Application Note SC003: SeedCount SC3 Digital Image Analysis System Evaluation Report on Barley

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Background

The SeedCount Digital Image Analysis System uses scanner and software technology to predict a range of physical measurement quality characteristics on a subsample of grain. An earlier version of SeedCount was previously evaluated by this laboratory in 2003 for a number of barley grain quality parameters:- test weight, kernel weight, grain plumpness, barley germ end staining (blacktip), dockage (broken fractions of pearled grain) and correlation to Perten Single Kernel Characterisation System (SKCS) parameters. The investigation highlighted both encouraging results for some parameters while it was suggested others might require further development. The current evaluation was on the latest version, SeedCount SC3 (*software version 2.2.2), which features an updated scanner and software modules in addition to new seed-trays (currently available for wheat, barley and rice) incorporating colour correction calibration strips, designed to enable SeedCount to standardise the colour and brightness of every scan image. As SeedCount has a small sample requirement for analysis and has non-destructive testing, it has the potential for use as a quality assessment tool in plant breeding applications.

* Note – prior to evaluation the SeedCount Australian agent provided an updated seedcount.dat file which overcame an image cropping error in the region of the calibration strips.

Parameters Evaluated

SeedCount SC3 was evaluated on barley grain for SeedCount parameters kernel weight, test weight, screening distribution and blacktip (germ end kernel discolouration).

Samples

24 samples from a 2005 season DAFWA agronomy trial were used to evaluate SeedCount predictions for parameters test weight, kernel weight and screening distribution. Reference values for test weight and kernel weight were supplied by the agronomy program. Screenings values were determined manually by mechanical means using an industry standard sieving machine as per EBC/IOB specifications.

A sample expressing strong blacktip was selected from DAFWA barley archival samples and used for evaluating SeedCount blacktip estimation and repeatability. In addition two other samples, one sourced from a former DAFWA kernel discolouration project and one provided by WA grain handler CBH Ltd, both expressing strong blacktip symptoms, were used to create a set of blacktip standards over the range 2% - 100% blacktip. The blacktip samples were screened over a 2.2mm sieve and those retained above the screen and expressing strong blacktip were individually selected and combined in order to accumulate sufficient blacktip sample. These were then proportionately combined with a similarly screened clean bright barley sample provided by Joe White Maltings and made up to a total number of 500 grains for each standard over the range. Also, two samples expressing the globe (rounded) grain phenotype were selected from a UWA barley introductions trial and used to compare sievings distribution and test weight with SeedCount values for this morphologically unique grain shape.

Evaluation methodology

The 28.89ml sample cup (28.89ml and 25.90ml supplied) was used for loading samples onto the tray as it provided optimum tray coverage with the test samples. All samples were analysed in single-tray mode. Samples were loaded as per SeedCount manual instructions with doubles being separated into individual wells and grains in both sections pressed down to lay horizontally. Manual tray arrangement times varied depending on the sample and level of contamination but in general took between 1-5 minutes per tray. The agronomy set samples were tested as delivered without removal of dockage and broken grains to test the discrimination of the SeedCount system. Blacktip assessment samples were manually selected as whole samples and in some scans, samples lying ventral side up in the width section of the tray were manually turned to face dorsal side up to critically evaluate the SeedCount blacktip algorithm. Globe shaped samples were also tested with whole grains only. Some scans were analysed with less grain than would fill the sample cup in order to determine the minimum sample size required to return SeedCount prediction results.

Results

Kernel Weight

Table 1. Grain weight comparison.

