Paper introduces strategies for holotomography in advanced bio research

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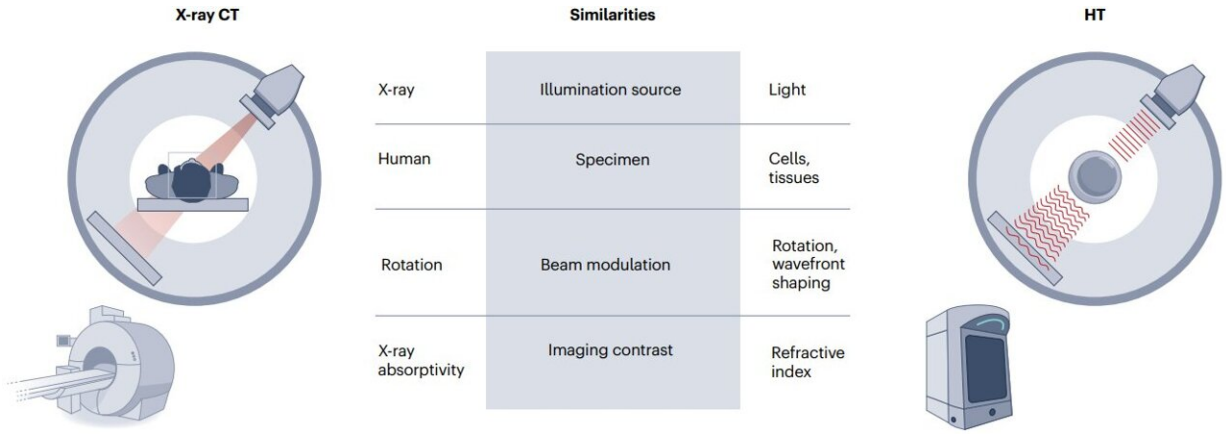


Schematic illustration of holotomography compared to X-ray CT. Similar to CT, they share the commonality of measuring the optical properties of an unlabeled specimen in three dimensions. Instead of X-rays, holotomography irradiates light in the visible range, and provides refractive index measurements of transparent specimens rather than absorptivity. While CT obtains three-dimensional information only through mechanical rotation of the irradiating light, holotomography can replace this by applying wavefront control technology in the visible range. Credit: KAIST Biomedical Optics Laboratory

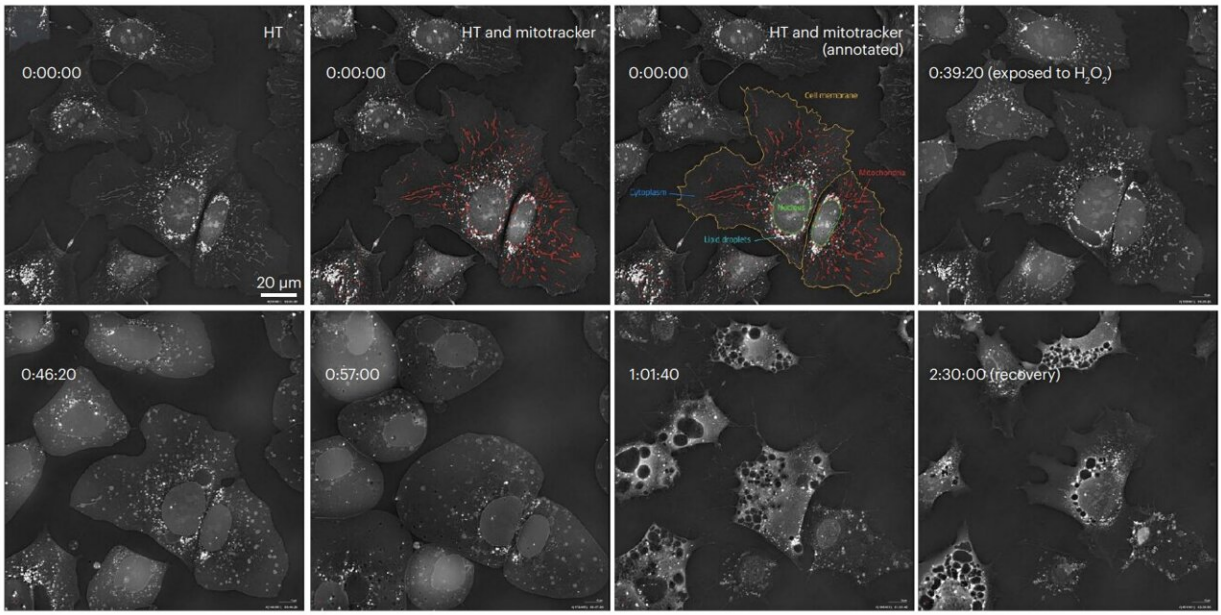
Measuring and analyzing three-dimensional (3D) images of live cells and tissues is considered crucial in advanced fields of biology and medicine. Organoids, which are 3D structures that mimic organs, are particular examples that significantly benefit 3D live imaging. Organoids provide

effective alternatives to animal testing in the drug development processes, and can rapidly determine personalized medicine. On the other hand, active research is ongoing to utilize organoids for organ replacement.

