A GUIDE TO NEAR INFRARED TECHNOLOGY

ABVista

Extraordinary science brought to life

CONTENTS

INTRODUCTION

Across the feed industry, companies recognize that a more targeted nutritional approach can help reduce production costs, and this is set to continue given the changes taking place within the global marketplace. Thus, feedstuff procurement and quality control are a major focus for feed manufacturers. These essential processes require a lot of resource management and constant monitoring and assessment, which all come with their associated costs.

Near-Infrared Spectroscopy (NIR) is a cost- and time-effective tool for evaluating the quality of feed ingredients and finished feed. Currently used for basic raw material and feed quality control, new advances in NIR software and hardware are set to deliver commercially viable systems capable of in-line and real-time monitoring of feedstuff and feed nutrient content and physical characteristics. This includes advances in calibrations which has extended the application of NIR analysis beyond traditional proximate analysis to the measurement of parameters such as the phytic-P levels in the diet.

At AB Vista we can work with you to evaluate your feed and feed ingredients using NIR technology. The enclosed chapters provide information on key areas relating to NIR.

DIFFERENCES BETWEEN NIR MACHINES

There are a number of different NIR instruments on the market; in general they can be split into 5 main groups according to how they generate spectra.

FT-NIR (Bruker, Thermo, Buchi)

A Fourier Transform (FT)-NIR instrument uses a system called an interferometer to collect a spectrum. The interferometer consists of a source, beam splitter, two mirrors, a laser and a detector. The energy goes from the source to the beam splitter, which splits the beam into two parts. One part is transmitted to a moving mirror; one part is reflected to a fixed mirror. A very precise laser wavelength in the system is used to control the moving mirror and also acts as an internal wavelength calibration. The two beams are reflected from the mirrors and recombined at the beam splitter. The beam from the moving mirror has travelled a different distance than the beam from the fixed mirror. When the beams are combined, some of the wavelengths recombine constructively and some destructively, which creates an interference pattern. This interference pattern is called an interferogram. This interferogram then goes from the beam splitter to the sample, where some energy is absorbed and some is transmitted. The transmitted portion reaches the detector. The detector reads information about every wavelength in the infrared range simultaneously. An algorithm called a Fourier transform is performed on the interferogram to convert it into a spectrum. One major benefit of FT-NIR is that the wavelength axis is very precise due to the internal laser. This means that transfer of calibrations between instruments is very good.

Dispersive Infrared Instruments (Foss, Unity)

Dispersive infrared instruments are sometimes called grating or scanning spectrometers. A dispersive infrared instrument also has a source and mirrors. The source energy is sent through both a sample and a reference path, through a chopper to moderate the energy reaching the detector, and directed to a diffraction grating. This grating is similar to a prism. It separates the wavelengths of light in the spectral range and directs each wavelength individually through a slit to the detector. Each wavelength is measured one at a time, with the slit monitoring the spectral bandwidth and the grating moving to select the wavelength being measured. This is then used to construct the spectrum. Dispersive instruments have been around a long time and therefore are widely used.

Diode array (Perten, Zeiss, Foss)

In a diode array spectrophotometer, a light source illuminates the sample with white light. Some of the light is absorbed (depending on the composition of the sample) and the rest is reflected. The light which is reflected hits a stationary grating, which separates the light by wavelength, converting the white light into a spectrum. Each wavelength is measured by a dedicated diode detector. With diode array technology all wavelengths are measured simultaneously, as each wavelength has a dedicated detector. One benefit of diode array technology is therefore the speed of measurement.

MEMS

The acronym MEMS (Micro-Electro-Mechanical Systems) is a rather generic catch-all term for any mechanical devices which is manufactured using silicon wafer technology derived from the semiconductor industry. The benefit of a MEMS system is that the size of the instrument can be greatly reduced, leading to the introduction of handheld NIR machines.

The portability of these devices provides great flexibility in which testing can take place and allows users to sample and test at many points in the process. MEMS systems are also extremely fast in collecting spectra, allowing many sub-samples to be scanned and averaged in the time it would take for a lab-based system to undertake a single scan. The downside is that they have smaller wavelength coverage and lower resolution, leading to a slightly higher error compared to the costlier lab-based instruments.

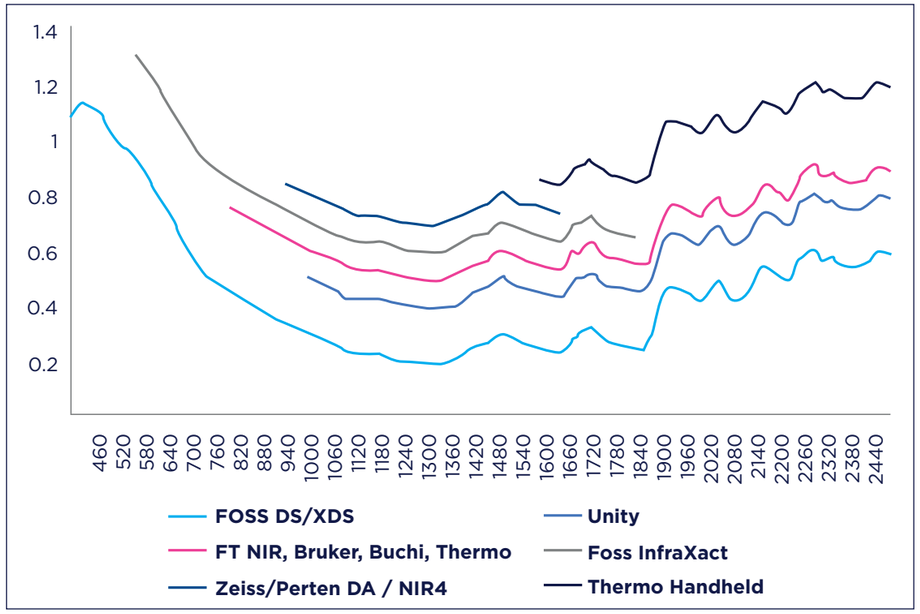
LVF (NIR4)

A linear variable filter (LVF) is a bandpass filter that has been intentionally wedged in one direction, providing a position-dependent dispersive optical element. LVF are ideally suited for use in compact instruments requiring high spectral resolution. The benefit of an LVF system is that the size of the instrument can be greatly reduced, providing flexibility and portability and allowing users to sample and test at many points in a production process.

Spectral range

The more critical issue is not how NIR machines generate a spectrum, but rather the range of wavelengths generated.

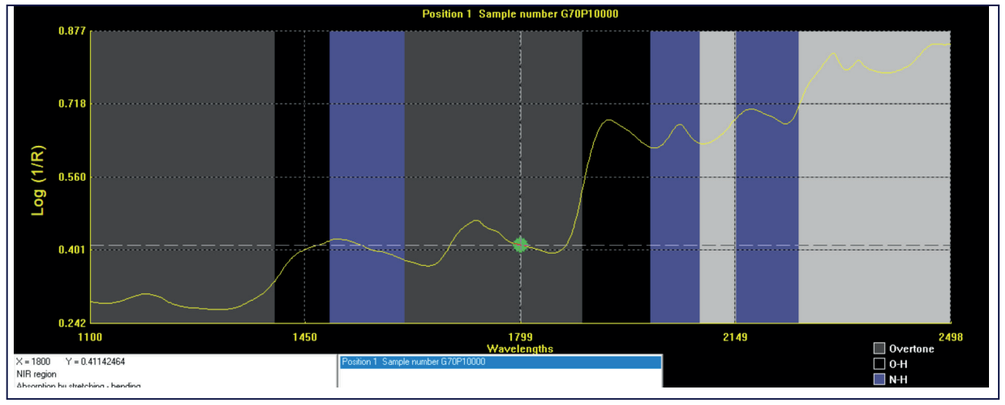
Figure 1: Wavelengths generated by different types of NIR machines.



