Serial sectioning in the SEM for three dimensional materials science

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ABSTRACT

Here we explore the range of serial sectioning techniques that have evolved over the past decade, providing a comprehensive toolkit for capturing rich 3D microstructures, chemistries and crystallographic information, with sub-micron resolution at volumes that extend out to mm³ or even cm³. In each case we consider the challenges associated with their application, the volumes they can analyze, the damage to the surface they impart, and their suitability for different materials. In certain cases these warrant hybrid methods, motivating workflows that leverage multiple sectioning modes within the same instrument. Finally, we provide a perspective on their future development, including advances in data collection, segmentation, registration, data fusion, and correlative microscopy. Furthermore, the exploitation of 3D techniques for a better understanding of existing materials, and the design of new ones, is discussed through their use in multiscale modelling, digital twinning, material informatics and machine learning frameworks.

1. Introduction

Materials science is focused on understanding the relationship between materials composition, processing, microstructure and properties. Much can be understood about the way materials behave from two dimensional (2D) views (usually cross-sections) of the microstructure of single phase and multiphase materials. However, many aspects cannot be fully analyzed from 2D cross sections, even if orthogonal sections are captured. Examples include the connectivity of pores or phases [1], the morphologies of crystalline grains [2,3] and the character of the boundaries between them [4], the shape and distribution of undesirable inclusions [5], and the trajectories of fibrous phases [6,7]. Full characterization requires 3D information, as does the accompanying modelling in 3D. Other important microstructural features are rare and unlikely to be found, or properly observed, in a 2D section of material selected at random. A 3D understanding is also important when considering materials systems and devices, for example 3D printed circuit boards, additively manufactured (3D printed) structures, coatings and membranes.

Another driver for 3D characterization is the fact that 3D modelling is becoming increasingly feasible across a range of scales, from the atomic scale (e.g. the atomic displacements introduced by radiation damage, or the molecular arrangements in a block copolymer), to the microstructure (be it the rafting of a nickel superalloy, or the domains within a ferroelectric material), to the macrostructure (e.g. a cross-ply composite laminate), to the component (e.g. an aircraft wing). Taken together with the emergence of additive manufacturing and other novel processing approaches, which give designers ever more freedom to design 3D hierarchical architectures [8,9] along the lines of those used in nature [10], it is critical that materials systems can be characterized in 3D at all relevant length scales. Such a framework provides the potential for a microstructurally-informed digital twin of a material across multiple scales [11], empowering refinement of manufacturing processes to control microstructures for optimal performance, or revealing degradation mechanisms that limit service to predict safe lifetimes.

In terms of 3D characterization techniques, those that are non-destructive, such as X-ray computed tomography (CT) [12], are often advantageous because the integrity of the sample is maintained and also because of the opportunity for temporally-resolved longitudinal studies, either as a function of manufacturing and processing, or in operation, which can be applied over long time periods in a time lapse, or over a shorter time via continuous monitoring. However, the spatial resolution, imaging contrast modes and chemical probes available non-destructively are limited compared to the information that can be extracted and combined from sequential 2D cross sections (e.g. grain substructures, chemical information etc.). In such cases, rich 3D datasets can be constructed by applying destructive serial sectioning methods. Here we focus on the application of serial sectioning to materials...
science, but most of the techniques are also relevant to earth and life sciences.

Although different methods for serial sectioning experimental setups exist, generally the idea is to create a sample pillar or other region of interest that has many adjacent free surfaces to enable easy sectioning and to limit the redeposition of sputtered material that can partially occlude the imaging window or other detector signals. The region of interest is then incrementally milled using either one or more ion or laser beam(s), or by mechanical means, while the exposed cross-sectioned surface is imaged so as to accumulate a dataset comprising information acquired on successive parallel slices.

To a large extent, the spatial resolution and the volume of data required determines the appropriate serial sectioning approach. Various sectioning techniques are compared in Fig. 1. Some sectioning methods are ill-suited for certain materials (e.g. ultramicrotomy for hard materials). The rate of data acquisition in relation to the volume of data acquired, and the maximum volume that can be analyzed or simulated, are often limits the choice of technique and the imaging modalities used in order to ensure a practicable experimental workflow. In terms of data acquisition rates, optical microscopy can often provide the fastest route for large volume datasets where grain orientation information or chemical gradient mapping is not required. This may become increasingly important as machine learning methods motivate a move toward much larger and broader data acquisition strategies, rather than collecting a few representative images. In this respect, it is also useful to consider how large a volume image is required to be statistically representative of the system of interest [13,14].

