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Research Paper

Shape Based Characterization of Nanoparticles – A Fuzzy Mathematical Approach

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This article presents a systematic study of the effect of size and shape on the spectral response of individual nanoparticles (NPs). When developing NPs as catalysts, their shape is very important. For a certain volume of material, nanoparticles make the best catalysts when they have a large surface area. It is a challenge to find the shape that has the largest surface area for its volume. The main focus of this paper is the interesting change in properties of the materials due to increase surface area to its volume. The particle shape contours were measured by transmission electron microscope with high resolution. These TEM images are analyzed with image clustering techniques and generalized shape theory that results the computational indicators for shape, degree of atomic compactness of NPs. These types of characterization help the researchers in size-based spectral tuning, biological labeling, and toxicity studies and suggest general protocols to address these problems.

Keywords: **Nanotechnology; Nanomaterial; Nanoimaging; Nano Synthesis; Fuzzy C-Means Clustering; Generalized Shape Theory and Metric**

Introduction

Nanometer ($1x10^{-9}$ m to $100x10^{-9}$ m) sized particles are of today's interests because of their shape and size-dependent (Karan *et al.*, 2012) physical properties. The chemical and physical properties of such aggregates, comprising only a few hundred atoms are in a transition region between the bulk and individual atomic or molecular properties (Dutta Majumder *et al*., 2007). By understanding size and shape related changes in these systems, it is hoped that advanced new materials can be developed together with a raft of new technologies (Bagchi, 2013). NPs with potentially useful size and shape (Majumder *et al*., 2011, Drexler, 1991) dependent properties have the advantage of ultra fine size, high surface area, useful interfacial defects and so are extensively utilized as key components in electronics and optical devices, pharmaceutics, paints, coatings, superconductors, semi conductors. In fact the reproducible preparation of shape controlled particles using the popular colloid-chemical approach is difficult. Using in-process measurement techniques and particle image analysis (Dutta Majumder, 1995), our research characterizes the synthesis of gold NPs improve compactness and spherical in shape.

Imaging beneath the surface of a sample has always been a challenge to microscopy as they cannot be seen in the traditional sense, but that should not prevent us from visualizing the nano world. The main goal of Nanotechnology (Wang *et al*., 2002; Zarur

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and Ying, 2000; Shi *et al.*, 1999) is to analyze and understand the properties of matter at the atomic and molecular level. Non-destructive, nanoscale characterization techniques are needed to understand both synthetic and biological materials (Andersen *et al.*, 2009; Douglas *et al.*, 2009). In this paper we propose new nanoscale image-based characterization techniques to analyze and synthesis nanoscale images that require algorithms to perform image analysis under extremely challenging conditions such as low signal-to-noise ratio and low resolution. To achieve this, we developed imaging tools that are able to enhance images, detect objects and features and analyze particle size and shape (Soo Ai *et al.*, 2012; Hieda *et al.*, 2008; Dutta Majumder 1995). Here we present the algorithms, describe their representative methods, and conclude with several promising directions of future investigation. Nano scale image processing allows us to understand unique properties of matter at atomic and molecular level spanning a wide range of applications in areas such as nano-bio-medicine, nano-chip manufacturing, material sciences, nano-agri-biotechnologies and environmental (Lehr *et al.*, 2011; Tarasenko *et al.*, 2010; Burello and Worth, 2011; Holden *et al.*, 2012; Greish *et al.*, 2012; Candeloro *et al.*, 2011; Donaldson *et al.*, 2004; Monteiro-Riviere and Tran, 2007).

Nanoscale Imaging Devices

Images at the nanoscale are obtained by measuring the reactive force resulting from the interaction of a nanoscale mechanical stylus-like probe with the specimen's surface. Atomic Force Microscopy (AFM) is an example of such a technique. In Fig. 1 of an image is limited by the radius of curvature of the scanning tip. Additionally, image artifacts can occur if the tip has two tips at the end rather than a

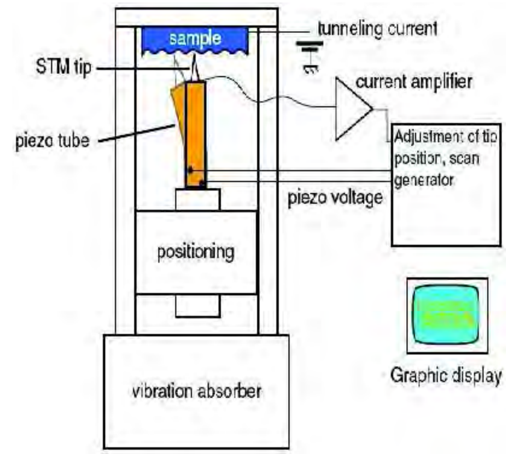


Fig. 2: Setup of a Scanning Tunneling Microscope

we illustrate the visualization range for the currently available major microscopes along with examples of structures that are visualized at those scales. For comparison purposes, the diagrams are also includes examples of the resolution range (Figs. 2 and 4) achieved by optical microscopy (Leung *et al.*, 2012; Ramachandra *et al.*, 2011; Schaper *et al.*, 2006; Yao and Wang, 2005).

