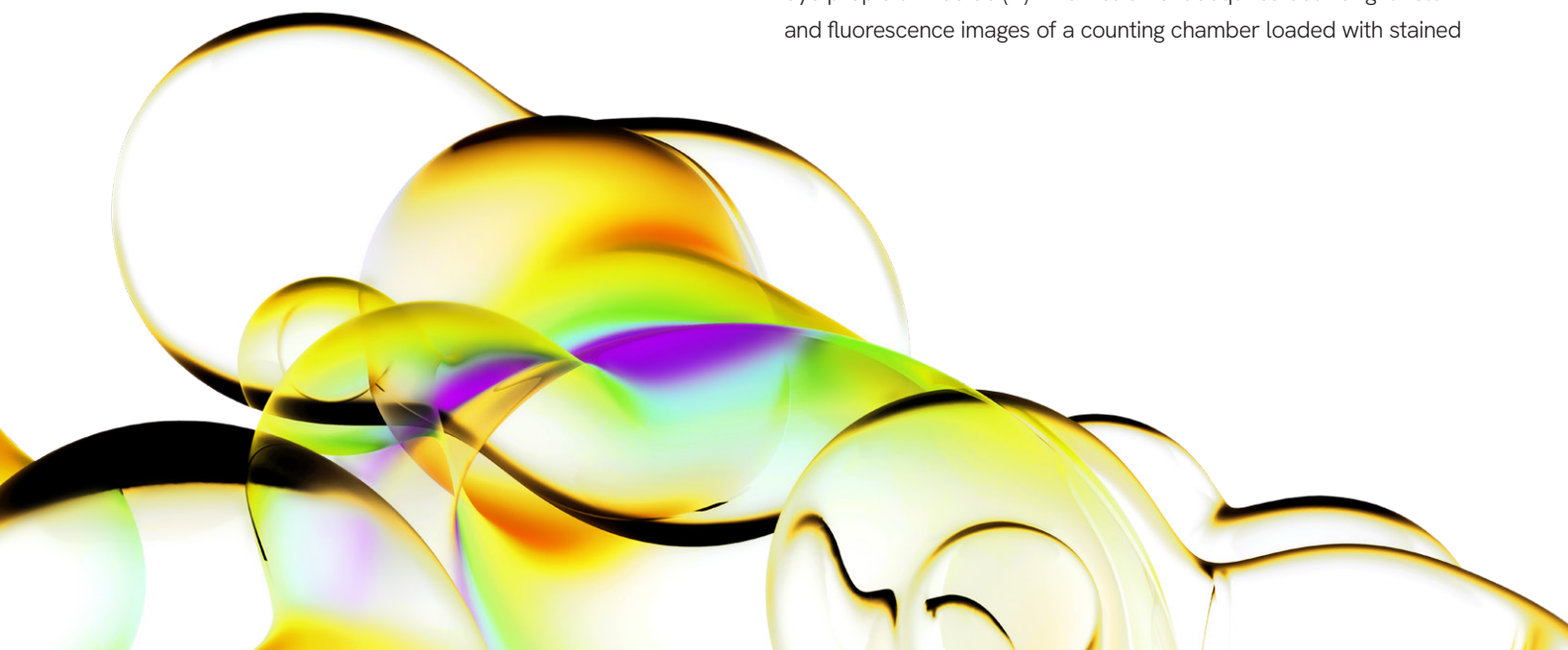
Yeast concentration and viability using image-based fluorescence analysis.

Introduction

Yeasts are an economically important organism used for ethanol production in the beverage and alternative fuels industries as well as a leavening agent in the baking industry. In addition, pathogenic strains of yeasts are involved in both plant and animal diseases. Concentration and viability determinations are routinely performed for quality control purposes in yeast production, fermentation processes, and fungicides research to monitor proliferation of pathogenic yeasts.

