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The rapidly changing face of electron microscopy



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ABSTRACT

This short but wide-ranging review is intended to convey to chemical physicists and others engaged in the interfaces between solid-state chemistry and solid-state physics the growing power and extensive applicability of multiple facets of the technique of electron microscopy.

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1. Introduction

Fifty years ago electron microscopy (EM) had already made a profound impact both on molecular biology and metallurgy. A negative staining method for high-resolution EM of viruses soon led to major progress in three-dimensional (3D) image reconstructions of small viruses [1–3]; and the reality of the existence, movement and importance of dislocations in crystalline solids were rendered visible by electron diffraction contrast [4]. Chemists, and to a lesser degree, materials scientists, were relatively slow to capitalise upon the power of EM; but the situation, for chemical physicists especially, has been utterly transformed in the last thirty years [5,6], and the pace of change in chemical electron microscopy is now very considerable.

Four years ago, two of us published a synoptic account [7], aimed at the community of chemical physicists in which, *inter alia*, we summarised the myriad array of structural and dynamic processes pertaining to biological, physical and engineering materials that was then retrievable (in unprecedented detail) using EM. During the short intervening time, there have been many other significant advances. As in multi-dimensional spectroscopy [8], where there have been rapidly improving spatial, spectral and temporal resolutions, so also is the case in multi-dimensional EM (MDEM). But there is also the extraordinary ease with which data may now be garnered. For example, modern scanning transmission electron microscopes (STEMs), used for the retrieval of energy-dispersive X-ray (EDX) and electron energy-loss (EEL) spectra, can routinely generate 'spectrum images' in which behind every image pixel lies a complete (EDX or EEL) spectrum. By recording a tilt series of such spectrum images, 4D 'spectrum tomograms' produce spectral (hence chemical) information at every real space voxel.

There is, however, another kind of 4D EM [9–11], which is the revolutionary one introduced by Zewail and co-workers that achieves ultra-fast EM at the femtosecond time-scale, while also reaching near atomic resolution. This remarkable technical advance has improved temporal resolution of imaging and spectra, generated by EM, by some 10 orders of magnitude compared with the video recording rates still used extensively by microscopists. Indeed, the Nobel Laureate Roger Kornberg, himself an inspired user of EM and other techniques in structural biology [12], has recently described the monograph entitled '4D Visualisation of Matter' [10] as 'a chronicle of an extraordinary journey of invention and discovery.'

We shall dwell only briefly in this short review on the 4D EM work of Zewail et al., despite the novelty of their photon-induced near-field electron microscopy (PINEM), with its other numerous advantages; and nor will we discuss aspects of electron holography with all its virtues [13], or indeed several other novel recent developments.

Before proceeding, however, we judge it pertinent to recall that electrons as primary radiation to interrogate condensed or fluidic matter are superior to both X-rays and neutrons, as demonstrated by Henderson in his seminal review [14]. He drew attention to the not widely appreciated fact that the amount of beam damage inflicted on the specimen per useful elastic event is 10^3 times as great for 1.5 Å wavelength X-rays than it is for 100 keV electrons. Even 1.8 Å wavelength neutrons are three times more damaging per useful elastic event [15]. It follows that, for the same amount of radiation damage, electrons yield the most useful information. It is also pertinent to recall that cryo-electron microscopy considerably diminishes beam damage. In addition, thanks to revolutionary recent advances in electron detector technology, the attractions of EM as a structural tool in molecular biology are fast outweighing the traditional 4-circle X-ray (or synchrotron based) diffraction methods. Even structures of extreme complexity, like large ribosomal subunits from human mitochondria (revealing 48 proteins) have

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been elucidated at 3.4 Å resolution using single-particle cryogenic EM [16,17].

So straightforward has it become, thanks to numerous technological and computational advances, to garner enormous amounts of digital information, that the era of 'big data' and the subject of data science have high relevance in modern EM. It is now almost mandatory to determine ahead of measurement the nature of the manageable information that one needs to collect. When one reflects that some 90% of all data in the world was created in the last two years alone, T.S. Elliot's thought springs to mind: 'Where is the wisdom we have lost in knowledge? Where is the knowledge we have lost in information?'

Earlier reviews from this laboratory [7,18–20] have described how extremely versatile EM has become in assessing structure at the atomic level, in exploring microstructures and their variety of artefacts, as well as in retrieving valence states of atoms and their dynamics from cryogenic temperatures upwards. What was not covered in those reviews was the way in which automation of specimen settings (tilts) and data retrieval has revolutionised access to the entire reciprocal space of objects under study with a consequential accumulation of a blizzard of new data. Not only is it feasible to conduct valency mapping in 3D, but it is also possible to chart the morphological dynamics of catalytically active microcrystallites and to open up a new era of 'crystal cartography'. Moreover, with aberration-corrected electron microscopes, it is readily feasible to identify single-atom (supported) catalysts; and these, in turn, have ushered in a new era of single-atom heterogeneous catalysis [21,22]. Mesoporous oxidic solids, with their large unit cell dimensions, and which are now of central importance in shape-selective and regio-selective heterogeneous catalysts, cannot be structurally defined by X-ray methods; but they can be structurally elucidated by EM [19,20,23].

2. Topics to be touched upon in this review

By means of a collection of representative illustrations, we outline some new frontiers in chemical physics and materials chemistry that can now be explored via MDEM. In doing so, we also summarise relevant complementary advances made in adjacent fields, such as signal processing, information theory and analytical science. In electron tomography (ET), for example, it may be essential to invoke compressed sensing (CS, see later), just as it is advantageous to do so in studies involving magnetic resonance [24].

Of growing importance in MDEM, where it is mandatory to process 'big data', is multivariate statistical analysis (MSA). This term actually encompasses many different, but related, approaches, including principal component analysis (PCA), independent component analysis (ICA) and non-negative matrix factorisation (NMF). In each case the raw spectrum, image or diffraction pattern, is decomposed into a series of 'basis' functions, each of which is weighted by a coefficient, or loading. The advantage of doing this becomes apparent if the number of statistically significant basis functions is small, for example perhaps fewer than 10 basis spectra from a data set composed of many tens of thousands! It is not obvious de novo, for a particular data set, which decomposition approach will be the most successful. This is an area of rapid progress and optimised MSA protocols for handling EM-related big data are now already of key importance.

