

Sampling Procedures with the Identity Raman Plate Reader

INTRODUCTION

The Identity Raman plate reader can easily accommodate samples in various physical forms, for analysis of liquids, gels, powders, tablets and solids. Switching between sample types is as easy as changing an adaptor in the plate holder of the Identity. This application note will describe the sampling methods, with example spectra, of many of these sample types.

Liquid samples

The default setup of the Identity is for analysis of liquids in the wells of microtiter plates. Simply fill the wells with approximately 200 μ l of liquid (for a 96-well plate), place the plate in the plate holder and load it into the reader. For this configuration the laser beam will focus approximately 2 mm above the plate bottom into the liquid, thereby discriminating against the spectrum of the plate material, as shown in Figure 1. Only material within the focus volume as indicated in

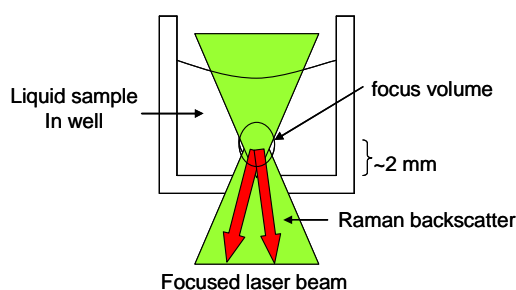


Figure 1. Laser focus into the well for analysis of liquids. The laser beam is focused approximately 2 mm into the liquid, with subsequent collection of the Raman backscatter from material in the focal volume. The 2 mm displacement of the laser focus from the plate material is sufficient to discriminate against the spectrum of the plate.

Figure 1 will contribute to the Raman spectrum. Routine collection of spectra of liquids in the wells can be performed with just a few seconds of integration time per well. Example spectra of organic solvents recorded on the Identity are displayed in Figure 2.

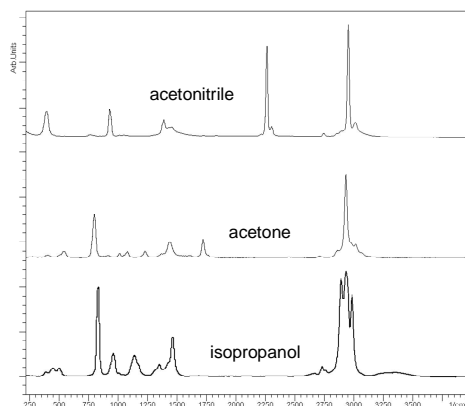


Figure 2. Raman spectra of isopropanol, acetone and acetonitrile (bottom to top) recorded with the Identity Raman plate reader in polystyrene 96-well plates with 532 nm laser excitation. The spectra are free from any contribution from the plate material.

APPLICATION NOTE

Powder samples

Powder samples as well can be analyzed in microtiter plates with the use of a powder adaptor (as shown in Figure 3) to raise the height of the plate, bringing the powder coating on the bottom of the plate into the laser focus as displayed in Figure 4.

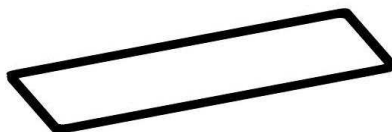


Figure 3. Powder adaptor for Identity Raman plate reader. The adaptor is a plastic frame with outside dimensions that match the format of standard microtiter plates. It is available in a range of thicknesses, from 1.8 to 2.2 mm, which when matched to a specific plate is used to raise the top surface of the well bottom to the focal point of the laser. Glass bottom plates are recommended for use with the powder adaptor to minimize the contribution of the plate material from the sample spectrum.

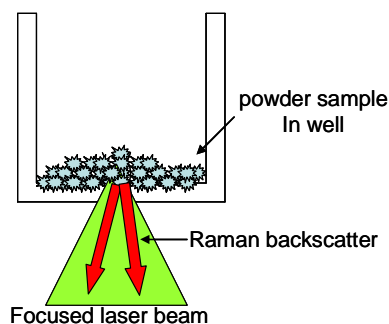


Figure 4. Schematic diagram of the laser focus into the powder at the bottom of the well with the use of the powder adaptor. The powder adaptor raises the height of the plate relative to the laser beam, bringing the top surface of the well bottom into the focus of the laser.

The plate height adjustment with the use of the powder adaptor is necessary because the Raman signal is highly attenuated with a focus 2 mm into the powder sample. The powder adaptor is used by placing it into the plate holder with the plate placed on top of it. Spectra of powdered materials obtained with and without the powder adaptor are shown in Figure 5, demonstrating the enhancement of the spectrum obtained with the powder adaptor.

