Macro to nano: a microscopy study of a wrought magnesium alloy after deformation

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Abstract
This educational paper aims to assist physics teachers in explaining the concepts and principles of material composition and deformation by describing how to perform a microscopy investigation of a deformed wrought magnesium alloy test piece. By examining the changes taking place at the different length scales from macro to nano it is possible to understand how changes in the microstructure influence the macroscopic appearance and properties. The microscopy techniques employed for the investigation of each length scale—light microscopy, scanning electron microscopy and transmission electron microscopy—are explained along with the procedures of specimen preparation. A background is given to help teachers explain the microstructure of magnesium, the role of magnesium alloys in modern industrial and technological contexts and the historical developments of microscopy. The paper can also be used to assist teachers in laboratory courses to describe the principles and methods of scientific experiment. The work is fully referenced and a reading list is included, making it suitable as a teaching resource for academic courses or for solo study.

Keywords: electron microscopy, magnesium, electron backscatter diffraction, length scales, specimen preparation, plastic deformation

(Some figures may appear in colour only in the online journal)
1. Introduction

The aim of this educational paper is to assist physics teachers in explaining and demonstrating the nature of matter from the macroscale to the nanoscale and how plastic deformation in a material can be investigated through these different scales using different types of microscopy. It describes how to perform a detailed microscopy investigation of a wrought magnesium alloy test bar subjected to a compressive stress to show what is actually happening inside the alloy when it undergoes plastic strain. Through explanations and diagrams the different types of microscopy are explained along with the scientific principles behind their operation and the requirements for specimen preparation. Insight obtained through such analysis helps in the understanding of material structures and behaviours and enables the development of materials with specific properties from the nanoscale up.

In physics education it is important to illustrate scientific principles with examples of where and how these principles apply in the real world [1], and where possible to involve students into a more active learning environment [2, 3]. Research of the cognitive and learning processes in students has revealed that classroom teaching can be more effective through hands-on activities, group discussions, group projects and graphical presentations [4, 5]. Trends in modern education are moving away from pure content-oriented teaching towards more student-centred teaching [6, 7]. Microscopy is an excellent way to demonstrate many of the key concepts of a modern physics course, especially those relating to the constitution of matter and to the theories of light and electron optics. This is particularly so when direct hands-on experience for students is possible [8]. All material things in our everyday environment, for example the desk that a student uses, the paper they write on and the pen they write with, have a microstructure, and using the scientific methods described in this paper it is possible to examine that microstructure down to the atomic level.

Students are often drawn to physics out of a sense of wonder as it promises to encapsulate the entirety of the known universe from the vastness of space and galaxy formation to the minutiae of atoms and subatomic particles. It is hoped that this paper captures some of that wonder as it takes the reader through a visualisation of the constitution of matter and the different methods necessary to achieve such a study. When a teacher can convey and transfer that sense of wonder to students it can inspire their curiosity and desire to learn. This paper is intended not only to explain the theory and methods of microscopy but also to provide a valuable companion document in the teaching of microscopy in the classroom or laboratory. It integrates with many of the more recent educational papers aimed at physics teachers at the university level such as the wave-particle nature of electrons [9, 10], the background of high resolution microscopy [11], the teaching of light and electrons [12], electron interference and diffraction [13], light and electron optics [14, 15], microscopy for lab courses [16] and new directions in electron microscopy [17]. Older educational papers remain a valuable resource for the fundamentals of electron microscopy, such as [18, 19], but a comprehensive demonstration of the complete microscopy procedures across the range of length scales is not presently available in the educational literature. Therefore the present paper can be of great value to teachers as it brings together the fields of optics, metallurgy, crystallography and materials engineering. Although aimed at university level physics, it is equally valuable for teachers of materials science and engineering.

Figure 1 shows an overview diagram of the different length scales from a magnesium alloy car wheel to the atomic structure of magnesium. This illustrates to students the key concept that matter is structured in an orderly fashion down to the smallest scales our instruments can penetrate. The paper is organised into two main sections: a background section describing the microstructure, properties and applications of magnesium, details of the
experiment and a historical perspective of microscopy, and a second section describing the microstructural investigation. Many of the concepts described in this study are complex and require a broad knowledge of material science and physics to properly grasp; to assist this, a reading list is included at the end of the paper and the reader is strongly advised to refer to these standard texts for a deeper explanation of the various principles involved.

2. Background

2.1. Historical and developmental perspective

A principle of physical phenomena is that the bulk properties of materials are derived from the microscopic structure and atomic nature of the elements and compounds from which the materials are constituted [20]. This has been recognised through empirical studies for millennia, but only in the last century has the experimental evidence been obtained to reveal a clearer insight as to how it works. For example, over two thousand years ago Wootz steel was manufactured in India by adding organic material such as wood and leaves into the crucible of molten iron to produce high carbon steel with special qualities of hardness and flexibility [21]. Recent investigation of such ancient artefacts has found that this process produced cementite and carbon nanotubes in the microstructure [22], which modified the properties of the iron. Wootz steel became the raw material for the famous Damascus steel used to make the highest quality blades of swords and knives during the middle ages. Likewise, the addition of chromium was found to improve the corrosion resistance of iron long before there was knowledge of the passivating effect of the chromium oxide surface layer [23].

Material science has flourished along with the development of microscopy, which gives the ability to examine and study microscopic structures. Although transparent crystals have been shaped into lenses for magnifying purposes since at least several centuries BC [24], the first optical microscope combining multiple convex lenses was developed in the latter part of the 16th century [25]. This opened up a whole new scale of the material world to investigation. The development of electron microscopy, beginning in the 1930s [26], has opened up further length scales below which light microscopy cannot penetrate. Light microscopy has a maximum resolution of around 250 nm but transmission electron microscopy (TEM) enables a resolution with aberration correction below 0.1 nm [27]. Early philosophical contentions of an atomic consistency of matter, extending as far back as the 5th century BC [28], were proven true in the 1950s when TEM was used to show beyond doubt the atomic structure of matter [29, 30]. While TEM enables the interior structure of materials to be examined, scanning electron microscopy (SEM), developed a few years later [31], enables high resolution study of material surfaces.
Greater understanding of microstructure has facilitated the development of new materials from the nanoscale up with properties designed-in by careful procedures such as micro alloying, thermal annealing and chemical vapour deposition. Lightweight engineering materials such as 3D graphene, carbon composites, and lightweight metals such as magnesium alloys are of special importance in the construction of components across a wide range of applications today. Because of their high strength-to-weight ratio magnesium alloys tend to be used in the digital, aeronautical and automobile industries allowing for overall weight reduction and reduced energy consumption [32]. The first major use of magnesium alloy in cars was in the 1938 Volkswagen Beetle which contained some 22 kg of the cast alloy [33]. However, magnesium oxidises, corrodes and ignites very easily, which means that handling and machining require special safety precautions, and practical applications are limited to inert environments or where special anti-corrosion coatings can be applied.

