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Abstract
Magnetic force microscopy (MFM) is a very powerful technique, which can potentially be used
to detect and localize the magnetic fields arising from nanoscopic magnetic domains, such as
magnetic nanoparticles. However, in order to achieve this, we must be able to use MFM to
discriminate between magnetic forces arising from the magnetic nanoparticles and nonmagnetic
forces from other particles and sample features. Unfortunately, MFM can show a significant
response even for nonmagnetic nanoparticles, giving rise to potentially misleading results. The
literature to date lacks evidence for MFM detection of magnetic nanoparticles with
nonmagnetic nanoparticles as a control.

In this work, we studied magnetite particles of two sizes and with a silica shell, and
compared them to nonmagnetic metallic and silica nanoparticles. We found that even on
conducting, grounded substrates, significant electrostatic interaction between atomic force
microscopy probes and nanoparticles can be detected, causing nonmagnetic signals that might
be mistaken for a true MFM response. Nevertheless, we show that MFM can be used to
discriminate between magnetic and nonmagnetic nanoparticles by using an electromagnetic
shielding technique or by analysis of the phase shift data. On the basis of our experimental
evidence we propose a methodology that enables MFM to be reliably used to study unknown
samples containing magnetic nanoparticles, and correctly interpret the data obtained.

S Online supplementary data available from stacks.iop.org/Nano/21/305706/mmedia

1. Introduction

Magnetic nanoparticles (MNPs) have a wide range of potential
and current applications in biomedicine including cell and
biomolecule sorting and separation, drug delivery, labelling
and magnetic hyperthermia therapy [1, 2]. For most of these
applications, it is necessary that the magnetic nanoparticles
(MNPs) interact with cells, typically with specific types of cells
located in the body, or *in vitro*. For drug delivery purposes it
can be advantageous to have the particles enter cells, whereas
for cell sorting or thermal therapy, external interaction with
a cell membrane is adequate. For therapeutic applications
the particles should be capable of targeting specific cells,
so that the particle's action will be delivered only where
required. It is therefore important for the development
of MNP applications that the location of the particles can

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be determined with high accuracy. Ideally, an imaging technique for development of these applications would give three-dimensional information, have nanometre resolution, and be able to specifically discriminate MNPs from other particles or biological materials. Magnetic force microscopy (MFM) fits these specifications, because it shares with atomic force microscopy (AFM) the three-dimensional nature of the information, and approaches the resolution of AFM, while enabling the probing of sample magnetization thus allowing discrimination of MNPs from background features. Like AFM, MFM allows the measurement of specimens under ambient conditions, meaning that cells can be examined in a hydrated state, while approaching the high resolution available in vacuum-only electronic microscopy techniques. Unfortunately, MFM can give a strong response even on nonmagnetic nanoparticles or under circumstances where no magnetic interaction would be expected, potentially giving rise to misleading results [3, 4]. Thus far, no MFM results have been shown using nonmagnetic particles as a control specimen.

Unlike in AFM, the probe in MFM is magnetized. Thus, in addition to the forces measured in AFM, in MFM the magnetic interactions between the fields of the probe and those of the sample are measured. In order to be able to measure these interactions, the probe is lifted a certain distance from the sample surface, otherwise short-range forces, which can be much stronger than magnetic interactions (such as van der Waals forces) will make measurement of the magnetic forces impossible. The most commonly used MFM implementation is the so-called 'lift mode' [5] in which a two-pass technique is used—each line in the image is measured twice, the first line measuring only sample topography, the second line being used to measure the magnetic fields at a fixed distance from the surface at each point. This is important because even at large lift heights, long-range forces (other than magnetic ones) might act on the probe. By maintaining the probe–sample distance constant, it is assumed that such nonmagnetic forces will remain constant, and thus only magnetic forces will be measured [6].

A recent publication discussed the application of MFM to detection and localization of superparamagnetic nanoparticles (SP-MNPs) [4]. In that paper, the successful imaging by MFM of clusters of small (<10 nm) SP-MNPs was demonstrated for the first time. SP-MNPs are an extremely important class of magnetic particles because due to the absence of remnant magnetization, they are magnetized only when a external magnetic field is applied, which is very useful for separation and therapeutic applications [7]. For the purposes of MFM imaging the ability to switch on and off the magnetization of the SP-MNPs by the application of an external field could be extremely useful in order to help understand the response of the MFM probe. However, it is possible that the field from a magnetized MFM probe could magnetize the sample itself, precluding the possibility to distinguish between particles' behaviour in and out of external fields. This would be particularly likely for SP-MNPs. Furthermore, there have been some puzzling results published, such as lift mode images of magnetite nanoparticles which show contrast that is not correlated with magnetic properties of the sample, and which

are described simply as fake MFM images, since the contrast seen in these images could not be properly explained [3]. So far, no study has been described that directly shows the difference seen between magnetic and nonmagnetic particles under the same conditions, meaning that interpretation of published MFM images of magnetic particles is difficult.

