Magnetic mapping of bio-inspired clusters of iron oxide nanoparticles
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Introduction

Iron oxide nanoparticles (IONs) hold widespread importance in biology. These include synthetic magnetite nanoparticles as well as naturally occurring ferrihydrite preset in ferritin. They can be used in a multitude of applications such as cellular labeling, sorting and tracking as well as targeted drug delivery and hyperthermia. The magnetic detection of these IONs is important in understanding their fate in biological samples. In order to accurately interpret magnetic signals from IONs, it is important to consider the aggregation state of nanoparticles ranging from the nanoscale to the mesoscale (< 1µm) and macroscale (> 1mm). Towards this goal, we aim to study both naturally derived and synthetic ION clusters. Naturally derived clusters were obtained by using deproteinated ferritin (the largest Iron storage protein present in the human body). A top-down microfabrication approach was used to pattern 10 µm sized clusters of iron oxide nanoparticles onto silicon wafers. The morphology, composition and magnetic properties of these ION assemblies were characterized by using magnetic force microscopy, the recently developed indirect magnetic force microscopy, analytical electron microscopy and superconducting quantum interference device magnetometry. Our studies indicate that clustering of IONs can significantly affect their magnetic detection.

Nanoparticle Imaging and Analysis

In order to fully understand the magnetic properties of mesoscale (<1µm) and macroscale (>1mm) iron oxide nanoparticle (ION) clusters, we use a variety of analytical methods. We use atomic force microscopy (AFM) techniques such as magnetic force microscopy (MFM) as well as analytical electron microscopy (AEM) to study the mesoscale cluster since these modalities are capable of imaging small structures with a very high resolution (in the order of nm). Magnetic resonance imaging (MRI) and superconducting quantum interference device (SQUID) magnetometry will be used to characterize ION magnetization on the macroscale.

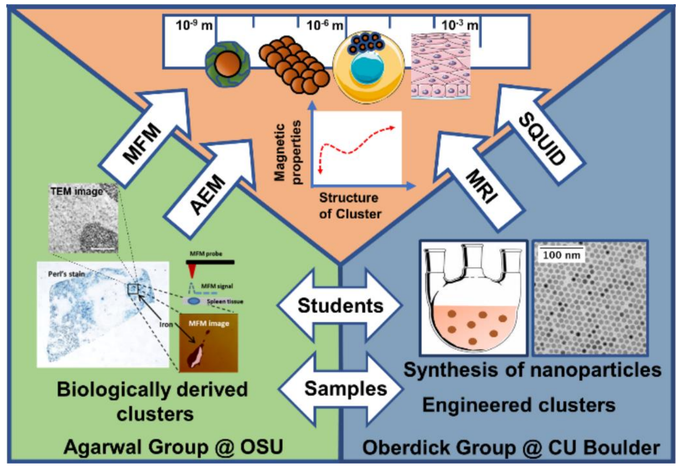

Figure 1: Visual representation of the particle analysis strategy.

Biologically Derived Iron Oxide Nanoparticles

There are two biologically derived sample types we will analyze in this study: lysosomes and deproteinated ferritin clusters. Unlike fabricated ION clusters, biologically derived clusters are not uniform in size, composition, etc., so this mesoscale (<1µm) analysis is essential. We will image lysosomes both in-situ and ex-situ using AEM and MFM. The iron rich lysosomes will be generated by culturing macrophages in an environment with a high iron concentration. To examine the lysosomes ex-situ they may be removed from the cells using a Lysosome Isolation Kit (Sigma-Aldrich, Catalog Number LYSISO1).

We will also analyze ferritin clusters using AEM and MFM techniques. However, we must first remove the biological material surrounding them as even the ferritin protein shell alone separates the ION clusters far enough apart to prevent them from generating a high magnetic moment. This inhibits an MFM probe's ability to detect them and results in a weak magnetic signal and a low-resolution phase image.

To remove the ferritin shell, we may use trypsin or high heat treatments to degrade it, leaving us with only the ION clusters contained within it (Figure 2). We may then immobilize the clusters and perform MFM imaging on them.

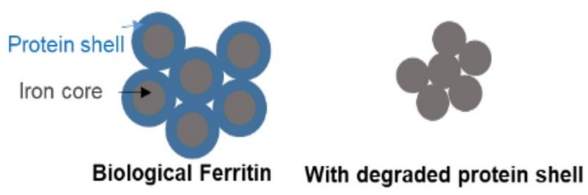

Figure 2: Visual representation of iron nanoparticle clusters with and without the ferritin protein shell

Microfabricated Nanoparticle Aggregates

The magnetization of magnetic nanoparticle aggregates changes as a function of several key parameters such as (1) nanoparticle size (2) aggregate size (3) aggregate shape and (4) density of nanoparticles. In order to systematically evaluate these effects, we developed a procedure for fabrication of photolithographically defined clusters of magnetic nanoparticles. The process gives us control of the geometry of clusters and enables exploration of magnetization of clusters as a function of their size and shape.

