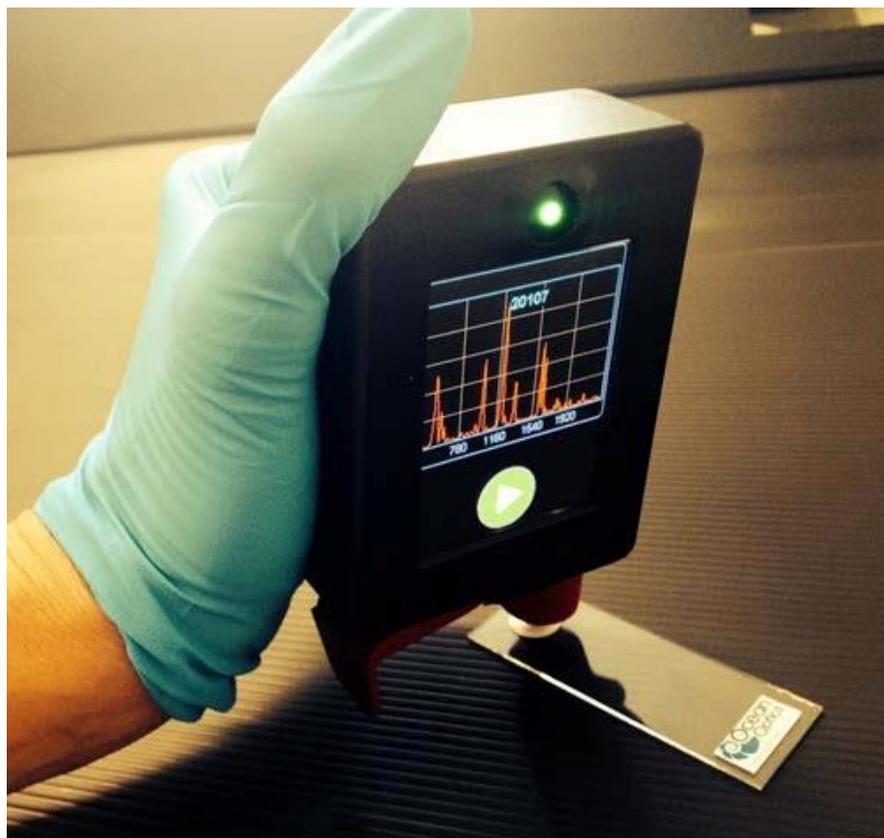




OCEAN OPTICS SERS User Manual



For Products: RAM-SERS-AU; -AG; -SP

Document: RAM-SERS-01-201607

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About This Manual

Document Purpose and Intended Audience

This document provides instructions for using Ocean Optics' Surface Enhanced Raman Scattering (SERS) Substrates.

Document Summary

Chapter	Description
Chapter 1: <i>Introduction</i>	Contains descriptive information about SERS and Raman spectroscopy.
Chapter 2: <i>Using SERS</i>	Provides use instructions.
Appendix A: <i>Specifications</i>	Contains technical specifications for the SERS products.

Product-Related Documentation

You can access documentation for Ocean Optics products by visiting our website at <http://www.oceanoptics.com>. Select *Support* *Technical Documents*, then choose the appropriate document from the available drop-down lists.

Warranty

Ocean Optics SERS is protected by a limited warranty against faulty manufacturing of goods. This warranty will not cover misuse or misadventure. Users are reminded that SERS is a technique that while suitable for many samples will not work for all. Ocean Optics takes no responsibility for unsuccessful use or application of the SERS product. This also applies to any customized or OEM applications of Ocean Optics' SERS substrates.

A 3-Year Warranty covers Ocean Optics miniature fiber optic spectrometers, light sources and sampling accessories – regardless of the application – from manufacturing defects. It also covers fibers and probes for a full 12 months. All OEM hardware is covered by a 12 month warranty. For more information about Ocean Optics Warranties, please see: <http://www.oceanoptics.com/warranty.asp>.

Introduction

What Is SERS?

In Surface Enhanced Raman Spectroscopy (SERS), analytes (your sample) are absorbed onto a noble metal (in this case either Au or Ag nanoparticles) surface prior to analysis in order to potentially enhance the Raman signal. By introducing the sample into a 3-Dimensional matrix, within which gold or silver nanoparticles have been deposited, and exciting with an excitation laser, the interaction between the SERS substrate and the sample, plus the excitation energy of the laser creates a plasmon resonant effect that can potentially amplify the Raman effect by many orders of magnitude. SERS enables ppb and even ppt-level detection of chemical and biological materials quickly and easily in the field. It also has many applications for pharmaceuticals, explosives, and tags for anti-counterfeiting.

SERS substrates have traditionally been fabricated using expensive lithography techniques and are not reusable, making cost a deterrent to use in mainstream applications. Ocean Optics' substrates offer equivalent or better performance than the competitor SERS products at a fraction of the price by using industrial deposition techniques to precisely deposit special nanoparticle ink onto a flexible substrate. One of the advantages of our SERS technology is that it is compatible with the Ocean Optics' full range of Raman spectrometers, meaning that portable measurement is possible with our IDRamini and IDRamini reader. Though peak intensity varies from substrate to substrate, peak ratio repeatability is very good, typically 5% or less. This provides for the substrates to be used both quantitatively and for simple identification purposes.

SERS sounds like the dream technique, so what's the catch? Not all samples are SERS active and users should be aware that SERS will not work out of the box for every sample. Because of our special method for manufacturing we can work with you to develop customized recipes for specific analytes, please contact us for more information. SERS can also shift the peaks relative to standard Raman spectra, i.e., a sample measured at high concentration without SERS may have different peak shifts when looked at with SERS. However, any change is consistent and so, therefore, is easily characterized. It does mean, however, that users requiring library matching should develop the library with SERS rather than adopting existing libraries for SERS applications.

Typical Applications of SERS

- **Detection of Explosives:** Explosives are clearly a big security threat. Being able to identify trace levels allows us to better screen for threats at security checkpoints or on the battlefield.
- **Detection of Narcotics:** Fast identification of Drugs in the field is a real boon for those combating drug related crime. Often only small residues of a sample can be collected from a crime scene. SERS also opens up a potential route for fast roadside drug screening using saliva samples.

- **Food Safety:** Certain additives such as Melamine found in milk powder or green malachite can be extremely harmful even at very low concentrations. Using SERS, we can qualify and quantify the level of dangerous trace elements in our food supply chain.
- **Anti-counterfeit tags:** High value products that are subject to duties & taxes are often the target of piracy and fraud. Petrol is one example. By adding a small amount of a SERS active taggant we can use this as an indicator of authenticity.
- **Biological Research:** Can be used to identify and characterize biological samples including identifying proteins, DNA and bacteria.

What Is Raman Spectroscopy?

Gold nanoparticles were mentioned above, and Raman spectroscopy really is like panning for gold! A wealth of information is there, if you can just sift through the rock, dirt, and sand obscuring it. The art to finding the gold in Raman spectra is the instrumentation, which must collect as many photons as possible while rejecting scattered laser light efficiently.

Raman spectra are generated when an incident photon from an excitation source interacts with a sample and rather than being simply (Rayleigh) scattered it communes with the vibrational energy of the sample molecule and is reflected as Raman (Stokes-shifted) light. By filtering out the background and detecting just the shifted light we obtain a fingerprint spectrum, packed with information about the atoms and structure of the molecule.

A good way to visualize the Raman effect is to imagine a ball bearing being dropped onto a drum. The drum starts to vibrate at its own frequency, and the ball bearing bounces off with slightly less energy (analogous to Stokes radiation). If the drum is already vibrating and the ball bearing hits at just the right time, the drum acts like a catapult to give energy to the ball bearing and it bounces off with even more energy (analogous to anti-Stokes radiation). The energy difference before and after the ball bearing strikes the drum provides information about the vibrational mode of the drum. [*Fundamentals of Molecular Spectroscopy, Banwell and McCash, John Wiley & Sons, Inc., New York, 1988*]

Ocean Optics' SERS Key Features

- **High sensitivity.** Substrates deliver great results and have demonstrated superior sensitivity for a range of analytes when tested against competitor substrates, all for an unbeatable price.
- **Great stability.** Highly stable substrates require no special handling and can be stored at room temperature.
- **Reliable reproducibility.** Highly reproducible and easily scaled manufacturing methods enable sensitive measurements at an affordable price. Our testing has shown reliable peak ratio reproducibility within 5%.
- **Customization.** Unique production techniques can be tailored to impart specificity to particular analytes (on demand) and custom form factors such as swabs and coatings.
- **Easy to use.** For great flexibility, substrates work reliably with the complete range of Ocean Optics Raman instruments. Simply drop your analyte onto the slide and use with a 532, 638 or 785 nm Raman setup.

