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Abstract. Nanoparticle metrology with atomic force microscope (AFM) aims to determine an average particle size from measurements of individual nanoparticles derived by image analysis. This constrains the statistical relevance of the measurement due to the limited number of particles which can be practically imaged and analyzed. Consequently, the number density of particles on samples prepared for particle measurement is an important parameter of sample preparation. A number density that is too low makes it difficult to obtain sufficient measurement statistics, whereas a number density that is too high can result in particle agglomeration on the substrate and limit the area of uncovered substrate that is required to obtain a reliable reference for measuring the particle height. We present imaging and measurement results of a particle number density gradient of 16 nm gold nanoparticles deposited using a gradual immersion process. Results demonstrate how samples with particle number density gradients can facilitate identification of an area on a sample with optimal particle number density for AFM particle metrology and thereby improve measurement efficiency and reliability. [DOI: 10.1117/1.JMM.11.1.011007]

Subject terms: nanoparticle; AFM; nanometrology; substrate functionalization; particle number density gradient.

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1 Introduction

The need for accurate and reliable dimensional measurement of nanoparticles is growing as their application in a diverse range of technologies becomes increasingly widespread. Research, development, and commercial application of nanoparticles is motivated by their often remarkable material properties which can differ markedly from the properties of their bulk form and are strongly related to particle size. Concurrent with this burgeoning in applications have been increasing concerns about the potential health effects of exposure to nanoparticles. It is likely that the use of nanoparticles in consumer products, their use and handling in the workplace, and their eventual environmental fate will become subject to standards and regulations which will need to be underpinned with measurements traceable to the International System of Units (SI).

A wide range of particle sizing techniques, along with nanoparticle reference materials, has been developed to meet the need for nanoparticle metrology. These include ensemble techniques such as dynamic light scattering and differential centrifugal sedimentation, and single particle techniques such as transmission and scanning electron microscopies. For measurements with these techniques to be traceable back to a realization of the definition of the SI metre, a traceability chain needs to be established.

The approach being taken at the National Measurement Institute, Australia (NMIA) is to link dimensional measurements of nanoparticles to the realization of the definition of the SI metre at NMIA via scanning probe microscopy.[1,2] One of the elements of this traceability chain is atomic force microscopy (AFM) measurement of monodisperse nanoparticles reference materials which can then be used to calibrate other particle sizing techniques.

A limitation of AFM is that it is a single particle technique, whereby the average particle size is determined from measurements of individual nanoparticles. Achieving statistically relevant measurements is contingent on the number of particles that can be reliably imaged and analyzed in a practical time.

This paper discusses the importance of sample preparation and particle number densities for nanoparticle metrology with AFM. Samples featuring controlled particle number density gradients can facilitate identification of sample areas with optimal number density for AFM metrology. Particle number density gradients samples of 16-nm-diameter gold nanoparticles have been prepared using a gradual immersion process. Images and measurement results demonstrate their potential for improving the efficiency and reliability of AFM particle metrology.

2 Imaging and Measuring Particles

2.1 *Fundamental Principles and Limitations*

The ideal microscopy for imaging and measuring nanoparticles can be imagined as one that could provide a three-dimensional (3-D) field of view so that the entire surface of each particle could be accurately mapped [Fig. 1(a)]. Such a microscopy technique could fully characterize the morphology of each particle and provide parameters for an ensemble of particles such as average surface area, volume, maximum and minimum dimensions, and diameter. Being a nonideal microscopy technique, AFM can only approach this imaginary technique.

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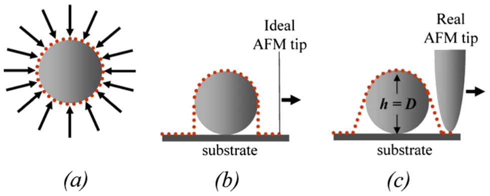


Fig. 1 (a) Imaginary ideal microscopy which maps the entire surface of the particle; (b) for AFM, the particle has to sit on a flat substrate, and only the upward facing surface is imaged; (c) the finite radius of the AFM tip results in a lateral broadening of the particle image. Therefore, assuming the particle is spherical, the diameter of the particle can most accurately be derived from its height.

