Lab-on-a-Chip Part 1 – Cell & Molecule Manipulation

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Cell & Molecule Manipulation

- **Methods to manipulate micro or nano scale objects:**
  - Optical
  - Acoustic
  - Electrical
  - Centrifugal force
  - Hydrodynamic
  - Magnetic
  - Surface modification

- **Examples**
  - Mechanical – barriers, porous membranes.
  - Impedance
  - Surface Acoustic Waves.
  - Dielectrophoresis.
  - Optical Tweezers and Scissors.
  - Electrowetting & Digital
  - Lab on a Disk – DNA, ELISA.
  - Surface Modification – plasma, CVD, laser, UV radiation, biofunctionalization, selective protein adsorption, and PEG/gold.
Mechanical

- A filter chamber fabricated by DRIE in a silicon substrate - 3 µm wide and 50 µm high pillars, and 2 µm spacing:

Permselective membranes for cell immunoisolation:
- High density uniform pores allow sufficient permeability to nutrients and hormones while preventing the passage of immunoglobulins.
- For example islet-cell transplantation.

Uniform pores can be micromachining in silicon.
Polyethylene terephthalate (PET) membranes may be machined with an excimer laser to produce pores as sieves.
Impedance Cell Sizing (Coulter principle)

- Cells passing through an aperture displace electrolyte and give rise to a change in impedance over the insulating wall.
- By giving the sensors a constant current, changes can be recorded by changes in voltage across the electrodes.
- Each cell crossing gives a pulse shaped response, the magnitude being related to the volume.
- Thousands of cells can be analyzed in a second.
When an appropriate frequency is applied to two electrodes present in a microfluidic channel, the impedance change in the detection volume can be used to distinguish between unbound beads and beads bound to pathogens.

Micrograph of chip in detection region. Channel depth/width of 19x40 micrometer.

Gain & Phase Characteristic

Top: impedance as a function of frequency
Bottom: phase as a function of frequency
(a) Electrical detection of cells in PBS buffer passing the detection volume. Top - raw measurement signal. Bottom - signal corrected for drift.
(b) Magnification of a measured impedance response for a passing yeast cell.
Left - Effective *diameter* extracted from measured impedance changes, with a normalized fit for populations smaller and larger than 1.5 μm.
Right - Impedance (blue) and optical signal (red) of beads and pathogens passing the detection volume.
Acoustic Forces

**Applications**
- Single cell isolation.
- Cell focusing and sorting.
- Cell washing and patterning.
- Cell–cell fusion and communication.
- Tissue engineering.

**Examples**
- Forces acting on cells and molecules in fluid suspension.
- Cell manipulation in 2D based on resonance, frequency change and nodes.
- Acoustic cell separator.

Rayleigh ("ray-lee") wave

Surface particles on the substrate move in elliptical paths having a similar surface-normal and surface-parallel component.
a, b) Moving a cell in 2D by altering the frequency in Hz scale. Since the frequency modulation is small in contrast to the resonance frequency, this moves the node position but keeps the resonance mode.
Effect of Altering Frequency…

c) For moving a trapped cell in a node in 2D pattern, the resonance frequency is shifted to the next resonance frequency which produces different nodal points. The trapped cells move to the adjacent nodes produced by a new frequency. (Note the LiNbO$_2$ substrate.)

a) Acoustic based-device for sorting the cells in different lines across the channel. By changing the nodal line, cells are sorted in different positions across the channel.

b) Pushing the fluid, carrying the cells toward the desired outlet by acoustic force.


Dielectrophoresis

- Motion of polarized (uncharged) particles in a nonuniform electric field.
- Dielectrophoretic forces depend on:
  - Charge of the particle (may be uncharged).
  - Geometry of the device.
  - Dielectric constant of the medium and particle.
  - Physiology of the particle.
- Uses:
  - Trapping, sorting, focusing, filtration, patterning, and assembly particles from 10nm to 100um.
  - Separating biological entities/particles suspended in a buffer medium.

Dielectrophoresis...

A. Uniform electric field
B. Nonuniform electric field.

A. Particle is more polarizable than the medium and it experiences net force toward the higher electric field ($E_{\text{high}}$) region. This process is known as pDEP.

