## 1. Purpose and scope

On the basis of VDI 3492, or for workplaces in accordance with DGUV Information 213-546 "Procedure for the separate determination of concentrations of inorganic fibres in work areas - Scanning electron microscopic method", a new measurement and analysis procedure for fibrous nanomaterials has been developed by the BAuA (FG 4.5), which is currently being validated. The method has not yet been standardized a norm. The basic principles are given in this standard operation procedure (SOP).
The SOP is used to determine the exposure to nanoscale fibrous materials at workplaces. For this purpose, an airflow with well-defined flow rate through a gold-coated track-etched membrane filter with the dust-laden air is established. The aerosol is deposited on the filter, which is subsequently analysed by scanning electron microscopy. All nanoscale fibrous or fibre-containing objects with WHO-dimensions[1], but with an extended diameter range of 20 nm < D < 3 µm, are counted und geometrically measured. Using the sampling parameters (volume flow rate and collection time) and the count number of identified fibrous or fibre-containing objects, the fibre number concentrations are extrapolated. Fibres with 1 µm < length < 5 µm are counted as a separate class but not further characterized in this SOP.

## 2. Responsibilities

The standard operating procedure applies to the laboratories in Berlin within research projects in the framework of dust and innovative materials. This SOP can be used as a basis for future standardization and standardization activities.

## 3. Description

### 3.1 Sampling at workplaces

Until a generally recognized standard is established, the measurements for determining the fibre number concentration of nano- and microscale fibre dusts are exploratory measurements based on TRGS 402.

Both stationary and personal samplers are used for measurement. Personal sampling with the PGP-FAP (37 mm filter) is carried out in accordance with the SOP "Workplace measurements with the PGP-FAP". Personal sampling with the sampling head according to VDI 3492 (25 mm filter) is carried out analogously. Pumps of the company Gilian are used.

For the stationary measurements the sampling devices PNA 3000 of the company APC Analytics as well as the BPP 4-8 of the company GBA Gesellschaft für Bioanalytik mbH are available. Other sampling pumps, for which an automatic control of the volume flow rate is guaranteed, can also be used. In particular, pumps are suitable for exposure measurement of nano- and microscale fibre dusts, which are easy to clean and can establish sufficient pressure drop to ensure maintenance of the flow for the chosen filters with a diameter of 25 mm and 400 nm pore size. In all other respects, the provisions in the SOP "Workplace Measurements with the PGP-FAP" apply.

Currently, gold-coated nuclepore track-etched membrane filters with 0.4 µm pore size and a diameter of 25 mm (manufacturer's specification) from the company APC are used (short: nuclepore filters).

For nanoscale fibre dusts, the following applies: The sampling duration and sampling volume must be selected, based on the knowledge of the general dust number concentration on site measured with suitable aerosol monitors. Criterion is a sufficiently high air volume per filter area after sampling to reach the required analytical detection limit (< 10,000 F/m³, preferably below 1,000 F/m³) and keeping the analysis practical.

Usually, background measurements, i.e. measurements without activities involving fibre materials, are performed. Possible deposited material is actively stirred up at the beginning of the background measurements. This way, contamination in the vicinity of the workplace can be determined.

[1] WHO criteria for critical fibre: Diameter D < 3 µm, length L > 5 µm, ratio D: L > 3:1

## 3.2 Preparation of the filter samples

The nulcepore filters are prepared in a clean environment (under local suction) onto a sample holder for SEM analysis. Filters with a diameter of 37 mm must first be cut to the appropriate size of the specimen holder, whereas filters with 25 mm diameter are kept in the original state

## 3.3 Scanning electron microscopic evaluation

The working conditions at the SEM, the counting rules as well as the classification scheme for the exposure assessment are carried out according to the provisional convention agreed with IFA and SUVA (Gefahrstoff - Reinhaltung der Luft, 78 (2018) Heft 5 und 6).

In the SEM, the first step is to take at least one overview image of the filter in order to make statements regarding e.g. damage, inhomogeneity in the deposition density or the presence of coarse dirt particles. Any noticeable problems are to be documented in the SEM laboratory notebook.

Afterwards, image acquisition takes place on the SEM (Hitachi SU8230), using the stage navigation software "TiNa". A project is created within "TiNa", in which, depending on the sampling conditions, a corresponding number of image acquisition positions are randomly generated on the sample. After sample realignment by the software, the randomly generated positions are approached one after the other by "TiNa". The first image acquisition is performed manually with the necessary acquisition parameters. For the operation of "TiNa", a manual from the software manufacturer is updated regularly.

To keep the time requirement low, the largest possible SEM images are acquired, preferably 20 MPixel images. The edge length of a pixel of the SEM image must be adapted according to the smallest geometrical element of the particles or fibres to be analysed, usually the diameter. It should be at least equal to the diameter of the thinnest relevant fibre in order to visualize all relevant fibres. The required lower diameter of at least 20 nm should be reliably detected.

