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EELS: A Tool For Investigating Biological Materials

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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 very powerful technique capable of providing chemical and electronic information from particular areas in the sample. Spatial information can be obtained using two 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 specimen. Concentrating on a particular ionization edge it is possible to build up images which show a two dimensional distribution of a particular element.

Unstained biological materials are traditionally difficult to analyze 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 when performing the experiment. Biological materials are almost entirely composed of carbon and in some areas they show other elements in small amounts. EELS is well suited to study such materials given its high sensitivity to light element and collection efficiency.

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 sample was fixed in formalin and glutaraldehyde , post fixed in osmium tetroxide and uranyl acetate, dehydrated in ethyl alcohols and propylene oxide and embedded in epoxy resin. The TEM sample was also coated with 5Å Carbon on both sides using the Gatan PECS®. This coating is extremely important in order to prevent any charging effect due to the electron beam travelling through the sample. TEM samples were kindly provided by Dr Wenlang Lin at Mayo Clinic in Jacksonville FL.

The same area in the TEM specimen was analyzed in both EFTEM and EELS SI modes. The TEM microscope used for this experiment is equipped with a LaB_6 electron source and in STEM mode a 10nm probe with a current density of 1.6nA/cm^2 was used. The area chosen for both experiments shows the presence of a feature that has been identified as a cell nucleus.

EFTEM results

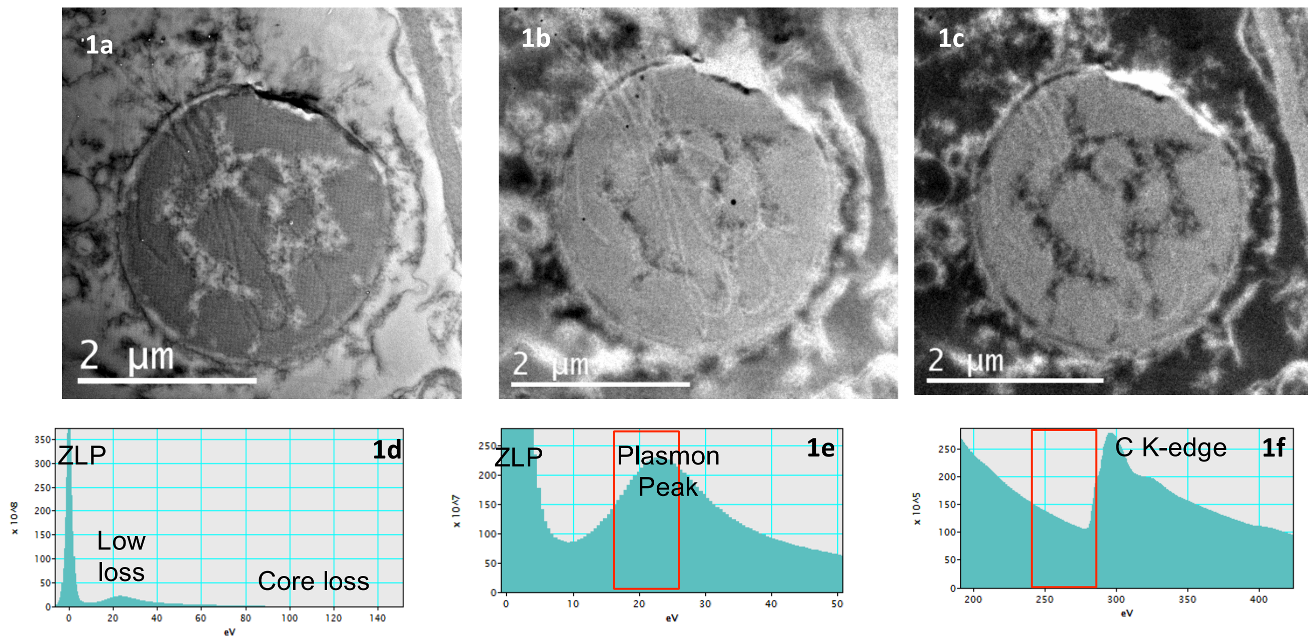
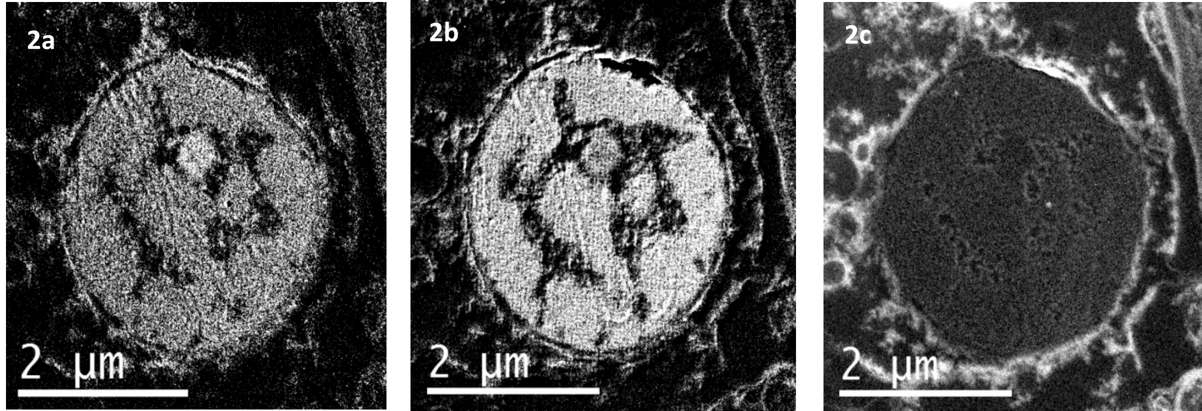


Figure 1a is an unfiltered TEM bright field image. All the scattered electrons both inelastically and elastically contribute to the image as shown in Figure 1d. Figure 1b is a plasmon filtered TEM image. Here only the electrons selected in the 25eV window placed in the plasmon region as shown in Figure 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.

