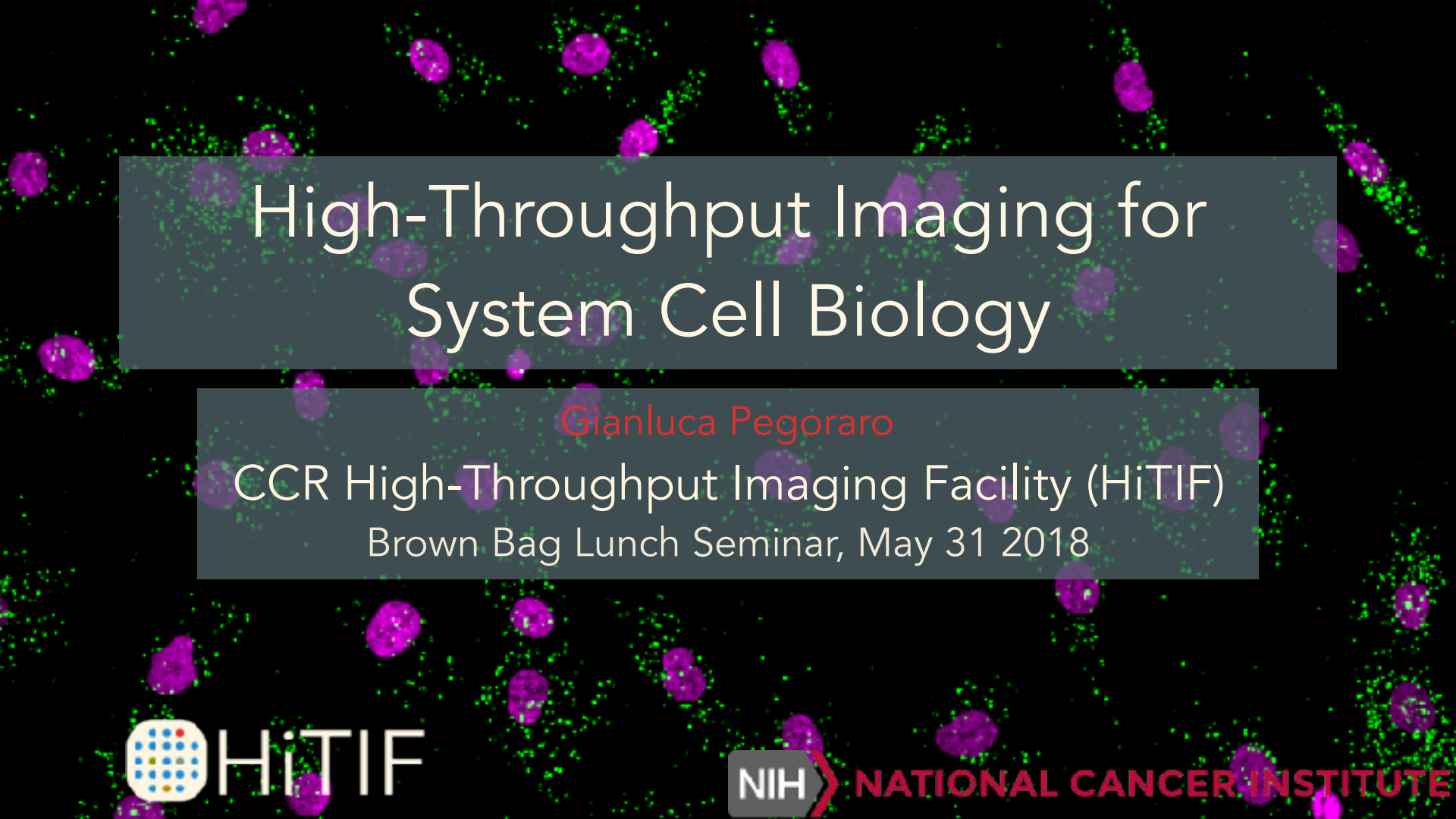


High-Throughput Imaging for System Cell Biology

Gianluca Pegoraro

CCR High-Throughput Imaging Facility (HiTIF)

