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Real-time 3D Tomographic Imaging of Biological Samples with Zooming to Features of Interest

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Imaging of biological samples is one of the most complex and challenging tasks in X-ray micro-tomography. The complexity is caused by several factors. First, in most cases the samples are unique and should not be destroyed during the tomographic experiment, prohibiting sample fragmentation into smaller parts fitting the detector field of view. Second, some biological samples are radiation sensitive and significant X-ray doze may change and destroy the internal structure, affecting the quality of reconstruction. Finally, non-destructive imaging of large samples by sequential scanning of different regions, requires complex projection data stitching procedures and leads to generating very large data volumes. To obtain tomographic reconstruction of such volumes in a reasonable time one needs to use High Performance Computer (HPC) resources based on several CPU and GPU computing nodes.

As an example of working with biological samples, we will present details of the scanning parameters used in a tomographic experiment for studying a feline spinal cord at sector 2-BM of the Advanced Photon Source (APS) at Argonne National Laboratory (ANL). The sample of the size 6.9 x 6. x 2.9 mm^3 was scanned with the resolution level (detector pixel size) of 0.69 µm. The data collection was carried out with the 'mosaic' approach, i.e. scanning with overlapping tiles each having the size of the detector field of view, with horizontal x vertical directions of (5 x 2) tiles. In each tile we collected a full tomographic data set with 6,000 projection angles and with 1.9s exposure time per projection, yielding 31.7 h total acquisition time for the mosaic scan. After projection stitching, the mosaic dataset had pixel sizes 6,000 x 10,000 x 2,900, which corresponds to 324 Gb of 16-bit data. Reconstruction was performed on a multi-GPU system and resulted in a 10,000 x 10,000 x 2,900 volume and 1.1 Tb of 32-bit data. It is worth to note that some of these data were not analyzed because features of interest were presented only in specific regions.

The complexity of tomographic experiments on modern synchrotrons motivated us to develop a new real-time 3D imaging monitoring instrument able to perform real-time tomographic reconstruction of three arbitrary slices across the sample, selecting representative regions of interest for high-resolution scanning. To reduce data volume, we also developed an on-demand data saving mechanisms for storing only the relevant projections acquired by a fast area detector sensor. The instrument has been developed at beamline 2-BM of the APS and includes an automatic lens changing mechanism developed by Optique Peter that allows for real-time zooming to a region of interest.

Real-time streaming reconstruction and visualization of three arbitrary slices through the sample is one of the tasks accomplished by the tomography instrument at beamline 2-BM. It is well-known that reconstruction of full 3D volumes is a time-consuming procedure typically done after finalizing CT data acquisition. The quality of reconstructed volumes is then verified by scrolling through slices in different axis, while more complex data analyses is typically carried out after the whole experiment is over. Therefore, instead of trying to reconstruct the full 3D volumes in real-time, we propose to reconstruct only 3 arbitrary slices (e.g. x, y, z ortho-slices) through the sample and allow users to choose these slices

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in real-time. Technically, reconstruction of a slice is straightforward and involves only the direct application of the following filtered back-projection (FBP) formula for a preset position in x, or y, or z,

$$f(x,y,z) = \sum_{i=1}^{N_\theta} g_{\theta_i}(x\,cos(\theta_i) + y\sin(\theta_i)\,, z)\,, \quad (1)$$

where g_{θ_i} is a filtered projection for angle $\theta_i, i = 1 \dots N_\theta$. If we assume that the number of discrete points in each x, y, z direction is of the order of N, then formula (1) can be directly evaluated with computational complexity $O(N_\theta N^3)$ in 3D case, and with complexity $O(3N_\theta N^2)$ for 3 arbitrary slices. If N is of the order of 1000, then 3 arbitrary slices can be reconstructed about 300 times faster than the whole 3D volume. This approach combined with extremely fast computations on NVidia GPUs guaranteed reconstruction of tomographic data from the detector in $\ll$ 1 second. The recovered slices are then concatenated into one 2D array and broadcasted for easy-to-use visualization in ImageJ, a tool very popular among biological users and familiar to most beamline users.

The streaming reconstruction system was integrated with an Optique Peter microscope to implement real-time zooming to regions of interest. The Optique Peter microscope system includes an automatic lens changer, see Figure 1. In the current setup we operate with 3 magnifications: 1.1x, 5x, 10x. However, lenses can be swapped depending on application needs. It is not difficult to see that zooming to the region of interest (ROI) can be done by moving the sample on the rotation stage and vertically using the x, z, y motors. As an example, consider zooming to the region of interest centered at (500, 1224, 1500) in a (2448, 2448, 2048) volume reconstructed from tomographic data acquired with 1.1x magnification. To find the new motor positions, we simply subtract half of the detector size in each dimension from the ROI center and multiply the result by the pixel size for 1.1x magnification. It follows that the x, z, y motors should be moved by (500-1224)/3.45×1.1 = -230.8 μm, (1224-1224)/3.45×1.1 = 0 μm, and (1500-1224)/3.45×1.1 = 88 μm, respectively. After changing the center, the ROI will be located in the middle of the detector image. The same procedure is applied when zooming is done between other magnifications.

