# The kinetics of Interferon-$\gamma$ induced RNA flow

Clayton W. Seitz

August 28, 2022

# Summary

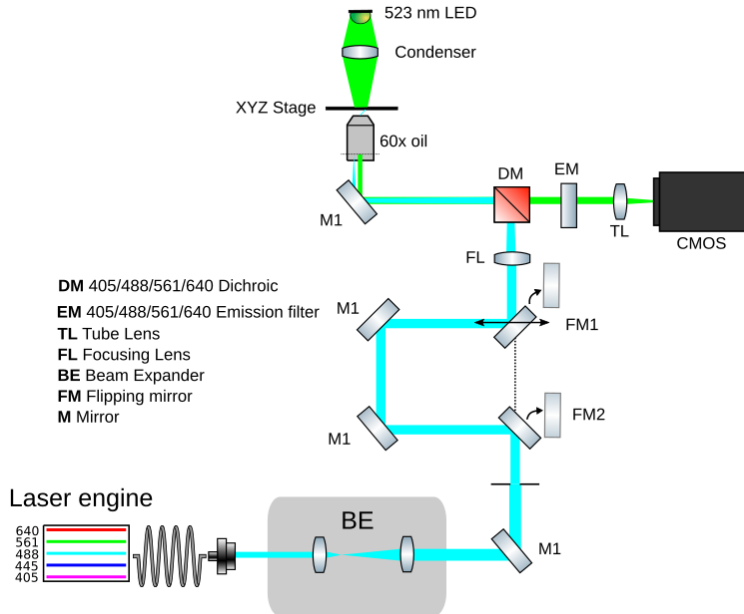Attempting to cover everything from imaging strategy to analysis method

**Part 1: Methods**
- ▶ Multiplexed imaging strategy
- ▶ Image stitching and registration
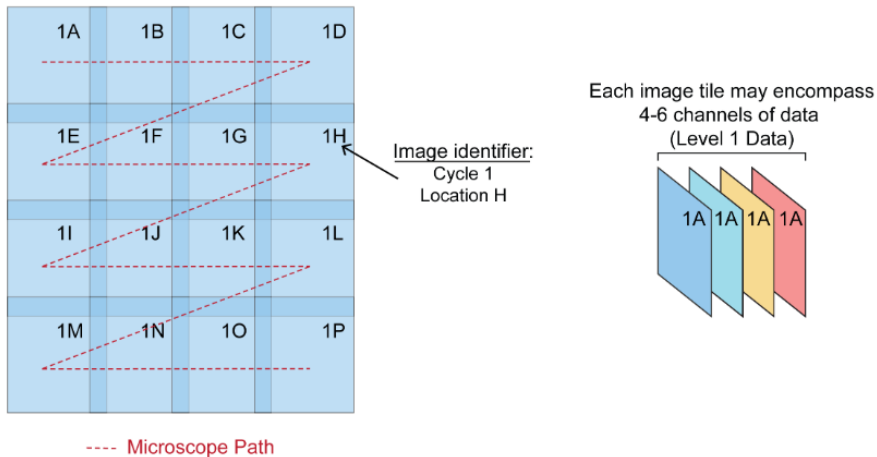- ▶ Recap of the compartment model for RNA flow

