INTEGRATED DETECTION and MANIPULATION of PARAMAGNETIC MICROSPHERES

Mark Tondra, NVE Corp. Eden Prairie, MN

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

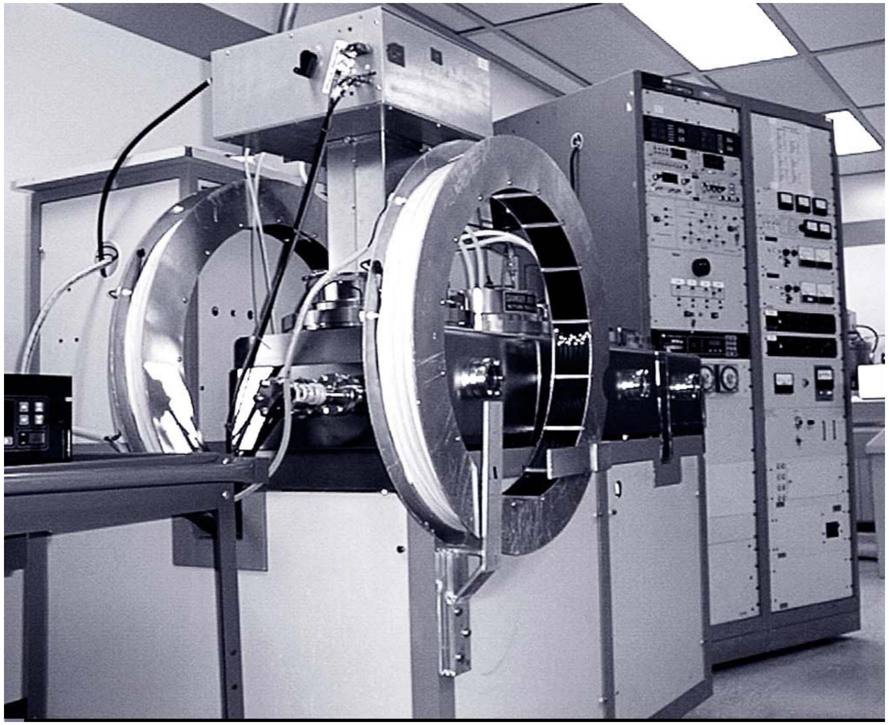
NVE Corporation

- Founded in 1989 as Nonvolatile Electronics
- Now traded on NASDAQ as NVEC
- About 60 employees
- 6000 ft^2 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

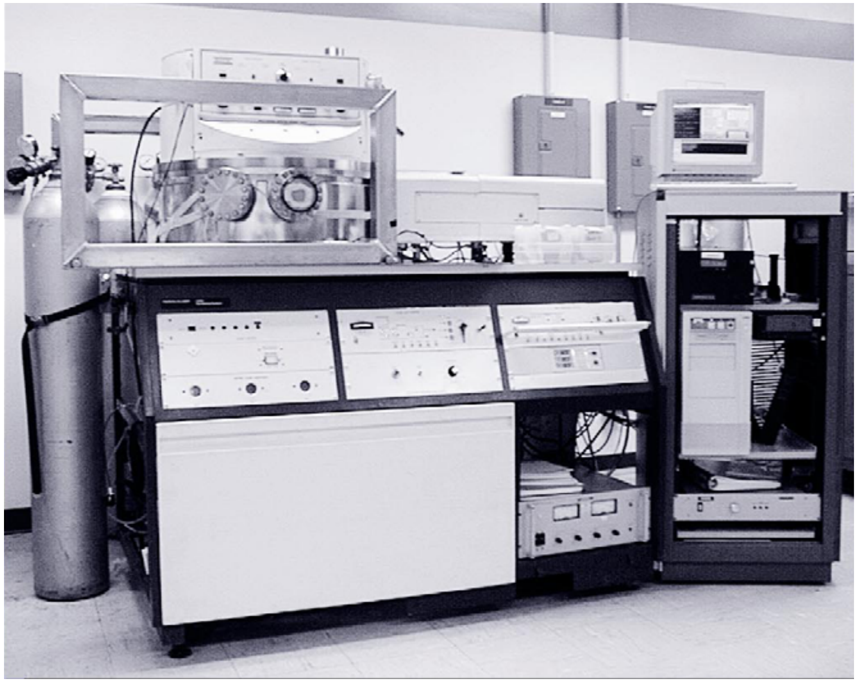
- Licensed by Honeywell in 1989 to produce Magnetoresistive Random Access Memory (MRAM)
- Introduced world's first Giant Magnetoresistance (GMR) product in 1996
- GMR Signal isolator product introduced in 1999
- Research & Development Thrusts
  - Biosensors
  - MRAM
  - Low-Field Magnetometers
  - Non-destructive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

NVE Fabrication Facility



Comptech

PE 2400



Acknowledgements

- Funded by NSF, DARPA

NVE:	ISU:	LSU – CAMD:	NRL:
Anthony Popple	Dr. Marc Porter	Zhengchung Peng	Lloyd Whitman
Bob Schneider	John Nordling	Kun Lian	Jack Rife
Kevin Jones	Rachel Millen	Josef Hormez	Cy Tamanaha
Loren Hudson	Nikola Pekas	Challa Kumar	Shaun Mulvaney
John Taylor	Toshi Kawaguchi		
Albrecht Jander			

www.nve.com/~markt/biomagnetics

Motivation for Current R&D Efforts

Military / Homeland Defense wants bioassays that are:

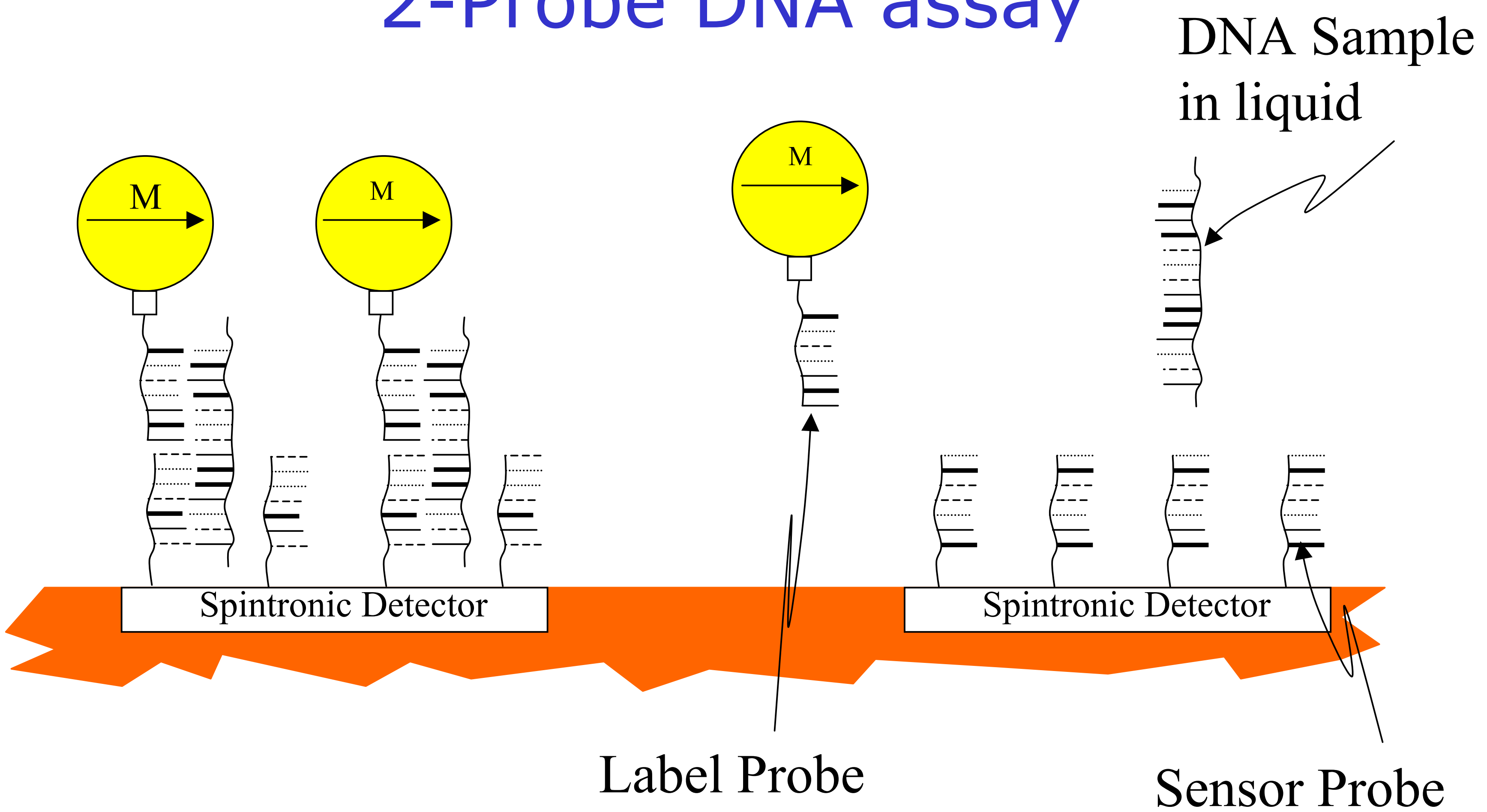
- Rugged
- Lightweight / handheld
- Cheap
- Rapid
- (Also: highly sensitive and specific, multi-functional, fool-proof, etc.)
- Readers, sensors, and fluidics must be mass-manufacturable.

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, << \$1 / mm^2

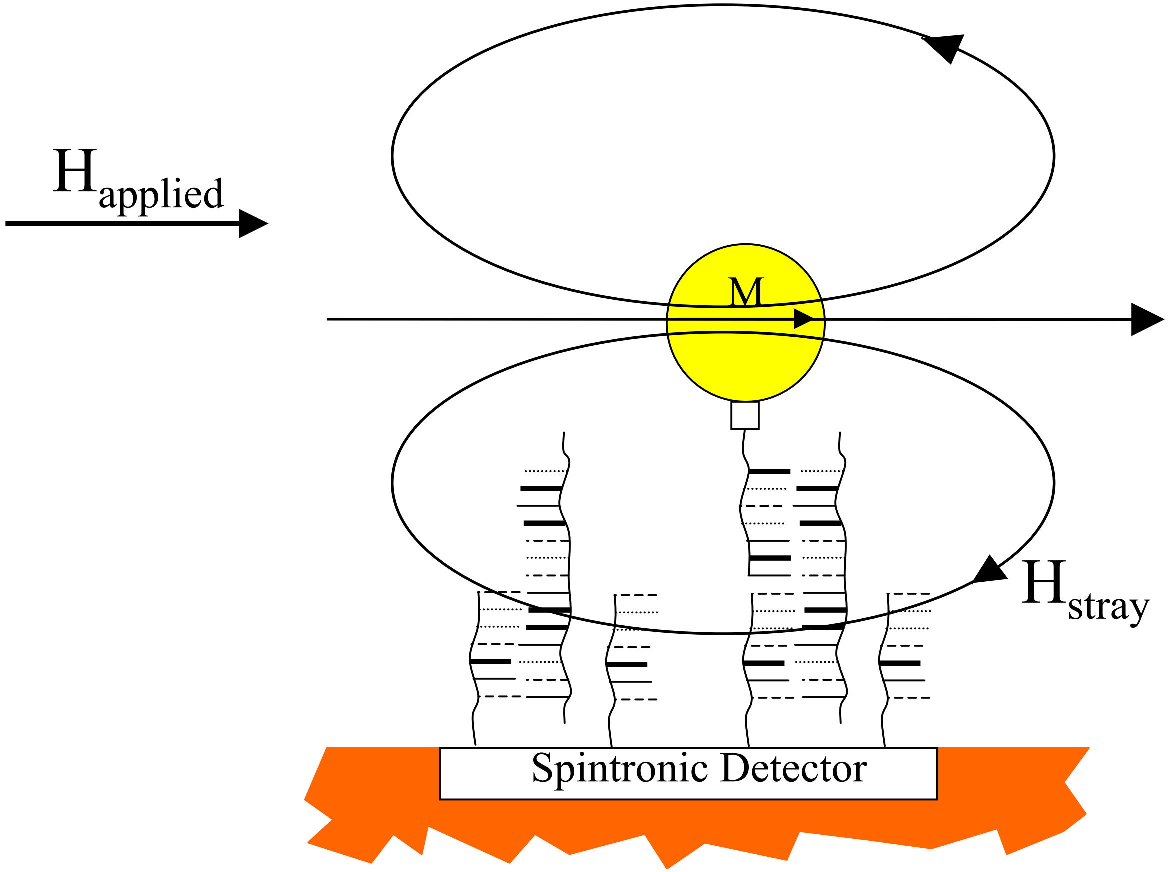
Magnetic Nanolabel Example:

2-Probe DNA assay



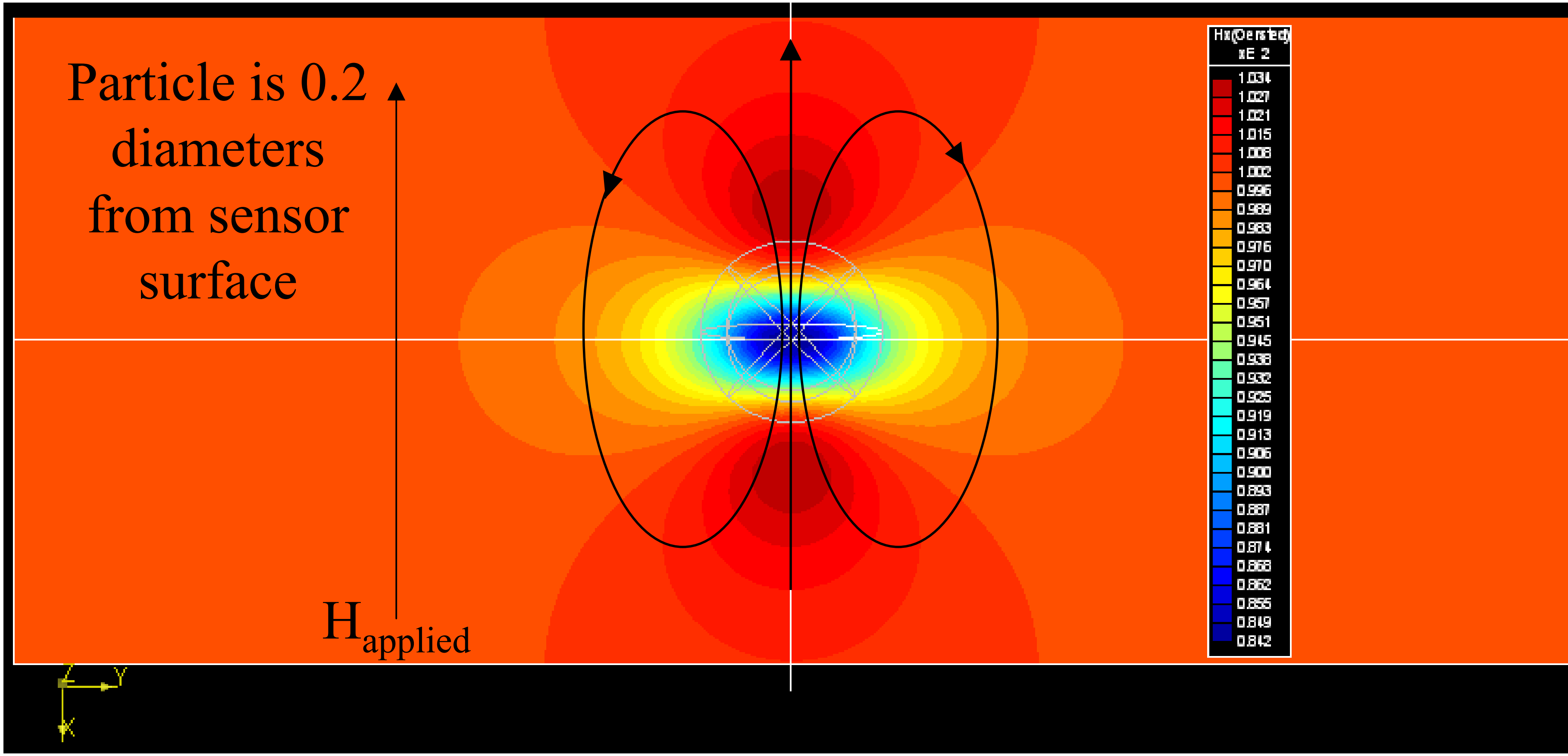
Stray Fields from Bound Magnetic Nanolabel

Detector sees $H_{Total} = H_{applied} + H_{stray}$ 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



