Additional support information for the Synergist Magazine article

Analysis of Wildfire and Structure Fire Combustion Residues: Microscopy Methods and Other Considerations. August, 2022

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OVERVIEW

The Synergist article "*Analysis of Wildfire and Structure Fire Combustion Residues: Microscopy Methods and Other Considerations*" (August, **bit.ly/syn2208fire**) was published to shed light and open an industry-wide dialogue on the advantages and limitations of various microscopical analysis methods, and the need to make the data provided by different laboratories more comparable. For publication purposes, the length and content of the Synergist article was limited. This meant a significant amount of support information, research work, and comparison photomicrographs illustrating the advantages and limitations of microscopy methods could not be included within the original article.

This paper provides the additional information describing the scientific limitations of Optical and Electron Microscopy analysis methods in general, and more specifically, the 2018 ASTM D6602–13 Method, "*Standard Practice for Sampling and Testing of Possible Carbon Black Fugitive Emissions or Other Environmental Particulate, or Both*", when applied to the specific purpose of analyzing settled particles associated with a fire-related event. The appropriate use and microscopical requirements for this purpose are described in the 2018 AIHA "*Technical Guide for Wildfire Impact Assessment for the Occupational and Environmental Health and Safety Professional*".

The analysis of wildfire (and structure fire) particles is a forensic investigation requiring a complete evaluation (including provenance) of the unaltered char, ash, soot, signature particles; and the preservation of their intact depositional patterns and transmitted and reflected light properties or "fingerprint". These light microscopical properties are required to visually differentiate *burned* from *unburned* particles. All of these factors are required to perform a confirmatory analysis of the source and level of smoke impact. Tape lift sampling is the most effective collection method to preserve the dust "fingerprint". Wipe and bulk dust sampling methods erase the ability to examine this "fingerprint". The technical ability to analyze this "fingerprint" also requires optical microscopical analyst training beyond what is prescribed in existing published methods including the ASTM D6602 Method.

Because no published ASTM, EPA, NIOSH or other methods exist for the specific purpose of evaluating the surface impact from a wildfire or structure fire event, the investigator is strongly encouraged to re-evaluate the laboratory they are using to ensure they provide the minimum optical microscopical requirements, analyst training, and reporting guidelines as outlined in the AIHA Technical Guide. This information should be available by requesting your laboratory's Standard Operating Procedure (SOP).

DISCUSSION

The Synergist article summarized the critical requirements and limitations of various sampling and microscopic analysis capabilities as they specifically apply to the forensic analysis of wildfire and structure fire residues. Some subtle differences in the sampling and microscopic analysis methods employed by various laboratories may appear insignificant, but they are not. Careful consideration must be used to incorporate the best combination of sampling and analysis methods. As the primary goal, the methods chosen must be able to consistently collect, predictably preserve, and reliably analyze the combustion particle properties and depositional patterns that may be associated with a fire-related event.

Direct sampling procedures (such as tape lifts) should be considered as the first and primary choice because they best preserve the intact spatial particle properties and depositional patterns found on the sampled surface. This allows the analyst to perform a direct examination and quantitative microscopical analysis with minimal particle alteration. Indirect sampling methods such as bulk, vacuum dust, or wipes mechanically remove the deposited particles collected using one sampling media, and then transfer the homogenized or agglomerated particles to a second or even third analysis media. This process can alter or destroy the spatial orientation and particle size distribution, remove semi-soluble or semi-volatile residues, and alter the quantitative information critical to understanding the source of the fire-related event. When indirect procedures are required in situations where direct methods cannot be practically employed, the investigator should understand there will be a loss of useful information. The investigator and laboratory need to also understand this useful information can include a wide range of qualitative and quantitative parameters, and that simply reporting the amount of char or soot in the sample is insufficient.

As anticipated, the Synergist article has generated significant awareness, and also controversy and challenges for investigators and laboratories that may have assumed their use of methods intended for other analytes (in the absence of standard published wildfire or structure fire methods), would provide reliable data as surrogate methods when used for the analysis of combustion residues, which they do not. The two most commonly cited methods, the EPA Method EPA/600/R-93/116 designed for the analysis of bulk asbestos samples, and the ASTM D6602-13 designed for carbon black, are analytically inadequate for the analysis of combustion residues by themselves. This is because they cannot reliably differentiate "*burned*" from "*unburned*" particles. The problem is, that when investigators and laboratories do not employ the primary sampling and very specific Optical Microscopy methods recommended in the 2018 AIHA Technical Guide, the resultant data may be reliable for some situations, but unreliable in others. This unknown variability can make data comparisons between different laboratories unreliable.

The industry accepted approach for the use of non-standard or "new" laboratory methods, as is the case for both wildfire and structure fire residue analysis, is spelled out in the International Standards (ISO/IEC) 17025-2017 Section 5.4. "*General requirements for the competence of testing and calibration laboratories*". Section 5.4.4 "*Non-standard methods*" spells out the requirements for laboratory method documentation. These same requirements are also described in the 2007 USEPA (EPA/600B-07/001) document entitled "*Guidance for Preparing Standard Operating Procedures. EPA QA/G-6*". Both of these documents clearly state that it is insufficient for a laboratory to simply claim they are using or following a general method even when they are using the method for its' intended purpose. This becomes even more problematic when the laboratory uses an analyte specific procedure for a purpose other than the one being tested (such as described above). The 2007 EPA SOP Guideline clearly states on page 2 "*SOPs are needed even when published methods are being utilized*". Additionally, laboratory "accreditation" for one scope of work (e.g. PLM analysis of asbestos) is not

applicable or sufficient by itself for another scope of work such as wildfire or structure fire analysis. "Accredited" laboratories are required to have an internal Standard Operating Procedure (SOP) specific to the analyte being tested. According to both ISO/IEC and EPA guidelines, "new" test and/or calibration methods should be developed prior to the tests and/or calibrations being performed, and should contain at least the following information:

