Surface-Enhanced Raman Spectroscopy:
Using Nanoparticles to Detect Trace
Amounts of Colorants in Works of Art

Federica Pozzi, Stephanie Zaleski, Francesca Casadio, Marco Leona,
John R. Lombardi and Richard P. Van Duyne

Abstract In recent years, powerful physical processes occurring in the vicinity of
nanoscale metal surfaces have been exploited in the art world for the detection of
trace amounts of colorants with surface-enhanced Raman spectroscopy (SERS). With
this technique, naturally occurring and man-made organic molecules used as
dyes and pigments in objects from antiquity to the present day are being detected
with high molecular specificity and unprecedented sensitivity. This chapter reviews
the broad spectrum of SERS analytical methodologies and instrumental improve-
ments that have been developed over the years in the field of cultural heritage
science, and discusses significant case studies within different types of works of art
and archaeological artifacts.

F. Pozzi (✉)
Department of Conservation, Solomon R. Guggenheim Museum, 1071 Fifth Ave,
New York, NY 10128, USA
e-mail: fpozzi@guggenheim.org

S. Zaleski · R.P. Van Duyne
Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston,
IL 60208, USA
e-mail: szaleski@u.northwestern.edu

R.P. Van Duyne
e-mail: vanduyne@northwestern.edu

F. Casadio
Department of Conservation, Art Institute of Chicago, 111 South Michigan Ave,
Chicago 60603, USA
e-mail: fcasadio@artic.edu

M. Leona
Department of Scientific Research, Metropolitan Museum of Art, 1000 Fifth Avenue,
New York, NY 10028, USA
e-mail: marco.leona@metmuseum.org

J.R. Lombardi
Department of Chemistry, City College of New York, 160 Convent Ave,
New York, NY 10031, USA
e-mail: lombardi@sci.ccny.cuny.edu

© Atlantis Press and the author(s) 2016
P. Dillmann et al. (eds.), Nanoscience and Cultural Heritage,
DOI 10.2991/978-94-6239-198-7_6

161
1 Introduction

Since the dawn of time, color has been used by humanity to beautify the world and convey thoughts and feelings through various means of artistic expression.

Among the colored materials employed since antiquity, pigments and colorants have played a key role in the dyeing of fabrics and creation of artworks, to embellish individuals, decorate objects of everyday use, and as a sign of hierarchical status. The term “pigment” is used to describe insoluble materials, organic or inorganic, which are ground to fine powder and suspended in a binder to form paint. On the other hand, the terms “dye” and “colorant” generally refer to organic substances that are soluble in the medium and show affinity for a substrate either inherently or through the action of additives. In textile dyeing, colorants may be applied to fiber substrates directly (direct dyes), after being solubilized with a reducing agent (vat dyes), or with the aid of bridging metal ions called mordants (mordant dyes). In painting, dyestuffs were typically precipitated onto an inert, insoluble, inorganic substrate to produce lake pigments with significantly improved lightfastness properties compared to the primary fugitive dyes.

Prior to the introduction of synthetic colorants in the second half of the 19th century, dyes were derived from a variety of natural sources ranging from roots, berries, bark and leaves to different types of organisms such as lichens, insects and shellfish (Mills and White 1987; Hofenk de Graaff et al. 2004; Cardon 2007). Most natural red dyes owe their color to anthraquinone chromophores and can be extracted from insects, as in the case of cochineal, kermes and lac dye, or plants, among which madder and brazilwood are numbered. Several blue and purple colorants, including indigo and Tyrian purple, consist of a mixture of indigotin-related substances obtained from plants or sea snails, while other dyes of similar shades, like orchil, are derived from different sources, such as lichens. As far as yellow colorants are concerned, flavonoids—occurring as glycosides in a great variety of botanical species—are their main chromophores, although a number of other molecules such as carotenoids, curcuminoids, naphthoquinones and galloytannins may be found as well. Besides reds, blues and yellows, other hues could be produced by using two or more dyes in combination: green shades, for instance, were traditionally obtained as mixtures of a blue and a yellow colorant. For this reason, historic tapestries that have been extensively exposed to light often show blue foliage as a result of the fading of the more fugitive yellow component of the mixture.

The fortuitous discovery of mauveine, the first synthetic dye, by William Perkin in 1856 prompted extensive chemical experimentation that led both to the synthesis of molecules previously derived from natural sources, such as alizarin and indigotin, and to the introduction of several improved artificial colors that have no counterparts in nature, thus initiating a rapid decline in the dominance of natural dyes in world markets. In addition to their use in textiles and artworks, those synthetic colorants that could be tested as safe have found wide application as
printing and pen inks, paint components, dyes for food, plastic and rubber, and cosmetic constituents (Mills and White 1987; Lewis 1988).

Scientific analysis of objects of artistic, historic and archaeological significance is key to reconstructing their story and elucidating the circumstances in which they have been created and, in some cases, have travelled through the centuries prior to ending up in the inquiring hands of conservators and scientists for in-depth technical examination. In particular, the identification of natural and synthetic colorants may help to clarify attribution, provenance and dating, technology of fabrication, trade routes and commercial transactions that may have allowed the usage of certain dyes far away from their native locations. Moreover, investigating the origin, nature and chemical behavior of the colored materials employed in the production of artworks and historical artifacts may shed new light on their original color and appearance, thus offering insight into the artist’s actual intentions and choices, the techniques used, and the dates ante quem and post quem the object was created, possibly even leading to the uncovering of falsifications and forgeries. Most importantly, a clear understanding of the materials used is essential to enable the object’s long-term preservation and to inform conservation treatments, as well as museum display and lending policies.

The identification of colorants from artworks and objects of archaeological, anthropological or historical value poses a whole set of analytical challenges. Recent advances in macro-X-ray fluorescence (macro-XRF) mapping (Dik et al. 2008; Alfeld et al. 2013a, b) and infrared (IR), ultraviolet/visible (UV/vis), and hyperspectral imaging (Delaney et al. 2010, 2014; Ricciardi et al. 2012; Dooley et al. 2013; Rosi et al. 2013) have opened up exciting opportunities to track the distribution of pigments over the entire surface of a painted object. However, X-ray techniques are most successful at mapping inorganic pigments and are not able to map organic compounds, with the notable exception of eosin (tetrabromo fluorescein, also known as geranium lake, a pigment extensively used by Vincent van Gogh). In hyperspectral imaging, the signal from dyes is invariably compounded with the spectral contributions of the binding medium and other paint components, so the identification of the organic colorant is possible only in rare cases. At the micro-scale, although recent research has been aiming at detecting and identifying dyes non-invasively in situ, to date the greatest chances of success still rely on the removal of one or more samples from the object under study. To complicate the analysis even further, the extraordinary tinting strength of many organic colorants caused them to be used in minute amounts in both historical textiles and works of art, where they are typically found in complex chemical environments, bound to cloth fibers by means of metal ions or embedded in paint layers in the form of lake pigments. As a result, the application of intensive sample pretreatment procedures often becomes a necessary step to isolate the colorants from their matrixes and achieve selective molecular identification. An additional obstacle is posed by the lack of permanence and susceptibility to deterioration of several colored organic materials that, upon exposure to air and light, undergo a number of chemical degradation processes leading to the formation of colorless species with a different molecular structure compared to the primary dye (Grosjean et al. 1988; Saunders
and Kirby 1994; Ferreira et al. 2001; Yoshizumi and Crews 2003; Ahn and Obendorf 2004, 2007; Cooksey and Sinclair 2005; Colombini et al. 2007; Clementi et al. 2007; Koperska et al. 2011; Manhita et al. 2011, 2013; Degano et al. 2011; Ramesova et al. 2012). Therefore, the amount of colorant available for analysis is often infinitesimal, challenging the limits of detection of many analytical techniques.

The analysis of organic colorants, made possible by the introduction of chromatographic and spectroscopic techniques, greatly benefited, in the past few decades, from both the improvement of pre-existing analytical procedures and the development of new cutting-edge instrumental methods. Initial attempts at detecting and identifying dyes in historical artifacts involved the use of absorbance UV/vis spectrophotometry (Taylor 1983; Wouters 1985) and thin-layer chromatography (TLC) (Masschelein-Kleiner and Heylen 1968; Schweppe 1993). However, in both cases, a sizable amount of colorant needs to be removed from the sample and brought into solution for analysis, thus requiring that the sample itself be of significant dimensions or deeply colored. Alternative non-invasive approaches by means of reflectance UV/vis spectrophotometry (Bacci et al. 1991; Montagner et al. 2011; Gulmini et al. 2013) and fluorimetry (Claro et al. 2008, 2010; Degano et al. 2009; Melo and Claro 2010; Romani et al. 2010; Clementi et al. 2014; Amat et al. 2015) have been also evaluated, occasionally leading to the successful characterization of pigments and colorants from medieval illuminations, paint cross sections, ancient textiles and wall paintings. Yet, electronic methods typically provide broad, featureless spectra that often appear to be nearly identical for distinct compounds of similar molecular classes and, in some cases, even for totally unrelated chemical groups. This, along with the ubiquitous additional contribution of binding media, fillers, additives, and all other sample components to the spectra, makes the interpretation extremely challenging. High-performance liquid chromatography (HPLC) remains the technique of choice for the characterization of organic colorants, allowing researchers to separate and identify dyes even in complex mixtures (Wouters 1985; Wouters and Verhecken 1989; Koren 1994; Halpine 1996; van Bommel et al. 2007). However, in this case, too, the amount of sample required for analysis may sometimes be prohibitive when dealing with museum objects for which permission to sample cannot be granted due to value, condition, or policy. While enabling the non-destructive characterization of colorants in minute samples or whole objects, vibrational methods such as Fourier-transform infrared (FTIR) (Gillard et al. 1994) and Raman/FT-Raman (Bell et al. 1997; de Oliveira et al. 2002; Edwards et al. 2003; Scherrer et al. 2009) spectroscopies were proven more suitable for the analysis of inorganic pigments and synthetic dyes. Raman spectroscopy, in particular, suffers from inherently weak signals and strong molecular fluorescence from natural dyestuffs that often obscures the Raman scattering signal even when using near-infrared (NIR) and IR excitation wavelengths to avoid fluorescence, such as 785 nm and above. Furthermore, because only sub-nanogram levels of dyes are needed to achieve intense coloration, Raman spectroscopy is often not sensitive enough to probe these materials, especially when they are embedded in complex matrixes such as textile fibers or artist’s paints.
Among the less sample-intensive techniques, surface-enhanced Raman spectroscopy (SERS) has allowed researchers to overcome many of the drawbacks described above, enabling successful detection and identification of colorants from artworks and historical artifacts with high molecular specificity, unparalleled sensitivity, and the lowest known limits of detection (Chen et al. 2007; Wustholz et al. 2009; Casadio et al. 2010). In SERS, organic molecules are adsorbed on noble metal nanostructures and can thus be probed due to the exceptional Raman scattering enhancement and fluorescence quenching provided by the substrate via chemical and electromagnetic effects (Fig. 1) (Lombardi and Birke 2009, 2012). It has been demonstrated that the SERS signal of ensemble-averaged molecules exhibits enhancements up to eight orders of magnitude over the normal Raman cross section. Since the initial discovery of SERS (Fleischmann et al. 1974) and the explanation of the observed phenomenon in the 1970s (Albrecht and Creighton 1977; Jeanmaire and Vanduyne 1977), the field has grown enormously and has been enriched by a wealth of experimental studies. In particular, in the past ten years, the potential of SERS for dyestuff identification in various art and archaeological applications has been increasingly harnessed, with a significant growth in the number of publications in this area.

