

## COMPUTATIONAL APPROACH FOR PARTICLE SIZE MEASUREMENT OF SILVER NANOPARTICLE FROM ELECTRON MICROSCOPIC IMAGE

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### ABSTRACT

**Objective:** To develop a simplified algorithm for determining the size and shape of the silver nanoparticles (AgNP) from Electron microscopic (EM) images using MATLAB.

**Method:** We examined the particle size of AgNP from the Transmission electron microscopic and Scanning electron microscopic image with uneven background. This approach based on image processing techniques in MATLAB.

**Results:** The user friendly Graphical User interface (GUI) tool was developed using MATLAB to measure the size of every particle from EM image. The mean particle size of AgNP calculated using this GUI tool by analyzing the N number of particle in the image.

**Conclusion:** This algorithm allows to measure the size of nanoparticles successfully were difficult to analyse by electron microscope with lack of in-built software. The step by step process leads to optimize the process of nanoparticles synthesis and facilitate to generate the needed statistical analysis for medical and other applications.

**Keywords:** Image processing, SEM, TEM, MATLAB.

### INTRODUCTION

In last few years, the production and the application of nanoparticles have been increasing extensively due to their unique physical and chemical properties. Nanoparticles with a diameter of about 100 nm are widely used as a carrier in target drug delivery application, as a biomarker in disease diagnosis and various medical fields [1-3]. Recent concerns have raise about health significance of nanoparticle and their related products. Since, the properties of the nanoparticle are directly dependents on their size and shape [4], and the particle size is desirable for the stability of nanoparticle [5]. Therefore, the accurate measurement of nanoparticle size distribution is essential for the quality and safety of Nano product. The nanoparticles size are measured by a complementary techniques like X-ray diffraction (XRD) and transmission electron microcopy (TEM), which provide the average information from a large number of particle instead of a single crystal leads to difficulty in result interpretation [6].

MATLAB is an advanced procedural language and user friendly environment for data analysis and mathematical computing function. MATLAB and the Image Processing Toolbox afford a wide collection of advanced image processing functions and interactive tools for enhancing and analyzing digital images. The toolbox functions implemented in the open MATLAB language is used to build up the customized algorithms [7].

The MATLAB figure makes the observer to improve and expand their article by setting interactive figures. It ensures a way to highlight their result in an image and easy to understand the significance of a research paper quickly. It helps to visualize research data for in-depth, and to use the primary data [8].

The Image processing techniques are easy to implement complex image process, mainly for fast prototyping. On the rear, it is difficult to understand how image processing works on hidden black boxes and make things more complicate truly. The following process like analysis, enhancement, and visualization is performed in each stage of image processing techniques [9]. In this article, we have reported a simplified algorithm for determining the size of the nanoparticles from electron microscopy (EM) image to provide statistical information from the SEM and TEM image using the GUI measure tool.

### MATERIALS AND METHODS

#### Synthesis and characterization of Sliver nanoparticles

Briefly, silver nanoparticles (AgNP) is synthesized by interacting  $10^{-2}M$  of silver nitrate ( $AgNO_3$ ) with *Citrus lemon* extract containing citrate as main reducing agent in the ratio of 1:4 for 4 hrs [10]. The broth containing AgNP was centrifuged at 10,000 rpm for 10 mins, and pellet obtained was redispersed in Millipore water and stored at  $4^{\circ}C$ . Thus, the obtained AgNPs Surface plasmon peak was monitored by UV-vis Spectra (Hitachi, U-2800) range from 200 nm to 800nm. The morphology of synthesized AgNPs were determined by Scanning Electron Microscopy (FEI sirion, Netherlands) at voltage of around 10KeV and Transmission Electron Microscopy (TEM) by placing a drop of diluted solution on copper grid at each regular interval of about 1 hour. The transmission electron micrographs were taken using Tecnai G-10.

