Examination of thin samples in TEM or STEM has been performed at hundreds of keV. This energy range offered high resolution but low contrasts which meant that tissue sections had to be contrasted with heavy metal salts. Recent TEM with aberration correctors preserve an acceptable resolution down to 20 keV and provide enhanced contrasts [1]. The LVTEM device is operated at 5 keV on samples thinner than 20 nm [2]. STEM attachments to SEMs have become widespread [3] profiting from an image contrast substantially increasing even for light elements at tens or units of keV. The methods for the preparation of ultrathin sections of various substances are capable of producing layers at and even below 10 nm [4,5] which enables one to further decrease the energy of the electrons provided the image resolution is maintained. When using the STEM technique virtually all transmitted electrons can be utilised for imaging, while in TEM we are restricted to using electrons capable of forming the final image at acceptable quality. This forces us to narrow the ranges of the angular and energy spreads of electrons that enter the image-forming lenses. Consequently, the STEM technique promises higher contrasts at comparable resolutions. Unlimited reduction of the energy of the illuminating electrons is possible by employing the cathode lens principle [6]. This consists of biasing the sample together with its holder (made flat on both sides) to a high negative potential that retards the incident electrons before they land on the sample surface and accelerates backscattered and transmitted electrons to their respective detectors above and below the sample (Fig. 1). Calculations have shown a final spot size only moderately extended even at units of eV so that quality-consistent micrographs can be recorded over the full energy scale [7].

Ultra-low-energy STEM at hundreds of eV can be successfully applied to the examination of ultrathin tissue sections free of any heavy metal salts (Fig. 2) [8] or to 2D crystals. Single atomic steps are revealed at high contrast on multilayer graphene samples and transmittance of electrons at tens or units of eV can serve as a tool for “counting” the graphene layers (Fig. 3).


Acknowledgement: Support by the Technology Agency of the Czech Republic under no. TE01020118 and the institutional support RVO:68081731 are acknowledged.
Fig. 1: Trajectories of signal electrons toward transmitted (TE) and backscattered (BSE) electron detectors and through-the-lens detector (TLD) with the specimen immersed in the field of the open objective lens (a), with the biased sample retarding the beam 11 times (b), and with a combination of both (c).

Fig. 2: Section of mouse heart muscle, free of osmium tetroxide post-fixation and any staining, estimated thickness 5 nm, micrograph taken at 500 eV (a), electron energy dependence of the average edge resolution (b), and electron energy dependence of the relative variance contrast (c).

Fig. 3: Commercial CVD multilayer graphene imaged at 220 eV (a), total transmittance of extremely slow electrons through varying number of graphene layers (b).