SampleID	GWT db	SeedCount TKW_Dry
05NO11 NO_1-1001	42.5	42.0
05NO11 NO_1-1002	35.4	34.0
05NO11 NO_1-1003	40.4	40.0
05NO11 NO_1-1004	40.9	41.0
05NO11 NO_1-1005	38.7	39.0
05NO11 NO_1-1006	38.9	40.0
05NO11 NO_1-1007	36.5	36.0
05NO11 NO_1-1008	36.7	37.0
05NO11 NO_1-1009	37.8	37.0
05NO11 NO_1-1010	40.4	42.0
05NO11 NO_1-1011	40.5	40.0
05NO11 NO_1-1012	40.2	39.0
05NO11 NO_1-1013	38.2	39.0
05NO11 NO_1-1014	32.6	32.0
05NO11 NO_1-1015	37.6	38.0
05NO11 NO_1-1016	38.9	39.0
05NO11 NO_1-1017	37.2	37.0
05NO11 NO_1-1018	37.3	39.0
05NO11 NO_1-1019	39.4	38.0
05NO11 NO_1-1020	35.1	36.0
05NO11 NO_1-1021	38.2	38.0
05NO11 NO_1-1022	39.8	40.0
05NO11 NO_1-1023	40.3	41.0
05NO11 NO_1-1024	38.4	40.0

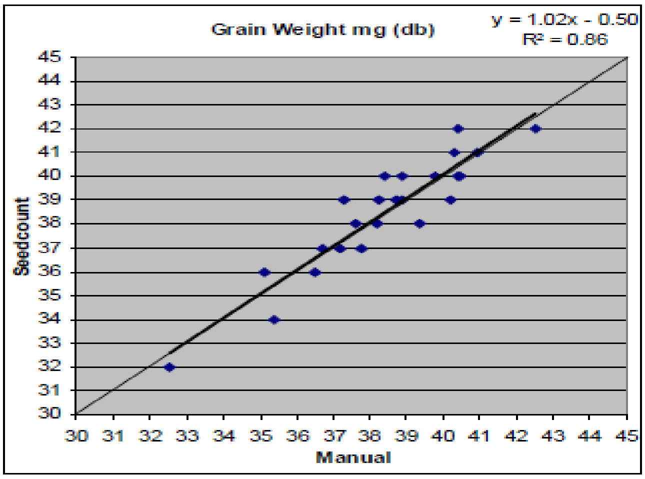


Figure 1. Grain weight comparison.

Test Weight

Table 2. Test weight comparison.

SampleID	HWT kg/hL	SeedCount Mini TW
05NO11 NO_1-1001	73.7	73.3
05NO11 NO_1-1002	69.5	69.6
05NO11 NO_1-1003	74.0	73.8
05NO11 NO_1-1004	74.2	72.6
05NO11 NO_1-1005	72.2	71.1
05NO11 NO_1-1006	72.6	71.4
05NO11 NO_1-1007	71.5	70.8
05NO11 NO_1-1008	72.5	70.0
05NO11 NO_1-1009	71.9	70.2
05NO11 NO_1-1010	71.9	71.4
05NO11 NO_1-1011	74.5	72.9
05NO11 NO_1-1012	73.9	72.3
05NO11 NO_1-1013	73.1	71.2
05NO11 NO_1-1014	69.1	68.7
05NO11 NO_1-1015	71.8	71.8
05NO11 NO_1-1016	72.6	71.6
05NO11 NO_1-1017	71.8	72.0
05NO11 NO_1-1018	73.7	72.0
05NO11 NO_1-1019	74.0	71.9
05NO11 NO_1-1020	71.5	70.4
05NO11 NO_1-1021	73.5	71.1
05NO11 NO_1-1022	74.9	72.9
05NO11 NO_1-1023	74.3	71.7
05NO11 NO_1-1024	73.2	72.0