Firstly, however, we focus on the primacy of STEM as the principal technique for effecting MDEM. There then follow sections dealing with ET, chemical MDEM, and also time-resolved MDEM. Finally the growth of crystallographic MDEM, which is well-suited to investigate relatively electron-beam sensitive organic molecular crystals, is outlined. [(The term multi-dimensional EM (MDEM)

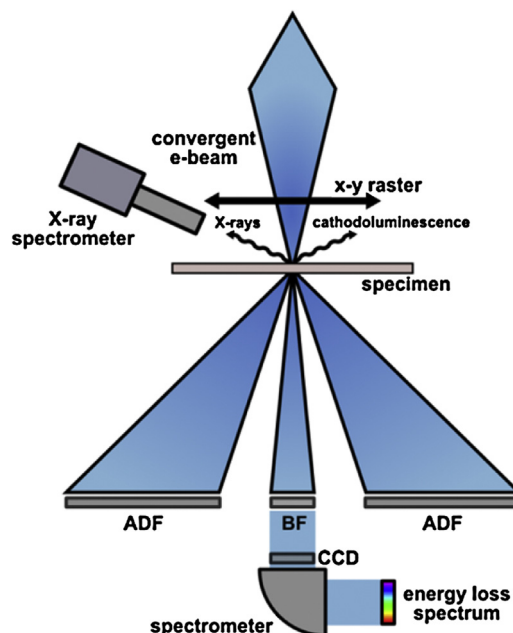


Figure 1. The scanning transmission electron microscope, salient electron beam-specimen interactions and associated detectors (ADF=annular dark-field, BF=bright field).

may offend the sensitivities of those who regard 4D EM – the 3 spatial plus time domain – as the only (justified rational) multi-dimensional EM. But 4D EM has already been used by others to mean something different – see for example, Gass et al., Ref. [25] below, who describe spectral tomography. For convenience and heuristic reasons we shall use MDEM in the sense subsumed in earlier sections of this review.])

3. The central role of STEM in MDEM

There are many imaging modes and associated analytical techniques available in electron microscopy that provide complementary information about structure, composition and chemico-physical properties from the micron to the atomic scale. One mode in particular, annular dark-field (ADF) STEM imaging, has proven to be invaluable in a great many circumstances, owing to its direct interpretability and atomic number contrast [13,26]. The now widespread availability of aberration-corrected electron optics has made atomically resolved images commonplace, a capability of great value, for example, for revealing the precise atomic architecture of nanoscale catalysts. Another great virtue of STEM is its amenability to the simultaneous collection of the multiple signals emanating from the specimen (Fig. 1).

As depicted in Figure 1, STEM images are acquired in a serial fashion in which the probe, typically sub-nm in diameter, dwells for a short time at a point on the specimen. The interaction of the beam as it passes through the specimen leads to a variety of signals arising both from elastic and inelastic processes. By scanning the beam across the specimen, each signal that arises from the interaction can be recorded with high spatial resolution, forming maps of the specimen's structure and composition – a form of nanoscale cartography! STEM therefore, with its ability to record multiple signals at each beam position, provides a central platform for MDEM, with the addition of further 'dimensions' provided by spectroscopy, diffraction and time resolution; hence STEM can reveal information in one of more of real space, reciprocal (diffraction) space, energy space and the temporal domain, see Figure 2.

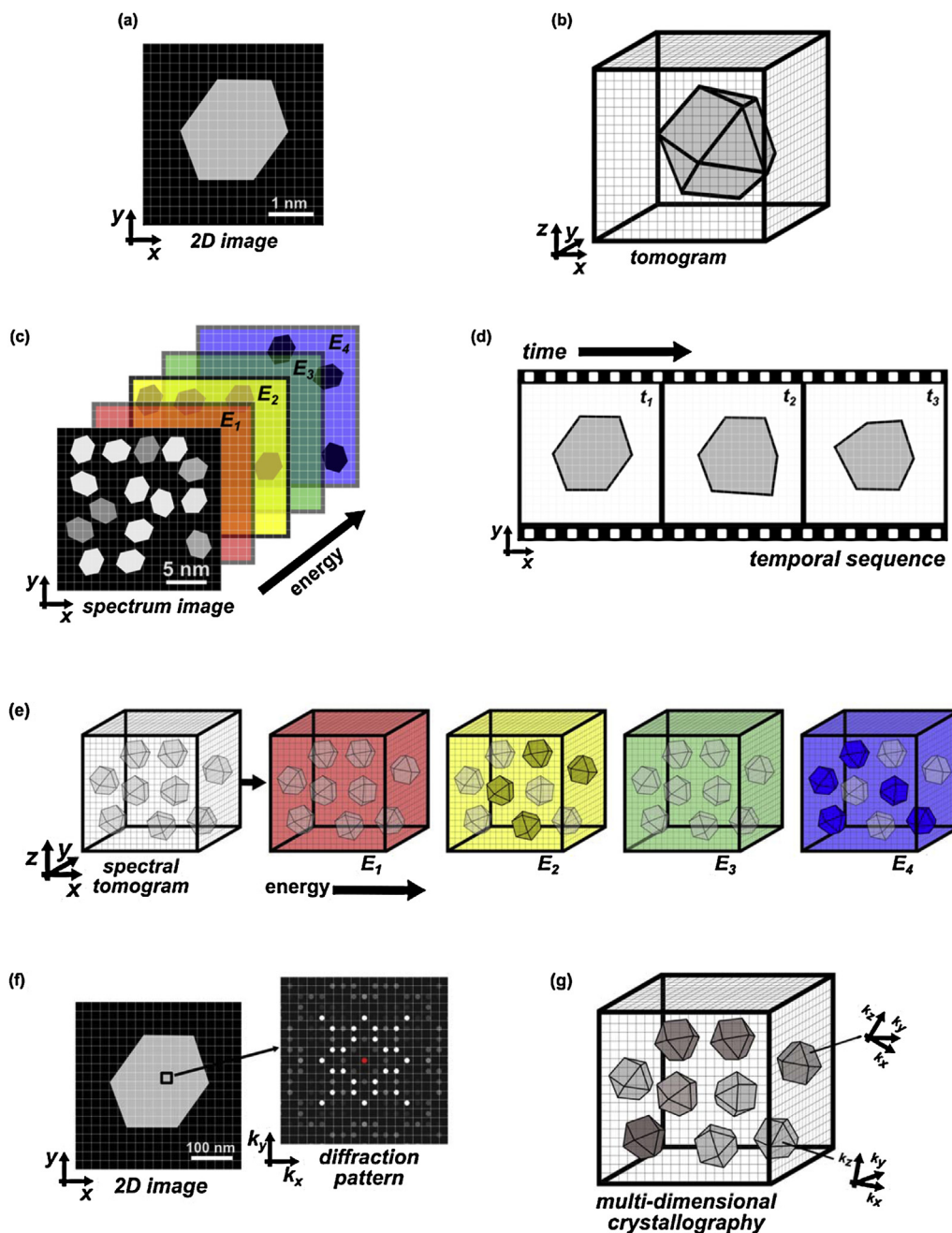


Figure 2. Forms of multi-dimensional electron microscopy: (a) 2D imaging, in which the image constitutes some form of integration through the specimen, typically yielding structural details; (b) 3D imaging, often accomplished by tilt series tomography to reveal 3D morphology; (c) spectrum imaging, in which at each pixel a full spectrum, or energy-loss series at chosen energy-loss windows ($E_1, E_2, \dots$), is recorded; (d) temporal image sequences, typically capturing response to a stimulus; (e) spectral tomography, entailing spatially resolved spectroscopy across all three spatial dimensions, i.e. 'voxel spectroscopy'; (f) diffraction mapping, in which a full (reciprocal space) diffraction pattern is recorded at each real space pixel; (g) multiple-dimensional crystallography, in which both 3D real and reciprocal space information is retrieved.

