Through-Hole Microarrays: A High Throughput Platform for Synthesis, Storage and Screening

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Cambridge, MA

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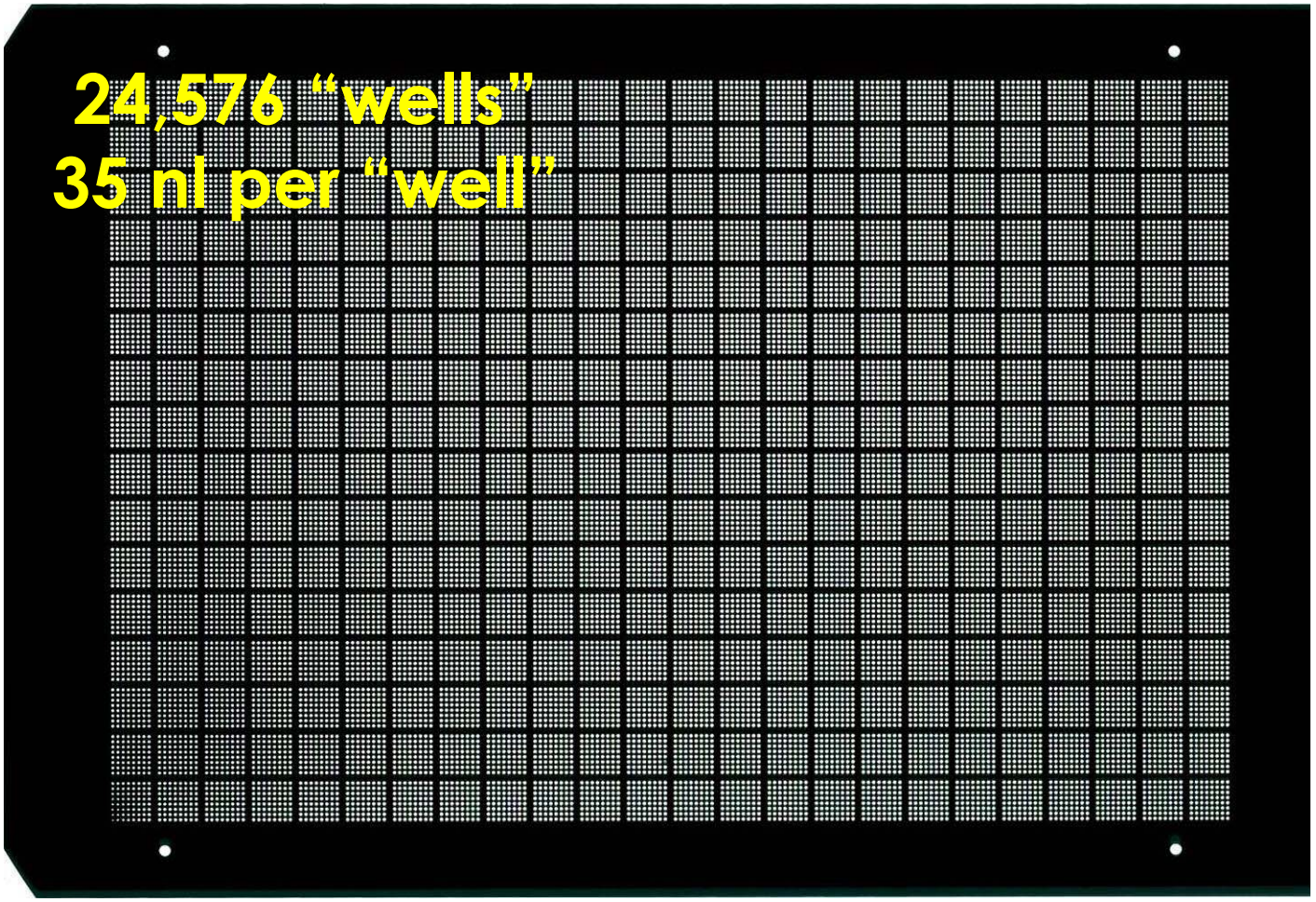
BioTrove Inc.

- Privately-held biotechnology company, commenced operations October 2000.

Platforms

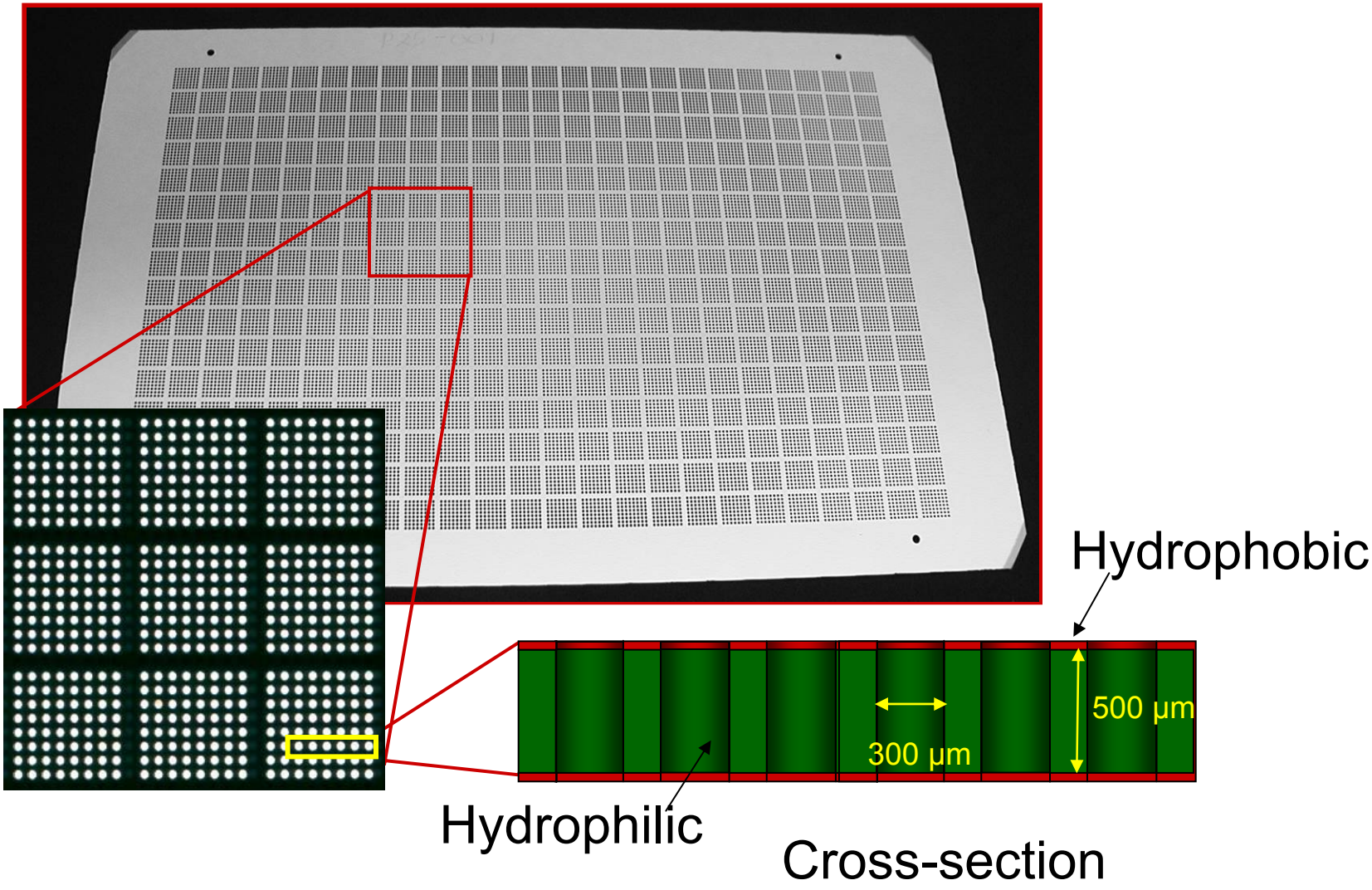
- **Living Chip™**
  - Technology developed at MIT and exclusively licensed to BioTrove.
  - Massively parallel nanofluidics.
  - High density nanoliter library storage and analysis
- **Lab-on-a-Tape™**
  - developed in-house and wholly owned by BioTrove.
  - Fast, automated serial assay initiation and analysis.
  - Detection of native molecular properties

