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:
Semipermeable Membranes…

- 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.

Impedance Detection of C. Albicans – van de Wouden…

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 unbound and bound beads and beads bound to pathogens.
### Impedance Detection of C. Albicans…

- (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.

### Effective Diameter from Impedance Changes…

- 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.

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By E.J. van der Wouden et al. / Procedia Engineering 25 (2011) 1465 – 1468
Surface Acoustic Waves – Gardner et. al.

Rayleigh ("ray-lee") wave

Surface particles on the substrate move in elliptical paths having a similar surface-normal and surface-parallel component.

Left) Gardner, J.W., VK Varadan and OO Awadelkarim, Microsensors, MEMS and Smart Devices edn. (Wiley 


Rayleigh (“ray-lee”) wave

Cell Manipulation in 2D & Resonance Frequency...

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.

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 LiNbO2 substrate.)

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 LiNbO2 substrate.)
Acoustic Cell Sorting – Ding et. al.

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.

Proposed Device – Jubery et. al.:

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 device.

Optical Tweezers and Scissors

- Laser tweezers:

Optical Forces on a Dielectric Particle...

Laser light of intensity \( I \) varies across space \( z \) 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 \( a \), 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.

**Electrowetting**

- **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.

**Surface tension** is a property of the liquid and depends 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.

**References:**
- Thomas Young lived from 1773 to 1829 and was an English scientist and researcher. Discovered interference of light.
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.

\[ \cos(\theta(V)) = \cos(\theta_0) + \frac{eLV}{D\varepsilon_0t^2} \]

\( \theta \) (theta) is the contact angle,
\( \theta_0 \) (theta-naught) is the equilibrium contact angle at \( V = 0 \),
\( V \) is the electric potential across the interface (V),
\( \varepsilon \) (epsilon) is the dielectric constant of the dielectric layer,
\( \varepsilon_0 \) (epsilon-naught) is the permittivity of a vacuum (8.85 \times 10^{-12} \text{ F/m})

(\text{where } F = \text{farad per m} \text{ and } t = \text{its thickness (m)})

A printed, paper-based active microfluidic chip actuates drops by electrowetting.

- Partially open (blue and red drops) and closed (green drop) forms.
- A modularly assembled platform.
- 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.
Centrifugal Microfluidics – CD Size Disks

- 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 reagent
  - No external pumps.
  - Volumes from nL to mL.
  - Channels, chambers and sensor matrices.
  - Bubble removal.
  - Metering and mixing.
  - Parallel operations.

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Lab-on-a-Disk: DNA – Mark et. al.

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.

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

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Carrier for Immunoassay...

Disposable test carrier for immunoassay: reagents are stored in aluminum pouches. If centrifugal force is applied, the pouches burst due to the increased hydrostatic pressure of the liquid.
Transfer of Magnetic Beads…

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

Nucleic Acid Analysis…

Process for the nucleic acid analysis. After extraction and amplification, the sample is divided into multiple chambers.

Lab-on-a-Disk: ELISA – Lee et al.…

Automated enzyme-linked immunosorbent 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.
ELISA...

Spin “Program” and Images

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, NH3, 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.
- UV Irradiation
  - Short-wavelength radiation in this range can be applied to the surface modification of fluorocarbon polymers.
- 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…


Obtained Contact Angle Modification…


Biofunctionalization…
Selective Protein Adsorption...

- **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...

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.
**LOC Materials…**

<table>
<thead>
<tr>
<th>Material</th>
<th>Aromatic</th>
<th>Aliphatic</th>
<th>Polymeric</th>
<th>Bioactive</th>
<th>Oligonucleotides</th>
<th>Other</th>
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<tbody>
<tr>
<td>Glass</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Polymer</td>
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<tr>
<td>Silicon</td>
<td>-</td>
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**Fabrication Costs…**

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Silicon</th>
<th>Glass</th>
<th>Polymer</th>
<th>Reduce Compatibility</th>
<th>Reagent Compatibility</th>
<th>Other</th>
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<tbody>
<tr>
<td>Electrochemical</td>
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<td>50%</td>
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<tr>
<td>Limiting Detection</td>
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<td>10%</td>
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<td>10%</td>
<td>-</td>
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<tr>
<td>Sensitivity</td>
<td>1000%</td>
<td>100%</td>
<td>0%</td>
<td>1000%</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Selectivity</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
<td>-</td>
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<tr>
<td>Customization</td>
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<td>50%</td>
<td>0%</td>
<td>100%</td>
<td>50%</td>
<td>-</td>
</tr>
</tbody>
</table>

**Plasma and CVD Techniques…**

**Table 5. Characters of Other Techniques in Synthetic Microfluidics**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Nanoparticle Synthesis</th>
<th>Siliconizing</th>
<th>Polyetherimide</th>
<th>Hybridization</th>
<th>Other</th>
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</thead>
<tbody>
<tr>
<td>CVD</td>
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<td>100%</td>
<td>0%</td>
<td>0%</td>
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<td>Plasma</td>
<td>0%</td>
<td>50%</td>
<td>0%</td>
<td>0%</td>
<td>-</td>
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<tr>
<td>Other Techniques</td>
<td>0%</td>
<td>10%</td>
<td>0%</td>
<td>0%</td>
<td>-</td>
</tr>
</tbody>
</table>
UV Irradiation and Sol-Gel…

Table 3. Characteristics of UV irradiation techniques in polymer microfluidics.

<table>
<thead>
<tr>
<th>Research group</th>
<th>Methodology</th>
<th>UV wavelength</th>
<th>Stability of coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schne et al. [10]</td>
<td>Coc</td>
<td>199</td>
<td>Acid group, dimethacrylates to PDMS bridge of heavier</td>
</tr>
<tr>
<td>Hinger et al. [18]</td>
<td>PVA</td>
<td>Laser UV curing</td>
<td>Not evaluated</td>
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<tr>
<td>Song et al. [17]</td>
<td>PEGDA</td>
<td>UV</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Busse et al. [13, 14]</td>
<td>PEGDA, PVA</td>
<td>UV, PVA</td>
<td>Not evaluated</td>
</tr>
</tbody>
</table>

Table 4. Characteristics of wet-etch techniques in polymer microfluidics.

<table>
<thead>
<tr>
<th>Research group</th>
<th>Printing method</th>
<th>Deposited material</th>
<th>Stability of coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tang et al. [12]</td>
<td>Inkjet</td>
<td>Glass frit from THM4</td>
<td>N/A</td>
</tr>
<tr>
<td>Hoda et al. [17]</td>
<td>Screen printing</td>
<td>Glass frit from THM4 and THM6</td>
<td>N/A</td>
</tr>
<tr>
<td>Brandt and Urbanov [11]</td>
<td>Inkjet</td>
<td>Glass frit from THM4 and THM6</td>
<td>N/A</td>
</tr>
</tbody>
</table>