CMOS Biochips for Point-of-Care Molecular Diagnostics

Arjang Hassibi
InSilixa, Inc.
Sunnyvale, CA USA
OVERVIEW

Molecular Diagnostics (MDx) and Stuff...
Diagnostics Information Flow (1)

Treatment options are based on diagnostics results

Visit

Basic Tests

Comprehensive Testing

Sample Preparation

Culture/DNA Sequencing/Chemical Analysis

Data Analysis

Point-of-Care

Core Facility

Treatment

Basic Tests

Comprehensive Testing

Sample Preparation

Culture/DNA Sequencing/Chemical Analysis

Data Analysis

Point-of-Care

Core Facility
Diagnostics Information Flow (2)

Actionable tests* take too long and are too expensive

Visit Not Actionable* Actionable*

Treatment $10’s $100’s

2-14 Days

* A conclusive test that requires no further testing
Molecular Diagnostics (MDx)

Highly actionable, but not mass-deployable

Urinary Tract Infection (7M Visits/Year)

- **0.5 Hour**
  - Dipstick Test ($30)
  - Infection (67% Accuracy)

- **2 Days**
  - Culture ($40-$100)
  - E-Coli Present (+99% Accuracy)

- **14 Days**
  - DNA Analysis (>500)
  - E-Coli Present Antibiotic Resistant Strain (99% Accuracy)

Respiratory Infection (85M Visits/Year)

- **0.5 Hour**
  - Throat Swab ($40)
  - Group A Strep (73% Accurate)

- **2 Days**
  - Culture ($40-$100)
  - Group A Strep Present (99% Accurate)

- **14 Days**
  - DNA Analysis (>500)
  - Group A Strep, H1N1 Flu, Pertussis, ...
  - (99% Accuracy)
MDx Problem Statement (1)

Identify molecular structures (e.g., DNA sequences) in presence of similar structures in a “dirty” biological environment

Example: *E-Coli* Identification

A unique sequence found only in pathogenic *E-Coli*

\[
\text{GTTTCGATTTCAGGTTTTTCAGGTTTTTGTTT} \text{ (28 bases)}
\]

\[
\text{Prob \{Random Occurrence\} } = \left(\frac{1}{4}\right)^N \bigg|_{N=28} \approx 1.388 \times 10^{-17}
\]
MDx Problem Statement (2)

Check specific DNA sequence for specific mutations that result in functional changes in the behavior of the organism.

Example: *E-Coli* Identification

---

**E-Coli Genome (47 Million bases)**

#### Resistant to Antibiotic (Super Bug)

\[ \text{.... GTCTTAGGCAGTTAAA} \]
\[ \text{CGTTTACGATGAAACACGGT} \]
\[ \text{ACGACGATTTTACAGGG...} \]

**Sensitive to Antibiotic (Normal Bug)**

\[ \text{.... GTCTTAGGCAGTTAAA} \]
\[ \text{CGTTTACGATAAACACGGT} \]
\[ \text{ACGACGATTTTACAGGG...} \]

---

TGA $\leftarrow$ TAA

Mutation
MDx Problem Statement (3)

For actionable infectious diseases MDx, 10’s to 100’s of unique DNA sequences and/or mutations should be detected.

Number of Unique DNA Targets

- Group A Strep
- Recurrent Urinary Tract Infections
- Respiratory Viral Infections
- HIV Detection/Monitoring
- Hospital Acquired Infections
- Tuberculosis (TB)
### Current State-of-the-Art

<table>
<thead>
<tr>
<th>TECHNOLOGY</th>
<th>PCR(^1)</th>
<th>DNA ARRAYS</th>
<th>DNA SEQUENCING</th>
<th>MDx NEED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument (Setup) Price</td>
<td>$20K -50K</td>
<td>$25K-75K</td>
<td>$100K-$700K</td>
<td>&lt; $1000</td>
</tr>
<tr>
<td>Price per Test(^2)</td>
<td>$80-$400</td>
<td>$200-$1000</td>
<td>$2.5K-$10K</td>
<td>&lt; $50</td>
</tr>
<tr>
<td>Max DNA Targets</td>
<td>6-20</td>
<td>20-1000</td>
<td>+1M</td>
<td>1000</td>
</tr>
<tr>
<td>Detection Accuracy</td>
<td>High</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>Test Time</td>
<td>2-3 hours</td>
<td>6-12 Hours</td>
<td>&gt; 2 Day</td>
<td>1 Hour</td>
</tr>
<tr>
<td>Fully-Automated</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Portable</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>PoC Compatible</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

1. Polymerase chain reaction
2. Excluding any service charge
Solution: InSilixa’s HYDRA-1K

Rapid (<1 hr), low-cost ($10’s), and simple (sample-to-answer) MDx to detect up to 1000 unique DNA sequences/targets.
TECHNOLOGY

HYDRA-1K System
CMOS Biochips

CMOS ICs that are enhanced and specifically packaged to function as high-performance molecular sensors.
Biosensing Pixels

Depending on the adopted chemistry, various detection modalities can be implemented in the pixels.

