Cellular Systems Biology Profiling in Drug Discovery and Development

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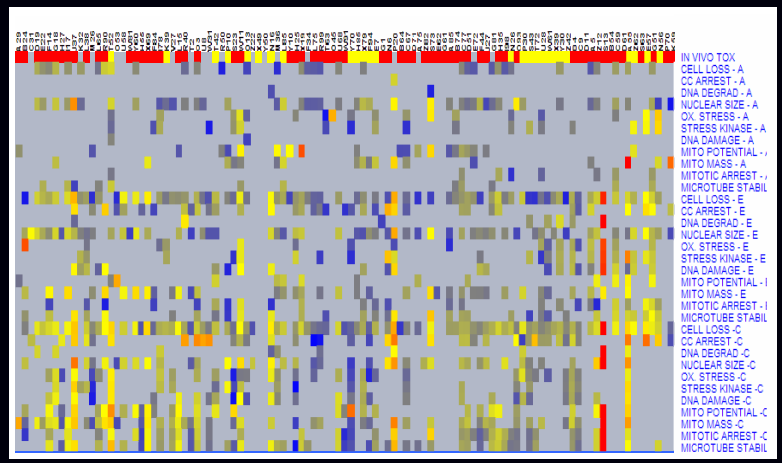

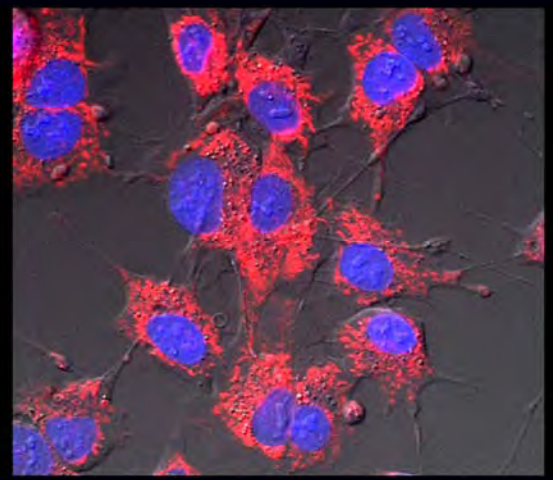

"Optimizing Drug Discovery, Development and Clinical Trials Through Cellular Systems Biology"

FDA/NIST Sponsored Workshop
In Vitro Analyses of Cell/Scaffold Products
December 2007

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Agenda

- Introduction to Cellumen and Cellular Systems Biology
- Cytotoxicity profiling as an example application of cellular systems biology

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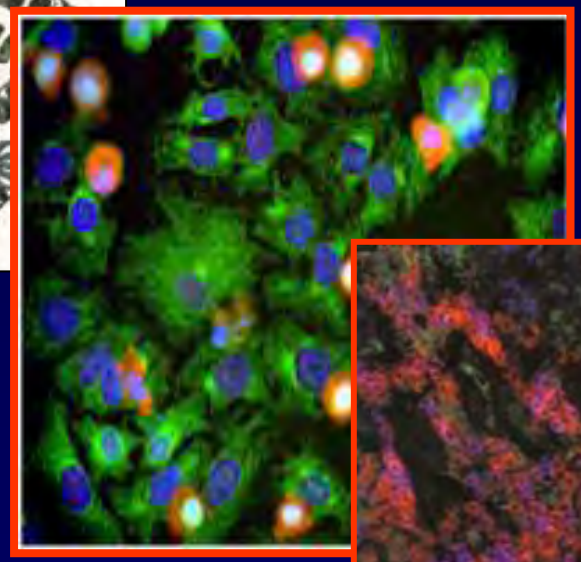
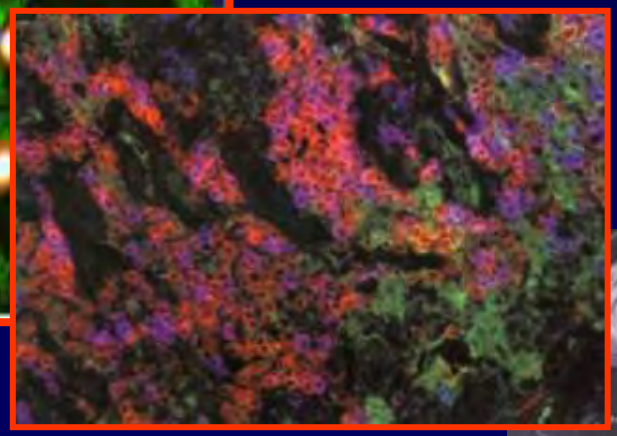
Exploring Cells and Tissues as Systems





“From Molecules to Man”

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The Challenge: Cell is An Integrated, Interacting Network

