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Experimental platform for optical sectioning in bright field microscopy

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Abstract

Bright-field (BR) microscopes are found everywhere, from the biggest metropolitan hospital to the local microbiology lab, since they comprise the most essential visualization tool in diagnostic pathology. However, BR microscopy can create only projection 2D-images, has finite depth of field and its resolution is diffraction limited. These problems have been solved by modern technologies, such as confocal and multiphoton excitation microscopy. However, the incorporation of such technologies in daily clinical practice as alternative to BR microscopy is more likely impossible due to the large complexity, the time consuming protocols and the extremely expensive cost. Thus, clinical routine still relies on the projection 2D diffracted-limited resolution BR microscopes. The aim of this study is to present the proof of concept of a methodology that enables the transformation of conventional BR microscopes in 3D sample imaging instruments by a/ rotating the specimen at limited angles around the focal plane, b/ acquiring projection measurements at each different angle and c/ reconstructing the whole 3D image of the sample using the concept of computed tomography. In this way, conventional BR microscopes found in every hospital, private clinic and local microbiology lab could be easily transformed into 3D optical slicing instruments of improved resolution, which will enable observing physicians to get access to information (visual and quantitative) that is, otherwise, undetected by the standard BR microscope. Preliminary experiments involve imaging of tissue samples with breast cancer on a Leica DM 2500 microscope coupled to a Leica DFC420 C digital camera.

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