Miniaturizing Microtiter Plate Technology



The Living Chip™ – A nanotiter plate

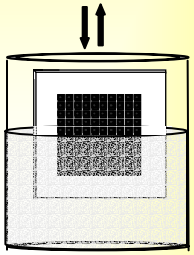
24,576 through-holes

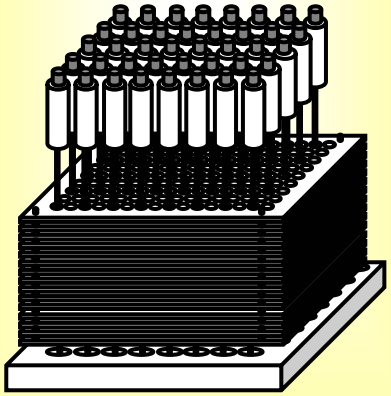


Living Chip™ Screening System

Chip Loading

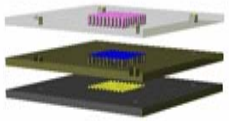
Reagent dispenser

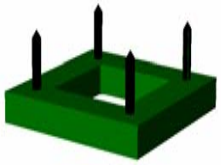




Reformatter

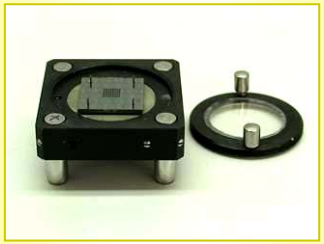
Mixing





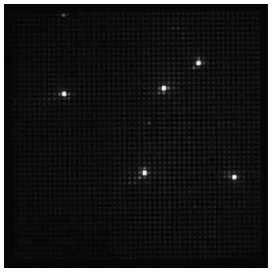
Stacking Jig

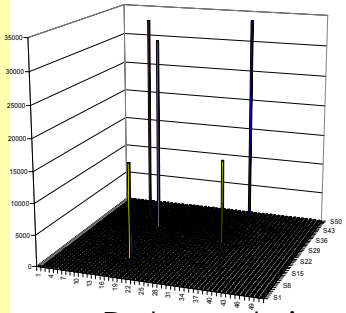
Incubation



Environmental Chamber

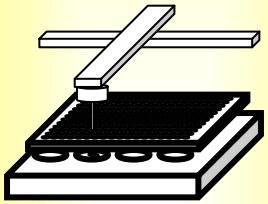
Imaging & Analysis





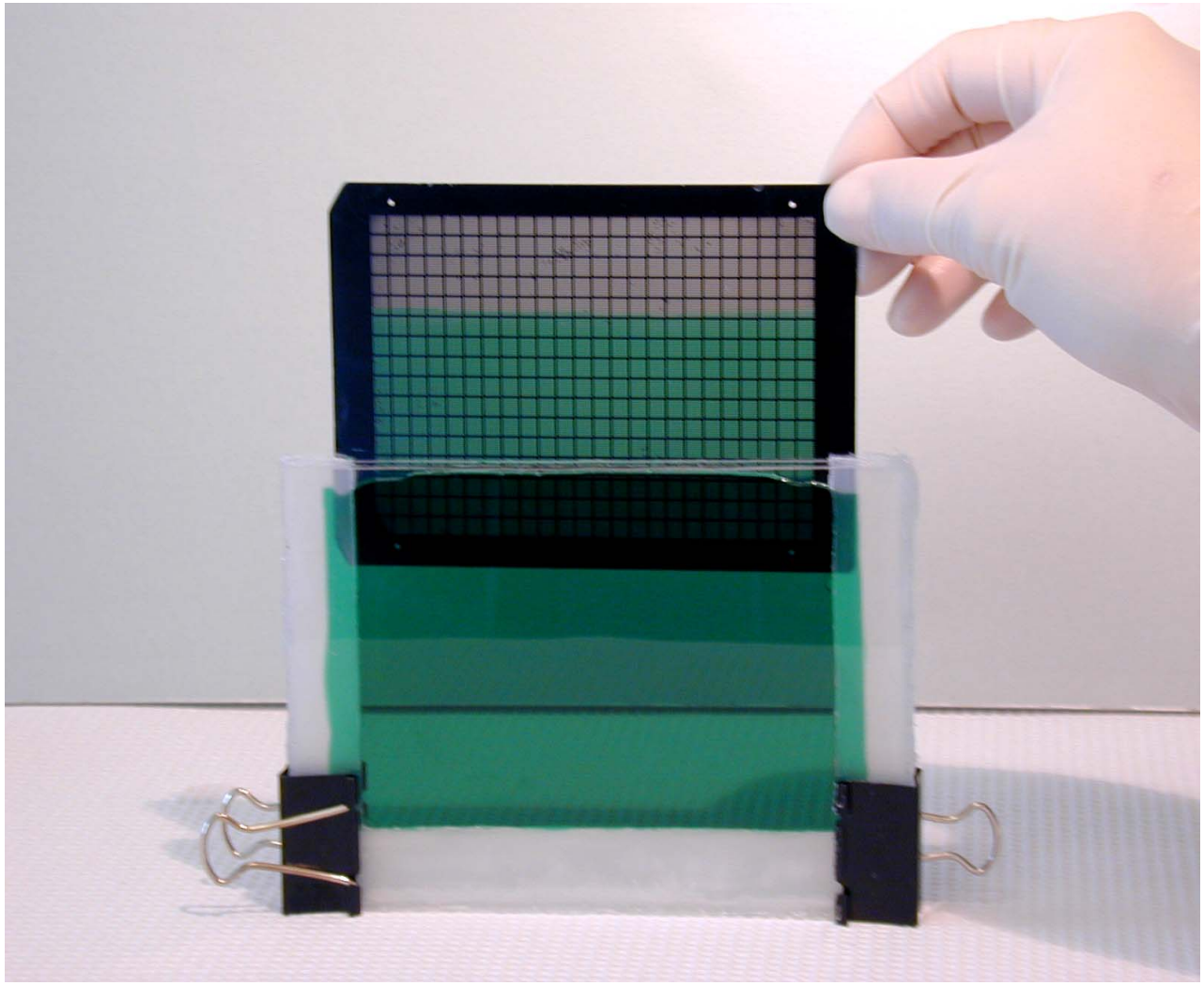
Data analysis software

Recovery

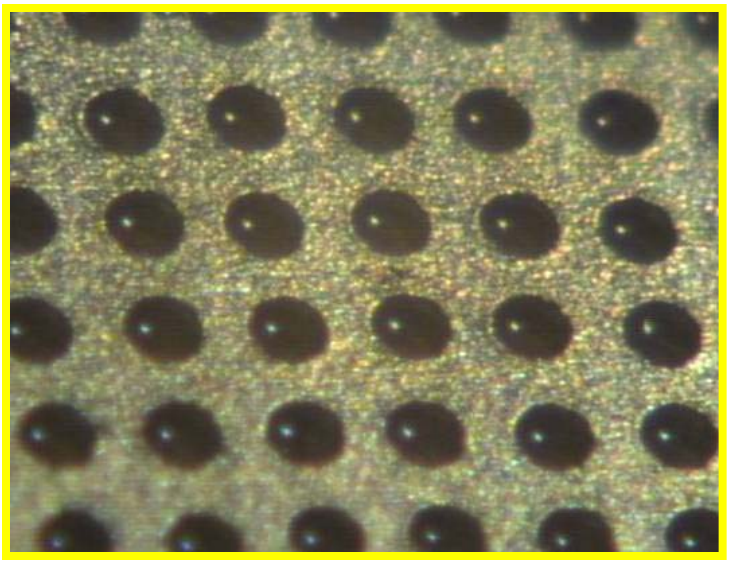


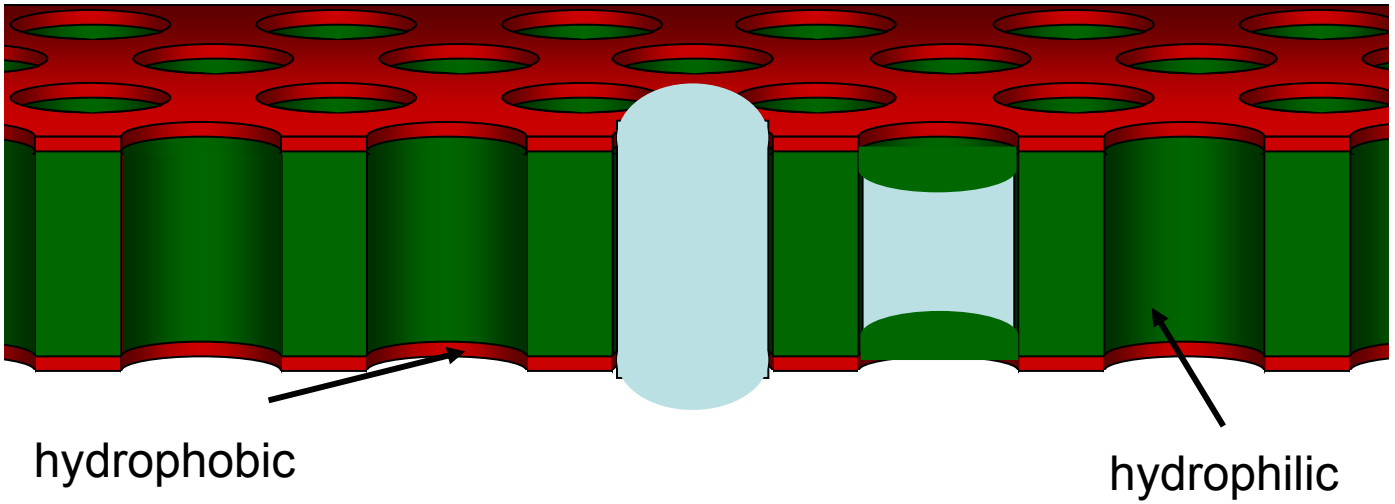
Cherry Picker

Dip Loading of Common Reagents

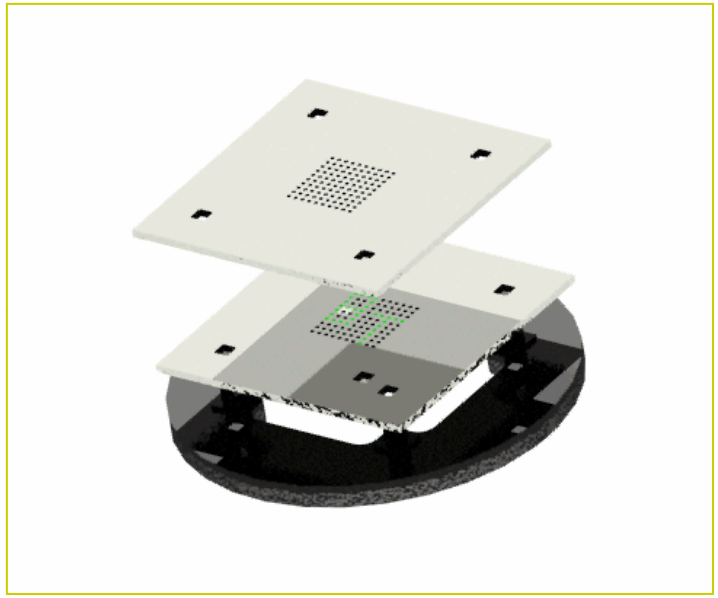


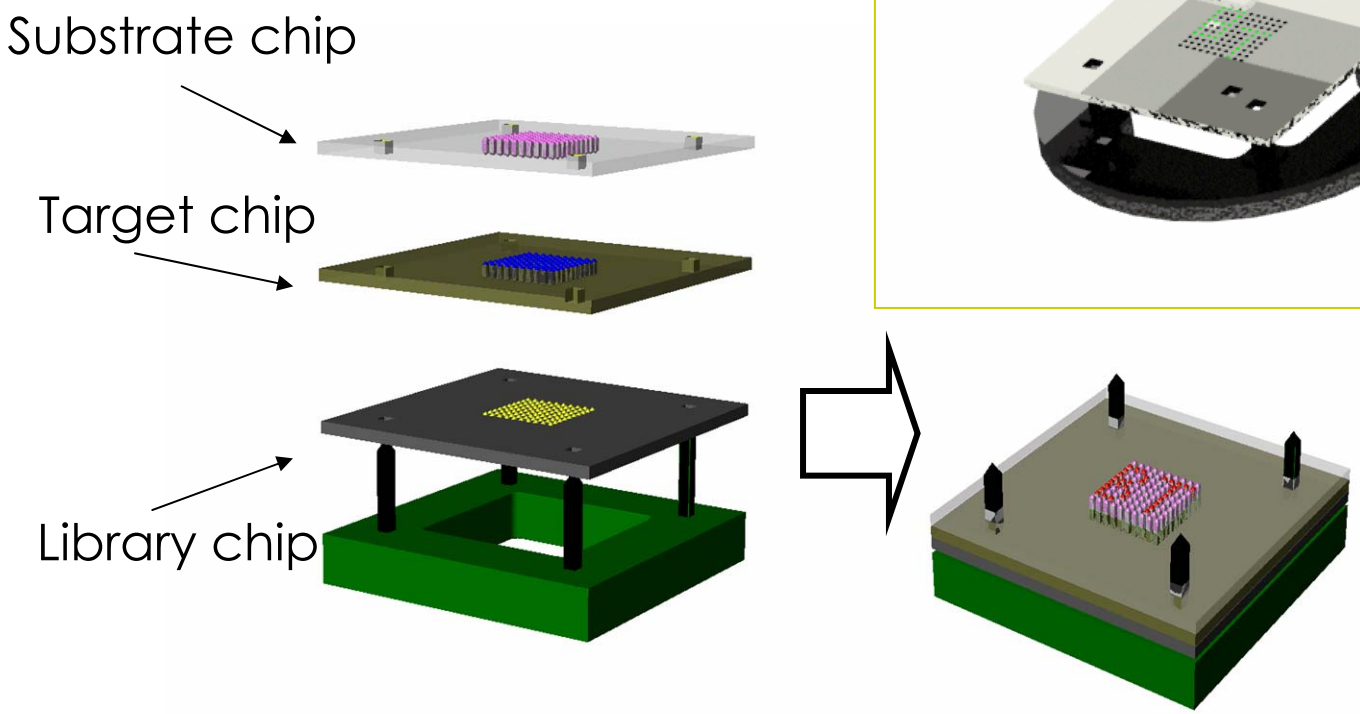
Hydrophobic exterior surfaces prevent chemical cross-talk between channels and produce positive menisci when the chips are filled.



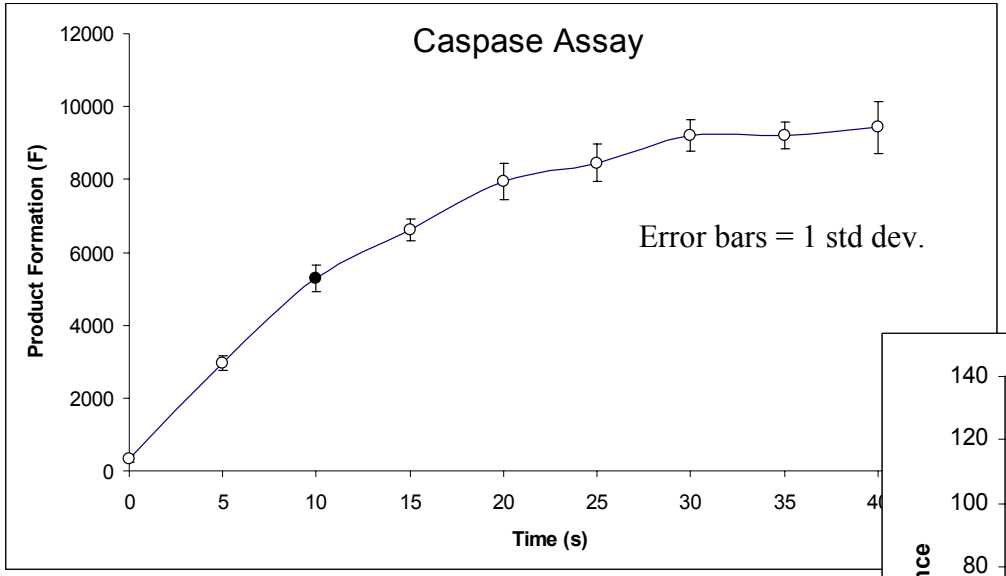


Massively Parallel Assay Initiation



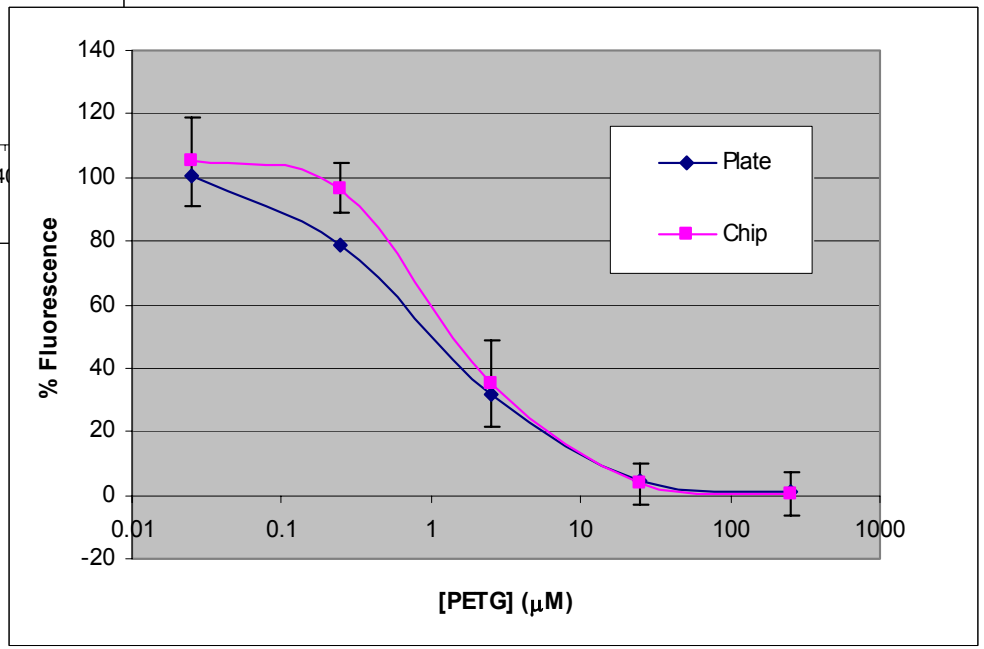


Assay Miniaturization



Z-prime = 0.72 @ 30% completion

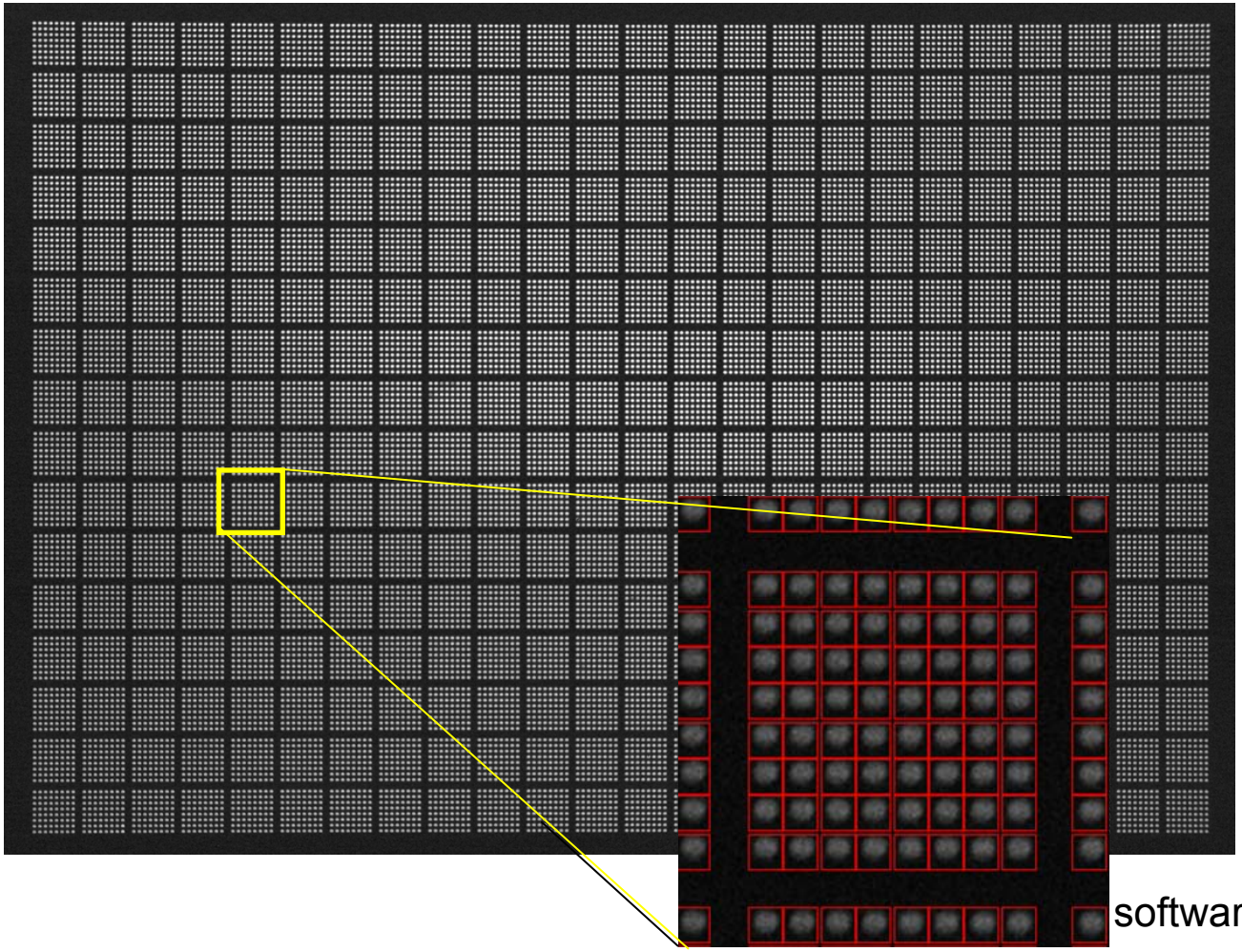
β-Galactosidase inhibition chip vs. plate



