Measurement of Low-Volume DNA Samples Using the Hellma TrayCell Fiber-Optic Device With the SmartSpec™ Plus Spectrophotometer

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Introduction

UV spectrophotometry is a widely used method for determining the concentration and purity of nucleic acid samples in the laboratory. Traditionally, large sample volumes (on the order of 50–100 µl) have been required to produce accurate measurements. This has been a drawback to the method since precious experimental RNA and DNA samples are often obtained only in low volumes. The recent introduction of spectrophotometers that have the ability to measure sample concentrations using low volumes has alleviated this problem, but at the cost of purchasing an expensive instrument.

The Hellma TrayCell fiber-optic device (Hellma USA, Inc.) can be used to analyze small volumes of biological samples, such as proteins and nucleic acids, on a standard spectrophotometer. The Hellma TrayCell device uses integrated beam deflection and fiber-optic cables to measure the sample directly on the surface of its optical window (Figure 1) (www.traycell.com). The Hellma TrayCell device comes with two caps, each providing a well-defined optical path (either 1 or 0.2 mm). The caps ensure reproducible measurements of samples because evaporation is minimized. They also enable the measurement of low-surface-tension samples that may otherwise be difficult to measure using small volumes. The 1 mm cap is optimized for low-concentration biological samples (<850 ng/µl) and requires 3–5 µl of sample. The 0.2 mm cap is designed for higher-concentration samples (up to 4,250 ng/µl), and uses as little

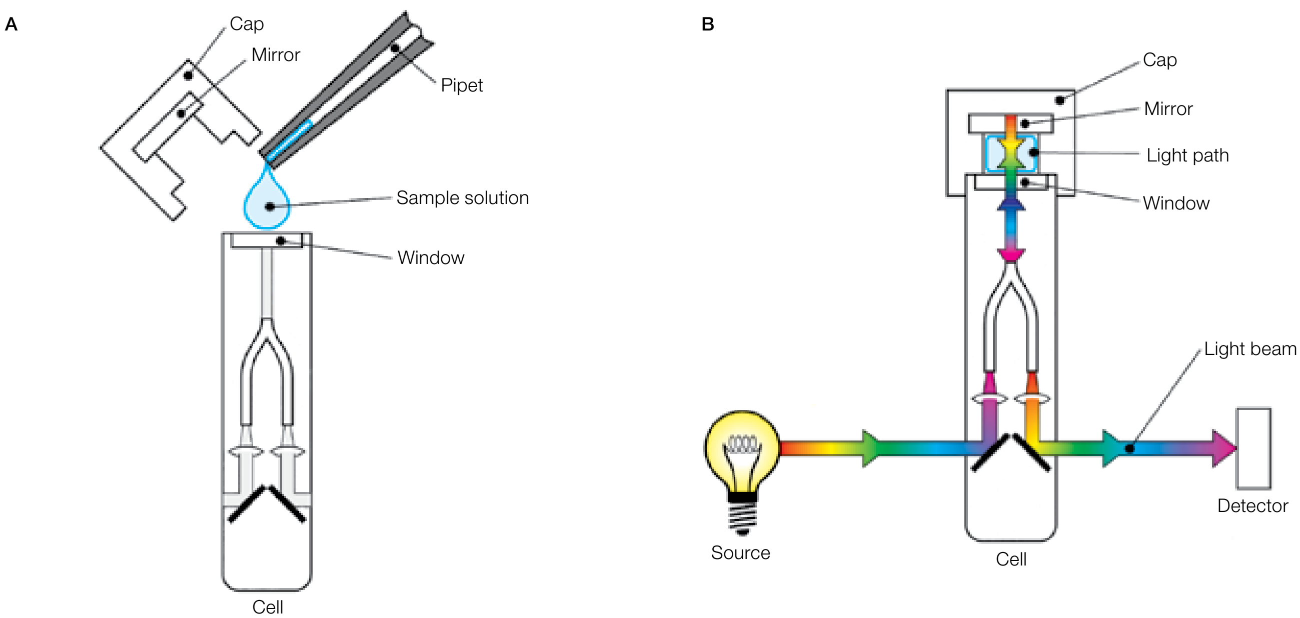

Fig. 1. Schematic representation of the Hellma TrayCell device. A, loading the device; **B**, device in the SmartSpec Plus spectrophotometer.

as 0.7 µl of sample to obtain a measurement. The dynamic range of the Hellma TrayCell device for double-stranded DNA (dsDNA) is reported to be 25–4, 250 ng/µl; however, the actual dynamic range may vary depending on the spectrophotometer being used

