Hierarchical Phase-Contrast Tomography: A Non-Destructive Multiscale Imaging Approach for Whole Human Organs

J. Brunet*[a,b], C. L. Walsh[a], P. Tafforeau[b], H. Dejea[b], A. C. Cook[c], A. Bellier[d], K. Engel[e], Danny D. Jonigk[f,g], M. Ackermann[g,h,i], P. D. Lee[a,j]

[a] Department of Mechanical Engineering, University College London, London, UK; [b] European Synchrotron Radiation Facility, Grenoble, France; [c] UCL Institute of Cardiovascular Science, London, UK; [d] Univ. Grenoble Alpes, Department of Anatomy (LADAF), Grenoble, France; [e] Siemens Healthineers, Erlangen, Germany; [f] Biomedical Research in Endstage and Obstructive Lung Disease Hannover, German Center for Lung Research (DZL), Hannover, Germany; [g] Institute of Pathology, Faculty of Medicine, RWTH Aachen University, Aachen, Germany; [h] Institute of Pathology and Molecular Pathology, Helios University Clinic Wuppertal, Universität Witten/Herdecke, Wuppertal, Germany; [i] Institute of Functional and Clinical Anatomy, University Medical Center of the Johannes Gutenberg–University Mainz, Mainz, Germany; [j] Research Complex at Harwell, Didcot, UK

ABSTRACT

Achieving cellular-resolution imaging of intact human organs is critical for improving our understanding of anatomy and pathology. Traditional clinical imaging and histological methods often fail to provide both global and detailed views of entire organs. Hierarchical Phase-Contrast Tomography (HiP-CT), an approach leveraging the ESRF's Extremely Brilliant Source upgrade to perform non-destructive, high-resolution imaging of intact human organs addresses these limitations. HiP-CT allows for whole organ scans at <20 μm/voxel, with localized zooms down to 2 μm/voxel, bridging the gap between clinical imaging and histology. This multi-scale capability enables detailed examination of anatomical structures and pathologies. We provide here the last developments of HiP-CT, along with various applications on human organs. HiP-CT has shown potential in research areas such as COVID-19 affected lungs and cardiac studies. Despite challenges like high radiation doses and data management, HiP-CT represents a significant advancement in biomedical imaging, with future research aiming to extend its application, correlative imaging upscale and downscale, and enhance data accessibility.

Keywords: Hierarchical Phase-Contrast Tomography (HiP-CT); Synchrotron X-ray computed tomography; Multiscale; Non-destructive Imaging; Human Organs; High-resolution Imaging; COVID-19; Cardiac

1. INTRODUCTION

Achieving detailed three-dimensional imaging of intact human organs, from the macroscopic scale down to cellular resolution, is a key challenge for better understanding human anatomy and pathology. Clinical imaging modalities, while useful for diagnostics, are often limited in bridging the gap between an overall structural overview and the detailed cellular architecture of the organ. Histological methods, although highly detailed, are destructive and limited in their ability to provide a holistic view of entire organs.

Hierarchical imaging using X-ray tomography represents an approach that addresses these limitations, it has long been utilized in fields such as bone research, where multiscale imaging techniques allow for the exploration of structural features across different length scales [1,2,3,4]. Recently, we extended the hierarchical imaging approach to large soft tissues. This technique, called Hierarchical Phase-Contrast Tomography (HiP-CT), leverages the increased X-ray spatial coherence provided by the ESRF's Extremely Brilliant Source upgrade to the world's first high energy forth generation source, along with advancements in sample preparation, beamline equipment, and scanning methodologies, to perform non-destructive imaging of whole human organs with unprecedented resolution. HiP-CT allows whole organ scans at 20 μm/voxel, with zooms down to 2 μm/voxel in localized areas anywhere, thus eliminating the need for physical sectioning. This multi-scale imaging capability bridges the gap between clinical imaging and histological techniques, enabling investigation of anatomical changes associated with various diseases. HiP-CT has been used in diverse research areas, notably in examining

Developments in X-Ray Tomography XV, edited by Bert Müller, Ge Wang,
Proc. of SPIE Vol. 13152, 1315216 · © 2024 SPIE
0277-786X · doi: 10.1117/12.3028717

structural modifications in lungs affected by COVID-19 [5,6] and in cardiac research providing new insights into the intricate structures and pathologies of the human heart [7].

