Determining Total Cell Count with MAVERICK Biomass Measurements

MAVERICK provides an in-line, Raman-based PAT solution for monitoring and control of glucose, lactate, and biomass in up to six bioreactors right out of the box.

Introduction

When managing a bioprocess, it is essential to monitor and control several critical process parameters (CPP) to maintain optimal cell culture conditions, ensure product quality, and maximize yield. Multivariate optical sensing technologies often require substantial expert configuration and set-up time that can take months or years, and significant expense.[1,2] MAVERICK offers the advantages of in-line Raman process analytical technology (PAT) without the cost and headaches inherent to the implementation of conventional process spectroscopy-based methods. Unlike conventional Raman analyzers, which rely on empirical calibration of spectral data to off-line reference measurements, MAVERICK utilizes a *de novo* algorithm that does not require empirical trial-and-error 'training' on bioprocess data.[3]

MAVERICK measures and reports "biomass units" (BMUs) via volumetric scattering and absorption of the culture media. This approach is similar to other indirect biomass-related measurements such as capacitance or optical density. Our data show that BMUs are linearly related to total cell densities via a simple conversion factor.

This document shows the linear correlation between BMUs and total cell densities as measured with a typical cell counter, plus how to convert BMUs obtained from MAVERICK into total cell densities.

Linearity between biomass measurements and total cell densities

To assess the linearity between biomass measurements using the MAVERICK and offline total cell densities, two cell lines (a CHO and a HEK293 cell line) were cultured as follows:

CHO culture:

- GS-CHO cells expressing a monoclonal antibody were cultured in 10L bioreactors (Sartorius Biostat B-DCU II with BioPAT DCU Tower) in a fed-batch process using chemically defined CD FortiCHO media and CD EfficientFeed C (Thermo Fisher Scientific). Cells were seeded at 0.3 million cells/mL in 7.9L of media and grown at 37°C. The dissolved oxygen set point was 30% and the pH set point 6.9 with a deadband of 0.03. EfficientFeed C was fed every other day at 4% v/v starting on day 3. Both media were supplemented with 4 mM L-glutamine. Glucose was fed as a bolus to 4 g/L when measurement had fallen below 2g/L.
- MAVERICK biomass measurements were continuously collected during the two-week culture using the stainless-steel immersion probe directly fitted into the bioreactor.
- Off-line measurements of total cell densities and viability were performed using a ViCELL XR (Beckman Coulter) daily.

HEK293 culture:

- HEK293 cells were cultured in a 3L bioreactor (Yokogawa BR1000) with 2.5 L working volume in either FreeStyle F17 Expression Medium (Thermo Fisher Scientific) and separately, in the CDM4HEK293 media (Cytiva). Glucose concentration was maintained at 1–2 g/L by daily sampling and subsequent feeding.
- MAVERICK measurements were collected during the 6-day culture using the stainless-steel immersion probe directly fitted into the bioreactor.
- Off-line measurements of total cell densities were taken using a Cellometer cell counter (Nexcelom) daily.

The CHO culture showed a maximum cell density of ~20 million cells per mL on day 8 as shown in Figure 1A. Viability started to decline from 95% between days 7 and 8 down to ~60% at day 14 (Figure 1A). The biomass values reported by the MAVERICK in the CHO and HEK293 cultures scale linearly (R^2 values ≥ 0.95) with the total cell count measured by Vi-CELL (Figure 1B), or Nexcelom Cellometer cell counter (Figures 1C and 1D).

Figure 1 (A)

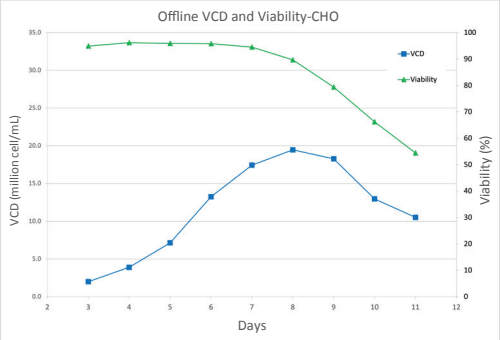

Figure 1 (B)

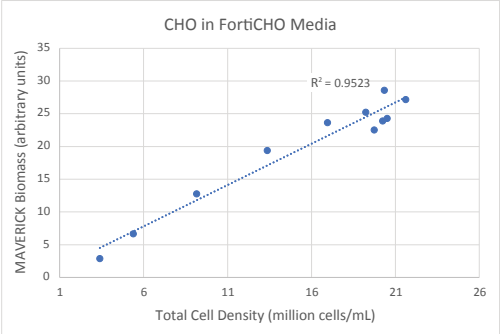

Figure 1 (C)

