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 abberrant 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

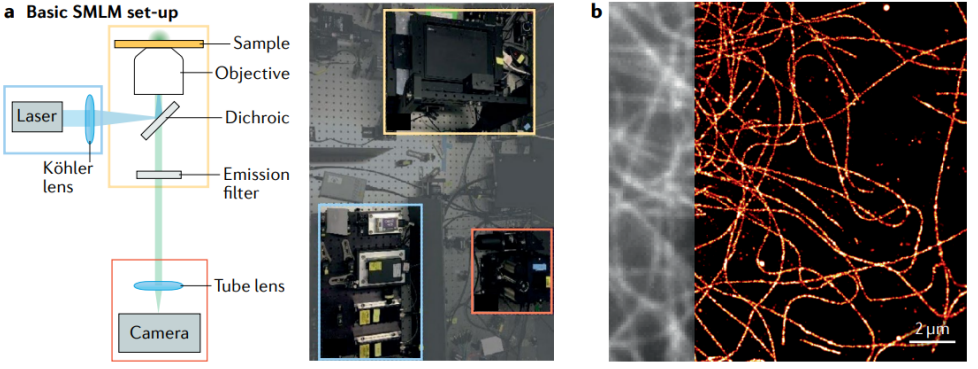
Int. J. Mol. Sci. 2022, 23(14), 8039;