AFM creates an image of a sample's surface topography by mapping a surface of constant tip-sample interaction. To image a nanoparticle and measure its dimensional properties, it is necessary for the particle to be sitting on a flat substrate. The AFM only images the upwards facing surface of the particle as a topographical surface feature [Fig. 1(b)]. A further limitation is that an AFM tip has a finite radius of curvature at its apex which is comparable with the dimensions of a nanoparticle. Since the image generated by scanning the tip across the particle represents a convolution of the tip shape with the particle topography, this results in a broadening of the apparent lateral dimensions of the particle image [Fig. 1(c)]. Reducing tip diameter reduces this effect; for example, single-walled carbon nanotubes with subnanometer diameter have been used as AFM tips achieving lateral resolutions down to 2 nm.[3]

2.2 *Deriving Particle Diameter from Height*

Given these limitations, it is advisable to derive the particle diameter from the difference in height between the level of the substrate and the apex of the particle image [Fig. 1(c)], assuming that the particle is spherical.[4] Nanoparticle reference materials are engineered for sphericity, so this assumption is not unreasonable. Furthermore, there are advantages in deriving the particle diameter from a height measurement: firstly, AFMs have a resolution in the vertical (out of plane) axis an order of magnitude better than their lateral resolution, and, secondly, a histogram method (see below) can be applied to readily obtain good statistics on the measurements derived from the AFM image.

The histogram method is effective for measuring height differences in AFM images and is one of the methods described and recommended in the VDI/VDE standard 2656, *Determination of geometrical quantities by using Scanning Probe Microscopes—Calibration of measurement systems*, for the calibration of step height transfer artefacts.[5] The method treats the vertical axis value of each of pixel in the AFM image as an individual height measurement. Given the large number of pixels in an AFM image, mean height values of nominally flat surfaces, such as substrates, can be determined with good statistical robustness.

For an idealized AFM image [Fig. 2(a)], with scan range $a \times a$, of a single particle with a diameter D, sitting on a perfectly flat substrate, the resulting histogram is shown in Fig. 2(b). The pixel count, $n(h)$, of the substrate peak in the histogram is: $n(0) \propto [a^2 - \pi(D/2)^2]$ and the distribution of the pixel heights from the particle is: $n(h) \propto \pi(Dh - h^2)$ between $h = D/2$ and $h = D$, where h is the pixel height. The actual numbers of pixel counts plotted in the histogram will depend on the number of pixels in the image and the binning in the histogram. For an actual AFM image [Fig. 2(c)] of a 100-nm nominal diameter particle, the histogram can only be approximated by this model. The substrate will not be perfectly flat and will appear as a broadened peak; the lateral broadening of the particle image will result in a distribution of pixel heights which fills in the gap between the peak corresponding to the substrate pixels and the particle pixels; the particle may not be perfectly spherical (for example, a flattened particle will result in a steeper height distribution curve); and imaging noise will be superimposed across the whole pixel height distribution. These effects can be seen in the distribution of pixel heights in Fig. 2(d), where the histogram shows a maximum height slightly less than the nominal 100 nm diameter of the particle.

3 Measurement of 16 nm Gold Nanoparticles in a Number Density Gradient

Sample preparation is critical for nanoparticle metrology. The aim is to deposit the nanoparticles from a liquid suspension onto an atomically flat or highly polished substrate so that particle heights can be unambiguously determined. Agglomeration of the particles, either before or after deposition, makes it difficult to resolve the heights of individual particles. Residue from the suspension medium may also obscure particles and the substrate.

