Published in final edited form as:

IEEE Trans Nucl Sci. 2015 October ; 62(5): 2310–2317. doi:10.1109/TNS.2015.2465380.

Element Mapping in Organic Samples Utilizing a Benchtop X-Ray Fluorescence Emission Tomography (XFET) System

A. Groll [Member IEEE],

Nuclear, Plasma, and Radiological Engineering, University of Illinois at Urbana-Champaign, IL 61801 USA

J. George,

Nuclear, Plasma, and Radiological Engineering, University of Illinois at Urbana-Champaign, IL 61801 USA

P. Vargas,

Department of Radiology, University of Chicago, IL 60637 USA, telephone: 773-702-6975

Dr. P.J. La Rivière [Member IEEE], and

Department of Radiology, University of Chicago, IL 60637 USA, telephone: 773-702-6975

Dr. L. J. Meng [Member IEEE]

Nuclear, Plasma, and Radiological Engineering, University of Illinois at Urbana-Champaign, IL 61801 USA, telephone: 217-333-7710

A. Groll: groll1@illinois.edu; J. George: jgeorge3@illinois.edu; P. Vargas: vargas@uchicago.edu; P.J. La Rivière: pjlarivi@uchicago.edu; L. J. Meng: ljmeng@illinois.edu

Abstract

X-ray fluorescence computed tomography (XFCT) is an emerging imaging modality that maps the three-dimensional distribution of elements, generally metals, in *ex vivo* specimens and potentially in living animals and humans. Building on our previous synchrotron-based work, we experimentally explored the use of a *benchtop* X-ray fluorescence computed tomography system for mapping trace-metal ions in biological samples. This system utilizes a scanning pencil-beam to stimulate the object and then relies on a detection system, with single or multiple slit apertures placed in front of position-sensitive X-ray detectors, to collect the fluorescence X-rays and to form 3-D elemental map without the need for tomographic imaging reconstruction. The technique was used to generate images of the elemental distributions of a triple-tube phantom and an osmium-stained zebrafish.

I. Introduction

It is well known that naturally occurring metal ions play important roles in disease, critical physiological function, and biological toxicity [1], [2]–[4]. In addition, heavy-metal constructs such as gold nanoparticles are being developed as imaging or therapeutic agents aimed at tumors and other lesions [5]–[7]. Both of these lines of research would benefit greatly from imaging methods allowing ex-vivo high-resolution mapping of metals in biological tissue.

Synchrotron-based X-ray fluorescence computed tomography (XFCT) has been explored as a way to map the distribution of trace metals in biological samples [1]. This technique typically employs a pencil beam of highly intense and monochromatic X-ray beam to illuminate the object. Among other interactions, the incident X-rays undergo photoelectric interactions with metal atoms, which will emit characteristic X-rays in response. The characteristic X-rays are collected by a non-imaging X-ray detector, and, in the absence of significant attenuation, the height or area of the measured characteristic X-ray peak can be directly related to the line integral through the elemental distribution map. The object is then scanned and rotated through the illumination beam in order to acquire a complete sinogram of line integrals in a first-generation tomographic acquisition [8]–[14]. A standard analytic or iterative CT algorithm can then be used for image reconstruction, although in the presence of attenuation, alternative models and methods are needed [15]. This first-generation geometry can achieve spatial resolutions limited only by the X-ray focusing optics, which can readily reach ~250 nm and can be pushed below 100 nm with specialized stabilization equipment. Naturally, the maximum size of the imaged specimen is limited by attenuation, dose, and imaging time considerations.

In recent years, several groups have begun to study the potential of developing a benchtop version of XFCT using conventional sources [6],[16],[10],[17]. The initial work has focused largely on organism-scale imaging of metal-based nanoparticles at multi-millimeter resolutions. Most of these approaches also use a variation of the first-generation CT geometry, with measurement of one line integral at a time.

