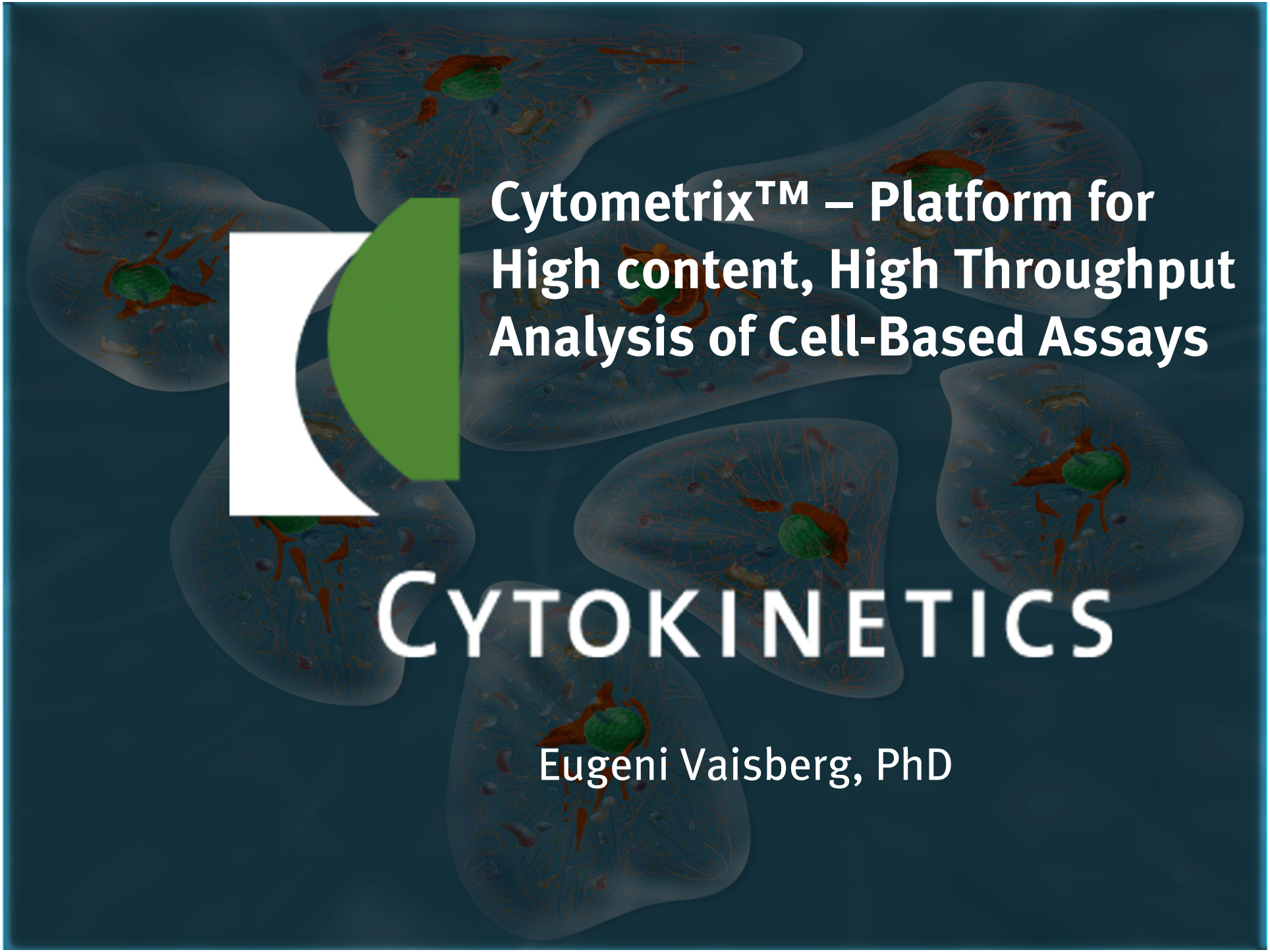

Cytometrix™ – Platform for High content, High Throughput Analysis of Cell-Based Assays

CYTOKINETICS

Eugeni Vaisberg, PhD

Overview

- Cytometrix analyses
- Examples of use
  - Characterization of on- and off-target compound effects
  - Compound profiling
  - Phenotype-based screening

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Multi-Level Cytometrix Analyses

- Image analysis and feature extraction
- Plate level standardization
- Cell population analysis
- Extensive automated and semi-automated quality control
- Data mining and high level analysis
  - Multiple applications – from primary screening to lead optimization, compound profiling, and prediction of clinical toxicities
- Analyses are designed to be resistant to process variations. No adjustments are necessary at run time.

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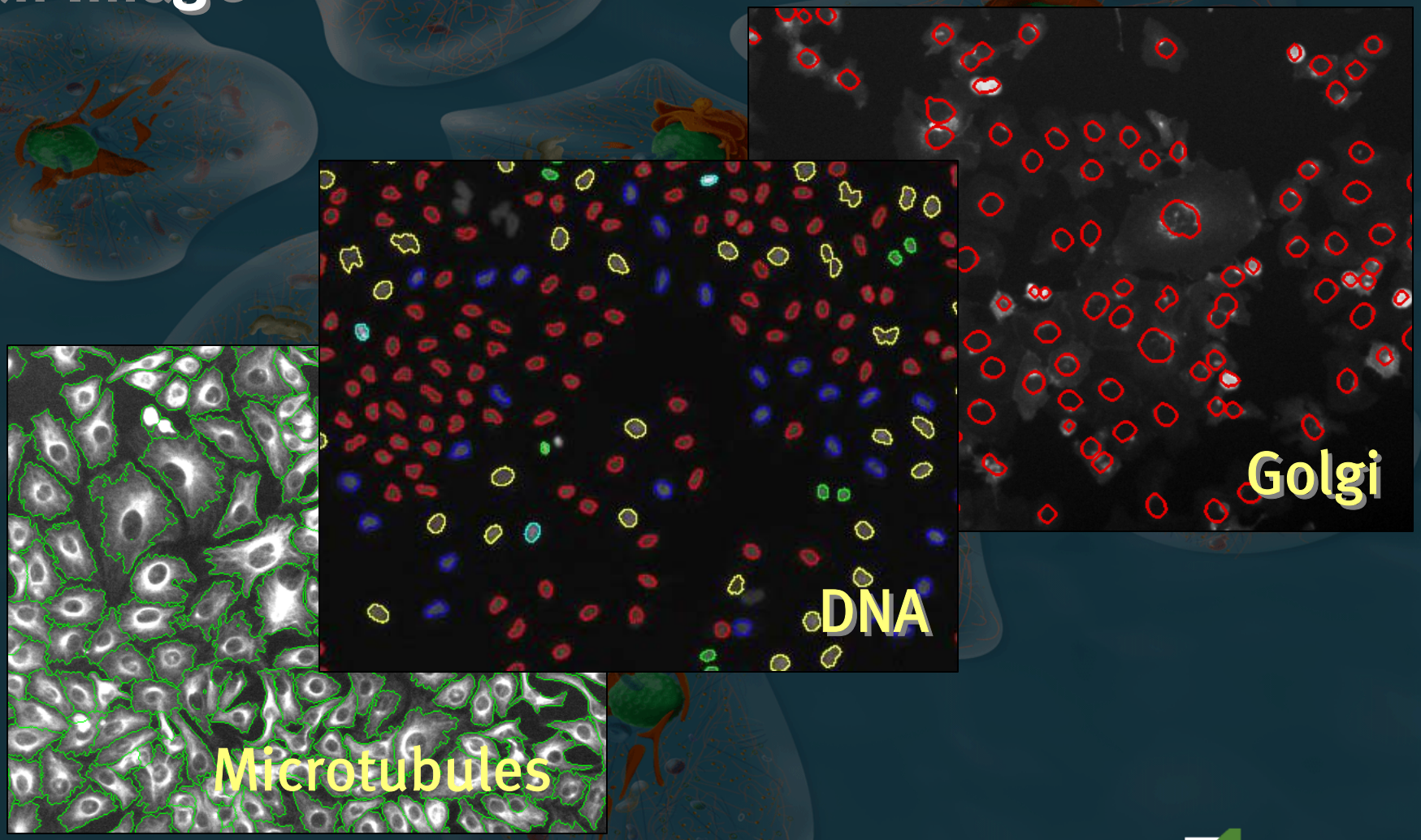
Features Are Extracted for Each Object in an Image



