Application note

- The NanoPhotometer™ Pearl -

Comparison of the performance characteristics of the New NanoPhotometer™ Pearl and the NanoDrop 2000c.

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Introduction

Spectrophotometers allow for quantification of nucleic acid and protein samples based on absorbance at 260nm and 280nm, respectively. Traditionally this method is performed in cuvette format which requires large sample volumes generally from 100µl - 3500µl. Technology advances have reduced sample volume needed which is necessary in life science applications where cost and sample availability are limited.

Implen GmbH has developed the NanoPhotometer™ Pearl (Fig. 1) to analyze ultra low sample volumes of 0.3µl while maintaining high accuracy, reproducibility and speed. The NanoPhotometer™ Pearl has been compared with respect to its performance characteristics to those of the alternative technology, the Thermo Scientific NanoDrop 2000c. The tests were designed to evaluate very low sample volume (0.3µl - 2µl) performance on DNA and proteins together with sample stability and overall speed of analysis.

Instrumentation

All tests were performed with freshly prepared samples under laboratory conditions at room temperature to avoid any thawing effects. The tests were performed under the described conditions by the same operator following the recommended operating procedures to eliminate any sample handling bias.

NanoDrop 2000c data were provided from instruments of Charité Berlin and University Hospital Ulm, both of which are new and within the calibration period (calibration check recommended every 6 months by the manufacturer). The NanoPhotometer™ Pearl was a new system and does not experience pathlength drift therefore requires no calibration. The pipette used was Eppendorf Research® plus variable 0.1µl - 2.5µl which was accurate for 0.3, 0.5, 1.0 and 2.0µl volumes and was within calibration period.

Submicroliter technology is made possible by reducing the pathlength from traditional 1cm cuvette format to 2mm, 1mm, 0.2mm 0.1mm, or 0.04mm with the NanoPhotometer™ Pearl or 1mm to 0.05mm with the NanoDrop 2000c. With understanding of Beer-Lambert Law (wikipedia.org/wiki/Lambert_beer), a wide detection range is possible without a need for sample dilution for readings over the linear range. The NanoPhotometer™ Pearl has a submicroliter detection range of 2 - 18,750ng/µl dsDNA and NanoDrop 2000c of 2 - 15,000ng/µl dsDNA.

Fig. 1. Implen NanoPhotometer™ Pearl and NanoPhotometer™ Pearl Design Edition

The NanoPhotometer™ Pearl and NanoDrop 2000c handle submicroliter volumes by completely different approaches. The NanoPhotometer™ Pearl employs Sample Compression Technology™ (Fig. 2 and implen.com/ nanophotometer/how-it-works.php) while the NanoDrop 2000c forms a surface tension dependent column (nanodrop.com/HowItWorks.aspx).

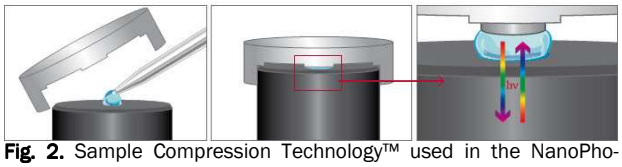

Fig. 2. Sample Compression Technology™ used in the NanoPhotometer™ Pearl.

Fig. 3. Handling of NanoPhotometer™ Pearl

DNA Quantification

A nominal 218ng/µl fish sperm dsDNA (Sigma) test sample was measured 10 times on both systems by applying a new sample each time to compare accuracy and reproducibility (Table 1, Fig. 3 + 4). A sample volume of 0.3µl, 1.0µl, and 2.0µl was used for the NanoPhotometer™ Pearl and 0.5µl, 1.0µl and 2.0µl for the NanoDrop 2000c.

Table 1. Fish Sperm dsDNA measured on NanoPhotometer™ Pearl and NanoDrop 2000c. Readings were performed ten times and averaged.

	Concentration Values (ng/µl)					
	NanoPhotometer™ Pearl			NanoDrop 2000c		
Volume	2.0µl	1.0µl	0.3µl	2.0µl	1.0µl	0.5µl
Mean	223.2ng/µl	223.3ng/µl	225.3ng/µl	225.6ng/µl	219.8ng/µl	220.5ng/µl
SD	1.32	2.36	2.06	2.89	2.48	4.42

The NanoPhotometer™ Pearl and the NanoDrop 2000c gave comparable results with 1.0µl and 2.0µl sample volumes with the NanoDrop 2000c having slightly higher standard deviation. Reproducibility differences are clear in smaller volumes when NanoDrop 2000c showed significant fluctuation in values with 0.5µl while the NanoPhotometer™ Pearl has reproducibility down to 0.3µl (Table 1, Fig. 4). The NanoPhotometer™ Pearl is able to measure down to 25ng/µl of dsDNA with a sample volume of 0.3µl while the NanoDrop 2000c using a 0.5µl sample volume measures down to 150ng/µl illustrating therefore that the NanoPhotometer™ Pearl is also significantly more sensitive with low volumes.

