Ultra-low-light CMOS biosensor complements microfluidics to achieve portable diagnostics

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Anitoa Systems, LLC
Enable Point-of-care Molecular Diagnostics

Rapid, precise diagnosis of infectious pathogens, so doctors can respond quickly with life-saving drugs and treatment
Today: Molecular Diagnostics (MDx) accurate, but not timely and accessible

Sample Collection

Transportation & Preservation

Batch Testing in Centralized Lab

Time to result: days to weeks
Point-of-care (POC) + MDx = Fast and actionable diagnostics

On-site detection w/portable instrument,

Sample Collection

Time to result: 30 min ~ 1.5 hours

Actionable diagnostics means:
• Distinguish bacterial vs. viral infections (flu, hepatitis)
• Detect drug-resistant mutations
• Quantitative when needed
• Fit into “symptom to treatment” timing window

Intl Micronano Conf. 2015, Amsterdam
Compact instrumentation is key…

- Current generation of MDx instruments are too bulky, expensive
  - *Microfluidics* alone fell short of enabling miniaturization
  - *Compact instrumentation with integrated and compact read out electronics is important* —> *need better biosensors*

Microfluidics allow diagnostic reactions in compact chip format

The instrument needs to be small too!

(Beckman Coulter’s Ampligrid System)
Landscape of molecular* bio-sensors

Sensitive and specificity

* Molecular means nucleic acid (DNA, RNA) or protein (antibody) molecules.
Introducing Anitoa ULS24 Ultra-low-light CMOS bio-imager

- **Ultra low-light sensitivity**
  - Detection threshold $\sim 3.0 \times 10^{-6}$ lux*
  - Low dark current, high SnR (>13dB at detection threshold)
  - Wide dynamic range (> 85dB)
  - 12-bit ADC, Digital interface through Serial Peripheral Interface (SPI)
  - Built-in junction temperature sensor
  - 150um pixels in 24 x 24 format
  - 3.3V and 1.8V power supply, **30mW max** power

* @ 550nm, 20nm bandwidth, 4s integration time
Intelligent Dark Current Management: Starts with high responsivity/low dark current photo-diodes. The readout circuit performs multimodal sensing to capture signal and noise information, the ADC and DSP takes advantage of the multi-modal information to achieve better noise cancellation.

**Intelligent Dark-current Management**

- **Readout**
- **AD**
- **ODSP**
- **Noise canceling filter**
- Multi-modal sensing
- Photodiodes w/ high responsivity

(process improvement: Target raw sensitivity and dark current)  
(analog circuitry innovation: Target elimination of reset noise and signal conditioning)  
(novel DSP algorithm: Target reduction of readout noise and fixed pattern noise)
Combining CMOS biosensor with Microfluidics

- CMOS biosensor and Microfluidics innovations enable compact molecular diagnostic instrumentation

- Miniaturization of optoelectronic sub-systems is the key
  - Ultra-low light CMOS biosensor complements Microfluidics

Multi-channel fluorescent imager powered by CMOS biosensor + Microfluidics
Putting it together - Anitoa’s portable Nucleic-Acid-Test (NAT) platform targeting infectious disease

Battery backed up Hand-held instrument

Microfluidics reaction bay

Connect to bioinformatics cloud

Features and benefits

Low-cost*, miniaturized design

Reliable. Good reproducibility

Low power, no moving parts, can be battery backed.

High sensitivity, high level of integration

Single chip* fluorescent and chemiluminescent imaging

(* 1 chip per channel)

(* no active component in consumable)
Anitoa ULS24 application performance data

a. dsDNA quantification; b. Chemiluminescence Immunoassay

Material: Chemiluminescence substrate for HRP.
Emission wavelength: 425nm
Integration time: 30s
Result: greater sensitivity and dynamic range than colorimetric method

dsDNA quantification with Qubit® quantitation reagent (Life Technologies).
Excitation light (LED): 470nm
Emission light: 525nm
Filters: Chroma OD6 band pass filters
Integration time: 100ms
Result: 500x more sensitive than A260 method

Fluorescence vs dsDNA concentration (ng in 200ul solution)

- Fluorescence
- Linear (Fluorescence)

Chemiluminescence signal vs enzyme concentration (ng/ml)

- $R^2 = 0.985$

A

B
Real time quantitative PCR with Anitoa ULS24 CMOS biosensor and microfluidics

Real time qPCR test data with Anitoa ULS 24

DNA template concentration:
- 10^3 X
- 10^2 X
- 10^1 X
- 10^0 X

qPCR test results: Detection and quantification of E. coli and HBV (incl. wild-type and drug-resistant variations: rtM204I, rtL180M) w/ 4 copies/reaction sensitivity, 10^9 dynamic range.
HBV drug-resistant mutations diagnostics with Anitoa ULS24 CMOS biosensor

- Important advantage of MDx is detection of drug-resistance mutations
  - …and predict drug reaction
  - Avoid further development of drug-resistance
  - Fluorescent wavelength multiplex instrument offers advantage

<table>
<thead>
<tr>
<th>HBV mutations</th>
<th>Lamivudine</th>
<th>Adefovir</th>
<th>Clevudine</th>
<th>Sebivo</th>
<th>Entecavir</th>
<th>Tenofovir</th>
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S: Sensitive  R: resistant  I: Intermediate

(* There is also the Interferon method, which shows broad sensitivity, but has more side effects, need injection.)

No more “shot-gun” approach in drug prescription.
POC MDx benefits to target applications

- Short symptom to treatment window
  (Actionable results on site)
- ICU urgent need
  (Avoid life threatening complications)
- Short Viral Sample Life
  (Use sample right away to Avoid false negative)

- Influenza A,B, H1N1, Swine Flu
- MRSA
- Hepatitis
Business case of POC MDx powered by Anitoa CMOS biosensor and microfluidics

<table>
<thead>
<tr>
<th></th>
<th>CMOS biosensor enabled POC MDx</th>
<th>1st gen POC (e.g. Cepheid GeneXpert, Biofire etc.)</th>
<th>Traditional MDx (Life Technology, Roche, Qiagen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deployment</td>
<td><strong>Point of Care</strong></td>
<td>Point of Care</td>
<td>Reference Lab</td>
</tr>
<tr>
<td>Size</td>
<td><strong>Handheld</strong></td>
<td>Bench top</td>
<td>Central Lab Equip.</td>
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<tr>
<td>Equip Cost</td>
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<td>Cost / test</td>
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<tr>
<td>Sample to result</td>
<td><strong>30min - 1.5hr</strong></td>
<td>1-2 Hours</td>
<td>days to weeks</td>
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Other identified applications of ultra-low light CMOS biosensor

1. Fluorescence Images Guided Surgery (FIGS)
2. Fluorescence or chemiluminescence-based Immunoassay/ELISA
3. Food safety, environment safety or bio-threat detection.
4. DNA or Protein microarray
5. Pyro-sequencing
6. Capillary electrophoresis
7. Cell sorting/Imaging flow cytometry /Circulating tumor cell detection
Summary and future plan

**Summary**

- Ultra-low-light CMOS biosensor combined with microfluidics technology enables compact and low-cost instrumentation for point-of-care molecular diagnostics.

**Future plan**

1. Further miniaturization of opto system
   - Smaller camera system for mobile integration
   - Direct coating and patterning of thin film filters on chip to achieve truly single chip multi-channel fluorescent imaging.

2. Create high speed variation of the chip
   - Targeting cell sorting and cancer screening applications

3. Further refinement of integrated opto-thermal-fluidic system platform
   - For handheld sample-to-answer MDx system
THANK YOU!

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