The data management mechanism in the proposed streaming model fully relies on using the EPICS AreaDetector [1] where a set of plugins running in their own threads allow for real-time processing of detector raw data. We implemented two independent pipelines for data processing. One pipeline works continuously and responsible for transferring projection data to the reconstruction engine with GPU. In case of a slow connection or limited computing resources, one can apply binning or crop data to smaller sizes. The second pipeline is associated with on-demand data capturing to an HDF5 file located on the machine controlling the detector. As opposed to the first pipeline, no data binning or cropping is applied in this case. Captured HDF5 files can be reconstructed offline using tomoPy [2] via its command-line interface tomopyCLI [3], or using a GPU-based reconstruction with tomocupyCLI [4].

To demonstrate the efficacy of the proposed instrument we consider a real-time reconstruction of different regions inside a large beetle sample. Green circles in the left part of Figure 2 shows approximate positions for scanning. The right panel demonstrates real-time reconstruction of 3 orthogonal slices through the sample at these positions. The features of interest in low resolution images (with magnification level 1.1x) are defined by intersection of black lines and marked by green rectangles for clarity. Black lines on each slice also indicate positions of other slices, e.g. slice z includes positions of slices x and y. High-resolution images (with magnification level 5x) are obtained in real time by

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automatic 3D zooming to the feature of interest inside green rectangles. Note that the images for the two magnifications are rescaled with different colorbars due to different levels of phase artifacts on tissue borders.

Scanning with 1.1x and 5x magnifications was performed with 0.05s and 0.1s exposure time, and with 360 and 720 projection angles per rotation, respectively. Such quick scanning allowed for very fast search of features of interest and enable saving of only relevant data. This approach is clearly more efficient in terms of data sizes and total acquisition time than the conventional scanning and stitching the whole large beetle sample in high resolution.

The proposed streaming reconstruction instrument also allows for exposure time, rotation speed, and angular step size to be changed at any time during the data streaming process. These new features open a completely new way to optimize data collection parameters. Instead of "blind" data capturing, one can select a relevant sample region directly looking at the streaming reconstruction, zoom into it, change exposure time and angular step to optimize reconstruction quality, and finally, capture data to an HDF5 file on-demand while streaming is still on-going. The on-demand data capturing feature should significantly reduce tomographic data sizes, while the opportunity to change magnification and acquisition parameters on-the-fly allows for decreasing radiation doze to the biological samples since low-resolution overview scans can be done very quickly. Additionally, the real-time X-ray tomographic microscopy we propose, opens new possibilities for *in-situ* characterization of micro-structure evolution in matter since it allows to implement real-time feedback on the sample changes with respect to environmental conditions [5].

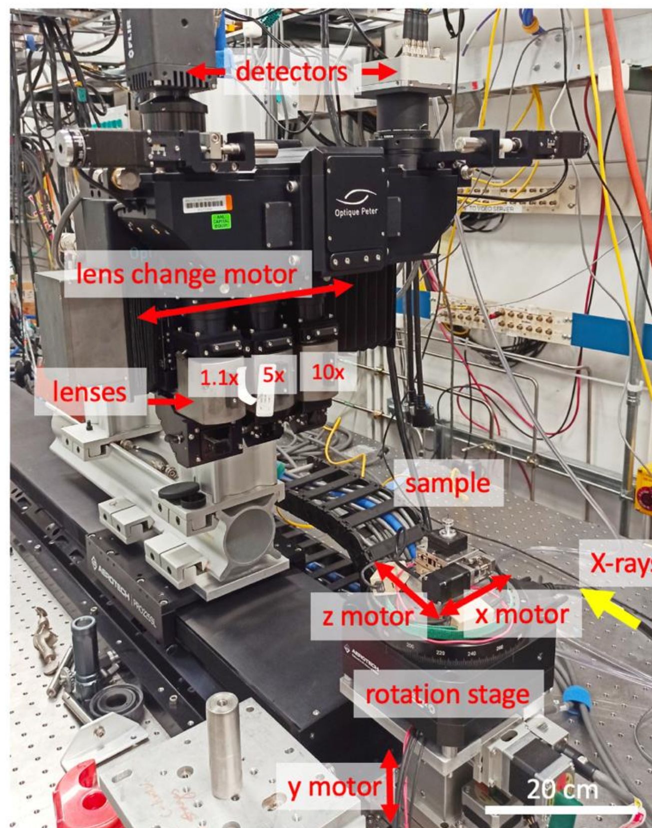

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Figure 1. The Optique Peter microscope system at beamline 2-BM of the Advanced Photon Source. 3D zooming to regions of interest is implemented by changing the magnification and by moving the sample with x, z motors on top of the rotation stage, vertical y motor under the stage.

