

High-Throughput Imaging Workflows for the Systematic Dissection of Cellular Pathways

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Interoperability of Web Computational Plugins, NIST, Dec 5 2019

High-Throughput Imaging (HTI)

Investigator

Experimental
perturbation

Imaging-based
cellular assay

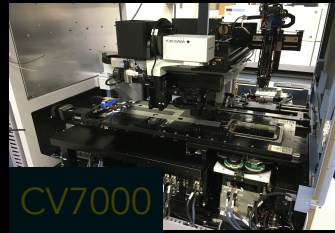
Phenotypic
change

HiTIF

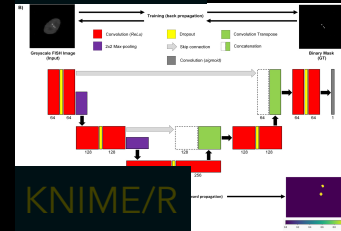
Automated
liquid handling



High-
throughput
microscopy



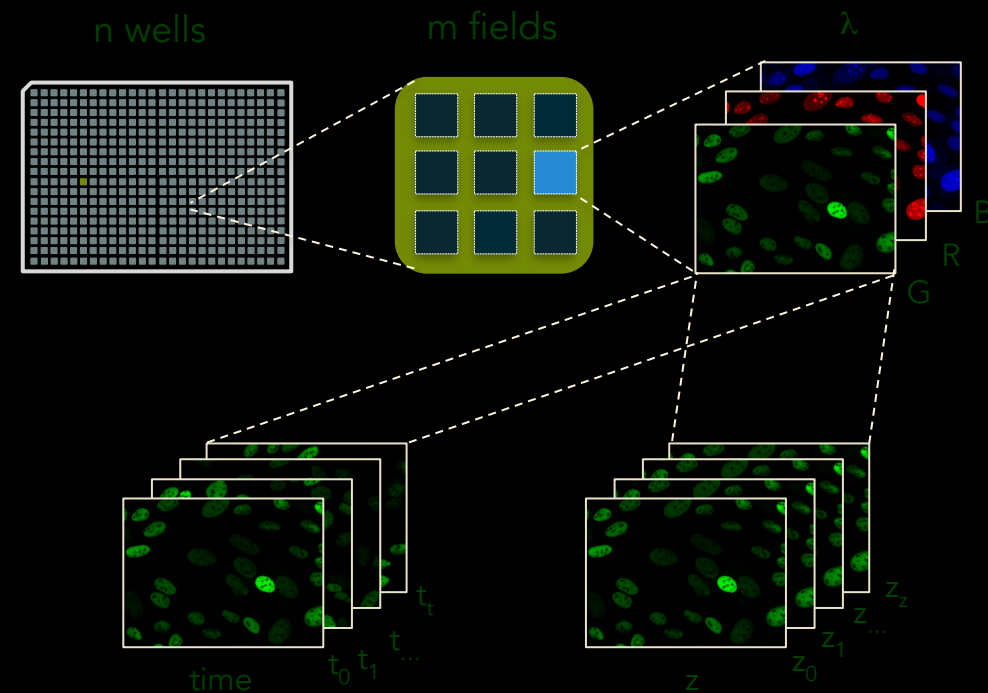
High-content
image analysis



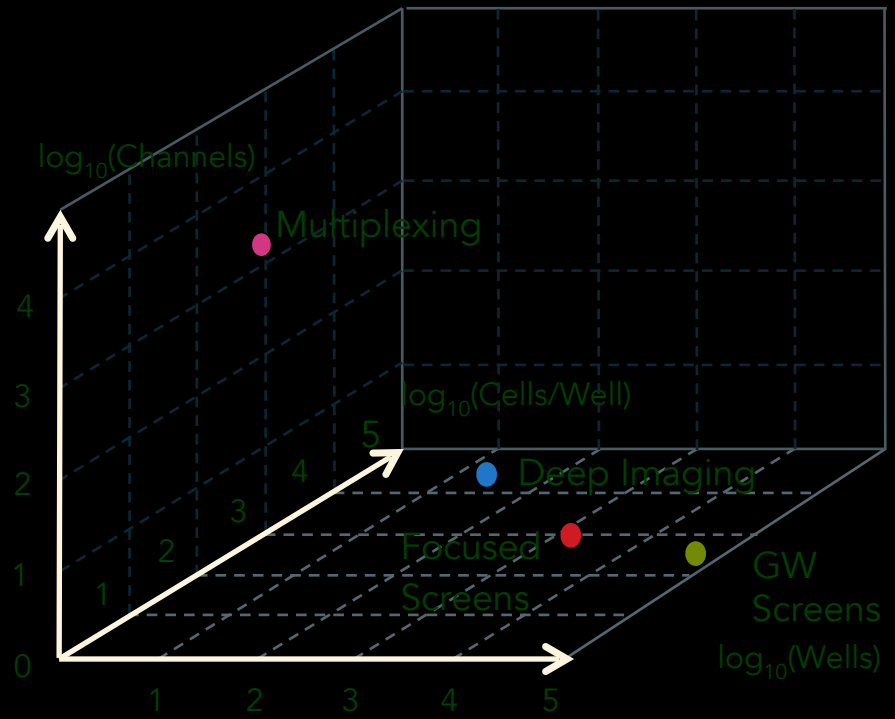
Up to:

- 10^4 Wells
- 10^4 Cells/Well
- 10^2 Feat./Cell

HTI Data Generation



$$2D \text{ images/day} = n * m * \lambda * z * t \approx \text{up to } 2 * 10^5$$

