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Correlative Synchrotron Micro-CT and FIB-SEM Imaging for the Analysis of Multifocal Pathologies

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Nowadays, the correlation of different imaging techniques with the aim of overcoming the single-method limits is getting increasingly widespread [1]. Correlative Light and Electron Microscopy (CLEM) is the most known correlative approach: it combines the strengths of Light Microscopy (LM, *i.e.* in vivo and in vitro analysis, temporal information of biological processes performed on large fields of view) with the Electron Microscopy ones (EM, *i.e.* resolution down to nm: ultrastructural information in a smaller field of view) [2]. Moreover, while LM can provide information on the three-dimensional (3D) organization of a biological sample, usually transmission electron microscopy (TEM) analysis is carried out on few tens of nanometers thin sections (2D) [3]. Therefore, when the ultrastructural characterization of a volume is required, time consuming and challenging serial sectioning procedures should be performed. For this reason, in the last decades many efforts have been invested in the development of simpler sectioning and new imaging methods that could give access to 3D high-resolution information, and today several techniques are available, such as focused ion beam-scanning electron microscopy (FIB-SEM) [4]. However, because of their intrinsic limitations, these new powerful techniques cannot be performed on entire organs, and they must be focused on previously selected (by means of LM, for example) regions of interest (ROIs).

We have recently described the correlative technique between synchrotron computed microtomography (micro-CT) and TEM: the non-destructive micro-CT imaging provides a 3D virtual map of the fixed, stained and embedded organ/tissue, where it is possible to identify with micrometric resolution ROIs to directly section and analyze through TEM [5]. This is particularly useful, for example, when the analysis pertains to those pathologies characterized by the multifocal localization of hallmarks into defined regions, which are not known *a priori*.

Here we report the correlative imaging micro-CT/FIB-SEM. In the micro-CT of Experimental Autoimmune Encephalomyelitis (EAE) rat spinal cord, model of Multiple Sclerosis (pathology characterized by demyelination and disseminated infiltration of immune cells within the nervous system), we could identify few perivascular ROIs (box in Figure 1A) where to focus the ultrastructural analysis through FIB-SEM. Here we could find and fully reconstruct an infiltrating monocyte, hallmark of EAE, demonstrating that the region was actively involved in the pathological process.

In general, the correlative microscopy here proposed could be used for the study of any biological sample, taking advantage from the highly informative 3D micro-CT map for the identification of ROIs and their 3D ultrastructural information provided by the FIB-SEM application.

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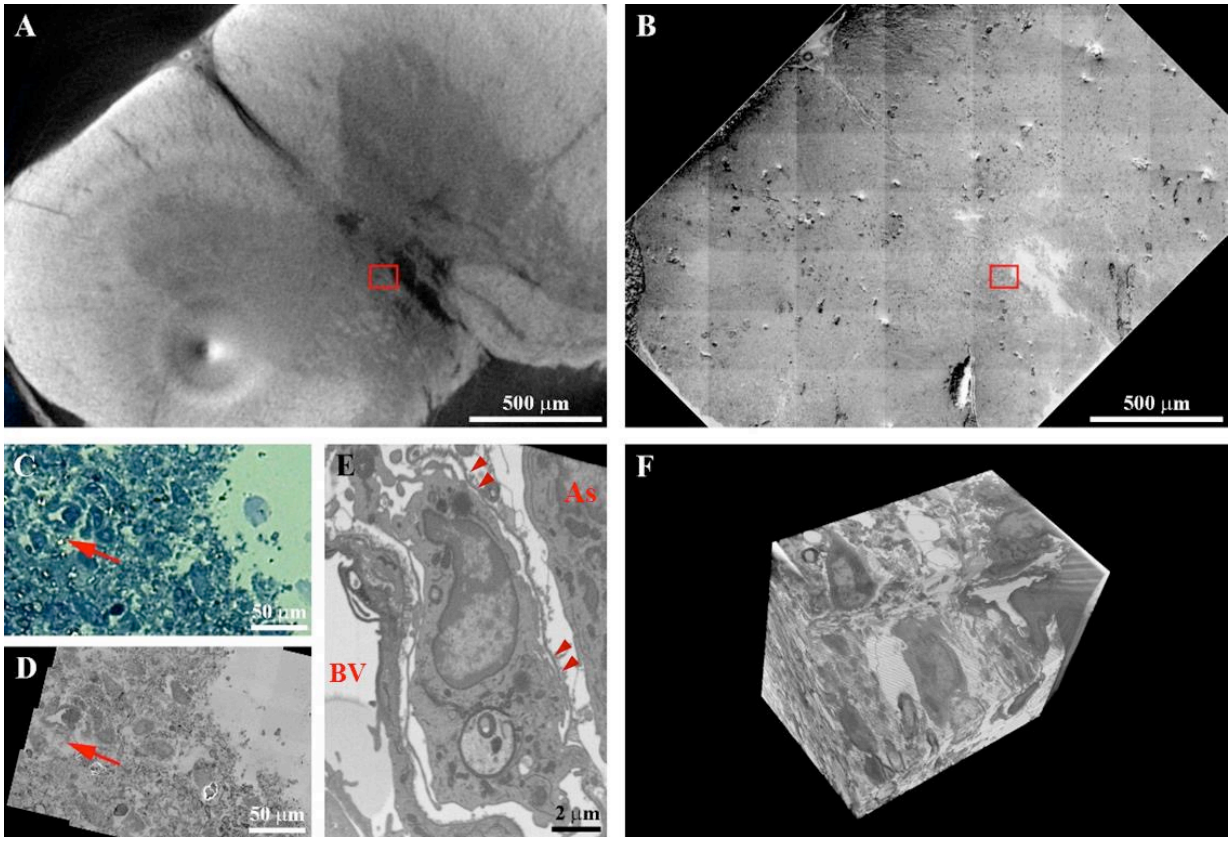

Figure 1. Correlative micro-CT and FIB-SEM. The same perivascular region (box) is identified in a micro-CT virtual slice (A), in EM (box in B and arrow in D) and in LM image (arrow in C). The FIB-SEM tomography was performed on this ROI, where it was possible to image an infiltrating monocyte (E), and fully reconstruct its volume (F). BV: blood vessel; As: astrocyte; arrowheads: basal membrane.

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