- Appropriate identification
- Scope
- Description of the type of item to be tested
- Parameters to be determined
- Apparatus and equipment, including technical performance requirements
- Reference standards and reference materials required
- Detailed description of the procedure
- Criteria and/or requirements for approval/rejection
- Data to be recorded and method of analysis and presentation
- Uncertainty of the procedure

Another issue that has not been adequately addressed by laboratories attempting to use the ASTM D6602-13 carbon black method for other purposes, is the inherent differences between manufactured carbon black and the lower temperature smoldering fire residues associated with wildfires and structure fires. In order to follow the guidance provided in the EPA or ISO/IEC guidelines, the laboratory is required to "*provide new test and/or calibration methods"* to address the "*uncertainty of the procedure*". This *uncertainty* when applying the TEM carbon black procedure to wildfire and structure fire residues is very high, and is described in the 2013 publication entitled "*Carbon Black vs. black carbon and other airborne materials containing elemental carbon: Physical and chemical distinctions"*. The ASTM Method works well for carbon black for very specific reasons. These same reasons make the TEM method unpredictable, and therefore unreliable, for other types of non-manufactured combustion particles. The differences between carbon black, open biomass burning particles (e.g., wildfires or controlled burns), and "traditional" diesel exhaust particulate are illustrated in Figure 1 on the following page below.

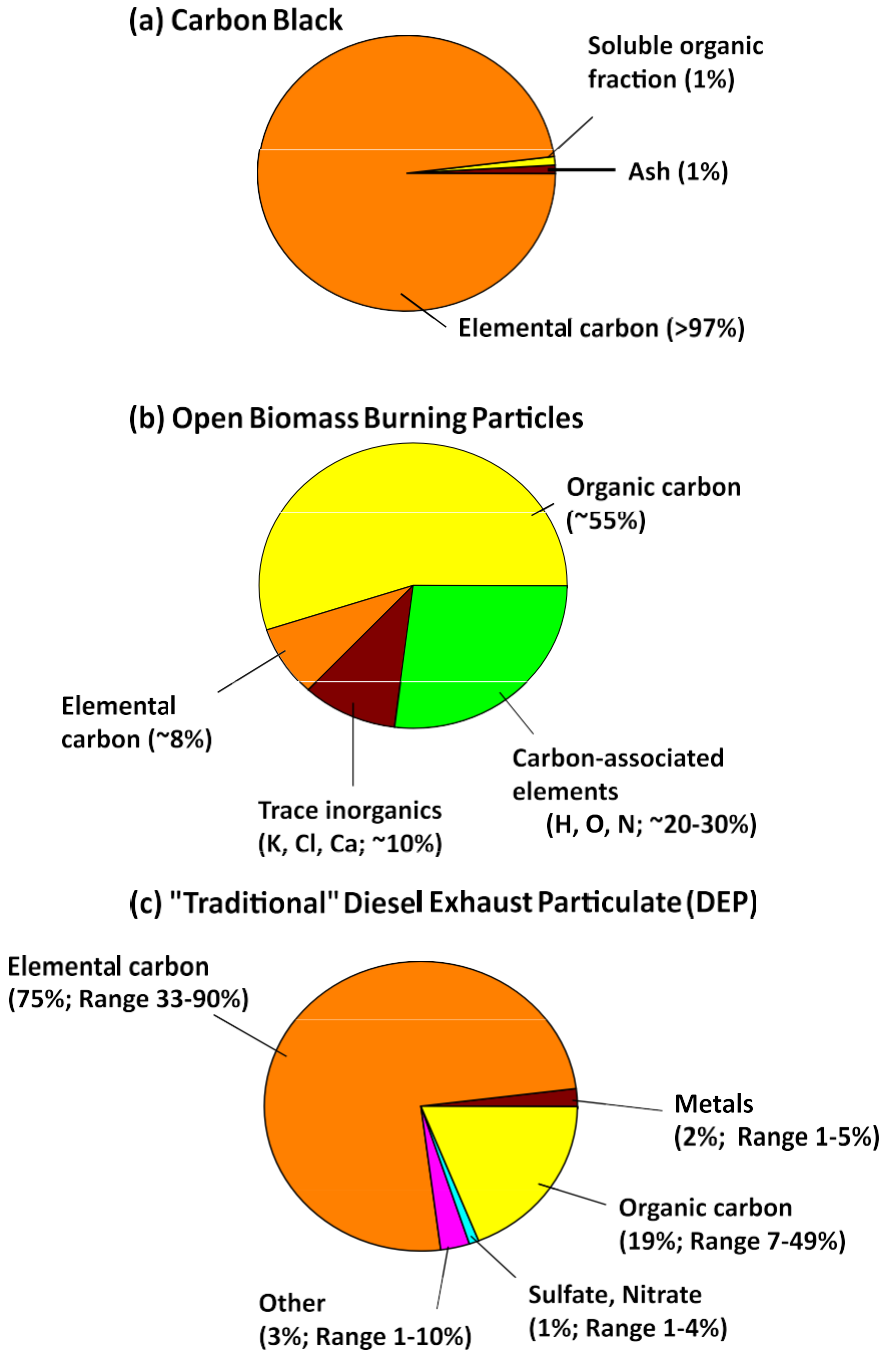


Figure 1. Comparison of chemical composition among different elemental carbon-containing particles. Elemental carbon-containing particles include (a) carbon black (data from Watson and Valberg, 2001) and two dominant forms of black carbon or "soot", namely (b) particulate emissions from open biomass burning (data from Reid et al., 2004) and (c) "traditional" diesel exhaust particulate representative of 1990s-era diesel engine technologies (data from US EPA, 2002).

