Microfluidic Analysis Systems

A. Scherer, H.P. Chou, M. Adams, F. Arnold M. Barbic S. Quake, Caltech

Microfluidic chips offer:

• High flexibility of integration
• Short analysis times
• Small analyte volumes

Supported by DARPA, NSF, and ARO
Microelectronic fabrication technology routinely makes far sub-wavelength structures (lithographic sizes <130nm)
Ant inspecting laser chip

Electron microscope image of laser chip inspected by a small black ant.

Next, we hope to use the laser chip to inspect the ant.
Replication molding of elastomer

1. Pour elastomer

3. Adhere to glass

Replication allows the fabrication of hermetically sealed flow channels for disposable microfluidic analysis systems.
RTV Replication

- Silicon Mold
- Pour Uncured Silicone Rubber
- Oven Bake
- Boil in HCl
- Trim Cured Rubber
- Finished Device
Advantages of Microfabricated Flow Systems

- Cheap
- Disposable
- No cross-contamination
- High sensitivity
- Can be integrated to define large and more functional systems
Distance between pneumatic and fluid channel (d) can be controlled during spinning.
On/Off Valves:

- Fluid channel: 100 µm
- Air control channel: 200 µm
- Flow medium: 1-µm beads.
Microvalves

- On/Off Valves:
- Switch Valves:

Switch Valve
100um x 100um x 10um
Peristaltic Pump

- Fluid In
- Fluid Out
- Air In/Out
- Vertical gap: 30 µm
Pumping Rate

![Graph showing the pumping rate as a function of frequency.]
Microfluidic Systems can Speed up Reactions

- Conventional DNA chips are *passive* devices, limited by diffusion
- Diffusion constant $D$ of 1-kbp DNA molecules is $10^{-7} \text{ cm}^2/\text{s}$
  - 0.4 mm in diameter in an hour
  - ~2 mm in diameter for a day

**Solution**: We use a micropump to *actively* move target DNA in the sample to pass all the hybridization probes several times
Multiple Disease Diagnosis
Chip Design

- Diagnostic photonic crystal cavities
- Fluid channel
  - a loop
- Switch valves
  - inputs
- On/Off valves
  - inlet/outlet
- Peristaltic pump
By using this rotary pump, it is possible to:

Flow fluids many times over sensor areas

Increase the speed of reactions

Mix reagents efficiently
Mixing of Fluorescent Beads
Surface Patterning

(a). biotin stripes;
(b). biotin/avidin checkboard pattern;
(c). 2-kbp DNA pattern on Surmodics slides.
Biotin/Avidin Model System

- **Diagnosis Pattern:** Biotin
- **Device:** MDD Chip
- **Target:** 1 µm Avidin-coated beads
- **Method:** Active Rotary Pumping

After 4-min pumping, most of beads were grabbed onto the biotin spots on the glass substrate.
Applications:

Disease diagnostic chips

High Throughput Screening

Cell Sorting
The serpentine valves can be closed, partitioning the chip into ~1000 different compartments. At the right of the figure the valve multiplexors are shown, which allow individual addressability of each column. The width of each channel is approximately 100 microns.

This chip has a main serpentine channel which can be filled with a mutant library, and then partitioned into 80 picoliter compartments. The compartments can be assayed, and then individual rows vented and recovered. This allows for ~30x enrichment per cycle on the chip.
Multiplexor Valves

Purge Buffer Input

Sample Fluid Input

Grey Layer: Fluid Layer

Black Layer: Control Layer

1000 compartments separated by valves
Reagents are mixed together

Products can be selectively removed

256 individually addressable chambers

Only 18 valve control lines connect this 1 inch chip to the outside world
Microfluidic cell manipulation

- Single chip functions can include
  - Cell culture and storage,
  - sorting,
  - purification, and
  - single cell lysis followed by
    - downstream molecular analysis
Cell Sorting Chip

valves

pump

Flow channel
Reversible Sorting

Measured with *E. Coli* cells expressing GFP

![Graph showing pump frequency vs. mm/sec and mean reversible time vs. frequency](image)

- Plot 1: Pump frequency vs. mm/sec
  - X-axis: Pump freq
  - Y-axis: mm/sec

- Plot 2: Mean reversible time vs. frequency
  - X-axis: Frequency (Hz)
  - Y-axis: Mean Reversible Time (msecs)
Multiple interrogation of the same cell at different times!!

