TECHNICAL NOTE

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Differences in bacterial optical density measurements between UV-Visible spectrophotometers

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Key words
Bacterial growth, *E. coli*, optical density (OD), OD600, UV-Vis spectrophotometers

Background

Optical density (OD) measurement of bacterial cultures is a common technique used in microbiology. While researchers have relied on UV-Visible spectrophotometers to make these measurements, 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.

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] To monitor bacteria growth a spectrophotometer is used to measure the optical density at 600 nm (OD600). Three of the more common applications where bacterial OD600 is used are:

- Determination and standardization of the optimal time to induce a culture during bacterial protein expression protocols
- Determination and standardization of the inoculum concentration for minimum inhibitory concentration (MIC) experiments
- Determination of the optimal time at which to harvest and prepare competent cells

Researchers and production facilities continue to rely on absorbance spectrophotometers to make these OD measurements.

Optical density, however, is not a measurement of absorbance as we understand it in colorimetric methods. It is a measurement of the reduction in transmittance caused by scattering from the bacterial suspension. The spectrophotometer converts this attenuated transmittance to absorbance just as it would in a colorimetric analysis.

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. Some components of the forward optical systems contribute to a difference in measured OD values:

- The distance between sample and detector
- The size and focal length of any collector lens used
- The area and sensitivity of the detector[5]

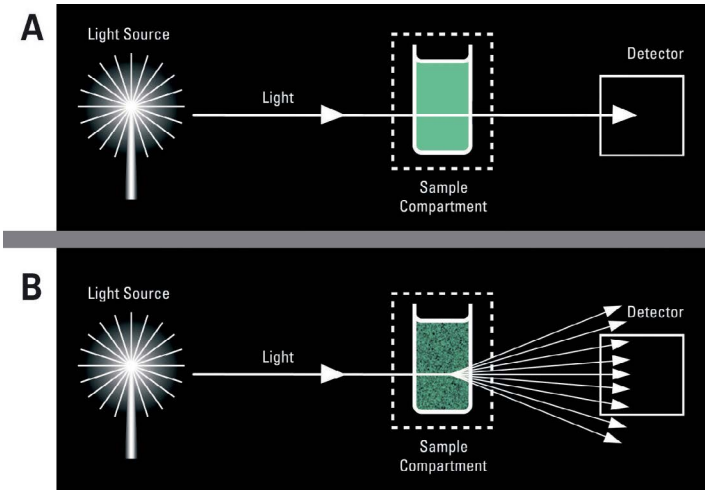

Figure 1: Light scattering in spectrophotometry

A. In a non-scattering sample, the attenuation in light transmission between the light source and the detector is caused by photochemical 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 scattering of light off the cells in suspension. This decrease in light reaching the detector creates the appearance of an increase in sample absorbance.

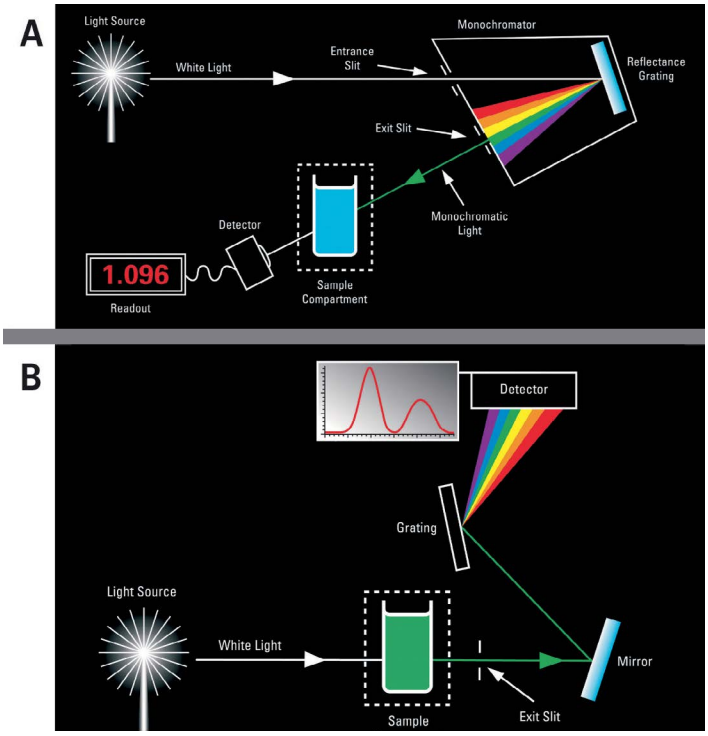

Figure 2: Differences between forward and reverse optical systems

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

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.

