Studies on artefacts induced in the specimen preparation routines of electron microscopy characterization

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Abstract. Artefacts are induced during the preparation of specimens for electron microscopic characterization. The present work is a study of the artefacts induced on worn surfaces and wear debris specimens of AA6061-B4C-Gr hybrid composites. The worn surfaces are prepared using routines such as Focused Ion Beam (FIB) milling and ion milling. Apart from these routines, a novel trial is made to prepare the worn surface using femtosecond laser machining. The wear debris is pelletized and sectioned using ultramicrotome. In femtosecond laser machining, FIB milling and ion milling operations, melting and re-solidification of ablated debris, re-deposition and melting of sputtered debris and formation of the amorphous layer, respectively are the major artefacts that diminish the accuracy of the inferences made on the microstructural characteristics of the specimen. In ultramicrotomy, knife marks, tearing of sections and absence of wear debris distribution reduce the effectiveness of the routine, making it impossible to derive any substantial inference from the micrographs.

1. Introduction
The methods by which the FIB milling, femtosecond laser machining, ultramicrotomy and ion milling are used to prepare specimens differ substantially. The following sections explain the complexities involved in these specimen preparation techniques. The purpose of selecting the routines to prepare specimens is described in the concluding paragraph of this section.

1.1. FIB Milling
Munroe [1] termed the FIB as the Swiss Army Knife of microscopy. The origin of FIB could be traced to the late 1970s, where the focused beam work was adapted from the Liquid Metal Ion Source (LMIS) when it was realised that sub-micrometer FIBs could be created using current density higher than 1 A cm$^{-2}$ [2]. Kitano et al. [3] have been one of the earliest workers to extend the application of FIB to non-semiconductor materials. Micrometer-sized nickel (Ni) powder particles were thinned using FIB and sub-micrometer sized grains with distinct grain boundaries were observed on the powder particles. In this study, FIB thinning provided large electron transparent area on the Ni powder specimen for High Resolution Electron Microscopy (HREM) imaging that is hard to obtain by conventional thinning methods. Uthayakumar et al. [4] milled a rectangular trench on the worn surface of Al-B4C-SiC hybrid composite in the direction perpendicular to that of sliding using FIB and observed substructure deformation and tribolayer. Slattery et al. [5] observed the fracture of Si needles in the subsurface (2-6 µm below the surface) by milling trenches on the worn surface of Al-Si alloy.

Some of the common FIB induced artefacts are the following:
1.1.1. Material re-deposition and amorphization. These artefacts compromise the microstructural integrity of the specimen that may lead to inaccurate inference. Studies revealed that surface damage layers were observed on the FIB milled FeAl-WC Metal Matrix Composite (MMC) specimens [6]. Formation of these layers could be linked to both the amorphization and material re-deposition. Surface amorphization of the specimen was caused by the energetic ions, and it reduced the contrast and resolution of the Transmission Electron Microscopy (TEM) image.

1.1.2. Implantation of Ga ion beam on features of interest of the specimen. Studies on polycrystalline Cu specimens subjected to FIB milling revealed the presence of Ga to a concentration of up to 20 atomic % at a depth of several nanometers below the surface [7].

1.1.3. Ion-induced damage in the specimen. Cairney et al. [8] thinned two different types of MMC specimens by using FIB milling and observed ion beam induced damage in the form of dislocations.

1.2. Femtosecond laser machining

The first industrial application of laser was for drilling in 1965 [9], and the micromachining with ultrashort laser pulses was first reported in 1987 [10]. A Femtosecond (fs) laser (1 fs = 10\(^{-15}\) sec) generates pulses in fs resolution. Femtosecond laser ablation of metal is precise, and mostly there is no heat-affected zone. Srinivasan et al. [11] observed that the polymethylmethacrylate (PMMA) can be etched using fs laser pulses without any thermal damage using low laser fluence in the range of 0.2–0.3 Jcm\(^{-2}\). The intralase fs laser irradiation is applied as an eye surgical system due to its precise ablation [12]. Also, fs laser is used to fabricate cardiovascular implants (stents) from biodegradable polymer materials [13]. Since fs laser processing is successfully applied where precision and quality are the key factors (e.g. eye surgery and stent fabrication) and as it ablates without any thermal damage (PMMA micromachining), its application is extended to prepare the specimens for electron microscopy characterization as a novel method.

