Fabricating Microfluidic Devices for High-Density Biological Assays

Todd Thorsen
Department of Mechanical Engineeering
MIT

Panamerican Advanced Studies Institute
Micro-Electro-Mechanical Systems
San Carlos de Bariloche, Argentina
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MIT Hatsopoulos Microfluids Laboratory

Outline for the Talk

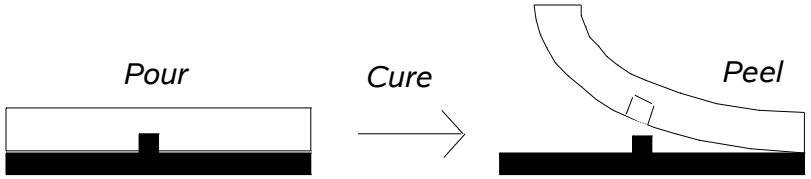
- Introduction to Soft Lithography
  - Chip Fabrication
  - Multilayer Technology
- High Density Cell-based Arrays
  - Microfluidic LSI
  - Detection Systems
  - Biochemical Applications
- Microfluidic DNA Arrays

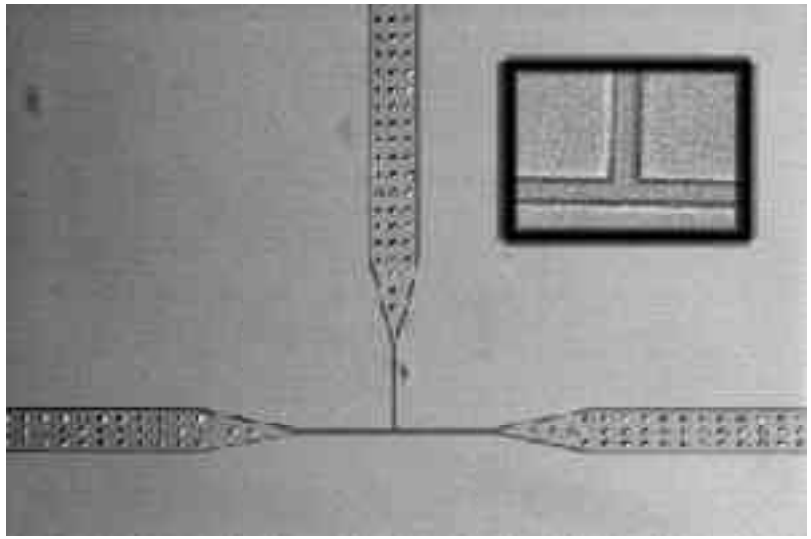
Introduction to Soft Lithography

- Inexpensive and rugged elastomeric materials: PDMS, polyurethane, etc.
- Easy and Forgiving Manufacturing Process
- Disposable and thus no cross contamination
- Simple Flow Design and Integration

Microfabrication

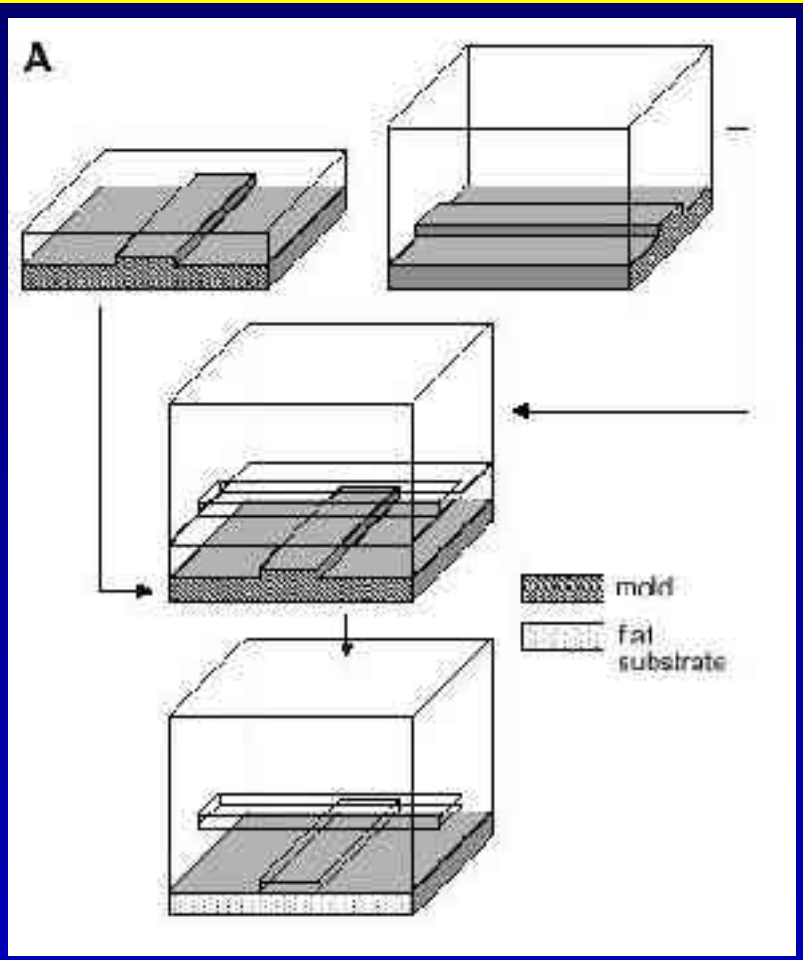
- Mold: Photoresist-patterned silicon wafer
  - Positive relief channel template
- Device: Elastomer
  - Cured on silicon mold
  - High fidelity negative replica of channels
  - Hermetically seals to coverslip





Valve fabrication – Multilayer Soft Lithography

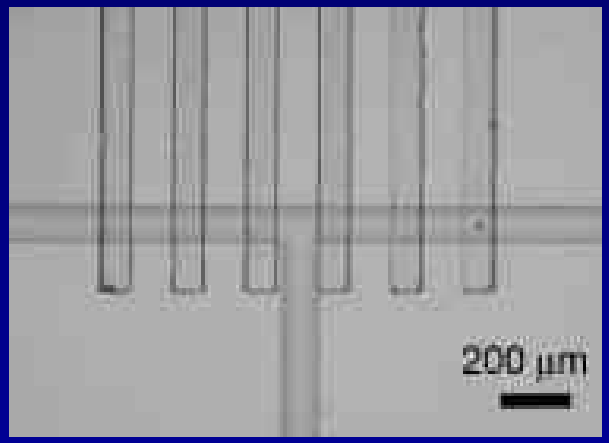
- Spin coat silicone over (A) rounded photoresist flow channels and (B) pour thick layer of silicone over control channels
- Primary Cure
- Punch and align
- Secondary secondary cure
- Pneumatic pressure in control layer deflects interface membrane between the two layer, creating a valve.