We described the principles of operation of some electron microscopes of our interest. A high-quality resolution for an STM is considered to be 0.1 nm lateral resolution and 0.01 nm depth resolution and with this resolution, individual atoms within a sample can easily be imaged and manipulated. The resolution

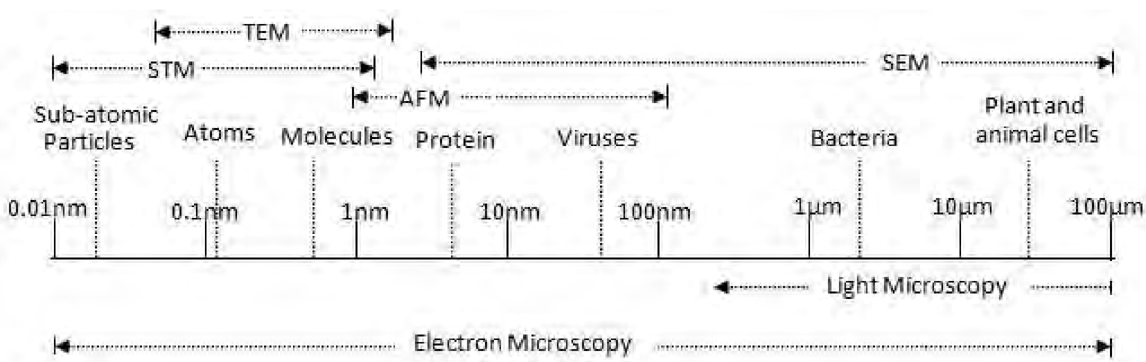


Fig. 1: Resolution range achieved by optical microscopy

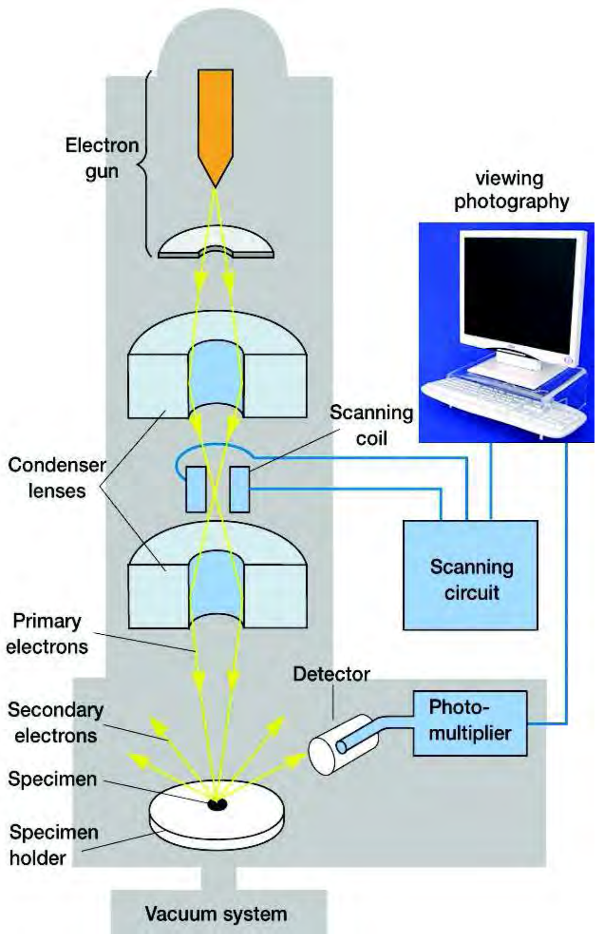


Fig. 3: Schematic Diagram of an SEM

single atom; this leads to "double-tip imaging," a situation in which both tips contribute to the tunneling.

Scanning Tunneling Microscope (STM)

The STM tip is mounted on a piezotube, which is deformed by applied electric fields. This deformation translates into lateral and vertical manipulation of the tip. Through an electronic feedback loop the position of the tip is adjusted according to the tunnelling current (constant current mode) and a two-dimensional current contour is recorded. This contour encodes all the information about the measurement.

The Scanning Electron Microscope (SEM)

SEM uses electrons reflected from the surface of a specimen to create a 3-dimensional image of specimen's surface features.

Atomic Force Microscopy (AFM)

Sample mounted on a piezoelectric scanner is scanned against a short tip and the cantilever deflection is usually measured using a laser deflection technique. The force (in contact mode) or the force gradient (in noncontact mode) is measured during scanning. Probe can touch the surface. Maintains a constant very small force.