We have been exploring an alternative geometry aimed at achieving ~100 micron resolution in ex vivo biological specimens [12],[13]. We call the approach X-ray fluorescence emission tomography (XFET) and it couples apertures typically seen in emission tomography with position-sensitive X-ray imaging spectrometers. It still employs a pencil beam of X-rays to excite the metal atoms in the object, but now the fluorescence x-rays are detected by an imaging detector placed behind a slit aperture. The emissions from a small segment of the illumination line are thus imaged onto a column of the detector and direct image “reconstruction” is possible without the need for solving an inverse problem.

In our previous work, we have carried out a series of Monte Carlo studies to demonstrate that in this regime the proposed XFET imaging geometries could significantly improve the sensitivity over the first-generation geometry [13]. We have also carried out a preliminary experimental study to demonstrate the feasibility of XFET with a synchrotron X-ray source [14]. The present paper demonstrates the translation of our approach to the benchtop and discusses methods used for performing spectroscopically accurate single-photon counting in a detector not natively designed for such work. XFET is a particularly attractive implementation for benchtop X-ray fluorescence imaging studies as it copes well with the reduced illumination intensities found in that environment. The transition away from synchrotrons and into the lab environment should result in a greater degree of freedom for the technology and presents opportunities for great refinement going forward.

II. Materials and Methods

A. Experimental Setup for the Benchtop XFET System

We have developed a prototype benchtop system (as sketched in Fig. 1) for imaging metal content within biological specimens. This system employs a dual-planar geometric design, as shown in Figs. 2 and 3. The lower-plane subsystem is for X-ray fluorescence imaging while the upper plane subsystem allows for standard radiography and computed tomography imaging, which provides useful information for alignment and attenuation correction. The sample must be translated between planes in order to take advantage of each portion of the experiment setup.

The lower-plane XFET subsystem contains the primary analytical X-ray source, an X-ray Optical Systems (XOS) X-Beam, whose X-rays are focused and monochromatized with a double-curved crystal monochromator and then collimated through a pinhole. This leads to a circular pencil-beam of X-rays of diameter approximately 100 μm . This source generates a flux of 1.55×10^7 photons/s/mm² of 17.5 keV (Mo K X-Rays) photons at a focal distance of 20.4 cm, or $\sim 1.2 \times 10^5$ photons/s within the 100 μm circular pencil beam.

For XFET data acquisition, we used a commercial X-ray CCD detector, Ikon-L (DO-936N), from Andor Technology [18]. The detector has 2048 $\times$ 2048 pixels of size 13.5 $\mu\text{m} \times 13.5 \mu\text{m}$, which leads to a total surface area of 2.76 cm $\times$ 2.76 cm. For this measurement, the vertical shift-speed was set to 76 μs with a horizontal shift frequency of 1 Mhz. We also used 2 $\times$ 2 pixel binning to optimize spectral performance. A single-slit aperture was placed in front of the Andor CCD detector, as shown in Fig. 2. The slit size was set and measured at $\sim 50 \mu\text{m}$.

The upper-plane CT subsystem, contains a standard polychromatic X-ray source from Oxford Instruments (Apogee 8000 series), which has a maximum tube potential of 50 kVp and a maximum tube current of 1.0 mA. The detector was a flat panel X-ray detector (Varian 1313 [19]) with 1024 $\times$ 1024 pixels 127 $\mu\text{m} \times 127 \mu\text{m}$ in size. The detector has a total active area of 13 cm $\times$ 13 cm. This detector is placed at 36.23 cm from the rotational sample, and the sample is placed 22.55 cm from the source which yields a source to detector distance of 58.78 cm. The sample holder itself is placed on a 5-dimension scanning system that is composed of 2 Newport stages, 2 Velmex stages, and a Micronix rotational stand. The necessity for a 5 dimensional system is derived from the need to control the CT magnification of the sample, the sample alignment with the CT, the rotational scanning during CT, the line by line scanning for fluorescence imaging, the fluorescence imaging magnification, and the sample alignment with the emission aperture of our Andor camera.

