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A New Workflow for x-ray fluorescence tomography: MAPSToTomoPy

Young Pyo Hong^a, Si Chen^b, and Chris Jacobsen^{b,a}

^a Department of Physics and Astronomy, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA

^b Advanced Photon Source, Argonne National Laboratory, 9700 S. Cass Ave., Argonne IL 60439-4837 USA

Abstract

X-ray fluorescence tomography involves the acquisition of a series of 2D x-ray fluorescence datasets between which a specimen is rotated. At the Advanced Photon Source at Argonne National Laboratory, the workflow at beamlines 2-ID-E and 21-ID-D (the Bionanoprobe, a cryogenic microscope system) has included the use of the program MAPS for obtaining elemental concentrations from 2D images, and the program TomoPy which was developed to include several tomographic reconstruction methods for x-ray transmission data. In the past, fluorescence projection images from an individual chemical element were hand-assembled into a 3D dataset for reconstruction using interactive tools such as ImageJ. We describe here the program MAPSToTomoPy, which provides a graphical user interface (GUI) to control a workflow between MAPS and TomoPy, with tools for visualizing the sinograms of projection image sequences from particular elements and to use these to help correct misalignments of the rotation axis. The program also provides an integrated output of the 3D distribution of the detected elements for subsequent 3D visualization packages.

Keywords

Tomography; Software; X-ray Imaging

1. INTRODUCTION

X-ray fluorescence microscopy provides high sensitivity for the study of trace metals,²⁷ which play an important role in normal cell function and in diseases^{12, 22, 28} and in environmental science.¹³ By collecting fluorescence images as a specimen is rotated, one can carry out a 3D reconstruction of elemental distributions in fluorescence tomography.^{5, 7, 21}

X-ray fluorescence tomography experiments at beamline 2-ID-E at the Advanced Photon source use a setup as shown in Fig. 1. Sample scans are done on a x - y raster grid, with x

horizontal and y vertical. Scans in the x direction are done in a fly scan mode, where data is acquired while the stage is in motion rather than in a move–stop–measure sequence.²⁰ X-ray fluorescence spectra are recorded using a SII Vortex energy dispersive detector, and differential phase contrast (DPC) signals are acquired simultaneously using a segmented transmission detector.¹⁹ Due to its strong signal-to-noise (SNR) ratio, DPC can be used for image registration.¹⁸ Data for each line are saved in a NC file format (www.unidata.ucar.edu/software/netcdf/), after which the entire scan plus metadata is saved in a locally-defined MDA (multidimensional archive) file format. The sample is then rotated and another 2D fluorescence scan is acquired.

2. DATA PROCESSING

At the end of a x-ray fluorescence tomography experiment at 2-ID-E, one is left with a set of files with the x-ray fluorescence detector spectrum recorded at each pixel, along with the differential phase contrast detector signals and other metadata. While there have been very nice demonstrations of fluorescence tomography using the raw fluorescence energy spectra,¹⁵ in most cases the raw data for each 2D projection image is first analyzed by the program MAPS.³¹ This program fits the energy spectrum recorded at each pixel to a set of x-ray fluorescence peaks plus background signals, delivering a HDF5 (www.hdfgroup.org) file with the concentration of each element measured in projection. The concentration is determined in comparison with a standard sample with known trace element concentration.

Computed tomography delivers a 3D view of an object from a set of projection images acquired at different sample rotation angles. Prior to the development of MAPSToTomoPy, this was done mainly by user-driven extraction of each element's concentration map into a TIFF image (partners.adobe.com/public/developer/tiff/), with the set of TIFF image projections for one element then loaded into ImageJ (imagej.nih.gov) for reconstruction. When it was seen that individual projections were misaligned due to rotation stage runout errors, those projections were shifted by hand and the reconstruction process was repeated. While functional, this was not a very convenient process and there were no good tools for rapid evaluation of data during an experiment such as through visualization of sinograms.

Our goal in developing MAPSToTomoPy was to create a convenient workflow for fluorescence tomography reconstructions and visualization (Fig. 2). To achieve this goal, we have drawn upon two key developments: the DataExchange schema⁴ for storing data in HDF5 files, and the program TomoPy¹⁶ which integrates a number of tomographic reconstruction algorithms into a single package written in the Python programming language. What MAPSToTomoPy does is to take the output of MAPS, and present the user with an easy way to look at sinograms from any of the elemental map projection series. Some alignment tools for correcting for rotation stage runout errors are already present, while others are under development. MAPSToTomoPy then calls on TomoPy to carry out the 3D reconstructions for each of the chemical elements, and stores the results together in a single HDF5 file for easy archiving and post-reconstruction analysis and visualization.

