Real-time monitoring of key bioprocess parameters using MAVERICK in-line device in Culture Biosciences' cloud-connected bioreactors

Introduction

Bioprocessing 4.0 aims to leverage advanced digital technologies, automation, and data-driven approaches to transform biopharmaceutical production. Culture Biosciences offers cloud-connected bioreactors and data science capabilities that enable the digital transformation of biomanufacturing processes for Process Development (PD). Culture Biosciences' bioreactors autonomously capture and ingest real-time data, via cloud-enabled controllers which can be monitored live or analyzed using Culture's Console™. Culture's real-time data analysis and modeling capabilities, working in conjunction with novel Process Analytical Technologies (PAT), enable clients to accelerate their biomanufacturing PD.

In an effort to enable innovative Bioprocessing 4.0 PD workflows using PAT methods, Culture Biosciences and 908 Devices formed a collaboration to test 908 Devices' MAVERICK® – a novel Raman spectroscopy-powered in-line PAT device – in Culture Biosciences' cloud-connected 250 mL bioreactors. MAVERICK provides real-time, in-line bioprocess analysis and control, right out of the box without the costs, complexities, and risks of conventional Raman approaches.

The vertically integrated hardware stack at Culture Biosciences enables rapid prototyping and modification of its proprietary bioreactors to support a client's process-specific PAT needs. Culture Biosciences' R&D - Hardware Team was able to modify the bioreactor

View of Culture Biosciences Cloud Bioreactor Lab with two reactors (288 and 289) setup with 908 Devices' MAVERICK device (left). Zoomed in view of the in-line probe inserted in a 250 mL vessel (right).

configuration and free up a bioreactor port to accommodate MAVERICK immersion probe, unlocking a new capability using real-time in-line Raman PAT to monitor PD bioprocesses.

Application Review and Examples:

In-line PAT measurements track dynamic cell culture condition changes

To test the cloud-connected biomanufacturing process with PAT capabilities, Chinese Hamster Ovary (CHO) cells were cultured in four 250 mL bioreactors using a standard fed-batch protocol. Two bioreactors were fitted with MAVERICK in-line immersion probes and two bioreactors were used as baseline experimental controls. MAVERICK measured glucose, lactate, and biomass in real-time continuously throughout the culture duration. Feed media, glucose and other nutrients were supplemented to the bioreactors following a fully-automated protocol, controlled by Culture Biosciences' cloud-connected proprietary controller and monitoring technology. Nutrient feeding steps can be activated manually or automatically, depending on the application and client's process need. In the protocol tested, supplemental feeding was activated using feedback from daily samples to respond to glucose levels in the bioreactor. Bioreactors were aseptically sampled at 24h intervals and analyzed for glucose, lactate, and other metabolites (Nova Biomedical BioProfile® FLEX2) and total cell density (Vi-CELL BLU). Following experiment completion (14 days), the data was analyzed using Culture's Console software and Application Programming Interface (API) to assess MAVERICK's performance within Culture's 250 mL bioreactor systems.

Glucose measurements show alignment between off-line data from Nova Biomedical BioProfile® FLEX2 and in-line PAT values from the MAVERICK system (RMSE[1] = 0.32 g/L). Figure 1 suggests that the MAVERICK PAT measurements were accurately reflecting the live culture conditions within the bioreactors. In-line PAT measurements can track the effect of feed and media addition events on process parameters, such as glucose concentration, (blue line) in real-time within the bioreactor environment. These dynamic cell culture condition changes are not captured in the off-line daily sample measurements (orange line).