2.2. Microstructure of magnesium

Magnesium is an alkaline earth metal (Group 2 of the Periodic table) with atomic number 12 and electron configuration $1s^22s^22p^63s^2$. In the solid state and due to the low electronegative potential, which is typical of metals, the outer shell $3s^2$ electrons become detached forming a delocalized electronic sea within which the magnesium cations are embedded [34]. The magnesium ions experience a mutual attractive electrostatic force to this electronic sea, which holds them together and produces high mechanical strength, while the electronic sea itself gives rise to the high electrical conductivity associated with metals. Metallic bonding mostly produces close packed structures at room temperature, face-centred cubic (fcc) and hexagonal close packed (hcp), the most efficient form of packing in terms of volume occupancy of atoms.

Figure 2 illustrates the structure of magnesium. Magnesium forms the hcp structure where each ion has 12 nearest neighbours (coordination number) and each unit cell contains exactly 6 atoms giving a space occupancy very close to the theoretical space occupancy for hcp of $\pi/(3\sqrt{2})$ or 74.048%. The space group denoting hcp symmetry is P63/mmc (number 194 in the Hermann–Mauguin notation), indicating two mirror planes (m) and one glide plane (c) of symmetry [35]. In the hexagonal unit cell (a) the interatomic spacing in the basal plane is $a = 0.32$ nm and the bi-planar spacing normal to the basal plane is $c = 0.52$ nm, where the ratio $c/a = 1.62$. The stacking sequence for hcp in the [0001] direction is denoted ABABA [36] as shown in (b) with alternate planes displaced by the vector $(a/\sqrt{3}) \cdot [10\overline{1}]$ (see appendix for details of the hexagonal coordinate system and the use of brackets in TEM...
notation) so that the atoms of one layer are located in the impression formed by the atoms of the previous layer. Because of the lightness of magnesium atoms (relative atomic mass $A_r = 24.3$ as compared to iron with $A_r = 55.9$) and their relatively large atomic radius (atomic radius $r = 150 \text{ pm}$ as compared to iron with $r = 140 \text{ pm}$), the bulk weight of magnesium is very low; in fact magnesium is the fifth lightest metallic element after lithium, sodium, calcium and potassium. Bulk magnesium is a polycrystalline solid (c) with grains ranging from a few microns to several hundred microns in size depending upon the processing method used in manufacture; each grain is an individual single crystal with its own orientation, as indicated by shades of grey.

Plastic deformation in a crystalline lattice mostly takes place by the production and slip of dislocations [37] where portions of the lattice slide over each other, advancing through the breaking and joining of individual atomic bonds, as a result of an applied stress. Certain slip planes and slip directions (Burgers vectors) are characteristic of specific lattice types and are known as slip systems. According to the von Mises criterion [38], five independent slip systems are required to accommodate arbitrary plastic deformation in polycrystalline metals. In magnesium only four lower order slip systems are available, and because of this twinning (shearing of the lattice rather than slip), which has a similar activation stress to the low order basal slip (slip on the basal plane), provides an additional independent deformation system to accommodate plastic strain [39]. Figure 3 illustrates these two modes of deformation when a lattice is subject to an arbitrary force (F). Extension twinning in magnesium, accounting for extension of the lattice along the c-axis, occurs on the \{10\I\} pyramidal planes and in the \{10\I\} directions, with an $86.3^\circ$ twinning angle ($\theta_t$) or rotation of the basal plane [40]. Contraction twinning, accounting for contraction of the lattice, and double twinning can also occur, but only in cases of severe deformation because of the higher activation stresses of these twinning modes. Fracture in magnesium tends to originate in regions with a high population of twins (twin bands) [41], and so the study of twinning is of particular importance in the development of magnesium alloys.

Figure 3. Illustration of the two main modes of plastic deformation in the magnesium lattice when subject to an arbitrary force (F): (a) dislocation slip and (b) extension twinning.
2.3. The magnesium–aluminium–zinc alloy AZ31B

The wrought magnesium alloy used in this study is the magnesium–aluminium–zinc alloy AZ31B. AZ31B is stronger and more easily machinable than pure magnesium, and because of its lightweight and excellent mechanical properties has many technological applications [42, 43]. It is usually prepared in sheet form and used for body panels in cars (e.g. the roof of the Porsche 911 GT3 RS). Nominally the alloy contains 3% aluminium (A) and 1% zinc (Z) (by weight) along with trace amounts of manganese, silicon and copper, hence its name AZ31B. These elements, together with the specific thermal treatments applied to the alloy during the casting process (indicated by B in the name), give improved mechanical properties in relation to pure magnesium. In particular, grain refinement (keeping the grain size to within 5–10 μm) strengthens the alloy by impeding the passage of dislocations between grains [44] and increases its ductility (the property of a metal that allows it to undergo plastic deformation without loss of strength or breaking). The presence of manganese improves corrosion resistance. Many of these improvements relate to the formation of intermetallic Al–Mn precipitates in the matrix during manufacture, which modify the nucleation and growth of grains and further impede the flow of dislocations. Plastic strain occurs through a combination of dislocation slip and twinning. Both are obstructed by the presence of grain boundaries, precipitates, solid solution dispersions as well as pre-existing dislocations and twins. The total yield strength (σy) can therefore be modelled as a sum of these individual components [45, 46]:

\[ \sigma_y = \sigma_0 + \sigma_{ss} + \sigma_{gb} + \sigma_{tb} + \sigma_d + \sigma_p, \]

where \( \sigma_0 \) is the intrinsic yield strength of the pure matrix, \( \sigma_{ss} \) is from solid solution of alloying elements, \( \sigma_{gb} \) is the strengthening effect of grain boundaries, \( \sigma_{tb} \) is the strengthening effect of twin boundaries, \( \sigma_d \) is strengthening by dislocations and \( \sigma_p \) strengthening by precipitates. \( \sigma_{gb} \) and \( \sigma_{tb} \) reflect the important principle of work hardening where metals become harder as a result of plastic deformation (i.e. the production of dislocations and twinning), which is exploited in the metal forging process to increase the strength of the metal.

