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Mapping of physiological and trace elements with X-PEEM

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Abstract. We present chemical mapping of *all* physiologically relevant elements at the subcellular level, as well as the trace element Gd. A broad energy range (60-1200 eV) is fundamental to investigate all the elements in the same microscopic locations. Concentrated elements (> 100 ppm) can be simply mapped by image ratio, while dilute species must be treated with great care to avoid artifacts. We propose a new method to obtain location maps of trace elements.

1.INTRODUCTION

Synchrotron spectromicroscopies now achieve the sensitivity and spatial resolution required to significantly contribute to biomedical studies. Many experiments focus on high-resolution at the cost of signal intensity and chemical sensitivity [1]. We discuss here an example of low-resolution, high-sensitivity experiments on cell microchemistry performed with X-ray PhotoElectron Emission spectroMicroscopy (X-PEEM) [2].

We use the SPHINX (Spectromicroscope for the PHotoelectron Imaging of Nanostructures with X-rays) X-PEEM from Elmitec (GmbH, Germany), mounted on the HERMON beamline of the Wisconsin-Synchrotron Radiation Center. SPHINX uses a magnetic lens column, to form a magnified image of the photoelectrons emitted by a specimen under soft x-ray illumination. The image magnification is continuously variable up to 10,000x, and the optimum lateral resolution is 5.5 nm, while the minimum detection limit is 80 ppm [3].

The detection of trace amounts of Gd is relevant for Gadolinium Neutron Capture Therapy (GdNCT) [2]. This element must be localized in the cell nuclei for the therapy to be successful [4]. We therefore optimized the sample preparations and data manipulation to achieve the greatest sensitivity to Gd and to positively identify the nuclei [5].

2.RESULTS AND DISCUSSION

Figure 1a presents the direct image of a group of four human glioblastoma cells cultured in vitro on a silicon substrate. The cells (MO59K cell line) were exposed to a gadolinium compound (100 μM HM-Gd-DOTA [5]) for 24 hours, washed, fixed, air dried, sputtered with 3 keV Ar ions for 10 minutes, then ashed for 120 hours in a UV/O_3 atmosphere. Despite the extensive sample preparation, the cell morphology appears unaltered, while the cell chemistry is preserved. Sputtering was adopted to access the inner portion of the cell bodies, otherwise not accessible to SPHINX (probe depth ~100 Å). Ashing selectively removes carbon while preserving the spatial distribution of all other elements[6,7]. In the present case, it was used to enhance the relative concentration of Gd which is present in trace amounts (often < 10 ppm and thus otherwise undetectable).

Figure 1b shows the distribution map of phosphorous obtained by digital ratio of two SPHINX image at 137 eV photon energy (on P 2p peak) and at 133 eV (pre-peak). Similarly the K, N, S, Na, Ca maps of figure 1c-g were obtained by the digital ratios of 297 and 296, 397 and 394, 178 and 176, 1049 and 1033, 346 and 345 eV images respectively.

The P map demonstrates that this element is present in higher concentration in the cell nuclei, as expected, because of the high density of phosphate groups in the DNA molecules. We typically use P maps to identify and outline the nuclei. The cell at the lower-center part of the image does not have a nucleus, as is frequently found in fast-cycling cancer cells which replicate with errors. The analysis of multiple elemental maps can be use to determine concentration ratios (e.g. [K]/[Na]) indicating that the cell is not in oxidative stress. These ratios are retained after fixing, sputtering and ashing.

