Update on Evaluation of Enhanced Darkfield Microscopy and Hyperspectral Mapping for Analysis of Airborne Nanoparticulate Collected on Filter-Based Media

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ABSTRACT

Current best-known methods for engineered nanomaterial (ENM) exposure assessment in occupational environments include the capture of airborne ENMs onto filter media. The current standard method for the analysis of filter media is direct visualization via transmission electron microscopy (TEM) for particle sizing, count, and morphology, often coupled with energy-dispersive x-ray spectroscopy (EDS) for compositional analysis. This method is low-throughput, expensive, and time- and resource-intensive. Enhanced darkfield microscopy (EDFM) with hyperspectral imaging (HSI) and mapping analysis is being evaluated as a high-throughput screening technique to rapidly identify ENMs of interest on filter media samples. We are exploing use of EDFM-HSI for the detection, characterization, and quantitation of ENMs caputred on filters with the intent to develop a standardized method and analytical protocols.

Keywords: mixed cellulose ester (MCE), classification, spectral angle mapper, reference spectral library, nanomaterials

1 INTRODUCTION

Workers who synthesize, handle, and/or dispose of engineered nanomaterials (ENMs) are the first to be potentially exposed to these materials by inhalation and/or through the skin. As such, it is critical to monitor their work environments for potential ENM emissions to which they may be exposed. Currently, exposure assessment methodology for ENMs includes capture of airborne ENMs onto filter media [1–3]. The standard method for the analysis of filter media is offline direct visualization via transmission electron microscopy (TEM) for particle sizing, count, and morphology, often coupled with compositional analysis, typically by energy-dispersive x-ray spectroscopy (EDS). TEM-EDS is low-throughput, expensive, and time- and resource-intensive. Guidance for the analysis of carbon nanotubes and fibers has recently been published, but this may not apply to all ENMs [4]. The current TEM-EDS methods for non-carbon nanotubes and/or fibers collected on filter media are based on methods for micron-sized asbestos [5,6], which may not be appropriate for analysis of ENMs. These limitations will hinder the timely compliance with any potential occupational exposure limits (OELs), thereby delaying the implementation of any necessary health and safety measures to protect worker health. A new rapid screening method is therefore urgently needed.

Enhanced darkfield microscopy (EDFM) with hyperspectral imaging (HSI) and mapping analysis have emerged as non-destructive methods for the direct visualization and analysis of ENMs in a variety of sample matrices [7–13]. It is now also being evaluated as a high-throughput screening technique to rapidly identify ENMs of interest on filter media samples from occupational exposure assessments. The CytoViva (CytoViva, Inc., Auburn, AL) EDFM-HSI system combines optical darkfield (DF) imaging and spectrophotometry to visualize and identify ENMs by capturing a spectrum from 400nm-1000nm at each pixel in a hyperspectral image [7,10,14]. ENMs can by identified by a mapping algorithm that compares the spectra from each pixel in a hyperspectral image to spectra from pixels known to correspond to the ENM of interest [7].

Initial work using silicon dioxide (silica; SiO_2) and titanium dioxide (TiO_2) ENMs captured on mixed cellulose ester (MCE) filter media have provided promising results. Here, we provide an update on ongoing work investigating the utility of EDFM-HSI for the rapid identification of silica and TiO_2 ENMs captured on MCE filter media.

2 MATERIALS & METHODS

2.1 Sample generation

MCE filters were exposed to ENMs. An aerosol of TiO_2 (mean diameter 21nm; AEROXIDE P 25, Fisher Scientific, Fair Lawn, NJ) was created via an aerosol generation system (NIOSH HELD, Morgantown, WV) using Leland pumps to create samples at a goal flow rate of 4LPM with loading concentrations of 32.8mg/m^3 and 19.4mg/m^3. TiO_2 exposure concentrations were chosen to reflect a high and moderate concentration from a larger sample set. An aerosol of silica NPs (10-20nm particle size; Sigma Aldrich, St. Louis, MO) was created via a Venturi aerosolization system (NIOSH DART; Cincinnati, OH) [15]. 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. Filters were exposed to 3.0mg/m^3, 2.0mg/m^3, or 1.5mg/m^3 silica NPs. Filter blanks for both materials and both exposure methods were prepared using filtered air only. Silica exposure concentrations were selected to reflect a low, moderate, and high concentration from a larger sample set.

2.2 Sample preparation for microscopy

Exposed MCE filters were prepared for EDFM-HSI. A portion of the filter (approximately 20-25% of the filter) was cut and placed on a cleanroom-cleaned glass microscopy slide (NEXTERION, SCHOTT North America, Inc., Tempe, AZ). A cleanroom-cleaned glass coverslip (NEXTERION) was placed on top of the filter but not adhered. Acetone (approximately 500µL) was pipetted in between the slide and coverslip to saturate and clear the filter portion. The coverslip was then sealed with clear nail polish. Cleanroom-cleaned glass microscopy slides and coverslips were used to minimize contamination of the filter samples.

