The objective of this paper is to present the application of a computational method that was recently developed to perform segmentation, inference and classification of partially overlapped nanoparticles (UECS). Recent discoveries in nanotechnology show that shape and distribution of nanoparticles are the key aspects of their properties. Adequate and proper classification of nanoparticles is nothing more than a problem of nanometrology. The challenge is to develop new measurement techniques and standards in view of future technological advances. Results show that the UECS method is significantly more effective than previous work available in the literature.

Palavras-chaves: Nanometrology, Image inference, Overlapped nanoparticles
1. Introduction

The functional behavior of nanoparticles is strongly linked to the morphology of these particles and this field of research, according to Kelly et al (2003), has long been studied in physical chemistry. Therefore, a precise classification of synthesized particles according to their size and shape is essential to characterize them in a given material and, hence, to evaluate physical characteristics such as hardness, resistance, etc.

Some researches have been focusing on optical properties of nanoparticles for years. It is known that the presence of gold colloidal nanoparticles in a glass is responsible for the reddish tones in a window. On the other hand, silver nanoparticles produce a yellowish tone. The nanoparticles shape effect has also been studied in polymer nanocomposites. According to Knauert et al. (2007), nanoparticles can influence properties of a polymer by a variety of mechanisms, especially the tensile strength or viscosity of the material.

The importance of nanoparticles’ shape is also shown in health science studies. Pal et al. (2007) presented results that indicate the presence of a dependency between silver nanoparticles’ shape with gram-negative organism E. coli. Chithrani et al. (2006) shows the dependence of the size of gold nanoparticles in mammalian cells when related to the movement of these elements. The rate of delivery or the ability of these structures, seen through nanofunctional images, is also studied.

Despite its importance, there are only a few studies on the automatic measurement of the morphology of the nanoparticles (McFARLAND & DUYNE, 2003; GLOTOV, 2008; CHEN & HO, 2008 and FISCHER et al, 2000). In these studies, the underlying assumption is that the hidden boundaries of nanoparticles are circular or elliptical. This assumption limits the applicability of these methods. The assumption relies on the fact that the current paradigm for crystals formation is that nanoparticles in stable shape is convex (WORTIS, 1988). Convexity is understood as follow, in Definition 1. For further details about convexity, see Harary & Nieminen (1981).

**Definition 1:** For any \( x, y \in S \), \( S \) is a convex set if

\[
(1 - \alpha)x + \alpha y \in S, \forall \alpha \in [0,1]
\]

Recent discoveries in nanotechnology show that shape and distribution of nanoparticles are the key aspects of their properties. Adequate and proper classification of particles is nothing more than a problem of nanometrology. Nanometrology is a subfield of metrology, concerned with the science of measurement at the nanoscale level. Nanometrology has a crucial role in order to produce nanomaterials and devices with a high degree of accuracy and reliability in nanomanufacturing. The challenge is to develop new measurement techniques and standards in view of future technological advances.

According to Park et al. (2012), there are two major difficulties in the analysis of nanoparticles morphological classification. The first one is the overlap. Often, there is a great amount of overlap in micrographics. These overlays hide the boundaries of the particles, making the process of individual recognition hard. Consequently, separate particles overlapped and/or make proper inferences about the hidden contours are the key to a precise
morphological analysis. The second difficulty is the large number of elements in each micrograph. It is not unusual to see 300-700 nanoparticles in a typical 1024x640nm image. The high number of elements in this analysis differentiates this problem from the traditional problems of segmentation, which focus on recognizing only one or a small number of complex structures. Another challenge is to perform this classification and analysis of nanoparticles in a short run time. For this purpose the method should be computationally efficient.

Morphological analysis is presented with a promise to solve three highly technical problems: *the problem of image segmentation*, ie, separating the particles individually in an aggregate of overlapped particles; *the problem of the inference of the contours*, ie, the recovery of missing parts of the separated particles, and finally, *the problem of shape classification* which involves classifying particles according to their shape and size (PARK et al., 2012).

The closest approach to this method is used to solve the problem of segmentation of bio-cells, ie, segmentation of multiple cells. This problem involves the separation of a single cell from a group of cells. The classical approach to the problem is the use of methods of morphological segmentation, represented by the watershed method and its variations (MALPICA et al., 1997; TEK et al, 2005; CHENG & RAJAPAKSE, 2009). This methodology usually provides good results, but that does not hold for inferences about the hidden parts of contours. Hence, it does not serve the ultimate goal of the morphological analysis.

