Multiplexed Diagnostics
Enabled by Silicon Photonics

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Disclosure of Interest

- Current Research Support
  - National Institutes of Health
    - National Cancer Institute
    - National Institute for General Medical Sciences
  - National Science Foundation
  - Mayo Illinois Alliance for Technology-Based Healthcare
  - Genalyte, Inc.

- Objective: To discuss an emerging point-of-care diagnostic platform based upon silicon photonic technology.

- Speakers Bureau
  - N/A

- Clinical Trials
  - N/A

- I hold options for stock and am a consultant and scientific advisory board member of Genalyte, Inc., a company developing in vitro diagnostic technologies.
Molecularly-targeted therapeutics and personalized medicine

- Patient-specific treatment of disease based upon a molecular diagnostic signature.

- Imatinib (Gleevec)
  - $4.7B for Novartis in 2012
  - $92K/patient/year
  - Tyrosine kinase inhibitor used to treat chronic myeloid leukemia (CML)
  - Targets the \textit{bcr-abl} fusion protein
    - Reciprocal translocation between chromosomes 9 and 22
    - Philadelphia chromosome
The important role of companion diagnostics

- Cancer is an extremely heterogeneous disease.
- Breast cancer
  - *BRCA1, BRCA2, Her2/neu, and many others*

- Trastuzumab (Herceptin)
  - Humanized mouse monoclonal antibody that binds to Her2/neu, which is overexpressed in ~20% of breast cancers
  - 95% 3-year survival rate
  - Developed by Genentech in collaboration with DAKO
    - IHC and FISH assays for Her2/neu++
  - Very expensive: ≥$70K treatment
Abundance of molecularly-targeted therapeutics are hitting the market

**Gefitinib (Iressa)**
EGFR inhibitor
*Non-small cell lung cancer*

**Vemurafenib**
B-Raf inhibitor
*Late stage melanoma*

**ABT-737**
Bcl-2 inhibitor
*Small cell lung cancer*

**PAC-1**
proscaspase-3 activator

**Nutlin-3**
MDM2 inhibitor

**PRIMA-1**
mutant p53 re-activator
Motivation:
Clinical diagnostics for personalized medicine

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Technology design ideals for *in vitro* diagnostics

- **Relevant sensitivity in clinical matrices**
  - Able to detect relevant levels of target molecules in native body fluids or tissue biopsy samples

- **Multiplexing capability**
  - Simultaneously able to measure 10s, 100s, or even 1000s of biomolecular signatures for improved diagnoses
    - Predictive, prognostic, and/or theragnostic biomarkers
    - Disease-altered signaling pathways

- **Biomolecular generality**
  - Applicability to the analysis of DNAs, RNAs, proteins, metabolites, etc.

- **Practicality of assay**
  - Fast time-to-result, assay simplicity, reagent cost/consumption

- **Manufacturability**
  - Cost effective and scalable to clinical demand
“Whispering gallery” resonators: High Q optical microcavities

Critically-coupled Si photonic microring resonators

- Fabricated on silicon-on-insulator wafers via deep UV lithographic processing with 10 nm precision and low sidewall roughness.
- Rings interrogated via on-chip linear waveguide located 200 nm from the microring.
**Critically-coupled Si photonic microring resonators**

- When the resonance condition in met, a strong optical field localizes on the microring due to constructive interference.
- Photons circulate many times giving a sample interaction length much larger than the geometric structure.

\[ m\lambda = 2\pi r n_{\text{eff}} \]
Biosensing with microcavity resonators: Label-free, real-time detection
Silicon-on-insulator microrings offer incredible scalability and measurement convenience

- Semiconductor processing
  - All optical components are monolithically incorporated into the top layer Si.
  - Commercial fabrication on 8” SOI wafers via deep UV lithography
    - 600+ sensor chips/wafer
    - Low chip cost; disposable sensing platform
  - Sensors scale to over 10,000/cm²
    - Redundant measurement increases precision; on-chip referencing.