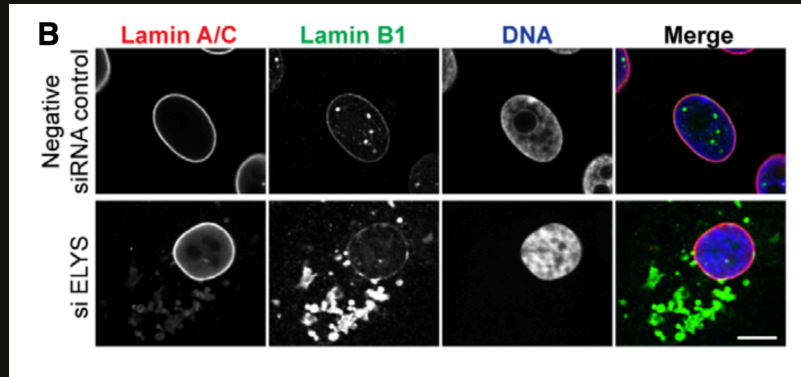


Typical Dataset Size: 5 - 500 GB

HTI Assay Formats

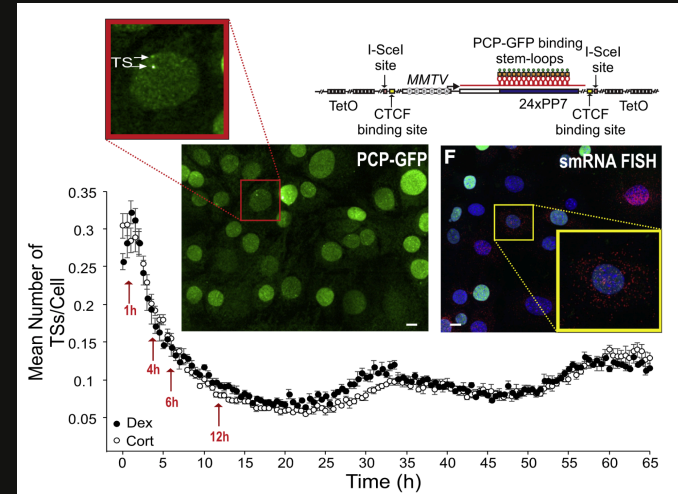
Focused Screens

(Jevtic, 2019; Liskovych, 2019; Baranes-Bachar, 2018; Veschi, 2017; Kubben, 2016; Shachar et al., 2015)



Deep Imaging

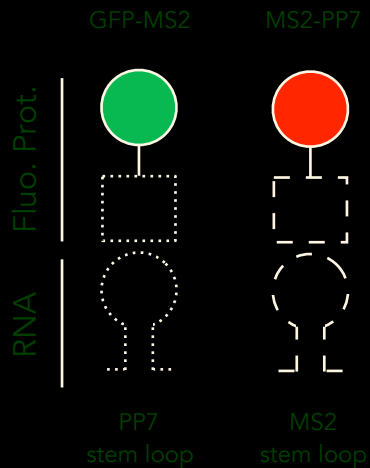
(Stavreva, 2019; Finn, 2019; Jowhar 2018; Zane, 2017; Burman, 2015a; Burman, 2015b)



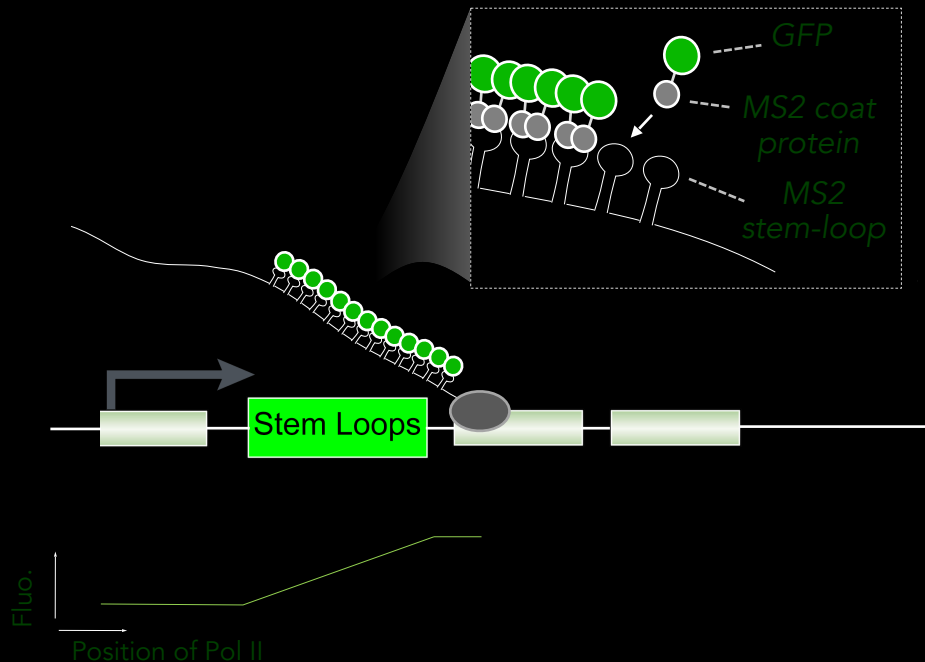
2. Imaging of Gene Expression in Live Cells