Despite these problems, and the existence of several more sophisticated techniques for magnetic characterization using AFM [8, 9], lift mode is the technique most commonly used for MFM, because it is relatively simple to implement and commonly gives high contrast even under ambient conditions. In lift mode, variations in phase, amplitude of resonant frequency are directly related to the local force derivative [10]. In the present study, the force derivative was assessed through the variation in phase shift due to the higher sensitivity of this signal. Unfortunately, while phase shift derived from lift mode images is commonly used to characterize magnetic nanostructures [4, 11–13], there has never been a systematic study of the response of this technique to the magnetization of the sample, and comparison of this response with that obtained with nonmagnetic samples. For this reason, the interpretation of the 'fake magnetic images' referred to above is difficult [14]. Furthermore, although it has been shown that coated magnetic particles might be imaged by MFM, no direct comparison of the distance-dependent response of coated with uncoated particles is available [12].

In this paper we report a detailed study of the response of MFM to magnetic and nonmagnetic nanoparticles. Specifically, in order to explain the results from previous works, we study the origin of the contrast detected when imaging nonmagnetic nanoparticles. Furthermore, we show how this contrast can be reduced by biasing of the AFM probe thus permitting clear imaging of the magnetic nanoparticles, without electrostatic interference. We also show how magnetic nanoparticles can be easily visualized when coated with nonmagnetic material, a highly useful feature for the measurement of MNPs in biological systems.

2. Experimental details

2.1. Nanoparticle preparation

To synthesize superparamagnetic nanoparticles, 0.71 g Fe(acac)$_3$ in 20 ml of phenyl ether was mixed with 2 ml of oleic acid and 2 ml of oleylamine under an argon atmosphere under vigorous stirring. 1,2-hexadecanediol (2.58 g) was added and the solution was refluxed for 2 h. After cooling to room temperature, absolute ethanol was added to mixture and the particles were magnetically separated with a permanent magnet [15]. To coat magnetite particles with silica, a modified version of a previously published procedure was used [16]. 140 μl of commercial magnetite nanoparticles at 5 mg ml^{-1} were added to a flask together with 2.36 ml of milliQ water, 2.475 ml of ethanol and 25 μl of TEOS. The flask was then placed in an ultrasound bath for 2 h and after that the nanoparticles were magnetically separated and resuspended in milliQ water. All reagents were purchased from Sigma-Aldrich and used as received.

2.2. Magnetic force microscopy

All magnetic force microscopy was carried out with a commercial AFM system (Veeco multimode with nanoscope IVa controller) under ambient conditions. All the results reported in this work were carried out with silicon cantilevers coated with a cobalt/chromium film (MESP probes from Veeco). The cantilevers had a resonant frequency (f_0) of approximately 75 kHz, and nominal spring constant of 2.8 N m^{-1}. The coating produced a coercivity of approximately 400 Oe. Other probes with similar properties (Nanosensors PPP-MFMR, $f_0 \approx$ 75 kHz) were tested and gave similar results. Magnetic fields were applied by either fixing the sample on top of a small NdFeB magnet (field approximately 1.2 T), or on top of the magnetic AFM scanner (field ≈0.2 T). Throughout this work, we used a nonmagnetic multimode AFM instrument; the AFM head and probe holder were constructed with nonmagnetic materials. For zero applied field experiments, we also used a scanner made with nonmagnetic materials. For applied field experiments, we tried using a scanner with built-in magnet, and described elsewhere [4]. We found no significant differences in the results using this technique to those obtained using the nonmagnetic scanner with the permanent NdFeB magnet under the sample. Images were collected in lift mode controlled by the software, and values of phase shift extracted from the images. Where needed, DC bias voltage was applied between the probe and the sample with the built-in facilities of the multimode. In these cases, the samples were deposited on conductive silicon supports, otherwise, freshly cleaved mica was used. All samples were deposited from solution and thoroughly dried before analysis. Magnetic nanoparticles were exposed to sonication before deposition to reduce clustering. In some experiments, we examined the same nanoparticles with different probes, to see the response without magnetized probes, or to repeat the experiment to determine the variability between different probes. In other cases, we needed to remove the samples to heat them, and then replace them and image the same particles afterwards. In order to do this, it was necessary to navigate back to exactly the same position on the sample, which is not a trivial task. This was helped by examining a cluster of magnetite nanoparticles with a distinctive shape, which was located near an optically visible feature. Using the inspection optical microscope attached to the AFM, along with an AFM scanner with approximately 150 μm scan range ('J' scanner, Veeco), we were able to reproducibly return to measure the same nanoparticles after changing the probe or swapping the sample.

