

# Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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# Outline

A phase separation model for transcriptional control

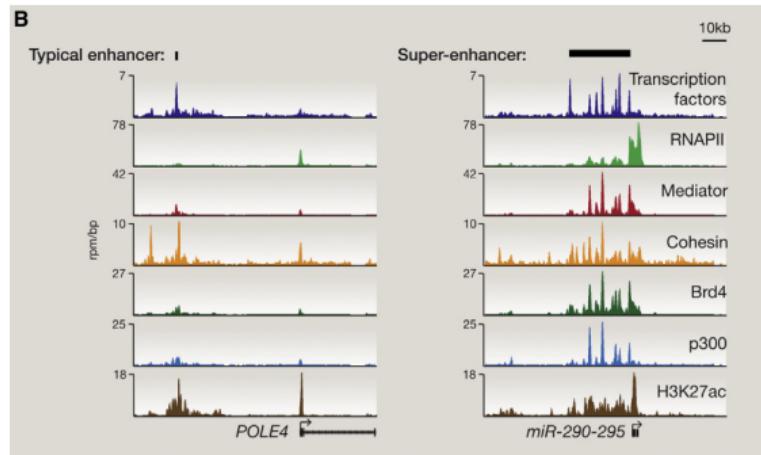
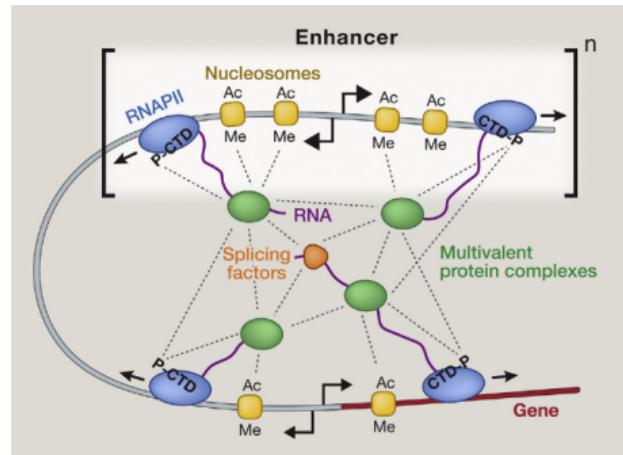
Super resolution imaging with *d*STORM

The time resolution of *d*STORM

Dense localization with deep learning

Results and future aims

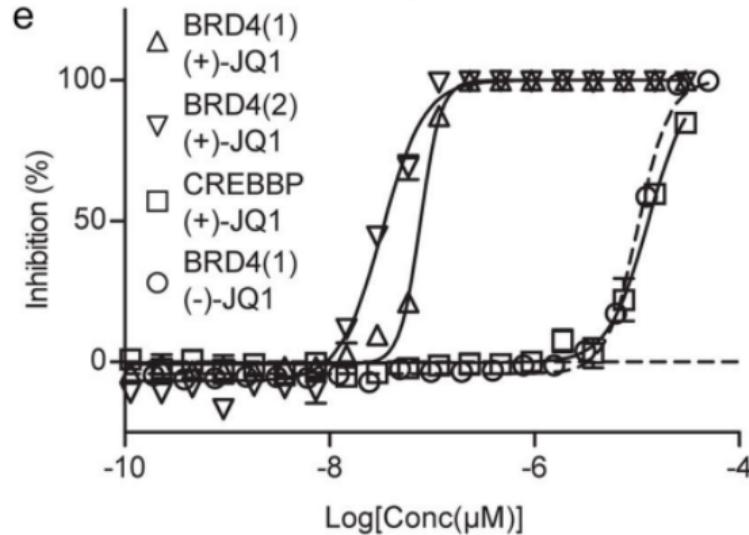
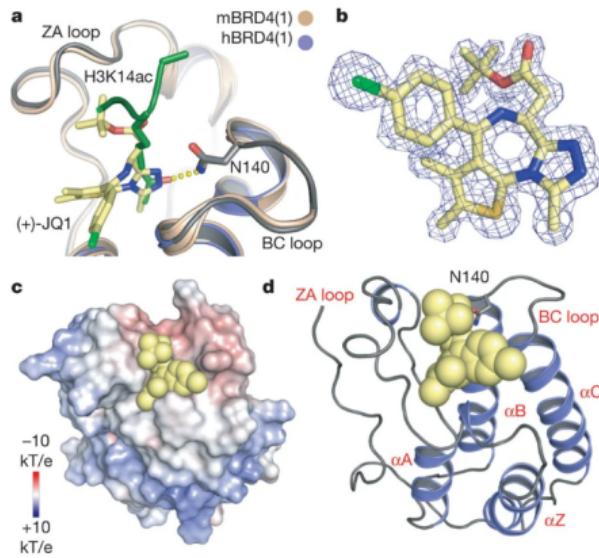
# A phase separation model for transcriptional control



Hnisz et al. *A phase separation model of transcriptional control*. Cell 2017

- ▶ Super-enhanced genes are regulated by large molecular assemblies
- ▶ Nucleosome clustering is poorly characterized at super-enhancers
- ▶ We study nucleosome clustering dynamics using super-resolution microscopy