Key Specifications

Table 1: SERS Substrate Product Details

Specification	RAM-SERS-AU	RAM-SERS-AG	RAM-SERS-SP
SERS Slide Dimensions	25.4 x 76.2 x 1 mm	25.4 x 76.2 x 1 mm	25.4 x 76.2 x 1 mm
SERS Active Area	5.5 mm diameter circle	5.5 mm diameter circle	4 x 4 mm square
SERS Active Chemistry	Gold (Au) Nanoparticles	Silver (Ag) Nanoparticles	Gold/Silver Film
Slide Material	Borosilicate Glass	Borosilicate Glass	Borosilicate Glass
Raman Excitation Wavelength	785 nm	532 nm	638 nm
Storage Lifetime	1.5 months	1 month	6 months
Reusable	No	No	Yes
Laser power	20 mW	20 mW	100 mW
Volume of analyte	15 μ L	15 μ L	10 μ L

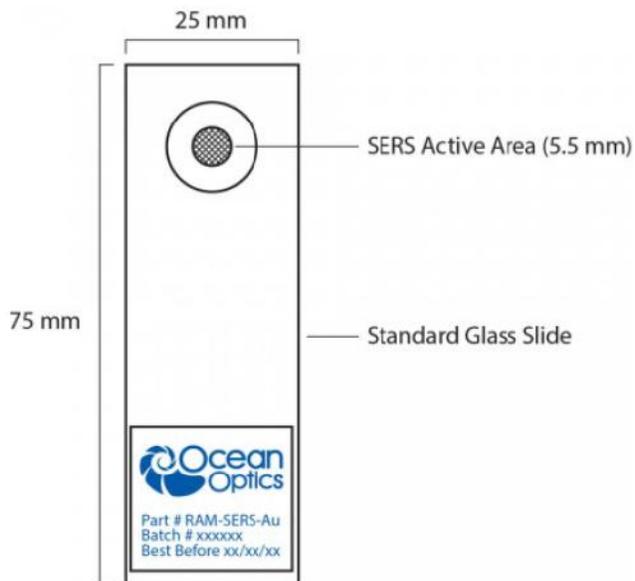
Table 2: Limit of Detection (LOD) for Some Commonly Used Analytes

Material	Why Do I Want to Detect Trace Levels?	LOD with QE-Pro*	
		RAM-SERS-AU	RAM-SERS-SP
BPE	BPE can be used as a taggant in fuel as well as in biological samples.	0.1 fg	30 fg
TNT	The threat of terrorism means the need for quick screening for trace levels of explosions is greater than ever.	5 ng	30 pg
Melamine	Poisonous to humans, especially babies and children at very low levels (<1 ppm).	5 pg	0.1 pg

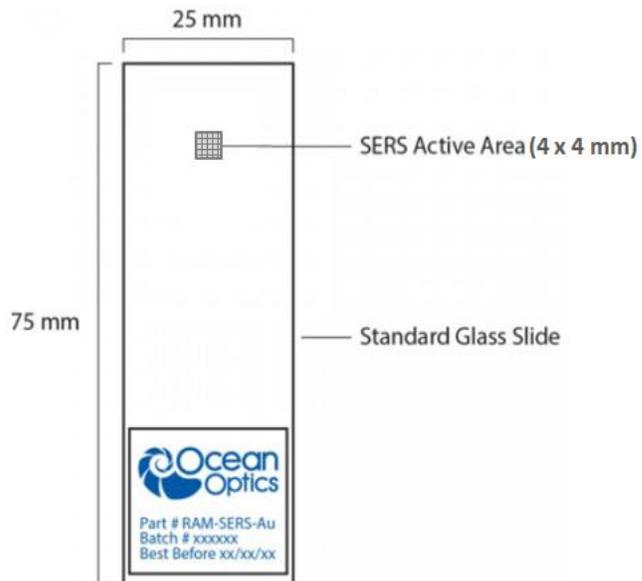
*Please note that the LOD will depend on the sample and spectrometer used and that these are provided as guidelines only.

Diagram

RAM-SERS-AU RAM-SERS-AG



RAM-SERS-SP



Package Contents

Each pack of RAM-SERS-AU-5, RAM-SERS-AG-5, and RAM-SERS-SP-5 comes with 5 individual substrates. Simply remove from the packaging and you're ready to get going.

Using SERS

Usage Notes

The following instructions are for RAM-SERS-AU (paper-based), RAM-SERS-AG (paper-based) and RAM-SERS-SP (glass-based) products. Instructions that are unique to each substrate will be indicated as needed.

- Use gloves during handling and ensure you are working in a clean environment. Never touch the active SERS area. As these are highly sensitive, any contamination has the potential to show up on your analytical spectra.
- Do not touch or apply pressure to the active SERS area as this may damage the nanoparticles or gold-silver film surface.
- For best results, use 15 μL of your analyte dissolved in the appropriate solvent for RAM-SERS-AU/RAM-SERS-AG and 10 μL for RAM-SERS-SP.
- Using a known reference solution is a good way to optimize your measurement and software settings. The RAM-SERS-AU/RAM-SERS-AG slides are one-time use, and should be discarded after the spectrum has been acquired. Reuse is not possible as once the sample has been absorbed onto the active area it is not possible to cleanse/remove it. However, RAM-SERS-SP slides can be reused one or two times after adequate rinsing. See [Reusability](#) for more details.

Solvent-Compatible Substrate and Adhesive

Some competitor products use plastics as the base material, which greatly limits the types of carrier solvents that can be used with those SERS products. Our use of optical-grade borosilicate glass allows for even the harshest of solvents to be used on the SERS chemistry.

The adhesive that holds the chemistry to the plate is a high-performance silicone-based adhesive compatible with a wide range of solvents.

Standard Measurement Protocol

Use clean gloves at all times when handling the SERS substrates. Remember SERS amplifies the Raman signal from trace levels and even small amounts of interfering samples may affect your measurement.

The measurement parameters can greatly affect the overall performance of the SERS substrate and these should be optimized carefully. Once optimized, this setup can be used for subsequent measurements with the same spectrometer type, laser and integration time enabling consistent and comparative data to be collected (see [Best Practices](#)).

Procedure

1. Open the foil shipping bag. The substrates are contained within the plastic slide holder for their protection.

2. Do not touch the SERS active region on the slide. Also do not allow the active area to come into contact with any other surface. This will help to avoid contamination.
3. It is important to keep the SERS substrates clean. Care should be taken in high humidity atmospheres as this may increase the chances of a non-analyte molecule being absorbed onto the substrate. Note also that the substrates should not be exposed to vapors or other potential contaminants that can adsorb onto the surface.
4. Introduce the analyte of interest directly onto the active area. We recommend drop casting or pipetting microliter amounts of testing analyte directly onto the SERS active area. If pipetting, your sample should be prepared using an appropriate solvent.
5. After the analyte has been deposited onto the SERS surface, the measurement can be obtained immediately. You may observe some time-dependent effects as the solvent evaporates. The rate of evaporation can be accelerated by the application of the laser energy. Allow the response to stabilize before recording the measurement results.

Slides should be disposed in standard glass waste containers. Please note that disposal protocol will be analyte dependent. Be sure to use an appropriate disposal method.

Best Practices

To ensure the best signal enhancement from the SERS substrate, we recommend that you observe the following parameters:

- **Focal distance** -- Ensure that the laser is focused on the sample and that the correct focal distance for the Raman system/probe is used. An X-Y stage can be used to adjust the focal distance to ensure that maximum response from the substrate is achieved. Once you have determined an appropriate distance you can fix the stage in position to maintain that focus for repeated measurements.
- **Laser power** -- The Raman response will also depend on the laser power. Too little and there will be no discernable signal, too much and you may damage the sample. The correct power depends on the substrate and spot size of the laser. For best results with RAM-SERS-AU/RAM-SERS-AG, use 102 W/cm^2 , which is equivalent to, for example, 20 mW for a 785 nm laser with a spot size of $158 \mu\text{m}$. Lower laser power densities are required for the paper-based substrates because they will burn at higher power densities. On the other hand, the RAM-SERS-SP substrates can withstand higher power densities without damage because they are made of out of glass. For these, we recommend $5 \times 10^3 \text{ W/cm}^2$, which is equivalent to 25 mW of 638 nm laser radiation over a $25 \mu\text{m}$ laser spot.
- **Integration time** -- As with the laser power, the integration time will depend on the measurement sample and conditions, the requirements of the application and the spectrometer used. Increasing integration time allows the collection of more Raman photons, thus increasing the signal. However, it can also increase background or fluorescence effects.

Storage

Each bag contains a box of 5 SERS substrates and a desiccant pouch to minimize humidity. While Ocean Optics SERS can be stored in normal conditions, we recommend the following to maximize shelf life and performance:

- Open the bag only when you are ready to use the first substrate.
- Ideal storage temperature is room temperature (15-25°C). If not using all 5 substrates within 24 hours, keep the remaining substrates in a dry environment such as a desiccator. A moisture-resistant Ziploc bag with the included desiccant pouch is also suitable.

- Use these in a clean environment and wear gloves during handling, ensuring never to touch the active SERS circle. As the SERS slides are highly sensitive, any contamination has the potential to show up in your results.
- Use all substrates before the published use by date. The products may continue to show results after this time but the sensitivity will be diminished.

Frequently Asked Questions

What kind of Raman equipment do I need to use with SERS substrates?

