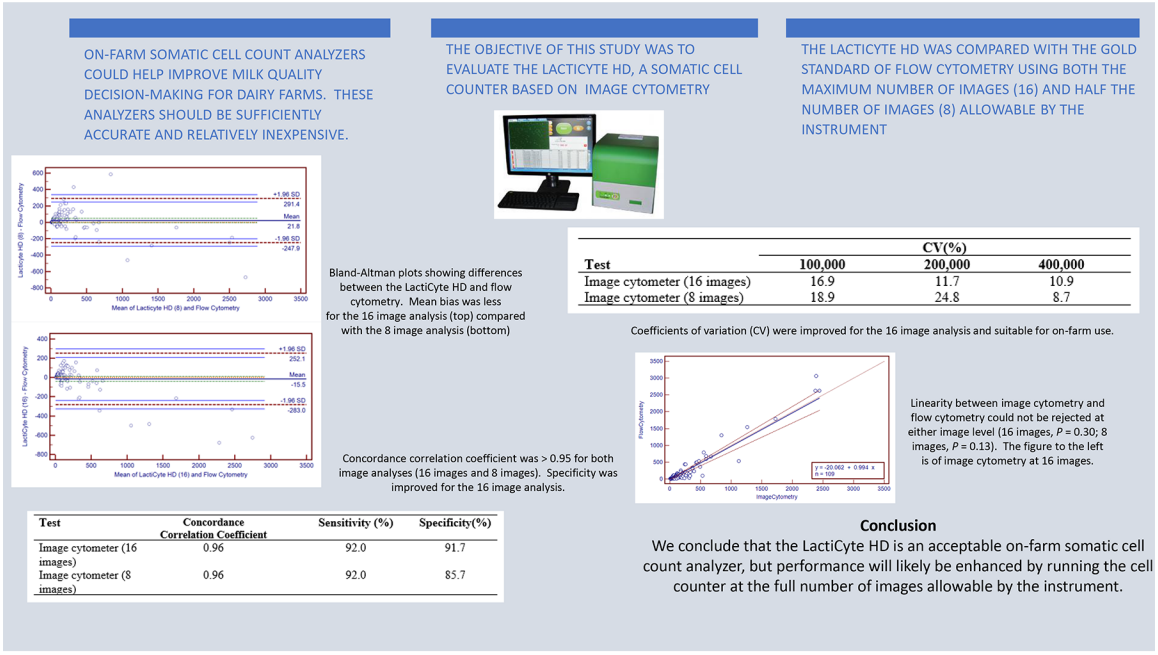
JDS Communications®
2023; 4:502–506

https://doi.org/10.3168/jdsc.2023-0380
Technical Note
Health, Behavior, and Well-being

Comparison of an image cytometry somatic cell count analyzer to a flow cytometry analyzer

A. Hubner,* N. Taechachokevivat, J. M. Grantz, K. D'Amico, A. Ueda, and R. C. Neves*

Graphical Abstract



Summary

This study compares the results of a benchtop somatic cell counter (LactiCyte HD, Page and Pedersen International Ltd., Hopkinton, MA) based on image cytometry with flow cytometry (Bentley SomaCount, Bentley Instruments, Chaska, MN). The counter utilizing image cytometry was analyzed at the full number of images (16) and half the number (8) allowed by the analyzer. The concordance correlation coefficient was 0.96 for both 16 and 8 images. Considering ≥200,000 cells/mL to be indicative of infection, the sensitivity and specificity of the LactiCyte HD at the full number of images were 92.0% and 91.7%, whereas the sensitivity and specificity of the analyzer at half the number of pictures were 92.0% and 85.7%, respectively. The coefficients of variation performed at 200,000 cells/mL were 11.7% and 24.8% when the analysis was carried out at full and half images, respectively.

Highlights

- Somatic cell count (SCC) performed by an image cytometer provided acceptable results for on-farm cell counting.
- The image cytometer had a moderate positive bias relative to flow cytometry.
- Acceptable sensitivity and specificity for the classification of milk with SCC ≥200,000 cells/mL were attained.
- True prevalence of infection (SCC ≥200,000 cells/mL) determined by flow cytometry was 22.9%.
- The image cytometer had increased precision when samples were analyzed at 16 images per slide over 8 images.

