Application Note: 52020

Application of Automated Data Collection to Surface-Enhanced Raman Scattering (SERS)

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Key Words

- Array Automation
- DXR/SERS Analysis Kits
- Gold Colloids
- Ink Analysis
- Laser Power Control
- MicroRNA
- OMNIC Software
- SERS
- Surface-Enhanced Raman Scattering
- TQ Analyst

Introduction

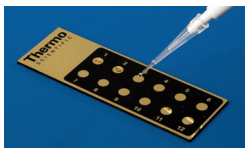
Surface-enhanced Raman scattering (SERS) is a technique that is gaining more use and popularity as an analytical technique. The potential for SERS to open up Raman spectroscopy to new sample types and analysis methods continues to grow. Some of the practical applications of SERS, including those of biological origin, involve samples that are often numerous in nature or require multiple instances of sampling for statistical verification. Individual SERS sampling may make the SERS technique too tedious to be practically applied. Combining multiple sample slides and Raman automation enables SERS to be used with real world sampling scenarios with speed and reduces human error. This application note discusses the use of automated collection and analysis for SERS and introduces the capabilities for high throughput, multi-sample analysis using the Thermo Scientific DXR Raman microscope.

Two different sample sets were analyzed to illustrate approaches to automated SERS sampling. The first sample set consisted of microRNA, which are small RNA molecules, typically in the size range of 21 to 25 nucleotides in length. Research has shown that microRNA are involved in gene expression, cell regulation, and as potential disease markers. Thus a high throughput method is required for analyzing large numbers of samples to identify if certain microRNA are present, and therefore a specific disease.

The second sample set consisted of 12 samples of ink on paper. Forensic analysis of inks on paper is important for the analysis of questioned documents to determine their authenticity, to determine what changes might have been made to the document and to help identify the ink(s) used.

Automating Data Acquisition and Management

A critical component of this work was Thermo Scientific OMNIC Array Automation software. Array Automation is used for the automated collection and analysis of multiple samples. Array Automation controls the movement of the motorized stage of a DXR Raman microscope, or the well-plate accessory of a Thermo Scientific DXR SmartRaman spectrometer, and coordinates the stage movement with the spectral data collection of the samples. Array Automation includes templates for many common multi-sample platforms, such as a 96 well-plate. New templates can be easily created in the software. A template for the 12-spot gold coated microscope slide used for SERS analysis, which is part of the DXR/SERS Analysis Kit, was also created.



Within Array Automation are several options for data collection. For each of the 12 positions on a slide, a single spectrum or a grid of spectra can be collected. If multiple spectra per position are desired, a grid of points, up to 13 by 13, can be collected for each position. Several parameters for grid collection are available; one is step size between points, with options ranging from 10 to 1000 microns. The overall grid size can range from 30 microns per side to at least 5000 microns per side, while the overall grid size will be limited by the size of the actual sample region, which is part of the template. When a grid is collected, the user can save either each individual spectrum or create an average spectrum for each location. For irregular samples or samples that don't fill the entire analysis area there are two options for optimal data collection: the software can either search for the strongest signal using a defined grid, or the user can manually search the sample and focus on each sample location before collection.

Data from a collection can be analyzed in the Array Automation software, in Thermo Scientific OMNIC software, or in the Thermo Scientific TQ Analyst chemometric software. Array Automation is also directly exportable to laboratory information management systems (LIMS). A large number of analysis techniques are available in Array Automation.

Metrics Available for Analysis in Array Automation

- Area above baseline
- Correlation
- Cluster analysis
- Group analysis
- Height above baseline
- Multivariate curve resolution
- Peak area
- Peak area ratio
- Peak height
- Peak height ratio
- Principal components
- Quantitative result

This note focuses primarily on the use of Array Automation for the collection of large data sets for multiple SERS samples such as microRNA.

Experimental

For the microRNA portion of this project, Thermo Scientific miRIDIAN microRNA samples were used. Samples arrived lyophilized in reaction tubes and were stored at -80°C until needed. Sample solutions were prepared by adding RNase-free water to the reaction vial; the vials were shaken to ensure dissolution of the entire sample that may have been on the walls of the vial. The final concentration of the solutions was approximately 1 microgram per microliter, as not all samples contained the same amount of microRNA. Each solution was separated into several aliquots and then frozen at -80 °C until needed.

