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Quantifying surface modification events from scanning force microscopy images

G. Roe [a], L. McDonnell [b,*]

[a] School of Science, Galway-Mayo Institute of Technology, Galway, Ireland
[b] Centre for Surface & Interface Analysis, Department of Applied Physics & Instrumentation, Cork Institute of Technology, Rossa Avenue, Cork, Ireland

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ABSTRACT

Scanning force microscopy (SFM) is widely used to monitor surfaces and surface modification processes. Some surface modification processes involve the addition (or removal) of discrete entities to (or from) a surface in circumstances where the absolute number of entities is related to some aspect of the process. A two-dimensional surface characterisation parameter – the surface area ratio (*SAR*) – was previously developed as a means of quantifying such modification and can be readily obtained from SFM images. Simulations have shown that the *SAR* parameter is superior for quantification purposes to conventional surface roughness parameters such as roughness average S_a, the area equivalent of R_a. Key features of *SAR* are as follows: its linear dependence with coverage; dependence of linearity slope on coverage mechanism; and its independence from the form, waviness or roughness of the underlying surface. A further advantage of this method is its simplicity given that the *SAR* parameter is readily obtained from SFM images. Simulations of adsorption onto flat surfaces have been validated using SFM images of polystyrene spheres adsorbed onto mica.

1. Introduction

Since its invention [1], scanning force microscopy (SFM) has been widely used to image surface topography and has proved to be an excellent technique to monitor surfaces and their modification. SFM offers a number of advantages over traditional microscopies: the potential for spatial resolution at the atomic scale; a lateral range that encompasses those of optical and electron microscopies; the ability to image in air, liquid or vacuum; and the absence of any technique-specific sample preparation requirements. In addition, image contrast in SFM is derived from actual height measurements of surface topography and thus three-dimensional shape and size data can be obtained directly from SFM images. This is unlike conventional optical and scanning electron microscopies where image contrast is generated by topographic slopes, thereby restricting metrology to lateral dimensions. Differences in the characterisation of surface topography by SEM and SFM are described elsewhere [e.g. 2] and will not be discussed further here.

When quantifying SFM images, it must be recognised that the heights of surface features can only be correctly measured when (i) the SFM tip has unrestricted access to at least one side of the feature and (ii) there is no distortion of the sample by the tip (or vice versa) during imaging. Of course, the SFM tip shape will always be added to the lateral dimensions of surface features and thus lateral quantification will require the SFM tip dimensions to be determined so that the images can be deconvoluted. Finally, images can only be validly quantified when the scanning force microscope has been calibrated.

Some surface modification processes involve adding (or removing) discrete entities to (or from) a surface in circumstances where the absolute number of entities, or topographic events, is related to some aspect of the process, for example, the adsorption of proteins [3] and in solid phase assays based on SFM detection of binding events [4–7]. There are two distinct approaches to quantifying the occurrence of such events within SFM images. The local approach uses particle detection software to isolate and then count each event [5–8], whilst the competing global approach interprets changes in three-dimensional surface roughness parameters calculated for the entire image [4,9].

With the particle detection approach, the first step is to isolate the events from the background by applying a planar threshold to the image. As image contrast within conventional optical and scanning electron microscopes is often dominated by the shorter wavelength slopes of the sample topography (the longer wavelengths that comprise the background being considerably suppressed during imaging), planar thresholding is often effective in separating the events from the background. However, such an approach is not well suited to SFM images as they generally have non-planar backgrounds that a planar threshold cannot

* Corresponding author. Tel.:+353 21 4326442; fax:+353 21 4345355.
E-mail address: liam.mcdonnell@cit.ie (L. McDonnell).

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distinguish from surface modification events, particularly when such events are relatively small. This is a similar problem to the difficulties in quantifying photographic images where non-uniform background lighting has been used [8]. The non-planar background in SFM images arises because image contrast is generated not just by the surface topography but also by surface form, sample tilt and the curved trajectory of the piezoelectric scanner. Thus, before a planar threshold can be applied to such SFM images these unwanted contrast contributions must be removed. Unfortunately, image processing procedures that identify and remove the longer-wavelength image background are quite complex, time consuming, generally very user intensive and often introduce image artifacts.

Using the global approach, there are several surface characterisation parameters available for consideration [9]. Average roughness S_a, root-mean-square roughness S_q, and the area equivalents of the linear surface characterisation parameters R_a and R_q are typical of the parameters routinely used to quantify surface roughness from SFM images [10]. The principal limitation in using the S_a or S_q parameters is that, by definition, they can only measure surface amplitudes. For example, two-dimensionally sinusoidal surfaces of identical peak amplitude have the same S_a or S_q values regardless of the wavelength of the surface. Of course, altering the spatial distribution of the amplitudes (from say sine to square), whilst retaining the same peak amplitude, will increase the values of the S_a and S_q parameters. However, such changes arise from the amplitude differences between the surfaces, rather than from the lateral differences, there being more higher amplitudes when the spatial distribution is changed from sine to square.

