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# Single Kernel Wheat NIR Analysis

Stephen R. Delwiche[1] and Floyd E. Dowell[2]
USDA-ARS
[1]Beltsville Agricultural Research Center
Building 303, BARC-East
Beltsville, Maryland 20705 USA
[2]U.S. Grain Marketing and Production Research Center
1515 College Avenue
Manhattan, Kansas 66502 USA

**ABSTRACT**

As popularity in whole grain near-infrared (NIR) analyzers has increased in the past ten years, so too has an interest in using this technology at the level of a single kernel of grain. This additional information can contribute to the revamping of U.S. grain standards so that more emphasis is placed on end-use quality. Research on the optical properties of single kernels of wheat has been underway in USDA laboratories at Beltsville, Maryland and Manhattan, Kansas. At Beltsville, single kernel research has consisted of wheat hardness, classification, and protein content by NIR transmittance, protein content and classification by NIR reflectance (1100-2500 nm), protein content of bulk samples by mathematical combination of single kernel protein predictions, and identification of scab-damaged kernels by hyperspectral image analysis. At Manhattan, single kernel NIR research has included refinement of the reflectance procedure to identify difficult-to-classify red and white kernels, prediction of protein content, detection of internal insect larvae infestation, detection of wheat scab, detection of vitreous kernels, and detection of heat damage, with all performed at near real-time conditions of the single kernel wheat characterization system (SKCS). These studies are discussed in context of their ramifications to official grading and classification, and to quality assessment through knowledge of kernel-to-kernel variability of intrinsic properties.

**SPEAKER PAPER**

## Introduction

Single kernel analysis of wheat gained interest in the 1980s with the desire for improving official classification procedures. At the time, the measurement of wheat hardness on a single kernel (SK) basis was considered useful for assignment of wheat class, and especially for detecting mixtures consisting of more than one contrasting hardness class. Ensuing from this need, the single kernel characterization system (SKCS) was developed by USDA-ARS engineers in Manhattan, Kansas (Martin et al. 1993), and later commercialized by Perten Instruments. The SKCS provides information on the hardness, moisture content, weight, and size of each kernel from a sample of 300 kernels. Because the hardness measurement is based on the force of crush, the method is destructive. Although some research on the use of near-infrared (NIR) spectroscopy for single seed analysis had been conducted on corn and soybeans (Finney and Norris 1978, Lamb and Hurburgh 1991, Orman and Schumann 1992), many of the advances of SK NIR research have occurred since the development of the SKCS. This is particularly true for wheat. With increased availability of diode array NIR spectrometers that are capable of collecting a spectrum in less than 1 second, researchers have seen the potential of coupling an NIR probe to the front end of the SKCS and operating this tandem device at a rate of 1 to 2 kernels per second. Coincident with this event has been a general increased interest in rapid nondestructive single seed analysis for use as a screening device for intrinsic properties analysis of breeders' lines and a means to spot check identity preserved lots. In the following sections, we offer descriptions of SK NIR wheat research performed in the past 8 years. However, before these are offered, more detail is provided on the need for SK properties assessment and potential use of NIR in such assessment.

**Why Single Kernel Analysis?**

*Classification and Grading* - U.S. wheat is divided into six unique classes: hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), hard white wheat (HWW), soft white wheat (SWW), and durum. For a lot to be classified into one of these six, at least 90% of the seed (from a dockage-free work sample that has been repetitively divided down to a 15 g portion) must be of one class. Otherwise, the lot is classified as mixed, or infrequently, unclassed. In either case, the lot's value is usually discounted. Further, wheat is graded into six levels: U.S. No. 1 (highest value) to U.S. No. 5, and U.S. Sample grade (lowest value). Each grade carries maximum allowable percentages of damaged kernels, foreign material, and shrunken and broken kernels (Table I). Grade is also based on the percentage of wheat from "contrasting classes." An example of two contrasting classes is hard white wheat and hard red winter wheat. Current USDA-GIPSA procedures for classification and grading a grain lot involve the manual (visual) inspection of a 15 g portion derived, through splitting, from systematic sampling of the grain lot itself. A typical inspection requires 10 to 15 minutes. Many of the categories that define damaged kernels and wheat from contrasting classes (as shown in Fig. 1) are amenable to NIR analysis.