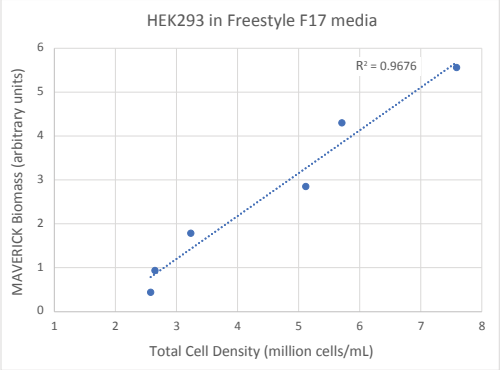

Figure 1 (D)

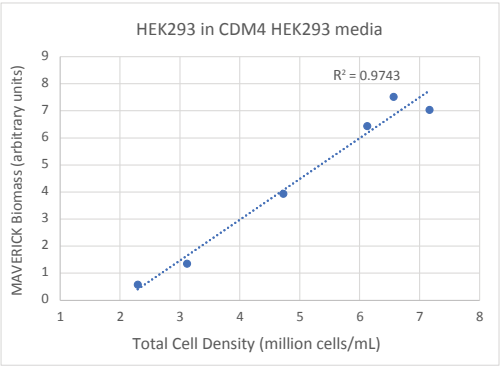

Figure 1. (A) Viable cell density and viability of CHO culture. (B), (C), & (D): Correlation between MAVERICK biomass data and offline total cell counts for CHO (B), HEK293 cells in Freestyle F17 media (C) and HEK293 cells in CDM4 HEK293 media (D).

Establishing a correlation between MAVERICK biomass measurement and process-specific total cell count

As can be seen in Figure 1, the relationship between MAVERICK biomass units (BMU) and offline total cell counts readings is linear. A conversion factor can be easily obtained by plotting multiple offline total cell density (TCD) readings to the MAVERICK BMU and calculating a regression line in Excel or similar software.

Alternatively, since the relationship is linear and zeroed as part of the MAVERICK calibration procedure, it is often sufficient to use one offline measurement with a cell count device midway through the culture, and determine a correction factor (CF) as:

$$MAVERICK\ CF = \frac{Reference\ TCD}{MAVERICK\ BMU}$$

Then any newly reported MAVERICK BMU can be converted to cell counts using the equation:

$$Estimated\ TCD = MAVERICK\ BMU \times MAVERICK\ CF$$

When applying this method to the example from Figure 1B (CHO culture), a single reference measurement at hour 68, allows one to estimate the subsequent cell counts with a mean absolute percent error of 8% (Figure 2). Therefore, MAVERICK BMUs provide an easy-to-obtain estimation of total cell densities. This simple method of correlating MAVERICK BMUs to total cell density is also available in the on-device software. Table 1 shows the estimated TCD calculated using this simple conversion factor applied to the MAVERICK BMUs.

Conclusions

MAVERICK provides an in-line, Raman-based PAT solution for monitoring and control of glucose, lactate, and biomass in up to six bioreactors right out of the box. Although a universal conversion factor for all cell cultures does not exist due to variables such as cell volume and shape, for most cell lines, a single conversion factor is adequate. With the MAVERICK monitoring of total cell count during bioprocess runs requires a simple one-point correlation enabling real-time monitoring of culture growth.

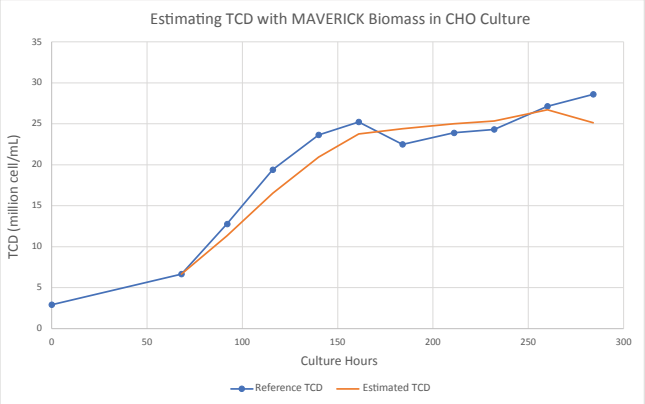

Figure 2. Estimated total cell densities in the CHO culture from MAVERICK biomass measurement. The reference TCD were obtained using an off-line ViCELL device.

Hour	MAVERICK BMU	Reference TCD	Estimated TCD	% Error estimated vs. reference TCD
0	3.40	2.9	-	
68*	5.38	6.65	6.65	
92	9.16	12.77	11.32	-11.4%
116	13.37	19.4	16.51	-14.9%
140	16.96	23.65	20.95	-11.4%
161	19.24	25.22	23.76	-5.8%
184	19.75	22.5	24.39	8.4%
211	20.25	23.9	25.01	4.6%
232	20.51	24.3	25.33	4.2%
260	21.63	27.15	26.71	-1.6%
284	20.36	28.59	25.14	-12.1%

*Table 1: MAVERICK BMUs (biomass units), reference (offline) total cell densities (TCD) and estimated TCD calculated using conversion factor CF based on a single reference measurement at hour 68 (marked with a *).*

Acknowledgements and References

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