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

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Motivation

Compliance with occupational exposure limits for respirable airborne fibres requires aerosol sampling and fibre counting by means of microscopic filter analysis [1]. Handling of industrially available toxicologically relevant nanofibres makes exposure assessment indispensable. This stimulates the development of automated image analysis approaches that are capable of detecting, tracking and morphologically characterizing nanofibres on filter samples. 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, pixel resolution and signal-to-noise ratio.

SEM Image Acquisition

We have developed SEM control software and implemented an automatic image acquisition workflow (Fig. 2) 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 gig pixels of data (Fig. 3).

Fig. 1: Left :SEM Hitachi SU8230, centre: Air lock of SEM, right: Sample on sample holder

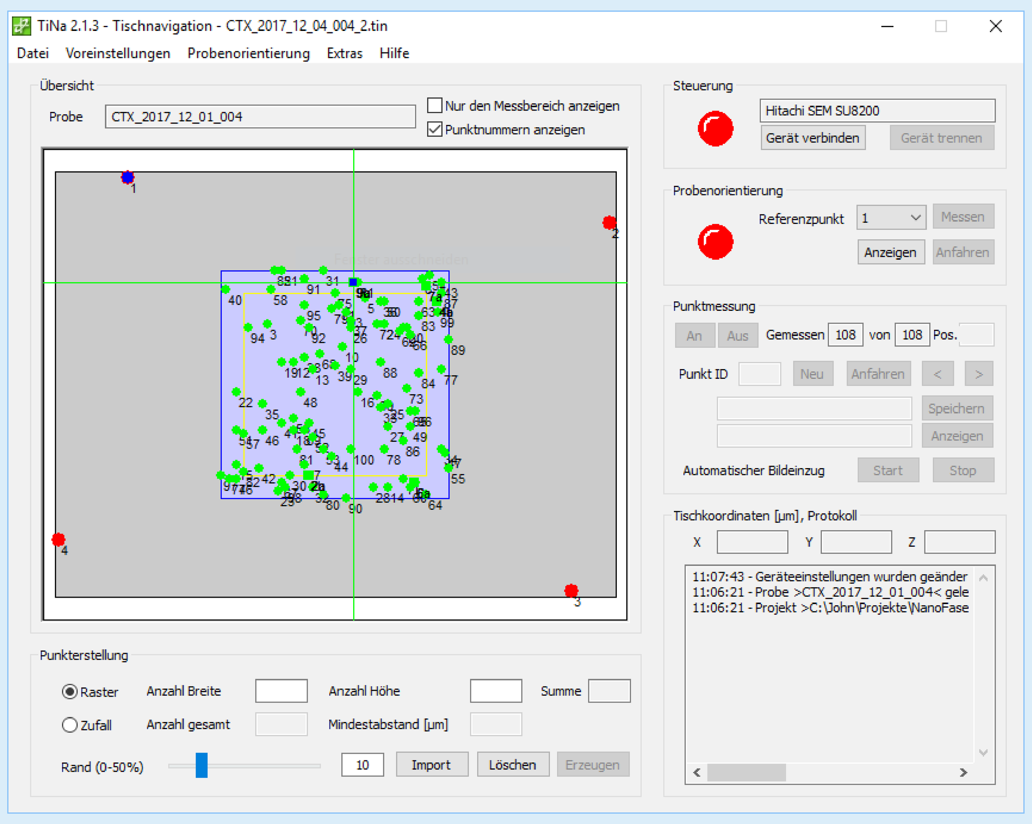