B. XFET Image Formation

The geometry of XFET image formation is shown schematically in Fig. 4. The pencil beam stimulates emission of characteristic X-rays as it passes through the object, depicted here as three cylindrical tubes corresponding to the physical test object to be described in the next section. The slit collimator creates a mapping between small segments along the pencil beam and vertical columns of the detector. A typical acquired slit image is shown in Fig. 5 where it can be observed only a fraction of the detector is actively used. Summing the

detected image vertically (i.e., collapsing over the rows) and reversing the resulting vector allows one to directly estimate the elemental distributions along the illuminated line, without the need to solve a tomographic reconstruction problem. By translating the object horizontally through the illumination beam, a 2D image of the slice can be built up, line by line. Vertical translation can allow for acquisition of additional slices.

The achievable spatial resolution for a given slice will be determined by the illumination beam extent (~100 micron spatial resolution as determined by the beam size) in one dimension and by the blurring introduced by the finite slit in the other. Due to the selected small sample size the highly focused and well collimated beam, changes in beam size across the sample are negligible. The specific geometry of the slit, camera, and tubes is illustrated in Fig. 6. The magnified extent of the slit in the beam plane is 62.9 microns.

C. Imaging Phantom and Osmium-Stained Zebrafish

This hardware was used to perform two separate experimental studies. The first imaging study involved a simple phantom comprising three capillary tubes filled with solutions: one a 25.0% (w/w) aqueous solution of sodium bromide, one a 25.0% (w/w) aqueous solution of iron (II) sulfate, and the last a 25.1% (w/w) aqueous solution of copper (II) nitrate. Each tube had an inner diameter of 0.798 mm and an outer diameter of 1.092 mm.

The second sample was a 5 day-postfertilization zebrafish embryo that was sacrificed, fixed, and stained with 1% osmium tetroxide and embedded in the resin Embed-812. The sample had been previously prepared for X-ray histology experiments. The zebrafish was approximately 2 mm in diameter and 14 mm tall. The resin encasement was approximately 3.4 mm in diameter.

The experiments were performed with 5000 frames per pencil beam illumination position with 2 seconds of exposure time per frame yielding a total of 5 hours per line when including readout time. To form a complete 2D image, the tube was translated linearly through 24 positions separated by 100 microns and the osmium stained zebrafish was translated through 12 positions separated by 100 microns.

D. X-Ray Photon Detection and Algorithm for Single Photon Counting

The Andor detector employed is not natively a photon-counting detector. To obtain energy information about individual events, we operate in a mode in which frames are read out rapidly enough that only a few isolated events are expected in each frame. This allows identification of individual X-ray events and determination of the deposited energy after careful correction and calibration.

In order to obtain an accurate energy response from the CCD detector, we have developed a single-photon counting routine to compensate for several physical factors that could degrade the energy information, and to determine the energy-deposition from each individual X-ray interaction. Given the small pixel size (13.5 m 13.5 m binned to 27 m 27 m) and the very thin depletion layer (15 m) of the CCD detector used in this experiment, X-ray photons interacting in the detector could result in signal charge being shared by multiple adjacent

pixels. In addition, long acquisitions could potentially lead the detector response to change with time or temperature.

The implemented single X-ray detection algorithm seeks to determine accurate single photon energy information as follows

1. 200 dark frames are collected from the CCD.
2. From the 200 background frames, a per-pixel average is calculated along with the average over all CCD pixels.
3. In order to minimize the influence of hot pixels, each per pixel average is compared to the overall CCD mean. The per pixel average is replaced with the CCD mean if the pixel average falls more than three standard deviations outside of the camera's noise. The resulting mean is used as the first offset subtraction.
4. A second iteration is performed after the initial offset subtraction. Each pixel in every background frame is reanalyzed. Because the noise of the camera follows a known Gaussian distribution, individual background frames for a given pixel with values which do not adhere to the distribution are not considered for just that pixel for that frame as they have either been influenced by background radiation or are hot (per frame behavior is not considered in the first iteration, only average behavior across all frames). A new true background is recalculated per pixel, and this offset is used.
5. Pixels which are constantly identified to not meet the criteria in the first and second iteration are recorded and have their offset calculated and subtracted to account for the hot behavior.
6. 2-D local maximum searching is performed across each data frame to identify single photon events. The sum over a 3×3 region around each maximum is computed.
7. The background is then continuously monitored to ensure no baseline drifting and corrected if so.

