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Final Summary Overview

NANOTRACE: Applications of subvisible to nanoscale particles in trace evidence

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PURPOSE OF THIS RESEARCH

Nanoparticles and other subvisible particles (10 nm to 100 µm) are present in nearly all forms of existing trace evidence, yet the overwhelming majority of trace examinations focus exclusively upon larger particles. In an era where highly engineered nanoscale materials are being introduced at increasing rates, it seems inconceivable that such materials are not being regularly examined as forensic evidence. While there are exceptions (e.g., GSR particles are generally less than 5 micrometers), the 100 micrometer boundary exists due to a variety of reasons that include a) the way in which evidence is collected; b) the methods used for isolating and preparing trace evidence; c) analytical methods that are not used to their full resolution, or in some cases, the lack of access to analytical methods with the resolution necessary to practically analyze smaller particles and feature; and d) a limited means for interpreting the significance of such particles.

The goal of this research is to systematically develop approaches for the isolation, analysis, and interpretation of particles in this size range, which would effectively equate the sensitivity of trace evidence to that of DNA analysis. While the smallest particles in this range will require higher resolution instrumentation, a wide range of particles can be characterized effectively by more effectively applying the suite of microanalytical methods present in most trace evidence laboratories today (stereomicroscopy, polarized light microscopy, and scanning electron microscopy). The potential value of smaller particles of traditional trace evidence, coupled with the increasing prevalence of engineered nanoscale particles and features, illustrates the clear need to develop a regular means by which evidence can be a) collected, b) prepared, c) analyzed and d) interpreted in the range of 10 nm to 100 µm. To this end, the research conducted under this grant focused on developing a framework upon which a typical forensic laboratory could improve their ability to find, handle, analyze, and interpret the significance of smaller particles in a practical manner.

PROJECT DESIGN AND METHODS

This research aims to establish the first systematic forensic approach to the characterization of subvisible (1-100 µm) through nanoscale (<1 µm) evidence. The approach was broken into four stages, as outlined below.

1. Recognition: A list of potential subvisible particles and nanoparticles that have the potential to be exploited as forensic evidence was compiled. Several broad categories were highlighted to be of the greatest interest, including glass microspheres, pigments, and polymers.

2. Development of isolation methods: Various isolation and collection methods were evaluated vis à vis typical forensic laboratory capabilities, including tape lifts, swabs, microvacuum filters, and loose soil/debris. As expected, the preferred method varied by particle types. Special attention was paid to background contamination in both the

matrix from which the material was sampled and the analysis environment itself.

3. Characterization and identification: Typical trace evidence laboratories have analytical capabilities that include stereomicroscopes, PLM, SEM/EDS, and micro-FTIR. This suite of existing instruments provides an excellent basis for the characterization of many types of subvisible particles. These, along with supplementary high-resolution methods (such as field emission SEM and transmission electron microscopy) and sample preparation methods were used to determine both the level of information and the limitations that may be expected when studying subvisible particles.

4. Interpretation: There is virtually no published information on the population of subvisible particles present in real-world forensic samples, nor is there data on the prevalence, transfer, and persistence of nanoparticles within a given substrate or environment. Our research examined samples, environments, and substrates in order to understand the potential for unintended contributions of subvisible particles. The results were considered in light of several topics, including the types of subvisible particles that can be identified in forensic samples from known environments, and the importance of measuring background levels of nanoparticles in the environment prior to rendering an opinion regarding the significance of any findings. In addition, a transfer study was performed to emphasize the ease of multi-level transfers that may occur with subvisible and nanoparticles.

Data Analysis and Findings

The following sections discuss various sub-projects conducted under this grant, the specific approach, an overview of the results, and the deliverable from each.

Nanoparticles and their Potential as Forensic Evidence

The use of nanotechnology and the engineering of nanomaterials has grown exponentially in the last decade and has found widespread application across a number of disciplines and commercial products. As such, nanoparticles and other subvisible particles are present in many forms of existing trace evidence (e.g., paint). For example, at the sub-visible level, the layer thickness of automotive paint is decreasing as paint technology develops. Layers of paint in an automotive finish are now routinely less than 10 µm. In addition, paint contains pigments and other materials such as colloidal silica which can be less than 10 micrometers in size. In some cases, coatings on pigment particles can be tens of nanometers in thickness. While these examples represent one material (automotive paint), similar examples exist in a wide range of particles. At present, however, the overwhelming majority of trace examinations focus exclusively upon particles and features greater than 100 µm. A literature review of potential nanoparticles was conducted to develop an understanding about the possible types of nanoparticles that might be encountered or exploited as forensic evidence. This list serves the additional purpose of identifying possible sources of nanoparticles, which provide insight into both questions of significance and background levels. This survey of nanoparticles has been arranged into a hierarchy to document the range of such particles. The list and its details of specific particle types formed the basis for the publications from this work.

