Technical note

Thermo Fisher SCIENTIFIC

High-throughput Raman analysis: Automated Sampling Array (ASA) for multi-sample measurements

Author
Robert Heintz

Industry/application:
This accessory supports automated multi-sample analysis for various applications, including qualifying finished products in pharmaceutical and other industries.

Products used:
Thermo Scientific™ Automatic Sampling Array accessory in conjunction with a Thermo Scientific™ DXR3 SmartRaman™+ Spectrometer

Goal:
To demonstrate the use of the Automatic Sampling Array accessory with customized sample holders for analyzing various sample types.

Key words:
Raman spectroscopy, finished products, multi-sample analysis, automated sample analysis, custom sample holders, tablets, vials, bottles, well plates, OPC UA

Key benefits:
Automated multi-sampling with customized sample holders for Raman analysis.

Introduction

Raman spectroscopy has been gaining attention for use in manufacturing processes. From qualifying incoming raw materials to reaction monitoring, all the way to quality assurance of finished goods, Raman spectroscopy is playing a bigger role than it did previously. Raman spectroscopy is especially suited for the analysis of finished products because it is not only fast and effective, but it is non-destructive and can often be used to analyze products within transparent containers and packaging. Products can be analyzed to confirm identity, purity, and chemical composition without compromising their integrity.

For Raman analysis to be practical within a manufacturing process, automated data collection from multiple samples is often required. Sampling flexibility with customizable sample holders is necessary to accommodate different types of products in various packaging. These practical requirements are the driving ideas behind the development of the Automatic Sampling Array (ASA) accessory for the Thermo Scientific™ DXR3 SmartRaman™+ Raman spectrometer.

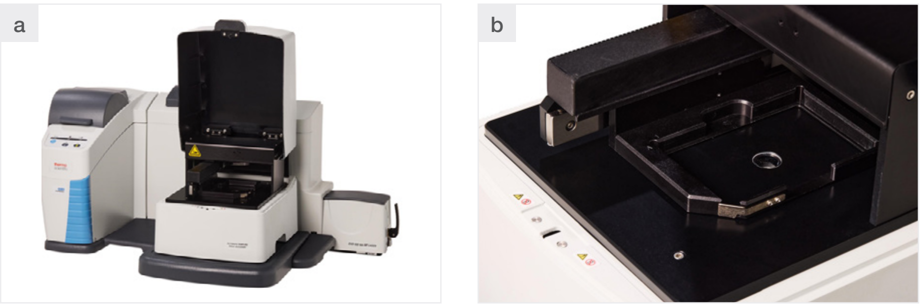

Figure 1. (a) DXR3 SmartRaman+ spectrometer with the ASA accessory installed; (b) ASA accessory with no sample holder.

thermo scientific

Experimental: accessory description

The Automatic Sampling Array (ASA) accessory is an exchangeable accessory that is used with the DXR3 SmartRaman+ spectrometer (see Figure 1). It utilizes an x-y stage with sample holders based on the standard dimensions for microplates (well plates) established by the Society for Laboratory Automation and Screening (SLAS) (formerly the Society of Biomolecular Screening (SBS)).[1,2] The outside dimensions are 127.76 mm (5.029 inches) x 85.48 mm (3.3654 inches). The locations and dimensions of wells within standard plates are also specified and vary with the number of wells (96, 384, 1536). Custom sample holders can be fabricated to fit these outside dimensions and may have unique layouts for accommodating different numbers and types of samples (examples are shown later in this document). The usable dimensions within the area that can be accessed for collecting spectra are 115.22 mm (4.536 inches) x 79.20 mm (3.118 inches). The focus is also adjustable from the top of the inner lip of the stage that supports the sample holders up to 8.5 mm above that position to accommodate different types of samples in various containers. The x, y, and z sampling positions are entered in the software in units of microns. The ASA accessories are calibrated at the factory to provide inter-autosampler positional reproducibility.

