Nanotechnology for Imaging and Therapy: Advances in in vivo Magnetic Nanoparticle Sensing

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Geisel School of Medicine
Dartmouth College
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Richard P. Feynman "There's Plenty of Room at the Bottom" (1959):
"The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom." … "Consider the possibility that we too can make a thing very small which does what we want – that we can manufacture an object that maneuvers at that level!"

Outline

Sensitive Detection
Magnetic Nanoparticle Imaging MPI
MSB Spectroscopy
MSB Measurements for Inflammation/Immune Monitoring:
- Molecular Concentration Measurements (in vivo ELISA)
- Phagocytic Activity - cell uptake
- Temperature

Disclosures:
- IP
- NIH/NCI 1U54CA151662 CCNE

Magnetic Particle Imaging, MPI

H drive field
Magnetization
Field Lines Produced by the Selection/Drive Coils
Pickup Coils
Field Free Point
Selection/Drive Coils
Uniform Alternating Field
Static Gradient Field

Magnetization

H drive field

Magnetization

Fourier Domain

No Harmonics In Applied Field

Detected Signal: Harmonics of the Derivative of Magnetization

Magnetic Particle Imaging, MPI

Large Gradient Field

Large Gradient Field

Zero Gradient Field

Magnetization Changes Resulting in Large Signal

Magnetization Saturated Over Time Resulting in No Signal

Magnetic Particle Imaging, MPI

Magnetic Particle Imaging, MPI

Magnetic Particle Imaging, MPI

MPI - Magnetic Particle Imaging:

• High Sensitivity by Detecting Harmonics
• Localization by Saturating Nanoparticles Outside Field-Free Point

Development Focused on:

• Low Noise Electronics - Good Filters
• Large Field Gradients*
• Nanoparticles with High Saturation & Neel Relaxation
Relaxation Mechanisms

Influence of the Microenvironment:
- Viscosity/Relaxation
- Temperature

$\tau_B = \frac{3nV_k}{k_B T}$

$\tau_N = \tau_0 e^{k_B T}$

Magnetic Particle Spectroscopy

MSB Magnetic Spectroscopy of Brownian Motion

1) Measures Harmonics – MPI Detection Sensitivity with
2) Larger Nanoparticles that Rotate Via Brownian Motion so the Signal Reflects the Microenvironment

Scaling-Uncertainty Measurements

Measured quantity that is monotonic function of the product of
1) an unknown and
2) an user controlled quantity.

Need not characterize either Functional form or Environment.
Scaling-Uncertainty Measurements

- Controlled Variable
- Measured Value (Can not control)

$M(a, b)$
Field Amplitude / Temperature
Field Frequency / Relaxation Time

$M(a_i, b_m) = M(a_{ref}, b_{ref})$
$a_m b_m = a_{ref} b_{ref}$

Calibration Sweep

Measure Harmonic Ratio

Product $- \omega L$

Harmonic Ratio


100nm Iron Oxide

4% Signal

5% Error

6% Error

7% Error

Scale Frequency Ratio $(-7)$

500 Hz

1000 Hz

1500 Hz

2000 Hz

2500 Hz

3000 Hz

3500 Hz

4000 Hz

4500 Hz

5000 Hz

5500 Hz

6000 Hz
Sensitivity to Changes in Relaxation

Master Variable Solution of the Langevin Equation

\[ A = \frac{\mu H}{k_B T \omega \tau} \]

Increasing \( \omega \)

300Hz \( A \sim 100 \)

1000Hz \( A \sim 30 \)

Sensitivity

Glycerol - Viscosity

IL-6 Sensitivity

Outline

• Sensitive Detection
  Magnetic Nanoparticle Imaging MPI
• MSB Spectroscopy
  **MSB Measurements:**
  • Molecular Concentration Measurements (*in vivo* ELISA)
  • Cell nanoparticle uptake
  • Temperature
Biological Markers

