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# Identification of Organic Colorants in Fibers, Paints, and Glazes by Surface Enhanced Raman Spectroscopy

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## CONSPECTUS

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Organic dyes extracted from plants, insects, and shellfish have been used for millennia in dyeing textiles and manufacturing colorants for painting. The economic push for dyes with high tinting strength, directly related to high extinction coefficients in the visible range, historically led to the selection of substances that could be used at low concentrations. But a desirable property for the colorist is a major problem for the analytical chemist; the identification of dyes in cultural heritage objects is extremely difficult. Techniques routinely used in the identification of inorganic pigments are generally not applicable to dyes: X-ray fluorescence because of the lack of an elemental signature, Raman spectroscopy because of the generally intense luminescence of dyes, and Fourier transform infrared spectroscopy because of the interference of binders and extenders. Traditionally, the identification of dyes has required relatively large samples (0.5−5 mm in diameter) for analysis by high-performance liquid chromatography. In this Account, we describe our efforts to develop practical approaches in identifying dyes in works of art from samples as small as 25 $\mu$m in diameter with surface-enhanced Raman scattering (SERS).

In SERS, the Raman scattering signal is greatly enhanced when organic molecules with large delocalized electron systems are adsorbed on atomically rough metallic substrates; fluorescence is concomitantly quenched. Recent nanotechnological advances in preparing and manipulating metallic particles have afforded staggering enhancement factors of up to $10^{14}$. SERS is thus an ideal technique for the analysis of dyes. Indeed, rhodamine 6G and crystal violet, two organic compounds used to demonstrate the sensitivity of SERS at the single-molecule level, were first synthesized as textile dyes in the second half of the 19th century.

In this Account, we examine the practical application of SERS to cultural heritage studies, including the selection of appropriate substrates, the development of analytical protocols, and the building of SERS spectral databases. We also consider theoretical studies on dyes of artistic interest.

Using SERS, we have successfully documented the earliest use of a madder lake pigment and the earliest occurrence of lac dye in European art. We have also found several examples of kermes and cochineal glazes, as well as madder, cochineal, methyl violet, and eosin lakes, from eras ranging from ancient Egypt to the 19th century. The ability to rapidly analyze very small samples with SERS makes it a particularly valuable tool in a museum context.

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## Introduction

Surface-enhanced Raman spectroscopy (SERS) is currently at the center of a powerful renaissance. Benefiting from the exponential growth and interdisciplinary nature of scientific investigations at the nanoscale level, hundreds of articles, journal special issues, and books are being published each year on this topic.[1,2]

The first observations of surface Raman signals from pyridine on electrochemically roughened Ag electrodes,[3] followed by the realization that they were enhanced by a factor of $10^6$ generated considerable excitement.[4,5] This phenomenon, now known as SERS, promised ultrasensitive detection and identification of molecules. That promise has now been realized. Ten years after the discovery of SERS, the first isolated identification of madder in a historical textile was reported, also using Ag electrodes as the SERS active substrate.[6] Today's developments in nanotechnology and nanofabrication of a wide variety of robust plasmonic substrates have allowed the achievement of enhancement factors up to $10^{14}$ allowing detailed vibrational fingerprinting down to the single molecule.[7] It is currently accepted that such enhancements are related to chemical and electromagnetic mechanisms,[8] although their relative importance is the object of a lively debate. The chemical mechanism implies resonance effects within the target molecule and charge-transfer effects between molecular orbitals and the conduction band of the noble metal substrate. The electromagnetic mechanism, believed by most to be the dominant contribution, is related to the collective oscillation of the conduction electrons in noble metals that creates a localized surface plasmon resonance (LSPR) induced by the incident laser light. The Raman signal of Raman active molecules in the vicinity of such LSPR is exponentially enhanced. All these phenomena endow SERS with extreme sensitivity.

It is only in the past 6 years that the potential of SERS for the ultrasensitive identification of molecules with otherwise weak inelastic scattering probabilities, such as natural organic materials used as artists' colorants, has been fully realized and exploited. In this Account, we describe our efforts to develop SERS as a sensitive and selective tool for the identification of dyes in works of art from samples as small as a single grain of pigment or a fragment of dyed fiber a few micrometers in length.

## Dye Analysis in Cultural Heritage

Natural dyes have been used since antiquity to color textiles and to manufacture pigments; the discovery of mauveine and the explosion of synthetic colorants chemistry[9,10] (Figure 1) in the second half of the 19th century further increased the importance of dyes. Due to their high tinting strength, organic dyes are usually present in very low concentrations in works of art, offering a substantial analytical challenge.

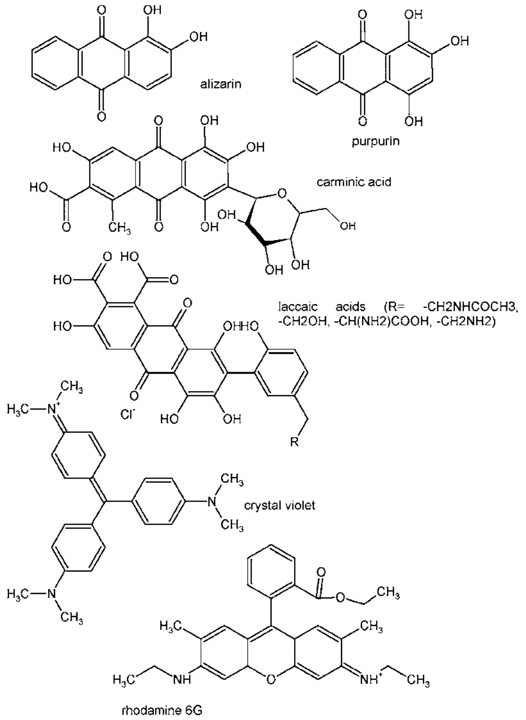

**FIGURE 1.** Some natural and synthetic dyes of key artistic interest.

Unambiguous, rapid, and ultrasensitive detection of natural and early synthetic dyestuffs is of paramount importance to address questions on the conservation, context, and chronology of works of art. Dye analysis is relevant to the long-term preservation of artworks because many colorants are not stable to prolonged light exposure.[11] Many works by Vincent Van Gogh (1853−1890) were painted with the bright pink pigment eosine (the K or Na salt of 2,4,5,7-tetrabromofluorescein, sold as an artist's pigment under the name "geranium lake"), which now appears completely faded.[12] Similarly, familiar masterpieces by artists such as Mary Cassatt (1844−1926) have lost some of their vibrancy and on occasion part of their meaning due to the fading of their cochineal carmine, aniline, and redwood dyes.[13] Even when the dyes are macroscopically faded, the detection of trace amounts of colorant, often preserved in interior painting layers or protected by cellulosic fibers in works of art on paper (Figure 2), can inform art historians and conservators on the original color scheme of artwork and provide new insights into the artist's intention.[14]

