At the end of the 19th Century, Pasteur’s research showed that alcohol production from glucose was linked directly to yeast. The fermentation property of yeast is utilized in the beer, wine, and ethanol production industries. For optimum yeast fermentation performance, percentage cell viability and concentration must be monitored. The Beckman Coulter Vi-CELL automates the labor intensive manual trypan blue viability method. In addition, the Vi-CELL reports accurate and precise cell concentrations.

**Equipment Used**

Beckman Coulter, Vi-CELL XR
Validated Vi-CELL XR reagent pack

**Instrument Settings**

The Vi-CELL XR provides the high resolution necessary for accurate results on yeast and other small cells. The Vi-CELL software includes the optimum parameters for a Yeast Cell Type which the user may select by a mouse click. The instrument settings for yeast cells are the following:

- **Min. Size (µm)** = 3
- **Max. Size (µm)** = 20
- **Number of Images** = 50
- **Aspirate Cycle** = 1
- **Mixing Cycle** = 3
- **Cell Brightness** = 85
- **Cell Sharpness** = 100
- **Viable Cell Spot Brightness** = 40
- **Viable Cell Spot Area** = 1
- **Min. Circularity** = 0
- **Decluster Degree** = High

**Results**

The results reported by the Vi-CELL are shown in the Results Section of Figure 1. The percent viability and concentration of this yeast analysis is 99.3% and 9.49 x 10⁶ cells/ml. In addition, the instrument reports viable cell concentration, mean diameter, cell circularity and size distribution. The Vi-CELL is linear from 50,000 to 10 million cells/ml.

**Conclusion**

The Vi-CELL provides automation of the manual method of yeast viability measurements employed in fermentation processes. The instrument removes the subjective nature of the manual test, providing objective results for each assay.
THE VI-CELL
The VI-CELL automates the widely accepted Trypan Blue Dye Exclusion method. The Vi-CELL combines the state of the art in imaging technology, proprietary algorithm and fluids management. At the heart of the Vi-CELL is the customized liquid handling system. This system, which allows sample aspiration, reagent handling and subsequent instrument cleaning, is fully automated. Once the cellular suspension has been aspirated and mixed with the trypan blue dye, it is pumped to the flow cell for imaging. The Vi-CELL can analyze up to 100 images for a given analysis increasing total volume from 15 to 30 times over the manual method with result in a less that 2.5 minutes.

MANUAL TRYPAN BLUE DYE EXCLUSION METHOD
As mentioned, the standard method for measuring cell viability is the Trypan Blue Dye Exclusion method. Trypan blue stain (0.4%) is mixed with an equal volume of cells. Viable cells, given their intact membranes, exclude the trypan blue stain; non-viable cells, membrane permeable, stain dark blue. The manual method, however, requires a technician, using a hemacytometer and microscope, to enumerate both stained and unstained cells and manually calculate the percent viability. In addition to being labor intensive, this technique has substantial accuracy error due to its subjective nature.

VI-CELL TECHNICAL SPECIFICATIONS

INSTRUMENT FUNCTION:
Concentration Range:
5 x 10^4 to 1 x 10^7 cells / mL
*Counting Accuracy: ± 6%

OPERATING SYSTEM:
Windows® 98
Windows® 2000
Windows® XP

INSTRUMENT TYPE:
Video imaging through a quartz flow cell

POWER REQUIREMENTS:
Power 50 watts
(65 Watts Max.)
Voltagers 100V, 120V,
220V or 240V 50/60 Hz

TEMPERATURE:
10° to 40° C (50° to 104° F)

WEIGHT:
11.3kg (25lb)

UNIT DIMENSIONS:
44.5cm (17.5”) height
38cm (15”) width
41cm (16”) depth

VI-CELL SERIES

<table>
<thead>
<tr>
<th>PN</th>
<th>AUTO SAMPLE</th>
<th>SIZE RANGE (µm)</th>
<th>SAMPLE VOLUME (mL)</th>
<th>ANALYSIS TIME (Min)</th>
<th>VIABILITY RANGE</th>
<th>IMAGING TECHNOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI-CELL XR</td>
<td>Yes</td>
<td>2-70</td>
<td>0.5</td>
<td>&lt;2.5</td>
<td>0-100</td>
<td>Auto-focus routine Firewire Camera 1394 X 1040 CCD array</td>
</tr>
<tr>
<td>VI-CELL AS</td>
<td>Yes</td>
<td>5-70</td>
<td>1.0</td>
<td>&lt;3.5</td>
<td>0-100</td>
<td>Manual focus routine Image frame grabber 640 X 480 CCD array</td>
</tr>
<tr>
<td>VI-CELL S</td>
<td>No</td>
<td>5-70</td>
<td>1.0</td>
<td>&lt;3.5</td>
<td>0-100</td>
<td>Manual focus routine Image frame grabber 640 X 480 CCD array</td>
</tr>
<tr>
<td>VI-CELL XR QUAD PACK</td>
<td>383722</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI-CELL AS, S QUAD PACK</td>
<td>38198</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI-CELL CONCENTRATION CONTROL</td>
<td>175478</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI-CELL FOCUS CONTROL</td>
<td>175474</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>