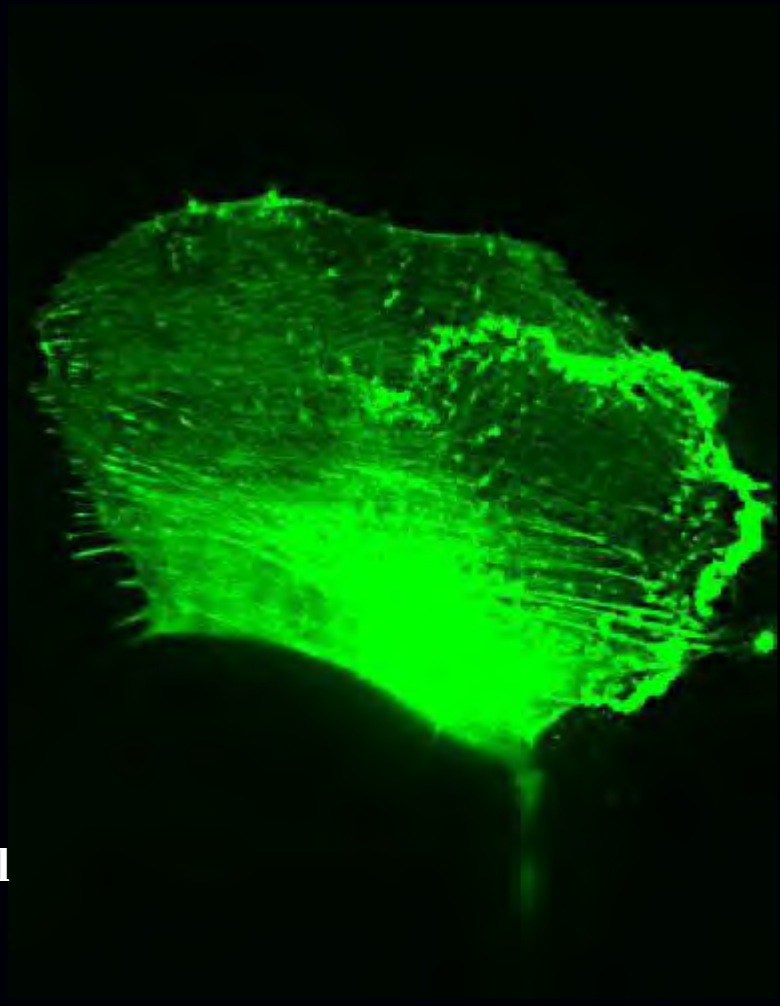
Gene Expression

Membrane Receptors

Membrane Pumps

Signaling Pathways

Post-translational Modifications



Cell Surface Attachments

Cytoskeletal Assembly-Disassembly

Cell Movements

Molecular Synthesis/ Degradation

Energy Metabolism

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Evolution of Cell-based Discovery

Emergence of Cellular Systems Biology

Cell population responses
HTS methods
Whole plate readers

"Simple" HCS
Automated cellular imaging
1st generation HCS readers/1-2 features measured

Multiplexed HCS
Automated cellular imaging
3-4 features with data management

Cellular Systems Biology
Optimally multiplexed cell and tissue imaging
advanced reagents, >10 features & classifier informatics

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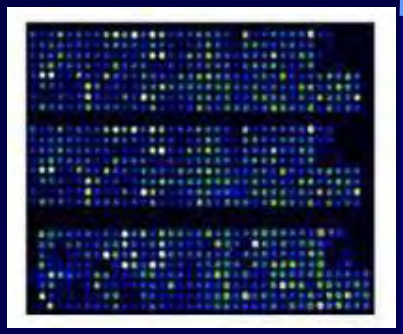
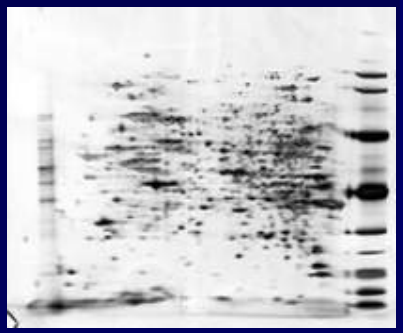
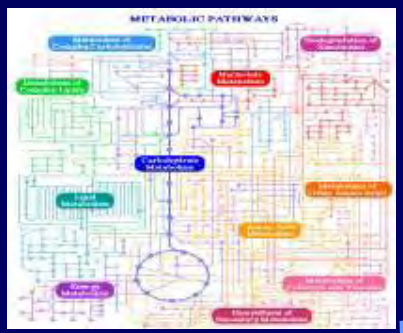
Cellular Systems Biology (CSB)™ Approach



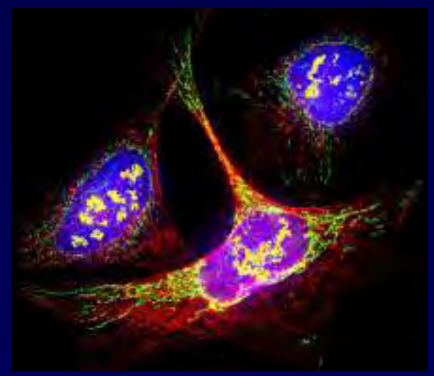




"omics" Information

The cell is an integrated and interacting network of genes, proteins & metabolic processes that gives rise to function

Functional Biomarkers



Cell Arrays

Tissue MicroArrays

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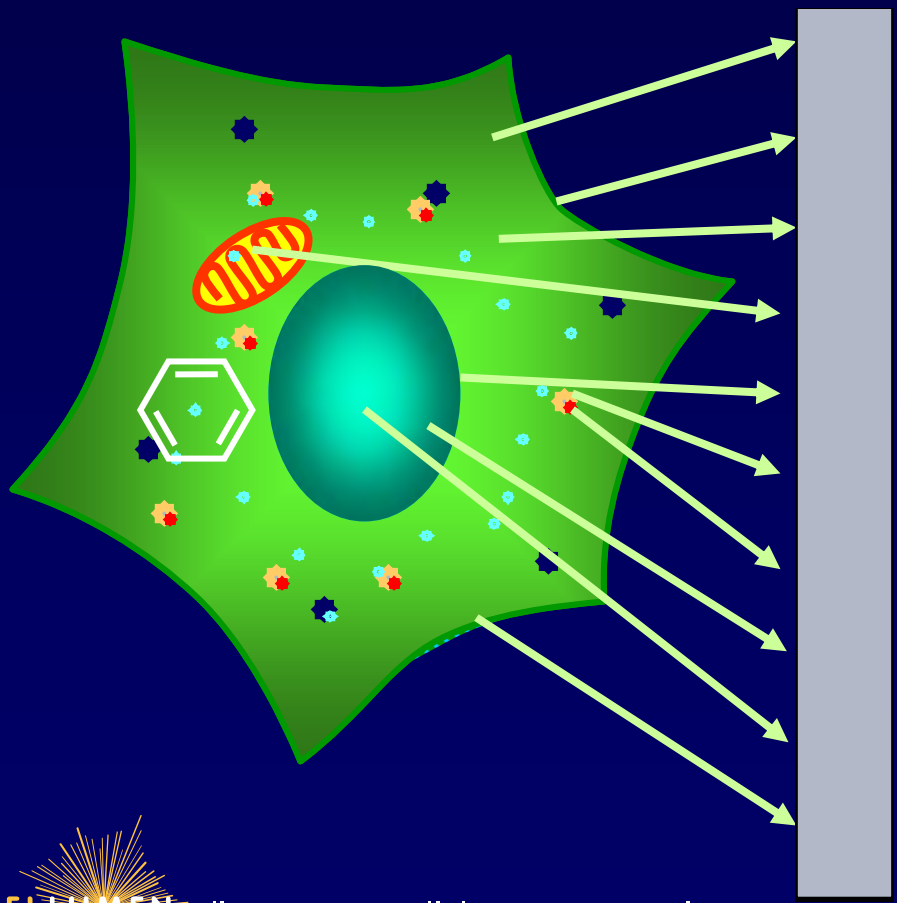
The Solution:
Cellular Systems Biology Profiling

