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Counting rules for carbon nanotubes and other nanoscale particles during SEM analysis

Abstract

When used for determining the concentration of carbon nanotubes (CNTs) in airborne dusts, scanning electron microscopic methods reach the limits of their capacity. This article describes the accompanying conditions that have to be controlled and optimized so that the dusts collected by active sampling on gold-coated nucleopore filters can be evaluated by scanning electron microscope analysis. The focus here is on enhancing the visibility of CNTs on filters. No criteria are as yet available for the counting of CNTs. The paper presents a strategy for discussion according to which CNTs can be evaluated in the context of the other particles occurring in dusts. Evaluation is performed in three stages. First, any large CNTs hairballs (particles > 20 µm) are identified at low magnification (200-fold). At the 2,000-fold magnification also specified for established fibre counting methods, smaller CNTs hairballs and fibre composite particles, for example, are detected (particles of 1 to 20 µm in size). Individual CNTs are then counted at a 20,000- to 50,000-fold magnification (particles < 100 nm and particles from 100 nm to 1 µm in size). Criteria for counting are proposed.

1 Introduction

Determining the concentration of nanoscale particles, particularly carbon nanotubes (CNTs), in workplace atmospheres is an essential aspect of prevention activity in such areas. At present, established procedures for this purpose do not exist. The descriptions below firstly address the issues and constraints associated with the determining of concentrations of CNTs and granular nanoscale particles by means of microscopic methods and present the proposals for analytical

parameters to be applied during scanning electron microscope (SEM) analysis. Secondly, they list possible counting rules for nanoscale particles in context with the other, accompanying dust fractions. The list is to be regarded as a maximum requirement.

2 Visibility of nanoparticles under the scanning electron microscope

In order for particles to be classified and counted, they must first be made visible. For nanoscale particles, this requirement is by no means trivial, since the boundary between visible and invisible is fluid. The visibility of objects (or more generally of structures) under the SEM is dependent upon the resolution that can be attained with the apparatus in question (i.e. the smallest interval that can be recognized as a boundary between two objects) and the contrast between the objects and the substrate. Under ideal conditions (maximum acceleration voltage, smallest aperture and smallest possible working distance), a modern field emission scanning electron microscope (FESEM) has a possible resolution of approximately 1 to 2 nm. This optimum resolution cannot be attained in practice, however: the ideal settings for good contrast are in some cases different to those for the ideal resolution. The contrast in images generated by means of secondary electron detectors is dependent upon the topography of the specimen, the difference in elementary composition between the object and the substrate (material contrast), and the type of secondary electrons employed for imaging (as a result of different detectors and/or detector settings).

3 Visibility of CNTs on gold-coated capillary pore membrane filters

The secondary electrons (SEs) used for imaging occur not only just below the surface of the specimen (SE1), but to a substantial degree at a certain depth below the surface (SE2). The penetration depth of the primary beam is therefore relevant. This in turn is dependent upon the energy of the primary electrons (i.e. the acceleration voltage) and the density of the penetrated material. At an acceleration voltage of 15 kV, a gold particle with a thickness of 100 nm absorbs virtually all primary electrons, whereas a carbon particle of the same size allows approximately 90% to pass. A carbon particle with a thickness of 100 nm, such as a CNT, on a gold substrate, such as a gold-coated capillary pore membrane filter, thus supplies a signal of [10% C + 90% Au]. Since gold emits approximately four times as many secondary electrons as carbon, the signal from the particle is therefore only approximately 10% weaker than that from the substrate, making the particle virtually invisible (**Figure 1a**).

Conversely, the signal from a gold particle of the same size on carbon [100% Au + 0% C] is 300% stronger than that from the substrate, and therefore clearly visible. In order for the beam not to pass (significantly) through a carbon particle with a thickness of 100 nm and for good contrast thus to be obtained to the (gold) substrate, the acceleration voltage must be substantially below 10 kV. This, however, yields a significantly weaker resolution and a poorer overall signal, i.e. one with stronger noise (**Figure 1b**).

In nanostructures, topographical contrast does not contribute substantially to the overall contrast. Substantially better images can, however, be obtained by means of an in-lens detector, since compared to the standard SE detector (Everhart-Thornley), the former gives greater consideration to the secondary electrons emitted close to the surface than to those emitted from a greater depth (**Figure 1c**). Nanoparticles are also even more easily recognizable at lower primary voltage with an in-lens detector (**Figure 1d**).