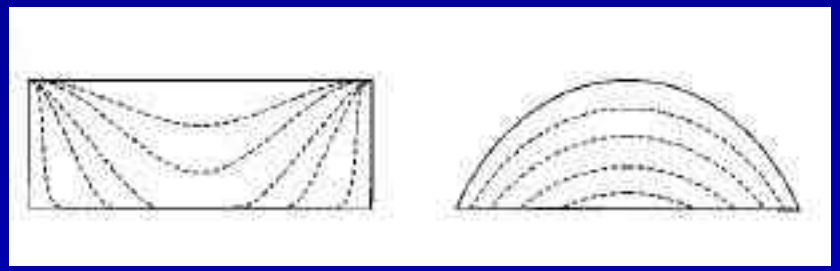


M. Unger, H.-P. Chou, T. Thorsen, A. Scherer, S.R. Quake, *Science*, **288**: 113-116 (2000).

Valve Geometry

- Geometry of microfluidic channel that you want to close off is critical
- Square- Inefficient closure/ leaky
- Round – Great closure





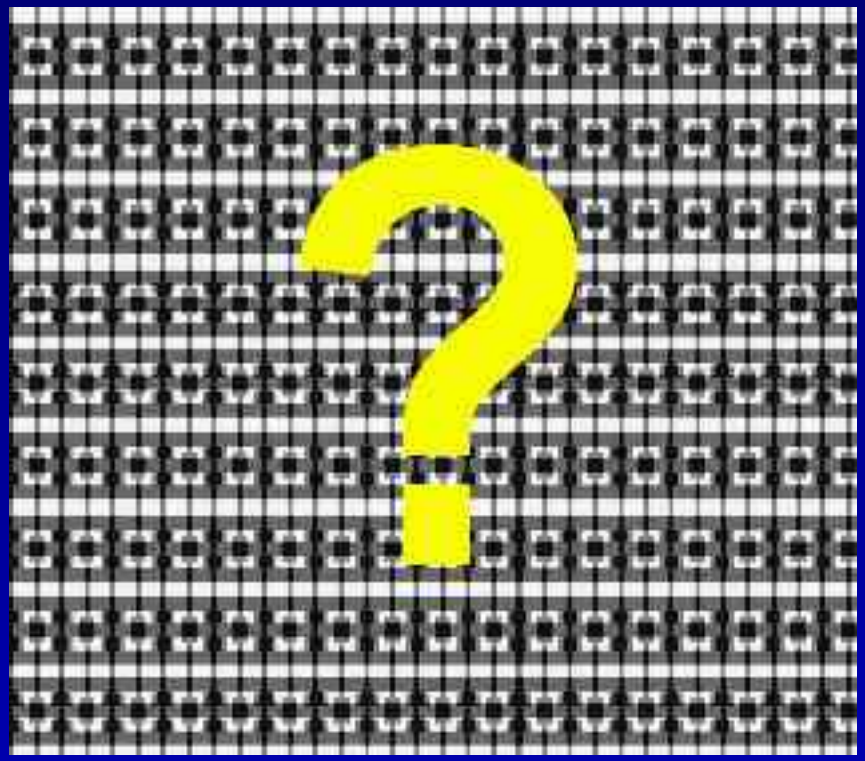
High Density Picoliter Volume Chips: Introduction and Objectives

-Valves used to compartmentalize cells/enzymes into small reaction chambers (picoliter scale)

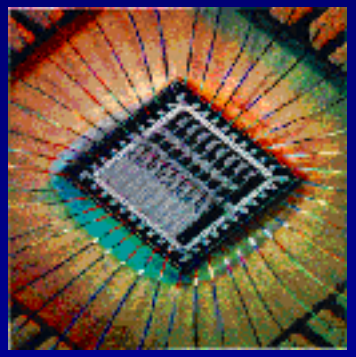
-Substrate can either be introduced with the enzyme or separately for controlled mixing

Microfluidic LSI

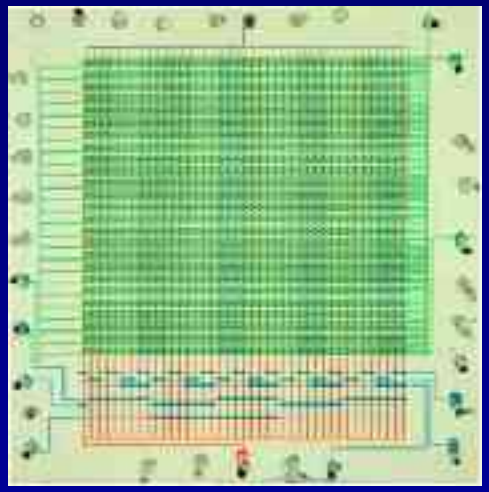
- So you have thousands of compartments on a single chip…How do you address them?
- An integrated control system is necessary for scalability