B. Particle is less polarizable than the medium, and the net force on the particle acts toward the lower electric field ($E_{\text{low}}$) region. This type of particle motion is known as nDEP.

a) A mixture of cells is introduced through inlet A and buffer is introduced through inlet B. The bulk fluid flow velocity through these inlets is 200 m/s.

b) Computational domain for simulation-considering particles. This domain is considered from the shaded region in the actual device.

c) Translocation path of cells in actual domain.
Optical Tweezers and Scissors

- Laser tweezers:
Laser light of intensity \((z)\) varies across space \((r)\) as shown in the graph, and is reflected and refracted at the interface of the particle.

Photons have momentum and so their redirection by interacting with the particle results in a momentum transfer to the particle.

Light is strongest at a \((a)\) resulting in movement towards the beam axis. The net result is shown by the blue arrow.
Applications…

a) Direct writing system.
b) Coupling with hollow optical fiber to target surface.
c) 3D patterning of multiple cell types.
Young’s equation (after Thomas Young who first proposed it in 1805) describes the simple balance of force between the liquid-solid, liquid-vapor, and solid-vapor interfacial surface energies of a droplet on a solid surface:

$$\gamma_{LG} \cos \theta + \gamma_{SL} = \gamma_{SG},$$

where

- $\gamma_{LG}$ (gamma liquid-gas) is the liquid-gas interfacial tension,
- $\gamma_{SL}$ (gamma solid-liquid) is the solid-liquid interfacial tension,
- $\gamma_{SG}$ (gamma solid-gas) is the solid-gas interfacial tension, and
- $\theta$ (theta) is the contact angle.

Thomas Young lived from 1773 to 1829 and was an English scientist and researcher. Discovered interference of light.
Surface tension is a property of the liquid and is dependent on temperature and the other fluid it is in contact with.

At the interface between a liquid and a gas, or between two immiscible liquids, forces develop in the liquid surface that causes the surface to behave as if a “membrane” were stretched over it.

This phenomenon is due to unbalanced cohesive forces acting on the liquid molecules at the fluid interface.

Surface tension is the intensity of molecular attraction per unit length along any line in the surface.
Digital Manipulation…

Electrowetting and electrocapillary - an externally added electrostatic charge will modify the surface tension or capillary forces at the fluid-surface interface.

Digital microfluidic circuit for manipulating samples and reagents. Division, transport and merging.

The effect of a potential $V$ on the contact angle is then determined by the following:

$$\cos \theta(V) - \cos \theta_o = \frac{\varepsilon_r \varepsilon_0}{2 \gamma_{LG} t} V^2,$$

where
- $\theta$ (theta) is the contact angle,
- $\theta_o$ (theta-nought) is the equilibrium contact angle at $V = 0$,
- $V$ is the electric potential across the interface ($V$),
- $\varepsilon_r$ (epsilon) the dielectric constant of the dielectric layer,
- $\varepsilon_0$ (epsilon) is the permittivity of a vacuum ($8.85 \times 10^{-12}$ F/m),
- (where F = farad per m) and
- $t$ is its thickness (m).
A printed, paper-based active microfluidic chip actuates drops by electrowetting.

a) Partially open (blue and red drops) and closed (green drop) forms.
b) A modularly assembled platform.
c) An integrated portable electrical control system consisting of the chip platform, the driving system, and the mobile-based wireless control system.

This system was able to detect glucose, dopamine and uric acid with its electrochemical sensors.
Applications

- Clinical chemistry.
- Immunodiagnostics
- Protein analysis.
- Cell handling.
- Molecular diagnostics.
- Food, water, and soil analysis.

Features

- Pneumatic energy for switching or pumping “inward”
- Pre-storage and release of reagents.
- No external pumps.
- Volumes from nL to mL.
- Channels, chambers and sensor matrices.
- Bubble removal.
- Metering and mixing.
- Parallel operations.

Example:
Nucleic acid based detection of pathogenic microorganisms and the immunoassay based detection of toxins.

Process:
1. Storage of liquids and lyophilized reagents on the LabDisk and their time-controlled release.

2. Transfer of sample material by the use of antibody-coated microbeads.

3. Aliquoting of sample material for simultaneous analysis on one LabDisk.
Disposable test carrier for immunoassay: reagents are stored in aluminium pouches. If centrifugal force is applied, the pouches burst due to the increased hydrostatic pressure of the liquid.