Carbon black is manufactured at high temperatures above 2,400°F and is dissimilar to the wide range and composition of soot and other burned particles that are generated at uncontrolled lower temperatures (i.e., 400 - 2,000°F) commonly found in wildfires and structure fires. Carbon black and diesel emissions are primarily composed of elemental carbon (>75% and >97% respectively), while open biomass burning particles contain very low concentrations of elemental carbon (~8%) and are primarily "organic" carbon and poly aromatic hydrocarbons (PAH's) with totally different volatility and solubility characteristics. These important

compositional differences significantly affect the reliability of detection when using Scanning or Transmission Electron Microscopy. This is due to the preparation procedures used, and the low vacuum pressures (causing sublimation) and high electron beam temperatures (especially TEM) used to perform the analysis. Laboratories (based on the ISO/IEC and EPA guidelines described above) are required to perform calibration tests to evaluate the effective recovery and detection of analytes other than carbon black if they are going to use it as test method for other purposes. There are simple validation tests that can be performed that will clearly show a significant and variable portion of the soot residues found in wildfires and structure fires will be lost in the initial sample preparation procedures.

Beyond the problems generated by using test methods designed for other analytes, there can be a very real communication and informational disconnect between what the investigator may need, and what information the laboratory is actually providing. There is a significant difference between the goals of wildfires and structure fire sampling, and what the different sampling and microscopy analysis methods may actually provide. The majority of fire related investigations inherently have a forensic multi-component approach; and the complexity of fire residues requires the laboratory to provide a multi-parameter analysis that addresses that complexity. In other words, simply reporting the percent level of char or soot may not answer the question or goal of the investigation.

In the absence of existing "standard" laboratory test methods, investigators need to ensure the capabilities, instrumental methodology, and data provided by their laboratory can effectively document an impact from a fire event. This can only be ascertained by examining the laboratory SOP (as previously described). SOPs are also required to understand and compare data from different laboratories. Laboratories should be continuously upgrading their procedures and analyst training to address the ever-increasing scope of fire-related investigations. Based on the sampling and analytical information in the 2018 AIHA Technical Guide, there were recommendations provided regarding the most effective microscopical methods, and the analytical data to be included in laboratory reports. Some laboratories are either not be fully aware of these recommendations, or have simply chosen to ignore their relevance and significance. A comprehensive laboratory analysis report (as part of the thorough site investigation) should be able to help answer some of the following questions:

Wildfires

- Are wildfire-related combustion residues present and elevated?
- Does the analysis differentiate background from potential event related sources?
- Does the analysis differentiate between vegetative and non-vegetative char and ash?
- Are significant wildfire indicator particles found (e.g., phytoliths, burned clays, fire retardant)?

Structure Fires

- Are in situ soot condensate deposition patterns present on the surface tested?
- If present, are the patterns consistent with a structure fire event?
- Are the char and ash particles consistent with a structure fire event?
- Are any structure fire indicator particles present (e.g., burned plastics, paper, synthetic fibers paint)?

To cite a quotation from Burt Rutan, it is best "***to use the lowest technology that will do the mission, not the highest***". For the analysis of wildfires, the consensus methodology described in the AIHA Technical Wildfire Guide (based on the input from over 20 experts and contributors) recommended the lowest technology possible, while still ensuring there are minimum requirements that will actually accomplish the ***mission***. There has been significant confusion over the actual ***mission*** of wildfire or structure fire testing, and the minimum testing requirements to perform this ***mission***. Some of the laboratories disputing the technical claims presented in the Synergist paper have been relying on microscopical methods designed for other purposes (i.e., PLM microscopy for asbestos, or TEM for the analysis of carbon black). This is most likely based on the assumption they will not have significant limitations for combustion particle analysis. In fact, neither of these methods provide the necessary capabilities required to analyze and quantify the wide range of wildfire or structure fire particle properties by themselves.

The goal of the microscopic analysis should be to rapidly detect and quantify fire-event related particles and depositional patterns; and differentiate them from the background debris and other combustion particles that are not associated with a fire-related event. Understanding this analysis goal or end-point is essential. This ability to rapidly recognize potentially "burned" from "unburned" particles in a field of hundreds of background particles can only be performed by using surface color and reflectivity through the use of reflected light darkfield microscopy. This requirement is a practical and critical limitation that precludes the use of electron microscopy (SEM or TEM) as the initial or primary method.

Electron Microscopes (SEM and TEM) produce black and white images, or more accurately stated, represent the electron contrast (light and dark), and not color. The SEM can employ automated particle analysis / dispersive X-ray analysis to differentiate the elemental chemistry of individual particles. This is typically used to differentiate soil particles, gunshot residue, construction minerals, and corrosion particles from other "carbonaceous" particles. An SEM equipped with differential pressure and using a low beam energy can reduce the loss of some semi-volatile soot condensates collected on tape lift samples, however, the identification and differentiation of carbon combustion particles from other non-combustion carbon particles is still only presumptive, and must be performed on an individual particle-by-particle basis. This requires the combined use of visual morphology and X-ray chemistry to identify the particle. The reported laboratory results are typically comprised of a series of individual pictures and X-ray spectra that only represent an infinitesimally small number of particles in the sample. Obtaining quantification of combustion-related (burned) particles in the sample is time-consuming, unreliable, and simply not practical. SEM analysis employs the "***highest***" level of technology, and yet, can only partially address the goal or *mission* of combustion particle analysis.