**Table I. Abridged List of Grade Requirements for U.S. Wheat[a]**

| Grade | Maximum Limits in Percent by Weight | | | | | |
|---|---|---|---|---|---|---|
| | A Damaged Kernels | B Foreign Material | C Shrunken or Broken Kernels | D Defects (= A+B+C) | E Contrasting Classes | F Total Wheat of Other Classes |
| U.S. No. 1 | 2.0 | 0.4 | 3.0 | 3.0 | 1.0 | 3.0 |
| U.S. No. 2 | 4.0 | 0.7 | 5.0 | 5.0 | 2.0 | 5.0 |
| U.S. No. 3 | 7.0 | 1.3 | 8.0 | 8.0 | 3.0 | 10.0 |
| U.S. No. 4 | 10.0 | 3.0 | 12.0 | 12.0 | 10.0 | 10.0 |
| U.S. No. 5 | 15.0 | 5.0 | 20.0 | 20.0 | 10.0 | 10.0 |
| Sample | Exceeds Limits for U.S. No. 5 | | | | | |

[a] Source: USDA/GIPSA/FGIS Grain Inspection Handbook: Book II: Wheat (6/1/97).

*Identification of infected kernels* - Kernels that are moldy not only contribute to the downgrading of wheat but also, if present in high enough number, may cause the concentration of an accompanying mycotoxin within the flour or ground grain to exceed recommended or mandated food safety limits. For example, the U.S. Food and Drug Administration (FDA) has established advisory levels for the concentration of deoxynivalenol (DON) of 1 ppm for finished wheat products for human consumption, 5 ppm for grain products destined for non-ruminant animals, and 10 ppm for grain products destined for ruminant animals and poultry. Conventional analytical procedures such as enzyme-linked immunosorbent assay (ELISA) tests are used for mycotoxin analysis. NIR instrumentation is generally not sensitive in the range of parts per million; however, mold-damaged kernels are typically much higher (by a factor of 100 or more) in concentration of the mycotoxin. Thus the NIR signal-to-noise from the mycotoxin in an infected kernel may be sufficiently strong for detection. Likewise, the mold itself may be detectable by NIR.

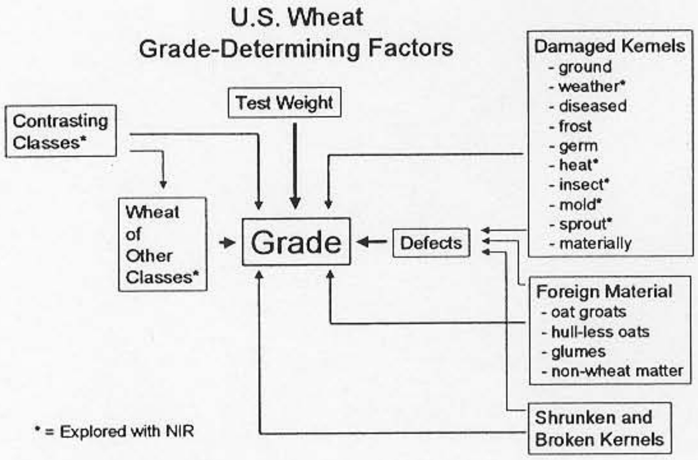


Figure 1. Features that determine the grade of U.S. wheat.

*Quality properties and their degree of variation* - Knowledge of the properties from individual kernels is potentially useful to processors because it provides a gauge of the consistency of the raw product. Kernel-to-kernel properties variation is caused at all stages of the growth and post harvest handling, that is, from the individual head to the blending operations at the country elevator and beyond. While variation in kernel hardness is commonly known to have an effect on milling properties, much less is known about whether variation in the intrinsic properties (e.g., protein, starch) has an effect on the processing characteristics or the quality of the final product.

