Maintaining cell lines in culture, under optimal growth conditions, is essential for production of recombinant proteins. The overall health of the culture, whether in a bioreactor or flask, is generally assessed by the determination of both cell concentration and percentage of viable cells.

Many cell culture facilities, expanding cells in bioreactors or flask cultures, use the standard, manual, trypan blue vital dye-exclusion cell-viability assay. Viable cells, given their intact plasma membranes, exclude the trypan blue stain; nonviable, membrane-permeable cells stain dark blue. This method requires that an operator be present, using a hemacytometer and light microscope to enumerate both the stained and unstained cells. The percentage viability is then calculated.

This manual technique has significant limitations. It is both time-consuming and labor-intensive, and observed variation in results can be significant among users analyzing the same cell sample due to the subjective nature of the determination. In addition, only several hundred cells are generally counted, which means statistical confidence is low.

The manual, trypan blue viability method was compared to the automated Vi-Cell™ instrument (Figure 1) from the Miami, FL, facility of Beckman Coulter (Fullerton, CA).

Instrument linearity was also measured using both 10-µm latex beads and Chinese hamster ovary (CHO) cells, a common cell line used for recombinant protein production. CHO and hybridoma cell lines were maintained in standard tissue culture media, at approximately 25%, 35%, 45%, 55%, 65%, 75%, 85%, and 95% viability.

The Vi-Cell demonstrated excellent correlation with respect to viability when comparing the manual and auto-
mated methods (Figure 2). The Vi-Cell demonstrated concentration linearity when tested with both latex beads (53,000–10,000,000/mL; Figure 3) and CHO cells (across concentrations of 60,000 to over 12,000,000 cells/mL; Figure 4).

**Automated Measurement**

The Vi-Cell automates the standard, manual, trypan blue vital dye-exclusion assay for cellular viability. With the Vi-Cell, cell samples are mixed with 0.4% trypan blue and aspirated from autosampler cups. Cells are then drawn through a flow cell and imaged with a CCD camera in real time. The images can be archived for subsequent viewing and reanalyses if desired.

Proprietary algorithms developed by Beckman Coulter determine which cells have absorbed trypan blue stain: cells absorbing trypan blue appear darker, and thus have lower gray-scale values. Cells with higher gray-scale values are considered viable. Imaging parameters, called cell types, define what is a cell and whether it is viable or nonviable. These standard operating methods ensure consistency. The software also includes features to monitor bioreactors and other cell culture processes.

The Vi-Cell is designed to comply with FDA 21 CFR Part 11 regulations on electronic records and signatures. Cell data is listed for each image and the total number of images viewed (usually 100). The size range of the Vi-Cell is 3–70 µm.

Figure 5 shows the cellular image screen. In addition to the percent-viability parameter, the Vi-Cell instrument also provides other valuable cell measurements. For example, both total- and viable-cell concentration, as well as size distribution and cell circularity, are assayed. Figure 6 shows a size-distribution graph of a CHO cell population.

**Bioprocessing**

The bioprocessing feature (Figure 7) of the Vi-Cell is particularly relevant to the recombinant protein production environment. Cells are routinely cultured, at relatively high concentrations, in bioreactors. A bioprocess may be set up for each bioreactor, and the dynamic, changing cell parameters may be
monitored over time. Thus, adverse culture conditions may be quickly detected and corrected.

The bioprocess adds the two additional values of cellular-growth rate and doubling time. An expanded version of any of the graphs listed on the bioprocess screen may be obtained by clicking on the expanded graph button in the top right-hand corner of the relevant graph. Figure 8 shows an expanded graph of a CHO cell viable-cell–diameter distribution.

Figure 7. Bioprocess feature

Figure 8. Expanded bioprocess graph

**Conclusion**

The Vi-Cell instrument has been designed to report percent viability, as well as other significant cell parameters, within less than three minutes. The Vi-Cell analyses significantly more cells than can a hemacytometer, thus providing much greater statistical confidence. It eliminates the operator-to-operator count variation inherent with the use of the hemacytometer. In addition, the Vi-Cell enables the end user to view real-time cell images and archive them for future analyses.

For biopharma facilities, a bioprocess may be created for each bioreactor, thus allowing pertinent cell parameters to be monitored easily over time, and the instrument was developed to comply with 21 CFR Part 11 regulations for electronic records.

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