Holographic histopathology enables fast, precise diagnostics

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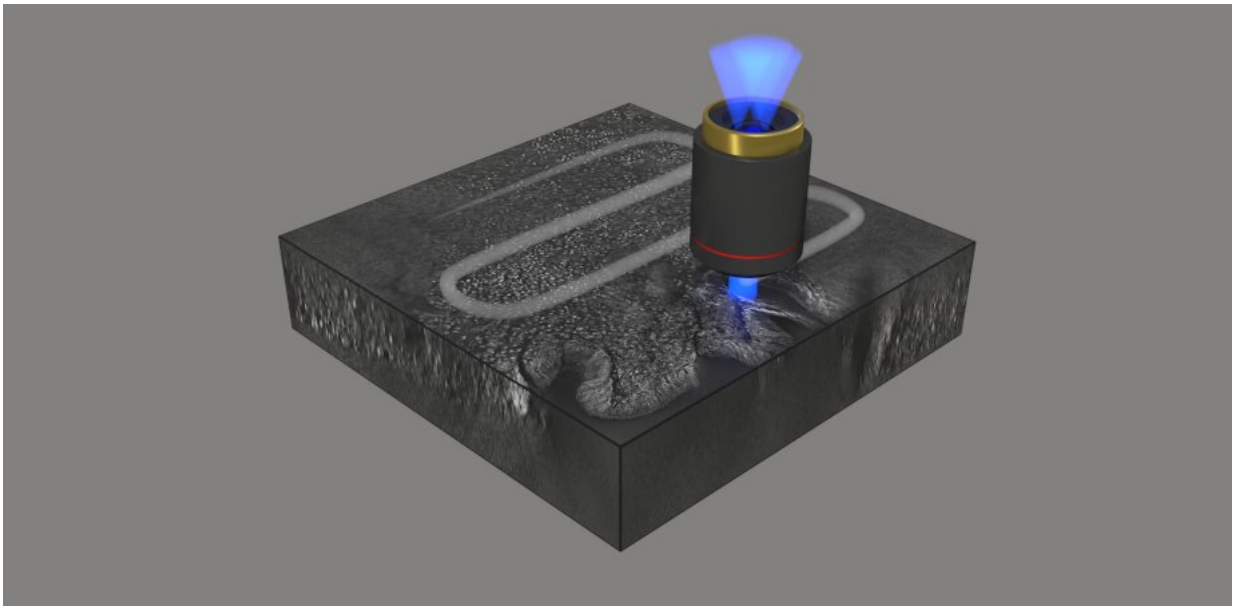


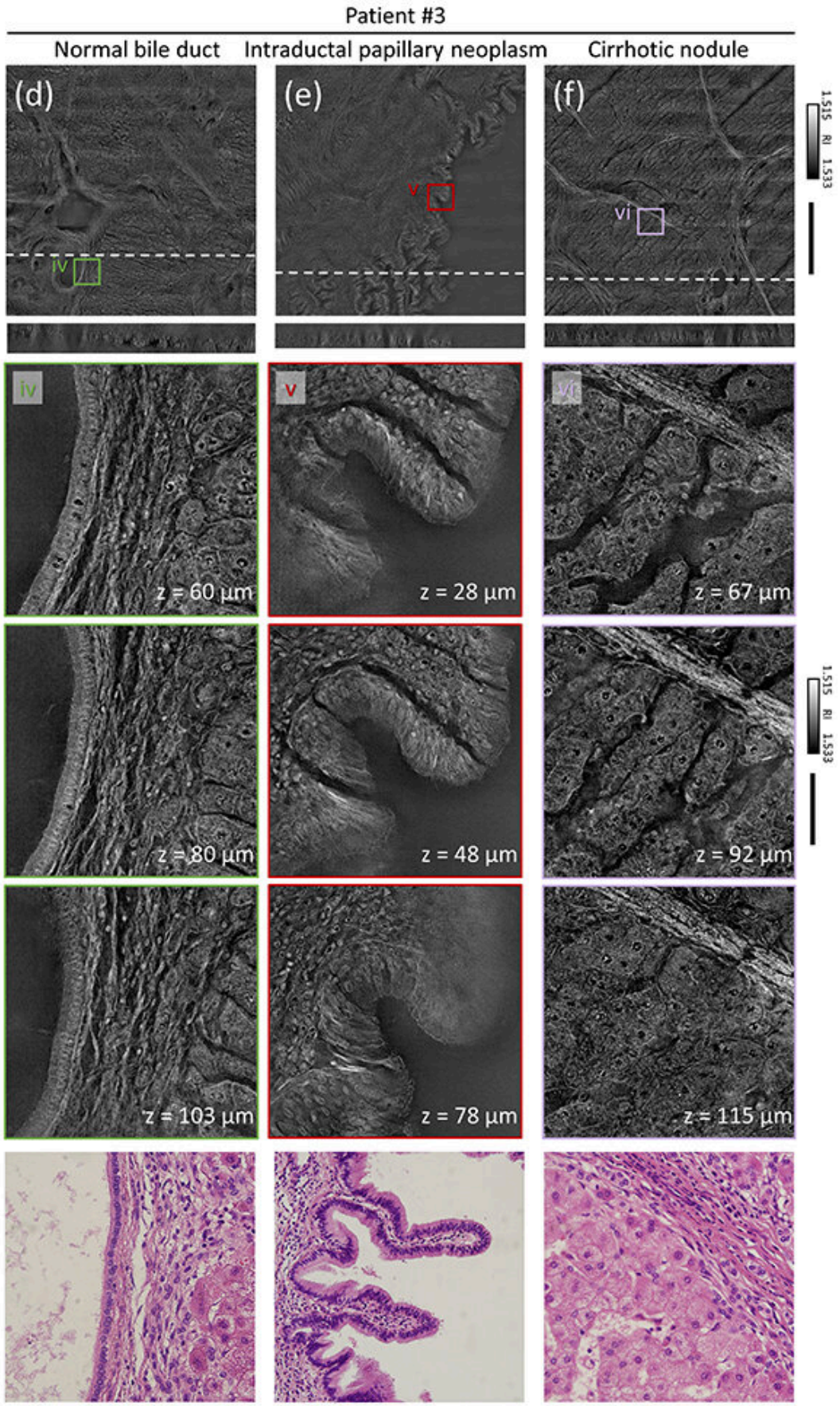
Schematic of the imaging of pathological tissue 3D structure by combining optical diffraction tomography and automated stitching. Credit: Hugonnet et al., doi 10.1117/1.AP.3.2.026004

