Electron backscatter diffraction (EBSD) sample preparation revisited: integrating traditional methods and new techniques in the last three decades

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ABSTRACT
Electron backscatter diffraction (EBSD) is an extremely powerful tool for analyzing the structure of matter. Sample preparation of heterogeneous materials as metallic alloys and minerals where grains with different physical and chemical characteristics has always been a challenge, being sometimes the main reason for the failure of the analysis. The present article intends to present to the reader a small survey of the most common EBSD sample preparation techniques and some of the newest advances in the area, as well references for further reading, though not being exhaustive as a review paper. Conventional techniques such as mechanical polishing and electropolishing as well as the more recent ion milling techniques using broad beam and the focused ion beam microscopes (FIB), are briefly presented and discussed.

Keywords: EBSD, FIB milling, thin foil preparation, minerals, alloys.

INTRODUCTION
Electron backscatter diffraction (EBSD) is a powerful analytical method that allows obtaining microtextural data such as grain/subgrain dimensions, local strain, point-to-point orientation, phase identification/distribution and texture analysis mainly in Materials Sciences, Geosciences and Engineering. These data are essentially based on Kikuchi patterns, which are collected within the first 100 nm of the material surface and demand flat and damage-free surfaces: the crystal lattice must be clean from contamination or amorphous layers and strain-free; except when strain analysis is the goal of the measurements [1-6]. The good visibility and well-defined aspect of those patterns allows judging how well prepared the sample was [7-9] and is essential for a good analysis. EBSD spectrometers are mainly coupled to SEM (scanning electron microscope) and sometimes can be integrated with an Energy Dispersive X-ray Spectrometer (EDS).

It is exactly the strength of this technique that makes the sample preparation difficult, grain boundaries,
heterogeneous grained materials with different hardness, sizes, orientations and chemical composition can be extremely challenging and makes the sample preparation procedures, details and protocols unique to each type or group of samples [10].

**The importance and the use of automated EBSD during the last three decades.**

The physical process on which EBSD analysis is based, was discovered and described in 1928 by Shoji Nishikawa and Seichi Kikuchi [11], after the observation of electron diffraction patterns through a thin mica plate. Several months later, the same authors confirmed those results from a cleaved face of a calcite crystal on photographic plates and described them as net planes encompassed by pairs of black and white lines due to selective reflection of the incident rays: diffraction through crystallographic planes of crystalline material [12]. This phenomenon was studied along the next decades [13-15], until the availability of a commercial scanning electron microscope (SEM) in the 1970s, which allowed using a phosphor screen and TV camera to record the Kikuchi patterns [16]. In 1973 Venables and Harland [17] described, for the first time, the Kikuchi diffraction patterns as electron backscatter diffraction (EBSD) obtained in a scanning electron microscope (SEM). In the 1980s, computer assisted indexing of EBSD patterns started to be used, but only in 1987, the first algorithms for indexing of the pattern and calculation of the crystal orientation were published and then improved in 1989 [18-19]; nonetheless, the EBSD was not completely and effectively automated.

The early 1990s were the landmark of fully automated pattern recognition and indexing methods for EBSD; in 1992, Stuart I. Wright developed these methods and published it in his PhD thesis [20] at Yale University. Later, all research groups converged on the use of Hough transform [21] as the most suitable procedure for obtaining the positions of the Kikuchi bands in the image; the use of Hough transform for EBSD indexing was established between 1992 and 1994 [22-23]. Figure 1 shows the number of articles based on automated EBSD usage that has been published between 1992-2019. The gradual increase of EBSD usage is related to the high quality of spatial resolution, as a consequence of the improvement of microscopes, detectors, computers and software, which allow identifying the phases, measuring their fraction and relationships, and determining the microstructural features of materials, such as grain size and morphology, boundary characteristics and crystallographic orientation; those features are directly correlated to their properties and performance (e.g. crystallographic orientation and grain size influences directly the mechanical properties of a material, such as the Young modulus). The understanding of crystallographic orientation is very important in both material design and processing. Moreover, EBSD technique is very versatile because it can be used on varied materials, such as metals and alloys, minerals, ceramics and semiconductors; essentially any crystalline material with grain sizes down to 10-50 nm [24-27].