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

The CytoViva EDFM-HSI system captures a spectrum (400nm-1000nm) from each pixel in a hyperspectral image with 2nm spectral resolution [7]. Optical DF images and hyperspectral images – also called datacubes – were collected as described previously [10,11,14,16,17]. Briefly, DF images and hyperspectral datacubes were acquired with a CytoViva EDFM-HSI system (CytoViva, Inc., Auburn, AL) mounted on an Olympus BX-43 microscope with an EDFM condenser. Images were obtained at 40x magnification using a DAGE optical camera system for DF images and a Pixelfly camera system for HSI that captures spectral data per pixel in the VNIR region. Ten DF images and 10 corresponding datacubes were captured per sample. Pixel resolution at 40x magnification with low spatial resolution is approximately 323nm x 323nm. Resolution was sacrificed in favor of increased area within the field of view (FOV). Images were captured randomly within the filter area for statistical purposes. ENMs were visible with EDFM as bright structures. DF images and hyperspectral datacubes were also collected for filter blanks. All datacubes were corrected for the spectra contributed by the microscope's light source. Following correction for the light source, all datacubes were spectrally subset from 450nm-725nm to remove noise at <450nm and >725nm for use with the mapping classification algorithm. All other classification models were used on lamp-corrected datacubes that were not spectrally subset.

2.4 Creation of reference spectral library (RSL)

A reference spectral library (RSL) was created for each ENM using the particle filtering method presented by Roth et al [14]. Briefly, spectra from pixels corresponding to the ENMs of interest were collected into preliminary spectral libraries (SLs) bsaed on their hyperspectral intensities, which were at least twice that of the background. The preliminary SLs were each then filtered against two datacubes captured of a filter blank. This filtering process removes spectra from the preliminary SLs that are duplicative of spectra found in the filter blanks, thereby correcting for the filter and potential contamination. The resulting corrected SLs are considered the RSLs and are used for pixel classification.

2.5 Pixel classification using the spectral angle mapper (SAM) algorithm

Following RSL creation and the capture of datacubes, each corrected datacube was mapped against its corresponding RSL using the spectral angle mapper (SAM) algorithm in the HSI software (ENVI 4.8, Harris Geospatial Solutions) [10,14,16,17]. The default SAM threshold (0.10 radians) was used. SAM compares the spectrum of each pixel to all the spectra in the RSL. A mapped image is created with a false coloration overlay that classifies pixels as ENM(+) or ENM(-), indicating the presence and location of the ENM of interest in the sample based on the SAM results.

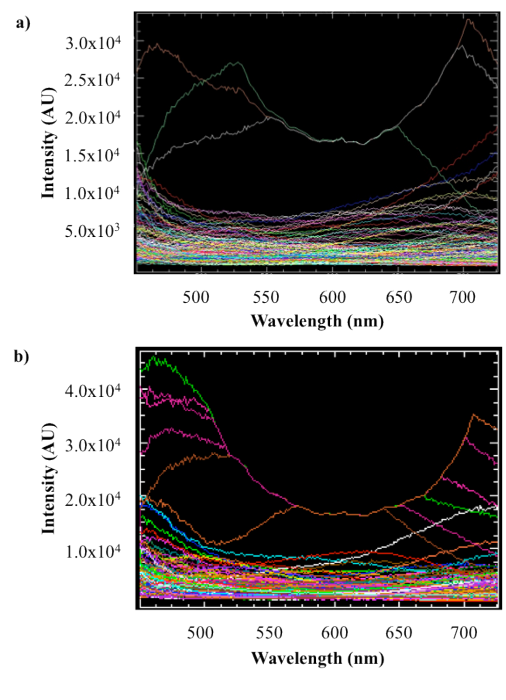

Figure 1: RSLs for pixel classification. a) silica; b) TiO_2.

3 PRELIMINARY RESULTS

RSLs were created for silica ENMs and for TiO_2 ENMs (Figure 1) from spectra collected from pixels corresponding to ENMs in positive control datacubes. The RSLs were used to map hyperspectral images to classify image pixels as ENM(+) or ENM(-), thereby locating and identifying the ENMs in the filter samples.

Silica ENMs were readily apparent by direct visualization with EDFM (Figure 2) at all three loading concentrations (1.5mg/m^3, 2.0mg/m^3, and 3.0mg/m^3). The RSL created from silica(+) pixels in a hyperspectral image of a 3.0mg/m^3 positive control sample was used to classify pixels as silica(+) or silica(-) in hyperspectral images, indicated in Figure 2 as an aqua false colortion overlay (bottom row).

