Exploiting the Dose-Rate Dependence of Radiolysis – a Future for Cryo-STEM ?

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X-ray measurements have revealed an *inverse* dose-rate dependence of mass loss from protein crystals at a temperature of 180 K [1] and also gradual *dark progression*, indicating diffusion as the causal process. Diffusion appears to be frozen out at 100 K [2]. Radiolysis in the TEM should be similar except that specimen thicknesses are of the order of 100 nm rather than microns, suggesting that mass loss will be rapid at 180 K and measurable even at temperatures around 100 K.

Indeed, previous EELS measurements [3] have indicated that nitrogen, oxygen and carbon are released from 40nm collodion films at 90 K, with an *inverse* dose-rate dependence. Above 0.03 A/cm^2, the dose-rate dependence became *direct* (higher radiation sensitivity at larger dose rate), which was attributed to specimen heating by the 1μm-diameter electron beam. Computer simulations indicated that the temperature rise is greatly reduced (for the same dose rate) by using a smaller-diameter electron beam.

Figure 1 shows EELS measurements of the relative thickness: $t/\lambda = \ln(I_t/I_o)$ of a 30nm film of Formvar (polyvinyl-formal) held at 92 K, plotted as a function of irradiation time for beam diameters of 330 nm and 66 nm and two very different current densities. We have subtracted a constant from each thickness to allow for components (*e.g.* carbon) that are removed much more slowly [4, 5]. The mass loss is seen to depend on irradiation time rather than accumulated dose, indicating an inverse dose-rate dependence with a characteristic time of $\tau_c = 12$ s. If the mass-loss rate is limited by diffusion to the top and bottom surfaces of the specimen (thickness t), the diffusion coefficient can be estimated as $(t/2)^2/\tau_c = 8 \times 10^{-14}$ cm^2/s at T = 92 K, corresponding to 2.5×10^{-5} cm^2/s for oxygen diffusion at room temperature (assuming an activation energy of 0.2 eV) which seems reasonable [6].

Scanning transmission microscopy involves a high dose rate and a small probe diameter, suggesting less mass loss than with conventional fixed-beam TEM, for the same amount of extracted information. The STEM measurements shown in Figure 2 again indicate a reduction in radiation sensitivity (larger characteristic dose D_c) with increasing dose rate. If this advantage translates into reduced structural damage, and especially if phase contrast can be achieved efficiently, STEM could be beneficial for cryo-EM studies of beam-sensitive specimens. Since frame time >> pixel dwell time, dark progression is likely but its effect can be eliminated from image data by performing a single scan [7].

