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A comparison of quantitative reconstruction techniques for PIXE-tomography analysis applied to biological samples

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ABSTRACT

The tomographic reconstruction of biological specimens requires robust algorithms, able to deal with low density contrast and low element concentrations. At the IST/ITN microprobe facility new GPU-accelerated reconstruction software, JPIXET, has been developed, which can significantly increase the speed of quantitative reconstruction of Proton Induced X-ray Emission Tomography (PIXE-T) data. It has a user-friendly graphical user interface for pre-processing, data analysis and reconstruction of PIXE-T and Scanning Transmission Ion Microscopy Tomography (STIM-T). The reconstruction of PIXE-T data is performed using either an algorithm based on a GPU-accelerated version of the Maximum Likelihood Expectation Maximisation (MLEM) method or a GPU-accelerated version of the Discrete Image Space Reconstruction Algorithm (DISRA) (Sakellariou (2001) [2]). The original DISRA, its accelerated version, and the MLEM algorithm, were compared for the reconstruction of a biological sample of *Caenorhabditis elegans* – a small worm. This sample was analysed at the microbeam line of the AIFIRA facility of CENBG, Bordeaux. A qualitative PIXE-T reconstruction was obtained using the CENBG software package TomoRebuild (Habchi et al. (2013) [6]). The effects of pre-processing and experimental conditions on the elemental concentrations are discussed.

1. Introduction

Proton Induced X-ray Emission Tomography (PIXE-T), in combination with Scanning Transmission Ion Microscopy Tomography (STIM-T) is used to produce quantitative 3D elemental concentration and mass density distributions in samples with a resolution of a few micrometres down to the sub-micrometre scale. This micro-structural characterization of biological samples is particularly challenging due to its low density contrast, sensitivity to beam radiation damage (which may induce morphological changes and mass loss), and potentially low levels of trace elements requiring significant irradiation time to acquire sufficient counts. Iterative reconstruction algorithms are required to compensate for the X-ray attenuation and non-linear X-ray production along the path of the beam [1].

The most complete iterative reconstruction algorithm for PIXE-T is the Discrete Image Space Reconstruction Algorithm (DISRA) developed by Sakellariou [2]. It is a quantitative algorithm that simulates PIXE-T and the X-ray attenuation for large solid angles. At every iteration, a local correction factor is applied, by taking the ratio of values at every voxel of the filtered back projections (FBP) of simulated and experimental projections. The noise induced by FBP is reduced by restricting possible values into discrete bands.

A slower convergence algorithm for PIXE-T is the MLEM algorithm. MLEM backprojects the difference in the simulated and experimental datasets at each iteration and applies this to correct the tomogram at each iteration. This does not require a projection range of 180° or 360° and in early PIXE-T experiments MLEM was employed using as few as only 4 projections for a 30×30 pixels image [3], far fewer than required by FBP-based algorithms, including DISRA. The range for 4 projections covered only 135°. However this used a symmetrical sample and assumed a point detector to calculate attenuation correction factors. MLEM has recently been employed for PIXE-T in Japan [4].

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MLEM and DISRA are both iterative techniques suiting different experimental conditions. A new data reduction software package for ion beam tomography was devised at IST/ITN in Lisbon, *i.e.* JPIXET [1]. This is a GPU(Graphics Processing Units)-accelerated software package for the pre-processing, analysis and reconstruction of data for PIXE and STIM tomography with a choice of iterative algorithms. Data may be reconstructed using: (1) DISRA, (2) GPU-accelerated DISRA and (3) GPU-accelerated MLEM algorithm. The accelerated versions implement a much faster simulation component, more accurate X-ray attenuation calculations and are capable of simulating overlapping pixels [1]. Data tables from the DISRA package are used with the addition of stopping powers updated from SRIM [5], including elemental and compound stopping powers for proton energies below 1 MeV. The GPU-accelerated algorithms were developed using the Common Unified Device Architecture (CUDA) to accelerate the reconstruction. CUDA is the NVIDIA GPU architecture to enable easy general purpose programming of GPUs and to enable massively parallel programming.

To test the algorithms with real data, and to look at the challenges with pre-processing and experimental conditions, quantitative reconstruction of a small worm, *Caenorhabditis elegans (C. elegans)*, was performed using the standard version of DISRA and the accelerated versions of DISRA and MLEM available in the JPIXET software. The data-sets were acquired at the AIFIRA facility of CENBG and a qualitative PIXE-T reconstruction was previously performed using the software TomoRebuild [6]. 3D STIM-T was performed before and after PIXE-T analysis.

