Market, Unmet Needs

- Worldwide *In-vitro diagnostics* ~$ 50 Billion and growing
- Nucleic Acid diagnostics ~$9 Billion
- Health, Security, Pathogen Detection, etc.
- Microbial testing
- Genotyping
- Viral load assays
- Blood screening

H. Siitari, Nucleic Acid Diagnostics Market
Dynamics
(1ns at 300 K)
Older Technologies

- Amplification is critical (PCR & alternatives, LCR, bDNA)

**Fig. 13.10** The black area indicates the section that is of interest in the original DNA (A). PCR begins by heating the DNA to 95°C to break the weak hydrogen bonds (vertical lines) that hold the complementary strands together. When the solution is cooled to 50°C, primers in the solution attach to the single strands of DNA and form hybrid strands (B). Beginning with the primers, the remaining portions of the DNA strands are replicated in the presence of DNA polymerase and free nucleotides to produce two new strands of DNA (C). The cycle of denaturation, hybridization, and replication is repeated many times (D–F) to produce additional copies of the region of interest. The arrows in B, D, and F indicate the direction in which replication occurs. The number of copies of the region of interest doubled between A and C (2 to 4) and again between C and E (4 to 8). There will be 16 copies after F is completed.
DNA-based Sensors

- DNA probe sequence (surface bound or not)
- Recognition of complementary nucleic acid sequence (analyte or target)
- Binding event converted into a signal (optical or electronic)
- Microfluidics to route samples, PCR, etc. (Lab-on-a-chip)

- Molecules: DNA & mRNA
- mRNA concentration indicates metabolic activity of a given gene (gene expression monitoring)
- DNA more stable than RNA, used more often
- All use hybridization (A:T [2], G:C [3])
- “Melting” temperature is broad for small sequences, also depends on sequence, ionic strength.

DNA-based Sensors

- Nearly all DNA sensors use immobilized nucleic acids
- ssDNA (8-70 bases) for genetic analysis
- dsDNA for protein & small-molecule recognition
- Measure change in mass, optical, electronic, electrochemical property
- NOT best for de-novo sequencing of DNA

Figure 1: General DNA biosensor design. Target DNA is captured at the recognition layer, and the resulting hybridization signal is transduced into a usable electronic signal for display and analysis. In the case of electronic and electrochemical biosensors, signal transduction is greatly simplified, because the incoming signal is already electronic in origin.
DNA arrays (Gene chips – Affymetrix)

- Each chip has multiple probes for the same gene or same fragment.
- Photolithographic technique for array fabrication
- Vast arrays + laser confocal scanning to measure
- Gene expression
  - Regulatory pathways
  - Confirm mechanism of action
  - Validate drug targets
  - Diagnostic procedures
- Genomic dna analysis
  - Whole or subset (SNP’s)

Information from Affymetrix
The lithographic mask set is used to represent the information on the array, and automated array tests verify the design by imaging the array in silico.

In design validation, the mask manufactured and aligned with a 1-inch-square quartz wafer. The mask set's patterns correspond to desired sequence of each probe and block or transmit light to wafer.

This synthesis is activated only at those where light is transmitted precisely and consistent feature width.

- When ultraviolet light shines through the mask, the 5'-ends of the oligonucleotides in the exposed areas of the substrate become deprotected in preparation for the chemical coupling with the nucleoside phosphoramidite monomer.

- Once the desired features have been activated, a solution containing a deoxynucleoside phosphoramidite monomer with a light-sensitive protecting group is flushed over the wafer's surface.

- In the next synthesis step, a different mask is placed over the wafer for another round of oligonucleotide deprotection and monomer coupling.
More on DNA microarrays

- Light-directed synthesis: Photolithography + solid-phase DNA synthesis
- ~ $10^6$ probes per chip (~ 1 sq. cm)
- Fluorescent tagged probe
- Illuminate through the glass

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**Table 3 • Array capacity and feature size**

<table>
<thead>
<tr>
<th>Feature Size</th>
<th>Expression</th>
<th>Sequence Analysis</th>
<th>Genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 μm</td>
<td>1600–6400 genes</td>
<td>8–16 kb</td>
<td>2000–4000 markers</td>
</tr>
<tr>
<td>20 μm</td>
<td>10,000–50,000 genes</td>
<td>50–100 kb</td>
<td>12,000–25,000 markers</td>
</tr>
<tr>
<td>2 μm</td>
<td>&gt;1 million genes</td>
<td>500–1,000 kb</td>
<td>1.2–2.5×10^6 markers</td>
</tr>
</tbody>
</table>

All numbers calculated for 1.28×1.28 cm arrays. aAssuming 4–20 probe pairs per gene. bAssuming 4–8 probes per basepair. cAssuming 6–32 probes per marker.

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DNA-based Sensors – components

- Probe synthesis
  - Pre-synthesized nucleic acid probes
  - Automated solid-phase DNA synthesizers (up to ~150 bases)
  - Labeling, end groups, etc. can be incorporated

- Immobilization of DNA probes
  - Relatively high coverage
  - Accessibility for hybridization
  - Covalent end-tethering is the best
  - Limit non-specific attachment of target
  - Substrate can be Au, SiO₂, Si (depends on transduction)
    - SiO₂: silanize glass, link end-derivatized DNA probe to organosilane monolayer
    - S-Au for gold, or direct linkage of S-DNA to Au (second alkanethiol needed to backfill)
  - 3D porous scaffolds, polymeric, silica gel

