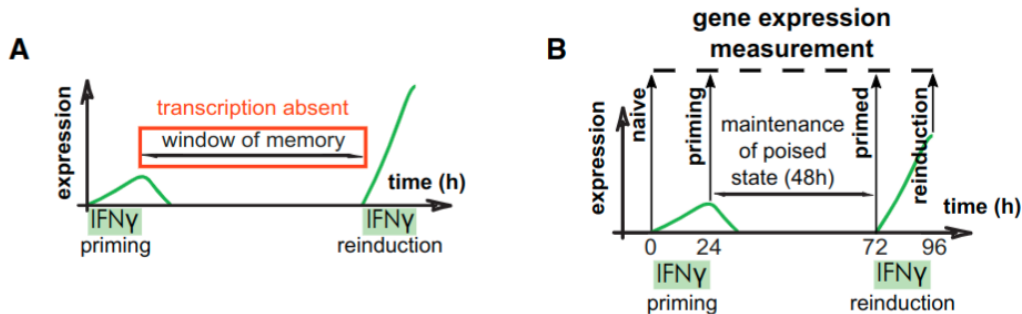


# Interferon- $\gamma$ induction of GBP5 in HeLa cells

Clayton W. Seitz

October 24, 2022

# The principle of Interferon- $\gamma$ induced transcriptional memory

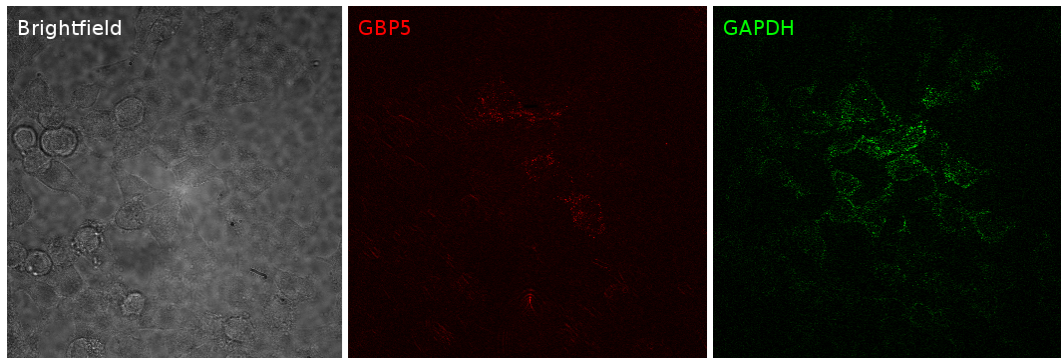


**Figure 1**

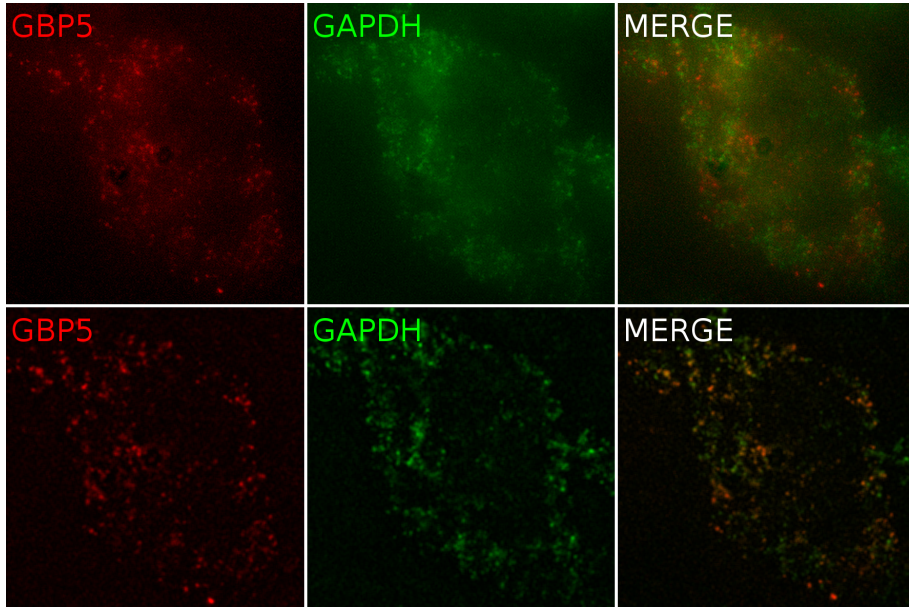
Siwek et al. *Activation of Clustered IFN $\gamma$  Target Genes Drives Cohesin-Controlled Transcriptional Memory*. Molecular Cell 2020

