Proteins and tissues' thin slices studied by CLEM in a SEM: how to prepare the most effective ITO substrates

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Correlative Light and Electron Microscopy (CLEM) performed in a Scanning Electron Microscope (SEM) is a technique that has been recently made much easier by the commercial availability of widefield fluorescence microscopes (FM) mountable in an SEM chamber. The workflow is composed of two steps: first the fields of view of the SEM and the FM are aligned exploiting the cathodoluminescence signal produced by the electron beam-glass interaction. Then a given area is imaged using the FM followed by the SEM, with this approach then allowing, other than standard CLEM methods, to directly correlate with high spatial precision the two images.

For CLEM studies, the samples have to be deposited on a substrate capable to provide both high transparency at the emission wavelength of the most common fluorescent markers and a high electrical conductivity to prevent charging effects under the electron beam. Indium Tin Oxide (ITO) coated glass substrates fulfill these requirements, allowing both SEM imaging and very low photon absorption in the visible range, resulting in a really effective correlative imaging also with biological samples. [1]

Glass-ITO substrates commercially available for various applications are quite expensive. Moreover, they are not yet optimised for CLEM studies: they can show an inhomogenous patchy surface which will contribute to the final CLEM images, see Fig. 1B. We have therefore studied alternative methods to prepare CLEM-optimized ITO substrates with better performance and lower cost by using methods conventionally available in most of EM labs, *i.e.*, magnetron (MS) and ion beam sputtering (IBS), respectively. This study is mainly aimed at getting glass-ITO supports with good electrical conductivity, high light transmittance and no visible superficial features, *i.e.*, with minimal roughness and granularity of the ITO layer. Different conditions were explored: 20 nm MS-ITO-films were deposited on glass using different sputtering gases, pressure and current, while 20 nm IBS ITO films were deposited using different ion-beam energy (4, 6, 8 and 10 keV). The as-deposited ITO films were then studied in terms of: light transmittance in the visible-NIR range, roughness, determined by Atomic Force Microscopy (AFM), and work-function by Scanning Kelvin Probe Microscopy (SKPM). These investigations were performed before and after annealing the glass-ITO supports upon inert atmosphere and exploiting a thermal range comprised between 200˚C and 400˚C. After investigating how the different deposition conditions and post-annealing treatments affect the final properties of the ITO coating, the best home-made glass-ITO substrate has been used for performing CLEM imaging of both protein and tissues' thin slices prepared for SEM observation, then compared with the same imaging performed by using a commercial glass-ITO support, showing the higher quality of home-made ITO thin films, whose deposition could be also carried out on different kinds of light beam transparent supports with limited cost. Figure 1 shows the CLEM imaging performed on a fluorescently labeled sample of protein fibrils (known as amyloid fibrils) deposited on both a home-made glass-ITO substrate prepared by IBS (Fig.1A) and a commercial one (Fig. 1B), highlighting the higher quality of the former with respect to the latter.

References

[1] Simona Rodighiero, Bruno Torre, Elisa Sogne, Roberta Ruffilli, Cinzia Cagnoli, Maura Francolini, Enzo Di Fabrizio, and Andrea Falqui: Correlative Scanning Electron and Confocal Microscopy Imaging of Labeled Cells Coated by Indium-Tin Oxide, Micr. Res. and Techn., 78: 433-443 (2015) doi: 10.1002/jemt.22492

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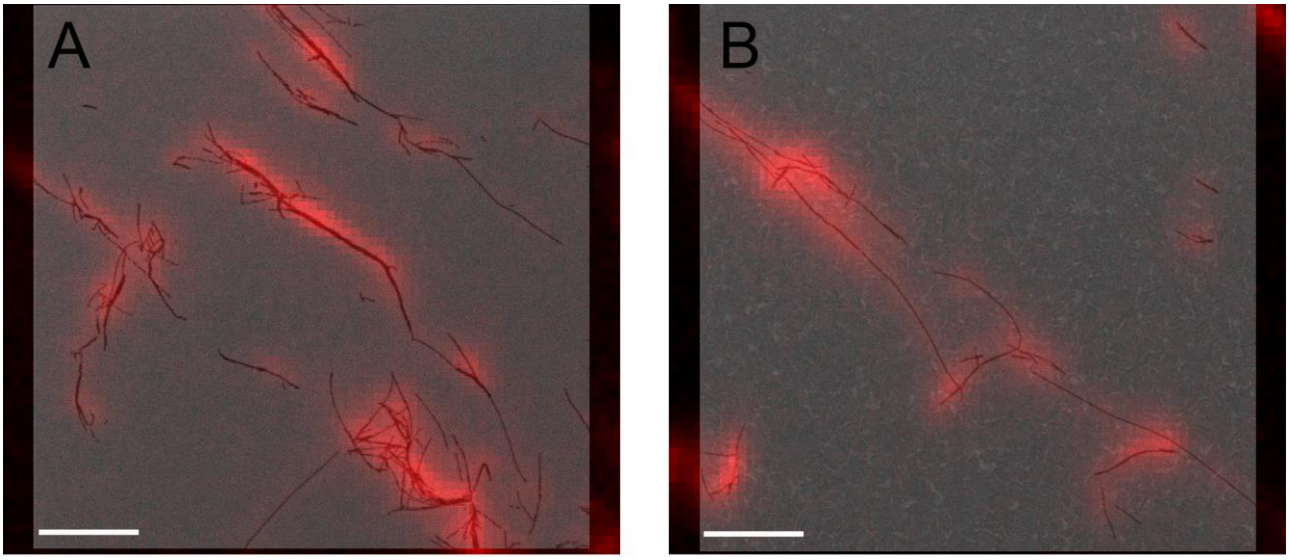

Figure 1: Comparison between two CLEM images of the α-synuclein fibrils labeled with Alexa Fluor 568 fluorophore deposited on an as-prepared 20 nm-thick film of ITO deposited by IBS (panel A) and a commercial one (panel B). The SEM and fluorescence images overlay is shown. The surface features observed on the commercial ITO substrate are not appearing on that deposited by IBS. Scale bar: 500 nm. Experimental conditions were as follows; SEM: 30 keV acceleration voltage, 40 us dwell time, 1024x1024 pixels; LM: 561/14 nm excitation, 607/34 nm emission, 3 s exposure time, 60x/1.4 NA objective lens.