

Rapid Quantification of Proteins in Complex Matrices using the DeNovix DS11 Microvolume Spectrophotometer

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Abstract

Recent advances in microvolume spectrometry have made significant impact on the workflow in life science laboratories by reducing the number of steps required in order to gain reliable data. Microvolume spectrophotometers require between 1-2 μL of sample and give highly accurate measurements in less than 20 seconds. During measurement, a droplet of sample is placed on a pedestal. An optical cell is formed when a top piece completing the light source assembly is placed onto the droplet and a column of fixed length is formed. This application has become the gold standard in nucleic acids research labs but has significant limitations for characterisation and quantification of complex protein samples due to frequent column breakage. These limitations are due to sample properties such as surface tension and viscosity. Until now, there has been little confidence in this technology for protein applications.

In this poster, we will introduce the DeNovix DS-11 as the next generation in microvolume spectrophotometry. It's patent pending SmartPath technology and proprietary algorithms ensure that a column is formed every time prior to measurement ensuring ultimate confidence in the quality of results for the first time for all nucleic acid and protein applications. In addition, it offers the widest dynamic range eliminating the need for sample dilution prior to measurement as previously required. We will also discuss the ground breaking EasyApps[®] software suite which comes as standard on the custom Android[™] tablet device, eliminating the need for an external PC. The small footprint of the DS-11 microvolume spectrophotometer (20 x 33 cm) makes it ideal for use in a busy multi-user environment, saving valuable bench space.

SmartPath[®] Technology

- The DS-11 proprietary SmartPath Technology in conjunction with our innovative microvolume design (patent pending) automatically uses the optimal path length when measuring a sample.
- Other microvolume spectrophotometers may offer multiple path lengths, but many require the user to guess which path length is needed prior to measuring the sample.
- The DS-11 uses real-time absorbance data to determine the best path length (ranging from 0.03-0.5 mm) to use for each measurement
- Pipetting errors, samples with low surface tension properties or unconditioned surfaces can cause 'unbridged' samples and is a real concern when using microvolume spectrophotometers



Figure 3: Correct pipetting technique and sample surface tension are critical requirements to minimise unbridged samples

Results and Discussion

The images from Figure 4 and the data from Figure 5 and Table 1 compares the performance of the DeNovix DS-11 and the NanoDrop[™] 2000 when measuring 5 replicates of 1 μL samples of four BSA dilutions. An Agilent 8453 spectrophotometer was used as a reference. The liquid connection between the surfaces on the DS-11 was always intact while the liquid connection between the surfaces of the NanoDrop 2000 was shown to frequently break.

The NanoDrop 2000 exhibited a very high degree of variability and inaccuracy compared to the DS-11. The DS-11 exhibited excellent reproducibility when measuring BSA samples on an unconditioned surface. The BSA dilutions measured on the unconditioned NanoDrop 2000 surface exhibited significant variability. The mean concentration for each dilution on the DS-11 corresponded very well to the reference spectrophotometer while the mean concentration on the NanoDrop 2000 was significantly lower due to column breakage.

Measure with full Confidence

Incorrect data associated with unbridged sample measurements is a common problem many users of microvolume spectrophotometers are unaware they are encountering. The DeNovix DS-11 uses proprietary algorithms and SmartPath Technology to eliminate any concerns associated with unbridged sample errors. This innovative feature makes the DS-11 the ideal spectrophotometer for laboratories using nucleic acids and proteins and enables the researcher to have the highest confidence that their measured data is always accurate.