4. Structural MDEM

In chemico-physical contexts, the extension of 2D structural EM (Fig. 2a) to fully 3D structural characterisation (Fig. 2b) has been greatly facilitated by STEM-based tomography [13,27]. In essence, ET entails recording a series of EM images in which the specimen is tilted to different angles. The series of images, each image of which constitutes a projection of the specimen (to a sufficient approximation), are then 'back-projected' (computationally) to generate a 3D reconstruction. The directly interpretable nature of the ADF signal in STEM makes it well-suited to 3D structural imaging of strongly scattering specimens which, following the first demonstrations ca.

15 years ago [28], led to its adoption across most of the chemico-physical sciences [13,27]. More recently, as addressed in the later sections of this review (and briefly elsewhere [18]), the increasing ability to couple STEM tomography with analytical and crystallographic signals is leading to a new era in MDEM.

Before proceeding however, we first discuss some of the notable recent advances in 3D structural EM. Many of these have been focused on increasing the fidelity of 3D reconstructions from limited data sets, especially for 'nano-metrology', atomic-resolution 3D imaging and the study of specimens whose sensitivity to the electron beam has hitherto precluded detailed investigation. In particular, there has recently been a shift towards 'smart'

methodologies, especially those exploiting prior knowledge. These methodologies can both improve the 3D reconstructions and reduce the amount of data (usually the number of images) that need be acquired in the first place.

The technique of discrete tomography was successfully introduced in ET by Batenburg and co-workers [29], and harnesses the assumption that the specimen is in some sense ‘discrete’, such as consisting of a small number of constituent phases (and therefore only a few discrete grey levels in the tomographic reconstruction), or that the specimen consists of discrete elements, such as atoms. A subsequent highly efficacious development has been the introduction of so-called ‘compressed sensing’ (CS) techniques in ET [30]. CS methods, grounded by fundamental paradigm shifts in sampling theory, harness versatile procedures of sparse approximation, as used ubiquitously in image compression. In essence, CS harnesses the fact that the important information in many signals can be captured in a form that is compact compared to the full signal (i.e. the signal can be represented ‘sparsely’), and incorporates this knowledge of sparsity into the sensing and reconstruction processes to reduce vastly the amount of information that needs to be recorded.

Figure 3a provides a clear example of the efficacy of CS-ET. Not only is the exterior morphology and concave region of an iron oxide nanoparticle more accurately reconstructed using the full set of tilt series images, but when the numbers of images used for reconstruction is reduced, the reconstructions from CS-ET remain remarkably robust, while the conventional reconstructions (obtained using the simultaneous iterative reconstruction technique, SIRT) degrade substantially [31]. The accurate 3D nano-metrology via CS-ET is a crucial characterisation of the novel iron oxide nanoparticles, the concave surface of which can destabilise nanoparticles that fit exactly into the concavity [32].

A significant strength of CS-ET is the ability to address different types of image content through choice of the appropriate sparsifying transform, making it applicable to the study of many types of nano- and atomic-scale structures and phenomena. As seen from the success of the JPEG image compression algorithm, familiar from digital photography, sparsity is prevalent in the macroscopic world; as revealed by (S)TEM, sparsity is prevalent at the nanoscale too. Importantly, whilst there are an increasing number of applications of CS across many scientific and engineering applications (for example, magnetic resonance imaging, computed tomography and fluorescence microscopy), notable progress is being made also in developing a deeper mathematical understanding of the technique, and in linking theory and practice through the development of optimised protocols [33]. Moreover, concepts of sparsity and CS are applicable in many other aspects of EM besides ET, and should prove especially valuable in reducing the electron dose to which specimens must be exposed but retaining high quality images and analyses.

Coupled with aberration-corrected optics, both discrete- and CS-based methods have played a pivotal role in pushing the achievable resolution of ET into the atomic regime, for example as applied to an embedded silver nanoparticle [34] and a gold nano-rod [35]. Another approach harnessing 3D Fourier filtering has recently disclosed atomic-scale details pertaining to dislocation structure in a platinum nanoparticle [36].

In addition, there has been considerable development in quantifying atomic resolution STEM images, and especially in the determination of the number of atoms in projected columns through careful measurement of the intensity of each atom column. These ‘atom counting’ results are often combined with prior knowledge about the nanoparticle morphology to generate a best fit model to the data, an example of which is illustrated in Figure 3b [37]. Whilst these methods often require strong assumptions to be made about the particle morphology in order to generate a 3D model, they are advantageous in requiring, in principle, only a

