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Reconstruction of ribosomal subunits and rDNA chromatin imaged by scanning force microscopy

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Scanning force microscopy (SFM) reveals surface topography by scanning a sharp tip in close proximity to the sample. Due to tip–sample interaction, artificial broadening of the real surface structure with the tip geometry occurs. One approach for image reconstruction is the use of calibration standards, preferably in the size range of the samples. In the present study, an image reconstruction method based on colloidal gold as a geometric standard was used to reconstruct SFM images of biomolecules. Sample and calibration standard size were in the nanometer range, and the standards were coadsorbed with the specimen. Raw and reconstructed images of the biomolecules were compared, and the reconstruction was characterized by difference images as well as determination of the difference volume. The application of image reconstruction based on colloidal gold as a calibration standard for SFM of biomolecules is discussed.

I. INTRODUCTION

Scanning force microscopy (SFM) uses a sharp tip for probing surface topography, by direct contact or tapping between tip and surface. The image of the surface can be distorted due to the geometry and localization of the tip normally incident upon the scanned surface region. If the tip size is in the range of the surface feature, artificial broadening occurs at steep surface corrugations generating region of indeterminacy below structures not accessible to the tip's apex. Many efforts have been directed toward minimization of the former effect by using sharper tips[1–4] or by reconstruction of the image after visualization.[5–7] Coadsorption of the specimen and the calibration standard allows the determination of the tip shape during the scanning procedure, therefore including dynamic changes of the tip shape occurring during scanning (e.g., adsorption of material, breakage). By reconstruction of the whole image using the determined tip structure, the broadening effects of the tip should be partially compensated, resulting in less image distortion. There is a need for characterization of reconstruction methods. Here is shown a quantitative description of the effect of reconstruction of visualized rDNA chromatin and ribosomal subunits based on the estimation of the apparent volume from the SFM image.[8]

II. MATERIALS AND METHODS

Ribosomal 50 S subunits were isolated from E. coli and purified,[9] prior to coadsorption with colloidal gold (24 nm) onto freshly cleaved mica. A 10 μ1 droplet was deposited on the mica for 1 min, prior to washing with 50 mM ammonium acetate and air drying.

Tetrahymena DNA chromatin was isolated and adsorbed as described.[10] Briefly, the chromatin was isolated from synchronized cells, purified in sucrose gradient, dialyzed against physiological buffer (containing 5 mM MgCl), and coadsorbed with colloidal gold (20 nm) onto freshly cleaved mica.

Scanning force microscopy of rDNA chromatin and ribosomal subunits was with an NanoScope III (Digital Instruments, DI, Santa Barbara, CA) and BioScope (DI), respectively. All imaging was in Tapping mode with silicon tapping tips (Nanoprobes, DI). The images were flattened prior to reconstruction. Reconstructed images were made with an algorithm previously described.[7]

The volume was estimated using NIH Image (NIH, Bethesda, MD) according to the procedure described

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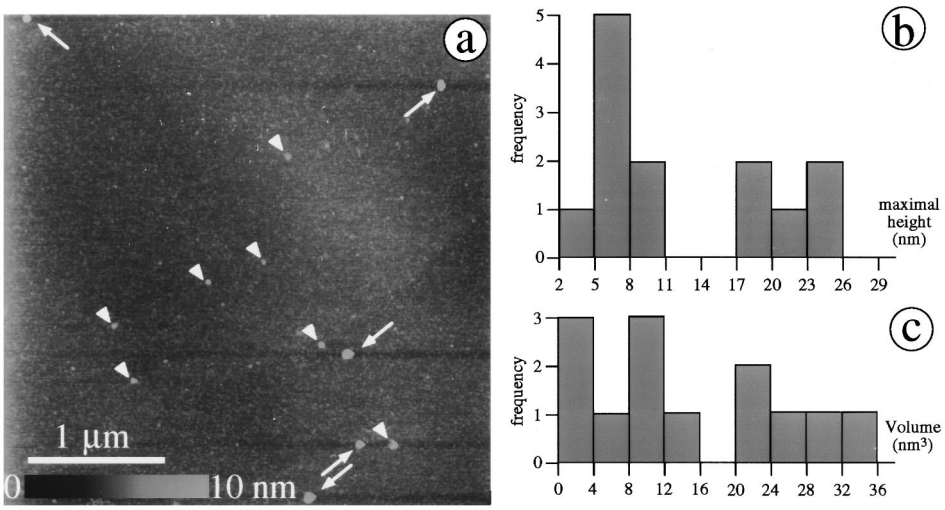