Attempting to employ TEM analysis for this same *mission* has even more severe limitations. Although the TEM has the highest theoretical image resolution of any microscopical method, employing automated particle analysis, or partial pressure vacuum and lower electron beam energies to prevent the loss of semi-volatile or heat sensitive particles in the sample is not feasible.

The advantages and limitations of Optical and Electron Microscopy methods are compared below in Figure 2. Examples photos of Optical and Electron Microscopes are given in Figure 3.

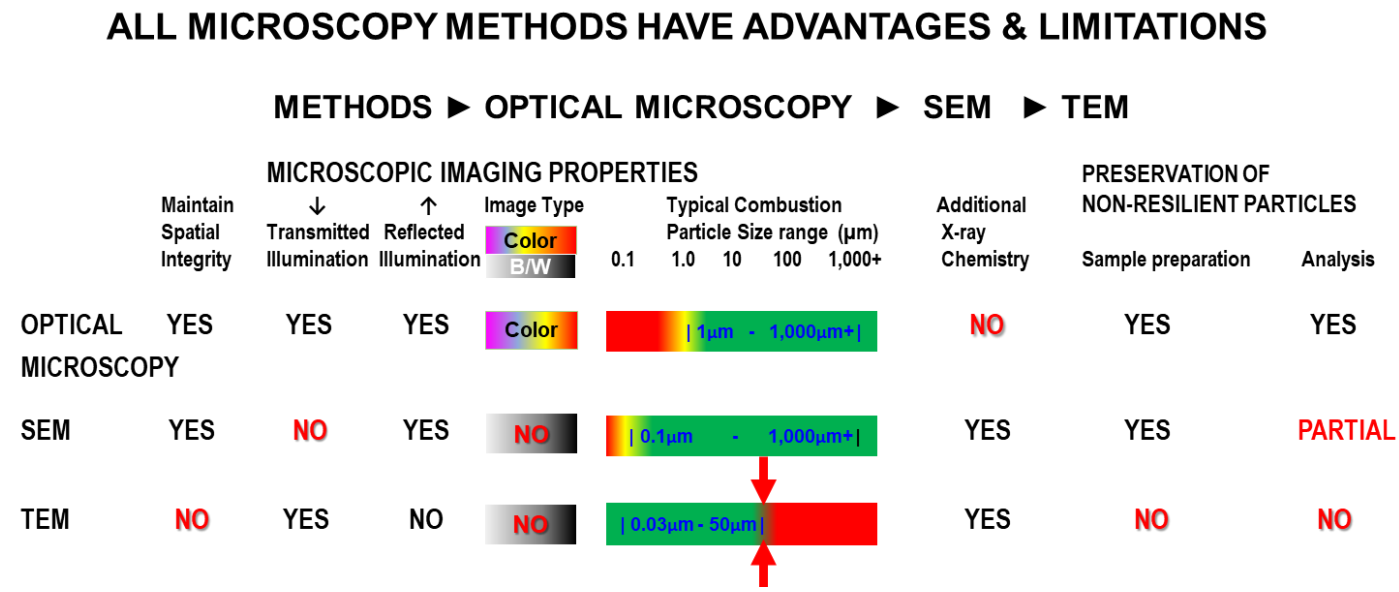


Figure 2. Illustrated Comparison of the Analysis and Sample Preservation Properties of Microscopy Methods.

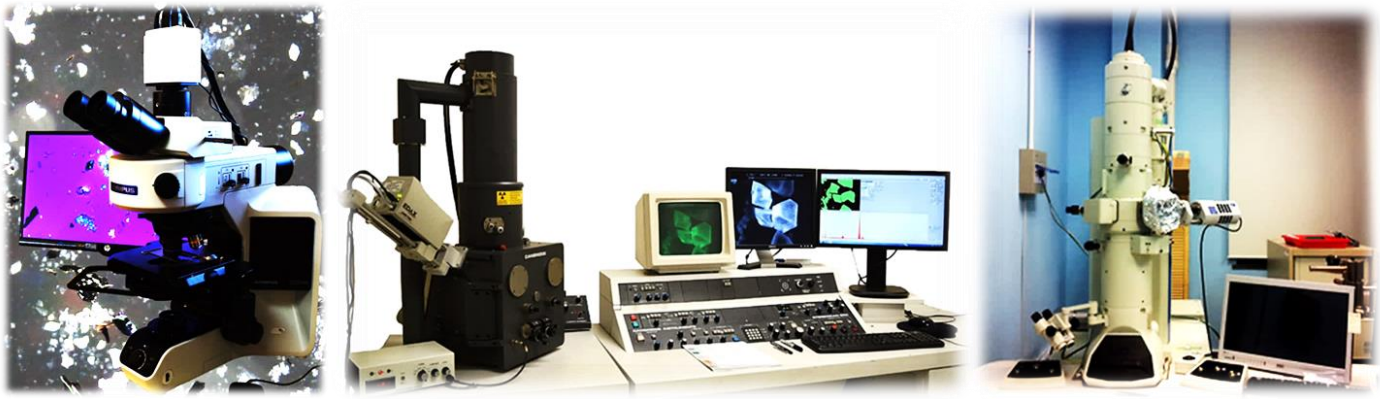


Figure 3. Examples of a properly equipped Optical Microscope, Scanning Electron Microscope, and Transmission Electron Microscope.

