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High Speed EELS and EFTEM Analysis across the Visual Cortex

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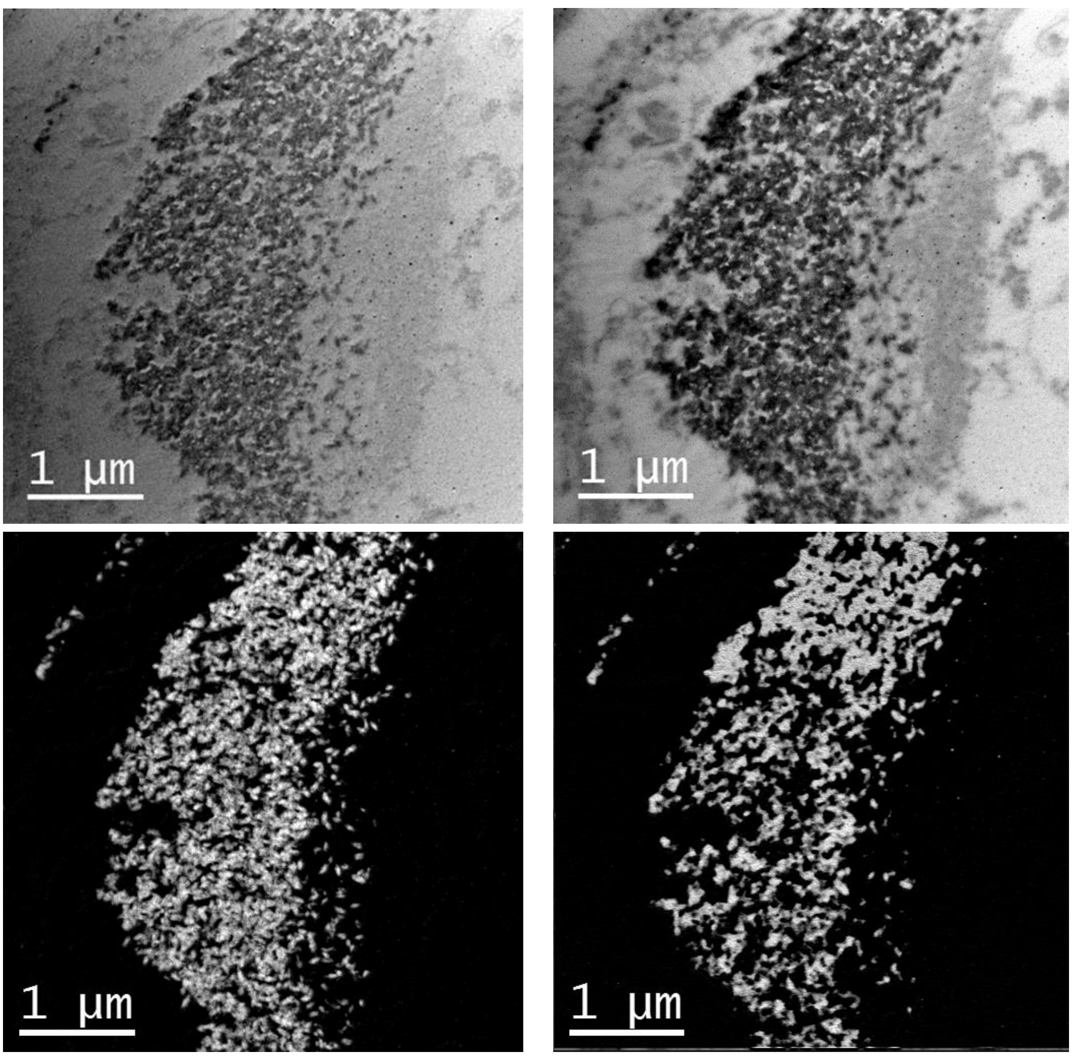
Unstained biological materials are traditionally difficult to analyze in the TEM as they show very little contrast and more importantly, they are quite susceptible to electron beam damage if extra care is not taken when performing the experiment. Biological materials are almost entirely composed of carbon but in some areas they show other elements in small amounts. Electron Energy Loss Spectroscopy (EELS) is well suited to studying such materials given EELS high sensitivity to light element and its high collection efficiency. The paper shows how quantitative information can be obtained from a region in a TEM sample across a blood vessel capillary region.

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) approach. EFTEM utilizes a special type of EELS spectrometer which has the capability to create images using only electrons that have lost a narrow range of energies after interacting 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. In this paper, 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, obtained from human autopsy, was fixed in formalin and glutaraldehyde, post fixed in osmium tetroxide and uranyl acetate, dehydrated in ethyl alcohols and propylene oxide embedded in epoxy resin. The TEM sample was also coated with 5Å carbon on both sides to prevent any charging effect due to the electron beam interacting with the sample. EFTEM and EELS STEM SI datasets 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 ER imaging filter and EELS spectrometer.

Figure 1 shows an unfiltered conventional TEM image of the region in the sample across a capillary blood vessel. Even though the TEM sample was slightly stained, the contrast shown in the unfiltered TEM image is poor. This is due to the fact that the sample is mainly made of carbon and other light elements such as O. However by removing the contribution of inelastic scattering as shown in Figure 2, the contrast can be greatly improved and hidden details and features are now revealed. These features are typically related to the varying composition and presence of other elements such as Ca and P as shown in Figures 3,4 respectively. These maps show the elemental distribution of Ca and P and were obtained using EFTEM spectrum imaging (EFTEM-SI), where a series of images are acquired while changing the position of the slit for each image. This method builds up an EELS spectrum energy plane by energy plane across a certain region of energy loss. Based on these EFTEM SI elemental maps, the Ca seems more diffused than the P. This is in good agreement with the EELS STEM analysis carried out across the same specimen in DualEELS mode where low- and core-loss regions of the EELS spectrum were simultaneously acquired generating an EELS spectrum that extends from 0eV to 1080eV. The relative concentrations of P and Ca seem to vary up to 3% and 5% respectively. Traces of Fe and Cu were also found in some localized regions across the analyzed area in the specimen.

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 chemical bonds and different oxidation states. This information is important in order to understand how the elements present interact with each other. Damage can be avoided by the capability of the GIF Quantum of acquiring EELS data with a very high spectral rate.



Figures 1-4 are unfiltered C-TEM image, zero-loss filtered image, Ca and P elemental maps respectively. The zero-loss filtered image was obtained by placing a 10eV slit so that the inelastic contribution that blurs the contrast in the TEM image can be removed. The Ca and P elemental maps were obtained using a EFTEM-SI procedure

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