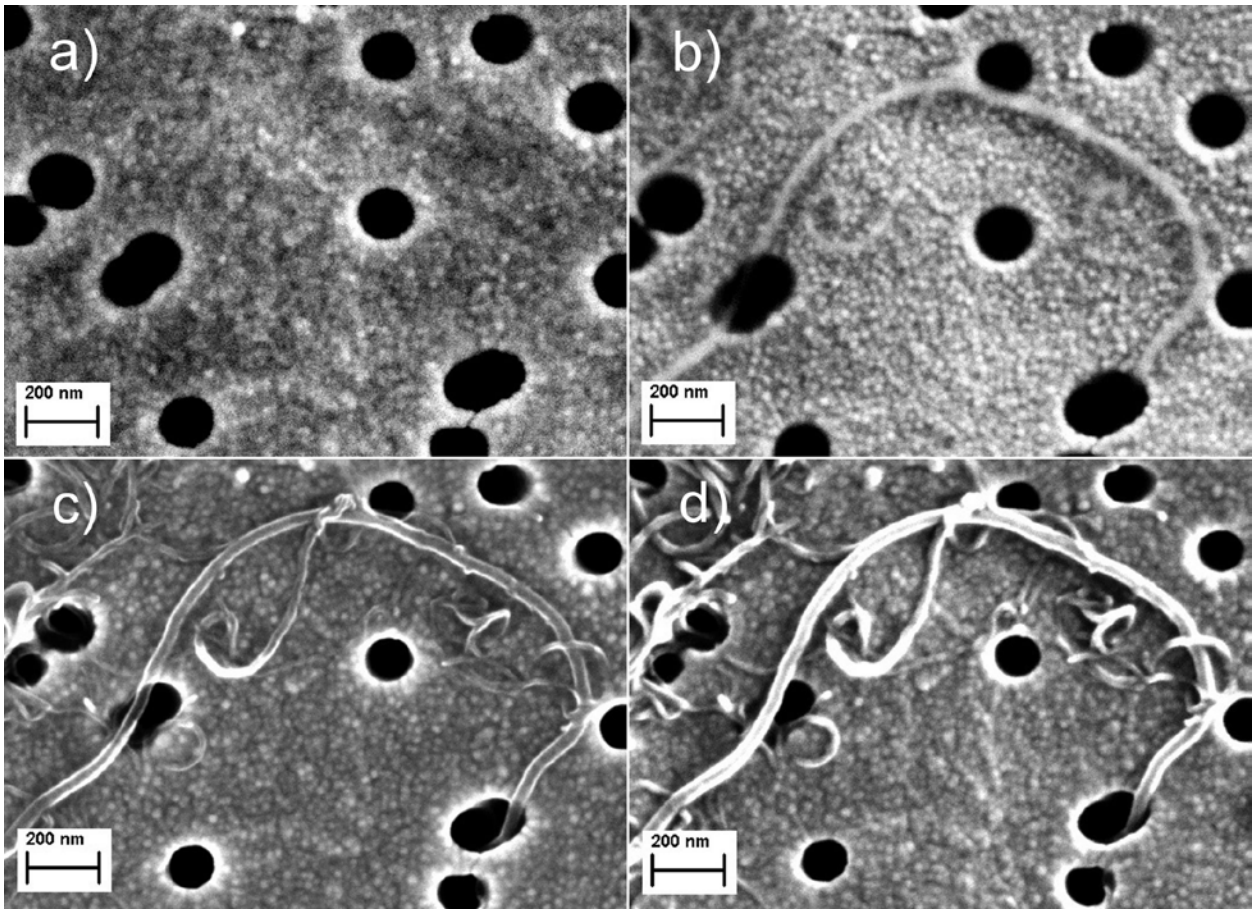

Figure 1:
CNTs on gold-coated capillary pore filters with different primary voltages and detectors;
a) SE 15 kV, b) SE 3 kV, c) in-lens 15 kV, d) in-lens 1 kV.

4 Visibility of nanoparticles on gold-coated capillary pore membrane filters

Gold-coated (sputter-coated) polycarbonate capillary pore membrane filters are frequently used as the specimen holders for SEM and energy-dispersive x-ray analysis (EDXA) of particles. Such filters have a very smooth gold surface with an even appearance at medium magnifications (e.g. 2,000x). The gold coating is necessary in order for the specimen to be made electrically conductive; otherwise it would become highly charged locally by the incident primary electrons, which would make imaging impossible. In addition, EDXA results in (virtually) no peak overlaps between gold and the other relevant elements, at least during the study of mineral dusts. Gold-coated capillary pore membrane filters are ideal for particle analyses in the micrometre range.