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Cell-Level Image Analysis

- Image preprocessing
  - Correction of platform-dependent image artifacts
- Segmentation
  - Robust identification of cells and cell domains
- Feature extraction
  - Shape, intensity, and texture-related features are extracted for each object identified in an image
  - Approximately 20 features for each object-marker combination
  - 50-80 features per cell
- Algorithms do not require “tuning” at run time
- Segmentation results and extracted features for each of the image objects are stored for subsequent analyses

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Well Level QC and Deselection

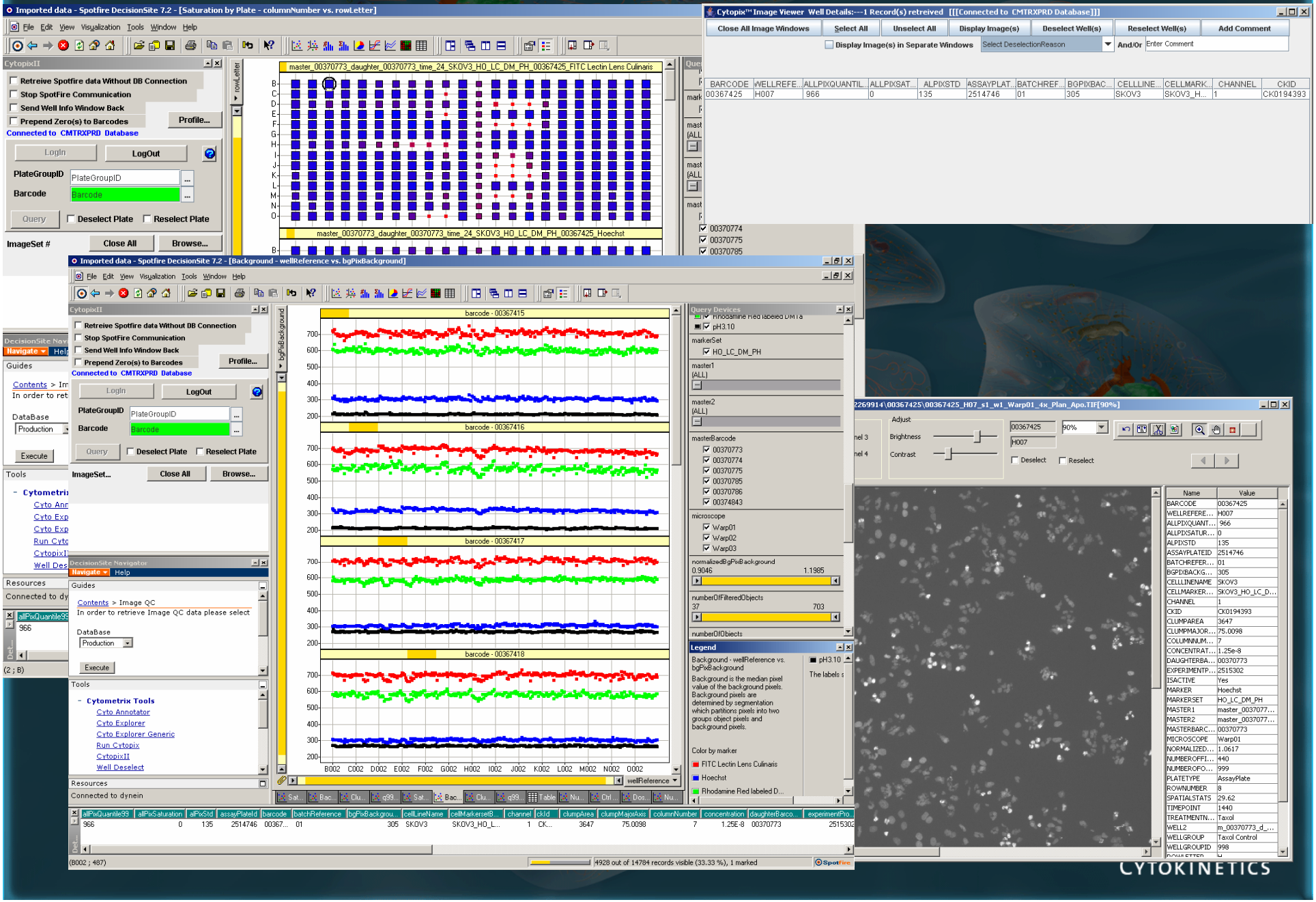
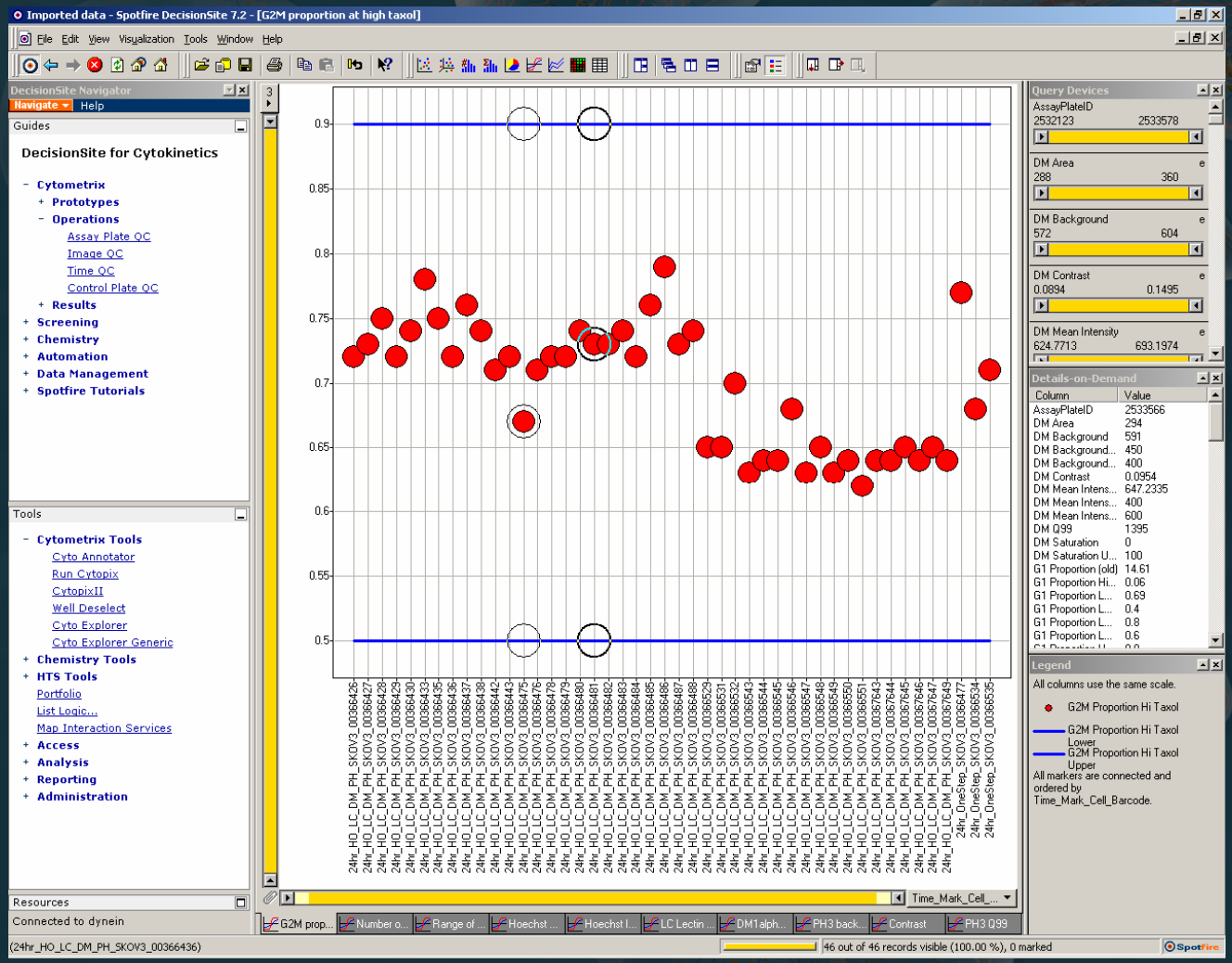


