Technical Note: 51874

Practical Applications of Surface-Enhanced Raman Scattering (SERS)

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Key Words

- Amino Acid l-alanine
- BPE
- Colloidal Substrates
- Frens Method
- Gold Colloids
- L-alanine
- Lee and Meisel Method
- Raman Laser Power
- SERS
- Silver Colloids
- Surface-Enhanced Raman Scattering
- *trans*-1,2-bis (4-pyridyl)ethylene

Introduction

Surface-Enhanced Raman Scattering (or Spectroscopy), commonly known as SERS, is a technique that extends the range of Raman applications to dilute samples and trace analysis, such as part per million level detection of a contaminant in water. SERS shows promise in the fields of biochemistry, forensics, food safety, threat detection, and medical diagnostics. Bacteria on food, trace evidence from a crime scene, cellular materials, or blood glucose are all samples where SERS have been investigated. If SERS is so useful, why isn't it more widespread as an analytical technique? This application note introduces the SERS technique and addresses questions about SERS and how it is applied to Raman.

What is SERS?

The SERS effect is achieved when an analyte is adsorbed onto or in close proximity to a prepared metal surface. The Raman excitation laser produces surface plasmons, (coherent electron oscillations), on the surface of the metal. These surface plasmons interact with the analyte to greatly enhance the Raman emission. Figure 1 depicts the SERS effect. To achieve the most effective enhancement, there must be resonance between the metal and the laser, making the correct pairing of substrate and laser of critical importance. The most commonly used metals for SERS are silver and gold, although research involving a wide variety of metals is ongoing. Other metals that have been shown to be useful include copper, platinum, and palladium. A very useful resource on SERS theory and applications is listed below as Reference 1 at the end of this technical note. Research has shown a signal enhancement of 10^4 or

more over traditional Raman sampling techniques (some researchers report an overall 10^{12} enhancement). An important component for a good SERS enhancement is roughness of the metal surface. If the surface is too smooth or flat there will not be effective surface plasmon generation. Surface roughness features are usually much smaller than the wavelength of the laser. For example, roughness features, or particle sizes, are commonly in the range of 20 to 100 nanometers for laser wavelengths in the range of 532 to 780 nanometers.

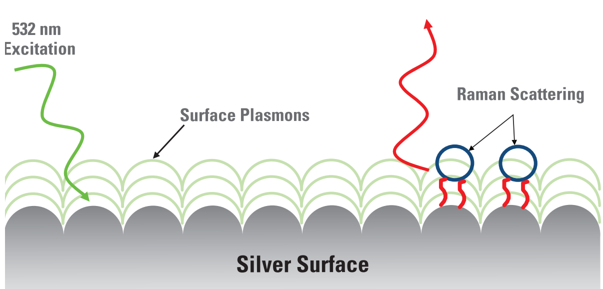

Figure 1: Diagram showing the SERS effect

The SERS Effect

Figure 2 shows the enhancement possible when using a SERS substrate. We chose a compound that is commonly used to demonstrate the SERS effect from a substrate, *trans*-1,2-bis(4-pyridyl)ethylene, BPE. The bottom spectrum is of BPE deposited on a bare slide, and the top spectrum is of the same amount of solution deposited on a SERS substrate. The difference between the two spectra is quite noticeable. The BPE sample not on the SERS substrate has only a few weak features, while the SERS spectrum has sharp, intense peaks. This illustrates what people are seeking from SERS: the ability to get a good spectrum from a dilute sample. Another goal is to get data that regular Raman, or other techniques, may not be able to deliver.