The Electronics Revolution

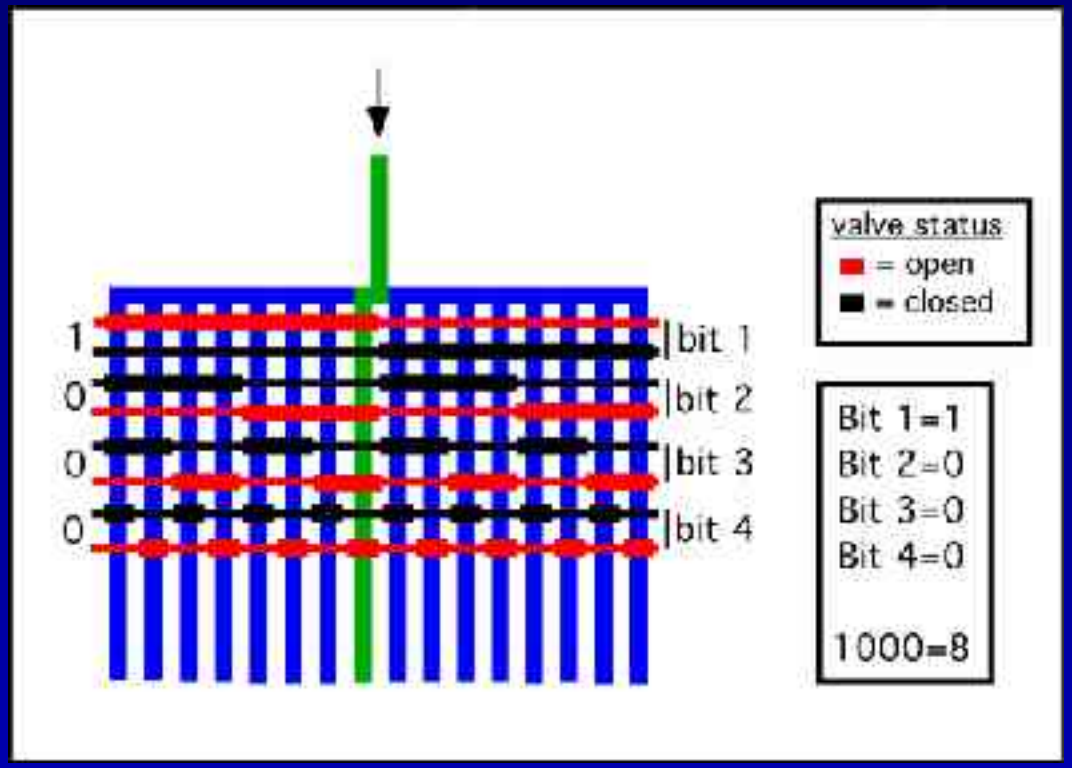


The Fluidic Revolution

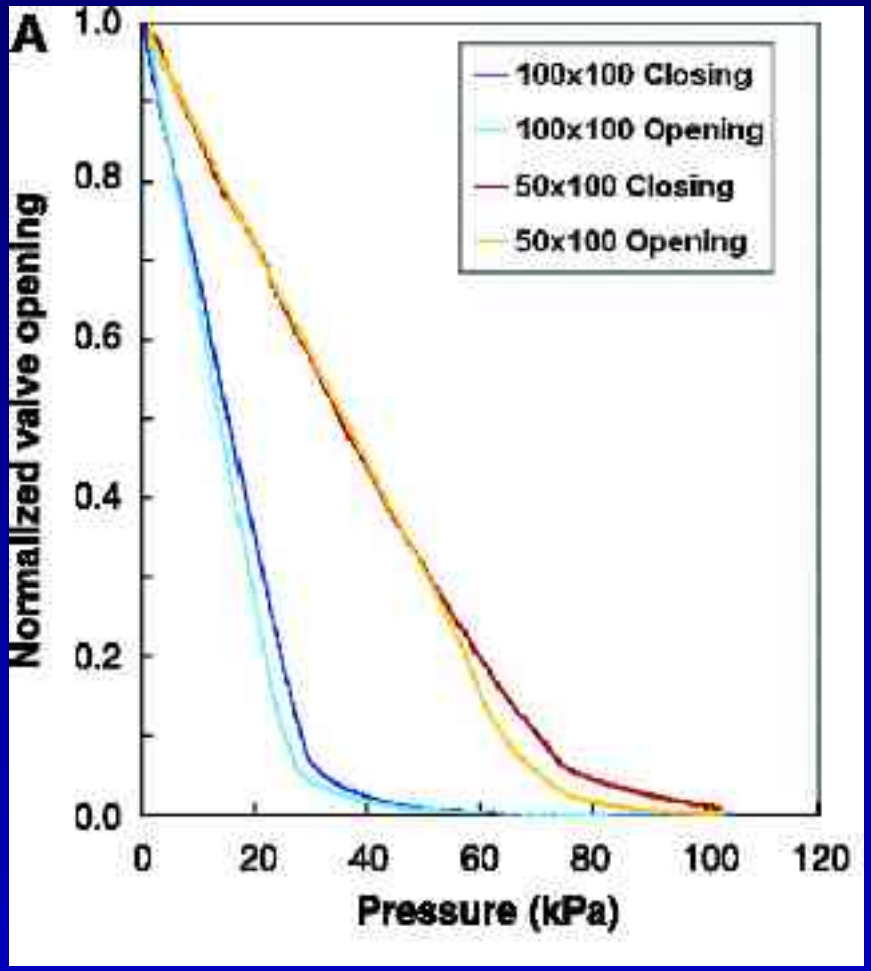


Multiplexor Valves: Combinatorial power

- $2\log_2 n$ valves for n fluid lines
- So….64 rows of fluid lines can be controlled with 12 valves
- Allows complex fluidic arrays to maintain small footprint