According to figure 1, a discrete number of articles based on automated EBSD usage has been published between 1992-2000. This progress continued along the 2000s decade. In 2006, Wilkinson et al. [28] improved the angular resolution to allow strain analysis of EBSD diffraction patterns and the determination of elastic distortion. However, during the 14th European Microscopy Congress, held in Aachen, in September of 2008 (see figure 1 in red), high-speed cameras with frame rates above 700 patterns per second were presented by Søfferud et al. [29]. Correlating those progresses with the increasing demand of EBSD works in areas such as Materials Sciences and Engineering, an interesting “boom” occurred during the transition between the years 2009-2011 (see figure 1 in blue): for the first time, the number of articles increased in the order of
almost a hundred. Since 2010 the reduction of the resolution to a nanometric scale has been progressively used, especially the use of lower accelerating voltages [30]. From 2012 to 2019 (see figure 1 in green), the number of EBSD published articles continuously increased mostly in the order of hundreds year by year. This can be explained by the progressive adoption of a conventional EBSD system in a field emission gun scanning electron microscope (FEG-SEM) with more user friendly software for both EBSD spectrometer and microscope, a more widespread use of transmission EBSD (t-EBSD) which allows obtaining diffraction patterns from thin foils with grain sizes in the order of 10 nm and their respective orientations [31-33] and the increase of EBSD 3D [1, 34].

Fig. 1. Evolution of EBSD published articles along the last three decades. Data for this graph was obtained from Web of Science [v.5.35] on September 3th 2020.

As t-EBSD is a technique that does not involve backscattered electrons, it has been designated transmission Kikuchi diffraction (TKD) and has been used as an interesting alternative to conventional EBSD [33]. For a good quality automated TKD analysis, samples need to be under 100 nm in thickness and can be prepared with the aid of electropolishing or ion milling, both conventional ion milling or in a focused ion beam microscope (FIB); the latter is more versatile because regions of interest down to 10 µm can be selected directly in the microscope. The sample is then mounted in a special sample holder so that the detector can be place behind the sample in respect to the incident beam. Simultaneous chemical analysis using dispersive X-ray spectroscopy (EDS) can also be performed and spatial resolution can be very effective for several materials, down to 2-10 nm [32-33, 35-36].

Sample Preparation Methods.
Each sample analyzed with EBSD demands its own preparation procedures. Conventional metallographic [37] procedures are the basis of all conventional EBSD sample preparation techniques, where final mechanical polishing, electropolishing or ion milling steps have to be added. t-EBSD on the other hand uses most of the sample preparation methods used for transmission electron microscopy, which are extensively discussed by Ayache
All the sample preparation procedures that will be described along this section are cited in Stojakovic [24], Vander Voort [37] and Ayache [38-39], and also refer to information compiled from websites of manufacturers of EBSD equipment [40-41] and manufacturers and suppliers of consumables and equipment for electron microscopy [42-44]. Figure 2 synthesizes the most common workflows based on bulk and thin foil specimen preparation which can be used for EBSD and t-EBSD sample preparation. We will discuss in more detail the conventional EBSD sample preparation.

Fig. 2. Workflow chart of the most common routines for EBSD sample preparation. On the left, conventional EBSD sample preparation workflow is shown. On the right, thin foil preparation workflow for t-EBDS is shown.

**Mechanical polishing.**

Basically, the mechanical polishing is composed of six main steps: sectioning, mounting or embedding, grinding, preliminary polishing and final polishing with colloidal silica. All that information was compiled and are described in agreement with the EBSD preparation procedures found in references [9-10, 40-47].

**Sectioning or cutting.**

Sectioning or cutting is the removal of a representative area from a studied material and is mainly performed by a wet abrasive cutting machine, composed of CBN (cubic boron nitride) or diamond cutting wheels. Sometimes a large sample or rock has to be cut prior to being brought into the sample preparation laboratory to be cut into a smaller piece. The first step to remember about cutting is to preserve the orientation of sample axes: in the case of metallic samples: rolling direction (a-axis), transverse direction (b-axis) and sample normal (c-axis) and in the case of minerals: the orientation in respect to the geological formation to be analyzed. The second one, is the size of the analyzed sample. It must be appropriate in order to allow mounting.
and the examination at SEM, normally about one centimeter. In order to preserve the microstructure of the sample, four variables must be strictly respected [47-50]: rotation speed, applied pressure, feed rate (e.g. the internal stresses that generate plastic deformations, such as the transformation-induced-plasticity - TRIP - effect on steels [51]) and cooling procedure must be carefully carried out. Aggressive cutting methods that generate heat or cause any type of deformation on the cut surface must be avoided. At this stage, the damage can be so severe, extending within the sample, and will not be removed by subsequent grinding and polishing processes. Additionally, heating can cause microstructural changes such as phase transformations or activation of precipitation/diffusion mechanisms [41, 52-53].