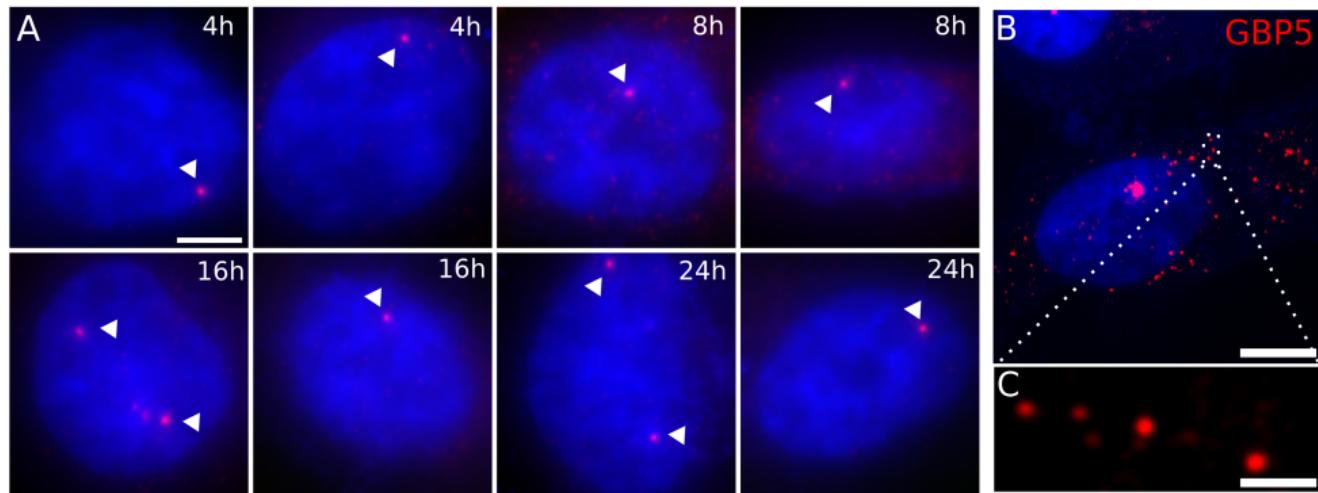
# (+)-JQ1 in complex with BRD4 protein



Filippakopoulos. Selective inhibition of BET bromodomains. *Nature*

- ▶ BRD4 is an interesting target since specific and non-specific inhibitors exist
- ▶ BET mimics including +JQ1 prevent binding of BRD4 to acetylated histones

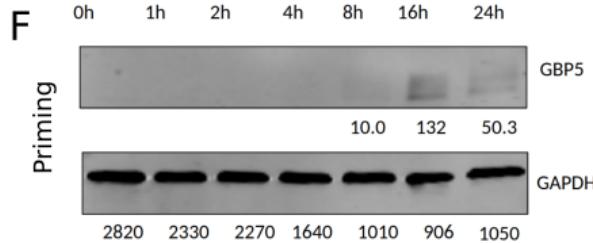
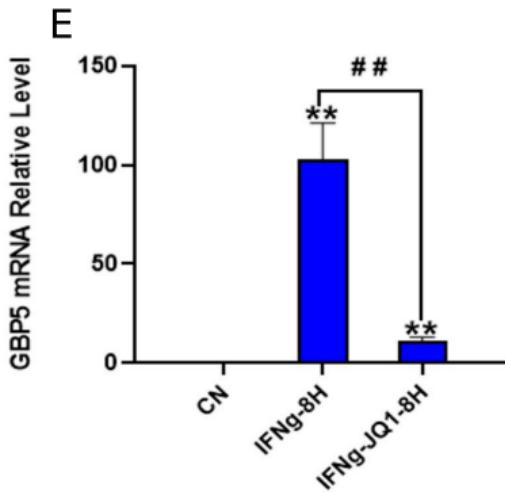
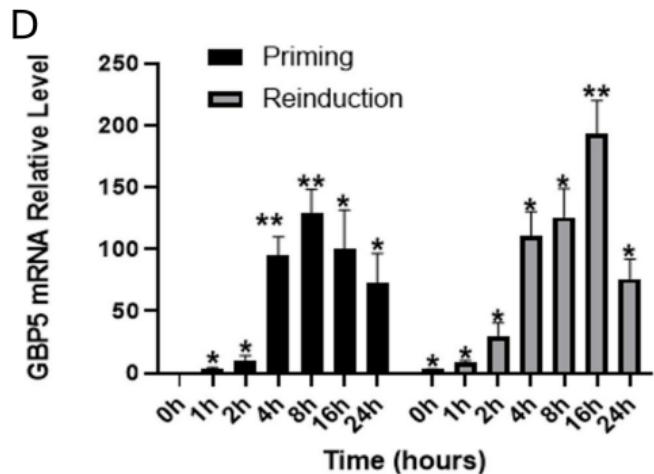
# Inhibition of a super-enhanced gene with JQ1



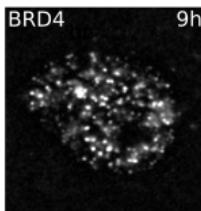
Blue - DAPI (binds DNA minor groove)

