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P1_4 The Importance of Absorption Corrections in Quantitative Transmission Electron Microscopy

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Abstract

This article discusses the effects that absorption has on quantitative transmission electron microscopy and assesses the validity of the assumption that such effects can be neglected if the specimen thickness is below 100nm. Calculations of the absorption correction factor are made for the example case of healthy soft tissue. The threshold thicknesses at which absorption becomes significant are found to be 149.9nm and 177.9nm for Carbon and Nitrogen respectively (with Oxygen as the comparison element). It is concluded that 100nm is an acceptable thickness assumption for a healthy tissue specimen based upon this evidence

Introduction

Transmission electron microscopes (TEMs) are commonly used for high resolution, high magnification examination of materials. A TEM fires a beam of powerful electrons at a thin sample; the variable transmission of electrons due to different element densities creates an image of the specimen. The addition of energy dispersive X-ray (EDX) detection systems can allow users to collect quantitative data about the chemical composition of a sample. EDX detection systems register the intensity of X-ray signals (generated by the interaction of the incident electron beam with atoms in the specimen) as a function of the X-ray energy. Each element has a unique set of X-ray energies so they can be easily identified from a spectrum. The intensity of each spectral peak is dependent upon the effects of atomic number, absorption and fluorescence, as well as specific TEM characteristics such as detector structure and electron beam energy. In many experimental situations, absorption and fluorescence are neglected, assuming that the sample is <100nm in thickness [1]. However the validity of this assumption for an experiment is rarely quantified [1] so would appear to be entirely arbitrary. This article seeks to determine whether or not this thickness assumption is valid in the case of absorption in a soft tissue specimen, where the correct chemical composition is important for medical diagnoses. In these calculations, absorption will be considered significant if $\geq 10\%$ [3].

Theory

The Cliff Lorimer k factor is commonly used when accuracy of quantitative data is important. It is a sensitivity factor which accounts for preferential detection of certain X-rays by the EDX system, as well as experimental conditions such as incident beam energy. It is defined [2] by the equation

$$\frac{C_A}{C_B} = k_{AB} \frac{I_A}{I_B} \quad (1)$$

where C_A and C_B are the concentrations of elements A and B in the sample, I_A and I_B are the detected X-ray intensities from elements A and B and k_{AB} is the Cliff Lorimer k factor. k_{AB} is dependent only upon the atomic number and TEM characteristics, since (1) was derived assuming that absorption and fluorescence are negligible for a thin sample [2].

X-rays produced by one element are absorbed by its surroundings; this can cause the detected intensities to be less than expected. The further the X-rays must travel the more likely they are to be absorbed; hence when samples are prepared for the TEM they are thinned as much as possible. The absorption correction factor (ACF) [3] is derived from the Beer-Lambert law which relates the incident X-ray intensity to the transmitted intensity. Applied to the Cliff Lorimer k factor from (1),

$$k_{AB}{}^* = k_{AB} \left[\frac{\int_0^{tcosec\alpha} e^{-(\mu/\rho)_{spec}^B \rho x} \, dx}{\int_0^{tcosec\alpha} e^{-(\mu/\rho)_{spec}^A \rho x} \, dx} \right] = k_{AB} \left[\left(\frac{(\mu/\rho)_{spec}^A}{(\mu/\rho)_{spec}^B} \right) \left(\frac{1 - e^{-(\mu/\rho)_{spec}^B \rho t cosec\alpha}}{1 - e^{-(\mu/\rho)_{spec}^A \rho t cosec\alpha}} \right) \right] \quad (2)$$

where the sections in square brackets are the ACFs. k_{AB}^* is an 'absorption corrected' k_{AB}, $(\mu/\rho)_{spec}^A$ and $(\mu/\rho)_{spec}^B$ are the mass absorption coefficients for elements A and B respectively in the specimen, ρ is the specimen density, x is the absorption path for a normal incident beam equal to *tcosecα* (where *t* is the thickness of the specimen) as defined geometrically in figure 1. The mass absorption coefficient of an element in a specimen is

Figure 1: Geometry used in (2)

$$\left(\frac{\mu}{\rho} \right)_{spec}^A = \sum_i w_i \left(\frac{\mu}{\rho} \right)_i^A \quad (3)$$

where w_i is the weight percentage composition of some element i in the specimen and $(\mu/\rho)_i^A$ is the mass absorption coefficient of X-rays from element A being absorbed by element i.
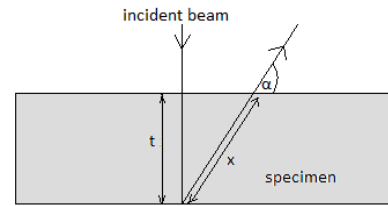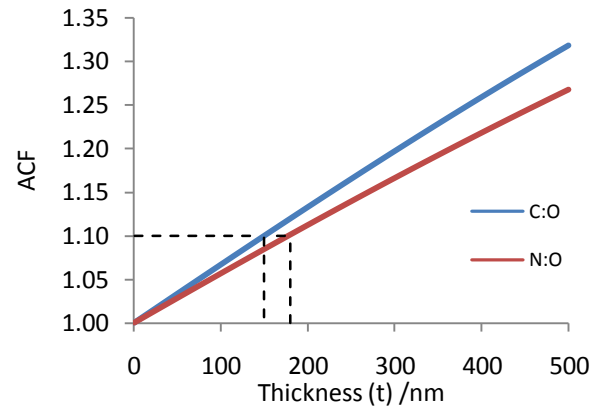
Results

Healthy soft tissue has weight percentages as follows [4]: Hydrogen (H) 0.101174, Carbon (C) 0.111, Nitrogen (N) 0.260, Oxygen (O) 0.761826. It is normal to use an abundant element as the comparison, labelled B in previous equations, so in this case oxygen shall assume this role. Using (3) and mass absorption coefficients for 200keV incident electrons and Kα emission lines [5], it is found that $(\mu/\rho)_{spec}^C = 4953.4 \, cm^2/g$, $(\mu/\rho)_{spec}^O = 2617.9 \, cm^2/g$ and $(\mu/\rho)_{spec}^N = 4600.9 \, cm^2/g$ (neglecting H as it is a very light

Graph 1: ACF as a function of thickness for soft tissue

element so difficult for TEM analysis [1]). Using the definition of the ACF in (2), and setting α to the maximum recommended angle of 10° [3] whilst varying *t* produces graph 1. The threshold thickness at which the ACF becomes significant (≥10% absorption [3]) for C:O is 149.9nm and for N:O, 177.9nm.

Conclusion

It has been determined that absorption effects for C:O and N:O in healthy soft tissue are only significant when the thickness exceeds 149.9nm and 177.9nm respectively. This is greater than the assumed thickness of 100nm, below which absorption effects are negligible. Therefore in this example calculation, it is reasonable to neglect absorption effects so long as the thickness does not exceed these threshold values. However, this threshold will change for other elements (with different atomic numbers and mass absorption coefficients) in different materials. In experimental TEM use, there is often a lot of uncertainty in thickness measurements due to specimen damage or preparation techniques; in these cases it would be advisable to include ACFs for greater reliability of results.

References

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