AFM image processing for estimating the number and volume of nanoparticles on a rough surface

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Abstract: This paper presents the method of computer automatic recognition and measurement of the number and volume of nanoparticles formed on a rough surface by smoothing, enhancement and segmentation of image processing. The grafted grains (nanoparticles) on polyethylene surface are taken as the example. This method uses shock filter enhancement and globally convex segmentation to separate the nanoparticles from the polymer substrate surface, and then the nanoparticles are extracted from the rough surface, the number and volume of nanoparticles on the rough surface are determined. By applying this method to analyze the surfaces irradiated for different time, the number and volume of grafted grains are obtained and they are consistent with the results obtained manually.

Key words: atomic force microscope, nanoparticles, rough surface, shock filter, global minimization.

1 Introduction

Nanoparticles are any types of microscopic particles with at least one dimension of the order of 100 nm or less, they exhibit new or enhanced size-dependent properties compared with larger particles of the same material. Due to a wide variety of potential applications in biomedical, optical, and electronic fields, nanoparticle has become an area of intense scientific research.

Atomic force microscope (AFM) has rapidly become an established technique

for characterizing nanoparticles. As one of the major advantages of AFM over traditional techniques such as SEM and TEM, AFM directly produces three dimensional images of observed objects. To simplify the observation and analysis, nanoparticles and other nanostructured materials are usually placed on silicon wafer or mica with extremely smooth surface (roughness less than 1 nm). Therefore, both qualitative and quantitative information on many physical properties including size, morphology, surface texture and roughness, and statistical information including size, surface area, and volume distributions can be easily determined with the programs accompanying AFM instruments which need only simple mathematic treatments.[1,2] However, in many real applications and very possibly more often, we have to study the nanoparticles on rough surfaces. Because of the complex structure of the substrate surface, it is rather difficult to obtain accurate information about the number, size and size distribution of nanoparticles on it. Existing programs for grain detection and size distribution of nanoparticles are often not able to correctly process such AFM images of a rough surface.[3] There are only a few studies on the analysis of nanoparticles on rough surfaces. Oikawa et al proposed a curvature-reconstruction method to estimate the sizes of particles by fitting sphere curvatures acquired from raw AFM data. By using a carbon nanotube (CNT) tip to measure gold nanoparticles with known sizes on the rough surface of dried cells, they found that the particle sizes could be estimated within 5%.[4] This curvature-reconstruction method can only be applied to nanoparticles with perfect spherical structures. Chuklanov proposed an algorithm for processing AFM data on the images of nanoparticles and constructing histograms of the size (diameter) distributions of particles.[3]

Figure 1: AFM height image of grafted HDPE surface. 2 μm scan, the contrast covers height variations in the 0-100 nm range. The grafting was carried out in 1 M GMA dichloromethane solution, 1 min irradiation.

We have studied the surface photografting of glycidyl methacrylate onto high-density polyethylene (HDPE) and the microstructure of the grafted chains with AFM.[5−7] The preparation of the grafted samples and the AFM experimental conditions are described in [5,6]. AFM experiments were performed using a Digital Instruments multimode AFM equipped with a Nanoscope IIIa controller (Digi-

tal Instruments, Santa Barbara, CA). The results were obtained in tapping mode AFM. A vertical engage 4842 JV-scanner and Si probes with a tip radius of about 15 nm were applied in all experiments. Figure 1 shows one of the AFM images obtained in our previous work. The HDPE surface is rough in nano-scale and with complex structures. It is impossible to prepare smooth polymer surface with a roughness less than 1 nm. The reasons are: (1) semicrystalline polymers such as HDPE usually have lamellar structures on their surfaces, the height of lamella is about 5-6 nm[5,8]. The strips in Figure 1 are the lamellar structures of a banded spherulite of crystallized HDPE, which consists of rows of granules; (2) the volume shrink of polymeric material during the preparation process usually induces rugged surface. When we were preparing the HDPE sample for AFM observations, silicon wafer was used as the molding plate, however, the maximum peak-to-valley height on the surface was usually several tens nanometers. The round or elliptical particles in Figure 1 are the grafted grains which consists of grafted polymer chains and with diameters in nanometers. In the initial stage of grafting reaction, the number of grains increases with grafting time[6], and the number and size of the grains vary with the solvent type[5]. To know the number and volume of the grains formed is important for the understanding of the microstructures of the grafted chains and the study of reaction kinetics in the very beginning of surface grafting polymerization. In these studies, we have to accumulate the quantitative information of the grains manually since there is no computing program can process such complex surface to obtain the number and volume of nanoparticles automatically.

