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Large area synchrotron X-ray fluorescence mapping of biological samples

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ABSTRACT: Large area mapping of inorganic material in biological samples has suffered severely from prohibitively long acquisition times. With the advent of new detector technology we can now generate statistically relevant information for studying cell populations, inter-variability and bioinorganic chemistry in large specimen. We have been implementing ultrafast synchrotron-based XRF mapping afforded by the MAIA detector for large area mapping of biological material. For example, a 2.5 million pixel map can be acquired in 3 hours, compared to a typical synchrotron XRF set-up needing over 1 month of uninterrupted beamtime. Of particular focus to us is the fate of metals and nanoparticles in cells, 3D tissue models and animal tissues. The large area scanning has for the first time provided statistically significant information on sufficiently large numbers of cells to provide data on intercellular variability in uptake of nanoparticles. Techniques such as flow cytometry generally require analysis of thousands of cells for statistically meaningful comparison, due to the large degree of variability. Large area XRF now gives comparable information in a quantifiable manner. Furthermore, we can now image localised deposition of nanoparticles in tissues that would be highly improbable to 'find' by typical XRF imaging. In addition, the ultra fast nature also makes it viable to conduct 3D XRF tomography over large dimensions.

This technology avails new opportunities in biomonitoring and understanding metal and nanoparticle fate ex-vivo. Following from this is extension to molecular imaging through specific anti-body targeted nanoparticles to label specific tissues and monitor cellular process or biological consequence.

KEYWORDS: Accelerator Applications; X-ray fluorescence (XRF) systems; X-ray detectors

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2014 JINST 9 C12040

Contents

2014 JINST 9 C12040

1 Introduction

XRF imaging is undergoing a paradigmatic shift with regard to experimental design and application in biology. This is being driven by ultrafast detector systems that are enabling rapid imaging, such as the MAIA detector implemented on bright synchrotron X-ray sources. On-the-fly detection and processing provides real time image output with effective acquisition times per pixel on the order of a few milliseconds. This speed is roughly 3 orders of magnitude faster than has conventionally been used at synchrotron facilities.

Biology is conventionally highly heterogeneous at every level. As such, analytics in biology utilise large statistics and population data. Imaging flow cytometry for instance can assess thousands of individual cells within an hour. Synchrotron XRF imaging has traditionally used detector systems such as multi element Germanium or Silicon drift detectors often requiring at least 1 second acquisition time per pixel for biologically relevant concentrations. As such, many published studies have compared populations on the order of 3 cells under each condition, each requiring several hours imaging time. Clearly, there is large disparity between the statistics necessary in biology and the capability of XRF imaging in such instances. However, a three-order improvement in acquisition speed is enabling adequate capability in expanding our knowledge in biological systems in a more statistically satisfactory way. This contribution acts to briefly overview new areas of research in the study of metals in animal tissues and cell benefitting from the ability to image larger areas and greater numbers of samples.

Details of the Maia detector are described in the literature and was created in a joint program between CSIRO, Australia, and Brookhaven National Laboratory, U.S.A., with subsequent commissioning at the Australian Synchrotron for XRF mapping [1]. The purpose of this contribution is to highlight the impact and future perspectives new detector technology is having in bio-related research.

2 Cellular imaging

Intercellular variability arises due to many factors such as cell lines, cycle, genetic homogeneity, stressors, and microenvironment. This leads to varying metabolism, cycling, and expression which

