Analyzing Differences in Bacterial Optical Density Measurements between Spectrophotometers

Abstract 1730

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Abstract

Background

Optical density (OD) measurement of bacterial cultures is a common technique used in microbiology. Researchers have primarily relied on spectrophotometers to make these measurements, however the measurement is actually based on the amount of light scattered by the culture rather than the amount of light absorbed. In their standard configuration, spectrophotometers are not optimized for light scattering measurements; commonly resulting in differences in measured absorbance between instruments.

Methods

This study investigated the effect of different spectrophotometer optical configurations on OD measurements of *E. coli* JM109 grown in batch culture. Spectrophotometers tested included reverse-optical systems utilizing array detection, traditional monochromator-based systems, and a monochromator-based system with an integrating sphere accessory (ISA). Growth curves were measured on each instrument by determining the optical density at 600 nm (OD600) and by counting CFU/mL. OD of McFarland standards was also measured in order to further characterize each optical system.

Results

OD data from spectrophotometers in the same optical configuration class were comparable. There was more variability between measurements at higher OD with the reverse optical systems, caused by lower stray light performance of these instruments. Monochromator-based systems produced superior precision at higher OD primarily due to better stray light rejection of the monochromatic versus polychromatic light from reverse optical systems. However, the reverse optical systems had a comparable dynamic range with respect to OD measurements. Use of an ISA resulted in data dissimilar from that produced with other systems because of its ability to capture nearly all of the forward scattered light. Measurement of McFarland standards confirmed these observations.

Conclusions

The ability of a spectrophotometer to make reliable light scattering measurements is highly dependent on its optical configuration; thus, spectrophotometers with different optical configurations show different OD measurements. Ideally, absorbance of highly scattering samples such as cell cultures is measured using an ISA in order to capture nearly all of the scattered light. Culture growth may be determined using OD600, however whenever changing spectrophotometers, a conversion factor should be calculated and applied.

Introduction

The standard phases of bacterial culture growth (lag, log, stationary, and death) are well documented, with the log phase recognized as the point where bacteria divide as rapidly as possible(1). Using a spectrophotometer to measure the optical density at 600 nm (OD600) of a bacterial culture to monitor bacterial growth has always been a central technique in microbiology. Three of the more common applications where bacterial OD600 is used are the following: (a) determination and standardization of the optimal time to induce a culture during bacterial protein expression protocols, (b) determination and standardization of the inoculum concentration for minimum inhibitory concentration (MIC) experiments, and (c) determination of the optimal time at which to harvest and prepare competent cells. Researchers continue to rely on absorbance spectrophotometers to make these OD measurements. Optical density, however, is not a measure of absorbance, but rather a measure of the light scattered by the bacterial suspension which manifests itself as absorbance (Figure 1). The effect that the optical configuration of a spectrophotometer has on optical density measurements has been well documented (2-4). Instruments with different optical configurations will measure different optical densities for the same bacterial suspension. Differences in the optical configuration of the spectrophotometer make the largest contribution to the observed differences. Forward optical systems employ monochromatic light for the measurement of absorbance where reverse optical systems utilize polychromatic radiation that is discriminated into individual wavelengths after it is passed through the sample (Figure 2). Some components of the forward optical systems contribute to a difference in measured OD values: (a) the distance between sample and detector, (b) the size and focal length of any collector lens used, and (c) the area and sensitivity of the detector (5). Despite current knowledge that different optical configurations will give different OD values, researchers continue to raise concerns about the differences in OD values seen among different spectrophotometers. In this study, we analyzed OD values obtained from several spectrophotometers possessing various optical configurations. We grew *E. coli* JM109 in batch culture and monitored the OD of the culture for 9.5 hours. We demonstrate the need to dilute cultures before measurement and show how to apply a conversion factor to the OD data in order to normalize differences in OD values due to the optical geometries found in different spectrophotometers.

Figure 1 *Light scattering in spectrophotometry.*

A In a non-scattering sample, the attenuation in light transmission between the light source and the detector is caused by the absorbance of light by the sample.

B In a scattering sample, (i.e., a bacterial suspension), the light reaching the detector is further reduced by the scattering of light. This decrease in light reaching the detector creates the illusion of an increase in sample absorbance.

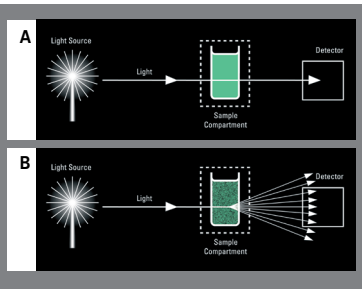
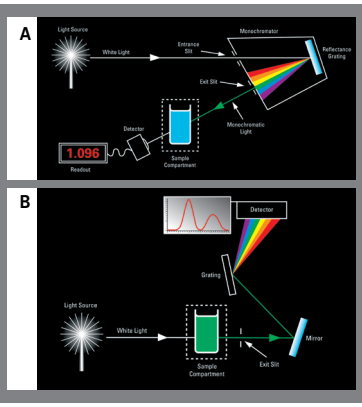


Figure 2 *Differences between forward and reverse optical systems.*

A In a forward optical system monochromatic light passes through the sample and then onto a detector.

