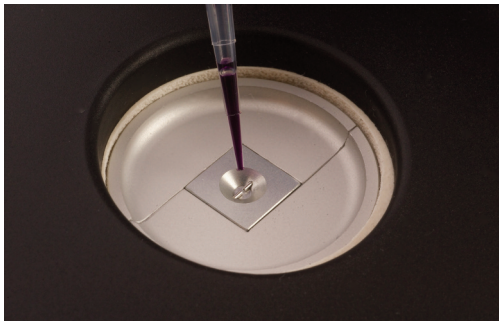
BioDrop™ µLITE dsDNA Application Note

Using a BioDrop µLITE spectrophotometer to measure the concentration of low volume samples of dsDNA

Micro-volume measurement of DNA is a routine application in many life science laboratories. Quantification and purity measurement of dsDNA is a key first step before performing experiments such as PCR, qPCR, Next Generation Sequencing, ChIP and ChIP Seq. The success of these experiments demands accurate and precise quantification of the dsDNA starting material. These experiments typically require highly concentrated solutions which are available only in small volumes. Typical concentrations of these popular assays can be found in Table 3. In addition, the high cost of the reagents makes accurate initial quantification even more crucial.

Current options for direct measurement of dsDNA are conventional spectrophotometers or dedicated micro-volume instruments. Conventional UV/Vis Spectroscopy requires sample dilution, adding time to the quantification method as well as increasing the possibility of error due to additional sample manipulation. Existing micro-volume instruments have multiple disadvantages, such as possible pathlength drift and inaccuracy in detection limits as well as issues such as contamination and bubbles disrupting analysis.

To address these issues, BioDrop has developed a novel micro-volume measurement device, the BioDrop µLITE. The BioDrop µLITE is a UV/Vis spectrophotometer which has a novel in-built sample port with a fixed 0.5mm pathlength. The fixed pathlength means that the instrument will continue to perform at the same reliabilty without the need to calibrate. The sample port improves the scientist's ability to measure low volume DNA, RNA, oligonucleotides and protein samples. Concentrations are obtained quickly using a colour touchscreen to select pre-programmed methods in the on-board software. Importantly, users do not have to perform time-consuming sample dilution.



A key application of direct micro-volume measurement is the quantification of dsDNA. To demonstrate the performance of the BioDrop µLITE, a series of experiments were performed to determine minimum sample volume, detection limit, reproducibility, linearity and sample carry over.

Table 1. Summary of Results	
DNA Detection Limit (ng/µL)	1.0
Minimum Volume (µL)	0.5
Maximum Concentration (ng/µL)	2,500
Reproducibility 100ng/µl (%CV)	1.0%
Reproducibility 1000ng/µl (%CV)	0.5%

Materials and Methods

BioDrop UV/Vis Spectrophotoscopy

The BioDrop μLITE is a split-beam, UV/Vis spectrophotometer which uses a Xenon light source and a 1024 CCD array detector. This technology delivers rapid, high quality measurements. Details of the techincal specifications can be found in Table 2.

The BioDrop μLITE delivers quick results: no warm-up time is required and the instrument is readily controlled using a high resolution colour touchscreen. Samples are pipetted directly into the in-built sample port and then measured.

Materials and Methods

Purified Salmon Sperm dsDNA (Sigma-Aldrich Item# D1626-250MG) was dissolved in HyPure Molecular Biology Grade H_2O directly before measurement using the BioDrop μLITE.

The 0.5mm pathlength of the μLITE spectrophotometer was selected from the drop-down menu of the on-board software. DNA concentrations were calculated automatically using the pre-programmed DNA quantification method: absorbance is measured over a wavescan from 230 to 320nm. Then the concentration of the dsDNA is calculated by subtracting the absorbance at 320nm from absorbance at 260nm and multiplying by the DNA concentration factor (50μg/mL) and the pathlength normalisation factor (20).

Multiple instruments were tested to determine the detection limit, minimum sample volume, linearity, reproducibility and carry over. Data representative of the test group is shown in Figures 1-3.

The dsDNA detection limit was determined by performing a series of twenty measurements in the in-built sample port using the DNA method in the on-board software.

Minimum sample volume was determined by performing a series of measurements using a 1μL microsyringe. The reproducibility of measurement at decreasing volume was determined by calculating the mean and standard deviation thereby confirming reliable measurements at the lowest possible volume.

Reproducibility of measurement was determined by performing ten measurements of 100 and 1000ng/μL dsDNA solutions and calculating the %CV from the mean and standard deviation of the calculated concentrations.

The linearity of the instrument was determined by plotting the measured concentration against the dilution factor of the measured solution. Linearity was represented by the least squares analysis of the line.

