Received: 18 April 2018 | Revised: 10 December 2018 | Accepted: 20 January 2019

DOI: 10.1002/jemt.23231

MICROSCOPY RESEARCH & TECHNIQUE WILEY

RESEARCH ARTICLE

Sample preparation method for visualization of nanoparticulate captured on mixed cellulose ester filter media by enhanced darkfield microscopy and hyperspectral imaging

Nicole M. Neu-Baker[1] | Adrienne C. Eastlake[2] | Sara A. Brenner[1]

[1]College of Nanoscale Science, Nanobioscience Constellation, State University of New York (SUNY) Polytechnic Institute, College of Nanoscale Science, New York

[2]Education and Information Division (EID), National Institute for Occupational Safety and Health (NIOSH), Cincinnati, Ohio

Correspondence
Nicole M. Neu-Baker MPH, State University of New York (SUNY) Polytechnic Institute, College of Nanoscale Science, Nanobioscience Constellation, 257 Fuller Road, Albany, NY 12203.
Email: nneu@sunypoly.edu

Review Editor: Alberto Diaspro

Funding information
National Institute for Occupational Safety and Health

Abstract

A significant hurdle in conducting effective health and safety hazard analysis and risk assessment for the nanotechnology workforce is the lack of a rapid method for the direct visualization and analysis of filter media used to sample nanomaterials from work environments that represent potential worker exposure. Current best-known methods include transmission electron microscopy (TEM) coupled with energy dispersive x-ray spectroscopy (EDS) for elemental identification. TEM-EDS is considerably time-, cost-, and resource-intensive, which may prevent timely health and safety recommendations and corrective actions. A rapid screening method is currently being explored using enhanced darkfield microscopy with hyperspectral imaging (EDFM-HSI). For this approach to be effective, rapid, and easy, sample preparation that is amenable to the analytical technique is needed. Here, we compare the sample preparation steps for mixed cellulose ester (MCE) filter media specified in NIOSH Method 7400—Asbestos and Other Fibers by Phase Contrast Microscopy (PCM)—against a new method, which involves saturation of the filter media with acetone. NIOSH Method 7400 was chosen as a starting point since it is an established technique for preparing transparent MCE filters for optical microscopy. Limitations in this method led to the development and comparison of a new method. The new method was faster, easier, and rendered filters more transparent, resulting in improved visualization and analysis of nanomaterials via EDFM-HSI. This new method is suitable for a rapid screening protocol due to its speed, ease of use, and the improvement in image acquisition and analysis.

KEYWORDS

acetone vaporization, engineered nanomaterial, exposure assessment, phase contrast microscopy

1 | INTRODUCTION

Hyperspectral imaging (HSI) has historically been used for geospatial and other large-scale analyses (Roth, Tahiliani, Neu-Baker, & Brenner, 2015; van der Meer et al., 2012). More recently, the technology has been applied to the analysis of nanoscale materials (Anderson et al., 2015; Badireddy, Budarz, Chellam, & Wiesner, 2012; Badireddy, Wiesner, & Liu, 2012; Dillon, Bezerra, Sosa Peña, Neu-Baker, & Brenner, 2017; Idelchik et al., 2016; More & Vince, 2015; Mortimer et al., 2014; Roth, Sosa Peña, Neu-Baker, Tahiliani, & Brenner, 2015; Roth, Tahiliani, et al., 2015; Shannahan, Sowrirajan, Persaud, Podila, & Brown, 2015; Sosa Peña et al., 2016; von der Kammer et al., 2012). The CytoViva (Auburn, AL) enhanced darkfield microscopy (EDFM) and hyperspectral imaging (HSI) system is increasingly used for analysis of nanoscale materials in a variety of biological and environmental

Abbreviations: DF, darkfield; EDFM, enhanced darkfield microscopy; EDS, energy-dispersive x-ray spectroscopy; FOV, field of view; HSI, hyperspectral imaging; MCE, mixed cellulose ester; MWCNT, multiwalled carbon nanotubes; NP, nanoparticle; PCM, phase contrast microscopy; REL, recommended exposure limit; TEM, transmission electron microscopy.

