Contrast of carbon in low-voltage electron microscopy - Monte-Carlo simulation

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Abstract. Low-voltage transmission electron microscopy (LVEM) using very low accelerating voltage \( \leq 5\) keV for observation of low density materials (unstained biological samples and organic thin films) has the advantage of high contrast due to the high scattering efficiency of atoms at low electron energy. The disadvantage of LVEM is the very low transmittable sample thickness. To quantify the transmittance of the samples and the contrast formation, we simulated the interaction of an electron beam with a thin carbon film by our Monte-Carlo code. As the result of our calculations, we obtained the spatial distribution of the transmitted electrons in conditions like those in LVEM, and we were able to find the dependence of the image contrast on the thickness and density of the samples and on the size of the microscope objective aperture. The critical thicknesses for elastic, inelastic and both types of scattering at a density of 2.0 g/cm\(^3\) and energy 5 keV were 17.7, 10.3 and 6.3 nm, respectively. The calculated dependences of the electron microscopical parameters show the limits of sample thickness in LVEM

1. Introduction

Low-voltage electron microscopy (LVEM) was recently developed for observing unstained biological samples and organic thin films, and is able to work in transmission, scanning transmission and reflection modes [1]. The main advantage of LVEM5 (Delong Instruments, Brno, Czech Republic) consists in the use of an accelerating voltage around 5 kV with final light microscope magnification of an electron-microscopical image. It delivers nearly twenty times more image contrast enhancement than a high voltage electron microscope using accelerating voltage 100 kV [2]. In the case of biological specimens, staining procedures can therefore be omitted. On the other hand, such lowly-accelerated primary electrons are able to pass only through ultra thin sections, below 20 nm in thickness. Electrons accelerated by energy 5 keV are able to scatter intensively on atoms with a low atomic number including atoms of resins, which also participate in scattering and contribute to image formation. This means that the microscope is very sensitive to specimen thickness.

In the theory of image contrast formation in an amorphous material, an absorption contrast is assumed, which depends on the thickness of the sample and on its atomic number and mass [3]. The logarithmic contrast in electron microscopy is usually defined as \( C_0 = \log N_0/N \), where \( N_0 \) and \( N \) are numbers of electrons (intensities) incident to the sample and those transferred through the objective aperture after sample transmission, respectively. The contrast of the sample can be characterized by the mass-thickness contrast parameter \( S = N_A \sigma_t/A \), where \( N_A \) is the Avogadro number, \( \sigma_t \) is the total scattering cross-
section of atoms in the specimen, and $A$ is atomic mass. Then, we can write for $C_0 = \rho x$, $\rho$ being the sample density, $x$ the geometrical thickness of the specimen and $\Delta C_0 = S(\Delta \rho \Delta x)$, $\Delta C_0$ and $\Delta \rho$ being the contrast and density differences at given thickness, respectively. Linear dependence of the contrast on the film thickness can be assumed for low density materials up to a certain critical thickness, given by the onset of multiple scattering. The characteristic “critical thickness” is defined as the thickness at which the electrons proceed on an average one elastic or inelastic scattering event during sample transmission. Next, the image is also deteriorated by inelastic scattering of the electrons, which is non-localized and decreases the image resolution.

MC simulation of the transmission of electrons through thin films, most of them at energies of 20 keV or more, was studied in [4]. In the low-energy region (0.2 - 5 keV), the coefficient of electron backscattering and the angular and energy distribution was studied, e.g., in [5]. The elastic process is characterised by the elastic differential cross-sections (DCS), often calculated using a static field approximation with relativistic partial wave analysis of the atomic potential. The atomic potential can be modified by using a muffin-tin model for atoms in the solid state [6]. Inelastic DCSs are often calculated using the theory of the dielectric response of matter to electron excitation. The inelastic mean free path (IMFP) can be obtained using e.g., the TPP-2 or TPP-2M formula [7]. Tougaard’s Universal cross-section [8] was developed for practical estimation of inelastic differential cross-sections, enabling analytical calculation of the loss function of materials in the form

$$f(W) = \frac{BW}{(C + W^2)^{\frac{3}{2}}}, \quad (1)$$

$B$ and $C$ being constants, $W$ being the energy loss. The angular distribution of inelastically scattered electrons is sometimes omitted, because the scattering angles are much smaller than the scattering angles in the case of elastic collisions. However, in TEM the low angle scattering is important. This scattering mechanism can be described by two ways; the first is plasmon scattering, when the scattering angle $\theta_m$ is between a minimum value $\theta_p$ [9]

$$\theta_p = \frac{W_p (E + E_0)}{E^2 + 2EE_0}, \quad (2)$$

and a maximum value $\theta_{max} = \sqrt{2 \theta_p}$. At the second type of scattering, electron-electron scattering with larger energy loss ($W \geq 100$ eV), the scattering angles $\theta_c$ are larger and they are given by the formula [10]

$$\sin^2 \theta_c = \frac{W}{E}. \quad (3)$$

The aim of this paper is to show the relation between image contrast and specimen thickness, which is important for defining the sample thickness directly from electron microscopical observation, and to estimate the critical thicknesses for elastic and inelastic scattering in a sample, which characterize the possibility to observe biological samples under given conditions.

