WIDE-FIELD DEEP ULTRAVIOLET SURFACE EXCITATION MICROSCOPE FOR 3-DIMENSIONAL BRAIN IMAGING

Deepa KASARAGOD, Meina ZHU, Hidenori AIZAWA
Department of Neurobiology, Graduate School of Biomedical & Health Sciences
Hiroshima University, Hiroshima, JAPAN
Email: deepa@hiroshima-u.ac.jp

Keywords: Block-face imaging, fluorescence microscope, whole brain imaging

Three dimensional (3D) cellular scale imaging techniques that allows for accurate quantification of smaller anatomical structures are important to improve our understanding of the brain architectural changes in the normal and the pathological conditions. For example, recently, it has been shown in the mouse depression model that there is a decrease in the volume of habenular structures [1]. Common 3D microscopy techniques include confocal, two-photon, light sheet imaging, etc. However, they consist of complex imaging units and are expensive. Recently, the imaging capability using deep ultraviolet light has been shown for block-face imaging of pathological samples for 2-D imaging [2-3]. It makes use of two aspects of deep UV light: 1). strong absorbance of the UV light in tissues and hence low penetration into the tissues compared to the other spectrum of light. 2). The property of deep UV light to excite conventional fluorescent dyes to emit light in the visible spectrum. Here, we report on a 3D wide-field imaging prototype using deep UV serial block-face imaging for imaging cellular components of brain using whole block tissue staining methods.

Our 3D wide-field imaging setup consists of UV light emitting diode at wavelength 285 nm in oblique incidence, a 10X/20X water immersion objectives and a color camera. The optical sectioning was achieved using UV surface excitation by limiting the fluorescence emission to tissue surface (20 μm or less in depth [3]). A custom motorised stage was built for wide-field imaging [4]. For the whole brain staining, tissue preparation steps were as follows: mice were anesthetized were transcardially perfused with PBS followed by 4% paraformaldehyde (PFA) dissolved in PBS. Excised brains were post-fixed in 4% PFA in PBS overnight. The mouse brains were stained for cell nuclei following the sucrose osmotic shock protocol [5]. For whole brain imaging, the sample was mounted on the Compresstome® (VF-700-OZ; Precisionary Instruments) vibrating microtome sample holder and was then embedded into 2% agarose solution for slicing. Serial sectioning was carried out after each wide-field cross-section imaging using this set up. A simplified set up that utilizes a single wavelength source for excitation of wide range of conventional dyes for 3D wide-field cellular resolution imaging towards quantitative imaging of the brain structures has been developed.

References:

[1] Yang et al., Three-dimensional analysis of mouse habenula subnuclei reveals reduced volume and gene expression in the lipopolysaccharide-mediated depression model, 2019, Exp. Neurobiol., 28(6): 709-719.

[2] Fereidouni et al., Microscopy with ultraviolet surface excitation for rapid slide-free histology, 2017, Nat Biomed Eng.,1: 957–966.

[3] Yoshitake et al., Rapid histopathological imaging of skin and breast cancer surgical specimens using immersion microscopy with ultraviolet surface excitation, 2018, Scientific Reports, 8: 4476.

[4] Campbell et al., Openstage: A Low-Cost Motorized Microscope Stage with Sub-Micron Positioning Accuracy, 2014, PLoS ONE, 9(2): e88977.

[5] Seiriki et al., High-Speed and Scalable Whole-Brain Imaging in Rodents and Primates, 2017, Neuron 94: 1085–1100.