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Comparison of an image cytometry somatic cell count analyzer to a flow cytometry analyzer

A. Hubner,* N. Taechachokevivat, J. M. Grantz, K. D'Amico, A. Ueda, and R. C. Neves*

Abstract: The objective of this study was to evaluate the performance of a small footprint benchtop somatic cell counter based on image cytometry (LactiCyte HD; Page and Pedersen International Ltd., Hopkinton, MA) against a flow cytometer employed at a regional dairy herd improvement (DHI) laboratory. Milk samples collected during monthly DHI testing were split into 2 samples. One sample was evaluated using flow cytometry (Bentley SomaCount FCM; Bentley Instruments, Chaska, MN) at the regional DHI laboratory, whereas the other was evaluated using image cytometry at 2 different image levels (full number of images, 16 pictures per slide; half number of images, 8 pictures per slide). Mean bias of the image cytometer at 16 images was −15,500 cells/mL, whereas at 8 images the bias was 21,800 cells/mL. When considering only cell counts ≤400,000 cells per mL, the bias for both imaging resolutions was positive, meaning the image cytometer read higher than the flow cytometer. Both imaging resolutions (16 and 8) had a concordance correlation coefficient greater than 0.95. Considering ≥200,000 cells/mL to be indicative of subclinical mammary gland infection, the sensitivity and specificity of the image cytometer at 16 images were 92.0% and 91.7%, whereas the sensitivity and specificity of the analyzer at 8 images were 92.0% and 85.7%, respectively. Method precision (repeatability; coefficients of variation) were calculated at 3 different somatic cell counts (100,000, 200,000, and 400,000 cells/mL) where each sample was run repeatedly 12 times. When analyzed at the full number of images the coefficients of variation were 16.9%, 11.7%, and 10.9% for 100,000, 200,000, and 400,000 cells/mL, respectively. Analysis at half the number of images resulted in coefficients of variation of 18.9%, 24.8%, and 8.7% for 100,000, 200,000, and 400,000 cells/mL. We conclude that the image cytometer is an acceptable somatic cell count analyzer for on-farm use for applications such as screening cows for microbiological testing, and that precision is superior when the analysis is performed at the full number of images allowed by the instrument.

Mastitis is the most common disease of modern dairy cows, and the detection of subclinical infections is generally performed by an analysis of SCC in milk using flow cytometry (Ruegg and Reinemann, 2002; Ruegg, 2017). Intramammary infections result in an influx of leukocytes, primarily neutrophils, into the mammary gland, which can greatly increase the number of somatic cells in the milk of infected glands (Schwarz et al., 2011). Subclinical infections are classified as an increase in somatic cells to ≥200,000 cells/mL from a composite milk sample with no visible abnormalities of the milk, and farms are most likely to be provided with this information via DHI testing (Dohoo and Leslie, 1991; Ruegg, 2017). International standards for somatic cell counting are established by the International Dairy Federation and International Committee for Animal Recording, and cell counters used by DHI laboratories should meet established specifications for accuracy (International Dairy Federation, 2023). For many farms, this testing occurs once per month and allows for interventions with individual cows such as culling of chronically infected cows (Norman et al., 2021). On-farm testing may be beneficial for several reasons, including selective cow testing between DHI sampling and testing of milk from individual mammary glands. Several benchtop and even handheld analyzers have been introduced to the market (Kandeel et al., 2019; Salvador et al., 2014). Analysis with these instruments should be accurate, repeatable, and relatively inexpensive. The cell counter cassette cost per sample of these analyzers range from <$1 to >$4 (US$), and prices on the upper end of this range are cost prohibitive for farms wanting to adopt recurrent testing for optimal decision-making (Page and Pedersen International Ltd., 2016; Kandeel et al., 2019). Therefore, the performance of analyzers that provide more economical testing needs to be critically evaluated.

