Spectrophotometric Quantification of Nucleic Acids

by Chris Voolstra, Anja Jungnickel, Lars Borrmann, Roland Kirchner, and Andrea Hube

The analysis of nucleic acids is part of the daily routine of the molecular biology laboratory. DNA and RNA concentrations; purity; and, in the case of modified nucleic acids, calculation of modification rates are mainly determined by UV-VIS spectroscopy. In standard UV-VIS spectrophotometers, cuvettes with a pathlength of 1 cm are generally used. This setup has limitations for nucleic acid measurements. Sample volumes of 50–1000 µL are required in most cases, with the need for sample dilution in order to stay in the linear range of the spectrophotometer. Depending on the application, the precious sample is usually not reusable after the measurement.

The LabelGuard™ Microliter Cell (**Implen GmbH,** Munich, Germany) is well equipped to handle these limitations. By choosing one of two lids with pathlengths of 1 or 0.2 mm, a virtual dilution factor of 1:10 or 1:50, without the need to physically dilute the sample, is achieved. In addition, the volumes needed for the measurements are 3 µL for the 1-mm lid, and less than 1 µL for the 0.2-mm lid. The cell offers high reproducibility, avoidance of dilution errors, and the possibility of retrieving the samples after measurement. In comparison to quartz glass cuvettes, use of the LabelGuard Cell saves time and money. For these reasons, it is a valuable analysis tool for such technologies as real-time PCR, laser capture microdissection, and microarray-based applications. In addition to these nucleic acid applications, it is also suitable for protein quantification, i.e., measurement of absorbency at 280 nm, bicinchoninic acid (BCA) assay, and Bradford and Lowry.

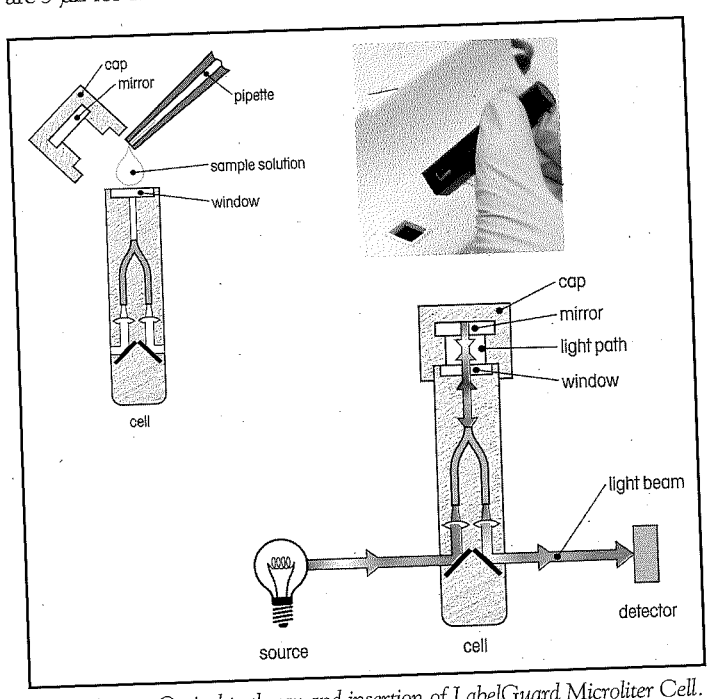

Figure 1 Optical pathway and insertion of LabelGuard Microliter Cell.

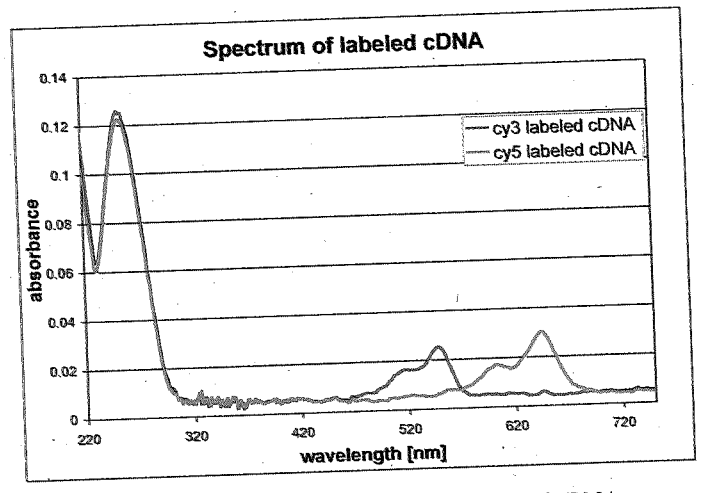

Figure 2 Samples of cyanine 3- and cyanine 5-labeled cDNA measured over a wavelength range of 220–750 nm with the SPECORD 50 (**Analytik Jena,** Jena, Germany).