DS11 Features

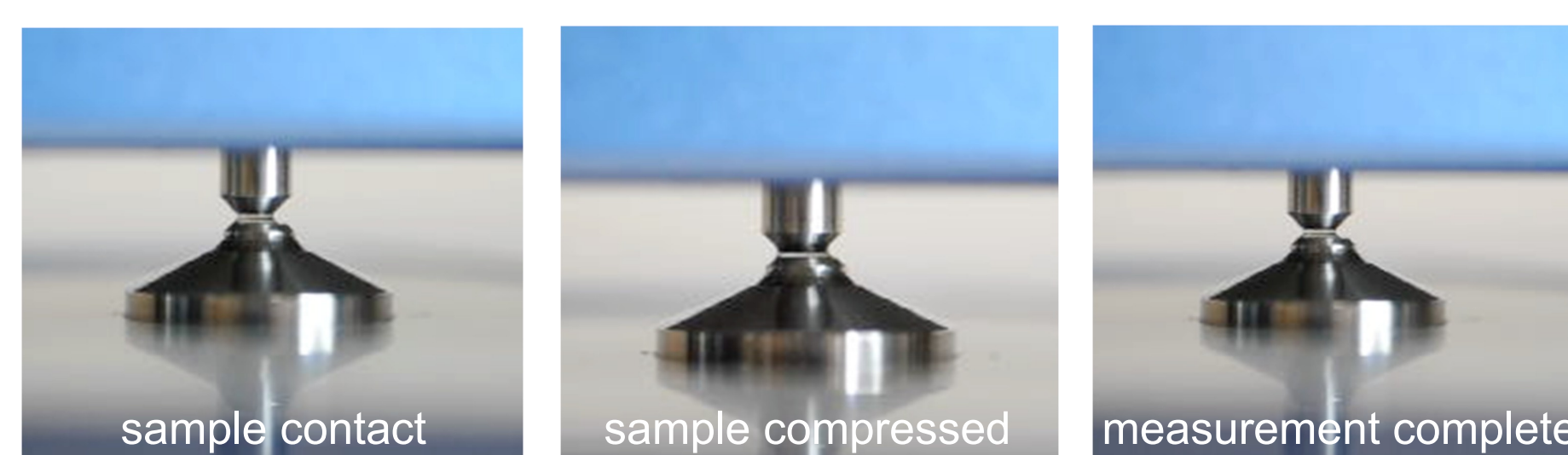
- Fully standalone system, no PC required
- 0.5 to 1.0 μL sample
- Measurement < 4 seconds
- Real time pathlength adjustment using patent pending SmartPath technology
- Ultra-short pathlength for high concentrations (0.03 to 0.5mm)
- Maximum concentration 750 mg/mL BSA; 25,000 ng/ μL dsDNA
- Footprint 20 cm x 33 cm, 2kg weight
- Onboard custom Android[™] operating system with EasyApps software
- Available in cuvette mode (DS-11+) for enhanced sensitivity and performance
- Wi-Fi, Ethernet, 3 USB ports, 8GB flash memory