Proprietary Cellular Biomarker Panels

Proprietary Profile Database



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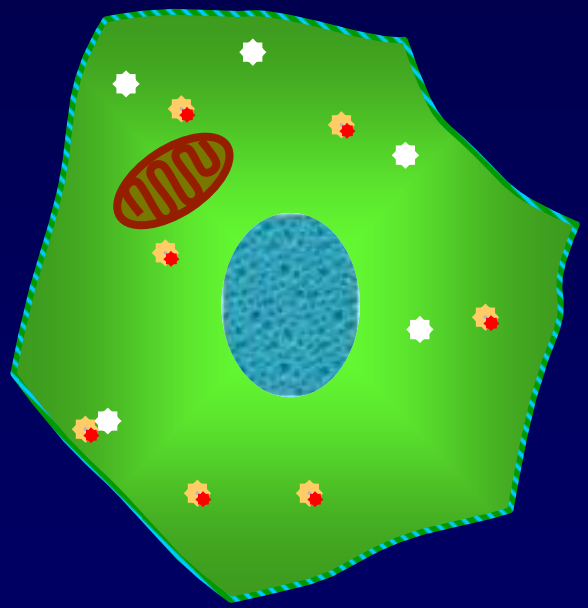
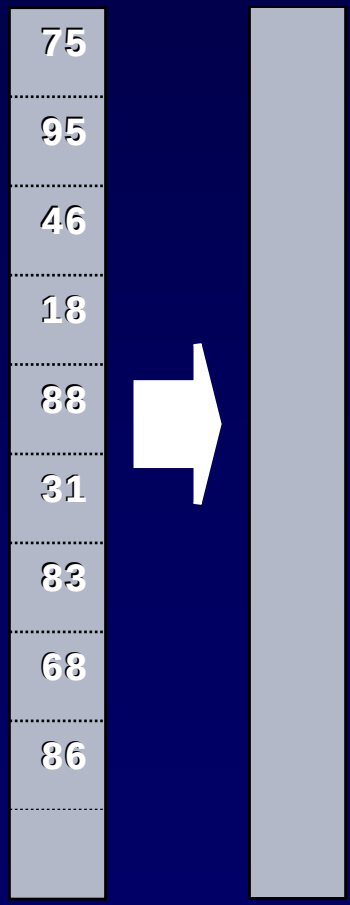
The Solution:
Cellular Systems Biology Profiling

Proprietary Cellular Biomarker Panels

Proprietary Profile Database

Classifiers





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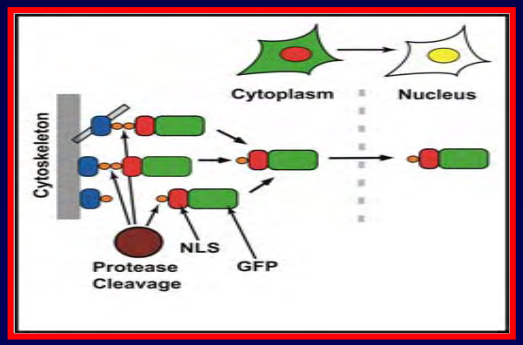
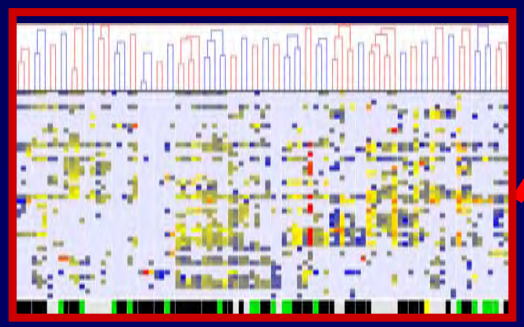
Tools of Cellular Systems Biology

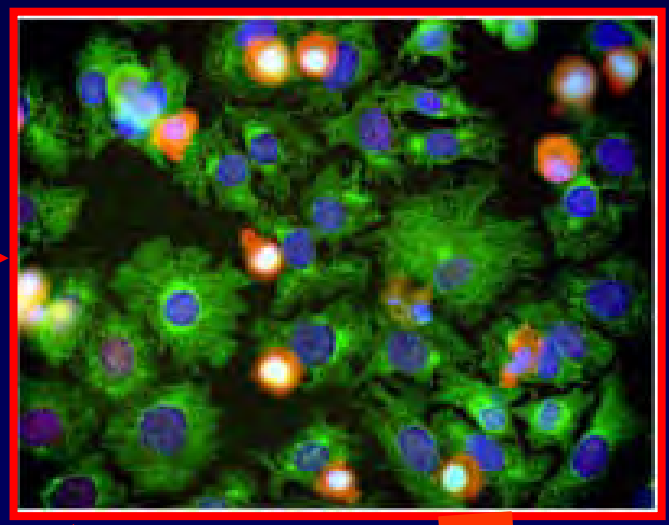
Existing Imagers



Reagents/Profiles



Informatics/Classifiers



- Cell "Models"
- Patient Cells/ Tissues

Systems Knowledge & Decisions

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Implementing Cellular Systems Biology (CSB)™

