Improving the Accuracy of Near-infrared Measurements Using Spectral Corrections: Back-reflection and Transfer Backgrounds

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Mid-infrared spectroscopists use spectral corrections on a daily basis to increase the accuracy of already highly-tuned techniques for component prediction. These include corrections for atmospheric water, CO_2, and attenuated total reflection (ATR). In the case of the ATR correction, differential absorptions arising from ATR crystal effects are scaled across the frequency range to improve the accuracy of an already-accepted and robust technique. UV-Vis spectroscopists have been using data corrections during collection since the advent of a background compartment that corrects for effects from cuvettes. Advanced users of FT-NIR (Fourier transform near-infrared) spectroscopy however, cannot currently use spectral techniques to automatically correct for various undesirable sampling effects. In the NIR region corrections such as atmospheric water and CO_2 are not very useful due to the relative insensitivity of NIR to these species compared to mid-IR. One of the main detriments to NIR data is sample or container-related effects like back-reflection from the face of a glass vial during measurement of the sample inside. A spectral correction that removes this spectral feature from the true sample response can significantly improve the accuracy of any NIR measurement.

Figures 1a and b: Thermo Scientific Antaris™ II FT-NIR analyzer (left) and Thermo Scientific Antaris MX FT-NIR analyzer with 4 channel multiplexing for fiber optic process monitoring (right).

The ability of NIR light to penetrate sampling media such as Petri dishes or serum vials is one of NIR's most important benefits. NIR energy can effectively travel through containers like polymeric drum liners or glass vials to analyze the materials inside whereas techniques like mid-infrared cannot. This makes a strong case to try and improve already-robust NIR technology by using a so-called "Dark Correction" that can improve data accuracy and linearity while ignoring effects from non-informative species such as glass. Spectral corrections in Thermo Scientific RESULT™ software provide even the intermediate NIR spectroscopist with new and advanced tools to remove unwanted spectral effects from many types of interfering media. Meanwhile, RESULT's signature ease-of-use remains unaffected as these tools can be implemented with no change in the front-end execution of daily NIR analysis.

A second benefit of NIR technology is the ability to monitor samples remotely using low-OH fiber optics, an impossible task for many other spectroscopic techniques. Once a NIR analyzer is in place in a process, the ability to

take new backgrounds is hampered by the processing schedule. It is still essential, however, to take environmental effects into account on a regular basis to keep any NIR method performing well. The result is a situation where backgrounds need to be taken but it is simply impractical to take them on the probe that is in process. A transfer background correction solves this problem by using backgrounds from one beampath (transmission, for example) in the absorbance spectrum of a second beampath (fiber optic). This technical note details applications for and implementation of dark and transfer background corrections for NIR spectroscopy using RESULT software.

The implementation of a spectral correction in RESULT is simple because it fits seamlessly into RESULT's existing spectral collection architecture. Using the Collect event in RESULT, the user can now create a Spectral Correction specification to accomplish any of the tasks noted above (Figure 2). The spectral correction specification works by allowing the user to do
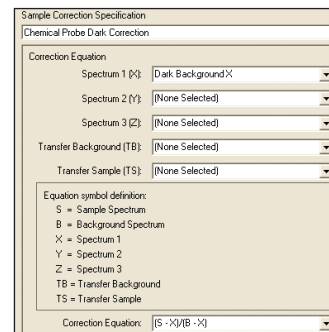