Therefore, the purpose of this work is to develop an algorithm for automatic processing AFM data to obtain the number and volume of nanoparticles on a rough surface.

2 Image Processing

The main idea of this work is to identify the grafted grains (nanoparticles) from the polymer substrate surface, and then determine the number and volume of nanoparticles. This method analyzes AFM images by image processing techniques and is performed in the MATLAB mathematical computing environment. The main steps

Figure 2: The original and processed AFM images with the original scan numbers as the units of X-Y axes. (a): the original image; (b): the smoothed image; (c): the resultant image by subtracting the smoothed image from the original one; (d): the enhanced image using the shock filter model.

are as follows:

(1)**Gaussian smoothing**

Due to the roughness and complexity of the polymer substrate surface, the original AFM images have non-uniform brightness and hence they are difficult to be processed. Figure 2(a) shows the same AFM image as that in Figure 1 but with the original scan numbers as the units of X-Y axes. Here we applied the low-pass Gaussian filter to exclude the influence of non-uniform brightness. A smoothed image u_s is a filtered version of the image $u : \Omega \rightarrow \mathbf{R}, \Omega = [0, 512] \times [0, 512]$, using the Gaussian filter G_σ [9]:

$$u_s(x, y) = u(x, y) * G_\sigma(x, y), \tag{1}$$

where a two-dimensional Gaussian function with the standard deviation σ is defined as

$$G_\sigma(x, y) = \frac{1}{2\pi\sigma^2} exp(-\frac{1}{2\sigma^2}(x^2 + y^2)). \tag{2}$$

Figure 2(b) is the smoothed image obtained by applying the Gaussian filter to Figure 2(a). Then, the subtraction of the smoothed image from the original one gives an image with uniform brightness [Figure 2(c)]. In other words, we elevate the valley bottom and make nanoparticles probably in one plane.

(2)**Shock filter enhancement**

The nanoparticles always have vague edge and can not be easily identified, hence we improved the clarity of nanoparticles by the Osher and Rudin shock filter model [10].

Two-dimensional shock filter model is

$$\begin{cases} u_t(t, x, y) = -|\nabla u| F(L(u)), \\ u(0, x, y) = u_0(x, y), \end{cases} \tag{3}$$

where

Figure 3: The globally convex segmentation results. (a): segmented nanoparticles; (b): histogram of the remaining polymer substrate, which creates a set of bins for each column, displaying each set in a separate color.

- $|\nabla u| = \sqrt{u_x^2 + u_y^2}$ is the propagation term;
- F is a Lipschitz continuous function satisfying $F(0) = 0, sign(s)F(s) > 0 (s \neq 0)$, for example, $F(s) = sign(s)$;
- L is a second-order edge operator. A simple choice for L is $L(u) = \Delta u = u_{xx} + u_{yy}$, another better choice for L,

$$L(u) = \frac{1}{|\nabla u|^2}(u_x^2 u_{xx} + 2u_x u_y u_{xy} + u_y^2 u_{yy}),$$

  which is invariant to x-y coordinate and corresponds to the second derivative of u in the direction of the normal to the isophotes.

The shock filter model creates strong discontinuities at the edge of nanoparticles and hence the nanoparticles on the substrate surface become obviously prominent, as shown in Figure 2(d).

(3)**Globally convex segmentation**

The difference between our objects of interest (nanoparticles) and the background (the polymer substrate) can be expressed as the distinctively different average gray value. If we can find a closed curve C, which can divide the image domain into the inside region Ω_1 and the outside one Ω_2 and the average gray in each part can exactly reflect the mean gray value differences between the nanoparticles and the polymer substrate, then this closed curve can be regarded as the boundary between nanoparticles and the polymer substrate.

Based on the above idea, we choose the globally convex segmentation proposed by Chan[11] and Bresson[12], which can avoid the local minimizers and the final solution is independent of the initial contours. The model is as follows

$$
\begin{aligned}
(\varphi^*, c_1^*, c_2^*) &= \underset{0\le\varphi\le 1, c_1, c_2}{argmin}\ E(\varphi, c_1, c_2) \\
E(\varphi, c_1, c_2) &= \mu \iint_\Omega |\nabla\varphi| dxdy + \lambda \iint_\Omega (u - c_1)^2 \varphi dxdy \\
&\quad + \lambda \iint_\Omega (u - c_2)^2 (1 - \varphi) dxdy
\end{aligned} \tag{4}
$$

where $\mu,\ \lambda \geq 0$ are fixed parameters, and $c1,\ c2 \in R$ represent the average gray value of the inside region Ω_1 and the outside one Ω_2, respectively.

