Application of Image Processing to Determine Size Distribution of Magnetic Nanoparticles

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Digital image processing has increasingly been implemented in nanostructural analysis and would be an ideal tool to characterize the morphology and position of self-assembled magnetic nanoparticles for high density recording. In this work, magnetic nanoparticles were synthesized by the modified polyol process using Fe(acac)3 and Pt(acac)2 as starting materials. Transmission electron microscope (TEM) images of as-synthesized products were inspected using an image processing procedure. Grayscale images (800 × 800 pixels, 72 dot per inch) were converted to binary images by using Otsu’s thresholding. Each particle was then detected by using the closing algorithm with disk structuring elements of 2 pixels, the Canny edge detection, and edge linking algorithm. Their centroid, diameter and area were subsequently evaluated. The degree of polydispersity of magnetic nanoparticles can then be compared using the size distribution from this image processing procedure.

Keywords: microscope image processing, magnetic nanoparticles, canny edge detector

1. Introduction

Images from optical and electron microscopes have been important parts in micro- and nanoscale characterizations. Morphology of structures can be studied but the qualitative analysis requires time-consuming visual inspections. With a recent advent in the field of digital image processing, such characterizations can be facilitated [1]. In a common image processing technique referred to as edge detection, objects are separated from the background by identifying the abrupt change in the intensity as an edge. After their boundaries are detected, the objects are counted and the size distribution can then be analyzed. Microscope image processing has been applied to a variety of complex features. For examples, titanium compressed films were studied [2] and the geometrical distribution of graphite particles in cast iron was evaluated [3] by using image segmentation on their optical micrographs. In the case of scanning electron microscope (SEM) images, morphologies of both microfiber [4] and particulate matter [5] were successfully analyzed. The implementation on SEM images was further improved by means of the scale space [6] and adaptive tuning [7] processes. Furthermore, the tomography of low-contrast superconducting materials captured by scanning transmission electron microscope (STEM) was quantified by digital image processing [8]. However, the intensity-based edge detection may become ineffective for images of nanostructures which have less contrast. Alternatively, Wajcik and Krapf employed Shannon entropy to detect and measure nanopores of diameter around 3 nm from transmission electron microscope (TEM) images [9].

Magnetic nanoparticles are under research and development for ultrahigh density recording and biomedical applications [10]. In prototypes of patterned media, the data are coded in forms of magnetization of magnetic nanoparticles regularly positioned on the substrate. Since the deviations in size, shape and position strongly affect the reading and writing of the data, the pattern inspection is compulsory. Because magnetic nanoparticles for recording capacity beyond terabit per square inch are smaller...
than 10 nm, either TEM or atomic force microscopy is used to maximize the magnification. Whereas edge detection algorithms were previously applied to count periodic magnetic micropillars in SEM micrographs [11], they cannot determine the size distribution and effectively eliminate the noise. Preprocessing and other additional steps are required to complement the image segmentation. The software packages, e.g. Image J, provide a rapid counting and size analysis of statistically significant number of nanoparticles [12]. However, these conventional programs are inappropriate for agglomerated particles because of the lack of high-contrast boundaries. In this work, an image processing procedure is developed based on the edge detection algorithm and tested on TEM images of self-assembled magnetic nanoparticles.

2. Experimental

Magnetic nanoparticles were synthesized from Fe(acac)$_3$ and Pt(acac)$_2$ in the polyol process which detailed in the previous work [13]. These nanoparticles were surface-modified by oleic acid/oleylamine and dispersed without sedimentation in n-hexane. Two samples have different concentrations of Fe(acac)$_3$, surfactants (oleic acid/oleylamine) and a reducing agent (potassium borohydride, KBH$_4$) as listed in Table 1. TEM images were taken from dried particles on carbon coated copper grid substrates using an accelerating voltage of 200 kV. The procedure of microscope image processing is summarized in Fig. 1. The grayscale TEM images (72 dot per inch, 800 × 800 pixels) were read by Matlab 7.11. The image was then converted to a binary image by using Otsu’s thresholding. The global threshold was automatically computed for each image based on the difference in the intensity of objects (i.e. nanoparticles) from the background. The closing process with disk structuring elements of 2 pixels was applied to remove noise included in the TEM image and enhance the edge. Each individual dot was then detected by using the Canny edge detector in the Image Processing Toolbox of Matlab. The Canny operator, one of the tools detecting edges in a very robust manner, is selected based on the comparison with other algorithms in the previous work [11]. The detected areas in the output from the Canny edge detector were then filled and the small unfilled objects of less than 200 pixels were subsequently removed. The filling process was required because the intensity of pixels surrounding the object was non-uniform. The pixel was added from one side to the other until the area was enclosed by pixels of comparable intensity. The centroid of each detected area can then be identified. To increase a performance of the detection, the process was further improved by the inclusion of an algorithm for repairing broken contour before the filling process in Step 5. Finally, the diameter and cross-sectional area of each detected nanoparticle were computed and compared with those obtained by inspecting an individual dot one-by-one on Photoshop CS5.

<p>| Table 1. Concentrations of reagents in the synthesis of magnetic nanoparticles. |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Reagents (mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe(acac)$_3$</td>
</tr>
<tr>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Fig. 1. Flowchart of the proposed image processing procedure.
3. Results and Discussion

From both original TEM images in Fig. 2, magnetic nanoparticles appear as dark dots on lighter background from the substrate. Although the sizes of nanoparticles in both samples are comparable, spheroidal nanoparticles in Sample 1 are apparently more uniform in size and shape. It follows that they exhibit clearer self-assembled tendency with the spacing slightly larger than their diameter. The conversion from grayscale to binary images in Fig. 3 gives rise to high contrast between black nanoparticles and the white substrate but several tiny ‘noise’ dots are introduced in the background. These noise dots are subsequently eliminated in the closing step as shown by Fig. 4. Most areas can then be detected by the Canny edge detector as shown in Fig. 5 corresponding to the nanoparticles with false detections of tiny noise dots inside some nanoparticles. After the successive steps of filling areas and removing small objects, some particles are obviously missing from Figs. 6 and 7 and are not account-

Fig. 2. Original TEM images of magnetic nanoparticles in (a) Sample 1 and (b) Sample 2.