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For the collection of SERS spectra, a DXR Raman microscope equipped with a 780 nm laser, brightfield/darkfield illumination, 20× microscope objective, and a motorized microscope stage was used. The samples were analyzed using 1 mW of laser power. Important to the analysis was the laser power control of the instrument, as it ensured that samples were not damaged by the laser during collection, and that the CCD detector of the instrument was not saturated by signal.

A DXR/SERS Analysis kit was used for the microRNA sample preparation. The SERS samples were prepared by mixing 2 microliters of one of the aqueous microRNA solutions with 2 microliters of the 70 nm gold colloid (also aqueous) using a micropipette. Two microliters of the resulting solution were then deposited onto one of the 12 spots of the gold slide (also part of the analysis kit). The samples were air dried before data collection. A verification solution, which is part of the DXR SERS analysis kit, was used in a preliminary test to verify that the combination of gold colloid particle size and laser wavelength could give a useful SERS response.

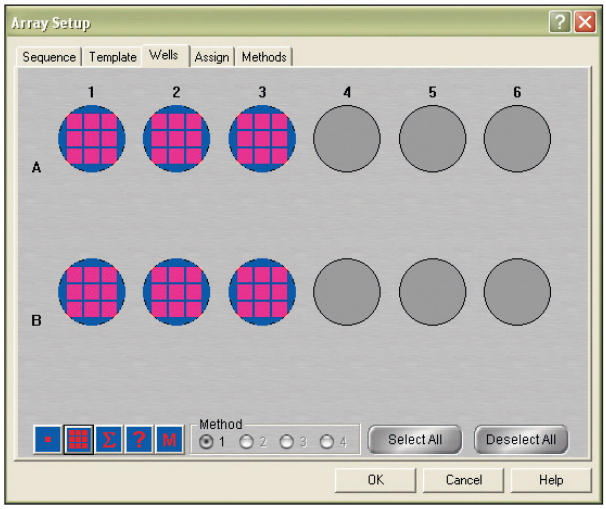

Figure 1: Array Automation set-up window showing the template for a 12-spot slide, the 6 spots on the left side of the slide (A1-A3 and B1-B3) have been selected for a multi-spectrum grid collection

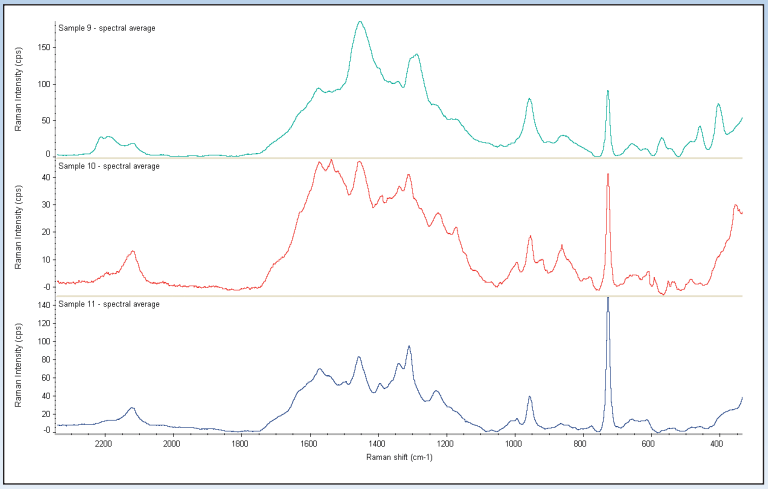

Figure 2: Average SERS spectra of selected microRNA samples (spectra were corrected using the fluorescence correction algorithm)

Data collection and analysis was performed using OMNIC™ software and Array Automation software. As mentioned earlier a software template for the 12 position gold slide was built and used for the spectral analysis of the slides.

A set of spectra was collected for each sample spot, with the collection set up and run using the Array Automation software. Figure 1 shows a screen capture of the set-up window for Array Automation showing the template for a 12-spot slide, with 6 spots selected for a grid collection. A square grid 650 microns on each side was collected, using a 50 micron step from point to point. This generated 169 spectra per spot on the slide.

