

Microarray Biochips for High-Throughput Metabolism-based Drug Screening

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Contents

- Drug metabolism & cytochromes P450 for drug screening
- Miniaturized drug metabolizing enzyme (DME) array (the Metabolizing Enzyme Toxicology Assay Chip, or MetaChip)
 - How to prepare the **MetaChip**: P450 sol-gel encapsulation
 - **MetaChip** platform coupled with human cancer cell monolayer for *in vitro* toxicology assay
- Miniaturized 3D cell-culture array (the Data Analysis Toxicology Assay Chip, or DataChip)
 - How to prepare the **DataChip**: human cancer cell encapsulation within collagen or alginate matrix as a drug screening target
 - **DataChip** platform coupled with **MetaChip** for metabolism-induced toxicity assay
- Application of the MetaChip for P450 inhibition study

Drug Discovery Processes

Success rate:

Target discovery

2M

Lead discovery

5K

Lead optimization

250

Preclinical evaluation

5

Clinical development

1

FDA approval

- Target identification/validation
 - Bioinformatics
 - Genomics
 - Proteomics

- Assay development
- Chemical library development
- High-throughput screening
 - Biochemical & cellular assays

- Structure-activity relationship
 - *In silico* evaluation
 - Lead derivatization
 - *In vitro* drug efficacy, toxicology & pharmacokinetics
 - *In vivo* efficacy

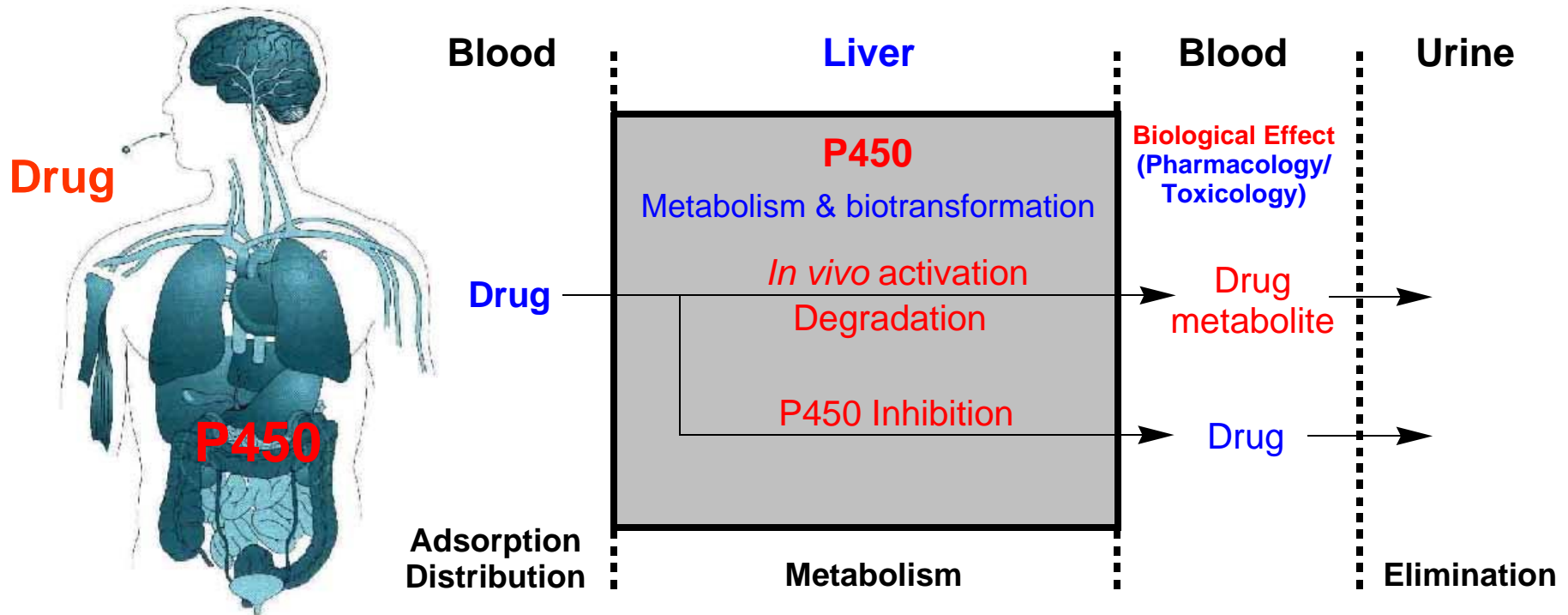
- Pharmacokinetics
- Pharmacodynamics
- Toxicity/safety pharmacology
 - ADME/Tox
- Drug formulation

- Phase I, II, III

∴ 15 year & 1.7B\$/drug

- Nearly **40% of drug candidates** fail in preclinical and clinical stage of drug development due to unanticipated **drug metabolism and toxicity**.
- **Current high-throughput screening** has **not** effectively integrated drug safety into the process of identifying high quality lead compounds.
- As a result, NIH and FDA indicated that **new high-throughput technologies** are needed for **drug candidate toxicity and human metabolism testing** in the early stages of drug development.

Cytochromes P450 & Drug Metabolism



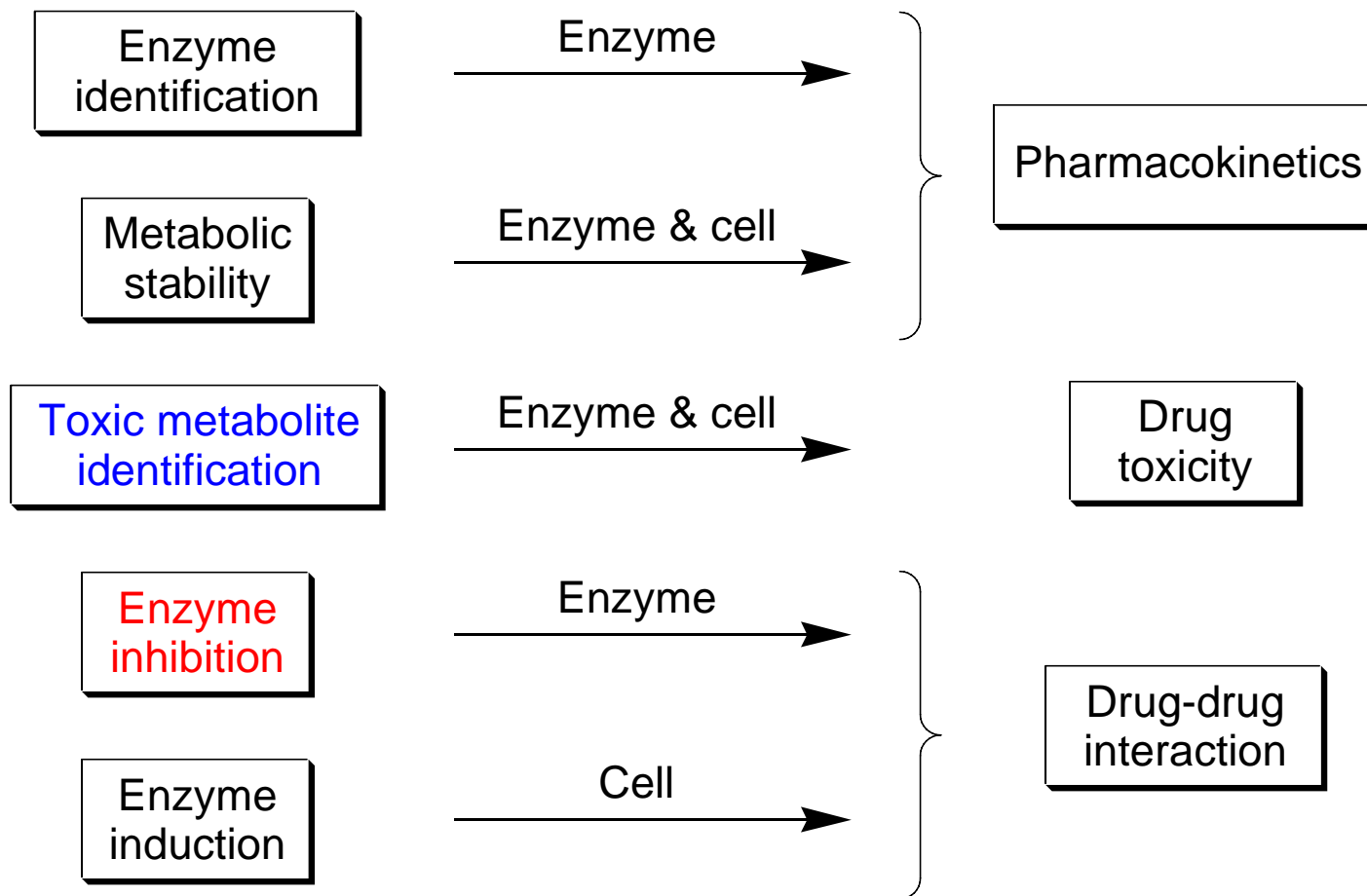
- P450 enzymes introduce an oxygen atom into drugs (oxidation).
- Catalyze the initial step in the metabolism of most drugs.
- More than 50 isoforms (e.g., 1A2, 2C9, 2D6, 2E1, 3A4, etc.) are known to exist in humans.



Drug metabolism & toxicology

Conventional *in vitro* Metabolism Study

Assay



Several metabolism-based studies (listed on the left-hand side) can be performed by either enzyme- or cell-based assays for different goals (listed on the right).

