Automated Online Solution for Vi-CELL XR Cell Viability Analyzers

Particle Characterization Application Note

Introduction

In the current bioprocessing environment, a significant unmet need exists for automated, real-time cell analysis. This need has been driven by several factors including: (1) the Food and Drug Administration's process analytical technology (PAT) and quality by design (QBD) initiatives[1,2]; (2) increased demand for high-throughput bioprocess development[3]; (3) the advent of high cell density cell culture and continuous processing manufacturing processes[4]; and (4) adaptations to industry workforce reductions[5] As a result of these factors, industry trends are moving toward the implementation of automated PAT strategies that are designed to bring analytics closer to the operation, eliminate gaps in real-time process monitoring and develop a more rapid, deeper understanding of the bioprocess.

In order for automated online cell analysis to be an effective process PAT tool, certain criteria need to be met. First, the technology should provide rapid and accurate cell analysis that performs as good as, or better than, the manual off-line analytical method. Second, it should reduce analytical variability and sample contamination due to manual sampling and operator-to-operator error[6] Third, it should facilitate a scale-independent strategy that allows the same analytical instrumentation to be used from the laboratory through production. Fourth, the technology should allow the integration of other third-party analyzers, i.e., HPLC, biochemistry analyzers, to facilitate a comprehensive PAT platform. Lastly, the real-time data generated by the cell analyzer should be seamlessly integrated into the process supervisory control and data acquisition (SCADA) or bioprocess management system.

Flownamics® has developed an automated online analytical solution for Vi-CELL XR cell viability analyzers that meets the aforementioned criteria. By integrating the Vi-CELL XR analyzer with the Seg-Flow® automated online sampling system, a robust, rapid cell analysis PAT platform is now available for real-time cell characterization of bioprocess cell cultures. This application note describes the attributes and performance of the Vi-CELL XR/Seg-Flow integrated system.

The Automated Online Analytical Solution

Automated Cell Culture Monitoring

The Vi-CELL XR cell viability analyzer coupled with the Seg-Flow automated online sampling system provides automated, online analytical solutions for improved bioprocess culture monitoring. The Seg-Flow system, which can be interfaced with almost any vessel type or scale, draws samples from one to eight bioreactor vessels and delivers each sample to the Vi-CELL XR instrument. Once the cell analysis is complete, the Seg-Flow system acquires and processes the Vi-CELL XR data to provide real-time monitoring of bioprocess cultures. Additionally, the Seg-Flow's FlowWeb™ OLE-for-process-control (OPC) technology provides seamless integration of the Vi-CELL XR data into any OPC-enabled SCADA or bioprocess management system for enhanced bioprocess monitoring and control. Figure 1 shows the Vi-CELL XR/Seg-Flow system.

The Seg-Flow system's patented segmented online sampling technology permits cell-containing samples to be delivered precisely and rapidly from the cell culture vessel to the Vi-CELL XR instrument. This functionality minimizes sample processing time as well as preserves the analytical fidelity of the Vi-CELL XR cell analyzer. For high cell density cultures requiring dilution prior to

Figure 1. The Vi-CELL XR/Seg-Flow 4800 automated online cell analysis system.

cell analysis, i.e., perfusion or concentrated fed-batch processes, an in-line sample dilution module is available as a bolt-on option for the Seg-Flow system. Also, the Seg-Flow's single-use fluid path technology facilitates easy product changeover between batches and aligns with current single-use practices widely adopted throughout the bioprocessing industry.

Integrated PAT Solution

In addition to being utilized for automated online cell analysis, the Vi-CELL XR/Seg-Flow system can be integrated with additional analytical instruments, i.e., biochemistry analyzers, HPLC/UHPLC, fraction collectors, etc., to provide a comprehensive PAT strategy for more effective bioprocess management.

Figure 2 illustrates a typical automated online cell culture process PAT strategy for providing real-time cell, nutrient and metabolite monitoring. In this scenario, the real-time Vi-CELL XR data is communicated to the SCADA system using the OPC communication protocol provided by the Seg-Flow's FlowWeb software platform. The Vi-CELL XR viable cell concentration data can be used by the SCADA system for activating or modulating the process feed controller for precise feed delivery.