- ▶ Guanylate binding proteins (GBPs) are a family of GTPases induced by IFN- $\gamma$
- ▶ BRD4 is directly involved in GBP gene expression

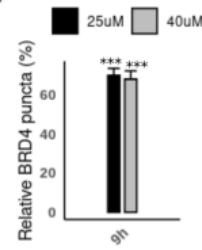
# Inhibition of a super-enhanced gene with JQ1



G

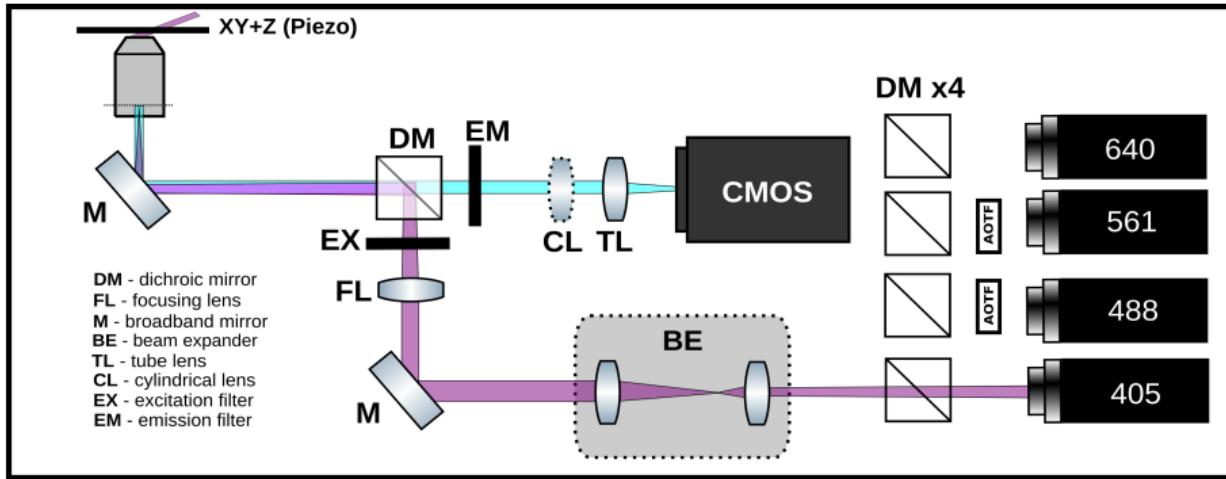


H



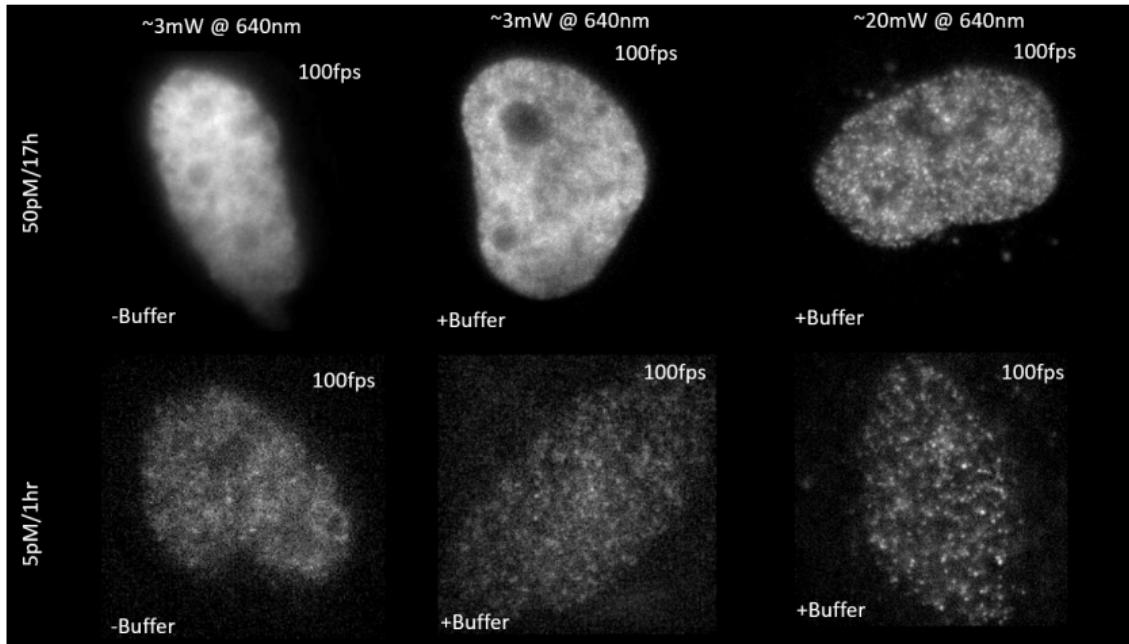
► \*: $P \leq 0.1$ , \*\*: $P \leq 0.01$