Finally, sample carry over was assessed by alternating measurements of water and 100 or 1000ng/μL dsDNA solutions. The in-built sample port was simply wiped with lint-free tissue in between measurements to evaluate the effectiveness of cleaning between samples.

Table 2. Technical Specifications

Display	5.7" colour capacitive touchscreen
Light Source	Pulsed Xenon lamp with 3 year warranty
Detector	1024 element CCD array
Wavelength Range	190 – 1100nm
Pathlength Accuracy	In-built sampling port has an accuracy of +/- 5mm
Wavelength Accuracy	± 2nm
Wavelength Reproducibility	± 1nm
Spectral Bandwidth	5nm
Stray Light	<0.5%T at 220nm and 340nm using $NaNO_2$
Absorbance Range	- 0.3A to 2.5A, 0 to 199%T
Absorbance Accuracy	±0.005A or 1% of the reading, whichever is the greater at 546nm
Absorbance Reproducibility	±0.003A (0 to 0.5A), ±0.007A (0.5 – 1.0A)
Noise	0.005A peak to peak 0.002A RMS
Output	USB port for USB memory stick
Power input	90-250 V, 50/60Hz, Max 30VA
Dimensions	420 x 260 x 185mm
Weight	approx. 3kg
Life Science PC Software	DNA, RNA, oligo, dye labelling efficiency, Tm calculation, direct UV and colorimetric protein methods
Software Languages	English, French, German, Spanish & Simplified Chinese

Results

Linearity of Measurement of the BioDrop µLITE

A range of DNA concentrations were measured to determine the linear range of the in-built sample port. Each sample concentration was measured five times. The mean was calculated for each concentration and then plotted against the dilution factor. The BioDrop µLITE demonstrated excellent linearity of up to 2500ng/µL as shown by the R^2 value of 0.9998. The R^2 value refers to the accuracy of measurement within a linear line, in which measurements displaying clear linearity exhibit an R^2 value of 0.9-1.0.

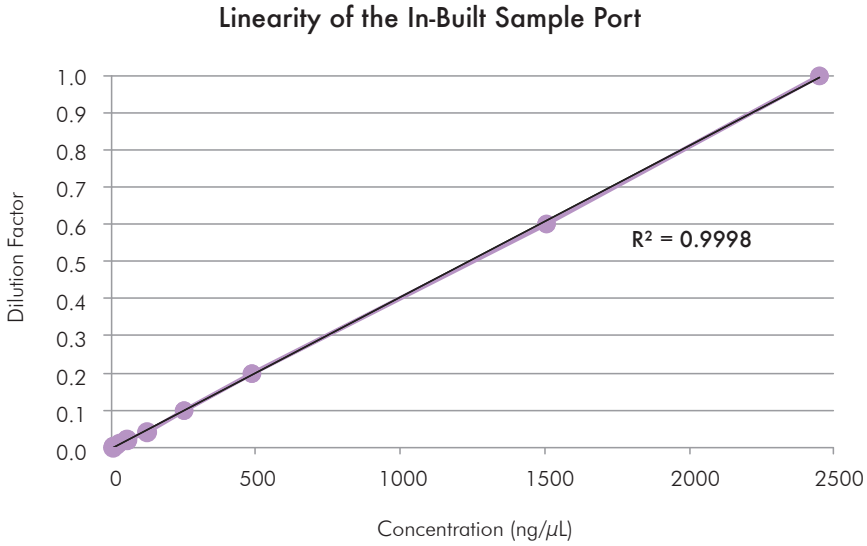


Figure 1: Linearity of the in-built sample port was determined by plotting concentration against the respective dilution factor.

dsDNA Detection Limit of the BioDrop µLITE

The detection limit of dsDNA is an important specification which allows the user to have confidence in the absorbance measurements of low concentration samples. A low detection limit allows scientists to use the minimal amount of sample for measurement thereby allowing for more sample to be used for subsequent experiments. The detection limit of the BioDrop µLITE was shown repeatedly to be less than 1ng/µL, a best-in-class specification, as shown in Figure 2.

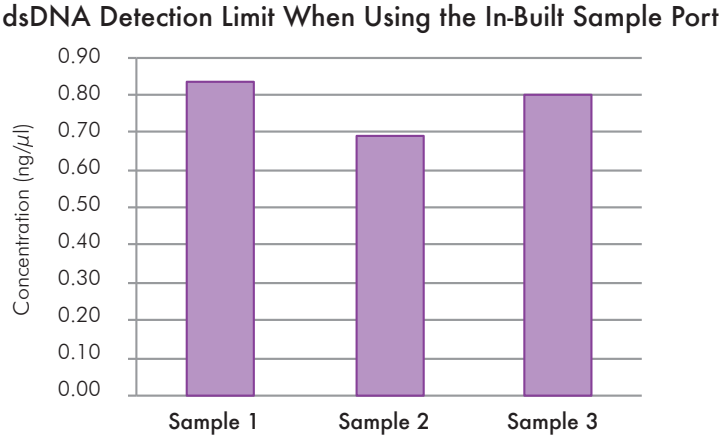


Figure 2: Detection limit of three independent in-built sample ports were analysed.

