A Quantitative Investigation of Biological Materials using EELS

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Introduction

Electron Energy Loss Spectroscopy (EELS) is the analysis of the energy distribution of the electrons that have passed through a thin sample and have interacted with it inelastically. EELS is a powerful technique capable of providing chemical and electronic information from particular areas in the sample. Spatial information can be obtained using two different approaches: the first method is to combine EELS with a scanning transmission electron microscope (STEM) where the electron probe is scanned across a selected area in the sample and an EELS spectrum is collected point by point across the scan giving a Spectrum Image (SI). The second method is to use Energy Filtering Transmission Electron Microscopy (EFTEM). EFTEM utilizes a special spectrometer which has the capability to filter the energy of the electrons that have interacted with the spectrometer with the specimen. Concentrating on a particular ionisation edge it is possible to build up images which show a two dimensional distribution of a particular element.

Why EELS for Biological materials

Unstained biological materials are traditionally difficult to analyse in the TEM as they show very little contrast and more importantly they are quite sensitive to the electron beam. The sample can be easily damaged by the electron beam if extra care is not taken while performing the experiment. Biological materials are almost entirely composed of carbon and in some areas they show other elements in small concentrations. EELS is very well suited to study such materials given its high sensitivity to light elements and collection efficiency.

Experimental methods

Here both EFTEM and EELS SI approaches have been used to reveal the elemental distribution across a selected area in a sample obtained from human autopsy tissue. The TEM sample was coated with 5Å of C on both sides using the Gatan PECS®. This coating is extremely important in order prevent the sample from any charging effect that might happen when the electron beam interact with the sample. EFTEM and EELS SI were acquired using a JEOL 2010 equipped with a LaB_6 electron source and STEM capability. Attached to the bottom of the microscope was a Gatan GIF Quantum® 965ER spectrometer. Some of the features available in this new spectrometer are: DualEELS™ capability that allows two different energy regions of the EELS spectrum to be recorded simultaneously under the same experimental conditions [1,2]; a 2K CCD camera that allows recording EELS spectra with an energy range that extends up to 2000eV and 4000eV in DualEELS™ mode; high-speed spectroscopy that allows the acquisition of over 1000 spectra per second; a 5th order aberration lens system corrected allowing large (>100mrad) to be used while maintaining the energy resolution below 1eV. Both techniques were used to investigate the same region in the sample across a capillary vessel.

EFTEM approach

As said above, TEM images from biological samples show very little contrast. However as shown here by energy filtering, the contrast in the TEM image is greatly improved and details which seem to be lost in the unfiltered image are now visible as shown below in Figures 1a-c.