Materials and methods

Strain

E. coli JM-109-*end* A1, *rec*A1, *gyr*A96, *thi, hsd*R17, (r_k^-, m_k^+), *rec*A1, *sup*E44, (*lac-pro* AB), [F'*tra* D36, *pro* AB, *laq* I^qZ M15] (Promega, L2001)

Spectrophotometers

Thermo Scientific™ UV-Visible spectrophotometers (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 2 L 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 600 nm was measured on all the instruments listed below in Table 1.

A second 10 mm cuvette was prepared with an aliquot of the batch culture diluted in LB medium to an OD600 of approximately 0.5. 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 by using 1 cm pathlength cuvettes.

Instrument	Optical system	Optical geometry	Light source(s)	Detector
Thermo Scientific™ NanoDrop™ 2000c	Reverse optic		Xenon flashlamp	CMOS array
Thermo Scientific™ SPECTRONIC™ 200	Reverse optic		Tungsten lamp	CMOS array
Thermo Scientific™ BioMate™ 160	Forward optic	Dual beam	Xenon flashlamp	Silicon photodiodes
Thermo Scientific™ Evolution™ 260 Bio	Forward optic	Double beam	Xenon flashlamp	Silicon photodiodes
Thermo Scientific™ Evolution™ 350	Forward optic	Double beam	Xenon flashlamp	Silicon photodiodes

Table 1: Thermo Scientific spectrophotometers

Results

OD600 data was collected from undiluted and diluted cultures and representative growth curves are shown in Figure 3. OD600 values from diluted solutions were multiplied by the dilution factor and compared to undiluted samples. Divergence 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 160 Spectrophotometer, which has a forward optical system but a dual beam optical geometry, shows a systematically lower OD600 measurement than indicated by the plate counting method. This is most likely due to its unique optical geometry. The McFarland data shows a similar trend as was observed with the *E. coli* growth curves; similar optical systems grouped together (Figure 5).

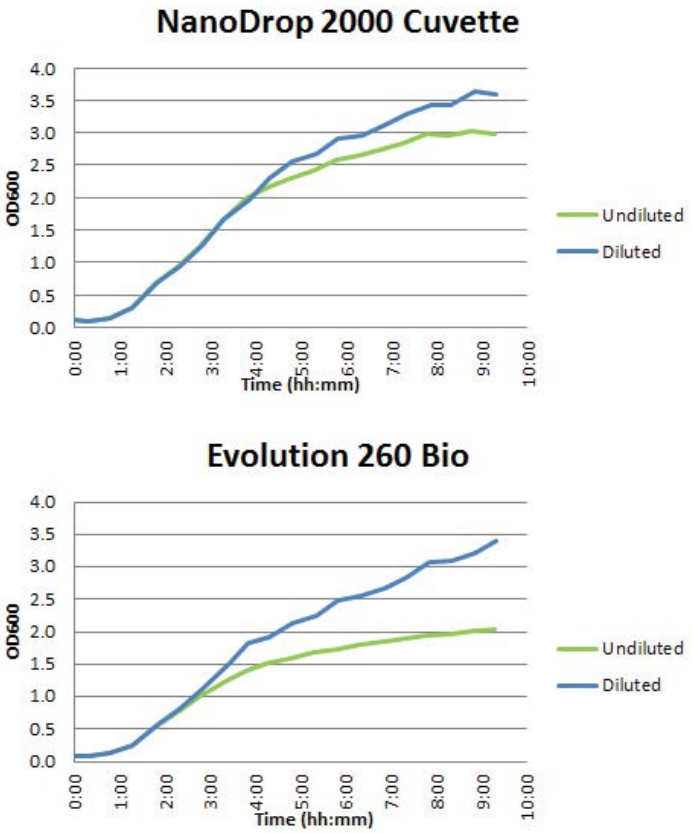

Figure 3: Comparison of growth curves of *E. coli* JM109 defined by measuring OD600 of diluted or undiluted bacterial samples. OD600 measurements were performed on the Evolution 260 Bio instruments. OD measurements were carried out every 30 minutes for 9.5 hours. Blue lines represent the OD600 from diluted 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.