This sum of this information has been compiled into a publication on the topic of "Nanoparticles and their Potential as Forensic Evidence." The article is undergoing final review and is being held to allow for the submission of several of the other articles described below, which will be referenced in this article. Ultimately, this article is intended to be a primer for forensic scientists looking to develop nanoparticles as trace evidence.

Processes for the Preparation and Analysis of Nanoparticles

One of the limiting factors to the use of subvisible and nanoparticle evidence is that laboratories do not have approaches in place for finding and preparing such particles. As a part of this grant, we have defined protocols for two different processes: one aimed at subvisible particles and another aimed at nanoparticles.

The subvisible particle work focuses on finding and isolating such particles from tape lifts, one of the more commonly used trace evidence collection substrates. While these lifts are often searched for paint, glass, hair and fibers, evidence is typically located by means of the unaided eye or low magnification stereomicroscopy. It is then isolated with the aid of a stereomicroscope. Smaller particles, at the subvisible (typically 1-100 µm) or even nanoscale size range, may contain a wealth of information; however, they are rarely exploited due to a combination of their small size and the fact that they are embedded in adhesive. When captured on or in the tape adhesive, it can be challenging to visualize, much less isolate, analyze and exploit this smaller scale evidence due to the lack of an optimized mounting medium, the birefringence of tape backing (which can interfere with polarized light microscopy), and the presence of an adhesive that can interfere with isolation and further analyses. To work around these constraints, we have assembled a method utilizing a three-stage approach that permits successively better visualization of such particles while still on a tape lift and a low temperature ashing (LTA or plasma ashing) process by which particles can be isolated from a tape matrix for more detailed microanalysis. This approach has been written up and submitted for publication as "The preparation of tape lift samples for the study of subvisible and nanoparticles."

When focusing exclusively on nanoscale particles, particularly those in or on substrates other than tape, other approaches to sample preparation may be utilized. Based upon our research into nanoparticles in soil evidence, we have developed a procedure for the isolation, sample preparation, and scanning electron microanalysis of these isolated nanoparticles. Examples of the types of nanoparticles that are observed in samples from various environments are presented, along with the information such nanoparticles provide regarding their potential source. For example, some particles only exist at the nano-level and would otherwise go undetected, such as pyrogenic silica, which may be used as a filler in cement and other building materials, or as an anti-caking agent for aerosolized biological weapons. However, nanoparticles may also provide an additional level of comparison, and in some cases, mirror what is found in larger size fractions. In the study of environmental samples, many particles present in larger size fractions are also observed in the sub-micrometer sized fraction.

This illustrates that even when ordinary trace evidence is not present, nanoscale trace evidence may be. This approach and the results of this portion of the research has been written up for submission to the journal "Geoscience" for a special issue on "Forensic Geoscience." It will be submitted for publication in late December 2018.

Individual Particle Types

Before it was possible to assemble the generalized approaches to subvisible and nanoparticle analysis discussed above, it was necessary to explore these topics as they relate to specific types of particles. Several different materials were explored, which included: glass microspheres, toner particles, 3D printer dust, and free titanium dioxide. Each of these particle types was selected for a different reason, as detailed below:

- Glass microspheres span a range of sizes from a few micrometers to several millimeters. They were selected because glass is a commonly examined type of trace evidence, yet microspheres, which are used in an immense range of applications from road paint to cosmetics, are almost entirely missed as trace evidence due to their small size and (typical) transparency.

- Toner particles were selected as an organic particle type that is in the low micrometer to submicrometer size and contains highly engineered nanoscale features. Toner particles are also extremely common due to the prevalence of laser printers and copiers. One of the goals of this analysis was to understand whether such particles and their nanoscale properties could provide probative information.

- 3D printer dust is of topical interest due to the recent court cases involving petitions for the free distribution of 3D printed weapons plans. This work studied the release of 3D printer dust and included the characterization and identification of 3D printer dust particles produced by printers using the two most common thermoplastic filaments: polylactic acid (PLA) and acrylonitrile butadiene styrene (ABS).