High resolution (x-y: 2-10 nm, z: 0.1 nm) Suitable for all surfaces. In contrast to the micro-fabrication

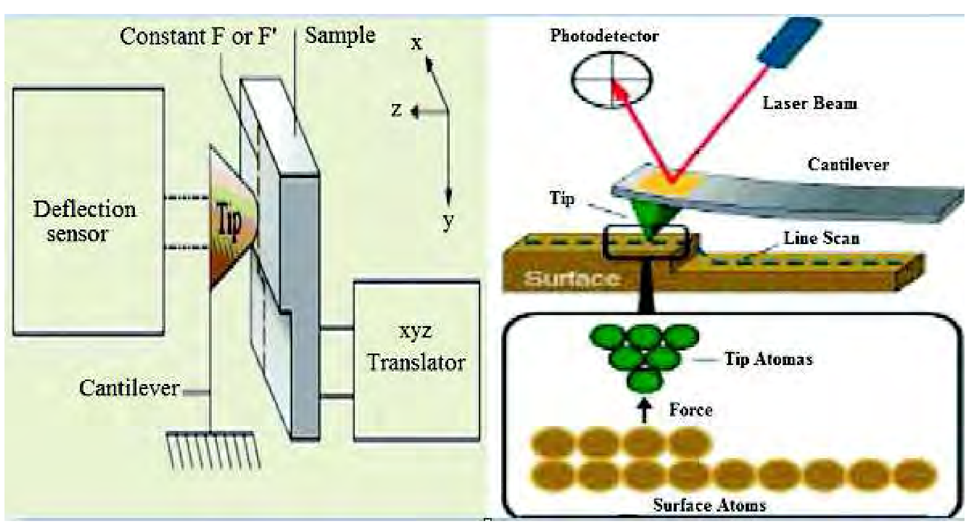


Fig. 4: Schematic Diagram of an AFM

technology as in semiconductor and MEMS (Micro Electromechanical Systems) industries nano fabrication utilize “top down“ and “bottom up“ methods. Shape, surface area, ratios of volume and surface area are very important properties along with self assembly. Structures are self assembled by taking advantage of these and some not yet fully known properties, that are being inspired by ‘nature’ as biological structures are typically self assembled at the nano scale range using molecular interactions such as Van Der Walls Force, Electrostatic force, Hydrophobic, Hydrogen bond.

Materials and Methods

Thiol, aspartic acid, Citrate protected gold nanoparticles of different size and shape were synthesized in the laboratory. Transmission Electron Microscopy (TEM) and spectroscopy study for Imaging and particle size distribution of Gold NPs were performed to observe the topology of the particles. These images are processed by fuzzy logic based clustering techniques. Mean diameter and compactness are measured automatically through algorithms by counting pixels belonging to every cluster. Surface(S) to Volume (V) ratio was measured considering the NPs are spherical in nature. Packing density is obtained by measuring the imperfections/ defects from its segmented TEM images. Results are observed, compared with existing method and chemical measurement and manual counting.

Nano scale Image Data Clustering Using Fuzzy C-Means (FCM)

TEM Image data of Gold NPs were (20 nm) classified using fuzzy C-Means clustering (James C. Bezdek, 1984; Karan *et al*., 2011) algorithm to separate the particle from its background, identify the defect and also the molecular arrangement inside the particle and developed an iterative optimization procedure for classification. Let X = $\{X_1, X_2, \ldots X_n\}$ be a set of samples to be clustered into *c* classes. Here we consider color as a feature for classification in RGB (red, green, blue) color space. The criterion function used for the clustering process is

$$J(V) = \sum_{k=1}^{n} \sum_{x_k \in C_1} | x_k - v_i |^2 \tag{1}$$

Where v_i, is the sample mean or the center of samples of cluster *i*, and $V = \{v_1, ..., v_c\}$. To improve the similarity of the samples in each cluster, we can minimize this criterion function so that all samples are more compactly distributed around their cluster centers. Membership values (µ’s) are assigned as per FCM algorithm.

In summary, the c-means clustering procedure consists of the following steps:

- S-1: Determine the number of clusters c.
- S-2: Partition the input samples into c clusters based on an approximation. If no rule of approximation exists, the Samples can be partitioned randomly.
- S-3: Compute the Cluster Centers
- S-4: Assign each input sample to the class of the closed cluster center.
- Repeat steps 3 and 4 until no change in *J* can be made and the algorithm converges.

For cluster validity, we consider three types of measures: partition coefficient, partition entropy and compactness and separation validity function.

Shape Based Image Registration of Gold NP’s

The perception of shape has been used for pattern recognition, computer vision, shape analysis (Soo Ai *et al*., 2012; Dutta Majumder 1995), and image registration. Here we proposed a generalized method of shape analysis and shape based similarity measures, shape distance and shape metric to measure the NPs shape. The shape of an object can be defined as a subset X in R^2 if (a) X is closed and bounded, (b) Interior of X is non-empty and connected and (c) Closure property holds on interior of X. This representation of shape remains invariant with respect to translation, rotation and scaling. Moreover another object Y in R^2 is of same shape to object X R^2 if it preserves translation, rotation and scaling invariance.

