Journal of Electron Spectroscopy and Related Phenomena 170 (2009) 1–3

Contents lists available at ScienceDirect

Journal of Electron Spectroscopy and Related Phenomena

journal homepage: www.elsevier.com/locate/elspec

Editorial

Introduction: Special issue on radiation damage

1. Microscopy with ionizing radiation

Radiation damage sets the ultimate limit on information that can be obtained in microscopy using any ionizing probe, whether it be VUV light, charged particles, or X rays. In fact, early pioneers in electron microscopy were worried that their struggle to apply the new instrument to biology might be defeated by radiation damage. Writing in the preface to László Márton's book *Early History of the Electron Microscope* [1], Dennis Gabor summarized the initial beliefs of the pioneers, as well as the outcome of the struggle, in the following words:

> The first pioneers believed as I did that if not everything, almost everything would burn to a cinder under the electron beam. So Knoll and Ruska started with a few things that would not "burn": wires of platinum and tungsten; and Marton impregnated his first organic preparates with osmium. "Let it burn, but let us look at the cinder." The great discovery dawned on them gradually, in the course of several years. The electron microscope is similar to the light microscope in its basic design, but entirely different in its action, that is to say in the way it forms contrast in the image. Absorption played almost no part. The main source of contrast was scattering and the first to recognize this fact clearly was Bill Marton. He was also the first to open thereby the way to the biological applications of the electron microscopee [2]. But even this was not the end of the surprises, because in very thin objects where even scattering was too weak, phase contrast took over.

From a modern perspective, we might summarize the above by saying that for atomic resolution imaging, electron microscopes often exploit an interference between elastically scattered and unscattered electrons, while at longer length scales a refractive-like phase shift due to the inner potential is exploited [3]. Damage arises from the fact that, depending on the accelerating voltage and material under study, several inelastic scattering events occur per elastic scatter, and these inelastic events deposit 30–60 eV of energy each.

With X-ray photons, the situation is somewhat different. Especially at photon energies below about 10 keV, photoelectric absorption dominates over elastic scattering, so that scattering-based contrast for single atom imaging is exceedingly challenging. Indeed, back in 1970, Breedlove and Trammel [4] pointed out that the energy deposited per elastically scattered electron is only a few times 10^2 eV, while the energy deposited per elastically scattered ångstrom-wavelength X ray is in the range of 10^6 eV; since bonds are broken at energies of about 10^1 eV, this seemed to doom the prospects for atomic resolution microscopy of single organic molecules [4], a conclusion that was later echoed by Henderson [5]. Of course a frequently employed solution is to divide the required dose for high resolution imaging among many identical molecules, such as is done in X-ray crystallography or in single-particle electron microscopy, or in proposed schemes for molecular structure determination using X-ray free-electron lasers [6]. In fact, today's X-ray microscopes work not at atomic resolution but at length scales of tens of nanometers, where single atom scattering is not the relevant parameter; instead, one considers absorption and phase contrast in a more standard optical model, and at these length scales and for micrometer or thicker samples X-ray microscopes often offer significant advantages of lower radiation dose for equivalent resolution images (see [7,8], or for calculations that include phase contrast in X-ray microscopy see [9,10]). A similar story applies to X-ray microprobes, which offer significantly lower dose per detection sensitivity relative to other approaches [11–13].

2. Radiation dose

Radiation damage effects depend directly on the dose, which is the amount of ionizing energy absorbed per unit mass. Using dose, rather than energy per area or some other measure, one can successfully translate photoresist exposures between electron and X-ray beams, and observe similar degrees of molecular dissociation in microscopy and crystallography experiments (see for example [14]). While some older literature reports dose in units of 100 ergs per gram or Rad, the SI unit is Joules per kilogram = Gray (1 Gray = 100 Rad). In radiation biology, an additional correction factor for relative biological effectiveness or RBE is added so that one has exposures in Sieverts = Gray · RBE or REM = Rad ·RBE. However, RBE $\simeq$ 1 for both X-rays and electrons so RBE is usually ignored in favor of dose.