The literature shows that certain methods for active contour can, potentially, solve the three problems reported. However, Hodneland et al (2009) and Zhang & Matuszewski (2009) state that the current technology is time prohibitive for the use of existing methods. The time demanded for large number of nanoparticles analysis in a micrograph, visible in nanoscale, makes this a challenge.

Thus, the objective of this paper is to present the application of a computational method that was recently developed to perform segmentation, inference and classification of partially overlapped nanoparticles (PARK et al., 2012). Results show that this method is significantly more effective than previous work available in the literature. The algorithm by Park et al. (2012) runs in two stages. The first stage solves the segmentation problem by decomposing the cluster particles. The second stage addresses, together, the problem of inference on the hidden contours and shape classification. The description of the algorithm is presented in section 2. Section 3 provides comparative results among the method used and the ones reported in the literature. Section 4 shows results of an application of the method to images of nanoparticles found on specialized papers available on the Internet and Section 5 presents final remarks and indicate future areas for research.

2. Segmentation, inference and classification of partially overlapping nanoparticles algorithm

This approach consists of a segmentation, inference and classification of nanoparticles method in two phases or stages. At first, to segment a large cluster of overlapping nanoparticles and, then, the boundaries through B-splines having multiple forms as guide can be built. In doing so, it is not necessary to coordinate hundreds of contours simultaneously ensuring better computational efficiency than the methods of multi-phase active contour proposed by Hodneland et al. (2009) and Zhang & Matuszewski (2009). The innovation of this method is the modification of existing morphological erosion process. Convex theoretical
justification for this separation capacity can be found in Park et al. (2012). Furthermore, the method of association between the edge segments and nanoparticles markers so as to construct evidence to delineate the individual objects was developed. Ultimately, these inputs are used as evidence to adjust contours using B-splines guided by multiple forms of reference (boundaries inference) as well as determine which best adheres to the evidence (shape classification).

2.1. Stage one

According to theory, nanoparticles are inclined to be convex (Wortis, 1998). Thus, in the first stage it is necessary to deal with the problem of convex decomposition. This segmentation task can be accomplished in two steps: find the markers of each individual particle and obtain the evidence to delineate the convex boundary of each piece individually.

Suppose you have a set of overlapping objects in a binary silhouette. Each binary silhouette can be easily obtained because micrographs have a high signal-noise rate (Figure 1b). Once nanoparticles have only convex morphology, the binary silhouette can be defined as the union of individual convex nanoparticles.

One possible way to obtain markers for each particular object is to use the method of morphological erosion, which consists in applying a set of subtraction operators called Minkowski, according to the mathematical morphology theory. A repeated application of this operator disconnects the junctions of overlapping objects (Figure 1c). One can apply this technique to each object a step before it is completely removed. Thus, one can accurately identify a marker for each nanoparticle, assuming that each one is convex and a nanoparticle is not fully contained in the other. In order to avoid over-segmentation, where the same nanoparticle has more than one marker, a second criterion was created for interrupting the replication of morphological erosion process. This criterion consists to compare the size of nanoparticles, as defined by Rosenfeld (1985, p. 72), with an arbitrary threshold. Results showed that for values among 0.2 and 0.3 are obtained good results in real nanoparticles images.

Since the markers have been obtained, each and every mark edge (pixels) is extracted from the image, which are associated with the markers previously identified. This edge-association brand label is used as evidence of contour. The association occurs through a distance measure.
and consists of two elements: the distance between the mark and the edge marker and the direction of the gradient vector of the mark edge (Note that the assumption here states that the nanoparticle is convex). An example of this procedure can be seen in Figure 2.

2.2. Stage two
Suppose you have a set of boundary marks, continuous or not, for each marker. The literature shows that previous studies consider only one type of a predetermined reference. However, consider that a nanoparticle can present several different shapes (all convex) and you want to perform, with multiple forms of reference simultaneously, the inference about contours overlaid and shape classification.

The contour of a nanoparticle can be assumed to be a uniform periodic B-spline curve of order $d$ with $p$ checkpoints, for $t \in [0,1]$.

$$f_i(t) = \sum_{h=0}^{p-1} \phi_{h,d}(t)p_{i,h},$$  

(1)

where $t$ is the parameter to identify a point on the curve, $\phi_{h,d}$ is the $h$th periodic B-spline, and $p_{i,h} \in \mathbb{R}^2$ is the $h$th control point. Suppose that $e_{i,j}$ is a noise observation of $f_i(t)$ at a B-spline parameter value $t_{i,j}$, i.e.

$$e_{i,j} = f_i(t_{i,j}) + \varepsilon_{i,j}, \varepsilon_{i,j} \sim \mathcal{N}(0, \sigma^2 I_2),$$  