Vi-CELL XR/Seg-Flow Performance Evaluation

Purpose

The purpose of the evaluation was to ensure that the Vi-CELL XR analytical fidelity would be preserved when integrated with the Seg-Flow system. The experiment was designed to mimic the typical number of samplings performed during a 2–4 week cell culture process (1–2 samplings/day), as well as exceed the sample size requirements for the Vi-CELL XR average cell concentration specification (n=20).[7]

Methods & Materials

A Vi-CELL XR cell analyzer was integrated with a Seg-Flow automated online sampling and feed control system to perform the automated online cell analysis. This integration allowed the Seg-Flow system to: (1) remotely control the Vi-CELL XR; (2) provide the automated sample delivery to the Vi-CELL XR instrument for real-time cell analysis; and (3) retrieve the analytical data from the Vi-CELL XR analyzer. Additionally, the integration permitted manual sample analysis in between automated sampling cycles.
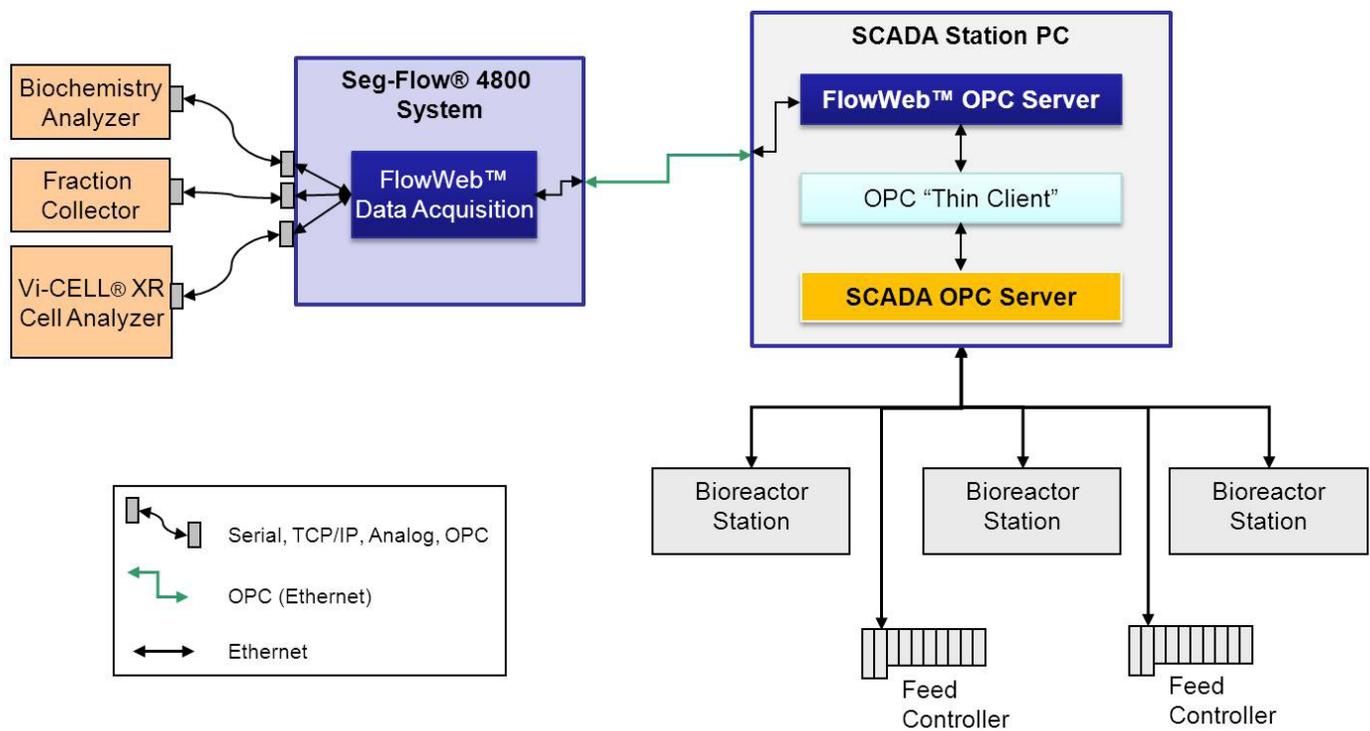
Figure 2. Architecture for the Vi-CELL XR/Seg-Flow integrated bioprocess PAT solution. The Vi-CELL XR/Seg-Flow interface can be integrated with additional third-party analytical technologies to provide a complete PAT solution for cell culture monitoring and control.

Cell concentration standards—which consisted of glass bead suspensions in 20% ethyl alcohol—were used in lieu of live cell cultures due to cell cultivation limitations at the testing facility. Concentration standards of 0.4 and 0.7×10^6 cells/mL, respectively, were used for the study.

