Applications Note

TruCell

Accurate Growth Rate Prediction of Fed Batch Mammalian Cell Culture Runs with In-Line Cell Density Monitors

Introduction and Motivation In mammalian cell culture, accurate prediction of the actual cell mass growth rate is a critical parameter in determining the success of a run and the yield of the batch. Knowing the growth rate is most critical early on in the run, when the culture is more fragile and has not fully stabilized. Thus, the early stage growth rate is a critical input into any decision that determines the time and resources invested into a specific cell culture run. Early termination of bad runs saves personnel and reactor time, allows earlier recovery in a campaign, and improves the overall economics of production.

Traditionally, the growth rate is predicted using off-line laboratory cell count values. Unfortunately, the low accuracy of laboratory cell counting methods, especially at low cell concentrations, and the inherent time delay in off-line measurements, makes early stage growth rate prediction using traditional off-line lab methods extremely difficult and unreliable. In this whitepaper, we will demonstrate how to overcome the limitations of traditional off-line lab methods by leveraging real-time, in-line cell density measurements to make growth rate predictions.

Relative Inaccuracy of Growth Rates Based on Off-Line Laboratory Cell Counting

Accurate off-line measurements (such as dry cell weight [DCW]) require significant analysis time, and do not offer the possibility of feedback input to the process (i.e., the run is well underway or almost finished by the time that results are available). Less accurate, but faster off-line laboratory cell counting measurements such as optical density [OD] typically suffer from dilution errors, sampling errors, and the general inaccuracy of spectrophotometers at low optical densities (such as those found in early batch samples from a cell culture run).

In-line optical density measurements, on the other hand, provide real-time information on total cell density and naturally lend themselves to providing real-time cell growth rates (i.e., the cell growth rate is simply given by the first derivative of the cell density curve). Moreover, laser-based sensors such as the Finesse TruCell, achieve high signal-to-noise ratios in measuring cell density, and can achieve excellent measurement accuracy and precision, even at low cell concentrations.

Figure 1 compares an in-line cell density measurement (using the TruCell monitor) with a traditional off-line cell density measurement during a growth run. The average noise of the in-line measurement was 0.002 MM cells/mL, whereas the average noise of the off-line measurement was 0.165 MM cells/mL. The in-line optical measurement is more precise overall, and clearly outperforms the off-line measurement at low cell concentrations. Moreover, the in-line measurement provides a continuous data stream while the off-line data points are separated by at least 5 hours. It follows, therefore, that the growth rate predicted using in-line measurement data will be much more accurate than that calculated from the off-line data.