The LactiCyte HD (Page and Pedersen International Ltd., Hopkinton, MA) is a somatic cell counter utilizing image cytometry and such analyzers offer the opportunity to be used at a smaller scale while maintaining low costs (cytometer cassette costs <$1.00/sample). Image cytometry uses a fluorescent dyeing technique to dye individual cells and magnifies them to produce an image that undergoes automated counting via a charge-coupled camera (Page and Pedersen International Ltd., 2016). The analyzer can produce 4, 8, or 16 images of a sample, followed by reporting a mean number of cells per milliliter. To the best of the authors' knowledge, the LactiCyte HD cell counter has not yet been independently evaluated for its performance against flow cytometry. The objective of this study was to determine the performance of the image cytometer compared with a flow cytometer used at a regional DHI laboratory for milk somatic cell counting. We hypothesized that the mean bias of the image cytometer would be less than 25,000 cells/mL.

We conducted 2 experiments using image cytometry. Experiment 1 was conducted to evaluate the performance of the analyzer compared with flow cytometry (Bentley SomaCount FCM, Bentley Instruments, Chaska, MN). A priori, agreement between methods was considered acceptable if the Lin's concordance correlation coefficient was ≥0.95 (Lin et al., 2005; Rodrigues et al., 2009) and

Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN 47907. *Corresponding authors: amhubner@purdue.edu and rcneves@purdue.edu. © 2023, The Authors. Published by Elsevier Inc. and Fass Inc. on behalf of the American Dairy Science Association®. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/). Received February 06, 2023. Accepted May 08, 2023.

the average bias ≤25,000 cells/mL. The second experiment was conducted to evaluate the analyzer's repeatability.

Milk samples were collected during monthly DHI sample collection at a dairy farm milking 5,000 Holstein cows. Daily milk production on this farm was 39.4 kg per cow. The mean bulk tank SCC of the farm for the month before sampling day was 192,000 cells/mL. Milk was collected using a proportional sampler (a plastic jar that collects milk throughout the milking process and is inserted into the milk hose between the milking unit and the milk pipeline). The jar was removed after a cow completed the milking process, and the sample was agitated and divided between 2 vials. One vial contained bronopol as a preservative and this sample was transported to the DHI laboratory for further analysis, which occurred the following day. The DHI laboratory testing met specifications of the International Dairy Federation for SCC testing (International Dairy Federation, 2023). The second sample, without bronopol, was transported to the location of the image cytometer for analysis, and the analysis was performed within 3 h of collection.

A convenience sample from a single pen of cows was collected. Cows from this pen were used based on previous sampling indicating that the mean pen-level SCC and percent of cows with subclinical mastitis would be approximately 200,000 cells/mL and between 12% and 25%, respectively, thereby reasonably representing average herds in the United States. Parities 2 through 5 were represented in the samples collected.

Analysis with image cytometry was performed based on the manufacturer's recommendations. After vortexing the sample, 100 µL of milk was pipetted into a 1.5-mL vial that contained lyophilized dye. Samples were allowed to incubate for 3 min with vortexing occurring every 45 to 60 s. After incubation, 8 µL of the sample containing dye was pipetted into 1 of 4 sample wells on the cassette provided by the manufacturer. Four samples were prepared concurrently, and when the cassette had all 4 samples loaded, it was placed into the image cytometer. Samples were analyzed at both the 16-image setting (**16-I**) and the 8-image setting (**8-I**). For experiment 2, repeatability was performed in samples containing approximately 100,000, 200,000, and 400,000 cells/mL and analyzed 12 times each at both 16-I and 8-I (cells/mL determined by flow cytometry: 89,000, 187,000, and 504,000 cells/mL).

Sample size was calculated using the MedCalc software (MedCalc Software, Ostend, Belgium) for a Bland-Altman analysis considering an α error probability of 5% and power of 80% while assuming an expected mean difference between testing methods of 25,000 cells/mL and a standard deviation of 50,000 cells/mL. This resulted in a minimum sample size of 83 samples. Deming regression, Passing and Bablok regression, Bland-Altman plots, sensitivity and specificity, positive and negative predictive value, and coefficients of variation were determined using MedCalc software. Sensitivity and specificity were determined using a 200,000 cells/mL cell count threshold.

