

# Rigidity of the Microscopic Environment Surrounding the Binding Site of Magnetic Nanoparticles

J. B. Weaver<sup>1</sup>, and A. M. Rauwerdink<sup>2</sup>

<sup>1</sup>Radiology, Dartmouth-Hitchcock Medical Center, Lebanon, NH, United States, <sup>2</sup>Thayer School of Engineering, Dartmouth College, Hanover, NH, United States

## Introduction:

Methods of evaluating the microscopic stiffness of cells have been very productive. The stiffness of the cellular cytoskeleton and the extra-cellular matrix have been linked to key factors determining patient survival including cell motility and metastasis<sup>1</sup>, and angiogenesis<sup>2</sup>. However, current methods of measuring stiffness on a microscopic scale are generally limited to *in vitro* use.

A new method related to magnetic particle imaging (MPI)<sup>5</sup> has been used to measure properties influenced by the rotational Brownian motion of magnetic nanoparticles<sup>3,4</sup>. The method is called magnetic spectroscopy of nanoparticle Brownian motion (MSB) and has been used to measure temperature with high accuracy<sup>4</sup>. It uses the nonlinearities in magnetization induced by a sinusoidal applied magnetic field. The nanoparticles are the unique source of nonlinearities so the sensitivity should be high. *In vivo* MPI in mice suggest the method is sensitive to 10 nanograms of iron oxide nanoparticles<sup>6</sup>. The nonlinearities in the nanoparticle magnetization are produced by the inability of the nanoparticle magnetizations to align with the applied field because of Brownian motion. The ratio of the fifth and third harmonics is a useful measure of the shape of the magnetization: it is very sensitive to nonlinearities and it is concentration independent.

We report here that MSB is sensitive to the stiffness of the microscopic environment surrounding the sites where the streptavidin functionalized nanoparticles are bound to the matrix.

## Methods:

A uniform gel mixed with biotinylated BSA formed the matrix. Changing the percentage of gelatin changed the stiffness of the matrix. Iron oxide nanoparticles (25 nm in diameter) functionalized with streptavidin were spread on the gel surfaces. The unbound nanoparticles were removed by washing the surfaces of the gels with buffer solution. The biotin-streptavidin bond is the strongest antibody bond with binding energies similar to covalent bonds. The MSB signals were recorded for free nanoparticles in solution and for nanoparticles bound to gels with 15%, 20% and 25% gelatin (13.2 kPa, 17.6 kPa, 22.0 kPa respectively).

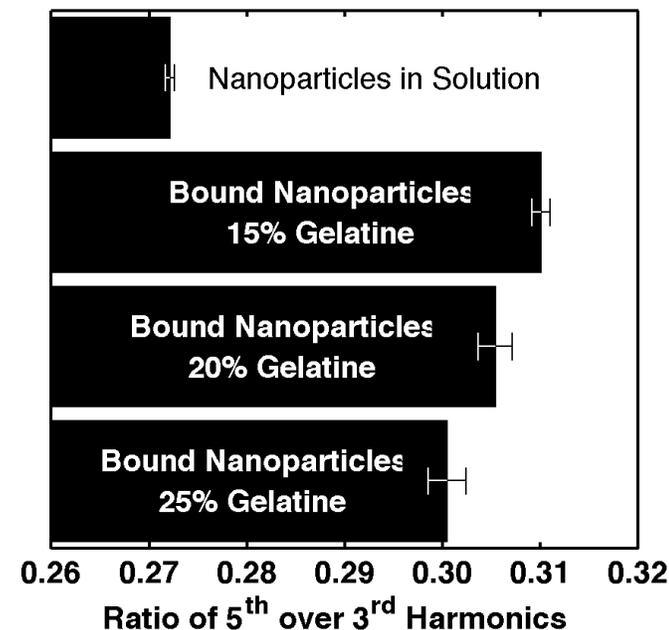
**Results:** The MSB signal increased significantly when the nanoparticles were bound to the gels. In

addition, the MSB signal was significantly different for different gel stiffness: the p-values were 0.0005 and 0.0074 for the differences between the 15% and 20% gels and the 20% and 25% gels respectively.

**Conclusions:** The MSB signal reflects the bound state of the nanoparticles as well as the stiffness of the matrix to which they are bound. The microscopic rigidity of the microscopic region surrounding the binding site is reflected in the MSB signal if the binding affinity is constant. This method is a potential contrast mechanism for MPI as well as a method of exploring mechanical properties of materials on a microscopic scale *in vivo*.

## References:

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**Figure 1:** The ratio of the 5<sup>th</sup> harmonic over the 3<sup>rd</sup> harmonic for nanoparticles in solution and bound to gels of three concentrations of gelatin. The nanoparticles were 25 nm iron oxide and coated in streptavidin, which bound the biotinylated BSA imbedded within the gels.