

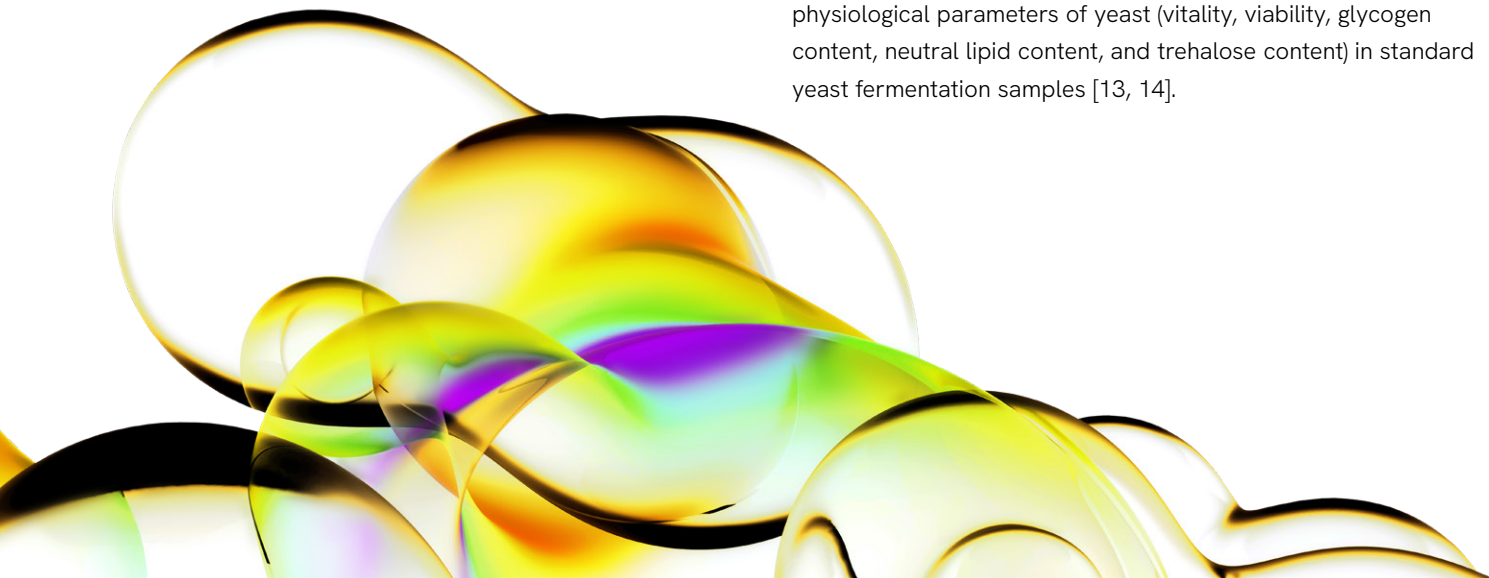
# Measuring physiological and metabolic characteristics of yeast health for beer fermentation samples using the Cellometer Vision.

## Introduction

Characteristics such as the viability, vitality, glycogen, neutral lipid, and trehalose content of yeast samples must be measured to better understand changes in physiological and metabolic status during fermentation [4-9]. Monitoring changes of these parameters during fermentation can improve current production processes, and a subset of these parameters (glycogen, neutral lipid, and trehalose content) has been shown to play an important role in predicting yeast viability both during and after fermentation [10, 11].

“Viability” ascertains whether the yeast cell is alive or dead, which can be determined by membrane integrity stains. We define “vitality” as a yeast cell’s metabolic activity as measured by dyes such as methylene blue, carboxyfluorescein diacetate and Calcein AM, which become fluorescent when acetate residues are cleaved by active esterase enzymes inside the cell. [8]. Glycogen controls the functionality of yeast during fermentation [9, 12] and provides the energy and carbohydrates required for sterol and lipid synthesis. Neutral lipids are energy-rich molecules stored in yeast that are synthesized early in the lag phase of fermentation due to stress, but not in the logarithmic phase. Fatty acids in neutral lipids cannot be metabolized, thus the level of neutral lipid remains consistent or increases during fermentation [9]. Trehalose is a disaccharide that supplies energy during the cell cycle. It can also protect yeast against high alcohol concentration, heat, dehydration, oxidation, pH, and osmotic stress. Similar to neutral lipids, trehalose’s protective attributes may allow for prolonged ethanol production during fermentation, which can potentially reduce overall production time.

