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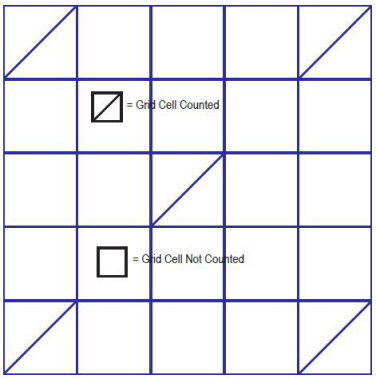
APPLICATION NOTE

Yeast Viability Measurements in Fermentation Studies

OVERVIEW

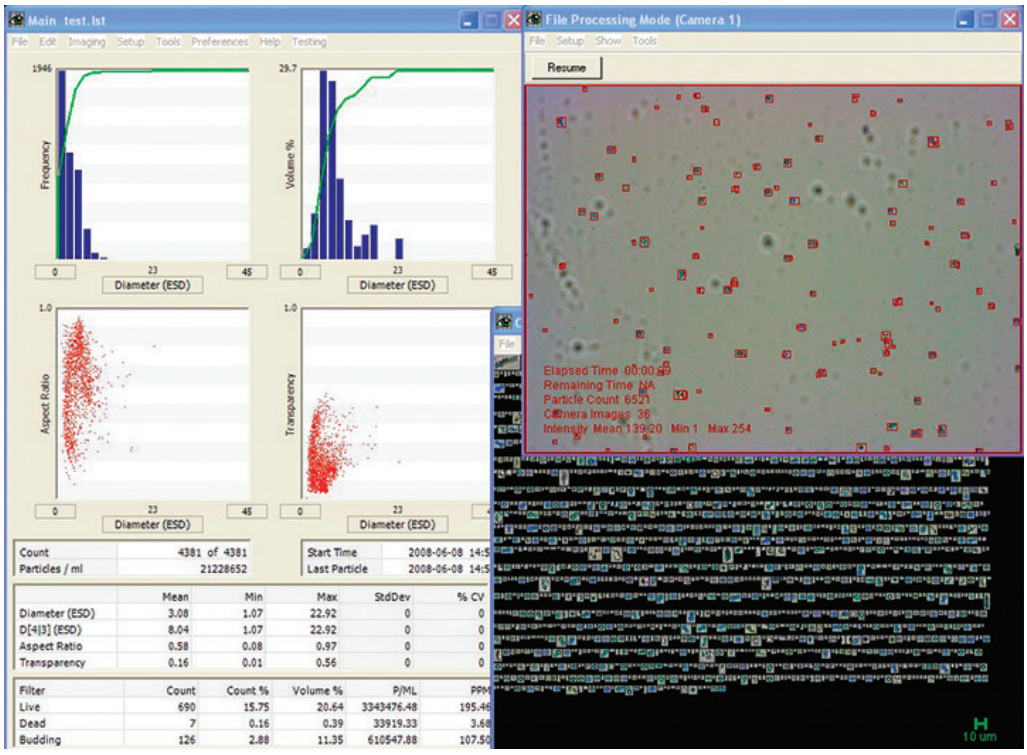
An important component of fermentation processes is to continually monitor yeast growth and viability. The most common method for doing this is using the ASBC hemocytometer count method. In this method, samples are taken from the fermentation vessel, stained with methylene blue, and then counted manually under a microscope using a hemocytometer.



While this method is well known and documented, it is, at best, an estimate based upon a very small sample count. The hemocytometer, when viewed under a microscope, presents a grid of measurement areas as seen at right.

Because of the time involved for an operator to do manual counting, only a small number of actual grid cells are counted, with the results then being interpolated as an average number. Not only is the sample size very small, which yields low statistical significance, but it is known that up to 25% error can be introduced merely by "operator interpretation".

It was desired to develop a method for making the yeast counts more precise, increase the statistical significance by looking at a larger sample, and to eliminate the time and potential operator error for this procedure.



METHOD

The FlowCam is ideally suited to automate this process. It can image, count and measure thousands of individual yeast cells in the time it takes for an operator to count only tens of cells using the hemocytometer method. The VisualSpreadsheet© software automatically produces a count of live, dead and budding yeast cells without any operator being involved. This normalizes out human error, and provides extremely precise and repeatable results. Furthermore, the numbers have a much higher statistical significance due to the larger data populations obtained by the FlowCam.

