



## 3-Dimensional Analysis of Microstructure Images Acquired through Serial Sectioning of a Material

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**Abstract---** *It is established by metallurgical experts that the careful study of microstructure of material is the most important step in material manufacturing. Generally, the study of microstructure is accomplished using stereological relations applied on two dimensional (2D) microstructure images to provide three-dimensional (3D) information. But classical 2D methods determine the information from microstructure images to a limited extent. The ongoing developments in image processing, computer vision and 3D analysis methods along with development in automatic serial sectioning devices have made 3D analysis of microstructure images of material much more practical and viable [1]. The serial sectioning is a technique that slices the material, layer by layer for imaging and their analysis. The 3D methods are inevitable in such applications those need precise insight information about the material.*

*In this paper, a novel automatic 3D method of digital image analysis for deriving important quantitative information from the microstructure images of a material, that are acquired through serial sectioning method has been proposed. The derived quantitative information of microstructure includes number of graphite grains, mean diameter, mean aspect ratio, mean length and mean volume of graphite grains. The results obtained by the proposed method are compared with the results obtained by classical 2D method based on stereological relations. The results of proposed method are accurate and close to practical limits and much superior to the results obtained by their counterpart 2D methods. The proposed method is expected to be useful in manufacturing and quality control practices.*

**Keywords:** 3-Dimensional microstructure analysis, 2D classical methods, serial sectioning, graphite grains.

### I. INTRODUCTION

The microstructure properties of graphite grains, namely, mean diameter, mean aspect ratio, mean length and mean volume of graphite grains are strongly related to the mechanical properties of the material [1,12,15,16,17]. Microstructure properties derived by characterization and analysis of microstructure images provide vital information for manufacturing and quality control practices. The analysis of microstructure images is accomplished by two ways, namely, 2D and 3D analysis. The 2D analysis techniques use single polished sections of material surface for acquiring microstructure images and apply the classical stereological relations for deriving quantitative information that define indirectly 3D structures [12,15].

On the other hand, in 3D metallographic techniques, a series of 2D images are acquired by serial sectioning of material and these images are used to reveal the internal 3D structures of materials that lie buried within an opaque material. The 3D microstructure data has utmost importance because it reveals significant geometric and topological quantities, which cannot be determined easily with the help of 2D images and classical stereological methods [1,2,5,18]. The literature on microstructure analysis indicates that the 3D analysis of microstructure is attempted for the first time in 1918 by Forsman's to understand the 3D structure of pearlite [2]. Similar significant efforts are made in 1962, by Hillert and Lange [3]. They produced a motion picture of serial section to show the true 3D structure of an entire pearlite colony in material microstructure. In 1964, Eichen has studied growth of ferrite by measuring the changing length of plates with increasing depth through serial sections [4]. In 1965, Hopkins and Kraft [5], in 1967 Howbolt and Brown [6], in the same year, Barrett and Yust showed the interconnectivity of voids in a sintered copper powder [7], Ziolkowski in 1985, used "mikrotom" to perform a serial sectioning study of grain boundary precipitates in an alpha/beta brass alloy [8].

Most of the aforementioned 3D analysis methods are manual based and their results were represented by hand-drawn sketches and graphical plots of length versus depth. They have provided results that are close to accurate results. Recent advancement in image processing [14] and 3D visualization capabilities, along with development of automatic sectioning devices, has made 3D analysis of serial sections much more practical. It is ongoing continuous joint effort of material science and computer vision experts that enabled to derive complete and unbiased data of microstructure. The field of materials characterization is enormously developing and inculcating methods that provide precise microstructural information in three-dimensions. For the first time in 1991, Hull et al., were among the first to use computer software to contain 3D wire-frame drawings of microstructure features [9]. Brystrzycki and Przetakiewicz in 1992 have used similar technique to study sizes and shapes of annealing twins in Ni-Mn alloy [10].

