

# **Automated Image Analysis and Artificial Intelligence applied to microscopic images: a new methodology for the morphological characterisation of nano-particles**

Juan López de Uralde

S<sup>3</sup>Lab. Laboratory for Smartness, Semantics and Securit. Universidad de Deusto

Avda. Universidades, 24, 48007 Bilbao, Spain

Email: [jlopezdeuralde@deusto.es](mailto:jlopezdeuralde@deusto.es)

Teresa Guraya

Escuela Universitaria de Ingeniería Técnica Industrial

Universidad del País Vasco

Plaza La Casilla, 3. 48012 Bilbao, Spain

Email: [teresa.guraya@ehu.es](mailto:teresa.guraya@ehu.es)

Ana Okariz

Escuela Universitaria de Ingeniería Técnica Industrial

Universidad del País Vasco

Plaza La Casilla, 3. 48012 Bilbao, Spain

Email: [ana.okariz@ehu.es](mailto:ana.okariz@ehu.es)

Enkarni Gomez

Escuela Universitaria de Ingeniería Técnica de Minas y Obras Públicas

Universidad del País Vasco

Colina Beurko s/n, Barakaldo, Spain

Email: [enkarni.gomez@ehu.es](mailto:enkarni.gomez@ehu.es)

Agustín Zubillaga

S<sup>3</sup>Lab. Laboratory for Smartness, Semantics and Securit. Universidad de Deusto

Avda. Universidades, 24, 48007 Bilbao, Spain

Email: [azubillaga@deusto.es](mailto:azubillaga@deusto.es)

Igor Santos

S<sup>3</sup>Lab. Laboratory for Smartness, Semantics and Securit. Universidad de Deusto

Avda. Universidades, 24, 48007 Bilbao, Spain

Email: [isantos@ehu.es](mailto:isantos@ehu.es)

# Automated Image Analysis and Artificial Intelligence applied to microscopic images: a new methodology for the morphological characterization of nano-particles

López-de-Uralde, J.<sup>c</sup>, Guraya, T.<sup>a</sup>, Okariz, A.<sup>a</sup>, Gómez, E.<sup>b</sup>, Zubillaga A.<sup>c</sup>, Santos, I.<sup>c</sup>

<sup>a</sup> Industrial Engineering Technical School, University of the Basque Country, Bilbao, Spain

<sup>b</sup> Mining Engineering Technical School, University of the Basque Country, Barakaldo, Spain

<sup>c</sup> S<sup>3</sup>Lab. University of Deusto, Bilbao, Spain

## Abstract

Exhaustive and accurate filler characterisation is desirable for rubber behaviour modelling. In this work new customized software is developed for this task. The application makes conventional characterisation combined with automated aggregate shape classification (branched, linear, ellipsoidal or spheroidal). The suitability of the software has been tested with carbon black images acquired with TEM, SEM and AFM microscopes. Results show good performance in image segmentation and the classifier gave 84% of positives in category classification and a great AUC of 0.94. Sample preparation and sampling seem to be critical. Sample preparation and sampling seem to be critical.

**Keywords:** carbon black, morphological characterisation, automated image analysis, automated classifier

## Introduction

Filled rubber is probably the most extensively nanoparticle reinforced material being used for years. Filler addition increases modulus, strength, fatigue or tear resistance but the actual nature of reinforcement mechanisms remains under debate.

Kohls<sup>1</sup> reviewed many attempts to model filled rubber. There is agreement in the importance of filler characteristics, mainly structure. Filler single units are formed by spherical particles of the same radius  $r_p$ , fused together to create aggregates; aggregates agglomerate and build up a network when added to rubber over the percolation threshold. More recently Koga<sup>2</sup> proposed a new model of hierarchical structure in which the smallest unit of the CB filler had a spherical shape and consisted of about nine primary CB particles fused together. Figure 1 shows a graphic representation of the size of each level for carbon black structure. There are two approaches to characterise carbon black; fractal and Euclidean. The Euclidean approach is more extensively used and is defined by the ASTM 3849-02 standard<sup>3</sup>. Additionally to geometrical parameters defined by the standard, Herd<sup>4</sup> pointed out the interest of an aggregate classification attending to four different shapes, as it is shown in figure 2.