APP NOTE CONTINUED ON REVERSE

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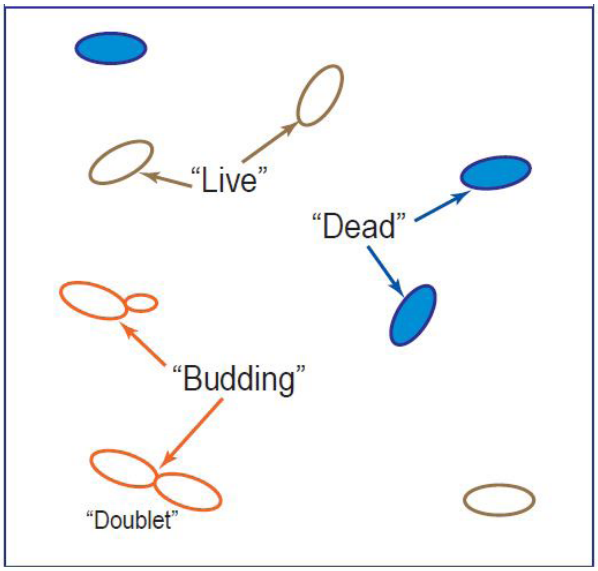
The yeast samples are taken from the fermentation vessel and prepared just as they are for the hemocytometer method by staining with methylene blue. The sample is then run through the FlowCam in autoimage mode at seven frames per second as it flows through the flow cell. Every yeast cell is imaged, stored and measured during acquisition.

As seen above, the FlowCam automatically captures each yeast cell as a single stored image from the fluid flow. During image capture, up to 26 different spatial and gray-scale measurements are recorded and indexed to the individual cell images.

When the yeast cells are stained with the methylene blue, dead cells will uptake the stain, causing them to appear blue to the camera. The diagram below shows how the cells would be counted in the hemocytometer.



For the FlowCam, differentiating between the live and dead cells is quite straightforward, and is based primarily on the "average blue" value recorded for the cell image (along with several shape measurements). The "budding" cells present a bit more difficult challenge, however, due to the fact that the resolution needed to accurately differentiate a single "live" cell from a "budding" cell is much higher than can be obtained with the FlowCam.

However, a simple solution to this is to simply look for "doublets", which are two yeast cells which have already "budded" and about to separate. The key thing we are looking for when counting "budding" cells is that the yeast is still viable and growing. So, to measure "budding", we simply filter for the "doublets", and then count each one of these as two "live" cells, and one "budding". The trend is the important measurement, not the absolute number.

RESULTS AND CONCLUSIONS

The images on the next page show how the FlowCam automatically calculates the concentration of live, dead and budding yeast cells. A total of 8,709 yeast cells were automatically characterized by the FlowCam in 35 seconds. Unlike the hemocytometer counts, this is not an estimate based upon extrapolation, rather it is a real count. The FlowCam also automatically calculates the concentration for each cell type as part of the process.

This large amount of data makes the FlowCam results much more statistically significant. And because of the elimination of human interpretation, the FlowCam results show extreme precision over multiple runs, with generally as small as 1% variability.

As stated previously, the filters to be used for characterizing the yeasts only need to be defined once. After the filters are defined, they can be re-used for all subsequent samples. The filters are easily defined in VisualSpreadsheet; the operator merely identifies particle images of the desired type by clicking on them, and then instructs the software to save these as a filter. The filter then simply looks for "similar" particles using statistical pattern recognition. From that point on, the analysis is entirely automated.

