



ENM TUTORIALS

Image descriptor calculation web tool

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MAIN AUTHOR:	Philip Doganis, Marios Kotsiandris
PARTNER:	NTUA
CONTACT DETAILS:	filippos@mail.ntua.gr +30 210 772 3236
AUTHORS:	Philip Doganis, Marios Kotsiandris



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1. INTRODUCTION

The images taken by Electron or Fluorescence microscopy are valuable sources of information that depict the actual structure and shape of the nanomaterial. To this end, we introduce a web tool, which provides the user with a systematic framework for the automated analysis of microscopy images of nanomaterials and the calculation of nanoparticle descriptors. The term *Descriptors* or factors has been adopted to refer to significant properties of nanoparticles, as they describe not only the morphology of the nanoparticle but also its behavior. The only data someone can extract from digital images are relevant to the shape, size and color of the nanoparticle. Nanoparticles come in a variety of sizes and shapes, ranging from simple to highly complex. As such attributes may have great influence on their functionality, some characteristic measurements of nanoparticle size should be defined in order to achieve proper characterization of size and shape.

The scope of this tutorial lies mainly in providing a guide to members of the nanotoxicology community that wish to derive nanomaterial descriptors from microscopy images of nanomaterials using the image descriptor calculation web tool presented here.

The web application has been developed in Java and is based on the open source ImageJ software. Its input consists of microscopy nanomaterial images, as well as user-defined parameters regarding the choice of applied filter and descriptors to be calculated. The output comprises a table with the calculated descriptor values for each particle and the average value for all particles of the image, which can be produced in various easily transferable formats. It should be noted here that image descriptor calculations will also be made available as RESTful web services that can be accessed from systems of any architecture that follows the tool's RESTful API. The user interface is hosted in the following address: <http://app.jaqpot.org:8880/imageAnalysis/>. The source code can be found at: <https://github.com/enanomapper/imageAnalysis>.

For the sake of brevity, technical details on the programming and additional remarks on the functionality of the tool were omitted but will be included in Deliverable 4.2 of eNanoMapper (which will be made publicly available at <http://enanomapper.net/>), alongside with a further analysis of features.

Currently, the applicability of the tool covers gold and metal oxide nanoparticles, but the application can be easily extended to cover other types of nanomaterials, such as carbon nanotubes. The tool is released with a user interface and has been made available as a web service, thus allowing its integration into larger applications, such as the eNanoMapper Computational infrastructure, and facilitating the utilization of the produced descriptors for nanoQSAR modelling.

The descriptors calculated will be described next, followed by a presentation of the application workflow.

2. APPLICATION DETAILS

2.1. DESCRIPTORS – STANDARD AND CUSTOM

The descriptors currently implemented in this application are provided by ImageJ¹ or have been added by us based on current bibliography¹. In order to provide a better breakdown of the choices provided in this application, a list of the descriptors and a small analysis for each of them is presented¹. A small reminder for the following list would be that all measurements are focused inside the nanoparticles. That being said, the “selection” mentioned below currently refers to the nanoparticle itself:

- **Area:** The area of selection in square pixels. This can be provided in calibrated units if a calibration of the image is provided.
- **Bounding Rectangle:** The smallest rectangle enclosing the selection. Uses the headings BX, BY, Width and Height, where BX and BY are the coordinates of the upper left corner of the rectangle.
- **Centre of Mass:** This is the brightness-weighted average of the x and y coordinates all pixels in the image or selection. Uses the XM and YM headings. These coordinates are the first order spatial moments.
- **Centroid:** The central point of the selection. This is the average of the x and y coordinates of all of the pixels in the image or selection. Uses the X and Y Results table headings.
- **Feret Diameter:** The longest distance between any two points along the selection boundary, also known as maximum caliper. Uses the Feret heading. FeretAngle (0-180 degrees) is the angle between the Feret diameter and a line parallel to the x-axis of the image. MinFeret is the minimum caliper diameter. The starting coordinates of the Feret diameter (FeretX and FeretY) are also displayed. The DrawFeretDiameter macro draws the Feret diameter of the current selection.
- **Fit Ellipse:** Fit an ellipse to the selection. Uses the headings Major, Minor and Angle. Major and Minor are the primary and secondary axes of the best fitting ellipse. Angle (0-180 degrees) is the angle between the primary axis and a line parallel to the x-axis of the image. The coordinates of the centre of the ellipse are displayed as X and Y if Centroid is checked. Note that ImageJ cannot calculate the length of either the major or the minor axis if Pixel Aspect Ratio in the Set Scale dialog is not 1.0.
- **Integrated Density:** Calculates and displays two values: "IntDen" (the product of Area and Mean Grey Value) and "RawIntDen" (the sum of the pixel values in the image or selection).

