Application Note

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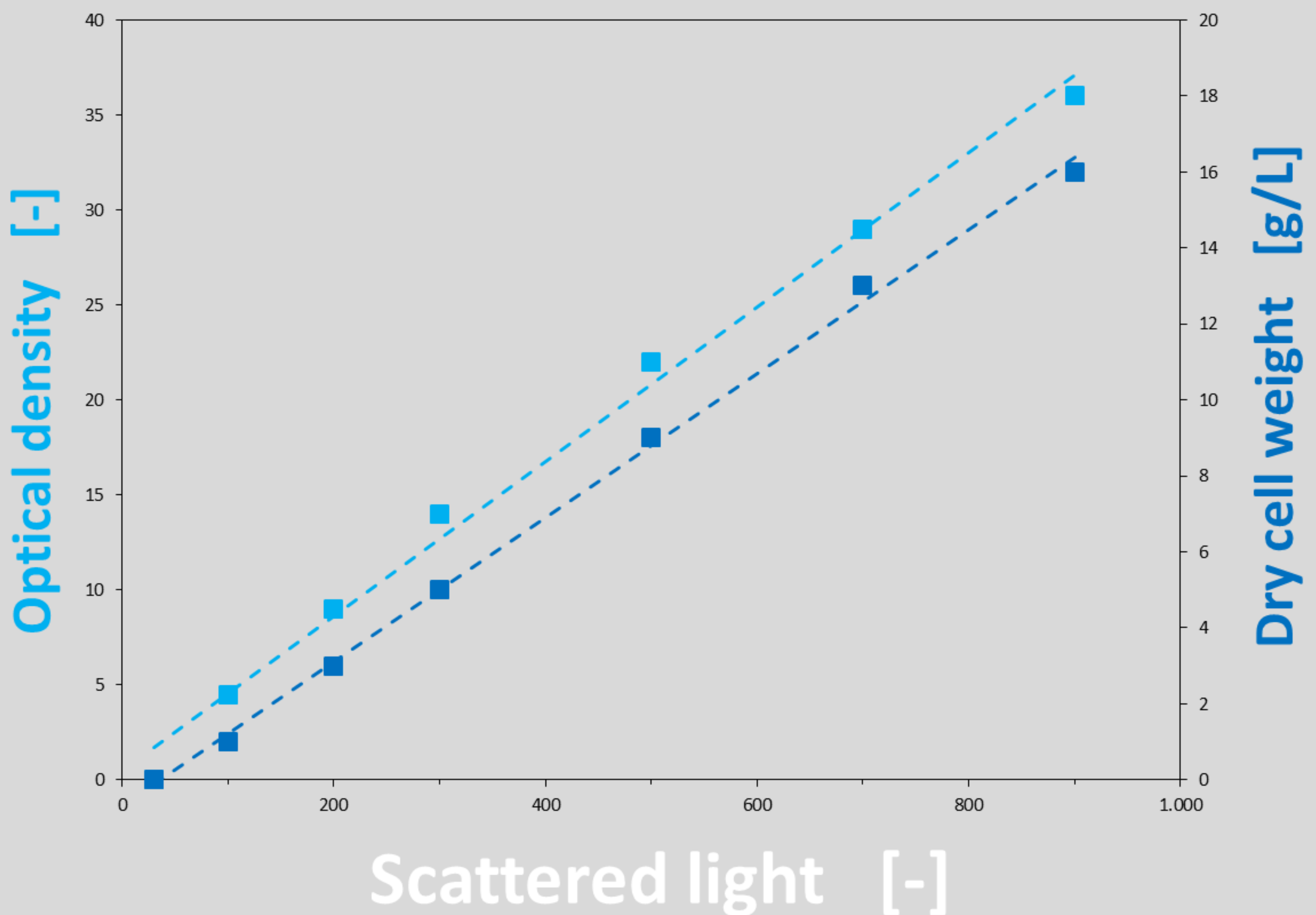
The scattered light signal: Calibration of biomass



Introduction

The innovative BioLector® device measures biomass in the form of scattered light intensities. Consequentially, it is not only possible to monitor biomass concentration online in microtiter plates, but also noninvasively and parallel in all wells. Conventionally, biomass analysis is performed offline after taking a sample. This is laborious and naturally confined to certain time points. Additionally, sampling is somewhat unpractical for fermentations in shaken microtiter plates due to small working volumes.

