# AUTOMATED DETECTION, TRACKING AND CHARACTERIZATION OF TOXICOLOGICALLY RELEVANT NANOSCALE FIBRES IN SCANNING ELECTRON MICROSCOPE IMAGES

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## ABSTRACT

An automated approach for the detection, tracking and morphological characterization of nanofibres is presented. It acquires and analyses scanning electron microscopic (SEM) images to determine the length, width and tortuosity of previously airborne nanofibres after sampling on filters. It allows identifying specific morphological fibre properties that are made responsible for toxicological hazard. Due to the low recommended occupational exposure limits of 10000 nanofibres per cubic metre and the high magnification necessary for imaging, only automated image acquisition and analysis will enable to routinely assess the release of hazardous nanoscale fibres. This task therefore deserves further attention of the image analysis research community.

## 1. INTRODUCTION

Compliance with occupational exposure limits for respirable airborne fibres requires aerosol sampling and fibre counting by means of microscopic filter analysis. For asbestos-like microscale fibrous materials, such compliance testing is performed by a microscope operator in real time according to well-established testing protocols. Handing of industrially available toxicologically relevant nanofibres makes exposure assessment indispensable. Compared to microscale fibres, the workload required for exposure limit testing of 10-times thinner nanofibres is – due to higher necessary magnification – at least a factor of 100 higher. This stimulates the development of automated image analysis approaches that are capable of detecting, tracking and morphologically characterizing nanofibres on filter samples. Morphological information of individual fibres is needed to identify and count the toxicologically relevant fibre fraction according to WHO-criteria (diameter < 3 µm, length > 5 µm and aspect > 3:1). However, resolving both width and length of high-aspect ratio fibres requires developing fibre width estimation algorithms that consider physical processes of SEM image generation as well as careful selection of imaging parameters, including image area, pixel resolution and signal-to-noise ratio. Fibre tracking algorithms must be capable of disentangling fibres at fibre crossing and may provide valuable information on fibre rigidity that is believed to govern nanofibre-related health hazards.

## 2. MATERIALS AND METHODS

Toxicologically relevant carbon nanotubes were deposited with reproducible degree of fibre individualization and concentration on track-etch membrane filters using a fluidized bed aerosol generator [1]. We have developed SEM control software and implemented an automatic image acquisition workflow that starts by orienting filter samples using sample-specific reference points on each imaged filter. It allows autonomous generation of SEM image data with nanoscale resolution and several gigapixels of data.

Automated detection and tracking of imaged nanoscale fibres is performed after an initial image segmentation step using classical [2] or machine learning-based thresholding, depending on substrate type.

For each segment, the surrounding polygon is determined to derive first estimates of fibre widths and fibre backbone pixels using distance histograms of all neighbouring polygon points to orthogonally lying edge points. The backbone data points are approximated with circle segments that are continuously connected using clothoids to avoid discontinuous course of curvature. This way, the fibre's curvature development is regularized by imposing a constraint on the maximum admitted circle curvature.

Nanofibre widths are determined on the basis of statistical analyses of the surrounding filter noise characteristics to identify significant signal levels that indicate outermost edges of a fibre.

## 3. CONCLUSION

The developed algorithms are embedded in an easy-to-use and versatile editor that allows also for manual evaluation of images. This enabled assessing the accuracy and reliability of the automated approach in comparison to human analysis. Future work will focus on developing self-learning classification algorithms.

## REFERENCES

[1] Broßell, D, et al. (2019). Assessment of nanofibre dustiness by means of vibro-fluidization. Powder Technology 342: 491-508.

[2] Zack G, Rogers W, Latt S (1977) Automatic measurement of sister chromatid exchange frequency. J Histochem Cytochem 25:741–753.