Brown Bag Lunch Seminar, May 31 2018

HiTIF

NIH NATIONAL CANCER INSTITUTE

Outline

1. Introduction to HiTIF/HTI
    i. High-Throughput Microscopy
    ii. High-Content Image Analysis
2. Image Analysis Pipeline for HT live cell tracking
3. HT Imaging of RNA

High-Throughput Imaging (HTI)

Investigator

Experimental perturbation → Imaging-based cellular assay → Phenotypic change

HiTIF

Automated liquid handling

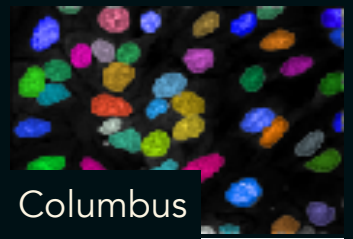
Janus

EL406

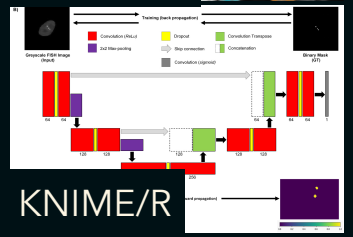
High-throughput microscopy

CV7000

High-content image analysis



Columbus

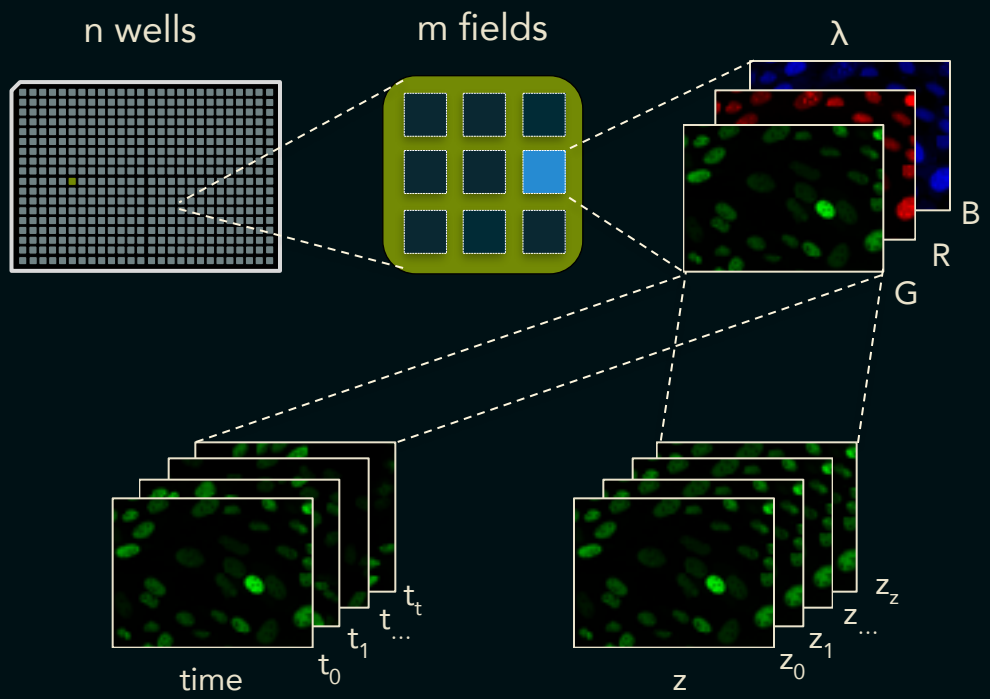


KNIME/R

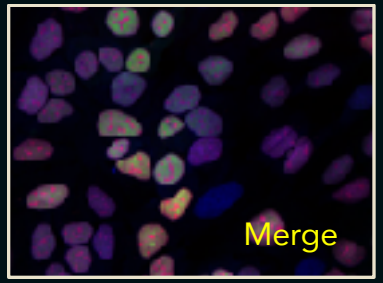
Up to:

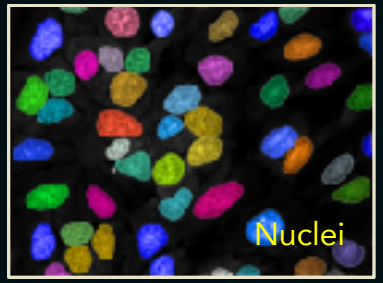
- 10^4 Wells
- 10^4 Cells/Well
- 10^2 Feat./Cell

