

**Improving the Quality of HCS
Assays with Optimized System S/N,
Multiplexing, Statistics, Informatics
and Systems Cell Biology**

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SBS
2005

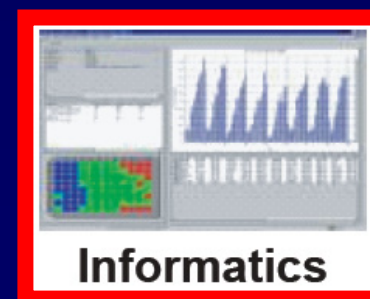


Topics

1. HCS is a Systems Engineering Challenge (S/N)
2. Some Key Factors that Impact HCS Data Quality before Applying Algorithms (Trade-Offs)
3. Multiplexing Adds Power and Complexity
4. Statistics and Informatics are Critical Tools
5. Systems Cell Biology is the Next Step in HCS



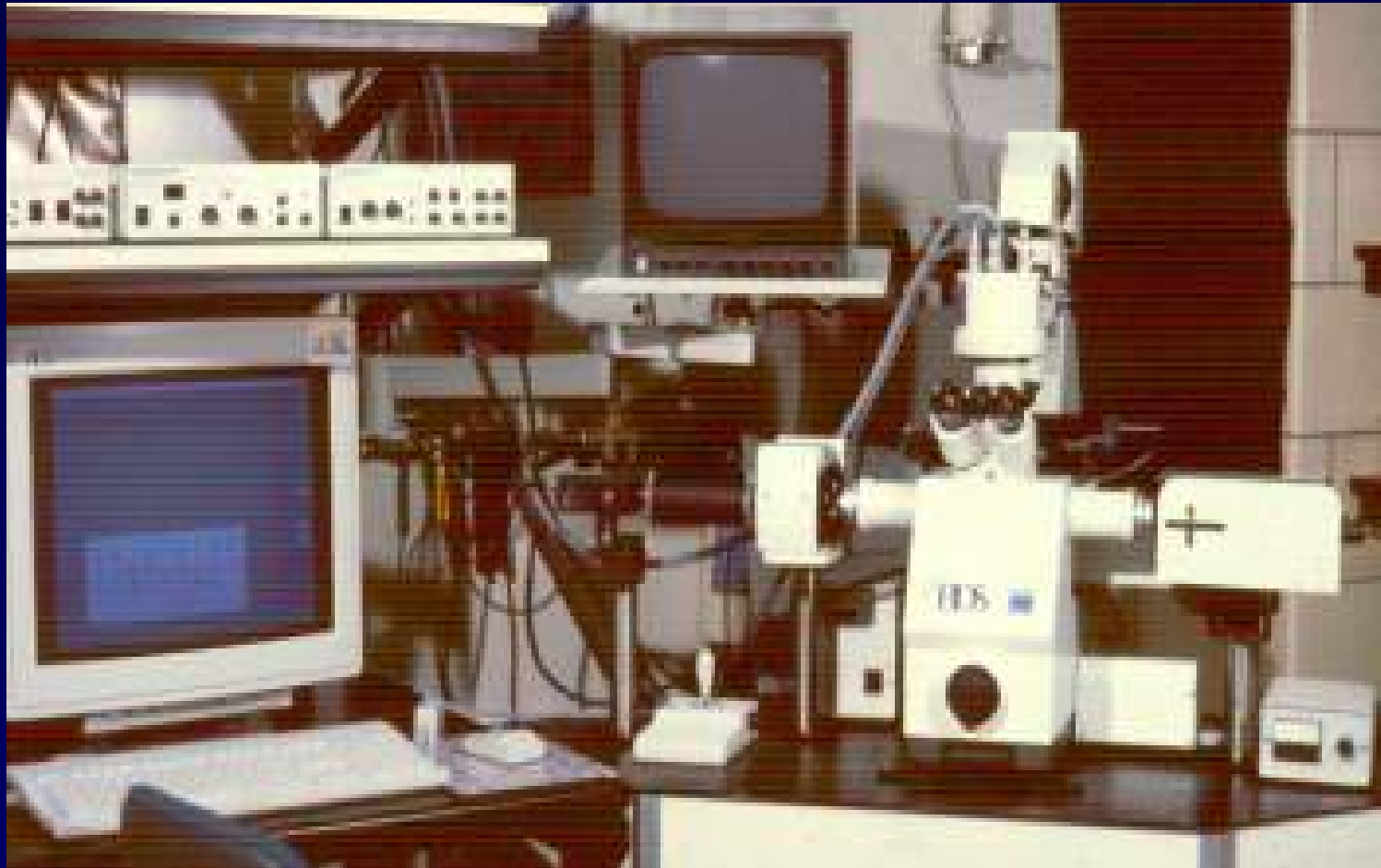
HCS: A Systems Engineering Challenge



Each of these components contributes to the reliability and variability in assay results.



