NANOMETER-SCALE INVESTIGATIONS BY ATOMIC FORCE MICROSCOPY INTO THE EFFECT OF DIFFERENT TREATMENTS ON THE SURFACE STRUCTURE OF HAIR

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Abstract

Objective   To investigate the effect of different washing regimes on the surface of human hair at the nanometer scale – comparable to the size of typical deposits left behind by commercial products.

Methods     Atomic Force Microscopy (AFM) and related techniques.

Results     It can be directly seen that washing hair using commercial hair-care products removes deposits that naturally form on the shaft, revealing the underlying structure of the hair, whilst in many cases leaving new deposits behind. The spatial distribution of these deposits is explored and quantified. The spatial distribution of the surface charge of pristine hair is mapped and the electrical screening effect of deposits is directly observed. We also show that the roughness of the treated hair depends directly on the type of product used, with a marked difference between shampoo and conditioner. Some products leave isolated deposits behind, whereas others leave layers of material behind which wet the hair surface.

Conclusion  Atomic force microscopy and the related techniques we have employed in a forensic approach are able to distinguish between different hair care products on the basis of the deposits they leave behind. This opens up the capability of further analysis tools to complement already existing techniques.
INTRODUCTION

Personal care products are used by billions of people across the globe on a daily basis, and the corresponding worldwide market is worth billions of dollars. In the UK alone, the haircare market is worth approximately £1.5 billion per annum\(^1\), with a current growth rate of around 5%. A variety of characterization tools are employed by the industry to determine the effect and efficacy of these products to help develop improved formulations. Nonetheless, consumers generally tend to trust whatever is written on the container, to buy from known brands, and are strongly influenced by advertising. The factors determining which products a given consumer purchases include the brand, the cost, the scent and the perceived benefit. Hair shafts have traditionally been studied using scanning or transmission electron microscopy (SEM or TEM)\(^2,3\), but these are not appropriate in some cases as they require a conductive coating to be deposited on the sample which can remove or damage any detritus on the surface that may be of interest. They also require a high vacuum, which may alter the structure of the shaft’s outer layers. Atomic force microscopy (AFM) has been used since the late 1990s to study the structure of the surface of hair and the effect of various washing and treatment regimes, typically from a tribological standpoint\(^4-6\). In this article, we present the use of AFM in a variety of functional imaging modes to garner more detailed information and at smaller length scales than has been presented before, where the effects of washing can be clearly seen and different products can be directly compared. A key advantage of this approach, apart from the high spatial resolution afforded by AFM techniques, is that it is possible to map the spatial distribution of deposits, allowing one to assess the efficacy of any given treatment, alongside other traditional analysis techniques. The specific measurements that we use are (i) topographic imaging in intermittent-contact (tapping) mode, (ii) particle size and roughness analysis on topographic images, (iii) adhesion force mapping and (iv) Kelvin Probe Force Microscopy (KPFM) which allows us to visualize electric charge on the surface. Through appropriate application of these techniques, it is possible to gain significant insights into the effects of washing on hair. It must however be borne in mind that AFM typically can only image areas up from a few hundred square nm to a few hundred square microns, and
the age and ethnicity of the subject, the imaging location relative to the root end, its location on the head, the washing and care history, the environment, the hardness of the water, and many other factors make it clear that a single AFM image of one hair is not and cannot be considered to be representative of the entire head of hair or how it responds to a given treatment. For that reason, in this article, we show the type of measurements that are possible, how to interpret them, and we show representative data that has been collected and has been found to be repeatable on different hairs from the same sample when treated in the same way.

The structure of hair is shown in Figure 1. The surface consists of a hard barrier comprising lamellar keratinized cuticles, which have a scaly appearance. There are typically 5-10 layers of cuticles, each having a thickness in the range 300-600 nm, and are bound together by a cell membrane complex. The cuticles themselves have a layered structure: starting from the bottom (inside) is the endocuticle which is a soft, disordered layer with low cystine content (typically < 3%) followed by the exocuticle which is hard, with a higher cystine content (typically ~15%), the A-layer, which is roughly 50-70 nm thick, consists of around 30% cystine and acts as a mechanical and chemical barrier, and finally the epicuticle which is a chemically resistant barrier, around 13 nm thick as determined by TEM experiments to which a lubricating fatty acid 18-MEA (18-methyl eicosanoic acid) layer, known as the B-layer, is covalently attached. The endocuticle is rough and irregular, the exocuticle has a characteristic “woodgrain” pattern comprising a series of ripples several nm thick, oriented along the hair axis, and the A-layer and those above are smooth with no discernable features. The origin of the ripples on the exocuticle is unclear although the fact that they are parallel to the hair axis indicates that they arise due to the stress associated with repeated swelling and/or shrinkage of the entire shaft radially, compounded by the layer structure. The ripple topology is like a fingerprint, unique to each individual hair, with clear characteristics that are species-specific.

