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# TABLE OF CONTENTS

February 2024  
Volume 39 | Number 2

## COLUMNS

- 08 Molecular Spectroscopy Workbench**  
Monitoring Chemical Changes by Raman Spectroscopy  
Fran Adar
- 12 Chemometrics in Spectroscopy**  
Data Transforms in Chemometric Calibrations, Part 4A:  
Continuous-Wavelength Spectra and Discrete-Wavelength Models  
Howard Mark and Jerome Workman, Jr.
- 32 Icons of Spectroscopy**  
Peter Griffiths: Icon of Infrared Spectroscopy  
Jerome Workman, Jr.



Cover Art:  
Predicting the harvest time of Cabernet Sauvignon grapes using NIR spectroscopy.

## SPECIAL FEATURES

- 28 Featured Article**  
Analytical Biology: An Emerging Discipline for the Future:  
Menglu Li and Katsumasa Fujita
- 36 Pittcon Preview**  
What to Expect from the 75th Annual Conference  
Spectroscopy Staff

## PEER-REVIEWED RESEARCH

- 18 Prediction of the Harvest Time of Cabernet Sauvignon Grapes Using Near-Infrared Spectroscopy**  
Yijia Luo, Jingrui Zhao, He Zhu, Xiaohan Li, Juan Dong, and Jingtao Sun

## APPLICATION NOTES

- |    |                                   |    |                          |
|----|-----------------------------------|----|--------------------------|
| 38 | Endress + Hauser USA              | 40 | PIKE Technologies, Inc.  |
| 39 | Harrick Scientific Products, Inc. | 41 | Oxford Instruments WITec |

## DEPARTMENTS

- Note from the CEO ..... 6
- Products and Resources ..... 42

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## Note from the CEO

**Mike Hennessy Jr.**  
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**S**pectroscopy readers, I am thrilled to present to you our February 2024 Annual Pittcon Issue, packed with insightful articles that delve into the diverse realms of spectroscopy. In this edition, we bring you a blend of expertise, innovation, and the celebration of iconic figures in the field.

First up is Fran Adar's column, "Molecular Spectroscopy Workbench," where she takes us on a journey through the fascinating world of Raman spectroscopy. In "Monitoring Chemical Changes by Raman Spectroscopy," Fran discusses the real-time insights this technique offers during sometimes hazardous polymerization experiments, using the cure of a commercial epoxy as an educational example.

In the realm of chemometrics, Howard Mark and Jerome Workman, Jr. present the fourth part of their series, "Data Transforms in Chemometric Calibrations." They explore algorithms rarely used in practice, explaining those associated with continuous and discrete wavelength spectra.

We follow this with a peer-reviewed article, "Prediction of the Harvest Time of *Cabernet Sauvignon* Grapes Using Near-Infrared Spectroscopy," by Yijia Luo and associates from Shihezi University, in Shihezi, China, demonstrates the application of near-infrared spectroscopy in determining the optimal harvest time of *Cabernet Sauvignon* grapes, offering precision and efficiency.

Shifting gears, Menglu Li and Katsumasa Fujita present a thought-provoking featured article, "Analytical Biology—An Emerging Discipline for the Future." They explore the development of analytical biology, a promising discipline that integrates various research fields to provide a holistic view of biological phenomena.

Lastly, don't miss the Icons of Spectroscopy Laureates series, where Jerome Workman, Jr. honors Professor Peter R. Griffiths in "Peter Griffiths: Icon of Infrared Spectroscopy." This series pays tribute to influential figures selected by our Editorial Advisory Board and editors, showcasing the luminaries who have shaped the field over the past century.

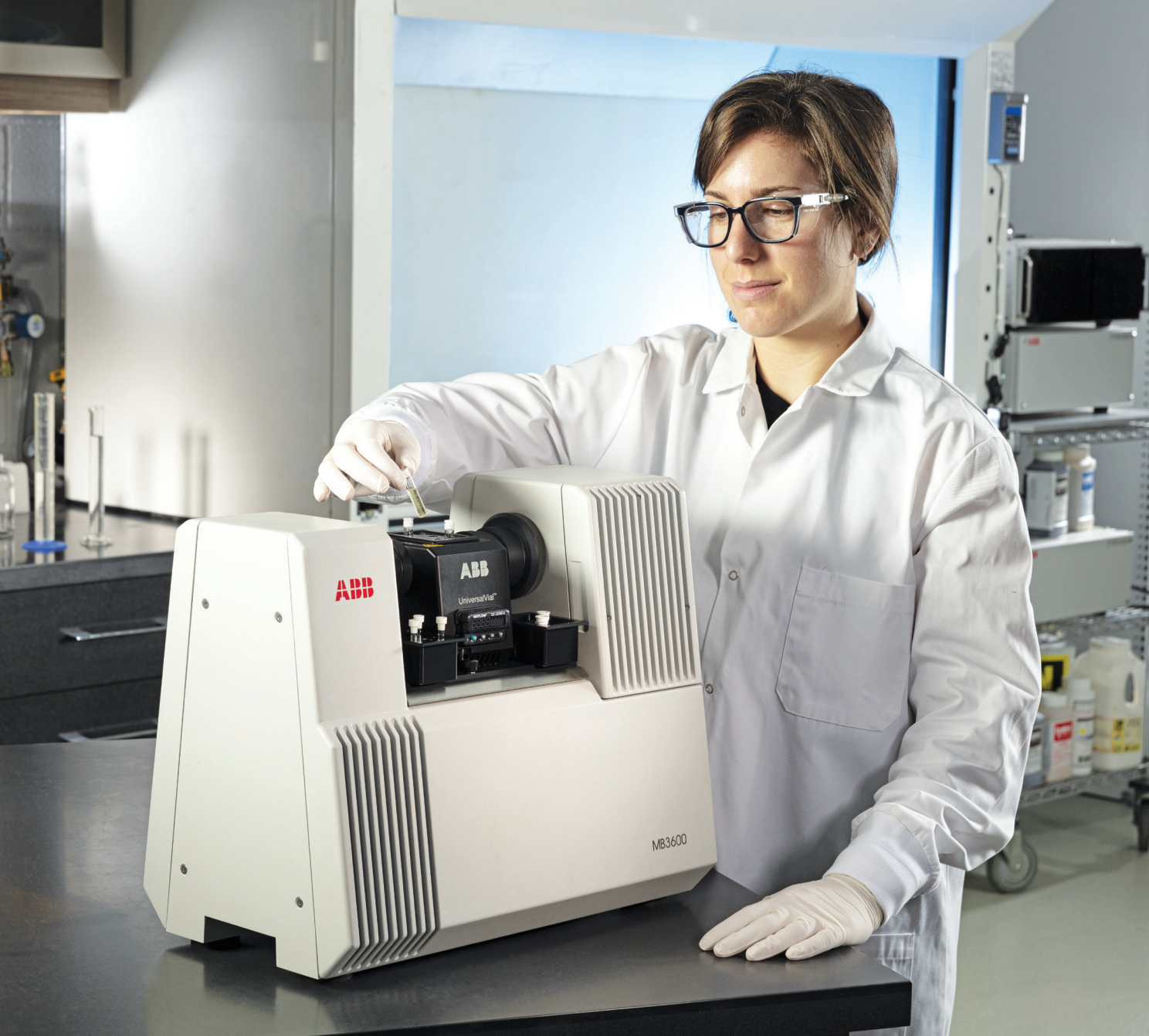
We hope you find this issue as enriching and enlightening as we do.

Happy reading!

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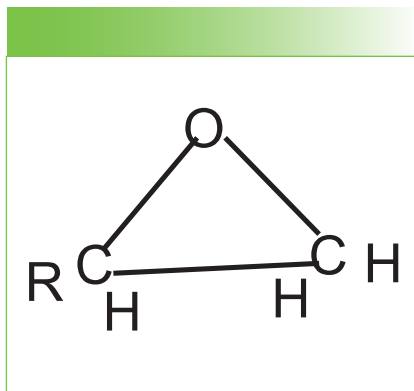


# Monitoring Chemical Changes by Raman Spectroscopy

Fran Adar

By now, it is well known that Raman spectroscopy provides information on the chemical composition of materials, and that said information can be made available in real time—that is, during a reaction. While many types of reactions can be monitored, one of the most important types is polymerization. Polymerization reactions typically involve the loss of a carbon double bond as the chain length is increased, and because the signals from  $\pi$  electrons are strong, the ability to follow this reaction until the end is quite good. However, setting up a polymerization experiment for demonstration purposes can be hazardous. Here, we will show the chemical and spectral changes that occur during the cure of a commercial epoxy.

**A**n epoxy is a three-membered ring containing two carbon atoms and one oxygen atom (see Figure 1). The ring has a characteristic Raman band near  $1275\text{ cm}^{-1}$  which can be followed as the epoxy is cured. Different epoxy formulations have different *R* groups selected for the desired properties. The epoxy that we studied here is a fast-curing Gorilla brand epoxy. Because it is a fast-cure, we wanted to start measurements after mixing as rapidly as possible. The product, which comes in a two-chamber syringe, was used according to the manufacturer's instructions; we



**FIGURE 1:** Molecular configuration of the epoxy ring.

deposited the two components on a stainless steel slide, after which they were thoroughly mixed and moved to the site for Raman measurements. Figure 2 shows how the measurements were done with a probe remote from the MacroRAM benchtop Raman spectrometer. By analogy, it is clear that polymerization measurements can be done in a polymerization reactor by selecting the most effective type of probe (immersion or through-a-window) to monitor the reaction. Figure 3 shows a close up of the laser light focusing on the epoxy on the stainless steel slide. The excitation used for these measurements was the 785-nm diode laser.

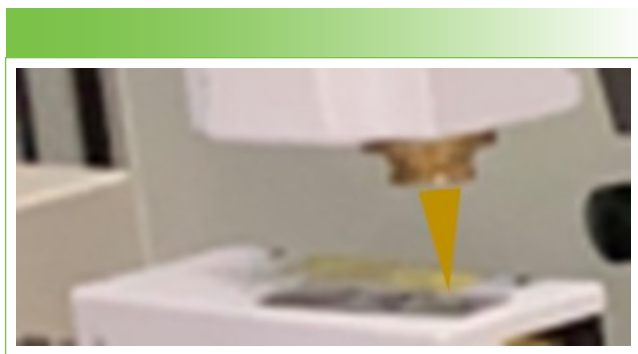
## Results

The system was set up to make measurements over a 4-h period in order to capture changes occurring over a longer period than the rapid cure. The entire spectral data set is shown in Figure 4. While the relative intensity between  $1200$  and  $1300\text{ cm}^{-1}$  were of interest to monitor the epoxy ring opening (1), the spectra also show clear evidence for an aro-





**FIGURE 2:** The scheme under which the measurements of the epoxy cure were made. The instrument and computer are on one side of the room and the Raman probe is mounted on the other with the fiber optic cables crossing the room. The sample position at the focal point of the Raman probe is noted by the red ellipse on the left.



**FIGURE 3:** Close-up view of the laser focused on the epoxy sample.

matic ring (doublet near  $1600\text{ cm}^{-1}$  and aromatic CH stretch near  $3060\text{ cm}^{-1}$ ), as well as an SH stretch above  $2500\text{ cm}^{-1}$ .

We chose to use classical least squares (CLS) to analyze this data set. Figure 5 shows a spectrum from the beginning of the acquisition in blue, from the intermediate phase in green, and from the end in red. The changes that we will focus on are the changes in relative intensity between  $1200$  and  $1300\text{ cm}^{-1}$  and the loss of the band above  $2500\text{ cm}^{-1}$ . Using these two spectra, the software can calculate and plot the scores for each spectrum as a function of time, as illustrated in Figure 6.

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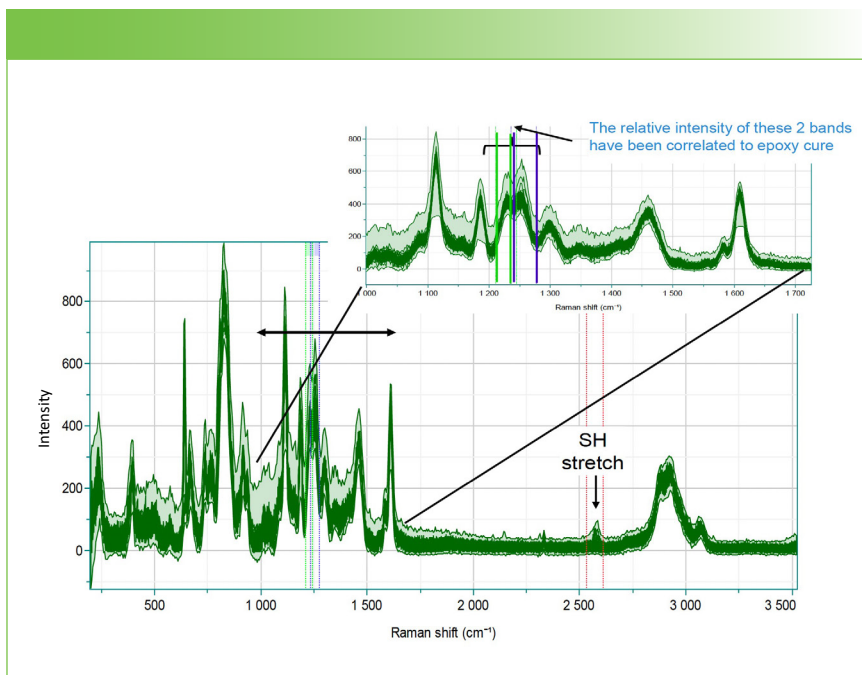
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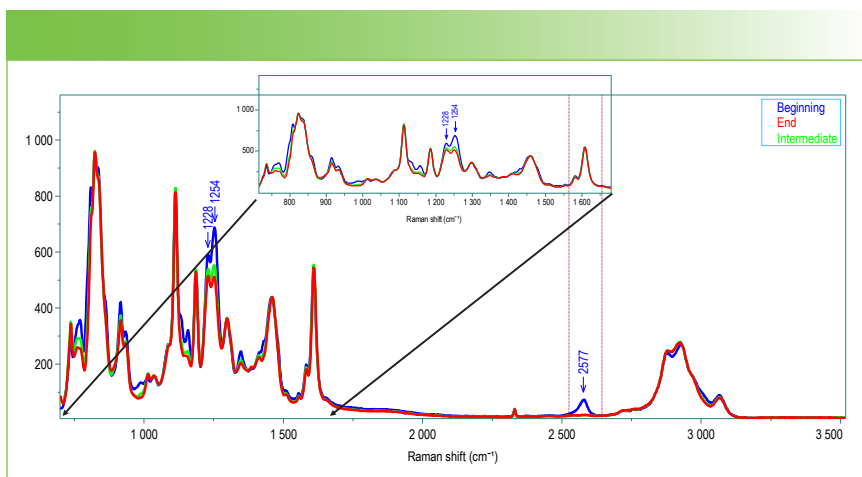
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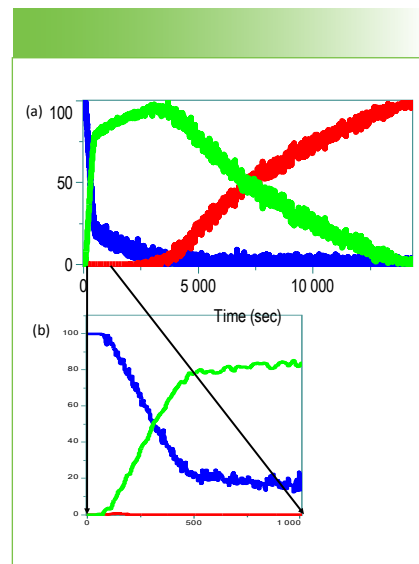
**FIGURE 4:** Spectra acquired over a 4 h period of the fast-curing Gorilla brand epoxy.