# Rare HeLa cell GBP5 expression @ 24h after reinduction with IFN- $\gamma$

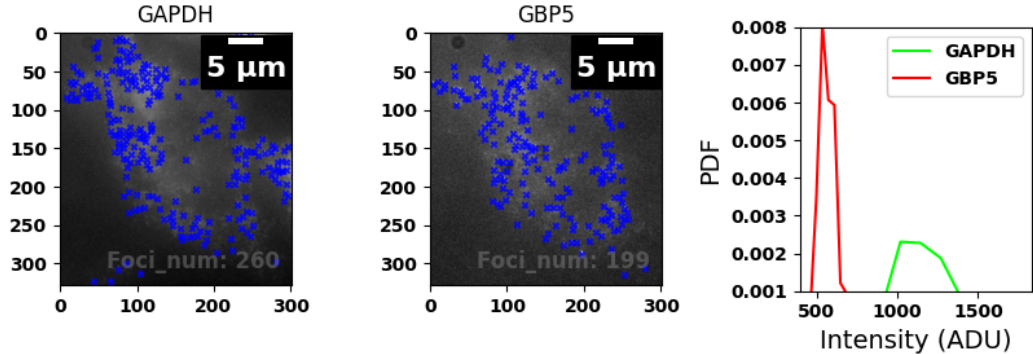
Single max intensity projection from a 10x10x9 (2mm x 2mm x 8um) tiled acquisition



## Rare HeLa cell GBP5 expression @ 24h after reinduction with IFN- $\gamma$



## Intensity histogram for rare GBP5 expression



- ▶ Very few ( $\sim 1\%$ ) reinduced cells express GBP5, but those that do express at high levels (relative to GAPDH)
- ▶ Control sample shows little to no GBP5 expression

# Comments on ergodicity of transcription

## 1. Priming leads to more transcription



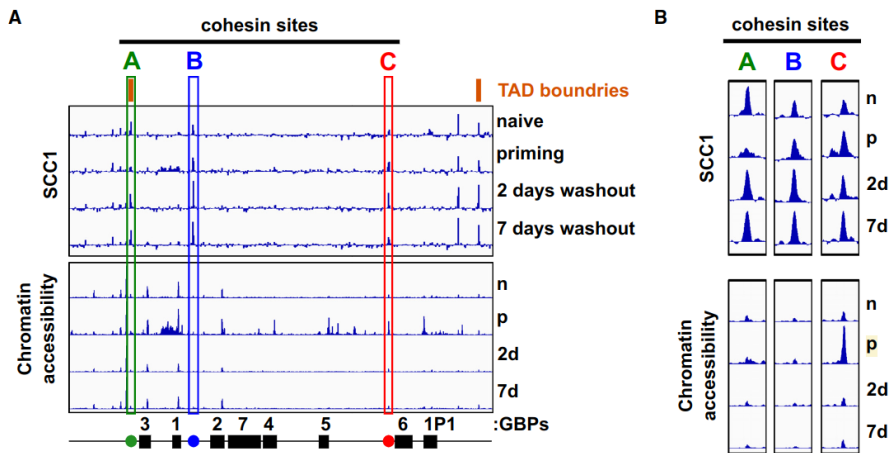
## 2. Priming leads to more cells transcribing



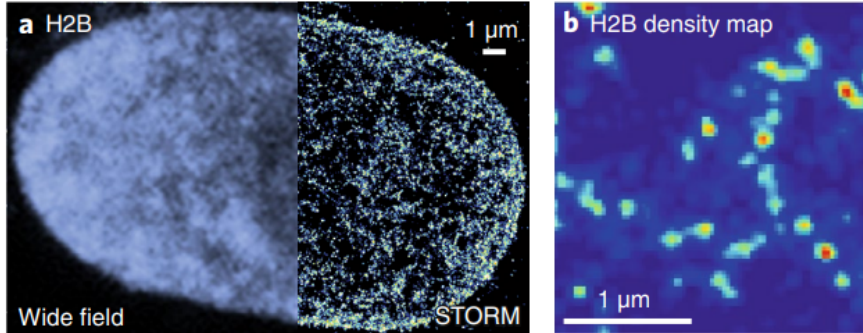
- ▶ RNA flow cannot apply to non-ergodic systems (yet ergodicity is often assumed)
- ▶ Previous work suggests that  $\text{IFN-}\gamma$  induces epigenetic changes at the GBP5 locus
- ▶ What is the epigenetic change? Is the epigenetic change all or nothing?