It should be pointed out that accurate filler characterisation appears to be critical for modelling. The powerful microscopic techniques available seem to be a good choice to extract exhaustive and accurate results<sup>5</sup>. Due to technique limitations, the methodology uses 2D projections of 3D objects and stereological principles (i.e. the three-dimensional interpretation of two-dimensionally observed objects) are used to estimate the geometrical parameters.

In this work three carbon black grades will be characterised following the ASTM standard requirements. Analysis will be applied to images captured with Transmission

Electron Microscope (TEM), Scanning Electron Microscope (SEM) and Atomic Force Microscope (AFM). For each grade and technique, morphological features will be extracted. Differences in relation to technique and the effect of shape on results will be commented. Also, the three carbon black grades will be transformed with an EPDM rubber; differences in morphological parameters will also be commented.

To accomplish the task, two customized software applications have been developed and integrated together in order to provide exhaustive and accurate results. One application was designed for automated analysis of SEM, TEM and AFM images of carbon black aggregates (AIA application). The second one was a machine learning aided application designed for automated morphological classification of aggregates (AAC application). The integrated program runs under MatLab environment and has been applied to make all characterisations. The software generates an output file with results and a simple statistical analysis. The file can be treated by any other commercial statistical software.

## **Methodology and software verification**

### **Materials and microscopic techniques**

The carbon black grades chosen for the study were Vulcan XC72R, Vulcan XC605 and CSX 691, all of them supplied by Cabot S.A., Spain. (Grade characteristics in Table 1).

Images for morphological characterisation were collected with a Hitachi S4800 Scanning Electron Microscope operating at 15KeV, a Philips CM120 Transmission Electron Microscope operating at 120 KeV and a Digital Instruments Nanoscope IIIa Atomic Force Microscope operating in a tapping mode. Around 1000 aggregates of each sample were captured (although ASTM standard defines a minimum of 2000 aggregates to get meaningful results).

Morphological characterization of the grades was carried out on both, CB powder in the dry state and after being mixed with an EPDM rubber. For the powder in the dry state, CB powder was dissolved in chloroform and dispersed with an ultrasonic probe following ASTM standard. Dissolution was dropped on a carbon covered TEM grid, on a polished SEM holder and on an AFM silicon wafer holder for image acquisition. Image magnification at each microscope was selected to get the pixel size required in the standard.

EPDM compounds were prepared with a small two roll-mill lab-system. Formulation is given in Table 2. After milling, compound was hot pressed vulcanization at 170°C. Ultrathin sections of about 100-150 nm thickness were prepared with a Leica EM EU6 ultramicrotome coupled to a cryostage Leica FU6 equipped with a diamond knife cooled at -60 °C and keeping the sample cooled at -130 °C. Slices were transferred to a TEM grid for observation. The remaining ultra-smooth surfaces were used for AFM and SEM observations. Prior to SEM observation surfaces were metalized with a few nanometers thickness Pt coating.

### **Integrated AIA/AAC software description and verification**

Images collected were processed with AIA/AAC application. Machine learning algorithm of the classifier should be trained with a few hundred of pre-classified aggregates.

Basically the algorithm follows the operations required by the Standard Test Method for Carbon Black to analyse images captured by transmission electron microscopes; i.e. background/noise elimination, thresholding, erosion and dilation. Application was customized for the specific characteristics of the images being processed. Figure 3 shows, as example, the result of the treatment applied to several TEM or SEM aggregate images of different grades. It can be said that the program draws the aggregate silhouette with high accuracy.

Based on the output image of the previous phase, some geometric features are extracted and a training vector  $v = (v_1; v_2; \dots; v_n)$  containing all these characteristics is generated per aggregate. The collection of vectors provides the learning dataset for the classification system. This methodology for aggregate shape categorization improves the method proposed by Herd, where classification is made according to ranges of the ratio L:W, being L and W the longest and shortest Ferets<sup>4</sup>. Fifteen different machine learning algorithms were tested. In order to check the suitability of each machine learning algorithm; the percent of correctly classified instances and the area under the ROC curve (AUC), that establishes the relation between false negatives and false positives, were evaluated. The best option, gave an accuracy of 84% and an AUC value of 0.94. Exhaustive details of the customized software were described in a previous work<sup>6</sup>.