The concentration standard solutions were mixed in their respective glass vessels using a high agitation rate throughout the duration of the experiment. The Seg-Flow system was programmed to automatically draw samples from each vessel at 30 minute intervals over a 12-hour duration. After each automated analysis, cleaning and rinse cycles were performed by the Seg-Flow system to eliminate any sample residue from the Seg-Flow fluid path. The cleaning cycle, using a 70% ethanol solution, was followed by a deionized water rinse to clean the Seg-Flow tubing for the following automated analysis.

Manual samples were obtained within five minutes after each automated analyses and were analyzed using the same Vi-CELL XR analyzer employed to perform the automated online analyses. A total of 25 analyses were performed for each sample set. Each sample set was evaluated for total cell concentration (TCC), viable cell concentration (VCC) and cell viability. All Vi-CELL XR cell viability and cellular parameter measurements were performed using a default Vi-CELL XR cell type protocol. For data analysis, the mean and standard deviation of each parameter were calculated.

Results

For bioprocessing applications, three common analytical parameters monitored for cell culture performance are TCC, VCC and cell viability—with VCC and cell viability being the most closely monitored analyses throughout the cell culture process.[8] Based on this industrial application, the TCC, VCC and cell viability were used as the performance metrics for the Vi-CELL XR/Seg-Flow integration evaluation.

The VCC profiles and VCC mean values for the automated and manual analyses for the high and low cell concentration standards are shown in Figures 3 and 4A, respectively. As shown by the analytical profiles, no significant variation between the automated and manual samples was observed for the VCC analysis throughout the duration of the evaluation. Additionally, a ±2.4% average concentration accuracy (n=25) for the automated VCC analytical method was achieved by the Vi-CELL XR/Seg-Flow system, which met the Vi-CELL XR average cell concentration specification of ± 3.0 %.
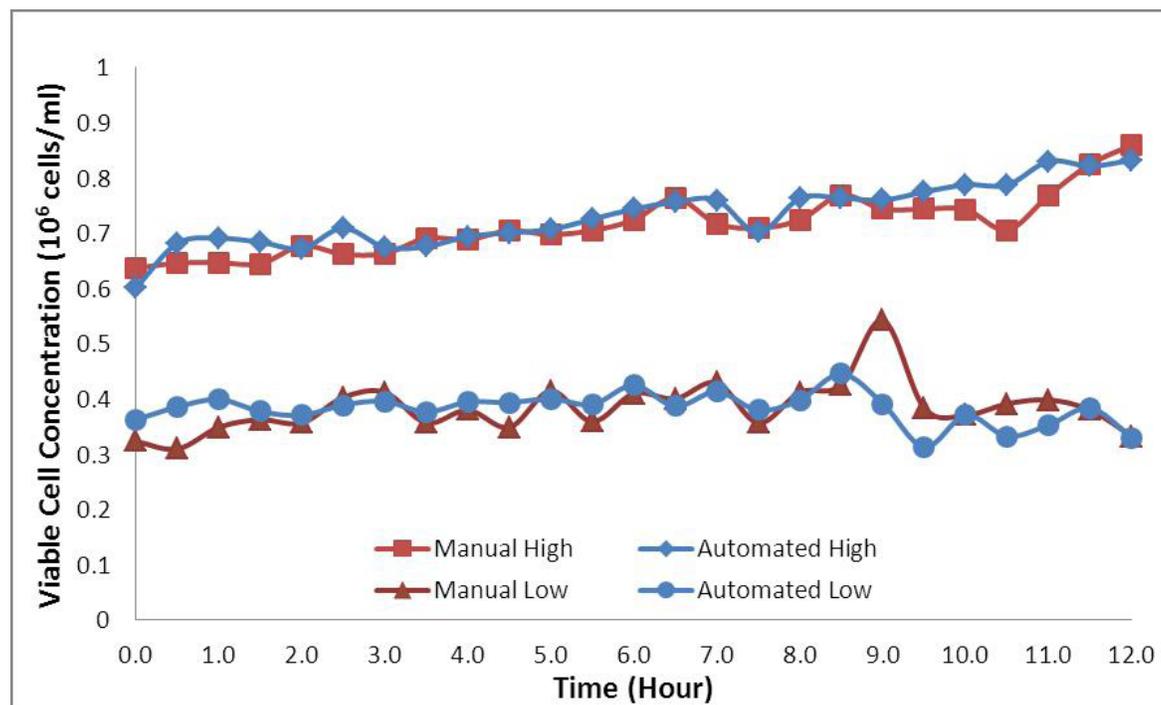
Figure 3. Viable cell concentration profiles for the high (0.7×10^6 cells/mL) and low (0.4×10^6 cells/mL) concentration standards. Automated and manual samples are shown in blue and red, respectively.
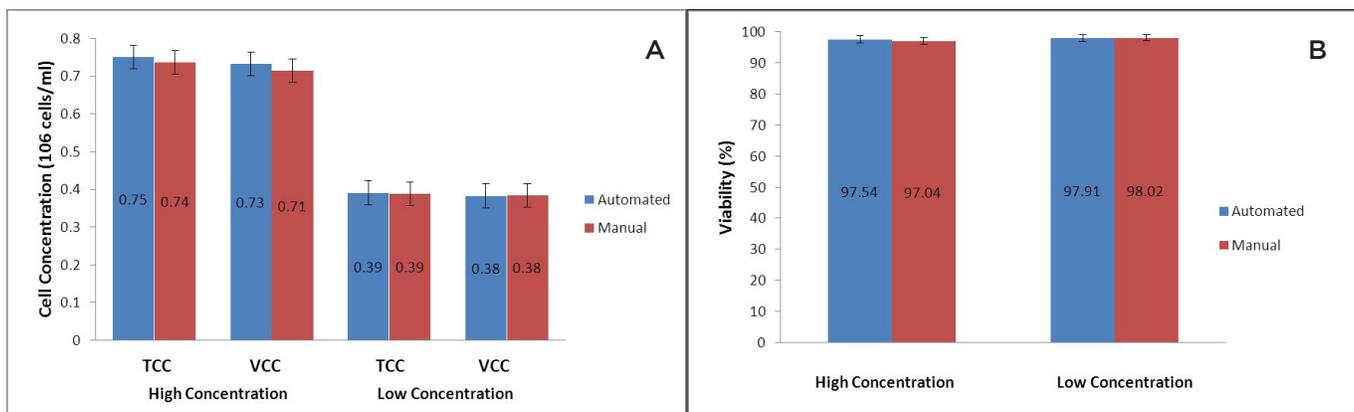
Figure 4. (A) Total and viable cell concentrations, and **(B)** viability for the high and low concentration standards. Each bar represents the mean of 25 analyses with error bars of one standard deviation. Mean TCC, VCC and % viability data for the automated and manual samples are shown in blue and red, respectively.

