poisson approximation depends on SNR

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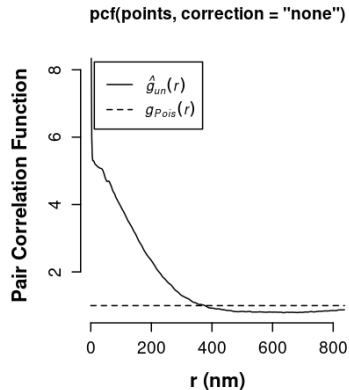

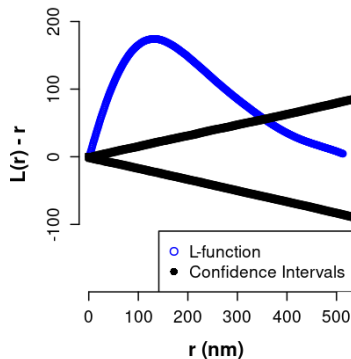Using the approximation we can write

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

Estimator precision sets the resolution limit in localization microscopy

▶ MLE can approach the CRLB on simulated isolated emitter data

Resolution is dependent on photoswitching kinetics

pcf(points, correction = "none")

▶ MLE can approach the CRLB on simulated isolated emitter data

Resolution is dependent on photoswitching kinetics

Define α as the *detection probability* with threshold $\delta << \Delta$

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

The number of molecules within the diffraction limit is $K\left(\frac{\lambda}{2\mathrm{NA}}\right)$. then $\alpha K\left(\frac{\lambda}{2\mathrm{NA}}\right)$ are detected, on average. Higher density gives more errors but decreases the SR frame duration Δ_{SR}. Thus we define
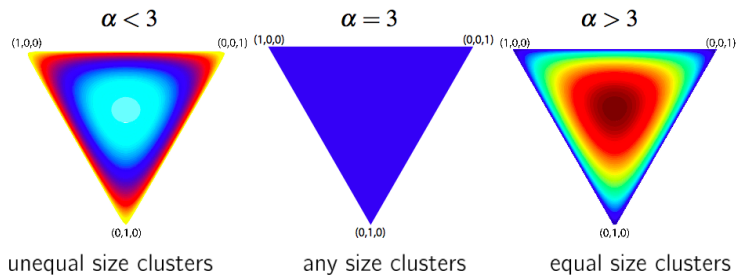
$$D = \alpha K \left(\frac{\lambda}{2\mathrm{NA}} \right) \quad T = \left(\Delta_{SR} + \frac{2N}{\log(1 - \alpha)} \right)^2$$

Resolution is dependent on photoswitching kinetics
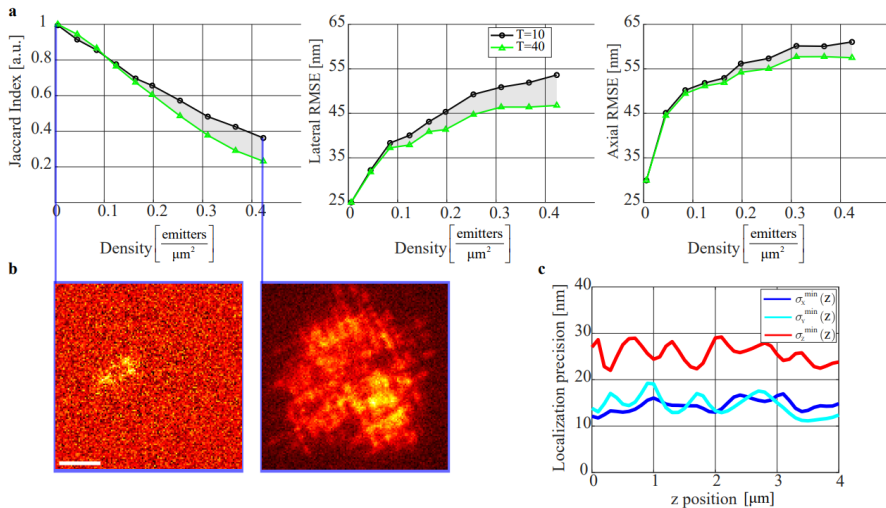
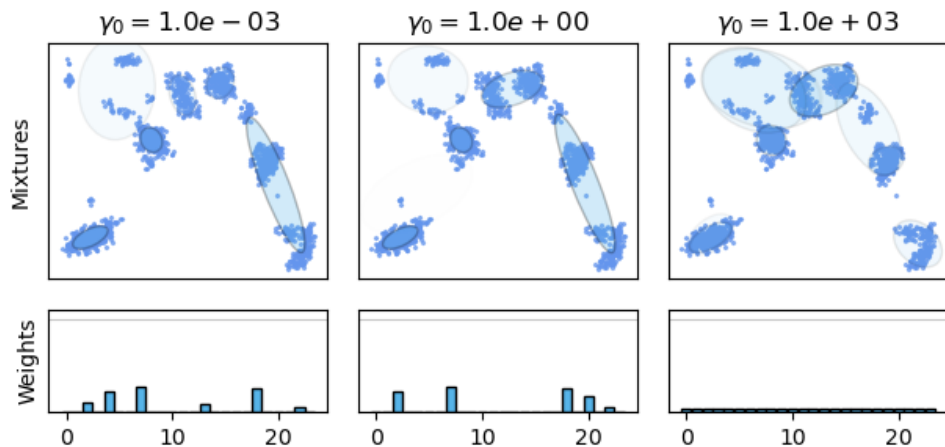# Deep learning enables accurate 3D localization and single molecule tracking

Dirichlet process Gaussian mixture model (DPGMM)

$\alpha < 3$ — unequal size clusters

$\alpha = 3$ — any size clusters

$\alpha > 3$ — 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