In term of set these three transformations can be represented as

Translation : $Y = \{(x + a), (y + b): x, y \;\; X\}$ (2)

Rotation : $Y = \{P1().P2()X\}$ where P1 & P2 are rotation around x and y axes. (3)

Scaling : $Y = \{(kx, ky): x, y \;\; X\}$ (4)

Distance d_1 between shape X and Y in F is defined as follows:

$$d_1(X,Y)=m_2[(X-Y)(Y-X)] \quad (5)$$

where m_2 is Lebesgue measure in R^2 and d_1 satisfies following rules: (i) $d_1(X,Y)\ 0$, (ii) $d_1(X,Y) = 0$ if and only if $X = Y$ (iii) $d_1(X,Y) = d_1(Y,X)$ and (iv) $d_1(X,Y) + d_1(Y,Z) \;\; d_1(X, Z)$. We consider, two nano particles are of same shape if and only if one of the image is translation, scaling and rotation of other.

- Shape extraction and similarity measure

To extract the feature of the boundary of the Region of Interest (ROI) it is helpful to represent the closed contour with a set of direction. The direction code may be taken among "n" selected points on the contour, which has same distance between any two consecutive points. The direction d makes an angle 45 with direction i, where real number d 1 to 8 and i = (1,2,..8). Let $d_m = (d_{ij})$, j = 1 to n where m = A, B are the contour starting from each reference point A and B and are denoted by d_A and d_B respectively (Fig. 5). If d_2 is a rotation of d_1 then $d_2 = d_1 +$ for any real number. For all j we can write $d_2 = d_1 + j$ and the distance function D, in terms of the direction code between the contour of interest and the model is defined as:

$$D(d_1 d_2)=\sum_{J=1}^{n} \min((d_{1j} d_{2j}),8-(d_{1j} d_{2j})) \quad (6)$$

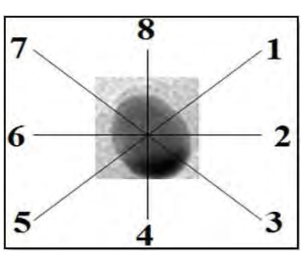


Fig. 5 Chain code representation

The normalized value of D is D/n and the shape similarity measure between the two shapes is given by = 1-D/n, smaller value of D indicates higher degree of similarity.

Experimental Procedure and Results

Nanoparticles Synthesis

- Synthesis of thiol protected gold nanoparticle

Aqueous solution of $HAuCl_4$ is mixed with solution of ToABr in toluene. Vigorous stirring causes transfer of $HAuCl_4$ into the organic layer. Dodecanethiol is added to the organic phase followed by addition of aqueous solution of NaBH4 is slowly added with vigorous stirring. Aqueous solution of sodium borohydride is slowly added with vigorous stirring. A deep brown coloured solution appears at the interface. Excess ethanol is added to the separated brown coloured solution and kept overnight which causes precipitation of GNP. The ethanol solution is filtered with nylon filter and the precipitate is re-dispersed in toluene. Imaging of NPs and its spectral responses are shown in Fig. 6.

- Synthesis of aspartic acid protected gold nanoparticles

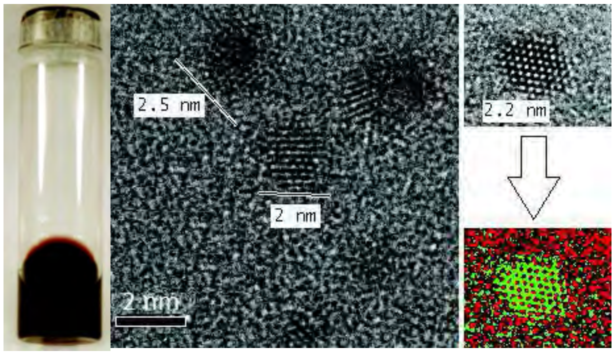
90 ml of 10^{-4}M aqueous solution of chloroauric acid is prepared. The solution is heated up to boiling condition. 10 ml of 10^{-2}M aspartic acid solution is added to the boiling solution. Aspartic acid acts as the reducing agent. The reduction process is continued under constant stirring. Heating is stopped. The reduction of the metal ions is evident with appearance of red color. Imaging of NPs and its spectral responses are shown in Fig. 7.

- Synthesis of Citrate capped gold nanoparticle

50 ml of 1mM aqueous solution of chloroauric acid is prepared. The solution is heated up to boiling under reflux condition. 5 ml of 1% tri sodium citrate dihydrate solution is added to the boiling solution. Sodium citrate here reduces the gold chloride. Stirring was continued until the color of the solution gradually changed from faint yellowish to clear to grey to purple to deep purple, and finally wine-red. Negatively charged citrate ions were absorbed onto the GNPs,

introducing the surface charge that repels the particles and prevents them from aggregation. Imaging of NPs and its spectral responses are shown in Fig. 8.