Beyond consideration of spatial resolution and total characterized volume, it is also important to consider the imaging modality or modalities required to obtain the information needed for a given problem. For metallurgical samples, the use of etchants can help provide varying forms of contrast in optical microscopy, including imaging of grain boundaries or specific phases (e.g. differentiating martensite from ferrite). For biological samples, samples can be dyed, immuno-labelled, or treated using genetically encoded fluorescent proteins. Multiple fluorescent tags can be identified simultaneously using light sources with different colors to highlight and identify specific molecules and study their biological roles [15]. For scanning and transmission electron microscopy (SEM/TEM), heavy element stains are required in life sciences applications to give sufficient tissue contrast [16]. Charging can also be an issue for polymers and biological samples; in-chamber coating has been employed to counter this, albeit at the expense of a longer, and more complex, acquisition process [17]. More conventional approaches include embedding the sample in a conducting material and limiting the electron dose. When imaging in the SEM, backscattered electron (BSE) imaging usually provides superior contrast to secondary electron (SE) imaging because serial sectioning aims to minimise topological contrast. However the BSE signal is also generally weaker than the SE signal, requiring longer dwell times to form an image. Furthermore, BSE and SE images can be challenging to segment in multiphase materials where the atomic-density-driven contrast is weak. Nevertheless, SE and BSE imaging also generally yield the finest obtainable resolution in a given instrument. In the SEM, other imaging modalities are also available, including electron backscattered diffraction (EBSD) mapping to reveal crystalline phases and their orientations, and energy dispersive X-ray spectroscopy (EDS, also referred to as EDX) for chemical element mapping. These SEM imaging modalities have been transformed in the recent past with advances in detector technology. For instance, detectors developed for synchrotrons are now being used for EBSD and transmission Kikuchi diffraction (TKD) [18,19]. Furthermore, detectors and modalities developed for the TEM are now being applied to transmission SEM (tSEM) [20–22].

EBSD microanalytical capabilities for crystalline materials continue to become more sensitive [18,19,23] and tremendously faster, with EBSD collection speeds now ranging from 3000–5000 frames per second achievable in commercial systems such as the Oxford Symmetry camera and EDAX Velocity camera. These improvements are driving the use of EBSD during serial sectioning for the mapping of grain orientations and subgrain misorientation gradients over ever-larger areas while enabling fast collection times critical for 3D sectioning techniques. For example, a 1 mm field of view can now be acquired at a 1 μm spatial resolution in 3–6 min. EBSD camera binning modes are frequently used to improve camera frame rates, which can reduce the detector resolution to 50–100 pixels on edge, requiring advanced re-indexing methods such as dictionary indexing [24,25] and EMSphInx [26] to capture the morphological details of small recrystallized grains.

Fig. 1. The relationship between voxel resolution, dataset volume and typical data storage requirements for SEM-based serial sectioning techniques. The out of plane resolution is assumed to be equivalent to the serial sectioning slice thickness. Data usage lines are for cubic voxels with 1–2 bytes per voxel (8 or 16 bit images). For reference, electron backscatter diffraction (EBSD) data saved with raw patterns takes up roughly 4 orders of magnitude more storage per voxel.
and subgrain orientation gradients that result from myriad material processing steps [27].

EDS is still by far the slowest data collection modality, but acquisition speeds also continue to improve, with a greater sensitivity to light elements. EDS can require on the order of 10 GB per square mm at 1 μm spatial resolution [13] if one retains the full EDS spectrum at every voxel. The EDS signal is generated from a micron-scaled interaction volume when using 10–30 kV electrons, which puts a practical lower bound limit on the resolution and consequently limits the required data storage volumes.

