From early routine near infrared spectroscopy to instrumental development to InSb chemical imaging of biological materials

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I am pleased to have the opportunity to come back to Bangkok, home of several of my former graduate students, for the 14th International Conference on Near Infrared (NIR) Spectroscopy. I have been charged with presenting the history of NIR from its beginnings to the present time. The medical and biological applications cited in the program title were the result of technological progress from a modest but very practical start. Instrumental analysis has been my academic specialty throughout my teaching career. When I was a chemistry teacher at the University of Illinois, Springfield, I visited the DICKEY-john factory as part of my spectroscopy teaching duties and subsequently formulated plans for building a scanning near-infrared grating mono-chromator instrument at Springfield. But then the telephone rang and I was recruited by Kansas State University (KSU) in the College of Agriculture. That is how I became involved with wheat protein analysis.

The early 1970s involved analogue computers tweaked by potentiometer adjustments, rapidly moving filter assemblies, and hot run source lamps, which burned out after fairly short periods of use. Early analogue instruments included the Neotec GRAIN Quality Analyser (GQA) and the DICKEY-john Grain Analysis Computer (GAC), both of which had continuously moving parts with either a Ferris wheel or a carousel type filter mount assembly. Some of the components did not conform to military specifications, and their characteristics varied at different temperatures. The Neotec GQA had filter angular dependence and a low duty cycle, and the GAC had a higher duty cycle and more signal to work with. Early experimental frustration included analogue computers that drifted and therefore required periodic adjustment.

By 1976, a stabilised filter instrument was used to follow the Kansas wheat harvest to test farmers' wheat samples in a mobile laboratory, prior to intermingling in a country elevator. A stabilised GAC emerged called the InfraAlyser 2.5 of Technicon Industrial Systems. The DICKEY-john (original equipment manufacturer) instrument, marketed by Technicon, was modified to enable easier electronic adjustment. Formerly, a person would fly out from the factory with a soldering iron and a pocketful of resistors and solder appropriate resistors into the circuit to develop or change the calibration. With the new instrument and a laboratory in a van, we followed the wheat harvest in 1976 in Kansas, where US wheat production is centred. We used nearby

Oklahoma wheat for the initial calibration. Starting close to the Oklahoma border, we moved our portable laboratory in a north-westerly direction over a period of three to four weeks. A highway sign proclaimed, "ON-SITE RAPID WHEAT PROTEIN TEST, Kansas State University." Wheat testing was featured on the front page of several daily Kansas newspapers. We removed any debris from the wheat samples brought in by farmers before grinding the sample and packing it into the cup. The protein percent was reported to the farmer and its importance for bread quality wheat and exportation was discussed on the spot.

Results of this venture included **(a)** location, in Kansas, at harvest of a welcome amount of much needed high protein wheat suitable for bread-making following depletion of previously stored crop year stocks and **(b)** because each of the samples analysed by NIR in the van was sent overnight to KSU for duplicate Kjeldahl analysis (reported the next day), confidence was built for future routine NIR protein determination. At each new location, we updated the calibration by including recent data. By the end of the project, we had analysed 1900 samples. Calibration improved with the expanded data-base, and accuracy was within the Kjeldahl precision. Furthermore, because we tested many known wheat varieties with consistently good results, we dispelled the notion that application to different varieties might be a problem. This was important because many wheat varieties are grown in the US. This built our confidence, and that of other workers, in the practical capability of NIR.

(c) The following year, NIR was adopted by the Kansas Grain Inspection Service and the U.S. Federal Grain Inspection Service. **(d)** Also, a 20-minute documentary video of the Kansas NIR testing was produced that was shown to farm groups and at the 1976 Technicon International Congress in New York. Later, the audio was translated into different languages, and the documentary was shown around the world. **(e)** An immediate result of testing experimental wheats harvested that year was that one Kansas wheat breeder discarded germplasm from all of his low protein wheat cultivars and reorganised efforts toward higher protein.

(f) In support of the Kansas Agricultural Experiment Station (KAES) breeding program, the "Infrared Wheat Protein Screening" project was established in 1977 to analyse 20,000 experimental wheats per year. This resulted in a 2.5% (absolute) increase in protein over ten years without reducing yield for new varieties released by the Kansas Agricultural Experiment Station. It also resulted in the protein level being raised without reducing the yield. It was well known that breeding could increase either yield or protein and it was widely assumed that you could not have both. With NIR screening we were, in effect, able to double the effective lifetime of our Kansas breeders, who, in the past, harvested and then replanted WITHOUT protein analysis data. We actually made history in Kansas. The concept of breeding to increase either protein OR yield was no longer true. Handling the volume of samples between winter wheat spring harvest and fall planting require accelerated analysis. We devised a system that eliminated the sample cup, required use of the operators' two hands and one foot, and was capable of 5,000 wheat protein analyses in a 24-hour period.