In 1994, in a significant effort, Wieland et al. have used SEM images of aluminum-manganese alloy for 3D analysis [11]. Though the effort required for serial sectioning and subsequent 3D analysis may always be a concern but it is

acceptable because of the capability of 3D analysis methods, which provide important new insights into microstructural evolution that led to new avenues of research [18]. In this paper, an automated method for 3D analysis of microstructure of materials is proposed, with a focus for assessing the size and other quantitative information of graphite grains based on microstructure images acquired using serial sectioning of a material sample. The serial sectioning technique is described below:

### Serial Sectioning

The serial sectioning is the technique that involves slicing the material layer by layer out of a material sample, followed by imaging each layer using microscope and camera, and then analyzing each image of successive layers [1]. The series of images acquired through serial sectioning of the material and 3D reconstruction of material image are depicted in Fig.1. For opaque materials, serial sectioning has been the most widely used method to acquire raw 3D characterization data at the macro-to-micro scale. The serial sectioning experiments are conceptually simple, being composed of two steps that are iteratively repeated until completion of the experiment. The first is to prepare a nominally flat surface, which can be accomplished by a variety of methods – a non-inclusive list includes cutting, polishing, ablating, etching, and sputtering – where ideally a constant depth of material removal has occurred between each section. The second step is to collect two-dimensional (2D) characterization data after each section has been prepared, although data could also be collected continually during material removal depending on the particular sectioning method that is employed. After collection of the series of 2D data files, computer software programs are used to construct a 3D array of the characterization data that can be subsequently rendered as an image or analyzed for morphological or topological parameters. The 2D characterization data collected during a serial sectioning experiment can be comprised of number of different types and/or quantities of information. For example, in the particular case of characterizing grain microstructures, this could consist of using optical microscopy to image the structure of etched grain boundaries.

## II. MATERIALS USED

Cast iron (2%-C) bars were chosen for metallographic evaluation. The samples were polished using standard mechanical techniques using silicon carbide abrasives in accordance with ASTM standard E3-01. The mounted specimens were final-polished using colloidal silica media with a 0.05  $\mu\text{m}$  particle size material, which is serial sectioned and then the microstructure images of resolution 400x300 pixels are acquired using light optical microscope.

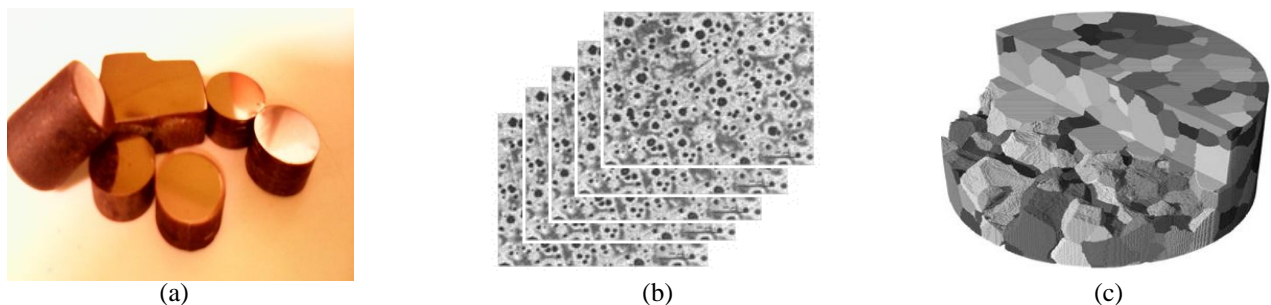


Fig.1: (a) Cast iron material pieces, (b) Stack of serial sections of material and (c) 3D simulated-reconstructed material image.

## III. PROPOSED SYSTEM

The sample is cut from the top and polished by following serial sectioning technique. In the proposed system, a square region of interest is selected from the sample by indenting four equally spaced marks as shown in (Fig.2), called fields. Using this subjective criterion, the size of the microstructural region of interest was taken as approximately 250 X 250  $\mu\text{m}$ , depth is of approximately 160  $\mu\text{m}$  and the size of each field is 50x50  $\mu\text{m}$ . The thickness of each slice is of 1.0  $\mu\text{m}$ . Then, the exposed surface is polished using silicon carbide abrasives.

The mounted specimens were final-polished using colloidal silica media with a 0.05  $\mu\text{m}$  particle size and microstructure image of 400x300 pixels is acquired from each of the five different fields of surface using light optical microscope. The process is repeated to get 60 images from each field by serial sectioning of material from top to bottom. A total of 300 microstructure sectional images are acquired. The probable noise in microstructure images is filtered by applying selective median switching filter [13].