Results: steps for using the ASA accessory

Defining sampling positions

The first step in using the ASA accessory is to define the sampling locations that will be used for collecting sample spectra. This depends on the sample holder and can be a single spot or large array of locations depending on the sample holder format. The software has an interactive screen for generating and storing templates for different types of sample holders. Figure 2 shows the setup for an 8-position vial holder.* The points are defined, and the accessory file is saved so it can be recalled and used anytime. Sampling locations for each vial can be determined by adjusting the stage position to optimize the Raman signal at each point. If the spacing on the sample holder is known, then once the coordinates of at least one position is established, the other sampling locations can be calculated.

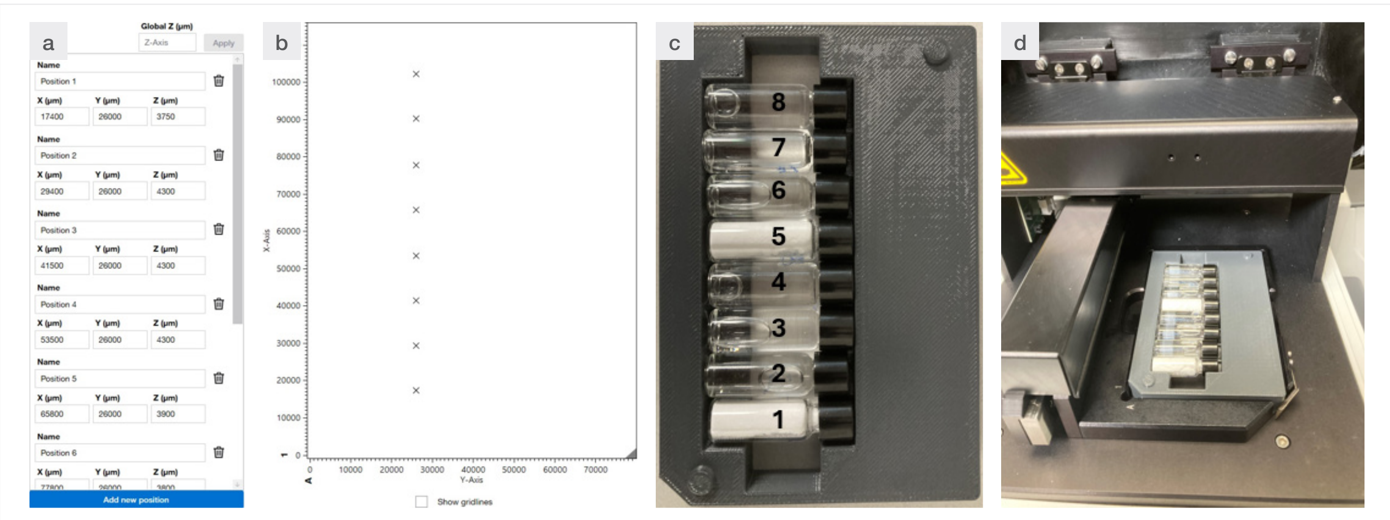

Figure 2. (a) Coordinates (x,y,z) for each defined sampling location. (b) graphically defining and displaying sampling locations. (c) 8-position sample holder for vials.* (c) sample holder in the ASA.

