INTEGRATED DETECTION and MANIPULATION of PARAMAGNETIC MICROSPHERES

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EMD Biosciences / Estapor Meeting, June 17, 2005

• Magnetic Biosensor Concept: Biochemical binding + magnetic detection

• Magnetoresistive biosensor fabrication

• Detection and Manipulation Examples: Scanning, Immobilized and Flowing Labels, Flow Sorting
NVE Corporation

- Founded in 1989 as Nonvolatile Electronics
- Now traded on NASDAQ as NVEC
- About 60 employees
- 6000 ft² clean room (class 100)
- Specialize in integrated magnetoresistive devices
- FY ’05 revenues ~$12M
  - Magnetic Sensors [biomedical, industrial]
  - Digital signal couplers
  - Contract and Govt. Research and Development
NVE Highlights

• Licensed by Honeywell in 1989 to produce Magnetoresistive Random Access Memory (MRAM)
• Introduced world’s first Giant Magnetoresistance (GMR) product in 1996
• GMR Signal isolator product introduced in 1999
• Research & Development Thrusts
  – Biosensors
  – MRAM
  – Low-Field Magnetometers
  – Non-destructive Evaluation
  – Electronics and systems
• About 60 employees
NVE Fabrication Facility

Comptech

PE 2400
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www.nve.com/~markt/biomagnetics
Motivation for Current R&D Efforts

Military / Homeland Defense wants bioassays that are:

• Rugged
• Lightweight / handheld
• Cheap
• Rapid
(Also: highly sensitive and specific, multi-functional, fool-proof, etc.)
• Readers, sensors, and fluidics must be mass-manufacturable.
Status of Development

• Developing some great magnetic detection tools
• Working with excellent university and Lab. Partners
• Looking for commercial partners to provide access to the marketplace for specific diagnostic products
• Mass-manufacturable sensor process, $\langle\langle 1 / \text{mm}^2$
Magnetic Nanolabel Example:

2-Probe DNA assay

DNA Sample in liquid

Spintronic Detector

Label Probe

Sensor Probe
Stray Fields from Bound Magnetic Nanolabel

Detector sees $H_{\text{Total}} = H_{\text{applied}} + H_{\text{stray}}$ along a designed sense-axis
Model prediction for single label – top view

Total \((H_{app} + H_{stray})\) sense-axis field in sensor plane from single particle

Particle is 0.2 diameters from sensor surface

\(H_{applied}\)
Detector Design Parameters

• 1 – Field of view: (Sensor Area) x (Label Diameter)
• 2 – Dynamic Range: Max / Min # of labels detectable
• 3 – Sensitivity: microvolts / label
• 4 – Noise: (microvolts / Hz^{0.5}) x (Bandwidth(Hz))^{0.5}
GMR Biosensor Detection Capabilities

1 - Single label detection – small sensor required

2 – Combine many small sensors together to make 200 micron diameter sensor (match spot size)

3 – Field of view: (Sensor Area) x (Label Diameter)

4 – Dynamic Range of detected labels can be >> 3 logs
Resistance \( \propto R_0 + \sin \theta \)
Resistance when labels are present

\[ \text{Resistance} \propto R_0 + \sin \theta \]

Signal is resistance difference
Magnetoresistive Material Structures

Resistance change due to Spin Dependent Electron Transport

SpinValve (pinned sandwich)

AF Pinning Layer

Magnetic
(Co,Fe, Ni & alloys)

Non-magnetic
(Cu & alloys, Al$_2$O$_3$)

Sandwich

Multilayer

2 µm

10 nm

20 µm
Sensors produced in semiconductor wafer fab

- Layer by layer deposition, photolithography, etch
- 1 150mm diameter wafer can deliver >10,000 sensors
- Integrated circuitry is easily added to basic sensor
SU-8 Lid using wafer-scale bonding method

SU-8 Lid

Spintronic Sensor

SU-8 Channels

Silicon Wafer

Metal 2 1.8 um

1.8 um

=2.5 pm BCB

20Å silicon nitride

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Reference resistors for field and temperature compensation

2 Sense resistors that experience the sample or field being measured

Reference resistors do not experience sample

\[ V_{out} = V_{out+} - V_{out-} \]. If there is nothing to make the sense resistors behave differently than the refs, \( V_{out} = 0 \) Volts
Layout: Define Sensing Region

100 \mu m \times 100 \mu m sense region
Layout: Place Sense Resistors

Place $R_{\text{sense1}}$
The two sense resistors are interwoven to allow them to sense the same region and experience the same excitation field.
Layout: Place Reference Resistors

Add two identical $R_{ref}$
Layout: Add Al Interconnect

Add interconnection metal (Aluminum)
Define sensing region by selective etching and surface chemistry
A bridge configuration is used for the GMR circuit to reduce baseline drift. The two sensor bridge doubles the signal of the traditional single sensor bridge.