*Sorting* - At the commercial level in the United States, single seed sorting based on visible light is currently used in the peanut and rice industries for removing moldy, damaged, and discolored seeds. Although probably not needed in commercial wheat processing, SK wheat sorting has application in plant breeding programs. Nondestructive SK analysis of the trait upon which selection is being made has the potential for hastening the development of new lines. For example, Silvela (1989) demonstrated that selection based on single kernel analysis was more efficient than conventional (whole plant) analysis when breeding for high oil corn. In the past few years a commercial instrument known as the Seed Meister (Brimrose Co., Baltimore, MD) has been marketed for the sorting (up to six bins) of corn, soybeans, peanuts, and seeds of similar size, according to their levels of oil, protein, moisture, and starch contents.

**Advances in Hardware Technology**

Until slightly more than 10 years ago, NIR instruments could be grouped into two categories, filter-based and scanning monochromators. In the first category, between 5 and 25 narrow band-pass filters were commonly used in an instrument, with the selection of the pass band for each filter dependent on the analyte (e.g., protein, moisture, oil) of interest. Later modifications of the filter design such as tilting the filter several degrees with respect to the optical axis permitted the collection of radiation at wavelengths shifted from the pass band. In the second category, moving gratings, moving prisms, or their combination were used to disperse radiation into a continuum across the NIR region, whereupon the reflected or transmitted energy was sensed at uniform wavelength increments by a stationary detector. With either category, collection time for a spectrum was typically 30 to 60 seconds. With the advent of diode array detectors, acoustical-optical tunable filters, liquid crystal tunable filters, and Fourier transform NIR spectrometers, the time needed to collect a spectrum has dropped substantially (albeit at the expense of increased noise). Diode array data collection times of 0.5 to 1 second yield spectra from individual wheat kernels that are suitable for the macro constituents such as protein and moisture contents (Psotka et al. 2000), color (Dowell 1998), and internal insect detection (Dowell et al. 1998).

**Advances in Software**

Coincident with the increase in performance of the personal computer, NIR calibration equations have increased in capability (and complexity). Sole reliance on multiple linear regression analysis has given way to principal component regression, partial least squares analysis, locally weighted regression, and artificial neural networks. Although usually applied to transmittance or reflectance spectra of intact or ground bulk samples, each technique is also applicable to SK analysis.

## Recent Single Kernel NIR Studies

**Hardness**

Delwiche (1993) examined the relationship between wheat hardness and NIR transmittance (850-1050 nm) of single wheat kernels. Working with the same set of reference standards as used in defining the NIR reflectance hardness scale (AACC 2000) (from which the SKCS hardness scale is based), Delwiche determined that SK NIR transmittance was sensitive to wheat hardness to the extent of the correlation between hardness and vitreousness. Soft wheat, which tended to show greater variation in vitreousness from kernel to kernel, also showed greater variation in hardness as predicted by NIR transmittance. It was thought that transmittance readings were not directly sensitive to the low-molecular weight proteins that, in action with the starch granule surface determine hardness.

**Protein Content**

Using the SK transmittance procedure, Delwiche (1995) developed PLS equations for protein content (N x 5.7) of single wheat kernels. Whole kernel combustion was used as the reference method. With separate calibrations developed for each of the six U.S. market wheat classes, the standard error of performance (SEP) ranged from 0.4 to 0.9% protein on test sets that possessed standard deviations of reference protein ranging from 1.3 to 2.4%. A drawback of this procedure was the care needed in hand alignment of the kernel placed within a neoprene aperture. Careful alignment was needed to minimize stray light (caused by an imperfect light seal) from reaching the detector.