Typical Metabolism and Toxicity Assays

- *In vitro* test: simple and straightforward but relatively large assay volume required
 - Isolated and purified cytochromes P450
 - Liver microsomes
 - Suspended and monolayer hepatocytes
 - Tissue slices
- *In vivo* test: difficult and time-consuming but more accurate
 - Animals: rodents, dogs, primates



Alternative approach

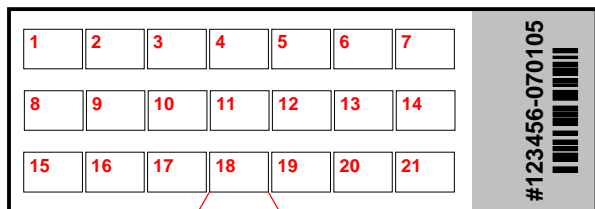
- *In vitro* **microarray biochip** test: simple, small, and fast
 - DNA chip
 - **Protein chip** (antibody array, [enzyme array](#), etc.)
 - **Cell chip** (cell pattern by photolithography, [3D cell array](#), etc.)
 - Tissue chip

Solidus' Microarray Chip Technology Platform

Metabolizing enzyme toxicology assay chip

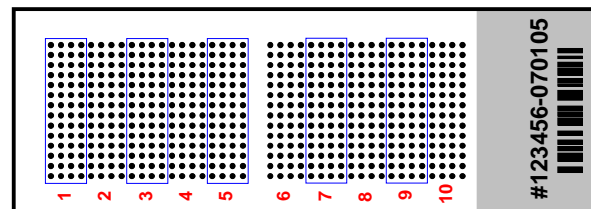
Data analysis toxicology assay chip

MetaChip



P450 spot array

DataChip



Cell spot array

Combined Chips

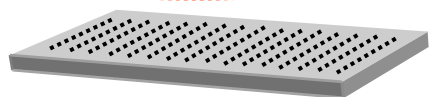
- **P450 inhibition**
- **Enzyme identification**
- **Metabolic stability**
- **Metabolism-generated toxicity**
- **Cellular toxicity**
- **Enzyme induction**

High-throughput human metabolism screening

Preparation of Sol-gel-based **MetaChip** for Metabolism-Induced Toxicity

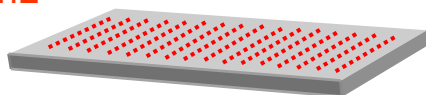
MetaChip Technology for Metabolism-Induced Toxicity

Mimic what occurs in the human liver
(Metabolite synthesis & P450 inhibition)



P450 chip

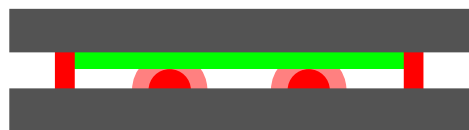
Size of slide: 2.5 x 5.7 cm²
Spots: 500 – 5000
Diameter: 100 – 800 μm
Volume: 5 – 100 nL



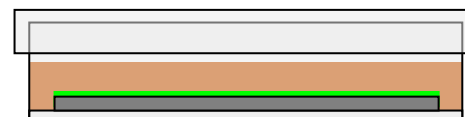
Spot with solution of drug/lead

Drug metabolite

Chamber slide



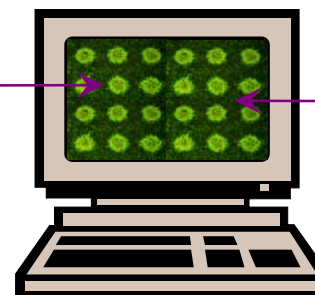
Contact with cells (Stamping)



Incubation of cells

Stain cells
for viability

Analysis
(scanner/microscope)



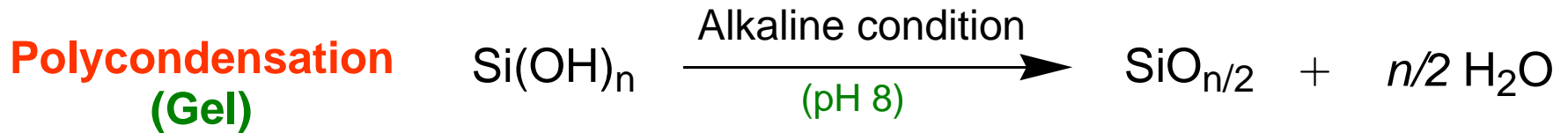
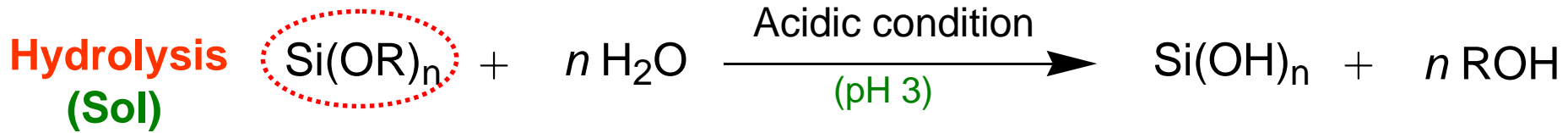
Dead cell
(yellow-red)

Live cell
(dark green)

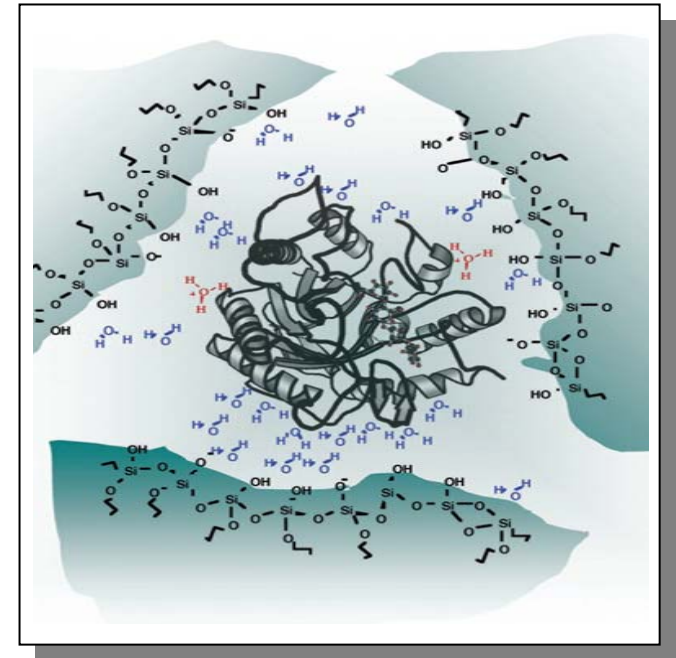
Dead cells correspond to active
compounds in microarray

How to test the cytotoxicity of drug metabolites?

Sol-gel Enzyme Immobilization



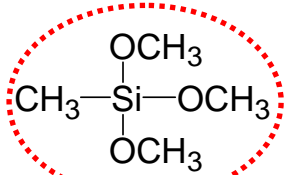
- Sol-gel formation
 - Silicone precursor hydrolysis
 - Mixing enzyme with sol solution
 - Gelation by condensation
- Mild and stabilizing enzyme encapsulation method
- Optically transparent
- Chemically and mechanically stable
- Tunable porosity



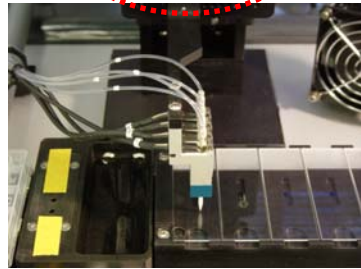
P450 encapsulation was extended to collagen and alginate gels.

P450 Encapsulation in Sol-gel

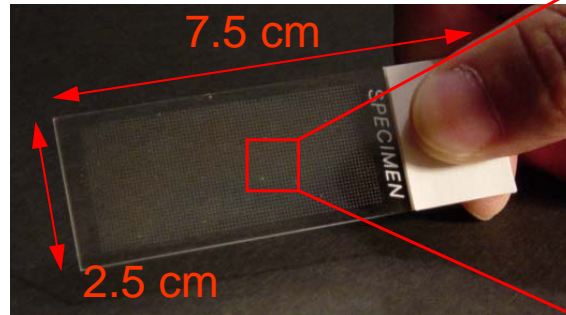
Methyltrimethoxysilane (MTMOS) sol-gel containing P450



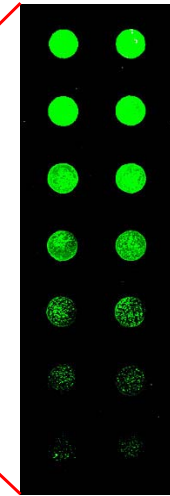
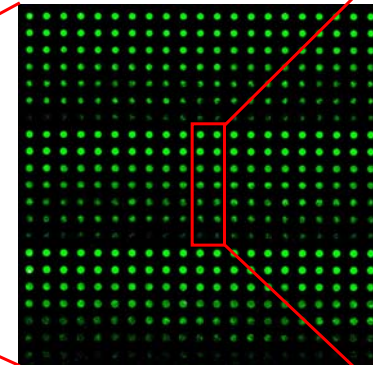
Spot diameter: 200 μm
Spot-to-spot distance: 700 μm



Microarrayer

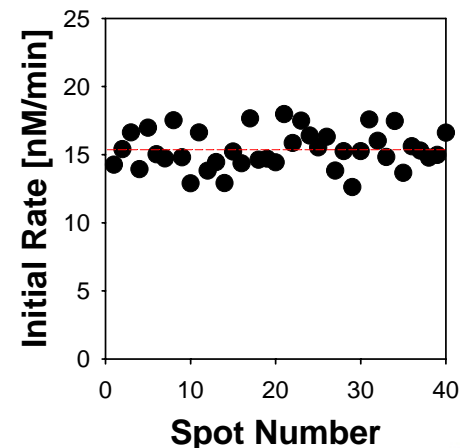


5 nL P450 sol-gel array
(2,856 spot)



Substrate

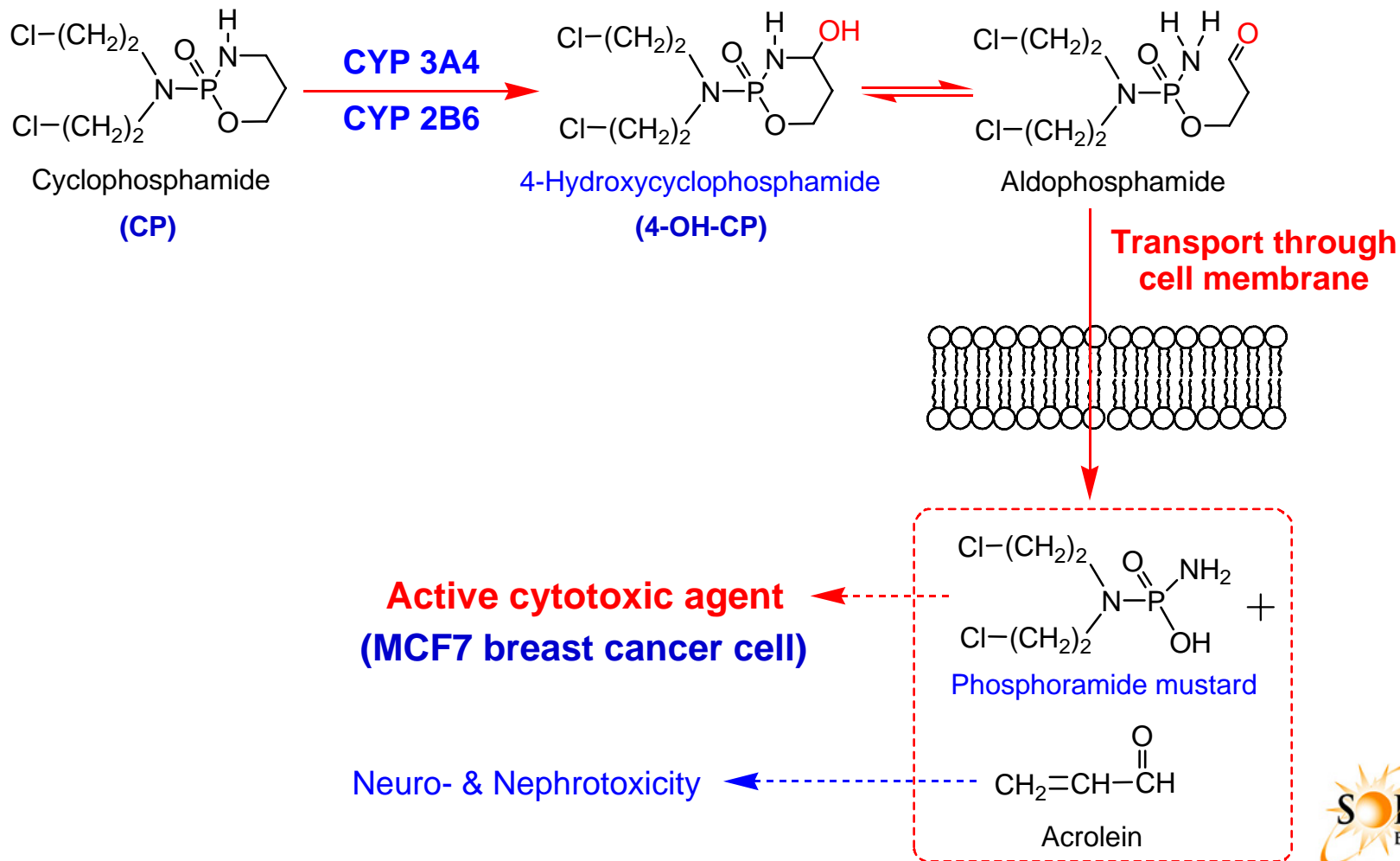
- Scale down to 5 nL.
- P450 encapsulated in sol gel matrices retained high activity (1/3 of native enzyme).
- P450 encapsulated in collagen & alginate matrices retained near native activity.