- Sensing GMRs – interdigitated $R_{S1}$ and $R_{S2}$
- Reference GMRs – $R_{R1}$ and $R_{R2}$
Die-Holding Printed Circuit Board

“Diving Board”

**Design Basics**

- 24 pin surface mount edge connector
- Narrow die area for fitting between in the excitation magnet gap

Wire bonds are potted such that sense pad is still exposed
Magnetic Excitation Module

• 8 On-board signal preamps
• Jumpers for sensor channels
• Jumpers for coil driver
BioMagnetIC System

- A to D card in laptop
- Pocket-sized excitation module
- Disposable sensor cartridges
- Adaptable device development platform
- R vs. H plots
- Vout vs. time
Magnetic Bioassay: Scanned Sample Mode
Sample Stick Experiment

- External field at 150 Oe
- Sample is held near the GMR (~50 µm) and moved across sensing area
The chemistry of self-assembled thiolate monolayers on gold is extremely versatile.

- The thiol head group binds strongly to the Au surface.
- The tail functional group can be tailored for desired sensor specificity.

![Diagram showing the structure of a self-assembled thiolate monolayer with labels for Thiol Head Group, Organic Spacer, and Tail Functional Group.](image)
NHS-Biotin reacts with primary amines to form an amide bond.
Assay Labeling with Magnetic Particles:

Biochemistry

Polystyrene MP

Streptavidin Monolayer (SA)

Biotin

Put Your Probe HERE

Polystyrene MP

Au Substrate

Au Substrate

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Magnetic Particle Binding Optimization

- Dynabeads® MyOne™ Streptavidin
- Superparamagnetic polystyrene beads (26% iron)
- Monolayer of streptavidin covalently attached to bead surface
- 10 mg/mL in phosphate buffered saline (pH 7.4, 0.01% Tween 20, 0.09% NaN3)

MP binding was studied to optimize binding procedures and conditions

*20 µL of 2 mg/mL MP on each sample
GMR Signal vs. MP Concentration

- Limit of detection (3x SNB) is 0.005 MP/µm², or 215 MP per gold square.
Best Sensitivity: Bind directly on detector
1) Single label detection is possible
2) >3 decades of dynamic range
3) Better than 1 fMolar with fluidics
4) Magnetism enhances specificity
In-Flow Integrated Detection of Magnetic Droplets
GMR Sensing of Magnetic Picodroplets

- Picoliter-sized droplets of ferrofluid formed at a fluidic junction
- GMR sensitivity 0.07%/Oe
- Wheatstone bridge configuration
**Ferrofluid Plug Formation**

- **Flow rate 1.0 µL/min**
- **Plugs formed at approx. 500 Hz**

- **Flow rate 0.2 µL/min**
- **Plugs formed at approx. 50 Hz**
• Two reference and two sensing GMRs configured as a Wheatstone bridge

• Channel passes over sensing GMRs
**Simulation**

Using “Amperes” magnetic modeling software

**Plug dimensions:**
- 13 µm wide
- 18 µm deep
- 85 µm long

**FerroTec 307**
- 10nm Co particles
- ~1% by volume

**H_x in Sensor Plane, Flowing Ferrofluid Plug**

- Effective GMR sensor area
- \( H_{\text{applied}} = 15 \) Oersted
- X direction,
  - \( \parallel \) GMR sense axis,
  - \( \parallel \) direction of flow
Direct Flow Velocity Monitoring

Excitation field 15 Oe; Flow rate 250 nL/min; 1.2% magnetite v/v

Velocity determined by cross-correlating the signals from two bridges
In-Flow Manipulation of Magnetic Particles
Diverter Design and Fabrication

- A uniform external field magnetizes particles
- Current lines induce field gradients of $10^2$-$10^3$ T/m
- Resulting force diverts particles to a desired channel
Magnetic Flow Sorting Experiments

Bangs Labs, 28% magnetite, 1 µm
Flow rate: 6 nL/min
85% of the beads in desired channel
Device Packaging with PDMS

- Electrical connections
- PDMS Fluidic connections
- Optical access
SU-8 Lid using wafer-scale bonding method