(2)

where the parameter value $t_{i,j}$ is unknown and it needs to be estimated. It lies in a two-dimensional problem of parameter estimation. The procedure suggests by Park et al (2012) is as follows: given a set of contour evidences for a set of markers $\{e_{11}, e_{12}, \ldots, e_{im}\}$

a) Find a convex hull inscribing the contour evidences by the Qhull algorithm (BARBER et al, 1996);

b) Sequentially order all points on the convex hull in counter-clock wise (or clock-wise) into $q_0, \ldots, q_t$ and then parameterize the points by the chord-length parameterization. The parameter $t_1$ for $q_1$ is assigned as
The biggest advantage of this parameterization is its simplicity and computational efficiency. Moreover, the parameterization is not affected by existing noise on the evidence located within the nanoparticle. For further details about chord-length parameterization, see Epstein (1976).

### 3. Comparative Study

This section shows a comparison between the method proposed by Park et al. (2012) – called UECS, and four state-of-the-art methods: marker-controller watershed segmentation – MCWS (MALPICA et al, 1997), normalized-cut – N-Cut (SHI & MALIK, 2000), multi-phase active contour – MPAC (VESE & CHAN, 2002) e sliding band filter – SBF (QUELHAS et al., 2010). We chose real micrographic images obtained by electronic synthesis of gold nanoparticles, subdivided into groups according to different levels of overlap. In low-degree levels, nanoparticles have virtually no overlap. In the medium-degree level, nanoparticles are overlapping but not to the point of being contained one in another one, which is bound to all methods. The highest level of overlap violates the assumption that the nanoparticles in one cluster are not completely hidden, or are completely contained one in another.

Table 1 shows the average deviation from the number of nanoparticles recognized by each method previously mentioned and the actual number of nanoparticles in each of the 12 images was analyzed. It is observed that the proposed method has a lower average deviation than other methods, that is, on average the observed errors in the counting method are smaller than the other methods observed. Furthermore, the results demonstrate that the deviations are less variable than others, showing that the method is also more consistent.

The UECS method correctly identified one marker per particle for most cases. In some cases, with high level of overlap, the UECS method received a single marker for more than one nanoparticle. Despite the association between markers and the evidences of contour presenting satisfactory results, sometimes noises were incorrectly classified as valid evidence of boundary. The results of the inference procedure also showed satisfactory results, when analyzed against the original image.

<table>
<thead>
<tr>
<th>Overlapping level</th>
<th>Number of images</th>
<th>Number of particles per image</th>
<th>Average Absolute Deviation (±std. dev.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>UECS</td>
</tr>
<tr>
<td>Low</td>
<td>3</td>
<td>76, 307, 28</td>
<td>2.67 (±4.62)</td>
</tr>
<tr>
<td>Moderate</td>
<td>6</td>
<td>28, 52, 459, 19, 108, 29</td>
<td>6.50 (±7.69)</td>
</tr>
<tr>
<td>High</td>
<td>3</td>
<td>63, 44, 45</td>
<td>10.33 (±1.53)</td>
</tr>
</tbody>
</table>

Source: Determined from the results of Park et al. (2012)

In summary, when compared with other methods, the UECS method showed better performance than the others. Figure 3 shows an example of analysis made by the five different methods. It can be clearly observed sub-segmentation in MPAC and N-cut methods,
as well as over-segmentation in MCWS method due to their sensitivity to noise. The estimation of the contours through the SBF method is still crude, but fitting a curve can smooth this. SBF was also unable to classify some particles.

![Image](image.png)

Source: Park et al. (2012)

Figure 3 – Results of inference about the contours

Considering the accuracy in the shape classification, the UECS method was compared with a human rate. Some incorrect classifications were observed. These errors, according to authors occur due to lack of evidence of contour and can be solved by a more efficient parameterization system. Nevertheless, the method presents a robust classification ability.

4. Application over images from Internet papers

In order to illustrate the results obtained by the method, we selected two images of nanoparticles obtained by transmission electron microscopes (TEM), published in an article available on the Internet (GRESH et al, 2011). The images treated are showed in Figure 4.

It is observed that the system produces satisfactory results in the segmentation and contours inference of nanoparticles, especially in a low level of overlapping. It is observed that, near the limits of the image, there is the incidence of major failures in the nanoparticles characterization, in particular when they are limited by the edge image. It is expected due to lack of edge markers in this context according to Park et al. (2012).