Ocean Optics SERS substrates are designed to work with all Ocean Optics Raman equipment. This includes a range of modular solutions where a spectrometer can be combined with a modular laser and a probe. It also includes a range of integrated solutions for handheld, benchtop and microscope applications.

How do I get my sample onto the active area of the SERS substrate?

If not already in solution, we recommend preparing your sample in solution with an appropriate solvent. SERS will work with dry powders or other trace amounts of solid samples but solution will generate the most reliable results as it allows the analyte to be absorbed uniformly throughout the SERS active area. Once in solution, we recommend adding 15 μL to the RAM-SERS-AU/RAM-SERS-AG substrates and 10 μL to the RAM-SERS-SP for best results.

How do I know if my sample will be SERS active?

You don't! It's difficult to know ahead of time whether your sample will work with standard SERS. It all depends on the relationship between the gold nanoparticles and your analyte. By adjusting the size and density of the nanoparticles it is possible to tune SERS to work better/worse for particular analytes. It is also possible to dope the substrate with linking molecules that help bind the analyte to the nanoparticles. It is important to recall, however, that SERS will not make a non-active Raman sample active. This means no metals, salts or molecules which do not meet the rules of symmetry that make a molecule Raman active.

Which excitation laser wavelength should I use for gold SERS (RAM-SERS-AU)?

While there is no fixed rule, in general the gold-based SERS have shown best results using 785nm lasers.

Many applications of SERS mention silver, do you have silver SERS and which laser wavelength should I use?

Yes, we offer a silver nanoparticle version of the paper substrate. However, these substrates tend to oxidize when exposed to the environment much faster than the gold substrates. Hence, the SERS performance of the silver substrates begins to degrade 2-3 weeks after receiving them. While gold is generally used with 785 nm excitation lasers, silver generally works best with 532 nm. However, like with the gold, there is no firm rule here.

What does "...particles will oxidize and this causes a reduction in the performance of the substrate as it ages," mean? Do they just lose effectiveness (sensitivity)? How can I tell when this has happened?

All pure metals will oxidize eventually – but some are worse than others while others such as gold are quite slow. The oxide layer is very stable (molecules like settling in that state). This tough oxide layer on top of your nanoparticles destroys the resonance SERS effect because the analyte cannot interact with the pure metal nanostructures. You can't tell by looking at it which is why we have a use by date on the slide.

Is there any way to slow the aging process caused by oxidation?

We continue to investigate ways to improve lifetime without dramatically increasing cost. Minimizing the conditions that increase the rate of oxidation can help prolong the lifetime of your substrates. This includes minimizing humidity (which also ups the chance of contamination) and keeping at a stable temperature.

I've opened the bag and done some measurements but still have some substrates left. How should I store these?

After you've opened the bag you can protect remaining substrates by storing them with desiccant in a resealable bag. We also recommend keeping them in a sealed container to avoid risk of contamination.

Can I reuse my Substrates?

For the gold and silver paper substrates: no they cannot be reused, once you have made your measurement it is close to impossible to remove the analyte from the substrate. Luckily, unlike many other SERS products, you won't damage your bank account buying more. However, for the RAM-SERS-SP glass-based substrates, they can be washed once and reused before the signal begins to diminish.

Appendix A

Specifications

Overview

This appendix contains specification information for the SERS product, including the following:

- [*Performance Specifications*](#)
 - [*Substrate Repeatability*](#)
 - [*Laser Power Dependence*](#)
 - [*Concentration Dependence*](#)
 - [*Examples of Analytes*](#)
 - [*Reusability*](#)
- [*Limits of Detection Across Optical Benches*](#) (QE Pro, Maya Series and USB4000 spectrometer benches, as well as the IDRaman mini and the IDRaman reader)

Also see [*Key Specifications*](#).

Performance Specifications

Description of Tests Performed

Specification tests were carried out using our gold nanoparticle paper-based SERS substrates (RAM-SERS-AU) and glass-based gold/silver alloy nanosponge SERS substrates (RAM-SERS-SP) across a range of production batches. The QE *Pro* bench and 785nm laser were used for the performance characterization of RAM-SERS-AU, whereas the IDRaman reader with 638 nm excitation was used for RAM-SERS-SP. The integration times and laser powers used are indicated in the text and figures as needed. Please note that different analyte scenarios may have more optimal settings. All raw data can be made available upon request.

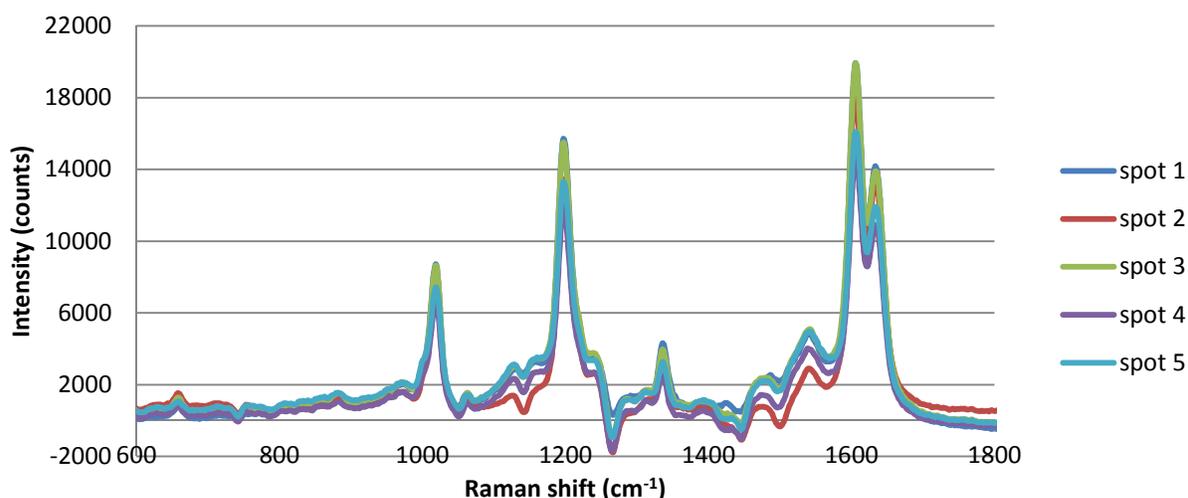
Substrate Repeatability

In order to demonstrate the reproducibility of SERS measurements for each substrate, spectra were collected on five different spots within one substrate and then one spot on six different substrates. The fuel marker 1,2-bis(4-pyridyl)ethylene (BPE) was used for RAM-SERS-AU, and the antifungal dye crystal violet (CV) was used for RAM-SERS-SP.

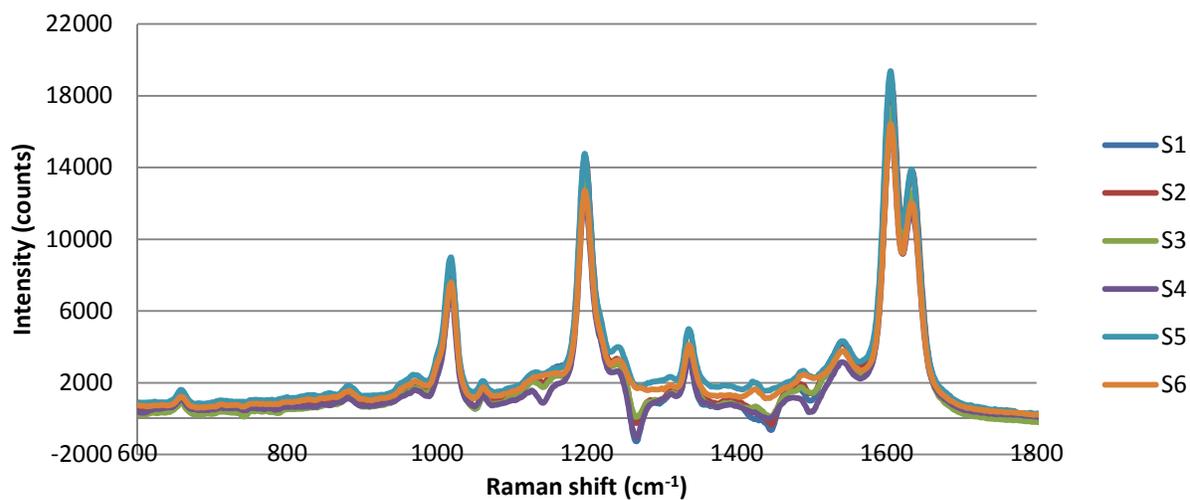
While the presence of peaks is critical in determining whether a species is or isn't present, the relative ratios of those peaks are also important for verification, and can even be used as a means of determining approximate concentration in some cases. The ratio between the peaks at 1605 and 1632 cm^{-1} were used for BPE and the ratio between the peaks at 915 and 1376 cm^{-1} were used for crystal violet. The percent relative standard deviation (standard deviation/average x 100) was calculated for the peak intensity ratio and raw peak intensity for the different substrates.

