Using Digital Image Analysis for Assessing the Quality of Wheat and Barley

Bruce Gordon Armstrong

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School of Science and Engineering
University of Ballarat
PO Box 663
University Drive, Mt. Helen
Ballarat, Victoria 3353
Australia

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Abstract

This thesis explores the issues involved in developing a relatively low-cost Digital Imaging Analysis (DIA) system for the quality assessment of wheat and barley using commonly available equipment. It also explores the capability of such a system to provide rapid and accurate assessments.

The research confirms that it is possible to devise such a system using flatbed scanners for image capture and conventional personal computers for the image analysis. However, it is necessary to modify the scanners, place them in a cabinet and develop special indented trays to hold the grain samples for optimal results. It is also necessary to develop complex software to undertake the analysis.

The small sample sizes and non-destructive DIA methods will be especially beneficial to grain breeders and others who only have limited amounts of grain to work with.

The DIA system developed (SeedCount) is capable of making very accurate counts of grain, and thus produces accurate thousand kernel weight assessments. Initially these counts were totally dependent on a novel (now patented) counting algorithm. The system can also make accurate morphological measurements of the kernel length, width and area that are limited in accuracy primarily by the image's resolution. Other sample attributes such as kernel shape (aspect ratios, ovality), dockage material and crease locations can also be assessed.

The novel bi-modal indented tray developed during this process also allows direct measurement of the kernel thickness, which was previously not possible. These measurements allow three-dimensional models of the kernels to be developed that can be used to assess the kernel's roundness, mass and screening assortment group with considerable accuracy.

Statement of Authorship

Except where acknowledged below or explicit reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma. No other person's work has been relied upon or used without due acknowledgement in the main text and bibliography of the thesis.

Portions of chapters four and five were presented at the 51st Royal Australian Chemistry Institute-Cereal Chemistry Division Conference held on the 9th to 13th of September 2001 at Coogee, New South Wales. The accompanying paper has been peer-reviewed and published in the Cereal Chemistry Division's Conference Proceedings (Armstrong et al., 2001).

Portions of chapters six and seven were presented at the joint 11th Barley Technical Symposium and 53rd Royal Australian Chemistry Institute-Cereal Chemistry Division Conference held on the 7th to 10th of September 2003 at Glenelg, South Australia. Two papers were published in the Barley Technical Symposium Proceedings. The papers were also peer-reviewed and published in the Cereal Chemistry Division's Conference Proceedings (Armstrong et al., 2003; Dines and Armstrong, 2003). Some of this material was also presented at the University of Ballarat Annual Research Conference 2003.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

AACC	American Association of Cereal Chemists
ABS	Australian Bureau of Statistics
ANN	Artificial Neural Networks
AOAC	Association of Official Analytical Chemists
ASBC	American Society of Brewing Chemists
AWB	Australian Wheat Board
CCD	Charge Coupled Device
CE	capillary electrophoresis
CW	Chondrometer Weight
DNA	Deoxyribonucleic Acid
EBC	European Brewing Convention
ES	Efficiency (Screening)
FGIS	Federal Grain Inspection Service
HPLC	High Pressure Liquid Chromatography
HW	Hectoliter Weight
HWE	Hot Water Extract
IoB	Institute of Brewing
JWM	Joe White Maltings
KW	Kernel Weight
MBIBTC	Malting and Brewing Industry Barley Technical Committee
MHW	Mini-Hectoliter Weight
NIR	Near Infra-red Reflectance (spectrometer)
NLWRA	National Land and Water Resources Audit
OT	Overtails
PHG	Pre-Harvest Germination
SE	Screening Equivalents
SEE	Standard Error of the Estimate
TKW	Thousand-Kernel Weight
TR	Thinness Ratio
TW	Test Weight

1 Introductory Summary

1.1 Background and Context

This research project focuses on wheat and barley, which are the two largest cereal crops in Australia. To provide some idea of the size of this industry, the Australian 2000 wheat crop had a value of 4,831 million dollars. The barley crop, though smaller, in 2000 was valued at 865 million dollars (Australian Bureau of Statistics (ABS), 2002).

Around 75% of the grain produced in Australia is exported and earns the country approximately $6 billion a year. Over half of the grain exports are wheat. Australia only produces about 3% of the total world wheat, but our high national export level comprises 15% of the international wheat trade as assessed by the National Land and Water Resources Audit (NLWRA, 2001).

Various wheat cultivars are needed for many different end uses. At one of the extremes of the range are soft, low protein wheat cultivars used for biscuit and cake flour. Hard wheats, with high protein levels and strong glutens that are suitable for bread and noodles are another extreme. Wheat that is below specification for human use is usually channeled into the stock feed industry, while prime hard wheat attracts a price premium.

Barley can be used directly as human food, used as a stock feed or malted for use in production of wort for beer or malt extract. Malting grade barley can be difficult to produce and thus attracts a price premium for growers.

Wheat and barley are of enormous economic importance to Australia. Virtually all of our domestically consumed cereals are grown and processed here. In addition to our whole grain exports, Australia also exports wheat flour, malted barley and a few consumer-ready items, thus exporting value-added products as well.

To improve, or even to simply maintain, the importance of the cereal grain industry in Australia, we need to constantly improve the quality of our cultivars. Quality

improvements can only happen efficiently if we have rapid and accurate tests to assess cereal quality (Duijnhouwer, Grashoff and Angelino, 1993). There is a long history of grain testing, and that history reveals the massive improvements in grain quality testing as technology has become more sophisticated (Hudson, 1960; Miskelly, 2003; Wrigley, 2000).

There is also a need to improve current methods for matching each cultivar to its optimum end-use. Such end-use quality testing will be enhanced by the continued development of rapid methods that can predict the processing and end-use suitability of the cultivars. Of course the ability to accurately identify the cultivar and track the grain lot throughout the food chain is essential.

Better understanding of the crop's growth and maturation requirements and improved harvest and post-harvest storage methods can enhance the quality of crops produced from existing cultivars. However, there is a limit to how far this approach can go. New cultivars with better genetics are essential for Australia to keep abreast of their major competitors.

The introduction of new cultivars is crucial to the viability of our cereal industry for several major reasons:

- To match tightly specified parameters required for specific products.
- To provide disease resistance to new diseases or improved resistance to existing diseases.
- Expand the borders of suitable growing areas by reducing the impact of poor soil and challenging environmental factors.
- Increase yields and/or quality.

1.2 Statement of the Problem

The overall aim of this project is to develop processes to assess cereal quality rapidly and economically, while only requiring small quantities of seed. The quantity of seed required is an important consideration for grain breeders in particular. Presently small

samples of seed must be significantly amplified before comprehensive testing can be performed. This is a major problem for the original cultivar breeders as they often only have a few grams of seed after their first harvest. It takes them several years to grow enough material to undertake even basic conventional quality analysis.

Similarly, cereal research organizations importing new cultivars from overseas are often restricted by quarantine requirements to small samples. Neither of these groups wishes to see most of their precious seed ground into flour for analysis. In addition to reducing the sample size, it is also desirable to be able to test the grain without damaging it so it can be returned to the breeding program.

Though sample size is less important to commercial grain dealers, small sample sizes are still desirable as it allows them to reduce the size of their sample storage facility.

1.3 Potential Outcomes

Possible solutions to these specific problems being addressed by this project are:

1. To determine if a digital image analysis (DIA) system can accurately and quickly count wheat and/or barley kernels, allowing rapid thousand-kernel weight analysis.

2. To develop test methods based on kernel properties derived from DIA that can be used to assess kernel shape and perhaps help identify kernel type and cultivars.

3. To determine if a DIA system can be developed that can replace the relatively slow method of mechanically sieving grain to assess screening fractions.

4. To determine if these tests can be used to predict the flour yield of wheat and the soluble hot-water extract of malted barley.

The potential outcomes of this research are new procedures to rapidly evaluate germplasm for characteristics required by the final users. Rapid analysis of smaller

samples will provide cost savings by decreasing the time and resources required for the detection of such characteristics. These outcomes can have an impact on the grain industry throughout, and possibly beyond, Australia.

This project, through decreasing the seed sample sizes needed for analysis and the non-destructive nature of the tests, may reduce cultivar selection time by one or two years. The methods developed in this project should be applicable to other cereal breeders and agricultural trial centers. It may well be that some of the new methods will also be applicable to bulk grain handlers and other commercial grain users.

2 Literature Review

2.1 Introduction

Grain users have various needs. Some users require high energy feeds for livestock. Millers need grains capable of producing flours suited to various baking requirements and maltsters must have malting quality barley.

In this chapter studies will be made of the morphology and structure of these grains, how these properties match with various end uses and how quality assessment helps to match the grain to its ideal use. Particular attention will be given to the role of digital image analysis in grain quality determinations.

The thesis will briefly examine the traditional methods of grain analysis and investigate the new instrumental methods that will be replacing many of these techniques over the next decade.

2.2 Characteristics of Barley

Barley, *Hordeum vulgare L.*, like many cereals, is an annual broad-acre monocotyledon crop that is grown widely throughout Australia, particularly in the southern states. It frequently has weak straw and needs to be harvested quickly when ready to minimise lodging and weather damage (Hessayon, 1982). However, the grain must be dry to ensure that the grain stores well. For this reason, Victorian grain standards dictate that the moisture content of harvested grain must be twelve percent or less (Vicgrain, 1999).

Barley grows in two-row or six-row cultivars. The rows refer to the number of columns of grain running along the head (See Figure 2.1 for an example of a two-row malting barley). All malting-grade barley grown in Australia is two-rowed.

Figure 2.1 Barley Head, Two-Row, Franklin

There are numerous characteristics of barley that make it one of the most widely grown cereals in the world. Agronomic characteristics contributing to this popularity are its ability to grow and yield well in a vast range of climatic and soil conditions (Gilmour, 2000). This agronomic robustness is augmented by the versatility of the grain, which is used for human and animal food and also in the malting, brewing and distilling industries. The morphology and composition of the grain will be discussed to help understand this versatility and explore why many of the common barley quality tests are important in determining the end use of the barley.

2.2.1 Morphology and Composition of Barley

Two barley kernels, each showing opposite sides of typical kernels, are displayed in Figure 2.2. A transverse section of a barley kernel is shown in Figure 2.3, taken from Stuart (1997). As the various components of the kernel are discussed, please refer to these figures to identify their location. The discussion will tend to work from the outside to the centre of the seed.

Figure 2.2 Barley viewed from the Ventral (crease visible) and Dorsal Sides

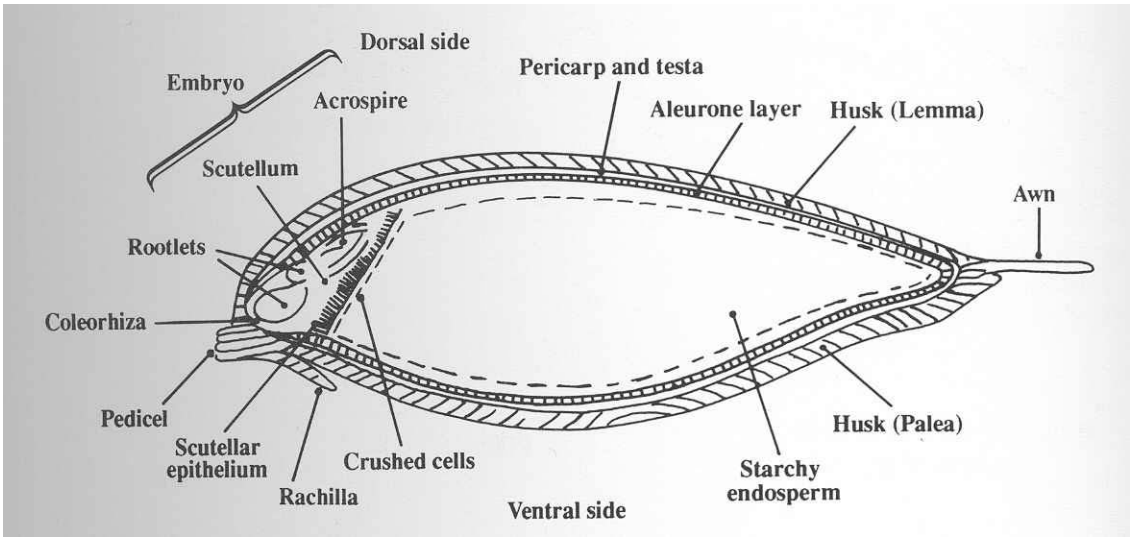

Figure 2.3 Transverse Section of Barley

Husk: Harvested barley generally has its husk, derived from the floral envelope, attached to the kernel as shown in Figure 2.2. The husk on the ventral side of the kernel is called the palea, while the husk on the dorsal side is called the lemma (Hoseney, 1986). The pedicel is the scar tissue left from where the kernel was attached to the rachis, which is the “stem” section of the head that all the kernels were attached to. The husk forms 7 to 13% of the dry weight of the grain. It is dead tissue composed of indigestible fibre and protects the kernel and the acrospire from damage (Stuart, 1997). The husk is useful in the brewing industry where it forms a filter bed for straining the wort and is a good

source of roughage in animal feed (Goldsmith and Shears, 2001). It is the husk that carries most of the kernel's microbial contamination load and is discoloured by weathering and fungi in unfavourable seasons or when harvested too late (van Nierop, Cameron-Clarke and Axcell, 2004).

There are some hull-less varieties, but they comprise only a small portion of the Australian barley industry and are rarely used for malting. Hull-less barley is more commonly grown in countries where barley is used extensively as human food.

Awn: This is essentially an extension of the husk, which projects from the husk on the end of the seed opposite to the embryo. It is typically many times longer than the kernel itself as seen in Figure 2.1, but it is broken and shortened to a stub in the harvesting and seed cleaning stages.

Pericarp and Testa: These thin layers, just beneath the husk, also protect the kernel from damage. As these layers age, they allow moisture and oxygen to penetrate into the embryo and permit the seed to germinate.

Aleurone Layer: The aleurone layer lies inside the pericarp and testa layers. This layer, two or three cells thick, stores oils and proteinaceous enzymes. When triggered by moisture, oxygen and hormones from the embryo, the cells are activated and release hydrolytic enzymes that break down the cell walls and the starches in the endosperm.

The pericarp, testa and aleurone layer together comprise the bran.

Endosperm: The endosperm makes up 70 to 80% of the dry weight of the kernel. It is the storehouse of the kernel. When mature it is packed with large dead cells filled with large and small starch granules in a protein matrix. The cell walls are composed of approximately 75% (1-3, 1-4) beta-glucan and 20% arabinoxylan (Stuart and Gooden, 2001). High malting quality endosperm has a white "mealy" appearance, while high protein endosperm has a translucent greyish "steely" appearance (Briggs, 1978). The whitish appearance is caused by air spaces in the endosperm. The more open structure

facilitates the movement of moisture, oxygen and enzymes through the endosperm, allowing rapid and even modification during malting. There is evidence that the extent and strength of bonding between the starch granules and the protein matrix also affect malting quality (Brennan et al., 1996).

Embryo: The rest of the kernel is generically called the embryo. It is a complex of living tissues that initiate germination and produce the roots and stems of the growing plant. It comprises 2 to 5% of the dry weight of the kernel. Millers refer to this portion of the kernel as the germ or pollard.

2.2.2 Germination

Growth is initiated when the kernel absorbs sufficient moisture to solubilize and activate various enzymes and enough oxygen to provide the high energy levels needed to sustain growth. The key hormones initiating germination are the gibberellin growth hormones, particularly the gibberellic acid GA_3. In addition to their effects within the embryo, these hormones move into and along the aleurone layer where they trigger the production, activation and release of beta-glucanase and xylanase, which break down the cell walls in the endosperm. They also initiate the production of proteases and diastase enzymes in the aleurone. The proteases degrade the protein matrix, while the diastase break down the endosperm starch into simple sugars that can be utilised by the embryo. The most important diastatic enzymes are alpha- and beta-amylase and limit dextrinase (Stuart and Gooden, 2001; Stuart, 1997). Maltsters refer to this process of converting the starchy endosperm into sugar as modification. Modification begins near the scutellum (the membrane between the embryo and the endosperm) and the aleurone layer and slowly moves deeper into the endosperm. The last portion of the endosperm to be modified is the central area the furthest from the embryo (Briggs, 1978, pg 13). It follows logically from this that large kernels take longer to modify than small kernels as the enzymes travel further from the aleurone layer to the centre of the endosperm in larger kernels.

Coleorhiza, Rootlets and Chit: The coleorhiza is the root sheath of the embryo. As germination continues, the rootlets begin to grow, pushing the coleorhiza out through the

husk. It initially appears as a single white protrusion near the pedicel that is called a chit. The chit is the first easily observed evidence that the kernel is germinating. The rootlets eventually break through the coleorhiza and continue to grow as several separate rootlets.

Acrospire: The acrospire, or coleoptile, begins growth by extending along the dorsal side of the kernel beneath the lemma. After several days it becomes longer than the kernel and protrudes from the awn end. If germination continues, the acrospire will become the initial stem and leaf of the barley plant.

2.3 Malted Barley

Most of the barley grown in the world is used directly for food, either for humans or for animal feed. Barley grown for these purposes is generally high yielding and often has a high protein content. However, feed barley purchasers will accept any protein level above 8 % as they are usually more interested in barley as an energy source than a protein source (Swan, 2000).

Swan states that malting grade barley needs protein in the 9.5 to 11 percent range. This range differs somewhat from that of the Malting and Brewing Industry Barley Technical Committee's (MBIBTC) criteria for selecting new malting varieties, which is 10 to 12 percent (MBIBTC, 2001). Higher protein levels reduce the amount of fermentable sugars that can be produced from the barley by replacing some of the starch with protein. High protein malts tend to produce poorly filtering, cloudy beer, while low protein malt has insufficient diastatic enzymes for efficient starch conversion and a poor head (Edney, 1996). Obviously there is a need for protein tests to decide if a barley sample's protein content falls within the acceptable malting range. These tests will be discussed later.

Malting consists of steeping (soaking) and germinating the barley to initiate the enzymatic degradation of the protein matrix and cell walls and the conversion of starch into simple sugars. Kilning (roasting) the germinated barley halts the conversion and preserves the malt by reducing the moisture content to about 4%. When used for brewing beer, the malt is crushed and mashed (mixed) with warm water to complete the

modification of the starches and release the sugars. After filtering, the resulting sugary solution (wort) is converted into alcohol by yeast during fermentation. Malting the barley is necessary as yeast is unable to utilise starch to produce alcohol.

Malting-grade barley generally undergoes the same tests as feed barley, such as moisture content, protein and sieving tests, but the acceptance criteria are different. The barley tests will be discussed in section 2.6. Malting barley also requires additional specialised tests to ensure that it is suitable for malting and brewing. Many of these tests require the barley to be micro-malted so the quality of its malt can be examined. As it takes approximately six days to malt barley (micro-malting is not quicker than commercial malting, it is simply small-scale malting), there is a real need for tests that can predict the malt quality of a barley cultivar without malting it.

The malting process is manipulated to produce malt with properties matching the malt users' specifications as closely as possible. The usual aim is to produce malt with as high a fermentable extract as possible while still retaining a useful level of diastase and minimising mass losses due to leaching and excessive rootlet and acrospire growth.

Maltsters can only produce high quality malt if they have homogeneous malting grade barley. This means that they need to begin with uniform sized kernels of a single cultivar that performs in a consistent manner during malting (Edney, 1996). Blended grain lots that contain different cultivars can create major malting problems for them. Screening assortments, cultivar identification and micro-malting tests are necessary to help them ensure that they have a suitable lot of barley.

Although barley is the most commonly malted cereal, other malting candidates are wheat, sorghum and millet (Agu and Palmer, 1998; Igyor, Ogbonna and Palmer, 1998; Muoria, Linden and Bechtel, 1998).

2.3.1 Micro-malting

Micro-malting allows maltsters to test the response of small grain samples under various malting conditions to help them optimise the performance of the grain before processing a full batch of commercial malt. These tests are especially useful when a new cultivar or the new season's barley is first available. The micro-malting can confirm if the barley's germination inhibition period is over and determine the barley's optimum malting conditions.

Traditionally, micro-malting was performed by the "stocking malting" method. A sample of the lot to be tested was placed in a strong mesh bag. The bag was then placed into the steep vessel and processed along with a large batch of commercial malt. Though this test had some value, the barley in the stocking could not be turned correctly nor did it allow the maltster to vary the malting parameters to suit the sample being tested (Briggs, 1978, pg 532).

The development of computer-controlled micromalters during the 1980's permitted accurate testing and tailoring of malting conditions to suit each lot of barley (Wrigley, 2000). Modern micromalters, such as those manufactured by Phoenix Biosystems and Joe White Maltings, are completely automatic and perform steeping, germination and kilning all in one chamber. The standard Joe White system can run batches containing from 250 grams to eight kilograms. A typical micro-malting process is given in the MBIBTC guidelines (2001).

2.3.2 Malt Quality Tests

Many of the malt tests specifically gauge the barley's suitability for beer production. A unique test given by the European Brewing Convention (EBC) for whole malted barley is the friability test, which crushes the kernels to estimate their ability to break down into fine particles (EBC, 2003, Method 4.15). Another malt test is the Calcofluor modification test (EBC, 2003, method 4.14). The remaining tests are performed on the mash (mix of ground malt and water) or wort (the liquor extracted from the mash).

2.3.3 Mash and Wort Tests

Currently the most important quality tests made on the mash and the wort are: hot-water extract (how much of the crushed malt can be solubilised in hot water), fermentability (how much of the extracted material can be fermented), viscosity, soluble nitrogen, free amino nitrogen, diastatic power, percent mealy, homogeneity and colour. Diastatic power is a measure of how much active starch-reducing enzyme is left in the kilned malt. Percent mealy is the percentage of endosperm that has an opaque, easily hydrated structure. Homogeneity is a measure of how evenly a barley lot malts. There are standard procedures for all of these tests given by the American Society of Brewing Chemists (ASBC), EBC and the Institute of Brewing (IoB) (ASBC, 1992; EBC, 2003; IoB, 1997).

2.4 Characteristics of Wheat

Wheat (*Triticum aestivum L.*) is more commonly grown in Australia than barley. It has longer growing and grain filling periods than barley and tends to be higher yielding. Most of the agronomic comments made for barley also apply to wheat. The principal differences are that wheat has a stiffer straw, is almost universally a six-row crop and produces hull-less kernels when harvested and threshed. Genetically, modern commercial bread wheat is quite different from barley as the wheat tends to be hexaploid, having six copies of its deoxyribonucleic acid (DNA) in each cell (Palmer, 1989).

Wheat often has a higher protein content than barley. Wheat protein also has a higher percentage of gluten, the complex sheet-forming proteins that are responsible for trapping gas in the dough, allowing leavening to occur (Charley, 1982; Shewry, 1996).

2.5 Current Use of Wheat and Barley in Australia

2.5.1 Feed

Wheat and barley are major ingredients in the animal feed industry. The proportion of our annual crops of these grains used in the feed industry depends on a complex mix of factors including the size of the livestock industry and the availability and price of these

grains relative to other feed ingredients (Hafi and Rodriguez, 2000). Table 2.1, sourced from the Australian Bureau of Agricultural and Resource Economics (Hafi and Connell, 2003), shows the importance of these grains in 2003. It is evident that the feed industry is a major user of Australian barley.

Table 2.1 Use of Grains in the Feed Industry in 2003-04

	Kilotonnes	% of Total Feed	% of Total Grain Production
Wheat	2854	26.2	26.1
Barley	1573	14.5	43.3

2.5.2 Milling

The milling industry in Australia is predominately dedicated to the milling of wheat. Very little wheat is eaten domestically as whole kernels.

The normal milling process begins by cleaning the grain to remove contaminants such as stones, pinched grain (thrus) and other non-millable material. Next is a conditioning step where the grain is wetted until it reaches a specific moisture content over 24 hours. This process makes it easier to remove the bran from the endosperm as large, non-adhering flakes. Finally the milling begins, with the grain being fed through a series of breaking and milling rollers with different roller surfaces and steadily decreasing roller gaps. The material is sieved throughout the process, allowing the removal of both flour by-products such as bran and pollard and specific milling fractions such as kibble and semolina. Where appropriate, material is fed back into the system to maximise the white flour yield (McMaster, Moss and Southan, 2000).

As was the case with micro-malting, small-scale test milling, commonly performed with a Buhler test mill, is not able to greatly reduce the time required for the milling process. Though there are fewer rollers and sieving steps in a test mill, it uses essentially the same process. There is still a 24-hour delay to condition the kernels and it then takes about forty-five minutes to mill the sample. The sample size required is also considerable,

requiring approximately two kilograms of grain. There are also some problems with inter-laboratory correlations with the test milling results (Mugford and Southan, 2003). The test mill sample size and throughput have recently been improved substantially by the introduction of the Quadrumat Junior mills (Morrison, Pleming and Allen, 2001). Morrison *et al* were able to reduce the sample size to 20 grams and the actual milling time to less than ten minutes without a significant loss in accuracy. The conditioning time was unchanged.

When barley or rice are milled, special de-hulling equipment is required to rub off the hulls (or glumes) before milling begins.

The large sample size is a problem for wheat breeders, while the time required is inconvenient for grain dealers and millers as well as for breeders. Because of these difficulties, some methods to predict the milling yield of wheat have been developed with varying success (Ali, 1968; Dexter and Symons, 2000; Dines, 2001). This thesis makes a contribution to this effort.

Milling procedures and grain lot selection vary with the intended end-use of the grain being milled. There are many end-uses that require different flours such as durum semolina for pasta, various types of noodles, bread flour, cake flour, biscuit flour, etc. More complex testing is demonstrating that the quantity and specific blend of proteins and starches in the grain are critical to these different uses (Batey, Skylas and Wrigley, 2002; Shewry, 1996; Siriamornpun et al., 2001).

As with barley, cultivar identification is needed to make sure the correct wheat is being used. Millers also need to know the kernel thickness distribution of the grain lot too as variations in the kernel sizes change how the grain performs during milling (Webb et al., 2000). They, like maltsters, prefer kernels with uniform dimensions.

2.5.3 Malting

The malting process was outlined previously. Most malt made in Australia is prepared from barley and used for beer production, with a small amount used in the distillery industry and in malted milk drinks such as Milo. Small quantities of wheat are malted for use in wheat beers.

2.5.4 Other Uses

Other grain treatments used in Australia are largely limited to polishing, which is used to grind the bran and aleurone layers off pearled barley and white rice. Polishing in Australia tends to take off more material than in some countries in an attempt to remove most of the embryo, as its high fat levels contribute to rancidity and off flavours on storage (Blakeney, 2004).

This thesis, because of space constraints, will not discuss the final end-use of the various grains, malts, flours, etc in making beer, bread and so forth.

2.6 Analysis of Wheat and Barley

Rapid, accurate and non-subjective grain tests help growers and processors agree on a fair price and ensure that the grain is used for the most appropriate purpose. Usually a battery of tests is required as each test contributes to a more comprehensive assessment of the grain. The tests also guide breeders when selecting new cultivars.

2.6.1 Sampling

2.6.1.1 Binomial Sampling Considerations

Sample size is a critical factor in cereal analysis, especially when examining morphological aspects of the cereal. In many cases, the sample is being taken to assess binomial properties of the grain lot, such as what percentage of the grain lot is/is not undersize, etc. Binomial comparisons are more sensitive to sampling issues such as representative sampling and sample size than simple measurements such as the average

kernel weight of a sample. But as binomial properties are frequently tested, their mathematical sampling properties are a useful introduction to sampling issues.

Figure 2.4, based on the work of Berstein (1971) and Wrigley & Baxter (1974) illustrates the sampling size concerns. It shows the mathematical accuracy limitations of sub-sampling a simple binomial distribution. As one example, the sample can be regarded as a mixture of grain that is less than 2.0 mm thick and grain that is 2.0 mm thick or thicker. This mixture, if the actual level of the less than 2.0 mm group counts is 5% of the total, and the total is about 225 counts, can only be sampled with an accuracy of +/- 3% at a confidence level of 95% (Figure 2.4). Though the maximum accuracy decreases rapidly with smaller sample sizes, it only increases slowly with increased sample sizes. For example, increasing the sample size to 500 only increases the maximum accuracy to 1.9%. Even with a sample of 3000 counts, the maximum accuracy is still 0.8%. To make matters more difficult, as the proportion of the admixture increases, the maximum error also increases (data not shown).

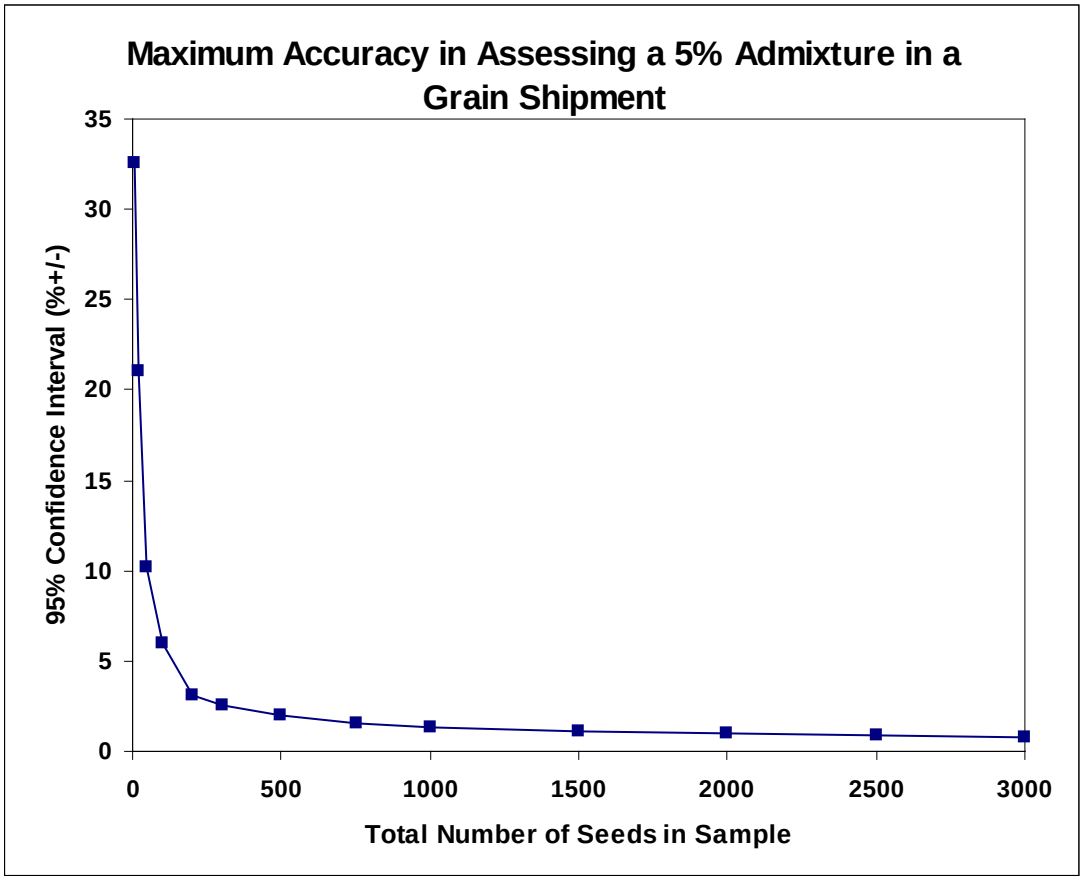

Figure 2.4 Binomial Distribution Limits on Sampling Accuracy

The sampling accuracy issue directly relates to how accurately any sampling system can estimate the proportions of different material in a mixture. It is obvious that the larger a sample size that can be used, the greater the potential accuracy of the system. It is also obvious that with poor sampling techniques, the actual accuracy will be worse than these mathematical optimums.

2.6.1.2 Small-scale Sampling

The use of sampling devices is of paramount importance in obtaining representative sub-samples when working with sub-samples that are a small percentage of the original grain lot (Parker, Bauwin and Ryan, 1982, pg 3). D. E. Briggs (1978, pg 175) goes so far as to say “Without due care in sampling, subsequent analyses are valueless.”.

The need for accurate sampling methods arises when grain is handled and transported as these processes cause segregation based on differences in both density and particle size. With increased handling, the segregation increases. A sample that has been transferred, shaken while being transported, re-transferred, etc will tend to have lighter material rise to the top and the smaller, denser seeds will settle to the bottom. As well as these natural problems, the possibility of deliberate segregation (eg, a layer of high quality seed covering poorer quality seed) needs to be addressed. A good sampling system will deal with all of these sources of error and produce consistent, representative sub-samples.

Traditional methods of removing or reducing samples.

In this project, sample (plot) masses ranging from 250 grams to ten kilograms are commonly encountered. For most tests, a blended sample representing one cultivar derived from two to four plots is used. The sampling methods used must be able to produce accurate, reproducible sub-samples from these plot samples. As the analytical testing uses samples varying from two kilograms (test milling) to less than a gram (enzyme testing), the sampling equipment needs to provide large variations in the ratio of input to output sample size.

Quartering Irons

The earliest representative method of sub-sampling was the use of quartering irons. These are made from two vertical strips of metal joined at their centers to form a cross. The sample is cut by being poured onto the join, and is distributed more or less equally into the four quarters. To obtain a small sub-sample, one quarter is retained each time and requartered by the above process until the desired mass is reached. This method improves the consistency of the sample by ensuring that each sample includes representative portions from the edge to the center of the grain mound.

Attaching a funnel to the quartering irons would reduce possible errors due to the pouring process itself (ie – lighter portions of the grain may tend to be carried into the more distant quarter or uneven distribution can be caused by not pouring exactly onto the

center of the join). The funnel would force the reblending and then a symmetrical distribution of the seed into the four quarters.

Quartering irons are simple to make and allow reduction down to 1/64th of the original mass in three passes. However, they require substantial bench space and it is tedious to collect the grain fractions from the bench top after each split.

Riffler

The riffler, also known as a Blom sample divider, consists of a box containing a series of chutes, the outlet of each one facing the opposite direction to its immediate neighbours. Three identical rectangular boxes are also part of this device. The sample to be sub-divided is placed into one box and the other two boxes are placed under opposite sides of the riffler. The sample is then poured into the top of the riffler and approximately half of the sample is collected in each of the two sub-sample boxes. Depending on the number of sub-samples or amount of sub-sample reduction needed, the process is repeated until the desired result is achieved. For example, to reduce a 6.4 kg sample to a 100 g sub-sample requires 5 passes through the riffler.

The riffler uses less bench space than the irons and the split samples fall directly into containers, making it easier to use. The design is also superior to the irons in that it is less prone to sample segregation during processing, giving more representative samples than the irons. However, it can only reduce the sample mass by half with each pass, so it requires more cuts than the irons to achieve similar reductions.

Boerner Divider

The Boerner divider is in essence a more sophisticated version of the riffler. This device uses a series of slots set around the edge of large cone. The slots lead alternately to either an inner or outer funnel below the main cone. These funnels then feed into separate receptacles. This design minimises any sample segregation due to sample pouring methods, but is, like the riffler, restricted to 50 % reduction of the sample with each pass.

Sampling Spears

Small scale sampling spears designed to take small samples from a bag or bucket full of grain have been used for many decades, and probably for many centuries. Early versions of bag spears were simply hollow tubes with an open pointed end that was inserted into the side or top of a bag. Modern spears have different opening shapes, internal dividers or rotating sleeves to enhance representative sampling as illustrated in the European Brewery Convention Analytica methods of analysis handbook (EBC, 2003). The Analytica also contains schematic diagrams of the previous three types of sample dividers.

The spears have the advantage of producing a small sub-sample of approximately the same volume from an initial sample of varying volume in a single step. Spears with different internal diameters and varying the distance that the spear is inserted into the bag or bucket allows considerable control of the sub-sample volume. The spear design developed by this author for SeedCount^{TM} Digital Image Analysis (DIA) sampling is detailed in section 3.4.2.2.

2.6.1.3 Bulk Sampling Devices

This research did not require the removal of representative samples from very large containers such as truckloads or shipping containers containing many tonnes of seed. These large-scale methods include larger and more complex sampling spears and pelicans and other devices for sampling grain while being transferred. They are thoroughly covered in such publications as the United States Department of Agriculture Federal Grain Inspection Service (FGIS) Grain Inspection Handbook (Book 1) (FGIS, 1997) and Mechanical Sampling Systems Handbook (FGIS, 1995).

One more comment needs to be made. The bulk sampling devices listed above generally produce sub-samples that are still large enough to fill a bucket or small bag. There is still scope for poor sampling when removing a further sub-sample from this material for use in a particular test. The author observed this personally while at a grain receivals depot near Toowoomba, Queensland. The receivals operator carefully used a vacuum assisted

spear to remove five cores of grain from a truck. The grain was transferred to a bucket. The operator was then faced with the dilemma of sub-sampling: would he either scoop his half-liter for the test weight test directly from the bucket, thereby selecting mostly material from the last spear-full of grain, or would he stir the material in the bucket to mix the spearloads, and risk segregating the grain in the stirring process? He chose the latter path. Discussions with the person taking us on the tour of the site revealed that the bulk receiver organisation had tried previously to prevent this problem by issuing rifflers to each site. They found that within two weeks none of the operators would use the rifflers as it slowed down their truck throughput too much (Leaman, 2002). It is this author's opinion that they should instead consider using a short sampling-spear designed to extract the required amount of grain through the depth of the bucket in a single operation.

2.6.2 Moisture Content

Moisture is one of the critical quality parameters of grain. Not only is low moisture required to prevent the grain deteriorating in storage, the moisture level has other ramifications. If the grain is too dry before harvest, head shattering and kernel breakage will increase. Specific moisture levels are required for some grain uses. As the moisture content of the grain falls, the percentages of the other components increase. Because of these interlocked relationships, many of the other cereal component tests are reported either on a dry or a standardised moisture percentage basis. The most common methods of measuring grain moisture are detailed below:

2.6.2.1 Oven

The official method of moisture determination is still oven drying for most of the cereal industry. For example, the Institute of Brewing (IoB) moisture method 1.2 (IoB, 1997) begins by grinding and weighing out a grain sample of about 5 grams. The sample is then dried in a 131^{o}C oven for two hours, cooled in a desiccator and reweighed. The weight loss is assumed to be the moisture content of the original sample. The EBC method is identical. The American Society of Brewing Chemists (ASBC) method 5

(ASBC, 1992) varies from the IoB by reducing the sample mass and drying time both by half.

However, the delay imposed while grinding the sample and drying and cooling the grist are not compatible with the rapid decisions needed by the bulk grain handlers receival points. Their operators typically only have three minutes to test and decide what to do with the truckload of grain in front of them. A variety of rapid moisture measuring devices have been developed to meet this need. Most of these devices need careful calibration against the primary standard of oven-dried moisture determinations.

2.6.2.2 Infrared Driers

One method of providing faster moisture determinations is the use of high-speed infrared lamp driers with built-in balances. These systems are essentially an extension of the oven method. They still require the grain to be ground before use. However, they automatically weigh the as-is sample, dry the grist with infrared lamps and as soon as the sample weight stabilises, calculate the sample moisture and print out the result. Excluding milling, they can calculate the grain moisture in about five minutes (Denver Instruments, 2004).

2.6.2.3 Electronic

Electrical moisture meters generally measure the dielectrical properties of the grain using resistance or capacitance. The instruments often produce a result within a minute, but need separate calibrations for each type of grain. The results are affected by grain density and temperature and can be inaccurate (Alizaga, Zeledón and Jiménez, 1994). There are many manufacturers of these instruments, which are often inexpensive and portable. FGIS use a Motomco moisture meter (FGIS, 1997). New microwave dielectric instruments are being developed which may overcome these problems (Nelson and Trabelsi, 2002).

2.6.2.4 Near Infrared Spectroscopy

The Australian bulk grain handlers use near infrared reflectance (NIR) or transmission (NIT) spectroscopy instruments to determine the grain moisture at their receival points. These instruments can be accurately calibrated and the newer systems can work on whole grains, removing the delay involved in grinding the grain before testing can proceed. In most cases a result can be displayed within 45 seconds. NIRS, covering both NIR and transmittance NIT is a common term for these instruments.

These systems do not actually dry the grain, but rather measure the absorption at particular wavelengths in the reflected/transmitted light and use this to calculate the amount of moisture present in the sample. Common cereal NIRS spectrometers are those manufactured by Foss and Perten. The whole grain instruments are essentially non-destructive, thus allowing the sample to be used for other testing too. However, they often require at least 200 ml of sample for an analysis (Perten, 2004).

2.6.3 Protein

Protein is an essential nutrient for all living things. It is also a critical part of cereals, where its quantity and quality dictate the final use of both wheat and barley (Anderson and Blechl, 2000; Dexter and Symons, 2000; Garcia del Moral et al., 1998; Larroque et al., 2000) .

A great deal of research over the past fifty years has been dedicated to isolating various proteins, including those functioning as enzymes, and determining their characteristics and effects under various conditions (Batey, Skylas and Wrigley, 2002; Larroque et al., 2000). However, this advanced work is not used in this thesis as the visible light DIA systems cannot measure protein. Only the common bulk protein measurement systems will be mentioned, as some effort has been made to correlate the DIA properties with flour and extract yields, which are linked to total protein levels (Berman et al., 1996; Garcia del Moral et al., 1998).

2.6.3.1 Kjeldahl

Kjeldahl is the classic system of protein determination. Its use for cereals is detailed in the IoB Method 1.8. It uses concentrated sulphuric acid, a catalyst and heat to dissolve the cereal sample. The digest is then made alkaline with sodium hydroxide and distilled to remove the ammonia produced as a digestion product from the protein. The amount of ammonia is determined by titration and used, with a conversion factor, to estimate the amount of protein in the original sample. The process involves extremely corrosive chemicals and is being replaced by the Dumas method as the standard method. There is also a direct alkaline distillation version developed specifically for cereals (RACI-CCD, 2003).

2.6.3.2 Dumas

The Dumas combustion method burns the sample in a high temperature oven in pure oxygen. The nitrogen oxides are reduced to nitrogen gas and quantified with a thermal conductivity detector. It is both safer and faster than the Kjeldahl method. A general Dumas method is given in EBC Analytica Method 3.3.2.

2.6.3.3 Near Infrared Reflectance Spectroscopy

NIRS uses the absorption of various molecular bonds to quantify the amount of protein in the sample, similar to its use for quantifying moisture. The instrument uses either a series of filters or a monochromator to select the optimal wavelengths. NIRS needs to be extensively calibrated against either Dumas or Kjeldahl nitrogen results, as detailed in the EBC Analytica Method 3.13. As a NIR instrument can also estimate the sample's protein at the same time as assessing its moisture, there is virtually no extra work required to make this assessment.

2.6.3.4 Digital Image Analysis

The charge-coupled devices (CCD) used as light detectors in video cameras and scanners are very sensitive to near infrared radiation. The lamps used in these applications are also

strong emitters of such light. These facts suggest that the systems could potentially be used as NIRS to measure protein and moisture too. However, standard DIA systems use special filters to limit them to visible light wavelengths and thus they are not able to estimate cereal protein content. Color systems use a more complex set of filters than greyscale systems. They use three-row banks of CCDs with a red, green or blue filter in front of each bank. The RGB outputs approximate the color of each pixel (Kodak, 2003).

Though it may be possible to couple one of the newer diode array NIRS to a DIA system to add NIRS functions to it, this has not been attempted in this project.

2.6.4 Kernel Weight

Plant breeders, maltsters and the rice industry commonly use the thousand-kernel weight (TKW) test because it provides useful information on seed morphology. Kernel Weight (KW) is, quite expectedly, the weight of a kernel of grain expressed in milligrams. The Thousand Kernel Weight (TKW) is really the same measure, but expressed as grams per thousand kernels. Thus a TKW of 36 grams equates to a KW of 36 milligrams. A common alternative name is Thousand Corn Weight (IoB, 1997).

Knowing a grain lot's TKW is valuable to maltsters and millers as high TKW kernels are plumper (ie thicker), malt and/or mill more evenly and have a higher flour or extract yield (Burger and LaBerge, 1985). This relationship is partially accounted for by the work of Crewe and Jones (1951) who demonstrated that the thickness of wheat bran remains stable over a wide range of KW. This means that larger and plumper grains (which have a higher KW) contain a higher proportion of endosperm as will be discussed in section 6.1.6. More endosperm means more flour. Dexter, Matsuo and Martin (1987) showed that there was a highly significant ($P < 0.01$) relationship between durum wheat's KW and its milling performance and spaghetti quality. Each one milligram increase in KW matched with a 0.5% increase in semolina yield.

The high TKW grains also produce more attractive malt (Stuart, 1998). TKWs assist breeders in selecting large kernel cultivars and permit growers to calculate their optimum

sowing rates (Schwarz and Horsley, 1995). The instant appeal of large, heavy kernels in many markets makes a high KW a desirable property.

Some studies have found a significant correlation between KW and flour yield (Ali, 1968; Evers et al., 1990).

The sampling method and the sample size used in determining TKW is critical. Hudson (1960) reported that individual random samples of 1000 kernels from an ungraded bulk sample could vary by as much as 12% from the "true" TKW for the entire bulk sample. He found that it was necessary to examine samples of 5000 kernels to achieve accuracies within 4% of the "true" TKW. This thesis will test his claim that variations would be this large and investigate this author's suggestion that at least part of these variations were due to poor sampling methods.

2.6.4.1 Hand-Counting TKW

Hand-counting the kernels for a TKW determination is tedious and time-consuming. Using a seed tray, which has indents to hold 100 kernels, speeds up the process and reduces the tedium, but it still requires 10 to 15 minutes to count the standard 40 grams of seed for barley TKWs using the IoB method 1.3 (IoB, 1997). As the method requires this to be done in duplicate, the time required is actually 20 to 30 minutes. For a typical barley lot, each 40-gram sample contains approximately 1000 kernels. The EBC method is identical. Both methods specify the removal of trash and broken kernels from the count and mass.

Many labs find the IoB process much too time consuming and use abbreviated versions of this method. The American Society of Brewing Chemists (Method Barley 2-D) condones using a single 15-gram sample to determine the TKW, claiming that "it is impractical to count out by hand 1000 kernels" (ASBC, 1992). This sample would be less than 400 kernels. Contrary to Hudson, they report only a 0.3 to 0.7% reproducibility error. The author strongly suspects that they have achieved their low errors by merely

sending around a specific 15-gram sample for each lab to weigh and count. If there is no sub-sampling required, it is impossible to test for sub-sampling errors.

This suspicion is virtually confirmed by the IoB's reported reproducibility error of 2.01 on a grain lot with an actual KW of 41, which is an error of +/- 4.9% while assessing sub-samples of almost 2000 kernels. Though the IoB result is better than Hudson would suggest, it bears little resemblance to the ASBC results.

2.6.4.2 Mechanical TKW

Laboratories that make frequent TKW determinations usually use electromechanical seed counters such as the Numigral or Countador counters. Indeed, the new EBC method recommends the use of such a counter (EBC, 2003). These counters can be used in either a full-count mode that counts everything put into it or in a countdown mode that stops at a specified number of kernels. The rice industry commonly uses a count-down method to count out 1000 kernels which are then weighed to give the TKW directly.

Though these systems are much less operator-intensive than hand-counting, they cannot remove the trash and broken kernels. To achieve accurate results the operator must still perform this step manually. It is also possible that when working in countdown mode the vibration of the counter will segregate the kernels and therefore preferentially count the smaller and denser seeds. Placing the minimum amount of seed required into the counter can reduce this possibility. This source of error is totally eliminated when the counter is used in the full-count mode.

2.6.4.3 Single Kernel Characterisation System

The Perten Single Kernel Characterization System 4100 (SKCS 4100) can analyse the kernel weight, diameter, hardness and moisture content of 300 individual kernels of wheat or barley in three minutes. The individual kernel by kernel analysis allows the SKCS to determine the uniformity of a grain sample (Perten). It is, however, ultimately a destructive test as it finally crushes the kernel to assess its hardness.

As the system individually counts and weighs the kernels it should be able to produce both an accurate average kernel weight and KW distribution of its 300-kernel sample. It is, however, limited in its applicability to the bulk grain lot by its small sample size. Dexter and Symons (2000) produce SKCS KW data, but do not attempt to correlate it with any objective KW standard, so there is no indication of how accurate the SKCS is. They also compare SKCS and DIA systems, but do not calculate a DIA KW, so there is no indication of the relative accuracy of the two methods.

Dexter & Symons (2000) found that the milling performance of durum wheat kernels with a weight below 40 mg was poor. They also found that the SKCS KW was strongly correlated to the durum wheat ash and color yields.

SKCS output has also been used to estimate kernel density and durum vitreosity (Nielsen, Pedersen and Munck, 2003; Perten).

2.6.4.4 Digital Imaging

Weighing the kernels poses no difficulties for any of these KW methods as accurate analytical balances are readily available. The major differences between the various methods occur in their counting accuracy. Digital image analysis (DIA) has the potential to count the kernels rapidly and accurately, but clumps of touching kernels are difficult to resolve into individual kernels for counting. Simply ignoring the touching grain results in inaccurate TKWs because only part of the sample mass is actually used in the count. DIA systems have been developed that use V-corrugated conveyer belts (GrainCheck, distributed by Foss Tecator, Sweden) or vacuum-assisted tray-filling equipment (SPY Grain Grader by Maztech, Canada) to physically separate the seeds (Nutech Analytical, 2003). These systems can work reasonably well, but they still have some touching seed problems and their specialised hardware makes the systems very expensive.

Another approach to DIA counting is to use commonly available computers and flatbed scanners and develop an algorithm that will count all of the single and touching grains in a randomly distributed sample. Shatadal (1994) developed a shape recognition algorithm

that digitally cut apart touching grains with 93% accuracy. Though this is an impressive achievement, it falls far short of the 100% accuracy the grain industry would like.

A different software approach to separating touching seeds developed by the author and his co-workers on the SeedCount DIA project will be presented later in this thesis.

Another approach to improving the count accuracy of DIA systems is to use an indented tray similar to the hand-counting trays that physically separate each kernel. This approach was used by Edney, Bassily, & Symons (1998), who had a 50 kernel tray. Foss use a circular indented tray in their new Cervitec 1625 Grain inspector (Foss, 2004) and SeedCount further developed the indented tray idea with a novel bi-modal indented tray developed by this author. Details of the SeedCount indented tray system will be presented later.

These separation systems are not perfect and sometimes more than one kernel, or broken kernels, will sit in one indent. Methods of identifying and eliminating the multiple and broken kernels as well as dockage material are required to achieve perfect counts. Some of the methods used by SeedCount to detect these errors will be discussed in sections 5.3.5.4 to 5.3.5.7.

2.6.5 Hectolitre Weight

2.6.5.1 Standard Test Weight

The hectoliter weight is really a measure of grain packing density. It is also known as the test weight (TW) or occasionally specific weight (SW), which can use different units to the metric hectolitre weight. The United States equivalent is the bushel weight. The metric measure is, as the name suggests, the weight in kilograms of one hundred liters of grain. However, one hundred litres of grain is an awkward amount to handle, and the Australian grain industry instead uses a chondrometer to estimate the hectoliter weight (HW). The chondrometer is essentially two tubes that jointly hold one liter of grain. The bottom section has a slide that can be inserted across it and captures exactly 500 ml of

grain. This half-liter is used to estimate the hectoliter weight (Vicgrain, 1999, Sections 2.4 and 2.5).

Although HW is sensitive to kernel size, packing and settling issues, it provides a rough estimate of the grain sample's specific density. The general idea is that denser grain is of higher quality than lighter grain (Nielsen, Pedersen and Munck, 2003).