Plate-Level QC and Deselection



Based on positive and negative plate controls
Examples of features used for QC:

- cell count
- proportion of live cells
- proportion of fuzzy cells
- background intensity for a channel
- object intensity for a channel
- contrast for a marker
- feature variation for control wells

etc

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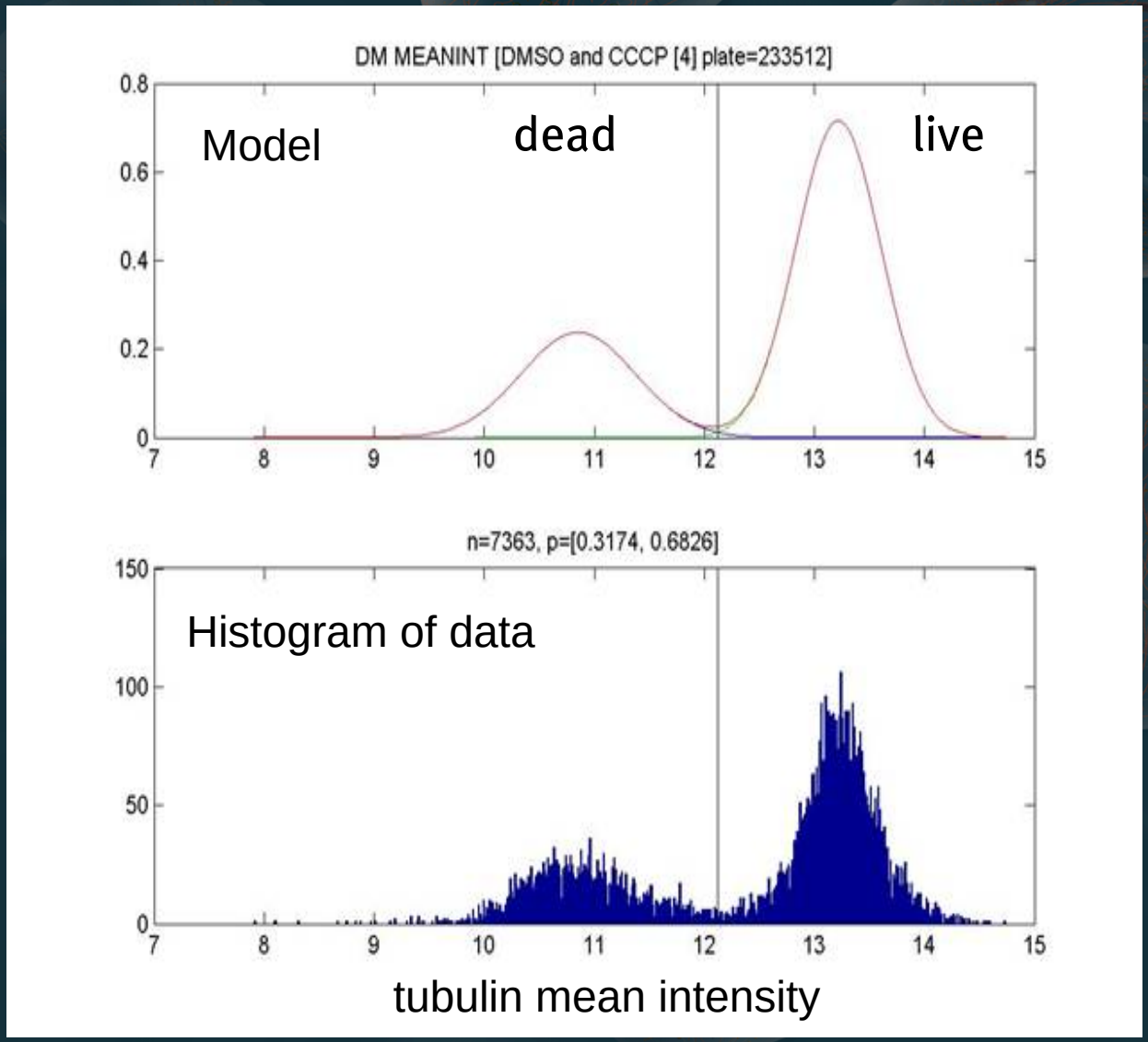
Focus on Sub-Populations of Cells (live/dead classification example)



Model is built on cells from multiple positive (CCCP) and negative (DMSO) controls.

