Integration of Optical Biosensor Systems for Point of Use

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Movement to Point of Use: Drivers

I. Public concerns
II. Immediacy: Eliminate sample transportation
III. New concepts for molecular recognition
IV. Integration of microfluidics and optics/electronics
V. Simplified fabrication technologies
VI. Miniaturization in electronics & communication
VII. Systems integration choices
I. Public Concerns*

- Medical awareness—point of care
- Diagnostics for developing countries
- Homeland security
- Clean drinking water
- Other toxic materials, nanotechnology "threat"
- Energy

USER DRIVES SYSTEM DESIGN

*Grant funding/market opportunity
NRL Automated Biosensors 1996-2012

- Lifepoint Impact
- Fast 2000
- Analyte 2000 on UAV
- BioHawk
- Fast 6000
- RAPTOR Plus
- CT-Array Biosensor
- mBio
- Signalyte
- Leopard
- UUV mounted immunosensor
II. Immediacy: Eliminate sample transportation

- Medical
  - Hospital bedside
  - Doctor’s office
  - Home
  - Remote telemedicine
- Environmental monitoring: pollution & climate change
- Food safety from source to store
- Homeland security and military operations
Autonomous Mobile Biosensor Systems

Spinoffs: Portable cyclone air samplers and fiber optic biosensors (Research International) Robust air sampler for BioWatch

Future: Swarms

• Remote identification
• 10 lb payload
  • Ram air cyclone
  • 4-fiber biosensor
  • Lyophilized antibodies
• Assays at 5 min intervals
• Real-time data transfer
• Successful demos at Dugway Proving Ground

Microarray Immunosensors

Concept

Sandwich

Glass slide

- Fluorophore
- Detector antibody
- Antigen (Analyte)
- Capture antibody
- Biotin
- Avidin

NRL Developmental Prototype

- Small camera
- 0.4 ft³ box
- <10 lb.
- External computer control
- Moldable reservoirs

2005-2012 Commercial Products

CT-Array Biosensor

mBio

Leopard


Golden et al., 1999, SPIE 3602, 132
Golden et al., 1999, SPIE 3602, 132
IBM concept for $1 multiplexed POC immunosensor for whole blood
Published by Gervais et al. in Adv. Mater. 2011, 23, H151–H176
III. New concepts for molecular recognition and signal generation

- Single domain antibodies
- Anti-microbial peptides
- Carbohydrates
- Designer peptides
Single-domain Antibodies & Fragments

- SdAb are stable: refold after exposure
  - Heat as high as 95°C
  - Solvent
- Papers by Ellen Goldman & George Anderson

Llama SdAb’s heated to 95°C, Cooled to 25°C
Tested for binding to SEB
Antimicrobial Peptides for Semi-selective Recognition

- Naturally occurring peptides - innate immune system
- 12-45 amino acids
- Disrupt microbial membranes
- Semi-selective binding
  - ID based on pattern of binding

Carbohydrate-Target Interactions

Nathan Sharon & Halina Lis in *Scientific American* January 1993, 82-89
Carbohydrate Binding of Toxins

IV. Integration of microfluidics with optics/electronics

• Single structure provides multiple functions

• Optical and fluidic components integrated into single substrate

• Low energy electronic components for data acquisition and transmission

• Compatibility with personal electronics
Integrating fluidic channel, sensor surface, and waveguide for signal collection


Signalyte
CreatvMicrotech
Fluid Focusing using Laminar Streams
Concept of “Soft Boundaries”

Fluids focus fluids

Fluids focus light.

Howell et al. (2008) Lab Chip, 8, 1097 - 1103.
Hydrodynamic Focusing of Conductance

Current Electrode

Permanent Magnets

Current Electrode

Fluidic Channel

Sense Electrodes

Captured 5µ Beads

Inlet 1

Inlet 2

Low-Conductivity Confinement Flow

Cells Specifically Bound

Outlet

Ionic Buffer

Before

After

Graph showing voltage (mV) vs. flow-rate ratio with data points for magnetic beads and no beads.

