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How to Read and Calculate Diameter Size from Electron Microscopy Images

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A B S T R A C T S

Scanning electron microscopy (SEM) and Transmission Electron Microscopy (TEM) is a chemical instrument that can be used to evaluate the characteristics of material. Techniques can be used to determine the morphology and diameter size of material. However, until now there has been no publications that describes in detail to read and interpret the electron microscopy images of both SEM and TEM. The purpose of this paper is to demonstrate the steps how to read and calculate the electron microscopy images based on the level of difficulties: (1) simple shape, (2) fairly complex shape, (3) very complex shape. This paper has the potential to be used as standard information of how to read and calculate diameter size of material from Electron Microscopy Images.

1. INTRODUCTION

Scanning electron microscope (SEM) and transmission electron microscope are the important analytical techniques that can be used to characterize nanoparticles and porous materials. Scanning electron microscope is used to generate surface images of objects. The specimen is at the microscopic level. It is by using High-energy electrons in the beam of light. The electrons emitted by the beam then Interact with the atomic structure of the specimen and generate a topographic map. The electron beam produces different types of electrons, including secondary scattering and backscattering, and discussed in more detail below (Velasco and Velasco, 2013).

In a transmission electron microscope, an electron beam is passed through sample to form the final image. TEM consists of three main parts: an illumination system (consisting of an electron gun and a condenser lens, which generates and forms an electron beam), the specimen stage where the TEM sample is entered. The sample in most cases it is placed on a copper frame which is covered with very thin holes carbon film. The sample being examined should be as thin as possible so that the electrons are get through it and imaging systems. Consisting of several lenses: objective lens, that is forming the first image of the electron transmitted through the sample, an intermediate lens that is responsible for magnification over a large range up to 106 and to assist diffraction noise formation on the display screen, and the projector lens that forms the final image on the phosphor screen or on a CCD camera (charge coupled device) (Jenei, 2012).

There have been many explanations on how to analyze SEM and TEM images. However, in general the article has not explained in detail the steps how to analyze the electron microscopy characterization results. In fact, it is necessary to have a detailed understanding for every student and scientist, especially those who conduct research in the field of chemistry.

However, many studies have describes the application of electron microscopy in the analysis of the morphology and diameter size of the particle. While the reading and calculating of the electron microscopy were not described in detail. Visual analysis makes it difficult to obtain quantitative data information, especially if the number of material to be analyzed is large, irregular in shape, and objects that overlap one another. Therefore, in this paper, we disscusses and explains how to read and calculate diameter size of material from electron microscopy images through step-by-step exposure in determining simple shape, fairly complex shape, and complex shape of material. We believe that this paper can be used as basic knowledge for students and scientists in understanding and analyzing SEM and TEM images.

2. CURRENT KNOWLEDGE FOR UNDERSTANDING SEM AND TEM IMAGES

2.1 SEM and TEM image analysis result

Identify the morphological and topographic shapes on the SEM images is carried out based on the signals given by the atoms in the sample. The signal appears when the electron beam from the electron source is accelerated using a positive electric potential interacting with the atoms in the sample (source). These signals are secondary electrons and backscattered electons.

Secondary electron excited by a charged beam of high energy particles, such as the position of electrons or ions when the light beam enters the sample for surface imaging in charged particle beam of SEM. They can quantitatively measure surface dimension with

subnanometer resolution and intuitive image interpretation. The electromagnetic environment and sample properties are related with the emission and detection of secondary electron varies (Zhou *et al.,* 2016).

This signal results from inelastic interactions between the sample's primary electron beam and has a lower energy than backscattered electrons. The Everhart-Thornley detector is the most frequently used device for detecting SE signals, placed on the side of the electron chamber at a point to increase the efficiency of detecting secondary electron signals.

Backscattered electron are beam electrons that have been scattered deeper in the sample. It can provide atomic number comparison, where the difference in signal strength is related to the local difference in average atomic number (Talbot and White, 2013). The signal generated and detected by the detector is the result of the elastic collision of the electrons with the atoms in the sample which results in changes in electron trajectories. A commonly used detector is a solid state detector, placed on top of the sample and concentrically onto the electron beam to maximize the use of the signal generated and detected by the detector as a result of the elastic collision of electrons with atoms in the sample which results in changes in electron trajectories. A commonly used detector is a solid state detector, placed on top of the sample and concentrically onto the electron beam to maximize the collection of the back scatted electron.

It is different compared when identifying morphological and topographic shapes in TEM images, which are based on the process of shooting electrons from the source of electrons through small and thin samples (the electrons can penetrate to the sample) are assisted by objective lenses and the image of the sample arrangement is captured by a fluorescent screen. The types of signals generated from this process include:

- Diffraction contrast
- Phase contrast
- Mass / thickness contrast
- Electron diffraction
- Characteristic X-Ray (EDX)
- Electron Energy Loss Spectroscopy (EELS)
- Scanning Transmission Electron Microscopy (STEM)

In determining the average size of the sample, be it pore size, particles, fibers, etc., in SEM or TEM images, a Feret diameter analysis is generally carried out. Ferret method is a measurement of the size of an object along a specified direction. Generally, it can be defined as the distance between two parallel planes that limit objects parallel to the direction (caliper diameter). This metric is used to analyze the granularity, for example in a microscope, it is applied to the projection of a 3D object on a 2D plane. In this case, the Feret diameter is defined as the distance between two parallel tangents, not the distance between planes. (Merkus, 2009). Diameter ferret can calculated by equation:

$$\frac{d_{max}+d_{min}}{2} \qquad (1) \text{ (Gupta and Yan, 2006)}$$

Where:

- d_{max} measured at an angle of 90 degrees to that of the maximum Feret diameter.

- d_{min} measured at an angle of 90 degrees to that of the minimum Feret diameter.

Calculation of the area and perimeter of each shape in the sample using the ferret method can use several formulas as shown in **Table 1.** The diameter equivalent of the un-spherical shaped can calculate with the equate diameter value on the sphere with the same area, can be calculated using the formula.

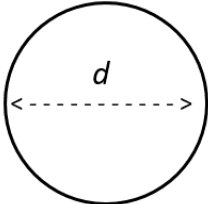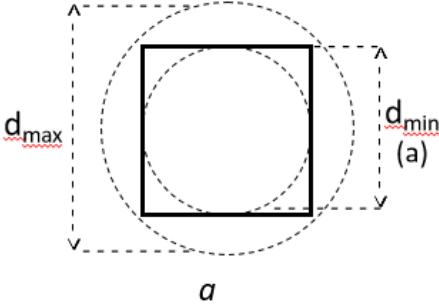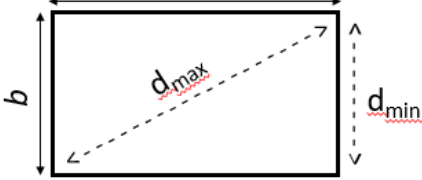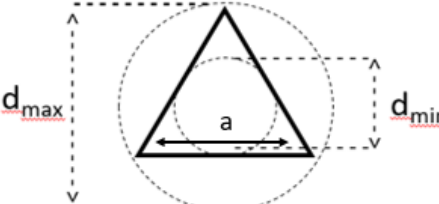$$d_{a=} 2\sqrt{\frac{A}{\pi}}$$
(2)(M. Li *et al.*, 2005)

or,
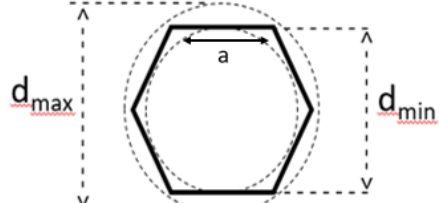
$$d_a = \sqrt{4\,A\,/\,\pi}$$
(3)(Bagheri *et al.*, 2015)

where:

A = projection area

The calculation of the shape factor for each particle shape such as spherecity, circularity, aspect ratio, and chunkiness can be calculated using the formula in **Table 2**.