**Immunotherapy effectiveness:**
- Cytokine signaling changes – canine model TNF-$\gamma$

**Infection Markers:**
- Increased cytokine signaling
- Increased phagocytic activity
- Increased metabolic activity (increased temperature)

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*in vivo ELISA*

Microscopic probes to measure quantitative concentrations *in vivo* over time

- Free NPs with unrestricted rotational motion
- Bound NPs with restricted rotational motion

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**Concentration Measurements**

Electron Microscopy

- Zetasizer hydrodynamic diameters
- Nanoparticle counts

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**in vivo ELISA - Concentration Measurements**

Sensitivities with current apparatus:
- ssDNA: 0.1 nM
- Streptavidin: 0.15 nM
- MMP-9: <2 nM
- Thrombin: 4 nM
- VEGF: <20 nM

ssDNA in Excised Kidney & in blood: <0.4 nM

**using 150 μg NPs**

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**a) New geometry & b) epoxied windings**

**Thrombin**

Old Apparatus: 1 nM
New Apparatus: 1 pM, 1 fM, 0.1 fM

p-value: 0, 0.077, 0.30

Sensitivity increased by factor of $10^4$.

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**First in vivo MSB measurements of cytokine concentrations.**

Show increased IL-6 secondary to bacterial infection.

IL-6 sensitive NPs in control mouse with NO infection.
IL-6 sensitive NPs in mouse with infection.
Concentrations of Important Signaling Molecules

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>aM</td>
<td>IM</td>
</tr>
<tr>
<td>IM</td>
<td>pM</td>
</tr>
<tr>
<td>pM</td>
<td>nM</td>
</tr>
<tr>
<td>nM</td>
<td>µM</td>
</tr>
</tbody>
</table>

**Maximum Sensitivity of Important Methods**

- PSA
- IL-1, TNF, IFN
- IL-6

**Methods in Development**

- MSB
- ELISA

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**in vitro Cell Uptake**

TEM images of nanoparticles in solution with MTG-B cells.

- (a) 5 minutes post addition of nanoparticles
- (b) 5 hours post addition of nanoparticles

Nanoparticles are:
- (a) outside cells
- (b) in vesicles and on cell surface.

Adam M. Rauwerdink & J.B. Weaver
Andrew J. Giustini & P.J. Hoopes

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**in vitro Cell Uptake**

MCF-7 Uptake

- less than

BT-474 Uptake


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### in vivo Tumor Uptake Studies

Harmonic Ratio vs Incubation Time in Tissue

1. Direct Injection
2. Incubation
3. Tumor Excision
4. Tumor Division
5. MSB Measurement
6. Pathology

Adam M. Rauwerdink (J.B. Weaver)
Andrew J. Giustini (P.J. Hoopes)

### Activity of Phagocytes in Blood

**No blood**
**25µl blood**
**50µl blood**
**100µl blood**
**150µl blood**

**Time Following Injection (sec)**

**Relaxation Time (ms)**

Daniel Reeves, Irina Renard, Xiaojuan Zhang (J.B. Weaver)
Seiko T. Brown (S. N. Fiering)

### Ovarian Cancer Early Detection

Method: 24 hrs post & NP inj in mice

**PS3/Kras Tg**
- Increased uptake by ovarian cancer

**WT Control**
- Uniform Signal

Identify Surgical Site Infections
UHMWP Temperature Probe Material from Anna Samia's lab, Case Western Reserve

Small (Neel Relaxing) Nanoparticles Embedded in UHMWP

- Solid (large signal)
- Biologically inert
- Localized
- Mean Error 0.4°

Conclusions:

• MSB can monitor local immune/inflammatory responses
  • Cytokine concentrations
  • Phagocytic activity - cell uptake
  • Temperature.
• MSB Sensitivity can be further optimized.

