Bridging Mesoscale Nucleosome Organization and Dynamics with Super Resolution Microscopy

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Introduction and Approach

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

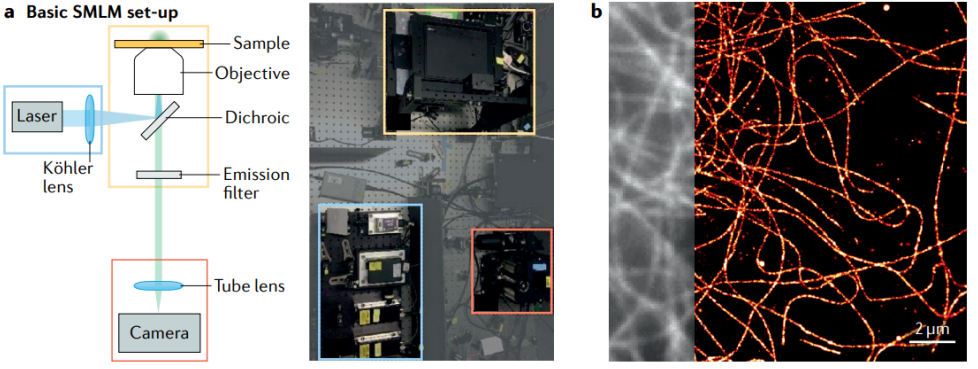
- Liquid-liquid phase separation (LLPS) is a major organizer of cellular biochemistry
- Recent work highlights the importance of CTCF-dependent transcriptional condensates in determining cell fates

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

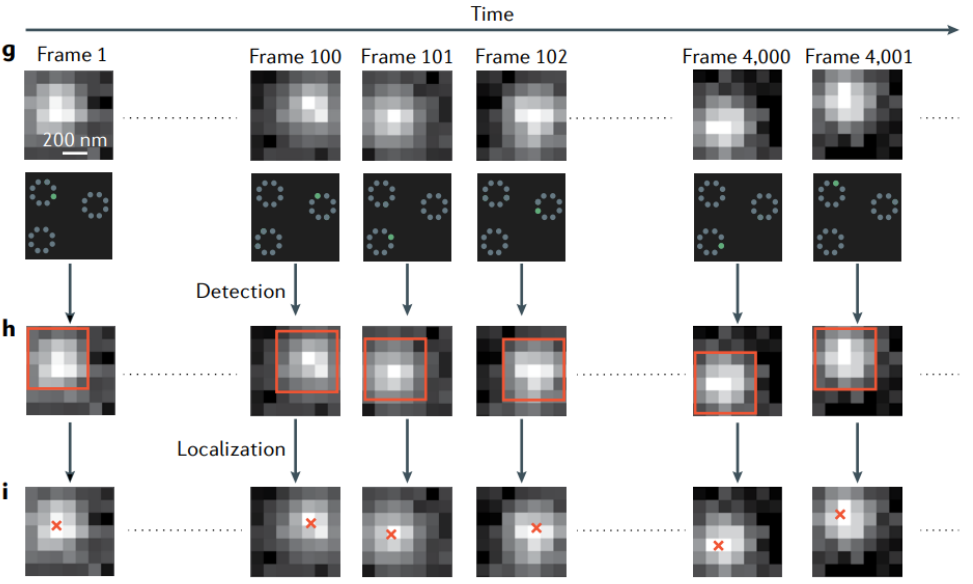
Formulate the basic research question and introduce the approach using major results from section 3