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

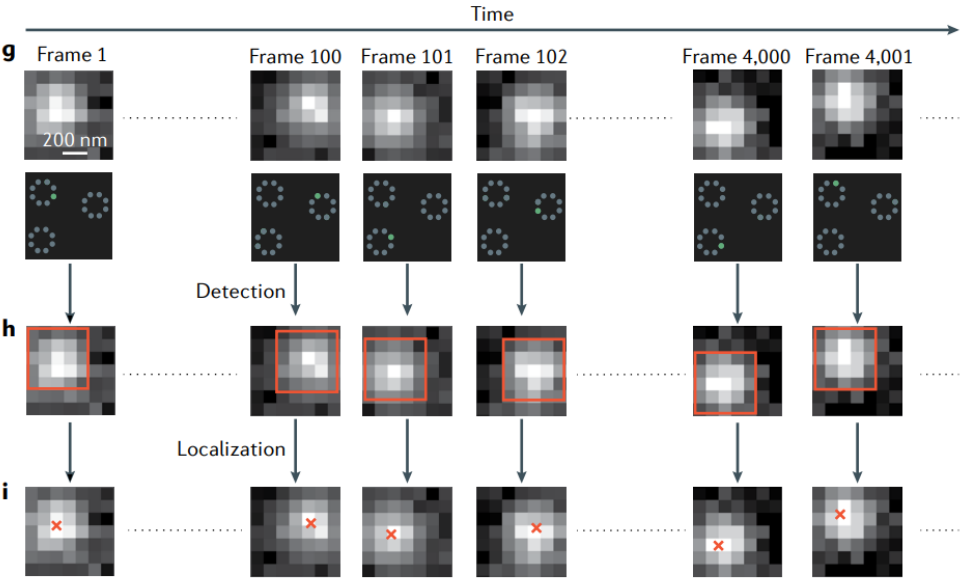
Methods

Direct stochastic optical reconstruction microscopy

Direct stochastic optical reconstruction microscopy

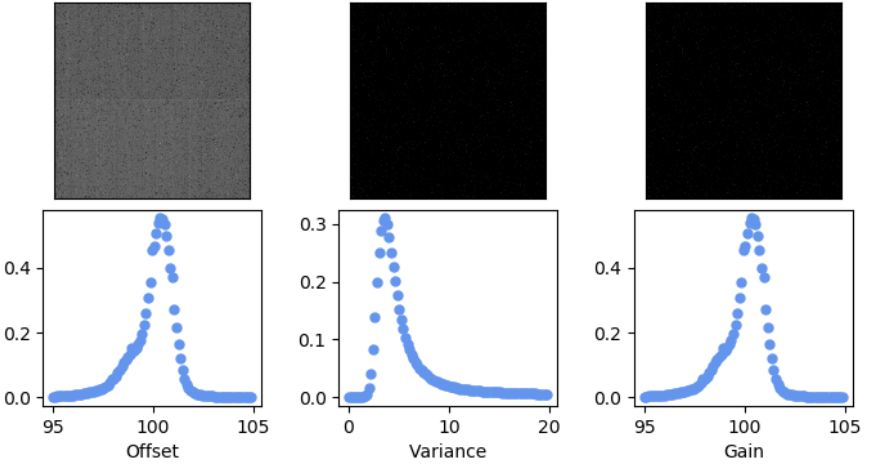


Direct stochastic optical reconstruction microscopy



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

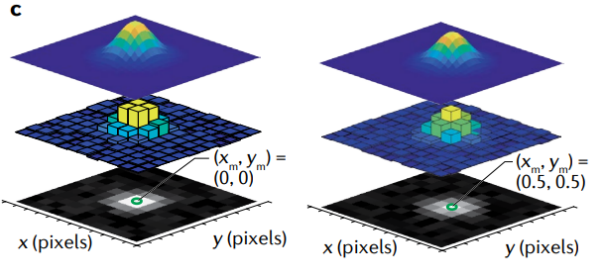
Localization: $\theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$

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

η – quantum efficiency

N_0 – emission rate

Δ – exposure time

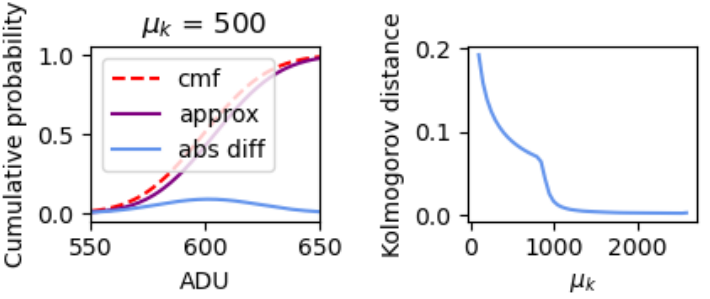


$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi}\sigma_k} e^{-\frac{(H_k - g_k q - o_k)}{2\sigma_k^2}}$$

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

Quality of the Poisson approximation depends on SNR

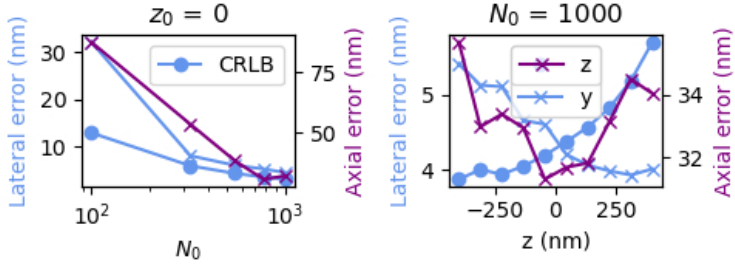
$P(H_k|\theta) \approx \mathrm{Poisson}(\mu_k + \sigma_k^2)$ for $N_0 > 500$ asssuming $\Delta = 100$ms



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

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

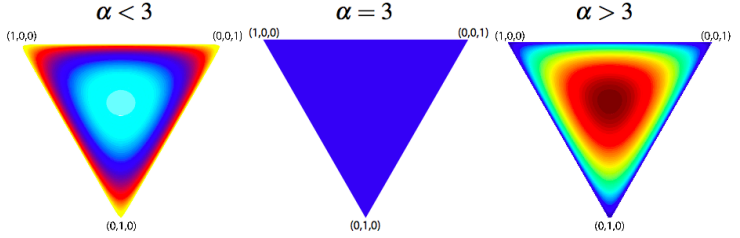
$$\alpha = \int_{\delta}^{\Delta} \left(\sum_{n=0}^{\infty} Q(N=n)\psi(t|n;\vec{k}) \right) dt \approx \mathop{\mathbb{E}}_{t\sim P}(\mathbb{I}[t>\delta])$$

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

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

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

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