Detection Elements

- Readout
  - Macroscopic fluorescence, diffraction, or interference
  - Optical bar-coding
    - Example: quantum dot-loaded microsphere capture agents
      - QDs show size-dependent luminescence
        - Narrow emission bands from a common excitation wavelength
        - Stable against photobleaching
      - Approach:
        - Load polymer microspheres with different amounts of several colors of QDs to obtain a unique fluorescence signature
          - 6 colors at 10 possible intensities allows for > $10^6$ possible ‘codes’
        - Capture molecule on surface of beads grabs labeled analyte

Figure 2. Fluorescence micrograph of a mixture of CdSe/ZnS QD-tagged beads emitting single-color signals at 494, 506, 547, 575, and 611 nm. The beads were spread and immobilized on a polylysine-coated glass slide, which caused a slight clustering effect. See Experimental Protocol for detailed conditions.

Figure 1. A. Schematic illustration of optical codes based on seven colors and methods for detecting those codes. B. Micrograph of laser-scanned fluorescence microsphere in which several laser beams are used to excite different microspheres and detect fluorescent microspheres using a quantitative spectrograph. (Han et al., 2001)
Excitation of bar-code and target fluorochrome by same wavelength

Microscope-based spectrophotometer for detection of emission spectra from individual beads

**Figure 5. Schematic illustration of DNA hybridization assays using QD-tagged beads.** Probe oligos (No. 1–4) were conjugated to the beads by cross-linking, and target oligos (No. 1–4) were detected with a blue fluorescent dye such as Cascade Blue. After hybridization, nonspecific molecules and excess reagents were removed by washing. For multiplexed assays, the oligo lengths and sequences were optimized so that all probes had similar melting temperatures ($T_m = 69–89^\circ C$) and hybridization kinetics (30 min). See legend in Figure 6 for the sequences used.

- **Optical absorption (colorimetric)**
  - what

- **Surface plasmon resonance and SPR arrays**
  - Developed commercially later 1980’s (Cooper 2002)
  - Typically, receptor is immobilized and free ligand is passed over sensor chip
    - Both ways possible, small ligands simply interfere with binding if immobilized

---

**Lecture 19 – Biosensors**

**Figure 2.** Typical set-up for an SPR biosensor. Surface plasmon resonance (SPR) detects changes in the refractive index in the immediate vicinity of the immobilized layer on the sensor chip. SPR is observed as a sharp shadow in the reflected light from the surface at an angle that is dependent on the mass of material at the surface. The SPR angle shifts from 10° (in the lower left-hand diagram) when biomolecules bind to the surface and change the mass of the surface layer. This change in resonant angle can be monitored non-invasively in real-time as a plot of resonance signal (proportional to mass change) versus time.

**Box 2.** | **Coupling methods for receptor immobilization: non-covalent attachment**

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
</table>
| Biotin–streptavidin-conjugation methods | These can be used to capture biotinylated receptors (as in). The multiplexed binding of streptavidin on each face of the sensor allows immobilized ligands to be studied in the same field of view. This method is highly efficient and results in stable complexes, but in effect, irreversible. It is commonly used to immobilize E. coli membrane and oligonucleotides.
| Monoclonal antibodies | These can be immobilized via a solid support by means of either coupling in OAS or EDC. Epoxy-tagged surface proteins can then be directly and irreversibly coupled to the sensor microtrenches through the antibody-epitope interaction. (Cooper 2002). Commonly used antigens, for example, include protein, monoclonal antibodies, and Fab fragments.
| Metal-coordinating groups | Conjugates such as amine-reactive (EDAC) and carboxyl reactive (S-NHS) have been widely used for direct immobilization of C+H, and 99% His-tagged receptors. (Cooper 2002). The amine affinity of the Glutamine-NP-derivatized sensor interaction means that there is sometimes considerable loss in the level of immobilized receptors. For this reason, anti-His monoclonal antibodies are often used in stable, covalently immobilized conjugates of the target receptor.

(Cooper 2002)
Lecture 19 – Biosensors

Biochip sensor chips

**Figure 1** Coupling methods for receptor immobilization: covalent attachment

- Immobilization is a key step in the sensor surface. It is of particular importance to the design of functional sensor arrays.

- The coupling method must be efficient, provide a stable attachment between the immobilized receptor and the sensor surface.

- For oriented immobilization, for example, the sensors can be functionalized with surface plasmon resonance (SPR) functional groups to react with the capture molecule.

- Various examples of covalent immobilization methods are shown in the figure.

- **a**: Coupling via oxime formation.
- **b**: Coupling via isothiocyanate (NHS-activated amine).
- **c**: Coupling via thiol-disulfide exchange.

- **Note**: These coupling procedures are typically used in combination with biotinylated molecules to allow for immobilization of proteins or enzymes on the sensor surface.

- **Ankylosing spondylitis (AS)** can be treated with commercially available functional linking reagents to effectively immobilize the sensor.