Figure 1: The DeNovix DS-11 and DS-11+ models are available in three colours

Bridged Versus Unbridged Liquid Columns

DeNovix DS-11



Nanodrop[™] 2000

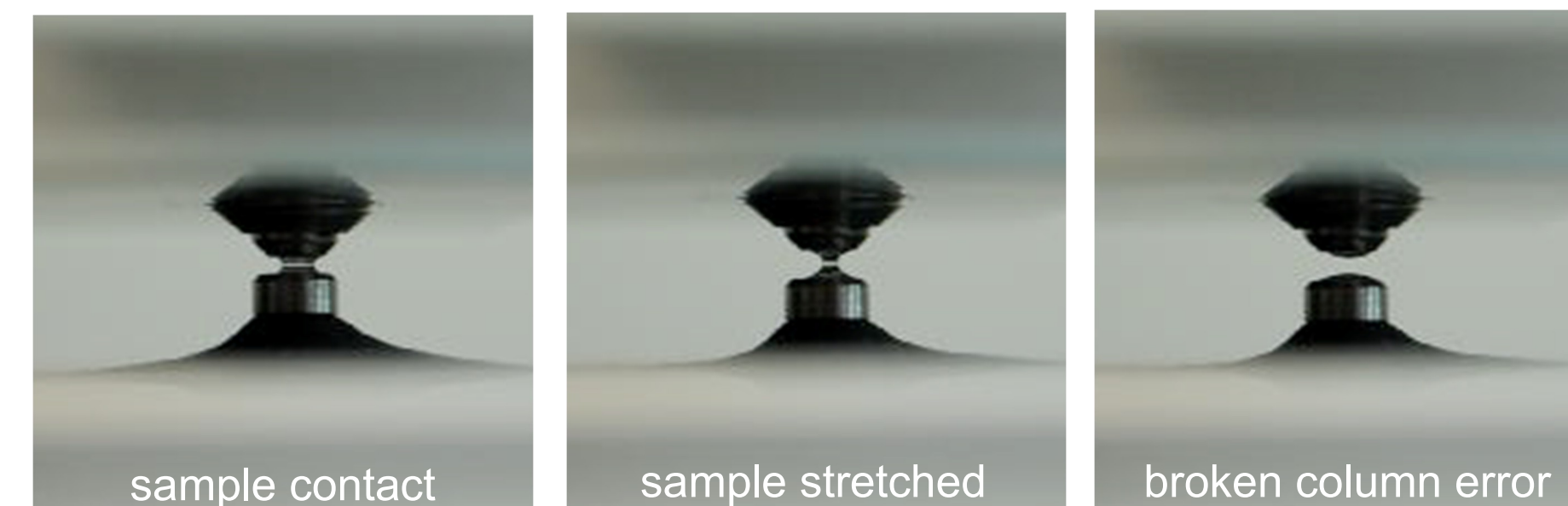


Figure 4: The DeNovix DS-11 compresses a sample droplet and maintains the liquid bridge between measurement surfaces. The NanoDrop 2000 stretches a sample droplet to a 1 mm gap which may result in broken columns and inaccurate results

Protein Applications with the DeNovix DS-11

Protein applications perfectly suited for the DS-11 include:

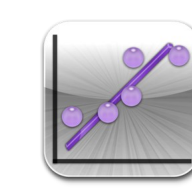
- Crystallography
- Membrane protein studies
- 2D electrophoresis
- Protein arrays
- Antibody production

Custom Protein Apps



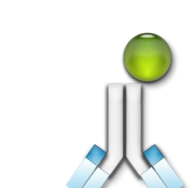
Protein A₂₈₀ App

- Purified protein production
- Broadest dynamic range
- 280 nm analysis for determination of specific protein using E1% factor
- Input, save and recall sample specific protein E1% values



Colorimetric App

- Quantification of purified protein samples or protein mixture
- Bradford, BCA, Protein 660, Lowry Assays



Labelled protein App

- Application for labelled protein probes quantification and quality control
- Absorbance information can be used for degree of labelling
- Results obtained include protein concentration (mg/mL) and dye label with absorbance for both



Kinetic App (DS-11+ model only)