Similar IC50 values in 200 times smaller assay volume!

$IC50_{Plate} = 1.0\ \mu M$
$IC50_{Chip} = 1.3\ \mu M$

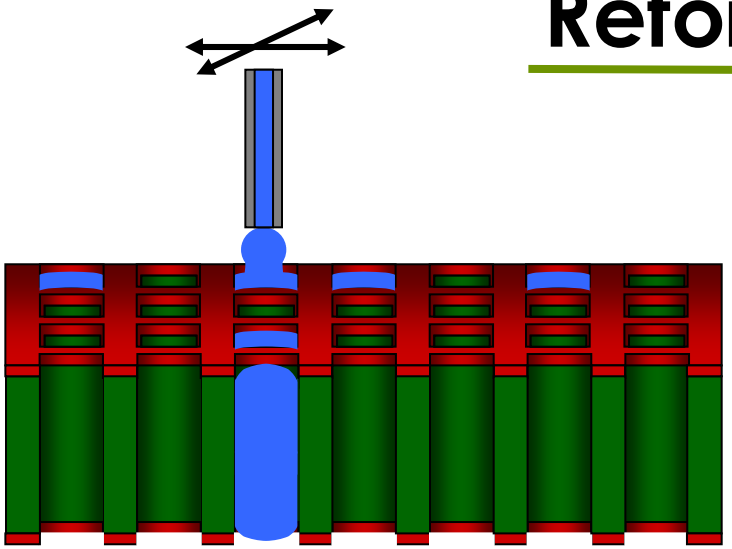
Imaging

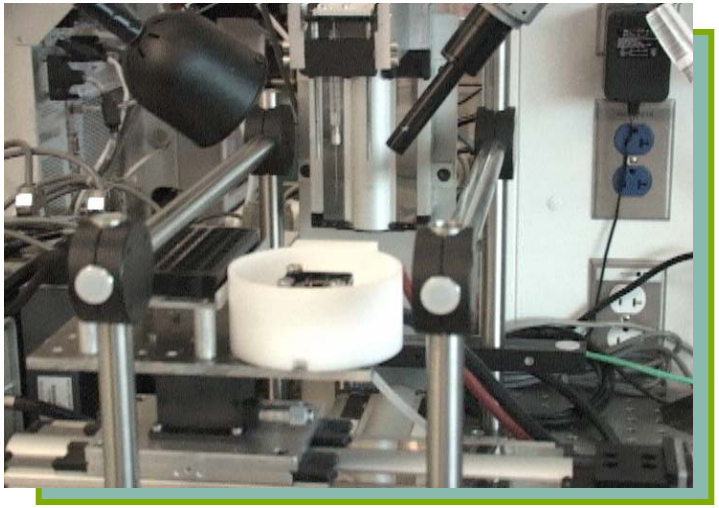


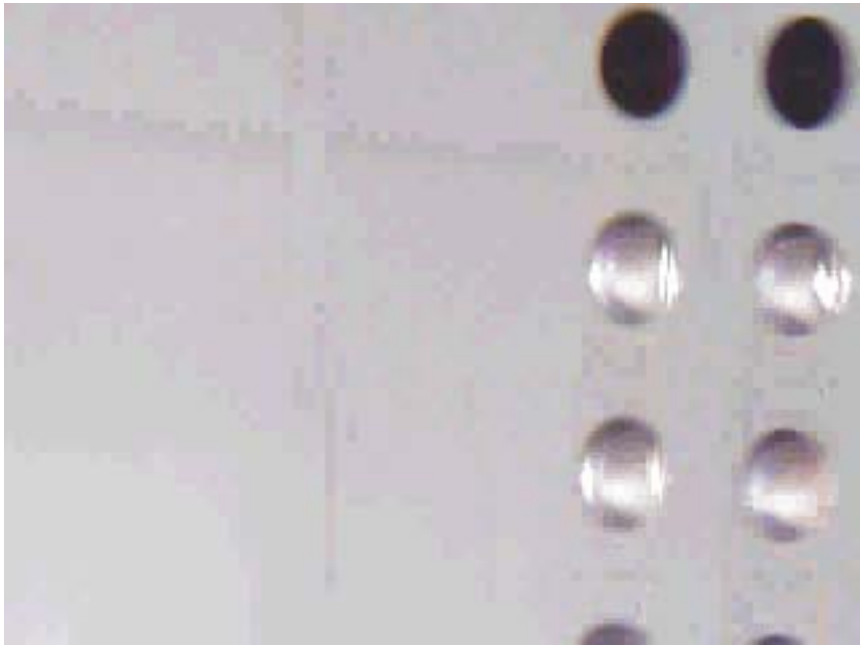
software grid fit

Fluorescence image taken an array loaded with 1 μM hydrolyzed fluorescein conjugate casein.

Reformatting

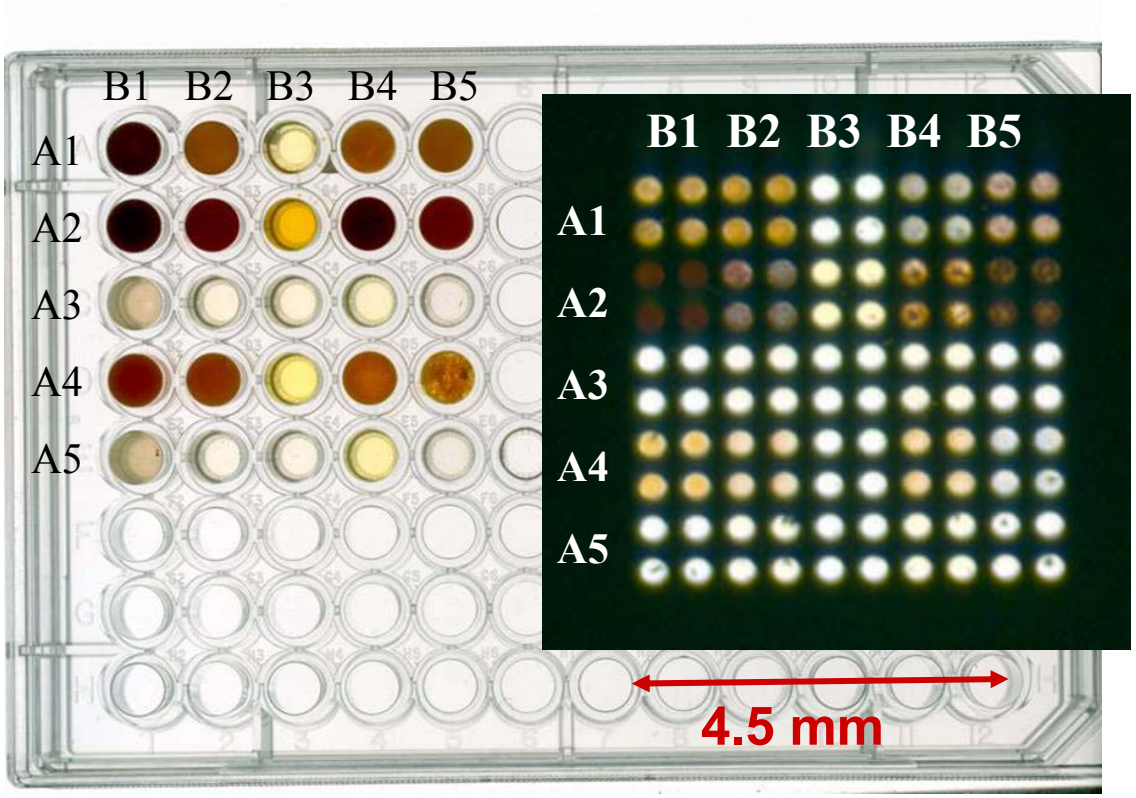






Assay Development Reformatter.

Combinatorial Synthesis Demo



A1 2-nitrobenzaldehyde

A2 5-nitro-2-furaldehyde

A3 glucose

A4 4-nitrobenzaldehyde

A5 aminomethyl coumarin

B1 4-bromophenylhydrazine hydrochloride

B2 4-cyanophenylhydrazine hydrochloride

B3 aminoguanidine bicarbonate

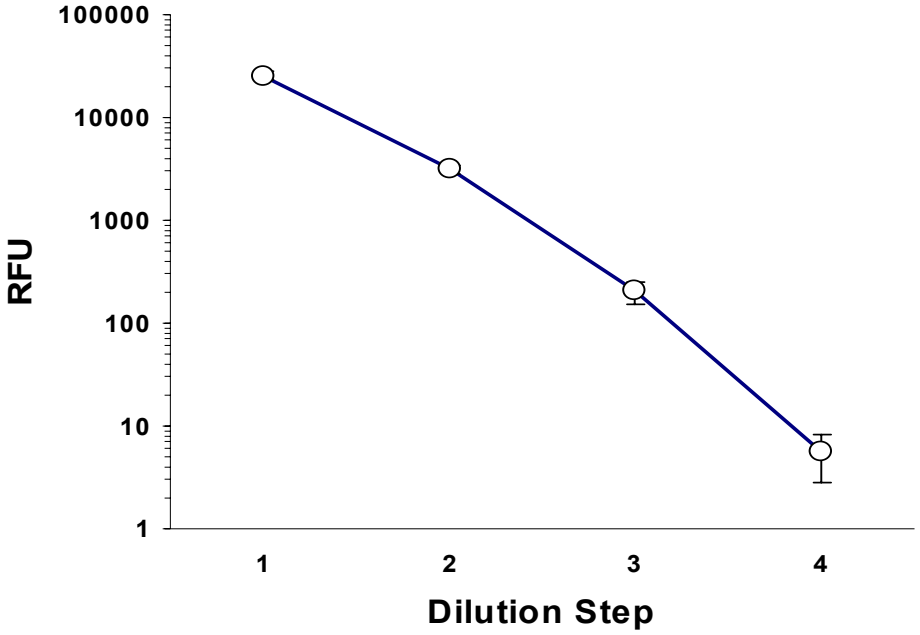
B4 3-nitrophenylhydrazine hydrochloride

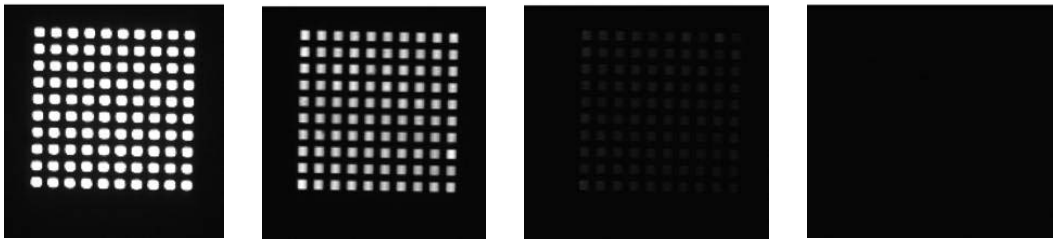
B5 2,4-dichlorophenylhydrazine hydrochloride

A small (25-member) hydrazone library was produced in a 100-channel chip by reacting aqueous solutions of 5 aldehydes with 5 hydrazines (see *J. Chem. Ed.*, **78**, 784 (2001)).