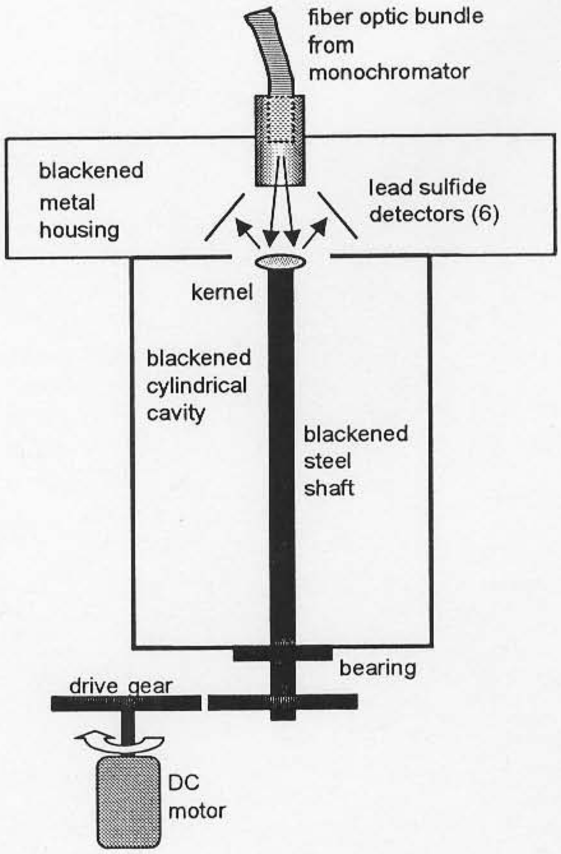


Figure 2 Single kernel reflectance assembly for a bench top analytical spectrometer.

Single kernel reflectance was later considered as an alternative to SK transmittance for protein content measurement (Delwiche 1998). Kernel alignment in a reflectance measurement (Fig. 2) was not as critical as in a transmittance measurement, making SK reflectance more suitable for automation. More than 300 commercial wheat samples, representing five U.S. classes (durum excluded) were used to develop protein content models, based on reflectance (1100-1400 nm). Model performance (shown for HWW in Fig. 3), with SEP ranging from 0.46 to 0.72% protein (depending on wheat class), was similar to corresponding SK transmittance models. One surprising result was that it was possible to develop calibrations that involved all five wheat classes simultaneously, without loss in model accuracy. Based on a test set of 10 kernels from each of 168 independent samples from the five classes, the SEP was 0.54% protein. The time needed for scanning a kernel (about 20 seconds), while reasonable for analytical work, was considered too long to be practical for use in commerce. Psotka et al. (2000) recently demonstrated the ability of a diode array spectrometer to measure SK protein content at scan rates of approximately 1 kernel per second.

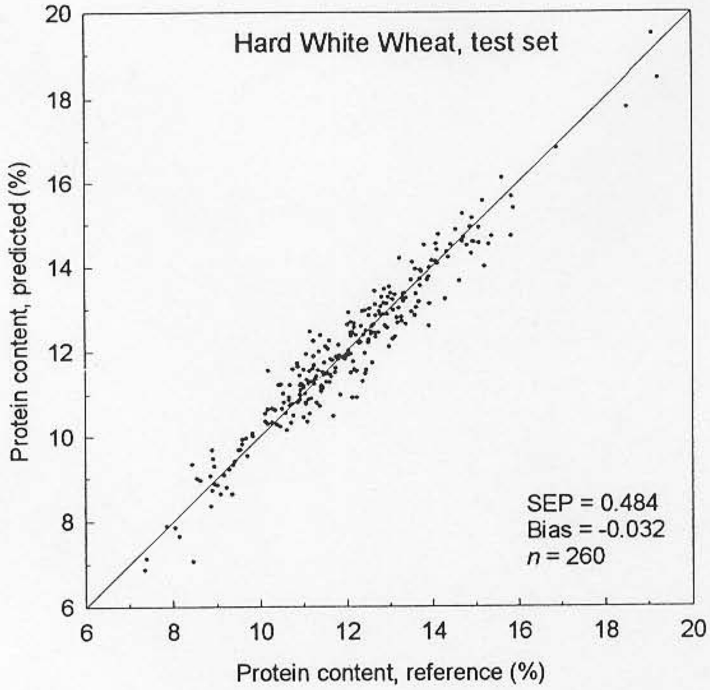


Figure 3. Example of model performance for single kernel NIR reflectance protein content.

Delwiche and Hruschka (2000) used the single kernel spectra from Delwiche's (1998) study to demonstrate the ability to develop models for prediction of the average protein content of a bulk sample, using weighted averages of single kernel spectra. By examining the sources of error, they found that the average of 100 single kernel protein measurements provided comparable performance to a conventional bulk sample NIR instrument.