In the ink analysis section of this project, samples were prepared by collecting twelve different ink sources (pens); eight black inks, three blue inks, and one red ink. A paper template was created that matched the size and dimensions of our 12-spot microscope slide. Ink samples were deposited on specific spots by simply writing on the paper. The template was attached to a microscope slide, by taping along the edges. For SERS analysis a silver colloid solution was prepared using the standard citrate Lee & Meisel method.[1] A total of 12 microliters of silver colloid were applied to each ink spot, in a series of three microliter aliquots, with time allowed between each application for the samples to dry. Untreated ink samples were also prepared and analyzed for comparison.

Spectra of the treated and untreated samples were collected using the DXR Raman microscope, this time equipped with a 532 nm laser, 10× objective, motorized microscope stage, and brightfield/darkfield illumination. The laser power used was 2 mW, with a 25 micron slit aperture. 30 one second scans were collected per sampling location. For the red ink sample a lower laser power was necessary due to strong background fluorescence, thus a 0.2 mW laser power was used, coupled with 30 scans that were each 0.2 seconds long. The 12-spot microscope slide template in Array Automation was used, with a 13 × 13 step grid per sample spot with a 50 micron step between sampling locations, resulting in a total of 169 spectra over a sample area of 650 microns by 650 microns, overall more than 2000 spectra were collected for each slide.

Results and Discussion

MicroRNA Analysis

Figure 2 shows the average SERS spectra of three of the microRNA samples that were analyzed. Each spectrum is the result of averaging a set of spectra collected on a microRNA SERS spot on the 12-spot slide. In Array Automation each individual spectrum from a grid can be displayed or analyzed, or the software can collect all the spectra for a sample spot and create an average, in this work individual spectra were collected. As can be seen in Figure 2 the spectra share many similarities, but there are noticeable spectral differences between the samples. Some spectral similarities are expected due to the samples being made of similar nucleotides, however the different arrangements of the nucleotides are

what lead to the noticeable spectral differences. This is one of the benefits of Raman and SERS. Figure 3 shows the results of a 12-spot analysis and how Array Automation displays all the data points, with the squares color coded based on an analysis algorithm. In this figure it was area above baseline for each spectrum, the analysis method can be changed and a new set of results will be displayed for a quick visual analysis.

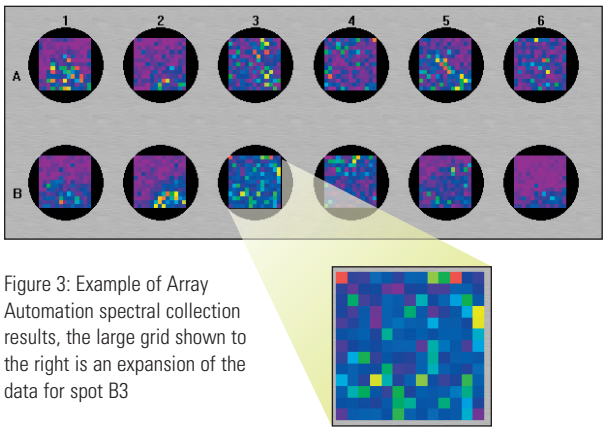

Figure 3: Example of Array Automation spectral collection results, the large grid shown to the right is an expansion of the data for spot B3

For one analysis a slide was prepared using duplicate sets of the six samples. One set of samples was designated as a known set and one set was designated as an unknown set. The sample layout is shown in Figure 4. For this data set a spectral average was generated for each sample spot. Spectra from the known samples were used to build a spectral library, using the standard OMNIC software. The spectra from the unknown samples were then run against the library of knowns. Figure 5 shows the result of one of the library searches. Two things of note from the figure: the first is the high percentage match from the library search, and secondly, is how well the spectra of the two samples match visually. Sample to sample reproducibility is very important for any good analytical method.