The principle of operation of the BioLector® device in regard to biomass is explained in this application note as well as factors that may influence experimental results. Furthermore, correlation to traditional biomass measurements is demonstrated, including the integration of a linear calibration in the BioLection software.

Operating principle

First, some basic information about scattered light measurement in the BioLector® is needed. The principle is displayed in Figure 1.

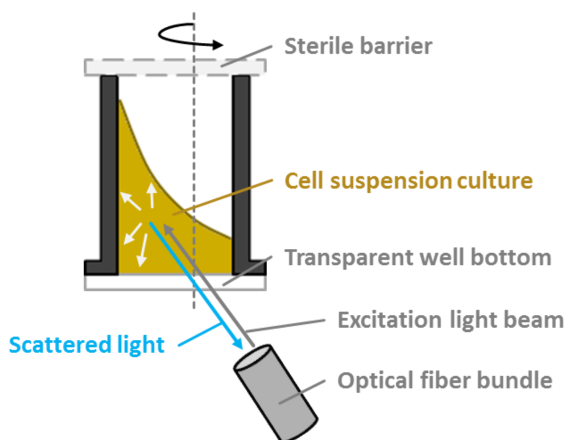


Figure 1: Measuring principle of scattered light.

Excitation light at 620 nm is beamed from an optical fiber bundle into the suspension culture. The light is scattered by particles (= cells) in the culture medium. It spreads in all directions, also toward the optical fiber. Here the back-scattered light is collected and guided to a photomultiplier.

The quantity of scattered light is proportional to the biomass concentration of the cell suspension culture. The detector sensitivity (device dependent) is adaptable *via* the parameter named "Gain". The gain is a non-linear, unit-free factor from 1 (low) to 100 (highest sensitivity of the photo detector).

The gathered data depend on several influence factors in the experimental setup. These include medium appearance, shaking frequency, filling volume, state of cells, gain and the device itself. Additionally, the transparent bottom of a plate should neither be touched nor scratched.

Factors of influence

If insoluble particles are suspended in the fermentation medium, the offset value of the measured data is increased. Large components (e.g. precipitates or cell aggregates) cause noisy signals. In that instance, fluorescent signals of metabolism like NAD(P)H or riboflavin often provide a solution.

Shaking frequency and filling volume have to be regarded together: Please read up on the maximal filling volumes in the data sheets of your used type of microtiter plate. Low filling volumes are not appropriate at high shaking frequencies. The excitation light might be reflected from well wall or bottom rather than transmitted through the media. To avoid these effects, use a minimal filling volume of 800 µL in 48 well microtiter plates for cultivations. Also, reflections may appear at a very low scattering content, which is especially important for cell culture. Further, at shaking frequencies below 600 rpm the cells might not be completely suspended.

Besides the main influence of biomass concentration, the morphology of the suspended cells has to be taken into account. Small, roundish cells scatter light stronger than big and elongated ones. In Figure 2 the scattered light signal of a cultivation of plant cells is shown. In the expo-

nential growth phase the cell form is more or less round, but after depletion of the C-source they elongate and the signal decreases.