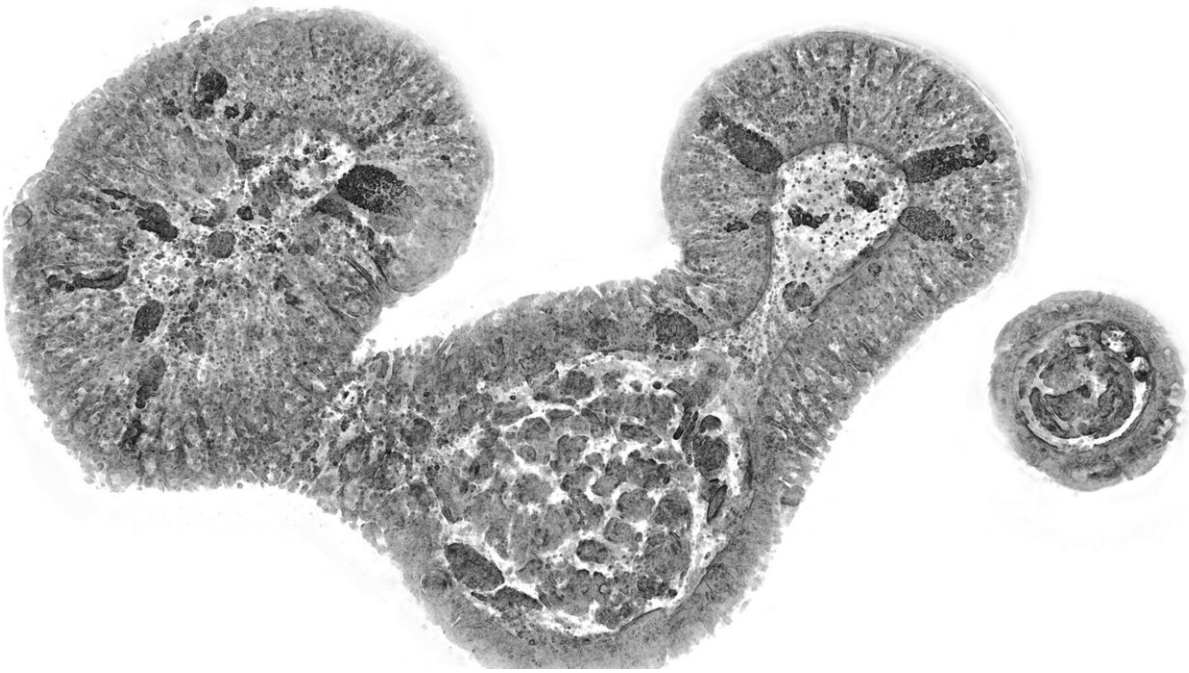
Organelle-level observation of 3D biological specimens such as organoids and stem cell colonies without staining or preprocessing holds significant implications for both innovating basic research and bioindustrial applications related to regenerative medicine and bioindustrial applications.

Holotomography (HT) is a 3D optical microscopy that implements 3D reconstruction analogous to that of X-ray computed tomography (CT). Although HT and CT share a similar theoretical background, HT facilitates high-resolution examination inside cells and tissues, instead of the human body.

HT obtains 3D images of cells and tissues at the organelle level without chemical or genetic labeling, thus overcoming various challenges of existing methods in bio research and industry. Its potential is highlighted in research fields where sample physiology must not be disrupted, such as regenerative medicine, personalized medicine, and infertility treatment.



Label-free 3D imaging of diverse live cells. Time-lapse image of Hep3B cells illustrating subcellular morphology changes upon H2O2 treatment, followed by cellular recovery after returning to the regular cell culture medium. Credit: KAIST Biomedical Optics Laboratory



Various types of cells and organelles that make up the imaging barrier of a living intestinal organoid can be observed using holotomography. Credit: KAIST Biomedical Optics Laboratory

This paper introduces the advantages and broad applicability of HT to biomedical researchers, while presenting an overview of principles and future technical challenges to optical researchers. It showcases various cases of applying HT in studies such as 3D biology, regenerative medicine, and cancer research, as well as suggesting future optical development.

Also, it categorizes HT based on the light source, to describe the principles, limitations, and improvements of each category in detail. In particular, the paper addresses strategies for deepening cell and organoid studies by introducing artificial intelligence (AI) to HT. The research is published in the journal *Nature Reviews Methods Primers*.

Due to its potential to drive advanced bioindustry, HT is attracting interest and investment from universities and corporates worldwide. The KAIST research team has been leading this international field by developing core technologies and carrying out key application research throughout the last decade.

This paper was co-authored by Dr. Geon Kim from KAIST Research Center for Natural Sciences, Professor Ki-Jun Yoon's team from the Department of Biological Sciences, Director Bon-Kyoung Koo's team from the Institute for Basic Science (IBS) Center for Genome Engineering, and Dr. Seongsoo Lee's team from the Korea Basic Science Institute (KBSI).

More information: Geon Kim et al, Holotomography, *Nature Reviews Methods Primers* (2024). DOI: 10.1038/s43586-024-00327-1

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