**FIGURE 5:** First (blue), intermediate (green), and last (red) spectra from epoxy cure. These spectra are used to construct the scores plots seen in Figure 6. Note the spectral regions where the intensity is decreasing from blue to green to red.

Measurements were made of the starting materials before cure (not shown) and confirmed that there is an -SH group in one of the components before the reaction. Not only is the -SH group disappearing during the initial 400 sec, but the ring opening continues to progress. The aromatic bands relative to the CH<sub>2</sub> and CH<sub>3</sub> stretches 2700 to 3000 cm<sup>-1</sup> and deformations

1400-1460 cm<sup>-1</sup> do not change much, which means that the aromatic groups are not participating in the cure, and are probably added for other reasons. Note also that there is no intense sharp band near 1000 cm<sup>-1</sup>, which would indicate a singly substituted or (1,2 or 1,3) substituted ring. This means that the aromatic is probably a 1,4 substituted ring.



**FIGURE 6:** (a) Scores plot over the 4 hours of the epoxy cure. (b) Note that while the most rapid changes are occurring during the first 400 sec, slow changes continue over the 4 hours. The -SH group disappears within the first 500 sec while the epoxy group changes continue slowly over the 4 hours.

## Summary

This simple set of measurements demonstrates how easy it is to follow a chemical reaction using a remote Raman probe and a simple spectrograph. In this case, it is clear that a sulfhydryl group seen in early spectra is a critical component for the rapid phase of the reaction, consistent with the known properties of -SH groups to enable crosslinking.

## References

- (1) Merad, L.; et al. In-situ Monitoring of the Curing of Epoxy Resins by Raman Spectroscopy. *Polym. Test.* **2009**, *28*, 42-45. DOI: [10.1016/j.polymertesting.2008.10.006](https://doi.org/10.1016/j.polymertesting.2008.10.006)

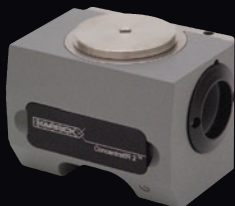


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# Data Transforms in Chemometric Calibrations, Part 4A: Continuous-Wavelength Spectra and Discrete-Wavelength Models

Howard Mark and Jerome Workman, Jr.

In this column and its successor, we describe and explain some algorithms and data transforms beyond those commonly used. We present and discuss algorithms that are rarely, if ever, used in practice, despite having been described in the literature. These comprise algorithms used in conjunction with continuous spectra, as well as those used with discrete spectra.

**W**e've previously published columns to present the common algorithms used for spectroscopic analysis, usually with an emphasis on near-infrared (NIR) quantitative analysis, although many algorithms were included to represent other types of spectroscopy (1,2). Those columns were broad in scope, but somewhat narrower in detail (we can't include everything about everything, after all!). In this column (and possibly the next one or two), we present more details about some algorithms that are rarely seen or used, but deserve more attention than they have previously been accorded. We hope that by making information about them available, some scientists will be piqued to investigate them further, or at least try them out and compare results obtained from them with the results from the more common or more popular algorithms.

Our previous columns about data transforms (3,4) discussed data transformations that were intended to enhance calibration performance results based on spectra representing a small number of discrete (non-continuous) wavelengths, most commonly implemented through the use of a small number of interference filters. That class of data analysis algorithms was initially developed because early NIR spectrometer

designs were often based on the use of discrete interference filters to select the wavelengths at which measurements of optical energy would be performed. While we digressed from that theme for a few columns, we always intended to return to it, and with this column we carry out that intention. While there is a measurement technology (the use of tilting filters; see [5]) that represents an intermediate case (tilting an interference filter enables the filter to pass a small range of wavelengths near the nominal wavelength [the designated wavelength it passes when perpendicular to the optical beam]; see page 82 in [6] for a graphical explanation, or [5] for the mathematics behind it all). In most applications, optical data is collected either discretely (as mentioned above) or "quasi-continuously" (that is, collected at closely spaced wavelengths over the range of wavelengths representing the spectrum).

We began this set of columns by discussing data transforms suited to calibrations based on spectral data at discrete wavelengths, and we will eventually return to those types of transforms, but now we interrupt that exposition in order to discuss data transforms better suited to continuous spectra. We believe that those are of more interest to our readers than the

others, for the simple reason that the corresponding algorithms are in more common use. We will, however, eventually go back to finish our discussion of data transforms for discrete data; there are some enormously interesting and important things to learn about them, including the fact that “improving the performance” includes the case where the performance being improved is that of a second instrument using the same calibration model. In other words, while often discussed separately (as we’ve done ourselves [7,8]), “calibration transfer” is (in one sense, at least “simply”) another application of the underlying concept of modifying a data set to achieve a specific goal for the calibration model. In some cases, the modification of the data is incorporated into, or indeed consists of, modification of the calibration algorithm. Sometimes, it’s hard to tell the difference, so here we will not attempt to make the distinction, but rather discuss the various methodologies that have been applied to the problem without further categorizing them. Where we have discussed a particular approach in a previous column, we will simply reference that column and, if appropriate, include any further explanations we feel necessary to understanding the modeling procedure. A summary of these methods has been presented at the 2020 Eastern Analytical Symposium (9), and some of our discussion here is extracted from that presentation.

### Hardware Approach

The hardware approach consists of matching instruments so well that the same calibrations would be developed on any of them individually. This is one of the topics that has been discussed previously in a recent series of our columns (10–14). The difficulty that arises is that the inevitable presence of noise in the spectral data, combined with the inherent tendency for the various wavelengths in the NIR spectra of a data set to be highly correlated, creates a problem. This problem is illustrated in one of our relatively recent columns (3). Figure 1 of that column, on page 18 of that

issue and reproduced as Figure 1 in this column, displays how when spectral data at two (or more) wavelengths are inter-correlated, the three-dimensional (and higher-dimensional) relationships are circularly symmetric. Because of this effect, the calculated calibration plane (or *hyper-plane*) is rotationally unstable around the axis of the higher-dimensional figure, and small changes in the error structure of the data can cause large changes in the orientation of the calibration plane; therefore,

the coefficients representing that calibration plane are similarly unstable. Thus, no matter how well the hardware is matched, the inherent residual noise of the measurement process will make the calibration models appear to be different, even though this is only due to the irreducible noise of the measurement process. This instability of the calibration model was the bane of the early NIR developers, and led to many attempts to avoid or minimize it. These attempts gave rise to the software



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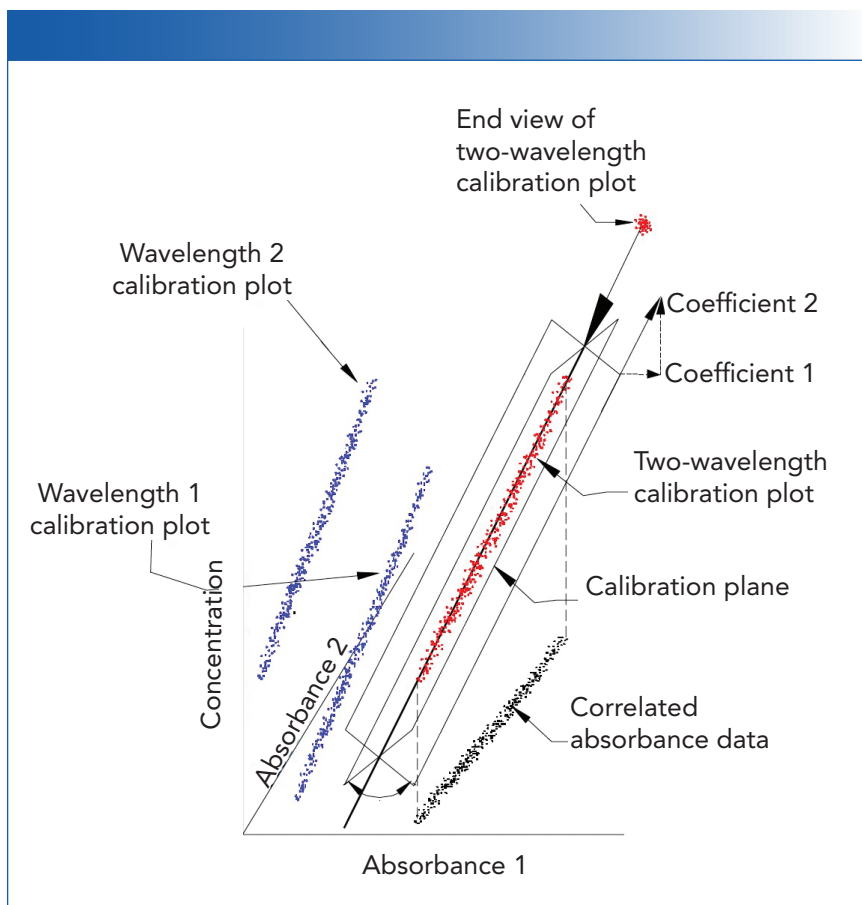
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**FIGURE 1:** Illustration of the effect of intercorrelation of the calibration data on the determination of the calibration coefficients (reproduced from [3]).

developments that are the forerunners of several of the modern data transformations still in use today.

### Software Approach

The software approach consists of algorithms that create the same models from similar data. The instability of the modeling process described above was not understood or appreciated for a long time. Other problems also afflicted the model development process, and it was often unclear which underlying effect was creating calibration difficulties in any particular case. One of the key problematic phenomena was the fact that most sample types of interest in the early days were (and still are, for that matter) solids, but not solid blocks of the material comprising the sample. Rather, the sample often consisted of fine powders composed of the sample material ground to a collec-

tion of particles that, with rare exceptions, could be characterized as being comprised of a mixture of particles in the size range from dust to sand. This created a problem in that when the sample is presented to an instrument, there is a large random component in what the instrument "sees." If such a sample is removed from the holder used to contain the sample, presented to the instrument, then replaced in the holder, different facets of the individual particles of sample interact differently with the NIR radiation and thereby produce a different set of measured values of reflectance at the various wavelength used for the same sample. Examples of the "Particle Size Effect" (sometimes also called the "Repack Effect") abound in the literature; several examples can be found in various editions of the *Handbook of Near-Infrared Analysis*, for example (6).

There was little the average scientist could do to directly affect either of these uncontrollable variables. What was possible, however, was to attempt devising algorithms that, as part of the calibration procedure, could empirically correct or compensate for the uncontrollable fluctuations. Many such algorithms were developed over the years, and we include descriptions of several of them in this (and our subsequent) column. We have already presented some of these algorithms, intended for use in conjunction with discrete wavelengths in predecessor columns (3,4) in this series. Now, we will begin describing algorithms intended for use in conjunction with wavelengths measured quasi-continuously over a range (spectra).

Note that while we list some of the algorithms here under particular headings, that listing reflects the fact that there may be a theoretical connection between the algorithm and the phenomenon it is being used to correct or compensate for. In practice, various scientists have used one or more of these algorithms to improve their calibration results in the face of any of the problem areas affecting their NIR calibrations. Sometimes, these transforms are used completely empirically to improve calibrations, even when the cause of the difficulty is unknown. An unfortunate side effect of this concept, verging on misuse of the algorithms, is the tendency of some scientists to attribute any improvement in the performance of a calibration (using the proposed data transform) to the effect of that proposed data transform. The possibility of other differences in the two cases (with and without the application of the proposed data transform) is not even considered. The possibility of other effects on the data, even a different contribution of the inevitable random noise, is completely ignored. Thus, while that approach may solve an immediate problem, it is lousy (although not quite junk) science.

We now present several algorithms that have been proposed by various scientists to mitigate the problems encountered when performing NIR calibration studies. Some of these were generated in the early days of chemometrics applied

to NIR analysis. Indeed, some of them predate the development of NIR analysis itself and even of chemometrics. Some were developed by mathematicians who were using multivariate methods to solve problems in physics, chemistry and other sciences. Of course they did not call it "chemometrics;" it was done under the umbrella of the branch of mathematics called "statistics." But the math was the same, regardless of the label.

With the advent of the modern chemometric algorithms, those initial early methods quickly fell into disuse and, to some extent, into disrepute. They came to be surrounded by an aura of being "primitive," "old-fashioned," not being up to modern standards, possibly incapable of solving modern problems. Maybe, but that's unfortunate. Scientists in those days had plenty of difficult and complicated problems to solve, and they solved them. Without having a computer and a "magic" algorithm handy to do their thinking for them, they had to study the systems causing their difficulties and learn to understand them—to know what was going on "under the hood," so to speak, so they could make intelligent guesses as to when and why they failed and how to make corrections. That sort of thinking, however, has become somewhat of a "lost art" nowadays, mainly the lost art of thinking about what we're doing, and replacing thinking with computer processing. The only case we know of in recent times that reflects the historic approach is one that we discuss below: the work of Karl Norris.

In our list here, we include some of those older algorithms. Just because they've fallen into disuse does not mean that they are useless. Indeed, by avoiding being overly complicated, some of them are easier to use and to understand, both conceptually and by their effect on calibration results. We start with the simpler ones and move on to more advanced algorithms. Some of these manipulations are, arguably, not so much data transformations as they are modifications of the calibration algorithm. Being by far the most complicated and difficult-to-understand, we do not include the principal

component regression (PCR) and partial least squares (PLS) algorithms here. We, as well as others, have presented and discussed both of those algorithms previously, in earlier columns of our series.

### CLS (Classical Least Squares)

The classical least squares (CLS) algorithm is arguably the oldest algorithm used for spectroscopic calibration, being developed directly from the application of Beer's law. In the earliest days, when both the instruments and the mathematics available were still very primitive, calibrating a spectroscopic instrument was more akin to calibrating a thermometer than a spectrometer. A single wavelength would be chosen. The scientist involved would develop a univariate relationship between the absorbance values of the sample at that wavelength, versus a measure of concentration of the desired analyte. In those days computers were not available either, and a "calibration" would often consist of a graph manually drawn on graph paper. We've discussed CLS in a series of previous columns starting with (15), another series beginning with (16), and a book on the topic (17). There were numerous limitations of this calibration method, not the least of which was the fact that it could be applied only to a clear liquid sample containing a single absorbing analyte in a non-absorbing solvent. As soon as a second absorbing material was added to the solution, it would interfere with the measurement of the intended analyte. In this case, a second measurement at a different wavelength would enable a correction to be made for the interfering material (17). In this case, it might then even be possible to measure the amount of both materials in the sample.