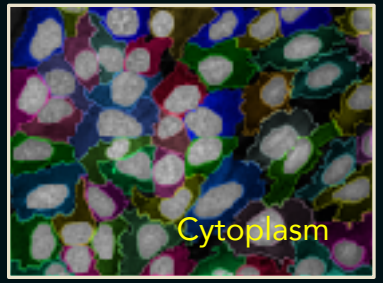
High-Throughput Acquisition and Analysis

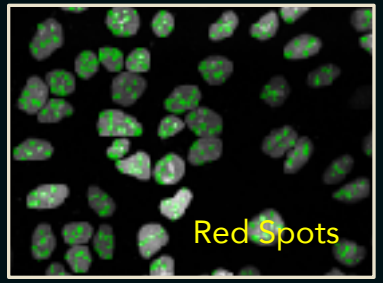


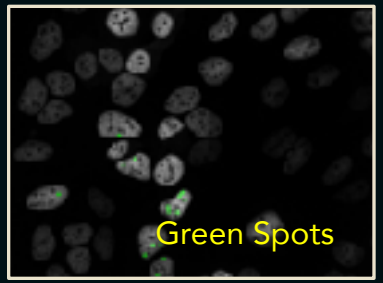
2D images/day = n * m * λ * z * t ≈ *up to* 10^5