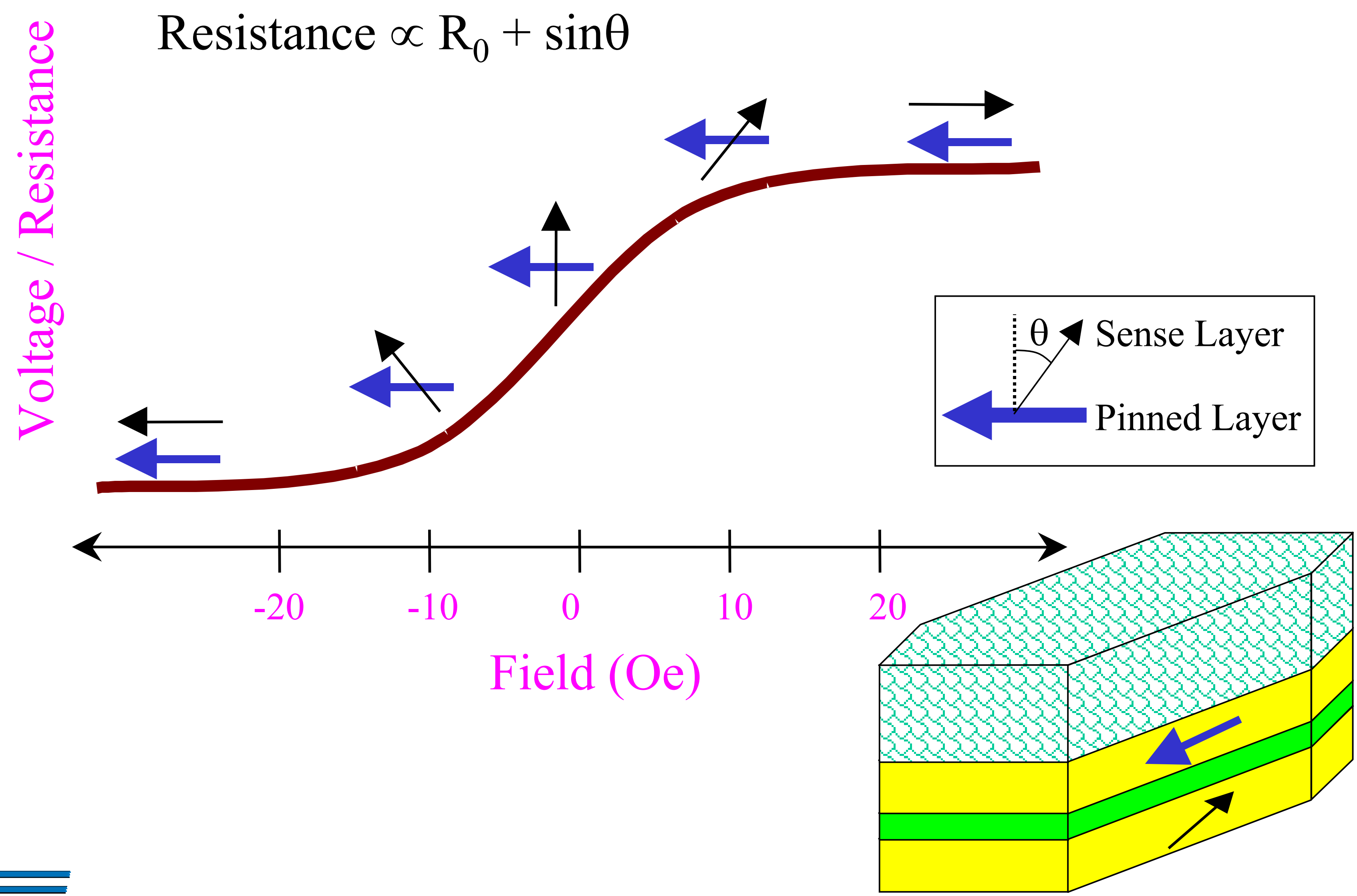
Detector Design Parameters

- 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 $(Bandwidth(Hz))^{0.5}$

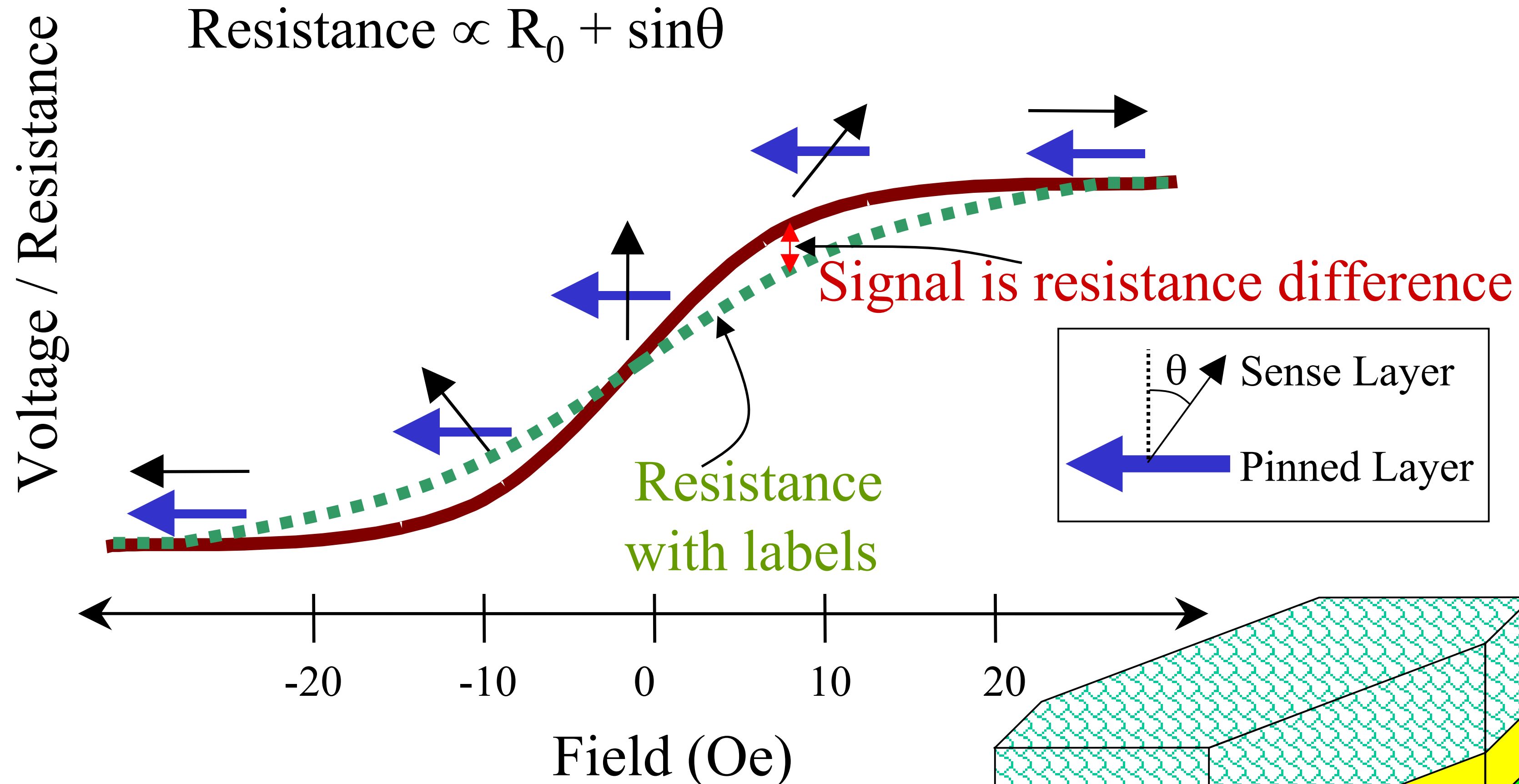
GMR Biosensor Detection Capabilities

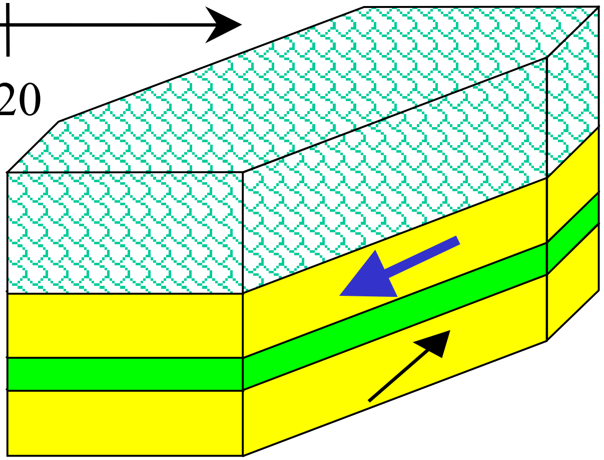
- 1 - Single label detection – small sensor required
- 2 – Combine many small sensors together to make 200 micron diameter sensor (match spot size)
- 3 – Field of view: (Sensor Area) x (*Label Diameter*)
- 4 – Dynamic Range of detected labels can be >> 3 logs

Idealized Spin Valve Transfer Curve



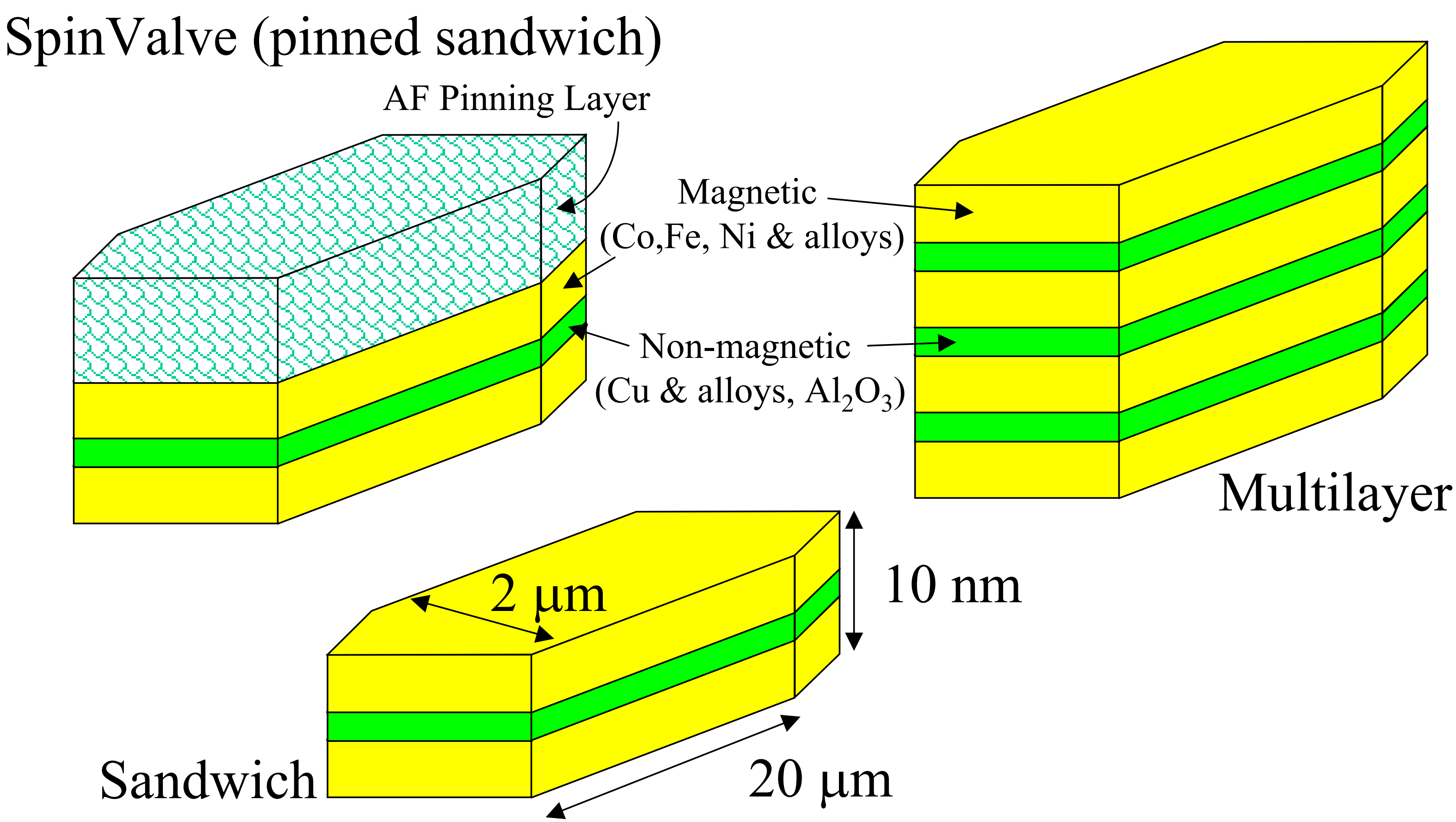
Resistance when labels are present





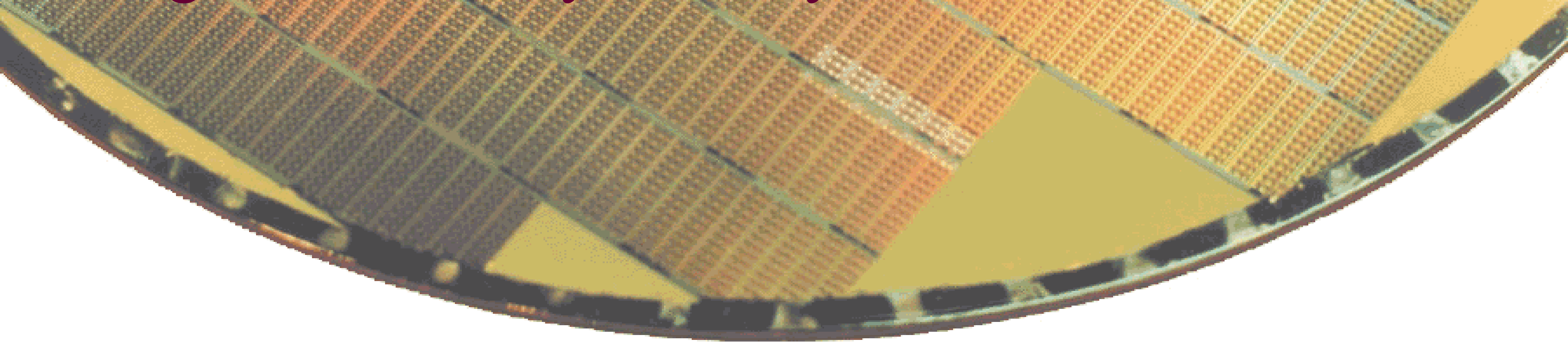
Magnetoresistive Material Structures

Resistance change due to Spin Dependent Electron Transport

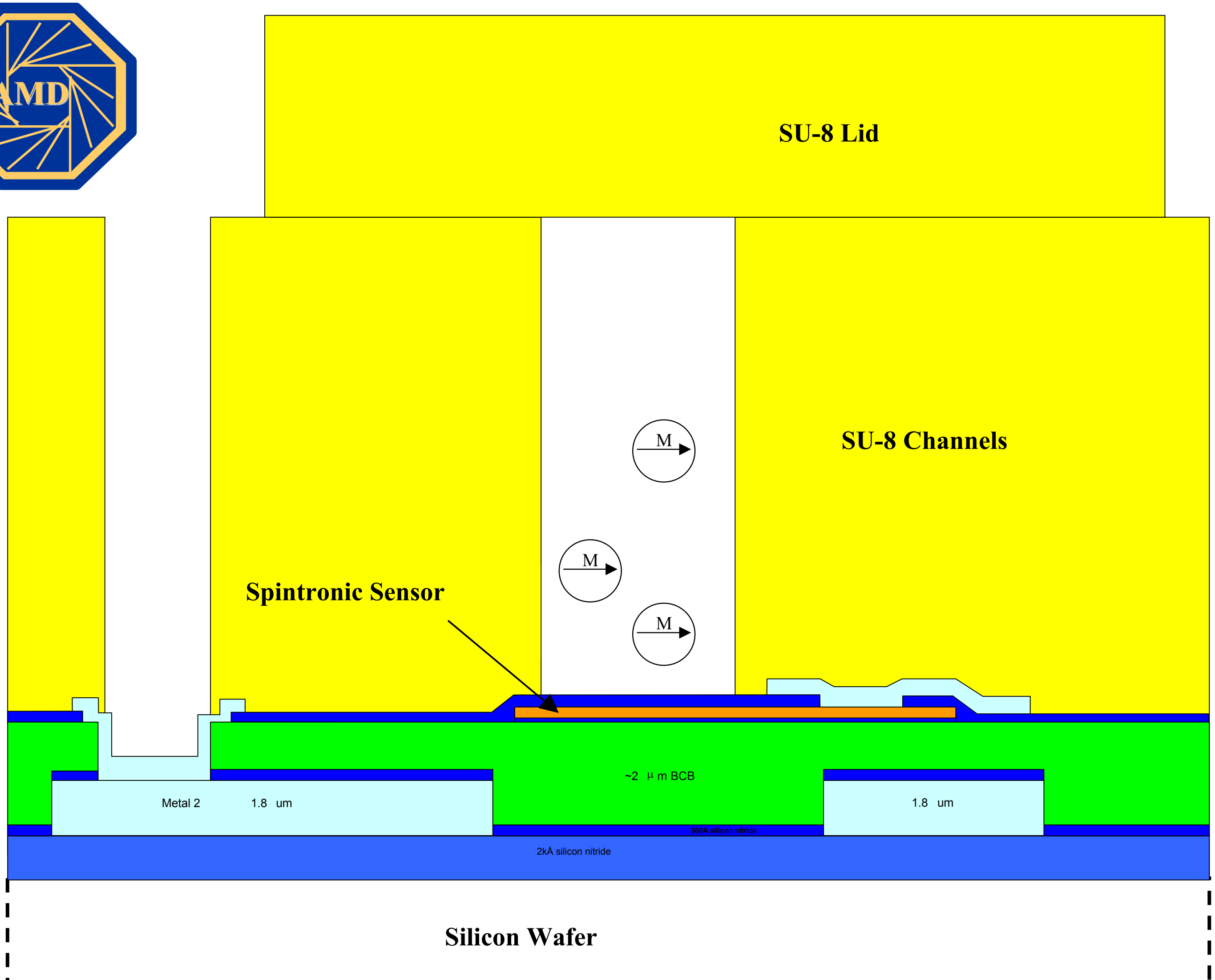


Sensors produced in semicond. wafer fab

- 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



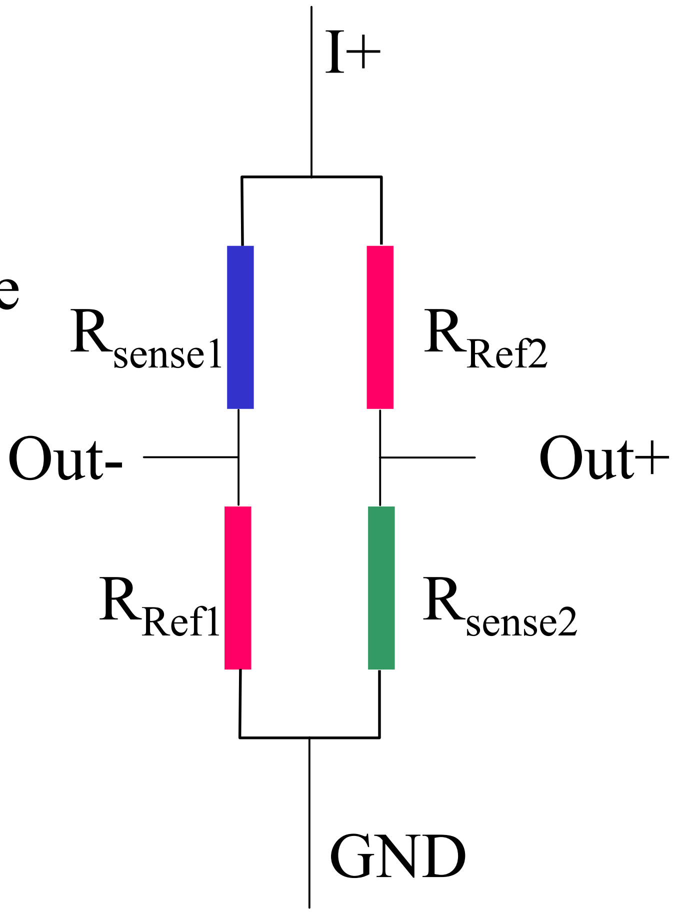
Wheatstone Bridge Design

Reference resistors for field and temperature compensation

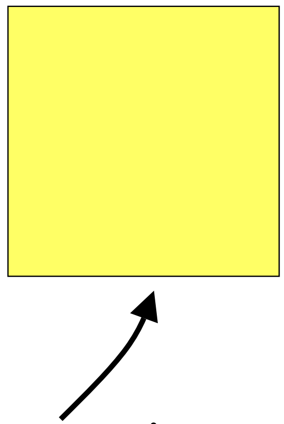
2 Sense resistors that experience the sample or field being measured

Reference resistors do not experience sample

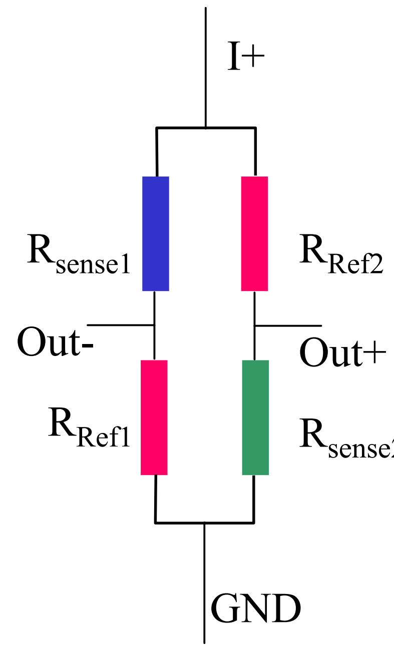
Vout = Out+ - Out-. If there is nothing to make the sense resistors behave differently than the refs, Vout = 0 Volts



