Scanning Electron Microscopy Analysis (SEM) of Nanoparticle Samples - Analysis of ESP/NAS Substrates using Scanning Electron Microscopy

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Content

1 Scope

This standard operation procedure describes the standard routine to be used for analyzing samples of airborne nanoobjects (def. of objects according to DIN CEN ISO/TS 27687) on suitable substrates and of particle samples (not necessarily airborne) intended for statistical analysis of particle sizes. This SOP is intended to be used for an intensive screening of Scanning Electron Microscopy (SEM) samples. A Quantitative analysis with regard to the size of the particles can be conducted, but a quantitative analysis with regard to the concentration of (airborne) particles is not covered with this analysis.

This SOP is developed based on the necessities given and experienced gained within several different projects (e.g. BMBF: CarboSafe, CarboLifeCycle, nanoGEM, EU-FP7: MARINA, UBA: Pr. 26897). The procedures described are subject to be changed and/or amended for special analytical needs.

2 Introduction

After sampling on suitable substrates (see e.g. SOP-M-Sampling for sampling of airborne nanoobjects, nanoGEM) these samples were analyzed with scanning electron microscopy (SEM) to provide images of nanoparticles and their agglomerates in sufficient resolution. Depending on the size of the particles and the microscope used a certain magnification has to be used to provide images with clearly identifiable particles. To give an overview of the sampling pattern also magnification series should be provided for a complete analysis of the substrate. A possible chemical analysis of the sampled particles using energy dispersive x-rays (EDX) may be performed on representative single particles or agglomerates to unambiguously identify their composition. Not all particles present have to be chemically analyzed but the choice of particles should be representative for the particles present.

The given information in this SOP is intended to be used for nearly homogenously covered substrates and substrates where the deposition is governed by a central spot with gradually decreasing particle concentration. This prerequisite can be supposed for substrates obtained via ESP/NAS sampling applying a central circular electrode. Substrates with obviously very inhomogeneous particle deposition are not covered within this SOP in the sense that a representative analysis is not possible by the described routines.

3 Procedure for analysis of substrates

The following sub-chapters describe the general procedure to obtain information on the particles present on a given sample. Emphasis is given on representative image acquisition to avoid any bias by the operator of the analysis. The described procedures allow a representative screening of the samples. If a quantitative analysis is necessary it has to be decided whether sampling artefacts might play a role. This is usually the case for airborne particle samples and thus the number of image fields has to be adapted according to the mean number of particles present within the images fields to match with the needed number of (single) particles necessary for statistical analysis. If sampling artefacts do not play a role a simplified procedure for image acquisition can be used, see chapter 4.

3.1 Sample adjustment

For a defined and reproducible analysis of a given sample an unambiguous mark (e.g. by using a SEM pen) has to be placed on the substrate under investigation. In case of rectangular samples the upper left corner might be used; in case of circular samples any position at the edge can be marked. Depending on the position of the mark the stage of the SEM is adjusted (regarding especially rotation of the SEM stage) and subsequently the middle of the substrate is placed central under the electron beam by moving the stage in x- and y-direction. This then defines the zero-point for the coordinate system of the sample. Depending on the aim of the analysis either only this position is used for further analysis or a more complete screening of the substrate is conducted e.g. at five different positions of the sample.

Figure 1 shows an example for a circular substrate with five distinct measurement positions.

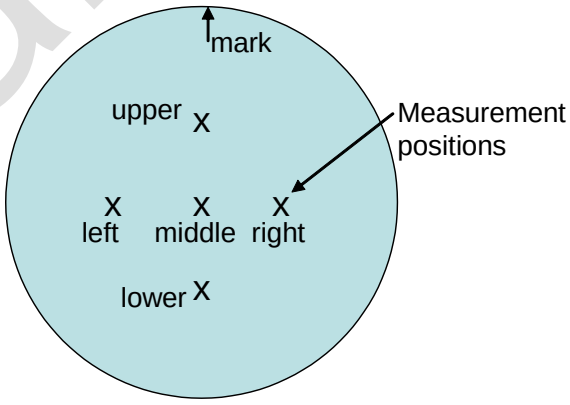

Fig. 1: Example for scanning positions on circular substrate

3.2 Scanning procedure and naming of SEM images

After the adjustment of the sample within the SEM usually five different areas are studied in-depth. This analysis covers images at lower magnifications (zoom), details obtained at high magnifications (details) and the routinely scanning of a certain sample area (scan) to obtain representative images.

To unambiguously name the images obtained a prefix in the name according to the position under investigation is to be used:

p0_0 = middle area
p0_5 = upper area
p0_-5 = lower area
P-5_0 = left area
p5_0 = right area

The numbers indicate the distance (in mm) from the central sample region in x- and y-axis. Investigations per position include a zoom series of images to show the general appearance of the sample with typical magnifications of 50x, 100x, 250x, 500x, 1kx, 2.5kx, 5kx, 10kx, 25kx, 50kx, 100kx. Also, details of particles of interest can be obtained.

Example for naming of images: NAME-Of-SAMPLE-p0_2-zoom-01
The last two numbers indicate the image number. Following indicators for the different imaging purposes should be used:

- zoom: indicating the zoom-series
- detail: indicating that special images at high magnification are obtained
- scan: indicating the routinely obtained images of the different sample position

The positions for the zoom-series and scan-series should be adapted for inhomogeneous samples or samples with strongly varying particle size to obtain a representative overview of the sample. Scan speed of the SEM should be set to obtain high quality images for “zoom” and “detail” and medium quality images for “scan” to lower the time necessary for analysis.

The “scan” images are obtained at a given (usually high) magnification based on a scanning scheme which tries to avoid slippage of the sample stage leading to overlapping images.