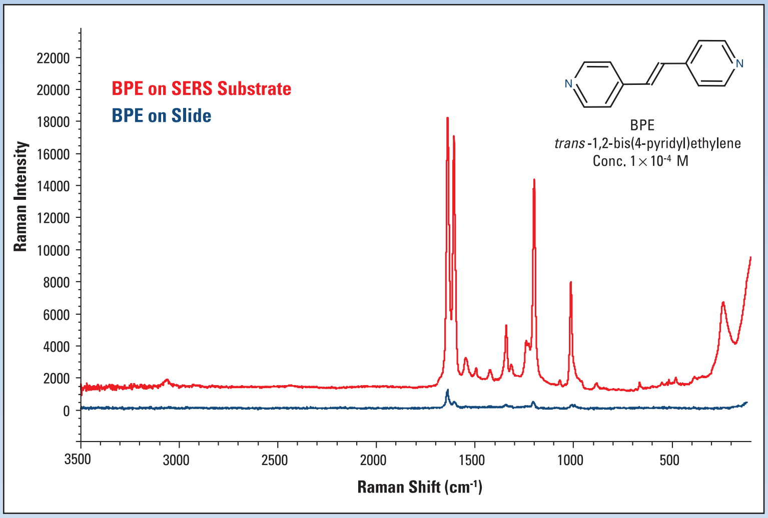

Figure 2: Comparison of Raman spectrum of a BPE solution on a plain surface and on a SERS substrate. The bottom spectrum, in blue, is the Raman spectrum of BPE deposited on a slide and allowed to dry. The top spectrum, in red, is the spectrum of BPE deposited on a SERS substrate and allowed to dry. BPE concentration was 1×10^{-4} molar, and the data was collected using 780 nm excitation at 1 mW.

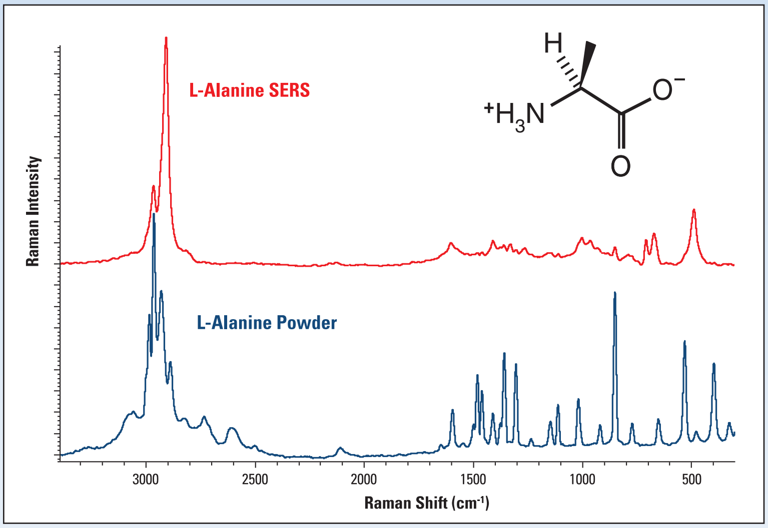

Figure 3: A comparison of l-alanine spectra obtained from bulk powder, shown on the bottom in blue, and from SERS of a dilute solution dried onto a silver SERS substrate, shown on the top in red. Both spectra were obtained using a 633 nm laser. The zwitterion ion form of l-alanine is shown on the top right corner.

Considerations When Performing SERS

When doing SERS, one thing to be mindful of is that it can give information beyond what a bulk Raman spectrum might deliver.

One of the ways that SERS can be used is to investigate how an analyte interacts with a surface, by how it adsorbs or binds to the surface and the geometry of the interaction. Because there may be different types of surface interaction, the spectra that result from SERS may be different than those from a bulk Raman analysis. An example of this is shown in Figure 3, which illustrates the Raman and SERS analysis of the amino acid l-alanine. Similar peaks are present in both spectra, but there are also significant differences, including peaks that have different heights, different shape, peak shifting, and new peaks. L-alanine has three different ionic forms depending on the pH of the solution: a cation, an anion, or a zwitterion. The metal substrates may carry an overall charge, which will influence how the analyte interacts with the substrate, and will influence which part of the analyte interacts with the substrate.

The SERS enhancement can be significant, so it is common that lower laser power is needed, as too much power can lead to a signal that overwhelms an instrument detector. Too much laser power could also potentially damage or degrade the substrate.

SERS Substrates

Typically used SERS substrates are either in the form of a colloidal metal solution, or are prepared as a flat surface, such as a microscope slide, with a metal layer deposited on top.

Colloidal substrates are metal nanoparticles suspended in solution, with the nanoparticles ranging in size from 20 to 100 nanometers in diameter, or length, depending on the particle shape.