In this paper, we computed the minimizer of (4) with the alternate minimization algorithm[13]:

- Fix φ^n and update c_1^n, c_2^n as follows

$$
c_1^n = \frac{\iint_\Omega u(x,y)\varphi^n(x,y)dxdy}{\iint_\Omega \varphi^n(x,y)dxdy}, \quad c_2^n = \frac{\iint_\Omega u(x,y)(1-\varphi^n(x,y))dxdy}{\iint_\Omega (1-\varphi^n(x,y))dxdy}
$$

- Fix c_1^n, c_2^n and update φ^{n+1} with Bregman iteration approach

$$
\begin{cases}
(\varphi^{n+1}, d^{n+1}) = \underset{\varphi\in[0,1], d}{argmin} \iint_\Omega |d| + \lambda h_r \varphi^n + \frac{\mu}{2}|d - \nabla\varphi^n - b^n|^2 dxdy \\
b^{n+1} = b^n + \nabla\varphi^{n+1} - d^{n+1}
\end{cases} \tag{5}
$$

  where $h_r = (u - c_1^n)^2 - (u - c_2^n)^2$.

Then, the final active contour is given by the boundary of the set:

$$
\{(x,y) \in \Omega \mid \varphi^{final}(x,y) > 0.5\} \tag{6}
$$

Figure 3 displays the results of segmentation. Figure 3(a) shows that all the nanoparticles are abstracted from the polymer substrate surface. Figure 3(b) is a histogram of the remaining polymer substrate and it shows that most of the raised and sunken parts of the remaining polymer substrate are within $\pm 5nm$, and hence the interpolation can be considered as the statistic substrate surface.

(4)**Estimating the number and volume of nanoparticles**

After the smoothing, enhancement and segmentation of AFM image processing, we can identify and separate the nanoparticles from the rough polymer surface.

Figure 4: The constructed images of grafted surfaces. (a) and (b) are constructed by placing particles with known sizes on a rough surface; (c) and (d) are constructed by establishing the partial differential equation model.

The number of nanoparticles can be obtained by using the connected component labeling algorithm, and the volume of each nanoparticle can be obtained by analyzing them with the original image data.

The detailed steps of estimating the number and volume of nanoparticles are as follows:

1. Load the nanoparticles image after segmentation, and then binarize the image;
2. Scan the image pixels one by one from left to right and top to bottom, determine the neighborhood relations between the pixels, and then give the same mark to the pixels in the same connected region;
3. Till all the connected regions are marked, the number of connected regions should be the number of nanoparticles.
4. Analyze the different connected regions with the original image data respectively, the bottom of each region may be considered as the underside of that nanoparticle. To sum the volumes of each nanoparticle can get the total volume of nanoparticles.

(5)**Verifying the accuracy of this method**

We have constructed two types of images, as shown in Figure 4. Figure 4 (a) and (b) are the model images that contain spherical particles with known sizes randomly placed on a rough surface. Figure 4 (c) and (d) are constructed by establishing the partial differential equation model to simulate the process of grafting reaction and the formation of grafted nanoparticles on a rough surface. We are not going to talk about the simulation method in detail since it is not very relevant to the topic of this paper. The constructed grafted surface images are similar to the real AFM ones. Because the exact numbers and volumes of the particles in the constructed images can be obtained, the constructed images can serve as ideal models to test the preciseness and errors of this evaluation method.

Table 1 shows the volumes of the nanoparticles in Figure 4 directly calculated from the structures and those estimated by this method. The volumes estimated by

Figure 5: (a) Histogram of the size distribution of nanoparticles and (b) the change of the height with the diameter of nanoparticles.

this method are slightly lower than those directly calculated from structures. The possible reason is the error introduced in calculating the statistic substrate surface [Figure 3(b)]. The grafted grains are mushroom-shaped, a little decrease in the height (especially from the bottom side) could lead to a significant decrease in the volume. For Figure 4 (a) and (b), the accuracy of this method is beyond 90%, because these two images are very regular and the containing particles are standard spherical shape. Even though there are more inevitable errors in the Figure 4 (c) and (d), the accuracy of this method is still higher than 80% for these two images. Since the heights of the nanoparticles in the constructed images are only about 10 nm, which are lower than those in the real cases, we believe that the accuracy of this measurement method should be higher when dealing with our AFM images.

Table 1: The accuracy of this measurement method.