After training, integrated AIA/AAC application processed the series of aggregate images. Aggregates will be classified by categories and filler features will be extracted and post-processed by a statistical commercial program SPSS.

## **Results**

### **Image acquisition microscope techniques**

Figures 4 to 7 show some examples of images captured with the three microscopes. The aim of this work was to check software capacities.

### **Aggregates categorization**

Table 3 summarizes results obtained with the automated aggregate classifier. It is not the aim of this work to discuss in detail neither differences between grades nor differences due to milling process. These results are analysed to evaluate the software capacities with different techniques and different samples.

Grade data supplied by Cabot (Table 1) shows increasing structure values for structure measurements: CSX (96), Vulcan XC605 (150) Vulcan XC72R (174). It should be reflected in an increasing percentage of branched aggregates<sup>4</sup>. When SEM images are analysed the branched class is dominant in the three grades; but similar branched fractions are found for the three grades. Comparing these results to those obtained from TEM image analysis, leads to high differences. Percentage of branched aggregates falls down dramatically but the expected tendency is satisfied. There is not an apparent reason for these differences on technique because the object of study is the same, so an exhaustive review of the process was done. It was found that the number of aggregates correctly segmented is too low in most cases so results do not have statistical significance.

As consequence, much more attention must be focused into sample preparing, sampling and image acquisition parameters. It should be carefully discussed if pixel sized (magnification) can be changed from that defined by the standard.

### **Aggregates characterization**

The lack of statistical significance of results made exhaustive statistical analysis time waste. In the next future it is expected to get information in changes occurring in carbon black structure due to milling and vulcanizing processes.

### **Conclusions**

New customized software for carbon black grades characterisation has been developed and tested. The software integrates two applications. The one named AIA (automated image analysis) has been specifically designed for analysis of SEM, TEM and AFM images, varying threshold parameters. The software generates an output file that can be imported and post-processed by any commercial statistical software. The other application, named AAC, is an automated aggregate classifier designed specifically for carbon black. As result, shapes distribution can be measured and aggregates grouped attending to this feature; this way, new characterisations can be made by each shape and evaluate contributions of each shape to the overall morphological parameters.

AFM does not produce useful images. Technique is very time-expensive and images analysis does not give good results. So AFM is discarded as characterization technique for carbon black grades. SEM and TEM technique seem to be suitable for carbon black characterisation. But sample preparing and image sampling must be carefully done. It must be taken into account that high number of aggregates can be lost during the segmentation process. At this moment, it can not be distinguish between SEM and TEM techniques in terms of efficacy and efficiency.

The interest of the software can be extended to other materials containing nano-reinforcements with the necessary customized changes. In fact, it is being applied to the study of biopolymers reinforced with nano-clays and silica (filler with different morphology).

### **Acknowledgements**

This work has been supported by the University of Basque Country with the NUPV09/03 incentive for investigation and the project UEGV09/C19 of the Basque Government. We thank Mikel Salazar for the carbon black 3D simulation images created for this paper. Technical and human support provided by SGIker, specifically the EM laboratory of the Analytical Microscopy and High Resolution in Biomedicine General Service, is gratefully acknowledged.

### **References**

- <sup>1</sup> D. J. Kohls, G. Beaucage Current Opinion in Solid State and Materials Science, 6, 183–194
- <sup>2</sup> T. Koga, T. Hashimoto, M. Takenaka, K. Aizawa, N. Amino, M. Nakamura, D. Yamaguchi and S. Koizumi| Macromolecule, 41, 453-464
- <sup>3</sup> ASTM D3849-02 - Standard Test Method for Carbon Black - Morphological Characterization of Carbon Black Using Electron Microscopy (2002). Testing method.