Reproducibility and Minimal Carry Over Between Samples

Measurements were made to determine the amount of carry over of dsDNA between samples. A simple wipe with a lint-free tissue is sufficient to reduce sample carry over to non-detectable amounts as shown in Figure 3.

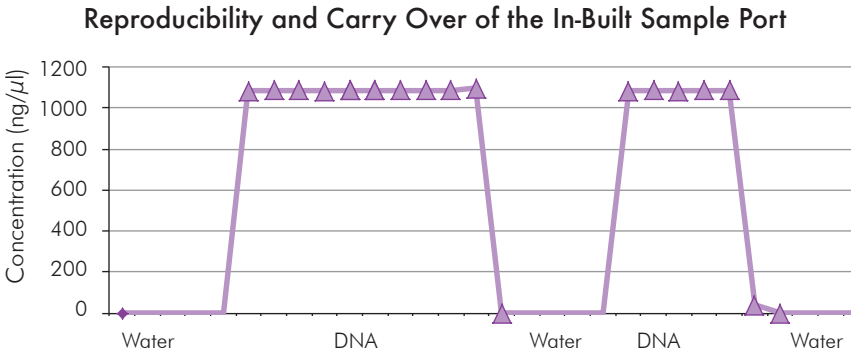


Figure 3: DNA carry over was analysed by measuring sample concentrations, which were immediately followed by water to show reduced DNA carry over.

Minimum Volume Sampling

Another key measurement for micro-volume quantification of dsDNA, is the lowest volume that can be pipetted and measured to achieve reproducible and accurate results. For this purpose, a 1µl microsyringe was used to pipette 1000ng/µL. Figure 4 demonstrates that the minimum sample volume that can be pipetted on to the in-built sample port is 0.5µl whilst maintaining excellent measurement performance thereby preserving precious sample.

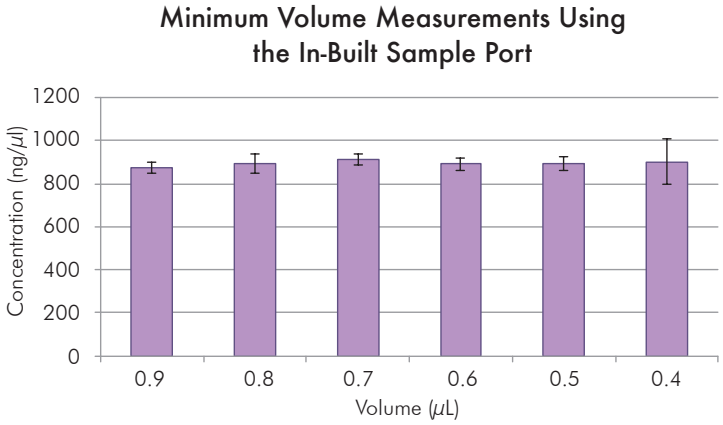
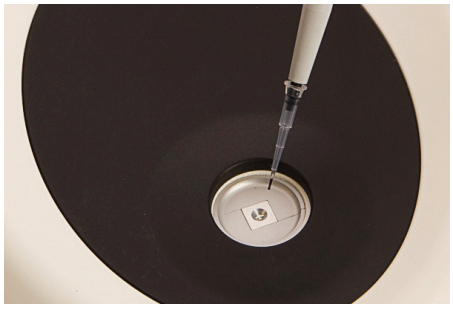


Figure 4: Minimum volume measurements of <1µl were analysed and found to be 0.5µl, ideal for downstream applications where sample volume can be a limitation.

Importance of Nucleic Acid Micro-volume Quantification

Table 3 highlights the key downstream applications for nucleic acids following quantification. These assays often require high sample concentrations and importantly, low volume.



Key downstream applications such as ChIP Sequence, transfections, DNA vaccines, qPCR and microarrays are used in industrial, academic and clinical laboratories.

Table 3. Key Downstream Molecular Assays Requiring DNA quantification	
ChIP Sequence	10ng/30µl (0.3ng/mL)
Sequencing	125ng/20µl (6ng/mL)
Next Generation Sequencing	10ng/µl
Transfections	5-30µg/100µl
DNA Vaccines	0.5-2mg
PCR	2ng/µl
qPCR	200ng/100µl
DNA Microarray	>2µg
siRNA	7.5µg/mL
Chromosomal Capture Conformation	2-3ng/µl

Reproducibility of BioDrop µLITE

Reproducibility of measurement is an important feature of most laboratory instruments. The performance of the BioDrop µLITE was consistent when measuring 100ng/µl as well as 1000ng/µl as shown in Table 4.