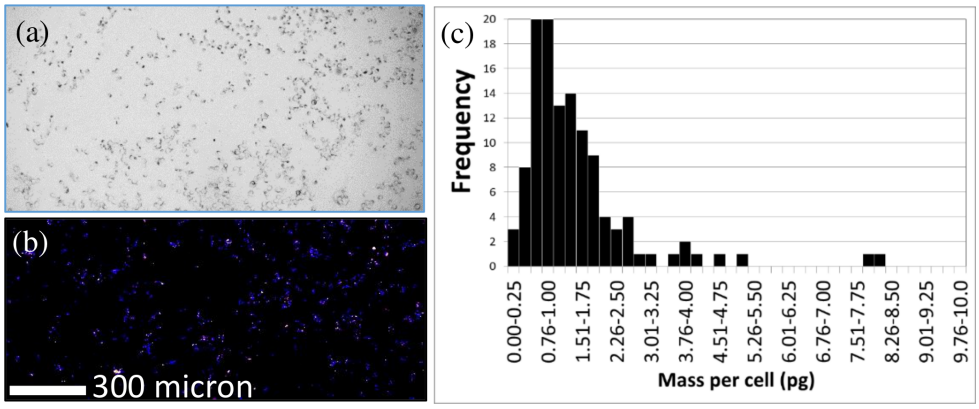

Figure 1. A breast cancer cell line cultured (a) in the presence of gold nanoparticles. Cultures were imaged for Au (b) and the quantity of gold in every individual cell was determined (c). This data indicated that less than 50% of the cells had actually internalized nanoparticles and the intercellular variability was much greater than may be expected with the majority of cells containing a nanoparticle content within a range of one order of magnitude.

influence cellular response and interaction. Additionally, heterogeneity in the exposure of cells to localized concentrations, gradients and inter-cellular dependencies also vary cellular interactions and uptake of biomolecules or other exogenous material such as nanoparticles. Ultimately, this impacts homeostasis, morbidity and delivery of various therapeutics. In recent years, particular interest has grown in the delivery and fate of nanoparticle-based therapies and diagnostics. Of significance to the efficiency and efficacy is the homogeneity of delivery and fate at the intended site. Nanoparticle design greatly impacts fate [2], but often is not characterized on an individual cell level. Fluorescent markers can be used however add to complexity and may influence surface interactions. It is also very challenging to quantify the number of the markers in an analysis area by optical means. Synchrotron XRF however offers quantitative imaging of inorganic materials with subcellular resolution and often, no requirement for labeling or staining. Figure 1 shows an example of a cell culture and a representative XRF image of the gold distribution after exposure to, and uptake of gold nanoparticles. Less than 50% of the cells had internalized nanoparticles. Individual cells could subsequently be analyzed for the quantified amount of gold which is represented in the histogram given in figure 1c. It is immediately obvious that there exists massive heterogeneity between cells. Individual cells cultured under identical conditions can take up quantities of nanoparticles an order of magnitude different, if at all. This will have significant impact on therapeutic intentions. Furthermore, it can be expected that studying differences between test variables will not be distinguishable without the analysis of large numbers of cells. In this case, the XRF image required 3 hrs acquisition with 2×2 micron pixels for an image of 3 x 1.5 mm. Acquiring such statistics via XRF imaging with conventional detector technology is simply infeasible.

XRF imaging has been further applied to 3D cell models where the delivery of drugs or nanoparticles could be imaged in sections of 'spheroid' models that are used to simulate a tumour mass [3, 4]. This information is acquired from statistically relevant areas and numbers of samples which is supporting the design of drug and nanomedicine formulations.

2014 JINST 9 C12040

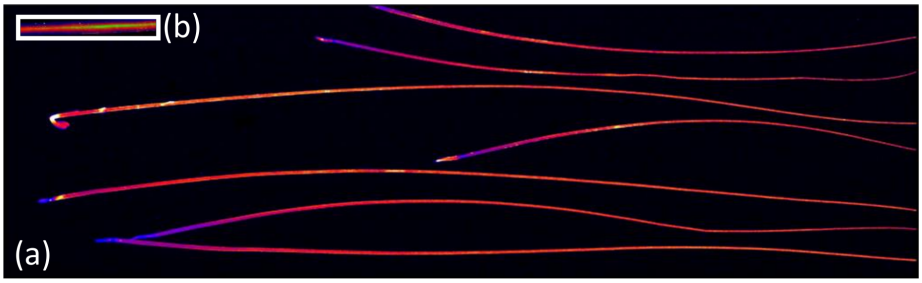

Figure 2. A large area XRF map of Zn in a selection of mouse whiskers (a). In a separate scan using a conventional detector and a comparable acquisition the inset image was produced and is provided to give a direct comparison between the new and conventional technologies (b). The colour scale indicates an increasing concentration from black to blue, purple, red, yellow, and white being the maximum.