Improve efficacy and decrease toxicity
of leads, clinical candidates & drugs

Drug Discovery → **Drug Development** → **Clinical Trials**

Cellular Models of Disease

Cytotoxicity Profiling

Patient Sample Profiling

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Cellumen Solution

CellCiphr™ Cellular Models of Disease

- Disease-relevant cellular models, biosensors, assays & profiles to improve quality and quantity of lead compounds
- Profiles & tools for important, previously intractable targets
- Flags off-target effects at the earliest stage

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Cellumen Solution

Drug Discovery → **Drug Development** → Clinical Trials

CellCiphr™ Cytotoxicity Profiles

- Identifies potential toxicity before entering expensive pre-clinical testing
- Enables prioritization of lead compounds

Partners

MILLIPORE CHA US EPA

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Cellumen Solution

Drug Discovery → Drug Development → **Clinical Trials**

CellCiphr™ Patient Sample Profile R&D

- Improves trial enrollment and therefore new drug candidate efficacy by stratifying patients with profiles of the patient's own samples
- Proprietary panels of cellular biomarkers can also serve as diagnostic tools to improve treatment selection

Collaborative Partner

MayoClinic.com
Tools for healthier lives

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Cytotoxicity Profiling

CellCiphr™ Cytotoxicity Profile

CellCiphr™ Advantages:

- Systems Biology approach monitors multiple functions, time points and doses
- Fully leverages the sensitivity and throughput of HCS
- Validated to HTS standards with 384-well capacity & extensive QC
- Classifier software for simplification and predictivity
- Delivers insights on mechanism of action

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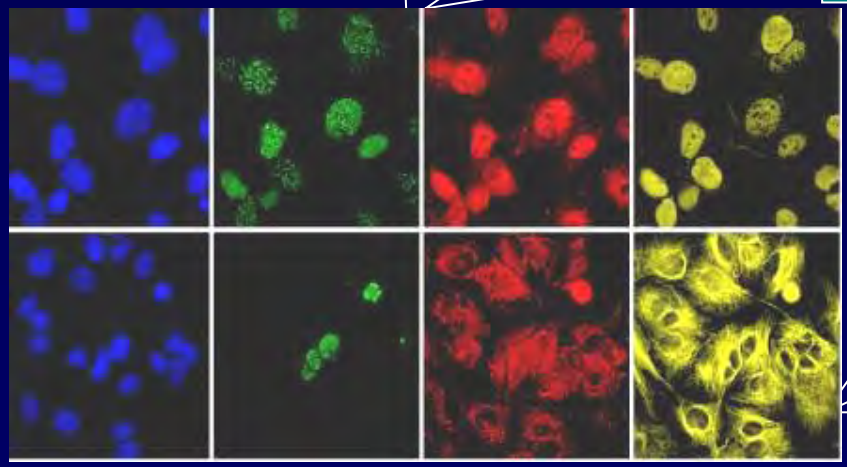
CellCiphr™ Cytotoxicity Panel #1

Panel 1 design* **(Collaboration with Millipore)**

- **Multiple mechanisms of toxicity**
- **Acute, early & chronic exposure**
- **384-well capacity**
- **HepG2**



Stress Pathway Activation

Organelle Functions

Oxidative Stress

DNA Damage

Cell Cycle

CSK Integrity

*Cellumen patents pending

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CellCiphr™ Cytotox Panel #1 Features HepG2 Cells

Cell Features	Measurement	Reagents
Cell Loss Cell Cycle Arrest DNA Degradation Nuclear Size	Cell Number DNA Content DNA Structure Nuclear Area	Hoechst 33342
Oxidative Stress	Histone H2A.X Phosphorylation	anti-phospho-histone H2A.X Antibody
Stress Kinase Activation	c-Jun Phosphorylation	anti-phospho-c-jun Antibody
DNA Damage Response	p53 activation	anti-p53 Antibody
Mitochondrial Function I	Mitochondrial memb. potential	MitoTracker Red
Mitochondrial Function II	Mitochondrial mass	MitoTracker Red
Mitosis marker	Histone H3 Phosphorylation	anti-phospho-histone H3 Antibody
Microtubule CSK	Microtubule stability	anti-α-tubulin Antibody

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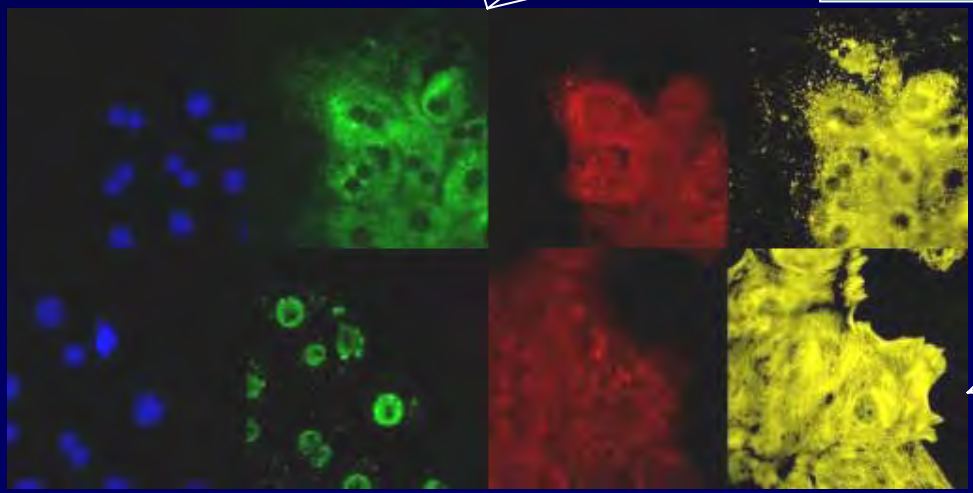
CellCiphr™ Cytotoxicity Panel #2

Panel 2 design*:

- Primary Rat Hepatocytes
- Multiple mechanisms of toxicity
- 11 parameters
- Acute, Early, 48h exposure
- Fixed endpoint, 384-well capacity