Figure 4 illustrates the twin-roll casting process used in the manufacture of AZ31B alloy. Molten alloy flows under the force of gravity through a hole in the furnace floor (a) onto a cooling plate, then into a tundish and finally between two large rotating drums which squeeze the malleable alloy into a flat sheet as it cools to room temperature. The drums are generally water-cooled steel with copper coating to aid thermal dissipation and prevent contamination of the sheet with iron. The final sheet (b) is ground using a large scale grinding process directly after rolling to produce high tolerance thickness and low surface roughness. The method of twin-roll casting was invented by Sir Henry Bessemer in 1857 for casting sheets of
stainless steel [47]. The alloy sheet used in this study was obtained from Magnesium Flachprodukte GmbH in Freiberg [48] and measured 1.5 m × 0.65 m × 3 mm. The density of magnesium is 1740 kg m$^{-3}$, which gives the sheet a mass of approximately 5 kg.

Because of the proneness of magnesium to oxidise, it is important to wear gloves when handling the alloy otherwise the surface becomes brown and stained. The low hardness of magnesium (250 MPa Brinell or 2.25 Mohs) means that it is also easily scratched. Because of the flammability of magnesium, care should be taken when machining solid pieces: the powder or swarf can ignite when heated close to its melting temperature of 650 °C. It is good practice to wear latex gloves and protective eyeglasses when working with magnesium alloys, especially when using chemicals for polishing or etching. The material safety data sheet provided by the manufacturer gives important information about handling and usage of the alloy.

Oxidation is a major problem when preparing specimens from AZ31B for microscopy. Upon exposure to water or air, brown patches of magnesium oxide form on the surface within seconds. Whereas in many metals the oxide layer is passivating (i.e. it forms a continuous layer that prevents further oxidation), in the case of magnesium the oxide layer is porous allowing continual oxidation and leading to heavy corrosion. A piece of magnesium left in methanol overnight will completely dissolve. In practice it was found that oxidation of specimens could be minimised by using dehydrated solvents rather than regular solvents or water and by storing the specimens in a desiccator.

2.4. Mechanical testing procedure

Design of components must take into account the likely stresses and environmental conditions of practical applications, and therefore testing is an important part of the development process. Figure 5 shows the test bar (a) and experimental procedure (b) for the compression test. The test bar was cut from the sheet using a standard workshop mill equipped with a very sharp cutting tool and air cooling. The standard shape provides a large contact area at each
end and a reduced cross-section in the middle that concentrates the applied stress. To facilitate the identification of any surface changes as a result of the compression test, the test bar was polished after milling using 4000 grit silicon carbide paper followed by 1 μm diamond particles in ethanol on a soft flock polishing cloth. The compression test was carried out using a hydraulic test rig that gripped the test bar at each end and applied a force \( F \) in the plane of the test bar. A contact extensometer was used to measure and control strain, and a load cell was used to measure the applied force. A single compression of \( \varepsilon = -0.3\% \) strain was made at a strain rate of \( \dot{\varepsilon} = 1 \times 10^{-4} \text{s}^{-1} \), where strain is defined as extension per unit length \( \varepsilon = \Delta l / l \) and compressive and tensile strains as positive and negative, respectively.

### 3. Microscopy investigation from macro to nano

#### 3.1. From metres to millimetres (visual inspection)

The length scale range observable with the naked eye is where we start this journey through the length scales. Investigating bulk samples at this length scale is a process of visual inspection. Visual inspection is a first stage in any microscopy investigation, providing an overview of the sample and can be used to identify specific areas of interest for more detailed investigation. Generally in microscopy, a sample is a small quantity of a larger bulk or population that is deemed to be representative of the whole, while a specimen is prepared from a sample to specific dimensions suitable for analysis.

A critical stage of any microscopy investigation is specimen preparation. The specimen must meet the requirements of size, cleanliness and surface finish in order for the investigation to proceed and to be effective. As a rule of thumb, the more detailed the investigation the more stringent and demanding are the requirements for specimen preparation. For visual inspection the test bar simply needs to be cleaned. As water causes rapid oxidation of magnesium, cleaning is best made using a soft cloth and a dehydrated solvent such as 99.9% ethanol.

Figure 6 shows an image of the test bar after the compression test recorded using a digital camera in macro mode. Using the highest resolution setting on the camera (usually denoted FINE) produces a better quality image that can be enlarged later to reveal more details of the surface. After the compression test, a transverse band approximately 10 mm wide, is clearly visible on the test bar. This shows that the test bar underwent plastic deformation and is consistent with the band of twinned grains reported by Denk et al [41], and is referred to here.
as the plastically deformed region (PDR). The PDR extends to the top and bottom surfaces of the bar as well as the sides, which suggests that it is a bulk phenomenon that has extended out to the surface. The area enclosed by a red square was selected for further examination at higher magnification and extracted from the test bar using a Struers Minitom sectioning machine set to the lowest speed and force to avoid excessive heating of the sample.

3.2. From millimetres to microns (light microscopy)

The lower boundary to the human visual faculty is generally considered to be an object 50 μm in size at 250 mm distance from the eye: equivalent to a speck of dust or the width of a human hair. A loupe can be used to inspect surfaces and reveal features down to a few tens of microns, but to make a detailed investigation below the millimetre scale and to record and analyse images, a light microscope is required. According to Rayleighs study of diffraction \[49\], the minimum resolvable detail or resolution \(\Delta\) of a lens is related to the wavelength \(\lambda\) of the image-forming ray travelling through a medium with refractive index \(n\) by the Rayleigh criterion:

\[
\Delta = \frac{0.61\lambda}{n \cdot \sin(\alpha)},
\]

where \(n \cdot \sin(\alpha)\) is the numerical aperture (NA). The Rayleigh criterion is often referred to as the diffraction limit, as it is the spatial limit below which a perfect aberration free lens cannot resolve its image two adjacent points in real space. Using blue light and an apochromatic (aberration corrected) objective lens with NA = 0.95 (corresponding to \(n = 1\) for air and collection semi-angle subtended by the object at the objective lens \(\alpha = 80^\circ\)), the minimum resolvable distance is approximately 250 nm.