**Classification**

Since the early 1990's, several researchers have examined the potential of visible or NIR SK spectroscopy for wheat classification, with particular interest in distinguishing red and white wheat. NIR research on red vs. white has been underway in Australia (Ronalds and Blakeney 1995), Canada (McCaig et al. 1993), and the United States (described below).

In the United States, red wheat and white wheat represent contrasting classes and therefore are subject to the rules of grading mentioned above. On a test set of 1590 SK spectra that represented 192 red wheat samples and 126 white wheat samples drawn from a market survey of U.S. wheat from one year's harvest, Delwiche and Massie (1996) demonstrated that SK reflectance in the 550 to 800 nm region could achieve classification accuracies in excess of 96 percent. Although longer wavelength regions were examined, the indicated region was most successful, attesting to the sensitivity of this region to the pigmentation of the seed.

From the genetic standpoint, white wheat occurs when all three of the alleles responsible for seed coat color are recessive. The red condition occurs in the presence of at least one dominant R allele, and intensifies with the presence of additional dominant R alleles. Visual identification of color is hampered by kernel disease, weathering, and excessive rain. For difficult to classify kernels, soaking in 5% (w/v) solution of NaOH and blotting kernels dry before visual or NIR analysis improves classification success (Dowell 1997). With soaking, Dowell was able to lower the misclassification rate for difficult to classify kernels to 2%, based on the reflectance (400-700 nm) of hand-oriented kernels. When the same spectrometer was used in a semi-automated mode of kernel placement (four seconds per randomly oriented kernel), the misclassification rates were still very low (<1%) but the complexity of the NIR models increased, as more PLS factors were used (Dowell 1998). Additional work at Dowell's laboratory on red vs. white classification was performed by Wang et al. (1999a,b,c). By developing separate two- to four-class PLS-based models for the number of dominant R alleles, Wang et al. (1999a) determined that classification was most difficult when attempting to distinguish 1R and 2R genotypes. Kernel size variation also adversely affected model accuracy, though with first or second derivative spectral pretreatment, the model's sensitivity to the size effect was reduced (Wang et al. 1999c).

**Mold**

Dowell et al. (1999) used a SK probe to measure concentrations of DON and ergosterol (an indicator of fungal invasion) within individual wheat kernels. GIPSA's Board of Appeals and Review first categorized the kernels as being scab-damaged or sound. Sound kernels were further divided into two groups: those with no visible scab and those with slight scab, whose levels were not sufficiently high to meet GIPSA's criterion for scab damage. Referenced to HPLC-determined concentrations of DON, the NIR PLS models identified more kernels with DON than did visual inspection. The standard error of 40 ppm for the PLS model of DON was well below the average DON concentration (105 ppm) by reference analysis of the scab-damaged kernels. This suggests that the NIR probe is more sensitive to scab detection than visual inspection.

Recently, Delwiche and Kim (2000) reported on the detection of scab-damaged wheat kernels by machine vision. A custom-made hyperspectral (425-860 nm at 3.7-nm intervals) imaging system gathered images of non-touching kernels from three HRS wheat varieties ('Grandin', 'Gunner', and 'Oxen'). Thirty-two normal and 32 scab-damaged kernels were selected to represent each variety. From a search of wavelengths that could be used to differentiate the two classes (normal vs. scab), a linear discriminant function was constructed from the best R(wavelength1)/R(wavelength2), based on the assumption of a multivariate normal distribution for each class and the pooling of the covariance matrix across the two classes. For a function developed using all three varieties, the ratio R(568 nm)/R(715 nm) produced a misclassification error (determined by cross validation) that averaged between 2 and 17%, depending on wheat variety. Figure 4 shows the ratio image of the most well behaved variety (Grandin), for which only 1 of 64 kernels was misclassified. When modeling was limited to one variety at a time, the misclassification error (averaging 0 to 12.5%, depending on variety) was less. Figure 5 shows the best ratio, R(605 nm)/R(733 nm), that was associated with a one-variety model for Grandin, for which perfect classification was attained. The variety (Gunner) that was most difficult to categorize by visual analysis was also the one with the highest misclassification error. With expansion to the testing of more varieties, a two-to-four wavelength machine vision system appears to be a feasible alternative to manual inspection.