Table 1. Equivalent diameters equation based on an equivalent cross-sectional Area

Equivalent cross-sectional Area	Shape	Area	Perimeter
	Spherical	$\frac{1}{4}\pi d^2$	πd
	Cube	a^2	$4a$
	Rectangular	ab	$2a + 2b$
	Triangular	$\frac{1}{4}\sqrt{3}\,a^2$	$3a$
	Hexagonal	$1.5\sqrt{3}\,a^2$	$6a$

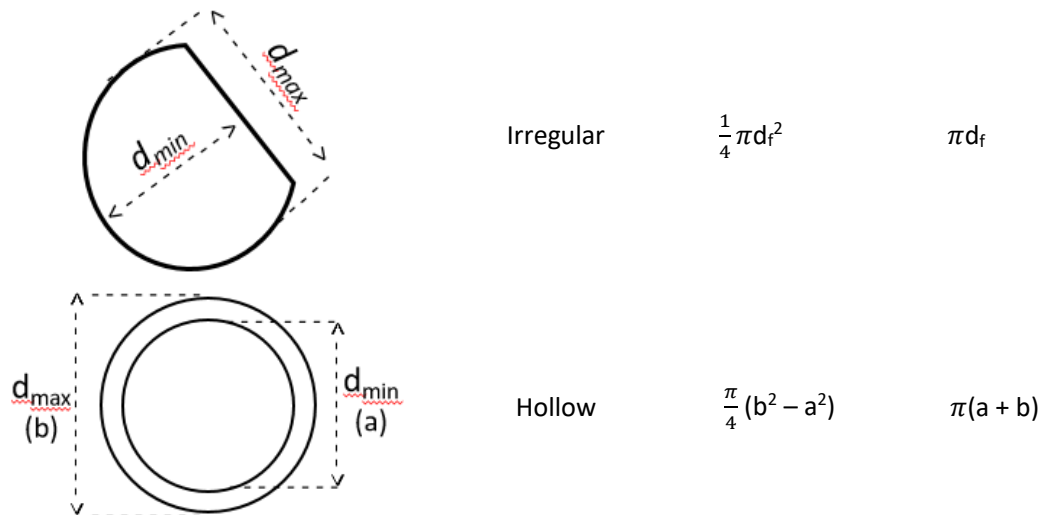|		
Irregular	$\frac{1}{4}\pi d_f^2$	πd_f
Hollow	$\frac{\pi}{4}(b^2 - a^2)$	$\pi(a + b)$

Table 2. Equation for sphericity, circularity, aspect ratio, and chunkiness from some references

Shape Factor	Equation No	Description	Refference
Spherecity $(\varphi) = \frac{4\pi A}{P^2} = \left(\frac{d_a}{d_p}\right)^2$ Circularity $(\sigma) = \frac{4A}{\pi F_{max}^2}$ Aspect Ratio $(AR) = \frac{d_{max}}{d_{min}}$ Chunkiness $(Ch) = \frac{1}{AR} = \frac{d_{min}}{d_{max}}$	(4) (5) (6) (7)	Non-spherical shape	(M. Li *et al.,* 2005)
Spherecity $(\varphi) = \frac{A_{sph}}{A_p}$ Circularity $(\sigma) = \frac{\varphi P_p}{P_{mp}}$ $A_p = \pi\left[\frac{(d_1.d_m)^k + (d_1.d_s)^k + (d_m.d_s)^k}{3}\right]^{1/k}$ $A_{sph} = 4\pi\left(\frac{d_p}{2}\right)^2$	(8) (9)	Non-spherical shape A_{sph} = Surface area of the volume-equivalent sphere A_p = Surface area of the actual particle d_1 = Long axis of the particle d_m = Medium axis of the particle d_s = Short axis of the particle d_p = Particle size inscribed circle	(Y. Wang *et al.,* 2018)
Circularity $(\sigma) = \sqrt{D_i/D_c}$ Circularity $(\sigma) = \frac{4\pi A}{P^2}$	(10)	D_i ≡ diameter of the largest inscribed circle D_c ≡ diameter of the smallest circumscribed circle A = projection area P = projection perimeter S_n = nominal surface area (surface	(Bagheri *et al.,* 2015)
Spherecity $(\varphi) = \frac{Sn}{S} = \frac{(36\pi V^2)^{1/3}}{s}$	(11)	area of a sphere having the same volume as the object) S = actual surface area of the object	(Wadell, 1932)

Spherecity $(\varphi) = \frac{\rho_{is}}{\rho_{es}}$	(12)	ρ_{is} = maximum inscribed sphere ρ_{es} = minimum enclosing sphere	(Bullard and Garboczi, 2013)
Spherecity $(\varphi) = \sqrt[3]{\frac{thickness \, x \, breadth}{length^2}}$	(13)		(Mora and Kwan, 2000)

2.2 SEM Image

1.2.1 Step-by-step Morphology Analysis Procedure

There are three steps to determine sample morphology from the SEM images as follows:

1) Step 1: Identify the type of detector when obtaining particle morphology in the SEM image. The sample morphology obtained using a secondary electron detector or a backscattered electron detector.

2) Step 2: Identify to the bright contrast and dark contrast of the SEM image. This is important to do to determine which object to analyze.

3) Step 3: Determine the shape of the object (be it particles, pores, fibers, etc.) by looking at the output in the form of an image resulting from SEM characterization. These shapes can be spherical, non-spherical, amorphous, hexagonal, octagonal, triangular, rectangular and circular shape (Wienke *et al.*, 1995).

1.2.2 Step-by-step the Scale Bar and Diameter ferret size Analysis Procedure

There are four steps in analyzing scale bar of the sample form of the SEM images as follows:

1) Step 1: Identify of the bar scales on SEM images. Generally, the scale bar is written at the bottom right of the image constructed with short lines or bars and shows the scale value of the lines listed.

2) Step 2: Identify diameter size of the particle by comparing with the scale bar.

3) Step 3: Calculate diameter ferret maximum (d_{max}) and diameter ferret minimum ($d_{min)}$ of the particle by comparing the ratio of the size of the particle diameter to the size on the scale bar.

4) Step 4: Calculate the diameter ferret (average diameter of the sample) by using equation 1 as presented as $\frac{d_{max}+d_{min}}{2}$.

1.2.3 Step-by-step Diameter equivalent and shape factor Analysis Procedure

There are three steps in determine diameter equivalent and shape factor analysis of the sample from the SEM images as follows:

1) Step 1: Calculate the Area and Perimeter of each shape by using equation in **Table 1** according to the shape of the sample.

2) Step 2: Calculate the diameter equivalent of the un-spherical shaped with the equate diameter value on the sphere with the same area by using equation 3 as presented as $d_a = \sqrt{4 \, A \, / \, \pi}$.

3) Step 3: Calculate of the shape factor for each particle shape such as sphericity by using equation 4 as presented as $\frac{4\pi A}{P^2}$, circularity by using equation 5 as presented as $\frac{4A}{\pi\,F_{max}{}^2}$, aspect ratio by using equation 6 as presented as $\frac{d_{max}}{d_{min}}$, and chunkiness by using equation 7 as presented as $\frac{d_{min}}{d_{max}}$.

2.3 TEM Image

2.3.1 Step-by-step Morphology Analysis Procedure

There are two steps in analyzing and interpreting the sample form of the TEM images as follows:

1) Step 1: Identify the type of signal when obtaining sample morphology based on TEM images. Make sure the signal type when obtaining TEM images is phase contrast signals. Thus, using electron contrast under low-dose conditions for imaging is recommended (Z. Liu *et al.*, 2020).

2) Step 2: Identify the bright contrast and dark contrast of the TEM image. This is important to do to determine which object to analyze.

3) Step 3: Determine the morphology of the sample by looking at the output in the form of an image resulting from TEM characterization. The morphological form of the sample can be spherical, non-spherical, amorphous, hexagonal, octagonal, triangular, rectangular and circular shape (Wienke *et al.,* 1995).

2.3.2 Step-by-step the Scale Bar and Diameter ferret size Analysis Procedure

There are four steps in analyzing scale bar of the sample form of the TEM images as follows:

1) Step 1: Identify of the bar scales on TEM images. Generally, the scale bar is written at the bottom right of the image constructed with short lines or bars and shows the scale value of the lines listed.

2) Step 2: Identify diameter size of the particle by comparing with the scale bar.

3) Step 3: Calculate diameter ferret maximum (d_{max}) and diameter ferret minimum (d_{min}) of the particle by comparing the ratio of the size of the particle diameter to the size on the scale bar.

4) Step 4: Calculate the diameter ferret (average diameter of the sample) by using equation 1 as presented as $\frac{d_{max}+d_{min}}{2}$.