<sup>4</sup> Herd, C., McDonald, G., Hess, W.: Rubber Chemistry and Technology, 65 (1), 107-129.

<sup>5</sup> L. Conzatti, G. Costa, F. Falqui and A. Turturro, Microscopic Imaging of rubber compounds, *Rubber Technologist's Handbook, Volume 2*, Ed.; J. White; S.K. De.; K. Naskar., 2009, Smithers Rapra Technology

<sup>6</sup> J- Lopez-de-Uralde, I. Ruizy, I. Santos, A. Zubillagay, P. G. Bringasy, A. Okariz, and T. Guraya, Lecture Notes in Computer Science, 6262, 185-193.

## List of figure captions

Figure 1. Graphic representation of the size of each level for carbon black filler: a) particles; b) aggregate; c) agglomerate

Figure 2. Morphological categories for carbon black aggregates

Figure 3. Segmented SEM and TEM aggregates images

Figure 4. SEM images: Vulcan XC605 grade: a) in the dry state b) filled EPDM

Figure 5. TEM images: Vulcan XC72R grade: a) in the dry state. b) filled EPDM

Figure 6. AFM images: Vulcan XC72R in the dry state: a) amplitude; b) phase

Figure 7. AFM images: Filled EPDM with CSX691 grade: a) amplitude; b) phase

## Tables

Table1. Carbon black grades

	I <sub>2</sub> AN (mg·g <sup>-1</sup> )	OAN (ml·100 g <sup>-1</sup> )
Vulcan XC72R	253	174
Vulcan XC605	90	150
CSX691O	15.4	96

Table2. Formulation used for filled EPDM compounds.

	phr
EPDM	100
CB	30
ZnO	5.0
stearic acid	2.0
paraffinic oil	10
TMTD	1.0
MBTS	1.5
sulphur	1.0

Table 3. Aggregate category fractions for CB grades/techniques/dry state-vulcanised

	% spheroidal		% ellipsoidal		% linear		% branched	
	SEM	TEM	SEM	TEM	SEM	TEM	SEM	TEM
Vulcan XC72R	1	6	2	34	32	25	65	37
CSX691	4	12	10	70	23	5	64	18
CSX691+EPDM	X	18	X	67	X	8	X	7
Vulcan XC605	1	4	4	39	32	24	63	33
Vulcan XC605+EPDM	X	9	X	41	X	15	X	35