Currently, the SEM is operated with the following measurement settings:

- Acceleration voltage: 3 kV

- Working distance: 6 mm

- Pixels: 5120 x 3840 pixels, corresponding to an image size of approx. 20 MPixel

- Point resolution (pixel size): 8.3 nm

The number of images to be analysed on the SEM is selected ("TiNa" setting) so that the analytical detection limit is below 10,000 F/m³, preferably below 1,000 F/m³ (the upper limit of the 95$^{th}$ confidence interval). Criterion is the effective air volume that has been sampled and analysed.The following Excel template is used for calculation:

*REM-Nachweisgrenze für Filterproben 20180724_auto_ok.xlsm* (*Q:\fb_4\fb45\REM-Bilder\Allgemein\ REM-Nachweisgrenze für Filterproben 20180724_auto_ok.xlsm*)

Since the superimposition of the scale and the magnification factor proved to be disturbing for the subsequent image evaluation, this information is only embedded in the first image, which is rejected for the particle analysis. The remaining images are acquired without scale bar.

The sample labelling or image file naming and image traceability is performed according to section 3.2 of the procedural instruction "Sample Labelling and Traceability".

The identification, counting and geometrical characterization of the fibres is performed on the set of saved SEM images. If fibre objects matching the WHO-criteria are found but cannot be identified as the material handled at the workplace solely on the basis of their morphological properties recognizable on the image, an additional analysis is performed by the SEM with EDX at higher resolution if needed. A relocation of the sample position can be accomplished by the software TiNa.

## 3.4 Evaluation of the exposure measurements

The software "FibreDetect" is available for the analysis of the images with regard to fibre counting or their morphology. Handling and evaluation procedure is described in the SOP "Characterization of fibrous (nano)materials using FibreDetect". The fibre morphology counting categories shown in tables 1 and 2 are applied and their geometrical properties measured. The results are listed in an export file. Objects matching the WHO criteria are allocated to a special category. Size categories and designations of the different classes of nanoscale fibrous or fibre containing objects with a diameter D < 3 µm are listed in Table 1. The specific counting rules are described in Section 3.4.2.

### 3.4.1 Categorization of the fibres

The allocation of fibrous objects to the different morphological classes is carried out according to Table 1. Examples are found in Table 2.

**Table 1: Size categories and names of the various classes of nanoscale fibrous or fibre containing objects with a Diameter 0.02 µm <D < 3 µm.**

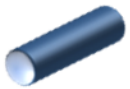
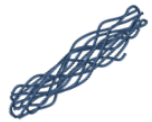
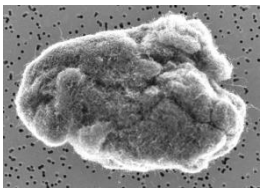
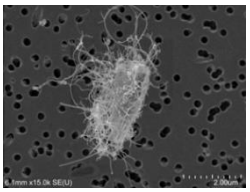
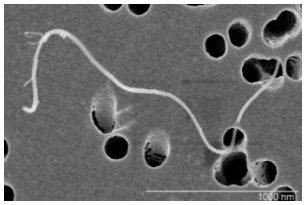
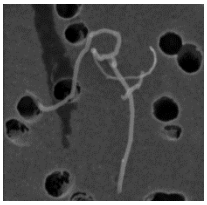
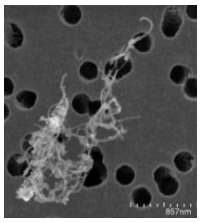
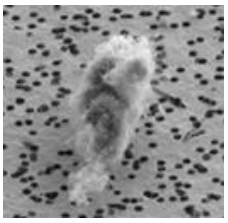
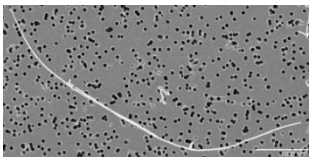
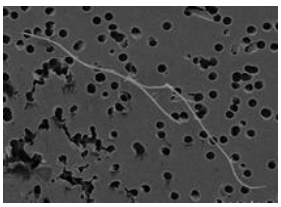
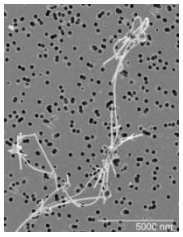
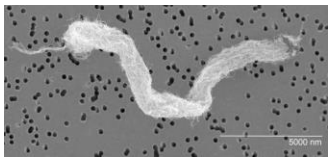
| | **Individual objects** | | **Fibre-Cluster** | **Fibre agglomerates** | |
|---|---|---|---|---|---|
| | **the particle recognisable** | **Course can be followed from the beginning to the end of the fibre** | **Partially countable agglomerated objects** | **Non-countable agglomerated objects** | |
| **Low-Aspect Ratio** *L:D* < 3:1 | **LARPO** | | **LARFC** | **LARFA** | **Low-Aspect Ratio** *L:D* < 3:1 |
| | Individual particle object  | | Partially countable agglomerated objects with collective LAR  | Non-countable agglomerated objects with collective LAR  | |
| **High-Aspect Ratio** 3:1 ≤ *L:D* 20 nm ≤ *D* ≤ 3 µm | | **HARFO** | **HARFC** | **HARFA** | **High-Aspect Ratio** 3:1 ≤ *L:D* 20 nm ≤ *D* ≤ 3 µm |
| | | Individual HAR fibre object  | Partially countable agglomerated object with collective HAR  | Not individually countable agglomerated object with collective HAR  | |
| **WHO-analogue AR** 3:1 ≤ *L:D* 20 nm ≤ *D* ≤ 3 µm | | **WHOFO** | **WHOFC** | **WHOFA** | **WHO-analogue AR** 3:1 ≤ *L:D* 20 nm ≤ *D* ≤ 3 µm |
| | | Individual WHO-analogue fibre object  | Partially countable agglomerated object with collective WHO-analogue aspect ratio  | Non-individually countable agglomerated object with collective, WHO-analogue aspect ratio  | |
| **Counting rules** | **Ignore LARPOs!** | | **Ignore LAR/HAR/WHOFCs as such!** | **Ignore LARFAs with L ≤ 1 µm!** | **Counting rules** |
| | | **Mention HARFOs with L ≤ 1 µm!** | **Mention HARFOs in LAR-/HARFC with L ≤ 1 µm!** | **Mention HARFAs with L ≤ 1 µm!** | |
| | | **Count HARFOs with 1 < L < 5 µm separately!** | **Count HARFOs in LAR-/HARFC with 1 < L < 5 µm separately!*** | **Count HARFAs with 1 < L < 5 µm separately!** | |
| | | **WHOFOs count!** | **WHOFOs in LAR-/HAR-/WHOFC count!*** | **WHOFAs count!** | |
| | | | ***: An upper limit can be set for the maximum fibres to be counted in a cluster.** | | |

