

## High Sensitivity Trace Detection using SERS-Active Microtiter Plates with the Identity Raman Plate Reader

### Introduction

Raman spectroscopy is useful for the analysis of bulk liquids and solids due to its ability to produce chemical “fingerprints” of materials with unique spectral signatures. Such spectra can be used to identify chemical unknowns, to act as a quality control check of known compounds, or to determine the crystalline structure of solids, to name a few applications. The ability of Raman spectroscopy to perform trace analysis, however, is limited because the spontaneous Raman process is inherently weak, with typical detection limits in the pph or ppt range unless enhancement techniques are utilized. One method of increasing Raman sensitivity is surface-enhanced Raman spectroscopy (SERS), which can enhance Raman scattering by six orders of magnitude or more, improving detection limits to the ppb range. A detailed description of the SERS process is beyond the scope of this article, but suffice it to say that in SERS the Raman spectra of compounds attached to metal nano-particles are greatly enhanced by the plasmonic field generated by the metal particles. The reader is directed to a select group of references (1-9) for a more thorough description on the SERS process and theory.

A few previous reports have described SERS substrate preparation techniques for use with microplates (10-13), but all of these reported measurements employed unique SERS-active substrates prepared by the researchers, as well as custom built Raman spectrometer systems or Raman microscope systems. Many laboratories that have the need to measure trace components in high throughput, however, may not have the expertise to synthesize the SERS substrates with high reproducibility, nor the budget to acquire the high performance Raman instrumentation described in the previous research. Here we describe the use of commercially available SERS-active microtiter plates with the Identity, a novel low cost Raman microtiter plate reader, for high throughput trace analysis measurements, and show results which demonstrate the enhancement in the Raman spectrum that can be achieved in this format.

### Experimental and Instrumentation

Benzenethiol and benzoic acid were obtained from Sigma Aldrich (Allentown, PA), and were used as is or diluted in HPLC grade methanol for normal Raman and SERS measurements.

All measurements were performed with a Digilab Identity Raman plate reader. The Identity, shown in Figure 1, is configurable with either a 532 nm or 785 nm laser and spectrometer with a Peltier-cooled CCD array detector capable of  $<10\text{ cm}^{-1}$  spectral resolution. The Identity supports standard clear, flat bottom 96 and 384 well microtiter plates, as well as custom plate formats. The laser is focused through the bottom of the plate into the well for the analysis of liquids. Powders or coatings on the bottom of the well can be analyzed by placing the plate on a plate shim to raise the bottom of the well to the laser focal point. Raman scattering is collected in an  $180^\circ$  backscatter configuration. Glass bottom plates are typically recommended for measurement of solids or coatings to reduce the interference in the spectrum from the plate material. The Identity includes an x-y motorized stage for automatic analysis of the well positions defined in the control software plate map, a screenshot for which is shown in Figure 2.

## APPLICATION NOTE



Figure 1. Digilab Identity Raman Microplate Reader

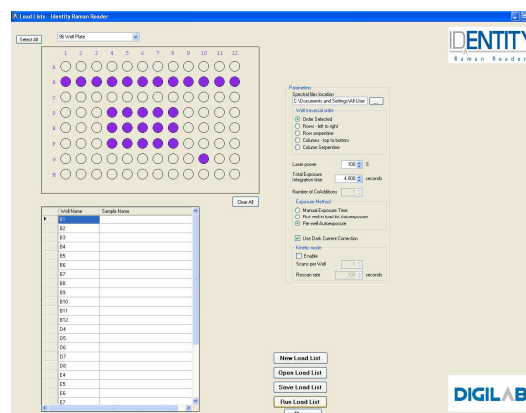


Figure 2. Identity load list view for creation of scan methods.

Well locations are selected by either clicking on individual wells, by clicking and dragging a rectangle over the desired wells with the mouse, or by clicking on Select All to scan all well locations. Laser power is set as a percentage of total laser power, and integration time can be set manually for all well locations, or automatically with the autoexposure selection. With autoexposure, the integration time is set by collecting a short pre-scan of the sample, from which the strongest peak in the Raman spectrum is determined to estimate the integration time necessary to nearly fill the dynamic range of the digitizer. If the estimated integration time is less than the entered exposure time, Raman scans are coadded to give the total exposure time.