RAM-SERS-AU

Repeatability of one RAM-SERS-AU substrate
785 nm, 3 s, 15 mW, 0.1 ppm BPE

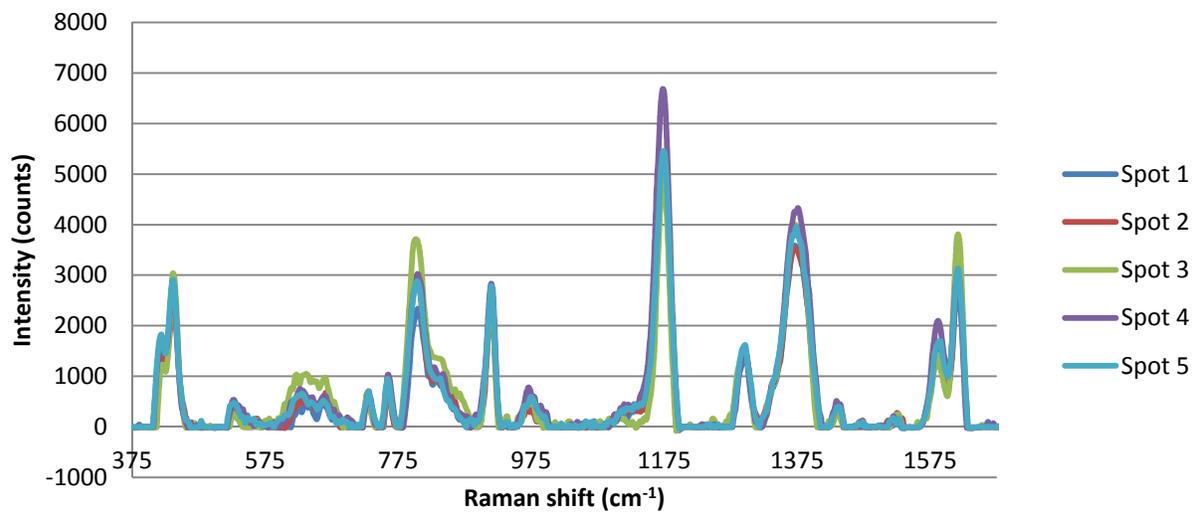


Repeatability between RAM-SERS-AU substrates 785 nm, 3s, 15 mW, 0.1 ppm BPE



RAM-SERS-SP

Repeatability of one RAM-SERS-SP substrate 638 nm, 1s, 21.2 mW, 1 ppm Crystal Violet



**Repeatability between RAM-SERS-SP substrates
638 nm, 1s, 21.2 mW, 1 ppm Crystal Violet**

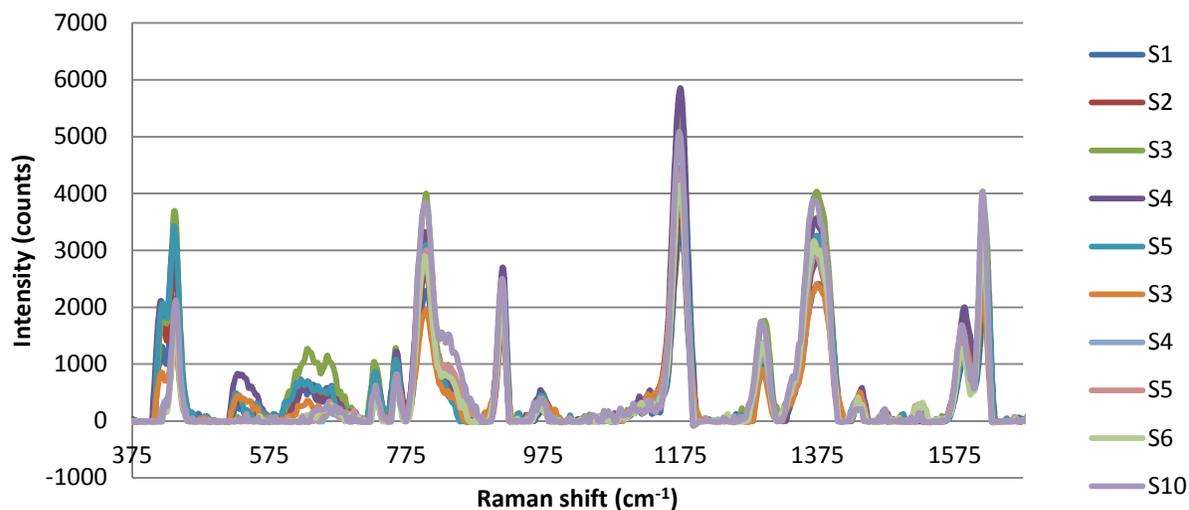


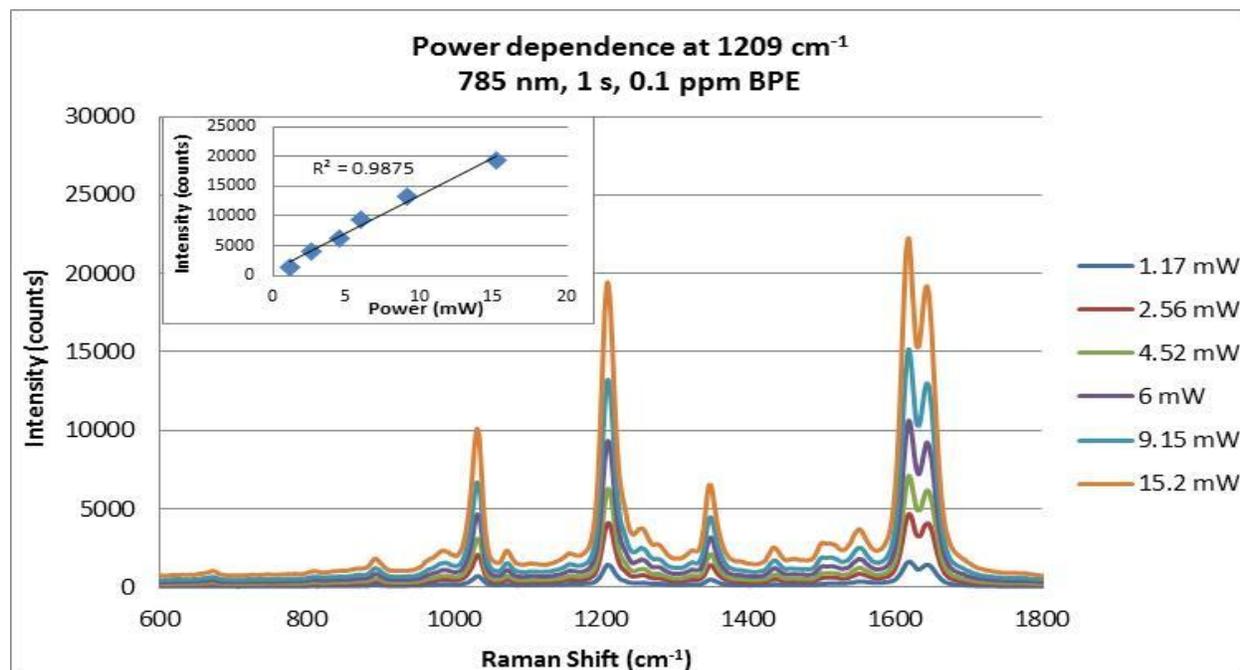
Table 3: Percent Relative Standard Deviaton (% RSD) Values for SERS Substrates

Substrate	Peak Intensity Ratio Repeatability	Raw Peak Intensity Repeatability
One RAM-SERS-AU	BPE (1605 cm ⁻¹ /1632 cm ⁻¹): 2.5% RSD	BPE (1605 cm ⁻¹): 13% RSD
6 RAM-SERS-AU	BPE (1605 cm ⁻¹ /1632 cm ⁻¹): 1% RSD	BPE (1605 cm ⁻¹): 7% RSD
One RAM-SERS-SP	CV (915 cm ⁻¹ /1376 cm ⁻¹): 4% RSD	CV (1175 cm ⁻¹): 13% RSD
6 RAM-SERS-SP	CV (915 cm ⁻¹ /1376 cm ⁻¹): 7% RSD	CV (1175 cm ⁻¹): 8% RSD