A total of 109 samples were collected. Of the 109 samples, the interquartile range of cells per mL was 162,000 (25,000 to 187,000 cells/mL) and 25 samples were ≥200,000 cells/mL as determined by flow cytometry. These samples were classified as positive for an infection prevalence of 22.9%. The median cells per milliliter for flow cytometry was 77,000. Among the samples analyzed at 16-I, 30 were classified as positive (30 of 109 samples, 27.5%). Among the samples analyzed at 8-I, 36 were classified as positive (36 of 109 samples, 33.0%).

Test agreement between flow cytometry and image cytometry at both image levels (16 and 8) is shown in Figure 1. Analysis performed at 16-I resulted in a smaller bias compared with 8-I (16-I: −15,500 cells/mL, 95% CI: −41,400 to 10,400 cells/mL; 8-I: 21,800 cells/mL, 95% CI: −4,400 to 47,900 cells/mL). However, at high cell counts (≥400,000 cells/mL) both resolutions read lower than flow cytometry. When considering only samples ≤400,000 cells/mL, both resolutions resulted in a positive bias, meaning the test method read higher than the flow cytometer. The magnitude of the bias for samples ≤400,000 cells/mL was smaller for the 16-I analysis compared with 8-I (16-I: 24,200 cells/mL, 95% CI: 13,400 to 34,900 cells/mL; 8-I: 49,300 cells/mL, 95% CI: 33,500 to 65,100 cells/mL). Test agreement between flow cytometry and image cytometry at both image levels (16 and 8) considering only samples ≤400,000 cells/mL is shown in Figure 1 and includes results from 93 samples.

Concordance correlation coefficients comparing image cytometry to flow cytometry were 0.96 for 16-I (95% CI: 0.95 to 0.96) and 0.96 for 8-I (95% CI: 0.94 to 0.97) (Table 1). Deming and Passing and Bablok regression are presented in Figure 2 for both imaging levels. Deming regression for 16-I indicates that there is a proportional and constant difference between the 2 methods (slope: 1.3, 95% CI: 1.2 to 1.4; intercept: −50.6, 95% CI: −70.2 to −31.0). Deming regression for 8-I also indicates that there is a proportional and constant difference between the 2 methods (slope: 1.2, 95% CI: 1.1 to 1.3; intercept: −73.0, 95% CI: −102.4 to −43.6). For the Passing and Bablok regression, no significant deviation from linearity was found at either image level (16-I: slope: 1.1, 95% CI: 1.1 to 1.2; intercept: −25.6, 95% CI: −35.8 to −15.7, $P = 0.30$; 8-I: slope: 0.9, 95% CI: 0.8 to 1.1; intercept: −20.1, 95% CI: −30.6 to −7.0, $P = 0.13$). Altogether, it is evident that the test method presents greater bias for samples with cell counts greater than 400,000 cells/mL and it is a limitation. We did not test the agreement of diluted samples with high SCC; it is possible that this would result in a decrease in biases between methods, though it needs to be tested. Additionally, a thorough vortexing of the original sample collected by the proportional sampler, instead of just manual agitation, may have resulted in greater accuracy comparing flow and image cytometry; however, this was not feasible under the on-farm conditions and is a shortcoming of the current study.

Considering all 109 samples, sensitivity and specificity were 92.0% and 91.7%, respectively, for samples analyzed at 16-I, whereas sensitivity and specificity were 92.0% and 85.7%, respectively, for samples analyzed at 8-I (Table 1). For this set of 109 samples, the positive and negative predictive values at 16-I were 76.7% and 97.5%, whereas the positive and negative predictive values at 8-I were 65.7% and 97.3%, respectively.

