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3D X-ray Insights into Human Tissue

Understanding physiological functions and pathological processes at the level of tissues and organs requires a plethora of advanced imaging techniques which are continuously evolving and which may take equal advantage of new optical concepts and technology, as well as progress in sample preparation, labeling chemistry or image processing. Imaging of human tissues at cellular and sub-cellular resolution is the realm of classical histology. For this purpose, the tissue samples obtained by surgical intervention or from a post mortem autopsy are cut into thin sections, stained and observed in an optical microscope. Images are obtained only of two-dimensional sections but not of the entire three-dimensional (3D) volume, at least not with isotropic resolution. In order to visualize and to quantify the cytoarchitecture in 3D, even deep in the tissue or organ, X-ray phase-contrast computerized tomography (XPCT) has recently emerged as a powerful tool for quantitative and fully digital 3D virtual histology.

Using highly coherent synchrotron radiation (SR), XPCT can be implemented at sub-micron resolution with a throughput of tissue volume on the order of 1 mm^3/min, capitalizing on progress in coherent X-ray optics [1], instrumentation and phase retrieval algorithms [2, 3]. In a multi-scale approach, parallel and cone beam illumination can be combined to cover a wide range of scales [4], see Fig. 1. Nano-focusing and inline holographic or ptychographic phasing reach resolution values even below $r \cong 50$ nm, but at this level the steep dose-resolution relationship $D \propto r^{-4}$ requires careful mitigation of radiation damage, and therefore essentially relies on stringent fixation and metal staining. Adapting protocols for volume staining from electron microscopy such as osmium-thiocarbohydrazide-osmium (OTO), XPCT (which at high resolution is also denoted as holo-tomography) could become a new tool for neuronal connectomics [5]. Contrarily, for a more moderate resolution comparable to visible light microscopy, XPCT can be performed on unstained and chemically fixed tissues. In particular, formalin fixed and paraffin embedded (FFPE) tissue, which is the standard preservation in clinical pathology, is fully compatible with XPCT. Advantages with respect to conventional histology are the 3D and large volume capability of XPCT. Since the technique is non-destructive and fully compatible with standard clinical pathology, correlative histology studies can be performed. Synchrotron based virtual histology and histopathology have already supported research on cardiovascular diseases (Fig. 1), neurodegeneration (Fig. 2), cancer, lung diseases, as well as infectious diseases, by providing 3D quantitative imaging of the pathological processes [6 - 10].

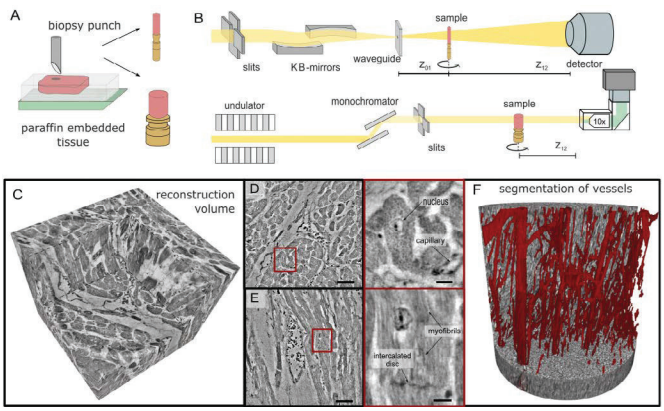

Fig. 1: XPCT using synchrotron radiation for 3D imaging of human heart tissue. (A) A biopsy punch is taken from a tissue block. (B) A sample cylinder with 1 mm radius is well suited for a high-resolution scan with cone beam synchrotron radiation (holo-tomography). Larger tissue volumes are scanned with a parallel beam; the sample can be stepped through the beam and sub-tomograms are stitched. (C) Reconstructed cube of ~350 µm side length of unstained human heart tissue, with (C, D) virtual sections showing cytoarchitecture components of interest (scale bars: (left) 50 µm/(right) 10 µm.) (F) Segmentation, rendering and analysis, here shown for the example of vasculature. Small capillaries in heart muscle are identified and analysed. Adapted from [10].

