

Medical > Ex Vivo > Other material

20

Confocal mosaicing microscopy of human skin ex vivo: spectral analysis for digital staining to simulate histology-like appearance.

Bini J, Spain J, Nehal K, Hazelwood V, DiMarzio C, Rajadhyaksha M.; J Biomed Opt. 2011 Jul;16(7):076008. DOI: 101117/1.3596742

ABSTRACT

Confocal mosaicing microscopy enables rapid imaging of large areas of fresh tissue, without the processing that is necessary for conventional histology. Mosaicing may offer a means to perform rapid histology at the bedside. A possible barrier toward clinical acceptance is that the mosaics are based on a single mode of grayscale contrast and appear black and white, whereas histology is based on two stains (hematoxylin for nuclei, eosin for cellular cytoplasm and dermis) and appears purple and pink. Toward addressing this barrier, we report advances in digital staining: fluorescence mosaics that show only nuclei, are digitally stained purple and overlaid on reflectance mosaics, which show only cellular cytoplasm and dermis, and are digitally stained pink. With digital staining, the appearance of confocal mosaics mimics the appearance of histology. Using multispectral analysis and color matching functions, red, green, and blue (RGB) components of hematoxylin and eosin stains in tissue were determined. The resulting RGB components were then applied in a linear algorithm to transform fluorescence and reflectance contrast in confocal mosaics to the absorbance contrast seen in pathology. Optimization of staining with acridine orange showed improved quality of digitally stained mosaics, with good correlation to the corresponding histology.