B In a reverse optical system, polychromatic light passes through the sample, discriminated into individual wavelengths and measured on an array detector.



Materials and Methods:

Strain: *E. coli* JM109-endA1, recA1, gyrA96, thi, hsdR17 (rk–, mk+), relA1, supE44, (lac-proAB), [F´ traD36, proAB, laqIqZ M15] (Promega, L2001)

Spectrophotometers: Thermo Scientific spectrophotometers used are described in Table 1.

Growth Curve: A 50 mL overnight *E. coli* JM109 culture was grown in a 250 mL baffled flask (16 hr, 250 rpm, 37°C) in Luria Bertani (LB) Broth. A batch culture was prepared by transferring 12 mL of the overnight culture in 600 mL of pre-warmed (37°C) LB media in a 2L baffled flask. The batch culture was grown for a total of 9.5 hours.

Culture Sampling: All spectrophotometers were blanked using LB broth. Every 30 minutes, a 5 mL aliquot was sampled from the batch culture. A 3 mL aliquot of undiluted culture was transferred to a 10 mm cuvette, and the optical density at 600nm was measured on all the instruments listed above in Table 1. A second 10 mm cuvette was prepared with an aliquot of the batch culture diluted in LB medium. This second OD measurement was measured to ensure that the optical density of the culture remained within the dynamic OD range of the spectrophotometers.

Viable Counts: At each 30-minute interval, an aliquot of the sample was also used to perform serial dilutions. Dilutions were plated on LB Agar plates and incubated at 37°C overnight. Colonies were counted to determine bacterial cell count (CFU/ml) at each time point.

McFarland Standards: OD600 measurements of aliquots from four McFarland standards (Remel, R20421, 1.0 – 4.0) were measured on each instrument (Table 1) by using 1cm pathlength cuvettes.

Table 1 *Thermo Scientific Spectrophotometers*

Instrument	Optical System	Optical Geometry	Light Source(s)	Detector
Evolution Array	Reverse optic		Deuterium and tungsten lamps	Photodiode Array
NanoDrop 2000c	Reverse optic		Xenon flashlamp	CMOS Array
SPECTRONIC 200	Reverse optic		Tungsten lamp	CCD Array
Evolution 260 Bio	Forward optic	Double Beam	Xenon flashlamp	Silicon Photodiodes
Evolution 300	Forward optic	Double Beam	Xenon flashlamp	Silicon Photodiodes
BioMate 3S	Forward optic	Dual Beam	Xenon flashlamp	Silicon Photodiodes

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Results

OD600 data collected from the undiluted and diluted cultures is shown in Figure 3. OD600 values from diluted solutions were multiplied by the dilution factor and compared to undiluted samples. Divergance of the two plots illustrates that different optical configurations will have different dynamic ranges with respect to optical density measurements. When the corrected OD values for each spectrophotometer and the cell counts were compared, we found that instruments with similar optical systems produced similar OD curves over time (Figure 4). The BioMate 3s, which has a forward optical system but a dual-beam optical geometry, diverges the most from the group of instruments. The McFarland data shows a similar trend as was observed with the *E. coli* growth curves; similar optical systems grouped together (Figure 5). The ISA produced much lower optical densities using the McFarland standards (Figure 6).

Figure 3 *Comparison of growth curves of* E. coli *JM109 defined by measuring OD600 of diluted or undiluted bacterial samples. OD600 measurements were performed on the Evolution 260 Bio and the Evolution Array instruments. OD measurements were carried out every 30 minutes for 9.5 hours. Blue lines represent the OD600 from diluted culture samples. Samples were diluted so they were within the dynamic range of the optical system. Green lines represent the OD600 of undiluted culture samples.*

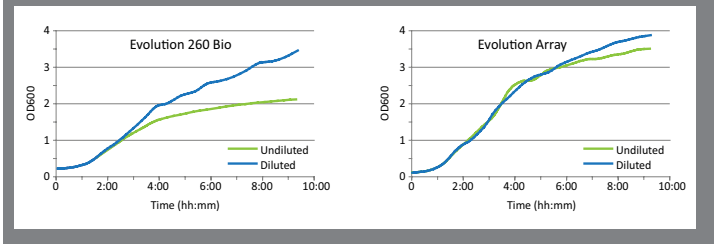


Figure 4 *Growth curves of* E. coli *JM109 obtained from corrected OD values. The OD600 was measured on all instruments every 30 minutes for 9.5 hours. The OD600 data was corrected by the dilution factor used for OD measurement. Samples were diluted so they were within the linear range of the optical system. The corrected OD was calculated by multiplying the OD by the dilution factor. Then it was plotted along side the viable cell count data.*