Figure 2: The implementation of spectral corrections in RESULT software is called a "Correction Specification" and is part of the normal data collection routine.
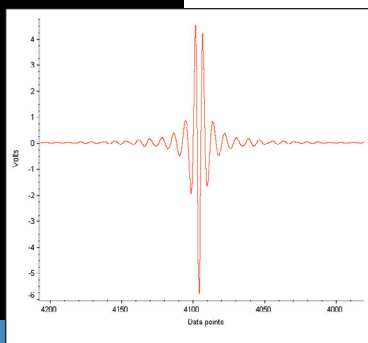
mathematical spectral manipulations (see Basics of Spectral Mathematics below) using any of a set of 6 different spectra or single beams labeled B, S, X, Y, TB, and TS. These letters are simply variables that act as stand-ins for spectra (single beam or ratioed) within the framework of the spectral math equation. So doing math on a spectrum is just like doing basic algebra. These 6 spectra can either be ratioed spectra (absorbance or %T) in certain cases or, more commonly, single beam spectra. X, Y, TB, and TS are unique because they are spectra saved from previous collects or from archived data. S and B are the current collection's sample (S) and background (B) single beams. Using some or all of these 6 spectra in a correction specification, RESULT software can calculate the desired functions using what can be referred to as spectral mathematics.

Basics of Spectral Mathematics

In order to discuss how RESULT software's spectral corrections can improve the accuracy of NIR methods, it is necessary to begin with a primer on spectral data types and basic spectral math.

The raw spectral information for any FT spectrometer is an interferogram (Figure 3). An interferogram is the result of passing a broadband light source through, in this case, a Michelson interferometer. Each point in an interferogram

contains information from all the frequencies of light – like playing all the keys on a piano at the same time. The interferogram is first passed through the sample beampath with no sample material in place which gives the background interferogram. This process removes any spectroscopic effects from the environment or the instrument. Next, the sample is placed in the beam in order to absorb the vibrational frequencies characteristic of the sample. Once the detector receives the interferograms (background and sample) both are deconvoluted using an algorithm called the fast Fourier transform. This results in single beam spectra (Figures 4a and 4b) that show the response of the instrument and sample as a function of frequency. Finally, these two single beam spectra, the background and the sample, are ratioed together mathematically to give the commonly known transmittance spectrum (Figure 5a). The -log of a %T spectrum yields an absorbance spectrum (Figure 5b).

In the above process we have carried out one of the most basic types of spectral math – where one set of data is divided by (ratioed to) another. Ratioing is the process of taking each data point from a sample single beam curve and dividing its value by that of the background single beam curve (I/I_o). This is possible because the measured spectra are not continuous functions, but are actually made up of discrete data points that are connected by a line. Although this line has no mathematical or scientific value, it serves to make spectroscopic data easier for us to see and use. For the two single beams under discussion, they can be represented in Microsoft® Excel® as a simple set of data with frequency in the first column and single beam energy in the second (Figure 6). If you divide the energy for the sample single beam by the energy for the background single beam for each frequency
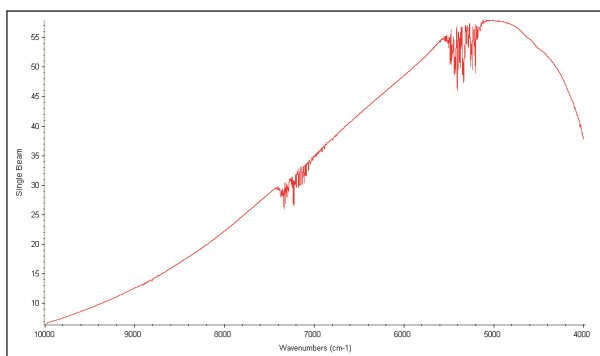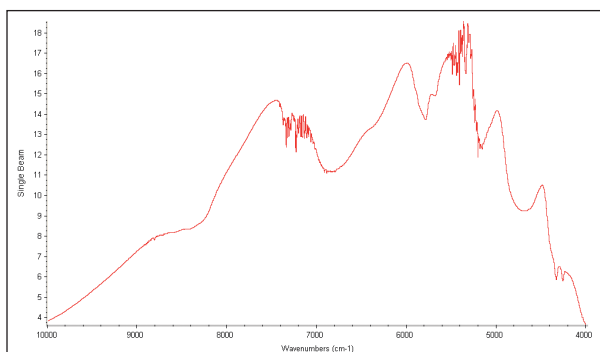