Direct stochastic optical reconstruction microscopy

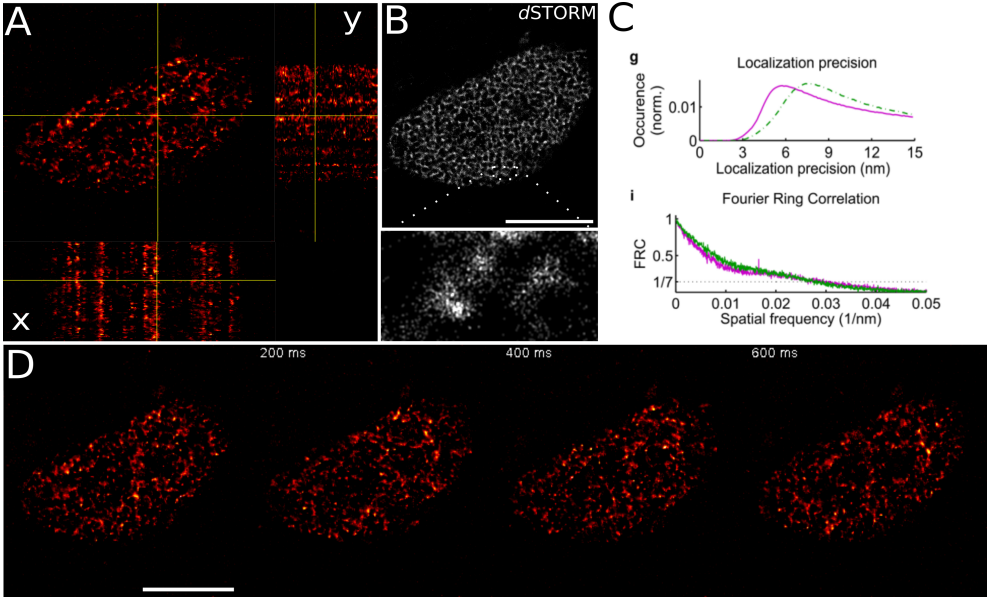
Direct stochastic optical reconstruction microscopy



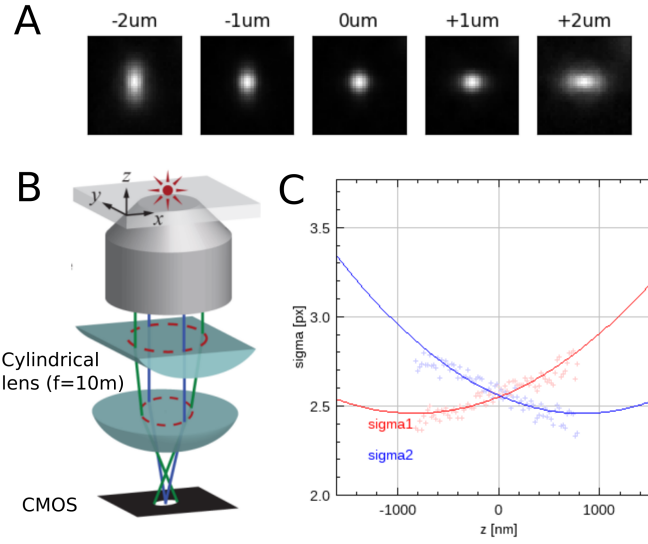
Direct stochastic optical reconstruction microscopy



Super-Resolution imaging of H2B in living Hela cells

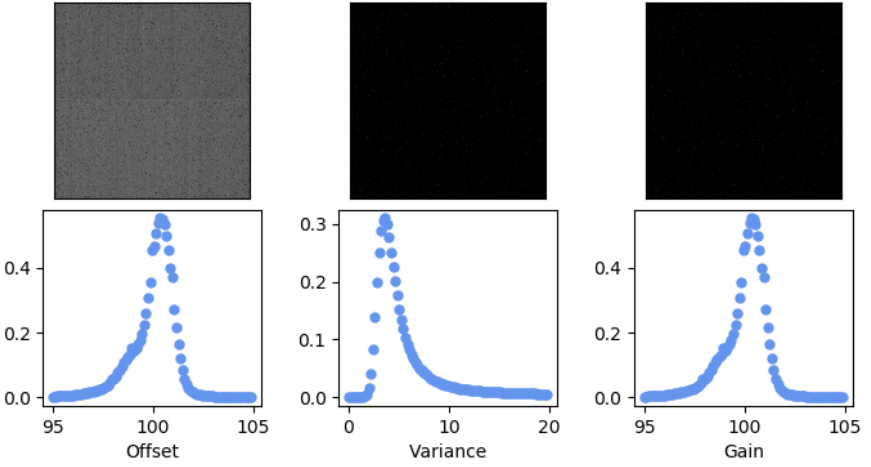


Point spread function engineering for three-dimensional imaging



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

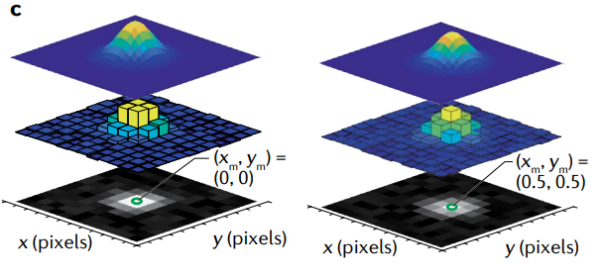
Localization: $\theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$

