Developing a Rapid Screening Method for Direct Visualization of Nanoparticles Captured on Filter Media During Occupational Exposure Assessments

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ABSTRACT

Best-known methods for ENM exposure assessment are based on using real-time direct-reading instruments (DRIs) to measure airborne particle counts and filter-based methods collected in tandem for off-line direct visualization by transmission electron microscopy (TEM) and for elemental analysis.[1] Direct visualization by TEM is based on methods developed two decades ago for micron-sized asbestos[2,3] and is low-throughput, expensive, and time- and resource-intensive. Therefore, the overarching goal of this project is to rapidly advance exposure assessment for the nanotechnology workforce by developing and testing a new protocol using enhanced darkfield microscopy (EDFM) with hyperspectral imaging (HSI) and mapping. EDFM-HSI combines imaging and spectrophotometry to identify, locate, and map ENMs within a sample based on the unique spectral response. Preliminary work has explored the use of EDFM-HSI for the analysis of silicon dioxide nanoparticles (NPs) captured on mixed cellulose ester (MCE) filter media.

Keywords: mixed cellulose ester (MCE), darkfield microscopy, hyperspectral imaging, spectral angle mapper, reference spectral library

1. INTRODUCTION

As engineered nanomaterials (ENMs) are increasingly incorporated into manufacturing processes and consumer goods, the nanotechnology workforce is also growing, with 6 million workers anticipated by 2020, of which 2 million are projected to work in the U.S.[4] Risk assessment for nanotechnology workers is still in its infancy since occupational exposure assessment strategies and physiologic and health outcomes of occupational exposure to ENMs have not yet been well characterized.

Current best-known methods for ENM exposure assessment in the workplace are based on using air sampling tools to characterize and quantify ENMs; these include real-time direct-reading instruments (DRIs) to measure airborne particle counts and filter-based methods collected in tandem for off-line direct visualization by transmission electron microscopy (TEM) and for elemental analysis.[1] The current methods for direct visualization of ENMs by TEM are based on those developed two decades ago for micron-sized asbestos[2,3] and are not appropriate for real-world ENM exposures. Further, TEM is low-throughput, expensive, and time- and resource-intensive. In addition, it must be coupled with another technique (e.g., energy dispersive x-ray spectroscopy [EDS]) for elemental identification, further contributing to the time- and cost-intensity of this BKM. Therefore, the overarching goal of this project is to rapidly advance exposure assessment for the nanotechnology workforce by developing and testing a new protocol for expedited filter-based sample analysis.

Hyperspectral imaging (HSI) and mapping is an established technique for large-scale applications,[5] such as remote sensing,[6] food safety,[7] and geology, and is now being used for the analysis of nanoscale materials.[8] Coupled with enhanced darkfield microscopy (EDFM), it has been used to identify ENMs in a variety of biological and environmental matrices[8] and shows great promise as a rapid screening tool for direct visualization of filter media from occupational exposure assessments. The CytoViva (CytoViva, Inc., Auburn, AL) EDFM-HSI system utilizes oblique angle illumination to enhance signal-to-noise of nanomaterials[9] and combines imaging and spectrophotometry using advanced optics and algorithms to capture a spectrum from 400nm-1000nm for each pixel in a hyperspectral image.[9,8,10–14] Using HSI software, it is possible to identify, locate, and map ENMs within a sample based on its unique spectral response. Spectral profiles for known ENMs are collected into reference spectral libraries (RSLs), which are used to identify those ENMs in other samples through a mapping algorithm.[10,11] Based on mapping results, an estimation of ENM concentration and size may be obtained.

Initial EDFM-HSI work was conducted in a collaboration between the National Institute for Occupational Safety and Health (NIOSH) and CytoViva, Inc. (Auburn, AL).[15] Mitsui-7 multi-walled carbon nanotubes (MWCNTs) were aerosolized onto mixed cellulose ester (MCE) filter media and analyzed by TEM and EDFM-HSI. The EDFM-HSI results were compared to the TEM results: an approximate linear relationship was found between MWCNT loading concentration and MWCNT count (R^2 = 0.9979), based on EDFM-HSI data, which corresponded with the linear relationship that resulted from electron microscopy (SEM: R^2 = 0.9848; TEM: R^2 = 0.9761).[15] Based on these initial results, EDFM-HSI is a promising method for the direct visualization and identification of NPs on filter media and is poised to serve as a rapid screening method that can be used to identify which filter samples, if any, truly need additional, more intensive analysis by electron microscopy.