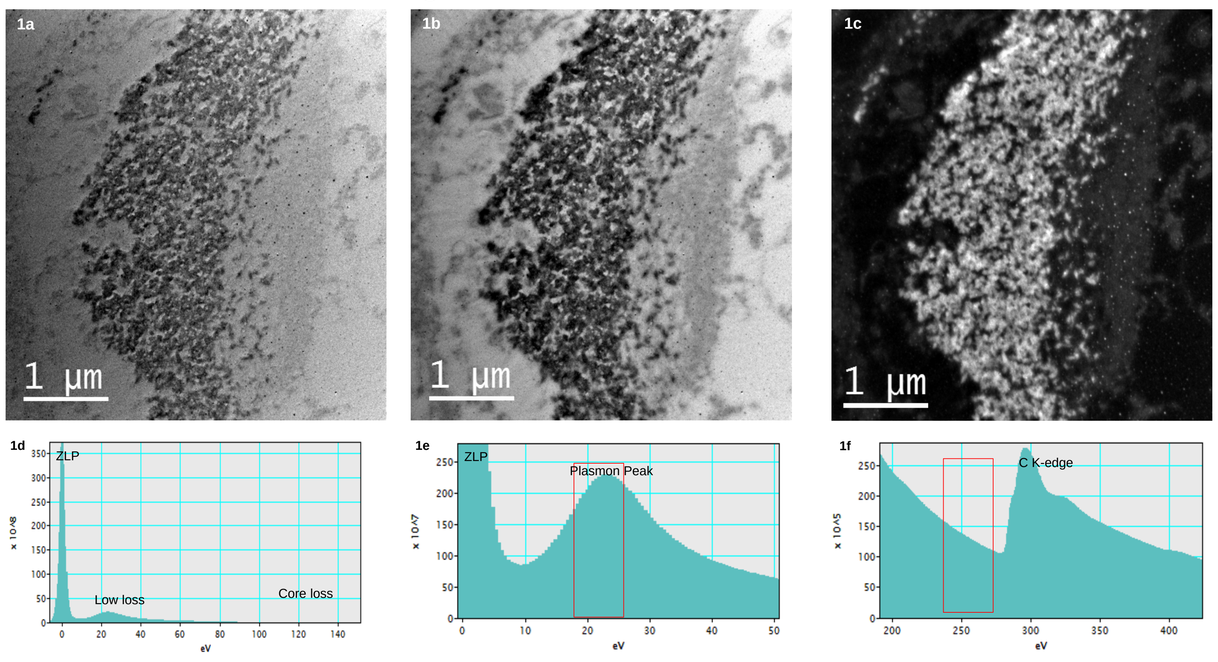


Figure 1a is an unfiltered TEM bright field image. All the scattered electrons both inelastically and elastically contribute to the image as shown in Figure 1d. Figure 1b is a plasmon filtered TEM image. Here only electrons selected within the 25eV window placed in the plasmon region as shown in Figure 1e contribute to the image. Figure 1c is obtained by placing the 25eV slit in the region of the EELS spectrum prior to the C K-edge as shown in Figure 1f. The position of the slit in the EELS spectrum determines which electrons contribute to the image.

EFTEM SI results

In standard EFTEM two or three images are used to obtain the elemental distribution. The EELS spectrum is not revealed. The background is removed by dividing the post edge image by the pre-edge image in the case of the jump ratio method. In the case of the three method the background is obtained from the two pre-edge images and then removed from the post-edge image. In the EFTEM-SI a complete series of images is acquired scanning the slit across a well defined region of the EELS spectrum. The main advantage of EFTEM-SI over the standard EFTEM is that for each point of the SI it is possible to extract a full EELS spectrum therefore the data can be processed using the same tools available for standard EELS.

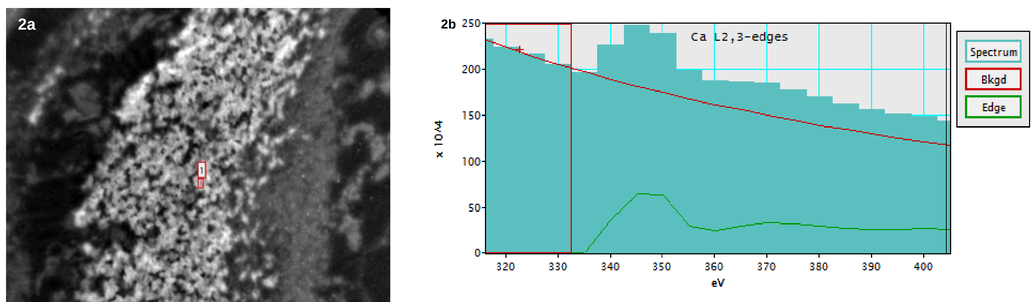
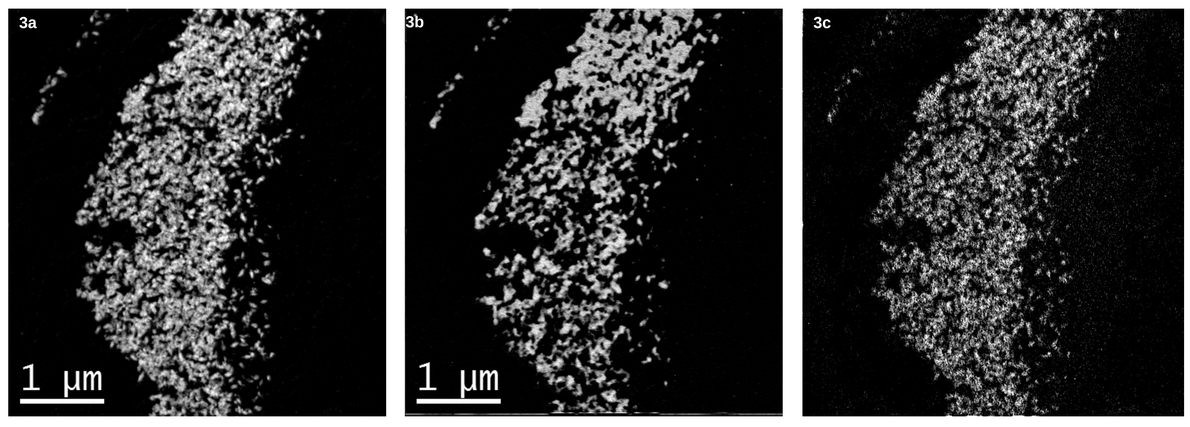


Figure 2a is EFTEM SI dataset acquired scanning a 10eV every 5eV across the region of the EELS spectrum from 310eV to 420eV. Figure 2b is a EELS spectrum extracted from the region in the red mark in the EFTEM SI in Figure 2a. The Ca $L_{2,3}$-edges at 346ev is visible and can be extracted by placing a power law background



Figures 3a-c are the Ca $L_{2,3}$-edges, the P $L_{2,3}$-edges and the N K-edge elemental maps respectively. The Ca seems more diffused than the P across the capillary wall vessel region. Such enhanced contrast can only be possible by performing EFTEM SI.

