Direct and Indirect Magnetic Force Microscopy (MFM) in Histology

Gunjan Agarwal

Mechanical and Aerospace Engineering

THE OHIO STATE UNIVERSITY

Biomagnetism

Sources

- **Super-paramagnetic: Iron**
  - **Ferritin, transferritin, hemoglobin, NTBI**
- **Diamagnetic: Calcium**

Tissues

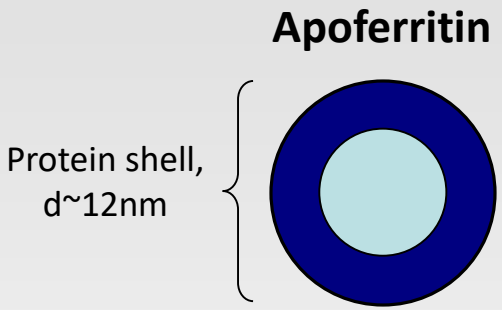
- **Liver (ferrihydrite)**
- **Spleen (ferrihydrite)**
- **Serum (?)**
- **Acute injury (ferrihydrite)**
- **Chronic plaques (maghemite)**

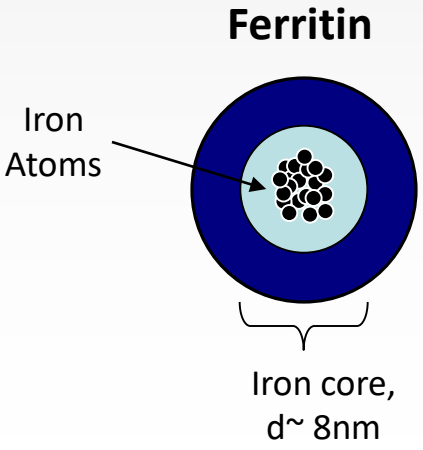
Ferritin

- The major iron storage protein in biological systems

- Globular protein complex (480 kDa): 24 subunits including heavy (H-Ferritin) and light (L-Ferritin) chains

- Exists as:
  - Apoferritin (without iron)
  - Holoferritin (with iron)
  - Iron core is mostly ferrihydrite, up to 4500 atoms
  - Superparamagnetic in nature





Diagnostic Tests for Iron

Non-invasive:

- MRI (liver, spleen, plaques)
- Biosusceptometry
- Serum proteins (ferritin, transferrin)
- Serum iron

Invasive

- Histochemical stains:
  - Perl's: (Fe^{3+})
  - Turnbull: (Fe^{2+}),
- Immunohistochemistry (ferritin)
- Analytical TEM

Problems:

- Mismatch between Non-invasive and invasive mapping
- Total iron is measured (size, density, oxidation state unknown)
- Total ferritin is measured (apo vs. holo ferritin unknown)
- Mismatch between iron vs. ferritin mapping
- Chemical environment is different in non vs. invasive imaging (eg. fixatives)

Our goal

Bridge the gap between Invasive and Non-invasive approaches for iron characterization in tissues

Non-invasive (Imaging)
(MRI, Biosusceptometry)

Invasive (Histology)
(Histochemical stains, TEM)

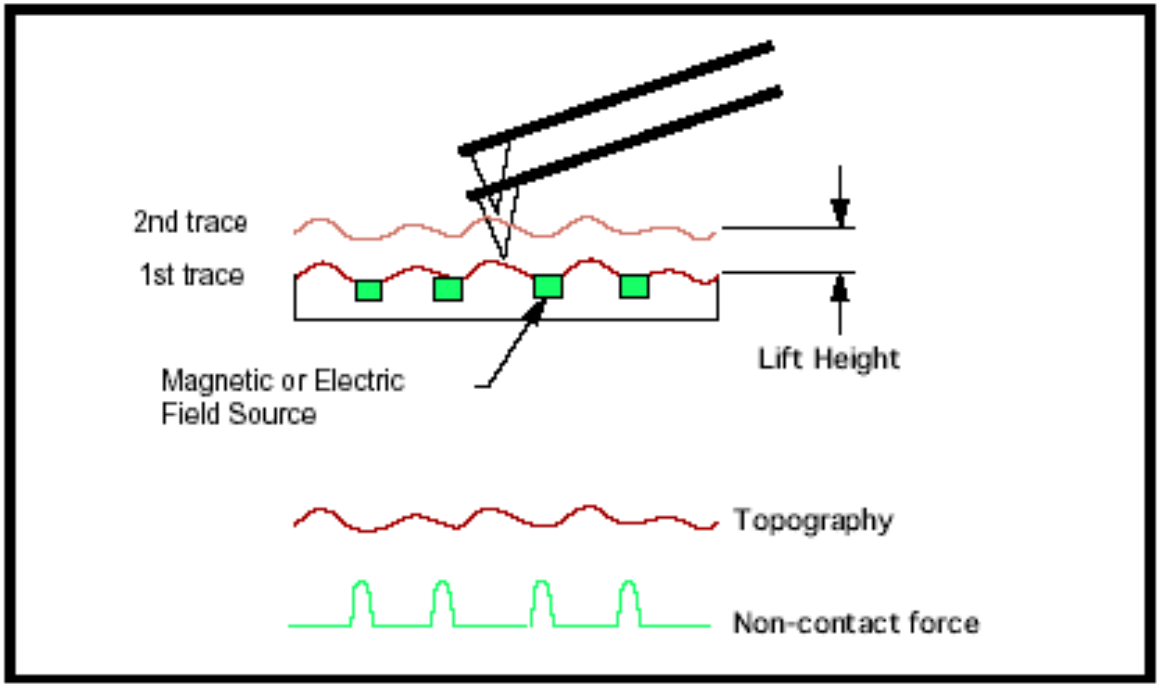
Magnetic properties ⟷ **Chemical properties**

??

↓

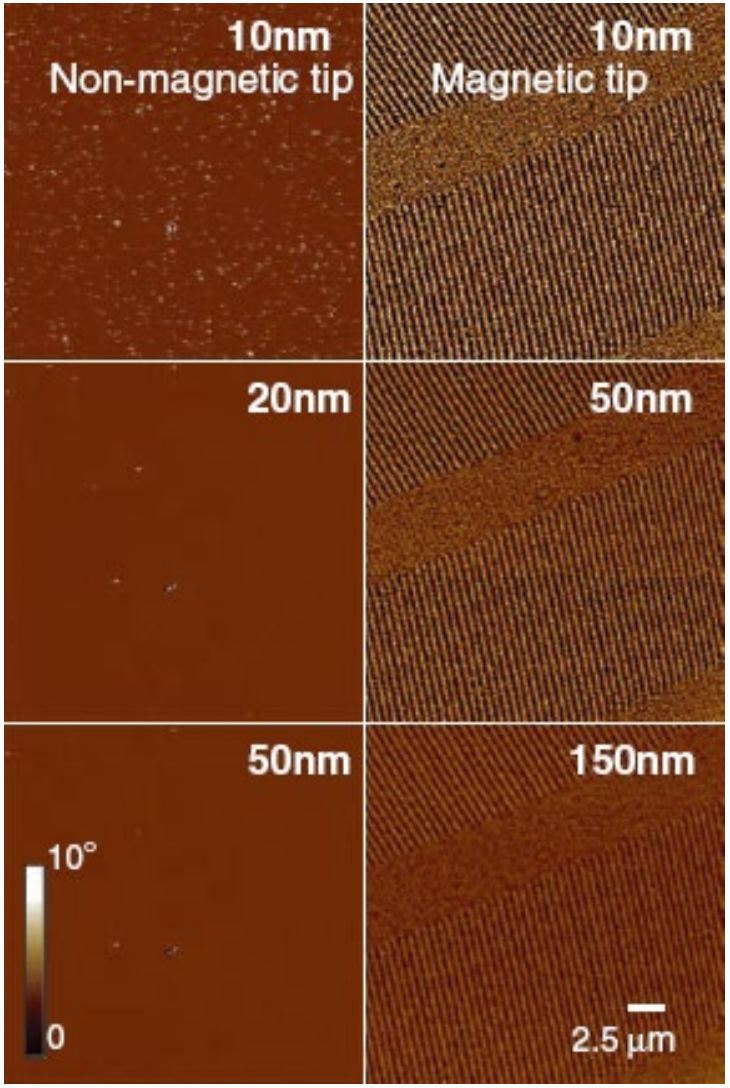
Magnetic Force Microscopy

Magnetic Force Microscopy



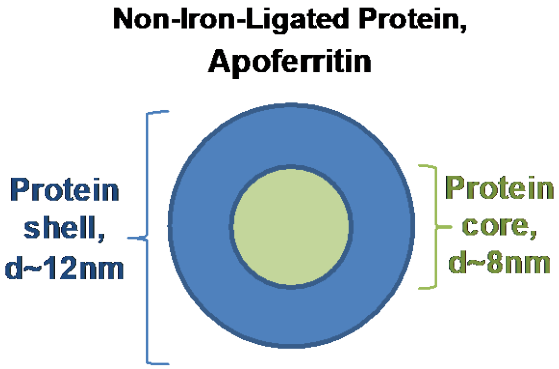
- High sensitivity
- High Spatial resolution
- Label free
- Available on Commercial AFMs