Proof of Concept

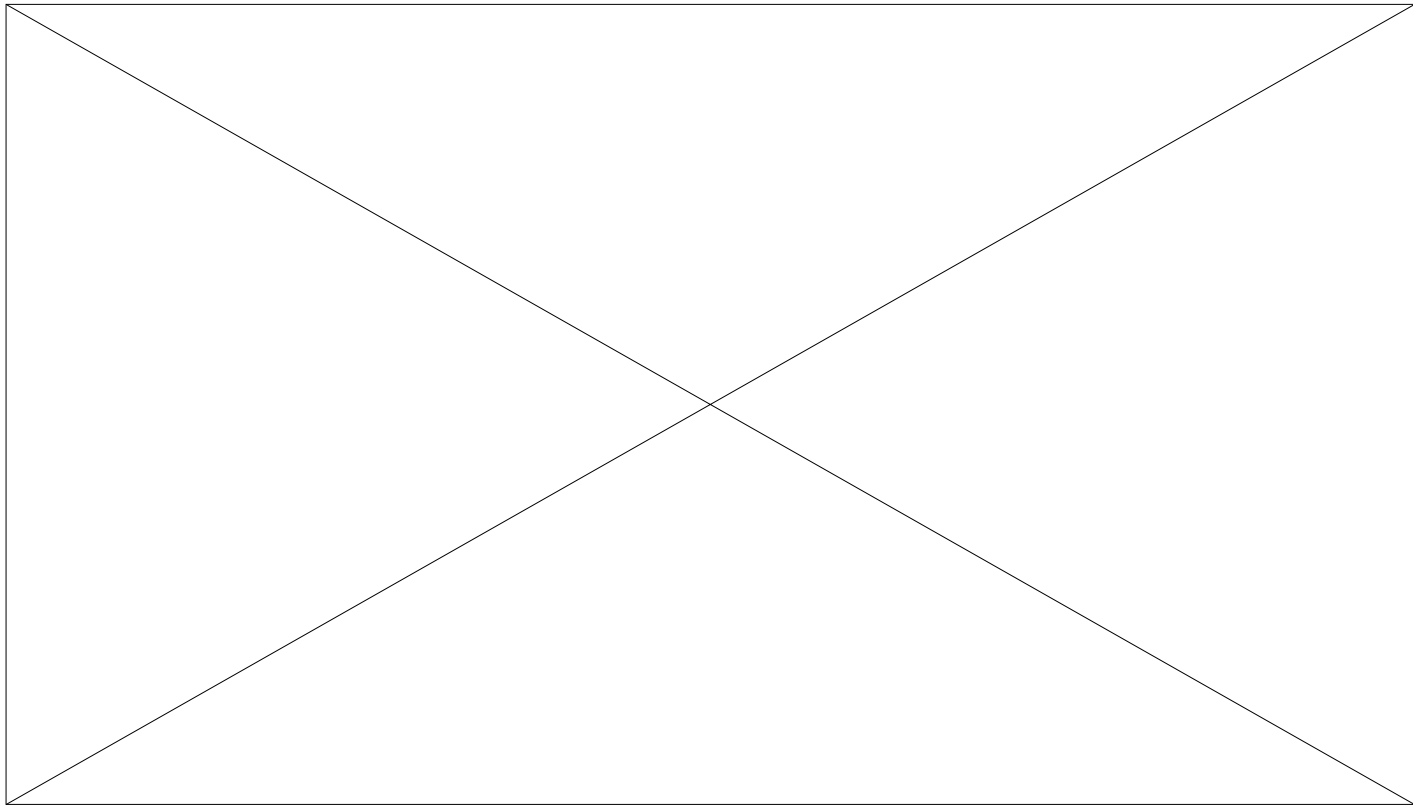
- Activated by P450 metabolism (prodrugs, protoxicants, etc.)
- Screened against available cell lines
- Medically relevant

Cyclophosphamide prodrug activation

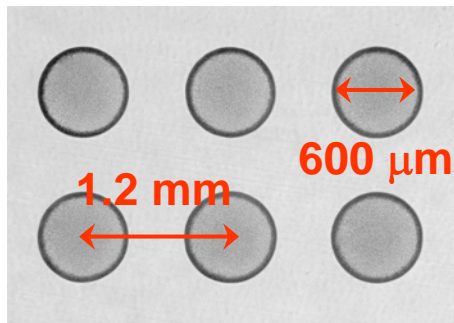


MetaChip Coupled with MCF7 Cell

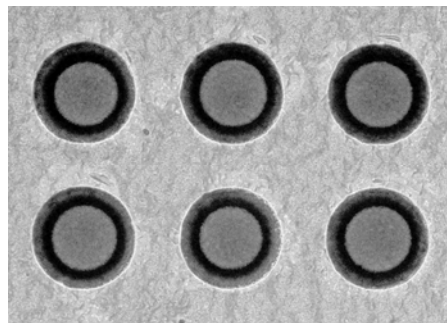
Stamping
Technique



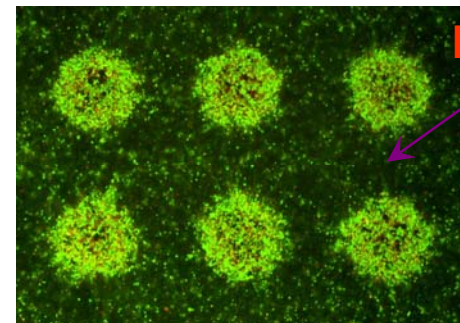
525 spots
(30 nL)



After sol-gel spotting



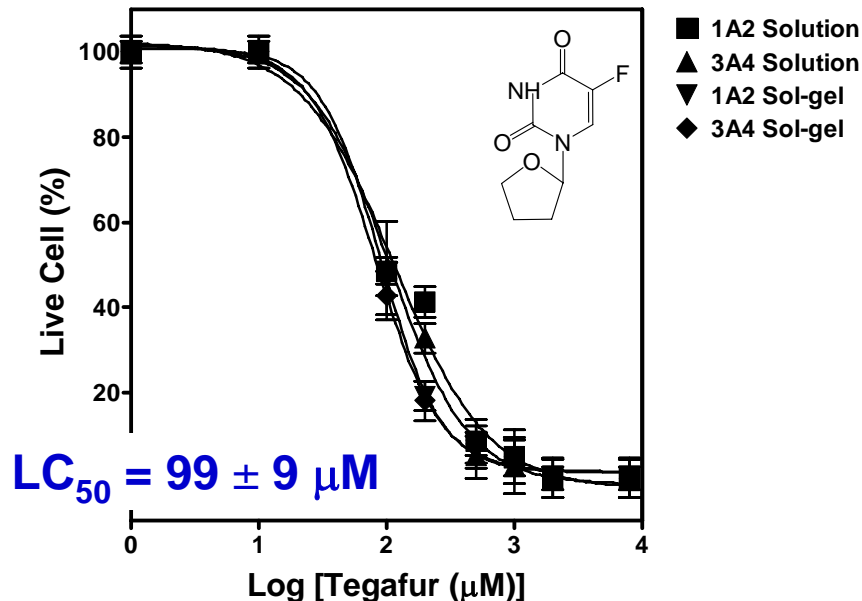
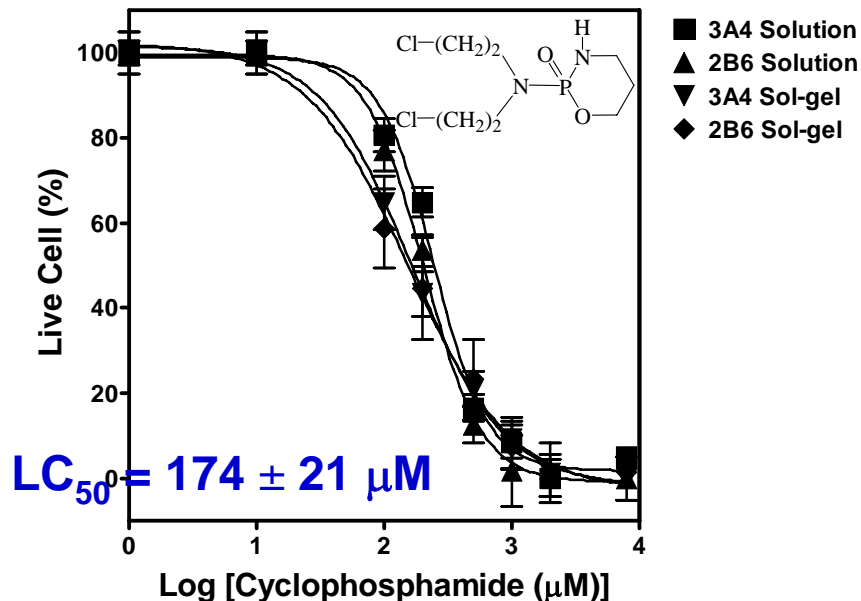
After stamping