Critical Knowledge Before HCS- 1980's



Factors Affecting HCS Data Quality

S/N Issues Before Applying Algorithms

- **Reagents (includes cells)**
 - QE, ϵ , concentration of fluorophore, copy # of target to label
 - Photobleaching, phototoxicity
 - Spectral bandwidth and overlap
 - Cell autofluorescence
 - Reagent impact on “normal” cell physiology (e.g. expression level of FP-target)
- **Sample Preparation**
 - Uniformity of cell adherence to substrate
 - Liquid handling consistency
 - Fixation and labeling quality and consistency
 - Live cell environmental control
 - Microplate design and quality



Factors Affecting HCS Data Quality

- **Instrumentation**
 - Signal to noise ratio
 - Optics (NA, magnification, depth of field, detector sampling frequency)
 - System noise (light source fluctuations, detector readout, ...)
 - Focus reliability
 - Filter bandwidth, blocking and overlap
 - Environmental control (Is room temperature acceptable?)
 - Speed (throughput) requirements vs. Trade-Offs
 - Standards
- ✳ **Must Maximize System-Wide S/N Before Applying Any Algorithm**



Multiplexed HCS Assays

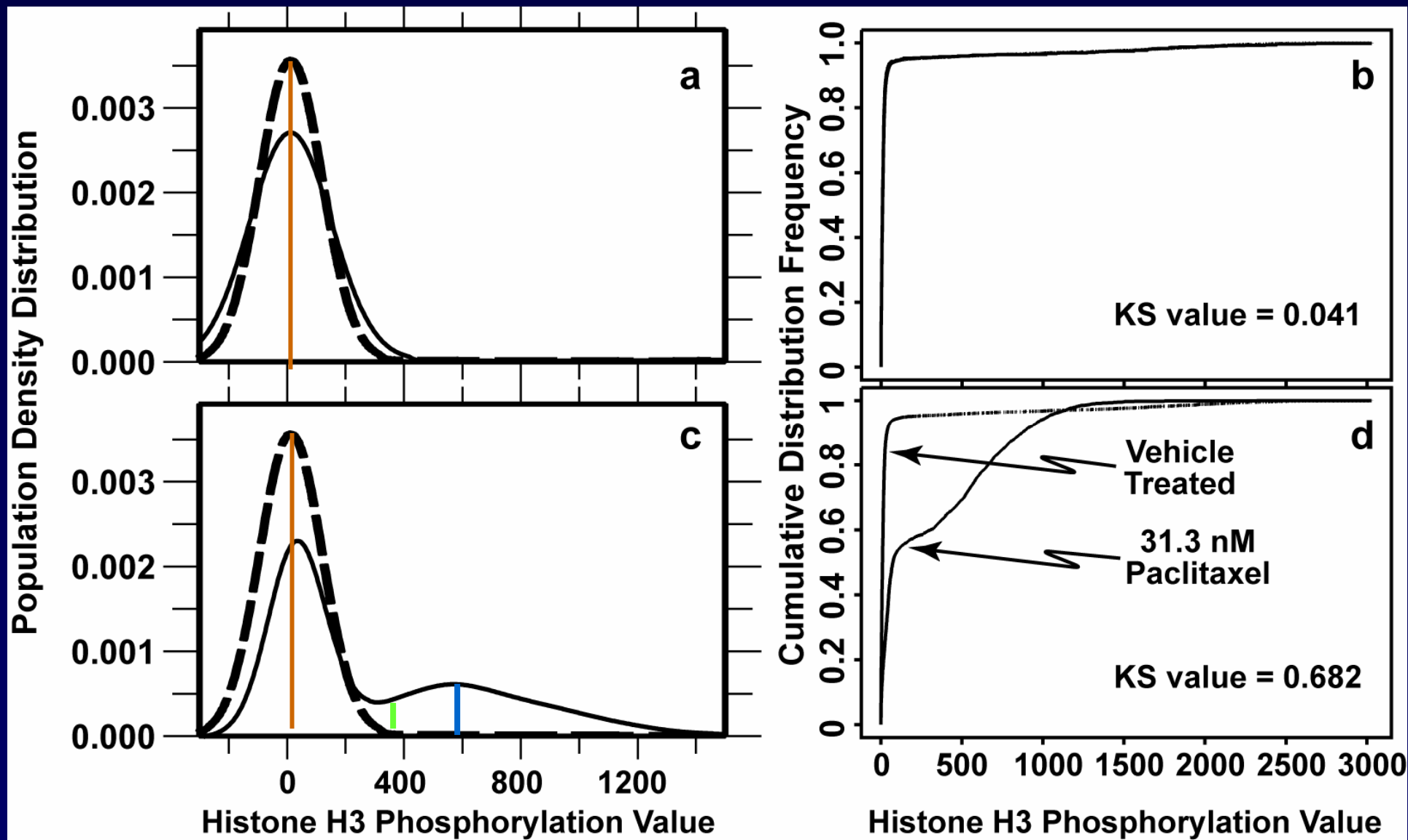
Can We Identify a Microtubule Modulating Compound and Concentration that Will Maximally Induce Apoptosis, While Minimally Perturbing Other Key Cellular Processes?