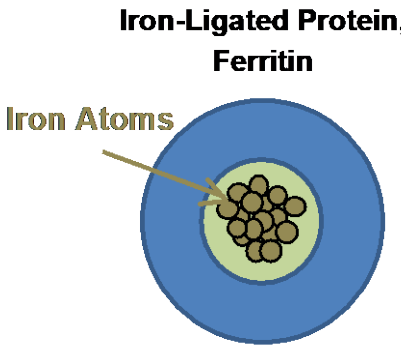
Direct MFM of a Magnetic Tape

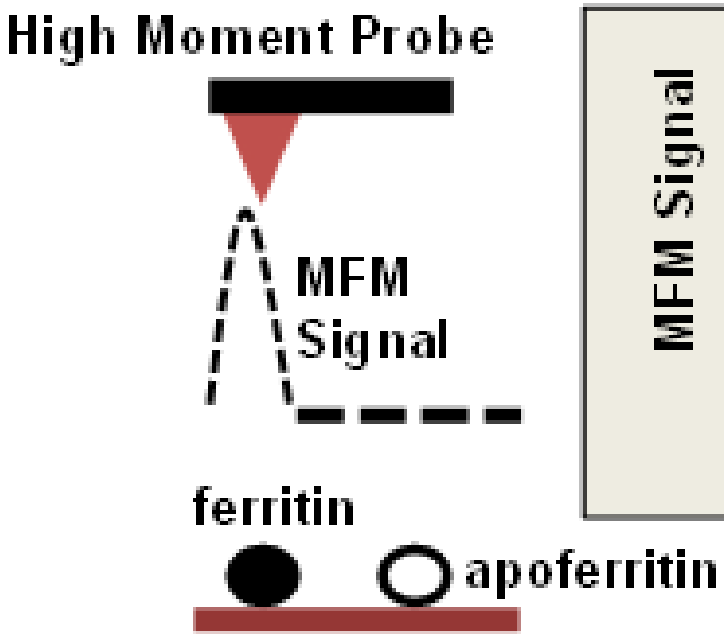


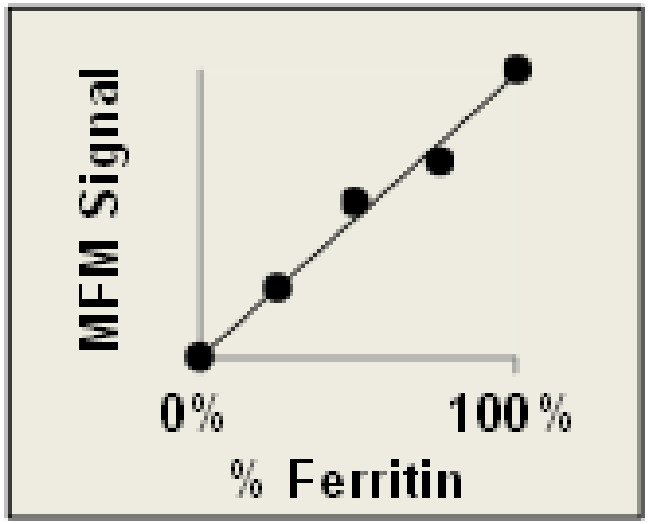
Savla et al, Israel J. of Chem., 2008

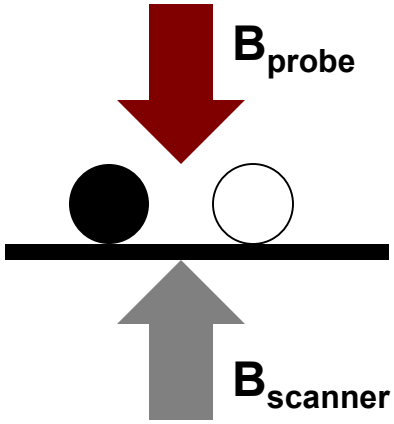
Direct MFM of Ferritin



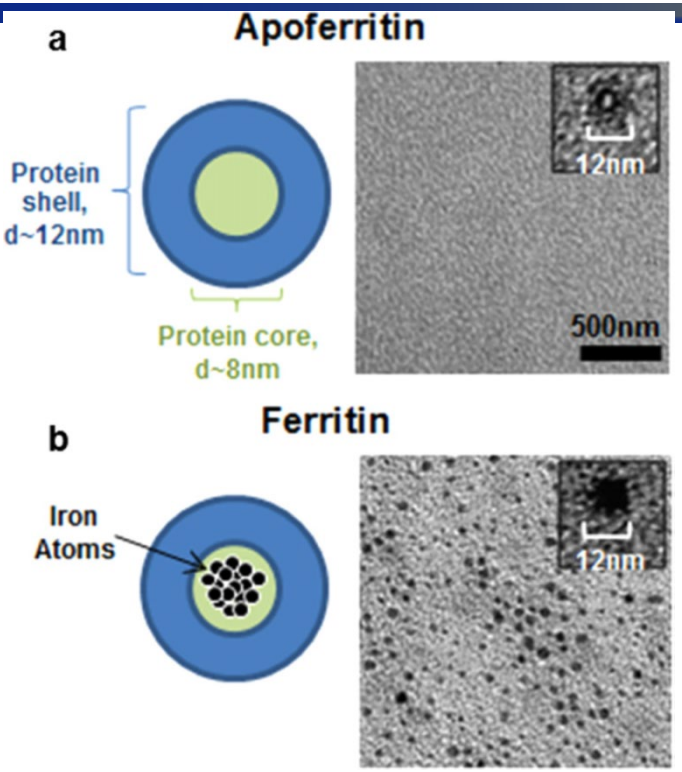


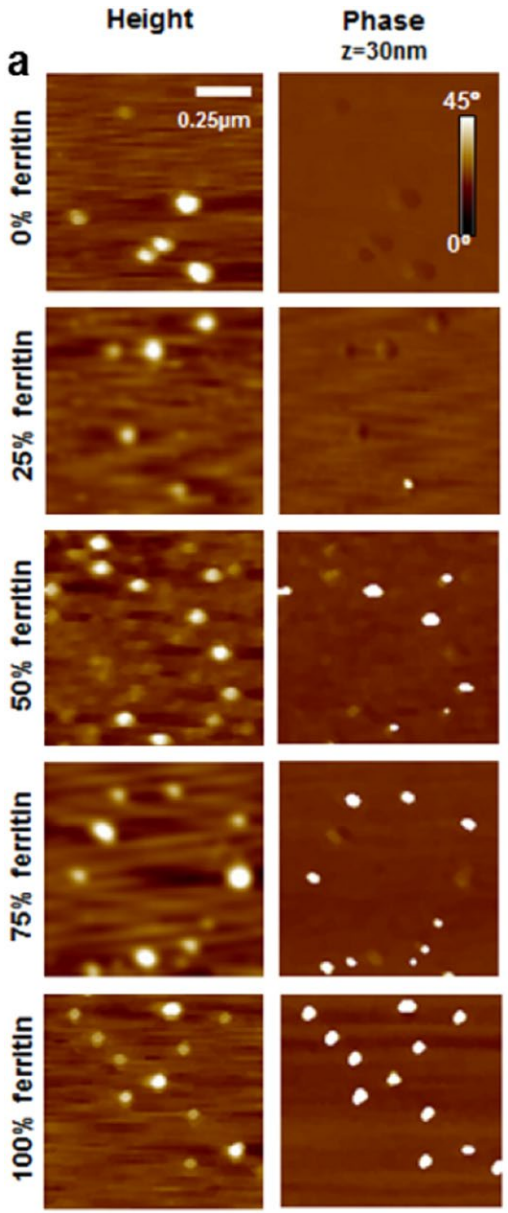


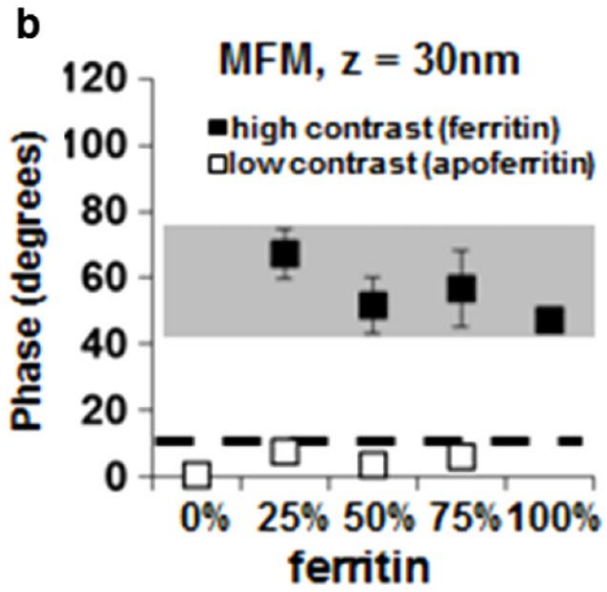


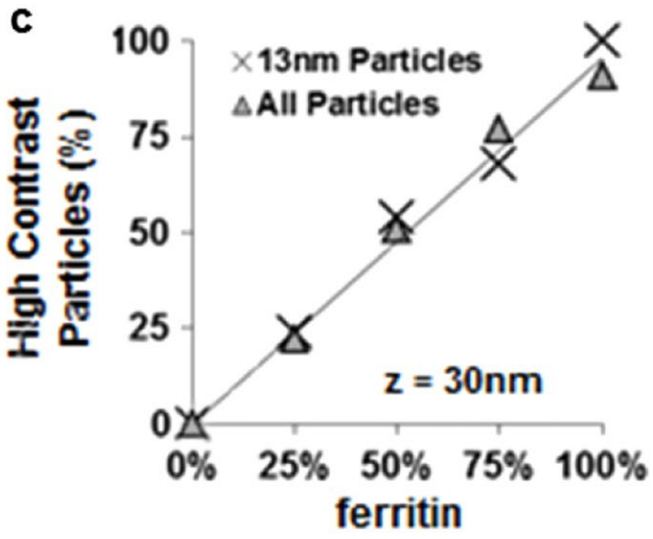


Direct MFM of Ferritin and Apoferritin









Nocera et al,

Nanotechnology (2014)

MFM in Histology

Experimental system:

Rodent spleen and spinal cord

Experimental approach:

Light microscopy

- Tissue sections (~ 5 μm thick, in 4% PF) on glass
- Histochemical stain (Perl's)
- Magnetic Force Microscopy (MFM)
  - ASYMFMHM probes
  - Multimode AFM (Nanoscope 3a controller)

Electron microscopy

- Transmission electron microscopy (TEM)
- Energy Dispersive Spectroscopy (EDS)
- Electron energy loss spectroscopy (EELS)

MFM of Healthy Tissue

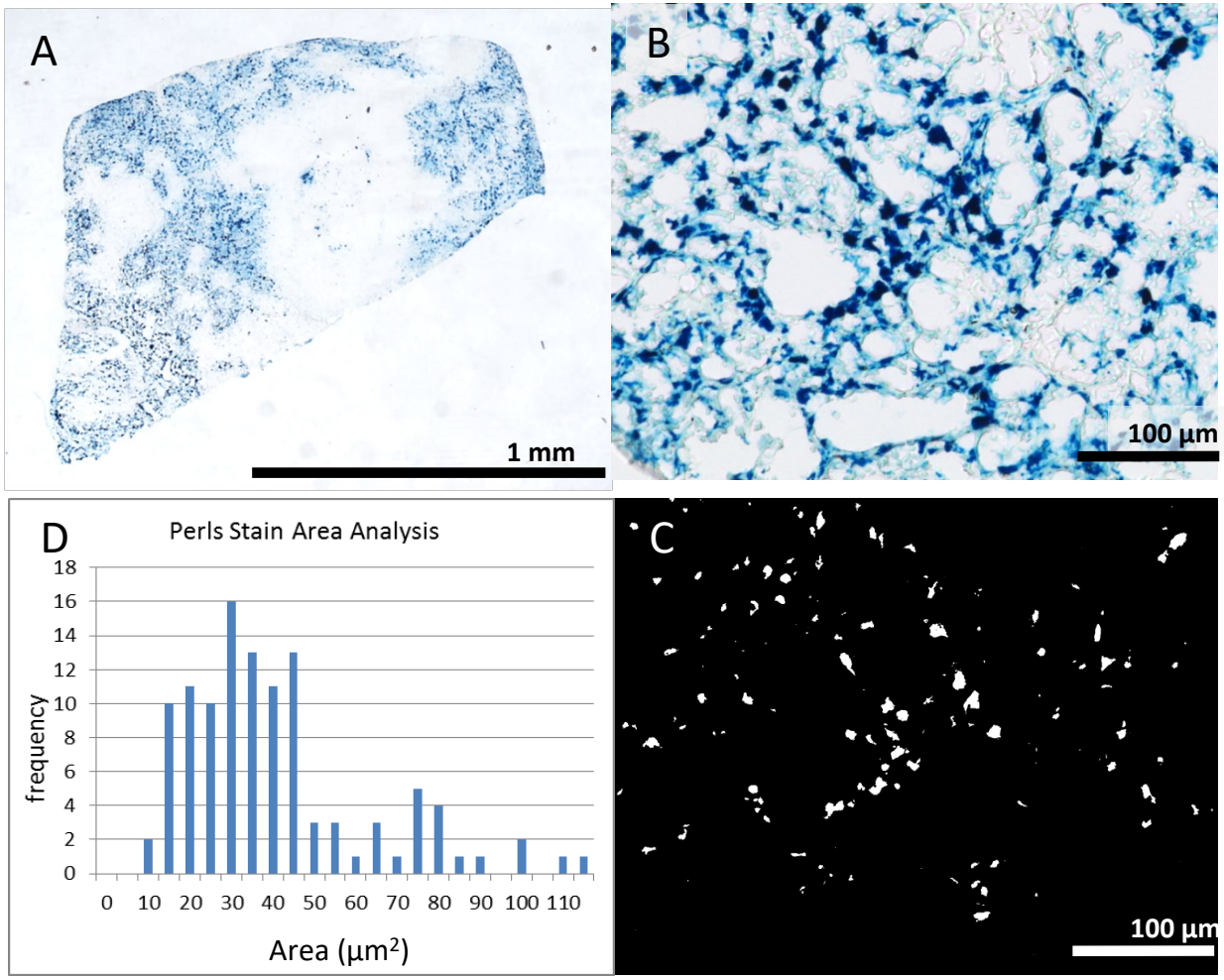
Objectives:

Effect of chemical fixatives

Detection of MFM signal

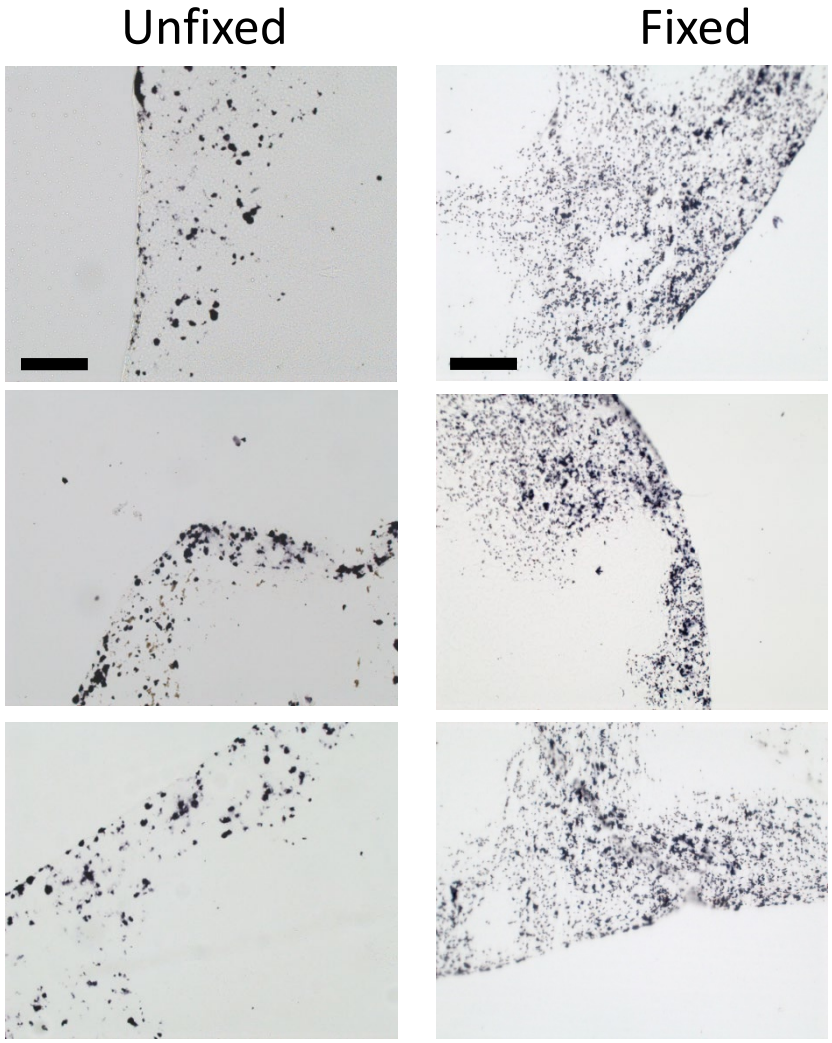
Verification of MFM signal

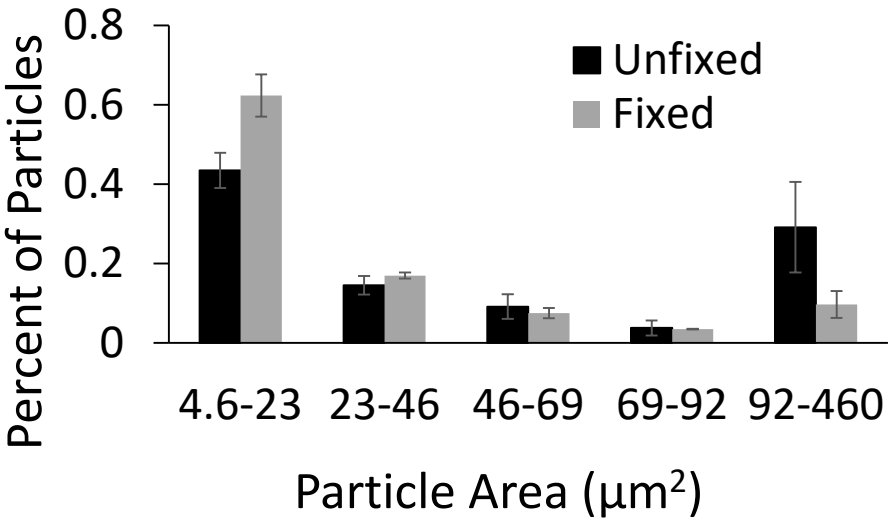
Rodent spleen: Perl's staining

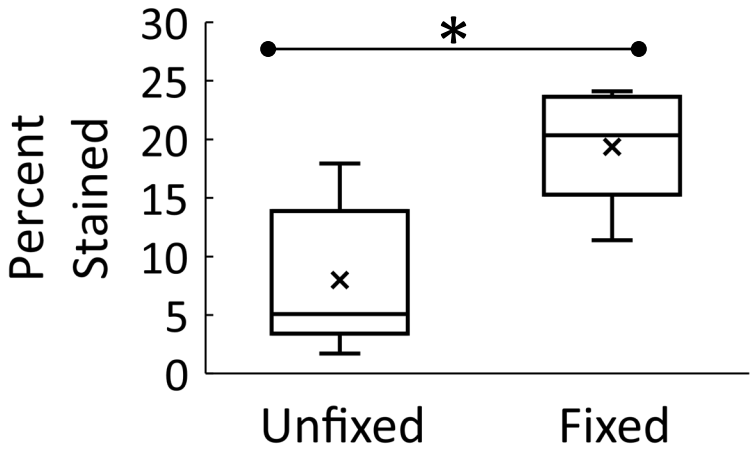


Size(z) of intensely stained regions ~ 20 to 40 µm²

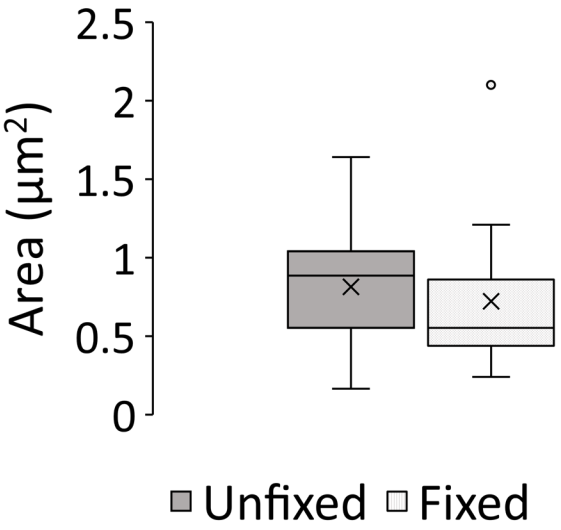
Rodent spleen (effect of fixative): Perl's staining