The number density of particles deposited on the substrate is also an important parameter which affects the particle measurement. It is necessary to image and measure enough particles to achieve sufficient measurement statistics. For example, the AFM protocol for gold nanoparticles in a recent

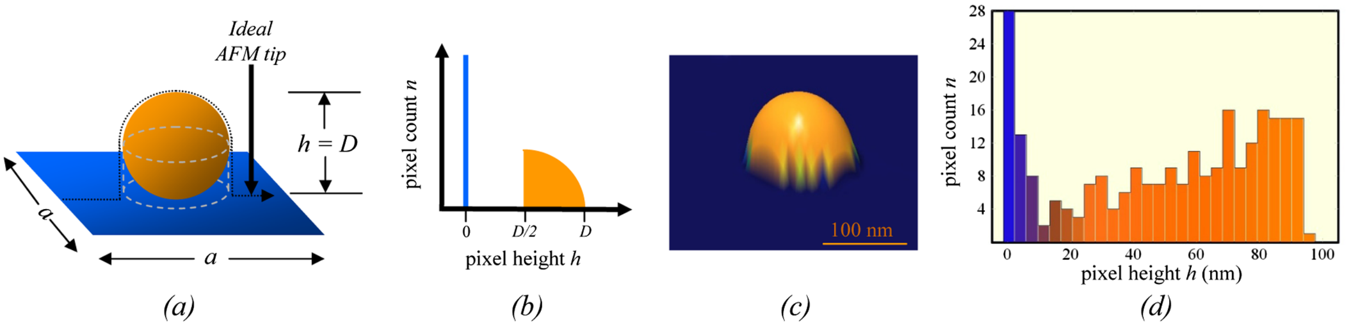


Fig. 2 (a) Ideal model of an AFM image of a particle on a flat substrate; (b) histogram showing the distribution of pixel heights of the particle imaged in (a), with the pixels of the substrate in blue and the pixels from the particle in orange; (c) rendered 3-D image of an actual AFM scan of a particle with a nominal diameter of 100 nm; and (d) histogram showing the distribution of pixel heights of the particle image in (c).

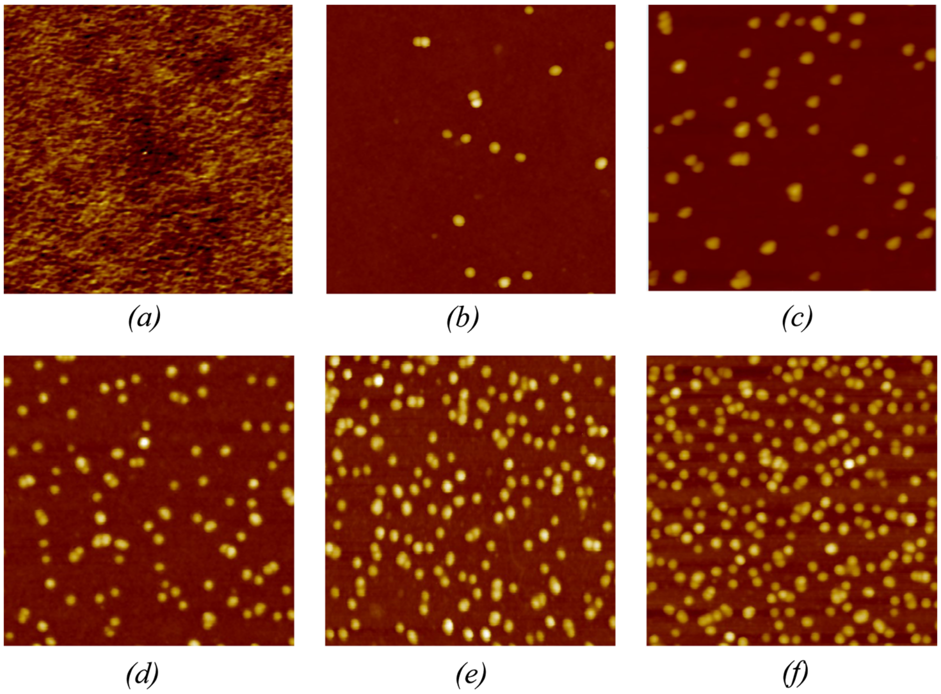


Fig. 3 Images acquired with 1 μm × 1 μm scan range of regions across the gradient sample: (a) approximately 4 mm from the edge of the sample, there are no particles and only the substrate topography is visible (color scale: black = –1.0 nm to yellow = 1.0 nm), (b) at ~5 mm, there are 14 particles/clusters, (c) at ~6 mm, there are 41 particles/clusters, (d) at ~8 mm, there are 81 particles/clusters, (e) at ~10 mm, there are 137 particles/clusters, and (f) at ~12 mm, there are 177 particles/clusters [color scale for images (b) to (f): red = 0 nm to white = ~25 nm].