In this overview, we will describe a 2012 study that demonstrates the use of Cellometer® Vision to examine five physiological parameters of yeast (vitality, viability, glycogen content, neutral lipid content, and trehalose content) in standard yeast fermentation samples [13, 14].



## Materials and methods

### Preparation of yeast fermentation samples

Single colonies of *S. cerevisiae* EBY100 were inoculated into liquid YPD (Difco). Samples of the diluted cultures were collected at 5-, 10-, 25-, and 30-h time points to measure nutritional parameters during the lag, logarithmic, stationary, and nutrient-deprived phases of growth, respectively.

### Fluorescent staining for five physiological parameters

For each time point sample: a solution of acridine orange and propidium iodide was used to measure the viability, a CFDA solution was added to measure vitality, an acriflavine solution was added to assess glycogen, Nile red was used to analyze neutral lipid content, and Con A-FITC was added to measure trehalose content. All stained samples were analyzed by image cytometry in quadruplicate.

### Fluorescent imaging of yeast fermentation samples over four time points

Three different user-changeable fluorescence optics modules (FOM) were used according to the respective fluorescent stains. VB-535-402 was used for detection of AO, CFDA, acriflavine, and Con A-FITC. VB-595-402 and VB-660-502 were used for the Nile red and PI stains, respectively.

Brightfield imaging was used to locate and focus on the yeast cells. After focusing, fluorescent images were captured and analyzed using the Cellometer software. FCS Express™ 4 Flow Cytometry software (De Novo Software) was used for interactive data analysis and presentation of target cell populations.

## Results

**Figure 1. Viability images.**

- Viability was measured by the number of AO and PI positive yeast in each sample and calculated by using the mathematical formula,  $(\# \text{AO}^+ \text{ cells}) / (\# \text{AO}^+ \text{ cells} + \# \text{PI}^+ \text{ cells})$ . Fluorescent images (pseudo-colored green and red) consistently show a large number of AO-positive cells at each time point.