We believe that the potential applications are many and diverse:
  • Cancer therapy monitoring
  • Early infection identification
  • Early cancer detection

Current NP Students
Yipeng Shi, Physics

Microbiology and Immunology
Prof. Steven Fiering
Prof. Brent Berwin

Radiology
Prof. Shefali Guri
Venkata Nemani

Thayer School of Engineering
Prof. Ryan Halter
Prof. Alex Hartov
Prof. Keith Paulsen

Surgery
Prof. Jack Kuehlert
Dr. Sohail Mirza

Former Weaver NP Students/Staff
Dr. Daniel Reeves
Dr. Xiaojuan Zhang
Dr. Inna Ferman

Dr. Adam Rauwerdink
Eva Kuehlert

Richard P. Feynman “There’s Plenty of Room at the Bottom” (1959): “The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom.” … “Consider the possibility that we too can make a thing very small which does what we want – that we can manufacture an object that maneuvers at that level!”

1) NIH - Centers for Cancer Nanotechnology Excellence 1U54CA151662-01
2) Norris Cotton Cancer Center
3) Department of Radiology
MSB\textsuperscript{1} (or MPS) employs the signal used in MPI to characterize the microenvironment:

- **Temperature\textsuperscript{1-4}**
- **Viscosity\textsuperscript{5, 6}**
- **Cell Phagocytic Activity\textsuperscript{7}**
- **Signaling Molecules\textsuperscript{8, 9}**
- **Local Matrix Rigidity\textsuperscript{10, 11}**

Applications we are pursuing in temporal order:

- **Monitor Temperature During Hyperthermia Treatment**
- **Ovarian Cancer Screening**
- **\textit{in vivo} ELISA for Immunotherapy Monitoring**
- **Early Identification of Surgical Site Infections**
Conclusions:
• NP signals can be localized using a perpendicular magnetization induced by a static perpendicular field.
• Gradients in:
  a) in-line and b) perpendicular fields can be used to achieve excellent conditioning (condition numbers <10).
• In-line geometry is fast but requires larger dynamic fields.
• Perpendicular geometry requires small dynamic fields but is slower.
• pMPI is worth further study.

Temperature Estimation Accuracy:


1) NIH - Centers for Cancer Nanotechnology Excellence 1U54CA151662-01
2) Norris Cotton Cancer Center, Prouty Funds
3) Department of Radiology
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2) Norris Cotton Cancer Center, Prouty Grant
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Phase Angle Scaling with Viscosity

New Apparatus Geometry

Thrombin

Old Apparatus: 1 nM
New Apparatus: 1 pM, 1 fM, 0.1 fM
p-value: 0, 0.077, 0.30
Sensitivity increased by factor of $10^6$

Perpendicular Apparatus Geometry

With Soft-Iron Core - 3rd Gen

Apparatus Comparison

<table>
<thead>
<tr>
<th>Generation</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feedthrough</td>
<td>2.5 nV</td>
<td>0.3 nV</td>
<td>0.04 nV</td>
</tr>
<tr>
<td>Standard Deviation in Feedthrough</td>
<td>0.2 nV</td>
<td>0.02 nV</td>
<td>0.006 nV</td>
</tr>
<tr>
<td>Sensitivity (grams iron)</td>
<td>90 µg</td>
<td>100 ng</td>
<td>&lt; 10 ng</td>
</tr>
<tr>
<td>Concentration Sensitivity (Molar)</td>
<td>1 nM</td>
<td>1 fM</td>
<td>?</td>
</tr>
</tbody>
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First in vivo MSB measurements of cytokine concentrations. Show increased IL-6 secondary to bacterial infection.

a) New geometry & b) epoxied windings

IL-6 sensitive NPs in control mouse with NO infection
IL-6 sensitive NPs in mouse with infection

All zero crossings same orientation – even harmonics
Same orientation for each zero crossing – signal is at the even harmonics