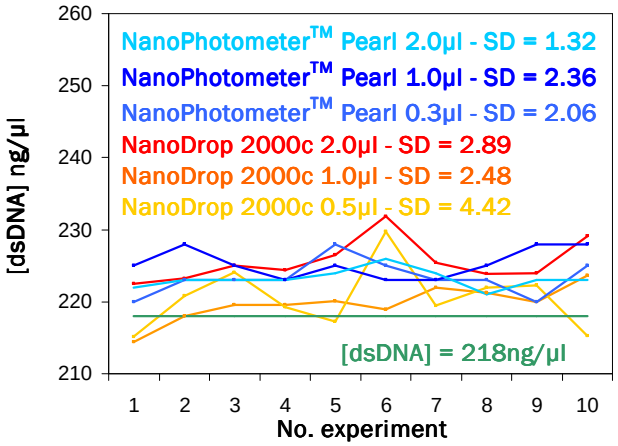

Fig. 4. Reproducibility of Fish Sperm dsDNA measurement and concentration calculations on NanoPhotometer™ Pearl and NanoDrop 2000c.

Protein Quantification

A nominal 59.8 mg/ml of a BSA (BDH Prolabo) sample was measured 10 times on both systems by applying a new sample each time to compare accuracy and reproducibility for 0.5µl sample volumes (Fig. 5, Table 2). A second set of samples was measured which had been diluted with Glycerol (10%) (Fig. 6, Table 2). Glycerol is a common stabilizing agent and also aids dispensing. The buffer used was 1xPBS for both samples and blank.

The NanoDrop 2000c showed significant variation for BSA samples with 0.5µl volume (Fig. 5) therefore the sample was diluted with glycerol and 1.0µl sample volume was used (Fig 6). Although 1.0µl improved the performance on the NanoDrop 2000c the results were still variable. In the tests the NanoPhotometer™ Pearl has performed more reproducibly.

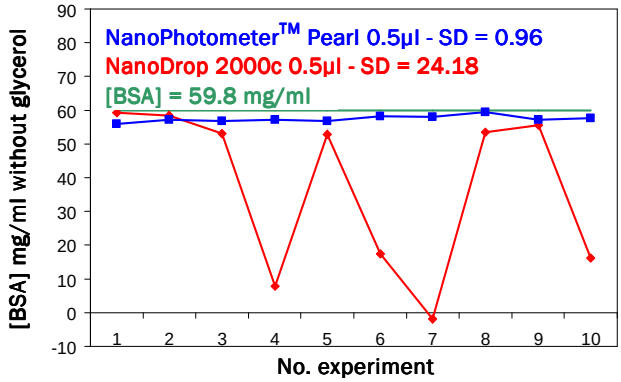

Fig. 5. Comparison of measurements from NanoPhotometer™ Pearl and NanoDrop 2000c with high protein concentrations (59.8mg/ml) and 0.5µl sample volume.

Table 2. BSA in 1xPBS without (BSA: 59.8mg/ml) and with (BSA: 52.6mg/ml) glycerol measured with NanoPhotometer™ Pearl and NanoDrop 2000c

	Concentration Values (mg/ml)	
without glycerol	**NanoPhotometer™ Pearl 0.5µl**	**NanoDrop 2000c 0.5µl**
Mean	57.49mg/ml	37.22mg/ml
SD	0.96	24.18
with 10% glycerol	**NanoPhotometer™ Pearl 0.5µl**	**NanoDrop 2000c 1.0µl**
Mean	53.09mg/ml	57.12mg/ml
SD	0.96	21.90

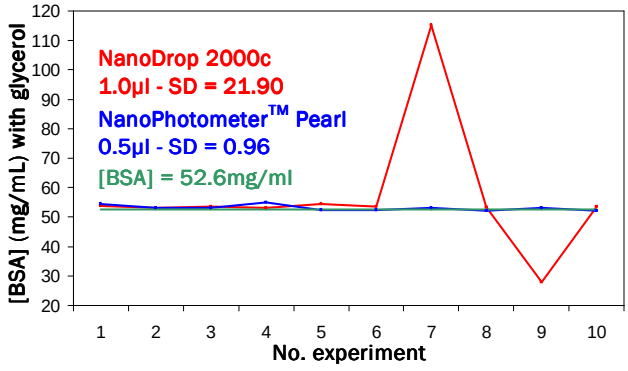

Fig. 6. High BSA concentration (52.6mg/ml) measurements with NanoPhotometer™ Pearl and NanoDrop 2000c containing 10% glycerol.

Stability over Time

To establish reading stability over time, measurements on the same applied sample are performed over 60 seconds in 15 second increments. First a blank and sample of deionized water (dH_2O) were measured over time (Fig. 7), and secondly a normal procedure of blank and then dsDNA were measured over time. Sample volumes were 1.0µl.