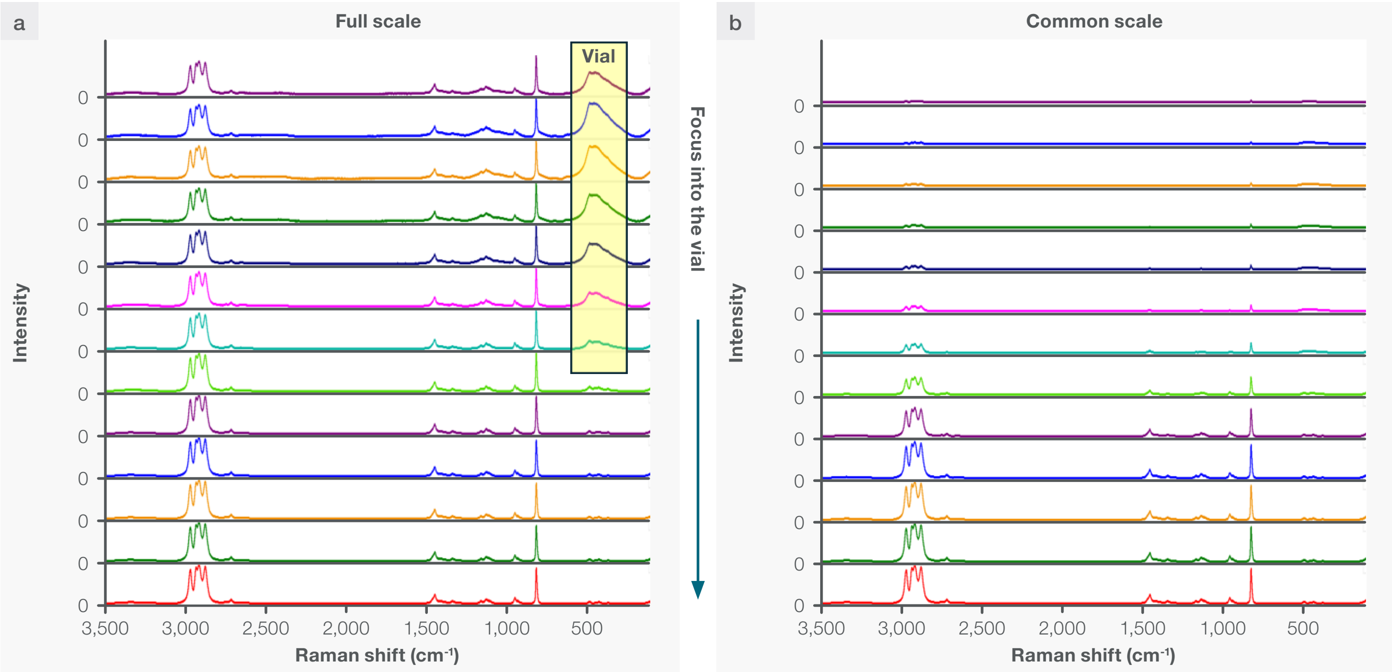

Figure 3. Raman spectra at different focal positions with the focal position increasing to focus further into the vial from top to bottom of the stack. (a) Spectra displayed as full scale for each spectrum with the area highlighted in yellow indicating the contribution from the glass vial; (b) the same spectra shown in common scale (the same scale for all the spectra) showing intensity differences.

Defining the focus (z)

The focus (z position) can be adjusted at each location for different sample types (different types of vials, liquids, solids, etc.), or the same z value can be used for all the samples. With vials, it is important to adjust the focus to measure within the vial and not to focus on the glass walls. An example of this is shown in Figure 3. As the z value is changed to focus deeper into the sample, the Raman peaks from the glass decrease and the peaks from the sample within the vial increase. The figure shows the same spectra both using a full scale and common scale because the Raman intensity from the isopropanol within the vial is much more intense than the glass. The sample peaks will typically increase in intensity as the laser is focused on and into the sample and may plateau a bit before starting to slowly decrease as focusing further into the sample results in a loss of Raman photons. The autofocus option automatically adjusts the z (focus) positions to maximize the Raman intensity, so the operator does not need to do this manually. The data in Figure 3 was collected using a 532 nm laser; however if a 785 nm laser is used, fluorescence from the glass vials might be observed (≈1100-2000 cm^{-1}) and will typically be more intense than the Raman peaks from the glass. This may add additional complications in optimizing the focal position, including the use of the autofocus option.