Conventional AFM experiments on hair have tended to concentrate on measuring cuticle step heights and the distance between steps, and have been used to observe the exocuticle, endocuticle and A/B layers. Statistical studies of step heights have been found to be a useful analysis tool in investigations of the effect of various haircare products. It is known that certain treatments, including bleaching, cause swelling of the cuticles, which can lead to damage of the entire hair shaft, and with the increasing need for substantiated claims, it is important to have reliable measurement protocols,
which AFM fulfills. More subtle chemical or physical damage to the cuticle surface can be observed and classified using AFM. In this article, we show that useful information can be obtained by looking at much smaller scales at the deposits left behind on hair after washing/conditioning.

MATERIALS & METHODS

Hair samples 1 cm long and taken near the distal end from a number of brown-haired Caucasian individuals were collected and subjected to a variety of treatments typical of everyday use. The hair had been unwashed for 2 days to allow some build-up of grease and particulates. In all cases, the swatches of hair were handled using latex gloves and were washed using water at 35 °C, and were subsequently left to dry naturally in a closed environment for 1 hr before any imaging. All samples were imaged before any treatments were carried out to accurately assess the effect of the treatments. One sample was simply rinsed with water without any further treatment and subsequently imaged, whereas further samples were then treated with either commercial shampoo, 2-in-1 shampoo and conditioner, conditioner or dish-washing liquid. In all cases, the gross structure of the cuticle surfaces on the scale of tens of microns was imaged followed by finer imaging on the scale of 1-5 microns where the rms (root mean square) roughness of the hair in between cuticle edges was measured. The combination of imaging and roughness measurements is important as some products leave deposits that wet the hair surface as a thin film with a resulting smooth appearance, whereas others do not wet it well, leaving globules of material behind. A roughness measurement alone could incorrectly indicate that more material is left behind in the latter case. The hair samples were fixed to metal discs using conductive sticky carbon pads that are typically used for mounting of specimens in scanning electron microscopes as they are stable and exhibit negligible drift or creep. The fibres were lowered onto the pads and pressed into place using tweezers, while being monitored in an optical microscope to ensure that the fibres did not roll on the pad and hence pick up any contamination from the adhesive. The measurements were carried out on two different AFM systems under ambient conditions. The fine topographic imaging and roughness measurements were performed using a Cambridgenano CN6000 SPM, whereas the largest scale images, adhesion force maps and KPFM measurements were performed using an NT-MDT solver pro-M AFM. The KPFM
and adhesion force measurements were performed using Pt-coated Si cantilevers from Nanosensors whereas for the rest of the measurements, plain Si cantilevers from Budgetsensors were used. All cantilevers had a reflective Al coating on the back side, and when operated in tapping-mode, had a free oscillation amplitude of the order 10 nm. In Figure 2, we show an optical micrograph of a hair fibre with the AFM tip in position, in the CN6000 SPM. Both microscopes are accompanied by a 2 micron resolution optical imaging system, assisting one in laterally moving the sample relative to the tip to access any desired location.

