New trends of X-ray microscopy in Biology

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X-ray microscopy techniques are emerging as powerful and complementary tools for sub-micron investigations. Soft X-ray microscopy traditionally offers the possibility to form direct images of thick hydrated biological material in near-native environment, at a spatial resolution well beyond that achievable with visible light microscopy. Natural contrast is available in the soft X-ray region, in the so-called “water-window”, due to the presence of absorption edges of the major constituents (C, N, O). Recent advances in manufacturing techniques have enlarged the accessible energy range of micro-focusing optics and offer new applications in a broad range of disciplines. In particular, X-ray fluorescence (XRF) radiation stimulated with monochromatic beam in the 1-20 keV range is very well suited for microanalysis. In this energy range zone-plates can still produce sub-micron probes and the sensitivity of XRF is about three orders of magnitude better than obtainable with electron probes. This allows not only a tremendous reduction of the dose required per unit of detected mass but also opens the possibility of detection of trace elements. These factors combined with the capability of imaging relatively thick samples in a wet environment make XRF a very attractive tool for microanalysis. The main unique attributes of X-ray microscopy in the “multi-keV” energy range are: i) The access to K-absorption edges and fluorescence emission lines of medium-light elements and L,M - edges of heavy materials allows micro-spectroscopy (e.g. XANES), chemical mapping, trace element mapping and specimen labelling with high spatial resolution. ii) The higher penetration depth compared to soft X-rays allows imaging of thick samples, in particular in their natural, wet environment. iii) Large focal lengths and depths of focus give suitable conditions for specific sample environments and X-ray tomography.

After a brief description of the intruments, examples of applications will be presented. These latter can be split into two major categories: elemental or chemical state mapping at tissue and/or sub-cellular scales. For instance the distribution of calcium in hair, which may reflect the prevalence to certain diseases, has been mapped, revealing its co-localization with lipids (1). X-ray microscopy has also proven to provide unique information in the study of the carcinogenesis of chromium (2). Cross-sections of testicular tissues from mice exposed to chromium were analysed, demonstrating the presence of Cr in the tunica albuginea and in particular cells of the interstitial tissue, where the Cr concentration below 5 µg/g dry mass in average could not be mapped with other methods. At the cellular scale, mapping of the spatial distribution of Cr oxidation states has been performed on cells exposed in vitro to carcinogenic concentrations of soluble and insoluble Cr(VI). So far no other micro-analytical techniques were sensitive enough to perform chemical state mapping at so weak a concentration. Last, X-ray micro-spectroscopy in the multi-keV range offers unique possibilities for studying the biological roles of metals ions. This will be illustrated by the localization and speciation of vanadium in blood cells from ascidians, which are marine animals able to concentrate sea water vanadium by a mechanism which is still unknown (3).

Finally, further developments related to biological applications will concern the integration of a cryo-chamber into the microscope chamber and, the development of original phase contrast techniques to make possible correlation between morphological and chemical information in weakly absorbing biological samples.

These projects have been developed in collaboration with: (1) J. Doucet, LURE, (2) G. Devès, LCNAB, CNRS UMR 5084, Gradignan, France, and K. Kasprzak, L. Anderson, Laboratory of Comparative Carcinogenesis, National Cancer Institute, USA, (3) H. Kihara, Kansai Medical University, Hirakata, Japan, and H. Michibata, Mukaishima Marine Laboratory, Hiroshima, Japan.