In principle, this process could be extended to more analytes in the sample (using three wavelengths to measure three absorbing ingredients in the mixtures, for example), but practical problems crop up. It's still limited to liquid mixtures (at that time, at least). The ingredients must be noninteracting. For example, an attempt to apply this method

to mixtures of water, ethanol, and acetic acid failed due to spectral distortions caused by hydrogen bonding (18). There are also questions about which wavelengths should be used and how should they be selected, among others. One solution to these difficulties was to use all the wavelengths in the spectrum. This algorithm became known in the spectroscopic community as the CLS algorithm, wherein the spectrum of a mixture of absorbing materials is considered as the sum of the absorbance spectra of the components of the mixture; the coefficients of the spectra of the ingredients represent the volume fractions of each of the various ingredients in the mixture. We've described and discussed this approach in a series of past columns (16,18–27), as well as it having been published in the formal literature (28). (Parenthetically, we note that, simultaneously, the multiple linear regression [MLR] algorithm became known to the spectroscopic community as the inverse least squares [ILS] algorithm; eventually, good sense prevailed, and the proliferation of terminology and abbreviations was cut short by the community's decision that use of the term ILS was to be deprecated. The change in terminology does not affect the utility of the algorithms, however.)

### Karl Norris' Derivative-Ratio Technique for Reducing Spectral Data to One Wavelength

While the application of derivative transforms of spectral data ( $d^nA/d\lambda^n$ , where  $n$  is generally between 1 and 4) has been in use almost as long as the technology of NIR analysis itself has been available, there is a growing recognition of the limitations of that data transform. Indeed, we ourselves recently published a critique of that data transform. One criticism is based on the fact that with digitized data, attempts to do so invariably result in an attempt to calculate 0/0, which is an error condition. Thus, one cannot actually calculate a derivative since that requires the wavelength difference between data points being used  $\rightarrow 0$ , a condition that cannot be achieved with digitized data. At best, the

difference between adjacent digitized data points must be used for the calculation, as an approximation to the derivative. The behavior of the approximation under these conditions has been previously examined (29–33). Nevertheless, Karl Norris and Bill Hruschka very successfully programmed their computer to perform the calculations needed to implement calibrations using “derivative ratios,” which are the quotients of the derivative approximations. This program would also vary the wavelengths at which these various “derivatives” and their quotients were calculated, then perform the calibration calculations using the resulting “derivative” quotients instead of the original absorbance values. The program enables the user to automatically select the wavelengths to use, and also has an interactive mode where the user can use his intuition to “guide” the wavelength search.

It was undoubtedly this capability that enabled Karl to routinely win the “Software Shootout” competition of the Chambersburg Conference (formally the International Diffuse Reflection Conference [IDRC], held every other year at Wilson College in Chambersburg, Pennsylvania), to the point where the conference organizers asked Karl to refrain from entering the competition just to give the other competitors a chance to win!

The heart of the algorithm was a two-step process: First, compute “derivatives” of the spectrum centered at all available wavelengths. Secondly, calculate the ratios (or quotients) of those derivatives at selected wavelengths. Those quotients were then used instead of the original spectral data in an MLR-type calculation to create the calibration model.

The original implementation of this algorithm was created on the computer in Karl’s U.S. Department of Agriculture/Agricultural Research Service (USDA/ARS) laboratory using a proprietary FORTRAN compiler. Since the work was all done under the auspices of the USDA/ARS, it was all in the public domain, and Karl intended to make his code available for free to any interested scientist, and especially to students. The use of

FORTRAN, however, created a stumbling block to that intention since most modern (Windows-based) computers did not have compatible compilers. Karl therefore collaborated with David Hopkins to convert his and Bill’s FORTRAN programs to an equivalent set of MATLAB programs, which Karl and David also intended to make available. With the death of Karl and the sudden unexpected death of David, with the assistance of David’s wife, a consortium of their friends were able to “rescue” the MATLAB code, and intend to make it available as Karl and David intended, although the details of how this will be accomplished have not been worked out.

### Post-Calibration Augmented-Components Methodology

During the period from the late 1990s through the early 2000s, David Haaland and coworkers, working at Sandia National Laboratories in Albuquerque, New Mexico, devised a most ingenious algorithm to improve calibration (strictly speaking, prediction) performance for aqueous solutions of urea, creatinine and sodium chloride (NaCl). It started with the realization that, when performing a spectroscopic calibration experiment, the “common knowledge” said that only the known and measured properties of the samples can be used for relating the spectroscopy to the chemistry (34–36). Other (unmeasured) properties (such as temperature, pH, or other chemical variations of the samples) that can potentially affect the measurement process are typically ignored. In this particular case, the unmeasured property of interest to these scientists is temperature. Since the measurements involved were made in aqueous solutions, the effect of temperature on the spectra of the samples can severely interfere with the desired spectrum of the analyte. In the author’s words, “The unmodeled spectral component... is the temperature of the solution, which is a relatively large source of unmodeled spectral interference in the near-infrared spectra of the unknown samples. Of course, temperature by itself does not have a spectrum,

but temperature exerts its influence on the samples by modifying the spectra of the samples” (35). The authors then proceed to introduce the use of PLS to extract a “temperature” spectrum from their aqueous solutions. Similarly, the authors also hypothesized the possibility of determining and removing the effect on the spectra of uncontrollable variations in the instrument.

The authors then demonstrated the ability of adding the “spectrum” of the temperature changes to the set of principal components to improve the performance of their calibration model. They were then able to apply their concept to other calibration algorithms, in particular to the CLS algorithm. An interesting sidelight is how Haaland and Melgaard (35) and your current authors independently developed similar graphic presentations of the development of the CLS algorithm. Haaland and Melgaard (35), however, also extended the concept to include spectra of unknown interferences. Since the CLS algorithm can be inverted and used to reconstruct the spectra of the mixtures and also of the mixture components, the difference between the reconstructed spectrum of a mixture and the actual measured spectrum of that mixture represents the “spectrum” of the unmeasured and previously unmodeled properties of the mixtures as described in their earlier paper (34). It is then a relatively straightforward exercise to add the “spectra” of the previously undetermined interfering phenomena (such as, for example, temperature) to the calibration model to correct for those interferences, without the need to re-measure the spectra of all the samples. This new algorithm was designated as the prediction-augmented classical least squares (PACLS) algorithm.

Haaland and coauthors then further extended their concept in two directions: 1) to produce a “hybrid” algorithm of CLS + PLS + PACLS, and 2) to expand the applicability of these new algorithms to maintenance, as well as development of spectroscopic calibrations. We don’t have space here to describe all these developments in detail, but an interested reader can easily find more information about





#### SCAN THE CODE

To read the full version of this article, with all tables and references.



them in the literature (34,35). Nevertheless, we note that these authors made a major contribution to the conceptual basis for various chemometric algorithms, particularly the use of PCR and PLS.

To go back into mathematical history, we find that mathematicians Brook Taylor and Jean-Baptiste Joseph Fourier (yes, he of the Fourier transform) developed what we now call Taylor series and Fourier series, respectively. What these two mathematical constructs have in common is their ability to approximate any arbitrary (although "well-behaved") function to any desired degree of precision by including sufficiently many basis functions in the calculation (the "basis functions" are polynomials in the case of Taylor series, and trigonometric functions [sines and cosines] in the case of Fourier series). Other mathematicians (for example, Bessel and Legendre) demonstrated that other mathematical constructs had that same capability, to reconstruct (approximate to any desired degree of

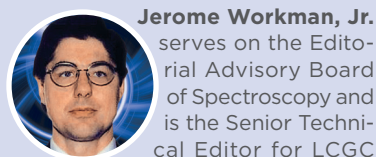
precision) any mathematical function through the use of a suitably chosen set of basis functions.

There are several more algorithms that are interesting and potentially useful, and that may be beneficial to an NIR practitioner who wants to investigate different ways to analyze data and produce superior calibration models. We will discuss some of these in a forthcoming Part B to this column.

A final word: If any of our readers are, or become, aware of the appearance of any of these algorithms, or the ones to

be published on our next column, in the literature, we would appreciate your letting us know about those appearances, ideally with a full literature citation.

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# Prediction of the Harvest Time of *Cabernet Sauvignon* Grapes Using Near-Infrared Spectroscopy

Yijia Luo, Jingrui Zhao, He Zhu, Xiaohan Li, Juan Dong, and Jingtao Sun

Harvest time assessment during the grape-ripening process can provide meaningful information for vineyard harvest scheduling. The purpose of this study was to investigate the identification of the harvest time of grape clusters using near-infrared (NIR) spectroscopy. During the harvest season from September to October 2019, bunches of *Cabernet Sauvignon* grapes were examined. Before establishing two classification models, namely partial least-squares discriminant analysis (PLS-DA) and support vector machine (SVM) models, raw spectra were processed by different pre-processing methods, including multiplicative signal correction (MSC), mean-centering, the standard normal variable (SNV), and the Savitzky-Golay method. Competitive adaptive weighted sampling (CARS) and the successive projections algorithm (SPA) were employed to select the optimal wavenumbers. The results indicate that NIR spectroscopy is a potentially promising approach for the rapid identification of different harvest times of *Cabernet Sauvignon* grapes, and the proposed technique is helpful for the prediction of ripened and over-ripened *Cabernet Sauvignon* grapes during the harvest time.

**C***abernet Sauvignon* grapes are the most widely planted wine grapes worldwide. They are a high quality wine grape variety, with strong adaptability, vigorous growth, and strong aging potential (1). The ideal harvest time of grapes is the key to obtaining high quality wines; as the saying in the wine industry goes, “seven points for raw materials and three points for fermented,” highlighting the importance of the grape harvest. The grape composition at harvest plays an important role in the future wine quality, and it is necessary for winemakers to carefully control the grape quality parameters during grape ripening, especially in the late stage and harvest period (2).

As a rule of thumb, grapes picked too early cannot reach a mature state, resulting in insufficient taste and aroma development; the taste of wine fermented at this stage is often green and astringent. The longer the grapes mature on the vine, the better the taste, but wine made with overripe grapes will have an overripe taste. Therefore, a suitable grape harvest period can ensure the production of high quality wine.

For generations, the determination of the grape harvest period has been primarily based on time and experience,

and the fruit is monitored close to the presumed harvest date via various ripening parameters—for example, the soluble solids content (SSC), total acid content, and total phenol content (3). Growers decide when to pick grapes primarily by sensory evaluation based on experience. Because human judgment is always subjective, artificial experience cannot provide completely suitable identification of the harvest time of *Cabernet Sauvignon* grapes. Particularly, in the case of a large sample size, taste and visual fatigue are prone to occur, and the accuracy of assessment is relatively low. Moreover, while *Cabernet Sauvignon* grapes can be evaluated via destructive methods, they are characterized by several disadvantages. Via these techniques, analysis is performed on only a few samples that are often not completely representative of the variability within the fruit lots. Additionally, when destructive analysis techniques are adopted, it is not possible to monitor the physiological changes of the same samples over the entire ripening period. It is therefore necessary to utilize a rapid, reliable, and non-destructive analytical tool to determine the harvest time of *Cabernet Sauvignon* grapes to ultimately improve the competitiveness of the grape-growing industry.

Compared with traditional physical and chemical methods, spectroscopy provides a faster, more convenient, and more accurate non-destructive analysis method for the determinant of fruit harvest times. Near-infrared spectroscopy (NIR) technology has been widely applied in food evaluation in recent years (4,5). There are many newly published studies that have investigated the potential ability of NIR to predict the internal quality characteristics, such as the SSC, pH, and total phenol content, of various fruits such as pears, strawberries, and apples (6–8). Additionally, some researchers have attempted to apply NIR as a non-destructive method for the qualitative analysis of fruits, and the majority of this research is concentrated on the prediction of several varieties (9,10) and several geographical origins (11,12).

However, NIR results always include substantial information produced from sample characteristics, as well as environmental and instrumental effects. Therefore, it is necessary to construct a robust model using selected information variables to improve both the modeling efficiency and prediction accuracy. Moreover, variable selection can simplify the model, yield better explanations, and reduce the cost of the measurement system. Many variable selection methods have been exploited to achieve these goals—among them, competitive adaptive weighted sampling (CARS) is a commonly used method (13). Variable selection is also applied to improve the performance of models established by genetic algorithms (GA) (14). The successive projections algorithm (SPA) has also been identified as an important method for the enhancement of model performance (15).

Although the application of NIR in the evaluation of fruit quality is increasing, little research has sought to analyze the harvest time of *Cabernet Sauvignon* grapes by NIR. In particular, there have been few studies on the identification of *Cabernet Sauvignon* grapes at different harvest stages via the combination of NIR and wavenumbers screening.

The present work aims to investigate the use of NIR for the harvest time discrimination of *Cabernet Sauvignon* grapes. With the aim of harvest time discrimination, a partial least-squares discriminant analysis (PLS-DA) model and a support vector machine (SVM) model were established in combination with CARS and the SPA for use on NIR spectral data.

## Materials and Methods

### Grape Samples

*Cabernet Sauvignon* grapes had been collected from a vineyard located in Shihezi City, Xinjiang Uygur Autonomous Region in China; the harvest was performed through five different stages. The first harvest time was started at September 16, 2019; three samplings were performed on September 23, September 30, and October 7, and the fifth harvest was performed on October 14 after the grape ripening. Sampling intervals were weekly. Each time, forty bunches were randomly collected from different vines. The bunches were stored in a cooler filled with ice to keep the berries fresh until transport

to the laboratory. *Cabernet Sauvignon* grapes were separated of the bunches with scissor, and each 10 grapes were individually into a group, which was regarded as representing the whole bunch of grapes. In addition, 200 bunches of grapes from the five harvest periods were divided into two subgroups; 150 samples were allocated in the calibration set and 50 samples were allocated in the validation set. Grape fruits should be kept to equilibrate to room temperature (25 °C) before spectral analysis.

### Instruments and Analysis

Spectral acquisitions of *Cabernet Sauvignon* grapes were performed by a TANGO-RFT-NIR spectrometer (Bruker Optics) in a reflectance mode at room temperature, with a resolution of 8 cm<sup>-1</sup> and a wavenumbers range of 12,500 to 4000 cm<sup>-1</sup>. *Cabernet Sauvignon* grape was placed in the sample cup of the spectrometer with a diameter of 3 cm. In the process of collecting the sample spectra, it was necessary to scan the air background regularly. Each sample (10 berries) was measured in triplicate, and the average spectra of three spectra were processed. The sample cup was cleaned with distilled water and dried with cleaning tissue paper between measurements. The operation of the spectrometer and the acquisition of the initial spectra were carried out with OPUS software (OPUS v. 7.0 for Microsoft, Bruker Optics).