The most-scalable method for characterizing large volumes in 3D is mechanical sectioning with optical microscopy, which enables incorporation of polishing and etching procedures. Etchants can be judiciously selected to reveal grain boundaries as well as specific phases, such as ferrite and austenite in steels that can be difficult to distinguish in an SEM. Optical serial sectioning was initially developed as a manual technique, but the results can be inaccurate and manual collection is inherently laborious. Fully-automated mechanical polishing serial sectioning setups have been developed to pass samples repeatedly between a sectioning instrument (polishing or cutting), etching station, and a microscope for imaging processes. Polishing and etching protocols can be tailored to limit damage to tens to hundreds of nanometers [28] or even ~50 nm in single crystal silicon [29]. This method is limited to the practical spatial resolution of optical microscopy (~0.25 μm), but large volumes can be sectioned (cm³-scaled), although volumes on the order of several hundred microns on edge are typically characterized by these approaches. We will not consider serial sectioning for optical microscopy or mechanical polishing serial sectioning in depth in this paper and the reader is directed to Rowenhorst et al. [30] and Uchic et al. [31] for additional information.

When considering serial sectioning techniques, it is often the case that the in-plane (x,y) resolution, which is determined by the imaging mode, is better than the resolution along the sectioning direction, typically defined by the slice thickness and capabilities of the sample stages. Depending on the technique, slice thickness may be controlled by stage movements and/or beam steering and alignments of the beam between slices based on fiducial marks. Consequently it is often worthwhile to choose the most appropriate sample orientation so as to best tolerate the reduced resolution along the slicing direction. When considering the spatial resolution, the physical origin of the imaging signal should also be taken into account, particularly in SEM imaging, where the accelerating voltage and sample geometry relative to a detector will affect the interaction volume [32]. For example, the interaction depth for EDS signal is on the order of a micron at 30 kV for nickel, whereas SE or BSE interaction volumes can be 10–100’s of nm deep. Consequently, if block face sectioning is used (whereby the remaining block is analyzed rather than the slice removed) the penetration depth will determine the depth resolution for high electron accelerating voltages, whereas the slice depth will limit it for low operating voltages [33].

Another key aspect when embarking on serial sectioning is to consider how to section the sample without creating significant artefacts. This is particularly true of soft solids for which the integrity of the sample needs to be maintained during the sectioning and imaging process (e.g. via cryo methods or resin infiltration), but also due to artefacts such as curtaining and redeposition associated with pores and cracks. The material response to specific sectioning techniques must be considered when choosing the experimental workflow, as some materials are more amenable to processes such as ion milling than others.

In this paper, we take a critical look at the techniques available for obtaining rich 3D datasets as well as provide some perspective on future developments, as well as their exploitation for better understanding existing materials and designing new ones.

2. In-SEM serial sectioning techniques

Here we consider the suite of serial sectioning methods employed for 3D characterization within an SEM across a range of scales (Fig. 1), providing an overview of the different methods, their appropriateness for different types of materials, their merits, and their practical limitations. Our aim is to help the experimenter to develop effective workflows and serial sectioning procedures to obtain 3D information about microstructure, chemistry, and defects at a scale appropriate to the task at hand.

2.1. Dual beam Ga FIB-SEM

Dual focused ion beam - scanning electron microscopes (FIB-SEMs) were originally commercialized for semiconductor-industry milling and patterning applications using a gallium FIB source. However, their application space has grown extensively to include 3D serial sectioning, TEM lamella preparation and sample liftout, micromachining, failure analysis and others. In essence, Ga FIB milling utilizes a liquid metal ion source (LMIS) to generate a tightly focused ion beam with currents ranging from picoamps to 50–100 nanoamps at accelerating voltages of 1–30 kV. The small beam spot sizes can be used to sputter away extremely thin (10–50 nm) layers of material, but it does so at relatively slow sputter rates (maximum removal of ~20 μm³/s). This results in Ga FIB-SEMs being useful for the serial sectioning of volumes up to roughly 50 × 50 × 50 μm [34], with typical slice thicknesses around 30 nm.

Over the years, Ga FIB has been applied to many different materials, including a wide range of semiconductor materials, metals, geological samples, and more recently a variety of biological materials under cryo- or fixed conditions. However, gallium is shown to be a poor sputterer of organic materials so only small volume datasets are practical. Ceramics and diamond are also very slow to mill, often making anything but the smallest amount of milling of these materials impractical. Non-conductive materials can be coated but charging issues have limited its application in such cases. The advent of cryogenic stages has also enabled the study of battery electrode materials via vitrification of the electrolyte, where Ga FIB sectioning has revealed the complex dendrite morphologies (in Fig. 2) that control performance and safety in lithium-metal systems [35].

