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Imaging Biological Specimens by STEM-in-SEM and Comparison with TEM

Erich Müller[1*], Milena Hugenschmidt[1,2], Heike Störmer[1], Lukas Grünewald[1], Susanne Fritsch-Decker[3], Carsten Weiss[3] and Dagmar Gerthsen[1,2]

[1.] Karlsruhe Institute of Technology (KIT), Laboratory for Electron Microscopy (LEM), Engesserstraße 7, 76131 Karlsruhe, Germany.
[2.] Karlsruhe Institute of Technology (KIT), 3DMM2O - Cluster of Excellence (EXC-2082/1 – 390761711), 76131 Karlsruhe, Germany.
[3.] Karlsruhe Institute of Technology (KIT), Institute of Biological and Chemical Systems-Biological Information Processing, Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany.
* Corresponding author: erich.mueller@kit.edu

Scanning transmission electron microscopy in scanning electron microscopes (STEM-in-SEM) is well competitive compared to transmission electron microscopy (TEM). The lower primary electron energies (typically $\leq$ 30 keV) yield a higher image contrast for weakly scattering materials [1]. Although sample damage by radiolysis is enhanced at lower energies, knock-on damage vanishes below a threshold energy that lies in most cases well above 30 keV [2]. Practical arguments for STEM-in-SEM are the widespread availability of scanning electron microscopes, which can be equipped with a STEM detector and a multiple sample holder suited for transmission imaging [3], flexible change of instrumental parameters like primary electron energy and detection angles, lower maintenance costs and the more straightforward use of SEM instruments compared to TEM instruments. In addition, STEM imaging can be combined with secondary or backscattered electron imaging, rendering STEM-in-SEM a correlative imaging technique for imaging both surface and subsurface features [4].

In this work, we explore the applicability of STEM-in-SEM to analyze biological samples using human A549 lung carcinoma cells exposed to nanoparticles (NPs) as an example. In previous work, those cells were investigated by backscattered electron microscopy [5]. Two main characteristics, the contrast-to-noise-ratio (CNR) and spatial resolution of bright field (BF) STEM images at 30 keV, are discussed and compared to 200 keV TEM images of the same region of interest of the sample. Since the contrast in TEM does not depend only on the projected mass-thickness of the sample but also on defocus and aperture settings, the beam convergence and detection angles were chosen such that similar image signals can be expected due to the reciprocity theorem for STEM and TEM [6]. Series of TEM images with increasing defocus values up to 1.5 µm values were recorded. Figure 1 shows the same region of a microtome section of a cell that has been exposed to silica NP, imaged by 200 keV TEM and 30 keV STEM-in-SEM. The TEM image in Figure 1a was taken in focus. A line scan (1 px wide) through one of the dark particles, shown below the micrograph, reveals a more pronounced noise, quantified by the contrast-to-noise ratio

$$\mathrm{CNR} = \frac{I_1 - I_2}{\sqrt{\sigma_1 + \sigma_2}} = 3.1$$

with the background intensity I_1 outside the particle, the particle intensity I_2 and the standard deviation σ_1, σ_2 of these intensities [7]. Figure 1b shows the 30 keV BF-STEM image of the sample region and reveals reduced noise, as visible from a higher CNR = 17.8.

Another essential characteristic is spatial resolution. It is found that the better contrast at low spatial frequencies in TEM is achieved in this case with a defocus $\Delta f = 1.5$ µm. A line scan over a small feature (Figure 2a) reveals a resolved feature size of 1.3 nm, estimated by the full-width-at-half-

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maximum (FWHM) of the intensity curve. No additional image in the BF-STEM case is needed and therefore Figure 2b is an enlarged section from Figure 1b. The same feature shows an estimated feature size of 1.8 nm here.
The possibility to obtain good CNR and spatial resolution from one single image in BF-STEM compensates for the fact that some thinning of the specimen is observed in BF-STEM. In contrast, no change is visible in TEM, expectedly due to reduced radiolysis at higher electron energies.

Our results show that STEM-in-SEM is a powerful technique for analyzing biological specimens like microtome slices of NP exposed cells. It combines good CNR values with a spatial resolution that can compete with classical TEM. However, its main advantage is its availability and more straightforward operation of the SEM instruments, multiple sample handling, and flexible change of instrumental settings.