The cutting wheels must be carefully selected in order to obtain an optimal sectioning for metallic materials: for the nonferrous ones, it is recommended SiC wheels; and for the ferrous ones, Al₂O₃ wheels due to their less extension of oxidation reactions. In addition, for other type of materials (not referring to nonferrous or ferrous), the designated “hard wheels” are appropriated for cutting soft materials (e.g. > HRC 35-50 - hardness Rockwell C), while the “soft wheels” are suitable for hard materials (e.g. > HRC 60 - hardness Rockwell C) [42-43, 48]. Generally, for samples with size of less than 25 mm, it is recommended to use a low speed (< 600 rpm) and high precision wheels (< 300 µm), in order to cause minimal damage to the sample surface during the polishing process [42-43, 50].

Mounting or embedding.
In this process, the sample is mounted or embedded in a solid medium for grinding and polishing. Mounting processes can be divided into two types: hot and cold. The hot one is subjected to temperatures of ~180-200 °C and occurs under pressure in a mounting press, where the sample is placed in a cylinder together with an appropriate mounting resin (e.g. thermoplastic resins, such as bakelite resins). The main features of hot mounting are: sufficient hardness to protect the edges of the sample; good adhesion in order to avoid gaps between the mounting material and the sample surface; not suitable for fragile, brittle and heat sensitive materials; the exact tolerance for the diameter of the mount is respected; parallel top and bottom surfaces are more easily scanned across large specimens; and high stability in vacuum conditions (no contamination caused by out-gassing or vapor).

Cold mounting or embedding is when a resin (e.g. epoxy, such as 2-hour epoxy resin [54]) is mixed with a hardener (e.g. duriplastic hardener, such as 2-hour fast cure hardener [54]), in order to provide the mounting compound; then, the polymerization process occurs and form the mounted sample. The main features of cold mounting are: minimum shrinkage (i.e., is when the resin shrinks away from the sample surface during curing) and better adhesion to the sample; and suitable for fragile, brittle and heat sensitive materials [40-41].

For EBSD there is no specification for the type of mounting materials. In case of performing hot mounting, it is recommended to use a conductive medium (copper or graphite filler [54]) in order to eliminate charging and drift problems. On the other hand, for cold mounting, the sample can be made conductive by the use of a mixture of resin with carbon powder. Non-conductive resins can also be used when mounting larger pieces of material, however conductive paints as carbon or silver, or one sided metal tapes, which do not out-gass under vacuum [44, 54-55] can be used to completely cover the non-conductive resin.

Grinding.
This process allows removing the deformation layer introduced during sectioning, producing a flat surface for examination. Grinding process can be divided into two steps: coarse and fine grinding. During the first step, the
damage (caused by sectioning) should be removed, which can be accomplished with the aid of grinding papers covered with silica carbide (SiC), diamond, alumina ($\text{Al}_2\text{O}_3$) or cubic boron nitride (CBN) abrasives. The second step consists in the reduction of damage and surface roughness of samples to a degree that is suitable for polishing. Usual abrasives are SiC and alumina covered papers, or diamond covered disks. For the first one, the process is composed of several steps and starts with the use of 180 or 240 grit SiC disk-paper and gradually changes to down to 1200 grit SiC; later, those papers must be discarded in order to maintain the removal of grinding remnants. Water is normally used as a lubricant in order to remove remaining material and avoid heating and thus the formation of an amorphous layer of 1-100 nm thickness on the surface, such as the Beilby layer [56-58]. In the case of oxidizing samples special lubricants should be used. Furthermore, it is recommended to accurately examine the surface of the sample with the aid of an optical microscope; damage induced during grinding may be invisible in the polished surface, but will be enough to prevent the EBSD-pattern formation.

