

Solidus Bioscience's MetaChip Technology for High-Throughput *In Vitro* Assessment of Chemical and Drug Candidate Toxicity

S●OLIDUS

A new venture that enhances the ability of cosmetics and chemicals producers to effectively and efficiently adopt *in vitro* toxicity testing and replace the use of animals as required by new legislation in Europe and emerging regulations worldwide

Ensuring the development of safer drugs, cosmetics, and chemicals...faster than ever

- Founded in August 2002 by Jonathan S. Dordick (Rensselaer Polytechnic Institute) and Douglas S. Clark (U.C. Berkeley)
- Technology patented - U.S. 7,267,958 “Biocatalytic Solgel Microarrays”
- Two additional patent applications pending
- Received \$3.1 M in STTR grants from NIH and NSF
- Raised over \$1 M in industrial contracts
- Seven employees

Key Drug Withdrawals

Name	Approved	Indication	Problems	Withdrawn
Tysabri	2004	MS	CNS demyleinating	2005
Bextra	2001	Analgesic	Cardiovascular/Skin	2005
Vioxx	1999	Analgesic	Cardiovascular	2004
Baycol	1997	Hyper-cholesterol	Muscle damage	2001
Propulsid	1993	Heartburn	Heart arrhythmias	2000
Troglitazone	1997	Type 2 diabetes	Hepatotoxicity	2000
Seldane	1985	Antihistamine	Heart arrhythmias	1998
Redux	1996	Obesity	Heart valve abnormalities	1997

In several cases here and elsewhere, the impact of metabolism (including reactive metabolite formation) cannot be ignored.

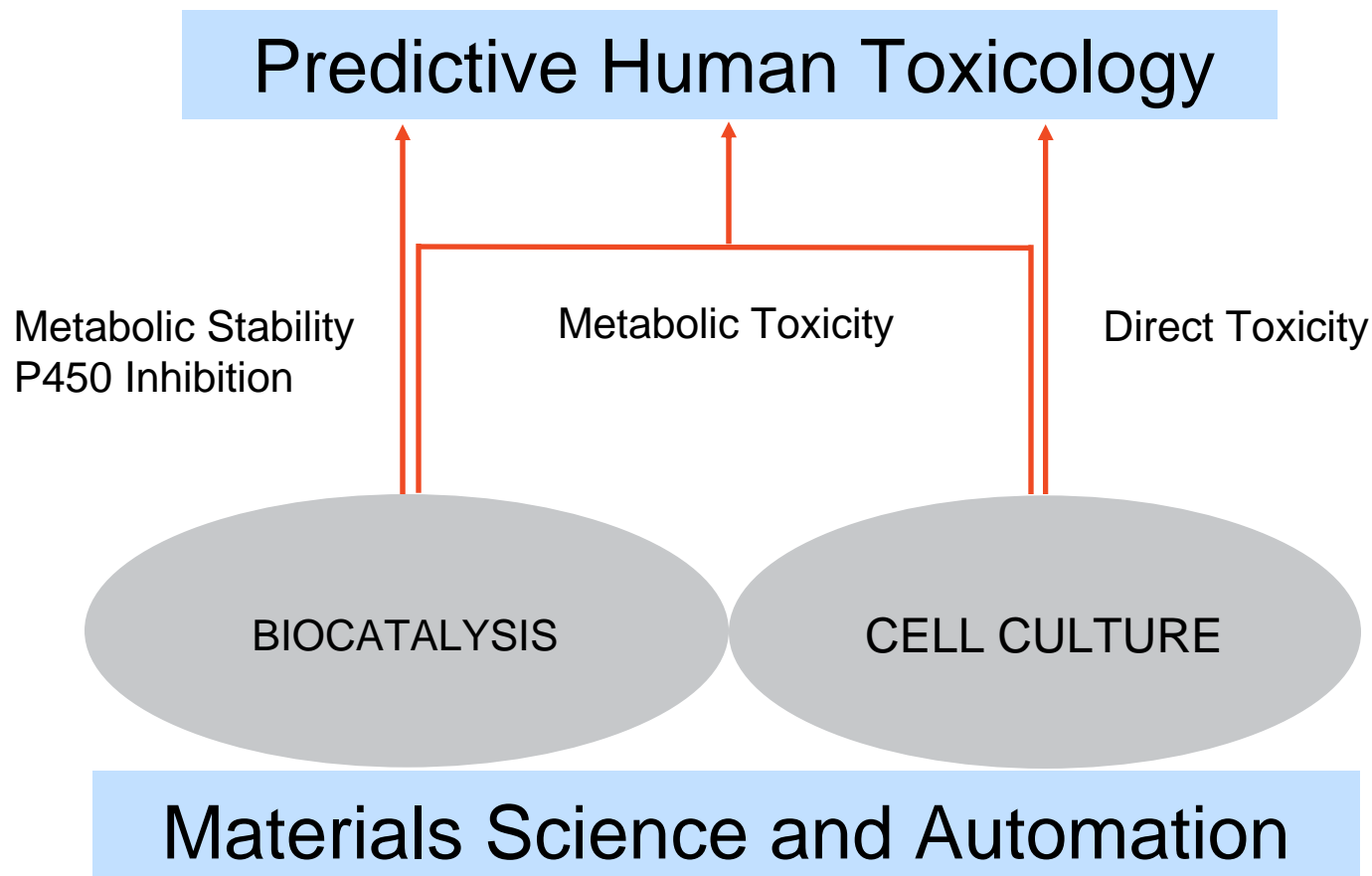
Toxicity Not Limited to Pharmaceuticals

- **Pharmaceuticals**
 - FDA (and related)
- **Cosmetics**
 - European Union's 7th Amendment
- **Cosmeceuticals**
 - 7th Amendment
 - FDA?
- **Chemicals**
 - EU's REACH regulations
 - EPA's ToxCast
- **Nutraceuticals**
- **Nanomaterials**
 - Carbon nanotubes
 - Buckyballs
 - Metal and metallic oxide nanoparticles

- Solidus is developing products that will satisfy current and emerging regulatory requirements
- Solidus' products will enable cosmetics and cosmeceutical companies to conform to 7th Amendment regulations of the EU
- Solidus' products will enable chemical and agrochemical companies to satisfy EU REACH legislation
- Solidus' products will enable pharmaceutical companies to lower the cost and improve the yield of developing new chemical entities

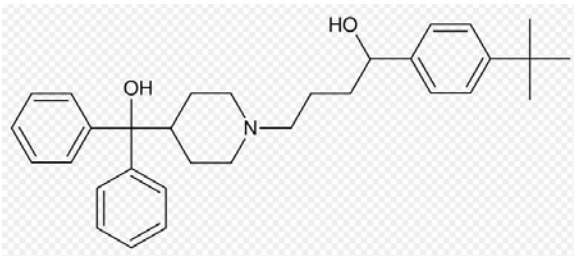
Enabling Foundation of Solidus' Technology

SOLIDUS

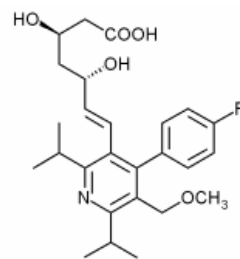


CYP450 Inhibition

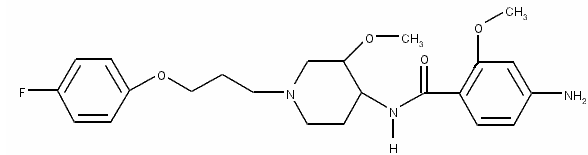
- Over 90% of all major drugs are metabolized by three CYP450 isozymes: CYP3A4, CYP2D6, and CYP2C9
- CYP450s can be inhibited by drugs in three ways: competitive, non-competitive, and irreversible inhibition
- CYP450 inhibition causes serious Drug-Drug Interactions (DDIs), which cost up to \$100 billion and cause serious complications in 7% of all hospitalized patients in the U.S.