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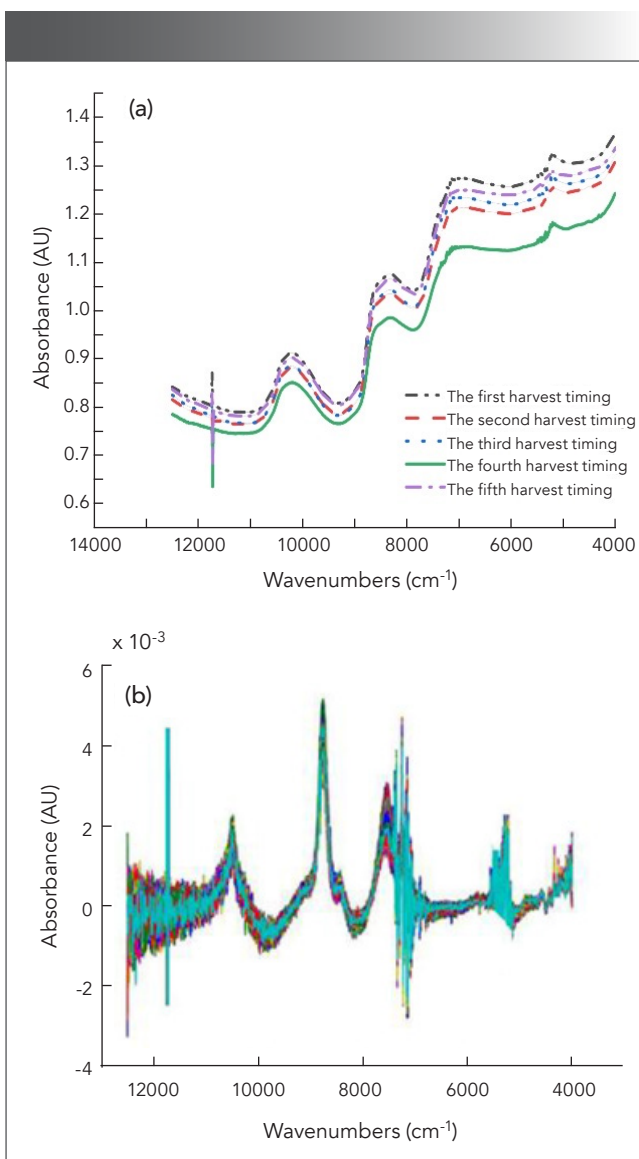
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**FIGURE 1:** (a) Original average spectra of *Cabernet Sauvignon* grape samples (b) Reflectance spectra of *Cabernet Sauvignon* grape at five different harvest periods obtained after Savitzky-Golay first derivative.

### Data Analysis

NIR spectroscopy data usually contains a lot of information. However, the data acquired from spectrometers include background information and noise besides sample information. It is necessary to preprocess spectral data for establishing accurate and stable calibration models. Before establishing the models, raw spectra were pretreated by multiplicative signal correction (MSC) (16), mean centering, standard normal variable (SNV) (17), and Savitzky-Golay first derivative (18) to improve spectral quality. Furthermore, the number of spectra data used for the establishment of the qualitative models was very large. Due to this, to get models with a smaller number of input wavenumber variables, char-

acteristic variable selection was performed by CARS and SPA to reduce invalid spectral information and speed up model calculation. *Cabernet Sauvignon* grapes were separated into calibration and prediction sets at a fixed ratio of 3:1 at each harvest time. Finally, the best supervised discriminant models were carried out with the selected variables applying PLS-DA and SVM for discriminating the harvest timing of *Cabernet Sauvignon* grapes. The procedure of these spectral preprocessing methods are performed in the Matlab2019a with spectral preprocessing methods toolbox available at <http://www.libpls.net>.

### Characteristic Variable Selection

In this study, CARS, SPA and their synergic methods were used to select effective wavenumbers for building reliable identification models of noise reduction spectra, realizing the rapid and accurate discriminating of harvest timing of *Cabernet Sauvignon* grape. The effects of CARS and SPA methods on dimensional reduction of spectral data were compared in the preliminary screening process. In the second screening, synergic method of CARS and SPA were compared.

CARS algorithm evaluates the contribution of each wavenumbers variable in the models according to the absolute values of regression coefficients of partial least square model. CARS is used to select a subset of  $N$  variables by implementation of  $N$  sampling runs iteratively (19). Monte Carlo resampling uses an iterative and competitive way to select a certain number of variables subsets from a fixed proportion of sample sequences (20). In each resampling, this method is used to select sensitive spectral variables with larger absolute regression coefficients in the calibration model, and the number of selected variables is determined by the exponential decreasing function. Finally, the subset with the lowest root mean square error of cross validation (RMSECV) is chosen (21). More details on the steps involved in CARS application can be found in previous studies (22).

Successive projections algorithm (SPA) is a new wavenumbers variable selection method for multivariate calibration. This technique is a forward selection algorithm aimed to decrease the total number of wavenumber variables used for establishing models. In the SPA algorithm, candidate subsets of variables with minimum collinearity are generated and evaluated by the value of root mean square error obtained from validation set of MLR calibration, and the uninformative variables are then removed by a variable elimination procedure without significant loss of prediction capability (23). More details on the steps involved in SPA application can be found in previous studies (24). The procedure of SPA and CARS are performed in the Matlab2019a with related toolbox available at <http://www.libpls.net>.

The technique is a forward variable selection method with the aim of reducing the number of wavenumber variables used in modeling, reducing the linear relationship by calculating the projections of other variables, and enhancing the

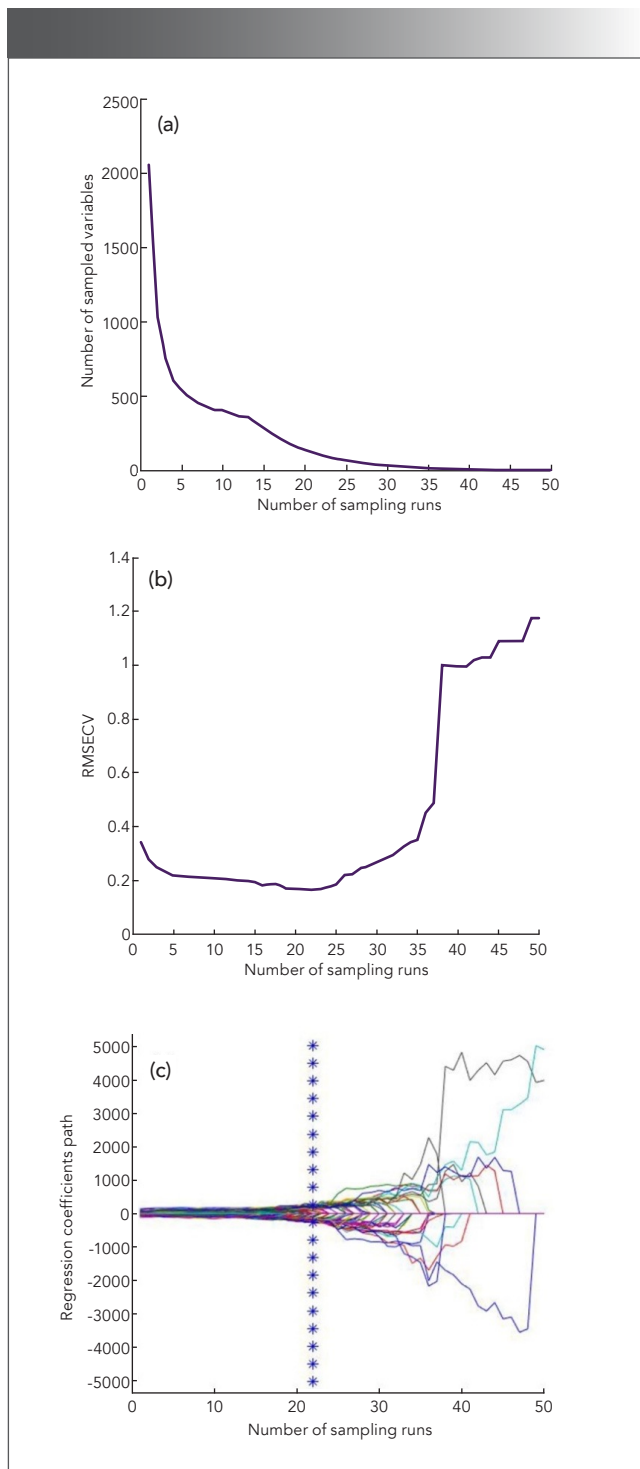
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**FIGURE 2:** (a) The changing trend of the number of sampled variables with the increase of sampling runs, (b) root-mean-square error of cross-validation values with the increasing of sampling runs, and (c) regression coefficients path by competitive adaptive reweighted sampling for harvest periods of *Cabernet Sauvignon*. Abbreviations: RMSECV, root-mean-square error of cross-validation.

**TABLE I:** Discriminant analysis models for different harvest time of *Cabernet Sauvignon* grapes using the full acquired spectra

Pretreatment	Models	Calibration Set		Prediction Set	
		False	Accuracy (%)	False	Accuracy (%)
Raw	PLS-DA	36	76	18	64
	SVM	0	100	5	90
MSC	PLS-DA	48	68	16	68
	SVM	0	100	6	88
SNV	PLS-DA	24	84	10	80
	SVM	0	100	6	88
SG+first derivative	PLS-DA	0	100	5	90
	SVM	0	100	0	100

Abbreviations: MSC: multiplicative signal correction, SNV: standardized normal variate, SG: Savitzky-Golay.

**TABLE II:** The result of PLS-DA and SVM models with different wavenumber selection algorithms on harvest time of *Cabernet Sauvignon* grapes

Variable Selections	Variable Number	Models	Calibration Set		Prediction Set	
			False	Accuracy (%)	False	Accuracy (%)
CARS	121	PLS-DA	0	100	3	94
		SVM	0	100	1	98
SPA	42	PLS-DA	48	68	25	50
		SVM	0	100	10	80
CARS-SPA	51	PLS-DA	0	100	2	96
		SVM	0	100	2	96

Abbreviations: CARS: competitive adaptive reweighted sampling, SPA: successive projections algorithm, PLS-DA: partial least squares-discriminant analysis, SVM: support vector machine.

performance of multiple linear regression by minimizing the collinearity in the calibration set (25). The above characteristic wavenumbers optimization method can remove the system noise in the spectral acquisition process and screen out the key information in the spectral region, improving the predictive ability of modeling (26). For the CARS algorithm, Monte Carlo sampling number were set to 50. The parameters of SPA were set as follows: The number of initial population, window width, mutation rate, and genetic iterations were 64, 5, 0.05, and 100, respectively. In the case of SPA, the range of variables selection was set as 1–50.

**PLS-DA**

Partial least squares discrimination analysis (PLS-DA) is a simple and stable linear classification method in multivariate analysis combining the characteris-

tics of partial least squares regression and the discriminant ability of classification technology. In fact, the PLS-DA model and the PLS regression model are implemented using the same principle. In short, the PLS-DA model is a classification problem transformed from one of regression, using classification variables to replace concentration variables. PLS-DA decomposes the spectral matrix and category matrix at the same time, strengthening the effect of category information in spectral decomposition and making the extracted spectral information most relevant to the category. PLS-DA accomplishes a rotation of the projection to latent variables focusing on class separation. A matrix of artificial variables, assuming a discrete numerical value (zero or one), was used as y data. The y dummy matrix is constructed to ensure that

the value of the objects belonging to the class corresponds to one, and the value of all other objects corresponds to zero (27). All the classification rules were evaluated using a cross-validation leave-more-out procedure using five cancellation groups (28), and the PLS-DA cutoff value for samples discrimination was fixed at 0.5. More specific information about the algorithm can be found in the provided reference (29). The procedure of PLS-DA is performed in the Matlab2019a with libPLS toolbox available at <http://www.libpls.net>.

**SVM**

Support vector machine (SVM) is a new nonlinear method proposed in accordance with the idea of structural risk minimization, and has been widely used in qualitative discrimination and regression analysis (30). The basic principle of SVM classification is to establish decision boundaries in feature space to separate data belonging to different categories. The main advantage of SVM algorithm is to use kernel function to solve the qualitative discrimination problem, which not only can reduce the complexity of the model, but also minimize the prediction error of the model. Generally, there are three classical kernel functions: polynomial kernel function, radial basis function (RBF) kernel function, and sigmoid kernel function. Among these three functions, the structure of RBF kernel function is the simplest and fastest computation. Therefore, only RBF kernel function was attempted in this work. More specific information about the SVM algorithm can be found in the provided reference (31). The procedure of SVM is performed in the Matlab2019a with SVM toolbox available at <https://www.csdn.net>.

**Results and Discussion  
Spectral Analysis**

Figure 1 presents the average measured raw absorbance spectra and preprocessed absorbance spectra of the grapes from the five harvest dates of September 16, September 23, September 30, October 7, and October

14, 2019. The raw spectra obtained from the *Cabernet Sauvignon* grapes include characteristic information. The stretching and bending vibrations of functional groups, such as C-H, O-H, and N-H, are related to the growth stage of grapes and affect the NIR spectral trend. The chemical ingredients and contents of grapes at different stages can influence the absorption intensity and sites of different groups. These functional groups are the basis of the composition of *Cabernet Sauvignon*, and are essential for the identification of the harvest period. As exhibited in Figure 1a, the *Cabernet Sauvignon* grapes from different harvest times had similar trends, but slightly different reflectance.

The wavenumbers at 10,800 and 10,200  $\text{cm}^{-1}$  may be associated with the OH groups in carbohydrates and water (32). The maximum absorption at 8340  $\text{cm}^{-1}$  was related to the second overtone of the C-H stretch. Another peak at 5200  $\text{cm}^{-1}$  was due to the combination of C-O deformation and the first overtone of OH stretching (33). Local maxima were observed around 9300, 7880, and 7150  $\text{cm}^{-1}$ . Additionally, the O-H functional groups in the water of *Cabernet Sauvignon* grapes produced absorption peaks at 9300 and 7150  $\text{cm}^{-1}$ . The Savitzky-Golay first-derivative method was used to eliminate the effects of baseline drift and scattering in the sample information for further spectral analysis (Figure 1b).

#### Full Spectra with PLS-DA and SVM

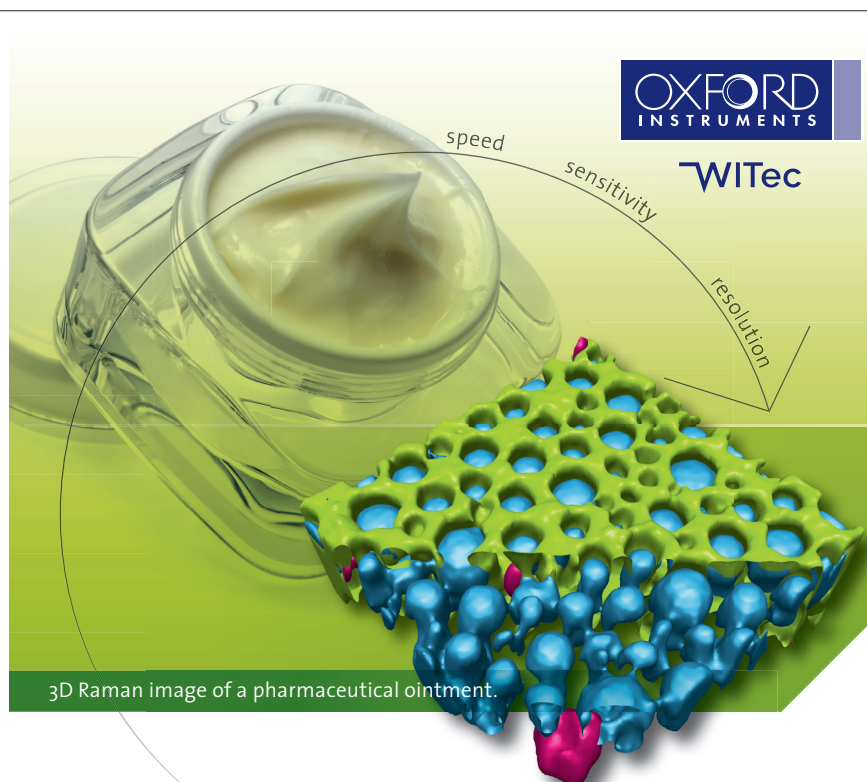
After using the previously cited pretreatment approaches, discriminant analysis models were established by applying the PLS-DA and SVM models for the identification of the harvest time of *Cabernet Sauvignon* grapes.