ASTM intercomparison required the measurement of at least 100 particles.[6] If the particle number density on the prepared sample is too low, it is necessary to resort to either impractically large scan ranges which can compromise both image resolution and image quality by requiring fast scan speeds, or scanning an impractically large number of sample areas. Alternatively, if the particle number density on the substrate is too high, then it is likely that particles will have agglomerated on the substrate, and it may become impossible to image a sufficient area of uncovered substrate to reliably determine the reference level for the particle height measurements.

It is advantageous if the nanoparticles can be deposited in a number density gradient across a substrate so that a region of the sample can be located with an optimal number density for AFM particle metrology. This can improve the efficiency of nanoparticle metrology with AFM by reducing the likelihood of needing to prepare and examine multiple samples with different uniform particle number densities.

3.1 *Sample Preparation*

Number density gradients of gold nanoparticles were created by first depositing a thin film of plasma polymer allylamine on 13-mm-diameter glass cover slips followed by time-controlled electrostatic binding the gold nanoparticles and the amine functionalized surface. Plasma polymerization was carried out in a custom-built reactor described elsewhere[7] using a 13.56 MHz plasma generator. Deposition of allylamine was carried out at a flow rate of 10 standard cubic centimeters per minute, power of 10 W, and time of deposition of 4 min. These conditions result in nitrogen-rich films with a thickness of about 20 nm, as previously

Table 1 Results from the series of images with particles in Fig. 3. The number of particles in Figs. 3(b)–3(d) was insufficient to determine statistically reliable values of mean and standard deviation.

Image	Location	No. of detected particles/clusters	Particle height (nm) Mean	Particle height (nm) SD	No. of individual particles	Particle height (nm) Mean	Particle height (nm) SD
3(b)	~5 mm	14	—	—	12	—	—
3(c)	~6 mm	41	—	—	31	—	—
3(d)	~8 mm	81	—	—	66	—	—
3(e)	~10 mm	137	19.9	4.0	108	19.9	3.2
3(f)	~12 mm	177	18.2	3.3	125	17.7	3.3

reported. Before deposition, all substrates were cleaned by oxygen plasma for 2 min using a power of 20 W.

The nominally 16-nm-diameter gold nanoparticles were synthesized by reduction of 50 ml of 0.01% $HAuCl_4$ solution by adding 1 ml of a 1% sodium citrate solution at 100 °C. After cooling the resulting suspension to room temperature, the particles were surface-modified with 2-mercaptosuccinic acid.[8]

Over a period of 2 h, the functionalized amine substrates were gradually dipped into the undiluted suspension of 16 nm gold nanoparticles. The process was controlled by a Zaber T-LSR* linear motion drive operated with Zaber software.[9] The particle number density gradient resulted from the length of time the substrate was exposed to the gold nanoparticle suspension as it was gradually immersed. The high-density end of the substrate received the maximum length exposure whilst the low-density end received the least.