Coefficients of variation are reported in Table 1 and were higher for the samples analyzed at 8-I except for the samples at 400,000 cells/mL, which were slightly lower for 8-I than for those analyzed at 16-I (16:I: 10.9%; 8-I: 8.7%). The coefficient of variation for 16-I at 200,000 cells/mL was 11.7%, and this is likely to be the most important cut-point when SCC analysis is performed. It should be noted that we were unable to replicate the coefficients of variation reported by the manufacturer, which are 2% and 5%. Further, the

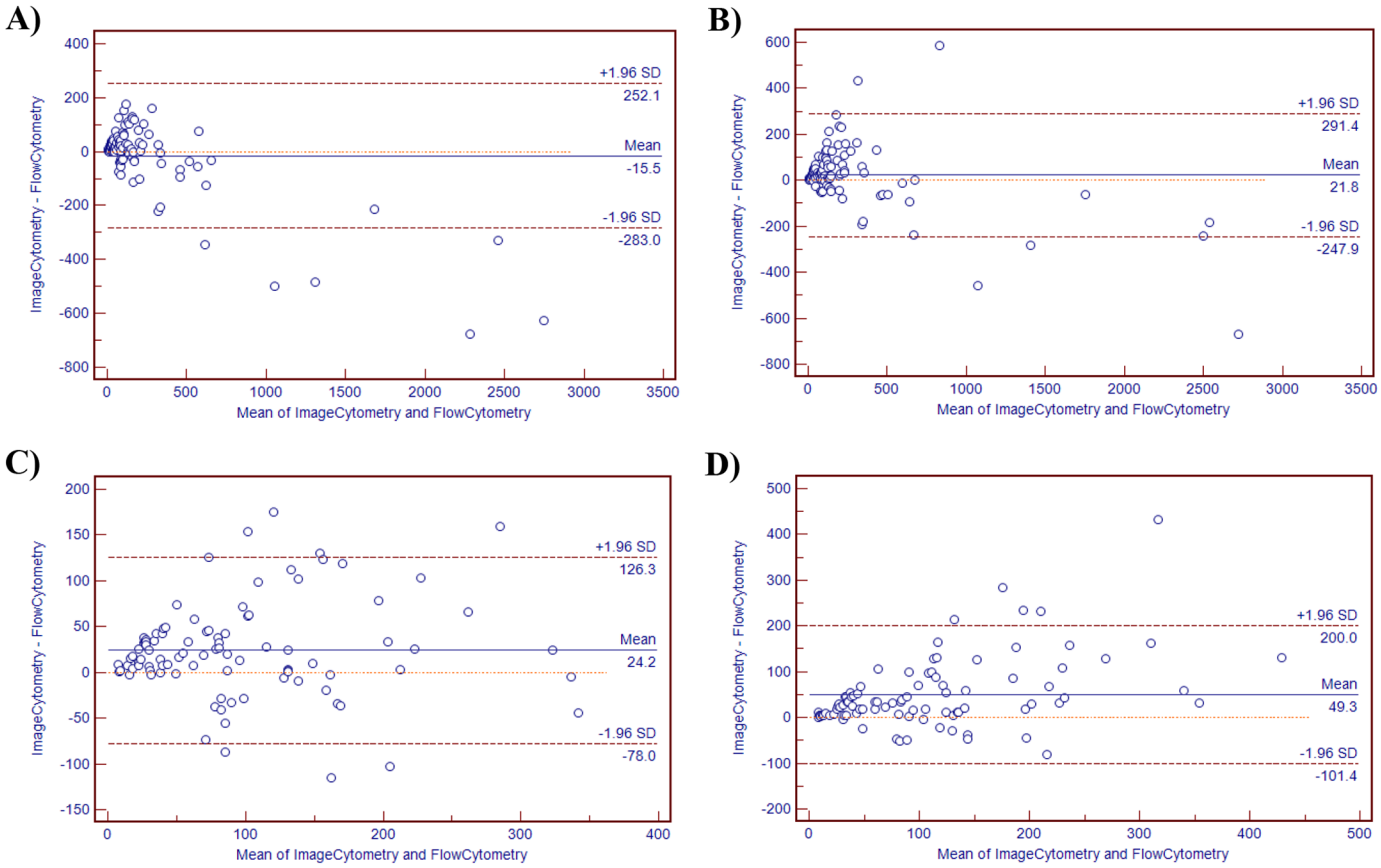

Figure 1. Differences in SCC (×1,000) determined by flow cytometry and image cytometry. (A) Analysis of the image cytometer at 16 images compared with flow cytometry. (B) Analysis of the image cytometer at 8 images compared with flow cytometry. (C) Analysis of the image cytometer at 16 images compared with flow cytometry for samples ≤400,000. (D) Analysis of the image cytometer at 8 images compared with flow cytometry for samples ≤400,000.

coefficients of variation for the image cytometer were greater than those recommended by the International Dairy Federation for disc or flow cytometers.