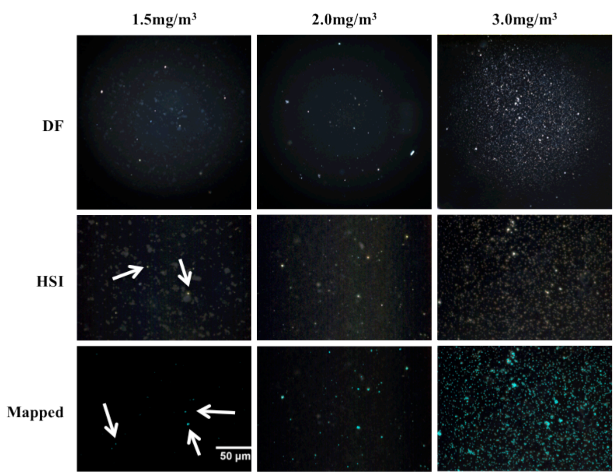

Figure 2: Silica ENMs on MCE filters. Top: DF; middle: HSI; bottom: map showing silica(+) in aqua.

TiO_2 ENMs were also easily visualized with EDFM (Figure 3) at both loading concentrations (19.4mg/m^3 and 32.8mg/m^3). The RSL created from TiO_2(+) pixels in a hyperspectral image of a 32.8mg/m^3 positive control sample was used to classify pixels as TiO_2(+) or TiO_2(-) in hyperspectral images, indicated in Figure 3 as a green false coloration overlay (bottom row).

4 DISCUSSION & CONCLUSIONS

This work continues initial work evaluating EDFM-HSI for the identification of silica ENMs captured on MCE filters by expanding the sample set to include additional samples at lower loading concentrations. Additionally, we report here preliminary work evaluating this technique for the identification of TiO_2 ENMs captured on MCE filters. Work to date has demonstrated that EDFM-HSI can easily visualize silica and TiO_2 ENMs captured on MCE filters and, moreover, hyperspectral data can be used to classify ENM(+) and ENM(-) pixels for these materials of interest. Imaging and analysis of additional filters at other exposure concentrations is ongoing, as is electron microscopy on selected samples to compare to EDFM-HSI data. We will also determine the limit of detection (LOD) for these materials using this EDFM-HSI system.

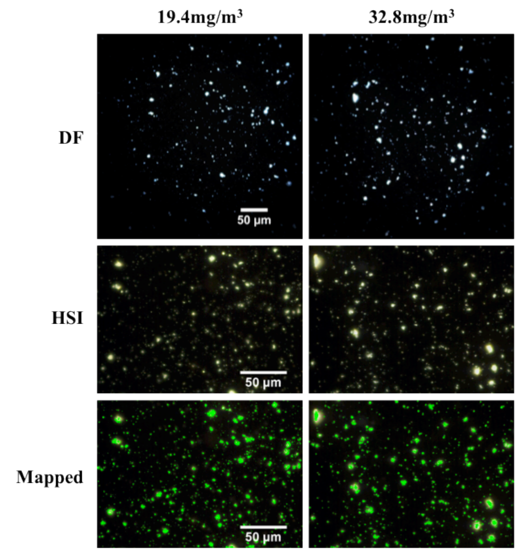

Figure 3: TiO_2 ENMs on MCE filters. Top: DF; middle: HSI; bottom: map showing TiO_2(+) in green.

EDFM-HSI shows promise for the rapid screening of ENMs captured on filter media. EDFM provides high signal-to-noise optical images, facilitating direct visualization. HSI provides spectral data that can be used to identify and characterize ENMs. This data can be further exploited: a comparison of classification algorithms using hyperspectral data is underway for multiwalled carbon nanotubes (MWCNTs) captured on MCE media.

There is need for additional evaluation tools when performing comprehensive exposure assessments of facilities that are handling emergent materials, such as ENMs, especially those that emphasize high-throughput, timely analysis. These evaluation tools must also be less expensive than the status quo. Analysis of the EDFM-HSI data obtained in this study will provide much needed insight into the potential future use of this method to determine the count, identification, and concentration of ENMs on field-collected filter-based samples. Future directions include expanding the EDFM-HSI protocol to additional filter media (i.e., polycarbonate) and to other ENMs, including real world mixed-material exposures from field sampling.

ACKNOWLEDGMENTS

The authors thank CytoViva, Inc. (Auburn, AL) for technical support and guidance. This work was funded by a CDC-NIOSH contract to N. Neu-Baker.

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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