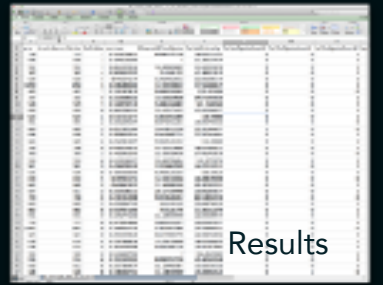
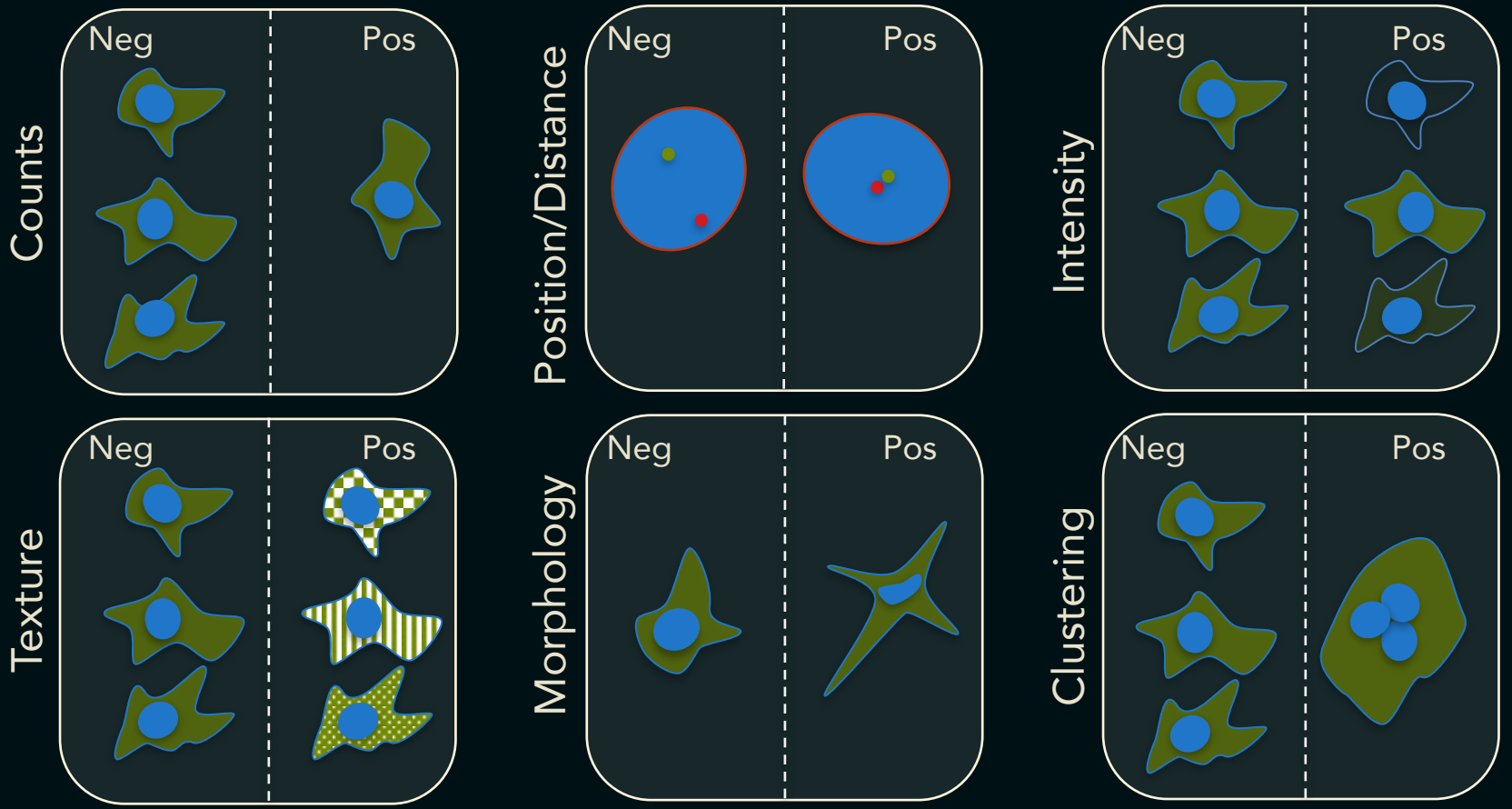
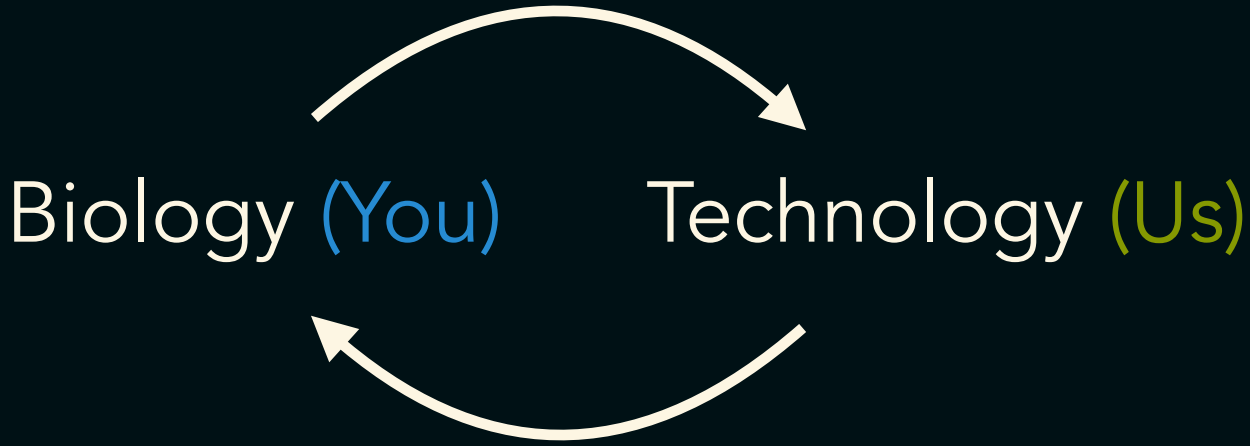


Image Analysis Features Classes



HiTIF Mission

HiTIF collaborates with CCR and NIH Investigators and provides them with the necessary expertise, instrumentation and software to perform systematic cell biology experiments via high-throughput imaging (HTI)



Perturbation Reagents Libraries

- Chromatin (521 genes)
- DNA Damage (582 genes)
- Nuclear Envelope (346 genes)
- Ubiquitin Ligases (~600 genes)
- Kinases/Phosphatases (~700 genes)
- Proteasome Pathways (~1,000 genes)
- Genome (~19,000 Genes)*
- Chromatin-related (300 Compounds)*