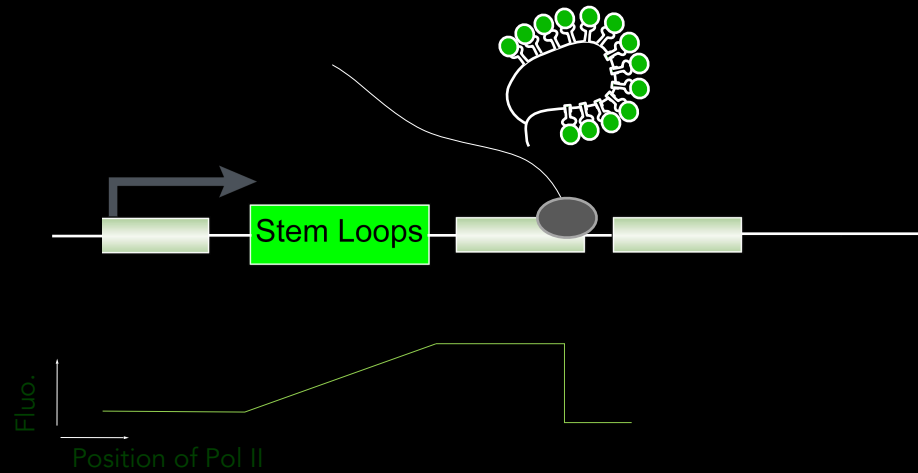
Yihan Wan
Larson Lab, NCI



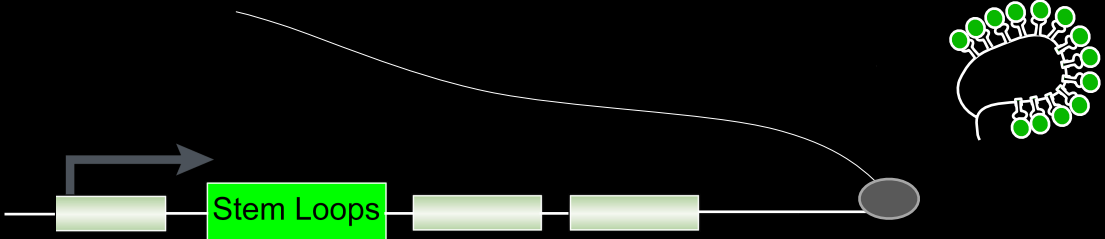
Bertrand, *Mol. Cell*, 1998
Femino, *Science*, 1998
Fusco, *Curr. Biol.*, 2003
Shav-Tal, *Science*, 2004
Chubb, *Curr. Biol.*, 2006
Larson, *Science*, 2011



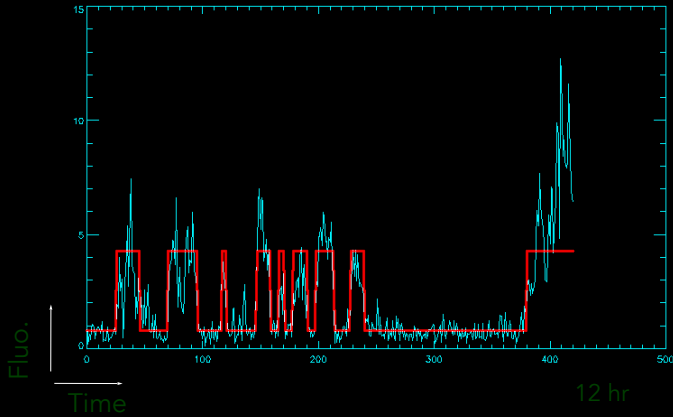
Imaging of Single Molecule mRNA Particles



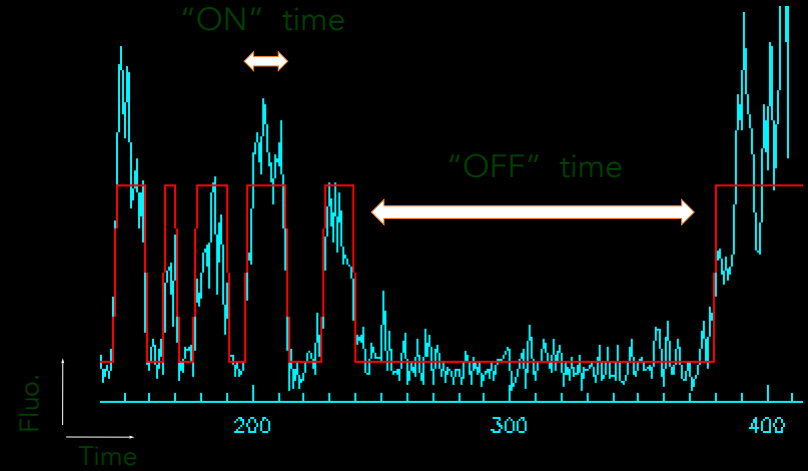
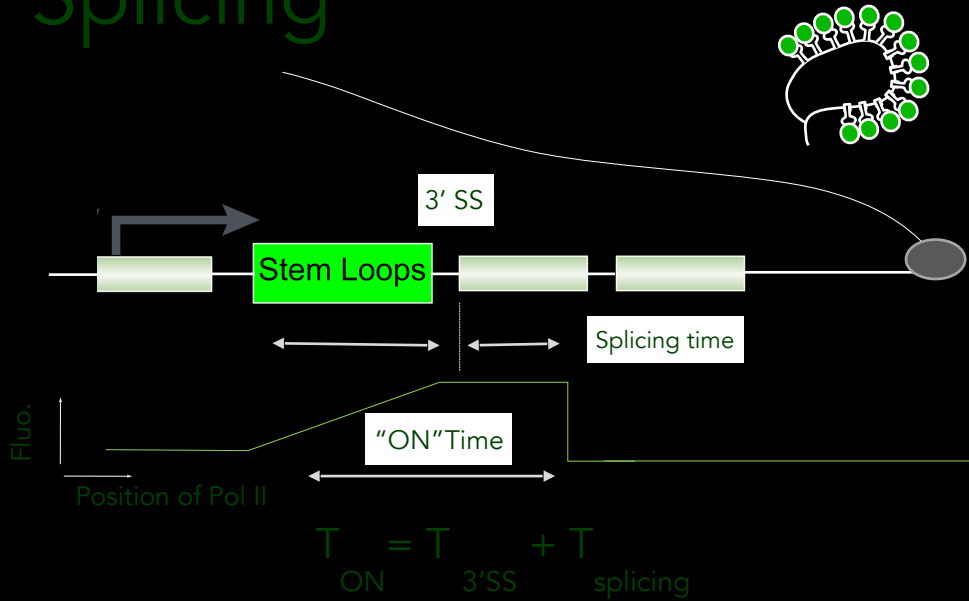
RNA Tagging Measures Transcription and Splicing