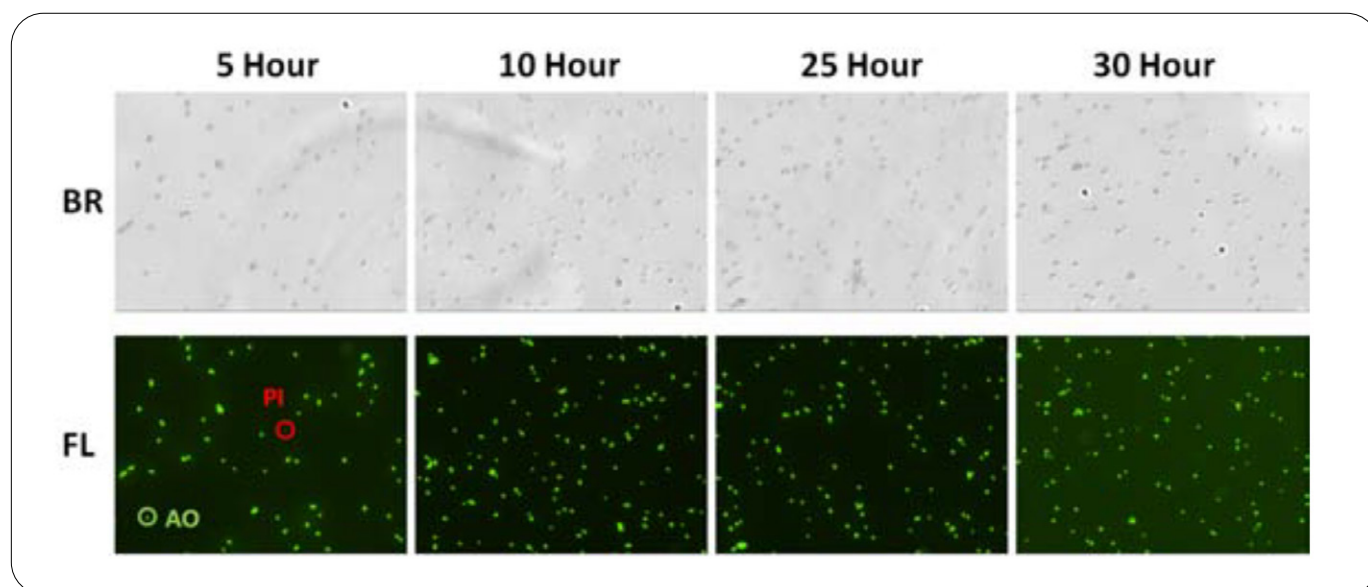


Figure 1: Brightfield (top) and fluorescent images of AO (in green)/PI (in red)-stained yeast to assess viability at the four time points.

**Figure 2. Kinetic plot for percent positive staining and mean fluorescent intensity**

- **Viability** percentages (2a; AO/PI) were consistent at ~99%.
- **Vitality** detection (2a; CFDA) showed a large reduction in metabolic activity in the cell populations over the course of their growth cycle. Active yeast percentages (2a) decreased from 31.1% to 3.2% as expected, as yeast become inactive due to lack of nutrients. In general, although fermenting yeast display high viability, this does not necessarily correspond to the vitality of yeast cells.
- **Glycogen** content (2a; acriflavine) decreased from 90.0% to 30.8%. The mean acriflavine fluorescence intensity also decreased with respect to fermentation time (2b), which indicated that glycogen was actively broken down during fermentation.
- **Neutral lipid** positive yeast (2a; Nile red) increased from 70.8% in lag phase to 87.6% during logarithmic phase before gradually decreasing to 71.9% at the 30-h time point.
- **Trehalose** positive yeast (2a; Con A-FITC) first increased from 26.10% to 51.74% and then decreased to 42.80% at the 30-h time point.

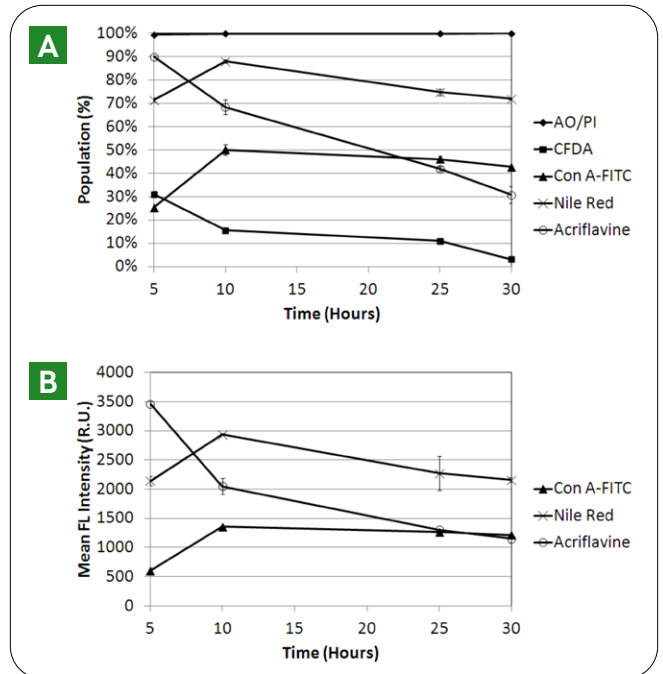


Figure 2: Kinetic plot of physiological changes during a 30-h culture period. a) Changes in highly fluorescent positive yeast populations for AO/PI, CFDA, acriflavine, Nile red, and Con A-FITC. b) The mean fluorescence intensity of acriflavine, Nile red, and Con A-FITC-stained yeast.

**Figure 3. Vitality images.**

- The fluorescent images shown here (pseudo-colored green) demonstrate decreasing vitality (as measured by CFDA fluorescence) as the yeast culture progressed to 30 h.