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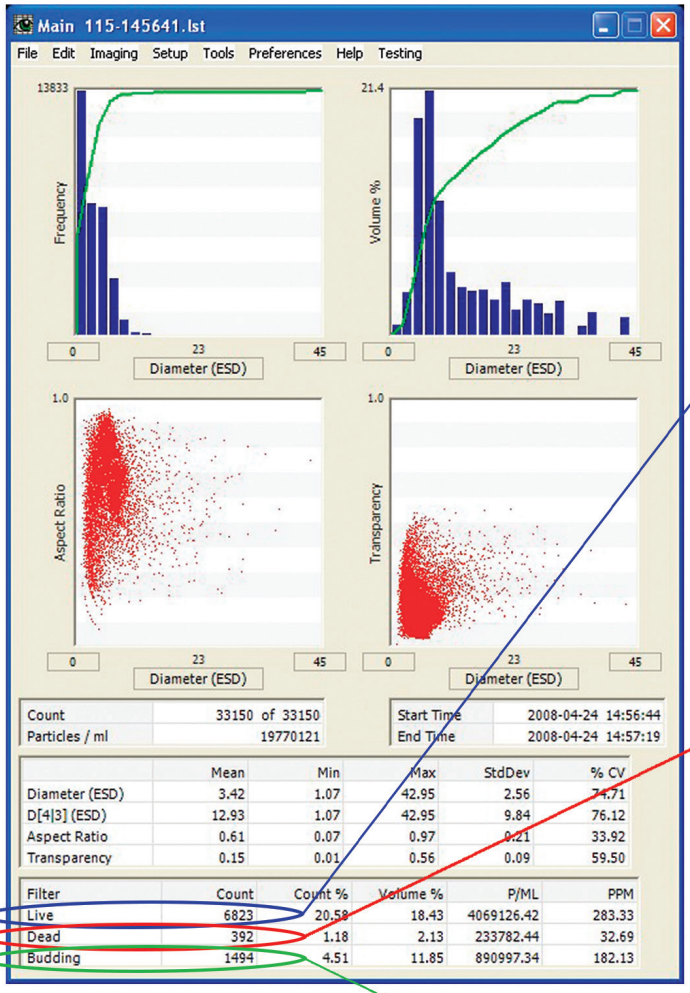
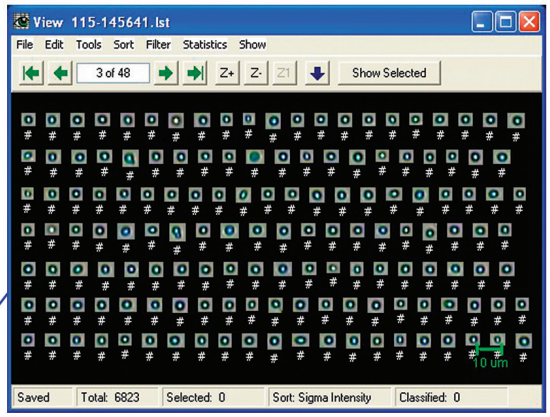
Yeast Viability Measurements in Fermentation Studies

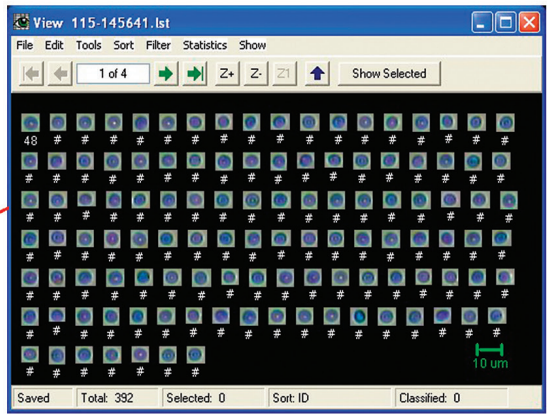


Total time to acquire,
measure and characterize
8,709 cells: 39 seconds



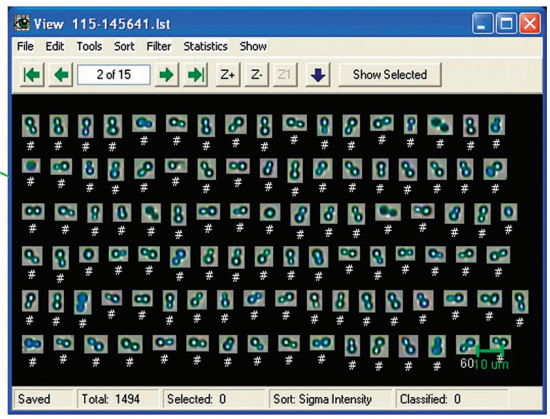
Live Count: 6,823

Concentration: 4.07M cells/ml



Dead Count: 392

Concentration: 234K cells/ml



Budding Count: 1,494

Concentration: 891K cells/ml

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