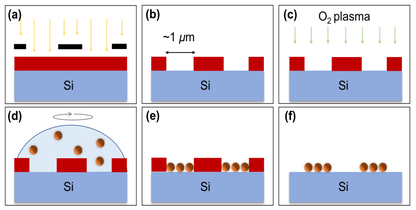

Figure 3: Process flow for producing micropatterned clusters. (a) Expose photoresist (red). (b) Develop photoresist to form ~1 micrometer wells. (c) Oxygen plasma to improve wettability on Si and remove excess photoresist. (d) Spin coat iron oxide nanoparticle solution. (e) Dry wafer. (f) Remove photoresist.

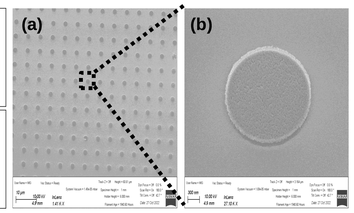

Figure 4: (a) Scanning electron microscope (SEM) image of an array of clusters on Si substrate. (b) Magnified image of single cluster comprised of many ~10 nm Fe_3O_4 NPs. Disc is ~1.9 micrometers wide and ~0.14 micrometers tall.

The magnetization of complex nanoparticle mixtures is a function of the shape of individual particles, as well as the global shape of the aggregates. It has been suggested[1,2] that the magnetic susceptibility of complicated particle mixtures can be described by an effective demagnetizing factor, $D = D_{NP}(1 - \emptyset_p) + D_{cluster}\emptyset_p$, where D_{NP} is the demagnetizing factor of individual NPs, $D_{cluster}$ is the demagnetizing factor of the shape of the cluster and $\emptyset_p$ is the packing fraction of NPs.

We are studying the validity of this formula by micropatterning disc-shaped aggregates filled with Fe_3O_4 nanoparticles (Figure 4). Then we are measuring the magnetic moment of substrates in an in-plane and out-of-plane geometry (Figure 5a, 5b) using superconducting quantum interference device (SQUID) magnetometry. The demagnetizing factor changes for each orientation of the micropatterned discs and has an effect on the DC magnetization (Figure 5c).

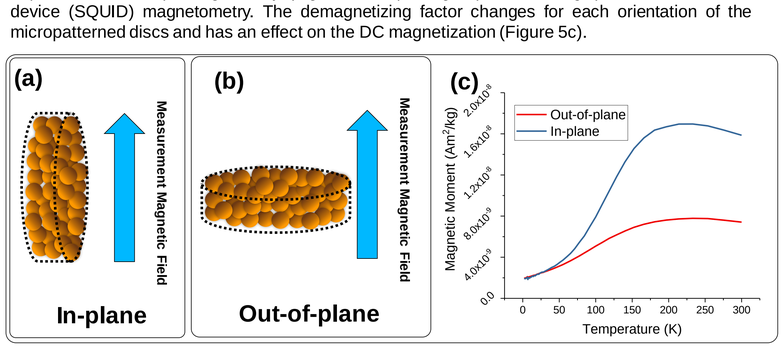

Figure 5: (a) In-plane measurement geometry. (b) Out-of-plane measurement geometry. (c) DC magnetic moment of micropatterned substrate (zero-field-cooled curve) as a function of temperature for in-plane and out-of-plane orientations.

Future work will examine the magnetic susceptibility of the discs as a function of relevant geometric parameters in order to better understand the magnetization of nanoparticle clusters. We plan on changing the effective packing fraction of discs by filling molds with a combination of magnetic (Fe_3O_4) and non-magnetic (Au) nanoparticles.

We are currently in the process of generating both biologically-derived and microfabricated ION clusters. Before using microfabricated clusters in our study, we analyzed them for their size accuracy, uniformity, etc. To do this, we performed MFM and AFM (Figure 6) on them. MFM allowed us to check for uniform magnetic properties amongst the clusters and analyze the strength of the magnetic forces by varying the lift height of the MFM probe, while AFM lets us study the cluster height profiles.

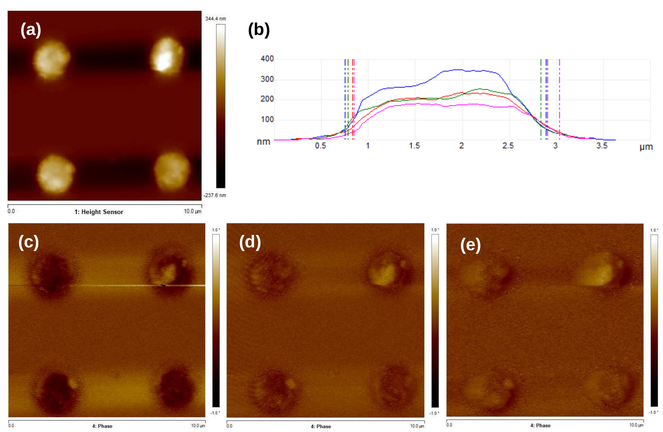

Figure 6: Magnetic force microscopy (MFM) analysis of microfabricated nanoparticle clusters including (a) topography image, (b) cluster height profiles and MFM at (c) 50nm, (d) 100nm and (e) 150nm probe lift heights