The 96-well SERS-active microtiter plates consisted of ~ 1 mm thick coating of a patented (10) silver-doped sol-gel chemistry on glass bottom microtiter plates. The height of the microplate was adjusted to bring the SERS coating into the focal point of the laser, as shown schematically in Figure 3. Approximately 100-200  $\mu\text{L}$  of each analyte in solution was added to individual SERS-active well for analysis. The analytes were allowed to passively diffuse into the porous sol-gel and to the immobilized silver particles.

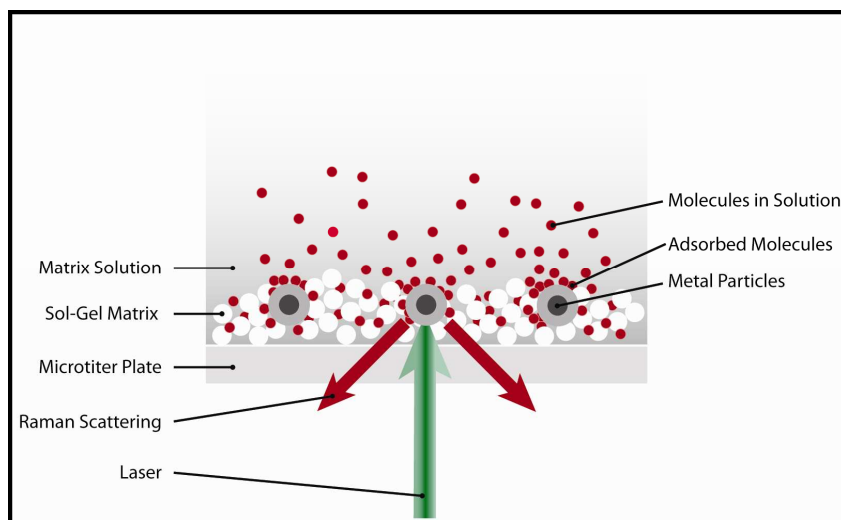


Figure 3. Schematic diagram of the Ag-colloid SERS coating on the well surface of a clear, flat bottom 96 well microtiter plate, showing the laser focus into the coating to obtain the SERS spectrum of analytes in solution that diffuse into the coating.

Spectra of benzenethiol, benzoic acid and 2,4-dinitrotoluene were collected using a 532 nm laser at 30 mW laser power and a 10 second total exposure time using the autoexposure setting. Each

## APPLICATION NOTE

spectrum was corrected using a dark current collected with the same integration time and number of coadditions as the sample spectrum. For these measurements total per well measurement time is thus approximately 20 seconds for collection of the sample spectrum and dark current spectrum, and the total time to scan an entire 96 well plate would be approximately 34 minutes. Sample diffusion occurs from the time the sample is added to the well to the time the plate is mounted for scanning, and does not add to the overall analysis time.

## Results and Discussion

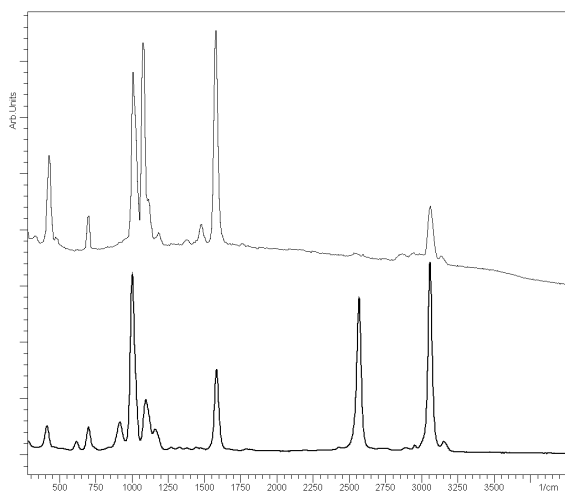


Figure 4. SERS (top) and Raman (bottom) spectra of 10 ppm in water and neat benzenethiol respectively. Both spectra were collected with a 532 nm laser at 30 mW laser power and 10 second measurement time.