MS2 loops inserted in endogenous gene



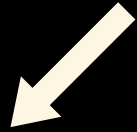
RNA Tagging Measures Transcription and Splicing



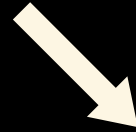
Throughput: 1 gene per year

Design of a Live HT Single Cell Assay

AIM: Increase the Throughput of
Transcription Dynamics Measurements



Live HT
Microscopy
Platform



HT Tracking of Nuclei and RNA-
binding-protein Spots

High-Throughput Live Cell Imaging Acquisition

Yokogawa CV7000S



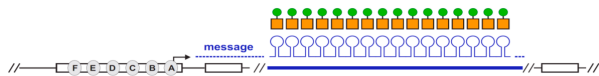
- 96-well plates
- 40X air or 60X water obj.
- Projected 5 -15 slices Z-stack
- Frame Interval: 100 s
- Up to 6 Wells, 6 Fields
- Up to 680 frames (~ 19 hrs)
- ~20,000 images
- 1276x1076 pixels

HT Live Imaging of GR Transcription at Single Sites



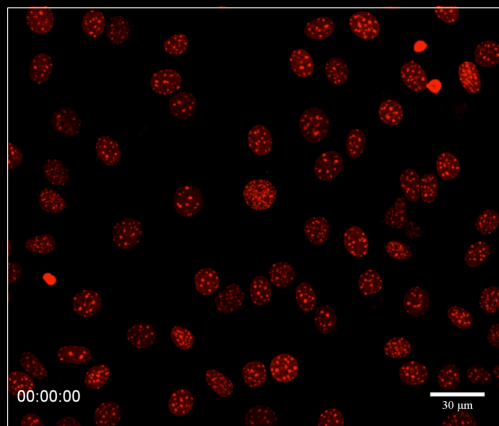
Diana Stavreva
Hager Lab, NCI

Integrated MMTV
promoter with PP7 cassette

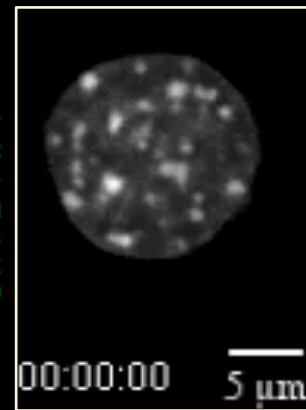


Responsive to Glucocorticoid
Receptor (GR) binding hormones

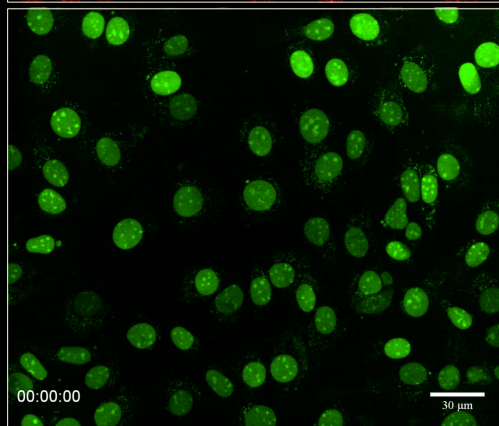
SiR-DNA



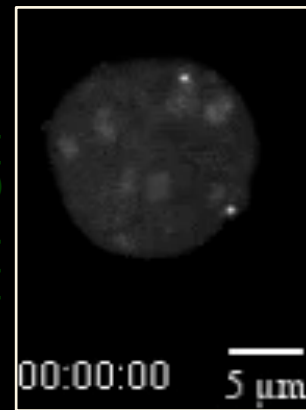
SiR-DNA



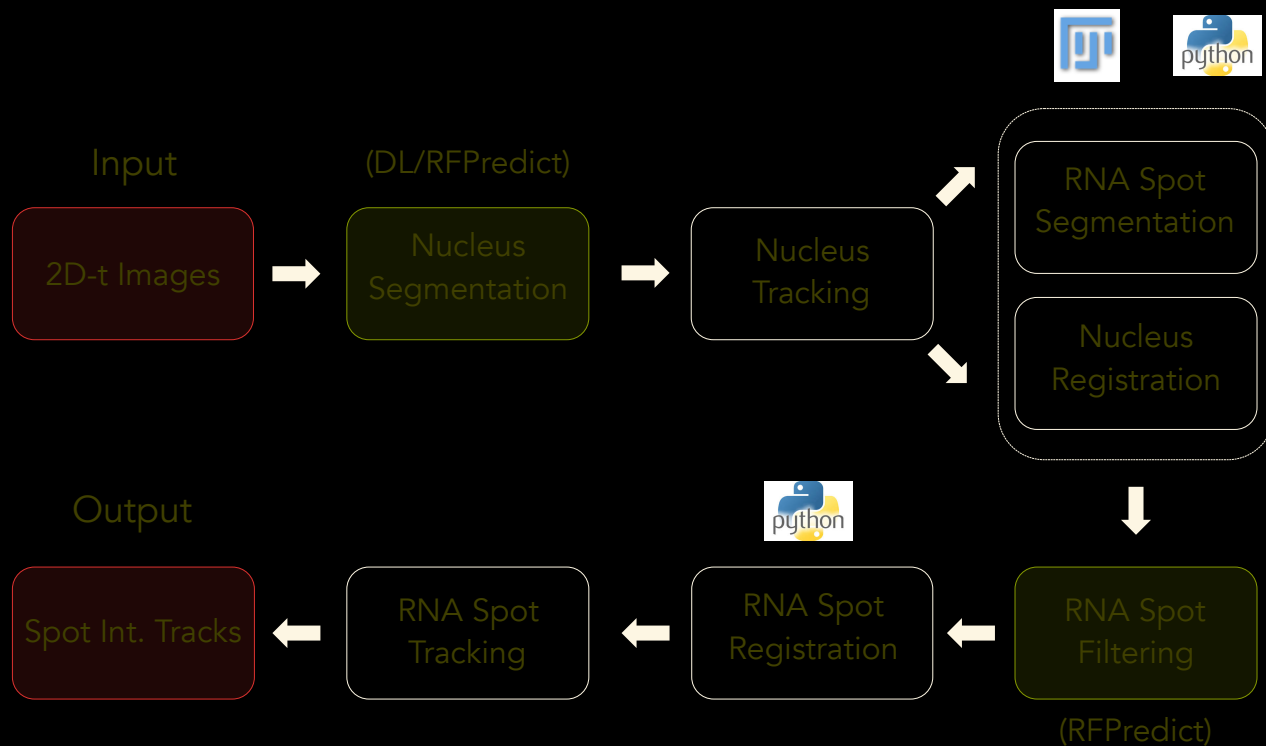
PP7-GFP



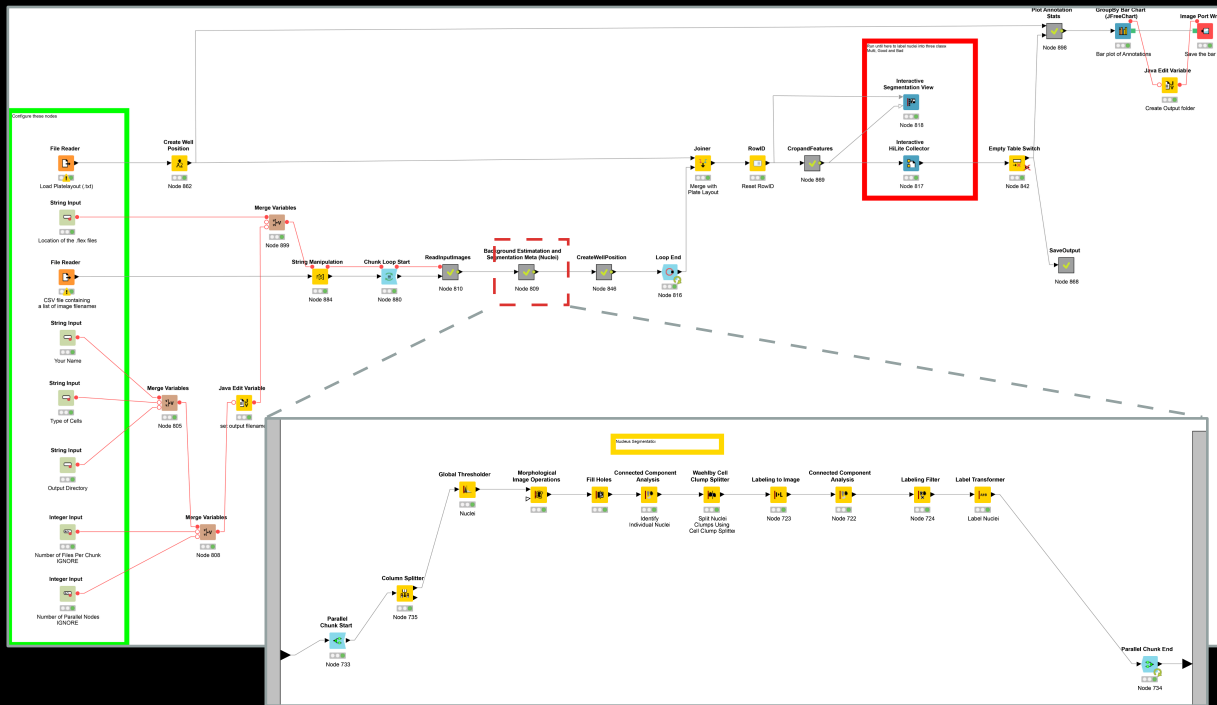
PP7-GFP



HCA Pipeline Workflow



KNIME for High-Content Image Analysis



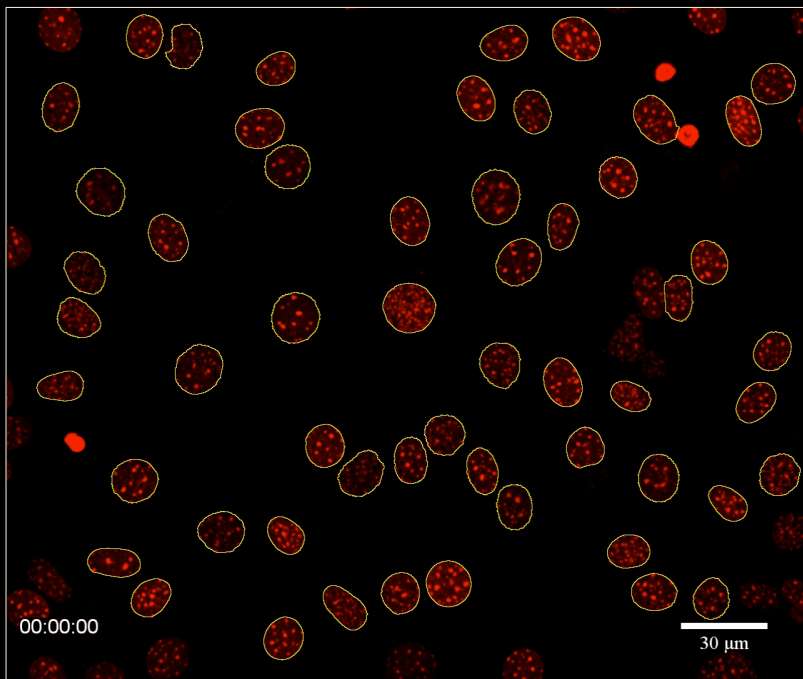
- Open source
- GUI / Web* / Headless
- Platform agnostic:
 - Desktop (Mac/PC)
 - Windows Server
 - Linux HPC
- Reusable image processing code:
 - KNIP
 - ImageJ
 - Matlab/Python/Etc