"RawIntDen" is only available in ImageJ 1.44c or later. "IntDen" and "RawIntDen" values are the same for uncalibrated image. The Dot Blot Analysis example demonstrates how to use this option to analyse a dot blot assay.

- **Kurtosis:** The fourth order moment about the mean.
- **Mean Grey Value:** Average grey value within the selection. In this value the sum of all pixels within the selection is divided by the count of pixels. If the image is not in grey scale then a transformation is made according to the following formula: $grey = 0.299red + 0.587green + 0.114blue$.
- **Median:** The median value of the pixels in the image or selection.
- **Min and Max Grey Levels:** The highest and lowest grey scale values appearing in the selection.
- **Modal Grey Value:** Stands for the most frequently occurring grey value within the selection.
- **Perimeter:** The length of the outer boundary of the selection.
- **Shape Descriptors (previously Circularity):** Calculate and display the following shape descriptors: Circularity, Aspect Ratio, Roundness and Solidity.
- **Skewness:** The third order moment about the mean. The documentation for the Moment Calculator plugin explains how to interpret spatial moments.
- **Standard Deviation:** The deviation of the grey values used to count the mean grey value.

A number of additional descriptors proposed in the literature (Gajewicz et. al., 2015, please also see Table 4, D4.1) have also been included in the application (porosity, sphericity, surface diameter, equivalent volume to surface, equivalent volume diameter). Some of these features (sphericity, equivalent volume to surface, equivalent volume diameter) use the **volume** of nanoparticles as a common base. It is well established that the automated calculation of volume from two dimension images not feasible in most cases and can only be performed when objects have specific angles. Therefore, the volume-related descriptors use **estimations** rather than **calculations** of volume. This estimation is based on the following assumption: *Given the circularity of an object, when it surpasses a defined threshold, it can be assumed that it tends to become a circle. In the opposite case it can be assumed that it tends to become a cylinder.* The threshold has been set empirically and is open to discussion and optimization. After characterizing a nanoparticle as circular or cylindrical, a volume calculation is made. If a nanoparticle is characterized as circular, the Feret diameter of the nanoparticle is used to calculate an equivalent sphere volume. In the opposite case, a cylinder equivalent volume is calculated in similar fashion. Considering the above explanation, the custom volume based descriptors are the following: Volume, Volume to Surface, Sphericity, Surface Diameter, Volume Diameter.

2.2. FILTERS

Five predefined filters for grey images have been included in the application: Huang, Triangle, Internodes, Isodata and IJ_IsoData (for more information, please consult ImageJ User Guide v1.46 by Ferreira&Rasband). Figure 1 shows the resulting images that occur after the application of three different filters to an original image (top left). It can be seen that these filters have clarified the elements in the image and can thus assist in the identification of distinct nanoparticles or agglomerates.