As shown in Figures 4A and 4B, the mean TCC and cell viability values were equivalent for both the automated and manual cell analyses. The TCC average concentration accuracy and the cell viability percent difference were ±2.0% and ±0.5 %, respectively—further illustrating the analytical precision of the Vi-CELL XR/Seg-Flow integrated system.

Conclusions

An automated, online solution has been developed for enabling Vi-CELL XR cell viability analyzers to perform real-time cell analyses for improved bioprocess monitoring and control. The Seg-Flow automated online sampling system provides flexible "plug-and-play" analytical options for the Vi-CELL XR analyzer. Through the Seg-Flow system interface, the Vi-CELL XR can be used as a stand-alone online cell analyzer or it can be integrated with other third-party analytical instruments to provide a comprehensive, automated PAT platform for enhancing process understanding. The Vi-CELL XR/Seg-Flow system preserves the Vi-CELL XR analytical fidelity, can be interfaced with multiple bioreactors, and allows the integrated Vi-CELL XR instrument to be used for manual analysis.

Authors

William Miller, Director, *Product Development*

Lucas Schimmelpfenning, *Applications Engineer*

Michael Biksacky, *President*

Flownamics Analytical Instruments, Inc. Madison, WI USA

References

1. FDA Guidance for Industry, PAT—*A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance.* September, 2004.

2. FDA Guidance for Industry, Q8 (R2) *Pharmaceutical Development.* November, 2009.

3. Carrier T, Heldin E, Ahnfelt M, Brekkan E, Hassett R, Peppers S, Rodrigo G, Van Slyke G and Zhao D. High-Throughput Technologies in Bioprocess Development. *Encyclopedia of Industrial Biotechnology.* 1–75: (2010).

4. Rios M. Developing an Integrated Continuous Bioprocessing Platform. *BioProcess International.* 10–11S; 30–33: (2012).

5. Skibo A. Managing Cost Without Sacrificing Quality. *BioProcess International.* 10–11; 30–33: (2012).

6. FDA Guidance for Industry, *Q&A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients.* August, 2001.

7. Beckman-Coulter, Inc. *Vi-CELL XR Cell Viability Analyzer Reference Manual.* Fullerton, CA: 2008.

8. Scott C. Biophysical Analysis of Living Cells. *BioProcess International.* 11–1; 24–27: 2013.

BECKMAN COULTER

Life Sciences

For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at www.beckmancoulter.com