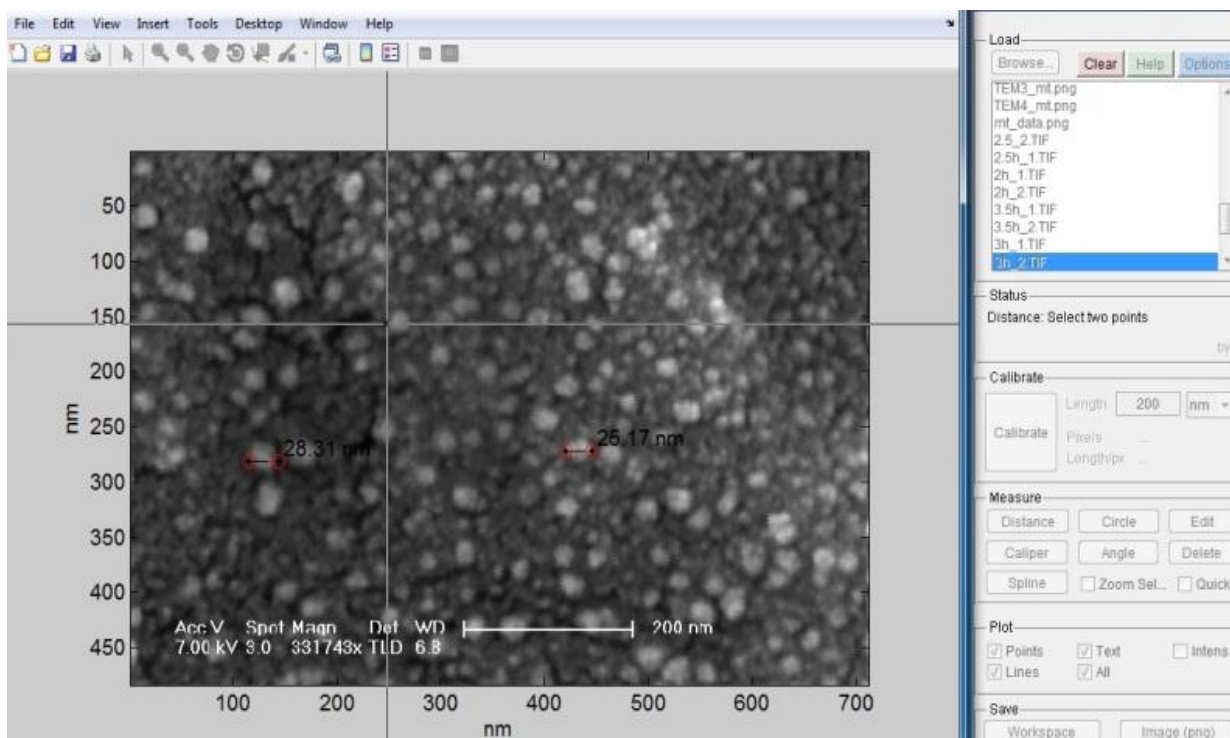
#### Image processing

In Image processing, the pixel region tool provided by the MATLAB 7.10.0.49, R2010a to set on each pixel region over image and displayed in the image tool. The major group of pixels that are to be displayed is viewed in extreme close-up using the pixel region tool in a new window. The current position of the pixel region in the specified image can be approximate by checking the pixel information given at the below status bar of the tool. In this manner, we found the x and y-coordinates of pixels in the board image coordinate system.

### RESULTS AND DISCUSSIONS

#### Measure Tool

This measure tool is projected to aid measuring the size of particles in an image. To measure the image it needs to have a little illustration to calibrate the pixel to duration ratio on, e.g. scale bar, ruler. The two files in GUI (Graphical User Interface) used are, i) measuretool.m - the code to run the file in MATLAB and ii) measuretool.fig - displays the GUI measure tool with frame.



**Fig. 1:** Graphical user interface of a program that allows user to measure selected particles size. The image shows how the nanoparticles are been measured using GUI.

**Image processing toolbox**

The tool is planned for high quality measurements and is therefore made approximately tools like "imline" from the image processing toolbox. These tools are terrific which allows user to select, zoom, re-adjust, and then confirm your selection. As a result, all measurements require several "clicks", the first click can be fast, and allows placing the measurement tool, where subsequent to the tool can be customized using its control points, when complete double click on the tool to confirm the selection. Finally, it is vital that when complete sequence of "clicks" is finished before a new measurement is started, otherwise the GUI will end less elegantly.

In this GUI measure tool, a set of plotting options can be established, which is enabled by setting it to 'on' (or off) visual objects. It process on points, lines and text not quit to enable or disable it. If it is enabled then measurements from all images in the "List" are shown concurrently, when disabled then only it shows the measurements of the current image. Intensity process on the text switches from the spatial capacity to the standard intensity of the pixels below the measured object. For Distance, the intensity is calculated by plotting the pixel values on discretized points of the measured line, and averages those. For circle, the average intensity is calculated for every pixel within the circle. Distance along the measurement and the second column the corresponding intensity.