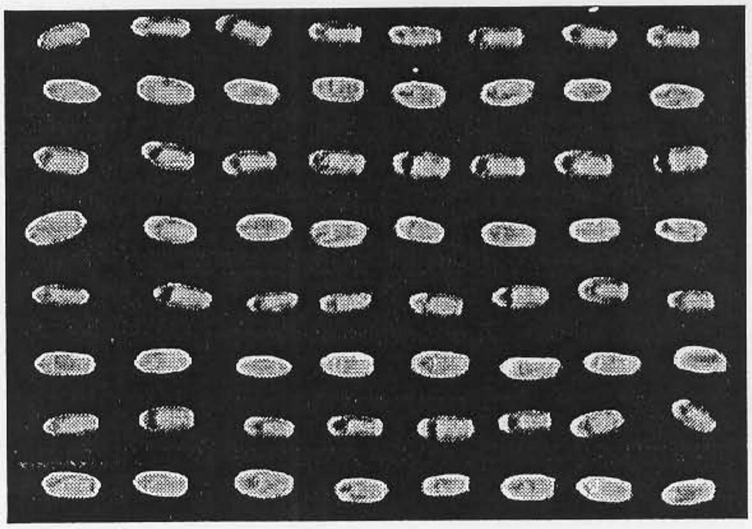

Figure 4. Image formed as the ratio of reflectance images at two wavelengths, $R(\lambda_1) / R(\lambda_2)$. Starting at top of image, rows alternate between sound and scab-damaged kernels. $\lambda_1$ = 568 nm, $\lambda_2$ = 715 nm.

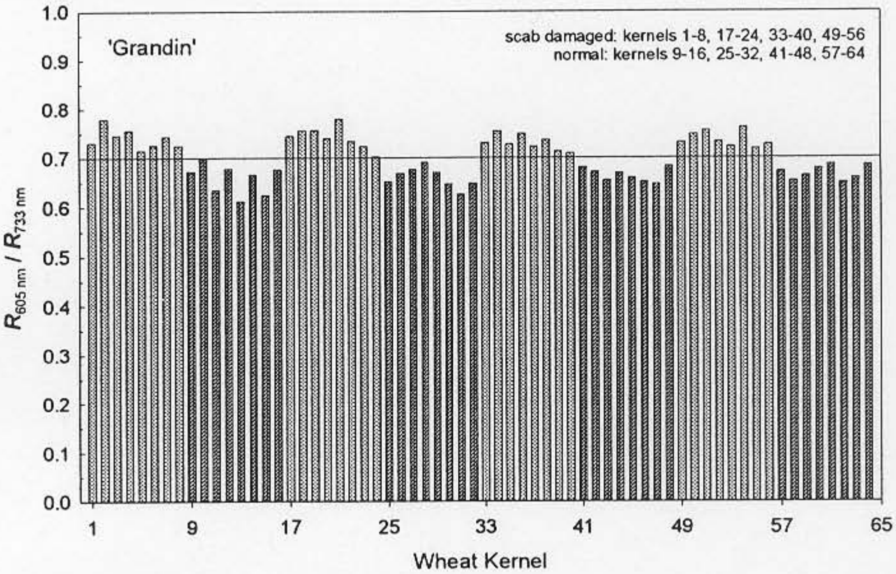


Figure 5. Ratio of reflectance images at two wavelengths, $R(\lambda_1) / R(\lambda_2)$. Horizontal line represents the boundary between sound (normal) and scab-damaged kernels.

