Optimalization of immunogold labeling on thin resin sections after cryofixation

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Various means for obtaining high-yield immunogold labelling together with fine ultrastructural details and spatial statistics evaluation of immunogold labelling will be presented and discussed.

First, various resins (Lowicryl, LR White, LR Gold, Unicryl) used for embedding were compared for their ability to reveal antigens for immunogold labelling on their surface. Their surfaces after cutting with a diamond knife were observed using atomic force microscopy (Fig. 1), and results statistically analyzed.

Second, we introduce the use of LR White resin in combination with high-pressure freezing and freeze-substitution. The ultrastructural preservation was excellent, and the immunolabelling signal was ~4-13 times higher in high pressure frozen than in chemically fixed cells. We conclude that the LR White resin in combination with HPF/FS can be successfully used for fine ultrastructural immunocytochemistry [1]. Various protocols influencing the immunolabelling effficiancy will be discussed.

Third, methods will be presented which allow one to detect clustering or colocalization of antigens/gold particles using the distribution of distances between them, and to delineate the borders of cellular compartments, which are defined by immunogold labelling of specific molecules even if they are morphologically inconspicuous (Fig. 2). A complete set of tools is now available for detecting reliably gold particles in EM images, to evaluate statistically the observed immunogold labelling patterns (clustering, colocalization, compartments), and to produce a convenient output for publications of results. The plug-ins are useful addition for image analysis software which accompanies modern CCD cameras for electron microscopes. These plug-ins are available at our web-site http://nucleus.img.cas.cz/gold [2,3]. Further progress in 3D analysis of ultrastructural tomography data and in the multiple labelling using novel nanoparticles will be presented.

1. V. Strádalová et al., Histochem. Cell Biol. 130 (2008) p. 1047
2. A.A. Philimonenko et al., J. Struct. Biol. **132** (2000) p. 201
3. C. Schöfer et al., J. Struct. Biol. **147** (2004) p. 128
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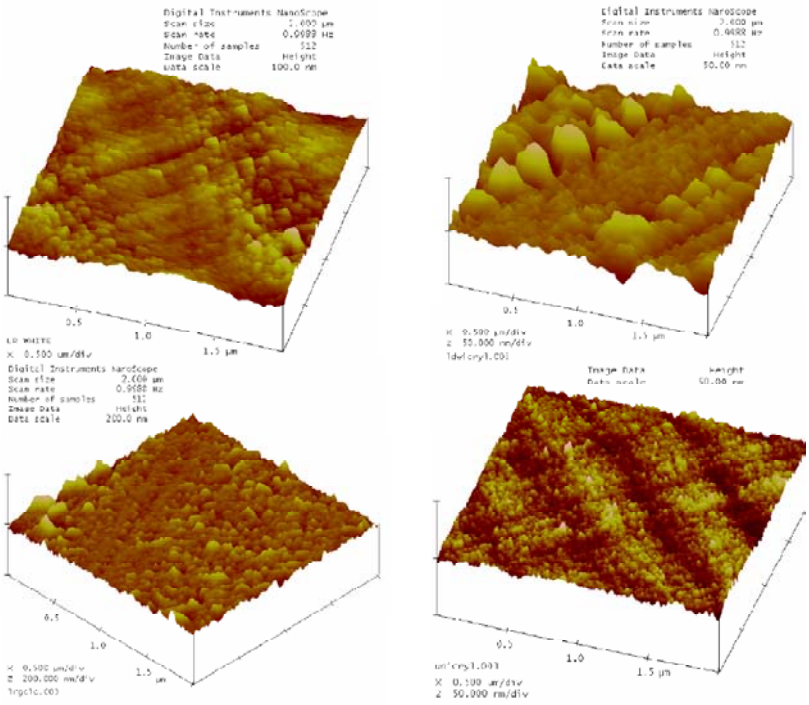

Figure 1. AFM evaluation of surface parameters of various resins after cutting. Clockwise from the top left: LR White, Lowicryl, LR Gold, Unicryl. Statistical evaluation did not reveal significant differences of the roughness factor and surface area between various resins. (Note that the large structures visible are artefacts caused by the knife.)

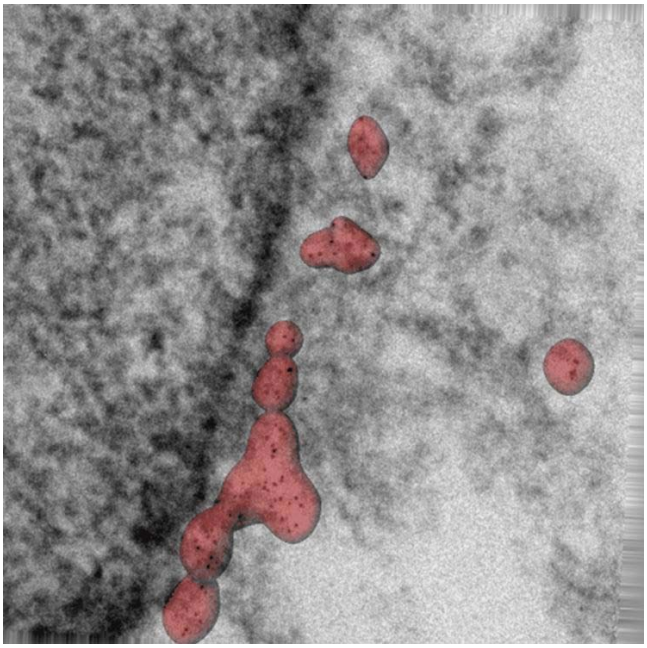

Figure 2. An example of 3D-reconstruction of intracellular structures based on spatially evaluated immunogold labeling intensities after pre-embedment labelling.

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