Replicating/Diluting

Rapid Dilution Series

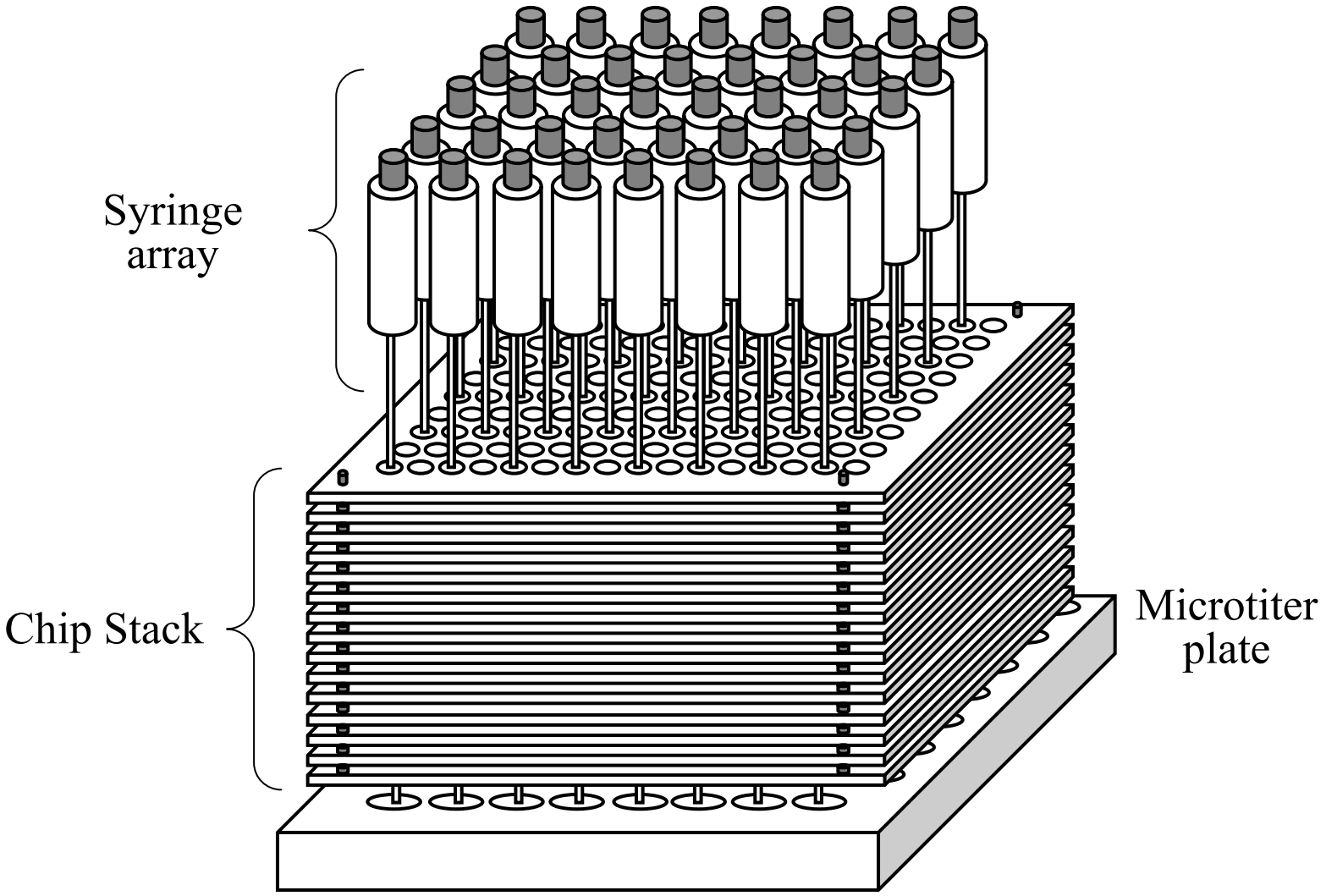




Master

Daughters

Parallel Reformatting

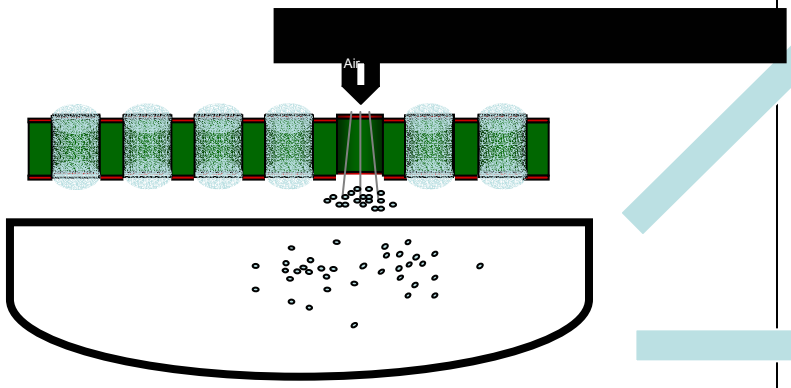


Sample Recovery: Picker

Sample Recovery- Puffing

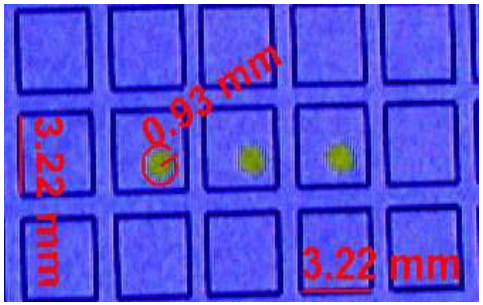
Blow Patterns

Puffer

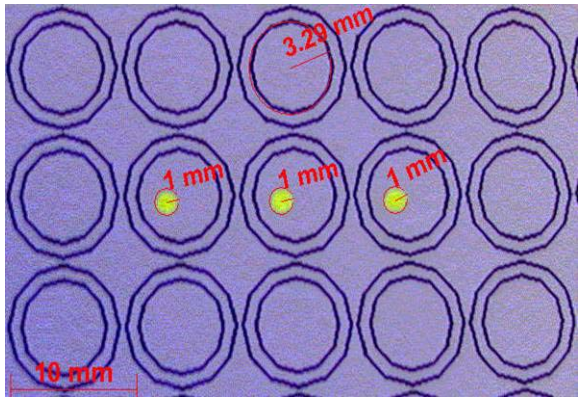


well of 96-well plate

384 well plate

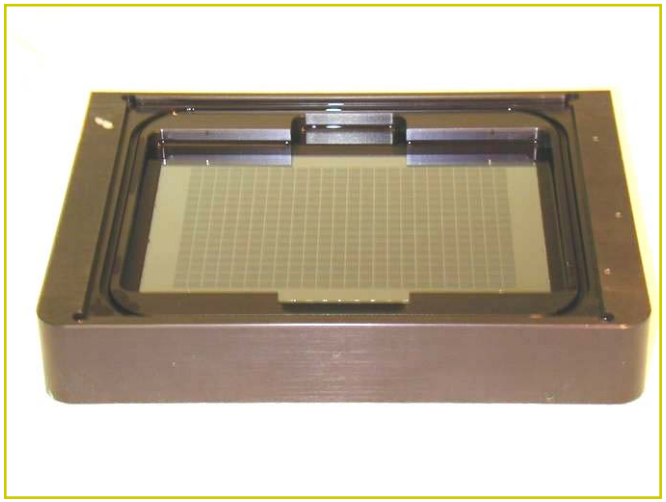


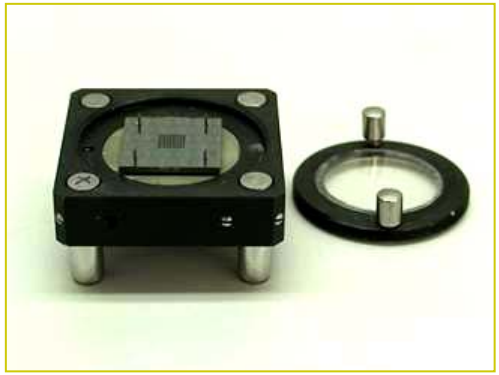
96 well plate



Environmental Control

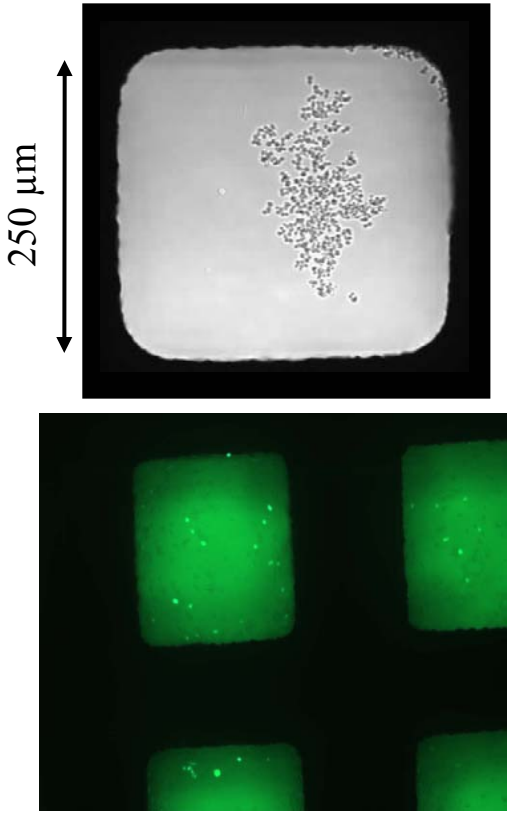
Wetbox





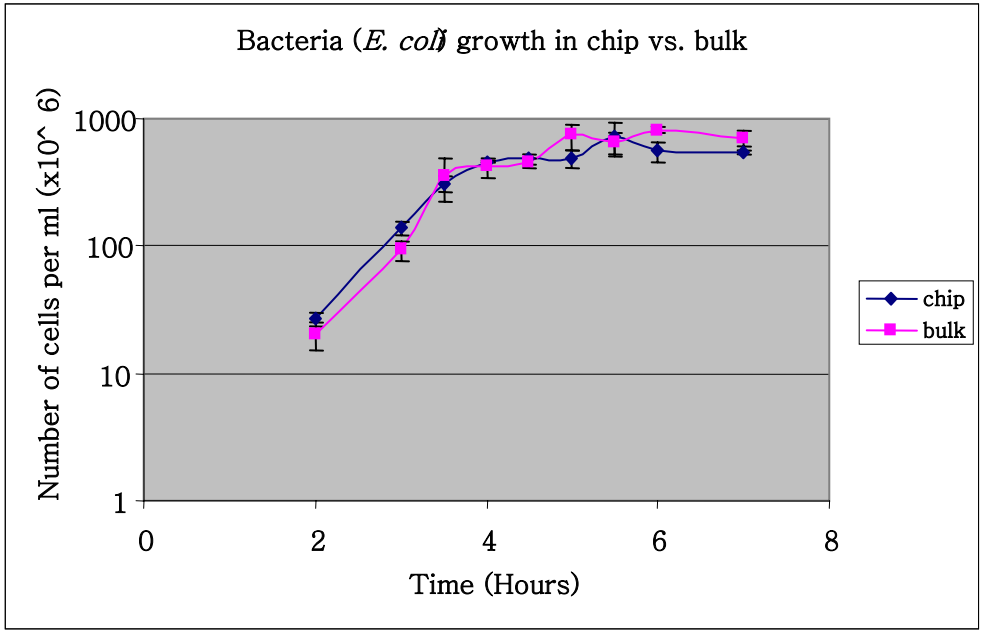
Jigs

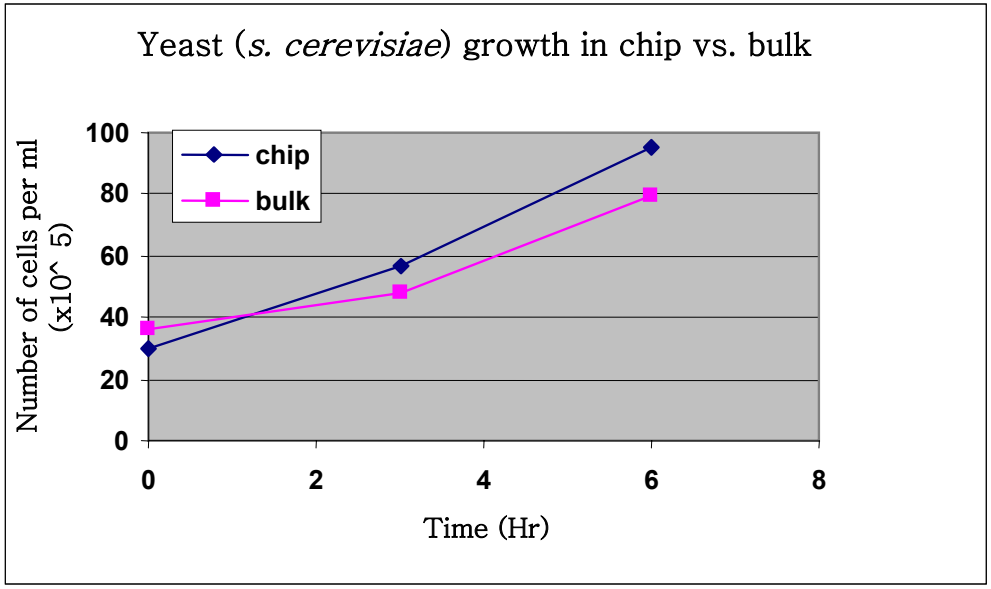
Cell Culture



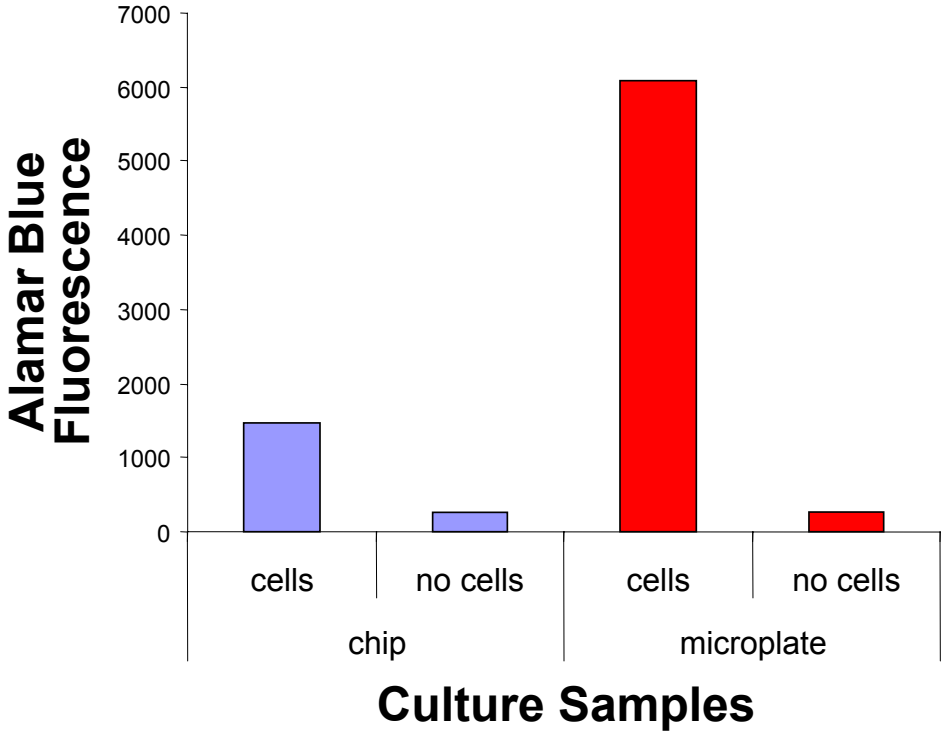
Yeast cells (*S. cerevisiae*) growing in single channels.

