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3D chemical mapping of intracellular granules of bacteria associated to the deep sea tube worm *Riftia pachyptila*.

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Energy-filtering transmission electron microscopy (EFTEM) allows computing elemental distributions of chemicals out of a sequence of energy filtered images. Combined with electron tomography, EFTEM is becoming a powerful tool to calculate 3D chemical maps for mesoscopic structures. We present a new software (EFTET-J) designed to compute three-dimensional chemical maps. EFTET-J includes background subtraction for 3D-chemical mapping and integrates reconstruction algorithms based on the public tomographic package IMOD.

EFTET-J has been applied to the analysis of 3D Fe distribution of intracellular granules from deep sea vent organisms (Gaill, 1993). Such organisms are facing extreme environmental conditions including fluctuations of harsh physical (temperature, pressure) and chemical conditions (high concentration of sulfur and iron in the vent fluid). One of the paradigmatic vent organisms, the so-called tubeworm *Riftia pachyptila* is protected by a specific extracellular matrix (ECM) composed of chitin associated to proteins. Microorganisms forming islands are colonizing the tube wall of this animal (Gaill, 1993). High number of these bacteria present electron-dense inclusions composed of ferric derivates which appear as circular structures of 100 nm diameter (Lechaire et al., 2002). Semi-thin sections of bacteria dwelling in the tube wall were

analysed on a LEO 912 equipped in cryomicroscopy to determine the 3D elemental distribution of these granules. Results obtained on these microorganisms has demonstrated the capacity of EFTET-J to compute 3D iron maps of intracellular inclusions located at different levels on a tomogram from semi-thin sections.

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