2.3.3 Step-by-step Diameter equivalent and shape factor Analysis Procedure

1) Step 1: Calculate the Area and Perimeter of each shape by using equation in **Table 1** according to the shape of the sample.

2) Step 2: Calculate the diameter equivalent of the un-spherical shaped with the equate diameter value on the sphere with the same area by using equation 3 as presented as d_a = $\sqrt{4\,A\,/\,\pi}$.

3) Step 3: Calculate of the shape factor for each particle shape such as sphericity by using equation 4 as presented as $\frac{4\pi A}{P^2}$, circularity by using equation 5 as presented as $\frac{4A}{\pi\,F_{max}{}^2}$, aspect ratio by using equation 6 as presented as $\frac{d_{max}}{d_{min}}$, and chunkiness by using equation 7 as presented as $\frac{d_{min}}{d_{max}}$.

3. EXPERIMENTAL METHOD

In order to understand how to read and calculate the electron microscopy images, this paper will explain how to read and calculate the electron microscopy images through step-by-step approach in determining morphology and diameter size of material with simple shape, fairly complex shape, and complex shape. The simple shape that used are is spherical, the fairly complex shape that used are: cube and rectangular. The complex shape that used are: irregular shape and hollow shape.

A short experimental procedure by (Eaton *et al.*, 2017) for spherical shape analysis by TEM image of Silica (Si2) nanoparticle synthesized via the hot-injection method. In addition, the cube shape prepared by (Y. Liu and Walker, 2010) is a bimetallic gold-copper bimetallic synthesized through the reduction process of Cu(acac)2 and gold chloride (HAuCl4). Meanwhile, (W. Li and Shao, 2009) shows a rectangular shape based in the TEM image of aerosol particles from the brown hazes in northern China. In short, (Jin *et al.*, 2019), synthesize triangular Gadolinium Oxide Nanoplates for Precise Excision of Cancer for theranostic agent for effective diagnosis and therapy of neuroblastoma. Triangular gadolinium oxide nanoplates are obtained by reaction of Gd (NO3) with BSA at a specified pH, temperature, and time. Meanwhile, (M. Wang *et al.*, 2008), shows ZnO crystal with a hexagonal morphological shape. ZnO crystal are synthesized under the influence of solvent composition control in a water-ethanol solvent system to dissolve zinc acetate and hexamethylenetetramine.

In short, (Sujka and Jamroz, 2013), shows SEM micrograph of native corn without any treatment on corn structure as the effect of ultrasonication. Meanwhile, for hollow images based on TEM micrograph by (Hah *et al.*, 2003) are hollow silica particles (scale bar: 200 nm) prepared via a two-step method in aqueous solution and were selective soluble in organic solvent.

4. RESULT AND DISCUSSION

4.1 Electron image analysis of simple shape

4.1.1 Spherical Shape
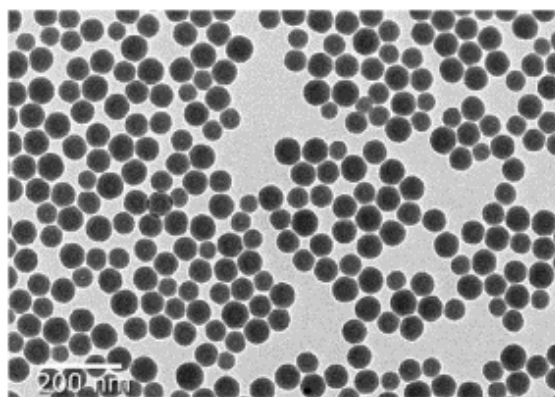
4.1.1.1 Morphology Analysis

Figure 1. Silica (Si_2) nanoparticle images from TEM (Eaton *et al.*, 2017)

Determination of the morphological shape of the sample is carried out in **Figure 10** on the Silica (Si_2) nanoparticle sample. Based on the steps in determining the morphological shape of the sample from the TEM image, the following conclusions are drawn:

1) Step 1: Identify the type of signal when obtaining sample morphology based on TEM images. **Figure 10** is determined by using the large of electron beam to avoid significant beam effect.

2) Step 2: Identify to the light contrast and dark contrast of the TEM image. **Figure 10** in the dark contrast section shows the silica nanoparticles.

3) Step 3: Determine the morphological form of the sample by looking at the image result from TEM characterization. The morphological shape of the Silica (Si_2) nanoparticle sample shows a perfect spherical shape as shown in **Figure 1.**

4.1.1.2 The Scale Bar and Diameter size Analysis Procedure

There are four steps in analyzing scale bar and calculate diameter size of the sample from the TEM images as follows:

1) Step 1: Identify of the scales bar on TEM images. On that scale (represented by white short bars) in **Figure 1** it shows that bar in the image is equal to 200 nm in size.

2) Step 2: Identify diameter size of the particle by comparing with the scale bar. It can be seen from **Figure 1** that the particle size in the image is shorter than the scale bar so that the particle size is smaller than 200 nm.

3) Step 3: Calculating the diameter as shown in **Figure 2** by comparing the ratio of the size of the particle diameter to the size on the scale bar. Measurements were made for each particle**.** Calculations are carried out on one axis, this is because the particle shape is assumed to be a perfect spherical.
Step 4**:** Calculate the average diameter of the sample using the Feret Diameter analysis. Measurements were made on 26 particles giving an average particle diameter size of Silica (Si_2) nanoparticle is 57.62 nm, as shown in **Table 3**.
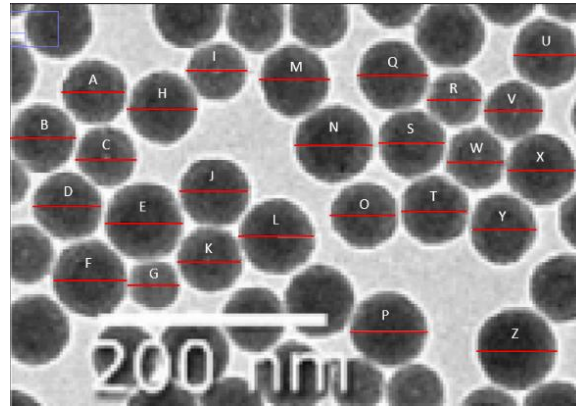
Figure 2. Measurements the horizontal ferret diameter (Dfh) of Silica (Si$_2$) nanoparticle images from TEM

Table 3. Result of Silica Nanoparticle diameter measurements

Particle	Ratio	Particle Size (nm)	Particle	Ratio	Particle Size (nm)
A	1 : 0.27	54.545	N	1 : 0.33	66.434
B	1 : 0.29	57.343	O	1 : 0.28	56.643
C	1 : 0.25	51.049	P	1 : 0.33	66.434
D	1 : 0.30	60.140	Q	1 : 0.30	59.441
E	1 : 0.33	65.734	R	1 : 0.23	46.853
F	1 : 0.32	63.636	S	1 : 0.29	58.741
G	1 : 0.22	43.357	T	1 : 0.29	58.741
H	1 : 0.30	59.441	U	1 : 0.29	58.042
I	1 : 0.28	55.245	V	1 : 0.25	50.350
J	1 : 0.30	60.839	W	1 : 0.25	49.650
K	1 : 0.28	56.643	X	1 : 0.29	58.741
L	1 : 0.33	65.039	Y	1 : 0.28	56.643
M	1 : 0.29	58.741	Z	1 : 0.35	69.231

4.2 Electron image analysis of fairly complex shape

4.2.1   Cube shape

4.2.1.1 Morphology Analysis

Determination of the morphological shape of the sample is carried out in **Figure 6** of the AuCu$_3$ nanocubes sample. Based on the steps in determining the morphological shape of the sample from the SEM image, the following conclusions are drawn:

1) Step 1: Identify the type of detector when obtaining particle morphology in the SEM image. The morphology of the sample in **Figure 6** is obtained using a secondary electron detector.

2) Step 2: Identify to the light contrast and dark contrast of the SEM image. **Figure 6** in the light contrast section shows the AuCu$_3$ nanocubes.