Seldane®

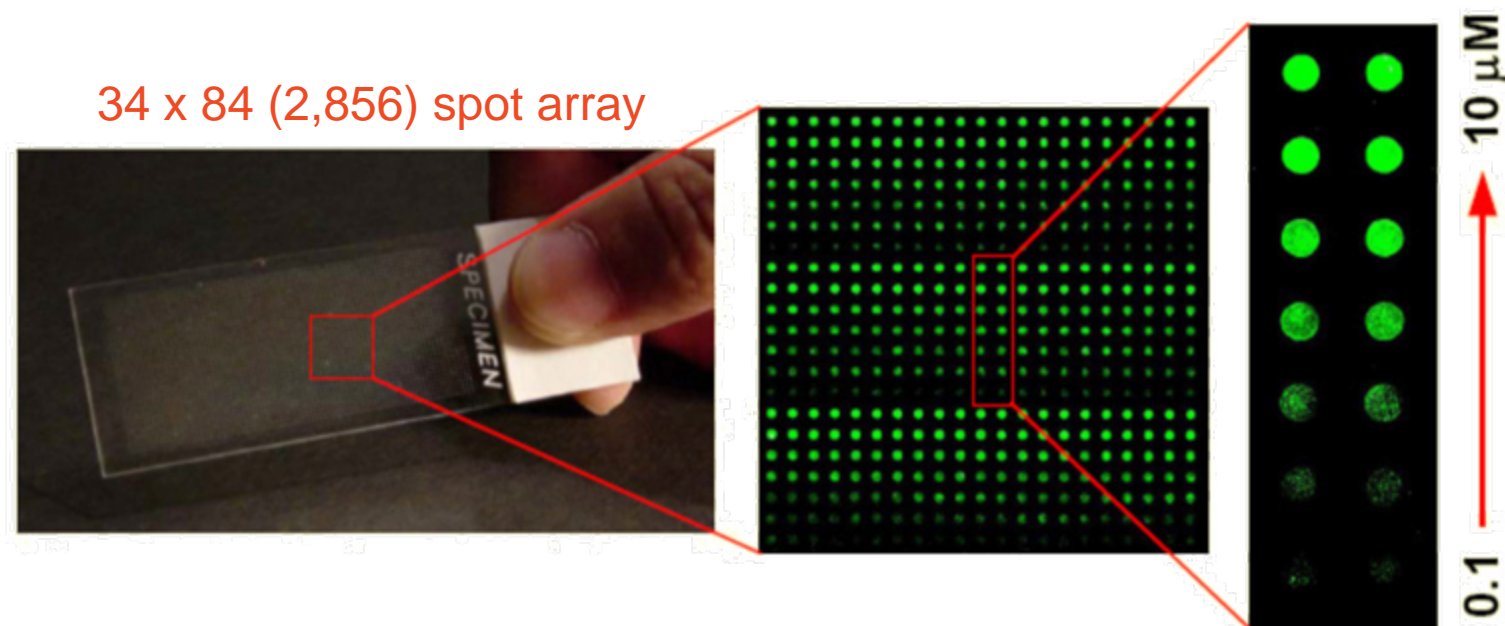


Baycol®



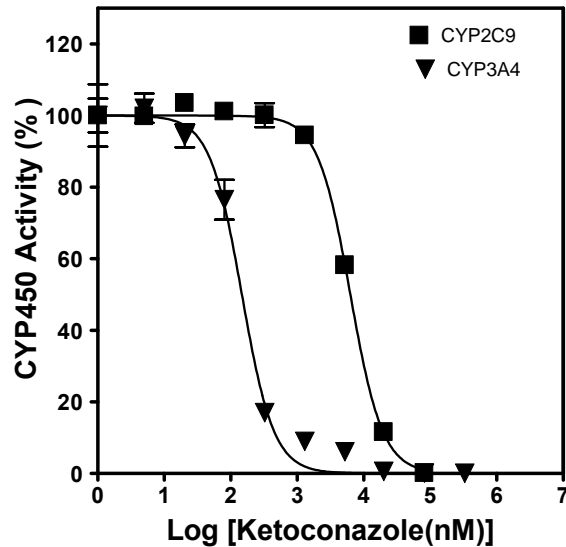
Propulsid®

The MetaChip



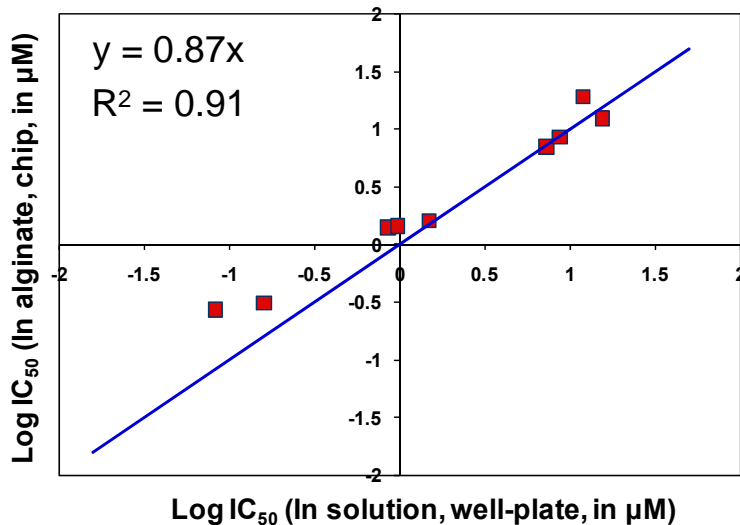
- Nano-liter scale: Save reagents and test compound
- IC_{50} values comparable to well-based methods
- Highly effective for determining IC_{50} values of reactive metabolites
- Rapid and high throughput

CYP450 Correlations

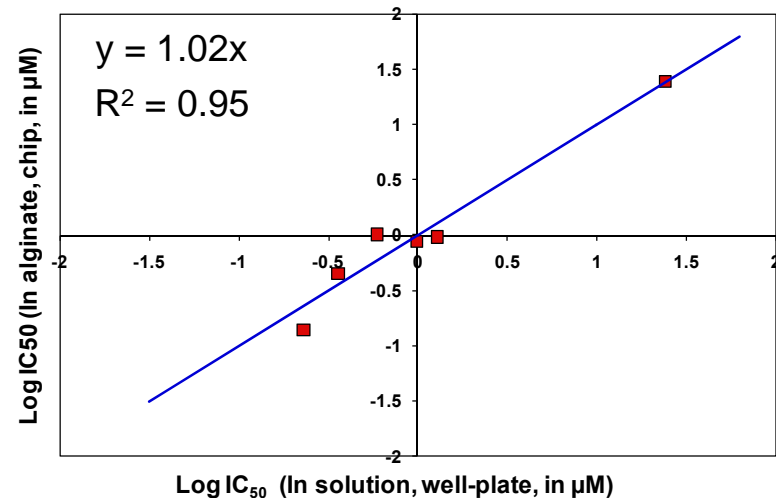


- CYP450 inhibition of human 3A4 and 2C9
- MetaChip consists of 20 nL alginate spots
- Solution assays performed in 384-well plates
- CYP3A4: clotrimazole; ketoconazole; miconazole; nifedipine; erythromycin; terfenadine; verapamil; ethynylestradiol; fluconazole
- CYP2C9: clotrimazole; sulfaphenazole; nifedipine; nicardipine; miconazole; ketoconazole