- Si transparency window at 1550 nm overlaps with telecom c-band
  - High speed and precision tunable lasers
Arrays of rings (up to 128/chip) are serially interrogated using fast scanning mirrors and fast sweeping laser.

Fully automated analyses: Integrated fluid handling, plate sipping, and flow control.

Disposable cartridges with pre-functionalized sensor chips and chip autoloader available.

Detection of a cancer biomarker: Carcinoembryonic antigen (CEA)

- Carcinoembryonic antigen (CEA) is a 185 kDa protein secreted into the serum.
- Tumor marker for colorectal, pancreatic, ovarian, esophageal, and thyroid cancers.

Rapid, kinetics-based quantitation of CEA

- A plot of the initial slope versus concentration gives a linear relationship, in contrast to endpoint-based quantitation.
  - Simple calibration, fast detection, 3+ order of magnitude dynamic range

\[ \frac{d\theta}{dt} = k_a C e^{-t(k_aC + k_d)} \]
\[ \lim_{t \to 0} \frac{d\theta}{dt} = k_a C \]

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Initial Slope (pm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1183</td>
<td>2.5</td>
</tr>
<tr>
<td>575</td>
<td>2.0</td>
</tr>
<tr>
<td>228</td>
<td>1.5</td>
</tr>
<tr>
<td>114</td>
<td>1.0</td>
</tr>
<tr>
<td>45</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\[ y = 0.019x + 0.017 \]
\[ R^2 = 0.997 \]

- Unknown A: 90 ± 2 ng/mL (91 ng/mL)
- Unknown B: 18 ± 1 ng/mL (17 ng/mL)

Rapid, kinetics-based quantitation of CEA

- A plot of the initial slope versus concentration gives a linear relationship, in contrast to endpoint-based quantitation.
  - Simple calibration, fast detection, 3+ order of magnitude dynamic range

The microring limit of detection, ~2 ng/mL (10 pM), is consistent with CEA ELISA (1 ng/mL).
Detection is accomplished in one step and in less than 10 minutes using pre-calibrated sensors (ELISA = 3+ hours).

Unknown A: 90 ± 2 ng/mL (91 ng/mL)
Unknown B: 18 ± 1 ng/mL (17 ng/mL)

Sensor arrays can be fabricated using conventional microspotting technologies.

Non-cross reactive, 5-plex immunoassay chip:
- : cancer biomarkers
- †: inflammatory biomarkers

Multiplexed, quantitative expression profiling

**Table:**

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>150 ng/mL</th>
<th>100 ng/mL</th>
<th>50 ng/mL</th>
<th>20 ng/mL</th>
<th>10 ng/mL</th>
<th>0 ng/mL</th>
<th>Unknowns</th>
</tr>
</thead>
</table>

**Graphs:**

- **Unknown A**
- **Unknown B**
- **Unknown C**

**Reference:**

Generality of microring resonators for quantitative bioanalysis

proteins

DNA
Anal. Chem., 2011
JSTQE, 2010

miRNAs
Anal. Chem., 2011;

Viruses

Real-time surface polymerization/deposition
J. Am. Chem. Soc., 2011;

mRNA and tmRNA
Anal. Chem., 2012;
Challenge: Multiplexed detection of biomarker panel in serum

Secondary and tertiary binding events can be monitored to extend dynamic range.

1 hour assay time
Dynamic range $> 10^6$
Limit of detection $\approx 30$ pg/mL (200 fM)
Redundant detection reduces false positives

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Enzymatic signal enhancement provides a route towards lower detection limits

- Horseradish peroxidase appended to 2° antibody or 3° reagent catalytically oxidizes 4-chloro-1-napthol to insoluble 4-chloro-1-naphthon.
  - Deposition is highly localized
  - Extends down to the stochastic limit

Adapted from:

Quantitative detection using enzymatic signal enhancement

- Generally applicable and multiplex compatible.
- Extends LODs to ≤ 1 pg/mL

*Figure showing relative shift over time for different concentrations of IL-6 and IL-2.*

