TRANSMISSION OF VERY SLOW ELECTRONS AS A DIAGNOSTIC TOOL

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Abstract

The penetration of electrons through solids is retarded by sequences of their interactions with the matter in which the electron changes its direction of motion and loses its energy. Inelastic collisions, the intensity of which reaches a maximum at around 50 electronvolts (eV) and drops steeply on both sides of this fuzzy threshold, are decisive for the penetration of electrons. Transmission microscopy (TEM or STEM) observes thin samples of tens to hundreds of nanometres in thickness by passing electrons of energies of tens to hundreds of kiloelectronvolts through them. The range below 50 eV has recently been utilized in the examination of surfaces with reflected electrons, where high image resolution is achieved thanks to the retardation of electrons close to the sample surface in the “cathode lens”. In this lens, the role of the cathode is played by the sample itself, biased to a high negative potential. This principle can also be utilized in the transmission mode with samples of a thickness at and below 10 nm. This method has recently been implemented and verified on graphene samples prepared by various methods. The results have made it possible to diagnose the continuity and quality of the graphene flakes. Furthermore, series of experiments have been performed involving the observation of ultrathin tissue sections with electrons decelerated to about 500 eV and less, where they provide an image contrast of the cell ultrastructure much higher than that provided by traditional microscopic modes.

Key words:

Electron microscopy, slow electrons, STEM, graphene, ultrathin tissue sections

1. INTRODUCTION

Since the beginnings of electron microscopy in the 1930s, the key parameter used to compare various types and trademarks of devices has been image resolution, although contrast mechanisms and the interpretation of micrographs have also been continuously investigated. In order to improve resolution, the energy of the primary electrons has been increased to hundreds of keV or even units of MeV, thereby ensuring the shorter wavelength of the electrons and, therefore, reduced diffraction error, while producing a focused probe in scanning systems or imaging the specimen with transmitted electrons emanating from its surface. In the transmission modes, both scanning and directly imaging versions, the high energy of the illuminating electrons enables them to pass thicker, easier prepared samples. However, the recent introduction of aberration correctors has substantially weakened the resolution argument as regards determination of the suitable energy of electrons, and the penetrability of samples alone remains important for transmission microscopes. At the same time, the emphasis on resolution is being gradually replaced by concentration on detection and contrast formation issues and an understanding of contrast mechanisms.

When decreasing the energy of incident electrons we get them penetrating shallower in the solid targets and transmitting thinner films and the range shortens faster than linearly with falling energy. The reason for this is the increasing rate of scattering events, both inelastic and elastic, which cause energy losses and deviations in the direction of motion of the injected electron. Because these scattering events are sources of information about the sample, we obtain accrued information borne by image-forming signal electrons originating from a thinner layer of the material. When employing electrons backscattered from the specimen surface, we get enhanced surface sensitivity at low energies while the transmitted electrons provide high contrasts from thinner and thinner samples. The plots in Fig. 1 indicate the minimum information depth for scattered electrons at around 50 eV.

