Lecture 14: Electrokinetic-driven Microfluidics

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Electrokinetic Effects

- Electroosmosis
  - Electrokinetic flow is generated when electrodes are placed in the reservoirs at each end of a channel and activated to generate electrical current through the channel. Under these conditions, fluids of the appropriate type will move (be pumped) by a process known as “electro-osmosis.”

- Electrophoresis
  - Another electrokinetic phenomenon known as “electrophoresis” also occurs in the channels. This is the movement of charged molecules or particles in an electric field.
  - Electrophoresis can be used to move molecules in solution or to separate molecules with very subtle differences.
  - Electrophoresis and electro-osmosis generally occur at the same time in channels. However, there are proprietary techniques for minimizing either force while maintaining the other, as appropriate, for a given application.

- EP superimposed on EOF
  - Fluid transport by EO
  - Separation by EP

- Electrophoretic speed usually much smaller than EOF!
  - All ions (and neutrals) migrate to cathode
  - In some applications electroosmosis is suppressed by
    - Surface modification
    - Buffer solutions (pH)

Gel Electrophoresis

- A research technique used to separate molecules (or fragments of a molecule) according to size. Upon electrical stimulation, smaller fragments of a molecule will move faster through the gel than larger fragments. The process is typically done to separate DNA fragments after the DNA has been cut with restriction enzymes.

1. Restriction enzymes cleave DNA into smaller segments of various sizes.
2. DNA segments are loaded into wells in a porous gel. The gel floats in a buffer solution within a chamber between two electrodes.
3. When an electric current is passed through the chamber, DNA fragments move toward the positively-charged cathode.
4. Smaller DNA segments move faster and farther than larger DNA segments.

http://www.mrc.dtu.dk/Research/BKMS/MTFSD/research%20topics/EO-pumps.aspx,
http://karstenolaf.free.fr

http://www.stanford.edu/group/hopes/diagnsis/gentest/s7.html
### [Electrokinetic] Gel Electrophoresis

**Agarose Gel**
- A polysaccharide polymer material, generally extracted from seaweed.
- The molecules are extremely water-soluble due to their large number of hydroxyl groups, and the solutions tend to be low-melting point aqueous gels. Sheets of agarose gels are readily prepared by pouring the warm, liquid solution into a mold, and are frequently used in molecular biology for the separation of large molecules by electrophoresis. A wide range of different agaroses, of varying molecular weights and properties are commercially available for this purpose.

**Ethidium Bromide**
- An intercalating agent commonly used as a nucleic acid stain in molecular biology laboratories for techniques such as agarose gel electrophoresis.
- When exposed to ultraviolet light, it will fluoresce with a red-orange color, intensifying almost 20-fold after binding to DNA.

### [Electrokinetic] Capillary Electrophoresis

**Capillary Electrophoresis (CE)**
- A family of techniques used to separate a variety of compounds. These analyses, all driven by an electric field, are performed in narrow tubes and can result in the rapid separation of many hundreds of different compounds. The versatility and number of ways that CE can be used means that almost all molecules, and even whole organisms can be separated using the powerful methods.

**Advantages**
- has very high efficiencies, meaning hundreds of components can be separated at the same time
- requires minute amounts of sample
- is easily automated
- can be used quantitatively
- consumes limited amounts of reagents
- can separate almost all molecules (DNAs, proteins, ions, cells, ...)

### [Electrokinetic] Microchip CE

**Microfabricated Channels**
- Quartz
- Glass
- Polymers

**Benefits**
- Improved Data Quality and Accuracy
- Improved Sensitivity
- Reduced Reagent and Labor Cost
- High Speed due to the shorter separation length
- Faster Assay Development
- Expanded Individual Researcher Capability
- Improved Enterprise-Wide Productivity

### [Bioanalyzer] Case Study: Agilent 2100 Bioanalyzer

**Bioanalyzer 2100 (By Agilent)**
- Lab-on-a-chip microfluidics-based platform
- Solutions for the analysis of DNA, RNA, Proteins, Cells
- Within 30 minutes delivering automated, high quality digital data

**LabChip (By Caliper Lifesciences)**
- Microchannel electrophoresis
- Miniaturized fluid pathways ensure short run times
- Micro-fabricated chips yield better reproducibility than conventional technologies
- Microscale format minimizes sample and reagent consumption
[Bioanalyzer] On-chip Electrophoresis

1. The sample moves through the microchannels from the sample well
2. The sample is injected into the separation channel
3. Sample components are electrophoretically separated
4. Components are detected by their fluorescence and translated into gel-like images (bands) and electropherograms

http://www.agilent.com/chem/labonachip

[Bioanalyzer] Hardware

- Chip
- Electrode Cartridge for electrophoretic assays
- Pressure Cartridge for flow cytometric assays

[Bioanalyzer] Applications

- Electrophoretic Assays
  - DNA
  - Protein
- Flow Cytometric Assays
  - Apoptosis/Red to Blue
  - GFP (Green Fluorescence Protein)
  - RNA
  - Antibody Staining/Blue to Red
  - Generic/Checkout Beads, siRNA Transfection Viability

Digital data in 30 minutes
- Automated data analysis
- Digital data can be filed in a database or shared
- No user-dependent data interpretation

http://www.agilent.com/chem/labonachip
**[Bioanalyzer] Movie Demos**

- Demo 1: Microchip CE
- Demo 2: 2100 Bioanalyzer Set-up demo
  - Sample analysis made simple – handling and interpreting data in three quick and easy steps. This animation shows how the Agilent 2100 bioanalyzer integrates sample handling, separation, detection and data analysis all on one platform - it's as easy as 1, 2, 3.
- Demo 3: Typical Workflow Demo
  - One platform. One workflow. One example – The 4 steps of a typical gene expression workflow.
  - This animation including speaker text, portrays the role of an Agilent 2100 bioanalyzer in a typical gene expression workflow. It focuses on RNA isolation, gene expression analysis, protein expression and protein purification, and shows how the 2100 bioanalyzer is an obvious fit in each stage of the workflow.
- Demo 4: LabChip Nucleic acids assay run
  - The assay run shown here is the same for each DNA or RNA assay kit.
  - Each sample is moved sequentially from its well to the central separation microchannel. As the fragments move down the central separation channel, they separate by size, finally passing the detection point.
- Demo 5: Cell LabChip Data Evaluation
  - The animation shows how easy data evaluation with the Cell Fluorescence software is. The 3 types of cell events are displayed in histograms or dot-plots. The final result is achieved in only 3 simple steps. No complex fluorescence compensation is needed and marker setting for definition of cell populations can be done by anyone in the lab.

**[Bioanalyzer] LabChip vs Gel Electrophoresis**

Comparison of gel-like image using the Agilent 2100 bioanalyzer with an agarose gel-scan

**[Electrokinetic] Strengths / Challenges**

**Strengths**
- Pulse free pumping
- No moving parts
- No "dispersion" of EOF plug
- "band broadening" widely suppressed
- Usable for various applications
- High degree of miniaturization and automatization
  - parallelization / multiplexing
- Small dead volumes
- Small capillary diameter
  - Large surface-to-volume ratio
  - Reduced sample volume and volume of mobile phase
  - Improved dissipation of heat
  - Operation at higher voltages possible
  - Faster separations
  - Higher efficiency

**Challenges**
- Reduction of injection volumes …
- Need high performance detection technologies
- Accumulation of charge carrier
- Gradient in pH-value builds up (problem in long separation channels)
- Streaming current counteracts external electric field
- Long term actuation / long term stability
- Gas bubbles are generated due to electrolysis at the electrodes
- pH changes over time changes the operation conditions