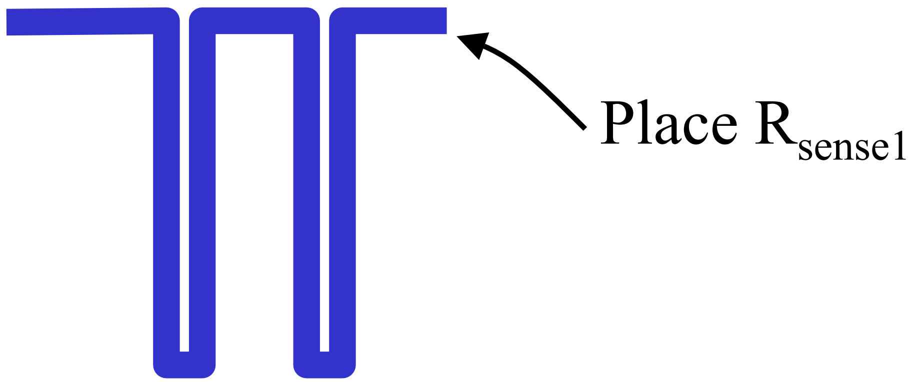
Layout: Define Sensing Region

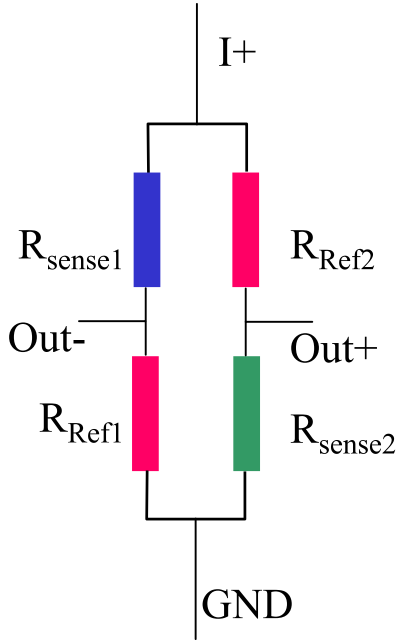


100 µm x 100 µm sense region

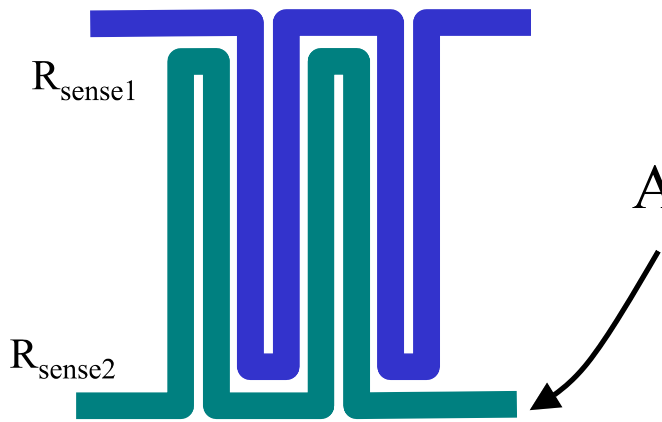


Layout: Place Sense Resistors

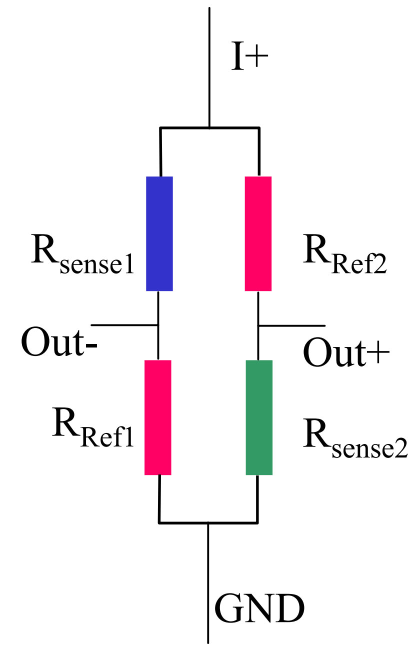




Layout: Place Sense Resistors

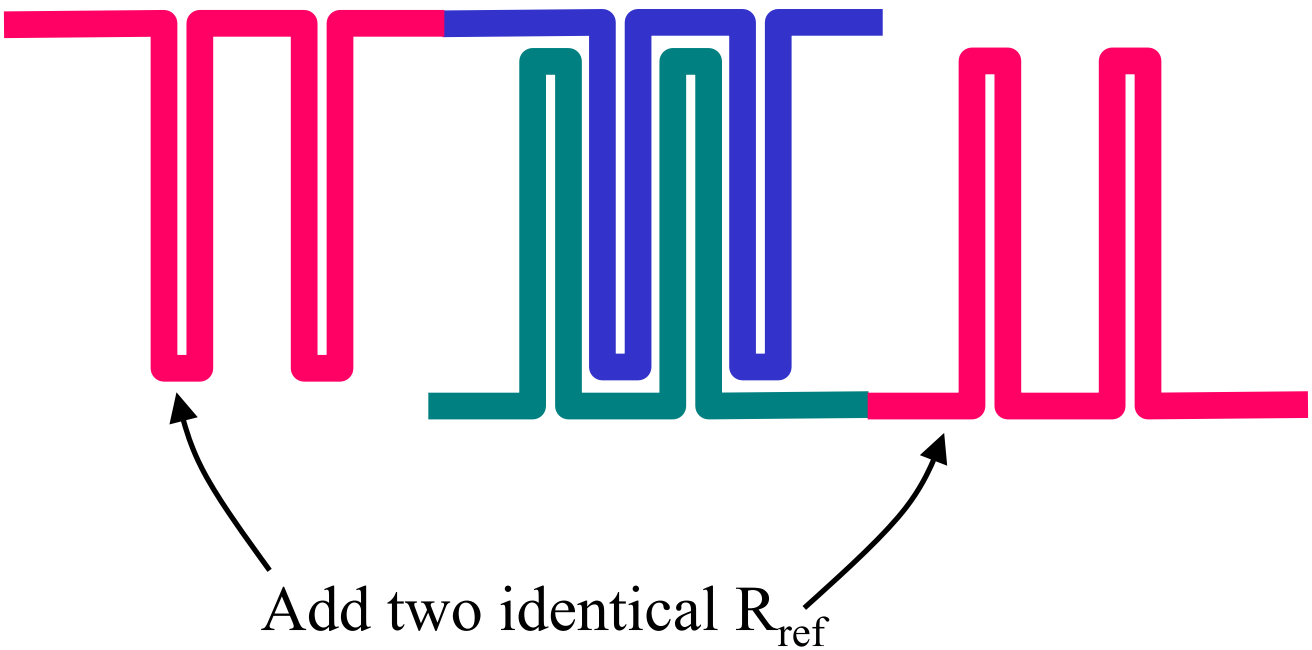


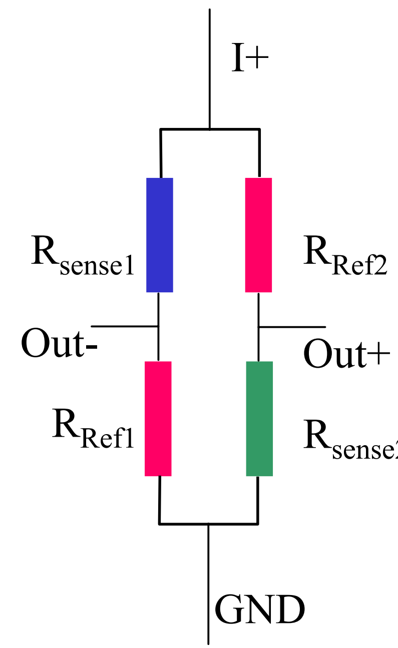
Add identical R_{sense2}



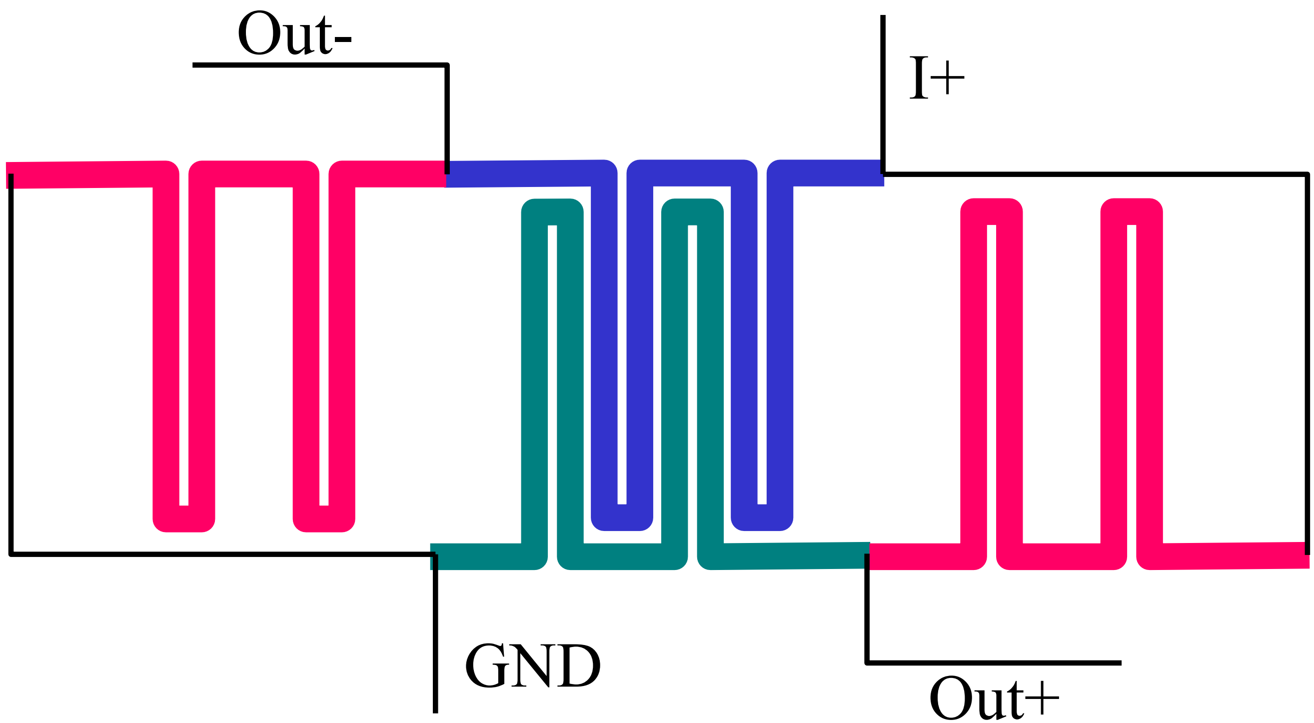
The two sense resistors are interwoven to allow them to sense the same region and experience the same excitation field.

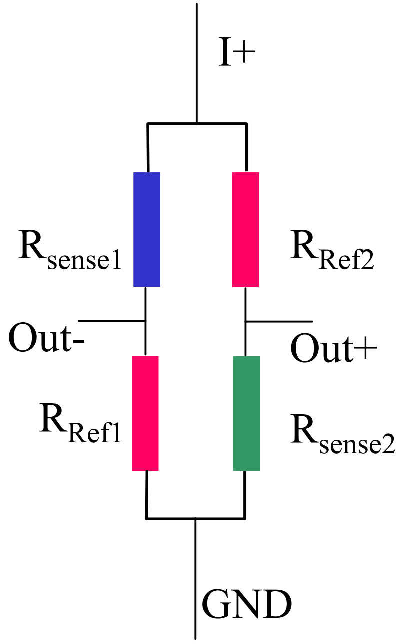
Layout: Place Reference Resistors





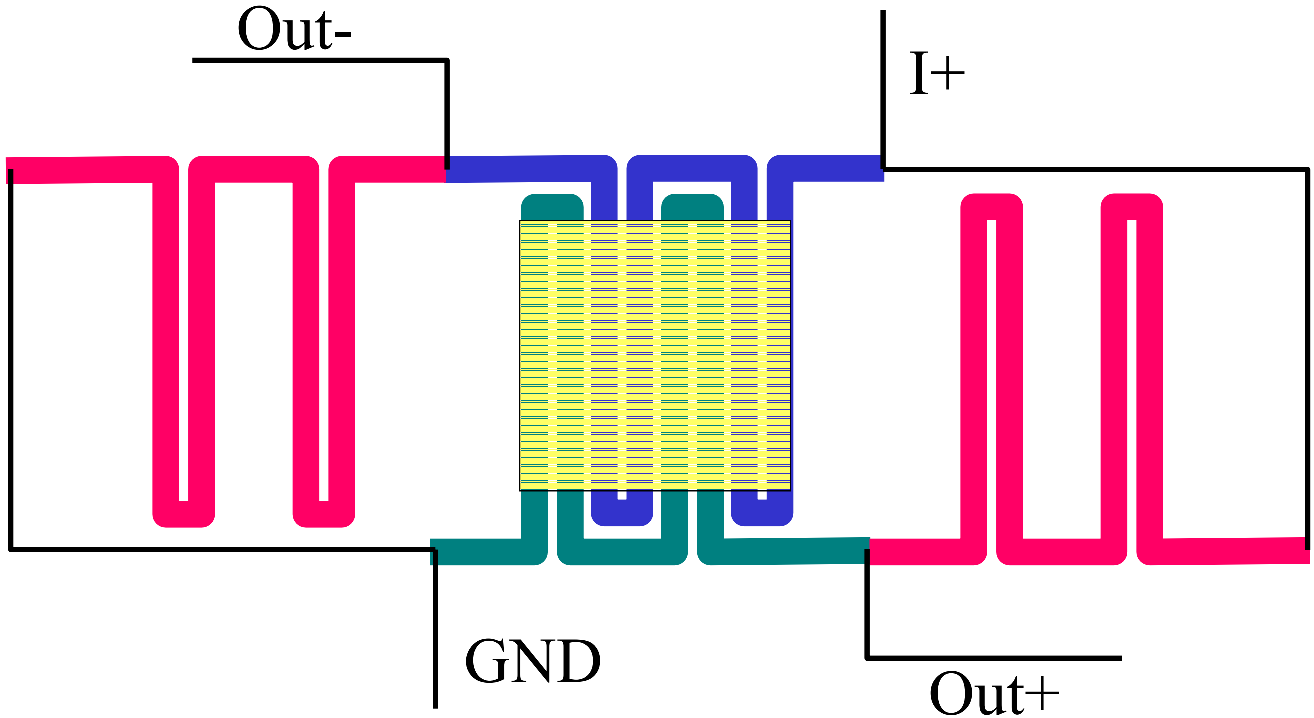
Layout: Add Al Interconnect

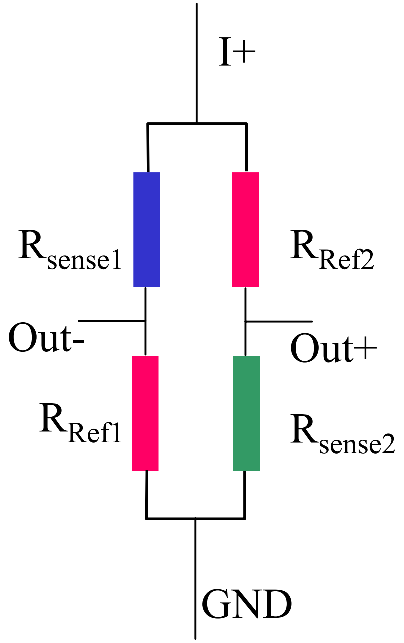




Add interconnection metal (Aluminum)

Layout: Add sense region

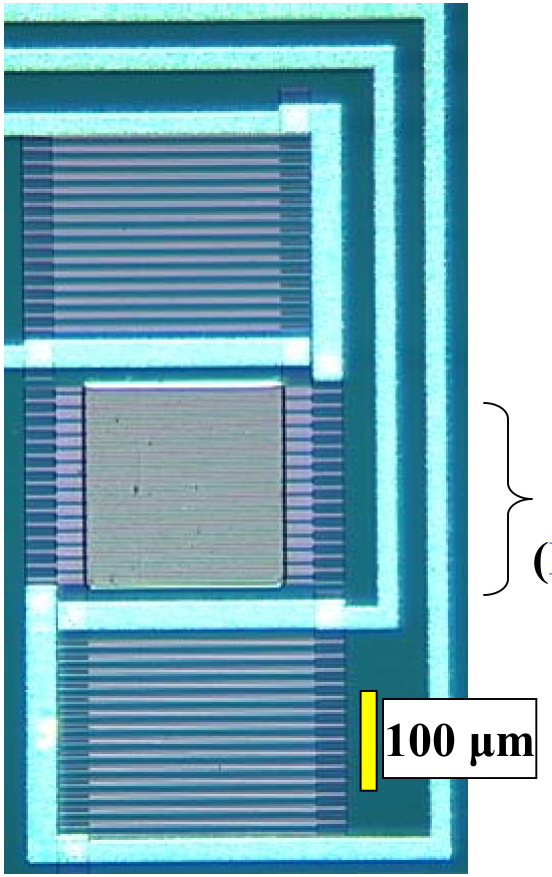


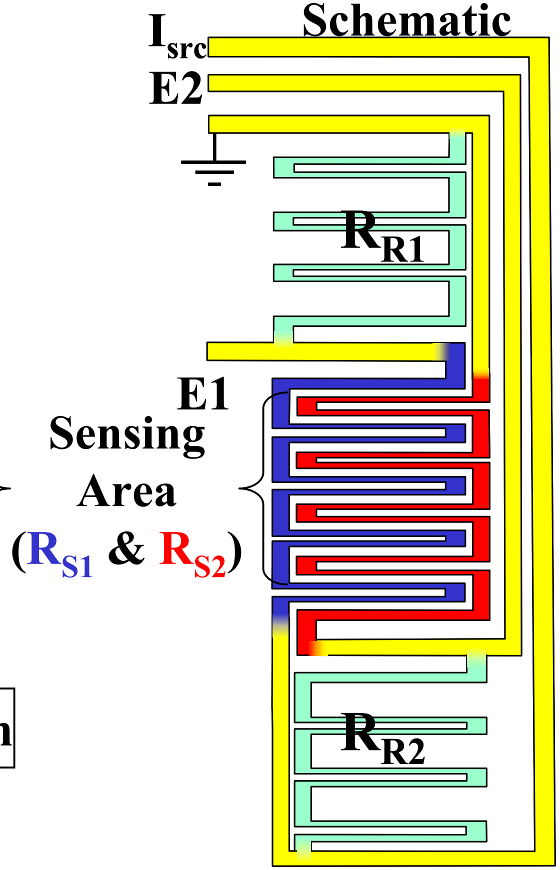


Define sensing region by selective etching and surface chemistry

GMR Chip Design

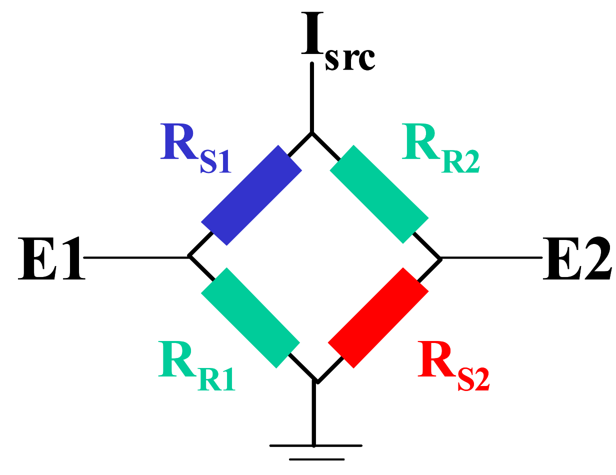
Actual Sensor





IOWA STATE UNIVERSITY

Bridge Circuit



- **Sensing GMRs – interdigitated R_{S1} and R_{S2}**
- **Reference GMRs – R_{R1} and R_{R2}**

- **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.**

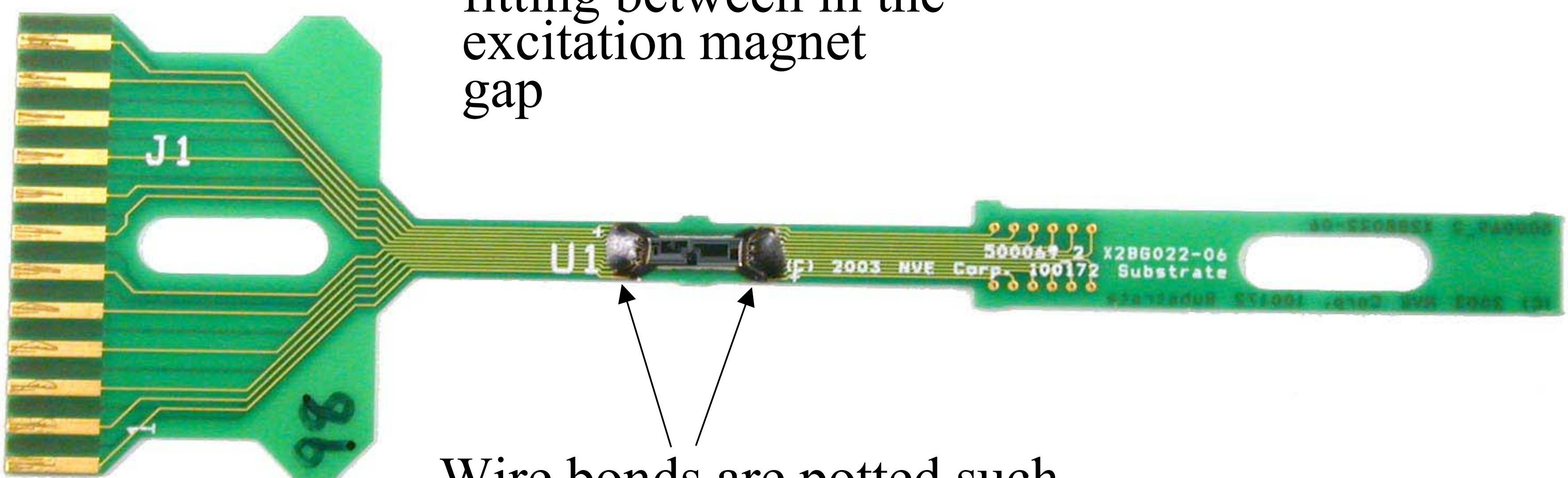
Die-Holding Printed Circuit Board

"Diving Board"