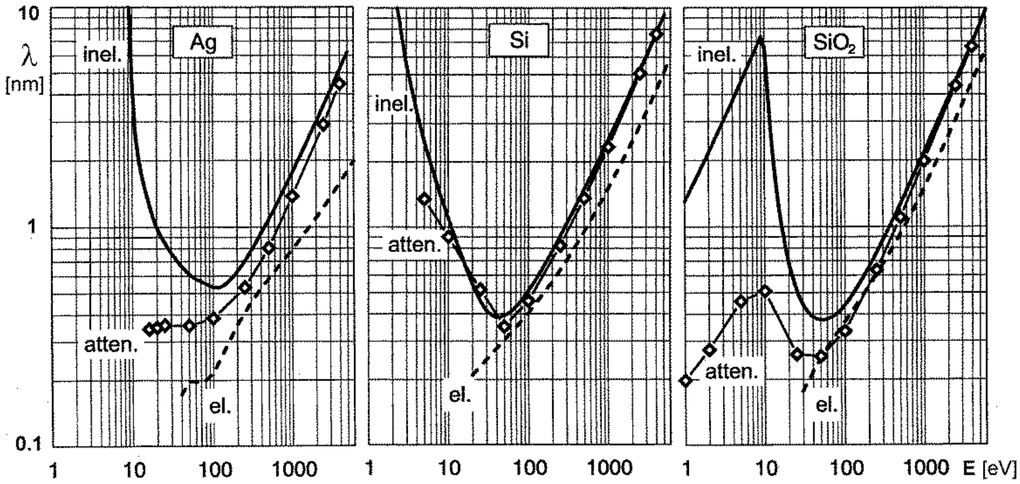

Fig. 1 Elastic (el.) and inelastic (inel.) mean free paths and attenuation lengths (atten.) for Ag, Si and SiO_2, calculated by a program simulating electron scattering in solid targets [1].

In fact, only the inelastic mean free path of electrons extends again after shortening to below 1 nm at 50 eV, seemingly suggesting the possibility of performing transmission microscopy at units or tens of eV with samples of thicknesses otherwise corresponding to tens of keV. However, the elastic mean free path plot does not turn upwards. This quantity is not plotted in Fig. 1 to below 50 eV with the explanation that the traditional simulating algorithms based on Mott cross-sections overestimate the scattering rate and should be replaced with quasi-elastic scattering on phonons. In addition, such slow electrons already deviate from the statistics of particle scattering and have to be considered as Bloch waves of electrons behaving in the environment of the valence and conduction bands and energy gaps. Altogether, elastic scattering of electrons below 50 eV is difficult to simulate and experimental data should be preferred [2]. This data proves the lowest energy range really suitable for electron penetration experiments, i.e. also for ultralow energy TEM or STEM [3,4]. Crystalline targets, with their easy motion routes along directions of a high density of electron states, are more promising.

This paper describes experiments with scanning transmission electron microscopy (STEM) performed below 500 eV down to units of eV on graphene and ultrathin tissue sections. The aim is to demonstrate an image resolution comparable with conventional microscopy though available with dramatically enhanced image contrast and information content.

2. MICROSCOPY WITH VERY SLOW ELECTRONS

The idea of creating a microscopic image with electrons emitted from the sample at very low energies comes from the early years of electron microscopy development – a review can be found in Ref. 5. The sample in the emission electron microscope plays the role of the source of electrons excited with photons, heat, ions and also electrons. The sample is immersed in a strong electric field accelerating the emitted electrons toward an objective lens forming the first intermediate image. It is extremely interesting that the main aberration coefficients of the combination of the accelerating field and objective lens, the spherical and chromatic aberrations of the lowest third order, are at a first approximation inversely proportional to the field strength above the surface [6]. More precisely, they are proportional to the ratio l/k where l is the length of the above-surface field and k is the ratio of the primary energy of the electrons to their energy just at impact on the sample surface, i.e. the landing energy. This means, in turn, that the resolution improves when the energy of the electrons is decreased, i.e. a dependence opposite to that valid for a conventional microscope without an axial electric field. We call the arrangement of the sample biased to a high negative potential in order to produce the above-surface field the cathode lens. Figure 2 shows an example of the calculated size of the primary beam spot for a cathode lens combined with a high quality magnetic objective lens. Obviously, the image resolution is perfectly acceptable even down to units of eV.

