Accurate, Reproducible dsDNA Quantitation Using the Small-Volume BioSpec-nano Spectrophotometer

Introduction
The BioSpec-nano is a state-of-the-art UV-VIS Spectrophotometer for life sciences, ideal for quantitation of nucleic acids or fluorescently-labeled nucleic acids. Completely automated, it offers such features as ‘Drop and Click’ sample mounting-measurement, and cleaning. Manual operations such as moving the optical fiber vertically or cleaning the parts in contact with the liquid are unnecessary. This communication presents data on the accuracy and reproducibility of the BioSpec-nano for dsDNA quantitation.

Experimental Conditions
All measurements were carried out using double stranded calf thymus DNA (Sigma, USA). Data here represent the entire specified range of 50 – 3700 ng/μL for 0.2 mm path length and 15- 1000 ng/ μL for 0.7 mm path length. Samples (1 or 2 ul) were placed on the pedestal using a small volume pipette. Each measurement took 3 seconds. Data analysis was carried out using the built-in software. The formula used was: sample concentration = dilution factor X (OD260-OD320) X Nucleic acid concentration factor. The nucleic acid concentration factor was set in accordance with the analyte selected1. The automatic wiper feature was used to clean the pedestal surface. Carryover data analysis was carried out by using molecular biology grade H2O (Sigma, USA) after 2 automated wipes. All measurements were repeated six or more times.

In order to compare the results obtained from BioSpec-nano to a 1 nm bandwidth instrument, a Shimadzu UV1800 spectrophotometer was used. A 10 mm cuvette was used for all analyses. Each concentration was repeated thrice or more.

Results
Table 1 represents the data collected at 0.7 mm and 0.2 mm pathlengths. The mean of 6 individual measurements is represented along with the standard deviation. Mean carryover data is shown for each set of measurements. Results for the 0.7 mm pathlength clearly indicate that for very low concentration of dsDNA (15- 150 ng/ μL) reproducibility within 0.8 ng/ μL is achievable. The carryover data shows that even at very high concentrations of 2500 ng/ μL and above there is less than 2 ng/ μL residue after 2 cleaning cycles.
Concentration data obtained from measurements using the BioSpec-nano was compared with values calculated from known concentrations of DNA. Table 2 shows the comparison between theoretical values, and concentration values measured using the UV1800 and the BioSpec-nano. It is evident from the results that there is very good agreement between the results obtained from the UV-1800, BioSpec-nano and the estimated concentrations, which further supports the suitability of this instrument for small volume DNA quantitation.

### Summary
Concentration measured using a BioSpec-nano is precise and highly reproducible. Automated movement of the fiber vertically creates a reproducible path length each time. The unique wiper feature not only improves the throughput of the measurement, it also eliminates the need for manual cleaning of the pedestals, resulting in negligible carry over between measurements.

### References