As described above, two major limitations preclude SEM or TEM from being used as a "primary" method of choice: 1). their inability to differentiate color, and 2). the potential sample loss of semi-soluble or semi-volatile particles during the preparation and analysis procedures (i.e., being placed in a vacuum, and heated by the electron beam during analysis). Neither Electron Microscopy method can visually differentiate "burned" vegetation and soot depositional patterns from "unburned" particles in the sample. As examples, the differences between images provided by Optical Reflected light Darkfield Microscopy (RLDF), and Scanning Electron Microscopy (SEM) are illustrated below. TEM cannot be performed as a part of these side-by-side comparisons because it is incapable of directly analyzing tape lift samples, the analysis of the larger char or ash particles; or directly analyzing the soot "fingerprint" patterns as found on the tested surface.

A. Partially charred (burned) vegetation (RLDF)

B. Partially charred (burned vegetation (SEM)

The first example shows a partially charred vegetation fragment observed in Reflected Light Darkfield Microscopy (A), and the exact same area in Scanning Electron Microscopy (B). The charred area of the vegetation fragment is invisible in the black and white image generated by the SEM.

C. Structure Fire Soot Deposition (RLDF)

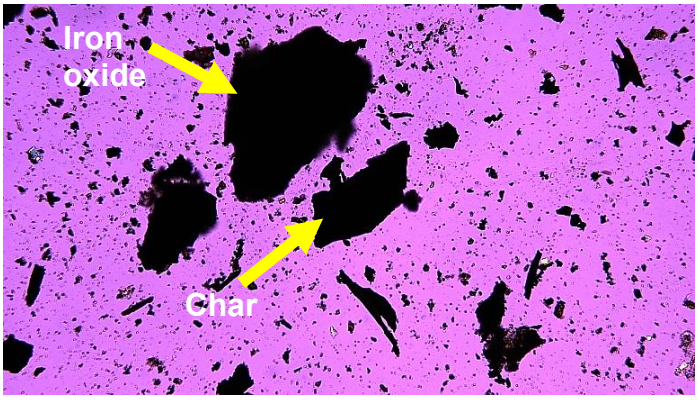
D. Structure Fire Soot Deposition (SEM)

The second example shows the exact same area of soot depositional patterns collected from a structure fire using Optical Microscopy (C), and SEM (D). Two human hairs were placed in a cross-wise position to show the location and relative size of the deposited soot patterns. The black soot deposition patterns observed in the RLDF Optical Microscopy image, are again completely invisible in the electron contrast image provided by the SEM. As discussed on page 5, in order to estimate the potential concentration of soot immediately obvious in image (C), the SEM analyst would have to analyze hundreds of the individual particles and X-ray spectra from the SEM image (D) to arrive at a similar observation or conclusion.

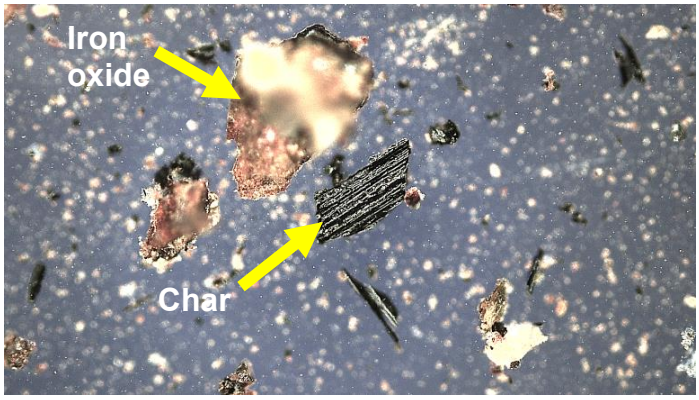
As discussed in the Synergist article, a standard PLM transmitted light microscope by itself is inadequate for the analysis of combustion particles, and the results should only be considered presumptive. For the results to be considered confirmatory, the reflected light properties (reflectivity, surface texture, and color) are also required to differentiate "burned" from "unburned" particles. The 2018 AIHA Technical Guide states *"Light microscopy includes bright-field transmitted, polarized light, and dark-field reflected illumination as a*

minimum capability." Some laboratories are still providing combustion particle analyses without these microscopical capabilities.

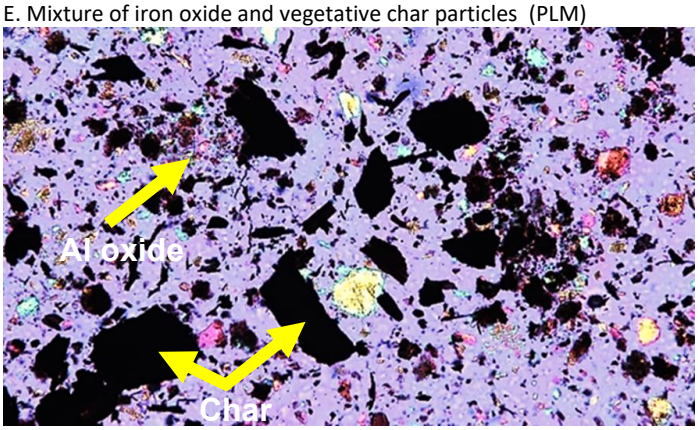
The following micrographs illustrate the importance of using a light microscope equipped with simultaneous reflected and transmitted light illumination modes. Micrographs (E-J) show examples of mixed leaf, grass, and bark vegetation char mixed with common look-alike corrosion materials and tire wear rubber encountered in indoor samples. The micrographs show the exact same areas using transmitted (PLM) and reflected light darkfield illumination (RLDF). Micrographs (K-L) illustrate the difficulty encountered when using transmitted polarized light (alone), to differentiate Char from the decayed vegetation found in Miracle-gro® potting soil.



