

COMPACT LASER SCANNING CONFOCAL MICROSCOPE

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In the frame of ESA General Support Technology Program, a compact confocal laser scanning microscope has been developed for 3D fluorescence imaging of biological samples.

The microscope permits normal confocal mode operation with 488nm wavelength excitation source and fluorescence lifetime imaging (FLIM) with 630nm wavelength excitation source. Fluorescence signals emitted following excitation from each of the source are detected on dedicated photomultiplier tubes (PMT). Proper optical signal separation and filtering is performed by means of dedicated filters dichroic separators. The software and hardware further include the specific imaging modes that are fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP).

In addition to this dual wavelength fluorescence imaging mode, the microscope includes a transmission imaging mode. The contrast technique implemented is differential imaging contrast (DIC). Both fluorescence and DIC imaging can be acquired simultaneously. The source chosen for the DIC is a near infrared LED. This choice permits the decoupling of DIC and fluorescence signals by a dichroic cold mirror. The DIC interference is issued from combination of Wollaston prism in conjugated planes. Contrary to most commercial DIC, the interference is performed in an image plane of the back focal plane of the microscope objective, with standard Wollaston prism (while most often Nomarsky prisms are used).

The FOV is 450nm x 450nm. The optics chosen for the microscope objective and tube lens system are Zeiss elements, as being the most compact microscopy system with a tube lens focal length of 160mm. The microscope objective is water immersion type with NA = 0.8, coverslip corrected, mounted in inversion mode. The opto-mechanical assembly is constructed on the two sides of the breadboard shall not exceeding 387x327x574 mm³.

A motorized sample transportation stage permits sample positioning in x,y,z, with unidimensional repeatability of 0.1µm. In this poster, the design, performances and limitations of the compact confocal microscope are presented. Examples of application on biological samples are shown.