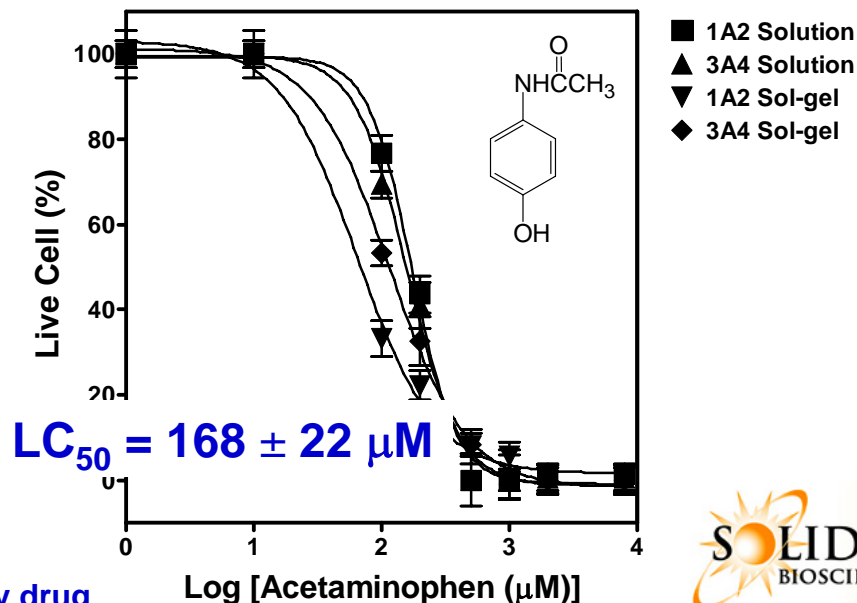
After cell staining

MCF7 cell

P450 Metabolism: Sol-gel MetaChip & 2D MCF7 Culture



– Reference: [Lee, M.Y., Park, C.B., Clark, D.S. and Dordick, J.S., Metabolizing enzyme toxicology assay chip \(MetaChip\) for high-throughput microscale toxicity analyses, Proceedings of the National Academy of Sciences of the United States of America \(PNAS\), 102\(4\), 983-987 \(2005\) \[Featured as cover article\]](#)

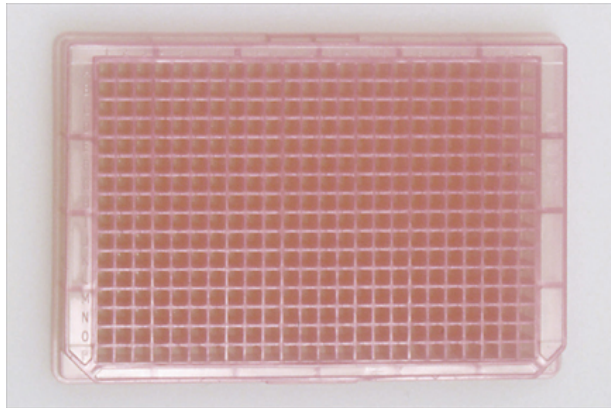


LC₅₀: Lethal concentration where 50% of cell death occurred by drug

Preparation of Hydrogel-based **DataChip** for Toxicology Assay

Expanding Microarray Techniques to 3D Cell Cultures

Conventional high-throughput approach



384-well plate platform

Size of plate: 8.5 x 12.7 cm²

Number of wells: 384

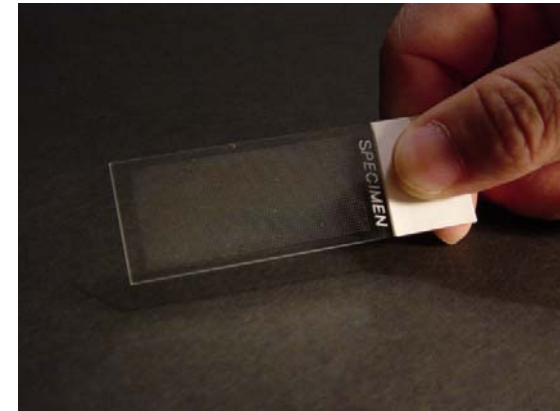
Well size: 3.5 x 3.5 mm²

Working volume: 10 – 100 µL

Cells: 2D culture



Solidus platform



3D DataChip platform

Size of slide: 2.5 x 5.7 cm²

Number of spots: 500 – 2000

Spot diameter: 100 – 800 µm

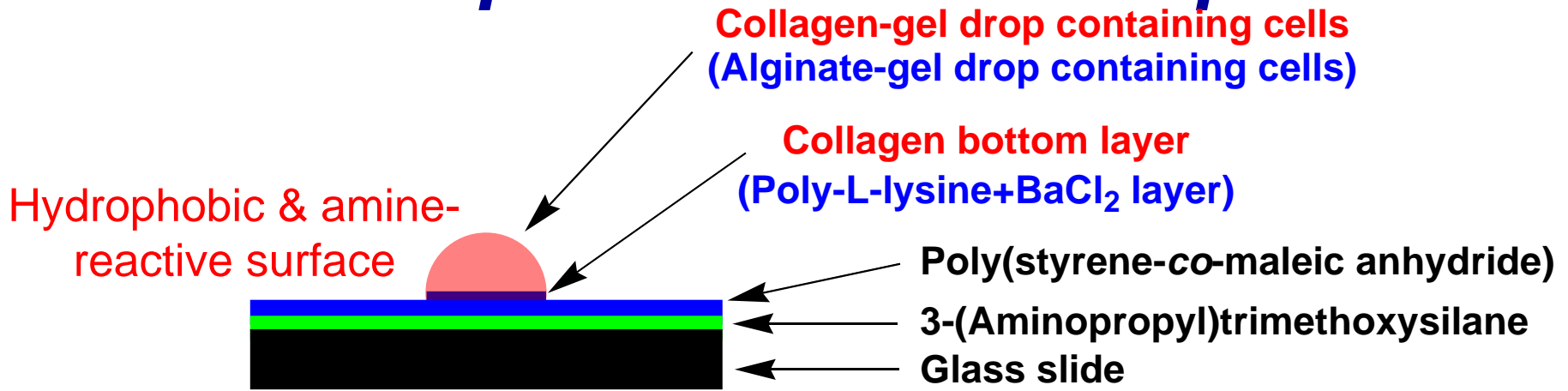
Spot volume: 10 – 100 nL

Cells: 3D culture

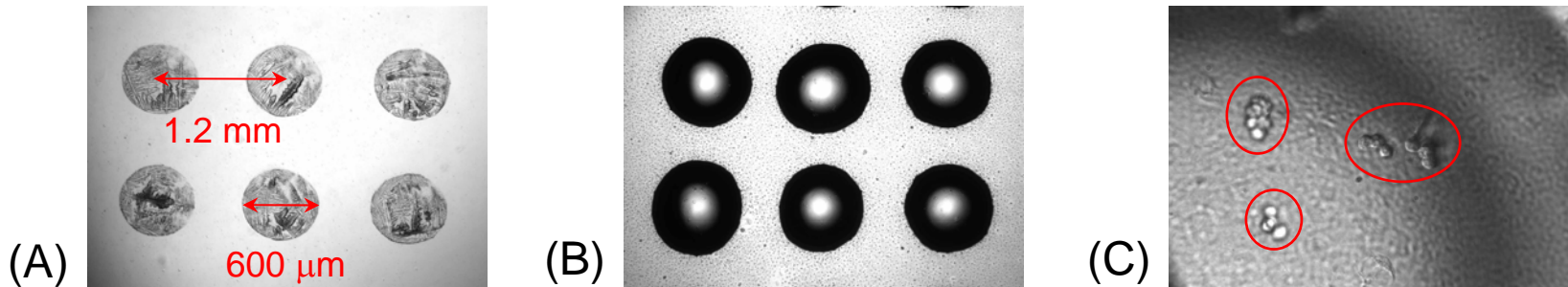
Why 3D Cell Culture Systems?

- Many cells of normal and malignant origin lose some of their phenotypic properties when grown *in vitro* as monolayer or suspension cultures.
 - The formation of tissue-like structures is highly inhibited in monolayer culture due to the strong affinity of cells to most artificial surfaces and the restriction to a 2D space, severely limiting intercellular contacts and interactions.
- Current attempts to maintain specific biochemical and morphological features of human cells similar to the corresponding tissue *in vivo*:
 - Culturing primary tissues or isolating early passage primary cells
 - Culturing established cell lines on specially manufactured growth surfaces and/or in controlled monolayer systems
 - Propagating established cell lines in 3D culture systems

Preparation of DataChip

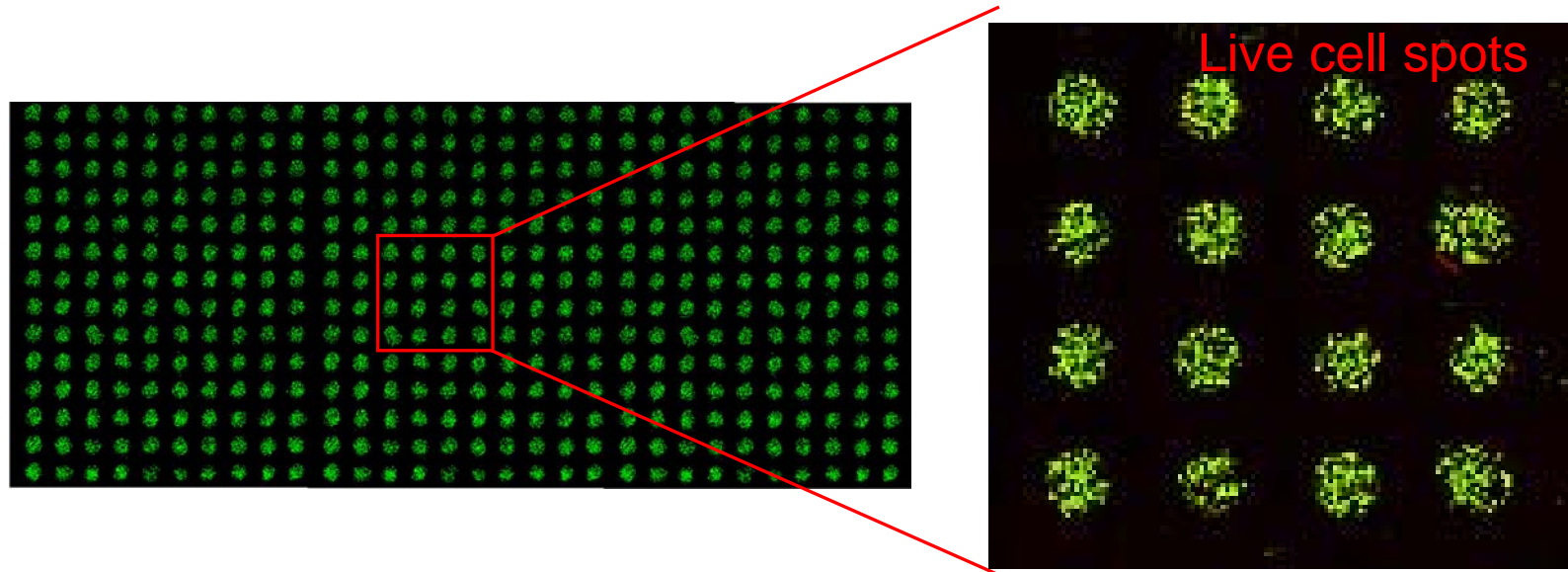


Preparation of collagen-gel drop (or alginate-gel drop) containing cells on a glass slide treated with 3-(aminopropyl) trimethoxysilane (APTMS) and poly(styrene-co-maleic anhydride) (PS-MA)