There is some evidence to support this contention. Dexter *et al* (1987) showed that there was a strong ($P < 0.01$) relationship between durum wheat's test weight and its milling performance and spaghetti quality. Each 1 kg increase in TW correlated with a 0.7% increase in semolina yield. Evidence will also be presented later in this thesis that supports a link between HW and increased flour yields for ordinary wheat.

In commercial use, the classic two-section chondrometer is usually replaced by a simple cylinder with the same internal diameter as the CBH chondrometer and a 500 ml volume. Rather than a horizontal slide to separate the correct volume, the excess is simply struck off with a vertical scraper. The grain is poured directly into the cylinder. These simplifications make the test weights more prone to variations due to grain consolidation differences (Ge, Zhang and Britton, 2000).

2.6.5.2 Small-scale Test Weights

Small-scale TW measurements have been trialled for some time. Harris and Sibbitt (1942) developed a system which used 100 gram samples and a 50 ml stainless steel measuring cylinder. Dexter's team used a version of this system in their durum studies.

The second and later versions of SeedCount included small-scale containers for measuring the kernel volume to be used in the indented trays. The SeedCount results are presented in section 7.3.3.1.

Troccoli & di Fonzo (1999) developed a test weight system that worked with only 25 kernels, and found that their TW correlated negatively and significantly with DIA-

derived kernel length and perimeter for the same kernels. They also found that two other kernel shape factors, aspect ratio and a circularity shape factor, correlated positively with TW. Though their results are interesting, it is difficult to expect much reliability in the formula they derived to relate these properties due to their tiny sample size.

2.6.6 Screening Assortments

Screens containing holes of a specified shape can be used to separate grain by their physical dimensions. Screening a sample through more than one sieve results in a series of fractions called a screening assortment (ASBC, 1992).

Screening assortments have a long history as a grain quality test, and are still vital today (Henry, 1990; MBIBTC, 2001). They are able to separate foreign material from the grain of interest on the basis of size (eg, large dockage material like whiteheads, backbone and straw will be retained on a screen that will allow the grain to pass through, while small weed seeds will pass through another screen that retains the grain). Screens of intermediate opening sizes can then separate the grain itself into various fractions. Knowing the size distribution of the grain is valuable to maltsters as they prefer grain with a homogenous size as this produces a more uniform malt (Edney, 1996).

2.6.6.1 Mechanical

In its simplest, and likely its earliest, form, screening consists of a woven mesh sieve attached to a frame. Due to the non-spherical shape of most cereals and difficulties with maintaining the mesh spacings, whole kernel screens were soon manufactured from sheet metal with precise slots punched through the metal. Depending on the level of information required, at times only a single screen will be used. Examples are slots 2.00 mm wide for determining the “screenings”, “thins” or “thrus” for wheat and 2.50 mm wide slots for determining “plump” kernels of barley (FGIS, 1997; Vicgrain, 1999). In the first instance, it is the material that passes through the screen that is measured. It must be less than a specified amount for the grain lot to make a particular grade. For the barley, it is the material that remains on the screen that is of most interest. In this case the percentage retained must exceed the grade requirements (Vicgrain, 1999). As will be

discussed in the DIA kernel thickness results (sections 6.1.4 and 6.3.6), these screening separations are based on the kernel thickness.

Some users require more complete information on the sample's kernel thickness distributions. This is normally performed with a stack of screens of descending slot width as the kernels move down the stack. The Institute of Brewing method 1.13 for screening barley specifies the use of three screens including the slot width (2.80, 2.50 and 2.20 mm) and length (25 mm) for each screen, the overall screen size (430 by 150 mm), the sample size (100 g) and the shaking method (horizontally reciprocating), speed (310 oscillations per minute) and duration (5 minutes). The results are reported as percent by mass in each screening fraction (IoB, 1997). As an example, the grain that has passed through the 2.8 mm screen but can't get through the 2.5 mm screen makes up the 2.8 to 2.5 mm group.

MBIBTC recommends a simpler two screen sieving test that is widely used in the malting industry in Australia. These screens have 2.50 and 2.20 mm wide slots and use a 500 ml sample. They require at least 85% by weight of the barley to be retained on the 2.5 mm screen (the Gradings) and no more than 3% by weight can pass through the 2.2 mm screen (the Screenings). They do not specify the screen area, agitation type or time (MBIBTC, 2001, Section 5).

The IoB method contrasts sharply with the common method used in the wheat industry: 500 ml of wheat is placed on a 300 mm diameter screen with 12 by 2.00 mm wide slots and shaken manually or in a tipping oscillation for 20 cycles by an Agtator (Vicgrain, 1999). This takes about 35 seconds.

These two methods are compared in Table 2.2.

Table 2.2 Comparisons of Standard Barley and Wheat Screening Methods

	Screen Area (m^2)	Shake Duration (sec)	Sample Mass (g)	Screen Number	Screen Efficiency
Barley	0.06	300	100	3	**6.5**
Wheat	0.07	35	400	1	**0.62**

The table has an entry that, in the author's opinion, effectively compares the screening efficiency of the two methods. The formula the author developed to produce these values is:

$$ES = \frac{A*T}{M*N}$$

Where:

ES = Efficiency, Screening

A = Screen Area (M^2)

T = Sieving time (seconds)

M = Sample Mass (grams)

N = Number of screens

The rationale for the formula is this:

The efficiency of a screening system is reflected by its ability to separate the material placed in it by its minimum dimension, which is the kernel thickness. (Kernel length and width are defined as the largest and second largest orthogonal dimensions.) Assuming that different systems have equivalent slot designs and shaking methods, they can be compared on the basis of their other properties.

The remaining principal factors reducing the effectiveness of the screen system relate to the access the kernels in the sample being sieved have to the slots in the screen. Increasing the screen area and/or the shaking time will increase the screening effectiveness by improving kernel access to the slots. Conversely, increasing the sample

mass will decrease the access that each kernel will have to the screen slots. Indeed, increasing the sample mass can even reduce the number of available slots in the screen by "blinding' some of the slots when kernels become lodged in them. The use of a stack of screens converts the process into a series of sequential screening operations, effectively reducing screening time on the lower screens by requiring the thinner material to pass through the upper screens first.

Though it could well be argued that specific weightings need to be applied to each factor, the general form of the equation seems valid. There are also questions about the effectiveness of the two different shaking actions and the movement characteristics of barley versus wheat. However, the fact that the IoB Barley method has an ES ten times that of the Vicgrain wheat method does make one wonder how effective the wheat method really is. It is widely known in the wheat industry that the Vicgrain screening method does not have time to completely separate the thins from the sample, but instead relies on some reproducibility which occurs as a result of their consistent use of that method (Leaman, 2002).

It is of interest to note that in their 2002-2003 Wheat Receival Standards that Australian Wheat Board now requires 40 shakes (AWB Limited, 2002). Conversely, few malt labs follow a full IoB screening process, usually reducing the shaking time to two or three minutes to speed up the test.

In the author's experience, the five-minute IoB method fits well with the time taken to clean, weigh and record the material collected from all of the screen fractions. This makes it a smooth process when screening a series of samples. The observation suggests that the five minutes is really a pragmatic time, rather than a required time, for complete separation of the screening groups.

Perhaps the most significant part of the screening assortment testing is its accuracy. This data is given in Table 2.3, reproduced from IoB Method Barley 1.13 and based on results from 9 laboratories.

Table 2.3 IoB Screening Assortment Accuracy

	Mean % by mass	Range	r(95)	R(95)
>2.8 mm	60	22.3 to 79.7	3.49	9.12
2.8 to 2.5	30.3	15.9 to 45.3	2.53	8.79
2.5 to 2.2	12.6	3.0 to 24.4	1.75	2.84
<2.2 mm	6.1	1.0 to 9.5	1.7	2.34

Three items stand out in this Table:

1. As the mean for each screening group increases, the error increases.
2. The inter-lab (R) errors are much larger than the intra-lab (r) errors (eg. 9.12 vs 3.49 for the >2.8 mm group).
3. The errors are quite large. – An error of 9% on a sample with a mean of 60% makes it difficult to compare grain lots with confidence.

The increasing error as the proportion of the sample being segregated increases is consistent with statistical theory (Berstein, 1971; Wrigley and Batey, 1995; Zar, 1984). The intra-lab errors are also consistent with binomial distribution theory when one considers that the 100 gram sample used contains an average of about 2500 kernels.

The larger inter-lab errors suggest that there are some definite issues arising between the labs. The most likely error source here is equipment variation, as laboratory staff involved in inter-lab testing usually take extra precautions to follow their industry standard procedures very thoroughly. The equipment variations that are most likely to affect the results are slot width problems and sieve movement errors (eg worn slots too wide, action too gentle, time too short, etc).

None the less, the official IoB method sets the benchmark for barley screening that other systems must attempt to match or exceed. In total contrast to the IoB, AWB Limited insist that their screening method must be reproducible to within 0.38% when running one of their reference wheat samples. The accuracy differences are probably largely explained by the screen slot width tolerances in the two systems. The Institute of

Brewing are content with the width being within 0.03 mm (IoB, 1997). AWB Limited insists on a maximum error of 0.01mm in the slot width for their field pans and 0.005mm in their reference pans (AWB Limited, 2002). Adding the Width tolerances (W) into the Screening Efficiency equation results in:

$$ES = \frac{A*T}{M*N*W}$$

This addition radically changes the relative efficiencies of the various methods as demonstrated in Table 2.4:

Table 2.4 Expanded Comparisons of Screening Methods

	Screen Area (m^2)	Shake Duration (sec)	Sample Mass (g)	Screens	Width Tolerance (mm)	Screen Efficiency
Barley IoB	0.06	300	100	3	0.03	215
Barley MBIBTC	0.06	120	400	2	0.03	32
Wheat VG	0.07	35	400	1	0.01	62
Wheat AWB	0.07	68	400	1	0.005	240

Wheat VG is the VicGrain wheat method, while the AWB reference method is quoted. Some assumptions are built into this table: The MBIBTC method is postulated as using IoB specification screens and shakers, but reducing agitation time to two minutes. The Vicgrain method is assumed to use standard AWB specification screens. Table 2.4 gives some support for the high accuracy of the AWB reference method, but it almost certainly underestimates the importance of the width tolerance. It also suggests that the MBIBTC screening method may not be very accurate if the assumptions used are correct: the IoB slot width specifications are not precise enough. The author suspects that the IoB shaking method, which is probably too gentle for such a large sample load, will compound their problems.

SKCS and DIA are perhaps the only alternative methods of calculating the screening assortments.

2.6.6.2 Single Kernel Characterisation System

The Perten Single Kernel Characterization System 4100 (SKCS 4100) can analyse the kernel hardness, weight, diameter and moisture content of 300 individual kernels in three minutes. The instrument provides an objective measurement of kernel hardness by crushing the kernel. The individual kernel analysis allows the SKCS to determine the uniformity of a grain sample (Perten, ; RACI-CCD, 2003).

The SKCS can measure kernel diameter and weight on a kernel by kernel basis, permitting estimates of the screening assortment (Dexter and Symons, 2000). However, the SKCS kernel "diameter" is a somewhat ambiguous measurement, as was the data table Dexter and Symons provide. None the less, there appears to be some correlation with the actual screenings.

Dexter & Symons (2000) used both an SKCS and a crude digital imaging system. They reported a poor correlation between the DIA kernel width and SKCS kernel diameter ($r = 0.58$). Without further facts, it is impossible to tell if this was due to inherent differences between the two parameters or insufficient sample sizes. However, their description of the kernel spreading for their DIA images as "randomly scattered" and with sample sizes of only 50 kernels their imaging results could probably be improved. They concluded that the DIA system was faster than the SKCS and better for determining the percentage of small kernels in the sample.

This use of the SKCS diameters was expanded by Lambe & Morris (2001). They found that the SKCS diameter tended to be about 0.5 mm less than the kernel diameter when measured with callipers. The author is still not sure what they mean by diameter. –is it the kernel width, thickness or a mixture of these? Lambe & Morris found a strong correlation between the SKCS diameter and wheat screenings ($R^2 = 0.88$). Though this is a helpful indication of screenings, as will be shown in the Screening Equivalent chapter

of this thesis, a much stronger correlation is needed to provide an industrially acceptable screenings prediction as shown by the AWB screening standards (AWB Limited, 2002).

2.6.6.3 Digital Image Analysis

Initial results and work by others suggest that DIA has the potential to generate accurate screenings equivalents. Kuhbauch & Bestajovsky (1989) were among the first to explore this possibility for barley. The SeedCount™ DIA system also has the possibility of making a multi-group assessment in less than a minute, thus cutting the normal IoB screening assortment time back to one-fifth or less (Armstrong, Armstrong and Weiss, 2004).

Converting this potential into an accurate, functioning system is a complex matter. DIA cannot physically separate the kernels into distinct groups that can be weighed and assigned a percent mass of the total. Nor can the typical DIA systems available even produce images of the kernel thickness, because the kernels tend to lie on the imaging trays with their width and length displayed and their thickness dimension facing towards the imaging device, and therefore effectively invisible (Dexter and Symons, 2000; Gebhardt, Rasmusson and Fulcher, 1993). To calculate screening assortments, called "screening estimates" hereafter, requires the DIA system to assess the thickness of the kernels, assign a mass to the kernel and then to group the kernels and calculate the percentage by mass in each group. The difficulty of this is illustrated by a related attempt by Nielsen *et al* (2003) to calculate kernel density using the kernel "volume" generated by GrainCheck from its two dimensional DIA data. GrainCheck does this by 'rotating' the kernel image area through 180 degrees. They found that the volume estimate was too inexact to be useful.

As the standard IoB method also stipulates that all material that is not whole or broken barley kernels is to be rejected, the DIA system also needs to be able to recognise the foreign material and remove it and its mass from the screening estimates (IoB, 1997).

The complexity is such that there are only a few papers on other DIA systems attempting to make a screening estimate. One attempt to match DIA data to screenings was made by Dexter & Symons (2000), using a basic imaging system. Like their report on the SKCS data, it is difficult to extract much more information from their tables than the observation that there is a tendency for the material held on wider-gapped screens to have larger apparent areas and wider kernels. They suggest that the likely cause of a general tendency for the DIA kernels to be wider than their mechanical screening thickness is the tendency of the kernels to lie "on the side rather than upright".

A surprisingly successful DIA attempt to predict kernel plumpness determined by a 2.5 mm screen is reported by Svensson, Egelberg, Peterson & Oste (1998) using a GrainCheck system. They claim a correlation (r) of 0.97 and a Standard Error of Prediction of 2.41. As kernel plumpness was not, to this author's knowledge, promoted as a feature of the GrainCheck systems, one must wonder if the matches they achieved only held for the barley samples used in the test, though they did report validating it on four different barley samples. They also restricted their correlation to the single 2.5 mm screen, rather than an array of screens as attempted in this thesis. The robustness of their correlations are contradicted by another study performed on a GrainCheck instrument (van Laarhoven, Douma and Angelino, 1997). They found that their 2.5 mm barley screening estimates would only work moderately well for the specific barley cultivar the correlations were developed for, with their best r^2 correlation being 0.835. When they tried to develop a "universal" barley screening estimate their r^2 correlation fell to 0.615. Even if the kernels can be made to display their thickness, DIA will be hard pressed to resolve the thickness with sufficient accuracy, as even at 300 dots per inch (dpi) the width of each "dot" is effectively 0.085 mm. Despite the difficulties, this author has attempted to develop and evaluate two different DIA screening estimate systems, as will be detailed in chapter seven.

2.6.7 Kernel Morphology

Kernel morphology is a very broad field, ranging from the overall dimensions and shape of a kernel right down to the smallest structures contained in each cell of that kernel. The

finest structures obviously require investigation by electron microscopy (Armstrong, 1997; Dang and Copeland, 2002; Fukui and Kakeda, 1990).

Generally the most easily observed morphological features are those that are the most commonly used for cultivar identification (Ferns et al., 1975; Fitzsimmons, 1979; Wrigley and Batey, 1995). In the usual case of predicting grain quality with morphological features, all the worker has available is the threshed grain so only kernel morphological features can be used. Some of these properties, such as kernel weight and screening assortments (essentially kernel size sorted by thickness) have already been discussed.

Many other aspects of kernel morphology and cultivar identification have been studied, both manually and using DIA (Gebhardt, Rasmusson and Fulcher, 1993; Symons and Fulcher, 1988). The more important of these will be discussed now.

2.6.7.1 Kernel Length

One very easily measured kernel property is kernel length. This can be measured with either callipers or DIA. It presents little difficulty for DIA as it is fairly straightforward to find the most distant points on the grain perimeter and measure the distance between them. The only real restriction in the DIA measurement accuracy is the precision of the optical system used and the pixel size (derived from the dots per inch (dpi) of the image selected).

Most current DIA systems use approximately 300 dpi (Both the SPY and SeedCount systems use 300 dpi and the Cervitec uses 320 dpi, although van Dalen (2004) is using 200 dpi). The Cervitec system is probably operating at the maximum resolution of its CCD cameras. The current SeedCount system is using a modified Epson 1660 Perfection scanner for image capture that is capable of 1600 dpi optical resolution. However, at 1600 dpi resolution the depth of field becomes quite small, probably in the order of 1 mm. This makes it impossible to keep the entire seed in sharp focus. The SPY image capture system is also based on scanner technology and probably has capabilities and

limitations that are similar to SeedCount. The effect of image resolution on the image pixel width and the resultant image data generated are shown in Table 2.5.

Table 2.5 Image Resolution's Effect on Pixel Width and Image Data

Resolution (dots /inch)	100	300	600	900	1200	1600
Pixel Width (mm)	0.254	0.085	0.042	0.028	0.021	0.016
Image Data (relative to 100 dpi)	1	9	36	81	144	256

The reasons behind the popularity of 300 dpi images in whole grain image analysis are revealed in Table 2.5. As the resolution is increased, the pixel width decreases proportionally: double the resolution and the pixel width is halved. But the image size, and the amount of data needing processing, increases as the square of the resolution as this change affects the image both horizontally and vertically. Moving from 100 to 900 dpi cuts the pixel width down to $1/9^{th}$ of the original width (0.028 mm), which is comparable to the IoB width tolerances of 0.03 mm. However, this same resolution change increases the image size by 81 times. The processing time will certainly increase by even more than the 81 times as the computer runs low on memory and the number of inter-pixel combinations skyrockets. To put this in concrete terms, a 100 dpi 2 megabyte image that requires 15 seconds to process would become be a 900 dpi 162 megabyte image requiring at least 20 minutes to process. As Graincorp only allocates a total of five minutes for all processing for each load at their receivals points, this resolution is clearly impractical (Orman, Lees and Hare, 2000).

Though it is probable that in a few years the image capture devices, computers and software will have advanced enough to cope easily with these image sizes and cut the time back to a few seconds again, for now they are simply too ponderous.

These resolution issues apply equally to all DIA-determined properties. They also affect older DIA information, as data from a few years ago, for the same reasons, was then effectively limited to 100 dpi greyscale images. Though this was adequate for grain counting, it was not very accurate for kernel dimensions and color-based analysis was extremely slow (Sapirstein, 1993). It is therefore fair to say that at this time DIA systems can resolve kernel length to approximately 0.085 mm.

Length is measured by all DIA grain systems. Majumdar & Jayas (2000a) differentiate between the rectangular kernel length and the major axis length. SeedCount uses the latter as it is more precise.

2.6.7.2 Kernel Width

DIA kernel width measurements can be somewhat more complex than length measurements as there are different methods of assessing this. Methods commonly used include the rectangular box width, minimum arc length from the centroid of the kernel, longest orthogonal line pair taken from longest axis of the kernel, etc (Armstrong, Armstrong and Weiss, 2004; Luo, Jayas and Symons, 1999; Majumdar and Jayas, 2000a).

The minimum arc method tends to produce undersize measurements on damaged grain as only the distance to the bottom of the missing section is measured. Conversely the box method produces excessively large widths if the kernel is not perfectly orientated either horizontally or vertically. SeedCount uses the longest orthogonal line pair method.

2.6.7.3 Kernel Area

Kernel Area is essentially a DIA grain measure as it is very tedious to measure by conventional means. Kernel area is easy to measure with Digital Image Analysis as the cross-sectional area of the seeds on the tray can be readily seen (Dexter and Symons, 2000). On a flat tray the seeds tend to lie in the most stable position: -as flat as possible.

This means that the narrowest dimension of the seed is usually the vertical height of the seed on the seed tray.

It would appear that kernel area should be a straightforward measurement. However, the process used to discriminate the kernel from its background in the image can affect the kernel area strongly. Some processes are highly dependent on lighting and kernel placement, as will be shown in section 4.4.4. The use of coloured backgrounds linked to colour dependent segmentation algorithms providing edge-patching routines makes this process more precise than the older greyscale thresholding methods, as discussed in sections 4.4.4.5 and 5.3.5.3. This precision is enhanced by using kernel presentation methods that produce low percentages of touching kernels. The kernel discrimination issue also affects the kernel orthogonal dimension measurements.

None the less, most DIA systems calibrate their kernel segmentation process to adjust for any edge erosion or tray inclusion. The results may not be identical with other imaging systems, but as virtually all DIA systems rely on capturing their own images, this is not usually a problem.

The typical seed position makes it possible to determine the longitudinal cross-sectional area of the seed. Simply dividing these areas into a number of groups gives the user some idea of the seed area distribution and thus indirectly the sample's kernel size distribution.

Though the seed-area based divisions have their own inherent value, it is unlikely that the grain industry will adapt them as a major quality measure to replace mechanical screenings. Their reason for this is sound. The standard screenings fractions are not simply an anachronism based on the ability to make screens. It is a reflection of the industry's commercial processes. Both maltsters and millers physically screen the grain before they continue with their processes. The smallest fraction is usually sold off as stock feed. The millers often separate the retained grain into various screened streams that are put through mills with different roller gaps designed to maximise the conversion of grain into flour (Palmer, 1989). Likewise, maltsters can screen the barley into various

streams that receive slightly different treatment (eg, the larger fractions are steeped, germinated and kilned for longer than the smaller fractions) (Briggs, 1978).

Because of the industry-wide practice of screening, it is necessary for DIA systems to convert their cross-sectional area based fractions into screening equivalents. If possible, this should be done to provide equivalents for the full range of screens typically used. As most receivals depots only perform a single screen test due to time restrictions, the DIA system would provide users with additional information on their grain (AWB Limited, 2002; Vicgrain, 1999).

2.6.7.4 Kernel Perimeter

Following a kernel's perimeter is a complex affair. Once the software has made its tray/kernel segmentation decisions, it remains essentially a matter of a pixel by pixel 'walk' around the edge of the kernel. The kernel perimeter needs to be defined before the more complex length, width or shape measurements can be made.

2.6.7.5 Two-dimensional Combinations

The above data can be combined to make various judgements about the grain. As one example, Sapirstein & Kohler (1999) were able to class wheat with sample sets of 300 or more kernels using only their perimeter, length, width and area measurements and their combinations.

2.6.7.6 Aspect Ratio

Aspect ratios are the most obvious combination of the above data. They are calculated by comparing the kernel width to length, using DIA or callipers (Edney, Bassily and Symons, 1998). Various combinations of the above measuring methods are used and some authors prefer using length/width (Majumdar and Jayas, 2000a; Maztech, 2003) rather than width/length (Agrovision, 1996; Armstrong, Armstrong and Weiss, 2004; Gebhardt, Rasmusson and Fulcher, 1993).

2.6.7.7 Thinness Ratio

Majumdar & Jayas (2000a) extracted a number of other relationships and interrelationships from their images. One is especially worthy of mention as it is related to this thesis. They referred to it as the "Thinness Ratio" (TR). It is also used in the SPY system where it is called "Roundness" (Maztech, 2003). As this thesis reserves Roundness to identify a three-dimensional property, Majumdar's terminology will be used. The thinness ratio equation is:

$$TR = \frac{P^2}{4 * \pi * A}$$

Where:

P = Perimeter

A = Area

The equation has the virtue of being independent of kernel size. A circular object would have a TR of 1, while a long, thin shape would have a TR of 2 or 3. An object with a convoluted perimeter would have a higher TR than a smooth-edged object, even if both had the same aspect ratio.

GrainCheck had a "Roundness" option too (Agrovision, 1996, pg 27). It was also based on the 2D image, and consisted of superimposing a circle over the kernel centre that was large enough to totally enclose the kernel. This resulted in an area that ranged from 0.05 to 1.0. The resultant value would be very sensitive to any projections from the kernel. This may be equivalent to the "circularity factor" referred to by Troccoli & di Fonzo (1999) earlier in section 2.6.4.1.

As Majumdar & Jayas (2000d) demonstrated, supplementing the 2-D morphology data with color and texture data derived from the kernel images allowed much higher classification accuracy.

2.6.7.8 Crease Detection

Color and texture data can be used to detect the presence of, and the position of, the kernel crease. This knowledge can be quite valuable as it helps to identify the orientation of the kernel. This is especially useful in determining screening equivalents as it indicates whether a kernel is correctly orientated or not. It also has implications for blackpoint detection, as the discoloration typically is strongest over the embryo area on the opposite side of the kernel to the crease (Allen, Pleming and Pan, 2001).

2.6.7.9 Three-Dimensional Morphology

As noted above, three-dimensional morphology is not easily performed with digital image analysis. But this does not mean it is not of interest.

A fully mechanical analysis of kernel density based on comparing the kernel volume and weight with a cube defined by calliper measurements of its orthogonal dimensions was conducted recently to provide additional morphological information (Nelson, 2002). The cube comparison is very crude and of doubtful value, but the paper does illustrate the continuing need for research on kernel volume and density.

Some attempts at three-dimensional DIA grain analysis using laser imaging have been made. They are limited to their use of a single wavelength and cannot provide color data, so the above researchers had to combine the laser images with conventional images. This combination permitted quite good (>98%) discrimination between wheat and foreign material (Chen, Chiang and Pomeranz, 1989) and 94% accurate discrimination of two wheat cultivars (Thomson and Pomeranz, 1991). It is likely that as more affordable, accurate, rapid and capable three-dimensional laser-based scanners become available, fully three-dimensional data could be used to quantify grain lots. 3D scanners are becoming common in engineering firms that copy complex shapes and reverse engineer products (ShapeGrabber, 2004).

2.6.7.10 Thickness

One of the principal three-dimensional parameters needed is kernel thickness, as discussed in section 6.1.4. Thickness is needed to produce accurate screening estimates, and is a major theme of this thesis. For a kernel viewed from its edge, that is one that is rotated 90 degrees on its major axis, the thickness is in essence the width of what is seen.

A rather novel approach to imaging the kernel thickness was made by Kim, Jo, Kim & Sung (1997), who developed a tray with two 55 degree mirrors attached, allowing the end and side of the kernel to be viewed at the same time as its dorsal surface. They were able to extract data from the three images of each kernel and use it to distinguish rice grown in different countries with 94% efficiency. However, their process was limited to examining a single kernel at a time, a process much too slow for industrial use.

The other DIA parameters mentioned in sections 2.6.7.1 to 2.6.7.9 can also be measured for such a seed. These parameters can be combined to produce a three-dimensional model of the seed as will be used later in this thesis.

2.6.8 Vitreousness

An important property of durum wheat in particular is vitreousness. Vitreous kernels produce more semolina than non-vitreous kernels, attracting a price bonus for vitreous durum. Vitreous kernels have darkish glassy appearance, while non-vitreous kernels have a white-yellow chalky appearance. Mottled kernels have both glassy and chalky regions. The chalky endosperm regions tend to have less densely packed starch granules with air spaces between the granules (Symons, Van Schepdael and Dexter, 2003).

Currently vitreousness is determined by a subjective manual visual method that is prone to dispute. There have been a number of attempts made to determine durum vitreousness (or semolina yield) with objective instrumental methods (Dexter and Symons, 2000; Orman-McLean, Lees and Hare, 2001; Sissons, Sissons and Smith, 2002). One attempt using the SPY DIA grain grader is reported by Orman, Lees, & Hare (2000). The SPY system was unable to measure the vitreousness with the accuracy achieved by the human

grader. The system was also rejected on the basis of it being too awkward to use and far too slow, taking almost twenty minutes per sample.

Another attempt to assess vitreous durum kernels by 'machine vision' was made by Symons, Van Schepdael & Dexter (2003). They reported matches that were often within 3% of the manual assessment, a major improvement over the SPY results. They also claimed their method was 'high speed'. However, as they recommended using 500 kernels for a test, and imaged the kernels in groups of 50, which had to all be hand-separated and arranged, this author has serious doubts about how fast it was. They neglected to give a quantitative analysis time. One other interesting attempt was made with two GrainCheck systems by Wang, Dowell, & Zhang (2003). They achieved classification matches of about 85%.

The SeedCount system has not yet been used to assess vitreousness, though it is probable that this will be explored at a later date. As the SeedCount system is far faster and easier to use than the SPY system (as shown below), if sufficient accuracy can be achieved it may prove to be useful for this analysis.

2.6.9 Cultivar Identification

Identifying cultivars is vital for grain breeders and is essential for ensuring payment to them under the Plant Breeder's rights (Wilson, 2001). Cultivar, or more generally variety, identification is also necessary to ensure that the correct cultivar is being placed into the appropriate class of grain (eg. Should the variety Babbler be placed into Australian Prime White (APH) class of wheat?) (Williams and Cracknell, 2001).

Cultivar identification is a difficult task to perform by simple kernel morphology and basic quality tests, especially when dealing with closely related cultivars. Such tests, in the hands of an experienced breeder, may well be able to identify the family group to which the cultivar belongs by examining the combination of characteristics the cultivar presents, as shown in classical Australian identification handbooks (Ferns et al., 1975; Fitzsimmons, 1979). Morphology is still being widely used for classification of grain

today, but generally only at the level of grain type and commercial class, such as Canadian Western Red Spring wheat (Majumdar and Jayas, 2000a; Majumdar and Jayas, 2000b).

DIA can make some of the measurements easier to come by, and even add two or three new parameters to the mix as shown in section 2.6.7. But even so, a complex matrix including germination and growth habits as well as kernel morphology is needed to make more than rudimentary identifications.

Randal Giroux (1999), in his overview of cultivar identification methods, said "While the use of digital imaging for wheat class discrimination and quality evaluation shows strong potential, the potential of digital imaging for varietal identification in barley remains unclear. The most significant factor that limits imaging is the large variability in seed characteristics within a pure line and the significant effects of environment on some of these seed characteristics." These difficulties have led to more precise cultivar identification methods, summarised in *Identification of Food Grain Varieties* (Wrigley, 1995) and briefly detailed below.

2.6.9.1 High Pressure Liquid Chromatography

Glen Fox (1994) investigated the use of High Pressure Liquid Chromatography (HPLC) for identifying cultivars. He eventually concluded that the water-soluble proteins were too limited for reliable identifications. Other workers found that that the alcohol-soluble proteins showed more promise (Allison and Bain, 1986). Larroque et al (2000) and Larroque et al (2003) reported continuing improvements of these HPLC methods.

2.6.9.2 Protein Electrophoresis

Electrophoresis has shown considerable utility as a cultivar identification method. At this time the official EBC method for identifying barley varieties is polyacrylamide gel electrophoresis of alcohol-soluble proteins (EBC, 2003, Method 3.12). The EBC method acknowledges that it cannot positively identify all varieties, and recommends using

morphological analysis for these cases. Capillary electrophoresis (CE) of gliadin proteins is widely used for identifying wheat and triticale cultivars (Capelli et al., 1998; Nakkote et al., 1999; Siriamornpun et al., 2002). New automated CE equipment is also available (Uthayakumaran et al., 2003).

2.6.9.3 DNA Markers

Over the past ten years a great deal of work has gone into finding unique sections of DNA that can be used to identify each wheat and barley variety (Ablett et al., 2001b; Ma et al., 2001; Zhang et al., 2001). Microsatellite DNA markers are already being used to identify rice cultivars (Ablett et al., 2001a). The continuing development of rapid automated DNA replication and matching equipment is making this system more viable each year (Gale, Ma and Zhang, 2002).

Although it is virtually certain that ultimately DNA methods will become the only authoritative method for cultivar identification, morphological identification is still helpful at this time. Even when the DNA methods and markers are fully developed, morphology will still play a role in cultivar selection procedures. DIA morphological data will form part of that morphological picture, as illustrated later in this thesis.

2.6.10 Defect Detection

In addition to placing kernels into the correct class or cultivar, there is also a need to detect and quantify defective material in the sample. The visual defect classification methods require identification of foreign grains, such as oats and weed seeds in a barley sample. They also require identification of insects and insect-damaged grain (AWB Limited, 2002). A number of defects are listed, many of which have been identified by DIA with different levels of success.

It is likely that DIA could eventually have a major role in identifying foreign material in grain lots, including grains, insects and weeds, as the technology matures. One attempt at

identifying insects in grain has already been made (Davies, Chambers and Ridgway, 2002).

2.6.10.1 Broken Kernels

A significant quality issue is broken kernels, where generally any kernel that is less than ¾ of a whole kernel is classed as broken (EBC, 2003; FGIS, 1997). This is a critical issue for maltsters and for planting seed for farmers, as badly broken grain does not germinate well if at all.

SeedCount currently assesses its samples for broken kernels, as detailed later in this thesis (Armstrong, Armstrong and Weiss, 2004). Other DIA systems also assess this defect, generally on a morphological basis (kernel length, area, aspect ratio, etc) (Johnsson, 2003; Luo, Jayas and Symons, 1999).

2.6.10.2 Pre-harvest Germination

Pre-harvest germination (PHG) and sprouting damage are important quality considerations that are currently tested by falling number (FN), rapid visco analyser (RVA) and direct enzyme testing (Elliot, Leung and Bason, 2000). The FN and RVA test for starch paste damage that can indicate high amylase activity resulting from PHG.

Visual examination is initially used to decide if PHG has occurred and further testing is required, so it is possible that DIA systems could detect sprouted grains by the morphological changes which sprouting causes to the embryo area of the seed. Indeed there is a DIA system that is being used to identify grain germination via rootlets that may eventually be able to detect the early stages of PHG (van Laarhoven, Angelino and Douma, 1999). Tanabata et al. (2004) have developed a DIA system that records the details of rice germination. Thomson & Pomeranz (1991) found that their 3D laser imaging system was able to identify 90% of germinated kernels due to morphology changes in the embryo region. The SeedCount DIA system does not attempt to test for PHG yet, so this issue will not be pursued in this thesis.

Germination is a desired outcome in breeding trials and malt production. Calcofluor tests are used in the malting industry to assess the extent of the malt modification during germination. DIA has been applied to Calcofluor tests to quantify the results and also to test for PHG (Reinikainen et al., 1996).

2.6.10.3 Blackpoint

Blackpoint is a kernel-staining defect of wheat that occurs over the embryo. The same defect in barley is frequently called blacktip. It appears to be a kernel developmental defect triggered by high humidity and temperatures during the kernel-filling stage and manifests as a brown to black discoloration of the kernel bran and husk (Mrva and Mares, 2000). This makes the grain look unappealing to buyers and can result in dark specks in the resulting flour (Mares, Wang and Baydoun, 2000). It does not appear to have any other negative effects on flour quality (Allen, Pleming and Pan, 2001). It is currently assessed visually, using a clear two-sided indented tray that holds three hundred kernels (AWB Limited, 2002). Some attempts have been made to assess blackpoint with DIA (Johnsson, 2003; Luo, Jayas and Symons, 1999).

2.6.10.4 Other Defects

There are a number of other grain defects that are assessed visually and thus have the possibility of being assessed by DIA. Some of the defects that have already been assessed by DIA with varied success are mildew, fusarium, grass-green, frosted, heated, midge and smudge (Johnsson, 2003; Luo, Jayas and Symons, 1999).

2.6.11 General Digital Image Analysis Issues

Many DIA (sometimes referred to as Machine Vision) issues have been explored during the discussion of DIA in the various tests listed above. There are a number of other DIA issues that have not been discussed yet, but need to be mentioned briefly.

Many of the morphology and classification issues that arise in cereal analysis are similar to other biological systems, including medical imaging, where discriminating shapes and measuring them are also required. Due to these similarities, texts written for these areas are often useful in cereal DIA too, such as: (Sapiro, 2001; Seul, O'Gorman and Sammon, 2000; Soille, 1999). Paul Rosin (2003) presents a series of algorithms for detecting and measuring elliptical, rectangular and triangular shapes in images that have applications in cereal work. Lezoray, Elmoataz, & Cardot (2003) have developed a new color object recognition algorithm that may be useful for grain defect classification.

One such issue is the debate between using direct “statistical” analysis for kernel classification or using artificial neural networks (ANN) for the analysis. Luo, Jayas and Symons (1999) presented evidence supporting the superiority of ANN for both classifying grain by type (achieving a 97.8% correct classification) and by defects within a class. In a series of excellent papers, Majumdar & Jayas (2000a; 2000b; 2000c; 2000d) then achieved a 99.8% correct classification of the same material using a direct parametric statistical method. They demonstrated that the selection of specific morphological, color and textural features for use in the classification process was critical.

A great deal of DIA work is being conducted on rice, including detection of degree of milling (Lui et al., 1998), cracked and chalky defects (Reece and Blakeney, 1993; van Dalen, 2004; Wan, Lin and Chiou, 2002).

Several groups are also working on developing automatic DIA inspection systems. Wan (2002) produced a sophisticated machine capable of processing 1900 kernels per minute.

2.7 Conclusions

Wheat and barley are essential cereals used throughout the world. The variety of their uses has led to a large number of quality tests. The number and complexity of these tests is continuing to grow as users become more discriminating and demand more appropriate grain for their particular purpose. Within this testing framework, there is an expanding

body of literature detailing the maturation of Digital Image Analysis as a useful quality assessment tool.

The work presented in this thesis will, it is hoped, help to expand the utility and acceptability of DIA in grain analysis.

3 Conceptual Framework, Materials and Methodology

3.1 Introduction

A great deal of work has been put into developing numerous methods to assess the quality of wheat and barley. Every test procedure has to meet at least these two requirements before it is accepted for common use:

- It must address a specific quality issue that is important to at least one segment of the grain industry.
- The test results must be highly correlated with the quality issue and be accurate, precise and repeatable.

Test procedures that are also quick, easy to use, safe, objective, non-destructive, environmentally sound and economical are also far more likely to be widely used than tests that lack these properties. New tests for existing quality parameters that improve some or all of these requirements and properties have a good prospect of replacing the older procedures.

The intention of this project is to develop new test procedures and evaluate their effectiveness against current procedures.

3.2 Research Questions

This project has one fundamental question: Can a Digital Image Analysis (DIA) system be developed that can offer effective grain quality screening procedures? There have been a number of attempts to produce such a system over the last two or three decades as detailed in chapter two. DIA systems are inherently safe, objective, non-destructive and environmentally sound as they do not use hazardous chemicals, the results are highly repeatable and the samples do not need to be milled before use. So far none of these DIA systems have found wide acceptance in the grain industry, in most cases due to one or more of the following faults: high cost, low accuracy, too slow and/or difficult to use.

Producing a successful DIA system will require the rectification of these faults and the broadening of DIA grain system capabilities. This rectification means that a number of specific issues need to be addressed in this thesis:

1. Can a reliable and useful grain image capture system be made from commonly available digital imaging equipment, thereby reducing the hardware cost of the systems?

2. Can a Digital Image Analysis (DIA) system be developed that can accurately count touching clusters of grain, thus allowing the creation of a DIA thousand kernel weight method?

3. Can a DIA system be developed that can directly measure the thickness of the kernels?

4. Can a DIA system be used to generate an estimate of the three-dimensional properties (roundness) of grain?

5. Can a DIA system be developed that can estimate the kernel mass and thus the screening equivalents by percent mass of a grain sample?

6. Can the DIA values of barley be used to predict the hot water extract of its malt or the DIA values of wheat predict its flour extraction rate?

7. Can software and a method of grain presentation be developed that are both quick and easy to use?

Testing methods that can make accurate determinations of various quantitative and qualitative traits (morphology, chemical profiles and malting characteristics) utilizing small quantities of seed are of special interest to plant breeders as they frequently only have small amounts of their new cereal lines. Traditionally, the visual tests have required tedious manual examination, while the other properties have required medium-scale

chemical tests using the standard procedures of the American Association of Cereal Chemists, Association of Official Analytical Chemists, the Institute of Brewing and VicGrain (AACC, 1995; AOAC, 1997; IoB, 1997; Vicgrain, 1999). Recent work suggests that near-infrared spectroscopy may also be applicable in this area (Roberts, Workman and Reeves, 2004).

Many of the conventional tests were assessed during the first year of the project using larger seed amounts of currently commercial varieties and a small selection of newly introduced cultivars. The selection of the best procedure involves two steps: The first step is optimizing each of the test methods that analyze the same parameter. The second step is the statistical comparison of the various optimized test results to decide which test needs the minimum sample size to provide acceptable differentiation of the grain lots.

Ideally, new micro-sampling procedures would show an identical ranking of the varieties with similar means and variances of results as would be available from larger-scale sampling. One objective of this investigation will be to determine the smallest sample required to give meaningful results so that the seed available can be used to maximum potential. Where possible, non-destructive tests such as Digital Image Analysis will be substituted so there will be no loss of fertile seeds.

A major contribution that the project will offer to breeders is the ability to make accurate decisions on new accessions at an early stage when seed quantities are still limited. These decisions will fast track the introduction procedure leading to the earlier adoption of superior genetics by the growers and end users.

Bulk grain handlers will be more interested in the ease of use, speed and accuracy of new tests than in the quantity of seed required for the test.

3.3 Materials

3.3.1 Wheat Samples

Initially, Wrightson Research and Allied Mills provided commercial winter wheat cultivars (Brennan, Gordon, Kellelac, Meering and Silver Star). Screened (>2.8 mm and <2.2 mm) sub-samples of Kellelac were included to test the limitations of the various counting and morphology methods.

These wheats were also used for developing other tests. The sample set was expanded with material from the Queensland (20 cultivars) and New South Wales Departments of Agriculture (16 cultivars), the University of Adelaide, Waite Campus (7 cultivars) and additional material from Allied Mills (72 cultivars). Wrightson Research also provided more samples (38 cultivars), many of them being experimental cultivars, grown throughout Victoria and southern New South Wales. Eventually material from three different growing seasons (2000, 2001 and 2002) was incorporated in the sample set and also used for confirmation of the Kernel Weight calibrations.

3.3.2 Barley Samples

Numerous barley and a few malt samples were analysed. The commercial barley cultivars used included Aspen, Century, Franklin, Gairdner and Parwan. Wrightson Research also provided over 100 grain lots of commercial and experimental cultivars grown at several locations and over three seasons (2000, 2001 and 2002) throughout Victoria. Joe White Maltings (JWM) provided additional commercial barley samples (38 grain lots) that were grown in New South Wales, Victoria, South Australia and Western Australia. The JWM material was mostly composed of Franklin and Gairdner crops. Screened (>2.8 mm and <2.2 mm) sub-samples of Gairdner were used to test the limits of the counting methods when processing barley.

The malt samples were produced from the above barley bulk samples. Some samples were malted commercially by JWM and others were malted in the University of Ballarat's micro-malter using MBIBTC standard malting protocols (MBIBTC, 2001).

The individually measured kernels used in the morphology work were taken from the above grain samples. They are discussed in more detail in section 6.3.1.

The author has been requested, for commercial reasons, not to divulge the names of the various experimental cultivars used.

Chemicals

All chemicals required for testing were of analytical grade and supplied by either BDH, ChemSupply, Tecator or Sigma-Aldrich.

3.4 Methodology

3.4.1 Seed Cleaning

In most cases the seed was cleaned before being analysed. This applied especially to the material sourced from Wrightson Research as their mini-scale harvester was set up to retain virtually all of the grain, thereby also retaining substantial amounts of chaff and other rubbish. Some of the smaller plots were also hand-harvested and threshed with a Peltz portable thresher. The threshed seed was cleaned with an Andrews and Bevins portable seed cleaner.

3.4.1.1 Seed Cleaner Operation

The uncleaned sample was placed in a hopper on the top of the cleaner. The hopper fed the sample across a reciprocating sloping perforated screen. The material that did not pass through this screen (mostly unthreshed heads and stalks) was collected in a side hopper. The material that did pass through the screen was then fed over a finer reciprocating sloping perforated screen. The fine material (mostly dust, small weed seeds and kernel fragments) that passed through this screen was collected in another side hopper. The material remaining on the fine screen then fell down an aspiration chute. The light material (chaff and stalks) was blown up and out of the back of the machine. The heavier material fell down the chute and collected in the clean grain tray at the bottom of the machine.

3.4.1.2 Wheat Cleaning

Wheat samples were cleaned using a top screen with 7.5 mm diameter circular holes and a bottom screen with 1.2 mm diameter circular holes. The seed feed control lever was adjusted to give an average feed rate of approximately 2 kg-min^{-1}. The aspirator airflow control slides on the sides of the seed cleaner were adjusted to ensure that none of the grain was being lost but most of the light trash was blown off. The coarse trash bin (Overtails) was checked after each sample. If the clean sample weighed less than two kilos the coarse trash would be rubbed to release more seed and run through the seed cleaner again. Cleaned seed that still contained a lot of trash would be rerun. Final removal of seed still in husks, etc was achieved by shaking the cleaned seed tray backwards and forwards until the rubbish grouped into a discrete clump that was then removed. The screens were cleaned after each sample by removing the cleaned seed tray and then rubbing the tops of the screens with a 570 by 20 by 20 mm board. The fine rubbish tray was checked periodically to ensure that only dust, grass and small weed seeds were being removed in the cleaning process.

3.4.1.3 Barley Cleaning

Barley was cleaned using the same equipment used for wheat cleaning. The principal changes were the use of a bottom screen with larger openings (1.5 mm diameter circular holes) and increasing the aspirator airflow. The barley samples did not clean as well as the wheat samples due to the greater presence of the awns and rachis, mostly due to the retention of the husk on the kernels. The results were improved by "de-awning" the sample in a small rubbish bin by agitating it vigorously with a broom handle and running each sample through the seed cleaner twice.

3.4.2 Sampling Devices

The use of sampling devices was of paramount importance in obtaining representative sub-samples when working with a small portion of the original sample.

In this thesis, grain lots were supplied by breeders and commercial processors and varied from 100 grams to 60 kilograms, though most samples were less than twenty kilograms. Twenty kilograms of barley is essentially a small bag of grain, while a bucket full of wheat weighs about ten kilograms. As many of the tests required less than 50 grams of seed, a reliable method of sub-sampling this sample range was needed.

3.4.2.1 Electro-mechanical Flip-flop Sample Divider

As the need for an adaptable sampling system became obvious, a unique variable split electro-mechanical sampling device was developed. The device used a digitally controlled flip-flop lever to vary the split from about 90 to 10%. This device would allow a 1% split to be made in two passes. Though effective, it proved to be too noisy for long-term use and was abandoned.

3.4.2.2 Bag-Sampling Spear

The flip-flop divider was mostly replaced with a specially designed bag-sampling spear that is now sold as part of the SeedCount^{TM} system. It has a solid nose cone and a series of rectangular holes to ensure that a representative sample is taken, as illustrated in Figures 3.1 and 3.2. The spear will hold up to 60 ml of sample. This is sufficient for the DIA tests and also for a number of other small-scale quality tests. The following usage instructions are taken from the SeedCount User Manual, mostly written by this author (Armstrong, Armstrong and Weiss, 2004):

To use the Spear:

1. Open the bag or bucket from which the sample is to be taken.
2. Hold the spear by the open, intact end of the tube with the filling cut-outs facing down.
3. Push the spear diagonally down to the bottom of the bag or bucket.
4. Rotate the spear a half-turn so the cut-outs now face up.
5. Shake the spear gently three or four times to fill the tube (See Figure 3.1).

6. The spear needs to only be approximately half full when removed from the bag as shown in Figure 3.2.
7. Invert the spear carefully and pour the contents into the Sample Cup as explained in the Scanning and Analysis section of Common Procedures.

Figure 3.2 Filled Spear

Figure 3.1 Spear Ready to Invert and Fill Cup

The sampling spear could perhaps be made more effective by adding an internal tube that could be progressively removed to ensure that the material near the bottom of the container has full access to the tube before material from higher in the bag falls down the tube. Larger sub-samples required for standard testing such as micro-malting and hectoliter weights were made with the riffler, as explained in sections 2.6.1 and 3.4.2.

3.4.2.3 DIA Sample Cup

For the digital image tray samples, a small sample cup is used to measure the sample volume needed. The cup is filled from the spear and levelled with a flat striker as shown in Figure 3.3.

Figure 3.3 Levelling the DIA Sample Cup

3.4.3 General Analytical Tests

The initial analysis methods used were the standard methods of the American Association of Cereal Chemists (AACC, 1995), the American Society of Brewing Chemists (ASBC, 1992), European Brewing Convention (EBC, 2003), Institute of Brewing (IoB, 1997) and Vicgrain (Vicgrain, 1999). Where necessary, these tests were modified for use with the Digital Imaging systems as noted below.

3.4.3.1 Moisture Content and Grinding

Wheat grinding was done on a Cyclotec 1093 sample mill (Tecator) using a 1 mm screen. Barley was ground on a Buhler Miag DLFU disk-type lab mill set for a fine (0.2 mm) grind as specified by EBC method 3.2 (EBC, 2003). The ground material was used for moisture, protein and falling number determinations.
Moisture was determined by the IoB oven method 1.2, using a Thermoline 0100FD digitally controlled laboratory oven. Samples were weighed with an AND ER-180A analytical balance accurate to 0.1 mg.

3.4.3.2 Screening Assortment

Screening assortment for barley was determined using the IOB method 1.13 on a Pfeuffer Sortimat screening system using EBC compliant 2.80, 2.50 and 2.20 mm slotted screens. The 2.00 mm screening was performed on a non-certified 300 mm diameter Grainline 2.00 mm slotted screen and shaken by a Retsch 3D laboratory sieve shaker set for a medium shaking action. The Retsch sieve shaker had been modified to take the 300 mm diameter Grainline screens.

The 2.00 mm screening for wheat was performed using the Vicgrain method 2.7 using an Agtator mechanical shaker with an AWB certified field slotted screen (AWB Limited, 2002). The 1.60 mm screen was not certified. The other wheat screening fractions were made using the EBC barley screening equipment. Both of the uncertified screens (the Grainline 2.00 and 1.60 mm screens) were checked with “Stainless” digital callipers and found to be accurate to 0.01 mm (within the IoB specification of +/- 0.03 mm (IoB, 1997)). The callipers themselves were checked against engineering calibration blocks and found to be accurate to 0.01 mm for distances less than 30 mm.

Aside from the 2.00 mm wheat screen, all others were shaken for one minute per screen in the stack. The Retsch shaker, as it has a somewhat different shaking action to both the Agtator and Sortimat units, was tested at longer shake times and it was found that virtually no change occurred as a result of increasing shake times beyond one minute per screen.

After the bi-modal indented tray was introduced with SeedCount version 2, the number of available tray indents dictated the sample size. This reduced the sample to approximately 33 ml in most cases as detailed in the following section.

3.4.3.3 Hectoliter Weight

Standard test (hectoliter) weight was determined with a Co-operative Bulk Handling (CBH – Western Australia) chondrometer using Vicgrain method 2.4.