However, fs laser processing of aluminum poses challenges such as re-deposition of ablated debris, surface roughening and melting and re-casting due to its low melting point and high thermal conductivity [14]. Zhu et al. [15] examined the influence of fs laser on 25 and 15µm thick aluminum (Al) foils by drilling sub-10 micrometer microholes using 60 fs Ti:Sapphire laser pulses at an energy fluence of 15 Jcm\(^{-2}\) in the air. The bottom surface of the microholes was irregular and rough. A Small amount of re-solidified molten droplets was also observed around the microholes. Vorobyev et al. [16] reported that a fraction of incident fs laser pulse energy dissipates into the bulk material and remains inside as the residual thermal energy which is considered undesirable since it adversely affects the service life and properties of the material.

1.3. Ultramicrotomy

Ultramicrotomy is a specimen preparation routine to produce ultra-thin sections of 60-100 nm thickness by producing a microcrack on the specimen during sectioning that propagates progressively into the specimen through the regions offering minimal resistance [17]. The knife edge induces the microcrack on the specimen. Specimen preparation by ultramicrotomy consumes less time compared to that of the ion beam routines, and geometrically complex specimens that are difficult to cut into discs of 3 mm diameter could be prepared using ultramicrotomy [18]. Malis and Steele [19] stated that specimens prepared using ion milling and electrochemical etching methods can give unreliable chemical inference when subjected to spectroscopy characterization. However, this problem does not arise with the use of ultramicrotomy wherein the thinning process is based on mechanical sectioning. For the same reason, there is no surface amorphization of the specimen too. Ultramicrotomy induces molecular bond breakage in a section [19]. Riedl et al. [20] observed the sectioning of nanocrystalline Cu-Nb powder particles embedded in the epoxy resin. Extensive deposition of the epoxy resin was observed over the sections after sectioning.
1.4. Dimple grinding and Ion milling
Before ion milling, the specimen is pre-thinned using dimple grinding technique to reduce the specimen thickness.

1.4.1. Dimple Grinding. A dimple grinder uses mechanical abrasion to grind a concave shaped impression or dimple at the center of the specimen to reduce its thickness to 5-10 µm at the center [17] and hence the time required to thin the specimen using ion milling is reduced [21].

1.4.2. Ion Milling. After pre-thinning, the specimen is subjected to ion milling in which it is thinned to the state of electron transparency [17]. A beam of ions with the energy of about 1-10 keV bombards the specimen surface to sputter the atoms out of the specimen [22] which is rotated to ensure continued cylindrical milling. Artefacts are induced in the specimen due to ion milling. In the case of milling a multi-phase specimen (Z39 steel) with the milling angle 10°, the specimen surface gets roughened due to inhomogeneous constituent phases (hard and soft constituent phases) of the steel specimen [17]. During ion milling, there is a probability of material re-deposition caused by the sputtered debris falling back onto the specimen surface. Carter et al. [23] found a thin amorphous layer formed at the interface of Si-SiC structure due to ion milling that made it impossible to analyze the Si-SiC interface. Apart from these, some of the other ion milling induced artefacts include, chemical diffusion, thermal and irradiation damages, possibility of cracking of the specimen disc due to the loss of its mechanical strength and perforation of blunt holes that has limited electron transparency.