Some instruments go into the visible region (400-1000nm), but this is not important unless you want to measure pigments.

Generally the NIR region is considered to be 1100nm-2500nm. The spectra in this region are made up of overtones and combination bands, which result in a replicated spectrum. Some instruments only measure in the overtone region, whilst some provide information over the whole spectrum. Our findings show that instruments that cover the full wavelength range provide more accurate and reliable results.

Figure 1: Example spectrum.



Sample Presentation

The majority of NIR instruments on the market have a variety of accessories for presenting different types of samples. These vary considerably between instruments, having worked with all the instrument over a number of years, there is no clear winner. However, the newer instruments Bruker, Buchi, Foss XDS, Unity Top Loader, present the sample horizontally rather than the older Foss instruments that work with a vertical transport. The horizontal transport system has been found to be more user friendly. Most NIR instruments have really been designed for solids; however, the Bruker MPA (Multi-Purpose Analyser) can be configured to do both solids and liquids on the same instrument.

Cost of Ownership

For all instruments, the ongoing costs will be for the source lamp (generally these last for 1 year; all are easy to self-fit and will cost between $100- $300). NIR cups are seen as consumables and use a high quality quartz glass; breakages are rare but can be costly. Most manufacturers would recommend an annual service, cost of which will vary depending on the supplier. The dispersive instruments have some moving parts that may need replacing over the life of the instruments; FT-NIR have an internal laser that is seen as a consumable. The general life of an NIR instrument is high, and you should be able to rely on one for at least 10 years.

Software

Again all instruments come with their own proprietary software. This is usually split into two parts, the operating system & user interface and the calibration software. Prices vary between vendors, but substantial saving can be made by purchasing ready made calibrations rather than buying the software to produce them internally.

Conclusion

The best combination of accuracy, reliability and future proofing is provided by lab-based full range scanning NIR instruments. However, they can be more difficult to use and tend to have more complex software. The portable systems often provide a much easier user interface and have the benefit of being able to be taken around the production process. Cost is obviously an important driver, as is support from the manufacturer. Once an NIR is installed and in use, it will become a vital part of the quality control system in place. It is essential that the supplier chosen is able to react quickly should the NIR breakdown or require updating. We would expect a supplier to be able to react and resolve a problem within 24-48 hrs. This can vary between suppliers and countries, so it is important that these issues are resolved or understood prior to purchase.

SPECTRAL RANGE

Due to the repetitive nature of NIR spectra, there is always a degree of redundancy due to N-H, O-H and C-H bonds absorbing at different wavelengths all at the same time. The consequence of this redundancy is that in many cases it becomes possible to extract similar information from different parts of the spectra.

This is one of the reasons behind the diversity of NIR machines in the market. There are many optical and digital differences between these instruments, but one of the most noticeable differences is that they operate at different wavelength ranges.

NIR covers the wavelength spectrum from 750 to 2500 nm, but some instrument types have a limited bandwidth and don't cover this range fully (Figure 1). Sometimes the range of the machine extends beyond the NIR range. For example, some instruments have a second detector that extend the spectral range into the visible domain, below 700 nm, which can help in measuring some quality parameters such as colour.

This doesn't mean that all of these instruments have equal performance. There are many other factors involved in performance of an NIR instrument, some of which have nothing to do with the instrument itself! For example, the quality of the NIR calibration model can dramatically influence the performance. However, access to a full scanning range NIR spectrometer can help in achieving a better prediction accuracy and precision.

Figure 1: Some of the main types of NIR instruments in the market and their associated spectral range.

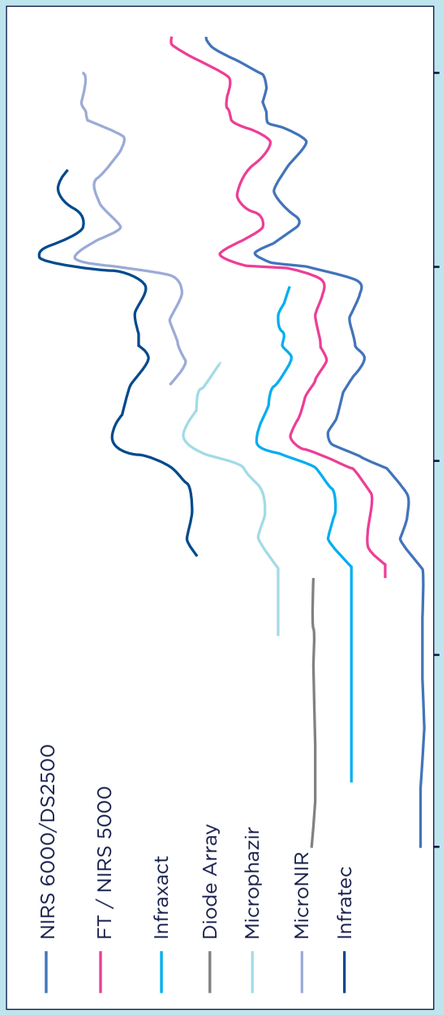


Figure 1: Specification of different types of NIR machines.

Manufacturer	Instrument	Type	Wavelength Range	Whole seeds	Ground / Powders	Wet Samples	Liquids
Foss	XDS	Dispersive	400nm-2500nm	Natural Product cell	Quarter / ring cup	Natural Product cell	Transflectance
	NIRS 6500	Dispersive	400nm-2500nm	Natural Product cell	Quarter / ring cup	Natural Product cell	Transflectance
	DS2500	Dispersive	400nm-2500nm	Large rotating cup	Small Rotating cup	Large rotating cup	Transflectance
	Infraxact	Dispersive	570nm-1850nm	Large rotating cup	Small Rotating cup	Large rotating cup	Not Available
Bruker	Matrix I	Fourier Transform	780nm-2500nm	Large rotating cup	Small Rotating cup	Large rotating cup	Transflectance
	MPA	Fourier Transform	780nm-2500nm	Large rotating cup	Small Rotating cup	Large rotating cup	Vials / Transmission
	Tango	Fourier Transform	780nm-2500nm	Large rotating cup	Small Rotating cup	Large rotating cup	Transflectance
Buchi	N500	Fourier Transform	1000nm 2500nm	Deep Rotating cup	Petri Dish	Deep Rotating cup	Transflectance
	NIR Master	Fourier Transform	1000nm-2500nm	Deep Rotating cup	Petri Dish	Deep Rotating cup	Transflectance
Unity	SpectraStar	Dispersive	1200nm-2400nm	Large rotating cup	Small Rotating cup	Large rotating cup	Not Available
	SpectraStar XL	Dispersive	600nm-2500nm	Large rotating cup	Small Rotating cup	Large rotating cup	Vials / Transmission
Perten/Zeiss /Foss	DA7200 / Corona / DA1650	Diode Array	950nm-1650nm	Large rotating cup	Small Rotating cup	Large rotating cup	Not Available
Thermo	Antaris	Fourier Transform	833nm-2500nm	Large Cup	Small Cup	Large Cup	Transflectance
Various	Handheld	MEMS	Varous	Direct Contact	Direct contact	Direct contact	Transflectance
AB Vista	NIR4	LVF	950-1650nm	Direct Contact with applicator	Direct Contact with applicator	Direct Contact with applicator	Transflectance