- Surface passivation with silica can be used to produce a monolayer of immobilized reagent.

- **OTC**: Oligo-thymidine ODT, Oligo-Cy3, Cy5, and Cy5-diacetate are labels for nucleic acids. Cy3, Cy5, and Cy5-diacetate are suitable for visualization of DNA. Oligo-thymidine acid (ODT) is a useful linker for immobilizing DNA or proteins, while Cy3 and Cy5-diacetate provide fluorescence labels for detection of specific DNA or protein interactions.
Advantages/disadvantages
- **Pros**
  - Fast measurements
  - Sensitive
- **Cons**
  - Cannot perform detection on turbid solutions

Electrochemical

**Electrochemical readouts**
- Conductometric
  - Measure changes in the conductance of the biological component arising between a pair of metal electrodes due to e.g. metabolism
- Potentiometric
  - Measure electrical potential difference between a sample and reference electrode
  - Monitor the accumulation of charge at zero current created by selective binding at the electrode surface

Lecture 19 – Biosensors
Electrode may be selective for certain ions or gases
- E.g. F\textsuperscript{-}, I\textsuperscript{-}, CN\textsuperscript{-}, Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, H\textsuperscript{+}, NH\textsubscript{4}\textsuperscript{+}
- CO\textsubscript{2}, NH\textsubscript{3}

Amperometric
- Measure current generated by electrochemical oxidation or reduction of electroactive species at a constant applied potential

Electrochemical detection:

<table>
<thead>
<tr>
<th>Electroactive species</th>
<th>ISFET - ion-sensitive field-effect transistors</th>
</tr>
</thead>
<tbody>
<tr>
<td>K\textsuperscript{+}</td>
<td>Polymeric membrane/polymer glass</td>
</tr>
<tr>
<td>Na\textsuperscript{+}</td>
<td>Polymeric membrane/polymer glass</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+}</td>
<td>Polymeric membrane/polymer glass</td>
</tr>
<tr>
<td>NH\textsubscript{4}\textsuperscript{+}</td>
<td>Polymeric membrane/polymer glass</td>
</tr>
<tr>
<td>CO\textsubscript{2}</td>
<td>Polymeric membrane/polymer glass</td>
</tr>
<tr>
<td>Cl\textsuperscript{-}</td>
<td>Polymeric membrane/polymer glass</td>
</tr>
<tr>
<td>NH\textsubscript{3}</td>
<td>Polymeric membrane/polymer glass</td>
</tr>
<tr>
<td>NO\textsubscript{2}</td>
<td>Polymeric membrane/polymer glass</td>
</tr>
<tr>
<td>NO\textsubscript{3}</td>
<td>Polymeric membrane/polymer glass</td>
</tr>
</tbody>
</table>

Common pH-modifying enzymatic reactions:

- Glucose oxidase: D-glucose + O\textsubscript{2} $\rightarrow$ D-glucose-1,5-lactone + H\textsubscript{2}O + H\textsuperscript{+} \[6.3\]
- Penicillinase: penicillin $\rightarrow$ penicillic acid + H\textsuperscript{+} \[6.4\]
- Urease (pH 6.0): H\textsubscript{2}NCONH\textsubscript{2} + H\textsubscript{2}O + 2H\textsuperscript{+} $\rightarrow$ 2NH\textsuperscript{4}\textsuperscript{+} + CO\textsubscript{2} \[6.5\]
- Urease (pH 9.5): H\textsubscript{2}NCONH\textsubscript{2} + 2H\textsuperscript{+} $\rightarrow$ 2NH\textsubscript{3} + HCO\textsubscript{3}\textsuperscript{-} + H\textsuperscript{+} \[6.6\]
- Lipase: neutral lipids + H\textsubscript{2}O $\rightarrow$ glycerol + fatty acids + H\textsuperscript{+} \[6.7\]

Advantages/disadvantages

- Pros
  - Fast measurements
  - Sensitive
    - Low detection limits typically ~ 10\textsuperscript{-9} M
  - Ability to perform measurements on turbid/opaque solutions

- Cons
  - PH-sensing mechanisms require weakly buffered or non-buffered solutions

Calorimetric readouts

- Measurement of heat generated by an enzymatic reaction
  - Typically utilize thermistors to transform heat into an electrical signal

Lecture 19 – Biosensors
Calorimetric detection:

Schematic diagram of a calorimetric biosensor. The sample stream (a) passes through the outer insulated box (b) to the heat exchanger (c) within an aluminium block (d). From there, it flows past the reference thermistor (e) and into the packed bed bioreactor (f, 1ml volume), containing the biocatalyst, where the reaction occurs. The change in temperature is determined by the thermistor (g) and the solution passed to waste (h). External electronics (l) determines the difference in the resistance, and hence temperature, between the thermistors.