In this paper, we will explore the principles and applications of HiP-CT, highlighting its capabilities, recent advances, and the significant impact it is expected to have on medical imaging and related fields.

2. METHODS

2.1 Sample Preparation

Multiple organs, including the brain, heart, lungs, and kidney, were obtained from bodies donated to Laboratoire d'Anatomie des Alpes Françaises (LADAF), following French legislation for body donation. After embalmment of the body, the organs are dissected and immersed in 4% formalin to preserve tissue structure, preventing degradation even during long-term storage. The organs then undergo partial dehydration through a series of alcohol baths with progressively increasing concentrations. This process minimizes tissue shrinkage. Between successive alcohol baths, the specimens are degassed to remove as much bubbles and dissolved gas as possible. This step is crucial as nucleation of bubbles during scanning can significantly reduce image quality. Subsequently, the organs are mounted in a cylindrical plastic container filled with a mixture of ethanol and crushed agar gel, which immobilizes the sample to prevent motion artifacts during scanning. For more information, see Brunet et al. [8]

2.2 HiP-CT imaging

Organs are imaged at the ESRF's BM18 beamline using HiP-CT. This beamline was designed for imaging large sample using propagation-based phase-contrast by combining a large (350 x 17 mm) and high energy beam, with high coherence (see Fig. 1). The high-coherence allow for larger propagation distances to be used without blurring the image. The distance between the X-ray source and the sample is 178 meters. For HiP-CT, a polychromatic quasi-parallel beam is used, optimized through a combination of filters (typically composed of molybdenum, silver, glassy carbon, and sapphire), with an average energy typically ranging from 90 to 126 keV. The sample itself is used as an X-ray filter, and its position is matched with the beam horizontal power profile to maximize the dynamic range of the data. HiP-CT uses a hierarchical approach, beginning with full-field tomography of the entire organ at an isotropic voxel size of approximately 20 µm. This is followed by local tomographic zooms in regions of interest, achieving voxel sizes down to approximately 2 µm. For the full-field scan of the entire organ, propagation distances ranging from 20 to 30 meters are used, while for the local tomography zooms, distances ranging from 2 to 10 meters are employed. For imaging large columns, an automated *z*-series is performed. The vertical field of view per scan ranges from 10 mm for whole organ to 5 mm to high resolution zooms. For each local organ scan, conducted in a sealed container, a reference scan of an equivalent container filled with 70% ethanol (for ethanol-prepared organs) or water (in case of formalin preparation) is performed. This reference scan served as a beam reference for flat-field correction, performed every 100 projections, to mitigate low-frequency artifacts associated with local tomography and effectively remove beam-hardening effects.