Internal Insects

Post-harvest grain losses caused by pests and poor storage practices total more then $1 billion per year in the U.S. Grain is inspected for the presence of insect damage and adult insects, but insect larvae inside kernels are not visually detectable. If wheat containing hidden insects is stored, the larvae will emerge as adults and further degrade the quality of the wheat. If the wheat is milled with hidden insects, then they will contribute to insect fragments in flour. Dowell et al. (1998) used an automated single kernel NIR system to detect the presence of hidden insects in wheat. They showed that larger larval growth stages (3rd and 4th instars) of angoumois grain moth, lesser grain borer, and rice weevil could be detected with 95% confidence. Figure 6 shows the ability of the NIR system to detect hidden insects and predict the larval size.

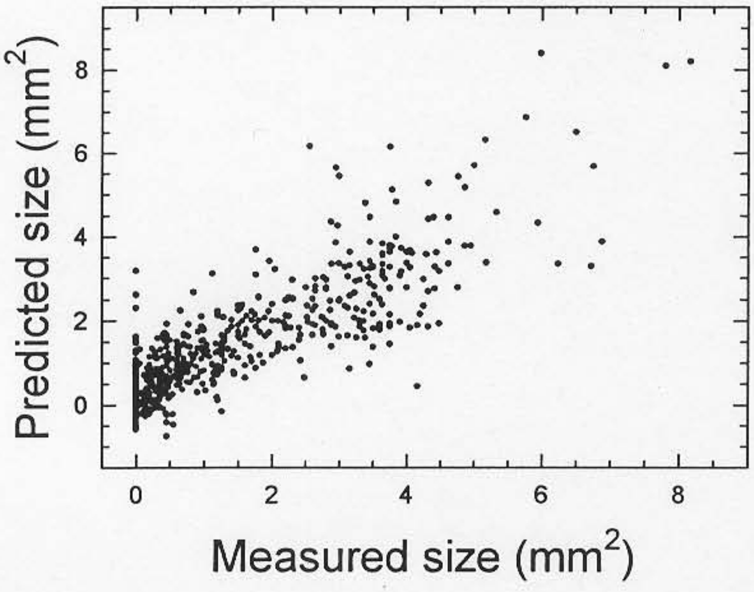


Figure 6 Size of larvae within a wheat kernel: NIR-predicted vs. actual size.

**Vitreous Kernels**

The vitreousness of durum wheat is used by the wheat industry as an indicator of milling and cooking quality. The current visual method of determining vitreousness is subjective and classification results between inspectors and countries vary widely. Thus, the use of near-infrared (NIR) spectroscopy to objectively classify vitreous and non-vitreous single kernels was investigated by Dowell (2000). Results showed that classification of obviously vitreous or non-vitreous kernels by the NIR procedure agreed almost perfectly with inspector classifications. However, when difficult-to-classify vitreous and non-vitreous kernels were included in the analysis, the NIR procedure agreed with inspectors on only 75% of kernels. While the classification of difficult kernels by NIR spectroscopy did not match well with inspector classifications, this NIR procedure quantifies vitreousness and thus may provide an objective classification means that could reduce inspector-to-inspector variability. Classifications appear to be due, at least in part, to scattering effects and to starch and protein differences between vitreous and non-vitreous kernels.

**Future Work**

Single kernel NIR research continues at the USDA Manhattan and Beltsville laboratories. Grade-determining factors, such as heat damage (Wang et al. 2001), hold promise for their adaptability to NIR measurement. Recent work in Manhattan has demonstrated the applicability of SK NIR to the detection of fumonisin in corn (Dowell et al. 2001). Infected kernels having greater than 100 ppm fumonisin or healthy kernels with less than 10 ppm fumonisin are most easily distinguished. At Beltsville, work continues on scab damage detection by segmentation of individual kernels in hyperspectral images.

**References**


AACC. 2000, Wheat hardness as determined by near-infrared reflectance. Approved Methods of the AACC: Method 39-70A, 10th ed. St. Paul, MN.

Delwiche, S.R. 1993. Measurement of single-kernel wheat hardness using near-infrared transmittance. Trans. ASAE 36:1431-1437.

Delwiche, S.R. 1995. Single wheat kernel analysis by near-infrared transmittance: Protein content. Cereal Chem. 72:11-16.

