World Academy of Science, Engineering and Technology
International Journal of Mathematical and Computational Sciences
Vol:14, No:12, 2020

3D Label-Free Bioimaging of Native Tissue with Selective Plane Illumination Optical Microscopy

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Abstract : Biomedical imaging of native tissue using light offers the potential to obtain excellent structural and functional information in a non-invasive manner with good temporal resolution. Image contrast can be derived from intrinsic absorption, fluorescence, or scatter, or through the use of extrinsic contrast. A major challenge in applying optical microscopy to in vivo tissue imaging is the effects of light attenuation which limits light penetration depth and achievable imaging resolution. Recently Selective Plane Illumination Microscopy (SPIM) has been used to map the 3D distribution of fluorophores dispersed in biological structures. In this approach, a focused sheet of light is used to illuminate the sample from the side to excite fluorophores within the sample of interest. Images are formed based on detection of fluorescence emission orthogonal to the illumination axis. By scanning the sample along the detection axis and acquiring a stack of images, 3D volumes can be obtained. The combination of rapid image acquisition speeds with the low photon dose to samples optical sectioning provides SPIM is an attractive approach for imaging biological samples in 3D. To date all implementations of SPIM rely on the use of fluorescence reporters be that endogenous or exogenous. This approach has the disadvantage that in the case of exogenous probes the specimens are altered from their native stage rendering them unsuitable for in vivo studies and in general fluorescence emission is weak and transient. Here we present for the first time to our knowledge a label-free implementation of SPIM that has downstream applications in the clinical setting. The experimental set up used in this work incorporates both label-free and fluorescent illumination arms in addition to a high specification camera that can be partitioned for simultaneous imaging of both fluorescent emission and scattered light from intrinsic sources of optical contrast in the sample being studied. This work first involved calibration of the imaging system and validation of the label-free method with well characterised fluorescent microbeads embedded in agarose gel. 3D constructs of mammalian cells cultured in agarose gel with varying cell concentrations were then imaged. A time course study to track cell proliferation in the 3D construct was also carried out and finally a native tissue sample was imaged. For each sample multiple images were obtained by scanning the sample along the axis of detection and 3D maps reconstructed. The results obtained validated label-free SPIM as a viable approach for imaging cells in a 3D gel construct and native tissue. This technique has the potential use in a near-patient environment that can provide results quickly and be implemented in an easy to use manner to provide more information with improved spatial resolution and depth penetration than current approaches.

Keywords : bioimaging, optics, selective plane illumination microscopy, tissue imaging
Conference Title : ICSRD 2020 : International Conference on Scientific Research and Development
Conference Location : Chicago, United States
Conference Dates : December 12-13, 2020