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Correlation Microscopy: Bridging the Gap between Light- and Cryo-Electron Microscopy

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Cryo-electron tomography (cryo-ET) of frozen, hydrated, biological samples on carbon-coated copper grids is a powerful technique for quasi *in vivo* studies of cellular structures with nanometer-scale resolution [1]. A major inconvenience of this method exists in the difficulty of locating and unequivocally identifying the structures of interest within a ice embedded sample. Additionally, kinetic experiments are almost impossible, due to the fact that the information about the dynamic state in vitrified samples has been lost. By establishing a direct correlation between cryo-fluorescence microscopy and cryo-electron microscopy (cryo-EM), we propose an effective way to overcome these limitations, opening the way for a wide spectrum of novel applications. Our concept of correlation microscopy is based on the possibility of identifying and determining the position of fluorescently-labelled structures in vitrified samples directly on the TEM grid by means of cryo-fluorescence microscopy, and the recovery of these positions during subsequent investigations with cryo-EM.

The entire vitrified grid can be imaged and mapped with epifluorescence under cryo-conditions thanks to a new designed and homemade cryo-holder for TEM grids, adapted to a fully automated, inverted light microscope (Zeiss Axiovert 200) with long working-distance objectives (20x and 40x magnification). The cryo-holder is devised to keep the sample at liquid nitrogen temperature (lN_2) and it is efficiently isolated from the external environment to ensure a thermal equilibrium and to prevent undesirable contamination during investigations. The absolute coordinates of the area of interest on the grid can be determined and recorded by cryo-light microscopy and directly recovered in the electron microscope with a MatLab-based program, integrated within the TOM toolbox [2].

Fluorescently labelled neurons grown on carbon-coated TEM grids have been embedded in vitreous ice and successfully used for first studies. In the case of thin specimens suitable for cryo-electron tomography (where the thickness of the amorphous ice does not exceed 200-400 nm), the resolution is comparable to investigations in buffer solutions. The ‘blurring’ of the fluorescence signal and the decrease in resolution is directly related to the thickness of the ice, which can be estimated from focusing and transmitted light measurements.

Our current applications involve the localisation of synapses by immunolabelling of glutamate receptors with fluorescent dyes and the distribution of fluorescent markers in vitreous cryo-sections. Future applications will involve labelling of extra-cellular molecules, structures and receptors with Quantum Dots [3], which are ideal candidates for correlation microscopy due to their fluorescence and electron-dense characteristics, which allow visualisation and targeting of the label both with cryo-light microscopy and cryo-EM, especially cryo-ET.

References

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[2] S. Nickell et al., Journal of Structural Biology (2005) in press (available online)
[3] D. R. Larson et al., Science 300, 1434-1436 (2003)
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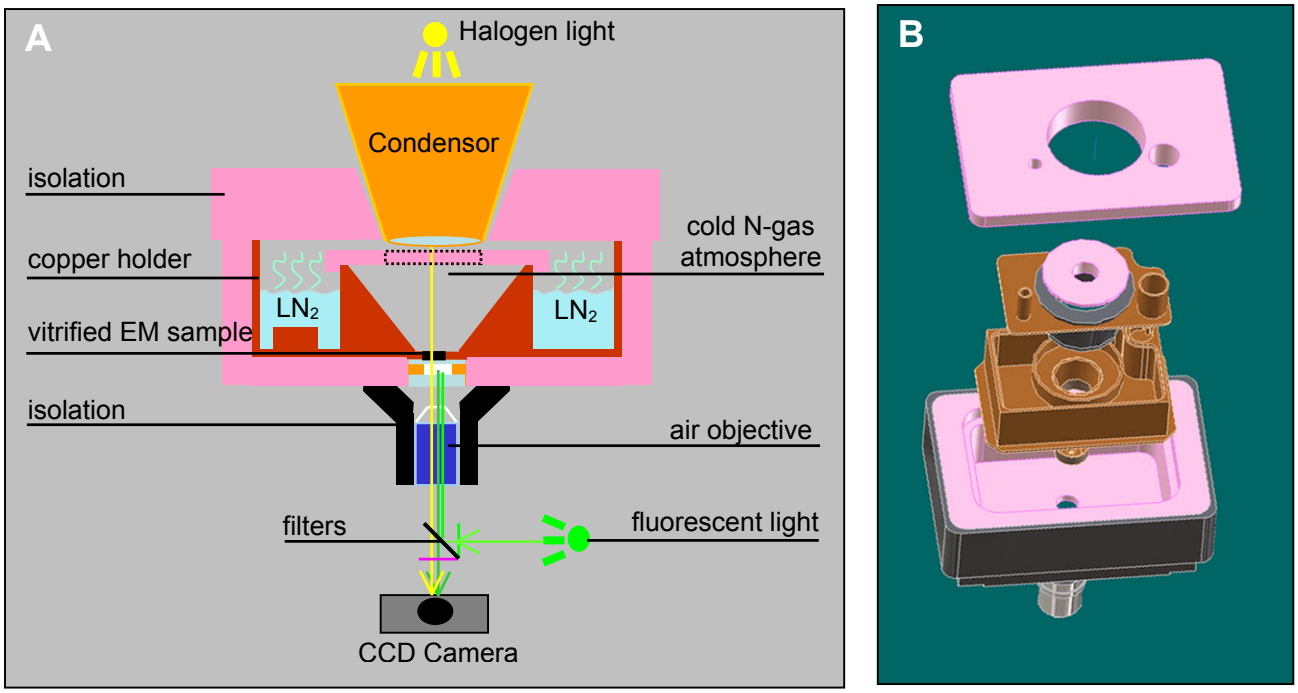