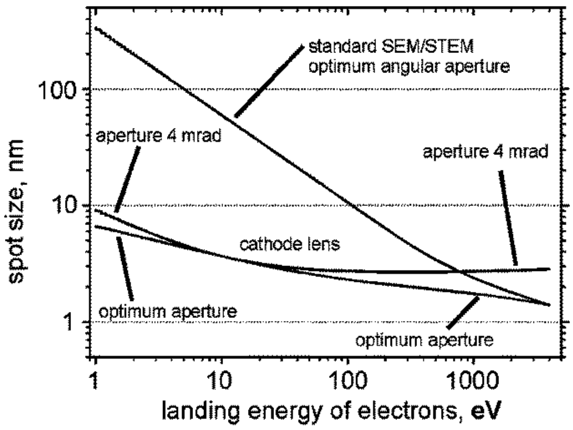

Fig. 2 Example for a SEM/STEM with aberrations C_S = 7.4 mm and C_C = 2.5 mm, primary energy 4 keV, working distance 4 mm, energy spread of the beam 100 meV, gun brightness 10^8 $Acm^{-2}sr^{-1}$, beam current 10 pA, anode bore 1 mm; Barth-Kruit summation rule for confusion discs - encircled current fraction of 50%. The optimum aperture is calculated for each energy separately.

Having the sample at a high negative potential that retards the primary electrons before their impact, we can insert two detectors at ground potential above and below the sample. The above-sample detector acquires the backscattered electrons, while the below-sample detector collects the transmitted electrons. Both electron fluxes are accelerated in the electric field caused by the sample bias and also collimated toward the optical axis. In this way we also obtain, in addition to excellent resolution, very high collection efficiency and amplification of the signal flux detected either via photons or electron-hole pairs.

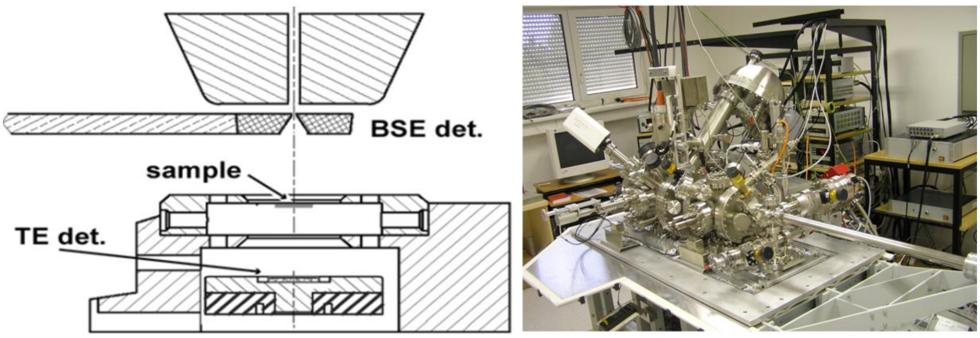

Fig. 3 Arrangement of detectors around the sample in an ultrahigh vacuum SLEEM at ISI Brno (left); outer view of the complete device equipped with a spectrometer of Auger electrons (right).

Practical implementation has proven extremely advantageous when inserting a detector of the backscattered electron (BSE) type just below the objective lens [6]. The small central bore of this detector enables the primary beam to pass, and the detector itself, which preferably consists of a single crystal detector disc, also plays the role of the anode of the cathode lens (see Figure 3). The below-sample detector of transmitted electrons (TE) may be split into coaxial rings for dark-field and high-angle-annular dark-field signal ranges, surrounding the central spot acquiring the bright-field signal.

3. RESULTS AND DISCUSSION

If the energy range of units of keV requires a sample thickness not exceeding 20 nm [7,8], films in units of nm can be expected to be transmittable at hundreds of eV and below. The first experiments were performed on a 3 nm film of gold deposited on a holey carbon support (Figure 4). Micrographs in the range of tens of eV confirmed the expected very high thickness contrast. However, the majority of structure details were identified as holes in a discontinuous foil, which probably disqualifies sputtering as a technology capable of producing samples suitable for very low energy STEM.