The detection of specific colorants can also provide important information about the historical context of the works of

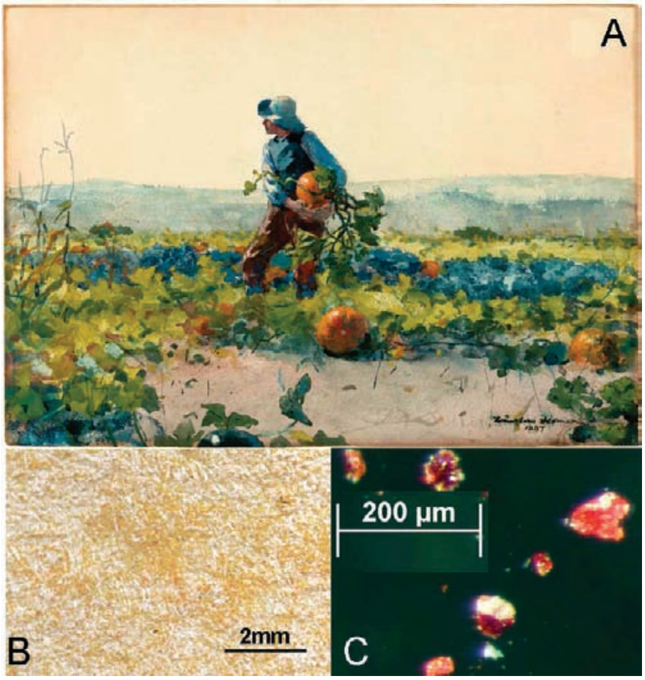

**FIGURE 2.** (A) Winslow Homer "For to Be a Farmer's Boy" 1887 (Gift of Mrs. George T. Langhorne in memory of Edward Carson Waller, AIC 1963.760). This image had long puzzled scholars due to the seemingly unfinished and flat sky in a highly finished work. (B) Optical stereomicrograph showing an area at the upper left corner of the watercolor, displaying a few colored particles trapped under the paper fibers. (C) Photomicrograph of pigment grains taken from panel B. SER spectra identified the lake pigments as Indian purple (a cochineal carmine lake precipitated with copper salts) and purple madder.

art or the technological ingenuity of the people who created them or can assist in elucidating trade routes in antiquity. Distinguishing cochineal (from *Dactylopius coccus* Costa), kermes (from *Kermes vermilio* Planchon), and lac dye (from *Kerria lacca* Kerr) whose chromophores are carminic, kermesic, and laccaic acids, respectively, is extremely relevant in art historical research because kermes from Southern Europe and lac dye from South East Asia were widely used until the 16th century when cochineal, a dyestuff with much higher dye yields, was imported from the Americas. Lastly, dye identification can provide useful clues to the origin and dates *ante quem* or *post quem* an artifact was created, possibly leading to the uncovering of forgeries.

Major constraints for the analysis of organic dyes in works of art are the weak scattering and high fluorescence of the biomolecules, invariably incorporated in matrices such as paint layers or historic textiles that are themselves chemically complex. Dyes are often incorporated into an inorganic substrate (alumina, calcium carbonate or sulfate minerals, clays, etc.) to make a lake that can be used as a pigment or fixed to textile fibers by means of bridging inorganic ions (mordanting), requiring extraction of the colorant prior to analysis. Finally, limited (a few hundred micrometers or less) or no sampling at all is typically allowed from works of art, and because the dyestuff is present in very high dilution, noninvasive or ultrasensitive techniques of analysis are preferred. UV–vis absorbance spectroscopy,[15] fluorimetry,[16,17] FTIR,[18] NIR,[19] and Raman spectroscopy have all been investigated for dye analysis. However, electronic spectroscopy methods tend to have poor specificity, and data interpretation is challenging for all the above-mentioned spectroscopic methods. Normal Raman spectroscopy has been successfully used to characterize mineral pigments and some 19th to 20th century synthetic colorants.[20–22] In general however, the fluorescence of the majority of dyes, or of the matrices in which they are found, is a major obstacle to normal Raman detection.

To date, high-performance liquid chromatography (HPLC) has demonstrated the most consistent results for dye analysis.[23,24] While chromatographic methods are highly selective, sensitive, and specific, they require the removal of approximately 1 mg of sample or 0.5–5 mm of dyed fiber, which is a very large amount for rare and priceless works of art.

SERS significantly reduces the amount of material needed for analysis compared with HPLC, so when the genus of the plant or species of insects used to derive the colorant is not an important question to address or when the sample size required for HPLC is not available, SERS provides a very powerful analytical alternative for art applications.

## SERS Research in Cultural Heritage

Although SERS has been around for almost four decades, it is only recently that sustained efforts have been devoted to its application to cultural heritage objects: these are the subject of recent reviews.[7,25] Most work to date has been carried out on reference materials, leading to the publication of high-quality, detailed spectra of anthraquinones, flavonoids, and indigoid dyes.[26–30] Fewer are the studies on alkaloids, curcumin, redwoods, orchil dyes, and melanin sepia,[31–33] but researchers are constantly expanding the range of dyes probed with SERS, so this gap will probably be filled soon. In addition to providing useful reference spectra, these studies have also investigated aspects such as complexation geometry, influence of pH, and orientation of the analyzed molecules with respect to the noble metal plasmonic substrate.

Because the orientation of the molecule with respect to the SERS-active surface results in selective enhancement of certain peaks, interpretation of the spectra can be very difficult. While *ab initio* computational methods can be used to assign normal modes and interpret SERS data,[27,29,31,32] spectral databases of reference colorants and an enhanced understanding of the interactions of the dyestuffs of interest with various SERS-active substrates are necessary. Going from microscope slide to museum case introduces further challenges and brings about the ambitious goal of obtaining high-quality spectra on single pigment grains or minuscule clippings of textile fragments from actual art objects, which are extremely complex systems.