In order for nanoscale particles, for example around and below 50 nm, to be made visible and still to be analysed morphologically albeit with limitations, magnification of around 20,000x to 50,000x is required. At this level of magnification, the grain structure of the sputter layer is clearly visible; the grain size is in the order of 30 to 50 nm. Individual granular particles below 50 nm can be distinguished poorly or not at all from the gold grains (**Figure 2**). The granularity is only weakly dependent upon the layer depth and process parameters of the sputter process. Weaker sputtering yields a slightly better surface. For granular nanoscale particles, the visibility threshold on gold-coated capillary pore membrane filters can thus be assumed to be around 30 to 40 nm.

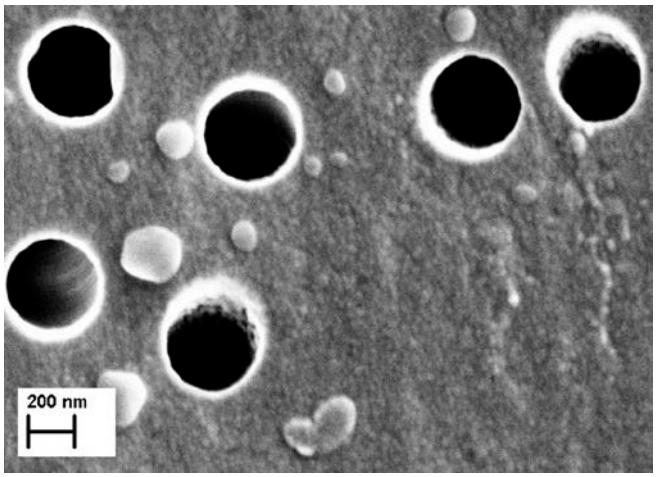

Figure 2:
Nano particles on gold-coated capillary pore membrane filters.

Long fibrous particles (nanotubes) are however still visible even when they are thinner than 30 nm. CNTs can be detected particularly well when they stick out from larger particles, agglomerates/aggregates or clusters and the filter substrate lies outside the focal plane. The thinnest visible CNTs in **Figure 3** are approximately 8 nm in thickness. CNTs at the boundaries of larger particles of composite materials ("hairs") are thus clearly visible, whereas CNTs on the

surface of such particles cannot always be readily distinguished from steps or edges of the particle itself (**Figure 4**).

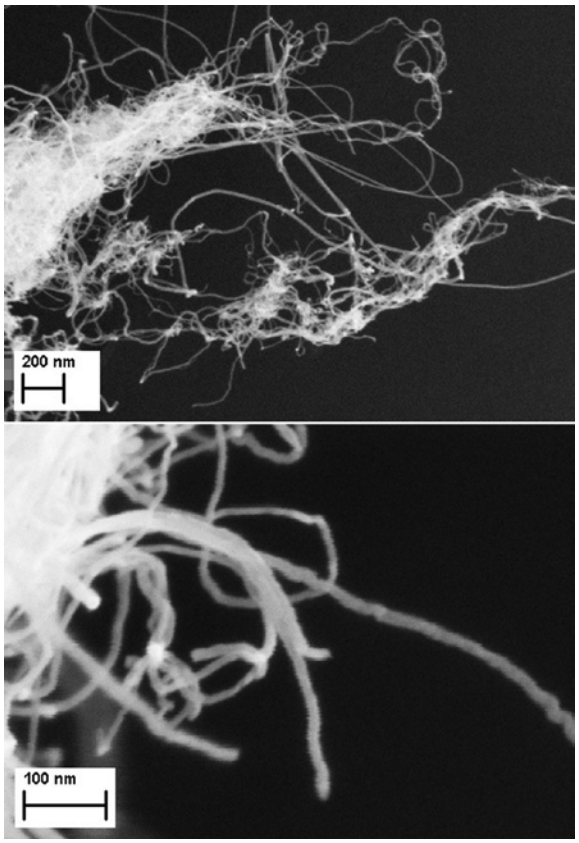

Figure 3:
CNTs at the edge of a cluster.