CYP3A4

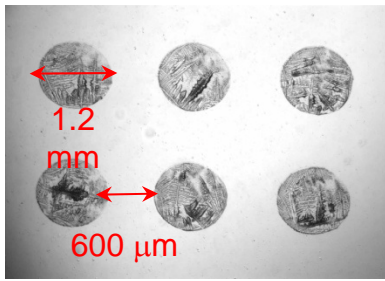


CYP2C9



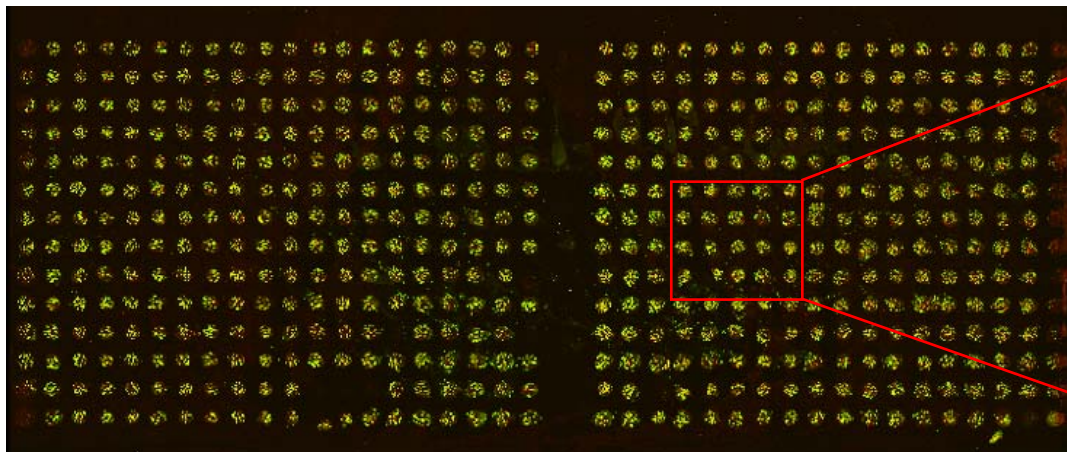
The DataChip

Alginate spots

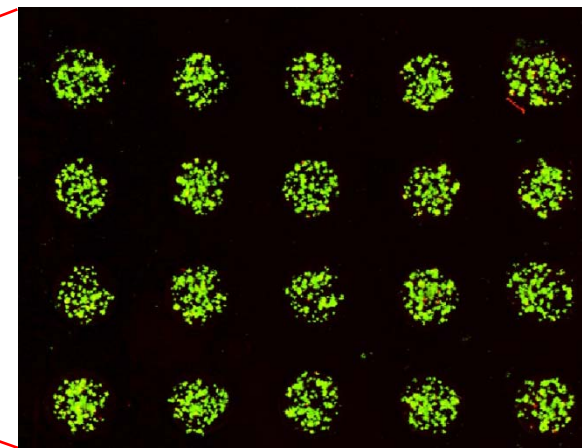


- Cell growth in 3D matrix similar to *in vivo*
- Gel-encapsulated cells printed with a microarrayer
- 20-nL spots, 20 cells/spot, 1,080 spots/slide
- Glass slides modified for robust attachment of hemispherical alginate spots

DataChip



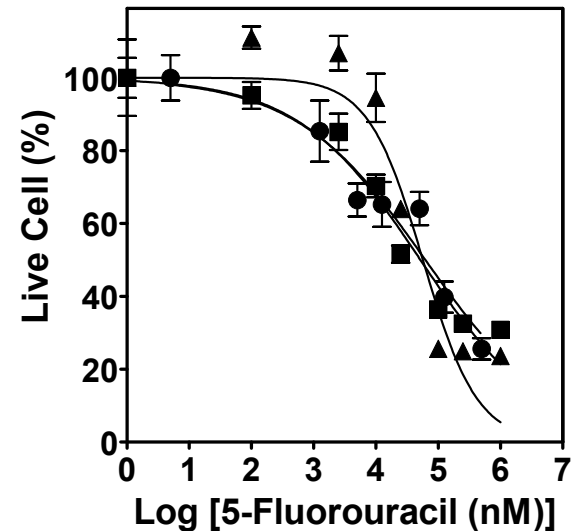
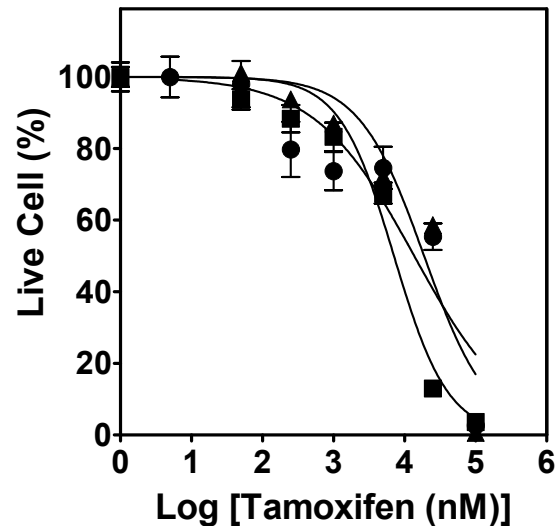
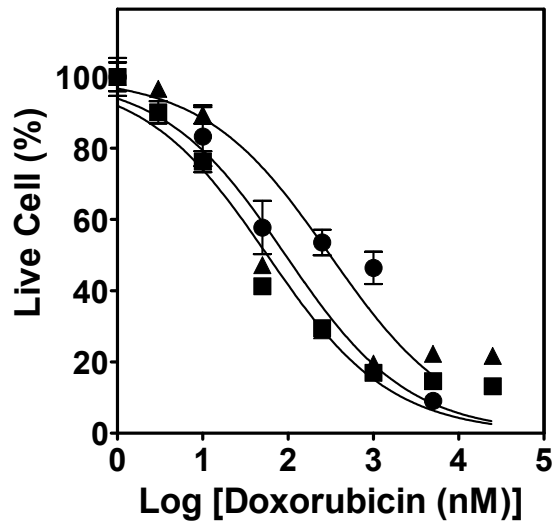
Live cell spots



Comparison of DataChip to 2D and 3D Cell Cultures

Dose response curves for doxorubicin:

(●) DataChip, (▲) 3D 96-well plate, and (■) 2D 96-well plate



IC₅₀ values in various formats (μM)

Drug	96-well plate (2D)	96-well plate (3D)	Collagen DataChip	Alginate DataChip
Doxorubicin	0.05 ± 0.00	0.09 ± 0.01	0.22 ± 0.04	0.19 ± 0.08
5-Fluorouracil	51.5 ± 3.63	56.8 ± 5.09	62.2 ± 11.7	84.6 ± 18.7
Tamoxifen	7.09 ± 0.24	18.9 ± 0.94	13.7 ± 2.32	13.3 ± 4.80

