

Bridging Mesoscale Nucleosome Organization and Dynamics with Super Resolution Microscopy

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Introduction

Genome organization in eukaryotes

- ▶ The eukaryotic genome has hierarchical structure
- ▶ This structure is highly variable and often aberrant in disease

Finn et al., Science 365, 998 (2019)

A phase separation model for transcriptional control

- ▶ Liquid-liquid phase separation (LLPS) is a major organizer of cellular biochemistry
- ▶ Recent work highlights the importance of CTCF-dependent transcriptional condensates in determining cell fates

Int. J. Mol. Sci. 2022, 23(14), 8039;

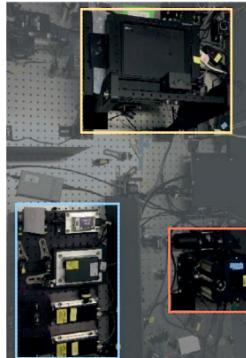
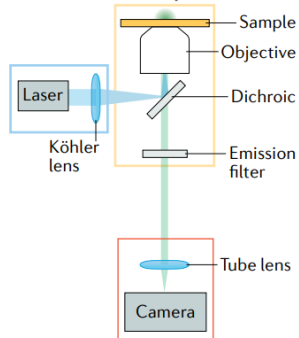
Formulate the basic research question and introduce the approach using major results from section 3

Methods

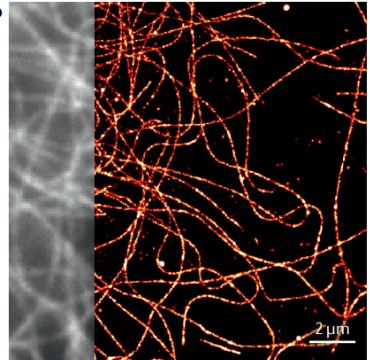
Direct stochastic optical reconstruction microscopy