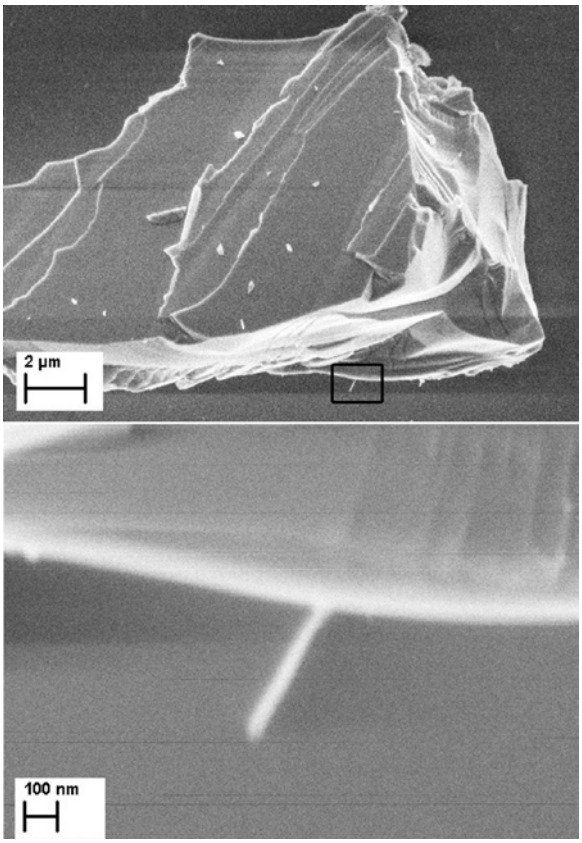

Figure 4:
Inorganic particles of a composite material with CNT sticking out.

5 Alternative specimen holders

Polished silicon discs yield a very smooth substrate for SEM imaging. This also enables granular particles in the order of magnitude of the gold structures to be made visible. During imaging of carbon particles, the contrast is better on this structureless surface than on gold, and at low acceleration voltages the (low) conductivity of the silicon is sufficient to prevent charges from forming. The best images of ultra-small granular particles < 20 nm are obtained with specimen holders consisting of TEM grids (**Figure 5**).

The visibility is also related to the proportion of the airborne particles precipitated onto the specimen holder in the first instance. The air to be analysed is not drawn through the silicon plates; instead, they are charged electrostatically or by impaction. No linear relationship exists in this case between the particle concentration in the atmosphere and the loading of the specimen holder. The same applies to the majority of TEM grids; only Holey grids through which the air is drawn can be used for quantitative measurements, if at all. Capillary pore membrane

filters also present problems when the particles to be analysed are smaller than the pore width. **Figure 6** shows by way of example that particle losses must be anticipated.

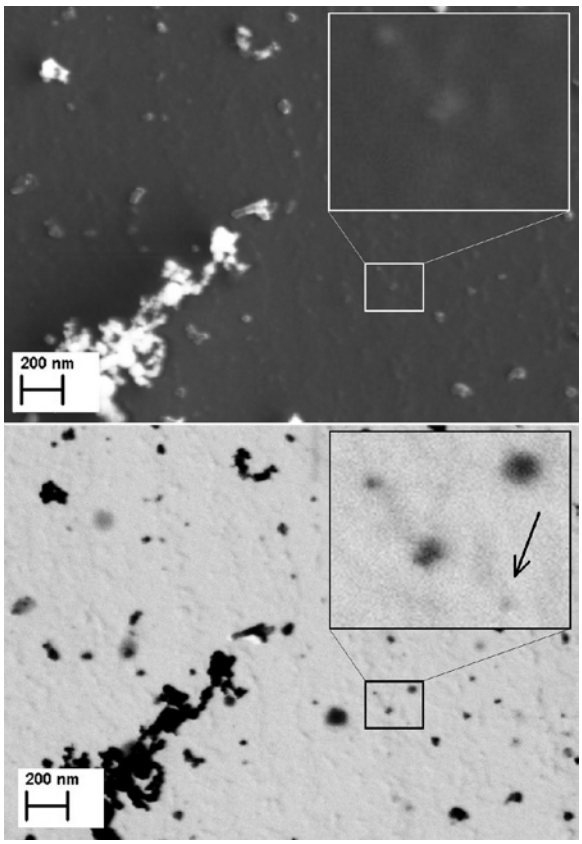

Figure 5:
Nanoparticles on TEM grid, SE image (in-lens) and transmission image.

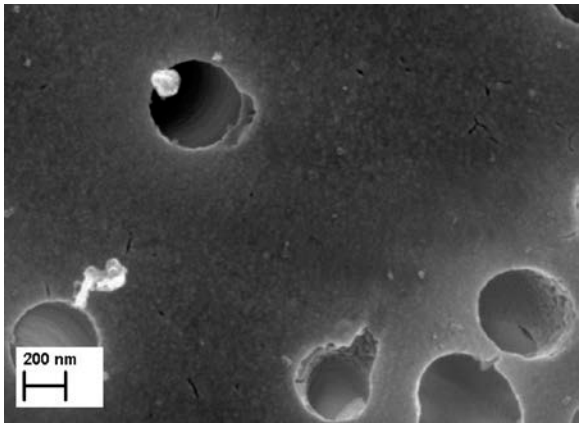

Figure 6:
A particle with a size of 100 nm is almost sucked through the pore.