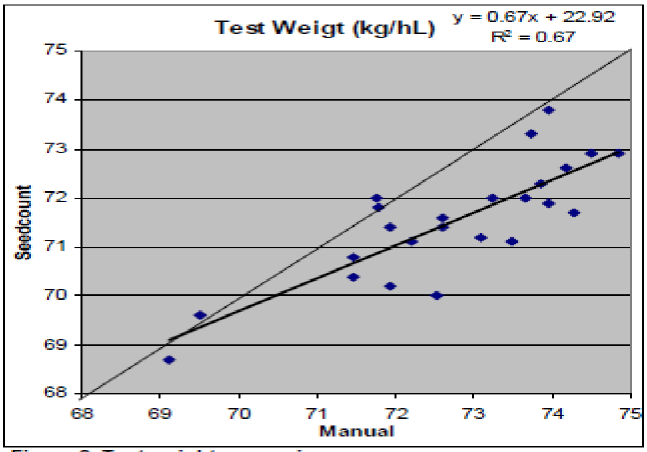


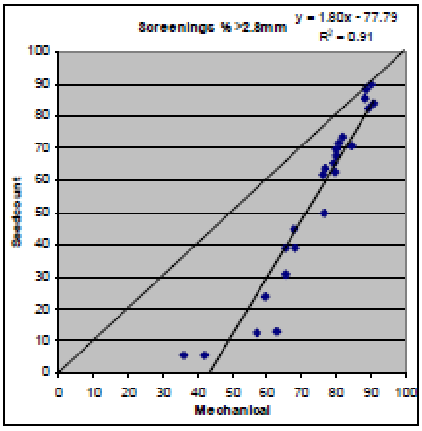
Figure 2. Test weight comparison

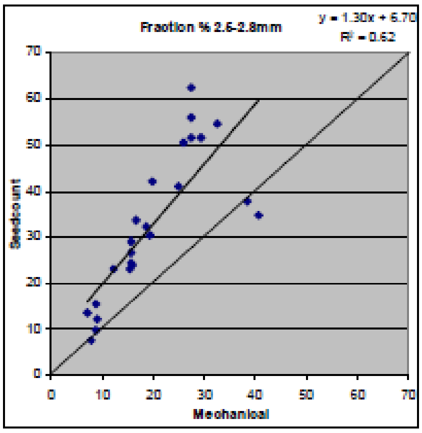
Screening Distribution

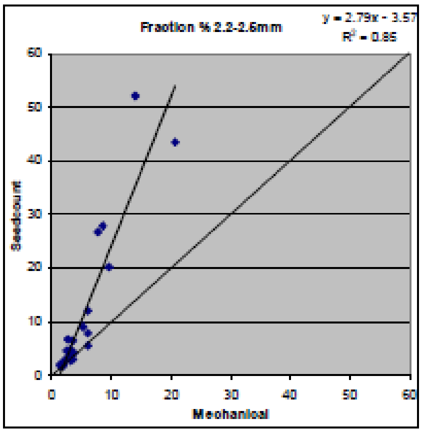
Table 3. Screening distribution comparison.

Mechanical	%>2.8mm	%2.5-2.8mm	%2.2-2.5mm	%<2.5mm	%<2.2mm
05NO11 NO_1-1001	88.7	8.9	1.8	2.3	0.5
05NO11 NO_1-1002	59.5	29.3	9.7	11.3	1.6
05NO11 NO_1-1003	90.6	7.1	1.8	2.2	0.4
05NO11 NO_1-1004	89.3	8.8	1.4	1.9	0.5
05NO11 NO_1-1005	67.7	24.9	6.0	7.3	1.3
05NO11 NO_1-1006	84.1	12.3	2.5	3.7	1.2
05NO11 NO_1-1007	56.8	32.6	8.5	10.6	2.0
05NO11 NO_1-1008	68.0	25.9	5.2	6.0	0.9
05NO11 NO_1-1009	62.6	27.5	7.7	9.9	2.2
05NO11 NO_1-1010	89.9	7.7	1.5	2.4	0.9
05NO11 NO_1-1011	88.2	9.1	1.9	2.7	0.8
05NO11 NO_1-1012	80.5	16.0	3.0	3.4	0.5
05NO11 NO_1-1013	80.0	15.7	3.1	4.3	1.2
05NO11 NO_1-1014	35.9	38.4	20.6	25.7	5.0
05NO11 NO_1-1015	79.9	15.7	3.2	4.4	1.2
05NO11 NO_1-1016	79.3	15.6	3.8	5.1	1.3
05NO11 NO_1-1017	65.1	27.5	6.1	7.3	1.2
05NO11 NO_1-1018	76.1	19.2	3.5	4.6	1.1
05NO11 NO_1-1019	76.7	18.6	3.6	4.6	1.0
05NO11 NO_1-1020	42.1	40.7	14.0	17.1	3.1
05NO11 NO_1-1021	76.5	19.8	2.8	3.6	0.9
05NO11 NO_1-1022	81.6	15.5	2.4	2.8	0.5
05NO11 NO_1-1023	79.4	16.5	3.2	4.0	0.8
05NO11 NO_1-1024	65.3	27.3	6.0	7.3	1.3