matrices (Anderson et al., 2015; Badireddy, Budarz, et al., 2012; Badireddy, Wiesner, & Liu, 2012; Dillon et al., 2017; Idelchik et al., 2016; Mercer et al., 2011; Mercer et al., 2013; More & Vince, 2015; Mortimer et al., 2014; Roth, Sosa Peña, et al., 2015; Roth, Tahiliani, et al., 2015; Shannahan et al., 2015; Sosa Peña et al., 2016; von der Kammer et al., 2012). Spectral data collected from pixels in a hyperspectral image may be used to identify materials of interest (Badireddy, Wiesner, & Liu, 2012; Husain et al., 2015; Idelchik et al., 2016; Idelchik et al., 2018; Mortimer et al., 2014; Sosa Peña et al., 2016), which cannot be achieved with transmission electron microscopy (TEM) or phase contrast microscopy (PCM) alone. Additionally, this modality is amenable to nanoparticle (NP) visualization and analysis since it easily detects NPs on a dark background from light scattered by the particles. Since NPs have dimensions that are less than the wavelength of visible light (Stark, 2011) with closely packed atoms, there is greater light scattering. The CytoViva enhanced dark-field microscopy with hyperspectral imaging (EDFM-HSI) system detects scattered light in the sample and, thus, NPs stand out from the surrounding matrix with high contrast. However, EDFM-HSI examination of NPs captured on filter media poses a problem due to the scattering of light by the filter itself. This significantly reduces the contrast between NPs and filter, thereby affecting the ability to detect and analyze NPs in the filter section. The methods described here considerably reduce the light scattering of the filter media, enabling the detection of NPs by EDFM-HSI. As such, this modality shows promise as a rapid screening tool for occupational exposure samples of airborne nanoparticulate collected on filter media.

Current best-known methods for analysis of filter media from exposure assessments include off-line direct visualization by transmission electron microscopy (TEM) (CDC-NIOSH, 1994a), often coupled with energy-dispersive x-ray spectroscopy (EDS) for compositional analysis. Since TEM-EDS is costly and time-intensive, and since the analytical methods for TEM-EDS of filter-captured particulate were developed using informal and qualitative approaches for nanomaterials, there is an urgent need for a rapid screening protocol for the direct visualization and identification of NPs. While EDFM-HSI cannot replace TEM-EDS, it is poised to fill the role of a rapid screening tool due to its ability to quickly visualize and identify NPs captured on filter media and identify which samples might truly benefit from more intensive analysis by TEM-EDS. If TEM-EDS continues to be used as the gold standard for direct visualization of NPs from occupational exposure assessments, the high volume of samples expected will cripple any attempts to make timely health and safety recommendations, whether for an individual company or industry or for the entire nanotechnology workforce. This new analytical protocol will therefore expedite time-to-knowledge.

An effective rapid screening protocol must also include a rapid sample preparation method. NIOSH Method 7400—Asbestos and Other Fibers by PCM (CDC-NIOSH, 1994b)—was identified as a potential method for preparing filters for EDFM-HSI since it is an established, published method for making mixed cellulose ester (MCE) filters transparent for optical microscopy. However, while NIOSH Method 7400 is appropriate for clearing filter media for PCM analysis, it was determined that it is not ideal for clearing MCE filter media for EDFM-HSI since it does not effectively render the filter transparent. Instead, it leaves an opaque background that interferes with the visualization and analysis of NPs and particularly of less reflective NPs, such as silica, where the hyperspectral data associated with the silica NPs is similar to that of the background. As such, a new sample preparation method for clearing MCE filters is needed.

Here, we compare the filter sample preparation technique described in NIOSH Method 7400 (CDC-NIOSH, 1994b) to a new method that uses acetone to saturate the MCE filter media, with particular attention paid to: (a) ease of use, (b) speed of preparation, and (c) quality of HSI images and data. This new acetone saturation method expands on work previously presented (Beach, 2012).

2 | MATERIALS AND METHODS

2.1 | MCE filter media exposed to silica NPs

An aerosol of silica NPs (Sigma Aldrich, St. Louis, MO; 10–20 nm in diameter) was created via a Venturi aerosolization system (NIOSH DART; Cincinnati, OH) (Evans, Turkevich, Roettgers, Deye, & Baron, 2013). This is a reproducible method that produces samples that resemble high-energy particle dispersal in the workplace. Nanoscale silica powder was placed into an exterior holding tube that is attached to the holding chamber. Air is pulled through the holding tube at a given volumetric flow rate (Q = 60 L/min), resulting in a flow rate of approximately 70 m/s. The aerosolized product in the chamber is then pulled through two different filter samples onto 37 mm-diameter filters. One sample is the respirable fraction pulled through a size-selective GK2.69 cyclone (BGI Mesa Labs, Butler, NJ), operating at flow rate of 4.2 L/min and having a 50% cut point of 4.5 mm. The other total inhalable particle sample is collected at a flow rate of 2 L/min through a closed-face cassette with no size classification. Duplicate filter samples (n = 8) were created by introducing 3.0 mg silica NP into the Venturi chamber. Filter blank samples (n = 2) were collected using the Venturi system, but without introduction of product into the chamber.

