Quantification of nano-scale carbon structure by HRTEM and lattice fringe analysis

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ABSTRACT

An image analysis algorithm is applied to materials – for characterization of solid-state structure on a nanometer scale using model carbon materials. Nanoscale carbons in the form of “soots” offer ease of demonstration while being relevant to human health and climate concerns. Demonstrated here is an image analysis algorithm applied to three nanoscaled carbon materials: a disordered soot, and two highly ordered soots featuring flat or curved atomic layer planes. Nanostructure parameters consisting of graphene layers’ length and tortuosity are extracted from high-resolution transmission electron microscopy images. The algorithm is composed of two major parts: a) image processing that generates a skeletonized binary image, and b) characterization that generates statistics on length and tortuosity based on the skeletonized image of the graphene layers. Algorithm robustness for variations in image processing parameters of contrast and threshold is demonstrated by similarity of output distributions of disordered diesel engine produced soot. Algorithm processing range was illustrated using highly ordered soots with flat (graphitic) and curved lattice nanostructure. Time resolved image analysis of an image sequence for polyhedral onions under electron irradiation demonstrate algorithm utility for tracking solid-state transformations.

Keywords: lattice fringe analysis; carbon nanostructure; tortuosity; thermal annealing; electron beam irradiation

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1. Introduction

Image analysis for structure has application to materials science. Lattice structure measured on the nanometer scale is termed “nanostructure”. It represents the physical dimensions associated with crystalline lattice planes of the material. These measures include length, curvature or tortuosity and separation distance between opposing planes. The chemical reactivity of carbon forms such as fullerenes, nanotubes and even ordinary soot has been shown to depend upon the nanostructure (Rouzaud and Oberlin, 1989; Neeft et al., 1997). Processing and application performance for these purposeful materials will depend upon their surface chemistry. Environmental and health impacts for incidental carbon materials in the form of soot also depend upon surface chemistry. Across these carbon forms, surface chemistry is dependent upon nanostructure (Vander Wal et al., 2010).

The nanostructure of carbons can vary substantially – dependent upon formation conditions such as temperature and time (Vander Wal and Tomasek, 2004; Hays and Vander Wal, 2007; Vander Wal et al., 2011) – which are highly source specific. For example, carbons derived from different pitches have long been known to be either graphitizable or nongraphitizable (Oberlin, 1984). Yehliu et al. (2012) showed the impact of fuel on the initial nanostructure of diesel soot and its subsequent reactivity. Gaddam and Vander Wal (2013) showed the impact of engine operating conditions on the gasoline soot nanostructure. As motivation these and other previous studies have shown the effect of nanostructure on the oxidative reactivity of the soot and commercial carbons (Ishiguro et al., 1991; Vander Wal and Tomasek, 2003; Song et al., 2006; Vander Wal et al., 2007; Müller et al., 2007; Jaramillo et al., 2014). To date a variety of methods have been routinely used to characterize the nanostructure of carbons, including Raman spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM).

X-ray diffraction has proven value in deriving carbon crystallite structure of a bulk material sample; it lacks the ability to examine localized, nano-quantities of sample (Franklin, 1950; Iwashita et al., 1993; Fujimoto et al., 1994; Iwashita et al., 2004). Comprehensive characterization and distinction of different types of soot by Raman spectroscopy is feasible, but the data processing, reproducibility of spectra and statistical uncertainty of spectral parameters determined by curve fitting still remains a controversial issue in the literature (Sadezky et al., 2005; Al-Qurashi and Boehman, 2008; Atribak et al., 2010; Pahalagedara et al., 2012). In contrast to XRD and Raman spectroscopy, high-resolution transmission electron microscopy (HRTEM) can reveal the crystalline structure of carbon, i.e., its nanostructure at an atomic level (Oberlin, 1984; Heidenreich et al., 1968; Vander Wal and Choi, 1999; Ishiguro et al., 1997). Such TEM images provide a direct measure of the length, curvature, and orientation of the carbon lamellae at any particular depth in the material as determined by the focal plane (Vander Wal, 1997).