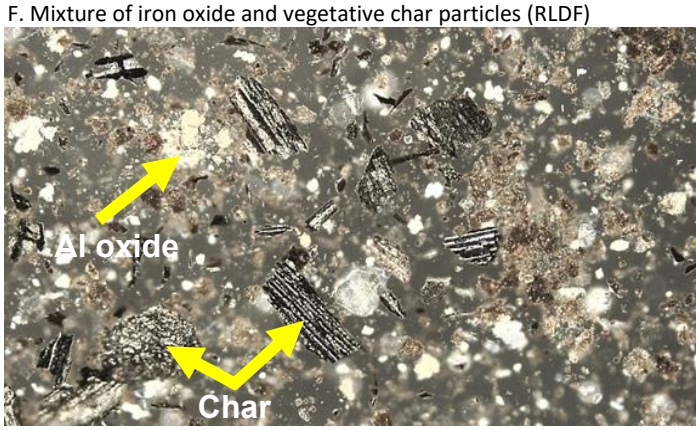
E. Mixture of iron oxide and vegetative char particles (PLM)



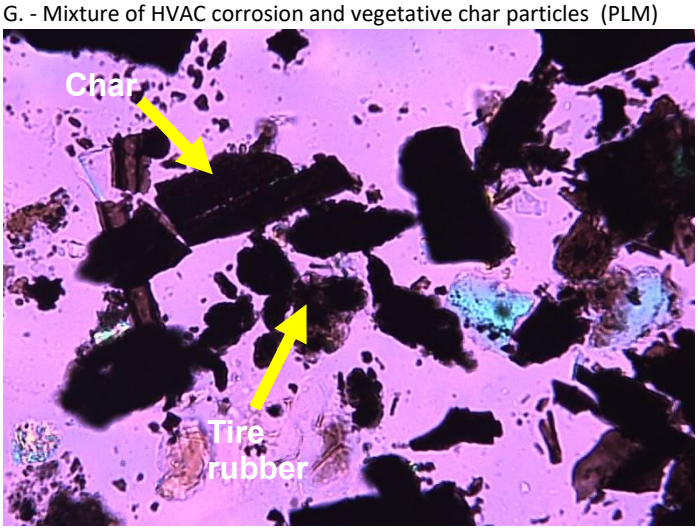
F. Mixture of iron oxide and vegetative char particles (RLDF)



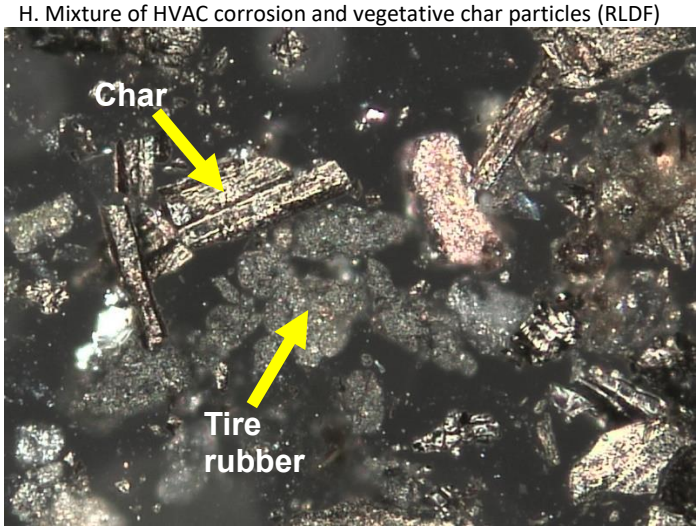
G. - Mixture of HVAC corrosion and vegetative char particles (PLM)



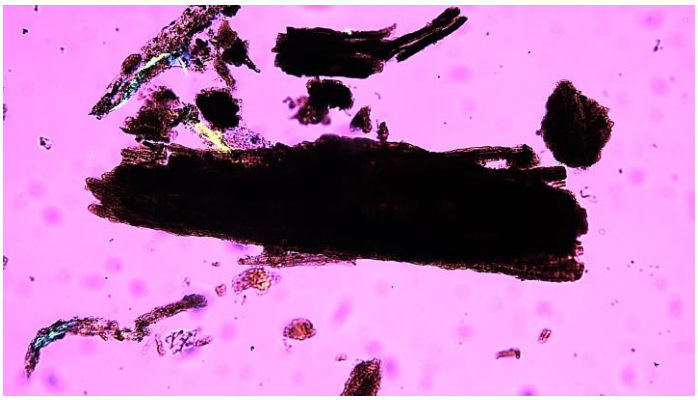
H. Mixture of HVAC corrosion and vegetative char particles (RLDF)



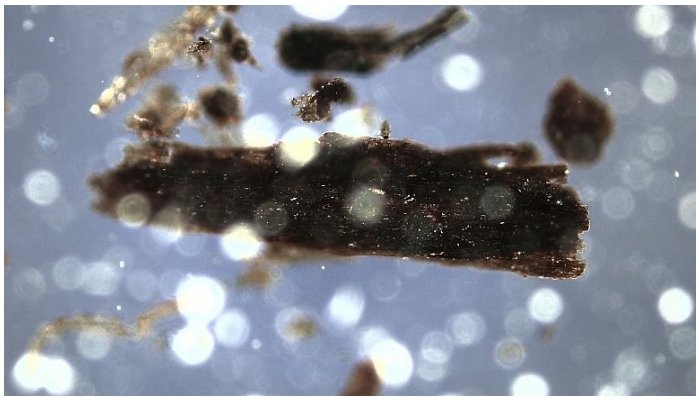
I. Mixture of tire wear rubber and vegetative char particles (PLM)