Figure 1 Lab Grab Sample versus TruCell Measurements of Total Cell Density

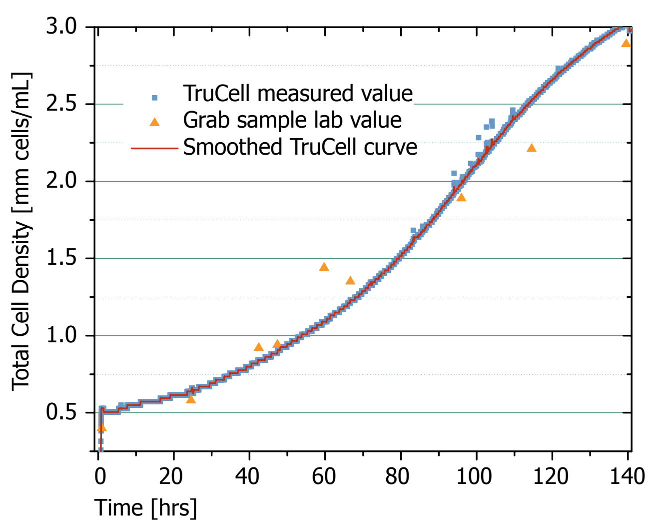


Figure 2a
Comparison of off-line OD and in-line TruCell AU

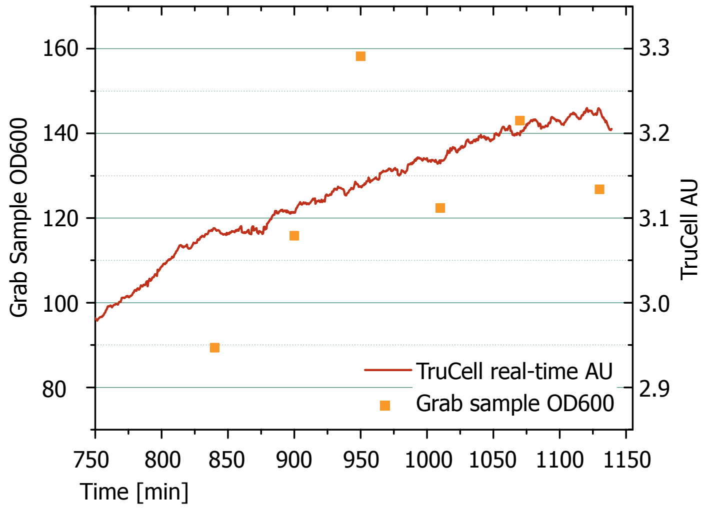


Figure 2b
Comparison of off-line DCW and in-line TruCell AU

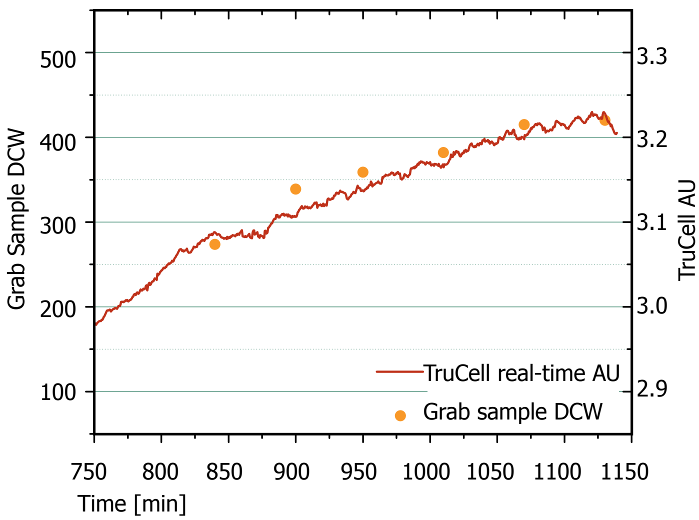
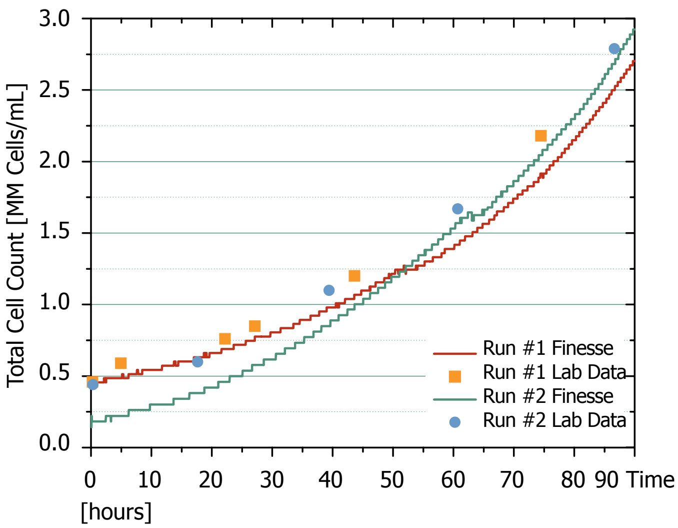


Figure 3
Growth Rate Characterization for Mammalian Runs



TruCell Run #1 Growth Rate: 1.81 x 10^{-2} MM cells/mL/hr
TruCell Run #2 Growth Rate: 2.40 x 10^{-2} MM cells/mL/hr
Lab Run #1 Growth Rate: 2.10 x 10^{-2} MM cells/mL/hr
Lab Run #2 Growth Rate: 2.48 x 10^{-2} MM cells/mL/hr

Figure 2 shows the difference in accuracy and precision between an off-line optical OD measurement and an off-line DCW measurement, when both are compared to in-line measurements. The DCW measurement is much more precise, and is in excellent agreement with the in-line measurement. Unfortunately, DCW analysis often takes a week or more, with the results arriving long after the fermentation run has been completed! Thus, Figure 2 illustrates that in-line cell density monitors provide an excellent alternative to off-line DCW measurements, with the added benefit that the data is available in real-time.

Advanced Monitoring and Control of Cell Density Growth Rate

Two consecutive Chinese hamster ovary (CHO) cell runs that have significantly different early stage growth rates are shown in Figure 3. The early stage growth rate for each run was estimated using both in-line (TruCell) and off-line methods:

- In-line: 1.81 x 10-2 MM cells/mL/hour for the first run and 2.40 x 10-2 MM cells/mL/hour for the second run
- Off-line: 2.10 x 10-2 MM cells/mL/hour for the first run and 2.48 x 10-2 MM cells/mL/hour for the second run.

According to the off-line measurements the growth rates were only slightly different between the two runs. Therefore, the two runs look very similar when only intermittent laboratory grab samples were available to predict the growth rate. Yet, in reality, the endpoint of the first run was a failed culture that stalled, and was aborted before successful harvest and expression, while the endpoint of the second run was a successful culture in excess of 11.0 MM cells/mL. Clearly, the predictive power of the grab sample approach is rather limited. In contrast, the in-line method computes that the growth rate of the second run is 33% higher than that of the first run. This difference is more than twice that obtained using the off-line method. Thus, the in-line method indicates that the two runs are very different, and that the growth rate of the second run is clearly superior.

Moreover, the real-time, in-line measurement could produce sufficiently conclusive data within the first 48 hours (2 days) of the run, and thereby save at least 100 hours (4 days) of run time. In other words, by using a TruCell monitor, cell culture productivity (or zero-yield run risk reduction) could be improved by 66%.

Accurately Monitor Early-Stage Growth Rates using Optical Density Measurements

Using advanced in-line optical (cell) density monitoring, researchers and engineers can develop and implement more advanced control strategies for controlling the reactor environment in order to improve the yield and repeatability of fermentation and cell culture processes. Specifically, we recommend using our TruCell probe to improve early stage determination of cell density growth rates for improving overall productivity.

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