**Table 1: Results of particle size analysis by GUI tool from SEM image**

Image	Particle Counts	Particle Size (nm)	Mean particle size (nm)
A	1	130	100.99
	2	110	
	3	75	
	4	130	
	5	94.45	
	6	72.5	
	7	95	
B	1	207.99	202.9
	2	222.22	
	3	184.62	
	4	192.15	
	5	200.04	
	6	210.68	
C	1	67.31	78.948
	2	92.4	
	3	103.55	
	4	93.2	
	5	101.64	
	6	93.2	
	7	67.31	
	8	51.78	
	9	51.78	
	10	67.31	

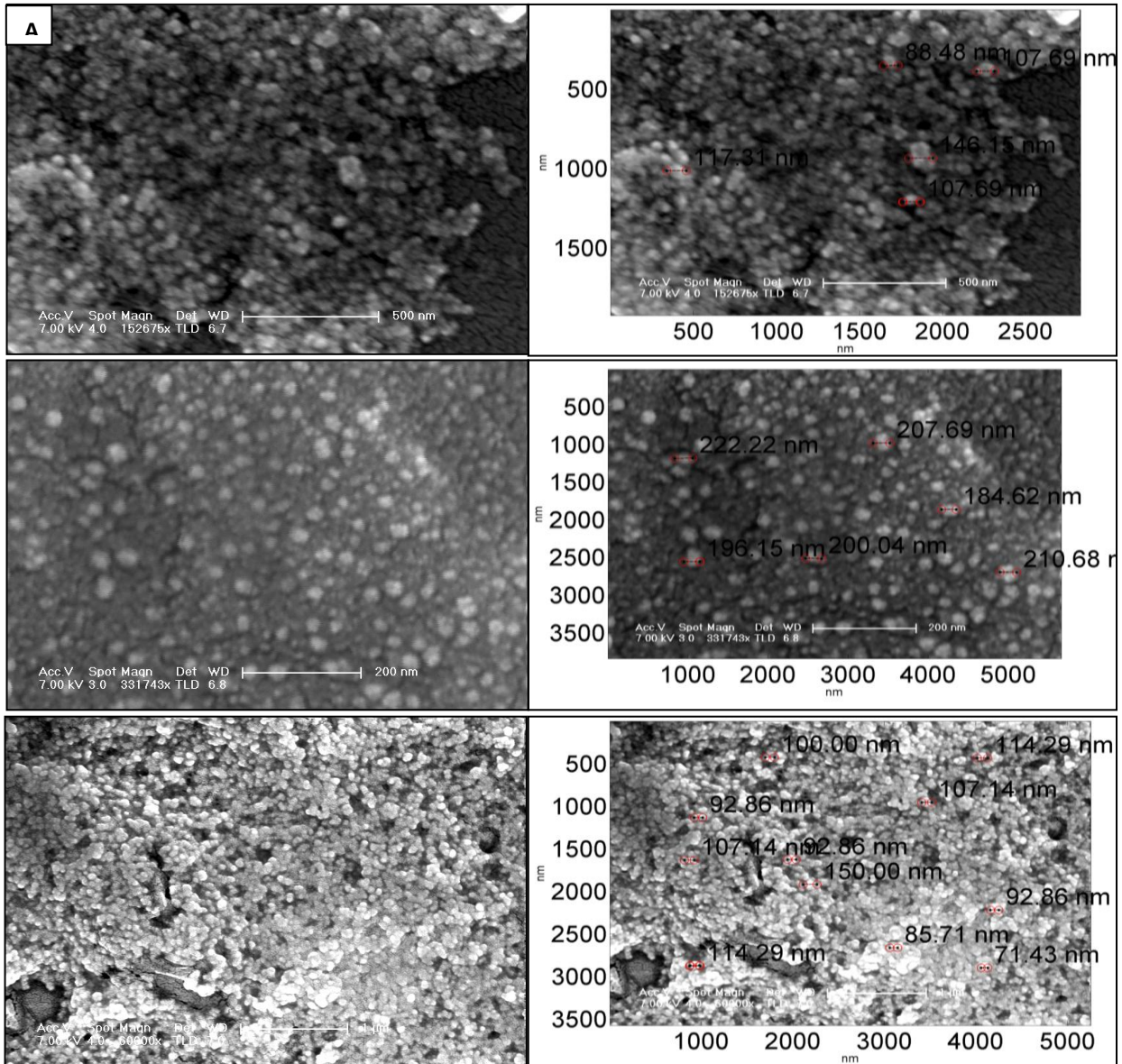


Fig. 2: SEM image of synthesized AgNPs taken at interval of A) 2 hr, B) 3 hr, C) 4 hr and the corresponding overlay of particle size measured by GUI tool in MATLAB image have been added to the original images.

Table 2: Results of particle size analysis by GUI tool from TEM image

Image	Particle Count	Particle Size (nm)	Mean Particle Size (nm)
D	1	43.01	48.88
	2	57.34	
	3	57.79	
	4	57.34	
	5	36.84	
	6	40.96	
E	1	50	49.22
	2	40.62	
	3	43.76	
	4	43.75	
	5	60.94	
	6	37.5	
	7	50	
	8	67.19	
F	1	84.45	116.09
	2	109.09	
	3	106.06	
	4	187.88	
	5	100	
	6	109.09	

From the SEM and TEM image specified in fig 2 and 3, the particle size of AgNPs are measured using the GUI measure tool and the values are been tabulated in table 1 and table 2. The mean sizes of particles are calculated and the values are listed in the above tables respectively.