<u>Design Basics</u>

24 pin surface mount edge connector

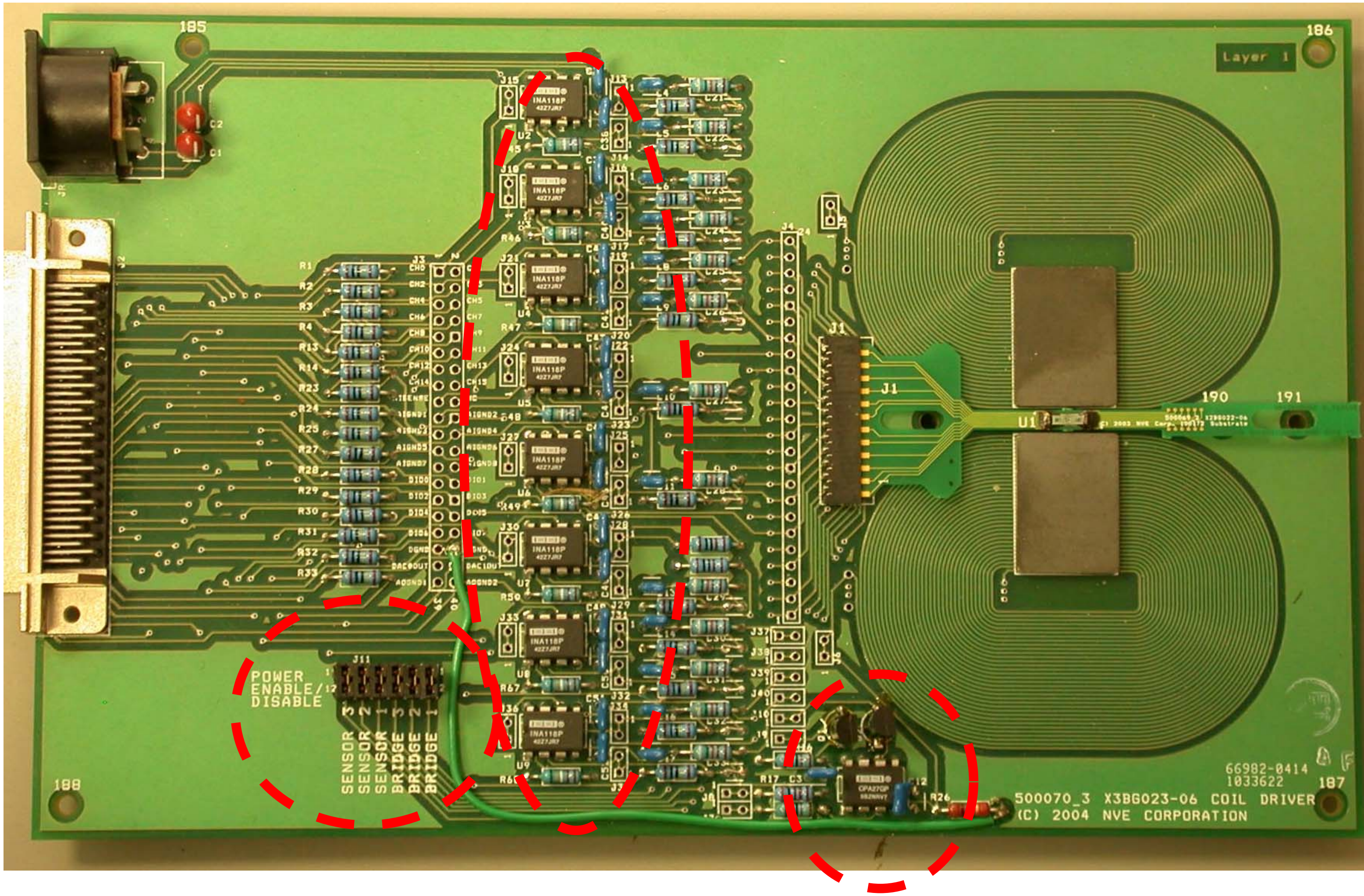
Narrow die area for fitting between in the excitation magnet gap



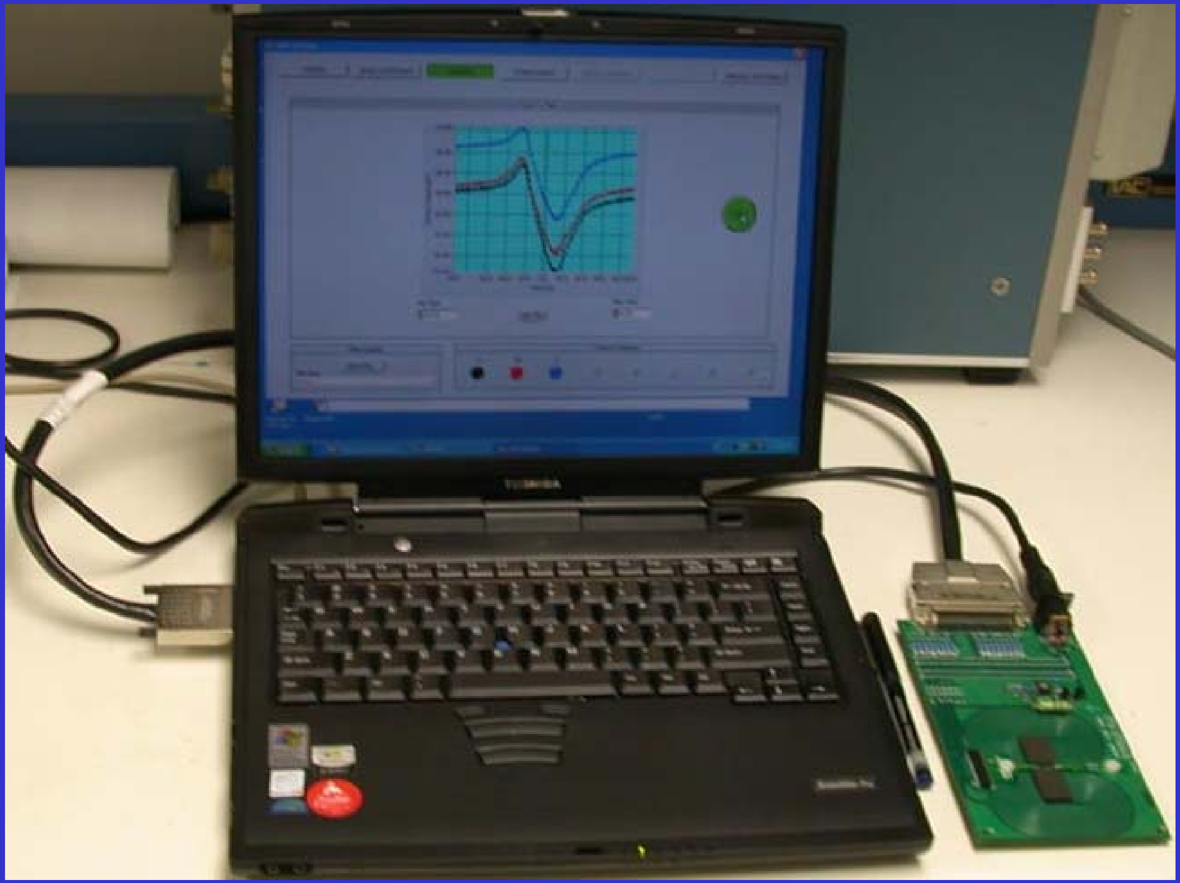
Wire bonds are potted such that sense pad is still exposed

Magnetic Excitation Module

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



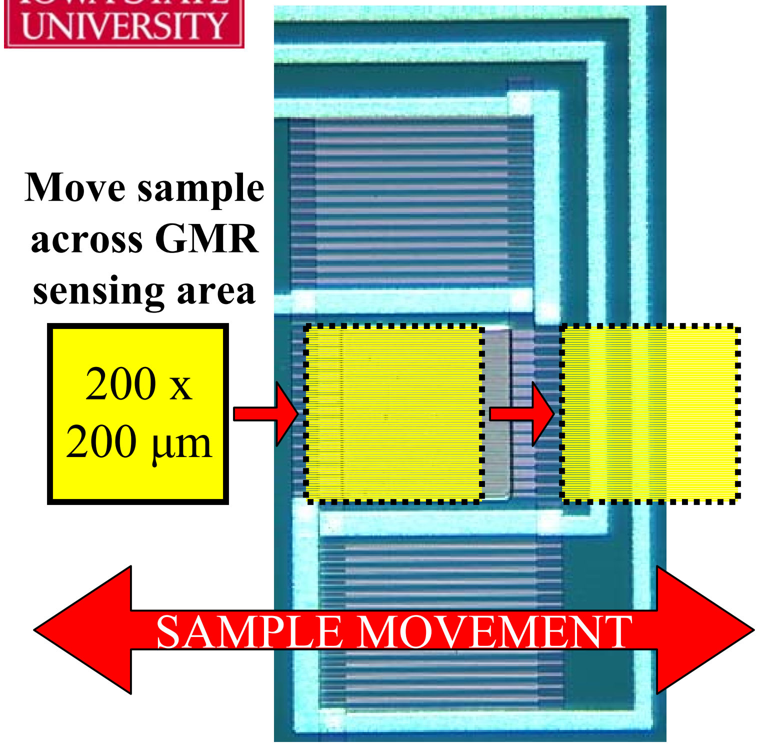
BioMagnetIC System

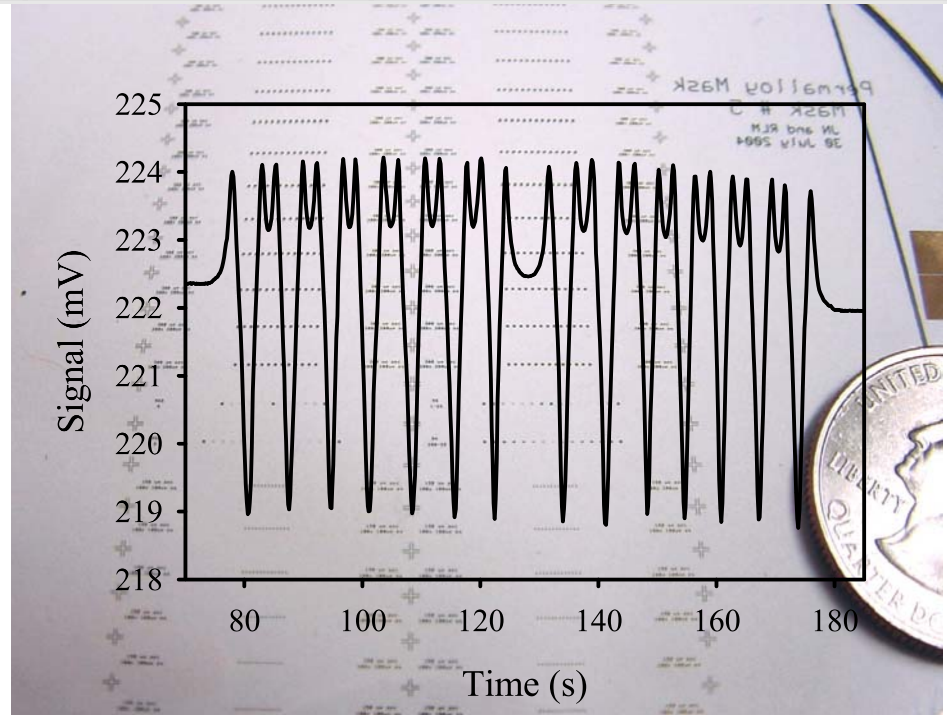


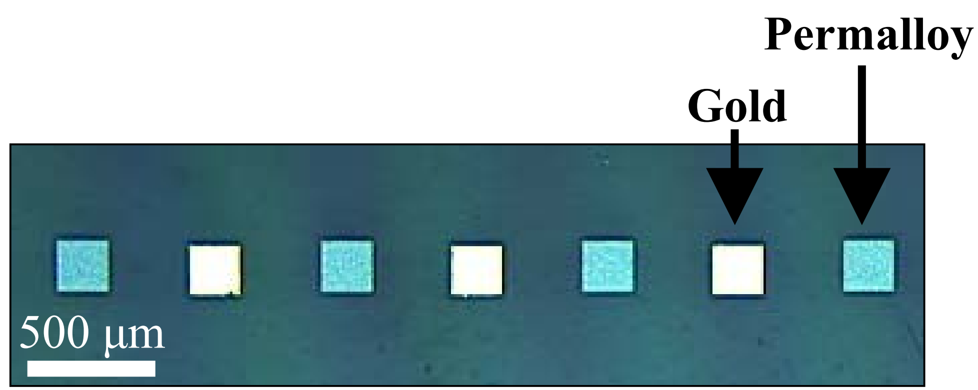
- A to D card in laptop
- Pocket-sized excitation module
- Disposable sensor cartridges
- Adaptable device development platform
- R vs. H plots
- Vout vs. time

Magnetic Bioassay: Scanned Sample Mode

Sample Stick Experiment





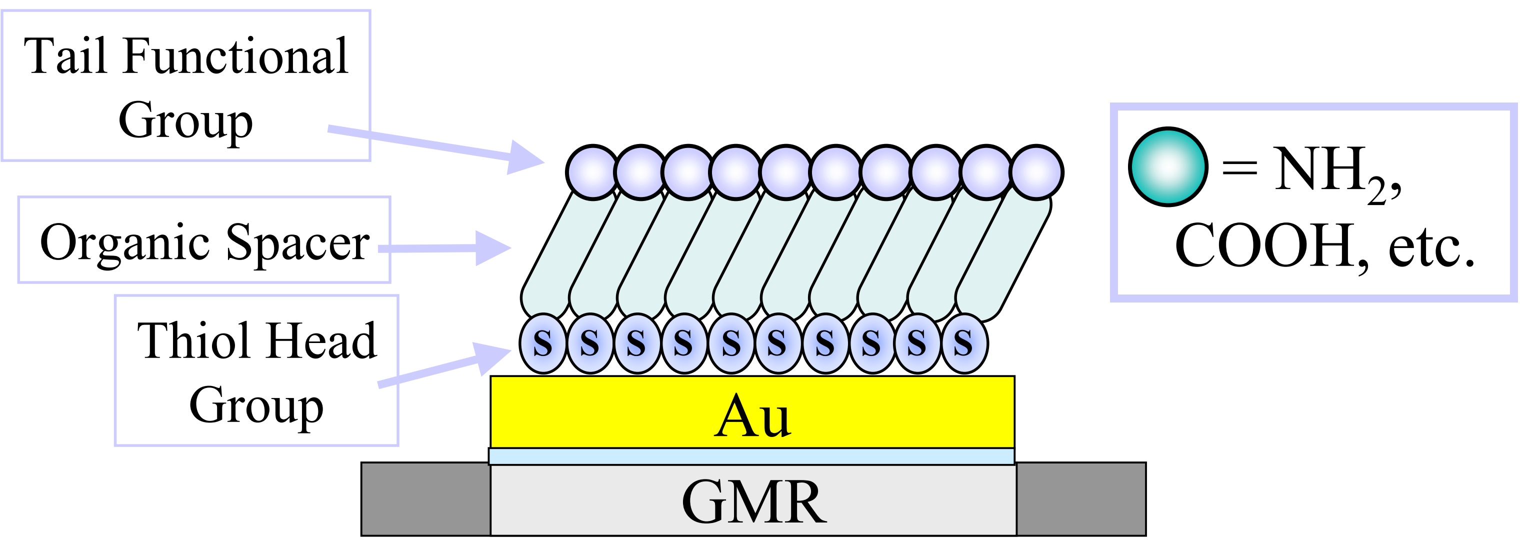


- External field at 150 Oe
- Sample is held near the GMR (~50 µm) and moved across sensing area