- Titanium dioxide represents the most common white pigment and a submicron sized particle. Both free and bound (in a matrix) titanium dioxide is found in a diverse range of applications. Titanium dioxide was explored as a proxy for other nanoparticles in order to evaluate nanoparticle sample preparation approaches, and to understand if the most common white pigment is prevalent in background environmental samples.

Ultimately, each of these individual particle type studies have been written up for publication. More detail about each of these topics follows.

Glass Microspheres

Microspheres are used in a variety of applications and span a range of length scales (~1 µm to 1 mm), compositions (*e.g.* silica, soda-lime glass) and morphologies (*e.g.* solid or hollow). They are found in a variety of commonly encountered products, yet their size, transparency, and shape can make them difficult to find or easy to overlook. Their presence either as free

particles or as particles embedded within a matrix is indicative of certain products or activities. This research focuses on the ways in which these particles can be recognized, characterized, compared, and interpreted in a forensic science context. Microspheres may be used comparatively (to associate samples) or in an investigative fashion (to provide constraints on the source of unknown dusts, residues, or other materials containing such particles).
Over fifty examples of microspheres used in various products have been characterized, and include details about their size, elemental composition, morphology, relative refractive index, and autofluorescence. The results indicate that microspheres can be located in products and grouped into various categories based upon the above characteristics. Furthermore, the specific properties of individual microspheres can be used to constrain the potential end-use of such products, making them useful not only in comparison but also in investigative matters.

Copy and Printer Toner Particles

To explore the potential evidentiary value of toner particles, a total of 53 toner samples were collected from known printer cartridges and characterized by various microanalytical techniques to establish the properties most useful for recognition. Samples were also collected from various locations in the laboratory to assess the incidence of toner particles in different environments. By light microscopy, toner can be recognized on the basis of particle size and shape, as well as color. Further examination of the micromorphology in the FE-SEM reveals characteristic morphologies and differences in surface texture and shape among toner sources. Raman microspectroscopy provided chemical identification of the pigment (or pigment class), and in some cases also gave indication of the polymer component. While blue and black pigments remained constant among toner varieties that were studied (copper phthalocyanine and carbon black, respectively), variation in yellow and magenta pigments was observed. Analysis of dust samples collected from various environments demonstrated that while toner is consistently present near printers, it also can be found on surfaces at increasing distances from the printer. Ultimately, toner particles can be located, characterized, and to some extent, discriminated, using a suite of microanalytical methods applied to samples that could be encountered in forensic casework.

3D Printer Particles

Dust samples were collected from a total of 36 Fused Deposition Modeling (FDM) 3D printers, which deposit successive layers of thermoplastic polymers to create three-dimensional objects. The printers represent a number of makes, models, and filaments (polymers) used in both academia and industry. Sample collection parameters have been established, including the evaluation and efficacy of the matrix (cotton vs. polyester flocked swabs) and particle release strategies (scraping vs. sonicating). Dust samples of the two primary 3D printer polymers, polylactic acid (PLA) and acrylonitrile butadiene styrene (ABS), have been analyzed and characterized with the polarized light microscope (PLM), fluorescence microscope, field emission scanning electron microscope (FE-SEM), energy-dispersive x-ray spectroscopy (EDS), Fourier-transform infrared microspectrometer (FTIR), Raman microspectrometer, and pyroprobe-gas chromatograph/mass spectrometer (py-GC/MS). ABS particles can be detected in dust samples using the typical forensic microscopical and instrumental techniques mentioned above. However, PLA particles could only be detected chemically via py-GC/MS.

There are indications the PLA particles are present in the dusts, but the particles are too small for conclusive identifications.

Titanium Dioxide

Although it is a naturally-occurring mineral, TiO_2 also exists as a manufactured product that has seen widespread use as a filler, pigment, delustrant, and photocatalyst in paints, paper, plastics, foodstuffs, and coatings. Experimental protocols have been developed to optimize sample collection, processing, and identification techniques for submicron TiO_2 particles in dusts and soils. Background levels have been described for various environments and examples of soils from our collections. The established protocol was applied to microvacuum filter samples and tape lifts of dusts (typical forensic lab collection matrices) in order to provide information on the occurrence, morphologies, and particle size distributions of TiO_2 for a particular type of dust analyzed from a given environment.