The SmartSpec™ Plus spectrophotometer from Bio-Rad Laboratories, Inc. is an accurate and dependable UV/visible scanning benchtop spectrophotometer with an accessible user interface that instantly calculates sample concentrations and nucleic acid purity. In its standard configuration, the SmartSpec Plus spectrophotometer requires that samples be diluted to 50–100 ng/µl and a minimum sample volume of 50 µl is used with trUView™ cuvettes, resulting in accurate and precise photometery within its linear range. Coupling the SmartSpec Plus spectrophotometer with the Hellma TrayCell fiber-optic device combines the precision and accuracy of the instrument with the convenience of using low sample volumes for measuring DNA and RNA concentrations.

In this study, we determined the dynamic range of dsDNA concentration measurements using the Hellma TrayCell device with the SmartSpec Plus spectrophotometer. The results were compared to the same samples analyzed using either the SmartSpec Plus spectrophotometer with trUView cuvettes and 50 µl sample, or a competitor spectrophotometer that requires only 1 µl of sample and has a reported dynamic range of 2–3,700 ng/µl for dsDNA. We found that the Hellma TrayCell device increased the linear dynamic range of the SmartSpec Plus spectrophotometer to 25–3,000 ng/µl. The competitor spectrophotometer is limited to use with low-volume (1–2 µl) samples. Adding the Hellma TrayCell device to the SmartSpec Plus spectrophotometer adds low-volume capacity to the system, expanding the accurate measurement range to 1–50 µl of sample.

Methods

To determine the dynamic range of the Hellma TrayCell device, we made a dilution series of sheared human genomic DNA (Sigma-Aldrich) with calculated concentrations from 4,000 ng/µl down to 3.125 ng/µl. Absorbance at 260 and 280 nm was measured ten times for each dilution using either the SmartSpec Plus spectrophotometer or the competitor spectrophotometer. All readings using the Hellma TrayCell device were performed with 3 µl of sample, and both the 1 and 0.2 mm caps were used for each point in the dilution series. For comparison, 1 µl of each dsDNA dilution was measured on the competitor spectrophotometer.

As a positive control, 50 µl of each dsDNA dilution was measured with trUView cuvettes (standard cuvettes with a 1 cm pathlength) in the SmartSpec Plus spectrophotometer. For the sample set, dsDNA samples with calculated concentrations >100 ng/µl were further diluted to ensure that samples were within the linear range of the SmartSpec Plus spectrophotometer. The measured concentration was then multiplied by the appropriate dilution factor to obtain the actual concentration of the sample. This procedure also served to confirm the accuracy of the calculated concentrations of the dsDNA dilution series.

Data were analyzed by comparing the calculated DNA concentration to the measured concentration for each dilution and instrument/fiber-optic device combination tested. The average concentration and percent coefficients of variation (%CV) for each set of measurements were calculated and used to determine the precision and dynamic range of each tested instrument/fiber-optic device combination.

Results

Both the Hellma TrayCell device and the competitor spectrophotometer were able to accurately measure dsDNA concentrations over a wide range without requiring dilution of the original sample (Table 1). We found that the Hellma TrayCell device extended the dynamic range of the SmartSpec Plus spectrophotometer from 50–100 ng/µl to 25–3,000 ng/µl of dsDNA. The range of the Hellma TrayCell device was comparable to that of the competitor spectrophotometer, which was 3.125–1,000 ng/µl in our study.

Data collected using the SmartSpec Plus spectrophotometer with trUView cuvettes served as a positive control in these experiments. Samples measured using this traditional method had to be diluted to <100 ng/µl to be within the linear range of the photometer. Therefore, if the calculated concentrations were accurate, then plotting the measured vs. the calculated concentration of dsDNA would give a slope of 1.0. The actual slope was 1.05 (Figure 2), indicating that the calculated dsDNA concentrations were accurate and could be used as the reference concentrations for the data analysis. The Hellma TrayCell device with the SmartSpec Plus spectrophotometer outperformed the competitor spectrophotometer for accuracy and had a slope of 0.86. The competitor spectrophotometer had the poorest correlation between measured and calculated DNA concentrations with a slope of 0.73.

