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 bcr-abl fusion protein
      • Reciprocal translocation between chromosomes 9 and 22
      • Philadelphia chromosome

  • Molecularly-targeted therapeutics and personalized medicine
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

Motivation: Clinical diagnostics for personalized medicine

[Image of molecular structures and clinical diagnostic flowchart]
Motivation:
Clinical diagnostics for personalized medicine

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.

- When the resonance condition is 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.

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
- Si transparency window at 1550 nm overlaps with telecom c-band
  - High speed and precision tunable lasers
- Low chip cost; disposable sensing platform
- Sensors scale to over 10,000/cm²
- Redundant measurement increases precision; on-chip referencing.

Genalyte Maverick Platform

- 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

![Graph showing kinetics-based quantitation](image)

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


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- The microring limit of detection, ~2 ng/mL (20 pM), is consistent with CEA ELISA (2 ng/mL). Detection is accomplished in one step and in less than 10 minutes using pre-calibrated sensors (ELISA = 3+ hours).

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- Unknown B: 18 ± 1 ng/mL (17 ng/mL)


Sensor arrays can be fabricated using conventional microspotting technologies

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![Image of sensor arrays](image)

Non-cross reactive, 5-plex immunoassay chip
- cancer biomarkers
  - PA
  - IL-6
  - APTT
  - GSA
  - TNF-α

**Multiplexed, quantitative expression profiling**

![Graph showing multiplexed expression profiling](image)

**Generality of microring resonators for quantitative bioanalysis**

![Graph showing microring resonator analysis](image)

**Challenge: Multiplexed detection of biomarker panel in serum**

![Graph showing biomarker detection](image)
Secondary and tertiary binding events can be monitored to extend dynamic range

Dynamic range > 10^6

Limit of detection = 30 pg/mL (200 fM)

Redundant detection reduces false positives

Limit of detection ≈ 30 pg/mL (200 fM)

Redundant detection reduces false positives

Enzymatic signal enhancement provides a route towards lower detection limits

- Horseradish peroxidase appended to 2° antibody or 3° reagent catalytically oxidizes 4-chloro-1-naphthol to insoluble 4-chloro-1-naphthol.
  - 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.

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>Prostate</td>
<td>~1 ng/mL</td>
<td>28 kDa</td>
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<td>Alpha-fetoprotein (AFP)</td>
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<td>Activated Leukocyte Cell Adhesion Molecule (ALCAM)</td>
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<tr>
<td>Cancer Antigen 125 (CA-125)</td>
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<td>Cancer Antigen 19-9 (CA 19-9)</td>
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<tr>
<td>Cancer Antigen 15-3 (CA 15-3)</td>
<td></td>
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<tr>
<td>Osteopontin</td>
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</tbody>
</table>

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

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

Maverick Detection System

Technology Commercialization

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

Life Sciences —— Clinical Diagnostics

Genalyte

Maverick Detection System

Multiple Autoimmune Disease Panel Companion Diagnostics

Maverick AutoArray Multiplex Assay

2 Sample Channels per Chip

4 samples per analysis

16 Analytes per Sample Channel

12 Chips per AutoArray
Maverick DETECTION SYSTEM Custom Multiplex Assays

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

24 samples x 16 analytes
12 samples x 32 analytes
1 sample x 384 analytes

Monitor up to 384 binding interactions in a single assay

Maverick DETECTION SYSTEM Spend Time Doing Research Not Assays

1. Load undiluted sample into reagent vial.
2. Pipette to mix.
3. Insert pressurized reagent vial and strip away instrument.
4. Press Start. The remainder of assay run is fully automated.

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

Custom Multiplex

Analytes
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