HRTEM images were often interpreted qualitatively, before the advent of computer-aided image processing algorithms (Ishiguro et al., 1991). TEM image analysis of carbonaceous materials evolved further with the advent of the modern image processing and pattern recognition techniques. Palotas et al. (1996) applied band-pass filtering in the Fourier space and suggested the importance of using the optimum intensity threshold values. Sharma et al. (1999) utilized a step filter to reduce noise, followed by the mathematical separation and reconnection of fringe layers. Shim et al. (2000) developed a semi-quantitative technique (binarization, band-pass filter, thresholding, skeletonization) that could yield fringe length and tortuosity, as well as other quantitative parameters such as mode,

degree and orientational order. Galvez et al. (2002) applied top-hat transformation to reduce possible artifacts due to intensity variations, and also eliminated fringes that are smaller than 0.25 nm. Goel et al. (2002) defined a curvature parameter based on electron microscope images of soot from fullerene forming flames. Due to this similarity of molecular origin(s), curvature in nanostructure is alternatively referred to as “fullerenic” nanostructure (Goel et al., 2002). The curvature parameter is defined as the actual arc length divided by the circumference of a virtual circle ($\pi \times$ arc diameter). Vander Wal et al. (2004, 2005) advanced lattice fringe analysis by using a geometrically complete set of image processing and geometrical parameters. The robustness of this algorithm was demonstrated by analyzing a series of carbon soots possessing different degrees of graphitic structure, synthesized at different heat-treatment temperatures. Yehliu et al. (2011a, 2011b) further developed and implemented a HRTEM image analysis algorithm using commercial software (Matlab[®]) that includes image processing and lattice fringe analysis functions to distinguish carbon nanostructure between diesel soots. More recently several atomistic models are also being actively investigated to quantify soot nanostructure (Teini et al., 2011; Leyssale et al., 2012; Toth et al., 2013).

A number of analytical results are already published in the literature studying carbon nanostructure by image processing. The focus of most of these algorithms is the development of the lattice fringe analysis algorithm itself, or the application of the algorithm to quantify the nanostructure of few types of nanoscale carbons. Yet tests of these algorithms for consistency against model materials offering structural diversity are few. Moreover discussion of output dependence upon variation of processing parameters – necessary to accommodate changes in image contrast and threshold is absent. Against this background, we present tests of our lattice fringe analysis algorithm (Yehliu et al., 2011a) for its robustness with respect to a) image processing parameters and b) geometric flexibility for diverse nanoscale structure – using model carbon materials in the form of “soots”. Finally a novel application is demonstrated by applying the algorithm to track the changes during a solid-state transformation of a graphitic nanoscale carbon irradiated by the electron-beam within a high-resolution transmission electron microscope.

2. Materials and methods

2.1. Materials

The primary goal of the lattice fringe analysis algorithm is to quantify the nanostructure of carbon materials including soots. To examine the dependence of image processing parameters, an ordinary soot from a diesel engine is illustrated here. The reference diesel soot is a NIST standard material (SRM 2975), collected from an industrial diesel powered forklift. Diesel soot is chosen because of its environmental and human health impacts, and relevance of its nanostructure to these. Three different image processing cases were studied that include an ideal reference case, non-contrast enhancement and a manually adjusted binarization threshold case. Each of these cases is summarized in Table I.

The second study uses arc soot that is synthesized using the carbon arc method (Krätschmer et al., 1990). To further graphitize the arc soot, ~20 mg of arc soot was heat-treated in a RD-G[®] high temperature furnace using a graphite crucible. This furnace was operated under vacuum, to maintain temperature stability and reproducibility. The temperature was inferred from an optical pyrometer. The heat treatment was a near-trapezoid function of time, with a soak time at elevated temperature of 2000 °C for one hour.