6 Determining of the element composition by means of EDXA

Certain conditions must be met before energy-dispersive x-ray analysis (EDXA) can be used to determine the quantitative components of chemical elements in particles:

- The excitation energy (primary voltage) must be at least twice the energy of the highest peak to be identified. For the detection of carbon, an excitation energy of at least 0.6 keV is required; for titanium, at least 9 keV (K_α = 4.5 keV); for copper, at least

16 keV (K_α = 8 keV); and for lead, 21 keV (L_α = 10.6 keV). In many cases however, the L_α or M_α lines can be used for identification, even for heavy elements, in which case an excitation energy of approximately 4 keV is sufficient. The peaks of these lines of the heavy elements overlap the peaks of the K_α lines of lighter elements, however.

- The ideal working distance for EDXA is always greater than that for optimum imaging of nanoscale particles, and is in the order of 6 to 10 mm for a modern SEM. In favourable cases, analysis is still possible at a somewhat lower working distance. At a working distance of 1 to 2 mm (for optimum image quality and visibility of the smallest particles) the x-ray detector is blind, however.
- Since the x-rays are produced not at the surface of the specimen but at a certain depth (which is dependent upon the primary voltage), virtually only the x-ray radiation of the substrate would be measured in the case of a very small particle, and barely the particle itself. The limit lies at a particle size of approximately 200 nm, or somewhat lower in favourable cases.

A chemical element present in a single nanoscale particle cannot therefore be identified with the use of SEM. If however larger aggregates/agglomerates of morphologically similar particles are present on the specimen in addition to discrete particles, they can be exploited for identification.

7 Recommendations for analysis

For analysis of capillary pore membrane filters loaded with CNTs a modern SEM with field-emission cathode (FESEM) is recommended; the resolutions required for nanoscale particles are unlikely to be reached easily in an SEM with tungsten cathode. For determining of the elements – which is necessary for example for distinction between CNTs and chrysotile fibrils – an energy-dispersive x-ray microanalysis system with light-element detector is required.

For the search for coarser particles, 200x magnification at a relatively large working distance is sufficient (Everhart-Thornley secondary electron detector). For particles and fibres in the order of magnitude of WHO fibres, a magnification of 2,000x should be used at the most favourable working distance for EDXA; the primary voltage should be 15 kV.

A magnification of 20,000x to 50,000x is required for the detection of nanoscale particles and CNTs. The necessary conditions for resolution and contrast can be met in this case only by means of an in-lens detector. Optimum images are possible at a primary voltage of substantially below 10 kV at a working distance of a few millimetres.

At an excitation voltage of 4 kV, EDXA is also able to detect carbon in CNTs with a thickness of 50 nm, since at such a low voltage the electron beam no longer significantly penetrates the gold layer of the capillary pore membrane filter and the carbon in the filter material thus barely emits x-rays. At 4 kV, the magnesium K peak can also be registered, thereby permitting differentiation between CNTs and chrysotile fibrils. The silicon K peak however cannot be separated from the gold M lines.

8 Proposed counting rules

Besides the constraints described above upon the visibility of nanoscale particles during SEM analysis, a further problem that is presented is that of the definitions for differentiation. The formulation of counting rules for nanoscale particles requires clear definitions; these, however. do not yet exist or are still the subject of controversial debate.

The relevant morphological criteria for the evaluation of nanoparticles from the perspective of occupational hygiene have also not yet been conclusively defined. If the analysis of air samples is limited to a few narrowly defined criteria, and if as a result certain nanoscale particles are determined only selectively, the results may be of severely limited benefit during subsequent analyses. Documentation of the most far-reaching qualitative and quantitative recording possible of particles is therefore considered important for the microscopic analysis of air samples from areas in which tasks involving nanomaterials are performed.

Based upon this approach, a phased procedure is proposed below for detection by scanning electron microscope of the nanoparticles in air samples. This proposal constitutes a maximum requirement by way of which ideally all particle types occurring in dusts are detected, i.e. including the coarser dusts accompanying the nanoparticles. The procedure is designed such that the relevant parts of the catalogue of requirements can be applied during analysis in a given case.

