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Comparisons of a new digital imaging technique for yeast cell counting and viability assessment with traditional counting methods

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Abstract

Brewing of beer relies heavily on the viability of yeast added. Accurate cell counts and assessments of cell viability are therefore vital throughout the brewing process to ensure a consistent quality end product. Classically this is performed manually, using a haemocytometer. A high amount of human error is associated with this method, due to variability in counting and inter-operator differences. In order to overcome these difficulties, automated cell counting techniques have entered the market. In this study, we compare a new automated yeast cell counting instrument using bright field microscopic images with traditional microscopy. We also compare this with live cell counts from a radio-frequency impedance based instrument. Errors, accuracy and time for analyses of the three methods were evaluated.

Figure 1 Countstar machine with slide being inserted into port.

Countstar Introduction

Countstar Yeast automates the cell counting procedure through digital image analysis of 20µl samples. It uses traditional dyes methylene blue or methylene violet to determine live, total and dead cell count along with viability (expressed as %mortality), average diameter, compact and %aggregation. These dyes stain non-viable cells their respective colour and leave live cells colourless. This instrument uses individually packaged disposable plastic slides, each containing 5 separate chambers to lower costs and waste. Once the sample is loaded, it should be left 3-5 minutes to settle and allow the stain to penetrate. Following a prompt, the instrument takes around 10 seconds to analyse an image. This machine requires no regular maintenance, will save vital time in the laboratory and is considered to reduce potential sources for human error associated with manual cell counts.

Aims

The aim of this study was to determine whether this new technology was a reliable and accurate way to perform cell counts and assess viability of samples, by comparing results with manual haemocytometer counts. Comparisons were also made with a radio-frequency impedance technology in regards to analysis time and errors accompanying these.

Methods

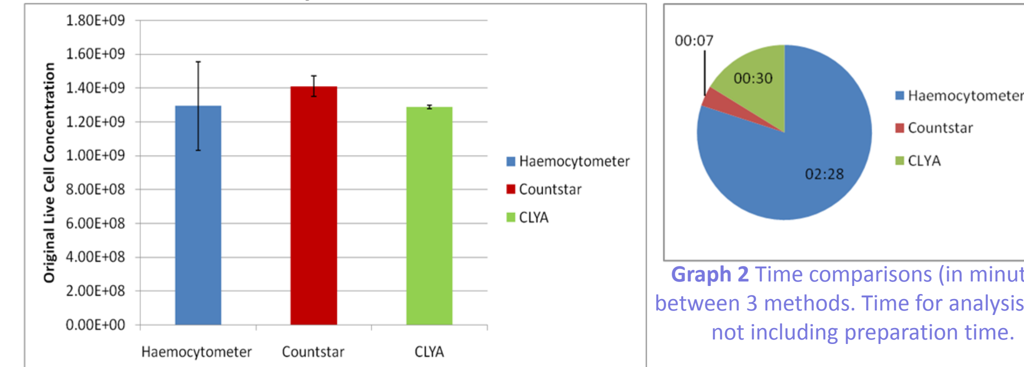
All experiments were carried out using methylene blue unless otherwise stated and solutions were diluted to approximately 1x10^{7} cells/ml. Experiments for this analysis included: Comparisons between the Countstar, Haemocytometer and CLYA*; Serial dilutions; Size comparisons; Repeatability; and Inter-operator differences.

*CLYA (Compact Lab Yeast Analyser) is an Aber Instruments Ltd. laboratory instrument that measures capacitance which is correlated to live cell concentration.

Figure 2 Countstar, software and dongle shown. Top right image of disposable 5 chamber slide with sample.

Results

Countstar vs haemocytometer vs CLYA

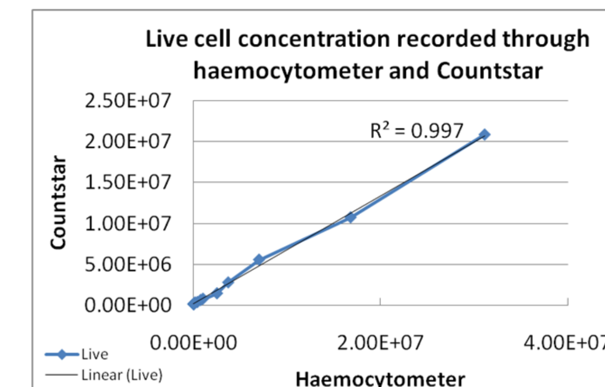


Graph 1 Live cell counts from Haemocytometer, Countstar and CLYA with error bars. 10^{-2} dilutions were used for haemocytometer and Countstar. NB. The CLYA was calibrated against the haemocytometer reading.

Graph 2 Time comparisons (in minutes) between 3 methods. Time for analysis only not including preparation time.

Similar live cell counts recorded for all methods. Reduced error was seen with the Countstar (Sd:5.98e7cells/ml) and the CLYA (Sd:1.08e7cells/ml) when compared with the haemocytometer repeats (Sd:2.63e8 cells/ml).

Serial dilutions

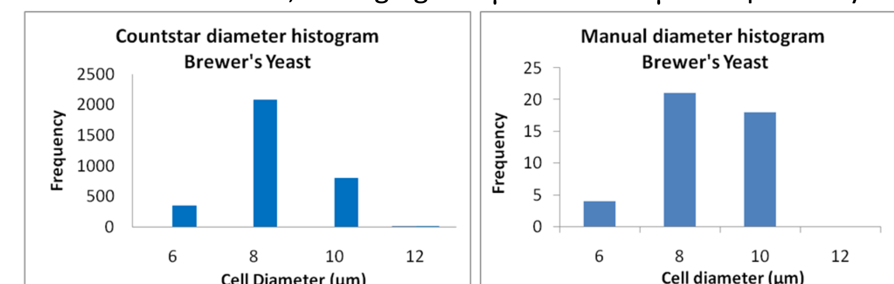


Graph 3 Correlation between live cell counts recorded with Countstar and haemocytometer. Samples taken from same solution, diluted 50% each time to cover range of Countstar.