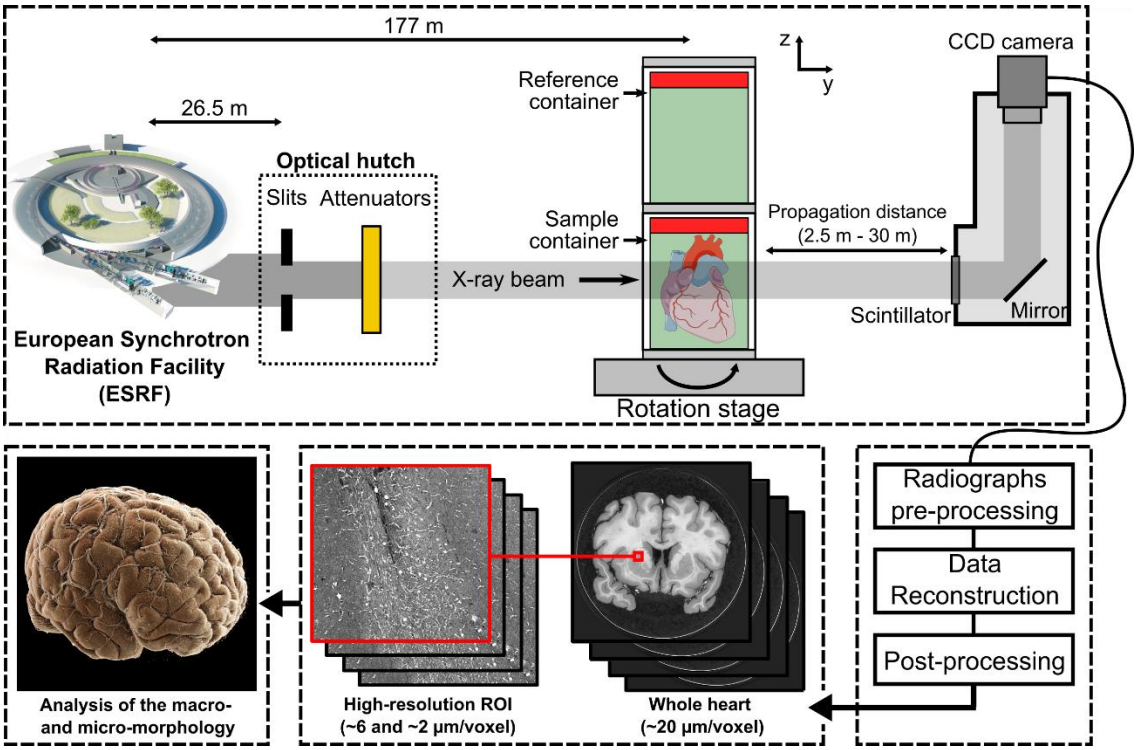

Figure 1. Schematic of hierarchical phase-contrast tomography (HiP-CT) setup and data processing pipeline. Modified from Brunet et al. [7].

2.3 Data processing

After pre-processing the projections with the angular flat-fields from the reference scan, a 3D volume is reconstructed with PyHST2 software [9] using a filtered back-projection algorithm combined with single-distance phase retrieval coupled with a 2D unsharp mask. Post reconstruction, the scans are concatenated vertically, followed by ring artefact correction using an improved version of the Lyckegaard *et al.* algorithm [10]. The reconstructed data volumes vary significantly in size, ranging from 100 GB to 15 TB, necessitating the use of high-performance computing resources for effective processing and visualization. The datasets generated are publicly available and can be explored via the Neuroglancer web interface [7] on the Human Organ Atlas website (https://human-organ-atlas.esrf.eu).

3. WHOLE ORGAN IMAGING WITH HIP-CT

We applied HiP-CT to image various whole organs, including lungs, hearts, brains, kidneys, and spleens from multiple donors with histological resolution [12]. As shown by Figure 2, the technique captures the entirety of each organ at an initial voxel size between 12 and 24µm depending on the organ size, providing a comprehensive macroscopic overview with high contrast, allowing for clear observation of anatomical boundaries. Subsequently, higher resolution scans down to 2 µm per voxel (1.4 µm for the small organs) allow for detailed examination of specific regions of interest (VOIs), revealing intricate microstructures (Figure 2C).