3. MAPSTOTOMOPY

As the workflow of Fig. 2 indicates, MAPSToTomoPy expects MAPS to have first generated projection images of elemental concentration. This is done in an automated fashion, triggered by the arrival of new MDA files in a specified data-collecting directory.

When several 2D projections are available, MAPSToTomoPy provides a listing of the files and their rotation angles, allowing a researcher to select all the files or deselect a subset. It then reads in the individual projection files of dimension $[Z, y, x]$ (where Z represents the chemical element, with $Z=20$ for calcium, for example), and constructs in memory a new array $[Z, \theta, y, x]$ where θ is the rotation angle and an alignment array $[\theta, y_0, x_0]$ of the required shift of each projection image to place it on the correct common axis of rotation. The initial values of $[\theta, y_0, x_0]$ are zero, but they can be saved and re-loaded in case there is a need to optimize the alignment in successive program runs based on information gained from a 3D reconstruction.

With an assembled dataset thus constructed in memory, one can first explore the raw data for a particular element Z by using a slide bar to control the scrolling through various projection angles θ . For each projection, a histogram of the intensity distribution is shown at left, which can be used to guide the selection of brightness and contrast scalings.

The next step is to construct and display a sinogram for a particular element. This is done by specifying a particular row y' in the image and by selecting a specific element Z , and building from $[Z, \theta, y', x]$ a 2D image $[x, \theta]$ which produces the sinogram shown at left in Fig. 3. This provides an experimenter with a quick visual check on the quality of the data acquired thus far, and one can often recognize rotation axis runout errors “by eye” if the selected row y' from one angle θ appears to be displaced in x relative to its neighbors. Besides helping an experimenter to be able to visualize these runout errors, MAPSToTomoPy provides the following approaches to try to identify the runout errors $[\theta, y_0, x_0]$ so they can be corrected prior to 3D image reconstruction:

1. Cross correlations¹⁴ allow one to align two images of an identical object, and with small enough rotation changes it provides a good estimate of the relative shift of one projection relative to its neighbor. Letting $\mathcal{F}\{\}$ represent a forward Fourier transform and $\mathcal{F}^{-1}\{\}$ represent its inverse, the magnitude of the cross correlation coefficient $|c(x, y)|$ between two images is given by

$$|c(x, y)| = \left| \mathcal{F}^{-1} \{ (\mathcal{F}\{f\})^* \cdot \mathcal{F}\{g\} \} \right| \quad (1)$$

where $*$ denotes the complex conjugate. The position $[x, y]$ where $|c|$ is maximized gives the relative shift between the two images, and thus provides a good estimate of the shift $[x_0, y_0]$ that must be applied to the second projection image in order to align it onto a common rotation axis.

2. Phase correlation²³ also estimates the translation shift. Phase correlation starts from a element-wise product of the Fourier transform of one image and the complex conjugate of Fourier transform of another image. An inverse Fourier transform is

then carried out, and the position of the maximum is again used to find the offset between the two images:

$$|p| = \left| \mathcal{F}^{-1} \left\{ \frac{\mathcal{F}\{f\} \cdot \mathcal{F}\{g\}^*}{|\mathcal{F}\{f\} \cdot \mathcal{F}\{g\}^*|} \right\} \right|$$

The phase correlation often gives better results than cross correlation because it is invariant to translation, rotation and scale. As with cross correlation, this approach expects the two adjacent-in-tilt images to be very similar to each other; when large angular increments are used, this may not always be the case.

3. Another way to align projections is to identify center of hotspots, or small regions with high intensity. The centers of these hotspots are fitted to a sine curve (representing the sinogram of a single object), and the deviation of each hotspot location from the overall sine curve can then be used to find that projection's offset in one dimension. Rivers *et al.* have used sinogram fitting to align to the center of mass of the entire sample²⁴ which is a strategy well-matched to samples with cylindrical symmetry like rock cores.

No matter which method is used, the updated “best current guess” of the runout shift $[\theta, y_0, x_0]$ is stored in the MAPSToTomoPy output file so that it can be used again (or improved upon) in subsequent analyses of the same data set.