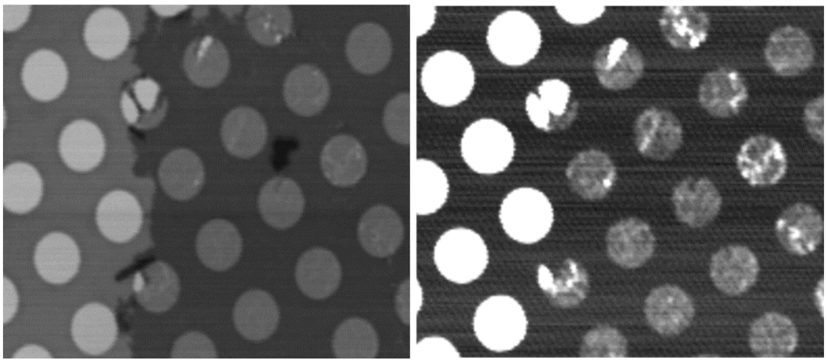

Fig. 4. A 3 nm gold film, prepared by magnetron sputtering and deposited on the Quantifoil; electron energy 3 keV (left) and 10 eV (right), the diameter of the holes in the supporting carbon foil is 2 μm [9].

The first experiments also revealed one peculiar property of the arrangement with a biased sample, namely an incoherent contribution to the signal of transmitted electrons produced by secondary electrons released near the bottom surface of the sample and accelerated in the cathode lens field after emission. This spurious signal reached its maximum intensity at 300 to 400 eV and caused sample transmittance apparently exceeding 100 % [9]. There is no way at hand of suppressing this phenomenon, though the secondaries are at least concentrated near the optical axis in the bright field channel and do not influence the dark field signals.

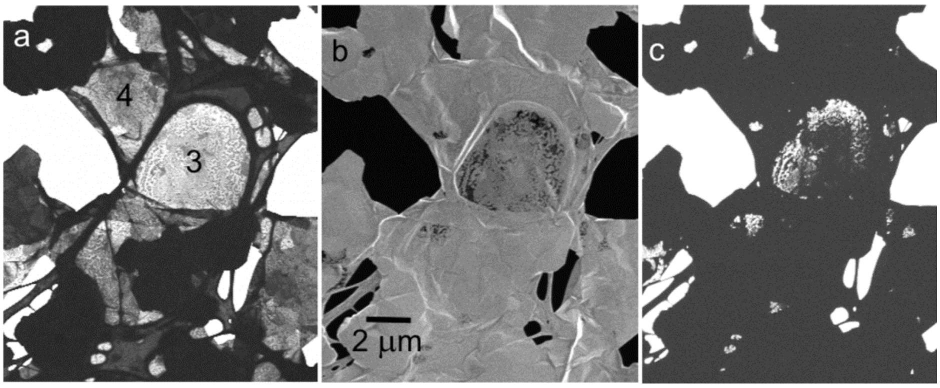

Fig. 5. Low energy electron micrographs of CVD graphene: transmitted electrons at 1 keV (a), backscattered electrons at 1 keV (b), and transmitted electrons at 5 eV (c) [10]. Flake no. 3 has Raman characteristics of single-layer graphene and flake no. 4 is near to these properties.

Obviously 2D crystals, in particular graphene, are an extremely suitable family of samples for demonstration experiments. We have examined graphene samples from two sources – one was a personal delivery of a liquid phase defoliated graphene by K. Novoselov and A. Geim, while the other was commercial CVD graphene^{TM} (see www.graphene-supermarket.com). Both samples were found to be composed of submicrometer-sized flakes, including those identified as single-layer graphene by Raman microspectroscopy (incapable of resolving details below 1 to 2 μm) (Figure 5).

When measuring the electron flux transmitted through single-layer graphene sites we found it reaching a maximum at 5 eV [4]. This property may prove utilizable as a criterion for recognizing the number of graphene layers at a much higher lateral resolution than Raman spectromicroscopy can offer.

The enormously wide scope of practice and application of TEM and STEM in biology and medicine offers a promising area of implementation for any innovations achieved in these two diagnostic and analytic technologies. The extremely successful development of low-voltage TEM [11] operated at or around 5 keV has proven itself an efficient tool in biomedicine thanks to greatly enhanced contrasts achieved in this energy range, enabling one to avoid the staining of tissue sections [7]. It suggests the possibility of continuing to lower the electron energy by employing the cathode lens principle with the aim of preserving image resolution and further increasing image contrast.