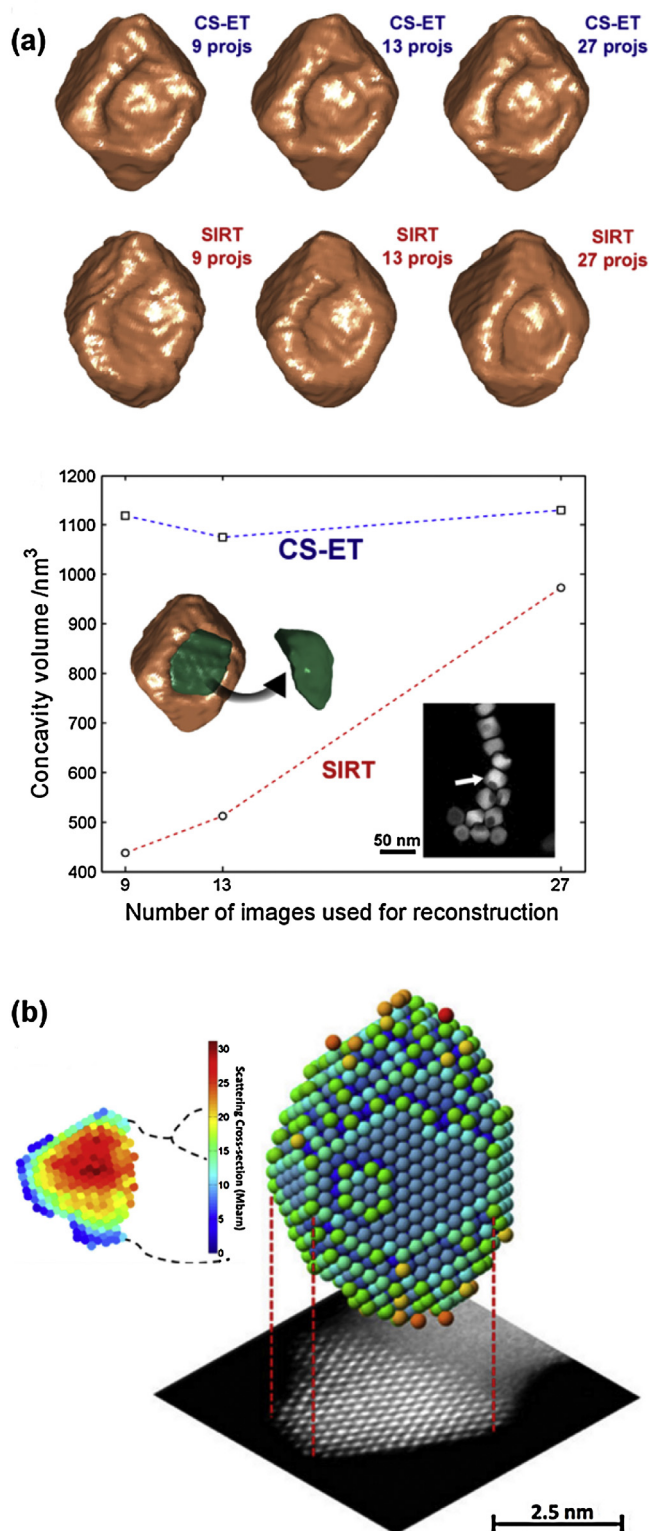


Figure 3. (a) (Top) Surface renderings of the reconstructed 3D morphology of an iron oxide nanoparticle, obtained using CS-ET or the conventional SIRT algorithm, and using reduced subsets of the tilt series images. (Bottom) The fidelity of the reconstructions further assessed by analysing the reconstructed concavity volume, which is quantified consistently using CS-ET. The nanoparticle analysed is indicated by the arrow in the inset ADF-STEM image [31]. (b) 3D reconstruction of the atom positions in a platinum nanoparticle, obtained by quantifying the image to determine the number of atoms in each projected atomic column and using the results in prior knowledge constrained molecular dynamics relaxation [37].

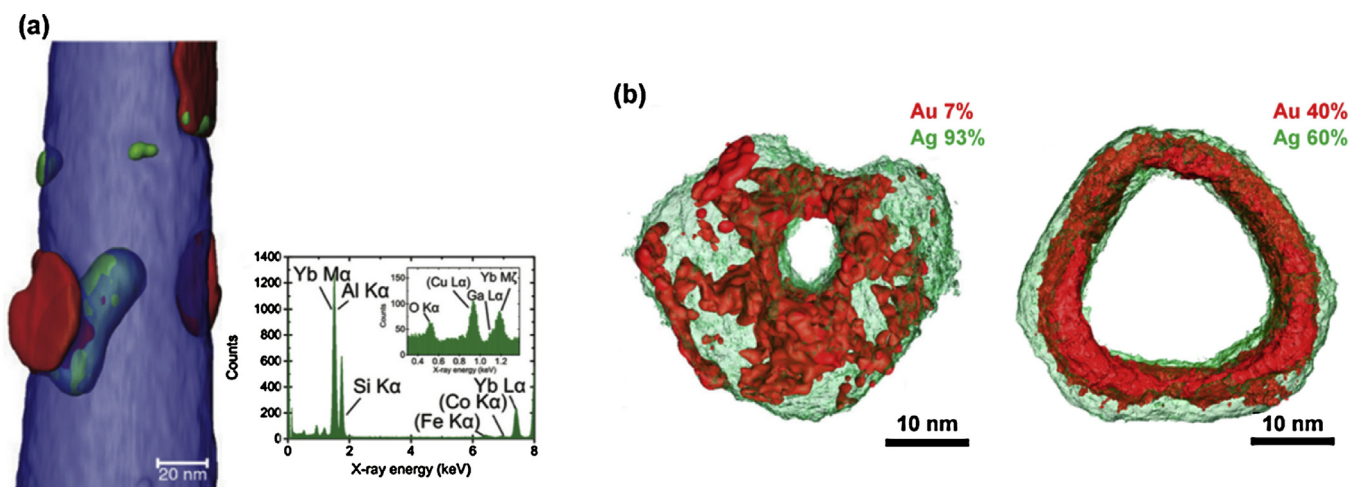


Figure 4. (a) (Left) Surface rendered 3D elemental maps of yttrium (green), silicon (red) and aluminium (blue) from an Al-5 wt%Si alloy (6100 ppm Yb), obtained from STEM-EELS and STEM-EDXS tomography; (right) local EDX volume spectrum obtained from the yttrium-rich precipitates [39]. (b) Surface rendered 3D elemental maps of Ag-rich (left) and more Au-rich (right) bimetallic hollow nanoparticles, revealing irregular Au surface segregation in the former compared to uniform Ag surface segregation in the latter [42].

single image and therefore offer the prospect of analysing many nanoparticles and tracking changes in 3D atomic structure over time. The availability of aberration-corrected microscopes has also enabled single-atom catalysts to be developed [21,22].

5. Chemical MDEM

The point-wise recording of spectroscopic signals in STEM, to form ‘spectrum-images’ (Fig. 2c), is most commonly performed with EDX spectroscopy (EDXS) and EEL spectroscopy (EELS), providing 2D real space maps with a third dimension containing spectral information. Both EDXS and EELS have benefited recently from advances in detector technology, thus facilitating faster more efficient recording.

For the case of EDXS, large solid angles (ca. 1 sr) are now available for X-ray collection, often with multiple detectors placed around the sample. Extending such analysis to 3D using tomographic acquisition, the geometry introduced recently by the FEI Company, in which four detectors are symmetrically disposed about the sample, is especially advantageous. To avoid serious shadowing artefacts however, one pair of detectors must be used when tilting clockwise, the other pair when tilting anticlockwise [38].

In EELS, the correction of higher order aberrations in the spectrometer optics has led to larger collection angles and thus improved efficiency, and the increase in read-out speed and improved versatility in how the CCD chip is used has led to very fast acquisition times, ca. 1000 spectra per second.

These new spectrometers and detectors have facilitated rapid, high quality 2D mapping of chemical properties, but have advanced also spatially resolved spectroscopy in three dimensions, as shown in Figure 2e. Indeed, if both EDX and EEL spectra are recorded simultaneously and employed for tomographic reconstruction, as recently undertaken by Haberfelner et al. [39], one can readily appreciate the abundance of data and the analytical flexibility afforded by such multi-dimensional microscopy.

Such 4D spectrum-tomographic data sets can be processed in two ways. Firstly, the spectrum-image data at each tilt in the series is used to generate a tilt series of 2D chemical maps (e.g. [39,40]), and therefore to reconstruct 3D chemical maps for each element. Alternatively, it is possible to reconstruct the volume at every energy increment, thus preserving the full spectrum at each voxel, a method sometimes known as ‘volume-spectroscopy’. The principle of the technique was first demonstrated using parallel beam

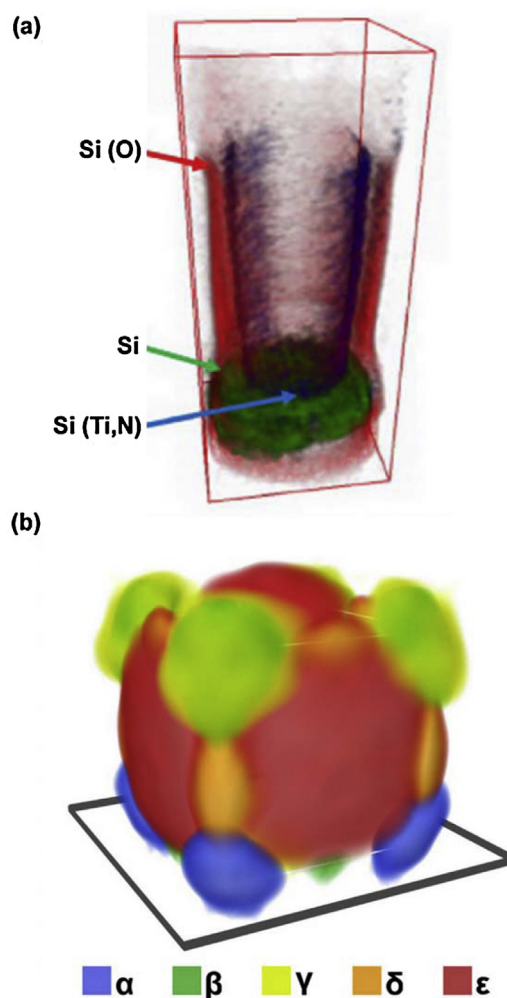


Figure 5. (a) Volumetric chemical state mapping, from STEM-EELS tomography, of silicon in a W-to-Si contact from a semiconductor device [40]. (b) 3D rendering of surface plasmon modes of a silver nanocube, reconstructed using STEM-EELS tomography, NMF and CS-ET [45].