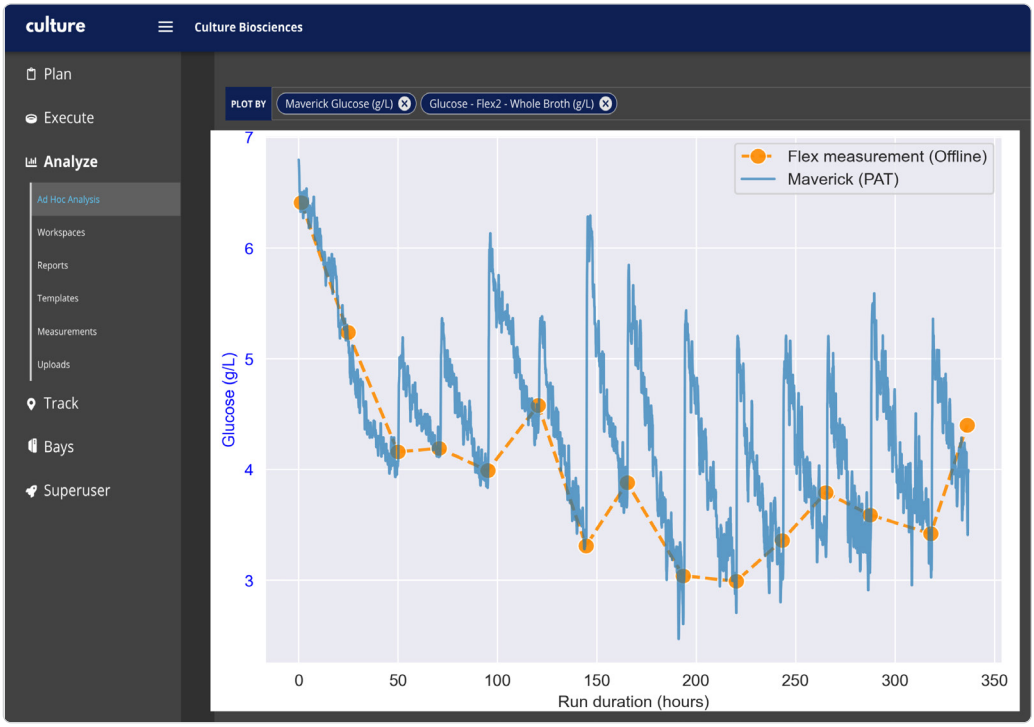

Figure 1. In-line glucose measurements in a Culture Biosciences bioreactor using MAVERICK PAT (blue) and daily offline measurements using Nova Biomedical BioProfile® FLEX2 (orange). Intuitive, automated visualization in Culture Biosciences Console.

Culture Biosciences' bioreactors enable accurate volume monitoring through cloud-connected sensors and controllers in conjunction with a data aggregation backend. The real-time monitoring of this data enables accurate tracking of all volume changing events in the bioreactor throughout the time-course of experiment. From the generated feed volume data set, feed additions explain the increase in glucose concentration seen with in-line MAVERICK PAT measurements (Figure 2).