For each local maximum (a single photon event on the detector surface), this method yields the detector x-position, the y-position, the peak energy value, the summed energy value, and the source frame number. Figure 7 shows an example of the single photon events on the detector plane.

Plotting the spectra using the summed energy values derived from the single photon counting algorithm reveals that processing is necessary to improve energy resolution. Fig. 8 shows a scatter plot of the summed energy vs the peak energy divided by the summed energy. It can be seen that as the ratio deviates from 1, which represents full energy deposition into a single pixel, the peak shifts downward. The peak shifting needs to be corrected for to achieve the ideal horizontal alignment visible in the bottom of Fig. 8.

The correction is done by partitioning the x-axis into a selected number of bins. In this case, 20 bins were used, and the copper K-alpha peak was selected as the reference peak for the

correction. In each bin the peak maximum position of the copper K-alpha peak was located through Gaussian fitting along the y-axis. As a result of doing this, the offset from the unity case is known per bin. Therefore, all ratios can be vertically shifted with respect to the unity ratio to align the spectrum, and ultimately correct for charge-sharing.

E. Calibration and Attenuation Correction

Calibration and attenuation correction are essential for converting the raw count images to quantitative maps of elemental density. To do so, we use the method described in Ref. 14, with notational changes to reflect the fact that we are tracking multiple fluorescence lines for some elements. Refer to Table 1 for the complete quantities of the following equation.

For element denoted Z (e.g. bromine) the average number $y_{Z_{I_j}}^{Pix}$ of counts observed in the line I_j (e.g.. the line K_α) in pixel Pix is given by

$$y_{Z_{I_j}}^{Pix} = [I_0 A t F_{Pix}(\vec{\mu}_{inc})] \left[\left(\frac{\tau}{\rho} \right)_Z \rho_{Pix}^Z \Delta x p_I \omega_I v_j \right] \left[\varepsilon_{Z_{I_j}} \Omega G_{Pix}(\vec{\mu}_{Z_{I_j}}) \right] \quad (1)$$

The first term in square brackets represents the number of incident photons reaching the pixel of interest. The second term represents the fraction of those photons that lead to emission of a characteristic X-ray in line I_j of element Z . The third term represent the fraction of those emitted X-rays that are detected. The goal is, of course, to solve for ρ_{Pix}^Z , the density of element Z in pixel Pix .

Attenuation correction is critically important in XFET imaging of low- Z elements like copper and bromine in millimeter-size specimens. There is substantial attenuation of the low-energy fluorescence X-rays as they exit sample toward the detector and this must be accounted for to obtain quantitatively and qualitatively accurate images. This is represented by the factor $G_{Pix}(\vec{\mu}_{Z_{I_j}})$ in the equation, which depends on the attenuation map at the energy of the characteristic X-ray line I_j of element Z . Attenuation of the incident beam as it travels through the sample should also be accounted for, and this is represented by the factor $F_{Pix}(\vec{\mu}_{inc})$, which depends on the attenuation map at the incident beam energy. This is generally a smaller effect than fluorescence attenuation since the incident beam energy is necessarily higher than that of all the fluorescence emissions.

In our synchrotron-based implementation we were able to obtain an attenuation map at the monochromatic incident beam energy to use as input for attenuation correction. This map could be appropriately scaled to the energy of each fluorescence emission line with provision made for crossing K edges of significant elements. This was described in detail in Ref. [14]. Our current setup does not allow for acquisition of monochromatic attenuation maps and we are still developing an attenuation correction algorithm that is able to make use of the attenuation map that can be obtained by use of the polychromatic CT subsystem. To correct the present data, we employed the prior knowledge that the objects comprised known glass tubes containing unknown solutions. We formed a Compton scatter image from the Compton spectral energies windowed from 16 to 17.5 keV; this image is shown in Fig. 11.

We used this image to identify the tube centers and then segmented the images into annular glass and circular lumen components. The element contained in each lumen could be readily identified from preliminary reconstructions. Each lumen was thus identified as a solution of known elemental composition but unknown density/concentration. Making an initial guess of elemental density allowed us to calculate preliminary attenuation maps using tabulated values of X-ray attenuation coefficients for elements. We then used Eqn. 1 iteratively to solve for the unknown densities, updating the attenuation maps as we updated the densities. The algorithm can readily account for mixtures of fluorescent materials as well.

