Unique optical performance of the Cary 50 UV-Vis Spectrophotometer ideal for analyzing microvolume samples

Technical Overview

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Introduction

Given the increasing demand for simple, rapid and non-destructive methods to analyze microliter volumes of chemical and biological samples, we have previously demonstrated that the Cary 50 UV-Vis spectrophotometer fitted with an ultra-microvolume cuvette can accurately measure concentration values of 4 µL samples of DNA. Given the occasional need to a) remove and replace the ultra-microvolume cuvette for practical purposes and b) analyze lower still concentrations of biochemical samples, in this paper we extend our initial observations to evaluate the reproducibility and light throughput of the ultra-microvolume cuvette when fitted to the Cary 50 UV-Vis spectrophotometer.

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Summary

- The ultra-microvolume cuvette fitted to a Cary 50 UV-Vis spectrophotometer is ideal for the measurement of microliter (<4 µL) sample volumes
- Significantly higher light throughput in the Cary 50 compared to a competitor's system means higher photometric range
- Removal and replacement of the ultra-microvolume cuvette for cleaning or other purpose has negligible affects on data, meaning excellent reproducibility

Aim

To evaluate the reproducibility and light throughput of a Cary 50 UV-Vis spectrophotometer fitted with an ultra-microvolume cuvette for accurate and reproducible absorbance measurements of microvolume liquid samples.

Materials and methods

An ultra-microvolume cuvette was fitted into a Cary Eclipse cell holder base in the sample chamber of a Cary 50 (Figure 1).

Figure 1. Cary 50 Bio UV-Vis spectrophotometer sample chamber fitted with a ultra-microvolume cuvette

The cell holder is optimized for the ultra-microvolume cuvette product on account of enhanced tilt and height adjustment capabilities compared to the standard Cary 50 cell holder. The ultra-microvolume cuvette was aligned vertically and horizontally to optimize light thoughput at 500 nm using the Align application module in the Cary WinUV Bio software. Adjustments were made in the cuvette holder in order to obtain light transmission values greater than 25% at 500 nm, relative to transmission readings taken at 100%T (with air at ambient laboratory temperature in the sample beam).

Results

For comparative purposes only, data presented in Figure 2 show a single transmission scan throughout the wavelength range 250–1000 nm using a competitor's conventional UV-Vis spectrophotometer properly fitted with an ultra-microvolume cuvette. Air was initially scanned at the same wavelengths as a baseline. Data in Figure 3 show the results from the same test using the Cary 50 UV-Vis spectrophomometer, however in this case the ultra-microvolume cuvette was repeatedly removed and replaced five times from the cell holder base to assess reproducibility.

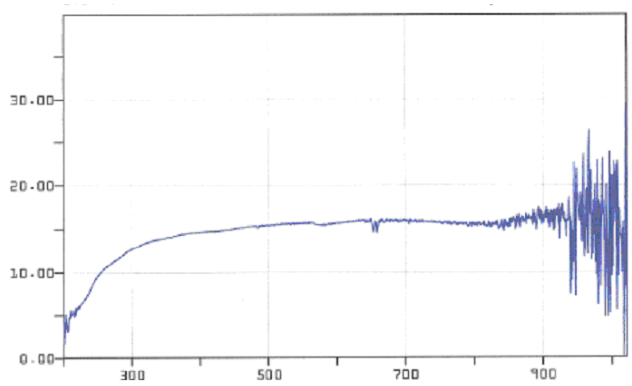

Figure 2. Reproducibility of wavelength scans of 4 µL samples of DNA, scanned over the UV region using the ultra-microvolume cuvette fitted to a conventional, competing UV-Vis spectrophotometer, not equipped with Agilent's xenon flashlamp optical design. Note noise in NIR region