Fig. 3. Binary images of (a) Sample 1 and (b) Sample 2 converted by using the Otsu’s thresholding.

Fig. 4. Outputs from the closing step for (a) Sample 1 and (b) Sample 2.

Fig. 5. Outputs from edge detection using Canny operator for (a) Sample 1 and (b) Sample 2.

Fig. 6. Images of (a) Sample 1 and (b) Sample 2 after the filling area step.

Fig. 7. Images of (a) Sample 1 and (b) Sample 2 after removing small objects.
Table 2. Mean and standard deviations of diameter and cross-sectional area of magnetic nanoparticles obtained from the one-by-one inspection and image processing procedures (with and without edge linking algorithm).

<table>
<thead>
<tr>
<th>Sample</th>
<th>One-by-one inspection</th>
<th>Image processing procedure</th>
<th>Image processing procedure with edge linking algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of particles</td>
<td>Diameter (nm)</td>
<td>Area (nm$^2$)</td>
</tr>
<tr>
<td>1</td>
<td>594</td>
<td>4.3 ± 0.8</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>2</td>
<td>590</td>
<td>4.7 ± 1.1</td>
<td>16 ± 6</td>
</tr>
</tbody>
</table>

Fig. 8. (Color online) Final outputs with computed size for (a) Sample 1 and (b) Sample 2.

Sample 2 (average diameter 4.8 nm, cross-sectional area 18 nm$^2$). Moreover, the higher standard deviations of diameter and area indicate a larger morphological variation. The difference in size distribution is likely rooted from the inclusion of the reducing agent in the synthesis. With the presence of KBH$_4$ in Sample 2, the Fe(acac)$_3$ may be effectively reduced but the uniform size distribution is apparently deteriorated.

The results from the proposed image processing procedure are in good agreement with the one-by-one inspection procedure.
tion of original TEM images. As compared in Table 2, the number of detections by the image processing may be much lower but both methods yield comparable mean diameters around 4 nm. Similar to the conclusion from the image processing, the one-by-one inspection indicates a larger degree of polydispersity in Sample 2. The log-normal distributions of the cross-sectional areas of nanoparticles in both samples are shown in Fig. 9. The difference in size between the two samples is increased in the one-by-one inspection which can be related to a higher number of particles involved. In the image processing, 189 nanoparticles which mostly have a large deviation in size and shape from the rest in Sample 2 are excluded from the calculation. For each sample, the difference in the mean area from the two different methods is higher than that in the diameter. Nevertheless, the sizes from the one-by-one inspection remain smaller than those obtained from the image processing. This trend underlines the capability of the image processing algorithm to differentiate the blurry edge from the background.

The number of particle detection can be improved by a careful selection of threshold value for the edge detector in Step 4. The automatic selection of threshold value for the Canny edge detector introduces broken edges as shown in Fig. 10. The effect of the threshold value on the image processing result was then studied. The edge linking algorithm was also applied to connect the broken contour with just one pixel gap due to the selection of threshold value. This algorithm scans through an input binary image to find the broken points, and applies a lookup table to repair the edge pixel upon its eight-neighborhood pixels. The Canny edge detector employs an expanded threshold set in the so-called hysteresis thresholding operation, where a significant edge is defined as a connected series of pixels with the edge magnitude of at least one member exceeding an upper threshold \( t_{2} \), and with the magnitudes of the other members exceeding a lower threshold \( t_{1} \). There are a large number of possible combinations between these threshold parameters. Each parameter corresponds to a different resulting edge profile. The image processing results under various setting of the hysteresis thresholds are compared in Fig. 11. The lower hysteresis threshold ranges from \( t_{1} = 0.1-0.8 \) and the upper threshold \( t_{2} = t_{1} + 0.5 \) is used. It can be observed from Fig. 11 that the image processing is sensitive to the choice of \( t_{1} \) and \( t_{2} \). The lower values of \( t_{1} \) and \( t_{2} \) reveal more details but, at the same time, cause more false positive detections. It follows that the number of particles detected by the program is greater than that of the one-by-one inspection. On the other hand, higher values of thresholds lead to missed features. The application of edge linking algorithm significantly improves the detection performance. With

![Fig. 10. (Color online) Examples of broken edges introduced by the Canny edge detection.](image)

![Fig. 11. (Color online) Number of nanoparticles detected for (a) Sample 1 and (b) Sample 2. Hysteresis thresholds are \( t_{1} = 0.1-0.8 \) and \( t_{2} = t_{1} + 0.5 \).](image)
the selected thresholds, this result can be compared with the result of the one-by-one inspection method in Table 2. In Fig. 12, the particle profiles detected by the current approach are comparable to those under the user supervision of the conventional visual inspection method.

4. Conclusions

It is demonstrated that the TEM images of magnetic nanoparticles with average diameter of less than 5 nm can be analyzed using the image processing procedure based on the Canny edge detector and edge linking algorithm. The binary image processing with Otsu’ thresholding, closing and filling steps are also needed to reduce noise and enhance the image. Two images of nanoparticles with unequal size distributions caused by the reducing agent are quantitatively differentiated. This method gives rise to larger diameter and cross-sectional area of nanoparticles than those obtained from the one-by-one inspection.

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