Probing phase transitions of DNA-protein condensates using single molecule localization microscopy

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June 22, 2023

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

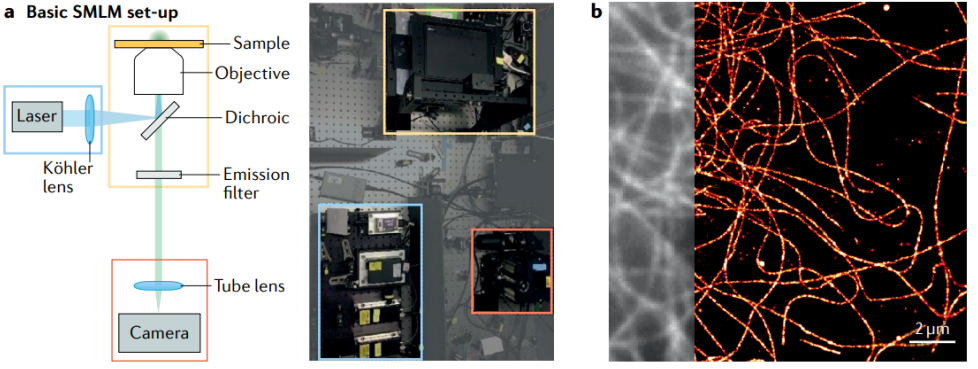
Int. J. Mol. Sci. 2022, 23(14), 8039;

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

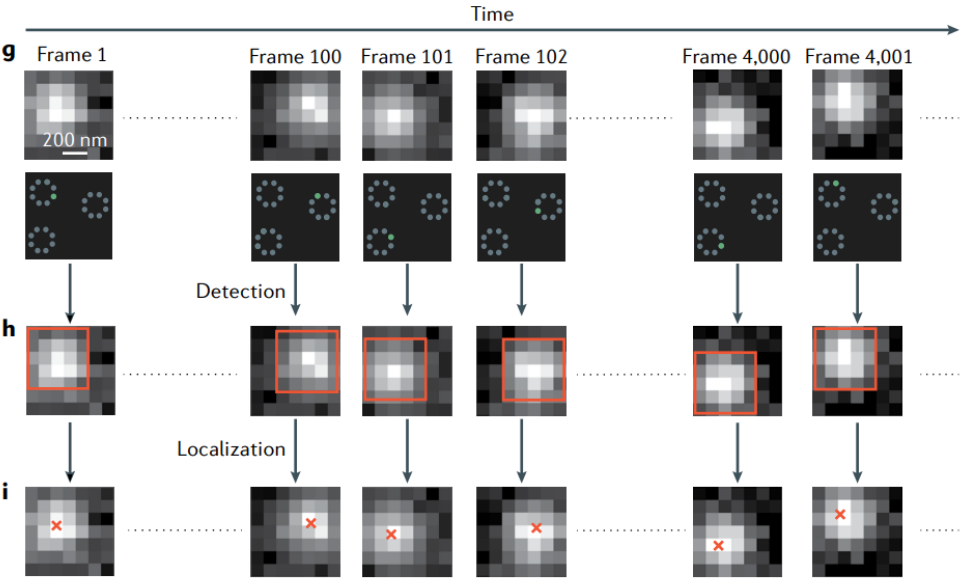
Widefield/HILO fluorescence microscope

Direct stochastic optical reconstruction microscopy

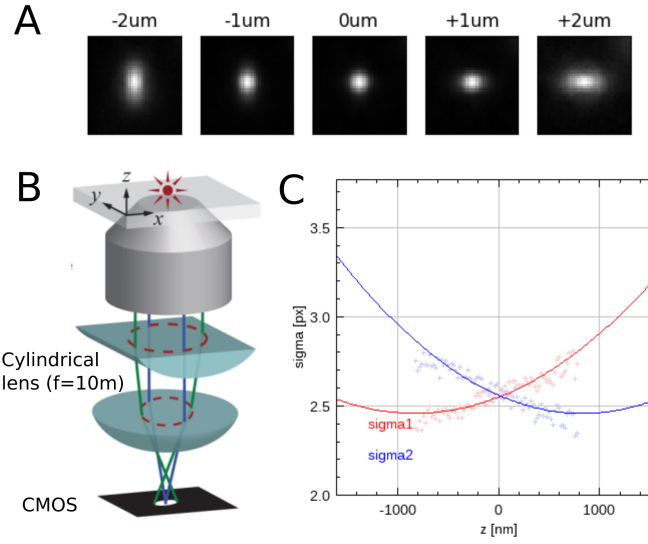
Direct stochastic optical reconstruction microscopy