* Cellumen patents pending

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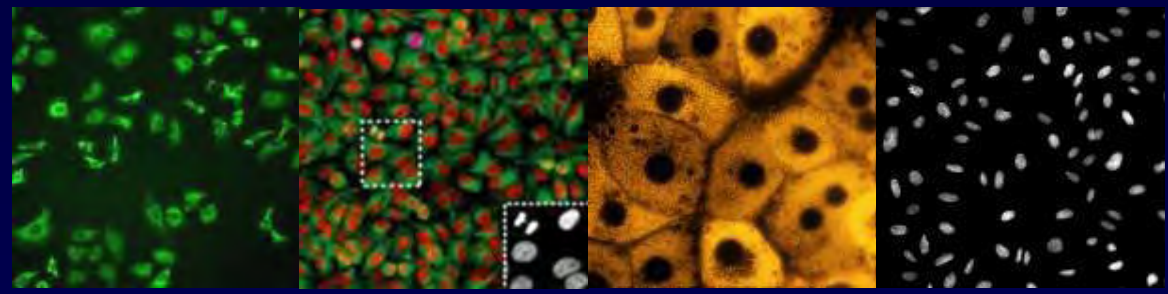
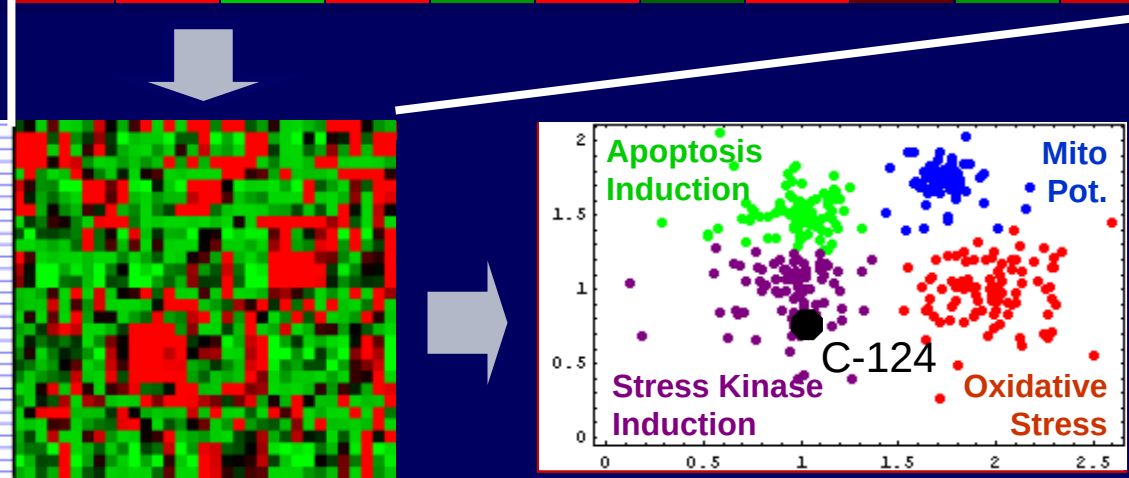
CellCiphr™ Analysis



Acute Profile
Early Profile
Chronic Profile



Clusters of compounds based on CellCiphr Profiles

Library of CellCiphr Profiles

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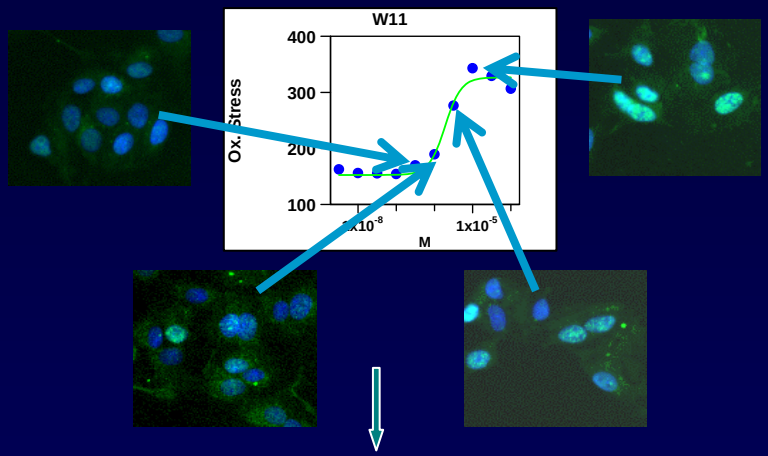
CSB Imaging and Data Analysis

A.



11 features measured in EACH cell; 3 time points; 10 pt dose-response, curve fit the data

Tabulate AC50 values

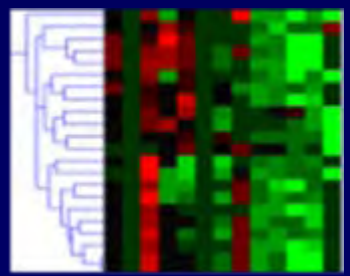
B.

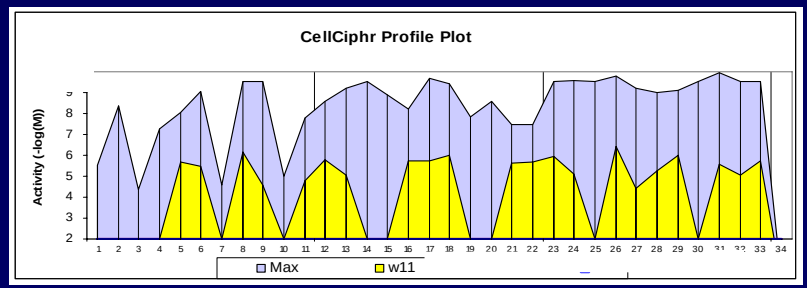
Cmpd	Cell Loss	CC Arrest	DNA Degrad	Nuclear Size	Ox. Stress	Stress Kinase	DNA Damage	Mito Potential	Mito Mass	Mitotic Arrest	Microtube Stability
Plate	Acute	Acute	Acute	Acute	Acute	Acute	Acute	Acute	Acute	Acute	Acute
T42											
R50	3.40							4.52			
P10				4.92				3.89	5.04		
S23					4.80	4.75			4.41		
W11					5.66	5.45		6.15	4.57		4.80
S13	3.82				4.18		3.80				