FIG. 1. Scanning force microscopy of ribosomal subunits coadsorbed with colloidal gold onto mica. The distribution of the maximal height and the volume of the particles in (a) was determined [(b),(c)] and used for classification into two classes, representing ribosomal subunits (arrowheads) and colloidal gold particles (arrows), respectively.

elsewhere.[8] The volume elements of the specimen were isolated from the background by using an interactively created mask. Then, all volume elements were added, and the resulting volume corrected by subtracting the background level.

The volume per nucleosome was determined by counting the nucleosomes in the image of rDNA chromatin and dividing the fiber volume by this number. The volume of nucleosomes of the extended nucleosomal chain (''beads on a string'') was measured by applying the volume measurement algorithm to samples described previously.[11] The volume based on crystallographic data was obtained by using the crystallographic dimensions[12] and assuming a cylindrical shape for the nucleosomal core. The core DNA was also estimated as a cylinder, with a diameter of 2 nm.

III. RESULTS

A. Ribosomal subunits

SFM of ribosomal subunits coadsorbed with colloidal gold onto mica resulted typically in an image as shown in Fig. 1(a). Round particles were distributed over the surface, clearly distinguished from the background. Particles of different size were observed, and measurement of the maximal height resulted in two classes of particles [Fig. 1(b)]. This discrimination is supported by the determination of the apparent volume; again the two classes were found [Fig. 1(c)]. Colloidal gold particles with diameters of ~24 nm were used, which should result in measured heights of similar values.[13] Therefore, the taller particles were assigned as gold, identifying the lower as ribosomal subunits. Control experiments with gold particles adsorbed in the absence of the ribosomal subunits supported this identification; usually, no particles with heights below 15 nm (e.g., preparation artifacts) were observed in such experiments (data not shown). The gold particles exhibit a round structure, as do most of the ribosomal subunits. Only occasionally the subunits are adsorbed in an extended structure [marked by the arrowhead in lower right corner of Fig. 1(a)].

A high resolution image of gold particles and ribosomal subunits is shown in Fig. 2(a); the inset contains a cross section of the marked pair of particles (r_1, g_1) along the drawn line. This image was used for image reconstruction. The tip shape was reconstructed referring to the gold particle g_1. The image [Fig. 2(a)] was then reconstructed based on the determined tip shape; the reconstructed image is shown in Fig. 2(b). The occurrence of streaks on high features [pointers in Fig. 2(b)] is a common phenomenon caused by this reconstruction algorithm. All particles exhibit a reduction in size compared to the original image in (a), as expected due to subtracting of the tip broadening. Figure 2(c) shows the difference image between the original (a) and the reconstructed (b) image, illustrating the effect of reconstruction. The difference signal reflects the shape of the particles. The ribosomal particles are hardly visible, but the cross section (inset) gives an impression of the dimensions.