Direct stochastic optical reconstruction microscopy



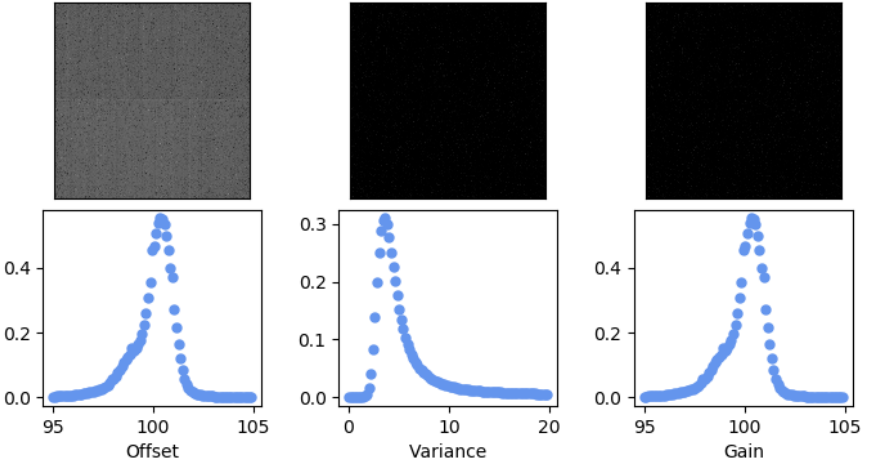
Point spread function engineering for three-dimensional imaging



Generative models and inference for SMLM

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

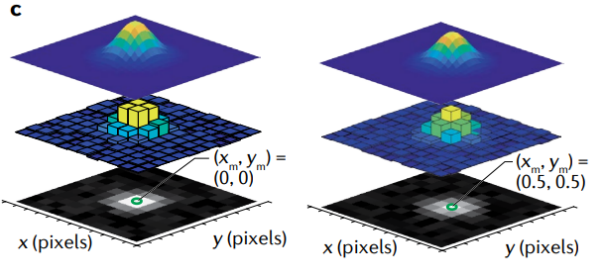
Localization: $\theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$

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

η – quantum efficiency

N_0 – emission rate

Δ – exposure time

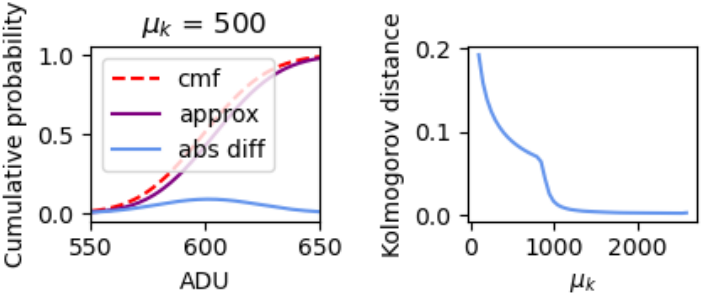


$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi}\sigma_k} e^{-\frac{(H_k - g_k q - o_k)}{2\sigma_k^2}}$$

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

Quality of the Poisson approximation depends on SNR

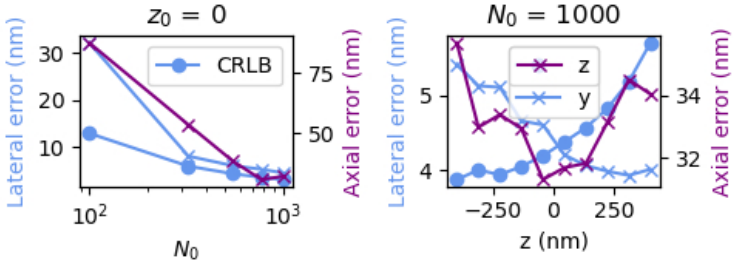
$P(H_k|\theta) \approx \mathrm{Poisson}(\mu_k + \sigma_k^2)$ for $N_0 > 500$ asssuming $\Delta = 100$ms