(A) Microscopic photograph of collagen bottom layer (30 nL, 560 spots) on the PS-MA-treated slide, **(B)** Collagen-gel drops containing MCF7 cells (60 nL, 560 spots) on the bottom layer, and **(C)** Magnified MCF7 cells within the collagen-gel drop

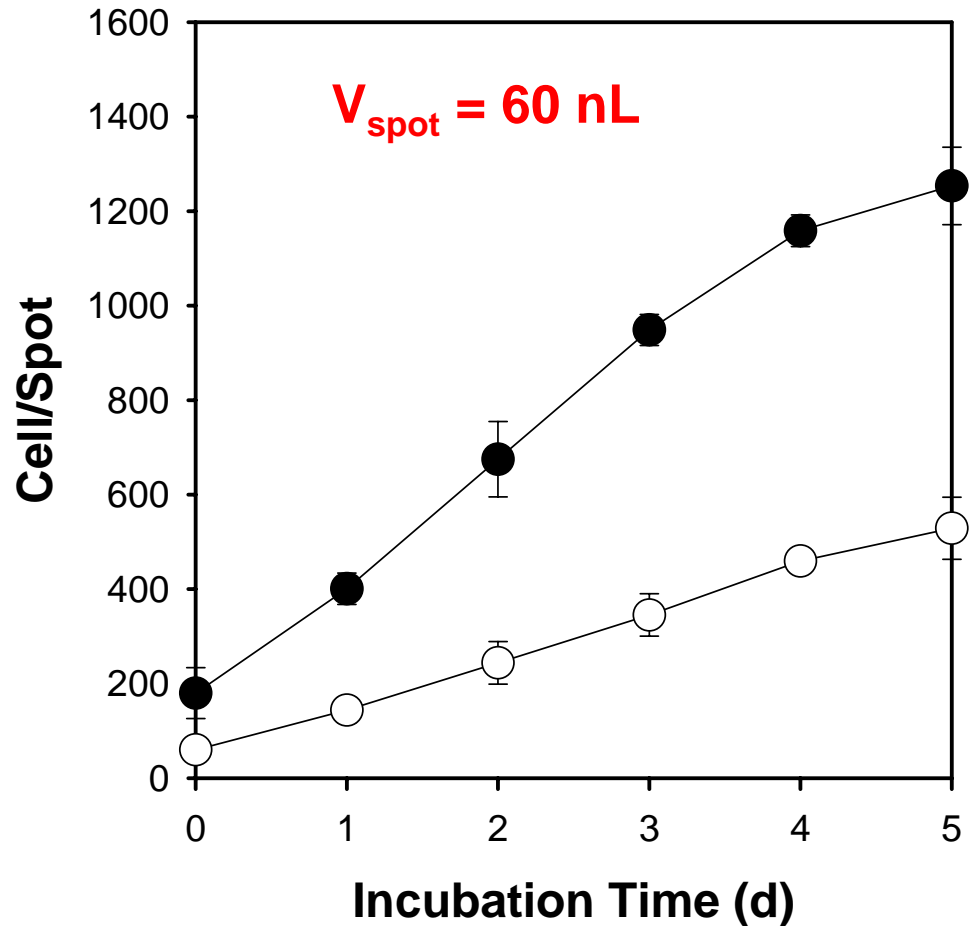
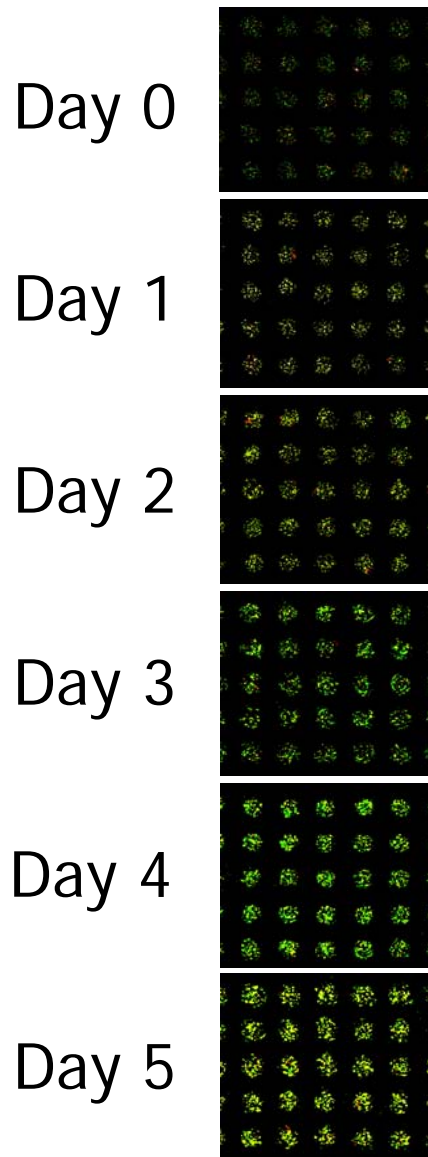
Scanning Images of Collagen-Gel Drop



Scanning picture of collagen-gel drops containing MCF7 cells.

- Gel-encapsulated cells printed with a microarrayer
- Green dots represent live cells in the collagen-gel drop.
- 60-nL spots, 180 cells/spot, 560 spots/slide
- Glass slides modified for robust attachment of hemispherical collagen-gel spots

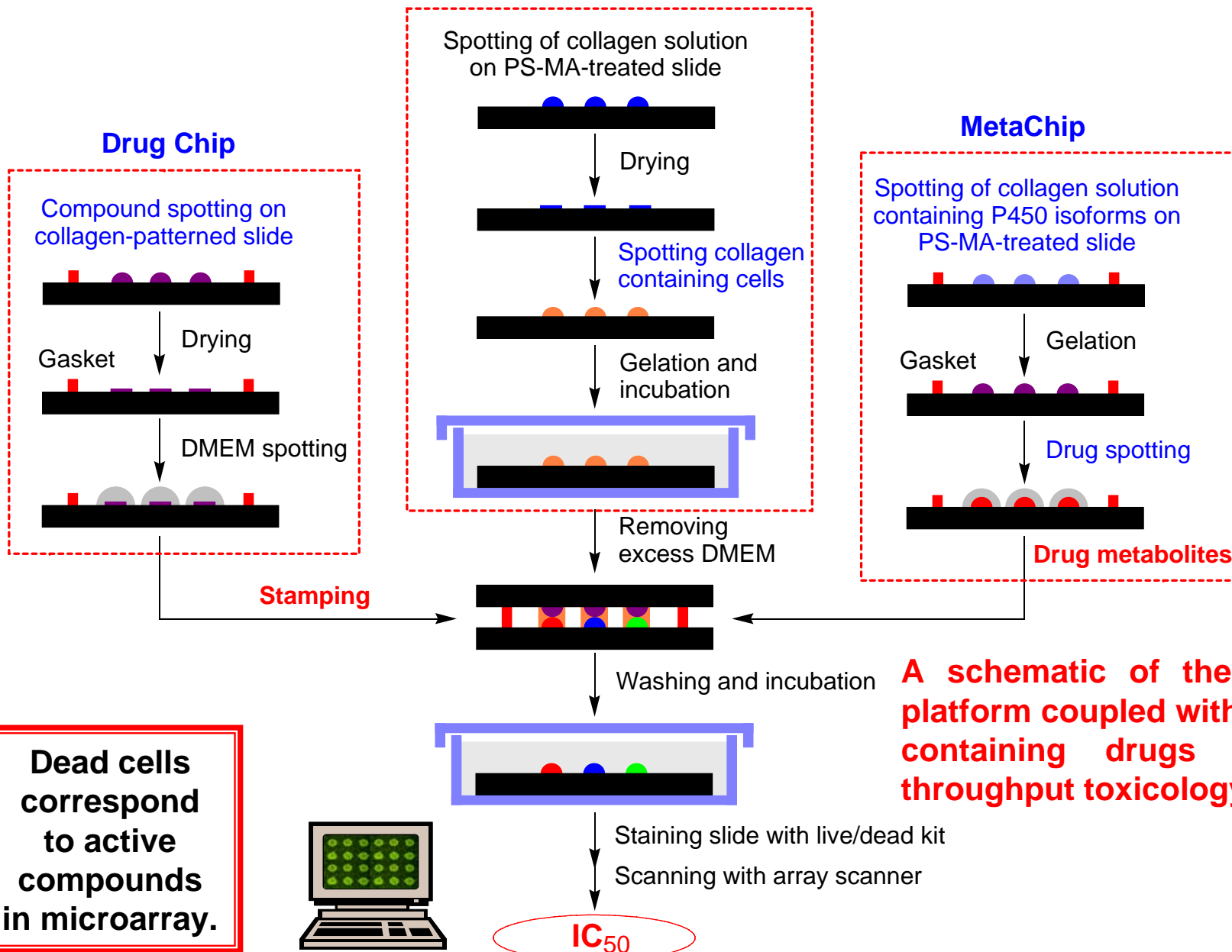
MCF7 Cell Growth in DataChip



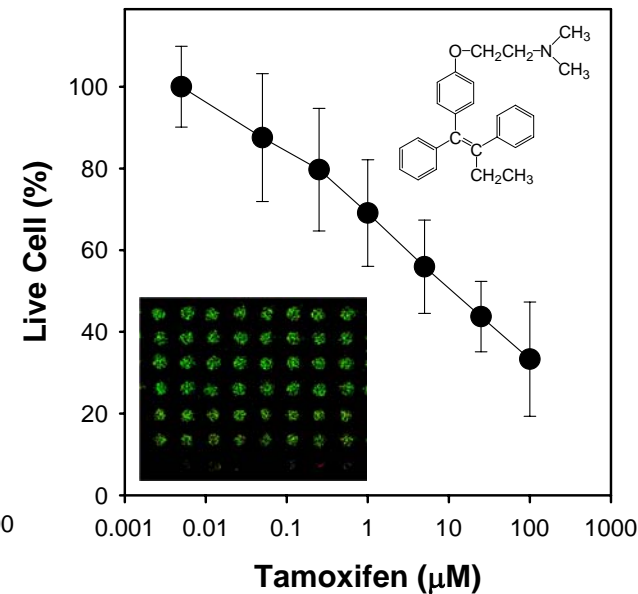
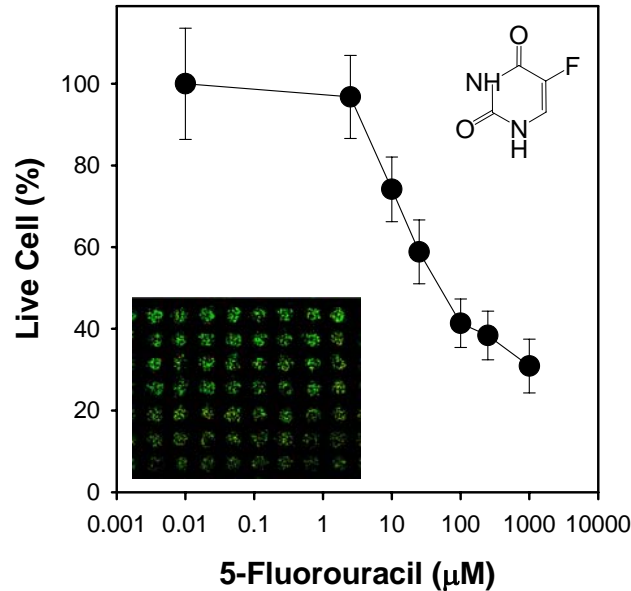
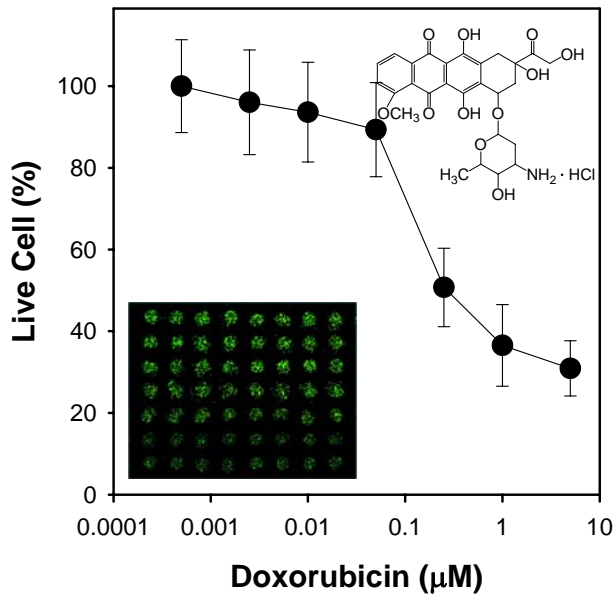
Similarly, cell encapsulation was extended to alginate gels (with BaCl_2 & poly-L-lysine).