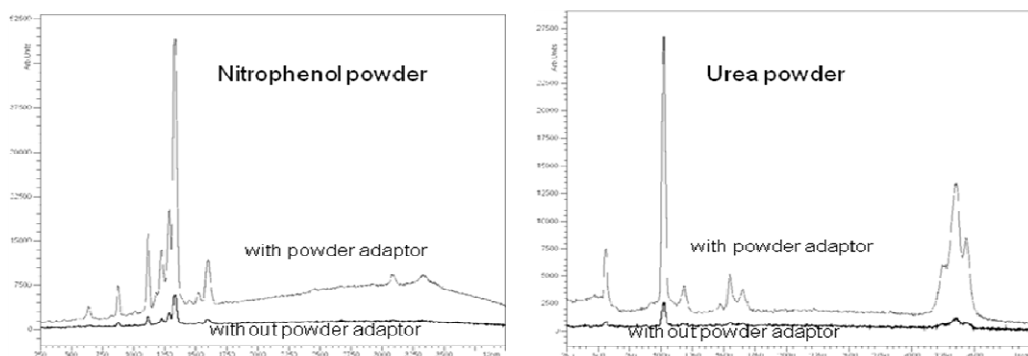


Figure 5. Spectra of nitrophenol powder (left) and urea powder (right) collected with (top) and without (bottom) the powder adaptor in the Identity plate reader with 532 nm laser excitation. Spectra of powdered samples obtained with the powder adaptor are enhanced by approximately a factor of 10 compared to those obtained without it.

APPLICATION NOTE

Tablets

For the measurement of compressed tablets, a tablet plate is available for the Identity reader. Instead of focusing the beam to the top of the bottom well, the tablet plate is designed with a set of machined dimples in a plastic plate, with holes in the center of each dimple, allowing the laser to focus to the bottom surface of the tablet without passing through any plate material. The tablet plate and a schematic cross-section showing the focus of the laser onto the tablet surface is displayed in Figure 6. The tablet holder can hold 32 tablets of either circular or oblong shape.

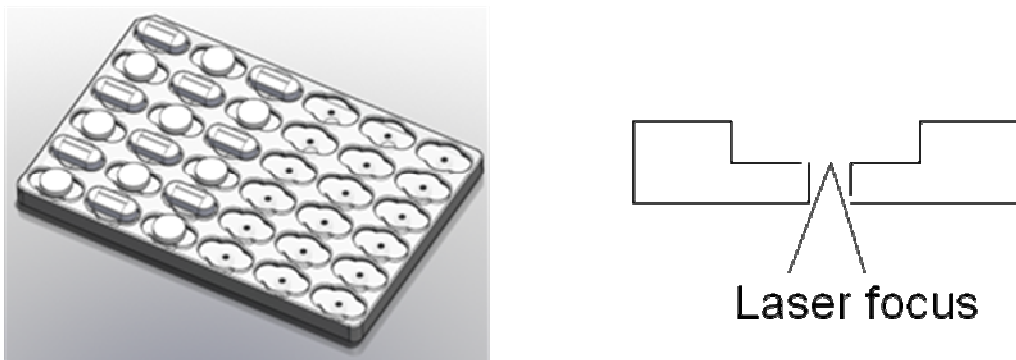


Figure 6. Tablet holder (left) partially filled with circular and oblong tablets, and cross-section of one well (right) showing the focus of the laser onto the bottom surface of the tablet without passing through any plate material.

Representative spectra of over-the-counter pharmaceutical tablets are displayed in Figure 7 using the tablet holder in the Identity with 785 nm laser excitation.