2. METHODS

2.1 Sample generation

An aerosol of silicon dioxide NPs (silica, SiO_2; 10-20nm particle size; Sigma Aldrich, St. Louis, MO) was created via a Venturi aerosolization system (NIOSH DART; Cincinnati, OH; **Figure 1**).[16] Silica powder is placed into an exterior holding tube attached to the holding chamber. Air is pulled through the holding tube at a given volumetric flow rate (Q = 60L/min), resulting in a flow rate of approximately 70m/s. The aerosolized product in the chamber is then pulled through two different filter samples onto 37mm diameter MCE filters. One sample is pulled through a cyclone and the other through a closed-face cassette and collected simultaneously. Blank filter samples were collected using the Venturi system, but without introduction of product into the chamber.

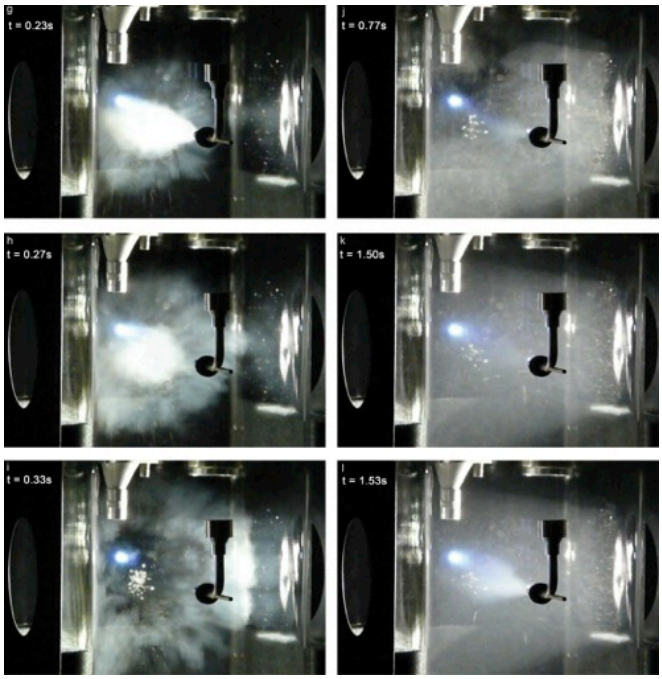

Figure 1. Sequential photographs of dispersion of silica nanomaterial powder in the Venturi aerosolization chamber (NIOSH DART; Cincinnati, OH).[16]

2.2 Sample preparation for microscopy

Exposed MCE filters were prepared for EDFM-HSI. A portion of the filter was cut and placed on a cleanroom-cleaned glass microscopy slide (NEXTERION). A cleanroom-cleaned glass coverslip (NEXTERION) was placed on top of the filter but was not adhered. Acetone (approx. 1mL) was pipetted in between the slide and coverslip to saturate and clear the filter portion. The coverslip was sealed with clear nail polish.

2.3 Enhanced darkfield microscopy (EDFM) and hyperspectral imaging (HSI)

Optical darkfield (DF) images and hyperspectral datacubes – images containing spatial and spectral data – were captured as previously described.[10] Briefly, DF images and hyperspectral datacubes were obtained with a 40x air objective, capturing 10 DF images and 10 corresponding datacubes per sample in areas where silica NPs were seen as bright spots against a dark background. DF images and hyperspectral datacubes were also collected for a filter blank. All datacubes were corrected for the spectra contributed by the light source. Following correction for the light source, all datacubes were spectrally subset from 450nm-725nm to remove noise at <450nm and >725nm.

2.4 Creation of reference spectral library (RSL)

A reference spectral library (RSL) was created for the silica NPs following the particle filtering method presented by Roth et al.[10] Briefly, spectra from pixels corresponding to silica NPs were collected into a preliminary spectral library based on their hyperspectral intensity. The preliminary spectral library was then filtered against two datacubes taken of the filter blank. This filtering process removes spectra from the preliminary spectral library that are duplicative of spectra found in the blank, thereby correcting for the filter and potential contamination. The resulting corrected spectral library is considered the RSL.

2.5 Hyperspectral mapping

Following the identification of silica NPs via EDFM and the capture of hyperspectral datacubes, each datacube was mapped against the RSL using the spectral angle mapper (SAM) algorithm in the HSI software (ENVI 4.8, Harris Geospatial Solutions).[10–13] The default SAM threshold (0.10 radians) was used. A mapped image is created to indicate the presence and location of silica NPs based on the SAM