Preliminary polishing.

Polishing consists of removing the remaining deformations and scratches caused during grinding processes and mainly intends to obtain highly flat and reflective surfaces in the samples before they can be observed in the SEM. In order to be properly polished, material properties of samples and appropriated types of abrasives, suspension mediums, polishing cloths and rotational speed and lubricants must be well known (e.g. alumina abrasives with some diamond solutions for harder materials such as steels [59]). Generally, preliminary polishing is started with the aid of a hard cloth with a coarse abrasive (e.g. for better flatness) and finished with a soft cloth with a fine abrasive (e.g. for better reflectivity). In addition, the polishing process requires accuracy in order to avoid the abrasion of different areas at different rates, originating “relief” or undulations formed on polished surfaces. Polishing by automated equipment is currently the most recommended for the preparation of material samples to be analyzed by EBSD [42-43]. Factors such as reproducibility of polishing steps, consistency in the application of operating parameters and the ability to process several samples at the same time, not only reduced the preparation time of these samples, but also considerably improved the final result of the polished surface of the sample and drastically reduced the effects of the cutting process [47-48]. In particular samples of pure composition or which have a high ductility, find in automated polishing a reliable alternative for the preparation of preliminary polishing. Automated polishing equipment works with a series of control parameters. We can mention the force applied to the sample against grinding papers or polishing cloths, speed of rotation of sandpaper or polishing cloths, quantity of lubricant and abrasive deposited [49-50]. In this stage of sample preparation by polishing, they are greatly influenced by the crystalline structure of the sample material. For materials that have a crystalline structure in FCC arrangement, they are very ductile and show more damage in each sanding and polishing step, than samples that have a HCP and BCC crystalline arrangement, which demonstrate a less ductile behavior. This is because the crystalline structures in FCC arrangement have 12 sliding planes, BCC has 6 sliding planes, and the HCP is restricted to only 3 sliding planes [48]. The average grain size of a sample and its heterogeneity of phases and compositions can have a direct influence on the roughness of the sample surface. Therefore, for crystallographic and microstructural reasons, the choice of the polishing parameters of the equipment and its abrasive steps (sandpaper and polishing cloths) must be strongly considered in the elaboration of a
work routine, involving control parameters, to polish a sample for the purpose of EBSD analysis [47].

**Final polishing with colloidal silica.**

Polishing with colloidal silica is the additional and required final mechanical polishing stage for EBSD analyses. Colloidal silica is a solution composed of negatively charged particles of SiO$_2$, with $8 < \text{pH} < 11$, which typical size is 0.05 μm [60-61]. Moreover, this step is the combination of the effect of mechanical polishing with slight chemical etching, removing most of the remaining “reliefs” and is very effective on ceramic and geological samples. Finally, the solution must be promptly washed away with water during the last seconds of polishing, in order to avoid being left to dry in the polishing cloths because colloidal silica crystallizes very fast [61]. For samples with high ductility, such as metals and their alloys, final polishing for EBSD is done using a vibratory polisher. This equipment consists of a polishing machine that has a polishing bowl that is lined with a very soft polishing cloth (velvet) and the polished face of the sample is placed under a support and set to move freely over the surface of the cloth, vibrating at a frequency 7200 cycles per minute, in low amplitude, in a solution of colloidal silica (0.02 μm) or colloidal alumina (0.05 μm) [42]. This vibratory movement produces a very effective polishing action, causing the least possible crystalline deformation and leaving free of stresses that greatly increase the chances of indexing Kikuchi patterns. As with preliminary polishing, vibrational polishing parameters must be taken into account according to the crystalline nature of the metallic material [50].