III. Results

A. X-Ray Photon Counting

The results of the spectral correction algorithms for the tube phantom can be seen in Fig. 9. The raw, uncorrected spectrum is shown in the top panel, and the corrected spectrum shown in the lower panel. For bromine, these comprise a K_{α} peak at ~ 11.9 keV (the $K_{\alpha 1}$ and $K_{\alpha 2}$ peaks at 11.924 keV and 11.877 keV, respectively, cannot be distinguished at this energy resolution), and the $K_{\beta 1}$ peak at 13.2 keV. For copper, we see the copper K_{α} peak (a blend of $K_{\alpha 1}$ at 8.048 keV and $K_{\alpha 2}$ at 8.027 keV) and the $K_{\beta 1}$ peak at 8.905 keV. Finally, for iron, only the joint K_{α} peak is barely visible at 6.4 keV. The tube containing iron, which has the lowest emission energies of the three elements, was inadvertently placed furthest from the detector and thus it suffered tremendous attenuation effects. Therefore, no further mention of iron is made.

In addition to being more distinct after correction, the various peaks are also more quantitatively accurate. The intensity of the bromine K_{α} emissions should be about 7.3 times higher than the K_{β} emissions. After correction, the K_{α} peak is about 6 times higher than the K_{β} peak, consistent with expectations. Finally, in addition to the characteristic X-ray peaks, there is a distinct single Compton scatter peak at 16–17.5 keV, spanning a relatively broad range of energies because of the range of scattering angles compatible with the system geometry. There is also a broad but low multiple Compton scatter background.

The second experimental study, with the osmium-stained fish, produced the raw and corrected spectra in Fig. 10. The osmium characteristic X-rays seen in this spectrum are the L-peaks, since the K-edge of osmium is well above the 17.5 keV beam energy, while the L edges are in the 10–13 keV range. The peaks observed are L_1 (7.82 keV), L_{α} (8.91 keV), L_{β} (10.35 keV), and L_{γ} (12.09 keV). The scattering peak was again observable.

B. Imaging Triple Tube Phantom and Osmium Zebrafish

The scatter image for the triple tube phantom is shown in Fig. 11. This is the image that was used to generate the attenuation map. The raw and corrected bromine and copper images are shown in Fig. 12. The uncorrected images, subfigures A and C in Fig. 12, have very strong vertical attenuation gradients because the slit is located at the bottom of these images, so X-rays emitted toward the top of each tube must pass through more material to reach the detector. There is also a distinct horizontal attenuation gradient because the incident beam, which travels from right to left in these figures, is attenuated as it travels across the specimen. The calibrated and attenuation corrected images, subfigures B and D, are seen to

be much more uniform. In addition, an artifactual source of high counts seen in the uncorrected copper image is greatly suppressed after appropriate calibration and correction. The source of this anomaly is not entirely clear.

We calculated the average and standard deviations of the elemental concentrations within the lumen of the tubes and the results are reported in Table 2. The expected concentrations of the metals are obtained using standard reference tables for the density of aqueous solutions of sodium bromide and copper (II) sulfate along with calculated mass fractions of the metals. The measured quantities are higher than expected by about 30–50%. The discrepancy could be explained by one or more of the following three factors: (1) We have not attempted to account for the presence of multiple Compton scatter, which produces a broad background in the energy spectrum, artificially elevating the elemental peaks. This can be accounted for in the future through the use of advanced spectral-fitting algorithms. (2) The estimated and calculated values for some of the calibration parameters (especially beam flux, solid angle, and detector efficiency) in Table 1 may have been inaccurate. This could be addressed by performing calibration using elemental standards of known concentration, which could allow for elimination of some of these factors. (3) The attenuation correction process may be overestimating the amount of attenuation. This process will be improved through development of an algorithm that can exploit the polychromatic CT data, possibly through a dual-energy acquisition that will allow for better quantitation of energy-dependent attenuation effects. It is difficult at this point to determine how effective these three improvements will be. To the extent that any of these errors are additive and independent of the actual concentration of elements present, they could swamp the true signal from a trace element present in much lower concentration than was used here. However, careful scatter estimation, calibration, and attenuation correction are widely used in conventional point scanning x-ray fluorescence measurements and allow for detection of parts per million concentrations of trace elements.