EM algorithm fits 2 normal densities and computes estimates of proportions of cells with different intensity of tubulin staining

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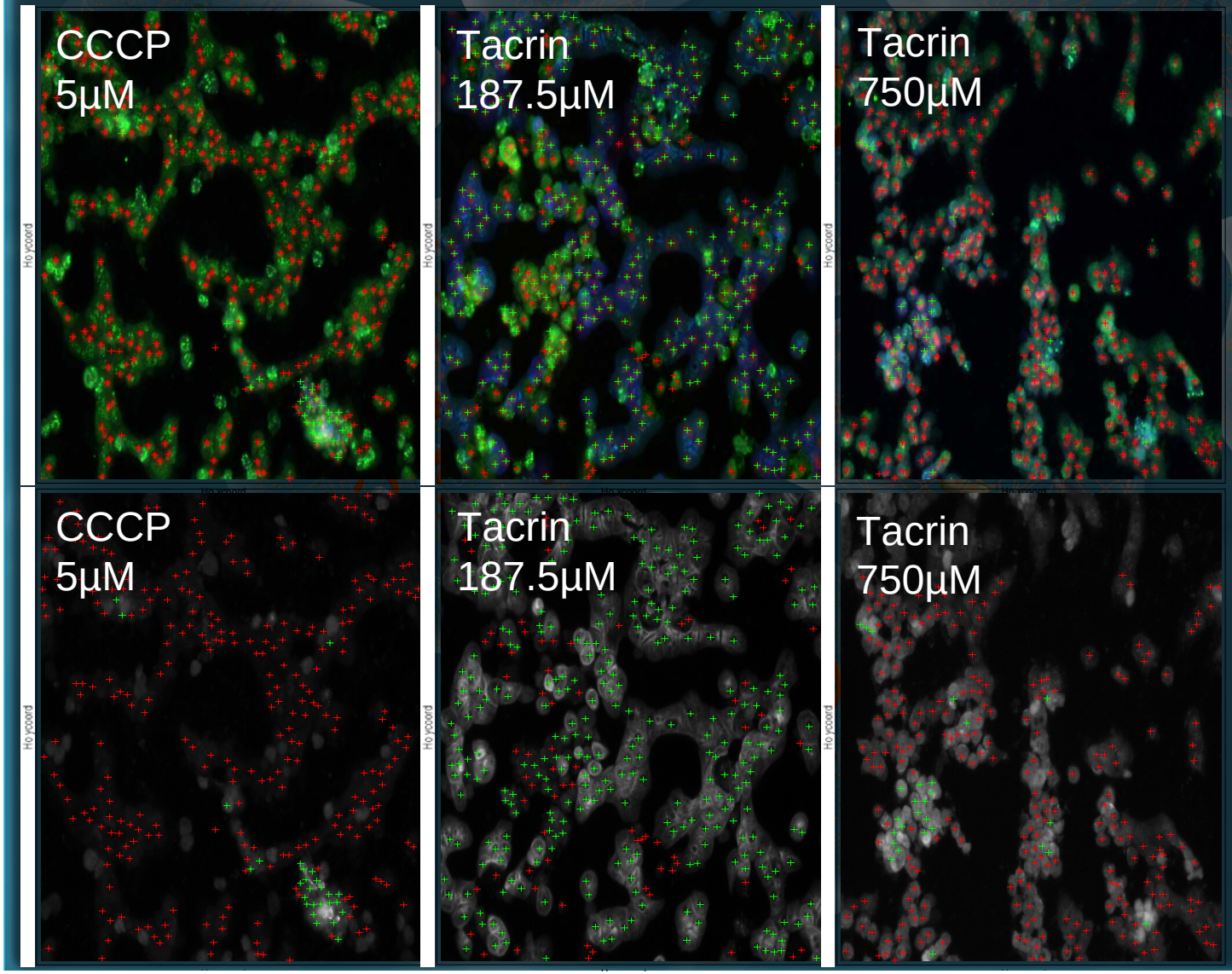
Focus on Sub-Populations of cells (live/dead classification)



Resulting model is applied to every cell on a plate

This allows to analyze properties of live and dead cells independently

+ Dead

+ Live

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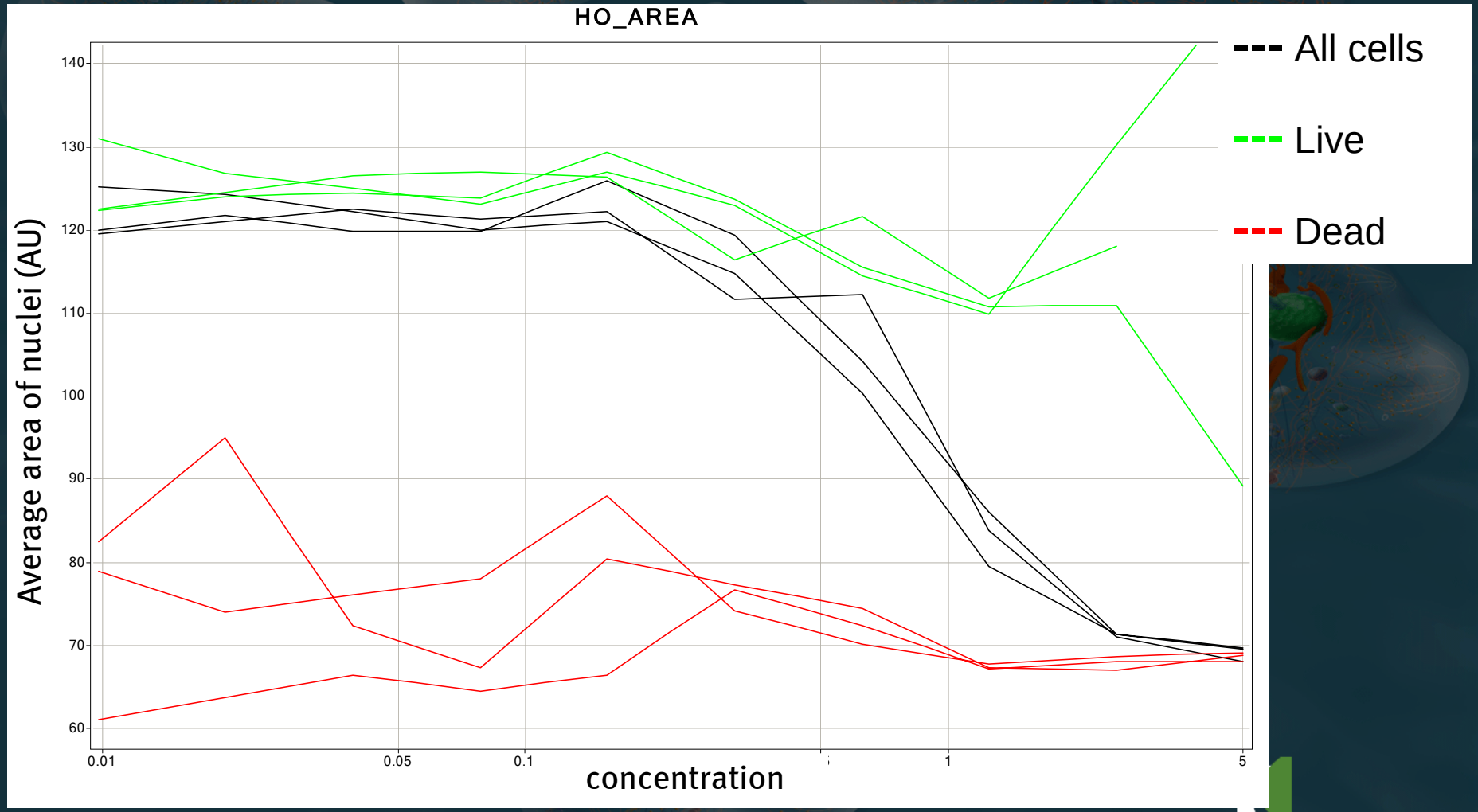
CCCP-induced changes in nuclear size in rat hepatocytes