Initially, the greatest effort in the field of SERS for art and archaeology aimed at the characterization of reference materials, with special attention being paid to natural red colorants: alizarin, purpurin, carminic and laccaic acids, and related anthraquinones have been the subject of several studies in the scientific literature (Shadi et al. 2004; Cañamares et al. 2004, 2006a; Chen et al. 2006; Cañamares and Leona 2007; Baran et al. 2009; Rambaldi et al. 2015). Articles about the detection of flavonoids (Jurasekova et al. 2006, 2008, 2012; Wang et al. 2007; Teslova et al. 2007; Cañamares et al. 2009; Corredor et al. 2009; Mayhew et al. 2013), indigoids (Bruni et al. 2010; Oakley et al. 2012), and dyes belonging to other molecular classes (Leona and Lombardi 2007; Cañamares et al. 2008a, b, 2010, 2014;
Geiman et al. 2009; Chang et al. 2009; Xie et al. 2012; Bruni et al. 2011a; Mayhew et al. 2013; Benedetti et al. 2014; Greeneltch et al. 2012; Doherty et al. 2014; Cañamares and Lombardi 2015; Zaffino et al. 2015) can also be found in the literature. In addition to providing valuable reference spectra for identification purposes, these works have explored aspects such as the influence of pH on the resulting spectral patterns, and the binding geometry and relative orientation of the molecules examined with respect to the metal surface. Computational methods such as density functional theory (DFT) have also been used to calculate theoretical spectra and assign the normal modes of vibration to the Raman bands, thus supporting the interpretation of SERS data.

At the same time, extensive research has focused on the improvement and optimization of the technique as an analytical tool to be used for the detection and identification of dyes in samples of artistic and historical value that may be very complex, altered or degraded. In this context, identifying faded colorants in artworks, and investigating the nature and causes of the corresponding degradation processes is essential to implement measures to slow down or altogether prevent future fading and to shed new light on how certain artifacts might have looked like right after completion. SERS has offered insight into important examples of such processes, such as the fading of madder- and cochineal-based purples and reds in watercolors by the American painter Winslow Homer (1836–1910) (Brosseau et al. 2011) and in masterpieces by the French Impressionist Pierre-Auguste Renoir (1841–1919) (Collins et al. 2014; Pozzi et al. 2014a) and post-Impressionist Vincent van Gogh (1853–1890) (Vellekoop et al. 2013). In all these cases, the detection and identification of faded dyes with SERS allowed researchers to confirm that the current colorless or severely discolored appearance of the artworks is the result of a fading process, and to put forward hypotheses on how the masterpieces might have looked like right after the artists painted them.

Recent advancements in the SERS technique that will be discussed in this chapter include the comparison of various metal substrates, such as silver colloids and silver films over nanospheres (AgFONs), and evaluation of their performances; the development of non-invasive approaches involving the use of SERS-active removable substrates; the adaptation of pre-existing methodologies of sample treatment and analysis, such as laser ablation and tip-enhanced Raman spectroscopy (TERS), to the specific needs of the cultural heritage field; and the combination of SERS with separation techniques, such as TLC and microfluidics, to resolve dye mixtures.

Complemented by case studies drawn from important collections in the United States, this chapter will demonstrate the tremendous advances and high applicability of SERS, as well as outline areas of future development for a technique that is now solidly established as a powerful means of colorants’ investigation in the field of art and archaeology.
2 SERS Substrates and Analytical Methodologies

Since the discovery of the surface-enhanced Raman effect in the 1970s, metal substrates of different types and forms have been developed for various SERS applications, beginning with roughened silver electrodes, then moving onto colloidal nanoparticles and solid surfaces with tailored thickness and nanostructured features. Accurate choice of the metal substrate is of utmost importance to ensure adequate measurement efficiency and high reproducibility of the spectra collected. While several metals including gold, copper, platinum, palladium and aluminum have been tested over the years, the best performances are expected from silver owing to its dielectric function and localized surface plasmon resonance (LSPR) spanning from the UV to the NIR/IR regions, i.e. the range of wavelengths typically used in SERS experiments for analyte excitation. Although incessant exploration in the field of high enhancing metal surfaces has provided practitioners with a continuously growing number of choices, substrates used in cultural heritage applied research must meet additional requirements of quick preparation, relatively low cost and straightforward use, as few museum laboratories have the extensive sample preparation capabilities of university-based facilities. Additionally, researchers are also striving to develop SERS substrates that could be used to identify colorants in situ without the need of removing even the smallest of samples: this would be crucial, for instance, in the case of works on paper or modern and contemporary art, from which sampling is normally more problematic.

The substrates and methodological approaches that have been used over the years for SERS analysis of art objects and archaeological artifacts are described in the following.

2.1 Colloidal Nanoparticles

Colloidal silver nanoparticles are by far the most widely used SERS substrate for art applications. The most popular method to synthesize silver colloids is by chemical reduction of a silver salt, typically silver nitrate, with trisodium citrate at boiling temperature (Lee and Meisel 1982). The Lee and Meisel method usually generates silver nanoparticles, mostly nanospheres and nanorods, in the 3–80 nm diameter range with a fairly broad visible absorption near 430 nm. Particle size, shape, and resulting plasmonic properties can be fine-tuned by suitable choice of the metal, reducing agent and stabilizer, chemicals concentration, addition rate, and temperature. In addition to testing alternative reducing agents, such as hydroxylamine (Leopold and Lendl 2003) and borohydride (Creighton et al. 1979), research groups all over the world have evaluated how different aggregants—including potassium nitrate (Cañamares et al. 2004), poly-L-lysine (Shadi et al. 2004), sodium chloride (Cañamares et al. 2008b) and perchlorate (Bruni et al. 2011a)—may promote the association of nanoparticles in clusters of various dimensions, where localized
regions of intense electromagnetic fields, the so-called hot spots, are responsible for a giant enhancement of the Raman signal (Ringe et al. 2013). An alternative to Lee-Meisel colloids involves a synthetic protocol based on the glucose-assisted reduction of silver sulfate in the presence of sodium citrate as a capping agent (Leona 2009). In this case, the use of a microwave oven equipped with pressure and temperature control has afforded silver colloids with a significantly narrower particle size range (3–10 nm) and absorption band, leading to increased stability of the substrate itself and more reproducible SERS performances.

The main drawback of citrate-capped silver nanoparticles lies in the occasional occurrence of spurious bands in SERS spectra that are due to competitive adsorption of citrate ions and related oxidation products onto the metal surface (Leona et al. 2006; Brosseau et al. 2009a; Bruni et al. 2010). This issue may be circumvented by adopting alternative techniques for the production of colloids, such as laser ablation and photoreduction. More popular than the first, based on the extraction of metallic nanoparticles from a silver plate induced by high intensity laser pulses (Cañamares et al. 2008c), is the fabrication of Ag substrates by laser photoreduction of a silver nitrate solution (Cañamares et al. 2007; Jurasekova et al. 2008, 2010; Retko et al. 2014). In both approaches, the absence of reducing or capping agents prevents disruptive interference (Cañamares et al. 2008c); however, the prolonged in situ irradiation required may raise concerns when these methods are applied to the analysis of samples from actual artworks due to the risk of thermal degradation and formation of carbon-rich phases.

In order to increase the applicability of SERS-active colloids to the analysis of complex samples from works of art, a useful pretreatment step has also been introduced involving a gas-solid hydrolysis performed by exposing microscopic samples to hydrofluoric acid (HF) vapor for 5 min in a closed polyethylene microchamber (Fig. 2). Specifically developed for mordant dyes and lake pigments, such pretreatment aims at hydrolyzing the chemical bond that the organic colorant forms with the fabric or inorganic portion of the lake through bridging metal ions. Compared to previously common extraction approaches based on the use of heated acids or alkali (Wouters 1985; Tiedemann and Yang 1995), the HF hydrolysis exploits milder experimental conditions and may be considered extractionless in that it does not involve physical detachment of the target dye from the sample under study. Simply, a small amount of colorant is released, in situ, from the surface of the dyed object becoming available for coupling to the silver nanoparticles. This methodology has proven critical to attain highly reproducible, conclusive dye identification in many instances, and has delivered superior results for the analysis of paints and glaze layers (Pozzi et al. 2012a, 2014a; Zaffino et al. 2014).