DataChip Cytotoxicity Platform

DataChip



DataChip Platform Coupled with Drugs

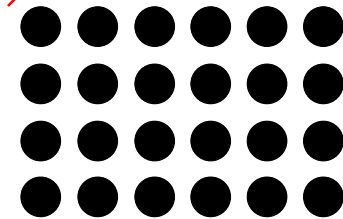
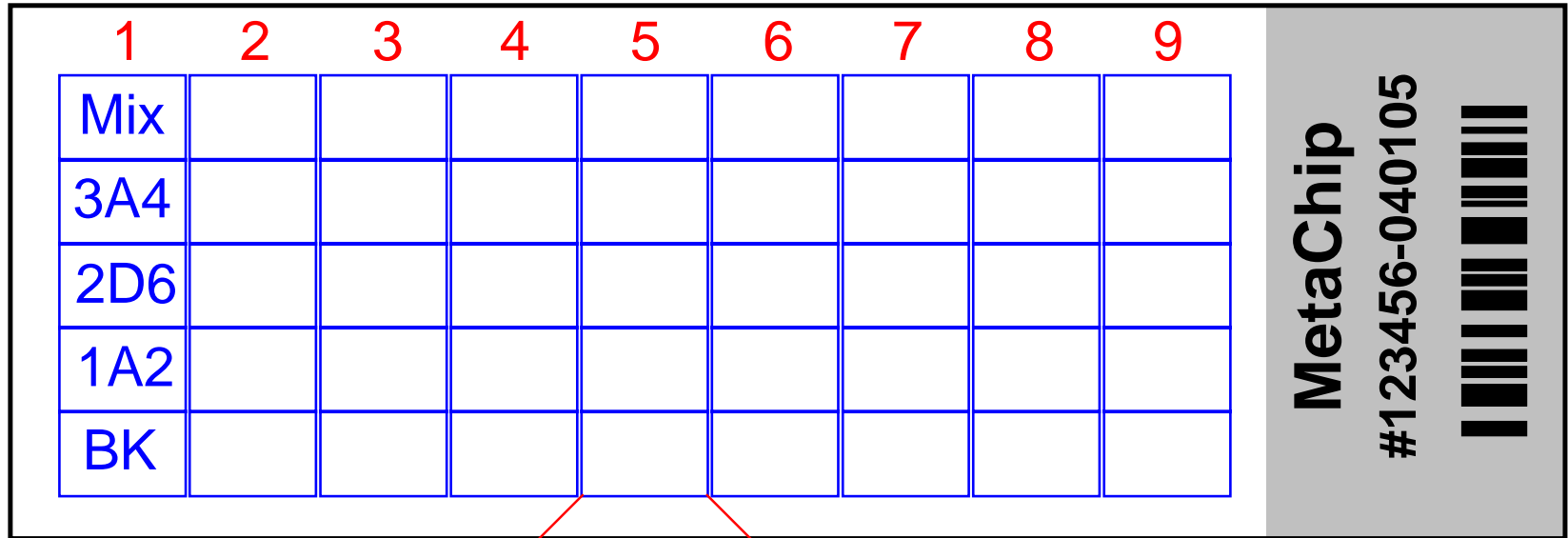


Effect of anti-cancer drug concentration on the growth inhibition of **MCF7 breast cancer cells** in 60 nL of the collagen-gel drop (6 h stamping + 2 h washing + 3 d incubation).

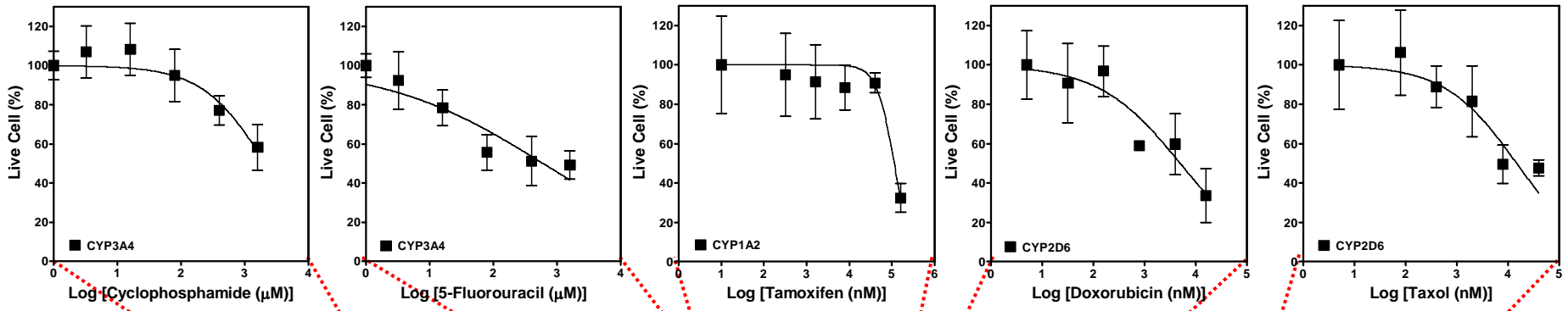
Drugs	Solubility	2D Cell in 96-well plate	3D Cell in 96-well plate	3D Cell in DataChip
Doxorubicin	Soluble in water	110 nM	240 nM	250 nM
5-Fluorouracil	Soluble in water	52 μM	57 μM	62 μM
Tamoxifen	Soluble in DMSO	7 μM	19 μM	10 μM

IC50: Inhibition concentration where 50% of cell growth inhibited by drug

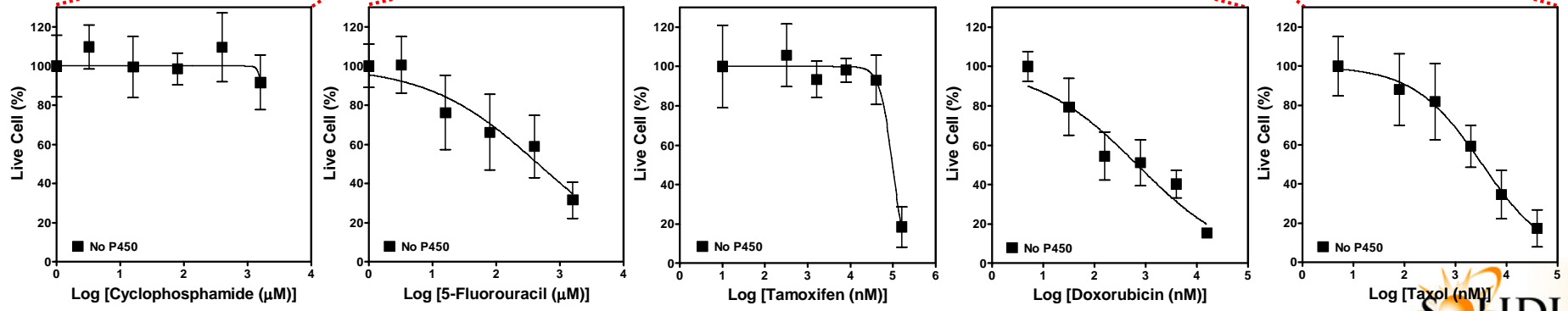
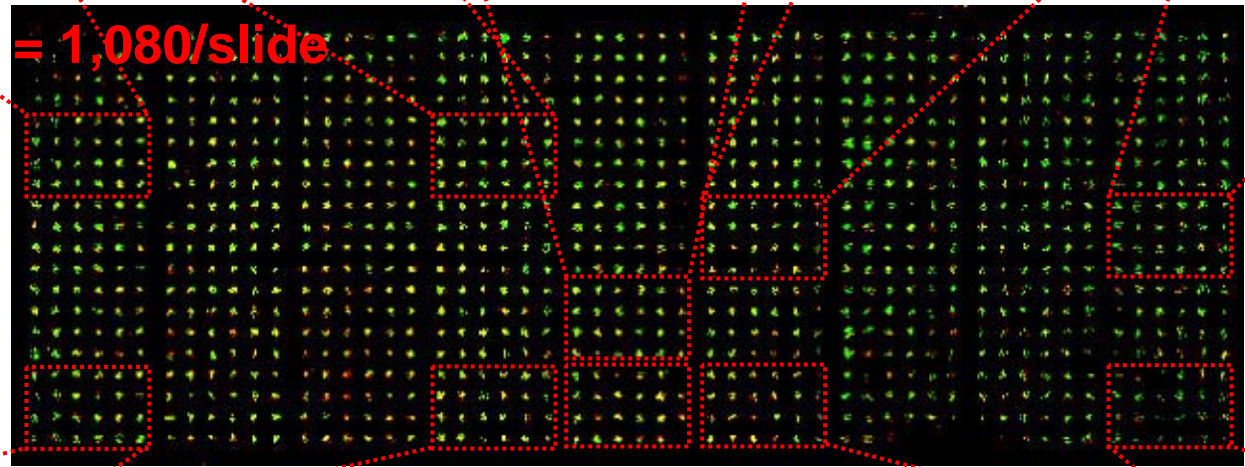
MetaChip Coupled with DataChip



A schematic of **MetaChip** layout ($20 \times 54 = 1,080$ spot array). A 20 nL of drug solutions (9 compounds per slide, 6 concentrations per cluster; 0 – 2000 μM) are printed onto each 10 nL of P450 spots (5 different P450 clusters containing no-P450 (BK), CYP1A2, CYP2D6, CYP3A4, and P450 mixture) for stamping on the complementary **DataChip** (20 nL, 1,080 spots).