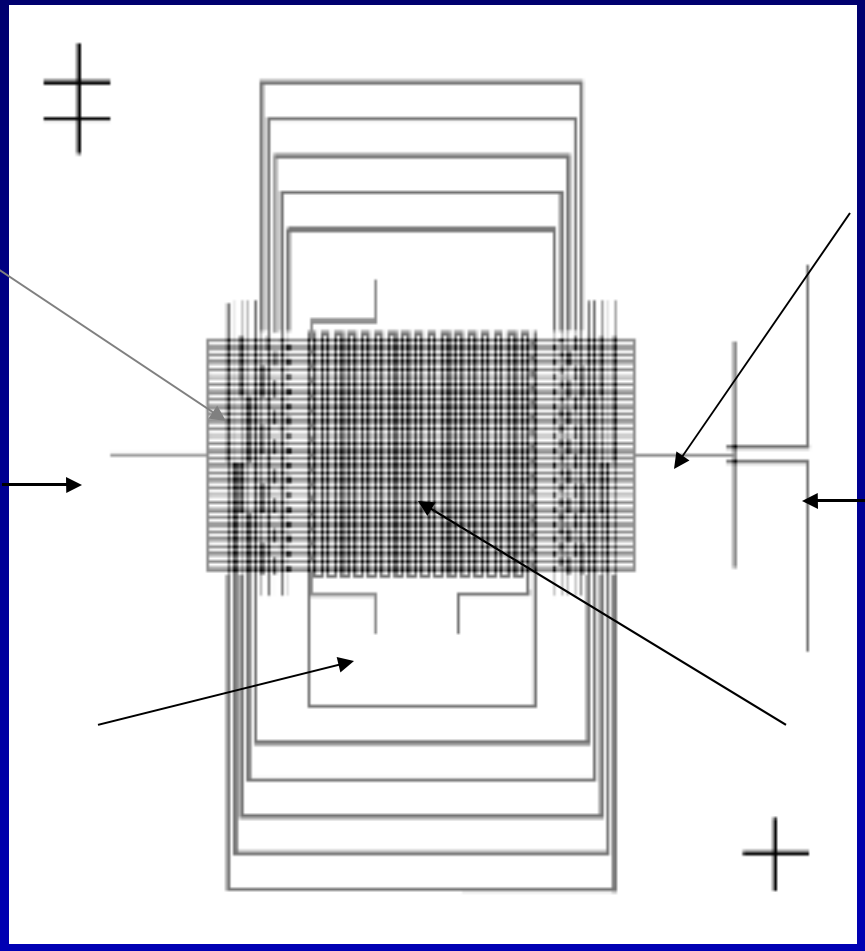
Valve response to pneumatic pressure



-Multiplexors work because interconnects do not close flow channels

-At 50 kPa, 100x100µm valve closes while 50x100µm valve remains open

First Generation Multilayer Chips: Serpentine Models



Multiplexor Valves

Purge Buffer Input

Sample Fluid Input

Single Row Sorting Junction

Sorting Valves

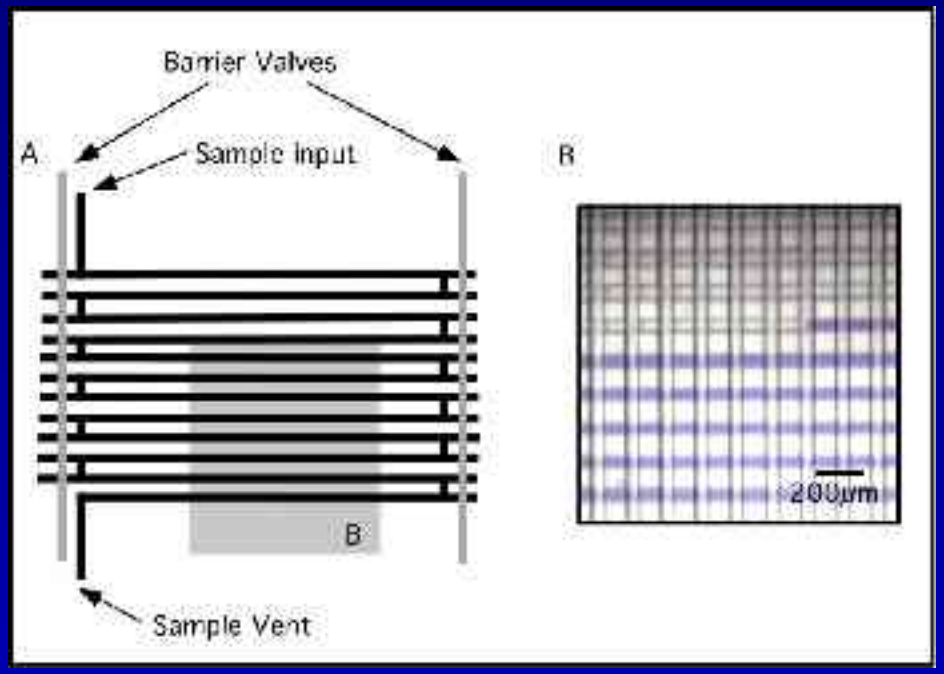
High Density Chamber Array

Grey Layer: Fluid Layer

Black Layer: Control Layer

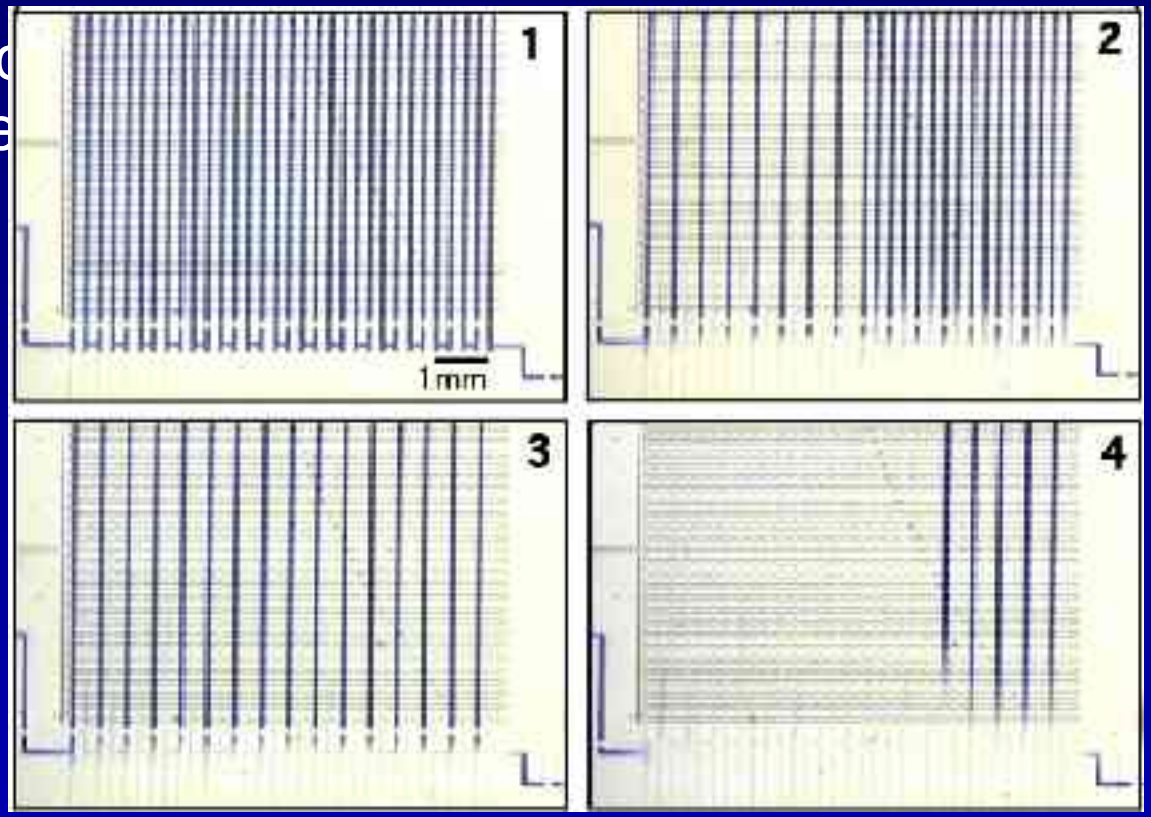
Compartmentalization

- Fluid injected into central serpentine channel partitioned off into ~80 picoliter sections by applying pressure to top control line



Chip Purging: Single Row Addressability

- -Each row controlled by multiplexed valve combination
- -Rows of cells/enzymes can be purged and collected
- -Chip useful for enrichment of rare events

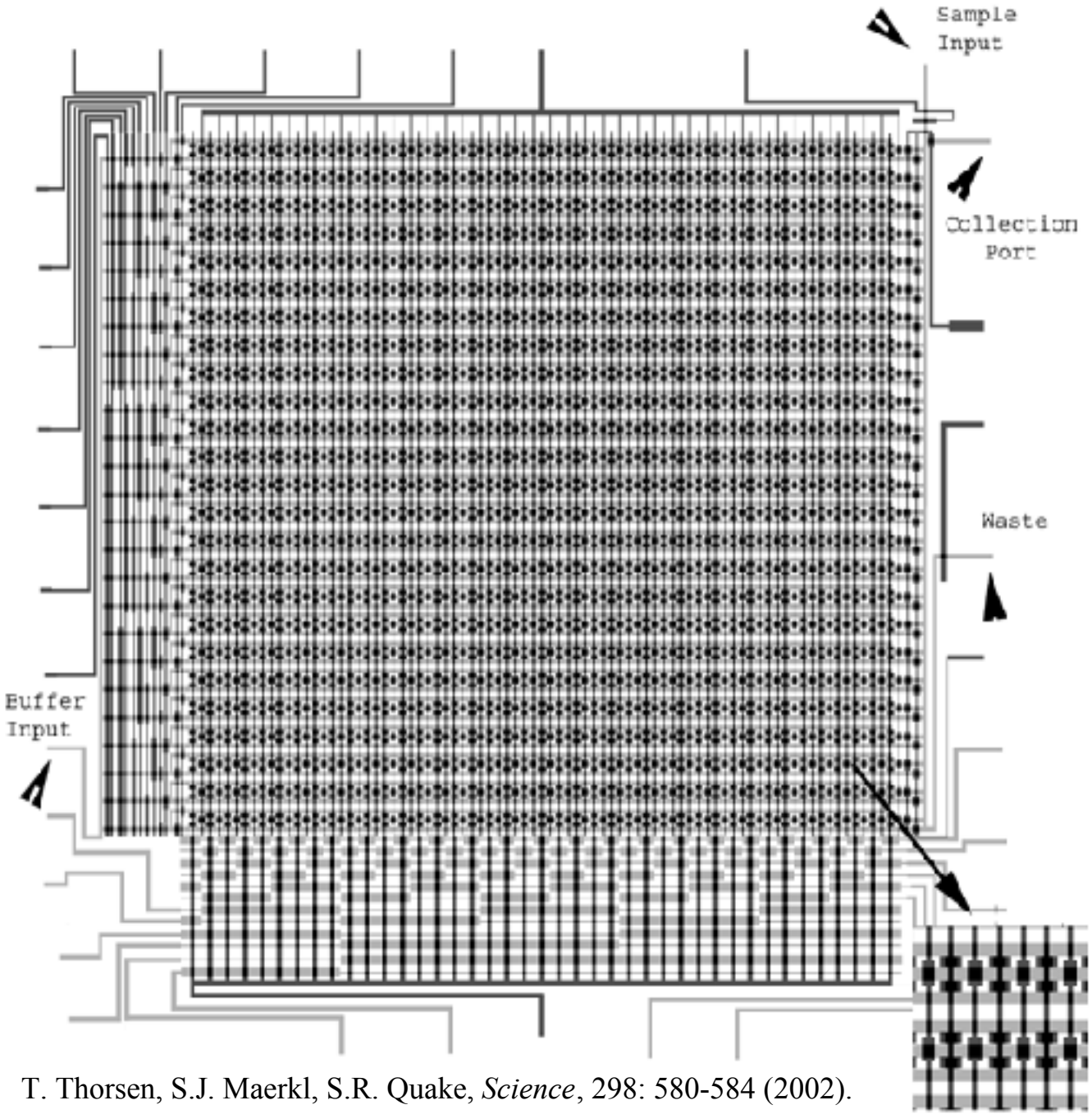


Second Generation Multiwell Chips: Single Well Addressability

- Each well can be addressed, removing contents without sorting, using multiple valves combined with external pressure
- Picoliter well volumes
- Highly parallel screening format