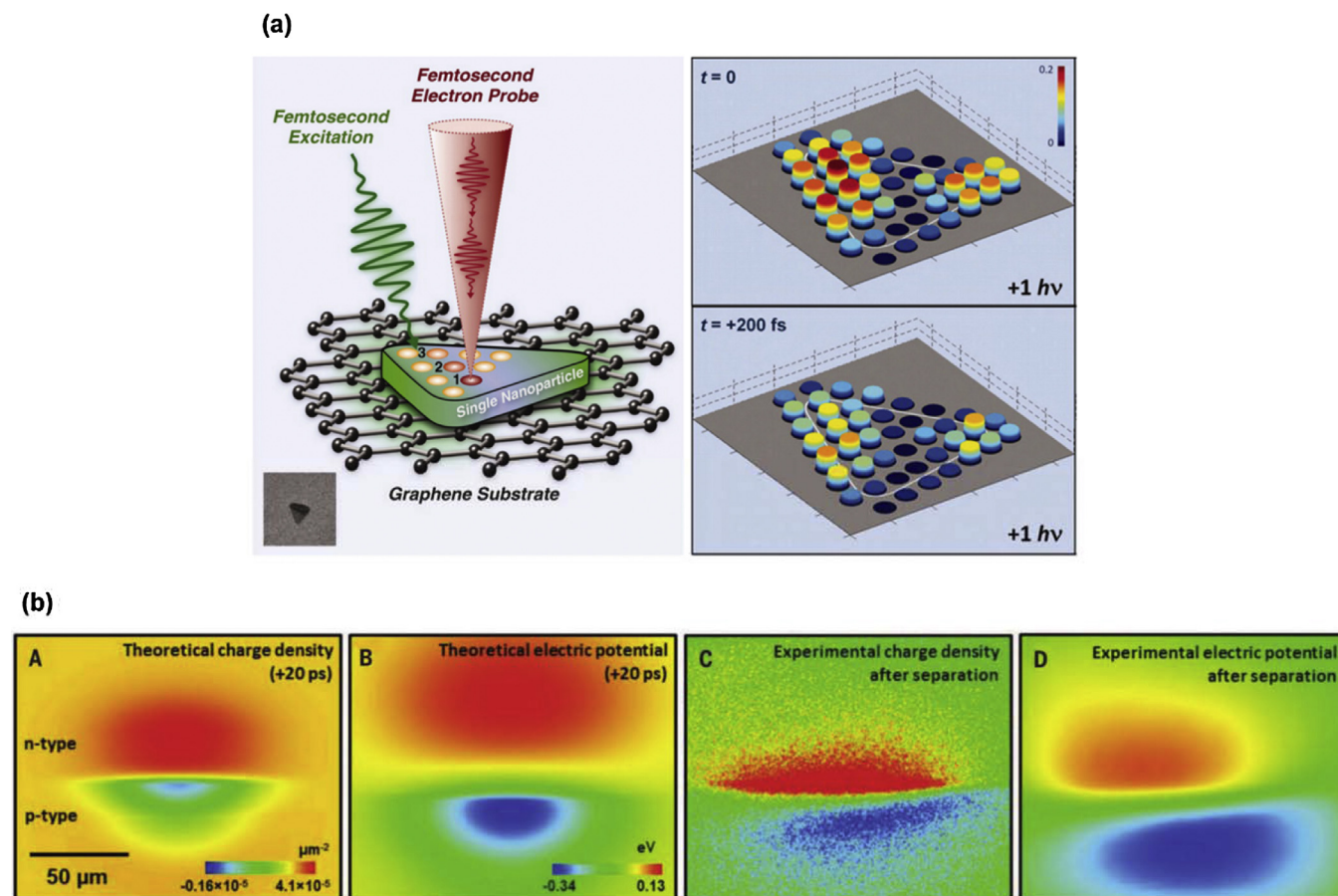


Figure 6. (a) Ultra-fast spectrum imaging of a silver nano-triangle, mapping plasmon field decay via STEM electron energy-gain spectroscopy [48]. (b) 4D (temporal) SEM imaging of carrier interface dynamics in p–n junctions [51].

energy-filtered TEM [25], has more recently been implemented in STEM mode [39,41], and a comparison of the two approaches has been investigated recently [39] (see Fig. 4a).

The pertinence of spectral tomography in understanding and optimising nanoscale catalysts is exemplified in Figure 4b, where elemental segregation within individual bimetallic silver-gold hollow nanocatalysts is revealed by STEM-EDXS tomography [42]. This firm evidence of a transition from Au surface segregation (Fig. 4b, left nanoparticle) to Ag surface segregation (Fig. 4b, right nanoparticle) as Au content increases enabled interpretation of changes in catalytic performance in a three-component coupling reaction among cyclohexane carboxyaldehyde, piperidine and phenylacetylene.

The very nature of chemical MDEM, and the acquisition of multiple spectroscopic signals, leads to a huge amount of data recorded for each experiment. As such, an increasingly important aspect of the analysis is how best to handle such ‘big data’ sets and to optimise data reduction. As we mentioned before, MSA is being increasingly adopted by the EM community to tackle this data problem. In chemical MDEM, for each spectrum-image, an MSA decomposition leads to a series of basis spectra and loading ‘maps’ whose pixel intensities are the weighting coefficients. For PCA, perhaps the most common approach, the basis spectra are ranked (as a scree plot) in terms of the variance (or information content) contained within each basis spectrum – ideally it is then clear which basis spectra to keep and which to discard as ‘noise’. For ICA, the decomposition provides components that are statistically independent (or uncorrelated) and in favourable cases, can lead to physically interpretable components, which

resemble phase-specific EDX spectra, and loadings that appear to yield readily interpretable maps of the different phases present in the specimen [43].