The focus of the criteria for analysis lies upon CNTs. These are primarily fibres; from an occupational hygiene perspective however, nanotubes – despite their fibrous geometry – do not necessarily satisfy the WHO criteria[1][1], according to which respirable fibres have to date been counted and distinguished from granular particles. Whereas CNTs satisfy the WHO criteria in their diameter and length-to-diameter aspect ratio, they do not always reach the required minimum length of 5 µm. In addition it is questionable whether CNTs – in contrast to asbestos

[1] WHO criteria for the differentiation of respirable fibres: diameter < 3 µm, length > 5 µm, length-to-diameter aspect ratio > 3 : 1

fibres – should generally be regarded as rigid fibres which cannot be incorporated by alveolar macrophages at a length of > 5 µm. Furthermore, CNTs with a length of > 5 µm may also assume a coiled form, in which case their greatest measurable dimension may be substantially below 5 µm. A simple count of CNTs is also exacerbated by the fact that they can occur as hairballs which may well attain or exceed a dimension of 20 µm more (such as "Baytubes" or "Graphistrength"). Particles in the magnitude of several µm are also observed during the application of composite materials containing CNT components from which CNTs stick out (nanofibre composite material particles).

Accordingly, for detection of the particle types associated with CNTs, SEM analysis would have to be performed in at least three stages of magnification (see **Table 1**).

Table 1:
Particle dimension and proposed magnification for analysis during SEM analysis of air samples from areas in which tasks are performed involving nanomaterials, particularly CNTs.

Particle dimension	Typical kind of particles	Suggested magnification for SEM-analysis
> ca. 20 µm	- hairballs of CNTs (e.g. “Baytubes”, “Graphistrength”) - in addition granular particles and fibres	200 : 1
x µm	- dust particles emitted from CNT composite materials (e.g. “Zentallium”) - in addition granular particles and WHO fibres	2,000 : 1
x to 100 nm	- *one-dimensional*: flaky particles (e.g. „Graphen“), nanoscale surface structure of coarser particles - *two-dimensional*: CNTs and other nanofibres - *three-dimensional*: “granular” nanoparticles - agglomerates und aggregates of nanoscale particles - in addition particles with size from 100 up to 1,000 nm	20,000 : 1 to 50,000 : 1

The incidence of larger CNT hairballs can be studied at a magnification of 200x. At the 2,000x magnification typical for determination of the WHO fibre concentration, it would be necessary to check whether exposure arises to particles from CNT composite materials. For the identification of both CNT hairballs and particles of CNT composites, the particles must be examined at a greater magnification (felt-like structure with visibly discrete CNTs in the case of CNT hairballs; discrete CNTs sticking out from particles of composites). Finally, the individual nanoscale particles and their aggregates and agglomerates are counted at a magnification of 20,000x to 50,000x.

The division into particle types shown in Table 1 is intended in the first instance to be schematic; it is to some degree arbitrary and should be modified on a case-by-case basis. CNT hairballs for example may also exhibit particle sizes below 20 µm. Owing to their very low density of approximately 0.1 g/cm³ however, it must be considered that CNT hairballs of a particle size of up to approximately 30 µm may, albeit with decreasing probability, still form part of the respirable dust fraction and thus be capable of reaching the lower parts of the respiratory tract[2]. Use of an established system for fibre dust measurements (such as the PGP-FAP with 37 mm filter) and a flow rate of at least 2 l/minute ensures that particles up to an aerodynamic diameter of approximately 30 µm are sampled.

Certain boundary conditions must be set out for the analysis which is to be performed in three stages. Corresponding proposals are made below. The descriptive terms are summarized in **Table 2**. The stages of analysis are summarized in **Table 3** and described in greater detail below.

Table 2:
Parameters of the particles and definitions.

parameter	**definition**
length (L)	rectified visible length of a fibre or a particle
diameter (D)	mean visible width of a fibre or a particle
particle size (G)	maximum extent of a particle or a fibre (diameter of the surrounding circle)
granular particle	particle with L : D ≤ 3 : 1
fibre	particle with L : D ≥ 3 : 1
composite particle	particles which are emitted of a used or treated composite material at a working place, e.g. carbon fibre composite materiel including a CNT portion

[2] CNT hairballs typically have a density of approximately 0.12 to 0.17 g/cm³ (e.g. [2]). Accordingly, CNT hairballs with particle sizes of 12 to 14 µm and 24 to 29 µm have an aerodynamic diameter of 5 to 10 µm respectively.