UNDERSTANDING NIR SPECTRA

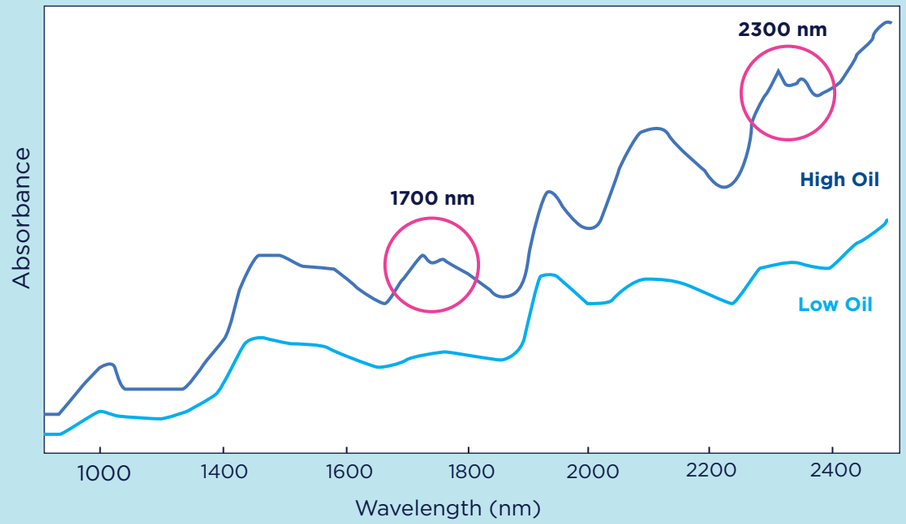
NIR spectra are notoriously difficult to interpret mainly due to broad, overlapping and non-specific nature of the absorption bands. Nevertheless, there is a wealth of information hidden in the peaks and troughs of these spectra, to the extent that they are called finger prints. A great deal can be learned, with some basic understanding of NIR spectral features. This skill is also useful in many situations, ranging from trouble shooting through to classification and outlier detection.

Section 1: Oil

Oil (or fat) has very characteristic absorption bands that are easily identified in NIR spectra, especially when oil concentration is high in a sample. Oil bands appear as distinct duplets at two regions in the NIR range: the region around 1700 nm and that around 2300 nm. The left branch of the duplet is normally more prominent. At lower concentrations of oil, the duplet at 1700 nm tends to get smoothed out, turning into a little lump.

An example below shows spectra form two agricultural samples one with high oil content (dark blue line) and the other one with very little oil (light blue line).

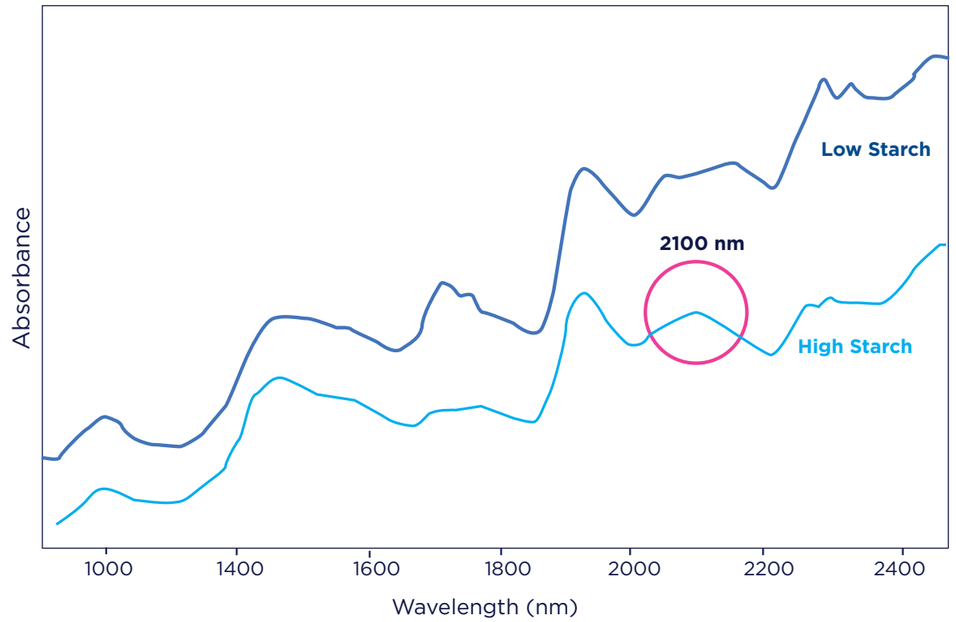
Figure 1: Oil band in NIR spectrum.



Section 2: Starch

In many cases, it is fairly straightforward to spot a high starch content in your sample if you look carefully at its NIR spectrum. Figure 2 shows spectra from two feed samples, with low (1%, dark blue line) or high (60%, light blue line) starch contents.

Figure 2: Starch band in NIR spectrum.



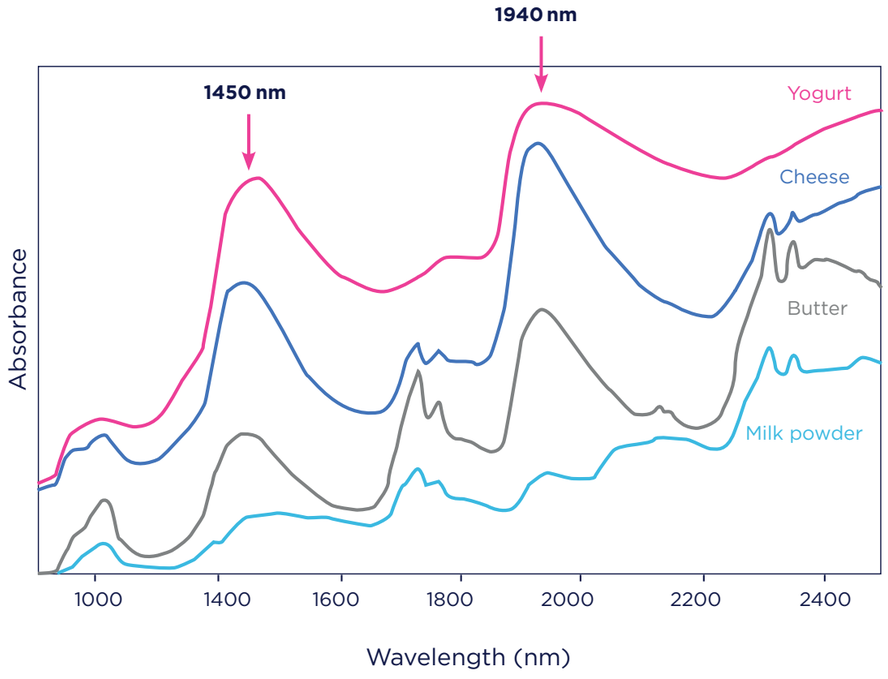
The smooth bump around 2100 nm is where the starch band is located. Notice the dip created by the lack of starch in the blue spectrum, exposing protein shoulders on either side.