Gallium FIB sources are relatively efficient at sputtering materials such as silicon, but ion implantation is common and can result in phase transformations [36], such as amorphization in silicon [37] or austenite to ferrite/martensite transformations in steel [38,39]. The extent of damage is proportional to the total ion dose, but for edge-on milling of silicon and austenitic steels, damage depths from Ga FIB are generally small.

![Fig. 2. Cryo SEM Ga FIB serial sectioning of lithium metal dendrites. Images courtesy of Springer Nature Limited and Zachman et al. [35].](image-url)
on the order of 10–30 nm \[39–41\]. These deleterious effects can be mitigated by reducing the accelerating voltage and the irradiation dosage, and controlling the incidence angle of the beam with regard to the region of interest. Protective platinum caps are often deposited on the FIB beam-incident face of the sample to reduce curtaining artefacts (vertical features aligned with the FIB beam resulting from an edge profile) \[42,43\] and to provide a barrier to normal incidence of the ion beam at the top surface.

Artefacts and damage from sectioning, especially in biological samples, can limit the accessible volume, and previous studies such as in Fig. 3 have relied on statistical analysis and smaller model systems to draw conclusions about larger-scale structures \[44\]. The difficulty in milling biological materials by Ga FIB also tends to limit the amount of automated image analysis, which often relies on manual segmentation that is typically more accurate, but is also extremely laborious and does not scale well for larger data volumes \[44\].

2.2. Dual beam Xenon PFIB-SEM

Over the last ten years, magnetically enhanced inductively-coupled Xenon plasma ion sources (ICP) have been shown to deliver smaller spot sizes at much higher beam currents than Ga LMIS sources. Currents in the \(\mu\)A range make it feasible to collect volumes on the order of \(300 \times 300 \times 300\) \(\mu\)m over timescales similar to those to make \(50 \times 50 \times 50\) \(\mu\)m volumes using a Ga FIB. In most respects, the sectioning process and many of the issues carry over from the Ga FIB method.

As edge-on methods, curtaining can be a problem in both the Ga FIB and Xe PFIB techniques, but good surface quality can be achieved by employing two different milling directions that are each a few degrees to either side of the vertical milling direction and applied for alternate slices (so-called rocking milling). Other milling strategies such as spin milling have recently been proposed \[45\] and have been used to gather and reconstruct relatively large 3D datasets, shown in Fig. 4, from Zr-based bulk metallic glasses containing dendritic structures. Controlling the level of damage incurred during milling is critical to utilize the increased milling rate. The depth of amorphous damage in silicon has been recorded by TEM as \(~22\) nm at 30 kV, and \(3.1\) nm at 5 kV for grazing incidence milling using Ga FIB and \(~13\) nm at 30 kV and \(2.4\) nm at 5 kV using Xe \[46\]. Furthermore, the damage level as

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**Fig. 3.** Ga FIB dataset of human muscle tendon. Manual segmentation was required to track individual fibers throughout the collected volume. (a) Individual cross section (scale bar equal to 1 \(\mu\)m). (b) Manual reconstruction of fibers. Images courtesy of Elsevier and Svensson et al. \[44\].

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**Fig. 4.** (a) Zr-based bulk metallic glass containing crystalline dendrites serial sectioned by PFIB spin milling \[45\] at \(0.5^\circ\) glancing angle, 30 kV / 500 nA over \(1200 \times 1200 \times 4.65\) \(\mu\)m containing 260 sections, total milling time \(\approx52\) h. (b) PFIB prepared cross section at 30 kV and 59 nA over \(120 \times 100 \mu\)m area imaged by through-lens secondary electrons of the same sample in (a) showing the microstructure and surface quality of the raw slice. (c) 3D Volume rendering of the PFIB serial sectioning showing two dendrites with interlocked dendritic grains.
recorded by EBSD pattern quality was also better despite the 20–60 times higher beam current (Fig. 5). For example, in a WC-Co hard metal it is possible to generate the same quality EBSD patterns from a PFIB cross section using 59–180 nA as those generated by a 1 nA cross-section in a Ga FIB.

2.3. Broad ion beam

Argon ion beam milling has been used for many years to thin TEM samples to electron transparency with minimal damage. More recently, broad ion beam (BIB) milling has been used to produce low damage surfaces for 2D analysis. Argon BIB milling utilises low accelerating voltages ≤ 5 kV but very high currents from 100's nA to mA's. This has been extended through the coupling of a BIB mill to an automated transfer system to enable serial sectioning over large areas [47,48] that are shown as 3D reconstructions in Fig. 6 for a calcite particle embedded in a geological sample. The BIB characteristically mills over a very large area (~4 mm) [48], albeit with a concave profile.