Fig. 2: Screenshot SEM control software TiNa

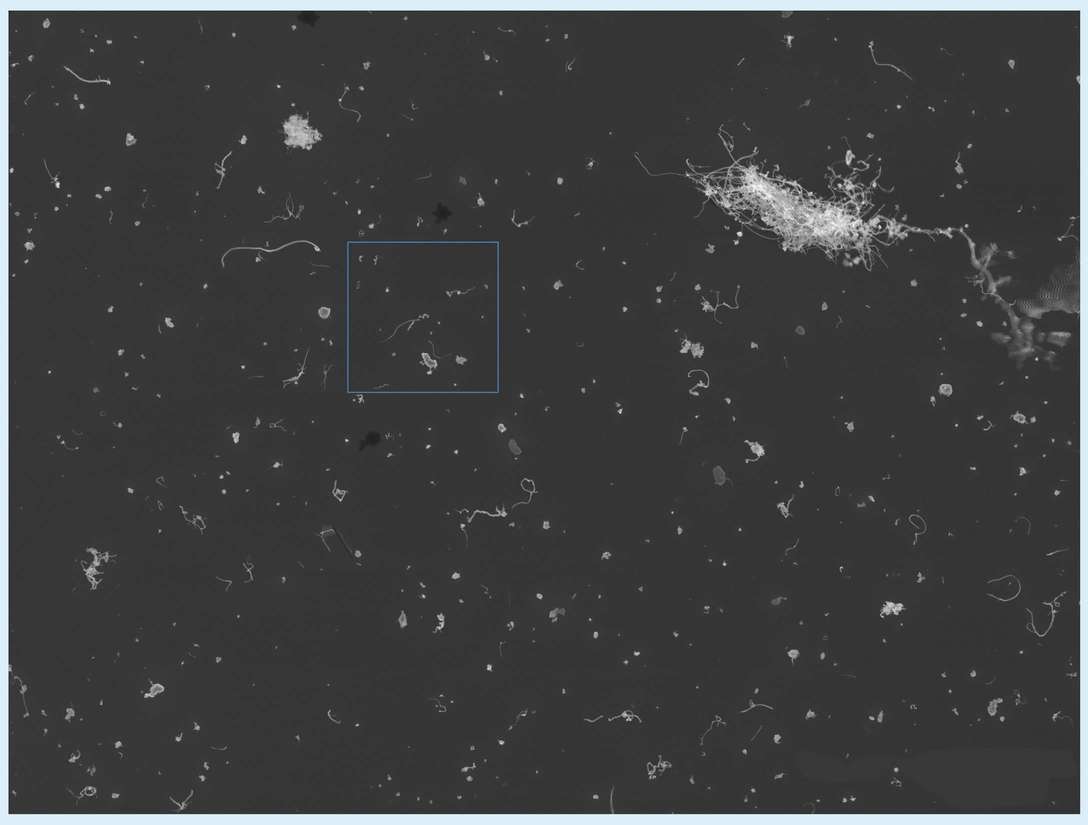

Fig. 3: Acquired SEM image of fibres

Automated detection and tracking of fibres

We have developed a software to detect fibres automatically in the acquired SEM images (Fig. 8 FibreDetect). The first step is image segmentation (Fig. 4) using classical [2] threshold methods. For each segment, the surrounding polygon is determined to derive first estimates of fibre widths and backbone pixels using distance histograms of all neighbouring polygon points to orthogonally lying edge points. The backbone data points are transformed into an adjusted B-spline and then approximated with circle segments that are continuously connected using clothoids to avoid a discontinuous course of curvature (Fig. 5). Fibre 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 (Fig. 6).

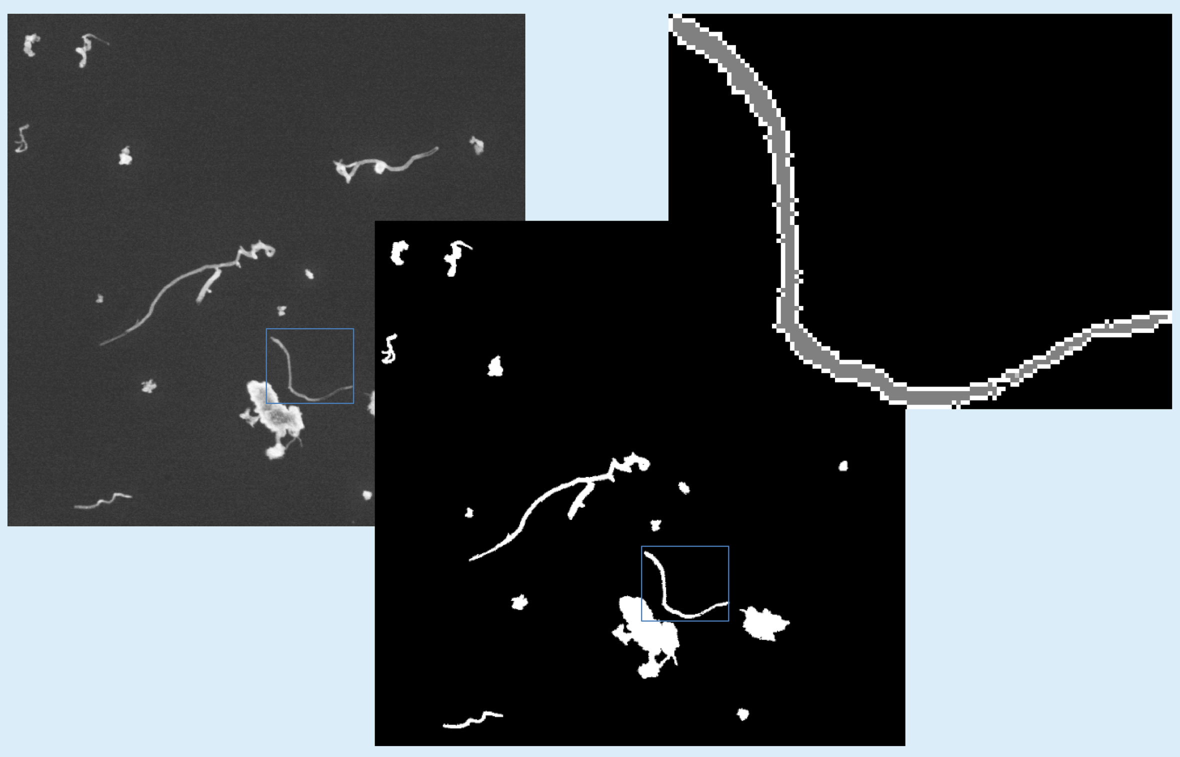

Fig. 4: Left :Section of SEM image, centre: Segmentation, right: Segmented object