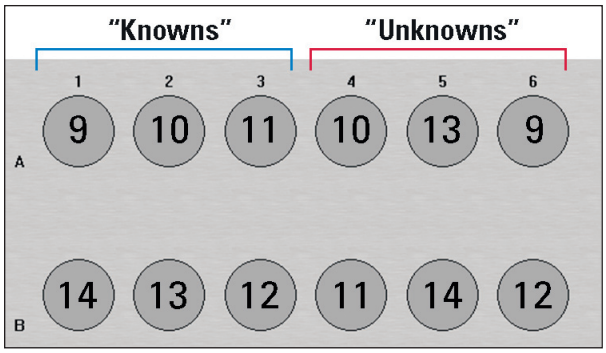

Figure 4: Showing the layout of microRNA samples, samples designated as knowns are on the left side, and samples designated as unknowns are on the right side, spots are labeled with the particular microRNA sample number

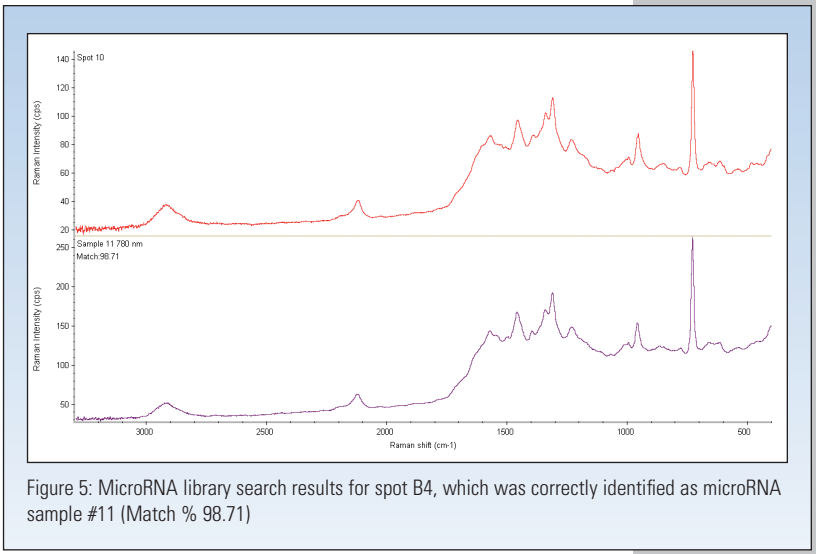

Figure 5: MicroRNA library search results for spot B4, which was correctly identified as microRNA sample #11 (Match % 98.71)

Ink on Paper Analysis

There were two goals for the ink analysis, one was to use the spectra from the ink samples to show that SERS is useful in the analysis of inks on paper and the second goal was to develop a method to discriminate all the inks, particularly those that are very similar in composition such as one black ink versus another. Figure 6 shows the Array Automation results for the analysis of the 12 samples of inks on paper treated with silver colloid, also analyzed was a set of untreated ink samples. As mentioned previously each spot contains 169 spectra, in a 13 by 13 grid. By collecting such a large number of spectra a better data set can be generated because different fiber orientations, ink coverage, and other variances can be included in the statistical analysis. And in the case of the SERS analysis any "hot spots" or "dead spots" can be averaged out so as not to skew the results.

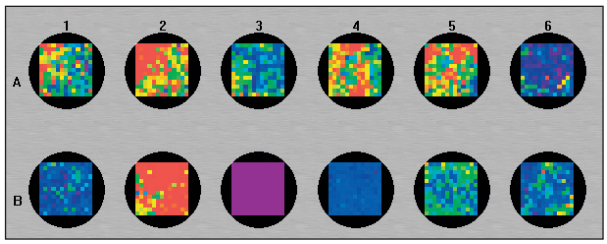

Figure 6: Array Automation results for the silver treated ink on paper SERS analysis

In comparison to the untreated samples, the application of the silver colloid to the ink samples showed a very strong increase in signal. Figure 7 illustrates the comparison of the spectral averages of an untreated ink sample versus a SERS ink sample. The Raman signal for the untreated sample appears to be a nearly flat line when compared to the SERS signal. All of the inks analyzed showed a similar response, a result of a dual mechanism of fluorescence reduction and signal enhancement. Figure 8 shows the average spectra for the treated and untreated red ink samples.