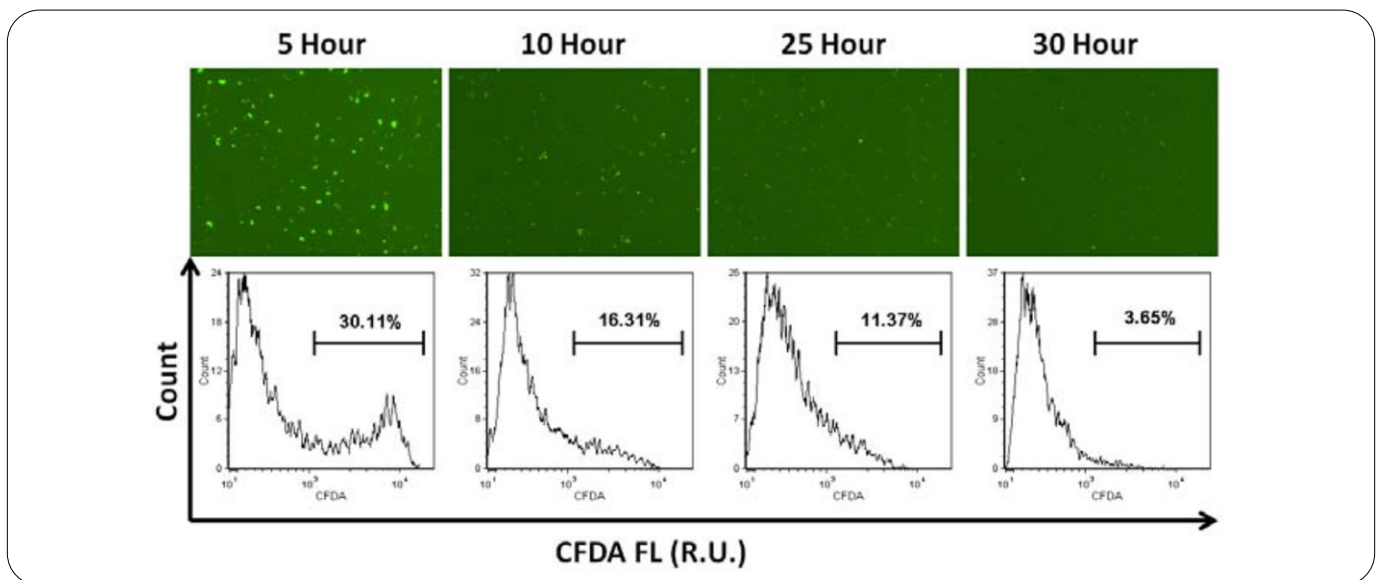


Figure 3: Fluorescent images and corresponding histograms of the counts of CFDA-stained yeast to assess yeast vitality at each time point.

**Figure 4. Glycogen content images.**

- The pseudo-colored fluorescent images show glycogen content (as measured by acriflavine) where reduction in fluorescence intensities throughout the growth period is clearly visible, and consistent with previous findings for

glycogen catabolism in yeast cells during growth [3, 9, 11]. In addition, this trend was directly correlated to vitality, as measured by CFDA.

