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# Applications and Future Prospects for X-ray Fluorescence Microprobe Analysis

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X-ray fluorescence microscopy is ideally suited for trace metal profiling and quantification due to its inherent elemental sensitivity of ~ 0.1-10 parts per million (ppm). A finely focused x-ray beam of 5-30 keV is used to excite characteristic x-ray emissions from a specimen, and the total metal concentration can be measured directly without any labeling with fluorescent sensors. Currently, a spatial resolution of ~ 200 nm is achieved routinely at the 2-ID-D station of the APS, with a minimum detection limit as low as 3 attograms for zinc ($2.7 \times 10^4$ atoms) within one second of data acquisition time. Typically, images of as many as 10-15 different elements are acquired simultaneously, thus ensuring complete alignment between various elemental images. The large penetration depth of x-rays allows the study of whole cells without sectioning, and is suitable for tissue sections of > 10 μm thickness, as well as hydrated/frozen samples. In addition, the possibility of performing micro-XANES analysis at discrete locations enables the oxidation state for the element of interest to be determined. These capabilities, which are complementary to optical and electron microscopy, have been utilized in studies of single bacterial cells (Fig. 1).

Recently, spot sizes as small as 30 x 50 $nm^2$ have been reported in the hard x-ray regime [2]. It is anticipated that focusing to < 5 nm with high efficiency will be possible [3]. The main limitation at that point will be radiation damage to the specimen. Generally, higher levels of resolution are associated with higher doses; however, this is not the case for XRF microprobe analysis. For example, in order to detect 100 localized zinc atoms within a 100-nm thick biological specimen, a 200-nm probe would need to deliver $2 \times 10^{11}$ Gy to the sample, whereas a 5-nm probe would need to deliver $3 \times 10^8$ Gy (see Fig. 2). This surprising result stems from the fact that, while the fluorescence signal increases as the probe size becomes smaller, the spectral background stays constant. Assuming a dose of $10^{10}$ Gy can be tolerated by properly cryo-protected specimens without significant mass loss or morphological changes [5], the detection limit for a 5-nm probe may be as low as a few zinc atoms. Thus, with further improvements to focusing optics, future fluorescence nanoprobes may achieve both high resolution and sensitivity needed for mapping individual metalloproteins and metal-containing macromolecules in biological specimens [5].

References

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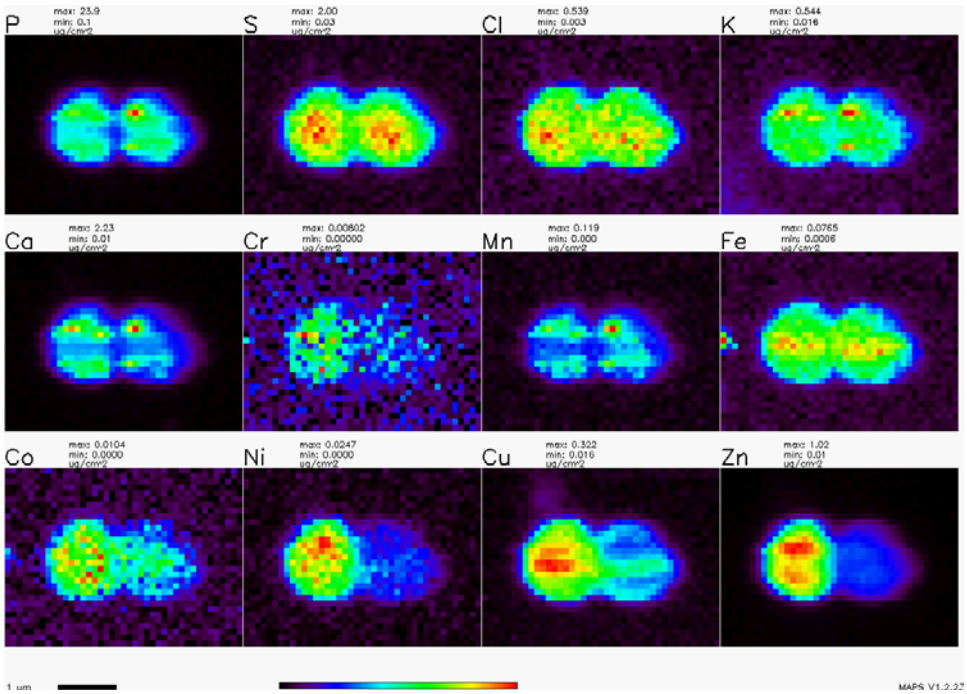


FIG. 1. Submicron-based x-ray fluorescence microprobe analysis of a single *Deinococcus radiodurans* tetracoccus, revealing the distribution of elements within the group of four cells [1]. The element distribution images are plotted to different scales designated by a single color-box, where red represents the highest concentration and black the lowest. The scan area was 4 x 3 $\mu m^2$.

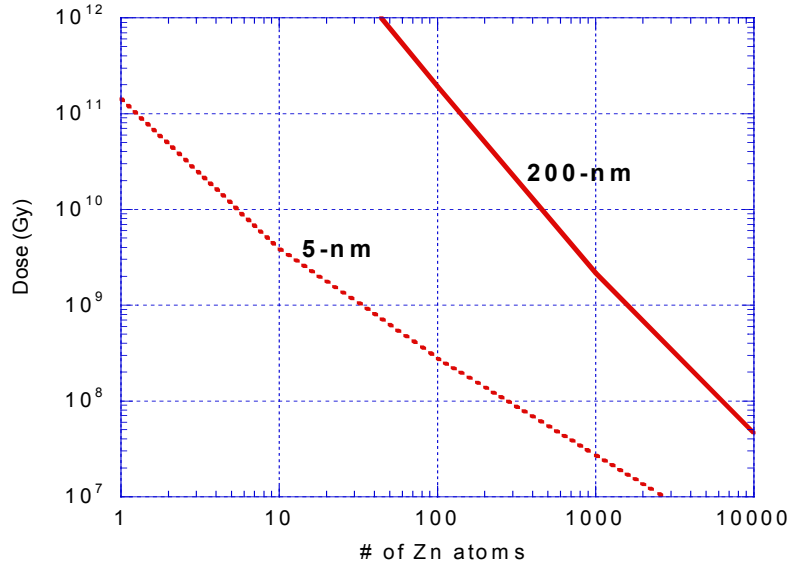


FIG. 2. Dose required to detect a fixed number of localized Zn atoms at the focus of a 200-nm (solid curve) and a 5-nm (dotted curve) probe. The calculation assumed the number of photons (E = 10 keV) at the focus spot was constant, and the specimen consisted of low-Z materials with density = 1 $gm/cm^3$ and thickness = 100 nm. The characteristic Zn $K_\alpha$ x-ray fluorescence was collected by a conventional energy dispersive detector. The criterion for detection is that the integrated Zn $K_\alpha$ signal-to-background ratio has to be ≥ 3.