As a face-on milling method, Ar-BIB is not restricted to the edge of the sample but can be undertaken anywhere on the top surface. Nevertheless, only an area hundreds of microns in diameter is sufficiently flat for serial section tomography. The method can remove very thin (10 nm) layers and since the milled region is typically much broader than it is deep, it is best suited to cases where only the near surface region needs to be probed in 3D. Previous work studying combined approaches of femtosecond laser treatment and subsequent Ar milling at 5 kV and 700 nA at a 15° glancing angle resulted in amorphization in single crystal silicon that was roughly 30 nm deep [49].

As illustrated by the quality of the EBSD patterns in Fig. 5, the level of damage introduced by BIB milling is low, even compared to Ga and Xe plasma FIB. The main drawbacks of BIB are speed, the difficulty of maintaining a flat surface over areas larger than a few hundred microns in area, and the difficulty of preserving the morphology of holes and cracks during milling. Nevertheless, BIB could conceivably be integrated with a faster sectioning method and applied as a final damage removing finishing step.

2.4. Laser based serial sectioning

Lasers of varying pulse width have been used to remove material across a number of microscope platforms, including SEMs, FIB-SEMs and optical microscopes. Until recently, continuous wave lasers and nanosecond-pulse lasers have been used primarily for coarse sample sectioning and milling. This is because the associated damage depth precluded removing thin layers of material with low damage during serial sectioning. Nanosecond lasers have been used on FIB-SEMs that utilize a load lock system between the laser machining chamber and the SEM chamber [50], resulting in roughly tens of microns of repeatability during sample transfer between two chambers [51]. These nanosecond laser FIB-SEM systems have been used for targeted feature extraction, bulk material removal and micromachining, and failure analysis [52]. However, the precision of load lock stages limits these systems to coarse serial sectioning applications.

Early serial sectioning work used femtosecond lasers to remove material with the sample face-on to the laser beam [53], which requires precise control of the laser fluence for controlled material removal. Subsequent iterations have incorporated scanning mirrors with the beam incidence parallel to the sample surface (i.e. edge-on). Initially, optical microscopy was used to capture surface information and rudimentary laser induced breakdown spectroscopy (LIBS) setups were demonstrated to capture chemical information [53]. Prototype TriBeam (laser-FIB-SEM) systems have been developed [54–56] using femtosecond lasers to remove material, while leveraging the microanalytical capabilities of the SEM for a wide range of imaging modalities and exploiting the FIB beam for final surface cleanup where necessary.

![Fig. 5. EBSD patterns for (a) Ar BIB, (b) Xe plasma FIB, and (c) Ga FIB [46] as well as (d) Laser TriBeam for the WC phase in a WC-Co hard metal. The BIB prepared EBSD pattern is much better defined because of the lower levels of damage introduced by the Ar broad ion beam compared to those prepared by Xe or Ga ions or the femtosecond laser.](image1)

![Fig. 6. (a) A 40 μm × 40 μm × 5.3 μm (voxel resolution of 240 × 240 × 20 nm) volume of interest prepared by BIB milling containing a calcite particle and (b) the 3D rendering of the calcite particle shows good alignment of the 266 slices.](image2)
Recently, a TriBeam (femtosecond laser-PFIB-SEM) system has been developed by Thermo Fisher Scientific on the Xe Plasma FIB Helios platform, resulting in a system capable of laser sectioning at rates 4–5 orders of magnitude faster than a Ga FIB and subsequent surface cleanup at rates 10–20 times faster than a Ga FIB [56]. These systems couple all the detectors and the electron, photon and ion beams into a single vacuum chamber, utilizing robust shutters to shield sensitive components during material removal. The sample is attached to a high resolution 5-axis stage such that sub-micron sections can be removed while ensuring that the sample can repeatedly return to different imaging positions.