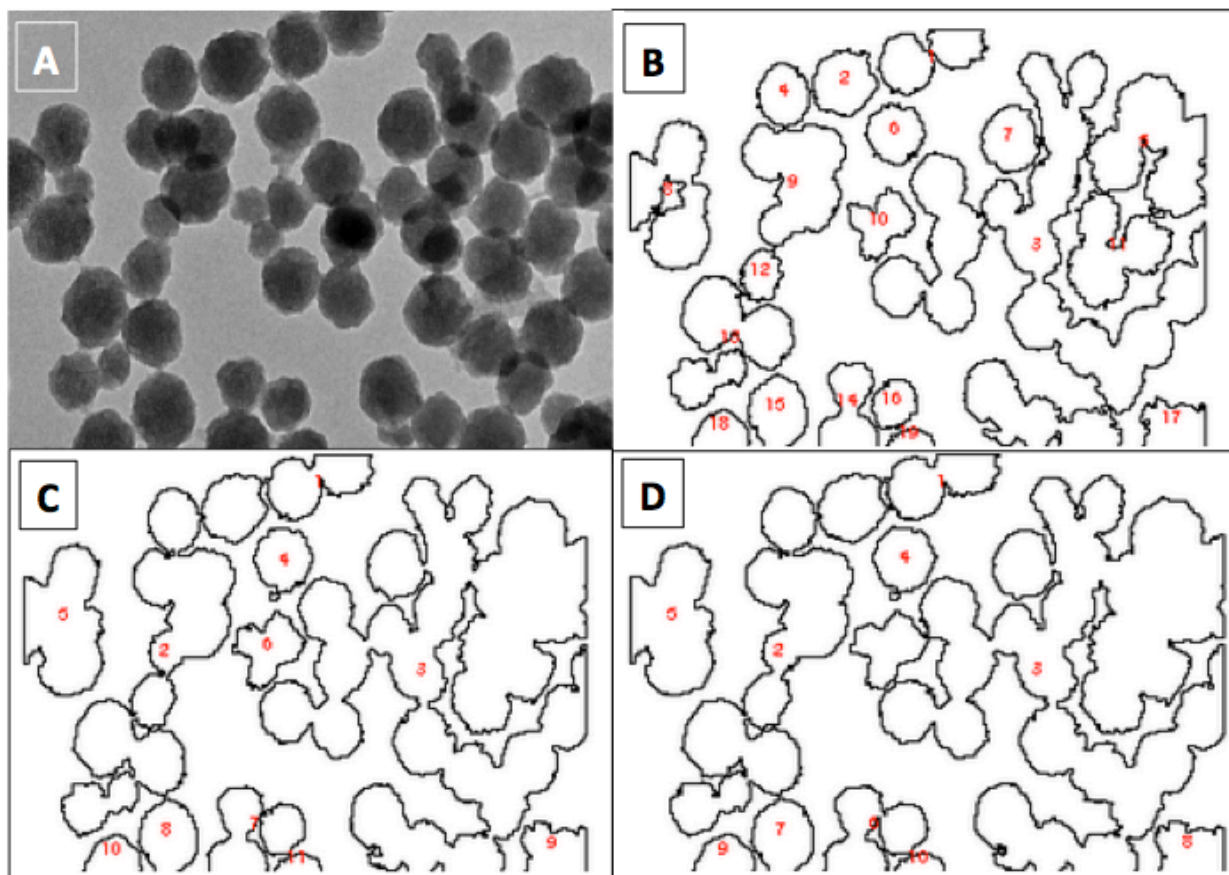


Figure 1 A: Original TEM image. Images with identified numbered particles after application of filter: B: Huang, C: Intermodes, D: Isodata. Image from Taylor-Pashow et. al. 2011.

2.3. APPLICATION WORKFLOW

The following paragraphs host a more detailed presentation of the workflow of the application. For each stage, information about its components and functionality will be provided.