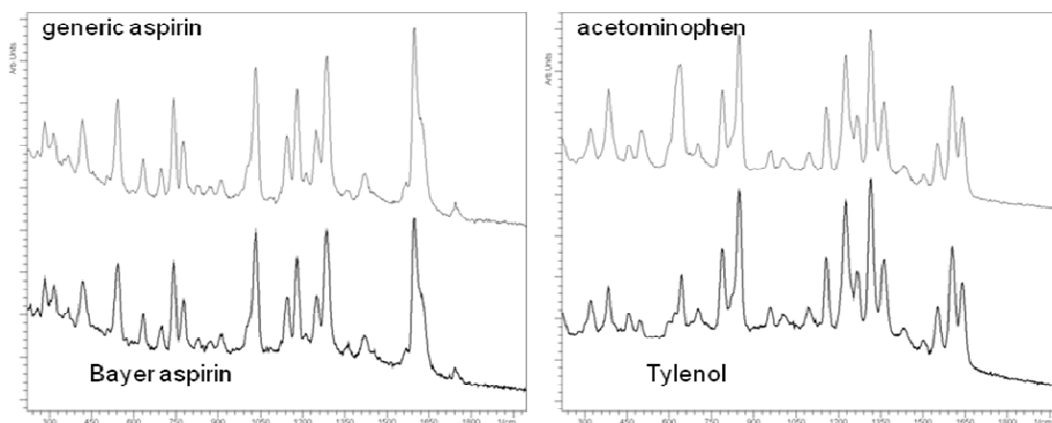


Figure 7. Spectra of over-the-counter pharmaceutical tablets collected with the tablet holder in the Identity plate reader. Spectra of Bayer aspirin (bottom) and generic aspirin (top) are displayed in the left panel, and spectra of Tylenol (Bottom) and generic acetaminophen (top) are displayed in the right panel. All spectra were collected using the Identity with 785 nm laser excitation.

Other Solids

Other solids, such as plastic sheets or films, can be measured using the solids sample plate. The solids sample plate is similar in concept to the tablet plate, with the exception that the plate is flat with 96 holes drilled through it to allow placement of solid samples over the small holes. As in the tablet plate, the bottom surface of the sample will be at the focal point of the laser for ideal sampling geometry, as shown in Figure 8.

APPLICATION NOTE

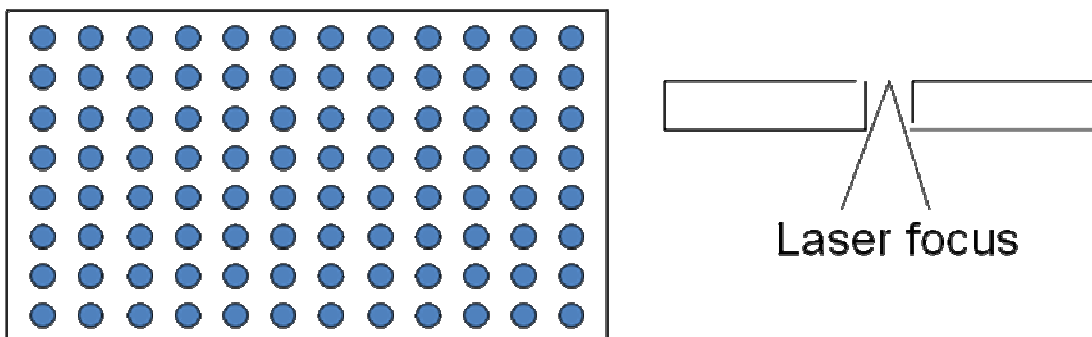


Figure 8. 96-hole Solids sample plate (left) and cross-section of one hole position (right) showing the focus of the laser onto the bottom surface of the solid sample without passing through any plate material.

Spectra of three plastic sheets collected on a 532 nm Identity system using the solids sample plate is displayed in Figure 9.

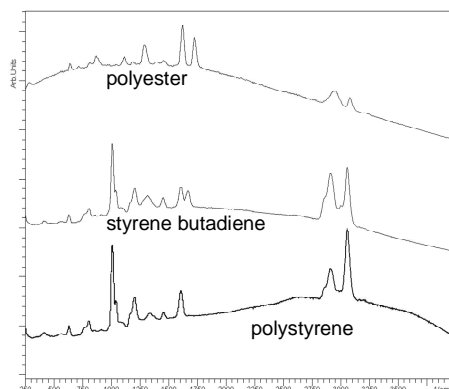


Figure 9. Spectra of polystyrene, styrene-butadiene, and polyester (bottom to top) collected using the solids sample plate in the Identity reader with 532 nm laser excitation.

Conclusion

The Identity Raman plate reader permits easy, high throughput sampling of a variety of sample forms, whether they be liquid, powdered, tablet or solid, with easy changeover using specially designed adaptor plates in the plate holder of the Identity. No adjustments, such as the focus of the laser, of the spectrometer are necessary to accomplish this task, so spectrometer performance is never compromised. High quality Raman spectra can be collected by users from a wide range of backgrounds, from the skilled spectroscopist to the laboratory technician.

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