Technical specifications

The optical pathway of the cell is designed for optimum measurement results using very low sample volumes (*Figure 1*). The sample is pipetted directly onto the center of the measuring window. Due to the integrated beam deflection and use of fiber optic light conductors, the sample can be measured directly on the measurement window. Use of the 1- or 0.2-mm lid creates a liquid column of defined pathlength of 1 and 0.2 mm, respectively. This generates virtual dilution factors of 1:10 or 1:50 (in comparison to a standard 1-cm cuvette measurement), which saves time and avoids dilution errors. The required sample volume for the 1-mm lid is 3–5 µL; for the 0.2-mm lid it is 0.7–4 µL. The mean dynamic range strongly depends on the type of photometer used. With the LabelGuard Microliter Cell, the average dynamic range for double-stranded DNA (dsDNA) is between 2 ng/µL and 5.000 ng/µL. The cell has a footprint of 12.5–12.5 mm, similar to standard cuvettes. The center height of the measuring window is adjustable, with adapters to the most commonly used center heights. Therefore, the cell is compatible with most commercially available spectrophotometers. The wavelength range is 190–1100 nm (*Figure 2*).

Experimental specifications

Different approaches were selected to validate the performance of the LabelGuard Microliter Cell. Linearity was determined over a broad concentration range with the SPECORD 210 spectrophotometer (**Analytik Jena**) (*Figure 3*). A dilution series of dsDNA was measured in triplicate at 260 nm. The resulting data show very good linearity (R^2 = 0.998) over a wide concentration range. Similar experiments performed with the SPEKOL 1300 photometer (**Analytik Jena**) and the BioPhotometer (**Eppendorf AG,** Hamburg, Germany) confirm this high linearity (data available upon request).

The reproducibility of the cell was tested by measuring the concentrations of plasmid DNA and oligonucleotide probes on a BioPhotometer at 260 nm. The concentration of the plasmid DNA, measured with the 1-mm lid, had an absorbance of 0.05 (27 µg/mL) at the lower detection limit. For the 0.2-mm lid, a sample concentration of 188 µg/mL was chosen, and this resulted in an absorbance of 0.07. With each lid, 14 independent measurements were carried out with 3 µL and 1 µL of sample volume, respectively. The coefficient of variation for the 1-mm lid was 2.0%, and for the 0.2-mm lid, 1.2%. With a DNA oligonucleotide probe (20 mer, **Metabion,** Martinsried, Germany), eight independent measurements were performed with sample volumes ranging from 700 nL to 3 µL using the 0.2-mm lid (*Table 1*). Despite the broad range of different sample volumes, the coefficient of variation of 3.1% is extremely convincing.

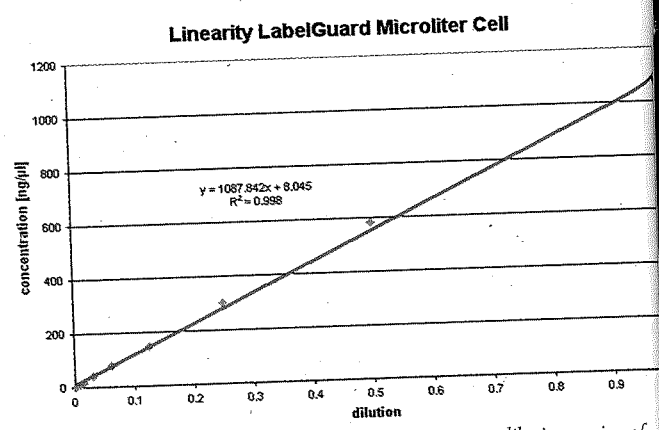

Figure 3 Linearity as determined by measuring a dilution series of dsDNA over a broad range of concentrations with the SPECORD 210 spectrophotometer.

