Technical Note 202

Counting Yeast Cells for the Brewing Industry

Introduction

Many fermentation processes require accurate yeast concentration and viability measurements prior to their start, and active monitoring of these same metrics during the fermentation. The focus of this technical note is to give a detailed description on how to quantify yeast cells on CellDrop™ Automated Cell Counters using the DeNovix Yeast Assay Kit.

Many yeast samples from brewing contain extracellular debris and are small in size, making them difficult to count using a traditional hemocytometer or brightfield cell counter. This technical note will provide the information needed to accurately count yeast concentration and viability using the fluorescent dyes fluorescein diacetate (FDA) and propidium iodide (PI).

Materials and Methods

Measurements were performed using the DeNovix Yeast Assay kit. Fresh *S. cerevisiae* sample cultured at room temperature in YPD media was diluted to approximately 2×10^6 cells/mL with assay buffer. For each measurement, 18 µL of yeast sample was combined with 1 µL of FDA and 1 µL of PI solutions. The reaction solution was mixed well and incubated at room temperature in the dark for 15 minutes. The sample was mixed by pipetting up and down before dispensing 10 µL into the CellDrop counting chamber.

Viability analysis was performed with the CellDrop FL in the Yeast app, which uses green and red filters to count live cells fluorescing green with FDA, and dead cells fluorescing red with PI. The CellDrop Yeast App default protocol was used for the count.

Note: Protocol optimization may be required for different yeast strains

Protocol Settings

CellDrop EasyApps® are designed to count a variety of cell lines with minimal customization. If necessary, the user has the ability to optimize count settings after a count has been taken or develop a new protocol. Protocols include several powerful settings to enable proper counting off all yeast cells in a sample while ignoring any debris that could compromise the count data. See Tech Note 189 – Count Settings for additional details.

Optimizing Count Settings

Count settings may be edited after a count is performed by pressing “Optimize Settings” on the results screen. This feature allows a user to assess what effect a parameter change has on cell count versus the previous parameter/count. Though some adjustments may still be required, the recommended settings for counting *S. cerevisiae* sample shown in Figure 1 are as follows:

Count Application	Yeast
Chamber Height	100 µm
Dilution Factor	1.11
Diameter(min)	2 µm
Diameter (max)	10 µm
Live Roundness	1
Dead Roundness	1
Green Fluorescence Threshold	1
Red Fluorescence Threshold	1

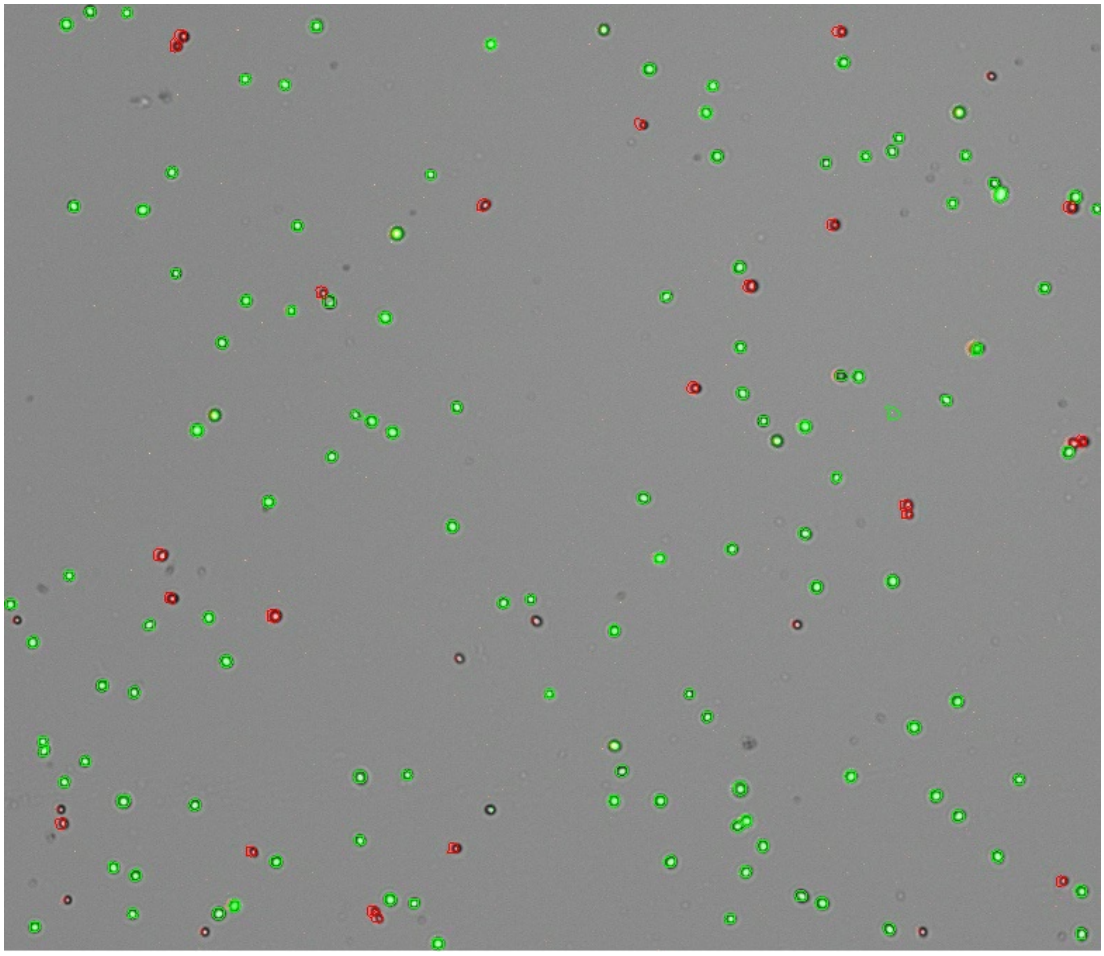

Figure 1: Zoomed result image of counted yeast cells in the Yeast app. Cells fluorescing green are live and cells fluorescing red are dead, additional uncounted debris can also be observed.

Summary

Automated counting of yeast samples on the CellDrop FL removes operator variability from the process and speeds up the workflow. The Yeast app reports data for total, live, and dead cell counts in terms of counted cells and cells/mL, as well as mean cell diameter and percent viability. The CellDrop FL with dual color fluorescence has powerful counting algorithms and customizable count settings to enable accurate counting and viability determination of a wide variety of cell types.

Further information:

Technical Note 185 – Yeast Assay (FDA/PI) Protocol

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