A simple algorithm for measuring particle size distributions on an uneven background from TEM images

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A B S T R A C T
Nanoparticles have a wide range of applications in science and technology. Their sizes are often measured using transmission electron microscopy (TEM) or X-ray diffraction. Here, we describe a simple computer algorithm for measuring particle size distributions from TEM images in the presence of an uneven background. The approach is based on adaptive thresholding, making use of local threshold values that change with spatial coordinate. The algorithm allows particles to be detected and characterized with greater accuracy than using more conventional methods, in which a global threshold is used. Its application to images of heterogeneous catalysts is presented.

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1. Introduction
Nanoparticles are used in a wide range of applications as a result of their chemical, optical, magnetic, mechanical, thermal and electronic properties. They are frequently dispersed on oxides or on carbonaceous supports and are often used as active phases in heterogeneous catalysts. Their sizes are commonly linked directly to their catalytic activity [1], with different crystal nucleation and growth processes giving rise to different particle size distributions (PSDs). For example, the catalytic treatment of supported metals can lead to a change in metal surface area as a result of processes such as sintering, resulting in a decrease in exposed surface area and hence in catalytic activity [2].

PSDs are measured routinely in industrial environments, with the requirement that they should be statistically meaningful. The adsorption technique for the determination of metal particle size is based on the fact that over an appropriate temperature range certain gases such as ethylene, carbon monoxide, oxygen and hydrogen form a chemisorbed monolayer on the surface of transition metals. It is an easy and simple experimental technique. The surface of the metal area can be inferred from the amount of adsorbed gas in combination with the metal content of the supported catalyst, only if assumptions are made about the particle shape (normally assumed to be spherical or cubic) [3].

The two complementary techniques that are typically used are X-ray diffraction and transmission electron microscopy (TEM). X-ray diffraction can be used to provide averaged information from large numbers of particles, but the interpretation of the results can be difficult, especially if the particles are not single crystals. TEM measurements rely on the acquisition of images and subsequent digital processing from typically no more than a few hundreds of particles [4,5]. However, even using digital image processing tools, the quantification of the sizes and distributions of nanoparticles using TEM is difficult task. First, for supported catalysts, the particle sizes of interest are in the range of nanometers. The detection and analysis of small aggregates that are supported on amorphous or crystalline substrates is difficult, especially when the particle size approaches that of phase contrast arising from the support [6,7]. Second, this difficulty is exacerbated by the fact that the clusters may be present at different heights, they may be overlapped by other particles and the support itself may be thick or rough. Although the visibility of metal nanoparticles can often be enhanced by recording bright-field images slightly away from Gaussian focus, it is not possible to perform accurate size measurements from such images because the image resolution is then poorer. Inferences from bright-field TEM images are also complicated by diffraction effects, particularly as small particles possess large reciprocal-space shape functions that can be intersected by the Ewald sphere at large tilts from zone axes [8].

In order to provide statistically meaningful size distributions from TEM images, many particles should be analyzed. Manual segmentation of TEM images can be time-consuming because, in most cases, it is difficult to analyze an image locally to obtain only the desired information from the particles. In order to address these issues, we have developed a simple image processing algorithm.
that makes use of a locally varying threshold. Examples of its application to TEM images of heterogeneous catalysts are presented for particles that exhibit diffraction contrast and are supported on a substrate that has a complex three-dimensional morphology. Although only the analysis of bright-field TEM images is described here, the approach is equally applicable to the interpretation of high-angle annular dark-field images of supported nanoparticles.

2. Adaptive segmentation

Image segmentation is an essential preliminary step in most automated pattern-recognition and scene analysis problems. Segmentation is used to subdivide an image into its constituent regions, and its accuracy determines the eventual success or failure of computerized analysis procedures. TEM images are now usually acquired digitally, e.g., on 1024 × 1024 pixel arrays. Fig. 1 shows an example of a bright-field TEM image of Pt catalyst nanoparticles supported on carbon black, from which the detection, measurement and classification of the sizes of the particles are of interest.