2. Computational Details

In our Monte-Carlo code, the elastic DCSs were calculated by the ELSEPA code [6] (Elastic Scattering of Electrons and Positrons by Atoms), using Dirac-Hartree-Fock with the muffin-tin atomic model. For the simulation the IMFP (in equations denoted as $\lambda_{im}$), we used values given by the TPP-2 formula or those obtained from our MC evaluation of absolute Elastic Peak Electron Spectroscopy. For calculating the energy loss distribution we modified Tougaard’s model of the Universal cross-section of electron inelastic scattering $f(E,W)$ [5], so we were able to find the values of $C$ for several electron energies between 0.1 and 5.0 keV. Then we found the functional dependence of $C$ on electron energy by regression. The approximate formula is $C = 571.64 \ln(E) - 2615.9 \{C[eV^2], E[eV]\}$, compared with our results; that, obtained by comparing with TPP-2 is slightly different. The former was used in our calculations. Energy loss functions calculated by this way have a reasonable shape with a maximum at about 20 - 30 eV. The amount of energy loss in an inelastic collision was obtained from the energy loss distribution by the standard Monte Carlo procedure. Then, the scattering angle was calculated either for
electron-electron scattering according to equation (3) or for plasmon scattering according to equation (2). The energy of bulk plasmon is \( W_p = 25.9 \text{ eV} \) [11].

The LVEM 5 electron microscope has working energy 5 keV and two objective apertures 30 and 50 \( \mu \text{m} \) in diameter (angular apertures 0.68 and 1.14 deg, respectively). The energy for the simulation was the same, and the values of the apertures sizes for calculation were taken as 0.62 and 1.2 deg, respectively. We studied pure carbon with density between values \( \rho = 2.34 - 1.60 \text{ gcm}^{-3} \). The hard limits of the calculation were set to 1 000 000 primary electrons. Below the energy of 0.1 keV the electron is practically stopped and is regarded as absorbed. The other details are as in usual MC codes.

3. Results and discussion

The MC results on electron transmission show an approximately exponentially decreased number of electrons without scattering with sample thickness, and the different geometrical thicknesses are given by three values of carbon density (figure 1). Figure 2 shows the change in logarithmic contrast \( C \) for electrons with output angle \( \vartheta \leq 11 \text{ mrad} \) with thickness. A very low degree of nonlinearity appeared for these dependences up to 60 nm. The dependences are very similar for the two models of scattering angle distribution for inelastic scattering.

Figure 1. Dependence of electron transmission through the film on film thickness. Figure 2. Dependence of logarithmic contrast on film thickness.

The characteristic “critical thicknesses” were obtained from the dependence on thickness of the relative collision number for electrons transmitted through the film (table 1). Because the density of a real carbon film is assumed to be higher than the density of the biological samples (about 1.6 gcm\(^{-3}\)), the calculated values should be the limits of microscopic observations in LVEM.

| DENSITY [g/cm\(^{-3}\)] | REGRESSION EQUATION FOR ELASTIC COLLISIONS | TRANSPARENT THICKNESS [nm] |
|-------------------------|------------------------------------------|-----------------------------|
| 2.34                    | \( y = 0.0001x^2 + 0.0614x + 0.0478 \)   | 15.0 8.9 5.5                |
| 2.00                    | \( y = 0.0001x^2 + 0.051x + 0.0683 \)    | 17.7 10.3 6.3               |
| 1.60                    | \( y = 2E-05x^2 + 0.0444x + 0.039 \)    | 21.4 12.8 7.9               |

The results showing the influence of objective aperture size are shown in figure 3. The logarithmic contrast \( C \) for all thicknesses scarcely depends on the objective aperture up to tens of milliradians, then it starts to decrease. This is due to high intensity in the zero collision peak, because
as late as at this objective aperture size the number of scattered electrons is comparable with the zero collision peak. Again, this result is the same for both models of angular distribution of inelastically scattered electrons. Finally, figure 4 shows the dependence of the cumulative electron number (not including the zero collision peak) of electrons transmitted through the film into the aperture. Only here did a difference appear between the models of the angular distribution of inelastically scattered electrons. The calculated thickness and the angular dependences of the contrast in figures 1 and 2 agree with the assumed shape at the energy and in the thickness range used here. The dependences in figure 3 show the limits of the increase in contrast due to decreasing objective aperture size.

4. Conclusion

Very good agreement with theoretical assumptions was found for the calculated angular distribution of elastically transmitted electrons in the given energy and thickness range. Testing by measurement is necessary, but we believe the agreement of the experimental and calculated thickness and aperture dependences of transmitted electrons can be improved by implementing other models of energy losses into the code.

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