Solidus' Technology Platform

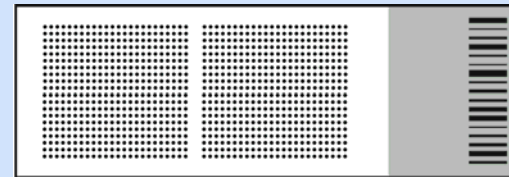
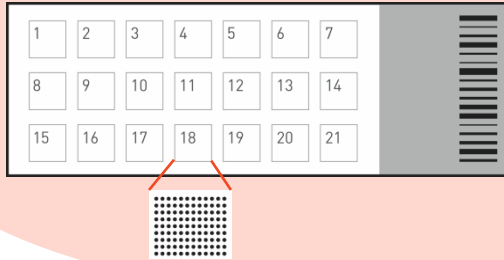
Metabolizing enzyme
toxicology assay chip

Data analysis
toxicology assay chip

MetaChip

DataChip

Combined
Chips



- P450 inhibition
- Enzyme induction
- Metabolic stability

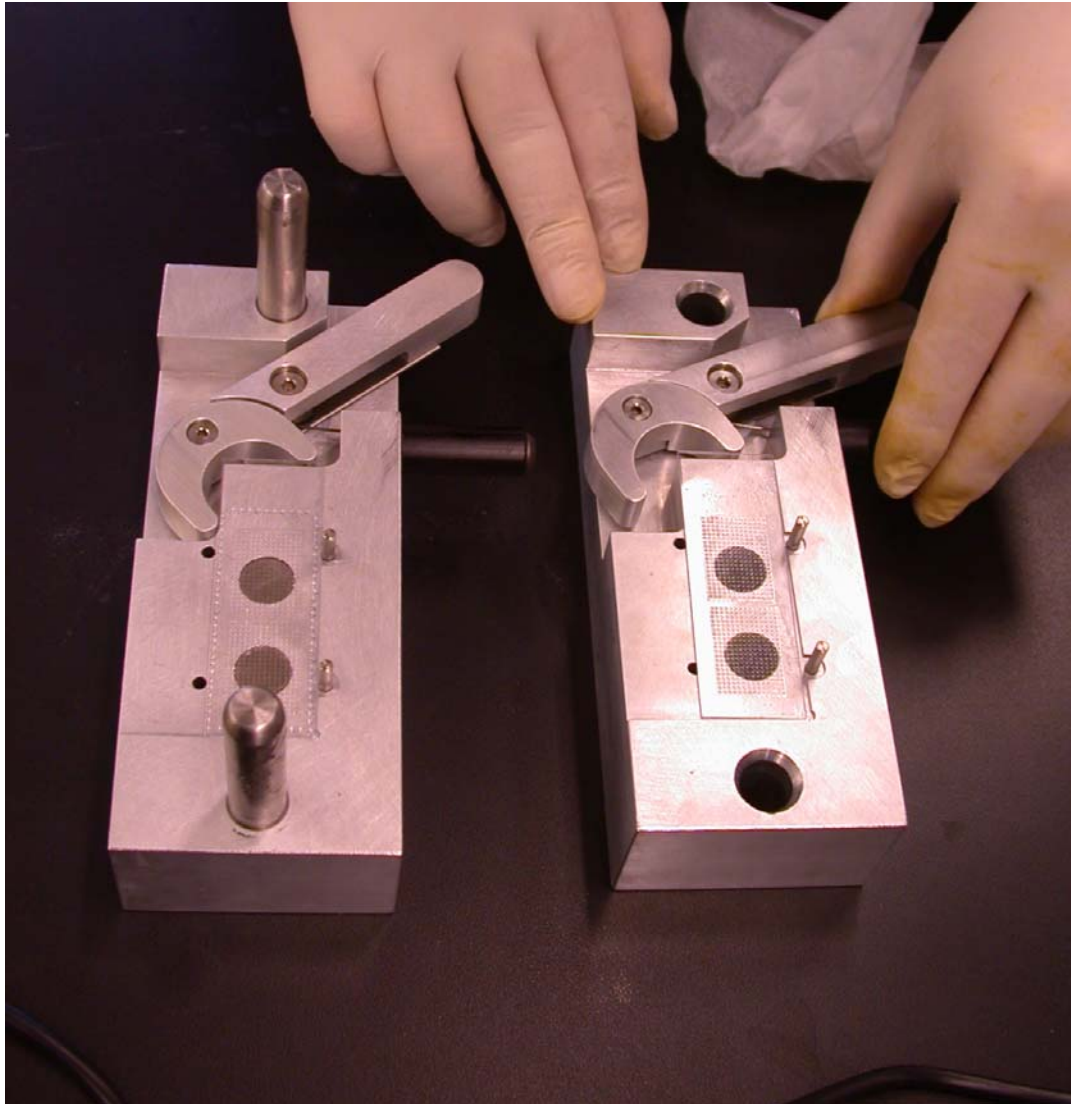
- Metabolism-generated toxicity

- Cellular toxicity
- Enzyme induction