Collecting spectra

Once the sampling locations have been defined, the accessory file can be saved and recalled for use as needed. In the software interface, the user can move to different sample locations and collect spectral data (see Figure 4). To automate data collection, it is necessary to utilize the OPC UA connectivity available with the DXR3 SmartRaman+ spectrometer. The OPC UA server can be used with a customer supplied OPC UA client to automate data collection and to manage data use and storage. Details concerning the use of the OPC UA server for data collection will be covered in a different technical note.

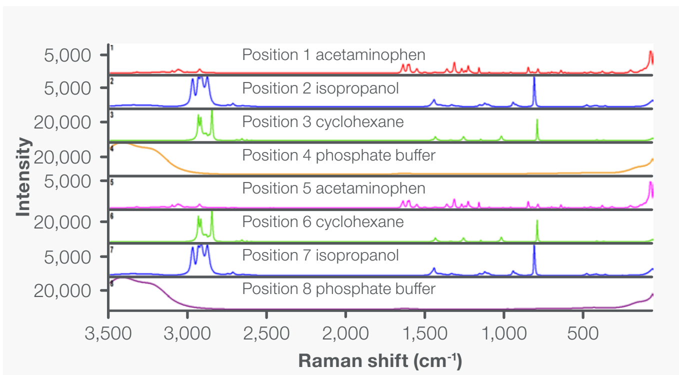

Figure 4. Raman spectra from all 8 positions (vials) in the 8-position sample holder (shown in Figure 2(c)).

Examples of different types of samples and holders

Tablet holder*

Samples do not have to be in vials or well plates. Figure 5 shows a sample holder designed for tablets that can accommodate up to 35 tablets.* The accessory template can be generated with defined sampling locations and then Raman spectra from each of the tablets can be collected (see Figure 6). This could be used for doing quality checks on tablets. It might even be possible to analyze tablets right through blister packs if the packaging is transparent, and an appropriate sample holder is fabricated.