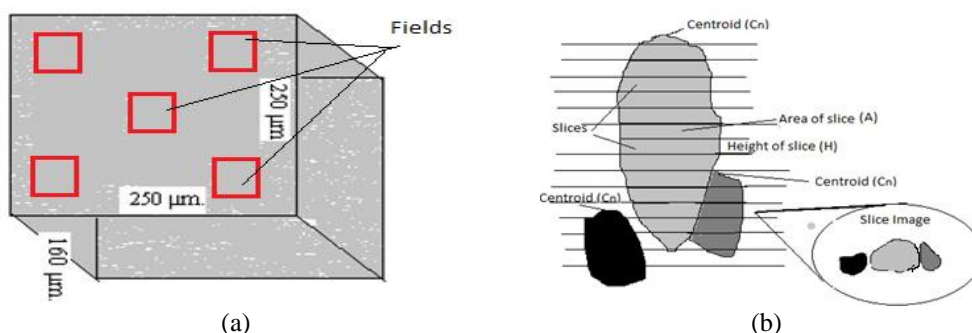


Fig.2: (a) The 5 distinct fields on surface of the material sample marked for acquiring microstructure images using light optical microscope, (b) A sketch depicting graphite grains in slice image.

The sample microstructure images and respective filtered images are shown in Fig.3. Then, each microstructure image is segmented using Otsu's segmentation technique [19] and each black region which is potentially a graphite grain is labeled. The centroid of each labeled graphite particle is registered. The centroid of each of the grain will serve as index in quantification process. The centroid value of a graphite grain, say,  $C_n$  in  $n^{th}$  and  $(n+1)^{th}$  image indicates it is single grain.

If a graphite particle with centroid  $C_n$  present in  $n^{th}$  image and the same  $C_n$  is not found in  $(n+1)^{th}$  image, then it indicates that the grain is completed in the  $n^{th}$  image. If, after a gap of image(s), a graphite grain with same centroid  $C_n$  is found in subsequent image, then it will be regarded as new graphite grain and its measurements are separately accounted. The framework for the proposed system is shown in the Fig. 4. The volume of each of the particle is determined using Eq.1:

$$\text{Volume of graphite grain with } C_n = \Sigma = \left( \frac{A_n + A_{n+1}}{2} \right) * H * N \quad (1)$$

where,  $A_n$  and  $A_{n+1}$  are area of graphite grain in the  $n^{th}$  slice and  $(n+1)^{th}$  slice, respectively,  $H$  is height (or depth) of each slice and  $N$  is number of slices in which the graphite grain appeared.

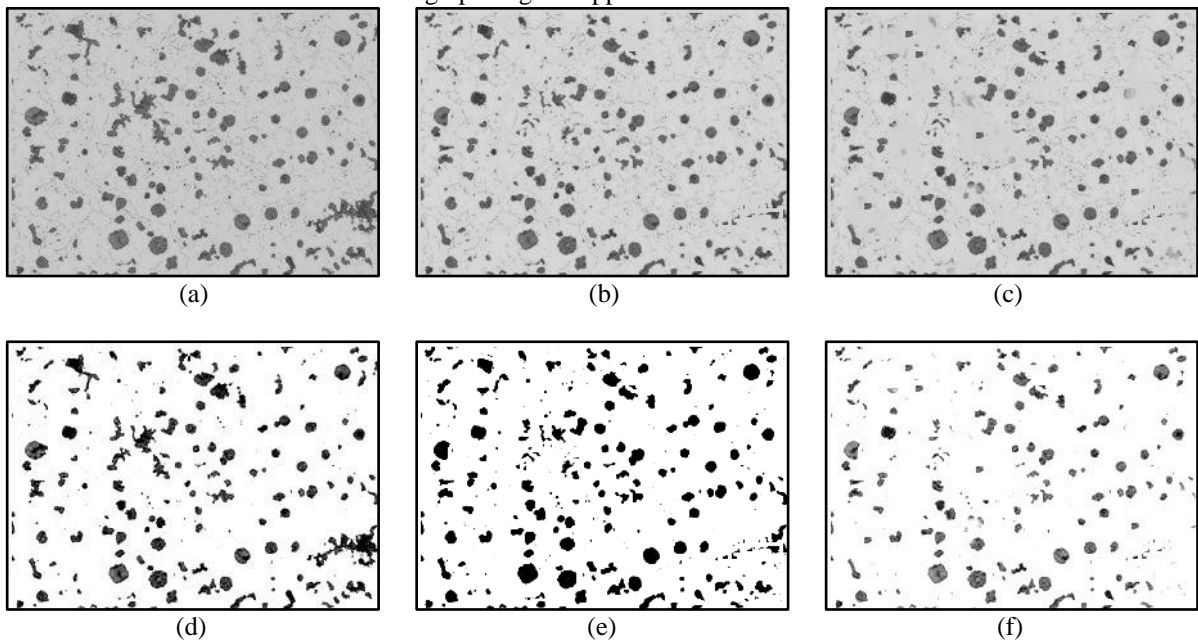


Fig.3:(a)-(c)Microstructure images of sections of cast iron material acquired by serial sectioning, (d)-(f) Results of de-noising and segmentation of microstructure images in (a)-(c).