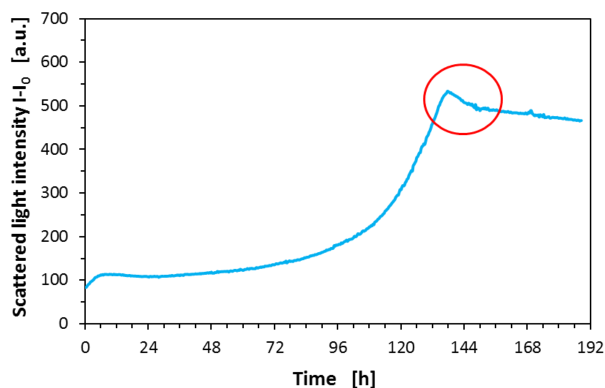


Figure 2: Cultivation of suspended plant cells.

Hence, a calibration is only applicable to the state of the used cells used. If data for significantly different cell states are required, more than one calibration should be carried out for meaningful results.

Calibration of biomass

To produce the biomass required to correlate traditional biomass measurement to scattered light, cultivate the cells of interest in the usual way and harvest them in the exponential growth phase. If needed, they may be centrifuged for concentration. Prepare a dilution series with fermentation medium. The volume of each dilution step has to be sufficient to measure the traditional biomass value (OD_{600}, CDW) and to fill the microtiter plate. A concentration range from inoculation to the estimated end of fermentation is recommended. Five different concentrations and one medium blank ought to be measured at least. Use two wells in the microtiter plate for each dilution step. If no other filling volume is needed for the experiment, use 1000 µL. Afterwards seal the plate with a gas impermeable film to prevent growth during recording. Place the microtiter plate into the BioLector® and start an experiment by proceeding BioLection HMI "Start Assistant" as usual. Use your corresponding experimental setup or create a new protocol. Enter a shaking frequency of at least 800 rpm and select a layout which includes all involved wells. Enter multiple "Biomass" filters with different gains in "Filter Settings". A suitable setup for most bacteria and yeasts are gains 10, 15, 20, 25 to 30. Choose higher gains for the monitoring of larger cells (e.g. 20, 30, 40 and 50). Start the BioLector® protocol and record data for a minimum of six cycles.

Meanwhile determine the biomass concentration offline. Please consider the following suggestions in your practical workflow:

- Use the same medium as in scattered light determination to prepare your dilution series. Otherwise the offset value of the calibration is shifted (see Figure 5).
- If growth is slow, no dilution series may be needed at all. Simply replicate the usual fermentation in multiple wells of one microtiter plate and take in-process samples ("sacrifice wells").

Evaluation

Load the measurement file with BioLection software. If the average of the scattered light signals at the highest biomass concentration unit is 600 (see Figure 3), the gain is suitable.

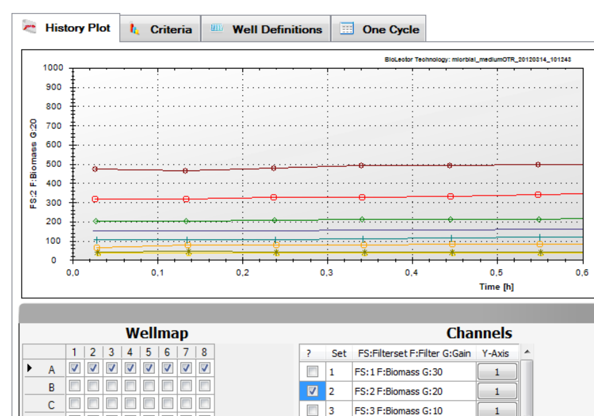


Figure 3: Measured data shown in the BioLection.

BioLection "Criteria" functions assist in further analysis. Check "Raw Data" ("Evaluation Criteria")

and choose the elected gain from filter set drop-down menu, e.g. “FS:2 F:Biomass G:20”. Also select all used wells at “Wellmap”. Tick the “Average Value” box and enter the “Time Interval”, which you want to examine. Draw only data in steady state into consideration and always neglect the first measuring cycle. Click onto “Top10”, “Top20” or “All” button to create a labeled bar chart (Figure 4). Duplicates ought to be averaged manually. Check the represented values for plausibility and proceed.