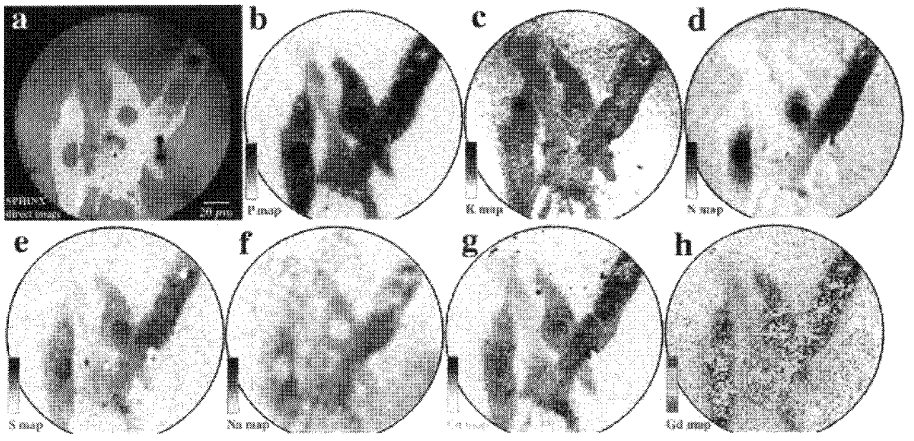

Figure 1. (a) Photoelectron micrograph acquired with SPHINX at 353 eV photon energy, showing 4 human glioblastoma cells. (b) P map acquired on the same 4 cells, demonstrating higher P concentration (darker color) in cell nuclei. (c) K map. (d) N map: N is also higher in nuclei, as is S (e), while Na is lower in nuclei (f). Calcium is slightly more concentrated in the perinuclear region (g). (h) Gd location map, binned 4 x 4 with respect to the other maps.

Figure 1h shows the Gd location map obtained via more sophisticated processing. Gd, present in much lower concentrations than the physiological elements, cannot reliably be mapped with image ratioing, because of unevenly distributed single pixel noise. We therefore consider the signal vs. noise in the spectra from every pixel in a stack of images, or "movie", acquired while scanning the photon energy. For Gd movies, we collect 81 averaged images, 1 every 1 eV, between 1220-1140 eV. We then "play" the movie away from the source, and extract a spectrum from each pixel. The Gd spectrum is normalized by a fitted 7th order polynomial, obtained by masking the Gd 3d peaks. The RMS noise is measured in the pre-edge region, and the integrated Gd peak intensity (Peak Area) is measured across a 12 eV energy window, as shown in Fig 2. If the spectrum from the pixel satisfies the criterion Peak Area / RMS >10, then the Peak Area is scaled and displayed in a spectrum color image; if the criterion is not satisfied, the data point is discarded, and Gd is considered non-detectable in that pixel. To save processing time, the movies

are binned 4x4 (the Gd pixel size is then 1.4 x 1.4 = 2 μm^2 in Fig.1h). This processing allows inter-comparison of local Gd concentrations between different cells or subcellular structures.

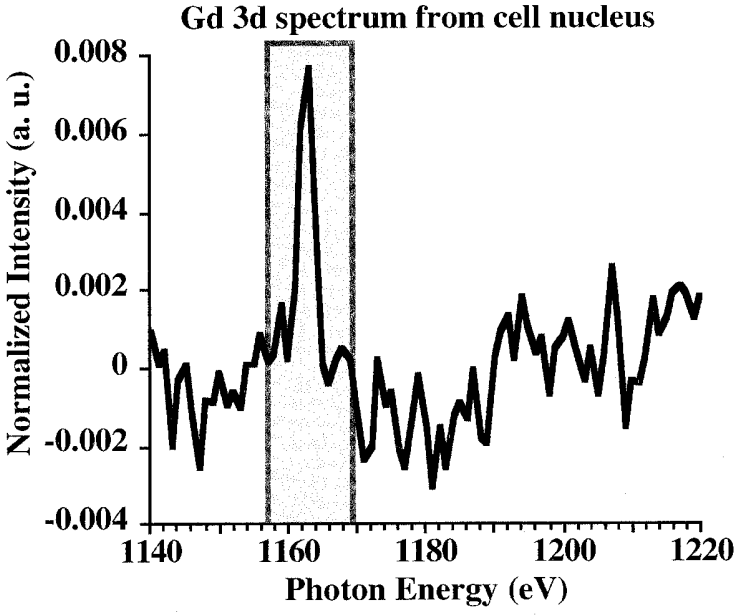

Figure 2. Gd 3d spectrum extracted from the same area of Fig. 1, from the nucleus of center cell. In this normalized spectrum the Gd $3d_{5/2}$ absorption peak is evident at 1163 eV. The shaded region between 1157 and 1169 eV indicates the energy range in which the peak area was measured for the Gd map of Fig. 1h.

3. CONCLUSIONS

Trace elements which have thus far represented a challenge for spectromicroscopy analysis, can now be detected and localized on a microscopic scale. By extracting single pixel spectra and displaying the true signal intensity, artifacts and pixel noise induced false positives are removed. This procedure is unnecessary for mapping most elements physiologically present in cells as presented.

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