Table 3:
Parameters for analysis of the particle count in stages 1 to 3.

parameter	**stage 1**	**stage 2**	**stage 3**
magnification	200 : 1	2,000 : 1	20,000 : 1 up to 50,000 : 1
object with G in µm	≥ 20	1 to 20	approximately 0.05 to 1 *
fibre with D in µm	≥ 3	0.2 to 3 **	< 0.2

* the minimum size of granular particles deposited on a gold-coated capillary pore membrane filter

** the class border at 0.2 µm results from the limitation of the reference measurement method of WHO for the determination of WHO fibre concentration

8.1 First analysis stage

The objects found are analysed individually qualitatively at 2,000x magnification.

- Granular particles: compact discrete particles, agglomerates/aggregates, composite particles, CNT hairballs and other particles[3] are to be distinguished and counted.
- Fibres: distinction must be drawn between carbon fibres/textile glass fibres (parallel edges, constant diameter), mineral wools (variable diameter, curved fibres), other inorganic fibres (such as mineral fibres) and other fibres.
- Composite particles: granular particles which may originate from composites (the main constituent to be determined by means of EDXA)[4] are to be tested for CNTs sticking out (examination of the lateral particle boundary at up to 20,000x magnification). Where composite particles with visible CNTs are present: documentation of the particle size and of the number of visible CNTs.
- CNT hairballs: identification of the hairball structure at 2,000x to 20,000x magnification and documentation of the particle size of the hairball.
- Criterion for abortion of analysis at this stage: the area to be analysed is 20 mm². Once over 50 objects (composite particles, hairballs) containing CNTs have been counted, analysis can be aborted. An area of at least 5 mm² must however be analysed.

[3] Particles that cannot be clearly assigned to one of the other categories. These include granular particles or fibres to which CNTs are agglomerated.

[4] The main component of composites is for example carbon in the case of carbon fibre composites or aluminium in the case of Zentallium.

Evaluation may comprise, in the first instance, marking of the positions of all relevant objects during assessment at 200x magnification, followed by selective viewing of these positions in turn at 2,000x magnification for identification of the objects.

8.2 Second analysis stage

The objects found are analysed individually qualitatively.

- Granular particles: compact discrete particles, agglomerates/aggregates, composite particles, CNT hairballs and other particles are to be distinguished and counted.
- Fibres: distinction is to be made between asbestos, fibrous fragments of carbon fibres/textile glass fibres (requires determining of the product fibre by means of EDXA), other inorganic fibres (e.g. mineral fibres), possibly product fibres (by means of EDXA with the use of reference material) and other fibres. The counting rules to BGI 505-46 [4] are to be applied. CNT hairballs which satisfy the fibre dimensions of the WHO must be counted separately.
- Composite particles: granular particles which may originate from composites (the main constituent to be determined by means of EDXA)[4] are to be examined for CNTs sticking out (examination of the lateral particle boundary at up to 20,000x magnification). Where composite particles with visible CNTs are present, documentation of the particle size and of the number of visible CNTs.
- CNT hairballs: identification of the hairball structure at 2,000x to 20,000x magnification and documentation of the particle size of the hairball.
- Criterion for abortion of analysis at this stage: the area to be analysed is 0.5 mm². Once over 50 objects (composite particles, hairballs) containing CNTs have been counted, analysis can be aborted. An area of at least 0.15 mm² must however be analysed.

8.3 Third analysis stage

- Granular particles: four particle types must be counted separately (see **Figure 7**).
- a) Primary particles of 0.05 µm ≤ G ≤ 0.1 µm
  b) Primary particles of 0.1 µm < G < 1 µm
  c) Agglomerates/aggregates containing primary particles ≤ 0.1 µm
  d) Agglomerates/aggregates containing no primary particles ≤ 0.1 µm

If possible, the geometry of the particles (spherical, angular, etc.) should be documented.

- Fibres: counting of CNTs according to the following criteria (see **Figure 8**):
  - Individual CNTs are counted. G (determined), L (measured or estimated) and D (determined) respectively are stated.
  - The number of CNTs of L > 5 µm is stated separately.
  - Overlapping CNTs are counted separately if they can still be distinguished from each other.
  - Should the overlap of fibres consist of an agglomerate that can no longer be differentiated, it is counted as a cluster; G (estimated from the size of the agglomerated region) and the number of CNT ends sticking out are stated in this case.
- Criterion for abortion of analysis at this stage: the area to be analysed is 0.005 mm² in size. Once over 50 objects containing CNTs (fibres, clusters) have been counted, analysis can be aborted. An area of at least 0.0015 mm² must however be analysed.