An alternative approach involves the use of colloidal pastes produced by centrifugation and concentration of nanoparticle suspensions. These pastes have been successfully used, in some cases, for the direct, extractionless, non-hydrolysis detection and characterization of organic colorants from textile fibers, pastels and watercolors (Brosseau et al. 2009a, b, 2011; Idone et al. 2013).
Although most of the studies mentioned so far have been carried out on loose samples removed from works of art, a limited number of proof-of-concept works recently published in the literature have also reported initial attempts at applying colloids directly on cross sections from paintings, with the goal of obtaining spatially-resolved data (Idone et al. 2014; Retko et al. 2014; Frano et al. 2014).

Fig. 2 SERS analysis of a red lake oil paint sample with Ag colloids upon HF hydrolysis treatment. Step (1) a microscopic paint specimen is removed from a painting by means of a scalpel blade and placed on a polyethylene holder; step (2) the sample is exposed to HF vapor at room temperature for 5 min in a closed polyethylene microchamber; step (3) 0.8 µL of Ag colloids and 0.1 µL of aggregant, typically 0.5 M KNO₃, are deposited onto the sample and SERS analysis is performed before evaporation of the droplet.
2.2 Solid-State SERS Substrates: Silver Films Over Nanospheres (AgFONs)

The second most common SERS substrate aside from colloidal nanoparticles consists of lithographically fabricated metal surfaces. A variety of lithographic fabrication methods exist, such as electron beam lithography (EBL), focused ion beam (FIB) milling, and nanosphere lithography (NSL) (Fan et al. 2011). However, most of these techniques require costly equipment, which can be difficult to access in a museum setting, and have therefore seen limited use in the field. NSL is a cost-efficient and facile method of fabricating SERS substrates. NSL masks are obtained by dropcasting commercial polystyrene or silica microspheres onto a cleaned glass coverslip and allowed to dry in a hexagonal close packed array. A 200-nm layer of metal, typically Ag or Au, is then deposited through thermal vapor deposition on the microsphere mask to create the SERS substrate, commonly known as a film over nanospheres (FON) (Fig. 3). FONs have been shown to have high uniformity over a large area and their LSPR, readily tunable by changing the microsphere size, can be easily matched with the laser excitation wavelength (Sharma et al. 2013). When optimized, FONs have enhancement factors (EFs) on the order of $10^6$–$10^8$.

The ease of fabrication, tunability, and high EFs of FONs make them ideal substrates for the identification of artists’ materials with SERS. The first use of AgFONs in cultural heritage science was to identify and characterize the red dyestuffs alizarin, purpurin, lac, cochineal and their mixtures (Whitney et al. 2006). In this work, AgFONs were optimized in three excitation wavelength regimes relative to the electronic resonance of the dyes: pre-resonant (632.8 nm), resonant

![Fig. 3 Representative scanning electron microscopy (SEM) image of a FON. A 200-nm Ag layer is deposited on the surface of hexagonal close packed silica microspheres. The high enhancement from the FON originates from the formation of nanopillar-like structures on the deposited metal surface, which leads a high number of SERS hot spots on the Ag surface (SEM image courtesy of Dr. Anne-Isabelle Henry, Van Duyne group, Northwestern University)]
(532 nm) and non-resonant (785 nm). It was found that the SERS signal was two orders of magnitude higher in the resonant regime compared to the non-resonant wavelength range, highlighting the importance of the resonance contribution to the SERS enhancement and the usefulness of a tunable SERS substrate. Additionally, the authors successfully resolved various binary dye mixtures in the pre-resonant wavelength regime. Whitney et al. (2007) demonstrated that exciting on electronic resonance with the dye in question can yield greater SERS signal, but it can also lead to photodegradation and possible fluorescence from binding media. Recent work examined the EFs of AgFONs optimized for NIR and IR wavelengths and Ag colloidal nanoparticles to identify eosin Y, an early modern synthetic dye, which was extensively used by Vincent van Gogh and sold under the name of geranium lake (Greeneltch et al. 2012). The use of NIR and IR laser excitation is beneficial for analyzing cultural heritage samples as compared to visible excitation because it minimizes photodegradation and interfering fluorescence. The authors observed that AgFONs EF using 1064 nm excitation was 20 times greater than that using 785 nm excitation. In addition, when compared to Lee and Meisel Ag colloidal nanoparticles, FONs were found to provide EFs greater by two orders of magnitude and more uniform signal.

Notwithstanding their ease of fabrication, high enhancement, uniformity, and tunability, AgFONs have been only occasionally used for practical applications of SERS to the identification of artists’ colorants. One of the main hurdles in their widespread use may be that the dyestuff needs to be brought into solution by extraction from the sample, while colloids and colloidal pastes can be applied directly onto a solid sample with or without HF pretreatment.

3 Innovative SERS Approaches

A significant portion of the recent research in the field of SERS for cultural heritage has focused on the development of new methods that can be deployed in situ or approaches that are endowed with enhanced spatial resolution. The most promising methodological developments to date are reported in the following.

3.1 Laser Ablation (LA)—SERS

A substantial drawback of most approaches to SERS for analyzing cultural heritage samples is their lack of spatial resolution. Samples smaller than 50–100 μm are difficult to extract in a controlled and precisely localized way, and, in the case in which drops of colloid are deposited directly on the sample by hand (Idone et al. 2013) the spatial resolution is ultimately limited by the size of the drop, i.e. about
0.5–1 mm in diameter with the most common pipettes. Even when using inkjet devices to deliver the colloidal nanoparticles (Benedetti et al. 2014) the spatial resolution does not go below 50–80 μm. Londero et al. improved the applicability of SERS analysis towards complex organic samples, while simultaneously reaching high spatial resolution, by combining laser ablation microsampling with SERS detection on a silver nanoisland film in a vacuum chamber (Londero et al. 2013). They successfully demonstrated spatial resolution of 5 μm and sensitivity down to 120 attomol on a test sample created by vacuum depositing a film of copper phthalocyanine on a quartz disc. In their setup, a visible laser pulse from an optical parametric oscillator (OPO) is focused on a target inside a vacuum chamber with sufficient intensity to ionize the sample and produce a plasma. The plasma heats the ionized region via an inverse Bremsstrahlung process and causes the material to explode outward as a plume of vapor. The vapor deposits onto a SERS active substrate, the silver nanoisland-coated bottom surface of the chamber window, where it can be interrogated by a read laser (Fig. 4).

Laser ablation has another advantage over the use of colloids besides spatial resolution: as it relies on vapor deposition rather than diffusion through water for the analyte to adsorb on the plasmonic substrate, it can be used on water insoluble pigments with excellent results. In their work, Londero et al. (2013) showed this on the pigments quinacridone and quinacridone quinone, and their mixture Pigment Orange 48.

An improved version of the ablation setup (Cesaratto et al. 2014) features UV ablation. By ablating in the UV, specifically at 355 nm, i.e. at a wavelength where virtually all organic materials are highly absorbing, vaporization is confined to approximately 1-μm depth. Even with extremely simple UV optics, Cesaratto and coworkers were able to show significant advantages over visible ablation. A cross section taken from the red area of a 16th-century decorated dish shows that two separate red paint layers exist on the plate (Fig. 5). The top layer is composed of sparse carbon particle embedded in a red glaze, while the bottom one is a lighter red glaze. The cross section was ablated in three different areas, with a minimum spot size about 13-μm wide (as determined by atomic force microscopy after the analysis). The two different layers could be characterized separately and without contamination one from the other, and the red colorant in the very top thin layer could be easily distinguished from the carbon particles. This is particularly critical given graphite’s ability to displace colorants on the SERS substrate, which can result in it being the only detectable component. The bottom layer was identified as a madder-based lake, while the upper one gave a SERS spectrum compatible with an unidentified synthetic organic colorant. The results reinforced suspicions of a modern addition raised from previous visual inspection of the object. This kind of work would not have been possible with the conventional approaches to SERS because of the complexity of the layering structure, or indeed, with any alternative technique, given that samples suitable for HPLC or liquid chromatography/mass spectrometry (LC/MS) could not have been obtained from such thin layers.
3.2 Inkjet Nanoparticle Delivery SERS

An interesting alternative to traditional approaches that involves removing a sample and treating it with a colloid in a separate stage was presented by Benedetti et al. (2014). Using inkjet technology to reproducibly deliver microdroplets of silver nanoparticles either on the specimen to be analyzed by SERS or on a work of art,
the authors of this work were able to demonstrate quasi non-destructive analytical capabilities. Droplets of approximately 50–80 μm were deposited directly onto a Japanese woodblock print with a piezoelectric inkjet head mounted on the Raman microscope and aimed at the focal point of the microscope objective. The suitability of either thermal or inkjet printing heads to deliver silver colloids for SERS analysis was demonstrated as well (Leona and Tague 2010). Although the droplets of silver nanoparticles cannot be removed from the substrate when dry, they are of such small diameter that they are nearly invisible to the naked eye.

Fig. 5  a A 16th-century decorated dish and microscope images of a cross section from the decoration (sample DD), with the ablated craters A, B and C. b SERS spectrum of the UV-ablated crater A in the main layer. Signals of reference madder lake were detected at 1283 and 1321 cm$^{-1}$, highlighted in the graph with an asterisk. c SERS spectra of the UV-ablated craters B and C in the top layer
3.3 Detachable SERS Substrates

While SERS analysis, unlike ordinary Raman, cannot be carried out in an entirely non-invasive way, several researchers have investigated quasi non-invasive analytical approaches based on the use of gels. Gels have been used for years in conservation to remove surface contamination from works of art in a localized and selective way. A gel confines the action of a solvent or cleaning agent to the area of the object with which it is in contact, preventing the spread of any material—either the cleaning agents or the materials targeted for removal—through and across the artwork (Carretti et al. 2010; Baglioni et al. 2015). For SERS applications, a gel could substitute the colloid drop traditionally used as the medium for the target dye and the plasmonic nanoparticles to interact; alternatively, a gel loaded with appropriate reagents could be used to perform a mild extraction of the target analyte from its substrate, for subsequent analysis with a silver colloid deposited on the gel.