2.2 | MCE filter media exposed to multiwalled carbon nanotubes

An aerosol of Nanocyl NC 70000 multiwalled carbon nanotubes (MWCNTs) (Nanocyl SA, Belgium; average diameter of 9.5 nm; average length of 1.5 μm) was created via an aerosol generation system (NIOSH HELD; Morgantown, WV). This system is capable of sustaining an aerosol concentration of 0.5 mg/m^3. Leland pumps were used to create MCE filter samples at a goal flow rate of 4 L/min. Loading concentrations included 17.91 mg/m^3 [equivalent to 8 hr time-weighted average (TWA) of 3.7303 μg/m^3); 3.07 mg/m^3 (equivalent to 8 hr TWA of 1.279 μg/m^3); and a filter blank exposed to filtered air only.

2.3 | Sample preparation using NIOSH method 7400: Asbestos and other fibers by PCM

The objective of the NIOSH Method 7400 is to prepare a transparent MCE filter media sample by collapsing the filter using acetone vapor and triacetin (CDC-NIOSH, 1994b). A wedge (approximately 20–25%) of the MCE filter is cut with a clean scalpel and placed onto a cleanroom-cleaned glass microscopy slide (NEXTERION, SCHOTT North America, Inc., Tempe, AZ). A Small Wonder Acetone Vaporizer (Wonder Makers

Environmental, Inc., Kalamazoo, MI), or "hot block," was warmed to approximately 70°C, the optimal temperature for this method. The glass microscopy slide with the filter portion is then inserted into the chamber. Approximately 250 ml acetone is slowly pipetted into the inlet of the hot block. The pipet and slide are removed after 3–5 s, at which point the filter is expected to be cleared. Triacetin (3–3.5 μl) is immediately pipetted onto the filter. The filter is then coverslipped with cleanroom-cleaned coverslip (NEXTERION) and sealed with clear nail polish. A total of 16 filters (nine exposed to silica, four exposed to MWCNTs, three blanks) were prepared by this method.

2.4 | Sample preparation using new method designed for enhanced darkfield microscopy with hyperspectral imaging

A wedge (approximately 20–25%) of the MCE filter is cut with a clean scalpel and placed onto a cleanroom-cleaned glass microscopy slide (NEXTERION, SCHOTT North America, Inc., Tempe, AZ). A cleanroom-cleaned glass coverslip (NEXTERION) is placed on top of the filter but not adhered. Using forceps, the coverslip is held in place while approximately 500 μl acetone is pipetted in between the coverslip and slide to completely saturate the filter until the filter becomes transparent (Figure 1). It is necessary to sandwich the filter between the coverslip and slide so that the filter remains flat against the slide and does not curl up or wrinkle when saturated with acetone. If bubbles appear, forceps may be used to gently press down on the coverslip to dislodge the bubbles, taking care not to laterally move the coverslip, which may also disrupt the filter. The coverslip is then sealed with clear nail polish. Liquid acetone should be present under the coverslip when it is sealed. A total of 16 filters (nine exposed to silica, four exposed to MWCNTs, three blanks) were prepared by this method.

2.5 | Imaging by EDFM-HSI

Filter samples were imaged by EDFM as previously described (Dillon et al., 2017; Guttenberg et al., 2016; Idelchik et al., 2016; Roth, Sosa Peña, et al., 2015; Sosa Peña et al., 2016). Briefly, filter samples were visualized with a CytoViva EDFM-HSI system (CytoViva, Inc., Auburn, AL) mounted on an Olympus BX-43 microscope with an EDFM condenser. The source (Fiber-Lite DC-950, 150 W quartz halogen light source with 0–100% intensity control; Dolan-Jenner Industries, Boxborough, MA) brightness was kept constant at 75% power for all image acquisition. Darkfield (DF) images were captured with an optical camera (DAGE-MTI, Michigan City, IN) mounted to the microscope. DF images were captured at 40x magnification to optimize the number of nanostructures in a given field of view (FOV). For each DF image captured, a hyperspectral image was also captured as previously described (Dillon et al., 2017; Idelchik et al., 2016; Roth, Sosa Peña, et al., 2015; Sosa Peña et al., 2016) Briefly, hyperspectral images were collected using a Pixelfly hyperspectral camera that captures a spectrum from 400 nm to 1,000 nm in each pixel. Hyperspectral images (501 lines) were captured at 40x to correspond with the 40x magnification DF images. Exposure times for hyperspectral image acquisition ranged from 0.2 s/line for samples prepared with the new method to over 1.5 s/line for samples prepared by NIOSH 7400.

