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 commercial version of this latter is available. Here, we propose an open and versatile light sheet based instrumentation which can easily be implemented on current fluorescence macroscopes, Many microscopy facilities and laboratories 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" and was tested on four different macroscopes available on the market.

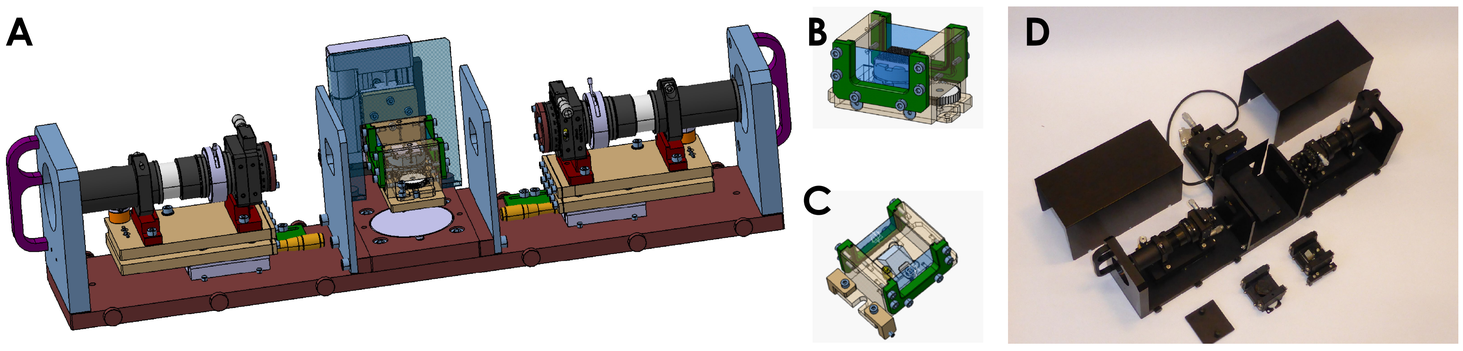
Architecture of the illumination module

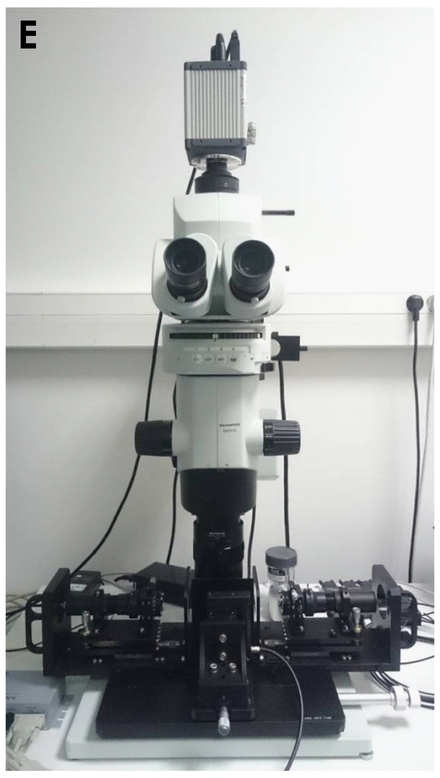
A modular and compact design

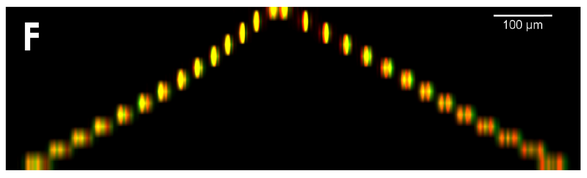
This system consists in a plug and play cylindrical-lens based dual illumination unit. Optical fibers coming from external laser combiners can directly be plugged on the unit through two FC/PC fiber ports.

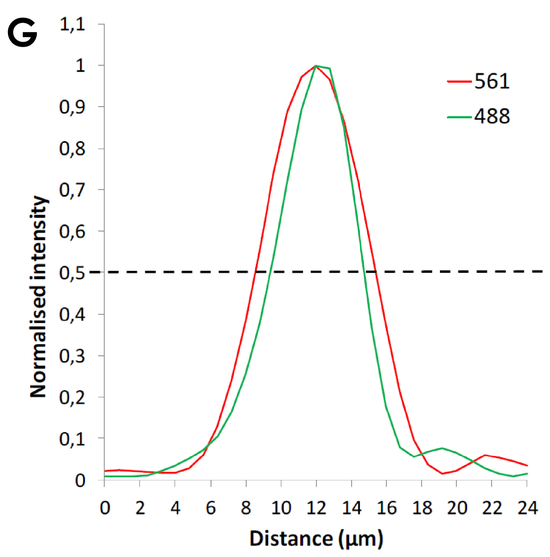
Illumination unit (A,D)

Each monomode fiber is plugged onto one illumination unit at the focal plane of an achromatic doublet (AC254-075-A-ML Thorlabs). The parallel beam emerging from this lens is projected onto a cylindrical doublet (ACY254-100-A Thorlabs) through an iris diaphragm. The two light-sheet obtained are facing each other, equidistant from the sample cuvette (B), crossed by the beams. The sample holder is mounted on a fast vertical translation stage (M-122.2DD, Physik Instruments).









Integration on a macroscope(E)

The unit was evaluated on different commercial macroscopes (MVX10 Olympus, Leica Z16, AxiozoomV16 Carl Zeiss, AZ100 Nikon) equiped with a sCMOS (ORCA Flash4 Hamamatsu) or a CCD camera (ORCA R2 Hamamatsu, CoolSNAP ES Roper Scientific). The system available in our facility was built on an MVX10 (E) with a four lasers bench (405,488,561,638nm, LBX-4C Oxxius) with a dual fiber output. In the last version the trinocular tube and cube turret of the MVX10 were replaced by a high-speed 4 position wheel (Lambda 10-B, Sutter Instruments) and a lens tube. All the hardware is piloted by the MicroManager software [4].

Co-alignment of the light-sheets (F,G)

The focalisation and co-alignment of the light sheets on the sample is done by the translation (M-UMR5_16 linear stage, BM11.16 Micrometer Newport) and tilting (AJS100-0.5 adjustement screw)of the illumination unit. This alignment is helped by a specially-designed alignment cuvette containing a knife-edge prism (KRPB4-15-550 Optosigma). An orthogonal view of the 488nm and 561nm light-sheets at different positions of the prism (F) indicates the quality of the alignment and allows a measurement of the axial resolution of the system (G). This alignment can be refined on the sample observed with the help of a beanshell script to do a dynamic overlay of the fluorescence images obtained by a sequential right/left illumination in MicroManager.

Portfolio

A first mono beam version of the prototype proved its efficiency on gliobastoma imaging in whole mice brains [5] and lymph nodes in pancreas [6] cleared by the 3Disco method.

A) Mouse pancreas with Langherans islets stained with Alexa488 (iDisco clearing method)
B) Vascularisation of a mouse cutaneous tumor stained with WGA Alexa647 (iDisco clearing method)
C) Thy1.1 EYFP mouse brain (ultimate Disco clearing method)
D) RFP Drosophilia (3Disco clearing method)

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