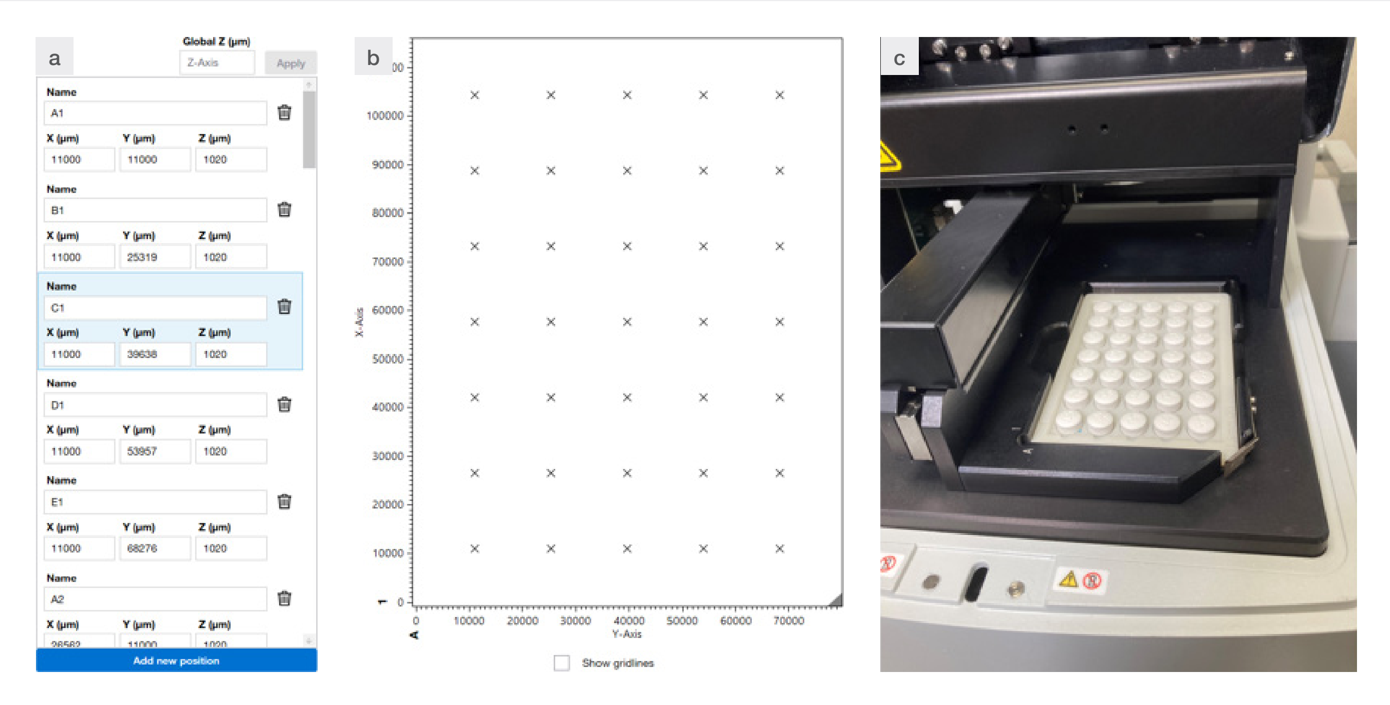

Figure 5. (a) Coordinates (x,y,z) for each defined sampling location (35); (b) graphically defining and displaying sampling locations (35); (c) 35-position tablet holder in the ASA.*

