Cryo-fluorescence imaging and CLEM on an inverted microscope platform with convenient sample access

M. Schwertner[1], K. Prakash[2], P. Timmins[3], M. Shaw[2]

[1]Linkam Scientific Instruments Ltd., Unit 8, Epsom Downs Metro Centre, Tadworth, KT20 5LR, United Kingdom; email: michaelschwertner@linkam.co.uk; [2]National Physical Laboratory, Hampton Road, Teddington, TW11 0LW, UK; [3]Aurox Ltd, Unit F4, Culham Science Centre, Abingdon, Oxfordshire, OX14 3DB, UK;

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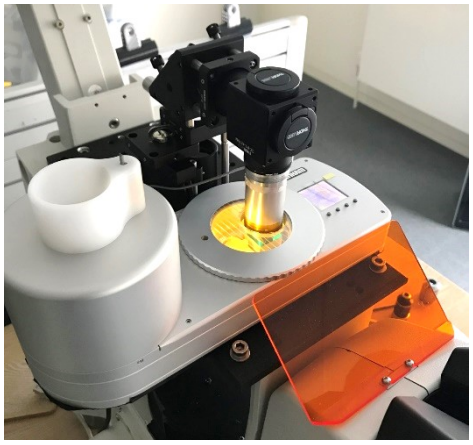
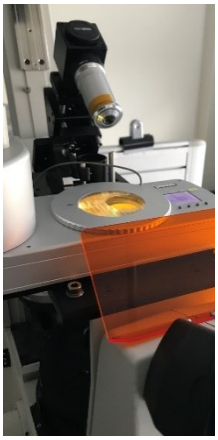
Cryo-imaging of biological samples embedded in vitrified ice has unique advantages and the development and implementation of cryo-EM and single particle analysis led to a Nobel Prize in 2017 [1]. More recently cryo-EM was also used for research into the structure and mechanisms of the coronavirus [2].

Fluorescence cryo-imaging in confocal and widefield modes can support the electron microscopy workflow in several ways. Firstly, in the form of CLEM (Correlative Light and Electron Microscopy), where fluorescence imaging of samples allows the use of specific and sensitive markers not available in EM. CLEM also limits sample EM beam damage through pre-identification of ROI's in a light microscope (LM) image with subsequent matching of coordinates. Secondly, cryo-imaging in the LM allows sample pre-screening and assessment of preparation quality saving costly EM time.





Current cryo-stage designs for fluorescence are typically for upright microscope setups and cannot be integrated with the inverted widefield or confocal fluorescence systems commonly found in bio-labs. While inverted-upright conversion optics are available [3], typical implementations do not allow straightforward sample loading. Here we describe a prototype for the integration of a CMS196V3 (upright) cryo-stage into an inverted microscope system (Nikon Eclipse Ti-E with Aurox Clarity Laser-Free Confocal) with a tilt mechanism for the direct loading of cryo-samples. In this talk we will present example image data along with PSF measurements to assess the optical performance of the conversion system.

We anticipate that the convenience of sample loading combined with integration into more common microscope systems can lead to a wider adaptation of cryo-fluorescence and CLEM.

[1] https://www.nature.com/news/cryo-electron-microscopy-wins-chemistry-nobel-1.22738
[2] G. Wolff et al., Science 10.1126/science.abd3629 (2020).
[3] http://lsmtech.com/