A SIMPLIFIED OPTICAL SCHEME FOR HIGH RESOLUTION SERIAL BLOCK-FACE FLUORESCENCE MICROSCOPY FOR 3-DIMENSIONAL MORPHOMETRY OF RODENT BRAINS

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Systems neuroscience aim to understand the complexities underlying the microscale organization and their effects at the level of organisms manifested as functional and behavioural attributes. There is a need for microscopy tool that allows for high throughput 3-dimensional (3D) morphological assessment of mouse disease models towards understanding the neural connectivity and its manifestation at the macroscale [1]. Although classical approaches have combined tissue clearing and whole block staining techniques with the confocal, two-photon or light sheet microscopy to this extent, for warrant of being complex and expensive, large scale and easy deployment is not a feasible option.

We present a simplified optical scheme for serial block-face fluorescence microscopy using deep ultraviolet (DUV) illumination for 3D morphological assessment of smaller anatomical structures of the brain with subcellular resolution. Optical sectioning is achieved on block-face imaging mode owing to the surface excitation ability of the deep ultraviolet light due to its low penetration in the biological tissues. This was first utilized for 2d imaging to obtain histology like virtual images from fluorescence microscopy [2-3]. Recently, we have shown the versatility of this DUV microscope for a variety of applications for fluorescence based brain imaging on par with the conventional imaging widely used [4]. 3D-DUV microscope integrates a tissue slicer for serial imaging. Arduino microcontroller based custom developed translation stage is used for wide-field imaging. In this presentation, we shall discuss the optical design, hardware and software interface as well as the whole brain staining techniques utilized for automated 3-dimensional imaging using DUV fluorescence microscope. 3D morphological assessment of habenula which is a small relay nucleus that plays a critical role in a diverse behaviour aspects as well as in depression shall also be presented.

References:

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