Complex devices in, for example, the semiconductor industry may have the same element (e.g. silicon) incorporated in different forms with associated differing electronic and chemico-physical properties. EELS-based chemical MDEM is sensitive to those properties via information encoded in the shape of the ionisation edges seen in core-loss spectra. As an example, Figure 5a shows a 3D map of the different bonding states of silicon (elemental, oxide, silicide) seen in a semiconductor device [40], differentiated through a least-squares matching of edge shapes with EEL spectra from a reference data set. Likewise, the sensitivity of catalytic performance to exact chemical species (and valency) on the catalyst surface requires 3D chemical mapping with nanoscale (or better) resolution, and core-loss EEL spectrum tomography meets that requirement. As a recent example, the 3D surface chemistry of ceria nanoparticles was investigated using ‘chemical shifts’ of the Ce M_{45} edge energy seen in EEL spectra and combined with ET reconstruction to show the 3D distribution of Ce^{3+} and Ce^{4+} ions [41].

The optical properties of excited metal nanoparticles are governed to a large extent by the phenomenon of localised surface plasmon resonances (LSPRs) and the associated local enhancement of induced electromagnetic fields. That enhancement, which can be orders of magnitude, has led to many possible applications including plasmonic solar cells, enhanced photocatalysis, single-molecule sensors and surface-enhanced Raman spectroscopy [44]. The precise interplay between the field enhancement and the nanoparticle shape and size was explored in a recent EELS-based tomography

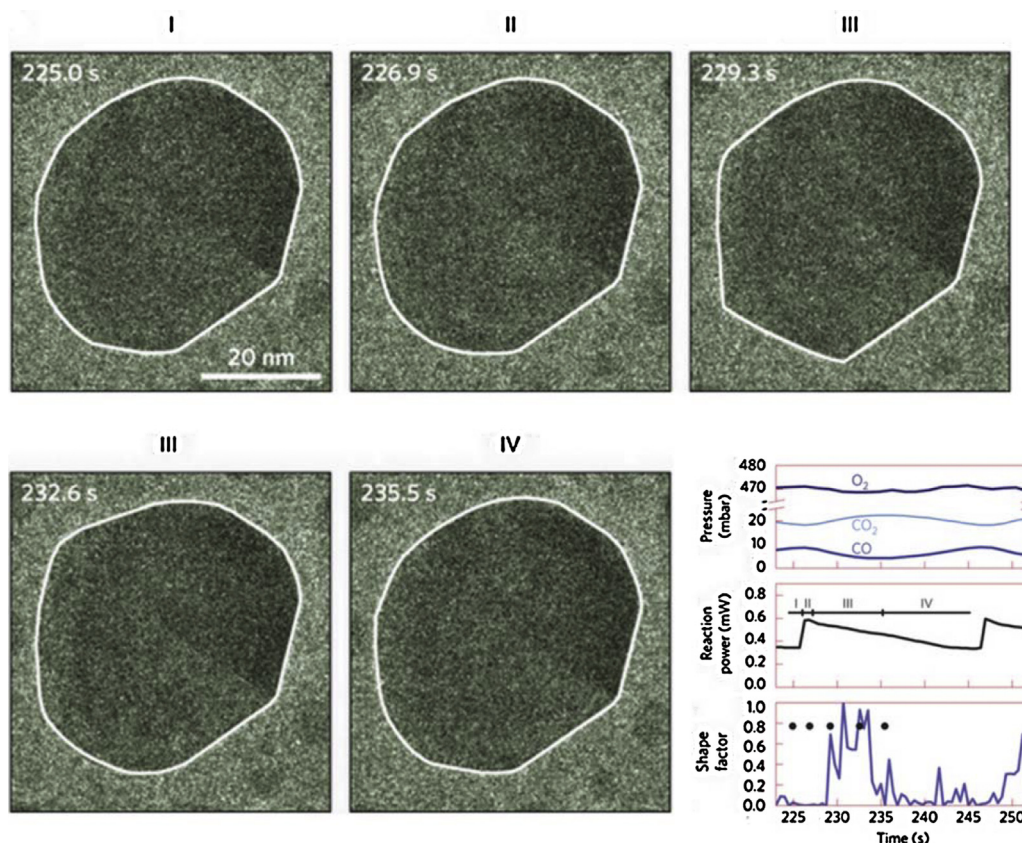


Figure 7. Correlation of reaction power and platinum nanocatalyst shape via in situ TEM, wherein the nanoparticle is seen to fluctuate between rounded and faceted morphology, related to periodic changes in CO oxidation [53].

study of a silver nanocube [45]. Figure 5b shows how by using a tilt series of EELS spectrum-images it is possible to map in 3D the individual LSPR modes. To extract the modes, the EELS data set, acquired with high energy resolution (ca. 100 meV) using an electron-energy monochromator, was decomposed using NMF (which imposes positivity) from which the basis functions and loadings can be interpreted as individual LSPR mode spectra, and their corresponding real space excitations, respectively. Through a number of valid approximations, the loading maps were interpreted as projections of a mode-specific induced potential and a full 3D reconstruction was possible using CS-ET methods.

Here we note in passing the development of a new STEM instrument with a novel monochromator for EELS [46] that provides an energy resolution of ca. 10 meV (comparable to that possible using the Zewail pump-probe approach [9–11]), coupled with aberration-corrected optics to maintain a small electron probe diameter and high electron current density. This high energy resolution opens up the possibility of undertaking vibrational spectroscopy at high spatial resolution, with the potential for studying both lattice vibrations in crystals and molecular dynamics, providing deep insights into fundamental chemico-physical behaviour. Combined, ultra-high resolution EELS and aberration-corrected imaging electron microscopically should, in principle, test the validity of the theoretical (computed) model for the selective nanocluster $\text{Cu}_4\text{Ru}_{12}\text{C}_2$ anchored on silica and described by Bromley et al. [47].

6. Time-resolved MDEM

MDEM incorporating a time dimension (Fig. 2d) and the application of stimuli to specimens in situ (such as heat, reactive gases,

liquid environments or deformation) offers the opportunity to track dynamic processes with nano- or atomic-scale spatial resolution. The aforementioned use of stroboscopic pump-probe methods by Zewail and co-workers can provide access to the ultra-fast (femtosecond) regime and therefore a whole range of significant transient phenomena in chemical physics contexts [9–11]. Figure 6a, for example, shows the sub-particle spatio-temporal mapping of the decay of the LSPRs of a silver nano-triangle [48], mapped in this case by electron energy-gain spectroscopy (EEGS). Unlike the LSPR study in Figure 5b, with such high temporal resolution it is possible to reveal directly the plasmon wave, how it interacts with the nanoparticle boundary, and the formation of the resonance. Zewail et al. have also evaluated elegantly by their 4D EM both the biomechanics of DNA structures [49] and illuminated our knowledge of amyloid formation [50].

Ultra-fast EM may be implemented also in a scanning electron microscope (SEM), and a recent study revealing carrier dynamics at the interface of a p–n junction warrants particular mention [51] in revealing a completely new model for carrier motion. After carrier photoexcitation the conventional drift-diffusion model greatly underestimates the true extent of carrier motion at very short timescales. The carrier positions indicate an initial ballistic transport with velocities of $\sim 10^6 \text{ ms}^{-1}$ and furthermore only minority carriers were seen to cross the junction leading to a gated localisation of charges. As shown in Figure 6b, the distribution of charge density (and associated electric potential) around the p–n junction is on a length scale of tens of μm after only 20 ps. Such distributions should take microseconds to form, based on conventional models, highlighting the power of high temporal resolution for revealing new dynamic phenomena. Ultra-fast EM has also been combined with tomography to study time-resolved 3D motion of a multi-walled carbon nanotube [52].