3) Step 3: Determine the morphological form of the sample by looking at the image result from SEM characterization. The morphological shape of AuCu$_3$ nanocubes shows a cube shape as shown in **Figure 3.**

4.2.1.2 The Scale Bar and Diameter ferret size Analysis Procedure

There are four steps in analyzing scale bar of the sample form of the SEM images as follows:

1) Step 1: Identify of the bar scales on SEM images. On that scale (represented by white stripes) in F**igure 3** it shows that scale by stripes in the image is equal to 100 nm in size.

2) Step 2: Identify diameter size of the particle by comparing with the scale bar. It can be seen from F**igure 3** that the particle size in the image is shorter than the scale bar so that the particle size is smaller than 100 nm.

3) Step 3: Calculate diameter ferret maksimum and diameter ferret minimum by comparing the ratio of the size of the particle diameter to the size on the scale bar. Measurements were made for each cube as shown in **Table 4**.

4) Step 4**:** Calculate the diameter ferret (average diameter of the sample) by using equation 1 as presented as $\frac{d_{max}+d_{min}}{2}$. The value is obtained by comparing the ratio of the size of the particle diameter to the size on the scale bar. Measurements were made on 10 cubes giving an average diameter equivalent size of AuCu$_3$ nanocubes is 49.06 nm as shown in **Table 4.**
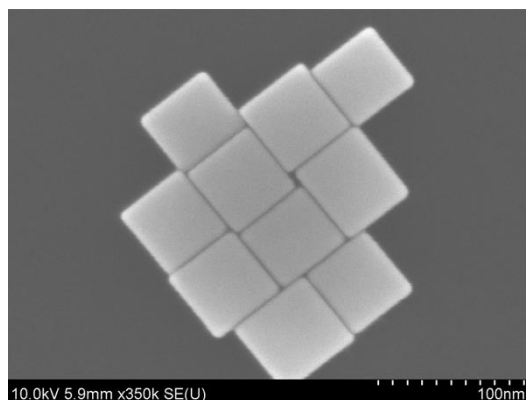

Fig. 3 SEM image of AuCu$_3$ nanocubes (Y. Liu and Walker, 2010)
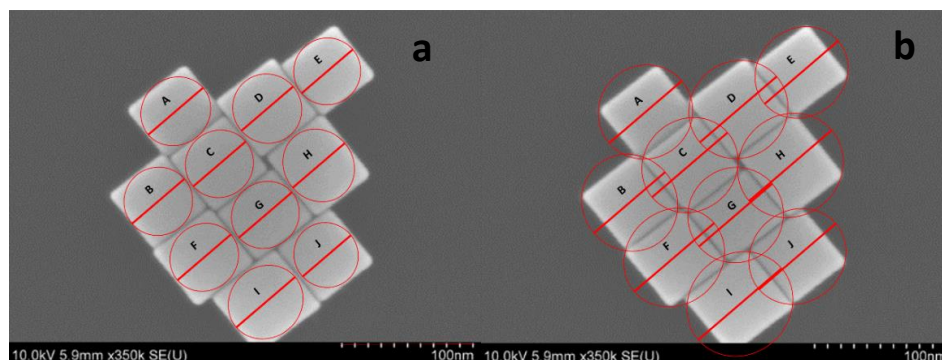

Figure. 4 Measurements (a) minimum diameter (d$_{min}$), (b) maximum diameter (d$_{max}$) of AuCu$_3$ nanocubes images from SEM.

4.1.2.3 Step-by-step Diameter equivalent and shape factor Analysis Procedure

1) Step 1: Calculate length side (a) as shown in **Figure 5** by comparing the ratio of the size of the cube to the size on the scale bar. Measurements were made for each cube as shown in **Table 5**.

2) Step 2: Calculate the Area and Perimeter of each shape by using equation in **Table 1** according to the shape of the sample as presented as $(A = a^2)$ and $(P = 4a)$. Measurements were made for each cube as shown in **Table 5**.

3) Step 3: Calculate the diameter equivalent of the un-spherical shaped with the equate diameter value on the sphere with the same area by using equation 3 for d_a and the same perimeter by using equation 4 for d_p . Measurements were made on 10 cubes giving an average diameter equivalent area size of $AuCu_3$ nanocubes is 48.10 nm as shown in **Table 5.**

4) Step 4: Calculate of the shape factor for each particle shape such as sphericity by using equation 4, circularity by using equation 5, aspect ratio by using equation 6, and chunkiness by using equation 7. Measurements were made for each cube as shown in **Table 5**.

Table 4. Result of cube diameter Ferret measurements

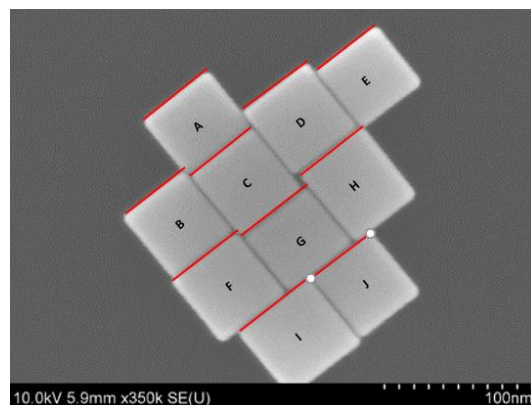| No. of Cube	Dmin		Dmax		Diameter Ferret (nm)
	Ratio	Length (nm)	Ratio	Length (nm)	
A	1 : 0.42	41.60	1 : 0.55	55.13	48.37
B	1 : 0.40	39.82	1 : 0.54	53.94	46.88
C	1 : 0.40	39.82	1 : 0.57	57.21	48.51
D	1 : 0.39	39.23	1 : 0.58	58.25	48.74
E	1 : 0.39	38.78	1 : 0.56	55.72	47.25
F	1 : 0.41	40.56	1 : 0.60	60.48	50.52
G	1 : 0.40	40.27	1 : 0.56	55.57	47.92
H	1 : 0.40	39.52	1 : 0.63	62.56	51.04
I	1 : 0.45	44.73	1 : 0.63	63.15	53.94
J	1 : 0.37	36.70	1 : 0.58	58.25	47.47

Figure 5. Measurements length side (a) of $AuCu_3$ nanocubes images from SEM.

4.2.2 Rectangular shape

4.2.2.1 Morphology Analysis

Determination of the morphological shape of the sample is carried out in **Figure** 7 on the Zn-rich coatings with a rectangular Pb-rich sample. Based on the steps in determining the morphological shape of the sample from the TEM image, the following conclusions are drawn:

1) Step 1: Identify the type of signal when obtaining sample morphology based on TEM images. **Figure 6** is determined by using the large of electron beam to avoid significant beam effect.
2) Step 2: Identify to the light contrast and dark contrast of the TEM image. **Figure 6** in the dark contrast section shows the Zn-rich coatings with a rectangular Pb-rich.
3) Step 3: Determine the morphological form of the sample by looking at the image result from TEM characterization. The morphological shape of rich coatings with a rectangular Pb-rich sample shows a rectangular shape as shown in **Figure 6.**

Table 5. Result of cube Equivalent diameter and shape factor measurements

| a (nm)	A (nm²)	P (nm)	d_a (nm)	φ	σ	AR	Ch
43.02	1851.00	172.09	48.56	0.79	0.78	1.33	0.75
40.26	1621.00	161.05	45.44	0.79	0.71	1.35	0.74
40.99	1680.05	163.95	46.26	0.79	0.65	1.44	0.70
43.75	1914.06	175.00	49.38	0.79	0.72	1.48	0.67
39.10	1528.72	156.40	44.13	0.79	0.63	1.44	0.70
44.33	1965.27	177.33	50.04	0.79	0.68	1.49	0.67
45.06	2030.24	180.23	50.86	0.79	0.84	1.38	0.72
42.01	1764.49	168.02	47.41	0.79	0.57	1.58	0.63
46.80	2190.46	187.21	52.82	0.79	0.70	1.41	0.71
40.84	1668.15	163.37	46.10	0.79	0.63	1.59	0.63
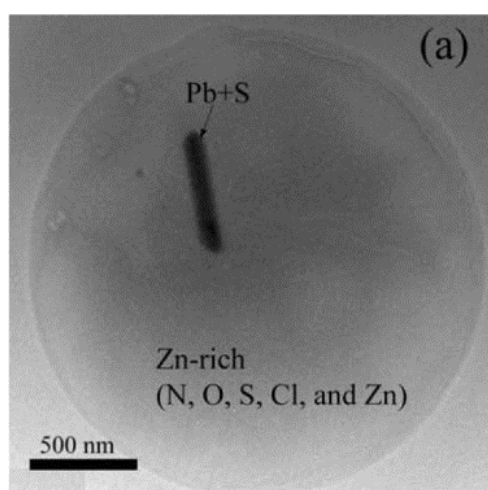
Figure 6. TEM image of Zn-rich coatings with a rectangular Pb-rich (W. Li and Shao, 2009)