3.2 *Imaging the Particle Number Density Gradient*

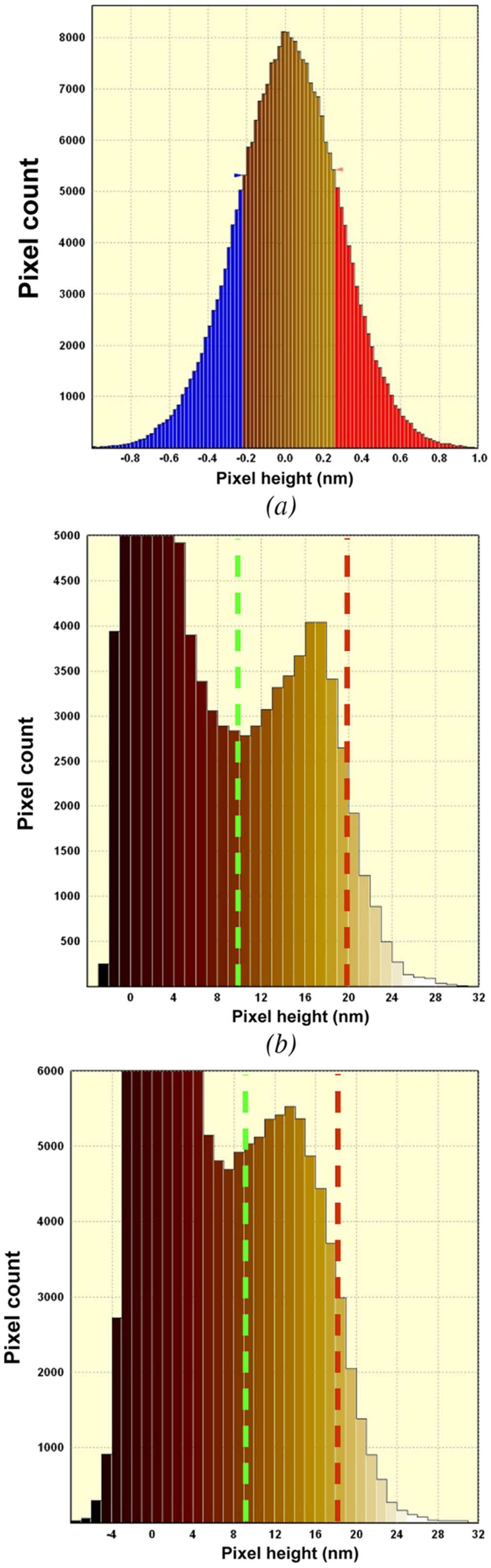
Images were acquired with an Asylum Research MFP-3D AFM using intermittent contact mode. Initial image processing included an iterative flattening process involving a first-order line flatten, calculating a mask, followed by another first-order line flatten, and repeating the process until there was no perceptible change in the image. Image analysis was performed using the Scanning Probe Image Processor (SPIP™) software produced by Image Metrology A/S.[10]

The sequence of 1 μm × 1 μm scan range images in Figs. 3(a)–3(e) illustrates how the particle number density increases across the sample. At the first position, (a), approximately 4 mm from the edge of the substrate, there are no particles and the image (with an amplified color scale) reveals the texture of the functionalized substrate. The histogram of this image [Fig. 4(a)] indicates that the height variation of the substrate is Gaussian with a standard distribution of ~0.22 nm. At the 5 mm point, (b), there are a few particles, and the density can be seen to increase in the successive images (c) to (f). Some particle agglomeration is visible in the images, however, this is limited to doublet and triplet particle clusters.

3.3 *Image Analysis and Particle Height Measurement*

Each of the images with particles, Figs. 3(b)–3(f), was analyzed using SPIP™'s particle analysis function to measure the heights of the particles. The particle analysis function uses a masking process to identify particles on the substrate and then calculates a wide range of particle size parameters for each particle, and measurement statistics for the set. Most of the parameters, however, rely on the accurate lateral measurement of the particles. The particle analysis function was used here to identify the height of the highest pixel of each identified particle.

Close examination of 3-D renderings of the images showed that the imaged particles were clearly round with no obvious faceting, so the assumption of spherical particles is valid. Prior to analysis of each image, the reference height



(c)

Fig. 4 (a) Histogram of images Fig. 3(a) showing the spread of heights for the functionalized substrate with SD ≈ 0.22 nm highlighted, (b) histogram of image Fig. 3(e), and (c) histogram of image Fig. 3(f). The red dotted line in (b) and (c) indicates the mean particle height values in Table 1 including clusters, the green dotted line indicates half the mean particle heights which should correspond to the $h = D/2$ point in Fig. 2(b).

