# Scanning transmission type imaging and analysis (EDX) of protein supported metallic nanoparticles

## in a field emission scanning electron microscope

**Wilhelm Habicht, Silke Behrens, Nikolaos Boukis and Eckhard Dinjus**

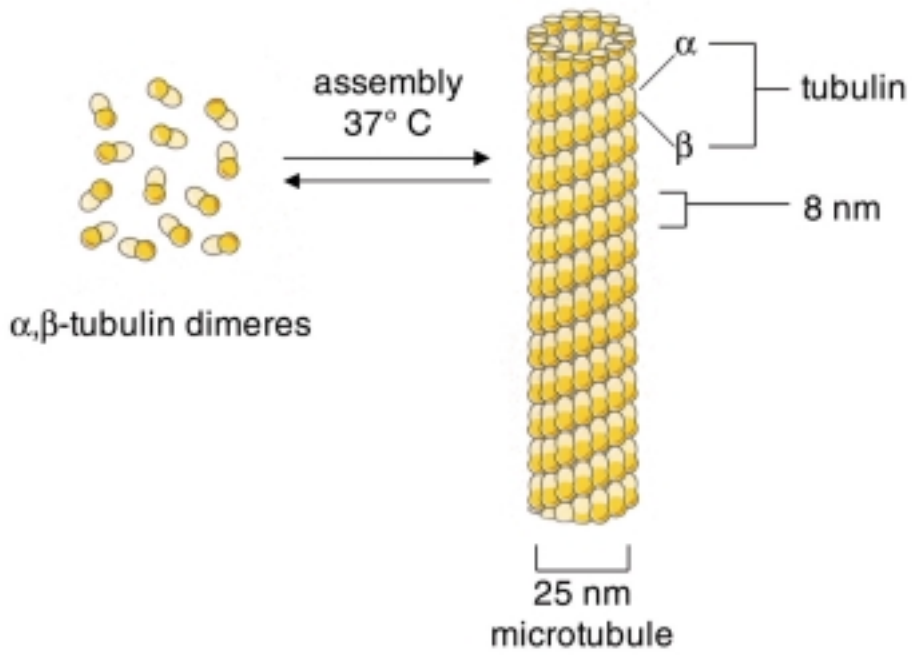


**Fig. 1: Microtubules are formed by a process of self-assembly at physiological conditions**

### Introduction

Highly ordered protein assemblies like microtubules (MT) can serve as template for the synthesis and support of noble metal nanoparticles (Pd, Au) in the lower nanometer size range [1, 2]. The surface to volume ratio increases continuously with decreasing particle size. Nanoparticles are therefore well suited as active component for heterogeneous catalysts. The catalytic properties of metallic nanoparticles are influenced by their particle size and size distribution. Usually, the highest catalytic activity is observed for nanodisperse particles in the 1–6 nanometer size range.

**Keywords**

**nanoparticles, catalysis, SEM, EDX**

The microtubules, serving as template, are biological macromolecules forming hollow cylinders of a high aspect ratio with an outer diameter of around 25 nm and a length of several micrometers. In nature, microtubules are components of the cytoskeleton and essential for, among other functions, organization and transport processes inside the cell. Microtubules are formed by a process of self-assembly and built by usually thirteen protofilaments consisting of α,β–tubulin heterodimers finally resulting in their typical microtubule structure (Figs. 1,2), [3]. Due to the high number of functional groups as active nucleation sites, microtubules can serve as an excellent template for nucleation of mono- and bimetallic noble metal clusters. For that reason they can immobilize and furthermore avoid particle agglomeration. It has been demonstrated that such an unusual configuration performs well in spe-

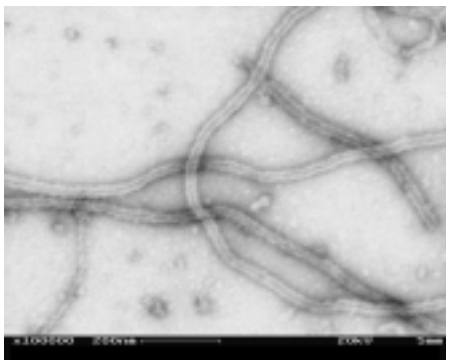

**Fig. 2: Scanning transmission electron micrograph of microtubules, negatively stained with uranyl acetate solution, prepared on a carbon filmed TEM grid, 20 kV, m = 100,000x**