- Probe length (15-40) and coverage matters (10^{12} vs 10^{14} for regular SAMs)
Figure 5 Electrochemical assay for mismatches through DNA-mediated charge transport. On the right is shown an electrode modified with well-matched duplex DNA. Current flows through the well-stacked DNA to reduce methylene blue (MB⁺) intercalated near the top of the film, to leucomethylene blue (LB). LB goes on to reduce ferricyanide in solution, thereby regenerating MB⁺ catalytically, leading to an amplification of the hybridization signal. In the case of a DNA film containing mismatched duplexes (left), current flow through the DNA duplex is attenuated, MB⁺ is not reduced, and the catalytic signal is lost.
**Electrochemical DNA Sensors**

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redox</td>
<td>Moderate to high sensitivity (femtomoles of target); well suited to multiple-target detection; samples remain unaltered</td>
<td>Chemical labeling step required; method used; sequence variation</td>
</tr>
<tr>
<td>Based</td>
<td>Extremely sensitive (femtomole to zeptomole range, $10^{-15}$ to $10^{-21}$ moles); well suited to multiple-target detection with different nanoparticles</td>
<td>Many development steps in a robustness of surface structure usually destroyed</td>
</tr>
<tr>
<td>Label</td>
<td>Highly sensitive (femtomole range) and simple assay; requires no labeling; uniquely well suited for mismatch detection; sequence independent; amenable to multiplexing; applicable to DNA-protein sensing step</td>
<td>Biochemical preparation of target</td>
</tr>
</tbody>
</table>

Optical Detection: DNA-coated Au particles

Mirkin group, Northwestern University
Nanosphere corp.
the mechanically
duplexes are
developing solution
to deposit
gold labels\textsuperscript{53,54}. A
silver and
adsorbed metal
solution limits down to
\(5 \times 10^8\) molecules, in
solution\textsuperscript{55}.

Baculis\textsuperscript{56} have exploited this technique to
unmodified target DNA. Gold nanoparticles are then hybridized to the unbound portion of the target, leading to a
device (See Figure 7). In their approach, electrodes with gaps
in the electrode leads is
laid upon the substrate between the
electrode leads. The
oligonucleotide capture strand in each electrode gap. Adapted from ref. 42

Figure 7 High-sensitivity conductivity assay. Probe DNA immobilized in a set of electrodes is hybridized to a portion of the unmodified target DNA. Gold nanoparticles are then hybridized to the unbound portion of the target, leading to a change in the gap. Silver metal is precipitated onto the gold nanoparticles, improving the resistance by lowering the resistance across the electrode gap. If the target DNA is captured, silver is not deposited across the gap, and the resistance is high. This strategy has been extended to produce an array of electrode pairs with an oligonucleotide capture strand in each electrode gap. Adapted from ref. 42.

target capture is signaled by a sharp drop in
target concentration, probably involving the
because each microparticle is order of \(10^{11}\) FCA molecules.

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Quantum dots replace fluorescent dyes

Quantum dots (CdS, CdSe) + silica + probe

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Molecular beacons consist of a DNA hairpin functionalized at one end with a fluorophore and at the other with a quenching agent. In the absence of the target DNA sequence, the quencher is brought in close proximity to the fluorophore, and no signal is generated. Addition of the target sequence leads to hairpin unfolding, concomitant duplex formation, and signal generation.
Molecular Beacons

No-reagents, only target!
No labeling of target
Negative: high signal without analyte:
sensing small change in big signal

Basic Physics of Semiconductor Devices-MOSFET

• MOSFET: Metal Oxide Semiconductor Field Effect Transistor
• Substrate can be p-type or n-type
• Gate conducting “polysilicon” (metal-like)
• Gate oxide (SiO₂): thin < 2 nm
• Source & Drain: n-type conducting diffusion layers

Operation
• Depletion regions at np junctions
• Any voltage across => no current
• +ve voltage on the gate attracts electrons into the p-region channel under the gate: form an inversion layer.
• Now apply a source-drain voltage: get a current. We have a switch.

http://www.mitre.org/work/electronics.shtml
Carbon nanotube FET
(towards a sensor!)

Sami Rosenblatt, Yuval Yaish, Jiwoong Park, Jeff Gore, Vera Sazonova, Paul L. McEuen
High performance electrolyte gated carbon nanotube transistors
Nanoletters 2002 2(8) 869-872
Relevant Commercial Sensors

Infineon (ChemFET)
Carbon Nanotube-Based Biosensor

The conductivity of a Carbon Nanotube can be affected by the covalent or electrostatic binding of analytes to the sidewall.

Two possible architectures:
Protein detection (CNT device)
20 molecules!

Salah Boussaad (DuPont)
Figure 1. (A) Schematic of a sensor device consisting of a SiNW (yellow) and a microfluidic channel (green), where the arrows indicate the direction of sample flow. (B) The SiNW surface with PNA receptor. (C) PNA–DNA duplex formation.
Figure 2. (A) Real-time conductance response from a SiNW device functionalized with PNA receptor. The arrow marks the point in time when the 60 fM WT DNA sample was added. The inset shows a SEM image of a typical SiNW device with source (S) and a drain (D) indicated; scale bar is 1 μm. (B) Time dependent conductance in DNA-free solution; the arrow indicates the point in time when a new solution sample was added. (C) Conductance versus time for SiNW with linked PNA receptor in the presence of 100 fM MU DNA sample (1) and following addition (arrow) of a second 100 fM MU DNA sample. (D) Conductance versus time for PNA-functionalized NW device during flow of DNA-free solution (1), 100 fM MU DNA (2), DNA-free solution (3), and 100 fM WT DNA (4).
Other Sensor Designs

Surface Plasmon Resonance

- Surface sensitive: measures refractive index change because of adsorption (usually on Au)
- No labeling required

Mass Sensing

- Surface Acoustic Waves
Microcantilever

- Micromachined cantilevers
- Au on one side
- Coat with probe (no label)
- Hybridization causes bending (surface stress)
- Measure by laser reflection