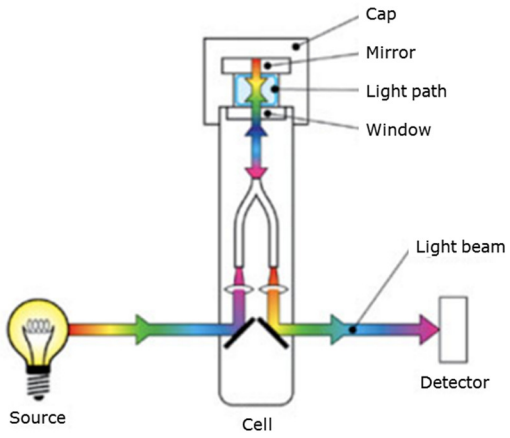
GENOVA BIO AND TRAYCELL

Using the Traycell with the Genova Bio

Introduction

One of the most common applications of a UV/Visible spectrophotometer is to measure the concentration of nucleic acids. Often there is very little sample available and the researcher may not wish to dilute it further in order give sufficient volume for measurement in a standard quartz cuvette. Ultra-micro quartz cuvettes are available which will allow as little as 20µl of sample to be measured directly, however they can be quite difficult to use to achieve reproducible results and if the sample needs to be recovered, there may be issues of contamination. An alternative approach, if the sample is of a sufficient concentration, is to use a TrayCell.

The TrayCell (Figure 1) is a fibre-optic, ultra-micro cell designed for measurements of extremely small sample volumes of DNA, RNA or protein. The dimensions of the TrayCell are equivalent to a standard 10mm path length cuvette and so will fit in all Jenway spectrophotometers; it is also extremely easy to use.



The TrayCell is first positioned in the cell holder of the spectrophotometer and a droplet of sample is placed on an optical window on top the surface of the cell. A cap containing a mirror is then fitted over the top. The type of cap used determines the path length through which the light passes.

Light from the spectrophotometer lamp is directed to the sample in the cap by optical fibres; the light returned by the sample is reflected off the mirror in the cap and passed down a second optical fibre to the detector.

***Figure 1**: Schematic diagram of the light path in the TrayCell.*

Once the measurement has been made, the cap is removed and the sample can be recovered if required. The window and cap are then gently cleaned using a lint-free swab or wipe. The TrayCell remains in the cell holder during all stages; this ensures that the aperture remains in an identical position for each measurement for increased reproducibility.

Virtual dilution

Two caps (1mm and 0.2mm) are supplied with the TrayCell which create defined optical light paths of 1mm and 0.2mm respectively. Caps giving 2mm or 0.1mm path length are also available as accessories. The reduced path length (compared to a standard 10mm cuvette) generates a virtual dilution of the sample with a dilution factor of 1:10 for a 1mm cap or 1:50 for a 0.2mm cap. This allows the user to measure the sample and recover it

without dilution. However the user should be aware that this means that a sample giving an absorbance reading of 1.0 in a 10mm path length cuvette will give an absorbance of 0.1 using the 1mm cap and 0.02 with the 0.2mm cap. Therefore it is important that the sample to be measured using the TrayCell is sufficiently concentrated to give a reading of at least 0.025 absorbance units for reproducible measurements.

In this application note we demonstrate DNA concentration measurement using the TrayCell in the Jenway Genova Bio spectrophotometer and compare the results with those from a standard 10mm path length micro cuvette.

Methods

A 1000µg/ml solution of genomic DNA was diluted with nuclease-free water to give a range of concentrations down to 1µg/ml. Samples of these dilutions were measured using the TrayCell with 1mm and 0.2mm caps (5µl and 2µl samples respectively) and the UV/Vis plastic micro cuvettes, part code 035 143 (100µl samples). The Genova Bio is fited as standard with the micro cuvette holder (630 304) to prevent excess light scattering through the side walls of the plastic micro cuvettes.

Measurements in the Genova Bio were made using the dsDNA mode with background correction enabled at 320nm. The method set up menu also allows entry of sample dilution. This was used to correct for the "virtual" dilution factors of the TrayCell caps. For the 10mm path length cuvettes, the dilution factor was set to 1; for the 1mm cap it was set to 1+9 (dilution factor of 10) and for the 0.2mm cap it was set to 1+49 (dilution factor of 50). Concentrations were recorded as µg/ml.