Graph showing impedance difference (Ohms) vs. flow rate ratio with a quadratic fit equation and R² value.
Polymer Optics Integrated with Microfluidics

Parallel processing without walls  
Polymer diode array  
Integrated system

Integrated Laser Diodes as Light Sources

Laser transfer and embedding can also be used for edge emitter laser diodes (sources) and photodiodes (detectors) as well as microlenses and prisms required for the complete miniaturized sensor package (A. Pique et al.)
LED Array by Laser Direct Write on Polyimide

The bare die LEDs are 100 µm thick and the polyimide substrate is ~125 µm thick.
Alberto Pique, NRL
Integration of Microfluidic Valves

Reviewed in Gervais et al. in Adv. Mater. 2011, 23, H151–H176 (Fig 6).
Integration of Microfluidic Pumps

From Gervais et al. in Adv. Mater. 2011, 23, H151–H176 (Fig. 7)
Immunodiagnostics liked to GPS and telecommunications

Explosive detection using unmanned underwater vehicle (Remus)

- Displacement immunoassay on a chip
- No reagent additions
- Continuous monitoring for days
- Assays directly in sea water
- Optical readout transmitted to PDA
- Coordinated with onboard GPS data

Anne Kusterbeck, PI (NRL)
V. Simplified Fabrication Technologies

- Soft lithography
- CNC milling
- Laser ablation
- Laser direct write
- Hot embossing
Materials

Hard:
- Glass
- Silicon
- Thermoplastics

• Soft:
  - PDMS
  - Hydrogels
  - Teflon
  - Thermoplastics
  - Paper

Surface modification:
- antifouling
- hydrophilic/hydrophobic
- tethers for biomolecules
- metal-coatings
- textured
VI. Miniaturization in electronics & communication

- Personal communication
- Personal data access (e.g. iPhone, iPad)
- Cloud computing
- Agile networks
- On-chip energy sources
VII. Systems Integration Choices

Choices dictated by application and user. Choices are interconnected. Results must be accurate, timely & actionable.

- Integrated or off-chip optics/electronics
- Option of on-chip pumps and valves
- Automated sample processing
- Automated analysis
- Integrated data processing
- Telecommunications for data transfer
Examples of System Choices

• Automated detection using antibody array
  – MBio diagnostics for resource-limited settings
  – Leopard array immunosensor for food safety

• Microflow cytometry
  – Analysis using coded beads
    • Multiplexed detection of biothreat agents
    • Clinical diagnostics or environmental monitoring
  – Continuous monitoring of marine algae
• MBio Diagnostics, Inc., Boulder, CO
• One sample, panel of results, in minutes
  – Multiplexed assays at point-of-care
  – Blood: HIV, Hepatitis, …
  – Respiratory: Flu, Strep, …
  – Cardiac: Troponin, …
  – Cell counting: CD4, …

Disposable reagents, fluidics, waveguide
Handheld reusable optics, electronics
Inexpensive, battery operated

© 2011 MBio Diagnostics, Inc.
MBio Multiplexed Serology System
Antenatal Screening Panel

- HIV
- Hepatitis
- Syphilis

Replace three rapid tests

- >1 million deaths: congenital HIV & syphilis
- Trial: Antenatal clinics in Kenya, 2700 patients

© 2011 MBio Diagnostics, Inc.
MBio Cell Counting System

Three registered images
Differential staining

Brightfield
Fluor1; CD3+
Fluor2; CD4+

0.5 mm

© 2011 MBio Diagnostics, Inc.
MBio CD4+ Cell Count
Current Clinical Sample Data

MBio CD4+ Count (cells/ul)
CD4+ Count (cells/ul)
Flow Cytometry (BD FACSCalibur)
MBio
Identity
Passing Bablok
Contact:

• Chris Myatt, CEO
  – chris.myatt@mbiodx.com

• Mike Lochhead, Vice President
  – mike.lochhead@mbiodx.com
Opportunity: Food Safety
Hanson Technologies’ OmniFresh 1000™

- Same automation as NRL Array Biosensor
- Integrated with large-volume concentrator
- Screens entire produce lots
- Continuously samples produce wash
- Results in 2 hours
- Large samples (100-300 gallons)
- Concentrates and then detects microorganisms using array biosensor
- Two highly successful validation pilots completed on production washing machines
Microflow Cytometry based on Hydrodynamic Focusing

- Grooves direct sheath fluid above and below core stream
- Simulations and confocal images match closely
- Number of chevrons determines height of core
- Relative flow rates determines width of core
Confocal Validation of Core Geometry
Multiplexed Immunoassays

Coded Beads

2 Fluorescent ID Tags
Light Scatter
Phycoerythrin Tracer

Assays for detection of multiple targets

Different antibodies on each bead enables deeply multiplex detection

Capture bead + Bioagent + Labeled antibody → Color analysis
Cholera toxin assay

![Graph showing the relationship between toxin concentration and assay output using Microcytometer and Luminex bead 81: CTX. The graph illustrates the response of the assay to different concentrations of cholera toxin.]
NRL 12-Plex Assay for *E. coli* O157:H7

- 50 – g α Listeria
- 54 – chicken IgY (+ control)
- 56 – g α *E. coli* O157:H7
- 58 – g α Salmonella
- 71 – r α Ricin toxin
- 75 – BSA (- control)
- 77 – m α *F. tularensis*
- 79 – r α *Y. pestis*
- 81 – r α Cholera toxin
- 92 – m α SEB
- 96 – g α *B. anthracis*
- 100 – r α Shigella
## Detection Limits

**Bead Sets and Limits of Detection**

<table>
<thead>
<tr>
<th>Bead ID</th>
<th>Analyte</th>
<th>Detection Limits-buffer</th>
<th>Detection Limits – 10% serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td><em>Listeria</em></td>
<td>1x10⁵ (cells/ml)</td>
<td>1x10⁶ (cells/ml)</td>
</tr>
<tr>
<td>54</td>
<td>Chicken IgY</td>
<td>Positive Control</td>
<td>Positive Control</td>
</tr>
<tr>
<td>56</td>
<td><em>E.Coli</em></td>
<td>1x10⁴ (cells/ml)</td>
<td>1x10⁴ (cells/ml)</td>
</tr>
<tr>
<td>58</td>
<td><em>Salmonella</em></td>
<td>1x10⁵ (cells/ml)</td>
<td>1x10⁶ (cells/ml)</td>
</tr>
<tr>
<td>71</td>
<td>Ricin</td>
<td>1x10⁻² (ng/ml)</td>
<td>Not determined</td>
</tr>
<tr>
<td>75</td>
<td>BSA</td>
<td>Negative Control</td>
<td>Negative Control</td>
</tr>
<tr>
<td>77</td>
<td><em>F. tularensis</em></td>
<td>1x 10⁵ (cells/ml)</td>
<td>1x 10⁵ (cells/ml)</td>
</tr>
<tr>
<td>79</td>
<td><em>Y. Pestis</em></td>
<td>1x 10⁵ (cells/ml)</td>
<td>Not determined</td>
</tr>
<tr>
<td>81</td>
<td>Cholera toxin</td>
<td>1x10⁻¹ (ng/ml)</td>
<td>Not determined</td>
</tr>
<tr>
<td>92</td>
<td>SEB</td>
<td>1x10⁰ (ng/ml)</td>
<td>Not determined</td>
</tr>
<tr>
<td>96</td>
<td><em>B. Anthracis</em></td>
<td>1x 10⁴ (cells/ml)</td>
<td>1x 10⁵ (cells/ml)</td>
</tr>
<tr>
<td>100</td>
<td><em>Shigella</em></td>
<td>1x 10⁵ (cells/ml)</td>
<td>1x 10⁵ (cells/ml)</td>
</tr>
</tbody>
</table>
E. coli Assay