Direct stochastic optical reconstruction microscopy

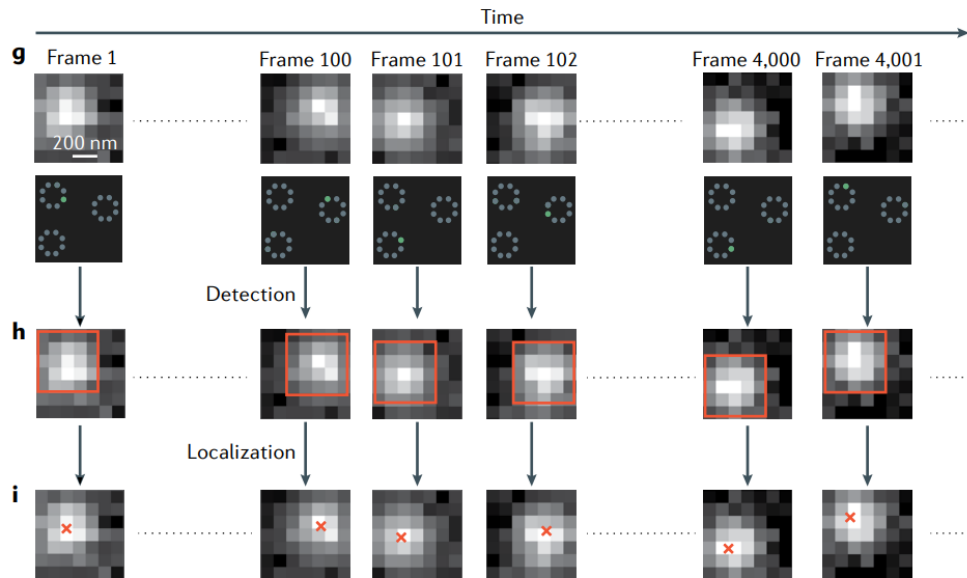
a Basic SMLM set-up



b

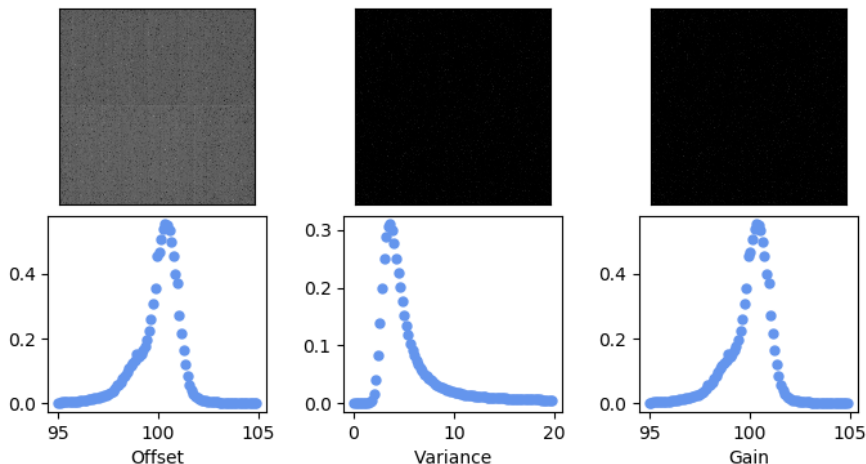


Direct stochastic optical reconstruction microscopy



Readout noise of sCMOS cameras

Hamamatsu ORCA v3 CMOS, air cooled to -10C



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

Maximum likelihood localization of an isolated fluorescent emitter

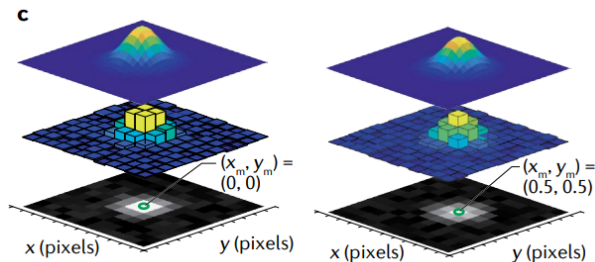
Localization: $\theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$

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

η – quantum efficiency

N_0 – emission rate

Δ – exposure time

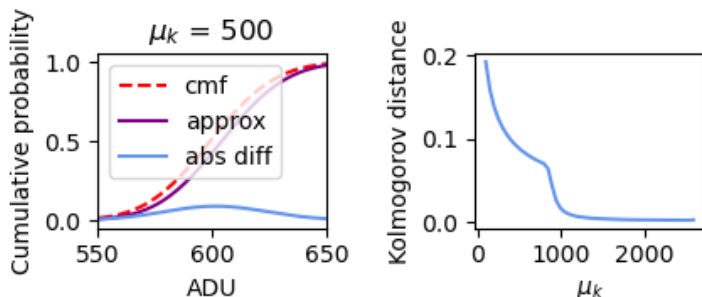


$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi\sigma_k}} e^{-\frac{(H_k - g_k q - o_k)^2}{2\sigma_k^2}}$$

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

Quality of the Poisson approximation depends on SNR

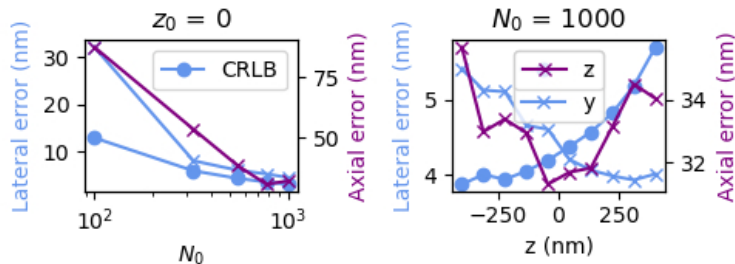
$P(H_k|\theta) \approx \text{Poisson}(\mu_k + \sigma_k^2)$ for $N_0 > 500$ assuming $\Delta = 100\text{ms}$



Using the approximation we can write

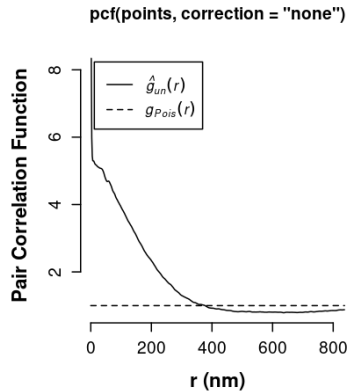
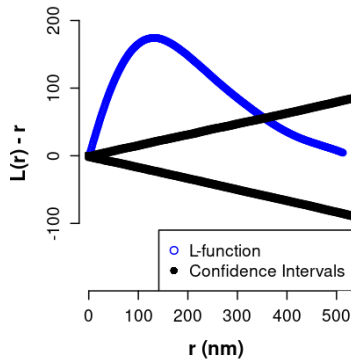
$$\ell(\vec{H}|\theta) = -\log \prod_k \frac{e^{-(\mu'_k)} (\mu'_k)^{n_k}}{n_k!} = \sum_k \log n_k! + \mu'_k - n_k \log (\mu'_k)$$