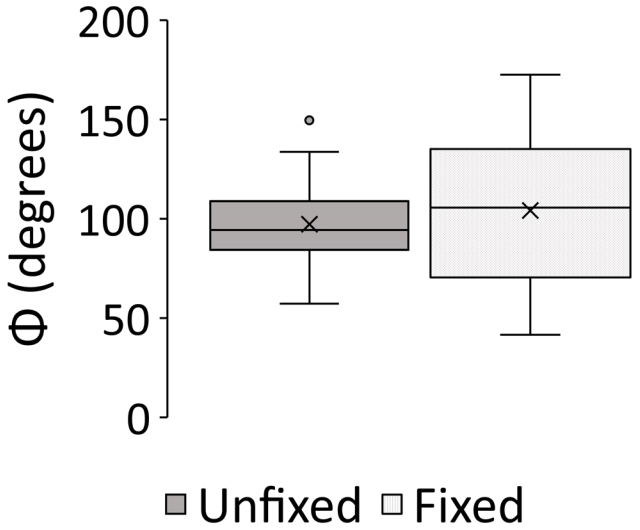




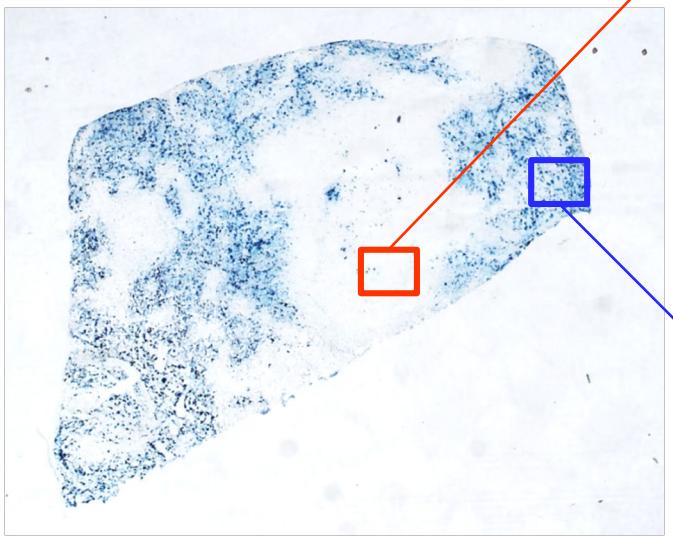


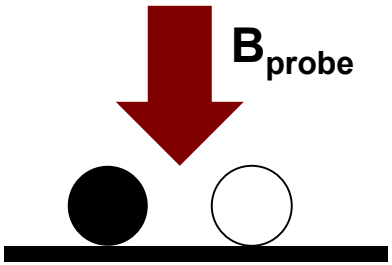
MFM of fixed and unfixed tissue

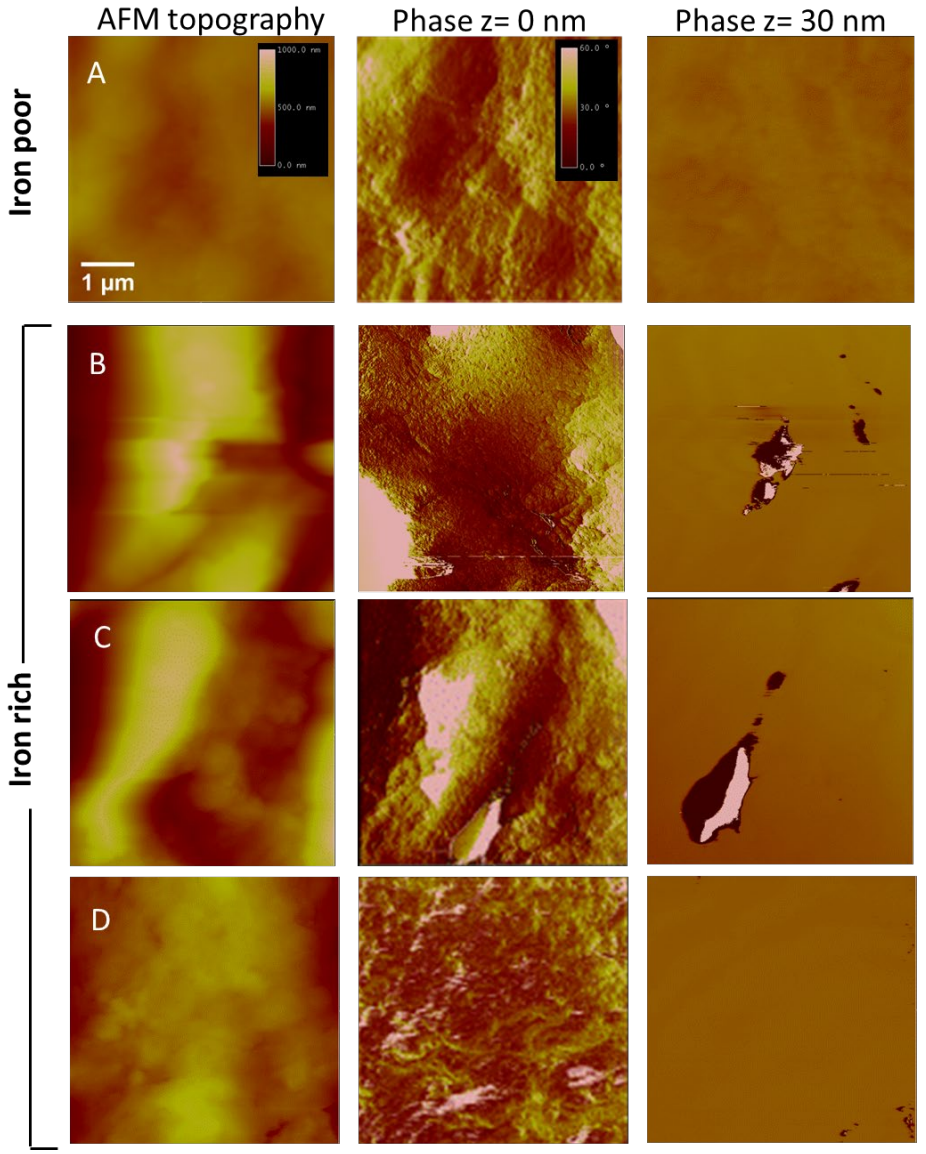




Detection of MFM signal

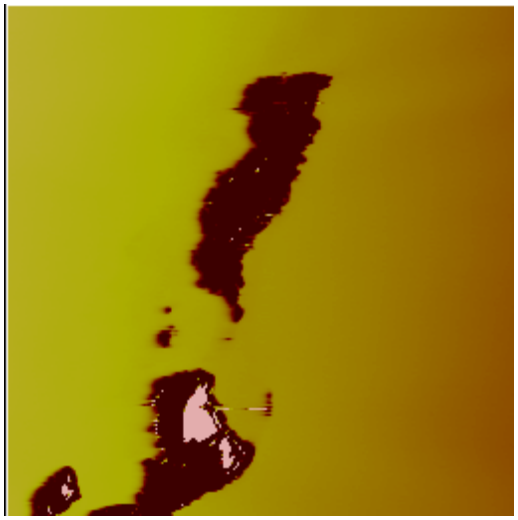




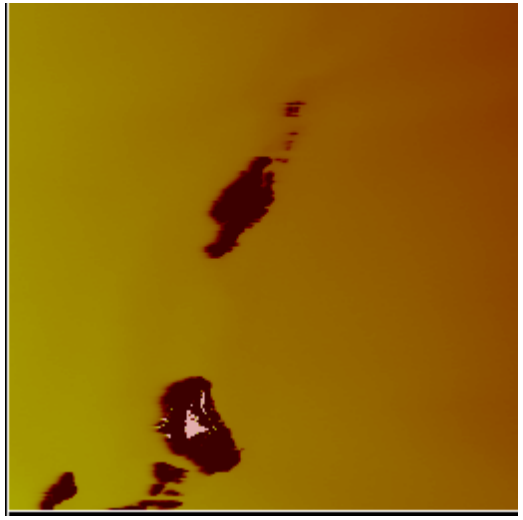


Long range detection

MFM probe



z = 30



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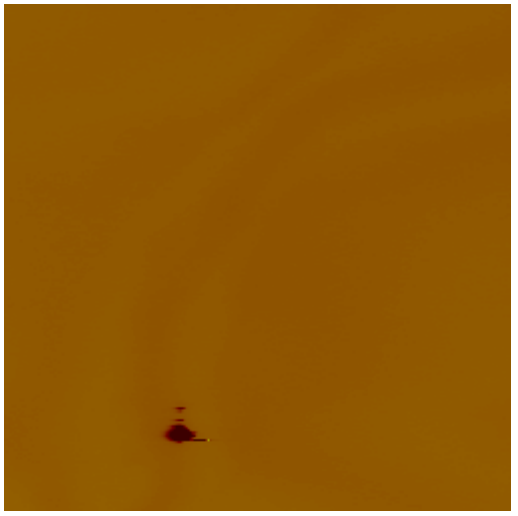
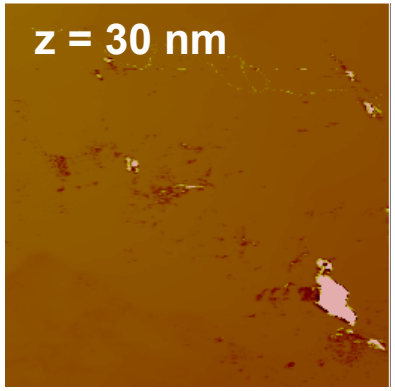
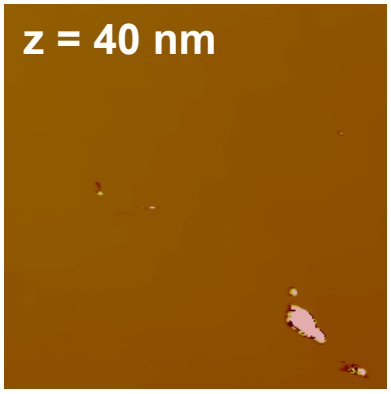
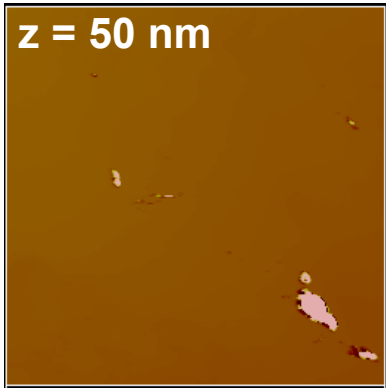


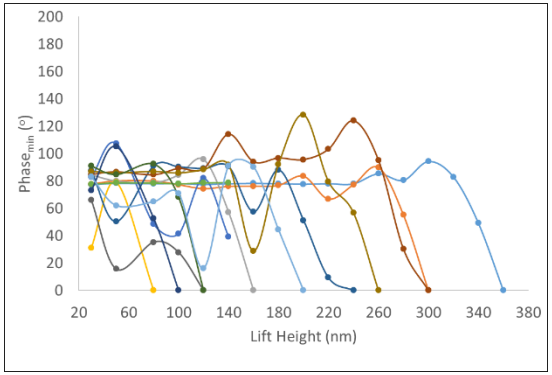
90 nm

AFM probe

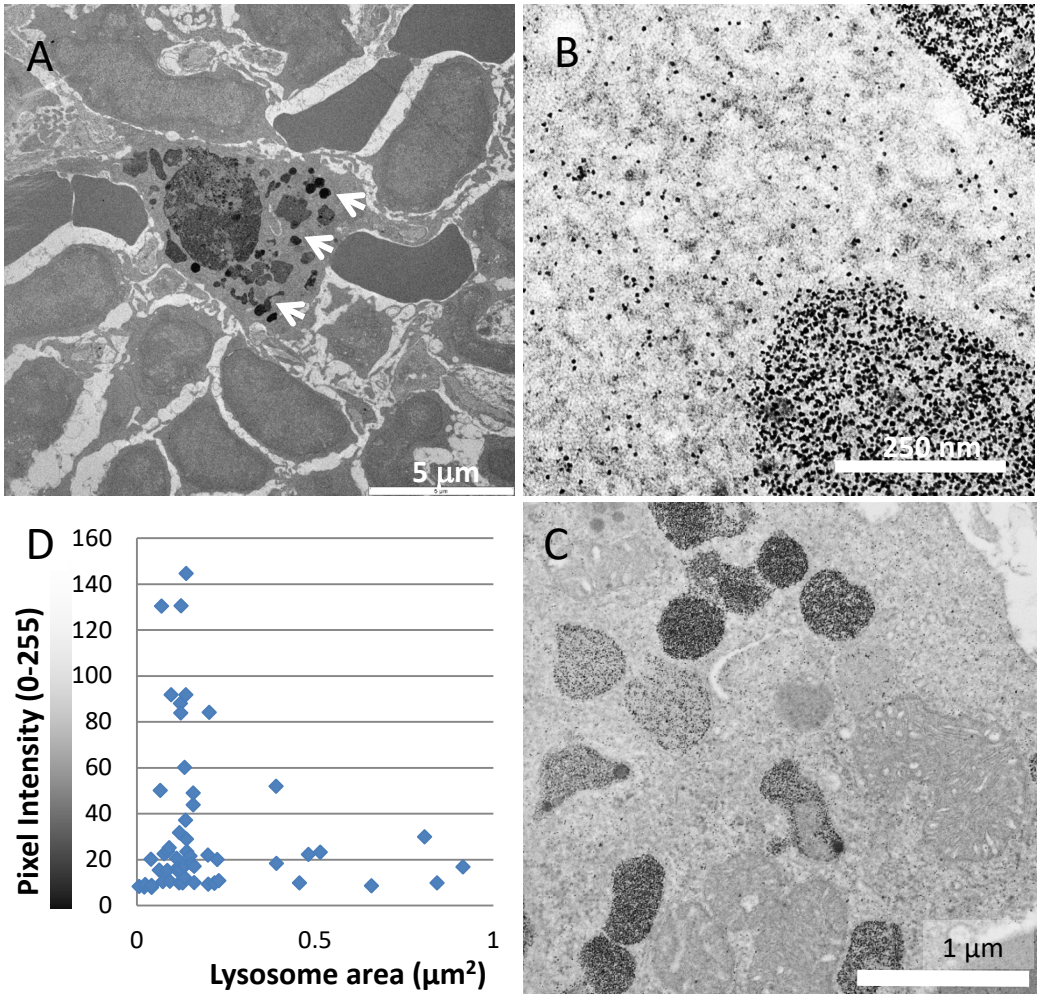






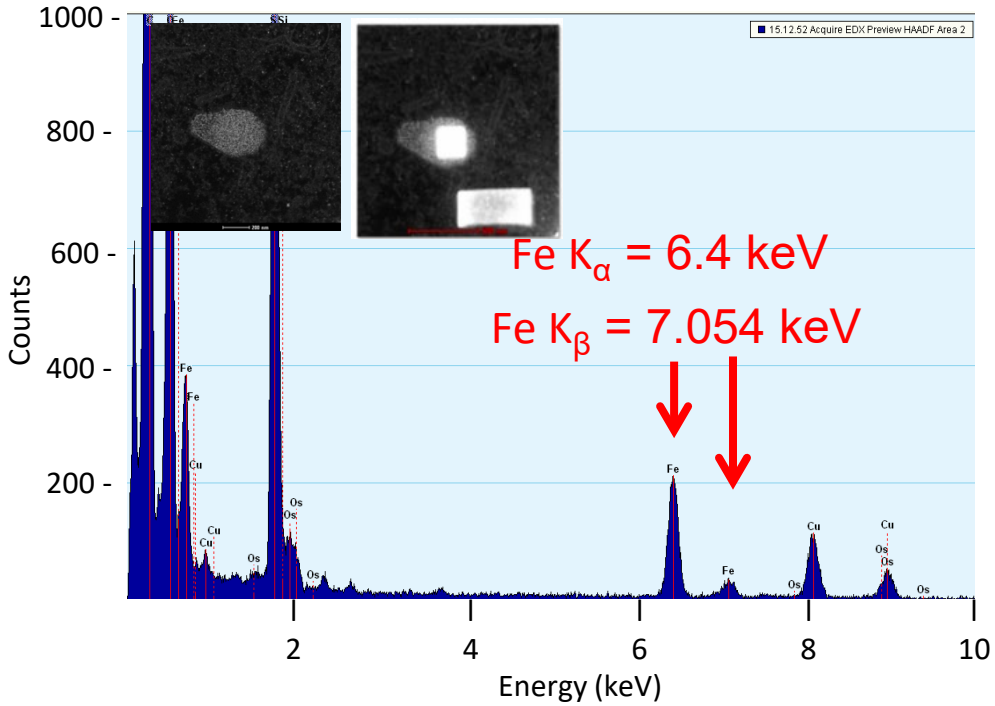


Verification of MFM signal: TEM



Size(z) of iron-rich lysosomes < 0.2 µm²

Energy Dispersive Spectroscopy



- MFM signal obtained from lysosomes (regions ~ **< 0.2 μm²)**
- No MFM signal from mono-disperse cytoplasmic ferritin

MFM of Healthy Tissue

Objectives:

Effect of chemical fixatives

MFM signal is not affected by fixatives

Detection of MFM signal

MFM signal present in iron-rich regions

AFM (non-MFM) probe cannot detect MFM signal

Verification of MFM signal

Size of MFM signal corresponds to iron rich lysosomes

Blissett et al, *Nanomedicine: NBM*, 2017

Walsh et al, *J. Mag. & Mag Mater* (in review)

MFM of Diseased Tissue

Objective:

Is there a difference in the **quality** and **quantity** of iron in healthy vs. diseased tissue?

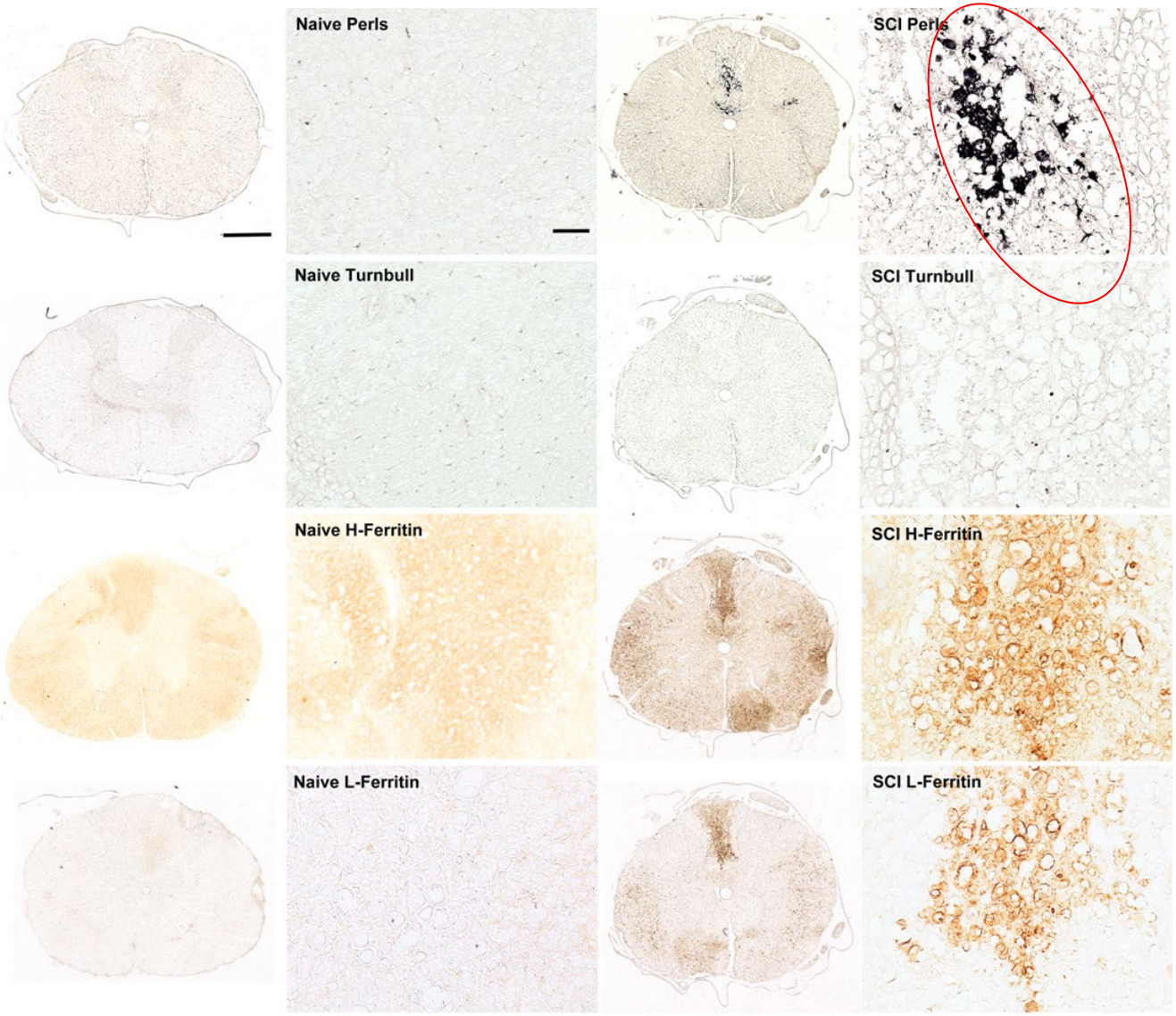
Experimental system:

Rodent model of acute injury (spinal cord injury)

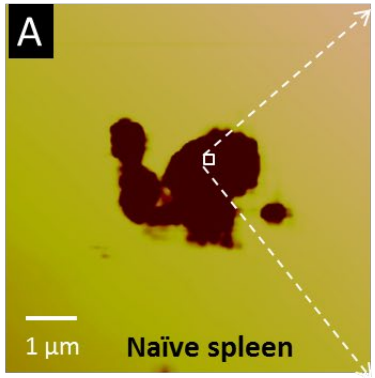
Rats: Naiive (healthy) and Injured (diseased)

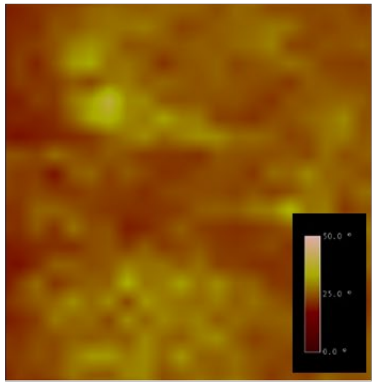
Tissues analyzed: spleen, spinal cord

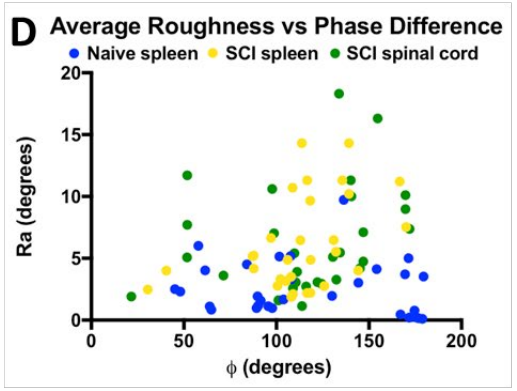
Perls' Stain (spinal cord)

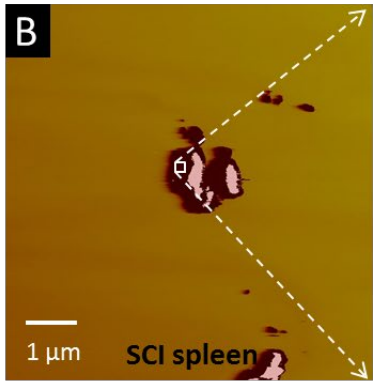


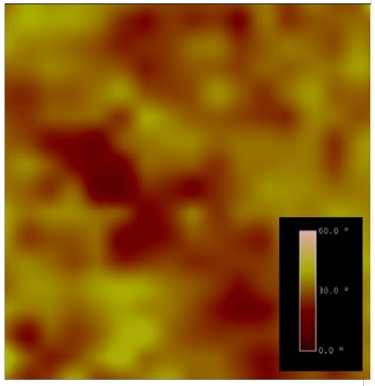
MFM analysis

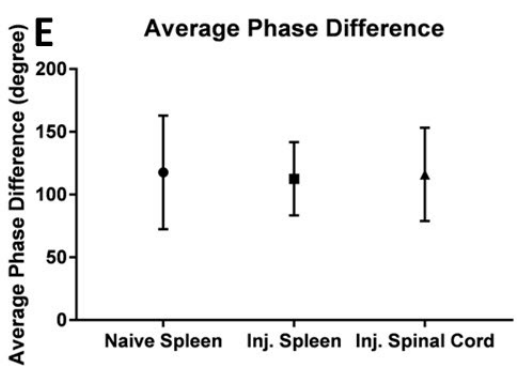


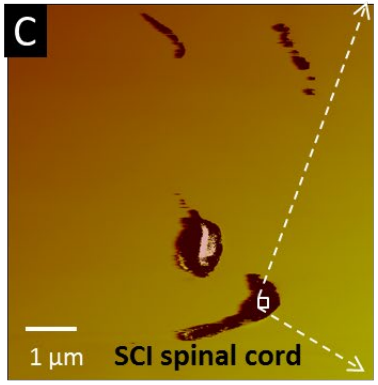


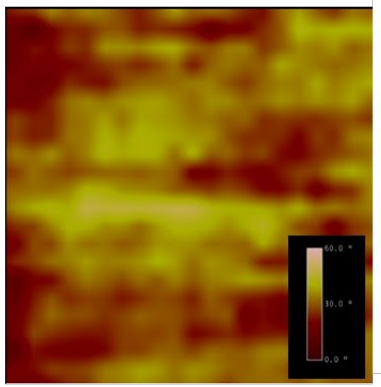


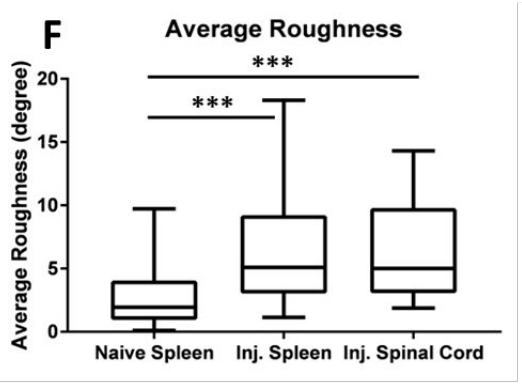




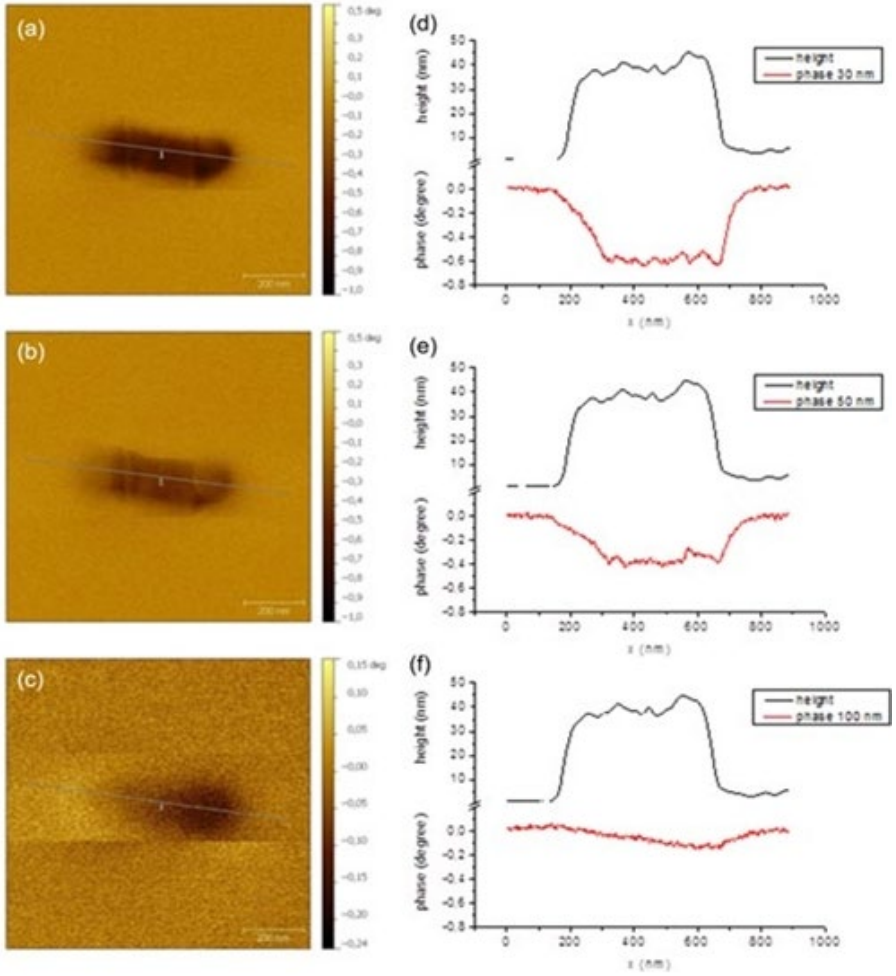


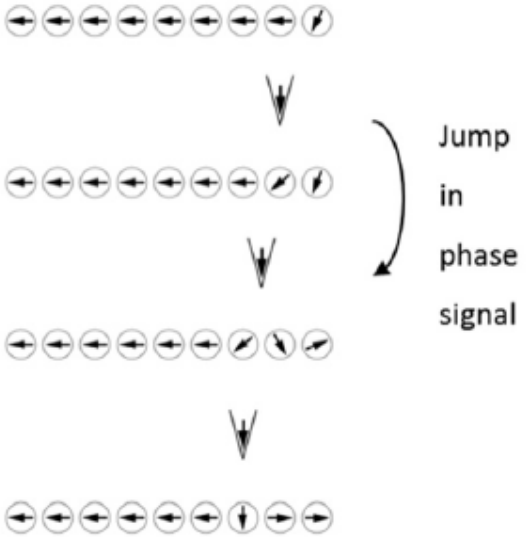






Roughness of MFM signal





Interparticle interactions also affect MFM signal

Magnetic force imaging of a chain of biogenic magnetite and Monte Carlo analysis of tip–particle interaction
André Körnig et al 2014 J. Phys. D: Appl. Phys. 47 235403 doi:10.1088/0022-3727/47/23/235403