The zebrafish data was analyzed in a similar fashion. The scatter image is shown in Fig. 13. Its extent corresponds to the extent of the embedding resin, and was used to establish the attenuation map, which was again assumed to comprise contributions from a known matrix component and an unknown amount of osmium. The estimated attenuation map was updated along with the estimate of the osmium distribution. Four spectral peaks were generated from the osmium stain. These peaks were isolated to form the raw osmium image of Fig. 14(a). This energy window isolated the axial profile of the zebrafish. Finally, the calibrated and attenuation corrected image is shown in Fig 14(b). The osmium distribution is more uniform after attenuation correction, although a slight left-right asymmetry is still visible, indicating the need for further improvements in calibration and attenuation correction procedures. Quantitation of the osmium concentration of the fish is $0.31 \pm 0.09 \text{ g/cm}^3$ and is consistent with the $0.22 \pm 0.03 \text{ g/cm}^3$ we had previously measured using synchrotron-based L-edge subtraction, a gold standard for metal quantitation.

IV. Conclusions and Future Work

We have experimentally demonstrated the feasibility of performing X-ray fluorescence CT images with a relatively low-intensity laboratory X-ray source and an emission tomography

system utilizing a position sensitive X-ray spectrometer coupled to single slit aperture. Compared to the conventional first-generation XFCT geometry, the XFET geometry explored here offers two major advantages. First, it allows one to build up real-time 3-D tomographic elemental mapping *without* the need for tomographic image reconstruction. Second, since the origin of each detected fluorescence X-ray photon can be determined directly, this imaging geometry provides a very efficient way for collecting imaging information. Compared to the use of the traditional line-by-line scanning geometry used in synchrotron-based XFCT studies, the imaging geometry reported here could offer identical image quality with a much reduced dose rate to the object being imaged. It would also allow for a much shorter imaging time with compared to the use of the conventional XFCT imaging geometry (with the same incident X-ray beam) [14].

Despite using concentrations that are approximately two to three orders of magnitude higher than expected for trace metals in biological tissue, the current prototype XFET system has extremely long imaging times. The current prototype XFET system has extremely long imaging times, owing to the low intensity of the X-ray source and a relatively inefficient detection system using a regular CCD camera and a single slit aperture. In order to develop a more practical laboratory XFET system, we will use a rotating anode X-ray source, equipped with Mo target and a monochromator to generate a fine pencil beam of monochromatic X-rays. This will increase flux and reduce imaging time by at least one to two orders of magnitude. The single-slit aperture will be replaced by multiple-slit apertures as only 1/3 of current detector is being utilized, this can be seen in Fig. 5, thereby using more of the sensor area and further improving the detection efficiency of the imaging system. Thirdly, while the CCD detector currently in use has excellent imaging resolution and energy resolution, it does suffer from a slow readout speed and relatively low detection efficiency for X-rays above 10 keV. Further improvement in the X-ray detector would also help to improve the overall efficiency of the detection system for capturing and identifying fluorescence X-rays from the object.

Acknowledgments

This work was supported in part by NIH award R01 EB017293. The first author would like to thank University of Illinois's senior undergraduate design group, including Nick Backes, Brent Cross, Ashley Farnan, Jessica Hsu, and Robert Peterson for their help pushing the project during its infancy phase. The University of Illinois's Mechanical Engineering machine shop members created some the parts necessary for developing the project.

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Fig. 1. Overview of experimental setup with each individual component visible. The polychromatic CT source is situated directly on top of the monochromatic XOS source.

Fig. 2. Overview of experimental setup with each individual component visible. The monochromatic analytical source and polychromatic source are overlaid. The source to sample (SS) and the sample to detector (SD) are labeled.

Fig. 3. Alternative view of the experimental setup. The 50 μm slit emission aperture is visible and the central sample holder is present with a zebrafish sample in place.