J. Mixture of tire wear rubber and vegetative char particles (RLDF)



K - Miracle-Gro® potting mix - Decayed vegetation (PLM)



L. Miracle-Gro® potting mix - Decayed vegetation (RLDF)

As can be seen in examples E-L, Transmitted Polarized Light illumination is incapable by itself of differentiating "burned" vegetation char particles, from other "unburned" environmental particles with similar morphology. The micrograph comparisons clearly show that transmitted light (PLM) illumination in general (and defined as a screening tool in the ASTM D6602-13 Method in Section 7), cannot reliably differentiate char from iron oxide (E-F), aluminum oxide corrosion (G-H), roadway tire wear rubber (I-J), or even the decayed vegetative fragments found in potting soil (K-L). Decayed potting soil vegetation fragments can easily be differentiated from char by its' very low surface reflectivity and texture when using reflected light darkfield illumination.

The Light Microscopy illumination requirements prescribed in the ASTM D6602 Method do not meet the minimum capability specified in the AIHA Technical Guide for the analysis of wildfire particles. The ASTM "*Apparatus*" (Section 7.2) is rudimentary and only specifies a "*Light Stereo Microscope, capable of at least 40x magnification*", and a "*Polarized Light Microscope, equipped with objectives at least in the 10 to 40x range of magnification*". The ASTM Method lacks the simultaneous capability of transmitted and reflected light darkfield illumination required in the AIHA Technical Guide. These limitations are not a deficiency of the ASTM Method for its intended purpose as a "screening" tool.

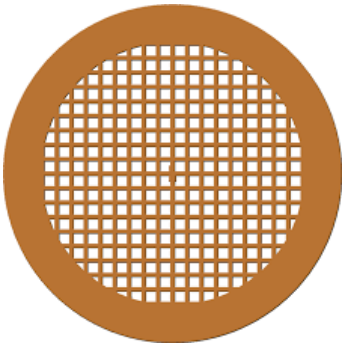
The Light Microscopy techniques used in the ASTM Method were never designed to be used as a primary method to identify or quantify "*other*" combustion particles in the first place. It was designed to provide an initial overview or *screening* procedure of the sample bulk composition. "*Section 7. Examination by Optical Microscopy, Page 3, Line 2, "Summary of the Test Method – This Method is a screening test method that provides an overview of the bulk composition of the sample through examination under a light microscope*". Referencing the ASTM D6602 procedure as the primary basis or analytical method used for wildfires or structure fire analyses is simply insufficient and inappropriate.

Similar confusion exists with some laboratories substituting the theoretically "***highest***" and most expensive technology (i.e., Transmission Electron Microscopy) for "soot" analysis, rather than the appropriate minimum capabilities required to perform the ***mission***, or inspection objective. As an elaboration on information provided in the Synergist article, there is a significant disconnect and misunderstanding regarding the purpose and application of the ASTM D6602 Method. It is the correct generally accepted method to identify the presence of carbon black based on the sub-micron nodule morphology and X-ray elemental analysis. However, it is ill-suited for the specific forensic purpose of determining a qualitative or quantitative impact from an actual fire event. In other words, the scope or mission for the ASTM D6602 Method is significantly different. As referenced in the Synergist article, the first sentence in the ASTM D6602 Method Scope, Section

1.0, clearly states, "*This practice covers sampling and testing for distinguishing ASTM type carbon black, in the N1000 to N900 series, from other environmental particulates*". The method was not intended to be used as a quantitative analysis procedure for combustion soot particles, nor was it designed to analyze the surface settling patterns generated in uncontrolled and lower temperature structure fires.

The physical sample limitations and the configuration and operational requirements of a TEM are ill-suited for the analysis of "large", fragile, semi-soluble, or semi-volatile char or ash particles. The usable sample sizes are also very small and need to be put into perspective. The maximum usable sample size is defined by the diameter of the copper grid (3mm) used as the sample substrate in all TEMs, and the very small amount of dust that can be placed on top of the grid without overloading or obscuring the visibility of particles. The actual size of a TEM grid (3mm in diameter) is shown at end of this sentence. ► 

According to the ASTM Method, *Section 8.3. Procedures*, only a small sub-sample of the "soiled" area of a wipe sample is placed in a vial or test tube with 10-20 milliliters (ml) of solvent and ultrasonicated. From this suspension, a small aliquot comprised of 5-10 micro-liters (µl) representing less than 1/10,000 of the collect wipe sample is pipetted dropwise onto a carbon coated TEM sample grid and allowed to dry. The resulting soot nodules and aciniform "micro-patterns" observed in the TEM analysis are not representative of the macro depositional patterns found on the impacted surface. Furthermore, the aciniform or aggregate pattern of soot nodules can be real or simply an artifact produced by particle aggregation during the solvent drying process.