1. R. Skomski et al., *IEEE Trans. On Magnetics,* (2007).
2. P. S. Normile et al., *Appl. Phys. Lett.,* (2016).

Indirect Magnetic Force Microscopy

Indirect magnetic force microscopy (ID-MFM) is a recently developed magnetic force microscopy (MFM) technique. Traditional MFM, also known as direct MFM (D-MFM), involves a magnetic probe making two passes over an immobilized sample. The first pass images the topography of a sample by tapping its surface with the tip of the probe, and the second pass scans the sample (at a designated lift height) to detect magnetic forces to develop a phase image (Figure 1a). ID-MFM involves performing MFM on an ultrathin membrane which has an immobilized sample on the other side of it (Figure 1b)

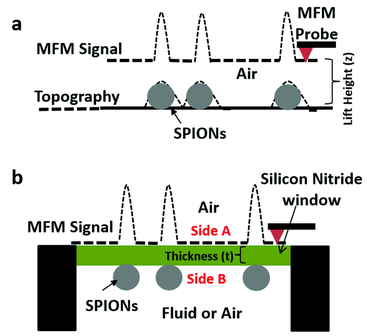

Figure 1: Schematic of (a) direct magnetic force microscopy (D-MFM) and (b) indirect magnetic force microscopy (ID-MFM)[3]

ID-MFM has several advantages compared to traditional MFM. It can scan a sample faster as it only requires one pass to generate an image. It also minimizes topographical cross-talk (i.e the roughness of the sample surface has a minimal influence on the phase image). The sample can also be isolated in an environmental that is separate and distinct from the probe, opening possibilities for scanning fluid-filled or biological samples.

If a transparent membrane is used (i.e. silicon nitride), samples imaged using ID-MFM may also be imaged using visual light and electron optic imaging techniques such as fluorescent microscopy and transmission electron microscopy (Figure 2a/b). Previous works have shown success in the detection of iron oxide nanoparticles (IONs) using ID-MFM (Figure 2c/d).

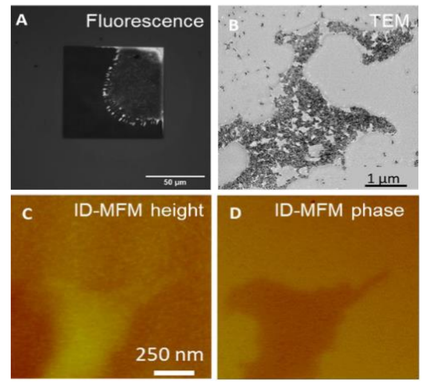

Figure 2: Multimodal imaging of fluorescently labeled SPIONs fixed on side B of a silicon nitride window: (a) Fluorescent microscopy, (b) TEM, (c) ID-MFM height imaging (first pass), (d) ID-MFM phase imaging (second pass)[3]

Moving forward, we will optimize the MFM probe magnetization and membrane thickness in order to generate highly accurate ID-MFM images as this modality is currently limited in its ability to detect small SPION clusters (<10 nanoparticles).

Potential Applications in Energy and Sustainability

To synthesize iron oxide nanoparticles (IONs), we may use "green" processes which utilize non-toxic and environmentally friendly materials such as ferric chloride and papaya leaf extract[4]. Using a sophisticated microfluidic device, we can monitor the growth of the IONs during synthesis with indirect magnetic force microscopy (ID-MFM).

Metal oxides are currently used in a variety of chemical reactions for purposes such as depollution, biomass conversion, green chemistry, photocatalysis, etc[5]. In the future, the techniques we are developing/optimizing, such as ID-MFM and photolithography can be used to:

(a) micropattern metal oxide particles on a substrate for localized catalysis
(b) evaluate the structural and magnetic properties of those patterns using atomic force microscopy (AFM) and magnetic force microscopy (MFM)

In contrast to biological applications, where nanoparticles with high saturation magnetization and low remnant magnetization (magnetically soft) are desired, energy storage requires nanoparticles with high coercivity (magnetically hard) and anisotropy. Thus novel strategies for synthesis as well as creation of composite assemblies consisting of soft and hard particles need to be employed for energy storage. I would like to apply the modeling and characterization expertise acquired during my research for design, synthesis of characterization of nanoparticles for energy storage applications.

3. K. Walsh et. al., *Nanoscale Adv.,* (2019).
4. S.H. Bhuiyan et al. *Heliyon*. (2020).
5. J.C. Védrine. *Catalysts*. (2017).