The most common method for determining yeast cell number and viability is manual counting on a standard microscope using a hemacytometer and methylene blue. One advantage of this method is that visual inspection of each sample allows the operator to check for contamination, presence of interfering debris, and obvious dilution errors. A major disadvantage is that the manual method is laborious, error-prone and the data acquired is not easily traceable. Although the equipments used to perform manual counting are relatively inexpensive, the cost of human labor and counting errors can be high enough to render manual counting less than practical in a production facility where accuracy, consistency, and record-keeping are highly desirable.

Here we describe an automated, image-based cytometry method that is rapid, highly accurate, and consistent for determining concentration and viability of yeasts. This method utilizes the Cellometer® X2 instrument for image-acquisition and associated software for image analysis and data management. Viability can concurrently be determined by staining with the fluorescent viability dye propidium iodide (PI). The instrument acquires both bright field and fluorescence images of a counting chamber loaded with stained



yeast samples. Total cell numbers are counted by the software using the bright field images while the viability of each counted cell is determined by analysis of the fluorescence images of the same fields of view. The performance of the Cellometer X2 system with respect to accuracy and reliability for determining cell concentration and viability is presented here.

Experimental procedure

Cell concentration measurements

A concentrated yeast suspension was made by mixing approximately 10 mg of dehydrated Muntons active brewing yeast (Muntons Plc., Stowmarket, Suffolk, UK) in 500 µL of distilled water at room temperature. The mixture was vortexed for 15 s followed by incubation at room temperature for 5 min to allow yeast to rehydrate. Six serial two-fold dilutions were made in distilled water. Samples were vortexed for 5 s before pipetting 20 µL into Cellometer counting chambers and the cells were allowed to settle for 1 min prior to counting on a Cellometer X2 instrument. Images were focused such that the center of each cell appeared bright while the edges were dark and sharp in the live image view. Cell counting parameters for size and contrast enhancement were optimized by examining the counted images to ensure that all individual cells were counted, clumps of cells were accurately declustered, and debris was ignored by the software. Six separate aliquots of each serial dilution were loaded into separate counting chambers and counted in succession to determine the mean concentration of each serial dilution and the corresponding coefficient of variation.

Cell viability measurements

Fresh yeast stored at 4 °C were rehydrated as above and diluted to approximately 2 x 10^7 cells/mL, which was used as a source of live yeast. This live yeast suspension was split into two aliquots and each placed in separate 50 mL tubes. One tube of yeast suspension was heat-killed by incubation in a 100 °C water bath for 10 min while the other tube was left at room temperature. Systematic mixing of heat-killed yeast into the live yeast suspension resulted in yeast samples ranging from high to low viability. For each live/dead yeast mixture, aliquots of 80 µL yeast suspension were mixed with 20 µL of a stock solution of PI at 200 µg/mL to give a final concentration of 40 µg/mL PI. Stained yeast samples (n=6 for each level of viability) were loaded into Cellometer counting chambers, allowed to settle for 5 min, and then imaged and counted on the Cellometer X2. Images were acquired using bright field optics and the VC-660-502 fluorescence optics module (excitation 540 nm and emission 600 nm). PI is a DNA-binding dye that is excluded by live cells, but enters dead cells with compromised membranes. The dead cells can then be imaged with the above fluorescence optics module. Total cell number was determined by counting of all the yeast in the bright field images and dead cell number by analysis of the corresponding fluorescent images. Live cell number was determined by subtracting dead cell number from total cell number, and viability was determined by dividing the calculated live cell number by total cell number and expressed as a percentage.

Results and discussion

Cell concentration linear range and reliability

Serial two-fold dilutions of rehydrated yeast were counted on the Cellometer X2 to determine the linear range for reliable yeast concentration measurements. The range of concentration that can be measured on the instrument is dependent on the size of the cell being counted (i.e. the range decreases as cell size increases). The Muntons strain is approximately 4.5 µm in diameter when rehydrated, and was selected for this experiment due to its intermediate size when compared to other yeast strains such as those used for lager beer or wine production. The upper limit for accurate yeast counting, as determined by examining counted images, represented a four-fold dilution of the original concentrated yeast suspension (Dilution 2) at 6.6 x 10^7 cells/mL. Serial dilutions of this sample were counted until the lower limit was reached (Dilution 6) at a concentration of 4.17 x 10^6 cells/mL.