## "One Size Does Not Fit All": Tailored SERS Substrates for Art Analysis

The practical application of SERS to cultural heritage studies has required extensive design and testing of optimized plasmonic nanostructured surfaces and colloids for analysis of various classes of art materials. The ultimate goal is to achieve dramatic fluorescence quenching and significant enhancement of the weak Raman scattering effect for the target analytes, while minimizing the amount of sample material required and its handling. In our work, we have developed parallel approaches that have led to the successful detection of microscopic amounts of biomolecules in extremely aged and complex matrices like archeological objects, faded pastels, and glaze layers in paintings.

**Solid State Substrates: Silver Island Films (AgIFs) and Silver Films over Nanospheres (AgFONs).** The use of silver island films (AgIFs) as SERS substrates to identify and characterize several reference red dyes has been demonstrated.[28,34] Moving from model systems to real world applications, however, requires optimization of the methodology. The nonuniformity of AgIFs substrates hinders the acquisition of consistent spectra, thus rendering collection of high-quality data a time-consuming operation. A significant improvement is offered by the use of silver films over nanospheres (AgFONs), which offer high reproducibility and extreme tunability, as demonstrated also for the quantitative detection of analytes such as biowarfare agents (anthrax) and glucose.[35−37] AgFON fabrication involves drop-coating polystyrene or $SiO_2$ nanospheres onto a clean glass substrate and then depositing ∼200 nm of Ag over the nanospheres. AgFONs not only are easily fabricated and economical but are composed of a highly ordered, uniform surface, which provides highly consistent SER spectra. The LSPR of a AgFON can be tuned simply by changing the size of the nanospheres, ensuring that they can be excited with various laser wavelengths and easily matched with the wavelength of visible absorption maximum of the studied dyes, giving rise to surface-enhanced resonance Raman effects (SERRS).

We have used these substrates for the detection of subnanogram quantities of the red dyes alizarin, carminic acid, and laccaic acid individually and in mixtures. Pairing a red laser excitation line ($\lambda_0 = 632.8$ nm) with AgFONs fabricated with 390 nm diameter $SiO_2$ spheres and a green laser line ($\lambda_0 = 532.15$ nm) with 300 nm diameter $SiO_2$ spheres, we achieved preresonance and resonance conditions, respectively, for these red dyes, leading to a 2 orders of magnitude enhancement of the Raman signal, compared with nonresonant measurements performed with a 785 nm laser excitation line coupled with 550 nm diameter $SiO_2$ spheres.[38] We also used AgFONs to analyze crocin, alizarin, purpurin, and carminic acid reference dyestuffs;[39] however when compared with the spectra obtained from the same dyes with silver colloids deposited on silica gels, the AgFON spectra displayed larger fluorescence backgrounds and inferior band resolution. The tunability advantage of the AgFONs in fact is often offset by substrate contamination with carbon arising during the silver deposition phase. It can be hypothesized that the organic dyes have lower binding affinity than the carbon contamination, which competes with the target molecules to occupy surface active sites (Figure 3). Contamination issues notwithstanding, the use of AgFONs is worth further exploration, given the extreme tunability of the substrates: promising strategies for cleaning substrates have recently been reviewed.[40]

**Silver Colloids.** Silver colloids have been by far the most popular substrate for the identification of dyes in cultural heritage objects with SERS. By use of mostly the standard Lee−Meisel citrate-reduced Ag colloids[41] or modifications thereof,[42] versatile SERS substrates can be easily prepared and used by any museum conservation departments with access to a Raman microscope.

Silver colloids have been used at the Metropolitan Museum of Art to investigate samples from textiles, works of art on paper, polychrome objects, and paintings, ranging in age from antiquity to the 19th century. While most of the work carried out at the Metropolitan Museum on mordant dyes and lake pigment has been based on a preliminary sample treatment step (a gas−solid nonextractive hydrolysis of the dye−metal complex carried out by exposing the sample to a HF saturated atmosphere), recent work at the Art Institute has successfully demonstrated that the

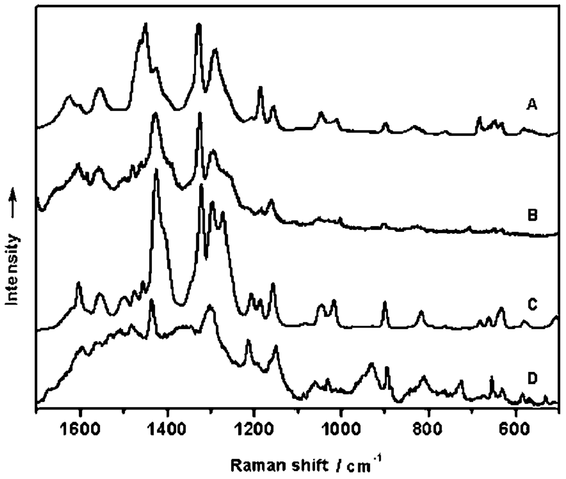

**FIGURE 3.** Comparison of AgFON and Ag colloid: (A) alizarin (1.0 × $10^{-3}$ M) on silica gel substrate with citrate-reduced silver colloids; (B) alizarin (1.0 × $10^{-3}$ M) on AgFON substrate fabricated with 390 nm $SiO_2$ spheres; (C) alizarin-dyed reference fiber (wool); (D) alizarin present in a minute fragment from a Peruvian textile (A.D. 800−1350; AIC 1955.613). Spectra C and D were obtained by direct application on the fiber of a colloidal paste obtained by centrifugation of Ag colloids prepared via the standard Lee and Meisel procedure. $\lambda_0 = 632.8$ nm, 5 $\mu$W power at the sample, 1 s acquisition for all spectra.

textile fibers can also be analyzed by direct application of colloids, without pretreatment. Direct SERS with citrate-reduced silver colloids was used to identify lac dye on a red woolen fiber from an Ottoman carpet dating to the late 16th/early 17th century (AIC 1964.554; gift of Mrs. Siegfried G. Schmidt). From a historical perspective, this result is important because it documents a rare and early finding of a European textile dyed with the Asian colorant lac. Similarly, other investigators have developed methods to synthesize colloids directly on the sample prior to analysis with immobilized photoreduced Ag nanoparticles obtained by prolonged laser irradiation of $AgNO_3$ solution.[43] The direct deposition of citrate-reduced colloids may better preserve the integrity of the substrate by dramatically reducing the risk of photooxidative degradation of the dyes induced by long laser irradiation. Ultimately though the pretreatment approach does increase the sensitivity of the method, making it possible to address more problematic samples, such as paints and glazes, in which the dye is diluted and dispersed in a vehicle such as a drying oil or a protein binder. The use of silver colloids finally is also extremely well suited for applications of hyphenated techniques such as thin layer chromatography (TLC) SERS that allow separation and molecular speciation of colorants in mixtures.[39,44]