Table 4. Reproducibility BioDrop µLITE	
Concentration	%CV
100ng/µl	1%
1000ng/µl	0.5%

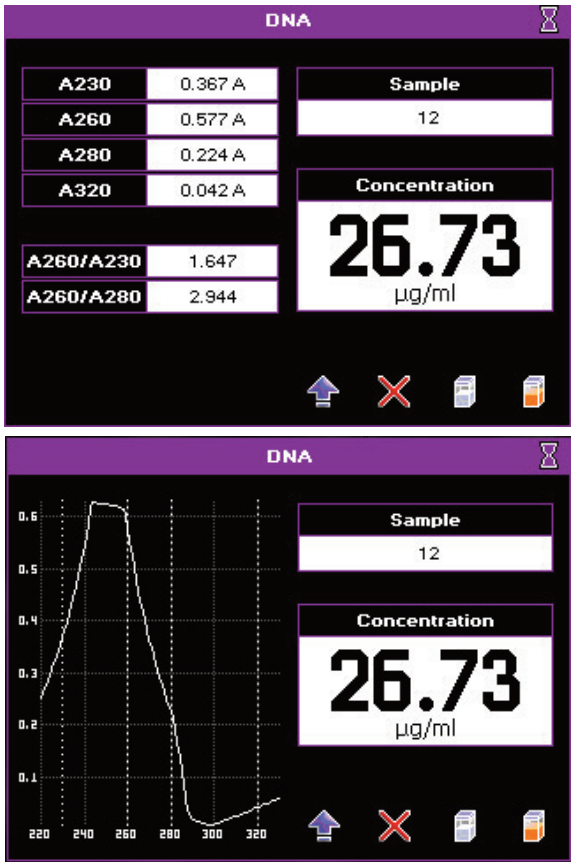
On-Board Software

BioDrop μLITE is dedicated micro-volume instruments controlled via a high-resolution touch screen for stand-alone analysis. The on-board software is both user friendly and easy to navigate. The software provides both quantitative and qualitative data analysis making analysis as well as measurement both simple and efficient.

Raw absorbance readings are measured at these key wavelengths are used to calculate the concentration of DNA and ratios of absorbances at 230nm and 280nm to 260nm are used to indicate the purity of the sample:

1. A230nm–Absorbance at this wavelength suggests the presence of sample purification contaminants such as phenol and chloroform.
2. A260nm–Peak absorbance for DNA.
3. A280nm–Peak absorbance for protein.
4. A320nm–Background measurement.

Using a wavelength scan gives the scientist a feel for the purity of the sample as well as highlighting the absorbance peak at 260nm).



BioDrop Resolution Software

BioDrop Resolution Life Science is a powerful and intuitive PC software package that is included with the BioDrop μLITE. The software features a Life Science module which contains methods for DNA, RNA, oligonucleotides and protein analysis. Table 5 summarises the key features of BioDrop Resolution Software.

BioDrop also provides an optional CFR module for laboratories requiring full 21 CFR part 11. Features included in this version allow restricted access for specified groups and administration rights.

Table 5. BioDrop Resolution Software

Life Science Features	DNA, RNA, oligonucleotides, and protein measurements which include: 1. Absorbance 2. Purity 3. Concentration
Method Developer	Design your own experimental protocol. Tailor your analysis to suit your requirements.
Quick Read	Measure the absorbance of unknown samples prior to further experimentation.
Quick Scan	Measure a wavelength scan of samples of unknown absorbances to confirm peak wavelengths.
Cy dye analysis	Absorbance measurements to assess the labelling efficiency of DNA and/or protein samples.

Conclusion

The BioDrop μLITE is a novel, dedicated micro-volume instrument which represents a clear improvement over existing instrumentation. The in-built sample port has no moving parts which delivers a linear measurement range of up to 2,500ng/μL. The exceptional pathlength accuracy of +/- 5μm guarantees excellent measurements. An unrivalled dsDNA detection limit of 1ng/μL enables measurement of low concentration as well as low volume samples. In addition, the in-built sample port is easy to use and sample volumes as low as 0.5μl volumes of dsDNA can be pipetted and measured accurately. Importantly, it is easy to clean thereby preventing sample carry over as shown by reproducibility studies when sampling between DNA and reference water samples. The BioDrop μLITE offers a new standard of excellence in micro-volume measurement and you never need to calibrate.

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