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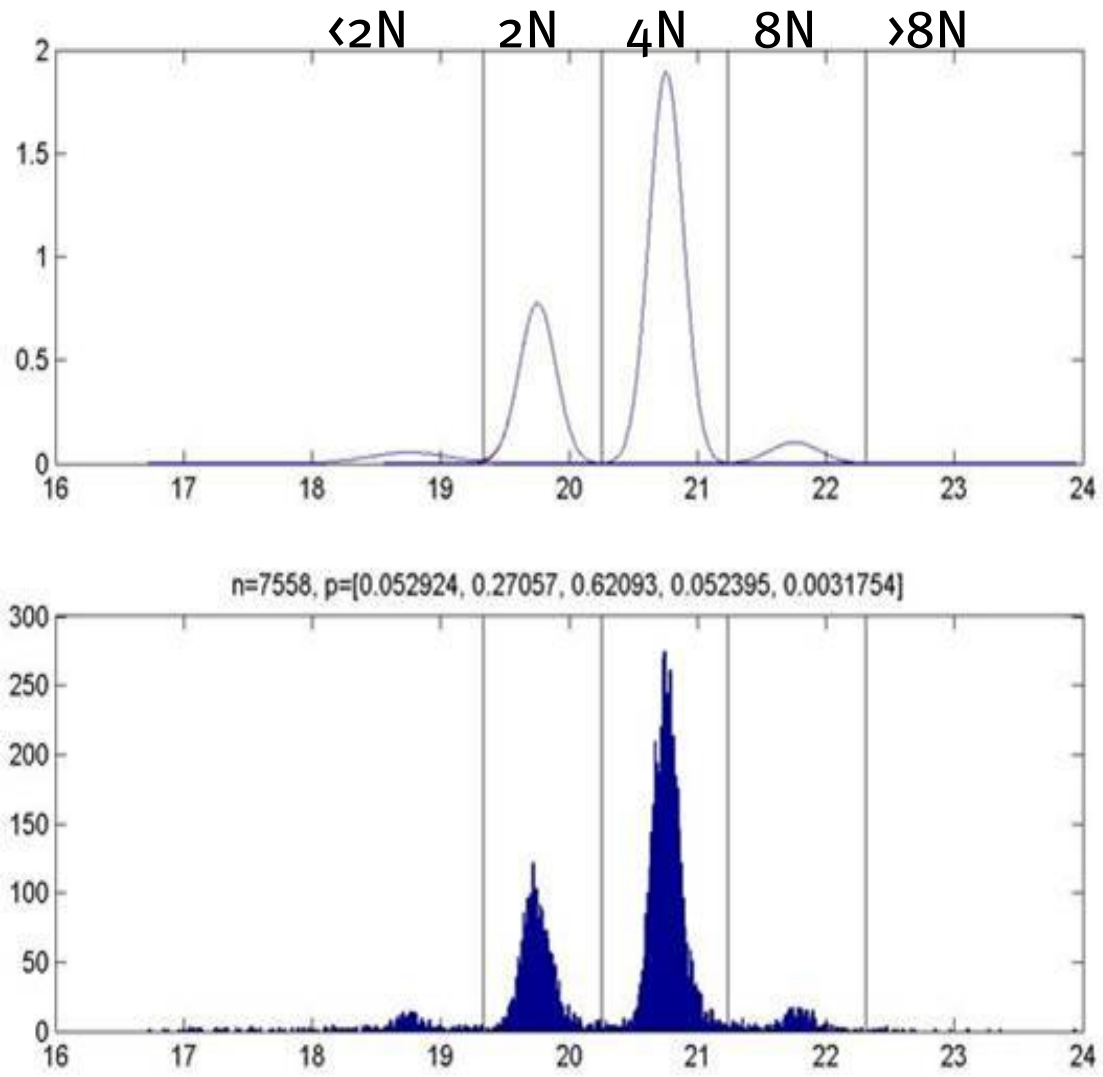
Identification of cells with different ploidy cell cycle



EM algorithm fits 5 normal densities and computes estimates of proportions of cells with different ploidy.

Similar approach is used to identify cells at different stages of cell cycle.