- Cuvette based measurement
- 37-45°C measurement range

EasyApps[®] Software

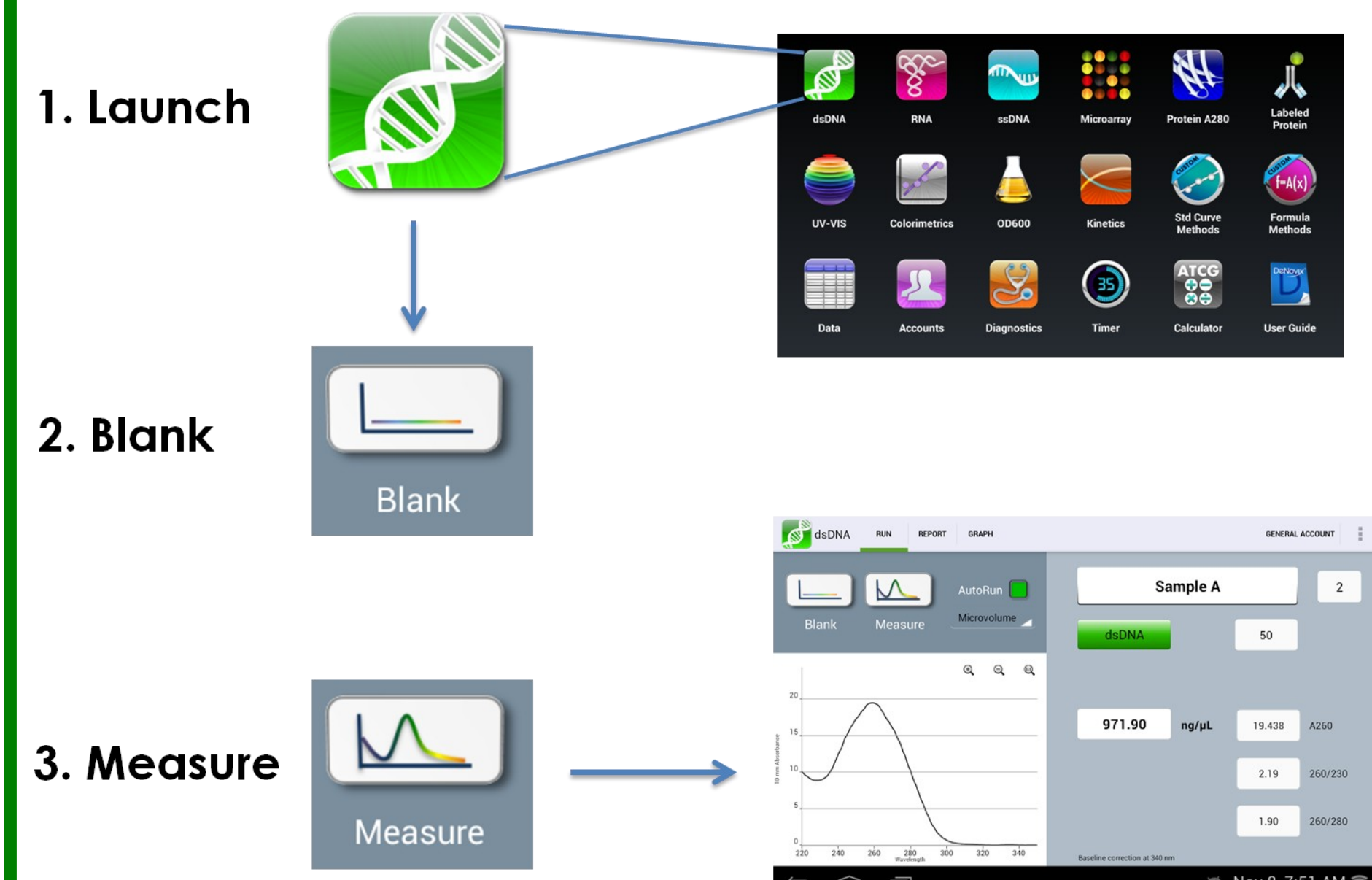
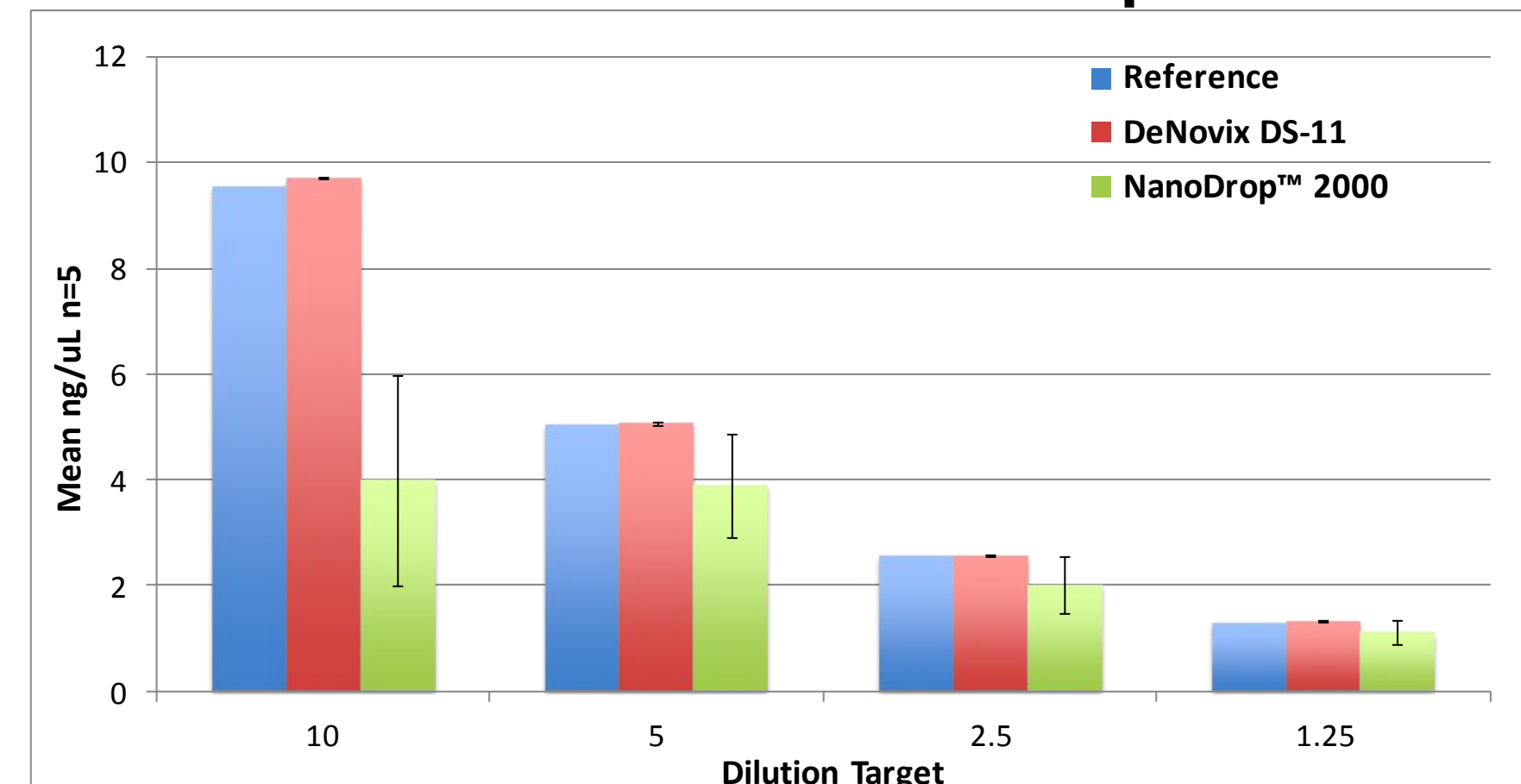