Gel-based colloidal substrates for SERS analysis were first explored by Bell and Spence (2001) and by Farquharson and Maksymiuk (2003) without, however, a specific focus on quasi non-invasive sampling of works of art. The first application of gels to SERS analysis of artworks is due to Leona, who used cross-linked hydroxyacrylate gels for non-destructive dye extraction from paper and textiles (Leona 2008; Leona et al. 2011). The cross-linked gels were effectively used as solid-phase microextraction substrates, and SERS analysis was performed by depositing silver colloids on the gels after removal from the work of art. The method was tested on the Metropolitan Museum’s Unicorn Tapestries (1495–1505), enabling the identification of madder, and on a 19th-century Japanese woodblock print, where methyl violet was detected (Fig. 6).

A different approach was followed by Doherty et al. (2011) using methyl cellulose as the gel medium and Lofrumento et al. (2013), Platania et al. (2015) using

---

**Fig. 6** a Use of hydroxygels to extract minute amounts of ink from a ball point pen tracing on paper. The region of extraction is barely distinguishable from the area from which no extraction takes place. b Sekigahara Homare no Gaika (A poem about the battle of Sekigahara) by Toyoharu Kunichika, 1892. Woodblock print on paper, triptych, each sheet originally oban size (27 × 39 cm), slightly trimmed. Private collection
agar. Both groups incorporated the silver colloid directly in the gel substrate to be put in contact with the work of art. The agar gel approach was further refined by Platania et al. (2014) by adding ethylenediaminetetraacetic acid (EDTA)—a chelating agent—to the gel, thus improving the extraction of a variety of dyes from substrates such as oil paints, printed and dyed textiles. The agar gel seems to be one of the most promising approaches because, due to its shrinking upon drying, it causes the nanoparticles to come into close contact, inducing the occurrence of SERS active hot spots. Additionally, agar gels are increasingly used for conservation treatments and are, therefore, widely accepted in the field.

### 3.4 Tip-Enhanced Raman Spectroscopy (TERS)

Tip-enhanced Raman spectroscopy (TERS) is a promising non-destructive analog to SERS, which combines the nanometer spatial resolution of scanning probe microscopy (SPM) and the chemical sensitivity of SERS. A TERS experiment can be performed using one of two variants of SPM: scanning tunneling microscopy (STM) and atomic force microscopy (AFM). In an STM experiment, a voltage is applied to the scanning probe tip that comes in tunneling contact with the sample. An STM image is produced by measuring the changes in the tunneling current as a function of tip position across the sample. Despite its sub-nanometer scale spatial resolution, STM is sample-limited due to the requirement that the substrate of interest must be conductive and therefore cannot be used to analyze most materials from works of art. Alternatively, in an AFM experiment, the scanning probe tip is on a cantilever which makes contact with the surface of interest; changes in tip height due to local surface topography are measured to produce an AFM image. While the AFM tip does make contact with the sample, the tip can be readily removed from the sample surface, which is advantageous when analyzing sensitive cultural heritage samples. The surface generality of AFM is an advantage over STM and has been used to probe a wide range of surfaces such as DNA strands, amyloid fibrils, cell membranes and polymer films, as highlighted in a recent review (Schmid et al. 2013). Moreover, the surface generality of AFM makes it amenable to analyze surfaces relevant to cultural heritage such as cross sections, paint films, works on paper including watercolors, and textile fragments.

The scanning probe tip is at the heart of a TERS experiment, as it is both the source of the nanometer scale spatial resolution as well as the plasmonic enhancing surface. A TERS-active scanning probe is fabricated by electrochemical etching of Ag or Au wire for STM, or by thermal deposition of an Ag or Au metal film onto a commercial Si cantilever for AFM.

The methodology of a TERS experiment is illustrated in Fig. 7. First, the plasmonic tip is brought in close contact with the sample surface of interest. Laser light is then directed towards and focused onto the tip-sample junction. Low laser powers on the order of microwatts are often used in TERS experiments because of the tight focusing of the laser at the probe tip apex, which can be a beneficial feature
when analyzing artworks prone to thermal and photodegradation. The illumination of the tip induces an enhanced electromagnetic field around the probe tip apex, and only molecules directly beneath the tip experience Raman scattering enhancement. Then, the probe can either remain stationary in a single location while TERS spectra are acquired, or it can scan across the surface of interest. The intensity of a specific Raman band as a function of tip position on the surface can be used to produce a TERS spectral map. Lastly, in order to ensure the TERS signal acquired is not due to contamination of the scanning tip or normal Raman or SERS of the substrate, a ‘withdrawn’ or ‘retracted’ spectrum is typically acquired with the plasmonic tip far from the surface of interest.

The first proof-of-principle AFM-TERS study for the non-invasive identification of dyestuffs of significance for the art field was recently reported (Kurouski et al. 2014). In this work, the authors analyzed a reference sample consisting of Kinwashi paper dyed with indigo, and were able to successfully acquire TERS spectra of the

Fig. 7 Schematic of an experimental setup for AFM-TERS. The sample of interest is placed underneath the AFM scan head (inset). After the sample is put in contact with the AFM tip, the laser light is brought up through a microscope objective and focused at the apex of the Ag-coated AFM cantilever tip, and a TERS approached signal is acquired. The sample is then withdrawn from the tip and spectra are collected to ensure that no normal Raman or SERS signal is present or any tip contamination occurred.
blue colorant on the paper support. When the dyed paper spectra were compared to a reference of indigo on an Au film, they observed missing vibrational bands and band shifts in the dyed paper spectral patterns, likely indicating the occurring of complex dye-substrate interactions. It was also found that signature peaks of cellulose and glucose, two major constituents of paper, were present in the TERS spectra. In order to demonstrate the feasibility of TERS for analysis of artworks, TERS spectra were obtained from a historic 19th-century manuscript fragment with iron gall ink and compared to that of Kinwashi paper freshly dyed with iron gall ink. The authors found that some vibrational modes of gallic acid, a primary component of iron gall ink, were only observed in either Kinwashi paper or the manuscript, which was attributed to the complex chemical composition of the ink and paper and to the interaction of gallic acid with the paper substrate. This work demonstrates the feasibility and strong future potential for TERS to non-invasively probe artworks with the high sensitivity of SERS. At present, the main limitations of the technique are that it is highly specialized and mostly available in university research labs (although commercial instruments have started to appear on the market in the past 5 years); in addition, when used in transmission mode, samples need to be semi-transparent to be able to be analyzed and they have to be limited in size to what the sample chamber can accommodate. These drawbacks can be overcome by working in reflectance mode, but to date no applications to cultural heritage materials have been described in the literature.

4 Resolving Dye Mixtures

Although SERS has successfully reduced the sample size requirements with respect to HPLC, it is still limited by its inability to physically separate dye mixtures. An immediate consequence of this is that, in the case of dye mixtures, only the signals of the main component are typically detected due to discrepancies in resonance, solubility, affinity for the metal surface, SERS cross section, or a combination of two or more of these factors (Shadi et al. 2004). This represents a real limitation, as it is well known from documentary sources that, throughout history, dyes were often used in combination to achieve particular color shades both in painting and textile dyeing (Cardon 2007). Several studies describing applications of the SERS technique to the analysis of artworks and historical textiles have described instances in which, while the presence of multiple colorants had been assessed by HPLC, only one could be detected by SERS (Leona and Lombardi 2007; Pozzi et al. 2012b). On the other hand, recent contributions to the scientific literature have offered proof of the simultaneous SERS detection of binary mixtures of dyes in multiple-component commercial pigments (Londero et al. 2013), fibers from historical textiles (Idone et al. 2013), and oil paint samples (Pozzi et al. 2014a). This has prompted researchers to pursue an extensive investigation of the capabilities of
SERS to concurrently detect and identify several dyes in combination and, at the same time, has encouraged alternative, more efficient ways to accomplish analysis of mixtures of organic colorants by SERS upon physical separation of the individual constituents.

4.1 Binary Mixtures in Solutions

While a detailed evaluation of the SERS technique’s ability to detect single dyes has been the subject of comprehensive studies in the past decade, to date very little work has focused specifically on the identification of individual components of dye mixtures. Thus far, spectra of binary combinations of reference alizarin, purpurin and lac dye have been presented within very preliminary studies (Whitney et al. 2007; Van Elslande et al. 2008; Jurasekova et al. 2010), some of which have offered an initial assessment of suitable metal substrates and ideal experimental conditions, along with summary considerations on marker bands, adsorption geometries, and relative detection limits of the colorants examined. These early studies have been significantly expanded by a more systematic work recently carried out at the Art Institute of Chicago. This work aims to determine the capabilities of SERS in terms of distinguishing a wider number of dyes, including alizarin, purpurin, carminic acid, laccaic acid and brazilein, in series of historically relevant mixtures, both for reference solutions and in actual samples such as mock-up red lake oil paints. First, SERS spectra of binary mixtures of the commercial colorants in various relative proportions and concentration comparable to that observed in museum objects were recorded on two different SERS substrates, i.e. Lee-Meisel citrate-reduced silver colloids and AgFONs. This aimed to qualitatively establish relative detection limits for each dye when present in solution alongside another colorant, and to gain a deeper understanding of how different metal substrates and analytical methodologies may affect dye identification. Second, binary mixtures of madder, cochineal and brazilwood were analyzed with SERS in red lake oil paint reconstructions prepared according to 19th-century historical recipes. For this set of samples, analysis was performed after hydrolysis with HF and only employed Lee-Meisel silver colloids as SERS-active substrate. Preliminary results from experiments on reference solutions have shown that, in many cases, the spectral contribution of the second dye in the mixture remains undetected unless it is present in high relative proportions (usually 1:1). A typical example, in this regard, is offered by mixtures of alizarin and carminic acid: for both colloids and FONs, the latter component becomes detectable in the 1:1 mixture, while the contribution of alizarin is predominant even in the 1:10 solution, i.e. in cases where the relative amounts of carminic acid are 10 times as high as those of alizarin. In addition, remarkable differences were observed between the two substrates both in terms of contribution of each dye in the mixture to the SERS spectrum and, in some cases, for what concerns the position and relative intensities of bands. This is likely due to the experiments’ different incubation times, enhancing in the case of colloidal
nanoparticles the preferential adsorption of one of the two dyes possibly due to the factors listed above as well as to differences in the surface chemistry of the metal substrates used. Overall, these new results underscore the need for more extensive experimentation in the area of hyphenated techniques that may enable the combined separation of colorants (e.g. via TLC or microfluidics) and their SERS detection in artworks.