SeedCount mini-hectoliter weight (MHW) volumes were determined with cups of volumes from 26.8 to 34.8 ml. The cups were made from 38.7 mm internal diameter PVC pipe and end caps. The cups were filled to overflowing and then leveled with a plastic striker as shown in Figure 3.3, taken from the SeedCount Users Manual (Armstrong, Armstrong and Weiss, 2004). Mass was determined with an AND HF-3000G top-loading balance accurate to 0.01 grams.

3.4.3.4 Protein

Wheat, barley and malt protein was determined by the Kjeldahl total nitrogen method (IOB Method 1.8) using a Buchi 435 12 tube digester and a Buchi 323 distillation unit with Tecator selenium catalyst tablets.

The DIA systems used in this project were not able to assess the sample protein. The SeedCount system was only able to accept the protein values as a manual operator input and add them to the sample data.

3.4.3.5 Falling Numbers

Falling number (wheat) determines the cultivar's starch paste strength and damage due to post-harvest α-amylase activity. These determinations were done on a Falling Numbers machine (Perten) using the AACC moisture corrected method (AACC, 1995). This data was not included in the thesis as the project thrust moved to DIA and there is not an

equivalent DIA method. It was initially intended to compare this data with Rapid Visco Analyzer data.

3.4.3.6 Malting

Micro-malting was performed in various subdivided containers in a Joe White Maltings micromalter following MBIBTC protocols, but modified to include a six hour air rest during the steeping phase (MBIBTC, 2001). As the compartment size was reduced with an internal stainless steel baffle to allow micro-malting of small breeder's samples, the turning frequency and the number of rotations in each "turn" was increased to minimize the matting of the germinating barley.

Commercially malted barley was provided by Joe White Maltings and had been malted in accordance with their normal malting methods (Joe White Maltings, 2002).

Malt quality testing protocols followed standard IoB and EBC methods. Tests performed were diastatic power, hot water extract (EBC method), colour, protein, moisture and fermentability. With the exceptions of moisture and protein, these tests were undertaken by JWM and will only be discussed when the results tie into other test procedures developed in this thesis.

3.4.3.7 Flour Milling

John Dines of Allied Mills Toowoomba performed the wheat flour yield tests on a Buhler test mill in accordance with their standard test milling procedures (Allied Mills, 2003). This test required approximately two kilograms of wheat for each run.

3.4.4 Thousand Kernel Weight Determination

Thousand Kernel Weight was determined by IoB method 1.3, except where otherwise stated. The main deviations from this method occurred with the DIA systems when the full 40 grams of sample required by the IoB method could not be accommodated due to

overloading the imaging trays. For the preliminary wheat thousand-kernel work, three sub-samples were taken from each bulk sample. The sub-samples were counted in duplicate for each method. Screened (>2.8 mm and <2.2 mm) sub-samples of Kellelac were included to test the limitations of the various counting and morphology methods. For the barley TKW work, initially three sub-samples were taken from representative grain lots and counted in duplicate. Screened (>2.8 mm and <2.2 mm) sub-samples of Gairdner were used to test the limits of the counting methods.

3.4.4.1 Mass Measurement

The mass was measured with an AND HF-3000G balance to the nearest 10 milligrams for all of the counting methods. The mass in milligrams was divided by the kernel count to arrive at the thousand-kernel weight in grams. Alternatively, the same number in milligrams was the average kernel weight.

3.4.4.2 Hand Counting

Hand counting was initially performed without seed trays. Counting without the trays was made more accurate by using a wooden stick (approximately 115 by 11 by 2 mm) to align the seeds into groups of 5. Ten groups were then combined to make clumps of 50 seeds. Once all the seeds were grouped, it was easy to arrive at a total count.

3.4.4.3 Hand counting with Indented Trays

Hand counting with indented trays used three different trays, each with one hundred indents. The barley tray was a standard commercial barley hand-counting tray. The author manufactured the wheat counting trays as commercial indented wheat trays could not be found. One wheat tray had smaller indents than the other. The tray with the smaller indents was used for counting wheat samples with smaller kernels and vice versa. Each tray was filled, shaken and the excess kernels brushed and shaken out. Single seeds in an indent were ignored. Extra (multiple) kernels in an indent were added to the count and empty indents were removed from the count. The final number was added to 100 to

determine the actual number of seeds in the trayful (eg: +5 multiples – 10 empties + 100 = 95). A bamboo skewer was used to nudge the seeds to ensure that a smaller seed was not hidden at the bottom of the indents. Whole and broken kernels were counted separately. The counts were recorded and summed to arrive at the total count.

3.4.4.4 Electromechanical Counting

Electromechanical counting was performed on a Numigral 1900-1101 and a Kirby KL9 counter using the methods recommended by their manufacturers. The feed speed was kept low on both counters to maximize their accuracy. This was easily controlled on the Numigral 1900 by altering its vibration rate dial. Optimal results were obtained by setting it so that it took about eight minutes to count a 40-gram sample.

The Kirby did not have a mechanical feed system and relied on careful, but unavoidably somewhat erratic, hand feeding to count accurately. If the feed rate became too high, the Kirby faulted and reset the count value to zero. After some experimentation, the most consistent feed rate for the Kirby was achieved by drilling a small hole in the corner of a plastic lunch box and shaking the kernels through this hole into the Kirby's feed funnel.

3.4.4.5 Digital Image Analysis Counting

Scion Image is a freeware program provided by Scion Corporation (http://www.scioncorp.com/). Scion Image is the PC version of Image, a Macintosh program produced by the United States' National Institute of Health. Weiss Enterprises (http://www.seedcount.com.au) is developing the SeedCount DIA system in association with the author and the University of Ballarat. SeedCount (Version 1) and Scion Image were run under Windows 98SE on a PC with a Pentium II 300mhz processor and 64 megabytes of memory. Images were mainly acquired from a Hewlett Packard Scanjet 5300C scanner.

The DIA kernel samples were weighed to the nearest 10 milligrams and presented for image capture. Several methods of distributing the kernels were trialled. They were:

1. **Direct:** The sample was placed directly onto the scanner glass using a hollow rectangular positioning frame and the scanner was "shaken" to distribute the kernels. A sample mass of about 35 grams of wheat and 30 grams of barley was found to be as large a sample as could be placed on the scanner without reducing the percentage of single seeds to less than 25%. These maximum sample masses also applied to Options 2 and 3.
2. **Combed Tray:** The sample was poured into a clear, flat-bottomed tray with angled sides. The kernels were shaken and spread with a coarse-toothed comb to maximise the percentage of single kernels. The teeth on the comb were 15 mm apart.
3. **Screened Tray:** The kernels were spread across a wire grid or screen inserted into the clear tray to "position" the seeds separately. The wire had a 1 mm diameter and was spaced on a 9 by 5 mm grid for barley. The screen was then lifted out and the image made from beneath the tray as in the Combed Tray. For the above three approaches a minimum of 25% single grains was needed to produce accurate counts. Placing a black acrylic cover lined with non-reflective black cloth over the scanner and tray provided a contrasting background for methods 1, 2 and 3 (Figure 4.2).
4. **Indented Tray:** A large indented tray was used in Version 2 of SeedCount (Figure 4.6). It was superficially like a hand-counting indented tray. A cup of known volume was overfilled, levelled, weighed and poured into the indented tray. The tray was then shaken and brushed to distribute the grains into the indents so only a few grains were still present as multiples. The tray was then loaded into the SeedCount scanner cabinet drawer and imaged from above with a specially modified scanner. The cup method resulted in sample masses of roughly 23 grams for wheat and 21 grams for barley. The SeedCount User's Manual, on the CD supplied with this thesis, details the use of this tray and its scanner cabinet (Armstrong, Armstrong and Weiss, 2004).

When the seed distribution was completed, the tray was scanned and the image transferred to the computer.

A number of different brands and models of scanner were trialled. Version 1 of SeedCount used Canon FB310, Hewlett-Packard Scanjet 5300C, Mustek 600EP and Plustek UT-12 scanners. Version 2 used HP Scanjet 5400C and Epson 1660 Photo Perfection Scanners.

A variety of software approaches were applied to counting the kernels:

1. **Scion Image:** Analysis with Scion Image required the user to invert the image and apply a threshold to the kernel image, erode the edges of the images and then count the resultant separate blobs. The wheat and barley macros the author developed and used in Scion Image to automate this process are attached as Appendix 1.
2. **SeedCount Version 1** used the Direct, Combed and Screened flat-bottomed presentation trays and low-resolution 100 dpi 8 bit greyscale images (the same trays and images were also used for Scion Image). The scan was automatically inverted, cropped and loaded into SeedCount. SeedCount applied a threshold to identify the kernels, but did not erode the images. Instead it used a special algorithm to count the kernels as explained in section 5.3.5.3. The sample mass was input, enabling the software to calculate the TKW on an as-is moisture basis. If the sample's moisture content was also input, the dry weight TKW could be calculated. The data was appended to a text file that could be easily converted into a spreadsheet.
3. **SeedCount Version 2**: In later versions of SeedCount (2.x) the images were made of kernels in the unique indented trays at 300 dpi, 24 bit color resolution. The images were no longer inverted and the tray background was removed by color selection to isolate the kernels. Some of the barley counts were made with this version of SeedCount. Version 2 images the tray from above. The development of this hardware and these procedures is covered more fully in Chapter 4.

The wheat TKWs in this thesis were calculated on a dry weight basis. Some barley TKWs are given on an as-is basis and noted as such.

3.4.4.6 Time Measurement

The average time taken for the various counting methods was recorded using a standard lab timer (Electronic Clock-Timer Model 870A) that had a ‘count-up’ function and was accurate to within one second a day. A series of runs were made with each method and the average times calculated. The timer was also used for checking the time when required for other tests too.

3.4.5 Kernel Morphological and DIA Screening Methods

3.4.5.1 Indented Tray

Unique indented trays (patent pending) were developed for barley and wheat with two distinct sections. One section of the tray held the sample laying “flat” in wide indents with the crease facing up or down. This section of the tray resembles conventional manual seed counting trays. The novel patented section was designed to hold its part of the grain sample “on-edge” in narrow, deep indents holding the kernel so the crease was facing to the side of the indent. This section was intended to allow direct measurement of the kernel’s thickness. Throughout this thesis, the kernel thickness is defined as the smallest of the kernel’s three orthogonal dimensions. For most cultivars this is the dimension passing from the dorsal to ventral surfaces of the seed. The development of these trays is detailed in section 4.4.4.

3.4.5.2 Calliper Measurements

Wheat and barley kernels were selected to provide a broad range of sizes and shapes. They were individually weighed using an AND ER-180A analytical balance with an accuracy of 0.1 milligrams. Their length, width and thickness were measured with “Stainless” brand 150 mm hardened digital callipers to 0.01 mm. The kernels were placed in the calliper jaws in the required orientation for each measurement and the jaws were closed until they were just tight enough to hold the kernels when the callipers were lifted. This method reduced the measurement difficulties caused by excessive compression of the grains while measuring them.

3.4.5.3 DIA Measurements

As the kernels were measured with the callipers, they were placed in order into specific columns of pockets in the "flat-edge" tray so seed by seed measurement comparisons could be made. First the seeds were inserted into indents in the narrow "on-edge" section of the tray. They were placed in individual pockets, but the author tried to make their placement in the indent itself random so the results would be comparable to a randomly filled tray of seeds. The tray and kernels were scanned using the SeedCount DIA system.

The images were then digitally analysed. The tray-masking function separated each seed 'blob' from the tray. The software counted each blob. Then it zoomed in on each blob. Every pixel in the kernel "blob" was counted to determine its area. Another function followed the edge of the kernel and found the two most distant points on the perimeter. This was recorded as the kernel length. It then drew a line between these points and ran a series of perpendicular lines from it. The longest pair of lines was the kernel width.

The kernels were then moved, seed by seed, to the wide "flat" section of the tray where they were again lined up in columns so their identity could be traced. They were analysed as above. This generated data on each blob's length, width and area. (In the narrow section, the blob's width was actually the kernel's thickness.) The wide and narrow "on-edge" DIA data for each seed was combined manually in Microsoft Excel 2000^{R} and compared to its calliper measurements. Microsoft Excel and SPSS were used to generate a series of multivariate equations that predict each kernel's thickness and roundness. The equation output was compared with conventional test data wherever possible. Curve Expert version 1.3 (**http://www.ebicom.net/~dhyams/cvxpt.htm**) was also used to test the data for non-linear correlations.

When the individual kernel work was finished, large-scale aggregate values for each cultivar were also made. These were made by placing a "cupful" of material in the tray as detailed in section 3.4.2.3. The trays were then scanned and analysed.

3.4.5.4 Crease Detection

Crease detection was required in Version 2 of SeedCount to determine if the seeds were lying correctly in the narrow and wide sections of the tray. This was done by scanning down through a slice at the centre of each kernel and looking for textural changes in the image that indicated a crease. The location of the crease was recorded as a number from 0 to 100, referring respectively to the top and bottom of the kernel.

3.4.5.5 Aspect Ratio

The aspect ratio was defined and measured as the kernel's width divided by its length. Only kernels in the wide section of the tray were used for this determination. Kernels with a crease within 20 percent of the seed's top (0 to 20) or bottom (80 to 100) edge were excluded as this orientation indicated that the seed was not laying flat in the indent. The reasons for excluding the narrow section kernels are explained in section 6.3.2.

3.4.5.6 Ovality

Length, width and kernel area data was derived from the same grain samples and images mentioned above. Area data was only derived by DIA. The mathematical oval area was calculated for both the calliper and DIA othogonal measurements using the standard Ellipse Area Formula $A = \pi\left(\frac{Length}{2}\right)\left(\frac{Width}{2}\right)$. The Ovality values were generated by dividing the DIA kernel area by the calculated area. These values were then checked to see if different grain types and cultivars had significantly different shapes and if these shapes had a correlation with the cultivar's yield.

3.4.5.7 Roundness

For the initial roundness calibrations, the same seeds were used sequentially in both the edge-on and flat sections of the trays. This meant that each kernel's orthogonal axis (length, width and thickness) could be measured by DIA as well as by the callipers. This

data was used to generate roundness values. The roundness values were calculated using the following dimensionless equation:

$$Roundness = \frac{\frac{Width}{Length} + \frac{Thickness}{Length} + \frac{Thickness}{Width}}{3}$$

The individually measured wheat and barley kernels were compared with their respective DIA values and the wheat and barley SeedCount Roundness adjustment equations were generated. The adjusted Roundness values were tested to see if they were accurate across the range of cultivars used.

The roundness concept was then scaled up for use with normal trayfuls of seeds where there were simultaneously different seeds in the flat and edge-on sections of the tray. This process required the creation of "Virtual 3D seeds". This process attempted to mimic the use of identity-preserved seeds in each side of the tray by matching the seeds in the opposite sections of the tray. The seeds were matched by aligning the smallest seed by area in the edge-on section with the smallest seed in the flat section, etc until all the seeds were matched. Only valid seeds that were correctly positioned in each section were used. Multi-cluster seeds were rejected. Examples of invalid single seeds in the narrow section were seeds that were lying width-wise across the indents or tilted up on one end. Invalid seeds in the wide section were seeds that were lying on their edge in the indents. Where there were different numbers of seeds in the two sections, the data of some seeds were repeated for the section with fewer seeds until all were matched. The replicated seeds were carefully chosen to minimise any biasing of the matching process. If the seed number difference between the two sections was too large, the image was rejected and the operator was requested to refill the tray in a more balanced manner.

The Virtual 3D seed concept was tested on the identity-tracked seeds and used for calculating roundness, kernel mass, thickness adjustments and screening equivalents.

The average cultivar roundness values were then checked to see if different grain types and cultivars had significantly different roundness and if the roundness had a correlation with the cultivar's yield. Twenty-nine wheat and 26 barley cultivars were used.

3.4.5.8 Screening Equivalents

As detailed above, virtual 3D seeds were created to link the data between the wide and narrow sections of the tray. The linked data was then used to calculate thickness adjustments and the kernel mass using commercially non-disclosable algorithms developed by the author.

Once these values were generated, each virtual kernel was allocated to its appropriate screening group by its adjusted thickness. For example, a 2.4 mm thick virtual kernel was allocated to the 2.2 to 2.5 mm group. The virtual kernel's mass was also allocated to that group. When all of the kernels were allocated, the mass in each group and the total mass were calculated. This allowed the percentage by mass of each group to be calculated. The complete structure of all of the screening groups and their percent mass was referred to as the Screening Equivalents to distinguish them from the mechanically determined Screening assortments.

4 Development of the SeedCount Digital Image Analysis System

4.1 Introduction

The promise of digital image analysis (DIA), mentioned in Chapter 2, resulted in its selection as a major tool to enhance the evaluation of the visual physical properties of whole grain cereals during this study. Problems of speed, cost, ease of use and in some cases poor accuracy in existing systems (as detailed in sections 1.2, 2.6.4.4, 2.6.6.3, 2.6.7.10, etc) inspired the development of a new DIA system for use in the grain industry.

The entire DIA system developed as part of this project began as an attempt to improve on the current methods of finding the kernel weight. Finding an accurate average kernel weight requires an accurate method of counting the kernels. Hand counting, even when using an indented grain tray, is slow and tedious. Though mechanical counters make the process less tedious, they are still slow to use. Early in the process of determining kernel weights, a colleague at the University of Ballarat (Paul Brass) suggested using a DIA system. Initial DIA work with Scion Image and Image Pro Plus was not promising. This frustration led to the development of the SeedCount^{TM} DIA system.

SeedCount is unique in its use of a modified scanner coupled to an indented seed tray. This system simplifies sample presentation and allows rapid, high quality image acquisition while also reducing total system costs.

Software development was also unique, resulting in the development of a new algorithm for seed counting which has been successfully patented. The novel indented tray, for which a patent application has been lodged, enables a new method of directly measuring seed thickness with DIA. Until the development of this indented tray, this was not practical. Blackpoint (and blacktip) determination is also being investigated, which

should result in a new DIA method that not only counts the number of seeds with blackpoint but also rates the severity of the affected seeds.

SeedCount (the trade name for this DIA system) detected and counted the single grains in the sample using an algorithm initially based on the patented MACE^{TM} software (US Patent 6,243,486 B1 -Weiss Associates). MACE was developed to count cells in histology and microbial colony forming units in petri dishes. (Demonstration versions of this program are available at http://www.colonycount.com). MACE was capable of separating the seed "blobs" from their background without destroying the greyscale information stored in the seeds. Though not perfect for seeds, the MACE results were a major improvement over the counts derived from the other DIA software packages trialled. The results encouraged the author and Marvin Weiss to modify and extend the software for seed counting and grain quality analysis tasks, resulting in a patent covering its new counting method (US patent 6,418,180 B1, Weiss Enterprises). As development continued, the SeedCount DIA system proved capable of determining other parameters in addition to the KW.

4.2 Hardware

The electronic hardware used in this DIA system in mid 2002 consisted of a scanner (Hewlett-Packard Scanjet 5400C) and a Pentium based personal computer running Windows 98. This equipment was connected via a Universal Serial Bus (USB ver 1.1) interface that allowed the rapid transfer of 100 dot per inch (dpi) grayscale images from the scanner to the computer. The introduction of faster computers and USB 2.0 scanners in late 2002 led to the substitution of an Epson Perfection 1660 Photo scanner and the image quality was increased to 300 dpi 24 bit colour images.

Initially, grain was simply spread on the scanner glass. This allowed clear images to be made, but it required some expertise to spread the grain evenly on the glass. It was awkward to remove the grain from the scanner after scanning. Non-electronic hardware was then developed to simplify this task. The first trays had bevelled sides and a flat, clear scratch-resistant polycarbonate bottom. This device allowed the grain to be scanned

through the bottom of the tray. The tray made it easier to load and spread the grain sample without grain loss. A comb with widely-spaced teeth helped distribute the grain in the tray. The tray could be easily removed from the scanner and the seed poured into a receptacle after scanning. More will be said on this tray version below.

Most of the kernel weight software development was made while using this type of flat-bottomed tray. Later a more advanced tray and grain sample presentation system was developed as detailed in section 4.4.

4.3 Software Interface

SeedCount's user interface was specifically designed for grain industry operators so it would be easy to use and clearly present the results of each scan analysis. A screen shot of the interface as it was in September 2001 is included as Figure 4.1.

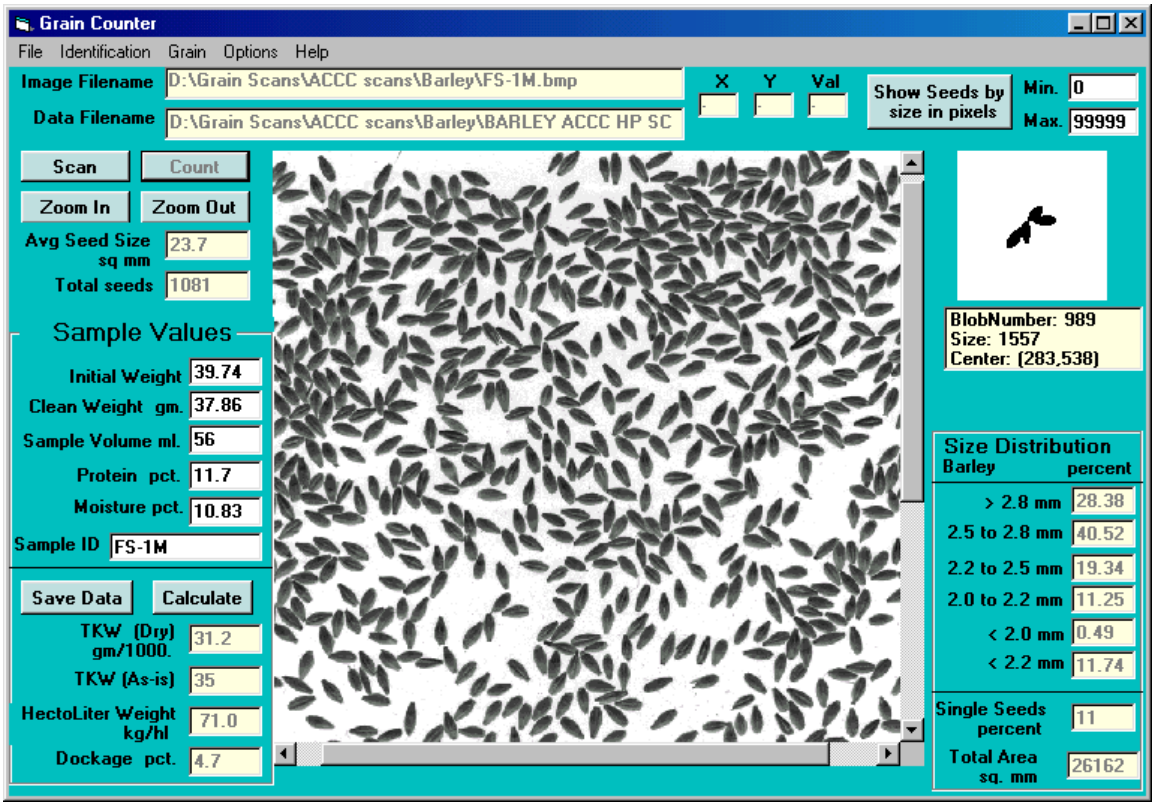

Figure 4.1 SeedCount Main Screen at September 2001

As can be seen from the screen shot, SeedCount was by then attempting to determine the average area, kernel weight, aspect ratio (plumpness), test (hectoliter) weight and screening assortment for each sample, using 100 dpi greyscale images. A small cluster of touching kernels is displayed in the upper right portion of the interface. The rest of this thesis examines how these tests were developed and follows them through to their current status.

4.4 Seed Presentation

One of the first problems to be solved was how to quickly and easily arrange the seeds to produce scans that could be successfully analysed by DIA software. The kernel presentation methods were briefly presented in section 3.4.4.5. The rationale behind their development is presented here.

4.4.1 Direct on Glass

At first, a set mass of seeds (40 grams) was simply poured onto the glass surface of the scanner. This process was not satisfactory as some seeds tended to bounce off the scanner. Making a simple hollow rectangle to retain the seeds on the scanner glass stopped this loss of seed, but the kernels still tended to clump together. The seeds were then separated to some extent with a custom-made comb that had five teeth set 20 mm apart. The comb helped distribute the kernels evenly across the entire surface of the glass. The scanner was then physically shaken gently to ensure that no seeds were lying on other seeds and to maximise the number of single seeds (i.e.- seeds which were not touching other seeds). As will become clear below, the more single seeds in the scan, the more reliable the results become. The hollow frame was then removed and the seeds were scanned.

A highly contrasting background was required to sharply distinguish the grain from the background. As wheat and barley grains tend to be a pale yellow colour, a matt black background was used. Experimentation demonstrated that an elevated cover lined with

“blacker than black” cloth from Edmund Scientific (USA) provided an excellent background while allowing easy access to the scanner.

Though this direct-on-glass method was simple and provided sharp, clear images, it had several major defects. One defect was simply that it was awkward to collect the grain off the scanner glass after scanning. Another problem was that shaking the scanner would likely reduce its working life. Yet another defect was that no matter how careful the operator was, there was still a high percentage of touching kernels.

4.4.2 Wire Mesh Insert

One attempt to increase the percentage of single seeds involved the use of a hollow rectangle fitted with a wire mesh with rectangular holes about the size of a barley seed. The intention was to brush the seeds into the holes in the mesh and lift it up without disturbing the seeds, thus creating a pattern of aligned and mostly single seeds. It was found that as the tray was lifted, many seeds would move and create small clusters of kernels. Other kernels would remain caught in the mesh. As section 5.3.6 shows, this approach was not very successful at increasing single seed percentages.

4.4.3 Flat-Bottomed Tray

Developing a clear-bottomed plastic seed tray solved two of the presentation problems. The grain could now be poured into the tray, spread and shaken, placed in the scanner, scanned and then poured back into its container. Although distributing the seeds properly in the tray took an experienced operator ten or fifteen seconds, it was not difficult. However, the new tray was not able to increase the percentage of single seeds. This version of the imaging system, including the tray insert and comb on top of the cover, can be seen in Figure 4.2.

Figure 4.2 Clear-bottomed Tray with sample, ready for scanning

The tray essentially covered the scanner glass, with vertical ends and sloping sides to avoid tray side interference in the scan due to the angle at which the scanner optics operate along the edges of the glass area. This angle is due to the reflected light from the scanner imaging area needing to pass through a set of folding mirrors and a centrally located lens in the scanner's moving optical carriage assembly before reaching the Charge-Coupled Device array (CCD). The tray shape can be seen in Figure 4.2.

However, the presence of the clear hard-coated 4mm polycarbonate tray bottom between the glass and the grain reduced the sharpness and brightness of the image slightly. It also made the grain appear slightly smaller in the lateral direction due to the increased distance from the scanner lens.

Though these methods of seed presentation worked reasonably well for counting the seeds, they had two serious faults when trying to find screenings equivalents or perform

more complex seed analysis. One fault was the inability to directly view the seeds edge-on. The importance of this is discussed in section 6.3.6.

The other fault was the low percentage of single seeds mentioned above. A totally unique and different approach was required to solve these two problems.

4.4.4 Indented Trays

The new seed presentation concept was to develop a tray with specially shaped indents that could hold the seeds on edge (edge-on). Imaging the seeds from above, instead of from below, eliminated the distortion caused by the tray. This made it possible to directly measure the thickness of the seeds.

Based on calliper measurements of a number of large and small barley kernels, the profile of the indent was determined. It had to be small enough at the bottom of the groove to cradle the smallest kernels and broad enough at the top to accept the thickest seeds. But it must still be narrow enough to exclude seeds from entering the groove flat on their backs and also shallow enough to minimise the number of hidden doubles caused by a small seed being overlaid by a larger seed.

4.4.4.1 Grooved Tray

Presenting the kernels edge-on was most easily done with simple Vee shaped grooves. However, when analysing barley, the grooves often filled up with seeds that aligned themselves with one end pointing up and partially over the neighbouring seed, as can be seen in Figure 4.3. Thus the image revealed a long string of overlapping seeds. Though the thickness of these kernels could be measured, it was impossible to accurately measure the kernel's length and area. It was also quite difficult to move the seeds along the grooves to separate them. The overlapping limited the usefulness of this tray design.

Figure 4.3 Grooved Tray Detail Showing Many Overlapping Barley Kernels

4.4.4.2 Edge-on Indents

The Vee-grooved concept was extended to incorporate a modified version of the individual pocket principal used in hand-counting seed trays as seen in Figure 4.4. This design largely overcame the overlapping problem, though some doubling up of kernels in individual indents still occurred. It was also apparent that having a section with pockets designed, like the hand-count tray, to display the seeds lying dorsal or ventral surface up, would maximise the number of single seeds in the scan. This tray design improved the accuracy of the seed counts. It also increased the data available for other analysis, as multi-kernel clusters could not be used for detailed analysis.

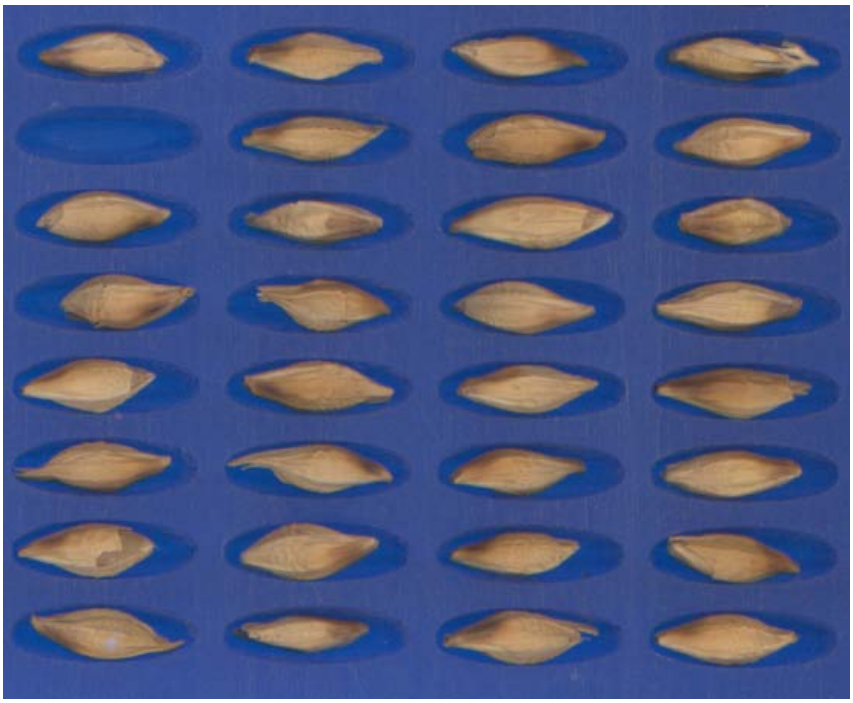

Figure 4.4 Narrow Indents, Individual Pockets - Barley Sample Shown

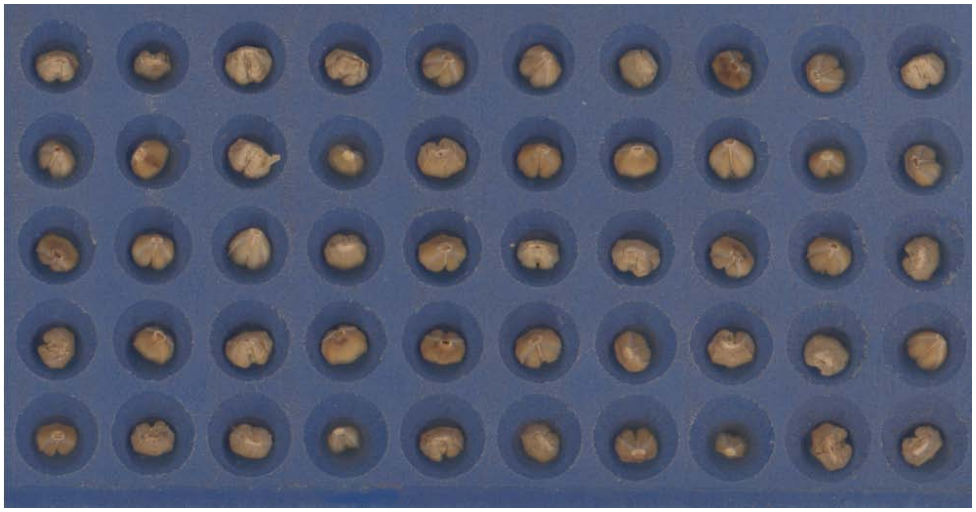

Figure 4.5 Barley Kernels in End-on Holes

4.4.4.3 End-on Holes

An extension of the edge-on indent concept was end-on holes. In these indents the kernels were placed into round, tapered holes shaped and oriented to present what was

effectively a "cross-section" of the seed to the scanner lens, as seen in Figure 4.5. This allowed the determination of both the width and thickness of each kernel. Very few doubles occurred in these holes, but they were somewhat more difficult to fill than the larger indents. Though the kernels in the end-on holes generally presented well, a significant number of seeds were not correctly aligned in the holes and gave excessively large dimensions when analysed. The end-on orientation made it difficult to identify such seeds and this approach was abandoned.

4.4.4.4 Tray Production

The complex indents and high precision (errors need to be less than 0.1 mm) required to make these trays necessitated their manufacture with a CNC (computer numerically controlled) router. As both the programming of the router and cutting of the tray was very expensive, the routed tray was used as a master and extra trays were cast in plastic resins. Figure 4.6 shows a cast tray. The tray incorporates two different types and several sizes of indents. This arrangement is explained in detail in section 6.3.2.

4.4.4.5 Tray Colour

The tray colour was selected to maximise the difference between the sample kernels and the tray. This difference facilitated designing a software algorithm to separate the tray from the kernels. It was also desirable to produce a tray that allowed light to penetrate into the tray to reduce shadowing of the edges of kernels deep in the tray. For these reasons a translucent blue tray was produced (Figure 4.6).

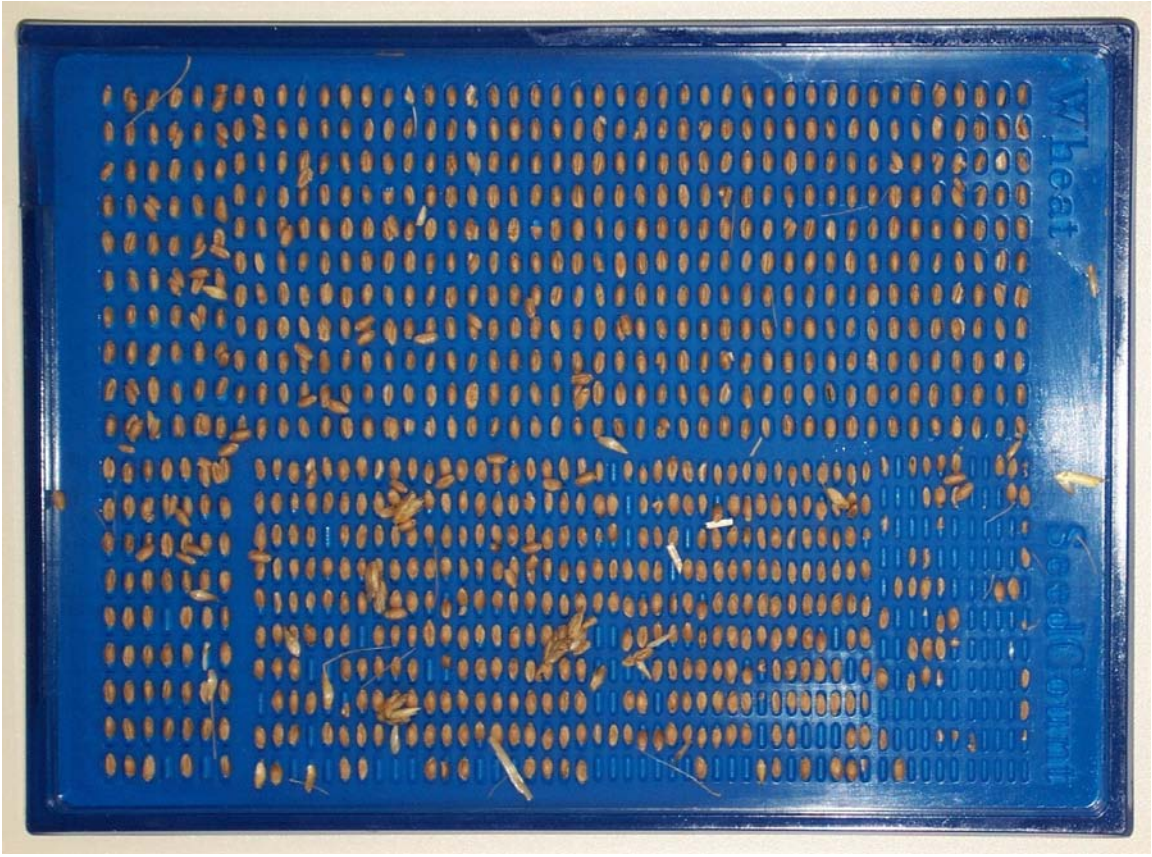

Figure 4.6 Bi-Modal Wheat Tray

4.4.4.6 Backlighting

As some of the indents were quite deep, especially the end-on holes, it was thought that backlighting might be essential. Backlit images could also be useful for revealing chalkiness in rice (Wan, Lin and Chiou, 2002), vitreousness in durum wheat (Xie et al., 2004) and steeliness in barley (Fulcher and Churchill, 1999). A camera box was designed and built that allowed backlighting of the trays. Electrofluorescent panels from Edmundson Scientific (USA), similar to those used to backlight LCD screens, were trialled as their even light output and thinness should be ideal for backlighting. It was found that even running at their maximum light output levels they were not bright enough for this purpose. A 35-watt 350mm diameter fluorescent tube was used instead. Interposing a 5mm thick layer of "white opal" acrylic sheet made the backlighting sufficiently uniform for this purpose.

4.4.4.7 Reflective Back

Another approach to reducing shadowing in the tray indents was to produce a tray with a reflective back. Three approaches were tested. They were a chemically-formed silver back using the silver mirror test (Vogel, 1954), a cast-in-place aluminium foil backing and a white painted backing. The white backing proved to be quite effective and was simpler to produce than the other two backings, so it was selected as the preferred backing.

4.5 Imaging Methods

There are currently three readily available methods of capturing digital images: Video cameras, digital still cameras and scanners. Initially flatbed scanners were used as the kernels could simply be placed on the scanner glass and imaged. However, when the indented trays were developed, standard scanners were no longer effective as distortions caused by imaging through the indents from beneath the tray made the images almost useless. It became necessary to find a way to image the trays from above.

4.5.1 Video Cameras

Video cameras, linked to image grabbers that convert the analogue camera output into still digital images, are often used to transfer images to computers for analysis. The cameras are fast, but the image quality, and especially the colour information, is poor. The number of available pixels for each image is also limited. For these reasons, video cameras were rejected.

4.5.2 Digital Cameras

Digital cameras offered better image colour quality and more pixels per image. A camera-based system also had the advantage of being able to image the trays from above. A Kodak DC4800 camera was selected for use as the initial intention was to produce 200 dpi 24 bit colour images. At its highest resolution setting (3.1 megapixels) the camera was capable of imaging an area large enough to hold six hundred to eight hundred

kernels of barley. Though the camera could capture the image rapidly, transferring the image to the computer for analysis required about 35 seconds. As this delay was comparable to the scanners available at that time, the camera was investigated for SeedCount image capture use.

4.5.2.1 Camera Box

To make accurate, comparable images, it was necessary to mount the camera in a fixed position and to have consistent lighting for the tray. The lighting was achieved by mounting two 20 watt fluorescent tubes in the camera box in a position where they provided even lighting across the tray but did not reflect light directly back into the camera lens. The camera was positioned at a height that produced 200 dpi images of the tray. The camera mount on the top of the box allowed the operator to change the distance to the tray and align the camera for optimal images. This arrangement is shown in Figure 4.7 with the front off the box. Later a backlighting lamp and translucent divider were installed in the bottom section. The backlighting section was also used with scanners by mounting the scanner upside down over the tray cut-out.

It was discovered that the Kodak DC4800 camera used was not able to focus sharply at the distance required to make 200 dpi images as shown in Figure 4.8. At about the same time, it was decided to increase the image resolution to 300 dpi for enhanced image quality. The camera's 3.1 megapixels were not sufficient to make large enough images for the required number of seeds at that resolution. The digital camera system was abandoned and the effort returned to using scanner based systems.

Figure 4.7 Digital Camera Box with Front Removed

Backlighting can be placed into section where ruler is seen.

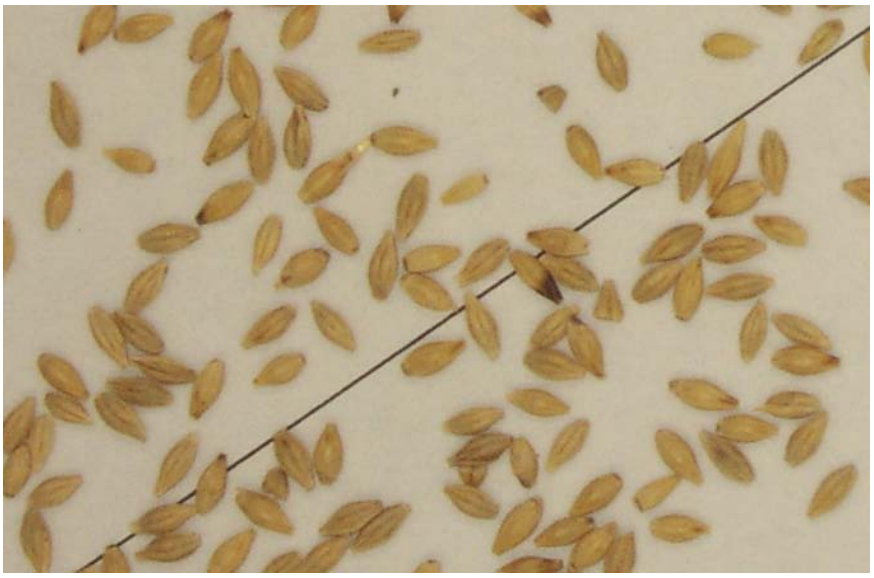

Figure 4.8 Barley Imaged at 200dpi with a DC4800 Digital Camera

4.5.3 Scanners

Scanners produce large, high-resolution images with excellent colour sensitivity at reasonable speeds. They also provide their own illumination. When it was decided to use 300 dpi images to provide better resolution for determining the seed thickness, scanners were the only systems able to make full-tray scans in one pass. However, they have fixed focal planes with a relatively small depth of field and are designed to work with the glass uppermost. This orientation, as noted above, could no longer be used with the indented trays as the imaging needed to be made from above the trays.

To use the scanners upside down, the carriage guide mechanism was modified to support the carriage in that position. It was also necessary to modify the scanner's optics so it would be able to focus at the plane of the cereal sample in the tray. The extra distance from the scanner glass to the new focal plane at the tray surface reduced the illumination levels from the scanner lamp. The scanner lamp also only illuminated the kernels from one side of the grain, causing one side of the grain to be darker than the other. Initially mirrors were tested to correct these illumination problems. Though this helped, eventually two lamps were used, one on each side of the kernel that was being imaged.

With these alterations it was possible to produce high quality images of the grain in the tray. However, the increased distance to the kernels caused lateral distortion of the image, making the kernels appear more compact than they really were. This problem has been compensated for by the software. The scanner used at that time (a Hewlett-Packard Scanjet 5400) was fairly slow (at 38 seconds) at producing a full-tray 300 dpi color scan. High-speed scanners with USB 2 interfaces are now available. One of these, an Epson Perfection 1660 Photo scanner, was substituted for the HP 5400 and reduced the scan time to 11 seconds.

4.5.3.1 Tray Presentation

A convenient method of placing the tray in position under the scanner was needed. This was achieved by using a cabinet with a roller track drawer system to hold the tray. To get the tray around the curved cover on the HP 5400 scanner, a cam-actuated lift system was

developed to bring the tray up as close to the scanner glass as possible to minimise the light reduction and lateral distortion mentioned above.

The Epson scanner did not have a protruding lip, making it possible to eliminate the cam system entirely without any loss of image quality. This cabinet design can be seen in Figure 4.9.

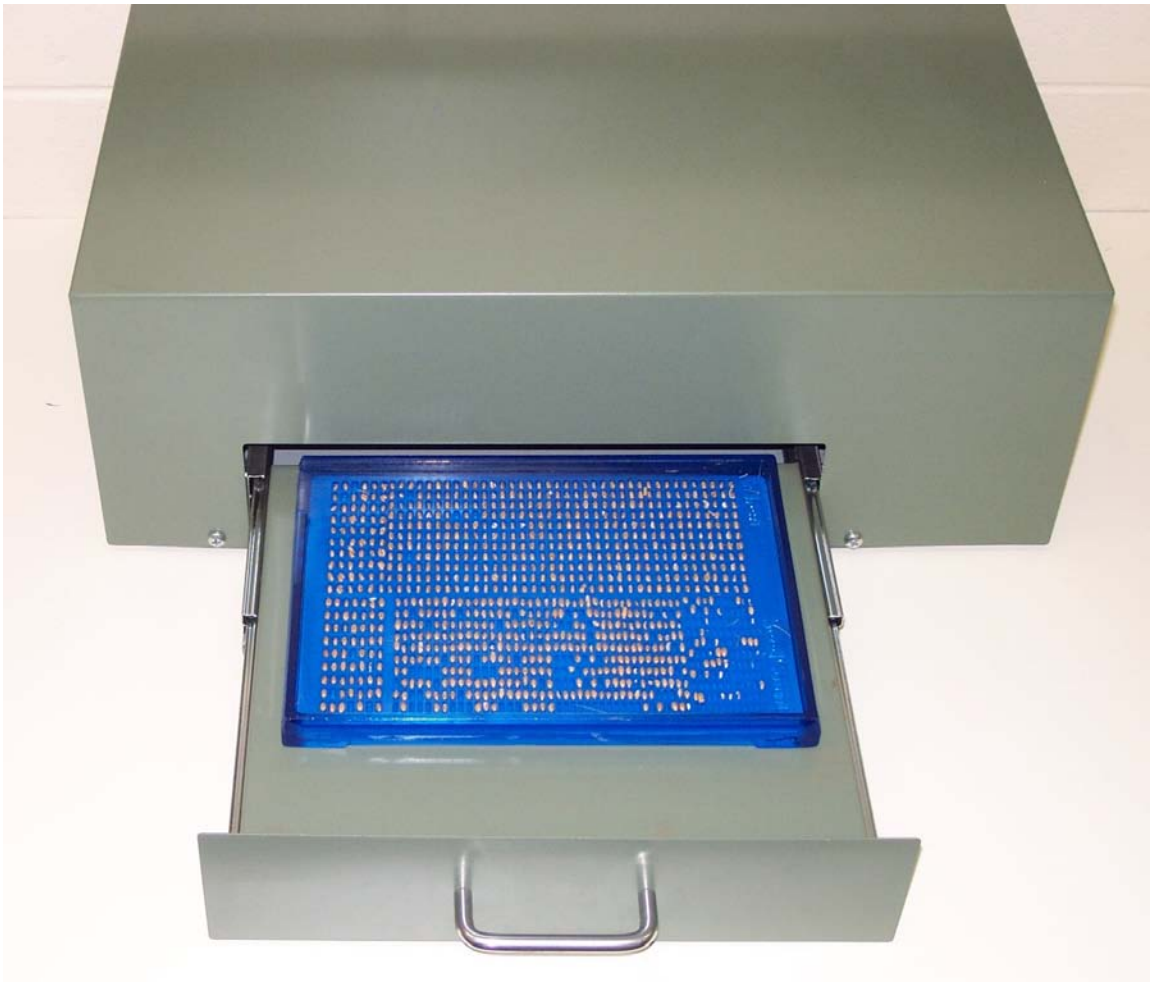

Figure 4.9 Scanner Cabinet With Loaded Tray Ready For Insertion And Scanning

The SeedCount version 2 main screen with a 300 dpi colour image displayed on it is included as Figure 4.10, showing the state of the program in October 2004. The highlighting shows kernels identified as having blackpoint, a feature that is not covered in this thesis.

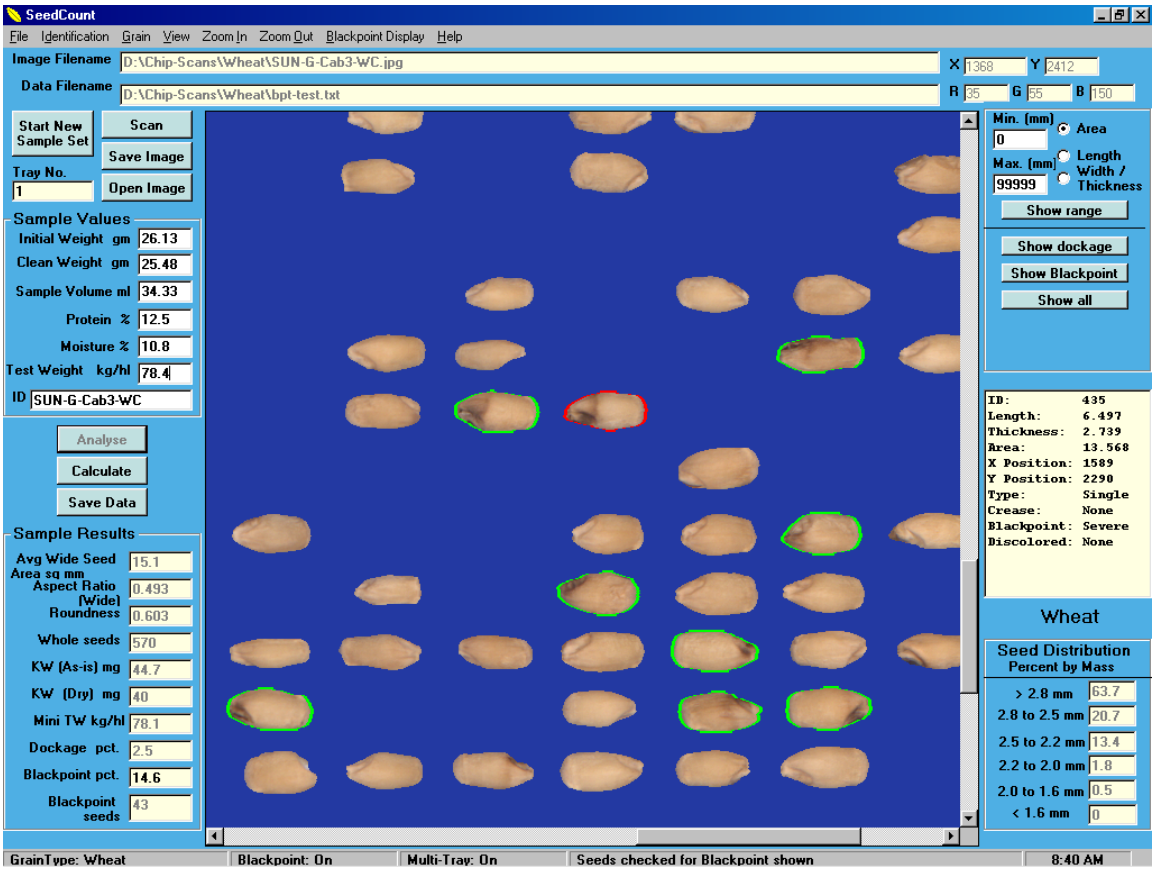

Figure 4.10 SeedCount Main Screen, October 2004

4.6 Conclusions

Extensive development of the DIA system has occurred throughout this project. Some of the development occurred in response to the release of new digital imaging equipment. Most of the development was internally driven as novel, and generally better, solutions to various imaging and analysis problems were pursued as outlined in this chapter.

