

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

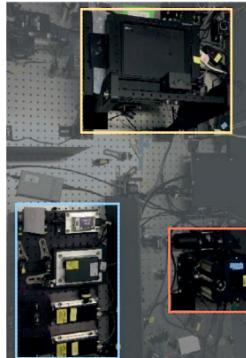
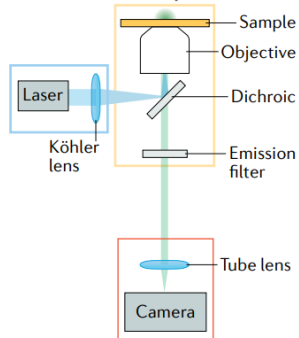
Formulate the basic research question and introduce the approach using major results from section 3

Widefield/HILO fluorescence microscope

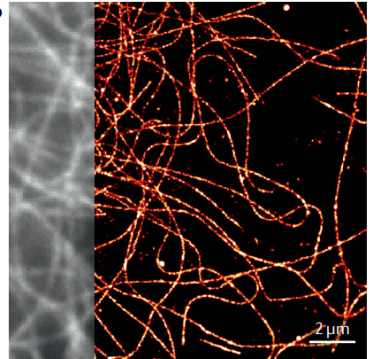
Direct stochastic optical reconstruction microscopy

Direct stochastic optical reconstruction microscopy

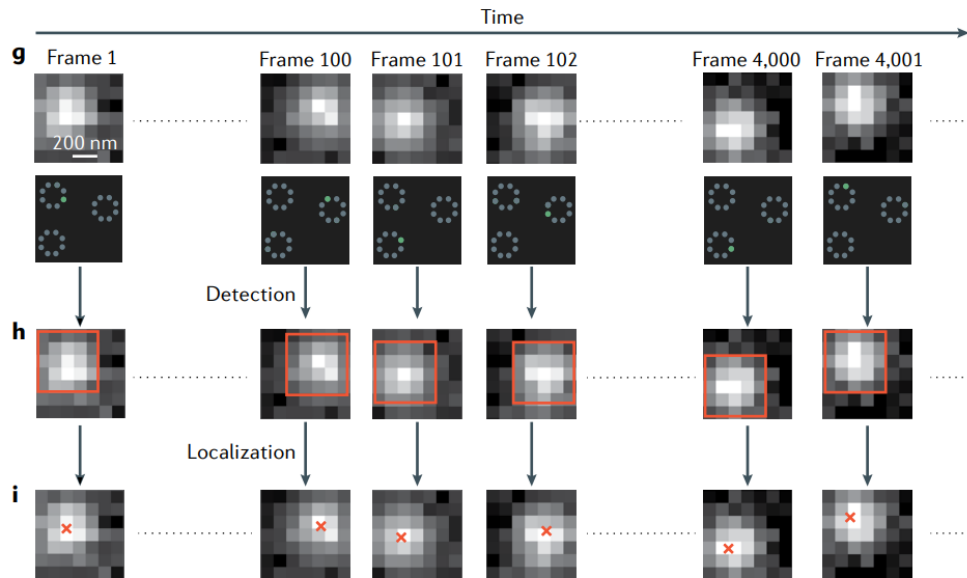
a Basic SMLM set-up



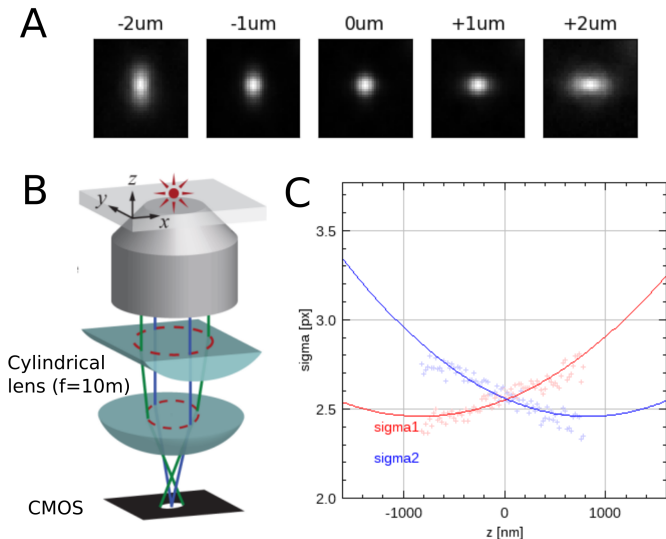
b



Direct stochastic optical reconstruction microscopy



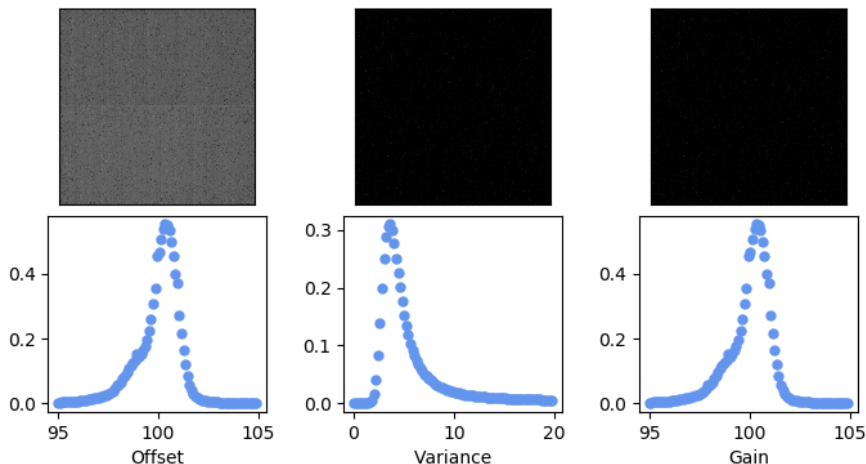
Point spread function engineering for three-dimensional imaging