Single Compartment High Density Chip

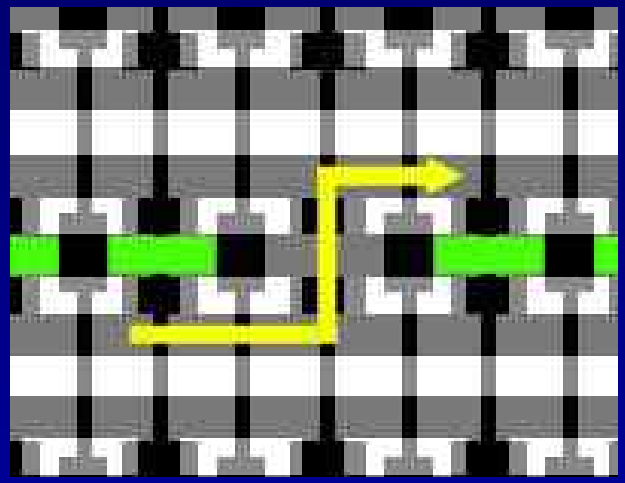
- 1000 wells
-200 pL/well
-Each well individually addressable

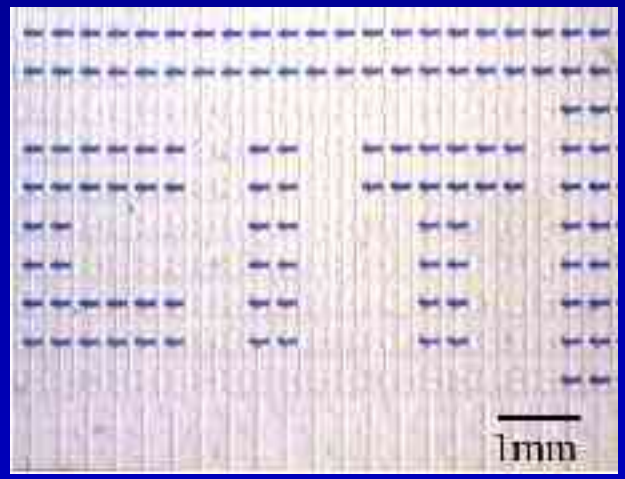


T. Thorsen, S.J. Maerkl, S.R. Quake, *Science*, 298: 580-584 (2002).

Single Compartment Addressing

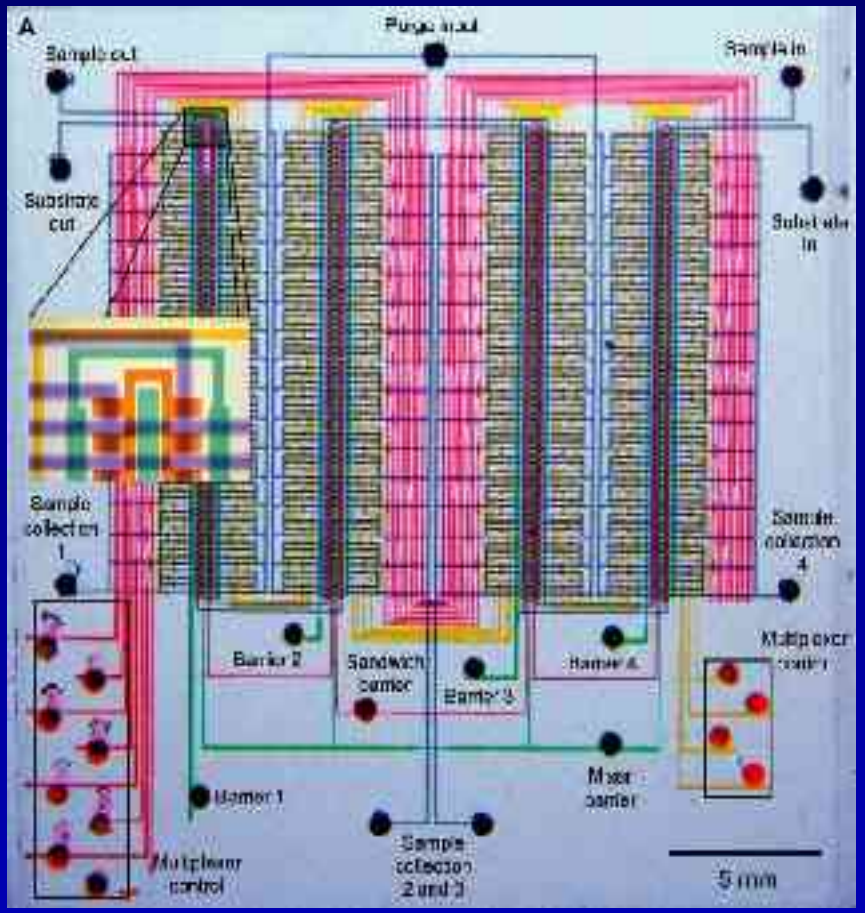
- Wells filled with bromophenol blue dye
- Purging accomplished by:
  - -releasing vertical valve pressure
  - -pressurizing fluid in local outflow line





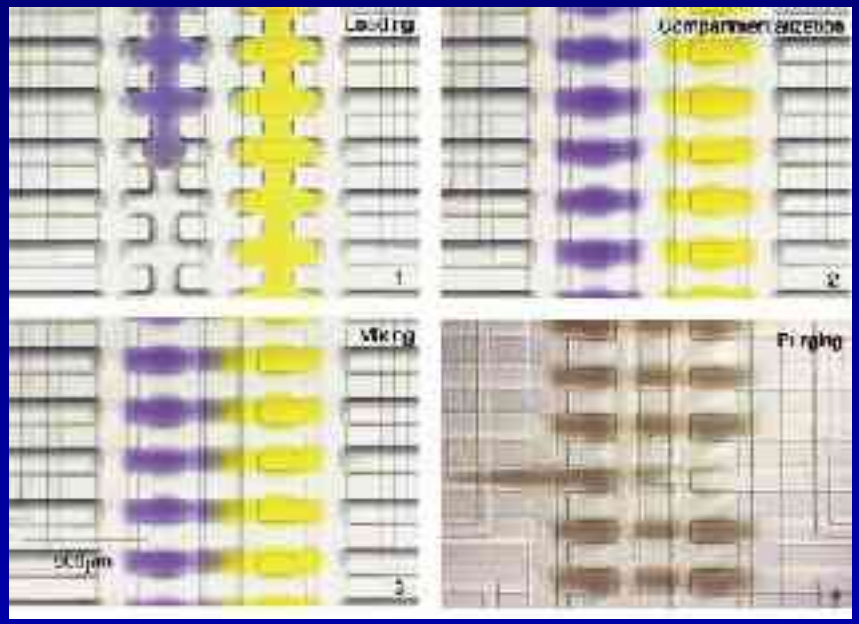
Complex Functionality - Mixing

- Elastomeric Valves in Multiplexed format can be used to construct sophisticated chips
- Dual sample chip with mixing functionality