The DIA system progressed from 100 dpi greyscale images of randomly distributed seeds on the scanner glass to 300 dpi full-colour images of seeds aligned in indented trays.

The following three results chapters detail how this DIA system has been developed and used to analyze the kernel weight, orthogonal dimensions, area, aspect ratio, roundness and screenings equivalents of grain.

5 Using Digital Image Analysis To Determine The Thousand Kernel Weight Of Barley, Malt And Wheat

5.1 Introduction

Rapid, accurate and non-subjective grain tests are required by grain breeders, growers, distributors and processors. These tests assist breeders to select promising new cultivars and allow those in the grain handling chain to agree on the correct classification and ensure that the grain is used for the most appropriate purpose. Common tests include protein, moisture, test (hectolitre) weight, screenings and visual inspections for pests, diseases and foreign matter (Vicgrain, 1999). Each test contributes to a more comprehensive assessment of the grain quality.

Plant breeders and maltsters also use the thousand-kernel weight (TKW) test, which provides additional information on seed morphology. The test indicates the average kernel weight, with the units expressed as grams per thousand seeds. The clean sample weight, measured to the nearest 10 milligrams, and the whole kernel count are required for this determination. TKW results are valuable to maltsters and millers as high TKW kernels are plumper, usually malt and/or mill more evenly and have a higher proportion of endosperm than small kernels (Briggs, 1978). The high TKW grains also produce more visually attractive malt (Stuart, 1998). TKWs assist breeders in selecting large kernel cultivars and permit growers to calculate their optimum sowing rates (Schwarz and Horsley, 1995).

Hand-counting the kernels for a TKW determination is tedious and time-consuming. Using a seed tray, which has indents to hold 100 kernels, speeds up the process and reduces the tedium, but it still requires 10 to 15 minutes to count each of the two lots of 40 grams of seed in one standard barley TKW (EBC, 2003; IoB, 1997). Laboratories that make frequent TKW determinations usually use electromechanical seed counters such as the Numigral or Countador or pill counters like the Kirby to hasten the process. These

machines can be useful but, as will be demonstrated, are still quite slow and in some cases are not very accurate.

Digital image analysis (DIA) can potentially count the kernels rapidly and accurately, but it has difficulty counting kernels that are touching others, as DIA generally regards all touching kernels as a single seed (actually a “blob”). Simply ignoring the touching grains would result in inaccurate TKWs because only part of the sample mass would be used in the count. DIA systems have been developed that use feed mechanisms (Cervitec), conveyor belts (GrainCheck) or vacuum assisted tray-filling systems (SPY Grain Grader) to physically separate most of the seeds. These systems can work reasonably well, but the specialised hardware required makes them very expensive.

Another approach to DIA is to use commonly available computers and flatbed scanners and develop an algorithm that will count all of the single and touching grains in a randomly distributed sample. Shatadal (1994), attempted to do this by developing a shape recognition algorithm that digitally cut apart touching grains. He was able to achieve 93% accuracy. This principle is used in the TrueGrade lentil DIA machine (Hinz Technologies, 2004).

5.2 Aims

This chapter investigates two other approaches to this problem:

- Developing macros that use Scion Image’s edge erosion routines to separate the touching kernels.
- The "SeedCount" approach, based on a novel counting algorithm coupled with special indented trays, for the counting of kernels.

The ultimate aim is to discover and verify a DIA kernel weight method that is accurate, reliable and fast.

This chapter concentrates on the various methods of counting kernels, as all these systems use the same method of determining the sample mass – a top-loading balance

accurate to 0.01 grams. Such a balance has an inherent error of less than 0.025% when weighing a 40-gram sample ((0.01*100)/40). This error is both consistent and insignificant when compared with counting errors, allowing it to be ignored in most TKW discussions. For example, an error of one seed in a count of 1000 kernels is an error of 1 * 100/1000 = 0.1%. This is the minimum counting error possible and is already 4 times the mass measurement error. The counting portion of the TKW determinations is therefore the portion of interest, as it is where the large errors can occur.

The first aim was to establish accurate counts for the samples used, an absolute requirement in any comparison of counting methods. The “gold standard” in counting accuracy was found by comparing duplicate counts for the same sample to see which method gave the most accurate and precise results.

The second aim was to compare the various counting methods against the “gold standard” and each other to discover the relative merits and deficiencies of these methods. The DIA counting systems were of special interest as they were being investigated as possible replacements for the existing methods.

5.3 Results and Discussion

Details of the materials and methods used are given in section 3.3. The same sub-samples were used in each counting method with the exception of some of the DIA indented tray samples that were not counted with the electromechanical counters or with Scion Image. The results are based on a minimum of 42 wheat, 30 barley and 24 malted barley sub-samples including replicates.

5.3.1 Hand-counted TKW

Hand-counting was performed with and without the indented manual count seed trays. The author performed most of the hand-counting, but 6 other people also counted some of the samples. Their counts were used as duplicates, and were recounted if there were large differences from the author’s count. These counts, and the counts made by other

methods, were converted to dry-weight TKWs by use of the sample mass and moisture determinations as detailed in sections 3.4.3.1 and 3.4.4.1.

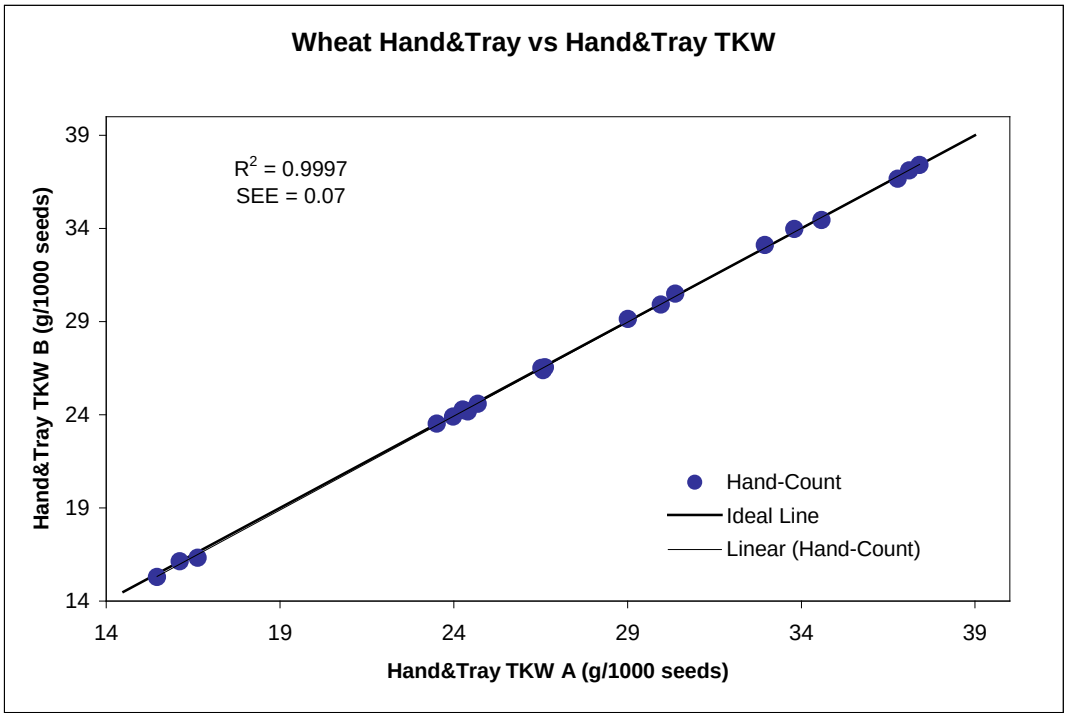

Figure 5.1 Hand-counting Wheat with Indented Trays

Comparison of Replicate TKW values determined by Hand-counting using Seed Trays.

The accuracy and precision of the principal results are illustrated on graphs that use a similar format for this section. This format will be explained for Figure 5.1, which shows the accuracy of TKWs calculated for wheat samples that have been hand-counted using indented seed trays. Seven cultivars were sub-sampled three times, producing 21 samples. Each sample was counted twice and contained an average of 1250 kernels weighing 36 grams. In Figure 5.1 the initial (A) and replicate (B) TKWs are plotted. The solid "ideal" line on each of the graphs matches "perfect" TKWs where the initial (X value) and replicate (Y value) TKW are identical (ie. Its R^2 value is 1.000). The Line of Best Fit (the dashed regression line, marked as Linear Hand-count, etc) is the actual Best Fit, which overlies the Ideal line on "perfect" correlations. The graphs and regressions were prepared with Microsoft™ Excel 2000.

The high correlation (R^2=0.99992) and small Standard Error of the Estimate (SEE = 0.07) as well as the almost perfect alignment of the linear best-fit and ideal lines in Figure 5.1 show that the Hand plus Tray counts are very accurate for wheat.

Figure 5.2 shows the results for barley (5 cultivars by 3 sub-samples by 2 replicates, avg of 938 kernels weighing 36.5 g). Figure 5.3 displays the malted barley results (4 cultivars by 3 sub-samples by 2 replicates, avg of 955 kernels weighing 34.5 g). The cultivars were selected to provide a wide range of TKWs. Like the wheat, they also demonstrated very high correlations and low SEE with the recounts and resultant TKWs.

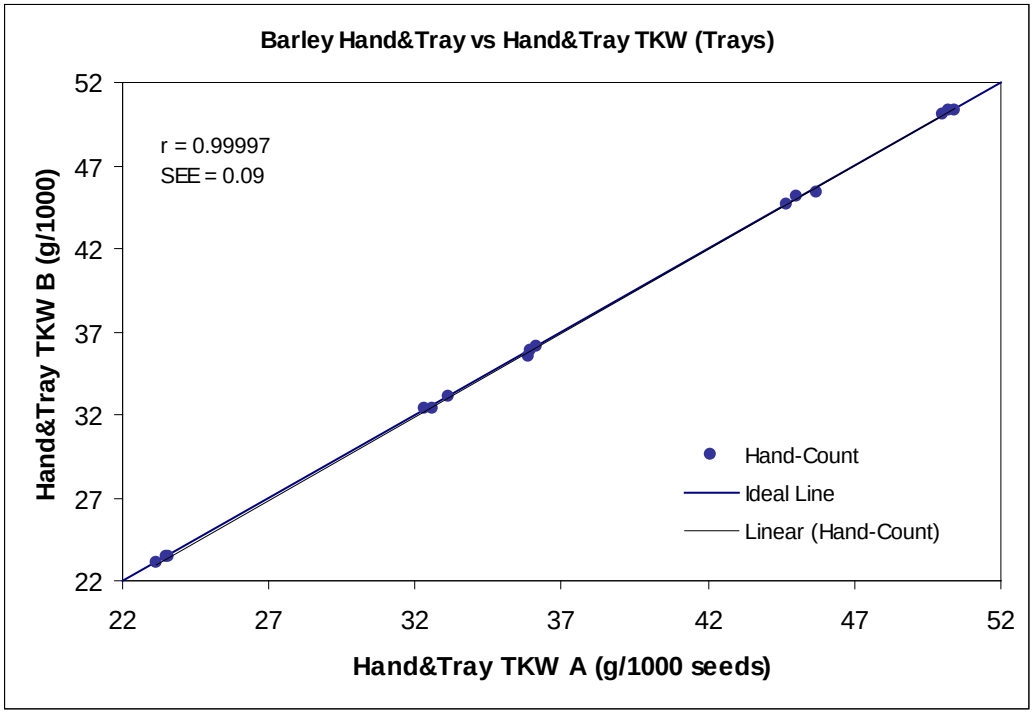

Figure 5.2 Hand-counting Barley with Indented Trays

Comparison of Replicate TKW values determined by Hand-counting using Seed Trays.

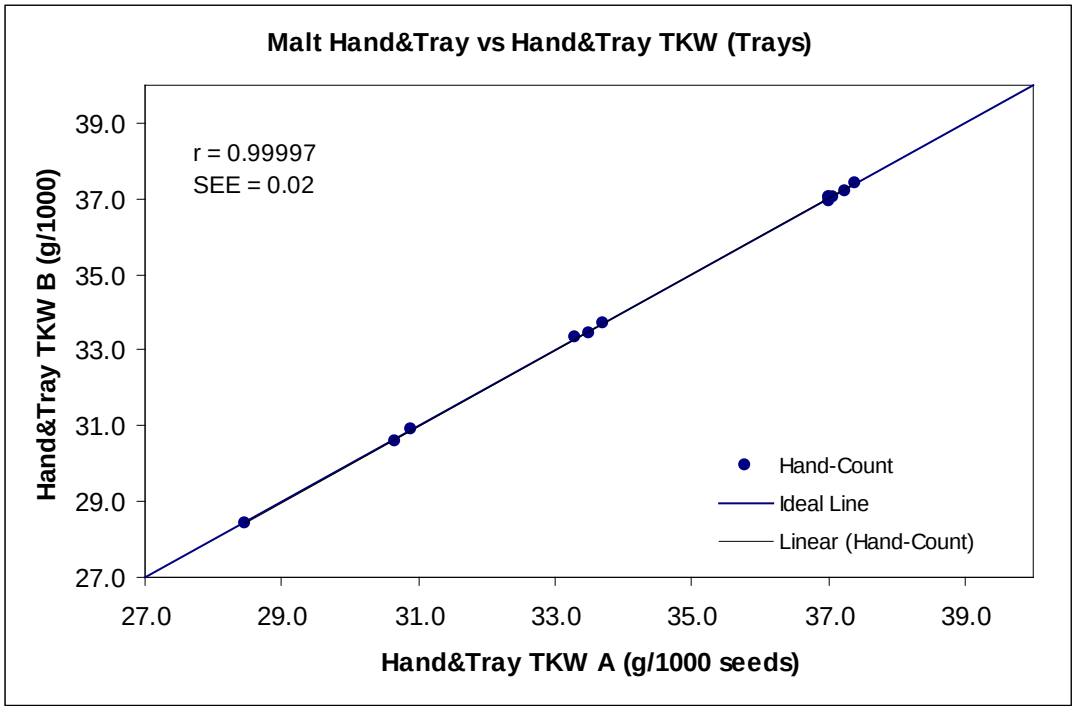

Figure 5.3 Hand-counting Malted Barley with Indented Trays

Comparison of Replicate TKW values determined by Hand-counting using Seed Trays.

The very high match in counts (and therefore in the resultant TKWs) for the hand-counting using indented trays has made these counts the baseline standard for comparisons with other methods. Where there were differences between the initial and replicate counts, as shown in Figures 5.1 to 5.3, additional counts were made to ensure that the baseline data was reliable. The verified results were then used for comparison purposes in the other graphs. The occasional differences found in this method were usually due to small seeds being covered by larger seeds in some indents. The person doing the counting had to move the larger kernels aside with a bamboo skewer to see if there was a hidden seed in the indent.

Please note that the people counting these samples were instructed to count accurately rather than rapidly when performing these counts. Lighting conditions were also excellent. These considerations also applied to the hand-counting that was performed without use of the indented trays. It is expected that people who were tired and

overworked, or who were working under poor lighting, would not achieve these accuracies.

The other possible contender for the baseline count was expected to be hand-counting without using the indented trays. In this case the counting was assisted by the use of small flat wooden sticks to group the kernels as counting proceeded. Figure 5.4 compares hand-only counts with tray-assisted hand-counts for wheat. The format of this graph is therefore somewhat different from Figure 5.1. In this case Hand A and Hand B are the two replicate hand-only counts and are both plotted on the Y axis as two separate series against the average verified hand-counted tray results which are plotted on the X axis. The line of best fit is that the first Hand-only TKWs (Hand A) vs the Hand-Tray results. The coefficient of determination (R^2) and the Standard Error of the Estimate (SEE) shown is for the data in both the A and B series. All of the remaining TKW graphs use this format.

As Figure 5.4 shows, hand-counting wheat without the use of the indented tray has a lower accuracy than when the tray is used. The decrease in accuracy can be seen in the deviation of some hand-counts from the ideal line. This trend is reflected in the decrease in the R^2 value to 0.9992, an increase in the SEE from 0.07 to 0.22 and a small deviation between the regression line and the ideal line. It is likely that the decrease in accuracy is due to the less structured counting method when not using the indented trays. Very similar results were obtained for the barley and malted barley hand-counts (data not shown).

As hand-counting without trays was less accurate than using the trays, hand-counting with indented trays was accepted for use as the “gold standard” baseline counting system. None the less, the accuracy of the hand-counted TKWs was still quite high.

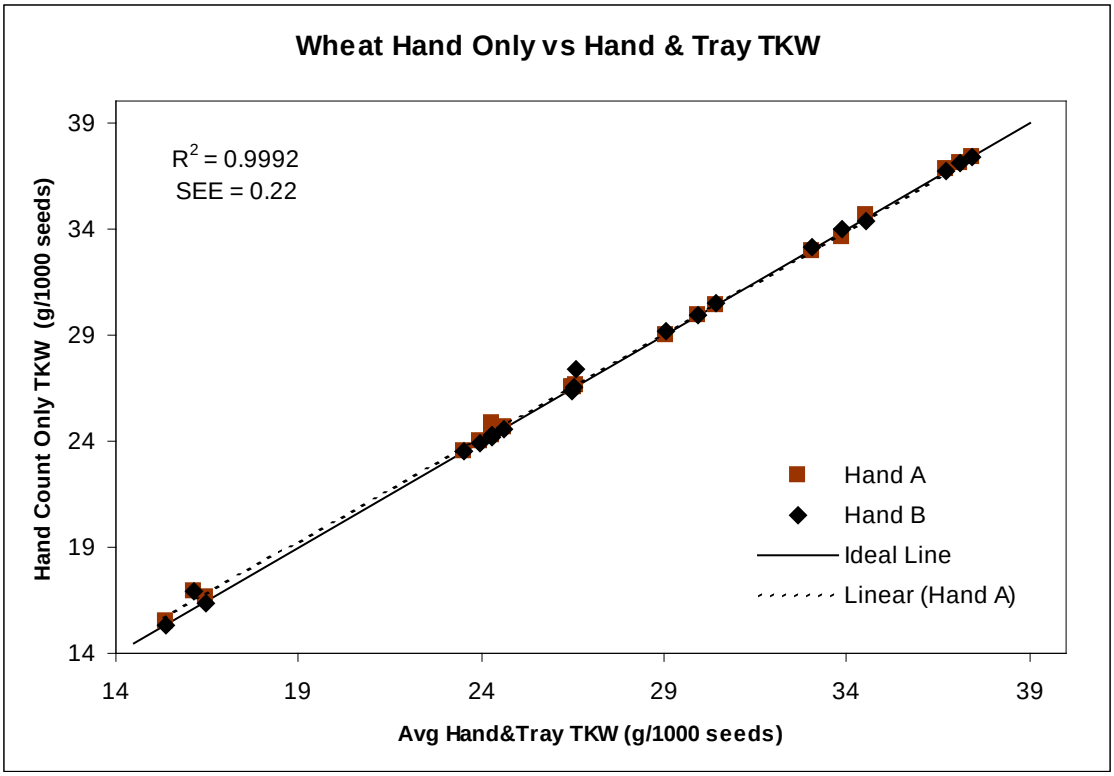

Figure 5.4 Hand-Only Wheat TKW

Compared with the average Hand-Count with indented trays.

5.3.2 Sampling Errors in Kernel Weight Determinations

In the Literature review it was mentioned that Hudson (1960) found that with a sample size of about 1000 kernels, the error in KW weight determinations could be up to 12%.

Figures 5.1 to 5.3 provide an opportunity to test Hudson's claims by checking the differences between the three sub-samples taken for each cultivar used in these graphs. Figure 5.1 is an excellent example of what can be expected. If all three sub-samples are identical, the three points should be virtually on top of each other, as can be seen by the "point" near the center of the graph. Alternatively, if the sub-samples were different, but the TKWs were accurate, there should be two or three points spread out along the ideal line, as can be seen near the bottom left of the graph. This linearity suggests that the

TKWs are more accurate than the sampling. But how large are the errors? Table 5.1 summarises the errors for these three graphs.

Table 5.1 Sampling Errors (Percent of Value)

	Avg Error	Max Error	Min Error	SEE
Barley	0.62	1.54	0.00	0.32
Malted Barley	1.13	5.12	0.08	0.64
Wheat	1.21	4.39	0.01	0.48
Average	0.99	3.68	0.03	0.48

It can be seen that the average error for all of these samples is less than one percent. The worst error (5.1%) was for a malted barley sample. As these samples averaged just over 1000 kernels each, it seems that Hudson was overly pessimistic about the required sample sizes. Though the above samples were taken carefully, they were only made with standard industry methods. The number of samples in this test is not large (48 samples), but it is sufficient to question the effectiveness of the sampling methods that Hudson based his conclusions on. The table also suggests that the sampling SEE for the bulk sample is 0.48 g/1000 kernels. This variance would be sufficiently accurate for most purposes, though it is substantially larger than the SEE of the actual TKW counting and weighing (<0.09 g/1000 kernels).

5.3.3 Electromechanically Counted TKWs

The samples counted electro-mechanically were the same samples used for the hand-counting.

Electromechanical (EM) counting was performed on a Numigral 1900 and a Kirby KL9 counter using the methods recommended by their manufacturers. The feed speed was kept low on both counters to maximize their accuracy.

Figures 5.5 and 5.6 respectively show the TKW results for wheat using the Numigral and Kirby electromechanical counters. As in the other graphs, these TKWs are compared to the average Hand plus Tray count.

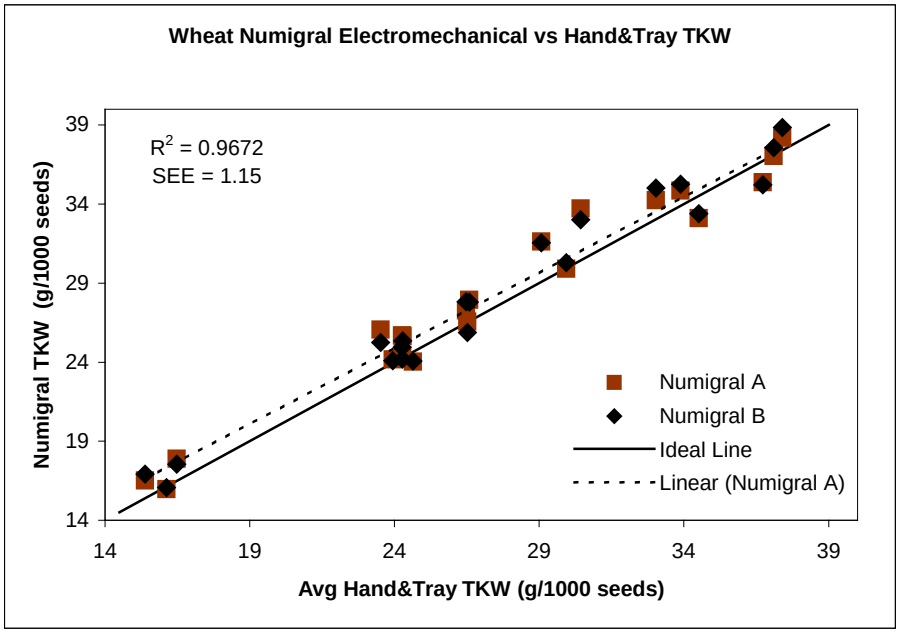

Figure 5.5 Numigral TKWs

Comparison of Replicate Numigral electromechanical counter TKW values with the average Hand-count using an indented tray results.

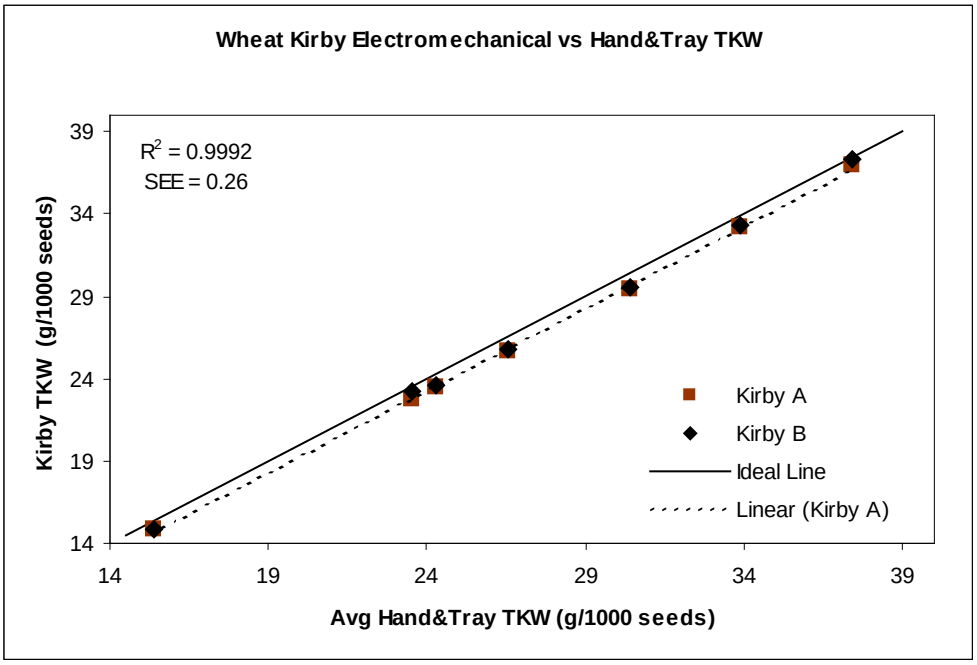

Figure 5.6 Kirby TKWs

Comparison of Replicate TKW values determined by a Kirby Electromechanical counter versus the Average of Hand-counting with Seed Tray values.

The graphs show that the two EM counters were clearly quite different in their counting results. The Numigral counter would erratically miss seeds and under-count, resulting in generally higher TKWs. The under-counting tended to be worse for samples with smaller kernels. This can be seen in the regression line of Figure 5.5, which is above the Ideal line, and deviates farther from the ideal line as the TKW decreases. The reproducibility of the Numigral was also lower than hand-counting, as can be seen by the frequent separations of the A and B series TKWs and the blowout in the SEE to 1.15 and the correlation reduction in R^2 to 0.967.

The Numigral counter also had a tendency to “eat” seeds while passing through the electronic sensor chute, causing them to be lost inside the casing. Because this resulted in irreversible changes in the samples, the Numigral counts were done last and the sample reweighed before the replicate count was performed to minimise the distortion caused by this fault in the instrument. Any attempt to increase the counting rate for the Numigral counter increased the number of missed seeds, further reducing its accuracy. As many

labs routinely reduce the Numigral counting time to about four minutes, the results shown in Fig 5.5 are likely better than the labs' usual accuracy (Agrifoods Victoria, 2001).

The Numigral operated by feeding kernels one by one down a chute and through photoelectric cells that triggered the counting. Presumably the under-counting occurred when two or more kernels struck the photocells at the same time, or overlapped sufficiently while passing through the cells to be counted as a single seed. Some of the "eaten" seeds may have passed into the cavities of the instrument before counting as well, but there were not enough of these kernels to fully account for the undercounting.

In contrast, the Kirby counter had a consistent, systematic tendency to over-count the kernels. This resulted in generally lower TKWs shown in Figure 5.6. The Kirby had much more consistency than the Numigral as can be seen in the close match in the A and B series counts and its relatively low SEE of 0.26. These results were quite surprising as the Kirby did not have a mechanical feed system and relied on careful, but unavoidably somewhat erratic, hand-feeding to count accurately. An average count time was approximately six and a half minutes. Due to availability restrictions, only 14 wheat and 12 barley samples were run on the Kirby.

It is difficult to know why the Kirby over-counted the kernels. Perhaps if the kernels were rotating end to end as they passed the photocell they may have been counted twice. Possibly a seed which passed the photocells with its long axis horizontal would have caused a larger light drop than a seed which passed the cell with its long axis vertical and was thus counted as two seeds. This theory could have been tested by counting spherical objects and seeing if the counts were more accurate.

Both of these counters, though used in grain quality labs, were assumed to count accurately and no correction equations were used to compensate for their peculiar counts. This approach should have been valid for the Numigral, which underwent regular NATA calibration testing. One can only think that it must have developed its "seed-eating" vice

since the last calibration. The Kirby unit had no labels on it to indicate that it had ever been calibrated.

5.3.4 Scion Image TKW

The images used for Scion Image and Versions 1.x of SeedCount were greyscale scans with 256 shades of grey made at a resolution of 100 dots per inch (dpi). A section of a typical kernel image used at that time is shown as Figure 5.7.

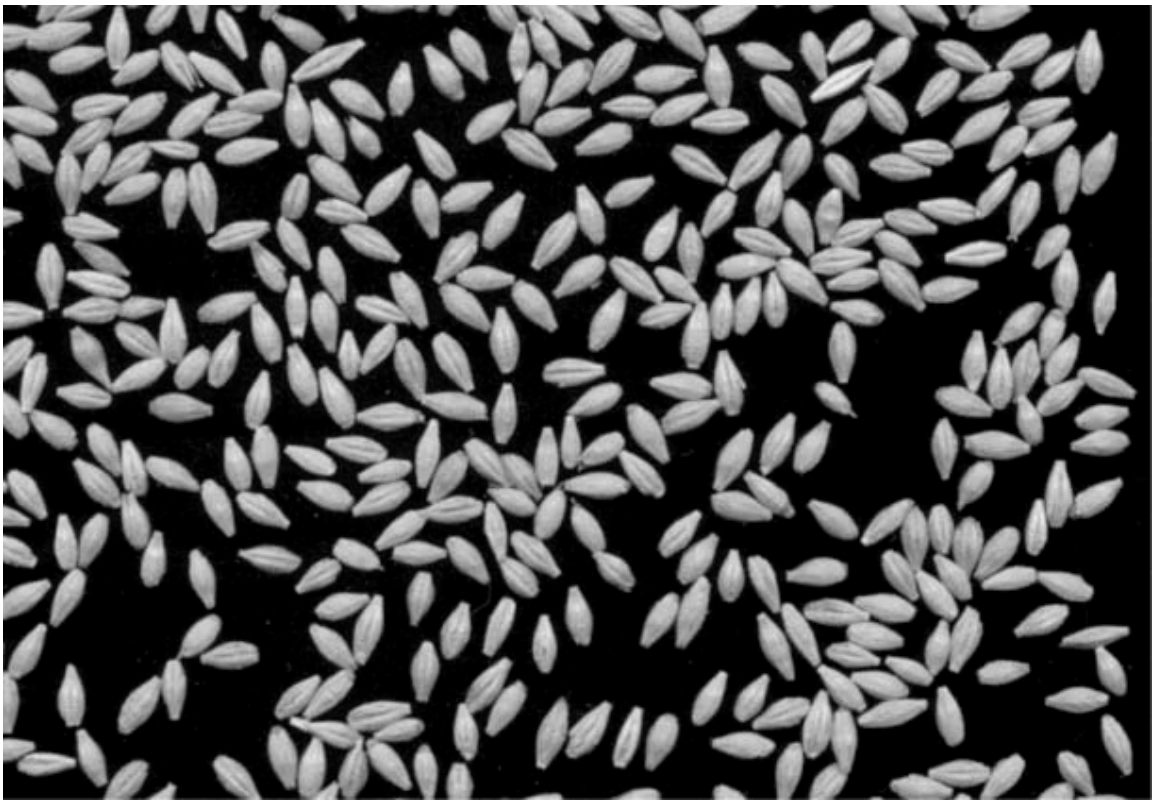

Figure 5.7 Franklin Barley

Detail from a 100 DPI greyscale image used in early SeedCount and Scion Image Analysis.

Initially the Digital Image Analysis counting was performed with Scion Image. It seemed that Scion Image (SI) should be able to count grain accurately as it was designed to count cells, which are also discrete oval objects. Many clusters of touching seeds were invariably created when the 40 grams of barley required by the IoB TKW test were

randomly placed on a scanner. It soon became apparent that the clusters were creating major problems for the software. Considerable effort was put into developing image manipulation sequences to separate the touching kernels. The best barley and wheat macros are presented in Appendix 1. Briefly, the procedure used was this:

First the image's greyscale values were inverted, so white pixels (dots) became black, pale gray became dark gray, etc. Then a greyscale level (threshold) was found that was effective at distinguishing the seeds from the background. The threshold was then used to convert the image into a binary black and white only image. Scion Image then separated the seeds by eroding the edges of the dark blobs (up to three times) and counted the resultant separate blobs that fell within a specified pixel range as single seeds.

However, the Scion Image counts were hampered by edge erosion problems as can be seen in Figure 5.8, which was eroded from Figure 5.6. Some eroded kernels remained connected together (see Ellipse 2 in Figure 5.8) while other kernels were cut into several pieces (as in Ellipse 1 in Figure 5.8). Frequently kernel creases would contribute to these cutting errors. These problems made accurate counts (and therefore accurate TKWs) very difficult with Scion Image.

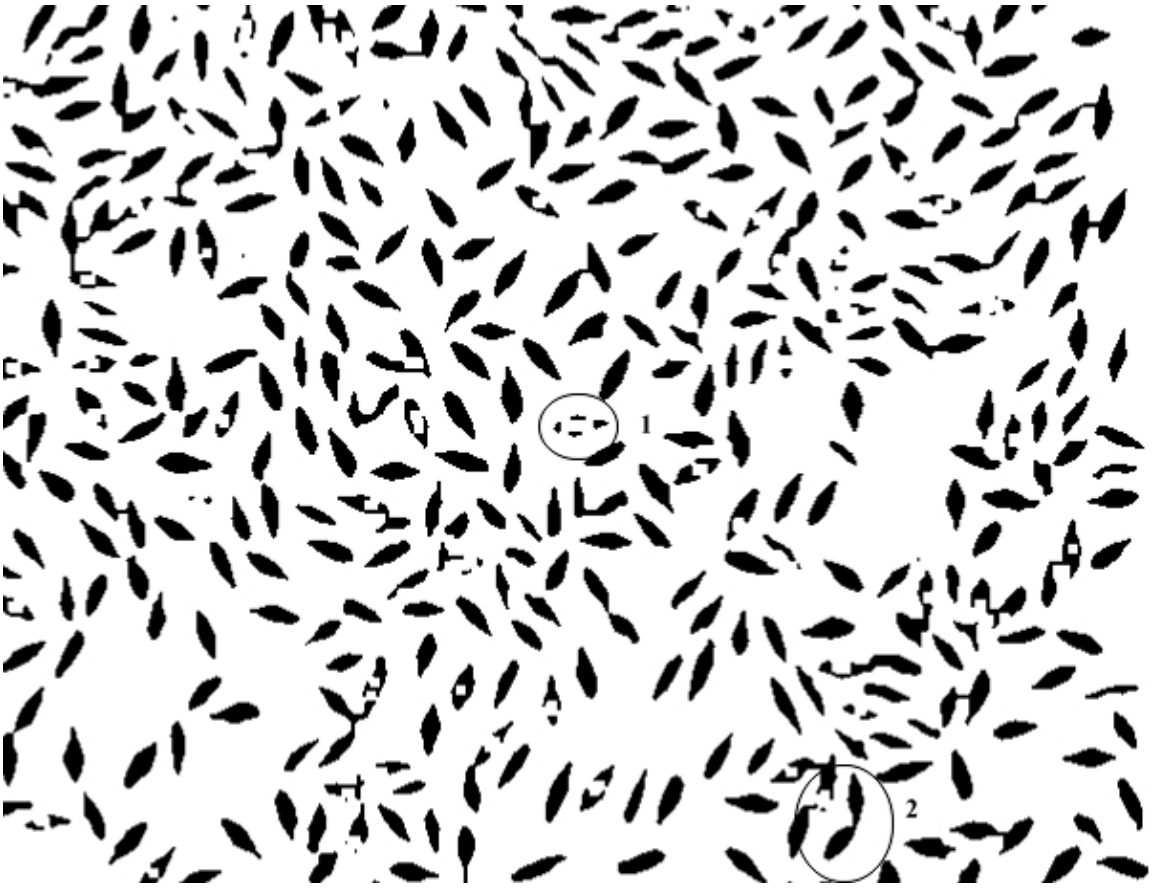

Figure 5.8 Eroded Franklin Image

Result of Scion Image Erosion Separations

The best that could be done with Scion Image was to attempt to balance the numbers of joined and split seeds to achieve a reasonable count. Though individual images could be tuned to allow accurate “counts”, the image needed to be manually counted and then have special settings used for it, making the method totally impractical. The extent of these problems can be seen in Figure 5.9. The SEE of 2.33 reflects the wide range of TKW errors generated by Scion Image. Comparing the ideal and regression lines in Figure 5.9 shows a clear crossover in Scion Image counting. It tends to under-count large kernelled grain because they have broader contact surfaces that are harder to separate. On the other extreme, the smaller grains are more readily separated and are easier to cut into pieces. Barley and malt results (data not shown) were similar to the wheat TKWs.

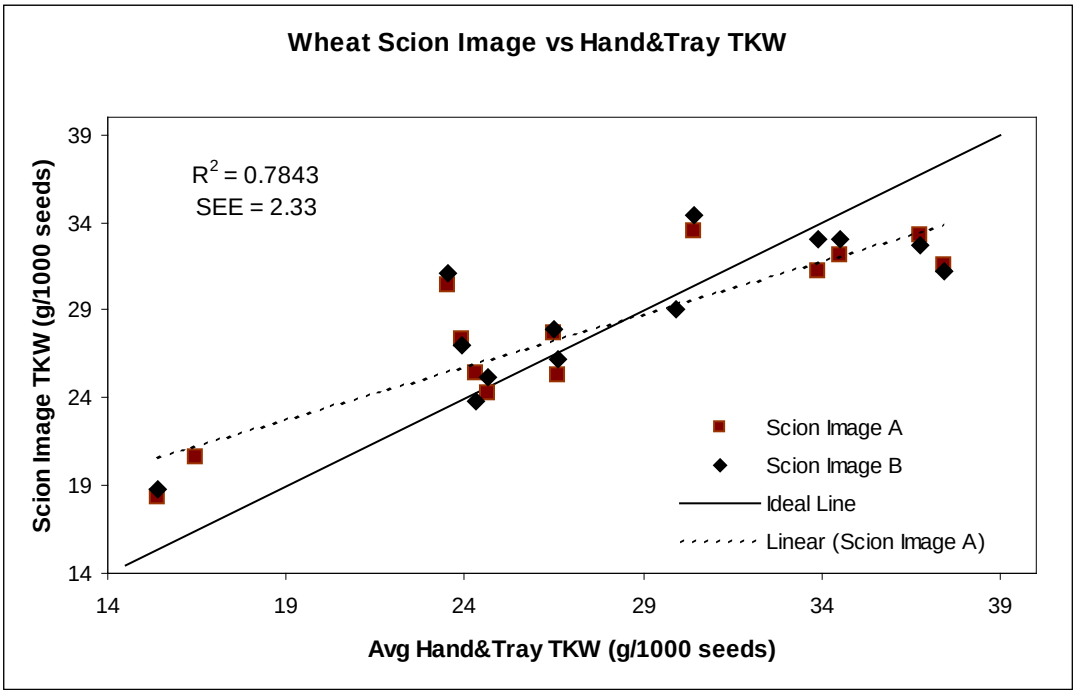

Figure 5.9 Scion Image TKW

Compared with indented tray hand-counting TKW.

The erosion process mutilates the kernel's length, width and area measurements, making it even less suitable for more detailed work than for merely counting the kernels. The binary thresholding is also unsuited to detailed DIA. It destroys the greyscale information that could have been utilized for more advanced analysis like detecting creases or blackpoint defects. Because of these handicaps, Scion Image was not used for any further DIA grain work.

A commercial image analysis package, Image Pro Plus by Media Cybernetics, was also trialled. It offered essentially the same methods to separate kernels as Scion Image. The counting accuracy obtained from it was very similar to the Scion Image results (data not shown).

5.3.5 SeedCount TKW

5.3.5.1 Flat Tray TKW Algorithm

The images used for Versions 1.x of SeedCount were identical to the uneroded Scion Image scans. They were greyscale scans with 256 shades of grey made at a resolution of 100 dots per inch (dpi) as shown in Figure 5.5.

As Scion Image was unable to accurately separate touching grains using a kernel erosion method, it was clear that a totally different way of counting kernels was needed. The SeedCount approach to the DIA multi-kernel cluster problem was unique. Rather than attempting to identify the kernels by cutting or eroding the various seed clusters, SeedCount used the inherent information in the clusters to count the seeds. This was done by identifying all of the single kernels in the image and calculating their average cross-sectional area as explained below.

The essence of the new algorithm, as used in Version 1 of SeedCount, was this:

- The seed sample is distributed in the tray as detailed in section 3.4.4.5 and scanned.

- The scan is loaded into SeedCount, and the greyscale values are inverted to convert the black background into white. The software then uses a greyscale threshold value to separate the background from the grains.

- The software sees the grains as “blobs”, with each blob consisting of a group of touching non-background pixels. A pixel is essentially a spot on the image. At 100 dpi there are 10,000 pixels in each square inch of image. The computer finds all the blobs and records the number of pixels in each blob.

- The software generates a histogram of the blobs (Figure 5.10). The material to the left of the first bell curve is debris and broken seeds. The first bell curve on the histogram is the single seeds; the next bell curve is the doubles, etc.

- The software finds the top end of the single seed bell curve and examines the edges of the blobs in this region to see if they are single or double seeds. Blobs with deep indents are marked as double seeds.

- The software then selects the single seed area of the histogram, counts the blobs in it and calculates the average blob area in pixels. This value is then used to estimate the number of seeds in each of the other, larger blobs that contain multiple seeds.

- The total number of seeds, the mass of the seeds and the moisture content of the seeds is used to calculate the thousand kernel weight of the grain on both a dry weight and “as-is” basis.

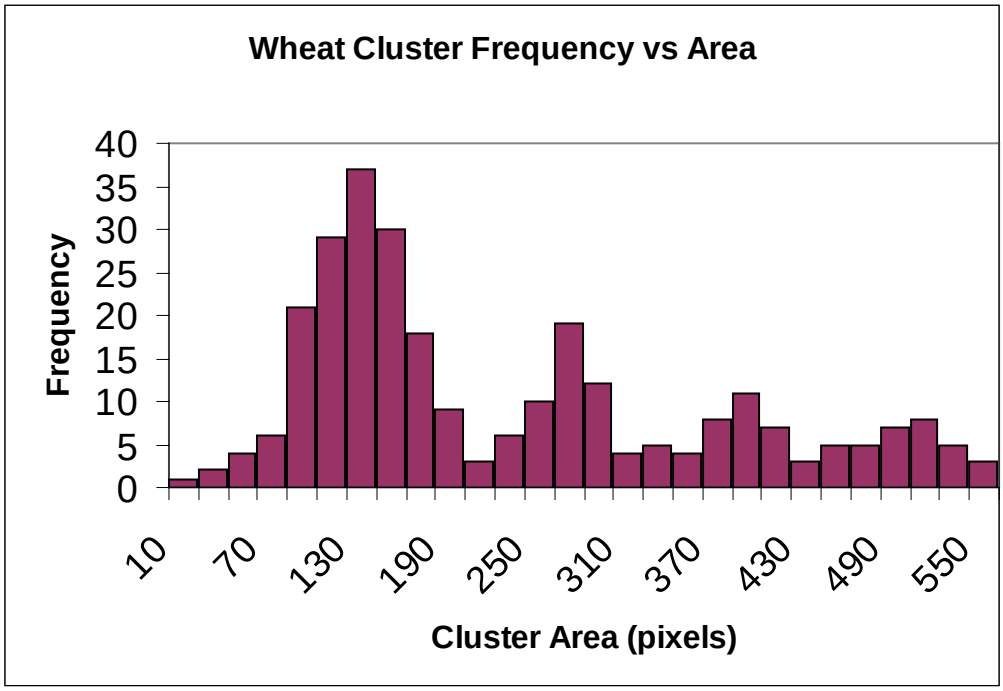

Figure 5.10 Distribution of Random Cluster Areas

This simplified histogram of a wheat sample shows the distinctive area clustering created by single, double, triple and quadruple seed clusters, also called blobs.

A typical flat tray of seeds contains about two hundred single seeds as well as numerous clusters containing two, three or more seeds. When the kernel area is placed into bins and plotted against the number of counts in each bin, there is a clear drop in frequency that separates the single seeds from the double seed clusters. As the number of seeds in the clusters increases, the peak counts for each cluster decreases and the size of the valley between each cluster groups also shrinks. SeedCount uses this valley to determine the area of an average single seed. This area can then be used to estimate the number of kernels in each of the multi-kernel clusters quite accurately. The counting algorithm is also patented (US patent 6,418,180 B1, Weiss Enterprises).

5.3.5.2 Flat Tray Results

The SeedCount DIA TKW results using a flat tray are summarised in Figures 5.11, 5.12 and 5.13, where they are compared to the baseline of Hand-tray TKWs. There is clearly a

vast improvement over the Scion Image results (Figure 5.9), as confirmed by the respective SEEs.

SeedCount's wheat results show a close fit to the ideal line (Figure 5.11) and high correlation with the baseline TKW (r=0.9992, SEE=0.25) demonstrates that it is both more accurate and more precise than the Numigral electromechanical counter (Figure 5.5; r=0.984, SEE=1.15).

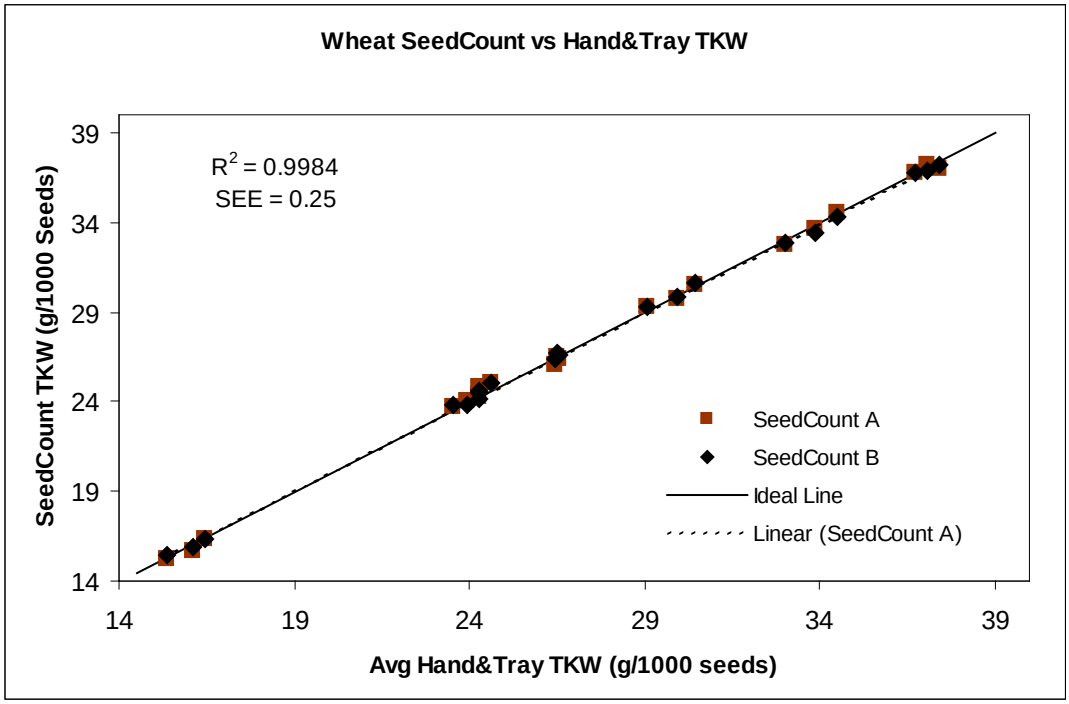

Figure 5.11 SeedCount Wheat TKW

Comparison of SeedCount Ver 1 Replicate TKW values versus the Average Hand-counting using Seed Tray values.

SeedCount barley TKWs (Figure 5.12) confirm that the counting algorithm also works well with barley, as shown by the excellent correlations (R^2 = 0.998) and low Standard errors (SEE = 0.39).

The malted barley TKWs, made with version 1, again show high correlations with the baseline data (Figure 5:13, R^2 = 0.996 and SEE = 0.25).

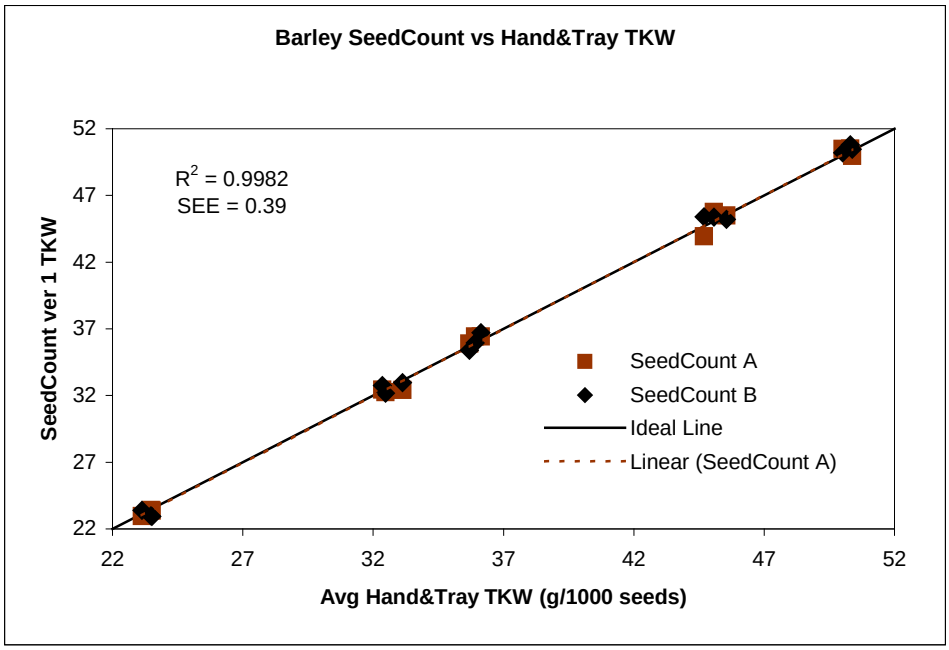

Figure 5.12 SeedCount Barley TKW

Comparison of SeedCount Ver 1 Replicate TKW values versus the Average Hand-counting using Seed Tray values.

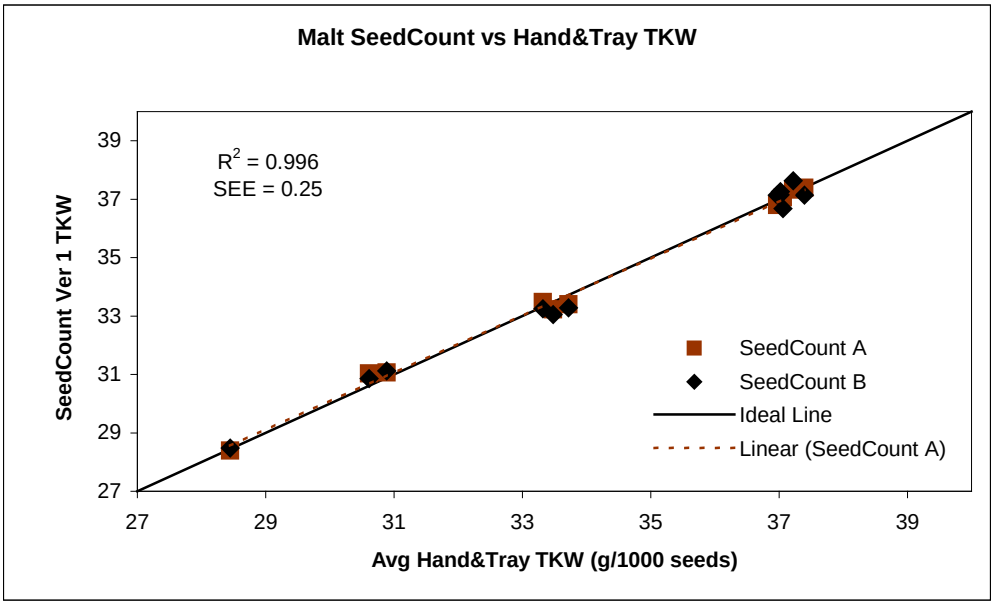

Figure 5.13 SeedCount Malted Barley TKW

Comparison of SeedCount Ver 1 Replicate TKW values versus the Average Hand-counting using Seed Tray values.