Mark, D., et al. 2012. Automated and miniaturized detection of biological threats with a centrifugal microfluidic system. *Smart Biomedical and Physiological Sensor Technology* 1x 8367, 83670E.
Transfer of Magnetic Beads…

The transfer of magnetic beads is automated by rotating the disk over a magnet.

Mark, D., et al. 2012. Automated and miniaturized detection of biological threats with a centrifugal microfluidic system. *Smart Biomedical and Physiological Sensor Technology* 1x 8367, 83670E.
Nucleic Acid Analysis…

Process for the nucleic acid analysis.

After extraction and amplification the sample is divided into multiple chambers.

Mark, D., et al. 2012. Automated and miniaturized detection of biological threats with a centrifugal microfluidic system. *Smart Biomedical and Physiological Sensor Technology* lx 8367, 83670E.
Automated enzyme-linked immuno-sorbent assay (ELISA) system.

Starting with whole blood, this microbead based system can measure the concentrations of the antigen and the antibody of Hepatitis B virus (HBV), HBsAg and Anti-HBs.

Compared to conventional ELISA, time was reduced from 2 hrs. to 30 min.
### Table 1  Spin program

<table>
<thead>
<tr>
<th>Spin No.</th>
<th>Speed (rpm)</th>
<th>Time (sec.)</th>
<th>Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3600</td>
<td>180</td>
<td>Plasma separation</td>
</tr>
<tr>
<td>2</td>
<td>2400</td>
<td>20</td>
<td>Transfer plasma into mixing chamber</td>
</tr>
<tr>
<td>3</td>
<td>+250–250</td>
<td>600</td>
<td>Mix beads, plasma &amp; detection probe</td>
</tr>
<tr>
<td>4</td>
<td>2400</td>
<td>35</td>
<td>Remove residue to waste chamber</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>2</td>
<td>Close 1st waste channel</td>
</tr>
<tr>
<td>6</td>
<td>2400</td>
<td>20</td>
<td>Transfer 1st washing buffer (150 μL) to mixing chamber</td>
</tr>
<tr>
<td>7</td>
<td>+250–250</td>
<td>20</td>
<td>Mixing beads and washing buffer</td>
</tr>
<tr>
<td>8</td>
<td>2400</td>
<td>35</td>
<td>Remove 1st washed residue</td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>2</td>
<td>Close 2nd waste channel</td>
</tr>
<tr>
<td>10–17</td>
<td></td>
<td>170</td>
<td>Repeat 6–9 twice for 2nd and 3rd washing step (each 100 μL)</td>
</tr>
<tr>
<td>18</td>
<td>2400</td>
<td>20</td>
<td>Transfer TMB to mixing chamber</td>
</tr>
<tr>
<td>19</td>
<td>+250–250</td>
<td>600</td>
<td>Mixing beads and TMB</td>
</tr>
<tr>
<td>20</td>
<td>2400</td>
<td>20</td>
<td>Transfer to stopping solution chamber</td>
</tr>
<tr>
<td>21</td>
<td>+250–250</td>
<td>20</td>
<td>Mixing with stopping solution</td>
</tr>
<tr>
<td>22</td>
<td>2400</td>
<td>20</td>
<td>Transfer to detection chamber</td>
</tr>
<tr>
<td>23</td>
<td>—</td>
<td>20</td>
<td>Detection</td>
</tr>
</tbody>
</table>

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[Images A to F showing steps of the spin program]
Surface Modification - Polymers

Functions of Surface Layers…

- Change of contact angle.
- Provision of functional groups for surface.
- Lowering surface energy.
- Immobilization (of molecular capture probes).
- Suppression of non-specific absorption/biofouling.
- Establishment of barrier properties to prevent swelling/dissolution.
- Gas permeability.
- Tuning of optical and thermal properties, dirt protection.
- Scratch resistance.

Global Deposition…

- **Plasma Activation.** (Ar, Ne, He, H₂, NH₂, CO, CO₂, O₂, H₂O, N₂, NO₂ and F₂)
  - e.g. Oxygen gas induces the formation of *hydrophilic* groups on the surface. (Although transient, as they quickly revert back.)

- **Chemical Vapor Deposition.**
  - Solvent-free integration of thin films and nanostructures.

- **UV Irradiation**
  - Short-wavelength radiation in this range can be applied to the surface modification of fluorocarbon polymers.
  - In UV light at low wavelengths (180–190 nm) acidic groups are created on the polymer surfaces that are available for patterned protein binding and cell adhesion.