Table I reports the prediction results of the PLS-DA and SVM models for the analyzed harvest period after applying different pretreatment methods. For the PLS-DA models, the best pretreatment was achieved via the use of the Savitzky-Golay first-derivative method, which yielded 90% accuracy on the pre-

dition set and 100% on the calibration set. For the SVM models, the application of the Savitzky-Golay first-derivative method also achieved the best pretreatment method, and yielded 100% accuracy on the prediction set and 100% on the calibration set. In recent research, via the use of pretreatment methods, Shao and associates (11) obtained similar results for distinguishing different types of Chinese quince fruit, and spe-

cifically achieved 98% accuracy on the prediction set.

It is therefore evident that more satisfactory results can be obtained based on some suitable pretreatment methods. The established predictive models that employ the pretreatment of the Savitzky-Golay first-derivative method exhibit sufficient robustness and precision for monitoring the harvest time of *Cabernet Sauvignon* grapes.



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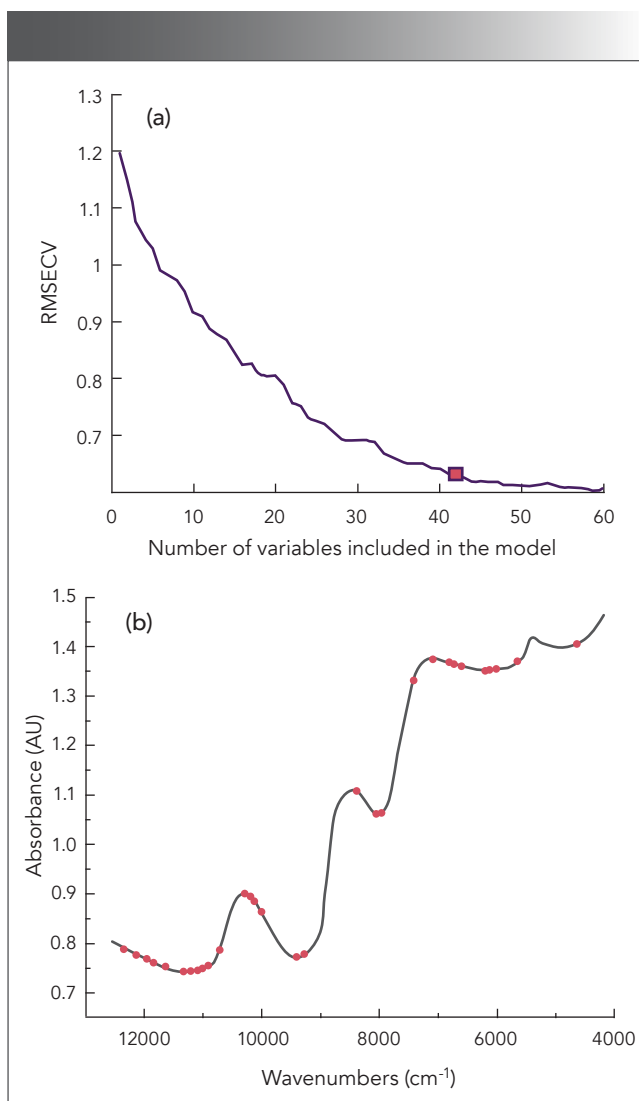
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**FIGURE 3:** (a) RMSECV plot and (b) distribution of variables selected by SPA for harvest periods of *Cabernet Sauvignon* grapes. Abbreviations: RMSECV, root-mean-square error of cross-validation. SPA, successive projections algorithm.

### Effective Wavenumber Selection

The effects of different wavenumber variables on the establishment of the models were not considered in the previous discussion. Because key variables have more powerful impacts on predictive models, variable selection methods were applied via the CARS and SPA approaches to determine the harvest time of *Cabernet Sauvignon* grapes. The models were again reconstructed with the PLS-DA and SVM algorithms, and the results are reported in Table II.

The CARS and SPA approaches achieved the efficient dimensionality reduction of the spectral wavenumbers in the initial screening, and the numbers of selected variables were 121 and 42, respectively.

Figure 2 presents the change of the number of variables selected, the root-mean-square error of cross-validation

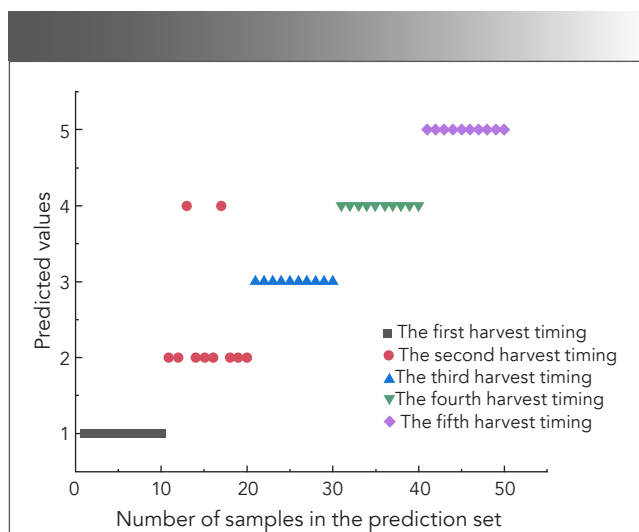
(RMSECV) values, and the regression coefficient path with the increase of the number of sampling run. It is evident that the number of selected variables decreased quickly and then slowed; these results were similar to those of a previous study, which revealed a process of fast selection followed by refined selection by CARS (34). The change trend of the RMSECV values was found to be consistent with that of the selected wavenumber variables, which can be attributed to the elimination of unnecessary wavenumbers, and then increased with the loss of information due to the removal of some key variables in the suitable subset. Figure 2c records the regression coefficient path of each wavenumber at different sampling. The best subset with the lowest RMSECV value is indicated by the vertical line denoted by an asterisk, and the number of selected wavenumbers was 121. Figure 3a presents the root-mean-square error (RMSE) distribution curve plot acquired by applying the SPA. It can be seen that the root-mean-square error of validation (RMSECV) value decreased sharply as the number of wavenumbers increased from 1 to 42, because at least 42 variables were required to resolve the spectral overlap characteristics of the sample. After that, the RMSECV value continued to decrease, but as the number of variables further increased, the improvement was very small, and the curve became stable. Figure 3b presents the distribution of 51 wavenumber variables selected by CARS combined with the SPA in the wavenumber range of 12,500–4000 cm<sup>-1</sup>, from which it can be seen that the variables were mainly distributed in the range of 12,500–12,000 cm<sup>-1</sup>.

### Results and Discussion of Different Identification Models

Based on the characteristic wavenumbers regions selected by the CARS and SPA methods, the PLS-DA and SVM models were respectively used to construct corresponding calibration and prediction models. For comparison, models were established over the whole spectra to predict the harvest time of *Cabernet Sauvignon* grapes. Table II reports the calibration and validation predictive results of models using different discrimination algorithms and wavenumber variable selection methods. The reconstructed models and their corresponding best mathematical pretreated methods were the same as those achieved using the whole spectra (Table I). In general, the simplicity and precision of the models were improved. This fact is evident by comparing the accuracies of the prediction and calibration sets of both the PLS-DA and SVM models using the whole spectra and those obtained via the use of the wavenumber variable selection methods. Hu and coauthors (35) demonstrated a similar situation, which proved that informative variable wavenumbers can be selected for the establishment of high-accuracy models. It can be clearly seen from Table II that the chemometrics results of the classification models using the CARS algorithm were better than those of the full-spectrum models, indicating that the CARS method can effectively select important variables. Fan and



associates (36) also used the CARS characteristic wavenumber screening method to establish a prediction model of the total acid content in vinegar, which achieved good prediction performance. The SPA selects the least number of wavenumbers, and the performance of the model established based on the wavenumber variables selected by the SPA was worse than that of the model established by the original spectra. The reason for this phenomenon may be that the SPA may have deleted some useful wavenumber variables from the entire spectrum, so its performance was not as good as that of the CARS method. This was confirmed by Wu and coauthors (37), who noticed that some wavenumber variables had little or no effect on the models and did not use them in model development, leading to a reduction in the amount of feature information. Moreover, a variable selection process was implemented in another study (38), and it was found that the performance of the model declined, possibly because the selection algorithm was applied to the spectral range with a low signal-to-noise ratio. The wavenumber variables selected by the CARS and SPA methods respectively accounted for 5.9% and 2% of the whole spectral variables. Although the performance of the models based on the wavenumber variables selected by the CARS method was better than that of the models based on the wavenumber variables selected by the SPA, the number of wavenumber variables selected by the CARS



**FIGURE 4:** The best results for discriminant analysis of harvest timing of *Cabernet Sauvignon* grapes in the prediction set by SG+1D-CARS-SPA-SVM model. Abbreviations: SG+1D, Savitzky-Golay first derivative. CARS, competitive adaptive reweighted sampling. SPA, successive projections algorithm. SVM: support vector machine.

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method was more than two times that selected by the SPA. To establish a model with fewer selected wavenumber variables and better performance, the number of wavenumber variables screened by CARS was screened again by the SPA. Finally, the number of wavenumber variables screened by CARS-SPA was 52, accounting for 2.5% of the total wavenumbers. From the perspective of constructing the models using the same wavenumber variable selection method, the identification models with CARS-SPA-PLS-DA and CARS-SPA-SVM respectively achieved better performance during calibration and verification. Although the CARS-SVM model achieved the best identification rate, the number of variables selected by the CARS-SPA method was less than one-half that selected by CARS, and the discrimination of the models based on CARS-SPA and CARS were not significantly different. A similar result was achieved by Qiu and associates (34), who predicted the total volatile basic nitrogen content with models developed using a combination of wavenumber variable selection methods. Furthermore, the models based on a combination of wavenumber variable selection approaches had better performance than those based on single approaches. Therefore, it is extremely important to select an appropriate feature wavenumber extraction method to improve the performance of the model. Moreover, the SVM models based on all three characteristic variable selection approaches (CARS, SPA, and CARS-SPA) exhibited satisfactory prediction results with an average identification rate of over 90%, and the classification precision of the prediction models for which variables were selected by CARS, SPA, and CARS-SPA were 97%, 80%, and 96%, respectively. The PLS-DA models that applied these methods achieved relatively poor prediction results, the corresponding performance indicator had an

average identification rate of 80% on the calibration set and prediction set, and the classification precision of the prediction models for which variables were selected by CARS, SPA and CARS-SPA were 94%, 50%, and 96%, respectively.

By comparison, the models combined with SVM performed better than those combined with PLS-DA. The reason for this phenomenon is the small amount of linear information in the spectral data or the small sample size used in this study. Non-linear models may have more advantages in dealing with some potentially nonlinear information, which is consistent with the findings of another study (39). The combination of the SVM qualitative identification algorithm with CARS-SPA achieved the best comprehensive identification performance; the identification rates on the calibration set and prediction set reached 100% and 96%, respectively, and two predicted samples were merely misidentified in the prediction set. These results outperformed those reported by González-Caballero and coauthors (40), who employed NIR spectroscopy (1600–2400 nm) associated with PLS-DA as a classification method to distinguish the ripening stages of *Cabernet Sauvignon* grape bunches and achieved 88% accuracy, thereby allowing selective harvest for the production of young, vintage, and soft red wines. Furthermore, similar results were achieved by Fernández-Novales and associates (41), who used NIR spectroscopy (700–1060 nm) and PLS-DA to differentiate red and white grapes, for which respective accuracy rates of 91.3% and 100% were achieved. The identification results of sample prediction based on the CARS-SPA-SVM model are presented in Figure 4.

From the identification results, it can be seen that two samples from the second harvest time were misjudged as samples from the fourth harvest time. Although identifying

different harvest times of *Cabernet Sauvignon* grapes is a complicated process that includes changes in various physical and chemical indicators within the grapes, the satisfactory prediction performance effectively supports the superiority of the algorithms. Overall, the obtained results suggest that the combination of NIR with a wavenumber variable selection method is promising and useful for the non-destructive evaluation of harvest times in one bunch of *Cabernet Sauvignon* grapes during on-vine ripening. Further research should be carried out on other grape varieties (both red and white) to perfect and support the developed approaches and to construct models for individual or global grape varieties.

## Conclusions

By applying the effective wavenumber variables selected by CARS and SPA, relatively good results were achieved in the discrimination of the harvest time of *Cabernet Sauvignon* grapes based on SVM, and the accuracy rate of harvest period discrimination reached 96% on the prediction set. This research provided an appropriate approach for grape farmers to discriminate the harvest time of each bunch of *Cabernet Sauvignon* grapes. Finally, the results achieved in this research may be applicable to only a specific region, and it is therefore necessary to further analyze the robustness of the developed model in different regions.

## Disclosure Statement

The authors report there are no conflicts of interest.

## Acknowledgments

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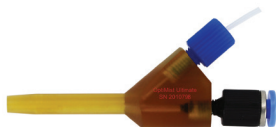
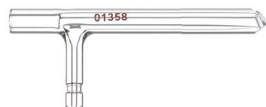
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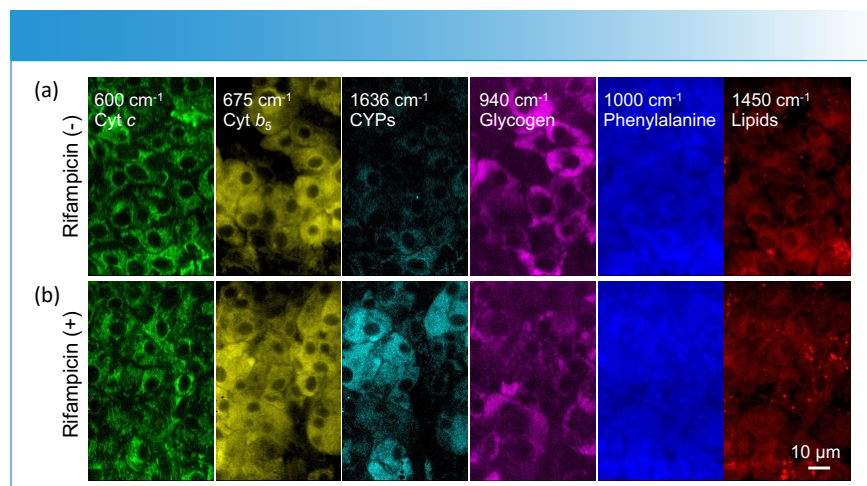
# Analytical Biology: An Emerging Discipline for the Future

Menglu Li and Katsumasa Fujita

The field of analytical chemistry is well established. It's time to develop the field of analytical biology—an emerging discipline that blends various research fields to provide a holistic view of biological phenomena.

**I**nterdisciplinary research at the intersection of physics, biology, and chemistry is attracting increasing interest and generating many innovative ideas. One example is the application of Raman spectroscopy to biological and medical research. Although the Raman effect was applied to chemical identification shortly after its discovery, it took nearly six decades for the first Raman spectrum of a live cell to be measured, because of the inherently low efficiency of Raman scattering and the complexity of biological samples (1,2). The later advances in laser technologies, such as solid-state ultrashort-pulse lasers (3,4), high-sensitivity detectors (5), and high-quality optical filters (6) have brought Raman spectroscopy into the realm of cell biology.