With the individual element files read in from the MAPS output and assembled into a $[Z, \theta, y, x]$ array based on the alignment shifts $[\theta, y_0, x_0]$, the next step is to send a $[\theta, y, x]$ data array from each elemental signal Z to TomoPy for 3D reconstruction. As a script-based reconstruction program, TomoPy can take an in-memory Python data array as its input, eliminating file output/input delays. MAPSToTomoPy does this for all elements, so that from the projection images $[Z, \theta, y, x]$ one arrives at a 3D reconstructed volume for each element of $[z, y, x]$. While TomoPy provides additional reconstruction approaches, for our fluorescence tomography experiments with a small number of widely spaced projection angles we often use the maximum likelihood–expectation maximum or MLEM algorithm⁸ as the default method. The only adjustable parameter for this algorithm is the number of iterations, so one can come up with a preliminary reconstruction in a relatively straightforward manner. The penalized maximum-likelihood PML algorithm² is often used for refinement, after a value of the penalty weighting parameter β is chosen.

One can then display as a 2D image the projection through the 3D reconstructed volume at a particular rotation angle, as shown at right in Fig. 3. The reconstructed volume is also written out to a set of TIFF files.

4. DEMONSTRATION WITH A TEST SPECIMEN

For developing techniques to correct for self-absorption of fluorescence signals in thick specimens, and to demonstrate the use of MAPSToTomoPy, we constructed a simple test structure. It consists of a glass rod (81% SiO₂ and 13% BO₃) of 200 μm diameter, with two wires wrapped on its side: one of 10 μm diameter W, and one of 10 μm diameter Au. This

test specimen was examined through a series of projection images involved 1750×51 pixels acquired with 200 nm step size, and a pixel acquisition time of 4 msec per pixel during fly scans. A total of 73 projection images was acquired over an angular range of 360 degrees, leading to a dataset best reconstructed by MLEM or PLM methods rather than standard filtered backprojection. The data were collected using an incident beam energy of 12.1 keV at beamline 2-ID-E at the APS. While this beam energy is high enough that the glass wire only absorbed 32 % of the beam through its center, the $1/e$ absorption length of Si K_α x-rays in the glass mixture is $5.84 \mu\text{m}$, so this dataset shows strong self-absorption in the Si fluorescence measurements. These data were affected by significant rotation stage runout errors, as shown in Fig. 4.

For this particular dataset, we used the Differential Phase Contrast (DPC) signal for correction of the rotation stage runout errors. The advantages of using DPC for this purpose include the fact that it provides a stronger signal-to-noise ratio because of the favorable ratio of phase to absorption in the x-ray refractive index of materials at multi-keV x-ray energies, and also because DPC is not affected by self-absorption of fluorescence within the sample.

Using the DPC signal, we used Canny's method of edge detection¹ to identify the edges of the wires and rod in each projection. After identifying the edge of the glass rod (which we knew to be quite circular), its center was found for each projection and the projection's shift $[\theta, x_0, y_0]$ was fixed to place this at the image center position in the horizontal direction (of course this approach cannot be used on more irregular object shapes, so the code for this was loaded as a plug-in rather than being "hard-coded" within MAPSToTomoPy). The uncorrected and corrected sinograms shown in Fig. 4 show good projection alignment using this approach.

With the dataset run-out error thus corrected, MAPSToTomoPy was used to manage the TomoPy reconstructions. As noted above, for a dataset with relatively few projections, Fourier-based approaches such as filtered backprojection as implemented in the "gridrec" algorithm¹¹ are not suitable and iterative methods such as MLEM and PML should be used. In this case, the MLEM algorithm was used with 3 iterations on the gold and tungsten signals, and 1 iteration on the silicon signal. The TIFF files of the reconstructed volume were then read into the visualization program VGStudioMax, leading to the combined rendering shown in Fig. 5. In this reconstruction, one can identify the gold and tungsten wires, while imperfections in the spectral fitting and background rejection of the strongly-self-absorbed Si signal lead to the appearance of silicon near these wires.

5. FUTURE PLAN

We outline here our plans for future development of MAPSToTomoPy.

5.1 Quantitative reconstruction

For our first developmental tests of MAPSToTomoPy, we have worked with non-quantified reconstructions while refining the workflow and alignment methods. However, x-ray fluorescence tomography is a quantitative technique.^{5, 24} Since MAPS uses a comparison of fluorescence signals from a reference standard for quantitation, one would need to either

measure or estimate the change in apparent concentration due to fluorescence self-absorption in the reference standard as it is rotated relative to the viewing angle of the fluorescence detector. In addition, items such as a sample holder might occlude some of the fluorescence signal at certain angles, and this must be corrected for. We are now exploring methods for making these corrections, and plan to incorporate them into MAPSToTomoPy.