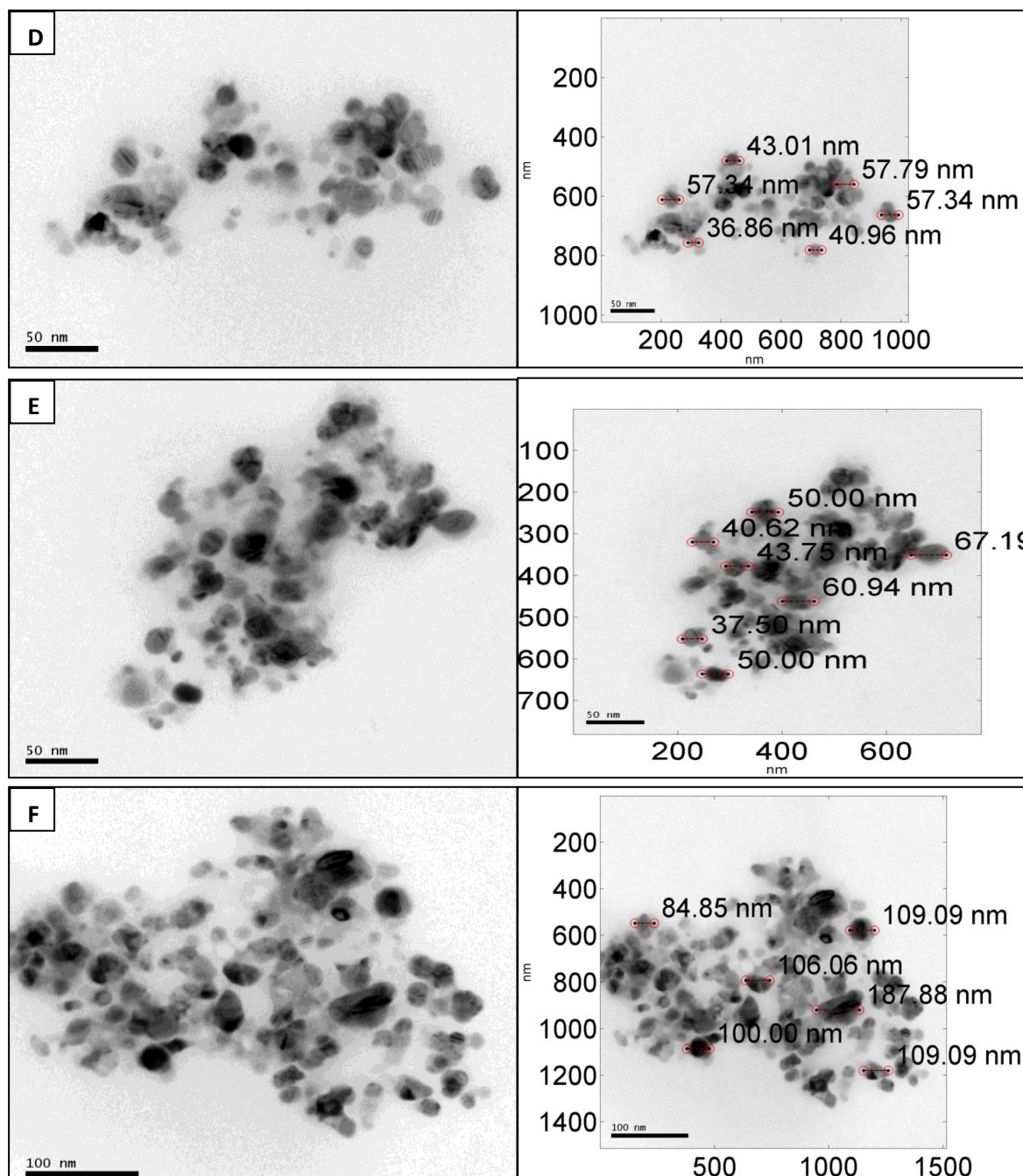


Fig. 3: TEM image of synthesized AgNPs taken at interval of D) 2 hr, E) 3 hr, F) 4 hr and the corresponding overlay of particle size measured by GUI tool in MATLAB image have been added to the original images.

## CONCLUSIONS

The research presents, automated procedure to set together size and shape of nanoparticles, to identify their boundaries in TEM or SEM image and specify the variations of shape with shape group and size of the nanoparticles. The advantage of this algorithm is to provide a tool to measure the size of nanoparticles EM images using MATLAB. This tool expedites the process to give information of nanoparticles, which would help to optimize the synthesis process of nanoparticles. The step by step process leads to the development of a unified framework for Nano-imaging community in future research and to measure the particle size from microscopic image with lack of in-built software.

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