Section 3: Moisture

Water is the easiest of all components to spot in the NIR spectra, unless your sample is very dry. Water molecules absorb the NIR light more than any other chemical species. This makes NIR a sensitive tool for the measurement of moisture. Water absorption bands are such broad and dominant features in the NIR spectra that sometimes they can swamp or mask the information from other components.

Two main absorption bands from liquid water are located around 1450nm and 1940nm. Figure 3 shows spectra of different dairy products with different moisture content (powder, 2%; butter, 15%; cheese, 50%; yoghurt, 80%). Notice the enlargement and broadening of moisture bands as the water content increases in the samples.

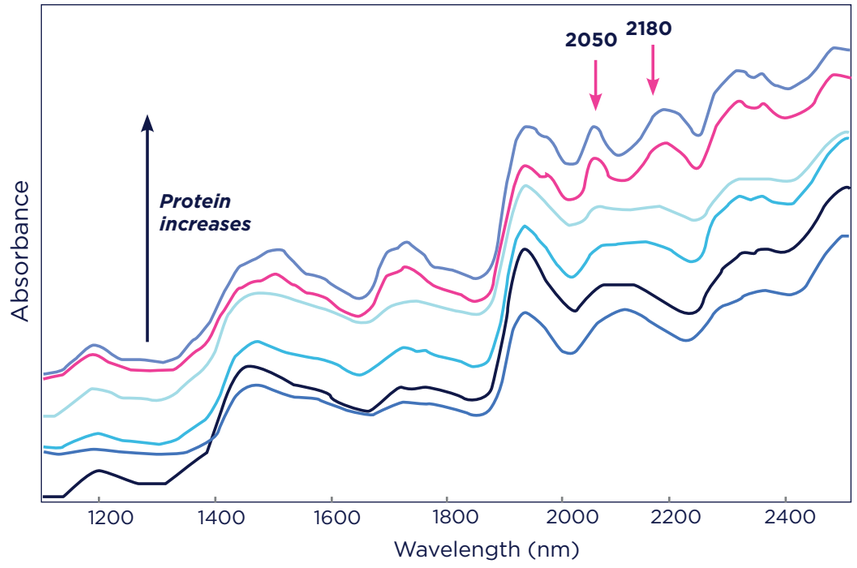
Figure 3: Moisture bands in NIR spectrum.



Section 4: Protein

Although difficult to spot at lower concentrations, protein manifests itself as two distinctive bands, at 2050 and 2180nm. These can be on either side of the starch band at 2100nm, resembling shoulders. The proximity of the protein and starch bands can often cause problems in the interpretation of starch content, with changes in protein content confused with changes in starch content.

Figure 4: Protein bands in NIR spectrum.



The above example shows a series of spectra with varying protein content, ranging from 10 to 60% and increasing from bottom to top. Starch content is consistent in all these spectra (~12%) although it appears to be changing. In this case, low starch content can be wrongly interpreted as high starch if protein content is also low, and high protein content can mask low starch content.

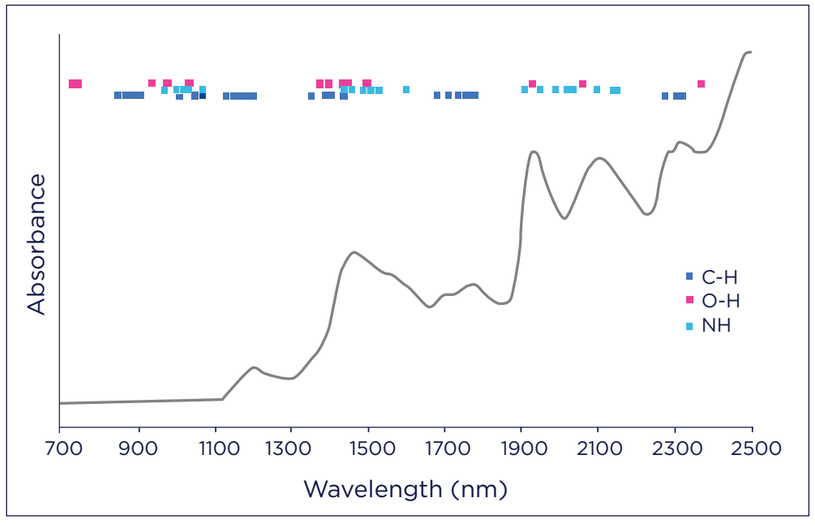
Thus, protein and starch need to be considered simultaneously in order to get an accurate prediction of both. This is one example why univariate (i.e. looking at one band only) analysis of NIR spectra can be misleading.

Section 5: Interpreting NIR spectra

It is important to highlight that, despite the characteristic peaks in NIR spectra, the interpretation is not as straightforward as it may seem due to the broad, overlapping and non-specific nature of their absorption bands. NIR bands are not related to any particular molecules (or chemical species). Rather they represent certain molecular bonds (mostly C-H, O-H and N-H). This means for example, any molecule with an N-H bond in its structure can potentially be confused with protein as it will overlap with NIR bands related to protein. Also, protein, oil and starch will all contribute to C-H bands at the same time.

This picture gets more complex when you notice that C-H, N-H and O-H bonds absorb in multiple locations in an NIR spectrum, in the form of overtones and combination bands. The spectrum is further complicated by the fact that C-H, N-H and O-H absorption bonds are pretty close to each other, as illustrated in Figure 5.

Figure 5: C-H, N-H and O-H can absorb in multiple overlapping locations in an NIR spectrum.



You only need to zoom in on any part of an NIR spectrum and you can't be totally sure what you are looking at as it can be any organic entity with N-H, O-H and C-H bonds in its structure. This is particularly true when dealing with feed samples containing lots of different organic components.

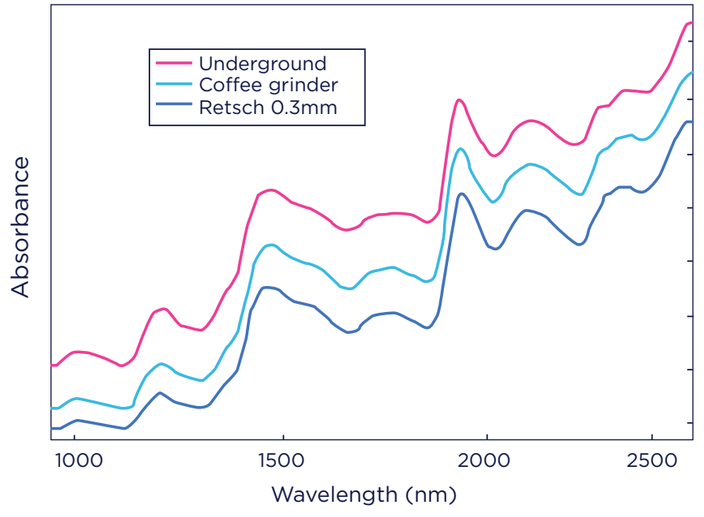
Section 6: Baseline shift

NIR spectra tell us about the chemical properties of the sample, and also reveal a great deal about the physical condition. One of the features of NIR spectra is that they often exhibit baseline shifts. This means that the spectra show an additive effect (i.e. is offset) along the absorption axis. This is usually indicative of variations due to particle size, packing density or porosity and even the presence of air bubbles.