Specimen preparation was made using 99.9% ethanol and either a filter paper, tissue or cotton bud, and additionally an ultra-sonic cleaner should be used to remove contamination and cleaning residue. Drying the specimen is best made using a flow of warm air (e.g. a hair dryer) as compressed air causes the specimen to become cold and the ambient air to condense on the surface, which then oxidises the magnesium. To reveal more detail of the grain structure, the surface was polished once more using a colloidal suspension of 0.04 μm SiO₂ particles in ethanol on a chemically resistant neoprene cloth and then lightly etched with a solution of 4% picric acid \(\text{C}_6\text{H}_3\text{N}_3\text{O}_7\) in ethanol, which preferentially etches interfaces such as grain and twin boundaries.

Figure 7 illustrates the investigation made using light microscopy. The Zeiss Axio Imager microscope is shown (a) along with a simplified ray diagram of the optical path between the light source, the specimen and the viewer (b) light is generated by a light emitting diode with colour temperature 5700–6500 K (i.e. spectral characteristic corresponding to that of an ideal black body radiator of that temperature), which spans the visible range but emphasises the high frequency blue end. Light is gathered and condensed into a beam by a condenser lens and reflected by a beam splitter (silvered glass) through an objective lens (so-called because it is close to the object) onto the specimen surface. Reflected light passes back up through the objective lens and beam splitter into the binocular or digital camera for viewing. The surface of the test bar across the border of the PDR from the selected area is shown before etching in (c) and after etching in (d). Outside the PDR the average grain size is measured as 5.1 ± 1.5 μm. Inside the PDR the elongated features are typical of twins. Individual grains generally contain multiple twins, and twins are observed to extend across grain boundaries. The average length of the twins is 6.8 ± 1.6 μm and they are aligned to within a few degrees of perpendicular to the long axis of the test piece. The small dark
features in the micrograph are Al–Mn precipitates, which due to their hardness have not been polished as smooth or as flat as the surrounding magnesium and therefore scatter light away from the optical path and appear darker. At this stage it is possible to confirm that the PDR is a band across the thin area of the test bar that has undergone plastic deformation by the mechanism of twinning to accommodate the strain imposed upon it by the compressive test stress. To obtain more information about the twins it is necessary to use a method capable of higher resolution and another method capable of determining lattice orientation.

3.3. From microns to nanometres (scanning electron microscopy)

To investigate the specimen below the resolution obtainable with visible light, SEM is used. Unlike light microscopy, which produces a magnified image of the specimen using photons, SEM generates an image by focusing and scanning an electron beam on the specimen surface. Based on the principle of wave-particle duality [50], the wavelength (λ) of an electron accelerated by an electric field of potential difference (V) is given by the de Broglie equation:

\[
\lambda = \frac{h}{m_{0}c} \left(2 \epsilon V m_{0} c^{2} + e^{2} V^{2}\right)^{-1/2},
\]

where \(h\) is Planck’s constant (\(6.62 \times 10^{-34} \text{ m}^{2} \text{ kg s}^{-1}\)), \(c\) is the speed of light in a vacuum (\(2.99 \times 10^{8} \text{ m s}^{-1}\)), \(e\) is the charge on an electron (\(1.602 \times 10^{-19} \text{ C}\)) and \(m_{0}\) is the rest mass of an electron (\(9.109 \times 10^{-31} \text{ kg}\)). Electrons accelerated through a potential difference of 20 kV (typical for metallographic studies) have an energy of 20 keV and an equivalent
wavelength of 8.6 pm. By bringing the specimen as close as possible to the objective lens, by using an in-lens secondary electron detector to increase the collection semi angle, by minimizing lens aberrations and by using a cold field-emission gun (CFEG) state-of-the-art microscopes can reach a resolution of around 0.6 nm (equation (2)). However, in most cases a practical resolution of around 10 nm is achievable, sufficient to examine the internal structure of grains but not to resolve individual molecules or atoms.

The same etched specimen was used as for light microscopy with no additional preparation. The specimen was mounted onto an aluminium SEM holder using a conductive carbon pad and copper tape to prevent electric charge accumulation from the electron beam during investigation, which if excessive (as on non-conductive specimens) can deflect the electron beam and degrade image quality.

Figure 8 illustrates the SEM investigation. The Zeiss Ultra Plus microscope (a) is shown along with a schematic of the instrument, (b) a diagram of electron-specimen interaction and (d) a detailed secondary-electron image of the specimen showing the grain structure and twins inside the PDR.
below the surface [51] (c). Within this ‘interaction volume’ a number of mechanisms are activated, but for SEM imaging the most important are secondary electrons and backscattered electrons. These are captured by the respective detectors, converted to an electric current and displayed on a computer screen to form an image of the surface. Backscattered electrons consist of electrons from the incident beam that are elastically scattered backwards by atomic nuclei in the specimen. Their production is highly sensitive to the mean atomic number and so can be used to investigate chemical variation across the specimen. Because of their relatively high energy they originate from within a few microns of the surface. Secondary electrons are emitted by inelastic scattering events between the incident electrons and electrons in the specimen. They have significantly lower energies than backscattered electrons and originate within the top 10 nm of the specimen making them suitable for investigating surface topography. The in-lens secondary electron detector is integrated into the objective lens to minimise the distance from the specimen and thereby maximise the NA and the imaging resolution. The test piece surface inside the PDR is shown by the secondary electron image (d). The twins appear in significantly more detail than in the light microscope study and smaller twins can be observed with the higher resolution. The Al–Mn precipitates, being significantly harder than the magnesium matrix, are proud of the surface after polishing; and this, combined with the slightly higher secondary electron emission due to higher atomic number of manganese, causes them to appear bright.

Grain orientation can be examined in an SEM using an electron backscatter diffraction (EBSD) detector and acquisition/analysis software [52]. Specimen preparation for EBSD has to be made very carefully as the surface needs to be flat, smooth and ideally damage free. To achieve this level of surface finish an additional polishing stage was used. A vibratory polishing machine was used with a colloidal suspension of 0.04 μm Al₂O₃ particles in ethanol on a neoprene cloth for 10 min.