B. rDNA chromatin

An SFM overview of isolated rDNA chromatin coadsorbed with colloidal gold reveals pieces of threadlike structures, occasionally building up a network [Fig. 3(a)]. Round particles (marked with g_1, g_2) with a height overwhelming the other structures were also observed. Due to their unique shape and the height of 15–20 nm, these particles were identified as colloidal gold. The threadlike structure represents the chromatin, as characterized by SFM elsewhere.[10] The gold particle g_2 was used for determining the tip shape, prior to the reconstruction of the image. The reconstructed image [Fig. 3(b)] exhibits some streaks on the gold particles, resulting from the reconstruction algorithm as mentioned above in the case of ribosomal subunits. The image features are

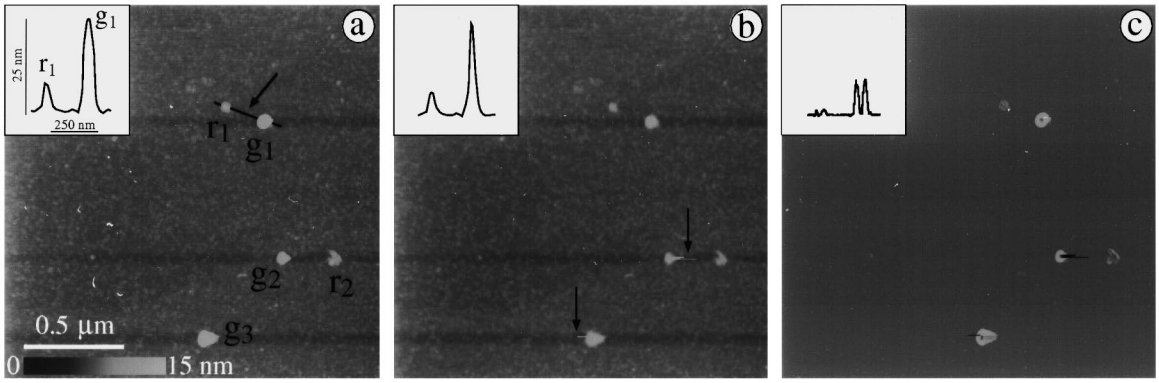

FIG. 2. Reconstruction of SFM's of ribosomal subunits. Lateral and height scales of all panels (including insets) are the same and are shown in (a). (a) A zoom of a region of Fig. 1(a) shows ribosomal subunits (r_1,r_2), coadsorbed with colloidal gold particles (g_1–g_3). The inset exhibits a cross section of r_1 and g_1 along the marked line in (a). (b) Reconstruction of the image shown in (a), using the tip shape determined referring to gold particle g_1. Streaks occurring are marked by arrows. (c) Difference image between original image (a) and reconstructed image (b), representing the effects of reconstruction.

slightly less prominent; the difference image between the original and the reconstructed one emphasizes the effect of reconstruction [Fig. 3(c)]. The difference image exhibits, besides the ring-shaped structure at the gold balls, a structure bordering the chromatin visualized in Fig. 3(a). Regions with a thicker and higher (brighter) bordering in the difference image correlate well with higher chromatin regions in the original image [arrows in Figs. 3(a) and 3(c)].

For a closer examination, a digital zoom of the rDNA chromatin specimen shown in Fig. 3(a) was reconstructed. The region around the gold particle g_2 was imaged with high resolution [Fig. 3(d)], and the tip shape determined referring