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*Certain commercial products are identified in this publication. Such identification does not imply recommendation or endorsement by the National Measurement Institute, Australia, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

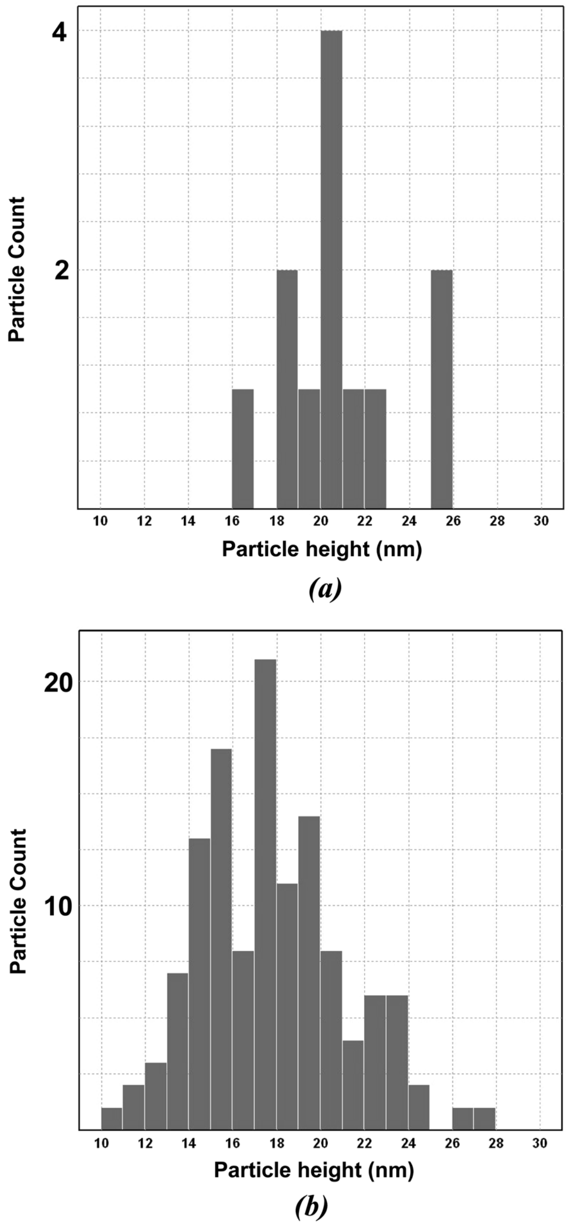

Fig. 5 Plots of the particle height distributions: (a) for the image taken at the ~5 mm position [Fig. 3(b)], the distribution is for only 12 individual particles, and (b) for the image taken at the ~12 mm position [Fig. 3(f)], the distribution is for 125 individual particles.

of the substrate was set to zero at the mean point of the substrate's histogram peak.

Results are presented in Table 1. Taking as a guide the AFM protocol for gold nanoparticles from the recent ASTM intercomparison[6], the mean and standard deviation of particle heights derived from Figs. 3(b)–3(d) were discarded because they contained fewer than 100 particles. With the particle number density at the ~5 mm location, it would have been necessary to increase the scan range to 9 μm^2 or take another eight 1 $\mu m \times 1$ μm scan range images to image and measure at least 100 individual particles.

Two measurements were derived from Figs. 3(e) and 3(f). The first measurement let the SPIP particle analysis function automatically identify particles and resulted in the inclusion of both individual particles and particle clusters. Whilst for the second measurement, particle clusters were excluded from the calculation so that only individual particles were included. With the relatively low level of agglomeration, there is little difference between the two sets of measurements.

The measured heights of the individual particles in the ~5-mm image and the ~12-mm image are plotted in Fig. 5. The figure clearly illustrates how measurement statistics improve with increased particle number density; however, these plots emphasize the necessity of imaging and measuring sufficient number of particles to achieve sufficient measurement statistics. Even with 125 individual particle measurements plotted in Fig. 5(b), there is neither an unambiguous Gaussian distribution nor an obvious peak to locate a mean particle size.

It should be noted that the images are based on a finite number of AFM scan lines. It is likely that the apex of each particle in an image does not exactly coincide with a scan line, so the exact value of D for each particle has not been determined. An approach to resolve this problem could be to fit a model shape, such as an ellipsoidal cap, to the image of each particle to locate its apex.