Figure 1: Schematic representations of the new designed cryo-holder for vitrified EM samples, adapted to a fully automated, inverted light microscope with long working-distance objectives (20x and 40x). **(A)** Cross-section of the cryo-holder setup. A specially designed copper holder keeps the grid at liquid nitrogen temperature. The grid is isolated from the external environment by a layer of Acetal and can be imaged both with transmitted and with fluorescent light. **(B)** 3-D view of the cryo-holder.

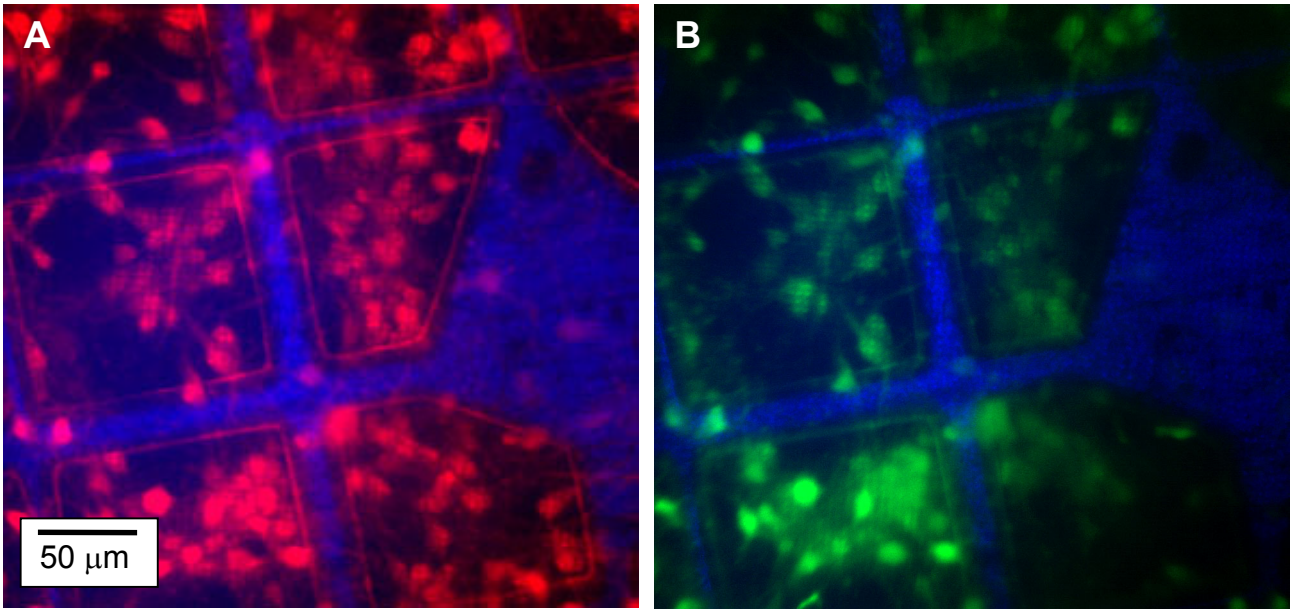

Figure 2: Epifluorescence images with 40x magnification of hippocampal neurons from rat embryos cultured on a TEM grid and live-stained with Fura-2 membrane-permeant calcium indicator. **(A)** Fluorescence image of the stained neurons before rapid freezing in liquid ethane. **(B)** Cryo-fluorescence image of the ice-embedded neurons at the same area of the grid as in (A). The sample was imaged while being kept at lN_2 in the cryo-holder represented in Fig. 1. Neuronal cell bodies as well as processes are clearly visible.

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