Spot density = 1,080/slide
(Hep3B cell)



Hepatotoxicity Test on DataChip/MetaChip

Growth inhibition of **Hep3B hepatoma cells** in 20 nL alginate-gel drop coupled with 10 nL P450 spot with drug (6 h stamping + 2 h washing + 3 d incubation).

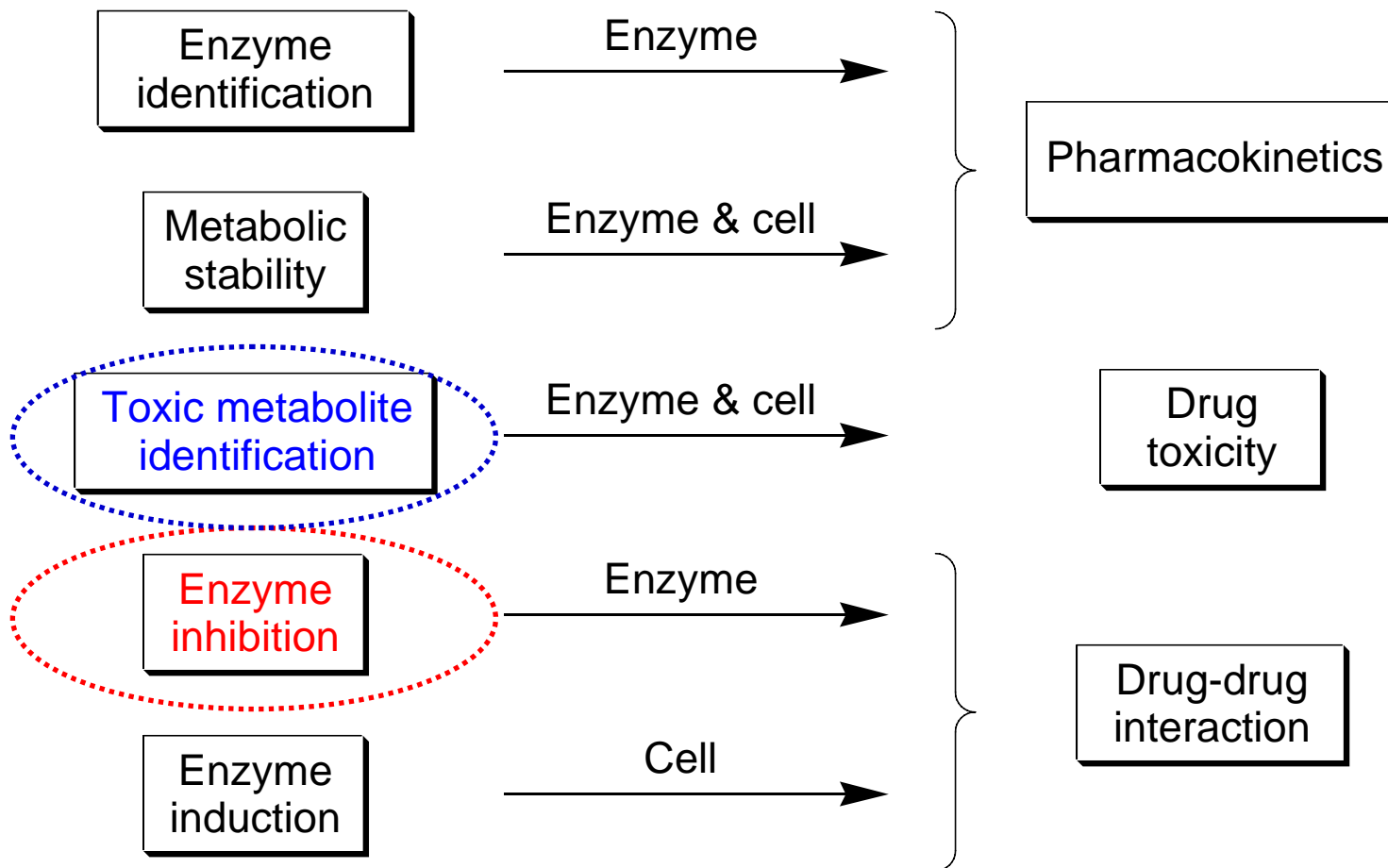
Drug	No P450	CYP1A2	CYP2D6	CYP3A4	P450 mixture
Cyclophosphamide	GT	GT	GT	2.1 ± 0.6 mM	GT
Acetaminophen	GT	2.6 ± 1.0 mM	GT	2.8 ± 1.2 mM	GT
5-Fluorouracil	190 ± 37 μM	218 ± 44 μM	131 ± 20 μM	349 ± 72 μM	436 ± 85 μM
Tamoxifen	83 ± 13 μM	112 ± 30 μM	28 ± 2 μM	398 ± 154 μM	63 ± 16 μM
Doxorubicin	0.7 ± 0.0 μM	1.0 ± 0.2 μM	4.9 ± 1.4 μM	19.3 ± 5.5 μM	9.7 ± 2.2 μM
Methotrexate	2.4 ± 0.6 μM	2.8 ± 0.7 μM	38 ± 12 μM	10.9 ± 2.8 μM	8.0 ± 3.0 μM
Taxol	3.3 ± 0.6 μM	4.2 ± 1.5 μM	14.7 ± 4.7 μM	6.5 ± 2.4 μM	5.5 ± 1.8 μM
Acetylsalicylic acid	GT	GT	GT	GT	GT
Amitriptyline	31 ± 2 μM	75 ± 14 μM	55 ± 10 μM	63 ± 12 μM	75 ± 9 μM
Digoxin	0.8 ± 0.1 μM	1.3 ± 0.1 μM	0.4 ± 0.0 μM	1.4 ± 0.1 μM	0.6 ± 0.0 μM
Theophylline	38 ± 8 μM	239 ± 56 μM	119 ± 26 μM	36 ± 11 μM	84 ± 22 μM
Propranolol	95 ± 13 μM	69 ± 10 μM	74 ± 9 μM	86 ± 13 μM	86 ± 11 μM
Paraquat	79 ± 12 μM	5.1 ± 0.3 μM	20 ± 2 μM	52 ± 9 μM	44 ± 6 μM

GT: The IC₅₀ value is greater than test concentration (= relatively nontoxic).

P450 Inhibition Study on Hydrogel-based MetaChip

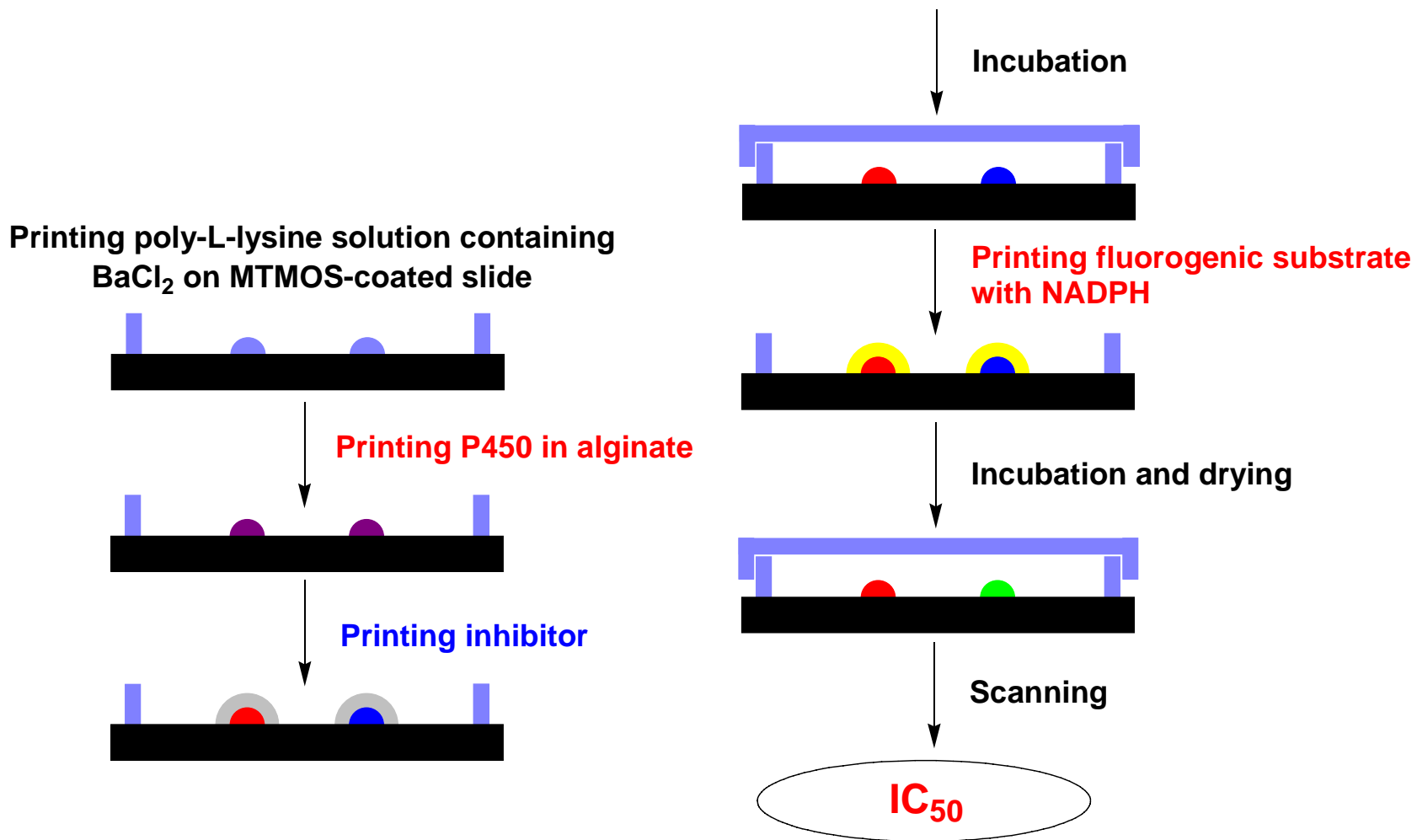
Conventional *in vitro* Metabolism Study