Image	Volume directly calculated from the structures ($\times 10^5$ nm^3)	Volume estimated by this method ($\times 10^5$ nm^3)	Accuracy
Figure 4(a)	1.17	1.09	93.2%
Figure 4(b)	1.18	1.12	94.9%
Figure 4(c)	3.14	2.53	80.5%
Figure 4(d)	2.66	2.23	83.8%

3 Results and Discussion

By applying the above method, the number of nanoparticles (grafted grains) in Figure 1 is calculated to be 269, and the total volume of them is 7.33×10^6 nm^3. The diameters and heights of the nanoparticles are obtained directly from the analyses of each nanoparticle. A histogram of the size distribution of nanoparticles is close to a normal distribution which shows in Figure 5(a), and Figure 5(b) plots the height against the diameter of the nanoparticles, it can be seen that the height of the nanoparticles increases almost linearly with the diameter of the nanoparticles. It is almost the same as that reported in [5]. The linear increase of the height

Figure 6: AFM height images of the HDPE samples grafted in 1 mol/L GMA acetone solution for different time. (a): 30 s; (b): 35 s; (c): 40 s. 2 μm scan, Z range: 0-50 nm.

Figure 7: The counted and calculated numbers (a) and the calculated volumes (b) on the grafted HDPE surfaces.

with the diameter of the nanoparticles is explained by the existence of grafting on the grafted chain, i.e., branching, which leads to the simultaneous increase of the size of the granules in all three dimensions.

To prove the validity of the method, here we used the AFM data obtained in our previous study [6] to calculate the numbers and volumes of grafted grains on the HDPE surfaces irradiated for different time. The preparation of the grafted samples and the AFM experimental conditions are described in [6]. The original AFM images of HDPE surface irradiated for 30, 35 and 40 s are shown in Figure 6.

Figure 7 shows the calculated results. The manually counted and the calculated numbers are presented in Figure 7(a). It is obvious that the number of grafted grains increases with increasing irradiation time. The calculated numbers are very close to the counted numbers especially when the irradiation time is longer (40s). The change of the calculated volume of grafted grains with irradiation time is shown in Figure 7(b), and the increase of volume with irradiation time is also observed. Since this is no significant increase in the size of nanoparticles in the images (Figure 6), the total volume of nanoparticles should be directly proportional to the number of nanopartilces. The increase of the calculated volume with irradiation time is consistent with that as expected.

In summary, we have developed an algorithm for processing AFM data to obtain the number and volume of nanoparticles on a rough surface. Shock filter enhancement and globally convex segmentation are used to separate nanoparticles from polymer substrate surface, then the number and volume of nanoparticles on the rough surface can be determined. By applying this method to analyze the surfaces irradiated for different time, the number and volume of grafted grains are obtained and they are reasonably consistent with the results obtained manually.

Although only grafted grains on HDPE surface were examined in this study, this method can also be applied to the estimation of other kinds of nanoparticles

on rough surfaces of different substrates. More accurate quantitative information about nanoparticles can be obtained with further improvements of this method.

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References

[1] J. Scalf, P. West, *http://nanoparticles.pacificnanotech.com* **2006**.

[2] R. Allabashi, W. Stach, A. de la Escosura-Muniz, L. Liste-Calleja, A. Merkoci, *J. Nanopart. Res.* **2009**; *11*, 2003.

[3] A. P. Chuklanov, S. A. Ziganshina, A. A. Bukharaev, *Surf. Interface Anal.* **2006**; *107*, 679.

[4] K. Oikawa, H. Kim, N. Watanabe, M. Shigeno, Y. Shirakawabe, K. Yasuda, *Ultramicroscopy* **2007**; *107*, 1061.

[5] H. L. Wang, H. R. Brown, *Polymer* **2007**; *48*, 477.

[6] H. L. Wang, *Chem. J. Chinese U.* **2006**; *27*, 1570.

[7] H. L. Wang, J. M. Han, *J. Colloid Interf. Sci.* **2009**; *333*, 171.

[8] Y. K. Godovsky, S. N. Magonov, *Langmuir* **2000**; *16*, 3549.

[9] M. Sonka, V. Hlavac, R. Boyle, *Thomson-Engineering* **2007**.

[10] S. Osher, L. I. Rudin, *SIAM J. Numer. Anal.* **1990**; *27*, 919.

[11] T. F. Chan, S. Esedoglu, M. Nikolova, *SIAM J. Appl. Math.* **2006**; *66*, 1632.

[12] X. Bresson, S. Esedoglu, P. Vandergheynst, J. Thiran, S. Osher, *J. Math. Imaging Vis.* **2007**; *28*, 151,

[13] T. Goldstein, X. Bresson, S. Osher, *CAM Report 09 - 06* **2009**.