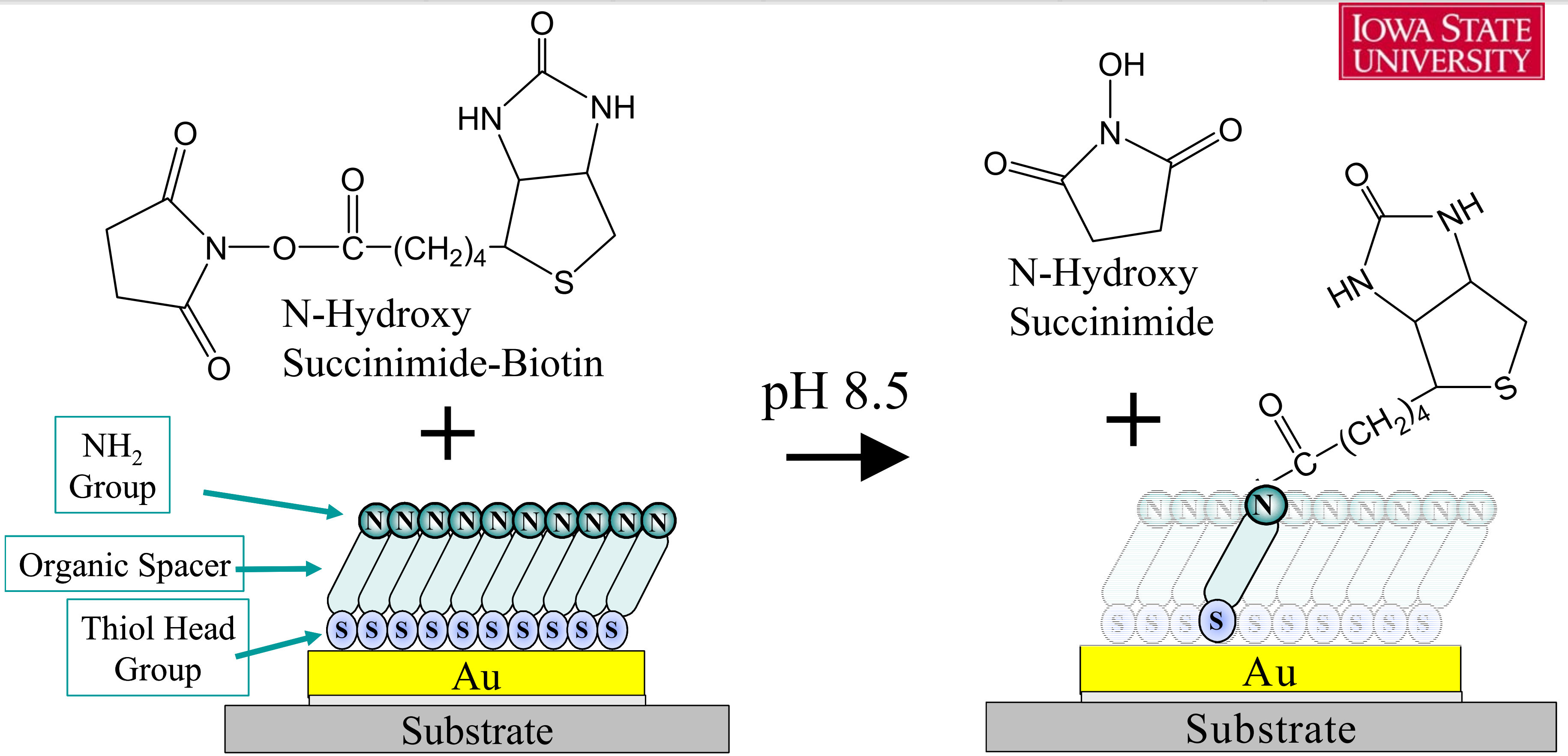
Surface Functionality & Versatility

IOWA STATE UNIVERSITY

- 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

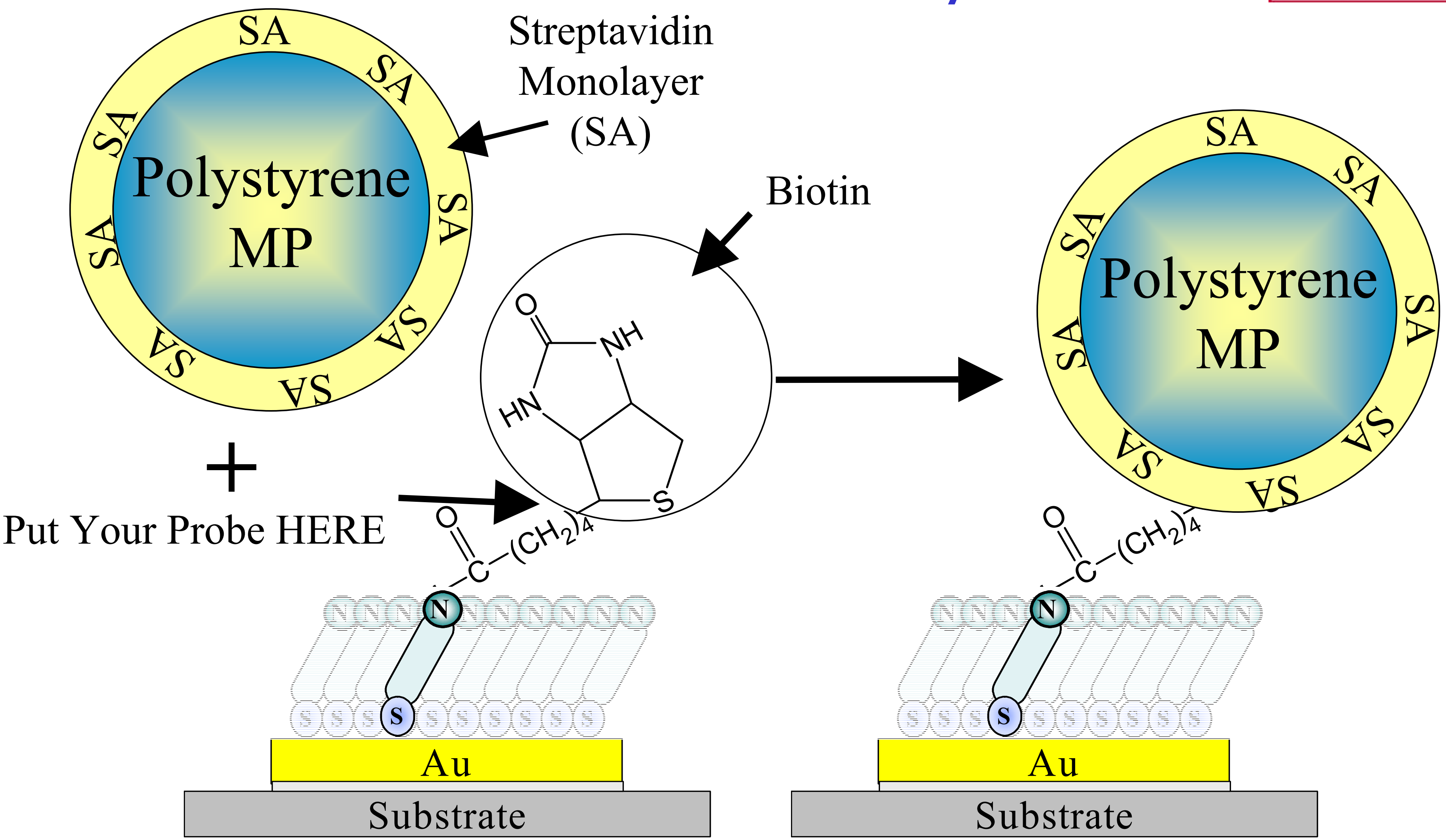


Surface Biotinylation



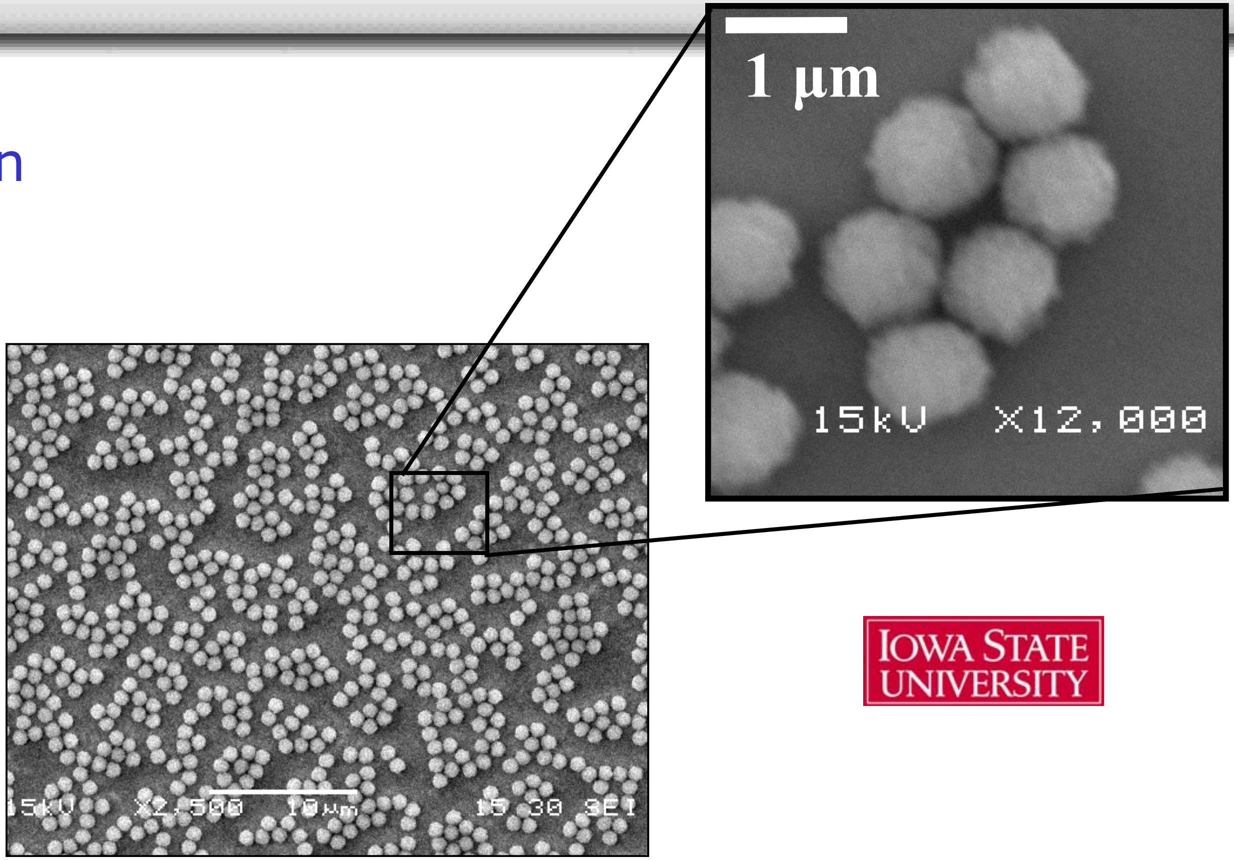
NHS-Biotin reacts with primary amines to form an amide bond.

Assay Labeling with Magnetic Particles: Biochemistry



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)

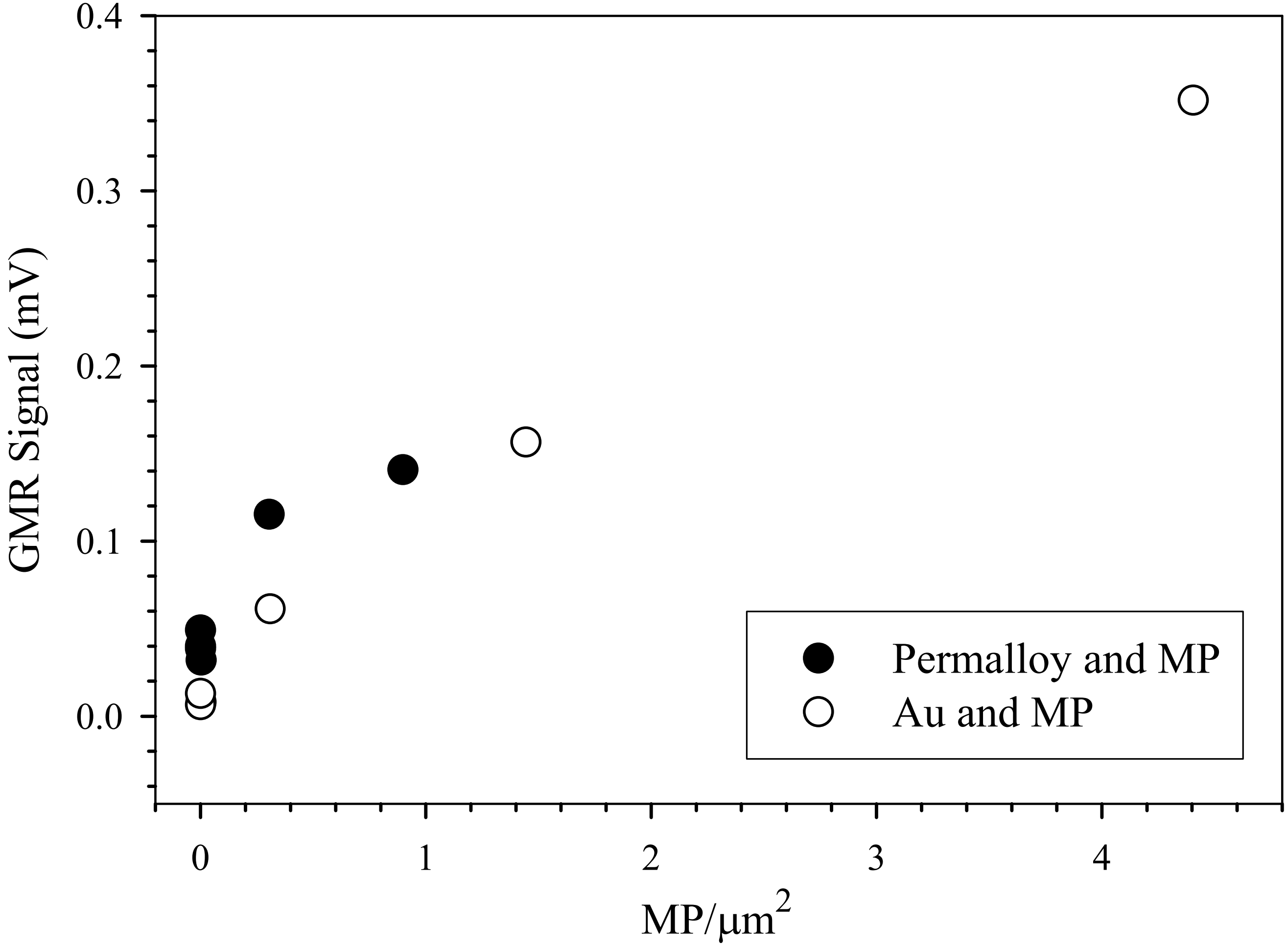


IOWA STATE UNIVERSITY

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

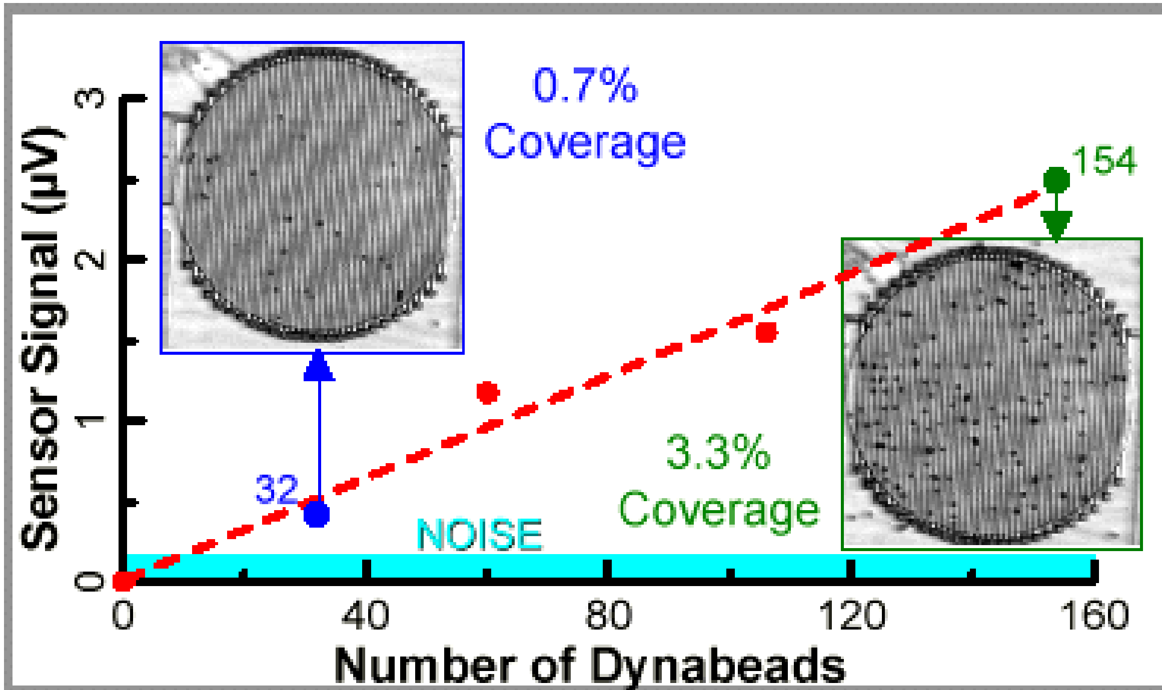


- **Limit of detection (3x SNB) is 0.005 MP/µm^2, or 215 MP per gold square.**

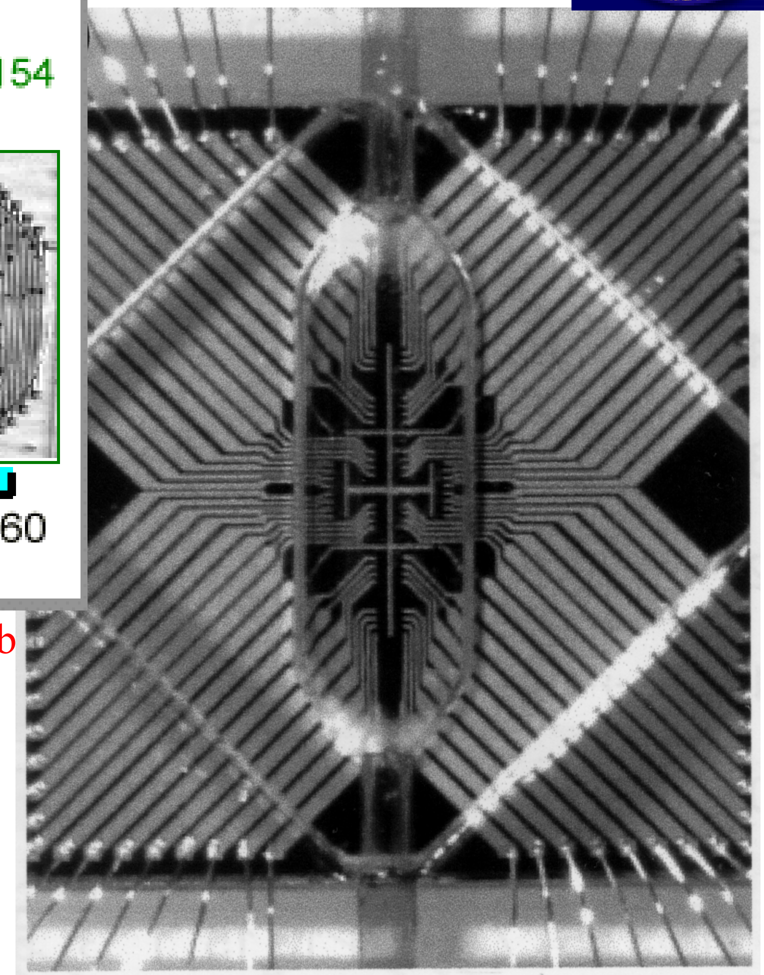
Best Sensitivity: Bind directly on detector

Magnetic Biosensor μArray: High Sensitivity

2.8 micron DynalBeads



Courtesy L. Whitman, Naval Research Lab

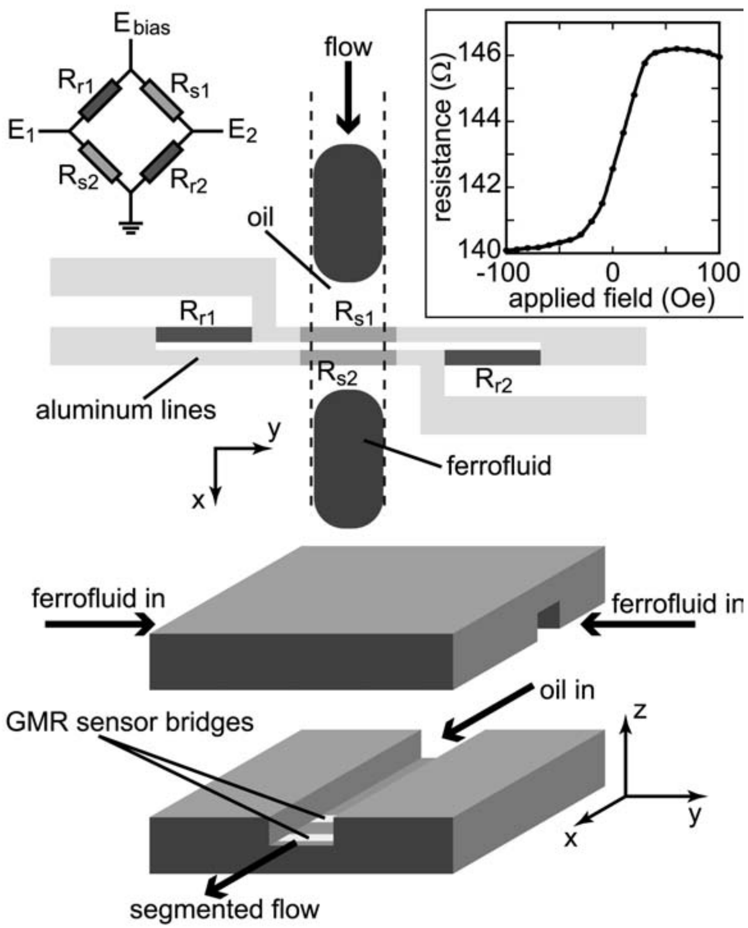


1) Single label detection is possible
2) >3 decades of dynamic range
3) Better than 1 fMolar with fluidics
4) Magnetism enhances specificity