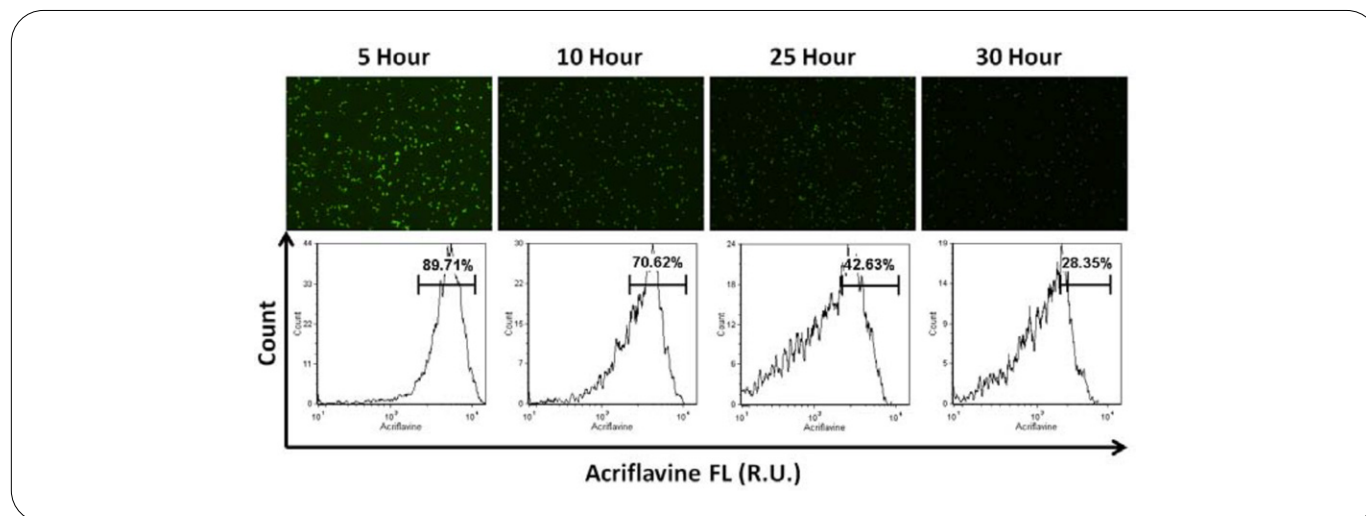


Figure 4: Fluorescent images and corresponding histograms of the counts of acriflavine-stained yeast to assess glycogen content at each time point.

**Figure 5. Neutral lipid content images.**

- The pseudo-colored fluorescent images show neutral lipid content (as measured by Nile red). Neutral lipid staining peaked during log phase growth, and decreased slightly in number and fluorescent intensity over the growth period. These results are consistent with previous work showing that neutral lipids are only synthesized during the beginning

of the growth phase, and do not break down as easily as glycogen [9]. According to previous work, neutral lipid content does increase under stress as well as prolonged fermentation for storage of energy [8, 15], but our results showed no significant accumulation of neutral lipids up to the 30-h time point.

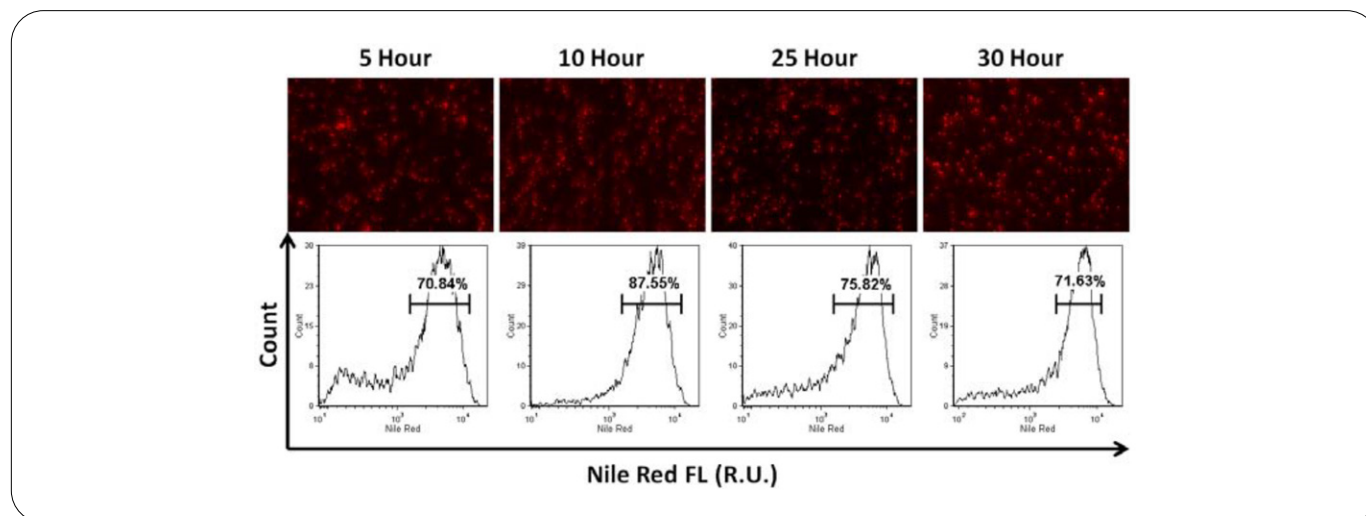


Figure 5: Fluorescent images and corresponding histograms of the counts of Nile red-stained yeast to assess neutral lipid content at each time point.