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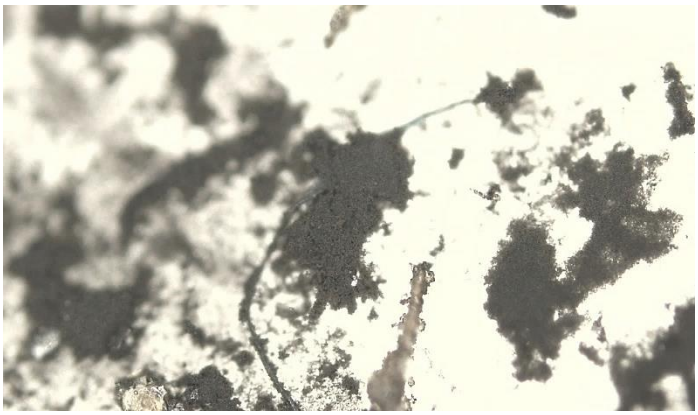
Another limitation is the maximum particle size that can be analyzed on the TEM sample grid. The viewable area is defined by the mesh openings of the 200-300 mesh sample grid. The TEM grid is essentially like a copper window screen where the only area that can be analyzed is the holes (open area) in the screen mesh. The grid opening can vary from 50-75µm restricting the maximum particle diameter that can be analyzed to significantly less 50µm. Combustion particles and the surface deposition patterns associated with wildfires and structure fires cover a significantly larger size range (typically 5-5,000µm), and can exceed the maximum size range analyzed by a TEM.

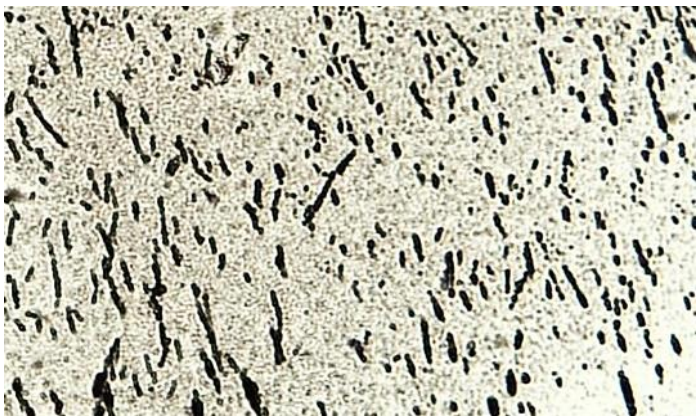
The thickness of collected particles is also another physical limitation for the TEM analysis. The TEM can only observe the internal structure of particles (similar to a black and white chest X-ray) with a maximum thickness of 2 micrometers.

The ASTM D6602 TEM Method is the perfect application to determine the presence of sub-micron carbon black particles, or particles with similar resilient properties. These properties include the ability to survive liquid ultrasonication, the high vacuum pressure and temperature of the electron beam; and require the high resolution of the TEM. Combustion particles and the deposition patterns associated with wildfires and structure fires typically do not have these same resilient properties, cover a significantly larger size range (typically 5-5,000µm), and exceed the maximum size range that can be analyzed by a TEM. Furthermore, the non-resilient soot residues produce diagnostic and easily recognizable deposition patterns when they impact building surfaces. These patterns are preserved with tape lifts, however, tape lifts cannot physically be placed into a TEM. The inability of the TEM to observe soot depositional patterns significantly limits its application as a diagnostic method for wildfire or structure fire analysis.

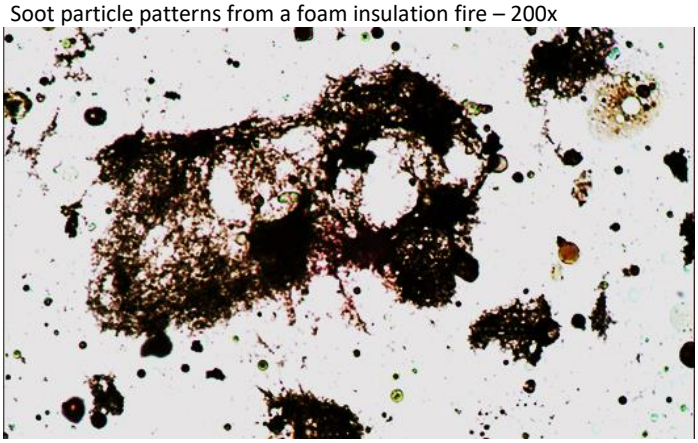
Examples of soot deposition patterns from different types of fires collected on tape lift samples and analyzed by Optical Microscopy are shown below:



Soot particle patterns from a foam insulation fire – 200x



Soot deposition patterns from smoldering PVC pipe – 500x



Soot deposition patterns from a cooking grease fire – 800x

Soot deposition patterns from candle wax and furnace puff back – 200x

It is important to note, the problems encountered analyzing certain types of semi-soluble and semi-volatile particles, does not diminish the value of using Electron Microscopy analysis for other types of particles found in dust samples.

For the practitioner or investigator that may be unaware of the microscopy limitations described in the Synergist article or this paper, especially those addressing using electron microscopy methods for soot analysis, simple validation experiments can be conducted by your laboratory to document the limitations the authors have described above. As previously indicated in this paper, the ISO/IEC guidelines require calibrations of "*the uncertainty or the procedure for estimating uncertainty*" as a part of any laboratory test method.

Finally, there are numerous peer-reviewed publications that address the microscopic evaluation of wildfires and other environmental combustion sources. These are provided on the following pages.

Synergist Response References

[1] ISO/IEC 17025. 2017, International Standard. "General Requirements For The Competence of Testing and Calibration Laboratories".

[2] EPA/600/B-07/001, 2007, "Guidance for Preparing Standard Operating Procedures (SOPs), EPA QA/G-6".

[3] ASTM D6602–13, reapproved 2018. "Standard Practice for Sampling and Testing of Possible Carbon Black Fugitive Emissions or Other Environmental Particulate, or Both".
[4] IESO / RIA Standard 6001, 2010. "Evaluation of Heating, Ventilation and Air Conditioning (HVAC) Interior Surfaces to Determine the Presence of Fire-Related Particulate as a Result of a Fire in a Structure".
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