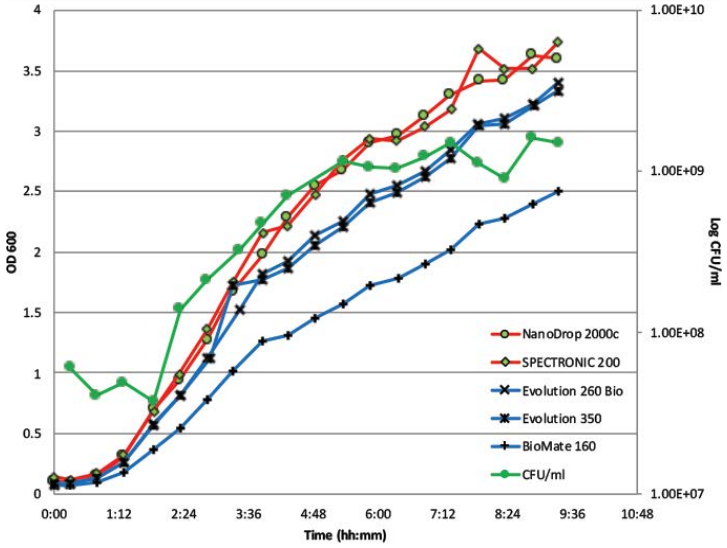

Figure 4: Growth curves of *E. coli* JM109 obtained from corrected OD values. The OD600 was measured on all instruments every 30 minutes for 9.5 hours. The OD600 data was corrected by the dilution factor used for OD measurement. Samples were diluted so they were within the linear range of the optical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

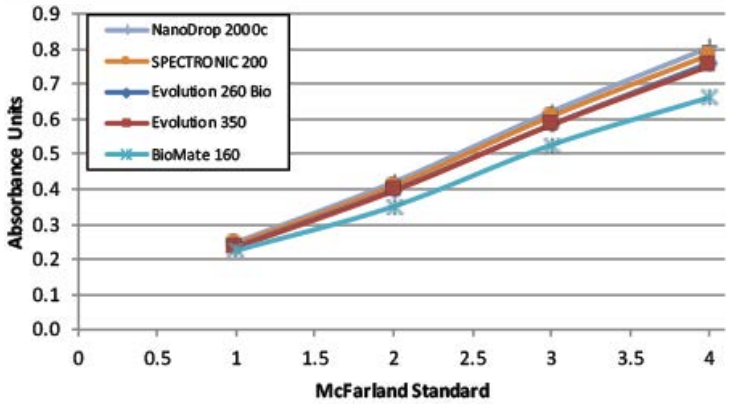

Figure 5: The OD600 of various McFarland standards measured on spectrophotometers listed in Table 1.

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.

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Figure 6 shows the OD of a culture measured on both an Evolution 260 Bio Spectrophotometer and a SPECTRONIC 200 Spectrophotometer. The OD ratio between the two instruments was calculated at each time point. The average of these ratios was calculated and used as a multiplication factor. For the Evolution 260 Bio Spectrophotometer, the calculated conversion factor was 1.22. Application of this conversion factor to the OD data normalizes the data and supports comparison between both instruments.

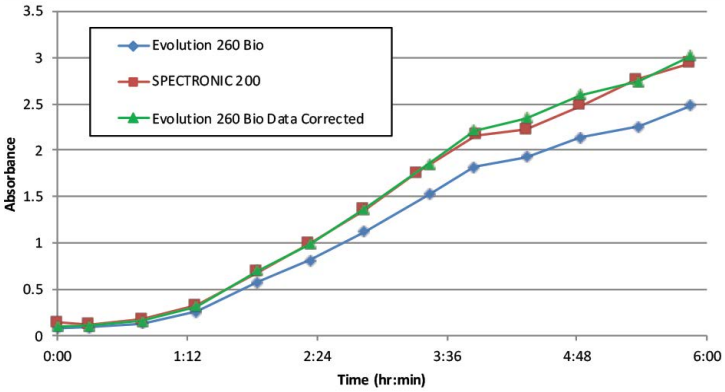

Figure 6: Application of a conversion factor to compare OD600 data from two different spectrophotometers. Blue = original Evolution 260 Bio data; Green = Evolution 260 Bio data × factor of 1.22.

$$\frac{\text{Current instrument OD}}{\text{New instrument OD}} = \text{Conversion factor}$$

Figure 7: Equation calculation for conversion factor between spectrophotometers.

For the most accurate conversion factor two things are important:

- All OD measurements used to calculate these values are from the corrected OD data (Figure 3)
- Take the average of the multiple conversion factors that are calculated across the log range of the OD measurements

Conclusion

When calculating OD measurements, it is critical to determine the optical density performance and dynamic range for your spectrophotometer. If OD readings fall outside the dynamic range of the instrument, 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 of NanoDrop 2000c Spectrophotometer and the forward optical system, dual-beam BioMate 160 Spectrophotometer.

Finally, we present how to calculate a conversion factor that can be used to normalize the OD data in order for appropriate comparisons to be made between spectrophotometers (Figure 7). 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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