The detection of particles in a TEM image is usually performed by thresholding the entire picture at once using a single “global threshold” value. Individual pixels in the image are then marked as “object” pixels if their value is greater (or smaller) than a chosen threshold intensity and as “background” pixels otherwise. This approach works well if all of the particles have a sufficiently different intensity from that of the background. However, it often fails because of local changes in intensity, as demonstrated by Fig. 1. Once a binary (thresholded) image is obtained, an opening operator (erosion followed by dilation) can be used to smooth the boundaries by removing small protrusions, to break narrow isthmuses and to remove regions that are smaller than the size of a chosen structuring element. Choosing the size of the kernel is possible to set a minimum size of particles to be detected. The image is then analyzed in order to measure and count the particles [9,10]. Unfortunately, in most cases of practical interest (e.g., Fig. 1), it is difficult to find a unique value for thresholding the entire picture correctly, and only a fraction of the particles in the image is outlined correctly in the binary image.

In order to address these issues, a method for improving the thresholding step before processing such images is described here. The method is based on an “automatic local thresholding” algorithm, which is applied to sub-regions of the image sequentially. An approach is already described in the literature [11,12]. The individual steps in the program include:

1. Selection of how many sub-divisions to use.
2. Cutting of sub-images from the original image (Fig. 2 shows how an experimental image can be subdivided for processing).
3. Thresholding of each sub-image. The output from this procedure is a binary image.
4. Opening (dilation plus erosion) with a chosen kernel size.
5. Combination and analysis of the processed sub-images. The resulting binary image is processed, particles counted and the boundaries are overlapped onto the original image to check the results.

The algorithm assumes that each sub-image to be thresholded contains two classes of pixels (e.g., foreground and background) and determines the optimal threshold automatically, in one of the several ways. The simplest way consists of (1) scaling the intensities in each sub-image and (2) selecting a threshold value equal to the median intensity range in each sub-image. These methods and a similar iterative approach, which is robust against noise, are described in [10]. After initial segmentation (e.g., using the mid-point between the minimum and the maximum intensities), the average of the intensities in each group of pixels is used to refine the threshold value. This procedure is repeated until the difference between successive threshold values is smaller than a pre-defined value. A more sophisticated approach, which is known as “Otsu’s method” can be used to determine the optimal threshold separating two classes of pixels so that the combined spread (intra-class variance) of the foreground and background pixels is minimized [13].

The algorithm was implemented using Matlab® software. A simplified version of the code is given in the Appendix. The algorithm requires only two input parameters: the number of sub-divisions and the size of the kernel used for the opening operation. In the examples described below, Otsu’s method was used for thresholding the sub-images, using the existing Matlab function graythresh().

Fig. 1. Bright-field TEM image of Pt catalyst nanoparticles on a c-support, illustrating the fact that local specimen thickness variations (e.g., between regions 1, 2 and 3) and diffraction contrast can affect the ease with which nanometer-sized metal particles can be detected and characterized.
Fig. 2 illustrates how to subdivide an image of Pt particles supported on a carbon black. In this example, $1 \times 1$, $4 \times 4$ and $30 \times 30$ square sub-divisions were chosen, with the right column showing the corresponding segmented particles. As the number of sub-divisions is increased, a greater number of particles are detected and outlined correctly. For the calculation of the particles size it was assumed the diameter of a circle with the same area as the particle. Fig. 3A and B show histograms of the measured particles sizes for $1 \times 1$ and $30 \times 30$ sub-divisions and best-fitting curves. The shapes of the PSDs are different. For no sub-division (using a global threshold) larger particles are detected (although they were not taken into account for fitting) and the number of particles analyzed are smaller. By contrast, when adaptive thresholding, the maximum measured particle size is smaller, the number of particles analyzed is greater, and most importantly, the shape follows the distribution expected for this type of sample. Fig. 3C shows the dependence of several statistical parameters (maximum size, number of counts, mean size and standard deviation) showing that as the number of sub-divisions is increased the number of particles analyzed increases, while the maximum size, the standard deviation and the mean size all decrease. When the size of the sub-division used is comparable to the particle size (in this example,
Fig. 3. (A, B) Histograms of PSDs measured from images (A) and (C) in Fig. 2. (C) Dependence of several statistical parameters on the number of sub-divisions used.

