Sample Analysis Report

Number 49

Accuracy in Microvolume Measurements. Investigating the Precision and Reproducibility of Cary 50, 300 and 4000 series Instruments for Microlitre samples

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Introduction

The Very small beam geometry, 1.5 nm bandwidth, and very high intensity flash source of the Cary 50, coupled with a long pathlength Czerny-Turner monochromator design and ultra-fast scanning capability, make the Cary 50 a uniquely powerful instrument when coupled to fiberoptics microvolume devices such as the Hellma Traycell. The ease of use is further enhanced due to the room-light immunity of the system, which removes the need to close the sample compartment when taking a reading. The standard Cary 50 microcell holder provides sufficient precision of alignment to easily interchange between microcell and conventional cuvettes.

The Cary 50 uses a Xenon Flash source lamp, which is so durable it should never need replacement. The instrument requires no warm-up time, and the light beam, although intense during the sub-microsecond flash, is unlikely to photo-degrade sensitive samples as the average intensity is low, and the sample is only ever illuminated with monochromatic light.

Figure 1 The **Award winning Cary 50** is an exceptionally versatile instrument

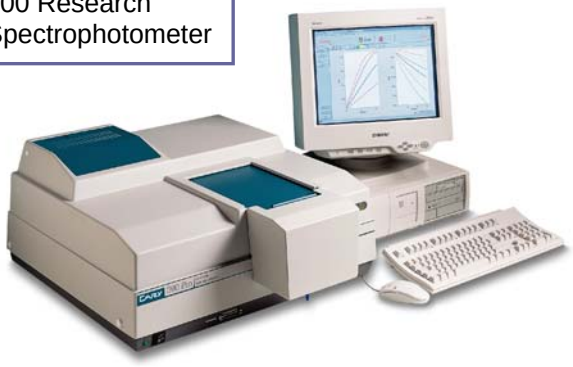
Figure 2 The Hellma Traycell provides a simple and very precise solution to low-volume measurements, when used in the Cary Series of Instruments

All Microvolume analysis has limits as the pathlength of the sample is much reduced from the normal 1 cm path of conventional spectrophotometers. However the Limitations of the Cary 50 are only seen when the concentration of DNA in the sample is very low. For SS DNA the lower detection limit is in the order of 1 ug/ul. At 5 ug/ul the quantitation algorithm shows errors of only about 3%, but the scan data is difficult to interpret due to noise approaching 20% of signal. (Figure 1)

Where samples contain less DNA than about 2-3ug/ul, the Cary 50 will provide reasonable quantitation but noisy scan data. In this case it is may be necessary to move to a higher performance spectrophotometer platform to get the highest quality results.

The Cary 300 (Fig 3) is a high precision research grade spectrophotometer, which is capable of reading to 0.0002 Abs in open beam and to a maximum absorbance in excess of 5 Abs. With the Hellma Traycell the light throughput is in the order of 6% open beam. This still leaves a large reserve of power for measuring up to 4 Abs in 1mm pathlength cells (equivalent to 40 Abs normal 1 cm pathlength.)

Figure 3 The Cary 300 Research Spectrophotometer



The Cary 4000 (Figure 4) represents the pinnacle of UV/Vis spectrophotometer design. True dual monochromation with the unrivalled Littrow Double out-of-plane monochromator provides an ability to read to in excess of 9 Abs, whilst the exceptional stability of the system confines noise on scan baselines to about 0.0001 Abs (at 0 Abs) even when used with the Hellma Traycell. This allows scanning of samples containing as little as 0.5 ug/ul ssDNA (500 Picogrammes/ul) with approx 10% quantitation error at these levels. Greater concentrations of DNA are measured with ease. (Results Figure 2-4)

Figure 4 The Cary 4000 Reference Grade Spectrophotometer

Methods

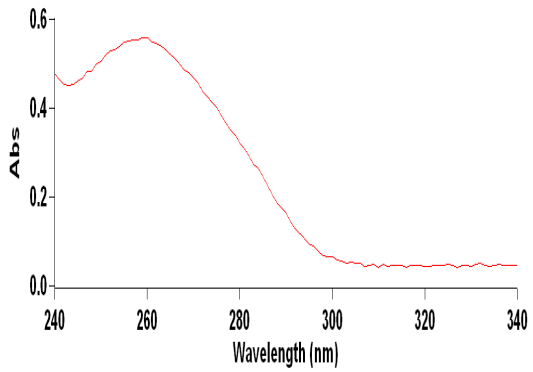
The Cary 50 was aligned for use with the Traycell accessory using the inbuilt Align software module and the adjustable cuvette base pin and holder pitch screws. (See Alignment and Installation guide for details) Best dual beam transmission values in the order of 25% were obtained after alignment. Removal and replacement of the TrayCell, or removal and replacement of the cuvette holder assembly caused no significant change in this baseline reading.