For the final study, graphitized carbon polyhedral onions were synthesized in a high temperature furnace using a graphite crucible under a He atmosphere at 3000 °C for a one-hour duration. The carbon polyhedral onions, deposited on lacey C/Cu grid, were introduced into the ultra-high vacuum (UHV) of the TEM, operating at an accelerating voltage of 200 kV. The electron beam continuously irradiated a particular particle/region, at a current density of 512 pA/cm². Images were subsequently acquired at time intervals of 2 and 5 minutes to observe the nanostructural changes.

2.2. TEM Characterization

A 200-kV field emission TEM (JEOL® EM-2010F) was used to take high-resolution bright field images. Nano-scale carbon materials were deposited on a lacey C/Cu grid by depositing a drop of the sample suspension, created by sonication in ethanol. Images reported in this work were acquired at a nominal instrument magnification of 500,000×. All images were acquired in Gatan® Digital Micrograph with a slow scan CCD camera (2048 × 2048 pixels) using a condenser aperture size of 70 µm and an exposure time between 0.5 and 1 s. Typically ten aggregates or more from at least five locations were surveyed to establish the consistency of the observations and ensure accurate representations.

2.3. Lattice fringe analysis

Quantification of the HRTEM images by lattice fringe analysis provides several statistical metrics describing the nanostructure order (Vander Wal et al., 2004; Yehliu et al., 2011a). The term nanostructure is used here to refer to the graphene layer plane dimensions, their tortuosity and relative orientation as observed in the HRTEM images. The parameters extracted from the skeleton images are denoted as fringe length, and fringe tortuosity. Fringe length is a measure of the lateral extent of the atomic carbon layer planes, whereas fringe tortuosity is a measure of their curvature reflecting the content of odd numbered five- and seven-membered carbon rings.

Digital images were processed using custom algorithms developed in-house and coded in Matlab®. The method is composed of digital image processing and fringe characterization. The image processing algorithm mainly includes ten steps as appropriately discussed below. Complete details on image analysis algorithm hierarchy (and the four major subroutines) is discussed elsewhere (Yehliu, 2010; Yehliu et al., 2011a).

2.3.1. Negative Transformation

In the bright-field TEM image, because the fringes block or scatter the incident electron beam, the carbon layer segments appear as dark lines. In a gray-scale digital image, a darker pixel corresponds to a lower intensity value, whereas a brighter pixel has a higher intensity value. A negative transformation is applied as a first step to yield consistency among graphene layers, the background and the numerical values of the pixels (Gonzalez and Woods, 2008). Figure 1b shows the negative image of the original HRTEM image (Figure 1a).

2.3.2. Region of interest (ROI) selection

Particles overlap, irrelevant objects (scale bar, lacey carbon, etc.), and highly noisy regions such as the particle cores, distort the fringe properties of interest. For this reason, the algorithm permits the user to select a region of interest (ROI) as shown in Figure 1b for further processing. Subsequent histogram processing for contrast enhancement applies to the selected ROI but not the whole image, as the pixels outside the ROI are forced to the lowest intensity level, 0.

2.3.3. Contrast enhancement

The user of the program can use histogram equalization to improve the contrast of the ROI. Performing histogram equalization expands the image intensity to the full gray scale thereby improving the contrast. Regarding TEM analysis of nano-scale carbons, improving contrast in the ROI can increase the intensity difference between a fringe and a background pixel, and thereby reduces the complexity when performing the following image processing steps of binarization and skeletonization.

2.3.4. Gaussian lowpass filter

In the previous step, while improving the fringe contrast, the background noise may also be elevated. A lowpass filter is applied in order to attenuate the high-frequency noise (Gonzalez and Woods, 2008). Following past work (Sharma et al., 1999; Shim et al., 2000), a spatial Gaussian lowpass filter is implemented in the frequency domain. In the present algorithm, the user inputs filter mask size (f_{size}) as 11 and standard deviation (σ) as 1.0. Besides reducing the noise level, the low pass filter can smooth the image and break fringes. This type of artifacts can be fully repaired using a lowpass Gaussian filter with morphological operation (described in Section 2.3.7).