http://www.sbu.ac.uk/biology/enztech/calorimetric.html

Lecture 19 – Biosensors
Piezoelectric \textsuperscript{8,9}

- Based on quartz crystal microbalance detection
  - Crystal is oscillated at a defined frequency by an oscillating applied voltage
    - Shear deformation induced as dipoles in crystal seek to align with direction of electric field
    - Deformation typically 10-100 nm for AT-cut crystals operating in freq. range of 1-10 MHz
  - Binding of analyte to surface changes mass of crystal and alter oscillation frequency

- Figure below from: [www-bond.chem.monash.edu.au/theses/ Graeme%20Snook/Chapter1.pdf](http://www-bond.chem.monash.edu.au/theses/ Graeme%20Snook/Chapter1.pdf)

Piezoelectric detection:
Quartz crystal microbalance

[Diagram]

http://www.q-sense.com/main.qcmd_tech.html
Piezoelectric detection:  
Quartz crystal microbalance

Detecting HIV virions:

Figure 3. Signal linearity with particle numbers. (A) Serial 10-fold dilutions of HIV1 gD in PBS. A sample volume of 1 ml (O) or 40 µl (D) was incubated for 40 min at room temperature on a QCM disk coated with anti-gD IgG mAb. The dashed line represents the noise floor. (B) Representative REVS spectrum corresponding to dissociation of a single virion in PBS from an anti-gD IgG mAb-coated QCM disk. The peak can be seen at -7.4 V.

(Cooper et al. 2001)
SPR Arrays

- External analysis/detection
- Optical method

Cell- and tissue-based biosensors (Stenger 2001, Gross 1997)

General concepts

- Why cell-based biosensors?
  - Known ultrasensitivity of cells:
    - Olfactory neurons respond to single odorant molecules
    - Retinal neurons triggered by single photons
    - T cells triggered by single antigenic peptides (Irvine 2002)
  - Ability to ‘integrate’ cellular or tissue response to compounds
    - Detect functionality of compound in addition to its chemical presence
      - i.e. tell the difference between a dead and live virus

- Cell-based biosensors are based on a primary transducer (the cell) and secondary transducer (device which converts cellular/biochemical response into a detectable signal)
  - Secondary transducer may be electrical or optical
- Detection of arbitrary targets
  - Transfect cells with receptors to introduce responsiveness of e.g. neuronal cells to a chosen compound
- Basis of electrical secondary transducers
  - Example cell types
    - Neurons

Lecture 19 – Biosensors
Non-sensory neurons grown in culture outside of normal homeostasis and the insulation of the blood-brain barrier behave in a ‘sensory’ manner (Gross 1997)

- Cardiomyocytes
  - Generate electric signals in a substance-specific and concentration-dependent manner
  - Signals generated can be monitored by microelectrodes

Microphysiometer\(^1\)\(^,\)\(^2\)

- Measures changes in extracellular acidification rate: pH changes associated with alterations in ATP consumption by cells (metabolism)
- Extremely sensitive readout of changes in cell metabolism
- EXAMPLE OF HARDING MCCONNELL’S WORK WITH T CELLS

Relative advantages and disadvantages of cell-based sensors

- Pros
  - Cell-based sensors may utilize the ability of cells to respond to complex mixtures of signals in a unique way
  - May provide alternatives to animal testing in the future
- Cons
  - Issues of maintaining cell viability and reproducibility in measurements

Patterning cells for sensing\(^3\)

- Techniques used:
  - Photolithography
  - Microcontact printing (soft lithography)
  - Microfluidic patterning
  - Membrane lift-off

Lecture 19 – Biosensors
**soft lithography and self-assembled monolayers**

- Techniques based on the formation of gold (or other metal)-thiol bonds and spontaneous assembly of close-packed alkyl chain structures on a surface

**Tissue analogs**

- Any papers out on the liver chip?  GRIFFITH LAB

**In vitro toxicity studies: tissue biosensors**

- Shown below is a model of the pharmacology of naphthalene
  - Tissue distribution and toxic chemistry outlined is a multi-organ, multi-compartment phenomenon
  - Potential methodology: Animal-on-a-chip
    - 2 cm x 2 cm Si chip
    - designed to have ratio of organ compartment size and liquid residence times physiologically realistic
    - minimum 10K cells per compartment to facilitate analysis of chemicals and enzyme activity
    - physiologic hydrodynamic shear stress values
Lecture 19 – Biosensors

Figure 1. Reaction scheme for naphthalene and its products.

(Quick and Shuler 1999)

Figure 4. (a) Microscopic CCA system with four chambers. The dimensions (w x l x d) of the chambers are: lung 2 mm x 2 mm x 20 μm; liver 3.5 mm x 4.6 mm x 20 μm; other tissue 0.4 mm x 109 mm x 100 μm; fat 0.42 mm x 50.6 mm x 100 μm. Cells are cultured as monolayers on the silicon surfaces modified by adsorption of polylysine and collagen (b).

(Park and Shuler 2003)
In vivo detection

- Biofouling typically limits lifetime of in vivo measurements to 1-2 days
  - Inflammation
  - Fibrosis
  - Loss of vasculature

References