These trials have demonstrated that version 1 of SeedCount is able to accurately count wheat samples ranging from 40 to 1700 seeds. Malted barley and barley samples, due to the larger kernels, are restricted to approximately 1100 kernels. The average sample sizes in the above examples were 1250 wheat, 908 barley and 955 malted barley kernels.

5.3.5.3 Indented Tray Results

The indented tray has the benefit of separating the kernels, so there are only a few multiple seed clusters in an image. However, from a KW perspective, the indented tray has the disadvantage of reducing the number of kernels that can be included in each image. Version 2, using the indented wheat tray, can count samples from a few seeds up to 1052 wheat seeds. For barley, version 2 is restricted by the indented barley tray to a maximum of 658 kernels.

Wheat SeedCount indented tray correlations are based on 12 cultivars run in duplicate, with an average whole seed count of 703 kernels (Figure 5.14). Despite the average count reduction from version 1, the correlation (now 0.999) and SEE both improved (0.19).

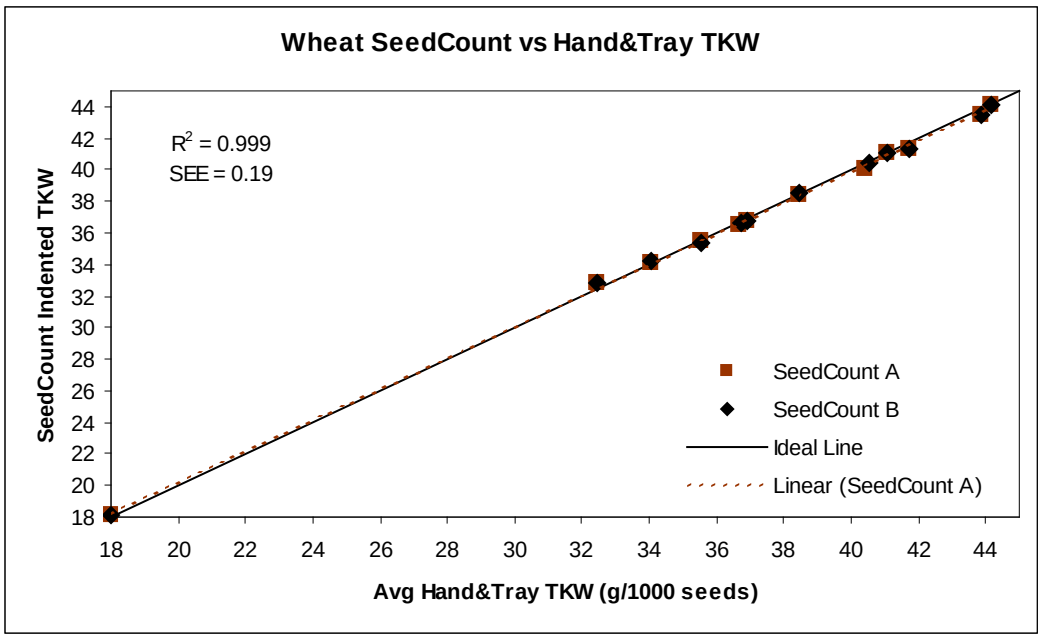

Figure 5.14 SeedCount Ver 2 Comparison with Average Hand-Tray TKW

The barley TKW SeedCount data was generated using Version 2 of SeedCount, using the patented indented trays and 300 dpi colour scans as discussed in the Methods and DIA Development chapters. Once again the average number of kernels in each sample dropped (this time to 492). SeedCount retained, and even slightly improved, its high correlation with the Baseline data (Figure 5.15, $R^2 = 0.999$ and SEE = 0.32) compared to Version 1.

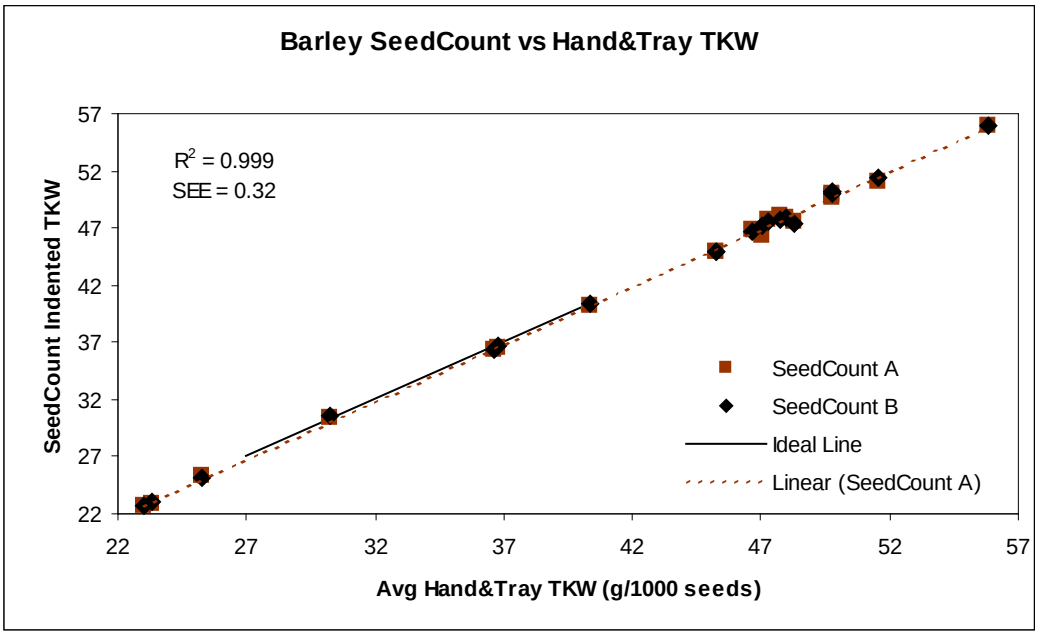

Figure 5.15 SeedCount Ver 2 Barley TKWs

Compared with Average Hand-counting values using Seed Trays.

The Version 2 malt results were similar to the barley results, but were based on a small data set (3 cultivars by 2 replicates). The data has not been shown.

The SeedCount graphs illustrate its ability to produce highly accurate TKWs for wheat, barley and malted barley using both Versions 1 and 2 of SeedCount with both flat and indented trays. The success of the flat tray method confirms the validity of the kernel counting algorithm used for touching seeds.

The use of the new indented trays in Version 2 of the SeedCount system improves on the TKW values obtained with Version 1 because there is a much higher percentage of single seeds, although the underlying counting method is unchanged. The accuracy was also increased by the introduction of methods to identify and isolate various "defects" in the sample. Principal among these are:

5.3.5.4 Beard Removal

Detecting kernels with long beards (awns), including kernels connected by the awns, required the development of a complex algorithm. It operates by finding “long” kernels and searching for the narrow beard section of the kernel(s). The beard section is isolated and removed from the kernel(s). The remaining portion of the kernel is retained for counting and further analysis. When the beard overlaps other seeds, the various seeds are separated, counted and retained. The attached beards are obviously more of an issue with barley due to its attached husks.

5.3.5.5 Debris

As with Version 1, large pieces of debris are manually removed by the operator. The initial and post-manual cleaning masses are entered into the system to allow for this material to be added to the dockage calculations. Broken kernels, loose awns and other small debris are automatically detected, quantified and then removed from the analysis. The calculated mass of this debris is also removed from the total mass. This process ensures that the debris does not affect the average kernel weight. The debris is added to the dockage calculations.

5.3.5.6 Foreign Seeds

Weed seeds with a size, color or shape significantly different from the cereal sample can be detected, recorded and separated from the kernel weight calculations.

5.3.5.7 Multiple Fragments

The algorithm for detecting blobs containing multiple touching fragments such as two broken fragments or a whole seed and broken seed was expanded to improve its versatility and accuracy, using algorithms similar to those used to detect double kernels.

5.3.6 Summary of the Various TKW Methods

The relative accuracy of the different TKW counting methods is compared to the 'gold standard' method of hand-counting assisted with an indented tray in Table 5.2. The results include wheat, barley and malted barley TKWs. The methods are listed by descending accuracy. The hand plus tray series A are correlated against the hand plus tray series B. The other methods are correlated against the average of the two hand plus tray series.

Table 5.2 Comparison of Various Counting Method Accuracies

Method	Correlation	Std Error
Hand plus Tray	1.0000	0.065
Hand Only	0.9995	0.299
SeedCount (DIA)	***0.9994***	***0.289***
Kirby (EM)	0.9987	0.459
Numigral (EM)	0.9900	1.186
Scion Image (DIA)	0.9058	2.495

Hand-counting assisted with indented trays is the most accurate counting method for performing TKWs tested (SEE = 0.065). However, it is probable that in a lab where many samples need hand-counting for TKWs the count accuracy would quickly deteriorate. Indeed, the ASBC standard method uses smaller samples, with lower accuracy, simply to reduce the tedium and operator time involved in these tests (ASBC, 1992). Hand-counting without using the indented trays resulted in a drop in accuracy, but still provided excellent accuracy (r = 0.9995, SEE = 0.299). The Kirby and Numigral

electromechanical counters had usable accuracy (r = 0.9987 and 0.990, SEE = 0.459 and 1.186 respectively).

The Scion Image erosion TKW method had inadequate accuracy (r = 0.906, SEE = 2.5), eliminating it as a useful TKW method. The SeedCount DIA system was able to match the excellent accuracy of the hand-counting only method (r = 0.9994, SEE = 0.289). The comparison confirms that SeedCount has a robust counting method that can be used to replace hand-counting or electro-mechanical counters for determining TKWs.

5.3.7 Grain Type and Cultivar Discrimination

The possibility of using TKW for distinguishing grain type and/or cultivar was also explored briefly. Table 5.3 shows the TKWs for six barley cultivars grown in three locations in Victoria in 2000.

Table 5.3 Effect of Growing Location on Barley TKW

Balliang		Leigh Creek		Yatchaw	
Variety Code	TKW (As-Is)	Variety Code	TKW (As-Is)	Variety Code	TKW (As-Is)
FN	35.23	FN	25.56	FN	27.23
14	39.12	PN	31.32	PN	30.07
PN	39.77	104	32.41	104	32.61
104	42.59	14	32.79	14	32.73
N81	45.77	N81	34.94	N81	33.63
GR	50.09	GR	37.38	GR	36.03
Average	**42.09**	**Average**	**32.40**	**Average**	**32.05**

For each location, the cultivars are listed in order of ascending TKW. Table 5.3 highlights the influence of growing conditions on the final kernel weight. Though Leigh Creek and Yatchaw produced kernels of approximately the same weight for each variety, at Balliang the kernels were all much heavier. This suggests that merely having a kernel weight is of little use for cultivar identification. However, in each location the relative

ranking of each cultivar by TKW is similar, with FN (Franklin) having the lowest kernel weight and GR (Gairdner) the highest. The rankings for some of the more average cultivars lose order for Balliang, suggesting that this ranking system is of limited value.

The wheat TKW rankings for 2000 are given in Table 5.4. This time 11 cultivars are included in the data set, but are again ranked by TKW by location. Wallenbeen is in New South Wales, while the other two locations are in Victoria. This sample set reveals light, medium and heavy TKW locations, again confirming that simple TKW values are of little use for cultivar identification. The value of the ranking method is also thrown in doubt. It can be seen that Brennan (BN) is the heaviest cultivar in the two Victorian locations, but only second heaviest in the third. Similarly, Declic (DC) was the lightest and second lightest at the Victorian locations, but 5th lightest at Wallenbeen. The ranking of the other cultivars is usually even more chaotic. The results show that using TKW ranking for cultivar ID would not be a reliable method.

Table 5.4 Effect of Growing Location on Wheat TKW

Byaduk		Leigh Creek		Wallenbeen	
Variety Code	**TKW (As-Is)**	**Variety Code**	**TKW (As-Is)**	**Variety Code**	**TKW (As-Is)**
DC	21.8	C6	23.4	GN	28.3
C6	22.0	DC	27.2	P7	31.8
GN	24.9	GN	27.2	C6	31.9
N0	24.9	N0	29.1	N9	31.9
N9	26.3	9A	31.6	DC	32.5
N7	28.2	P7	32.5	N0	32.9
N1	28.2	N9	32.8	C7	34.7
9A	30.0	N1	34.0	N1	35.2
P7	31.1	C7	34.9	N7	36.2
C7	31.5	N7	36.0	BN	39.4
BN	33.3	BN	37.9	9A	41.1
Average	**27.5**	**Average**	**31.5**	**Average**	**34.2**

Tables 5.3 and 5.4 also reveal the range of TKWs for barley was 25.6 to 50.1, while wheat ranged from 21.8 to 41.1. Though barley tends to have heavier kernels, there is

obviously a very large overlap in these two ranges, indicating that TKWs would not be a reliable method to discriminate between barley and wheat.

5.3.8 Yield Predictions

Barley: Hot water extract (HWE) predictions based on KW for the Joe White Maltings sample set would not be useful as they only had a very weak negative correlation ($r = -0.15$). KW had moderate positive correlations with percent mealy ($r = 0.43$, $p<0.01$) and homogeneity ($r = 0.46$, $p<0.02$) and a strong correlation with screening gradings >2.8 mm ($r = 0.93$, $p<0.001$, data not shown). It is possible that KW could be used with other factors in predicting these properties.

Wheat: KW was moderately correlated with clean flour yield for the Allied Mills sample set ($r = 0.42$, $p<0.01$). KW could be used with other factors to predict flour yield, as its SEE (6.4) when used alone is too imprecise (data not shown).

5.3.9 Effect of Kernel Presentation on DIA Counting

The accuracy of the SeedCount counting method depends on having a high percentage of single seeds and small clusters. As the seeds tend to form large clusters when poured into the trays, a substantial amount of work was put into testing methods that might increase the percentage of single seeds.

The SeedCount results shown above were achieved using either the clear, flat-bottomed tray (for SeedCount version 1) or the indented blue tray used in SeedCount version 2. As discussed in section 4.4, three presentation methods were used in Version 1 of SeedCount:

1. **Direct:** The seeds were placed directly on the scanner glass (also used for the Scion Image scans).
2. **Combed:** Seeds were poured into a clear, flat-bottomed tray and shaken and combed to separate the seeds (most of the version 1 results presented above used this method). The tray was placed on the scanner.

3. **Screened:** As in 2, but the seeds were brushed over a wire grid in the tray that was removed before scanning.

Table 5.5 summarises the effect that the various presentation methods had on SeedCount accuracy. Data for wheat, barley and malted barley were combined to produce this table.

Table 5.5 Effect of Kernel Presentation on SeedCount's TKW Accuracy

Presentation	Scanner Position	% Single Seeds	Correlation	Standard Error
Direct	Beneath Seeds	27	0.9994	0.324
Combed Tray	Beneath Seeds	32	0.9993	0.306
Screened Tray	Beneath Seeds	36	0.9994	0.320
Indented Tray	Above Seeds	98	0.9995	0.285

SeedCount KW is correlated with the Hand plus Tray "gold standard" KW. Table 5.5 indicates that the method of distributing and imaging the samples had only a small effect on SeedCount's accuracy. Even the massive increase in the percentage of single kernels resulting from use of the indented tray only made a modest improvement in SeedCount's TKW accuracy. Three points should be noted here:

In version 2 the sample sizes are smaller due to the number of indents available (eg barley samples are typically about 23 grams rather than the 40 grams used in version 1). Smaller samples typically result in poorer correlations. That this has not happened suggests that the version 2 presentation method is superior.

In Version 2 of SeedCount only whole seeds are counted, whereas version 1 counted all objects. As the IoB method stipulates that only whole seeds should be counted, it is likely that this has also contributed to the improvement in the version 2 results.

The indented tray presentation method would have had a beneficial effect on the Scion Image TKW as it would not have been necessary to erode the images to separate the kernels. However, it would still have been very slow and would not have been able to

count any remaining multiples correctly. A Scion Image process similar to this was trialled for rice (van Dalen, 2004).

5.3.10 Scanner Comparisons

As the initial concept was to develop a low-cost DIA system that could use standard scanners, several brands of scanners were tested to see if the SeedCount software could be coupled with virtually any scanner. As most scanner manufacturers replace their models every 9 to 12 months, some flexibility in the scanner used would also be desirable.

Table 5.6 summarises the evaluation of the various scanners. The scanners are listed from oldest to newest. The correlations and Standard Errors compare the listed scanner's SeedCount TKWs with the Hand-Tray baseline values for the same wheat samples.

Table 5.6 Scanner Comparisons

Scanner	Speed (Sec)	Tray Type	Dpi	Correlation	SEE
Mustek 600 EP	65	Flat	100	0.9996	0.262
Canon FB 310	53	Flat	100	0.9993	0.347
HP Scanjet 5300C	36	Flat	100	0.9993	0.306
Plustek UT-12	27	Flat	100	0.9993	0.306
HP Scanjet 5400C	38	Indented	300	0.9995	0.288
Epson Perfection 1660	16	Indented	300	0.9995	0.285

The scanners used with the flat trays under SeedCount Version 1 had a larger influence on the results than those used with version 2 as can be seen in the variations in the Standard Errors in Table 5.6. This is almost certainly due to the superior software method of isolating the kernels used in the second version. In version 1 the seed separation was influenced by the image brightness produced by the various scanners. Version 2 does not use this brightness threshold and thus does not have this problem. It uses a colour algorithm to distinguish the seeds from the tray.

Some improvement would also be due to the higher percentage of separate seeds in the indented trays. Version 1 of the software was tuned with the Mustek scanner, which explains why it produced the best results with that scanner. It is probable that if Version 1 had been retuned specifically for each scanner, they would all have produced almost identical count results. None the less, the differences in SEE are small compared to other methods such as the electromechanical counters.

These results suggest that both versions of SeedCount are reasonably robust and scanner-independent, at least for counting and TKW purposes. Though the replacement of the scanners is awkward as some recalibration work is required for optimal performance, the newer models are usually faster and more reliable than the earlier scanners. Changing scanners is more complex for Version 2 as modifications must be made to the scanners to enable them to work correctly upside down.

The scanner speeds and analysis times will be discussed in the following section.

5.3.11 Time Considerations

As with all analytical methods, the time that is taken to obtain a result is critical. This is especially the case at a grains receivals depot during harvest time. During harvest all of the farmers need to harvest their grain as rapidly as possible to minimize loss due to seed drop (grain falling from the heads onto the ground) or wet weather (which can cause pre-harvest sprouting damage that lowers the value of the crop). These factors result in a long line of loaded grain trucks at the receival points. Each driver is under pressure to get back to the paddock to take another load and thus allow the harvesters to continue their work. This pressure is often transferred to the operator at the receivals. Most depots attempt to sample, test and grade each truckload in three to five minutes (Orman, Lees and Hare, 2000). The time allowed for all the actual testing is often only about two minutes. The time the operator can devote to the TKW determinations can only be a small portion of that time.

A comparison of the speed of the various counting methods was undertaken (Table 5.7). The parameters summarised in Table 5.7 are combined results for wheat, barley and malt analysis for all methods except SeedCount, which has different tray filling times for wheat and barley. The run cycle times are how long it takes to completely process each sample when a series of samples are being run. The run cycle allows some things to happen simultaneously when possible. For example, when using SeedCount, the operator can be sub-sampling while the previous sample is being scanned and can then empty the tray and refill it with the new sub-sample while the previous one is being analysed. This also applies to Scion Image and the Numigral. The other systems are completely manual and no simultaneous operations can occur. The operator time is the measure of how much input the operator needs to make for each sample. The methods are listed in order of their accuracy (Table 5.2).

Table 5.7 Speed Comparison of Counting Methods

Method	Sampling (min)	Tray Filling (min)	Scanning, Data Entry	Analysis (min)	Run Cycle (min)	Operator Input (min)
Hand plus Tray	0.7	2.3	0.1	10.2	13.3	13.3
Hand Only	0.7	0	0.1	18	18.8	18.8
SeedCount Wheat	***0.7***	***0.67***	***0.4***	***0.75***	***1.6***	***1.6***
SeedCount Barley	0.7	1.67	0.4	0.7	2.6	2.6
Kirby (EM)	0.7	0	0.1	6.6	7.4	7.4
Numigral (EM)	0.7	0	0.1	8.6	8.7	0.8
Scion Image (DIA)	0.7	0.67	0.4	6.4	6.5	1.8

SeedCount wheat trays can be filled in approximately 40 seconds. As this is roughly the same time that it takes the program to analyse the previous image, there is a rapid rollover from one sample to the next. The barley tray is more difficult to fill, mainly due to a tendency for some kernels to stand on their ends in the narrow section of the tray. Most of these kernels need to be levelled by hand. The barley tray can take up to 100 seconds to fill, making it the rate-limiting step in barley analysis and increasing the total

run cycle time to over two minutes. It is expected that better tray design will largely eliminate this problem and reduce the barley tray filling time to nearly that of the wheat trays. Tray filling for the hand-counting method consists of eight to ten fills per sample as the trays only hold 100 seeds each time. The Scion image system uses the simple clear-bottomed tray, which takes about as long to prepare as the indented DIA wheat tray.

Run cycle times show that the SeedCount wheat and barley are easily the fastest methods (1.6 and 2.6 min respectively) and can fit within the required receival stand times. The Operator input times tell a somewhat different story: The Numigral counter has a smaller operator input per analysis (0.8 min) provided the sample does not need hand cleaning and the operator has other work they can do while waiting for the counter to finish. This situation would make the Numigral acceptable under laboratory conditions, but totally unsuitable for receivals use. Though the Scion Image system looks like it could have similar usage as the Numigral, Table 5.2 indicates that it is too inaccurate for lab or receivals use.

Despite our rejection of Scion Image, van Dalen (2004) used it with images of rice kernels that were individually hand separated and obtained reasonable measurements with it. It is unlikely that such an approach will be much, if any, quicker than hand-counting the samples using an indented tray.

Table 5.7 does not show that SeedCount also produces a series of other assessments and saves the data in a spreadsheet compatible format during the same run cycle period. Some of the other assessments are kernel length, width, thickness, area, aspect ratio, roundness, hectolitre weight and screening assortment. None of the other TKW methods can do that.

5.4 Conclusions

Digital Image Analysis, using the SeedCount software, is capable of rapidly determining precise TKWs.

As it can take about 13 minutes to hand-count a 40 gram sample of wheat for a TKW determination, even when using an indented tray, TKWs have not been included in the tests performed at wheat and barley receivals stands. Even EM counters are too slow for this environment at 8.7 (Numigral) and 6.7 (Kirby) minutes. Though the Numigral could be run faster and the sub-sample size reduced to make the count quicker, this would reduce the accuracy of the TKW. However, it would still be too slow. The SeedCount DIA system has broken the three-minute "time barrier", making TKWs a useable test for grain receivals.

As time is critical, faster imaging methods are essential for DIA to succeed. As pointed out in section 4.5.3, scanners are rapidly becoming faster. This process is illustrated in the Speed column of Table 5.6 where as one moves down the column the scanners become newer. However, there are more improvements here than first meets the eye. When changing from the flat to indented trays, there is also a change from 100 dpi greyscale to 300 dpi colour images. The information in each pixel is tripled to provide the colour (there are three channels -red, green and blue- and each channel has 256 levels, the same as the greyscale channel). There is also a nine-fold increase in the number of pixels in each image as both the number of pixel rows and columns in an image of the same area are tripled. So overall there is 27 times as much data to be acquired and processed in the new images used in the Version 2 images when compared to the Version 1 images.

Thus it can be seen that the Epson 1660, processing a high-resolution colour image in 16 seconds, is actually 110 times faster than the Mustek processing a low-resolution greyscale image in 65 seconds. Processing the data from the respective images carries the same handicap of a vastly increased workload.

Fortunately the processing speed of desktop computers has also grown dramatically in recent years, allowing the time required to analyse these images to remain manageable. The SeedCount image analysis times in Table 5.4 are for a Version 2 system with an Epson Scanner and include the time required to fill the tray, scan and analyse it. It is the average time taken for a sequence of 26 samples. The average mass for these samples

was just over 22 grams. The results for the other methods are for the earlier sample sets that had samples with an average mass of 36 grams. On this basis it would not be unreasonable to insist that the SeedCount times should be doubled. Even if this is done it is still obvious that the SeedCount system, with a twin analysis time of 3.2 minutes is clearly faster than any of the other methods.

The average cycle time, using the other methods, ranged from 18 minutes for hand-counting to 6.4 minutes for Scion Image. Though the images were produced with the slow Mustek scanner, most of its analysis time was taken up in processing the image. The Scion Image software was very slow in comparison with SeedCount, which performs its image analysis in less than 40 seconds. Though part of this time difference was due to SeedCount 2 running on a faster computer, the difference in computing power would have been more than offset by the extra information in the Version 2 images. The long Scion Image processing times are almost certainly due to the use of an inefficient programming language.

Kernel Weights cannot be used to reliably rank cultivars or to separate wheat from barley. Heavier kernels tend to yield more flour, but TKWs have no link to HWE.

DIA can be used for much more than simple TKWs. Many aspects of kernel morphology and cultivar identification have been studied using DIA (Gebhardt, Rasmusson and Fulcher, 1993; Symons and Fulcher, 1988). Initial results of the author and work by others suggest that DIA has the potential to generate accurate screenings equivalents (Kuhbauch and Bestajovsky, 1989), identify kernel staining and color (Luo, Jayas and Symons, 1999). Some of these aspects of DIA will be investigated in the following chapters.

6 Using Digital Image Analysis Systems for Estimating Kernel Length, Width, Aspect Ratio, Area, Ovality, Thickness and Roundness

6.1 Introduction

Maltsters and millers prefer large, plump grains. As will be shown below, these plump grains should contain a higher percentage of endosperm, which usually means a higher yield of white flour or soluble extract from the grain (Burger and LaBerge, 1985). The traditional method of screening the grain is to use an array of sieves of diminishing slot widths. The thickness is the smallest of the grain's linear dimensions, so screening is achieved by shaking the grain through the slots based entirely on its thickness. These multi-tray screening assortments provide useful information about the sample's size distribution, which is linked to the potential yield and is therefore of commercial significance.

Millers prefer grain with consistent thickness so they can optimise their roller gaps while milling (Posner and Hibbs, 1997). Similarly maltsters desire uniformly-sized grain as it produces homogenous malt (Edney, 1996). Barley containing diverse-sized grain results in over-conversion of the smaller grain and under-conversion of the larger grain during malting. The multi-screen assortment data is useful for predicting a grain batch's homogeneity as well as the percentage of plump grain. However, making these multi-screen assortments is time-consuming and they are usually limited to sorting the grain (by thickness only) into at most 4 or 5 groups. Rapid Digital Image Analysis (DIA) methods capable of providing more detailed data using a variety of kernel parameters may yield additional helpful information when assessing grain quality.

At this point it will be useful to define the measurement nomenclature used. The three orthogonal grain dimensions are regarded as being:
Length: The longest (primary) dimension of the seed, almost always running from the embryo to the distal end.

Width: The maximum distance across the seed that is perpendicular to the length. It is usually perpendicular to the plane from the seed's primary axis to the seed crease.
Thickness: This is the distance across the seed that is perpendicular to both the length and width. Throughout this thesis, the kernel thickness is defined as the smallest of the kernel's three cardinal dimensions, in accordance with standard industry nomenclature. For most cultivars thickness is the dimension passing from the dorsal to ventral surfaces of the seed, as can be seen in Figure 6.1. Discussion of this aspect of the research assumes that the kernels have this standard shape as opposed to the "over-square" varieties which are higher than they are "wide" when resting ventral side down (Ferns et al., 1975).

It is difficult to estimate the kernel thickness and screen gradings accurately with standard DIA systems (Gebhardt, Rasmusson and Fulcher, 1993). This is because these systems cannot 'see' the kernel thickness. On a flat surface the seeds are the most stable laying with their width and length horizontal to the surface and their thickness vertical to the surface (as shown by the left seed in Figure 6:1, viewed from above). This orientation gives the seed its lowest possible potential energy. This orientation is referred to as "wide-on", "flat", or simply "wide" hereafter. For most cereal varieties and cultivars this means the seed lays with either its ventral or dorsal surface down and the outline of its frontal plane can be seen. On a flat tray it is unusual for a seed to lie on its side with the seed width vertical to the surface (this orientation is referred to as "narrow" or "edge-on" hereafter and is illustrated by the right seed in Figure 6:1). Generally the seed crease is facing left or right for an edge-on seed, revealing the outline of its sagittal plane. Therefore in the normal laying position the kernels display their length and width rather than their thickness when viewed from above (as shown by the left seed in Figure 6:1).

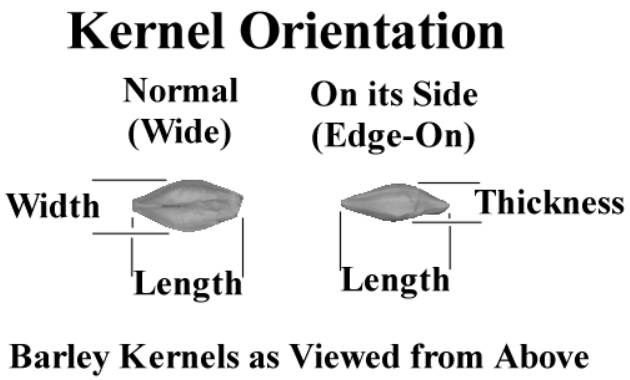

Figure 6.1 Kernel Orientation and Definitions

Developing an indented tray to hold some kernels in an "edge-on" position would approximate the orientation in which they slide through the screens. This would enable direct DIA thickness measurements that could be used to assign each seed to its appropriate screening fraction. This chapter presents one attempt to develop such a tray and assesses its usefulness in estimating the thickness of wheat and barley.

While assessing this data, five different kernel parameters were examined to see if any, or all, of them provided data that could be used to distinguish grain cultivars or predict flour or soluble extract yield. These parameters were:

6.1.1 Aspect Ratio

Aspect ratio is the kernel's width to length ratio as measured by DIA or with callipers, and will be used in this manner throughout this thesis. Some researchers refer to this as F-shape (Edney, Bassily and Symons, 1998). Occasionally aspect ratio is determined by comparing the kernel's length to width ratio.

6.1.2 Kernel Area

Kernel area, effectively its frontal or sagittal section, can be determined by looking at the kernel's image. It is difficult to measure the seed area aside from DIA techniques due to the complex shape of the kernels.

6.1.3 Kernel Ovality

Kernel ovality is a novel parameter that assesses how closely the area of the frontal section of the seed, as displayed in the wide section of the tray, approaches the area of a perfect oval with the same length and width as the kernel. This parameter is only measured on seeds laying in a crease up or down position.

None of the approaches suggested so far provide a comprehensive indication of the two-dimensional, let alone the three-dimensional shape of the kernel, but may still be useful parameters.

6.1.4 Thickness

Kernel thickness is often indirectly referred to as plumpness. It is typically determined by screening (eg plump barley consists of all kernels thicker than 2.5 mm). Although plumpness is reported as the percentage by mass of kernels with this property, the kernel's inclusion in this group is determined by its thickness rather than its width, length or mass.

However, thickness measurements are only one aspect of the kernel's properties. This chapter will, after exploring each property individually, combine the various properties to provide a broader, and hopefully more robust, model of the actual kernels being studied.

6.1.5 Roundness

Kernel roundness is another novel parameter proposed in this thesis. As there does not appear to be a usable definition of kernel roundness in the literature, it was necessary to

devise a definition. The following novel definition of kernel roundness is proposed and has been used throughout this thesis:

$$\text{Roundness} = \frac{(\text{Width/Length} + \text{Thickness/Length} + \text{Thickness/Width})}{3}$$

The formula summarises more detailed kernel information than all of the above approaches.

Measuring individual kernels with callipers is slow and tedious. DIA, coupled with a bi-modal indented seed tray, has the potential to rapidly measure hundreds of kernels in a few seconds. If DIA systems can accurately measure kernel thickness, it would make roundness measurements a readily available kernel quality parameter. Sections of this chapter investigate whether the SeedCount DIA system can accurately estimate roundness, and whether roundness, as a measure of kernel shape, also has an effect on grain performance when malting or milling.

6.1.6 Yield Estimates

Yield has different meanings for maltsters and millers. Maltsters define their yield as the soluble extract. This is the percentage of the malt, by mass, which is converted into water-soluble material by the malting and mashing processes. Millers define their yield as the milling extraction rate. It is the percentage of their cereal, generally wheat, which can be converted into clean white flour. In both cases their yield is derived primarily from the starchy endosperm of the cereal.

6.1.6.1 Theoretical Yields

Cereals are essentially composed of an endosperm core wrapped with an aleurone, bran and husk layer. If the thickness of these layers are constant, it follows that larger and rounder seeds should have higher percentages of endosperm. Crewe and Jones (1951) demonstrated that the thickness of cereal bran was stable for a broad range of kernel

sizes. This result was applied to simplified barley shapes in Table 6.1. It is assumed that the husk and bran is uniformly 0.08 mm thick. The analysis predicted that larger and rounder kernels will contain a higher percentage of endosperm and embryo (Table 6.1) and thus proportionally more flour/extract should be available. The effect of the embryo on the yield is ignored by assuming that it will always be the same percentage of the total kernel.

Table 6.1 Theoretical Effect of Kernel Volume and Shape on Endosperm

Shape	Length	Width	Thickness	Volume	Percent Endosperm and embryo	Percent Husk and Bran	Roundness Value
Thin Barley	6.8	2.10	1.40	10.47	87.1	12.9	0.394
Average Barley	8.00	3.30	2.40	33.18	91.8	8.2	0.480
Round Barley	9.70	4.10	3.20	66.64	93.5	6.5	0.511
Sphere	4.57	4.57	4.57	49.86	93.6	6.4	1.000

It can be seen that increases in kernel volume and roundness should be linked to increases in the percentage of endosperm, including the embryo, which climbs from 87.1 to 93.5 %, a gain of 6.4%. The calculations also indicate that there is only a small gain available from making barley even rounder than our current roundest barley. Data will be presented to see if these theoretical improvements actually correlate with real yield gains.

6.2 Aims

- To develop and test a bi-modal tray that will allow direct DIA measurement of kernel thickness in the Narrow (Edge-on) section of a tray.

- To investigate kernel aspect ratio, area, ovality, thickness and roundness and see if they can be used to identify kernel cultivars.

- To test the ability of the DIA aspect ratios, area, ovality and roundness to predict flour and soluble extract yields.

6.3 Results and Discussion

6.3.1 Sample Set

As detailed in the Materials and Methods chapter, 255 barley kernels from 14 cultivars and 205 wheat seeds from 10 cultivars were individually weighed and their length, width and thickness measured with digital callipers. The kernels were then arranged in the appropriate SeedCount tray, scanned and digitally analysed. Each seed's flat and narrow (edge-on) DIA data was combined and compared to its calliper measurements. All DIA data in this chapter was taken from 300 dpi images. Microsoft Excel and SPSS were used to generate multivariate regressions that compare each kernel's thickness and roundness with the manual measurements.

Measurements of individual kernels of malted barley were not undertaken as this was deemed to be of little commercial interest. It was also thought that the result would essentially duplicate the unmalted barley results.

Large-scale aggregate values for particular cultivars (as detailed in the methods chapter) were also calculated and tested for correlations with their flour or extract yield. The aggregation method and values will be discussed in detail in the sections 6.3.3.3 and 6.3.7.5.

6.3.2 Indented Tray

Attempts to directly measure the kernel thickness using DIA led to the development of a new type of tray. The unique indented trays (patent pending US-2004-0052398-A1) were designed with two distinct sections. One section of the tray held the sample laying "flat" in wide indents with the crease facing up or down. This section of the tray resembles conventional manual seed counting trays as used in Chapter 5 for determining kernel weights. The novel patented (pending) Edge-on section holds its part of the grain sample on its edge in narrow, deep indents. The kernel was usually wedged in the indent such that the crease was facing to the side of the indent as shown in the two right columns in Figure 6.2. This tray section was intended to allow direct measurement of the kernel's

thickness. The success of this approach will be discussed in the thickness section of this chapter. The two left columns of Figure 6.2 show seeds in a typical “wide” or “flat” indent. It can be seen by comparing these two sections that when the seed is laying ventral side down, the kernel is quite wide, while when laying with the crease to the side, the kernel appears to be relatively narrow.

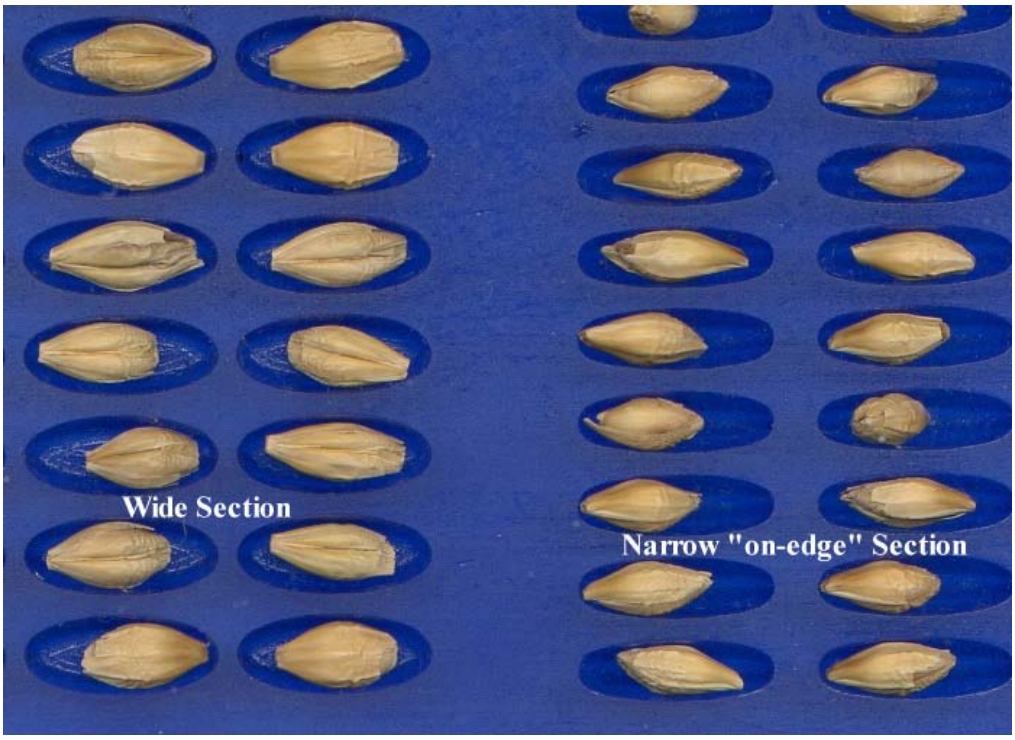

Figure 6.2 Indented Tray Detail

The bi-modal tray was not necessary for the aspect ratio, wide area and ovality work, but was essential for the generation of thickness and roundness data.

6.3.3 Length, Width and Aspect Ratios

Aspect ratio was investigated as it was thought that it may have a strong correlation with kernel size and cultivar shape and therefore may be useful for estimating extraction yields and cultivar identification. As mentioned above, aspect ratio is the kernel width divided by its length. Thus kernels with larger aspect ratios tend to be “rounder” than

"thinner" kernels with low aspect ratios. Circular kernels would have an aspect ratio of 1.0.

Kernels in the narrow section of the tray were not used to generate aspect ratios. This was due to the difficulty in getting the longitudinal axis of the seeds to lie horizontally in the deeper "narrow" indents. Seeds would often "tilt", resulting in one of their ends projecting up out of the indent at various angles (Figure 6.2). Barley, with its longer points and deeper narrow indents, was especially prone to this problem. This orientation defect had minimal impact on the kernel thickness measurements. However, it did have the effect of shortening the kernel length and distorting the aspect ratio. In contrast, the wide section of the tray had wider indents with flatter bottoms that favoured the kernels lying flat, preventing this orientation problem.

6.3.3.1 Kernel Length and Width

Before looking at the DIA aspect ratio work, it was first necessary to investigate whether the SeedCount DIA system could accurately measure the kernel length and width used to generate the kernel aspect ratios. This data is presented in Figures 6.3 and 6.4 for wheat and Figures 6.5 and 6.6 for barley. The DIA system is, with 95% confidence, capable of assessing the kernel length within about 0.27 mm and the kernel width within about 0.16 mm. These estimates are not as accurate as the digital callipers, which can assess these values within 0.02 mm. However, the DIA values are much faster and easier to attain and are accurate enough for most comparative work as the errors are only a small fraction of the range of kernel values. For example, barley lengths in this data set vary from 6.15 to 12.25 mm. The 0.27 mm error is only 4% of the total range of kernel lengths.

The higher measurement correlations for barley were not expected. It had been thought that the additional errors caused by loose or split barley husks would produce erroneous DIA measurements. Callipers easily compress these damaged husks, but the DIA system can only work with its apparent width for each kernel. One loose-husked Franklin barley kernel can be seen in Figures 6.5 and 6.6, appearing well below the general cluster of results in both cases as the husk compressed in both length and width.

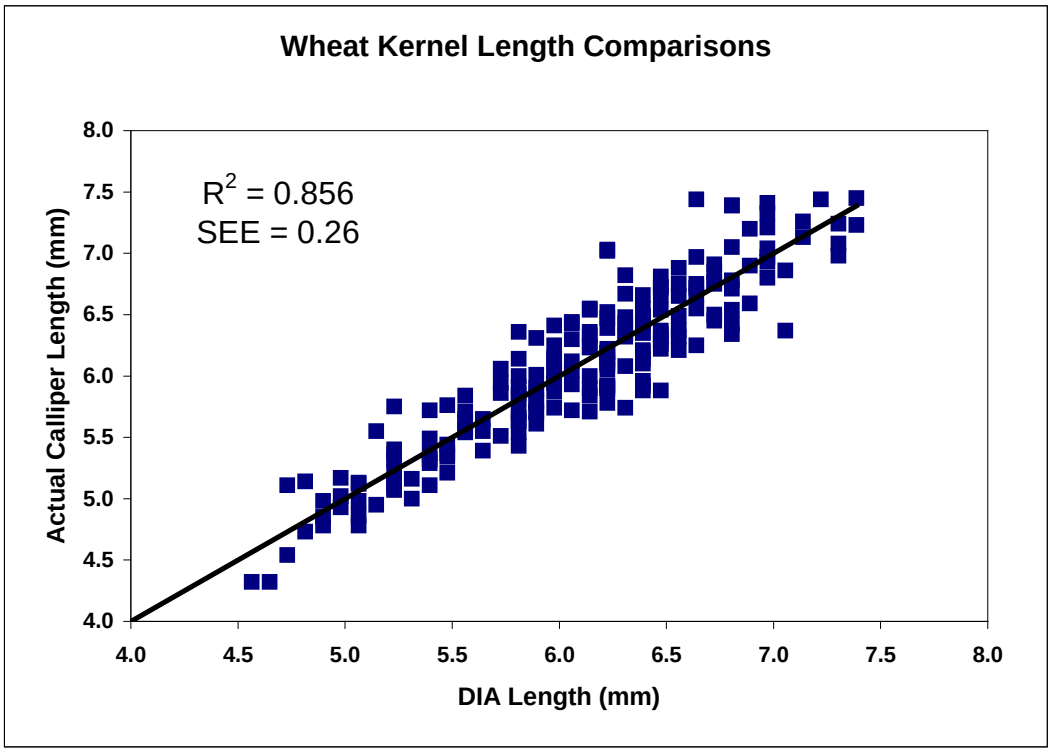

Figure 6.3 Wheat Kernel Lengths

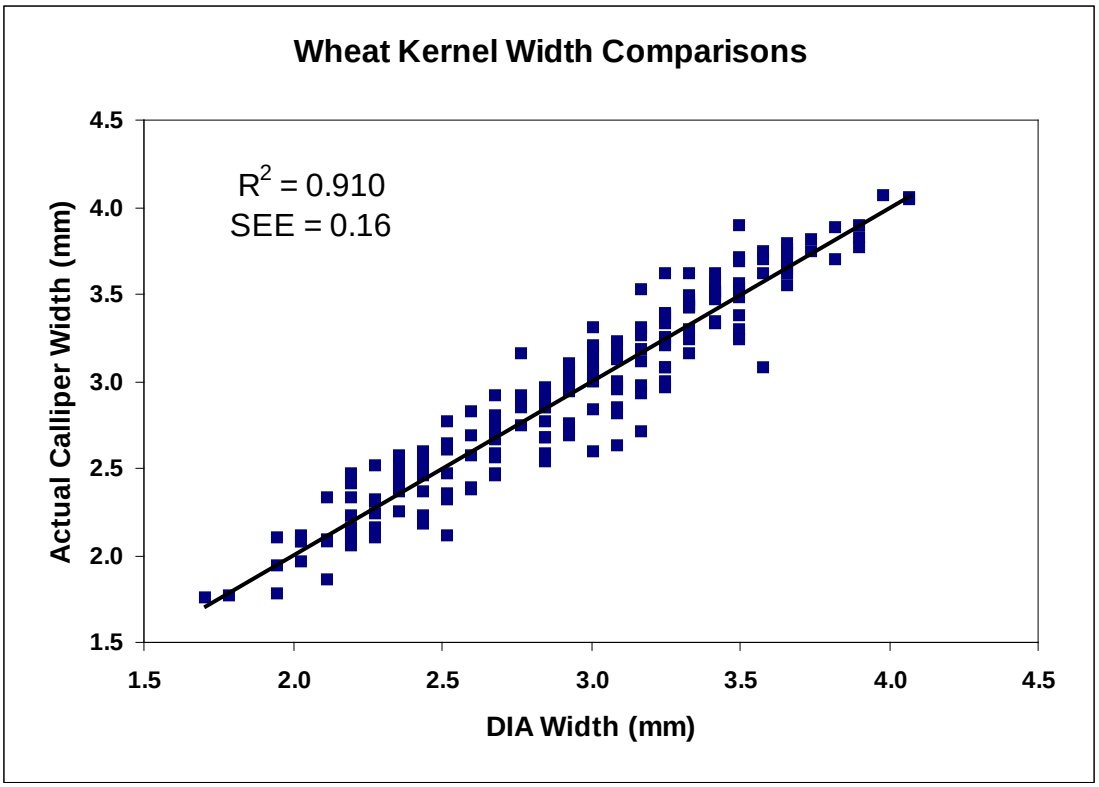

Figure 6.4 Wheat Kernel Widths

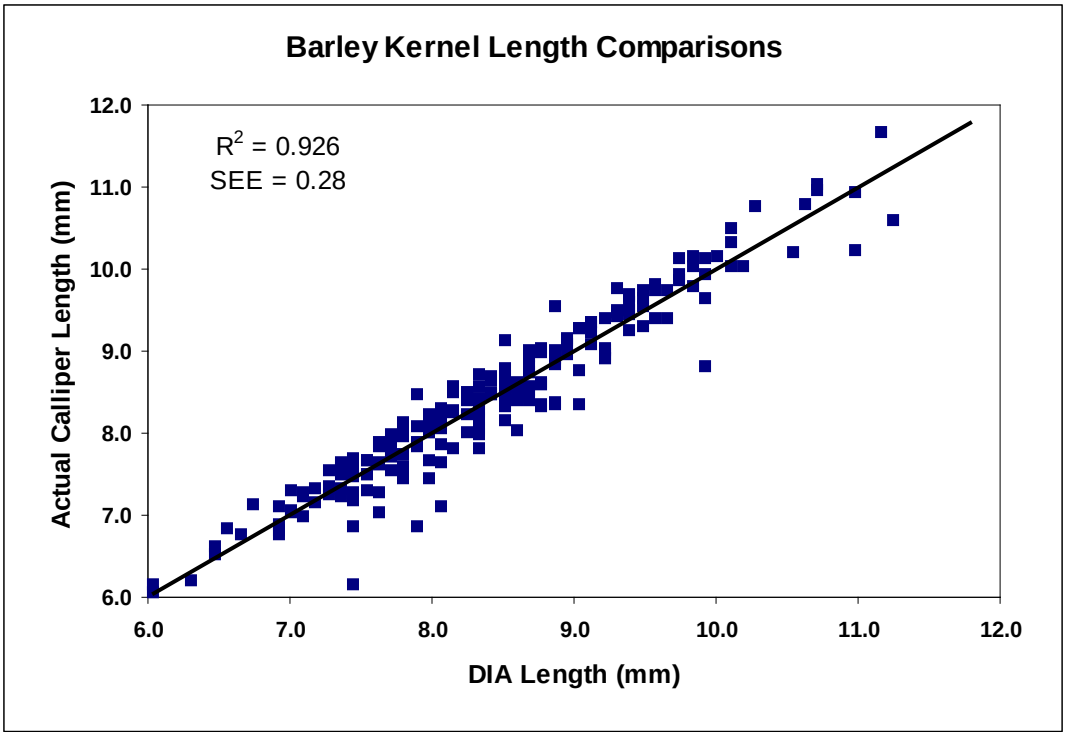

Figure 6.5 Barley Kernel Lengths

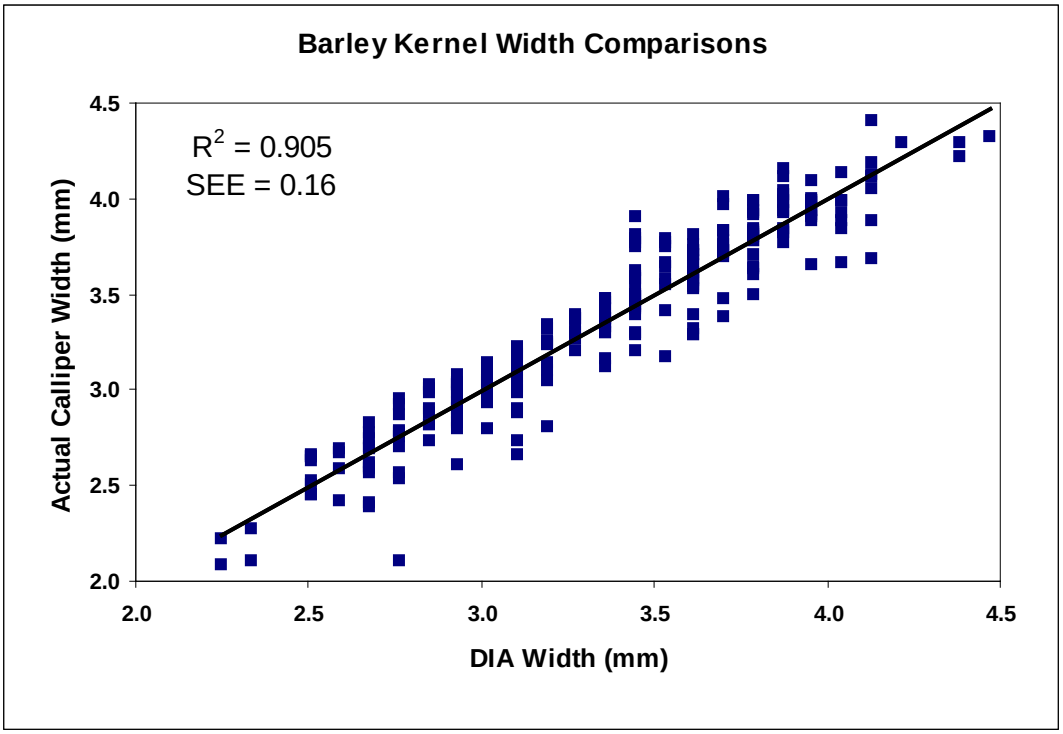

Figure 6.6 Barley Kernel Widths

The vertical "blockiness" of these graphs is due to the 300 dpi image pixel size. The image resolution is a major contributor to the DIA inaccuracies as is discussed in section 6.3.6.

