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Comparison of fluorescence methods for determining yeast viability using a novel automated image-based cell counting and viability system

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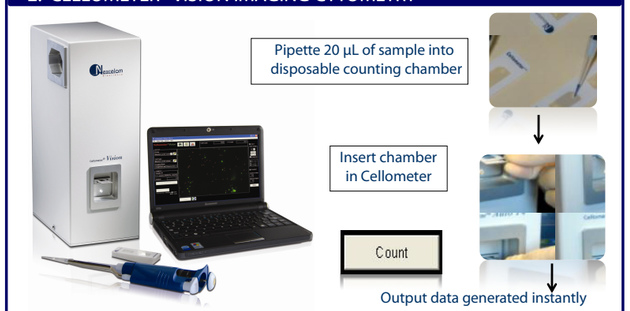
1. ABSTRACT

Yeast viability is an important parameter that can affect fermentation performance in a brewery, which can dictate the quality of the end product. Traditional methods for determining viability involve either counting cultured yeast colonies on agar or counting methylene blue-stained yeast cells using a hemacytometer and a microscope. Faster and more robust technologies such as flow cytometry or absorbance plate readers involve the use of fluorescent staining rather than colorimetric stains. Although standard practices, the traditional methods have some advantages but also some well-recognized drawbacks. The hemacytometer and microscope are an image-based technology, which allows counting of single or chain forming yeasts, but they are labor-intensive and prone to human error when evaluating multiple samples. Flow cytometry is an automated and high throughput cell counting technology, but it cannot be used for chain forming yeasts. Previous comparative studies have shown discrepancies in determining the appropriate yeast viability staining assay, which may be due to the differences and drawbacks of traditional and flow cytometry analysis technologies. Here we present a recently developed platform for bright field and fluorescence image-based cell counting and viability measurements that allows for direct comparisons between different fluorescent staining methods without the previously mentioned limitations of manual counting and flow cytometry. The system performs automated cell counting, which reduces assay time and is more objective, allowing for higher throughput analysis and more robust results. In addition, the imaging capability allows declustering of chain-forming yeasts, which improves the accuracy of the results in the presence of cellular aggregates. This system was used to compare three fluorescent viability stains: bis-(1,3-dibutylbarbituric acid) trimethine oxonol ($DiBAC_4(3)$, oxonol), propidium iodide (PI), and the magnesium salt of 8-anilino-1-naphthalenesulfonic acid (MgANS) on various yeast samples, which resulted in identical viability determinations for the same sample, in contrast to previous publications. We propose that this platform can be used for studies on different yeast strains and various culture conditions to determine the robustness of various staining methods with the goal of establishing a standard, optimized method for yeast viability measurements that is more reliable than the traditional methylene blue stain followed by manual counting.

2. CELLOMETER® VISION IMAGING CYTOMETRY



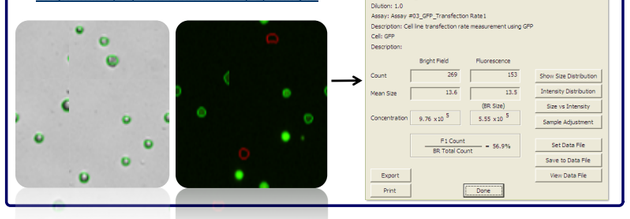
Pipette 20 µL of sample into disposable counting chamber

Insert chamber in Cellometer

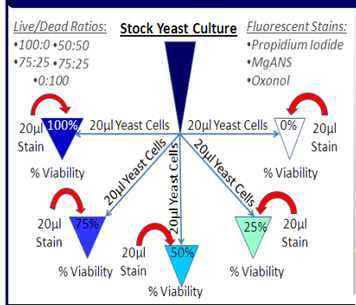
Count

Output data generated instantly

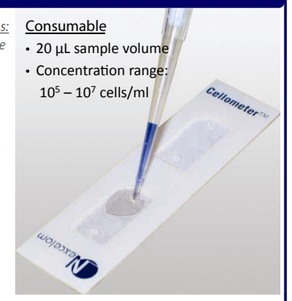
Bright-field (BR) and fluorescent (FL) images



3. YEAST VIABILITY EXPERIMENT PROCEDURE



Consumable
- 20 µL sample volume
- Concentration range: 10^5 – 10^7 cells/ml



Experimental Protocol

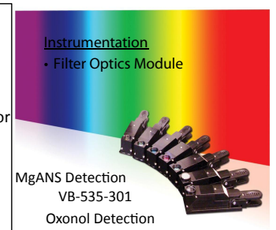
•Dried yeast pellets suspended in 6 ml PBS medium and allowed to rehydrate for 10 minutes.

•Half of the yeast cell solution was heat-killed for 20 minutes on a hot plate.