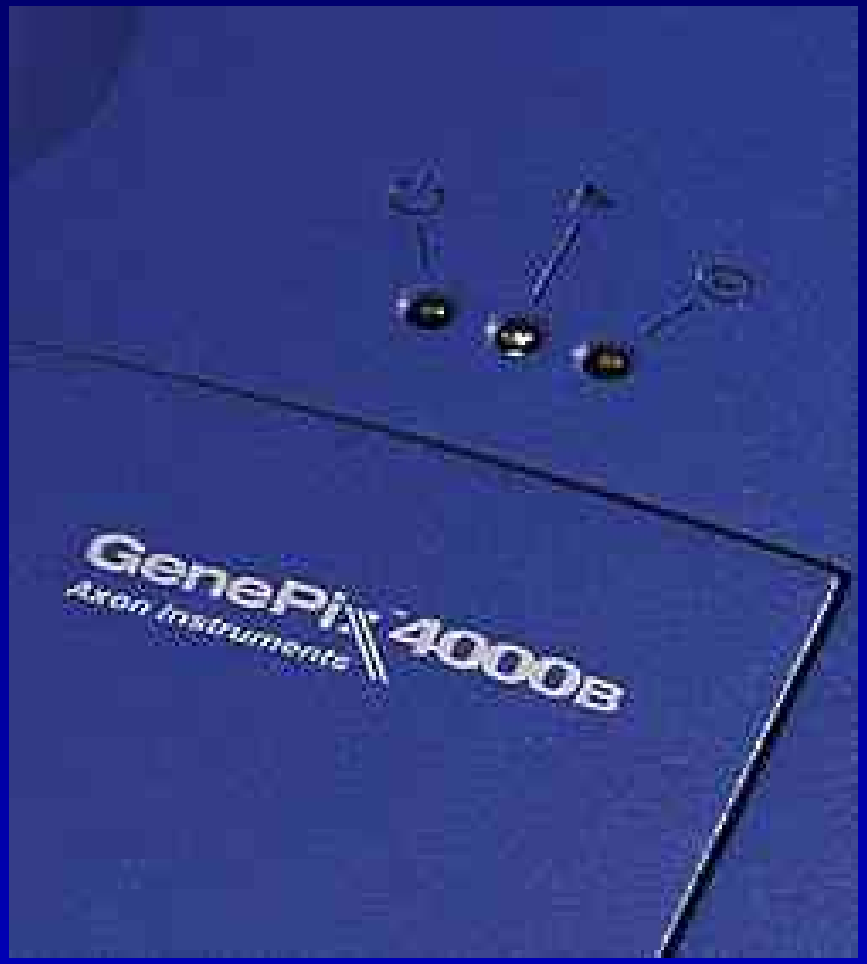
Mixing Mechanics

- Sequential chip addressing
  - Load
  - Compartmentalize
  - Mix
  - Recovery

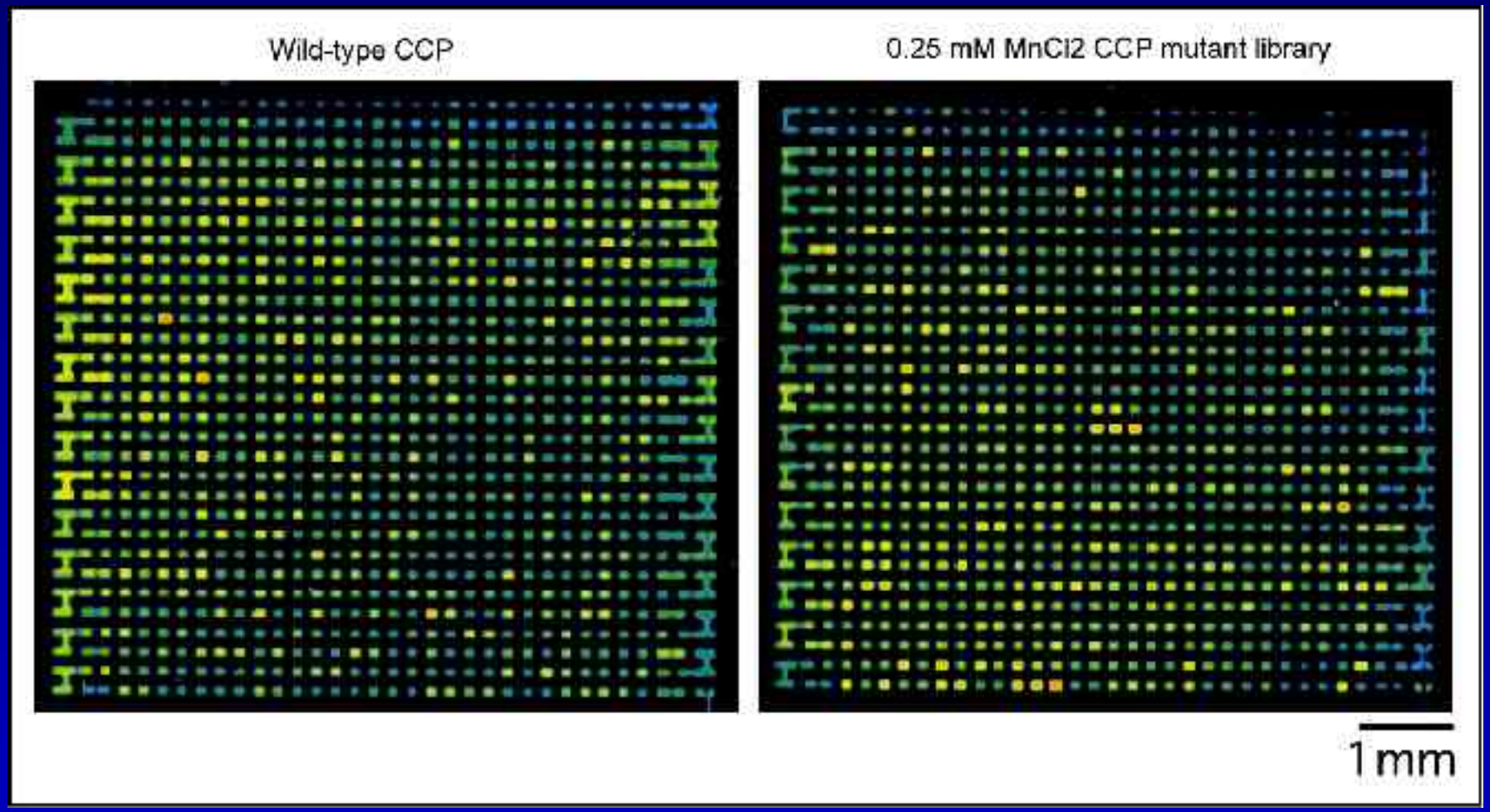


Axon Genechip Scanner

- Originally engineered for DNA arrays
- Dual wavelength diode laser scanner (523/635nm)
- 1” x 1” scan at 5 micron resolution in under 5 minutes
- No chip alignment necessary



