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# Soft X-ray Tomography: a Mesoscale Bio-Imaging Technique to Study Single Cells in 3D with Automated Segmentation Tools for Several Sub-Cellular Structures

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Soft X-ray microscopy has been developing for decades and is capable of imaging biological samples with faster speed and better resolutions than ever before due to the technical improvements in CCD detectors, X-ray optics and brighter light sources. Soft X-ray tomography (SXT) is a three-dimensional volume imaging technique that can capture single biological samples in minutes with a 35-50 nanometer isotropic resolution [1, 2]. The intrinsic contrast comes from organic components presented inside of the cells within the "water window" energy, called the linear absorption coefficient (LAC) [3]. Specifically, structures more densely packed with organic components will transmit the X-ray differently compared to less densely packed organelles and will thus be reported with a different LAC. In addition to the obtained morphological information, this results in quantifiable information of the bio-composition of the sub-cellular structures in the tomogram, which can subsequently be compared across several conditions [4]. Samples are imaged after cryo-fixation in the near-native state, meaning non-invasively and label-free. Eukaryotic cells, such as bacteria and yeast, as well as human stem cells and somatic cells, have been analyzed with SXT. Another advantage of SXT it the fast-imaging speed. Collection of, for instance, tens of yeast images can be done within minutes, making SXT a high-throughput imaging device [5].

Different organelles can be distinguished based on the LAC value: the cell membrane, lipids, mitochondria, vacuoles and the nucleus with its chromatin subtypes. Identification is followed by segmentation and 3D surface rendering of each of these sub-cellular structures (see Fig 1). On the reconstructed data, statistical analysis of for instance roundness, shapes, inter-organelle distances and LAC-intensity of the sub-cellular structures can then be performed. Manually annotating huge amount of data generated by SXT is time-consuming and prone to human subjectiveness, automatic segmentations are thus required. We are, therefore, developing machine-learning tools that leverage limited number of manual segmentations to train a classifier to automatically segment and annotate the objects. For example, we are using this method to understand how the spatial and mechanical interactions between organelles contribute to cytoplasmic patterning in yeast (in collaboration with Mary Mirvis, UCSF). Minimal amount training datasets imaged with SXT are in the pipeline to enable image-based modeling and biophysical simulations.

For mammalian cells, T-lymphocytes are an example of crawling cells that play a central role in cancer-related extravasation processes. Manual segmentation of the cell membrane is used to train a script to detect the surface evolution in high detail to accurately show the lamellipodia and filopodia on the cell surface. Furthermore, SXT is particularly useful to study the compacted degree between hetero- and euchromatin in the nucleus under different conditions and during differentiation or maturation processes [6] (see Fig 2). Nuclear envelope segmentations allow machine learning to automatically differentiate and segments the subtypes in an automated and objective LAC-based manner. The obtained isotropic

tomogram can be used for quantification studies of the chromatin, including the compaction and/or condensation, compartmentalization, distribution, and its interconnection.

In summary, SXT is a tool to image and quantitively analyze cellular structures in their most native state, where automatic segmentation is a critical point for quantification and high throughput of the samples.

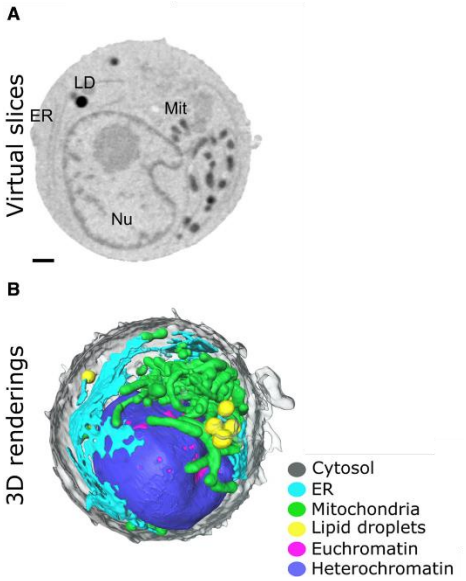


**Figure 1.** Virtual slice (A) and 3D rendering (B) of a B-lymphocytes shows the different sub-cellular organelles visible with soft X-ray tomography imaging. Scale bar is 1 μm. Image adapted from [7].

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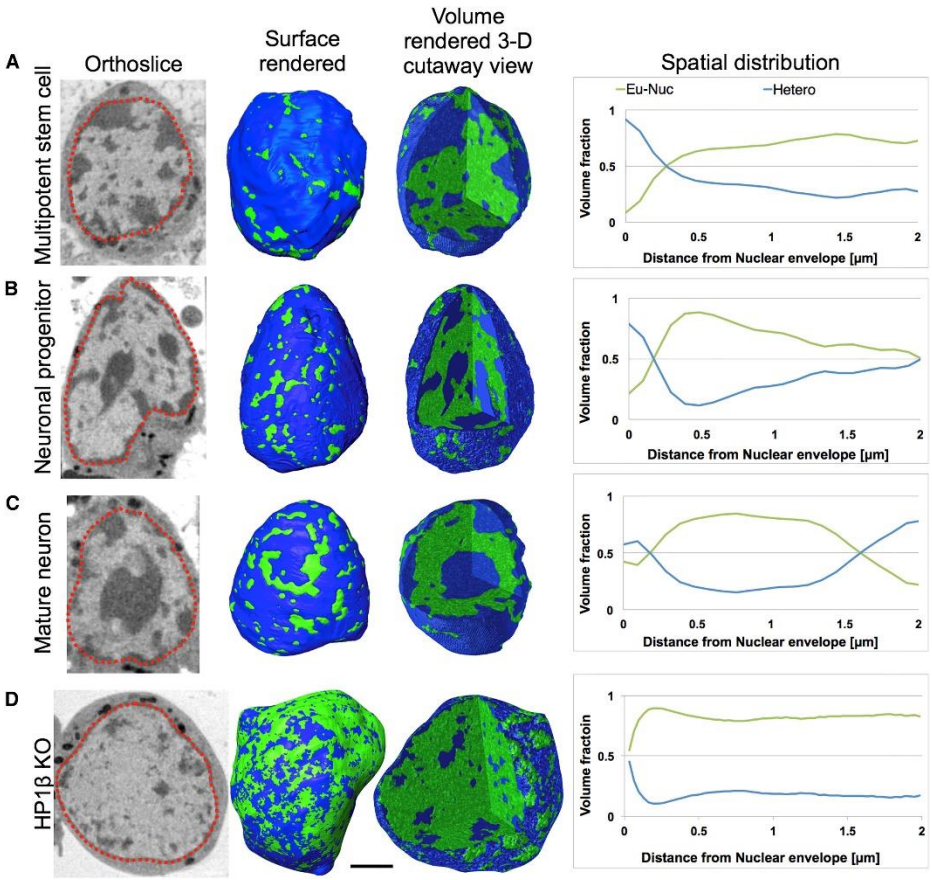


**Figure 2.** Spatial distribution of chromatin in different cell types. From [6].

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