In-Flow Integrated Detection of Magnetic Droplets

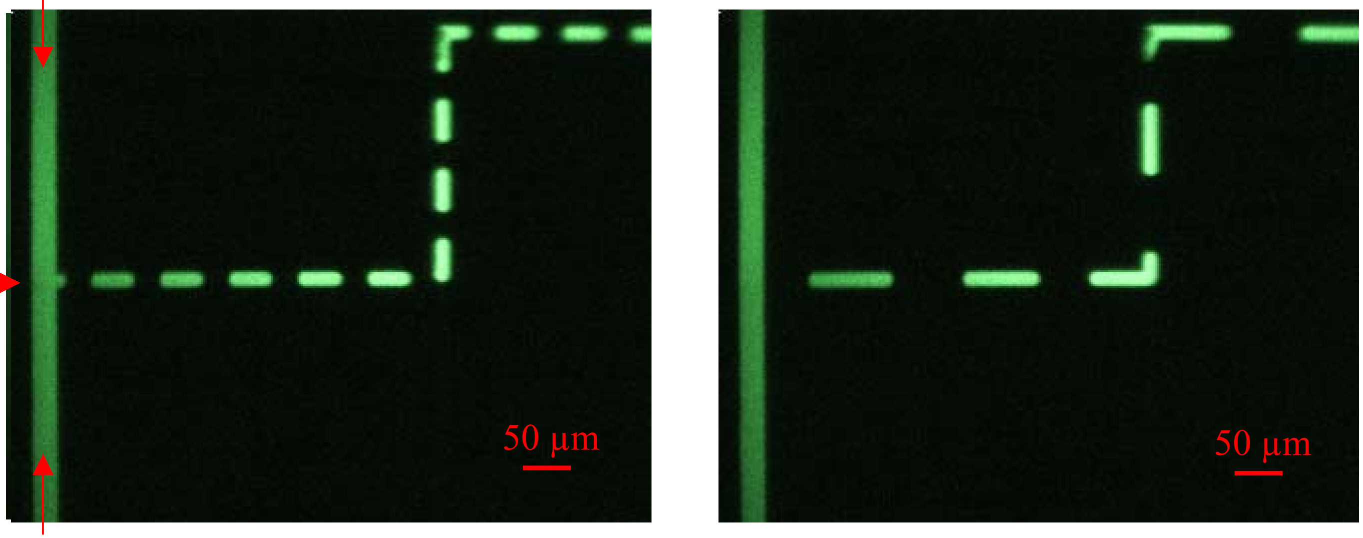
GMR Sensing of Magnetic Picodroplets

IOWA STATE UNIVERSITY



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

Ferrofluid Plug Formation

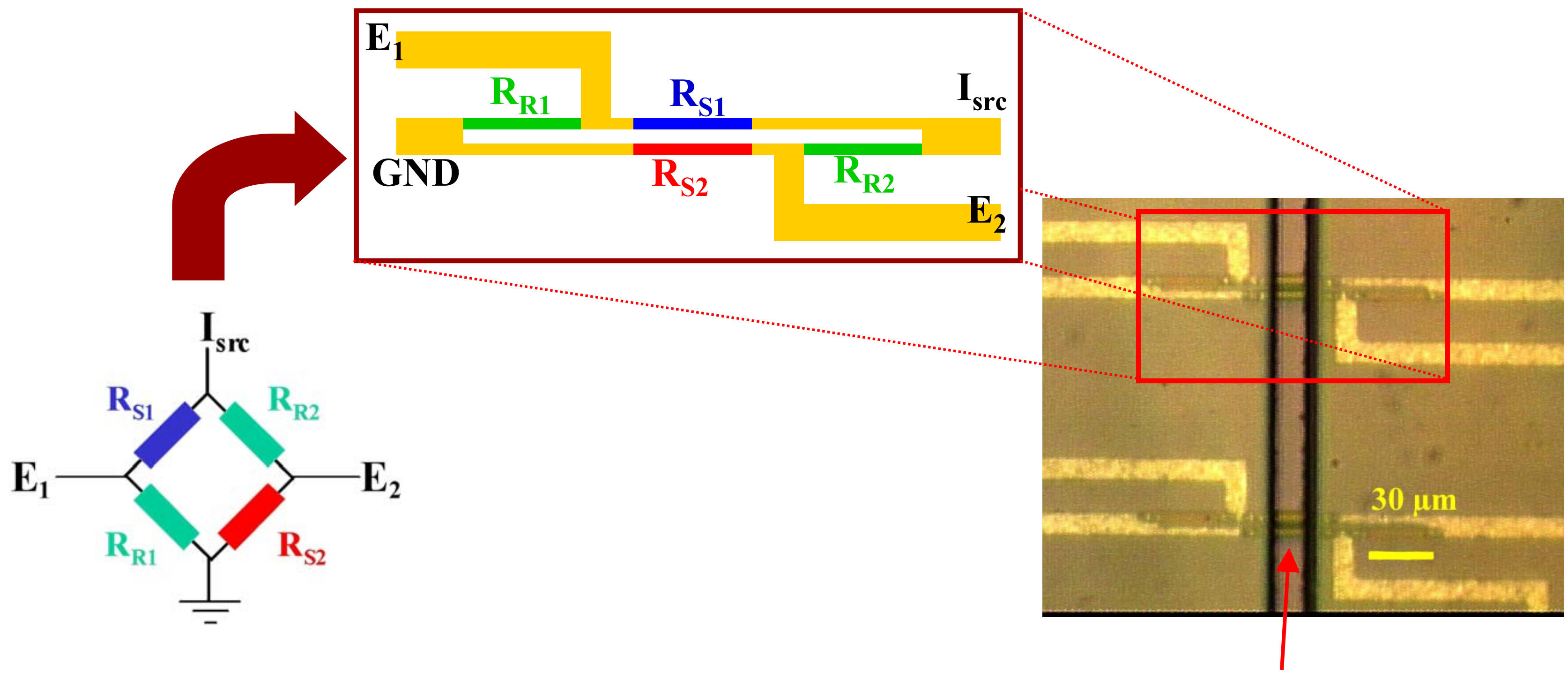


- Flow rate 1.0 µL/min
- Plugs formed at approx. 500 Hz

- Flow rate 0.2 µL/min
- Plugs formed at approx. 50 Hz

GMR Sensor Architecture

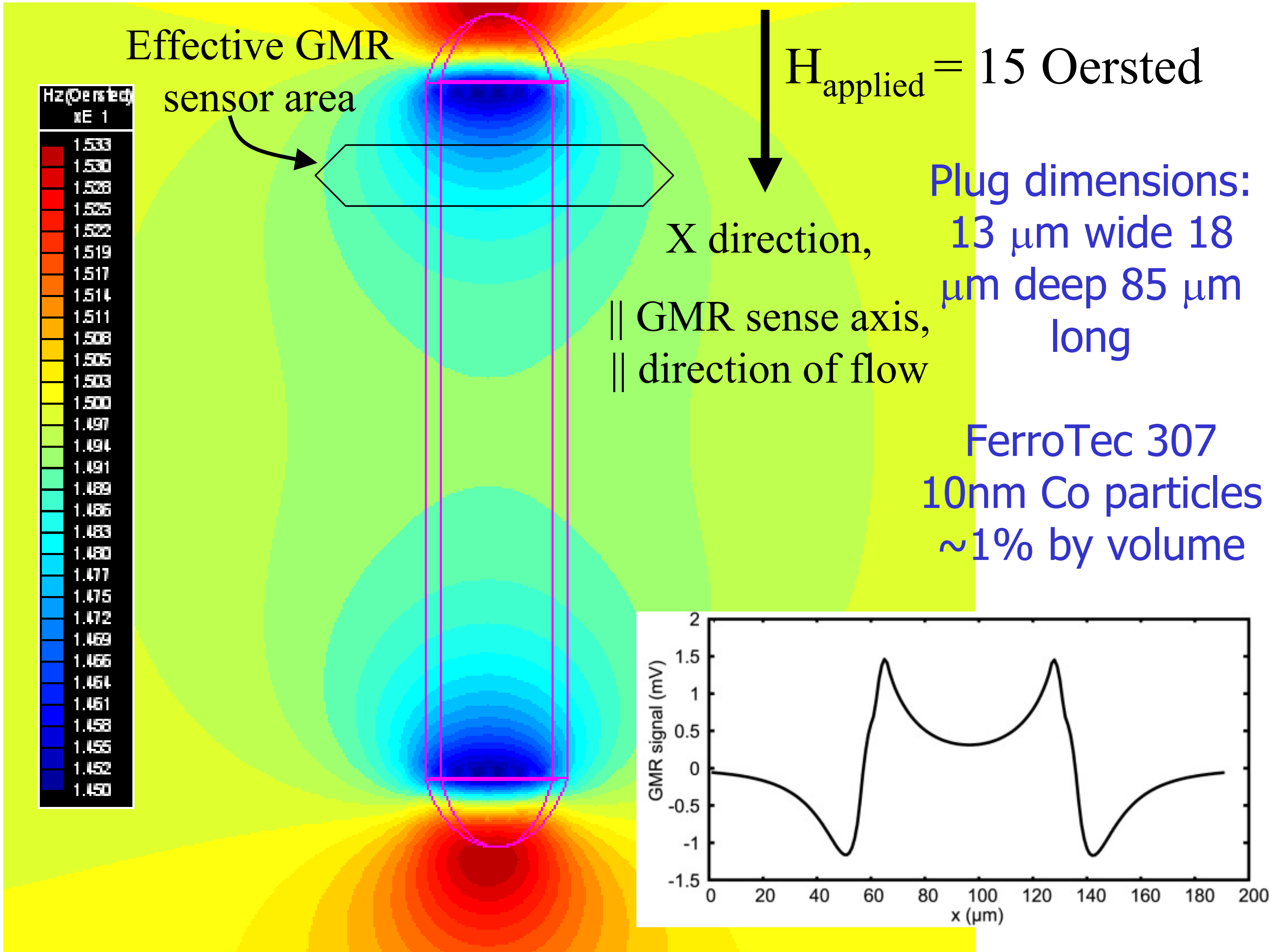
•Two reference and two sensing GMRs configured as a Wheatstone bridge



•Channel passes over sensing GMRs

H_x in Sensor Plane, Flowing Ferrofluid Plug

Simulation using "Amperes" magnetic modeling software

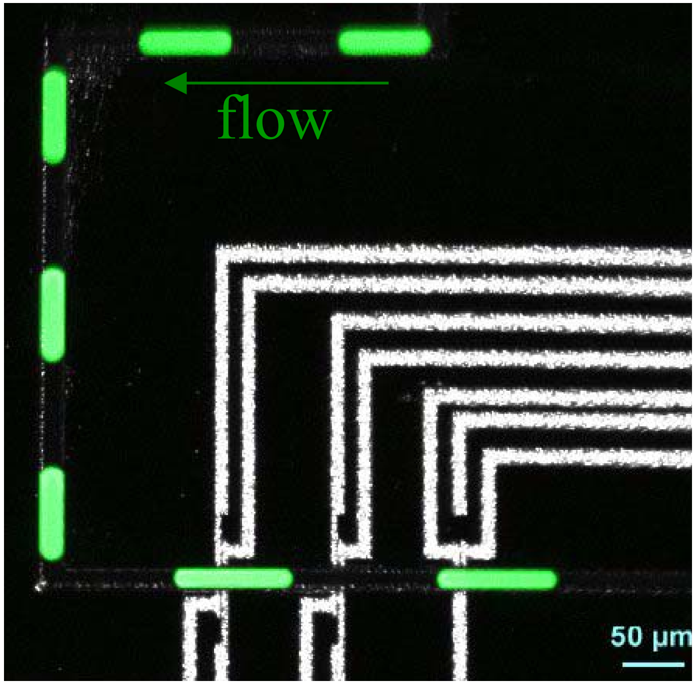


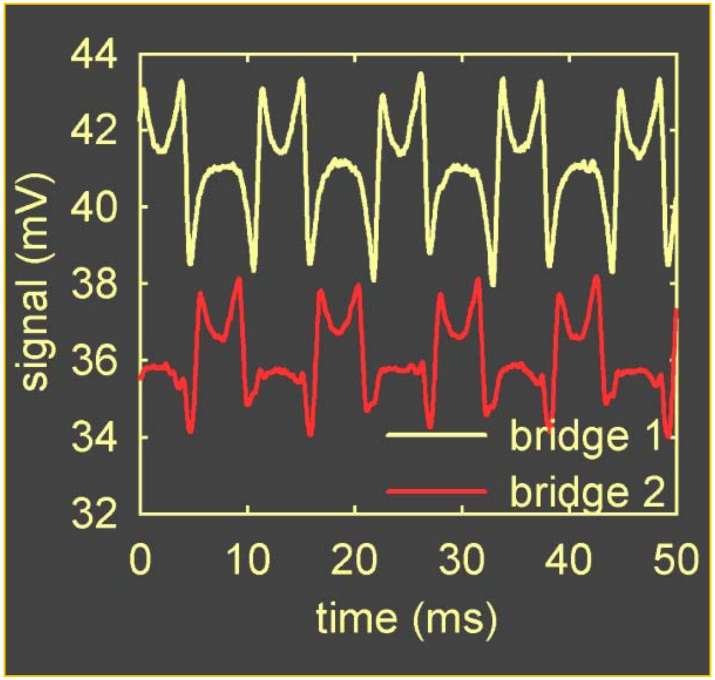
Plug dimensions: 13 μm wide 18 μm deep 85 μm long

FerroTec 307
10nm Co particles
~1% by volume

NVE

Direct Flow Velocity Monitoring





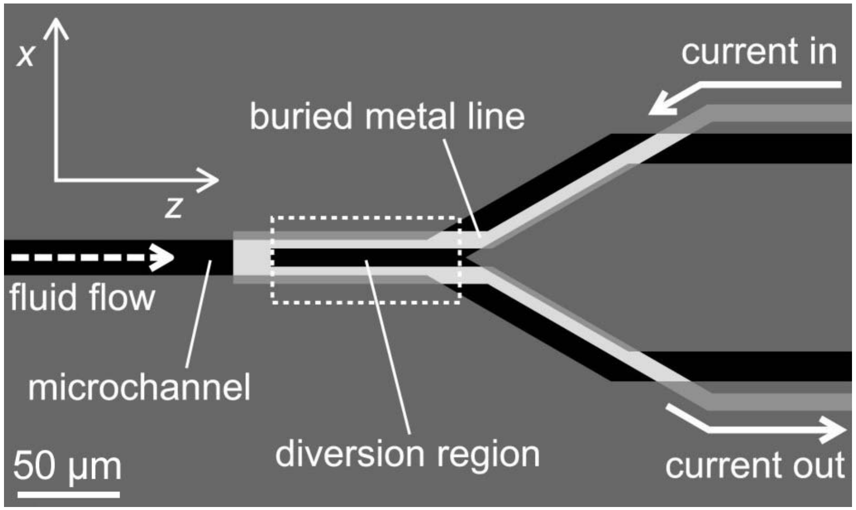
Excitation field 15 Oe; Flow rate 250 nL/min; 1.2% magnetite v/v

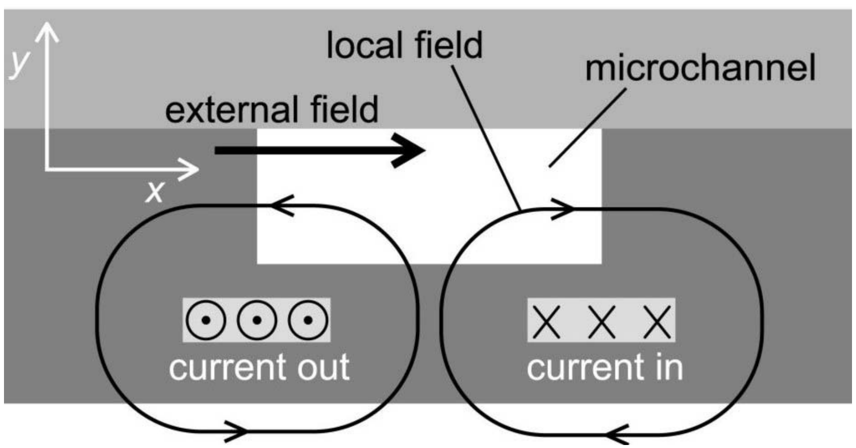
Velocity determined by cross-correlating the signals from two bridges

In-Flow Manipulation of Magnetic Particles

Diverter Design and Fabrication

IOWA STATE UNIVERSITY

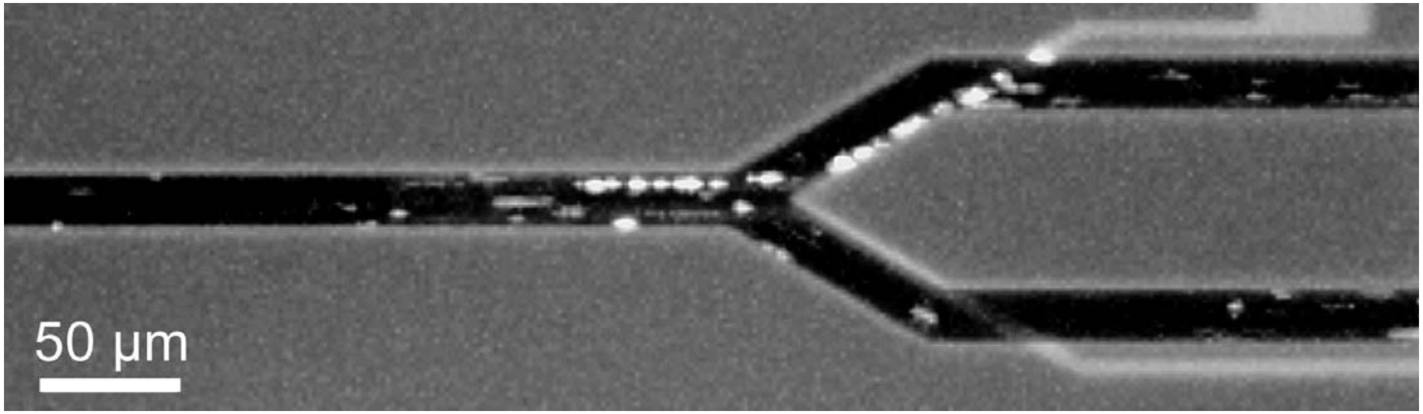


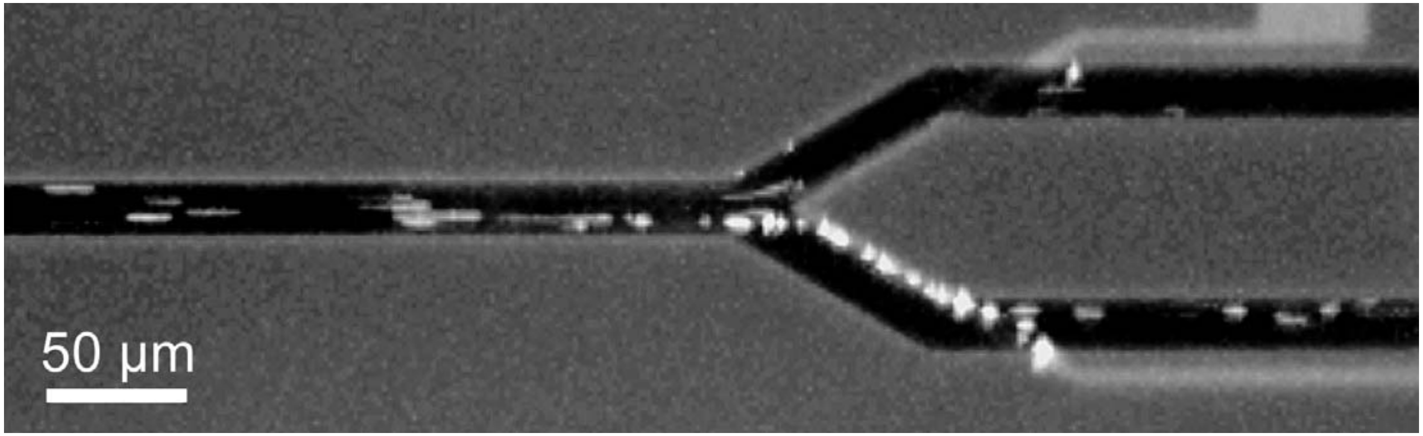


- A uniform external field magnetizes particles
- Current lines induce field gradients of 10^2-10^3 T/m
- Resulting force diverts particles to a desired channel

