Fabricating Microfluidic Devices for High-Density Biological Assays

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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.

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
- Grey Layer: Fluid Layer
- Black Layer: Control Layer
- Single Row Sorting Junction
- Sorting Valves
- High Density Chamber Array
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

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)
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
Microfluidic-Microtiter Array

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