The authors of the present work studied the tribological behavior of AA6061-B4Gr hybrid composites [24]. In studying the tribological behavior of AA6061-B4Gr hybrid composites, it is required to understand the phenomena such as subsurface plastic deformation, the formation of tribolayer, dynamic recrystallization, submicron crystalline structure of the worn surface and influence of wear mechanisms on the morphology of wear debris. To characterize the phenomena such as subsurface plastic deformation of the worn surface and formation of tribolayer, the specimens are prepared by FIB milling and femtosecond laser machining techniques. Dynamic recrystallization and sub-micron crystalline structure of the worn surface are studied by thinning the specimen to electron transparency that is carried out using ion milling technique. Also, the wear debris is prepared using ultramicrotomy to analyze the influence of wear mechanisms on the morphology of the wear debris. However, it is observed that artefacts are induced during the preparation of specimens for characterization. The objective of the present work is to discuss scientifically and draw significant results on the reasons and consequences of incurrence of the artefacts.

2. Materials
For the present study, materials that are prepared using different specimen preparation routines are worn surfaces and wear debris of AA6061-10 wt. % B4C-Gr hybrid composites. The worn surface of 2.5 wt. % Gr hybrid composite is prepared by FIB milling and femtosecond laser machining. The worn surface of 7.5 wt. % Gr hybrid composite is prepared by ion milling, and the wear debris of 5 wt. % Gr hybrid composite is prepared by ultramicrotomy.

3. Testing and Characterization Scheme
The hybrid composite pins of dimensions 30 mm height and 8 mm diameter are tested, as per ASTM G99-05(2010) Standard using pin-on-disc tribotester (Ducom, TR-20 LE) under atmospheric conditions (1 atm., 30 ± 1°C, and 60 ± 5% RH). The 2.5 wt. % Gr hybrid composite worn surface specimen is tested for a sliding speed of 2.5 m/s, applied load of 30 N, and sliding distance of 600 m. The wear debris of 5 wt. % Gr hybrid composite specimen is tested for the sliding distance of 1000 m, sliding speed of 1.5 m/s and applied load of 30 N. The 7.5 wt. % Gr hybrid composite worn surface specimen is tested for applied load of 50 N, sliding distance of 600 m and sliding speed of 1.5 m/s. The counterface used is EN31 bearing steel disk of hardness 65 HRC.
After the sliding wear tests, the worn surfaces and wear debris are prepared with different sample preparation routines. Slots are cut on the 2.5 wt. % Gr hybrid composite worn surface specimen using FIB milling equipment (Zeiss, Neon 40 EsBCrossBeam, available at Central Manufacturing Technology Institute, Bengaluru, India) and femtosecond laser machine (Clarke-MXR UMW 2110, available at Central Manufacturing Technology Institute, Bengaluru, India). Transmission Electron Microscope (TEM) (Jeol, JEM-1011, available at Rajiv Gandhi Centre for Biotechnology, Trivandrum, India) of acceleration voltage 100 keV is used to characterize the 5 wt. % Gr hybrid composite wear debris specimen. In the pelletizing procedure, centrifuge and thermomixer are used, and the sectioning is carried out using ultramicrotome (Leica, EM UC7, available at Vikram Sarabhai Space Centre, Trivandrum, India). The 7.5 wt. % Gr hybrid composite worn surface specimen is characterized using a Scanning Electron Microscope (SEM) (Hitachi, SU6600, available at authors affiliated institute) and High Resolution Transmission Electron Microscope (HRTEM) (Jeol, JEM-2100, available at PSG Institute of Advanced Studies, Coimbatore, India) of acceleration voltage 200 keV. The 7.5 wt. % Gr hybrid composite worn surface specimen is ion milled using Precision Ion Polishing System (PIPS) (Gatan, 691).

4. Results and Discussion
The artefacts induced in the 2.5 wt. % Gr and 7.5 wt. % Gr hybrid composite worn surface specimens and 5 wt. % Gr hybrid composite wear debris specimen that are prepared using the different specimen preparation routines is discussed in the following sections.

4.1. FIB milling induced artefacts
The worn surface of 2.5 wt. % Gr hybrid composite specimen which is subjected to FIB milling is shown in figure 1. A rectangular slot of size 4 x 5 µm with a depth of 3 µm is milled with Ga ion beam accelerated to the energy of 30 keV at a beam current of 2500 pA under vacuum. The slot is milled to observe subsurface deformation, reinforcement particles (B_{4}C and Gr) in the subsurface and formation of tribolayer. The stub is tilted to the position of 56° to view the walls of the slot such that the features of interest are revealed.