Estimator precision sets the resolution limit in localization microscopy



- MLE can approach the CRLB on simulated isolated emitter data

Resolution is dependent on photoswitching kinetics



- MLE can approach the CRLB on simulated isolated emitter data

Resolution is dependent on photoswitching kinetics

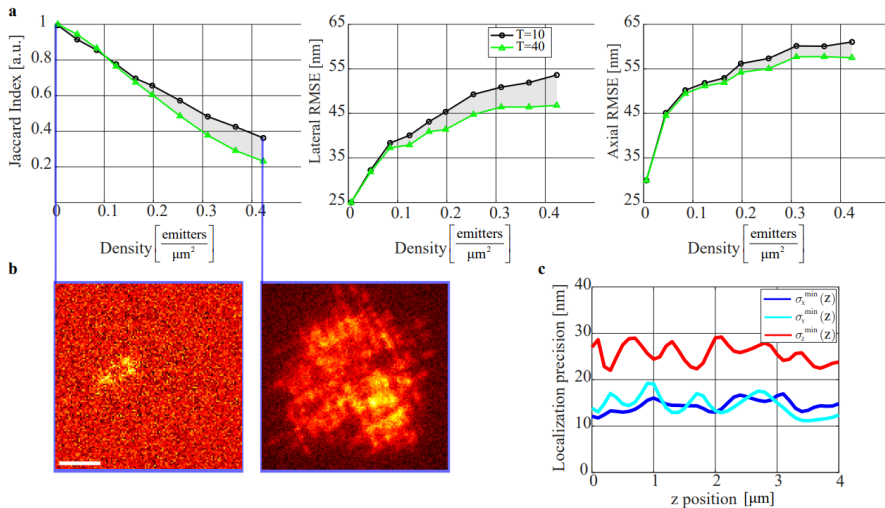
Define α as the *detection probability* with threshold $\delta \ll \Delta$

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

The number of molecules within the diffraction limit is $K \left(\frac{\lambda}{2\text{NA}} \right)$. then $\alpha K \left(\frac{\lambda}{2\text{NA}} \right)$ are detected, on average. Higher density gives more errors but decreases the SR frame duration Δ_{SR} . Thus we define

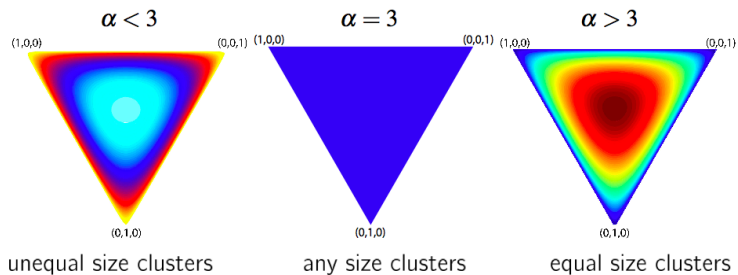
$$D = \alpha K \left(\frac{\lambda}{2\text{NA}} \right) \quad T = \left(\Delta_{SR} + \frac{2N}{\log(1-\alpha)} \right)^2$$

Resolution is dependent on photoswitching kinetics

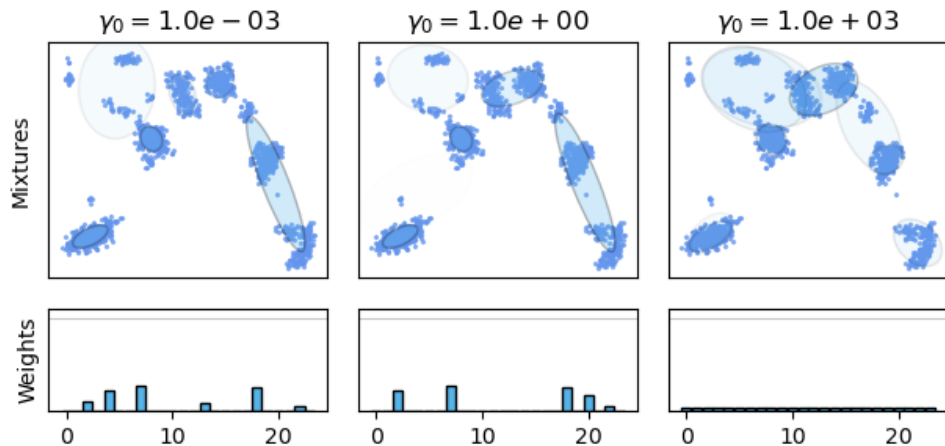


Deep learning enables accurate 3D localization and single molecule tracking

Dirichlet process Gaussian mixture model (DPGMM)



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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