Figure 9 illustrates the EBSD investigation. The specimen is mounted in the microscope chamber with a 20° inclination to the beam (a). The electron beam is scanned across the specimen surface and electrons that are backscattered by the specimen are diffracted by lattice planes in the top few nanometers of the surface. At each scanned point diffracted electrons exit the surface in a conical distribution and are imaged on the detector. Software is used to identify diffraction patterns (called Kikuchi maps) (b), which can be very faint, within the image, and these are indexed against the expected (in this case magnesium) compound and phase [53]. In this way the orientation of the lattice at each point is determined and an orientation map obtained. EBSD orientation maps generally have around a million data points. Noise reduction routines are used to eliminate non-indexed points by identifying them with surrounding points. An EBSD orientation map of the surface inside the PDR is shown in (c). The image shows grains mostly in shades of red and twins in shades of green and blue within the grains and sometimes extending over multiple grains. The different colours indicate different lattice orientations and red indicates that the majority of grains are aligned with the c-axis normal to the surface. This is called basal texture and is typical of the twin-roll casting process that forces the unit cells into that orientation at the surface. Green and blue indicate that the twins are tilted away from the parent grains, and analysis of orientation differences between neighbouring points quantifies the tilt as 86.3° ± 0.8° (d). This angle is characteristic of the shearing transformation of extension twins. To examine the microstructure inside the twins and the arrangement of atoms, it is necessary to use the method of microscopy capable of highest resolution.
3.4. From microns to sub-nanometres (transmission electron microscopy)

To investigate at the nanometre level and below, even to reveal single columns and planes of atoms, TEM is used. There are two main modes of TEM operation: conventional TEM (or CTEM) and scanning TEM (or STEM). In the present investigation only CTEM is used. For the typical accelerating voltage used in a modern TEM of \( V = 200 \text{kV}, \) the de Broglie equation (equation (3)) yields an electron wavelength of \( \lambda = 2.5 \text{ pm} \). Using this value and a typical collection semi-angle of 10 mrad, the Rayleigh criterion or diffraction limit is 0.15 nm. In the case of STEM, using a larger collection semi-angle and aberration correction lens system, the smallest resolvable distance can be brought below 0.1 nm. The current world’s best resolution obtained is 40.5 pm [54].

3.4.1. Specimen preparation. Specimen preparation for TEM is more complicated than for other methods of microscopy: in addition to requiring two smooth damage-free surfaces, the specimen must also be thin enough for electrons to pass through [55]. This is determined by the inelastic mean free path (IMFP) of electrons in the medium of the specimen. At 200 keV, the IMFP of an electron in metals is between 100 and 200 nm, with magnesium having an IMFP of 150 nm [56]. Therefore, in order for a sufficient intensity of electrons to pass through the specimen to form an image, the specimen needs to be less than 100 nm thick, and for best quality imaging less than 20 nm thick.

Figure 9. Electron backscatter diffraction is used to investigate grain orientation of a specimen in an SEM: (a) a schematic of the method, (b) an indexed Kikuchi map from magnesium, (c) an orientation map from inside the PDR and (d) a distribution of twin disorientations inside the PDR.
Figure 10 shows the different stages of specimen preparation used. The AZ31B specimen was first ground and polished to a thickness of 20 μm using an automated polishing machine with a range of abrasive diamond films from 16 to 0.1 μm particle size (a). The coarser grade abrasive was used first to remove the larger portion of the sample (B), while the remaining material was removed using finer grades (C). To minimize oxidation, the final polishing stage was performed in ethanol. After gluing the specimen onto a 3 mm diameter copper support ring with MBond 600 epoxy resin (b), as the thin magnesium foils tend to roll up otherwise, final thinning was made using a Gatan PIPS II ion polisher (c) with twin argon ion beams operated at 4 keV and ±5° inclination to the specimen surface, giving a removal rate of approximately 1 μm per minute. The final specimen (d) consisted of a square section of magnesium, with a hole in the centre approximately 200 μm in diameter, mounted on a copper ring. Around the edges of the hole, the material was sufficiently thin to be electron transparent.

3.4.2. Overview of the transmission electron microscope. Figure 11 shows (a) an image of the JEOL F200 transmission electron microscope and (b) a schematic of the three main
systems and the main working components. At the top of the evacuated column electrons are
generated in a single crystal tungsten filament, extracted through field emission and
accelerated through an electrostatic potential gradient of (typically) 200 kV. Electrons are
then condensed into a fine parallel beam using a condenser lens and incident upon the thin
specimen. The specimen is contained within a specimen holder that is inserted into the
column through a goniometer-airlock dual unit. The specimen at the end of the holder is
located on the optical axis of the microscope inside the objective lens and can be translated
and tilted using the goniometer. Electrons passing through the specimen undergo diffraction
by the lattice planes of the specimen and are recombined by the objective lens to form an
image, which is magnified using the projector lens onto a phosphor screen at the lower end of
the microscope column for viewing. By raising the phosphor screen the electrons expose a
special digital camera which combines a scintillator and CCD imaging cell to display the
image on a computer screen. The microscope is operated through a combination of manual
controls and a graphical user interface.

3.4.3. Electron diffraction. To fully appreciate the extensive functionality of TEM, and to
interpret results from an investigation, it is necessary to grasp the fundamentals of electron
diffraction. The theory of diffraction, developed by Bragg [57] (see figure 12), describes the
relationship between electron wavelength ($\lambda$), lattice plane spacing ($d$) and diffraction angle ($\theta$) for constructive interference of order $n$ (integer) as:

$$ n \lambda = 2d \cdot \sin \theta. $$

(4)

The difference in length between path A and path B after diffraction is $2d \sin \theta$, which must be
an integer multiple of the electron wavelength for constructive interference of the two beams
to occur. When $d = 0.3 \text{ nm}$ (typical plane spacing in inorganic solids) and $\lambda = 2.5 \text{ pm}$, for
the lowest order diffraction ($n = 1$), $\theta = 0.24^\circ$.

In the TEM, the electron beam is generally diffracted into many separate beams as it
passes through the specimen. These beams form a diffraction pattern, which can be viewed on
the phosphor screen or computer display. A great deal of information relating to the structure

Figure 11. The JEOL F200 transmission electron microscope (a) and a schematic of the instrument (b).
of the specimen can be obtained from the diffraction pattern, especially when a
crystallographic axis is parallel to the optical axis. In this case, the spot geometry can be
used to determine the compound and phase diffracting the beam.

### 3.4.4. Image contrast

Contrast in a TEM image is classified as either mass-thickness contrast, diffraction contrast or phase contrast [58]. Both mass-thickness and diffraction contrast correspond to a reduction in intensity from the incident beam and are combined under the single term amplitude contrast.