2.3.5. Top-hat transformation

In this study, a top-hat transformation was applied to correct the uneven illumination in HRTEM images (Galvez et al., 2002; Yehliu et al., 2011a). A disk was used as the structural element, whose radius was 5 pixels, which is larger than the apparent thickness of the individual graphene layers. Although the type and size of structural elements are both adjustable, all the three cases discussed in this paper process the images with a top-hat transformation with a disk structural element with diameter of five pixels.

2.3.6. Thresholding

In order to extract lattice fringes from the background, thresholding an image is necessary. In general, larger threshold values result in fewer fringes, whereas the identifiable fringes increase as the threshold value decreases. In the present algorithm, a reference threshold value is generated based on Otsu's method (Otsu, 1979), though the user can adjust the value, if necessary, according to the condition of the ROI and the thresholding result.

2.3.7. Morphological operation

Following binarization by Otsu's method, the resulting binary image might show features of thin protrusions, small holes and gaps that cause broken fringes after obtaining skeletons by a thinning algorithm. By applying the morphological closing structural element, the small holes are filled, and the discontinuities and branches are repaired. Morphological opening in particular removes protrusions of the T and Y shaped links (Gonzalez and Woods, 2008). From previous work (Yehliu et al., 2011a), a 2 × 2 pixel square has shown to retain maximum shorter lamella while reducing any artifacts resulting from noise.

2.3.8. Clearing fringes on the ROI border

Fringes present on the ROI border are incomplete fringes and can potentially impact the analysis result. By using the morphological reconstruction subroutine in Matlab®, the pixels of the fringes that touch the ROI border can be identified (Vincent, 1993).

2.3.9. Skeletonization

For further fringe characterization, skeletons of each fringe are extracted via a thinning (skeletonizing) process. In this study, a parallel thinning algorithm was implemented by using a built-in function in Matlab® (Lam et al., 1992). Using an iterative

process, the algorithm removes pixels from each fringe based on a pixel-deletion criterion until further changes cease.

2.3.10. Remove short length fringes (<0.5 nm)

After performing skeletonization, the program calculates fringe lengths, and permits the user to assign the minimum length. In the literature, the choice of the minimum fringe length varies (Shim et al., 2000; Rouzaud and Clinard, 2002; Yehliu et al., 2011a). In this study, fringes <0.5 nm are removed as these are hard to distinguish from noisy or non-crystalline structures.

The fringe characterization generated statistics on fringe length (L_a) and tortuosity based on the processed skeletonized image of the carbon lamellae. Histograms summarizing the distributions of fringe length, and fringe tortuosity were fit to a 2-parameter lognormal probability density function with median and standard deviation as the two adjustable parameters.

3. Results and discussion

3.1. Processing parameters

Figure 1a shows the original, eight-bit grayscale, bright-field HRTEM image of the diesel soot. The carbon lamellae (fringes) appear as dark lines in this bright-field image. This TEM image of the diesel soot shows a nanostructure commonly referred to as core-shell structure (Ishiguro et al., 1997). In this type of nanostructure, the inner core is composed of several fine particles that are covered by several surrounding carbon layers with a distorted structure. Quantification of this HRTEM image is performed using the lattice fringe analysis algorithm. To ensure that the higher intensity pixels correspond to the carbon lamellae, this TEM image is negatively transformed as shown in Figure 1b. The algorithm permits the user to select a region of interest (ROI) as shown by the marked area. This ROI contains sufficient and clear fringe information, which will be further processed by the image analysis algorithm to generate statistics about nanostructure order.