Fluorescent Detection Arrays*

High dynamic range (HDR) wavelength-specific $\Delta \Sigma$ photosensor in every pixel.

*Singh et al., VLSI Symp. (2011)
Biosensing Pixels

Depending on the adopted chemistry, various detection modalities can be implemented in the pixels.

Impedance Spectroscopy Arrays

Lock-in amplifier-based impedance measurement in every pixel

* Manickam et al., ISSCC (2010)
Biochips require a complex packaging/assembly process.

1. **CMOS Wafers**
   - Pad
   - Substrate
   - CMOS Die

2. **Dicing and Mounting**
   - Epoxy

3. **Wire-bonding**

4. **Surface Coating**
   - Linker Polymer
   - Si
   - CMOS Backend

5. **Probe Spotting**
   - Immobilized Probes
   - Probes
   - Pixel

6. **Wash and Surface Block**
   - Wash
   - Surface

7. **Fluidic Cap Assembly**
   - Fluidic Cap
   - Immobilized Probes
   - Pixel
Detection Process (1)

Chip (and the pixels) are exposed to the sample containing the targets

1. Insert into the “Reader”

2. Sample Insertion, 3. Thermal Cycling, and 4. Data Acquisition

5. Disposal
Detection Process (2)

Output data reports the DNA capturing events, as a function of time and temperature, at every biosensing pixel.

<table>
<thead>
<tr>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>t = 0</td>
<td>T = 95°C</td>
</tr>
<tr>
<td>t = 10 min</td>
<td>T = 45°C</td>
</tr>
<tr>
<td>t = 20 min</td>
<td>T = 45°C</td>
</tr>
<tr>
<td>t = 30 min</td>
<td>T = 45°C</td>
</tr>
<tr>
<td>t = 35 min</td>
<td>T = 85°C</td>
</tr>
</tbody>
</table>

Sample Insertion, Thermal Cycling, and Data Acquisition
HYDRA-1K Platform

A CMOS biochip system for point-of-care DNA analysis

HYDRA-1K Biochip Module ($30-$50): Disposable module that electronically detects up to 1000 (1K) unique DNA sequences and provides a digital output.

Data

Reader²:
Controls the functionality of HYDRA-1K chip and provides connectivity

Sample + Reagents

¹ Cost varies depending on the application
² Not the final design
HYDRA-1K: Open Platform for MDx

HYDRA-1K reagents, hardware, and software are designed to enable flexible application development.

CMOS Biochip

Standard Reagents and DNA Probes

Configurable FPGA-based Software/Hardware

Standard Image (Raw) Format

Standard (FASTA) Output File
**HYDRA-1K: DNA Sensor Array**

DNA sequence identification enabled by pixel-level DNA capturing and optical detection

Real-time detection of DNA-hybridization events in every pixel
HYDRA-1K: CMOS Biosensor Array

DNA sequence identification enabled by pixel-level DNA capturing and optical detection
HYDRA-1K: Integrated Pixels

Programmable +120 dB dynamic range photo-sensor and thermo-cycler are integrated in every pixel.
The biochip includes the 1024-element biosensor array, a 24-bit ΣΔ data converter, on-chip thermal controller, and power management system.
<table>
<thead>
<tr>
<th><strong>TECHNOLOGY</strong></th>
<th>IBM 6RF ($L_{\text{min}} = 0.25 , \mu\text{m}, 1P, 4M, 2.5V/5V$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ARRAY</strong></td>
<td>32 x 32 (1008 Biosens. + 16 Temp. Sens.)</td>
</tr>
<tr>
<td><strong>BIOSENSING PIXELS</strong></td>
<td>Fluorescence ($\lambda \sim [450 \text{ nm}, 700 \text{ nm}]$)</td>
</tr>
<tr>
<td><strong>DETECTION DYNAMIC RANGE</strong></td>
<td>145 dB ($I_{\text{ph}} \sim [0.5fA, 10nA]$)</td>
</tr>
<tr>
<td><strong>SPEED</strong></td>
<td>0.1-50 Frames/Sec (Programmable)</td>
</tr>
<tr>
<td><strong>RESOLUTION</strong></td>
<td>&gt;24 Bit</td>
</tr>
<tr>
<td><strong>POWER CONSUMPTION</strong></td>
<td>112 mW ($I_c = 45 \text{ mA @ 2.5V}$)</td>
</tr>
<tr>
<td><strong>HEATING/COOLING RATES</strong></td>
<td>(+4/-4)$^\circ$C/sec</td>
</tr>
<tr>
<td><strong>TEMPERATURE CONTROL ACCURACY</strong></td>
<td>0.25$^\circ$C/sec</td>
</tr>
<tr>
<td><strong>COST (INCLUDING ASSEMBLY)</strong></td>
<td>$30$-$50^*$</td>
</tr>
</tbody>
</table>

*Varies depending on the application*
EXAMPLE

*Mycobacterium Tuberculosis (MTB)* Detection
TB bacterium has a very specific DNA region with possible mutations that result in resistance to antibiotics.