- **Cell Cycle**
 - **Phospho-Histone H3**
 - **Nuclear Condensation**
 - **MT Stability**
 - **Apoptosis**
-
- **Cellomics ArrayScan (Target Activation and Cell Health Profiling)**
 - **Kolmogorov-Smirnov (KS Test) Statistics**
 - **Heat Maps and Distribution Maps**

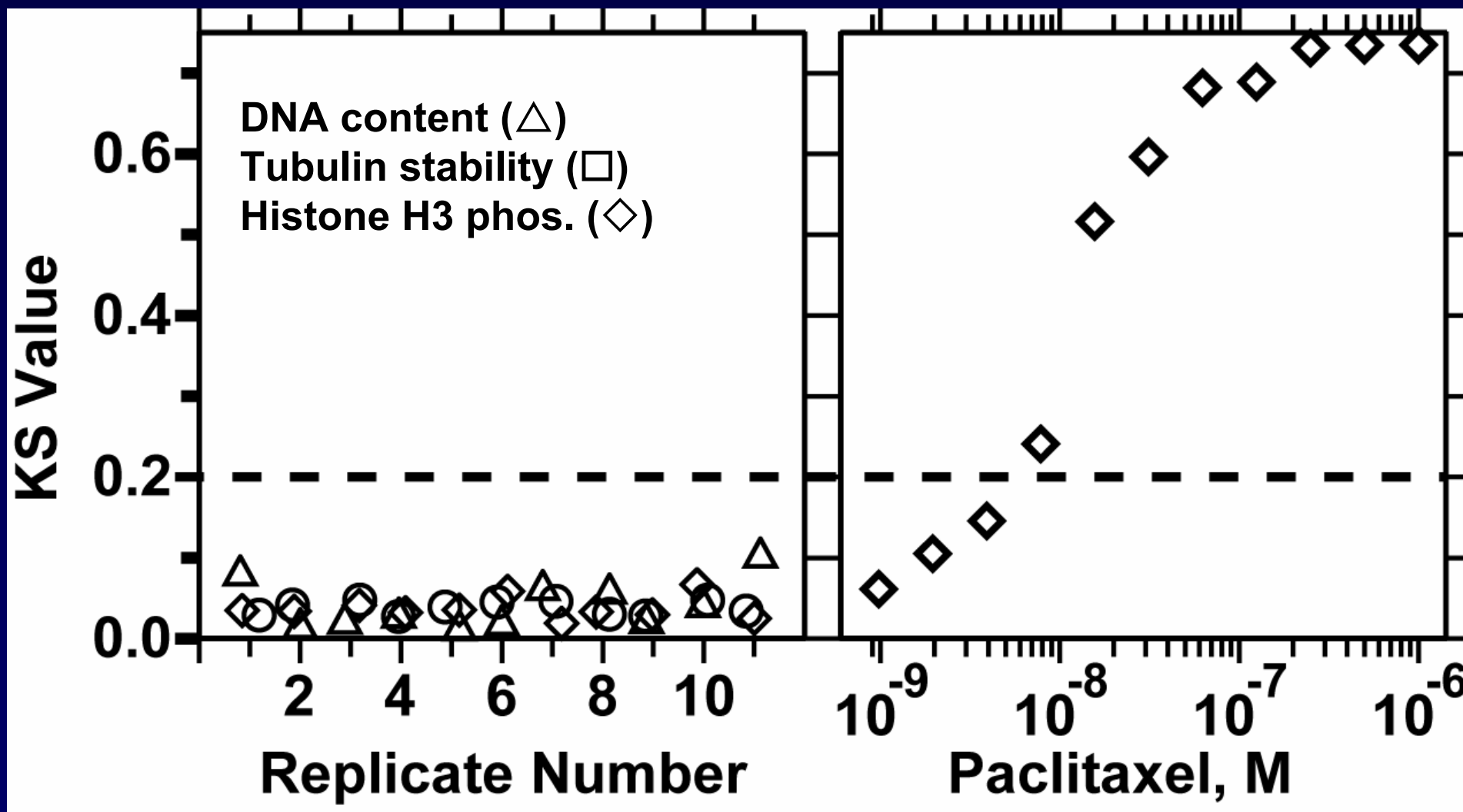
Giuliano et. al. (2005) ASSAY and Drug Dev.Tech.