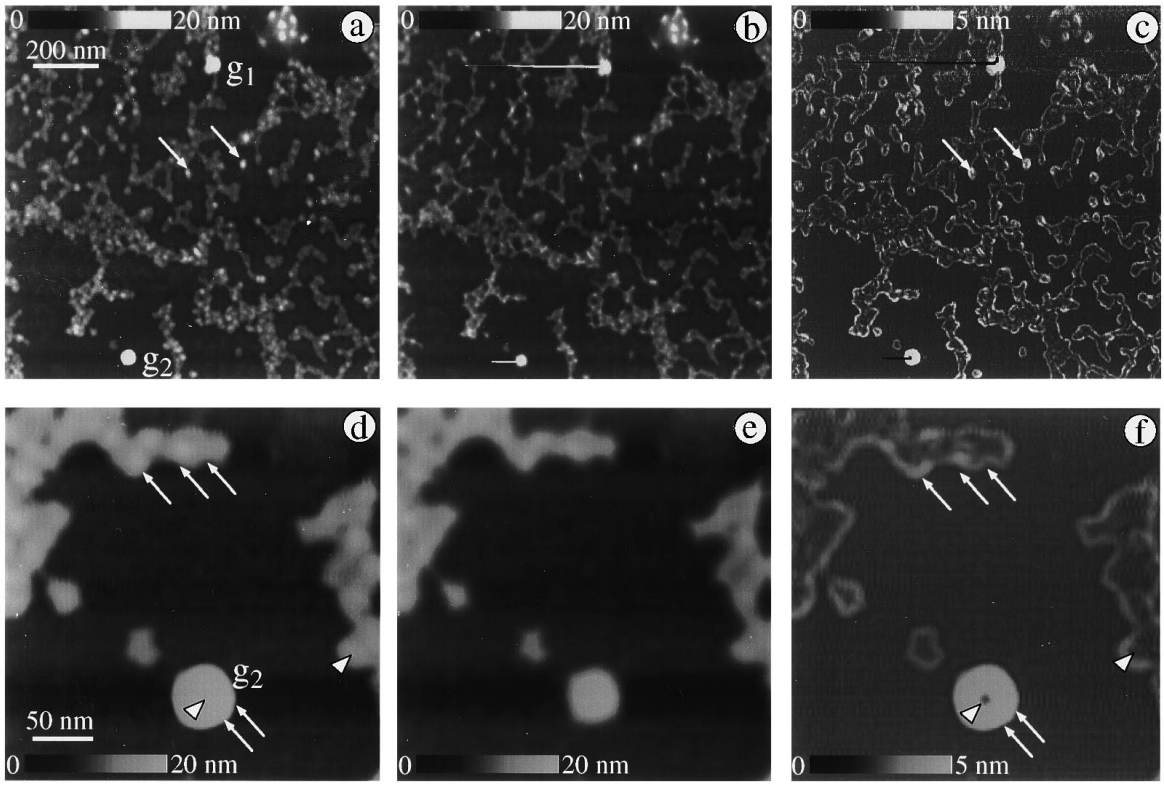

FIG. 3. Reconstruction of SFMs of rDNA chromatin. The lateral scales of the panels of one row are the same and shown in the first image of the raw. (a) SFM overview of rDNA chromatin coadsorbed with colloidal gold. The threadlike structures were identified as chromatin, whereas the two round protrusions (marked with g_1,g_2) represent colloidal gold particles. (b) Reconstruction of the image in (a), based on gold particle g_2. (c) Visualizing the reconstruction effect as the difference between the original (a) and the reconstructed (b) image. The arrows in (a) and (c) mark the location of high surface features, which are strongly affected by the reconstruction. (d) A digital zoom of the region around the gold particle g_2 in (a). Edges and top regions of surface features are marked in (a) and (c) by arrows and arrowheads, respectively. (b) Reconstruction of the image based on the gold particle g_2. (c) The effects of reconstruction visualized as the difference between (a) and (b), located at the edges of the structure (arrows). The higher regions (arrowheads) are barely affected.

to this gold particle. The reconstructed image [Fig. 3(e)] reveals a reduction in width of the gold particles as well as of the chromatin structures. The difference image [Fig. 3(f)] elucidates that the regions affected by the reconstruction are the bordering parts of the structures (arrows); the highest peaks are unaffected (arrowheads).

IV. DISCUSSION

Coadsorption of calibration standards with biomolecules should meet some requirements. First of all, the standard should allow calibration of the tip shape by a defined known structure. In the case of adsorbed colloidal gold, the structure is described well by electron and scanning force microscopy.[13,14] Distortions introduced by using deformed gold particles as standard can be avoided by a statistical approach that includes many particles. The size of the colloidal gold particles should be at least three time the typical height of the molecules to be reconstructed, because only the top third of the gold particle is imaged by the tip. Therefore, only about one third of the height of the gold particle goes into describing the tip shape. This applies to 24 nm gold particles coadsorbed with ribosomal subunits, as shown in the height distribution in Fig. 1(b) as well as in the cross section in Fig. 2(a) (inset). In the case of rDNA chromatin samples, the unique round shape of the gold particles highlights them among the chromatin fibers [Fig. 3(a)].