Imaging and Characterization of Capped Gold Nanoparticles



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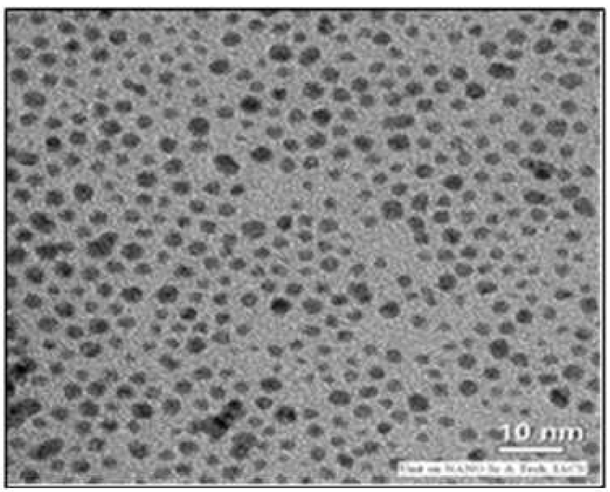

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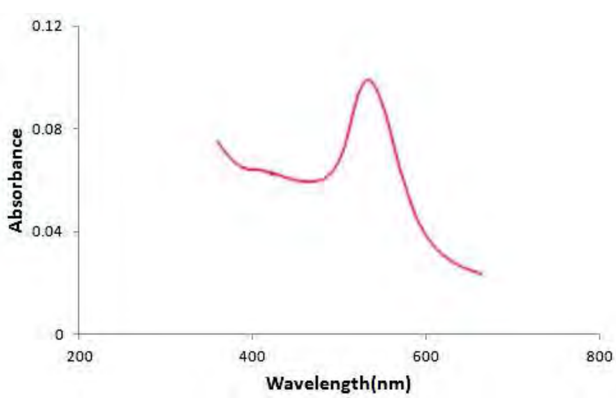
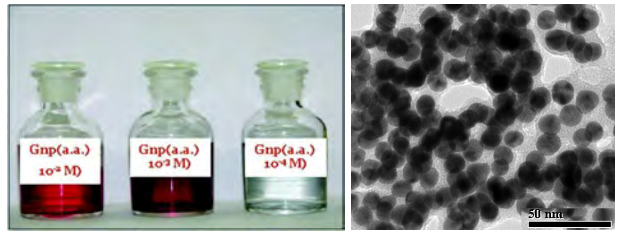


D

Fig. 6: A: Interface colour of thiol capped gold nanoparticle, B: TEM image of thiol capped GNP 2nm, C: TEM image of thiol capped GNP 3-5nm and D: Surface Plasmon peak of thiol capped gold nanoparticle



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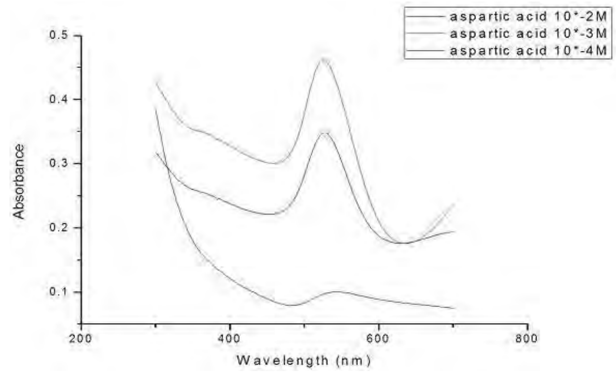

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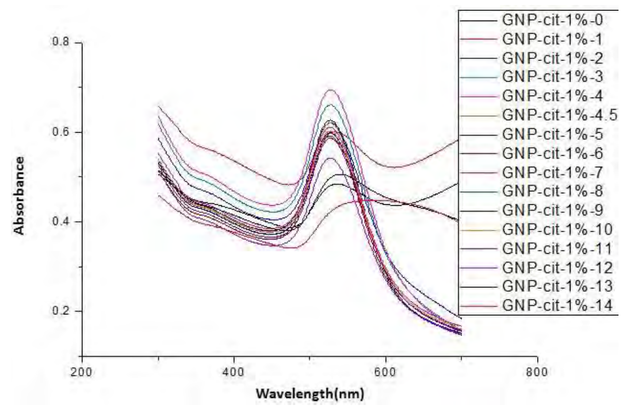
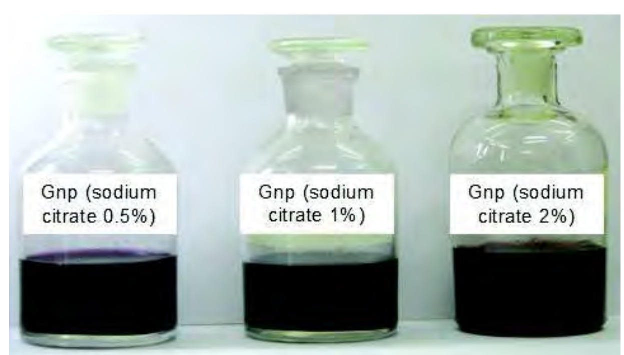