RESULTS & DISCUSSION

Untreated hair

In Fig. 3(a), we show a typical image of an unwashed hair shaft, where the exocuticle, cuticle edges and various deposits can be clearly seen. To highlight the features of interest, it is necessary to flatten the image to remove the curvature of the fibre, as shown in Fig. 3(b). To avoid imaging artefacts, all images are taken at the top of the cylindrical surface of the hair shaft rather than near the edge. Particulates and deposits are distributed all over the surface, as can also be seen by a side-lit representation of a section of hair, as shown in Fig. 3(c). In between the larger particulates, there are many smaller deposits as shown in Fig. 3(d), which is a zoom-in of a smaller area. The deposits shown here are typically 50-100 nm across, and 20-50 nm thick. In Fig. 3(e), we show a line profile taken from an AFM image (not shown), to illustrate a number of measurements that can be performed from a single image: (i) the average spacing between cuticle edges in this case is 7.8 microns, (ii) the average step height between cuticle edges is 200-400 nm and (iii) the deposits have an average height of around 100 nm. Across the more than 30 hair samples we investigated, the rms roughness on the flat regions between step edges before washing was found to be in the range 30-50 nm. In most cases, this level of contamination was too high to permit imaging of the fine-scale morphology of the hair surface, although a number of exceptions were noted, such as the area shown in Fig. 3(f). Here, the woodgrain texture of the exocuticle can be seen, as has been reported elsewhere. For the samples reported here, this consists of periodic grooves that are oriented along the long axis of the hair shaft, typically 2-20 nm deep, with period in the range 60-200 nm. For comparison to Fig. 3(e), Fig. 3 (g) shows a cross-section through a hair that has been cleaned (section taken from image shown in Fig. 6(a)), from which it is
apparent that there are no obvious deposits, given the flatness of the regions between cuticle edges.

In order to gain further insights into the nature of the deposits, and to understand if there is a specific interaction between them and the surface, we performed KPFM and force volume spectroscopy. KPFM measures the contact potential difference (CPD) between the AFM tip and the surface, so can be used to spatially map the distribution of charge on a surface, and although the resolution is a few tens of nm at best, this is comparable to the size of typical deposits on the hair, so is a meaningful technique to employ. Charges on the surface of hair give rise to problems with static, and need to be compensated either by being neutralized or being screened. Being able to visualize where these charges are is a useful analytical tool for testing how effectively the charge is being compensated by treatment with any given product. The KPFM measurements were performed in a two-pass technique in lift-mode, with a lift height of 25 nm. KPFM revealed that the CPD between the hair and the cantilever was 0.4-0.6 V. In Figs. 4(a) and (b), we show the topography and KPFM images of a small area, and in Fig. 4(c), the surface potential (in colour) is overlaid on the 3-d topography. It is known that the surface of hair has patches of negative charge, which can be dissipated either through condensation of water vapour under high relative humidity conditions or by application of conditioner, which has some positively charged components. In the images of untreated hair presented here, it is possible to see that the deposits naturally occurring on hair are associated with areas of reduced (i.e. less positive) surface potential. However, given the correlation between the shape of the features in the topography and the potential map, it is also a possibility that it is the deposits themselves that possess a lower surface potential than the hair surface they are on, and that this is where the overall negative charge on hair originates. A cross-section through both images (Fig. 4(d)) reveals the correlation, and that the modulation in surface potential associated with the deposits is of the order 70 mV. To test this further, an area of a freshly cleaned (by soaking in ethanol overnight and by rinsing in deionized water) hair surface was found where an endocuticle was exposed, as shown in Fig. 4(e). The corresponding KPFM image, shown in Fig. 4(f) reveals that the potential is indeed lower there by around 70 mV as compared to the surrounding areas, and that relative to the outer hair surface, the endocuticle itself is negatively charged.
We then investigated the deposits using force volume spectroscopy. In this measurement, the AFM cantilever is approached to the hair/deposit surface from a distance of a few hundred nm, into contact and then is pushed into the surface, without deforming it significantly. It is then withdrawn (retracted), and the force on the cantilever for both the approach and retract procedures is measured. This allows us to determine the adhesion force (the force necessary to pull the cantilever off the surface) and adhesion energy between the tip and the surface area of interest. Force volume spectroscopy on a the bare hair surface and on a deposit as shown in Fig. 4(g) and (h) respectively, shows that there is a 20% higher adhesive force and almost 4 times higher adhesion energy (the area of the attractive part of the retract force-distance curve) on a deposit as compared to the bare hair surface, and also the deposits are softer and can deform more, as can be seen by comparing the approach curves for both locations. The approach curve on the hair surface (Fig. 4(g)) shows that there is a long-range electrostatic force leading to the gradual downwards deflection of the cantilever before snap-in (as indicated by the thin arrow – this is the point at which the attractive force from the sample on the AFM cantilever is strong enough to overcome the stiffness of the cantilever, so it “snaps” into contact), followed by a conventional snap-in to contact and linear deflection/displacement behavior. The electrostatic force is due to the contact potential difference between the hair and the cantilever. The retract curve shows an adhesive force of the order 0.6 nN. By comparison, on the deposit (Fig. 4(f)), the approach curve shows no such snap-in, but instead shows deformation of the deposit. The retract curve shows a higher adhesive force of the order 0.9 nN, which is almost certainly due to the formation of a liquid bridge between the tip and the deposit which has to be pulled out by almost 80 nm from it’s original position before the cantilever can pull away.