Femtosecond laser material removal rates have a dependence on the laser fluence at the location of the irradiating beam, as has been shown in the extensive literature showing single pulse laser ablation crater sizes and depths. In many cases, abrupt transitions occur between low and high fluence ablation rates, which can be leveraged to remove material at much higher rates, albeit with the potential for greater material damage [55]. However, in all modes of femtosecond laser material removal, the rate is still many orders of magnitude faster than the ion beam methods discussed above. The removal rate can also be modified by selecting different femtosecond laser wavelengths, such as 515, 780, or 1030 nm [52,56] which may be varied to enhance energy absorption in a particular material. In practice, the removal rates are sufficiently fast such that the time required to remove a slice of material on the order of 1 mm × 1 mm × 1 μm will vary minimally (< 1 min per slice) between materials classes. Laser milling can be applied to a very wide range of material classes including semiconductors (Si, strontium titanate), metals (Ti alloys, Ni alloys) and composites (W-Cu, SiC-SiC) and biological materials (shown elsewhere [57]), as illustrated in Fig. 7.

The minimum slice thickness for femtosecond laser serial sectioning is controlled by the light induced periodic surface structures (LIPSS) texturing produced by the femtosecond laser. The preferential orientation of the LIPSS can be controlled by modifying the polarization and wavelength of the laser light. In most materials, the LIPSS are on the order of 100–250 nm and can be removed with a subsequent glancing angle FIB mill. Slice thicknesses down to 250 nm are achievable, but often require a simplified experimental setup where stage movements are limited to those needed to incrementally move the sample into the beam, or by keeping the sample static and performing the incremental movements with the beam steering optics. In general, slice thicknesses of 500 to 1500 nm are more routinely employed.

Laser-induced damage varies markedly with the pulse width of the laser beam and laser processing conditions. Damage essentially scales with pulse width, for example femtosecond pulsed lasers have a damaged region on the order of 50–250 nm depending on the material being irradiated [58]. In many metallic materials, the damaged region may contain an elevated dislocation density, whereas in GaN and Si very few dislocations are observed, although amorphization in Si has been reported [55,58]. In all materials, the LIPSS form in the irradiated regions [59], which have a functional dependence on their orientation and periodicity based on the polarization and wavelength of the irradiating laser beam and the material they are interacting with. Heat affected zones (HAZ) have been reported to be up to tens of microns deep for nanosecond lasers [60,61], decreasing to microns or sub-micron scale for picosecond and femtosecond lasers [60,62–64], respectively, and with a dependence on the laser processing conditions such as fluence and sample versus laser ablation geometry. Surfaces have been ablated and 3D datasets collected using low fluence and 515 nm wavelength femtosecond lasers in biological and soft materials without significant modification to the structure [57].

2.5. Serial ultramicrotomy

Ultramicrotomy, whereby a diamond knife cuts an ultra-thin (5 to
150 nm thick) section, is routinely used in the life sciences and polymer sciences to prepare specimens for transmission electron microscopy (TEM). The ultramicrotome can also be employed for 3D imaging using serial block face sectioning in the SEM (SBEM or SBFSEM). This method is able to prepare serial sections of area over 500 × 500 μm at rates of around 1 slice per second. A combination of a conventional SEM and a microtome was described as early as 1981 by Leighton [65], but serial sectioning was pioneered by Denk and Horstmann in 2004 [66]. A number of different commercial in-SEM automated serial microtome systems are now available. Somewhat harder materials, such as light metals and coatings, can be studied by serial ultramicrotomy, despite the greater challenge they present to the cutting tool. Array tomography [67], involves transferring the slices to tapes or slides that are imaged off-line. This has the advantage that the method, while destructive, retains all the slices for further analysis.

Biological materials in particular are well-suited to SBEM, provided they are sufficiently fixed, stained and embedded appropriately. SBEM has been successfully applied to study brain samples in rats [68] as well as humans [69], which help provide validation of existing structural models based on non-destructive evaluation.

A key concern is to minimise cutting artefacts which are generally more significant than for ion beam methods [33]. For soft heterogeneous materials, smearing or pull out of harder phases can occur [70]. For metallic and polymeric samples, plastic damage can be minimised by reducing the slice thickness and the rake angle presented by the tool - both of which reduce the applied stresses. This can further be improved by oscillating the knife as illustrated in Fig. 8 [71]. Damage can be minimised sufficiently for 3D EBSD analysis in many cases.