D

Fig. 7: A: Interface colour of aspartic acid capped gold nanoparticle, B: TEM image of aspartic acid capped GNP 13-20nm, C: Surface Plasmon peak of aspartic acid capped gold nanoparticle and D: pH stability study of aspartic acid capped gold nanoparticle



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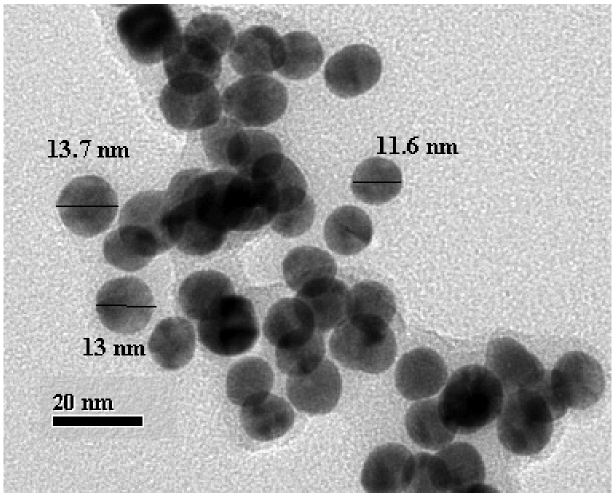

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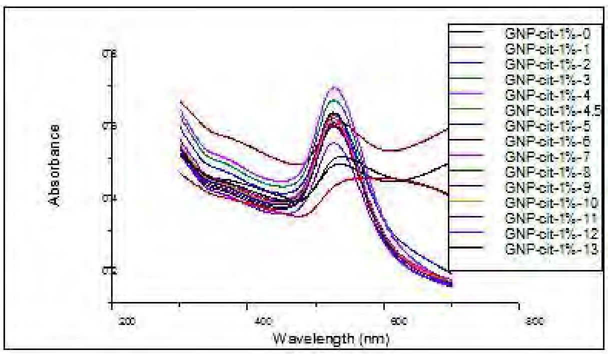
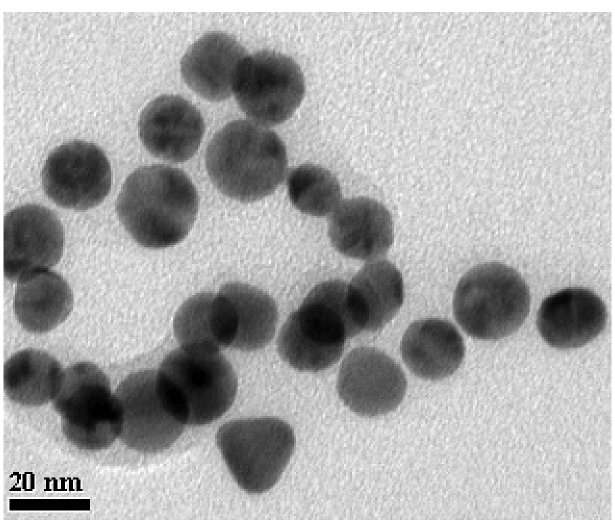


C

Fig. 8: A: Interface colour of Citrate capped gold nanoparticle, B: TEM image of Citrate capped gold nanoparticle, 11-13nm and C: pH stability study of Citrate capped gold nanoparticle



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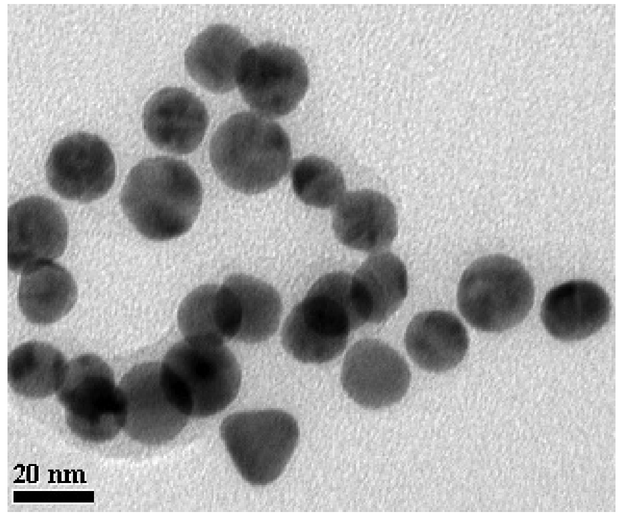