Raman spectroscopy offers unique capabilities, such as label-free and comprehensive detection of biological molecules in cells and tissues. Unlike infrared spectroscopy, Raman spectroscopy does not strongly interact with water and can provide molecular information under physiological conditions without damaging samples. Raman spectroscopy is used for a broad range of measurements ranging from general Raman spectral analysis to high-resolution Raman imaging with subcellular sensitivity. An example can be seen in Figure 1, which shows high-resolution Raman images of hepatocytes with and without the treatment of the drug rifampicin (7). In particular, high-speed Raman spectroscopic imaging, coupled



**FIGURE 1:** Raman images of different cellular components in hepatocytes (a) without and (b) with drug (rifampicin) treatment.



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with microscopy, has gained significant interest in the life sciences (8–10). Raman microscopy faithfully translates molecular information from biological samples, such as DNA, proteins, carotenoids, and lipids—and their intracellular balance—into spectra that can provide information about cells, tissues, and species. This comprehensive analysis of intracellular molecules can be employed to detect cell apoptosis and differentiation and to diagnose diseases such as cancer that have not been fully explained using traditional biological methods. This capability can pave the way for new approaches in cell and tissue analysis in medical diagnostics, regenerative medicine, and drug development.

In many applications, Raman microscopy is used to distinguish biological phenomena at the levels of biomolecules, cells, tissues, and even organisms. This approach is somewhat akin to analytical chemistry, which aims to analyze chemicals (that is to separate, identify, or quantify the chemistry of a sample in sufficient detail in order to gain a better understanding of its nature, structure, or function), but with this use of Raman spectroscopy and microscopy one aims to also analyze biological phenomena. From this perspective, these studies are shaping a new discipline that might be referred to as analytical biology. Although this term has rarely been used as a research field, its roots can be traced back to a 1950 book titled *Analytical Biology* by G. Sommerhoff (11) and a 1966 *Nature* article by I.J. Good (12). However, these early works focused more on mathematical and theoretical biology rather than the analysis of biological materials or phenomena. From our viewpoint, as shown in Figure 2, the field analytical biology would stand at the intersection of analysis and biology, indicating a research field that includes studies, instruments, and methods aimed at analyzing biological substances and phenomena.

The strategy in analytical biology is different from typical biological methods that are mainly based on reductionism. Raman microscopy offers a holistic and unbiased approach to analyzing multiple biological molecules simultaneously, al-

lowing for non-targeted analysis. This phenotypic and high-content strategy can complement target-oriented approaches. The concept of analytical biology also aligns with other state-of-the-art and emerging techniques, particularly omics approaches such as genomics, transcriptomics, proteomics, and metabolomics. With this strategy it becomes possible to first obtain an overarching view of a given biological phenomenon and then proceed to elucidate the underlying mechanisms using target-oriented

methodologies. This approach can be combined with chemical biology, which includes studies using multimodal analysis with targeted methods, such as those using Raman tags and probes (13). The relationship between these approaches can be seen in Figure 2.

Because biological phenomena are not solely associated with a single protein, holistic approaches like omics techniques have garnered interest for their potential to provide a comprehensive view of biological phenomena instead of



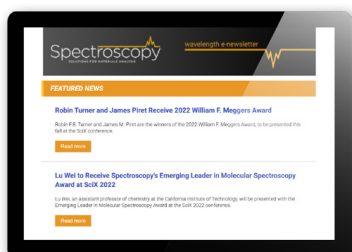
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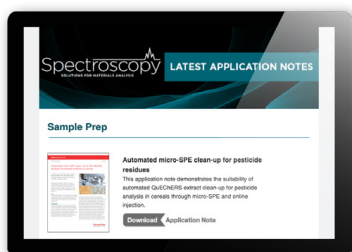
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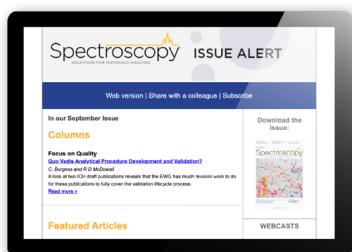
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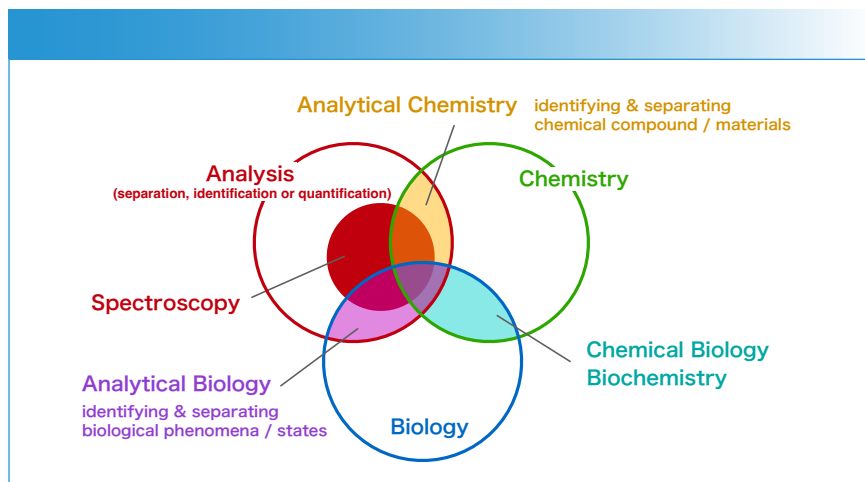
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focusing on a single or limited number of biomolecules. Unbiased and phenotypic screening has been found to be valuable for gaining a comprehensive understanding of complex phenomena such as multifaceted and multilevel hepatotoxicity. Analytical biology represents an emerging discipline that blends various research fields to provide a holistic view of biological phenomena. By incorporating spectroscopy and other cutting-edge techniques, analytical biology allows scientists to explore new aspects of biology and uncover deeper insights into the mechanisms behind complex biological processes. As this field continues to evolve, it promises to enrich our understanding of the intricacies of life and open new avenues for discovery in medicine, drug development, and related areas.

We came up with this idea of analytical biology when we encountered the difficulties in choosing a category of research on submitting our papers to journals. Throughout history, there are



**FIGURE 2:** The overlap of related disciplines: analytical biology stands between analysis and biology, just as analytical chemistry stands between analysis and chemistry, and as chemical biology stands between chemistry and biology.

numerous examples of new technologies paving the way for new research fields. We are excited by the prospect that studies in advanced spectroscopy may stand at the forefront of this exciting and emerging new field of analytical biology and all that it promises.

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# Peter Griffiths: Icon of Infrared Spectroscopy

Jerome Workman, Jr.

*Spectroscopy* is publishing a series of feature articles highlighting the lives and careers of the most influential spectroscopists over the past 100 years. These individuals were selected by our Editorial Advisory Board and team of editors to represent the leading figures in spectroscopy over the century. Our second featured Icon of *Spectroscopy* is Peter R. Griffiths.



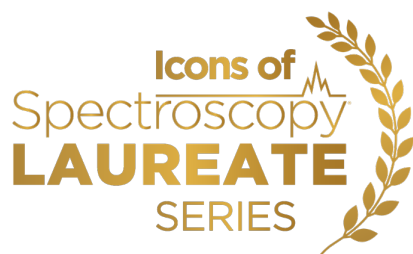
**Peter Griffiths** was born and raised in England, spending the first 25 years of his life there. His formal education includes a BA in 1964 and DPhil in physical chemistry in 1967, both at Oxford University. After graduating, Griffiths moved to Maryland and completed post-doctoral training at the University of Maryland from 1967 to 1969 under the supervision of the iconic Ellis R. Lippincott. After a brief industrial career with leading infrared (IR) technology companies, such as Digilab in Cambridge, Massachusetts and Sadtler Research Labs in Philadelphia, Pennsylvania, he joined the faculty of Ohio University in 1972, reaching the rank of Distinguished Professor. After ten years, he moved to the University of California, Riverside before accepting the chairmanship of the chemistry department of the University of Idaho,

from which he retired in 2008. As of 2008, Griffiths had entered status as professor and Chair Emeritus at the University of Idaho, and as of 2014, as Adjunct Professor of Chemistry at the University of Utah.

Griffiths has an impressive record of over 320 refereed publications, 14 books (three authored and 11 edited), 55 book chapters, one issued patent, four national committees served on, seven editorial boards, multiple named lectureships, and many of the most prestigious awards in spectroscopy. His impact has extended to mentoring many doctoral students and scientists over his years as a professor, lecturer, and short course instructor. Former students and collaborators, like Andrew Weakley, have made comments related to Griffith's influence on their careers.

"He pretty much made my career at this point in a big way. The reason why I have a consulting business is because of Peter, and the reason why I landed a postdoc at UC Davis at the Air Quality Research Center is because of Peter," Weakley said. "So thank you so much, Peter. We appreciate all that you've done for us."

Griffiths' primary focus lies in analytical vibrational spectroscopy,





notably in Fourier transform IR spectroscopy (FT-IR), supplemented by occasional investigations into Raman spectroscopy and gas, liquid, and supercritical fluid chromatography (SFC). His research group has delved into diverse areas within this field, including diffuse reflection (DR) spectroscopy, open-path atmospheric monitoring, and the integration of FT-IR spectrometers with different chromatographs such as gas chromatography (GC), high performance liquid chromatography (HPLC), and SFC.

Among Griffith's trademarks are his books on the subject of FT-IR, which have become the gold standard of FT-IR treatises. His first authored book on the subject, *Chemical Infrared Fourier Transform Spectroscopy*, was published in 1975 (1). His second edited work, *Transform Techniques in Chemistry*, was published in 1978 (2), and his most well-recognized book on FT-IR, coauthored with Jim de Haseth, is titled *Fourier Transform Infrared Spectrometry* (3).

"In our group, it's referred to as 'the Bible' that every student reads," said Rohit Bhargava at the University of Illinois, Urbana-Champaign, speaking at the SciX conference. "At Illinois, when we get promoted from assistant to associate professor, the library asks us for one book that has had a transformative influence on our lives. They buy that book in honor of that person getting promoted, and they place a little plaque. If you go to the University of Illinois library, there's a *Fourier Transform Infrared Spectrometry* book with a little plaque in it that tells you why it's there in that library."

Another highly impactful publication is the five-volume *Handbook of Vibrational Spectroscopy* edited by Griffiths and John Chalmers (4). The five volumes comprising this work consist of 46 sections, 239 chapters, 350 authors from 250 institutions, and 3572 pages, providing a cross-section of the knowledge and insight of many of the world's most experienced researchers in vibrational spectroscopy (5).

Among Griffith's honors and awards are: The Coblentz Award in Molecular Spectroscopy (1975), Distinguished Professor, Ohio University (1978), Spectroscopy Society of Pittsburgh Award (1985), New York SAS Gold Medal in Spectroscopy (1995), Fritz Prëgl Medal of Austrian Society for Analytical Chemistry (1995), University of Idaho Award for Excellence in Research or Creative Activity (1995), Honorary Membership, Society for Applied Spectroscopy (2001), Bomem Michelson Award in Molecular Spectroscopy (2003), Gerald S. Birth Award for Outstanding Work in Near-Infrared Spectroscopy (2004), Distinguished Faculty Award, University of Idaho College of Science (2006), Alexander von Humboldt Senior Research Fellowship (2006), Erskine Fellow, University of Canterbury, Christchurch, New Zealand (2008), Anachem Award in Analytical Spectroscopy (2012), an Honorary DSc

from the University of Idaho (2018), and the Ellis R. Lippincott Award in Molecular Spectroscopy (2023).

The Lippincott Award is a fitting, sunset tribute to the starry career of Griffiths and was awarded at last year's SciX conference in Sparks, Nevada. Griffiths first met Lippincott when he was a graduate student in "Tommy" Thomson's laboratory at Oxford which was attended by the major researchers in IR spectroscopy at the time, including not only Lippincott but also Richard (Dick) Lord.

"Since I am now 81 years of age, and I retired over 15 years ago, this award will not benefit me professionally to any great extent. On the other hand, from a personal standpoint it is tremendously gratifying," said Griffiths upon receiving the honor. "I believe that I am the only Lippincott Awardee to have worked in his laboratory, and I learned a great deal from my interaction with him."

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"As is the case for many week-long conferences, Wednesday afternoon was free and my fellow 'gopher,' Roger Lake, and I were asked to play a game of croquet with Lord and Lippincott. One year later, Roger was a post-doc in Dick Lord's lab at MIT, and I was in a similar position in Ellis's lab at the University of Maryland. Learning how a successful scientist operated by observation of Ellis Lippincott was critical to my career," Griffiths said (6).

Griffiths has served as president of both the Coblenz Society and the Society for Applied Spectroscopy. He served more than 30 years with the journal *Applied Spectroscopy* as an Associate Editor from 1981 to 2009, the Editor-in-Chief from 2009 to 2012, and the Editor from 2012 to 2018. Griffiths has been associated as an editor with other leading journals in analytical chemistry and spectroscopy, including *Spectroscopy and Spectral Analysis* (China) (1995–2012), *Spectrochimica Acta* (1992–2008), *Analytical and Bioanalytical Chemistry* (Germany) (1998–2010), *Journal of Analytical Sciences* (Japan) (1995–2005), *Analytical Chemistry* (1987–1989), and *Energy and Fuels* (1988–1991).

Although Griffiths has published many papers in *Applied Spectroscopy* (English Language), he also has published in the following journals (in alphabetical order): *Advances in Chromatography*, *Aerosol Science and Technology*, *Annali di Chimica* (Italian Journal of Analytical Chemistry), *Analytical and Bioanalytical Chemistry*, *Analytical Chemistry*, *American Pharmaceutical Review*, *Applied Optics*, *Applied Spectroscopy* (Chinese Language), *Applied Spectroscopy* (Japanese Language), *Applied Spectroscopy Reviews*, *Chromatographia*, *Computer Enhanced Spectroscopy*, *Energy & Fuels*, *Fresenius Journal of Analytical Chemistry*, *Fuel*, *Geochimica et Cosmochimica Acta*, *Institute of*

*Petroleum* (London), *International Journal of Environmental Analytical Chemistry*, *Journal of Catalysis*, *Journal of Chromatographic Science*, *Journal of Chromatography*, *Journal of Colloid and Interface Science*, *Journal of Environmental and Occupational Health*, *Journal of High Resolution Chromatography*, *Journal of Molecular Structure*, *Journal of Near Infrared Spectroscopy*, *Journal of Physical Chemistry*, *Journal of Physical Chemistry B*, *Langmuir*, *Microchemical Journal*, *Mikrochimica Acta*, *Mining, Metallurgy & Exploration*, *Optical Remote Sensing for Environmental and Process Monitoring*, *Oxford Dictionary of National Biography*, *Proceedings of the 11th International Conference on Fourier Transform Spectroscopy*, *Proceedings of the Royal Society (London)*, *Proceedings of the Society of Analytical Chemistry*, *Progress in Fourier Transform Spectroscopy*, *Spectrochimica Acta*, *Spectrochimica Acta (Far Infrared Spectra)*, *Spectroscopy*, *Spectroscopy and Spectral Analysis*, *Vacuum*, and *Vibrational Spectroscopy*.

Over the past 30 years, Griffiths has made many friends, connections, and collaborations having taught a week-long course on the interpretation of IR and Raman spectra at Bowdoin College, Maine. He has acted as a consultant to multiple companies, law firms, and technical organizations over his active years. His legacy and mentorship will be certain to continue for several more generations.

### Research Impact Book Chapters

A review of Griffith's CV and publications list demonstrates the broad range of research explored during his career (7), and his impact as a scientist is evidenced by the 55 book chapters spanning various aspects of spectroscopy and analytical chemistry.