FIB induced artefacts are observed in the form of re-deposition of sputtered debris inside the milled slot (marked with dotted arrow) and onto the walls (marked with arrow) of the milled slot.
the quality of chemical analysis of the specimen. The material re-deposition is thought to be minimized by milling with low beam current towards the final stages of milling [1]. This type of milling is called cleaning or polishing mills. Cairney et al. [8] suggested that during polishing mills the ion beam is rastered line by line towards the features of interest. So that the milling process concludes by milling at the very edge of the section (an object or shape to be produced by FIB milling) which in turn minimizes the material re-deposition.

4.2. Femtosecond laser machining induced artefacts

A rectangular slot is ablated on the worn surface of 2.5 wt. % Gr hybrid composite specimen as shown in figures 2(a) and (b) to observe the microstructural changes incurred in the subsurface due to the wear testing. The size of the rectangular slot is 20 x 25 µm with 20 µm depth. It is ablated by using an amplified Ti:sapphire laser system generating 60 fs pulses at a 1 kHz pulse repetition rate. The ablation is conducted under the laser energy fluence of 50 Jcm⁻². The bottom surface of the slot is seen to be roughened due to the re-deposition of the ablated debris which is melted and re-solidified on the bottom surface as shown in figure 2(a). Vorobyev et al. [26] suggested that the re-deposition of the ablated debris back onto the specimen causes its residual heating that leads to an increase in temperature of the bulk specimen. In figure 2(b), damage to the immediate region next to the slot wall (area in between the dotted lines) can be observed. The walls of the slot are seen to have lost its microstructural integrity as the slot edges at the bottom surface has become shallow and the right side wall of the slot is damaged severely by the crack (marked with dotted arrow) running on its surface.

![Figure 2. SEM micrographs showing (a) Re-deposition of ablated debris and (b) Damage of the immediate region next to the slot (area in between the dotted lines) and crack on the slot wall (marked with dotted arrow).](image)

All these artefacts make further characterization and analysis of the worn surface micromachined with femtosecond laser pulses, to be rather inaccurate. The size of the section to be processed plays a significant role in the induction of artefacts. Zhu et al. [15] observed that when the slot depth gets deeper, the ablation rate is reduced due to factors such as loss of pulse energy through the walls and the re-deposition of ablated debris. Selection of appropriate micromachining parameters based on the properties of the material and dimensions of the sections to be produced has a significant role in the minimization of artefacts.

4.3. Ultramicrotomy induced artefacts

To characterize the wear debris of 5 wt. % Gr hybrid composite specimen directly using TEM, the thickness of the wear debris should be sufficiently small to make it electron transparent. But, the wear debris used to be irregular in shape with significant thickness, which necessitates its thinning.
The wear debris is embedded using Diglycidyl Ether of Bisphenol-A (DGEBA) liquid epoxy resin by adapting the procedure detailed by Catelas et al. [27] with small changes. DGEBA epoxy resin has excellent mechanical and chemical resistance and high adhesive strength [28]. Methyl tetrahydrophthalic anhydride is used as the curative. The embedding procedure starts with suspending the wear debris in 100% ethanol in a microcentrifuge tube. Then, centrifuging is carried out in two stages as suggested by the above procedure. At first, centrifuging is done at 20,913 x g (14,000 rpm) for 30 min. The resulting supernatant is removed, and the wear debris is re-dispersed in a solution of 0.5 ml acetone and 0.5 ml liquid epoxy resin. The tube is then placed in a thermomixer for 16 hours under slow motion at room temperature. Centrifuging is then carried out for the second time by adapting the same parameters to achieve uniform distribution of wear debris which is followed by applying vacuum for 1 hour to evaporate the acetone. 1 ml of epoxy resin is again added into the tube that is kept in vacuum for 3 hours to remove the air bubbles and to ensure the evaporation of residual acetone. Polymerization is carried out for 2 hours at 90 °C and then for 4 hours at 150 °C. The excess resin layer covering the specimen block is removed by grinding with SiC abrasive sheet of 1500 grit size. The specimen is then thoroughly washed using ethanol to remove the traces of SiC particles and cut using a razor blade to obtain an area of about 0.5 mm$^2$. The specimen is fixed to the ultramicrotome arm for sectioning. Sectioning is carried out at a knife angle of 35 ° which is suitable for multi-phase materials [17]. As a common rule, if the knife angle is selected as 35 °, a cutting speed of 0.2 mm/sec and knife clearance angle of 6 ° is selected. Diamond knife is used for sectioning and the sliced sections float on the surface of the distilled water contained in the boat and is slowly scooped onto the copper grids.

