Analysis of Beam-Sensitive Materials by Electrons and X-rays

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Abstract

Structural and chemical analysis by electron and x-ray beams is compared, with emphasis on organic specimens and the limitations to spatial resolution that are imposed by radiation damage.

Key Words: TEM, x-ray, radiation damage, dose-limited resolution.

1. Introduction

Both electrons and x-rays are widely used for materials characterization, the atomic structure of most crystalline materials having been determined from diffraction of hard x-rays using laboratory sources or (with higher spatial resolution) synchrotron x-rays. Although x-ray beams cannot be focused down to atomic dimensions, diffractive imaging (recovering the phase of the diffracted electrons) allows the structural and morphological analysis of more complicated objects such as macromolecules. Near-edge (NEXAFS, XANES) and extended (EXAFS) fine structure of x-ray absorption edges helps further to determine the atomic structure of non-crystalline materials. Soft x-rays (often in the water window, 280 - 540 eV photon energy) can be focused by a zone plate, allowing chemical and elemental and analysis of light-element materials, including organic structures.

The transmission electron microscope (TEM) enables electron diffraction and direct imaging down to atomic resolution, the de Broglie wavelength being of subatomic dimensions for accelerating voltages above 10 kV. The TEM can also incorporate various forms of spectroscopy, including electron energy-loss spectroscopy (EELS) where ionization edges in the range 100 - 2000 eV energy loss are the equivalent of x-ray
absorption edges and can be used for elemental quantification or (via their fine structure) the determination atomic and electronic structure.

Various practical considerations influence the choice of electrons or x-rays. Hundreds of TEMs in the USA alone are capable of atomic resolution and many of these are equipped with EELS capability. State of the art TEMs (with aberration correction and a monochromated electron source) cost several million dollars and tend to be found in regional or national centers. Synchrotron x-ray sources are even more expensive, and there are a few dozen worldwide. X-ray measurements often take several hours whereas TEM images, diffraction patterns or spectra are usually recorded in seconds or minutes, in part because the goal of high spatial resolution makes specimen or beam drift more significant, although this drift can often be compensated electronically.

Soft x-rays can be focused to 20 nm but only with about 5% efficiency, whereas electrons with energy greater than 60 keV can be focused with high efficiency to atomic dimensions, or even down to 50 pm in an aberration-corrected instrument. Because of these small lateral dimensions and the thin specimen required, the TEM is the instrument of choice for analyzing very small volumes (TEM-EELS can detect less than $10^{-19}$ g of some elements) whereas x-rays are often better at measuring low concentrations. Although focused-ion-beam (FIB) instruments have eased the situation, the requirements for a thin (<1µm) TEM specimen are limiting for some materials and the ability use a microns-thick specimen in a synchrotron beam line makes the examination of materials in gaseous or liquid environments easier. However, environmental cells are also possible in the TEM and have recently become more practical, thanks to MEMS techniques and the possibility of liquid encapsulation in graphene (Wang et al., 2014).

Although electrons have the advantage in terms of focusing, many specimens are beam-sensitive and the spatial resolution of analysis is determined not by the optics of the system but by radiation damage. This damage can take various forms, depending on the nature of the specimen.

2. Radiation damage

X-rays and electrons are both forms of ionizing radiation and give rise to ionization damage, also called radiolysis. In this process, energy is removed from the incident beam
through inelastic interaction and the potential energy of the specimen is increased irreversibly. Incident electrons that are scattered inelastically by valence electrons lose typically 10 - 50 eV per inelastic event, while inner-shell excitation (important in heavier elements) can absorb hundreds of eV. For organic specimens, the mean energy loss is around 40 eV per inelastic event, with K-shell excitation accounting for around 20% of that value. Valence-electron excitation is largely a collective (plasmon) process but the plasmon decays rapidly, transferring its energy to single electrons and generating secondary electrons that travel through the specimen and cause further damage through inelastic scattering, accounting for 80% of the damage in polymethyl methacrylate.