Table 1. Comparison data for dsDNA concentrations.*

Concentration of dsDNA (ng/µl) Based on Serial Dilutions	SmartSpec Plus Spectrophotometer With Hellma TrayCell		Competitor Spectrophotometer		SmartSpec Plus Spectrophotometer With truView Cuvettes	
	dsDNA, ng/µl	%CV**	dsDNA, ng/µl	%CV	dsDNA, ng/µl	%CV
4,000	3,327.95	0.91	2,894.36	0.32	4,161	0.92
3,000	2,666.55	0.94	2,230.76	0.79	3,189	0.52
2,000	1,846.36	0.43	1,515.91	0.78	2,132	0.00
1,000	903.30	1.01	771.22	0.41	1,062	0.60
500	449.51	0.00	411.89	1.61	520.1	0.00
100	88.91	0.93	95.91	0.83	100.3	0.52
50	42.62	6.33	49.31	1.37	61.2	0.45
25	22.25	6.49	24.71	1.22	35.9	0.22
12.5	7.94	20.60	11.99	3.70	21.7	0.55
6.25	—	—	5.84	4.71	15.4	0.85
3.125	—	—	2.96	14.17	12.1	1.2

* The 1 mm cap of the Hellma TrayCell device was used to measure dsDNA concentrations between 3.125–500 ng/µl, while the 0.2 mm cap was used to measure the 1,000–4,000 ng/µl samples.

** n = 10.

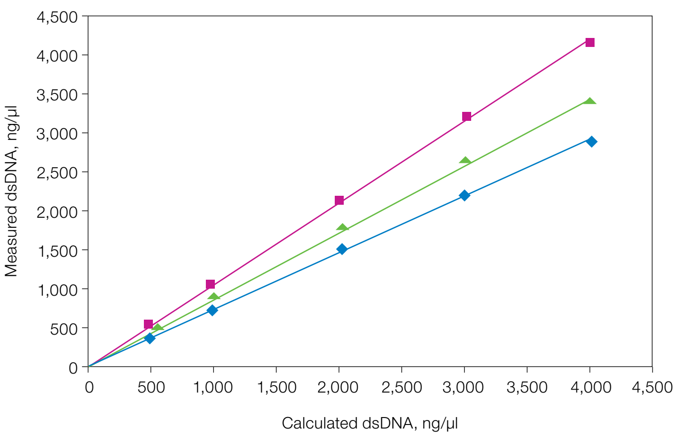

Fig. 2. Plot of calculated versus measured dsDNA concentrations. SmartSpec Plus spectrophotometer with Hellma TrayCell device (▲ slope = 0.86, r^2 = 0.9975), competitor spectrophotometer (◆ slope = 0.73, r^2 = 0.9994), and the SmartSpec Plus spectrophotometer with trUView cuvettes (■ slope =1.05, r^2 = 0.9998).

The Hellma TrayCell device accurately measured up to 3,000 ng/µl of dsDNA, while the competitor spectrophotometer was inaccurate above 1,000 ng/µl of dsDNA (Table 1). The Hellma TrayCell device was accurate when measuring as little as 25 ng/µl dsDNA; however, the %CV at this lower concentration was 6.49%. The competitor spectrophotometer was the most accurate for low concentrations of dsDNA, giving accurate readings at 3.125 ng/µl with a %CV <5% for concentrations as low as 6.25 ng/µl. It should be noted that even though the SmartSpec Plus spectrophotometer with standard cuvettes had only a small dynamic range compared to the other conditions tested, it had the highest precision with a %CV <1.2% in all cases.

Conclusions

The Hellma TrayCell fiber-optic device is designed for measuring low-volume samples and extending the dynamic range of traditional spectrophotometers. Since the Hellma TrayCell device is compatible with traditional cuvette-style spectrophotometers, the scientist retains the flexibility to perform cell density, colormetric, and simple kinetic assays that require a cuvette, while gaining the additional ability to perform low-volume concentration measurements. We tested the ability of the SmartSpec Plus spectrophotometer to measure a wide range of dsDNA concentrations when combined with the Hellma TrayCell device. We found that only 3 µl of sample are needed to accurately measure dsDNA concentrations ranging from 25–3,000 ng/µl. The Hellma TrayCell device is extremely user-friendly — it is compatible with standard spectrophotometers, does not have to be removed from the instrument between readings, and requires only a few seconds to clean between samples. This fiber-optic device is an excellent choice for measuring low-volume nucleic acids in conjunction with the SmartSpec Plus spectrophotometer, without the need for a costly new instrument.

Information in this tech note was current as of the date of writing (2007) and not necessarily the date this version (rev A, 2008) was published.

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