**Table 2:** Example images for the morphological differentiation of nanoscale fibre-containing or fibrous objects.

| **category LARFA** | |
|---|---|
| Agglomerates with an aspect ratio of less than 3:1 | |
|  |  |

| Objects that can be counted and length-determined as single fibres with a rectified length of 1 to 5 µm | | Agglomerates with an aspect ratio greater than 3:1 and a length of 1 to 5 µm | |
|---|---|---|---|
| **category HARFO** | **category HARFC** | **category HARFA** | |
|  |  |  |  |

| Objects that can be counted and length-determined as single fibres with a rectified length greater than 5 µm | | Agglomerates with an aspect ratio greater than 3:1 and a length greater than 5 µm | |
|---|---|---|---|
| **category WHOFO** | **category WHOFC** | **category WHOFA** | |
|  |  |  |  |

### 3.4.2 Counting the fibres

After the objects have been measured and categorized by FibreDetect, the export function of the software file can be used to export the number of objects in the different categories according to Table 2.

The following counting rules are used to determine the concentration of nanoscale fibrous particles according to the classification described above:

- For the purposes of this rule, any object is counted as a fibre or fibrous agglomerate if it has
  - a mean width of 20 nm < D < 3 µm and a length/width ratio L:D > 3:1 and
  - a rectified[2] length 1 µm < L < 5 µm

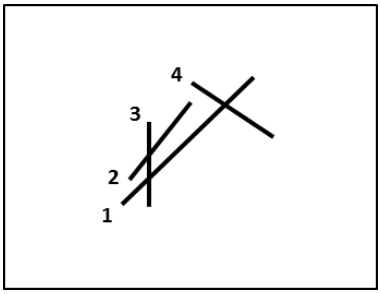
  → short nanofibre object or agglomerate or

[2] The arc length of the irregular line closely following the fibre over its full longitudinal axis.

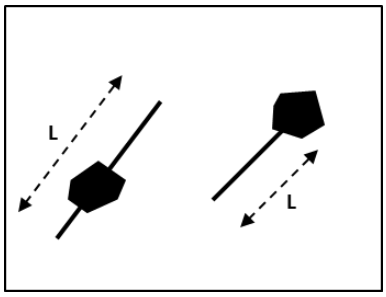
- a rectified length L > 5 µm

  → WHO analogue nanofibre object (FWHO) or agglomerate (AWHO).