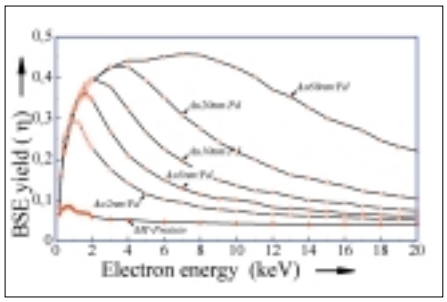


**Fig. 3: Backscattered electron yield η for different Pd/Au particle diameters as a function of electron energy (Monte Carlo simulation by MOCASIM)**

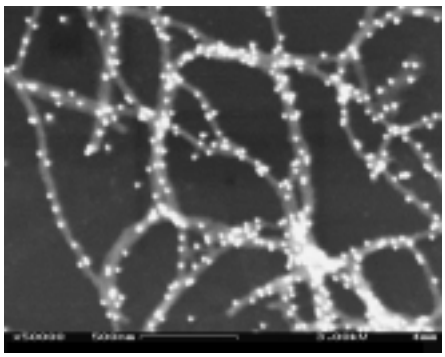

**Fig. 4: Secondary electron micrograph (inlens detector) of 20 nm Pd/Au particles at 3 kV beam voltage, m = 50,000x**

**Fig. 5: The modified TE detector mounted on the sample stage like a specimen stub; the Al TEM-grid holder is placed on top of the TE detector housing, the annular aluminium shield is on the left**

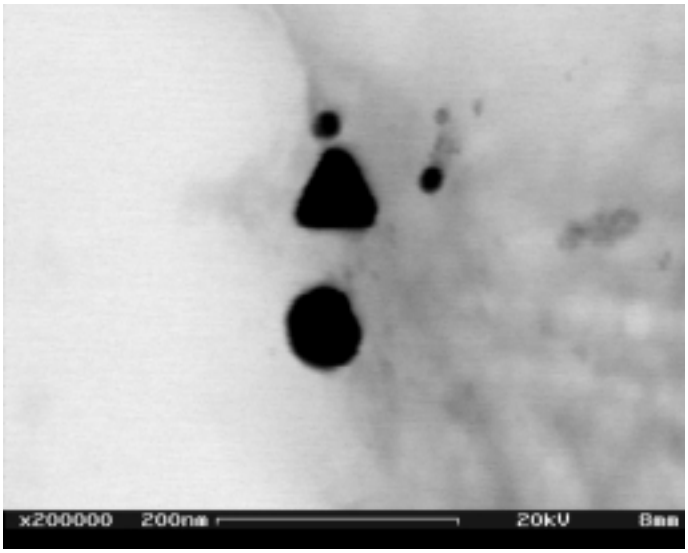


**Fig. 6: STEM micrograph of different shaped Pd/Au particles prepared on a carbon filmed TEM grid, 20 kV, m = 200,000x. The Pd/Au ratio of the triangular particle is 0.06 (by EDX)**

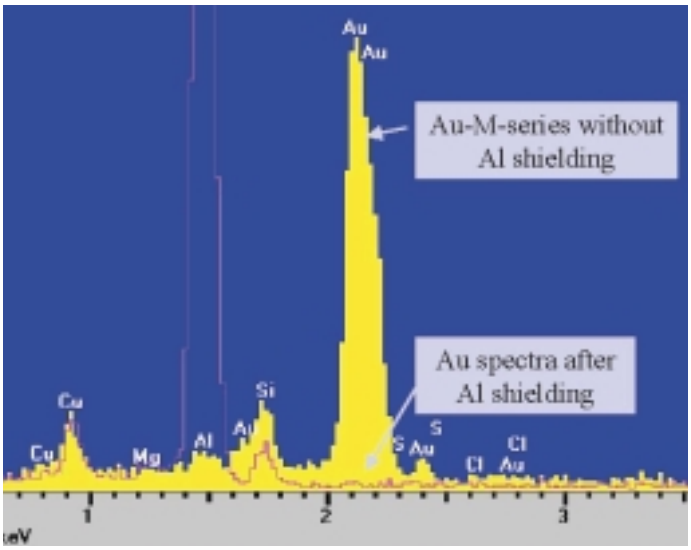


**Fig. 7: Effect of shielding the TE detector with aluminium; a comparison of Au M X-ray spectra collected with and without Al shield. Beam voltage = 20 kV, acquisition time 300 s**

cial catalytic reactions [2]. Beyond that, the potential of metal decorated microtubules as a new ultrasonic contrast agent for medical applications is investigated.