A summary of some key themes in his book chapter publications:

- *Spectroscopy Data Processing* (2017): Griffiths addresses the critical aspect of data processing in spectroscopy, emphasizing its significance in the interpretation and analysis of spectral information.
- *Gas Chromatography/Infrared Spectroscopy* (2016): Discussing the synergy between these two topics, Griffiths explores their combined applications.
- *Infrared Spectroscopy* (2016): Providing a comprehensive overview, this contribution to the *Encyclopedia of Analytical Science* highlights the role and significance of infrared spectroscopy in analytical research.
- *Infrared Mapping Below the Diffraction Limit* (2014): Delving into advanced techniques, Griffiths explores this topic by showcasing his engagement with cutting-edge developments.
- *Surface-Enhanced Infrared Absorption* (2013): Griffiths' investigation of this technique contributes to the understanding of this specialized area within spectroscopy.
- *Raman Spectroscopic Studies of Explosives and Precursors* (2012): Collaborating with other authors, Griffiths explores the applications and instrumentation of Raman spectroscopy in forensic science, emphasizing its role in studying explosives.
- *Detection of Melamine in Foodstuffs* (2010): Griffiths contributes to the understanding of vibrational spectroscopy's role in food science.
- *Introduction to the Theory and Instrumentation for Vibrational Spectroscopy* (2010): Griffiths provides foundational knowledge on vibrational spectroscopy, outlining theories and instrumentation critical for this analytical technique.
- *Sampling Techniques and Fiber-Optic Probes* (2010): Griffiths collaborates on the exploration



#### SCAN THE CODE

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of these techniques in vibrational spectroscopy, addressing practical aspects of data collection.

- *Continuum and Discontinuum Theories of Diffuse Reflection (2007)*: Contributing to the *Handbook of Near-Infrared Spectroscopy*, Griffiths explores theories of diffuse reflection, adding to the theoretical foundations of spectroscopy.
- *Dynamic Infrared Linear Dichroism of Polymers (2007)*: In a collaborative effort, Griffiths adds to the understanding of this topic as it relates to vibrational spectroscopy of polymers.
- *History, Trends, and Prognostications (2007)*: Summarizing historical evolution and future trends, Griffiths provides insights into the development and potential future directions of vibrational spectroscopy.
- *Infrared Spectroscopy (2005)*: Contributing to the *Encyclopedia of Analytical Science*, Griffiths provides a comprehensive chapter on infrared spectroscopy, emphasizing its analytical significance.

These summaries highlight the diversity and depth of Griffiths' contributions to spectroscopy, ranging from fundamental principles to advanced applications in various scientific domains. His extensive body of work reflects a commitment to advancing the understanding and application of spectroscopic techniques.

### Research Papers

Griffiths' research papers encompass an equally broad spectrum of topics, predominantly centered around the versatile applications and advancements in spectroscopy, with a specific focus on FT-IR spectroscopy. One overarching theme explores the application of spectroscopy in environmental monitoring, where papers examine the

utilization of FT-IR to monitor and quantify diverse environmental factors such as diesel particulate matter, silica in mine dust, greenhouse gases, atmospheric ammonia, methane, and nitrous oxide.

In the realm of mining and metallurgy, Griffiths' research extends to the characterization and monitoring of materials, including coal dust. Papers within this theme focus on quantifying elemental and organic carbon, estimating  $\alpha$ -quartz, and measuring silica. Another significant area involves the application of spectroscopy in chemistry and material science, with papers exploring topics such as the synthesis of graphene paper, electroless deposition of metals, surface-enhanced spectroscopy, and kinetic studies of chemical reactions.

Biographical and historical perspectives are presented in select papers, offering insights into figures like Norman Sheppard, the impact of papers in the journal *Applied Spectroscopy*, and the early days of FT-IR spectroscopy. Griffiths also contributes to the pharmaceutical and medical fields, with research papers focusing on mid-infrared (mid-IR) spectroscopy's application in the pharmaceutical industry and the estimation of  $\alpha$ -quartz in respirable coal mine dust.

Methodological and instrumentation developments are highlighted, covering advancements in FT-IR spectroscopy techniques such as ultra-rapid scanning, open-path FT-IR, and applications of neural networks. Chemical and physical studies are explored, involving investigations into the catalytic behavior of silver nanoparticles, the synthesis of graphene paper, and surface-enhanced Raman spectroscopy (SERS).

Instrumentation and data processing are significant themes, with papers addressing various aspects of these topics as they relate to

spectroscopy, including discussions on noise sources, baseline correction algorithms, and the characterization of silicones.

Examining the titles of research papers further reveals additional themes. Noteworthy topics include infrared spectroelectrochemical analysis of adsorbed hexacyanoferrate species, surface and solution species in the ferricyanide/ferrocyanide redox couple, near-infrared (NIR) spectrometry, surface-enhanced infrared absorption spectrometry, Raman spectra of explosive materials, capillary SFC/FT-IR, step-scan FT-IR spectrometry, SFC/Fourier transform spectrometry, open-path FT-IR spectrometers, and vibrational spectroscopic studies of asbestos, transitite, and concrete.

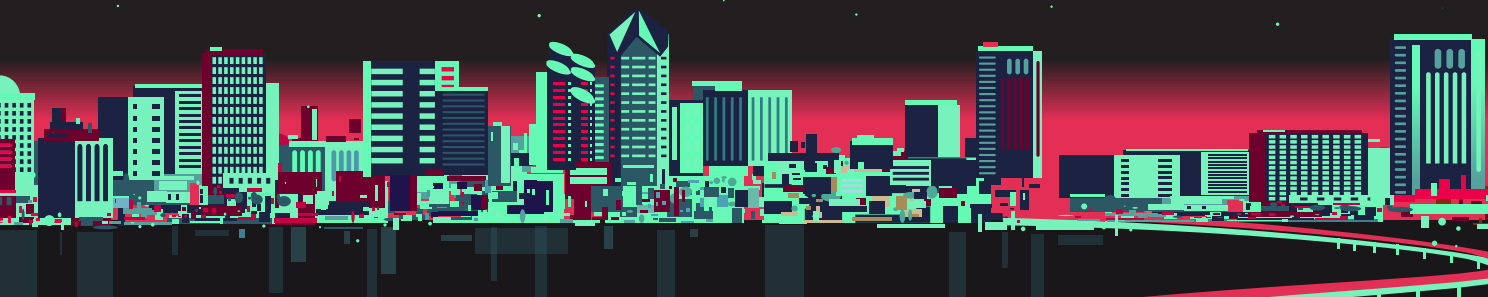
The remaining papers cover a diverse range of subjects, from noise sources in step-scan FT-IR spectrometry to baseline correction algorithms, automated baseline correction, characterization of silicones, feed-forward neural networks, and various applications of FT-IR in a variety of different fields.

Griffiths' extensive body of research reflects a rich diversity of topics within the field of spectroscopy, with a predominant emphasis on infrared and Raman spectroscopy, electrochemical analysis, and the integration of different analytical techniques. The research spans applications in environmental monitoring, mining, chemistry, material science, pharmaceuticals, and medical fields, showcasing the interdisciplinary nature of Griffiths' contributions to the scientific and spectroscopic communities.

*Some material related to summarizing the subject matter of 320 refereed publications of Griffiths was written with the help of artificial intelligence and has been carefully edited to ensure accuracy and clarity.*

# Pittcon Preview: What to Expect from the 75th Annual Conference

This year, the event will take place in San Diego and features several experts in spectroscopy.



*Spectroscopy Staff*

**T**he 75th Annual Pittcon Conference and Exposition is taking place from February 24–28, 2024, in San Diego, California. The event will feature numerous awards and talks from analytical scientists in industry, academia, and national laboratories.

The event will kick off with the Wallace H. Coulter lecture, which features an individual who has demonstrated a lifetime commitment and made important contributions that have had a significant impact on education, practice and research in laboratory science. Omowunmi Sadik, distinguished professor and director of The BioSMART Center at the New Jersey Institute of Technology (NJIT), will deliver the lecture this year. Sadik's talk will focus on the use of sustainable nanomaterials for understanding reaction mechanisms, small-scale synthesis, and biosensing.

The conference also includes several awards focused specifically on spectroscopy, including the Coblentz Society's Williams-Wright Award, which honors an industrial spectroscopist who has made significant contributions to vibrational spectroscopy while working in industry. The 2024 Williams-Wright Award winner is Jun Zhao, the director

of engineering at Metrohm Spectro. Zhao's work focuses on Raman spectroscopy instrumentation and methods.

The Pittsburgh Spectroscopy Award will also be presented at the conference. This award, established in 1957, is presented annually to a researcher who has demonstrated outstanding achievements in the field of spectroscopy. This year, Vicki H. Grassian, distinguished professor and distinguished chair in physical chemistry in the Department of Chemistry and Biochemistry at the University of California, San Diego, will be honored. Grassian's group focuses on the chemistry and impacts of environmental interfaces as it relates to atmospheric aerosols, engineered and geochemical nanomaterials, and indoor surfaces.

The conference also features several sessions focused on the latest advances in spectroscopy. A session led by Dmitry Kurouski, a professor at Texas A&M University and the winner of *Spectroscopy's* 2023 Emerging Leader in Molecular Spectroscopy Award, will focus on infrared and Raman chemical imaging at the nanoscale, atomic force microscopy-infrared spectroscopy (AFM-IR), and tip-enhanced Raman spectroscopy (TERS).

These are emerging analytical techniques that have single-molecule sensitivity and sub-nanometer spatial resolution. There will also be a session on novel spectroscopic approaches, which will feature speakers from industry and academia discussing techniques such as infrared correlation nanoscopy and Fourier transform infrared (FT-IR) spectroscopy.

Pittcon attendees also have the option to attend several networking workshops throughout the week. These sessions offer an opportunity to connect with other professionals who share similar interests, and will focus on hot topics in analytical chemistry, including cannabis and forensics.

Attendees can also network in the exhibit hall. This year, the event exhibit hall will include booths from companies such as PerkinElmer, Shimadzu Scientific Instruments, Metrohm, and more. Attendees can visit *Spectroscopy* at booth 1042 to speak with the editors and pick up a copy of the magazine.

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## Monitoring and Control of a Semi-Batch Polymerization with Raman Spectroscopy

### Introduction

Semi-batch reactions offer several advantages over traditional batch reactions, such as greater selectivity of products, gradual addition of reactants for better process control, and the ability to isolate product as it is formed. Polymerizations are often done in semi-batch mode because it offers the ability to carefully control the composition of the product in order to create high-performance materials with fine-tuned custom properties.

However, one significant challenge of semi-batch reactions is that the concentrations of the reactants constantly change, in both relative and absolute senses. If uncontrolled, this variation in the concentrations of reactants can easily result in unwanted properties in the product. So, this reaction mode requires continuous real-time monitoring of the concentrations of the reactants. Simply adding the reactants in the correct ratios is insufficient.

Additionally, batch-to-batch consistency is essential for high-performance materials with finely tailored properties. And in many manufacturing plants, the same process equipment must be capable of producing a wide variety of high-performance materials, so the process-control method must be both precise and flexible.

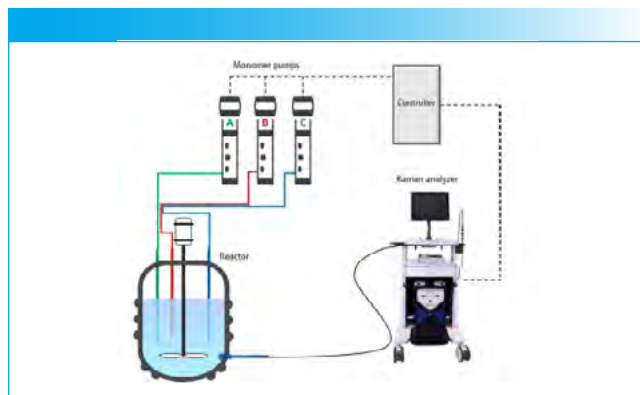
Raman spectroscopy offers several advantages for real-time process monitoring of semi-batch polymerizations: It can identify bands specific to particular functional groups, such as the vinyl and alkyl moieties common to polymerization monomers, and it offers both easy sample interfacing and fiber-optic coupling of the probe to the base unit for flexible placement of the base unit relative to the process line.

### Experimental

In this experiment, a semi-batch polymerization reaction was carried out using three proprietary monomers, designated monomers A, B, and C. A schematic of the system is shown in Figure 1.

A Raman analyzer was used to deliver and collect 785 nm near-infrared radiation from a laser. A noncontact optic was used to perform the analysis through a pressure- and temperature-rated sapphire viewport in the wall of the reactor. Each sample acquisition consisted of 60 seconds of illumination of the reactor contents using 125 mW of laser radiation followed by a 20 second pause. Five to seven data points from each monomer were used to generate quantitative Raman data for real-time process control.

In this reaction, a vinyl functional group in each of the three monomers changes to an alkyl group during the polymerization. Raman is sensitive to this, whereas traditional infrared spectroscopy is much less so. Chemometric modeling in this case was



**Figure 1: Schematic diagram of process control system for semi-batch polymerization**

based on the Raman signal for the vinyl region in all monomers, the C–H wag in monomers A and B, and the lactone region in monomer C.

### Results and Discussion

The polymerization reaction was performed under both open-loop (in which spectroscopy is used only for process monitoring, not for feedback control) and closed-loop (in which spectroscopy is used for process monitoring and real-time feedback control) conditions.

### Conclusion

Raman spectroscopy was demonstrated to be a simple, accurate, and effective method of process analysis for the purpose of real-time monitoring and control of a semibatch polymerization. Raman data were able to be used for real-time feedback control of the process to maintain ideal process conditions in a closed-loop reaction system and to produce a highly consistent product between batches, ensuring consistent quality of sensitive high-performance materials.

### References

- (1) Müller, J. et al. "Semi-Batch Polymerization Analysis by Multi-Point Raman Spectroscopy." *Process Analytical Technology*, September/October 2006, 1–6

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# Temperature Controlled Gas Cell and its Application

Harrick Scientific Products, Inc.

When studying the vibrational spectroscopy of organic molecules in the condensed phase, the functional groups' interactions with other species and neighboring molecules (solvent) can result in significant broadening of the rotational transitions, resulting in a featureless spectrum. However, in the gas phase, the molecules will be able to rotate and vibrate freely. Depending on the resolution of the spectrometer used, they will produce prominent peaks of the rotational lines for the various isotopic species present.

## Experimental

Synthesis of HCl (DCl) from acetyl chloride and H<sub>2</sub>O (D<sub>2</sub>O) was carried out after capturing an acetyl chloride background using a vacuum system operating at 50 torr with a reaction flask attached to the Harrick temperature-controlled gas cell (see Figure 1). Acetyl chloride is introduced to the reaction vessel via septum injection and is allowed to undergo phase transition into its vapor state, filling both the reaction vessel and the Harrick gas cell. The gas cell can then be isolated from the system and placed into a commercial FTIR (2.0 cm<sup>-1</sup> resolution, 32 scan average) to obtain a background. The cell can then be reintroduced into the vacuum system, and the synthesis of HCl (DCl) is initiated. H<sub>2</sub>O (D<sub>2</sub>O) is injected into the reaction vessel and allowed to react with the acetyl chloride producing HCl vapor. The HCl/acetyl chloride vapor will fill the gas cell and can then be isolated and removed from the system for analysis using the FTIR spectrometer.