The first page of the application is an index page (as presented in Figure 2). This index page is written in XHTML and includes the following actions:

1. Select a filter
2. Select full image analysis or identification and analysis of nanoparticles
3. Select the descriptors to be calculated
4. Upload a file and show preview of the processed file
5. Submit this form and receive the results

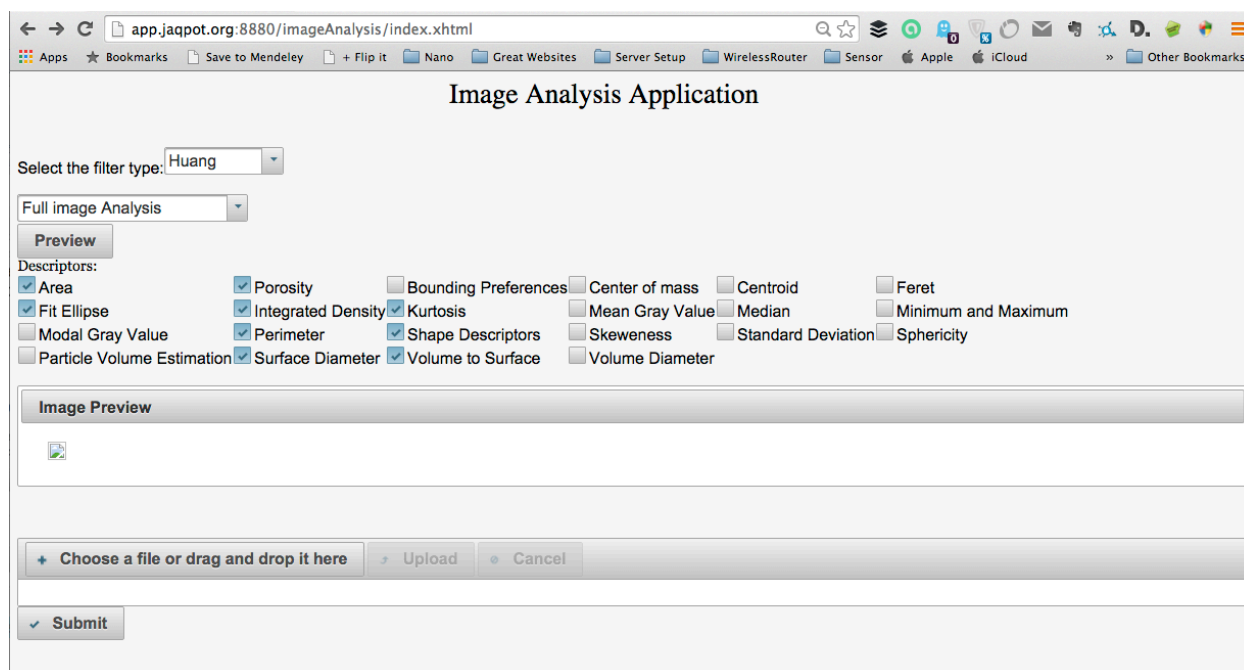


Figure 2-Application Index page

2.3.1 FILTER SELECTION

In this step, the user may select a filter among the five options mentioned in Section 2.2. Initially when a user selects a different threshold, a new image preview with the applied threshold is presented. This automation was made in order to provide the user with a direct presentation of the changes with the different selection.

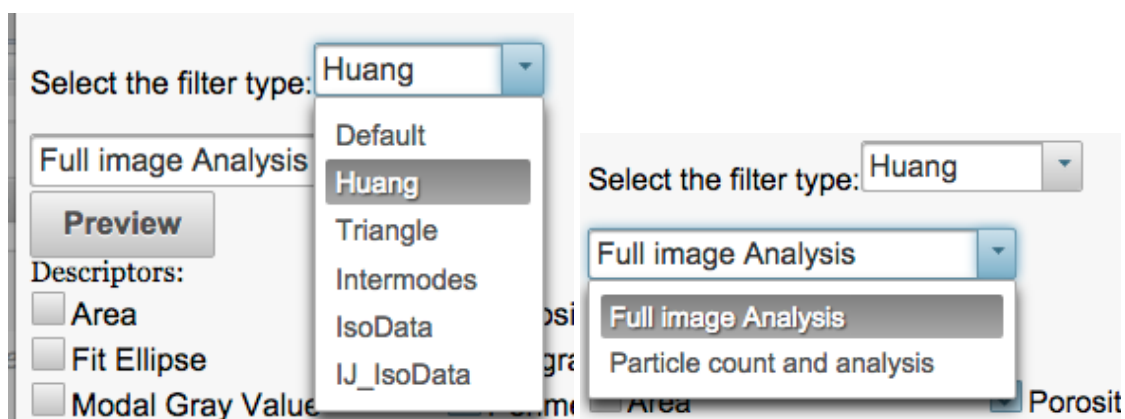


Figure 3- Filter and type of analysis Selection Fragment

2.3.2 CHOICE OF TYPE OF ANALYSIS

The user has two choices:

- **Full Image Analysis**
Analysis is performed on the entire image
- **Particle count and analysis**
The tool identifies individual nanoparticles and performs analysis on each one of them. In this case, results are given for each particle and for a virtual "average" particle that contains the average value of each property for all particles.

2.3.3 DESCRIPTOR SELECTION

In the next step, a user should select the desired measurements (descriptors). In order to enable this functionality a *Measurement Model* was created that contains an index with all the descriptors (default and custom) and each descriptor is assigned a unique key. A user can select multiple descriptors by clicking the relevant checkboxes. During the submission of the form, these selections are saved and mapped with the values of the default measurements; finally, a total measurement result is returned. In the following picture the list of available properties is shown, as they appear on the index page.

<input type="checkbox"/> Area	<input type="checkbox"/> Porosity	<input type="checkbox"/> Bounding Preferences	<input type="checkbox"/> Center of mass	<input type="checkbox"/> Centroid	<input type="checkbox"/> Feret
<input type="checkbox"/> Fit Ellipse	<input type="checkbox"/> Integrated Density	<input type="checkbox"/> Kurtosis	<input type="checkbox"/> Mean Gray Value	<input type="checkbox"/> Median	<input type="checkbox"/> Minimum and Maximum
<input type="checkbox"/> Modal Gray Value	<input type="checkbox"/> Perimeter	<input type="checkbox"/> Shape Descriptors	<input type="checkbox"/> Skewness	<input type="checkbox"/> Standard Deviation	<input type="checkbox"/> Sphericity
<input type="checkbox"/> Particle Volume Estimation	<input type="checkbox"/> Surface Diameter	<input type="checkbox"/> Volume to Surface	<input type="checkbox"/> Volume Diameter		

Figure 4-Measurement Selection Fragment

DESCRIPTOR MAPPING

In addition, due to the fact that more than one default descriptors are implemented and used inside the application, a descriptor mapper that matches the default and custom descriptors with user friendly names was created. This mapper is responsible to handle dependencies between separate descriptors. For example, when a user selects the volume descriptor that has a direct dependency to the Feret and Circularity descriptors, the mapper automatically changes the selected measurements in order to have complete data during the result process.

2.3.4 FILE UPLOAD AND PREVIEW

In this step, a user is able to upload a file to the server. This component provides not only the ability to open a file browser on the clients system, but also a client side validation of the files selected. Only image files (".png", ".gif", ".jpg" and ".jpeg") are accepted to be uploaded. Another main constraint is on the size limit of uploaded files, which is limited to a maximum of 10 MB, in order to avoid uploading huge files, which may create a traffic impediment for the server. When a file is selected for upload, an input stream buffer opens and begins to convert the file into a byte stream structure. During the final stage and if no problem or exception has appeared, a message is presented to the user informing him of the state of the file.

A TEM image of 30nm gold nanoparticles shown in Figure 5 was used as an example. The image was provided by the authors of Walkey et.al. 2014 and is available at http://app.jaqpot.org/imageRepo/10.1021_nn406018q/30nmWalkey.png.