10 Hz pump frequency

RAW DATA

75 Hz pump frequency

Future Plans

1. Accomplishing reversible sorting of cells $10^6$ in one hour
2. 30 times faster than the original electro-osmotic sorter
3. Study time course measurements of the same cell
4. Sorting mutant GFP libraries according to ratio of wavelengths

Waste

Collect

Diagram of cell sorting process.
<table>
<thead>
<tr>
<th>Device Number</th>
<th>Cells/mL</th>
<th>Time</th>
<th>Recovery</th>
<th>Enrichment</th>
<th>Cells sorted</th>
</tr>
</thead>
<tbody>
<tr>
<td>042800RTV</td>
<td>2.8x10^7</td>
<td>2 hours</td>
<td>48%</td>
<td>7</td>
<td>9,251</td>
</tr>
<tr>
<td>110300IIIIRT</td>
<td>26.3x10^7</td>
<td>2 hours</td>
<td>50%</td>
<td>35</td>
<td>79,200</td>
</tr>
<tr>
<td>110300IIIIRT</td>
<td>2.8x10^7</td>
<td>2 hours</td>
<td>21%</td>
<td>89</td>
<td>177,333</td>
</tr>
<tr>
<td>110300IRTV</td>
<td>9.7x10^7</td>
<td>3 hours</td>
<td>16%</td>
<td>43</td>
<td>40,922</td>
</tr>
<tr>
<td>110300IIISYL</td>
<td>2.6x10^7</td>
<td>2 hours</td>
<td>26%</td>
<td>13</td>
<td>15,600</td>
</tr>
<tr>
<td>110300IIISYL</td>
<td>1.3x10^8</td>
<td>3 hours</td>
<td>39%</td>
<td>83</td>
<td>479,381</td>
</tr>
</tbody>
</table>
Summary: Lab on a chip

- We have demonstrated pumping and valving of sub-picoliter fluid volumes.
- We have demonstrated rotary pumping and fast inline mixing within a ring-shaped fluid channel.
- Combining these capabilities, we can expect significant improvements in diagnostic rates and efficiency.
- We are now ready to construct monolithic fluidic devices with integrated optics and nanocavities.
Fluorescent DNA Sizing

- Stain DNA with fluorescent dyes
- The longer the DNA, the more dye molecules are attached => brighter emission.
Single Molecule Sizing System

- A Flow Cell
- Epi-fluorescent Optics System
- Computer Data Acquisition and Analysis
Key Technologies Behind SMS

- High Brightness Light Source
- Optical Filters
- Soft Lithography for Flow Channels
- Avalanche Photodiode Detectors (LA-APD)
- Very Bright DNA Intercalating Dye
  - Cyanine dyes: YOYO-1 and TOTO-1
Single Molecule Detection System

- Microfluidic chip
- PMT detector
- 532nm doubled YAG laser
- Dichroic mirror
- 12 inches
Typical Output Signal

\( \lambda \) DNA/ POPO-3/ Multi-PMT

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Signal (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>0.4</td>
</tr>
<tr>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>2.0</td>
<td>0.8</td>
</tr>
<tr>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>3.0</td>
<td>1.2</td>
</tr>
<tr>
<td>3.5</td>
<td>1.4</td>
</tr>
<tr>
<td>4.0</td>
<td>1.6</td>
</tr>
<tr>
<td>4.5</td>
<td>1.8</td>
</tr>
<tr>
<td>5.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Precision and Resolution

Linearity (Precision)
Lambda/HindIII

\[ R^2 = 0.9989 \]

Coefficients of Variance
Lambda/HindIII

C. V.
Integration of Detector Arrays for Cell and Molecule Sorting

Silicon substrate

Flowchannel

Electrical contacts

Detectors

Filters

460nm 31 layer Bragg filter

Absorbance (A.U.)
Absorption sensitivity

Bromophenol Blue Absorption

Concentration (uM)

Absorption %

Control group
CMOS Imager Data
Applications: Vis. Abs. Spectroscopy – Examples and Results-Orange G

Peak absorption wavelength for Orange G is 476nm.
Optical microcavities can be used to increase the effective pathlength of light
Sensitive detection is possible when using high Q microcavities constructed with high reflectivity mirrors
Lithographically defined microcavities can be used to form extremely small mode volumes
Small microcavity lasers have been developed as optical interconnect sources.
Spectroscopy of Picoliter Volumes: Integrated vertical cavity absorption spectrometers can decrease size, cost and sample volume required while significantly improving the sensitivity of the measurement.
Mirrors and active area are controlled by crystal growth.