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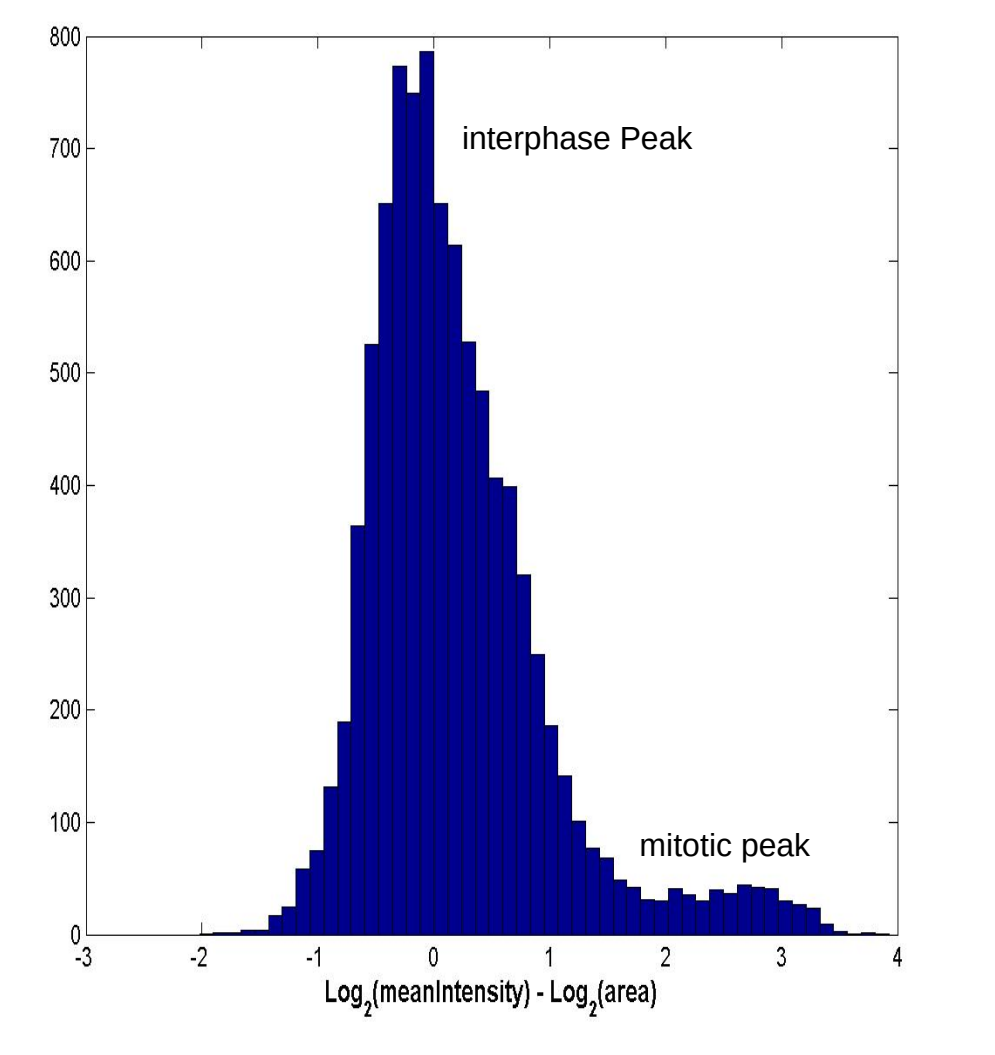
Identification of cells at different stages of cell cycle



EM algorithm fits 2 normal densities and computes estimates of proportions mitotic and interphase cells

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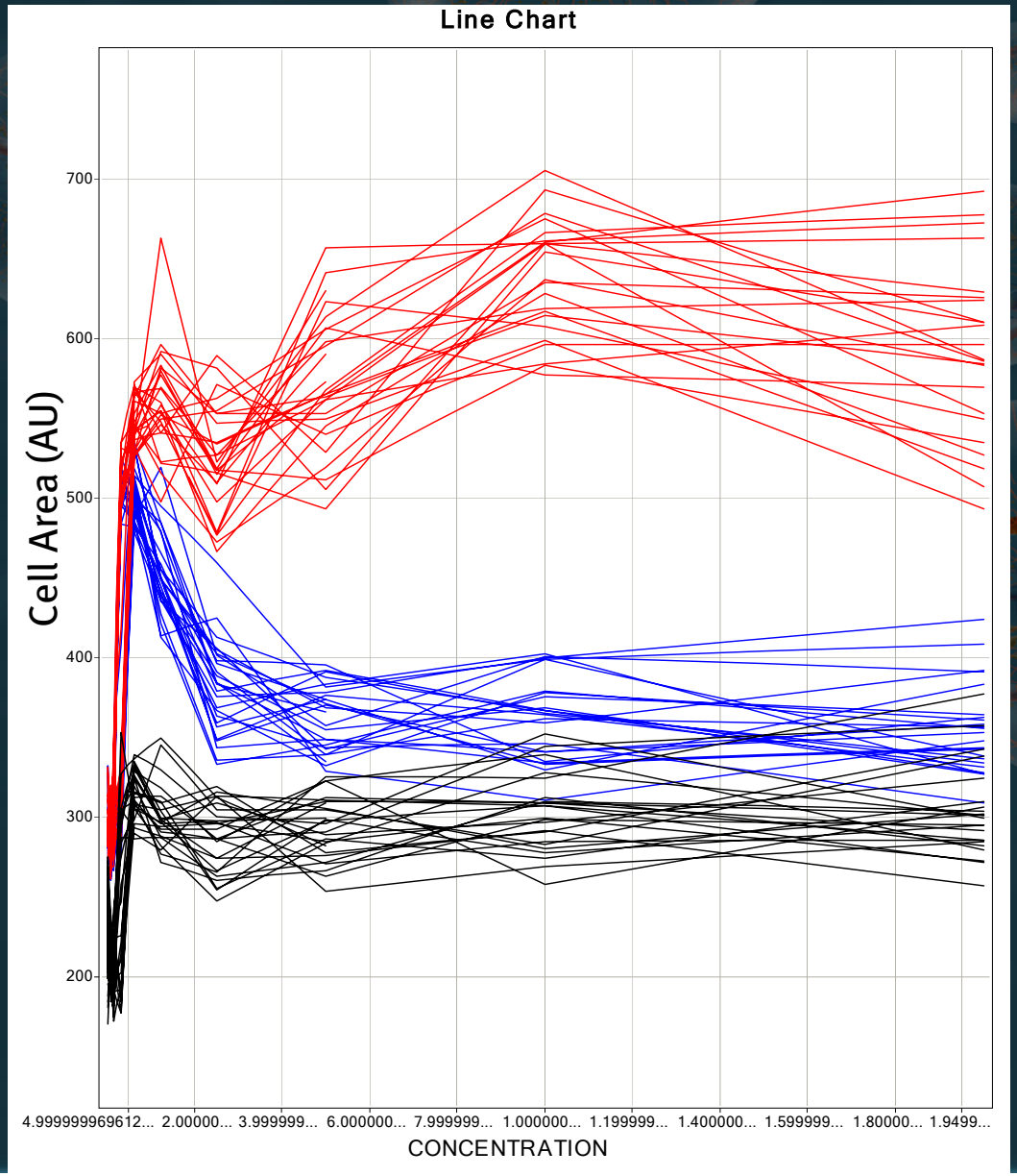
Higher quality results from subpopulation analysis



Apparent Taxol-induced decrease in cell size is due to a shift in cell cycle status (accumulation of mitotic cells).

