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An Improved Method for 3-Dimensional Analysis of Microstructure Images Acquired through Serial Sectioning of a Material

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Abstract--- Generally, the study of microstructure is accomplished using two dimensional (2D) microstructure images to provide three-dimensional (3D) information with the help of stereological relations. Although 2D analysis suffice the needs of many analysis but the information extracted through 2D methods is to a limited extent. For analysis of material microstructure images in 3D form a technique called, 'Serial Sectioning' is used. 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 that need precise insight information about the material. Such an effort is presented in [1]. In this paper, an improvement over the method discussed in [1] is presented by adopting a new method for determining the centroid of the graphite grains. 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 and the results reported in [1]. The results of proposed method are found to be accurate and more close to practical limits. The proposed method is expected to be useful in manufacturing and quality control practices.

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

I. INTRODUCTION

The customized manufacturing of cast iron is possible by the controlled mixture of graphite material (about 1.5 to 2.8%) to the ferrite at the molten state of material. The molten alloy (Ferrite+Graphite) finally allowed cooling down to room temperature. The shape and distribution of graphite grains have also a great role to play in material properties and thereby its application in manufacturing the end products. 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 [1,2,13,16,17,18]. Microstructure properties derived by characterization and analysis of microstructure images lend vital information for material manufacturing and quality control practices. The analysis of microstructure images is performed in two ways, namely, 2D and 3D analysis. The 2D analysis techniques use single polished sections of material surface for acquiring microstructure images and then the classical stereological relations are used for obtaining 3D quantitative information [13,16]. On the other hand, in 3D metallographic techniques, a series of 2D images are acquired by serial sectioning of material , which are used to reveal the internal 3D structures of materials embedded within an opaque material.

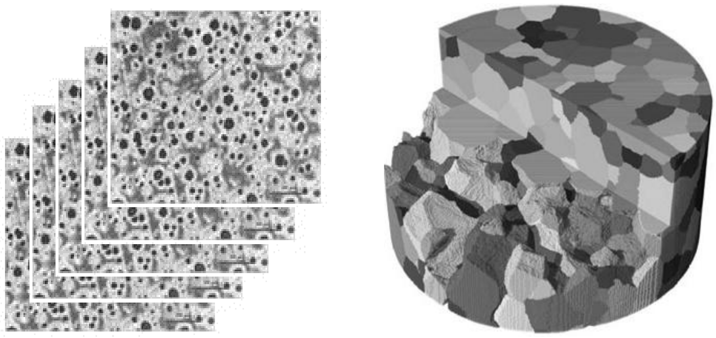
Serial Sectioning

The serial sectioning is comprises 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 [1,2]. 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 by imaging the material surface 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.

The 3D microstructure data is of 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 [1,2,3,6,19]. 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 [3]. Similar significant efforts are made in 1962, by Hillert and Lange [4]. 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 [5]. In 1965, Hopkins and Kraft [6], in 1967 Howbolt and Brown [7], in the same year, Barrett and Yust [8] showed the interconnectivity of voids in a sintered copper powder , Ziolkowski [9] , in 1985, used "mikrotom" to perform a serial sectioning study of grain boundary precipitates in an alpha/beta brass alloy . The advancement in image processing [15] and 3D visualization capabilities, along with development of automatic sectioning devices, has made 3D analysis of material samples much more practical. For the first time in 1991, Hull [10], used 3D wire-frame drawings of microstructure features for analysis. Brystrzycki and Przetakiewicz [11], in 1992 have used similar technique to study sizes and shapes of annealing twins in Ni-Mn alloy. Recently, an effort in 3D analysis of microstructure is presented in [1]. In the present paper, an improved method for 3D analysis of microstructure of materials is proposed by developing an improvised centroid estimation method, 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 section II contains the details of materials used. The section III describes the proposed method. The experiments results and discussions are presented in the section IV. The section V gives the conclusion

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 µm particle size material, which is serial sectioned and then the microstructure images of resolution 400x300 pixels are acquired using light optical microscope.