SeedCount	%>2.8mm	%2.5-2.8mm	%2.2-2.5mm	%<2.5mm	%<2.2mm
05NO11 NO_1-1001	88.2	9.8	2	2	0
05NO11 NO_1-1002	23.6	51.6	20.2	24.8	4.7
05NO11 NO_1-1003	84.1	13.7	2.1	2.1	0
05NO11 NO_1-1004	82.4	15.5	1.9	2.1	0.2
05NO11 NO_1-1005	44.8	40.9	12.2	14.3	2
05NO11 NO_1-1006	70.8	23.1	4.6	6.1	1.5
05NO11 NO_1-1007	12.5	54.6	27.8	32.9	5
05NO11 NO_1-1008	39	50.6	9.1	10.4	1.3
05NO11 NO_1-1009	12.8	56	26.7	31.2	4.5
05NO11 NO_1-1010	89.9	7.6	2.1	2.5	0.4
05NO11 NO_1-1011	85.6	12.2	1.9	2.2	0.4
05NO11 NO_1-1012	71.6	24	3.6	4.4	0.9
05NO11 NO_1-1013	69.5	24.5	4.3	6	1.6
05NO11 NO_1-1014	5.3	37.8	43.6	57	13.4
05NO11 NO_1-1015	67.7	26.6	4.8	5.7	0.9
05NO11 NO_1-1016	65.4	28.9	4.3	5.7	1.4
05NO11 NO_1-1017	38.8	51.5	8	9.7	1.7
05NO11 NO_1-1018	62	30.4	6.4	7.6	1.2
05NO11 NO_1-1019	63.9	32.3	3	3.8	0.8
05NO11 NO_1-1020	5.6	34.6	52	59.8	7.8
05NO11 NO_1-1021	49.7	42	6.8	8.3	1.4
05NO11 NO_1-1022	73.5	23	3	3.5	0.5
05NO11 NO_1-1023	62.6	33.6	2.8	3.8	1
05NO11 NO_1-1024	30.6	62.3	5.5	7.1	1.7







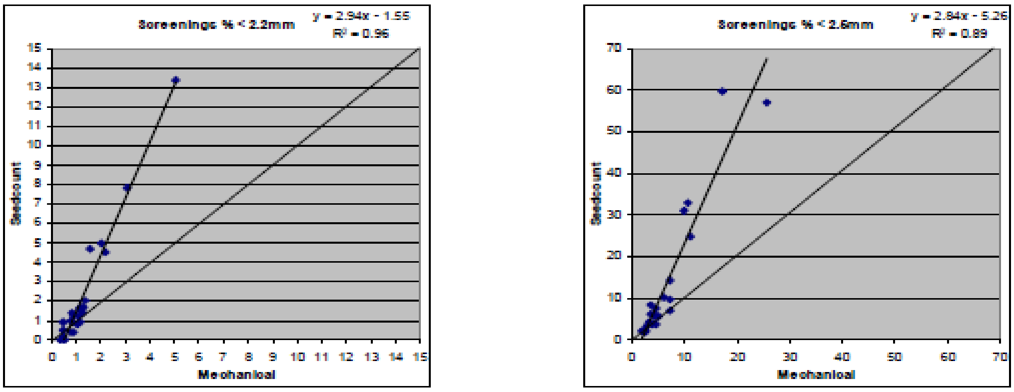


Figure 3. Screening distribution comparison.