Figure 3. TEM micrographs of the sections: (a) Knife marks (marked with dotted arrow) and (b) Wear debris (marked with arrow) and knife marks (marked with dotted arrow).

Figures 3(a) and (b) show the ultramicrotome sections. Knife marks (marked with dotted arrow) which are parallel and continuous are observed on the sections. Knife marks are produced due to the accumulation of microscale debris of the previous cuts on the knife edge. During sectioning this microscale debris come in contact with the section (rather than the knife edge), and makes scratches (knife marks) on it. Knife marks are minimized by cleaning the microscale debris deposited on the knife edge without damaging it. Wear debris (marked with arrow) hanging scantly on the section is observed in figure 3(b).
Wear debris agglomeration is observed on the section as shown in figure 4(a). Usually, pelletizing and sectioning reduces the wear debris agglomeration [29]. Wear debris agglomeration indicates that the centrifuging process has not been effective for uniformly distributing the wear debris. Also, the wear debris could have been adhered together loosely, during collection from the counterface after the wear testing, which acts as a hindrance to debris distribution. Optimizing the centrifuging speed and time can minimize the wear debris agglomeration. Figure 4(b) shows the varying thickness distribution of a section. A dotted curve in figure 4(b) distinguishes between the thick and the thin regions of the section. Ramalingam and Black [30] attributed the thickness variation to the generation of plastic deformation instabilities at the knife edge during sectioning. Wear debris (marked with dotted arrow) is observed on the right side of the section. Severe tearing of the sections is incurred as shown in figures 3(b), 4(a) and (b). Resin embedded ultramicrotome section is susceptible to high energy electron beam heating, as the resin is a poor heat conductor [19] which in turn, causes the breakdown of the embedding resin.

Figure 5 shows the chatter lines (marked with dotted arrow) that are incurred as a result of the high frequency vibrations generated between the knife and the specimen block. Sectioning parameters such as clamping pressure, sectioning speed and knife clearance angle influence the setting up of high frequency vibrations. Precise control of these parameters can mitigate the resulting artefact. It is observed from figures 3(a) and (b) and 5 that there is no distribution of wear debris on the sections. This can be overcome by re-trimming the specimen block to expose regions that hold more wear debris clusters.
It is noted that the wear debris consists of a combination of the constituent elements (matrix material-AA6061, reinforcement particles-B4C and Gr) of the hybrid composite. EDS spectrum reveals the constituent elements of the wear debris as Al 88.77, O 6.71, Mg 0.86, C 2.08 and Fe 1.57 by wt. %. As the knife edge comes in contact with the ductile matrix material debris during sectioning, the sectioning mechanism is shearing [19] and the section compression is the probable artefact. If the knife edge comes in contact with the brittle reinforcement debris, cleaving followed by wedge crack propagation is the sectioning mechanism, and the fracture of debris structure is the probable artefact. Hence, it is evident that the mechanism of sectioning and induction of artefacts differs by the ductile or brittle nature of the wear debris. This is a major complexity involved in the sectioning of wear debris of the hybrid composite using ultramicrotomy.

