Application Note

Counting Yeast Cells for Brewing and Wine Industries Can Be Facilitated by the Scepter™ 2.0 Handheld Automated Cell Counter

Introduction

Yeast cells are critical to the fermentation process. During fermentation, carbohydrates present in the wort (a water-based solution of grain sugars) are converted by yeast into alcohol, carbon dioxide, and numerous byproducts. There are several stages in the process at which analysis of the active yeast culture is advantageous. The first, and most critical point for cell enumeration, is during the pitching process. Pitching is the initiation of the fermentation process, in which a live yeast culture is introduced into the wort (Figure 1). Introduction of a consistent cell concentration is required for successful fermentation as well as to maintain batch-to-batch reproducibility. In addition, samples are often taken during fermentation proper. During this process, yeast cells undergo budding. As shown in Figure 1, yeast sampling is also done at the end of the fermentation process; recovery of yeast from the final product permits re-pitching (multiple uses of a single yeast stock). These samples will be significantly more complex than the starting content due to accumulation of protein. If not eliminated, this protein may interfere with accurate counting. Consistent harvesting and re-pitching practices ensure consistent fermentation and yeast performance over many cycles.

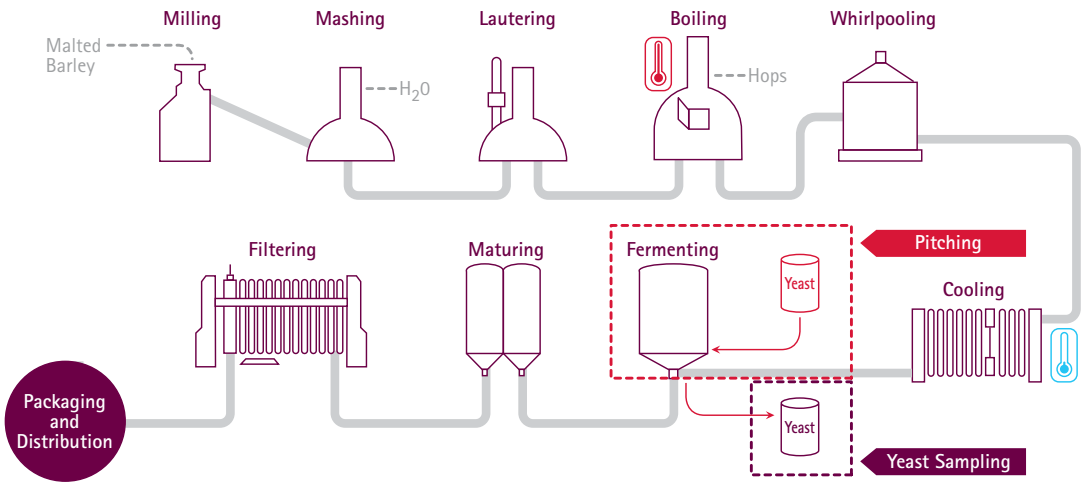


Figure 1.

The Brewing Process. Accurate counting of yeast cells is important during the pitching and yeast sampling steps of the brewing process.

