Technical Note

#2 Sample Compression Technology™

Introduction

The patented Sample Compression Technology™ provides unmatched precision and accuracy with reliable measurement geometry even for challenging samples including proteins and products in volatile solvents. This technology is especially designed for ultra-low sample volumes of 0.3 µl to 2 µl.

Principle of Small Volume Measurements

The sample concentration in UV/VIS spectroscopy is calculated based on the Beer-Lambert law:

$$c = A \times \frac{1}{\varepsilon} \times \frac{1}{d}$$

c = concentration (g/l)
A = absorbance value
Ɛ = extinction coefficient of sample (g*cm/l)
D = path length (cm)

This equation describes the correlation between concentration, absorbance and path length. Measurements need to be within the linear range for any spectrophotometer to be trustworthy and precise. For ease of use and to avoid manual dilution errors the NanoPhotometer® creates a virtual dilution of each sample. Depending on the concentration of the sample the system applies an automatic adjustment of the path length. The instrument always measures first the longer 0.67 mm path and if necessary as a second step the shorter 0.07 mm path.

Sample Compression

In comparison to other technologies, the NanoPhotometer® uses an optical geometry similar to a microscope setup. 0.3 µl – 2 µl of sample are applied to the pedestal, which acts as a “microscope slide”. The “cover glass” is a mirror embedded into the lid which when lowered squeezes the drop between two quartz surfaces (indicated by red lines in figure 1 & 2).

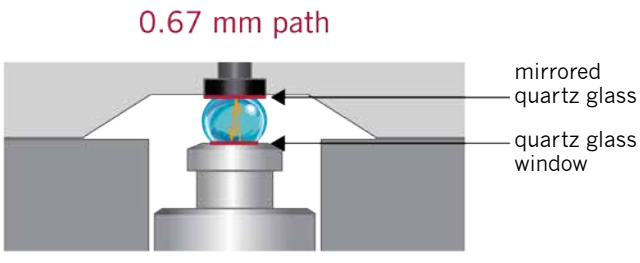

Figure 1: Sample drop squeezed between two quartz surfaces (0.67 mm path); light path indicated by orange arrows.

A capillary film, shaped into a precisely defined flat layer of even thickness, is formed eliminating the need for surface tension. At the same time the sealed micro-environment limits evaporation. From below, light shines through the sample, is reflected by the upper mirror and returned through the lower quartz window in the pedestal (see figure 1 orange arrows) onto the detector.

The actual distance between the two quartz surfaces is half of the path length as a result. This reduced distance has an immensely positive influence on the drop stability especially for challenging samples like proteins or samples with volatile solvents.

The NanoPhotometer® NP80/N60/N50 always reads the 0.67 mm path length first and only if the sample is not in the linear range, moves the pedestal to form the second shorter 0.07 mm path (figure 2).

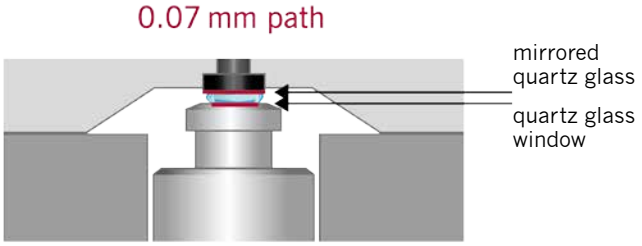

Figure 2: Sample drop squeezed between two quartz surfaces (0.07 mm path).

Contained Sample Environment

Sample Compression Technology™ has the added benefit of holding the sample in place and evaporation can be neglected. This sealed and protected microenvironment allows reliable protein measurements and the analysis of samples in volatile solvents. Even kinetic studies in small volumes are possible with this technology.

Conclusion

The patented Sample Compression Technology™ is key to obtain reliable and accurate readings from ultra-low volume samples. With the contained microenvironment geometry, the instrument is optimized for reliable performance in all climate zones and also is ideal for challenging studies like low surface tension proteins, volatile solvents or kinetics in a drop.

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