The study of non-repeatable (chemically irreversible) processes constitutes another distinct variant of MDEM, prominent aspects of which are the investigations of growth processes and catalytic reactions in situ. Dedicated environmental (S)TEMs include sophisticated differential pumping systems that allow the electron beam to propagate in high vacuum except for the small volume around the sample. Alternatively, novel MEMS-based holder design has produced ‘nanoreactors’, which incorporate thin membrane windows that separate the sample environment from the high vacuum environment of a conventional electron microscope.

Figure 7 highlights a recent example of a time-resolved in situ study to better understand the changes in the structure of catalytic nanoparticles in response to changes in the local gaseous environment. In this case, as the Pt nanoparticle converts CO to CO₂ in the presence of oxygen, the nanoparticle transforms from a spherical to a more faceted shape. As the CO conversion decreases, the nanoparticle transforms back to a more spherical shape. This process repeats to generate a remarkable structural oscillation that is intimately coupled to the CO conversion [53].

7. Crystallographic MDEM

As many technologically important chemico-physical systems are crystalline in nature, techniques that can map the local crystallography in two and three dimensions are of great value. By recording electron diffraction patterns at every pixel in a real space scan of the region of interest, a 4D data set is recorded that enables the local symmetry, orientation and phase to be determined in an automated fashion, with nanometre resolution.

As a first example, scanning convergent beam electron diffraction (CBED), with nm-sized probes, was used to study local symmetry fluctuations in the relaxor ferroelectric $(1-x)\text{Pb}(\text{Mg}_{1/3}\text{Nb}_{2/3})\text{O}_{3-x}\text{PbTiO}_3$ (PMN-PT) [54]. These systems are known for having complex domain structures (of the order of a few tens of nanometres) yielding tweed-like contrast in TEM images. The scanning CBED results provided direct experimental evidence for variations in ionic displacements and fluctuations in local polarisation at a scale much finer than any compositional variations found using EDXS.

Analysis of crystal orientations in polycrystalline and multiphase systems can now be accomplished routinely using the automated crystal orientation measurement (ACOM) method [55], in which electron diffraction patterns, often in precession mode to increase the extent of reciprocal space explored, and often using a convergent beam but with a modest convergence angle, are compared to a library of simulated patterns to facilitate rapid orientation determination. The high speed of the scan (100 frames per second is possible) ensures that the ACOM method can be used not only for metals, ceramics and semiconductors, but also as a ‘low-dose’ technique for beam-sensitive organic materials, even without cryo-cooling [56], and to monitor, for example, lithium migration in the re-chargeable lithium-ion battery material LiFePO_4 [57]. Conventional imaging and spectroscopy may struggle to identify the changes in the lithium content directly, but the lattice variations introduced by the loss (or gain) of lithium, make phase identification straightforward.

Nanocrystalline metals exhibit a range of chemical and physical properties that are different from, and, depending on the application, often superior to their bulk counterparts. An effective method to produce such materials is electrodeposition which can lead to a remarkable structural hierarchy in which nanocrystalline grains are arranged within mesoscale ‘colonies’. Figure 8a shows scanning diffraction (ACOM) maps at the boundary between two of these colonies [58]. The method is able to determine with high accuracy the orientation of each nanocrystalline grain, establish a clear

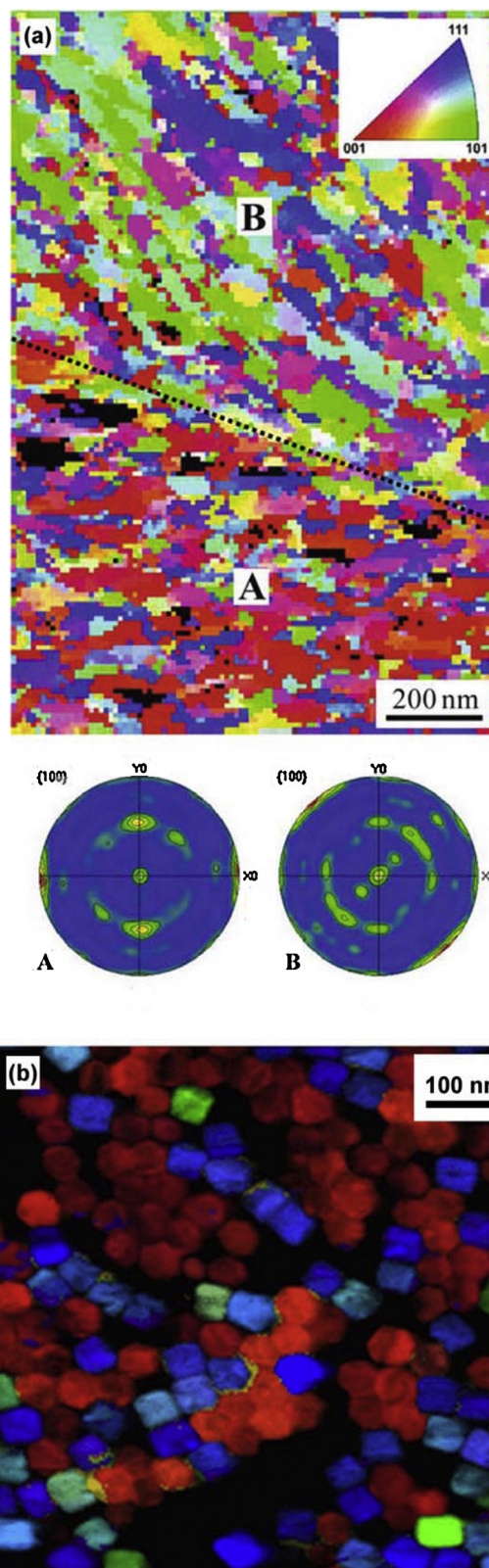


Figure 8. (a) ACOM orientation map of electrodeposited Ni-Fe nanoscale grains showing a distinct boundary between regions A and B, corresponding to two mesoscale ‘colonies’. The {100} pole figures indicate a pronounced fibre texture, with the $\langle 001 \rangle$ fibre axis approximately parallel to the local direction of grain elongation in both regions [58]. (b) ACOM orientation map of NaYF_4 nanoparticles. The hexagonal nanoparticles shown as ‘red’ correspond to an orientation with the c axis normal to the sample plane, those that are green and blue correspond to orientations with the c axis parallel to the sample plane [56].

fibre texture and thus the crystallographic relationship between the colonies.

Upconversion – the generation of radiation of higher energy from that of lower energy – often involves use of transition metal, lanthanide, or actinide ions doped into a solid-state host. Upconverting nanocrystals have attracted considerable attention recently due to their potential use as fluorescent targets for biological imaging. NaYF_4 , one of the most studied systems for this application, crystallises in two forms: cubic and hexagonal. By using ACOM each individual nanoparticle crystal structure can be identified and the orientations determined, see Figure 8b [56].