2.3. Magnetometry

The magnetic properties of the dried MNPs were studied using a commercial Quantum Design MPMS superconducting quantum interference device (SQUID) magnetometer. The hysteresis loops were run at 370, 300 and 5 K for a maximum applied magnetic field of 50 kOe. Temperature-dependent zero-field-cooled (ZFC) and field-cooled (FC) magnetization measurements were obtained for a range of temperatures between 5 and 380 K with an applied magnetic field of 140 Oe.

2.4. Transmission electron microscopy

Images were obtained using a HITACHI H-8100 instrument operating at 200 kV. The samples for TEM analysis were prepared by depositing 10 μl of nanoparticle solutions on carbon copper grids, washing twice with 10 μl of Millipore water, and air drying.

3. Results

3.1. Comparison of response to magnetic and nonmagnetic nanoparticles

In order to clearly distinguish between magnetic and nonmagnetic NPs, we analysed mixed systems, consisting of depositions of colloidal solutions of the two types of NPs. In the first case, we studied a mixture of gold and magnetite nanoparticles. We chose to use two populations with distinct sizes, so that the nature of the particles could be determined from the topographic scans obtained concurrently with the lift mode images. The results shown in figure 1 derive from a mixed system of gold nanoparticles (mean diameter 17 nm), and magnetite nanoparticles (mean diameter 50 nm); transmission electron microscopy (TEM) images showing the shape and size of these particles are included in the supporting information in figure S1 (available at stacks.iop.org/Nano/21/305706/mmedia).

In oscillating lift mode as used here, the contrast is formed by the change in effective spring constant of the cantilever caused by the interaction between the fields above the sample with the field from the probe. This causes a shift in the frequency of oscillation which is most commonly detected by the effect it has on the phase of oscillation [17, 18]. Other authors have observed a negative phase shift over small magnetic domains (usually represented in MFM images by a darker shade), due to attractive interactions between the probe and the magnetic domain [4, 19]. In addition, when the probe-magnetic domain distance is small, rather than being a unidirectional effect, the magnetically induced phase shift can have both positive (at the edges) and negative (in the centre) regions over a single magnetic domain [20]. However, some authors have also demonstrated positive phase shifts, or dipolar contrast, with half the contrast being negative, and half positive, although for nanoparticles this is typically found only when external fields are applied perpendicularly to the measurement direction [4, 12, 21]. Here, when fields were applied, they were always parallel to the measurement direction (perpendicular to the surface).