The Cary 300 was used with standard cell holders and aligned using the align module, the cell holder was modified by application of a small degree of pitch adjust which improved open beam throughput to 6% with the TrayCell present.

The Cary 4000 was used with standard cell holders, which provide sufficient precision of alignment without special adjustment. This is because the larger beam image of the Cary 4000 has uniform energy distribution, and although transmission with the Hellma TrayCell is only about 5% of open beam transmission, in the Cary 4000, the very large dynamic range of the Cary 4000 allows measurement with these levels of transmission without significant loss of precision.

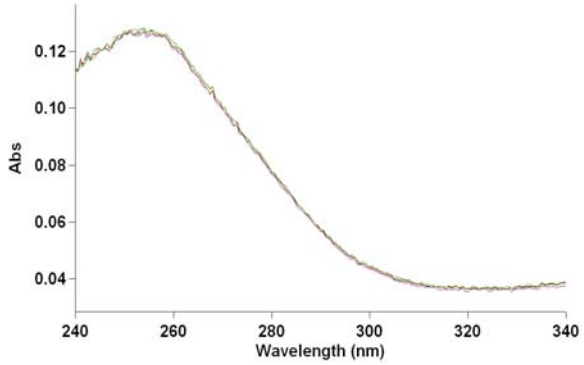
Results:

Results 1 Undiluted PCR DNA Cary 50 (1 ul 0.2 mm Pathlength)



The scan is within the normal 'Concentrated DNA' PCR product range and the quantitative precision can be expected to be better than 99% for this concentration.

Results 2 'Fake' DNA diluted to estimated 50 ug/ul in 4ul sample size



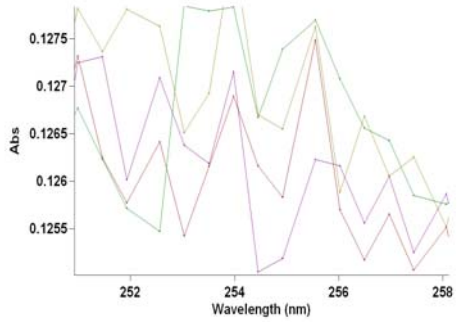
Results 3

DNA quantitation software report for the above scan.

Sample	A[260]	A[280]	Bg[320]	Ratio	Protein µg/ml	Nucleic Acid µg/ml
blank 1	0.0007	0.0004	0.0003	2.8319	-0.0957	0.2818
blank 2	0.0038	0.0027	0.0023	3.6430	-0.5184	0.9689
Fake DNA 1	0.1208	0.0766	0.0356	2.0774	-0.8715	52.1690
Fake DNA 2	0.1215	0.0774	0.0365	2.0793	-0.9282	52.0354
Fake DNA 3	0.1230	0.0772	0.0365	2.1269	-2.3886	52.9392
Fake DNA 4	0.1221	0.0777	0.0366	2.0788	-0.9151	52.3006

The scan shows four repeat samples overlaid, the variance between the quantititation result for 'DNA' content is a range of 52.03-52.93 (0.9ug/ul total variance) with a mean value of 52.35 ug/ul Variance approx 1%)

