Size Distribution of Gold and Paladium Nanoparticles measured in Cryo FESEM

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Scanning electron microscope equipped with cold field emission cathode and cryo-attachment for the observation of frozen specimens connects two advantages - high resolution and the observation of biological specimens containing water as close as possible to their native state. In connection with a high sensitive detection of backscattered electrons they would present an effective tool for observing of metal markers on a surface of biological specimens.

The Autrata detector with the yttrium aluminum garnet (YAG) scintillator activated by cerium [1] belongs to the family of detectors with the highest resolution allowing the observation of colloidal gold nano particles which are used most often as markers in imunolabeling procedures. In addition it can work even at low energies of primary electrons. Erlandsen et al. [2, 3] proved that 10 nm gold particles could be detected by means of this detector at FESEM working at room temperature with accelerating voltages of 2-5 keV. The most important parameter of colloidal nanoparticles used for the imunolocalisation is their size. In our study we would like to find out, if it is possible to use this type of detector at FESEM working at cryo-mode for the size determination of gold and paladium nanoparticles and to determine optimal conditions for its using in this mode.

Samples were prepared on either mica or carbon layered grids, with primarily bound JLA antibodies and secondarily bound IgM antimouse 12 nm gold particles. The control group contained all steps except the addition of the primary antibodies. One batch of grids was contrasted using uranyl acetate and lead citrate, with a second batch being left uncontrasted for comparison.

Grids were observed in a FE-SEM with an ALTO 2500 (GATAN) cryo attachment, with grids held in place on custom made targets.

Images were recorded in 8-bit greyscale at varying accelerating voltages for optimization and under room temperature (~20 °C) and cryo (liquid nitrogen −196 °C) conditions.

Recorded images of the gold nano particles were used to count the number of particles observed using the ImageJ program. The initial image was converted from 8-bit greyscale to black and white 2-bit, with adjustment of the threshold histogram to eccentuate particles. During this stage it is important to eliminate background noise from the image and to insure sharp definitions between individual particles. Particles were then counted using specifically predefined parameters (circularity, minimum size, maximum size).

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Recorded images of paladium nano particles were inverted and background noise removed to aid particle definition. In order to measure distances the scale had to be calibrated to pixles before the diameter of the particle could be measured.

Overall the observation of 12nm gold particles is feasable under both cryo and room temperature conditions. Room temperature observation has a clear advantage over cryo in levels of observed particles [Fig.1], but this does not completely dismiss the possibility for the facilitation of gold immunolabeling under cryo conditions.

An important component of immunolabeling is the ability to distinguish between particles of different diameters [Fig.2]. This allows for labeling of more than one component of the specimen at a time for observation. Currently, while this is possible in the FE-SEM there are still problems with specifically determining particle size due to charge build up on the edge of the particles. This leads the particles overall diameter to either increase or decrease according to the accelerating voltage set in the microscope.

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5. Supported by grant project of Academy of Sciences of Czech Republic No. KAN200520704 and Z60220518.

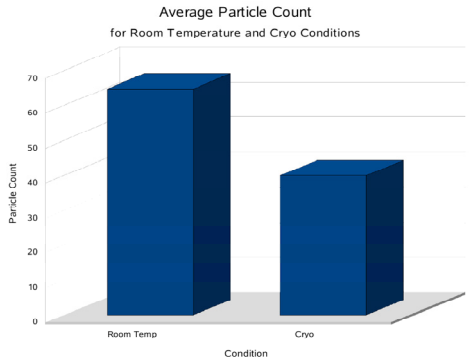

Figure 1. The average size of gold nano particles visulaized in SEM at 50,000x magnification. Left bar Room Temperature, right bar Cryo conditions.

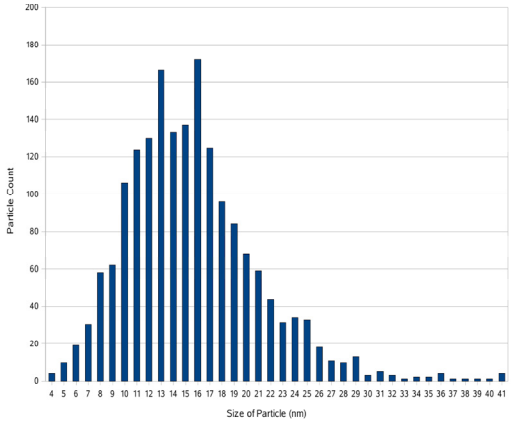

Figure 2. The distribution of nano sized particles in SEM at 100,000x magnification. Average size of particles measured where 16nm in diameter, with a range of 4nm to 41nm.