Figure 1 summarizes typical results from the sample produced by mixing magnetite and gold nanoparticles. Figure 1(A) shows an image obtained at 15 nm lift height. It is clear that both gold NPs (white arrows), and the magnetite NPs (black arrows) can be imaged by lift mode MFM under these conditions. Comparison of parts A and B show that the magnetite particles gave a far stronger response than the gold nanoparticles, and that at large lift heights the gold nanoparticles were invisible to the MFM, while the magnetite particles remained distinguishable from the background. Furthermore, by comparison of the shades
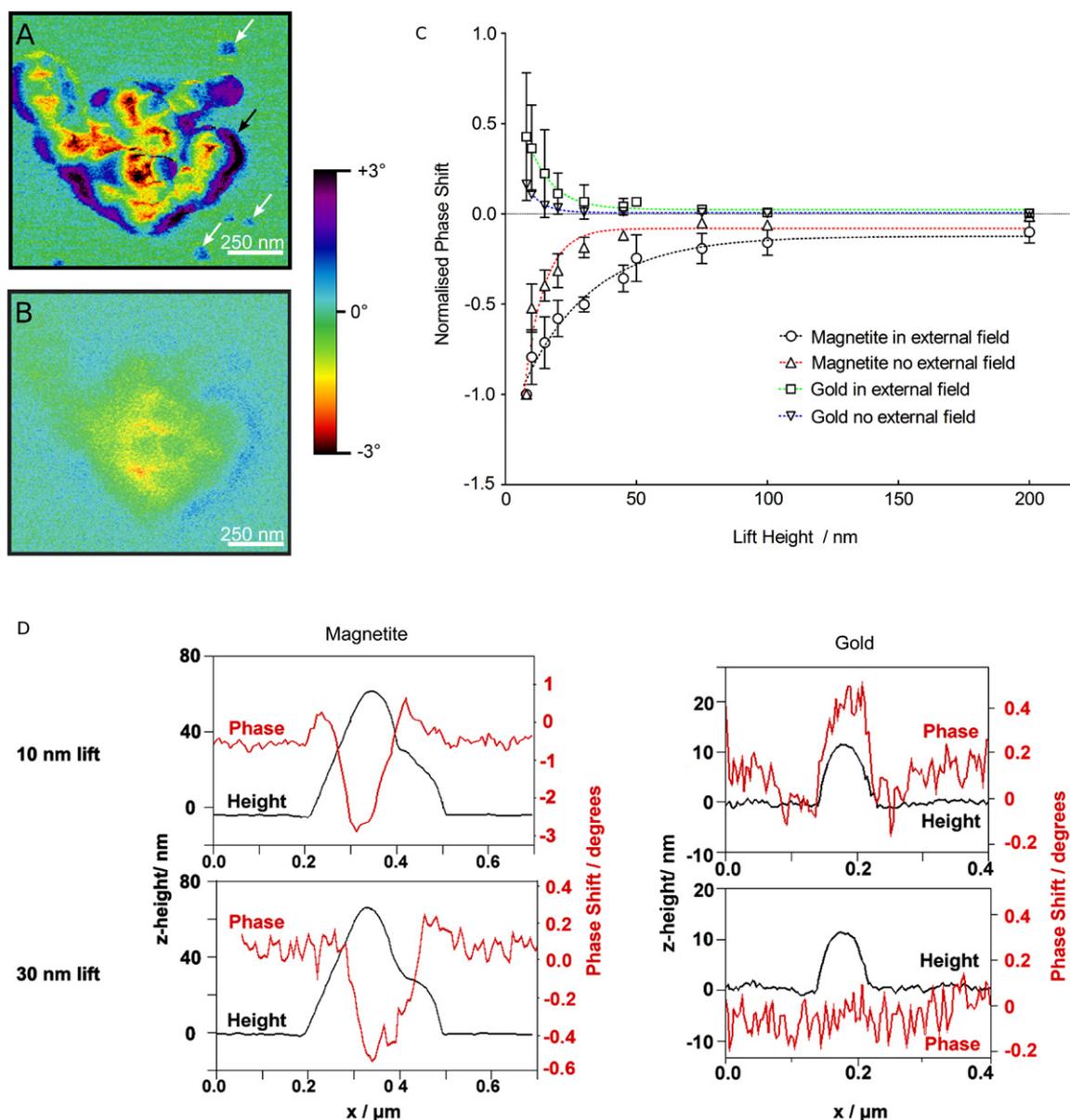
Figure 1. Left: MFM phase shift images at lift heights of 15 (A) and 100 nm (B) showing the same area containing a cluster of Fe_3O_4 NPs (black arrow) and scattered AuNPs (some examples with white arrows). (C) Phase shift response of the magnetite and gold nanoparticles in an external magnetic field, and in the absence of field. Mean values; error bars show standard deviation of results with different probes. Note that in some cases, error bars are hidden behind symbols. Dotted lines were added to guide the eye. (D) Line profiles through phase and height images of a small magnetite cluster and an individual gold nanoparticle (left and right, respectively). The profiles are from images obtained at lift heights of 10 and 30 nm on the top and bottom, respectively.

in the figure with the colour bar it is possible to see that the gold particles gave an almost entirely positive phase shift with no appreciable negative phase shift. On the other hand the large cluster of magnetite particles showed large negative shifts, as well as positive shifts around the edges of the particles. At the larger lift height, the positive phase shifting behaviour from the gold nanoparticles was greatly decreased, to the extent that the particles could not be observed in the image. In order to more fully characterize the phase shift response, images were measured at various lift heights from 10 to 200 nm. From these images, the phase shift was measured over the centre of both gold and magnetite

nanoparticles (the particle location was determined from the topographic images measured at the same time). Various sample preparations were examined, and the same particles examined with different probes. See section 2 for details on how this was done. Overall, the results were similar, but we found that the magnitude of the response varied greatly from one probe to another. Despite using probes from the same batch, and magnetizing them using the same technique their responses varied enormously. Presumably manufacturing variability leads to probes that have considerably different magnetic properties, as well as variations in probe geometry. If the response from any one particle measured with different
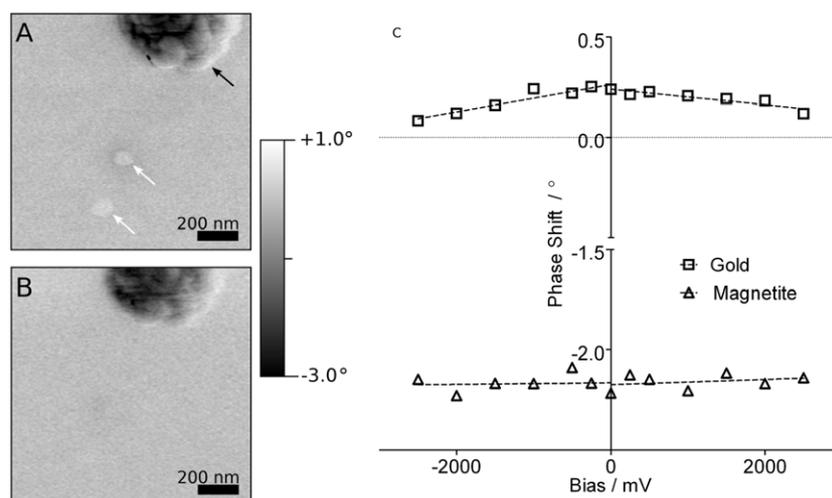
Figure 2. MFM lift mode images when biasing sample ((A) 0 V; (B) −2500 mV). White arrows show gold nanoparticles, and the black arrow a cluster of magnetite nanoparticles. (C) Plot of phase shift versus bias applied. Dotted lines show linear fits to the data.