In the case of x-rays, the main inelastic process below 10keV photon energy is the complete absorption of photons through the photoelectric effect. The absorbing atom or molecule is excited to a higher-energy state and may not return to its original ground state, indicating damage. However, the main radiolysis damage comes from the photoelectrons that are generated, with energies of several eV or even thousands of eV, which travel through the specimen, suffer inelastic scattering and create further damage.

A common assumption concerning radiolysis is that the amount of damage is proportional to the energy deposited in the specimen through inelastic interaction. This assumption is reflected in the measurement of radiation dose $G$ in Grays (Gy):

$$ G = \frac{D}{\rho} \frac{E_{av}}{\lambda_i} $$

Here $D$ is the fluence (incident electrons or photons per m$^2$), $\rho$ is the density in kg/m$^3$, $\lambda_i$ is the average distance (in m) that an electron travels before inelastic scattering (or a photon before absorption) and $E_{av}$ is the average energy exchange (in J) per inelastic or absorption event. For soft x-rays ($E_{av} = 500$ eV), the absorption length $\lambda_i$ is typically 1 µm in an organic material ($\rho = 1$ g/cm$^3 = 1000$ kg/m$^3$), giving $G$ (in MGy) = (0.08)$D$, where the fluence $D$ is in photons/nm$^2$. Electron microscopists employ a thin specimen and for convenience they measure dose as the fluence $D$. In Eq.(1), $E_{av}$ can be specified in eV if $D$ is expressed in C/m$^2$. For a typical organic or biological specimen, $E_{av} \sim 40$ eV and $\lambda_i = 100$ nm for 100keV electrons, giving $G$(MGy) = (0.4) D(C/m$^2$).

The dose needed to destroy the molecular structure of an organic specimen (the so-called critical dose, $G_c$) varies from a few MGy for aliphatic amino acids to about $10^5$
MGy for halogenated copper phthalocyanine. As a typical value, we will take $G_e = 40$ MGy (corresponding to $D_e = 10^2 \, \text{C/m}^2$ for 100keV electrons), which is also roughly the value for polymethyl methacrylate (PMMA).

In a conducting material, radiolysis is effectively quenched; the high density of free electrons ensures that holes created as a result of electron excitation are filled before the atom or molecule has time to reorganize. Therefore metals and semiconductors should not be damaged by x-rays. However, high-energy electrons carry appreciable momentum and high-angle elastic scattering by atomic nuclei can transfer several eV or tens of eV to each nucleus, giving rise to knock-on displacement damage. In the interior of a crystal, this damage takes the form of displacement of atoms from lattice sites, producing interstitial atoms and vacancies. At the surface of a specimen, fast electrons can displace atoms into the surrounding vacuum (electron-beam sputtering) or along the surface (radiation-enhanced surface diffusion). The knock-on effect takes place only if the maximum energy transfer (in a 180-degree collision) exceeds the so-called displacement energy, implying a threshold incident energy below which displacement does not occur. In organic materials, these thresholds are often below 100 keV, so the TEM does produce knock-on damage but the characteristic dose is of the order of $10^6$ MGy. In other words, the rate of knock-on damage is several orders of magnitude below that of radiolysis and can normally be neglected for organic specimens (Egerton, 2013).

3. Dose-Limited Resolution

For robust specimens (metals, some semiconductors and insulators) the spatial resolution of x-ray or electron-beam analysis is determined by the focusing optics. But for a radiation-sensitive specimen, including most organic and biological specimens, radiation damage imposes a more severe limit, known as the dose-limited resolution (DLR). The dimension $\delta$ of the smallest specimen region from which a useful signal can be obtained ($\Delta N$ recorded electrons or photons, above to the background level $N_b$ in neighboring pixels) depends on the required signal/noise ratio: $\text{SNR} = (\text{DQE})^{1/2} (\Delta N/N_{\text{shot}})$, where $N_{\text{shot}}$ is the shot-noise component, equal to $(N + N_b)^{1/2}$ according to Poisson statistics, and the detective quantum efficiency DQE takes account of noise added by the detector. The number of recorded particles is $N = F \, D \, \delta^2$, where D is the incident fluence and F is the
ratio of recorded to incident particles. Combining these equations, the dose-limited resolution $\delta$ is given by:

$$\delta^2 = \frac{N}{(FD)} = (\text{SNR})^2 \left( \frac{\text{DQE}}{C^2+3C+2}/C^2 \right)$$  \hspace{1cm} (2)$$

where $C = (N - N_0)/N_0 = \Delta N/N_0$ is the contrast between the recorded pixel and its neighbors, which is negative in the case of absorption contrast. Usually SNR is taken as 3 or 5 (Rose criterion) and for an ideal detector, where DQE = 1, Eq.(2) can be simplified to $\delta = (5/|C|)(FD/2)^{-1/2}$ for $C << 1$ (low-contrast image) and to $\delta = 5 (FD)^{1/2}$ for $C >> 1$ (the feature of interest is absent in adjacent pixels).

In the case of soft x-ray absorption spectroscopy or imaging in the water window ($E_{av} \sim 500$ eV, $\lambda_i \sim 1$ $\mu$m), a critical dose of $G_c \sim 40$ MGy translates into about 500 photons/nm². Assuming a 1$\mu$m-thick specimen and using data for PMMA at the oxygen K-absorption edge, $C = -0.52$ and $F = 0.19$; then if we take DQE = 0.5, Eq.(3) gives $\delta \sim 11.5$ nm for oxygen mapping. This result would indicate that radiation damage is not resolution-limiting in scanning-transmission x-ray microscopy (STXM), where the zone plate forms a focused probe of diameter > 20 nm the specimen. But in a fixed-beam mode with a zone plate of 5% efficiency between the specimen and detector, F is reduced to 0.01, giving $\delta = 52$ nm and indicating that resolution is now dose-limited.

The scanning transmission electron microscope (STEM) employs an annular dark-field detector to record dark-field images from electrons scattered through larger angles, for which the probability is relatively low. Even with an optimized detector, F in Eq.(2) is small, giving typical $\delta$ in excess of 4 nm for $G_c \sim 40$ MGy (Egerton 2013).

The fixed-beam TEM often operates with an objective aperture that gives diffraction contrast by absorbing scattered electrons. With increasing specimen thickness, the DLR improves initially (due to an increase in contrast C) but then gets worse because F falls due to electron absorption at the objective aperture. In the case of a boundary in a polymer ($G_c = 40$ MGy) where the density changes by 10%, for example, low kV gives better resolution for a very thin specimen but $\delta < 10$ nm requires a thicker specimen and higher accelerating voltage (Egerton, 2014).

To obtain better resolution from an organic sample, the objective aperture can be replaced by a phase plate that changes the phase of the scattered electrons relative to the
unscattered ones. With an ideal phase plate, Eq.(2) predicts near-atomic resolution (for 10% density change) for specimens thicker than 100 nm, even in a beam-sensitive specimen ($G_c = 40$ MGy) (Egerton, 2014). Phase plates currently suffer from charging and contamination problems but are gradually being improved (Glaeser, 2013a). It might in future be possible to extract high-resolution phase information in STEM mode, by recording diffraction information from each pixel and using ptychographic processing (Sader et al, 2010; D’Alfonso et al, 2014).

The understanding of many biological processes depends upon knowing the three-dimensional structure of macromolecules such as proteins. Howells et al. (2009) have calculated the dose required for 3-dimensional imaging (with SNR = 5) based on x-ray diffraction and compared this with the dose required for damage at the same resolution. Their data (see Fig. 1) showed that 10 nm resolution is obtainable from a single voxel; better resolution requires averaging over many identical units, for example by preparing a crystalline sample of protein.

![Figure 1. X-ray dose (crosses) and electron dose (open circles) required for SNR = 5 from a voxel of protein ($\rho = 1.35$ g/cm$^3$, $\lambda_i = 100$nm, $\lambda_e = 300$nm) of dimension $\delta$ (Howells et al., 2009). The dotted line represents damage doses measured by x-ray and electron diffraction; best resolution corresponds to its intersection with the dashed and solid lines.](image-url)
In Fig. 1 we show also the required dose of electrons, estimated from Eq.(2) assuming that a fraction \( \delta/\lambda_e \) of electrons are diffracted from each voxel (\( \lambda_e \) = elastic MFP = 300 nm) and detected with DQE = 1. Because of the strong diffraction of electrons, the required dose is substantially less than for x-rays (Henderson, 1995) and 1.5nm resolution becomes possible from measurement on a single molecule. Still better resolution is obtainable from a crystal, which explains the success of cryoelectron microscopy, based on phase-contrast imaging of frozen-hydrated materials. Longer-range effects of radiation damage (specimen warping and shrinkage) can be problematical but fast-readout direct-recording detectors have helped to alleviate this problem (Glaeser, 2013b).