H11	▼	fx	=(E11/B11)*100				
A	B	C	D	E	F	G	H
BKD Single Beam			Sample Single Beam			% Transmission Spectrum	
Frequency	Energy		Frequency	Energy		Frequency	%Transmission
3972.64	0.00		3972.64	0.00		3972.64	0.000
3976.50	0.00		3976.50	0.00		3976.50	0.000
3980.36	0.00		3980.36	0.00		3980.36	0.000
3984.21	0.00		3984.21	0.00		3984.21	0.000
3988.07	0.00		3988.07	0.00		3988.07	0.000
3991.93	0.00		3991.93	0.00		3991.93	0.000
3995.78	0.00		3995.78	0.00		3995.78	0.000
3999.64	37.79		3999.64	3.62		3999.64	9.587
4003.50	37.89		4003.50	3.63		4003.50	9.571
4007.35	37.77		4007.35	3.61		4007.35	9.570
4011.21	38.09		4011.21	3.65		4011.21	9.575
4015.07	38.55		4015.07	3.70		4015.07	9.597
4018.93	38.59		4018.93	3.71		4018.93	9.622
4022.78	38.82		4022.78	3.75		4022.78	9.652
4026.64	39.01		4026.64	3.78		4026.64	9.689
4030.50	39.49		4030.50	3.85		4030.50	9.740
4034.35	39.75		4034.35	3.89		4034.35	9.795
4038.21	40.04		4038.21	3.94		4038.21	9.851
4042.07	39.80		4042.07	3.95		4042.07	9.919
4045.92	39.98		4045.92	3.99		4045.92	9.992
4049.78	40.54		4049.78	4.08		4049.78	10.069
4053.64	40.83		4053.64	4.15		4053.64	10.157
4057.49	40.91		4057.49	4.19		4057.49	10.250
4061.35	41.09		4061.35	4.25		4061.35	10.351
4065.21	41.38		4065.21	4.33		4065.21	10.459
4069.07	41.60		4069.07	4.40		4069.07	10.578
4072.92	41.70		4072.92	4.47		4072.92	10.709
4076.78	41.77		4076.78	4.53		4076.78	10.847
4080.64	41.88		4080.64	4.60		4080.64	10.987
▶	▶I	BKD plus Sample SB					

Figure 6: Spectral data is simply a collection of x,y data points so a spreadsheet view of spectral math simplifies manipulations like ratioing (dividing) a single beam background by a single beam sample and multiplying by 100 to get a %T spectrum

and multiply by 100, what is left is the data that makes up a traditional %T spectrum. This data is shown at the right in Figure 6. If we take the negative log of this data, we have an absorbance spectrum.

Division is not the only type of math that can be performed on a single beam spectrum, ratioed spectrum or set of spectra. These data types can be squared, cubed, derivatized, added, and subtracted, to name a few examples. Almost any mathematical process that can be done in the realm of real numbers can be carried out on a spectrum or

Figure 4a: A single beam background that is the result of a Fourier transform of an interferogram

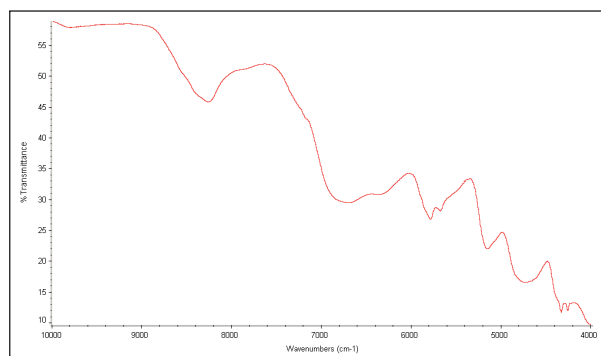Figure 5a: A % Transmission spectrum that is the result of dividing a sample spectrum by a background spectrum and multiplying by 100