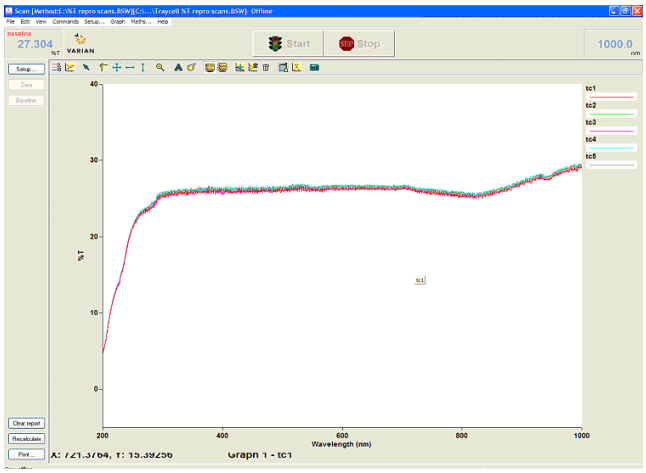

Figure 3. Reproducibility of wavelength scans of 4 µL samples of DNA, scanned over the UV region using the ultra-microvolume cuvette with the Cary 50 UV-Vis spectrophotometer

Table 1. Statistical data derived from raw reproducibility data given in Figure 3

No. Repeat Scans	Wavelength (nm)	Mean %T	StDev	%RSD
5	260	22.79	0.23	1.0
5	280	24.02	0.19	0.8
5	340	25.97	0.30	1.2
5	500	26.36	0.10	0.4
5	700	26.49	0.18	0.7
5	1000	29.31	0.25	0.9

Data shown in Figures 2 and 3 demonstrate that the Cary 50 fitted with the ultra-microvolume cuvette yields significantly higher %T values (>10% throughout the range 200–1000 nm) compared to %T values recorded using the ultra-microvolume cuvette fitted into a UV-Vis spectrophotometer from a competitor. Moreover, data in Table 1 show that repeatedly removing the ultra-microvolume cuvette from the cell holder and replacing it has negligible effects on the %T values recorded, thus demonstrating excellent reproducibility througout complete use of the ultra-microvolume cuvette. Comparing data in Figures 2 and 3 also clearly show that whilst there is excessive noise in the competitor's instrument in the NIR range 800–1000 nm, the Cary 50/ultra-microvolume cuvette system is well suited for sample measurements within the NIR range.

Discussion and conclusion

Quantitation of microliter volumes of DNA by UV-Vis spectrophotometry has a variety of benefits:

1. Low volumes - save on precious samples and reagents
2. Cleaning/purchase of quartz cuvettes or other vessels is not required
3. The method is non-destructive to samples
4. Accurate and reproducible measurements can be recorded in seconds
5. No chromophores or fluorophores are required to visualize the sample
6. No compromise in data quality compared to results recorded using larger volumes in a cuvette or microplate

In this short review, we evaluated some performance characteristics of the ultra-microvolume cuvette fitted to the Cary 50 UV-Vis spectrophotometer to confirm that a) light throughput and therefore photometric range of the Cary 50 system is far superior to that of a competitor and b) results are extremely reproducible (maximum RSD = 1.2% across the wavelength range 200–1000 nm) even when the ultra-microvolume cuvette was removed and replaced sequentially between wavelength scans.

We believe these observations indicate that the Cary 50/ultra-microvolume cuvette system is ideal for rapid, reproducible and accurate measurements of a broad range of concentrations of microliter volumes of DNA. It is the optical design of the Cary 50 instrument that makes these measurements possible, since unique xenon flashlamp dynamics and a millimeter beam size maximizes the light flux through the optical system of the ultra-microvolume cuvette. We now intend to further our investigations to explore the dynamic range of this instrument system using a variety of biomolecules and samples.

Acknowledgements

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References

1. Keighley, RA and Fyfe, DJ. (2005) Simple and rapid quantitation of microliter DNA samples using the Cary 50 UV-Vis spectrophotometer. Application note #91; www.agilent.com

www.agilent.com/chem

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