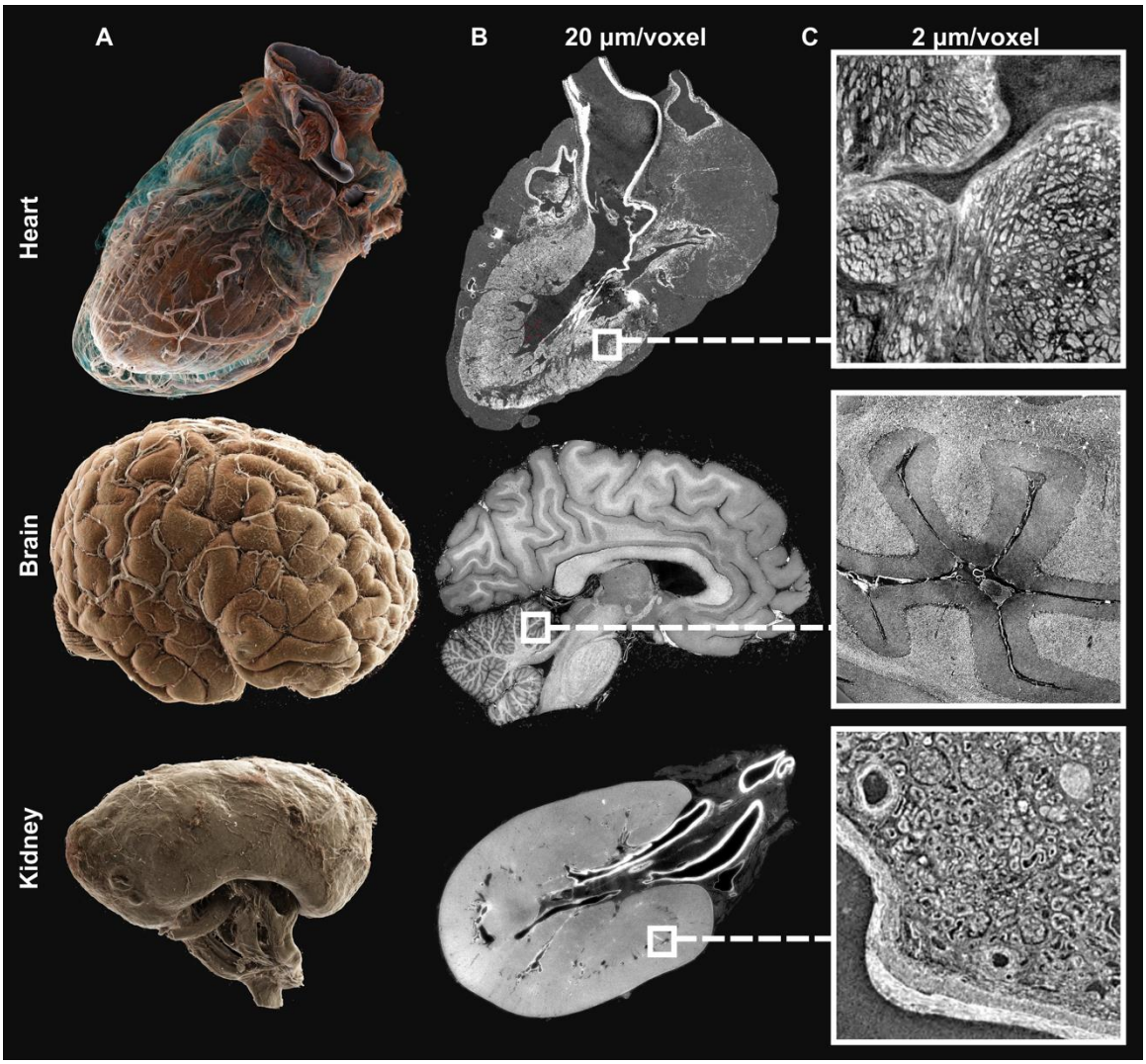

Figure 2. HiP-CT 3D imaging of intact human organs. (A) 3D cinematic rendering of whole organs. This rendering highlights the overall structure and anatomical features visible at a macroscopic scale. (B) Cross-sectional view of the HiP-CT reconstructed images at a voxel size *circa* 20 µm. (C) High-resolution zoom scans at a voxel size *circa* 2 µm. In C, the center of brain zoom is darker due to a small misalignment of the reference scan with the sample scan.