Transfer Characteristics of a Fluorescent Detection Spray

Given that most trace evidence that is processed at present is visible – either to the unaided eye or under a stereomicroscope, issues of contamination, background levels, and unintentional transfer are minimal. However, when dealing with populations of particles that cannot be visually observed by these approaches, these issues (contamination, background levels, and unintentional transfer) must be treated. Micronized zinc sulfide is commonly used as a fluorescent tracer in police investigations. Subvisible particles of zinc sulfide, ~2 – 5 µm in diameter, comprise the fluorescent indicator component, and these particles can be used as visual illustrations of contact transfer between two objects. Evidence of contact between objects may be visualized through illumination by ultraviolet light, which causes luminescence of the powder. This research provides a microanalytical characterization of the zinc sulfide particles, and offers approaches by which this powder (or other fine particles) may be detected and specifically identified in quantities from major to trace. The study also illustrates the ease of higher order cross transfers (at least up to the tenth order). The results of research have implications for both laboratory analysis and for the validity of police investigations have been explored. This work was published in Forensic Science International.

Databases and Collections

The above research has added to the PI's understanding of reference collections and databases. The importance of these tools to forensic work was catalyzed through a request by NIST to give a keynote talk on the importance of collections and databases in trace evidence. Using lessons from research from this grant, the talk was compiled into an article which has been accepted for publication in the Microscope journal.

Practical Incorporation of this Research into Bench-level Analyses

In addition to the particle-specific analyses, a forensic case study involving the investigation of a subvisible paint particle was performed as an example of how a forensic lab may begin to incorporate subvisible particles into current analysis schemes. Forensic paint comparisons are generally conducted on samples which, while small relative to their sources, are still visible to the unaided eye and are thus readily located and analyzed. However, a more detailed examination of candidate surfaces can yield questioned samples even when such traces are not visible to the unaided eye. While certain analytical details, such as layer structure or a pure FTIR spectrum may not be possible due to the limited size of such samples (as a result, for instance, of the transfer process), a detailed analysis can provide sufficient analytical data to arrive at a probative result. In one such case example, a 40 μm paint transfer was observed on the surface of another paint sample. Using a combination of stereomicroscopy, polarized light microscopy, infrared microspectroscopy, and Raman microspectroscopy, all conducted on a single, minute subsample (~20 μm), it was possible to state that the paint source was automotive in nature and to demonstrate several independent, positive points of comparison with a suspected source. This conclusion is based upon a combination of the polymer, microscopic texture, and pigment package (which included three specifically identified pigments). This approach demonstrates a) the potential for improved detection limits when searching for a questioned sample, b) the potential benefit of higher resolution analyses on samples that would be traditionally labeled as “sample-size limited,” and c) the value of case-specific interpretation over standardized, one-size-fits-all reporting templates. This practical example illustrates that such approaches are achievable by the bench-level forensic scientist, and that qualitative results can be interpreted and presented to the jury in a scientifically valid and forensically probative manner outside of a statistical paradigm of interpretation.

Scientific Research Papers and Presentations

Subvisible and nanoparticles comprise an entirely new class of forensic evidence, one which requires a new way of thinking about sample collection, analysis, and interpretation. As mentioned previously, the initial research design included the creation of an electronic database containing brief descriptions of subvisible and nanoparticles that may be encountered as forensic evidence. Because a short database entry would not allow for detailed explanations, comparisons between related or similar particles, or information about challenges and nuances that are distinct to certain particles, the database was reimagined as a collection of scientific research papers and presentations at forensic science conferences. In this way, the various approaches to nanoparticle analysis, including sample collection parameters, data interpretation, and background contaminant studies, can be explained in detail with figures, tables, and charts. A one-page database entry for a finite number of particles is simply too limiting, and could not fully capture every subvisible or nanoparticle that may be encountered by a forensic analyst. It is therefore more effective to offer detailed analyses of several different types of particles, including methods for incorporating this into typical forensic lab workflow, and to present that information in a research paper format. Thus, the forensic analyst may form an understanding of the potential value of all nanoparticles, and not be limited to those that are catalogued in a database. As such, 27 research papers and presentations at scientific meetings constitute the final product of this research project. A full listing is available in Appendix I.

Implications for Criminal Justice Policy and Practice in the United States

Presently, the subvisible particle class of evidence goes largely, if not entirely, unused. Its potential significance has already been recognized, as demonstrated through numerous cases analyzed by our laboratory and others that embrace this approach to trace evidence. It is clear that as the use of nanotechnology increases, nanoparticles and nano-features in and on microscopic particles of trace evidence will also continue to increase in both number and diversity. We believe that the program of research presented here offers a great deal of advancement towards addressing the major issues currently limiting the use of subvisible and nanoscale evidence in the forensic laboratory.