This baseline shift is a very common feature. For example, if you scan sub-samples of the same sample multiple times you may see that the resulting spectra are not completely overlaying each other. This can happen when the particle size or porosity in the sample increases, allowing part of the NIR light that is shone on the sample to escape through the gaps between particles and hence to reach the instrument detector. This translates into a higher absorption baseline. This is called a 'pseudo-absorbance', to differentiate it from real chemical absorption by the sample.

In Figure 6 the whole (unground) corn spectra shows a relatively high baseline (pink line). If we grind the sample with a coffee grinder, less light will escape through the sample gaps and reach the detector, hence the baseline shifts downwards (light blue line). Grinding the sample to a smaller particle size with a Retzch laboratory mill will cause the baseline to shift downwards even further, as shown by the dark blue line.

Figure 6: Baseline variation is indicative of particle size or porosity in the corn.



Baseline shifts are one of the reasons why pre-processing samples prior to taking NIR spectra is important. Pre-processing methods such as normalisation can minimise the physical variability in the spectra so that chemical information becomes more pronounced.

REPEATABILITY AND STANDARDISATION

Applying a repeatability file to ensure robust predictions

A good database for NIR modelling must contain data obtained from all the various conditions that you would expect to encounter during routine analysis. This is not always possible, for a number of reasons including time restrictions, lack of space for sample storage or geographical limitations. Other sources of variation come from carrying out analyses on different NIR machines, and the differences in the temperature of both the sample and the instrument at the time the analysis is carried out. A way to reduce the impact of this lack of sample information is to use a repeatability file.

A repeatability ('rep') file contains spectra of one or more samples scanned using different instruments and in different conditions. The goal of including a rep file in a calibration is to develop an equation that gives the same predicted value across all conditions represented in the scans; the equation is developed so that it is not sensitive to the rep file's spectral variation. A rep file is a clever way of introducing variability into calibrations without actually having to add reference values for the new samples. This reduces the worry of not having enough applicable samples to influence a large calibration set.

How is a rep file used in the calibration process?

In normal regression, the predicted value for the average spectrum is the average reference value. With a rep file, we want the average spectrum plus and minus the differences in the rep file spectra (i.e. standard deviation in the rep file) to still predict close to the average reference value.

This is done by centering and scaling each rep file (i.e. a series of spectra associated to a single sample scanned under different conditions) separately and assigning 0 as their reference values. The next step is to add these to the centred calibration dataset, followed by the usual regression. This process ensures that the regression coefficients calculated will minimise the prediction errors often seen under different conditions. Calibrations developed using rep files are usually slightly less accurate but offer much more reliable results when there are differences between instruments and assay conditions.

The standardisation process

The reason for standardising an instrument is to make its spectra match those of a master instrument. This is a particularly useful tool when a calibration built with data from a grating instrument is transferred to other similar instruments, removing a variable (differences between instruments) and ensuring more robust predictions.

This transformation is very important for getting the best performance in the secondary instrument, but it has some limitations. The standardisation process is only good at the time it was carried out but not after any changes have been made to the instrument, for example lamp repairs. In this scenario, another standardisation process must be carried out. Standardisation is usually more important in NIR equipment using grating technology, as opposed to fourier transformation.

References:

Westerhaus, M.O. "Improving repeatability of NIR calibrations across instruments." *Proceedings of the Third International Conference on Near Infrared Spectroscopy,* 1990.

Westerhaus, M.O. "Eastern Analytical Symposium Award for outstanding achievements in Near Infrared Spectroscopy: my contributions to Near Infrared Spectroscopy." (2014). NIR News, Vol 25 No 8. pg 16-20.

SAMPLING TECHNIQUE

Analysis is a critical part of quality control and sampling technique is a key part of ensuring representative analysis.

When gathering your sample, there are some golden rules.

- Take as many samples as you can
- Ensure samples are representative
- Compare like with like

Quantity as well as quality

The more samples you take the better. If you want to really know a subject, you do lots of research and gather as much knowledge as possible. The same applies to analysis - if you want to be confident in your results it is advisable to take more than one sample. With traditional wet chemistry this can be time consuming and expensive; NIR analysis allows a much greater number of samples to be analysed in a shorter time frame at a much lower cost.

Ensure samples are representative

Effective sampling allows you to take a smaller amount of the finished product and extrapolate that result to apply to the entire population. If your sample isn't representative of the whole population, the result will not represent the bulk of your material. While it is tempting to leave out the 'bad bits', and only take a sample of the 'good' material, this will cause your final result to be unrepresentative. One advantage of NIR is that you can analyse many samples and use the larger amount of information to build up a more accurate picture of your material.

Compare like with like

Make sure all equipment used during collection is clean and appropriate for the sample to be samples. Always make sure that the sampling method is the same when comparing results obtained from NIR with those from wet chemistry. Figure 1 illustrates the best way of gathering silage samples from across the face of a silage clamp.

Figure 1: Diagram illustrating how to gather samples from across the face of the silage clamp to give a representative analysis of the nutritional content of all of the forage across the face.

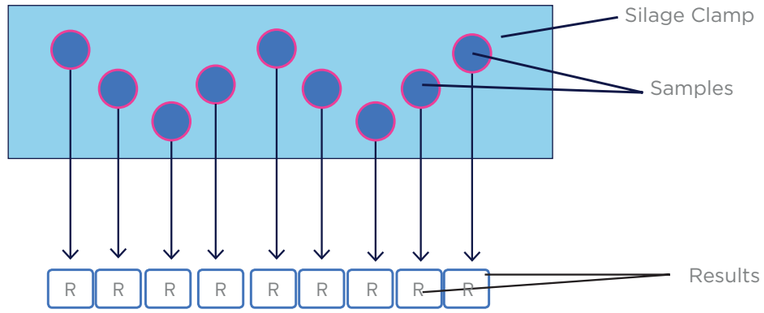
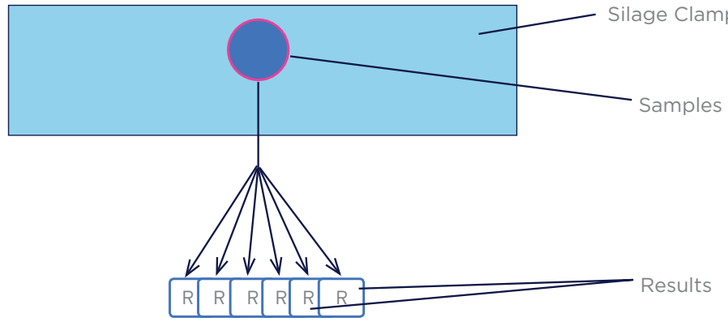


Figure 2: Diagram illustrating a non-representative sampling technique often used



As illustrated in figure 2, where only one area of the overall feed is sampled, the results produced cannot tell you about the quality of any other part of this feed. If this area of the feed, in this case the clamp, is being specifically analysed, then the sampling technique is fit for purpose. However, if this sampling technique is used to gain information about the silage in the whole clamp, it could be misleading. Splitting this one sample to provide more than one analysis result further compounds the problem and is not a true reflection of the forage held in the clamp. It would not be a fair comparison to look at the results from samples taken from methods shown in figure 1 and figure 2, as they have not been gathered in the same way. Any comparison would be misleading.

Furthermore, it is important to be consistent in your sampling technique if you want to compare data historically, and this is best served through following a standard operating procedure. If a consistent approach to sampling and analysis is used, trending and benchmarking can be carried out on the data to show the changes in nutritional value over time.