Figure 2 shows an example of a 7 x 7 scanning scheme with the according image numbering indicated. The center of this matrix corresponds to the sample position at which the zoom-series is obtained. The arrows in Figure 2 indicate the stage movement; the whole field is scanned in a meander. To minimize stage slipping in both directions the first image field is reached after the stages was moved one field beyond in each direction. In this way no slippage is obtained in y-direction and only a minimal slippage is obtained in x-direction. Naming of the images should be as follows: NAME-Of-SAMPLE-p0_2-scan-17 (example for marked field of view in Figure 2).

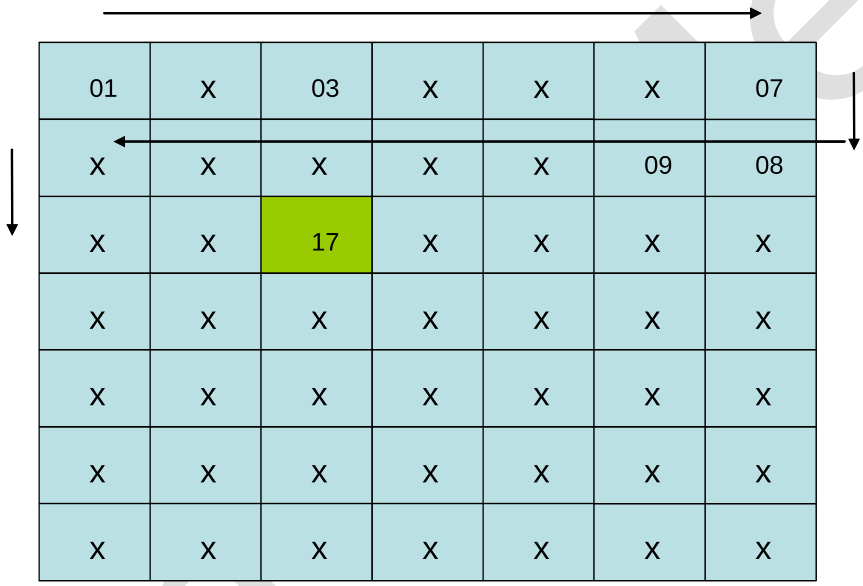

Fig. 2: Illustration of scanning routine

3.3 Necessary resolution and exemplary values for JEOL 7500F SEM (IUTA)

The necessary magnification and thus the obtained resolution depend on the particles to be imaged. In case of nanoparticles with a size of some ten nanometer a magnification of 50kx or more is necessary to provide sufficient details of the structures and allow the measurement of geometrical dimensions. In case of agglomerates the necessary magnification might be (much) less.

Following values are exemplarily shown with regard to an area scanned at 5kx and are valid for the JEOL 7500F SEM at IUTA. The actual values of the instruments used can be obtained by any image at the given magnification. Of course, the calibration of the SEM has to be routinely checked with an appropriate sample (e.g. Chessy sample).

The image size at 5kx magnification is appr. ~24 µm times~18 µm. Applying standard image parameters this image contains of 1280 times 1024 pixel. Thus, the pixel size at 5kx is appr. 18 - 19 nm, which also defines the maximum resolution of the image under optimal instrument settings. Values for other magnifications used for analysis have to be recorded and documented.

4 Particle size analysis

If the aim of the SEM analysis is to provide a particle size distribution (either primary particle sizes or agglomerate sizes), a sufficient number of particles has to be imaged at an appropriate magnification. The latter depends on the structure size, e.g. primary particles usually need a higher magnification than agglomerates. For a statistical meaningful analysis the size of at least 300 agglomerates / aggregates and of at least 500 primary particles has to be analyzed. For the analysis of agglomerate sizes the sample should not be overloaded (more than appr. 10% of the sample area covered with particles) to avoid biased results due to overlapping of particles.
Image analysis is not part of this SOP but it can either be done by using (semi-) automatic software or “by hand” using e.g. ImageJ (Freeware image processing program, available at: http://rsb.info.nih.gov/ij/, version 1.41 or higher) as an analysis tool.

To obtain the images for a subsequent particle size analysis the sample is positioned in a way that an area suitable for later analysis is in the center of the zoom series. The zoom series (see chapter 3.2) is conducted to show the overall particle burden and homogeneity of the sample. Furthermore, single particles should be imaged in high-resolution mode to document different particle morphologies. To conduct the statistical analysis a series of images is to be obtained at the necessary magnification, see chapter 3.3. After one image is obtained the sample is moved one field of view on the x-axis and the next image is to be obtained. The sample position can slightly be adjusted to cover particles completely which are otherwise cut at the image edges. To stay in the area of particle deposition the stitching of the images is done in a meander: starting from the first image at the sample centre the sample is moved only in x-direction for appr. 1 mm. Afterwards, the displacement is done on the y-axis for appr. 100 µm. The next images are obtained in the counter direction on the x-axis until a distance of 2 mm is covered, etc... In this way the sample is scanned until 500 particles have been imaged.

For the analysis of primary particle sizes (e.g. for nanopowders) the samples can be covered by a thick particle layer. In this case the zoom series is conducted followed by several images at a sufficiently high magnification to measure the particle size.

For circular particles one measurement of the particle diameter is sufficient; for irregular particles and agglomerates / aggregates the size is to be measured for two nearly perpendicular axes. Afterwards, the geometric mean value can be used to assign a particle diameter.

5 Quality control

The SEM has to be checked periodically (at least according to routine service intervals) especially with respect to size calibration of images. A suitable standard (chessy sample) should be used. Samples should be stored in a particle free atmosphere (special containments, use of parafilm for boxes, vacuum desiccator) under dry conditions. Vacuum storage is preferable. Transmission times from containment to the SEM have to be kept to a minimum.

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