Area-derived two-dimensional surface characterisation parameters respond to both lateral and amplitude differences and therefore reflect, more fully, the character of a surface, as well as the changes that occur therein. However, as surfaces with completely different wavelengths and amplitudes can have the same areas, such parameters do not uniquely describe a surface. Absolute surface area and S_{dr}, the developed surface ratio [9], are two examples of area-derived two-dimensional surface roughness parameters in current use. Previously [11], we introduced an alternative surface characterisation parameter – the surface area ratio, *SAR* – where *SAR* is the ratio of the absolute surface area of the substrate after surface modification (e.g. adsorption) to the absolute surface area of the substrate before surface modification. This definition is presented schematically in Fig. 1. Whilst we have demonstrated elsewhere [12] that the *SAR* parameter varies linearly with the number of ferritin molecules bound onto anti-ferritin sensor surfaces, a detailed study of how the *SAR* parameter is affected by the surface modification mechanism and by the substrate topography has not published.

In the work presented here, the *SAR* and S_a parameters were calculated from simulated SFM images as spheres were "adsorbed" on top of three different substrate topographies by three different coverage mechanisms. The results of the simulations were compared to the *SAR* and S_a values calculated from real SFM images of polystyrene spheres adsorbed onto mica. The good agreements obtained between the simulated and real images for this particular case and in the case of [12] have validated the *SAR* parameter as an effective method of quantifying surface modification events from SFM images.

2. Experimental

2.1. Preparation of monolayers of polystyrene spheres

Using the general method described by Van Cleef et al. [13], 1.07 μm diameter polystyrene (also referred to as latex) spheres (Sigma-Aldrich, Poole, England) were immobilised onto mica. Briefly, the polystyrene spheres were supplied in an aqueous suspension having a solids content of 10% and this solution was subsequently diluted with water in five steps within the range 1:10–1:100,000. A micropipette was used to deposit 0.1 ml aliquots of the diluted preparations onto freshly cleaved mica substrates (Agar Scientific, Stansted, England), which were then placed in a desiccator for 15–20 h.

2.2. Scanning force microscopy

Scanning force microscopy was performed in air using an Explorer^{TM} instrument (Veeco Instruments, Santa Barbara, USA) equipped with SPMLab 3.06.06 image acquisition and processing software. The mica substrates were fixed to magnetic stainless steel sample holders using double-sided adhesive tape and placed onto the magnetised holder of a manual XY sample manipulator. The polystyrene sphere samples were imaged in contact mode using rectangular silicon cantilevers (Nanosensors, Wetzlar, Germany) with a nominal force constant of 0.32 N/m (manufacturer's specification). The SFM tip radius was estimated experimentally to be approximately 20 nm. Images were typically acquired at line scan rates within the range 1.0–0.67 Hz. *SAR* and

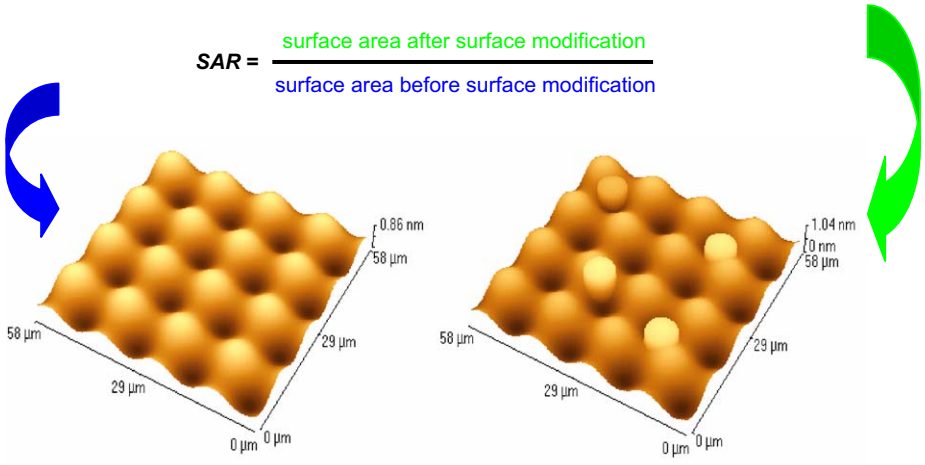

Fig. 1. Schematic representation of the definition of the *SAR* parameter.