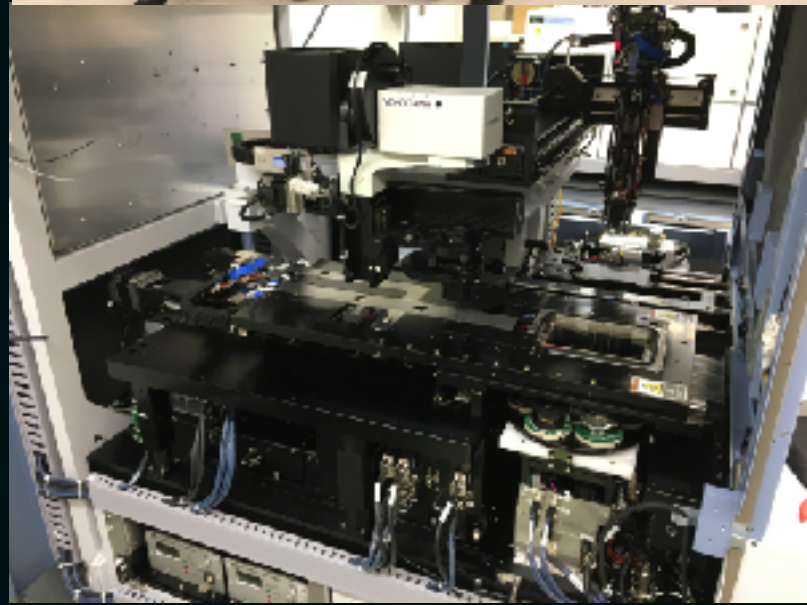
* : In collaboration with NCATS RNAi Screening Platform

