TRANSFORM YOUR MACROSCOPE INTO AN ULTRAMACROSCOPE FOR 3D IMAGING OF CLEARED ORGANS

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Serial thin sectioning of whole organs are commonly used to indirectly get the three-dimensional (3D) reconstruction of their histological structures. This approach requires intact sections as well as proper alignment of all the individual images [1]. In the 90's, light-sheet fluorescence imaging combined with clearing methods was firstly used to image whole guinea pig cochlea [2]. More recently, this approach was implemented for bigger samples with a dual illumination path, coupled with the development of clearing reagents [3]. A version of this system is commercially available. Here, we propose an open and versatile light sheet based instrumentation which can easily be implemented on commercial fluorescence macroscopes, Many microscopy facilities and labs already possess a detection unit *i.e.* commercial fluorescence macroscopes coupled with cameras. Our approach allows a cost effective upgrade of a pre-existing systems into "ultramacroscopes". It consists in a plug and play cylindrical-lens based dual illumination unit with a sample holder mounted on a fast vertical translation stage. Optical fibers coming from laser boxes can directly be plugged on the unit. The two light-sheets can be easily aligned using a specially-designed alignment cuvette and/or with the help of a dynamic overlay of the fluorescence images obtained by a sequential right/left illumination. All the hardware is piloted by the open source MicroManager software [4]. A previous version of the prototype proved its efficiency on gliobastoma imaging in whole mice brains cleared with 3Disco [5]. We now present our last 3D images obtained on this new prototype with 3Disco, iDisco and Ultimate Disco cleared organs.

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