4.4. Ion milling induced artefacts

A step by step specimen preparation routine has been adapted to characterize the worn surface of 7.5 wt. % Gr hybrid composite specimen using HRTEM. A specimen of 500 µm thickness is sectioned from the hybrid composite pin using a precision cut-off machine. The thickness of the specimen is reduced to 300 µm by manual polishing during which one side of the double sided adhesive tape is stuck with the specimen while its other side is stuck to the finger. The specimen is punched to a diameter of 3 mm by using a disc punch system. In the first pre-thinning phase, the disc specimen of 3 mm diameter is mounted on the specimen mount of the disc grinder and ground to a thickness of 70 µm. Dimple grinding (dimpling) is the final pre-thinning phase. The disc specimen is dimpled using phosphor bronze grinding wheel (15 mm diameter) to a thickness of 20 µm at the center. The dimpling load and speed are selected to be 25 g and 200 rpm, respectively. The diluted diamond polishing compound of 2 microns is made to be present in the dimpling region throughout the dimpling process. To reduce the thickness further (less than 20 µm) and to remove the mechanical damage induced due to dimpling, the grinding wheel is replaced with felt wheel, and the dimpling is continued through gentle polishing using the diamond paste of 1 micron for 2 min. The dimpling load and speed are selected to be 30 g and 200 rpm, respectively. Initially, dimpling was tried beyond 20 µm without changing the grinding wheel which resulted in breaking of the disc specimen as shown in figures 6(a) and (b). The zone of breaking (marked with dotted arrow) is shown in figure 6(a). A crack is also shown (marked with arrow) near the zone of breaking. Figure 6(b) shows a magnified image of the crack (marked with dotted arrow) running through the disc specimen surface.

Figure 6. SEM micrographs showing (a) Zone of breaking (marked with dotted arrow) and crack (marked with arrow) on the disc specimen and (b) Higher magnification image of crack (marked with dotted arrow) running through the disc specimen surface.
Figure 7. HRTEM bright-field micrographs showing amorphous layer on the surface of disc specimen (a) Low magnification micrograph and (b) High magnification micrograph

Here, only the unworn side of the disc specimen is processed using dimpling and ion milling. Ion milling is carried out using Ar ion beam by adapting 5° beam angle and 6 keV beam energy till the perforation is reached in the regions of interest of disc specimen. After ion milling, the disc specimen is characterized using HRTEM. The low magnification micrograph (figure 7(a)) and high magnification micrograph (figure 7(b)) illustrate a layer on the surface of the disc specimen. This layer made the surface of the disc specimen wholly featureless which is characteristic of the amorphous layer. Discussions on amorphization due to ion beam irradiation is based on the accumulation of a critical amount of lattice damage [31] due to which the crystalline structure of the specimen is transformed to the amorphous state. This lattice damage can incur in the form of an increase in point defect concentration [32]. Formation of amorphous layers on the surface of the specimen can be minimized by polishing with low energy ion beam during the finishing stages of ion milling.

5. Conclusions
In summarizing the artefacts induced in the worn surfaces (2.5 wt. % Gr and 7.5 wt. % Gr hybrid composite specimen) and wear debris (5 wt. % Gr hybrid composite specimen) that are prepared by different specimen preparation routines, the following inferences are observed:
1. In FIB milling, the sputtered debris does not get ejected into the vacuum, but rather re-deposited inside and on the walls of the milled slot that leads to the reduction in the milled depth.
2. In femtosecond laser machining, the bottom surface of the slot is roughened due to the melting and re-solidification of ablated debris. Crack is also formed on the wall of the slot.
3. In ultramicrotomy, knife marks are produced due to the accumulation of microscale debris on the knife edge and chatter lines are formed due to high frequency vibrations between knife and specimen block.
4. In ion milling, an amorphous layer is formed on the surface of the disc specimen due to the accumulation of a critical amount of lattice damage.

The above inferences make it evident that due to the artefacts induced in the specimen preparation routines, the micrographs obtained using electron microscopy techniques fail to illustrate proper results.

6. References
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