To verify for the variation in image processing parameters having an impact on the output distributions, the selected ROI is analyzed via different case scenarios. Although a user has the ability to adjust several of the image processing parameter values in the algorithm, here only three cases are discussed, as being most common. These are summarized in Table I. Case I represents the standard operating procedure for this lattice fringe analysis algorithm drawing on the work of Yehliu et al. (2011a). In this case the user adjusts none of the parameters. In Case II, the selected ROI is processed without histogram equalization or contrast enhancement of the negatively transformed image. In general improving contrast in the ROI can increase the intensity difference between a fringe and a background pixel, and thereby reduces the complexity when performing the next image processing steps of binarization and skeletonization. Of importance here is that the reference threshold value computed/determined based on Otsu's method is different compared to Case I. As thresholding an image is necessary in order to extract lattice fringes from the background, finally in Case III, the binarization threshold value is manually adjusted to 0.1 that is 25% lower than the Otsu's threshold value used in Case I.

Figure 2 shows the skeleton images in a magnified area of the selected ROI subject to different image processing parameters before performing skeletonization. In pattern recognition, the term skeleton denotes a representation of the structural shape of a plane region by a collective of thin arcs and curves. Figure 2b (Case II) shows a slightly fewer number of skeletons compared to Figure 2a (Case I) in the same magnified area. This is because as

the histogram is not equalized, the image intensity is not uniform, which might lead to some few fringes having similar intensity as the background and thus being excluded. Figure 2c (Case III) uses threshold value (0.1) that is 25% less than the Otsu's threshold value. Decreasing the threshold value increases the number of identifiable fringes, but the fringes also appear to become longer and begin to merge. The skeletons of each fringe are extracted for further statistical analysis.

Figure 3a shows the extracted lamellae length distributions for the three cases outlined in Table I. The similarity of the fringe length distributions confirms that the variation in image processing parameters has little to no impact on the output length distributions. Summarizing these distributions, median fringe length values were extracted from lognormal fits and are plotted in Figure 3b. The median fringe length for Case II is slightly smaller compared to the two other cases. This is in agreement with the skeleton image as shown in Figure 2b. In order to test if the differences in medians are significant, a test for difference between two medians is performed at a 95% confidence interval. The resultant t-statistic (1.81, two-tailed) suggests that there is insufficient evidence to infer that the median values differ, supporting the claim that image processing parameter variations has no effect on output statistics.

3.2. Thermal Annealing

Figure 4a shows the representative HRTEM image of unheated arc soot. This type of nanostructure without discernable crystalline structure is often termed as amorphous carbon, although very short lamellae are recognizable. However crystalline stacking or spatial order is completely absent in the unheated arc soot. This carbon material (arc soot) is heated in a furnace for extended period of time. Upon heating up to 2000 °C for one hour, the arc soot transformed into long tangled, interlocked lamellae enclosing large voids as shown in Figure 4b. At such high temperatures, cross-links are broken, lamellae mobility is enabled and free radicals are created, leading to lamellae growth. In contrast to other carbon blacks, the graphitized structure doesn't show any polyhedral shells containing multiple parallel stacks of graphitic layers comprising each wall of the polygon (Vander Wal et al., 2004, 2005). The initial nanostructure of the arc soot does not contain any outer shells or crystallites that could have potentially served as templates for such parallel stacking to evolve. Yet the visual comparison qualitatively verifies that the heated arc soot has well defined and recognizable lamellae.

To quantify the extent of graphitization upon heating of arc soot, HRTEM images are analyzed by the lattice fringe analysis algorithm. Consistent with the visual HRTEM observations, there is a progressive increase in structure as manifested by the broadened fringe length distribution (corresponding to longer lamellae) for the heated arc soot as shown in Figure 5a. Complimentary tortuosity distributions reveal that, upon thermal treatment, the arc soot exhibits decreased curvature in the lamellae as quantified by the narrowing of fringe tortuosity distribution shown in Figure 5b. These distributions clearly indicate heat treatment of arc soot increases lamellae length while simultaneously decreasing the fringe tortuosity – both metrics captured and quantified by the lattice fringe analysis algorithm.