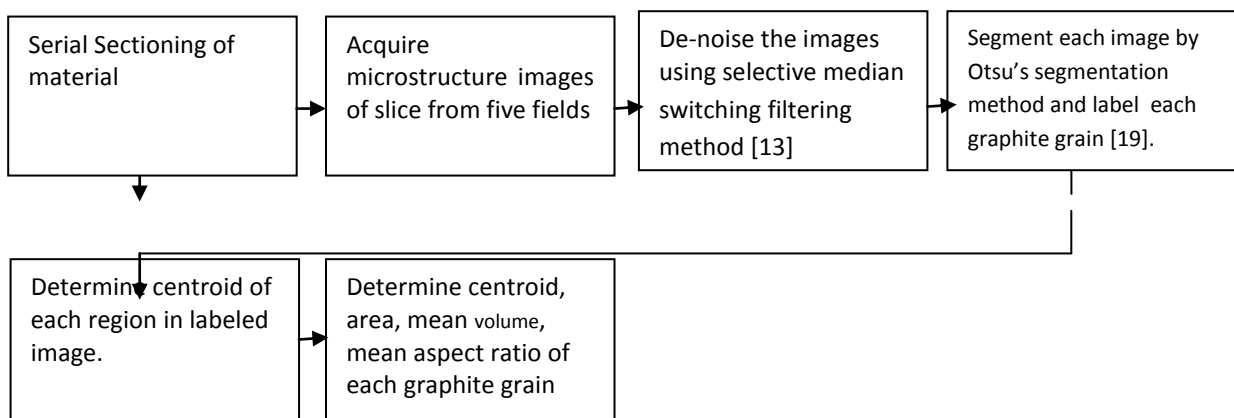


Fig.4: The proposed framework for the proposed system.

The algorithm for the proposed method of 3D analysis of microstructure images determining quantitative information.

**Algorithm 1:**

- Step 1: Input the RGB microstructure image of slice and convert it into grayscale image.
- Step 2: Apply 'selective median switching filter' method [13] for de-noising the image.
- Step 3: Segment the image using Otsu's segmentation method and obtain granular region and background region. Label the filtered and segmented image.

Step 4: Eliminate the border touching grains.

Step 5: Each labeled region is a graphite particle and register centroid ( $C_n$ ) of graphite particles.

Step 6: \*Determine the area of each graphite particle until the grain disappears from its centroid position. Record the area of each graphite grain.

Step 7: Repeat Step 1 to Step 6 for each microstructure image.

Step 8: Determine number of particles measured, the volume of each particle and then mean volume of graphite grains and aspect ratio of graphite grains.

\*If a graphite particle present in  $n^{th}$  image is untraced in  $(n+1)^{th}$  image at the respective centroid position, then it is understood that the grain is completely scanned for analysis. If a particle having the same (x-y) coordinates of disappeared particle then register the particle as new particle and record the area separately.

#### IV. EXPERIMENTAL RESULTS AND DISCUSSION

For the purpose of experimentation, the microstructure images of cast iron (2% C) bar samples (Fig.1(a)) are used. The 300 microstructure images are acquired from the material by serial sectioning. These images are analyzed both by classical 2D and proposed 3D analysis methods for comparison of the performance of both methods. The quantitative information of graphite grains is determined. The implementation of the proposed method has been done on a Pentium Dual Core computer system @ 2.6 GHz using MATLAB R2009b. The Tables I through V show area of five sample graphite grains in microstructure images of slices acquired by serial section method from five distinct fields. The Table VI shows quantitative information, namely, number of grains, mean diameter, mean length, mean aspect ratio and mean volume of material sample. The results obtained by proposed method are compared with the results obtained using 2D classical stereological method. It can be inferred from the comparison that the quantitative information determined by proposed 3D analysis method is more accurate and natural representative of microstructure than the classical 2D method. The 2D methods provide only approximate quantitative volumetric information about material properties. It is due to the fact that the 2D analysis is made only on few sample surface microstructure images.