3 Tissue imaging

Of greater significance for applied research is the analysis of clinically relevant tissues. Here, ultrafast XRF imaging may be applied to understanding metal association in various morbidities [5]. Such information can relate to disease etiology with relevance to early diagnosis and therapies, as well as understanding environmental exposure to metal pollutants [6], or understanding the fate of nanoparticles for delivery to tumour for example [7].

A large area image example of mouse whiskers is shown in figure 2 which was collected over a period of approximately 4 hours. The image shows longitudinal variation in the zinc content which is systemically altered as a result of certain morbidities [8, 9]. This study was examining changes in Zn with tumour development in an animal model. The image inset (figure 2b) is a representative image of a hair collected for approximately the same acquisition time using a solid state germanium detector. The new technology provides greater detail for larger areas and greater numbers of samples. Collecting data from multiple samples enables assigning errors and statistically distinguishing parameters. Furthermore, imaging large areas, of tissue for example, can help solve what can be described as a 'needle in a haystack'-type problems, where statistical probabilities for imaging a discrete phenomenon are low.

4 XRF tomography

Expanding spatial information to thick tissues beyond the ability of optical microscopies has further allure. The rapid image acquisition now facilities capturing 2D XRF images at multiple angles for computed tomographic reconstruction with full 3D elemental distribution over relatively large volumes. A reconstruction of a tumour spheroid-model is given in figure 3. In this case, the penetration of a gold nanoparticle formulation was assessed for its ability to penetrate the tumour model. The data can be used for quantitatively assessing distribution and various formulations in the optimising of nanomedicines. Again, the large degree of heterogeneity in biological samples is overcome by the ability to collect data over the entire volume of the model system. Furthermore in this scan, essential elements such as Fe, Cu and Zn were also observed. These elements play many roles in cellular level mechanisms of homeostasis however have also been linked as being potential prognostic factors in predicting survivability of patients.

2014 JINST 9 C12040

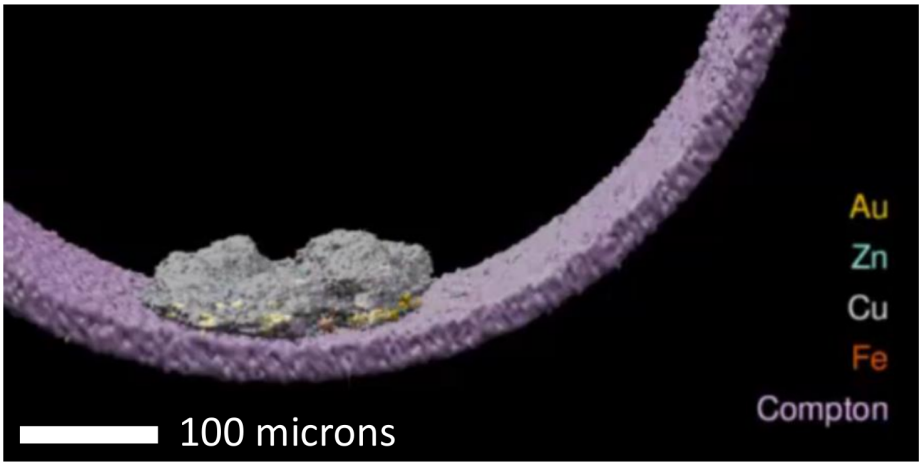

Figure 3. A 3D reconstruction of a tumour cell model adhered inside a capillary tube.

5 Summary

Ultrafast XRF imaging afforded by new detector technology is enabling immensely informative imaging power of metals in biological samples. This is providing statistical comparisons in what are inherently highly heterogeneous samples which rely on numerous variables. The opportunity to assess metals in biology quantitatively as a function of a large number of variables and of satisfactory statistics is offering exciting insights into metal and nanoparticle fate. This applies to various fields of research into disease etiology, exposure to pollutants and designing nanoparticle-based diagnostics and therapeutics. Ultrafast XRF mapping will also enable new approaches in forensic, toxicological and biomonitoring studies [10–12].

With regard to imaging metals in cellular and animal/human tissues, we anticipate that a wealth of new information will begin to emerge because of developments in detector technology such as the MAIA detector demonstrated here.

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2014 JINST 9 C12040

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