probes in terms of phase shift versus lift height graphs are plotted together, the curves showed high variability, and the maximum response can vary by 100% or more. Unfortunately, measurement of the magnetization of MFM probes in order to take account of this variable is not simple [22]. In this and most reported work, this parameter is effectively a mystery and one of the great limiting factors for quantitative MFM [9]. In order to standardize the response of the probes, we normalized the response on a particular magnetite particle, at 15 nm lift height (the lift height showing the greatest response), assuming that the difference found here was simply due to magnetization of the probe. For each data point, data from the same particle, but measured with at least different three probes was averaged. The results of this analysis, showing the response of both gold and magnetite particles and both with and without applied field are shown in figure 1(C). For comparison, sample raw data (not averaged or normalized) is included in the supporting information as figure S2 (available at stacks.iop.org/Nano/21/305706/mmedia), in order to show the variability. These values were obtained by extracting the phase information measured over the centre of individual particles, either gold or magnetite. The curves in figure 1(C) curves clearly show that the response from the gold particles gave a positive phase shift, while that from the magnetite particles was negative, and also much greater in size. It can also be seen that for these particles, the presence of an external field was not necessary to measure the magnetic response. In fact, the difference from one probe to another was far greater than the difference with and without field. However, figure 1 shows that on average there was a small but significant difference in the response from the magnetic nanoparticles with and without the field, in that in the presence of the applied external field, the response decayed more slowly with increasing lift height. In this case, the response remained appreciable in the images at heights greater than 50 nm, whereas without the field, at these heights it was hardly measurable. However, since the difference between the results was so little, we assume that when the field was not applied, the particles were magnetized

by the probe. Other authors have also reported magnetic response of large (>100 nm) magnetic nanoparticles outside of an applied field [12, 19, 23], although in these cases, remnant magnetization would also explain the results. In order to exclude this possibility we heated our particles to above their blocking temperature (determined by SQUID magnetometry to be 370 K), to remove any remnant magnetization and once again measured them without applying a field. This did not make any significant difference to the distribution or magnitude of the phase shift seen by MFM, further suggesting that our results were due to sample magnetization by the probe. Magnetometry results (see supporting information, figure S3 available at stacks.iop.org/Nano/21/305706/mmedia) from these nanoparticles indicated that they would have been highly magnetized (85–97% of saturation magnetization) in the 0.2–1.2 T field applied. Measuring the field strength of MFM probes is complicated [24], but other authors have calculated the probable field from the type of probes as we used [14]. Based on these field strength estimates, the external field from the probe would be expected to have had a similar effect to that of the large magnets (91% of the saturation magnetization). Thus, it is not surprising that the results when the particles were placed in an external field were very similar to those found with no external magnet. Given that the MFM probe touches the particle (when measuring the topography), the field strength applied by the MFM probe may have been as great as that we applied using larger magnets at a greater distance. Thus, in practical terms, no prior or concurrent magnetization is required to observe the magnetic field of this type of MNP by MFM.

3.2. Origin of nonmagnetic contrast

In order to determine whether the results for the nonmagnetic nanoparticles were due to electrostatic effects, the same experiment was performed on a conductive substrate, which was grounded, as was the conductive AFM probe—figure 2(A). This had no discernible effect, i.e. the ratio of the phase shift of
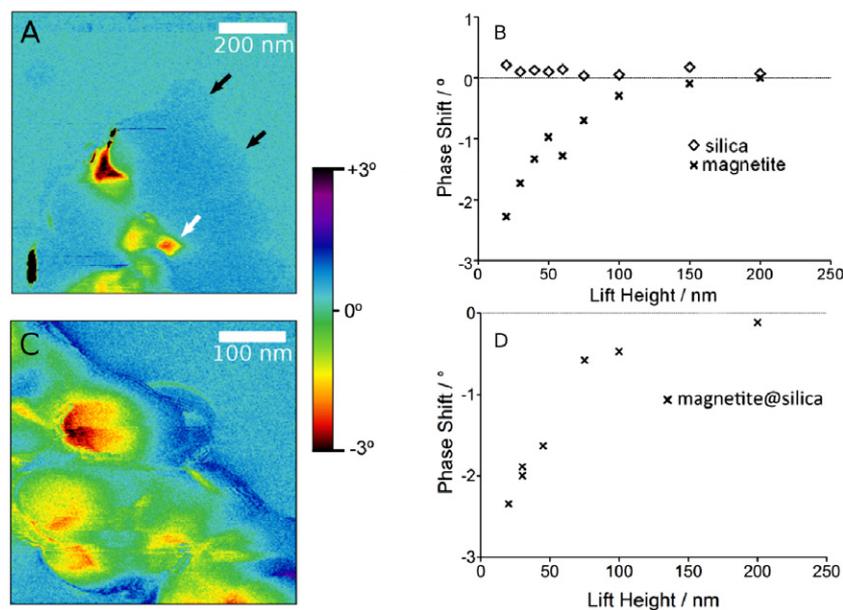
Figure 3. MFM data from silica/magnetite systems. (A) MFM phase shift image of a mixture of pure magnetite and pure silica particles. (B) Plot of phase shift versus lift height for this mixed system, showing data collected from silica and magnetite particles. (C) MFM phase shift image showing the response from magnetite/silica core:shell particles. (D) Plot of phase shift lift height data from these silica-coated particles.