Cell Loss	CC Arrest	DNA Degrad	Nuclear Size	Ox. Stress	Stress Kinase	DNA Damage	Mito Potential	Mito Mass	Mitotic Arrest	Microtube Stability
Early	Early	Early	Early	Early	Early	Early	Early	Early	Early	Early
5.18	4.85		4.00			4.80	5.45		5.00	4.40
4.57						3.44			3.61	3.67
4.28					3.63					
5.77	5.08			5.72	5.71	6.00			5.63	5.69
4.38										

Cell Loss	CC Arrest	DNA Degrad	Nuclear Size	Ox. Stress	Stress Kinase	DNA Damage	Mito Potential	Mito Mass	Mitotic Arrest	Microtube Stability
Chronic	Chronic	Chronic	Chronic	Chronic	Chronic	Chronic	Chronic	Chronic	Chronic	Chronic
5.26										
5.01			5.97		5.90					4.89
4.32	4.56			3.34	3.87	3.21	6.45	4.25		4.07
5.94	5.09		6.41	4.43	5.25	6.01		5.59	5.04	5.72
4.78	4.45			4.46		3.99				

C.





Quantitative and Visual Tools for Compound Profiling

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Cytotoxicity Profiling

CellCiphr™ Profiling Case Study

- Drug Safety Data for 137 compounds*
- Each compound scored on a scale of 0, 1, 2, 3, 4 for in vivo toxicity
- Compounds tested in CellCiphr HepG2 Panel 1 and CellCiphr Rat Hepatocyte Panel 2
- CellCiphr 1 automated analysis provided similarity to known controls, the group toxicity rank order and a safety index

* Includes 101 unknown (blinded) and 36 control compounds

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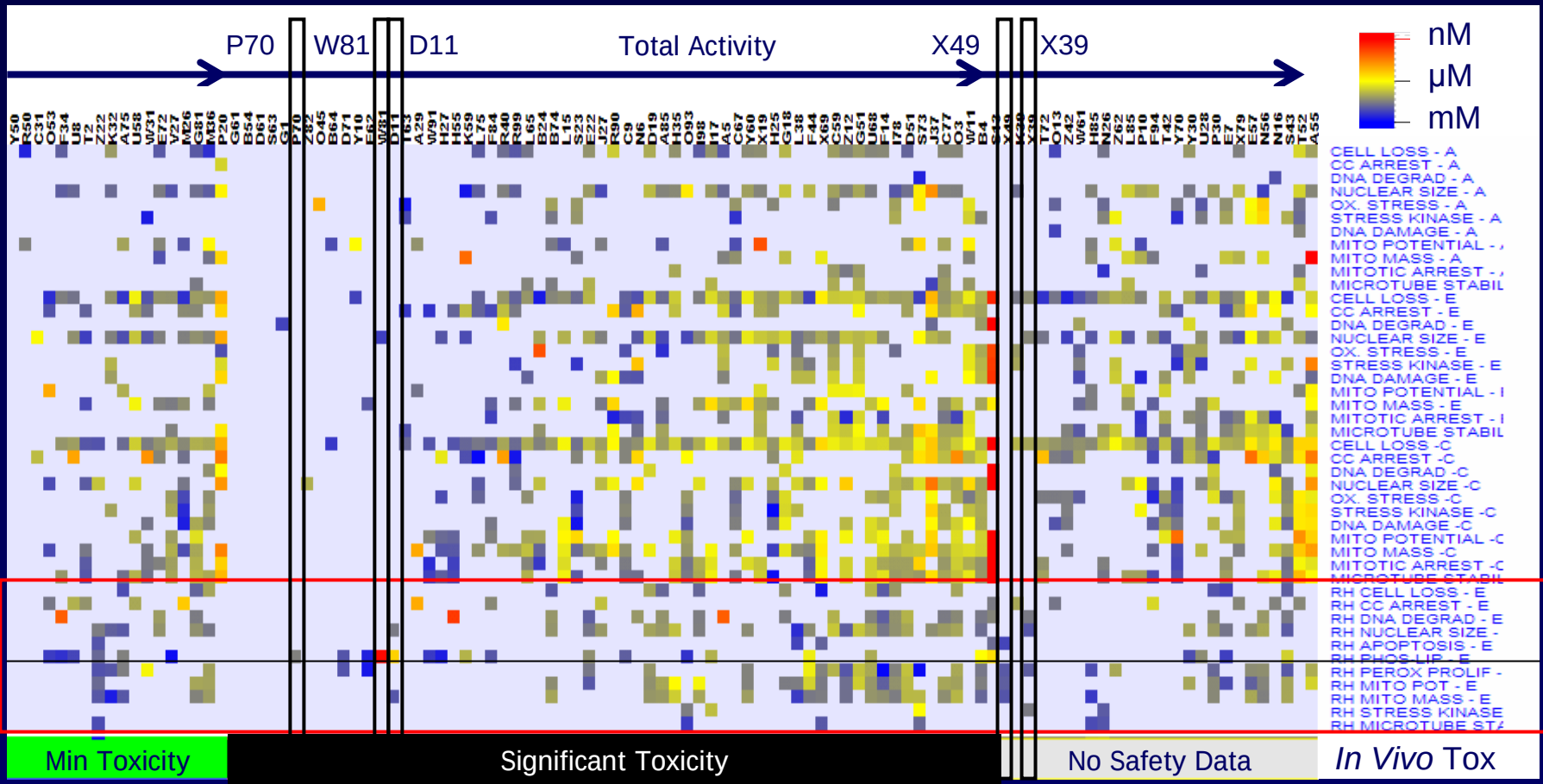
Combining CellCiphr™ Features from HepG2 and Rat Hepatocytes Optimizes Toxin Detection