## Recent SERS-Driven Discoveries from the Art World

While work on model systems now abounds, fewer are the published applications to actual works of art. The parallel explorations conducted in our laboratories have obtained successful results with a wide variety of art objects from different cultures, time periods, artistic techniques, and states of preservation (a notable example of practical application of SERS outside of our work is the identification of purpurin in Roman cosmetics by Van Elslande et al.[45]). Some examples of the work carried out on objects from the collections of The Metropolitan Museum of Art and of the Art Institute of Chicago are offered in the following sections.

**Textiles.** SERS cannot be compared with HPLC, in that it does not separate the various components of a dyestuff, yet its ability to rapidly analyze much smaller samples is remarkable in a museum context. In the course of the conservation of a 16th century Netherlandish tapestry ("The maiden's companion signals to the hunters", from "The hunt of the unicorn" series; South Netherlandish, ca. 1495−1505. The Metropolitan Museum of Art, 38.51,1.2; Gift of John D. Rockefeller, Jr., 1937), shown in Figure 4, a single wool fiber measuring 1 mm by approximately 50 $\mu$m was removed for analysis. After treatment with HF vapor, a drop of Lee−Meisel polydisperse colloid was added to the sample, and the laser beam was focused on a small silver-covered spot on the surface of the fiber with a 20× objective (Figure 4). The spectrum clearly shows the presence of alizarin (Figure 5).[26] In all, the analysis took less than 30 min.

**Archaeological Objects.** The potential of surface-enhanced Raman as a microanalytical technique is well demonstrated by the successful identification of the dyestuff madder in a 25 $\mu$m sample from an ancient Egyptian painted leather fragment (Figure 6). The increase in sensitivity necessary to handle such a small sample was obtained by working at resonant excitation with the pink dye employing a 488 nm laser, using a new monodisperse, highly sensitive colloid obtained by reproducible microwave-supported glucose reduction of silver sulfate with sodium citrate as a capping agent, and pretreating the sample by exposure to HF in a microreactor to hydrolyze the dye−metal complex and maximize dye adsorption on the colloid.[42] In this case, the technique is more properly identified as surface-enhanced resonance Raman scattering (SERRS). The results of the analysis clearly show the quality of the data obtained with the procedure. The archeological and historical significance of the discovery is also remarkable: the detection of madder in the 4000 year old

**FIGURE 4.** "The maiden's companion signals to the hunters", from "The hunt of the unicorn" series; South Netherlandish, ca. 1495−1505. The Metropolitan Museum of Art, 38.51,1.2; Gift of John D. Rockefeller Jr., 1937. Detail.

Middle Kingdom leather fragment represents the earliest evidence so far for the chemical knowledge necessary to obtain a dye from a plant source and manufacture a lake pigment from it.

**Pastels.** Nineteenth century pastel sticks are mixtures of pure pigments or lakes, inorganic fillers (calcium carbonate and sulfates, kaolin clays, quartz and other silicates, barium sulfate, etc.), and low concentrations of binders such as plant gums, glues, beeswax, or oils. Pastels are also rarely varnished, although occasionally fixatives such as tree resins and other media are used. These characteristics make them relatively simple systems that can lend themselves to the application of direct, extractionless SERS with citrate-reduced silver colloids on single grains of lake pigments.

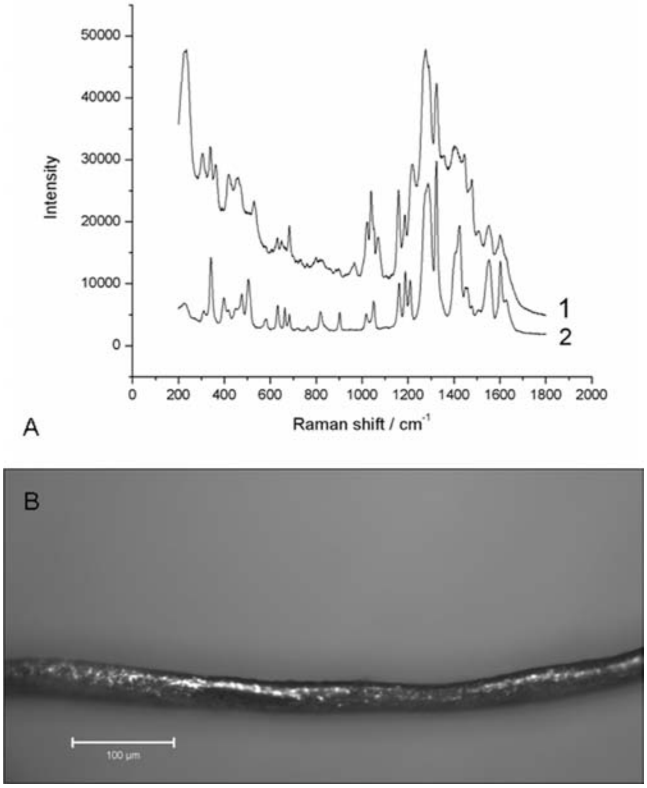

**FIGURE 5.** (A) Spectrum obtained from a HF-treated fiber sample from "The maiden's companion signals to the hunters" (1) and a HF-treated reference madder dyed fiber (2); $\lambda_0 = 785$ nm, 0.8 mW power (at laser), 20 s. (B) Reflected light micrograph of the fiber sample from "The maiden's companion signals to the hunters" treated with Ag colloid. The spectrum was obtained focusing the laser beam on a cluster of aggregated Ag nanoparticles (the highly reflective dots covering the fiber).