80mT - AMF
1mT perpendicular field
100 nm

Condition Number to code 128 pixels 5.4

Comparison: MPI and pMPI conditioning as a function of gradient


7/15/2015

**Condition Number to code 128 pixels** $10^{19}$

<table>
<thead>
<tr>
<th>AMF</th>
<th>Negative Gradient Field</th>
<th>Small Positive Gradient Field</th>
<th>Large Positive Gradient Field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**80mT - AMF, 75mT/FOV gradient, 0.1mT perpendicular field, 100nm**

**Scaling-Uncertainty Measurements**

$\psi(\alpha, \beta)$

Field Amplitude – Temperature
Field Frequency – Relaxation Time

$\beta_m = \frac{\alpha_{int}}{\alpha_m} \beta_o$

Can control $u$
Can not control $\beta_m$

**$\psi(\alpha_m, \beta_m)$**

$\alpha_m, \alpha_{int}, \alpha_i$
Change drive amplitude, $H_i$, at constant temperature, $T_o$, to generate calibration curve.

First measurement at a selected drive field, $H_o$, yields a value of $H_o/T_1$ from which the current temperature, $T_1$, can be found.

Subsequent measurements at the selected drive field yield values of $H_o/T_2$ from which the current temperature, $T_2$, can be found.

Temperature Measurement:

Magnetization a function of $\mu H/kT$: $M = M_o \left[ \tanh \left( \frac{\mu H}{kT} \right) \right]^{-3}$


**in vitro Cell Uptake**

TEM images of nanoparticles in solution with MTG-B cells.

(a) 5 minutes post addition of nanoparticles

(b) 5 hours post addition of nanoparticles

Nanoparticles are (a) outside cells and (b) in vesicles and on cell surface.

Adam M. Rauwerdink & J.B. Weaver
Andrew J. Giustini & P.J. Hoopes
Cells Uptake Measurements

Protein Binding Cell Surface and in Media

No Increased Binding

Invagination & Protein Binding

Cool Cells to Eliminate Invagination

in vivo Tumor Uptake Studies

Harmonic Ratio vs Incubation Time in Tissue

1. Direct Injection
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3. Tumor Excision
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Binding in Blood Over Time

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Saeo Y. Brown (S. N. Fiering)
MSB - Magnetic Relaxation

Applied Magnetic Field

Neel Relaxation
Magnetic Nanoparticles
Brownian motion

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Brownian motion

MSB - Magnetic Relaxation

Applied Magnetic Field

Brownian motion
**MSB - Magnetic Relaxation**

- **Applied Magnetic Field**
- Decreased temperature:
  - Slower Brownian motion
  - Slower Neel relaxation
- Increased viscosity:
  - Slower Brownian motion
- Increased binding energy:
  - Slower Brownian motion

**MSB - Magnetic Relaxation**

- **Applied Magnetic Field**
- Increased Binding Energy:
  - Slower Brownian motion

**MSB - Magnetic Relaxation**

- **Applied Magnetic Field**
- Increased Matrix Stiffness:
  - Even Slower Brownian Motion
Typical/Realistic Values

\[ I\dot{\Omega} = m \times B - 6\eta V\Omega + \sqrt{2DN} \]

Moment of inertia and angular acceleration

Magnetic moment and magnetic field \( \approx 10^5 \)

Viscosity volume and angular frequency \( \approx 10^5 \)

Gaussian noise and diffusion constant \( \approx 10^5 \)

\[ D = 6\eta V k_B T \]

\[ \langle N(t) \rangle = 0 \quad \langle N_i(t) N_j(t') \rangle = \delta_{ij} \delta(t - t') \]

Torques on a Brownian particle

\[ I\dot{\Omega} = m \times B - 6\eta V\Omega + \sqrt{2DN} \]

Inertia

Magnetic Torque \( \approx 10^5 \)

Viscous Drag \( \approx 10^5 \)

Thermal fluctuations \( \approx 10^5 \)

Magnetic Nanoparticle Simulations.