Table I Area of five sample graphite grains in microstructure images acquired by serial section method from field#1

Centriod $C_n$ of grains	Area of grains ( $\mu\text{m}$ )														
	Microstructure images														
	1	2	3	4	5	6	7	8	9	10	.....	58	59	60	
(176,13)	13	14	2	14	10	2	5	9	15	15	.....	2	NF	NF	
(322,8)	10	5	5	NF	NF	NF	*14	12	10	6	.....	NF	NF	NF	
(320,236)	NF	NF	*15	2	12	11	11	10	6	6	.....	8	NF	*6	
(123,171)	3	15	15	8	13	3	7	14	12	15	.....	10	9	6	
(197,99)	2	11	10	10	NF	NF	NF	NF	NF	NF	.....	NF	NF	NF	
.....															

Table II Area of five sample graphite grains in microstructure images acquired by serial section method from field #2

Centriod $C_n$ of grains	Area of grains ( $\mu\text{m}$ )														
	Microstructure images														
	1	2	3	4	5	6	7	8	9	10	.....	58	59	60	
(259,257)	10	2	5	9	15	15	3	15	15	8	.....	3	7	14	
(185,190)	9	15	15	3	15	15	8	13	3	7	.....	12	NF	*10	
(174,250)	2	5	9	15	NF	*3	7	15	8	13	.....	7	14	12	
(74,111)	12	15	10	1	13	15	11	12	12	6	.....	NF	NF	*2	
(397,94)	1	2	2	2	9	11	5	15	1	NF	.....	12	12	3	
....															

Table III Area of five sample graphite grains in microstructure images acquired by serial section method from field #3

Centriod $C_n$ of grains	Area of grains ( $\mu\text{m}$ )														
	Microstructure images														
	1	2	3	4	5	6	7	8	9	10	.....	58	59	60	
(78,246)	2	5	9	15	NF	*3	7	15	8	NF	.....	3	7	15	
(353,264)	12	15	10	1	13	15	11	12	12	6	.....	15	8	13	
(148,196)	1	5	1	2	13	1	NF	*2	2	7	.....	3	7	NF	
(18,34)	15	3	8	10	8	13	3	1	13	15	.....	15	11	12	
(231, 14)	12	15	10	1	12	15	10	12	12	6	.....	11	5	15	
.....															

Table IV Area of five sample graphite grains in microstructure images acquired by serial section method from field #4

Centriod $C_n$ of grains	Area of grains ( $\mu\text{m}$ )													
	Microstructure images													
	1	2	3	4	5	6	7	8	9	10	.....	58	59	60
(224,289)	6	7	9	12	9	3	7	15	8	NF	.....	NF	NF	*2
(347,18)	2	9	10	1	13	15	11	12	12	6	.....	3	4	9
(358,160)	1	2	2	2	9	11	5	15	1	NF	.....	12	2	NF
(32,220)	10	10	7	5	8	10	3	NF	8	NF	.....	7	5	8
(134,165)	NF	NF	NF	NF	*10	10	7	5	8	10	3	2	8	NF
.....														

Table V Area of five sample graphite grains in microstructure images acquired by serial section method from field #5

Centriod $C_n$ of grains	Area of grains													
	Microstructure images													
	1	2	3	4	5	6	7	8	9	10	.....	58	59	60
(267,21)	7	10	11	12	5	11	10	3	2	8	.....	15	9	4
(360,268)	5	8	9	10	10	7	5	8	10	3	.....	8	NF	*6
(390,265)	2	2	2	9	11	5	15	1	NF	NF	.....	NF	NF	NF
(388,175)	4	6	7	3	9	1	NF	*3	6	9	.....	4	3	2
(192,108)	1	NF	NF	NF	NF	*4	5	7	6	6	.....	15	12	6
.....														

Legend: NF-Not found in slice image, \* New grain started at same centroid

Table VI Comparison of quantitative results obtained by proposed 3d and classical 2d methods

Method	Number of graphite grains	Mean Diameter ( $\mu\text{m}$ )	Mean length ( $\mu\text{m}$ )	Mean aspect ratio	Mean Volume ( $\mu\text{m}^3$ )
2D method using stereological relations	180	$12.53 \pm 0.20$	$10.47 \pm 3.2$	$1.4 \pm 0.5$	1456
3D proposed method	132	$10.93 \pm 0.16$	$11.63 \pm 1.22$	$0.9 \pm 0.2$	1820

The Fig.5 shows the comparison of number of graphite grains examined and the mean volume of graphite grains computed by using classical 2D and proposed 3D methods from the material sample. The Fig. 6 shows comparison of quantification of graphite grain parameters computed by 2D and proposed 3D methods.