# Instrumentation for single molecule localization microscopy



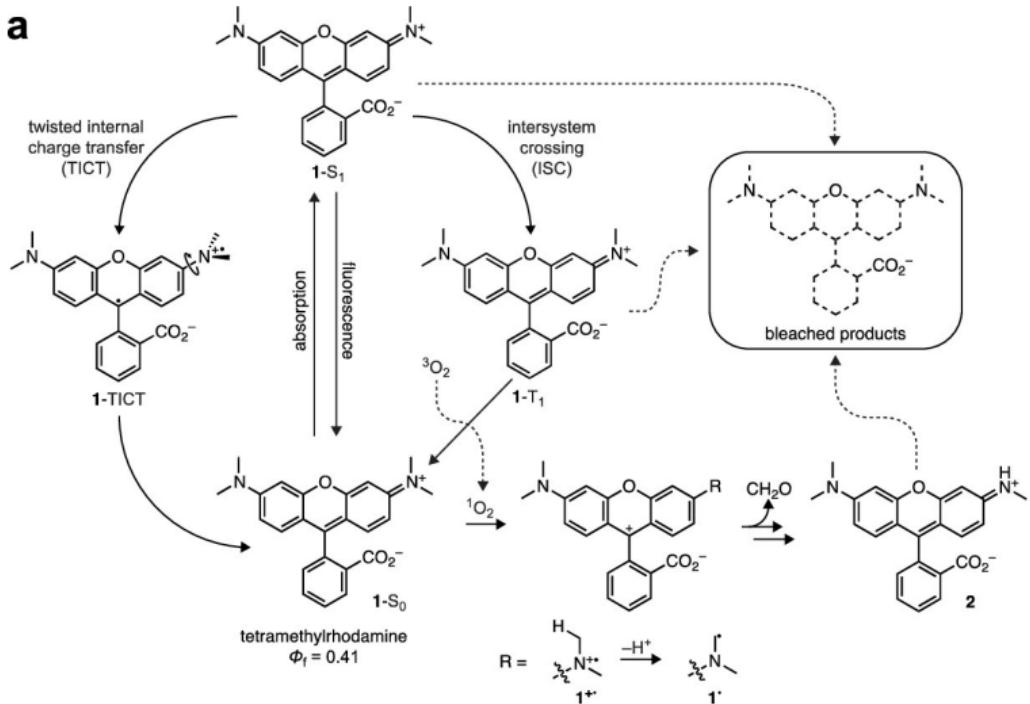
- ▶ Selectable widefield and oblique illumination
- ▶ Widefield useful for high throughput
- ▶ Oblique method illuminates a thin section of nuclei

# Dense labeling of histone H2B in fixed cells at RT



- ▶ Dense labeling of H2B-Halotag w/ fluorescent ligand JF646
- ▶ Reducing buffer is usually a primary thiol like cysteamine (MEA)
- ▶ Photoswitching of JF646 allows us to beat the diffraction limit

# Direct STORM: The photophysics of rhodamines



- ▶ Reduction of the T<sub>1</sub> state yields a dark, long-lived, and stable radical state

# Maximum likelihood localization of an isolated fluorescent emitter

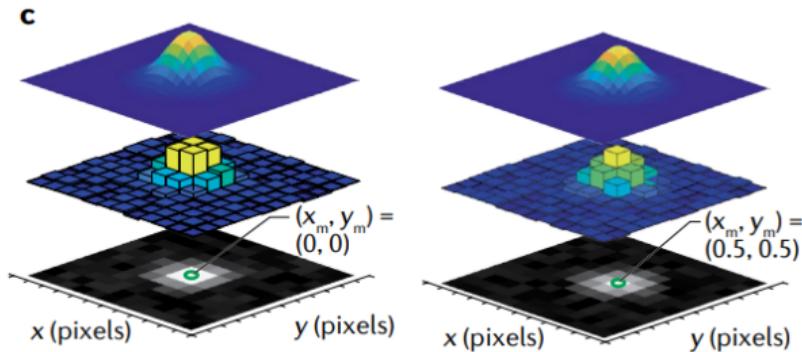
$$\text{Localization: } \theta^* = \underset{\theta}{\operatorname{argmax}} \prod_k P(H_k|\theta) = \underset{\theta}{\operatorname{argmin}} - \sum_k \log P(H_k|\theta)$$