**Part 2: Application**
- ▶ Gene selection based on RNA flow model
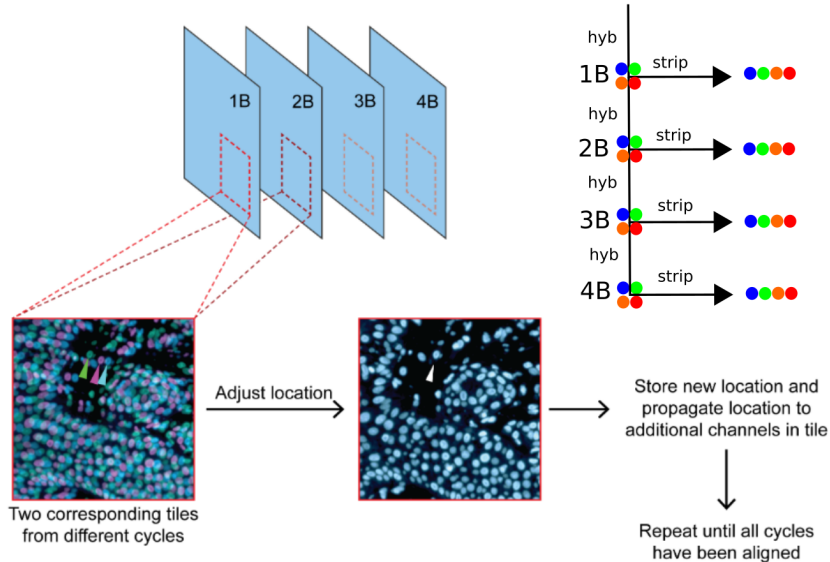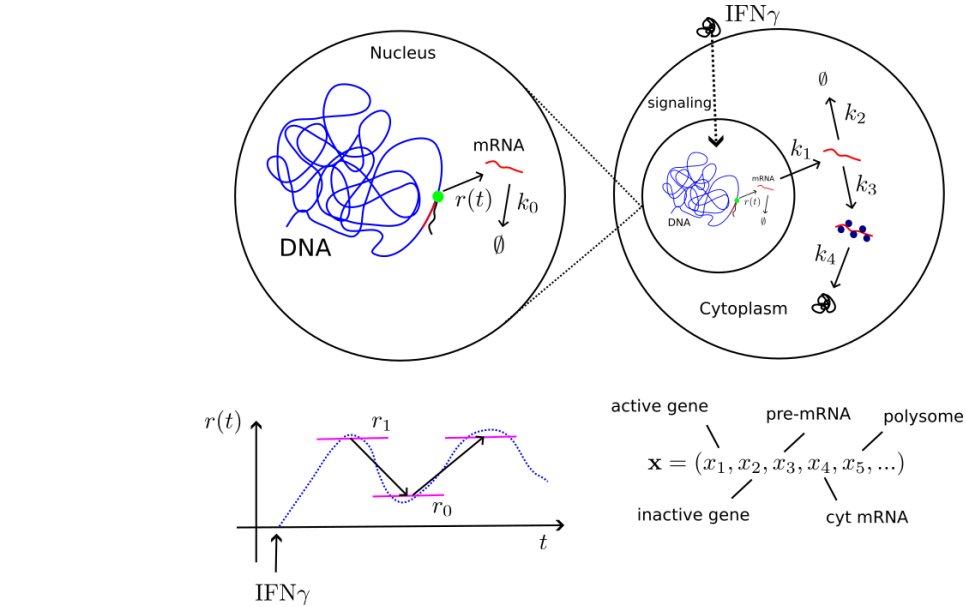- ▶ Brief comments on probe design

# Imaging setup

# 4-channel acquisition



Each image tile may encompass
4-6 channels of data
(Level 1 Data)

Image identifier:
Cycle 1
Location H

---- Microscope Path

*Courtesy of the Laboratory of Systems Pharmacology at Harvard*

# 16-channel acquisition and registration



Two corresponding tiles from different cycles

Adjust location

Store new location and propagate location to additional channels in tile

Repeat until all cycles have been aligned
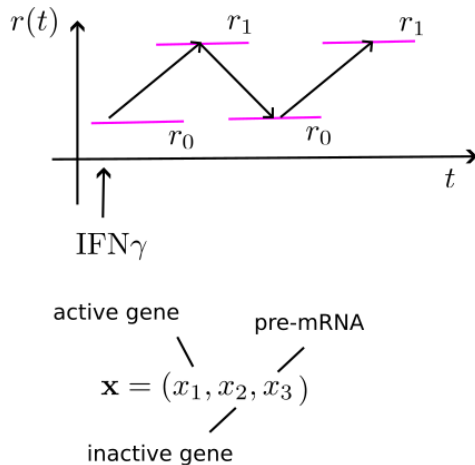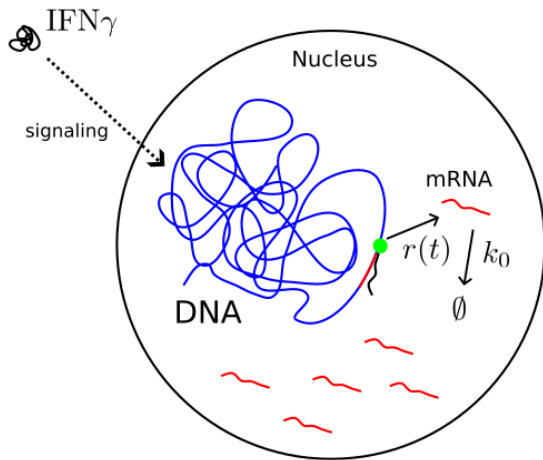
# A model for RNA Flow

# Starting small: telegraph model of gene expression



What biological parameter(s) determine $r(t)$?