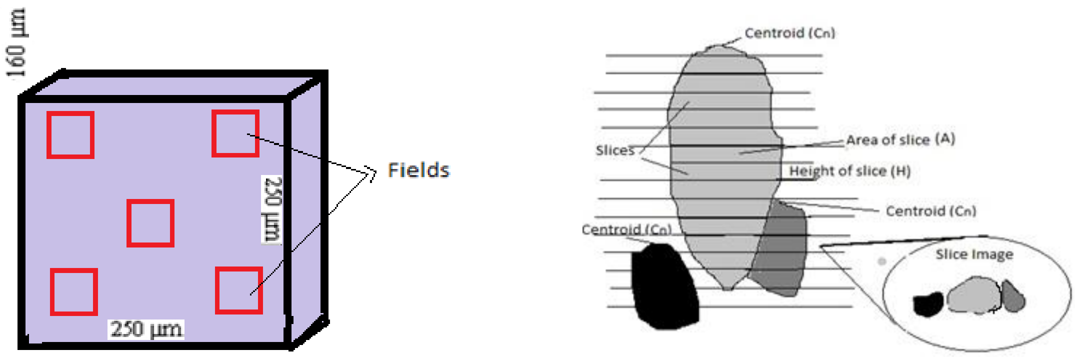
(a) (b)

Fig.1: (a) Stack of serial sections of material and (b) 3D simulated-reconstructed material image [1].

III. PROPOSED METHOD

As discussed in [1,2], the sample is cut from the top and polished by following serial sectioning technique. In the proposed method, a square region of interest is selected from the sample by indenting four equally spaced marks as shown in the Fig.2, called fields. Using this subjective criterion, the size of the microstructural region of interest was taken as approximately 250 X 250 µm, depth is of approximately 160 µm and the size of each field is 50x50 µm. The thickness of each slice is of 1.0 µm. Then, the exposed surface is polished using silicon carbide abrasives.

The mounted specimens were final-polished using colloidal silica media with a 0.05 µm 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 sec tional images are acquired. The probable noise in microstructure images is filtered by applying selective median switching filter [14].



(a) (b)

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 [1].

The sample microstructure images and respective filtered images are shown in the Fig.4. Then, each microstructure image is segmented using Otsu's segmentation technique [20] 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. In this work, the method used for determining the centroid is improvised. It is explained as following :

Improved centroid estimation

In [1] for determining the centroid of grain at nth layer the following condition is applied: 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. This method works well with the shapes that are cascading vertically on the same axis. However, the method for determining the centroid in [1] has great chances of missing the centroid in the case of grains having too irregular shape. As we move deep down to several layers, the centroid of irregular shaped grains will not be same for layer (n) and layer (n+1), the centroid obviously shifts in some direction as illustrated in the Fig. 3.

Therefore, the following condition is imposed , in place of the condition used for centroid in [1] , in order to confirm the exact centroid of grain and to know the continuity or end of grain that is under investigation:.

Consider , a graphite particle (grain) with centroid C_n is present in n^{th} image and C_{n+1} is centroid of grain is in $(n+1)^{th}$ image.

If $dist\ (C_{n+1}, C_n)\frac{1}{\sqrt{2}} < Diameter\ of\ grain$

Then ' it is same grain'

Else grain is not same and considered as, 'it is start of new grain'.