**R^2** values for different plots:
- R^2 = 0.9867
- R^2 = 0.9999
- R^2 = 0.9950

8-plex array to detect clinically-relevant levels of cancer serum biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cancer</th>
<th>Basal Levels</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoembryonic Antigen (CEA)</td>
<td>Colorectal</td>
<td>~2.5 ng/mL</td>
<td>180 kDa</td>
</tr>
<tr>
<td>Prostate Specific Antigen (PSA)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Alpha-fetoprotein (AFP)</td>
<td></td>
<td></td>
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<tr>
<td>Activated Leukocyte Cell Adhesion Molecule (ALCAM)</td>
<td></td>
<td></td>
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<tr>
<td>Cancer Antigen 125 (CA-125)</td>
<td></td>
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<td></td>
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<tr>
<td>Cancer Antigen 19-9 (CA 19-9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer Antigen 15-3 (CA 15-3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteopontin</td>
<td></td>
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</tbody>
</table>

- Screened 80 antibodies to develop an orthogonal 8-plex sensor chip

Adam Washburn and Winnie Shia
Differential biomarker levels correlate with organ-specific disease

* analyses in 1/3 diluted serum
Translational applications of microring resonators in progress

- Ultrasensitive detection of cardiac troponin and troponin-related biomarkers of acute and chronic cardiovascular diseases/disorders.
  - With Dr. Allan Jaffe @ the Mayo Clinic
- Inflammatory biomarker profiling to track comorbidities in the ICU setting, such as sepsis, delirium, acute lung and kidney injury.
  - With Dr. Karen White @ Carle Hospital, Urbana
- Validating liver and biliary duct cancer biomarkers.
  - With Dr. Lewis Roberts @ the Mayo Clinic
- Systems-on-a-chip: Simultaneous profiling of protein and miRNA signatures for prognostic glioma sub-classification.
  - With Dr. Mark Johnson @ BWH/HMS
Technology Commercialization

Founded in 2007
Team of ~50 people
Located in San Diego, CA

Life Sciences

Maverick Detection System
• Isotyped Immunogenicity Testing
• PK Assays

Genalyte
• Autoantibody Detection
• Cancer Biomarkers
• Custom Assay Development

Clinical Diagnostics

Dx

Multiplex Autoimmune Disease Panel Companion Diagnostics
Maverick Detection System

Maverick AutoArray Multiplex Assay

- 2 Sample Channels per Chip
- 4 Sensors per Analyte
- 16 Analytes per Sample Channel
- 12 Chips per AutoArray
- Fluidic Gasket
Custom Multiplex Assays

- Biomarkers
- PK
- Allergy
- Vaccines
- Autoimmune
- Isotyping

Monitor up to 384 binding interactions in a single assay

24 samples x 16 analytes
12 samples x 32 analytes
1 sample x 384 analytes
Spend Time Doing **Research** Not Assays

Start & Walk Away Automation

- No babysitting
- Saves technician time
- Reduces opportunity for error
Maverick
DETECTION SYSTEM

Pipeline

More Data...From a Smaller Sample...with Less Hands On Time

- ENA-4
- ENA-6
- Isotyping
- Type 1 Diabetes
- Immunogenicity
- Complement 1 & 2
- Rheumatoid Arthritis 1
- Rheumatoid Arthritis 2
- ANA-14
- Custom Multiplex

Genalyte
Conclusions

• The development of new multiparameter analyses technologies will greatly advance our clinical understanding of disease onset, progression, and treatment options. Personalized medicine will be tightly coupled to individualized diagnostics.

• We have pioneered a silicon photonic microring resonator-based platform as a scalable technology for the quantitative detection of disease-relevant protein and nucleic acid biomarkers, and have shown the ability to modularly construct multiplexed sensor arrays that can be highly automated and relatively rapid.

• Current efforts are directed toward further enhancing measurement sensitivity and assay speed, demonstrating higher levels of multiplexing, clinical translation, utilizing sensor technology to understand complex, interfacial chemical and biomolecular processes.
Acknowledgements