High-Throughput Human Metabolism Screening

Stamping Device for MetaChip/DataChip

Loads both MetaChip and DataChip

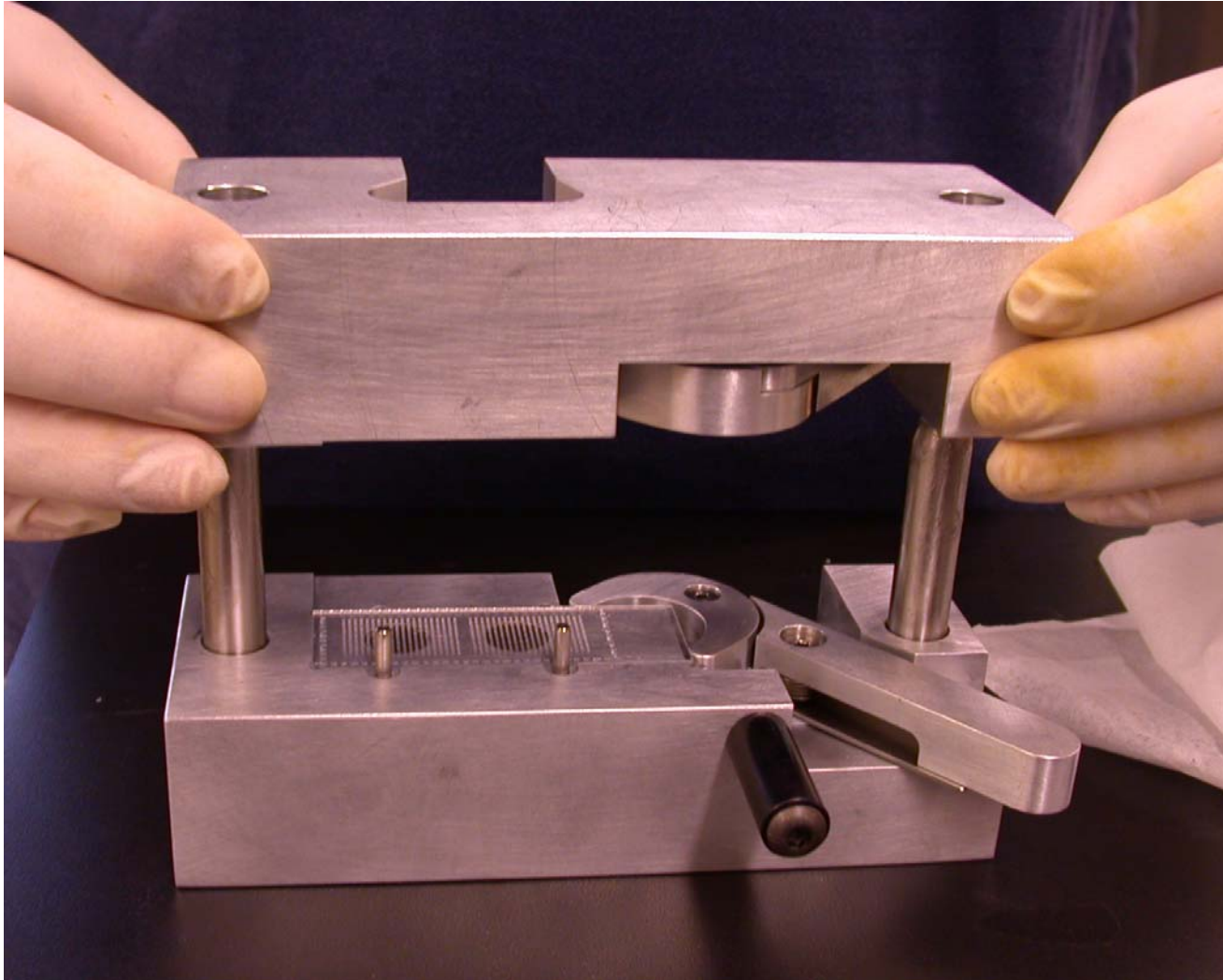


MetaChip loaded into top half of stamping device

DataChip loaded into top half of stamping device

Stamping Device for MetaChip/DataChip

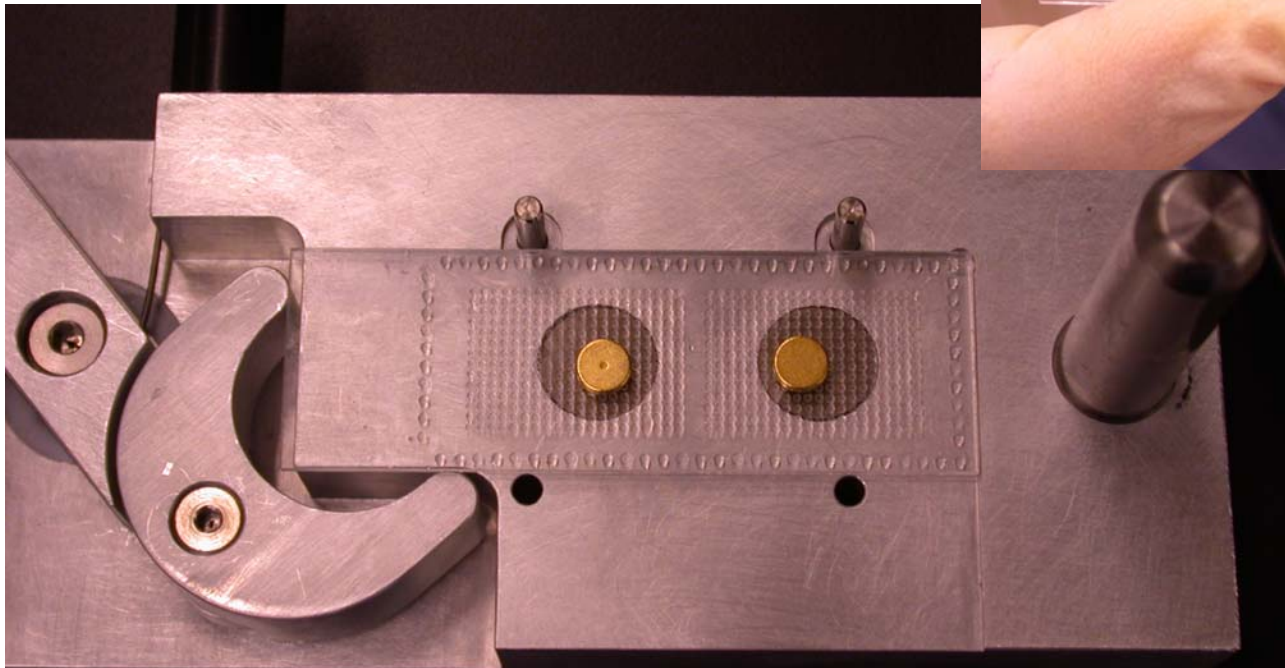
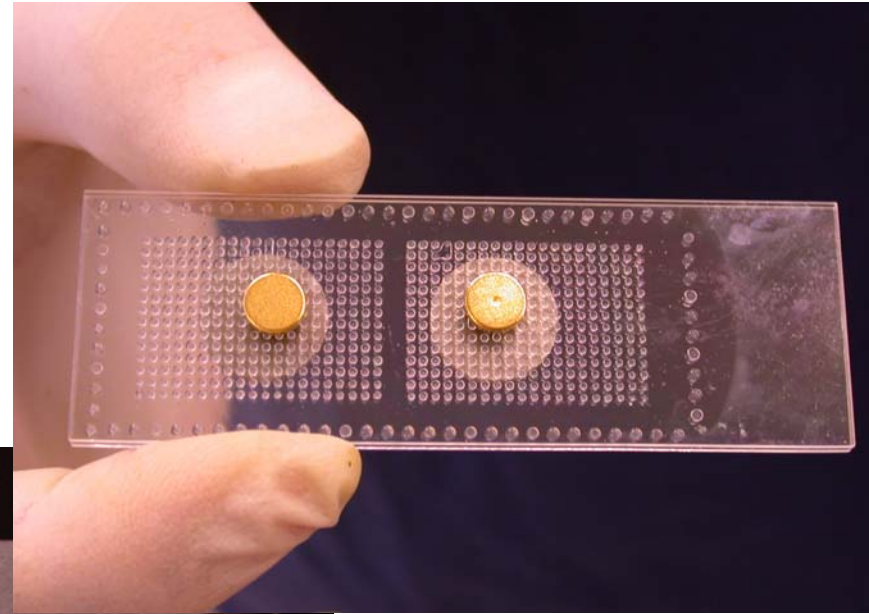
Pin-Guided Alignment for Precise Stamping