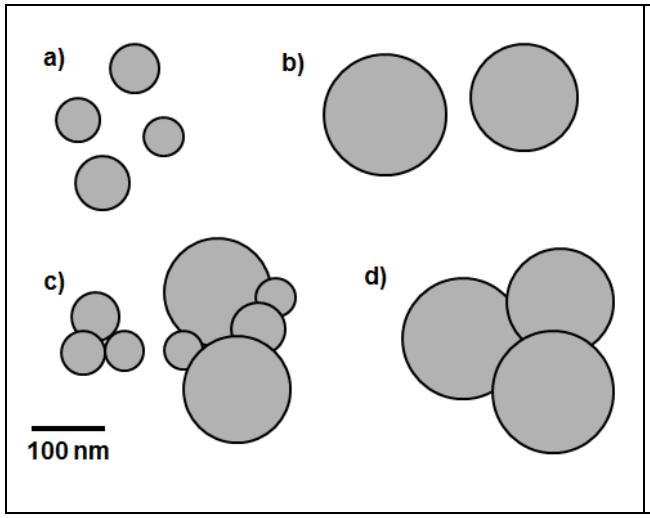

Figure 7:
Schematic diagram of categories for the counting of nanoscale granular particles, a) primary particles of 0.05 µm ≤ G ≤ 0.1 µm, b) primary particles of 0.1 µm < G < 1 µm, c) agglomerates/aggregates containing primary particles ≤ 0.1 µm, d) agglomerates/aggregates containing no primary particles ≤ 0.1 µm.

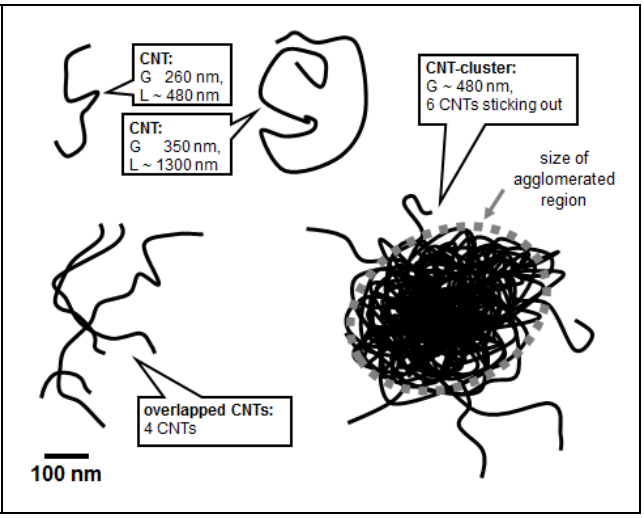

Figure 8:
Schematic diagram of the categories for the counting of CNTs. Distinction is made between discrete CNTs, overlapping CNTs and clusters of CNTs (dotted grey line: boundary of the agglomerated region of a CNT cluster for the estimation of G).

It must be considered that analysis of filters may not be possible owing to excessive loading. Experience gained with the use of a SEM analysis for determining the fibre concentration from filter specimens indicates that approximately one-eighth of the area of an image field is the maximum filter loading at which specimens can still be analysed. Should an image field exhibit a

higher loading, it is to be rejected. Once more than ten image fields in total have been rejected, the sample itself is deemed not suitable for analysis [4; 5]. This procedure can be adopted for stages 1 and 2 of the analysis proposed here. Experience has not yet been gained with the proposed stage 3. The filter must also be examined for homogeneous loading.

The level of the detection limit for counting of the different particles also differs between the three analysis stages. For determining the concentration of CNTs in accordance with stage 3 of the analysis at 20,000x magnification, a detection limit of 1.5 million CNTs/m³ is calculated mathematically based upon the boundary conditions of the established analysis methods (sampling in accordance with BGI 505-46 on a 37 mm capillary pore membrane filter with a sample air volume of 280 l) and an analysed surface area of 0.005 mm².

9 Future prospects

The criteria described here for the counting of nanoscale particles and the other dust particles arising in combination with them constitute a preliminary proposal for a classification of particles during microscopic analysis that is to be as comprehensive as possible. An attempt has been made here to differentiate between relevant particle fractions and classes and to permit detailed analysis at justifiable analytical effort. The intention is for a discussion to be launched with the objective of developing a catalogue of criteria, with the widest possible acceptance and scope for application, for the analysis of air samples for nanoscale particles with particular focus upon CNTs.

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