- **Sol-Gel**
  - Low reaction temperature
  - May provide improved bonding, barriers, corrosion protection.

- **Dynamic Coatings**
  - Surfactant solutions are pumped at a certain constant speed through the channel and physisorb to the channel surface. Eventual desorption from the surface.

Y-Mixer with Hydrophobic Coating...

Colored water showing wetting

Hydrophobically coated.

Ultra Short Laser Pulse Modification…

Hildenhagen, J. et al. 2013. Simultaneous Micro Structuring and Functionalization of Surfaces with Picosecond Laser. *International Conference on Optics in Precision Engineering and Nanotechnology (Icopen2013)* 8769, 87691D.
Obtained Contact Angle Modification...

Biofunctionalization...

Biofouling occurs as platelets, fibrinogen, IgG and albumin bind to sensors and other surfaces.

Foreign body giant cells (FBGC) may envelope surfaces in response to macrophages being drawn to areas of inflammation.

Poly(ethylene glycol) (PEG):
- A nontoxic, non-immunogenic and non-antigenic polymer may prevent these phenomena.
- Stable, non-fouling surfaces may be created by:
  - Chemical coupling reactions,
  - UV-induced graft polymerizations,
  - Self assembled monolayers (SAMs).
**PEG and Gold Surface Modification…**

Poly(ethylene glycol) (PEG)

Summary

- Methods to manipulate micro or nano scale objects:
  - Mechanical Barriers - barriers, porous membranes.
  - Impedance
  - Surface Acoustic Waves.
  - Dielectrophoresis.
  - Optical Tweezers and Scissors.
  - Electrowetting & Digital Manipulation
  - Lab on a Disk – DNA, ELSIA, Allergens.
  - Surface Modification – Plasma, CVD, Laser, UV radiation, Biofunctionalization, Selective Protein Adsorption, PEG/Gold.

- Appendix – LOC material comparison. Plasma, CVD, UV & sol-gel coating techniques.
## Table 3. Comparison of Typical Materials Used for the Fabrication of LOC Systems

<table>
<thead>
<tr>
<th>material</th>
<th>characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>silicon</td>
<td>rigid, high temperature resistance, ease of surface modifications (silanol), biocompatible, low unspecific binding, not gas-permeable, active fluidic system requires polymer hybrid device</td>
</tr>
<tr>
<td>ceramics</td>
<td>rigid, good mechanical, thermal and electrical properties</td>
</tr>
<tr>
<td>glass</td>
<td>rigid, transparent, ease of surface modification</td>
</tr>
<tr>
<td>poly(methyl methacrylate) (PMMA)</td>
<td>rigid, transparent, low water absorption</td>
</tr>
<tr>
<td>cyclic olefin copolymer (COC)</td>
<td>rigid, transparent, low water absorption</td>
</tr>
<tr>
<td>polystyrene (PS)</td>
<td>rigid, transparent</td>
</tr>
<tr>
<td>polycarbonate (PC)</td>
<td>rigid, transparent, highly heat resistant</td>
</tr>
<tr>
<td>polydimethylsiloxane (PDMS)</td>
<td>flexible, transparent, biocompatible, chemically inert, highly gas permeable</td>
</tr>
<tr>
<td>Whatman chromatography paper</td>
<td>rigid, pure cellulose, homogenous, reproducible, biocompatible</td>
</tr>
</tbody>
</table>

Fabrication Costs…

Table 4. Evaluation of Practicability of LOC Materials for Use in Biosensor-Related Applications

<table>
<thead>
<tr>
<th>applications</th>
<th>silicon/glass</th>
<th>elastomers</th>
<th>thermoplastics</th>
<th>paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>electrochemical detection</td>
<td>good</td>
<td>limited</td>
<td>moderate</td>
<td>moderate</td>
</tr>
<tr>
<td>cost of production</td>
<td>high</td>
<td>medium</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>reusability</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>disposable device use</td>
<td>expensive</td>
<td>good</td>
<td>good</td>
<td>good</td>
</tr>
<tr>
<td>online monitoring</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>
### Plasma and CVD Techniques

