Correlating SEM and Raman Imaging of Nucleopore Filter Sampled Nanofibers

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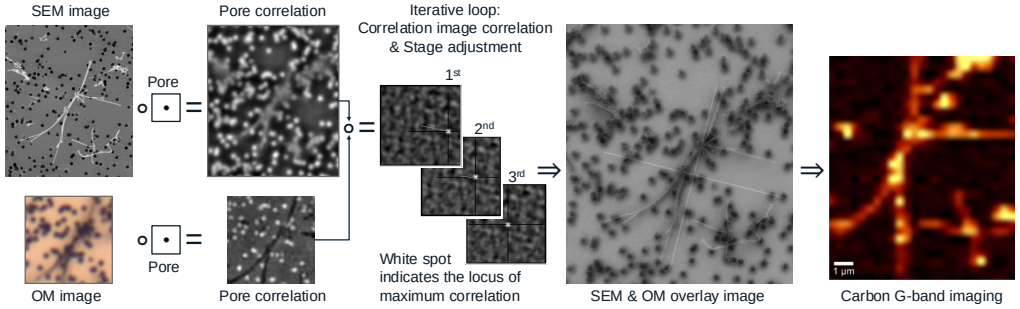
Introduction

In the light of the “Global Asbestos Disaster” [1], human exposure to high concentrations of respirable biopersitent fibers must be controlled. Reports on the asbestos-like pathogenic potential of rigid and biodurable nanofibers of multi-walled carbon nanotube type [2,3] make it necessary to determine concentrations of airborne nanofibers on a level of 10,000 fibers/m³. BAuA develops such techniques based on sampling aerosols on nucleopore filter membranes, followed by SEM imaging. For reliable SEM visualization of rigid nanofibers, resolutions of about 5−8 nm are required, resulting in about 2 gigapixels of SEM data. For every nanofiber recognized and localized on the filter, Raman-based chemical analysis is mandatory to distinguish natural fibers like cellulose from man-made biodurable fibers.

The high workload on SE and Raman microscopes motivated using not an SEM-integrated Raman but two independent instruments to parallelize the analyses. We have developed a method to adjust the x-y stepping motor stage of our Apyron at the fiber position with high accuracy to automatically analyze or image all candidates for biodurable fibers with Raman spectroscopy. This enables BAuA to check compliance with nanofiber exposure limits and to recommend health protection measures [4].

Methods

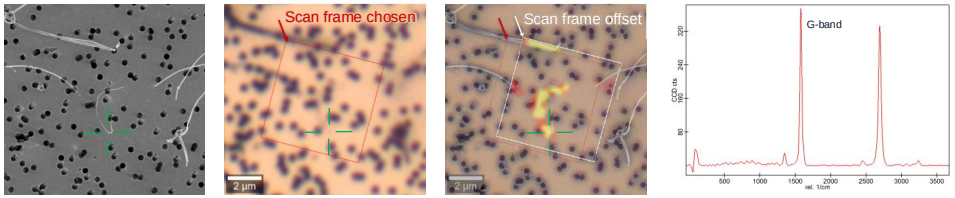
Using the Hitachi and WITec software APIs, a software was developed in C++ to control both the SU8230 SE and Apyron Raman microscopes. The software first automatically acquires focused SEM images at statistically random locations of a position-calibrated filter. The track-etched filter pores of 400 nm diameter can likewise be resolved in the Apyron using a 100× objective (0.75 NA) with a video magnification of 1160× and a pixel size of 56 nm. Correlation of both images with a “standard pore” kernel results in pore position-weighted images that are used for a normalized squared Euclidean distance correlation of the pore constellation in the vicinity of the nanoscale object to analyze.



Results

The initial filter positioning accuracy is about 5 µm and results from measurement errors of four calibration marks on the filter and movements of the filter membrane. The pore constellation pattern matching approach allows improving the positioning accuracy to 87±31 nm as determined from automatic addressing of 64 objects. This way, even optically non-resolvable nanoscale objects can be localized with high accuracy and submitted to Raman analysis and imaging.

However, after having started Raman imaging, unsupervised stage movement by the GUI or API from the frame-defining center to its top left edge currently causes scan area offsets in the order of a micron. A scan frame should thus be definable relative to a starting edge that can be addressed with high accuracy.



CNT position in SEM image | CNT position OM image | SEM, OM & G-band overlay | Spectrum taken at indicated position

Conclusion

- Unique constellation patterns of track-etched pores enable unambiguous high-resolution positioning
- The accuracy to locate an object in Raman is well below diffraction limit and confocal volume extension
- Even optically non-resolvable nanoscale objects become accessible for Raman analysis
- For characteristically patterned substrates, no integration of Raman into the SEM is necessary
- WITec’s microscope controller API gives full access to hardware features necessary to achieve not only image analysis and stage control but to implement even complex analysis workflows
- However, API functions should give access to the scan frame starting edge and stage speed control.

References

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2. Rittinghausen et al. DOI 10.1186/s12989-014-0059-z
3. Broßell et al. in DOI 10.20850/9781716632426 or 10.31219/osf.io/487wv
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