4.2 Thin-Layer Chromatography (TLC)—SERS

Combining efficient separation with the fingerprinting ability typical of vibrational spectroscopies, the coupling of SERS with chromatographic techniques such as TLC or HPLC provides an obvious means toward effective discrimination of multiple dye components in complex mixtures. In particular, TLC-SERS, whose first application was reported by Henzel in 1977 (Henzel 1977), has been used for a series of preliminary proof-of-concept studies recently undertaken in the cultural heritage field thanks to its ability to significantly reduce the amount of material, sophisticated equipment and time needed for analysis. The first example of use of TLC-SERS for the characterization of several organic colorants in combination was demonstrated by Brosseau et al. (2009b). In this work, the authors were able to discriminate single anthraquinoid dyes in a binary mixture in both reference solutions and extracts from a dyed wool fiber. In the same year, a second work published by a different research group described the separation and identification of dye components in ballpoint pen inks by TLC-SERS, highlighting the great potential of this technique in the field of forensics (Geiman et al. 2009). After these initial studies, applications of TLC-SERS were then extended to the analysis of mixtures of the main β-carboline alkaloids from the seeds of the Syrian rue plant (Peganum harmala), i.e. harmalol, harmaline, harmane and harmine, which are relevant as historical dyes and as drugs (Pozzi et al. 2013a), and to the separation and identification of the structurally related components of mauve, the first synthetic organic dyestuff (Cañamares et al. 2014). All these works share a common analytical procedure, in which solution aliquots of the dye extract or commercial material of interest were deposited onto a silica gel TLC plate using a glass capillary and eluted in a glass developing chamber by means of an appropriate solvent mixture. Then, the eluted spots were visualized and marked under ultraviolet light. SERS analysis was subsequently performed directly on the silica gel plate, upon deposition of a few microliters of Ag colloid with or without aggregant, after drying of the droplet or while the spots were still wet. In these approaches, the sometimes inefficient dye separation provided by the silica gel was compensated for by its chemical and vibrational inactivity compared to other substrates. Initial attempts to make the entire TLC plate SERS-active by both pre- and post-treatment with silver colloids resulted either in poor separation of the dyes and difficult spot identification, or in an uneven distribution of the nanoparticles on the plate surface. New avenues for methodological improvement include the application of AgI and other
silver halides onto gold-coated slides to form a new generation of SERS-active TLC substrates that have shown great promise thanks to their tunable structure, as well as superionic, optical, and complexing properties (Sciutto et al. 2015).

### 4.3 Microfluidics—SERS

An alternative method to simultaneously separate, detect and identify multiple analytes is offered by the development of a microfluidic-SERS platform. In this approach, the channels of the microfluidic-SERS device are functionalized with alkanethiol self-assembled monolayers (SAMs), all which have different functionalities. The polarity of the tail group can be adjusted to attract different classes of dyes, analogous to adjusting the mobile phase solvent polarity in chromatography. A microfluidic-SERS approach is not only tailorable, but also requires a small sample; the micrometer scale of the device channels typically involve sample volumes on the order of 100 µL to flow solution through the entire device. This is beneficial when working with samples that are limited in size or volume, which is often an issue in cultural heritage science. Previous work has shown that microfluidic-SERS devices can detect analytes with a concentration as low as 3 nM (Kim et al. 2014).

A microfluidic-SERS device developed by the Van Duyne group at Northwestern University consists of a SERS-active substrate, i.e. metal FONs, and a polydimethylsiloxane (PDMS) patterned channel mold. Device fabrication is illustrated in Fig. 8. First, the microfluidic devices are produced by lithographically patterning the channel design onto the AuFON SERS substrate with UV light cured photoresist. Next, the photoresist is removed, leaving behind the channel pattern directly on the SERS substrate. The patterned SERS-active substrate and the cured PDMS mold are exposed to oxygen plasma and fused together. Holes are punched in the PDMS to create the inlets and outlets for the channels, where solutions can be injected in a controlled manner via an infusion pump. Each individual channel is then functionalized with the alkanethiol of interest, and the channels are rinsed to remove excess or unbound reagent. Lastly, the target analyte is flown through the entire device and SERS measurements are taken on an inverted microscope setup by focusing the laser light at the plasmonic substrate surface.

In proof-of-principle experiments, the Van Duyne group has successfully fabricated microfluidic-SERS devices and explored the separation capability of alkanethiol SAMs on a SERS substrate. First, SERS spectra of a 100 µM alizarin solution in both a non-functionalized device and a SAM-functionalized device channel were acquired, and the characteristic SERS spectrum of the dye was successfully detected in both devices. Additionally, it was found that the PDMS has a unique Raman spectrum, which can be subtracted out from the SERS spectra to avoid interference. Non-microfluidic device FONs were then prepared in order to explore the capability of different terminated alkanethiol SAMs to separate different classes of dyestuffs. Two FONs were functionalized with a –CH₃ and –OH
terminated alkanethiol SAMs, HS–(CH$_2$)$_5$–CH$_3$ and HS–(CH$_2$)$_6$–OH. After thorough rinsing, the FONs were incubated in a 1:1 ratio mixture of dyes alizarin, an anthraquinone dye, and brazilein, a neoflavonoid dye. As shown in Fig. 8, the –CH$_3$ terminated SAM only shows SERS of alizarin, but the –OH SAM displays SERS signal characteristic of the mixture of alizarin and brazilein. The two proof-of-principle experiments demonstrate that alkanethiol SAM-functionalized microfluidic-SERS devices can be designed to selectively separate and detect different classes of dyes in mixtures.

5 Case Studies

Almost three decades after the first reported use of SERS for the analysis of art samples using a roughened silver electrode (Guineau and Guichard 1987), and particularly after the last decade of renewed and sustained interest in the technique, SERS has become an established and increasingly used method for the identification of organic colorants in art and archaeology. Following demonstration of the reproducibility of the results obtained on reference dyes, colored fibers, and lake pigments (Pozzi et al. 2013b), and upon publication of significant collections of reference spectra of anthraquinoids, flavonoids, carotenoids, and other classes of colorants widely encountered when analyzing art (Leona et al. 2006; Cañamares et al. 2006a, 2008b; Whitney et al. 2006; Cañamares and Leona 2007; Bruni et al.
2011a), SERS has become a powerful technique in the analyst’s toolbox to advance the knowledge and support the preservation of a wide range of art objects, as demonstrated in the following sections.

5.1 Textiles

Dyers and craftsmen have harnessed the coloring power of various insects and plants to dye textiles since the beginning of human history. Hence, it is natural that the first applications of SERS as a less sample-intensive alternative to HPLC for the analysis of colorants in art have been directed to the analysis of textiles. As early as 1987, Guineau and Guichard identified madder on a woolen thread after extraction with hydrochloric acid (HCl) and acetone using a roughened silver electrode (Guineau and Guichard 1987). Borrowing from the sample preparation approaches developed for HPLC, the most commonly followed analytical procedures to analyze historical textiles initially involved pretreatment of the sampled threads with a hot solution of HCl and methanol (MeOH) in water. Such pretreatment aimed to release the dyestuff from the complex with the metal cation that acts as a linker between the colorant and the fiber. This type of hydrolysis, however, is rather aggressive and may affect the proteinaceous or glycosidic structure of the fiber itself, bringing in solution much more than just the free dye. While not much of a concern for separation techniques such as HPLC, this is an impediment to efficient SERS analysis of colorants due to interfering components being included in the analysis. Thus, very early on researchers started to experiment with milder extraction methods (Leona et al. 2006) for textile fibers, such as treatment with 1:1 dimethylformamide and water with 1% EDTA, as originally proposed by Tiedemann and Yang (1995). An extraction procedure based on immersion of the fiber in 4 M HF, coupled with SERS on NaClO₄-aggregated silver colloids, allowed the identification of a yellow dye derived from the dried fruit rinds of pomegranate in archaeological woolen threads from the Libyan Sahara, a result confirmed by parallel analysis with gas chromatography/mass spectrometry (GC/MS) and HPLC (Bruni et al. 2011b). Moreover, Bruni and coworkers identified madder on a red woolen thread from the same burial site and Tyrian purple on an ancient bone from a 4th-century tomb in the basilica of S. Ambrose in Milan, where the colorant had likely transferred from the textile used to wrap the body. While it was not possible, in the first case, to identify madder upon HCl:MeOH extraction due to interference of the peptide residues from the hydrolyzed wool protein, a milder method based on immersion in HF led to the successful identification of the red anthraquinone colorant with SERS (Bruni et al. 2010).

Researchers have also been able to circumvent the issue of extraction of the dyestuff from colored fibers with a variety of alternative methodological approaches. Jurasekova et al. (2010) used laser photoreduction of an aqueous solution of silver nitrate to perform in situ SERS identification of alizarin and carminic acid on reference wool and linen fibers dyed with madder and cochineal. In the same work,
colorants belonging to the flavonoid family have also been identified in fibers dyed with dyer’s greenweed (Genista tinctoria L.), onionskin (Allium cepa), chilea (Baccharis sp.) and old fustic (Clorophora tinctoria L.) following traditional pre-Columbian recipes. This approach was also successfully applied to the identification of alizarin in an archaeological Coptic textile from Egypt dated to the 6th–8th century. Although the authors were able to detect Al-complexes of the dye-stuffs, in many cases the spectra reported appear on top of the bands of carbonaceous material between 1300 and 1600 \( \text{cm}^{-1} \), likely formed because of the laser’s thermal energy (dwelling for up to 20 min on the analyzed spot) and conductive silver nanoparticles.