## Synthesis of protein supported metallic nanoparticles

Following a bottom-up approach, the Pd nanoparticles are synthesized by reduction of the corresponding metal salts ($Pd^{2+}$). Bimetallic Pd/Au particles are prepared following a seed-growth mechanism: Au particles bond to microtubules served as nucleation seeds and are covered in a second step with a palladium layer [2].

## Experimental set-up

Specimen preparation:
After wetting a TEM grid with the solution and final air-dry, the thickness of the dehydrated microtubules is about 15 nm [4]. They are sufficiently stable for observation and analysis under SEM/EDS operating conditions (10–30 kV). All TEM grids (Cu, 400 mesh) are covered with a carbon film (5–10 nm).

## Equipment

The specimen are analyzed using a scanning electron microscope with a thermal field emission (Schottky) type cathode (FE-SEM), DSM 982 (LEO GmbH., Oberkochen, GER), equipped with an inlens (annular type) and a lateral (Everhart-Thornley type) secondary electron (SE) detector. Additionally, an external backscattered electron (BSE) detector (4 Quadrant low voltage type) is installed, and if required, the transmission electron (TE) detector can easily be mounted. Both detectors are Si diode types (K.E. Developments Ltd., GB). The microscope is interfaced to an energy dispersive X-ray spectrometer (EDS), Link Isis 300, with a high resolution 10 $mm^2$ Ge ultra thin window (UTW) detector (Oxford Instruments GmbH, Wiesbaden/Germany).

## Standard electron detection modes and X-ray analysis

Commonly, the microanalysis system is connected to the microscope. In the case of high atomic number (Z) material on a low Z support, particle detection is performed preferably by backscattered electron (BSE) measurements and subsequent energy dispersive X-ray (EDX) microanalysis. To avoid the influence of the substrate, the specimen are deposited onto thin film supports of low atomic number like carbon filmed TEM grids.

As a disadvantage, there is a diode inherent reducing sensitivity (signal to noise ratio) in backscattered electron detection with decreasing beam energy. The BSE yield $\eta$ is also a function of primary electron energy and specimen composition (atomic number) and therefore electron penetration depth, showing a maximum depending on particle diameter. For the specimen under consideration, the BSE yield $\eta$ rises with increasing acceleration voltage (beam energy) and increasing particle size (Fig. 3), [5].

In low voltage operation by secondary electron (SE) detection with an inlens detector good image contrast can also be obtained (Fig. 4). But at low primary electron energies (say below 5 keV) simultaneous X-ray excitation is, depending on the X-ray lines of interest, not possible or the detection is suffering from a low X-ray count rate [6]. Even resolution is getting poorer due to broadening of the electron probe diameter at decreasing beam energies. Furthermore, for the ease of use, it's desirable to operate the microscope and the connected microanalysis unit without shifting the working distance (WD = 8 mm) and without changing any beam parameters like excitation voltage and current. As a consequence, the conditions for image observation and X-ray spectra acquisition should be uniform and excitation energy should be equal or exceed 10 keV for the X-ray energies of interest (Pd $L\alpha_1$ = 2.839 keV and Au $M\alpha_1$ = 2.123 keV).

## Transmission electron detection in a scanning electron microscope (STEM)

An alternative to secondary or backscattered electron detection is the image formation via a transmission electron detector in a dedicated scanning electron microscope (STEM). To obtain scanning transmission type images as well as to gain contrast a special mounting device for TEM grids with a diode type detector beneath is installed. It can be mounted like a specimen stub instead and also adjusted to the electron beam. Such a detector is well placed for signal collection from weakly scattering small objects. They should be sufficiently thin to permit transmission of beam electrons. Depending on the composition (atomic number, density), the specimen thickness must generally less than 500 nm to obtain an useful penetration at typical SEM beam energies of 20–30 keV [7]. Then resolu-