•Different live/dead yeast cells were mixed at 100%/0%; 75%/25%; 50%/50%; 25%/75%; 0%/100%.

•Each set of dilutions was stained using Propidium Iodide, Oxonol, and MgANS to test the viability of the yeast cells at various ratios of live/dead.

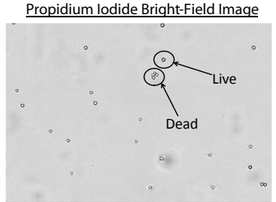
Instrumentation
- Filter Optics Module



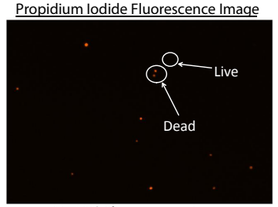
MgANS Detection VB-535-301
Oxonol Detection VB-535-301
Propidium Iodide Detection VB-595-501

4. BRIGHT-FIELD/FLUORESCENT IMAGES USING DIFFERENT STAINS

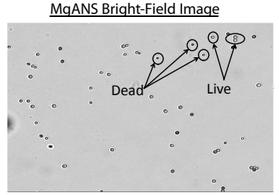
Propidium Iodide Bright-Field Image



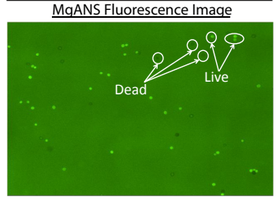
Propidium Iodide Fluorescence Image



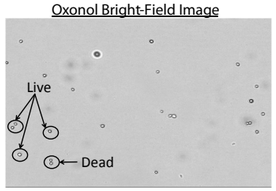
MgANS Bright-Field Image



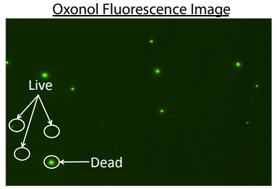
MgANS Fluorescence Image



Oxonol Bright-Field Image



Oxonol Fluorescence Image

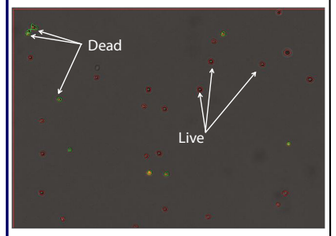


Fluorescence indicates Live/Dead Yeast Cells

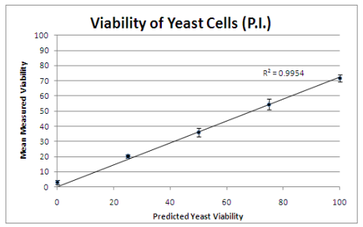
5. COMPARISON OF THREE FLUORESCENT STAINS AS VIABILITY MARKERS

Green/Red circles indicate counted live/dead yeast cells.

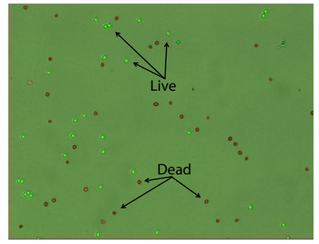
Propidium Iodide Combined Image



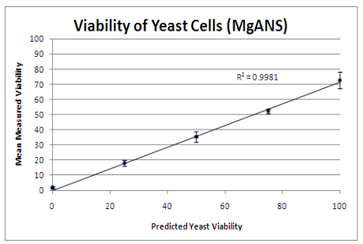
Propidium Iodide Viability Series Results



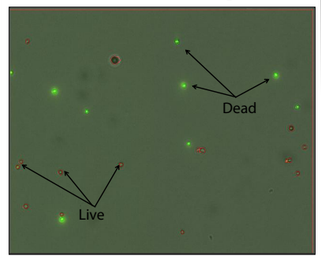
MgANS Combined Image



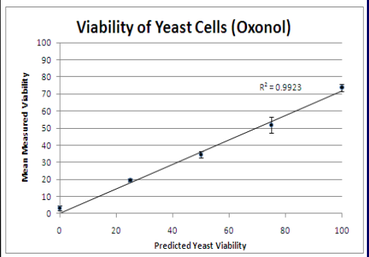
MgANS Viability Series Results



Oxonol Combined Image



Oxonol Viability Series Results



6. CONCLUSIONS

Propidium iodide, MgANS and oxonol were tested on the same yeast samples and resulted in identical viability measurements with a high correlation to predicted values, demonstrating the validity of these methods for determining yeast viability.

Contrary to previous reports, MgANS was found to stain the live cells and not the dead cells. This discrepancy will be investigated in future studies.

The system presented here is an appropriate platform for making direct comparisons between different fluorescence viability assays and thus can be used as a tool for determining the credibility of various other assays for determining yeast viability.