Excellent correlation between Countstar and haemocytometer readings through range of concentrations (R^2=0.997). Similar for Total cell concentration (R^2=0.996).

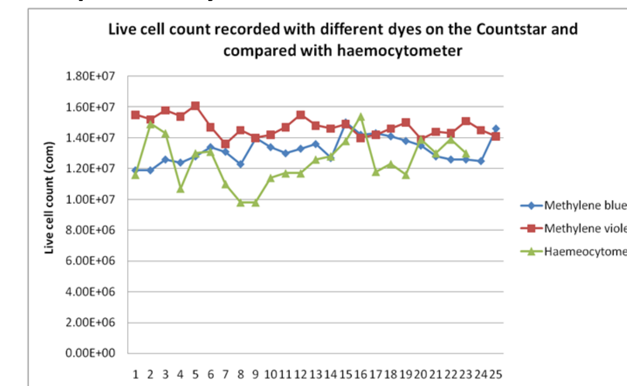
Comparisons, size histograms

Diameters measured by the Countstar corresponded well with those calculated with microscope using an eyepiece graticule (8.15; 7.97 µm respectively). Also true for smaller cells, averaging 6.52µm and 6.59µm respectively.



Graph 4 Diameter histograms recorded Countstar (i) and manually (ii).

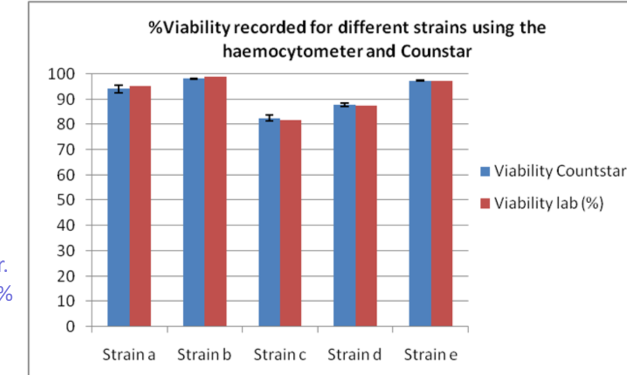
Repeatability test results



Graph 5 Repeatability for Countstar and haemocytometer. 25 samples recorded using the Countstar with methylene blue; methylene violet to check consistency with both dyes; and haemocytometer.

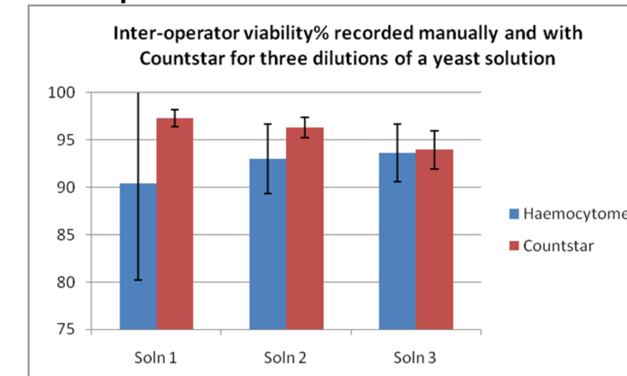
Live cell counts (in cells/ml) smaller deviation with Countstar (1.32x10^{7}±8.15x10^{5}; 1.47x10^{7}±6.17x10^{5}; 1.25x10^{7}±2.11x10^{6} respectively). Viability (in %) similar for three sets of data, with respective averages of: 96.86±1.15; 96.47±1.19; 96.96±1.53.

Yeast strains



Graph 6 Viability recorded for five strains of yeast from a major UK Brewery. Haemocytometer readings based on single reading from lab. 10 repeats for each strain analysed with Countstar. Good repeatability seen across all strains.

Inter-operator differences



Graph 7 Four operators recorded viability via manual cell count and Countstar. All operators performed three readings of each solution which were averaged. Overall averages were calculated for each solution. Error bars show variation between operator averages.

Conclusion

- Cost effective concept: Capable of analysing viability without more expensive fluorescence as with alternative equipment.
- Manual cell counting still the main substitute to use of hazardous fluorescent dyes.
- Reliable analysis of yeast cell populations, smaller error associated with Countstar than haemocytometer repeats.
- Serial dilution tests demonstrated excellent correlation with haemocytometer readings (R^2=0.997) .
- Analysis time significantly lower using the Cotstar.
- Able to accurately measure cells: Data corresponded well with diameters recorded manually and in a fraction of the time.
- User-friendly software automatically saves data, including images for future reference.

Tests performed demonstrate good correlation between Countstar Yeast and manual cell counts. The Countstar Yeast is able to accurately and reliably record a range of cell data, saving valuable time and potential for human error associated with manual cell counts.

Future work

Further investigation on the Countstar Yeast is ongoing within Aber Instruments and at brewing laboratories with multiple yeast strains. Testing needs to be carried out with highly flocculent yeasts, mixed yeast strains and with media where there is likely to be high levels of non-yeast particles with the same diameter range (e.g. some yeast based biofuel processes).

Acknowledgements

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