Sharing NIR Information

In 1983, I wrote an invited cover article in Analytical Chemistry[1] entitled "Near Infrared Reflectance Analysis: Sleeper Among Spectroscopic Techniques" to inform practicing analytical chemists of NIR as a new practical analytical method.

Short courses presented at scientific meetings, book chapters and other articles,[2,3] and recognition by the Society for Applied Spectroscopy, the American Society for Testing and Materials and the Council of NIR Spectroscopy also increased awareness of NIR.

In the 1980's, many new applications were developed mainly by using good analytical chemistry to provide new and more reliable laboratory reference methods, e.g. G.C. for moisture in dough and determination of ferulic acid content by HPLC to measure non-endosperm cell walls in flour milling fractions. A KSU PhD student from Bangkok (Vera Pussayanawin) developed and provided the HPLC ferulic acid analytical method.[4]
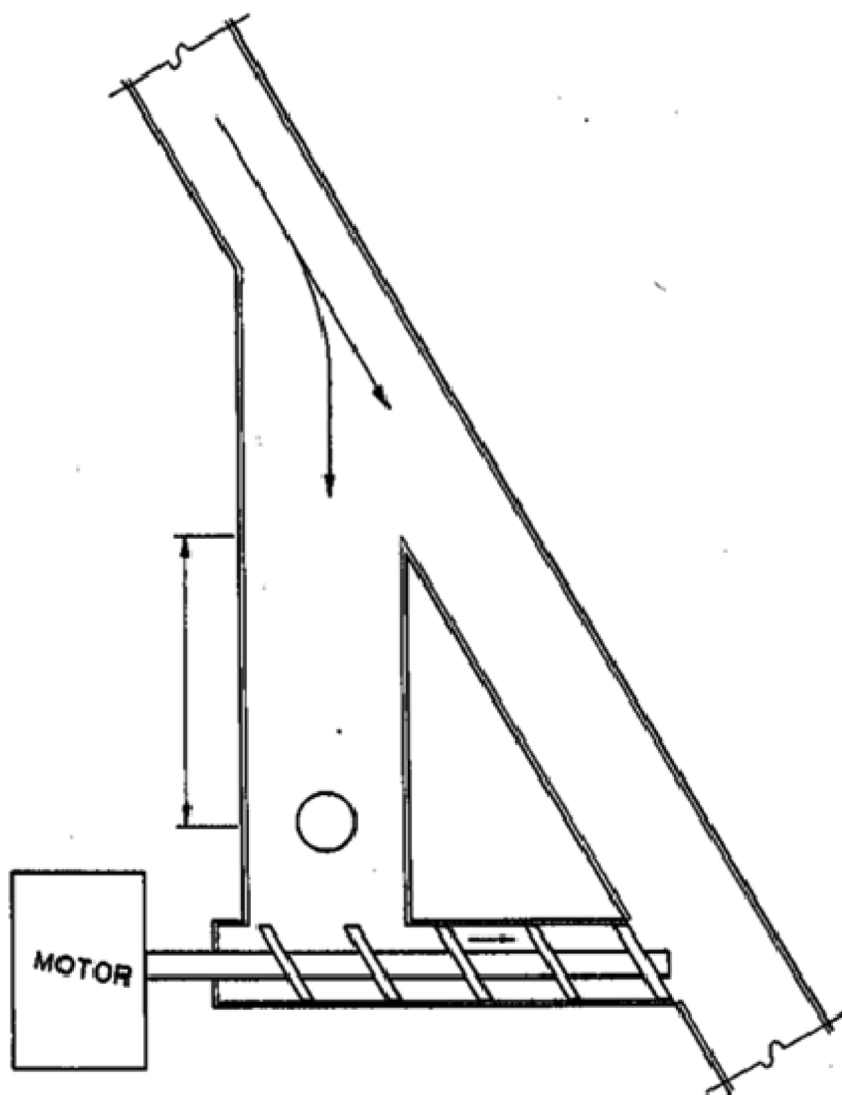
Figure 1. Online sampling system for KSU pilot flour mill, where product filling the vertical member is exposed to the window of a filter instrument optical head before being augured back into spouting.

In an effort to replace the ash test for flour, NIR scanning data from an experimental grating monochromometer instrument was compared with mid-IR data obtained using KBr pellet transmission. The same samples were run by both mid and near-IR, enabling tentative band assignments for NIR by relating them to the fundamental vibrations in a two-screen presentation at a San Francisco AACC meeting.[5] The subsequent year, at the International Bread Congress in Winnipeg, a talk titled "NIR flour QC" turned the tide in the cereal chemistry community, and each instrument company added a new filter for the determination of cellulose and hemicellulose.[6]

Design, construction, and testing of an on-line NIR system for use in the 5-story KSU pilot mill was achieved in cooperation with E.S. Posner, an experienced flour miller. The optical head of a commercial filter instrument was separated by a heavy-duty 108-foot cable assembly, and a custom designed sampling system (Figure 1) was connected to the aluminium spouting at two locations in the mill.