This research provides a foundation that permits immediate application to forensic casework, serves as a solid base upon which future research in this nano-trace evidence can develop, and provides guidance to trace evidence laboratories when considering the next generation of laboratory equipment purchases. In a broader sense, we believe that the use of smaller-scale trace evidence will increase the relevance of trace evidence sections in the criminal justice system. Finally, the fluorescent tracer work provides specific guidance for police and other investigators in better utilizing a common police tool (fluorescent tracer spray).

Contributions to Crime Laboratories

Subvisible particle analysis is, at present, generally limited to a handful of specialized laboratories or specialized categories of evidence (e.g., GSR). Given the prevalence of particles in this size range and the manufacturing developments impacting materials at the micro- and nano-scale, the importance of such particles and features will only become greater (e.g. paint layers less than 20 µm are common). This is intended to provide practical guidance to crime laboratories to assist them with developing ways to exploit these smaller particles. As detailed in Appendix I, the results of this research have been presented at a number of scientific meetings and workshops that crime laboratory personnel have attended. In addition, the results of this research will ultimately be published in peer-reviewed journals.

Appendix I: Scholarly Products

Abstracts for each of these papers are provided in Appendix II. The status of each publication is listed after the title.

Publications (including those in preparation or submitted)

Palenik, C.S., Brinsko-Beckert, K., Insana, J., and Palenik, S.J. (2018) Analytical and transfer characteristics of a fluorescent detection spray: Implications for subvisible and nanotrace particle transfers. Forensic Science International Volume 286, May 2018, 96-105. https://doi.org/10.1016/j.forsciint.2018.03.007.

Palenik, C.S. (accepted, 2018) The role of collections in Trace Evidence. The Microscope.

Palenik, C.S. and Palenik, S.J. (submitted) A process for the preparation of tape lift samples for the study of subvisible and nanoparticles. Forensic Science International.

Brinsko-Beckert, K.M., Palenik, C.S., and Palenik, S.J. (in final review). A method for the isolation and characterization of nanoparticles in the forensic laboratory. Submission to the Journal Geoscience – a special issue on Forensic Geology, due mid-December 2018.

Palenik, C.S., Groves, E.G., Insana, J., and Palenik, S.J. (in final review) Forensic analysis of subvisible paint particles. Submission to Journal of Forensic Science anticipated in mid-Dec 2018.

White, K.M. and Palenik, C.S. (in final review). A Forensic Study of Known Toner Particles. Submission anticipated in late December 2018.

Brinsko Beckert, K.M. and Palenik, C.S. (in final review). The Forensic Analysis of 3D Printer Dust. Submission anticipated in late-December 2018.

Brinsko Beckert, K.M. and Palenik, C.S. (in preparation). Nanoparticles as trace evidence: Recognition and exploitation in forensic laboratories. Submission anticipated in first quarter 2019.

Nytes, B.N. and Palenik, C.S. (in preparation) Applications of glass microspheres as forensic trace evidence. Submission anticipated in 2019.

Conference Presentations and Workshops

Palenik, C.S. (2019, planned) Nanotrace in forensic science. Forensic Technology Center of Excellence Webinar.

Palenik, C.S. (2018) Advanced topics in forensic microscopy. Talk given at the Bundeskriminalamt (German Federal Crime Laboratory) in Wiesbaden, Germany.

Palenik, C.S. (2018) Nanotrace Evidence in Forensic Investigations. National Association of Criminal Defense Lawyers. Making Sense of Science XI: Forensic Science and the Law. Las Vegas, NV.

Palenik, C.S. (2018) Applications of Raman Spectroscopy for Trace Evidence. American Academy of Forensic Science 70th Annual Scientific Meeting, Seattle, WA. Among other topics, the workshop will feature a lecture by Dr. Palenik on the applications of Raman microspectroscopy to subvisible particles.

Palenik, C.S. (2018) High Order Trace Transfers: Considerations for the analysis of subvisible and nanoparticles. American Academy of Forensic Science 70th Annual Scientific Meeting, Seattle, WA.

Palenik, C.S. (2018) Fulgurites in litigation. American Academy of Forensic Science 70th Annual Scientific Meeting, Seattle, WA.

Brinsko Beckert, K.M. and Palenik, C.S. (2018). Nanoparticles as trace evidence: Part I. Recognition and collection. Poster presentation at PittCon 2018, Orlando, FL.

Brinsko Beckert, K.M. and Palenik, C.S. (2018). The Forensic Analysis of 3D Printer Dust Particles. Poster presentation at PittCon 2018, Orlando, FL.