4.2.2.2 The Scale Bar and Diameter Ferret size Analysis Procedure

There are four steps in analyzing scale bar and calculate the diameter of the sample from the TEM images as follows:

1) Step 1: Identify of the scales bar on TEM images. On that scale (represented by white short bars) in **Figure 6** it shows that bar in the image is equal to 500 nm in size.
2) Step 2: Identify diameter size of the particle by comparing with the scale bar. It can be seen from **Figure 6** that one axis has a shorter size while the other axis has a larger size than scale bar (500 nm).
3) Step 3: Calculate diameter ferret maximum and diameter ferret minimum by comparing the ratio of the size of the particle diameter to the size on the scale bar. Measurements were made to one end only rectangular as shown in **Table 6.**
4) Step 4**:** Calculate the diameter ferret (average diameter of the sample) by using equation 1 as presented as $\frac{d_{max}+d_{min}}{2}$. The value is obtained by comparing the ratio of the size of the particle diameter to the size on the scale bar. Measurements giving a diameter equivalent size of Zn-rich coatings with a rectangular Pb-rich is 353.94nm as shown in **Table 6.**

4.2.2.3 Step-by-step Diameter equivalent and shape factor Analysis Procedure

1) Step 1: Calculate length side (a and b) as shown in **Figure 8** by comparing the ratio of the rectangular size to the scale bar. Measurements were made as shown in **Table 7**.
2) Step 2: Calculate the Area and Perimeter of each shape by using equation in **Table 1** according to the shape of the sample as presented as (A = ab) and (P = 2a + 2b). Measurements were made as shown in **Table 7.**
3) Step 3: Calculate the diameter equivalent of the un-spherical shaped with the equate diameter value on the sphere with the same area by using equation 3 for d_a giving a diameter equivalent area size of rectangular Pb-rich sample is 290.88 nm as shown in **Table 7.**
4) Step 4: Calculate of the shape factor such as sphericity by using equation 4, circularity by using equation 5, aspect ratio by using equation 6, and chunkiness by using equation 7. Measurements were made for each cube as shown in **Table 7**.

4.2.3   Triangular shape

4.2.3.1 Morphology Analysis

Determination of the morphological shape of the sample is carried out in **Figure 9** of PEG-modified GD_2O_3 TNs sample. Based on the steps in determining the morphological shape of the sample from the TEM image, the following conclusions are drawn:

1) Step 1: Step 1: Identify the type of signal when obtaining sample morphology based on TEM images. **Figure 9** is determined by using the large of electron beam to avoid significant beam effect.
2) Step 2: Identify to the light contrast and dark contrast of the TEM image. In **Figure 9** for the dark contrast shows the PEG-modified GD_2O_3.

3) Step 3: Determine the morphological form of the sample by looking at the image result from TEM characterization. The morphological shape of the PEG-modified GD$_2$O$_3$ sample shows triangular shape as shown in **Figure 9.**

4.2.3.2 The Scale Bar and Ferret Diameter size Analysis Procedure

There are four steps in analyzing scale bar of the sample form of the TEM images as follows:
1) Step 1: Identify of the scales bar on SEM images. On that scale (represented by white bar) in **Figure 9**, it shows that scale bar in the image is equal to 50 nm in size.
2) Step 2: Identify diameter size of the particle by comparing with the scale bar. It can be seen from **Figure 9** that the sample diameter size in the image is shorter than the scale bar, it means the particle size is smaller than 50 nm.
3) Step 3: Calculate ferret diameter maximum and diameter ferret minimum by comparing the ratio of diameter particle size to the scale bar. Measurements were made to each triangular as shown in **Table 8.**
1) Step 4**:** Calculate ferret diameter (average diameter of the sample) by using equation 1 as presented as $\frac{d_{max}+d_{min}}{2}$. The value is obtained by comparing the ratio particle diameter size to the scale bar. Measurements were made on 6 triangulars giving an average diameter equivalent size of AuCu$_3$ nanocubes is 32.51 nm as shown in **Table 8.**
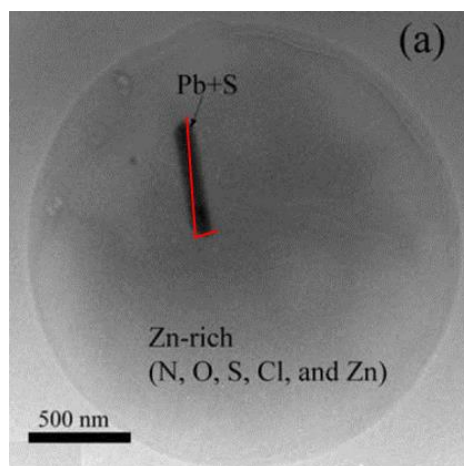

Figure 7. Measurements diameter size of Zn-rich coatings with a rectangular Pb-rich images from TEM.

Table 6. Result of cube diameter measurements

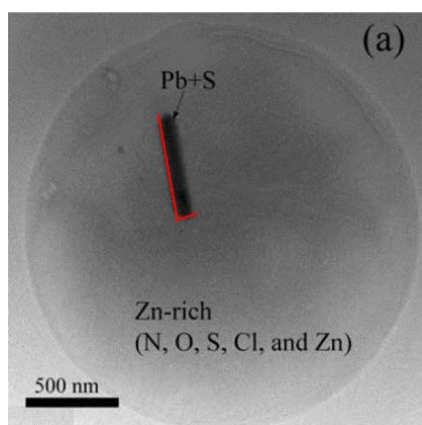| d max (a)		d min (b)		Diameter Ferret
Ratio	Length (nm)	Ratio	Length (nm)	(nm)
1 : 1.19	595.24	1 : 0.23	112.64 nm	353.94

Figure 8. Measurements length side (a and b) of AuCu₃ nanocubes images from SEM.

Table 7. Result of cube Equivalent diameter and shape factor measurements

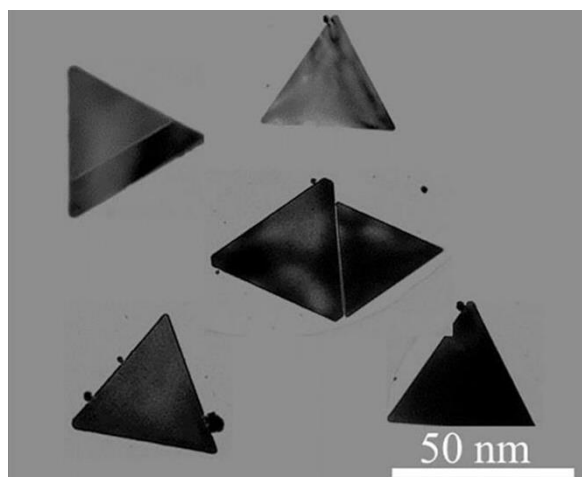| a (nm)	b (nm)	A (nm²)	P (nm)	d_a	φ	σ	AR	Ch
112.64	589.66	66419.3	1404.6	290.88	0.42	0.24	5.23	0.19