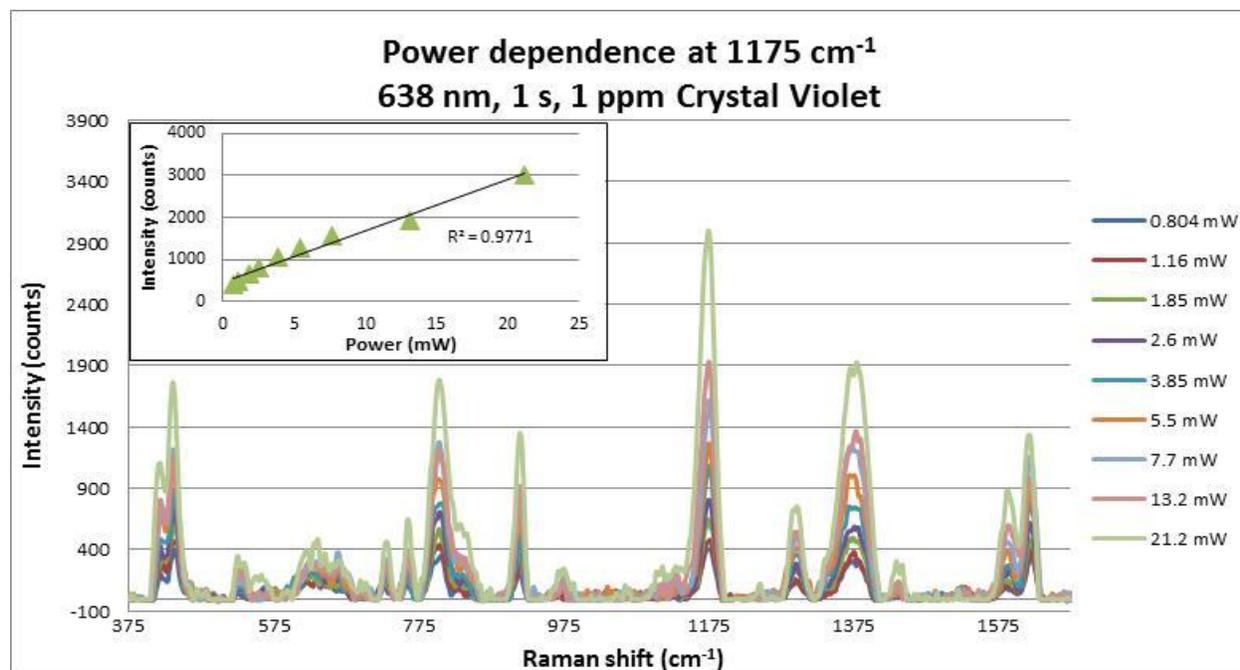
Laser Power Dependence

The intensity of the Raman signal should scale linearly with laser power. Hence, a laser power study was conducted on both substrates using BPE for RAM-SERS-AU and crystal violet for RAM-SERS-SP.

RAM-SERS-AU



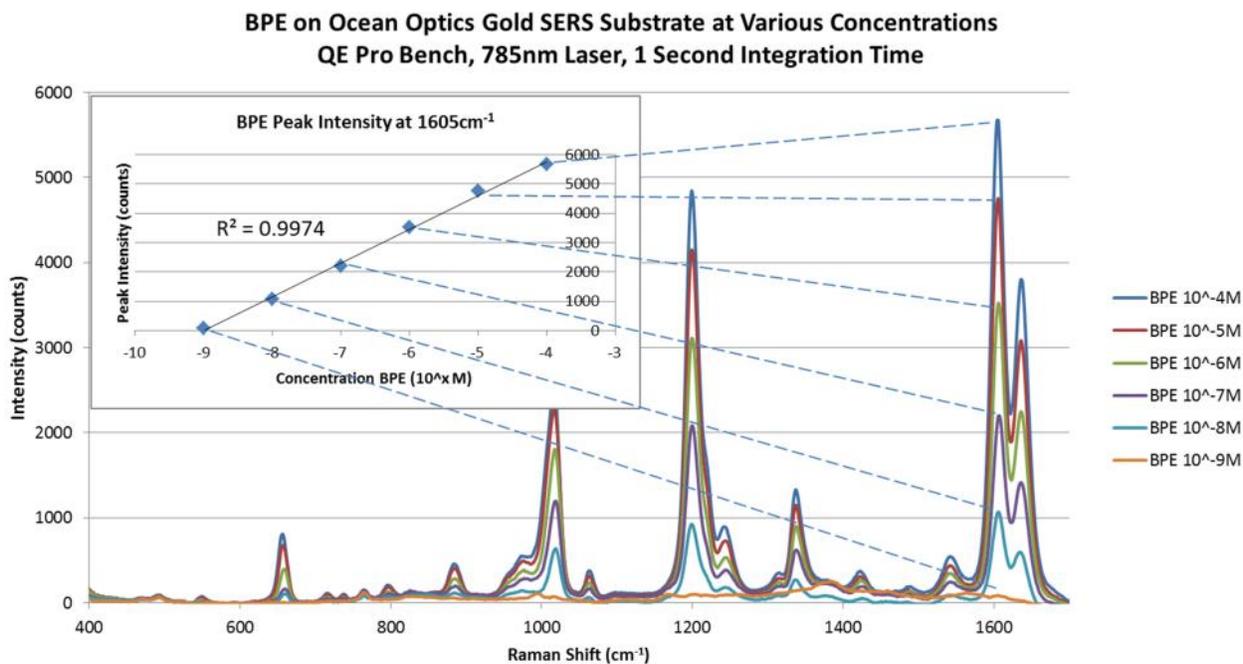
RAM-SERS-SP



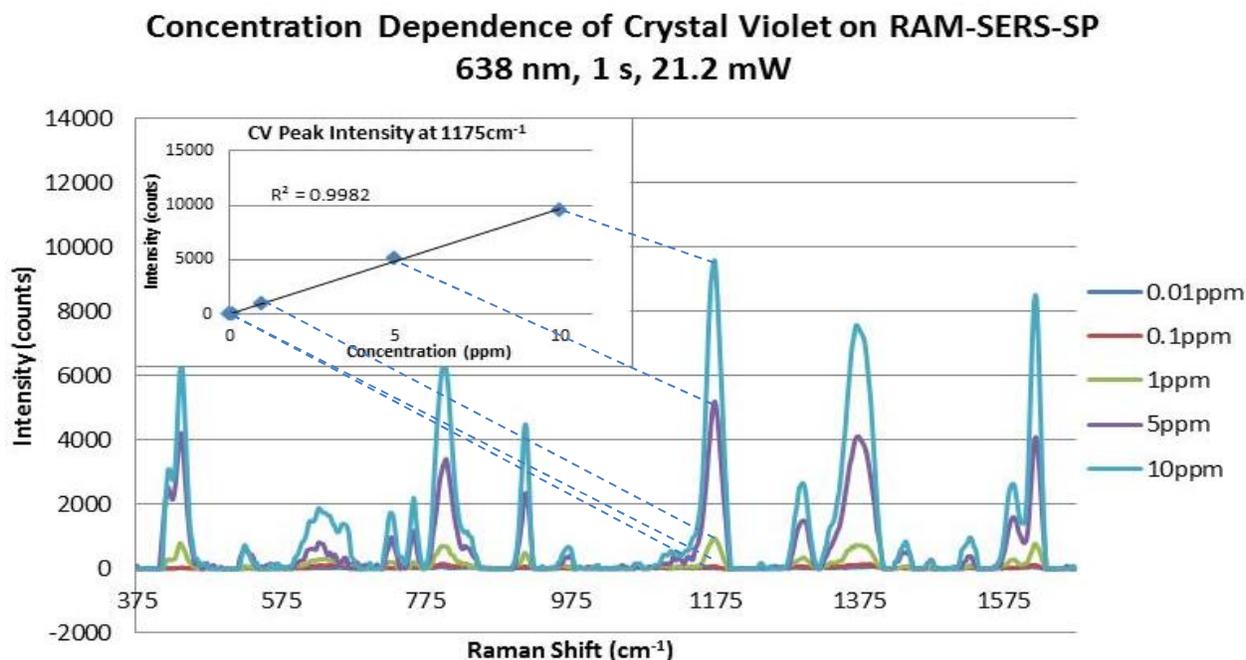
Concentration Dependence

The Raman peak intensity should also be concentration dependent. Various concentrations of BPE ranging from 10^{-9} M to 10^{-4} M (0.0002 to 18 ppm) were used for the RAM-SERS-AU substrate. Crystal violet having concentrations of 0.01 to 10 ppm were used for RAM-SERS-SP.