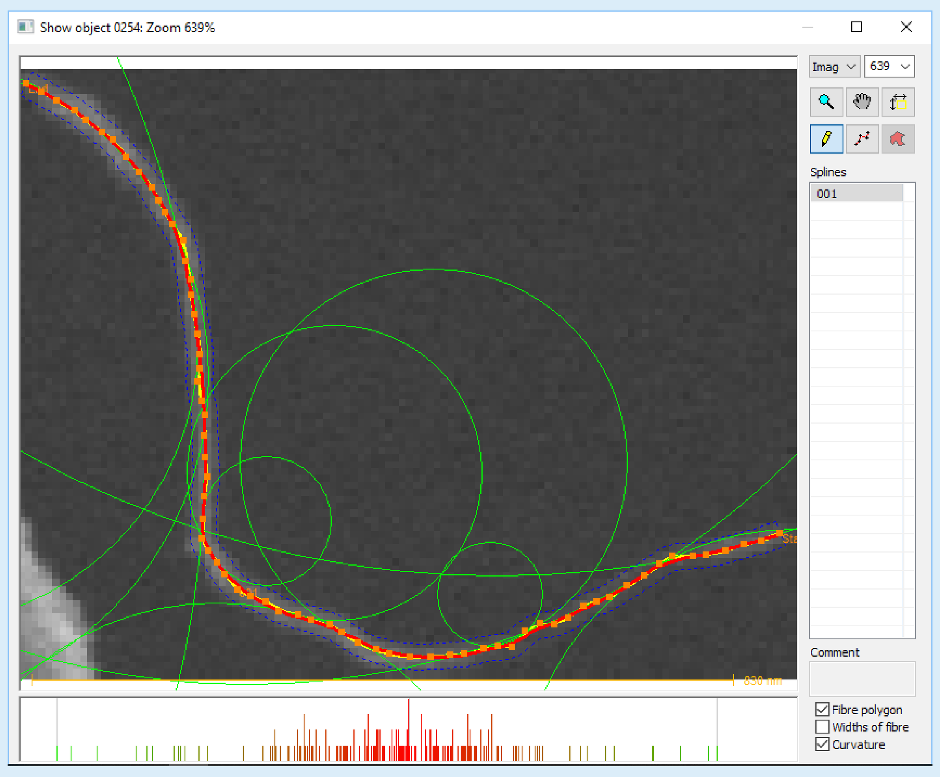

Fig. 5: Fitting circles (green), fibre backbone as spline (yellow) and line route (red)