**Table 1. Characteristics of plasma treatment techniques in polymer microfluidics.**

<table>
<thead>
<tr>
<th>Research group</th>
<th>Polymeric substrate</th>
<th>Type of gas and contact angle</th>
<th>Stability of the coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsougeni et al [98]</td>
<td>PMMA, PEEK</td>
<td>O$_2$, ≤5°</td>
<td>20 days (PMMA), 60 days (PEEK)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C$_2$F$_6$, 153°</td>
<td>Months to years</td>
</tr>
<tr>
<td>Tsereni et al [99]</td>
<td>PDMS</td>
<td>O$_2$, ≤5° Teflon-like coating + O$_2$, ~140°</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Vourdas et al [100]</td>
<td>PMMA + Si-containing photoresist</td>
<td>O$_2$, ≤5°</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>He et al [101]</td>
<td>PMMA</td>
<td>O$_2$, CYTOP, 120°</td>
<td>Not evaluated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O$_2$, CYTOP-polyaniline, 170°</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Subramanian et al [102]</td>
<td>PMMA, COC, PC</td>
<td>O$_2$, heptadecafluoro-1,1,2,2-tetrahydrodecyl trichlorosilane (HFTCS), ≥145°</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Maheshwari et al [103]</td>
<td>PDMS</td>
<td>O$_2$, ~8°</td>
<td>85% recovery after 5 days</td>
</tr>
<tr>
<td>Roy and Yue [104]</td>
<td>COC</td>
<td>O$_2$, + bPEI, 32°</td>
<td>Stable for ~5 days</td>
</tr>
<tr>
<td>Wang et al [105]</td>
<td>PDMS</td>
<td>Air + APTES, 106° Air + APTES + mPEG, 64°</td>
<td>4 weeks</td>
</tr>
</tbody>
</table>

**Table 2. Characteristics of CVD techniques in polymer microfluidics.**

<table>
<thead>
<tr>
<th>Research group</th>
<th>Polymeric substrate</th>
<th>Deposited material</th>
<th>Stability of the coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen and Lahn [108]</td>
<td>PDMS</td>
<td>Poly-(4-benzoxy-1-p-xylene-co-p-xylene)</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Riche et al [109]</td>
<td>PDMS</td>
<td>Poly(PFDA-co-EGDA)</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Dudek et al [110]</td>
<td>COP</td>
<td>SiO$_2$</td>
<td>27 weeks</td>
</tr>
<tr>
<td>Gandhiraman et al [111, 112]</td>
<td>COP</td>
<td>APTES, APTES + EDA, DEGDME, MPTMS</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Eichler et al [113]</td>
<td>COC, PC</td>
<td>HMDSO, TMS, TEOS, SiH$_4$</td>
<td>20 days</td>
</tr>
<tr>
<td>Joshi et al [114]</td>
<td>SU-8</td>
<td>Amine groups due to the pyrolytic dissociation of ammonia</td>
<td>Not evaluated</td>
</tr>
</tbody>
</table>
### Table 3. Characteristics of UV irradiation techniques in polymer microfluidics.

<table>
<thead>
<tr>
<th>Research group</th>
<th>Polymeric substrate</th>
<th>Wavelength (nm)</th>
<th>Stability of the coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schütte et al [119]</td>
<td>COC</td>
<td>185</td>
<td>Acid groups’ density decrease to 25% within 19 weeks</td>
</tr>
<tr>
<td>Pfleging et al [120]</td>
<td>PS</td>
<td>Laser: 193 Mercury lamp: 185</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Nagai et al [121]</td>
<td>PDMS</td>
<td>248</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Hozumi et al [122, 123]</td>
<td>PMMA, PS</td>
<td>172</td>
<td>PMMA: not evaluated. PS: only 100 kPa remains stable after 30 days</td>
</tr>
</tbody>
</table>

### Table 4. Characteristics of sol–gel techniques in polymer microfluidics.

<table>
<thead>
<tr>
<th>Research group</th>
<th>Polymeric substrate</th>
<th>Deposited material</th>
<th>Stability of the coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al [126]</td>
<td>PDMS</td>
<td>Glass layer from TEOS</td>
<td>No available data</td>
</tr>
<tr>
<td>Abate et al [125]</td>
<td>PDMS</td>
<td>Glass layer from TEOS and MTES</td>
<td></td>
</tr>
<tr>
<td>Roman and Culbertson [127]</td>
<td>PDMS</td>
<td>Glass layer from isopropoxide, zirconium isopropoxide and vanadium trisobutoxide oxide</td>
<td></td>
</tr>
</tbody>
</table>