Blacktip

Table 4. Blacktip repeatability evaluation.

SampleID	SeedCount Parameters									
	Whole Seeds	BP Mild %	BP Severe %	BP Total %	BP Test Num	Discolored %	mcws	mcts	mcdws	mcdws%
100% BP REP1	485	44.9	8.5	53.4	399	2.5	233	253	100	42.9
100% BP REP2	486	39.8	6.1	45.9	407	2.5	219	267	82	37.4
100% BP REP3	487	41.4	5	46.4	420	1.9	213	273	96	45.1
100% BP REP4	486	46.1	6.5	52.7	414	2.4	228	258	116	50.9
100% BP REP5	486	42.8	4.6	47.4	416	1.4	199	287	85	42.7
100% BP REP6	484	41.8	7.1	48.9	411	0.7	232	254	96	41.4
mean	485.7	42.8	6.3	49.1	411.2	1.9	220.7	265.3	95.8	43.4
SD	1.0	2.3	1.4	3.2	7.4	0.7	13.2	13.2	12.1	4.5
CV%	0.2	5.5	22.6	6.6	1.8	38.4	6.0	5.0	12.6	10.3
100% BP (all dorsal)	484	57.1	7.4	64.5	471	3	210	276	210	100

SD – standard deviation
CV% - coefficient of variation
mcws – manual count width section
mcts – manual count thickness section
mcdws – manual count (dorsal) width section
mcdws% - manual count (dorsal) width section %

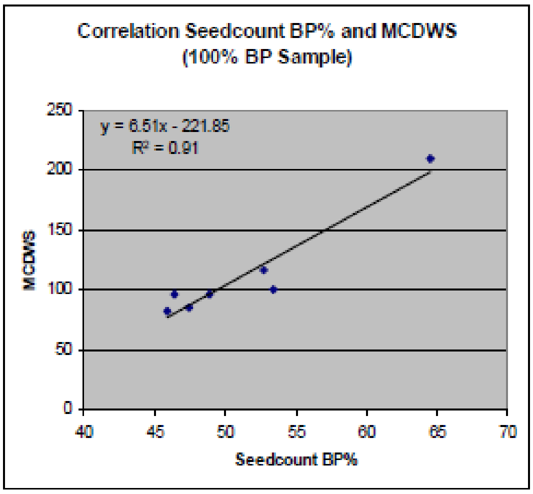


Figure 4. Relationship between Seedcount BP% and mcdws.

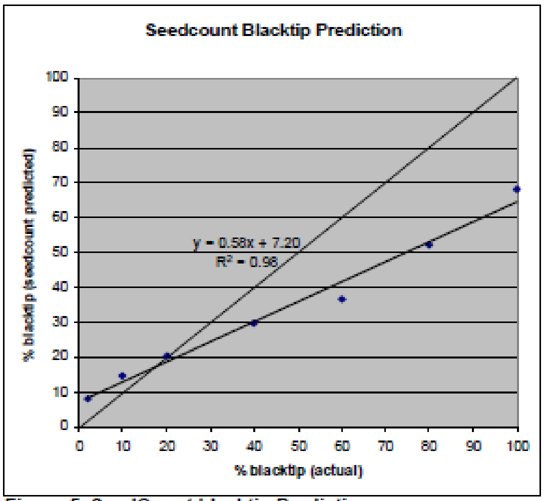


Figure 5. SeedCount blacktip Prediction.

Table 5. SeedCount blacktip prediction.

% blacktip (actual)	% blacktip SeedCount
2	8
10	14.7
20	20.4
40	29.6
60	36.5
80	52.4
100	68.2

Table 6. Determination of minimum grain requirement for SeedCount analysis.