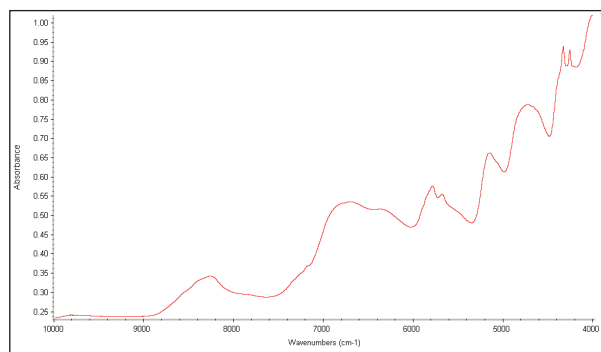Figure 4b: A sample single spectrum that has not yet been ratioed to a background spectrum

Figure 5b: An absorbance spectrum is the result of taking the -log of the %T spectrum in Figure 5a

spectra. In the case of NIR spectroscopy, this can help out with, as mentioned above, back-reflections from windows and ratioing different spectra against one sample single beam spectrum.

The Dark Background Corrections

When performing diffuse reflection or transmission experiments in NIR spectroscopy, many times there is a physical barrier between the instrument and the sample. This may be a vial or a Petri dish. This is useful for any type of close-contact diffuse scattering measurement as it prevents any sample from contaminating the optical path. The vial itself, however, presents a new spectroscopic challenge in measuring any sample – back-reflection. When the source beam impinges on a vial, the front face of that surface transmits most of the beam. But a small fraction of this beam reflects off the front or rear face of the vial or cuvette without hitting the sample and travels back in the direction from which it came in a process known as specular reflection (see Figure 7). This specular reflection does not provide any information about the sample. It does, however, contribute to the baseline of the spectral data that is acquired with the spectrum. Using a chemometric treatment such as second derivative smoothing to analyze near-infrared data, is an effective way to compensate for back-reflection in NIR spectroscopy. The incorporation of Spectral Corrections in RESULT software allows an even greater degree of control over variability from factors such as dirty or inhomogeneous glassware and cuvettes, resulting in methods that are more accurate and robust.
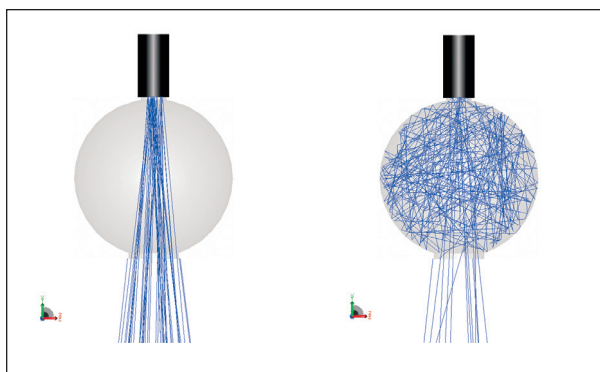

Figure 7: A graphical representation of the two types of light reflections that occur when a glass vial (dark cylinder) is placed on a collection optic such as an integrating sphere

The first dark background correction in RESULT is the simplest, **(S-X)/B**, where S is the current sample single beam spectrum, B is the current background single beam spectrum, and X is a single beam spectrum collected on the same beampath with a blank vial or cuvette in the way. In this case, X can be referred to as the "Dark" background because the light reaching the detector is not coming from a highly reflective background material like diffuse gold or Spectralon®, but from a very poorly reflecting material such as an empty glass vial. Relating this back to the concept of spectral math, the dark single beam spectrum is subtracted from the sample single beam spectrum, and this difference is then divided by the background single beam spectrum, yielding a dark-corrected, ratioed spectrum.

