

# **Serial Tiled Z-stacks (STZ) microscopy of the tumor microenvironment in optically cleared tissue**

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3D tissue anatomical structures at a single cell resolution can reveal the tumor microenvironment which is essential information to establish future diagnostic plans. Compare to a conventional serial 2D histopathology image stack, the 3D images of tissue cleared tumors in this report contains the complete information without section loss and could correlate tissue structures and targeted molecular marker in their naive 3D morphology. However, the optical penetration depth of an un-cleared tumor images is limited by  $< 30 \mu\text{m}$ , and the state of art tissue clearing technique already improves the optically transparent depth to a few millimeters. The optically transparent tumor is still not homogeneous, so the best imaging depth is still limited to  $\sim 200 \mu\text{m}$ . As a result, a serial tiled Z-stack (STZ) microscope is developed to provide a 3D image stack of  $> 200 \mu\text{m}$  in thickness, with an interactive slicing system and a confocal microscope; the image of whole tumor could be acquired by serial imaging loop, and the implementation of the onstage clearing process improves the imaging depth. An overlapping region between two imaging acquisition batches can avoid the sampling loss due to section. Although the data size of 3D images is much larger than of conventional 2D pathological slide images, the computer AI assisted auxiliary system can still provide 3D parameters to pathologists for better diagnoses.