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EFTEM and its Application in Cryo Electron Microscopy

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Dedicated to Prof. Elmar Zeitler on the occasion of his 75th birthday.

It is now nearly one decade since energy filtering transmission electron microscopy (EFTEM) became one of the favorite tools in the field of electron microscopy and especially in analytical electron microscopy (AEM) [1, 2]. This rapid development was not only a direct result of the invention and implementation of high performance imaging filters like the in-column omega-(Ω)-filter [3, 4] and the post-column GIF (Gatan Imaging Filter) [5] but it was also directly related to the accomplishments in computer sciences and subsequently in the development of suitable digital cameras (CCD cameras) [6-8]. Nowadays, energy filters are widespread in all the different scientific communities (e.g. material science, life science) because of its advantages for and in TEM investigations.

To understand the principle of energy filtering we will shortly describe the key concept with the already well-established three-dimensional information cube [9]. This cube represents a 3-dimensional data space, with the intensity I(x, y, ΔE), where x and y are the coordinates at the exit surface of the specimen and ΔE the energy loss. It can be accessed by different approaches. Using a focused probe in a scanning TEM (STEM) in combination with a spectrometer (e.g. an energy filter), we are able to record sequentially EEL-spectra (vertical columns of the cube) for each point on the specimen. On the other hand using a fixed beam TEM in combination with an energy filter, 2-dimensional images (horizontal planes) at a specific energy loss can be acquired at once. This is referred to as electron spectroscopic imaging (ESI) [1].

EFTEM allows us to separate the contributions from elastically and inelastically scattered electrons by inserting an energy-selective slit in the energy dispersive plane. While in material science the energy filter is mainly used for inelastic filtering, the life science community has set their priorities to elastic filtering. Inelastic filtering utilizes the ionization edges. Common methods are ratio mapping, elemental mapping and spectrum imaging [10-13]. These methods require high electron doses (up to ~ 100 000 e/nm^2) and are therefore not necessarily suitable for low-dose applications. However, elastic filtering or zero-loss filtering is mainly used in biological applications [14] and of course for diffraction studies. Inelastic filtering is, indeed, more ‘analytical’, but we cannot dismiss the advantages and the improvements made by elastic filtering.

In zero-loss imaging the slit (usually 5 to 20 eV wide) is centered at the zero-loss peak formed by the unscattered and elastically scattered electrons. Thus zero-loss filtering removes the contribution of inelastically scattered electrons, which will result in an increase in contrast and resolution. This is especially important for vitrified (ice embedded) biological samples [15], because of the increasing probability of inelastic scattering events with decreasing atomic number (and increasing sample thickness; typical sample thickness between 0.5 – 3 µm at 300kV and ~ 1µm at 120kV).

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Cryo-EM is used in our laboratory for single particle analysis but mainly for electron tomography [16, 17]. Tomography plays a key role in the revelation of the molecular machinery inside living cells, thus we have to rely on the best resolution we can get and therefore zero-loss filtering is an absolute necessity [18].

Additionally, most probable loss imaging or structure-sensitive imaging can be applied to biological samples as well. This technique is especially suitable for thick samples, for samples with different constituents (e.g. in biomineralization) and stained samples. Furthermore EFTEM can be applied to measure the specimen thickness fast and accurately [19, 20]. This is particularly useful for ice embedded specimens, e.g. to determine the local quality of the sample.

Until now, experiments and investigations have been performed on a Philips CM120 BioFilter and a Philips CM300 FEG transmission electron microscope. Both microscopes are equipped with a Gatan imaging filter (GIF) and a Gatan CCD Camera. At the moment we mainly use liquid nitrogen to keep the vitrified specimens at temperatures around ~ 90 K. In the near future we will be able to use a Tecnai F30 with helium cooling, approaching cooling temperatures around ~10 K. The prospects of using 'liquid' helium are encouraging, e.g. regarding the radiation 'resistance' [21]. It is proposed that the sensitivity to radiation damage will be reduced by a factor of 2 to 3, which will improve the window of opportunity, especially for electron tomography.

We will present and discuss our recent results in respect to our earlier work in EFTEM and cryo electron microscopy particularly electron tomography and we will discuss the future possibilities and the improvements, which can be obtained with helium cooling.

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