Comparing the reconstructed and original scanning force micrograph of ribosomal subunits, all round particles on the mica surface appear less prominent. Due to the topographic contrast of the SFM, this could indicate a decreased height of the reconstructed structures. However, measurements result in hardly altered maximal heights. This is demonstrated by the cross sections [insets in Figs. 2(a) and 2(b)], which on the other hand indicates a thinning of the structures by the reconstruction. This effect is expected, because most of the broadening occurs at steep surface structures due to contact with the tip edges, and a correction should result in a thinning of the sample. A qualitative description of the reconstruction effects is possible by the difference image between the original and the reconstructed one, revealing the locations of reconstruction at the specimen images [Fig. 2(c)]. The gold balls and the (barely visible) ribosomal subunits exhibit a structure along their borders, which represents the volume removed by the reconstruction. The cross section [Fig. 2(c), inset] shows that only the flanks of the structures are subject of the correction, resulting in the outlining structure. This pattern could also be observed in the reconstruction of rDNA chromatin (Fig. 3). For a quantitative description of reconstruction the volume of the chromatin was used, which was determined from the original and the reconstructed image [Fig. 4(a)]. The reconstructed chromatin has ~20% less volume than the original; this amount represents the broadening induced by the tip. Measurements based on tip shape determinations referring to other gold particles or done with other chromatin samples yield broadening effects in the same range (data not shown). The extent of broadening should be influenced by the height and steepness of surface

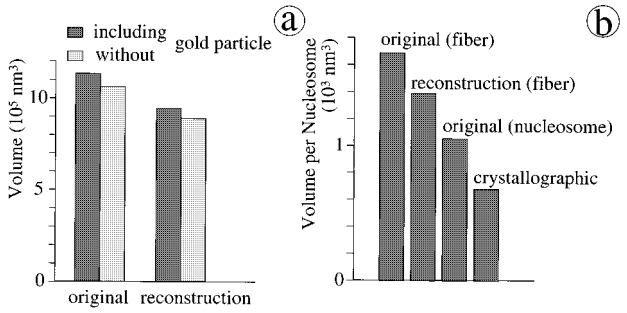

FIG. 4. Volume determination of rDNA chromatin from SFM images. (a) The volume of the rDNA chromatin in the original image [Fig. 3(a)] and the reconstruction [Fig. 3(b)] was measured. The dark bars represent the volume including the gold particles, the bright bars shows the volume after deleting the gold particles in the images. (b) The volume per nucleosome was determined for the original and reconstructed rDNA chromatin [Figs. 3(a) and 3(b), respectively]. For comparison, the volume of nucleosomes determined from images of an extended nucleosomal fiber (beads on a string, see Ref. 11) and calculated from the crystallographic determined dimensions (Ref. 12) are included.

structures. This was observed on higher chromatin structures [arrows in Fig. 3(a)], where the broadening effects are increased [Fig. 3(c)].

The volume per nucleosome exhibit the same amount of broadening [Fig. 4(b)]. Compared with the volume based on crystallographic data, the reconstructed volume is still higher. A likely explanation could be the region of indeterminacy, which cannot be accounted for by the reconstruction algorithm.[15] This region depends from the height of surface features; therefore, a comparison with the volume determined from extended nucleosomal fibers (where single nucleosomes can be measured) should be helpful. Determination of the nucleosome volume of such samples results indeed in a reduced volume compared to the original or even reconstructed rDNA chromatin fiber [Fig. 4(b)], supporting this explanation.

V. CONCLUSIONS

The reconstruction algorithm applied to biomolecules such as ribosomal subunits and chromatin fibers allowed a correction of the SFM images by removal of the structural broadening induced by the tip shape. Typical improvements were in the range of 20%, determined by the volume of the original and reconstructed image. The description of the volume changes became apparent as a tool for description of structural broadening effects and their correction. Images with small broadening due to tip effects should be characterized by a less prominent change in volume.

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