Enzymatic library screening: Cytochrome c peroxidase in *E. coli*

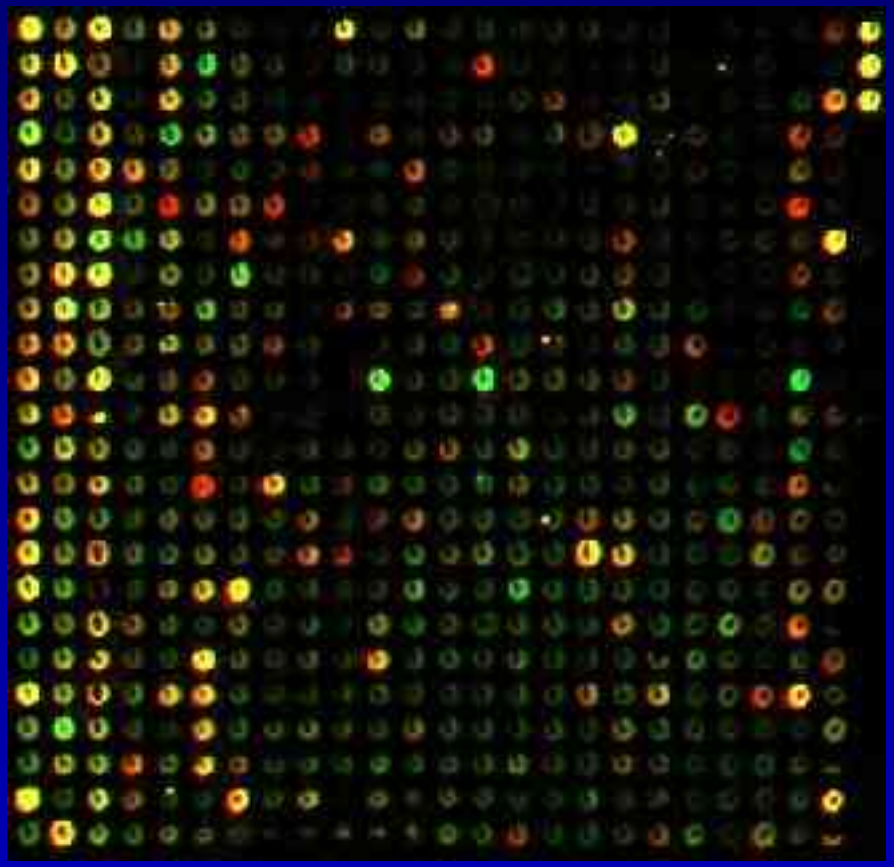


Microfluidic DNA Arrays

- Core Objectives
  - Low cost
  - Easy to use
  - Fast results
  - Sensitive
  - Flexible configuration

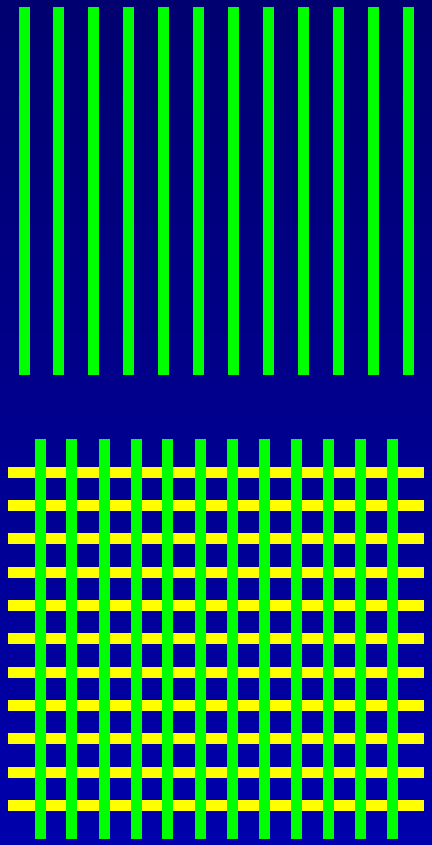
Existing DNA Microarray Technology

- **Expensive**
  - Requires high level of automation; robotic equipment
- **Low Sensitivity**
  - Probe has to find target DNA over entire chip surface



Microfluidic Strategy

- Pattern a glass slide with markers of interest in columns
- Expose individual samples in rows
- Observe hybridization at the intersections



10μm wide channels
(1/6 diameter of
Human hair)

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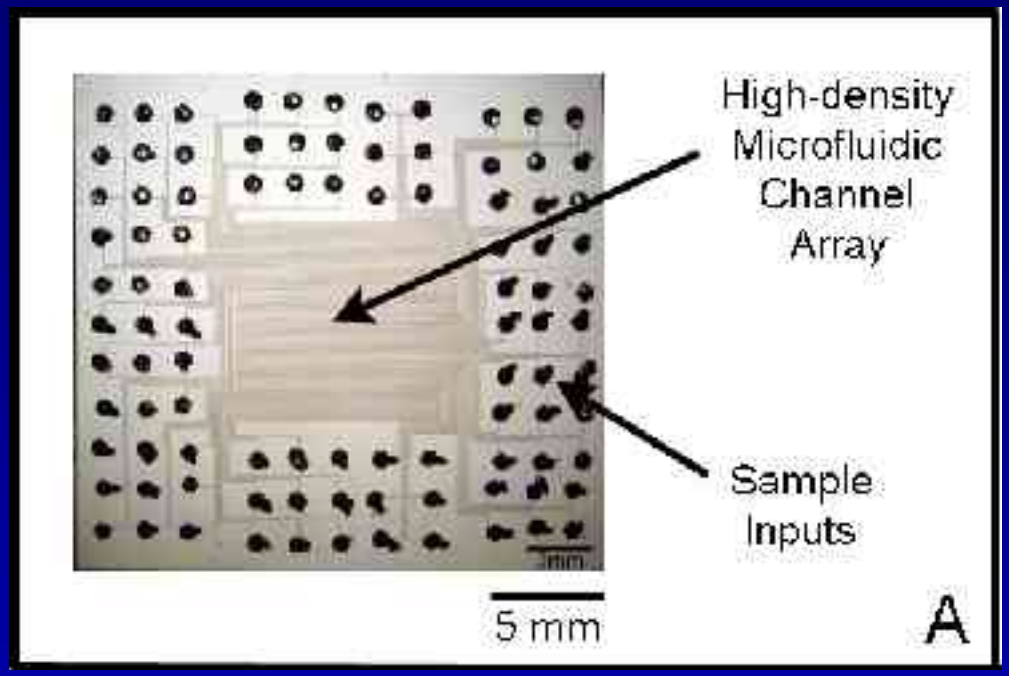
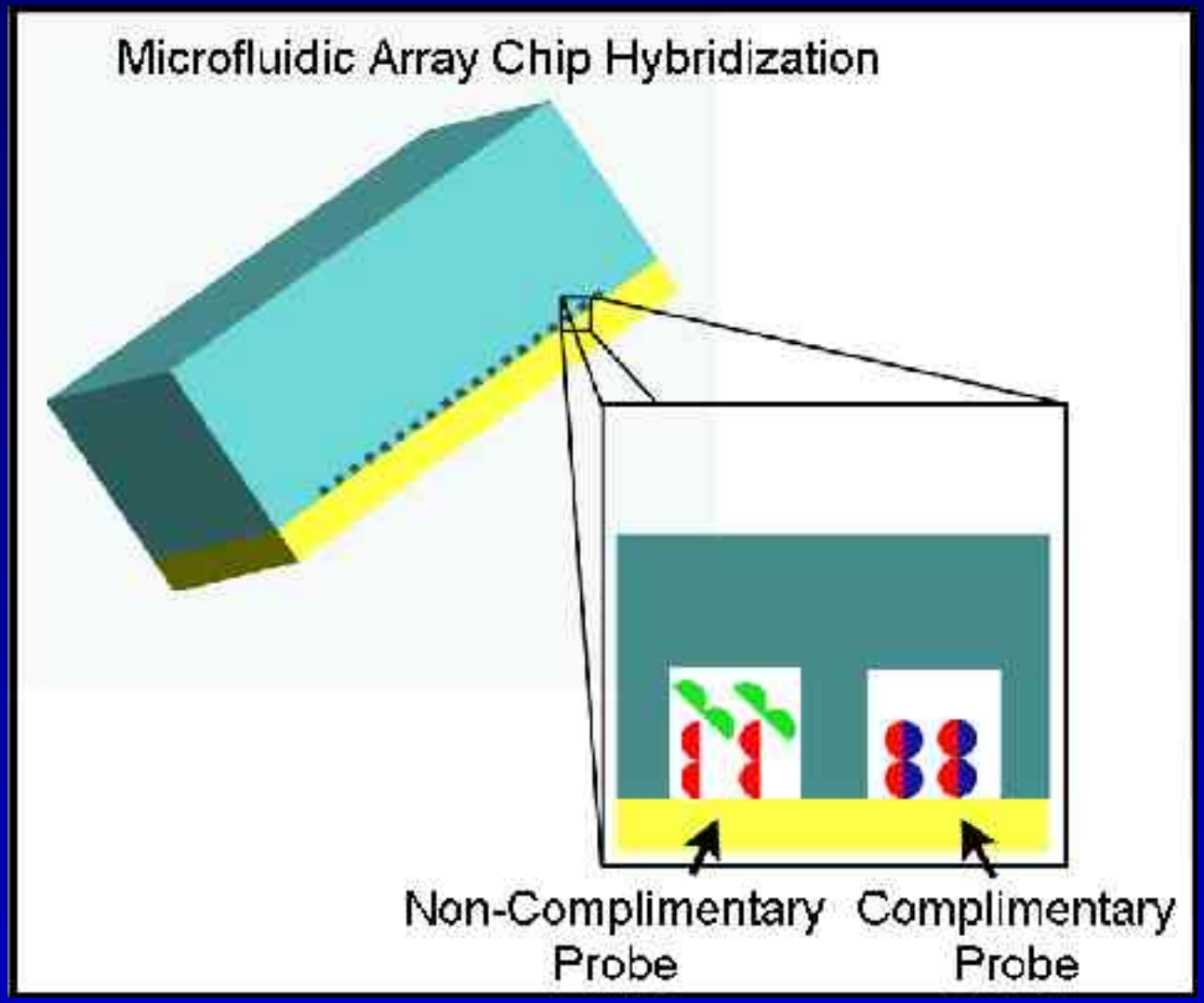
Deposition of Target DNA