Light emits perpendicular to the wafer surface.

Threshold currents as low as 10 µA have been reported.

VCSELs are presently used for fast optical interconnects.

J.L. Jewell, A. Scherer, S. McCall, J. Harbison
20,000 lasers can be fabricated into a 100x100 micrometer area.
Photonic Crystal membranes are constructed by lithography, ion etching, and chemical etching.
The defect cavity localizes light through total internal reflection at the air/slab interface and Bragg reflection from the 2D photonic crystal. The high-index slab ($n=3.4$) contains 4 QWs for gain, and is only 200 nm thick.
Room Temperature Lasing

Laser Spectrum of single defect

L-L response of PBG laser
Tuning of Photonic Crystal Lasers

- Wavelength tuning from 1500 - 1620 nm.
Design of a Tuneable High-Q Defect Cavity

The field intensity is maximum in the center of the 2-D cavity.

Such cavities can have maximum Q values of 30,000 (with linewidths of 0.05 nm at 1500 nm)
Examples of fabricated cavities
High Q Nanocavity Design

$p/d = 0.75$,  
$r/a = 0.275$,  
r defect $/a = 0.2$,  
$a = 20$,  
n = 3.4

$Q_{\text{max}} = 11000$
A high Q nanocavity is designed with a center hole for sensing molecules absorbing within a very narrow linewidth (Q ~ 10,000).

Photonic crystals may be used to concentrate light in small microcavities for optical spectroscopy.
Surface Plasmon Devices

- Small metal particles have been used to concentrate light to even smaller mode volumes and can be lithographically defined
Light localization vs. light extraction

Localization (guided and defect modes)
- nanocavities, waveguides

Extraction (leaky modes)
- LEDs
Photonic crystal structures for efficient light extraction

Motivation: build an efficient LED
- increase extraction efficiency
- increase spontaneous emission rate
- increase modulation speed
- improve directionality
- use electrical contact as light extractor

InGaAs QW
C-HH: 986nm
C-LH: 930nm
• **bad:** nonradiative
• **good:** large density of states (spontaneous emission enhancement)
• **bad:** penetration depth into semiconductor <20nm
Coupled surface plasmons

**ASP (TM_{-1})**

- Increased field intensity far from the metal surface
- Possible spont. em. enhancement of 4.5 times
- Still nonradiative

**SSP (TM_{0})**
**TE modes in metallic photonic crystals**

- **a = 250 nm**
- **|E|**
- **25 nm Ag**
- **90 nm GaAs**
- **200 nm Ag**
- **\( \eta_x \approx 33\% \)**
Fabrication procedure

1. Semiconductor membrane
   100nm AlAs
   GaAs substrate

2. Thick silver layer
   Semiconductor membrane
   100nm AlAs
   GaAs substrate

3. Thick silver layer
   Semiconductor membrane
   GaAs substrate

4. Semiconductor membrane
   Thick silver layer
   Silver coated Si substrate

5. Thin silver layer
   Semiconductor membrane
   Thick silver layer
   Silver coated Si substrate

6. Patterned PMMA layer
   Silver coated Si substrate

7. Patterned PMMA layer
   Thin silver layer
   Semiconductor membrane
   Thick silver layer
   Silver coated Si substrate

8. Thin patterned silver layer
   Semiconductor membrane
   Silver coated Si substrate
Measurement results

- 46x PL enhancement
- wafers with periodicities of 480nm and 650nm have spectra similar to unpatterned structure (c)
Colloidal Silver Metal Optics
Effects of Shape on Plasmon Resonance of Individual Colloidal Silver Nanoparticles
Ag half-cylinder on GaAs substrate

- \( r = 25 \text{nm} \)
- absorption losses in Ag included

B field intensity for the localized surface plasmon mode
Nanomagnets Tipped with Plasmon Resonators
Magnetic Micro-Manipulation
Electro-Magnetic Micro-Motor for Micro-Fluidics Applications

(a) Side View

XYZ Positioning Stage

Micro-Coils

Micro-Tips

3-Channel Current Driver

Positioning Stage

Magnetic Micro-Particle

(b) Top View

Phase relationship between currents in each micro-coil as a function of time.
Summary: Microfluidic Integration

⇒ Microfluidic pumps and valves can now be used to manipulate sub-picoliter volumes on a chip

⇒ Integration of this new ability with optical and electronic sensors allows the construction of new measurement tools

⇒ The concentration of light by dielectric nanocavities and metallic particles is a particularly interesting area of sensing