3 | RESULTS

3.1 | Filter clearing by each method

Both sample preparation methods were tested on MCE filters exposed to silica NPs. NIOSH Method 7400 was slower to execute, ranging from approximately 10–15 min for the first sample and approximately 3 min per subsequent sample. However, it often took upwards of 30 min if the vaporization chamber exceeded the optimal working temperature of 70°C and needed to cool down. Typically, only three samples could be prepared before needing to wait for the chamber to cool down to the optimal working temperature. The new method of liquid acetone saturation was much faster, taking a total of approximately 1–2 min per sample.

3.2 | Direct visualization and hyperspectral analysis of filters via EDFM-HSI

Filters prepared by either NIOSH Method 7400 or the new method were visualized by EDFM-HSI at 40x magnification. After sample

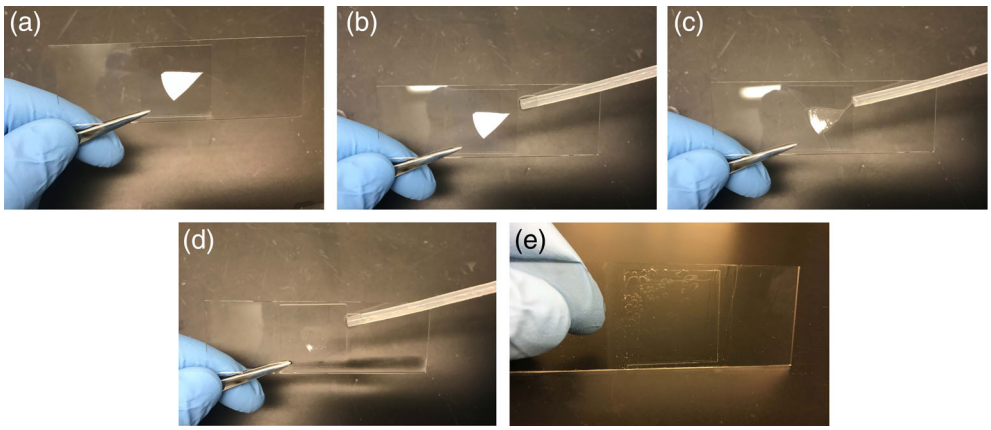

FIGURE 1 **New sample preparation method.** (a) A portion of an MCE filter exposed to silica NPs was cut with a clean scalpel, placed onto a cleanroom-cleaned glass microscopy slide with a cleanroom-cleaned glass coverslip placed on top, but not adhered. Forceps are used to gently hold the filter and coverslip in place to prevent the filter from curling. (b–d) Acetone (approximately 500 μl) is pipetted in between the slide and coverslip to saturate and clear the filter. (e) When the filter is completely saturated and cleared, the coverslip is sealed with clear nail polish. Photo credit: Ana Segarceanu (SUNY Polytechnic institute) [Color figure can be viewed at wileyonlinelibrary.com]

preparation, filters appeared transparent to the naked eye, regardless of which sample preparation was employed. However, on visualization by EDFM, it was apparent that NIOSH Method 7400 did not completely render the filter transparent; an opaque filter background was visible, which interferes with the visualization and analysis of less reflective materials, like silica. The new method of liquid acetone saturation cleared the filter to a greater extent than NIOSH Method 7400: there was no opaque filter background remaining in these samples, which facilitated visualization by EDFM (Figure 1).

Hyperspectral images of filters prepared via NIOSH Method 7400 showed low contrast between the silica NPs and the filter background, which remained somewhat opaque (Figure 2). This low contrast translated into low signal-to-noise: the overall light intensity was higher due to background scatter, but this rise in background light reduced the signal-to-noise of spectral features tied to NPs. Here, the spectra for the nanostructures were approximately 1,700 arbitrary units (AU) and the spectra for the background pixels were approximately 1,200 AU. Generally, background is considered to have spectral intensity of approximately 1,000 AU or less. Spectral intensities of 2,000 AU or greater, or intensities that are at least twice that of background, are ideal for pixels corresponding to NPs. To acquire a similar signal-to-noise to the new method using NIOSH 7400, the acquisition time per line must be increased by upwards of 7.5 times. This was possible for some samples prepared via NIOSH Method 7400, but this added a considerable amount of time to the overall imaging process and did not always yield improved results. Furthermore, any silica NP that was embedded deeper into the filter was more difficult to visualize via HSI due to the low contrast between the NPs and the filter. Conversely, when samples were prepared by the new method, there was increased contrast between the silica NPs (11,000 AU) and the background (200 AU; Figure 2) and the exposure time could remain low (~0.2 s), expediting analysis.