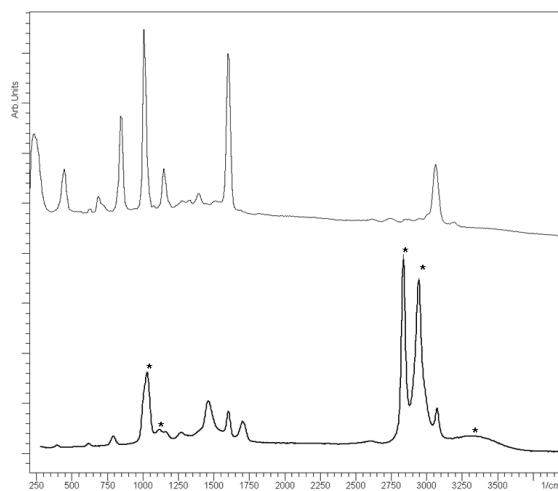


Figure 5. SERS (top) and Raman (bottom) spectra of 10 ppm and 10% benzoic acid in methanol, respectively. Methanol bands are indicated with (\*) in the bulk Raman spectrum. Both spectra were collected with a 532 nm laser at 30 mW and 10 second measurement time

The SERS spectrum of a solution of 10 ppm benzenethiol in methanol is compared to the Raman spectrum of neat benzenethiol in Figure 4. Benzenethiol is highly SERS-active, and is often used to establish SERS-activity for various SERS substrates. A brief examination of the normal Raman spectrum compared to the SERS spectrum demonstrates the changes in relative peak intensity and position to the spectrum that can be induced by the SERS effect. In the case of benzenethiol, sulfur forms a chemical bond with silver as substantiated by the disappearance of the  $2550\text{ cm}^{-1}$  peak due to thiol (S-H). Such a bond will also change the electron density and hence the bond strength of several vibrational modes and consequently their spectral peak positions, as well as intensities. Such bonding will also influence the proximity and orientation of the vibrational modes with respect to the metal surface and the plasmon field, again influencing peak intensity.

The goal of this preliminary study was to establish the ability of SERS-active microtiter plates and a Raman plate reader to perform high throughput analysis of trace chemicals, and only modest low concentrations were measured. Nevertheless, a comparison of the aromatic ring mode intensity at  $\sim 1580\text{ cm}^{-1}$  for the SERS of the 10 ppm sample and the Raman of the pure sample was used to estimate a  $3 \times 10^4$  enhancement factor. This modest enhancement factor underestimates the enhancements that can be expected at lower concentrations, as the SERS intensity is limited by the available silver surface area, which typically becomes saturated in the 1 to 10 ppm analyte concentration range. Furthermore, the signal-to-noise ratio suggests that

## APPLICATION NOTE

significantly lower concentrations, such as ppb, could easily be measured (as is true for all the spectra presented).

Next, benzoic acid, which represents a functional group common to many drugs, was examined. A similar comparison of the Raman to SERS spectra for benzoic acid in methanol at 10 wt% ( $10^5$  ppm) to 10 ppm, respectively, is displayed in Figure 5.

Raman peaks for methanol are marked with an asterisk. Since methanol is not SERS-active, its Raman peaks do not appear in the SERS spectrum. This also illustrates the benefits of laser excitation through the bottom of the plate, as opposed to from the top and through any solvent, which would then contribute to the SERS spectrum.

## Conclusion

The principle of high throughput trace analysis has been demonstrated with commercially available SERS-active 96 well microtiter plates in the Identity Raman plate reader. Quality spectra were obtained from samples that demonstrate the sensitivity achievable by SERS in a microplate format. Although only modest enhancement factors were estimated, this was attributed to the high concentration of the samples measured during this preliminary study, and sub ppm detection limits should be expected. This is also supported by the excellent signal-to-noise ratios for all of the SERS measurements. Finally, the speed of analysis, 10 seconds per well, suggests that the combination of SERS-active microtiter plates and a Raman reader has great potential for high-throughput trace analysis for many applications.

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