Magnetic Flow Sorting Experiments

IOWA STATE UNIVERSITY

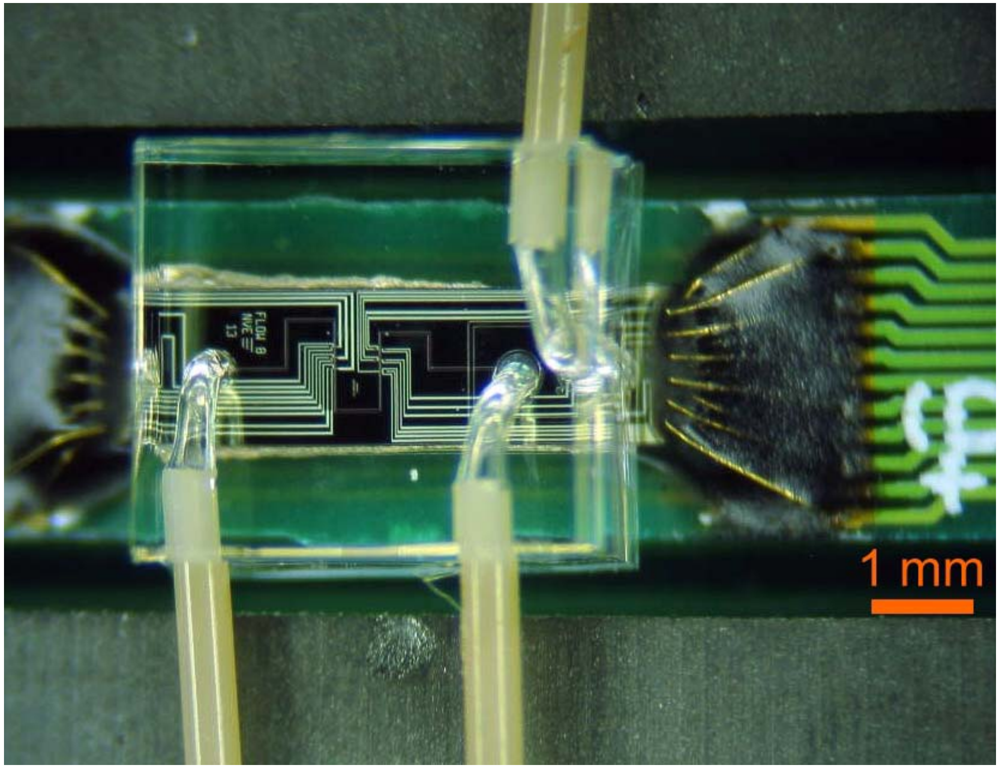




- Bangs Labs, 28% magnetite, 1 µm
- Flow rate: 6 nL/min
- 85% of the beads in desired channel

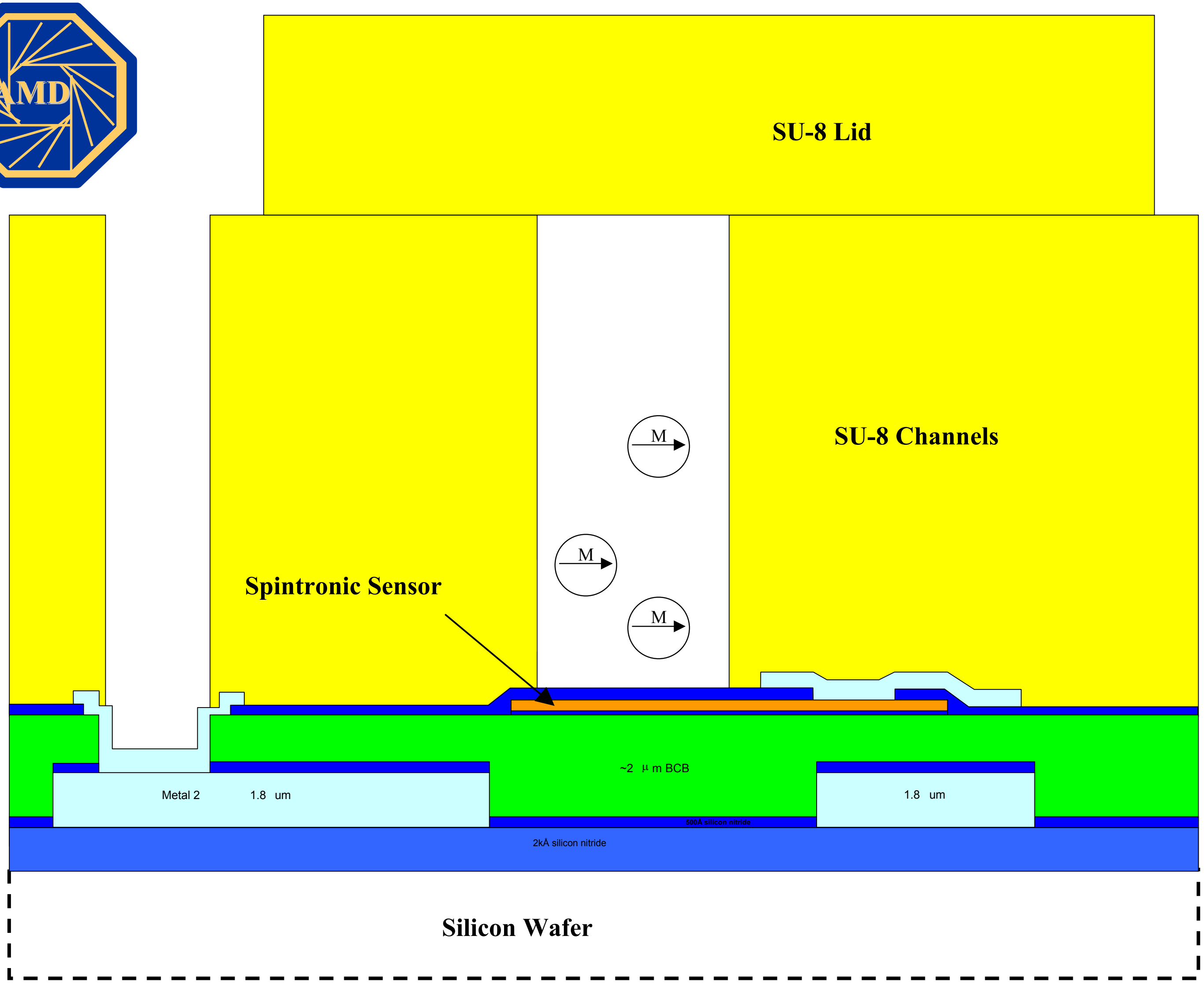
NVE

Device Packaging with PDMS

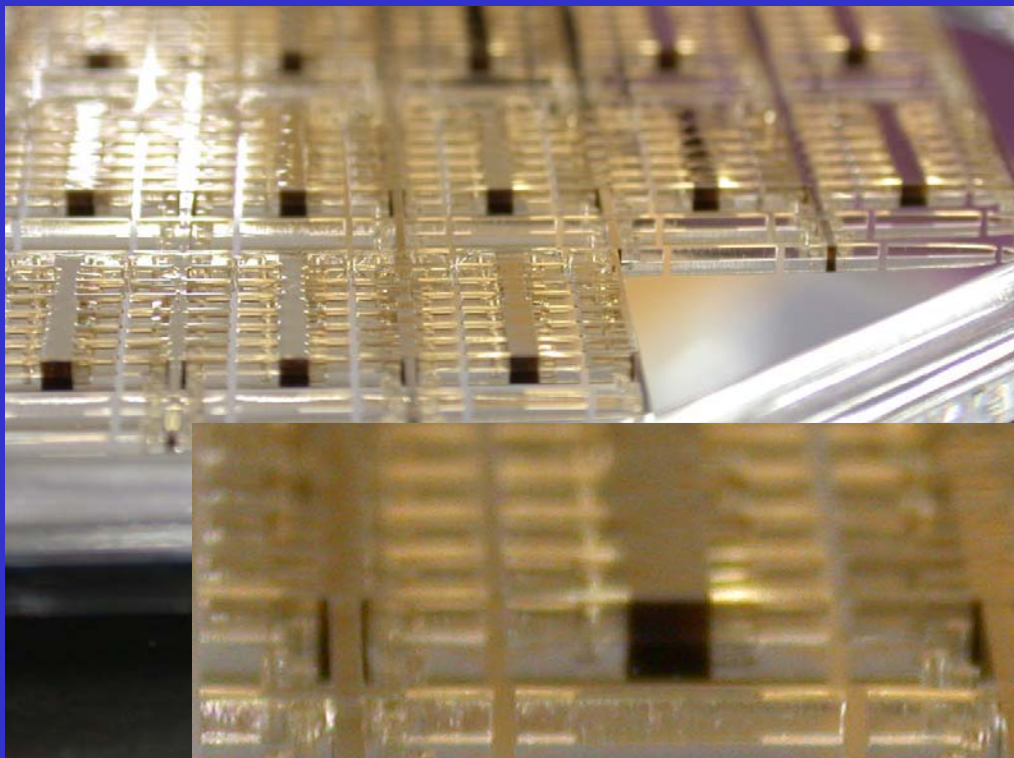


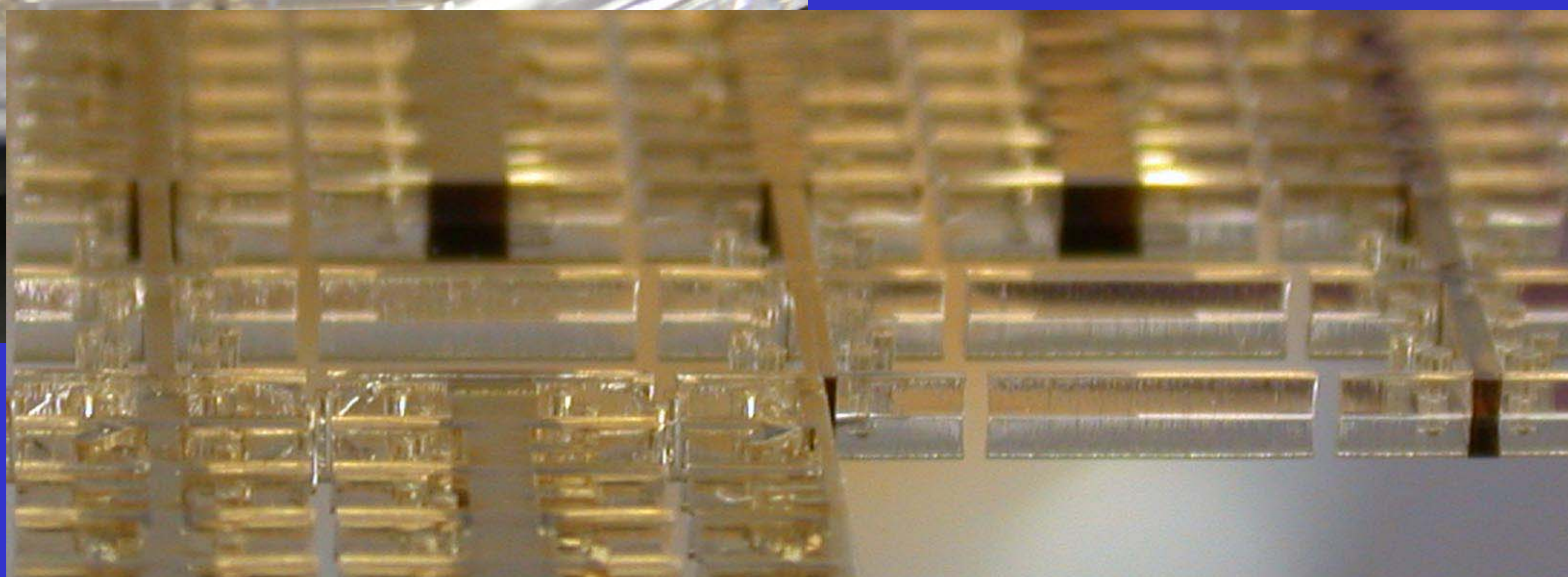
- Electrical connections
- PDMS Fluidic connections
- Optical access

SU-8 Lid using wafer-scale bonding method



SU-8 on GMR wafers

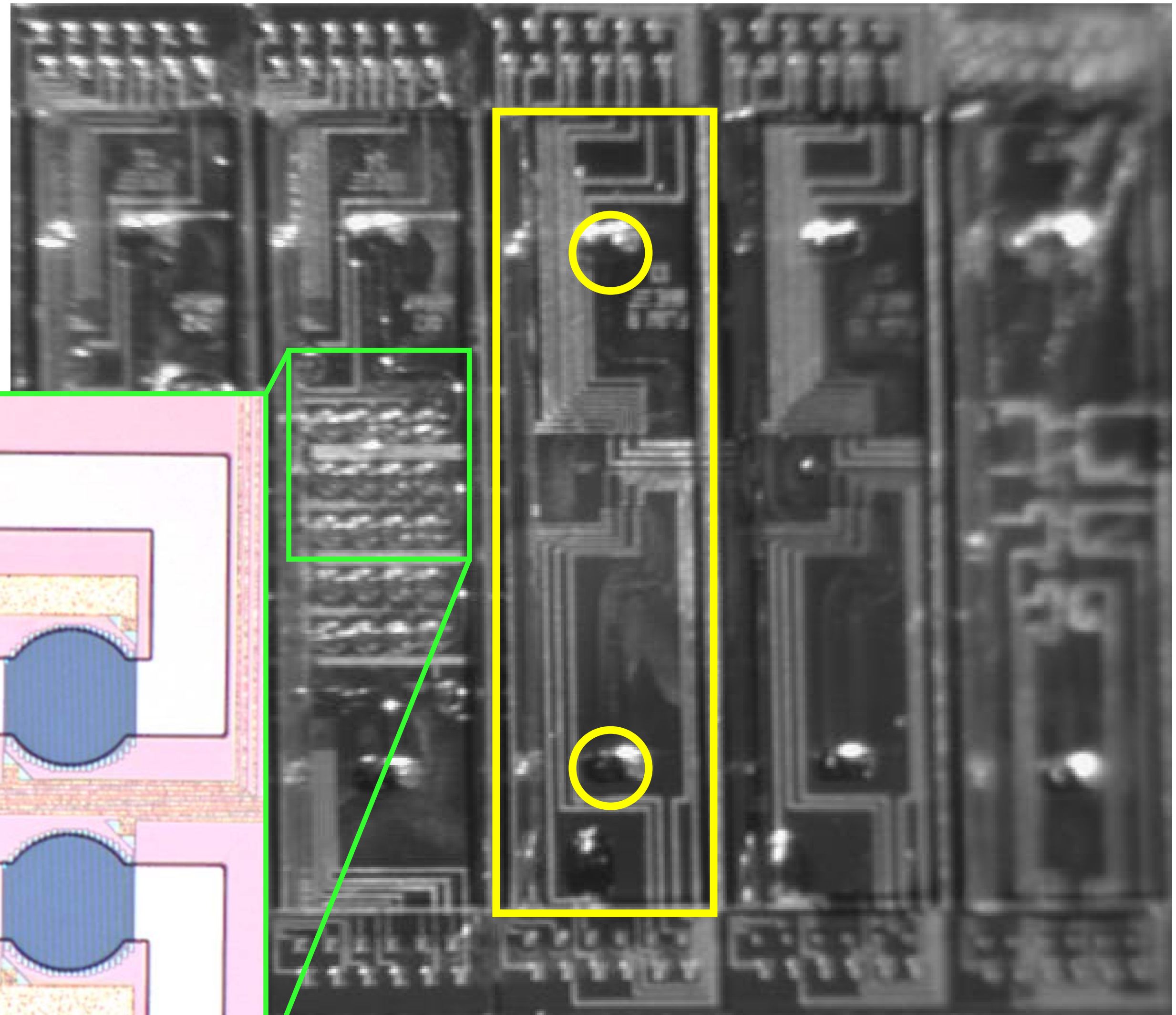


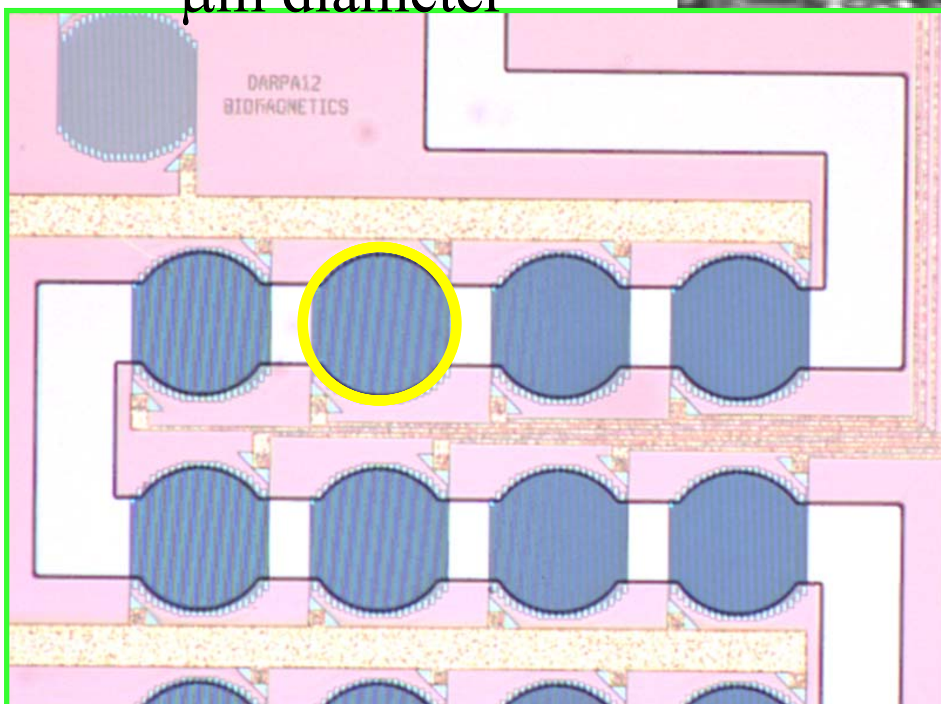


- Up to 860 μm thick
- Ultra-high aspect ratio
- Wafer level processing
- Rapid prototyping
- Adding: Multi-level, pick & place

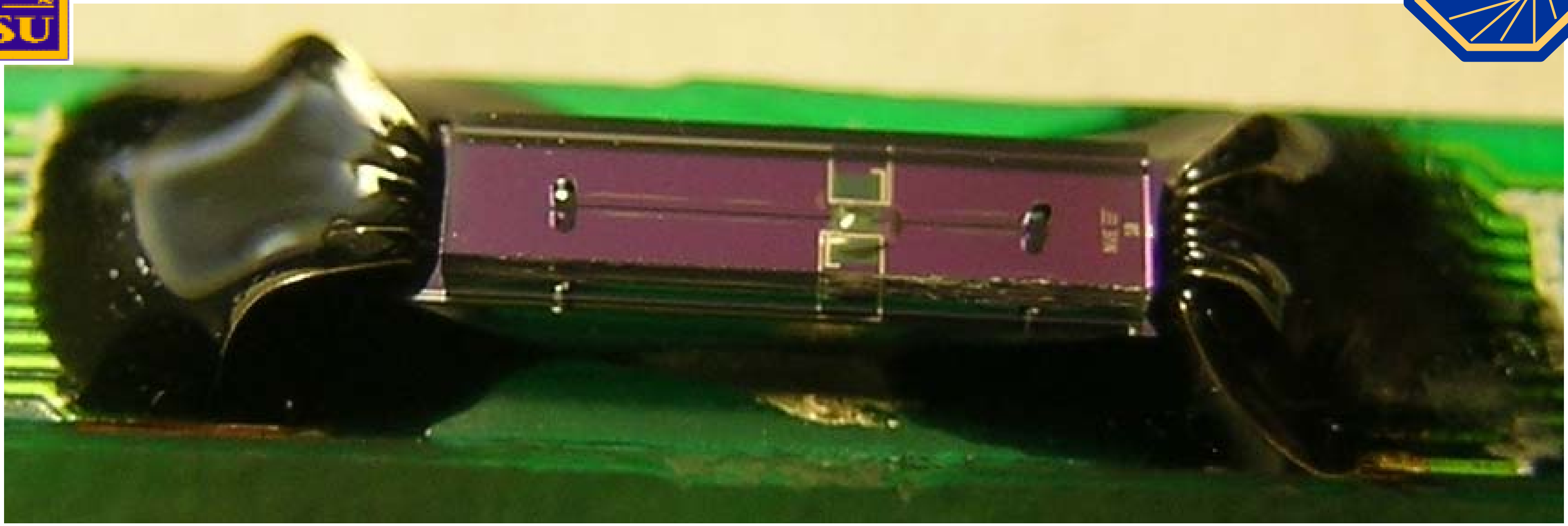
Covers with 100 micron ports on the wafer