Histology is the study of biological tissues at a microscopic level. Also called microscopic anatomy, histology is widely used to provide diagnosis of cancer and other diseases. For example, tissue samples obtained during surgery might help to determine whether further surgical action is needed, and further surgery may be avoided if a diagnosis can be rapidly obtained during an operation.

Traditional methods in histopathology are generally limited to thin specimens and require chemical processing of the tissue to provide sufficiently high contrast for imaging, which slows the process. A recent advance in histopathology eliminates the need for chemical staining and enables high-resolution imaging of thick tissue sections. As reported in *Advanced Photonics*, an international research team recently demonstrated a 3D label-free quantitative phase imaging technique that uses optical diffraction tomography to obtain volumetric imaging information. Automated stitching simplifies the image acquisition and analysis.

Optical diffraction tomography

Optical diffraction tomography is a microscopy technique for reconstructing the refractive index of a tissue sample from its scattered field images obtained with various illumination angles. It enables label-free high contrast visualization of transparent samples. The complex scattered field transmitted through the sample is first retrieved using off-axis holography, then the scattered fields obtained with various angle of illuminations are mapped in the Fourier space enabling the reconstruction of the sample refractive index.


Patient #3
Normal bile duct
Intraductal papillary neoplasm
Cirrhotic nodule
(d)
(e)
(f)
iv
v
vi
1.515 RI 1.533
z = 60 µm
z = 28 µm
z = 67 µm
z = 80 µm
z = 48 µm
z = 92 µm
z = 103 µm
z = 78 µm
z = 115 µm

Volumetric histopathology of unlabeled 100-µm-thick pancreas tissue sample from a patient with intraductal papillary neoplasm of bile duct in the liver. For the purpose of comparison, adjacent tissues were prepared in thin tissue slides with conventional H&E staining method. (the fifth row, 400x magnification). Credit: Hugonnet et al., doi 10.1117/1.AP.3.2.026004.

A recognized limitation of optical diffraction tomography is due to the complex distribution of refractive indexes, which results in significant optical aberration in the imaging of thick tissue. To overcome this limitation, the team used digital refocusing and automated stitching, enabling volumetric imaging of 100-um-thick tissues over a lateral field of view of 2 mm x 1.75 mm while maintaining a high resolution of 170 nm x 170 nm x 1400 nm. They demonstrated that simultaneous visualization of subcellular and mesoscopic structures in different tissues is enabled by high resolution combined with a wide field of view.

Fast, accurate histopathology

The researchers demonstrated the capacity of their novel method by imaging a variety of different cancer pathologies: pancreatic neuroendocrine tumor, intraepithelial neoplasia, and intraductal papillary neoplasm of bile duct. They imaged millimeter-scale, unstained, 100-µm-thick tissues at a subcellular 3D resolution, which enabled the visualization of individual cells and multicellular tissue architectures, comparable to images obtained with traditional chemically processed tissues. According to YongKuen Park, researcher at the Korea Advanced Institute of Science and Technology and senior author on the study, "The images obtained with the proposed method enabled clear visualization of different morphological features in the various tissues allowing for

recognition and diagnosis of precursor lesions and pathologies."

Park notes that further research is needed, but the results suggest great potential for fast, accurate histopathology during surgery: "More research is needed on sample preparation, reconstruction speed, and mitigation of multiple scattering. We expect optical diffraction tomography to provide faster and more precise diagnostics in histopathology and intraoperative pathology consultations."

More information: Herve Hugonnet et al, Multiscale label-free volumetric holographic histopathology of thick-tissue slides with subcellular resolution, *Advanced Photonics* (2021). DOI: 10.1117/1.AP.3.2.026004

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