#### 3.4.4.1. Mass-thickness contrast

With reference to figures 13 (a) and (b), regions of the specimen that contain heavier (higher atomic number) elements or are thicker absorb more electrons from the incident beam and therefore these regions appear darker in the image as the intensity of the transmitted electrons is less than surrounding regions. When the area of the specimen under investigation is less than 20 nm in thickness, the effect of mass-thickness variations upon the image are negligible. Close to the hole the TEM specimen usually appears bright as the foil there is thin enough to transmit a large intensity of electrons, away from the hole the specimen becomes thicker and the image appears darker. In specimen preparation it is aimed to achieve a large electron transparent area in order to maximise the area of specimen for investigation. Typically in a specimen prepared using PIPS, an area of several hundred square microns is usable.

#### 3.4.4.2. Diffraction contrast

With reference to figure 13(c), electrons diffracted with the same inclination converge at the back focal plane of the objective lens forming a diffraction pattern, while electrons from the same point of the specimen converge at the image plane to form an image [59]. The objective aperture in the back focal plane can therefore be used to include or exclude specific diffracted beams, giving contrast in the image. A smaller or larger aperture increases or decreases contrast, respectively. When the non-diffracted beam passes through the aperture, a bright field image is formed where the hole appears bright and diffracting planes dark. By tilting the incident beam using the beam tilt control, a specific diffracted beam can be made to pass along the optical axis and through the objective aperture forming a dark field image where the hole appears dark and the diffracting planes bright. This is particularly useful for examining orientation-related features such as coherency of
precipitates. Diffraction contrast is the main mode of investigation in the TEM where it is particularly useful for studying defects in the lattice.

3.4.4.3. Phase contrast. Phase contrast is also an effect of diffraction, but results from interference of diffracted beams rather than their exclusion. In figure 13(d) the electron beam, considered as a plane wave, undergoes diffraction by lattice planes as it passes through the specimen. With the objective aperture removed, these beams recombine to form an image with very little diffraction contrast, but because different beams take different paths through the specimen they have different phases. Phase contrast therefore arises due to constructive and destructive interference of these beams at the image plane and contains information about the crystalline nature of the specimen. At high magnification, the arrangement of atoms in the lattice can be measured to identify the elements and compounds of the specimen at that location and to identify lattice defects.

Figure 14 shows the results from the TEM investigation. The region of the test bar outside the PDR is shown in low magnification in (a) where the granular structure can be easily seen with grains (numbered 1–8) being approximately 5 μm in size. Grain size in TEM is always underestimated as the image presents only a cross-section plane view of grains; a geometrical correction can be used to obtain a more accurate value [60]. Within many of the
grains are dense networks of dislocations. These are not visible in SEM or EBSD, but in TEM they give rise to diffraction contrast by scattering the electron beam. No twinning was found outside the PDR. This is consistent with TEM study of the unstrained alloy sheet that revealed the presence of dislocations but no twinning, and indicates that the dislocations were formed during the twin-roll casting process where the higher temperature favoured additional slip systems. The small dark regions are Al–Mn precipitates: the higher average atomic number of these precipitates causes an increase in backscattering of the electron beam, and their extra hardness causes them to be slightly thicker than the matrix (being not so readily thinned in mechanical polishing and PIPS), and hence they appear dark in the image. The particularly low brightness of this small group of precipitates additionally suggests that their orientation is also strongly diffracting (i.e. a crystallographic axis is close to parallel to the incident electron

**Figure 14.** TEM images showing (a) the granular microstructure outside the PDR, (b) the distinct presence of lamella-like twins inside the PDR, and HRTEM images showing (c) the lattice of magnesium with the [2110] zone axis normal to the paper with directions, plane spacing and hexagonal unit cell marked and (d) details of the twin boundary, indicated by the white square in (b), viewed along the [2110] direction and sheared by $86.3^\circ$. 
The region of the test bar inside the PDR is shown in low magnification in (b). Twins, which appear as lenticular or lath-like shapes, are observed in high density throughout the PDR. The areas marked 1, 3 and 5 are the original matrix from the grain, while areas 2 and 4 are twinned regions. Stacking faults, inclined at 43° to the edges, are visible within the twins by diffraction contrast, sometimes spanning the entire width of the twin. In magnesium and other hcp metals, such as titanium or zinc, stacking faults are known to occur on the basal plane. A phase contrast high resolution (HR) TEM image of the magnesium matrix is shown in (c). The specimen is tilted so that the [2110] zone axis is parallel to the electron beam, normal to the page. The bright spots represent atom columns sideways on to the hexagonal unit cell with the c-axis in the plane of the page pointing upwards, which is confirmed by the 0.28 nm spacing of the (0110) planes and 0.26 nm spacing of the (0002) planes. The image (d) shows high resolution details of the twin boundary from the region indicated by the white square in (b). The actual region of the image is approximately 1/50 the size of the white square. The atom arrangements can be clearly seen across the twin border, which is highlighted by the shaded band across the image. The lattice of the twin is sheared by 86.3° in relation to that of the grain, as shown by the orientation of the hexagonal prisms, which represent the hcp unit cell. This is the expected angle for extension twins, in which {0112} the planes are the shared or intersecting twinning planes. In the image, the line of atoms central to the shaded band are on the (0112) plane, which is perpendicular to the page. This confirms the observations from EBSD experiments.

4. Conclusions

This study through the length scales shows the value of microscopy in investigating the structure and behaviour of matter. Plastic deformation observed in a magnesium alloy test bar as a result of a single compressive test is shown to correspond to structural changes in the alloy that originate within the individual grains. Rearrangement of atoms within grains by twinning is found to be the main process responsible for macroscopic plastic strain. Knowing this helps us understand the behaviour of magnesium components during applications and helps in the development of new alloys with improved mechanical properties such as yield strength and creep resistance. To make these observations, a sequence of microscopy investigations was used starting from visual inspection and working down in length scale to the observation of lattice planes and atoms. The level of difficulty of the investigation increases with decreasing length scale, as does the complexity of the equipment and the required financial investment. The study demonstrates how important it is to understand the intrinsic behaviour and properties of materials intended for practical application. This is crucial for the appropriate use of materials and is an important lesson to learn for physicists and engineers.

The electron microscope is actually a small linear particle accelerator and, akin to those larger ones at research centres such as CERN and Fermilab, it collides particles (electrons in this case) with a target (the specimen here) and uses a number of detectors to collect data from the experiment. The entire process of electron microscopy (TEM in particular) brings together a wide range of different scientific disciplines including those related to specimen preparation, data analysis, lens design, computer simulation, and in the more advanced cases even relativistic theoretical physics and quantum mechanics to properly model and explain the data. Additionally, electron microscopy has applications in a wide range of fields of materials science, including metals and alloys, ceramics, organic materials, polymers, and has played a large role in the development of nanotechnology especially nanoparticles, nanotubes, fullerenes and monolayer graphene.
The paper has been arranged so that it is a practical use to science teachers, can be used as a graphical source for slides in lectures, a source of important key literature, and as an overview of the process of microstructural investigation. It can be used in courses on physics, materials science or engineering.