Stamping Device for MetaChip/DataChip

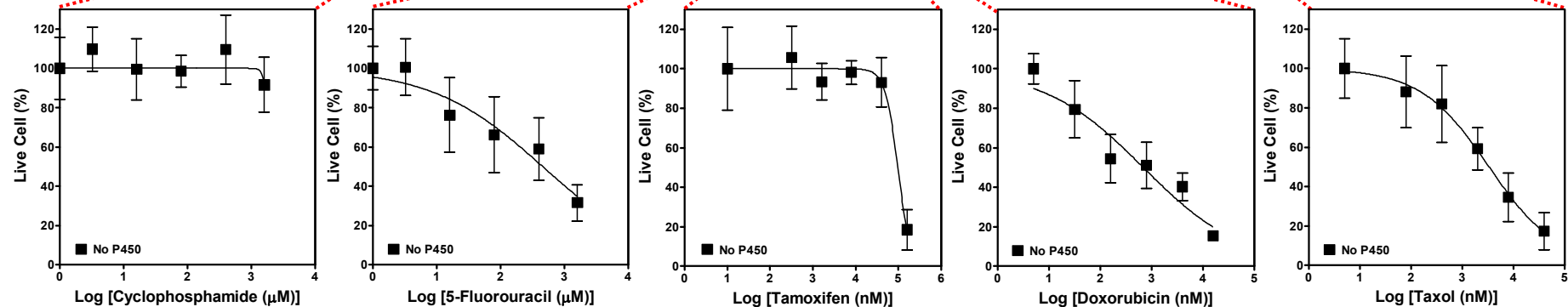
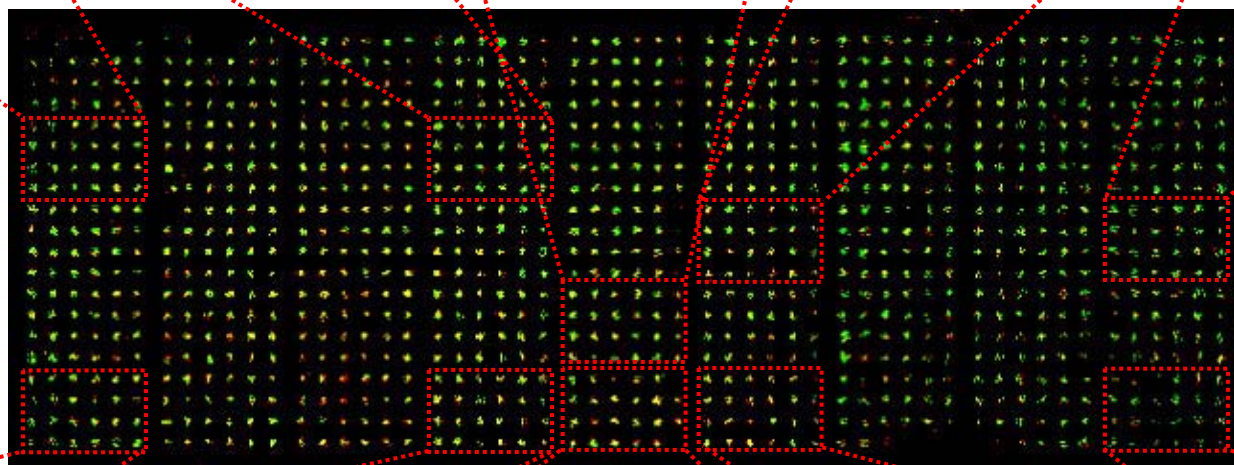
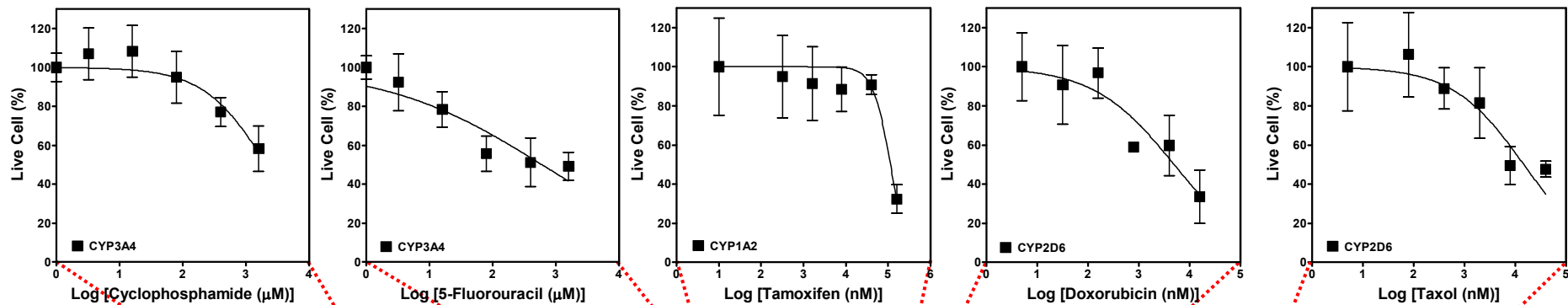
Removing Stamped Chips

- Magnets are placed in holes on top piece to secure the chips
- Top slide clip is released and the top of the device is lifted off



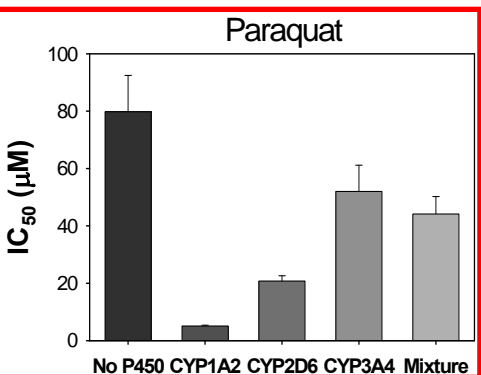
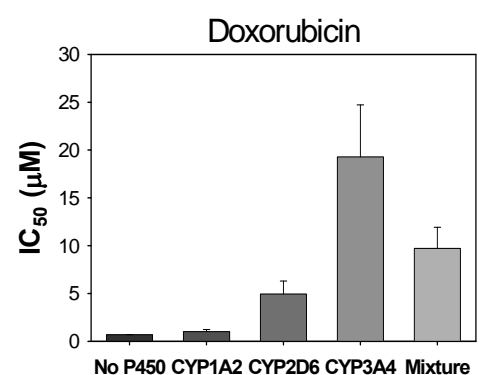
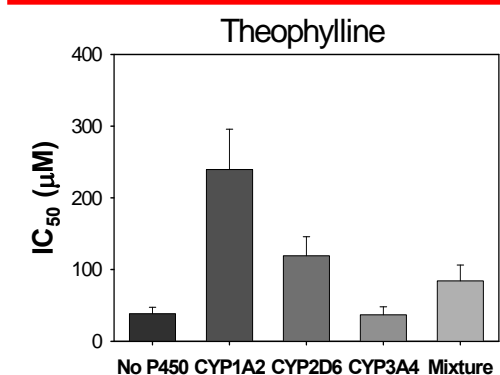
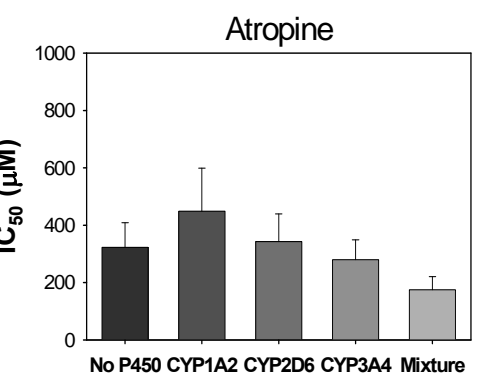
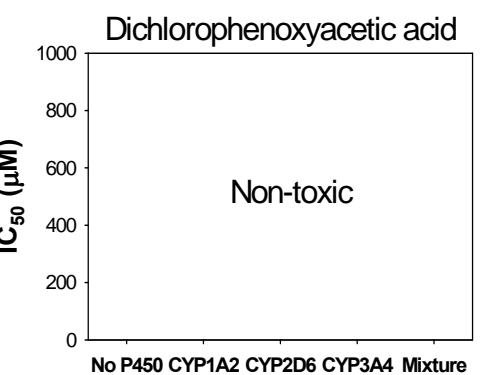
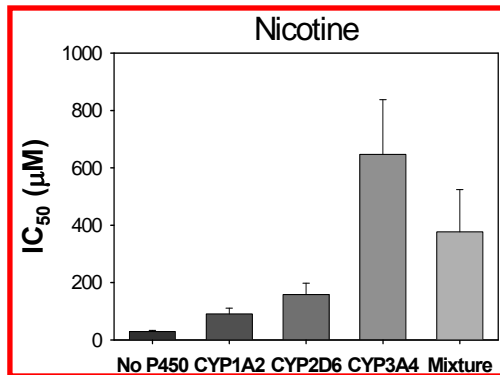
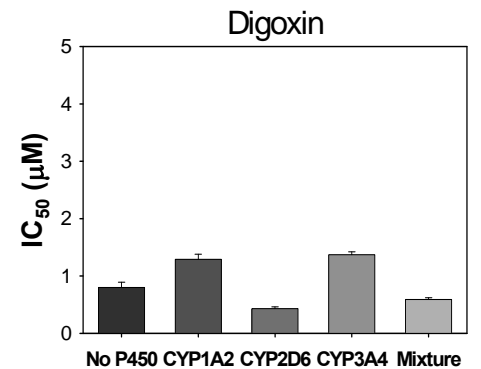
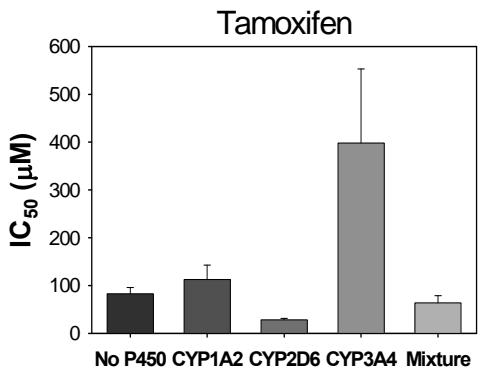
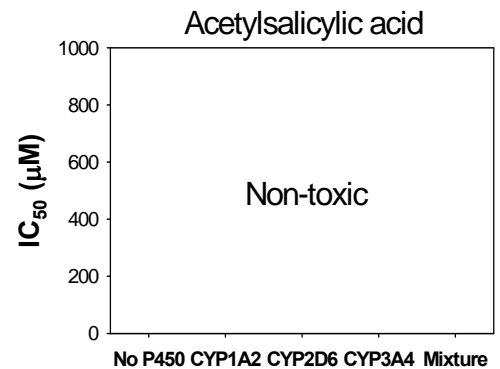
Chips can then be removed and incubated!

