031

A novel image analysis method for improving counting of chain-forming yeasts

jschier@harpoon.com
leo.chan@revvity.com

Jaime Schier[1], Joseph Thomas[1], Dmitry Kuksin[2], and Leo L. Chan[2]
[1]Mass Bay Brewing Co., Boston, MA 02210
[2]Revvity Health Sciences, Inc., 360 Merrimack St., Suite 200, Lawrence, MA 01843

UNITED WE BREW 2019
Master Brewers Conference

1. ABSTRACT

Breweries have been using a large variety of yeast strains for beer fermentation to produce unique flavors. It is highly important to directly count the yeast cells to monitor effects of cell concentrations on the beer products. Over the last decade, image cytometry has been used to rapidly and automatically measure yeast
concentration and viability throughout the fermentation. The ability to consistently assess yeast conditions during fermentation allows brewers to better characterize the health of the yeast organism as well as identify any issues to minimize risk of production batches. The image cytometry technologies can easily count individual yeast cells or decluster group of cells in proximity. However, these yeast strains can exhibit different morphologies as they propagate and ferment in the tanks. Yeast strains that are small chain-forming prove to be more difficult in utilizing image cytometry-based analysis, where segmentation may not accurately determine yeast cells in branches and sequences.

In this work, we utilized an improved image analysis algorithm in the Cellometer X2 image cytometer to increase the accuracy of yeast counting and viability measurement. The novel algorithm uses a special optical property that the small chain-forming yeasts exhibit to accurately segment them into individual cells. The algorithm was tested on regular non-chain-forming and chain-forming yeasts with the propidium iodide viability stain to produce cell concentration and viability results. The results were visually validated and demonstrated improved cell counting accuracy and consistency. In addition, the novel method can be used with other chain-forming or flocculated yeasts that are difficult to analyze.

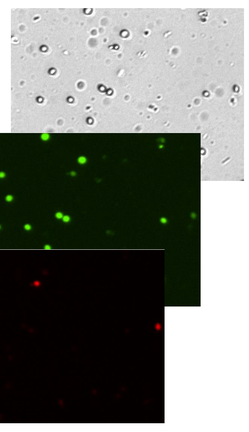
2. CURRENT METHODS FOR MEASURING YEAST COUNT & VIABILITY

Methods	Description	Known Issues
Hemacytometer	Manually counting yeast cells	•Time-consuming and tedious process •Requires experienced user for accurate counting
Fluorescence Microscopy	Visualization of fluorescently labeled yeast cells	•Qualitative observe instead of quantitative analysis •Not automated, low throughput
Flow-Based Analysis	•Quantitative analysis •Automated analysis	•Relatively expensive and high maintenance •Requires experienced user for proper operation •Cannot visually observe yeast cells

3. CELLOMETER IMAGE CYTOMETRY INSTRUMENTATION AND PROTOCOL

Pipette 20 µL of sample into disposable counting chamber

Insert chamber in Cellometer



Assay: Yeast AOPI Viability

Cell Type F1: Yeast AOPI Viability FL1
Cell Type F2: Yeast AOPI Viability FL2

Sample ID: 23h-1X-2
Dilution: 4.00

Results:

Count	Concentration
Total: 896	2.06x10^7 cells/mL
Live: 854	1.96x10^7 cells/mL
Dead: 42	9.71x10^5 cells/mL

Viability: 95.3%

Bright-field (BR) and Fluorescent (FL) images

- Cellometer image cytometer automatically counts live and dead yeasts in the sample using Acridine Orange and Propidium Iodide fluorescent stains

4. NEW ALGORITHM FOR COUNTING CHAIN-FORMING YEASTS

Chain-Forming Yeast | **Zoomed-in Image** | **Counted Image**

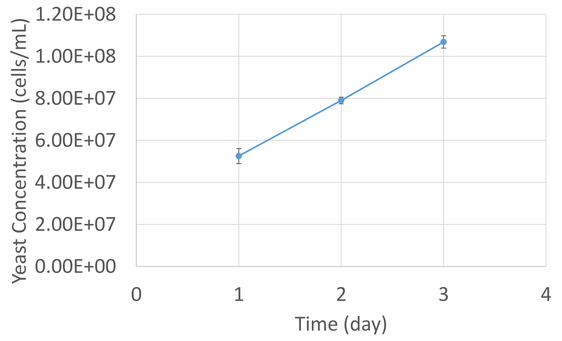
- Under standard imaging algorithm, the background is bright, the software will try to decluster the chain-forming yeasts
- If yeast cells are smaller, it makes it difficult to decluster
- The new imaging algorithm, the exposure is reduced, causing the background to decrease and increase brightness of yeasts
- The software can count individual yeast cells in the darker images

5. MONITORING CHAIN-FORMING YEAST COUNT THROUGH FERMENTATION

Day 0 | **Day 2** | **Day 5**

- A38 chain-forming yeasts at day 0 are very small and showed multiple chains, causing difficulty in standard imaging algorithm
- If the standard algorithm is used, then the cell count can be under-estimated
- As the yeast proliferated, the chains reduced, and cell size increased
- Therefore, in the later time points, the new algorithm may not be required

Chain-Forming Yeast Counting



6. CONCLUSION

- In conclusion, we have demonstrated the capability of using image cytometry to count chain-forming yeasts
- The new algorithm can be used for small chain-forming yeasts in early stage of fermentation, ensuring accurate pitching
- There may be application for maintaining more accurate cell counts of actively propagating yeast cultures as well

MASS. BAY BREWING CO.
Mass Bay Brewing Co., 306 Northern Avenue, Boston, MA 02210

Revvity Health Sciences, Inc., 360 Merrimack St., Suite 200, Lawrence, Massachusetts.