E. coli - Buffer normalized

E. coli - 10% serum normalized
Automated Sample Prep

Sample + Beads

Biotinylated Antibody Cocktail

PE-Streptavidin

Magnetic Trap

Howell et al. Patent Publication 20110188339, and international application PCT/US11/22942
Verbarg et al. 2012 Lab on a Chip. Advance Article
DOI: 10.1039/C2LC21189K
Changing Magnetic Field in Microchannel

Magnetic Gradient Magnitude

Mag Field Gradient (A/m²)

Distance (mm)

$10^7$
Capture and Release

MagTrap Capture Mode

Fluid Flow Direction
Automated Processing for *E. Coli* Detection

- Stronger signal in less time
- Less reagent required
- Preconcentration if desired
- Sample volumes flexible

Black bars: automated processing: 5 min + 3 min reagent exposures
Gray bars: manual processing with 5 min + 3 min reagent exposures
White bars: manual processing with 30 min + 30 min reagent exposures
Microflow Cytometer with Automated Sample Processing

- Can be used for:
  - sequential samples (clinical, food, grab)
  - periodic monitoring (air, water)
- On chip reagents, mixing, analysis
- Off chip magnets, optics, pumps, data processing
GE + NRL: Integrate Sample Prep with Microflow Cytometer on Disposable Chip for POC

Sample Preparation Module

- Reservoir 1
- Reservoir 2
- Magnetic Trap Port
- Waste Outlet

Microcytometer Module

- Sheath Fluid

Optical Detection Fluidic Control
Automated Microflow Cytometer System for Marine Algae: System considerations

- **Core Size** – ideally the diameter of the smallest expected particle.
  - Larger core size gives more throughput but poor variance
    *(Algae are submicron to hundreds of microns)*

- **Particle velocity** – higher velocity provides greater throughput.
  - Increases internal backpressures requires more power-intensive pumps
  - Faster signal processing requires more power-intensive microcontroller
    *(Bigger algae are very dilute)*

- **Detector Dynamic range** – Wider range improves algae discrimination
  - Requires decreasing electronic noise provided by more expensive/complex electronic parts
  - Alternative is to overlap detectors, which requires more space and power
    *(Scatter can be misleading due to inclusions in algae, colors critical)*

- **Resolution** – the best resolution occurs with 16 or more data points per peak.
  - Faster sample processing (more power-intensive electronics)
  - More data throughput (complex electronic design)
  - More expensive detectors
  - Extra data storage space
    *(Data must be stored on board)*

- **Sensitivity** – increase minimum light level detection
  - Complex optical schemes (expensive coatings and parts, can require a lot of space)
    *(Total system must fit in 8’inch diameter can)*
Microflow Cytometer to Detect Changes

Field trials in fall 2011
• Reconfigured for corrosion resistance
• Integrated in can for submersion
• Pressure tested
• Cage-deployed off Oregon coast
• Measured 3 distinct algal populations
• NO LEAKS
Undergoing further miniaturization

• 1 µ Synechococcus
• 12-85 µ Nitzschia d.
• 8-32 µThalassiosira

Optical Biosensors

• Current
  – Commercial biosensors available for point of use
  – Initial use by defense and diagnostics industries
  – Multiple commercial transitions

• Near term
  – Expanding sensing capability/information density
  – Miniaturization of automated systems

• Future
  – Small, inexpensive, easy-to-use sensors:
    for diagnostics, environmental monitoring, food & water safety
  – Biosensors for distributed operations:
    multiple targets, continuous monitoring, networked response
Thank You

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AND YOU FOR LISTENING!