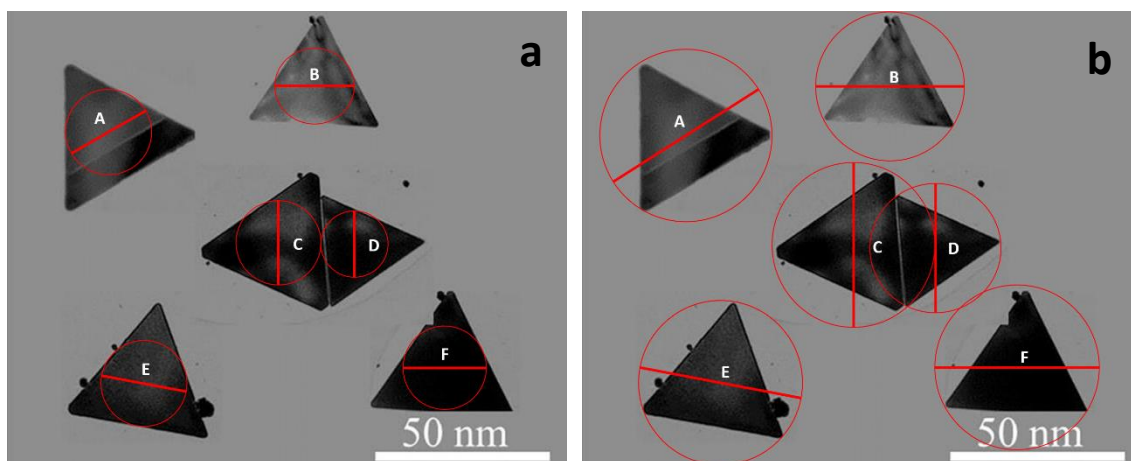**Figure 9.** TEM micrograph of PEG-modified GD₂O₃ TNs (Jin *et al.,* 2019)

Figure 10. Measurements (a) minimum diameter (d_{min}), (b) maximum diameter (d_{max}) of PEG-modified GD₂O₃ TNs

Table 8. Result of irregular diameter measurements

No. of Triangular	Dmin		Dmax		Ferret Diameter (nm)
	Ratio	Length (nm)	Ratio	Length (nm)	
A	1 : 0.38	19.24	1 : 0.83	41.50	30.37
B	1 : 0.38	19.24	1 : 0.85	42.51	30.87
C	1 : 0.48	24.06	1 : 0.94	46.97	35.52
D	1 : 0.38	18.88	1 : 0.74	36.89	27.88
E	1 : 0.48	24.14	1 : 0.92	46.04	35.09
F	1 : 0.48	23.78	1 : 0.94	46.90	35.34

4.2.3.3 Step-by-step Diameter equivalent and shape factor Analysis Procedure

1) Step 1: Calculate length side (a) as shown in **Figure 11** by comparing the ratio of the rectangular size to the scale bar. Measurements were made as shown in **Table 9**.

2) Step 2: Calculate the Area and Perimeter of each shape by using equation in **Table 1** according to the shape of the sample as presented as (A = $\frac{1}{4}\sqrt{3}$ a^2) and (P = 3a). Measurements were made as shown in **Table 9.**

3) Step 3: Calculate the diameter equivalent of the un-spherical shaped with the equate diameter value on the sphere with the same area by using equation 3 for d_a giving a diameter equivalent area size of PEG-modified GD_2O_3 TNs sample is 27.13 nm as shown in **Table 9.**

4) Step 4: Calculate of the shape factor such as sphericity by using equation 4, circularity by using equation 5, aspect ratio by using equation 6, and chunkiness by using equation 7. Measurements were made for each triangular as shown in **Table 9**.

4.2.4   Hexagonal shape

4.2.4.1 Morphology Analysis

Determination of the morphological shape of the sample is carried out in **Figure 12** of hexagonal ZnO nanodisks sample. Based on the steps in determining the morphological shape of the sample from the SEM image, the following conclusions are drawn:

1) Step 1: Identify the type of detector when obtaining particle morphology in the SEM image. The morphology of the sample in **Figure 12** is obtained using a secondary electron detector.

2) Step 2: Identify to the light contrast and dark contrast of the SEM image. In **Figure 12** for the light contrast shows the hexagonal ZnO nanodisks.

3) Step 3: Determine the morphological form of the sample by looking at the image result from SEM characterization. The morphological shape of the hexagonal ZnO nanodisks sample shows hexagonal shape as shown in **Figure 12.**

4.2.4.2 The Scale Bar and Diameter size Analysis Procedure

There are four steps in analyzing scale bar of the sample form of the SEM images as follows:

1) Step 1: Identify of the scales bar on SEM images. On that scale (represented by white bar) in **Figure 12**, it shows that scale bar in the image is equal to 1 µm in size.
2) Step 2: Identify diameter size of the particle by comparing with the scale bar. It can be seen from **Figure 11** that the most sample diameter size in the image is larger than the scale bar, it means the particle size is larger than 1 µm.
3) Step 3: Calculate ferret diameter maximum and ferret diameter minimum by comparing the ratio of diameter particle size to the scale bar. Measurements were made to each hexagonal as shown in **Table 10.**
4) Step 4**:** Calculate ferret diameter (average diameter of the sample) by using equation 1 as presented as $\frac{d_{max}+d_{min}}{2}$. The value is obtained by comparing the ratio particle diameter size to the scale bar. Measurements were made on 7 hexagonals giving an ferret diameter size of ZnO nanodisks is 1.11 µm as shown in **Table 10.**
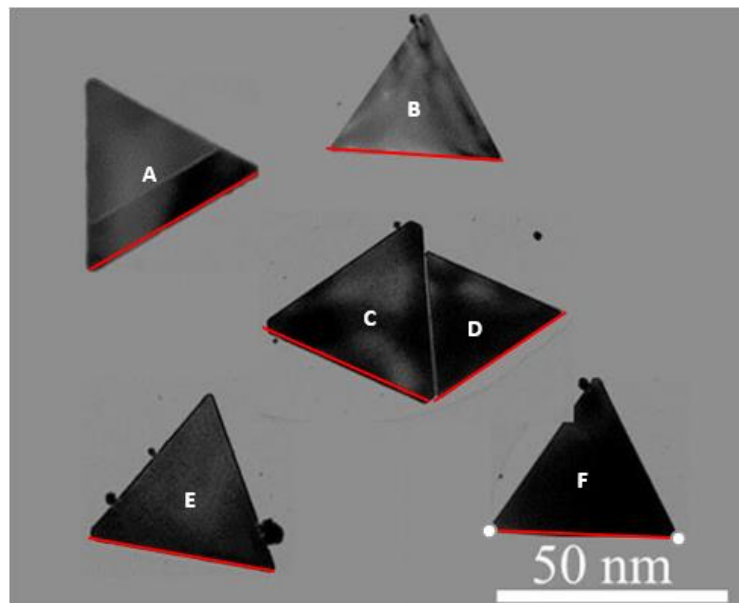

Figure 11. Measurements length side (a) of PEG-modified GD_2O_3 TNs images from SEM.

Table 9. Result of cube Equivalent diameter and shape factor measurements

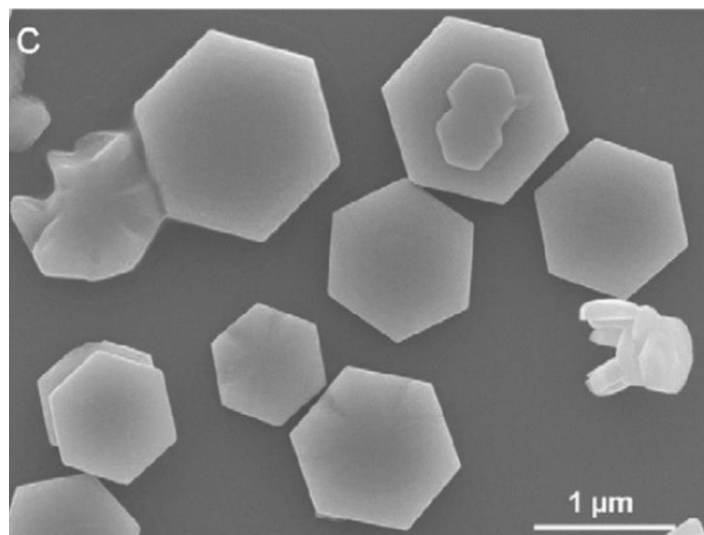| a (nm)	A (nm²)	P (nm)	d_a (nm)	φ	σ	AR	Ch
36.15	566.01	108.46	26.85	0.60	0.42	2.16	0.46
38.02	625.91	114.06	28.24	0.60	0.44	2.21	0.45
35.87	557.06	107.60	26.64	0.60	0.32	1.95	0.51
28.41	349.42	85.22	21.10	0.60	0.33	1.95	0.51
40.03	693.79	120.09	29.73	0.60	0.42	1.91	0.52
40.67	716.36	122.02	30.21	0.60	0.41	1.97	0.51