Table 1 **Reproducibility determined with oligonucleotide probes; sample volumes ranging from 700 nL to 3 µL were measured at 260 nm**

No.	µg/µL Oligo	A260	Volume
1	0.570	0.380	3 µL
2	0.576	0.384	2 µL
3	0.572	0.381	1 µL
4	0.585	0.390	1 µL
5	0.567	0.378	1 µL
6	0.620	0.413	1 µL
7	0.569	0.379	700 nL
8	0.570	0.380	700 nL
Mean	0.578	0.386	
SD	0.018	0.012	
%CV	3.1	3.1	

Since there are no disposables used with the LabelGuard Microliter Cell, the cleaning of the measurement window is important. Because all parts of the cell are highly accessible, the cleaning protocol is very straightforward. After the measurement, the cell remains in the cuvette holder. The lid is removed and the sample can be retrieved and stored for later use. The measurement window and lid should be cleaned with a fluff-free swab or wipe. If necessary, residual fluff should be removed completely from the measurement window and the lid with dry compressed air to ensure optimum performance. Alternate measurements of high DNA concentrations and buffer at 260 nm show that possible systematic carryover effects can be eliminated (*Figure 4*).

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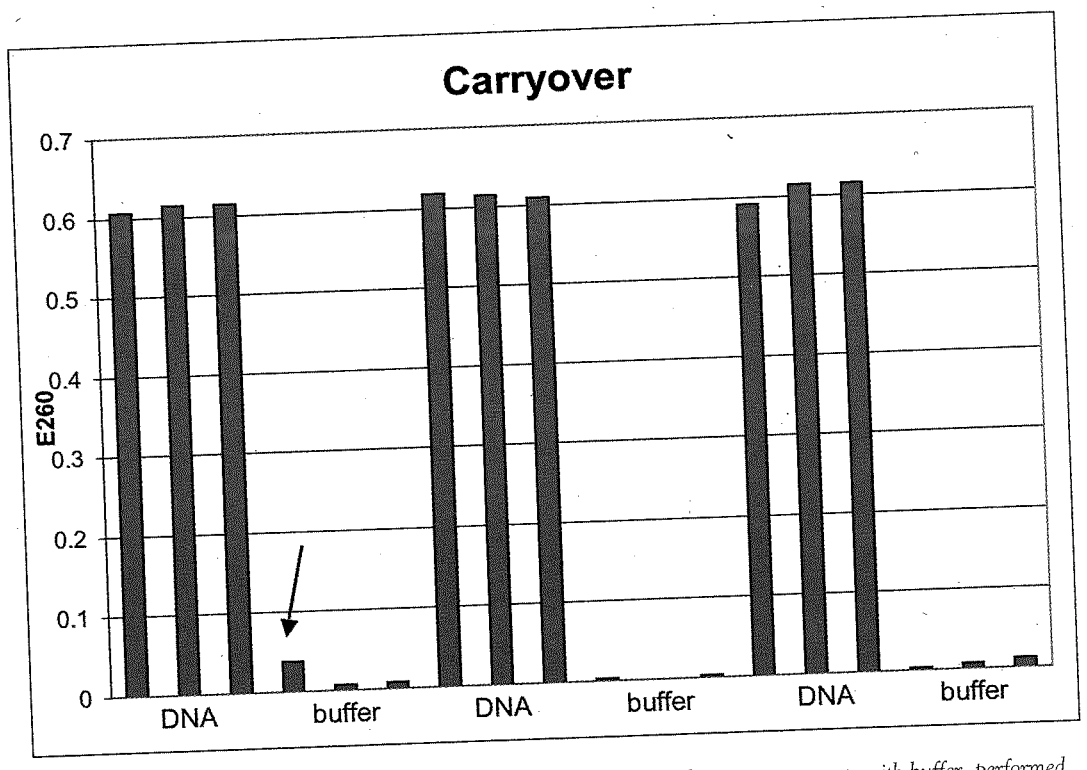

Figure 4 *Three measurements with high concentrations followed by three measurements with buffer, performed with the BioPhotometer. It is clear that there are no systematic carryover effects. Nevertheless, minimal sample carryover (6%) at the fourth measurement (arrow) was observed, demonstrating the importance of careful cleaning of the Microliter Cell.*