**Figure 6. Trehalose images.**

- The pseudo-colored images demonstrate fluorescence intensities of Con A-FITC-stained yeast. According to previous publications, trehalose is mobilized early during the growth period to provide energy for fermentation and then decreases over time as nutrients in the media have been depleted [4, 9, 11]. It has also been shown that as the

ethanol percentage in the environment increases, trehalose accumulates to protect the cells from toxic agents [4, 16]. Our results showed a gradual decrease in average fluorescence intensity up to the 30-h time point, which indicates that no significant trehalose storage had occurred.

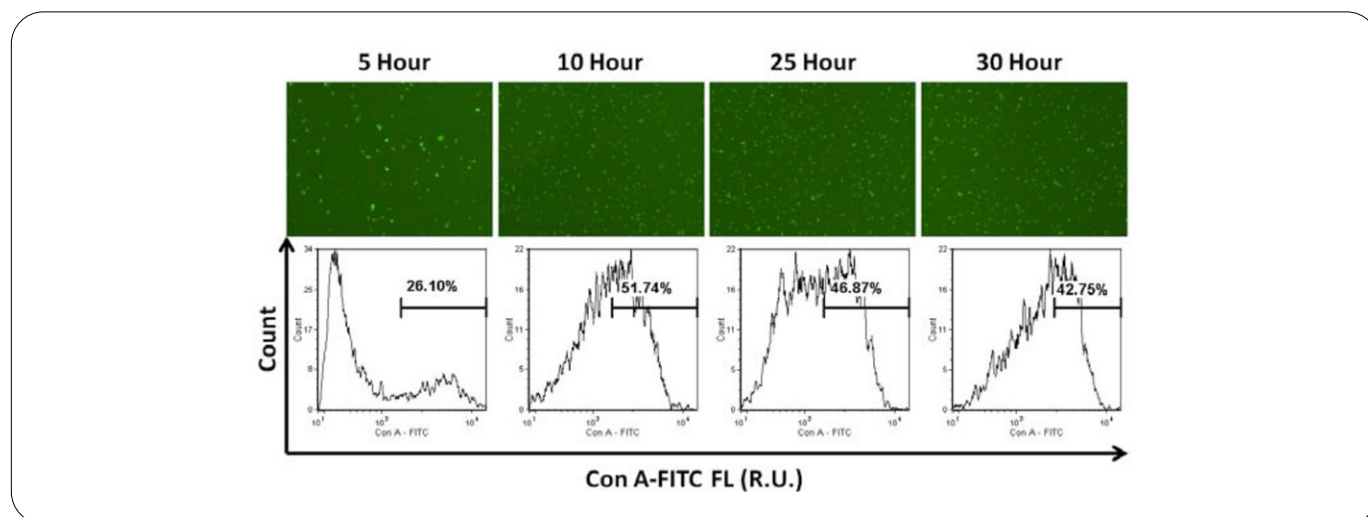


Figure 6: Fluorescent images and corresponding histograms of the counts of Con A-FITC-stained yeast to assess trehalose content at each time point.

**Conclusion**

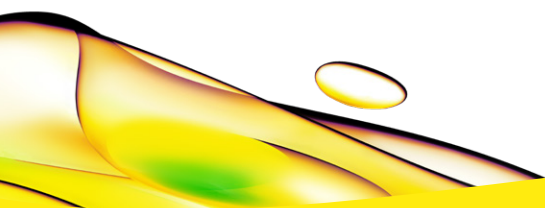
- This demonstrates a novel, efficient, convenient detection method for kinetic physiological measurements of *S. cerevisiae* using the Cellometer Vision image cytometer in standard fermentation samples. The ability to measure and optimize these physiological markers during fermentation can be used to improve factors such as consistency, flavor, and shelf life.
- This method addresses the training requirements and the challenges of automation, reproducibility, and affordability seen with current techniques.

**References**

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