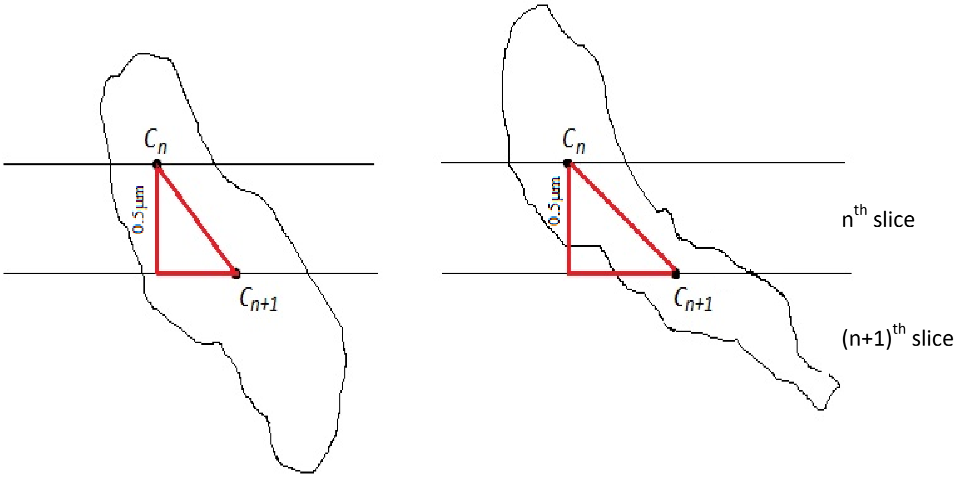
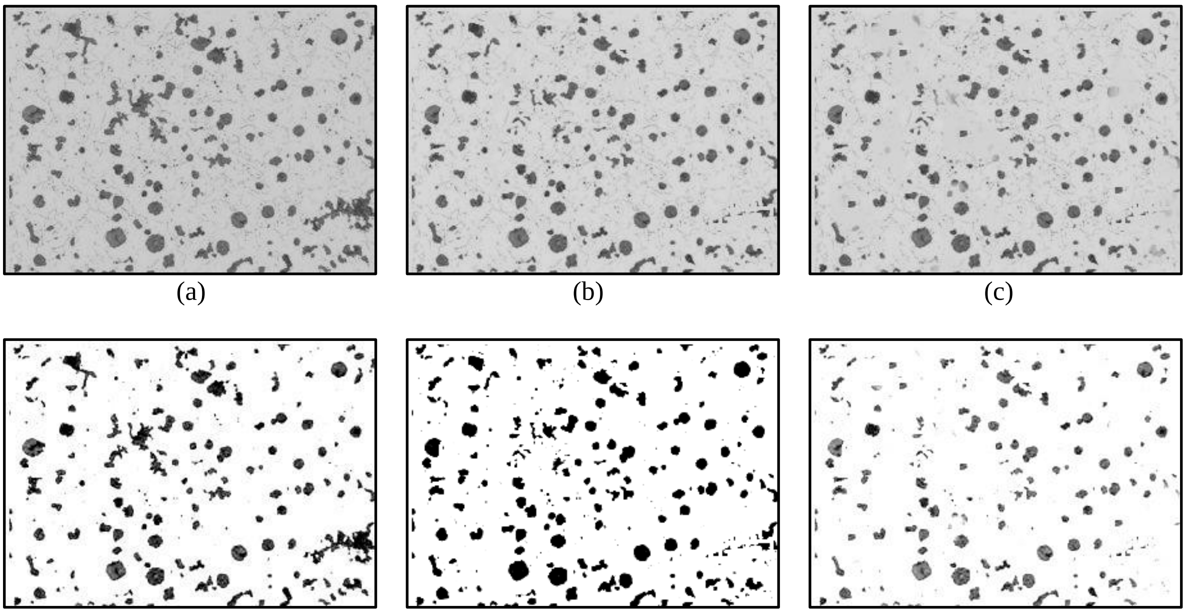


Fig. 3 The shift of centroid in grains in subsequent slices

The volume of each of the particle is determined using Eq.1:

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

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.



(a) (b) (c)

(d) (e) (f)

Fig.4:(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).

The framework for the proposed system is shown in the Fig. 5.