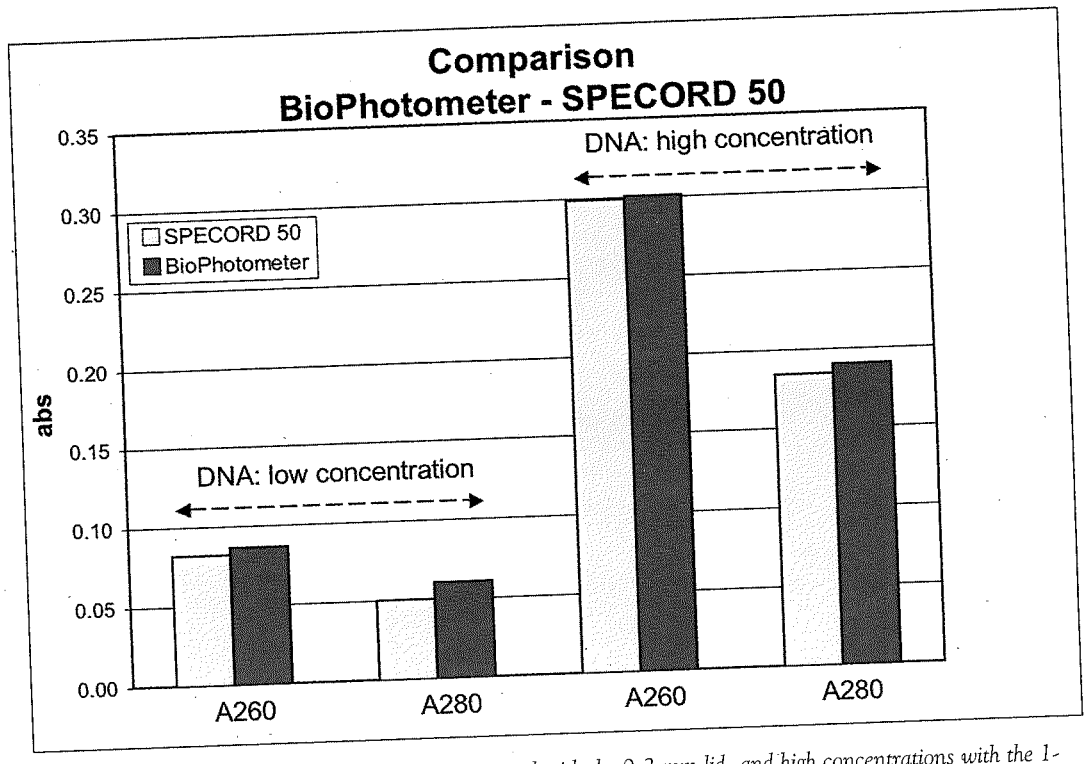

Figure 5 *Low DNA concentrations were measured with the 0.2-mm lid, and high concentrations with the 1-mm lid in triplicate. The resulting absorbance values of the low- and high-concentration measurements at 260 and 280 nm are very consistent and are independent of the spectrophotometer used.*

The correlation of measurements performed with a standard quartz cuvette or with the LabelGuard Microliter Cell was shown by measuring six different RNA samples at 260 nm with the BioPhotometer. Each measurement was carried out in triplicate with the cell or with the quartz cuvette, and the mean values were compared. Although the concentration range selected was in a very sensitive region, the values were absolutely comparable (data available upon request). To compare the compatibility of the LabelGuard with different spectrophotometers, the identical DNA sample was measured in triplicate with the BioPhotometer and the SPECORD 50. The obtained concentration values are comparable to a high degree (*Figure 5*).

Conclusion

The LabelGuard Microliter Cell enables the absolute reliable spectrophotometric analysis of ultralow-volume nucleic acid and protein samples. It can be used in most commercial spectrophotometers with sample volumes starting from less than 1 µL. By avoiding the necessary dilution of samples, possible error sources can be eliminated in comparison to standard glass or plastic cuvette measurements. The simple handling and cleaning procedure of the cell saves hands-on time and materials costs for spectrophotometric analysis.

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