RAM-SERS-AU



RAM-SERS-SP



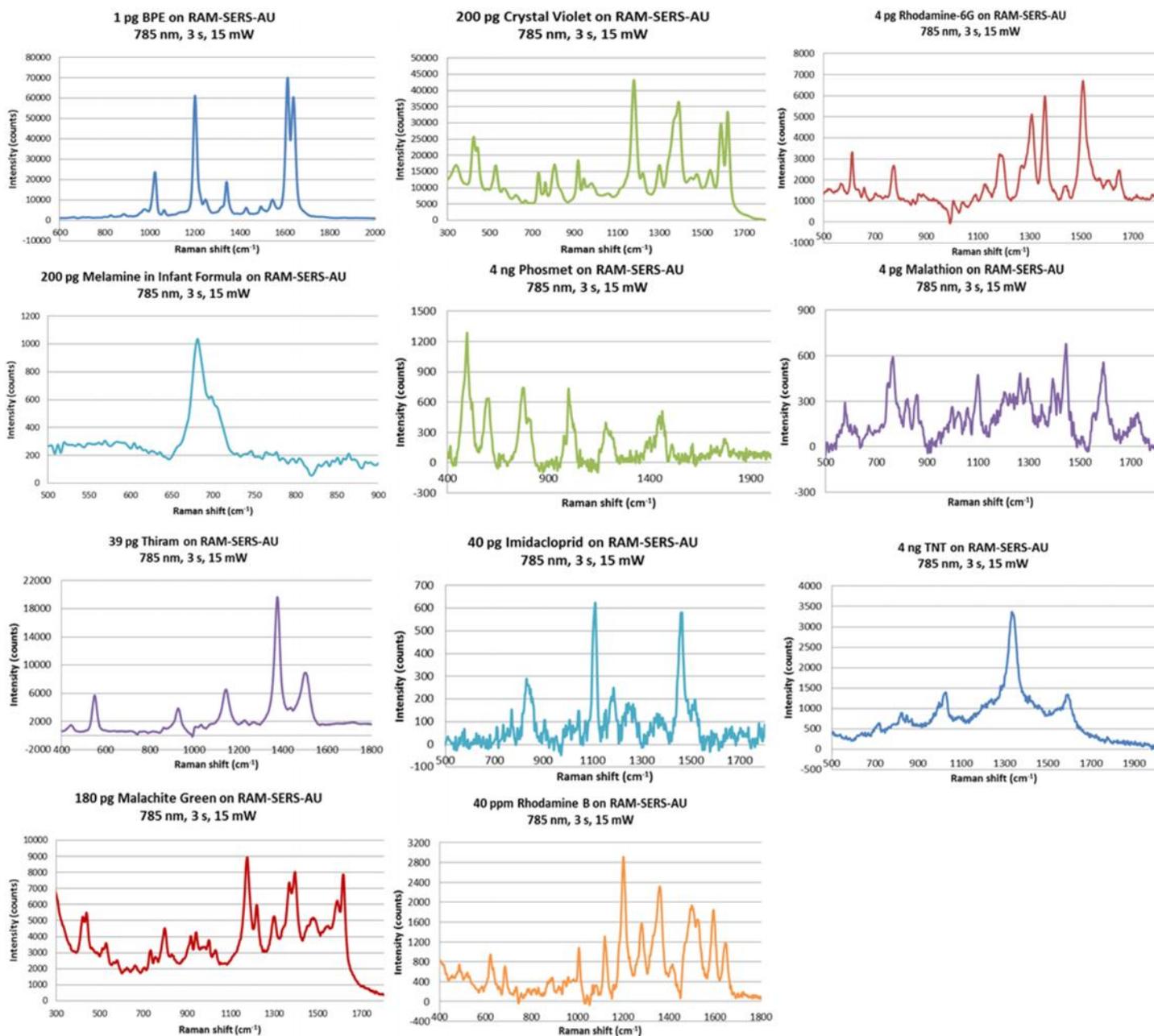
Examples of Analytes

The following figures show a few examples of different analytes that can be used with either of the SERS substrates. The table below categorizes these analytes.

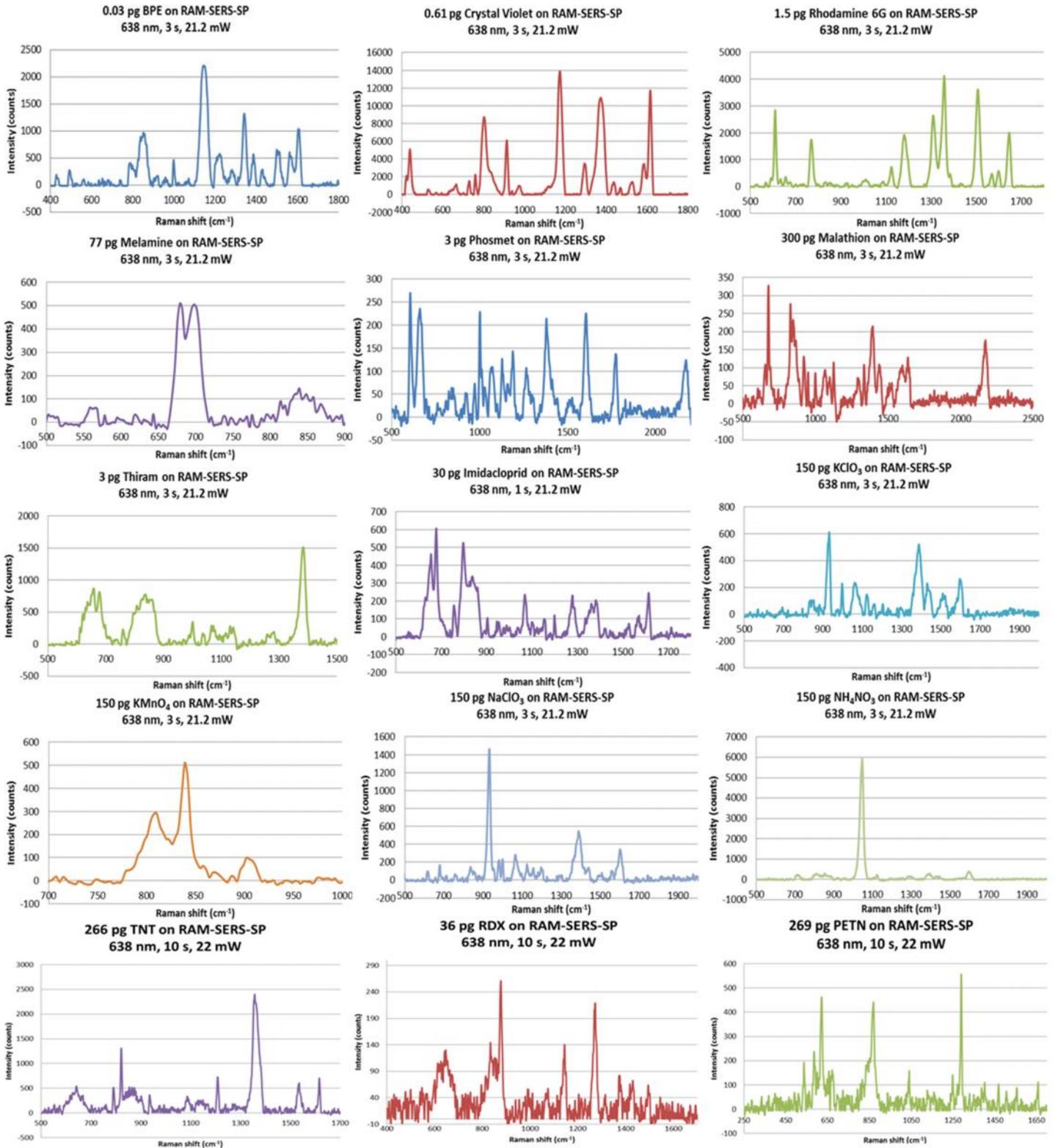
Table 4: Types of analytes for Raman analysis

Markers/Taggants	BPE
Pesticides, antifungal dyes	Phosmet, malathion, thiram, imidacloprid, crystal violet, malachite green
Explosives/precursors to make explosives	TNT, RDX, PETN, KMnO_4 , NaClO_3 , KClO_3 , NH_4NO_3
Dyes	Rhodamine 6G, rhodamine B

RAM-SERS-AU



RAM-SERS-SP

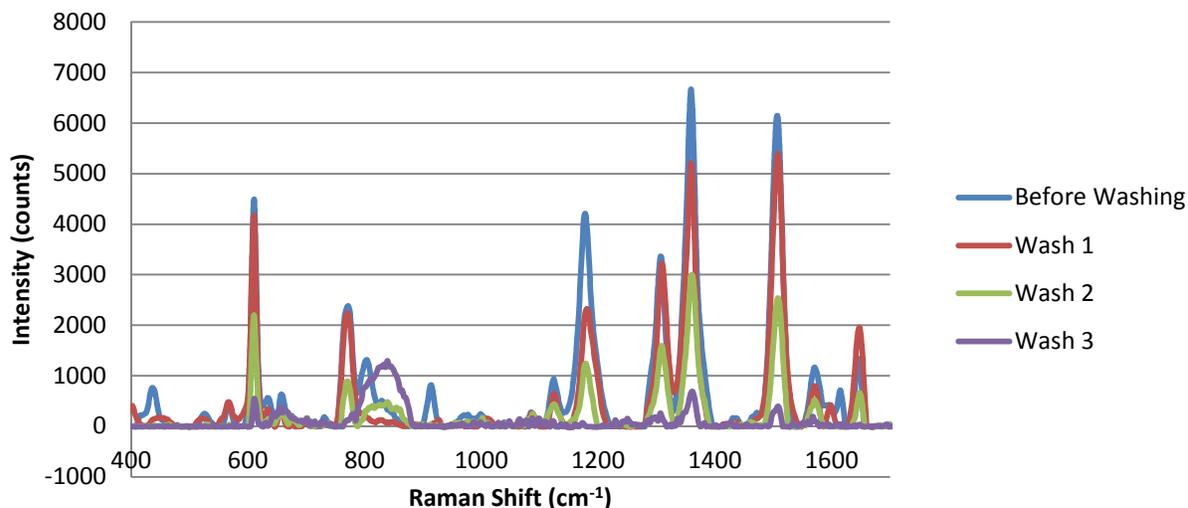


Reusability

RAM-SERS-SP substrates can be reused after sufficient washing with appropriate solvents. In the following example, 10 μL of 1 ppm Rhodamine 6G (Rh6G) in acetone was added to the substrate, and the SERS spectrum was collected using 1 second integration time, 21.2 mW of power, and 3 averages. To clean the substrate for reuse, it was placed in a vial with isopropyl alcohol and sonicated for 15 minutes, followed by sonicating in acetone for 15 minutes. In between each rinse cycle, the SERS signal was checked using the same conditions noted above to ensure no analyte remained on the surface. After one washing cycle, the intensity of the SERS signal decreases slightly. After 2 or 3 wash cycles, the SERS signal intensity significantly decreases, and after the third wash the deterioration of the gold/silver film is clearly visible. Rh6G was selected for the reusability study instead of crystal violet because it does not bind as strongly to the substrate, making the rinsing process easier.