**Electropolishing and chemical etching.**

Electropolishing is a polishing method that can improve the acquisition of high-quality EBSD patterns. It is a very effective and relatively cheap method and is also very useful for TEM analysis because it does not cause and/or removes the remnant deformations layers and surface irregularities observed after mechanical polishing [62-64]. The sample be polished/etched (when the metallic sample is dissolved) turns into an anode after being submerged into an electrolytic reaction cell containing a temperature-controlled electrolytic solution (mixtures of sulphuric acid and phosphoric acid and perchlorates with acetic anhydride [66-70]) is connected to an external power supply composed of a positive terminal of direct current (DC); and the cathode, which can be a stainless-steel plate [64]. The principle is simple: the external power supply provides current through the electrodes and then the metal of the anode is removed from the surface, becoming cations that move towards the cathode [64-66]. The quality of electropolishing results depend on the careful control of the applied current density, the accurate chemical composition of the electrolytic solution, the electrolyte temperature and anode/cathode distance. Moreover, it is recommended to assess the quality of the electropolished surface with the aid of optical microscope [64].

**Ion milling.**

Ionic milling or etching is the process of using an ion beam to remove material from a sample either for nanofabrication or for sample preparation. Ionic polishing or ion milling equipment are well known as the last sample preparation step for transmission electron microscopy (TEM) thin samples. This equipment uses mainly a collimated Ar$^+$ beam to slightly perforate the sample in order to create an extremely thin region at the border of the hole which is then observed in the microscope [38-39, 68-70]. The already thinned sample is mounted on a holder which can be N$_2$ cooled or not and the holder can rotate or oscillate while one or two Ar$^+$ beams are collimated onto the sample on a certain angle which normally varies from 20° down to 4° using energies from 500 V to 15 kV. Until recently, only
BalTec RES 101 was used also for EBSD sample preparation [71-72]. For several types of samples, ion milling has been considered more efficient than mechanical polishing with colloidal silica because it does not produce residues and thus allow obtaining better high resolution Kikuchi patterns. Moreover, it is also less hazardous and complex than electropolishing.

In the last decade, cross section polishers and specific ion-mill equipment for SEM and EBSD were developed by all major ion mill manufacturers, which traditionally worked only for TEM sample preparation, with quite good results for very different type of materials.

Walde et al. [73] analyzed the microstructure of a heat treated Aluminum powder alloy 6061 using a cross sectional polisher for 2 hours at 6 kV. Dankhazy [58] studied austenitic steel, an aluminum alloy, Cu, Ni and Ag high purity samples trying to determine the best milling parameters, using the image quality value (IQ) of the EBSD data. Except for steel, all alloys and pure elements had an optimal time between 6 and 7 minutes, steel took 26 minutes. The best angles were determined to be between 6 and 8 degrees and the accelerating voltage 10 kV. And they finished the experiment with limestone using an angle of 30° in respect to the Ar+ gun for 2 hours at 10 kV and a final polishing with 2 kV at 5° angle with the sample oscillating between ±30°. Abdolvand and Wilkinson [74] studied a zircaloy-2 HCP sample with excellent Kikuchi pattern quality enabling them to study residual stress fields. They used a dual 7.5 kV beam at 8° angle for 15 minutes.

Roessler et al. [75] characterized the microstructure of Portland cements using a Ar+ broad milling polisher and varying the milling parameters during the process: a first step using a flat tilt angle for 20-30 minutes and 4 kV and finishing with a higher tilt angle, about 30° using 6 kV for approximately 45 minutes. And Vieweg [76] studied the inductive hardening process on the martensitic structure of a 50CrMo4 steel.

As can be deduced, very different materials are being prepared in these new ion mill equipment where high tension, angles, movement of the sample and time have to be adjusted to each kind of sample, using sometimes more than one process to achieve the best quality surface preparation for EBSD.

In 2020, Mineta [77] was able to study highly oxidative surface materials, as Mg-Li alloys prepared by a cross section polisher and then analyzing it immediately in ultrahigh vacuum in a Field Emission Auger Microprobe with a EBSD spectrometer.

Thin foil sample preparation.

In 2002 Small [31] proposed a special sample holder to analyze nanoparticles with EBSD, deposited on a TEM copper grid which was mounted on a 2 mm thin carbon substrate. A decade later, 2012, Keller [32] proposed a similar way of acquiring EBSD patterns using a sample mounted on a TEM grid and tilting the sample in such a way that the EBSD patterns are obtained with the e-beam diffracting through the sample instead of being reflected by the sample; they called it t-EBSD.

Lateral resolution improved from the micrometer range into the nanometer range, down to 2 nm. This opened up a huge opportunity for analyzing structural information of larger areas in heterogeneous materials [33, 35]. Consequently, all methods originally used to prepare samples for transmission electron microscopy also can be used for t-EBSD. Ayache [38] is a major reference in TEM sample preparation in areas ranging from material science into life sciences.

