# Synchrotron X-ray Imaging of Soft Biological Tissues: Concepts, Uses, and Future Prospects

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## Abstracts

A flexible isotropic three-dimensional imaging method, synchrotron- based tomographic phase-contrast X-ray imaging (SRμCT or SRnCT) can be used to examine biological samples of any size, from single cells to human-sized objects. The extremely bright and coherent X- rays generated by a synchrotron light source are exploited by SRμCT and SRnCT. Through the use of phase contrast, this allows for quick data collection and improved image contrast for delicate biological materials. In this review, we give a summary of the fundamentals of the method, go over how biologists might use it, and offer a prediction for the development of this new biological methodology. We present the most recent developments in the field, including the imaging of entire human organs at micron resolution, the use of X-rays as a virtual histology tool.

Keywords: Phase-contrast imaging, Soft tissue imaging, Synchrotron, X-ray imaging.

## 1. Introduction

Recent developments in synchrotron hard X-ray imaging, however, have attempted to address this problem. With an emphasis on propagation-based phase-contrast tomography, we describe the physics behind synchrotron hard X-ray imaging in this Review. We go over the fundamentals of this method and how biologists might use it to advance their imaging-based studies. We cover current developments in the field, such as correlative imaging techniques, mapping of the neural connectome, and imaging of entire organs at a micron resolution. It's important to note that this method uses a lot of the same names and acronyms. Frits Zernike developed the initial idea of phase-contrast imaging using visible light more than 80 years ago (Ackermann, 2022).

Ulrich Bonse and Michael Hart first proposed X-ray phase-contrast imaging using a crystal interferometer in the 1960s. Atsushi Momose then used this technique in biology . Anatoly developed the fundamentals of free propagation phase-contrast imaging . Since then, numerous methods have been created based on this idea, and numerous tests have been carried out at

various synchrotron sites. The basic technology is the same even though the techniques vary in terms of the X-ray beam shape, propagation distance, number of distances, energy, coherence, and detector setups. For this Review, we refer to synchrotron radiation micro-computed tomography (SRμCT) or synchrotron radiation nano-computed tomography (SRnCT) ( Albers, 2021; Albers, 2023).

Synchrotron hard X-ray imaging principles:

Synchrotrons are a kind of particle accelerator (storage ring) that produces X-rays by accelerating charged particles, usually electrons. The continuous acceleration needed to keep the particles inside the roughly circular ring produces X-radiation. The introduction of magnets into the storage ring allows for the fine tuning of the energy of these synchrotron X-rays. The generated X-ray beams are guided down straight-line pipes while the charged electrons continue their trajectories around the ring (Archit, 2023).

Three characteristics of a synchrotron X-ray beam—high coherence, high collimation, and high brilliance are essential to its improved imaging capabilities. The oscillating X-rays being in phase is referred to as coherence. when every photon in a light beam is at the same location on the oscillation curve at any given moment. X-ray beams from a laboratory source are usually not coherent, though efforts are underway to make the beams more coherent. The term "collimation" describes the amount of beam divergence. Because light rays are parallel to one another when there is high collimation, the beam's size does not greatly expand as it moves. The intensity and directionality of an X-ray beam are described by brilliance. High brightness leads to quick data capture, while coherence and high collimation allow imaging of biological samples without staining the samples to enhance contrast ( Arhatari, 2021).

Phase-contrast X-ray tomography fundamentals:

By penetrating matter, X-rays allow for the non-destructive imaging of details within materials, meaning that you can see inside without having to rip it apart. This is in contrast to, for example, volume electron microscopy (vEM), which requires the sample to be sliced very thinly during or before the imaging process. Hard X-rays are utilized in order to adequately penetrate samples of a biologically significant size. Hard X-rays have a short wavelength because of their high photon energy. Because the sample only slightly modifies the phase signal, it is highly challenging to measure the phase changes across biological tissue interfaces at the sample point (Barbone,2021).

Both phase and absorption information are contained in the projection images after they are recorded. A procedure known as "phase retrieval" is used to recover the phase information. Edge detection and holographic phase contrast are currently the two primary subfields of propagation-based phase-contrast imaging. By acquiring a tomographic dataset at a single propagation distance, the edge detection mode—also known as the "direct-contrast regime" produces projections with significantly improved edge contrast (Beister, 2012).