During the comparison of the components of a historical pastelbox belonging to the artist Mary Cassatt (Boston Museum of Fine Arts) and one of her pastel drawings depicting a young girl (Mary Cassatt, pastel study, "Sketch of Margaret Sloane, Looking Right"; gift of Laura May Ripley, AIC 1992.158), the extensive use of carmine lake was identified, mixed with whites and extenders and other pigments to obtain pastel sticks ranging from pale pink to fuchsia to lavender to purple. The dye component alizarin was also observed in mauve pastel sticks, matching what was found on a mauve-colored stroke in the girl's white collar. Additionally, the early synthetic colorants rhodamine B and rhodamine 6G were identified in the pastel box. A spectrum closely related to $\beta$-naphthol or a monoazo dye was also recorded on the historical pastels, but it could not be more specifically assigned to a definitive colorant. This colorant was also found in a sam-

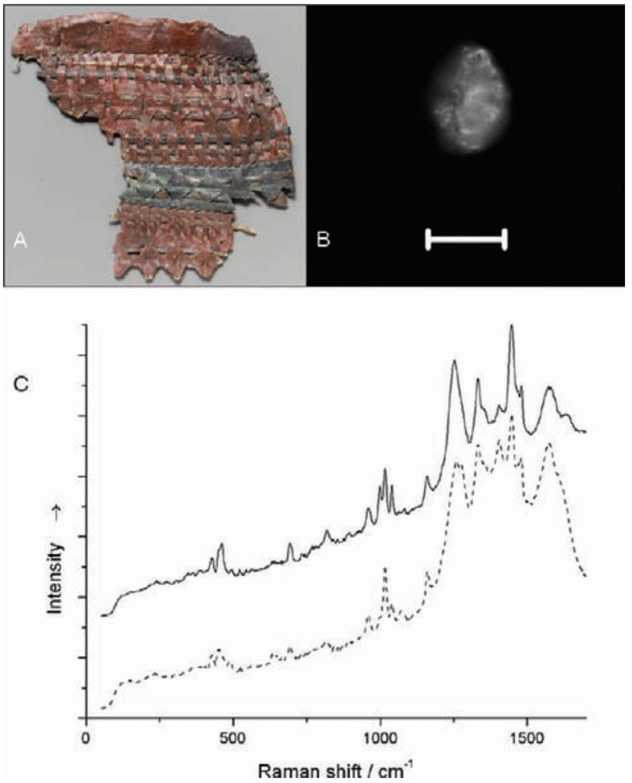

**FIGURE 6.** (A) Fragment of a quiver; Accession No. 28.3.5; Middle Kingdom, ca. 2124−1981 BC (H. 11 cm; W. 13 cm). MMA 1911−1912, Tomb MMA830, Thebes, el-Khokha, Upper Egypt; Rogers Fund, 1928. (B) Polarized reflected light photograph of sample removed from red painted area before HF treatment (scale bar = 20 $\mu$m). (C) SERRS spectrum of sample from Middle Kingdom leather quiver: solid line, spectrum of sample from red painted area; dashed line, spectrum of a 2nd century BC pink pigment from Corinth, Greece, previously identified by HPLC as a madder lake (mostly purpurin). Spectra were normalized and vertically shifted for ease of comparison, but no smoothing or baseline correction was employed. $\lambda_0 = 488$ nm, 0.25 mW power (at laser), 30 s.

ple from the fleshtone of the Mary Cassatt sketch (Figure 7).[46] The finding of similar colorants in the pastelbox and the sketch, especially some less common, early synthetic ones, is an important element should the authenticity of this artwork be called into question.

**Paintings.** Organic dyes are found in paintings mainly in two forms: as lake pigments, more or less used in the same way as inorganic pigments to create highly scattering and thus opaque layers, or as glazes, that is, fine dispersions of lakes into a transparent medium such as a drying oil. Glazes are translucent and are used to modify underlying colors and heighten the sense of depth in a painting. Both the presence of the oil matrix and the dilution of the dye represent substantial analytical challenges. The same method developed for the analysis of microscopic archeological samples was applied successfully to the analysis of glazes in paintings. While examining the painting "St. John the Baptist Bearing Witness" (Figure 8), attributed to the workshop of Francesco Granacci, Florence (ca. 1510), we removed a 50 $\mu$m sample of a red glaze. SERRS analysis of the sample revealed the presence of kermes,[42] a result consistent with the position of this anthraquinone dye as the main colorant for red glazes in Europe before the introduction of cochineal from the New World (Figure 8).

## Concluding Remarks

The work carried out in the last five years on the application of surface-enhanced Raman scattering to the analysis of cultural heritage has demonstrated the potential of the technique for the minimally invasive identification of artists' materials. In fact, the identification of dyes in ultramicroscopic samples of paints and glazes, at the level of specificity provided by Raman spectroscopy, is currently possible only by the methods described in this Account. SERS not only fills an important gap in the cultural heritage scientist toolbox: the application of SERS to works of art has emerged as the leading practical application of the technique.

At the Metropolitan Museum of Art and at the Art Institute of Chicago, SERS has been used in dozens of case studies involving textiles, polychrome objects, works of art on paper, and paintings. In several cases, particularly those involving textiles, SERS has been tested alongside high-performance liquid chromatography, providing an important validation for the emerging technique, but in many instances, SERS was the only technique that could be used due to the restrictions in sampling imposed by the nature of the objects studied.

SERS is still in its infancy as an analytical technique of rapid and routine applicability. While we have demonstrated that reproducible results can be easily obtained with little or no sample preparation, substantial work remains to be done to assemble robust and comprehensive spectral databases. In its current state, SERS does still require the removal of samples from works of art and suffers limitations in terms of spatial resolution. Opportunities for future research include the development of noninvasive approaches and the application of SERS to the analysis of single layers on stratigraphic cross sections. Bringing the probe directly to the artifact or sample without leaving residues could, for example, be achieved with tip-enhanced SERS (TERS) or advanced functionalized SERS-active optical fibers.