4 | DISCUSSION

The new sample preparation method proposed and described here is simple, fast, and generates better EDFM-HSI images and spectral data when compared to NIOSH Method 7400. This new method does not require specialized equipment, reagents, or extensive training, making

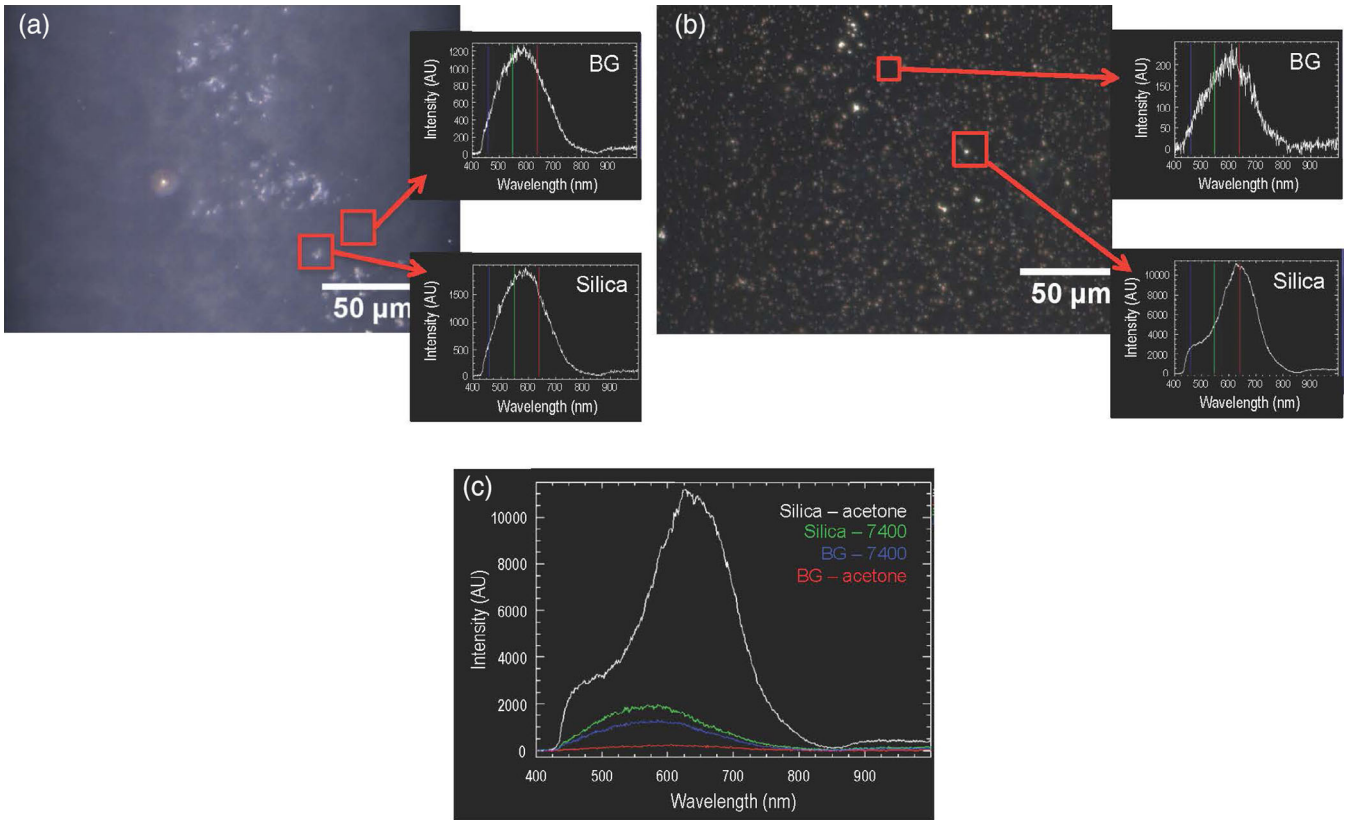

FIGURE 2 **Hyperspectral images of MCE filters prepared by each method.** A filter sample was prepared by each method. (a) MCE filter exposed to silica NPs prepared by NIOSH method 7400. Note the low contrast between the NPs (bright white spots) and background. (b) MCE filter exposed to silica NPs prepared by the new method of acetone saturation. Note the high contrast between the NPs (bright white spots) and background. Both images were captured at 40x magnification with exposure times of approximately 0.2 s/line. (c) Spectra from background (BG) pixels and silica NP pixels are shown: The maximum spectral intensity of the BG pixel prepared by NIOSH method 7400 is approximately 1,200 arbitrary units (AU) with noise, which is typical of background (blue spectrum); the maximum spectral intensity of the silica NP pixel prepared by NIOSH method 7400 in (a) is approximately 1,700 AU (green spectrum). Conversely, the maximum spectral intensity of the BG pixel from the filter prepared by the new method in (b) is approximately 200 AU with noise, which is typical of background, while the maximum spectral intensity of the silica NP pixel from the filter prepared by the new method is approximately 11,000 AU [Color figure can be viewed at wileyonlinelibrary.com]