![Image](image.png)

Figure 4 – Results of contours inference in two images from Gresh et al (2011)

The statistical results determined by the algorithm are shown in Table 2. This type of result can estimate not only the average size of the nanoparticles but also to determine its distribution. It is also useful to check stability for a given material through the distribution of
the nanoparticles. The consequence of being able to measure/estimate these aspects is the ability to predict the physical characteristics of the materials during the manufacturing process resulting in more control over what is being produced and on some of the physical characteristics of the finished product.

5. Final considerations

The main contribution of the proposed algorithm to the area of nanotechnology is its efficiency related to cost in the classification of nanoparticles. The costs of using this system is restricted to only obtain images through TEM, and software license to run the Matlab code, which certainly minimizes the high costs resulting from the use of sophisticated equipment. The characterization, as the shape and size of nanoparticles is obtained in a much more efficient and consistent manner when compared with other computational procedures such. The findings also showed that the method presents higher reliable results.

It is noteworthy that, according to the authors, through a more robust method of parameterization, a substantial gain in performance can be obtained from this tool. This also is indicative of the authors for future research. The implementation of a logical test in the algorithm to deal better with the borders of the images can also be implemented to correct faults identified in these portions, and consequently, improve the average performance overall.

<table>
<thead>
<tr>
<th>Nanoparticle number</th>
<th>Size (pixels)</th>
<th>Shape</th>
<th>Centroid point (x,y)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>1</td>
<td>41.573</td>
<td>Rectangle</td>
<td>25.125</td>
</tr>
<tr>
<td>2</td>
<td>45.577</td>
<td>Circle</td>
<td>48.226</td>
</tr>
<tr>
<td>3</td>
<td>47.864</td>
<td>Circle</td>
<td>80.692</td>
</tr>
<tr>
<td>4</td>
<td>44.359</td>
<td>Triangle</td>
<td>96.657</td>
</tr>
<tr>
<td>5</td>
<td>10.036</td>
<td>Rod</td>
<td>112.350</td>
</tr>
<tr>
<td>6</td>
<td>40.807</td>
<td>Triangle</td>
<td>145.250</td>
</tr>
<tr>
<td>7</td>
<td>47.528</td>
<td>Circle</td>
<td>176.440</td>
</tr>
<tr>
<td>8</td>
<td>57.447</td>
<td>Circle</td>
<td>204.370</td>
</tr>
<tr>
<td>9</td>
<td>49.203</td>
<td>Rod</td>
<td>189.580</td>
</tr>
<tr>
<td>10</td>
<td>35.009</td>
<td>Circle</td>
<td>217.220</td>
</tr>
<tr>
<td>11</td>
<td>45.287</td>
<td>Circle</td>
<td>215.310</td>
</tr>
<tr>
<td>12</td>
<td>72.787</td>
<td>Rod</td>
<td>278.840</td>
</tr>
<tr>
<td>13</td>
<td>39.960</td>
<td>Circle</td>
<td>292.610</td>
</tr>
<tr>
<td>14</td>
<td>38.630</td>
<td>Circle</td>
<td>314.180</td>
</tr>
<tr>
<td>15</td>
<td>43.545</td>
<td>Circle</td>
<td>325.860</td>
</tr>
<tr>
<td>16</td>
<td>38.806</td>
<td>Circle</td>
<td>364.220</td>
</tr>
<tr>
<td>17</td>
<td>31.404</td>
<td>Circle</td>
<td>401.560</td>
</tr>
<tr>
<td>18</td>
<td>45.349</td>
<td>Rod</td>
<td>405.550</td>
</tr>
</tbody>
</table>

Table 2 - Descriptive Results obtained by UECS algorithm for image 1 of Figure 6

Some constraints on the code, eg, the assumption that the intersection among more than two must be empty shows that there is still space for improvements. While there is not an automated solution, a semi-automatic procedure must be useful and certainly would ensure greater accuracy of results. Similarly, the image quality can generate misclassification. The code provides application of additional filters but this option still needs more documentation.
From the point of view of nanometrology, the results represent advancement in the field of measurement and shape classification of nanoparticles. Of course, discounting the errors of measurement and inference, it is certainly an important step in the process of pattern generation. These patterns can be studied as to their physical characteristics with low relative cost. This will lead to the best control of production, and consequently, higher quality aggregate products.

Acknowledgements

The author would like to acknowledge generous support of its sponsor. The author would also like to thank Dr. Chiwoo Park (Florida State University), Dr. Jianhua Z. Huang and Dr. Yu Ding (Texas A & M University) for having kindly provided the Matlab code of the algorithm, images, preliminary results, as well as allowing the use of all this information in the production of this paper. The author would like to thank Alan Sonntag too, for English review on this paper.

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