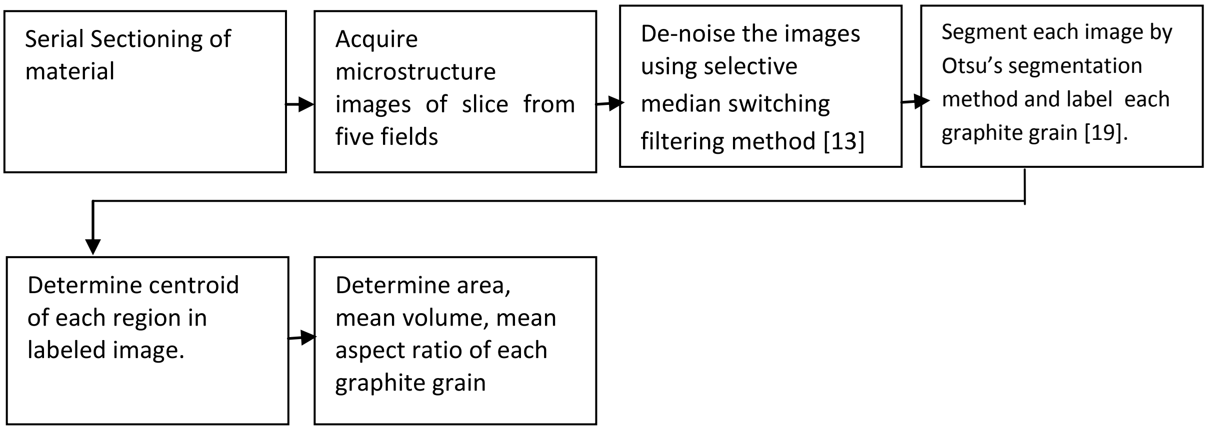


Fig.5: The framework for the proposed method.

The algorithm for the proposed method of 3D analysis of microstructure images determining quantitative information is given below:

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 [14] 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.
Step 6: Determine the centroid of particle based on the condition,
If dist $(\mathrm{C}_{n+1}, \mathrm{C}_n)\frac{1}{\sqrt{2}}$ < Diameter of grain *Then* 'it is same grain' *Else* grain is not same and considered as, ' it is start of new grain'.
Step 7: Register centroid (C_n) of graphite particles.
Step 8: Determine the area of each graphite particle until the grain disappears from its centroid position. Record the area of each graphite grain.
Step 9: Repeat Step 1 to Step 8 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.

IV. EXPERIMENTAL RESULTS AND DISCUSSIONS

Microstructure images of cast iron (2% C) bar samples (Fig.1(a)) are considered for experimentation. There are 300 microstructure images acquired from the material by serial sectioning. These images are analyzed by proposed 3D analysis method for comparison of the performance of methods discussed in [1]. 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 in [1]. It is observed that the quantitative information determined by proposed improved 3D analysis method is more accurate and natural representative of microstructure than the classical 2D method and 3D method in [1]. The improved centroid estimation method discussed in this paper is yields better results than the method in [1]. Also, 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. The improvement in results is shown in the tables. Due to erroneous detection of centroid in [1], some of the grains were abruptly discontinued (marked as NF). But, in the proposed method, such grains are continued to the correct depth of the grain. Significant improvement in computation of other grain measurements is also observed.

TABLE I AREA OF FIVE SAMPLE GRAPHITE GRAINS IN MICROSTRUCTURE IMAGES ACQUIRED BY SERIAL SECTION METHOD FROM FIELD#1

Centroid C_n of grains	**Area of grains (µm)**												
	Microstructure images												
	1	2	3	4	5	6	7	8	9	10	……	58	59	60

(176,13)	13	14	2	1 4	10	2	5	9	15	15	… …	2	N F	N F
(322,8)	10	5	5	3	N F	N F	*1 4	12	10	6	… …	N F	N F	N F
(320,236)	N F	N F	*1 5	2	12	11	11	10	6	6	… …	8	N F	*6
(123,171)	3	15	15	8	13	3	7	14	12	15	… …	10	9	6
(197,99)	2	11	10	1 0	8	6	NF	N F	N F	N F	… …	N F	N F	N F
…..														

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 (µm)**													
	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 (µm)**													
	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	4	……	3	7	15
(353,294)	12	15	10	1	13	15	11	12	12	6	……	15	8	13
(138,196)	1	5	1	2	13	1	NF	*2	2	7	……	3	7	NF
(16,34)	15	3	8	10	8	13	3	1	13	15	……	15	11	12
(222, 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 (µm)**													
	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	6	……..	7	NF	*2
(347,22)	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	5	……	7	5	8
(134,164)	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 (μm)**	**Mean length (μm)**	**Mean aspect ratio**	**Mean Volume (μm^3)**
2D method using stereological relations	180	12.53 ± 0.20	10.47 ± 3.2	1.4 ± 0.5	1456
3D method discussed in [1]	132	10.93 ± 0.16	11.63 ± 1.22	0.9 ± 0.2	1820
3D proposed method	122	9 ± 0.11	10.63 ± 0.5	0.8 ± 0.2	1910

The Fig.5 shows the comparison of number of graphite grains examined and the mean volume of graphite grains computed by using classical 2D method , 3D method in [1] and proposed 3D method from the material sample. The Fig. 6 shows comparison of quantification of graphite grain parameters computed by 2D method , 3D method in [1] and proposed 3D method.