Results 4 Detail from Scan apex at 260nm

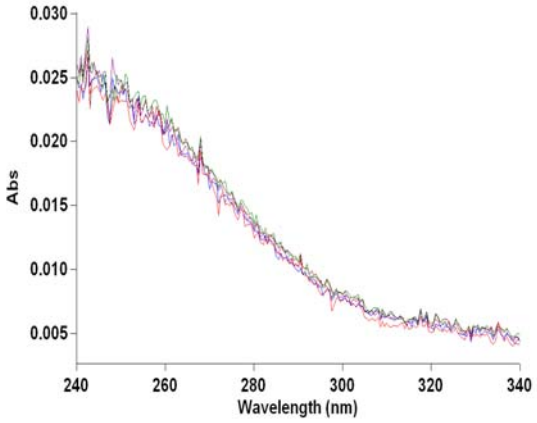


Variance between scans is lost in scan noise of approx 0.002A peak-peak

The report file includes all the calculation parameters applied to the raw data to determine the DNA concentration. The protein and Nucleic Acid 'factors' are user editable, and can be changed to make allowance for high (or low) GC ratio DNA samples or highly absorbent contaminant proteins.

Samples were then diluted tenfold and presented for analysis at more normal assay concentration. (in 4ul volume with 1 mm path cap) The samples were read, then re-presented after removal and cleaning of the TrayCell.

Results 5: Repeatability with very dilute samples

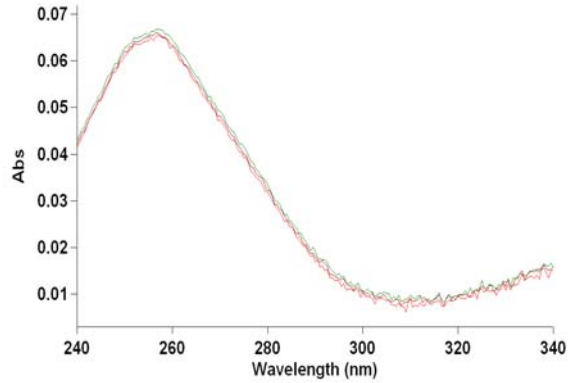


Results 6 DNA Quantitation Software for the above Scan

Sample	A[260]	A[280]	Bg[320]	Ratio	Protein µg/ml	Nucleic Acid µg/ml
Fake DNA 1	0.0207	0.0130	0.0055	2.0257	0.1352	9.3328
Fake DNA 2	0.0205	0.0133	0.0056	1.9359	0.6635	9.1265
Fake DNA 3	0.0214	0.0135	0.0058	2.0053	0.2595	9.5237

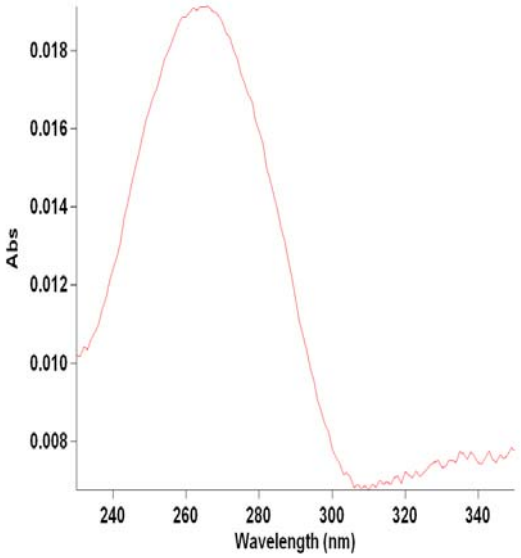
The sample had been diluted in water 1:6 The variance at the equivalent of 9 ug/ul DNA is 0.2 ug/ul or about 1/50.

Results 7 Reproducibility of result and comparison of standard sample with Cary 300 Research Grade Spectrophotometer.



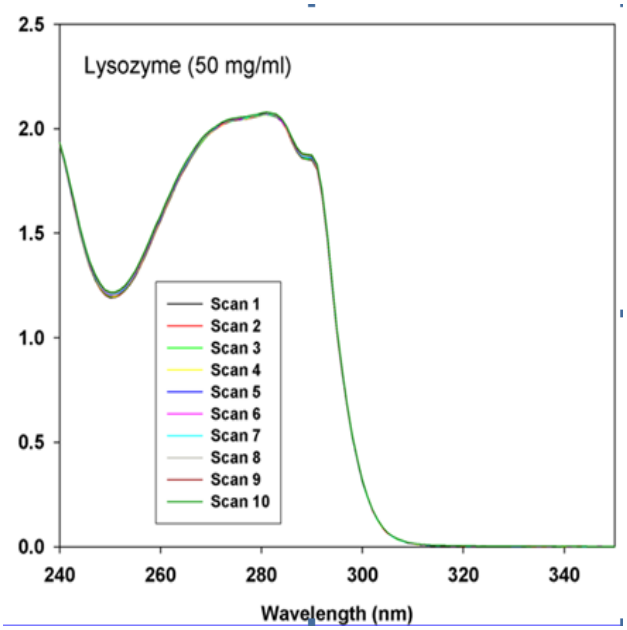
The results show the enhanced smoothness and precision of the Cary 300 despite the lower overall light throughput as a percent of normal beam. The reproducibility is within less than 1%. The precision of reading is slightly better than the Cary 50