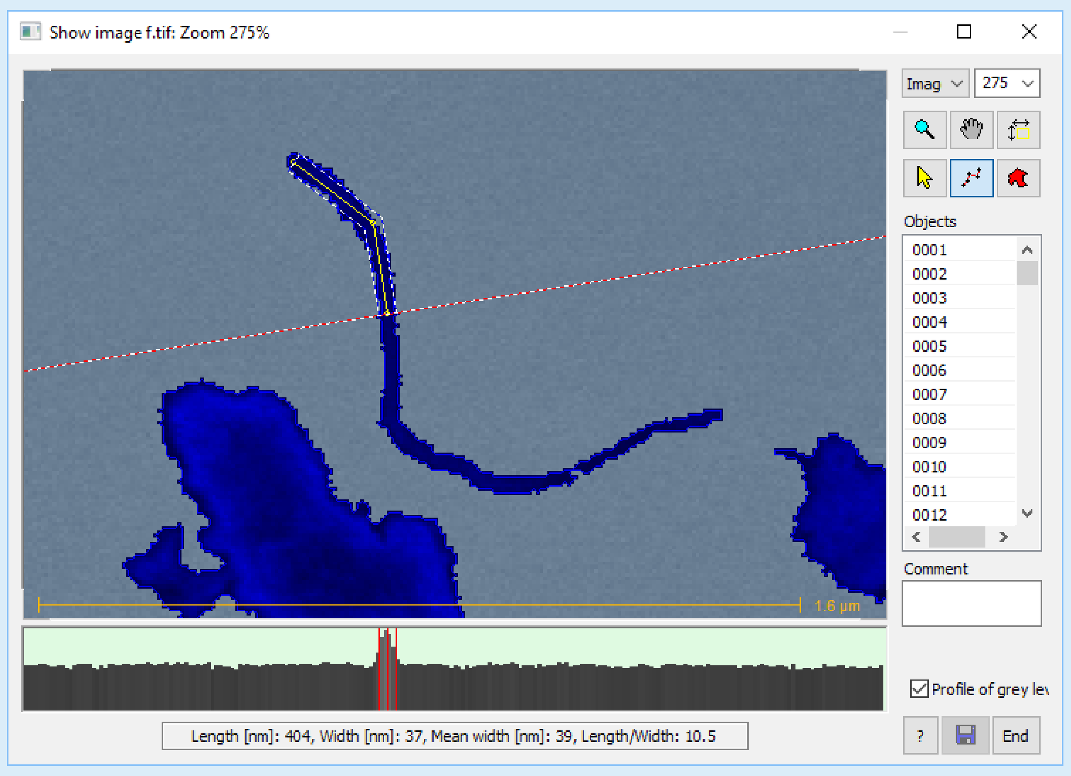

Fig. 6: Fibre width refinement using grey value statistics of the fibre and the surrounding substrate image area

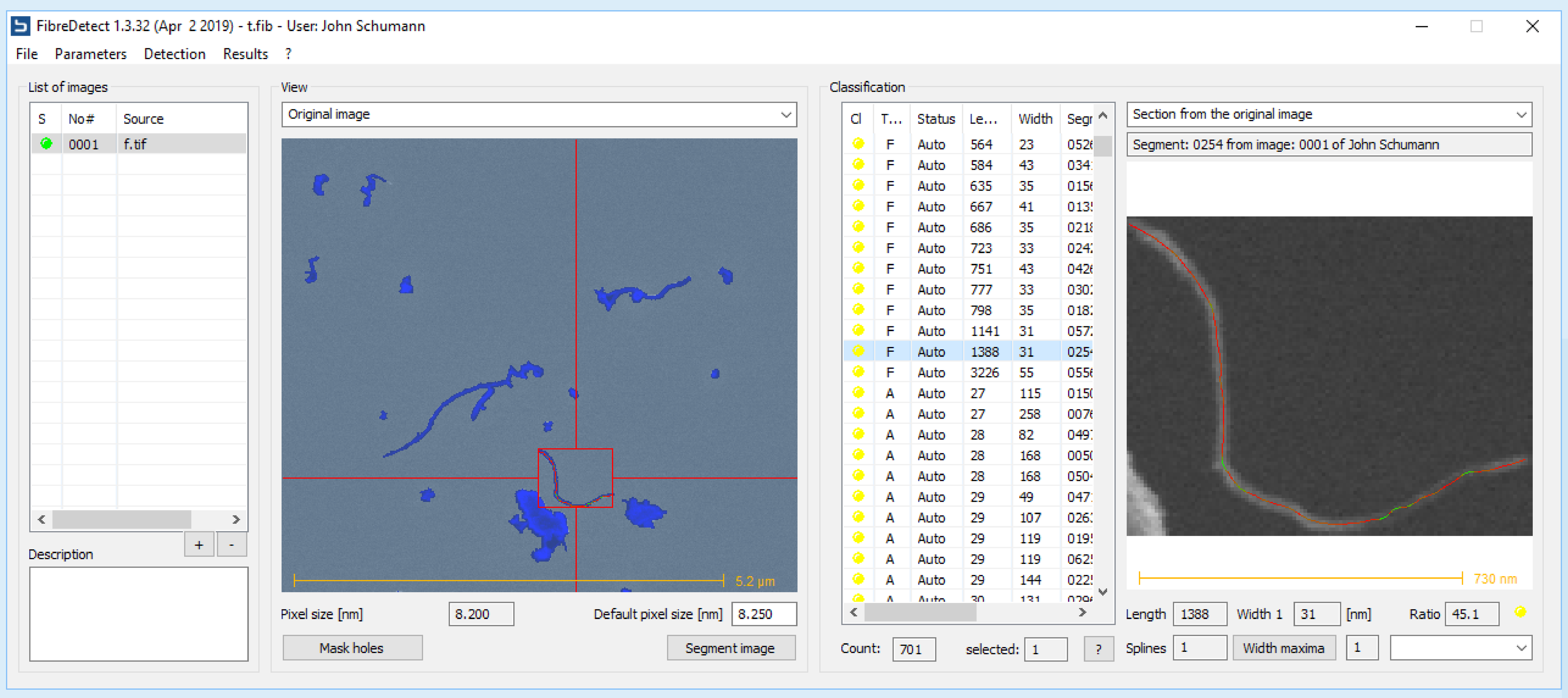

Fig. 8: Screenshot of the fibre detection and morphological analysis tool *FibreDetect*

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.

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