# Epigenetic changes at GBP genes after IFN- $\gamma$ treatment

Siwek et al. *Activation of Clustered IFN $\gamma$  Target Genes Drives Cohesin-Controlled Transcriptional Memory*. Molecular Cell 2020



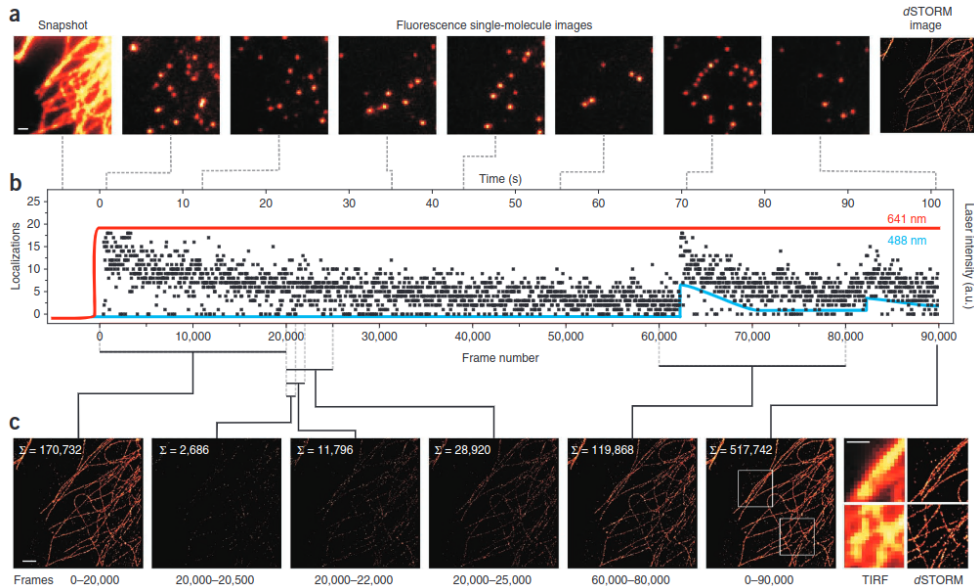
# STORM imaging of H2B



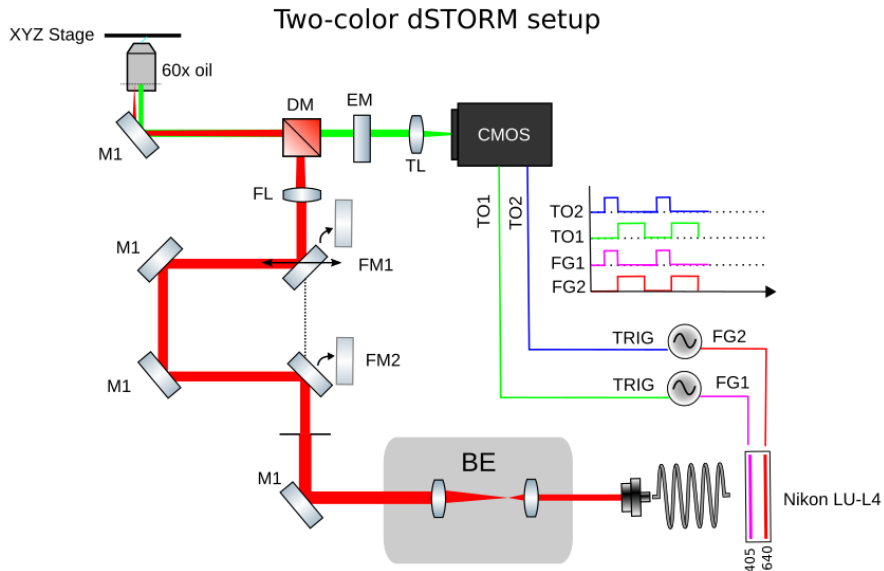
Lakadamyali et al. *Visualizing the genome in high resolution challenges our textbook understanding*. Nature Methods 2020



# Working principle of dSTORM



# Details on STORM timing setup



# Remaining questions on the application of STORM

If STORM works with the H2B-HaloTag

- ▶ How do we interpret H2B density maps?
- ▶ Can you correlate ChIP-seq/ATAC-seq data with H2B density?