Spot density = 1,080/slide; Hep3B Cells



Nine compounds, 5 P450s or mixtures, 6 concentrations, 4 replicates

Example Effects of Metabolism

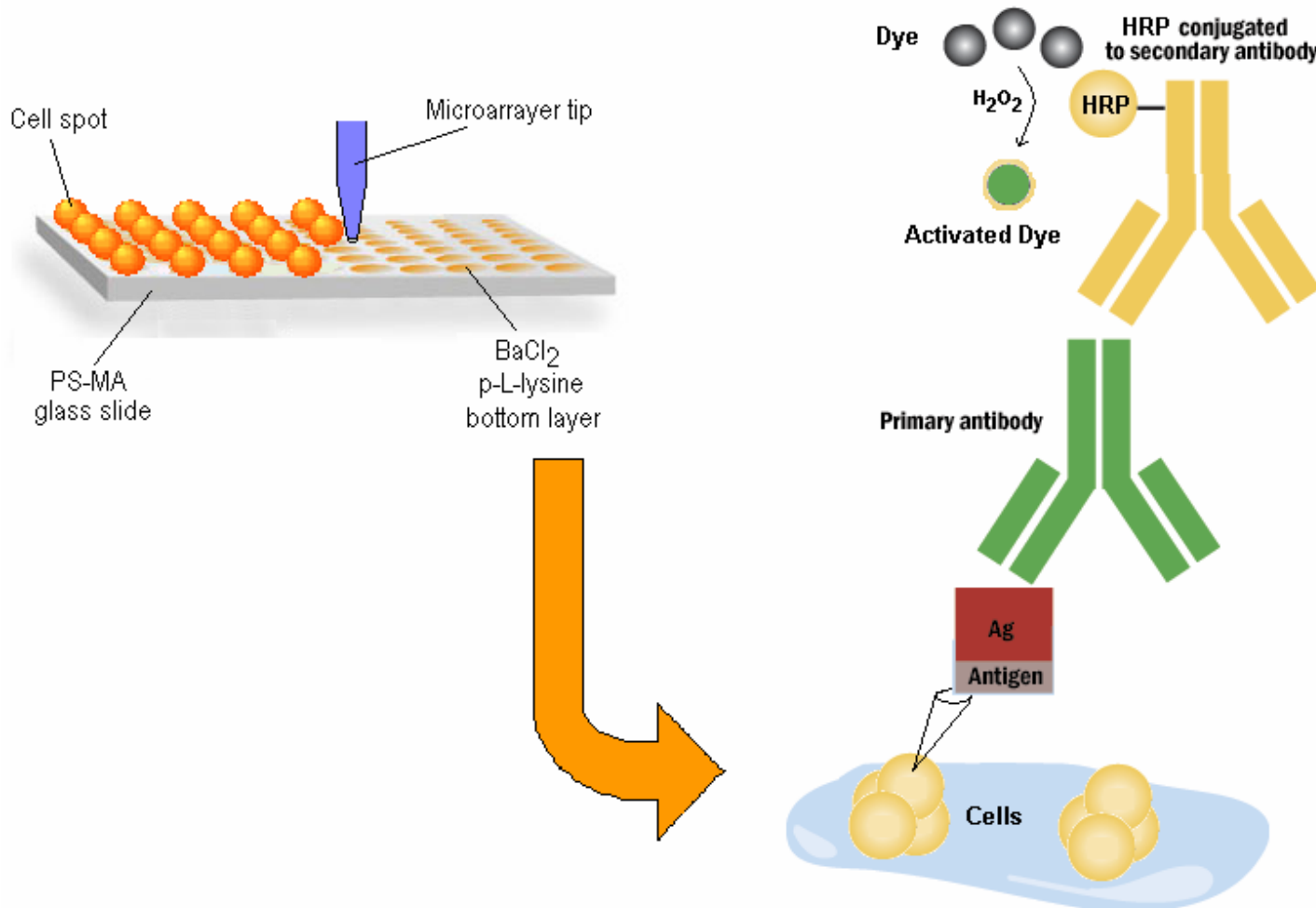


- 50 compounds were obtained from the client and analyzed by the MetaChip technology
- Three human cell types were used: Hep3B (hepatoma), MCF7 (breast carcinoma), and A293 (kidney adenocarcinoma)
- IC₅₀ values were obtained for the parent compounds and for metabolic products of different CYP450s, phase II enzymes, and their mixtures
- Pronounced effects of metabolism were observed for several compounds. Enhanced toxicity was observed in some cases and reduced toxicity in others
- A total of **27,000** IC₅₀ values were determined over a 2-month period