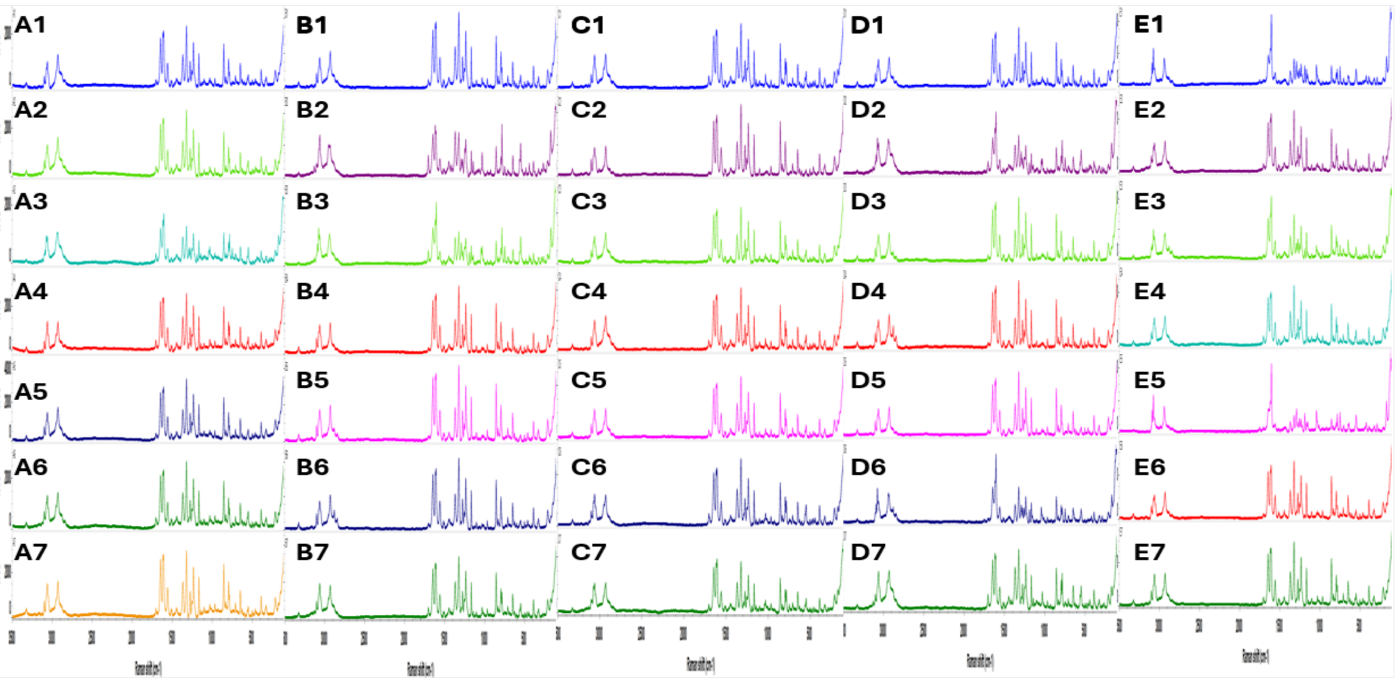

Figure 6. Raman spectra from all 35 tablets in the tablet holder (shown in Figure 5(c)).

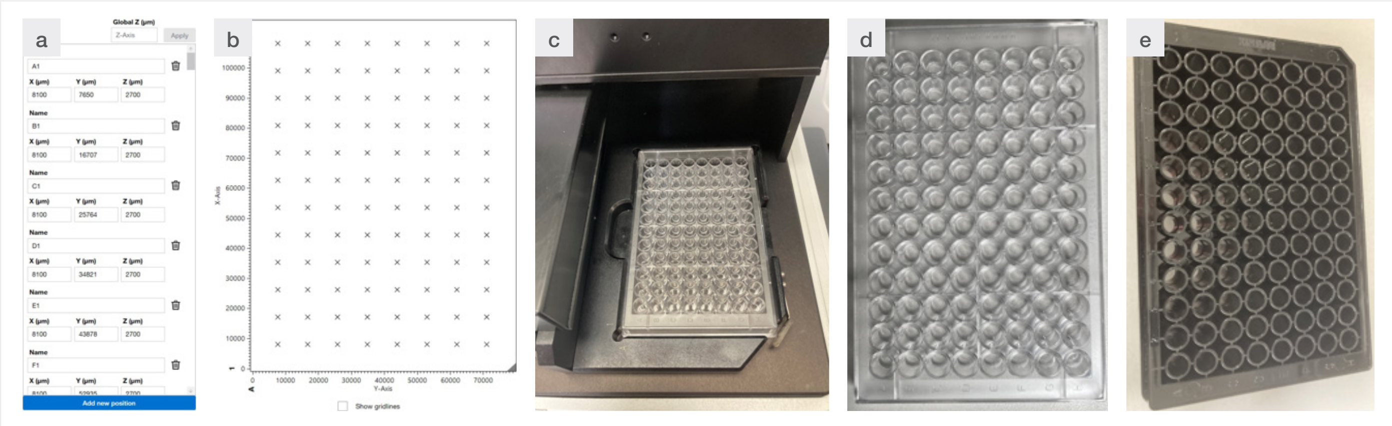

Figure 7. a) Coordinates (x,y,z) for each defined sampling location (96); (b) graphically defining and displaying sampling locations (96); (c) 96-position well plate in the ASA; (d) polystyrene 96 well plate; (e) 96 well plate with glass bottom.

Well plate

The accessory is based on microplate dimensions and thus it can accommodate different types of well plates. Figure 7 shows a standard 96 well plate. In this case, it is more efficient to establish the coordinates associated with one or more of the wells at the corners of the plate (A1, A12, H1, and H12) and then calculate the positions of the other wells. The laser comes from underneath the well plate and passes through the bottom of the well plate to get to the sample. If a polystyrene well plate is used, then there will likely be contributions from polystyrene in the Raman spectra of the samples. These contributions can be minimized by focusing into the sample but will likely not be completely eliminated. The desire to minimize polystyrene signals needs to be balanced with the fact that focusing further into a sample will start to degrade the Raman intensity. This can be seen in Figure 8 where the initial spectrum on the top represents the well plate itself, and the final spectrum represents the Raman spectrum of caffeine in a glass bottom well plate. In between the two representative spectra, the spectra show the effects of focusing deeper into the sample. The polystyrene peaks decrease in intensity but do not completely disappear. To get a spectrum without the polystyrene peaks, it will likely be necessary to subtract the polystyrene spectrum (see Figure 9). The polystyrene peaks may also interfere with the autofocus operation in that the software might optimize the polystyrene signal rather than the sample intensity. An easier solution would be to use well plates with glass bottoms to minimize spectral contributions from the well plates. Dealing with fluorescence from glass containers when using a 785 nm laser is a comparable situation to these interferences from the polystyrene well plates and may require a similar approach.