6.3.3.2 Individual Aspect Ratios

The aspect ratio data was initially tested for the individually measured kernels. It was found that the calliper and digitally measured kernel aspect ratios were strongly correlated (R^2=0.805, SEE=0.03 for wheat (Figure 6.7) and R^2=0.870, SEE=0.02 for barley (Figure 6.8), $p<0.01$) across this entire data set. The result validated obtaining aspect ratios by DIA.

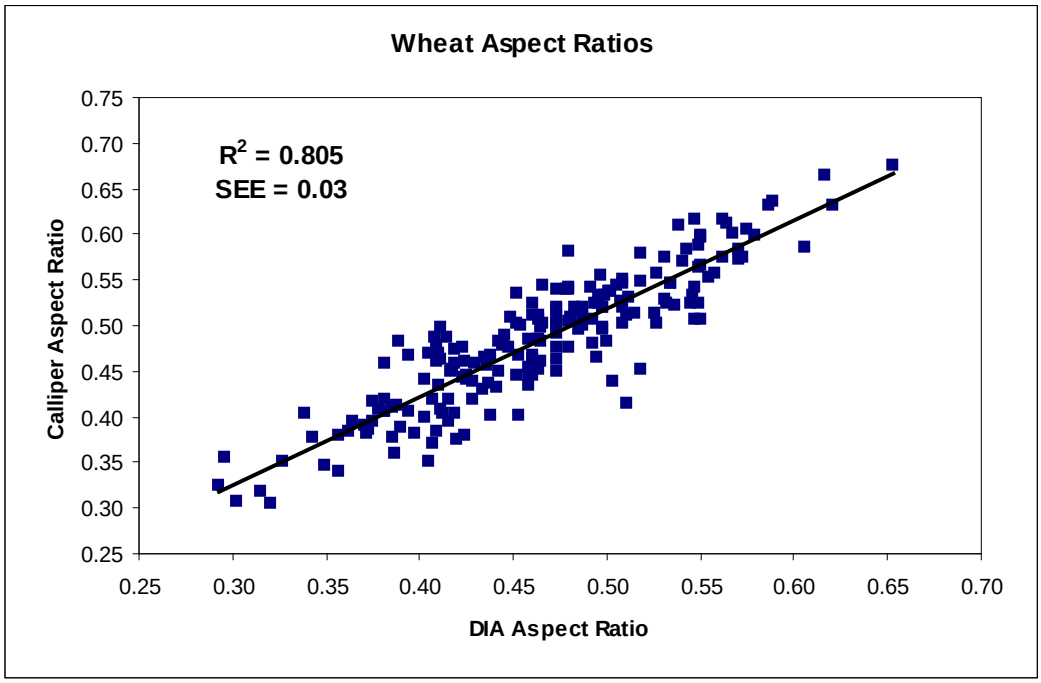

Figure 6.7 Wheat Aspect Ratios for Individually Measured Kernels

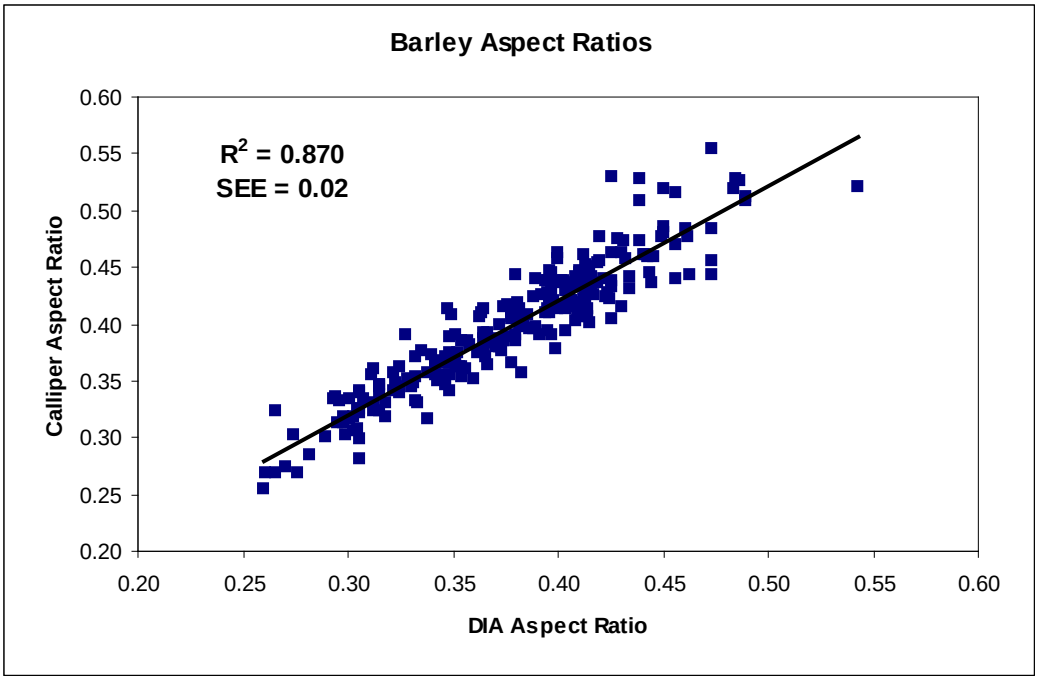

Figure 6.8 Barley Aspect Ratios for Individually Measured Kernels

There was a distinct and highly significant tendency for aspect ratios to increase as the kernels became wider ($r=0.78$, $p < 0.001$ for wheat, 0.70, $p < 0.001$ for barley) or heavier ($r=0.64$, $p < 0.01$ for wheat, but only 0.40, $p < 0.05$ for barley). These observations suggest that kernels become more circular as they get larger. This in turn suggests that the kernel lengths are less variable than the other dimensions when the kernels are filling for each variety. However, this data set is too small to confirm this theory.

6.3.3.3 Inter-cultivar Aspect Ratios

The inter-cultivar relationships were then explored. Significant differences between wheat cultivars were detected. The “roundest” cultivar was Brennan, with an average DIA aspect ratio of 0.58. The “narrowest” cultivar, with a DIA aspect ratio of 0.40 was a Declic crop grown on waterlogged land near Byaduk, Victoria. As the Declic plots grown at other locations had larger aspect ratios, this result indicates environmental effects on aspect ratios.

Genetic differences were, for some cultivars, stronger than the kernel size effects. For example, Kellelac samples which had been separated by screening (essentially kernel size segregation) into fractions less than 2.2 mm and greater than 2.8 mm thick had aspect ratios of 0.43 and 0.49 respectively. The Declic and Brennan cultivars (genetic differences) fell well outside this range.

However, when aspect ratios were determined for many common commercial wheat varieties, it became evident that most Australian commercial wheats are highly inter-related and the range of aspect ratios is usually quite small. A set of 72 wheat cultivars supplied by Allied Mills illustrates this commonality. The cultivars had an average aspect ratio of 0.52 and a standard deviation of only 0.021 across the entire sample set.

Average aspect ratios for the various cultivars in the individually measured barley dataset varied from 0.35 (B18) to 0.45 (L14). Unlike wheat, the kernel size seemed to be the principal property affecting the aspect ratio in barley. This is illustrated by a screen-segregated sample of Gairdner barley: kernels with a thickness of less than 2.2 mm had an average aspect ratio of 0.31, while those greater than 2.8 mm had an average aspect ratio of 0.42. This one cultivar thus covered nearly the entire range of aspect ratios for all barley cultivars.

Identification of barley cultivars on the basis of aspect ratios was not possible. Barley aspect ratios are strongly affected by the position at which the kernel awn has broken. Though there are possibly some cultivar-specific effects on awn breakage, such as tougher awns, the principal factor in retained awn length is almost certainly in the harvesting process, particularly the thresher settings and grain loading levels. The awn length would also be shortened by additional grain handling after harvest.

In summary, aspect ratios may be useful for segregating wheat into various major groups, but it is of little value in making cultivar-specific identifications. Aspect ratios are not able to segregate between barley cultivars at all.

6.3.3.4 Cereal Type Discrimination

Aspect ratios were also examined to see if they could distinguish between wheat and barley. The average aspect ratio data for a variety of cultivars is summarised in Table 6.2. The most significant feature of the table is that the average wheat and barley aspect ratios are separated by three standard deviations. Although wheat and barley separation is not total, the table indicates that aspect ratios should be generally useful for discriminating between wheat and barley. For this sample set, only 2 of the 29 wheat cultivars overlapped with the barley range, while 11 of the 26 barley cultivars overlapped with the wheat (data not shown). As this data set had some samples that were specifically selected for their extreme shapes, it is likely that a 'normal' data set would exhibit a more complete separation. This assumption is supported by the results of Majumdar & Jayas (2000a), who used the similar property of radius ratio (ratio of the longest to shortest arc taken from the centroid of the kernel) with considerable success.

Table 6.2 Average Aspect Ratios

	Average	Max	Min	StdDev
Barley	0.42	0.46	0.34	0.03
Wheat	0.51	0.57	0.43	0.03

6.3.3.5 Yield Estimates

The Allied Mills wheat sample set showed a significant correlation between cultivar aspect ratios and flour yield ($r = 0.56$, $p < 0.001$). However, the high standard error of the estimate (SEE = 5.9) indicated that the kernel aspect ratio alone would not be able to predict the flour yield with sufficient accuracy for commercial use.

The Joe White Maltings barley samples showed no significant correlation between the aspect ratios and soluble hot water malt extract ($r = -0.13$).

6.3.4 Kernel Area

Kernel area was investigated to see if it was correlated with kernel mass and other dimensional properties. The kernel area, width, and length were measured by DIA on the wide (frontal) kernel images derived from the "wide" section of the bi-modal indented tray as discussed in section 4.4.4. Likewise, the kernel narrow (sagittal) area and thickness were derived from kernel images in the narrow (edge-on) tray section (Table 6.3.

Table 6.3 Correlations (r) Between Kernel Wide Areas and Other Properties

	Narrow Area	**Width**	**Length**	**Thickness**	**Mass**
Barley Wide Area	**0.95**	0.87	0.84	0.80	**0.91**
Wheat Wide Area	**0.93**	**0.95**	0.85	0.85	**0.94**

Strong correlations were identified between the wide areas of both wheat and barley kernels with their narrow areas, width, length and mass (Table 6.3). This was not surprising as it is reasonable to expect seeds with a larger cross-sectional area to be larger in these other properties too.

Perhaps more surprising was that the wide areas were more highly correlated with kernel mass than with the seed's length or thickness. This indicates that the kernel wide area could be a useful predictor of kernel mass, a relationship explored in the next chapter.

Kernel Area, though easily measured with DIA systems, only yields the cross-sectional area of the kernel. It does not specify the kernel shape. For this reason it was expected to have little value in cultivar identification as kernel size varies as much with growing conditions as it does with cultivar. This was found to be the case.

Average wide kernel area showed a significant negative correlation with wheat protein by Leco ($r = -0.64$, $p < 0.01$), a result predicted by the theoretical shape (ie, larger kernels

have a higher endosperm content, causing a lower protein content as noted in Table 6.1.) Unexpectedly, this link did not lead to a significant correlation between kernel area and flour yield (r = 0.11). This result contrasted with that of Berman et al. (1996), who found a strong correlation.

Barley average area proved to have no significant correlation with either protein (r = -0.19) or malt extract (r = -0.05). The yield results will be discussed in detail in chapter 7.

6.3.5 Ovality

The ovality values were investigated to see if different cultivars had significantly different shapes and if these shapes had a correlation with the cultivar's yield. If clear differences were found, this value could be used for selection of high yielding grain lots. The data was also tested to see if ovality was linked to the kernel area, which may make ovality useful for kernel size classification.

Length, width and kernel area data was derived from the same grain samples and images mentioned in sections 6.2 and 6.3. Area data was derived by DIA only. The mathematical oval area was calculated for both the calliper and DIA axis measurements using the standard Ellipse Area Formula: $A = \pi\left(\frac{Length}{2}\right)\left(\frac{Width}{2}\right)$. Calliper and DIA oval areas were strongly correlated (eg r = 0.96 for wheat, data not shown). The Ovality values were generated by dividing the DIA kernel area by the calculated ellipse area.

Figure 6.9 illustrates the ovality concept. The outline of a typical barley kernel is overlaid on an ellipse of the same width and length as the kernel. It can be readily seen that the kernel's area will be less than the oval's area. However, it can also be seen that in the left half of the figure the area of the kernel is greater than that of ellipse. But in the right half, the kernel area is substantially less than that of the ellipse.

These non-symmetrical aspects of the kernel shape have some consequences for the ovality values. Kernels with quite different shapes can have similar ovality values. These more detailed shape issues may tend to "blur" the ovality results.

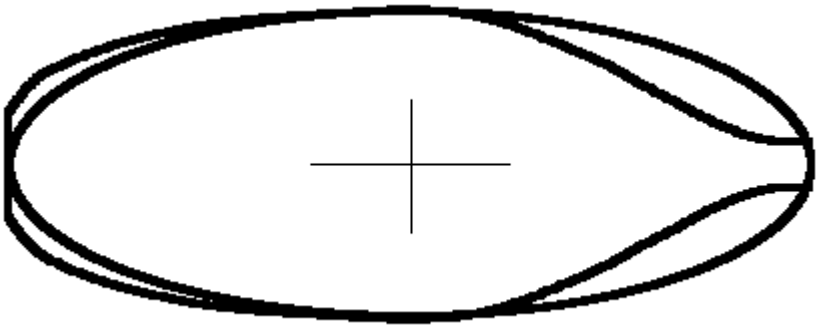

Figure 6.9 Ovality Principle: Barley Kernel Overlaid on its Ellipse

DIA and calliper derived ovality values were poorly correlated for individual kernels (Figure 6.10, $R^2 = 0.19$ for both wheat and barley). As the same area was used in both calculations, the poor correlation is due to amplification of the differences in the length and width measurements of the two methods. The results also show that some of the calliper ovality values were above 1.0 for short, squat kernels with blunt ends that were "squarer" than the ovality formula predicted.

DIA kernel ovality proved to be only loosely related to wide seed area ($r = 0.61$ for wheat and –0.50 for barley). The results indicate opposite trends for wheat and barley. Wheat tends to become more oval as the kernel area increases. As the seed area increases for barley, it tends to have a lower ovality. This appears to be linked to the presence of longer awns on many of these low ovality barley kernels. The 'Thinness Ratio' of Majumdar & Jayas (2000a) may have been more useful, but their ratio was not calculated by this version of SeedCount.

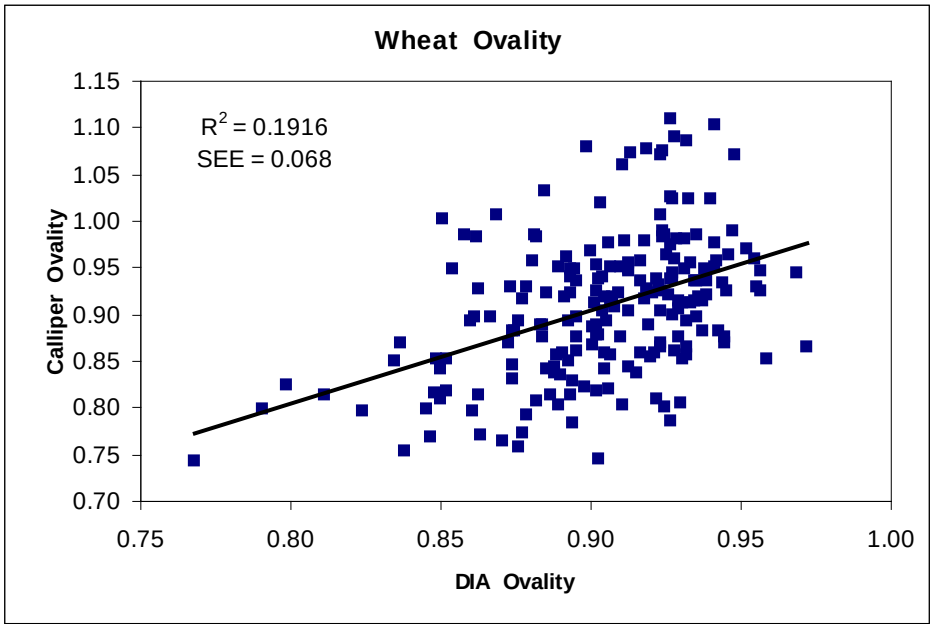

Figure 6.10 Wheat Ovality for individually Measured Kernels

Cultivar identification by DIA ovality was also disappointing. Though there was a weak correlation of ovality with cultivars, the average ovality for wheat cultivars only ranged from 0.88 to 0.93. The ovality range for barley cultivars was 0.91 to 0.99. The average standard deviation of the DIA ovality estimates was 0.04. No significantly different cultivars were found, making the ovality values essentially useless in cultivar identification for both barley and wheat. The general ovality difference between the wheat and barley ranges suggested that it may have some value in discriminating between these cereal types.

As Ovality had already failed to produce significant results at the cultivar shape level, there seemed no prospect of finding a distinctive correlation with flour or extract yield and this line of exploration was terminated.

6.3.6 Thickness

Kernel thickness has enormous importance in the grain industry due to the widespread use of slotted screens to separate the grain into discrete groups for further processing or even for rejection from processing entirely.

6.3.6.1 Thickness Estimates Using a Flat Tray

DIA estimations of kernel thickness are usually difficult to make. With a standard flat tray that presents the seeds in a wide-only orientation, the best that could be done was to calculate an estimated kernel thickness based on presumed correlations between thickness and kernel area, width and length. This approach was of limited value as the correlations between these properties were not high enough to allow accurate thickness estimates (Table 6.4).

Table 6.4 Correlations (r) Between Kernel Properties Using a Flat Tray

	Actual Thickness Barley	Actual Thickness Wheat
Actual Mass (mg)	0.94	0.92
Wide Area (mm^2)	0.77	0.87
DIA Length	0.40	0.70
DIA Width	0.92	0.85

The "Actual Thickness" measurements used to generate Table 6.4 were the calliper measurements. The relatively high correlations between kernel mass and thickness for both barley and wheat, though a very significant property of the kernels, was of little use in predicting kernel thickness in a DIA system. This was simply because normally the mass of a particular kernel is not known. However, once the kernel thickness was known, this relationship was definitely useful in predicting the kernel mass. This aspect will be explored in section 7.3.1.

There was a significant correlation between thickness and area for wheat, but it was not strong enough to permit accurate thickness estimates. The poor correlation between barley thickness and length ($r = 0.40$) was almost certainly due to length variations caused by the almost random breakage points of the awns. This conclusion was supported by the higher (though still mediocre) correlation between wheat thickness and length ($r = 0.70$).

One of the strongest correlations in Table 6.4 was that between barley thickness and width (r = 0.92). These properties proved to be highly related and offered the best possibility of predicting barley thickness using a flat tray. Unlike the length and area relationships, the thickness-width correlation was actually lower for wheat. Perhaps this was due to the greater shape variation in the wheat samples relative to barley. As mentioned above, there are a few over-square wheat cultivars and many more square wheat cultivars that have approximately equal widths to thicknesses. In contrast, the author has never seen any square, let alone over-square, barley cultivars.

The author initially tried to relate the frontal sectional area (FSA) of the single seeds to various gradings using the assumption that seeds with a larger area and greater width will also have a greater thickness. This assumption was tested by developing multivariate equations to convert the FSA and width into a seed thickness. The calculated thicknesses were then compared with the actual kernel thickness. The comparisons revealed that even the relationship of seed width to thickness was quite variable, especially from one cultivar to another. The results are illustrated in Figure 6.11 for wheat. The data points are arranged in ascending actual (calliper) thickness within each cultivar. Both the actual and calculated thickness are shown for each kernel. If both values are identical, the two markers will overlay each other, forming an eight-pointed star. This rarely happens for these calculated values, though they do have some correlation with the actual thicknesses (R^2 = 0.75, SEE = 0.24).

It can also be seen that these calculations work better for some cultivars than others. The experimental line KV5, beginning at seed number 111, correlated quite well. However, the thinner Brennan kernels, starting at seed number 155, all have drastically larger calculated thicknesses. The barley results were similar to the wheat.

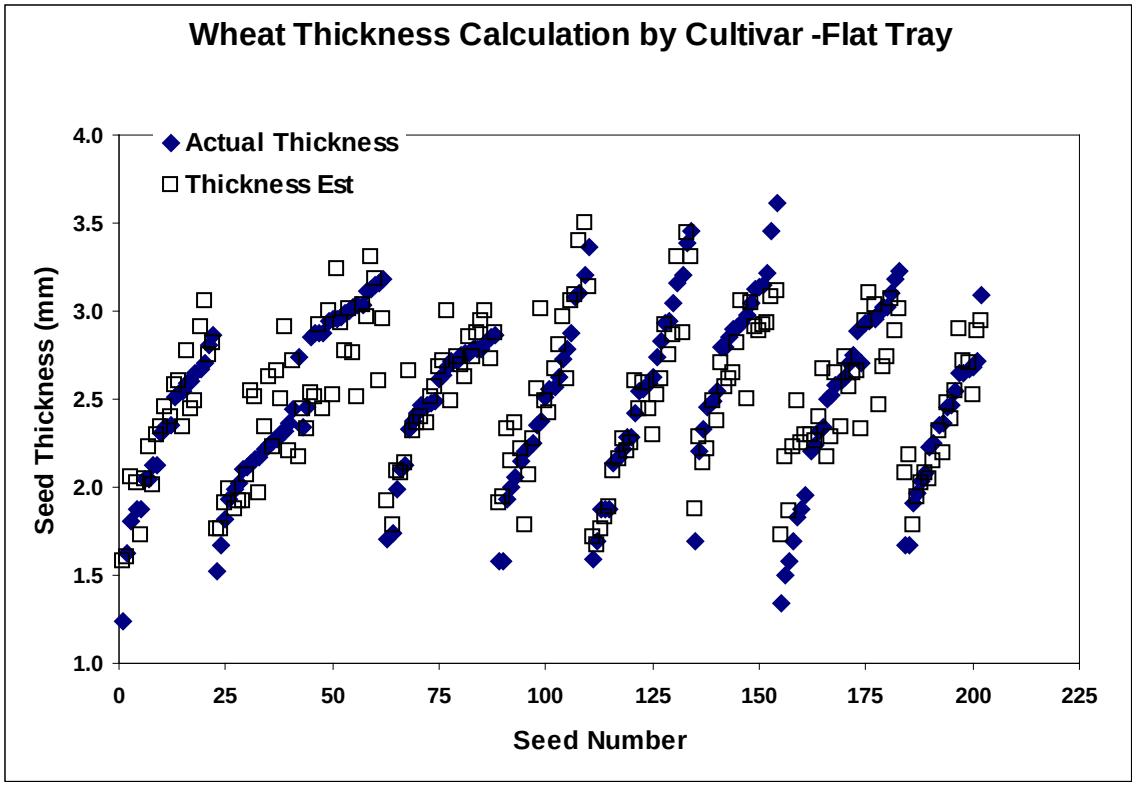

Figure 6.11 Wheat Thickness Calculation using Flat Tray DIA Area and Width

It seemed possible that extending the equations to include other factors that are related to the seed thickness such as the average kernel weight, aspect ratios, and overall seed area to mass ratio might result in better correlations. The mass-linked correlations were expected as previous testing (data not shown) had demonstrated that the specific seed density of each cultivar was largely uniform regardless of its seed size. Statistical correlations of over twenty cultivars of both wheat and barley were used to select which factors would be the most useful to correct the DIA thickness calculations. Though the correlations did improve modestly, the results were, in the opinion of Grainco Australia, still not accurate enough for commercial use (Wilson, 2002).

The obvious solutions to the DIA flat-tray thickness limitations were to devise either an optical system that could view the seeds from the side as well as from above or some method of holding the seeds on their sides so their thickness could be viewed directly from above (or below).

The novel approach used in this study was to devise specially shaped indents in a seed-imaging tray that could hold the seeds in an “edge-on” position for viewing from above. The remainder of this section examines the effectiveness of this type of tray.

6.3.6.2 Thickness Measurements using a bi-Modal Indented Tray

Several approaches to creating “thickness-viewing” indents were trialled as detailed in sections 4.4.4.1 to 4.4.4.3. Only results derived from the final indent system developed (section 4.4.4.4) are included in this and the following chapter. The narrow indent section of the tray held the kernels “edge-on” so their thickness could be directly imaged and measured by the DIA software. The wide section holds the other half of the sample lying “flat” in wide indents with the crease facing up or down. A full tray can be seen in Figure 4.6 and a detail from a tray in Figure 6.2. Separate trays were developed for barley and wheat.

It is useful to examine in more detail the commercial reality of kernel thickness measurements. Mechanical screenings assortments depend totally on the grain’s thickness and the configuration of the slotted screens. The kernel’s minimum linear dimension is often “through” the grain’s groove. However, the measurement through the bottom of the groove itself is usually less than the kernel’s apparent screening thickness. The reason for this is two-fold:

- In commercial practice, the screenings are made by passing the grain through a slotted screen which is made from sheet metal 1.5 to 3.3 mm thick. The kernel groove is usually narrower than this. When placed on a screen, a kernel with a minimum dimension of 2.1 mm measured from the bottom of the groove to the back of the seed will not be able to pass through a 2.2 mm slot. Figure 6.12 shows such a kernel only just fitting though a 2.4 mm slot in a 1.5 mm thick screen. The kernel’s long axis is aligned with the length of the slot.

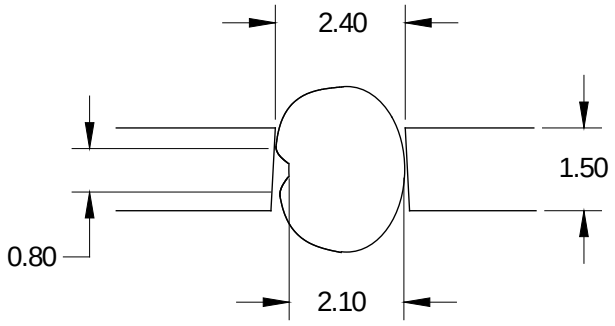

Figure 6.12 Typical Wheat Kernel Passing Through a 2.4 mm Slot in a 1.5 mm Thick Screen

- For research purposes, individual seeds were measured with digital vernier callipers. This was normally done using the flats of the jaws, which again will not fit into the bottom of the grain's groove. In practice, grain samples were first screened and then kernels were selected from each screening group and measured with callipers. It was found that only rarely did a calliper measurement place a seed outside its mechanical screening group. This finding validated the use of callipers in measuring the kernel thickness. In the example illustrated in Figure 6.12, the kernel would have a calliper thickness of about 2.38 mm.

So in measuring a kernel's thickness, like its length and width, it is the maximum measure in this direction that is needed. Though in rare instances some mis-shaped seeds will have a smaller measurement in a different direction to the three orthogonal dimensions, the maximum thickness measurement made with callipers will generally give results that compare well with mechanical screenings.

6.3.6.3 Individual Kernel Thickness Measurements

Initial testing and calibration work was performed on the individually weighed and measured kernels. The kernels were placed in the "flat-edge" tray and scanned with the SeedCount DIA system. To allow "three-dimensional" DIA data to be acquired for the

kernels, they were scanned with the seeds first in the narrow and then in the wide section. The seeds were individually placed, scanned, moved and rescanned as explained in section 6.3.7.

The images of the kernels were isolated from the tray image and digitally analysed. Each seed's directly measured edge-on DIA thickness was combined with other parameters in a similar manner to the flat-tray thickness estimates to generate a series of multivariate equations that predicts each kernel's thickness. Because of commercial confidentially agreements more details on the equations cannot be given.

The improvements in the DIA wheat thickness estimates made possible by the bi-modal tray are illustrated in Figure 6.13. The R^2 correlation jumped from 0.75 to 0.94, while the Standard Error of the Estimate dropped from 0.24 to 0.12 mm.

Barley thickness estimates have also improved dramatically, as shown in Figure 6.14.

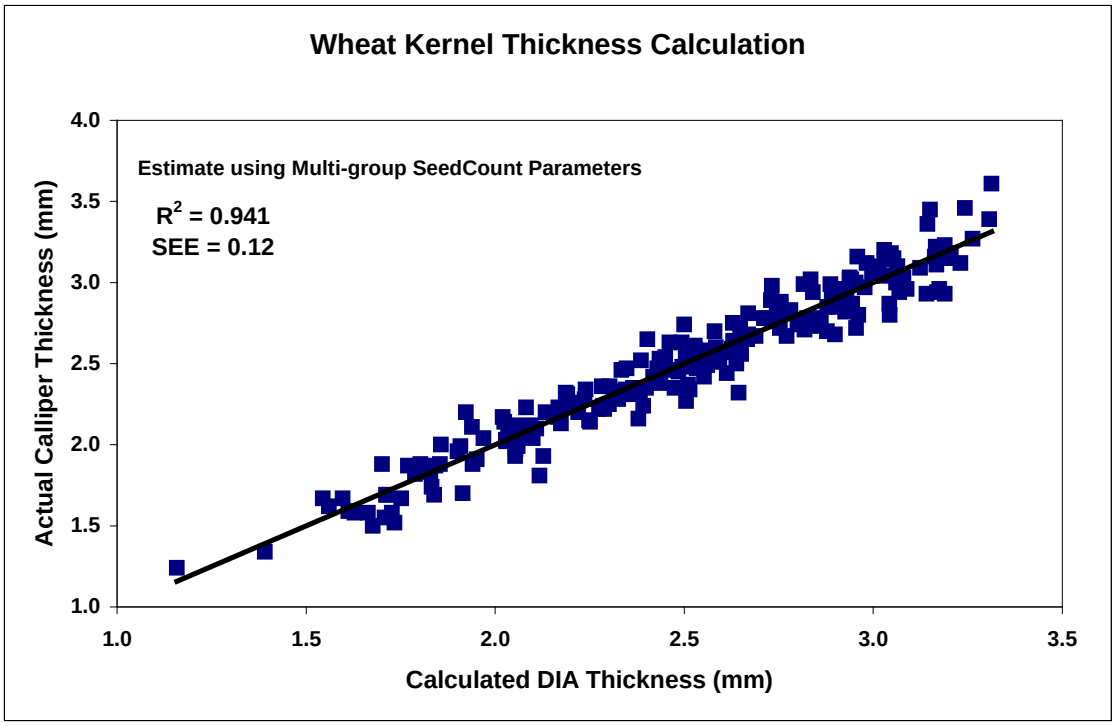

Figure 6.13 Wheat Narrow Indent DIA vs Calliper Thickness

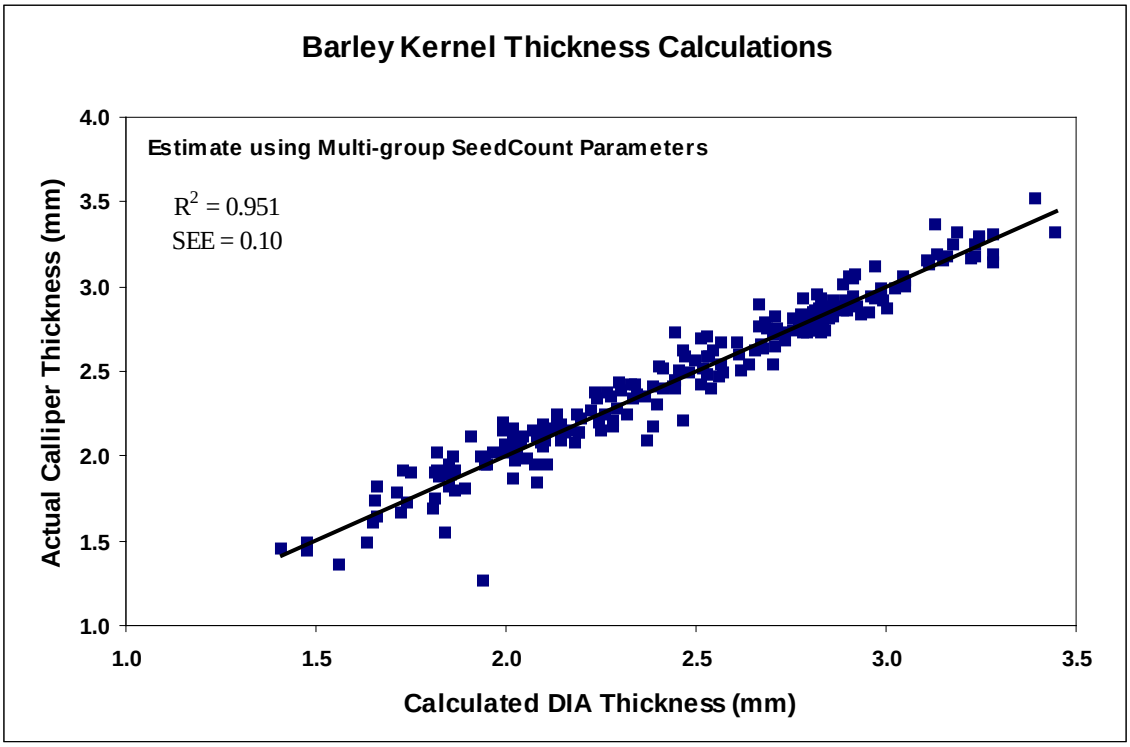

Figure 6.14 Barley Narrow Indent DIA vs Calliper Thickness

The above graphs illustrate the improvements in DIA thickness across the entire data set of individually measured seeds. However, they do not show how these DIA measurements cope with cultivar-specific measurements. The cultivar-specific measurements are shown in Figures 6.15 and 6.16, and indicate that SeedCount is now capable of accurately estimating the kernel thickness of all of these cultivars.

Performing thickness comparisons for bulk samples was not directly possible due to the impracticality of hand-measuring many thousands of kernels with callipers. However, this was in essence done in the following chapter on Screening Equivalents. As the screenings are based on kernel thickness, a comparison of the number of kernels within each grouping provides a crude comparison of their thicknesses.

Yield estimates for thicknesses will be dealt with in sections 7.3.2.4 and 7.3.2.5 as they are more effectively assessed as an aspect of the screenings groups.

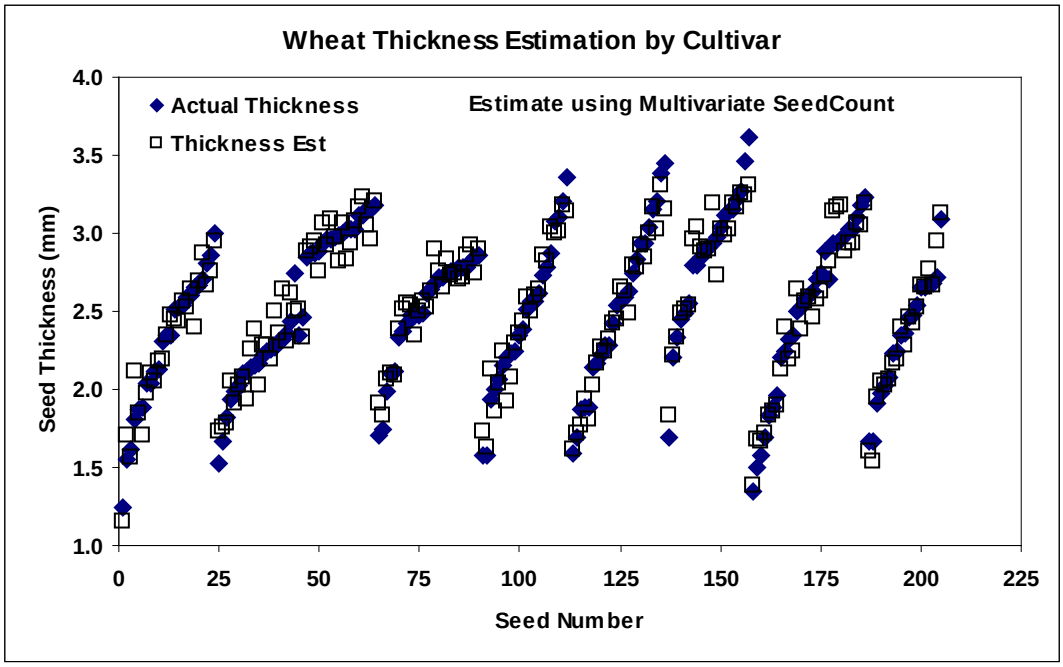

Figure 6.15 Wheat Thickness Estimate by Cultivar

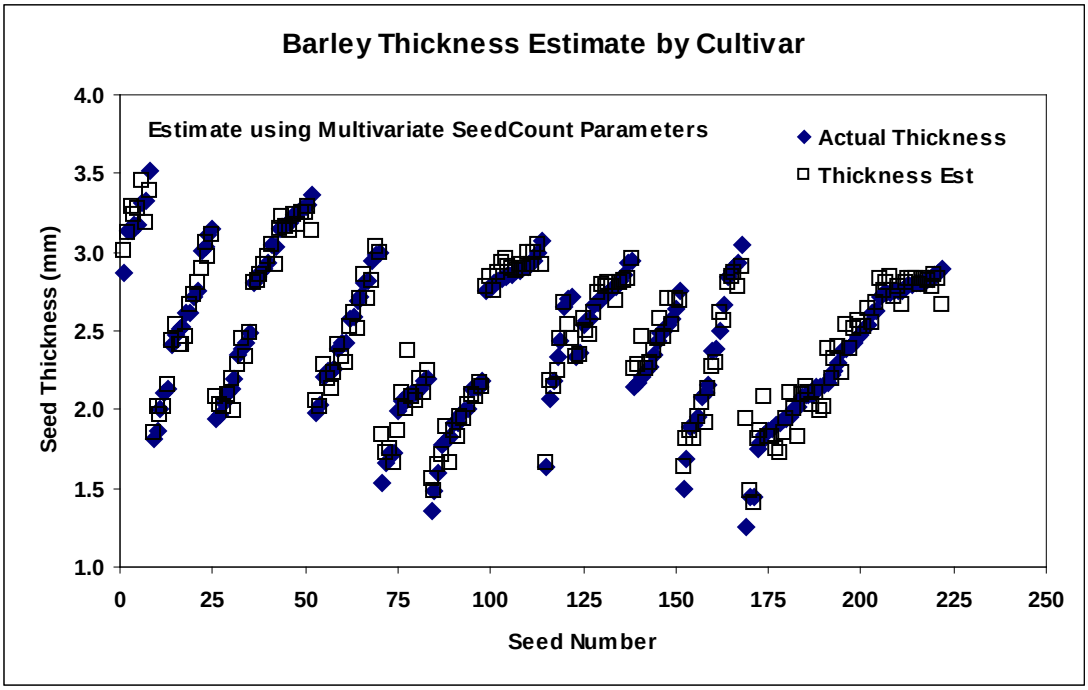

Figure 6.16 Barley Thickness Estimate by Cultivar

6.3.6.4 Thickness Conclusions

The Direct, Combed and Screened seed presentation methods given in Section 5.3.9 all had an intrinsic fault when used for determining kernel thickness: When placed on a flat tray, the grains usually assumed the most stable position, which was lying dorsally (crease down) or ventrally (crease up). This made it impossible to directly view the seed thickness. The narrow section of the bi-modal "Wide-Narrow" seed tray allowed direct observation of seed thickness in most cases.

The effectiveness of the narrow "edge-on" indents at displaying the kernel thickness was shown in Figure 6.13 and 6:14. They confirm that the bi-modal indented tray DIA system was able to assess the kernel thickness with reasonable accuracy.

There were some accuracy limitations in the directly measured DIA thicknesses due to the dimensions of each pixel. Even at a scan resolution of 300 dpi, the pixels were 0.085 mm across. The thickness of a kernel could not be estimated with greater accuracy than this. Even this accuracy was perhaps more than could always be claimed. Individual pixels along the edge of the kernels often included part of the seed edge and part of the tray. If the pixel was mostly seed, it would be retained as seed. If the pixel was mostly tray, the tray removal mask would remove it. Depending on how these pixels combined, the kernel thickness could be as much as a pixel more or less than it should be. However, across all the kernels in the tray these thickness errors would tend to balance each other.

There was another source of thickness error that was always directional. This error was the tendency of some seeds to be misaligned in the narrow indents. Rather than being perfectly vertical in the indent so the thickness could be correctly imaged, some kernels would tend to lie against one face of the indent or even lie horizontally across the top of the narrow indent. These orientation errors would result in overly large thicknesses. Functions were added to the software to minimise these errors. One function looked for the kernel crease. If the crease was visible, but not close to the edge of the kernel, the seed was not used for calculating properties like the average kernel thickness. Similarly, if the seed was lying horizontally, but dorsal side up, the kernel's aspect ratio and other

properties were used to assess its incorrect position and also remove it from thickness calculations.

However, seeds that were only slightly misaligned were difficult to detect. Their inclusion resulted in a small overall over-estimation of the kernel thicknesses. This error was corrected by an equation developed from the individually measured kernels for both wheat and barley, ensuring that the “average” kernel had the correct thickness. The effectiveness of the equation can be seen in the relatively small Standard Error for thickness (0.11) compared with kernel width (0.16) and length (0.27).

As the thickness range contained in a screening group can be as small as 0.2 mm (eg the 2.0 to 2.2 mm group), an error of about 0.1 mm for individual kernels is somewhat large. Using higher image resolutions of perhaps 600 dpi and further improvements in the tray design to reduce the number of misaligned kernels would reduce the standard error.

As scanners and computers become faster it is a certainty that higher resolution systems will be developed. The author has already designed better trays.

6.3.7 Roundness

6.3.7.1 General Roundness Considerations

Roundness is calculated from the seed’s thickness, width and length ratios and is a useful indicator of the grain shape. The roundness formula (section 6.1.5) has the advantage of being dimensionless. Seeds with the same shape will have the same roundness value, even if one seed is small and the other is large, as shown in Table 6.5.

Table 6.5 Shape vs Size Effects on Roundness

Sample	Length	Width	Thickness	Volume mm^3	Roundness
Small Mid-shape Barley	6.50	2.68	1.95	17.79	0.480
Average Mid-shape Barley	8.00	3.30	2.40	33.18	0.480
Large Mid-shape Barley	10.00	4.13	3.00	64.80	0.480

A broad range of kernels was measured to test the roundness concept. A representative selection of these seeds is listed in Table 6.6, which highlights changes in the roundness value due to varying kernel shapes. The samples are listed by ascending roundness, progressing from a long and thin grass seed to a perfect sphere. It is evident that the roundness equation confirms that barley is more elongated (less round) than wheat. For both barley and wheat, it is again evident that plump kernels are rounder than the thin screenings. The roundness equation appears to be useful for establishing the sphericity of cereals.

Table 6.6 Roundness Values of Selected Samples

Sample	Length	Width	Thick-ness	Aspect Ratio W/L	Ratio T/L	Ratio T/W	Round-ness	Aspect Ratio /Roundness
Grass Seed	6.50	1.70	0.75	0.26	0.12	0.44	0.27	0.96
Barley- screening	8.12	2.52	1.92	0.31	0.24	0.76	0.44	0.71
Barley-Plump	10.15	4.00	3.30	0.39	0.33	0.83	0.51	0.77
Wheat- screening	4.96	2.16	1.84	0.44	0.37	0.85	0.55	0.79
Wheat-Brennan	5.94	3.54	3.00	0.60	0.51	0.85	0.65	0.92
Red lentil	5.15	4.95	2.50	0.96	0.49	0.51	0.65	1.48
Chickpea-Kaniva	10.30	9.85	8.80	0.96	0.85	0.89	0.90	1.06
Sphere	4.57	4.57	4.57	1.00	1.00	1.00	1.00	1.00
Average	**7.30**	**4.10**	**3.16**	**0.56**	**0.41**	**0.73**	**0.57**	**0.95**

The aspect ratios tend to follow a similar sequence. The lentil and chickpea samples in Table 6.6 emphasize the utility of roundness values. Both have identical aspect ratios.

However, when their roundness values are compared, the lens-like lentil has a roundness of 0.65 compared to a value of 0.90 for the relatively spherical chickpea. They are clearly separated by their roundness values.

There are instances when the roundness value fails to distinguish kernel shapes. This can be seen in Table 6.6 by comparing the egg-shaped Brennan wheat kernel with the round but flat lentil. They both have a roundness value of 0.65. In this case another property is required to demonstrate that their 3D shapes are different. Unlike the lentil and chickpea comparison, it is their aspect ratios that are different. So it seems that both aspect ratio and roundness values are needed to allow discrimination of some kernel shapes.

These two parameters can be combined to produce another parameter: aspect ratio/roundness. This parameter can easily distinguish the wheat, lentil and chickpea. However, it has difficulty separating the plump wheat kernel (0.92) from a thin grass seed (0.96). So it seems that all of these values have a role to play in defining the shape of a seed and in distinguishing kernel types.

6.3.7.2 Barley and Wheat Roundness Values

The initial DIA roundness calibrations used the same seeds sequentially in both the edge-on and wide sections of the trays, as was done in the thickness section. This meant that each kernel's length, width and thickness could be measured by DIA as well as by the callipers. This data was combined and used to generate roundness values on a kernel-by-kernel basis. The barley and wheat results are shown in Figures 6.17 and 6.18. Individual seed roundness correlations between the DIA and calliper derived values were $r = 0.91$, std error of 0.02 for barley and $r = 0.81$, std error of 0.03 for wheat. Though the correlations were highly significant ($p<0.001$) the graphs and standard errors indicate that there was considerable spread between the two measurement methods, especially for the wheat roundness.

An attempt was made to rectify this by comparing the individual kernel calliper measurements with their respective DIA values. Wheat and barley dimension adjustment

regression equations were generated. The DIA roundness values were recalculated with the adjusted DIA measurements to see if the correlation improved. The correlations remained virtually unchanged, though the overall accuracy of the various DIA values improved.

The increase in the Standard Errors over the individual measurements indicates that the errors in each dimension tended to be additive. Given the Roundness formula, this is not an unexpected result.

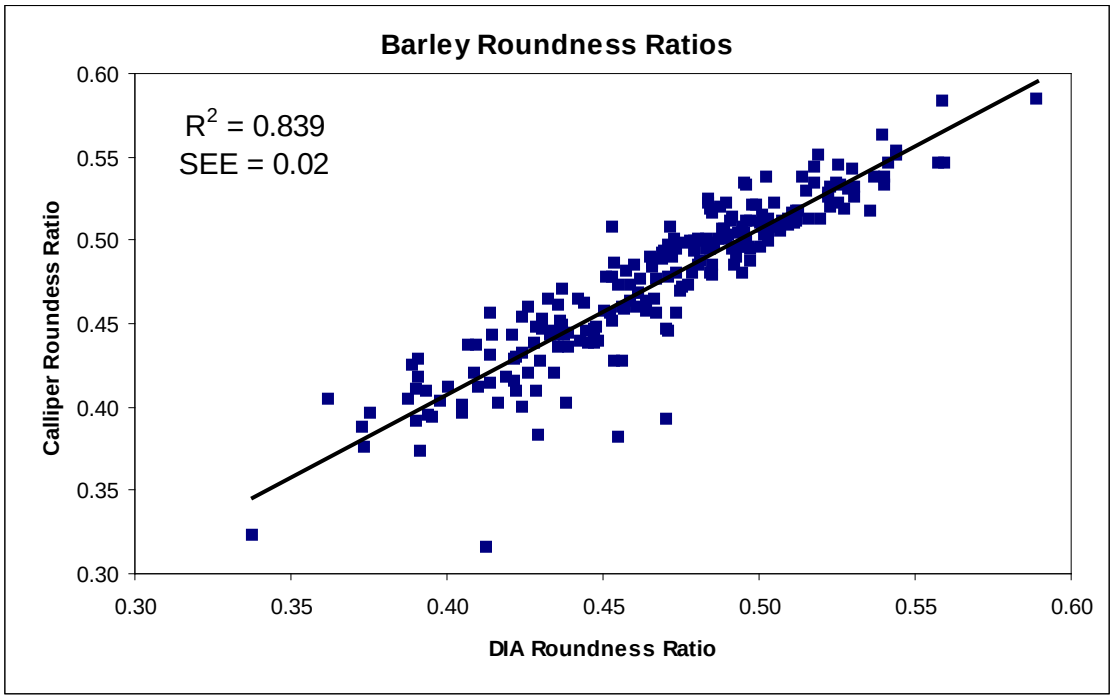

Figure 6.17 Barley DIA Roundness Values

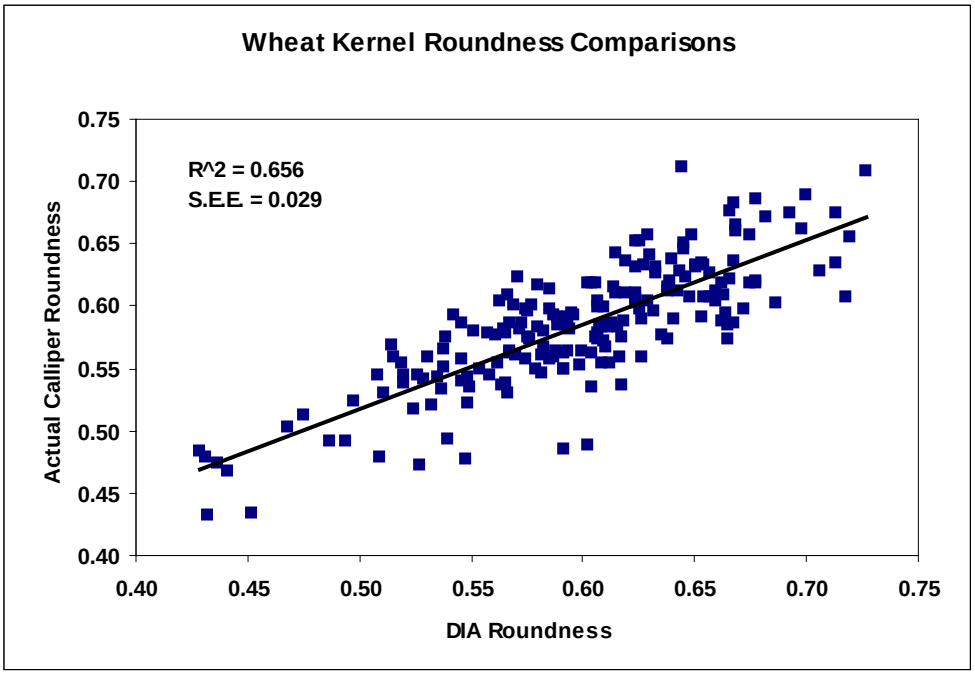

Figure 6.18 Wheat DIA Roundness Values

The roundness values for the individually measured kernels were then examined on a cultivar basis, with the wheat results summarised in Table 6.7. The values for each cultivar were composed from 12 to 54 individual kernels. For these cultivars, the R-squared correlations climbed to 0.9 and the Standard Error dropped to 0.011.