TEM images from biological samples show a very little contrast. By energy filtering, the contrast in the image is greatly improved and details which seem to be lost in the unfiltered image are now visible as shown in Figures 1b,c.

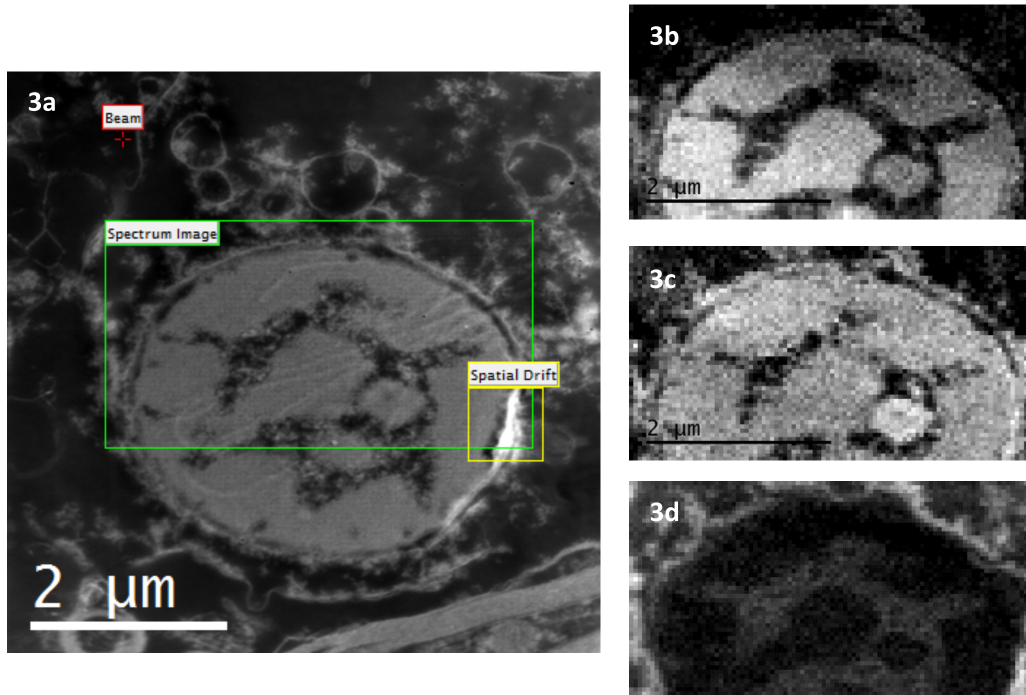
By selecting an energy window around an ionization edge, it is possible to obtain images where the intensity is correlated to the concentration of a specific element. The signal of this element is superimposed on other signals such as diffraction contrast, thickness variation, elastic contrast and most importantly the background. In general the background can be removed using two different approaches: the two window and the three window method. In the former, image is formed by simply dividing the filtered image acquired just after the ionization edge by the one acquired from the region before the edge. In the latter method, two images prior to the edge give the background contribution which is determined by the power law. The background removal prior to the ionization edge is largely influenced by the position and width of the slit. The choice of these two conditions determines the size of the background and therefore the error after its removal. The best way to tackle this problem is to acquire a series of images where the position of the slit for each image is scanned over a certain region in the EELS

spectrum. This is called EFTEM SI. In addition to pure filtered images, EFTEM SI stack contains spectroscopy information. An EELS spectrum can be extracted everywhere across the stack of images and the background can be placed accordingly giving the best fit.



Figures 2a,b,c show the N K-edge at 401eV, the P $L_{2,3}$-edges at 132eV and the Cl $L_{2,3}$-edges at 200eV elemental maps respectively. Each map was extracted in EFTEM SI mode. 10eV slit was scan over a region of the EELS spectrum between 90eV and 230eV. The P and Cl elemental maps are extracted from this EFTEM SI stack. The N elemental map was extracted from another EFTEM SI stack where 15eV slit was scan from 320eV to 430eV. 10eV and 15eV width slits were used as they represent the best compromise between two factors: the slit should be narrow enough to avoid edge overlap but it should be large enough to provide adequate counting statistics.

EELS SI results



Figures 3a is an ADF STEM image. The green box represents the area where the beam was scanned during the acquisition of the EELS SI. This area was divided into a series of pixels and an EELS spectrum was acquired pixel by pixel. Each edge can be easily extracted and mapped out. Each EELS spectrum was acquired using a very short acquisition time (<0.3seconds) and in sub pixel scanning mode which is available in Gatan Digiscan®. Here the beam is scanned across the pixel size during the acquisition of the EELS spectrum and as a result the dose is spread over the whole pixel size.

Figures 3b,c,d are the P $L_{2,3}$-edges, N K-edge and Cl $L_{2,3}$-edges elemental maps respectively.