EELS STEM Results

The EELS SI analysis was carried out in DualEELS™ mode where the low-loss region containing the Zero Loss Peak (ZLP) and the high-loss from 80eV to 1080eV. Having the low-loss region of the EELS spectrum acquired simultaneously under the same experimental conditions allows to obtain the absolute number of atoms and therefore the relative composition across the region analysed during the EELS SI. Relative compositional maps are shown below. The quantity of Ca and P seem to vary up to 4% and 15%. Traces of Fe and Cu can be observed. In particular the Fe seems to be uniformly distributed across the capillary wall vessel region within a concentration at around 2%. Quite peculiar it is the Cu that is found only in some localized regions with a maximum concentration of 5%.

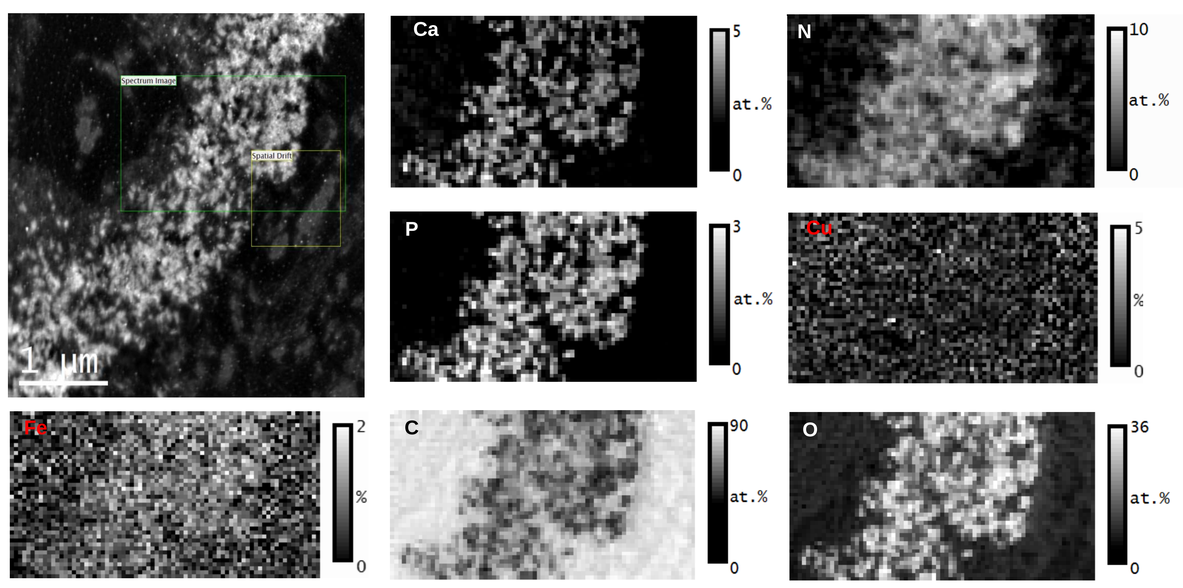


Figure 4a is the ADF STEM survey image, the green box is the area where the beam was scanned during the EELS SI acquisition. The relative elemental compositional maps are also reported above.

Conclusions

EELS has proved to be a valuable tool to obtain compositional information from biological samples. In addition to the composition, EELS can also give insight into the chemistry unveiling the nature of the chemical bonds and different oxidation states. This information is important in order to understand how the elements chemically interact with each other. Damage can be also avoided thanks to the capability of the GIF Quantum to acquire EELS spectra at a rate of over 1000 spectra per seconds. Thus, large datasets can be acquired in a very short time.

References

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[2] J Scott, P Thomas, M Mackenzie, S Mcfadzean, AJ Craven and WAP Nicholson, "Near-simultaneous EELS spectrum imaging, "Ultramicroscopy, vol. 108, pp 1586-1594, 2008

Acknowledgements

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