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

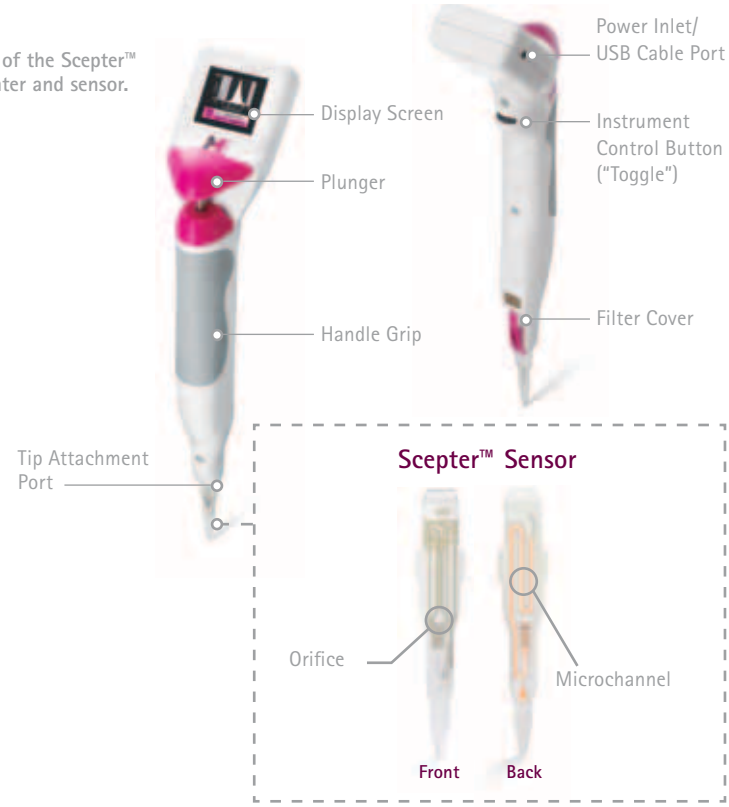
The Scepter™ cell counter offers an easy-to-use alternative to current methods of yeast cell counting. With the Scepter™ cell counter, EMD Millipore has captured the ease of automated instrumentation and accuracy of impedance-based counting using the Coulter principle in an affordable, handheld format (Figure 2). The instrumentation has been collapsed into a device the size of a pipette and uses a combination of analog and digital hardware for sensing, signal processing, data storage, and graphical display in the form of a histogram. The 40 µm- and 60 µm-aperture sensors placed at the tip of the instrument are engineered with a microfabricated, sensing zone that enables discrimination by cell size and cell volume at sub-micron and sub-picoliter resolution, respectively. Here, we outline the method for using Scepter™ counting for the enumeration of yeast cells.

Materials and Methods

Yeast Culture:

10 mg of dehydrated *Saccharomyces cerevisiae* (ATCC #7754) was added to 500 µL H_2O, vortexed 15 sec, and incubated for 5 min at room temperature to rehydrate. 100 µL of this stock was then used to initiate a culture in 250 mL Sabouraud Dextrose Broth. The culture was grown in a shaker (265 RPM) for 20 hours at 30 °C. Upon completion, a 50 mL aliquot was centrifuged, and the pellet was resuspended in 50 mL phosphate-buffered saline (PBS). Using the Coulter Counter® (Beckman Coulter), an initial concentration was determined for this sample. Theoretical concentrations were determined by dividing the initial concentration by dilution factors. The sample was then diluted to 1.5 x 10^6 cells/ mL and subsequent serial dilution in PBS was performed. PBS contains an optimal salt concentration for sufficient conductivity required for optimal counting performance. For each test, we used the recommended sample volume of 100 µL in a 1.5 mL microcentrifuge tube. Other tubes may not be able to accommodate the width of the sensor, or provide sufficient sample depth for the instrument to function properly. Since cells settle quickly, we kept the yeast cell suspension well mixed prior to testing to ensure reproducible counts.

Figure 2.
Diagram of the Scepter™ cell counter and sensor.



Scepter™ Cell Counting:

Operation of the Scepter™ cell counter is similar to using a standard laboratory pipette. The Scepter™ cell counter is turned on by depressing and holding the toggle on the back of the instrument. Once on, the instrument will prompt the user to attach a sensor. The Scepter™ unit displays detailed on-screen instructions for each step of the counting process. Briefly, depress the plunger and submerge the sensor into the solution. Next, release the plunger to draw 50 µL of cell suspension into the sensor. The Scepter™ cell counter detects each particle passing through the sensor's aperture, then calculates concentration and displays a histogram of cell diameter or volume on its screen.

Scepter™ Data Analysis:

The upper and lower limits of the histogram, called gates, are either set automatically based on the histogram profile, or can be set to the same gates used in the previous count. After the count is complete and the histogram is displayed on the instrument, the gates can be moved manually to fine-tune the analysis. Up to 72 histograms can be stored on the instrument itself. All test data files can also be uploaded to a computer and further analyzed using Scepter™ Software Pro.

Other Counting Methods:

For comparative studies, counts were also performed using the Z2 Coulter Counter® (Beckman Coulter) according to manufacturer's instructions using the same starting suspension and serially diluted samples.

Results

Samples of varying concentrations from a single overnight culture were used to assess the feasibility of counting yeast with a Scepter™ cell counter fitted with 40 µm aperture sensors. For all concentrations tested, yeast samples yielded interpretable histograms that could be gated and used to calculate yeast size and concentration. Examples of these histograms are shown in Figure 3.