White, K.M. and Palenik, C.S. (2018). A Forensic Study of Known Toner Particles. Poster presentation at PittCon 2018, Orlando, FL.

White, K.M. and Palenik, C.S. (2018). Applications of Glass Microspheres as Forensic Trace Evidence. Poster presentation at PittCon 2018, Orlando, FL.

White, K.M. and Palenik, C.S. (2017). A forensic study of known toner nanoparticles. Oral presentation at Inter/Micro 2017, Chicago, IL.

Brinsko Beckert, K.M. and Palenik, C.S. (2017). The forensic analysis of 3D printer dust particles. Oral presentation at Inter/Micro 2017, Chicago, IL.

Palenik, C.S. (2016). Counterfeit materials and their relation to forensic science. Oral presentation at the Interpol Forensic Science Managers Symposium, Lyon, France.

Palenik, C.S. (2016). Advanced Trace Evidence Analysis. Workshop presentations at the Asian Forensic Sciences Network Annual Meeting 2016, Bangkok, Thailand.

Palenik, C.S. (2016). The role of collections in trace evidence analysis. Oral presentation at the Trace Evidence Data Workshop: Improving Technology and Measurement in Forensic Science. National Institute of Standards and Technology (NIST) Forensic Science Research Program, Washington, D.C.

Palenik, S.J. and Palenik, C.S. (2016). The utilization of microscopy in developing investigative leads from the examination of microscopic trace evidence in forensic

investigations. Oral presentation at the Microscopy and Microanalysis 2016 Meeting, Dayton, OH.

Palenik, S.J. (2016). The role of fluorescence in the examination and analysis of dust traces. Oral presentation at Inter/Micro 2016, Chicago, IL.

Hargrave, K.H., Nytes, B.N., and Palenik, C.S. (2016). Applications of glass microspheres as forensic trace evidence. Oral presentation at Inter/Micro 2016, Chicago, IL.

White, K.M., and Palenik, C.S. (2016). Laser toner nanoparticles as forensic evidence. Oral presentation at Inter/Micro 2016, Chicago, IL.

Appendix II: Abstracts for Paper Submissions

Palenik, C.S., Brinsko-Beckert, K., Insana, J., and Palenik, S.J. (2018) Analytical and transfer characteristics of a fluorescent detection spray: Implications for subvisible and nanotrace particle transfers. Forensic Science International Volume 286, May 2018, 96-105.

Fluorescent detection sprays are applied to objects to elucidate evidence of contact. Billed as an invisible powder, evidence of contact between objects may be visualized through illumination by ultra-violet light, which causes the fluorescent tracer to luminesce. While the presence of the fluorescent powder on a suspect or object is often used as evidence of direct contact, the fine nature of the powder, which is comprised of sub-visible particles that are generally less than 10 µm in diameter, lends itself to higher-order transfers that do not necessarily involve the original object. Due to the small particle size and light-yellow color, the particles are generally invisible to the unaided eye in white light. This increases the opportunity for unwanted or unanticipated transfers (i.e., contamination). This article provides a microanalytical characterization of a common fluorescent tracer and the approaches by which this powder (or analogous powders) may be applied, detected, and specifically identified in quantities that range from major to trace. This research illustrates the ease of higher order cross-transfers (up to the 10th order) and the considerations necessary to maximize the evidentiary value of sub-visible particles and nanotraces, while minimizing the chances of cross-contamination.

Palenik, C.S. (accepted, 2018) The role of collections in Trace Evidence. The Microscope.

"The list is the origin of culture." This statement by author and philosopher Umberto Eco provides an indication of the central role that lists play in society. Certainly, lists and the physical collections upon which they are often based go back to the earliest days of forensic science. Even Sherlock Holmes enumerates "Upon the Distinction between the Ashes of the Various Tobaccos" in his fictional monograph. Yet while we live in a world more dedicated to organizing, characterizing, and standardizing than ever before, materials collections in trace evidence remain underdeveloped. There are certainly reasons for this, such as the difficulty of obtaining samples, organizing samples, a likely low frequency of direct casework application, and the expectation that errors will exist even in the most carefully curated collections. Yet, beyond the existential pleasure that many of us take in such collections, there is an inherent value both to casework and the continued development of the supporting science. Through our decades of experience curating various physical collections, applying these collections to cases, and firsthand experiences related to the limitations, uncertainties, and errors that may be associated with such collections, I hope to share our enthusiasm for the topic and the value it has brought to our own expertise in trace analysis.