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

$\eta$  – quantum efficiency

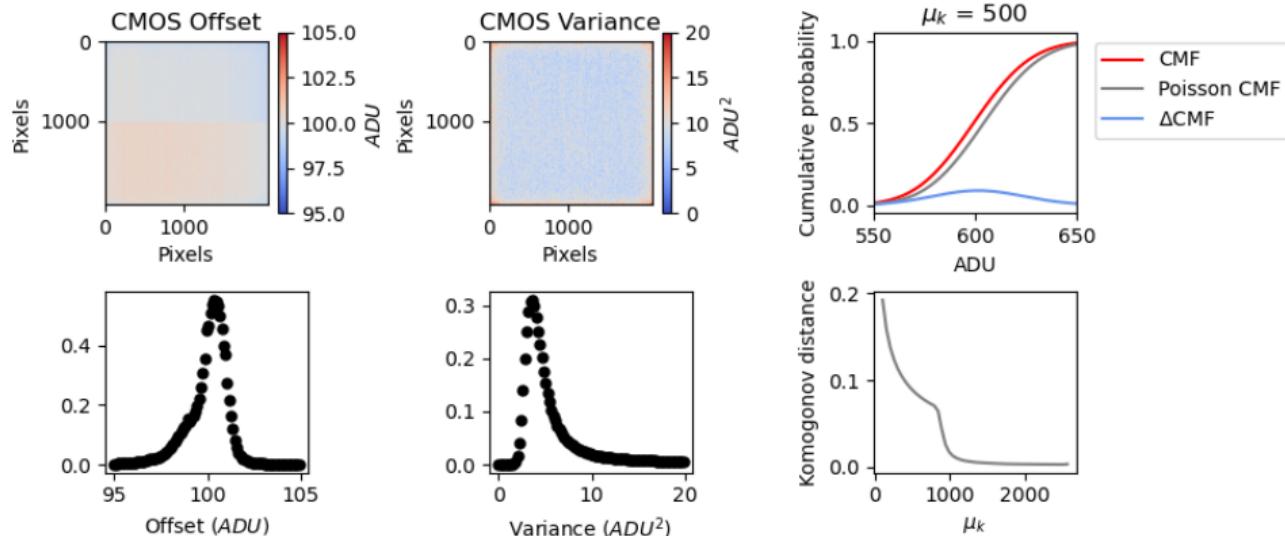
$N_0$  – photon count

$\Delta$  – exposure time



- Likelihood function can be directly optimized with gradient descent

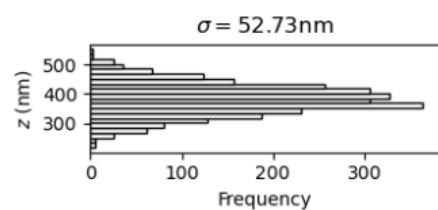
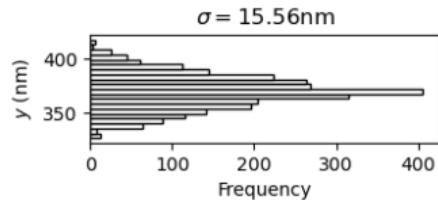
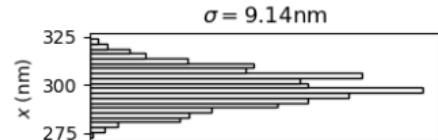
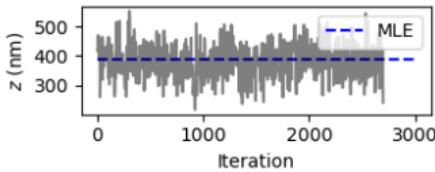
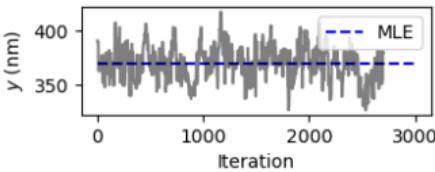
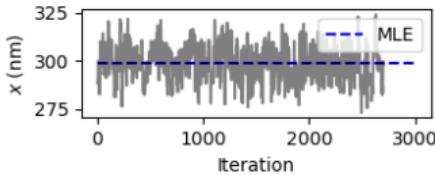
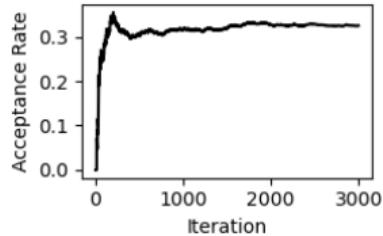
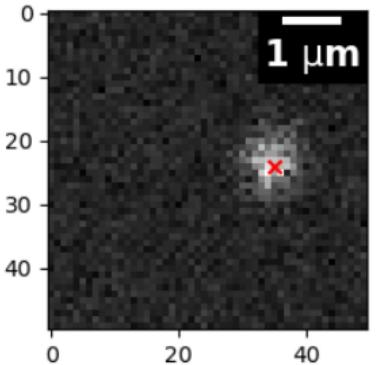
# A Poisson approximation at moderate SNR simplifies SMLM



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

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

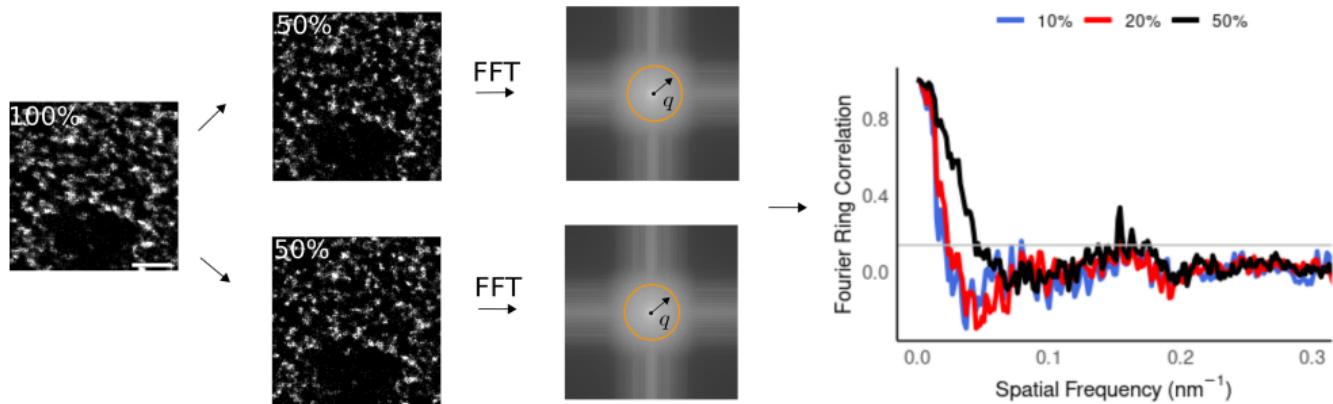
# Estimator precision in localization microscopy