	SeedCount Parameters					
SampleID	Whole Seeds	BP Mild %	BP Severe %	BP Total %	BP Test Num	Discolored %
100 grains	100	30.2	3.5	33.7	86	3.5
200 grains	200	41.4	8.3	49.7	169	1.2
300 grains	301	40.9	3.9	44.7	257	1.9
400 grains	401	49.7	5.9	55.6	338	3

Globe shaped grain evaluation

Table 7. Comparison of grain data for globe shaped barley grain.

Mechanical	HWT kg/hL	%>2.8	%2.5-2.8	%2.2-2.5	%<2.5	%<2.2
globe MB06-353	68.9	77.2	17.5	4.9	5.3	0.5
globe MB06-332b	70.1	98.8	1.0	0.3	0.3	0.0
SeedCount	Mini TW	%>2.8	%2.5-2.8	%2.2-2.5	%<2.5	%<2.2
globe MB06-353	68	72.4	21.5	5.1	6.1	1
globe MB06-332b	68.6	100	0	0	0	0

Discussion

Results for kernel weight evaluation (Table 1 and Figure 1) indicate that SeedCount SC3 was able to predict the reference values and could be used as an alternative to direct manual measurement. The results for test weight (Table 2 and Figure 2) show a lower correlation and increasing deviation from the 45^{o} line with higher values. Results would not be accurate enough to replace reference chondrometer results but may be useful for general estimation where only a small amount of sample grain is available.

For screening distribution, SeedCount determines screening fractions from "virtual grains" generated from pixel area data. The results (Table 3 and Figure 3) show reasonable correlations for most screening fractions however significant deviations from the 45^{o} line are indicated. All fractions apart from %>2.8mm show Seedcount to be overestimating mechanically derived values on this data set. SeedCount screening distribution might be useful for ranking estimates but would not be accurate enough to estimate absolute values.

The results for blacktip prediction (Table 4) indicate acceptable repeatability for BP total % (cv 6.6%), however the actual percentage of blacktip present (~100%) was greatly underestimated. Further investigation revealed that by manually turning the grains over in the width section of the tray (to lay in the dorsal-up orientation), increased the predicted percentage by approximately 15% to 65%. In addition the average amount of grains falling naturally in the dorsal-up orientation in the width section of the tray was determined to be only 43% (cv 10.3%). Although the instruction manual indicates that only dorsal-up grains are evaluated for blacktip, it was found that on most scans, some ventral-up grains (with ventral crease visible) were incorrectly selected and scored as not exhibiting blacktip. The relationship between SeedCount total blacktip % and manual count dorsal width section (mcdws) shows a strong correlation (Figure 4). Therefore it is postulated the remaining prediction error is in part attributed to incorrectly assigned grains in the thickness section of the tray where the blacktip region may not be clearly seen or incorrectly scored ventral-up grains in the width section. It is suggested that the blacktip module accuracy might be improved by only scoring grains in the width section of the tray.

The results for the manually assembled blacktip range of samples (Table 5 and Figure 5) indicate a good linear trend however the SeedCount tends to overestimate below 20% blacktip content and progressively underestimate above this level. The minimum quantity of grain required for SeedCount analysis (Table 6) was determined to be around 200 grains. Analysis of 100 grains resulted in warning error messages alerting to uneven grain distribution on the tray and screening distribution results were not calculated. Blacktip estimates were calculated on 100 grains however results indicate more uniform estimates are obtained with 200 grains or more.

The results of globe shaped grain evaluation (Table 7) indicate reasonable estimation of screening equivalents and HWT of moderately (MB06-353) and highly (MB06-332b) expressed forms of the globe shape phenotype.

Conclusion

The SeedCount SC3 evaluation showed kernel weight could be accurately estimated on barley grain. The estimates for test weight and screenings distribution would only be considered useful for indicative purposes or general ranking or where only small quantities of grain were available for analysis. The blacktip module estimates indicated acceptable repeatability for replicates however the amount was underestimated at levels above 20% and overestimated at levels below this. It was suggested that only scoring grains in the width section of the tray may reduce the error. The globe shape phenotype could be reasonably estimated for test weight and screenings.