An example of this function, using the Thermo Scientific Antaris II FT-NIR analyzer, uses the integrating sphere diffuse reflection beampath to analyze substances in vials, with the gold flag as an automatic internal background material. One common application is the analysis of

lyophilized materials in serum vials. Serum vials typically are made from thick glass that is heterogeneous. Incorporating a spectral correction in a workflow in RESULT would allow the user to factor the scattering effects of the serum vial out of their spectral measurements for those cases where a higher degree of accuracy is desired.

To set this correction up in RESULT, we create a workflow that first collects a single beam spectrum on the integrating sphere with only a blank serum vial on the sphere. This requires using an empty attenuation screen and a high gain setting of either 4 or 8. Stray light from the room will not affect this measurement because Fourier transform spectrometers are not affected by exogenous light like a dispersive instrument would be. This dark background will be the single beam spectrum we use for spectrum "X" in our correction. Figure 7 illustrates the path of NIR light from source to sample (left) and after both diffuse reflection and back reflection (right). Diffusely reflected light carries the information about your sample and is very valuable, while the back-reflections can adversely effect baselines and linearity.

The next step is to set up a Collect event in RESULT that collects a normal absorbance spectrum using the integrating sphere. The sample single beam in this case will be "S" in the correction specification, and the automatic internal background taken off the gold flag will be "B." We can then designate the single beam spectrum from the "Dark" background sphere collection as spectrum X, and RESULT automatically designates S and B. The product will be an absorbance spectrum that has been corrected for spectral reflections from a serum vial when using the integrating sphere with a gold background. Figure 8 shows both the corrected and uncorrected spectra with transmittance (%T divided by 100) as the y-axis scale. Since the corrected spectrum has had the dark single beam subtracted from it before ratioing, it has lower transmittance than the uncorrected spectrum.
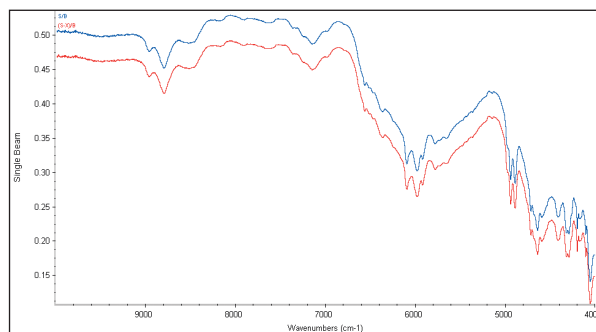

Figure 8: The result of a spectral correction. The uncorrected spectrum (S/B) is at top while the dark corrected spectrum **(S-X)/B** is below. X is the dark background taken through a blank serum vial to correct for glass back-reflections.

Sampling effects like different fiber optics or heterogeneous containers manifest themselves not only in the sample spectrum, but also in the background spectrum, effecting method performance. A natural extension of the **(S-X)/B** specification that will correct for back-reflections in this case, is one that takes the form **(S-X)/(B-X)** where the dark background, X, is subtracted from both the sample and background spectra. An application where this type of correction would be especially powerful is one where the baseline information was vital to the measurement itself. The textbook example of such an application is the use of NIR to predict particle size of fine powders like pharmaceutical excipients.

The procedure in this case involves correcting for back-reflection off a Petri dish that contains microcrystalline cellulose. Microcrystalline cellulose comes in several particle sizes to provide different properties in reaction and tabletting processes. Only the proper particle size can go into a specific process, so these are critical measurements. In order to improve the accuracy of the measurement, a dark background spectrum is taken through the Petri dish with no sample. This will become the "X" spectrum in the correction specification of **(S-X)/(B-X)**. The background is taken with the blank Petri dish in place, but it is now backed with a highly reflective material such as Spectralon. The sample spectrum is now taken with the microcrystalline cellulose in the Petri dish. To the operator running this analysis, the procedure will not change, but the measurements will be more robust and accurate due to the dark correction running behind the scenes.