## Results and Discussion

The IR spectra generated from the reaction of acetyl chloride and D<sub>2</sub>O using the Harrick temperature-controlled gas cell shown in Figure 2 can be identified as the vibrational spectrum for the transition between the ground state and the first excited state showing all of the rotational peaks in the two allowed branches (P and R).

## Conclusion

A crucial advantage of using the Harrick temperature-controlled gas cell is the clean contained environment that it provides. After each synthesis, the Harrick cell can be connected to the vacuum system and pumped out while heating the cell using an 80 °C liquid circulator to remove all traces of acetyl chloride from the cell. Failure to do this leads to significant

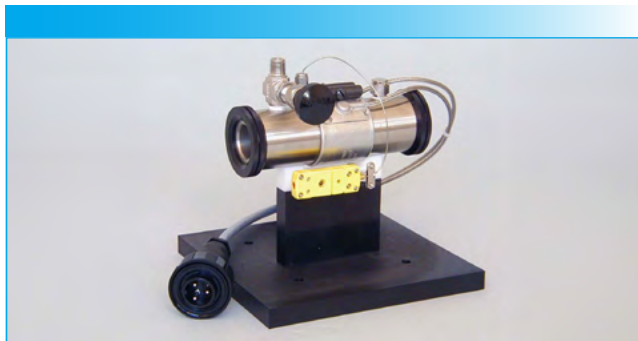


Figure 1: Temperature Controlled Gas Cell

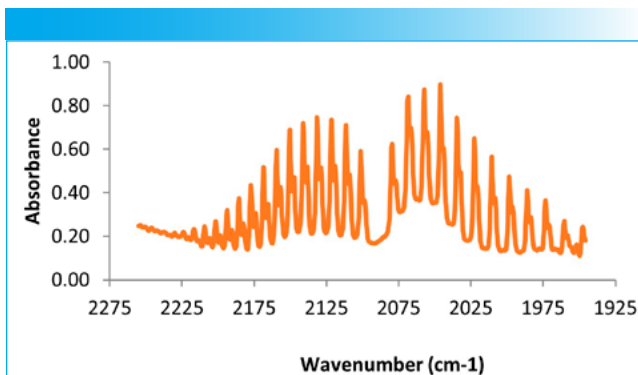


Figure 2: Transmission Spectrum of DCL.

degradation of a prior cell over the course of a few weeks.

When using reactants that produce non-ecofriendly product, exposure limits can be elevated. The gas cell provides a contained environment for the reaction to take place and also allows transport to the IR instrument for analysis limiting exposure to the technician or student. The Harrick gas cell provides an alternative for chemical work up for IR analysis and final product identification.

## Reference

- (1) Atkinson, D. Physical Chemistry Lab: RoVibrational Spectra of HCl and DCl. (adapted from "Vibrational-Rotational Spectra of HCl and DCl" in *Experiments in Physical Chemistry* by Garland, C. W.; Nibler, J. W.; D. P.) revised 1/17/2020.

**Harrick Scientific Products, Inc.**

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# Fringe-free Mid-IR Transmission Measurements of Films

Ellen Miseo, Miseo Consulting; Jenni Briggs, PIKE Technologies

In transmission, a polymer film was measured at Brewster's angle to eliminate interference fringing in the IR spectrum. This sampling technique is ideal for making quantitative measurements of low concentration components, such as additives.

How to present a sample to an FT-IR instrument is possibly the most confusing aspect of infrared analysis. Deciding what approach is the best for each problem requires understanding the experiment aims and the drawbacks of a technique.

Quantifying an additive in a polymer film is a good example. If you simply use ATR, you are only examining the sample surface. Also, due to the short pathlength of single reflection ATR, low concentration components often do not result in strong enough absorbance bands to be observed in the spectrum. Transmission measurements, on the other hand, offer a much longer pathlength. However, when the measurement is collected with the sample normal to the IR beam, a film having two parallel surfaces will result in a fringing pattern overlaid on the spectrum of the sample. This fringing will interfere with the peak height or area measurement needed to quantify an additive (1).

This application note explores how to collect an IR transmission spectrum of a film, which is free of fringing.

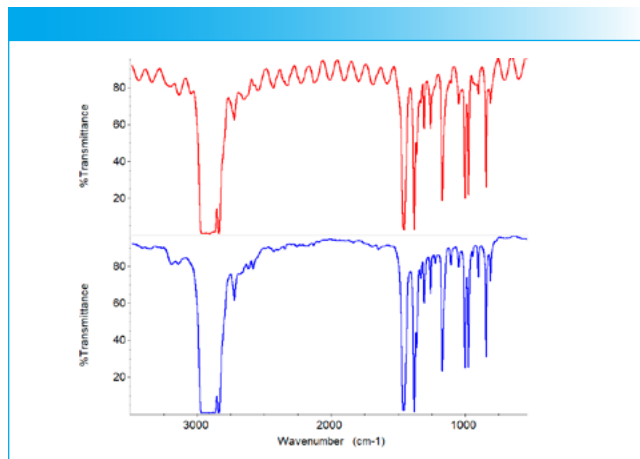
## Material and Methods

Mid-IR transmission spectra of a 30- $\mu$  polyethylene film was collected at angles from 0° (normal to the IR beam) to 60° using the automated RotatIR variable angle transmission accessory equipped with a ZnSe polarizer (PIKE Technologies). The polarizer was set to p-polarization. 16 scans were co-added at a resolution of 4  $\text{cm}^{-1}$ .

## Results

Figure 1 shows the transmission spectrum of a polyolefin polymer film exhibiting parallel surfaces and positioned normal to the IR beam (in red). Obvious fringing in the spectrum is present, which potentially masks bands of interest when the fringe magnitude is greater than the band magnitude or the fringe overlaps partially. Either of these occurrences interferes with quantitative analysis.

A simple solution to eliminate the fringes is to make the measurement at Brewster's angle (2). Brewster's angle is defined as the angle of incidence where light with p-polarization is perfectly transmitted through a transparent medium



**Figure 1: Polyethylene film spectra collected normal to the IR beam (red) and at Brewster's angle (blue).**

with no reflections. The film measured here exhibits no fringing when positioned at Brewster's angle, 52° (Figure 1; in blue).

Since Brewster's angle depends on the refractive index of the sample, it needs to be measured to find the angle where the fringes are eliminated. To measure the exact angle where the fringes disappear can be tedious. An automated sampling accessory, such as the RotatIR, is more efficient in determining Brewster's angle.

Although this example demonstrates the utility of the technique on a simple polyolefin, determining Brewster's angle has significant applications in optical material design and use.

## Conclusion

For films with two parallel surfaces, making transmission measurements at Brewster's angle offers a fringe-free IR spectrum.

## References

- (1) Konevskikh, T.; Ponomov, A.; Blümel, R.; Lukacs, R.; Kohler, A. Fringes in FTIR spectroscopy revisited: understanding and modelling fringes in infrared spectroscopy of thin films. *Analyst* **2015**, *140* (12), 3969-3980.
- (2) Clark, F. R. S.; Moffatt, D. J. The Elimination of Interference Fringes from Infrared Spectra. *Appl. Spectrosc.* **1978**, *32* (6), 547-549.

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## Raman Imaging for Large-Area Wafer Inspection

These measurements show how Raman imaging microscopy integrated with optical profilometry can investigate inhomogeneous doping, reveal areas of stress, and detect height variations over the surface of a 150 mm silicon carbide wafer.

### Introduction

Raman imaging is a powerful tool for research and quality control in the semiconductor industry as it can non-destructively acquire detailed, spatially-resolved information to determine the chemical composition of a sample, visualize component distribution, and characterize properties such as crystallinity, strain, stress or doping. This is particularly valuable for compound semiconductors, which often consist of multiple layers and complex structures.

The integration of an optical profilometer enables large-area measurements of entire wafers to be carried out while maintaining very high resolution, and advanced Raman microscopes can automate the process to deliver speed and consistency.

### Experimental Conditions

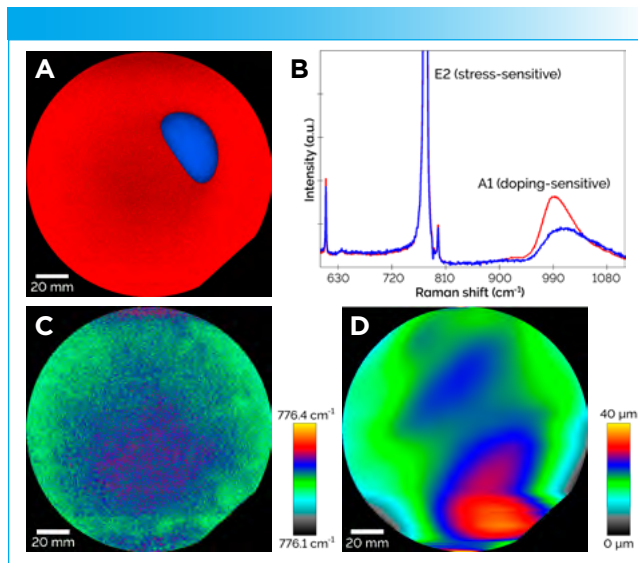
In this example, the complete surface of a 150 mm (6 inch) silicon carbide (SiC) wafer was imaged with Raman microscopy, using a 532 nm laser for excitation. The instrument employed was a WITec alpha300 Semiconductor Edition Raman system equipped with active vibration damping, WITec Suite data acquisition and post-processing software with TrueComponent Analysis™, the TrueSurface™ profilometry module for closed-loop focus stabilization, and a UHTS 600 ultrahigh-throughput spectrometer.

The sample was provided courtesy of the Fraunhofer Institute for Integrated Systems and Device Technology IISB, Erlangen, Germany.

### Results

The analysis showed that the doping concentration was not homogeneous over the full area. The highly sensitive spectrometer was able to detect peak shifts well below 0.01  $\text{cm}^{-1}$  and could thus reveal stress fields within the wafer.

TrueSurface recorded the wafer's topography simultaneously with the Raman data to compensate for height variations and obtain a sharp large-area image, which also facilitated the generation of a topographic map of the sample.



(Sample courtesy of the Fraunhofer Institute for Integrated Systems and Device Technology IISB, Erlangen, Germany.)

**Figure 1:** (A) Confocal Raman image of a 150 mm SiC wafer. TrueComponent Analysis identified two spectra, which mainly differed in the doping-sensitive A1 peak (ca. 990  $\text{cm}^{-1}$ ). The image reveals an oval region (blue) with a different doping concentration than the bulk wafer area (red). (B) Raman spectra of the two components identified. (C) Confocal Raman image of a 150 mm SiC wafer, color coded for the position of the stress-sensitive E2 peak (776  $\text{cm}^{-1}$ ). The image reveals a small, presumably stress-induced peak shift from the wafer's center toward its edge. (D) Topography of a 150 mm SiC wafer with height variations of up to 40  $\mu\text{m}$ .

### Conclusions

This study provides clear experimental evidence of the capabilities and advantages of Raman imaging microscopy combined with optical profilometry for the large-area analysis of compound semiconductor wafers. The distributions of doping concentration, material stress, and topographic variation were characterized and visualized across the complete surface of a 150 mm SiC wafer with exceptional resolution.

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### Diamond ATR accessory

The IRIS diamond attenuated total reflection (ATR) accessory from PIKE is designed for infrared (IR) sampling for powders, gels, liquids, solids, and more. According to the company, the accessory is suitable for research, QA/QC, and sample identification.

**PIKE Technologies**,  
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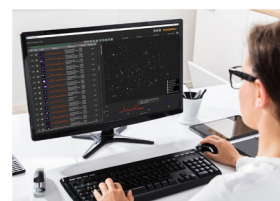


### Particle analysis module

Renishaw's particle analysis software module for its inVia confocal Raman microscope is designed to automate the microscope so that it can identify particles on images, and then chemically analyze them using Raman spectroscopy.

According to the company, the software provides chemical information on each particle and its morphology statistics.

**Renishaw**,  
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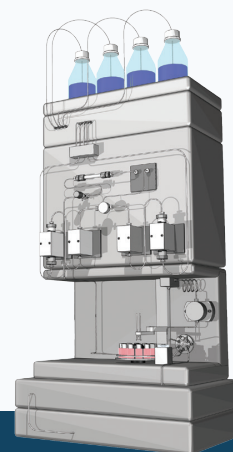
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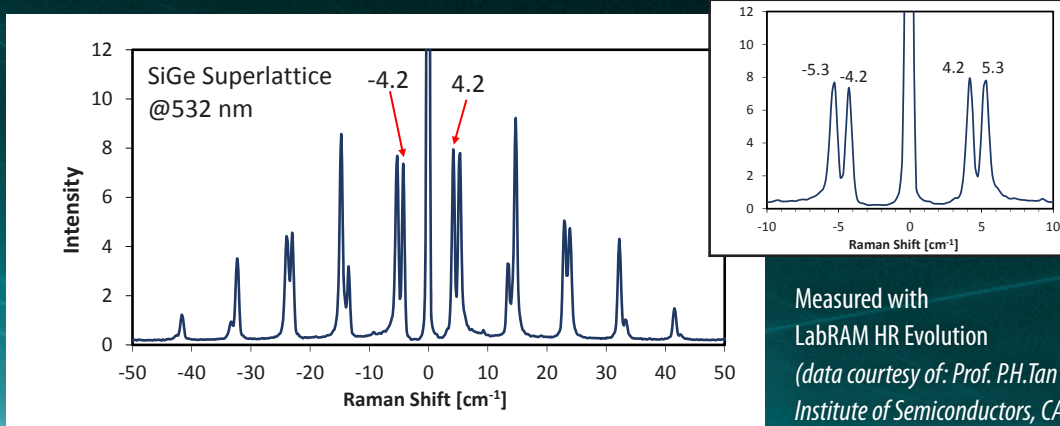
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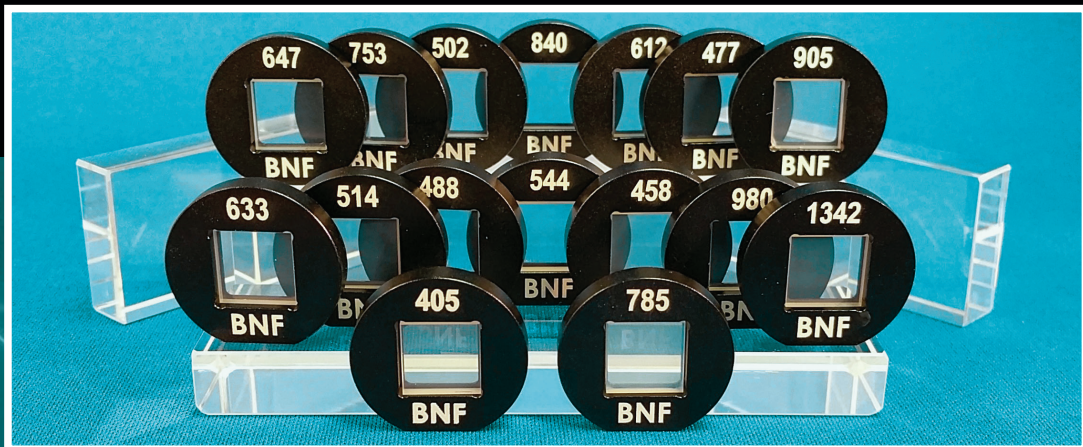
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