Some people choose to make their own colloid solutions, usually following a process such as the Lee and Meisel method for silver colloids[2] or the Frens method for gold colloids[3]. For better reliability and reproducibility you can purchase solutions of colloidal nanospheres (or other shapes and metals). Contact Thermo Fisher for more information.

Thermo Scientific DXR Raman Microscope

One sample preparation method uses a few microliters of the colloid solution which are applied to the sample or are mixed (50:50 for example) with the sample solution. The sample can be placed on a microscope slide and allowed to dry before analysis. Once prepared, the sample is then analyzed with the Raman instrument.

Metal surfaces prepared on microscope slides are very straightforward to use because no sample preparation is needed other than to deposit a small quantity (a few microliters) on the substrate surface and allow the solution to dry. These types of substrates can be more difficult to produce. Some basic methods involve depositing a colloid solution on the surface and allowing it to dry. More advanced methods involve some deposition process for the metal, and then possibly a step to roughen the surface for optimum surface plasmon generation, thus improving the SERS signal.

A third, less common technique is to embed the SERS substrate in a sol-gel. This is done by mixing metal nanoparticles in while producing the sol-gel or to make the sol-gel with a photoreactive chemical (silver containing for example), and then exposing the sol-gel matrix to the proper wavelength so that the chemical reacts and forms the nanoparticles *in situ*.

Choosing the proper SERS substrate is based on the type and form of sample that you are working with. Samples that are in solution can be deposited on a substrate slide, or mixed with a colloidal solution, and then analyzed as a solution or allowed to dry before analysis. For analyzing something like bacteria it may be necessary to mix the sample with a colloidal solution so that the colloid can interact with the bacteria, centrifuge down the sample, pour off the supernatant to remove unwanted components, and then either analyze the result or re-suspend the bacteria and colloid so it can be easily deposited on a slide.

For those starting to implement SERS in the laboratory, we recommend a quick and easy test to confirm you have the proper combination of metal substrate and laser for SERS analysis. A solution of BPE is an ideal test sample in that it has very distinct SERS spectrum, and is effective when using either a colloidal or a metal surface substrate. Using a standard solution such as BPE allows you to become comfortable with your experimental conditions and sampling techniques. When everything is working correctly, you will see best results using much lower laser powers (potentially down to 1 mW or lower) compared to normal Raman.

Advanced SERS techniques can involve the modification of the metal surface either by chemical reaction with a simple molecule and creating a surface layer on top of the metal, by introducing polymer layers for different chemical environments, or by adding functionalization onto the metal substrate. Functionalization might involve or include the use of antigens for the binding of specific antibodies, for example. These modifications add specificity to the substrate, allowing for more discriminate analysis of a specific analyte or analytes.

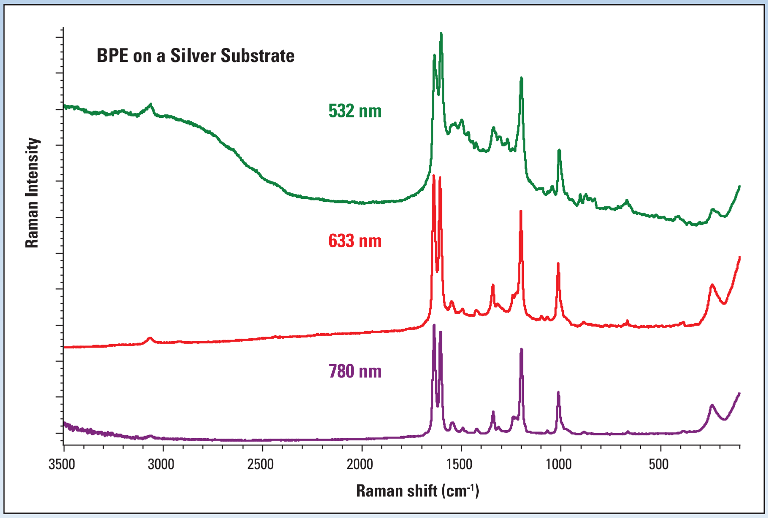

Figure 4: These are the spectra resulting from analyzing the same spot on a silver substrate with BPE on it, using three different lasers, 532, 633, and 780 nm. All the spectra were collected using the same collection conditions: 1 mW laser power at sample, 25 micron slit aperture, and 2 one-second exposures. All spectra are displayed on the same intensity scale, just vertically offset for display purposes.

