Deep learning-based single cell volume segmentation for soft X-ray microscopy data

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Background and Aims:
Soft X-ray microscopy within the water window spectral range offers non-destructive imaging capabilities with nanometer resolution and a penetration depth of up to 10 µm. This enables the investigation of biological samples in a near native state in three dimensions. The interpretation of X-ray microscopy images of biological cells can be challenging due to the similar absorption characteristics of carbon-based structures within these samples. To address this challenge, we have developed a contrast enhancement protocol, to improve the interpretability of the acquired images. Subsequently, an efficient segmentation technique based on deep learning is deployed for the extraction of biologically relevant information from the large amount of data generated by fast tomogram acquisition.

Methods:
We employed a laboratory-based soft X-ray microscope operating within the water window (500 eV photon energy). To enhance the contrast in acquired images, we developed a contrast enhancement protocol incorporating an adaptation of the Paganin filter as well as an unsharp masking filter. Additionally, we deployed a neural network model utilizing a U-Net architecture for the segmentation of THP-1 cell tomograms. This approach leverages the natural higher contrast of lipid membranes to facilitate segmentation.

Results:
The implementation of the contrast enhancement protocol resulted in improved contrast-to-noise ratios, thereby enhancing the interpretability of acquired images. Moreover, the neural network-based segmentation technique efficiently extracted biologically relevant information from tomograms, as confirmed by organelle volume calculations that align with tabular data. This segmentation can enable quantitative insights at the subcellular level, including the location and distances between different organelles, as well as their concentrations.

Conclusions:
Our study offers a comprehensive approach to soft X-ray microscopy analysis, addressing challenges in image interpretation and data segmentation. The developed contrast enhancement protocol and segmentation techniques facilitate efficient and accurate investigation of biological samples at the sub-cellular level. For the generalization of the segmentation process, increasing both the volume and quality of the data, as well as refining sample preparation, is necessary. Nevertheless, our advances contribute to analyse soft X-ray microscopy data more efficiently.

Keywords:

Soft X-ray microscopy; Tomography; Segmentation;

Reference:

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