In perspective, further research into the capabilities of SERS for powerful selective detection of low-concentration analytes, combined with robust nanofabrication techniques and a thorough exploration of the theoretical underpinnings of the SERS

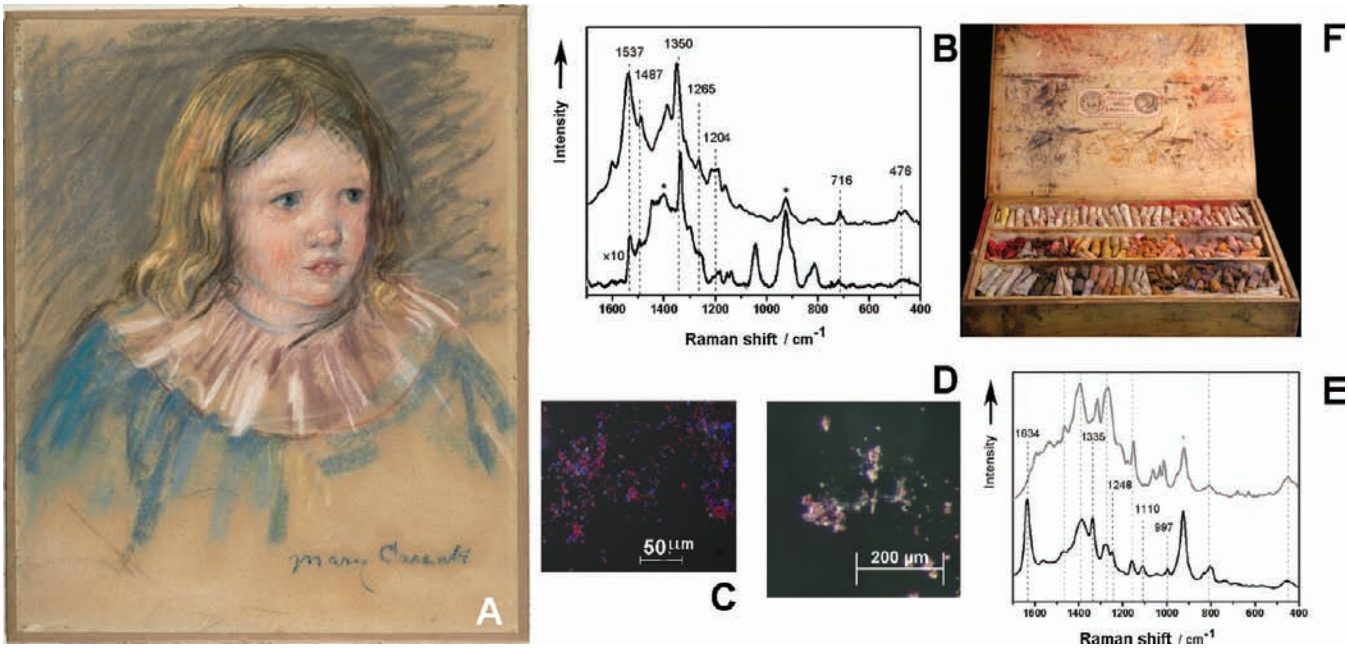

**FIGURE 7.** (A) Mary Cassatt, "Sketch of Margaret Sloane, looking right" (pastel on tan wove paper, Gift of Laura May Ripley, AIC 1992.158). (B) SERS spectra of (upper curve) pastel stick #7 and (lower curve) a pink sample of fleshtone in face from the Mary Cassatt's pastel. Peaks that are characteristic for a yet unidentified $\beta$-naphtol or azo red red pigment, which also appears in pastel stick #7, are labeled. (C) Photomicrograph of a small sample from pastel stick #7. (D) Photomicrograph of a sample removed from a mauve stroke in the sitter's broad collar. (E) SERS spectra of (upper curve) madder root (*Rubia tinctorum L.*); and (lower curve) the sample illustrated in panel D. Long dashed lines indicate peaks that are consistent with madder root dye, and dashed lines with peak position noted indicate bands that are consistent with pastel stick #14 containing rhodamine B and rhodamine 6G. (F) Mary Cassatt's pastel box, courtesy of the Boston Museum of Fine Arts, Conservation Department. Citrate bands are indicated on panels B and D with an asterisk.

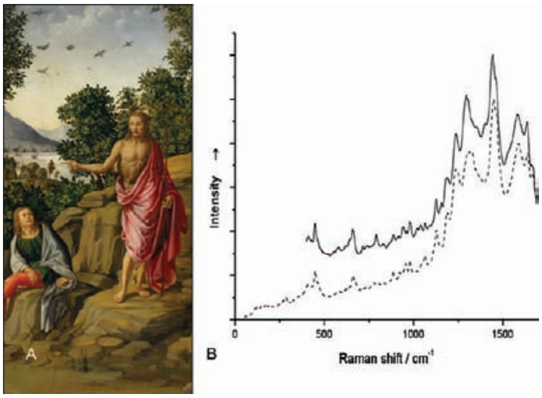

**FIGURE 8.** (A) St. John the Baptist Bearing Witness (detail). St. John the Baptist Bearing Witness. Accession no. 1970.134.2; workshop of Francesco Granacci, Florence (ca. 1510). 75.6 × 209.6 cm. Purchase, Gwynne Andrews, Harris Brisbane Dick, Dodge, Fletcher, and Rogers Funds, funds from various donors, Ella Morris de Peyster Gift, Mrs. Donald Oenslager Gift, and Gifts in memory of Robert Lehman, 1970. (B) SERRS spectrum of red glaze sample from St. John the Baptist Bearing Witness: solid line, spectrum of sample from red glaze; dashed line, spectrum of a reference sample of kermesic acid. Spectra were normalized and vertically shifted for ease of comparison, but no smoothing or baseline correction was employed. $\lambda_0 = 488$ nm, 0.25 mW power (at laser), 30 s.

effects for organic dyes may hopefully soon reach critical mass so that the technique can become not only an established part of the cultural heritage scientist's toolbox but an analytical technique of general applicability.

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**John R. Lombardi** was born in 1941 in Yonkers, New York. He attended Cornell University as an undergraduate and received his Ph.D. from Harvard University in 1967. He was assistant professor at the University of Illinois and is currently a Professor of Chemistry at The City College of New York. Other lines of interest include work on surface-enhanced Raman scattering on semiconductor quantum dots.

**Richard P. Van Duyne** is Charles E. and Emma H. Morrison Professor of Chemistry at Northwestern University. He received his Ph.D. from the University of North Carolina at Chapel Hill. His research interests include surface-enhanced Raman spectroscopy, nanosphere lithography, localized surface plasmon resonance spectroscopy, molecular plasmonics, spectroscopic methods for chemical and biological sensing, structure and function of biomolecules on surfaces, tip-enhanced Raman spectroscopy (TERS), ultrahigh vacuum scanning tunneling microscopy, ultrahigh vacuum surface science, Raman spectroscopy of mass-selected clusters, and application of SERS to the study of works of art.