KS Test of Paclitaxel Induction of Histone H3 Phosphorylation

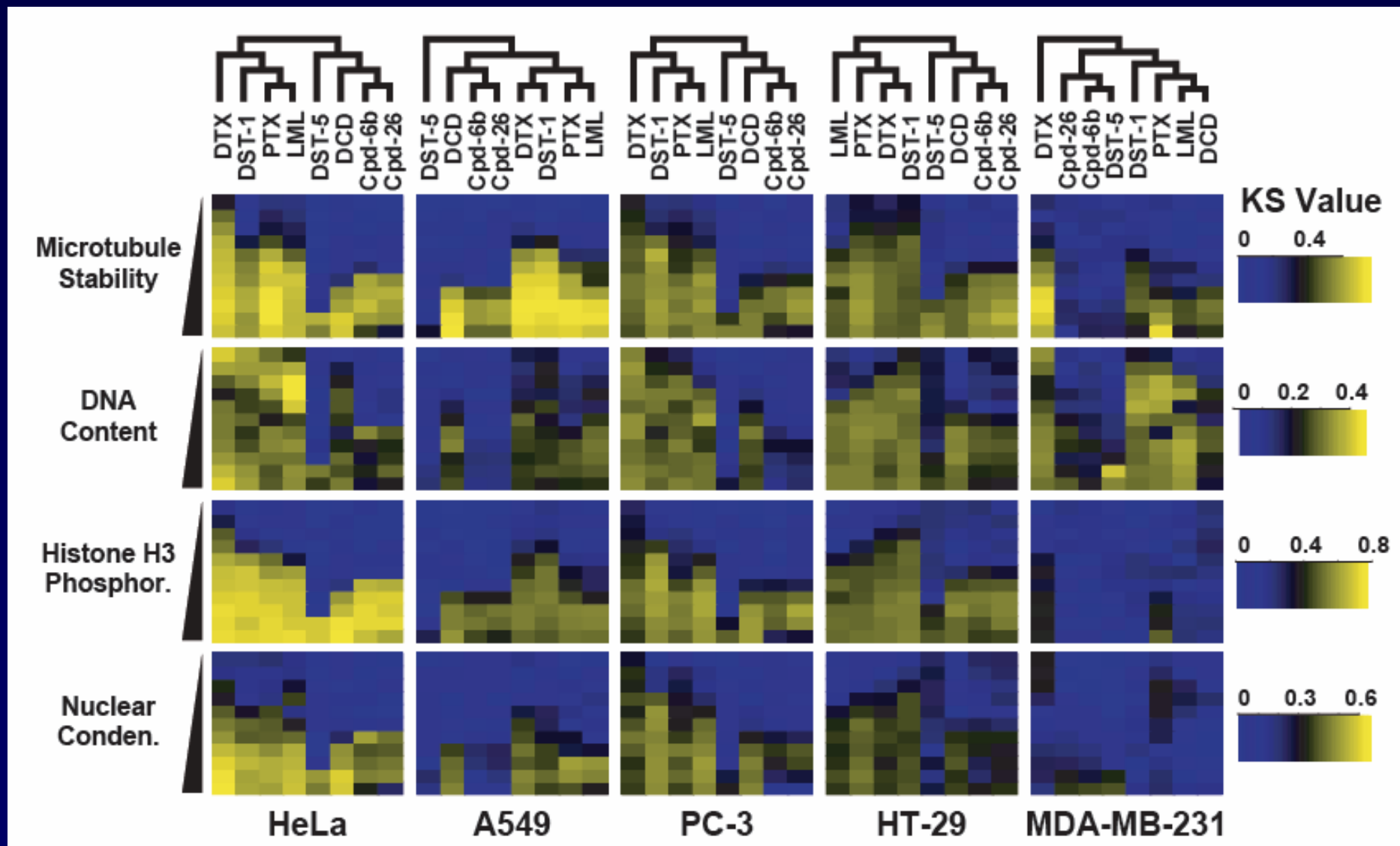


Applying KS Test to a Multiplexed Assay

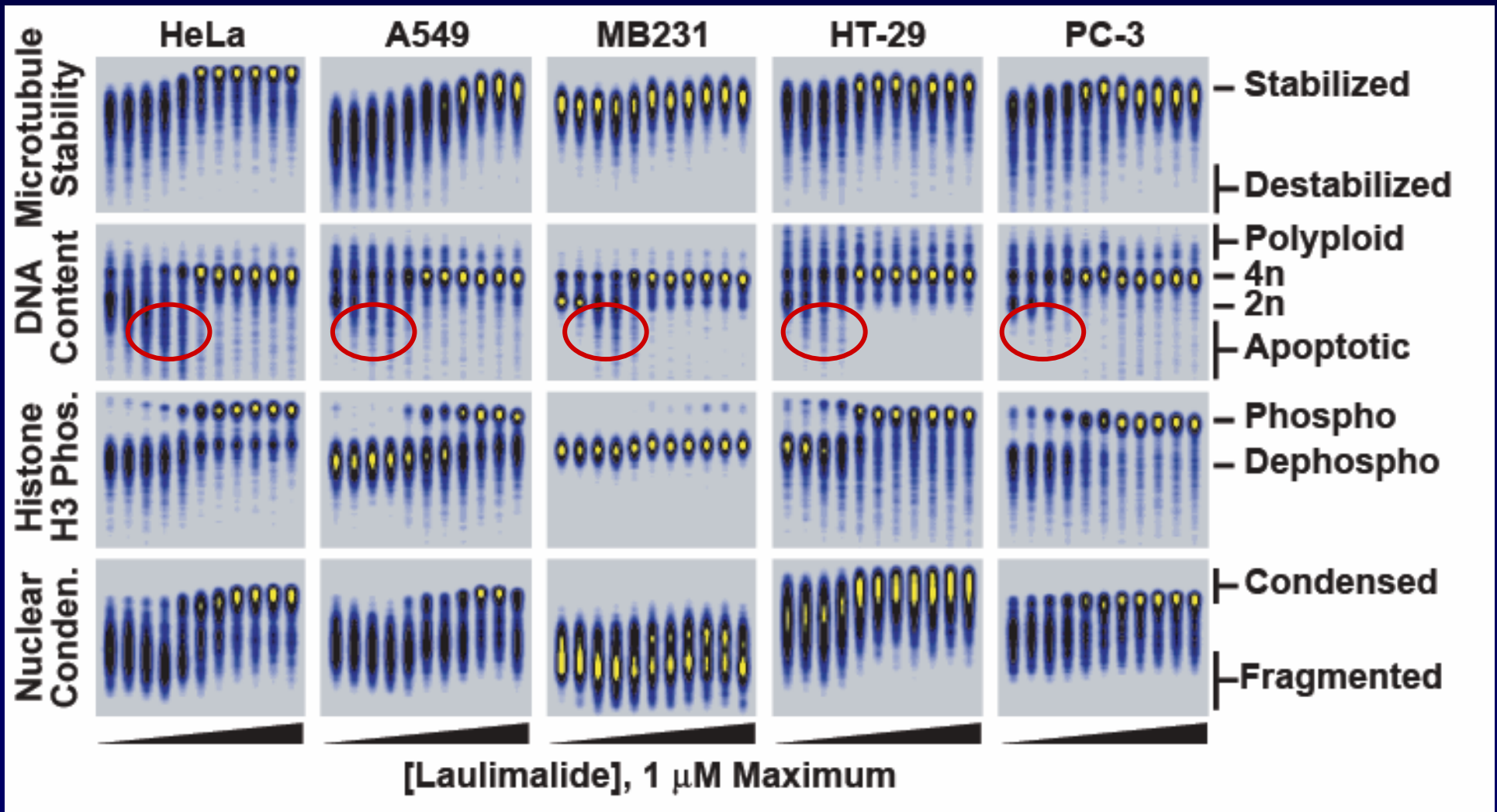


KS Heat Map & Cluster Analysis