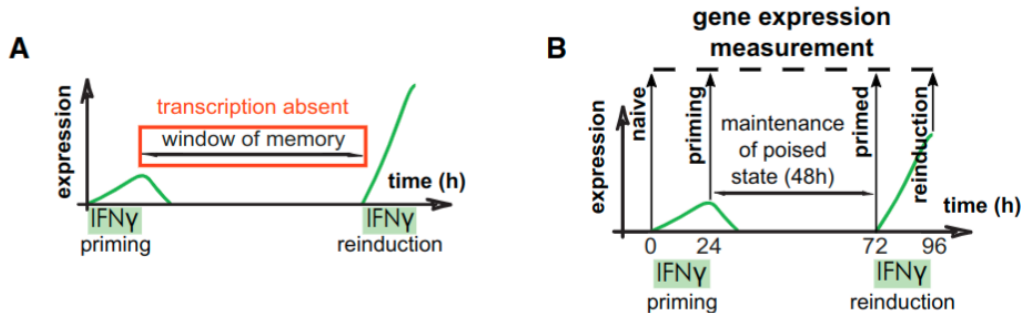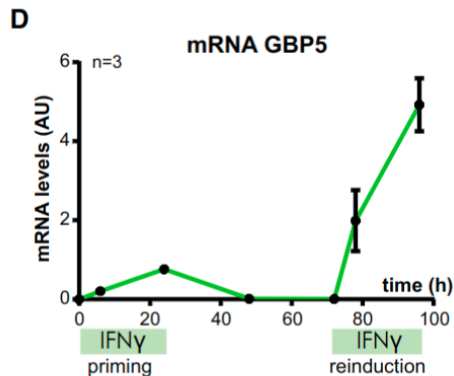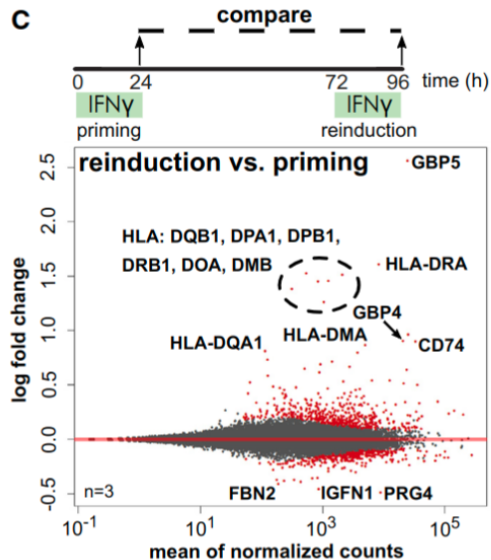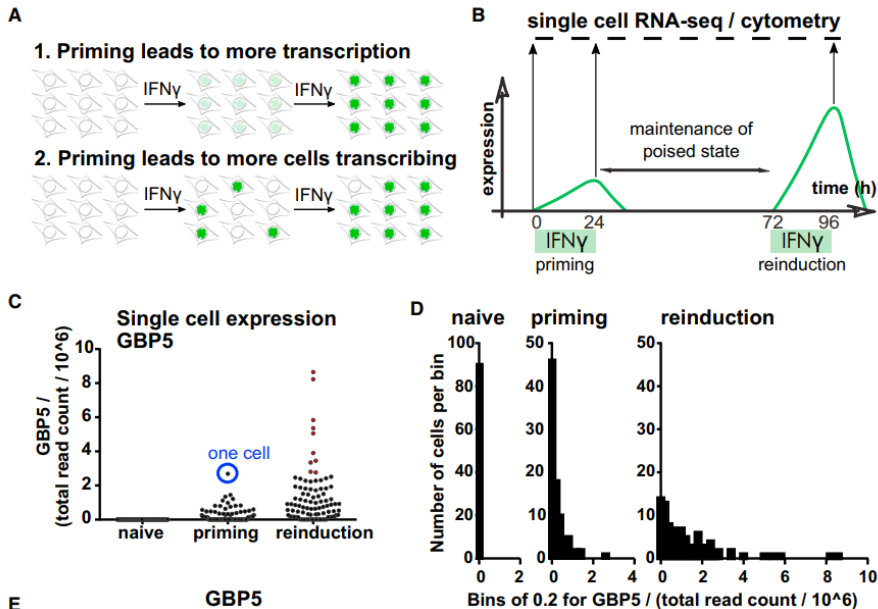# The principle of Interferon-γ induced transcriptional memory



**Figure 1**

Siwek et al. *Activation of Clustered IFNg Target Genes Drives Cohesin-Controlled Transcriptional Memory*. Molecular Cell 2020

# Total RNA-seq identifies GBP5 as a memorized gene

# Single-cell RNA-seq identifies GBP5 as a memorized gene



A

**1. Priming leads to more transcription**

**2. Priming leads to more cells transcribing**

B

single cell RNA-seq / cytometry

C

**Single cell expression GBP5**

one cell

D   naive   priming   reinduction

Bins of 0.2 for GBP5 / (total read count / 10^6)

E                        **GBP5**

# BLASTing probes for GPB5 and CD274 (control)