20-sensor array, 200 µm diameter

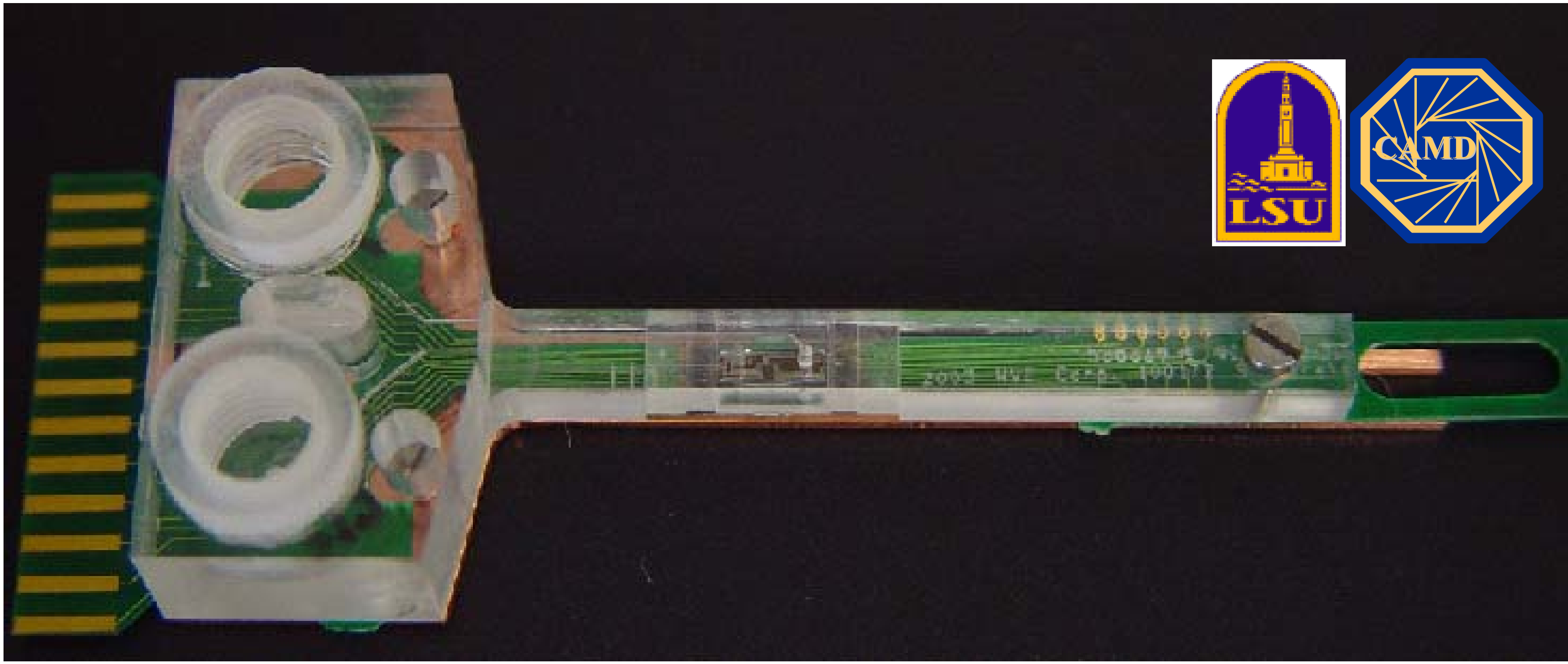




Dice, mount on circuit board, wire bond

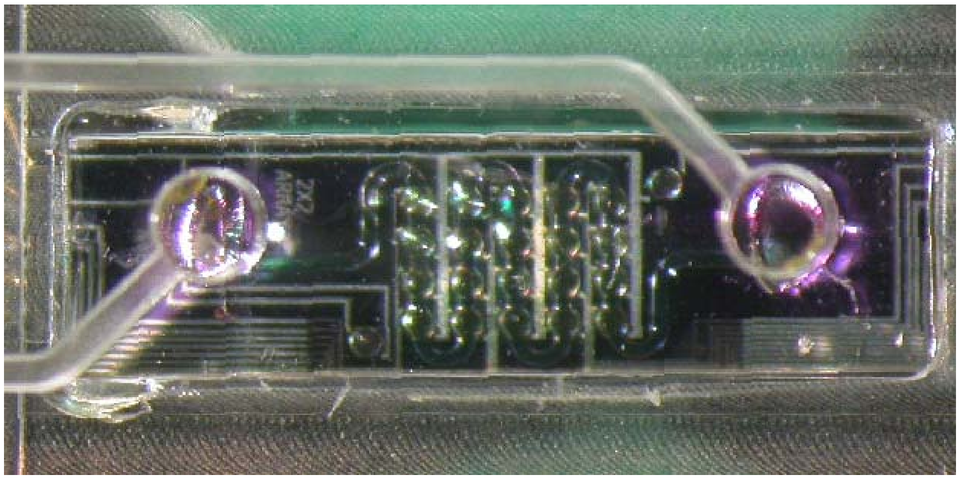


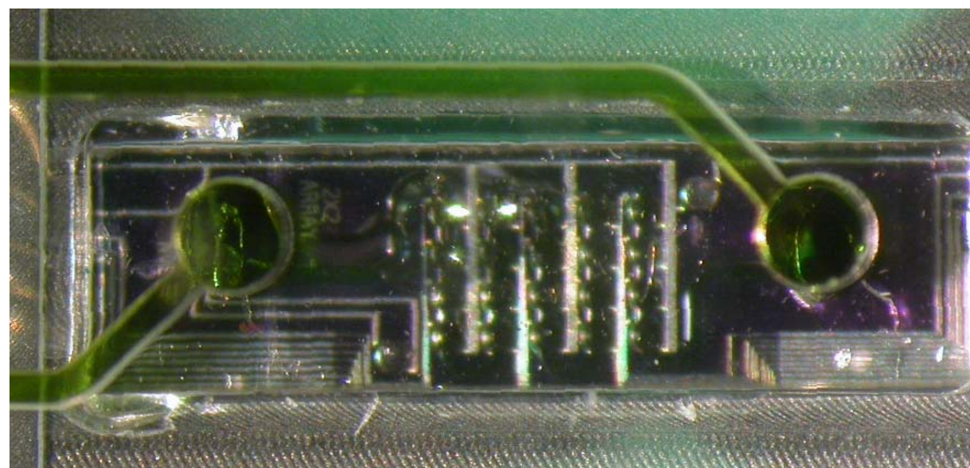
Ready to use fluidic / GMR chip



Ready for flow:

before and after pumping liquid





Detector Comparison

Magnetic	Photonic
Little background	Many light sources
Array is embedded in chip	Array must be scanned mechanically
Integrated single label detection is natural	Single label detection requires sophisticated optics
Less "colorful"	Narrow-band labels
Fields are not easily steered	Photons can be directed, diffracted, etc
Challenging fluidics integration	Challenging fluidics integration

Both are more sensitive than electrochemical

Comparison with Electric Manipulation

Magnetic	Electrophoresis or E-field
Not limited by buffers	Need special buffers
Little spurious magnetism	Many charged items
External magnetic field source	Easiest device to fabricate
Challenging fluidics integration	Challenging fluidics integration

Near-term Technical Goals

BioMagneticS **Program**

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

Next Generation

Ultimate:

Molecular counting and bead-by-bead manipulation

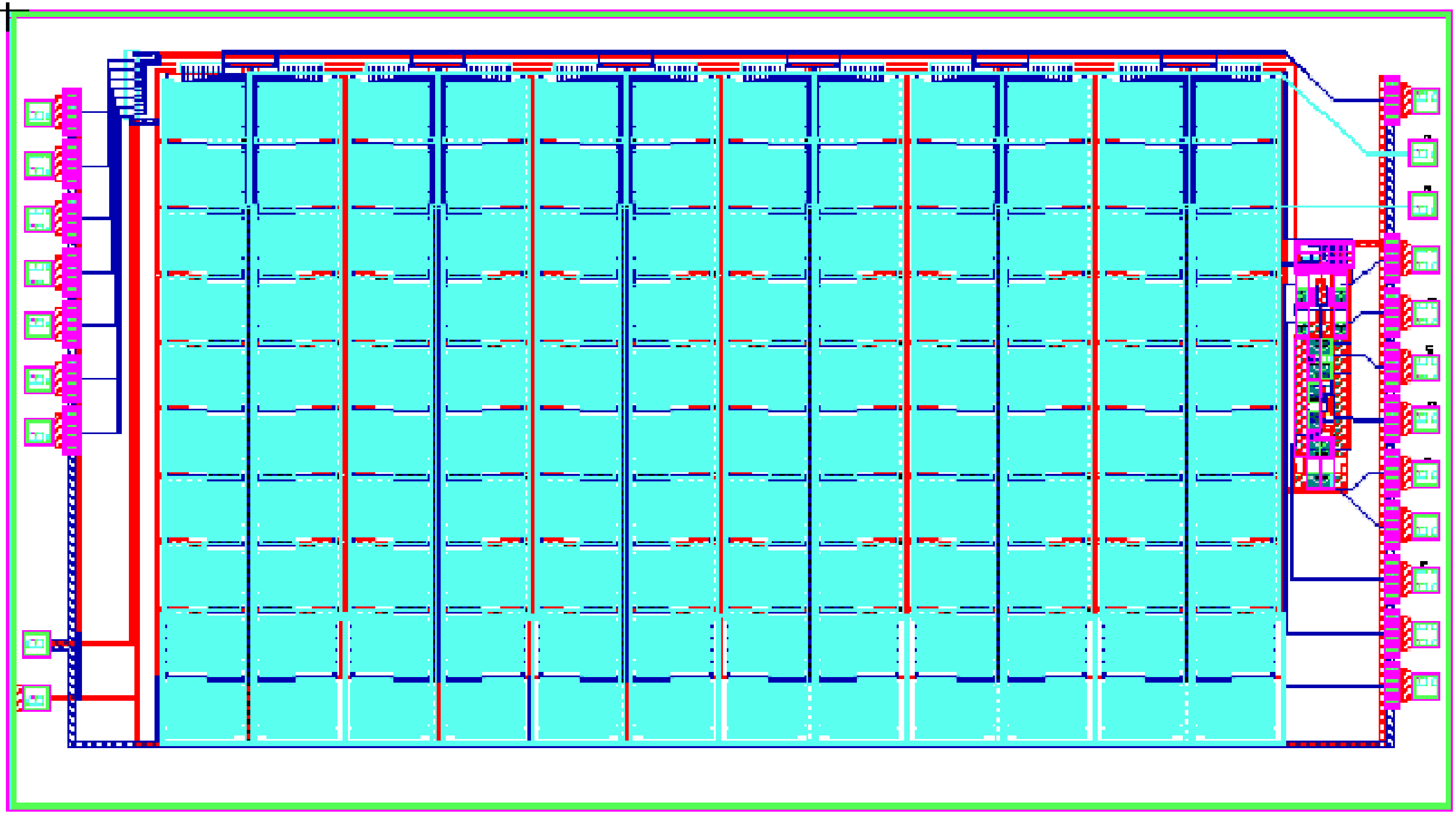
- Reduce label size to ~10 nm (more difficult detection)
- Use magnetics to enhance assay dynamics (mixing, capturing, binding hybridization, sorting)
- Increase system complexity by combining components
- Increase assay complexity with multiple analytes, sensors, and channels.

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 influence 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

Summary

•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

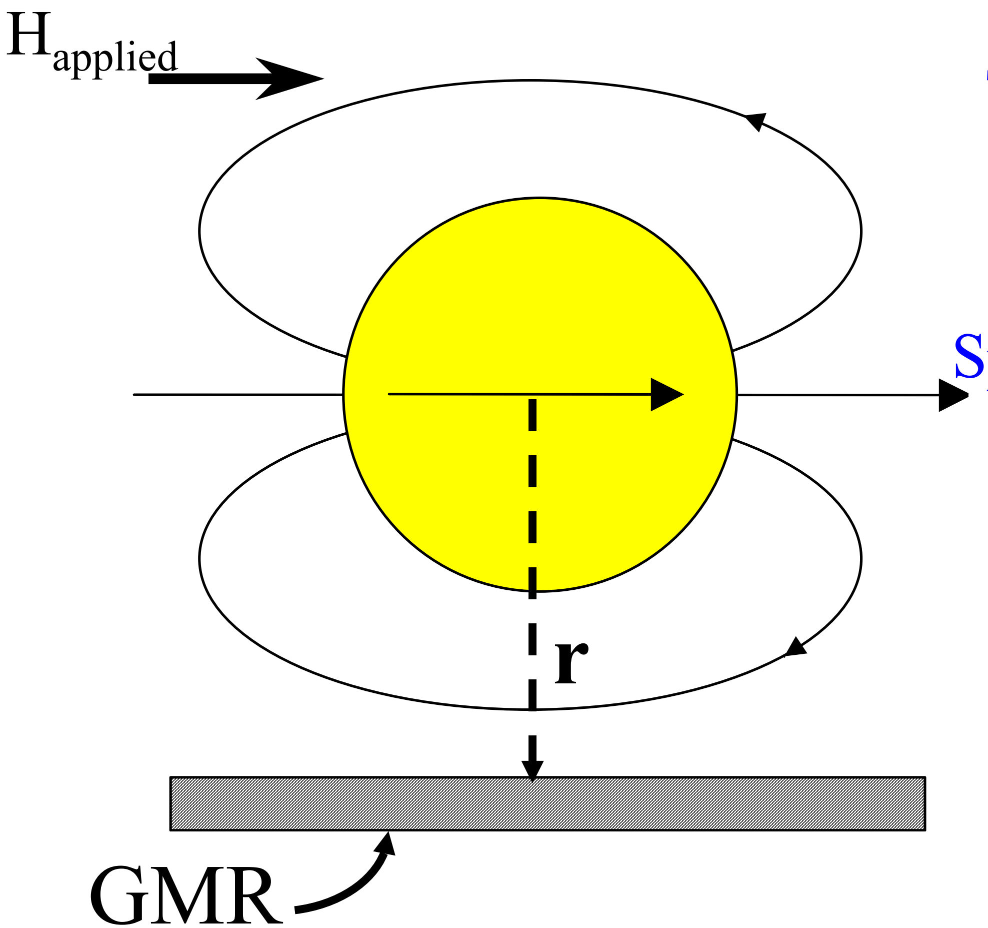
10 nm Labels should be detectable

- 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

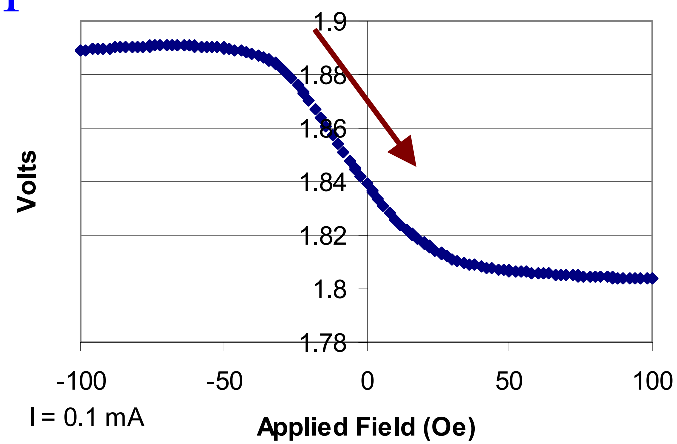
Stray Fields: in-plane excitation



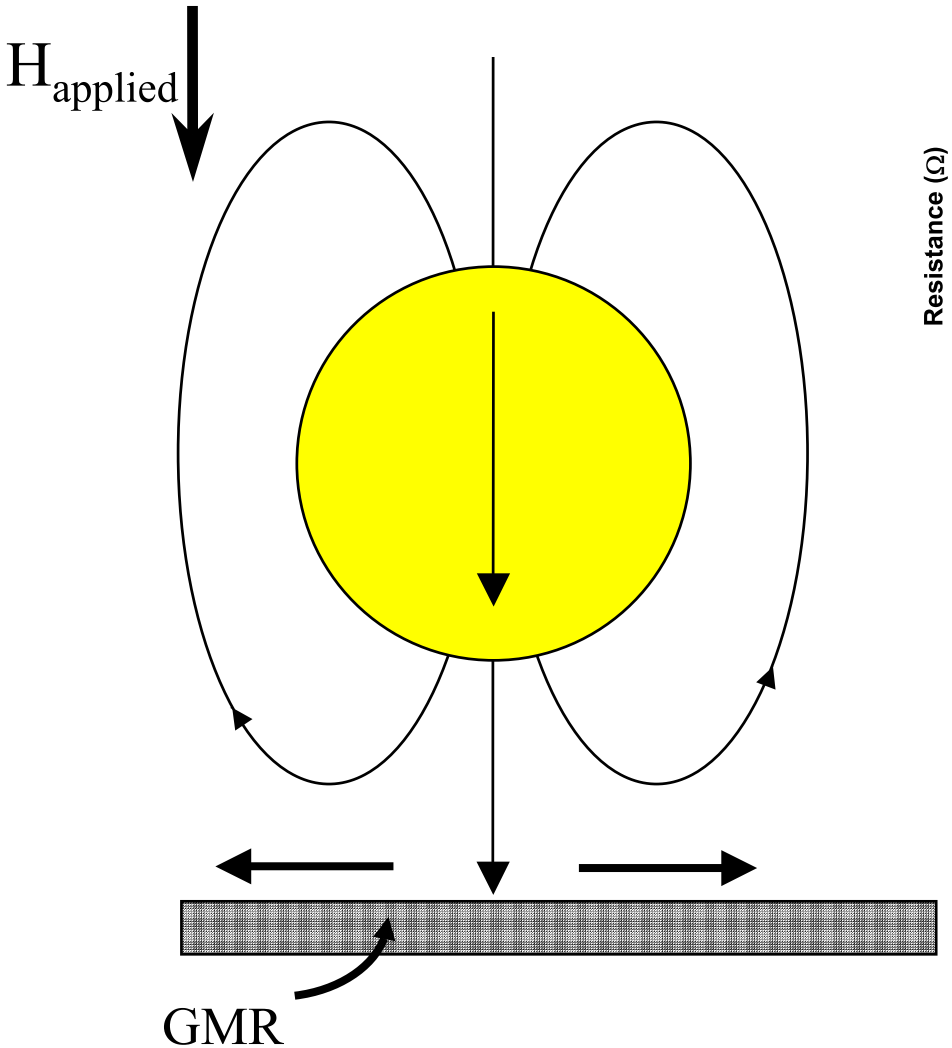
Need a sensor with bipolar output

The detected in-plane stray fields are in one direction

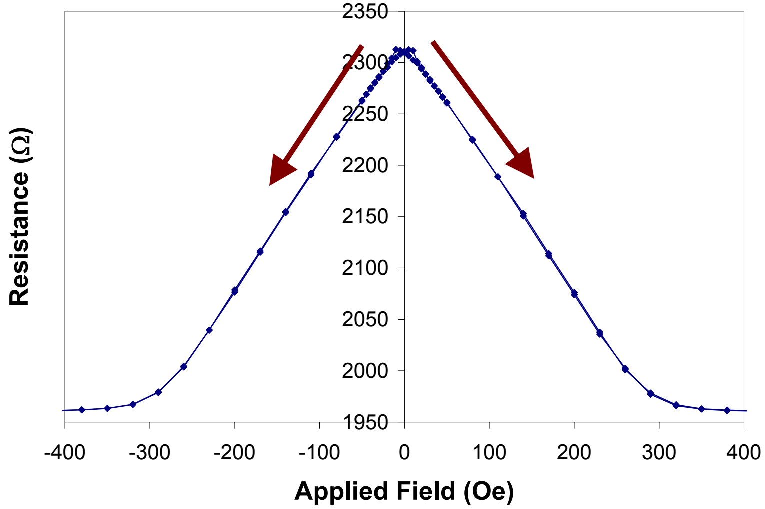
Spin Valve Transfer Function



Vertical Excitation



Multilayer Transfer Function



Need a sensor with unipolar output

The detected in-plane stray fields are in many directions