SU-8 Lid

Spintronic Sensor

Silicon Wafer

Metal 2 1.8 um

-2 Ω in BCB 1.8 um

20Å silicon nitride

SU-8 Channels
SU-8 on GMR wafers

Up to 860 µm thick
Ultra-high aspect ratio
Wafer level processing
Rapid prototyping
Adding: Multi-level, pick & place
Covers with 100 micron ports on the wafer

20-sensor array, 200 \( \mu m \) diameter
Dice, mount on circuit board, wire bond
Ready to use fluidic / GMR chip
Ready for flow: before and after pumping liquid
## Detector Comparison

<table>
<thead>
<tr>
<th>Magnetic</th>
<th>Photonic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Little background</td>
<td>Many light sources</td>
</tr>
<tr>
<td>Array is embedded in chip</td>
<td>Array must be scanned mechanically</td>
</tr>
<tr>
<td>Integrated single label detection is natural</td>
<td>Single label detection requires sophisticated optics</td>
</tr>
<tr>
<td>Less “colorful”</td>
<td>Narrow-band labels</td>
</tr>
<tr>
<td>Fields are not easily steered</td>
<td>Photons can be directed, diffracted, etc</td>
</tr>
<tr>
<td>Challenging fluidics integration</td>
<td>Challenging fluidics integration</td>
</tr>
</tbody>
</table>

Both are more sensitive than electrochemical
## Comparison with Electric Manipulation

<table>
<thead>
<tr>
<th>Magnetic</th>
<th>Electrophoresis or E-field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not limited by buffers</td>
<td>Need special buffers</td>
</tr>
<tr>
<td>Little spurious magnetism</td>
<td>Many charged items</td>
</tr>
<tr>
<td>External magnetic field source</td>
<td>Easiest device to fabricate</td>
</tr>
<tr>
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Near-term Technical Goals

**Program**

- Demonstrate Single Particle Detection
- Demonstrate a flow cytometer using magnetic labels and sorting
Next Generation

Ultimate:

Molecular counting and bead-by-bead manipulation

• Reduce label size to ~10 nm (more difficult detection)
• Use magnetics to enhance assay dynamics (mixing, capturing, binding hybridization, sorting)
• Increase system complexity by combining components
• Increase assay complexity with multiple analytes, sensors, and channels.
Biosensor array parameters

• First commercial array will be for Veterinary screening, ~ 20 sensors, 200 micron diameter, immunoassay (Seahawk Biosystems) (disposable cartridge with sensor inside)

• Largest array without integrated circuits is 68 sensors

• High density DNA chip format possible (1,000,000 sensors)

• Currently building a 96 sensor chip with integrated circuit switching
Integrated Circuit Design: 96-site array

0.35 micron TSMC wafers now in-house
Disposable sensor vs. sensor instrument

- Permanent sensor could scan lateral flow assays, glass slides, tapes, etc.
- Detection performance is greatly influenced by proximity (microns)
- Cost per sensor die is low, <<$1
- Typical GMR product retail cost ~$1 - $3
Possible Applications

• Portable high performance bioanalytical system for military
• Food and water safety (detect e-coli, salmonella, ..)
• Chemicals that can be bound magnetically
• Disposable DNA chip, gene expression
• Immunoassay

• Best apps would benefit from high-volume low-cost model
• Integrated microfluidic tools for detecting and sorting have been demonstrated

• Detection of immobilized and flowing labels demonstrated with wide dynamic range (> 3 decades)

• May open new applications in single molecule detection

• Manufacturable integrated microfluidics

• Magnetic detection system is ideal for high volume, low cost applications, and where portability and ruggedness are important (motivates the military funding)

www.nve.com/~markt/biomagnetics
10 nm Labels should be detectable

• Signal to noise is fine, hard to ascertain label location
• Dynamic range decreases with label size
• Some assays are better off with much larger detectors
• Get best of Dynamic Range and detection of small labels with an array of detectors
• Technical limits on small end are due to thermal magnetic stability of labels and detectors, fabrication, and characterization challenges, and chemical stability of labels.
Groups working on nanolabel detection

• S. Wang, Stanford
• M. Johnson, NRL
• P. Freitas, Portugal
• J. Hormez, LSU
• M. Porter, ISU
• M. Megans, Philips
Stray Fields: in-plane excitation

Need a sensor with bipolar output

The detected in-plane stray fields are in one direction

Spin Valve Transfer Function
Vertical Excitation

Need a sensor with unipolar output

The detected in-plane stray fields are in many directions

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