2. Method

2.1. Experimental

Tomography experiments were performed on a small worm, *C. elegans*, prepared and irradiated at the AIFIRA microbeam line of CENBG as fully detailed in [6]. STIM-T was performed of 254 slices of *C. elegans*. As each slice takes approximately 2 h to collect the data with PIXE-T to analyse the whole sample would take too long in view of the time available to use the beamline. Thus only isolated slices were analysed with PIXE-T. 2D PIXE analysis was performed to locate an ideal slice to analyse with PIXE-T, followed by the PIXE-T analysis. However it was observed that the sample shrunk slightly during the PIXE analysis. The initial mass loss is very rapid and mainly due to light matrix elements, especially H, as explained in Section 4. A further STIM-T analysis, of 128 slices, was performed. For STIM-T, the beam current is much lower (a few thousand ions per second, *i.e.* less than 1 fA) than for PIXE-T ($\sim$250 pA). For this reason STIM-T did not induce visible damage or morphological change to the sample. This was confirmed by the comparison of projection datasets and FBP reconstructions which demonstrated that the major morphological changes had already occurred during the PIXE analysis. For this reason the quantitative reconstruction was performed using the post-PIXE STIM-T analysis.

For both PIXE-T and STIM-T a 1.5 MeV beam was used and 100 projections were taken over a range of 180°. A scan width of 128 pixels across 100 μm was used producing voxel sizes of approximately $\sim$0.787 μm in width. For STIM-T, 128 slices over 100 μm were analysed, producing voxel sizes of $\sim$0.787 μm in height. For PIXE-T one of the slices is displayed (shown in Fig. 1). The STIM beam size was measured to be 300 nm across and 1 μm for PIXE. As the PIXE beam spot size was greater than the pixel width, the pixels overlapped. Therefore surrounding pixels may contribute to the X-ray count at a pixel. This is a particular problem at sharp boundaries where characteristic X-rays may be detected even though the element of interest is not present. A SiLi detector with an area of 80 mm^2 was placed at 22 mm from the sample at an angle of 135° to the beam. The beam current was $\sim$250 pA.

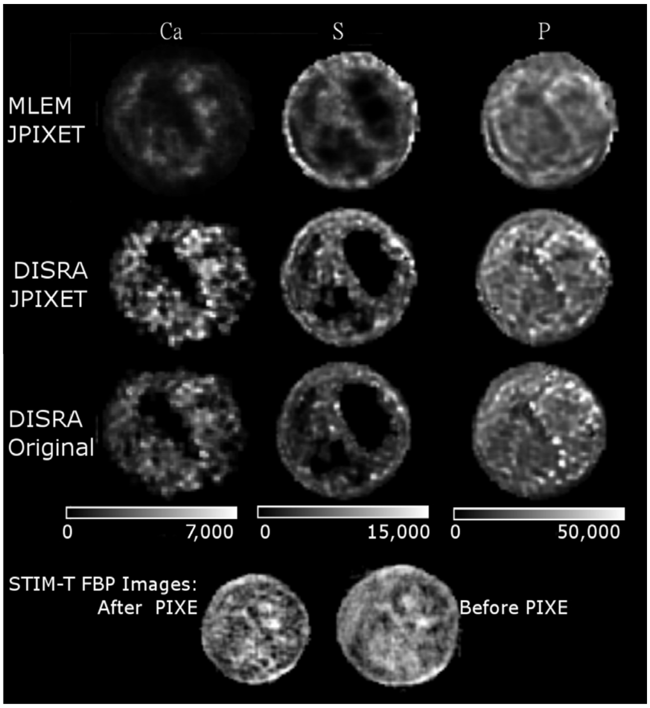

Fig. 1. Concentrations of the elements P, S and Ca using the original DISRA, the JPIXET accelerated version of DISRA and the JPIXET accelerated MLEM algorithm.

2.2. Preprocessing

TomoRebuild and JPIXET were used to preprocess the data. The STIM-T data was median filtered and the sinogram centred. An edge-detection algorithm was used to remove noise outside of the sample region. For PIXE-T, the counts for each element were mapped and then centred.

2.3. Reconstruction

The elements P, S and Ca were reconstructed using the original DISRA and the GPU-accelerated DISRA and MLEM algorithms. Reconstructions were performed on a computer with 4 GB RAM, Intel Core duo E4500 CPU (2.2 GHz) running Windows 7 64-bit. An NVIDIA Quadro 4000 graphics card with 2 GB GDDR5 of RAM and Compute Capability V2.0 was used for CUDA calculations.

For MLEM 30 iterations were performed, and for both versions of DISRA 10 iterations were performed. For MLEM some processing was performed after each iteration to reduce noise induced by the differences in the STIM-T and PIXE-T projection data. For MLEM, the reconstruction for 100 projections and 30 iterations took $\sim$90–110 min. For the original DISRA and for JPIXET version the reconstruction took $\sim$30–40 min. For all versions, the detector was segmented into 79 pixels however the attenuation calculations differ as explained in [1]. With only one slice analysed by PIXE, and with few detector segments (larger solid angles require significantly more segments), the time saved using CUDA was not significant in this case as the X-ray attenuation calculations were not such a large part of the reconstruction. Also, it was balanced by the computational overhead of moving the data between the computer and the graphics card.

To compare MLEM and DISRA with limited projections, reconstructions were performed with only 50, 25, 12 and 6 projections.