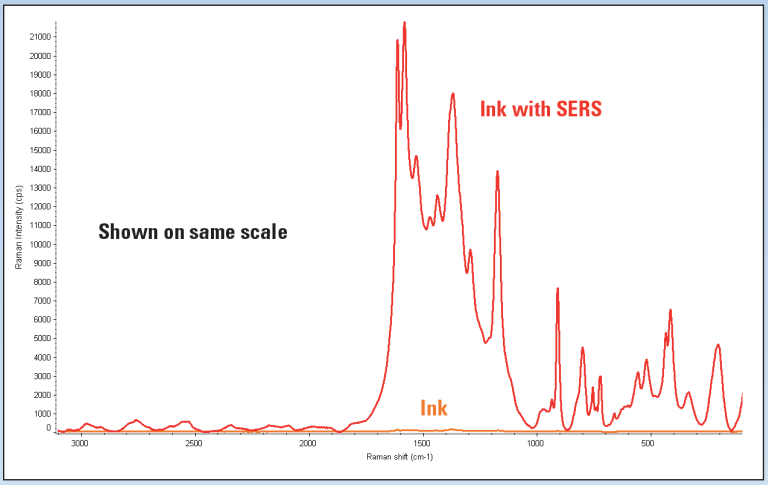

Figure 7: Spectral comparison of a Raman and SERS analysis of a black ink on paper, shown on the same intensity scale to illustrate the signal enhancement from SERS

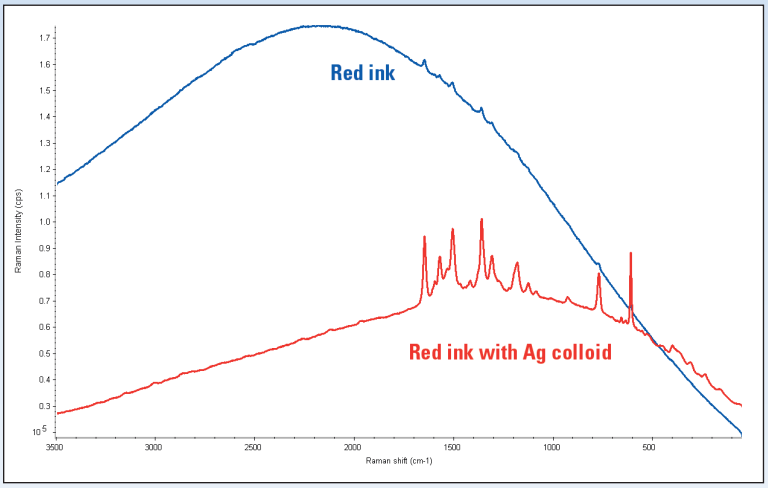

Figure 8: Spectral comparison of the Raman and SERS spectra of the red ink on paper

Spectra for both sets of ink samples (SERS and regular Raman) were loaded into the TQ Analyst™ software. TQ Analyst is used for complex data analysis, particularly large sets of data. It can be used for quantitative analysis, qualitative analysis, and large calibration sets. Data for ten of the ink samples were used for the chemometric analysis. The red ink was excluded as it required different collection parameters than the other samples, and one of the black inks was found to be the same formulation as one of the other samples so it was also excluded. A qualitative discriminant analysis method was constructed using the spectra. For effective comparison of the SERS and regular Raman data, the same parameters were used for both data sets. Figure 9 shows a principal component score plot for the untreated ink sample data, as can be seen there is not a clear separation of the various samples. There were 750 of the 1690 spectra misclassified, which is approximately 44% of the spectra, a very significant problem. The SERS spectra were analyzed using the same parameters, and a plot of the resulting principal component scores is shown in Figure 10. Now a much better separation of the samples can be seen, though the two dimensional representation does not adequately show the discrimination. There were 54 of the 1690 spectra misclassified, which is a much better 3% result. The misclassified spectra are from three black ink samples, which may have the same or very similar formulation. More ink samples could be analyzed using the same sampling and analysis parameters so that an even larger and more comprehensive model could be constructed for forensic ink analysis.