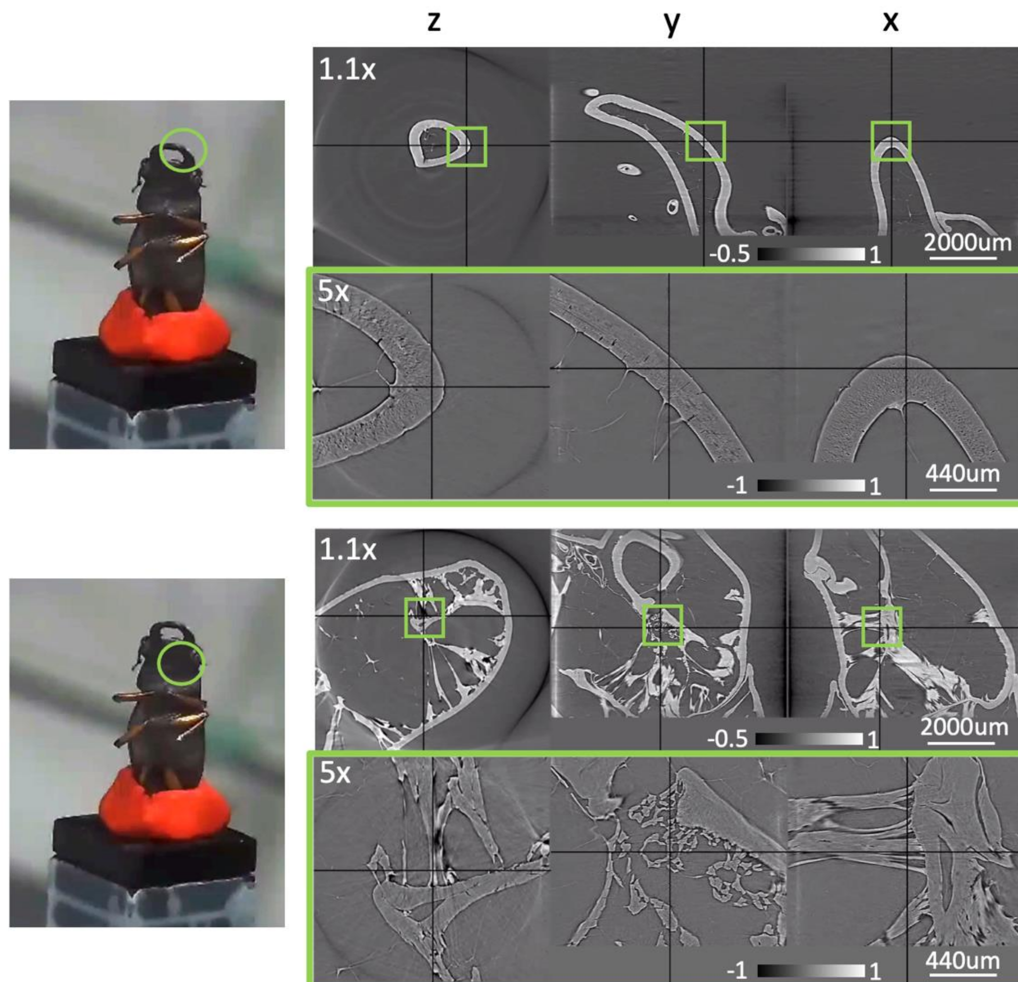

Figure 2. Real-time tomographic reconstruction of 3 ortho-slices through 2 different regions inside a beetle sample. The feature of interest was chosen in real-time with low-resolution reconstruction (magnification 1.1x) and corresponds to the intersection of black lines (also marked by green rectangles). Automatic 5x zooming to the feature of interest produced high-resolution images of the sample inside the green rectangle region.

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[5] This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility at Argonne National Laboratory and is based on research supported by the U.S. DOE Office of Science-Basic Energy Sciences, under Contract No. DE-AC02-06CH11357. The authors also acknowledge the group led by Dr. Chardon from the Department of Neuroscience at Northwestern University for collaborative work on the feline spinal cord imaging.

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