Results

DNA concentrations for each dilution were calculated by the Genova Bio software based on the default factor values and virtual dilution factor of the sample. In dsDNA mode the equation used by the Genova Bio is :

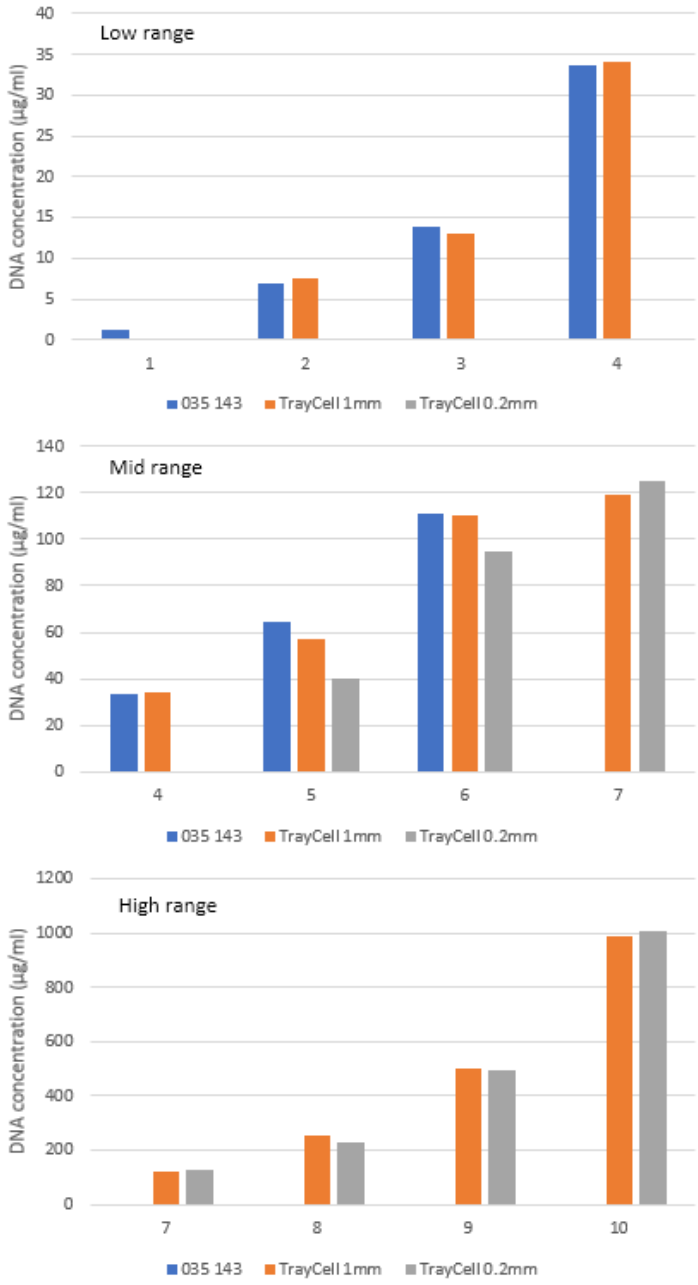
Concentration = ((Absλ1-AbsREF) x 50 x Dilution factor

Where:
Absλ1 = Abs260; AbsREF = Abs320;
Dilution factor = 1, 10 or 50 depending on cuvette/cap.

The values obtained from each cuvette are summarised in Table 1 and Figure 2.

Sample	035 143 cuvette	TrayCell 1mm	TrayCell 0.2mm
	DNA concentration (µg/ml)		
1	1.25	-	-
2	6.90	7.50	-
3	13.75	13.00	-
4	33.70	34.00	-
5	64.55	57.00	40.00
6	111.05	110.50	95.00
7	-	119.00	125.00
8	-	253.00	230.00
9	-	502.50	495.00
10	-	986.50	1007.50

***Table 1**: Measured DNA concentrations of each sample dilution using the TrayCell and micro volume cuvette. Shaded cells show results where A260 was <0.025.*



The results demonstrate that the TrayCell gives comparable values to the samples measured in conventional 10mm path length cuvettes up to the limit of the spectrophotometer absorbance range. The results also clearly show that due to the virtual dilution factor of the TrayCell caps, it is possible to measure DNA of a much higher concentration (greater than 125µg/ml) than standard cuvettes avoiding dilution of the sample.