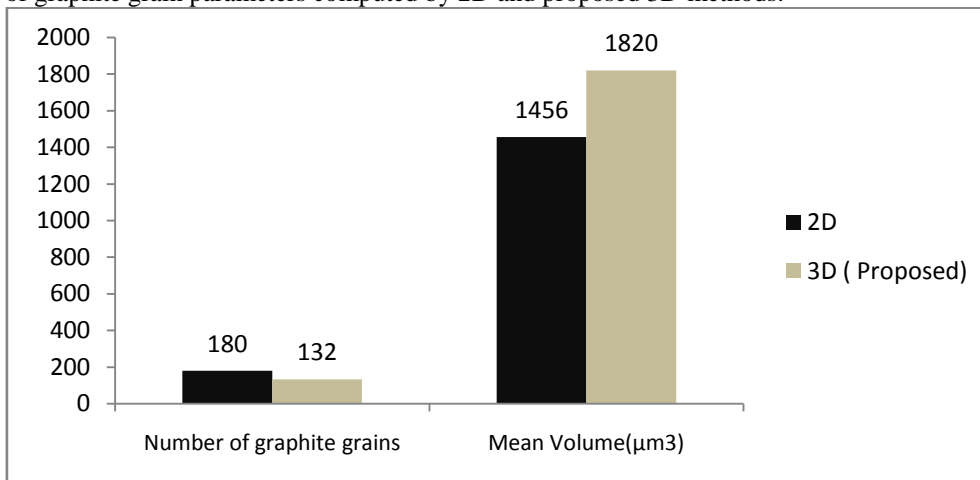


Fig. 5: Number of graphite grains examined and mean volume of graphite grains by 2D and proposed 3D methods from the material sample.

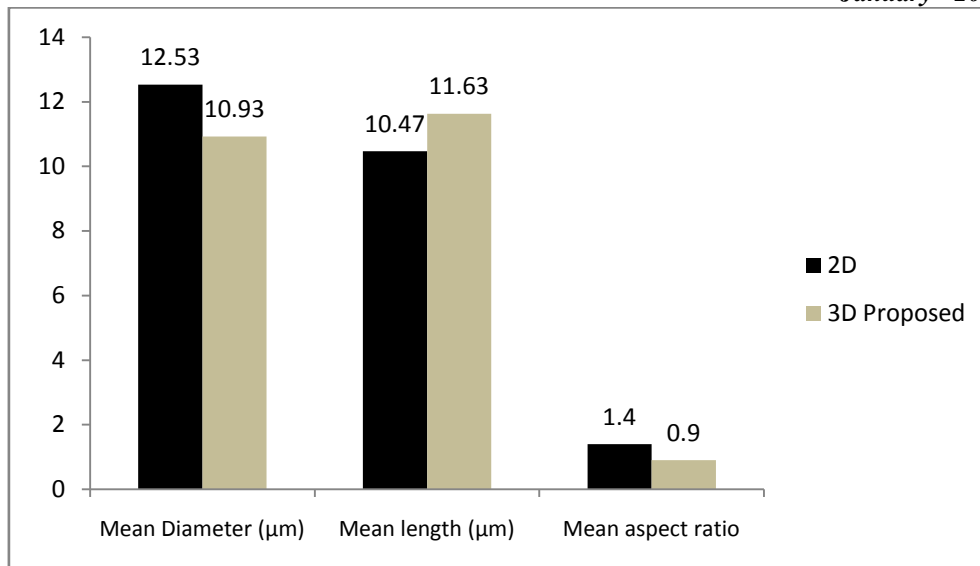


Fig. 6: Comparison of quantification of graphite grain parameters computed by 2D and proposed 3D methods

The results reported in this paper are validated with the mechanical properties of the end product manufactured using the sample material in consultation with metallurgy experts. It is seen that the mechanical properties are closer to the results obtained by proposed 3D method than the 2D classical method.

## V. CONCLUSIONS

In this paper, a novel, efficient and automatic 3D microstructure image analysis method for estimating graphite grain size and other parameters of volumetric estimation in cast iron material has been presented. Though the serial sectioning method needs considerable effort in preparing microstructure images for analysis, yet this effort is tolerable because of the fact that the 3D analysis method yields practically viable quantitative information along with very important new insights into microstructural evolution. The experimental results show that the proposed 3D method estimates accurate and superior quantitative information of graphite grains compared to classical 2D method. Therefore, the proposed 3D method has potential for applications in the field of material manufacturing industry.

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