UNCERTAINTY OF NIR RESULTS

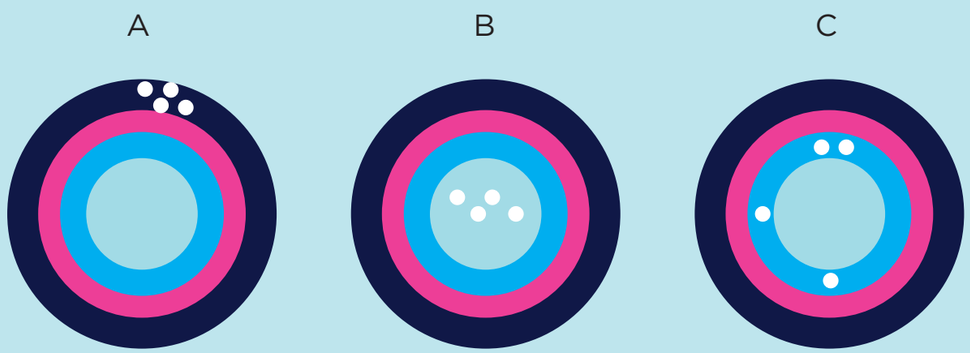
When an analytical test is performed on an ingredient, there is an inherent degree of variability that will have an effect on the results. For example, if the same sample is analyzed two or more times the likelihood is that the results will not be identical. This concept is true with most analytical tests; however, some tests have lower variability than others. Understanding the concept of variability is key to interpreting results and understanding analytical data.

There are two main causes of error in analytical results: systematic and random error. Systematic error determines the accuracy of the results produced and random error determines the precision.

The magnitude of the uncertainty depends mainly upon the method used (repeatability) and the operator, equipment and environment (reproducibility). Throughout this Technical Note there is reference to the ‘true’ value. This is the hypothetical correct result that could only be determined by averaging a number of repeat measurements.

Precision and Accuracy

Precision describes the typical view of error; in any set of results the ‘true’ value is somewhere in the middle. The uncertainty is variable from one determination to another, and can be due to factors such as inaccuracies in weighing and measuring. Accuracy is derived from systematic error in the method. In principle, systematic errors by their very nature (they remain unchanged when a measurement is made under the same conditions), can be corrected. Precision can be thought of as the ‘spread’ of the data and accuracy as the distance that the average value is away from the ‘true’ value. The diagram below shows a pictorial representation of the relationship between accuracy and precision.



A) Precise, not Accurate, B) Accurate and Precise, C) Accurate, not Precise

Repeatability and Reproducibility

If replicate analysis of a sample is performed by one operator on the same instrument under the same conditions, the variation between results is the repeatability. If a sample is split in two, sent to several different labs where it is analysed by the same method but different operator and equipment, the correlation between the results is the reproducibility. This is often referred to as ‘inter-lab error’. The error associated with reproducibility is always greater than that associated with repeatability.

Comparing Results

When comparing wet chemistry and NIR data, you should always consider the uncertainty that you expect between the individual results. This will depend on the method used and the sample tywpe analysed. If replicate results are within the expected inter-lab error, they are essentially the same result; it is only if the two results are outside this error that action, such as resampling or reanalysis, may be needed.

Example: Protein (%) of a wheat sample. The inter-lab error for this test is ±0.3%.

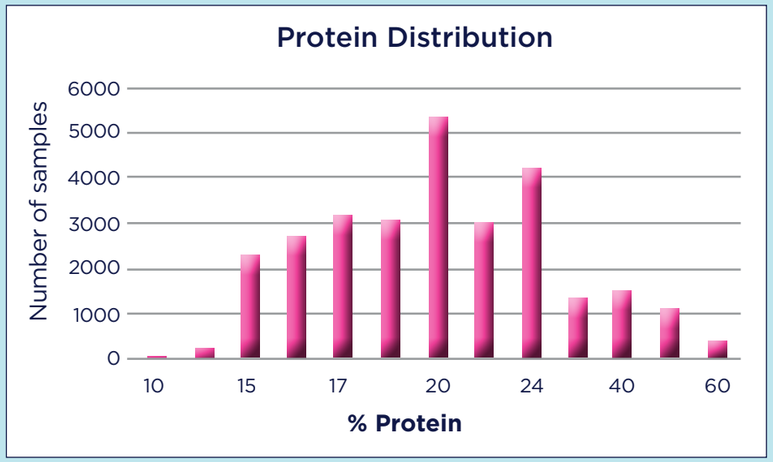
Reference	NIR	Comment
12.5	12.8	No statistical difference
12.5	12.9	Slight difference
12.5	13.0	Discernable difference

FINISHED FEED VARIABILITY

Least-cost feed formulation is widely practiced within the commercial feed industry for production animals. Least-cost formulation works on a matrix of nutritional specification, raw material availability and constraints, and raw material costs to provide a recipe that meets the animals' requirements at the lowest raw material cost. When the cost of one ingredient increases, lower cost ingredients may be used in order to meets the specification, thus providing the customer with the most economical feed. However this practice does cause concern to some when it comes to the use of NIR in predicting finished feeds. Can an NIR calibration predict the composition of a feed that can change in composition on a regular basis?

AB Vista's finished feed calibrations contain over 30,000 samples, with a large distribution across all nutrients. Figure 1 shows the sample distribution for finished feeds within our database based on protein value.

Figure 1: Sample distribution for finished feeds based on protein value.

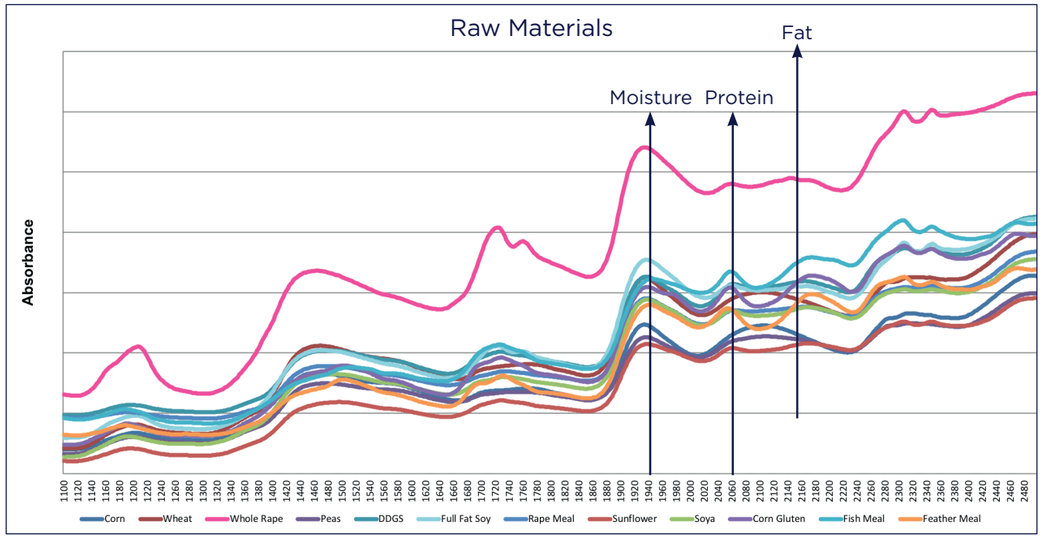


Atoms in compounds are held together by chemical bonds. Depending on the configurations of these bonds, they will emit characteristic vibrations when excited. When Near Infra-red energy is added to the system, the bonds will vibrate; the laws of physics constrain the vibrations that can occur, so only vibrations at certain frequencies are permitted.