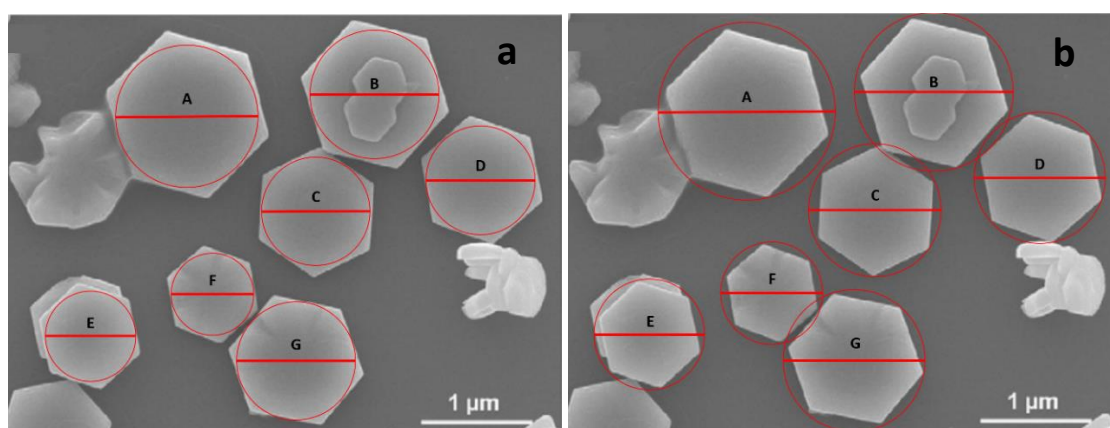**Figure 12.** SEM micrograph of hexagonal ZnO nanodisks (M. Wang *et al.,* 2008)

Figure 12 Measurements (a) minimum diameter (d_i), (b) maximum diameter (d_0) of hexagonal ZnO nanodisks

Table 10. Result of hexagonal diameter measurements

No. of hexagonal	Dmin Ratio	Dmin Length (µm)	Dmax Ratio	Dmax Length (µm)	Ferret Diameter (µm)
A	1 : 1.28	1.28	1 : 1.60	1.44	1.44
B	1 : 1.16	1.16	1 : 1.43	1.29	1.29
C	1 : 0.98	0.98	1 : 1.19	1.09	1.09
D	1 : 0.98	0.98	1 : 1.18	1.08	1.08
E	1 : 0.81	0.81	1 : 1.00	0.90	0.90
F	1 : 0.74	0.74	1 : 0.92	0.83	0.83
G	1 : 1.07	1.07	1 : 1.28	1.17	1.17

4.2.4.3 Step-by-step Diameter equivalent and shape factor Analysis Procedure

1) Step 1: Calculate length side (a) as shown in **Figure 11** by comparing the ratio of the rectangular size to the scale bar. Measurements were made as shown in **Table 11**.
2) Step 2: Calculate the Area and Perimeter of each shape by using equation in **Table 1** according to the shape of the sample as presented as (A = 1.5 $\sqrt{3}$ a²) and (P = 6a). Measurements were made as shown in **Table 11.**
3) Step 3: Calculate the diameter equivalent of the un-spherical shaped with the equate diameter value on the sphere with the same area by using equation 3 for d_a giving a diameter equivalent area size of ZnO nanodisks sample is 1.09 μm as shown in **Table 11.**
4) Step 4: Calculate of the shape factor such as sphericity by using equation 4, circularity by using equation 5, aspect ratio by using equation 6, and chunkiness by using equation 7. Measurements were made for each triangular as shown in **Table 11**.

4.3 Electron image analysis of fairly complex shape

4.3.1 Irregular shape

4.3.1.1. Morphology Analysis

Determination of the morphological shape of the sample is carried out in **Figure 19** on the native corn without any treatment sample. Based on the steps in determining the morphological shape of the sample from the SEM image, the following conclusions are drawn:

1) Step 1: Identify the type of detector when obtaining particle morphology in the SEM image. The morphology of the sample in **Figure 14** is obtained using a secondary electron detector.
2) Step 2: Identify to the light contrast and dark contrast of the SEM image. In **figure 14** for the dark contrast shows the native corn without any treatment.
3) Step 3: Determine the morphological form of the sample by looking at the image result from SEM characterization. The morphological shape of the native corn without any treatment sample shows a irregular shape as shown in **Figure 14.**

4.3.1.2. The Scale Bar and Diameter size Analysis Procedure

There are four steps in analyzing scale bar of the sample form of the TEM images as follows:

1) Step 1: Identify of the scales bar on SEM images. On that scale (represented by black short stripes) in **Figure 15**, it shows that scale bar in the image is equal to 20 μm in size.
2) Step 2: Identify diameter size of the particle by comparing with the scale bar. It can be seen from **Figure 15** that the particle size in the image is shorter than the scale bar, it means the particle size is smaller than 20 μm.
3) Step 3: Calculate ferret diameter maximum and ferret diameter minimum by comparing the ratio of diameter particle size to the scale bar. Measurements were made to each irregular particles as shown in **Table 12.**
4) Step 4**:** Calculate ferret diameter (average diameter of the sample) by using equation 1 as presented as $\frac{d_{max}+d_{min}}{2}$. The value is obtained by comparing the ratio particle diameter

size to the scale bar. Measurements were made on 7 irregulars giving an ferret diameter size of native corn without any treatment is native corn without any treatment as shown in **Table 12.**

4.3.2 Hollow shape

4.3.2.1 Morphology Analysis

Determination of the morphological shape of the sample is carried out in **Figure 11** of monodisperse hollow silica particles sample. Based on the steps in determining the morphological shape of the sample from the TEM image, the following conclusions are drawn:

1) Step 1: Identify the type of signal when obtaining sample morphology based on TEM images. **Figure 16** is determined by using the large of electron beam to avoid significant beam effect.
2) Step 2: Identify to the light contrast and dark contrast of the TEM image. In **Figure 16**, the dark contrast shows the monodisperse hollow silica particles.
3) Step 3: Determine the morphological form of the sample by looking at the image result from TEM characterization. The morphological shape of monodisperse hollow silica particles sample shows a hollow shape as shown in **Figure 16.**
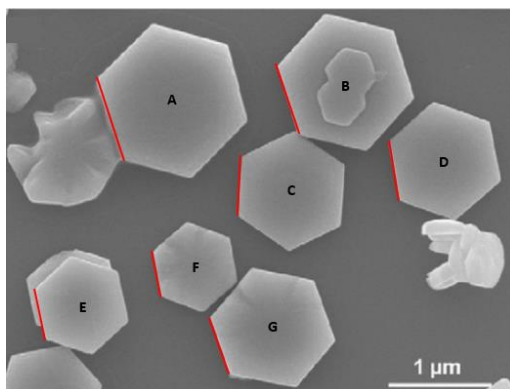

Figure 13. Measurements length side (a) of ZnO nanodisks images from SEM

Table 11. Result of cube Equivalent diameter and shape factor measurements

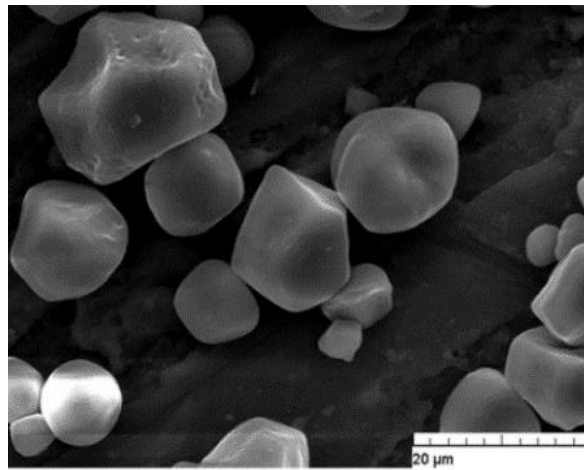| a (µm)	A (µm²)	P (µm)	d_a (µm)	φ	σ	AR	Ch
0.82	1.75	4.93	1.49	0.91	1.08	1.13	0.89
0.69	1.23	4.13	1.25	0.91	0.94	1.11	0.90
0.59	0.91	3.55	1.08	0.91	0.97	1.11	0.90
0.59	0.90	3.54	1.07	0.91	0.99	1.10	0.91
0.51	0.66	3.03	0.92	0.91	1.04	1.11	0.90
0.46	0.54	2.74	0.83	0.91	1.00	1.12	0.89
0.55	0.78	3.28	1.00	0.91	0.72	1.09	0.91