A milder, in situ, extractionless SERS method relies on the use of Ag nanoparticle pastes obtained by centrifugation of Lee-Meisel colloids that are deposited directly on textile fibers. These colloidal pastes were first described by Cañamares (Cañamares et al. 2006b). A few years later, Brosseau et al. applied them directly to reference wool fibers dyed with purpurin, carminic acid, madder, Cape Jasmine, as well as to samples from historical textiles (Brosseau et al. 2009a). Thus, they were able to identify purpurin on a silk thread from a 17th-century cover from Italy; cochineal in the red and pink silk fibers from a long shawl from France dated to the 19th century; and lac, native of India, on a wool thread from a Turkish carpet dated to the late 16th/early 17th century. Brosseau and coworkers also demonstrated the validity of this methodological approach by identifying curcumin in reference silk yarns dyed with turmeric, though a certain degree of photodegradation was observed in this case, as evidenced by the broad carbon bands on which diagnostic signals for the dyestuff were superimposed.

Interesting studies of alizarin, purpurin and madder include the work of Wustholz et al. (2009), who used pastes of silver colloids applied directly on a minute fragment of a Peruvian textile (800–1350 A.D.) to identify alizarin, likely derived from the native plant Galium corymbosum L. Rambaldi et al. (2015) used silver colloids with and without HF pretreatment to record SERS spectra of wool threads tinted with various species of madder (Oldenlandia umbellata L. and Rubia tinctorum L.), which led to the preferential detection of alizarin or pseudopurpurin—a minor component of the dyestuff mixture present in madder—depending on the specific botanical species employed for dyeing. Additionally, the silver-doped agar gel micro-extraction procedure described above was successfully applied by Lofrumento and coworkers on a pre-Columbian textile to detect alizarin after training the method on laboratory-dyed textiles treated with alum and purpurin, alizarin and caminic acid (Lofrumento et al. 2013). In an effort to identify areas with the highest metal coverage by monitoring the morphology and distribution of the applied silver nanoparticles, Prikhodko et al. (2015) recently developed a hyphenated SEM-Raman system to conduct SERS analysis on reference samples dyed with cochineal and madder.

A few works also concern themselves with lesser studied compounds. For example, in situ applications of silver colloids were exploited to characterize the colorants of wool threads dyed with materials traditionally used in Mexico, such as carminic acid from cochineal (Dactylopius coccus), achiote (Bixa orellana), muittle
(Justicia spicigera), zacatluxcalli (Cuscuta sp.), brazilwood (Caesalpinia echinata), and cempazuchitl (Tagetes erecta) (Casanova-González et al. 2012). Doherty et al. successfully employed SERS to identify different orchil species, i.e. Roccella tinctoria and Lasalia pustulata, on wool fibers both on reference samples and on a 16th-century purple dyed tapestry from Brussels. In this work, Lee-Meisel colloids aggregated with magnesium sulfate were directly deposited onto the threads, without any pretreatment (Doherty et al. 2014).

Early approaches availing themselves of an in situ hydrolysis pretreatment step include Leona and Lombardi’s demonstration of the use of HCl vapor to enhance detection of berberine on a small thread from a faded and weathered 17th-century silk textile from China, which had been previously analyzed with HPLC to reveal a mixture of berberine and safflower (Leona and Lombardi 2007).

The in situ, non-extractive hydrolysis with HF described above for the analysis of red lakes in paints (Fig. 1) has also been demonstrated to be of value for the analysis of textile samples, a first example of this being the identification of berberine on a Byzantine textile dated to the 11th century (Leona et al. 2006). The HF pretreatment has proven to be highly effective for the analysis of textile samples, with the notable exception of silk fibers (Pozzi et al. 2012a). These were found to undergo a significant degree of hydrolysis resulting in the release of proteinaceous by-products in the analyte solution, which interfere with the analysis by preferential adsorption on the silver nanoparticles. Despite the challenges encountered in the examination of silk fibers, the HF hydrolysis was still successfully applied to the analysis of both reference and historic silk samples. For example, a reference silk fiber dyed with weld, from the Reseda luteola dried plant, was treated with HF vapors and then analyzed with concentrated Ag colloids by Corredor et al. (2009), revealing the presence of luteolin. This method was also applied to the identification of reference silk dyed with extracts of Persian berries, demonstrating the identification of some diagnostic bands for quercetin, probably mixed with other flavonoids such as rhamnetin and kaempferol (Teslova et al. 2007). Other examples include the examination of reference samples of unmordanted and mordanted silk, as well as mordanted wool fibers dyed with Cape Jasmine, leading to the SERS identification of the main chromophores of this dye, the carotenoids crocetin and crocin (Cañamares et al. 2010). The authors noted, though, that in this case SERS analysis allowed the detection of the diagnostic bands for the dyestuffs even without the HF hydrolysis pretreatment step, likely because the two colorants identified also display a strong normal Raman spectrum.

Zaffino et al. (2014) extended the SERS analysis of colorants in textiles to NIR excitation, comparing HF pretreatment and non-hydrolysis procedures on a large set of reference dyes and dyed fibers, as well as historical samples from Chinese Ningxia (18th and 19th century) and Caucasian Kaitag (17th and 18th century) textiles. This work included the first instance of FT-SERS spectrum of an iron-gall dye on a sample from the latter (Zaffino et al. 2014). In one of the most extensive applications of the combined HF pretreatment and silver colloid SERS, Pozzi et al. examined an important corpus of Navajo blankets in the collection of the Art Institute of Chicago (Pozzi et al. 2014b), allowing the identification of the colorants.
in their various red woolen yarns in a selection of 8 19th-century blankets. While the presence of carminic acid was positively confirmed by SERS for fibers dyed with natural dyes, synthetic organic colorants were identified by normal Raman spectroscopy with 785 nm excitation, highlighting the use of Ponceau 4R and two beta-naphthol dyes. Results from this study had a major impact on the dating of some of the blankets examined, that had been given an earlier date based on stylistic grounds only (Fig. 9).

In many cases, it is advantageous to combine SERS with complementary methods of colorant identification and materials characterization. This multi-technique approach was followed, for example, in the analysis of Kaitag textiles from Russia (Pozzi et al. 2012b), where SERS was used in association with SEM/energy dispersive X-ray (EDX) analysis, visible reflectance spectroscopy, HPLC, and XRF spectrometry for a complete characterization of the textile fibers, with special focus on their colorants and deterioration products. The two dozen textiles analyzed, dating from the 17th to 18th century, were primarily made of cotton with silk embroideries and showed a variety of colorants. Of those, SERS identified madder for red; indigo for dark and light blue; weld (or another luteolin-based dye) for yellow; the same luteolin-based dye, or sometimes a different yellow one, in combination with indigo for green; tannins mordanted with iron for dark brown; again tannins, sometimes with the addition of indigo, for black. In this particular case study, the main limitation of SERS emerged during examination of those threads that were shown through the multi-technique analytical approach to be dyed with more than one dyestuffs, as only one component was detected by SERS.
To date, only a handful of examples have been reported on the successful SERS detection of multiple dyestuffs on a single textile fiber. One of these accounts was provided by Idone and coworkers, who performed direct, extractionless identification of both cochineal carmine and brazilwood on animal and vegetable fibers using colloidal pastes (Idone et al. 2013). The study focused on the much admired textiles produced in Italy in the early 20th century by artist Mariano Fortuny y de Madrazo (Granada, Spain, 1871—Venice, Italy, 1949). The samples dyed with both carmine and brazilwood, as identified by SERS, included two red cotton panels produced by the Società Anonima Fortuny and a silk thread of a red velvet panel made at the Palazzo Pesara-Orfei in Venice, the latter also displaying a Mariano Fortuny Deposè stamp.

5.2 Paintings

Although many of the technical and methodological improvements in the field of SERS for cultural heritage have only been tested on mock-up paint layers (Doherty et al. 2011, 2014; Platania et al. 2014; Retko et al. 2014), SERS is becoming increasingly popular in real-world case studies to detect and identify red dyes and lake pigments in samples from actual paintings. The first application in this context, reported in 2009, concerns the analysis of a 50-µm red glaze sample from St. John the Baptist Bearing Witness (ca. 1506–07), a painting from the workshop of Italian artist Francesco Granacci (Leona 2009). In this case, SERS analysis was performed on glucose-reduced citrate-capped silver nanoparticles with HF hydrolysis pretreatment of the sample, proving the effectiveness of this combined methodology for the ultra-sensitive analysis of medium-rich paint samples such as colorants dispersed in oil glazes. Remarkably, the identification of kermes in the specimen examined is consistent with this anthraquinoid dye being deemed as the main colorant for red glazes in Europe before the introduction of cochineal from the New World in the second decade of the 16th century. This analytical procedure, progressively grown into a standard protocol at the Metropolitan Museum of Art and other institutions, has then proven successful in various routine applications, including the characterization of madder lake in oil paint samples from The Card Players (1623–24) by the Dutch painter Jan Lievens and from an homonymous painting (1890–92) by Paul Cézanne (Pozzi et al. 2013b).

The first extractionless non-hydrolysis study of organic dyes in oil paintings was reported by Oakley et al. (2011), who employed colloidal pastes to examine red colorants in oil glazes from works belonging to the Colonial Williamsburg Foundation collection, i.e. Portrait of William Nelson (1748–50) by the earliest native-born American artist of European descent, Robert Feke, and Portrait of Isaac Barré (1766) by Sir Joshua Reynolds, a founder of the Royal Academy of Arts. In this case, in addition to providing a probe of local environment by means of correlated fluorescence measurements, the authors identified carmine lake in
samples from the two paintings, albeit showing poor reproducibility due to unevenness of the silver nanoparticle coating.