magnetite versus the phase shift over gold was approximately the same. However, upon applying a DC bias between the tip and the sample, considerable differences were observed. As can be seen in figure 2(B), applying a bias of 2500 mV to the probe effectively hid the gold nanoparticles from the image, while the intensity of the magnetite particles remained the same. The plot of bias versus phase shift (figure 2(C)) confirms that increasing biases decreased the contrast on the AuNPs (to as low as 33% of their unbiased values), while changing very little the contrast on the MNPs (97% of the unbiased value). Overall, these results suggest that electrostatic forces are responsible for the contrast seen on the gold nanoparticles and also suggest a way to reduce these contributions from MFM images. This technique enables 'cancelling' the electrostatic contribution from the nonmagnetic components, meaning that only the magnetic particles will show significant contrast in the MFM image (compare figures 2(A) and (B)). Based on the results seen here, we believe that the positive phase shift response seen on nonmagnetic nanoparticles, was due to a repulsive electrostatic interaction between equally charged probe and surfaces. On the other hand, since the probe could magnetize the samples, magnetic nanoparticles exhibited a mostly attractive interaction with the probe, exhibiting a negative phase shift over the centre of the particles.

These results show that the long-distance contrast seen on the gold nanoparticles was electrostatic in nature, but does not establish whether it depends on the nature of the surface material. In the context of applications that require nanoparticle entry into cells, it would be useful to be able to determine the location of MNPs even when they are localized in the subsurface of the cell. In principle this is possible for MFM; as can be seen in figure 1, magnetite nanoparticles can be detected from heights as great as 100–

200 nm. In addition, many nanoparticles are being produced with a core–shell structure with several advantages, including the ability to reduce Fe dissolution, increase stability, increase biocompatibility and enable new functionalities to be added to the particles. For such core–shell particles, MFM must be able to detect the field of the magnetic core through the coatings. In order to determine whether similar results could be obtained with nanoparticles where the magnetic domain was not present at the surface, we studied a core–shell system including magnetite nanoparticles. This system helped us to determine if a metallic surface is required for the electrostatic effects we observed with gold nanoparticles. The particles studied consisted of magnetite cores coated with a shell of approximately 2–3 nm of silica (magnetite/silica core:shell particles). TEM imaging (see supporting information figure S1 available at stacks.iop.org/Nano/21/305706/mmedia), showed that the core particles were mostly cubes, and were all covered by the silica coating. These particles were studied in the same way as for the gold/magnetite system and the results are shown in figure 3. As a control, a mixture of the core particles (i.e. pure magnetite) with pure silica spheres was also studied.

This system of mixed magnetite cores with pure silica spheres showed spatially varying responses, with strong negative contrast in some places, and weaker positive contrast in others (figure 3(A)). The negative contrast appears in small features of varying shapes, sometimes squarish; whereas the weaker positive contrast corresponds clearly with the large, circular features in the topography images (see the supporting information figure S4 available at stacks.iop.org/Nano/21/305706/mmedia), and was clearly due to the silica spheres which uniformly had spherical shapes (see TEM images in the supporting information available at stacks.iop.org/Nano/21/305706/mmedia). The response in phase shift versus distance

is shown in figure 3(B), which shows a similar response to the gold/magnetite system. The fact that overall the response was similar further shows the validity of the differentiation of magnetic from nonmagnetic forces in MFM via the sign of the phase shift. As may be seen from the topographic AFM and TEM images included in the supporting information, the silica particles were rather large, (mean diameter around 100 nm) as opposed to the smaller gold NPs (mean diameter 17 nm), thus indicating that the sign of the phase shift does not depend on particle size. It is clear from these results that nonmagnetic particles give a positive phase shift, whether made of silica or gold, and whether they are large or small.