Different chemical bonds (for example organic O–H, C–H and N–H bonds) vary in strength, and consequently the amount of energy required to make the bonds vibrate. This difference in energy requirement will be seen in a spectrum as a series of absorptions at different energy wavelengths (see Figure 2).

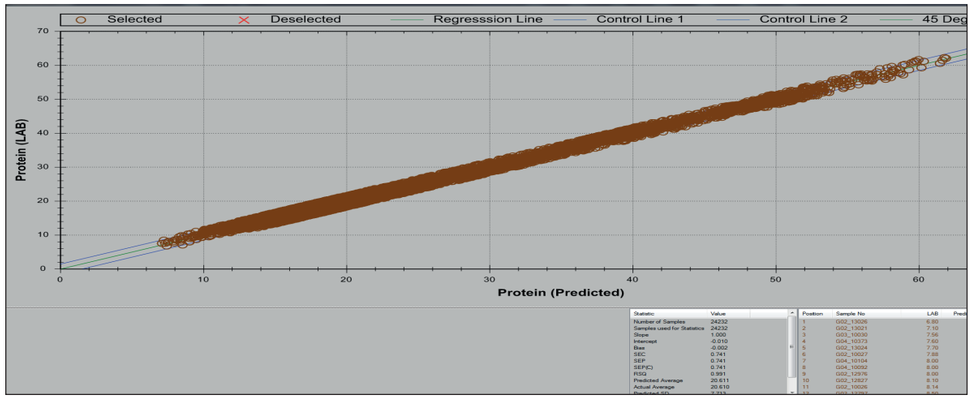
We can use information from the spectrum to build up a picture of the structure of the molecule. If we identify O-H, C-H and N-H bonds from the spectrum, we can deduce that the sample being analysed is protein. The basic make-up of protein is the same for all the different raw materials (C-O-H-N); therefore we can be confident we are measuring protein rather than the presence of a particular raw material.

Figure 2: The NIR spectra of different raw materials showing key molecular structures at specific wavelengths.



In fact the combination of this large database and highly variable nutrients provides an ideal matrix for NIR modelling, and the resulting calibration gives excellent performance as seen in Figure 3.

Figure 3: Protein NIR predictions (x axis) versus wet chemistry reference results for crude protein in finished feeds.



THE SIZE OF THE DATASET MATTERS

The benefit of a large NIR dataset over a smaller dataset is the robustness and accuracy of predictions for unknown samples. For feed and ingredients, the larger dataset will contain more information representing seasonal, geographical, varietal and recipe differences.

On the following pages are four graphical representations of the correlation between results for protein obtained from wet chemistry and NIR analysis.

Figure 1 shows results from a small set of samples correlated against predictions from an NIR calibration created from this small dataset. The correlation between the two is good. The correlation is also good when the same set of samples is plotted against predictions from an NIR calibration created using a large dataset such as found in AB Vista's calibrations (Figure 2). Figure 3 shows the correlation between results from a large set of samples and the corresponding NIR predictions based on a calibration created on a large dataset. In Figures 1, 2 and 3, the R2 value indicates strong correlation. However, plotting a large dataset of samples against predicted results from an NIR calibration developed from a small dataset results in the R2 value dropping significantly (Figure 4). This highlights the weakness of developing a calibration on a small dataset. A calibration developed from a large dataset will work on both the smaller dataset (Figure 2) even though the samples are not included in the calibration as well as on a large dataset (Figure 3). This highlights the value of the large dataset and how this can offer greater robustness and accuracy than when using smaller, limited datasets.

The correlation coefficient (R2) and Standard Error Predicted (SEP) values demonstrate that a calibration based on a smaller data set will perform poorly on a large data set where the variation of samples has not been included in the calibration database. This would be expected as NIR is a learning technique. If we have not taught the NIR what these samples look like, then it will never be able to predict them correctly, hence the poor correlation R2 value of 0.76 in Figure 4.

Calibrations created using large datasets are more robust than those based on smaller datasets. AB Vista's calibrations are based on over 350,000 samples gathered from different geographies over 25 years, making them both robust and accurate, providing confidence in results.

Figure 1: Samples: Small dataset Calibration: Small dataset
R2 = 0.97; SEP = 0.50

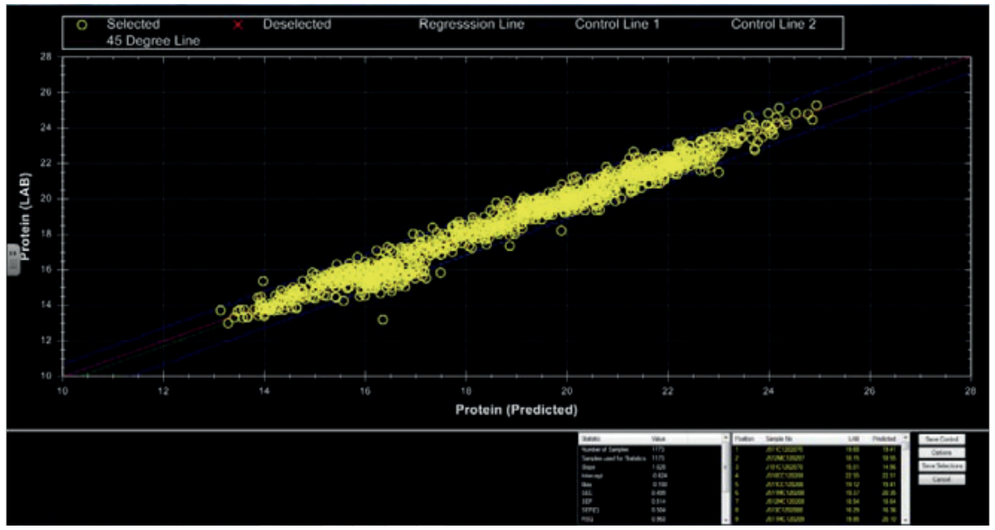


Figure 2: Samples: Small dataset Calibration: Large dataset (AB Vista)
R2 = 0.97; SEP = 0.49

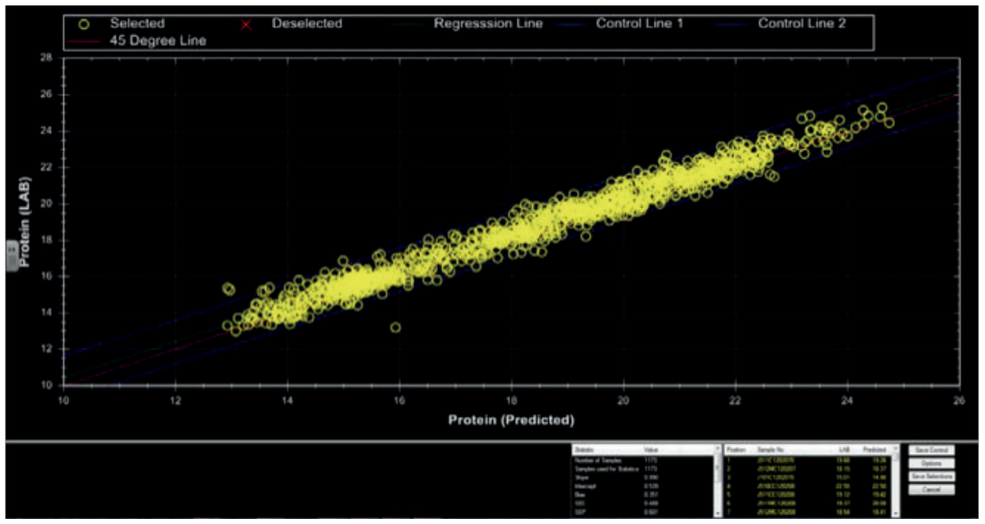


Figure 3: Samples: Large dataset Calibration: Large dataset (AB Vista)
R2 = 0.97; SEP = 0.70