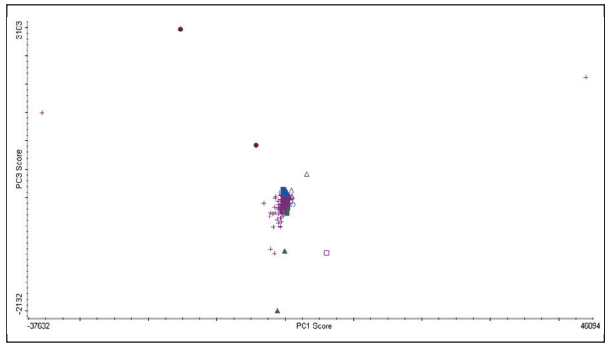

Figure 9: Principal component score plot for the untreated ink on paper results (Raman). There were 750 misclassified spectra out of 1690, 44.38% of the spectra

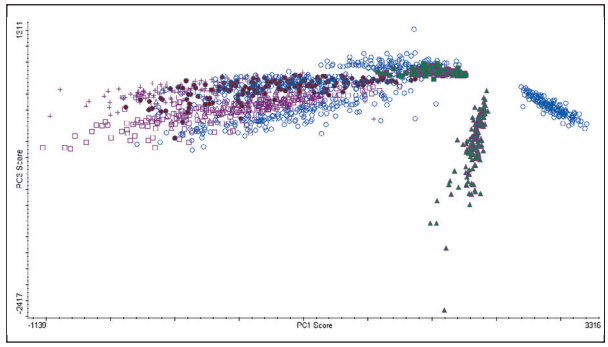

Figure 10: Principal component score plot for the silver treated ink on paper results (SERS). There were 54 misclassified spectra out of 1690, only 3.20% of the spectra

Automation

The ability to automate data collection is critical for any type of high throughput analysis. In the past, SERS data collection has typically been limited to single samples requiring hands-on work of the analyst to swap samples or analyze new areas of the same sample. The method presented in this note, using Array Automation, moves SERS forward as a technique towards the ultimate goal of a fast, reliable, reproducible method. Array Automation brings many benefits to the analysis process. Since multiple samples can be analyzed on one slide, less time is needed for sample swapping. Part of the time savings also include sample preparation, as preparing 12 samples at one time is much more efficient than preparing 12 individual samples. Large amounts of data can be collected and analyzed with one process and without constant user interaction, unlike individual samples where each data point has to be individually collected, then collated, and finally analyzed. As shown in this note more than 2000 spectra per slide could be collected with one analysis run and then analyzed by various means.

As can be seen in the results of the two experiments, SERS can be applied to different types of analysis, using different SERS substrates. Array Automation allows for the collection of a large amount of data that can be used to build spectral averages, for statistical models, or other types of data analysis. For microRNA analysis Array Automation could be used for the development of a diagnostic method where samples are tested for the presence of a specific microRNA related to a disease, and for forensic analysis Array can be used to collect ink data to the help in the identification of different inks used on a forged document.

Conclusion

In conclusion, automated data collection from the combination of DXR Raman microscope with SERS substrates (colloids from a DXR/SERS Kit, home-made SERS colloids, or SERS slides) and Array Automation software add-on for OMNIC moves SERS forward from a single sample analysis method to an automated high throughput analytical technique that has many potential future applications. The work shown in this note demonstrates the ability of a user to prepare up to 12 samples per slide, place the slide into the instrument, collect from one up to 169 spectra per sample, and then analyze that data with a variety of tools. This can all be done with one instrument and one suite of software tools.

References

1. Lee, P.C.; Meisel, D. J. Phys. Chem. 1982, 86, 3391.

Further Information

For more information on SERS please see our Technical Note 51874 "Practical Applications of Surface-Enhanced Raman Scattering (SERS)"

We also have recorded webinars on a variety of SERS and Raman applications that may be of interest. Please see this page: http://www.thermoscientific.com/ramanwebinars

For more information on microRNA please see the Tech Review "MicroRNAs: Review of Discovery, Biogenesis, and Research Areas" which can be found here: http://www.dharmacon.com/uploadedFiles/Home/Support_Center/Technical_Reviews/microrna-tech-review.pdf

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