- ▶ SMLM techniques are diffraction unlimited
- ▶ This makes them desirable for super-resolution, but they are slow

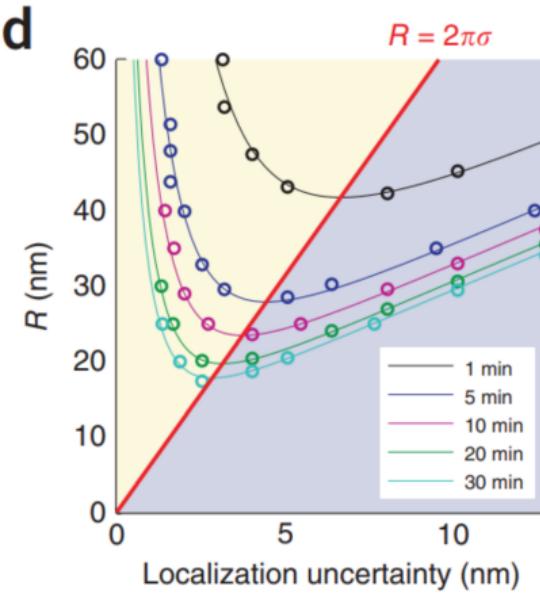
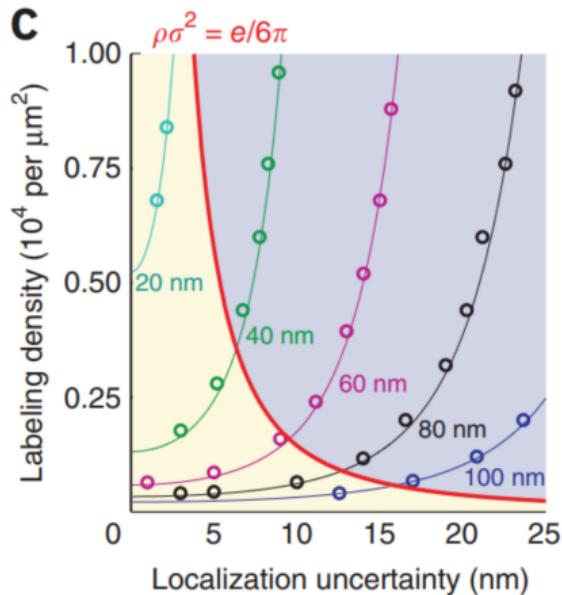
# Fourier ring correlation links spatial and temporal resolution

- We can view dSTORM as sampling from a density
- Reduced localizations increases time-resolution but degrades spatial resolution



$$\text{FRC}(q) = \frac{\sum_{\vec{q} \in \text{circle}} \tilde{f}_1(\vec{q}) \tilde{f}_2(\vec{q})^*}{\sqrt{\sum_{\vec{q} \in \text{circle}} |f_1(\vec{q})|^2} \sqrt{\sum_{\vec{q} \in \text{circle}} |f_2(\vec{q})|^2}}$$

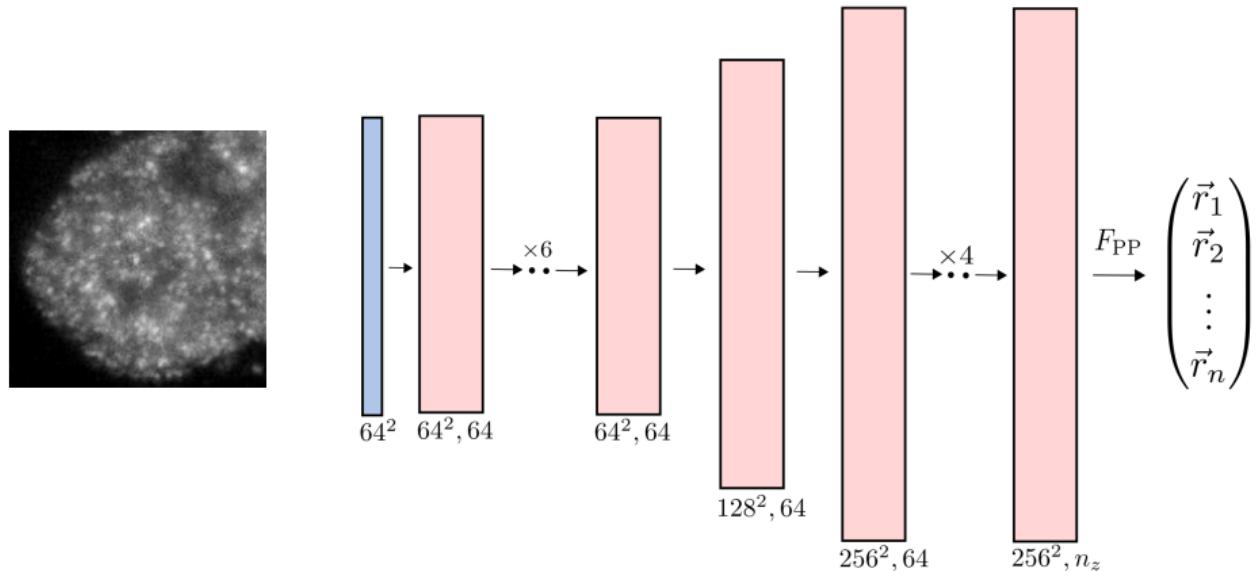
## Fourier ring correlation links spatial and temporal resolution



Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy. *Nature Methods* 2013

- ▶ Maintaining constant resolution with increasing uncertainty requires increasing density
- ▶ However, localization uncertainty is not independent of labeling density

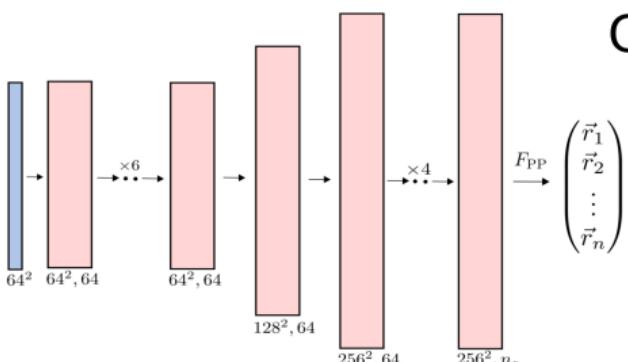
# Deep learning enables dense localization in two-dimensions



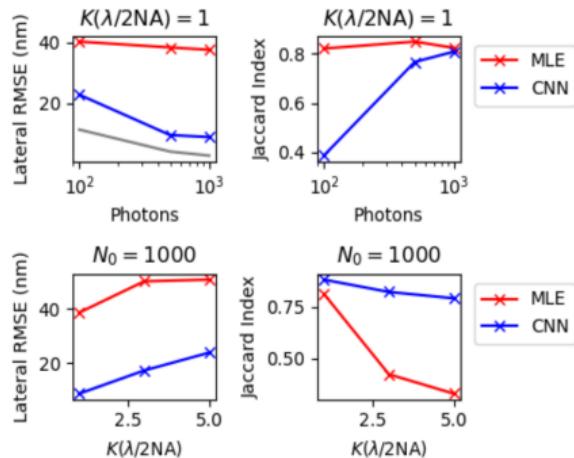
Localization is cast as semantic segmentation of the high resolution tensor:

# Estimator precision sets the resolution limit in localization microscopy

B

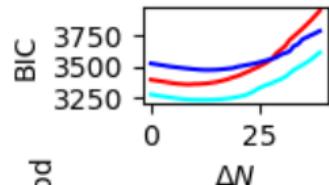
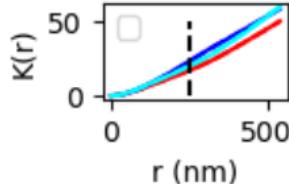
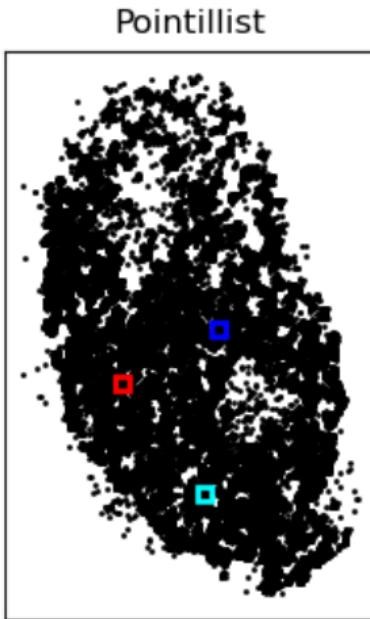
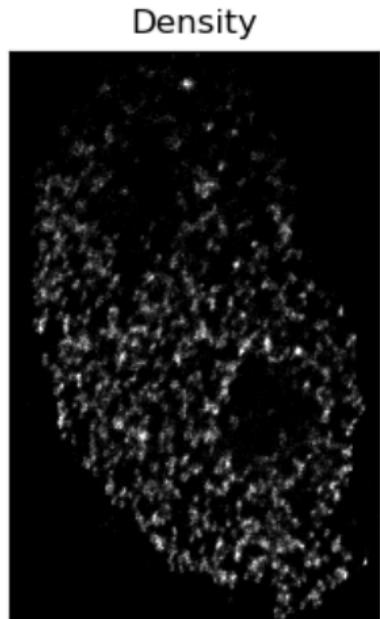