It is reasonable to assume from these results, and on the basis of standard binomial distribution theory as discussed in section 2.6.1.1, that increasing the sample size will lead to further improvements in accuracy.

Table 6.7 Calliper vs SeedCount DIA Roundness Values on a Cultivar Basis

Wheat Sample	Actual Calliper Roundness	Standardised SeedCount Roundness
KDC-WM Average	0.55	0.55
KL22-1-WM Average	0.57	0.56
KL28-3-WM Average	0.63	0.61
KV5-1-WM Average	0.58	0.60
W15-WM Average	0.56	0.56
W20-WM Average	0.60	0.58
W21-WM Average	0.60	0.61
WBN-2-WM Average	0.56	0.56
WBN-WM Average	0.66	0.65
WGN-WM Average	0.56	0.57

The DIA system can approximately replicate the calliper measured roundness values (Table 6.7). However, roundness alone is not sufficient to identify cultivars. As examples, two different cultivars grown in the same location and identified in the table as W20 and W21, have identical “Actual” (calliper-derived) roundness numbers. But two samples of the same cultivar (Brennan), grown in different locations (WBN-2 and WBN), have widely different roundness numbers.

6.3.7.3 Virtual Kernel Matching

Up to this point, all of the roundness numbers were generated for seeds whose identity had been tracked and matched across both sections of the trays. It was necessary to see if the virtual seed concept could produce similar results. The same seed images were used again, but this time they were matched using “virtual seed” alignment: the smallest seed by area in the edge-on section was linked with the smallest seed by area in the wide section, etc until all the seeds were matched.

Figures 6.19 and 6.20 illustrate the matching process for these kernels. The plots are based on the wide area rankings (smallest to largest area, numbered from 1 upwards for the first cultivar and then continuing through the other cultivars), with the narrow area ranking overlaid on them. Each cultivar forms a diagonal series of squares. Perfectly matched kernels will form an eight-pointed star.

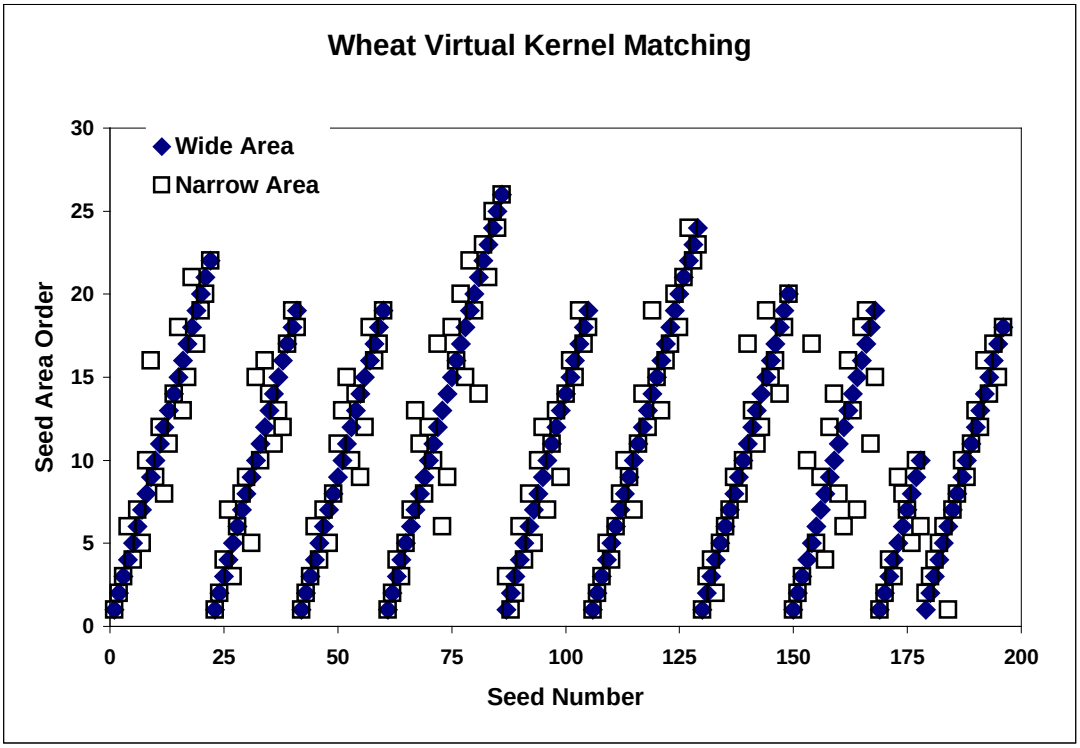

Figure 6.19 Virtual Seed Matching for Wheat

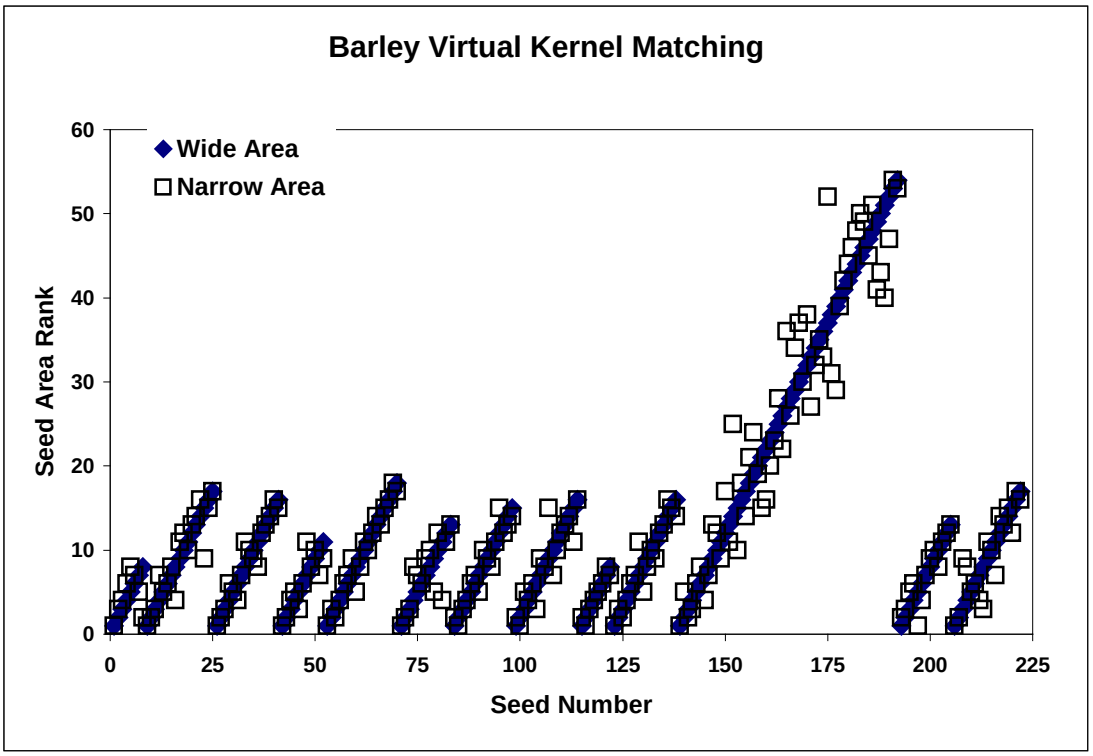

Figure 6.20 Virtual Seed Matching for Barley

The two figures reveal a better match for the wheat kernels than for the barley kernels. The mis-matches indicate that the correspondence between a kernel's wide and narrow area is not always uniform. It is also apparent that some cultivars are more uniform in this respect than others, examples being the wheat series beginning at kernel 150 (poorly correlated) and at 179 (highly correlated). The barley virtual seeds indicate that as the number of kernels in each series increases, it becomes more difficult to accurately match them (Figure 6.20, series beginning at kernel 139).

The important question is this: Do these mis-matches have a significant effect on virtual seed derived values? In this chapter the effect of the mis-matches on the roundness values will be examined. In the next chapter the mis-match effect on kernel mass calculations will be explored.

6.3.7.4 Individual Kernel Virtual Roundness

The roundness calculations for the 22 kernels of one wheat cultivar, ordered by actual (calliper measured) roundness are presented in Table 6.8. The seed-matched roundness values are those in which the wide and narrow seed data is positively matched by kernel identity. The area-matched roundness values use the "virtual seed" area-matching method. The table confirms the strong correlation (r = 0.65) between the actual and seed-matched roundness values. The correlation between the seed-matched and area-matched roundness numbers is somewhat lower (r = 0.47). Across the entire wheat data set the standard error between these values is 0.045. At a seed by seed level one's confidence in the roundness numbers generated by the "virtual seed" matching method would not be high.

Table 6.9 expands Table 6.8, comparing calliper-measured DIA values with SeedCount calculated values for both kernel-matched and area-matched roundness values. It can be seen that the average roundness values are essentially unchanged, as was shown in Table 6.8. This is due to the simple fact that each average value summarises the results of combining all of the seed measurements for that cultivar. No change would be expected at this level. As this is the level at which Roundness Numbers are reported in SeedCount, any virtual seed-matching errors have no end effect on its Roundness values.

The virtual seed concept was then scaled up for use with normal trayfuls of seeds where there were simultaneously different seeds in the wide and edge-on sections of the tray, as detailed in section 3.4.5.7. As the average Roundness values were independent of the accuracy of the virtual seed matching system, this was an acceptable process.

Table 6.8 Comparisons of Positive and Area Matched Roundness Calculations

Wide Seed Number	Actual Roundness	Seed-Matched DIA Roundness	Area-Matched DIA Roundness
1	0.43	0.45	0.45
2	0.49	0.59	0.57
3	0.49	0.49	0.51
4	0.52	0.52	0.53
5	0.52	0.50	0.54
6	0.54	0.51	0.58
7	0.54	0.52	0.60
8	0.55	0.55	0.55
9	0.55	0.58	0.51
10	0.55	0.61	0.62
11	0.56	0.53	0.49
12	0.56	0.57	0.54
13	0.57	0.54	0.57
14	0.57	0.61	0.56
15	0.58	0.61	0.60
16	0.58	0.56	0.62
17	0.58	0.56	0.56
18	0.58	0.55	0.58
19	0.59	0.54	0.54
20	0.60	0.57	0.56
21	0.62	0.58	0.59
22	0.63	0.64	0.53
Average	**0.55**	**0.55**	**0.55**
SEE		**0.034**	**0.040**

Table 6.9 Average Roundness Numbers

	Actual Roundness	Matched SeedCount Roundness	Area Matched Roundness
KDC-WM Average	0.55	0.55	0.55
KL22-1-WM Average	0.57	0.57	0.56
KL28-3-WM Average	0.63	0.64	0.64
KV5-1-WM Average	0.58	0.61	0.61
W15-WM Average	0.56	0.58	0.58
W20-WM Average	0.60	0.60	0.60
W21-WM Average	0.60	0.63	0.63
WBN-2-WM Average	0.56	0.56	0.56
WBN-WM Average	0.66	0.69	0.70
WGN-WM Average	0.56	0.59	0.59
Grand Average	**0.58**	**0.60**	**0.60**

6.3.7.5 Full-tray Virtual Roundness

The average roundness values of 29 wheat and 26 barley cultivars were determined to see if different cultivars had significantly different roundness (data not shown). Full-tray barley cultivars had roundness values ranging from 0.44 to 0.55 with a mean value of 0.51. The wheat cultivars had a mean roundness number of 0.62, ranging from 0.57 to 0.71. These values confirm that wheat is rounder than barley, the usual conclusion of anyone who works with grain. The interesting part of this data though is the complete Roundness separation of the barley and wheat cultivars used. This suggests that the roundness value could be a very useful tool for discriminating between wheat and barley in a contamination situation. As the cultivars used were selected to offer a broad range of the material used in Australia, it is possible that this separation at a roundness number of 0.56 may maintain its significance. This possibility was strengthened by the results for a second set of 42 wheat cultivars that had an average roundness of 0.60 and a range of 0.65 to 0.57.

Roundness also had a positive correlation (r = 0.55 for wheat and 0.83 for barley) with grain retained on the 2.8mm screen, indicating that samples with thicker grains also tend to be rounder. This roundness result supports the aspect ratio findings, to which they were correlated (r = 0.74 for wheat and 0.80 for barley).

It is the author's opinion that although the roundness values provide useful information on a kernel's shape, roundness cannot be used as a stand-alone parameter for positive cultivar identification in Australia as numerous cultivars can have quite similar shapes. This problem is further complicated by the fact that growing conditions can also affect kernel roundness, as has already been demonstrated with aspect ratios and roundness (See Table 6.7 and the comments below it). However, roundness can provide another small part of the matrix that will help in cultivar identification.

It is likely that kernel roundness numbers may be more useful in Canada due to their visual distinguishability requirements:

> "In Canada, wheat classes and grades are based upon end use quality. They are identified by their visual characteristics, called kernel visual distinguishability, KVD. New varieties must perform the same as or better than other varieties in the same class, and they must also look like other varieties within the same class. Similarly, grades within each class are visually distinguishable." (Canadian Grain Commission, 2003)

As Australia does not have a KVD requirement, it will be more difficult to separate classes here on a visual basis. The KVD also explains why many of the DIA wheat classification studies quoted in the literature review have been performed on Canadian wheat.

The roundness value is only weakly correlated with kernel weight (r=0.55 for wheat and 0.39 for barley). This means that roundness is a largely independent measure of the seed properties that is distinctly different from weight. Roundness therefore has the potential

to add additional and different information about a grain sample's possible performance to the other parameters.

Roundness was tested to see if this potential was real: did it have a correlation with the cultivar's yield?

6.3.7.6 Roundness Yield Predictions

Aspect ratios, wide areas, ovality and roundness were compared to milling extraction data for 42 wheat samples sourced from Queensland and New South Wales. Similarly, the barley data was compared to soluble malt extract data for 41 samples from Victoria, South Australia and Western Australia. Both sample sets were selected to cover an extensive flour yield/extract range. Standard test milling (Allied Mills, 2003) and malting and mashing (EBC, 2003; MBIBTC, 2001) protocols were followed.

Correlations between the DIA data and clean flour yields were explored and the results are displayed in Figure 6.21. Wheat flour extraction was positively correlated with roundness ($r = 0.60$, $p < 0.001$, std error = 6.7). This correlation was consistent, statistically significant and indicates a general trend towards higher flour yields with increasing kernel roundness. However, the large standard error of the estimate (6.7) means that roundness on its own was more of an indicator rather than an extremely impressive predictor of flour yield. This may in part be due to the size-independent nature of the roundness values that only reveal sphericity.

This study found no significant correlations between malted barley soluble hot-water extract and roundness ($r = -0.21$, see Figure 6.22). This may be due to overriding quality factors such as the 'maltability' and residual enzyme activity of the various barleys used (Garcia del Moral et al., 1998). Barley roundness cannot be used to predict the soluble extract yield of its malt.

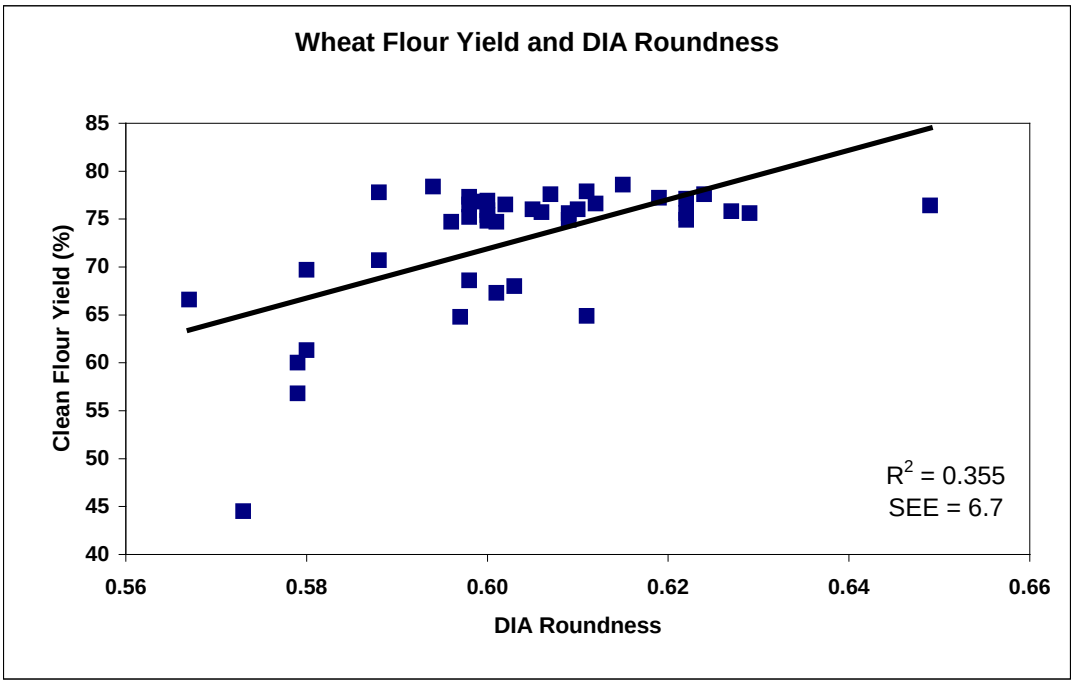

Figure 6.21 The Role of Roundness in Wheat Flour Yield

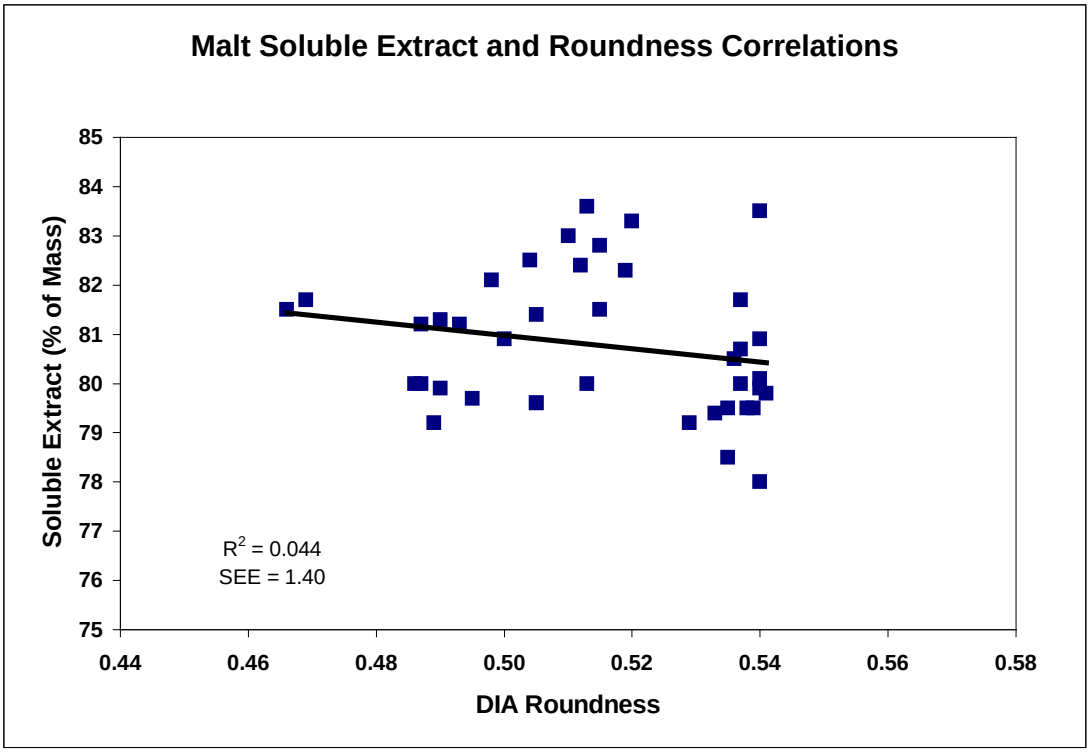

Figure 6.22 The Role of Roundness in Malted Barley Extract

6.3.8 Time Considerations

One other aspect of the orthogonal and area measurements is the time taken for the respective methods. Measuring the length, width and thickness of a kernel with digital callipers and recording the data takes approximately 45 seconds per seed. As an average bi-modal tray of wheat contains 750 kernels, this would take 45/3600 * 750/2 = 4.7 hours to measure 375 ‘virtual’ seeds.

Estimating the area of each seed without DIA is difficult. It can be done by projecting the seed onto a screen marked with a grid and counting the number of squares covered and converting this to an area. Using a pre-counted shape of coloured squares marked on the screen that is the average size and shape of most seeds of that type can speed up the counting. The seed projection needs to be aligned to lie over this coloured block. This process takes a minimum of 45 seconds a seed. For our average trayful of seed, we are again looking at more than 4.7 hours just to record the area of the kernels in the wide section of the tray. Once the orthogonal dimensions and kernel area have been determined and recorded, it is a simple matter to calculate the ovality and roundness in a spreadsheet, so no extra time is being added for these processes.

The SeedCount DIA can make these measurements, and a great number of others, plus calculate the means, standard deviations and the secondary combinations from these measures in less than 60 seconds including filling the tray. If more than one sample is being run in a series, the tray filling can be overlapped with the analysis time, reducing the time per sample to less than a minute. Using a value of sixty seconds per SeedCount run, this represents 60*100/4.7*2*3660 = 0.17% of the time otherwise required. Phrased another way, a long nine-hour day of work can be completed in one minute, and with much less stress to the operator.

6.4 Conclusions

The “wide-edge” indented tray based DIA system can assess kernel length, width, thickness and area, though with some loss of accuracy (+/- 0.01 mm for callipers,

+/- 0.11 mm for DIA.). This data can be used to generate aspect ratio, ovality, roundness and aspect ratio/roundness values. Though none of these values will allow definitive cultivar identification, they can help sort grain into kernel types and sometimes into general cultivar families within a type.

Average wide kernel area showed a significant negative correlation with wheat protein by Leco ($r = -0.64$), a result predicted by the theoretical shape (ie, a higher endosperm content in larger kernels necessarily means a lower protein content as noted in the Literature review.) Unexpectedly, this link did not lead to a significant correlation between kernel area and flour yield, which was only $r = 0.11$. Barley average area proved to have no significant correlation with either protein ($r = -0.19$) or extract ($r = -0.05$). This issue will be discussed in detail in section 7.3.2.5.

“Three-dimensional Virtual Seeds” can be used to predict average roundness values across a full sample. However they cannot be relied on to accurately match individual kernels when they are used in both sections of the tray.

Roundness is positively linked to wheat flour yield, but probably needs combining with other factors to usefully predict the yield. However, roundness cannot predict the soluble hot-water extract of malted barley.

DIA allows an enormous reduction in the time required to make these measurements. Over nine hours of work can be completed in one minute.

Further software and hardware development allowing the use of higher resolution scans, better trays and larger sample sizes will increase the accuracy of DIA systems.

7 Using Digital Image Analysis Systems for Estimating Kernel Mass, Screening Assortments and Yield

7.1 Introduction

Kernel size is an important factor affecting the ratio of endosperm to the total kernel mass. As the kernel becomes larger, the percentage of the kernel that is endosperm increases (See Table 6.1). For millers and maltsters, this should equate to, respectively, higher flour and higher soluble extract yields. There has been a long tradition among breeders, conscious of this relationship, to select for plumper (ie thicker) kernels.

DIA potentially offers a fast method to provide a full grading of the sample's seed thickness, which should be highly correlated with the mechanical screening assortments. Once achieved, this data could be extended to provide a full analysis of the grain's KW distribution.

Screening the grain through an array of sieves of diminishing slot widths provides useful information about the sample's size distribution based on the kernel thickness, and hence its potential yield and homogeneity. Chapter Six discussed how standard DIA systems cannot directly see the kernel thickness, which is the dimension on which the screening system operates (Gebhardt, Rasmusson and Fulcher, 1993). This is because seeds, when spread on a flat surface, display their length and width rather than their thickness. Chapter Six also showed how SeedCount's unique indented tray could hold some kernels in an "edge-on" position that closely matched the orientation in which they slide through the screens. This orientation enabled direct DIA thickness measurements. These measurements can now be used to assign each kernel to its appropriate screening fraction. If each kernel's mass can also be estimated, the masses can be summed and the percent mass of the kernels in each fraction can be determined. These calculated percentages will be referred to as Screening Equivalents.

It would be desirable to combine the data derived from both the wide and narrow sections of the tray to estimate the kernel mass and screening groups. However, as an individual seed can only be in one portion of the tray at a time, it was necessary to develop a method of linking the seeds in both sides of the tray to generate more comprehensive three-dimensional statistics. This task was performed for roundness estimates in Chapter Six by matching the seeds in the two sections by their increasing kernel area, thus creating the "virtual" 3D kernels. The same 'virtual' seed approach will be used in calculating the DIA-based Screening Equivalents.

7.2 Aims

This chapter examines whether a DIA system incorporating a bi-modal indented tray is capable of accurately estimating the kernel mass and hence the percent by mass screening assortment of wheat and barley. Finally, it attempts to establish a link between the DIA-determined grain properties and the grain's flour or soluble extract yield.

7.3 Results and Discussion

The materials and methods used to generate these results are set out in sections 3.3 and 3.4.5.

Estimating the Screening Equivalents with DIA depends on finding the grain's thickness and mass. Finding the thickness was covered in the previous chapter. This chapter begins with an investigation into SeedCount's ability to assess the kernel mass.

7.3.1 Mass Estimation

7.3.1.1 Individual Kernel Mass Estimates

As was done for the thickness work, initial calibrations were made with 255 barley kernels from 14 cultivars and 205 wheat seeds from 10 cultivars that had been individually weighed and measured with digital callipers. They were analysed with

SeedCount and the DIA data was combined to generate a series of multivariate equations that predict each kernel's mass and screenings group. These results were compared with the conventional data.

Kernel Mass estimation was mathematically somewhat more complex than thickness estimations for most samples as there was no individual DIA kernel mass to begin with. In the case of the individually weighed seeds, though, there was a known kernel mass to test the calculated mass results against. There was also the kernel area and the average kernel weight, calculated as set out in section 3.4.4.5, for the sample in the tray to provide a starting point.

7.3.1.2 Wide Section Mass Estimates

Initial attempts at calculating the kernel mass were made with Version 1 of SeedCount, which only allowed flat tray values to be used. The results, on a cultivar basis, are illustrated in Figure 7.1 for barley, using only the wide section of an indented tray. The calculated mass worked reasonably well for some cultivars. However, for many cultivars it tended to compress the mass estimates into a central region, thereby reducing the accuracy of the calculations. The compression is particularly noticeable for the cultivar beginning at seed number 167. Wheat results were similar (data not shown).

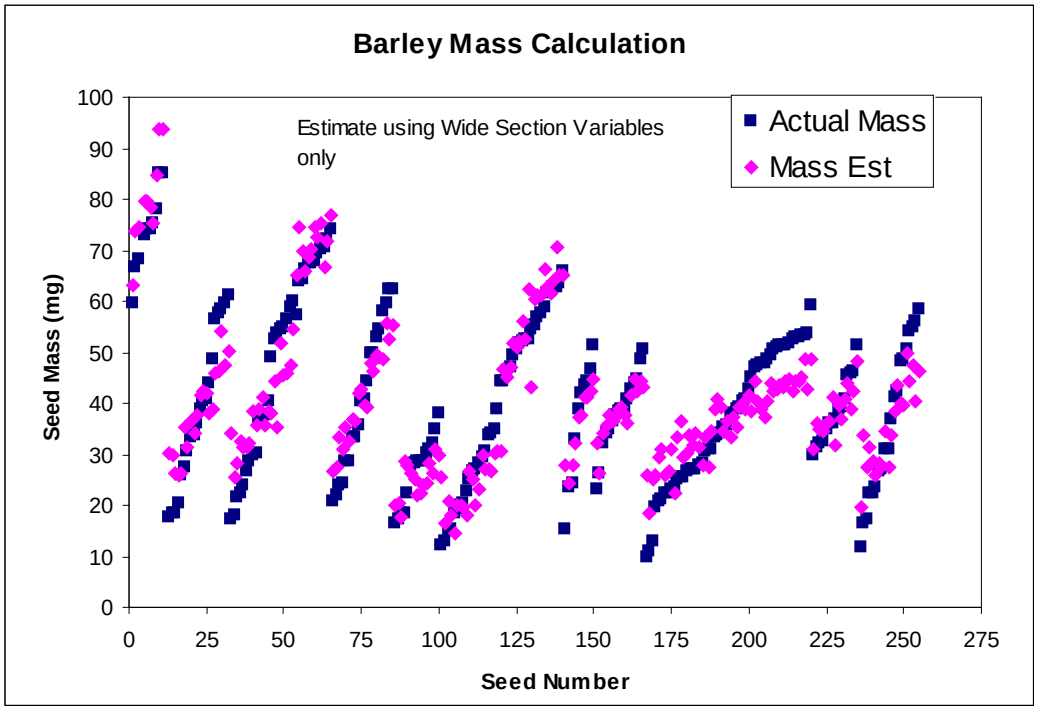

Figure 7.1 Simple Mass Estimate of Barley by Cultivar

7.3.1.3 Bi-Modal Tray Mass Estimates

A later series of correlations for Version 2 of SeedCount using both its wide and narrow section properties plus some other values generated by various combinations of these primary properties were prepared. Variables that were strongly correlated with the actual kernel mass were selected. Co-linear variables were removed and both linear and non-linear regressions were investigated to find an equation that could calculate the kernel mass using only data readily available to SeedCount. For commercial confidentiality reasons the author is not permitted to divulge the exact equation that was developed and used in SeedCount.

As the formula combined data derived from both the narrow and wide sections of the tray, the creation and use of virtual seeds was again required (section 6.3.7.3). SeedCount's ability to estimate the kernel mass of wheat is shown in Figure 7.2, while Figure 7.3 illustrates its effectiveness with barley. The multivariate calculations result in

estimates that correlate very well (R^2=0.965 for both wheat and barley) with the actual masses.

The formula worked with a wide variety of wheat and barley cultivars as illustrated in Figures 7.4 and 7.5, suggesting the algorithm is robust. In these figures each diagonally rising cluster of points is a different cultivar.

The formulas could not be directly bulk-tested on full-tray samples as doing so would, similar to bulk-testing the thickness estimates, require the individual weighing and tracking of many thousands of kernels. Instead, bulk-testing was undertaken indirectly via the screening equivalents as explained in section 7.3.2.2.

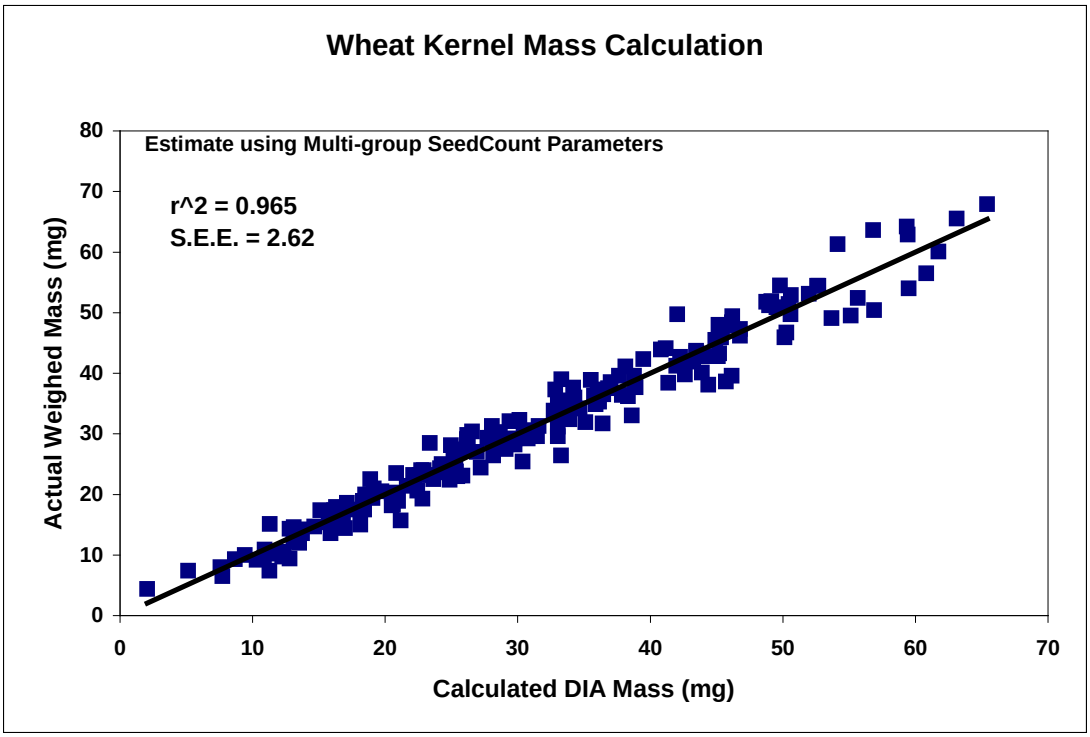

Figure 7.2 Calculated DIA Wheat Kernel Mass

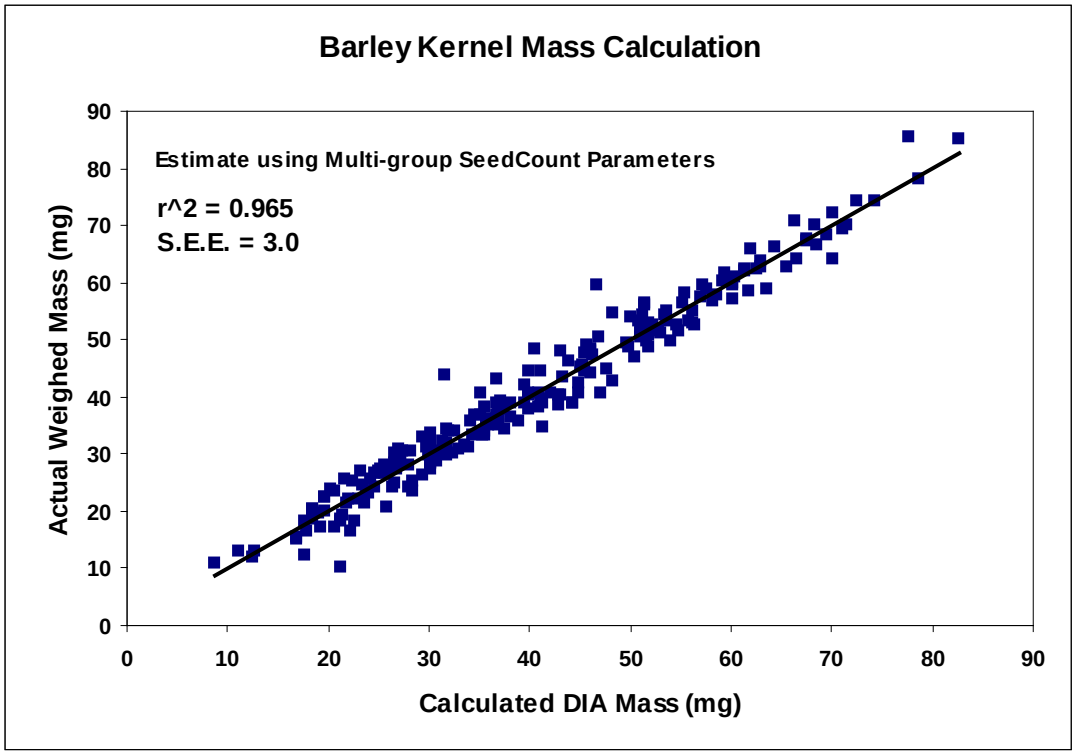

Figure 7.3 Calculated DIA Barley Kernel Mass

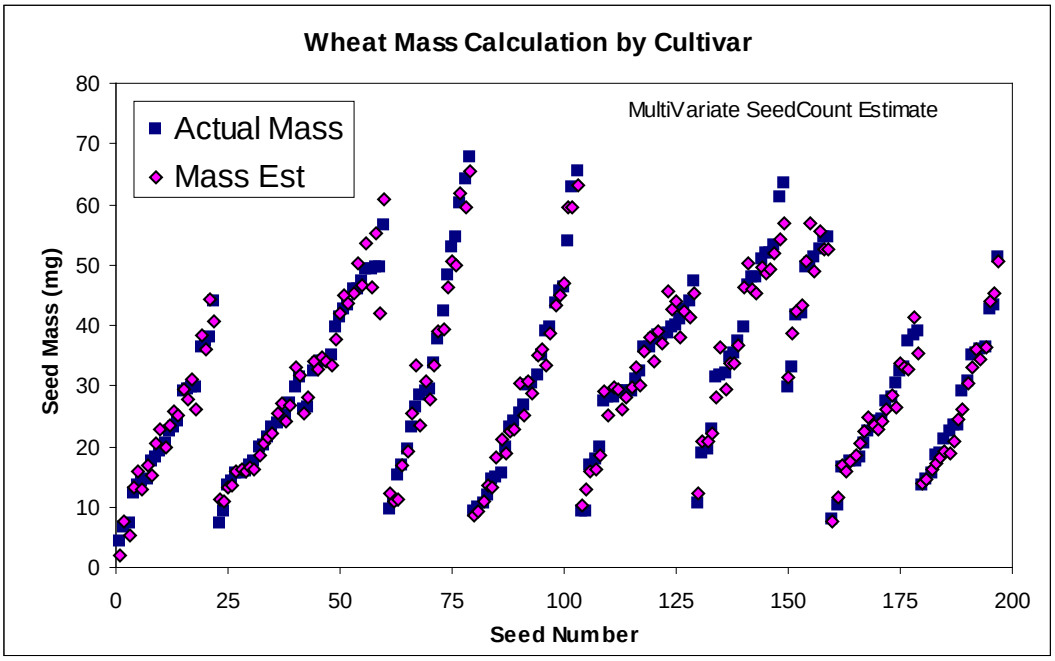

Figure 7.4 Wheat Kernel Mass Estimates by Cultivar

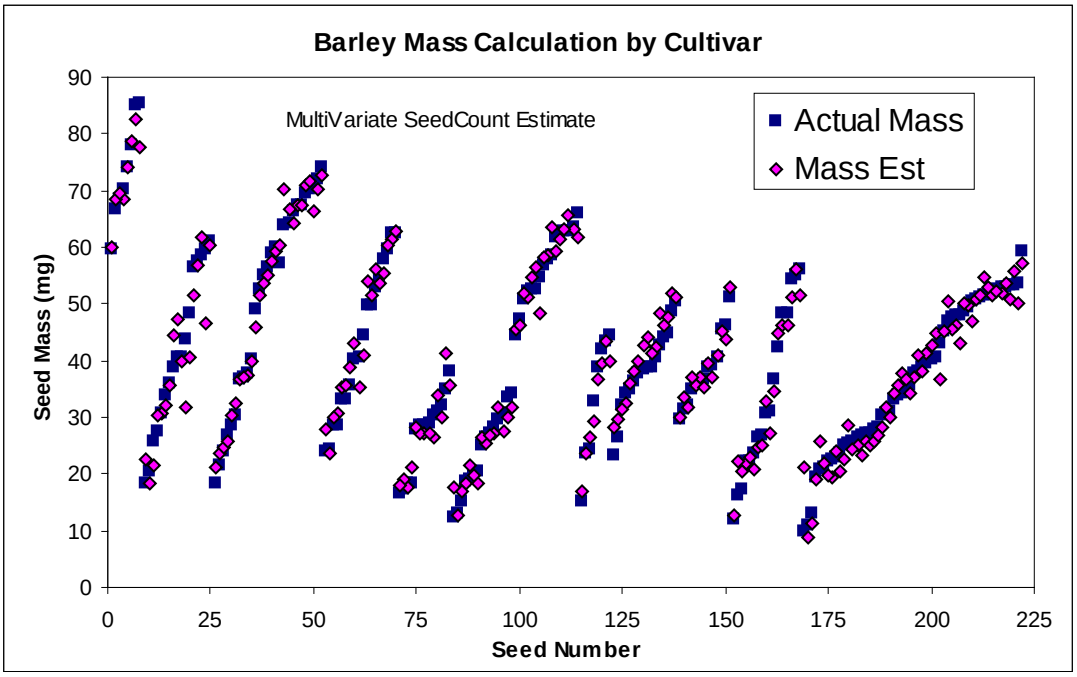

Figure 7.5 Barley Kernel Mass Estimates by Cultivar

7.3.1.4 Full-Tray Mass Estimates

Valuable information can be derived from graphs of the mass distribution of full-tray samples. Figure 7.6 shows the calculated masses for a Sun white wheat experimental breeding cultivar. There is clearly a problem with this sample shown by the separation of the grain into two distinct mass groups. If it was a commercial bulk grain sample, one would suspect that it was either a blend of two different crops or was grown in a field sown with two quite different wheat cultivars. As the sample is a carefully selected and handled breeding sample this is unlikely. It is more probable that it is showing the results of either part of the seed germinating well after the rest or frost-damage retarding the development of some of the kernels during head-filling.

The kernel mass graph could also reveal large numbers of broken grains or small weed contamination, which would show up near the left hand edge. In Figure 7.6 there were only 3 such fragments, suggesting it was a well-handled sample. Heavy items on the right

of the graph could be large foreign seeds or multiple grains. There are only four of these, again suggesting that the tray was filled correctly and the sample was relatively clean.

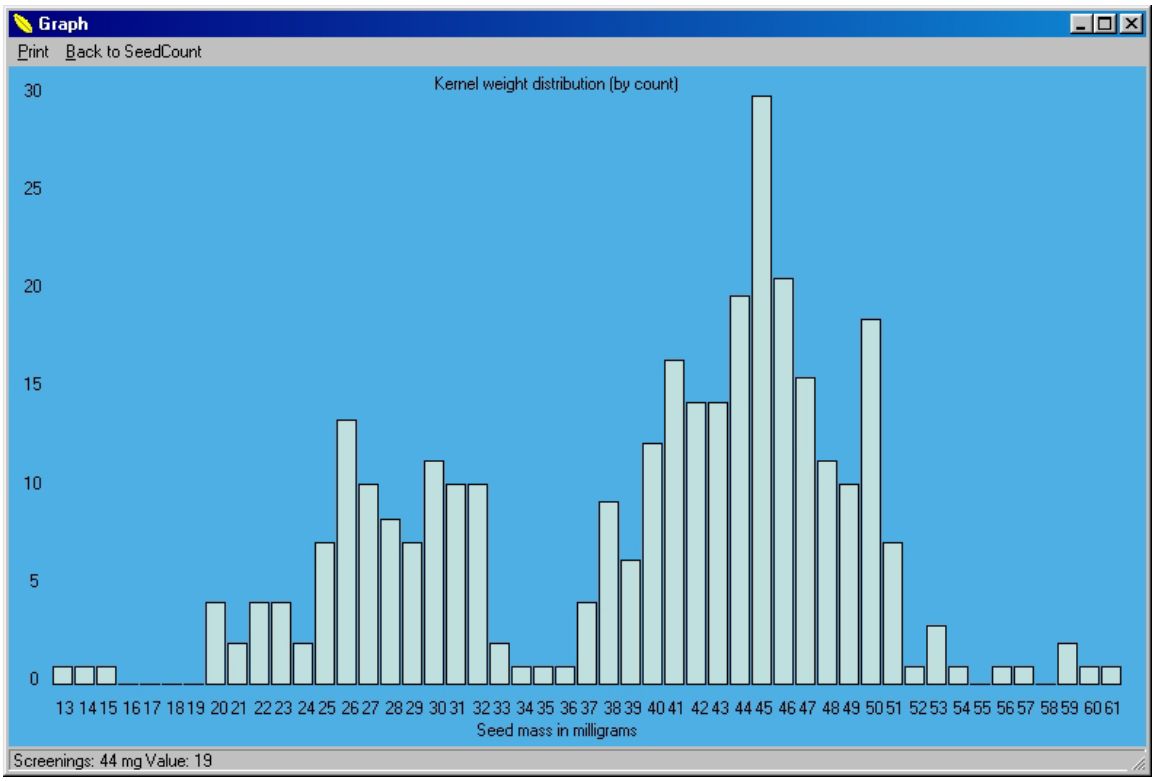

Figure 7.6 DIA Kernel Weight Distribution for a Sun Breeding Line Sample

7.3.1.5 Kernel Mass Time Considerations

Individual kernel mass data can only be acquired manually by weighing each kernel one by one. This takes approximately 15 seconds per kernel. The operator must place it on an analytical balance pan, close the door, wait for the balance to stabilise, record the kernel mass, open the door and remove the kernel. As there are about 750 kernels in an average tray of wheat, which produce 375 virtual seeds, the graph represents the equivalent of 94 minutes of manual work (ie 15/60 * 375 = 94).

SeedCount calculates these masses as part of its one-minute analysis, thereby reducing this to 1.1 percent of the time requirement (100* 1/94 = 1.1).

7.3.2 Screening Equivalents

7.3.2.1 Individual Kernel Screening Equivalents

SeedCount's ability to allocate the kernels into the correct screening groups was initially tested with the individually measured kernels. The results are summarised in Table 7.1, which shows the count of kernels that the calliper-measured (actual) thickness and the DIA-adjusted (calculated) thickness placed into each group. The overall counts are quite similar, with only the 2.2 mm breakpoint appearing to create problems for both the barley and wheat counts. The percent difference for each actual/calculated pair is based on the percent difference of the pair across all groups, rather than the difference within the one pair alone.

Table 7.1 Number of Barley and Wheat Kernels in Various Screening Groups

Screening Group	>2.8	2.8 to 2.5	2.5 to 2.2	2.2 to 2.0	< 2.0
Actual Barley	65	46	34	38	39
Calculated Barley	66	45	37	34	40
Percent Difference	**-0.5**	**0.5**	**-1.4**	**1.8**	**-0.5**
Actual Wheat	63	46	43	19	34
Calculated Wheat	65	46	37	24	33
Percent Difference	**-1.0**	**0.0**	**2.9**	**-2.4**	**0.5**

The distributions aren't as precise as Table 7.1 indicates when examined on a kernel identity basis. When the barley allocations were checked in this way, it was found that 177 of the 222 kernels were placed in their correct group. Twenty-one kernels had been inserted one group too low, and 24 kernels had been inserted one group too high. No kernels were more than one group out of alignment. The kernels that were placed in the wrong groups were generally those close to the boundary between two groups. None the less, the data supports a strong tendency for both wheat and barley kernels to be placed in their correct screening fraction.

The next and final phase in exploring the utility of screening equivalents was to determine if the masses assigned to these kernels in their groups corresponded with the actual screening groups. The results of this step are shown in Table 7.2.

Table 7.2 Screening Masses, Assortments and Equivalents

	>2.8	**2.8 to 2.5**	**2.5 to 2.2**	**2.2 to 2.0**	**< 2.0**
Actual Barley (mg)	3861.8	2006.5	1229.4	1074.2	818.9
Barley Assortment (%)	43.0	22.3	13.7	11.9	9.1
Calculated Barley (mg)	3909.5	1994.9	1304.8	945.0	837.0
Barley Equivalent (%)	43.5	22.2	14.5	10.5	9.3
Percent Difference	**-0.5**	**0.1**	**-0.8**	**1.4**	**-0.2**
Actual Wheat (mg)	2963.3	1575.3	1061.1	360.5	421.1
Wheat Assortment (%)	46.4	24.7	16.6	5.6	6.6
Calculated Wheat (mg)	3062.7	1519.8	953.2	433.8	411.9
Wheat Equivalent (%)	48.0	23.8	14.9	6.8	6.5
Percent Difference	**-1.6**	**0.9**	**1.7**	**-1.1**	**0.1**

The Table appears to be more complex than it really is. A walk-through of the barley data will clarify it. The Actual Barley row gives the sum in milligrams of the actual weighed mass of the kernels placed in each screening group by their calliper-measured thickness as shown in Table 7.1. The Barley Assortment row below it shows the result of converting these masses to Percent by Mass in each group. This is, of course, simply a standard Screening Assortment. The Calculated Barley row gives the sum in milligrams of the calculated mass of the kernels placed in each screening group by their calculated DIA thickness as shown in Table 7.1. Similar to the Barley Assortment row, the Barley Equivalent row shows these masses converted into Percent by Mass for each group. The Percent Difference row then highlights the differences between the Barley assortment and the Barley Equivalent values for each group. This process is duplicated for the Wheat data. As there was only one “sample” in this set at the group level, standard errors could not be calculated.

The group differences highlighted in Table 7.2 tend to be smaller than the group differences in Table 7.1, with the exception of wheat groups with a thickness of 2.5 mm or greater. These tables indicate that the screening equivalents are more accurate than their underlying kernel count distribution. This improvement reflects a general tendency in the calculated-DIA method to link the kernel thickness to the kernel mass. Therefore lighter kernels would tend to be demoted to the "thinner" screening groups and heavier kernels would be promoted to the "thicker" groups. These weightings have partially compensated for mis-grouping kernels, resulting in the general improvement in the screening equivalents.

Although Screening Equivalent errors of up to 1.7% are too large for commercial use, they would certainly be useful for rapid indicative tests of a sample's screening assortment, especially for plant breeders and others who need to run many hundreds of samples.

7.3.2.2 Full-Tray Screening Equivalents

The screening equivalent estimates finally needed testing on trays filled with kernels taken from single grain lots as this is mode that SeedCount would normally operate in. It was bulk tested on 26 barley and 28 wheat varieties with essentially full trays. The mechanical screenings were only determined once for each sample. The DIA results were determined in duplicate, using the same kernels as the mechanical screenings. The second DIA image was made by emptying the tray and refilling it with the same seeds. This process eliminated the sampling variations that would occur if using a different sub-sample each time. The trays can hold maximums of 658 barley or 1052 wheat kernels. The kernel data from the flat and on-edge sections was digitally combined to form three-dimensional virtual seeds. Each virtual seed's adjusted thickness, roundness, mass and screening group was calculated. These predictions were then compared to essentially standard screenings results for the same samples using certified screens (IoB, 1997, Method 1.13). The test differed from the standard IOB tests in that the sample size was reduced to the DIA trayful of seeds, weighing from 21 to 26 grams and containing an average of 500 barley or 762 wheat kernels.

The DIA thickness and virtual seed masses were used to generate the screenings groups, as detailed above. Tables 7.3 and 7.4 compare DIA screening equivalents to mechanical screening assortments for the bulk full-tray samples. Each duplicated run was assessed independently to produce the first four data rows of the tables. The last two rows contain data derived by analysing the two duplicate runs together.

Table 7.3 Barley Full-Tray Screening Equivalents

	> 2.8	**2.8 to 2.5**	**2.5 to 2.2**	**2.2 to 2.0**	**< 2.0**	**<2.5**
Barley Correlation	0.98	0.92	0.86	0.93	0.96	**0.993**
Barley Std Error	6.9	5.2	6.2	4.8	4.6	**4.2**
Avg Counts	99.9	43.4	26.7	21.6	35.0	--
Avg % Mass	55.2	18.9	9.8	7.7	8.5	--
Std Deviation	37.4	18.2	12.9	12.8	18.4	--
Dup Std Deviation	3.5	3.3	1.3	0.8	0.5	--
Avg Std Error	6.0	4.6	6.2	4.8	4.7	--
IoB Repeatablility	3.49	2.53	1.75	1.70*	--	**--**
IoB Reproducibility	9.12	8.79	2.84	2.34*	--	**--**

* Actually the total error for the 2.2 to 2.0 and <2.0 groups

The first two rows of Table 7.3 record the correlations (r) and standard errors between the screening assortments and the screening equivalents for barley. Table 7.3 has an extra column at the right containing the averaged results for all groups less than 2.5 mm. This was done to replicate the common commercial shortcut of using only a single 2.5 mm screen to determine kernel “plumpness”.