In whole organ imaging, HiP-CT can distinctly identify macroscopic features through anatomical location and morphology. For instance, the sulci and gyri of the cerebral cortex, the pelvis and calyces of the kidney, and the four chambers of the heart and associated coronary arteries are all clearly visible at the initial resolution (Figure 2A,B). HiP-CT also reveals functional units and specialized cells across different organs. As shown in Figure 2C, the layers of the cerebellum and microvasculature are identifiable. In the heart, structures such as cardiac muscle fibers and individual cardiomyocytes are visible. The kidney imaging shows the glomeruli along with its intricate capillary network. This hierarchical imaging capability facilitates the study of organ anatomy and pathology in unprecedented detail.

A large number of organ datasets with multiple zoom scans are downloadable on the Human Organ Atlas website (https://human-organ-atlas.esrf.eu). Furthermore, the Neuroglancer web interface enables online exploration of the whole dataset from anywhere with a web browser and internet connection.

4. DISCUSSION AND CONCLUSION

In this paper, we report the last advances of HiP-CT, a phase-contrast-based synchrotron X-ray tomography imaging technique using the high-energy capabilities of fourth-generation synchrotron sources. Our advancements enable detailed hierarchical 3D imaging of multiple intact human organs, maintaining high imaging quality from whole organs down to individual functional units and specialized cells in local zooms.

HiP-CT offers significant advantages, including non-destructive imaging, which allows for subsequent comparisons with other imaging techniques, such as histology, aiding in validation and HiP-CT image labeling. The hierarchical nature of HiP-CT addresses several limitations of synchrotron CT based methods by facilitating easy alignment of images at different resolutions from any location within the organ. This feature simplifies data visualization and interpretation and allows for the assessment of whether a high-resolution region is representative of the entire organ.

HiP-CT has demonstrated its translational potential in exploring the 3D microstructural underpinnings of various diseases. For instance, in a recent study, HiP-CT was used to investigate structural changes in the lungs of deceased donors infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [5,6], allowing for detailed visualization of the vascular alterations associated with the infection. These findings provided crucial insights into the mechanisms of COVID-19 pathology and highlighted the considerable translational potential of HiP-CT for biomedical applications. In another study, HiP-CT was used to map two entire hearts [7], one healthy and one pathological, revealing new insights into the cardiac conduction system, with potential applications in treating cardiovascular diseases such as arrhythmia.

Some limitations should be noted. first, HiP-CT is limited to ex-vivo studies due to the high radiation dose of the X-ray beam, which is unsuitable for living subjects, and to the necessity to use X-ray absorption homogenization by complete immersion and degassing. Additionally, the use of formalin and ethanol in sample preparation can cause tissue shrinkage. However, multiple ethanol baths of increasing concentration are used to mitigate this potential issue. The computational power and storage needed to manage the vast amounts of data generated by HiP-CT present another challenge, prompting the development of new methods to optimize data processing, storage efficiency, and accessibility for researchers.

Future research will focus on applying HiP-CT to a broader range of organs, enhancing our understanding of various anatomical and pathological conditions. The end goal is to image complete ex-vivo human bodies at 25 μm /voxel, thanks to continuous advancements in sample preparation, beamline equipment, and scanning methodologies. Furthermore, the open-access nature of HiP-CT data via platforms like the Human Organ Atlas will foster collaborative research, accelerating discoveries in medical science. Last but not least, having many samples imaged both using clinical techniques and HiP-CT should make possible to use the HiP-CT data to enhance *in fine* the quality of the data obtained by clinical imaging.

ACKNOWLEDGEMENTS

This project has been made possible in part by CZI grant DAF2020-225394 and grant DOI https://doi.org/10.37921/331542rbsqvn from the Chan Zuckerberg Initiative DAF, an advised fund of Silicon Valley Community Foundation (funder DOI 10.13039/100014989), the ESRF funding proposals md1252 and md1290, the Royal Academy of Engineering (CiET1819/10), and MRC (MR/R025673/1).