Giuliano et. al., (2004). J. Biomol. Screening 9:557-568

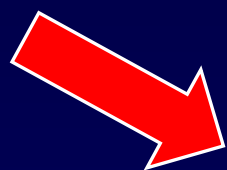


Distribution Map of Laulimalide Effects

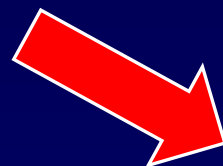


Systems Cell Biology Is the Next Step

HCS Assays



Multiplexed Assays



Systems Cell Biology



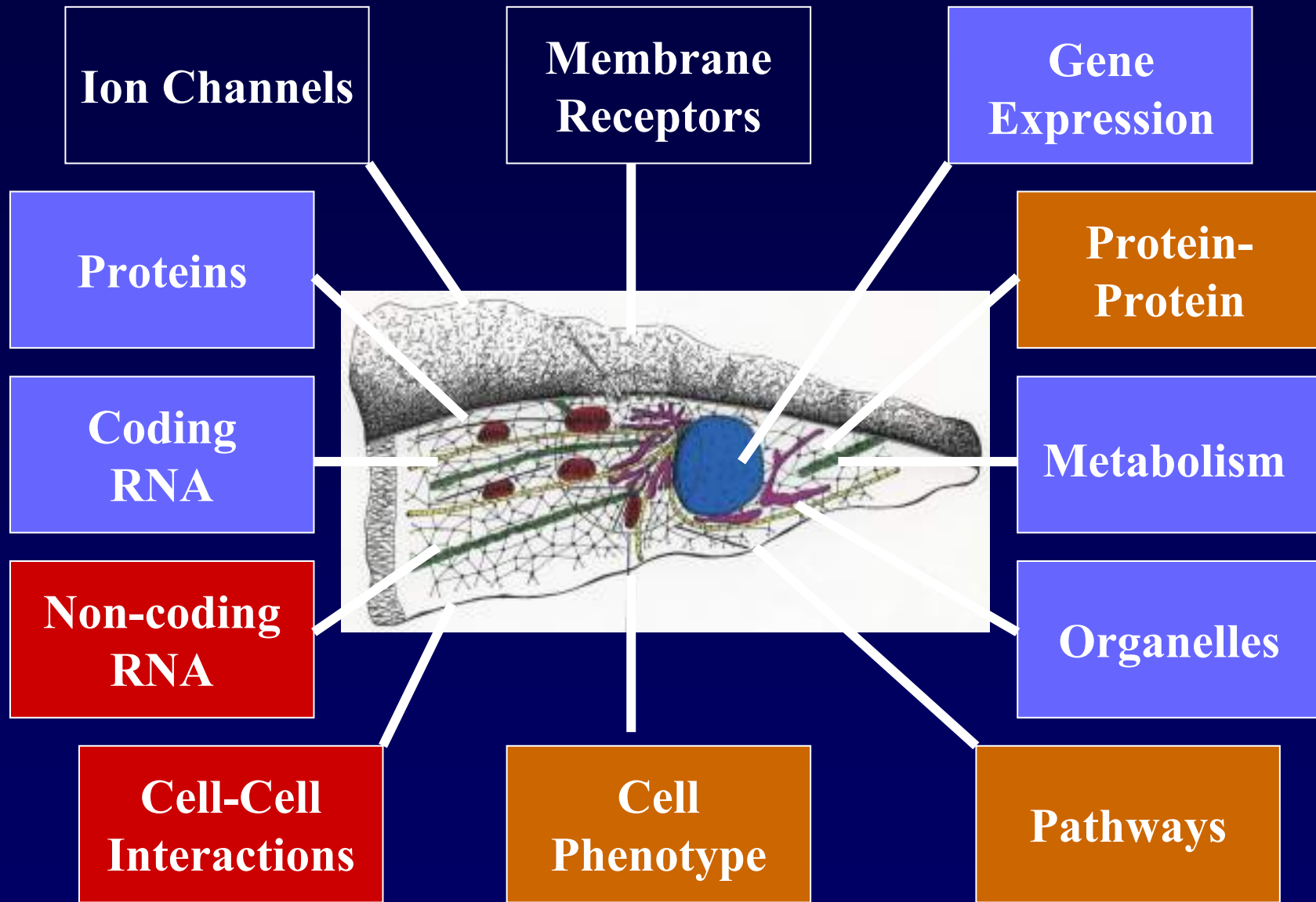
Systems Cell Biology

Beyond Single Targets and Pathways
for Improved Target Validation and Lead
Generation:

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Understanding how the integration of the complex biochemical and molecular processes, occurring in time and space within cells, are responsible for cell functions and their responses to environmental changes



Cells are Complex Systems



Measure and Manipulate



Key Elements of Systems Cell Biology

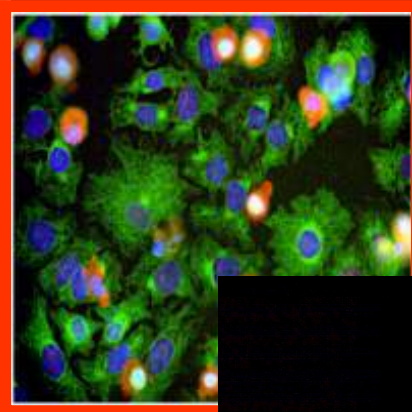
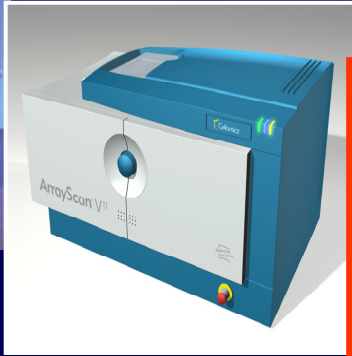
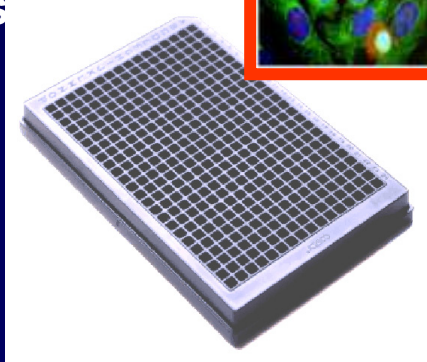
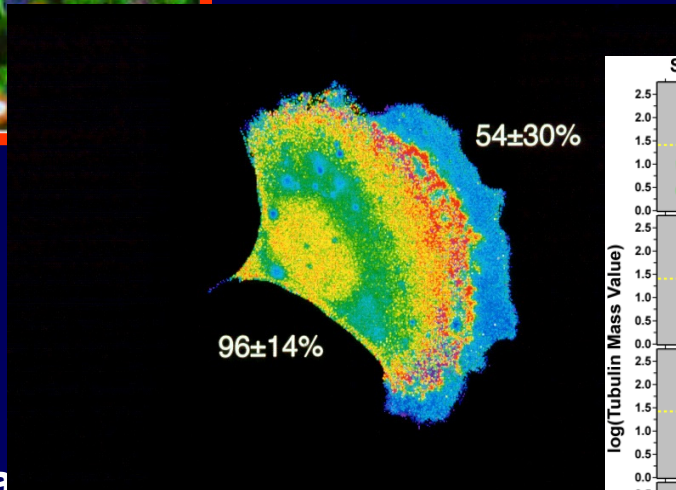