Hair that has been rinsed with water

In Fig. 5(a), we show a typical image of a hair shaft that has just been rinsed with water, from which it can be seen that the hair is still covered by deposits, and that unsurprisingly, the rinsing process has not removed them, although it did remove large, micron-scale particles. The rms roughness of the hair is found to be 33.4 nm with a mean deposit diameter of 200 nm, comparable to the unwashed hair.

Hair that has been washed with dishwashing liquid
In Fig. 5(b), we show the surface of a hair that has been treated with a commercially available amphiphilic detergent: dishwashing liquid. The rms roughness of the hair is reduced to 11.6 nm and the mean deposit diameter is reduced to 127 nm. From the image, it appears that the effect of this treatment is to simply break up the existing deposits into smaller ones which become more spread out without then removing them, resulting in an overall greasy texture.

*Hair that has been washed with commercial shampoo*

We have carried out measurements on a number of commercially-available shampoos, conditioners and 2-in-1 products, and found significant variations in the resulting morphology of the deposits left behind on the hair surface. The results of the shampoos can be categorized into three different classes depending on the deposit distribution: (i) very few deposits left behind, (ii) significant deposits left behind randomly distributed, not wetting the hair surface, and (iii) deposit left behind in the form of a layer wetting the hair surface. In all cases, the shampoos we tested resulted in fewer deposits than before washing, so can be considered as being effective at cleaning. Our use of rms roughness measurements is simply to be indicative of the quantity of deposits left behind, and is not meant as a figure of merit to say that any one product is better than another, as of course many shampoos are designed to leave deposits behind for repair or protection of the surface. We will now look at specific examples of each of cases (i)-(iii).

(i) *Very few deposits.*

A number of the shampoos we investigated left almost no deposits behind at all, and allowed the intrinsic structure of the surface of hair to be clearly observed. Examples are shown in Fig. 6. In Fig. 6(a), we show an area of a hair which is very smooth after washing, and in Fig. 6(b), we can see further detail on an area on such a hair showing the characteristic woodgrain striations on the exocuticle surface (labeled “Ex”), and the edge of an endocuticle (labeled “En”) as well as a small section of smooth epicuticle (labeled “Ep”). Fig. 6(c) shows a zoom-in of an area showing the fine structure of the surface, and Fig. 6(d) shows a line profile across this surface, showing that the period of the features on this part of this hair is in the range 60-200 nm, with corresponding variations in height in the range 2-20 nm, with an average value of approximately 7 nm. The rms roughness of
these essentially deposit-free exocuticle surfaces was found to be 4.2 nm. On
the smooth area at the top, although it is not clear whether the image is
seeing the top of the A-layer, the epicuticle, or the B-layer, the roughness is
extremely low at approximately 0.4 nm.

(ii) *Randomly distributed non-wetting deposits.*

In many cases, the appearance of the hair after washing was as shown
in Fig. 7(a), which is a high-end shampoo containing amino acids and which is
designed to leave residue behind. Small deposits with a mean diameter of 30-
60 nm and height 10 nm are seen everywhere on the surface, resulting in an
rms roughness of the hair of 14.2 nm. Another example of a different product
is shown in Fig. 7(b), where there are fewer deposits, typically 100 nm across
and 20 nm thick, but resulting in an overall rms roughness of the surface of 6.6
nm.

(iii) *Deposits left behind as a layer.*

We have observed cases in which the residue from a shampoo completely wets
the hair surface, forming a uniform layer just a few nm thick. An example of
this behavior is shown in Fig. 8(a), where monolayers and aggregates of
deposited material can be seen. From Fig. 8(b), the nature of the film of
deposit is more apparent. It is wetting the hair surface, forming a uniform
layer, and in some areas, the film is several layers thick. From the cross-
section in Fig. 8(c), the thickness of each layer is just 4-5 nm. A surface
roughness analysis of the flat regions of the molecular film reveals a rms
roughness of the order 0.15 nm, which will be limited by the tip radius of
curvature.

