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Imaging of Cellular Trace Metals by Fluorescence Scanning X-Ray Microscopes

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Third generation synchrotron radiation sources offer source brightness 8-9 orders of magnitude higher than laboratory x-ray sources, and it has become an essential structural biology tools for solving protein structures with Angstrom resolution using sophisticated x-ray crystallography techniques. However with recent advances in x-ray focusing optics, such as Fresnel zone plates and mirror optics, it has become possible to image in real space single cells [1] and bacteria [2]. Synchrotron x-rays can now be routinely focused to a spot size < 200 nm, with > 10^9 monochromatic x-ray photons per second at the focal spot [3]. This allows practical scanning x-ray microscopes to be built (Fig. 1). In particular, using the scanning x-ray microscope in x-ray fluorescence mode (analogous to SEM/EDX) provides a set of unique capabilities for biological microanalysis:

- Due to the large x-ray penetration length, minimal specimen preparation is required. In fact, this had been used for studying natural bacteria in a hydrated state (Fig. 2).
- Trace concentrations of < 1 part-per-million (ppm) are detectable for most transition and heavy metals. Co-localization of multiple elements can be directly imaged without any marker or enhancing agent.
- The spatial resolution is mostly determined by the quality of the focusing optics, rather than the specimen thickness.
- It can also reveal the chemical states, such as Cr (VI) vs Cr (III), Pt (IV) vs Pt (II), by performing micro-XAFS (x-ray absorption fine structure).
- Overlapping fluorescence lines from different elements may be separated by optimizing the incident x-ray energy (e.g. 12.8 keV x-rays will excite As and Se K_α, but not Pb L_α).

Applications had been performed on trace metals of exogenous and endogenous origins. In the former case, it is particularly useful for imaging small metal complexes such as cisplatin, chromium carcinogens, metal-containing non-steroidal anti-inflammatory drugs (e.g. copper-indomethacin), where attachment of biomarkers to the complexes is undesirable and may lead to possible alternation of cellular metabolism. In the latter case, it has been applied for studying the host environment in mycobacterial-infected macrophages, cell differentiation mediated by metalloproteins, and metal accumulation in marine protests. The high elemental sensitivity of this technique allowed these studies to be performed at naturally occurring concentrations.

References

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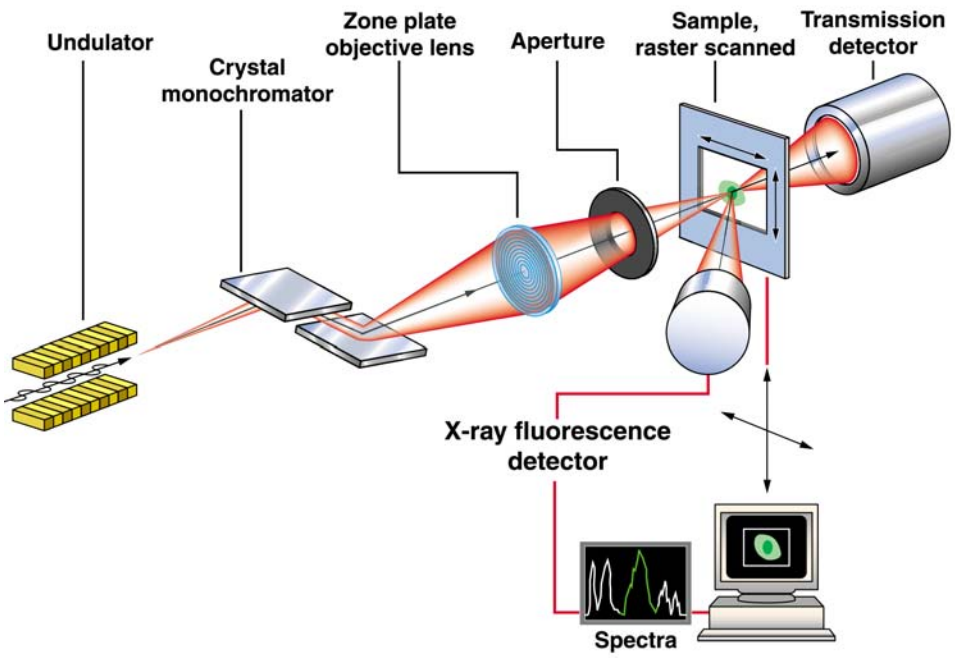

Fig. 1 Schematic of a scanning x-ray microscope in fluorescence mode.

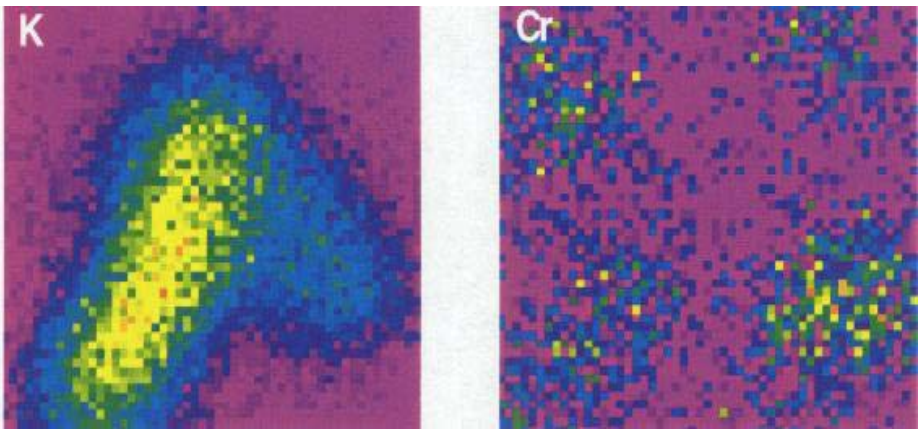

Fig. 2 X-ray fluorescence images of hydrated *Pseudomonas fluorescens* bacteria treated with chromium (VI) solution; elemental maps of potassium and chromium are shown. The image area is 5 x5 μm^2.

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