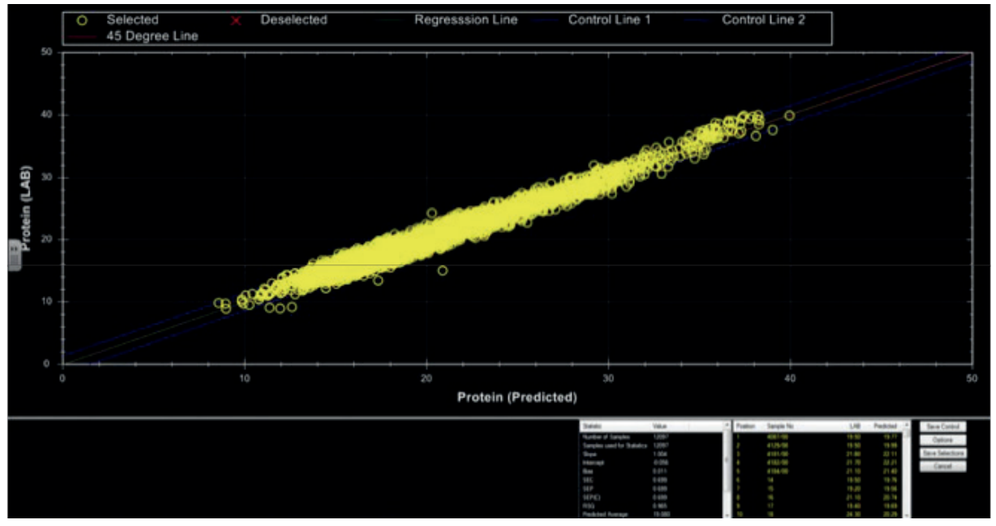
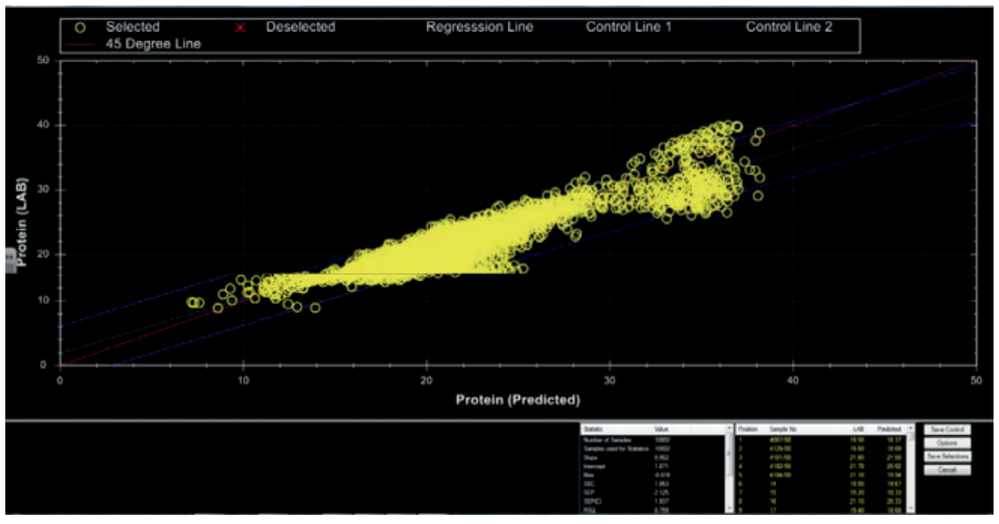


Figure 4: Samples: Large dataset Calibration: Small dataset
R2 = 0.76; SEP = 1.91

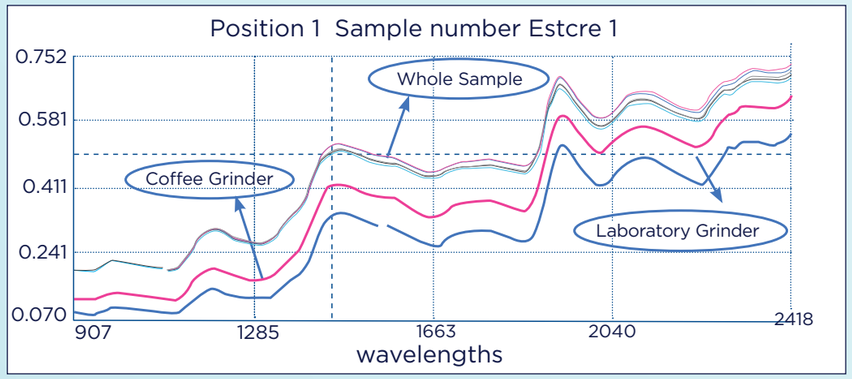


INFLUENCE OF PARTICLE SIZE ON NIR ANALYSIS

To determine the effect of sample particle size on NIR analysis, whole samples and samples ground with two different grinders were compared. A cereal sample was ground using either a laboratory or an ordinary coffee grinder. The whole and ground samples were analysed 20 times by repacking the sample between each scan.

There is a clear distinction between sample preparation methods, as seen in the grouping of the spectra (Figure 1). Spectra of ground samples tend to be more similar (the lines are closer together) than the spectra of whole samples (lines are more spread apart). Therefore the ground samples have a lower variability, due to the increased homogeneity of the samples following the grinding process.

Figure 1: Spectra of whole and ground samples.



Such spectral variation is likely to have an impact on the final NIR predictions. To test this theory, the composition of the spectra shown in Figure 1 were predicted. The Means, Standard Deviation (SD) and % Coefficient of Variation (C of V, calculated as the ratio of SD:Mean) for each parameter are displayed in Figure 2.

Figure 2: Statistics of analysis of a cereal sample as influenced by sample preparation method.

Results		Moisture	Fat EE	Fat AH	Protein	Fibre	Ash	Starch
	Mean	10.6	2.7	3.3	14.5	5.3	2.3	46.2
Whole	SD	0.36	0.28	0.17	0.41	0.35	0.10	1.58
	C of V	3.4%	10.7%	5.1%	2.8%	6.7%	4.5%	3.4%
Coffee Ground	SD	0.10	0.13	0.07	0.09	0.17	0.02	0.78
	C of V	1.0%	4.7%	2.2%	0.7%	3.3%	0.7%	1.7%
Laboratory Grinder	SD	0.05	0.03	0.03	0.06	0.08	0.01	0.24
	C of V	0.5%	1.1%	0.9%	0.4%	1.4%	0.5%	0.5%

The results show how homogeneity improves (lower %CV) when samples are ground, which translates to a higher repeatability of the results and therefore a more reliable NIR prediction. So while scanning whole samples will reduce analysis time, accuracy and precision should be the driving force in any analytical process. To achieve close to the same precision on whole material, the operator would need to take duplicate or triplicate scans.

For more information on how AB vista can work with you to evaluate your feed and feed ingredients, please contact us.

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