Medians and standard deviations were calculated from these histograms. The median fringe length increased from 0.72 nm to 1.05 nm for the heated arc soot, suggesting a 45% increase in the lamellae length. A test for difference between the two fringe length medians was performed, and the resultant t-statistic (-20.4) clearly suggests that there is sufficient evidence to support the

claim that heat treatment of arc soot lead to increase in lamellae length. The same is found to be true for the difference between the fringe tortuosity medians.

3.3. Electron-beam irradiation

To further test the sensitivity of the algorithm for curvature, a highly ordered, well graphitized nanoscale carbon was irradiated by high intensity electron-beam. Such carbons have hollow polyhedrons consisting of facets of the layer planes that are bent from one facet to another. Figure 6a shows a representative HRTEM image of the carbon polyhedral onion prior to electron beam irradiation. The lamellae on the perimeter are highly ordered and do not exhibit any curvature. An electron beam at 200 kV with a current density of 512 pA/cm² irradiated this particle. Subsequently HRTEM images were acquired at specific time intervals, 2 and 5 minutes, respectively. Electron-beam irradiation breaks bonds through electron excitations and also displaces atoms by so-called “knock-on damage”. As result the evolution of the nanostructure might be different compared to thermal annealing. As evidence, traditionally the e-beam irradiation leads to nested concentric lamella, analogous to an onion and referred to as such (Iijima, 1980; Ugarte, 1992; Oku et al., 2004). Although graphitic structure is the most stable allotropic form of carbon, e-beam exposure leads to lamellae fragmentation within the first few minutes as observed in Figure 6b. Thereafter continuous e-beam exposure increases the mobility of these shorter fragments, enabling vegetative lamellae growth. In contrast to the graphitization as generally observed in heat treatment studies, e-beam irradiation leads to a visible degree of curvature, as seen in Figure 6c.

The visual comparison in Fig. 6 qualitatively demonstrates the changes in carbon nanostructure induced by e-beam irradiation. In order to further quantify these solid-state transformations, the HRTEM images were analyzed by the lattice fringe analysis algorithm. Median fringe length and tortuosity values were extracted from the fitted lognormal distributions, at each time interval. Upon initial irradiation, the lamellae length decreased as shown in Figure 7a. This decrease in lamellae length is attributed to the bond breakage due to the high intensity of e-beam. With lamellae mobility enabled, lamellae reform, yielding a more stable and ordered lattice structure, partially recovering the lamellae length. On the other hand, tortuosity as shown in Figure 7b, continuously increased with increasing electron beam exposure, confirming the curvature transformation as observed visually, induced by the electron beam irradiation.

4. Conclusions

Three different nanoscale carbon materials were characterized for solid-state structure using a custom lattice fringe analysis algorithm. Nanostructure parameters of lamellae length and tortuosity were extracted by analysis of HRTEM images. The images of diesel engine produced soot were examined for variation in image analysis parameters to test the robustness of the algorithm. The three different image processing cases that include an ideal reference case, non-contrast enhancement and a manual threshold case had no significant effect on the output nanostructure statistics. The thermally annealed (2000 °C) arc soot transformed into a non-graphitic form hallmarked by extended, but tangled lamellae. Lattice fringe analysis of the TEM images showed a 45% increase in median lamellae length, quantifying the lamellae growth. Highly ordered polyhedral onions were electron irradiated within a TEM with images acquired at successive time intervals. The transformation from graphitic to curved nanostructure was confirmed by an increase in the fringe tortuosity statistical distributions.

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Table I. Summary of image processing parameters for the indicated cases.

Case	Contrast enhancement	Gaussian filter size (pixels)	Gaussian filter deviation (1/pixel)	Top-hat transformation (radius of disk in pixels)	Binarization threshold value	Morphological opening & closing (pixel square)
I	Yes	11	1	5	0.1333	2 x 2
II	No	11	1	5	0.0863	2 x 2
III	Yes	11	1	5	0.1	2 x 2

fig 1

fig .2

fig 3

fig 4

fig 5

fig 6

fig 7

graphical abstract