Considering test performance at cell counts ≤400,000 cells /mL, where more meaningful agreement is likely to be of greater value to the user, both resolution levels read higher than flow cytometry. This would be expected to result in a greater number of false positives and fewer false negatives. Differing circumstances for the user will dictate whether a test reading high versus low is preferred. Even in circumstances where a false positive may ultimately result in culling, a user may prefer fewer false negatives relative to false positives. This could be the case when screening cows for culturing to detect contagious mastitis organisms.

Another consideration for users of this analyzer, and other on-farm analyzers, is that samples for these counters are likely to be taken by stripping of milk at the beginning or end of the regular milking, and not from milk sampled throughout the milking process as is typical for DHI testing. This would likely artificially increase SCC, especially for those cows with high cell counts (Sarikaya and Bruckmaier, 2006). This should be taken into consideration by users of this, and other analyzers, when milk stripping is used for sample collection. Despite this shortcoming, several analyzers

Table 1. Concordance correlation coefficient, sensitivity, and specificity for the image cytometry cell counter (16 and 8 images) compared with flow cytometry; correlation coefficients (CV) of the image cytometer (16 and 8 images) at cell counts of approximately 100,000, 200,000, and 400,000 cells/mL[1]

No. of images	Concordance correlation coefficient	Sensitivity (%)	Specificity (%)	CV (%) 100,000	CV (%) 200,000	CV (%) 400,000
16	0.96	92.0	91.7	16.9	11.7	10.9
8	0.96	92.0	85.7	18.9	24.8	8.7

[1]For sensitivity and specificity, a cell count from flow cytometry of ≥200,000 cells/mL was considered positive for intramammary infection.