Adapted from: Jan Eglinger, KNIME Summit, Spring 2017

Nucleus Segmentation and Tracking

Segmentation



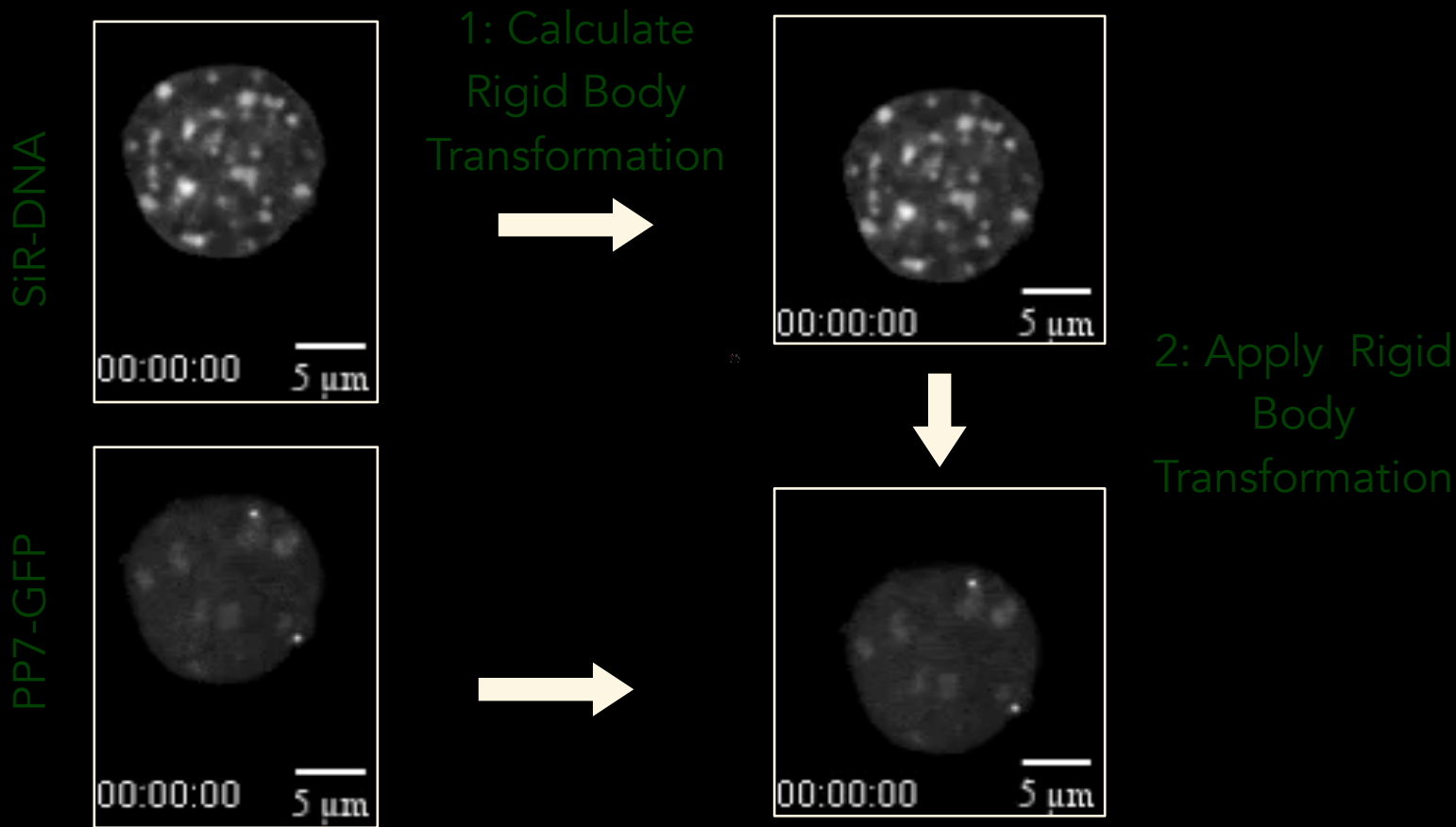
Seeded Watershed +
Random Forest Classifier