However, such crystallographic MDEM can be extended even further, firstly coupled to in situ studies to provide information as a function of time and/or strain, and secondly to provide an additional spatial dimension to yield genuine 3D crystallographic data. In situ crystallography at the nanoscale is especially important in order to study grain growth and the complex interplay of stress and microstructural changes. In Figure 9a we show examples of orientation maps which are essentially snapshots of nanoscale grain growth as a function of strain [59]. In this case a nanocrystalline gold film, prepared by magnetron sputtering, has undergone tensile strain and the deformation process understood by examination of grain size, grain orientation and rotation and twinning/detwinning both over a micron-sized area and locally using selected grains.

Whilst such orientation maps encode large amounts of crystallographic data, in reality most specimens are heterogeneous in three dimensions and so such maps are at best, projections of a complex 3D microstructure. There is therefore a pressing need to extend this analysis to incorporate the third spatial dimension. One approach [60], applied as a proof-of-principle to polycrystalline aluminium, is to use a series of hollow cone dark-field (HCDF) images in which, by varying systematically the cone and Azimuthal angles, grains with different orientations (and thus scattering to different parts of reciprocal space) can be differentiated and their orientation determined. By repeating at successive tilts, a full 3D orientation map may be reconstructed, see Figure 9b.

Rather than recording a tilt series of DF images, which is not particularly dose-efficient, an alternative approach is to instead record a tilt series of diffraction patterns. Such ‘diffraction tomography’ has been used to collect 3D diffraction data on small crystallites in order to determine the crystal structure [61], in some cases combining also with independent real space tomography reconstructions to associate 3D morphology and crystal structure [62,63]. However, by scanning the beam using ACOM, acquiring diffraction patterns directly at every pixel, at every tilt, it is possible to recover directly the 3D changes in crystallography within a volume of material. In general, any recorded pattern from a 3D heterogeneous sample is likely to be a mix of patterns from more than one phase or grain. In order to separate the individual diffraction signals from such a mix, MSA can again be used, this time to decompose the data set into basis, or component, diffraction patterns and associated real space loading maps. The tilt series of loading maps can be used to reconstruct a 3D real space picture of the phase (or grain) distribution, whilst the component patterns provide the local 3D orientation information [64,65].

Although to date such crystallographic MDEM has been applied only to metallic alloy systems, it could be used to investigate crystalline or partially crystalline organic, hybrid framework and molecular crystals if the total dose is kept to a minimum [66–68] and efficient direct electron detectors employed. This would open up a new avenue of research to investigate the interplay of microstructure, crystallography and chemico-physical properties in these important systems.

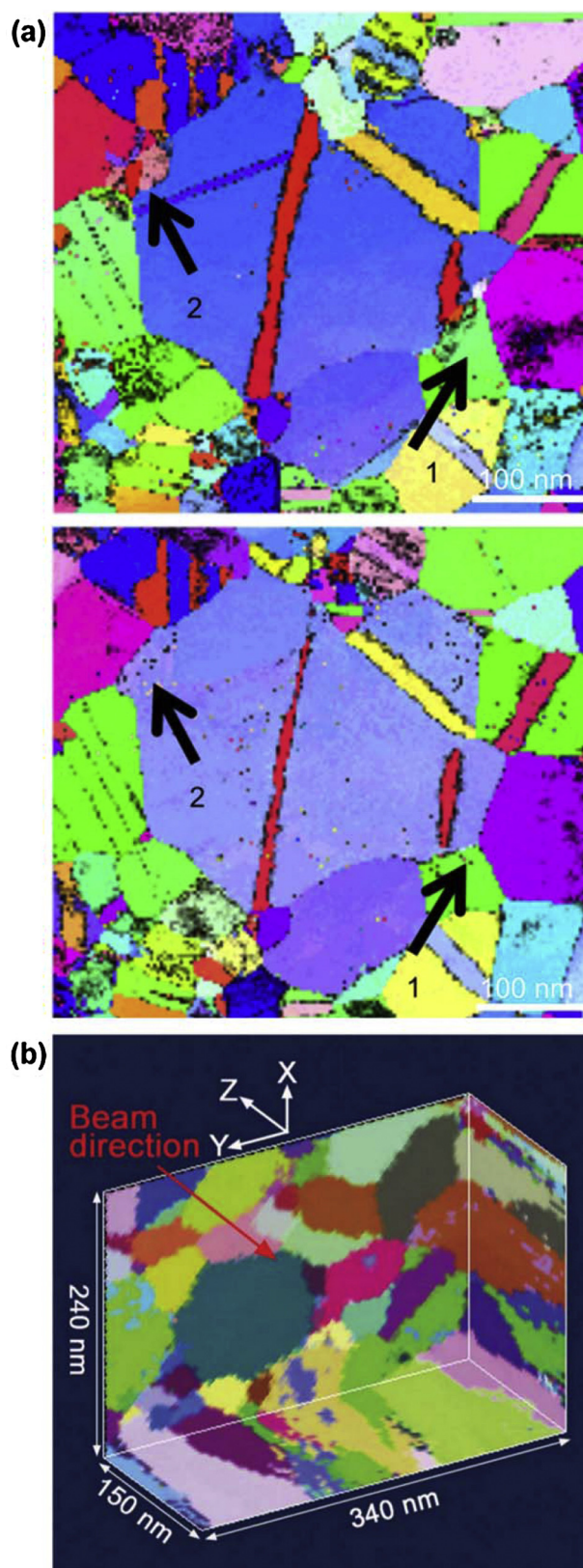


Figure 9. (a) Evolution of nanoscale microstructure under applied tensile stress. The top orientation map is before stress has been applied, the bottom one afterwards showing grain rotation and ripening phenomena. The colours represent crystal orientation [59]. (b) 3D grain-orientation map from a polycrystalline aluminium specimen, 150 nm thick, derived from many thousands of HCDF images. The colours here represent different crystal orientations with a tolerance of 2° [60].

8. Conclusions

The remarkable versatility of modern electron microscopy, coupled with the increase in computational power and sophisticated analytical methods, has led to the growth of multi-dimensional electron microscopy (MDEM). MDEM offers a way to investigate complex chemico-physical properties at the nanoscale by combining tomographic acquisition with spectroscopy, diffraction and time resolution. We have illustrated MDEM through a series of examples, highlighting the progress made and the novel information that can be gleaned from undertaking such experiments. The rise of MDEM is clear and the possibility to acquire increasingly vast amounts of data is growing. In this review, however, we highlight also one of the key problems that lies ahead, namely that of optimal data reduction and the need for rapid analysis. Unless we tackle that problem, we may indeed, as Eliot warns, through the increasing flood of data lose the ‘wisdom’ for which we strive.

Note added in proof

While this article was in press, we came across an innovatively novel paper by Chen, Alivisatos et al. (ACS Central Science, 2015, 1, 33–39), in which a generalized strategy is used to trace the trajectories of pairs and groups of nanocrystals moving in solution using in situ liquid phase TEM – see also Yuk, Alivisatos et al., Science, 2012, 336, 61–64.

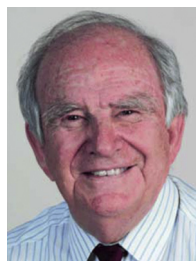
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