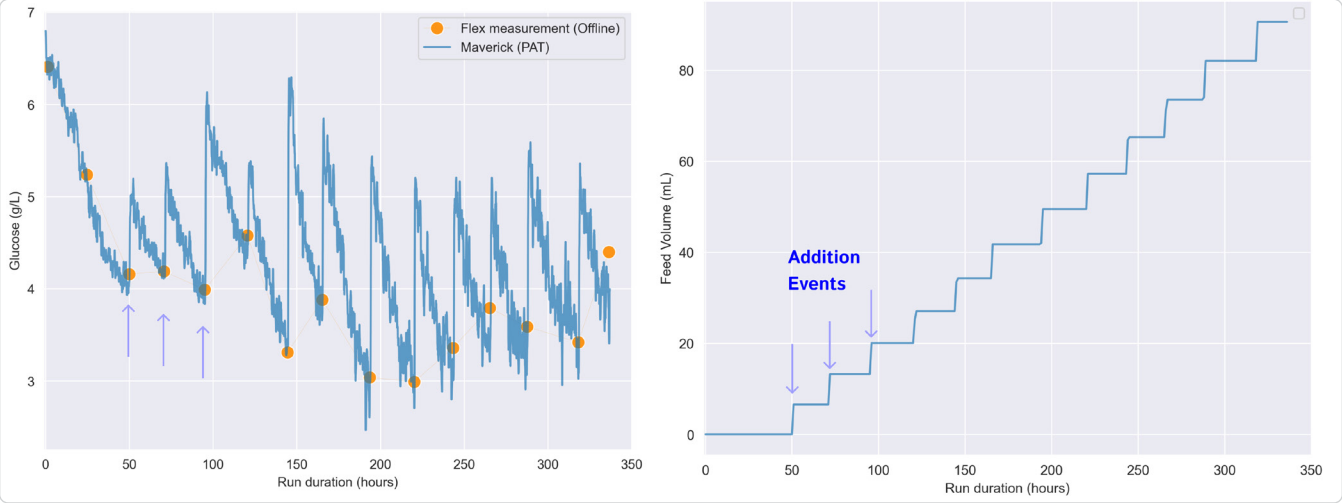

Figure 2. Glucose spikes measured by 908 Devices' MAVERICK coincide with feed pump data captured and processed by Culture Biosciences' cloud bioreactors. Visualization in Culture Biosciences Console.

Similar to the observed glucose measurements, lactate levels are accurately measured using the MAVERICK probe when compared to off-line analytics (RMSE = 0.29 g/L) (Figure 3) .

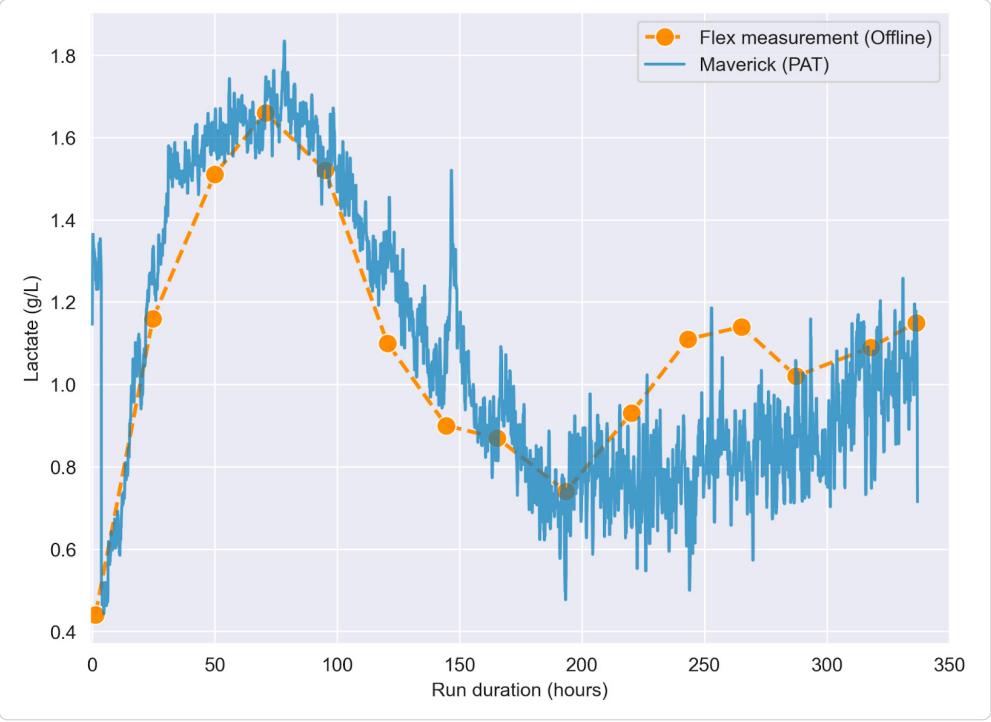

Figure 3. Lactate measured in-line by MAVERICK (blue) and through daily off-line samples (orange). Visualization in Culture Biosciences Console.

MAVERICK also provides real time monitoring of total biomass measurements; these values were compared to the off-line Vi-CELL BLU total cell density. Optical or spectroscopy-based biomass measurement methods typically require a calibration curve for a conversion into physical units. Figure 4 below shows the raw signal measurements from MAVERICK (in dimensionless biomass units, BMU) on the left side y-axis and Vi-CELL BLU measured total cell density (in million cells/mL) on the right side y-axis. The figure shows that the two curves are in agreement, indicating that MAVERICK is capable of effectively capturing the underlying physical signal. A divergence in the two measurements occurs beyond 250 hours, which can be attributed to the reduction of viable cells that occurs at the end of a typical mammalian cell culture experiment. The reduction in cell viability results in increased cell debris which may be a confounding variable for in-line PAT measurements, while the Vi-CELL BLU's visual measurements can be tuned to exclude lysed cells.