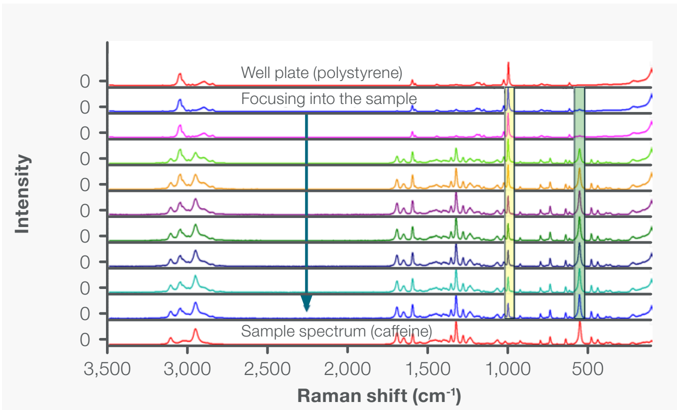

Figure 8. Stack of spectra with the focal point increasing so the focus is further into the well. The well is filled with caffeine powder. The top spectrum is the spectrum of the polystyrene well plate and the bottom spectrum is the spectrum of caffeine powder in a glass bottom well plate; (a) the yellow area shows the decrease in intensity of a polystyrene peak; (b) the green area shows the increase in intensity of a caffeine peak.

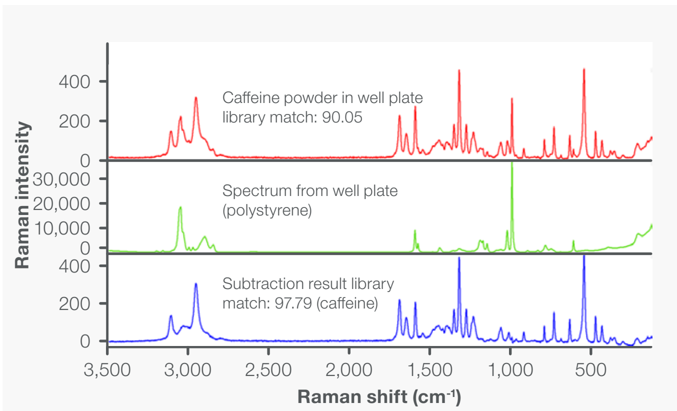

Figure 9. (a) Spectrum of caffeine powder in a polystyrene well plate. The spectrum still has contributions from the polystyrene, so the library match value is lower (90.05); (b) spectrum from the polystyrene well plate; (c) subtraction result where the polystyrene spectrum is subtracted from the top spectrum to give a spectrum mostly free of polystyrene contributions and this is reflected in the higher library match value (97.79).

Figure 10. (a) Bottle of hexadecane analyzed using the bottle holder shown;* (b) bottle of 3-aminopropyltriethoxysilane using the bottle holder shown.*

Bottle holder*

While the primary use of the ASA accessory is to facilitate data collection on multiple samples, it also can be used with single samples. Figure 10 shows the use of a bottle holder that can be used with larger bottles. In this case, there is no real automation in the data collection because a single position is used. However, a series of different locations within the open area of the sample holder could still be used if the user was trying to evaluate the homogeneity of a sample. While not applicable for liquid samples, it might be a consideration for solid samples.

Standards sample holders*

Sample holders can also be used to hold standards that are employed to check instrument performance. Figure 11 shows two of these types of sample holders. The holder on the right (image11(a)) includes a calcium carbonate window (1), a sample of silicon (2), and a polystyrene puck (3).* The Raman spectra shown in Figure 11 are from these three materials. The holder on the left in the figure (image 11(b)) is for a standard polystyrene card sample.