Challenges of Using SERS

As the previous section introduces, one of the challenges of implementing SERS is picking the right substrate for the sample. Earlier we mentioned that the best SERS enhancement comes from proper pairing of metal and laser. It should be noted that the size of roughness features is important. The laser generates the surface plasmons when it interacts with the metal, and the surface roughness features of the metal affect the strength of the surface plasmons and the resulting enhancement. If the laser and metal are not properly matched, the signal will be weak, noisy or not produce any enhancement at all. Figure 4 shows the result of using different wavelength lasers with a silver substrate. As can be seen in the figure, all the lasers produce a spectrum from the substrate, but the quality of the spectra varies noticeably. The spectrum from the 532 nm laser is noisier and has some broad features present. The 633 and 780 nm spectra are much better, but overall the 633 nm laser is the best choice for this substrate, with less noise, and higher signal intensity. The size and shape of the roughness features will affect the laser wavelength as well. For example, different diameters of spherical colloidal gold will require different laser wavelengths for optimal performance, the general trend is the larger the diameter of the particle the longer the laser wavelength.

Another challenge is making your own substrates. As mentioned earlier, there are published methods for making colloids, and methods for producing metal substrates on surfaces. When making colloids, it is imperative to have very clean glassware, ultrapure water and to be careful of reaction conditions when making the colloids, so that the proper particle formation and size is achieved. Improper conditions can result in a wide range of particle sizes, making them not as responsive to the laser wavelength chosen, or at worst there may be contamination or no colloid formation. Making the metal surfaces on a glass slide can involve expensive equipment and expertise. And lastly modifying the surface of a substrate may require advanced chemistry or techniques.

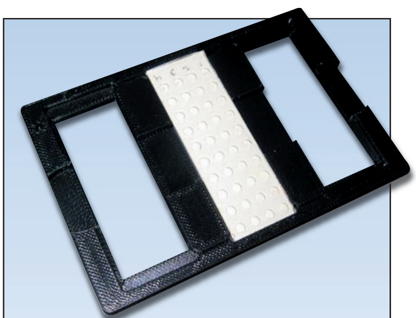

Figure 5: Multiple slide holder for SERS substrate slides, fits a motorized microscope stage on the DXR Raman microscope, or the motorized Well-Plate toolhead for our Universal Platform Accessory on the DXR SmartRaman spectrometer.

Thermo Scientific DXR Raman Instrumentation and SERS

We have brought together all the tools needed to make SERS accessible and effective, including SERS substrates, BPE standard solution, equipment, software, and sample holders. Combine these with our powerful, easy to use DXR Raman instruments and you can fully realize the power of SERS without having to become a surface scientist. Three laser wavelength options are available for the DXR Raman spectrometer, 532, 633, and 780 nm, which provides a wide range of choices for SERS substrates. Our DXR Raman instruments offer extensive laser power control, which is critical when performing SERS analysis. The laser power on a DXR Raman spectrometer is calibrated as power at the sample, and the laser power can be controlled in 0.1 mW steps, from maximum laser power down to 0.1 mW.

We have developed a slide holder for holding multiple SERS substrate slides, shown in Figure 5, which works with our Thermo Scientific DXR Raman microscope and Thermo Scientific DXR SmartRaman spectrometer. You can now perform high throughput SERS analysis through the combination of the slide holder with the Array Automation software add-on for our Thermo Scientific OMNIC software.

Thermo Scientific DXR SmartRaman Spectrometer

Our scientists can also assist with custom conjugation of substrates so that you can develop the most effective SERS substrate needed without having to do all the chemistry.

Conclusion

Surface-enhanced Raman scattering is a powerful technique that allows you to use Raman in new ways for existing samples, and opens the door to new sample types that initially may not have been ideal for Raman. SERS analysis can be easily combined with our Thermo Scientific DXR Raman microscope or DXR SmartRaman spectrometer and accessories. For information on instrumentation, SERS substrates, kits, custom conjugation, and other products please contact us.

References

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