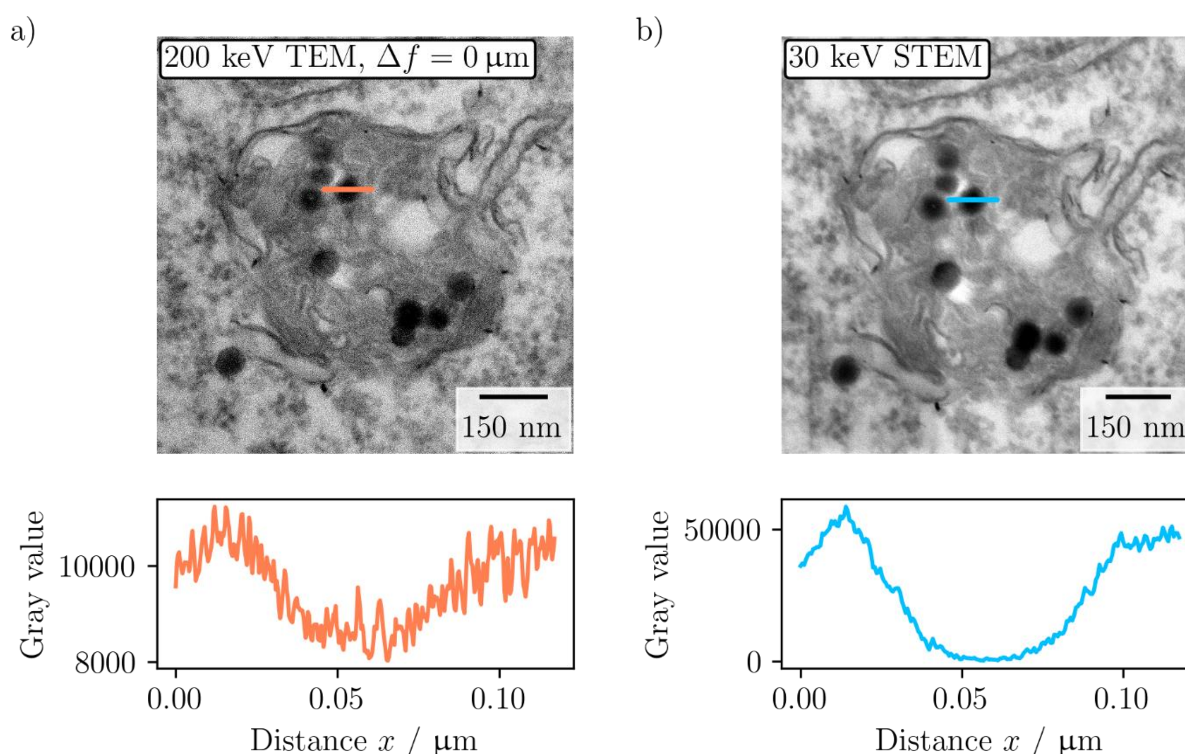

Figure 1. Comparison of the CNR of a 200 keV TEM image and a 30 keV BF-STEM image of the same area. a) TEM image recorded at a CM200 (Philips, equipped with a TVIPS TemCam-F416 CMOS camera), objective aperture 12.5 mrad, condenser (C2) aperture 4 mrad. b) BF-STEM image taken at a Helios GF FX (Thermo Fisher Scientific), convergence angle and detection angle 14 mrad.

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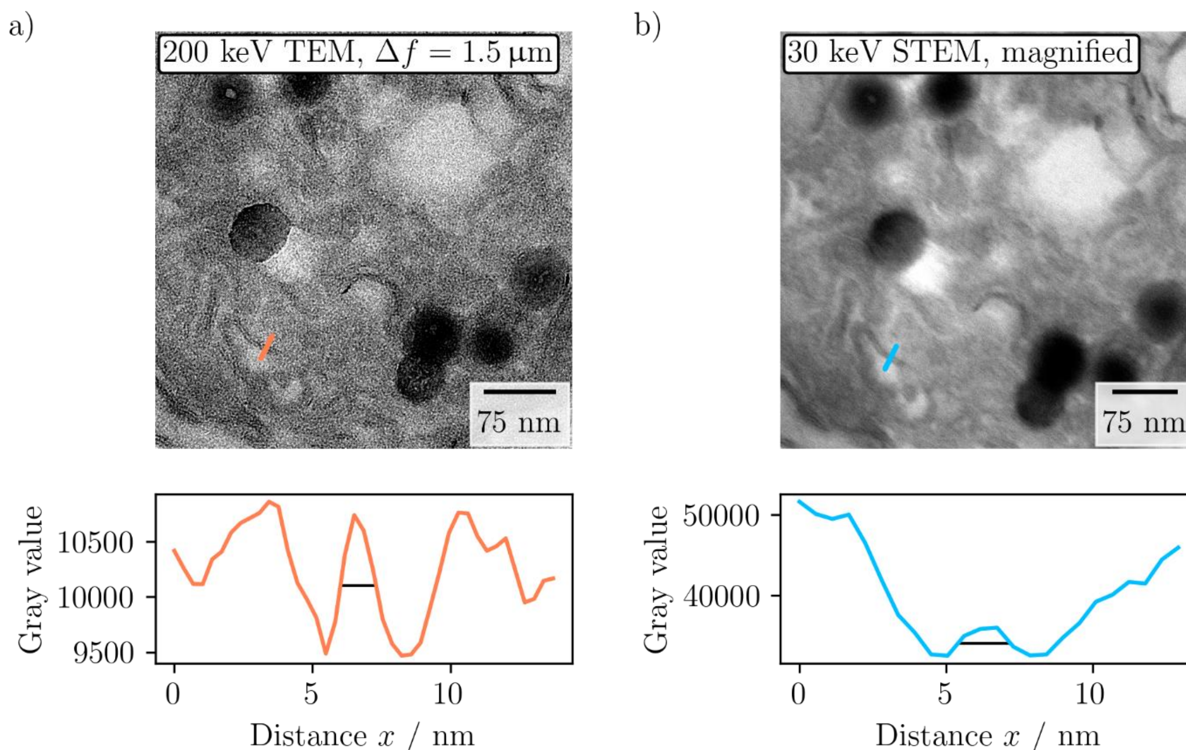

Figure 2. Comparison of the spatial resolution of a 200 keV TEM image and a 30 keV BF-STEM image of the same area. a) TEM image with strong defocus (1.5 µm) and a line scan (width 3 nm) of a small structure, revealing resolved feature size of 1.3 nm, estimated by the FWHM. b) The same BF-STEM image as in Figure 1a, magnified. The line scan shows a feature size of 1.8 nm.

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