Focused ion beam (FIB) milling.

The focused ion beam microscope was originally developed in the late 1970s basically for semiconductor
applications as failure analysis. Commercially available FIBs one decade later evolved into resourceful equipment for the semiconductor industry, nanofabrication and TEM sample preparation as can be read in a review carried out by Reyntjens [78]. Nowadays, a FIB has an ion column with a liquid metal ionic source (LMIS) with a collimation system to form a small ion probe, deposition gases and sometimes a lift-out system. Frequently a dual column: electron and ion can be found in commercial microscopes. A conventional dual FIB sample preparation process [79, 80] can be synthetized as following: a sample is mounted with silver paint on the sample holder or stub. Double sided carbon tape should be avoided because the sample will be tilted and normally slides off the glue of the tape. As grown films, cleaved samples or ground or polished samples which are somewhat flat are normally used. The region of interest (ROI) is searched for, using secondary electrons (SE) or backscattering electrons (BSE) images obtained with the e-column. A metallic protection layer, normally gold or platinum, is deposited onto the ROI up to 10/20 µm width. Two large trenches on each side of the ROI are milled with the ions, frequently Ga⁺ ions into a depth of approximately 100 µm, leaving a thin lamella in the middle of the trenches. This lamella is then cut out of the sample and welded onto a special FIB grid. In this process, the sample is tilted in order to set the right milling directions. A final polishing with low energy ions is then performed, also using small tilts to polish the sample on both sides. Common problems to FIB sample preparation are amorphization and selective milling due to the ion interaction process where milling rates are different for different materials [81, 82]. Matteson [82] studied the amorphization process in Si and Cu samples finding that a modification in the milling geometry could improve the quality of the EBSD patterns. Since then several other articles have been discussing modification of the conventional FIB sample preparation technique to avoid damage and amorphization [83-86]. The last decade, the use of ion source microscopes has been spreading [87-88], not only improving image quality, but also increasing the milling and nanofabrication options for different types of materials like graphene [89]. In 2012 Echlin [90] proposed a combination of an e-beam with Xe-beam and a pulsed laser system to prepare difficult samples.

**Serial sectioning, EBSD 3D or EBSD tomography.**

In 1999, Kral [52] performed a “manual” serial sectioning on a FeCMn alloy, by polishing off about 250 layers of material of approximately 0.2 µm thickness. Fiducial marks were used to enable alignment of all 250 images. At the same time, Rollet [91] also used a serial section to study triple junctions between grain boundaries of MgO. Zaefferer [92] in 2005 used a FIB to perform serial sectioning and EBSD data collection, though the process was not automatized at the time, as he states at the end of the paper: “Another important development will be the automatic 3D mapping of microstructures in FIB-SEM instruments. While the hardware already exists, the software for the display and analysis of 3D microstructures is still missing”. Situation which is already changed in 2006 when Konrad [93] was able to perform a serial sectioning and EBSD data acquisition on a Fe3Al-based alloy, which was first classically 2D analyzed and then 3D rendered for further inspection. Essentially the sample is prepared and mounted on the sample holder as for conventional EBSD and first EBSD data collection is performed. But then the ions are used to mill away a layer, exposing the material underneath which will again suffer an EBSD data collection and so forth. Finally, a set of EBSD data sets will have been collected which can then be rendered into a volume to be further analyzed. Several examples can be found on Raabe’s homepage [34] and also in the literature.
CONCLUSIONS

EBSD sample preparation methods as any other sample preparation methods are always a challenge, especially with heterogeneous and new materials being developed in the most different areas. Much has already been done and is available in the literature, which should always be consulted. However new materials and old materials which were not possible to be prepared until now, might be possible by applying different techniques or a combination of them.

One aspect which should also be kept in mind is the cost of the sample preparation; ionic milling, specially the FIB, seems to be a very useful technique. However, it is a rather expensive technique, concerning either the machine itself or maintenance costs. Summarizing, we expect faster and new sample preparation methods arising in the next years which will enable the analysis of samples which were not dreamt of, as material engineering is becoming a reality.

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