- DNA introduced into microchannels sealed to glass slide
- Rapid DNA deposition on glass surface occurs in seconds vs. days using current technology

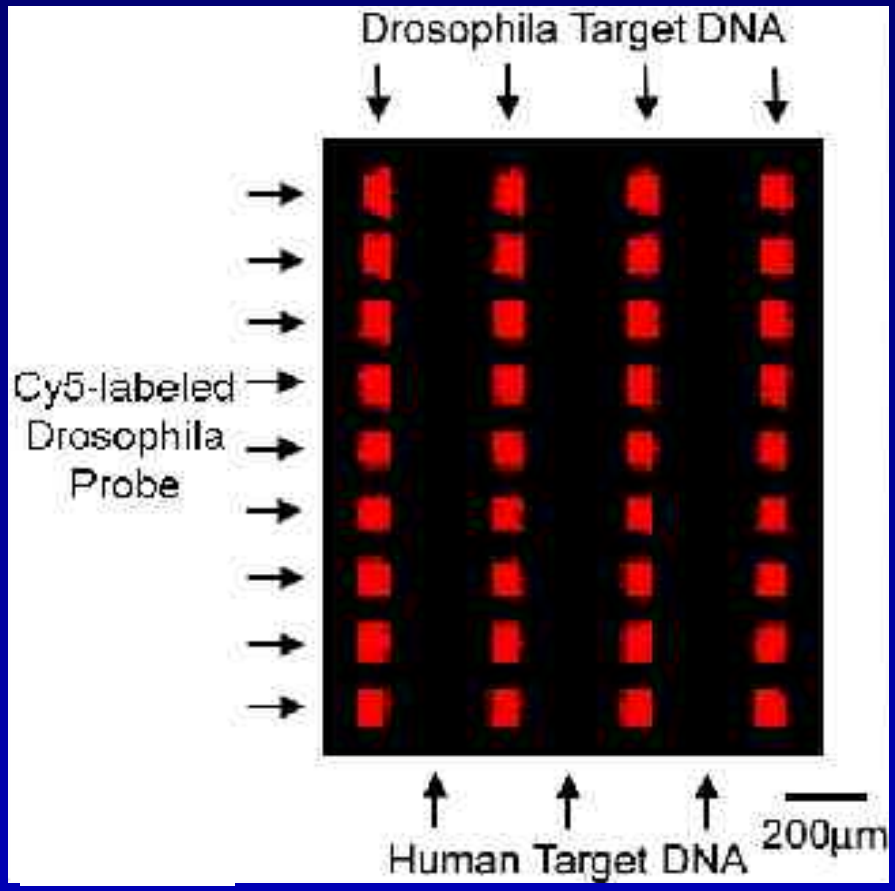


Microfluidic Array Hybridization



Microfluidic DNA Hybridization: Discrimination

- *Drosophila* (fruit fly) and human DNA targets
- Fluorescently-tagged *Drosophila* probe



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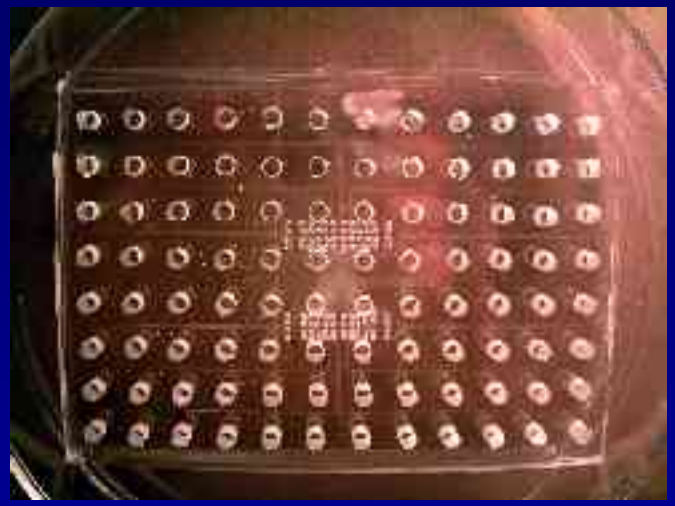
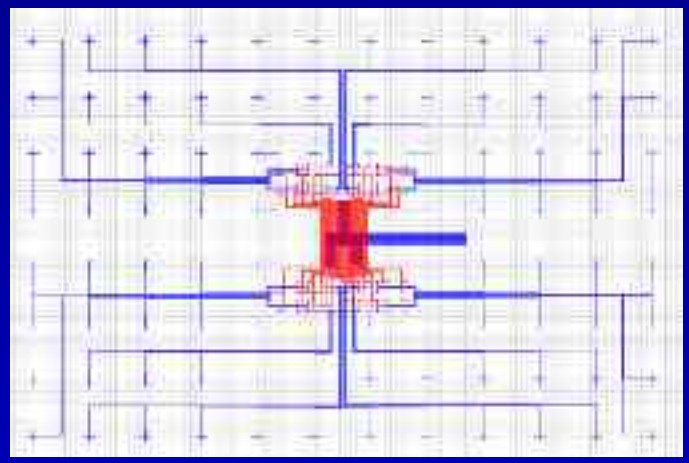
Microfluidic-Microtiter Array

- Standard 96-well microtiter plate (top)
- Microfluidic circuits print samples to slide
- One plate/ 10000 assays





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Macro-to-Micro Advantages

- Adapts to industry standard biological equipment (pipettors, microtiter plate loaders)
- Sample handling in the microliter range; easy for benchtop research in small and large laboratories

Future Opportunities

- High-throughput single-cell assays
- Low-cost genotyping
- Clinical diagnostics
  - Pathogen detection
  - Gene upregulation
- Chip integration (sensors/detectors)

Acknowledgements

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  - Dr. James Benn – Senior Visiting Scientist; Dept. of Mechanical Engineering
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