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## FOOTNOTES

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## REFERENCES

1 Aroca, R. *Surface-Enhanced Vibrational Spectroscopy*; John Wiley & Sons: Chichester, U.K., 2006.

2 Kneipp, K., Moskovits, M., Kneipp, H., Eds. *Surface-Enhanced Raman Scattering: Physics and Applications*; Topics in Applied Physics; Springer: New York, 2006.

3 Fleischmann, M. P.; Hendra, J.; McQuillan, A. J. Raman Spectra of Pyridine Adsorbed at a Silver Electrode. *Chem. Phys. Lett.* **1974**, *26*, 163–166.

4 Jeanmaire, D. L. L.; Van Duyne, R. P. Surface Raman Spectroelectrochemistry. *J. Electroanal. Chem. Interfacial Electrochem.* **1977**, *84*, 1–20.

5 Albrecht, M. G.; Creighton, J. A. Intense Raman Spectra of Pyridine at a Silver Electrode. *J. Am. Chem. Soc.* **1977**, *99*, 5215–5217.

6 Guineau, B.; Guichard, V. Identification des colorants organiques naturels par microspectrometrie Raman de resonance et par effet Raman exalte de surface (SERS), in, *ICOM Committee for Conservation: 8th triennial meeting, Sydney, Australia, 6—11 September, 1987 Preprints*; The Getty Conservation Institute: Marina del Rey, CA, 1987; Vol. II, pp 659—666.

7 Wustholz, K. L.; Brosseau, C. L.; Casadio, F.; Van Duyne, R. P. Surface-Enhanced Raman Spectroscopy of Dyes: From Single Molecules to the Artists' Canvas. *Phys. Chem. Chem. Phys.* **2009**, *11*, 7350–7359.

8 Lombardi, J. R.; Birke, R. L. A Unified View of Surface Enhanced Raman Scattering. *Acc. Chem. Res.* **2009**, *42*, 734–742.

9 Cardon, D. *Natural Dyes: Sources, Tradition, Technology and Science*; Archetype Publications Ltd.: London, 2007.

10 Venkataraman, K. *The Chemistry of Synthetic Dyes*; Academic Press: New York, 1952.

11 Saunders, D.; Kirby, J. Light-Induced Colour Changes in Red and Yellow Lake Pigments. *Natl. Gallery Tech. Bull.* **1994**, *15*, 79–97.

12 Burnstock, A.; Lanfear, I.; van den Berg, K. J.; Carlyle, L.; Clarke, M.; Hendriks, E.; Kirby, J. Comparison of the Fading and Surface Deterioration of Red Lake Pigments in Six Paintings by Vincent Van Gogh with Artificially Aged Paint Reconstructions. In *Preprints of the 14th triennial meeting of the ICOM Committee for Conservation*, James & James: London, 2005; Vol. I, pp 459—466.

13 Stratis, H. K. Innovation and Tradition in Mary Cassatt's Pastels: A Study of Her Methods and Materials. In *Mary Cassatt: Modern Woman*; The Art Institute of Chicago Harry N. Abrams Inc.: New York, 1998; pp 212—226.

14 Brosseau, C. L.; Casadio, F.; Van Duyne, R. P. Revealing the Invisible - Using Surface-Enhanced Raman Spectroscopy to Identify Minute Remnants of Color in Winslow Homer's Colorless Skies. *J. Raman Spectrosc.*, in press.

15 Karapanagiotis, L.; Valianou, L.; Daniilia, S.; Chryssoulakis, Y. Organic Dyes in Byzantine and Post-Byzantine Icons from Chalkidiki (Greece). *J. Cult. Herit.* **2007**, *8*, 294–298.

16 Claro, A.; Melo, M. J.; Schäfer, S.; de Melo, J. S.; Pina, F.; van den Berg, K. J.; Burnstock, A. The Use of Microspectrofluorimetry for the Characterization of Lake Pigments. *Talanta.* **2008**, *74*, 922–929.

17 Clementi, C.; Miliani, C.; Romani, A.; Santamaria, U.; Morresi, F.; Mlynarska, K.; Favaro, G. In-Situ Fluorimetry: A Powerful Non-invasive Diagnostic Technique for Natural Dyes Used in Artefacts: Part II Identification of Orcein and Indigo in Renaissance Tapestries. *Spectrochim. Acta, Part A.* **2009**, *71*, 2057–2062.

18 Gillard, R. D.; Hardman, S. M.; Thomas, R. G.; Watkinson, D. E. The Detection of Dyes by FTIR Microscopy. *Stud. Conserv.* **1994**, *39*, 187–192.

19 Bruni, S.; Caglio, S.; Guglielmi, V.; Poldi, G. The Joined Use of n.i. Spectroscopic Analyses - FTIR, Raman, Visible Reflectance Spectrometry and EDXRF - To Study Drawings and Illuminated Manuscripts. *Appl. Phys. A: Mater. Sci. Process.* **2008**, *92*, 103–108.

20 Bell, I. M.; Clark, R. J. H.; Gibbs, P. J. Raman spectroscopic library of natural and synthetic pigments (pre- ≈ 1850 AD). *Spectrosc. Acta, Part A* **1997**, *53*, 2159–2179.

21 Vandenabeele, P.; Moens, L.; Edwards, H. G. M.; Dams, R. Raman Spectroscopic Database of Azo Pigments and Application to Modern Art Studies. *J. Raman Spectrosc.* **2000**, *31*, 509–517.

22 Scherrer, N. C.; Zumbuehl, S.; Delavy, F.; Fritsch, A.; Kuehnen, R. Synthetic Organic Pigments of the 20th and 21st Century Relevant to Artist's Paints: Raman Spectra Reference Collection. *Spectrochim. Acta, Part A* **2009**, *73*, 505–524.

23 Wouters, J. High Performance Liquid Chromatography of Anthraquinones: Analysis of Plant and Insect Extracts and Dyed Textiles. *Stud. Conserv.* **1985**, *30*, 119–128.

24 Wouters, J.; Verhecken, A. The Coccid Insect Dyes: HPLC and Computerized Diode-Array Analysis of Dyed Yarns. *Stud. Conserv.* **1989**, *34*, 189.