Assay



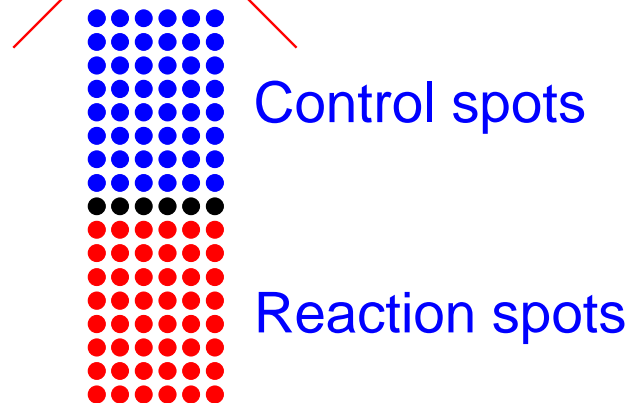
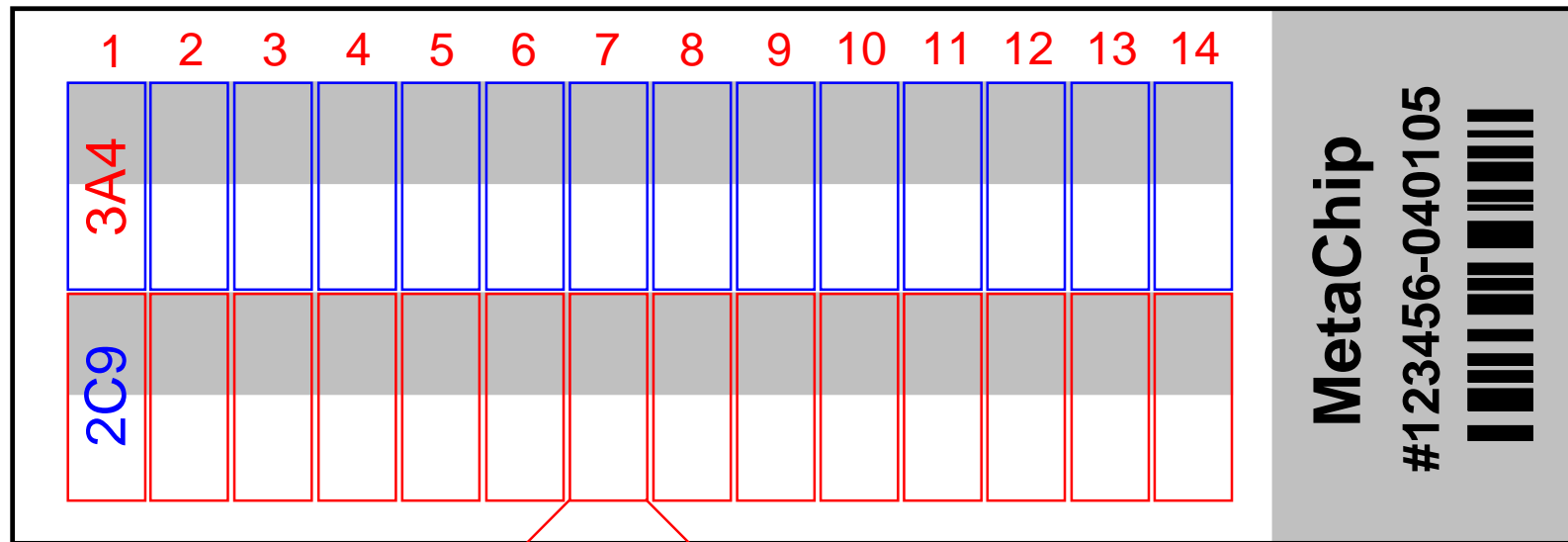
Several metabolism-based studies (listed on the left-hand side) can be performed by either enzyme- or cell-based assays for different goals (listed on the right).

MetaChip Inhibition Platform



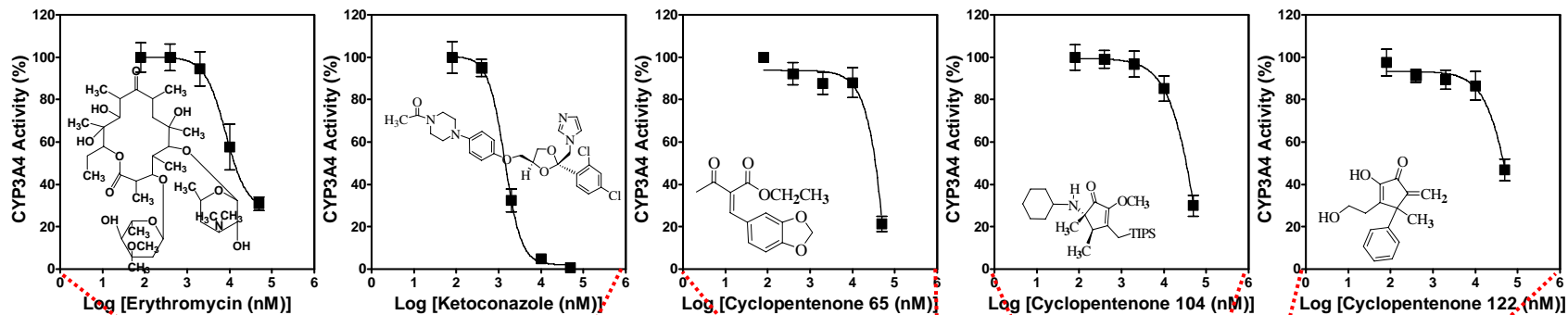
A schematic of the MetaChip platform for high-throughput P450 inhibition study

MetaChip Layout for P450 Inhibition



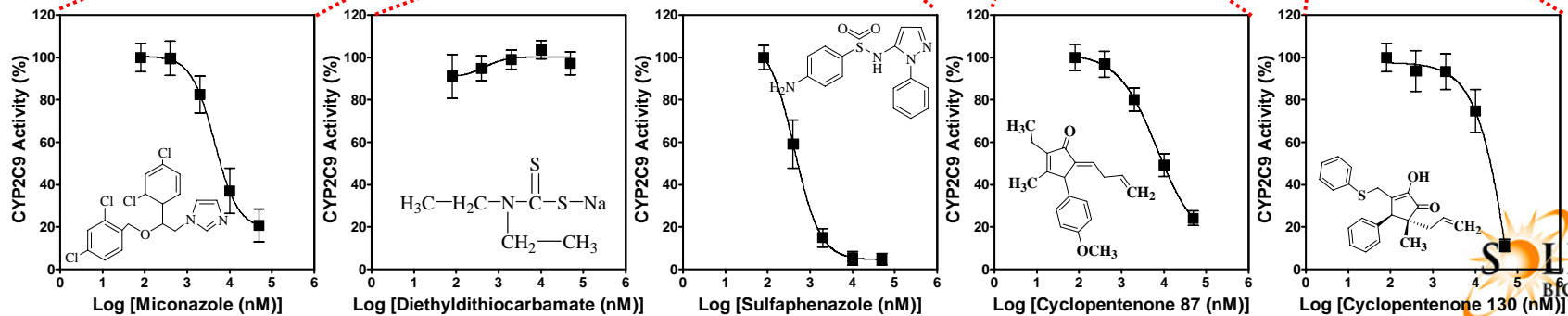
A schematic of MetaChip layout for P450 Inhibition ($34 \times 84 = 2856$ spot array). A 20 nL of inhibitor solutions (14 compounds per slide, 6 concentrations per cluster; 0 – 100 μ M) are printed onto each 10 nL of P450 spots (2 different P450 clusters containing CYP3A4 and CYP2C9).

P450 Inhibition for Drug-Drug Interaction



CYP3A4

CYP2C9



Summary of Key Solidus' Technology

- **High-density human P450 microarrays**
 - Active and stable P450s in volumes as low as 5 nL
 - Suitable for P450 activity screening, P450 inhibition screening, and metabolite generation and toxicity screening
- **High-density human cell culture microarrays**
 - 3D cell cultures in volumes as low as 20 nL
 - Multiple cell types (MCF7, A293, Hep3B, etc.) used
 - Suitable for assaying the cell-specific toxicity of drug candidates and their P450-generated metabolites

Acknowledgements

- **Funding**

- NIEHS (ES-012619)

- **Collaboration**

- Professor Jonathan S. Dordick in RPI: MetaChip development
 - ✓ Michael G. Hogg, Ph.D.
 - ✓ Moon-Il Kim, Ph.D.
 - ✓ Sumitra M. Sukumaran
- Professor Douglas S. Clark in UC Berkeley: DataChip development
 - ✓ Anand Kumar, Ph.D.
- Professor John Wen in RPI: MetaReader development
 - ✓ Glenn Saunders, P.E.

Drug Metabolizing Enzyme (DME)

- **Oxidative DMEs (Phase I reactions)**
 - **Cytochrome P450 (CYP450): Carbon oxidation**
 - Flavin-containing monooxygenase (FMO): N or S oxidation
 - Monoamine oxidase (MAO): Oxidative deamination
 - Alcohol dehydrogenase: Alcohol oxidation
 - Aldehyde dehydrogenase: Aldehyde oxidation
 - Aldehyde oxidase: Aldehyde oxidation
- **Conjugative DMEs (Phase II reactions)**
 - **UDP-glycosyltransferase (UGT): Glucuronidation**
 - **Glutathione S-transferase (GST): Glutathione conjugation**
 - Sulfotransferase (SULT): Sulfation
 - N-Acetyltransferase: Methylation
 - Acyl-CoA synthetase: Coenzyme A conjugation
 - Phosphotransferase: Phosphorylation

MetaChip Coupled with MCF7 Cell

Spotting of sol solution with P450
on hydrophobic glass slide



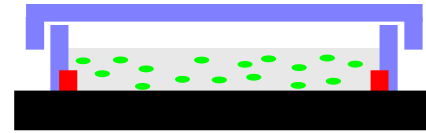
Gelation



Prodrug spotting



Chamber slide



Cell culture



Cell monolayer

Gasket

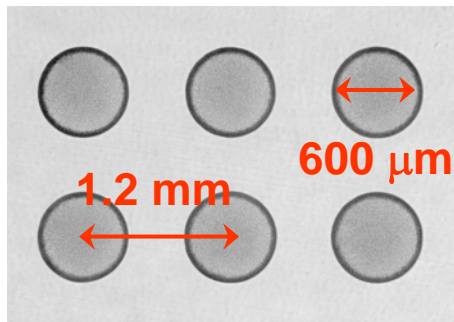


Stamping & Reaction

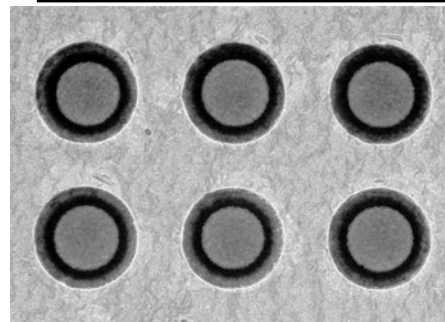


Stamping
Technique

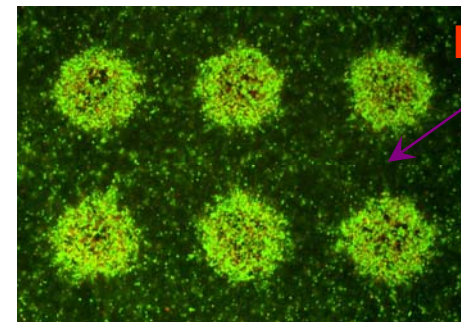
525 spots
(30 nL)



After sol-gel spotting



After stamping



MCF7 cell

After cell staining

MetaReader System