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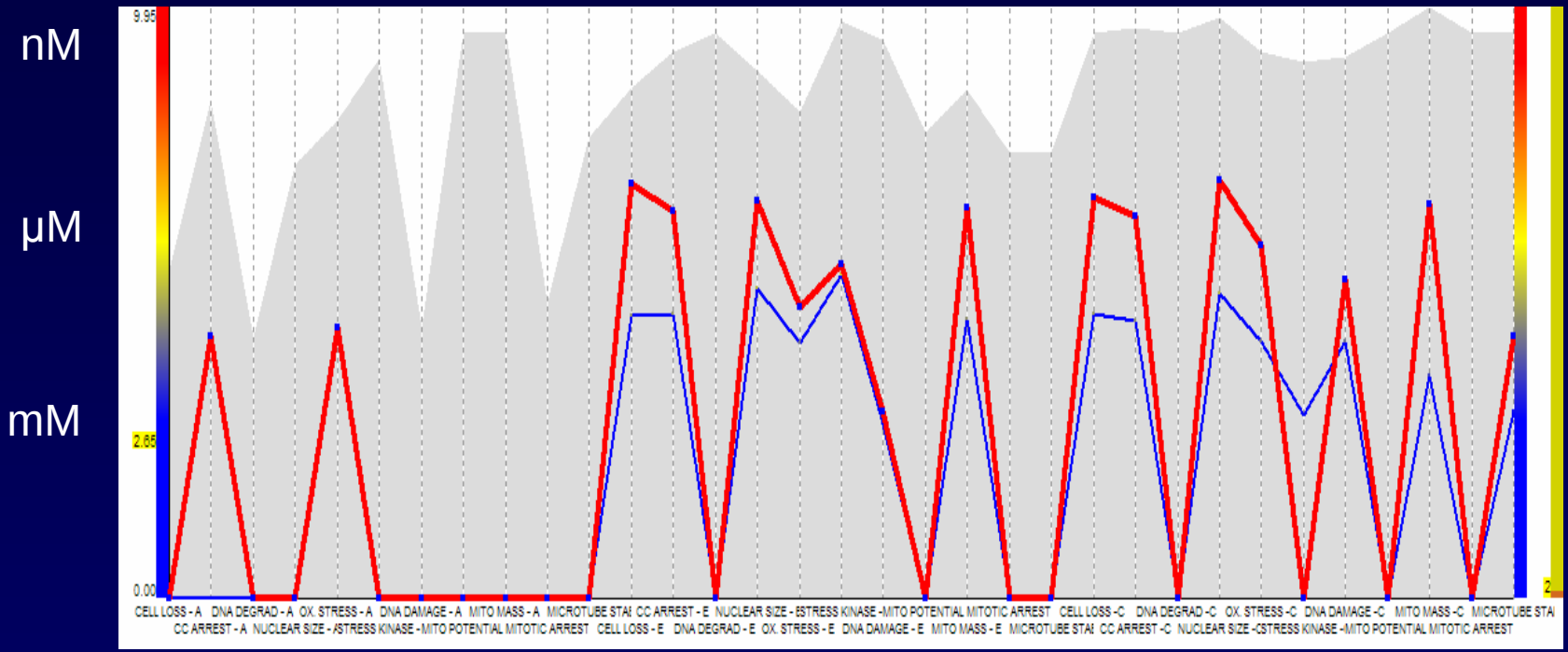
CellCiphr™ Similarity Profile



Compound	Differences from Etoposide (red)
H25 (blue)	- CC Arrest Acute
H25 (blue)	- Stress Kinase Chronic

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Classifier Design

- 137 compounds produced over 4500 dose-response curves
- Difficult to apply manual scoring methodology to handle analysis
- Assay data from compounds was used with in vivo scorings to construct a classifier to rank compound toxicity
- First generation classifier had improved performance over simple cytotoxicity assay

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Accuracy of CellCiphr Classifier 1

In vivo Toxicity	n	24 hr cell loss	CellCiphr Classifier
Significant	47	84%	100%
Moderate	66	65%	80%
Minimal	24	25%	46%
Overall accuracy	137	61%	82%

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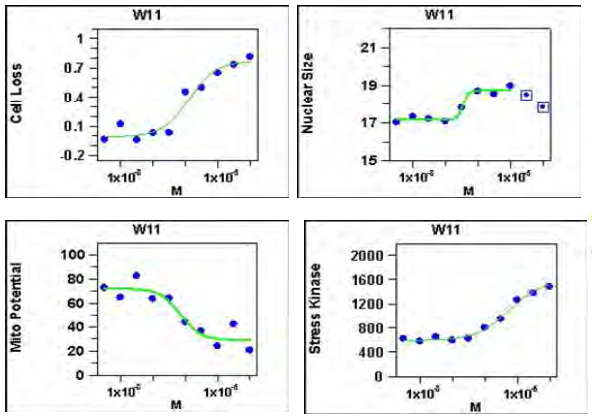
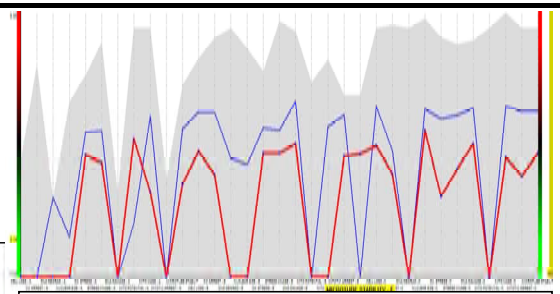
CellCiphr™ Report