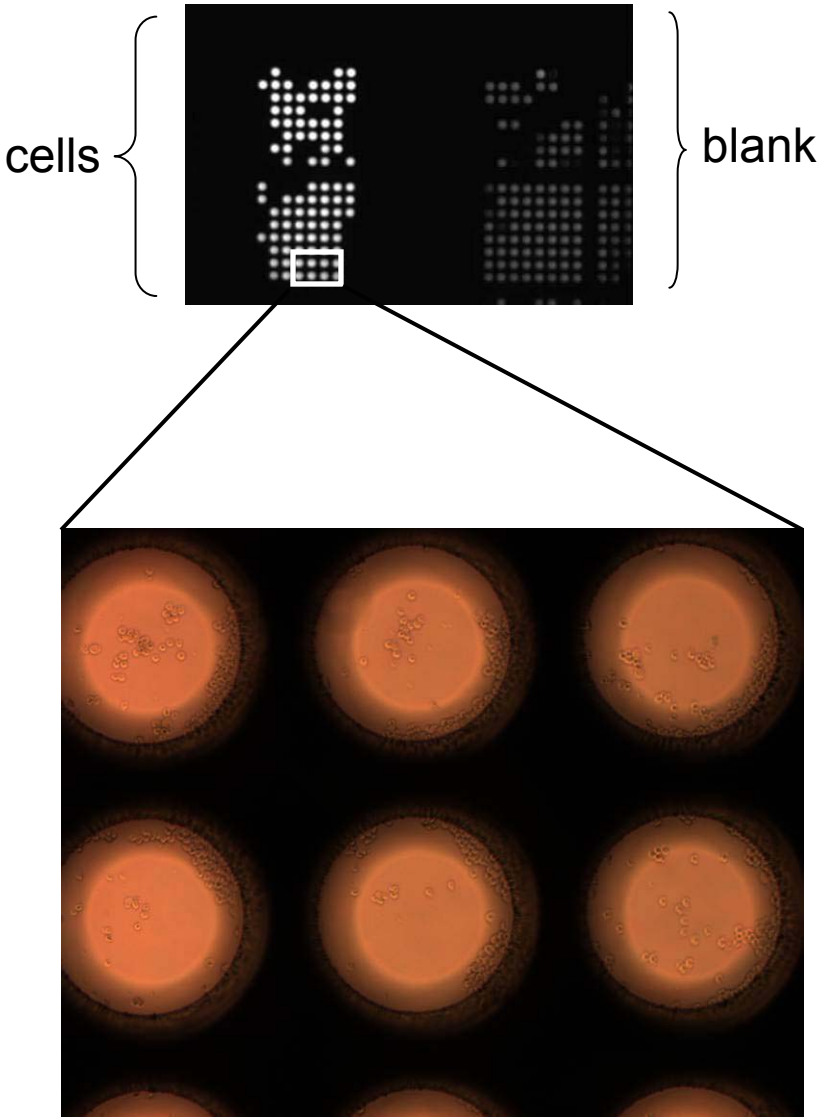
~1000 cells per channel
Cell viability assay: MitoTracker Green FM yeast mitochondrial stain





Mammalian Cell Culture

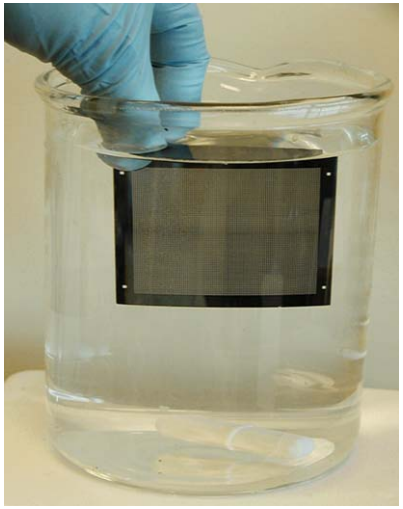




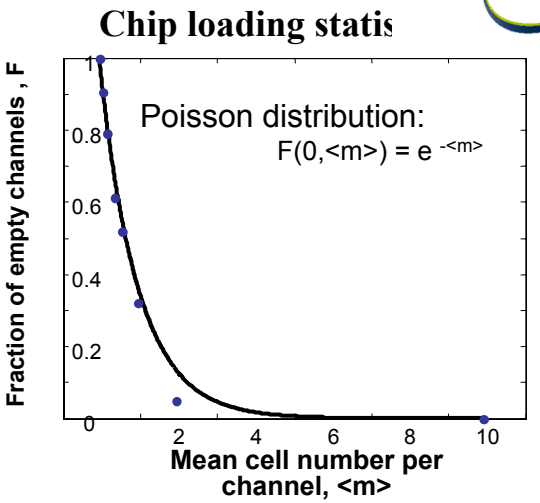
Jurkat Cell Culture

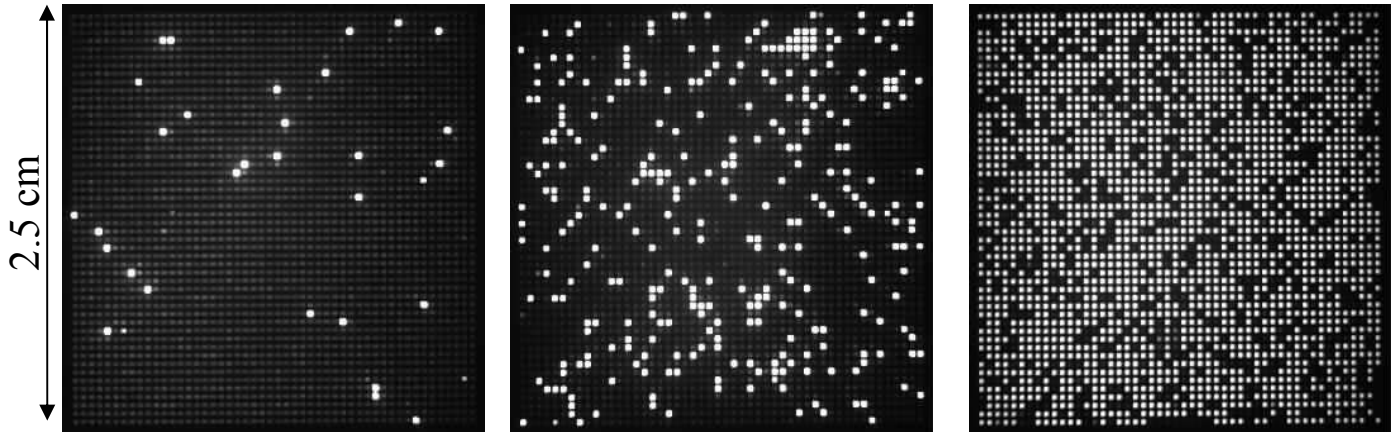
- 35 nl per channel cell culture volume
- 75 hrs cell culture

Dip Loading Statistics



Number of cells loaded into each channel follows a Poisson distribution!





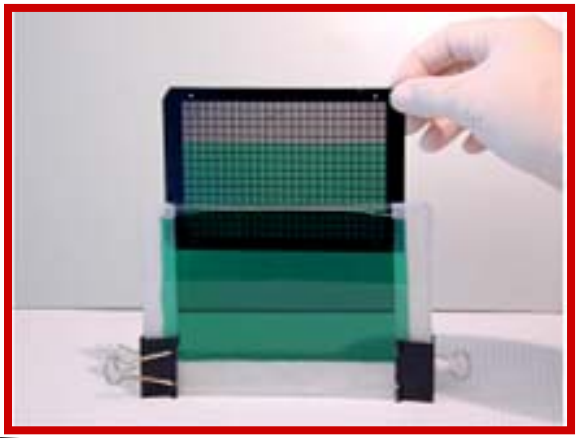
160 pos cells/mL
31 positives
1.24%

1600 pos cells/mL
310 positives
12.4%

16 000 pos cells/mL
1905 positives
76.2%

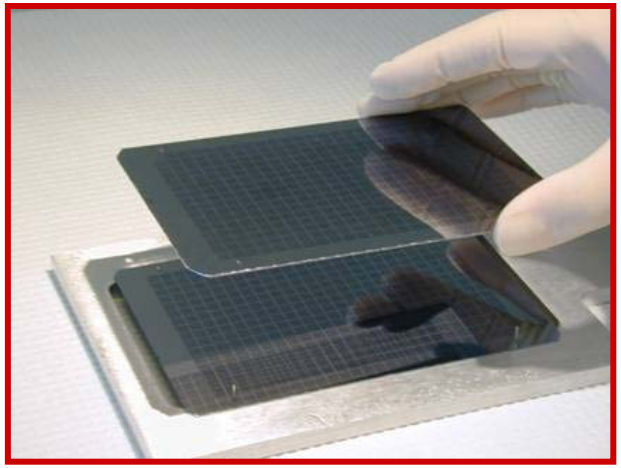
Biocatalyst Discovery

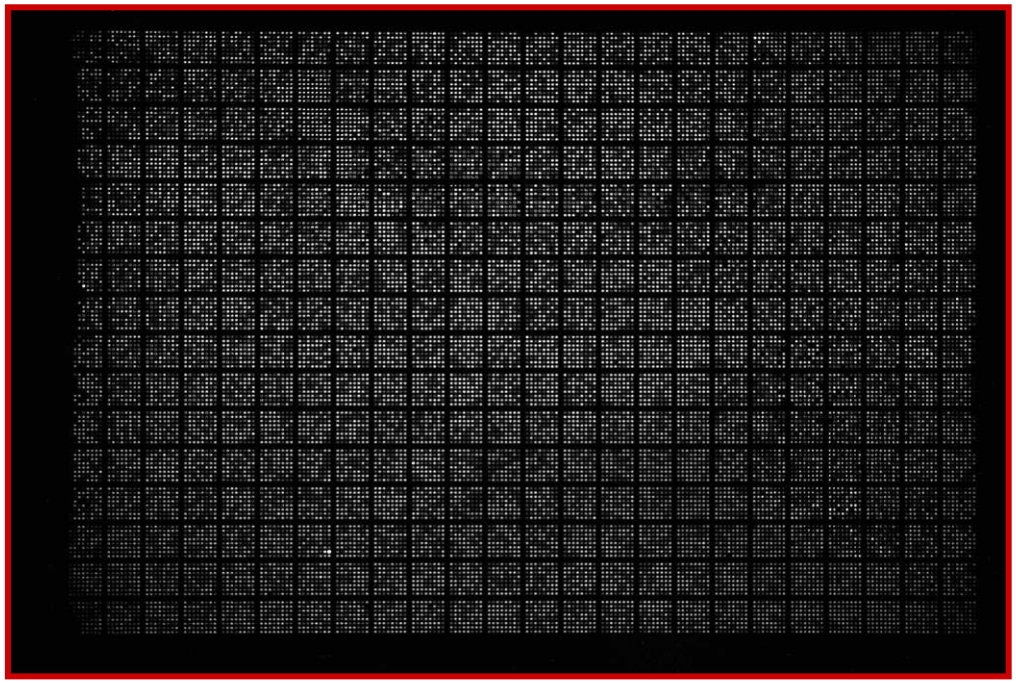
Dip load mutagenized bacillus library
~10^4 cells/mL or 0.5 cells/channel



Cell culture
(24 hrs @ 37°C)

Stack to add substrate
(10 μg/ml F:caesin)

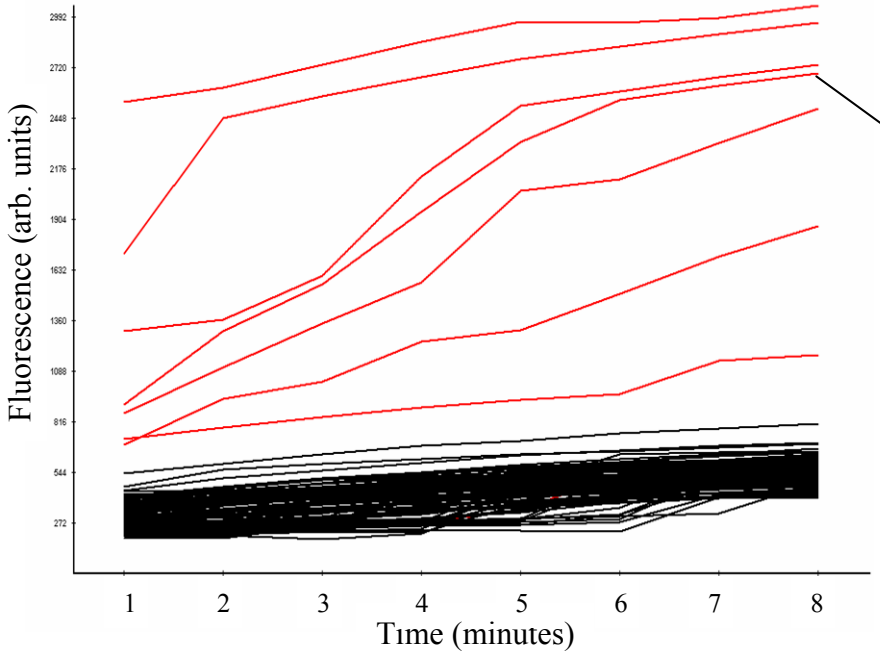




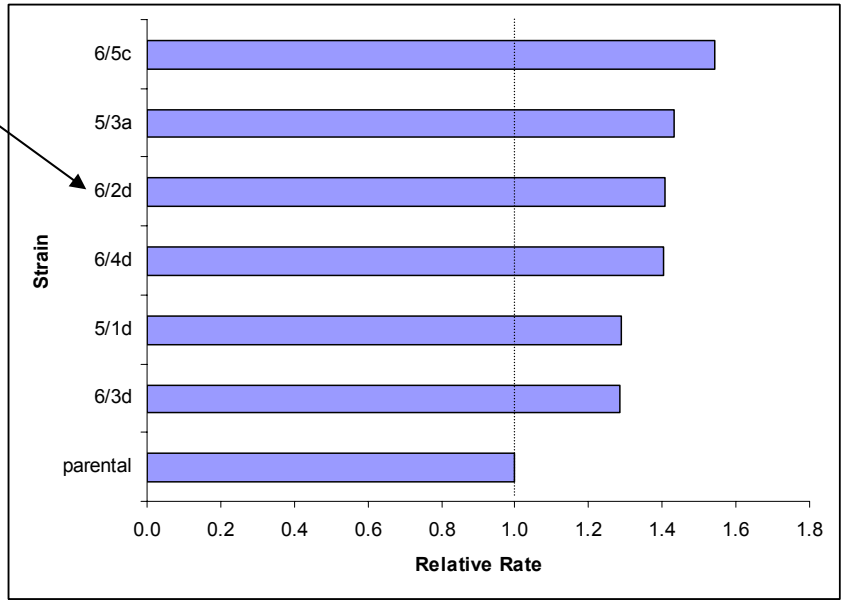
Mutants with increased protease activity yield greater fluorescence rate signal.