Conclusion

These experiments show how the flexibility and vertical integration of Culture's bioreactor platform allows for the rapid integration of PAT probes into 250 mL bioreactors. The data generated utilizing Culture Bioscience's bioreactors, cloud-based controllers and software Console together with MAVERICK, a novel Raman spectroscopy powered in-line PAT device, was found to track closely with off-line measurements for multiple critical process parameters. The use of PAT in bioreactors provides in-line, continuous data about cell culture conditions, thereby enhancing process understanding and enabling precise bioprocess monitoring and control.

References

1. Root mean squared error, RMSE using MAVERICK measurements of the same time point as BioProfile® FLEX2. $\sqrt{(\Sigma(y\_maverick - y\_flex2)^2 / n)}$

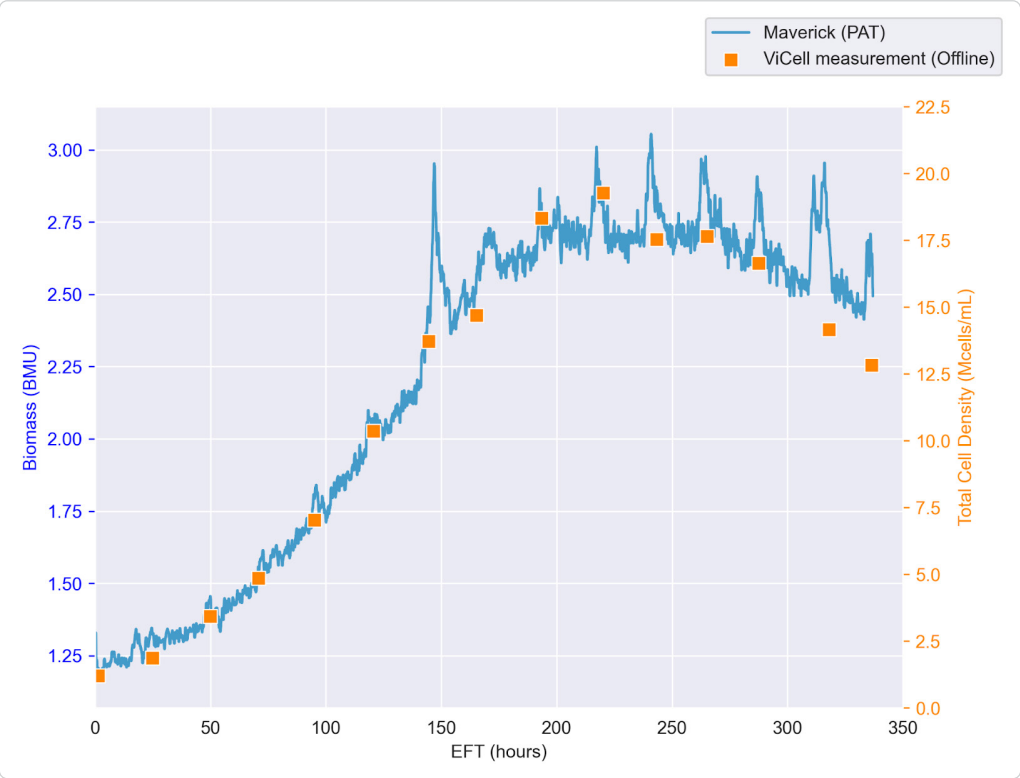

Figure 4. Biomass (arbitrary units) measured in-line by MAVERICK PAT (blue, left y-axis) compared against total cell density (million cells/mL) available from daily off-line measurements (orange, right y-axis). Visualization in Culture Biosciences Console.