The last dark correction in RESULT software is one in which dark noise from one accessory or beampath is subtracted from the sample single beam and dark noise from a different accessory or beampath is subtracted from the background. The form for this correction is **(S-X)/(B-Y)**. This correction improves robustness when using a dedicated external background channel (twin channel) for a fiber optic probe that remains in process and cannot be removed. This would be a very useful application for the above correction because the dark background would be removed from both beampaths so a background can be taken on a "mimic" of the real system without the added noise from differential back-reflections.

The Transfer Background Corrections – (S/X) and (S/B)/(X/Y)

The second family of spectral corrections in RESULT is called the Transfer Background Corrections. This group of spectral corrections allows the user to either ratio sample single beams to alternate backgrounds or to create a true background transfer function, freeing users from taking backgrounds on any specific beampath. If you have an instrument with multiple beampaths, a transfer correction provides the ability to use any beampath on the analyzer as an internal background channel. This functionality can, for example, loosen the requirement of collecting backgrounds on a fiber optic probe that is already dedicated to a process, allowing a user to collect a background on an Antaris transmission beampath and ratio it against a sample spectrum from a fiber. Another example of the value of a transfer function is an application where a fiber optic probe is dedicated as a background channel against a channel with a different probe. RESULT automatically creates a transfer function from archived spectra that will allow greater accuracy in using non-traditional backgrounds with multiple fiber channels.

The first transfer correction in RESULT is by far the simplest, **(S/X)**. This allows any sample single beam to be ratioed against (divided by) any background spectrum that has been collected previously. This could mean that a background that was taken in the same workflow or was stored in an accessible network location can be used as the background for a current collection event.

(S/X) is a useful correction because it allows the user to ratio a background collected on any Antaris beampath to a sample spectrum. This can be critical because when the analysis is taken to the sample, as in a remote fiber-optic measurement, there is no ability to stop and take a background without disrupting the process. In this case, using **(S/X)**, a RESULT user on an Antaris MX FT-NIR analyzer can collect a background on another channel without disrupting the more crucial process that is being monitored. The **(S/X)** correction is also extremely useful for applications such as in-line chemical batch reaction monitoring.

Spectroscopists can also create a powerful background transfer function, **(TS/TB)**, that addresses the difference between the two very different beampaths, and allows the user to take useful backgrounds that incorporate real-time environmental changes instead of simply loading a saved spectrum that does not reflect the current environment of the instrument. The specification for this correction in RESULT is **(S/B)/(TS/TB)**. This is among the most powerful corrections in the RESULT correction toolbox. RESULT automatically makes a correction factor that normalizes the differences between different background beampaths. An alternate form for this equation that is more demonstrative of its function is **S/[B*(TS/TB)]** which shows the effect the **(TS/TB)** transfer function has on the background. In collecting a ratioed spectrum using a dedicated fiber optic probe, for example, the user can now collect a background using a completely different beampath (either a different channel on the Antaris MX or a different beampath on the Antaris II) with the addition of a special normalization function.

This is where this correction differs from the simple **S/X** correction. The normalization function is composed of a ratio of two single beam spectra. The first, TS, was collected on the alternate beampath with no sample in the way while the second, TB, was collected on the sample's beampath with no sample in the beam. Provided these two spectra were collected at roughly the same time, RESULT can create an extremely effective transfer function that will make for a more robust method. The user can create a powerful transfer function that addresses the difference between the two very different beampaths and allows the user to take useful backgrounds that incorporate real-time environmental changes instead of simply loading a saved spectrum that does not reflect the current environment of the instrument.

Thermo Fisher Scientific has become the first near-infrared instrument vendor to offer automatic spectral corrections to factor out sample effects like those from serum vials or Petri dishes. In addition, one can use backgrounds not only from different beampaths on the same instrument, but *different* beampaths from different instruments and ratio them against a specific sample. These innovative tools allow RESULT users the latitude to improve the accuracy of already-robust methods.

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