The most obvious change from Tables 7.1 and 7.2 is the large increase in the errors. The average error across all the barley groups was 5.3. The 2.5 mm multi-group proved the most accurate grouping in Table 7.3 with a standard error of 4.2. The next three rows provide more details on the sample set analysed. The Average Count shows how many seeds were in the mean for each group. The Average Percent Mass indicates how much of the mass, across all of the samples, fell into each group. The Standard Deviation row

reveals the heterogeneity of the samples, which were specially selected to present a broad range of kernel sizes and shapes to the system for testing.

The Average Count reveals that across all groups there was an average of 226 “virtual” seeds in each sample set. As each virtual seed is composed of a narrow and a wide seed digitally “fused” together, this number compares well with the average total number of real seeds in this data set, which was 482. The low overall counts, as well as the even lower counts in some groups, such as the 2.2 to 2.0 mm group that on average only contained 22 seeds, raise concerns that the sample size may be too small. This issue was briefly considered in section 6.3.7.

The sampling issue directly relates to how accurately any method can estimate the proportions of different material in a mixture. It is obvious that the larger a sample size that can be used, the greater the potential accuracy of the system. The SeedCount system addresses this sample size issue by having a multi-tray mode in which the results of successive trayfuls of sub-samples from a particular grain lot are treated as a single analysis. The appropriate statistics are calculated and presented in its data file.

It can be claimed that the sub-sampling accuracy issue is not directly addressed by the screening data presented so far as it is simply reusing the same sample with different analytical techniques. This has been done deliberately to remove sub-sampling as a source of error. But Figure 2.4 illustrates how a few miscounts in a small data set have a much stronger affect on the final accuracy than the same number of miscounts would have in a larger data set. In such a case, increasing the sample size would reduce the errors. However, if the errors were systematic, increasing the sample size would also increase the number of errors and provide little or no improvement in accuracy. Comparing the duplicate DIA runs for each sample should either improve SeedCount’s accuracy or reveal the presence of systematic errors.

7.3.2.3 Screening Equivalent Effectiveness

The Duplicate Standard Deviation row of Table 7.3 indicates how effectively SeedCount reproduces data using the same kernels. The data shows that there can be considerable differences from one run to the next even using the same material in the trays. This is especially the case for material with thicknesses greater than 2.5 mm as they have a deviation of over 3. The following row shows the result of combining the duplicate runs and comparing that with the mechanical screening assortments to yield the Average Standard Errors. This row of Table 7.3 reveals little, if any, improvement over the comparisons based on individual runs. The author thinks that this is mainly due to faults in the tray design that make it difficult for larger kernels to seat correctly in the tray and to the resolution limitations of the 300 dpi images.

The final two rows of Table 7.3 show the results of IoB's assessment of their mechanical screenings. It can be seen that for the >2.8 and 2.8 to 2.5 mm groups, that the SeedCount accuracy is less than the intra-lab repeatability where the same person is using the same equipment to get the results. However, SeedCount is more accurate than mechanical screening for these groups when the result across different labs (reproducibility) is considered. This suggests that SeedCount screening equivalents may be commercially useful in the barley industry for determining the percentage of plump grains, at least until IoB and EBC tighten up their screen slot width tolerances.

The Wheat full-tray screening equivalents data (Table 7.4) contains two different groups to the barley data (Table 7.3). Creating the <1.6 mm group converted the <2.0 mm group of the barley table into the 2.0 to 1.6 mm and <1.6 mm groups. The less than 1.6 mm group is significant in the United States, where it is used to determine the simple screenings level, rather than the 2.0 mm screen used in Australia.

Table 7.4: Wheat Full-Tray Screening Equivalents

Slot Width (mm)	> 2.8	2.8 to 2.5	2.5 to 2.2	2.2 to 2.0	2.0 to 1.6	<1.6
Wheat Correlation	0.89	0.86	0.79	0.97	0.97	0.38
Wheat Std Error	12.1	6.8	7.8	1.6	1.1	0.4
Avg Counts	144.9	111.5	83.3	19.1	10.1	1.3
Avg % Mass	47.2	30.3	17.8	3.3	1.3	0.1
Std Deviation	27.1	13.5	14.8	5.2	2.3	0.2
Dup Std Deviation	2.5	2.8	1.8	0.7	0.3	0.1
Avg Std Error	12.1	6.5	7.9	1.3	0.9	0.4
AWB Std Error					0.38*	

* Tolerance for the “Less than 2.00 mm” groups combined together

Apart from these different groups, the Table layout is the same as Table 7.3. The results are quite similar to barley. Wheat fared somewhat better with an average standard error of 5.0. Despite the lower errors, the correlations were often somewhat lower. This is especially the case for the less than 1.6 mm group that had a correlation with the mechanical screenings of only 0.38. This is almost certainly due to the extremely small numbers of kernels in this group, which averaged only 1.3 seeds.

Despite the problems of the small <1.6 mm group, the general trend is for a much larger sample count, with an average total count of 370 virtual seeds out an average of 706 real seeds. The virtual kernel overshoot of the 50% real seed level is due to the matching system used by SeedCount that allows some kernels to be used more than once.

The final row of Table 7.4 lists the AWB tolerance for their 2.00 mm screenings at 0.38%. The thesis data was revisited to allow a direct comparison with the <2.00 mm AWB value, and produced a standard error of the estimate of 1.2%. Though SeedCount’s level of accuracy for this property is encouraging, it does not currently meet AWB’s requirements.

Despite larger counts in the thicker kernel groups, they tend to have the lower correlations with mechanical screenings. Suggestions as to why this may be so are presented below.

The duplicate Standard Deviations are generally somewhat lower than they were for the barley. This suggests that the wheat tray may be more effective at orientating the kernels correctly, or that the more uniform shape of the wheat is easier to analyse than the barley.

The standard errors based on the averaged duplicate values show little difference from the individually compared values. This, combined with the relatively small duplicate standard errors, suggests that the screening equivalent problems are systematic, and frequently cultivar based. A cultivar that analyses poorly once will tend to produce similar, though still poor, results on refilling the tray and being analysed again. One extreme example would be the wheat sample AM41, which had a mechanical screening >2.8 mm of 34.7% by mass. On the first DIA analysis, it was given a value of 14.8%. The replicate value was 17.4%. Possible reasons for this will be discussed below.

The most accurate gradings are for the most critical screens from a commercial perspective (the sum of < 2.5 mm grades for barley and the < 2.0 mm for wheat). The estimates are all highly significant ($p < 0.01$) and will be useful for breeders and others who need a quick estimate of the screening assortment.

Although the correlations are all highly significant, indicating that there is a real and substantial link between the DIA screening equivalents and the mechanical screening assortments, the standard errors for the screening equivalents were, at this stage of development, too large for them to have commercial applications. This is because commercial interests want their screening values to be reproducible within 0.5%. A standard error of 5, in this case equating to an error of 5%, is too inexact. A graphical representation of the accuracy for all groups bears out these concerns, as shown in Figures 7.7 and 7.8. Figure 7.7 shows all of the full-sample barley screening assortments and their respective screening equivalents overlaid on the same chart. Various marker

symbols are used to distinguish the different screening groups. Figure 7.8 illustrates the wheat screenings, treated in the same manner.

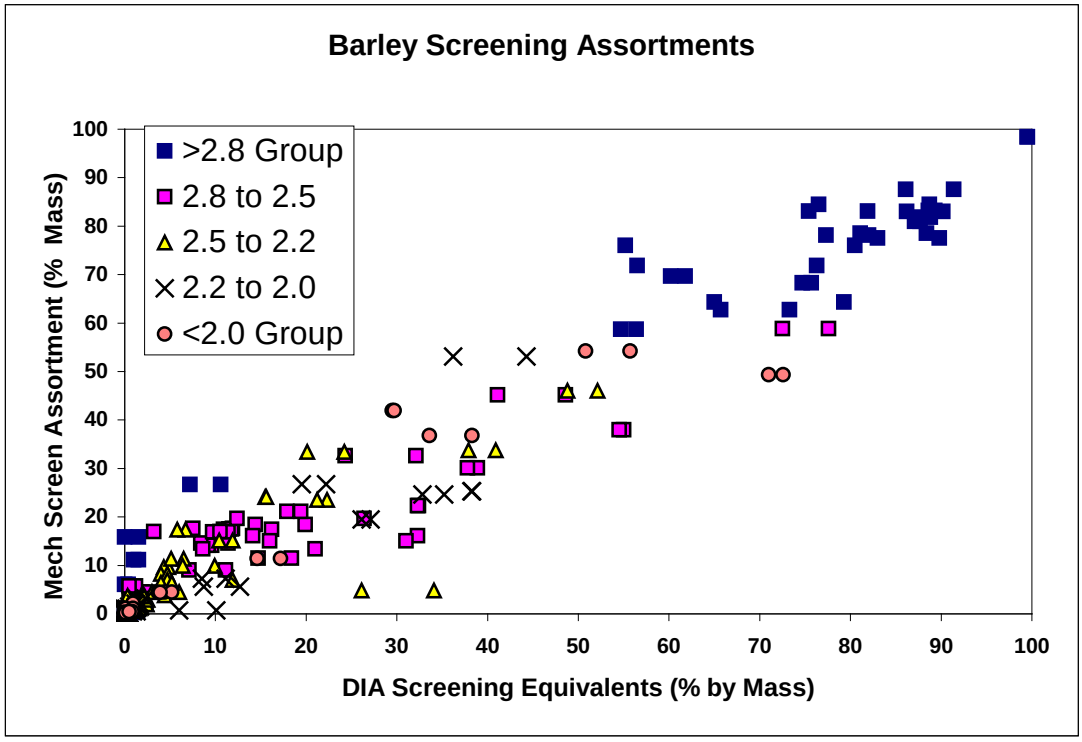

Figure 7.7 Barley DIA Screening Equivalents

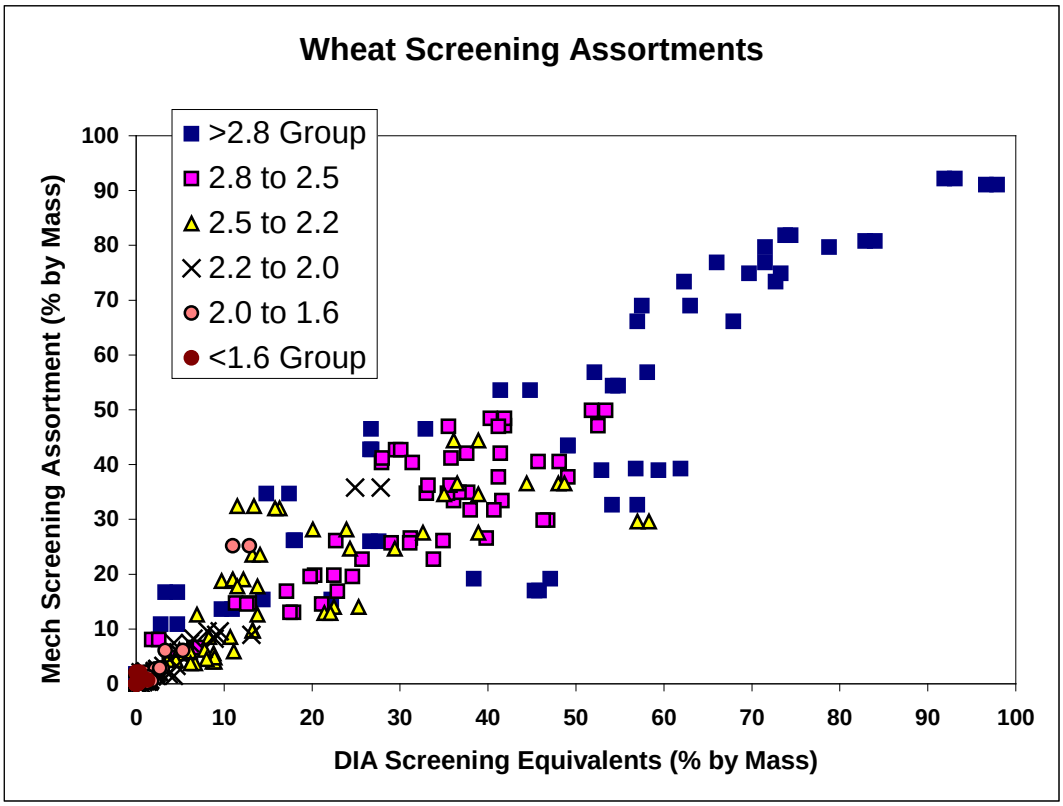

Figure 7.8 Wheat DIA Screening Equivalents

Figure 7.9 is a subset of Figure 7.8, showing only the groups containing eight percent or less by mass. Even at this scale the general match between the screening assortments and equivalents can be seen. This region is of particular concern to commercial wheat dealers as one of their critical quality criteria is that a maximum of 5% of the sample can be in the screening groups of less than 2.0 mm. These groups are marked with brown or orange spheres. It can be seen that of the 56 wheat tests, only 2 (replicates of the Kellelec sample KV2), fell close to the 5%. In one case (surrounded by a circle) SeedCount correctly scored the sample as having an excessive amount of under-thickness material. In the other case (surrounded by a rectangle), SeedCount let it slip through as containing only 3.3% under-size material.

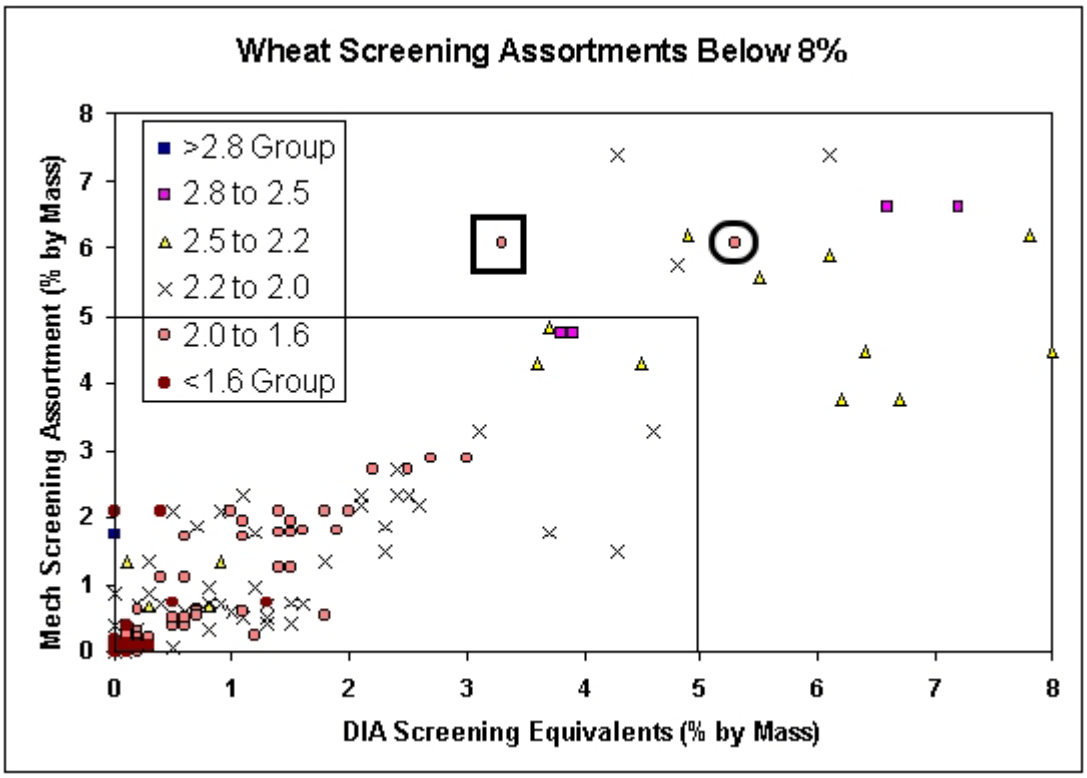

Figure 7.9 Wheat Screening Groups Containing 8% or Less of Sample

As pointed out with Figure 2.4, sampling issues are paramount when setting cut-off criteria that have serious financial ramifications for both the farmers and grain purchasers. The standard industry procedure requires a half-litre container full of grain to make the screening determination. The container holds approximately 10,000 kernels of wheat. This would allow a maximum accuracy of approximately 0.5%. In reality the accuracy is not this high, as blinding (blocking) of the screen and insufficient screening time can mean that part of the under-thickness material can often be retained on the screen. Alternatively, worn or inaccurate screens can allow excess material to pass through.

The largest errors generally occurred where many kernels in the sample had thicknesses near the breakpoint between two fractions. This can be seen with the help of Figure 7.10, taken from sample AM31. The figure shows the SeedCount assessed kernel thickness

placed into 0.1 mm wide bins. There are 213 values, with 39 counts in the largest bin of 3 to 3.1 mm.

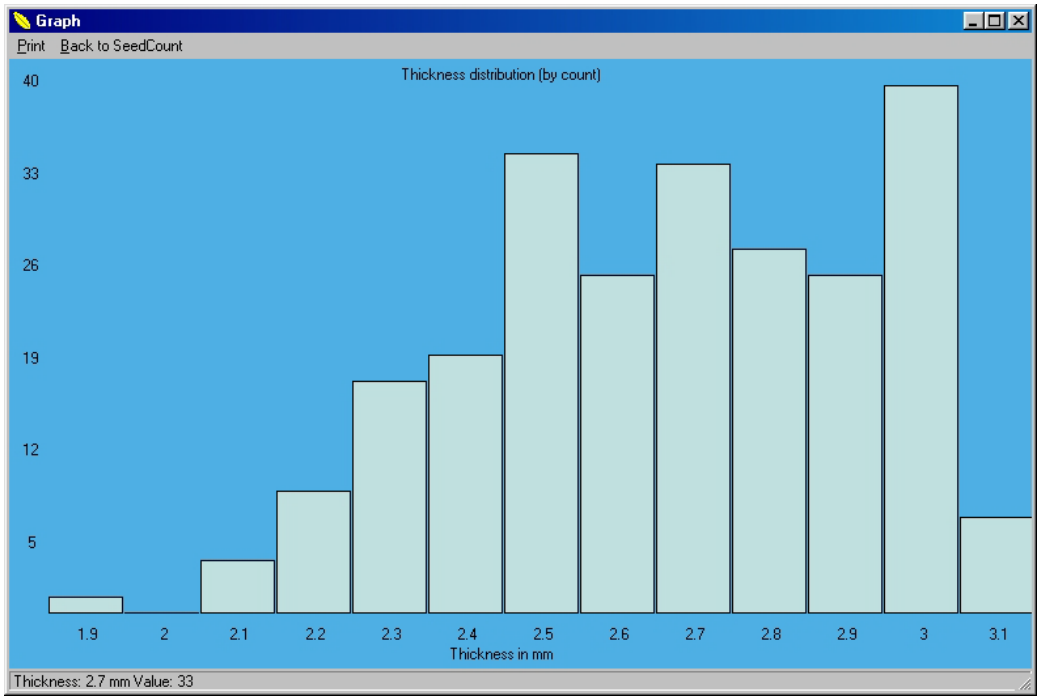

Figure 7.10 AM31 Thickness Distribution

Similarly there are 35 counts in the 2.5 mm bin and 19 in the 2.4 mm bin. Eleven of these 54 kernels are almost on the division line between the two groups. A small decrease of 0.03 mm in their thickness could push some seeds from the 2.5 mm bin into the 2.4 mm bin. This small change would have a dramatic effect on the Percent Mass in the 2.8 to 2.5 and the 2.5 to 2.2 mm groups (If a seed's thickness is greater than 2.50 mm it is counted in the 2.8 to 2.5 mm group). This has in fact happened in the duplicate run (not shown), and the 2.8 to 2.5 mm group dropped by 5% while the 2.5 to 2.2 mm group increased by this amount.

Figure 7.10 also illustrates the potential of DIA. SeedCount already has the capability of displaying its data in any format needed by the user. When the accuracy issues are resolved, thickness breakdowns in steps of 0.1 mm would be very useful for millers when assessing the most appropriate method of milling a grain lot. Indeed they could even use

this data to decide if they wish to purchase a particular grain lot with a broad range of kernel thicknesses.

7.3.2.4 Flour Yield Estimates

The DIA screening equivalents and milling extraction data were compared for 42 wheat samples sourced from Queensland and New South Wales. For clean wheat flour extraction, cultivars with a higher percentage of thick kernels showed a positive correlation with flour yield (eg: r = 0.54 for the 2.5 to 2.8 mm fraction, SEE = 5.96, $p < 0.001$, Figure 7.11). Cultivars with thinner kernels had a negative correlation with yield (eg: r = -0.59, SEE = 5.74 for the 2.0 to 2.2 mm fraction, Figure 7.12). The correlations were consistent, statistically significant and indicated a general trend towards higher flour yield with increasing kernel thickness.

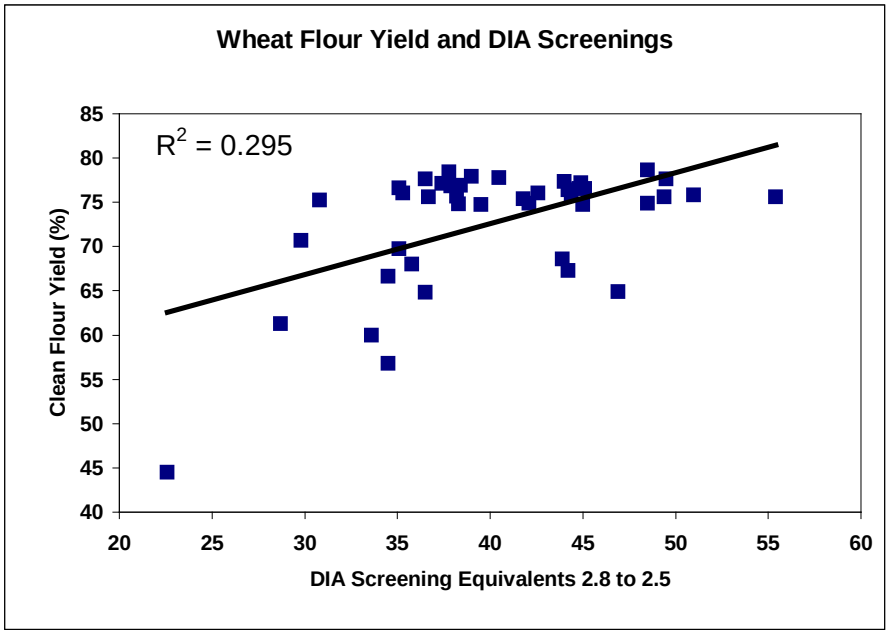

Figure 7.11 Flour Prediction by 2.8 to 2.5 mm Screening Equivalents

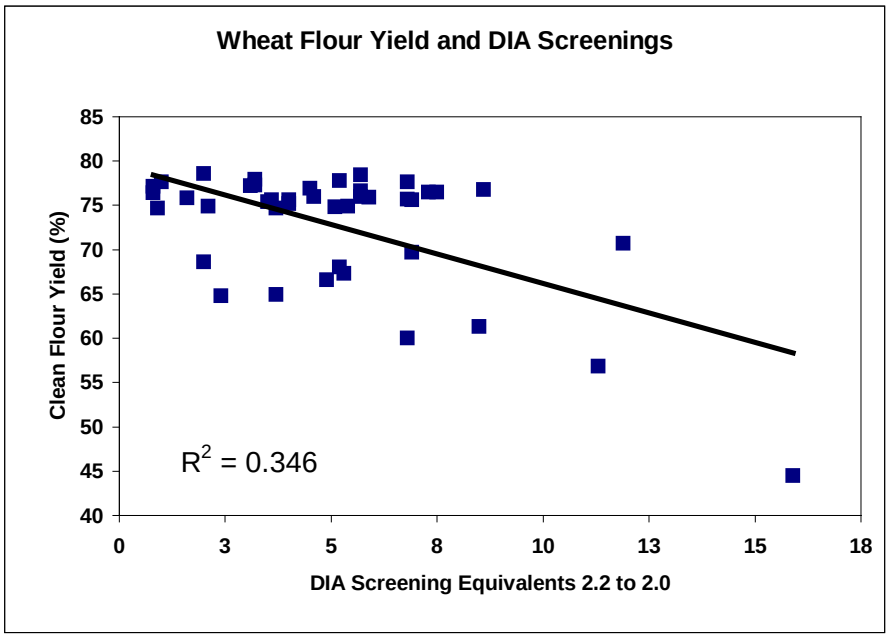

Figure 7.12 Flour Prediction by 2.2 to 2.0 mm Screening Equivalents

As with the roundness yield correlations (section 6.3.7.5), the screening equivalent correlations alone were not strong enough to produce commercially useful yield estimates. However, they may contribute to a more robust equation when used with other variables, as will be trialled below.

Recent work on milling efficiencies suggests that one of the most important properties controlling flour extraction rates is the separability of the endosperm and the bran (Mabille et al., 2003). The crease size and the bran thickness within the crease also influence the flour yield (Peyron et al., 2002). These factors may explain much of the deviations in the kernel size, roundness and screenings models used above.

A complicating factor in this data set was that the samples were cleaned as part of the standard quality testing and only the cleaned material was passed on for DIA imaging and analysis. This made it impossible to make meaningful correlations between the SeedCount hectolitre weights and dockage material and the standard tests for these properties.

7.3.2.5 Hot Water Extract Yield Estimates

Barley screening equivalents and soluble extract data were compared for 41 samples from Victoria, South Australia and Western Australia. The sample set was selected to cover an extensive hot water extract (HWE) range. Standard malting and mashing protocols (EBC, 2003) were followed.

Contrary to the usual prediction of higher malt extract yield from larger kernels, Edney, Bassily and Symons (1998) found "consistent and clear trends for smaller kernels to have higher malt extract and better modification (friability)." This trend was not found either. There were no significant correlations between the soluble hot-water malt extract and the barley screening equivalents in either direction, with the best correlation being r = 0.31 for the 2.5 to 2.8 mm group.

It is possible that the predicted additional endosperm available in the larger kernels may be poorly converted during malting due to their increased distance from the starch degrading enzymes, as suggested by Edney, Bassily and Symons (1998). This idea could be tested by seeing if complete conversion of the remaining starch was occurring during the mashing process. If conversion was complete, this idea would be eliminated as an explanation of the lack of correlation between the kernel size and available extract.

A malting barley prediction study by Garcia del Moral et al. (1998) also found that size and DIA parameters did not correlate well with HWE. They concluded that it was dependent on "1) grain physical composition, 2) grain enzymatic potential and 3) malting conditions". As 2 and 3 are not visible parameters, one should perhaps not be surprised that DIA properties were not highly correlated with HWE.

The only strong correlation with malt extract in this study was a negative correlation with protein (r=-0.67). If larger kernels contained higher percentages of endosperm as suggested, one would expect this to be confirmed by a negative correlation between kernel thickness and protein. Conversely, if protein was positively correlated with

increasing kernel thickness, it was possible that the extra protein would offset the expected increase in endosperm in the larger kernels.

Protein was not positively correlated with the screening equivalents, so it was not possible that there was a cross-elimination of screening equivalents correlations occurring that removed the link with malt extract. Protein was not negatively correlated with the screening equivalents either. These results in combination suggest that the most likely reason there were no correlations between screening equivalents, protein and soluble extract is that the percentage of endosperm in the kernels was quite stable across the entire sample set. If this is so, it suggests that either the assumption that the kernel husk and bran layers in barley have a constant thickness is not correct or that as the kernels get larger there is a higher proportion of protein deposited in the endosperm. Both of these propositions need testing.

7.3.2.6 Screening Time Considerations

As discussed in section 2.6.6.1, it is not unreasonable to require a one-minute shaking period per screen in a screen array for samples of 100 grams or less. One minute per screen is less than what is required by the EBC (2003) and more than what is required by the VicGrain (1999) methods.

As the screening assortments used in this thesis have four screens for the barley and five screens for the wheat, this equates to four and five minutes respectively for the mechanical screenings. It is assumed that the weighing and other associated tasks can be performed while the next sample is being screened, so the total time per assortment does not increase.

SeedCount can produce its wheat screening equivalent in approximately one and a half minutes each in an equivalent multi-sample situation where one sample is being weighed out and distributed in the tray while the previous sample is running. Barley takes about two and a half minutes per sample. This amounts to a seventy percent reduction in time for wheat and a 37 percent time reduction for barley.

If only a single screen split is required, SeedCount does not represent a saving in time unless one considers that a number of other tests are also conducted on the sample in the same analysis.

7.3.3 Multi-Variable Flour Yield Prediction

There was a possibility that the DIA derived values could be combined with other grain properties to produce a yield prediction formula of commercial significance. A successful search for other useful properties was undertaken.

7.3.3.1 Mini Hectoliter Weight

One property with a high correlation with flour yield was essentially a by-product of sample preparation for the DIA system: SeedCount's mini-Hectoliter Weight (MHW, r = 0.82). The mini SeedCount HW method used for these samples employed a 30.7 ml sample cup. The DIA grain sampling followed the standard SeedCount method as shown in section 3.4.2 (Armstrong, Armstrong and Weiss, 2004). As the volume was known and the sample mass was measured for each test, the data was used to calculate a Mini-Test Weight. Though this was not an intrinsic DIA test, it proved to correlate well with standard test weights (data not shown). As the volume was only incrementally smaller than Harris and Sibbitt's system (1942), this correlation was not an unexpected outcome.

7.3.3.2 Other Properties of Interest

Several other properties that are measured as parts of standard wheat quality assessments were identified as being strongly correlated with wheat flour yield. These tests were the standard 500 ml chondrometer weight (CW, r = 0.72), Dockage (r = -0.84, Comprised of Screenings Overtail (OT - large pieces of dockage material that will not pass through the screens) and Thrus (small dockage material that passes through a 2.0 mm slotted screen—usually referred to in this thesis as the <2.0 mm screening group)) and kernel hardness (r = 0.80). Standard chondrometer, screening, hardness and test milling (Allied Mills, 2003; Vicgrain, 1999) protocols were followed.

7.3.3.3 Flour Yield Equations

As noted above, Hardness and CW proved to be strongly and positively correlated to higher flour extractions. It appears that the denser, harder kernels allow more complete separation of the bran from the endosperm and better conversion of the endosperm into flour (Dines, 2001; Peyron et al., 2002).

MHW and CW are both strongly correlated with the clean wheat flour yield. Though the data is not shown, CW has an even higher correlation with the "dirty" wheat yield (r = 0.81), which is not surprising as CW was determined on the dirty wheat samples. Samples containing large amounts of unmillable low-density thrus and overtails would necessarily produce a lower yield of flour.

Wheat protein levels did not correlate highly with flour yield for this data set (r = -0.39). It was expected on the basis of the literature that protein and yield would have a strong negative correlation. It is possible that this sample set is too small and somewhat different correlations may have emerged from a larger sample set.

A multivariate approach to the prediction of milling yield resulted in an equation combining the effects of HW, Roundness, 2.0 to 2.2 mm Screenings, Hardness (in PSI), CW and Screening OT. Aspect ratios were removed from inclusion in the equation due to their co-linearity with Roundness, while the 2.8 to 2.5 mm screening equivalents were removed because of their interrelationship with the 2.2 to 2.0 mm screening equivalents. The Dirty Wheat Flour Extract was chosen as a target rather than the clean wheat as this made the formula directly applicable to assessing the milling quality of the bulk grain in the condition it arrives at a receivals point. Due to a number of missing "Thrus" values in the standard screenings data, it was decided to substitute the screening overtails for the total dockage data. The formula was:

Estimated Dirty Wheat flour Extract = -2.819 + 0.2092* MHW + 1.793 * Roundness – 0.4009 * Screening + 0.6252 * CW + 0.1044 * Hardness – 0.592 * Screening OT

The effectiveness of the prediction is shown in Figure 7.13, where it can be seen that this equation had an excellent correlation (R^2 = 0.91) and reasonable standard error (SEE = 2.9).

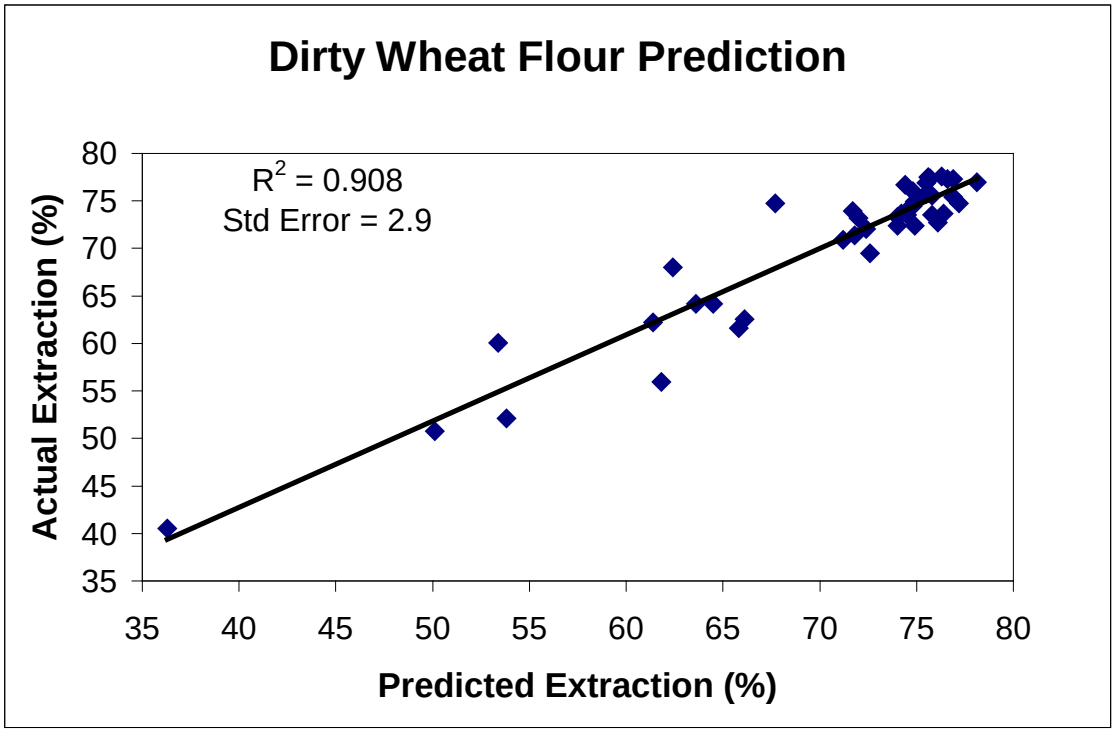

Figure 7.13 Multivariate Estimate of Wheat Flour Extraction

As well as the author's equation above, another formula was derived in conjunction with John Dines and presented at the 2003 Barley Technical Symposium/Cereal Chemistry Division conference (Dines and Armstrong, 2003). In this case the variables were selected automatically, based on a Best Subsets Regression against "Dirty Wheat Extraction" using the Minitab Statistical Package. The resultant multiple regression equation had an R^2 value of 0.917. The equation was:

Dirty Wheat Extraction = 29.6 + 21.1 * Roundness - 0.350 * (2.0 - 2.2 Screening) - 1.18 * Protein Leco + 0.474 * CW + 0.116 * Hardness + 0.376 * Dockage - 0.567 * Thrus

The essential differences in the two equations were the substitution of the MHW and the Screening Overtails with the Protein content (determined with a Leco instrument) and the DIA Dockage Percent. The latter equation has a somewhat poorer Standard Error than the former equation (SEE = 3.2 vs 2.9), though the difference is small.

As mentioned above, the wheat samples were mechanically cleaned during the Allied Mills tests and therefore before the SeedCount DIA testing. This resulted in some differences between the two data sets that would not normally be seen, especially in the Mini-Hectoliter Weight vs Chondrometer Weight and dockage Pct vs Screenings OT and Screening Thrus measurements.

Neither hardness nor chondrometer weight showed strong correlations with screening equivalents. But both hardness and CW proved to be strongly positively correlated with higher flour extractions and each other. It appears that the denser and harder kernels allow more complete separation of the bran from the endosperm and better conversion of the endosperm into flour. As these correlations were stronger than those between yield and screening equivalents, it may well be that kernel hardness is more important to flour yield than kernel size.

Barley remains uncorrelated to the DIA determined values and no attempts were made to develop an equation to link these apparently unrelated properties.

7.4 Conclusions

The “flat-edge” indented tray based DIA system can be used to assess kernel thickness. The thickness can be combined with other DIA data to estimate the kernel mass and the bulk screening assortment. As well as these values, SeedCount can assess the thousand-corn weight, as reported previously (Armstrong et al., 2001), and the sample’s Hectoliter Weight, cross-sectional area and dockage levels. Some of these values, coupled with the sample’s hardness, can be used to make predictions of the flour yield of wheat. A more extensive data set, including information on crease size and bran separability would make the equation more robust.

No robust hot-water extract-DIA equations for malting barley could be developed.

Neither wheat nor barley showed strong evidence of higher proportions of endosperm in larger kernels.

Further software and hardware development allowing the use of higher resolution scans and larger sample sizes will make DIA systems more accurate.

8 Conclusions, Implications and Recommendations

8.1 Conclusions and Implications

In section 3.2, seven research questions were proposed, based largely on perceived faults in current DIA systems and in part on the perceived potential of DIA. In this chapter a summary of the research results will be presented, structured around those questions. This summary will be followed by other conclusions and recommendations for further research.

1. **Can a reliable and useful grain image capture system be made from commonly available digital imaging equipment, thereby reducing the hardware cost of the systems?**

This project has demonstrated beyond any doubt that commonly available digital imaging equipment can be used to build a reliable and useful grain image capture system at a reasonable cost. In this case, it was shown that modern high-speed USB-2 based flatbed scanners can be used to capture high quality digital grain images. Although by the end of the project it was found necessary to make modifications to the scanners and to mount them in a cabinet, the cost of this work was minor. Compared with the cost of a high-resolution video camera, an illumination cabinet with a light level control system and a frame grabber, the scanner-based system is both remarkably capable and inexpensive.

The most expensive portion of the hardware to develop was the indented trays. The cost per tray will drop dramatically as the number of trays produced from each master increases. As the tray system developed for this project could be modified for use with many grain DIA systems, its development cost is not specifically counted against this project.

2. **Can a Digital Image Analysis (DIA) system be developed that can accurately count touching clusters of grain, thus allowing the creation of a DIA thousand kernel weight method?**

Hand-counting assisted by simple indented seed trays is the most accurate counting method for performing TKWs. Electromechanical counters provided acceptable accuracy at a slow counting rate.

The results shown in Section 5.3.4 clearly demonstrate that a DIA system can be, and has been, developed that can accurately count touching kernels of grain. The counting accuracy is achieved with a novel patented counting algorithm developed by this author and Marvin Weiss. The method works so well that it rivals the accuracy of hand-counting under the best conditions and is superior to the electromechanical counters tested in the thesis and to other DIA KWs reported in the literature. This counting accuracy results in very accurate TKWs.

3. **Can a DIA system be developed that can directly measure the thickness of the kernels?**

In section 6.3.4 it was demonstrated that the novel bi-modal indented trays are able to hold wheat and barley kernels “on-edge” and allow direct measurement of the kernel thickness. It was shown that this process works on a wide range of cultivars and allows thickness to be measured to an average accuracy of 0.11 mm. Increasing the image resolution and modifications to the tray design would further improve this accuracy.

The kernel thickness is essential for measuring kernel roundness and finding screening equivalents.

4. **Can a DIA system be used to generate an estimate of the three-dimensional roundness of grain?**

The author proposed a novel definition of 3D roundness. The key to producing these three-dimensional roundness values by DIA was being able to measure the kernel thickness. Section 6.3.5 demonstrated that this is now possible and the novel value can be easily measured by DIA. Individual seed roundness correlations between the DIA and calliper derived values were r = 0.91, std error of 0.02 for barley and r = 0.81, std error of 0.03 for wheat, indicating that the DIA roundness figures are strongly correlated with the calliper measurements. It was also demonstrated that the DIA measurements held their accuracy across a wide range of cultivars and when applied via a "virtual kernel" matching system.

The roundness values can be used to distinguish wheat from barley for both the calibration and validation sample sets.

5. **Can a DIA system be developed that can estimate the kernel mass and thus the screening equivalents by percent mass of a grain sample?**

Kernel mass estimates determined by DIA (section 7.3.1) were highly correlated with the actual mass on a kernel-by-kernel basis. This correlation held across a broad range of cultivars for both wheat (R^2= 0.965) and barley (also R^2 = 0.965). The principle was extended to the 'virtual kernel' matching method and again demonstrated that the kernel mass could be estimated accurately on a cultivar-independent basis (section 7.3.1.3).

SeedCount was able to predict screening groups by mass. The screening equivalent accuracy is given in Tables 7.3 and 7.4. The SEE for various barley groups varied from 6.9% for the >2.8 mm group to 4.2% for the <2.5 mm groups. It was also demonstrated that SeedCount screening equivalents are more reliable than IoB inter-lab mechanical screening tests for determining the percentage of plump kernels greater than 2.5 mm.

The wheat SEEs varied from 12.1% for the >2.8 mm group to 0.4% for the <1.6 mm group. The SEE for the <2.0 mm groups was 1.1 percent, which was greater than AWB's maximum allowable error of 0.38% for these groups. SeedCount's accuracy may be sufficient for wheat breeding assessments, but it is not accurate enough for commercial use.

6. **Can the DIA values of barley be used to predict the hot water extract of its malt or the DIA values of wheat predict its flour extraction rate?**

Barley:

No significant correlation was found between kernel weight (r = -0.15) or aspect ratios (r=-0.13) and soluble hot water malt extract for the Joe White Maltings barley samples. This study (Figure 6.22) found no significant correlations between malted barley soluble hot-water extract and roundness (r = -0.21). Nor were there any significant correlations between the hot-water extract and the barley screening equivalents, with the best correlation being r = 0.31 for the 2.5 to 2.8 mm group.

The DIA values were not able to predict the hot water extract of malted barley from the initial barley with any confidence. This finding was similar to that of Garcia del Moral et al. (1998) and may be due to overriding quality factors such as the 'maltability' and residual enzyme activity of the various barleys used.

Wheat:

Significant correlations were found between cultivar kernel weights and flour yield (r = 0.42, p<0.01, SEE = 6.4), and between aspect ratios and flour yield (r = 0.56, p < 0.001, SEE = 5.9) for the Allied Mills wheat sample set. Wheat flour extraction was also positively correlated to roundness (r = 0.60, p < 0.001, SEE 6.7) and screenings equivalents for cultivars with a higher percentage of thick kernels (eg: r = 0.54 for the 2.5 to 2.8 mm fraction, SEE = 5.96, p < 0.001, see Figure 7.12). However, the high standard error of these estimates indicated that the correlations alone would not be able to predict the flour yield with sufficient accuracy for commercial use. The DIA correlations were combined with the results of standard tests such as the 500 ml chondrometer weight,

dockage and kernel hardness to produce the milling yield prediction shown in Figure 7.14, where it can be seen that this equation had an excellent correlation ($R^2 = 0.91$) and reasonable standard error (SEE = 2.9).

7. **Can software and a method of grain presentation be developed that are both quick and easy to use?**

The final method of grain presentation used in SeedCount using the bimodal indented tray is reasonably quick and easy to perform.

Table 8.1 summarises the timesavings involved in the use of the SeedCount system compared with the manual methods it replaces. The time required for SeedCount for each test is simultaneous, while it is cumulative for the manual tests. It shows that SeedCount can do 673/60 = 11.2 hours of work in one minute. This represents an enormous saving in time and/or increase in information on each sample tested.

Table 8.1 Comparison of Cereal Analysis Time (Minutes)

Test	Kernel Weight	Orthogonal	Area	Mass	Screenings	Total
Manual	12.5	282	282	93	4	**673.5**
SeedCount	1	1	1	1	1	**1**

The software has been developed to be easy to use and provides many of the major output results directly on-screen. The system is designed to trap data input errors and maximise output clarity and comprehensiveness with minimal input from the operator. Verbal feedback from a number of beta testers of the system has confirmed that they find the software easy to use, but no formal study of this has been undertaken. Details of the method of use can be seen in the SeedCount Users Manual and experienced in the demonstration version of the software in the attached CD. Most users also find the trays easy to fill, but a few users report that manually filling the trays is tedious.

8.1.1 Other Conclusions

The “wide-edge” indented-tray DIA system can assess kernel length, width, thickness and area, though with some loss of accuracy (+/- 0.01 mm for callipers, +/- 0.11 mm for DIA.). This data can be used to generate aspect ratio, ovality, roundness and aspect ratio/roundness values. Though none of these values will allow definitive cultivar identification, they can help discriminate kernel types and sometimes even determine general cultivar families within a type.

Average wide kernel area showed a significant negative correlation with wheat protein by Leco ($r = -0.64$), a result predicted by the theoretical shape (ie, a higher endosperm content in larger kernels necessarily means a lower protein content as noted in section 6.1.6.1.) Unexpectedly, this link did not lead to a significant correlation between kernel area and flour yield, which was only $r = 0.11$. Barley average area proved to have no significant correlations with either protein ($r = -0.19$) or extract ($r = -0.05$).

“Three-dimensional Virtual Seeds” can be used to predict average values across a full sample. However they cannot be relied on to accurately match individual kernels when the kernels are used sequentially in both sections of the tray.

Further software and hardware development allowing the use of higher resolution scans and larger sample sizes will make DIA systems even more accurate, and therefore more useful for commercial quality assessments.

8.2 Recommendations

It is possible that the predicted additional endosperm available in the larger barley kernels may be poorly converted during malting due to its increased distance from the starch degrading enzymes, as suggested by Edney, Bassily and Symons (1998). Determining if complete conversion of the remaining starch was occurring during the mashing process could test this idea.

Trial higher resolution scans to see how effective they would be at increasing the accuracy of orthogonal measurements and screening equivalents.

Test the effect of the lateral position of the kernels in the trays to see if this alters their DIA measurements.

Explore the utility of the “thinness” equation used by Majumdar & Jayas (2000a) in discriminating kernel types.

Develop a more universal kernel type discrimination system.

Attempt more complex discriminations such as blackpoint and vitreousness using colour and textural properties.

Improve the bi-model tray indent shapes so they can hold the kernels more precisely.

Expand the range of grains that can be analysed with the SeedCount system.

9 Appendices

9.1 Appendix A: Scion Image Macros

Barley Macro (Barleycount.txt)

```
{Cereal counting example. Has been adapted for counting Barley.
The macro will probably need to be customized for other images.
Parameters that may need to be changed include number of Erode
iterations and min and max measurements.
Ensure that Analysis/Options Max measurements has been set to at least
2000 before using macro.
Designed for use with 256 shade grayscale images using 100 DPI
resolution}

var
     Area,Major,Minor:real;

Macro 'Kernel Sizes Only [S]'

begin
  Open('');
  Invert;
  SetThreshold(40);
  Wait(4);
 SetThreshold(60);
  Wait(4);
 SetThreshold(50);
  Wait(4);
  MakeBinary;
  SetBinaryCount(1);
     SetPrecision(1);
    SetParticleSize(90,450);
    SetOptions('Area,Major,Minor');
    SetScale(3.937, 'mm');
   AnalyzeParticles('reset,include,label');
   {Label added to help debug selection process}
  ShowResults;
       end;

Macro 'Count Kernels Only [C]'
{procedure CountKernels;}

begin
 {Open('C:\Program Files\Scion Image\Images\wheat2.tif');
   Wait(1);}
  Invert;
  { AutoThreshold;
     GetThresholds(lower,upper);
end}
SetOption;
Sharpen;
Convolve('C:\Program Files\Scion Image\Kernels\Ba17x17.txt');
```

```
 SetThreshold(35);
 { Wait(1);}
  MakeBinary;
  SetBinaryCount(1);
    Erode;
 {Wait(2);}
    Erode;
{ Wait(2);}
 SetBinaryCount(4);
    Erode;
     SetPrecision(1);
    SetParticleSize(10,10000);
    SetOptions('Area');
    SetScale(0,'pixel');
   AnalyzeParticles('reset');
     ShowResults;
  end;

Macro 'Kernel Sizes and Count [S]'

begin
   Open('');
  Invert;
     Duplicate('Temp.tif');
      SetThreshold(50);
  { MakeBinary;
  SetBinaryCount(1);
     SetPrecision(1);}
    SetParticleSize(90,450);
    SetOptions('Area,Major,Minor');
    SetScale(3.937, 'mm');
   AnalyzeParticles('reset,include');
   ShowResults;
     Export('');
       Dispose;
       Dispose;
Wait(4);
  Open('Temp.tif');
 SetThreshold(60);
 { Wait(4);}
  MakeBinary;
  SetBinaryCount(1);
    Erode;
    Erode;
    {Erode;}
       SetParticleSize(1,1000);
    SetOptions('Area');
    SetScale(0,'pixel');
   AnalyzeParticles('reset');
     ShowResults;
  end;
```

Wheat Macro (Wheatcount.txt)

```
{Cereal counting example. Has been adapted for counting Wheat.
The macro will probably need to be customized for other images.
Parameters that may need to be changed include number of Erode
iterations and min and max measurements.
Ensure that Analysis, Options, Max measurements is set to at least
2000.
Designed for use with 256 shade grayscale images using 100 DPI
resolution}

var
     Area,Major,Minor:real;

Macro 'Kernel Sizes Only [S]'

begin
  Open('');
  Invert;
  SetThreshold(40);
  Wait(4);
 SetThreshold(60);
  Wait(4);
 SetThreshold(50);
  Wait(4);
  MakeBinary;
  SetBinaryCount(1);
     SetPrecision(1);
    SetParticleSize(90,450);
    SetOptions('Area,Major,Minor');
    SetScale(3.937, 'mm');
   AnalyzeParticles('reset,include,label');
   {Label added to help debug selection process}
  ShowResults;
       end;

Macro 'Count Kernels Only [C]'
{procedure CountKernels;}

begin
 {Open('C:\Program Files\Scion Image\Images\wheat2.tif');
   Wait(1);}
  Invert;
  { AutoThreshold;
     GetThresholds(lower,upper);
end}
SetOption;
Sharpen;
Convolve('C:\Program Files\Scion Image\Kernels\Ba17x17.txt');
 SetThreshold(30);
 { Wait(1);}
  MakeBinary;
  SetBinaryCount(1);
    Erode;
SetBinaryCount(3);
    Erode;
```

```
  SetBinaryCount(4);
    Erode;
     SetPrecision(1);
    SetParticleSize(20,5000);
    SetOptions('Area');
    SetScale(0,'pixel');
   AnalyzeParticles('reset');
     ShowResults;
  end;

Macro 'Kernel Sizes and Count [S]'

begin
   Open('');
  Invert;
     Duplicate('Temp.tif');
      SetThreshold(50);
  { MakeBinary;
  SetBinaryCount(1);
     SetPrecision(1);}
    SetParticleSize(90,450);
    SetOptions('Area,Major,Minor');
    SetScale(3.937, 'mm');
   AnalyzeParticles('reset,include');
   ShowResults;
     Export('');
       Dispose;
       Dispose;
Wait(4);
  Open('Temp.tif');
 SetThreshold(60);
 { Wait(4);}
  MakeBinary;
  SetBinaryCount(1);
    Erode;
    Erode;
    {Erode;}
       SetParticleSize(1,1000);
    SetOptions('Area');
    SetScale(0,'pixel');
   AnalyzeParticles('reset');
     ShowResults;
  end;
```

9.2 Appendix B: SeedCount Demonstration Version CD

The CD also contains copies of the CCD presentations, sample images, the SeedCount Manual and Tutorial

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