Immunocytochemistry in Biological Electron Microscopy

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An important progress in biological electron microscopy in recent years was the introduction of preparation techniques which are ideally performed completely under low temperature conditions. Firstly, the biological tissue has to be cryofixed in order to maintain the complex integrity of the cell, trapping most of the solutes, macromolecules and membranes without segregation effects and damage caused by ice-crystallization. Secondly, the sensitive biological system has to be prepared for a fine structural and molecular analysis. This can be done in the frozen-hydrated state involving cryosectioning and freeze-fracture techniques or in the resin embedded state involving conventional thin sectioning. For the latter procedure, freeze substitution (FS) or freeze drying of the frozen material and embedding preferentially under low temperature conditions is necessary.

Firstly, this tutorium is focussed on the methodology of cryofixation, freeze substitution and low temperature resin embedding of plant specimens. The subsequent use of immunocytochemical techniques allow the collection of information on the quantitative and spatial distribution of cellular components and their involvement in cellular processes. Secondly, this tutorium gives an introduction in immunocytochemical techniques. In modern cell biology there is great interest in the subcellular localization of unbound and bound proteins with structural and/or enzymatic function by immunocytochemistry. In order to obtain reliable results some important criteria have to be considered: (a) maintenance of protein antigenicity (b) immobilization of the antigen (c) preservation of the cellular fine structure (d) accessibility of the antigens (e) fixation of fast processes. Visualisation of the antigen-antibody reaction is achieved by labelling one of the reactants directly or indirectly, with colloidal gold. The basis of