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Correlation of structure and mass via scanning transmission electron microscopy

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Scanning transmission electron microscopy (STEM) is known for its strength of offering high-resolution images [1] and tomographs of thick samples [2]. Beyond imaging, STEM enables the access to quantitative data, e.g. by electron energy loss spectroscopy [3]. Additionally, STEM also allows the mass determination of nano-scaled structures like organelles [4], or protein complexes [5]. Despite this technique was already introduced in 1962 by Zeitler and Bahr [6], only a few institutions worldwide established this method (for review see e.g. [7,8]). This might be owed to the excellent alternatives like quantitative mass spectrometry [9] or mass measurements utilizing nanochannel resonators [10]. Nevertheless, quantitative scanning transmission electron microscopy (q-STEM) offers still an advantage towards these techniques: the strong correlation between structure and quantitative data. For quantitative studies, like local thickness or mass measurements, commercial electron microscopes require modifications of the hardware as well as specific software packages for image processing and simulation of electron scattering.

Here, we present progress in the development of this analytical tool in terms of hard- and software extension as well as samples preparation. In our case, an “in-lens” high-resolution scanning electron microscope (S-5000, Hitachi Ltd., Japan) was equipped with a sensitive annular dark-field (ADF) detector. Consisting of a plastic scintillator with a time constant of 2.2 ns, a light pipe with high transparency, a ultra fast photomultiplier and a high-speed discriminator and counter (Figure 1 a)) [11,12] the ADF detector enables single electron counting. In combination with the dedicated software packages for image processing [13] and the electron scattering simulation [14], our system is capable to measure thicknesses up to approximate 7-fold mean free electron path λ within the specimen (e.g., 7λ at 30 keV carbon is approximate 180 nm) and molecular masses in the range of 100 kDa to a few GDa. Since the analysis requires a high level of purity of the specimen [15], we additionally investigated in a novel cryo high-vacuum transfer system for the contamination free transfer of freeze-dried samples (Figure 1 b)).

Therefore, our set-up seems to be well suited for the measurement of mass related parameters, such as mass of globular particles, mass per unit length, and mass per unit area of structures. With this progression we aim for interdisciplinary applications like simultaneous structure and mass thickness investigations of, for example, nanoparticles, hollow spheres, nanotubes, organic films and DNA-protein complexes [16].

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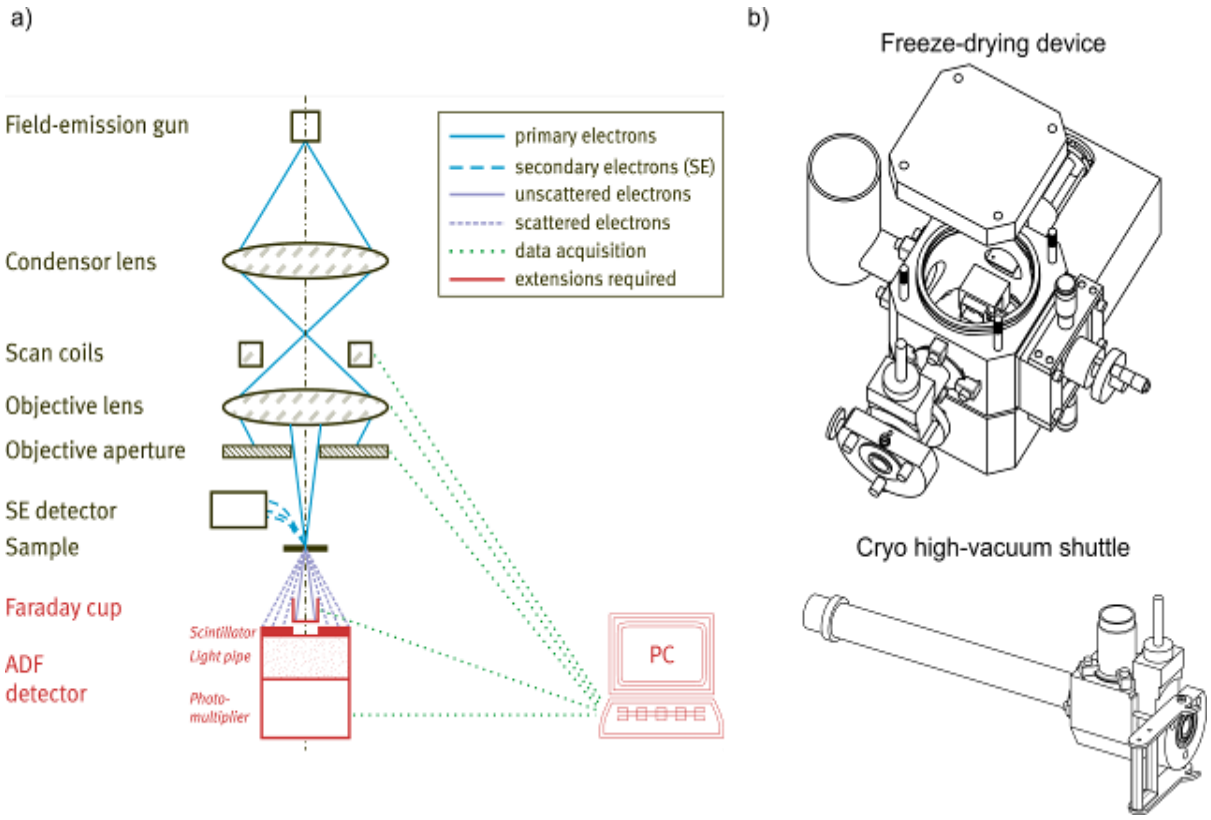

Figure 1. a) Schematic overview of the extended microscope and its main components. b) Schematic overview of the freeze-drying device and the cryo high-vacuum shuttle as well as the main components.