HIGH-PRECISION CORRELATIVE FLUORESCENCE AND ELECTRON CRYO MICROSCOPY

Pascale Schellenberger\textsuperscript{1}, Rainer Kaufmann\textsuperscript{1,2}, C. Alistair Siebert\textsuperscript{1}, Christoph Hagen\textsuperscript{1}, Harald Wodrich\textsuperscript{3}, Kay Grünwald\textsuperscript{1}

\textsuperscript{1}Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, OX3 7BN, UK
\textsuperscript{2}Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK
\textsuperscript{3}Microbiologie Fondamentale et Pathogénicité, MFP CNRS UMR 5234, University of Bordeaux SEGALEN 146 rue Leo Seignat, 33076 Bordeaux, France

E-mail: rainer@strubi.ox.ac.uk

**KEY WORDS:** fluorescent cryo microscopy, electron cryo microscopy, cellular tomography, fluorescent microspheres, correlative microscopy

Correlative light and electron microscopy (CLEM) is an emerging technique combining functional information provided by fluorescence microscopy (FM) with the high-resolution structural information of electron microscopy (EM). So far correlative cryo microscopy on frozen hydrated samples has not been reported below the micrometre accuracy. Here a method is presented that enables the correlation between fluorescently tagged proteins and electron cryo tomography (cryoET) data with a precision accuracy in the nanometre range. In particular, thin areas of vitrified whole cells are examined by correlative cryoFM and cryoET. We will present a workflow for cryoCLEM which includes the implementation of two independent electron dense fluorescent markers to improve the precision of the alignment, but also provides the ability of obtaining an estimate of the correlation accuracy for each individual object of interest. We will show the correlative workflow from plunge-freezing to cryoET step by step at the example of locating fluorescence-labelled adenovirus particles trafficking inside a cell, where we achieved an overall correlation accuracy in the range of ~ 60 nm.

With the main limiting factor being the number of photons detected from the fluorophores attached to one particle, the present method has the capability of reaching molecular precision for locating objects in cryoEM images identified with cryoFM.