Tracking



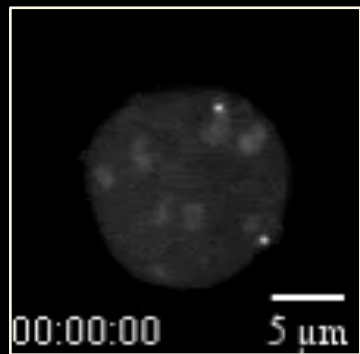
LAPTracker

Nucleus Registration



Spot Detection and Tracking

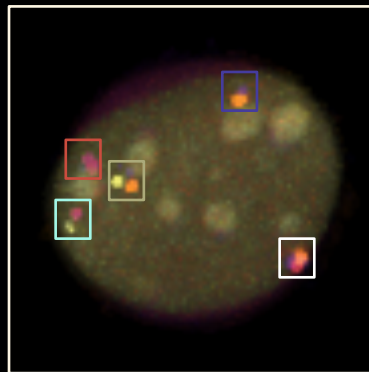
Registered Nucleus



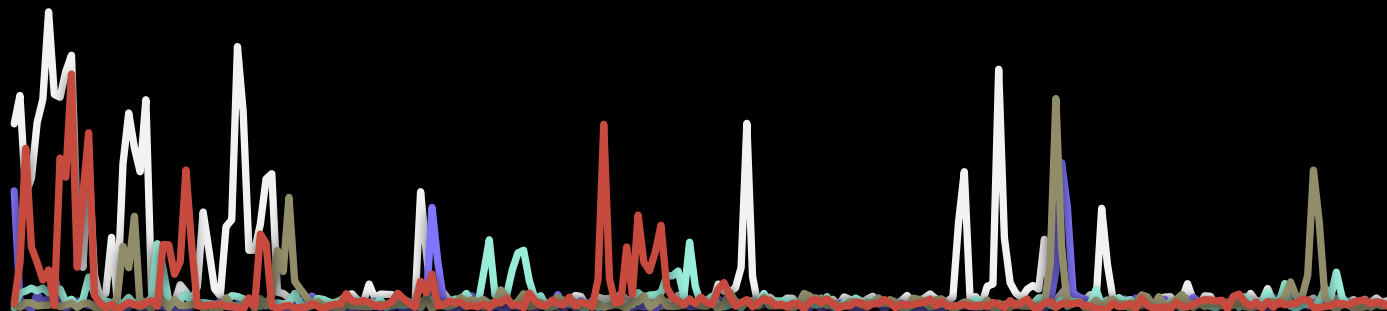
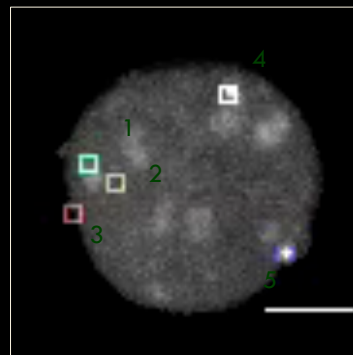
Spot Detection
+
LAPTracker



Temporal Coding



Interpolated Tracks

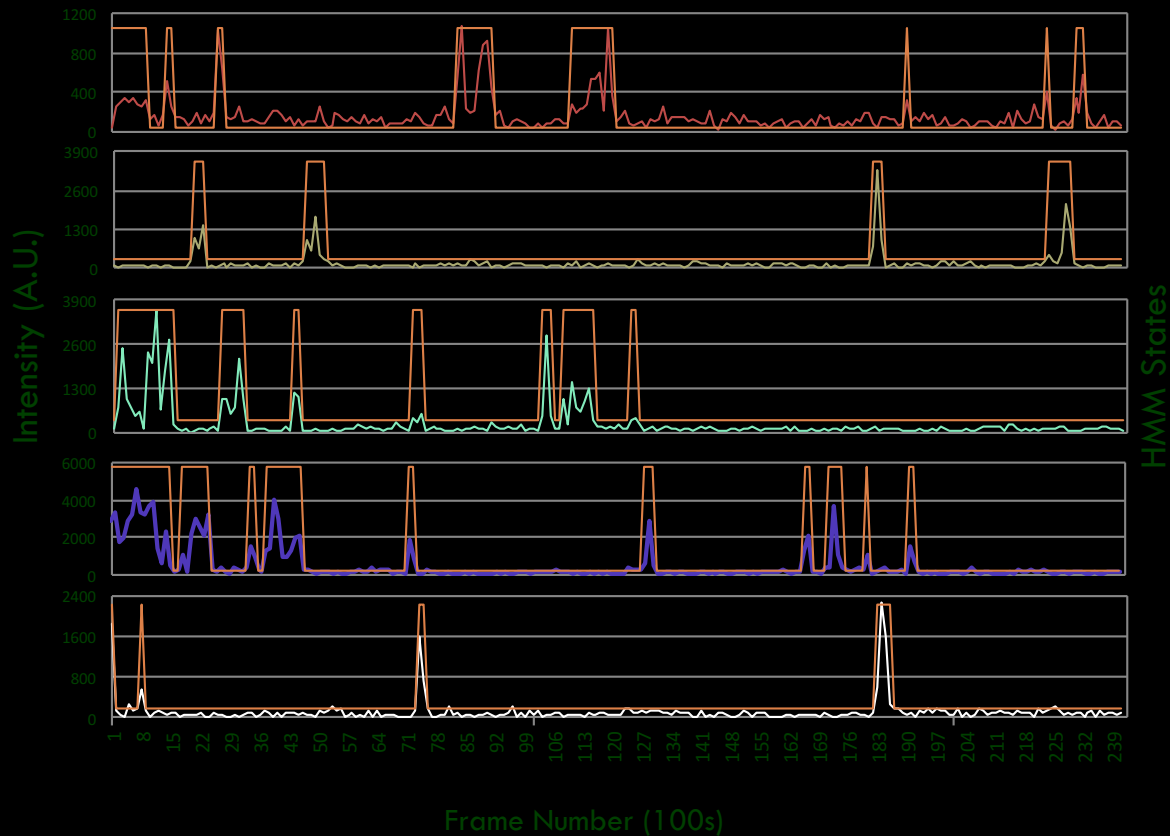
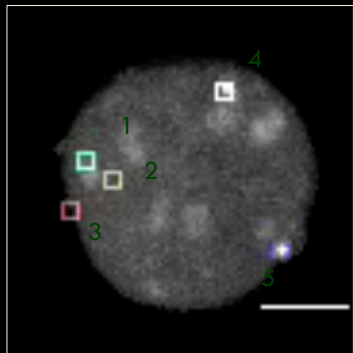