References:
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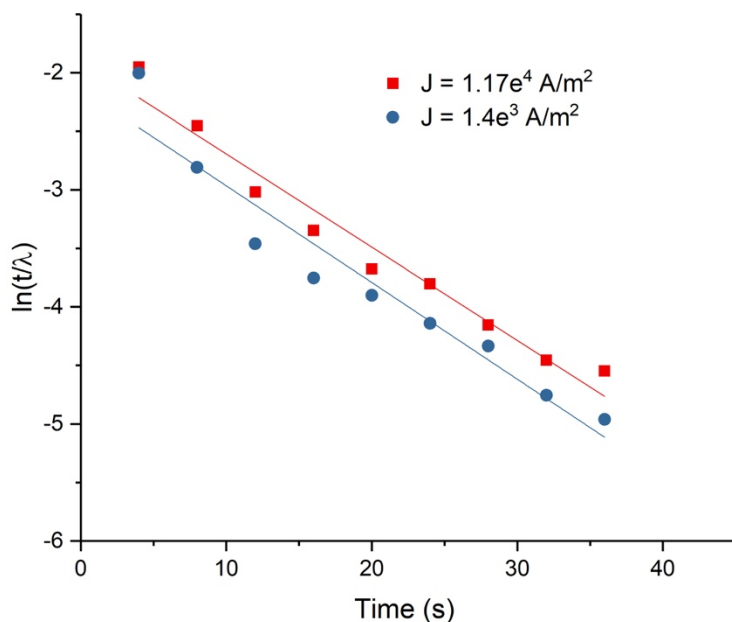[2] M. Warkentin et al., Acta Cryst. D **67** (2011), p. 792.
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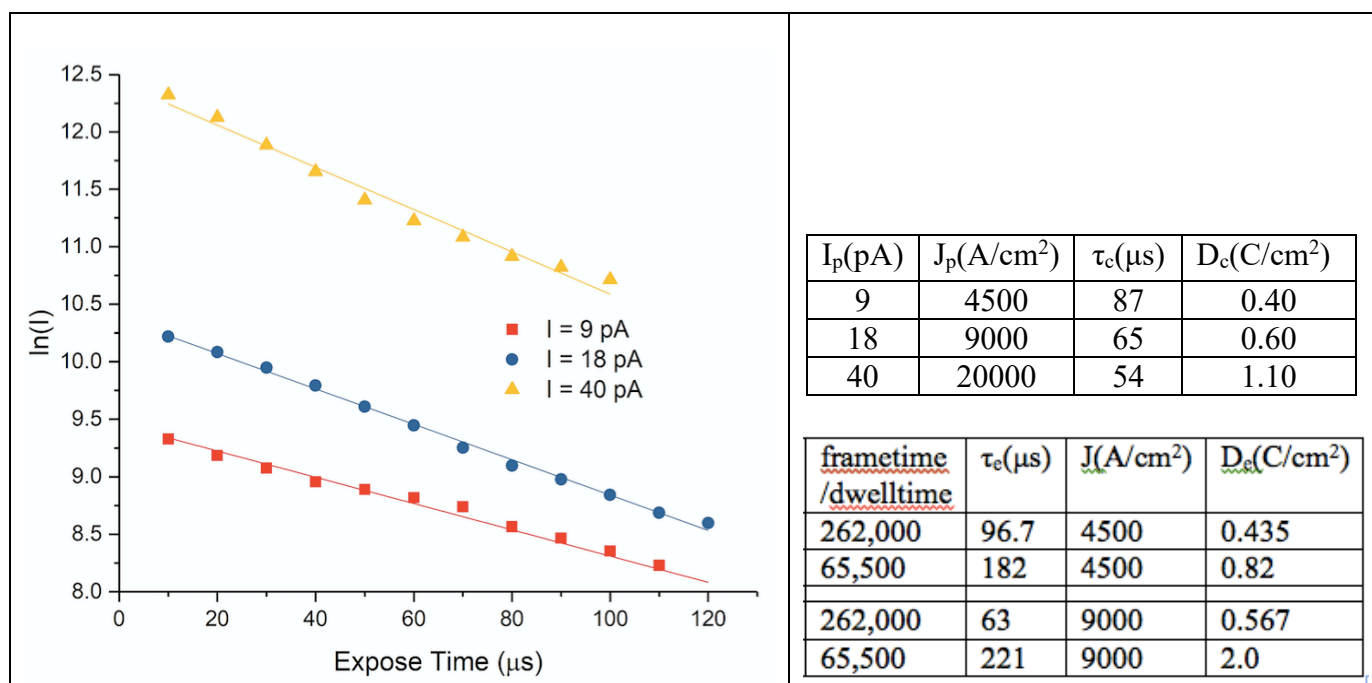[4] R.F. Egerton, J. Microscopy **118** (1980), p. 389.
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Figure 1. EELS thickness measurements for dose rates of 0.14 A/cm^2 (square data points, 330nm probe) and 1.17 A/cm^2 (triangles, 66nm probe). The slopes correspond to a characteristic time of τ_c = 12 s.

I_p(pA)	J_p(A/cm^2)	τ_c(µs)	D_c(C/cm^2)
9	4500	87	0.40
18	9000	65	0.60
40	20000	54	1.10

frametime /dwelltime	τ_e(µs)	J(A/cm^2)	D_e(C/cm^2)
262,000	96.7	4500	0.435
65,500	182	4500	0.82
262,000	63	9000	0.567
65,500	221	9000	2.0

Figure 2. Cryo-STEM (at 93 K) mass-loss measurements on Formvar, using a 0.5nm-diameter probe and 12 frames (512 × 512-pixel raster). The natural logarithm of the ADF signal is plotted against pixel exposure time. The Table shows probe current I_p, probe-current density J_p, characteristic time (τ_c = 1/slope on the graph) and the resulting characteristic dose D_c, which increases with increasing dose rate.