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Correlative Chemical Element Imaging in Cells Using Fluorescence Microscopy and Synchrotron X-ray Fluorescence

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Synchrotron radiation X-ray fluorescence (SXRF) microscopy holds the potential for fundamental breakthroughs in the understanding of biological systems because chemical element distributions can be evidenced at the sub-cellular level (<100 nm) with low detection limits (<100 ng/g). In addition to SXRF, it is also possible to perform X-ray Absorption Spectroscopy (XAS) which is based on the photo-electric effect. X-ray absorption near edge structure (XANES) provides information about oxidation state and molecular geometry of the elements. What is the distribution of trace metals in cells? Do some elements accumulate within sub-cellular organelles? What are the chemical species of the elements in these organelles? These are some of the fundamental questions that can be addressed using X-ray chemical element imaging and speciation with synchrotron radiation beams [1].

The combination of SXRF with other imaging techniques enables comparing trace element distributions with subcellular structures, i.e. organelles, or protein distributions. Correlative microscopy is the best solution to provide reliable information about such subcellular localization of the elements. Several methodological approaches are possible. For example, SXRF has been combined to immunofluorescence microscopy [2] or to transmission electron microscopy [3]. Live cell fluorescence imaging prior to SXRF is also an interesting combination since it needs minimal sample preparation and therefore native trace element distributions can be preserved [4] (Fig. 1).

Examples of application can be divided according to the type of labeling used for live cell fluorescence microscopy. Some applications did not use any labeling but the auto-fluorescence properties of the organelles, such as in the case of the cytoplasm and chloroplast of aquatic protist cells [5], or the natural fluorescence of iodinated-polymyxin antibiotic drugs [6]. Subcellular fluorescent imaging can be performed easily with organelle specific organic fluorescent dyes. A wide variety of fluorescent stains are commercially available for organelles. Mitochondrial, lysosomal and nuclear dyes have been used to identify precisely these organelles prior to SXRF imaging [7-10]. Fluorescent-tagging of proteins, i.g. with GFP (green fluorescent protein), have also been applied in combination with SXRF for organelle identification such as for the endoplasmic reticulum [10] or the Golgi apparatus [11] (Fig. 2). In this late example, SXRF imaging was complemented with X-ray absorption spectroscopy for element speciation of oxidation state. GFP-tagged proteins are also valuable tools to locate metallo-protein inclusions such as for example in the study of amytropohic lateral sclerosis with cells overexpressing the Cu,Zn superoxide dismutase [12]. Several studies have also reported the use of fluorescent metal sensors in the context of SXRF imaging at the single cell level, such as copper-selective metal sensors [13, 14] and zinc fluorophores [15].

This communication will review the applications of SXRF imaging combined to live cell fluorescence microscopy for element correlation with cellular structures or protein distributions. Protocols for optimal sample preparation and perspectives of instrumental developments for integrated correlative microscopy will be discussed.

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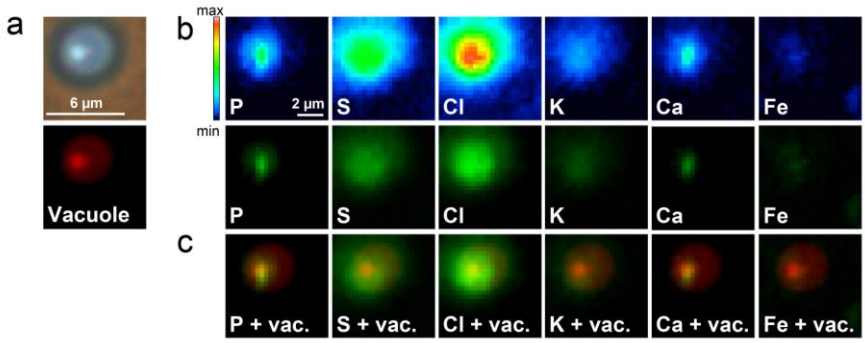

Figure 1. Correlative organelle fluorescence and SXRF microscopy of a yeast cell. a) yeast with Arg-CMAC labeled vacuoles (blue fluorescence), and in false color (red) for the purpose of correlative imaging; b) SXRF chemical element distribution (ESRF ID21) (multi color bar) and same distribution maps in green for red green correlation; c) correlative imaging of SXRF (green) and vacuole fluorescence (red) showing the accumulation of P, Cl, and Ca within the yeast vacuole. Reproduced from [4], with permission from Springer.

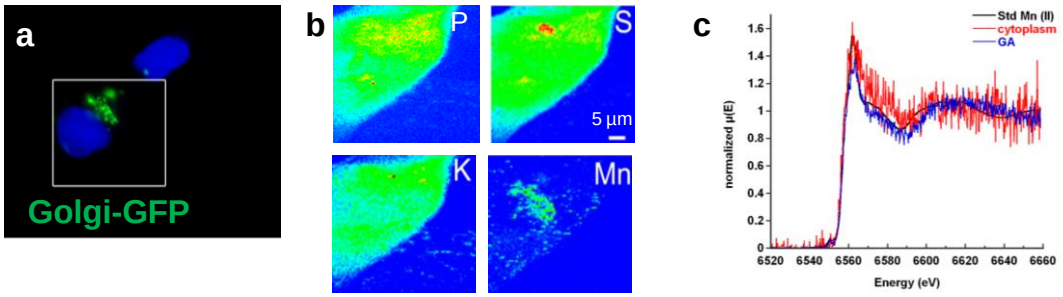

Figure 2. SXRF and XANES with organelle fluorescence identification a) live cell fluorescence imaging of Golgi apparatus (GFP, green) and nucleus (Hoechst dye, blue) in neuroblastic cells. b) SXRF element imaging (ESRF ID21 beamline) of the same cell after cryofixation (analysis performed at liquid nitrogen temp. in frozen hydrated state). c) Micro-XANES at Mn absorption K-edge in the cytoplasm and Golgi apparatus (GA) showing the presence of Mn(II) in both compartments. Adapted from [11].

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