Equations Describing Nanoparticles Relaxing via the Brownian Mechanism

\[ \frac{d\vec{m}}{dt} = \frac{1}{6\eta V} \left( \vec{m} \times \vec{H} + \sqrt{2D} \vec{N} \right) \times \vec{m} \]

\[ D = 6\eta V k_B T \]

1. Viscosity
2. Temperature

---

Master Variable Solution of the Langevin Equation

\[ A = \frac{\mu H}{k_B T \omega_T} \]

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Equations Describing Nanoparticles Relaxing via the Brownian Mechanism

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\[ D = 6\eta V k_B T \]

1. Viscosity
2. Temperature
Conclusions:

- Simulations show that NP signals can be localized using the perpendicular magnetization.

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In-Line: Gradients in In-Line Fields

Scaled differently
1mT perpendicular field

Signal Localization in Time

Signal Amplitude
Old Apparatus Geometry

Drive Coil (Vertical AC Field)
Sample
Pick up Coil
Balancing Coil in series with the pick-up coil
Drive Field Monitor Coil

Switched Capacitors
Power Amplifier
Phase-Lock Amplifier
Computer
ADC
Control Lines

Detected Signal: Harmonics of the Derivative of Mag
a) New geometry & b) epoxied windings
Old Apparatus - 20 microgram of NPs
New Apparatus - 100 nanogram of NPs
Increase in sensitivity by factor of >200

P-value – 2x10⁻⁵ at 100μg


Daniel Reeves


MSB - Magnetic Spectroscopy of Nanoparticle Brownian Motion

The harmonics are only produced by magnetic nanoparticles.
Nanogram sensitivities in vivo.

Higher harmonics decrease faster than the lower harmonics with increased relaxation time¹⁴ (increased viscosity or binding).
Higher harmonics decrease faster than the lower harmonics with increased relaxation time \(^1\)\(^-\)\(^6\) (increased viscosity or binding).

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### a) New geometry & b) epoxied windings

<table>
<thead>
<tr>
<th>Position</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-value</td>
<td>-2x10^{-5} at 100ng</td>
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**Old Apparatus**
- 20 microgram of NPs

**New Apparatus**
- 100 nanogram of NPs

Increase in sensitivity by factor of >200

### P-value – 2x10^{-5} at 100ng


### In vivo ELISA

- Free NPs with Unrestricted Rotational Motion
- Bound NPs with Restricted Rotational Motion

- Porous Walled Container
- Binding Domain
- Targeted Biomarker
- With Binding: Transition to Saturation Smoother Harmonics All Decrease Higher Harmonics Decrease More Than Lower Harmonics


The harmonics are only produced by magnetic nanoparticles. Nanogram sensitivities in vivo.

Fourier transform of the H field is a single peak because it is a pure sinusoid.

Fourier transform of the magnetization has many harmonics because of the “squared off” magnetization.

Higher harmonics decrease faster than the lower harmonics with increased relaxation time \(1^r\) (increased viscosity or binding).

Higher harmonics decrease faster than the lower harmonics with increased relaxation time \(^1\) (increased viscosity or binding).

Biotinylated Lysine Fixable Dextran Polymers (500,000 MW)

Streptavidin Conjugated Nanoparticles

Bound Nanoparticles

Rigid – Many cross-links

Soft – Few cross-links

The number of glutaraldehyde induced cross-links was used as a surrogate for material stiffness.

<table>
<thead>
<tr>
<th>Glutaraldehyde per Dextran</th>
<th>Harmonic Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.65</td>
</tr>
<tr>
<td>10</td>
<td>0.60</td>
</tr>
<tr>
<td>100</td>
<td>0.55</td>
</tr>
<tr>
<td>1,000</td>
<td>0.50</td>
</tr>
<tr>
<td>10,000</td>
<td>0.45</td>
</tr>
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490 Hz, 20.9 mT