*Hair that has been washed with 2-in-1 commercial shampoo & conditioner*

We have treated a number of hair samples with several different 2-in-1
products, as shown in Fig. 9. In Fig. 9(a), we show an image of a hair which has been
treated with one of these products. We can see that there are thick deposits distributed
along the hair everywhere apart from near the top of cuticle edges. The rms
roughness of these samples was found to be 18.5 nm on average. In Fig. 9(b), the
result of treating with another 2-in-1 product is shown – in this case, an area with an rms roughness of 27.5 nm.

*Hair that has been treated with conditioner*

Conditioner is often used as it is claimed that it moisturizes hair. This should not be mistaken for meaning that it hydrates hair, as it does not necessarily do so. It typically contains molecular species that adhere to the B-layer either chemically or to the charges in it, and either prevents moisture from escaping from the hair surface or attracts water from the atmosphere onto the hair. In Fig. 10 we show an image of hair that has been treated with a commercial conditioner after washing with a commercial shampoo. In between the two treatments, the hair roughness was measured as 6 nm, whereas after the conditioner was added, the roughness increased to 26.5 nm, comparable to the pre-washed value. It is clear from this image that the conditioner completely covers the entire surface, and therefore will act effectively as a protective barrier. Detailed experiments measuring the thickness distribution of conditioner have been reported elsewhere\textsuperscript{12, 13}.

A number of issues became apparent during the course of this work which should be considered. First and foremost, as has been said earlier, and by others, AFM-based experiments can reveal an unprecedented level of detail into the structure of hair and the effect and efficacy of different washing/conditioning products. However, given the variations in local properties (both mechanical, surface structural and chemical) from one location to another on a single hair and the variations from one part of the head to another, AFM alone is not a sufficient tool. However, as part of a larger analysis suite, AFM and related techniques have a significant amount to offer. The use of functional imaging has been shown here, in the form of adhesion mapping and surface potential mapping. Other techniques, such as force modulation and chemical force microscopy could be used to further reveal fine details in the structure of hair surfaces.

*Comparison between different types of treatment*

In Fig. 11, we have plotted the measured rms surface roughness for twenty different commercial haircare products, of which 13 are shampoos, all displaying a post-
treatment rms surface roughness below 10 nm, 2 of which are a conditioner or 2-in-1 shampoo/conditioner blend, with a post-treatment roughness of just under 30 nm, and 5 further products which are specifically designed to leave deposits behind to moisturize hair, protect against dandruff, repair it or protect it against continued heat or colouring treatments, with a roughness in the range 10 - 25 nm. The resulting rms roughness data shows clear delineation between these three groups of products, validating the use of AFM to distinguish between different products in this way. It is worth pointing out that conditioners have the overall effect of reducing hair friction (traditionally associated with roughness) on the macroscopic scale, making the hair easier to comb, style etc., and feel softer. However, our results show that at the nanoscale, surface roughness is increased by the application of conditioner. Therefore this effect of increased roughness on the nanoscale should not be confused with the beneficial effect of the reduction of “roughness” (i.e. friction) on the macroscopic scale conferred to hair by treatment with a conditioner.

CONCLUSIONS

We have carried out multiple AFM techniques on hair samples that have been washed/treated with a number of different shampoos/conditioners and 2-in-1 products, all of which are commercially available. We have shown that the exocuticle carries a negative charge, and that deposits on unwashed hair tend to have a negative charge and to be randomly distributed on the surface, with a mean roughness of up to 50 nm. We have shown that simply rinsing hair with distilled water removes particulates but causes the existing deposits to break up into smaller structures. Washing with shampoos reduces the roughness of hair + deposits to typically below 10 nm. The use of 2-in-1 products, conditioners or shampoos that are designed to treat hair in some way tends to result in large quantities of deposits that cover the entire surface, with roughnesses up to 30 nm. The measurement of surface roughness combined with images of the resulting surface + deposits can be used to systematically distinguish between the effect of different hair care products.
REFERENCES

1. Euromonitor international report “haircare in the UK”, (2013)
FIGURE CAPTIONS

Fig. 1. (a) The gross structure of hair. The medulla, which is not always present, runs through the centre, surrounded by the cortex which contains melanin and determines the hair colour. The cortex is surrounded by hard, scaly cuticles. (b) The layered structure of a cuticle.