Palenik, C.S. and Palenik, S.J. (submitted) A process for the preparation of tape lift samples for the study of subvisible and nanoparticles. Forensic Science International.

Tape lifts are commonly used as a substrate for the collection of microscopic trace evidence. While traces such as fibers, glass and paint can generally be readily recognized by stereomicroscopy, larger particles, such as these often represent a minor to trace fraction of the total particle load on a given lift. In contrast, smaller particles (broadly categorized as dust), which are frequently the dominant component on the tape, are rarely exploited as evidence. These particles may range in size from hundreds of micrometers down to nanometers and can span an even greater range of particle types. These minute particles are difficult, or sometimes impossible, to study by stereomicroscopy and a challenge to manipulate and isolate. In addition, when collected on tape, such particles become trapped in the adhesive, further complicating attempts to isolate all but the largest particles for further analyses. Here we present a practical process for improving the in situ microscopical analysis of dust particles collected on tape as well a method for isolating such particles for in-depth analysis. By adopting this approach, the largely ignored dust fraction, which includes subvisible and nanoscale particles, becomes more accessible for detailed examination and analysis.

A method for the isolation and characterization of nanoparticles in the forensic laboratory. (in final review). Submission to the Journal Geoscience – a special issue on Forensic Geology, due mid-December 2018.

Nanoparticles comprise a new subcategory of trace evidence that is often overlooked and underutilized in forensic science. Despite the fact that they are nearly ubiquitous in the environment and are found in a number of widely available and commonly used consumer products, from cosmetics to paint to food, they have rarely been exploited in casework. This may be due in part to a general lack of awareness regarding nanoparticles, as well as the fact that no protocols for their isolation and examination have yet been published in the context of forensic science. This paper details a procedure that may be employed in forensic laboratories to isolate nanoparticles for analysis and characterization, including recommended dispersion media, dilution requirements, and substrates. An atlas illustrating the types of nanoparticles that may be seen in soil samples is presented, along with information regarding the applicability, practicality, and use of nanoparticles as trace evidence.

Palenik, C.S., Groves, E.G., Insana, J., and Palenik, S.J. (in final review) Forensic analysis of subvisible paint particles. Submission to Journal of Forensic Science anticipated in mid-Dec 2018.

Forensic paint comparisons are generally conducted on samples which, while small relative to their source, are still visible to the unaided eye and are thus readily located and analyzed. Here we show that a more detailed examination of candidate transfer surfaces can capture materials (questioned samples), even when such traces are not visible to the unaided eye. While certain analytical details (such as the layer structure or a pure FTIR spectrum) may not be possible to collect due to the size and conditions of such samples, a detailed analysis of the sample characteristics that are analytically accessible may still provide sufficient analytical data to arrive at a probative result. Here we present the analysis of a suspected paint transfer, involving particles of paint as small as 40 µm in size. Using a combination of stereomicroscopy, polarized light microscopy, infrared microspectroscopy, Raman microspectroscopy, and SEM/EDS all conducted on a single, sub-sample of the original minute particle, it was possible to state that the paint source was automotive in nature and to demonstrate several independent, positive points of comparison with a suspected source. This conclusion is based upon a combination of the polymer compositions, microscopic texture, and pigment package (which included three specifically identified pigments in each sample). This work demonstrates a) the potential for improving detection limits when searching for a questioned sample, b) the potential benefits of higher resolution analyses on samples that would be traditionally labeled as "sample-size limited," and c) the value of case-specific interpretation over standardized, one-size fits all report templates.

White, K.M. and Palenik, C.S. (in final review). A Forensic Study of Known Toner Particles. Submission anticipated in late December 2018.

Modern printing toners represent a prime example of subvisible particles that can be easily transferred to hands, clothing, and other surfaces. To explore the potential evidentiary value of toner particles, toner samples were collected from known printer cartridges and characterized by various micro-analytical techniques to establish the properties most useful for recognition, identification, and comparison. Environmental samples (i.e., dust) were then collected from various locations at varying distances from toner-based printers, using both tape lifts and carbon adhesive stubs, to assess the possibility of detecting toner. By light microscopy, toner can be recognized on the basis of particle size and shape, as well as color. Further examination of the micromorphology in the field emission scanning electron microscope reveals characteristic morphologies and differences in surface texture and shape among toner sources. Raman spectroscopy provides chemical identification of the pigment (or pigment class) and, in some cases, also permits identification of the polymer component. While black and blue pigment chemistry remained constant among toner varieties that were studied (copper phthalocyanine and carbon black), variation in yellow and magenta pigments was observed. Analysis of dust samples collected from various environments demonstrated that while toner is consistently detectable in close proximity to printers (within 2 feet), it also can be detected in dust collected in nearby rooms. This research demonstrates that toner particles can be located, characterized, and discriminated, using a suite of microanalytical methods that are applicable to forensic casework.