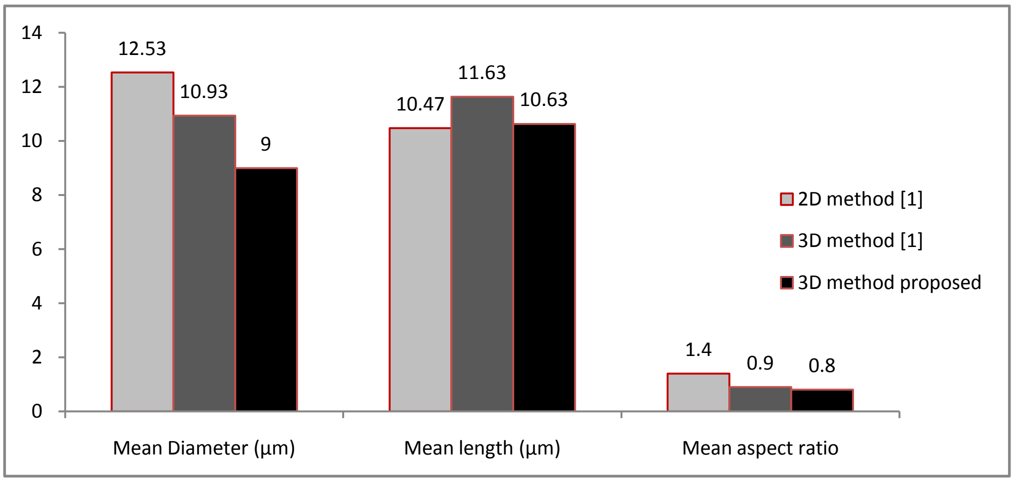


Fig. 5: Number of graphite grains examined and mean volume of graphite grains by 2D method , 3D method in [1] and proposed 3D method from the material sample.

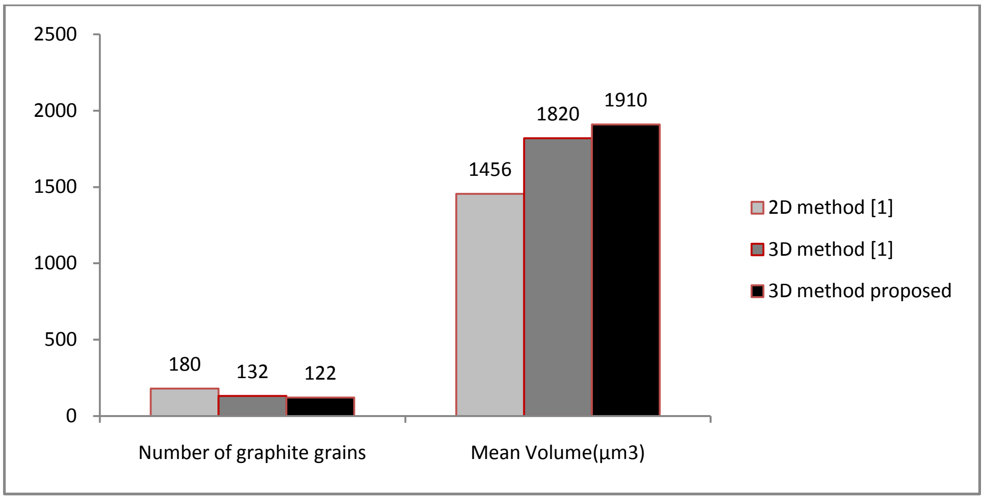


Fig. 6: Comparison of quantification of graphite grain parameters computed by 2D method , 3D method in [1] and proposed 3D method.

The experimental results are validated with the mechanical properties of the end product manufactured using the sample material in consultation with metallurgy experts. Close agreement between the mechanical properties of material with the results obtained by the proposed 3D method which is better than the methods discussed in [1].

V. CONCLUSIONS

In this paper, an improved 3D microstructure image analysis method for estimating graphite grain size and other parameters of volumetric estimation in cast iron material has been presented. A new method for determining the centroid of grains is presented. The experimental results show that the improved 3D method estimates quantitative information of graphite grains more accurately as compared to both classical 2D method and 3D method in [1]. Therefore, the improved new 3D method has potential for applications in the field of material manufacturing industry.

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