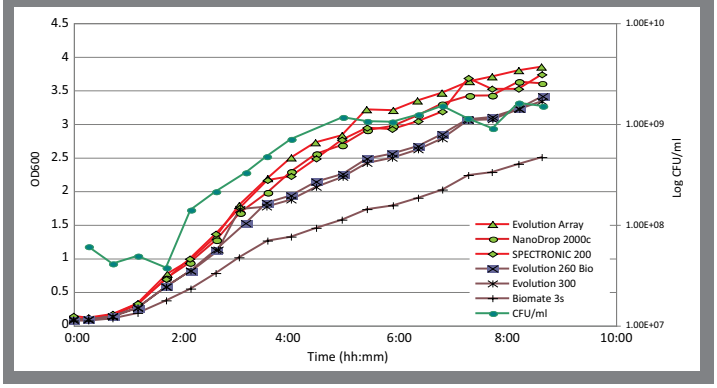


Figure 5 *The OD600 of various McFarland standards measured on spectrophotometers listed in Table 1.*

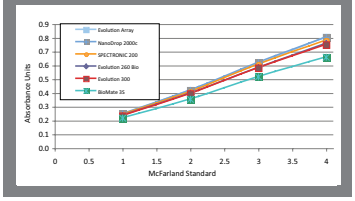
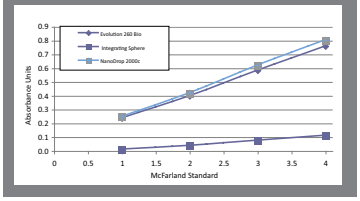


Figure 6 *Comparison of OD data measured on standard spectrophotometers and a spectrophotometer with an ISA. The OD600 of various McFarland standards was measured on an Evolution 260 Bio (dark blue), NanoDrop 2000c (light blue), and with an integrating sphere accessory (black). Significant differences in absorbance values were observed between the spectrophotometer with the ISA and standard instruments.*



Conversion Between Spectrophotometers

The variation in optical density observed between two instruments can make standardization of a protocol difficult. This is especially true when one laboratory tries to replicate data from another lab, but uses a different spectrophotometer, or when an aging spectrophotometer is replaced in the same lab.

Figure 3 shows the OD of a culture measured on both an Evolution 260 Bio and a BioMate 3S. The OD ratio between the two instruments was calculated at each time point. The OD600 ratio from each time point was determined and the average ratio was calculated and used as a multiplication factor. For the BioMate 3S the calculated conversion factor was 1.46. Application of this correction factor to the OD data normalizes the data and facilitates comparison between both instruments (Figure 7). To better facilitate agreement among spectrophotometers a correction factor is included in the local control software of the BioMate 3S spectrophotometer.

Figure 7 *Application of a conversion factor to compare OD600 data from two different spectrophotometers. Green = original BioMate 3S data; Red = BioMate 3S data x factor of 1.46.*

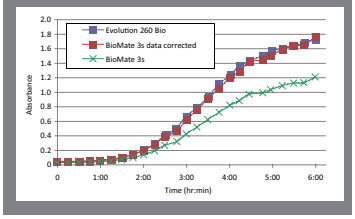


Figure 8 *Equation calculation for conversion factor between spectrophotometers. For the most accurate conversion factor two things are important; (1) All OD measurements used to calculate these values are from the corrected OD data (Figure 3), (2) take the average of multiple conversion factor that are calculated across a range of various OD measurements.*

$$\frac{\text{Current Instrument OD}}{\text{New Instrument OD}} = \text{Conversion Factor}$$

Conclusion:

- It is critical to determine the optical density performance and dynamic range for your spectrophotometer when calculating OD measurements. For the most accurate information about the growth curve it is important to dilute the cell suspension.
- OD measurements are highly dependent upon the optical system and geometry. Spectrophotometers with different optical systems and configurations will read different OD values for the same suspension. For example, there is substantial divergence between the reverse optical system Evolution Array and the forward optical system, dual-beam BioMate 3S.
- An integrating sphere accessory (ISA) captures nearly all of the forward scattered light, which results in much lower optical densities. This is due to the fact that scattered light which normally appears to be absorbed by the sample is actually collected by the ISA accessory. An ISA is ideal for measuring the absorbance of analytical components in highly scattering solutions; however it is ineffective at measuring the optical density of cell suspensions.
- Finally we present how to calculate a conversion factor that can be used to normalize the OD data so that appropriate comparisons can be made between spectrophotometers. It is important to note that this conversion factor is specific to a particular organism because the size and shape of the particle will affect the conversion factor.

References:

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