

## Accurately Monitor High Range (>350 OD) Bacterial Fermentation Using In-Line Cell Density

**Introduction and Motivation** In bacterial fermentation, the cell density concentration grows exponentially to a high end-point value. Real-time control of bacterial fermentation processes therefore requires sensors having a wide dynamic range. To date, there has been a lack of sensors having good precision, accuracy, and linearity, combined with a wide operating range.

Traditionally, the cell density concentration is determined by off-line laboratory cell density measurements such as OD<sup>600</sup> values. Unfortunately, the relatively low accuracy of these laboratory

methods, especially at high concentrations, and the inherent time delay in off-line measurements, makes advanced, real-time process control impractical with lab methods alone. Attempts have been made to measure *E. coli* concentrations at high OD<sup>600</sup> values on-line using optical absorption devices based on incoherent light sources such as lamps or light-emitting-diodes (LEDs). Unfortunately, these types of sensors have limited performance and dynamic range. In this whitepaper, we will demonstrate the superiority of monitoring bacterial fermentation processes using on-line, laser-based absorbance sensors.

### Attempts at Optical On-Line High Cell Density Monitors for *E. coli* Fermentation

Optical devices based on the measurement of light transmission have been proposed for monitoring high concentration fermentation slurries. These optical sensors typically use broadband incoherent light sources, such as filtered incandescent tungsten bulbs or NIR-LEDs in the 850 to 900 nm range. The source light travels through the sample by means of a physical gap, and is captured by a photodetector. The sensor measures the amount of light transmitted through the sample and computes the sample's reduction in light transmission. Owing to their simplicity, optical sensors are robust and generally well suited for such measurements.

In fermentation applications, however, the use of optical devices has been limited to date by their inability to accurately measure high cell density concentrations. Some fermentation processes can reach endpoint concentrations

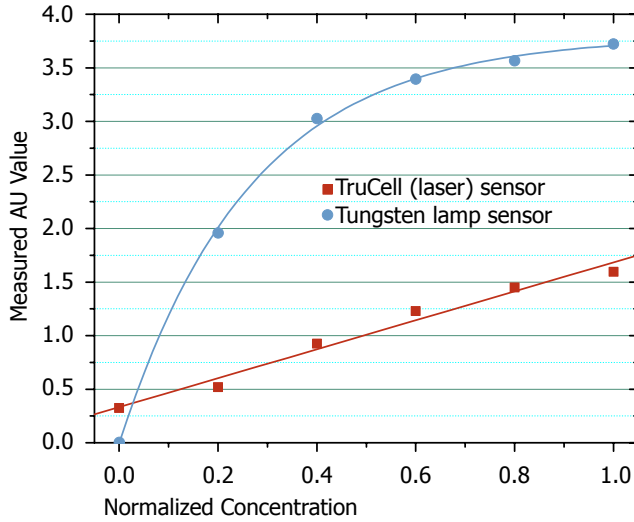
exceeding 350 OD, which corresponds to an extremely high reduction in light transmission. This means that the sample's appearance is very opaque (or dark), and few of the photons from the light source actually reach the detector. Because traditional optical devices rely on incoherent radiation light sources, the achievable power density (number of photons per area) of a collimated light beam at the wavelength of interest is low, and becomes even smaller after transmission through the fermentation sample. The photodetector signal is therefore very weak, and may become close to the dark current of the detector. In this case, the detector cannot respond as well, and the sensor signal will "saturate", and the response curve will flatten. In other words, the signal-to-noise ratio at the photodetector is poor, and the optical device cannot provide a precise or accurate signal.

### TruCell Laser-Based Fermentation Monitors

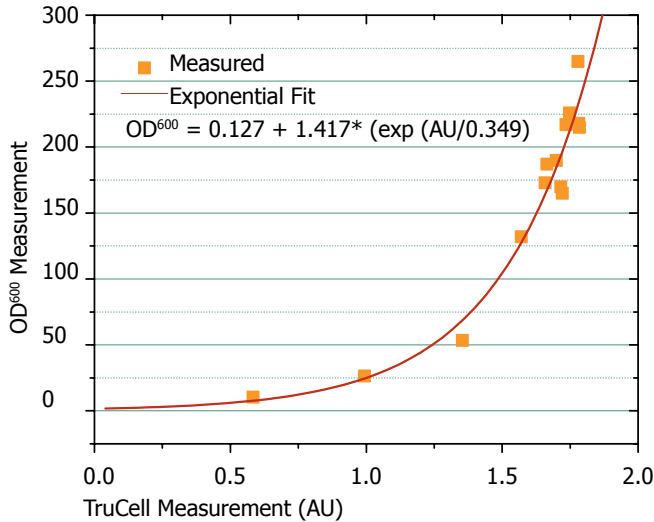
The Finesse TruCell monitor uses a laser for its light source. Lasers provide coherent radiation, which means that they have narrow emission bandwidths (< 10 nm), and are easily collimated to cylindrical beams. Because the laser emission is narrowband (unlike LEDs or lamps where at least 50% of the output power is lost by filtering to

a narrower emission bandwidth) and can be efficiently collimated (unlike in LEDs or lamps where at least 50% of the output power is lost), most of the laser light is available for transmission through the sample (compared to less than 25% for LEDs and lamps). For a given light source power, more photons are transmitted through optically dense

**Figure 1**  
Absorbance Response of two optical sensors using incoherent and laser light sources



**Figure 2**  
Correlation of TruCell AU to Laboratory OD<sup>600</sup> values for E. Coli Fermentation



(highly absorbing) sample media by the laser. Therefore, more photons reach the photodetector and produce a higher signal-to-noise ratio, which improves measurement precision and accuracy. The laser sensor won't saturate as quickly as the LED/lamp-based sensor.

Figure 1 shows the response of the Finesse TruCell laser-based sensor compared to a competing tungsten lamp-based optical sensor, when measuring the absorbance of a series of samples having increasing concentration. The tungsten lamp based sensor response saturates above a normalized concentration of 0.5 (i.e., the slope of the response curve is reduced to 1/7th of its value at low concentrations!). The saturation indicates that at high concentrations the sensor “runs out of photons,” and can no longer effectively respond to concentration changes.

In contrast, the TruCell sensor response does not show any signs of signal saturation over the entire test concentration range for this same sample. The TruCell response indicates that it has “photons to spare”, and will provide excellent signal-to-noise and precision in its measurements.

Figure 2 shows the response of a TruCell sensor to high concentrations of E. coli fermentation broth. The TruCell sensor response shows that for wide bacterial concentration ranges (with concentrations as high as 250 OD), the measured optical loss is a combination of absorption and scattering. The correlation between in-line TruCell AU and off-line laboratory OD<sup>600</sup> remains reproducible, because the sensor has good precision over a wide dynamic range. The correlation is exponential.

## Benefits of TruCell for Accurate Monitoring and Control of Bacterial Fermentation

Using advanced in-line optical cell density monitoring, control strategies for fermentation processes can be developed. These controls will provide better batch-to-batch repeatability and improve the yield per available reactor hour. We recommend using a TruCell, laser-based sensor.