A systematic work comparing SERS spectra of anthraquinoid red dyes from a wide variety of substrates obtained with or without HF hydrolysis confirmed how, in many cases, highly reproducible, conclusive dye identification in lake-containing glazes can only be achieved upon HF hydrolysis (Pozzi et al. 2012a). The high effectiveness of the HF treatment for SERS analysis of oil paints has been initially demonstrated on the aforementioned samples from Cézanne’s The Card Players, as well as paints from Henri Matisse’s The Young Sailor (1906), and Rembrandt’s Aristotle With a Bust of Homer (1653). Further proof of the efficacy of the HF treatment is offered in another article by Pozzi et al. (2014a), which examines a wide selection of red lake oil paint reconstructions prepared according to 19th-century historical recipes, proving that successful dye identification can be accomplished upon HF hydrolysis even when inorganic pigments, extenders, ground materials or binding media are associated with the red lake in the sample analyzed. This comprehensive work on reference materials was then extended, in the same paper, to the systematic analysis of samples from 19th-century French Impressionist and post-Impressionist paintings in the collection of the Art Institute of Chicago, including works by Manet, Pissarro, Renoir, Monet and Gauguin. This study demonstrated the pervasive use of mostly two types of red lakes by these artists: cochineal and madder. In the case of Renoir’s Chrysanthemums (1881–82), microscopic examination of the painting revealed the presence of a very thin, purple-colored overpaint on top of the original red lake. The two paint layers could be analyzed individually upon careful separation by means of a scalpel, revealing the use of cochineal as the original lake and Pigment Red 48:3 for the ~20-μm thick retouching (Fig. 10).

The first evidence of SERS identification of binary mixtures of red lakes in painting layers was offered in two instances, i.e. Renoir’s Woman at the Piano (1875–76) and Édouard Manet’s Woman Reading (1879–80). In the first case, a few particles with an orange fluorescence emission were spotted within a non-fluorescing red lake paint layer in a cross section sample removed from the painting. The identity of the two types of lake pigments could be determined by SERS: while the signals observed right after sample preparation were consistent with madder, after a few minutes the spectrum of carminic acid was recorded from the same sample. This observation most likely indicates that the alizarin and purpurin molecules originating from the fluorescing madder particles, which have a higher affinity for the silver substrate (Cañamares et al. 2006a), were adsorbed first on the nanoparticle surface. As aggregation proceeded, carminic acid from cochineal—present in significantly higher amounts in the sample within the non-fluorescing layer—then occupied the remaining surface sites, resulting in the carminic acid signals becoming predominant in the SERS spectrum. As to Manet’s Woman Reading, while the SERS data acquired from the corresponding samples did not match the individual reference spectra of commercial madder or cochineal lakes, a perfect correspondence was found with the spectrum of a red lake paint
reconstruction prepared as a mixture of cochineal and Kopp’s purpurin (a purpurin-rich extract of the madder lake) on aluminum sulfate (Pozzi et al. 2014a).

Beside successfully detecting red lakes in combination in a few cases, SERS was used by Oakley et al. (2012) upon treatment with sulfuric acid to probe mixtures of blue inorganic pigments and organic colorants, i.e. Prussian blue and indigo, in a single sample from the early 18th-century oil painting Portrait of Evelyn Byrd (1725–26).

Most recently, the applicability of SERS to the analysis of oil paints and lake glazes has been further expanded to include the examination of paint layers in cross section samples. Idone et al. (2014) used an extractionless non-hydrolysis approach to characterize highly fluorescing red lake pigments in a cross section from a 16th-century mural painting of Sant’Anna Metterza. Although the experiment was successful, the authors emphasized the need to repeat the experiment on a number of different areas in order to find suitable spots where clusters of aggregated nanoparticles give rise to a significantly improved signal enhancement. Frano et al.
(2014) identified carmine and madder lakes in cross sections of 18th- and 19th-century paintings by direct deposition of colloidal pastes. Although more complex and equipment-intensive, the coupling of UV laser ablation sampling with SERS detection on a vapor-deposited silver nanoisland film, described above, has recently led to the successful identification of kermes in samples from *The Incredulity of Saint Thomas* by Venetian master Luca Signorelli and *Adoration of the Shepherds* by Giorgione, both dated to the 15th century (Cesaratto et al. 2014).

### 5.3 Works of Art on Paper

Applications of SERS to the analysis of colorants used in works of art on paper are still rare. In graphic works, the pigment particles are typically bound with small quantities of aqueous media such as glue or gums, and mixtures of compounds tend to be simpler than for pigments used in paintings. Therefore, reported uses of SERS in this area have involved the direct deposition of colloidal pastes on small pigment particles removed from the artworks. To date, various authors have successfully performed SERS analysis on watercolors and pastels from the 19th century, illuminated manuscripts, and Japanese screens and woodblock prints.

Brosseau et al. (2009b) analyzed the colorants of late 19th-century pastel sticks that belonged to artist Mary Cassatt (1844–1926), ranging in color from pale pink to dark purple, and found dyestuffs that were also encountered on a pastel drawing by the artist in the collection of the Art Institute of Chicago. Analysis was performed with citrate-reduced Lee-Meisel colloids concentrated into a paste and deposited directly on individual pigment grains. Interestingly, the materials identified show the coexistence of traditional dyes with some newly introduced synthetic organic colorants in the palette of the artist, including carmine lake, madder, rhodamine B and 6G, and a beta-naphthol or monoazo dye.

Regarding the watercolor medium, Pozzi et al. (2013c) used the HF pretreatment to analyze reference historical lake pigments in series of watercolor washes included in historical catalogs of the famed English colorman Winsor and Newton, and reported identification of madder on a sample removed from *Silver Ball, Barge and Trees* (1930), a watercolor by Arthur Dove made with gouache, ink and charcoal (Pozzi et al. 2013b). SERS analysis of a sample taken from an extremely faded area of the sky of Winslow Homer’s watercolor *For to Be a Farmer’s Boy* (1887) found cochineal, Indian purple (a precipitate of cochineal carmine on copper substrate) and purple madder by comparison with reference spectra taken on a Winsor and Newton antique book showing swatches of original, unfaded watercolor samples (Brosseau et al. 2011). In Homer’s work, the red lakes were found to be mixed with vermilion and chrome yellow, which led to conclude that the sky must once have depicted a sunset or dawn scene. Based on this newly found
evidence, a decision was made to digitally recolorize an image of the work of art to offer scholars and the public the opportunity to view the work as the artist originally intended.

Several researchers have recently added SERS to the toolbox of analytical methods deployed to investigate illuminated manuscripts, artifacts that are generally studied mostly non-invasively and for which miniaturization of the samples analyzed is of paramount importance in light of the rarity of the illuminations and extremely thin layers of colored media. Castro et al. (2014) combined minimally invasive SERS with non-invasive microspectrofluorimetry to identify, upon HF hydrolysis, lac dye reds in 12th–13th-century medieval illuminations from Portugal. Aceto et al. (2015) successfully combined SERS and other micro-invasive techniques (such as matrix assisted laser desorption ionization-time of flight-mass spectrometry, and inductively coupled plasma-mass spectrometry) with FTIR, FT-Raman, fiber optic reflectance spectrophotometry, spectrofluorimetry, and XRF spectrometry to identify folium and orchil on various reference samples including dyed parchment—a mock up of the exceedingly rare purple codices. The authors claim that SERS was effective at identifying the colorants on the dyed parchment samples with and without extraction with formic acid, and report the first SERS spectrum of folium. El Bakkali et al. (2014) used UV/vis reflectance and fluorescence spectroscopies alongside SERS to identify carminic acid in red and pink inks on 19th-century manuscripts from Morocco. In a work by Leona and coworkers (featured in Sgamellotti et al. 2014), SERS on silver colloids was used to identify lac dye in the center of the iris petals in Ogata Kōrin’s masterpiece Irises at Yatsuhashi (1709 or later), a pair of Japanese screens from the early 18th century in the collection of the Metropolitan Museum of Art. The identification of a triaryl-methane dye, either methyl violet or crystal violet (the two dyes cannot be differentiated by SERS) on a Japanese print from 1892, Sekigahara Homare no Gaika (A poem about the battle of Sekigahara) by Toyoharu Kunichika, was also reported by Leona et al. as an example of both gel-sampling SERS and inkjet colloid delivery SERS.

Additional work on Japanese woodblock prints of the Meiji period (1868–1912) is being conducted at the Metropolitan Museum of Art. The two-step SERS protocol developed by Pozzi et al. (2012a) has been used to detect what are probably some of the earliest uses of magenta, cochineal, and eosine in Japan (Fig. 11) (Leona et al. 2015).

Of relevance to the analysis of graphic documents are the recent applications of SERS to study historical inks. Roldán et al. (2014) combined SERS and other analytical techniques to identify inks made with bistre. Among historic inks, sepia can be challenging to identify in old and aged samples because of the similarity of its spectral pattern with the profiles of carbon-based blacks. Centeno, Roldán, and respective coworkers (Centeno and Shamir 2008; Roldán et al. 2014) have studied this subject extensively and combined SERS with complementary analytical techniques to successfully characterize the synthetic chromophore in both reference and historical materials.
5.4 Other Applications

The ability to detect minute amounts of colorants makes SERS an ideal technique for the investigation of archaeological samples, which often contain only very small remnants of the original material and are heavily affected by contamination and aging. Hence, SERS may sometimes be the only analytical method that can be used to obtain dye information from these very ancient and often fragmentary artifacts. The first application of SERS to archaeological material was demonstrated by Van Elslande et al. (2008), who detected purpurin in Roman cosmetics. Subsequently, Leona (2009) described the earliest identification of madder dye in a 4000 years old Egyptian leather fragment. The identification of madder in archaeological samples including pink pigments from an excavation at Corinth (2nd century B.C.) and a statue of Caligula (Virginia Museum of Fine Art, 1st century A.D.) were reported by Pozzi et al. (2013b). Londero et al. (2013) also identified madder in a fragment from the trappings of an ancient Egyptian chariot of Amenhotep III (New Kingdom, 1390–1352 B.C.) pretreated with HF and analyzed with LA-SERS.