Compound: W11 Source: CHA Project	Measurable Effects	AC_{50} (M)	Time (hrs)
Maximum Tolerate Dose		n/a, 1.2E-5	1, 72
Earliest Toxic Indicator	Mitochondrial Potential	7.07E-07	24
Most Sensitive Toxic Indicator	Nuclear Size	3.90E-07	72
General Indicator of Toxicity	Cell Loss	1.5E-6, 1.3E-6	1, 72
	Nuclear Size	8.4E-6, 3.4E-7	1, 72
Mechanistic Indicators	Oxidative Stress	1.9E-6, 2.2E-6, 36.8E-6	1, 24, 72
	Stress Kinase	1.9E-6, 3.6E-6, 5.7E-6	1, 24, 72
	DNA Damage	993.0E-9, 982.4E-9	1, 72
	Mitosis Arrest	2.3E-6, 9.1E-6	1, 72
	Microtube Stability	2.0E-6, 16.0E-6, 1.9E-6	1, 24, 72
	Cell Cycle Arrest	8.4E-6, 8.0E-6	1, 72
	Mitochondrial Potential	7.07E-7	24
	Mitochondrial Mass	27.2E-6, 2.5E-6	24, 72

Correlation with Other Compounds	Compound	Correlation Coefficient
Similarity with Compounds in set	C9	0.59
	Vinblastine	0.70
	Nocodazole	0.69
Similarity with Toxin Cassette Compounds	Colcemid	0.60





CellCiphr™ Profile
W11 (red), Vinblastine (blue)

Rank Order 1/ Safety H
l d

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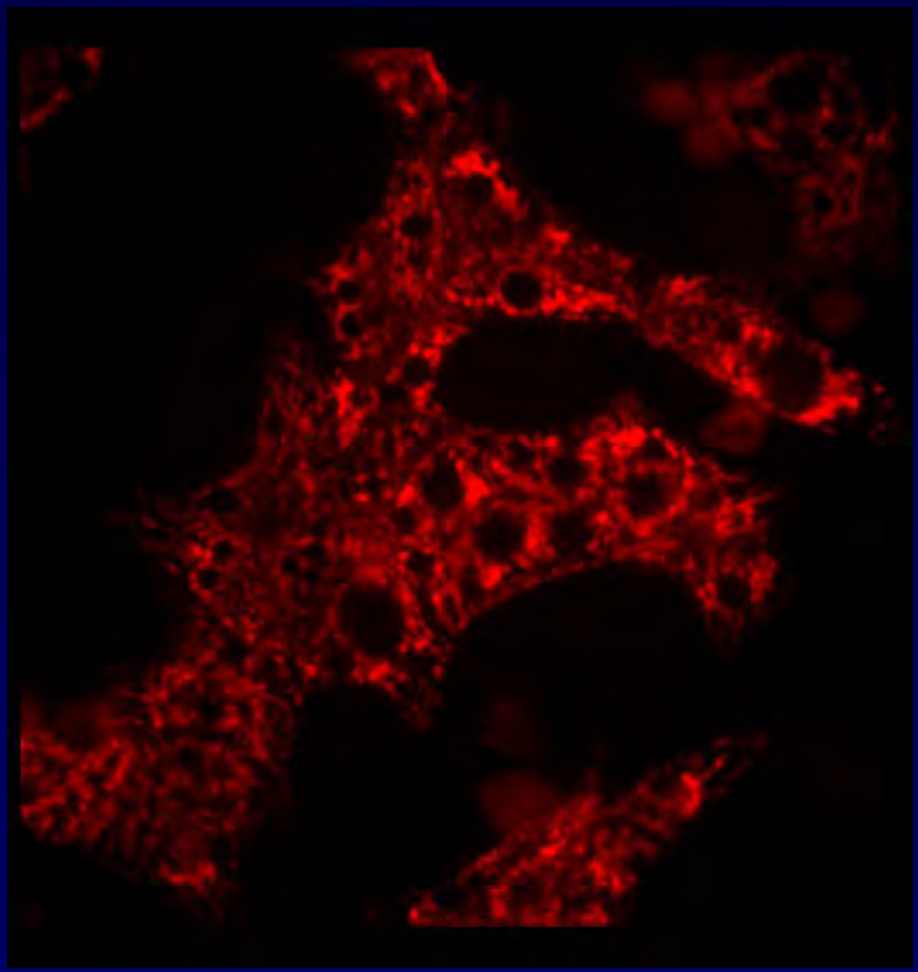
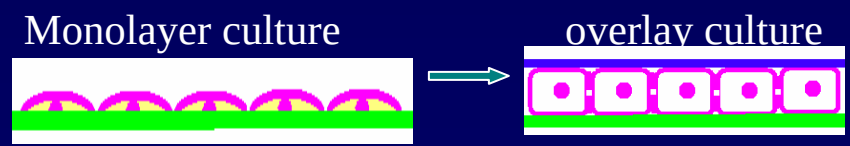
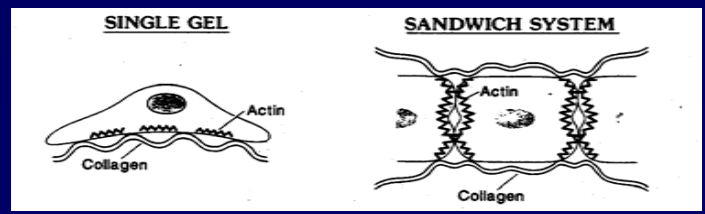
CellCiphr™ Panel 3: Rat Hepatobiliary



- Highly differentiated rat hepatocytes (sandwich culture)
- Chronic exposure (3 day +)
- 384 well capacity
- Functional assays for:
  - Cholestasis
  - Steatosis
  - Mitochondrial potential





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CellCiphr™ Panels in Development

Human and Rat 1° and Cell Lines

- Tissue selectivity panels
  - Neuronal cells
  - Cardiomyocytes
- Stem-cell derived cultures
- Co-culture/tissue engineered array models
- Kinetic, Live Cell Panels

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Summary

- The CSB approach to cell based discovery is being implemented to improve efficacy and decrease toxicity of leads, clinical candidates & drugs
  - Cellular models of disease
  - Cytotoxicity profiling
  - Patient sample profiling
- CSB based cytotoxicity profiling assays and classifiers are evolving to improve early decision making

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