Technical Note 168

OD600 Measurements

Introduction

The turbidity measurement of microbial cultures is a commonly used method to determine the growth phase or cell number in an actively growing culture. Most often, these determinations are done using a spectrophotometer to measure the absorbance at 600 nm. However, the measurements are a measure of light scattering rather than a measurement of absorbed light. OD_{600} values may differ amongst cell types using one instrument or when measuring the same cell type on different spectrophotometers.

The purpose of this note is to highlight sources of the potential differences observed in OD_{600} measurements between instruments and present performance data examples from the DeNovix® DS-11 Series.

Cell Types

For OD_{600} measurements, the light passing through the sample is scattered in random directions by cells suspended in the sample. This light scattering is a function of both the specific cell size and shape as well as the density of the cell suspension. Additionally, dead cells and cell debris may contribute to light scattering. Different cell types at the same density (eg. cells per mL) may result in different OD_{600} values when measured on the same instrument.

Optical Configurations

It is well known that optical configurations of spectrophotometers play a role in the light scatter detected by a specific instrument. Different OD_{600} values will be reported for the same bacterial culture when measured on spectrophotometers with different optical set-ups. An OD_{600} of 0.8 using one instrument can be reported as 0.5 on another without being incorrect on either unit.

Empirical Target Values

Many investigators rely on target OD_{600} values obtained from literature sources when harvesting microbial cell cultures or determining the correct density to inoculate a culture for protein expression studies. Unfortunately, these target values may not be appropriate for the combination of the cell type and instrument in use. It is recommended that target OD_{600} values be empirically determined using growth curves correlating with OD_{600} values with plate counts for each cell type when using a new spectrophotometer.

Conversion Factors

DeNovix DS-11 Series Spectrophotometers / Fluorometers enable the measurement of microbial cultures using either the 1 µL microvolume mode (optimal for higher density cultures) or various path length cuvette modes.

The DS-11 Microvolume mode enables much higher OD_{600} values using its patented automatic path length adjustment. Keeping in mind that the microvolume and cuvette modes use different optical configurations, it may be useful to establish a conversion factor when comparing values measured using the two modes.

$$\text{Factor} = \frac{OD_{600}\ \text{Spectrophotometer A}}{OD_{600}\ \text{Spectrophotometer B}}$$

It is best to determine the factor using measurements close to the desired target OD_{600} value. Account for dilutions as appropriate.

An equivalent factor may be determined and then applied to measurements made using different spectrophotometers.

Example Data

The data below is an example of how measuring the same light scattering sample on different spectrophotometers may result in different measured OD600 values (Tables 1 and 2).

A Formazin 4000NTU turbidity standard (Hach cat #246149) was diluted 7.7-fold to produce the initial stock solution. A series of 2-fold serial dilutions in water were then performed. Each solution was measured in triplicate using disposable cuvettes with 2 mL of the respective dilution. No baseline corrections were applied to the 600 nm measurements.

Table 1: Mean OD600 Values; n=3

Sample	Agilent 8453	DS-11+
Stock	1.1588	0.8376
Stock 1:2	0.5696	0.4245
Stock 1:4	0.2794	0.2173
Stock 1:8	0.1372	0.1125
Stock 1:16	0.0684	0.0594
Stock 1:32	0.0326	0.0310
Stock 1:64	0.0140	0.0158

Table 2: Precision of Replicate Measurements; n=3

Sample	Agilent 8453 % CV	DS-11+ % CV
Stock	0.61%	0.24%
Stock 1:2	0.82%	1.15%
Stock 1:4	1.61%	2.76%
Stock 1:8	0.63%	1.92%
Stock 1:16	0.91%	3.82%
Stock 1:32	5.03%	8.58%
Stock 1:64	11.17%	10.82%

Linear Range

Cuvette based instruments generally have an upper OD limit of around 1.5 for the pathlength measured. OD_{600} values this high may not be sufficient to cover the entire growth cycle of the culture and may not be linear in regards to growth. Cultures in the death phase may exhibit different light scattering behaviors that may affect the correlation between live cell density and measured OD_{600} values.

Therefore, careful dilutions of the culture must be made in order to plot the entire growth curve or determine the limit at which measurements no longer linearly correlate with live cell density.

In addition, although a linear relationship between measured OD_{600} values and specific cell densities may overlap in range on two different instruments, the slope of the linear line may differ. The data from Table 1 is graphed below (Figure 1) as an example of this paradigm.

When using conversion factors to correlate data from one instrument to another, it may be necessary to use different factors for specific OD ranges to compensate for the different slopes.

Note: Although the slopes differ, the R2 values for both instruments are > 0.9999.