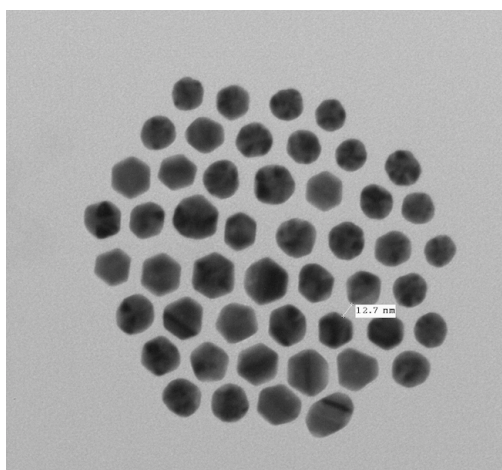


Figure 5-Original Image (Nanoparticles, 30nm, Gold)

The application of the Huang filter results in the preview shown in Figure 6:

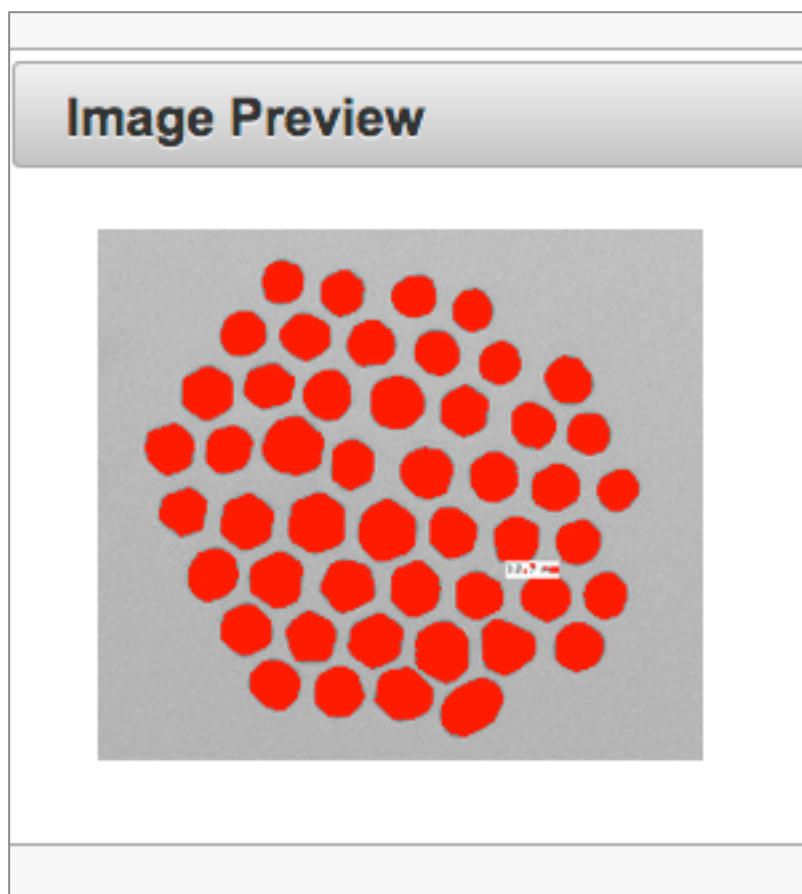


Figure 6 Image Preview with Huang filter

2.3.5 RESULTS PAGE

In this example the “Particle count and analysis” option and the full list of descriptors were chosen. After clicking on the “Submit” button, the image calculation process commences. During the process, selected measurements, filter and image files are fed into the Process helper. The results are saved to a CSV file and a parser transforms the CSV file into a list of Java models. This list is stored inside a Result model, which is passed through the session from the index page to the result page. The processed image and statistical data are also saved in the result model. The last command executed in the managed bean responsible for the index page is the redirection to the result page.

When the page has finished loading, the output of the processed image is shown at the top (Figure 8). Each nanoparticle recognised is shown with a red border and a unique counting number (index) within it. The image is shown in full scale for two main reasons. Firstly, scaling the image would make it impossible to display the nanoparticle numbers, which are important for the user. Secondly, a detailed image in the same dimensions as the original would allow the user to compare the two images.