Atomic resolution by direct imaging is not possible with x-rays because of the focusing problem, so structure must be determined from diffraction from a crystalline specimen. But some proteins cannot be crystallized and in response to this problem, very short (<100fs) spatially coherent pulses generated from a free electron laser have been employed in diffract-before-destroy measurements. A diffraction pattern (sufficient to determine specimen orientation) is obtained from a particle (macromolecule or small crystal) before it damages, then patterns from many orientations are combined to give sufficient resolution (< 0.5 nm) to determine protein structure (Spence et al., 2012).

Could a similar diffract-before-destroy technique work with fast electrons? One difference is that electrons have appreciable momentum that can create knock-on damage. But as remarked earlier, such damage may be negligible compared to ionization damage. More serious is the fact that electrons carry electrostatic charge, so Coulomb repulsion within the incident beam limits the current density and provides a trade-off between the number of electrons per pulse and the pulse duration. Radio-frequency electron sources can produce 100fs pulses with more than \( 10^5 \) electrons (Li et al., 2010; Muro’oka et al., 2011) and at these high current densities the primary repulsion comes from space charge rather than stochastic repulsion. The space-charge effect is reduced at higher electron energy because the electrostatic repulsion is largely compensated by magnetic attraction of the moving electrons. Ideally, space charge can be compensated by refocusing (Kruit and Jansen, 1997) but solenoid focusing appears to be limited to several \( \mu m \) for 2.5MeV electrons. If negligible damage occurs within 100 fs and if ten electrons must be diffracted from a single particle of diameter \( d = 10 \) nm, then 10 = \( (d/D)^2(10^5)(d/\lambda_e) \) and the beam diameter D needs to be no more than 200 nm. Use of a field-emission electron...
source, combined with the correction of spherical and chromatic aberration, might make phase-contrast (coherent) imaging possible on a sub-ns time scale (Armstrong et al., 2007; Reed et al., 2009) but this is clearly a major engineering challenge.

4. Diffractive Imaging

For observations of subtle structural changes in nano-objects, in particular aperiodic objects at femtosecond time scales, diffractive imaging is attractive because it requires no imaging lenses. Lens aberrations and beam crossovers (which can degrade temporal resolution) are thereby avoided and the spatial resolution is then diffraction- or dose-limited. In diffractive imaging, a coherent beam of electrons or x-rays strikes an object and generates one or more diffraction patterns, which are used to reconstruct an image via an iterative feedback algorithm (Weierstall et al., 2002). To achieve reliable reconstruction, a highly coherent incident beam is required because the technique uses wave interference to generate high-contrast interference patterns. Coherent waves must be generated at the source (photocathode, field emitter synchrotron, etc.) and the beam must maintain coherence until diffraction.