Data Analysis

Primary Screen



Secondary Screen



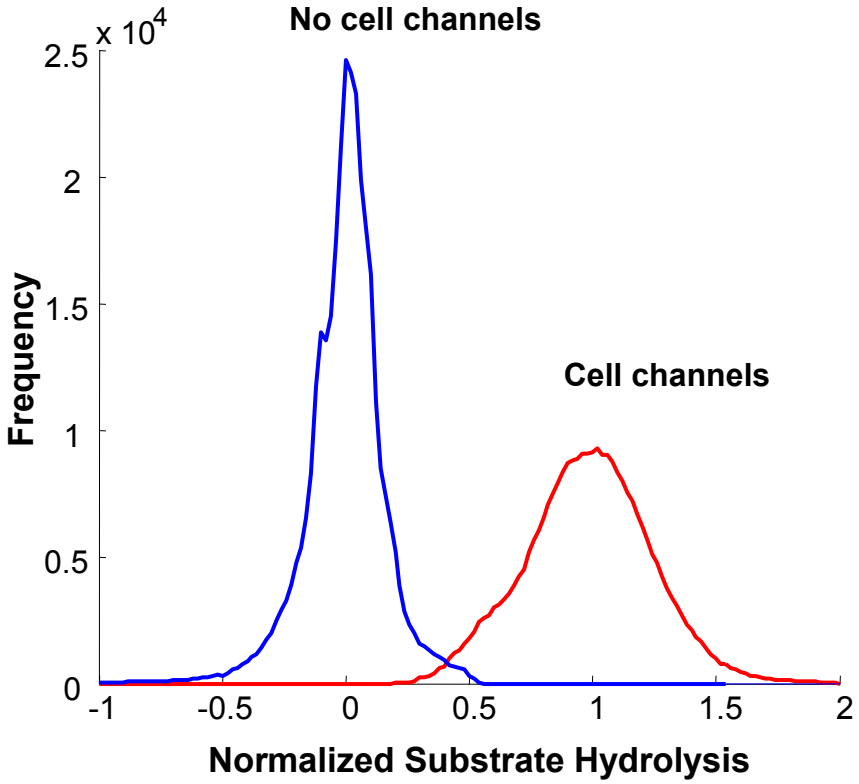
Primary Screen: Image data was processed to collect channel fluorescent intensity as a function of time. Reaction rates were then used to pick the top clones from each chip.

Secondary Screen: Top clone from each chip was purified, grown overnight in 96-well plate and the hydrolysis activity of the medium measured.

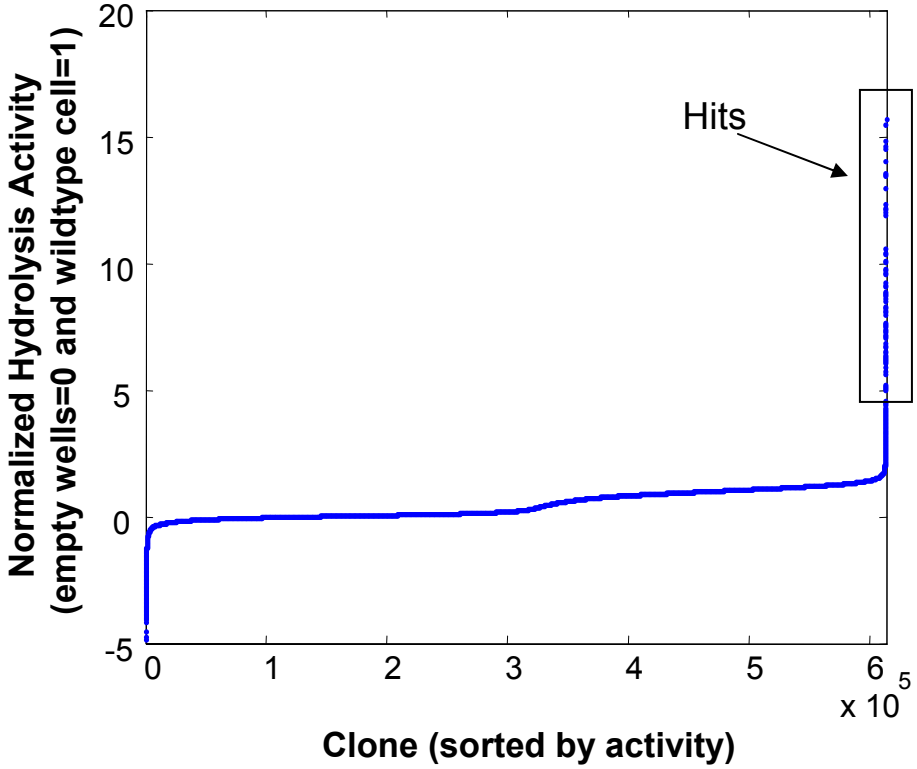
Results: Mutants with improved protease expression were isolated using the Living Chip.

Biocatalyst Library Activity

Activity Histogram



Hit Selection

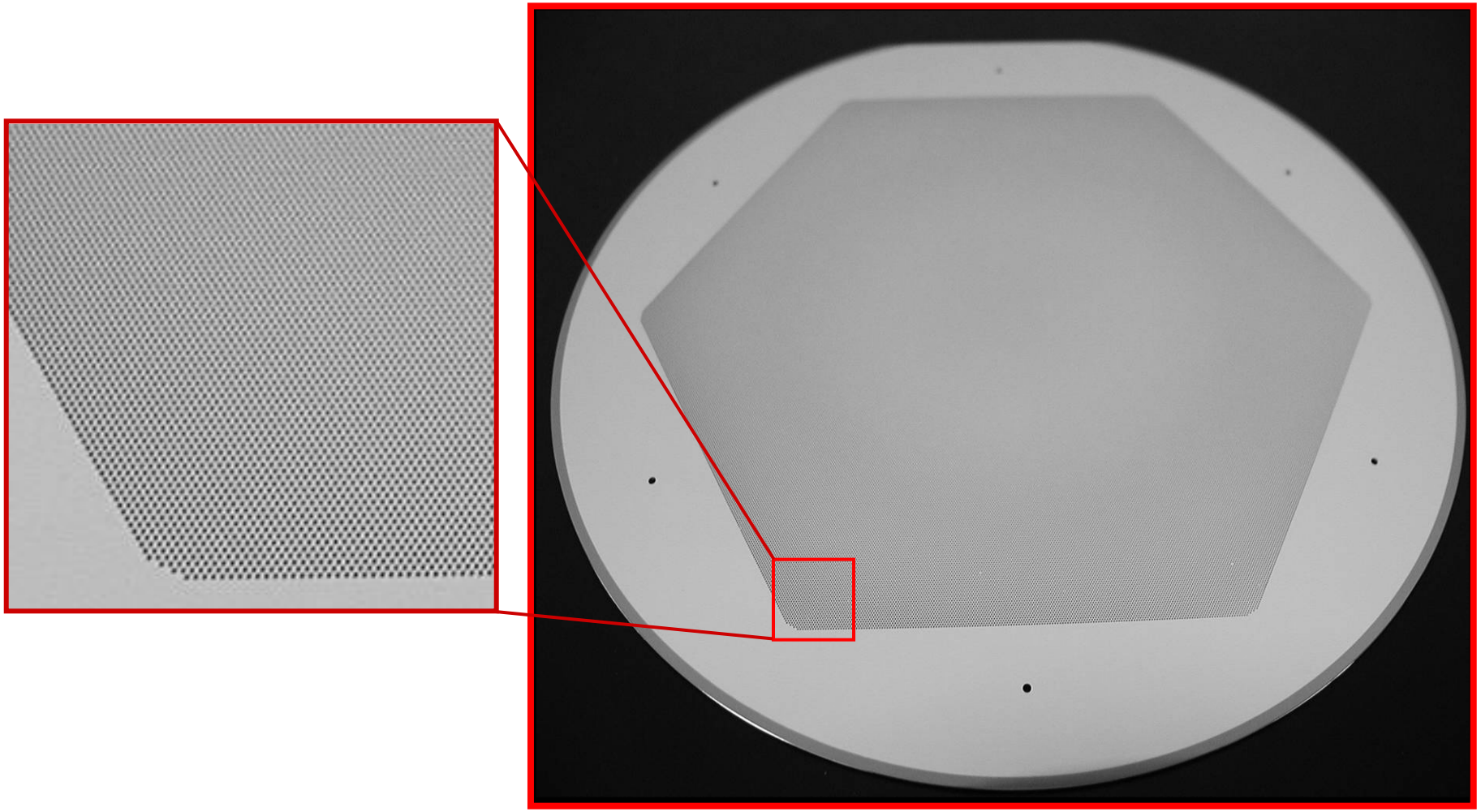


Per plate CVs for normalized cell positive population is 20%.

37 clones had activity 5 to15-fold above average.

Technology Scale-up

Next generation: 100,000 channel chip
= 260 384 well microtiter plates



Phage ELISA for Antibody Discovery

Chip #1-antigen

Coat channels :Dip-load chip with antigen

Wash and load with PBS

BLOCK

Chip #2-antibody

Dip load cells expressing (+) and (-) Fd phage

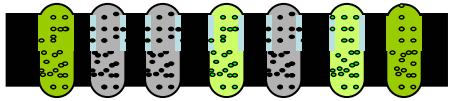
Grow overnight 30 °C

(-)-Fd Phage (non-specific)