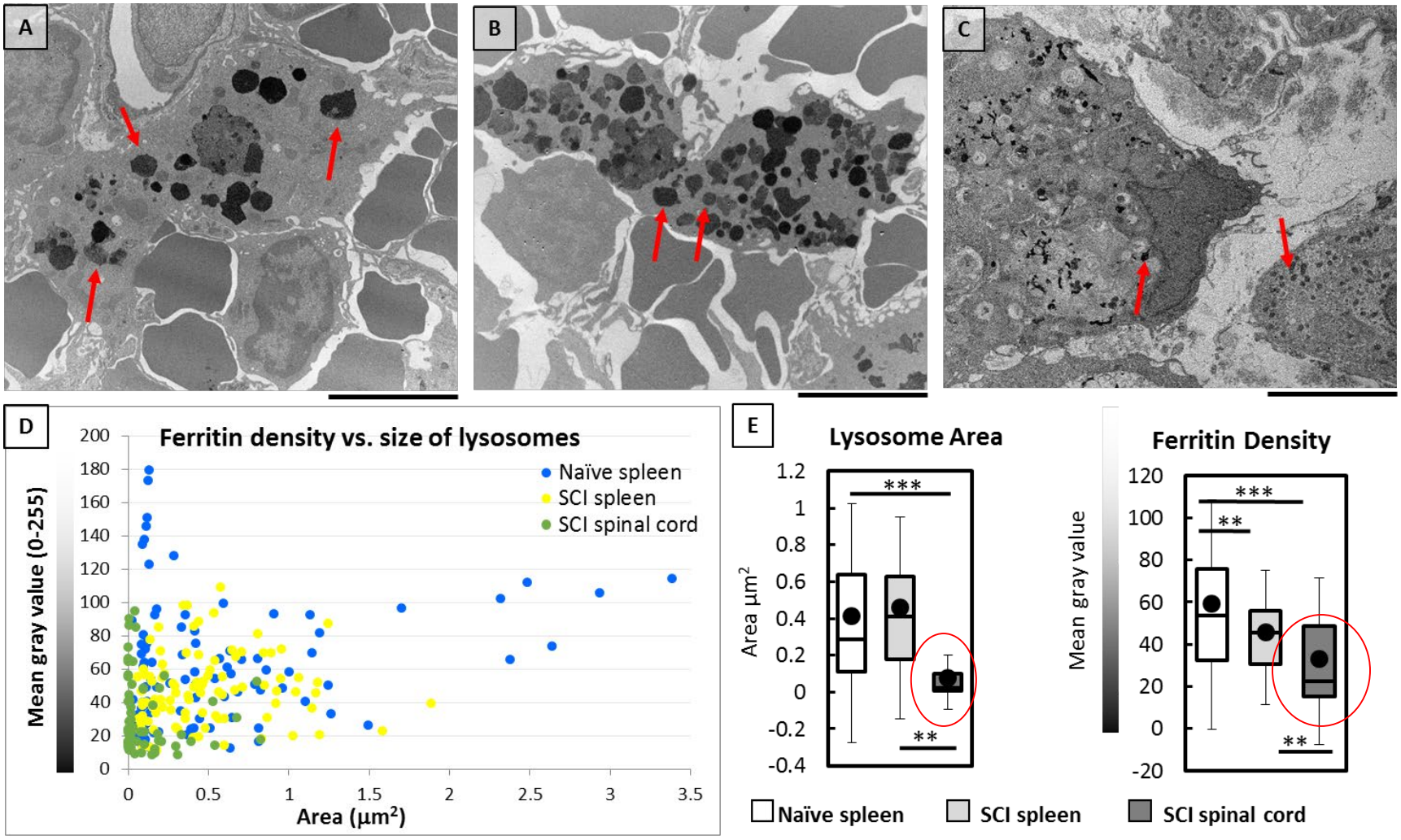
Parameters affecting MFM roughness

- Density of ferritin(iron)
- Size of ferritin (iron)
- Oxidation state of ferritin iron
  -Magnetite (Fe^{2+}) > Ferrihydrite (Fe^{3+})

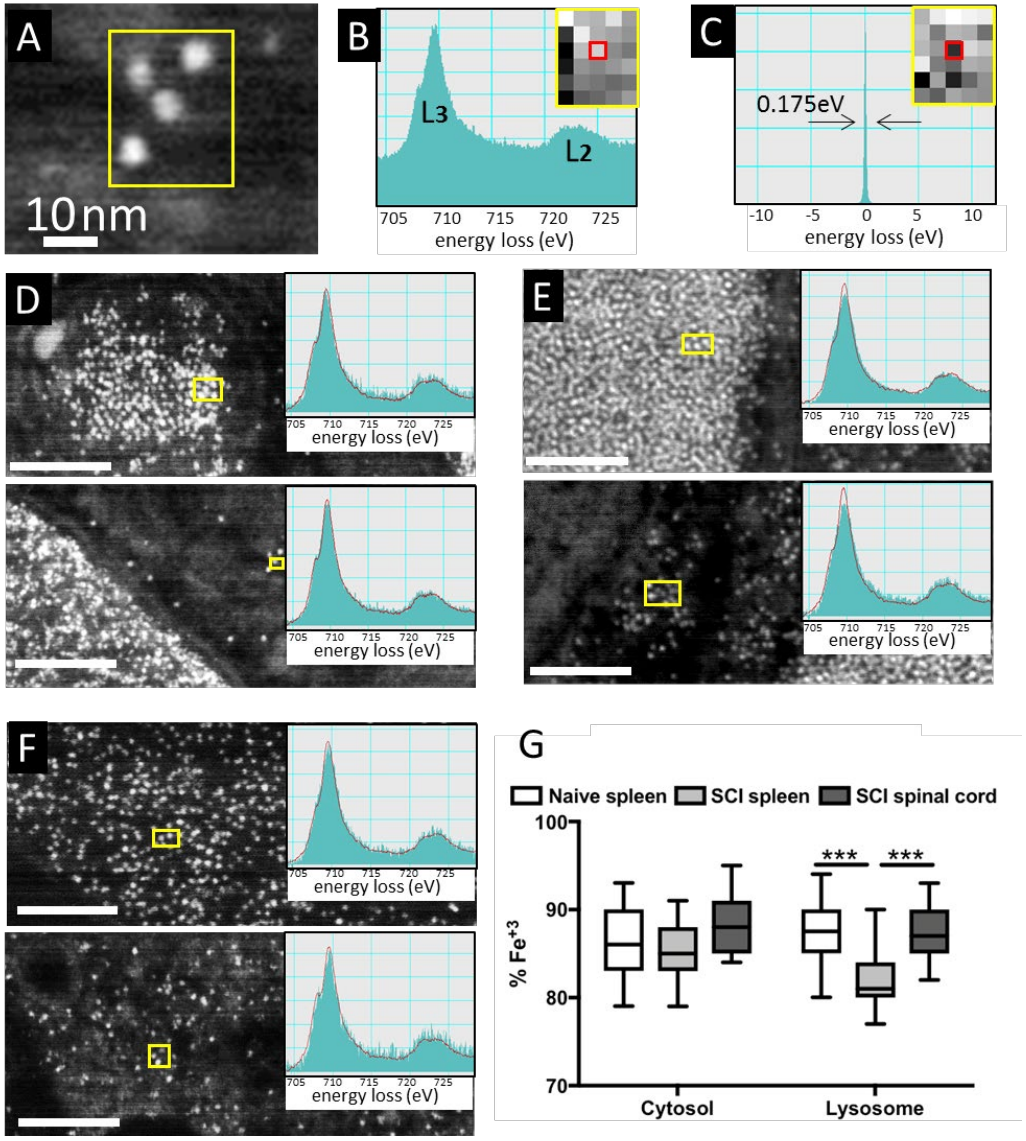
TEM analysis

EELS spectroscopy

TEM analysis: lysosome size and density



EELS analysis: oxidative state of iron



MFM analysis of diseased tissue

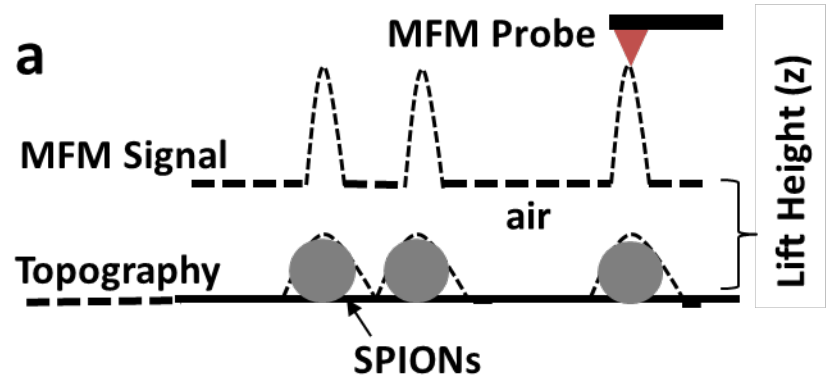
Objective:

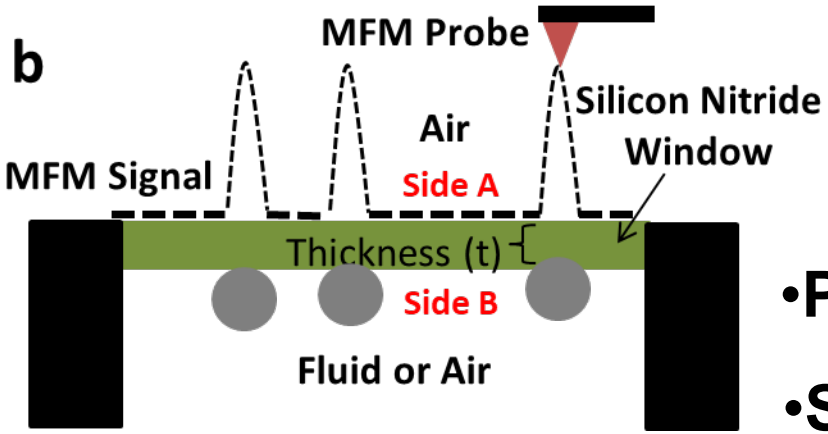
Is there a difference in the **quality** and **quantity** of iron in healthy vs. diseased tissue?

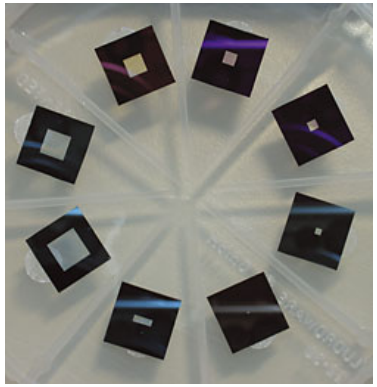
- **Size of lysosomes is reduced in injured tissues**
- **No major differences in oxidation state between injured and naïve animals**