Brinsko-Beckert, K.M. and Palenik, C.S. (in final review). The Forensic Analysis of 3D Printer Dust. Submission anticipated in late-December 2018.

3D printers are becoming increasingly efficient and economical, and thus more widespread and easily accessible to consumers and the general public. Previous research has documented the release of dust particles during the printing process. However, little is known about their morphology and other characteristic features. This study was undertaken as part of a federal research grant (NIJ Grant No. 2015-DN-BX-K033) to characterize these particles so that they may be collected, recognized, and analyzed appropriately. Samples were collected from a variety of 3D printers, representing both consumer- and commercial-grade models. These printers use thermoplastic filaments, typically polylactic acid (PLA) or acrylonitrile butadiene styrene (ABS), though others may be used (nylon, polyvinyl acetate, polyurethane, etc.).

Cotton or polyester-flocked swabs were used to collect dust from various surfaces within the printer chamber and surrounding areas up to 10 feet away. Particles produced from ABS filaments are most easily recognized based on color and rounded morphology via light microscopy; FTIR spectra of the particles confirmed the identification of the ABS polymer. Pigments and the ABS polymer matrix were also identified using Raman microspectroscopy. Dust from PLA printers consistently contained finer, submicron sized particles (relative to background levels) that could be observed by field emission scanning electron microscopy; however, the size of the particles precluded their specific identification as PLA. This presentation will detail the collection procedures employed to find, isolate, identify and compare 3D printer dust particles, and a discussion of their potential applications and limitations as forensic evidence.

Brinsko-Beckert, K.M. and Palenik, C.S. (in preparation). Nanoparticles as trace evidence: Recognition and exploitation in forensic laboratories. Submission anticipated in first quarter 2019.

The use of nanotechnology and engineering of nanomaterials has grown exponentially in the last decade, and has found widespread application across a number of disciplines, including biology, medicine, electronics, energy, optics, and materials manufacturing, among others. These nanoparticles and other subvisible particles are present in nearly all forms of existing trace evidence, yet currently the overwhelming majority of trace examinations focus exclusively upon larger particles. In this era where highly engineered nanoscale materials are being introduced at increasing rates, it is inconceivable that such materials are not being regularly examined as forensic evidence. Practical forensic research is currently being undertaken by the authors in order to systematically develop approaches for the isolation, analysis, and interpretation of particles on the nanoscale, effectively equating the sensitivity of trace evidence to that of DNA analysis. While the smallest particles in this range may require higher resolution instrumentation, the majority of these particles can be characterized effectively by applying the suite of microanalytical methods present in most trace evidence laboratories today (stereomicroscopy, polarized light microscopy, and scanning electron microscopy). Here we present the first part of our research: describing the relevance, classifications, and applications of nanoparticles, then following with information about how these particles can best be recognized and collected in a forensic science laboratory.

Nytes, B.N. and Palenik, C.S. (in preparation) Applications of glass microspheres as forensic trace evidence. Submission anticipated in 2019.

Microspheres are used in an increasing variety of applications, from personal care products to food to industrial applications. Glass microspheres represent a significant subset of the microsphere market and are encountered in cosmetics, paints, plastics, and building materials (among other applications). While they are used in a variety of consumer-grade products, their size, transparency, and shape can make them difficult to find or easy to overlook. For example, in solution, an isotropic, glass microspheres may be confused with an immiscible phase. Despite such difficulties, the size range (~5-1000 micrometers) and composition (glass), make them accessible and potentially useful indicators of products, activity, or associations. This presentation will cover the range of physical, optical and elemental characteristics of reference microspheres (obtained from manufacturers) and the ways in which glass microspheres can be located and characterized in industrial and consumer applications (e.g., cosmetics, spackle, and polymers). When present in dust microspheres may be encountered as free particles where they may be the sole basis of an association, or they may be encountered in a matrix (e.g., a polymer or ceramic) where they could be used to improve the significance of an association. The results from these analyses illustrate some of the ways in which microspheres can be located, characterized, and interpreted in the context of a forensic investigation.