Limitations for the TrayCell occur at lower concentrations. Based on the standard estimation of dsDNA that a 50µg/ml solution gives absorbance of 1.0 at 260nm and also that the lowest accurate reading is 0.025 Abs, the theoretical limits of the TrayCell for dsDNA are 13µg/ml when using the 1mm cap and 63µg/ml for the 0.2mm cap. Although Table 1 shows that it was possible to obtain results with lower concentrations than these, it should be noted that the actual absorbance values at 260nm were below 0.025 and are not reliable. Using a reference wavelength at 320nm does help to eliminate anomolous readings.

***Figure 2**: DNA concentrations of each dilution as measured in various cuvettes.*

A further experiment was performed to determine if there was any cross-over between samples, especially when reading from a high to a low concentration. Repeated samples of water and a highly concentrated sample were measured with a simple wipe of the read head and cap with a tissue between readings. The results shown in Figure 3 demonstrate no impact of the previous sample on the next. If it is required to recover the sample it would be recommended to wipe the TrayCell and cap with nuclease-free water and ethanol.

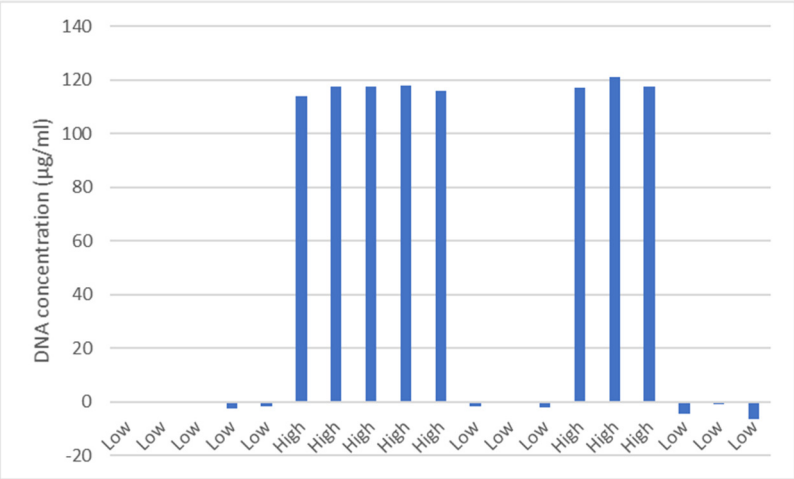


***Figure 3**: Repeated readings with water and a high concentration of DNA with simple wiping between each sample.*

Conclusions

The TrayCell is a useful tool for measuring the absorbance of samples where there is limited sample available or the sample needs to be recovered after measurement without dilution. The required sample volume for the 1mm cap is 3µl to 5µl and for the 0.2mm cap, 0.7µl to 4µl. Therefore if the sample is sufficiently concentrated, only a very small volume is required. Concentration values obtained with the TrayCell are comparable to those obtained with standard 10mm path length cuvettes. Further advantages include quick and easy cleaning and strictly defined path lengths meaning instrument misadjustment is impossible.

A minimum concentration of 13µg/ml dsDNA is required for the 1mm cap and 63µg/ml for the 0.2mm cap. The table below summarises the requirements for other nucleic acids.

Nucleic acids	Sample specific factor	1mm cap (factor 10)	0.2mm cap (factor 50)	Total measuring range*
dsDNA	50	13-850	63-4250	6-8500
ssDNA and Oligonucleotides	33	8-561	41-2805	4-5610
ssRNA	40	10-680	50-3400	4-5100

***Table 4**: Range of nucleic acid detection for the TrayCell with 1mm or 0.2mm caps. Sample specific factor refers to the concentration in µg/ml which gives an absorbance of 1 at 260nm in a 10mm path length. The values are based on an average spectrophotometer with a minimum absorbance at 260nm of approximately 0.025.*Total measuring range includes the optional caps of 2mm and 0.1mm.*

Applications where the TrayCell might be used could include:

1. Measurement of DNA stocks/standards for concentration and purity verification.
2. Measurement of oligonucleotides or PCR primers after reconstitution.
3. Measurement of plasmid DNA concentration after mini-prep extraction.
4. Determining purity and concentration of proteins.
5. All micro volume spectrophotometric measurements.
6. Other applications where absorbance levels are too high to be read in conventional 10mm path length cuvettes.