Figure 2: Custom methods on the DS-11 Android platform enable simple operation for all popular nucleic acid and protein applications

DeNovix DS-11 vs NanoDrop[™] 2000



Reference		DeNovix DS-11				Nanodrop 2000			
mg/mL	Agilent	mg/mL	%CV	%error	mg/mL	%CV	%error	mg/mL	%CV
9.53		9.71	0.3%	1.91%	3.99	49.9%	58.11%		
5.03		5.06	0.8%	0.55%	3.9	24.9%	22.49%		
2.56		2.56	0.8%	0.15%	2.01	26.7%	21.50%		
1.28		1.32	1.5%	3.20%	1.13	20.4%	11.56%		

Figure 5 and Table 1: Four separate BSA concentrations each measured five times on unconditioned DeNovix DS-11 and Nanodrop 2000 instruments

Other Applications on the DS-11



dsDNA

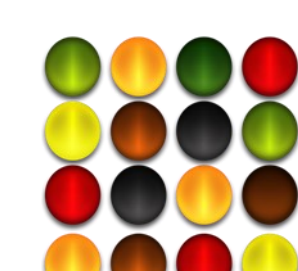


RNA



ssDNA

- Applications for nucleic acid quantification
- Broadest dynamic range on the market
- Direct UV analysis at 260 nm on purified samples
- Results obtained
 - Concentration (ng/ μL)
 - 260/280 and 260/230 purity ratios



Microarray App

- Application for labelled oligonucleotide probe quantification and QC
- Absorbance information can be used for Frequency of Incorporation
- Oligo concentration (ng/ μL), dye label concentration (μM) and absorbance data obtained



OD600 App

- Bacterial optical density measurements at 600 nm
- Cuvette mode on DS-11+ shows best performance for typical growth curve cell densities
- Microvolume shows best performance for high cell densities

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Further Information

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