Shrinkage of freeze-dried cryosections of cells : investigations by EFTEM and Cryo-CLEM

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Freeze-drying of cryosections of cells or tissues is considered to be the most efficient preparation for microanalysis purpose related to transmission electron microscopy since other preparation methods, including freeze-substitution, involves redistribution of mobile elements (1). Many analytical biological studies using freeze-dried cryosections have been published with different microanalysis methods (EDXS/STEM, EELS/STEM, EFTEM...) (Energy Dispersive X-ray Spectroscopy, Scanning Transmission Electron Microscopy, Electron Energy Loss Spectroscopy, Energy Filtered Transmission Electron Microscopy) (2,3,4). It allows the measurements of ions and water contents at the ultrastructural level. However, an important drawback is associated to freeze-drying: the shrinkage of the cryosections which is particularly problematic for water contents measurements by STEM dark field intensities measurements (5). It is then important to study shrinkage and especially differential shrinkage occurring in typical experimental conditions of freeze-drying. Reported values of lateral shrinkage vary from10% to 30% (6,7). Differential shrinkage has been reported between cellular compartments (8) and assumed to be direction dependent due to compression during cutting (9). Others studies report no substantial differential shrinkage (10). The aim of this presentation is the investigation of this phenomenon (11) by means of three different methods applied to both hydrated and dehydrated cryosections: direct distance measurements on fiducial points, thickness measurements by energy filtered transmission microscopy (EFTEM) and our original approach of cryo-correlative light electron microscopy (cryo-CLEM) (12). Measurements in our experimental conditions reveal a lateral shrinkage around 10 % but the most important result concerns the lack of differential shrinkage between most of the cellular compartments. Our results confirm the interest and validity of freeze-drying for sample preparation when analytical measurements at the subcellular compartments scale are concerned even if, due to collapse, high resolution imaging at the molecular level with atomic resolution prospect has to be performed in the frozen-hydrated state.

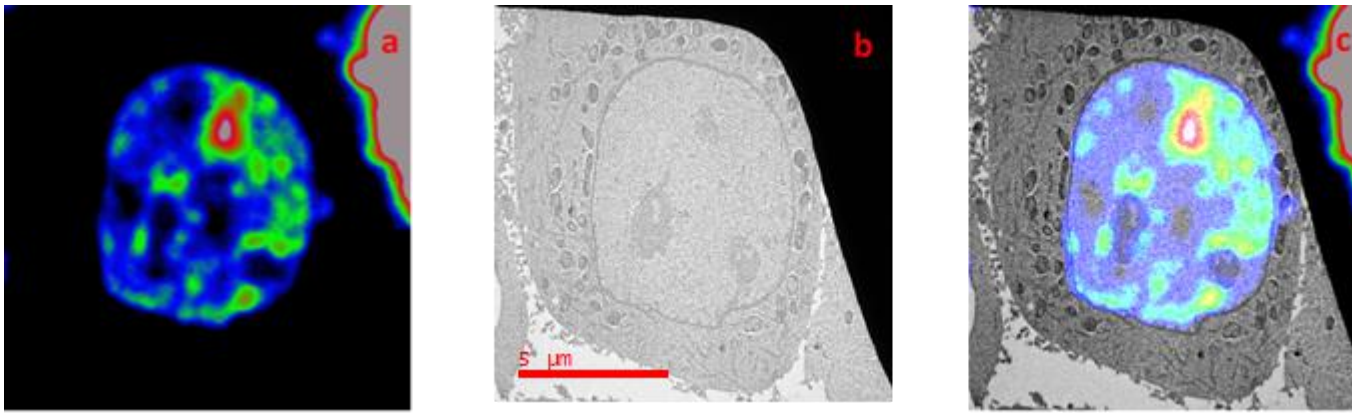

Figure 1: **(a)** Fluorescence image of a HeLa H2B-GFP cell in frozen-hydrated state. Fluorescence intensity of chromatin in the nucleus is coded in red, green and blue, corresponding to high, medium and low fluorescence intensity; **(b)** TEM image of the same cell in the freeze-dried state. **(c)** Merge of images (a) and (b).

Références

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