Hence, the RAM-SERS-SP substrates can be reused up to one time. **Please note that this is analyte dependent, where strongly bound analytes may require more rigorous washing techniques which could compromise the structure of the gold/silver film.**

**1 ppm Rhodamine-6G on RAM-SERS-SP after washing
638 nm, 1 s, 21.2 mW**

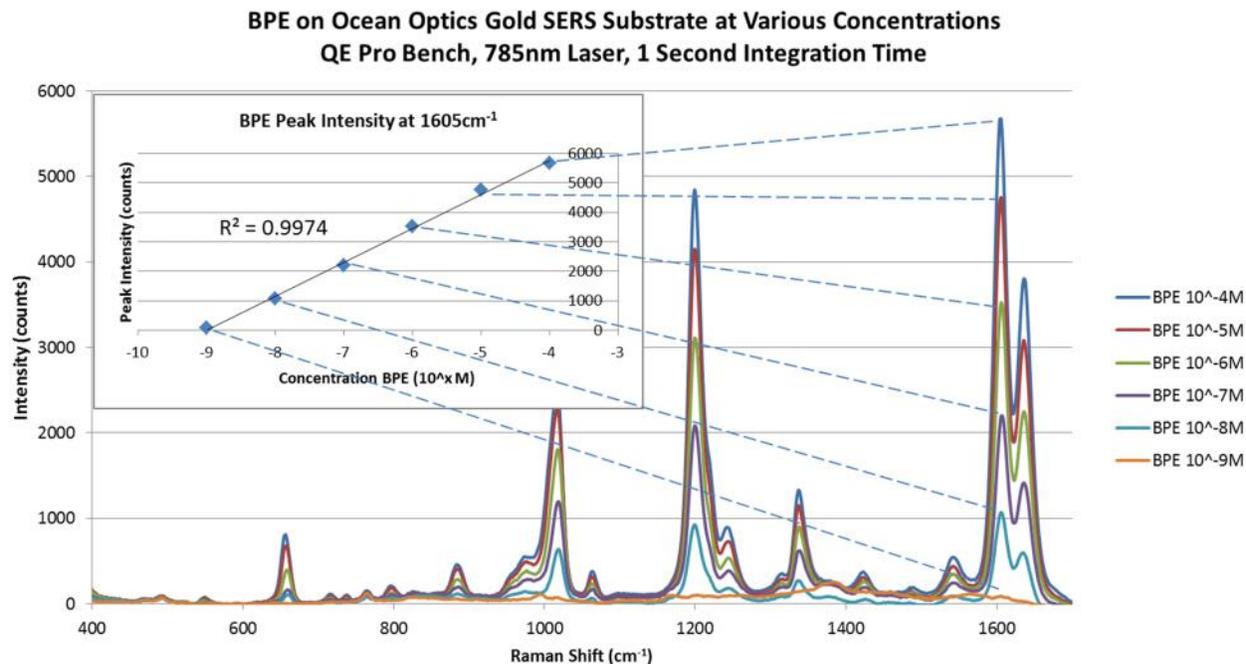


Limits of Detection across Optical Benches for RAM-SERS-AU

The following tests were carried out on the paper-based RAM-SERS-AU gold substrates. The Raman spectra of BPE (concentration ranging from 0.02 ppb to 18 ppm) was measured using 785 nm excitation and 1 second integration time with the following Ocean Optics spectrometers: QE Pro, Maya series, USB 4000, IDRaman Mini, and IDRaman reader.

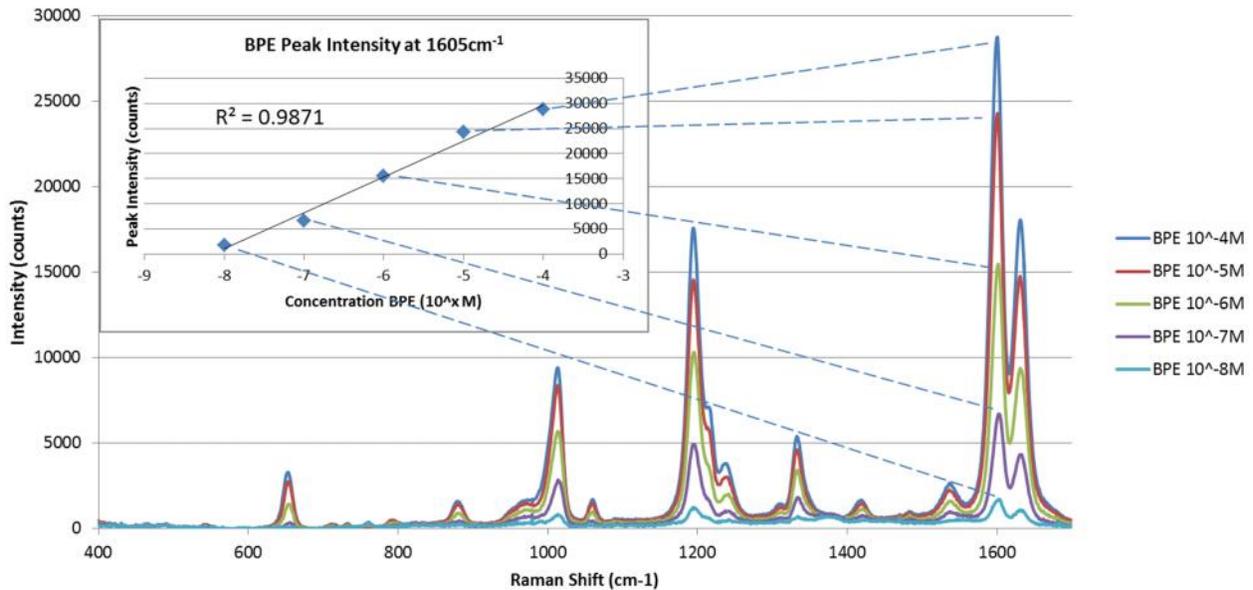
Note: The tests carried out using QE Pro, Maya and USB 4000 spectrometers utilized a separate 785 nm laser having a spot size of 158 μm . The IDRaman units are integrated units comprising of both laser and spectrometer and offer different spot sizes, 25 μm for the IDRaman reader and 2 mm for the IDRaman mini (the mini utilizes orbital raster scanning and distributes the laser spot over a ~ 2 mm area).

QE Pro



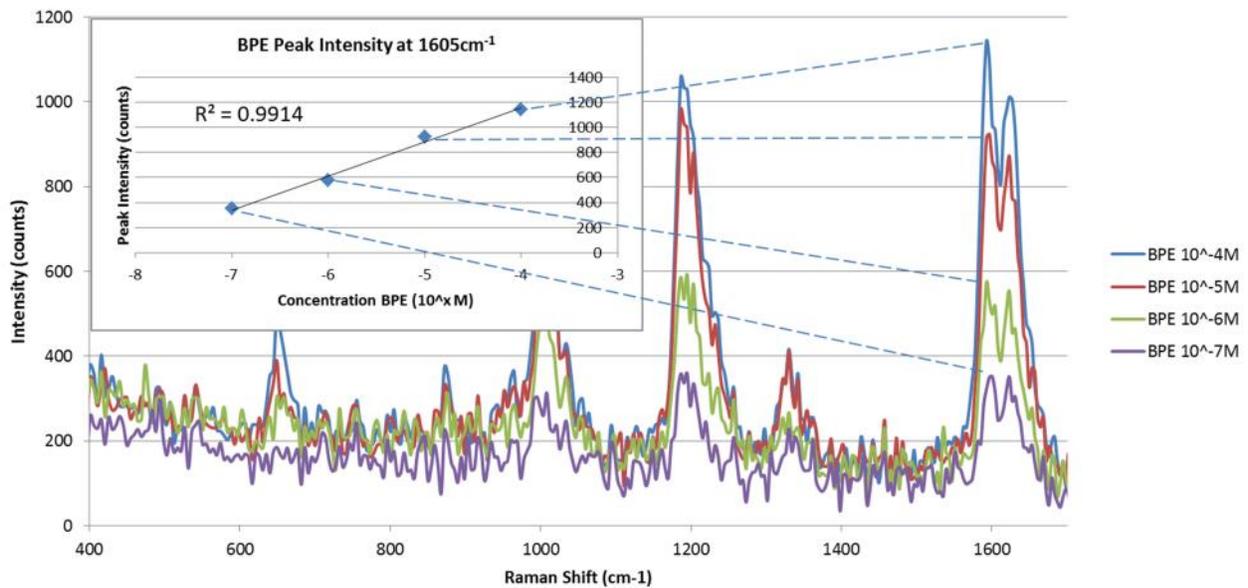
Maya Series

BPE on Ocean Optics Gold SERS Substrate at Various Concentrations
Maya Bench, 785nm Laser, 1 Second Integration Time