Figure 14. SEM micrograph of native corn without any treatment (Sujka and Jamroz, 2013)
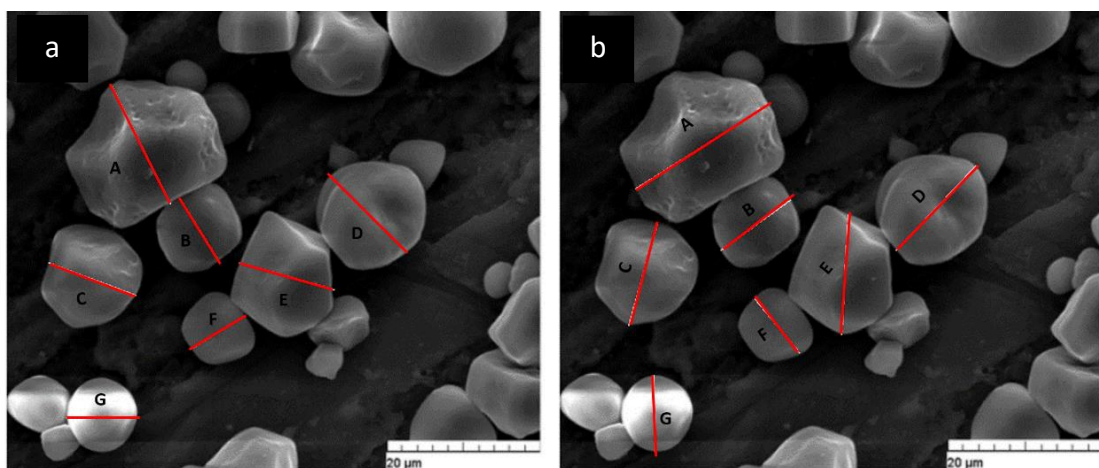

Figure 15. Measurements (a) minimum diameter (d_i), (b) maximum diameter (d_0) of native corn without any treatment sample images from SEM.

Table 12. Result of irregular diameter measurements

particle	d_0		d_i		Ferret diameter
	ratio	Length (nm)	ratio	Length (nm)	(µm)
A	1.02	20.31	0.86	17.23	18.768
B	0.56	11.14	0.49	9.71	10.427
C	0.68	13.70	0.61	12.24	12.9665
D	0.75	15.09	0.71	14.25	14.669
E	0.78	15.58	0.65	12.99	14.285
F	0.45	9.05	0.41	8.12	8.585
G	0.50	9.93	0.46	9.27	9.596

4.3.2.2 The Scale Bar and Diameter size Analysis Procedure

There are four steps in analyzing scale bar of the sample form of the TEM images as follows:

1) Step 1: Identify of the bar scales on TEM images. On that scale (represented by black short bars) in **Figure 1** it shows that bar in the image is equal to 200 nm in size.

2) Step 2: Identify diameter size of the particle by comparing with the scale bar. It can be seen from **Figure 16** that the diameter size in the image is larger than the scale bar. It means, the diameter size is larger than 200 nm.

3) Step 3: Calculate ferret diameter maximum and ferret diameter minimum by comparing the ratio of diameter particle size to the scale bar. Measurements were made to each irregular particles as shown in **Table 13.**

4) Step 4: Calculate ferret diameter (average diameter of the sample) by using equation 1 as presented as $\frac{d_{max}+d_{min}}{2}$. The value is obtained by comparing the ratio particle diameter size to the scale bar. Measurements were made on 7 monodisperse hollows giving an ferret diameter size of native corn without any treatment is 542.05 nm as shown in **Table 13.**
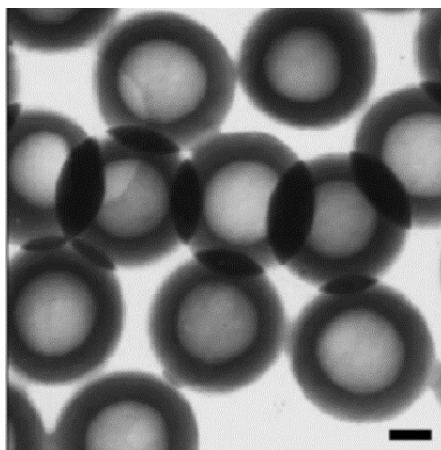

Figure 16. Monodisperse hollow silica particles (scale bar: 200 nm) (Hah *et al.,* 2003)
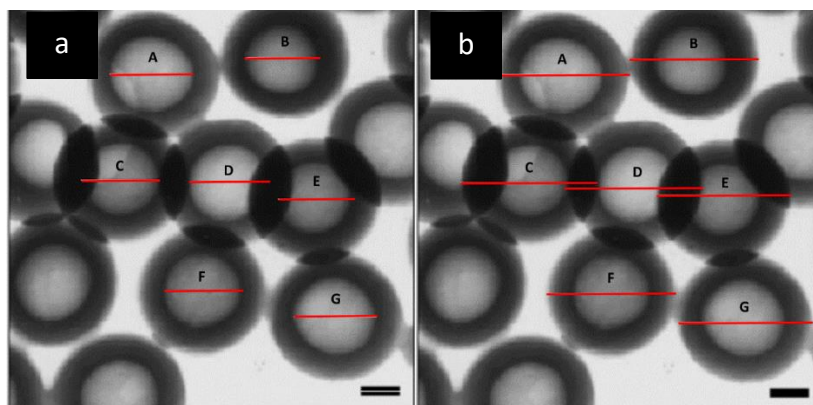

Figure 16. Measurements the (a) minimum diameter (d_i), (b) maximum diameter (d_0) of monodisperse hollow silica particles from TEM images.

4.3.2.3 Step-by-step diameter equivalent and shape factor analysis procedure

1) Step 1: Calculate minimum and maximum diameter as shown in **Figure 16** by comparing the ratio of the rectangular size to the scale bar. Measurements were made as shown in **Table 13**.

2) Step 2: Calculate the Area and Perimeter of each shape by using equation in **Table 1** according to the shape of the sample as presented as $(A = \frac{\pi}{4}(b^2 - a^2))$ and $(P = \pi(a + b))$. Measurements were made as shown in **Table 14.**

3) Step 3: Calculate the diameter equivalent of the un-spherical shaped with the equate diameter value on the sphere with the same area by using equation 3 for d_a giving a diameter equivalent area size of monodisperse hollow silica particles sample is 542.05 nm as shown in **Table 14.**

4) Step 4: Calculate of the shape factor such as sphericity by using equation 4, circularity by using equation 5, aspect ratio by using equation 6, and chunkiness by using equation 7. Measurements were made for each triangular as shown in **Table 14**.

Table 13. Result of particle diameter measurements

particle	d_i		d_0		Ferret diameter (µm)
	ratio	Length (nm)	ratio	Length (nm)	
1	1 : 2.10	420	1 : 3.36	665	515.58
2	1 : 1.77	355	1 : 3.20	645	538.52
3	1 : 1.98	395	1 : 3.45	690	565.75
4	1 : 1.93	385	1 : 3.43	685	566.57
5	1 : 1.83	365	1 : 3.35	670	561.85
6	1 : 1.95	390	1 : 3.23	645	513.74
7	1 : 2.08	415	1 : 3.38	675	532.35

Table 14. Result of cube Equivalent diameter and shape factor measurements

A (nm^2)	P (nm)	d_a (nm)	φ	σ	AR	Ch
208672.63	3406.90	515.58	0.23	0.60	1.58	0.63
227650.00	3140.00	538.52	0.29	0.70	1.82	0.55
251258.88	3406.90	565.75	0.27	0.67	1.75	0.57
251985.00	3359.80	566.57	0.28	0.68	1.78	0.56
247804.88	3249.90	561.85	0.29	0.70	1.84	0.54
207181.13	3249.90	513.74	0.25	0.63	1.65	0.60
222469.00	3422.60	532.35	0.24	0.62	1.63	0.61

5. CONCLUSION

This study describes the easiest way to understand electron microscopy image analysis. Various methods that can be used to analysis morphology and calculate diameter size of electron microscopy images have been presented in detail step-by-step. We believe that this paper can be used as basic knowledge for students and scientists in understanding how to analysis morphology and calculate diameter size of electron microscopy images.

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