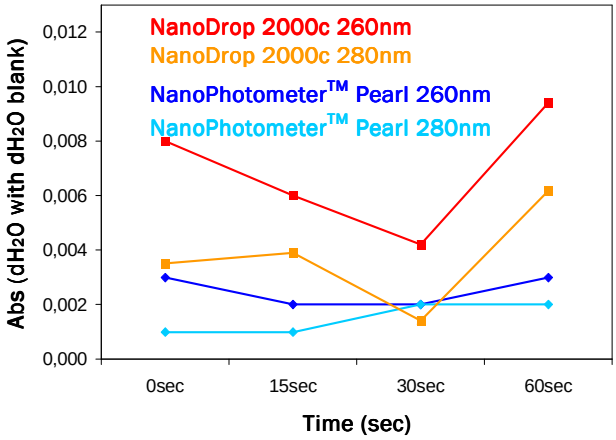

Fig. 7. Signal stability of the NanoPhotometer™ Pearl and NanoDrop 2000c with dH_2O blank measured at 260nm (dsDNA) and 280nm (BSA) over 60 seconds. The values were normalized to a pathlength of 1mm.

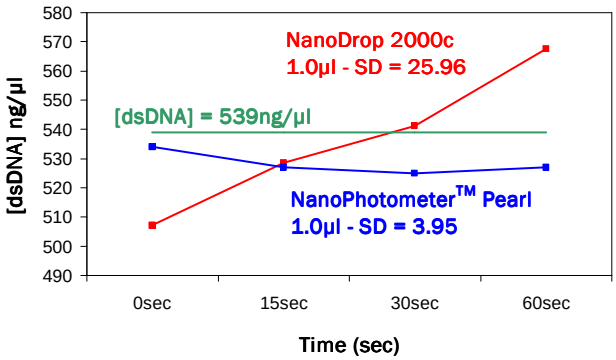

Fig. 8. Signal stability of NanoPhotometer™ Pearl and NanoDrop 2000c. The dsDNA sample was applied once and was measured at 0sec, 15sec, 30sec and 60sec.

The blank stability on the NanoPhotometer™ Pearl is superior which is due to the low noise dual detector based optical system. The NanoPhotometer™ Pearl is also shown to be significantly more reproducible measuring the same applied sample over time when compared with the NanoDrop 2000c. A system with poor sample stability requires care when reporting data and replicates should be performed on a regular basis to confirm the analysis. With the DNA sample the NanoDrop 2000c showed a steady increase in concentration over time which may be due to evaporation as the sample is exposed to the environment during measurement. With the NanoPhotometer™ Pearl the sample is enclosed under a lid to minimize evaporation and to prevent contamination. The sample may also be recovered if required for further analysis.

Speed of Analysis

The NanoPhotometer™ Pearl and the NanoDrop 2000c were timed from switch on to being ready for analysis, the time to take an individual reading, and the time taken to measure a blank (Table 3). The time required to clean the optical surfaces between samples was the same for both instruments.

Table 3. NanoPhotometer™ Pearl and NanoDrop 2000c speed of analysis measured by stopwatch.

	NanoPhotometer™ Pearl	NanoDrop 2000c
Time per measurement	3.5 s	6-8 s
Time for blank	3.5 s	15 s

The NanoPhotometer™ Pearl is faster with both sample and blank measurements as compared to the NanoDrop 2000c. Furthermore with the NanoPhotometer™ Pearl's mobile stand alone character it is ready to use after 5 sec of turning on. Since the startup time of the NanoDrop 2000c is mainly depending on the boot up time of the connected PC and corresponding application software, it is significantly longer. The NanoPhotometer™ Pearl, though capable of connecting to a computer, has an onboard display and can be configured with a thermal printer for direct printing or an SD card module for measurement data storage.

Conclusion

Submicroliter performance of the NanoPhotometer™ Pearl and NanoDrop 2000c was evaluated with regard to accuracy, reproducibility, sample stability, and speed of operation. In DNA analysis, the NanoPhotometer™ Pearl and Nanodrop 2000c both performed well with sample volumes of 1.0µl and above. At 0.5µl the NanoDrop 2000c showed a significant higher standard deviation of the results compared to the NanoPhotometer™ Pearl. Only the NanoPhotometer™ Pearl was reproducible in volumes down to 0.3µl.

With protein analysis, the NanoPhotometer™ Pearl was accurate to reference protein concentration and reproducible with very low standard deviation. The NanoDrop 2000c performed on protein analysis with high variability. Increasing sample volume and adding glycerol did not significantly improve the high variability with the NanoDrop 2000c.

Stability over time is superior with the NanoPhotometer™ Pearl both in regards to signal stability observed with the blank and less deviation of repeated measurements of the same applied sample over time. Operation time was also shown to be less with the NanoPhotometer™ Pearl in every category.

Acknowledgements

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