Table 1 shows the method required for the study of each length scale, the specimen preparation required and the type of information obtained. An indication is also given of the financial investment required for each method.

| Length scale | Method | Preparation | Information obtained | Approx. cost, € |
|--------------|--------|-------------|----------------------|---------------|
| Visual inspection | • Cleaning of the specimen surface using ethanol with a soft cloth or cotton bud to remove dirt and dust. | Bulk size and shape, surface appearance, colour, roughness and general condition. | N/A |
| Light microscopy | • Polishing to prepare a smooth surface. | Surface appearance and topography, roughness and defects such as pits and scratches, grain size and shape and precipitates. | €20–30 K |
| | • Etching to reveal grains and twin boundaries. | | |
| Scanning electron microscopy | • Sectioning of the bulk specimen to fit into the vacuum chamber. | Surface topography, elemental analysis (with x-ray detector) and grain orientation analysis (with EBSD system), grain size and shape and precipitate elemental analysis. | ~€300 K (up to €1 M with advanced detectors) |
| | • Polishing of the specimen surface. | | |
| | • Etching to reveal grains and twin boundaries. | | |
| Transmission electron microscopy | • Sectioning, grinding, polishing and bonding to a TEM support ring. | Microstructural and elemental analysis including grain size, phase, space group, plane spacing, lattice geometry and lattice defects. | ~€2 M |
| | • Ion-milling to <50 nm thick. | | |
| | • Electro-polishing is an alternative to ion-milling. | | |
| | • Plasma cleaning to remove atmospheric hydrocarbon contamination. | | |
cost of equipment required to make the investigations. Although investigation at each
length scale requires a discerning eye and the ability to pay attention to detail, the amount of
training and experience required to perform the investigation increases significantly with
decreasing length scale. Not only are the microscopes more complicated to use, but the
specimen preparation is more complex and interpretation of the results significantly more
demanding.

With a few hours of specific training, an undergraduate student can learn to use a light
microscope to observe fine details in a specimen. More advanced methods, such as dif-
ferential interference contrast and polarized light microscopy, can be picked up gradually.
A learning module covering one semester can easily give the necessary theoretical
background to become proficient in the basics of light microscopy. SEM requires a full day
of training including a hands-on training session, either individually or in a small group,
and a theoretical lesson to introduce the principles. The background of electron optics and
electron-solid interaction are complicated, and so a full capability of SEM is best inte-
grated with a science course including modules in these two areas. In the case of TEM, a
background in science is required (e.g. a degree in physics or materials science) as a basis
for using the instrument. Additionally, a full semester course is usually required in order to
learn the relevant aspects of electron optics, magnetic lens design, vacuum technology, the
theory of propagating waves, electron-specimen interactions, interference and diffraction.
A full week’s course is required for learning the manual controls and the computer
interface of the microscope, which give control of the key parameters such as the apertures,
beam energy, brightness, spot size, convergence angle, filament current and magnification.
In each case the learning process can continue over many years. New analytical
attachments are being developed frequently for instruments, adding to the range of mea-
surements that can be made using microscopes; and each new method has its own
theoretical background and principles of operation.

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‘n2m’ (nano-to-macro).

Appendix

The hexagonal coordinate system
Figure A1. A standard notation in the science of crystallography is that curved brackets \( \langle xxxx \rangle \) represent planes, square brackets \([ xxxx]\) represent directions, while \( \langle xxxx \rangle \) are used to represent families of directions and \( \{ xxxx\} \) families of planes.

Reading list

Born M and Wolf E 1997 *Principles of Optics* (Cambridge: Cambridge University Press)
Callister W D, Rethwisch D G 2009 *Materials Science and Engineering: An Introduction* (London: Wiley)
Williams D B and Carter C B 1996 *Transmission Electron Microscopy* (New York: Plenum Press)
Ayache J, Beaunier L, Boumendil J, Ehret G, Laub D 2010 *Sample Preparation Handbook for Transmission Electron Microscopy* (Berlin: Springer-Verlag)
Croft W J 2006 *Under the Microscope: A Brief History of Microscopy* (Singapore: World Scientific)
Borchardt-Ott W 2012 *Crystallography - An Introduction* (Berlin: Springer-Verlag)
Kittel K 2004 *Introduction to Solid State Physics: 8th Edition* (London: Wiley)
Ashcroft N W and Mermin N D 2003 *Solid State Physics* (New York: Brooks Cole)