Area of interphase cells increases with increased Taxol concentrations.

-- All cells
-- Interphase cells
-- Mitotic cells

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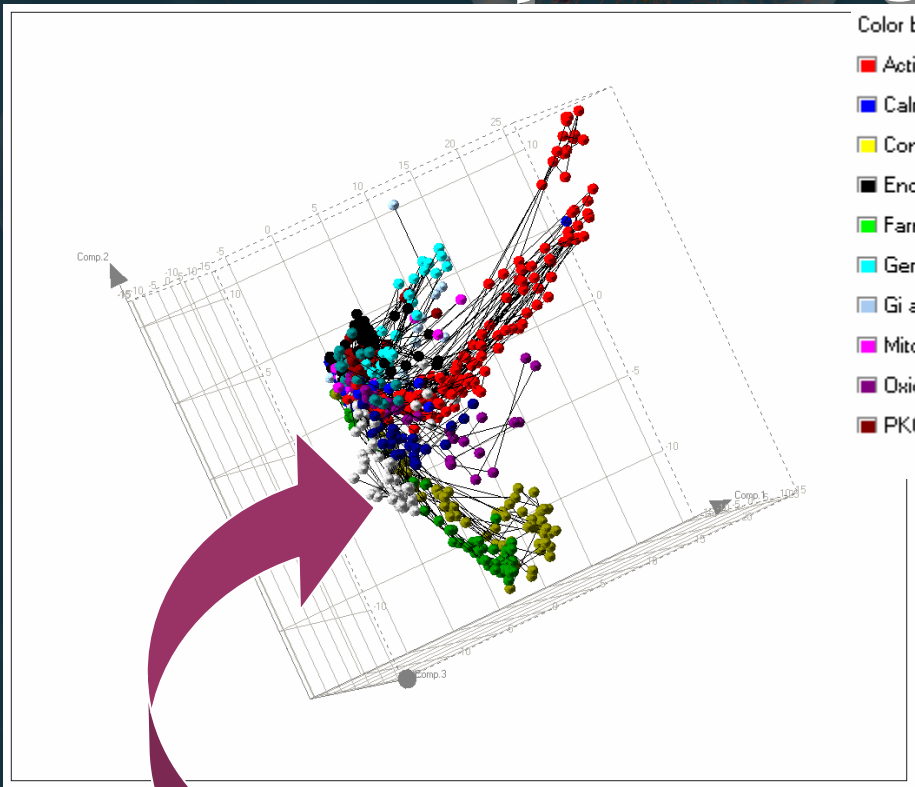
Cytometrix™ Fingerprints Represent Multiple Biological Phenotypes

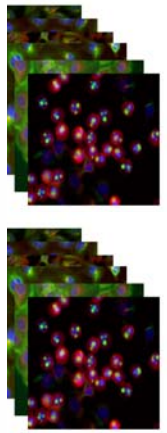


Color by MOA

- Actin inhibitor
- Calmodulin antagonist
- Control
- Endoplasmic reticulum Ca2...
- Farnesyltransferase inhibitor
- Geranylgeranyltransferase ...
- Gi and Go activator
- Mitochonrial ATPases unco...
- Oxidative Phosphorylation ...
- PKC activator
- Topoisomerase II inhibitor
- Tubulin destabilizer
- Tubulin stabilizer
- Vacuolar-type H+-ATPase i...
- p34cdc2/cyclin B inhibitor
- p38 MAP kinase inhibitor

Markers are connected by TR.GR.ID, and ordered by CONCENTRATION.

The labels show TREATMENTNAME



ach concentrations of a compound
is represented by 72 Images:
triplicates of
6 cell lines x 2 marker sets

0.607112	0.755361	0.402051	0.676631	0.493887	0.044729	0.744009	0.127986	0.164904	0.733524
0.654774	0.415216	0.890765	0.75875	0.94049	0.732281	0.148215	0.834312	0.984625	0.211816
0.141281	0.121671	0.813961	0.086101	0.050186	0.11433	0.454915	0.930839	0.982997	0.225689
0.280919	0.964064	0.468359	0.320258	0.678557	0.883461	0.706279	0.576434	0.370606	0.93283
0.648271	0.994139	0.488319	0.430738	0.189922	0.544443	0.535019	0.635496	0.413575	0.929208
0.913174	0.48984	0.930526	0.904631	0.594295	0.779588	0.965611	0.472232	0.002068	0.520181

60 values: 6 cell lines x
10 biological phenotypes

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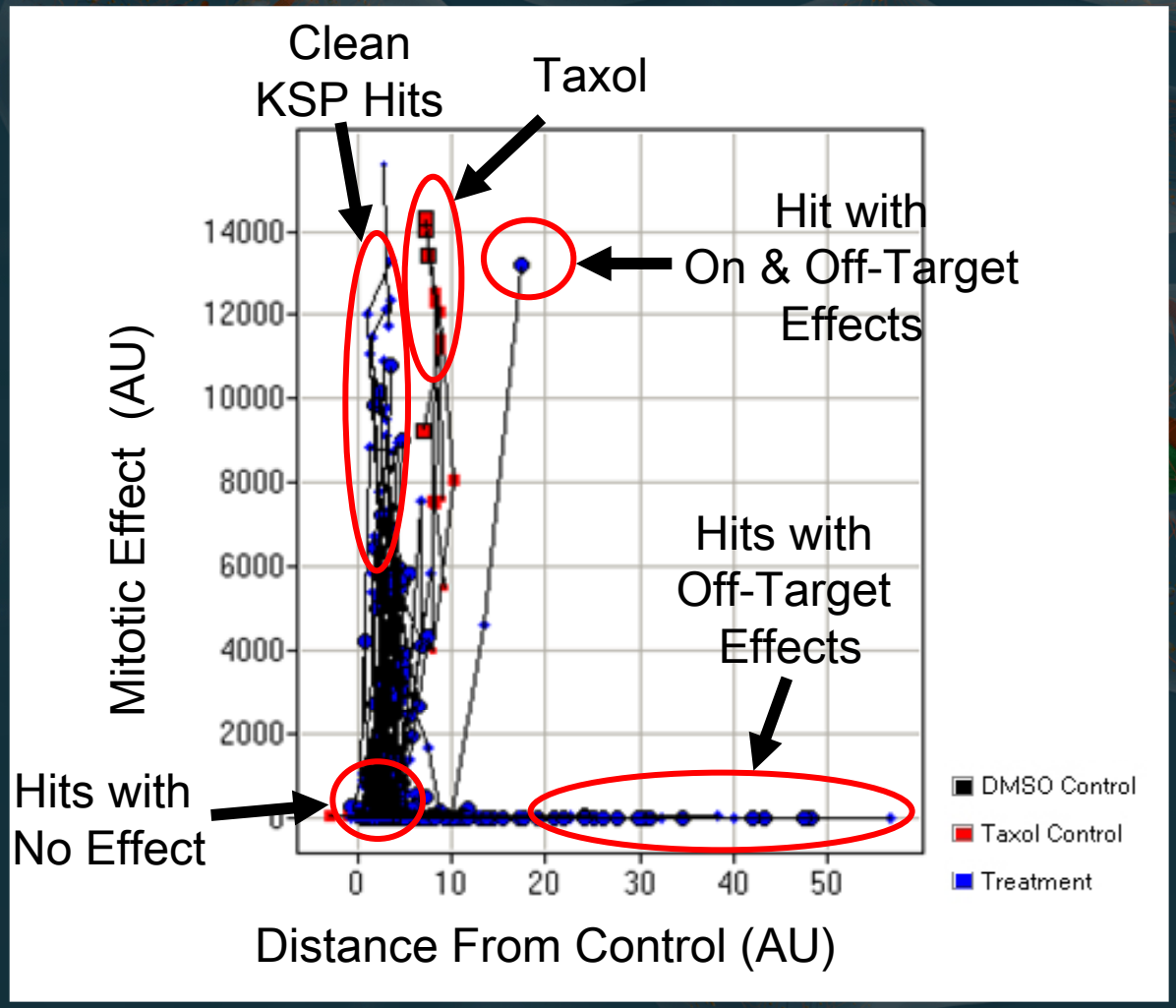
Prioritization of Biochemical KSP Hits by Measuring "On" vs. "Off" Target Cellular Effects