REFERENCES

[1] Müller, B., 2019, October. Recent trends in high-resolution hard x-ray tomography. In Developments in X-Ray Tomography XII (Vol. 11113, p. 1111302). SPIE.
[2] Velasco, A.A., Tanner, C., Schulz, G., Rodgers, G., von Jackowski, J., Humbel, M., Weitkamp, T., Bunn, H.T. and Müller, B., 2022, October. Hierarchical imaging of African bovid tooth cementum using X-ray microtomography. In Developments in X-Ray Tomography XIV (Vol. 12242, pp. 146-153). SPIE.

[3] Müller, R., 2009. Hierarchical microimaging of bone structure and function. Nature Reviews Rheumatology, 5(7), pp.373-381.
[4] Schulz, G., Götz, C., Deyhle, H., Müller-Gerbl, M., Zanette, I., Zdora, M.C., Khimchenko, A., Thalmann, P., Rack, A. and Müller, B., 2016, October. Hierarchical imaging of the human knee. In Developments in X-Ray Tomography X (Vol. 9967, pp. 41-52). SPIE.
[5] Ackermann, Max., Paul Tafforeau, Willi L. Wagner, Claire L. Walsh, Christopher Werlein, Mark P. Kühnel, Florian P. Länger et al. "The bronchial circulation in COVID-19 pneumonia." American Journal of Respiratory and Critical Care Medicine 205(1), 121-125 (2022). https://doi.org/10.1164/rccm.202103-0594IM
[6] Ackermann, M., Kamp, J. C., Werlein, C., Walsh, C. L., Stark, H., Prade, V., ... & Jonigk, D. D.. The fatal trajectory of pulmonary COVID-19 is driven by lobular ischemia and fibrotic remodelling. EBioMedicine, 85 (2022). https://doi.org/10.1016/j.ebiom.2022.104296
[7] Brunet, J., Cook, A. C., Walsh, C. L., Cranley, J., Tafforeau, P., Engel, K., ... & Lee, P. D.. Multidimensional Analysis of the Adult Human Heart in Health and Disease Using Hierarchical Phase-Contrast Tomography. Radiology, 312(1), e232731 (2024). https://doi.org/10.1148/radiol.232731
[8] Brunet, J., Walsh, C. L., Wagner, W. L., Bellier, A., Werlein, C., Marussi, S., ... & Tafforeau, P. Preparation of large biological samples for high-resolution, hierarchical, synchrotron phase-contrast tomography with multimodal imaging compatibility. Nature protocols, 18(5), 1441-1461 (2023). https://doi.org/10.1038/s41596-023-00804-z
[9] Mirone, A., Brun, E., Gouillart, E., Tafforeau, P., & Kieffer, J.. The PyHST2 hybrid distributed code for high speed tomographic reconstruction with iterative reconstruction and a priori knowledge capabilities. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 324, 41-48 (2014). https://doi.org/10.1016/j.nimb.2013.09.030
[10] Lyckegaard, A., Johnson, G., & Tafforeau, P.. Correction of ring artifacts in X-ray tomographic images. International Journal of Tomography and Statistics, 18(F11), 1-9 (2011).
[11] Maitin-Shepard J, Baden A.. Neuroglancer. GitHub. https://github.com/google/neuroglancer. Published 2021. Accessed July 20, 2024.
[12] Walsh, C. L., Tafforeau, P., Wagner, W. L., Jafree, D. J., Bellier, A., Werlein, C., ... & Lee, P. D.. Imaging intact human organs with local resolution of cellular structures using hierarchical phase-contrast tomography. Nature methods, 18(12), 1532-1541 (2021). https://doi.org/10.1038/s41592-021-01317-x