How much radiation dose does it take to destroy on average one bond per molecule in organic materials? The energy of a H–C bond is 80.9 kcal/mol, or 3.50 eV/bond. However, some bonds undergo recombination, and the excitation energy of other bonds in organic materials is higher. A better measure of molecular damage is provided by the G factor in radiation biology, which is the number of bonds broken per 100 eV of absorbed ionizing radiation. G factors of about 5 are common for many organic materials, so it takes more like 20 eV per atom to break a molecular bond. For carbon, this easily translates into a dose in Gray:

$$\frac{(20\,\mathrm{eV/atom})\cdot(N_A\,\mathrm{atoms/mol})\cdot(1.6\times10^{-19}\,\mathrm{J/eV})}{(12\,\mathrm{g/mol})\cdot(10^{-3}\,\mathrm{kg/g})} = 1.6\times10^{8}\,\mathrm{Gray} \quad (1)$$

In fact, this dose is quite close to the dose at which atomic resolution diffraction spots are seen to fade in protein crystallography exper-

0368-2048/$ – see front matter
doi:10.1016/j.elspec.2009.01.004

iments, indicating a reduction in bond-to-bond electron density correlations.

In reality, radiation exposures are often measured at an experimental site or specified in the literature not in terms of dose but in the form of incident particle fluence, or the time-integrated number of incident particles per unit area in the exposure. To convert the dose to Gray, one needs sample-dependent information. We provide expressions below (Eqs. (4) and (5)) that allow such conversions to be made easily.

The dose delivered to a sample illuminated by charged particles (such as in electron microscopy) can be obtained from the Bethe formula, the general form of which is ([3], Eq. 5.79):

$$\left|\frac{\mathrm{dE}}{\mathrm{d}x}\right| = \frac{e^4 N_A}{4\pi \in_0^2 E\beta^2}\overline{\left(\frac{Z}{A}\right)} \ln\left(\frac{\mathrm{E}\beta^2}{2\mathrm{J}}\right) \quad (2)$$

Here E is the incident particle energy, e is the charge on the electron, N_A is Avagadro's number, ϵ_0 is the permittivity of free space, Z and A are the atomic number and weight, respectively, β is the ratio of the particle velocity to the velocity of light and $J(\simeq 13.5\bar{Z}\,\mathrm{eV})$ is the mean ionization energy per atom. If the incident particle is an electron with an energy less than about 200 keV, then, in practical units, we have ([3], Eq. 10.2):

$$\left|\frac{\mathrm{d}E_e}{\mathrm{d}x}\right| = 7.8\times 10^4 \overline{\left(\frac{Z}{A}\right)} \frac{1}{E_e} \ln\left(\frac{E_e}{J}\right) \ \mathrm{eV}/\left(\mu\mathrm{g/cm^2}\right)/\mathrm{electron} \quad (3)$$

where energies are now measured in eV and distances in cm. We may regard Eq. (3) as expressing the energy deposited per unit mass (in eV per μg) by an electron fluence of one electron per cm^2. In other words it gives us the dose per unit fluence. Changing units to Gray (J/kg) for the dose, and electrons per $\mu\mathrm{m}^2$ for the incident fluence I_0, we finally have:

$$\mathrm{Dose\,(Gray)} = 1.25\times 10^3 I_0 \overline{\left(\frac{Z}{A}\right)} \frac{1}{E_e} In\left(\frac{E_e}{J}\right) \quad (4)$$

This expression is valid for conventional electron microscopes. However, for very high-energy electron microscopes (> 300 keV), the $1/E_e$ decrease of the dose per fluence levels off and is replaced by an energy-independent behavior characteristic of minimum-ionizing particles. It is also noteworthy that the dose per fluence does not depend on the density of the sample. This is because, for a given volume, both the energy deposited and the mass are proportional to the number of atoms in the volume. Finally, for organic materials at 100 kV a rough equivalence between fluence and dose is $1\,e^-/\mathrm{nm}^2 \simeq 3.2\times 10^4$ Gray [10].

The papers in this issue mainly concern X-ray microscopy. As noted before, at energies below about 20 keV photoelectric absorption completely dominates over energy deposition due to inelastic or Compton scattering. It is therefore quite straightforward to use the Lambert-Beer law $I = I_0 e^{-\mu t}$ to calculate the dose at depth t in terms of the linear absorption coefficient μ (μ^{-1} is also called the absorption length). Using tabulations of absorption length μ^{-1} in $\mu\mathrm{m}^{-1}$ such as those provided from Henke et al. [15] (available on the Center for X-ray Optics, Lawrence Berkeley National Laboratory web site www-cxro.lbl.gov/optical_constants), one can translate a fluence of I_0 into radiation dose as:

$$\mathrm{Dose(Gray)} = \frac{I_0 h\nu}{\mu^{-1}\rho} = 1.602\times 10^{-4}\frac{I_0(\mathrm{photons}/\mu\mathrm{m}^2)\cdot h\nu(\mathrm{eV})}{\mu^{-1}(\mu\mathrm{m})\cdot\rho(\mathrm{g/cm^3})} \quad (5)$$

where $h\nu$ is the energy per photon and ρ is the density. It should be noted that the absorption length μ^{-1} is inversely proportional to density ρ, so that dose depends simply on atomic absorption rather that density. Finally, this expression gives the so-called *skin dose* or dose on the first surface exposed to the X-ray beam; the dose deeper in the sample will be lower due to attenuation of the beam by overlying material, and furthermore it is based on the conservative assumption that no energy is carried off by photoelectrons escaping from the irradiated material.

3. Cryo methods to mitigate dose effects

Radiation damage sets significant limits on the study of room temperature specimens, especially if imaged in a hydrated state (some examples are shown in [16]). In 1974, Taylor and Glaeser demonstrated that cryogenic conditions could dramatically reduce the detrimental effects of radiation damage in electron crystallography [17] and microscopy [18] experiments. Cryo microscopy has long since become routine in many electron microscopy laboratories [19], where samples are rapidly frozen using plunging or high pressure freezing techniques prior to transport into the microscope. Cryo techniques have also become standard practice in protein crystallography (one review of common practices is provided by Garman [20]). In X-ray microscopy, cryo methods have been demonstrated by several groups [21–23]. While cryo methods are very effective in preventing mass loss [22] and the fading of diffraction spots, they do not function by preventing bond damage from happening. Instead, they function by locking the reaction products in position via a cage effect. As a result, they provide almost no improvement in the dose sensitivity of experiments measuring, for example, near-absorption-edge spectroscopic resonances in organic materials [24]. Thus cryo methods seem to halt the diffusion of radiation-produced free radicals and inhibit the loss of radiation-scissioned molecular groups, but the initial bond damage is not prevented.

4. The special issue: discussion

It is not our purpose here to review the now extensive literature on radiation damage and its effects, which has been done already by several authors [3,5,25–28]. There has also been a series of recent workshops on the subject, within the crystallography community, which have been reviewed by Garman and co-workers [29–32]. Rather, our purpose is to introduce the papers in this special issue of the Journal of Electron Spectroscopy and Related Phenomena and to advocate for more attention to the possible influences of damage in future imaging and spectroscopy experiments. Our experiences in processing articles submitted for the special issue have shown that the best studies were achieved when the possibilities of damage were considered before beginning the experiment. This means that routine measurement and recording of the parameters needed to calculate the dose after the experiment need to be planned and written into the standard experiment-control software from the start, and an accurate measurement of detector efficiency and other losses between the sample and detector needs to be obtained. The main reason this is so important is that questions as to whether damage may have played a role in a given result often only become evident after the experiment when attempts are made to analyze and understand the data.

Knowledge of the applied dose is always the starting point for investigating questions of damage to the sample. To allow comparison with the results of other investigators and to use the various assessments of the "maximum tolerable dose" D_{MT} that one can find in the literature, it is essential to express sample irradiation in terms of absorbed energy per mass, or dose in Gray. Using this measure, a compilation of results from both the literature and diffraction measurements made at the Advanced Light Source Synchrotron-Radiation Facility at the Lawrence Berkeley National Laboratory have been assembled by Howells et al. [14]. These results, from multiple radiation sources, were remarkably consistent and revealed the following useful and roughly linear relationship between D_{MT}

(defined as 50% fading of a diffraction spot) and the resolution d:

$$D_{MT}(\text{Gray}) \simeq 1.0 \times 10^8\, d(\text{nm}). \quad (6)$$

However, note that this relationship is followed most closely by dose effects resulting from changes in molecular bond correlations (for example in atom-resolution diffraction and crystallography, and spectroscopy techniques such as XANES and EXAFS); at longer length scales where mass preservation is the more relevant consideration, there is very little data available but there are some indications [22] that significantly higher doses can be tolerated.

5. Conclusion

The articles in this special issue deal with a range of phenomena involving the radiation damage that inevitably accompanies measurements made with ionizing radiation. We hope this special issue will help the community to understand radiation damage effects in greater detail and to appreciate the need for quantitative evaluation of their impact on any given measurement, as well as the need to mitigate their effect by improving the efficiency of experiments in terms of information acquired per unit dose.

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Available online 5 February 2009