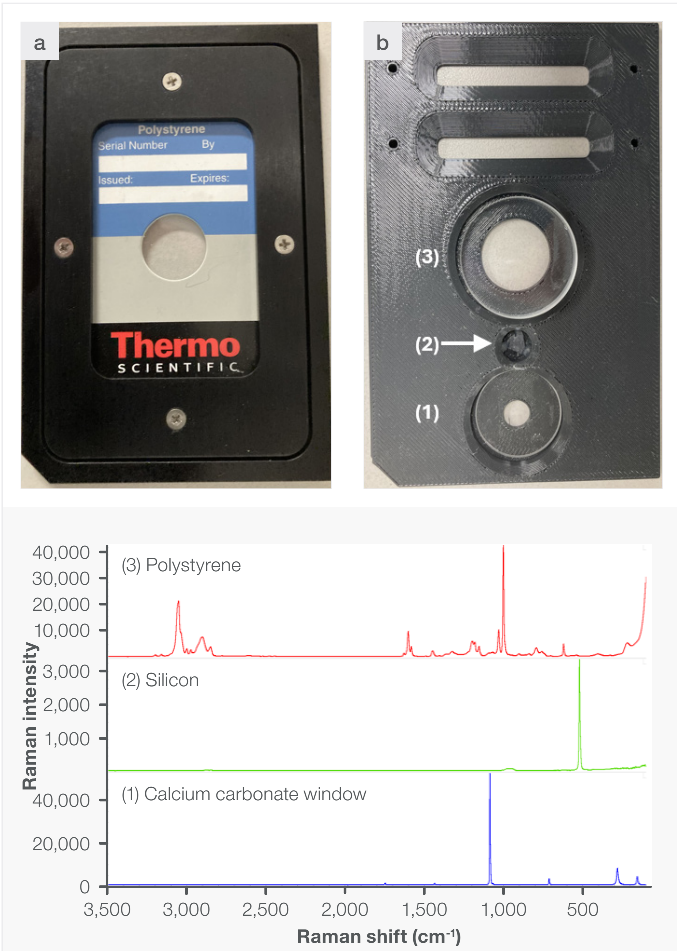

Figure 11. (a) A sample holder with three samples built-into it and used to check instrument performance (calcium carbonate window, silicon sample, and a polystyrene puck).* The spectra are shown in the figure; (b) holder for a polystyrene standard card (spectrum not shown).

Conclusion

The Thermo Scientific DXR3 SmartRaman+ spectrometer was designed to facilitate automated collection of multiple samples. The Automated Sampling Array (ASA) accessory allows for use of customized sample holders based on the standard dimensions established for well plates by the Society for Laboratory Automation and Screening (SLAS). Customized sample holders can accommodate samples in vials, bottles, tablets, and well plates as well as other formats for different types of samples.* The software allows for generating arrays of sampling locations for each type of sample holder, and these arrays may be saved and recalled for later use. OPC UA communications can be used to automate collection of spectral data from all the defined locations. The flexibility in accommodating different sample types and configurations makes the SmartRaman+ spectrometer with the ASA accessory a very useful tool supporting the qualification of finished products.

References

1. For Microplates – Footprint Dimensions, ANSI SLAS 1-2004 (R2012), American National Standards Institute (ANSI) and Society for Laboratory Automation and Screening (SLAS) 2011.
2. For Microplates – Well Positions ANSI SLAS 4-2004 (R2012), American National Standards Institute (ANSI) and Society for Laboratory Automation and Screening (SLAS) 2011.

* The sample and vial holders discussed in this note are not provided by Thermo Fisher Scientific. Users can create their own holders with outer dimensions for microplates (well plates) as established by the Society for Laboratory Automation and Screening (SLAS), formerly the Society of Biomolecular Screening (SBS).