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

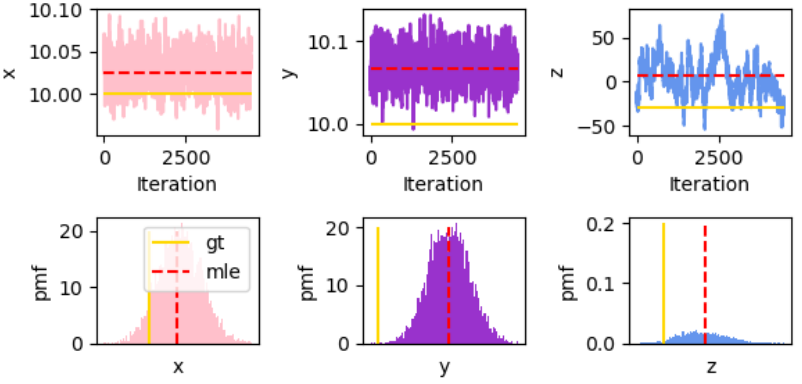


- MLE can approach the CRLB on simulated isolated emitter data
- MLE does not generalize well to dense time-series

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

Two-dimensional reconstruction of H2B in interphase Hela nuclei

Estimator precision is dependent on imaging conditions

We have shown how localization precision is affected by SNR, which is affected by the frame rate. However, localization precision is also independently determined by the density, which is affected by photoswitching rate constants. True density is unknown apriori, so, as a rule, we strive for the lowest density possible for a fixed set of rate constants. Lateral precision is less sensitive to SNR, so it is probably possible with our setup.

Is 3D reconstruction even possible in our current conditions?

To determine if 3D is possible even in principle, we should

- Measure the rate constants from isolated JF646 molecules in the cytoplasm
- Measure density recovered in 2D experiments
- Simulate 3D from a recovered 2D structure and determine under which conditions satisfactory precision and Jaccard index are met

Estimating uncertainty with gradient-based MCMC

Uncertainty as a function of incident photon count - or is this the same as estimator precision?

A deep learning framework for localization microscopy

Precise localization in dense regimes is intractable

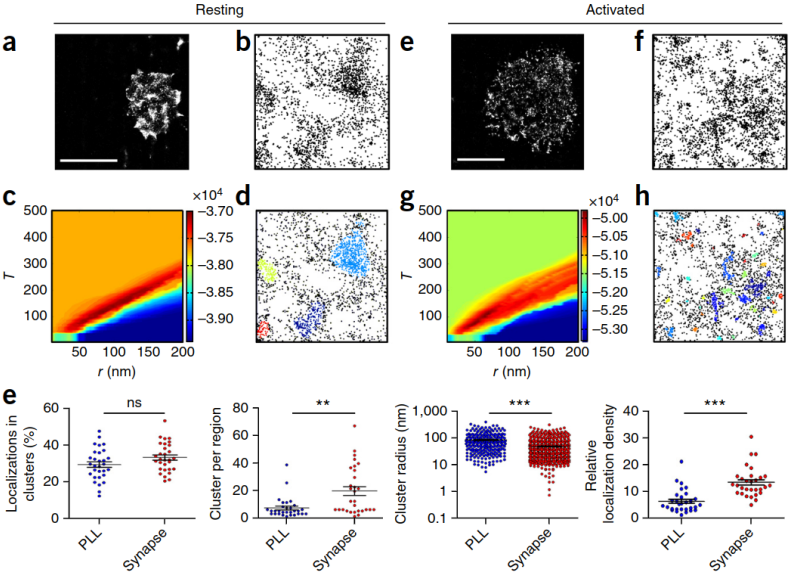
A deep learning framework for localization microscopy

Deep learning enables ultra-dense imaging for fast reconstruction

A Bayesian clustering algorithm on high density

Number of clusters is unknown apriori - Bayesian nonparametrics

Bayesian clustering algorithms on high density data



Nucleosome organization in MED1-BRD4 transcriptional condensates

Inducing GBP5 gene expression with Inteferon-γ

Colocalization of nascent GBP5 mRNA with phase separation markers

Colocalization of nascent GBP5 mRNA with phase separation markers

Costaining of H2B/BRD4/MED1 in interphase Hela cells

Cluster analysis of H2B at putative transcriptional condensates

Physical cluster analysis of H2B at putative transcriptional condensates