Few studies exist in the literature in relation to lake pigments used to decorate polychrome sculpture and furniture. Some notable examples include studies of red glazes in medieval polychrome sculpture at the Metropolitan Museum of Art, i.e. the detection of lac dye in a Spanish crucifix dating to 1150–1200, which is thought to be the earliest example of the use of lac dye in European sculpture (Pozzi et al. 2013b). From a slightly later date, ca. 1175–1200, the French Romanesque sculpture of a Virgin and Child in Majesty, made in Auvergne, France, is the earliest documented example of lac dye use in France (Leona 2009). These findings document trade from India, where the colorant originates from, in medieval times.

**Fig. 11** Hiroshige III, View of Benten on Nakanoshima in Shinobazu Pond, Ueno Park, 1881. Woodblock print triptych on paper. The *violet* is methyl *violet*, the lighter *red* on the robes is cochineal, and the *pink* of the *cherry* blossoms is eosine
and are of great historical importance. Daher et al. (2014) also used non-hydrolysis SERS on Ag colloids to identify carmine and madder lake in pink layers from French decorative arts objects such as a weaving shuttle and a commode dating from the 18th century.

Introduced in the mid-19th century as the first form of recording a photographic image on a plate, daguerreotypes by their very nature owe to the image-forming process the presence of silver/mercury nanoparticles on their surface. Hence, Centeno et al. (2008) exploited this structural and compositional property of the medium to diagnose the nature of a white degradation haze forming on daguerreotypes originating from the famed Southworth and Hawes studio. Silver chloride compounds and substituted aromatic compounds were identified by simply focusing a 785 nm laser onto the naturally nanostructured silver surface of the daguerreotypes, giving rise to the SERS effect. Because metallic silver can be redeposited when silver chlorides are exposed to UV/vis illumination, ultimately obfuscating the original image, the SERS data was instrumental to inform exhibition policies for the collection.

5.5 The Interface with Forensics: Modern Inks

The examination of questioned documents is an active area of interest for the application of Raman spectroscopy and SERS, which are considered of value in identifying and comparing the inks of questioned entries. Since it represents a relatively non-destructive technique, Raman spectroscopy promotes the identification of pigments and dyes, which often represent the most intense contributors to the Raman signal. While several reviews on the analysis of questioned documents discuss most of the recent literature concerning normal Raman spectroscopy (Braz et al. 2013; Calcerrada and Garcia-Ruiz 2015), the special contributions of SERS studies are presented here.

In recent years, the focus of forensic research on the characterization of pen inks has turned to ball point and gel inks. These are especially amenable to SERS studies since the dyes typically display a strong SERS enhancement, and the fluorescence interference that usually hampers normal Raman spectroscopy is readily suppressed by adsorption of the analyte on a metal substrate. A recent study on synthetic dyes compared systematically the discrimination capabilities of SERS with various excitation wavelengths (Geiman et al. 2009). Ten dyes representing classes commonly found in ink formulations were selected for the study: Acid Blue 1, Acid Orange 10, Acid Red 52, Aniline Blue, Crystal Violet, Methyl Violet, Pararosaniline, Rhodamine B, Sudan Black B, and Victoria Blue B. These dyes were studied at several excitation wavelengths, i.e. 633, 785 nm (with and without SERS), as well as FT-Raman 1064 nm. Among normal Raman techniques, only the FT-Raman produced spectra with fairly good signal intensity and signal-to-noise ratios, while 633 and 785 nm laser excitation caused high levels of fluorescence overwhelming the dye signals. SERS spectra were obtained using dilute solutions
of the dye molecules with silver colloids, and although some differences were encountered between SERS and the normal Raman spectra, presumably due to differences in the selection rules, a considerable net increase in signal intensity was observed. In a related study, Seifar et al. reported that methyl violet exhibited a strong resonance effect (SERS) at 514.5 and 457.9 nm, while other dyes were most easily observed with 785 nm excitation (Seifar et al. 2001). Bell and Spence (2001) have compared the discriminant advantages of SERS using in situ deposition of silver colloids and normal Raman spectroscopy combined with other analytical techniques commonly employed in document examination. Compared with various extraction methods such as TLC, they found that Raman techniques provided an equivalent and sometimes better discrimination, while being both rapid and non-destructive. SERS ensured an increased differentiation when compared with normal Raman spectroscopic techniques, but problems of reproducibility were obtained in several samples. Luo et al. (2013) employed gold nanoparticles as a SERS active substrate in order to analyze pens and printers/copiers inks in situ at 633 nm. Raza and Saha employed silver-doped agarose gel disks as SERS substrate for examining ballpoint inks, carrying out analysis at 785 and 514 nm excitation. The gel disks have been shown to extract the ink on small sections of the pen strokes. The dyes extracted into the gel medium were found to be stable for more than thirty days and were furthermore examined by attenuated total reflection (ATR)-FTIR in sequence (Raza and Saha 2013). White (2003) reported an interesting SERS experiment on a three year old bank check that was coated with silver colloid. Finally, Wagner and Clement (2001) described the use of SERS (silver colloid, 633 nm) on ballpoint inks as well as fluid inks of varying colors. A comparison of the discrimination power of normal Raman spectroscopy and SERS showed significant enhancement of the Raman signal using SERS. However, black ballpoint inks apparently lack characteristic features. They are most likely composed entirely of graphite, which usually displays only several broad peaks in normal Raman spectroscopy, and are difficult to characterize using SERS.

Only few reports have focused on ink jet/toner printers as analyzed by SERS. Rodger et al. have obtained SERS on a set of four different ink jet dyes on five different papers on silver colloid, at 514 and 633 nm, as well as FT-Raman at 1064 nm (Rodger et al. 2000). SERS was shown to be successful for identifying the dyes, while FT-Raman produced interference from the paper and filler as well. This was explained as due to the increased size of the area sampled, which most likely included some of the paper. Additionally, SERS is relatively insensitive to the ingredients in paper. Luo et al. (2013) have examined a gold colloid formulation on ink from printers and copiers as well as pen inks, although no information on the samples were provided.

Beside the popularity of pen and printers inks, security inks have a special forensic interest. They are largely used on banknotes (either as the ink used in the banknote itself or as an added stain during robberies), various official documents, and checks, by doping the material of interest with pigments or nanoparticles that exhibit unique optical properties such as fluorescence. Historically, security inks were formulated using materials doped with rare earth lanthanides elements such as
europium, terbium, ytterbium, thulium or erbium. These rare earths have unique spectral features when added to a normal ink, which can be clearly discriminated from any other attempted forgery or counterfeit. In recent years different patents have been issued which utilize SERS taggants or SERS active sols in security inks. SERS nanotags consist of a nanoparticle coated with a unique “reporter” molecule, which is then encapsulated by an inert silica or polymer layer. The outermost layer ensures nanoparticle stability over time and protection from environmental conditions, so that the taggants can be analyzed even after many years.

6 Conclusions and Future Outlook

It has taken the science of SERS over thirty years since inception for it to become a mature field of study. This is testament to the wide variety and applicability of its scope, as well as the potential for numerous applications covering a wide variety of disciplines. Furthermore, the theoretical underpinnings have engendered lively controversy, and have also led to connections to numerous other areas of scientific concern. Ultimately, the potent combination of high sensitivity and high resolution afforded by SERS makes it unique as an analytical tool, and provides numerous pathways for potential applications. In this chapter, we have reviewed the application of SERS to cultural heritage objects and, due to the similarity of analytical techniques and materials, to systems of interest to forensic science. It may be fairly said that even the above compilation is likely incomplete, and subject to augmentation in the near future.

Researchers have reached the hallmark of a robust field, which should be celebrated. However, this brings up the question as to where the field goes next. Certainly, some of the techniques discussed above are still in their infancy, such as TERS, laser ablation—SERS, and microfluidics—SERS. Therefore, considerable intensification of effort and progress should be expected in these areas. More common hybrid techniques such a TLC-SERS have the promise to allow SERS to become more multidimensional in its approach. More well-developed areas, such as substrate development or extraction methodology, have been of considerable interest for some time, and may not see such rapid change in the future. In any case, the exciting aspect of recent developments is that there is an increasing variety of new locales in which SERS is applied to cultural heritage and forensic science. This proliferation confirms that the applications described here are becoming more widely accepted, and of interest to a wider scientific community for routine-based use. We hope that this review will become a springboard for new researchers interested in advancing the field through their own ideas and unique applications.

Acknowledgments SZ, FC and RPVD would like to acknowledge the National Science Foundation (MRSEC NSF DMR-1121262, NSF CHE-1152547, and NSF CHE-1041812) and the Northwestern University/Art Institute of Chicago Center for Scientific Studies in the Arts (NU-ACCESS) for their support. Additionally, this work made use of the EPIC facility (NUANCE
Center-Northwestern University), which has received support from the MRSEC program (NSF DMR-1121262) at the Materials Research Center; the International Institute for Nanotechnology (IIN); and the State of Illinois, through the IIN. JL and ML thank the National Science Foundation (CHE-1402750) for funding. FC and FP are indebted to the Andrew W. Mellon Foundation for funding a postdoctoral research position and providing support for Conservation Science at the Art Institute of Chicago.

References


Cañamares MV, Lombardi J, Leona M (2009) Raman and surface enhanced Raman spectra of 7-hydroxyflavone and 3′,4′-dihydroxyflavone. E-PS, pp 81–88


Surface-Enhanced Raman Spectroscopy: Using Nanoparticles … 203