Plotting of the measured concentration versus the concentration factor (inverse of the dilution factor) resulted in a linear relationship with an R^2 of 0.9968. Thus the linear range for counting Muntons yeast (and other similarly sized strains around 4.5 µm in diameter) on the Cellometer X2 is between 4 x 10^6 and 6.6 x 10^7 cells/mL (Figure 1A).

Repeated measurements were performed on the sample to determine the reliability of counting by calculating the Coefficient of Variation (CV). Within the linear range for counting yeast on the Cellometer X2, the CV was between 4% and 13% (Figure 1B).

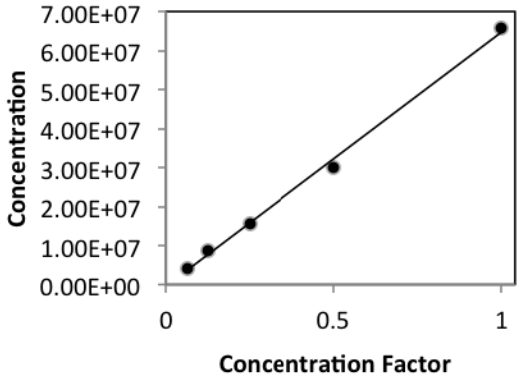
Cell viability measurement accuracy and reliability

Aliquots of the live yeast suspension were mixed with the heat-killed yeast to generate samples at various levels of viability. The measured viability of the live yeast sample was 78% while the heat-killed sample was 0%. Fractions of live and dead yeast were mixed to analyze intermediate viability levels.

The measured viability was plotted against the percent of heat-killed cells in the final mixture and gives a linear correlation with an R^2 of 0.9996. Comparison of predicted and measured viability showed a high degree of agreement and replicates of viability measurements had a standard deviation of ≤ 1.3% (Figure 2A,B). Taken together, viability measurements using PI on the Cellometer X2 are both highly reproducible and reliable.

Conclusion

A

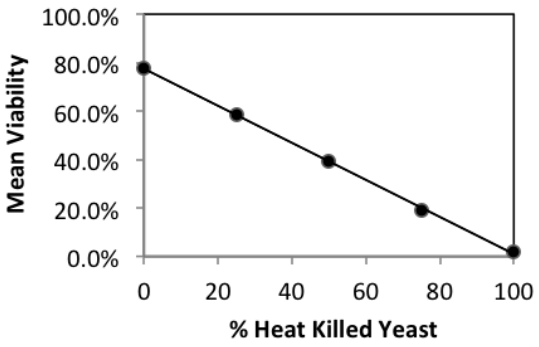


B

Dilution	Mean concenration	CV
2	6.58E+07	6%
3	2.99E+07	4%
4	1.55E+07	7%
5	8.84E+06	10%
6	4.17E+06	13%

Figure 1: Linear range of accurate yeast concentration measurements and corresponding CV values.

A



B

Live/dead	Predicted	Measured	St dev
100%/0%	-	78%	0.4%
75%/25%	59%	58%	1.2%
50%/50%	39%	39%	0.6%
25%/75%	20%	19%	1.3%
0%/100%	0%	0%	0.4%

Figure 2: Accuracy and reliability of viability measurements of live yeast mixed with various quantities of heat-killed yeast

Our data demonstrated high accuracy and reliability over a wide range of yeast concentrations and viabilities using the Cellometer X2. As an image-based system, it has the advantages of manual methods involving visual inspection while reducing labor intensive steps and including a data management component for ease of traceability. As such, it can be easily integrated into an existing manual counting work flow. The system presented here is an important tool for yeast concentration and viability measurements due to its greater throughput and robustness compared to manual counting with the potential to improve fermentation and yeast production performance in industrial settings.

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