B

Fig. 9: A: Original TEM Image of Gold nanoparticles 20nm, B: Segmented TEM Image of Gold nanoparticles 20 nm in 3 classes

Shape tracing and size distribution of Gold Nanoparticles using Fuzzy C-Means Clustering and Generalized Shape Theory

The digital image of gold nanoparticles have been analyzed with the help of FCM, to isolate the image from the background and generalized shape theory to

Table 1: Pixel value with membership after clustering into 3 classes, with cluster centre

R	G	B	Initial assignment			After 9th iterations		
			μ_1	μ_2	μ_3	μ_1	μ_2	μ_3
111	111	111	0.37	0.1	0.53	0.02	0.72	0.26
111	111	111	0.12	0.74	0.14	0.02	0.72	0.26
127	127	127	0.86	0.1	0.04	0	0.99	0
131	131	131	0.61	0.1	0.29	0	1	0
145	145	145	0.36	0.1	0.54	0.04	0.92	0.05
160	160	160	0.11	0.74	0.15	0.19	0.71	0.1
183	183	183	0.85	0.1	0.05	0.66	0.27	0.07
212	212	212	0.6	0.1	0.3	0.99	0	0
218	218	218	0.35	0.1	0.55	1	0	0
206	206	206	0.1	0.74	0.16	0.97	0.02	0.01
207	207	207	0.84	0.1	0.06	0.98	0.02	0.01
215	215	215	0.59	0.1	0.31	1	0	0

Cluster Centre

Color	V1	V2	V3
R	87.3	46.5	11.9
G	13.3	83.9	38.9
B	52.8	12.8	91.3

fins the shape as well as surface area as shown in Figs. 9 and 10. The defect, surface area and surface area to volume ratio have been calculated considering the pixel count, the result shown in Table 1 and in Figs. 11, 12.

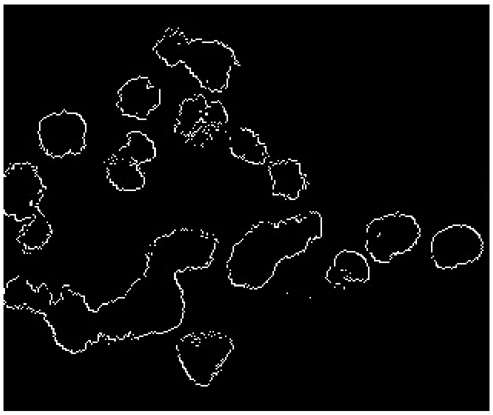


Fig. 10: Trace of Gold nanoparticles 20nm for its shape measurement in TEM image

Conclusion

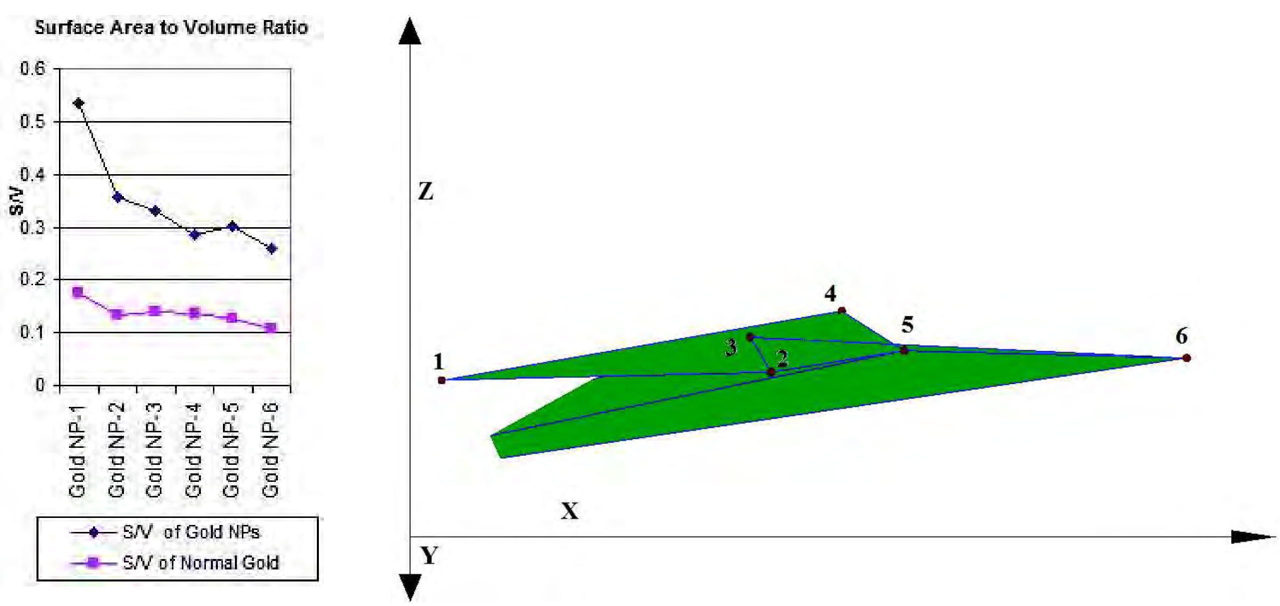
The process of isolating important regions of an image into components is generally referred as image segmentation. We have presented a novel and simple method for measurement of the size, shape and other spectral response of gold NPs. In this paper to isolate the gold NPs from its backgrounds and also to find the overlapping regions in the image we have applied fuzzy c-means clustering algorithm on TEM images as shown in Fig. 9A and B. Pixel wise measurement of the area and diameter of NPs gives accurate and better results in dimensioning process. Though we have concentrated on gold NPs, but our theory is general and can be applied on any type of classification problem. The Polymer based nanocomposites