As may be seen in figure 3(C) for the magnetite/silica core–shell particles, the MFM images highlighted the core–shell structure. At low lift heights, the particles showed positive contrast around the edges, but in the centre contained a domain of negative contrast. At higher lift heights, the positive regions were less well defined. Not every particle displayed these regions of negative contrast, suggesting some silica-only particles were present in the sample. In general, the response from these coated magnetite particles was very similar to that from the uncoated particles. The phase shift lift height relationship, as shown in figure 3(D), did not differ significantly from pure magnetite nanoparticles. As far as these experiments could show, response of MFM is not affected by the silica coating, and detection of the magnetite core of coated particles is rather In no other study of nanoparticles by MFM published so far, has there been a direct comparison of nonmagnetic with magnetic nanoparticles, instead only the response of magnetic particles has been shown [12]. Pacifico *et al* showed MFM images of silica particles with and without the presence of a magnetic core [12]. Without the magnetic core, the signal disappeared, and no contrast was seen at all. In light of the results presented here, where silica-only particles gave considerable contrast compare to the mica background, it seems likely, the lack of contrast observed by Pacifico *et al* was due to imaging of the nanoparticles only We would expect based on our results, that such silica shell only particles would show contrast compared to the sample substrate, or another material. On the other hand, compared to magnetic nanoparticles, silica particles show little contrast based on their topography alone as can be observed in figure 3(A). It is interesting to note that despite looking at different sample preparations, and samples prepared on different substrates, the positive phase shift response was always present for the gold or silica-only nanoparticles, and any magnetite-containing particles we examined exhibited a negative phase shift. It appears that the probe magnetized the samples in our case, since all magnetite particles examined were magnetized in the same direction. In lift mode MFM, the scan line that is sensitive to sample magnetization is preceded by a topographic scan in which the probe physically touches the samples. it is likely during this initial scan line, that the samples were magnetized. Thus, it is a fundamental property of lift mode MFM that the probe is likely to magnetize small magnetic domains. Thus, other, more complex modes of MFM where the probe does not approach the sample so closely may be more appropriate to measure the *in situ* magnetization of small

magnetic nanoparticles [6]. It has also been noted that the field from such commercial probes is rather inhomogeneous (it is not perpendicular to the sample surface), which can also induce distortions in the measurements of sample fields [6]. However, such complex techniques are unnecessary for the work presented here since our aim was to unambiguously detect the magnetic nanoparticles and discriminate them from nonmagnetic particles, rather than to characterize their magnetizations fully.

3.3. Nature of contrast on magnetic nanoparticles

Based on the results shown so far, it is clear that, even in the absence of an applied external magnetic field, there is a significant response from magnetic particles that is distinguishable from that from nonmagnetic particles (i.e. pure silica or gold particles), and that does not depend on the nature of the particle surface. However, based on the work described in section 2.1, one of the major issues of MFM, that is, *proving* that this response is due to the magnetic field from the particles, was not clearly achieved. Since we saw only a small difference when the external field was applied, it was not clear whether their external magnetization was required for MFM. Other work has addressed this issue, either by probing in the absence of an applied magnetic field, or by using a nonmagnetic probe to perform control experiments [4, 25, 26]. Unfortunately, this previous work has compared the results found with MFM probes to those found with completely different AFM probes— namely high stiffness probes having a resonant frequency of around 300 kHz [4]. This type of probe would be expected to be considerably less sensitive to weak forces than those typically used for MFM (which have resonant frequency around 70 kHz) as they are approximately 15 times stiffer. The low-frequency probes are typically used for MFM because they are more sensitive to weak magnetic fields. This leaves the possibility that the observed difference could have been due to different probe sensitivities. Therefore, we compared the results shown above with those obtained using a nonmagnetic probe with approximately the same resonant frequency and force constant. Again, 50 nm magnetite nanoparticles were studied. The results shown in figure 4 indicate that when using silicon probes, no significant negative phase shift was observed, and the results were rather similar to those seen over nonmagnetic metallic nanoparticles with a magnetized probe. This shows definitively that magnetization of the MFM probe is necessary to see the large negative phase shifts seen here on magnetic nanoparticles.