Fig. 2. Optical micrograph of AFM tip in place on a hair.

Fig. 3. (a) AFM image of unwashed human hair. Scale bar is 5 µm. (b) The same image after flattening, where the debris on the hair is now more prominent. (c) Side-lit 3-D representation of an area of hair revealing that particulates and deposits are relatively uniformly distributed across the hair surface. Scale bar is 3 µm. (d) Smaller scale image showing deposits on hair, away from particulates and cuticle edges. Scale bar is 200 nm. (e) Cross-section through topography of a 35 µm long section of hair with 4 cuticle edges in the range 200-500 nm thick with a lateral spacing approximately 7 µm, and where the deposits can be seen as ripples/bumps. (f) AFM topography image revealing woodgrain striations, characteristic of the exocuticle. (g) cross-section through a clean hair devoid of deposits, for comparison to Fig. 3(e).

Fig. 4. (a) AFM topography image of unwashed hair in a section away from cuticle edges and particulates, revealing deposits up to 60 nm high. (b) Kelvin Probe potential map of the same area showing decreases in surface potential corresponding to the location of the deposits. (c) Surface potential as a colour map overlaid on the 3-D topography to highlight the correlation between the two. (d) Cross-section through the line AA’ on topography and potential. (e) Topography image of a freshly cleaned hair surface revealing an exposed area of endocuticle, labeled “En” and (f) KPFM image of the same area showing that the exocuticle is negatively charged with a potential of around 70 mV lower than the epicuticle. (g) Force volume curve on a location of hair without any apparent deposits showing an adhesive force of 600 pN. The gradual downwards deflection of the cantilever on the approach curve (indicated by the arrow) is due to long-range electrostatic forces, and the point of snap-in is indicated by the thin arrow (h) Force volume curve on a deposit showing an adhesive force of 900 pN.
Fig. 5. (a) AFM image of hair that has just been rinsed with deionized water – the hair is covered with small deposits (which appear as bright spots in the image). Scale bar is 2 µm. (b) AFM image of hair that has been washed with dishwashing liquid. Scale bar is 2 µm.

Fig. 6. (a) AFM image of hair that has been cleaned with a shampoo that leaves very few deposits. Scale bar is 2 µm. (b) AFM image of a clean area of hair revealing the exocuticle (Ex) with its characteristic woodgrain striations, the Endocuticle (En) and the Epicuticle (Ep). (c) Zoom-in of woodgrain pattern on exocuticle showing its regularity. Scale bar is 100 nm. (d) Cross-section through image (c) showing variations in topography associated with woodgrain pattern.

Fig. 7. (a) AFM image of hair treated with shampoo containing amino acids revealing a large number of clusters of deposits. Scale bar is 1 µm. (b) Hair that has been treated with a shampoo that moisturizes. Scale bar is 2 µm.

Fig. 8. (a) AFM image of hair that has been washed with a shampoo that leaves a smooth layer of material behind. Scale bar is 200 nm. (b) Zoom-in of a 1 µm x 1 µm area showing the layer structure of the deposited material. Scale bar is 100 nm. (c) Cross-section through the area shown in Fig 8(b) showing that the mean height of the layers of residue is 4-5 nm.

Fig. 9. (a) AFM image of hair that has been washed with a 2-in-1 shampoo and conditioner revealing a large number of deposits of conditioner left behind. Scale bar is 4 µm. (b) Hair that has been treated with a different 2-in-1 product, also revealing a large number of deposits. Scale bar is 3 µm.

Fig. 10. AFM image of hair that has been washed, rinsed and then treated with a conditioner. A large number of deposits are evident, distributed relatively evenly across the entire hair. The scale bar is 4 µm.

Fig. 11. Plot of rms surface roughness of hair vs product number showing a clear distinction between Shampoos, conditioners (labeled “C”) and others (labeled “O”). The surface roughness prior to washing was in the range 30-50 nm, as shown by the shaded region. The data point that is circled is for an anti-dandruff shampoo.
Long-range electrostatic force

Snap-in
Large number of deposits ~ 200 nm across

Deposits ~ 130 nm across
FIGURE 6(A)
Distance (nm)
Height (nm)

FIGURE 8(C)

FIGURE 9(A)
**Product number**

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**Before washing**

**Shampoos**

- **C**
- **O**

**Product number**