# Figures

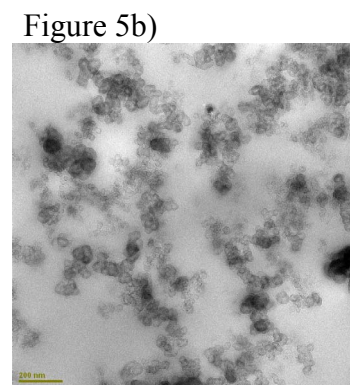
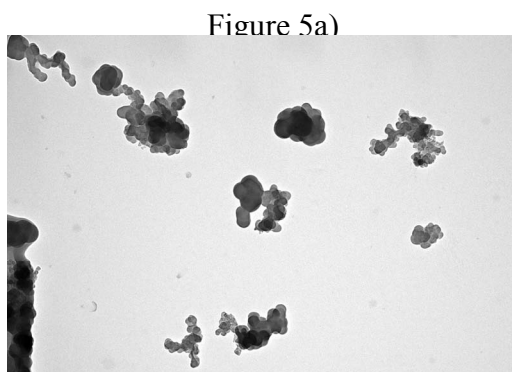
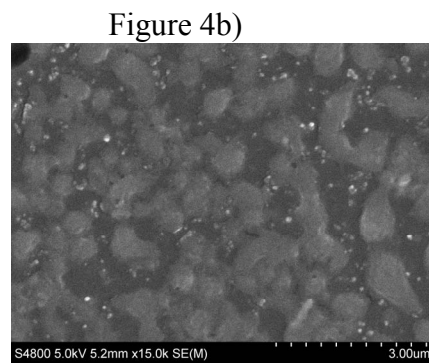
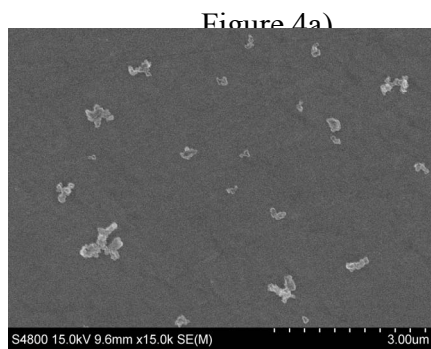
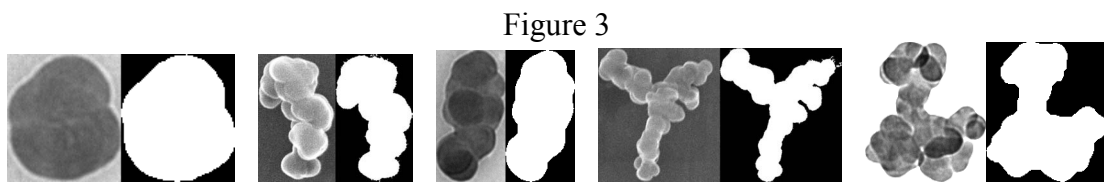
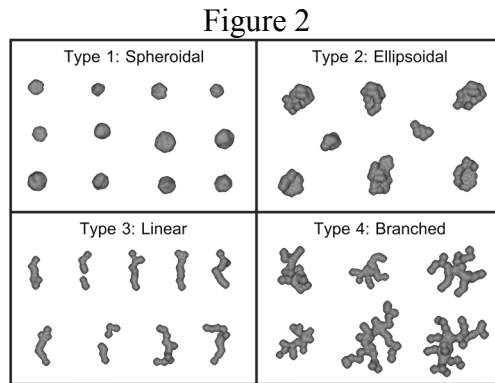
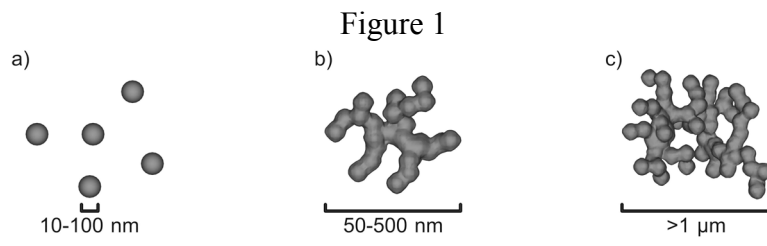


Figure 6a)

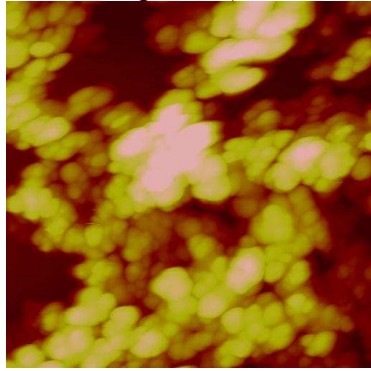


Figure 6b)

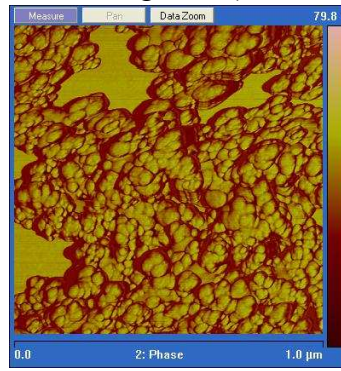


Figure 7a)

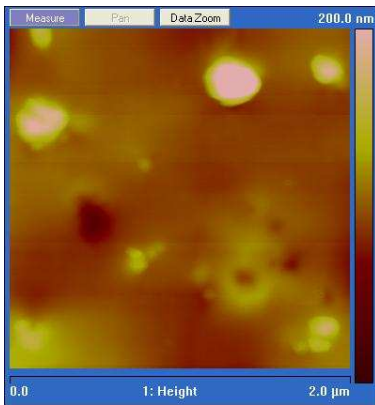


Figure 7b)