2.6. A serial sectioning toolkit

Taken together, the existing suite of in-SEM serial sectioning tools covers a very wide range of materials challenges, multi-modal data needs and lengthscales/resolutions. Fig. 9 provides an indication of the appropriateness of the different methods as applied to a spectrum of different materials. Alongside this, Table 1 gives an indication of the maximum sample sizes, material removal rates, typical slice thicknesses, and damage characteristic of each method. Damage can also be present in a number of other forms, depending on the serial sectioning method and material being investigated. For instance, chemical modification and strain can occur from ion implantation during Ga FIB and PFIB milling, dislocation injection can occur subsurface from femtosecond laser ablation [58], and SBEM/microtomy can produce mechanically damaged surfaces that are challenging to investigate using microanalytical techniques beyond SE/BSE imaging.

3. Future developments

3.1. Hybrid and multibeam milling strategies

The complexity of serial sectioning workflows is increasing in order to study a broader range of materials and material systems. For instance, multibeam approaches are being adopted in laser TriBeam systems to optimise the materials removal rate vs damage relationship by using the Ga FIB or Xe PFIB beam to subsequently clean-up the thin damage zones that may be introduced by the laser in certain materials, or when extremely high-quality EBSD data is required [55,56]. Furthermore, multigas species have been integrated into PFIB ion sources (oxygen, nitrogen, argon) [72], and the oxygen source is particularly useful for low damage sectioning of resin-embedded biological and soft material samples that were previously challenging to access. The argon source can be used to produce high quality, low damage sections in semiconductors [73] and the nitrogen source can be used for nitriding to harden sample surfaces.

3.2. Automated data collection and image segmentation

Currently, the process of collecting 3D datasets requires significant imaging expertise, especially if multiple modalities are collected, to form highly-multidimensional datasets, or data is collected across multiple scales or instruments. Greater automation of workflows is making the collection of serial section data much more routine, while new software will make it simpler to merge and co-visualise multiple datasets collected on the same region of interest. The BisQue cloud-based infrastructure [74–77] is one example software platform that tracks data provenance, enables datasets sharing, has integrated visualization, can perform data analysis in existing modules such as DREAM.3D [78] and cell profiler [79] or Python, and can perform full 3D reconstructions. 3D image datasets can easily be many hundreds of GB in size, with multi-modal datasets scaling to much larger sizes. Such large datasets present significant challenges if they are to be qualitatively appreciated or to have their microstructural features quantified. Conventionally, microscope images are segmented into phases or features through a series of image processing steps applied to each constituent 2D image using commercial or open source packages. This type of analysis is time-consuming and results are often highly-dependent on the user. Advances in machine learning and pattern recognition being made in other fields offer the promise of training algorithms to undertake image segmentation with relatively little supervised learning. Going forward, this will radically speed up and simplify the process of image segmentation and remove a significant barrier to the proliferation of 3D image characterization.
3.3. Correlative imaging

One of the advantages of in-SEM 3D characterization is the ability to apply multiple imaging/mapping modalities to the same region of interest, sometimes called correlative microscopy [80]. This is now routine in 2D, where scanning electron microscopy is combined with EDS mapping and EBSD to provide insights into the elemental distribution and the local crystallographic texture, respectively. This technique has been extended to 3D using serial sectioning to build up rich multidimensional datasets and is sometimes referred to as correlative tomography [81]. The ability to map with both EBSD and EDS detectors simultaneously via an API, albeit with a different mapping resolution for each detector, is critical for detectors with different interaction volumes (EBSD vs EDS) and different typical data collection dwell times. Currently, practical time limits usually force the collection of EBSD and EDS to have a unified spatial mapping grid size, therefore saving on redundant scanning time.

A good example of correlative microscopy is provided by the formation of butterfly defects in bearing steels [82], shown in Fig. 10. These defects tend to form subsurface around large roughly 20 μm inclusions. In this case, the presence of cracks are best viewed by BSE imaging, and the extent of the damage in the form of white etched matter (WEM) is observed as low-confidence or non-indexing regions in the EBSD data. Furthermore, the nature of the inclusion and the dissolution of carbides in the WEM can be mapped by EDS [82]. Merging multiple modalities can be difficult due to drift that can occur during the extended time periods and large stage tilt needed to acquire the EBSD pattern, introducing distortions into the 3D data that must be corrected.

Table 1
The comparison of the key characteristics for each serial sectioning technique.