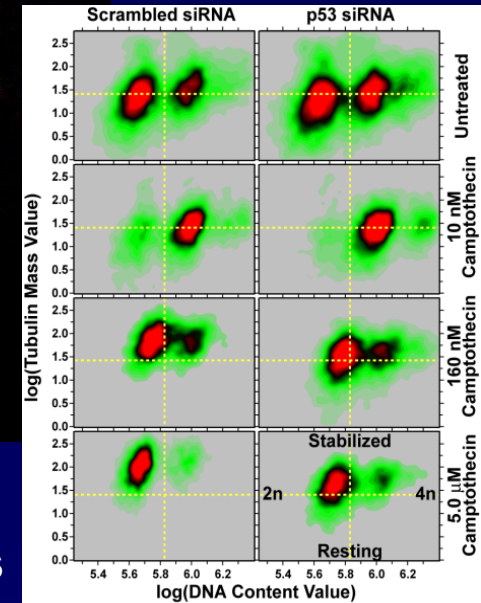
Plate Readers



Panels of Multiplexed Assays



New Measure and Manipulate Reagents

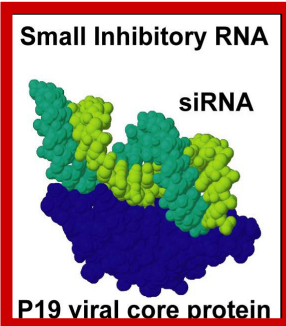


More Informatics



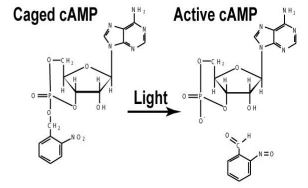
Reagents that Measure and Manipulate Cell and Constituent Functions

Small Inhibitory RNA




siRNA
P19 viral core protein

Caged Molecules



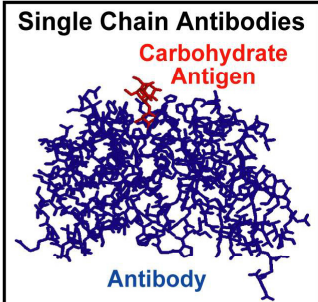
Caged cAMP Active cAMP
Light

Gene Switches



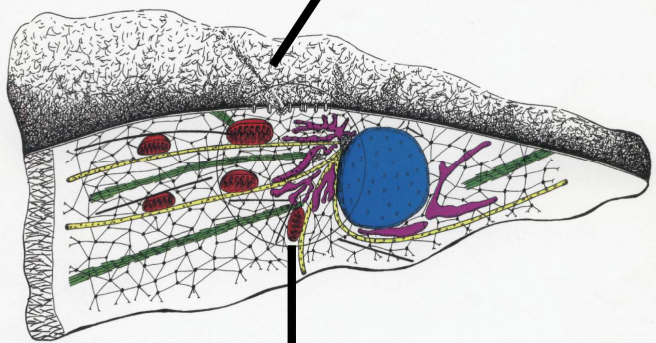
Switch
mRNA

Single Chain Antibodies



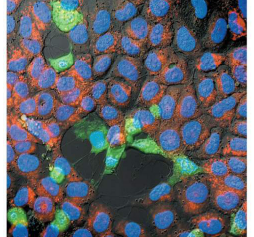
Carbohydrate Antigen
Antibody

Manipulate



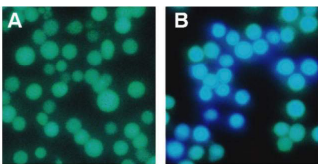
Measure

Fluorogenic Substrates



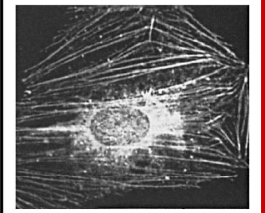
Caspase 3 Activation
Molecular Probes

Gene Expression



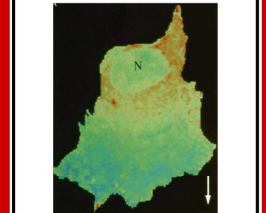
β -Lactamase gene reporter
Zlokarnik, G. et al. (1998)

Fluorescent Analogs



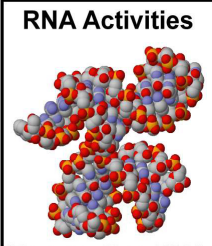
Non-muscle Actin

Fluorescent Protein Biosensors



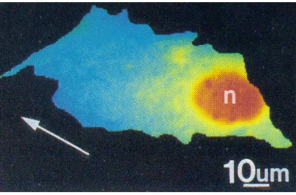
Myosin II Phosphor.

RNA Activities



Non-coding RNA

Physiological Indicators



Calcium Gradients
10 μ m

Cellumen Inc.

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Summary

1. **HCS System Component S/N is the Key to the Success of Imaging Algorithms**
2. **KS Test Yields Significant Multiplexed HCS Data Sets**
3. **Systems Cell Biology the Next Step in Cell-Based Discovery**
4. **Reagents that Measure and Manipulate Cell Constituents a Key to Systems Cell Biology**

***Taylor, Haskins and Giuliano (eds.) . High Content Screening: A Powerful Approach to Drug Discovery and Systems Cell Biology. Methods in Molecular Biology. Humana Press. (Early 2006)**