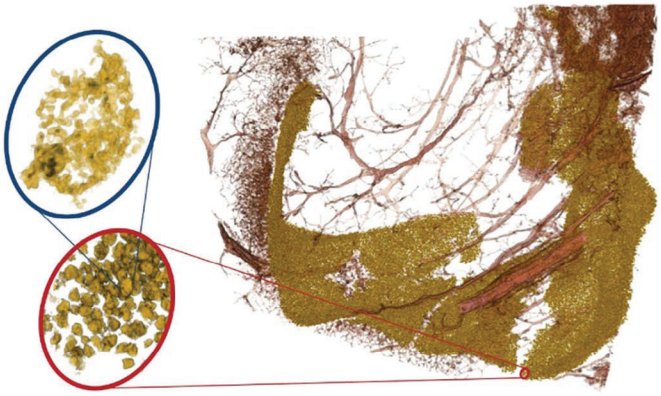

Fig. 2: Multiscale imaging of human brain tissue. 3D visualisation of the cytoarchitecture of the dentate gyrus in the hippocampus, reconstructed by XPCT. Neuronal cell nuclei are shown in yellow for the granule neurons in the dentate gyrus region of the hippocampus. Blood vessels are shown in red. By changing the X-ray optical magnification in the multi-scale recordings, one can zoom into regions-of-interest (red ovals). In these scans the resolution is high enough to resolve sub-structures of the nucleus, associated with different DNA packing regimes. Adapted from [8].

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Further Challenges: translation to laboratory µCT, functional labelling and automated segmentation/analysis

To fully unlock the potential of this method and to meet biomedical needs, we now need to meet the following challenges:
1.) Develop and implement specific labeling strategies, for example based on colloidal metals coupled to nanobodies.
2.) Increase contrast and resolution for XPCT at synchrotron sources [11], for example to reach the level required to reconstruct neuronal circuits, and more generally for sub-cellular physiology and pathophysiology.
3.) Leverage on the mathematical solutions and algorithms for inverse problems and machine learning, to optimize phase retrieval, tomographic reconstruction, segmentation, and more generally 3D image processing of bulky data.
4.) Translate the techniques from synchrotron sources to laboratory instruments (laboratory µCT) closer to a clinical and pre-clinically environment. First promising steps into this direction have been made [12].

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Prof. Dr. Tim Salditt

Tim Salditt is professor of experimental physics at the Institute for X-ray Physics of the University of Göttingen. He studied physics in Munich and Grenoble, and received his Ph.D. in 1995 for research in kinetic growth of surfaces and interfaces by diffuse X-ray scattering in the laboratory of Johann Peisl in Munich. In his postdoctoral work with Cyrus Safinya in Santa Barbara, he worked on the structure and interactions of lipid/DNA complexes. Driven by the motivation to study biomolecular assemblies also in the hierarchical and complex functional environment of cells and tissues, he then turned to coherent X-ray optics and developed X-ray waveguides for nanoscale holographic imaging and tomography. With his group, he operates a synchrotron instrument at the Deutsches Elektronen-Synchrotron DESY for holo-tomography and multiscale imaging of biological samples. Using a combination of nanodiffraction and advanced holographic phase retrieval, they can now study biomolecular assemblies and biological matter, from the molecular level to biological cells and tissues. Tim Salditt also works towards the goal of translating phase contrast tomography from synchrotron to laboratory instrumentation, to make high resolution X-ray phase contrast tomography more accessible for other biomedical researchers. As a principal investigator of the DFG Center for Excellence *Multi-Scale Bioimaging: from molecular machines to networks of excitable cells*, he engages in multiple collaborations with medical research groups. Tim Salditt is member of the Academy of Science in Göttingen and supports both DESY and the European X-ray free electron laser as a member of the scientific advisory committees.