Pixel height histograms of the images in Figs. 3(a), 3(e), and 3(f) are shown in Figs. 4(a), 4(b), and 4(c), respectively. The histogram in Fig. 4(a) is of the image at ~4 mm with no visible particles and illustrates the flatness of the substrate as a Gaussian height distribution with a standard deviation of ~0.22 nm. In the histograms of images with visible particles (4(b) from the image at ~10 mm and 4(c) from the image at ~12 mm), the overlaid red dotted line indicates the mean particle heights from Table 1 (the value including particle clusters), whereas the overlaid green dotted line indicates half the mean values which should correspond to the $h = D/2$ point in Fig. 2(b).

The mean particle height lines intersect the histograms between half and two-thirds the way up the trailing edge of the histogram in both histograms, and the half mean height lines cross in close proximity to the minimum point of the histogram between where the substrate peak dominates and where the particles dominate. This would suggest that for a set of particles with an approximately Gaussian distribution of heights, the mean height is approximately indicated by the half way point up the trailing edge of the histogram. The half mean height point occurs around a minimum rather than a maximum point as suggested by the model, which is probably due to the larger particles dominating the contribution at these points and to the added contribution of the lateral broadening of the particle images due to tip convolution.

4 Conclusion

This paper describes the preparation of samples with a particle number density gradient to facilitate the identification of a sample area with an optimal number density for AFM particle metrology and thereby improve measurement efficiency and reliability.

Samples with particle number density gradients were prepared by depositing 16-nm-diameter gold nanoparticles from suspension onto functionalized substrates using a gradual immersion process in which the resulting particle number density was related to immersion time. AFM images showed that the particles were deposited in an arrangement suitable for AFM particle metrology with predominantly individual particles deposited on the substrate. The observed agglomeration was mostly in the form of doublet and triplet clusters. Particle number density increased across the sample from 0 particles per μm^2 to 177 particles and particle clusters per μm^2. Mean particle heights were derived from analysis of two of the $1\ \mu m \times 1\ \mu m$ scan range images with over 100 individual particles and particle clusters. The mean particle heights derived from image analysis ranged from 17.7 nm to 19.9 nm with standard deviations ranging from 3.2 nm to 4.0 nm. The inclusion of particle clusters in the analyses of these two images did not significantly affect the mean particle height results.

These results demonstrate the ability of gradual immersion sample preparation to produce particle number density gradient samples suitable for AFM metrology. This approach has the potential for improving the efficiency and reliability of AFM nanoparticle metrology.

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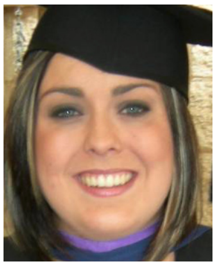

Renee V. Goreham is completing final year of her PhD at the Mawson Institute, University of South Australia in NanoBioTechnology. Her thesis is named "A method for generating density gradients of gold nanoparticles to study the effect of nanoscale topography on mammalian cells." Her main interests are determining the molecular mechanisms involved at the interface of biomaterials and the host tissue to improve current or create novel biomedical devices. She has a bachelor's degree in science (honours) and a bachelor's degree in technology (forensic and analytical techniques) from Flinders University.

Jan Herrmann heads the Nanometrology Section at the National Measurement Institute Australia (NMIA). His team develops physical standards, instruments, and methods for measurements at the nanometer length scale with a focus on the characterization of nanomaterials. In its particle characterization laboratory, the group has established a number of complementary techniques for measuring the properties of nanoparticle systems. This facility is underpinned by a metrological Scanning Probe Microscope which establishes traceability of dimensional measurements at the nanometre scale to Australia's primary length standards.

Åsa K. Jämting is a research scientist in the Nanometrology Section at the National Measurement Institute Australia where she has developed significant expertise in the measurement and characterization of a range of different particle systems with a focus on nanoparticles. This includes measuring particle size distributions and surface charge of particles in liquid suspensions, metrological evaluation of novel particle characterization instrumentation, and development of analysis techniques to optimize the performance of established characterization methods.