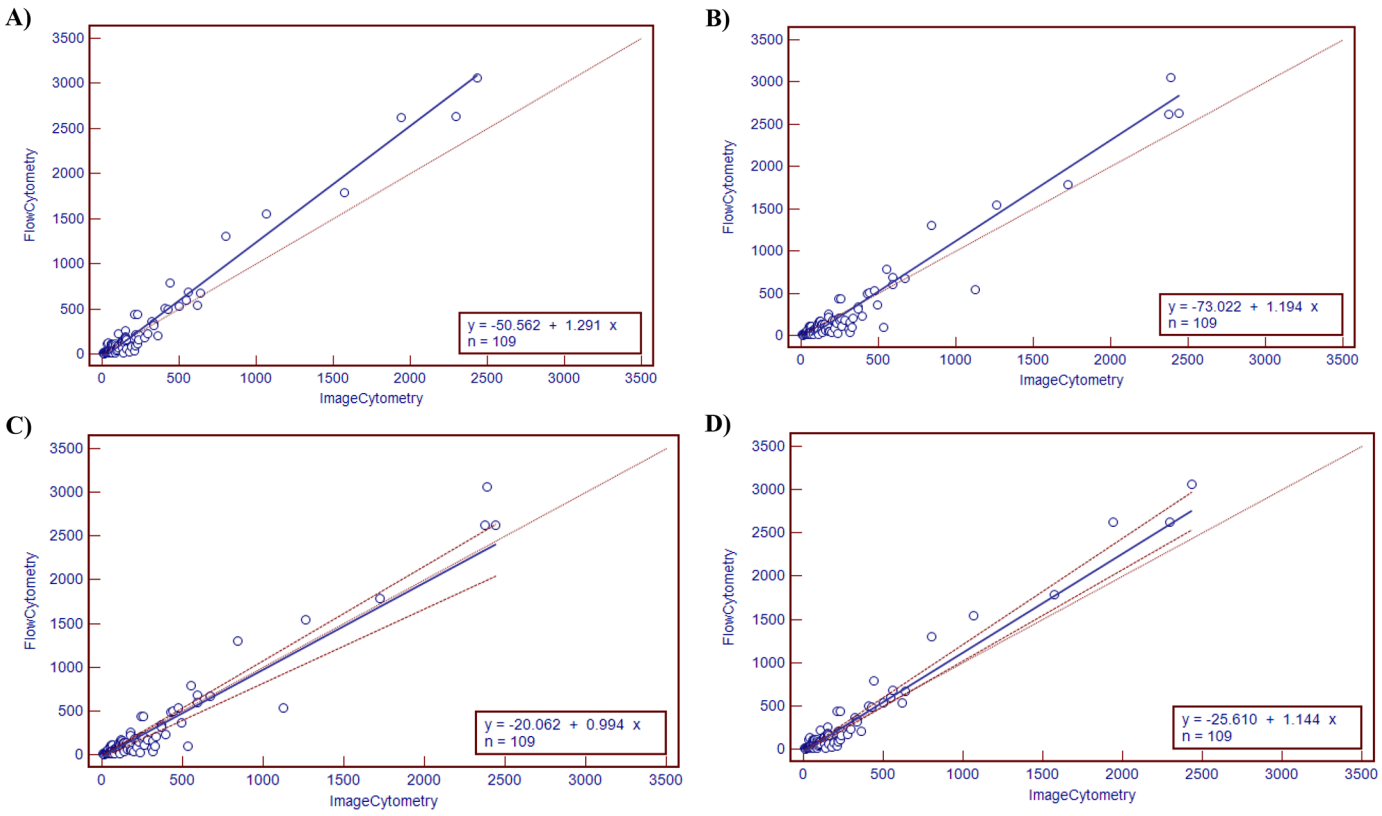

Figure 2. Deming and Passing and Bablok regression of image and flow cytometry. (A) Deming regression for image cytometry at 16 images. (B) Deming regression for image cytometry at 8 images. (C) Passing and Bablok regression at 16 images. (D) Passing and Bablok regression at 8 images.

continue to be present in the marketplace and it is likely, based on the cassette price of this analyzer, that it could be a feasible option for use by dairy farms.

Samples analyzed with the image cytometer could be evaluated in approximately 70 s per sample (16-I) and 40 s per sample (8-I) and the machine is not automated to run multiple samples. This may also be an important consideration for users as they determine the number of samples to evaluate and which cows to sample. The lack of automation means that the image cytometer is unlikely to replace DHI testing, but rather to act as a supplement to DHI testing.

Further work could examine the accuracy of the image cytometer and whether accuracy could be improved with differing milk temperatures or dilutions. Additional procedures that improve accuracy may help the image cytometer to be an acceptable test for a broad range of uses.

Repeatability was better when the analysis was performed at 16-I than at 8-I. Furthermore, specificity was higher for the analysis performed at 16-I than at 8-I. We conclude that the image cytometer is an acceptable on-farm SCC analyzer based on a concordance correlation coefficient ≥0.95 and would be appropriate for situations such as screening cows for further diagnostic testing. The performance of the image cytometer will likely be enhanced by running the cell counter at the full number of images allowable by the instrument.

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Notes

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Financial support for this study was provided by AH's faculty start-up funds.

All experimental procedures carried out in this study were approved by the Institutional Care and Use Committees of Purdue University (West Lafayette, IN; protocol 2205002269).

We thank the participating farm and the employees of Central Star Laboratories (Grand Ledge, MI) for allowing us to collect samples during DHI testing.

Author contributions were as follows: AH, conceptualization, data analyses, data collection, and manuscript preparation; NT, data collection and manuscript editing; JMG, data collection and manuscript editing; KD, data collection and manuscript editing; AU, data collection and manuscript editing; RCN, conceptualization, data analyses, and manuscript editing. All authors contributed to the article and approved the submitted version.

The authors have not stated any conflicts of interest.