Fig. 4. Conceptual representation of photons from a triple-tube sample propagating through the slit. Photons generate the expected flipped projection. Photons are only geometrically limited in the horizontal direction and are free to project anywhere along the vertical direction to produce the slit projection.

Fig. 5. Example projection of accumulated frames from the slit geometric setup discussed. The vertical projection that is generated by slit emission aperture is clearly visible.

Fig. 6.
Acquisition geometry showing key dimensions.

Fig. 7. Single photon event data from detector plane with two events which have been magnified. The summed 3×3 region and peak pixel are identified.

Fig. 8.

TOP: Plot of the ratio of peak/summed energy vs. summed energy for the triple tube sample.

BOTTOM: Corrected plot with improved resolution.

A. X-Ray Photon Counting

Fig. 9.

TOP: The uncorrected, raw triple tube spectrum from the CCD detector. BOTTOM: The Gaussian corrected spectrum which shows resolvable peaks.

Fig. 10.

TOP: The uncorrected osmium zebrafish spectrum. BOTTOM: Corrected spectrum. Multiple osmium peaks are visible along with the Compton scattering peak.

Fig. 11.

Compton peak image, collected from the corrected spectrum, of triple tube phantom obtained from 24 positional scans and mapped as 24 rows. The presented image, as well as those to follow, is cropped to only the portion of the detector which received enough events to form an image.

Fig 12.

(A) Raw counts bromine image. (B) Corrected and calibrated bromine image (C) Raw counts copper image. (D) Corrected and calibrated copper image. These images have been cropped to only the region of the detector which received enough counts to form an image as well. All images were derived from the Gaussian corrected spectrum.

Fig. 13. Compton scattering image collected from the zebrafish scan. This image was used to identify the boundaries of the embedding resin.

Fig. 14. Image data collected and mapped from the osmium zebrafish. (A) Raw counts osmium image. (B) Calibrated and attenuation-corrected image.

Table 1

Model parameters

Quantity	Definition	Units	Value for Br	Value for Cu
$y_{Z I_j}^{Pix}$	Detected number of photons in pixel for element (Z) and line (I_j) of interest	Number	NA: Measured quantity	NA: Measured quantity
I_0	Incident photon flux density	Number $s^{-1} cm^{-2}$	1.55×10^9	1.55×10^9
A	Beam area	cm^2	7.8×10^{-5}	7.8×10^{-5}
t	Dwell time	s	10,000	10,000
$F_{pix}(\vec{\mu}_{inc})$	Fraction of incident photons that reach pixel of interest without being attenuated	Dimensionless	Calculated from attenuation map at beam energy	Calculated from attenuation map at beam energy
$\left(\frac{\tau}{\rho}\right)_Z$	Photoelectric cross section for element Z at incident energy	cm^2/g	73.98	47.8
p_I	Participation fraction of shell I	Dimensionless	0.86	0.87
ρ_{pix}^Z	Density of element Z in pixel P_{ix}	g/cm^3	NA: Quantity being estimated	NA: Quantity being estimated
x	Voxel size	cm	0.0007	0.0007
	Fluorescence yield of shell I	Dimensionless	0.618 (K shell)	0.439 (K shell)
α	Branching ratio of detected line (s)	Dimensionless	0.88 (K_α), 0.12 (K_β)	0.9 (K_α), 0.1 (K_β)
$G_{pix}(\vec{\mu}_{I_j})$	Fraction of emitted X-rays that reach slit opening without being attenuated	Dimensionless	Calculated from attenuation map at beam energy (see text)	Calculated from attenuation map at beam energy (see text)
$\varepsilon_{Z I_j}$	Detector efficiency at emission energy	Dimensionless	0.1 (K_α), 0.08 (K_β)	0.3 (K_α), 0.2 (K_β)
	Geometric efficiency of detection	Dimensionless	0.0007	0.0007

TABLE 2

Expected and measured metal concentrations

	Expected (g/cm ³)	Measured (g/cm ³)
Bromine	0.24	0.31 ±0.06
Copper	0.11	0.17 ±0.04