Results 8 Dilute DNA sample in Cary 4000



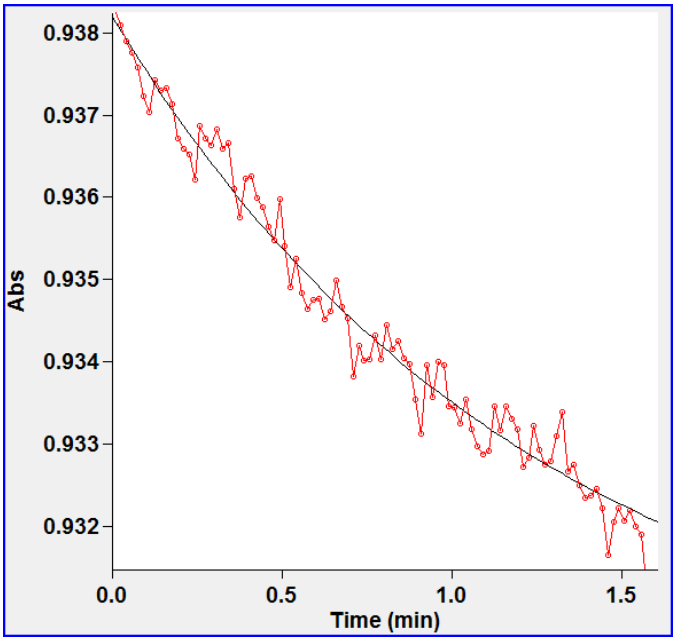
The optical performance of the Cary4000 far exceeds that of the Cary 50, however, in terms of DNA quantitation the error of 3-5% at 2ng/ul possible with the Cary 50 can be exceeded by the Cary 4000 but at these levels DNA quantitation is sufficiently precise for most analysis by Cary 50.

Results 8 Stability of readings. Repeat scans of a concentrated lysosyme solution in a Cary 4000.



Although there is a very slight change due to evaporative loss over the course of this experiment, nearly 40 minutes elapsed between the first and last scans. This shows that the TrayCell has enough resistance to evaporative change to enable kinetic experiments to be performed.

Results 9 Kinetics in Microvolumes A 4 ul Phosphite assay in a Traycell in Cary 4000



Tthe scale should be noted, this experiment resulted in a total change of absorbance from 0.938 to 0.932, a change of only 0.006 Abs in 1.5 minutes.

Discussion

The Cary 50 when used in conjuction with the Hellma TrayCell can reliably quantitate DNA down to 2-5ng/ul and provide viable scan data at concentrations to 5ng/ul. Where more demanding results are required it is possible to extend the capability using the Cary 4000 or Cary 300 spectrophotometers. The Cary 4000 can provide smooth scan data for nucleic acids down to sub nanogram samples in less than a microlitre. The design of the TrayCell prohibits evaporation and allows repeat readings to be taken, even with sub-microlitre samples.This also allows for the possibility of Microvolume enzyme assays.

This investigation compares the performance of the Cary 50, Cary 300 and Cary 4000 spectrophotometers for microvolume quantitation. The results show that although the Cary 4000 far exceeds the precision and reproducibility of the Cary 50, the Cary 300 only offers increased linear dynamic range over the Cary 50 with little improvement in noise or variance.

This result is to be expected since the efficiency (in terms of light throughput as a % of normal open beam energy) of the Hellma TrayCell is significantly less in normal beam image spectrophotometers than in the Cary 50. It is the size of the beam image, which provides the limits to performance in this type of assay. The Cary 4000 beam image is similar in shape and size to the Cary 300 but the Cary 4000 has such reserves of range that the reading is significantly better than the Cary 50.