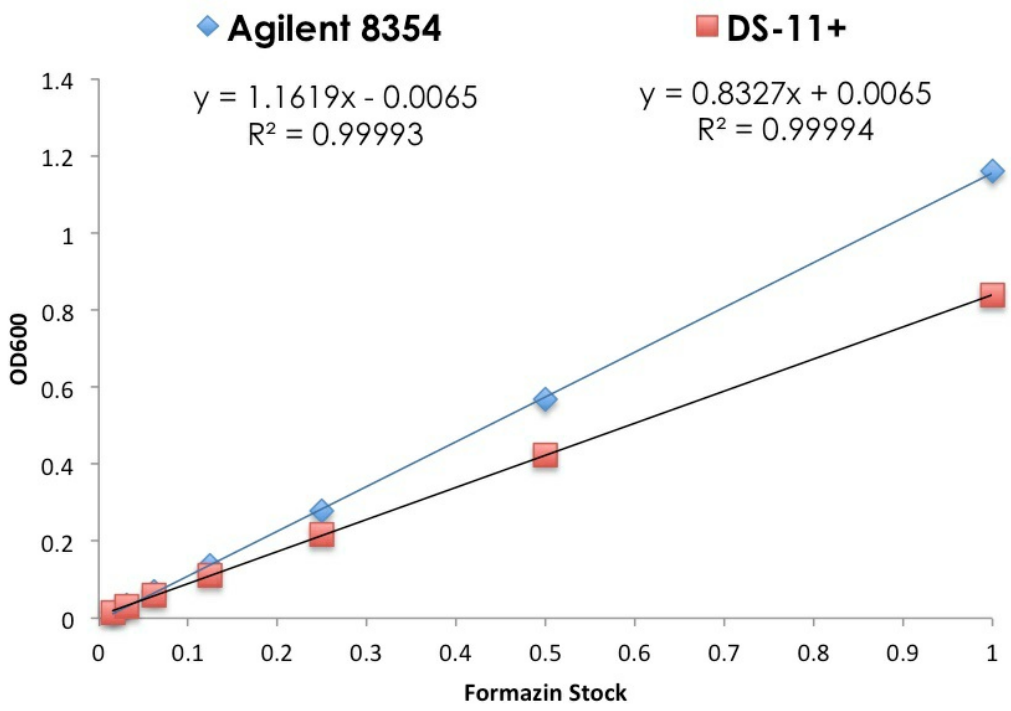

Figure 1: Linear relationship between Formazin standards and measured OD600 values.

OD600 Values and Cell Number

The OD_{600} app in the DS-11 + software allows the user to enter a cell number factor that will be multiplied by the measured OD_{600} value to calculate an approximate cells/mL concentration. As previously discussed, OD_{600} values are dependent on the size and shape of the cells from the solution being sampled as well as the cell density. An OD_{600} value of 1 might equal approximately 1 $x10^8$ cells for one cell type yet equal only 0.5 $x10^8$ cells for another.

To use this feature, it is recommended that appropriate cell number conversion factors be initially determined for each cell type. Construct a calibration curve for OD_{600} values correlated to cell numbers by colony forming units grown on agar plates. Be sure to multiply by dilution factors when appropriate.

Yeast cells are typically larger than bacterial cells. Therefore, an OD_{600} value of 1 will be generally be equivalent to fewer yeast cells than for bacterial cells.

Best Practices

To ensure the best results, target OD_{600} values should be empirically determined for each cell type for both the microvolume and cuvette modes.

Cuvette Mode (recommended)

- Ensure that the culture is well mixed and that cells have not settled prior to taking an aliquot from the suspension.
- Ensure that expected cell density is within the linear range of the selected DS-11+ cuvette mode.
- Use high quality plastic cuvettes or quartz cuvettes with Z-heights of 8.5 mm.
- Use clean cuvettes for each sample measurement. Clean cuvettes according to the manufacturer's recommended protocol.
- Ensure that the cuvette is inserted in the proper orientation.

Microvolume Mode

- Clean both sample measurement surfaces prior to making the Blank measurement.
- Ensure that the culture is well mixed and that cells have not settled prior to taking an aliquot from the suspension.
- Ensure that expected cell density is within the linear range of the selected DS-11 / DS-11+ Microvolume mode. Keep in mind that although the microvolume mode can measure samples with very high OD values, the culture itself might not demonstrate a linear response to high cell densities.
- Ensure that a full 1 µL sample is delivered to the sample surface for each measurement.
- Use a fresh aliquot for each measurement.
- Use a fresh tip to deliver each sample aliquot.
- Avoid introducing bubbles when pipetting samples onto the measurement surfaces.
- Use a dry lab wipe to remove the sample from both the top and bottom surfaces immediately after each measurement.

OD at Different Wavelengths

The Formula Methods app enables the creation of customized methods and formulas, including those for measuring OD at wavelengths other than 600 nm. OD at 650 nm, for example can be measured using the following parameters: **Analysis nm** = 650, **Baseline nm** = leave blank, **Min nm** = 550, **Max nm** = 700.

Summary

DeNovix DS-11 Series instruments enable the measurement of both yeast and microbial cultures using the preconfigured OD_{600} app.

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DeNovix Inc.
3411 Silverside Road
Wilmington, DE 19810, USA

Phone: +1.302-442-6911
Email: info@denovix.com
www.denovix.com