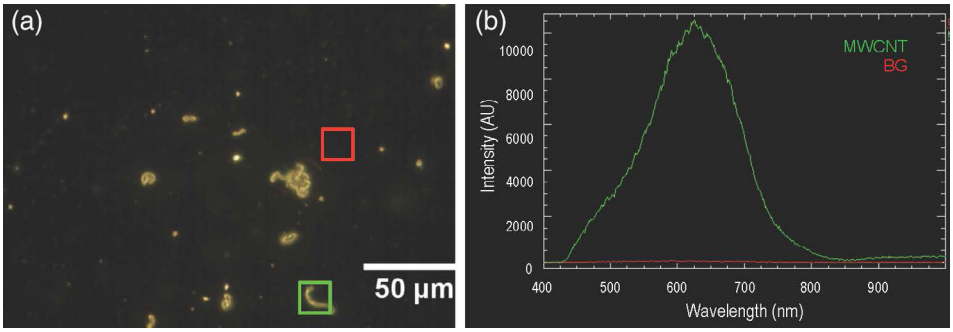

FIGURE 3 **Hyperspectral image of MWCNTs on MCE filter prepared with the new method.** An MCE filter was exposed to MWCNTs prepared by the new method presented here. (a) Note the high contrast between the MWCNTs (bright fiber structures) and black background. HSI image was captured at 40× magnification with exposure time of approximately 0.2 s/line. (b) Spectra from a background pixel (BG) and a MWCNT pixel are shown: The maximum spectral intensity of the BG pixel (red spectrum, corresponding to a pixel within the red box in the image) is approximately 80 AU with noise, which is typical of background; the maximum spectral intensity of the MWCNT pixel (green spectrum, corresponding to a MWCNT+ pixel within the green box in the image) is approximately 10,000 AU [Color figure can be viewed at wileyonlinelibrary.com]

it an ideal sample preparation technique for a rapid screening protocol. Acetone is a standard reagent in most research and commercial laboratories. Other glass microscopy slides and coverslips could potentially be used instead of the cleanroom-cleaned slides and coverslips (Mercer et al., 2017; Roth, Sosa Peña, et al., 2015) used here only if they have been shown to be free of contaminants, like dust or other particulates, that may interfere with analysis. In future work, various cleaning and decontamination methods will be assessed for their ability to appropriately remove particulates from microscopy slides. Additionally, while this new sample preparation method seems to provide better contrast without artifacts compared to traditional MCE filter preparation per NIOSH Method 7400, further work is needed to ensure that particulate is not lost using this technique. This method is also not NP-specific and can therefore be used for any MCE sample that has been used to collect nanoparticulate, from either lab-generated samples, as shown here, or from field sampling. The researchers are currently utilizing this sample preparation method for EDFM-HSI analysis of other ENMs on MCE filter media, including MWCNTs. To illustrate the applicability of the method for other NPs captured on MCE filters, Figure 3 shows MWCNTs on MCE filter media prepared using the new method.

Sandwiching the filter between the slide and coverslip and holding it with forceps while pipetting acetone in between is critical so that the filter does not curl or fold. In developing this sample preparation method, attempts were made to pipette a drop of acetone directly onto the filter, place the filter on top of a drop of acetone on a slide, and to pipette acetone in between the coverslip and slide without holding with forceps, but these methods all resulted in filters that would instantaneously fold, wrinkle, or curl, which would impede EDFM-HSI data acquisition. Clamping the coverslip to the slide may be done using a small clamping device instead of forceps may be appropriate to maintain consistent pressure; however, care must be taken not to exert too much pressure that would break the coverslip and/or prevent the flow of acetone. This is an area for further exploration.

As EDFM-HSI is increasingly utilized for nanoscale analysis of biological and environmental samples, it is therefore more accessible to the research community for other applications, such as for analysis of filter samples of airborne nanoparticulate collected for occupational exposure assessments. As such, commercial and research laboratories that utilize EDFM-HSI will also benefit from this new sample preparation method, since it is not only faster and easier than NIOSH Method 7400, but will also save money, in terms of cost of reagents and equipment as well as in person-hours. As EDFM-HSI is increasingly adopted—particularly for occupational exposure assessment applications—its advantages, coupled with ease of sample preparation, will yield more occupational exposure data that will be invaluable to the research community and will enable companies and industries to make informed decisions regarding protection of worker health.