A running mean of five consecutive readings was updated every 15 seconds on a monitor in the miller's office. The more scientific calibration for non-endosperm was based on HPLC fluorescence determination of bran content, a method developed in our laboratory.

The KSU system was operated for more than 18 months. Test point data by NIR was mimicked by downstream flour ash determination. Miller Posner chose the key intermediate product streams to monitor. We subsequently reported economic results from using our system in an AOM bulletin[7] and a chapter on the KSU on-line system was included in an American Chemical Society monograph on "Chemical Sensing".[8]

From the NIR scanning spectra of individual KSU pilot mill streams off line, we noted that the first middlings (pure endosperm) had no bands due to cellulose in the 2300–2360 nm region whereas the fifth break and sixth middlings showed evidence of non-endosperm in that region. Protein and moisture analysis of individual mill-streams was achieved with sorting. Appropriate calibration development and selection to enable quantitative determination required extensive data collection over multiple crop years.

Instrument Building

A new 1989 patented random wavelength access acousto-optic tuneable filter (AOTF) near infrared spectrometer with no moving parts, "The Fastest Gun in the West",[9] that was capable of two wavelength analysis in 8 msec, was the basis for two custom AOTF instruments built at KSU for dynamic testing of polymers and films. A lightweight, compact, low energy consumption version of the experimental KSU spectrometer was subsequently used for early space exploration attempts with the MARS rover. Commercial versions of the AOTF are now in use. Although the principle was not new, building an instrument worthy of quantitative use for introduction in 1986 was a challenge. The instrument featured programmable isophotonic operation to enhance S/N at low transmission, and a high-duty cycle was achieved by switching only between needed wavelengths (Figure 2).

The digital wavelength reproducibility was so good that it was possible to perform spectral subtraction.[10,11]
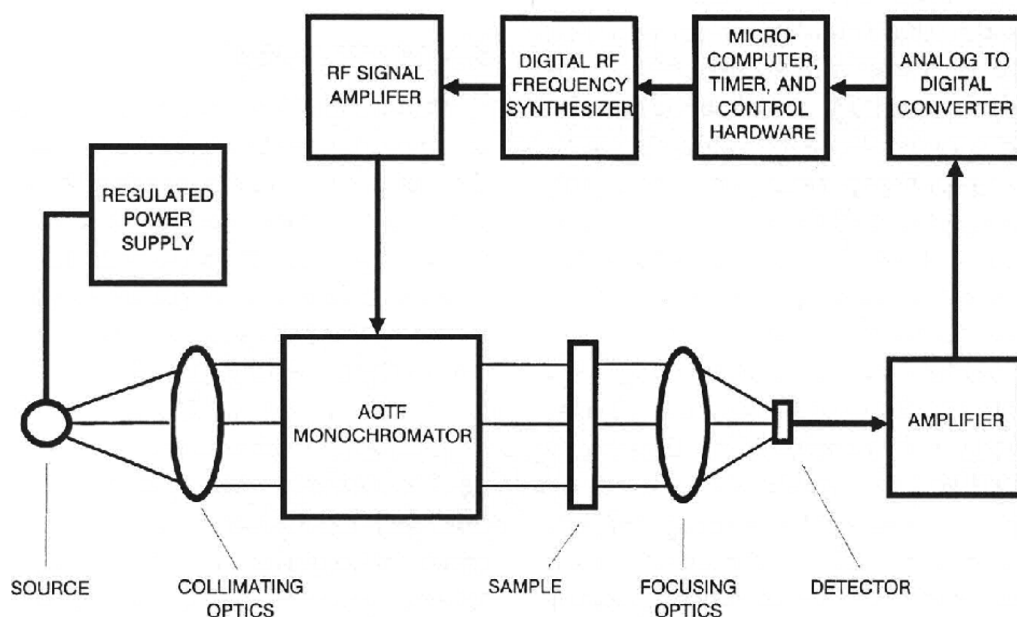
Figure 2. Diagram of the KSU research model acousto-optic tuneable filter NIR spectrometer. Note that ultrasonic waves from the frequency synthesiser are applied at a right angle to horizontal optical rays entering the AOTF crystal from the source for filtering.

Custom Instrument for Polymer Characterisation

We constructed a dynamic NIR-AOTF polymer testing device that differentiated between different batches of high-density polyethylene or between different compositional levels of a copolymer. In this system, a single computer controlled high-frequency mechanical perturbation and spectroscopic dynamic dichroic differentiation orientation response is described in a Review of Scientific Instruments article.[12] To determine the strength of wheat gluten, a home built, automated AOTF that generated select polarised wavelengths produced dichroic responses that revealed the relative gluten strength by the extent of molecular orientation produced from controlled work input via metered mechanical elongation.