Comparative performance

To assess the range and linearity of Scepter™ cell counter's performance, we plotted the measured concentration values (mean of 4 replicates) versus the corresponding theoretical concentrations across the dilution range. Identical samples were tested in

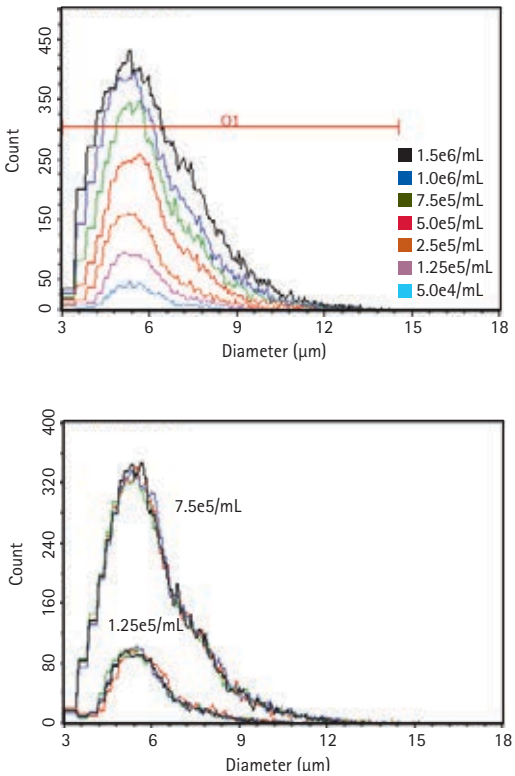


Figure 3.

Scepter™ Software Pro permits yeast cell analysis and display of single samples or in batch format through histogram overlays. The plot on the top is an overlay of histograms from each of the 7 steps in the dilution series. On the bottom, 4 replicates at each of two concentrations have been overlaid to demonstrate measurement reproducibility.

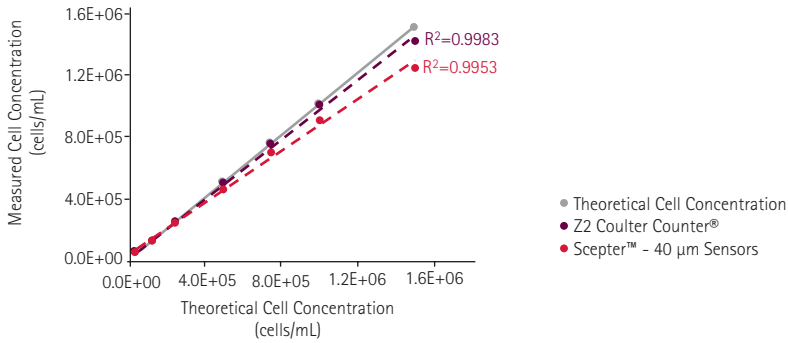


Figure 4.

For Scepter™ cell counter and Z2 Coulter Counter®, measured yeast cell concentrations are plotted against the theoretical concentrations. The solid gray line represents the theoretical values. For each device, the dotted lines represent best linear fit to the seven data points. Both platforms show a loss of linearity and counting accuracy with the increase in cell concentration.

Theoretical concentration (cells/mL)	Scepter™ Cell Counter with 40 µm sensors		Z2 Coulter Counter®	
	Conc. (cells/mL)	%CV	Conc. (cells/mL)	%CV
50,000	53,950	2.1	53,893	1.7
125,000	129,175	1.4	132,708	1.1
250,000	252,375	3.8	257,115	0.5
500,000	461,425	1.3	499,695	0.6
750,000	699,500	0.9	744,963	2.9
1,000,000	903,250	0.2	1,003,200	1.4
1,500,000	1,241,250	2.5	1,413,195	0.3

Table 1.
Comparative Analysis of Yeast Counts

parallel on the Coulter Counter® (Figure 4). While both instruments demonstrated high linearity in sample counting (Scepter™ R^2 0.995; Coulter Counter® R^2 0.998), each device lost accuracy at higher concentrations. For the Scepter™ cell counter, this result may be partially due to the fact that at higher concentrations, a small part of the histogram peak was not detected and cut off, leading to underestimation of the cell count. The precision in counting was determined by calculating the coefficients of variation (% CV) at each data point (Table 1). Overall, the two instruments showed equivalent performance.