(+)-Fd Phage

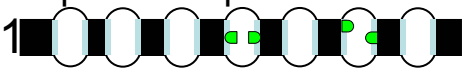
#1

#2



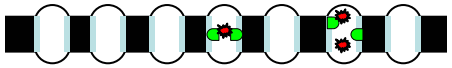
Wash and separate chips

#1



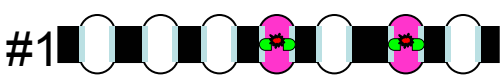
+ α-phage-HRP

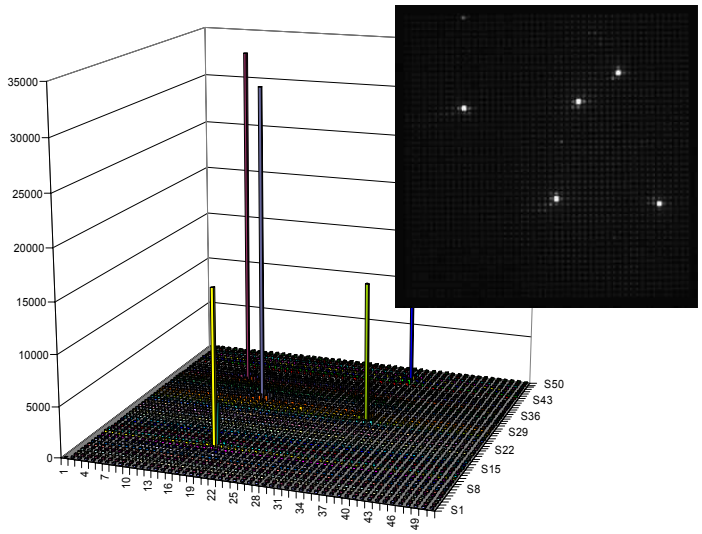
#1



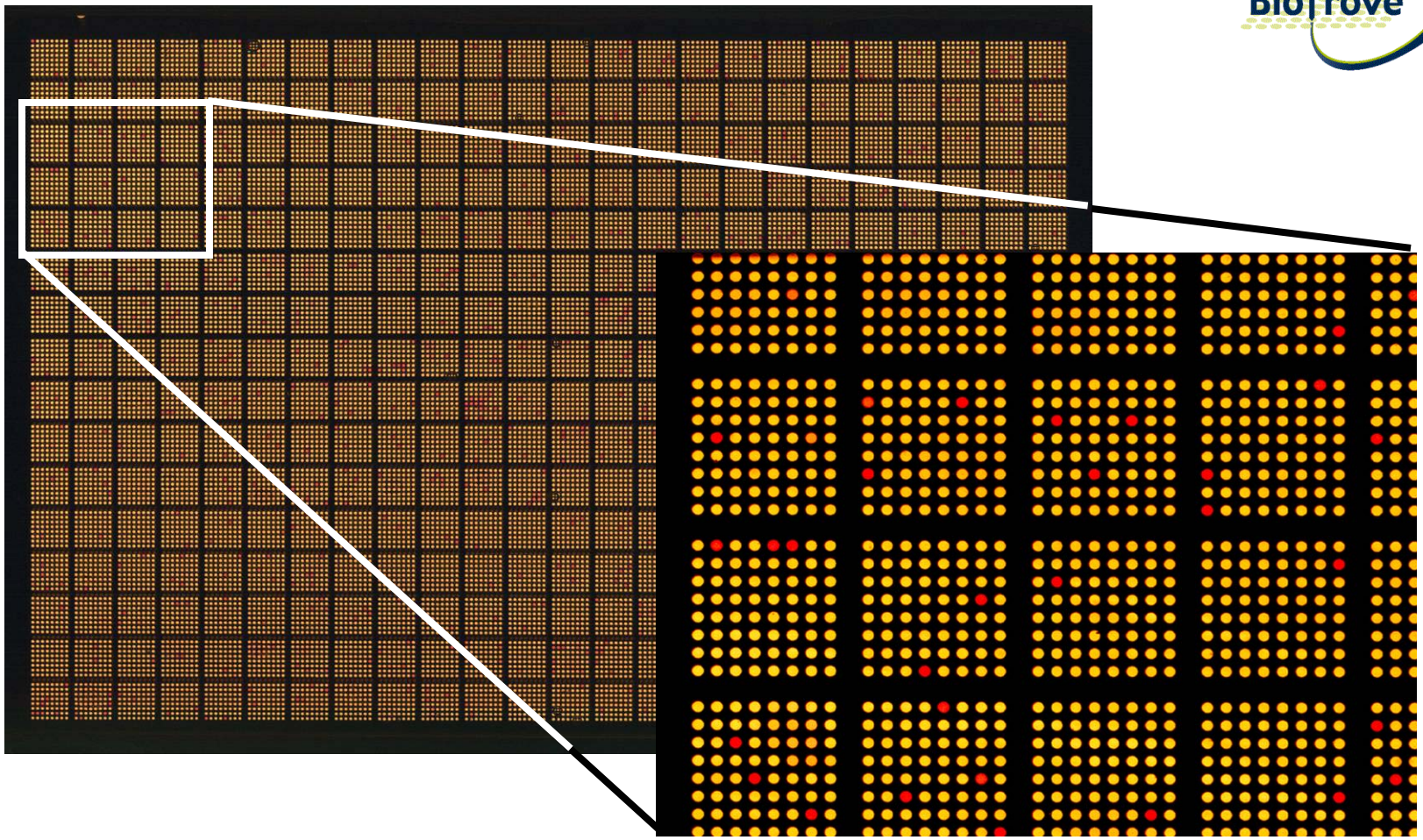
Wash and develop

#1



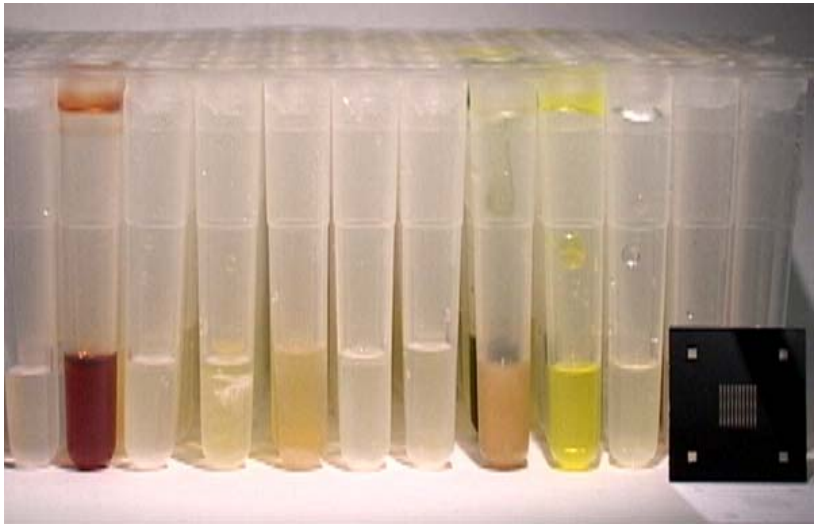


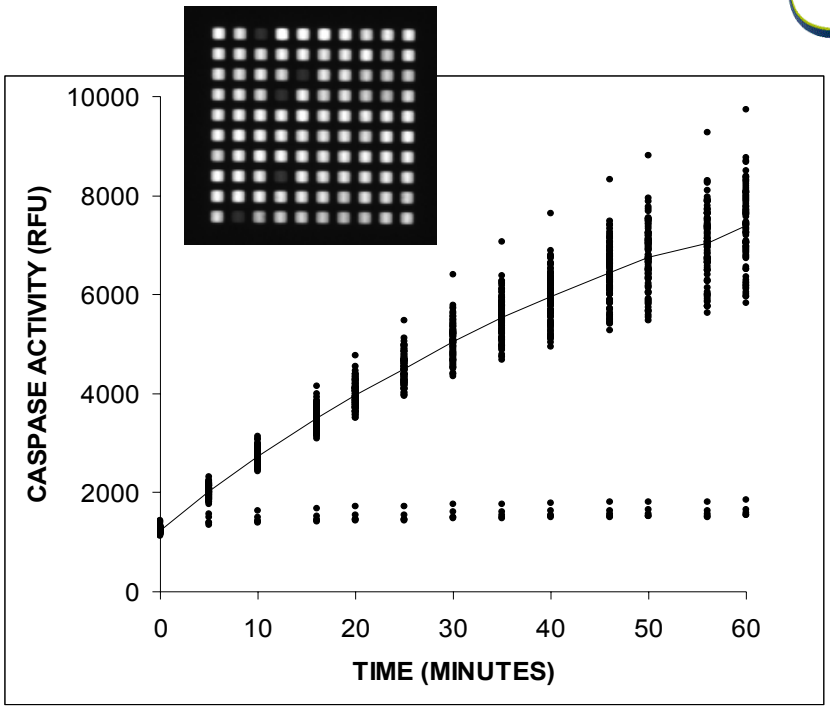
Ultra High Throughput ELISA



- Array is dip loaded from dilute *E. coli* suspension (2000 cells/ml) expressing recombinant human proteins..
- Clones were cultured for protein expression overnight.
- Expressed-protein adsorbed non-specifically to the activated walls of the channel and was subsequently probed using an HRP-conjugated antibody.
- The ELISA was developed with a fluorescent substrate before imaging.

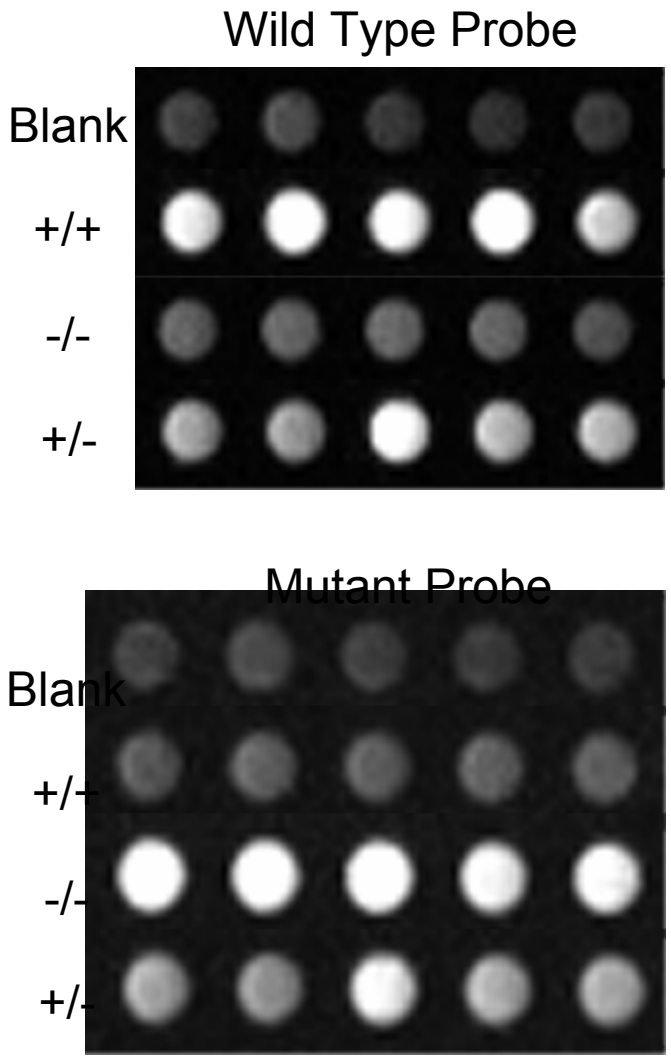
Small Molecule Library Screen



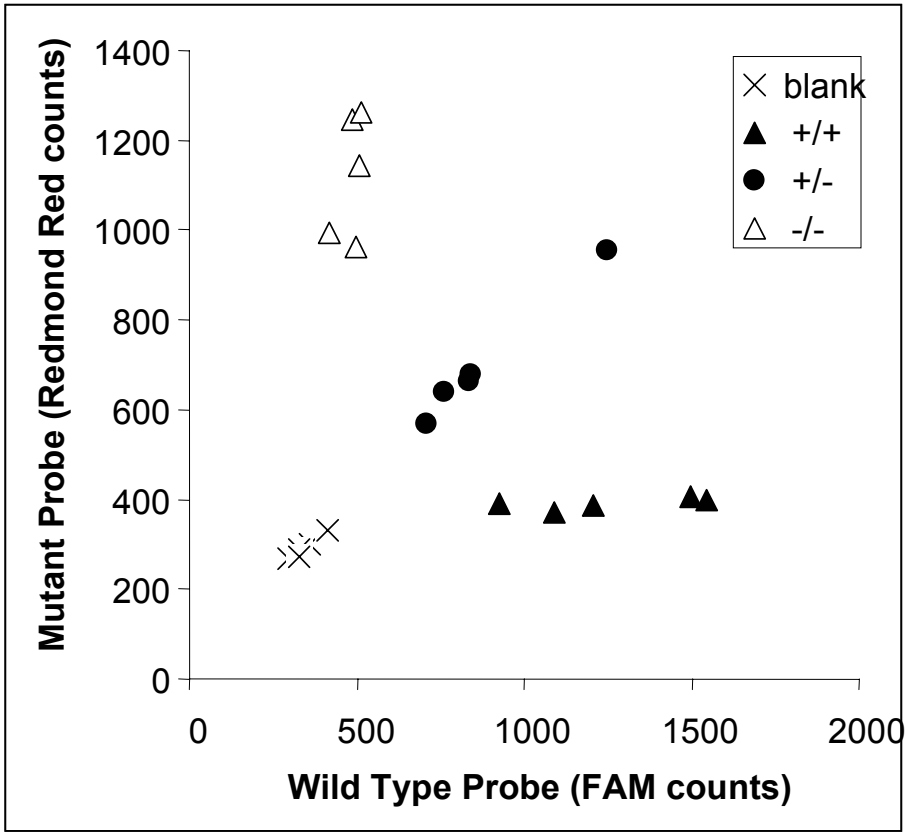


- 95 Chembridge compounds were diluted to 200 uM in Caspase Assay Buffer and reformatted into chip (56 nl/well).
- 200 uM Caspase inhibitor (DEVD) randomly inserted into five channels.
- Z-prime = 0.72 @ 30% completion.
- Signal/Buffer = 15
- Signal/Noise = 51

Genomic Assays



Third Wave Biplex Invader Assay



2.5 ng genomic DNA per channel (≅1000 copies)

Library Storage

Storage of a one million compound librar,

Living Chip

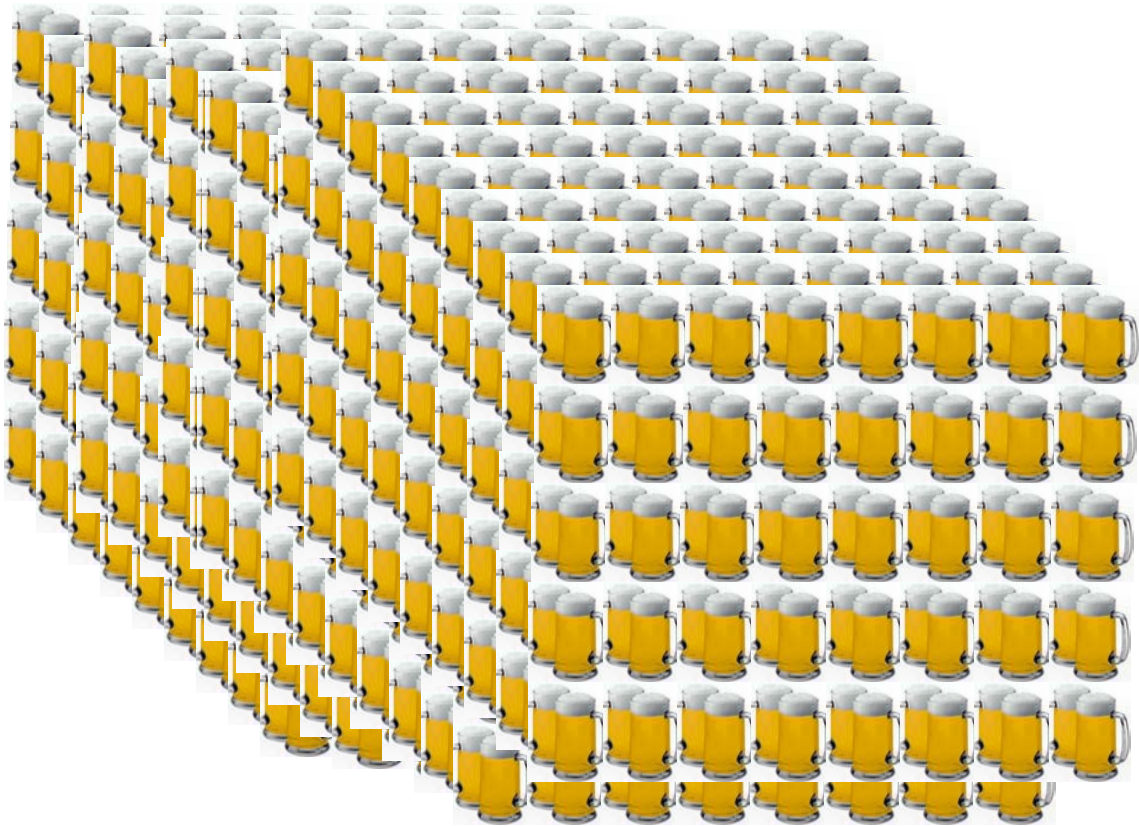
40 x 25k plates
50 nl per channel

10 copies → 0.0002 m^3
Total fluid volume = 0.5 liters

384-well plates

2,604 plates
25 µl per well

10 copies → 4 m^3
Total fluid volume = 250 liters

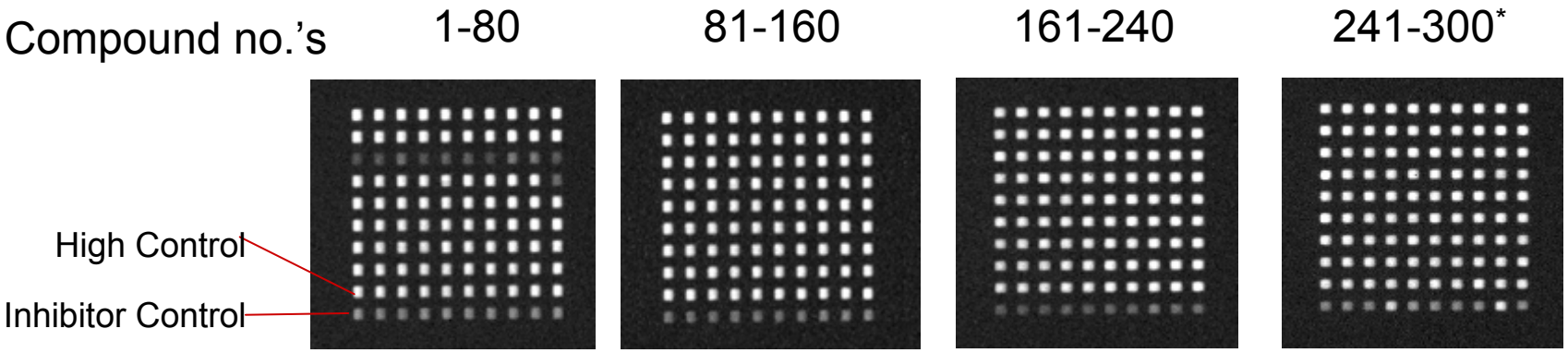


Chip Storage Advantages

- **High density, nanoliter storage**
  - reduces storage space needs and material waste
  - facilitates distribution of library
- **Ideal format for low temperature storage**
  - chips can be submerged directly into liquid nitrogen
  - affordable storage at low temperature, inert conditions.
  - small sample volumes freeze rapidly
  - libraries can be aliquoted into chips -> freeze/thaw only once!
- **Simple interface to microtiter plate systems**
  - provides for easy and rapid conversion.
- **Integrated screening**
  - screen directly from chip, or transfer to microtiter plate first.