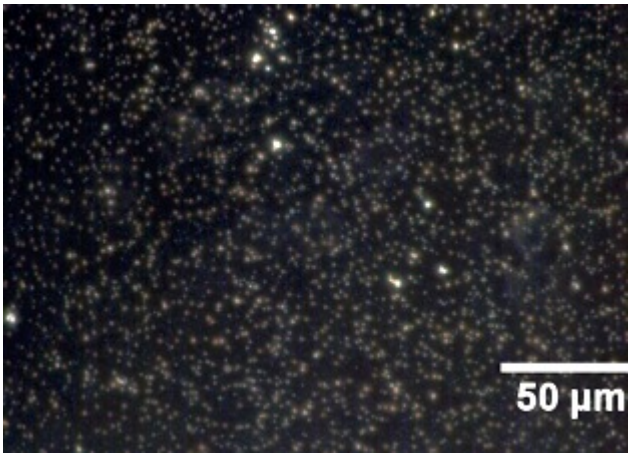

Figure 2. Hyperspectral datacube of silica NPs captured on MCE filter.

results. A false coloration overlay on the datacube indicates the pixels with spectra that match the RSL.

3. PRELIMINARY RESULTS

Sample preparation of filters for EDFM-HSI via saturation with acetone yields more consistently and more completely cleared filters and in less time than NIOSH Method 7400.[17] This method also improves the signal-to-noise with both EDFM and HSI, producing higher contrast DF images and hyperspectral datacubes. DF images and hyperspectral datacubes were captured from areas containing silica NPs. Silica NPs were easily visualized by EDFM as bright white spots on a dark black background (**Figure 2**). A RSL was created (**Figure 3**) and used to map to hyperspectral datacubes to identify locate silica NPs. Preliminary results indicate appropriate mapping of the RSL to silica NPs captured on MCE filter media (**Figure 4**).

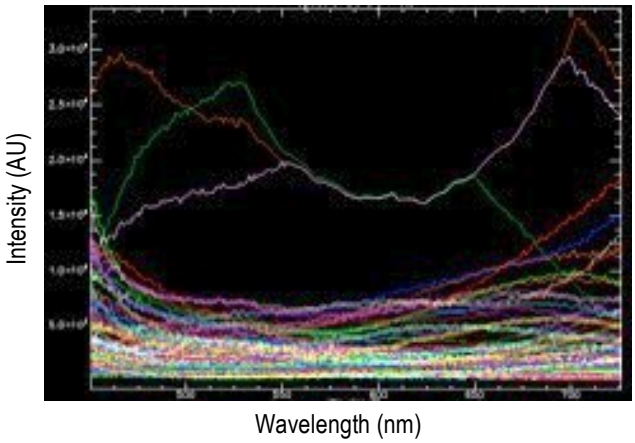

Figure 3. Reference spectral library (RSL) for silica NPs captured on MCE filter.

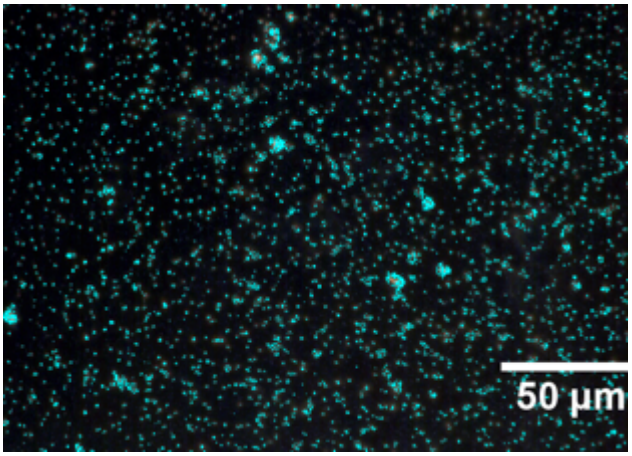

Figure 4. Hyperspectral datacube (seen in Fig. 1) mapped with silica NP RSL using SAM algorithm. Aqua false coloration overlay indicates pixels with spectra that match RSL spectra.

4. CONCLUSIONS

Results indicate that silica NPs can be easily visualized by EDFM and, moreover, can be specifically mapped using hyperspectral data. Sample preparation has been refined, with an improved, faster method presented here. Future directions include expanding the EDFM-HSI protocol to other filter media types (e.g., polycarbonate) and to other industrially relevant ENMs, including mixed material exposures from field sampling. Additionally, EDFM-HSI results will be compared to TEM data, the current gold standard for direct visualization of NPs on filter media. A method for semi- or relative quantitation of NP abundance is in progress. EDFM-HSI is poised to expedite analysis of NPs captured on filter media and identify only those samples that may warrant more intensive analysis by TEM. Furthermore, this method will allow for timely implementation of worker health and safety recommendations, if needed, as well as facilitate compliance with potential future occupational exposure limits (OELs) for nanomaterials.

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DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health (NIOSH). The instrumentation used in this report does not constitute endorsement by NIOSH. The authors declare no conflict of interest.

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