Automated liquid handling

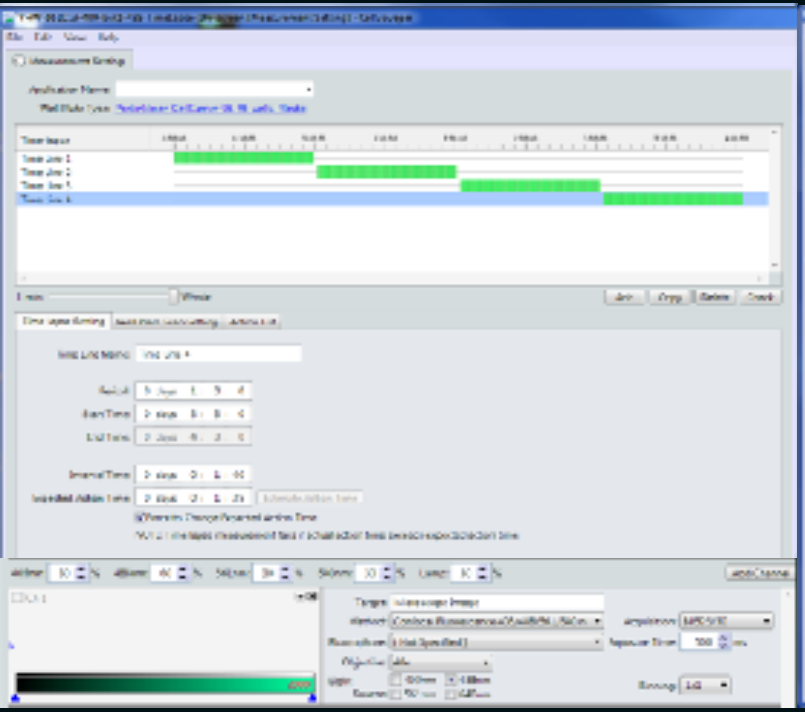
Yokogawa CV7000S

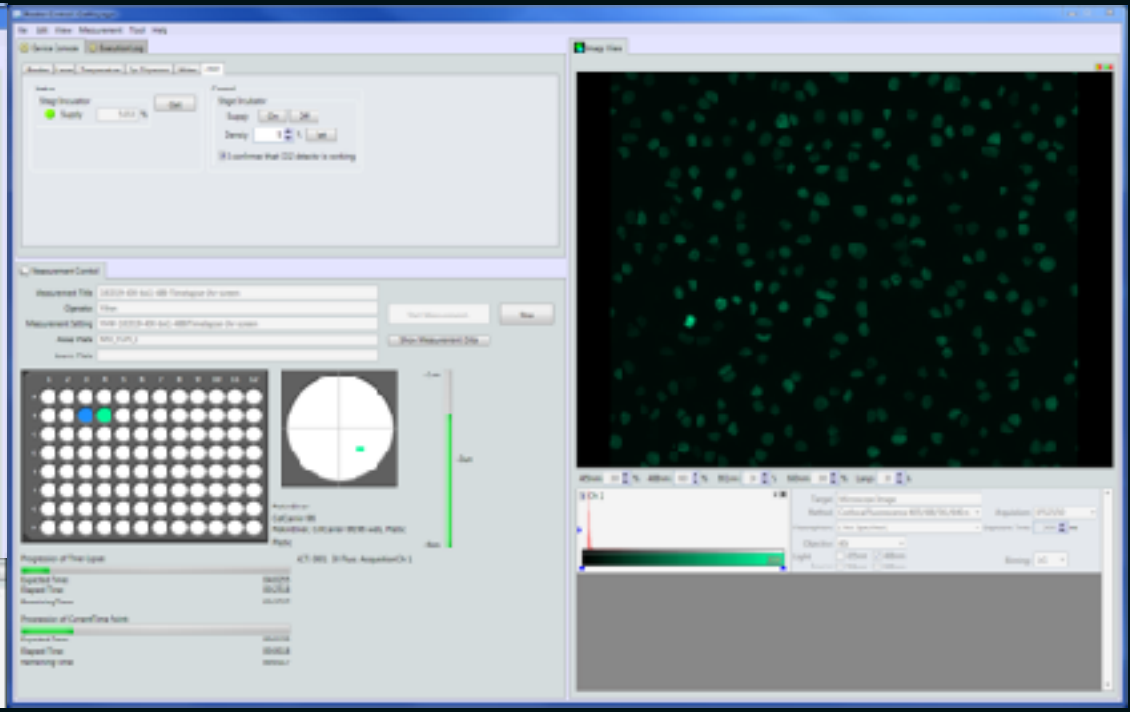
- CSU-W1 Nipkow spinning disk
- 2 sCMOS cameras (2556 X 2156 pixels)
- 4 Ex. Lasers: 405, 488, 561, 640 nm
- 4X, 10X, 20X (0.75), 40X (0.95), 60Xw (1.2) lenses
- Confocal, Epifluorescence, DPC, Brightfield
- Up to 72 hrs live cell imaging

High-throughput microscopy



Serial Time-Lapse Experiments





High-throughput microscopy

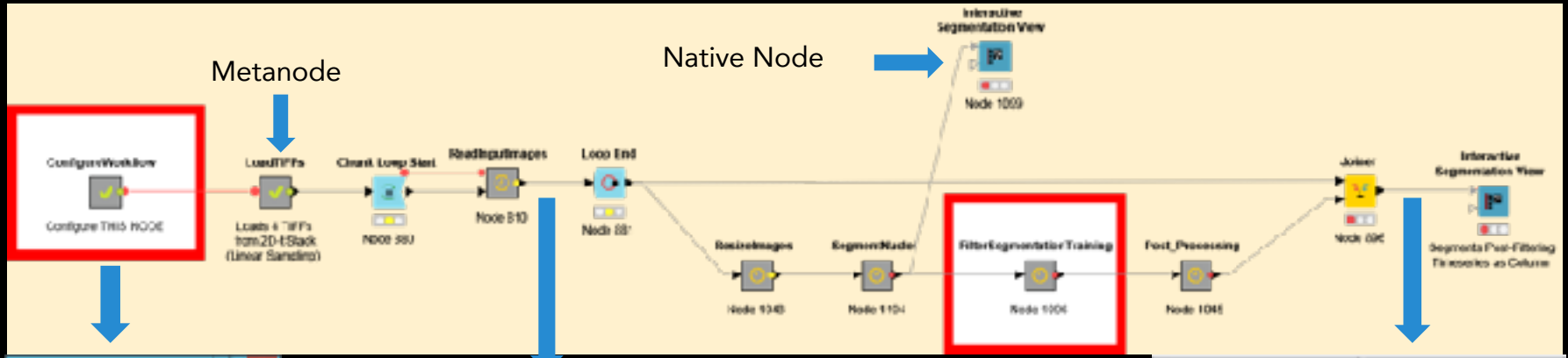
Open Source High-Content Analysis Platform

Adapted from: Jan Eglinger, KNIME Summit, Spring 2017

- Open source, reproducible
- Image processing (KNIP), data analysis, scripting nodes
- GUI (Workflow) and command line (Headless)
- Platform-independent: Desktop, Linux Server, HPC (Biowulf/NIH)