Cooperation with medical researchers involved neuroscience and cardiac specimens from animal models and human tissue. Divine intervention (cardiac event) provided the opportunity to examine my own blood glucose with an FT-NIR forehead probe while on a hospital treadmill and on another occasion, to analyse my own excised arterial plaque for lipid.[13] Recent approval for clinical use of a near-IR heart catheter and its commercialisation is an important heart health milestone. A massive lipid deposit inside the aorta wall of the mouse fed sucrose revealed the importance of future diagnostic use of a NIR optical catheter to interrogate the heart vessel wall for lipid content that would reveal potentially unstable plaque.[14] Lipid bands in the plaque excised from my artery were similar in size to those of the adversely affected mouse.
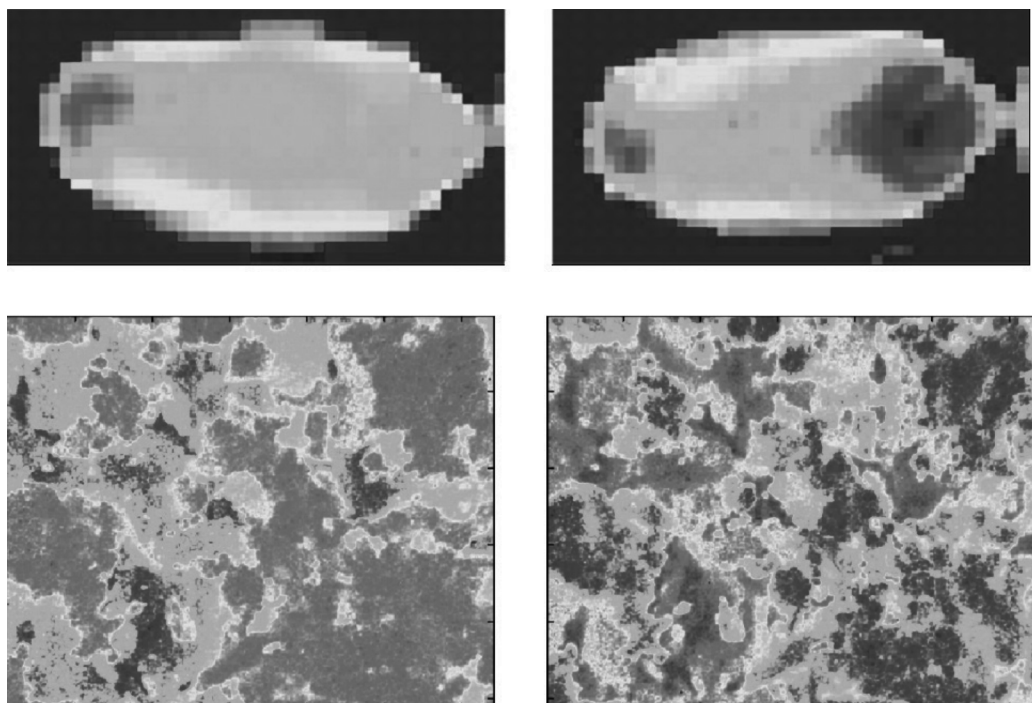
Figure 3. NIR chemical images of ungerminated and germinated wheat kernels (top left and right, respectively) and a binary mixture of ground corn and soybean meal contrasted by wavelength selectivity to highlight corn (bottom left) and soybean meal (bottom right).

"Back to the roots". Beginning in 1987, after introduction of the first IR microscope, we devoted much attention in our laboratory to *in situ* mid-IR imaging of wheat kernels and other biological materials[15,16] and have presented more than 150 lectures on IR microspectroscopy.

NIR array imaging answers the questions WHAT, WHERE, and HOW MUCH for mixtures of granular commodities. Designed for the pharmaceutical industry, it has also been used for non-destructive probing of whole wheat kernels for early detection of germination.[17,18] By using wavelength selective contrast, each individual pixel in the binary mixture is classified via discriminant analysis, and quantitation is done by counting pixels of a particular analyte eliminating the necessity of empirical calibration equations.

Our recent NIR imaging has included early non-destructive detection of germination,[17,18] solid mixture uniformity, flour milling unit efficiency,[19] mixed feed analysis,[20] distinguishing waxy from normal wheat kernels,[21] and horny endosperm content in corn. Figure 3 shows the difference between ungerminated and germinated wheat kernels, and illustrates the chemical image contrast between ground corn and soybean meal protein supplement (lower left and right, respectively).

Much has occurred since the early days of near infrared spectroscopy. Who knows what the future will bring?

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