3.4. Suitability of MFM to study single superparamagnetic nanoparticles

The results shown so far were based on nanoparticles with large magnetite cores (average diameters of about 50 nm), and clearly show a strong response from these particles. However, as discussed previously, a very important class of MNPs is that of superparamagnetic nanoparticles, which can consist of the same material as larger MNPs, but often much smaller diameters. Detection of SP-MNPs by MFM has been reported, but there is some doubt whether single SP-MNPs could be
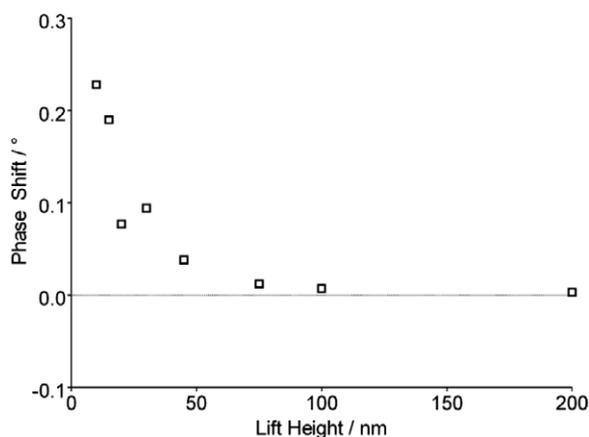
Figure 4. Plot of phase shift lift height response over a cluster of magnetic nanoparticles when measured with a nonmagnetic probe. Measured in an external applied field of 1.2 T.
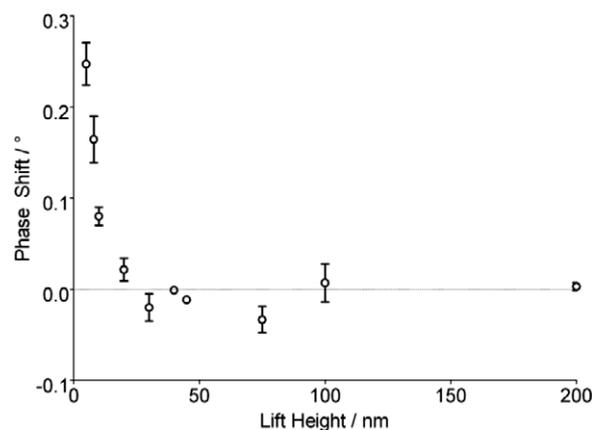
Figure 5. Plot of phase shift versus lift height response using a standard magnetic probe over single superparamagnetic (8 nm) nanoparticles. Measured in an external applied field of 1.2 T.

detected by MFM in ambient conditions at all, because the magnetic field from nanoparticles and therefore the phase shift that is detected in MFM depends very strongly on the particle diameter [21]. Based on modelling studies of the magnitude of the field from typical MFM probes, phase shift response from magnetite SP-MNPs <20 nm in diameter has been calculated to be below the thermal noise limit for MFM measurements [4, 14]. We therefore studied small (average diameter 8 nm) superparamagnetic nanoparticles by the same technique as for the large MNPs, in a strong external field (1.2 T), in order to ensure they were magnetized. A similar response to that found for nonmagnetic nanoparticles or when using a nonmagnetized probe on magnetic particles was observed (figure 5). The field we applied should have lead to 96% of saturation magnetization. Therefore, the field of magnetized single superparamagnetic nanoparticles is not detectable by MFM under ambient conditions, matching calculations made elsewhere [4]. Indeed, these particles were indistinguishable by MFM from similarly sized nonmagnetic particles. However, matching previous results [4], clusters of many superparamagnetic nanoparticles *were* visible, giving strong negative contrast (results not shown). This further shows that the difficulty in imaging these particles by MFM is simply due to their low field rather than another more fundamental problem.

4. Summary

In conclusion, we have confirmed that MFM is sensitive to the magnetic fields of magnetic nanoparticles with diameters around 40–60 nm. We showed for the first time, a direct comparison of the response of lift mode MFM to nonmagnetic (gold and silica) particles with that to magnetic (magnetite) nanoparticles. A response that might be, and has previously been, mistaken for a magnetic interaction can be detected from nonmagnetic nanoparticles of different materials and sizes. Such a response is inherent in the MFM technique and therefore could also be detected from magnetic particles whose field is too weak to be detected (i.e. individual

superparamagnetic nanoparticles with diameter <10 nm). The nonmagnetic interactions gave positive phase shifts, indicative of repulsive interactions with the MFM probe. Magnetic interactions were characterized by mostly negative phase shifts over the centres of the particles, with positive responses only at the edges. This difference in phase shift response can be used to characterize the nature of the interactions with the sample, and thus the nature of the sample itself. Since no previous work has compared the response of nonmagnetic nanoparticles with that from magnetic nanoparticles, it is only with the new results presented here, that MFM images of mixed systems, (i.e. those showing magnetic nanoparticles in the presence of other features), can be correctly interpreted. Particles such as those studied here do not require an external magnetic field in order to be studied greatly simplifying the experimental setup. Therefore, MFM is capable of discriminating between magnetic and nonmagnetic nanoparticles, but researchers who wish to show the magnetic response from their nanoparticles using MFM must ensure they observe negative phase shifts— attractive interactions, and not only positive ones. Using these considerations, magnetic particles can be easily distinguished from nonmagnetic particles, and thin surface coatings make no measurable difference to their response. The ability to definitively identify magnetic nanoparticles with this technique is expected to be extremely useful for present and future application of MNPs in biological systems.

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