5 | CONCLUSIONS

This new sample preparation method for visualization of NPs on MCE filters using EDFM-HSI is inexpensive, rapid, simple, and clears the filter more completely than NIOSH Method 7400, yielding images with increased signal relative to background, thereby improving HSI data quality. Further work is needed to ensure that particulate is not lost using this technique versus the NIOSH Method 7400 and to compare, in detail, HSI to TEM-EDS regarding specific fiber sizes for a given material. The current TEM-EDS methods for direct visualization of particulate captured on filter media from occupational exposure assessments present a significant hurdle for companies and industries to comply with current or future recommended exposure limits (RELs) or safety recommendations regarding potential exposure to ENMs. A new, rapid screening protocol is, therefore, critical to advance the state of the science. A simple, cost-, and time-effective sample preparation method must be incorporated into any new analytical protocols to expedite safety recommendations for the nanotechnology workforce and protect worker health.

ACKNOWLEDGMENTS

This research was financially supported by the National Institute for Occupational Safety and Health (NIOSH). The authors thank James

Beach, PhD and Jamie Uertz (CytoViva, Inc., Auburn, AL) for technical support and guidance. In addition, the authors are grateful to Charles Geraci, PhD, CIH (NIOSH); Laura Hodson, MSPH, CIH (NIOSH); Gary Roth, PhD (NIOSH); Robert Mercer, PhD (NIOSH); Alan Dozier, PhD (NIOSH); James Beach, PhD (CytoViva, Inc.); and Alan Segrave, PG (Maxxam Analytics) for their review of this manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

ORCID

Nicole M. Neu-Baker https://orcid.org/0000-0002-6820-8954

REFERENCES

Anderson, D. S., Patchin, E. S., Silva, R. M., Uyeminami, D. L., Sharmah, A., Guo, T., … Van Winkle, L. S. (2015). Influence of particle size on persistence and clearance of aerosolized silver nanoparticles in the rat lung. *Toxicological Sciences*, *144*, 366–381.

Badireddy, A. R., Budarz, J. F., Chellam, S., & Wiesner, M. R. (2012). Bacteriophage inactivation by UV-A illuminated fullerenes: Role of nanoparticle-virus association and biological targets. *Environmental Science & Technology*, *46*, 5963–5970.

Badireddy, A. R., Wiesner, M. R., & Liu, J. (2012). Detection, characterization, and abundance of engineered nanoparticles in complex waters by hyperspectral imagery with enhanced darkfield microscopy. *Environmental Science & Technology*, *46*, 10081–10088.

Beach, J. (2012). *Counting carbon nano-tubes on collapsed filter membrane by CytoViVa hyperspectral darkfield microscopy*. Albany, NY: Safe Nano Design Workshop.

CDC-NIOSH (1994a). Asbestos by TEM. Method 7402. In *NIOSH manual of analytical methods* (4th ed., p. 7402). Cincinnati, OH: National Institute for Occupational Safety and Health.

CDC-NIOSH (1994b). Asbestos and other fibers by PCM. Method 7400. In *NIOSH Manual of Analytical Methods* (p. 7400). Cincinnati, OH: National Institute for Occupational Safety and Health.

Dillon, J. C., Bezerra, L., Sosa Peña, M. P., Neu-Baker, N. M., & Brenner, S. A. (2017). Hyperspectral data influenced by sample matrix: The importance of building relevant reference spectral libraries to map materials of interest. *Microscopy Research and Technique*, *80*, 462–470.

Evans, D. E., Turkevich, L. A., Roettgers, C. T., Deye, G. J., & Baron, P. A. (2013). Dustiness of fine and nanoscale powders. *The Annals of Occupational Hygiene*, *57*, 261–277.

Guttenberg, M., Bezerra, L., Neu-Baker, N. M., Idelchik, M. P. S., Elder, A., Oberdörster, G., & Brenner, S. A. (2016). Biodistribution of inhaled metal oxide nanoparticles mimicking occupational exposure: A preliminary investigation using enhanced darkfield microscopy. *Journal of Biophotonics*, *9*, 987–993.

Husain, M., Wu, D., Saber, A. T., Decan, N., Jacobsen, N. R., Williams, A., … Halappanavar, S. (2015). Intratracheally instilled titanium dioxide nanoparticles translocate to heart and liver and activate complement cascade in the heart of C57BL/6 mice. *Nanotoxicology*, *9*(8), 1013–1022.