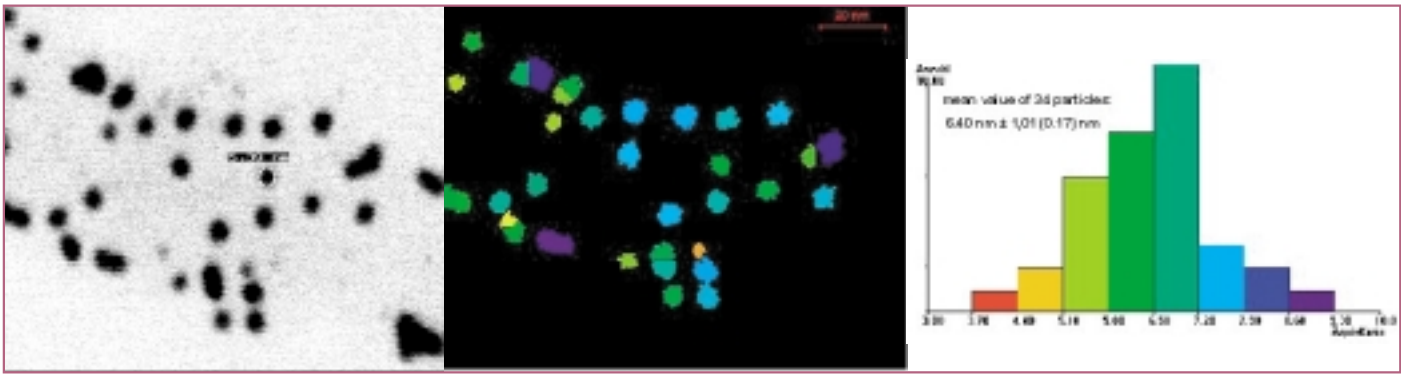


**Fig. 8: left: STEM micrograph of Pd/Au particles (5 nm) at an magnification of 800,000x, 20 kV beam voltage; middle: the same image after binary segmentation and particle classification; right: histogram of circle equivalent diameters vs. particle numbers**

tion is only determined by the incident probe diameter and remaining beam spreading effects. Also a remarkable improvement in the spatial resolution for X-ray analysis is obtained. Under 20 kV operating conditions, the dehydrated MT, compared to the metallic particles, are highly electron transparent and thus a higher contrast than with the commonly used backscattered electron detector is achieved. This facilitates statistical image analysis as well as spectra acquisition by EDS. X-ray analysis is performed while using the TE detector grid holder as a specimen support.

Unfortunately, the protective gold layer on the main parts of the TE device is a disadvantge in elemental analysis of the bimetallic gold/palladium particles. Excitation of X-rays in the protective Au layer caused by scattered beam electrons seriously interfere with the Au particle X-rays of interest and makes the determination of Pd/Au ratios difficult. Therefore, the commercial grid holder is replaced by an aluminium one and a small tubular shield is added inside the diode housing (Fig. 5). The observation of nanoparticles now is performed preferably in the transmission mode, because of an enhanced contrast compared to the backscattered electron image. After a specimen area of interest is selected, the corresponding BSE image is transferred immediately to the EDS unit as a base for further spectra acquisition. Any area or particle of interest can be analyzed successively. Depending on specimen conditions like mechanical stability, magnifications between 50,000x to 300,000x are easily achieved. An example of a STEM micrograph with different shaped Pd/Au particles at an intermediate enlargement (200,000x) is shown in Fig. 6. Even the maximum magnification of 800,000x is achieved in the microscope used. This is illustrated in a STEM micrograph of 5 nm Pd/Au nanoparticles deposited on a microtubule string as well as the subsequent statistical particle size determination (Fig. 7). A spot analysis at 20 kV operating voltage with a standard aperture size (30 µm), is performed at a typical probe current of 420 pA and acquisition times between 60 and 100 s [8].

## Results and conclusions

Metal particle detection in an organic dehydrated matrix is possible by means of a modified transmission electron detector in a dedicated FE-SEM with subsequent X-ray spectra acquisition by a high resolution Germanium detector. By modifying the TE detector with an Al shield, most of the interfering X-rays (> 99 %) are absorbed (Fig. 8). Thus X-ray spectra evaluation and the ratio determination of combined Pd/Au clusters is now achieved. The Schottky type electron gun of the FE-SEM provides sufficient beam current for the acquisition of X-ray spectra even with the standard aperture and 20 kV beam voltage. Due to the short counting times, no beam drift is observed and excessive sample stress is avoided. Fast switchover between different observation modes (BSE-TE) and spectra acquisition without changing of any microscope parameters is possible. Statistical image analysis (e.g. particle size determination) of the STEM micrographs owing to enhanced signal contrast is facilitated.

## Acknowledgements

The synthesis of the microtubules and the metallization has been performed in the Institut für Molekulare Biotechnologie, Jena, Germany. We gratefully acknowledge the collaboration of Marina Baum and Prof. E. Unger.

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