The Scepter™ cell counter's ability to accurately determine particle size comes from the precise diameter of the sensor's laser-drilled aperture. The 40 µm tip is capable of accurately measuring particles in the 4-16 µm range. Budding yeast (*S. cerevisiae*) are roughly spherical in shape with a diameter of 5-10 µm. To assess the accuracy of the Scepter™ cell counter in reporting yeast cell size, we compared cell diameters measured at the various sample dilutions (Figure 5). Overall, little variability was found in the measured size on either device (overall size ranges: Scepter™ 6.00-6.30; Z2 Coulter Counter® 5.92-6.41). However, for each device, we consistently found a slight size increase with increasing cell concentration on both platforms.

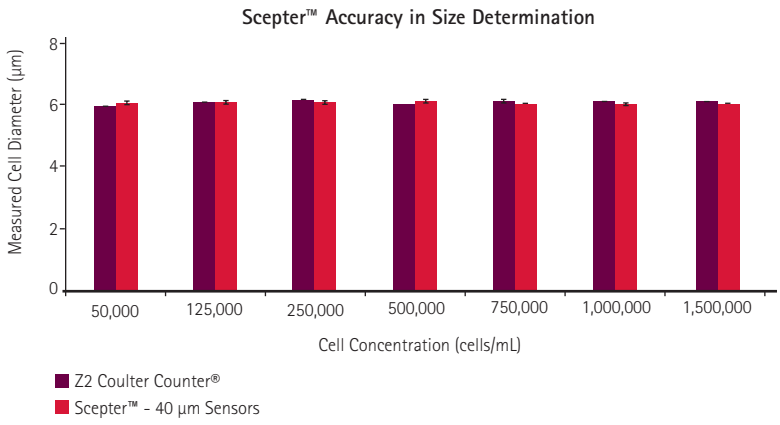


Figure 5.

A range of yeast cell concentrations (5 x 10^4 – 1.5 x 10^6) was counted on the Scepter™ and Coulter Counter® devices. Error bars represent standard deviation of 4 replicate measurements. Results demonstrate the accuracy and reproducibility of both methods in size determination.

Summary

As a tool for the brewing industry, the Scepter™ cell counter provides an inexpensive alternative with comparable performance to other automated cell counting instruments. This performance quality combined with Scepter™ cell counter's handheld and intuitive format suggest that the Scepter™ cell counter is well suited for the daily workflow of the brewing process, and can help ensure reproducibility of fermentation from batch to batch and over time.

As a testament to the utility of Scepter™ counting in the brewing industry, a spokesperson for the renowned Sinebrychoff Brewery in Finland (part of the Carlsberg Group) described the methods leading to the brewery's successful use of the Scepter™ cell counter:

"We use Scepter™ automatic cell counter to determine the number of yeast cells in our pitching yeast. Depending on the brewing yeast strain and the present condition of the yeast, the cell number count may vary. In general, the yeast sample has to be diluted 1:50,000; we use physiological saline as diluent. We used Auto gating method with 40 µm sensors, the range of measured particles were between 3-10 µm. The cell number in pitching yeast is used to monitor trends. Scepter™ is very fast and easy to handle, so it saves a lot of time counting yeast cells. Also, as Scepter™ gives very linear results, this means we can get consistent counts from person to person. In addition, the portability of the Scepter™ facilitates the counting."

Ordering Information

Description	Quantity	Catalog No.
Scepter™ 2.0 Handheld Automated Cell Counter		
with 40 µm Scepter™ Sensors (50 Pack)	1	PHCC20040
with 60 µm Scepter™ Sensors (50 Pack)	1	PHCC20060
Includes:		
Scepter™ Cell Counter	1	
Downloadable Scepter™ Software	1	
O-Rings	2	
Scepter™ Test Beads	1	PHCCBEADS
Scepter™ USB Cable	1	PHCCCABLE
Scepter™ Sensors, 60 µm	50	PHCC60050
	500	PHCC60500
Scepter™ Sensors, 40 µm	50	PHCC40050
	500	PHCC40500
Universal Power Adapter	1	PHCCPOWER
Scepter™ O-Ring Kit, includes 2 O-rings and 1 filter cover	1	PHCCOCLIP

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