Packing Density and S/V Measurement of Gold NP's (2nm)

NP ID	Origina lImage	Segmented Image (Cluster:2)	D	A	T	PD	S/V of Gold NPs	S/V of Normal Gold particle	S/V Increase Factor
GNP-1			489	439	928	0.325	0.537	0.175	3.07
GNP-2			794	840	1634	0.369	0.356	0.132	2.71
GNP-3			628	815	1443	0.424	0.330	0.140	2.36
GNP-4			594	926	1520	0.476	0.287	0.136	2.10
GNP-5			804	1002	1806	0.413	0.303	0.125	2.42
GNP-6			1054	1346	2400	0.420	0.258	0.109	2.38

GNP: Gold NP's , D: Defect Area , A: Actual Area, T: Total Area , PD: Packing Density

Fig. 11: Calculation Table for S/V of Gold NPs of 2nm range with its normal value



A B

Fig. 12: A: Comparison of S/V of Gold NPs of 2nm range with its normal value and B: Surface plot of Packing Density. X-Defect, Y-molecule occupied, Z-Packing Density

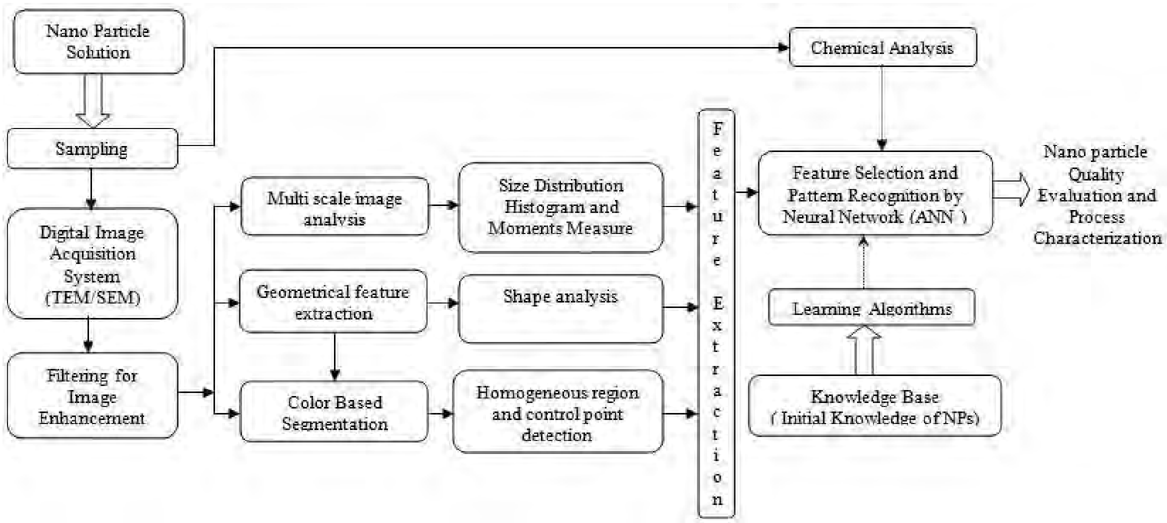


Fig. 13: Proposed architecture of NPs in process characterization

preparation (Evans *et al*., 2006; Park *et al*., 2012) offer the advantage of (a) a cluster or atomic level control, and (b) an efficient scale up for processing and production has a lot of potential application in nano structured material preparation. Here the size and shape of the nanoparticles are measured using fuzzy based clustering and shape analysis and gives better results. The high degree of particle size and shape measurement in the nm range suggest a successful application of this method to conclude the signature of (Walls *et al*., 1985; Chu, 2002) occurring in the nano particles, realization of colour filters, UV absorbers with particularly interesting performances. These values may be used as the input of Artificial Neural Networks (ANN) to characterized the synthesis process of gold NPs as proposed in Fig. 13. The ANN offers a successful tool for NPs preparation analysis and modeling. The flexibility offered by the choice of different polymeric agents as a future element of interest towards future advances in nano-optical and nano-bio applications.

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