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References

[1] Hernandez C, Ravn O and Forero-Shelton M 2014 Challenges in a physics course: introducing student-centred activities for increased learning *J. Univ. Teach. Learn. Pract.* 11 1–6
[2] Freeman S et al 2014 Active learning increases student performance in science, engineering, and mathematics *Proc. Natl Acad. Sci.* 111 8410–5
[3] Masters M F and Grove T T 2010 Active learning in intermediate optics through concept building laboratories Am. J. Phys. 78 485–91
[4] Council N R 2005 How Students Learn: History, Mathematics, and Science in the Classroom ed MS Donovan and JD Bransford (Washington, DC: The National Academies Press) p 632
[5] Council N R 2000 How People Learn: Brain, Mind, Experience, and School: Expanded Edition (Washington, DC: The National Academies Press) p 256
[6] Council N R 2015 Reaching Students: What Research Says About Effective Instruction in Undergraduate Science and Engineering ed N Kober (Washington, DC: The National Academies Press) p 256
[7] Council N R 2013 Adapting to a Changing World: Challenges and Opportunities in Undergraduate Physics Education (Washington, DC: The National Academies Press) p 142
[8] Read A J 1989 ‘Hands-on’ exhibits in physics education Am. J. Phys. 57 393–4
[9] Matteucci G et al 2008 An experiment on the particle–wave nature of electrons Eur. J. Phys. 30 217–26
[10] Matteucci G 1990 Electron wavelike behavior: a historical and experimental introduction Am. J. Phys. 58 1143
[11] Bechhoefer J 2015 What is superresolution microscopy? Am. J. Phys. 83 22–9
[12] Velentzas A 2014 Teaching diffraction of light and electrons: classroom analogies to classic experiments Phys. Teach. 52 493–6
[13] Frabboni S et al 2011 Two and three slit electron interference and diffraction experiments Am. J. Phys. 79 615–8
[14] Horsley S A R et al 2014 Revisiting the Bragg reflector to illustrate modern developments in optics Am. J. Phys. 82 206–13
[15] Ovalle V et al 2008 Studying charged particle optics: an undergraduate course Eur. J. Phys. 29 251–6
[16] Peidle J et al 2009 Inexpensive microscopy for introductory laboratory courses Am. J. Phys. 77 931–8
[17] Silverman M P, Strange W and Spence J C H 1995 The brightest beam in science: new directions in electron microscopy and interferometry Am. J. Phys. 63 800–13
[18] Hall C E and Weber R L 1954 Introduction to electron microscopy Am. J. Phys. 22 97–97
[19] Weber R L 1952 College courses in electron microscopy Am. J. Phys. 20 301–4
[20] Mittemeijer E J 2011 Fundamentals of Materials science: Microstructure–Property Relationships Using Metals as Model Systems (Berlin: Springer)
[21] Srinivasan S and Ranganathan S 2017 India’s legendary Wootz Steel: An Advanced Material of the Ancient World (Hyderabad: University Press)
[22] Verhoeven J D, Pendray A H and Dauksch W E 1998 The key role of impurities in ancient damascus steel blades J. Miner. 50 58–64
[23] Cobb H 2010 The History of Stainless Steel vol 166 (Novelty, OH: ASM International) pp 57–57
[24] Layard A H 1853 Discoveries Among the Ruins of Nineveh and Babylon (New York: Harper and brothers)
[25] Bradbury S 1968 The Microscope Past and Present (Oxford: Pergamon)
[26] Menter J W 1956 The direct study by electron microscopy of crystal lattices and their imperfections Proc. R. Soc. A 236 119–35
[27] Smith D J 2008 Ultimate resolution in the electron microscope? Mater. Today 11 30–8
[28] Taylor C C W 1999 The Atomists: Leucippus and Democritus (Toronto: University of Toronto Press)
[29] Spence J 2002 Achieving atomic resolution Mater. Today 5 20–33
[30] Jones E R and Childers R L 1984 Observational evidence for atoms Phys. Teach. 22 354–60
[31] Ruska E 1987 The development of the electron microscope and of electron microscopy Biosci. Rep. 7 607–29
[32] Kainer K U and Mordike B L 1999 Magnesium Alloys and their Applications (Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA)
[33] Kulekci M K 2007 Magnesium and its alloys applications in automotive industry Int. J. Adv. Manuf. Technol. 39 851–65
[34] Raynor G V 1960 The Physical Metallurgy of Magnesium and its Alloys (London: Pergamon)
[35] Glazer M and Burns G 2013 Space Groups for Solid State Scientists (Amsterdam: Elsevier)
[36] Rosenberg H M 1988 The Solid State (Oxford: Oxford University Press)
[37] Koehler J S 1942 On dislocation theory and the physical changes produced by plastic deformation *Am. J. Phys.* 10 275–85

[38] Jones R M 2010 *Deformation Theory of Plasticity* (Blacksburg, PA: Bull Ridge Publishing)

[39] Barnett M R 2007 Twinning and the ductility of magnesium alloys *Mater. Sci. Eng. A* 464 1–7

[40] Christian J W and Mahajan S 1995 Deformation twinning *Prog. Mater. Sci.* 39 1–157

[41] Denk J et al 2018 Concept of the highly strained volume for fatigue modeling of wrought magnesium alloys *Int. J. Fatigue* 117 283–91

[42] Mordike B L and Ebert T 2001 Magnesium: properties-applications-potential *Mater. Sci. Eng. A* 302 37–45

[43] Bettles C and Barnett M 2012 Advances in wrought magnesium alloys *Metals and Surface Engineering* (Oxford: Woodhead Publishing)

[44] Cao P, Qian M and StJohn D H 2005 Native grain refinement of magnesium alloys *Scr. Mater.* 53 841–4

[45] Whitmore L et al 2014 Yield strength prediction in Ni-base alloy 718Plus based on thermo-kinetic precipitation simulation *Mater. Sci. Eng. A* 608 114–22

[46] Hull J P 1999 The second industrial revolution and the staples frontier in Canada: rethinking knowledge and history *Scientia Canadensis* 18 22–37

[47] Kawalla R, Oswald M and Schmidt C 2008 New technology for the production of magnesium strips and sheets *J. Metalurgija* 47 195–8

[48] Rayleigh L 1874 On the manufacture and theory of diffraction-gratings *Phil. Mag. Sci.* 47 81–93

[49] Brogile L 1924 A tentative theory of light quanta. *Phil. Mag. Sci.* 47 446–58

[50] Matsuda K et al 2005 The potential of the scanning low energy electron microscope for the examination of aluminum based alloys and composites *J. Electron. Microsc.* 54, 109–17

[51] Schwartz A J et al 2009 *Electron Backscatter Diffraction in Materials Science* (New York: Springer)

[52] Maitland T 2004 Electron backscattered diffraction *Adv. Mater. Process.* (Novelty, OH: ASM International) p 34–6

[53] Morishita S, Ishikawa R, Kohno Y, Savada H, Shibata N and Ikuhara Y 2018 Attainment of 40.5 pm spatial resolution using 300 kV scanning transmission electron microscope equipped with fifth-order aberration corrector *Microscopy* 67 46–50

[54] Anderson R M and Walck S D 2014 Specimen preparation for transmission electron microscopy IV *MRS Symp. Proc.* (Cambridge: Cambridge University Press)

[55] Iakoubovskii K and Mitsuishi K 2009 Elastic scattering of 200 keV electrons in elemental solids: experimental observation of atomic-number-dependent oscillatory behavior *J. Phys.: Condens. Matter* 21 155402

[56] Bragg W H and Bragg W L 1913 The reflexion of x-rays by crystals *Proc. R. Soc.* A 428–38

[57] Ludwig R 1989 *Transmission Electron Microscopy: Physics of Image Formation and Microanalysis* (Berlin: Springer)

[58] Feynman R 1963 *Diffraction (The Feynman Lectures on Physics vol 1)* (Reading, MA: Addison-Wesley)

[59] Whitmore L et al 2012 Transmission electron microscopy of single and double aged 718 Plus superalloy *Mater. Sci. Eng. A* 534 413–23