Coherence of x-ray or electron waves is usually defined by a temporal (longitudinal) coherence length: \( \lambda_L = \frac{\lambda^2}{\Delta \lambda} = \frac{vh}{\Delta E} \) and a spatial (transverse) coherence length: \( \lambda_T = \frac{\lambda}{2\pi \theta} \), where \( \lambda \) is the de Broglie wavelength, \( v \) is the electron or photon speed, \( h \) = Planck constant; \( \Delta E \) and \( \theta \) are the energy and angular spread of the beam, respectively (De Graef, 2003). It has been shown that the coherence width of the incident beam needs to be approximately twice the lateral width of the object to be imaged (or the unit cell, in the case of a crystal), Shannon sampling having twice the spatial period of Bragg sampling. As the coherence width is reduced, the size of the Bragg peaks in reciprocal space grows until they overlap, leading to a reduced image resolution. The longitudinal coherence length is the distance over which two waves from the same source point with slightly different wavelengths \( \Delta \lambda \) will arrive completely out of phase. Small energy spread (small \( \Delta \lambda \)) is required to minimize loss of interference along the wave propagation direction and the loss of information due to the spread of information on detector pixels, especially when high energy x-ray or electrons are used to extend the diffraction limit (Spence and Howells, 2002; Spence et al., 2004).
For a third-generation synchrotron x-ray source, such as at the Advanced Photon Source, the typical angular spread of the beam is $2 \times 10^{-6}$ rad. If we assume a monochromated beam with a wavelength of $\lambda = 0.1$ nm and $\Delta \lambda / \lambda = 10^{-5}$, the longitudinal coherence length is about 5 $\mu$m and the transverse coherence length about 25 $\mu$m. In comparison, the ultrafast electron diffraction (UED) apparatus at BNL, equipped with an RF gun operated at 2.8 MeV and the best of its kind for time resolved experiments with electrons, has $\sim 10^5$ electrons per pulse and time resolution $\sim 100$ fs (Zhu et al., 2006). With a beam size of 300 $\mu$m on the sample, the temporal coherence length of the beam is about 2 nm and the spatial coherence length about 10 nm, likely insufficient for time-resolved diffractive imaging and at least 1000 times poorer than the coherent beams at the third-generation undulator source now routinely used for coherent diffractive imaging. In the case of an ultrafast electron microscopy (UEM) setup with a photocathode replacing the FEG gun in a commercial transmission electron microscope, it was estimated that a spatial coherence length $10^{-4}$ of the size of an object might be able to generate diffraction patterns with sufficient quality to reconstruct an image of the object (Piazza et al., 2014). Again, the damage problem is reduced if the specimen consists of a large crystal (Gao et al., 2013).

5. Secondary Damage

Although a complete absence of radiation damage is desirable, it may not be essential and depends on the resolution required. Secondary damage effects involving atom motion over more than atomic distances (e.g. diffusion) occur on longer time scale. Such processes include radical diffusion (taking ns or longer) and conformational or crystal relaxations, often requiring microseconds (Warkentin et al., 2011). They have recently been investigated by x-rays using detectors operating at ms timescales (Warkentin et al., 2013), while for electrons, direct-recording detectors can operate at 400 frames/sec (Li et al., 2013). Therefore a combination of fast (but not ultra-fast) detection and low specimen temperature may allow measurement before complete destruction has occurred, outrunning at least part of the damage. Many conformational changes in biological samples (including proteins) occur in the microsecond to millisecond time domain, suggesting that longer pulses could be used to determine secondary and tertiary structure, even if the primary (atomic) structure of the molecule is damaged.
The existence of secondary damage can give rise to a dose-rate dependence of damage, whenever the timescale of a damage process approaches or exceeds that of the measurement. Dose-rate dependence is commonly observed for inorganic materials where the dose rate (electron or x-ray fluence) can be relatively high because of the lower radiation sensitivity. There can even be a dose-rate threshold, below which no damage is observable (Salisbury et al., 1984; Jiang and Spence, 2012). Dose-rate dependence is not usually observed for organic materials, except in an aqueous environment where longer-range radical diffusion contributes secondary damage (Cherezov et al., 2002) or at low temperatures where diffusion is slow (Egerton and Rauf, 1999).

Dose-rate effects are designated as normal or inverse, depending on whether the radiation sensitivity (damage/dose ratio) increases or decreases with increasing dose rate. Both effects may take place in the same specimen; for example, the sensitivity may first decrease as the dose rate increases, due to limited diffusion speed, but then increase at high dose rate because of specimen heating by the beam (Egerton and Rauf, 1999).

The existence of a dose-rate effect could influence the choice of imaging mode. In fixed-beam microscopy, the dose rate is constant whereas in scanning microscopy (STEM, STXM), the dose rate within a focused probe is high during the pixel-dwell time but then falls to zero (for that pixel) during the remainder of the frame time. Scanning mode could therefore be advantageous if the specimen shows inverse dose-rate effect.

References