Blissett et al, *Scientific Reports*, 2018

Indirect MFM

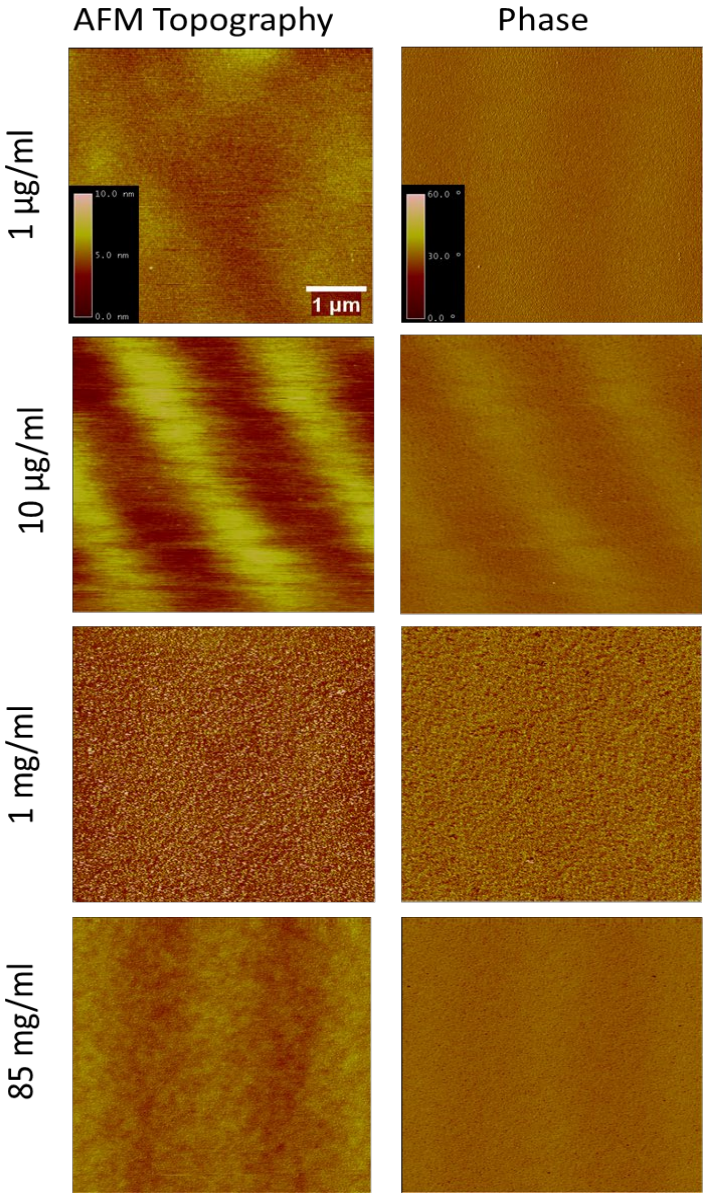


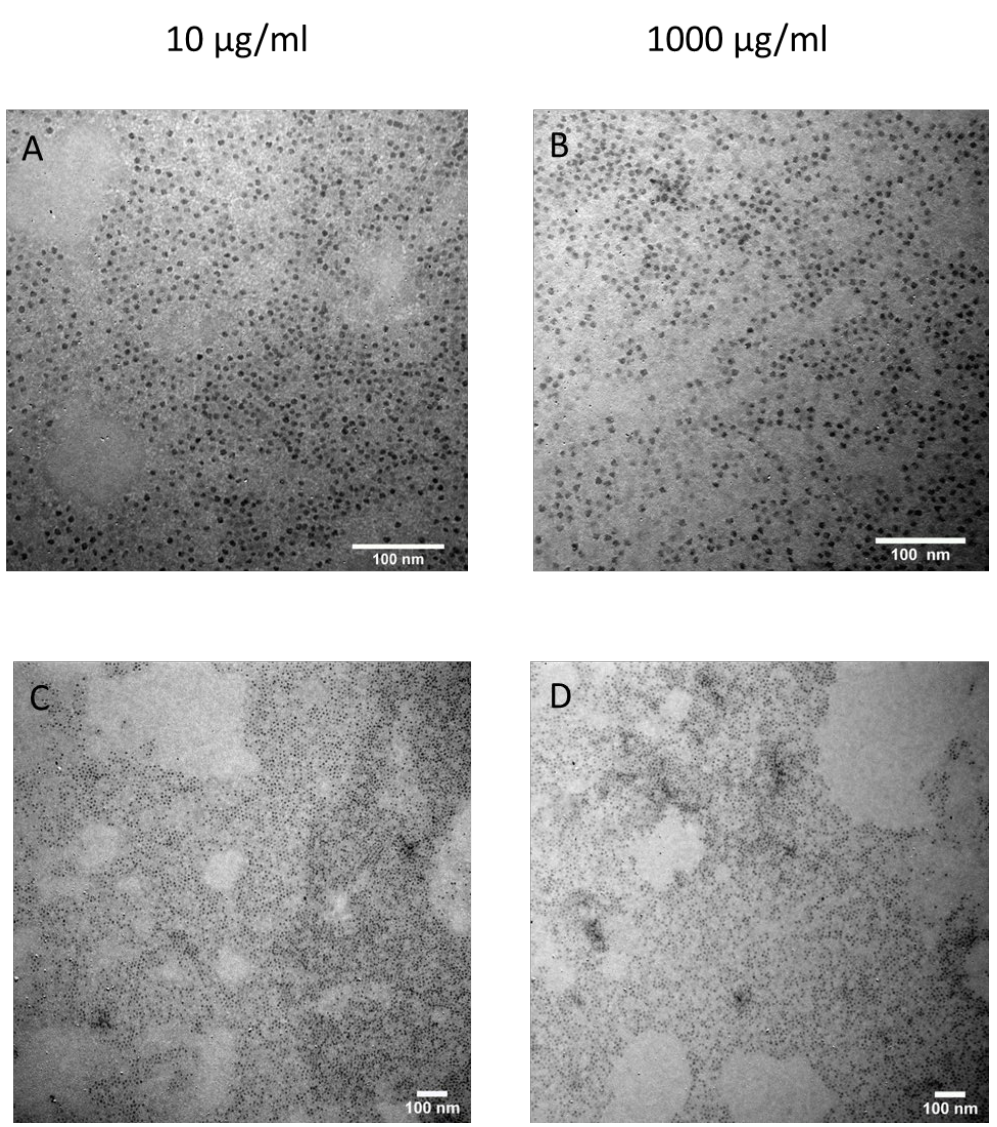




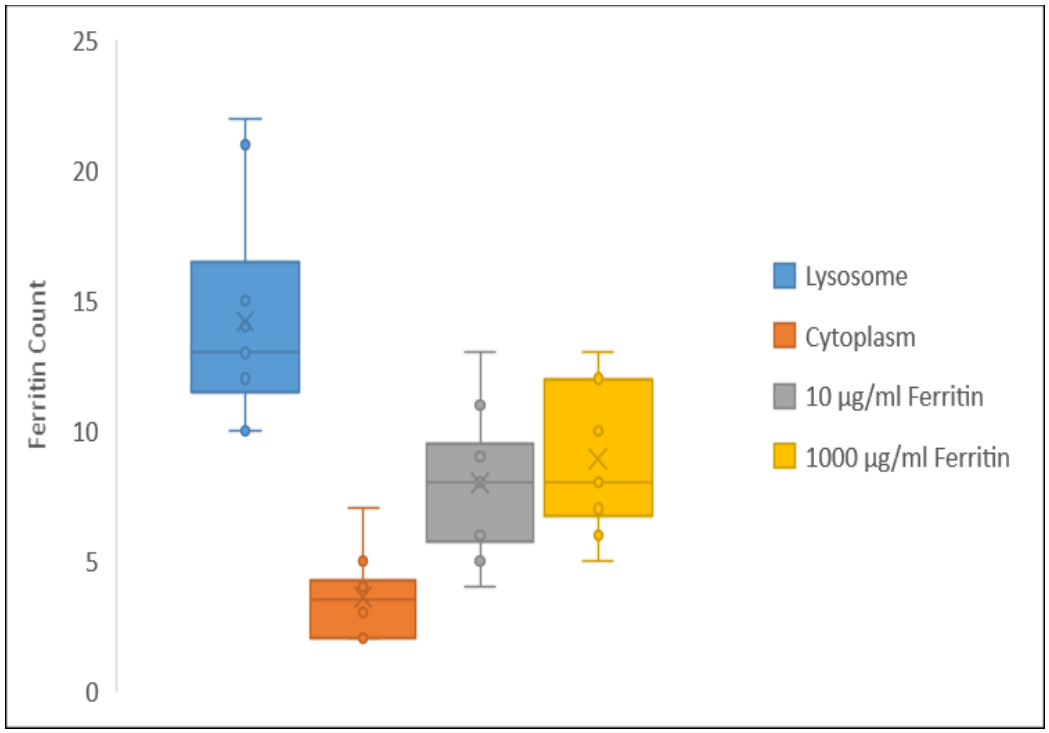
- Probe does not get contaminated
- Samples can be kept in a fluids
- Scanning at multiple lift heights not required
- Multimodal Imaging is possible (MFM, TEM, Light)

ID-MFM of ferritin





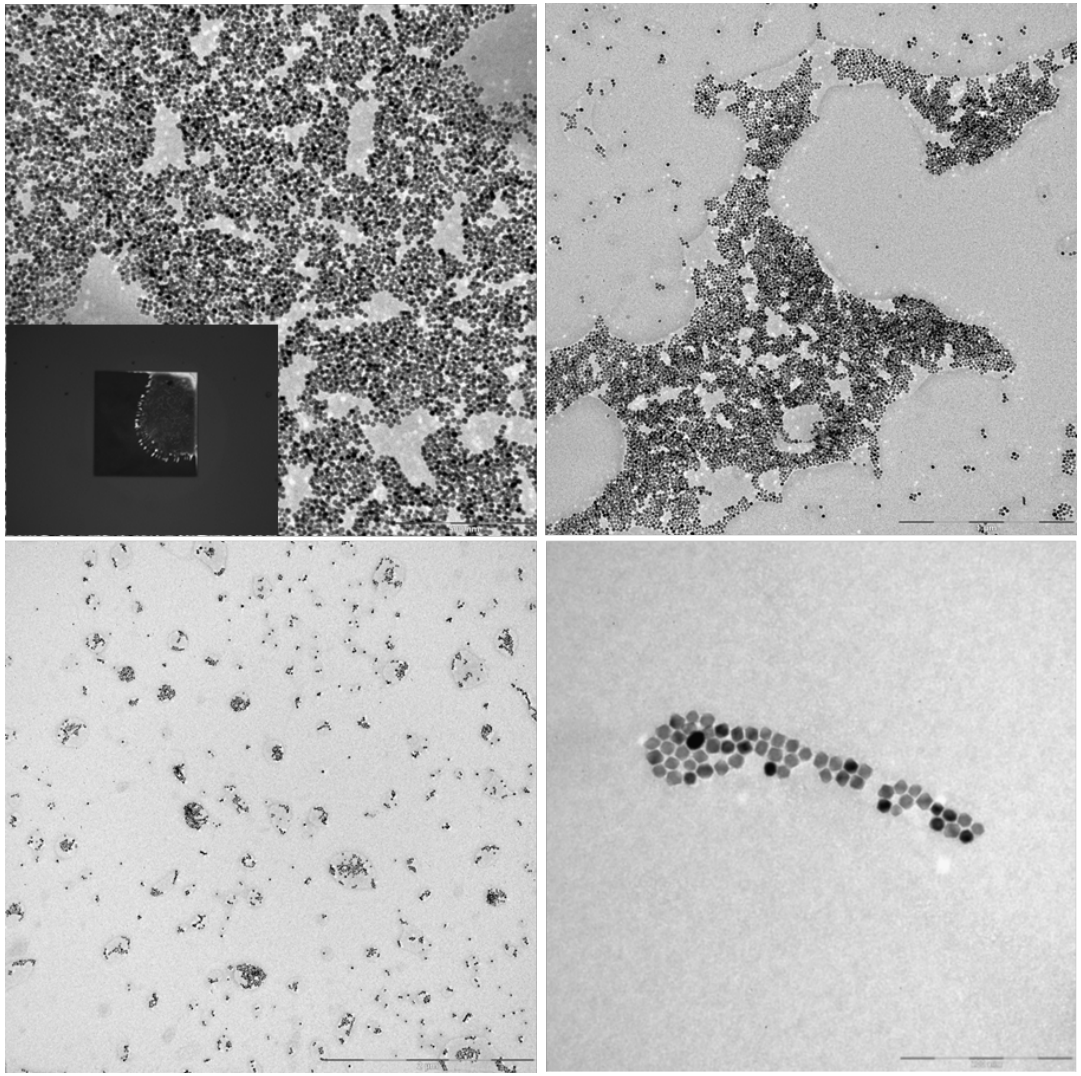
Ferritin density

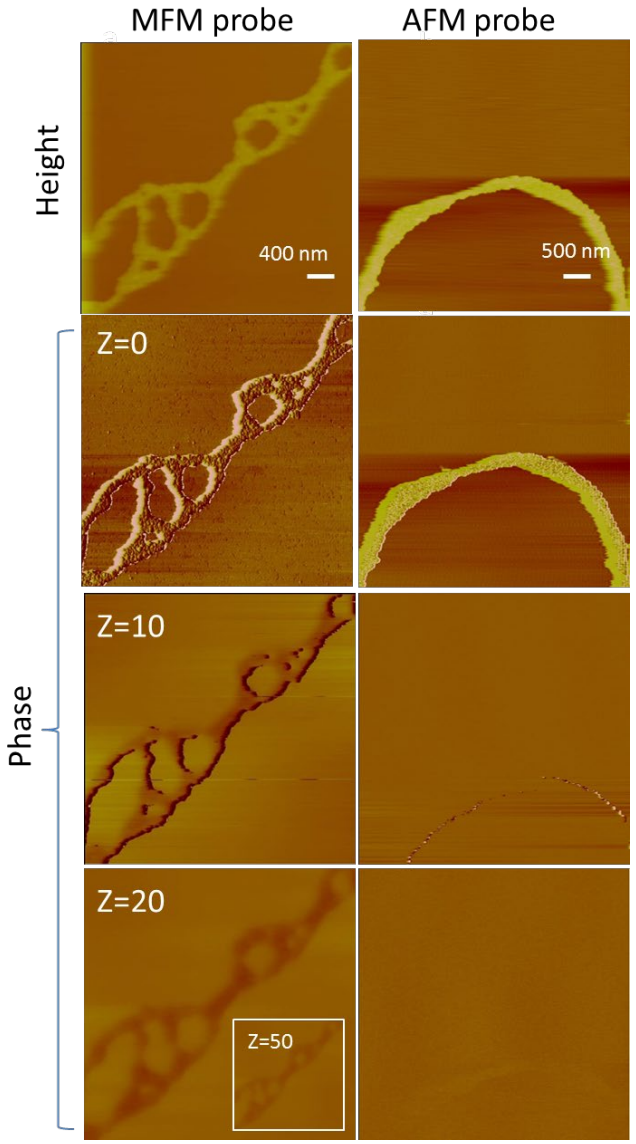


- Ferritin density in lysosomes (in-vivo) is much higher than that which can be achieved with purified ferritin (in-vitro)

- Direct MFM signal could only be obtained from lysosomal ferritin in tissue sections

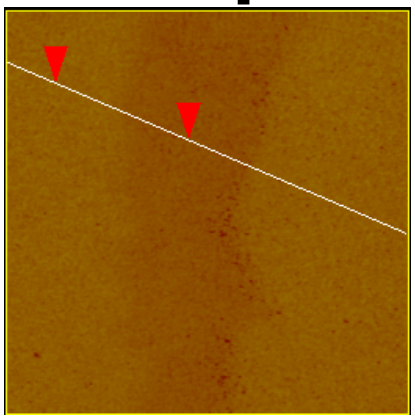
Direct MFM of iron oxide nanoparticles

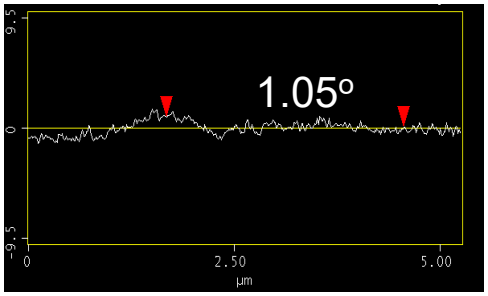




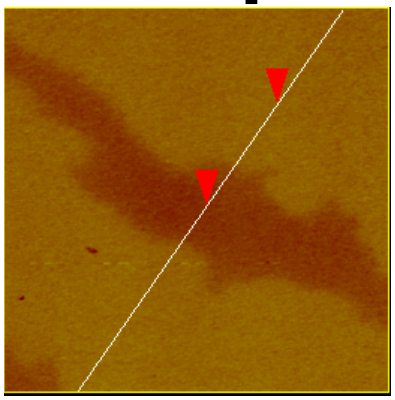
ID MFM (10 nm thick membrane)