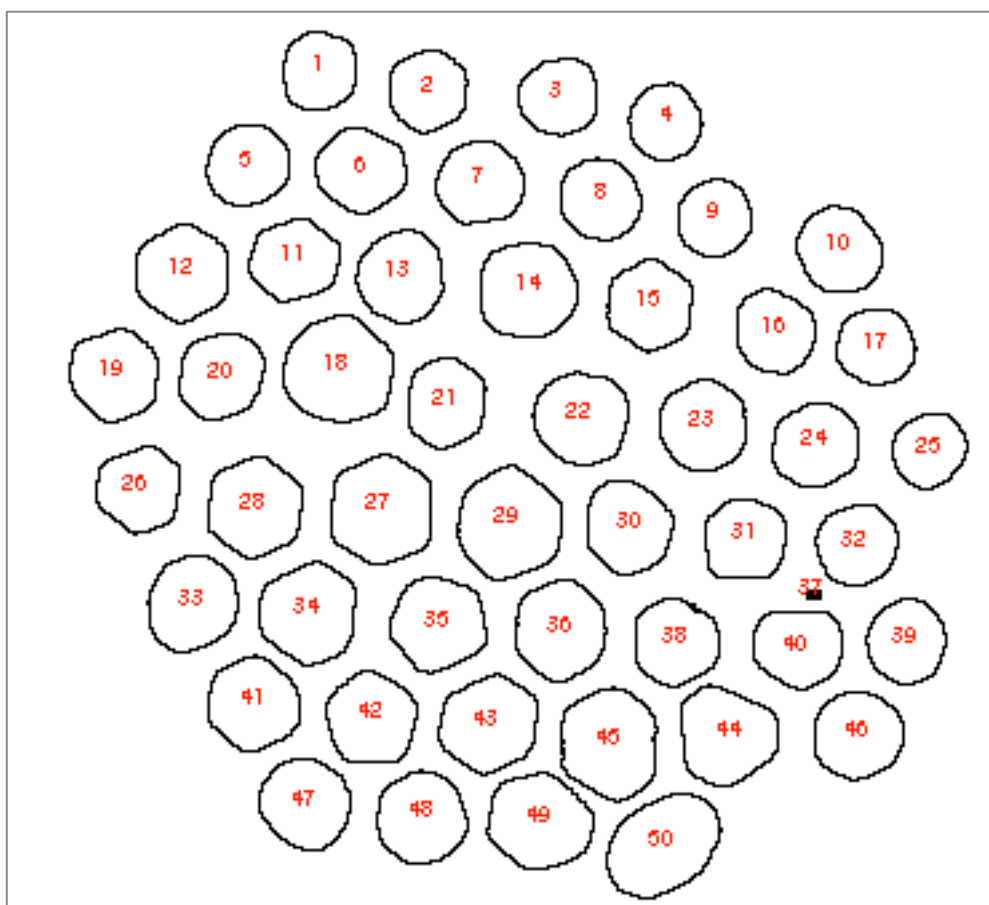


Figure 7-Nanoparticle outlines with index numbers, after image processing with Huang filter

Below the image, lies the result table (Figure 8). Rows of the data table correspond to the distinct nanoparticles that were identified in the image, whereas on the first row, a statistical virtual nanoparticle is shown, created by dynamically calculating the average value of each column.

List of Properties					
id	Min Feret	Feret Angle	Feret X	Feret Y	Area
Average Particle	31.235773	92.364944	194.8	186.08	845.32
1	27	37.235	114	46	651
2	28	77.276	164	60	684
3	28.771	159.228	201	42	663
4	26	76.430	252	70	595
5	30	31.264	86	81	730
6	30.866	163.610	125	69	803
7	30.420	11.976	170	82	784
8	29.698	143.842	218	76	710
9	27	68.552	270	106	620
10	30.423	142.125	308	93	784
11	29.120	16.390	100	112	774
12	34	82.304	74	131	957
13	32.556	80.272	156	131	856
14	35	152.103	188	108	1036
15	31.666	29.539	235	132	807
16	29	128.928	286	121	718
17	28	148.392	323	130	643
18	40	147.426	116	138	1262

Figure 8-Results table with calculated image descriptors

4. ACKNOWLEDGMENTS

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5. REFERENCES

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6. KEYWORDS

Enter tutorial's keywords