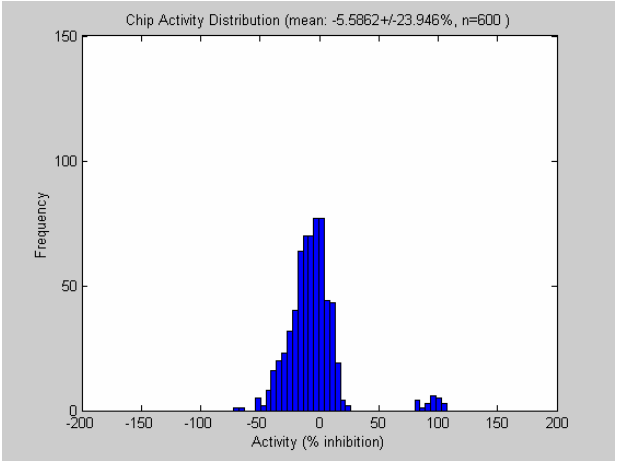
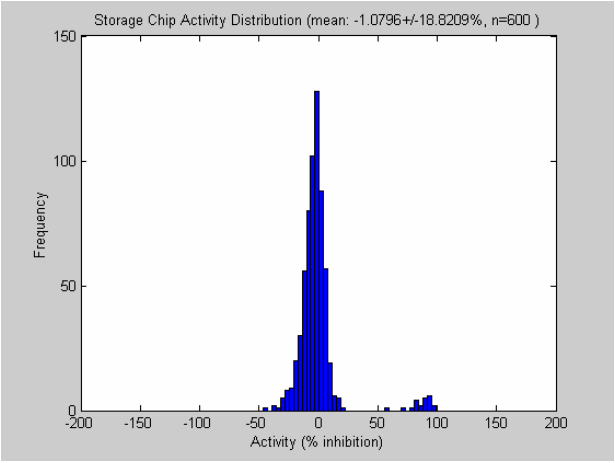
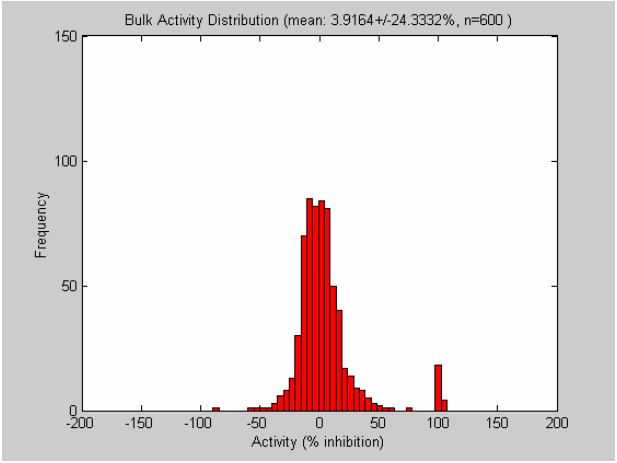
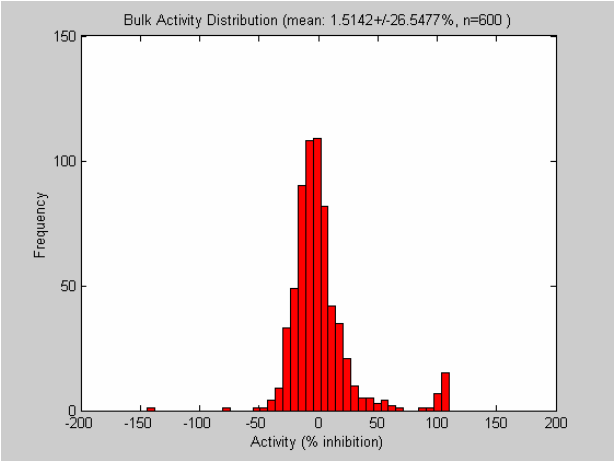
Example Protease Inhibitor Screen

- Target: UV FRET-labeled peptide.
- Z-prime consistently > 0.55 @ 30% inhibition.
- Three hundred compounds analyzed.
- Assay volume = 54 nanoliters.
- Storage for five days @ -20°C.



*Note: on last chip, inhibitor control was running low and two channels did not fully load

Distribution of Inhibition Data

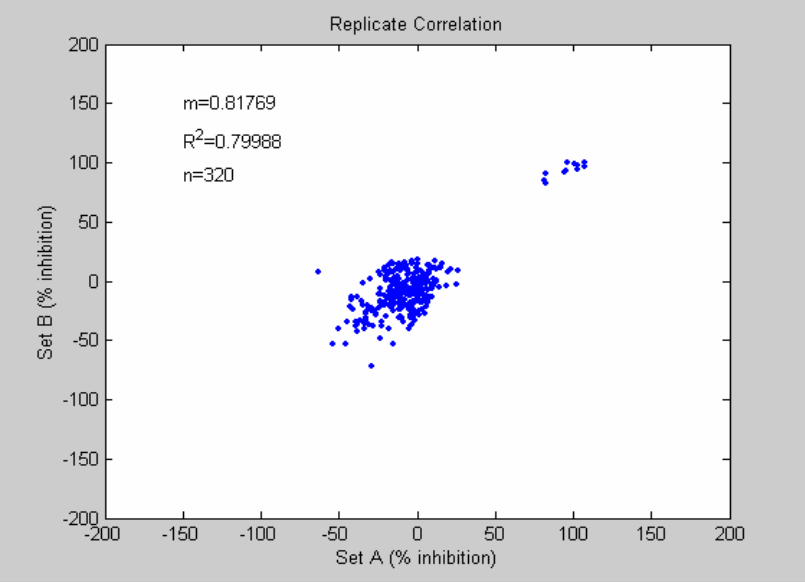
	Without Storage	With Storage
Chip		
Bulk		

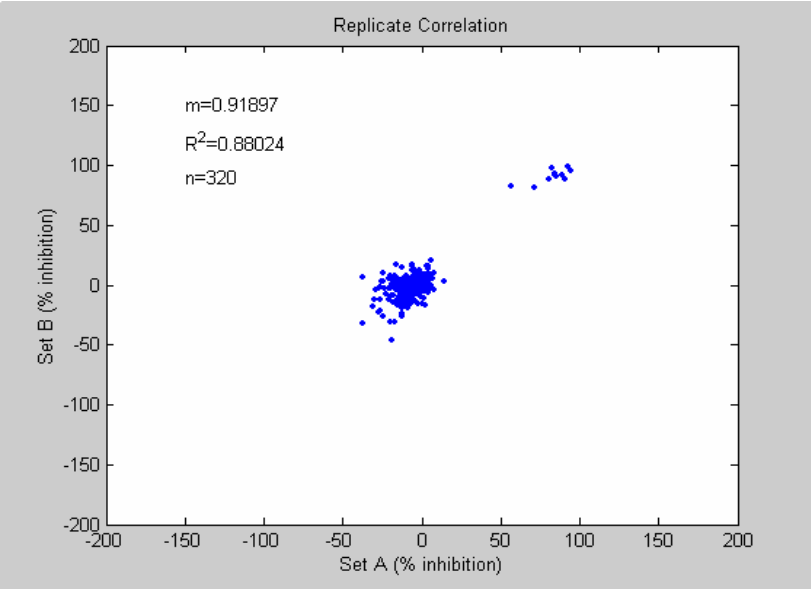
Correlation of duplicates

No storage

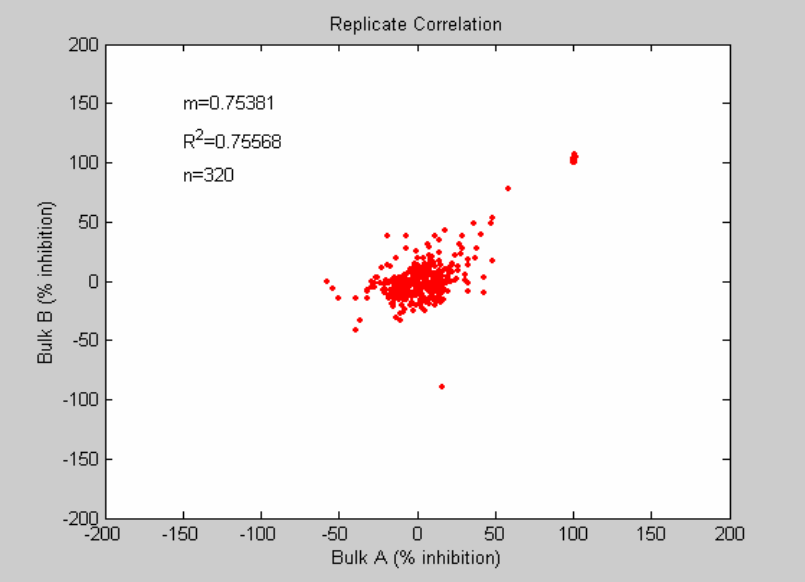
Storage

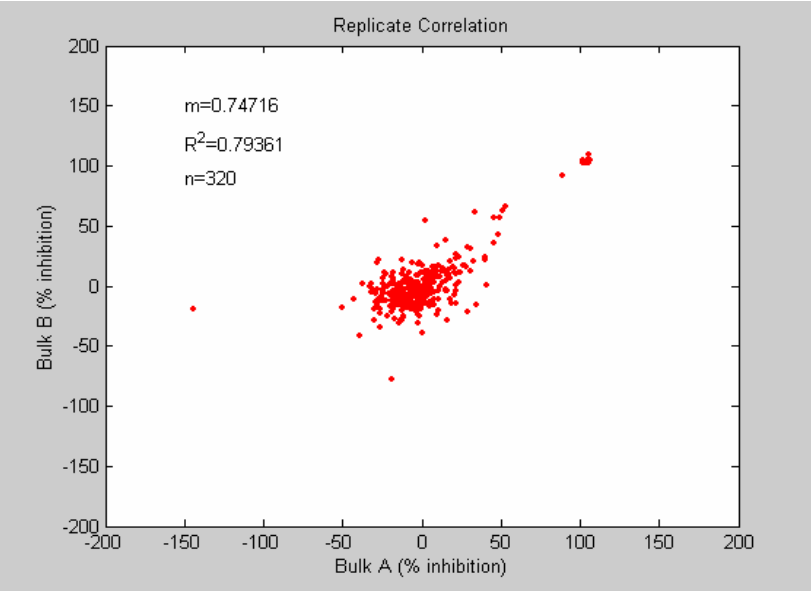
Chip





Bulk





Summary

Living Chip™

Massively parallel fluidics

An integrated, broadly applicable platform for nanovolume synthesis, storage and screening providing

- Isolated nanoliter reaction volumes.
- Simple automated interface with microplates.
- Assay flexibility and speed.
- Desktop-sized instrumentation.
- No evaporation.

Technology Attributes

- **Faster time to market**
  - Ultra high throughput (>10^6 meas./day)
  - Use less target -> Decreased time for target production.
- **Saves precious compound library, target and reagents.**
  - Isolated, nanoliter reaction volumes.
- **Easy access to locally stored libraries.**
  - Simple and automated interface to microplates.
- **Enables substantially larger libraries (> 10-fold)**
  - High density, nanovolume storage.
  - More lead compounds.
  - Data sets for building better predictive models.

Applications and Future Directions

- **Integrated Library Storage and Screening**
  - HT toxicity characterization of libraries (>10^6/day).
  - HT drug-drug interaction assays.
- **Molecular Discovery - Diversity Screening & Evolution**
  - Rapid analysis of large diversity libraries (>10^7 reactions/person/day).
- **Functional Genomics & Proteomics**
  - HT screening of gene knock-out & gene expression cell libraries.
- **Molecular Diagnostics**
  - Single chip genomic assays.
- **Process Optimization**
  - protein crystal growth
  - screen for drug polymorphs
- **Synthesis - Catalysis Discovery to Speed Chemistry**
  - Rapid lead optimization
  - Higher quality, more diverse libraries

Future Directions

- **Scale-Up of Reformatting**
  - Multiple copies of a 25K plate in < 1hr.
- **More Assay Modes**
  - Polarization, time-gated, radiometric, label-free detection.
  - ADME-Tox assays (CaCo2, Alamar Blue, others).
  - Rapid initiation (e.g. Fura-2)
- **Greater automation**
  - Screen 1 to 10 million data points per day.
- **On-chip Synthesis of molecules/probes**

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