High-content image analysis

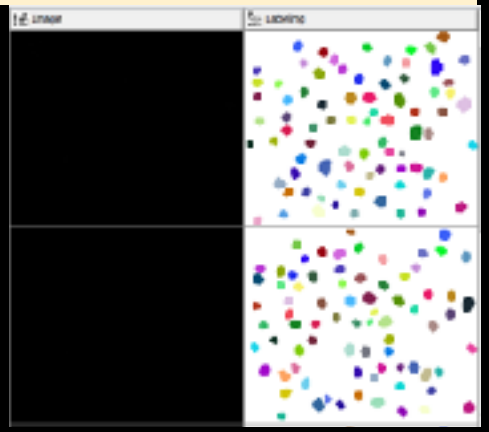
KNIME/KNIP for HCA



Chunking

Chunking

Parallel Processing



High-content image analysis

HiTIF Provides Advanced HTI Workflows

Automated liquid handling → **High-throughput microscopy** → **High-content image analysis**

- siRNA Library Management
- Automated siRNA transfections
- Semi-automated IF/FISH Methods

- High-magnification
- 3D SD Confocal
- 4-channels
- Live-cell

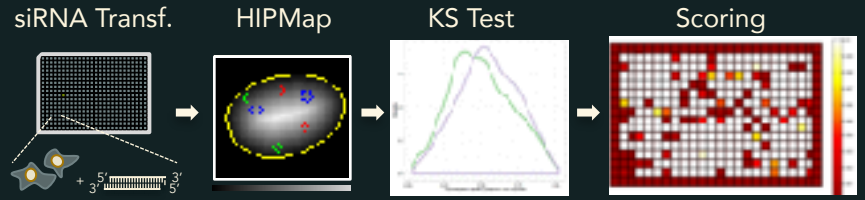
- Custom Image Analysis Pipelines
- Custom single-object data analysis
- siRNA Screen Stats

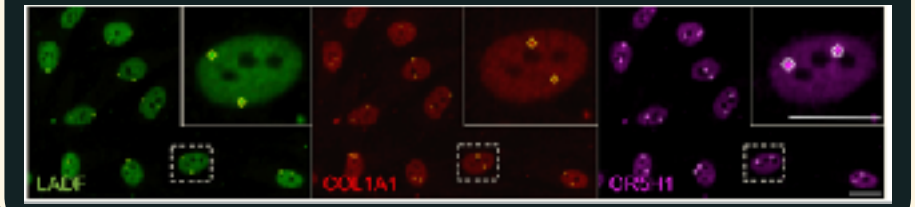
HiTIF Assays Formats

Focused siRNA Screens

Genomic locus positioning

(Shachar et al., 2015a, 2015b; Ji, 2016)

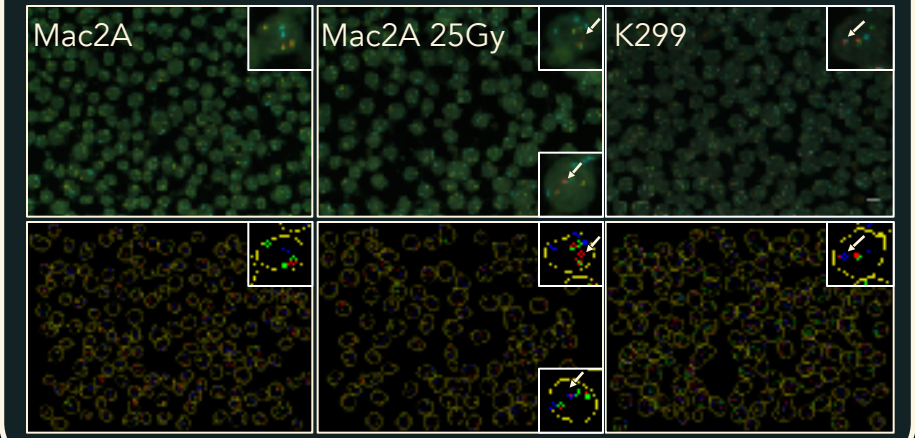




Hypothesis-based

Chromosomal translocations

(Roukos et. al, 2013; Burman et al., 2015a, 2015b)



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