Generative models and inference for SMLM

Readout noise of sCMOS cameras

Hamamatsu ORCA v3 CMOS, air cooled to -10C



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

Maximum likelihood localization of an isolated fluorescent emitter

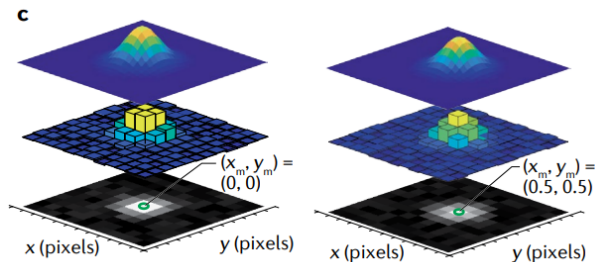
Localization: $\theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$

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

η – quantum efficiency

N_0 – emission rate

Δ – exposure time

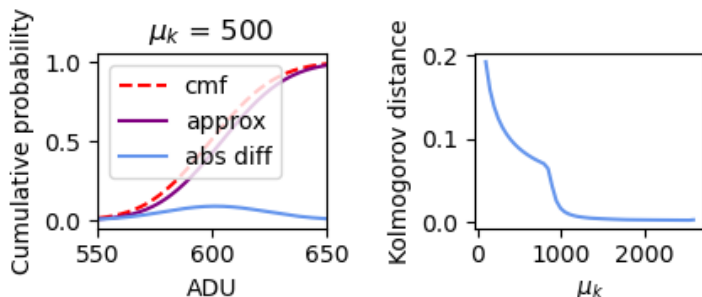


$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi\sigma_k}} e^{-\frac{(H_k - g_k q - o_k)^2}{2\sigma_k^2}}$$

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

Quality of the Poisson approximation depends on SNR

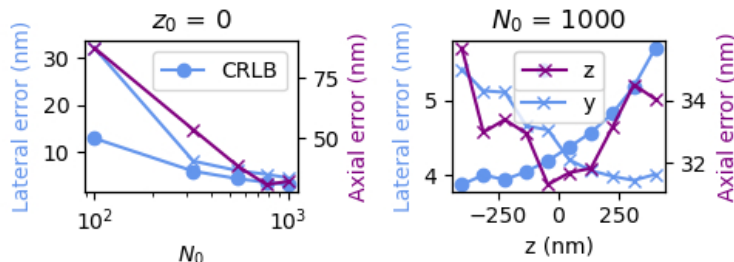
$P(H_k|\theta) \approx \text{Poisson}(\mu_k + \sigma_k^2)$ for $N_0 > 500$ assuming $\Delta = 100\text{ms}$



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

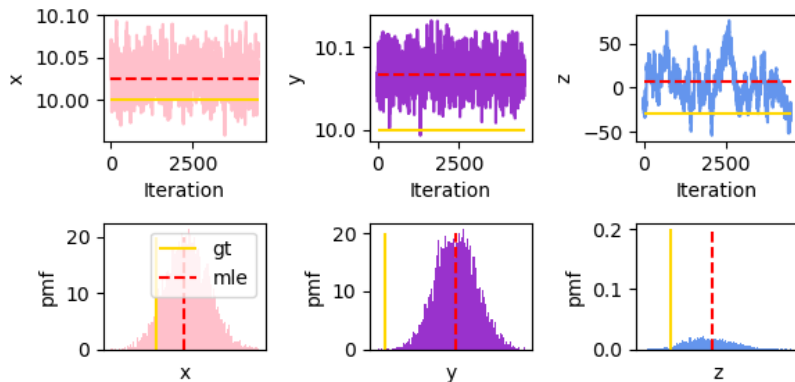


- MLE can approach the CRLB on simulated isolated emitter data
- MLE does not generalize well to dense time-series

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 $t \rightarrow \infty$

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

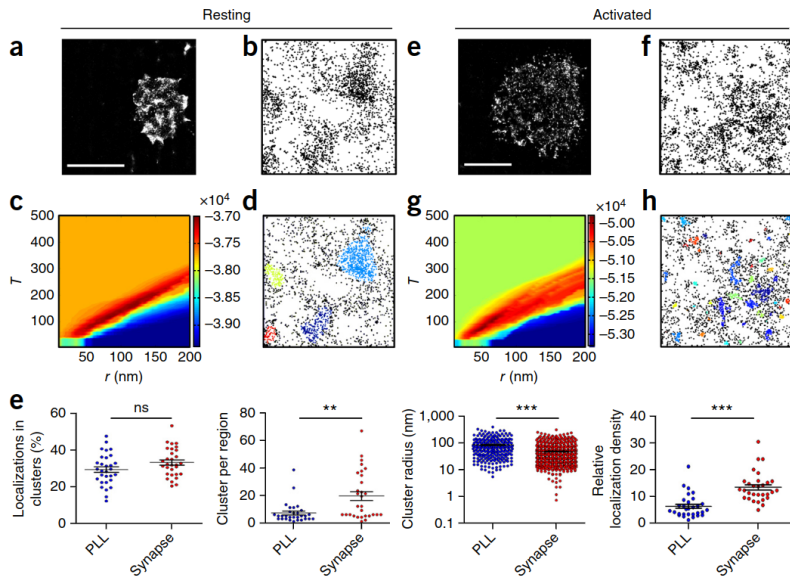
A deep learning framework for localization microscopy

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