Biological sample preparation for X-ray imaging:

There are various methods for preparing tissue samples for X-ray imaging. As of right now, no rigorous sample preparation procedure created especially for X-ray imaging exists. Instead,

current techniques used in clinical and scientific contexts have been adopted and modified for use in community imaging using synchrotrons. For instance, X-ray imaging is easily compatible with the traditional histological procedure of formalin fixation and paraffin embedding (FFPE), making it a very complimentary technique. Fixation, dehydration, and paraffinization provide a stable solid medium that does not heat up or move when exposed to X-ray beams, while also enhancing the natural tissue contrast. Additionally, samples that have been prepped for vEM, such as samples that have been resin- embedded or stained with heavy metals, can be imaged (Boergens, 2017).

Here, the tissue is further contrasted with the resin backdrop by staining such samples with osmium tetroxide. Since both of these preparations are solidly mounted, there are no negative effects when the samples are scanned repeatedly. Imaging is less common in liquid suspensions and gels, mostly because of the possibility of sample movement from heating, bubble formation, or gel deformation (Ackermann, 2022).

Uses of propagation-based synchrotron hard X-ray imaging in biology:

Hierarchical phase-contrast tomography (HiP-CT) is arguably one of the most prominent applications of synchrotron X-ray tomography in recent years. HiP-CT has been used to fixed full human organs using a highly coherent X-ray source over a range of resolutions, beginning at the whole-organ level with a voxel size of 25 µm and gradually zooming in with a final voxel size of 1.3 µm. The beamline hardware components, sample preparation, and mounting were all carefully chosen and calibrated to enable this approach. HiP-CT is carried out with two distinct detectors for the voxel ranges and a polychromatic beam in edge detection mode. Nonetheless, the public release of comprehensive multiresolution data is one of the project's greater accomplishments (Albers, 2021).

## 2. Recommendations:

Nowadays, published works utilizing laboratory-based X-ray data are the main source of information regarding the use of X-ray imaging as a targeting tool in, for instance, a vEM workflow. Both the quality of the data and the speed at which it can be gathered are greatly enhanced by shifting the gathering of X-ray data from the laboratory to the synchrotron. Both of these make it possible to photograph a huge number of samples, which has two advantages: first, it makes statistically sound population studies possible, which improves the demonstration of endemic variability in biology. Second, a process that might otherwise be insurmountable can be made more manageable by applying downstream sophisticated and time-consuming procedures, like vEM, to properly chosen ROIs.

It's important to be able to photograph fixed tissues that are suspended in liquid because it allows for post-data collection genetic analysis of the tissues. It has been demonstrated that the genetic information present in the cell is not destroyed throughout the X-ray data collection process.To obtain a multiplexing detailed perspective of the tissue as a whole, further steps will involve merging X-ray imaging with methods like spatial transcriptomics, immunofluorescence, and

others. The possibility of targeted personalized therapy is further enhanced by the collecting of this data.

## 3. Conclusion:

In Conclusion, For many thorough imaging studies, segmenting and analyzing characteristics of interest continues to be a challenge. Utilizing advancements in other imaging modalities like light and electron microscopy, a substantial amount of money is being invested in the use of artificial intelligence approaches. Naturally, there are difficulties, such as when samples are photographed when hydrated in liquid, which results in exceptionally low contrast in the X-ray data. The ability of the eye-brain interface to recognize feature boundaries is impressive, but it is computationally demanding. Furthermore, while the X-ray imaging data extends isotropically in 3D, the current approaches that are being built upon largely operate in a 2D area or a 2D+ volume.

But there is improvement, and the more complicated biological samples are imaged using X-ray technology, the more information may be made accessible to make things better. Overall, biological X-ray imaging of soft biological tissue is poised for an exponential rise in popularity, aided in particular by the speed of high-quality data acquisition, thanks to the synchrotron beam's significant phase component, particularly in the most recent fourth generation sources, and visionary biologists who are willing to try new imaging techniques.

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