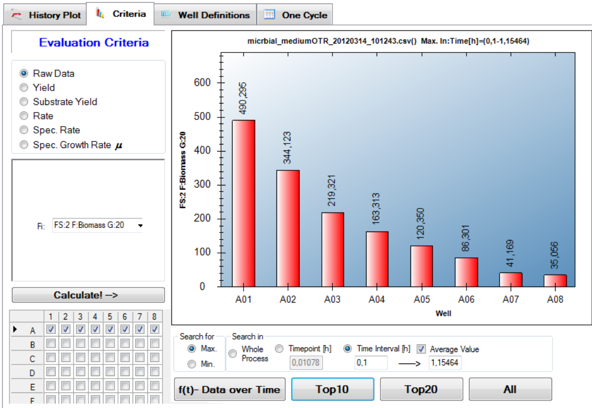


Figure 4: Bar chart of maximal concentration.

Calculations

Use a calculation program (e. g. MS Excel) to create a chart. Type the averaged scattered light values in the first column (x-axis) of a data sheet and traditional biomass units in the second or third column (y-axes). Mark the columns and insert an XY chart. Fit linear trend lines to the data sets. Their formulas and coefficients of determination (R^2) ought to be shown in the diagram. In Figure 5 optical density and cell dry weight are plotted versus the corresponding scattered light signals. A serial dilution with six steps and a medium blank were used. Direct proportionality of scattered light to the concentration of suspended cells defines the range of operation of the BioLector® over a wide range. For example, for *Pichia pastoris* a linear range from OD_{600} = 0.2 to 350 (Gain 5) and a CDW of up to 70 g/L was proven. The following example (Figure 5) shows the calibration data of a cultivation of *E. coli* in minimal medium at gain 20.

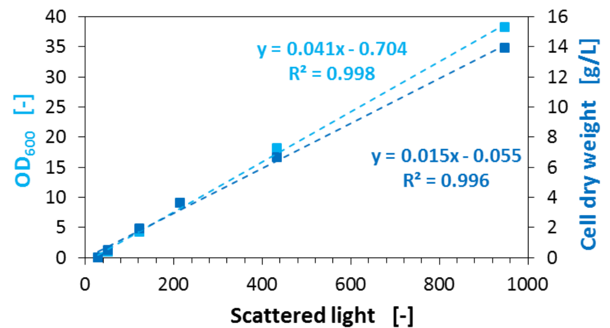


Figure 5: Biomass calibration plot.

A linear trend line has the following format:

$$y = \text{slope} \cdot x \; - \text{offset}$$

Offset reflects the baseline level of scattered light. Slope describes the proportion of offline biomass concentration unit (y) to scattered light (x). Enter the calculated data in BioLection software under “Calibration” and “Settings” (Figure 6). The software provides calibrated biomass (“Cal. Biomass”) automatically in “History Plot” tab (Figure 3).

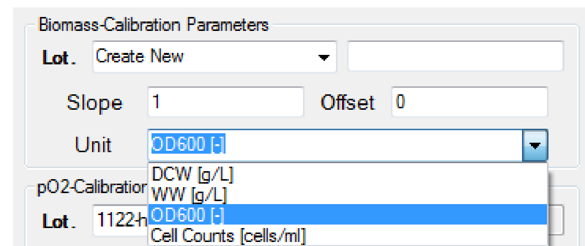


Figure 6: Calibration Settings in the BioLection.

Summary

The BioLector® device provides online-measurement of biomass concentration *via* scattered light. The scattered light signal is proportional to the suspended biomass. However experimental setup can influence the scattered light signal as well as cell-dependent factors like morphology. Yet, biomass concentration is typically stated in other units like optical density or cell dry weight. This can be brought into agreement by calibration. The values slope and offset of the trend line describe the correlation of the calibrated units.

Please visit the website for further information: www.m2p-labs.com, www.beckman.com

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