5.2 Quantitative concentration and self-absorption correction using phase contrast

Elemental concentration rather than content drives biochemical gradients, so quantitative concentration is an important parameter to quantify in 3D. For samples comprised of heavier elements, x-ray absorption images can be used to reconstruct the 3D mass distribution in a standard tomographic approach, and this can be used for self-absorption correction and concentration estimation.²¹ For hard x-ray imaging of samples dominated by lighter elements, absorption contrast can be very low while phase contrast delivers images with considerably higher contrast.³ One can recover quantitative images of projected mass using various processing methods applied to the differential phase contrast (DPC) signal,^{6, 19} or quantitative electron density reconstructions^{10, 30} of scattered coherent radiation in x-ray ptychography^{25, 29} for which data can be recorded while taking x-ray fluorescence scans.^{9, 26} In order to retrieve integrated phase contrast,⁶ normalization for signals from each segment of segmented detector is required. Currently, MAPS contains only analyzed DPC signals, but modifications are underway to record the raw signals as well so that MAPSToTomoPy can take care of signal normalization.

Phase contrast images also allow one to carry out projection alignment with better signal-to-noise as required for exploring dose fractionation,¹⁷ where a high-statistics 3D reconstruction is obtained from a series of low-statistics 2D images. The use of DPC signals has been shown to provide significantly more robust projection alignment signals than are available from the fluorescence data.¹⁸

5.3 Projection registration and run-out error correction

Projection registration about the rotation axis is the one of the key factors for successful 3D reconstructions. As noted above, one can use cross correlation, phase correlation, or sinusoid fitting methods to correct for some run-out errors. Another approach is to use optimization methods to explore various values of runout correction factors $[\theta, y_0, x_0]$ while seeking to minimize an error metric, or using entropy maximization. Since this requires multiple reconstructions to be carried out on the fluorescence tomography dataset, the in-memory workflow and data management capabilities of MAPSToTomoPy will help ease the exploration of these sorts of approaches.

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Figure 1.

X-ray fluorescence tomography setup at beamline 2-ID-E at the Advanced Photon Source at Argonne National Laboratory. A segmented transmission detector is located at end of the beam path, while an energy-dispersive detector for x-ray fluorescence signal collection is located perpendicular to the beam path. Figure courtesy of Stefan Vogt.

Figure 2.

Diagram of the MAPSToTomoPy data workflow. The process can be divided into two parts. Collection of 2D x-ray fluorescence datasets, and reduction to 2D elemental concentration maps, is done by the program MAPS. MAPSToTomoPy then collects these 2D projections, provides sinogram visualization and alignment tools, and organizes the use of TomoPy to obtain reconstructions of the 3D distributions of the chemical elements detected.

Figure 3.

Screen shots of MAPSToTomoPy in use. At left is shown a sinogram, where a single row y' from projection images of one element $\mathcal{Z}$ is shown, yielding an image with dimensions $[\theta, x]$. At right is shown a 2D projection $[y, x]$ from one particular viewing angle θ and for one particular element $\mathcal{Z}$, generated from the reconstructed volume.

Figure 4.

Sinograms of the test specimen, which consisted of a 200 μm diameter glass rod with one Au and one W wire (10 μm diameter) along its side. The top two images (A and B) show the Si fluorescence signal, while the bottom two images (C and D) show the differential phase contrast signal. The sinograms at left (A and C) are as-recorded, and show the effects of considerable runout error on the rotation stage used (as can be seen especially in the “waveiness” of the left edge of the Si cylinder, which was quite regular when inspected in a light microscope). The sinograms at right show the same data after determining a per-projection shift $[\theta, y_0, x_0]$ for each rotation angle, based on a fixed center position of glass rod.

Figure 5.

3D image reconstructed from a fluorescence tomography dataset. This image shows the gold wire (green), tungsten wire (red), and glass rod (blue). The reconstruction was done using the MLEM method using three iterations for the gold and tungsten signals, and one iteration for the silicon signal. Because of the very high degree of self absorption of the Si signal, the glass rod appears hollow; only fluorescence from near its edges was able to escape and reach the detector.