25 Chen, K.; Leona, M.; Vo-Dinh, T. Surface-Enhanced Raman Scattering for the Identification of Organic Pigments and Dyes in Works of Art and Cultural Heritage Material. *Sens. Rev.* **2007**, *27*, 109–120.

26 Leona, M.; Steger, J.; Ferloni, E. Application of Surface-Enhanced Raman Scattering Techniques to the Ultra-sensitive Identification of Natural Dyes in Works of Art. *J. Raman Spectrosc.* **2006**, *37*, 981–992.

27 Whitney, A. V.; Van Duyne, R. P.; Casadio, F. An Innovative Surface-Enhanced Raman Spectroscopy (SERS) Method for the Identification of Six Traditional Red Lakes and Dyestuffs. *J. Raman Spectrosc.* **2006**, *37*, 993–1002.

28 Whitnall, R., Shadi, I. T.; Chowdry, B. Z. Case Study: The Analysis of Dyes by SERRS. In *Raman Spectroscopy in Archaeology and Art History*; Edwards, H. G. M., Chalmers, J. M., Eds.; Royal Society of Chemistry: London, 2005; pp 152—165.

29 Cañamares, M. V.; Garcia-Ramos, J. V.; Domingo, C.; Sanchez-Cortes, S. Surface-Enhanced Raman Scattering Study of the Adsorption of the Anthraquinone Pigment Alizarin on Ag Nanoparticles. *J. Raman Spectrosc.* **2004**, *35*, 921–927.

30 Shadi, I. T.; Chowdhry, B. Z.; Snowden, M. J.; Withnall, R. Semi-quantitative Analysis of Alizarin and Purpurin by Surface-Enhanced Resonance Raman Spectroscopy (SERRS) Using Silver Colloids. *J. Raman Spectrosc.* **2004**, *35*, 800–807.

31 Leona, M.; Lombardi, J. R. Identification of Berberine in Archaeological Textiles by Surface Enhanced Raman Spectroscopy. *J. Raman Spectrosc.* **2007**, *38*, 853–85.

32 Canamares, M. V.; Lombardi, J. R.; Leona, M. Surface-Enhanced Raman Scattering of Protoberberine Alkaloids. *J. Raman Spectrosc.* **2008**, *39*, 1907–1914.

33 Centeno, S. A.; Shamir, J. Surface Enhanced Raman Scattering (SERS) and FTIR Characterization of the Sepia Melanin Pigment Used in Works of Art. *J. Mol. Struct.* **2008**, *873*, 149–159.

34 Whitney, A. V.; Van Duyne, R. P.; Casadio, F. Silver Island Films as Substrate for Surface-Enhanced Raman Spectroscopy (SERS): A Methodological Study on Their Application to Artists' Red Dyestuffs. *Proc. SPIE* **2005**, 117–126.

35 Stuart, D. A.; Yuen, J. M.; Shah, N. C.; Lyandres, O.; Yonzon, C. R.; Glucksberg, M. R.; Walsh, J. T.; Van Duyne, R. P. In Vivo Glucose Measurement by Surface-Enhanced Raman Spectroscopy. *Anal. Chem.* **2006**, *78*, 7211–7215.

36 Zhang, X.; Young, M. A.; Lyandres, O.; Van Duyne, R. P. Rapid Detection of an Anthrax Biomarker by Surface-Enhanced Raman Spectroscopy. *J. Am. Chem. Soc.* **2005**, *127*, 4484–4489.

37 Zhang, X.; Zhao, J.; Whitney, A. V.; Elam, J. W.; Van Duyne, R. P. Ultrastable Substrates for Surface-Enhanced Raman Spectroscopy: $Al_2O_3$ Overlayers Fabricated

by Atomic Layer Deposition Yield Improved Anthrax Biomarker Detection. *J. Am. Chem. Soc.* **2006**, *128*, 10304–10309.

38 Whitney, A. V.; Casadio, F.; Van Duyne, R. P. Identification and Characterization of Artists' Red Dyes and Their Mixtures by Surface-Enhanced Raman Spectroscopy. *Appl. Spectrosc.* **2007**, *61*, 994–1000.

39 Brosseau, C. L.; Gambardella, A.; Casadio, F.; Van Duyne, R. P.; Grzywacz, C.; Wouters, J. Ad-Hoc SERS Methodologies for the Detection of Artist Dyestuffs: Thin Layer Chromatography-Surface Enhanced Raman Spectroscopy (TLC-SERS) and In Situ On the Fiber Analysis. *Anal. Chem.* **2009**, *81*, 3056—3062.

40 Lin, X. M.; Cui, Y.; Xu, Y. H.; Ren, B.; Tian, Z. Q. Surface-Enhanced Raman Spectroscopy: Substrate-Related Issues. *Anal. Bioanal. Chem.* **2009**, *394*, 1729–1745.

41 Lee, P. C.; Meisel, D. Adsorption and Surface-Enhanced Raman of Dyes on Silver and Gold Sols. *J. Phys. Chem.* **1982**, *86*, 3391–3395.

42 Leona, M. Microanalysis of Organic Pigments and Glazes in Polychrome Works of Art by Surface-Enhanced Resonance Raman Scattering. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 14757–14762.

43 Jurasekova, Z.; Domingo, C.; Garcia-Ramos, J. V.; Sanchez-Cortes, S. In Situ Detection of Flavonoids in Weld-Dyed Wool and Silk Textiles by Surface-Enhanced Raman Scattering. *J. Raman Spectrosc.* **2008**, *39*, 1309–1312.

44 Geiman, I.; Leona, M.; Lombardi, J. R. Application of Raman Spectroscopy and SERS to the Analysis of Synthetic Dyes Found in Ballpoint inks. *J. Forensic Sci.* **2009**, *54*, 947–952.

45 Van Elslande, E.; Lecomte, S.; Le Ho, A. S. Micro-Raman Spectroscopy (MRS) and Surface-Enhanced Raman Scattering (SERS) on Organic Colorants in Archaeological Pigments. *J. Raman Spectrosc.* **2008**, *39*, 1001–1006.

46 Brosseau, C. L.; Rayner, K.; Casadio, F.; Van Duyne, R. P.; Grzywacz, C. M. Surface-Enhanced Raman Spectroscopy: An In-Situ Method To Identify Colorants in Various Artist Media. *Anal. Chem.* **2009**, *81*, 7443–7447.