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

η – quantum efficiency

N_0 – emission rate

Δ – exposure time

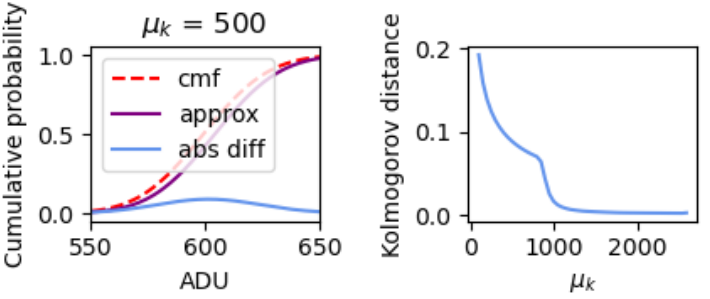


$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi}\sigma_k} e^{-\frac{(H_k - g_k q - o_k)}{2\sigma_k^2}}$$

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

Quality of the Poisson approximation depends on SNR

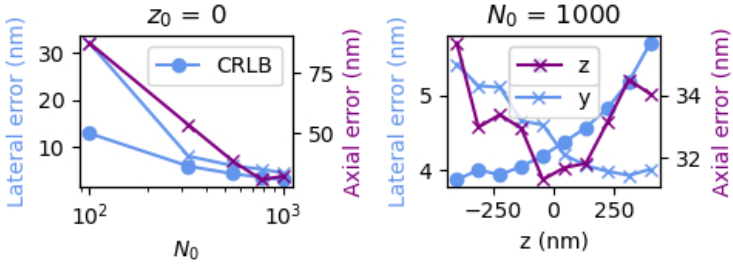
$P(H_k|\theta) \approx \mathrm{Poisson}(\mu_k + \sigma_k^2)$ for $N_0 > 500$ asssuming $\Delta = 100$ms



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

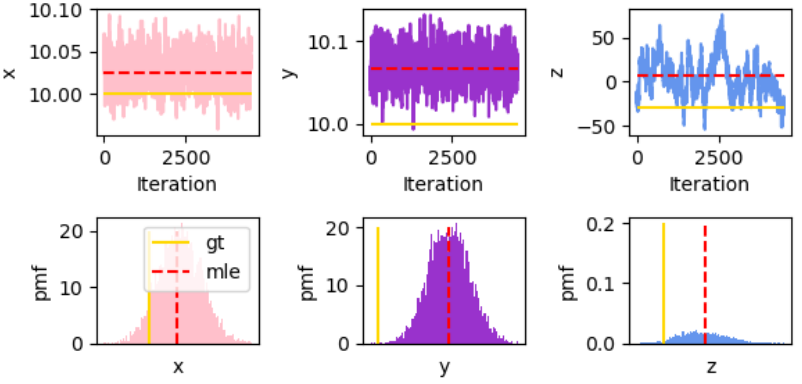


- MLE can approach the CRLB on simulated isolated emitter data
- MLE does not generalize well to dense time-series

Estimating uncertainty with gradient-based MCMC

Stochastic gradient langevin dynamics (SGLD):

$$dw = -\nabla \ell(\theta) dt + \epsilon \sqrt{\eta dt}, \quad \epsilon \sim \mathcal{N}(0, \sigma^2), \eta \propto dt$$



The diffusion samples from the posterior $P(\theta|\vec{H})$ as $t \to \infty$

Photoswitching kinetics of Janelia-Fluor 646 in thiol buffer

Resolution is dependent on photoswitching kinetics

The number of molecules within the diffraction limit is $K\left(\frac{\lambda}{2\mathrm{NA}}\right)$. If α is the *detection probability*, then $\alpha K\left(\frac{\lambda}{2\mathrm{NA}}\right)$ are detected, on average. We want to minimize

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

The second term contains $\frac{2N}{\log(1-\alpha)}$, which is the minimum number of frames needed to detect 99 percent of N molecules (which can be obtained from the geometric distribution). If we assume a two-state generator, then

$$P(t) = P(0)e^{Gt}$$

and $G\pi = 0$ gives $\pi = (\alpha, \beta) = \frac{1}{k}(k_{12}, k_{21})$ where $k = k_{12} + k_{21}$.

Deep learning enables accurate 3D localization and single molecule tracking

Mesoscale nucleosome organization and dynamics

Dirichlet process Gaussian mixture model (DPGMM)

GMM cluster analysis of H2B

Number of clusters is unknown apriori - Bayesian nonparametrics

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BRD4 associates with the small clusters

Besag's L-Function

Besag's L-Function and 3D diffusion