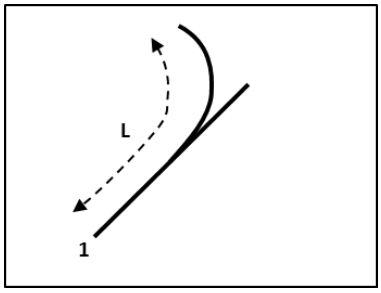
- Fibres with a length L < 1 µm are not counted but their presence on the filter in the report.→ very short nanofibres.
- Overlapping or crossing fibres (fibre clusters) are counted individually, if possible.



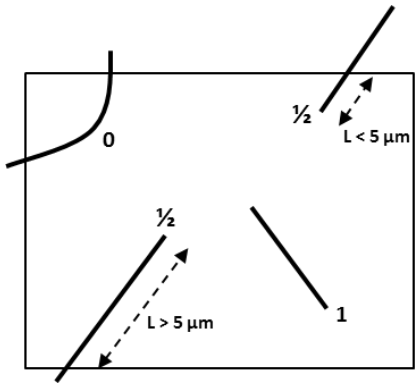

- If the number of intersecting fibres is to high so that they cannot be counted individually, the fibre bundle is counted as a fibre agglomerate if its enveloping dimensions meet the above criteria for length L, the average width of the entire object D < 3 µm, and the length-to-width ratio L:D.
- For single fibres and fibre agglomerates, the width and the measured rectified length are determined. For agglomerates, the longest possible curve is chosen for the measurement of the length, i.e. in case several curves can be fit over the agglomerate.
- For non-fibre-containing agglomerates with L:D < 3, the projected area as well as the perimeter are determined with the help of a polygon tool in FibreDetect.
- Bulges (such as those caused by resin or binder in artificial mineral fibres) are ignored.
- Fibres that are or appear to be attached to non-fibrous particles are treated as if the non-fibrous particles were not present. However, only the visible length of the fibres is considered unless the fibres pass through the particles and do not appear to be interrupted.




- A fibre that appears compact and undivided at one or more points along its length, but appears to divide (splice) into separate fibres at other points, is considered 1 fibre. The longest curve is chosen for length determination. Its width is measured in the un-spliced part.



- Fibres with both ends inside the counting field receive the counting weight 1.
- Fibres with only one end inside the counting field, are assigned the counting weight ½.
- Fibres and fibrous agglomerates that protrude from the counting field and whose measurable length is > 5 µm within the field of view are counted as WHO analogue objects.
- Fibres and fibrous agglomerates that extend outside the counting field and whose measurable length is < 5 µm are marked in FibreDetect and must be viewed again for characterization with "TiNa" on the SEM in order to be able to measure the actual length of the object.
- Fibres with both ends outside the counting field get the counting weight 0 and are not measured, but the export csv file includes the comment "many fibres with no end in the counting field" for the respective image.




- In FibreDetect, the objects found are assigned to a material based on their morphology using a drop-down list in the object classification window. The following materials are available for selection: product fibre, no product fibre and unknown for objects that cannot be clearly identified (later assignment). A corresponding material list can be imported.
- If more than one eighth of an image is covered by fibres or particles (overload), this count field is not considered and deleted from the image list in FibreDetect.
- If more than 10% of the images of a filter sample are considered to be overcrowded, this filter sample must be discarded.

### 3.4.3 Calculation of the fibre number concentrations

The method provides the extrapolated average fibre number concentration during sampling of nano- and microscale fibrous or fibre-containing objects with a diameter D < 3µm. The counting results for fibres and agglomerates matching the WHO criteria are used for the concentration calculation as follows:

$$C_{WHO} = \frac{n_{WHO} * A}{N * a * V}$$

$C_{WHO}$ Fibre number concentration of objects matching the WHO-criteria [F/m³]

$n_{WHO}$ Fibre count of objects matching the WHO-criteria per image

A Filter area [mm²]

N Number of images evaluated

a Area represented by one image [mm²]

V Sampled air volume [m³]

The calculation results are saved in an Excel file, so that the concentrations can be calculated more effectively. An additional Excel script provides the upper and lower limits of the 95% confidence interval for the compliance check of the exposure limit. The current version of the Excel script can be found at:

*Q:\fb_4\fb45\REM-Bilder\Allgemein\ REM-Nachweisgrenze für Filterproben 20180724_auto_ok.xlsm*

The result of the concentration calculation is saved as a copy in the corresponding folder of the workplace measurement.

### 3.5 Measurement report

Measurement reports must be prepared for the workplace measurements. A template and sample measurement reports can be found here:

*Q:\fb_4\fb45\Arbeitsplatzmessungen*

## 4. Applicable documents

- “Sample identification and traceability" process instruction
- Procedural instruction "Monitoring of measuring and test equipment”
- Procedural instruction "Quality records and QM documents”
- SOP "Workplace Measurements with the PGP-FAP”
- SOP "Characterization of fibrous (nano) materials using FibreDetect".
- Instruction manual "TiNa“
- Sampling protocol form