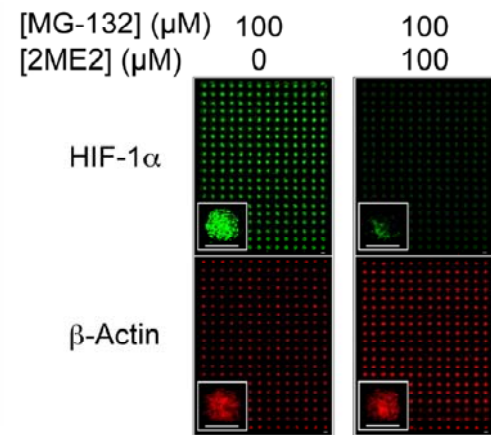
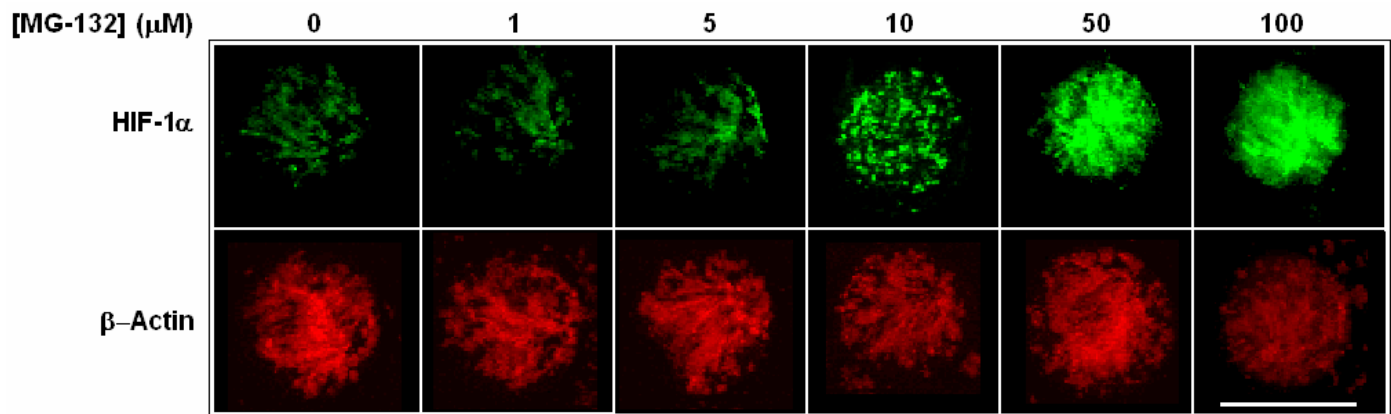
Predictions Require Multiple Endpoints

- **Cytotoxicity with:**
 - Transformed human and animal cells
 - Primary hepatocytes
 - Primary non-hepatocyte cells
- **Protein markers for:**
 - Inflammation
 - Angiogenesis
 - Apoptosis
 - Mitochondrial function
- **Genetic markers**

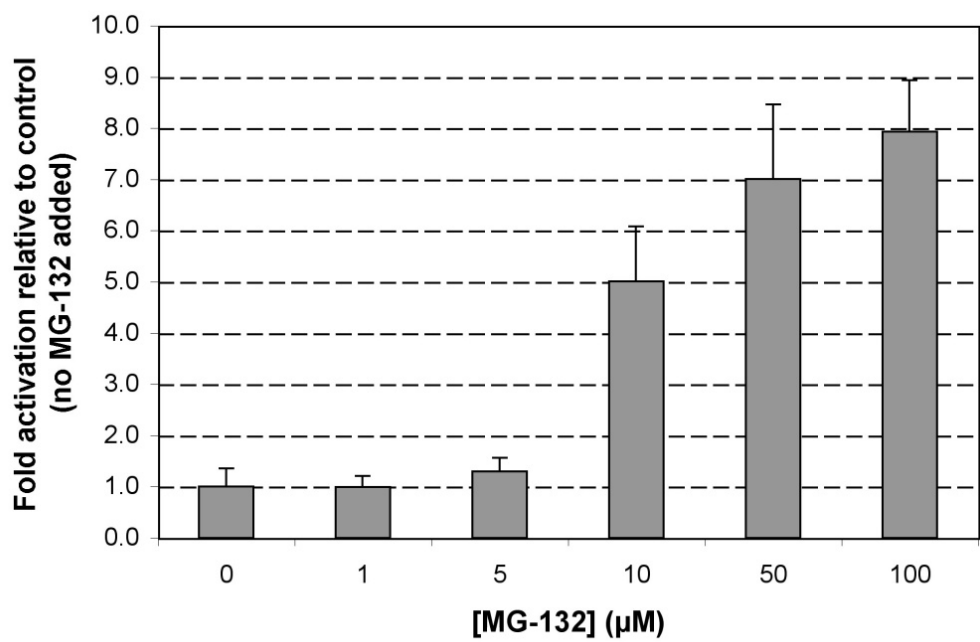
In-cell, On-Chip Immunofluorescence Assay for High-Content Screening



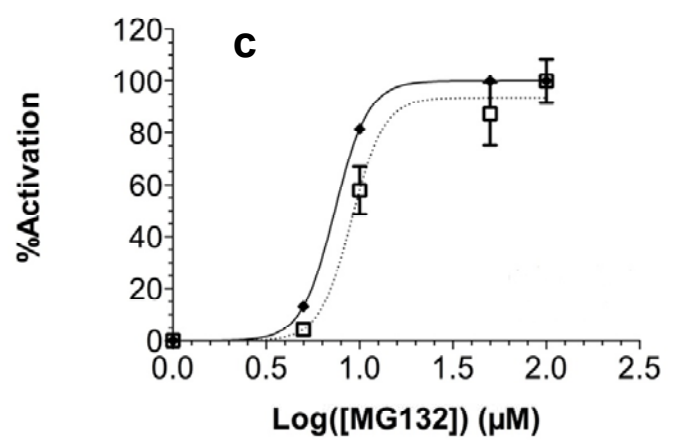
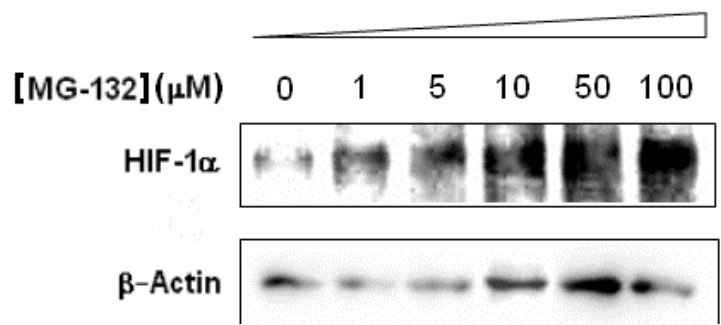
- Quantification of the alpha subunit of the hypoxia-inducible factor (HIF-1 α) after chemical stimulation of human pancreatic tumor cells encapsulated in 3D alginate spots



Scale bar: 800 μ m



Angiogenesis inducer (Hif-1 α inducer)



Benefits Powered by the Solidus Technology



Enables high-throughput human toxicology studies for drugs, cosmetics, ag products, and chemicals

The MetaChip:

- Provides accurate information rapidly on potential organ-specific toxicity of drug/ag/cosmetic/chemical candidates
- Mimics human metabolism by generating human metabolites and analyzes their inherent toxicity
- Determines P450 inhibition
- Reinforces compound safety indications via analysis of metabolism rates (“metabolic stability”)
- Requires very little compound (μg) for analysis, compatible with early stage screening

- **MetaChip** – CYP450 chip for detecting inhibition and metabolism-induced toxicity
- **DataChip** – 3D human cell chip for screening cytotoxicity
- **MesaChip** – Metabolic stability chip for measuring rates of compound metabolism
- **Multizyme Chip** – Multi-enzyme chip for improving desired properties of compounds
- **MetaReader** – Automated device for analyzing Solidus' chips