— Track 1
— Track 2
— Track 3
— Track 4
— Track 5

Two-state HMM Modeling

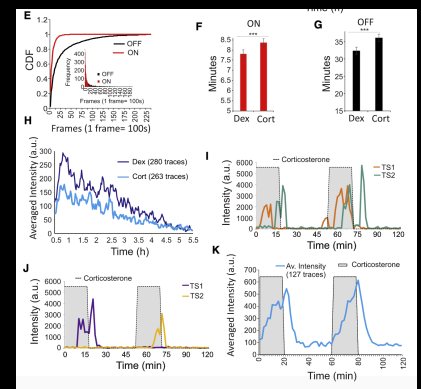
PP7-GFP

Interpolated Tracks

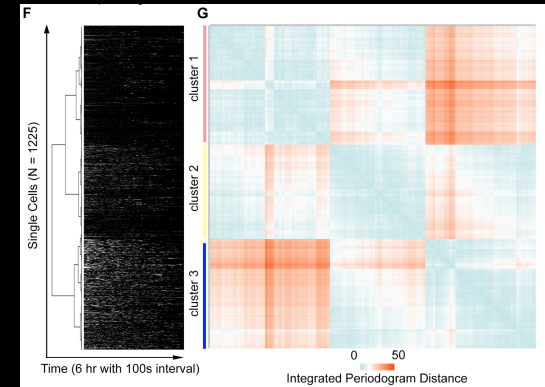


Workflow Applications

- Analysis of GR transcription dynamics (Stavreva *et al.*, Mol Cell, 2019)

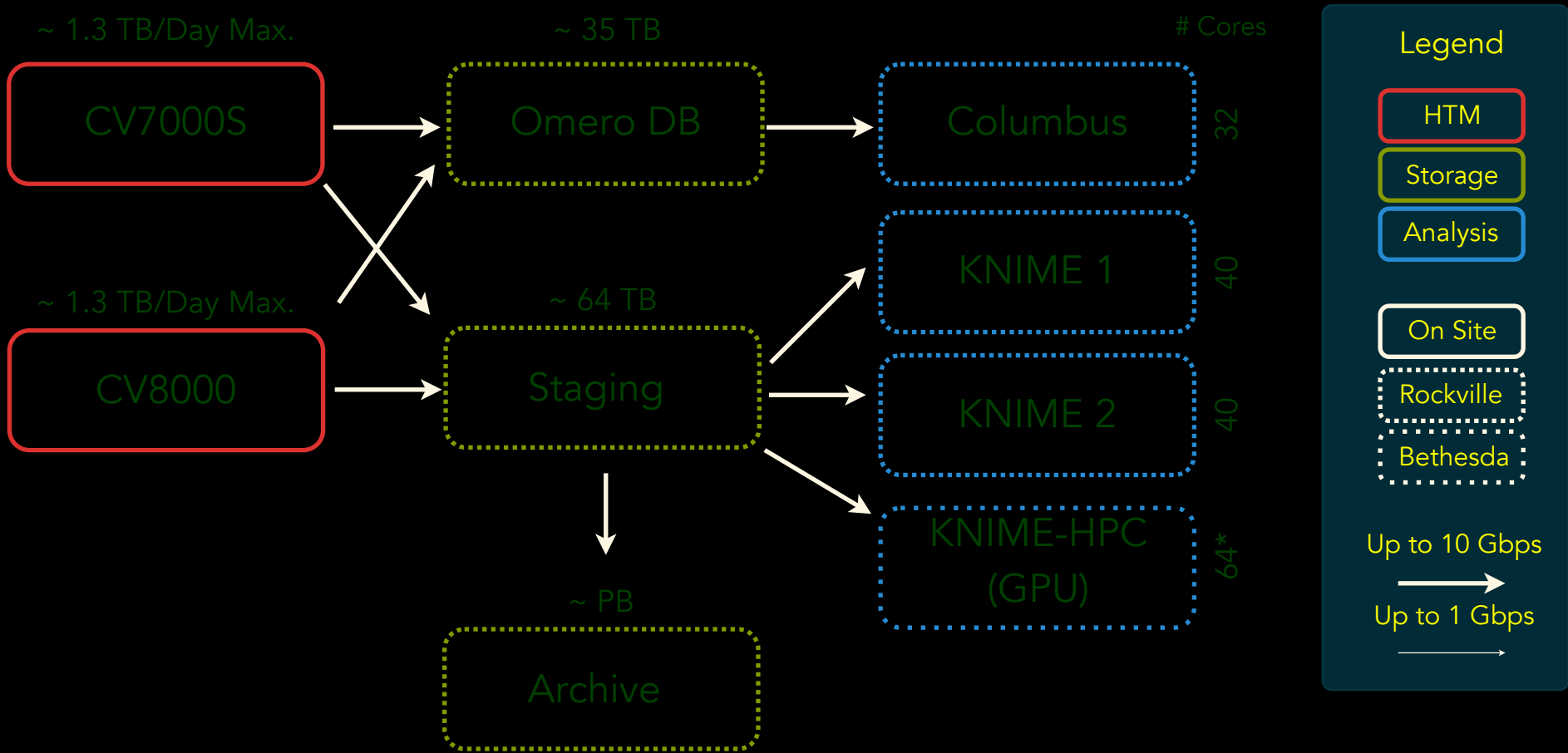


- Semi-genome wide Imaging of endogenous gene expression (Wan *et al.*, submitted)



- Secondary validation of RNA-FISH based chemical- and functional-genomics screens.

3. HiTIF Image Bioinformatics Infrastructure



Acknowledgements

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HiTIF

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