**Detecting Drug Resistant TB**

**Mycobacterium Tuberculosis (MTB)**

30 mutations within the *rpoB* gene that cause resistivity to Rifampicin.
DNA probes are designed to capture the wild-type and the mutant strains for every possible mutation.

**Mycobacterium Tuberculosis (MTB)**

**Probe Design Procedure**
## Probe Location within the Array

<table>
<thead>
<tr>
<th>Location</th>
<th>Wild-Type Probe</th>
<th>Mutated Strain Probe</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>L511P (M)</td>
<td>F514L (W)</td>
<td>N519D (M2)</td>
<td>H526D (M-L)</td>
</tr>
<tr>
<td>L511R (M)</td>
<td>F514L (M)</td>
<td>L521L (M)</td>
<td>H526E (M)</td>
</tr>
<tr>
<td>S512L (M)</td>
<td>D516Y (M1)</td>
<td>S522L (H)</td>
<td>H526R (M)</td>
</tr>
<tr>
<td>Q513L (M)</td>
<td>D516E (M1)</td>
<td>H526Y (W)</td>
<td>S531L (M2)</td>
</tr>
<tr>
<td>Q513E (M)</td>
<td>D516F (M)</td>
<td>H526Y (M2)</td>
<td>S531M (M1)</td>
</tr>
<tr>
<td>Control (1)</td>
<td>N518D (W)</td>
<td>H526N (M1)</td>
<td>S531W (M1)</td>
</tr>
<tr>
<td>Control (1-Cy)</td>
<td>N518D (M)</td>
<td>H526N (M2)</td>
<td>S531W (M2)</td>
</tr>
</tbody>
</table>
Capturing and DNA Melt-Curve Analysis

Wild-Type

Mutation
## Example Results

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Wild-Type TB</th>
<th>Strain-1</th>
<th>Strain-2</th>
<th>Strain-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>L511P</td>
<td>CTG</td>
<td>CTG</td>
<td>CCG</td>
<td>CCG</td>
</tr>
<tr>
<td>Q513L</td>
<td>CAA</td>
<td>CAA</td>
<td>CAA</td>
<td>CAA</td>
</tr>
<tr>
<td>F514L</td>
<td>TTC</td>
<td>TTC</td>
<td>TTC</td>
<td>TTC</td>
</tr>
<tr>
<td>D516F</td>
<td>GAC</td>
<td>GAC</td>
<td>TTC</td>
<td>TTC</td>
</tr>
<tr>
<td>N518D</td>
<td>AAC</td>
<td>AAC</td>
<td>AAC</td>
<td>No Call</td>
</tr>
<tr>
<td>L521L</td>
<td>CTG</td>
<td>CTG</td>
<td>CTG</td>
<td>CTG</td>
</tr>
<tr>
<td>H526Y</td>
<td>CAC</td>
<td>CAC</td>
<td>TAC</td>
<td>TAC</td>
</tr>
<tr>
<td>S531L</td>
<td>TCG</td>
<td>TCG</td>
<td>TCG</td>
<td>TTG</td>
</tr>
<tr>
<td>L533P</td>
<td>CTG</td>
<td>CTG</td>
<td>CTG</td>
<td>TTG</td>
</tr>
</tbody>
</table>

Sensitivity > 50 Copies-per-Sample

Strain Detection Success Rate = 97.22%
## Comparison

<table>
<thead>
<tr>
<th>TECHNOLOGY</th>
<th>PCR(^1)</th>
<th>DNA ARRAYS</th>
<th>DNA SEQUENCING</th>
<th>HYDRA-1K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument (Setup) Price</td>
<td>$20K -50K</td>
<td>$25K-75K</td>
<td>$100K-$700K</td>
<td>~$250</td>
</tr>
<tr>
<td>Price per Test(^2)</td>
<td>$80-$400</td>
<td>$200-$1000</td>
<td>$2.5K-$10K</td>
<td>$30-$50</td>
</tr>
<tr>
<td>Max DNA Targets</td>
<td>6-20</td>
<td>20-1000</td>
<td>+1M</td>
<td>1000</td>
</tr>
<tr>
<td>Detection Accuracy</td>
<td>High</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>Test Time</td>
<td>2-3 hours</td>
<td>6-12 Hours</td>
<td>&gt; 1 Day</td>
<td>1 Hour</td>
</tr>
<tr>
<td>Fully-Automated</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Portable</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>PoC Compatible</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

1. Polymerase chain reaction
2. Excluding any service charge
Small DNA Differences Matter

Albert Einstein (1879-1955) - Bobo the Chimp (1995-Now) = 1.5% DNA Difference