AU = Arbitrary Units

Assay Parameters

- Stains for DNA, Tubulin, Golgi, Phospho-Histone H3
- 4 compound concentrations
- 3 tumor cell lines
- 2 time points

Biological Features (examples)

- Cell cycle
- Nuclear morphology
- Cell shape
- Cytoskeletal morphology
- Golgi / intracellular transport

Distance From Control

- Multi-dimensional value
- Combines dozens of cellular features
- Interphase cells only

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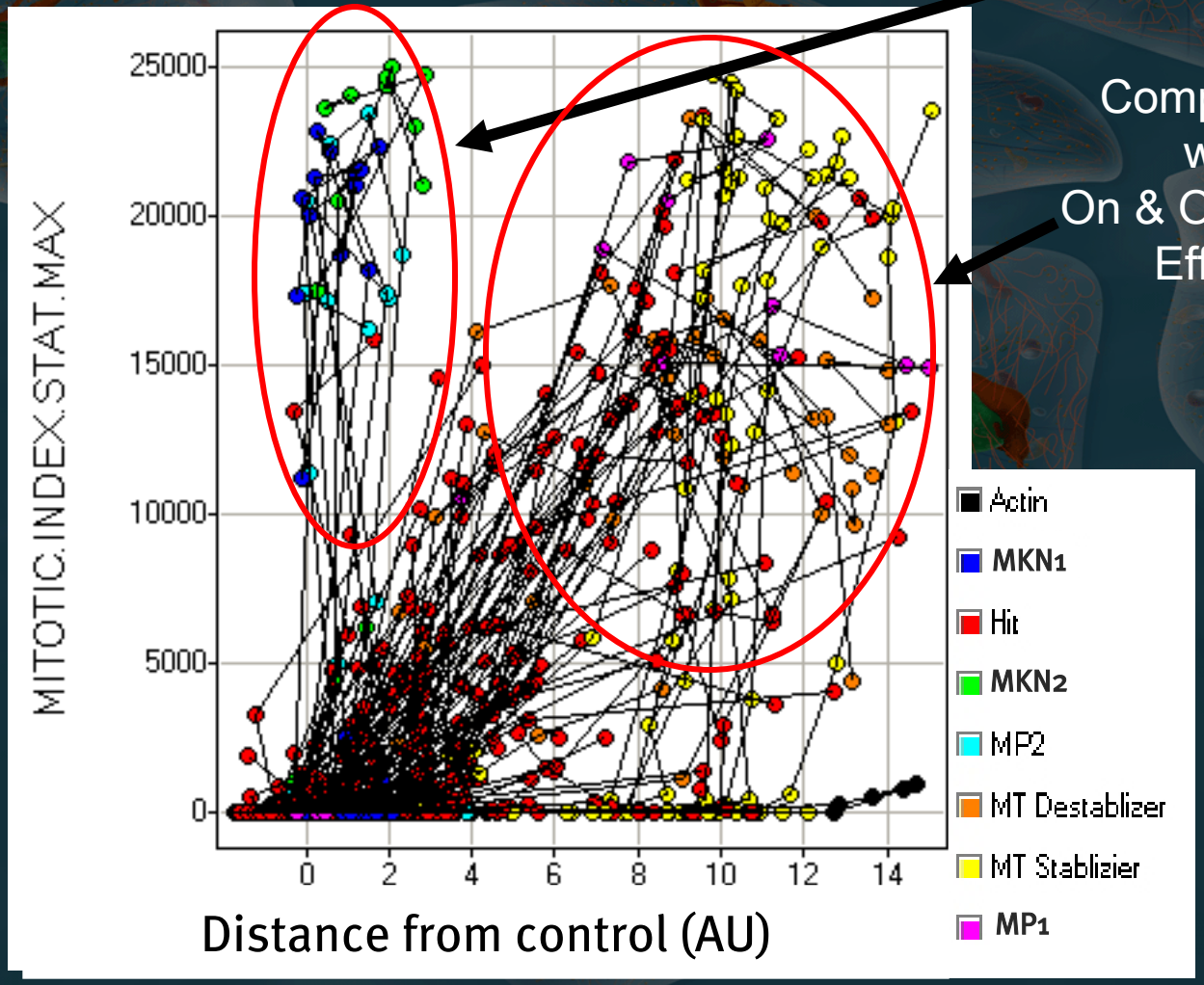
Combination of on- and off-target metrics identifies “clean” compounds



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Conclusions

- Robust biological image analysis platform with multiple applications in drug discovery
- Algorithms are designed to be resistant to process variations
- Extensive automated and semi-automated quality control and monitoring
- “High content” analyses allow broad characterization of compound effects

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Acknowledgements

Cindy Adams

Vadim Kutsyy

Ge Cong

Aibing Rao

Ke Yang

Reggie de la Rosa

Jay Trautman

Adrianne Lee

Lane Conn and CCL

Robb Moody

Lisa Belmont

Ken Wood

Gus Bergnes

Jade Dizon

Alvin Teodoro

Claudia Gallegos

Dale Snyder

Lane Conn

...and many others

Gurvinder Singh

Kelly Penhall-Wilson

Ping Ling

Eskinder Kiros

Bob Tang

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