Idelchik, M. P. S., Dillon, J., Abariute, L., Guttenberg, M. A., Segarceanu, A., Neu-Baker, N. M., & Brenner, S. A. (2018). Comparison of hyperspectral classification methods for the analysis of cerium oxide nanoparticles in histological and aqueous samples. *Journal of Microscopy*, *271*(1), 69–83.

Idelchik, M. P. S., Neu-Baker, N. M., Chandrasekaran, A., Friedman, A. J., Frame, M. D., & Brenner, S. A. (2016). Relative quantitation of metal oxide nanoparticles in a cutaneous exposure model using enhanced darkfield microscopy and hyperspectral mapping. *NanoImpact*, *3–4*, 12–21.

Mercer, R. R., Hubbs, A. F., Scabilloni, J. F., Wang, L., Battelli, L. A., Friend, S., … Porter, D. W. (2011). Pulmonary fibrotic response to aspiration of multi-walled carbon nanotubes. *Particle and Fibre Toxicology*, *8*(1), 11.

Mercer, R. R., Scabilloni, J. F., Hubbs, A. F., Wang, L., Battelli, L. A., McKinney, W., … Porter, D. W. (2013). Extrapulmonary transport of MWCNT following inhalation exposure. *Particle and Fibre Toxicology*, *10*(38), 13.

Mercer, R. R., Scabilloni, J. F., Wang, L., Battelli, L. A., Antonini, J. M., Roberts, J. R., … Hubbs, A. F. (2017). The fate of inhaled nanoparticles: Detection and measurement by enhanced dark-field microscopy. *Toxicologic Pathology*, *46*, 1–19.

More, S. S., & Vince, R. (2015). Hyperspectral imaging signatures detect amyloidopathy in Alzheimer's mouse retina well before onset of cognitive decline. *ACS Chemical Neuroscience*, *6*, 306–315.

Mortimer, M., Gogos, A., Bartolomé, N., Kahru, A., Bucheli, T. D., & Slaveykova, V. I. (2014). Potential of hyperspectral imaging microscopy for semi-quantitative analysis of nanoparticle uptake by protozoa. *Environmental Science & Technology*, *48*, 8760–8767.

Roth, G. A., Sosa Peña, M. P., Neu-Baker, N. M., Tahiliani, S., & Brenner, S. A. (2015). Identification of metal oxide nanoparticles in histological samples by enhanced darkfield microscopy and hyperspectral mapping. *Journal of Visualized Experiments*, *106*, e53317.

Roth, G. A., Tahiliani, S., Neu-Baker, N. M., & Brenner, S. A. (2015). Hyperspectral microscopy as an analytical tool for nanomaterials. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology*, *7*, 565–579.

Shannahan, J. H., Sowrirajan, H., Persaud, I., Podila, R., & Brown, J. M. (2015, 12 pages). Impact of silver and iron nanoparticle exposure on cholesterol uptake by macrophages. *Journal of Nanomaterials*, *2015*, 1–12.

Sosa Peña, M. P., Gottipati, A., Tahiliani, S., Neu-Baker, N. M., Frame, M. D., Friedman, A. J., & Brenner, S. A. (2016). Hyperspectral imaging of nanoparticles in biological samples: Simultaneous visualization and elemental identification. *Microscopy Research and Technique*, *79*, 349–358.

Stark, R. W. (2011). Nanomanipulation and nanorobotics with the atomic force microscope. In K. D. Sattler (Ed.), *Handbook of nanophysics: Nanomedicine and nanorobotics* (pp. 43-1–43-13). Boca Raton, FL: CRC Press, Taylor & Francis Group.

van der Meer, F. D., van der Werff, H. M. A., van Ruitenbeek, F. J. A., Hecker, C. A., Bakker, W. H., Noomen, M. F., … Woldai, T. (2012). Multi- and hyperspectral geologic remote sensing: A review. *International Journal of Applied Earth Observation and Geoinformation*, *14*, 112–128.

von der Kammer, F., Ferguson, P. L., Holden, P. A., Masion, A., Rogers, K. R., Klaine, S. J., … Unrine, J. M. (2012). Analysis of engineered nanomaterials in complex matrices (environment and biota): General considerations and conceptual case studies. *Environmental Toxicology and Chemistry*, *31*, 32–49.

How to cite this article: Neu-Baker NM, Eastlake AC, Brenner SA. Sample preparation method for visualization of nanoparticulate captured on mixed cellulose ester filter media by enhanced darkfield microscopy and hyperspectral imaging. *Microsc Res Tech*. 2019;82:878–883. https://doi.org/10.1002/jemt.23231