Fig. 4. Illustration of the application of global and local thresholds to the same bright-field TEM image of Pt nanocatalyst particles on a c-support. The curves show how the number of particles detected, their measured maximum and mean sizes and standard deviation vary with the number of sub-divisions used.
60 sub-divisions) the standard deviation and the number of counts both approach steady and apparently reliable values. However, Fig. 3C also shows the sensitivity of the measured parameters to the precise choice of the number of sub-divisions, and hence the care required even when using this approach.

Fig. 4 shows a second example of the application of the local thresholding algorithm, in which the same image is processed using both local and global thresholding. The same statistical parameters are plotted as a function of sub-divisions as in Fig. 3C. Although the improvement when using local thresholding is clear, Fig. 4 also illustrates the danger that even when the detection of the particles is optimised using the algorithm described above, some small and large particles remain undetected even when they can be distinguished by the eye. Also, some of the features that are detected occasionally do not correspond to particles. As a result, in order to improve the measurement of the PSD, the software was designed with an interactive user interface (Fig. 5), to allow squares with sizes selected by the user to be dragged to selected positions on the image in sequence and segmented using Otsu’s method. Alternatively, particles segmented already can be selected and deleted. This semi-automatic feature fully exploits the concept of adaptive thresholding, allowing the refinement of the results obtained using fully automatic division of the images. In Fig. 5, this interactive approach was used to detect Au particles supported on crystals of TiO2.

Although local thresholding is simple and powerful in itself, it can also be used as a first step before the application of more sophisticated algorithms that require seed points, such as watershed approaches or region-growing-based methods.

3. Conclusions

The measurement of particle size distributions from TEM images is often difficult, especially on an uneven background. Here, this problem is addressed by partitioning TEM micrographs into sub-images automatically and/or manually and segmenting them using an adaptive threshold referred to as Otsu’s method. Using this approach, images are analyzed with little human intervention and more accurately and objectively than when using a global threshold. As a natural extension of the concept, the results can be greatly improved by applying an adaptive threshold to interactively selected regions of the images.

Appendix

MATLAB® code for adaptive segmentation without interactivity.

%Selecting an image
[file,ruta]=uigetfile({‘*.bmp’;‘*.tif’},’Select a TEM image’)
cd(ruta);
fc=imread(file(:,:,1));

%Selecting subimages
[N,M]=size(fc);
ns=input(‘Number of divisions =’);
or=input(‘Minimum size =’);
x=fix(N/NS);
y=fix(M/NS);
for sx=1:x:N-x
for sy=1:y:M-y
sp=fc(sx:sx+x-1,sy:sy+y-1);
find_objects(sp,gsa,gsb);

%Thresholding
T=graythresh(sp);
spT=im2bw(sp,T);
g(sx:sx+x-1,sy:sy+y-1)=spT;
end
end

Fig. 5. Graphical user interface of a program that allows either automatic segmentation of an entire image or segmentation of user-selected regions with chosen sizes. The image shows particles of Au supported on crystalline TiO2 identified using the latter interactive approach.
g2 = imopen(imcomplement(g), strel('disk', 'or'));
[labeled, a] = bwlabel(g2, 4);
points = regionprops(labeled, 'Centroid', 'PixelList');
[B, L2, N2] = bwboundaries(labeled, 4, 'noholes');

% Draw segmented particles
imshow(fc);
hold on;
for s = 1:numel(points)
    boundary = B{s};
    if(s > a)
        plot(boundary(:, 2), boundary(:, 1), 'g', 'LineWidth', 1);
    else
        plot(boundary(:, 2), boundary(:, 1), 'r', 'LineWidth', 1);
    end
end
hold off

% Histogram
graindata = regionprops(labeled, 'basic');
areap = [graindata.Area];

% Draw segmented particles
imshow(fc);
hold on;
for s = 1:numel(points)
    boundary = B{s};
    if(s > a)
        plot(boundary(:, 2), boundary(:, 1), 'g', 'LineWidth', 1);
    else
        plot(boundary(:, 2), boundary(:, 1), 'r', 'LineWidth', 1);
    end
end
hold off

% Histogram
graindata = regionprops(labeled, 'basic');
areap = [graindata.Area];

References