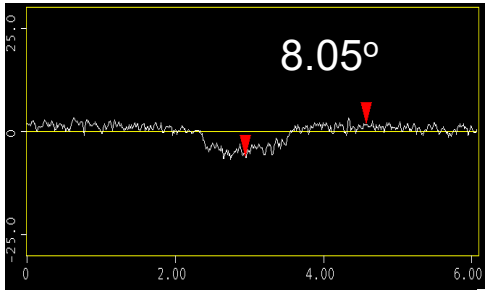
AFM probe

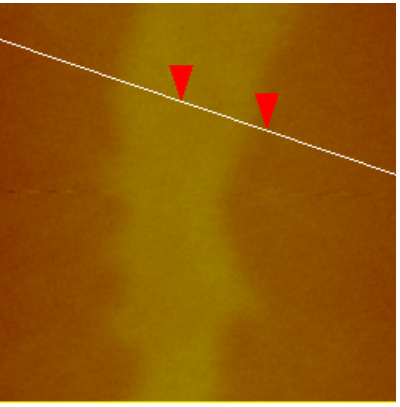


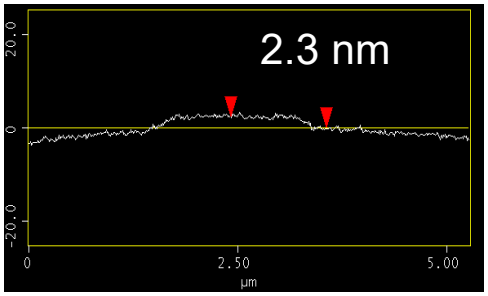


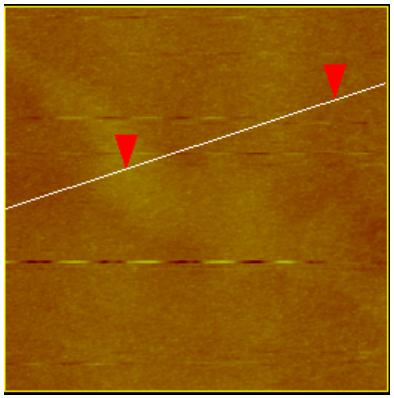
MFM probe

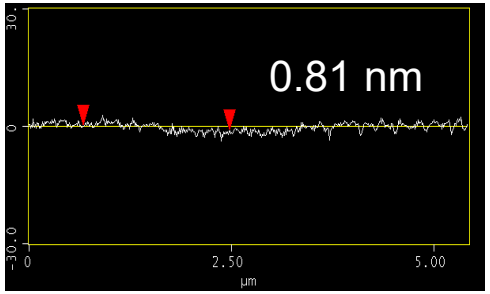




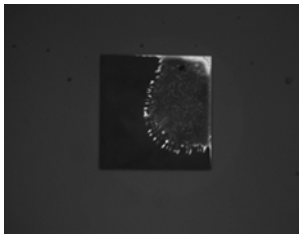


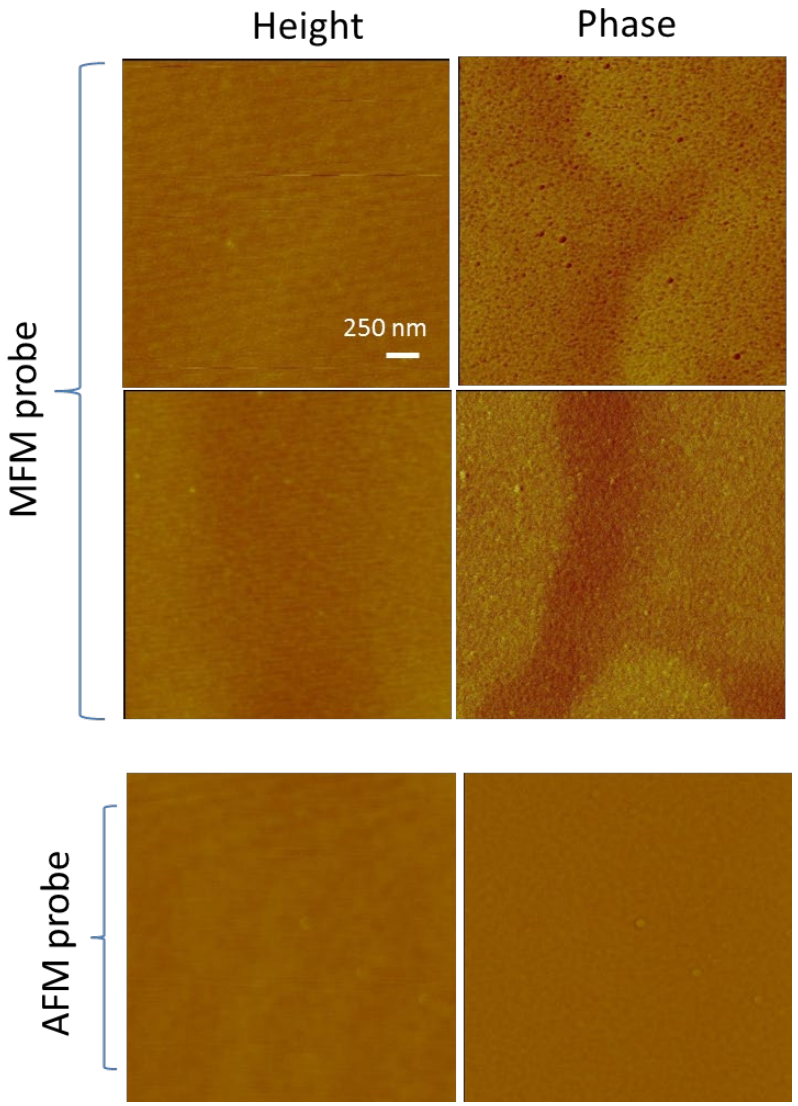




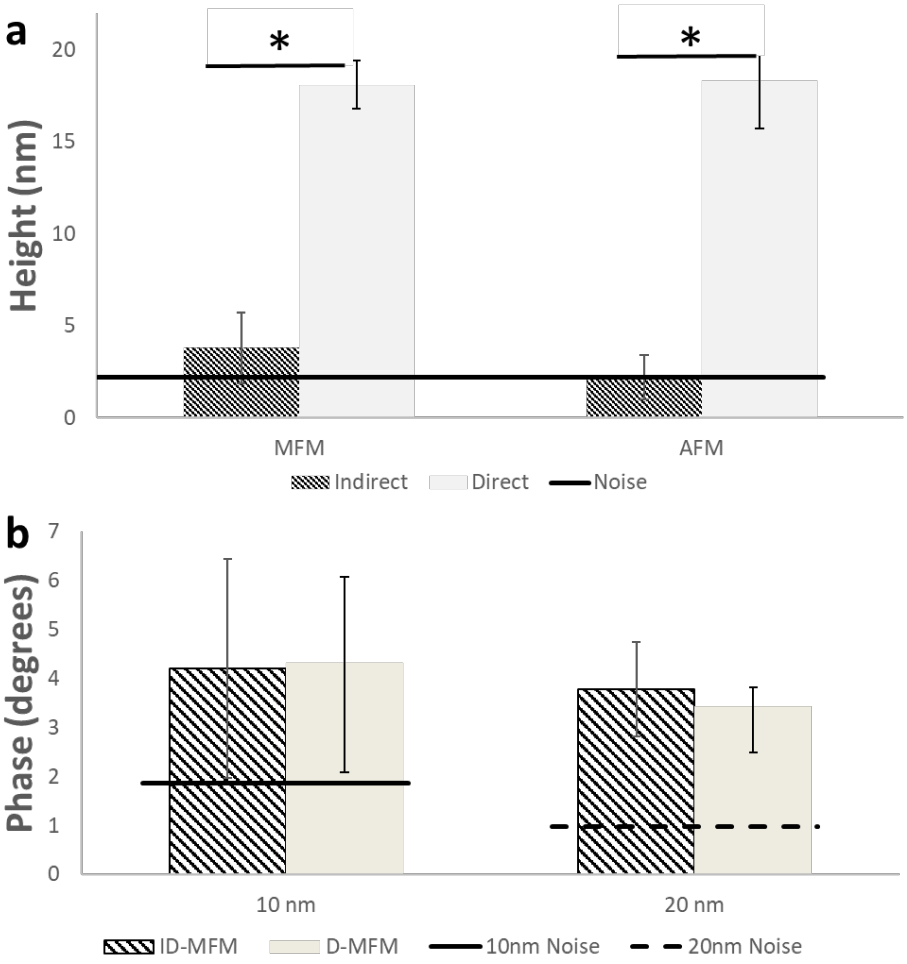


ID MFM (20 nm thick membrane)





Comparison of Direct and Indirect MFM

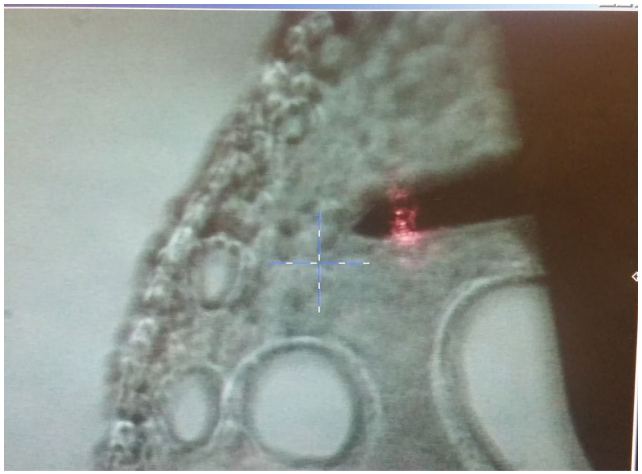


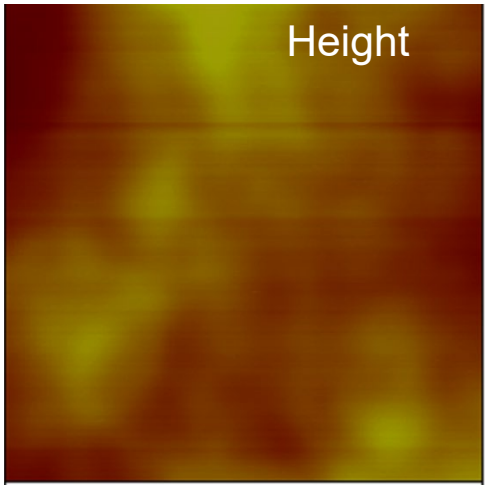
- Minimal contribution of surface topography
- Minimal contribution from van-der Waals interactions
- No compromise in strength of MFM signal
- Multimodal imaging possible for MFM, TEM and fluorescence microscopy

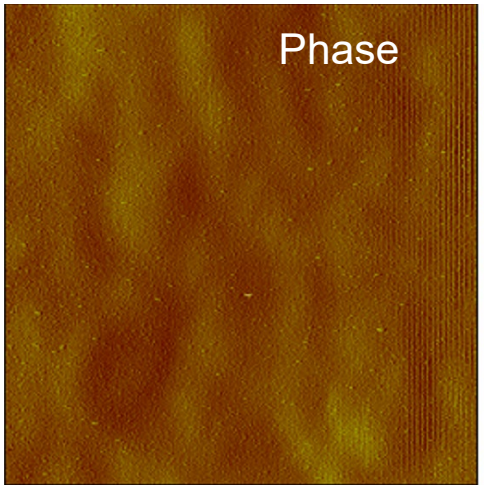
Sifford et al, *Nanoscale Advances*, 2019

Conclusions

- Both Direct and ID MFM can serve as high resolution, label free tools for iron-detection in histology
- MFM signal in tissues arises from clusters of ferritin(iron)
- ID MFM can serve as a artifact free, high-throughput, multimodal technique for iron-detection in histology
- ID-MFM can be adapted for samples in fluids







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