**Low energy STEM allows watching the conjugation process of nanoparticles with biomolecules.**

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Scanning transmission electron microscopy (STEM) represents the technique allowing the use of low energy primary electrons, in particular when implemented in a scanning electron microscope (SEM). The landing energy of electrons can be further drastically lowered by using the cathode lens with negatively biased sample [1]. In this configuration the resolving power is well preserved down to lowest landing energies. Insertion of the biased sample between two grounded detectors enables to detect all transmitted electrons (TE) in channels corresponding to the STEM detector rings (bright and dark eld or even high-angle annular dark eld) simultaneously with the backscattered electron signal (BSE).

In this study, we used low energy STEM for visualization of nanoparticles conjugated to biomolecules which are standardly used as primary or secondary antibodies in immunolabeling procedures. The suspension of conjugated nanoparticles was adhered to the copper grids covered with carbon supporting layers. Grids were treated by the glow discharge method to make them hydrophilic 2h prior to use. Part of the samples was negatively stained using 2% aqueous solution of uranyl acetate.

The microscopic examination was performed in the standard vacuum SEM Magellan 400L (FEI) equipped with a cathode lens and detectors of TE and BSE. We tested different values of the landing energy of electrons in the range from 1 to 30 keV, while we recorded simultaneously images in bright (BF) and dark (DF) field STEM and in backscattered electrons. Fig. 1 shows images of conjugated QDs recorded at 5 and 30/29 keV on stained/unstained specimens. It is clear that the decrease of the landing energy led to the image contrast grow, however mainly in the bright field STEM imaging. Let us note that the cathode lens field collimates some part of the scattered TE to the bright field detector. Micrographs at 5 keV of the unstained sample do not reveal the biomolecules either in BF or in DF frames but in the BSE image they are clearly visible. Because of increased rate of scattering at lower energy, the contrast bearing species probably escape to outside of the dark field TE detector ring; this is indicated with dark contrast of the QD even in the DF frame. Nevertheless, the staining procedure was inevitable for the visualization of biomolecules bound to metal nanoparticles at higher electron energies. Still, the envelopes of the quantum dots are visible in the BSE frame of the unstained sample. The combination of the image signals of transmitted and reected electrons proved to be advantageous because it facilitates the interpretation of images. [2]

[1] Frank L., Nebesarova J.,Vancova M., Patak A., Mullerova I.: Imaging of tissue sections with very slow electrons. Ultramicroscopy 148 (2015) 146-150

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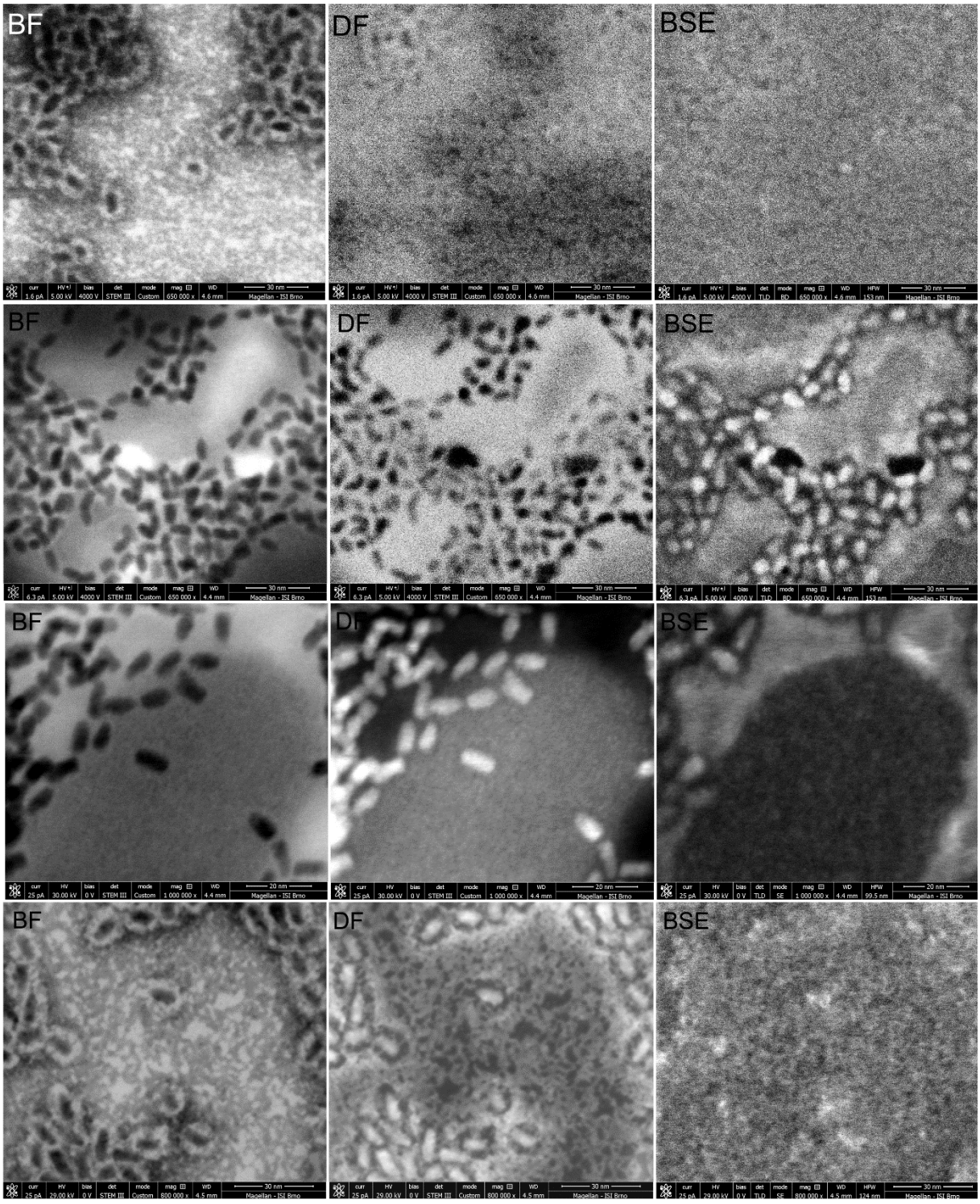


Fig.1. Images of Qdot™ 605 ITK™ Carboxyl Quantum Dots recorded on the stained/unstained sample at accelerating voltage of 5 keV and the sample potential -4 keV (the first/second row), on the unstained sample at 30 keV (the third row) and on the stained sample at 29 keV (the fourth row), both without any sample potential.