The experiments were performed on a piece of mouse heart muscle fixed in glutaraldehyde, dehydrated using the acetone sequence and embedded in a hard resin at elevated temperature. Ultrathin sections between 4 and 10 nm were cut with an ultramicrotome. It should be emphasised that, in certain series of samples, no staining with heavy metal salts was performed and post-fixation with osmium tetroxide was also avoided. The very first series of micrographs (Figure 6) demonstrates the added value provided by combining the STEM frames with the BSE signal enabling one to distinguish the thinnest sites of the sample from empty holes.

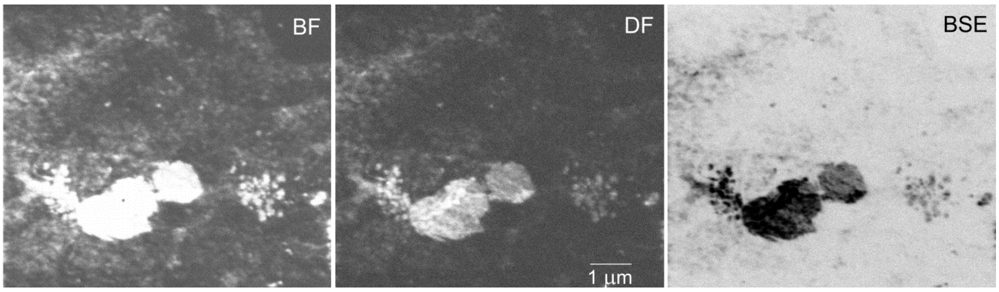

Fig. 6. Three simultaneously acquired image signals, namely transmitted electrons detected within polar angles of emission: BF ~ 0 to 75 mrad, DF ~ 86 to 366 mrad, and backscattered electrons (BSE); primary energy 4.5 keV, landing energy of electrons 500 eV, beam current 13 pA. Sample post-fixed with OsO4.

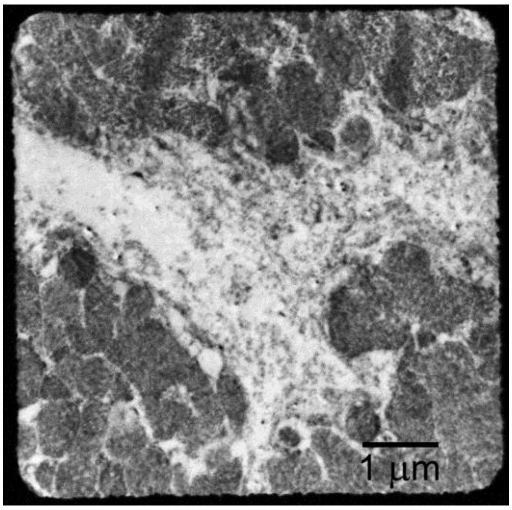

Fig. 7. Ultrathin section of mouse heart muscle prepared with no post-fixation or staining. STEM micrograph at 500 eV, bright-field image, beam current 13 pA.

A further series of experiments on samples free of any post-fixation or staining with heavy metal ions has confirmed the hope of obtaining a "standard" image resolution with the dramatically enhanced contrast of STEM micrographs (Figure 7). The thinnest samples can be observed down to tens of eV, though for now an energy of around 500 eV seems to be most recommendable.

4. CONCLUSIONS

Scanning Transmission Electron Microscopy has proven itself applicable down to a landing energy of electrons of hundreds of eV. Although the preparation of specimens presents certain challenges, the microscopic technique seems to be sufficiently well developed and ready to use. The first application examples have already provided useful results, namely a promising method of diagnostics of the morphology of graphene samples and a way of avoiding any heavy metal fixation or staining of tissue sections or other organic films.

ACKNOWLEDGEMENTS

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