C

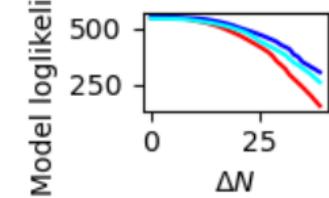


- ▶  $K(\lambda/2NA)$  is Ripley's K function at the diffraction limit ( $\lambda = 640\text{nm}$ )
- ▶ Convolutional neural networks (CNNs) approach the Cramer-Rao lower bound (gray)

# Chromatin nanodomains in a living Hela cell nucleus

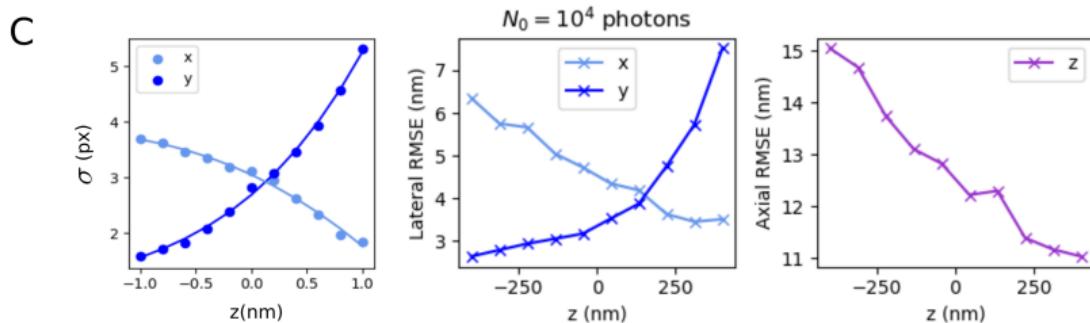
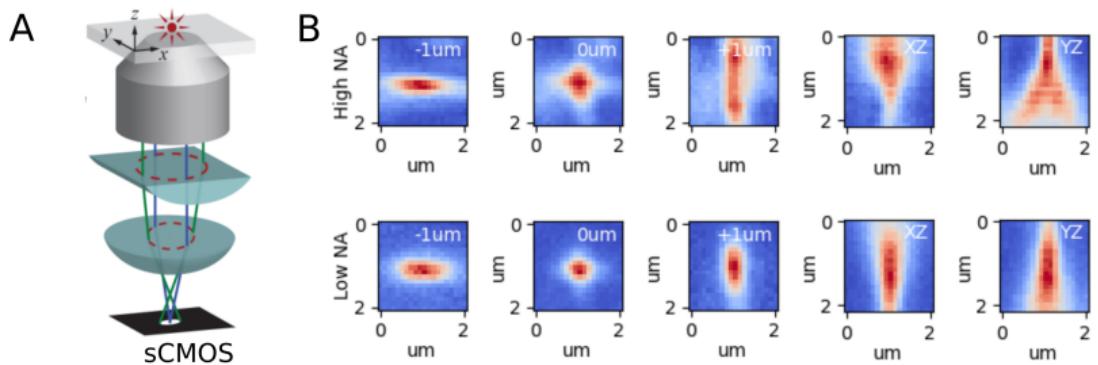


Model loglikelihood



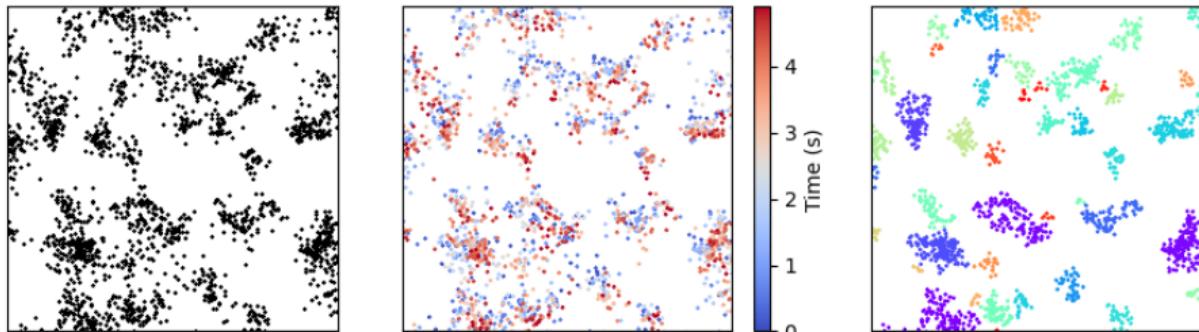
- ▶ Isotropic Gaussian KDE using 30x30nm bins
- ▶ Closest pairs are fused one at a time, until we minimized the BIC
- ▶ Likelihood is computed under a Gaussian Mixture Model (GMM)

## Astigmatism based three dimensional imaging



- ▶ A weak ( $f = 10\text{m}$ ) cylindrical lens breaks the axial symmetry of the PSF

## Future Aims



- ▶ Integrate 2D SMLM and 2D/3D single molecule tracking (Brownian dynamics in a potential)
- ▶ Determine suitable metrics for measuring cluster dynamics
- ▶ Perform fixed and live cell SR experiments on specific and non-specific inhibition of BRD4