Delwiche, S.R. 1998. Protein content of single kernels of wheat by near-infrared reflectance spectroscopy. J. Cereal Sci. 27:241-254.

Delwiche, S.R., and Hruschka, W.R. 2000. Protein content of bulk wheat from near-infrared reflectance of individual kernels. Cereal Chem. 77:86-88.

Delwiche, S.R., and Massie, D.R. 1996. Classification of wheat by visible and near-infrared reflectance from single kernels. Cereal Chem. 73:399-405.

Dowell, F. E. 1997. Effect of NaOH on visible wavelength spectra of single wheat kernels and color classification efficiency. Cereal Chem. 74:617-620.

Dowell, F. E. 1998. Automated color classification of single wheat kernels using visible and near-infrared reflectance. Cereal Chem. 75:142-144.

Dowell, F.E. 2000. Differentiating vitreous and nonvitreous durum wheat kernels by using near-infrared spectroscopy. Cereal Chem. 77:155-158.

Dowell, F.E., Throne, J.E., and Baker, J.E. 1998. Automated nondestructive detection of internal insect infestation of wheat kernels by using near-infrared reflectance spectroscopy. J. Econ. Entomol. 91:899-904.

Dowell, F.E., Ram, M.S., and Seitz, L.M. 1999. Predicting scab, vomitoxin, and ergosterol in single wheat kernels using near-infrared spectroscopy. Cereal Chem. 76:573-576.

Dowell, F.E., Pearson, T.C., Maghirang, E.B., Xie, F., and Wicklow, D.T. 2001. Reflectance and transmittance spectroscopy applied to detecting fumonisin in single corn kernels infected with fusarium verticillioides. Cereal Chem. (in review).

Finney, E.E. and Norris, K.H. 1978. Determination of moisture in corn kernels by near-infrared transmittance. Trans. ASAE 21:581-584.

Lamb, D.T. and Hurburgh, C.R. 1991. Moisture determination in single soybean seeds by near-infrared transmittance. Trans. ASAE 34:2123-2129.

Martin, C.R., Rousser, R., and Brabec, D.L. 1993. Development of a single-kernel wheat characterization system. Trans. ASAE 36:1399-1404.

McCaig, T.N., DePauw, R.M., and Williams, P.C. 1993. Assessing seed-coat color in a wheat breeding program with a NIR/VIS instrument. Can. J. Plant Sci. 73:535-539.

Orman, B.A. and Schumann, R.A. 1992. Nondestructive single-kernel oil determination of maize by near-infrared transmission spectroscopy. J. Am. Oil Chem. Soc. 69:1036-1038.

Psotka, J., Dowell, F.E., and Delwiche, S.R. 2000. Analysis of protein and moisture in single kernels of wheat using the single kernel characterization system and NIRS. Abstract presented at AACC Annual Meeting, Kansas City.

Ronalds, J.A., and Blakeney, A.B. 1995. Determination of grain colour by near infrared reflectance and near infrared transmittance spectroscopy. in: Leaping Ahead With Near Infrared Spectroscopy (Batten, G.D., Flinn, P.C., Welsh, L.A., and Blakeney, A.B., eds.) 148-153.

Silvela, L., Rodgers, R., Barrera, A., and Alexander, D.E. 1989. Effect of selection intensity and population size on percent oil in maize, *Zea mays* L. Theor. Appl. Genet. 78:298-304.

Wang, D., Dowell, F.E., and Lacey, R.E. 1999a. Predicting the number of dominant R alleles in single wheat kernels using visible and near-infrared reflectance spectra. Cereal Chem. 76:6-8.

Wang, D., Dowell, F.E. and Lacey, R.E. 1999b. Single wheat kernel color classification by using near-infrared reflectance spectra. Cereal Chem. 76:30-33.

Wang, D., Dowell, F.E., and Lacey, R.E. 1999c. Single wheat kernel size effects on near-infrared reflectance spectra and color classification. Cereal Chem. 76:34-37.

Wang, D., Dowell, F.E., and Chung, D.S. 2001. Assessment of heat-damaged wheat kernels using near-infrared spectroscopy. Cereal Chem. (in print).