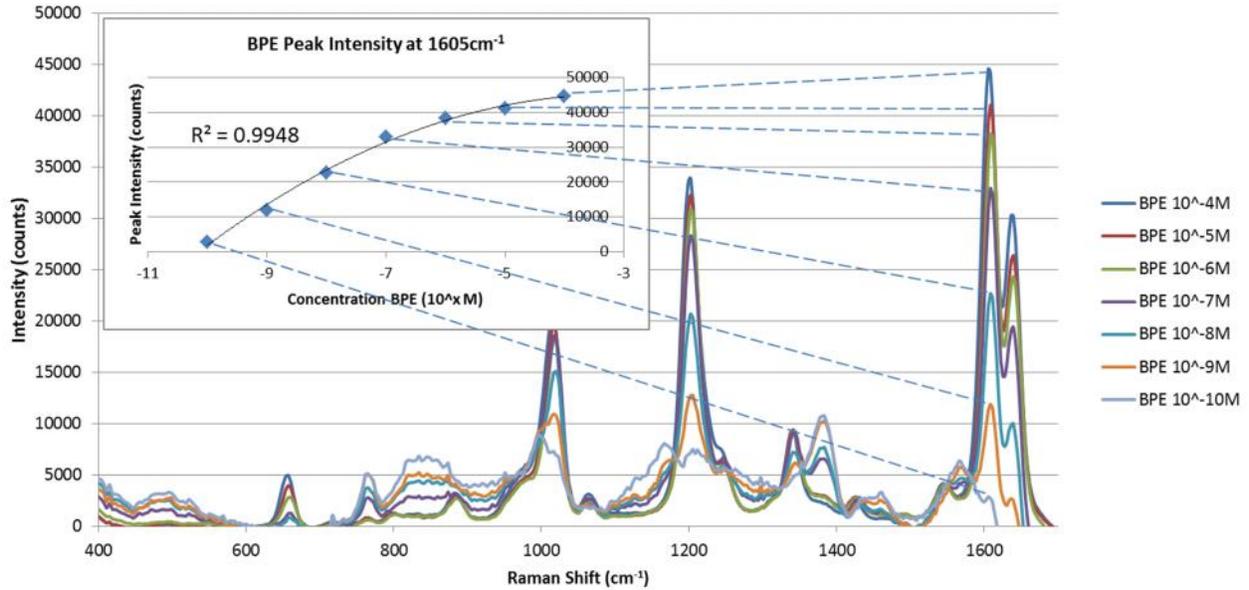
USB 4000

BPE on Ocean Optics Gold SERS Substrate at Various Concentrations
USB4000 Bench, 785nm Laser, 1 Second Integration Time



IDRaman mini

BPE on Ocean Optics Gold SERS Substrate at Various Concentrations
ID Raman Mini Reader, 785nm Laser, 1 Second Integration Time



IDRaman reader

BPE on Ocean Optics Gold SERS Substrate at Various Concentrations
ID Reader Bench, 785nm Laser, 1 Second Integration Time

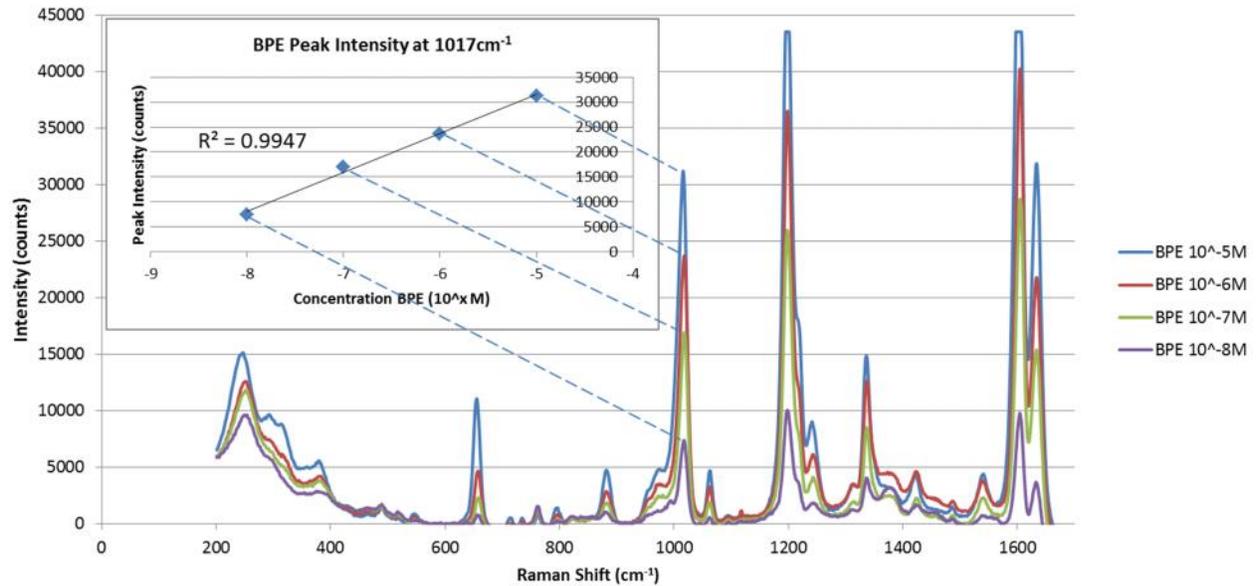


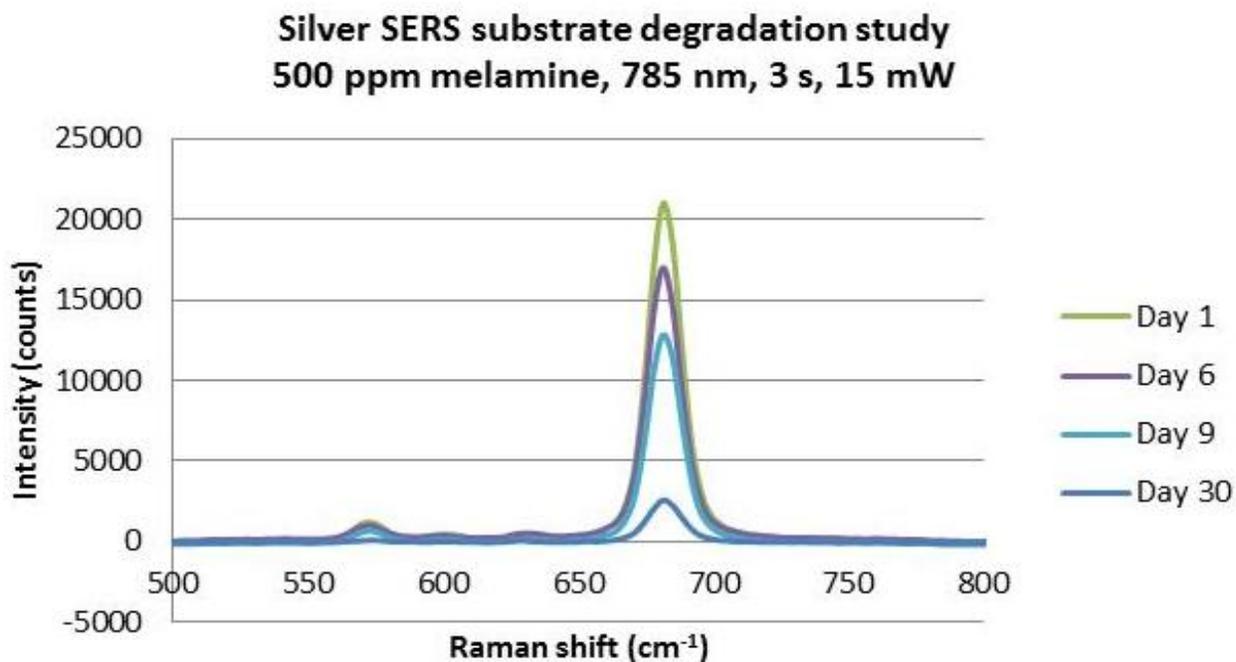
Table 5: Wavelength and SERS substrate dependence of some common analytes

Laser wavelength (nm)	RAM-SERS-AU	RAM-SERS-AG	RAM-SERS-SP
532	--	Rhodamine 6G	--
638	Malachite green, crystal violet	Rhodamine B	Explosives
785	BPE, E. coli, pesticides	Melamine	--

Note: This table shows which analytes have the strongest SERS signal when combined with a specific laser and SERS substrate, although most of these analytes will still give decent SERS signal with different combinations of the laser and SERS substrate. For example, 785 nm excitation with the silver substrate gives the highest SERS signal for melamine, but there is also sufficient SERS signal with 785 nm and the gold substrate or 638 nm and the nanosponge substrate.

Lifetime of Silver Substrates

The silver SERS substrates, which consist of silver nanoparticles (50-100 nm diameter) embedded in a quartz paper matrix, tend to oxidize when exposed to ambient conditions much faster than the gold substrates. The figure below shows that the SERS performance of the silver substrates markedly declines 30 days after the production date due to this oxidation.



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