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Fig. 9. A schematic showing the applicability of UMT (ultramicrotomy) or serial block face sectioning (SBEM) in the SEM, gallium FIB, xenon plasma focused ion beam (PFIB), argon broad ion beam (BIB), femtosecond laser ablation (TriBeam), and oxygen PFIB for the 3D sectioning of different materials and materials classes.
3.4. Multimodal dataset registration and fusion

The reconstruction of 3D serial sectioning data or the merging of datasets collected with different tools and modalities requires careful alignment of the image or data stack, which can be affected by distortions inherent to imaging modality. For instance, SEM imaging includes electron lens distortions, tilt distortions when imaging at high angles such as during EBSD [83], and signal sampling discrepancies that are a function of the geometry and the physical mechanisms that govern the generation of a signal (e.g. EDS vs BSE interaction volume).

Quantitative multimodal dataset registration approaches can be applied across characterization tools, such as between destructive (TriBeam) and non-destructive X-ray diffraction contrast tomography (DCT) or computed tomography (CT) techniques to leverage the advantages, and ameliorate the disadvantages, of both methods [2]. The correction of some component of these distortions and alignment of different imaging modalities has been addressed in 2D and 3D elsewhere using machine learning approaches [84] and the thin plate spline method [85], but requires careful consideration of the sample geometry and the ability to identify individual features in all considered modalities. Once data is quantitatively registered and fused, imaging modalities can be seamlessly queried, on a voxel by voxel basis, to gain insight into problems such as grain growth via time resolved data in strontium titanate [2,86].

Forward modeling approaches have been leveraged and should be expanded in the future to guide and predict the types of contrast mechanisms and imaging distortions that may be present for specific imaging modalities. De Graef's EMMsoft software package [87] provides a comprehensive microscope simulation framework that includes nearly all SEM and TEM imaging modalities as well as EDS, EBSD, and TKD. For instance, simulations of EBSD diffraction patterns using EMSsoft have been used for orientation indexing [24,26] and simulations of STEM imaging with the iSEM [21] helped guide predictions for defect imaging contrast. Furthermore, the proliferation of new hardware including sensor technologies also provides the opportunity for combining in situ processing information with ex situ 3D characterization to develop constant feedback process controls. This approach can be especially beneficial to additive manufacturing processes, where the design and processing space is extremely wide. Efforts to link sensor data recorded during manufacturing processes to porosity in final components has been particularly useful in identifying and managing these critical defects [88,89].

3.5. Microstructural informatics

Materials science has long focused on the development of processing-microstructure-property relationships with the aim of better understanding existing materials and designing new ones. This has in part driven the development of a very wide range of microscopy tools and mapping instruments from the nanoscale to the macroscale. Conventionally, the vast amounts of data obtained from these instruments has been collapsed into a few key microstructural variables (e.g. grain size, phase fractions, etc.) that are quantified and then correlated against materials processing parameters (deformation, thermal history, etc.) and/or to certain materials properties (e.g. strength, magnetisation, permittivity, etc.). 3D characterization methods are now able to provide vast amounts of data; one of the key tasks now will be how we represent these high-dimensional data sets in terms of the smallest set of variables that captures the essence of the data (i.e. the microstructural fingerprint) [11,90,91]. This would thus enable us both to store and access microstructural data in an efficient and cost effective manner, but more importantly, it enables us to incorporate microstructural data into materials informatics approaches to the design of new materials and the computational design and optimisation of manufacturing processes.

4. Conclusions

The serial sectioning techniques available for 3D data collection in the SEM have been presented with regard to the materials systems, data volumes, damage, and slicing resolution. The relatively newly developed serial sectioning techniques (PFIB, BIB, and TriBeam) now enable 3D data acquisition from a wide range of materials including metals, ceramics, polymers, geological specimens, and biological materials. Current serial sectioning techniques are often limited by the speed of microanalytical techniques, such as EDS and EBSD, but new high speed detectors are emerging with greater electron sensitivity. 3D experiments of the future are likely to require combined slicing and cleanup approaches in order to assess the full range of material challenges and microstructure that they contain. Software infrastructures will need to be extended to enable smart sampling, on-the-fly reconstruction and instrument feedback, and more integrated forward modelling approaches of imaging modalities. The proliferation of advanced 3D characterization tools will correspondingly require novel analytic frameworks to probe the fundamental nature of materials across all aspects of modern technology.

Acknowledgments

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