

### Introduction

The NanoPhotometer® NP80/N60/N50 is designed to provide accurate and reliable measurements for ultra-low volume samples of 0.3 µl to 2 µl. No recalibration is needed for the entire lifetime of the instrument. The unique True Path Technology™ is the backbone of this important feature, which creates a calibrated optical environment that does not change over time.

### Principle of Small Volume Measurements

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

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

$c$  = concentration (g/l)  
 $A$  = absorbance value  
 $\epsilon$  = 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 of 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 is applying an automatic adjustment of the path lengths. Therefore preciseness of these path lengths is key for accurate measurements.

### Precise Path Length Setting

True Path Technology™ works with two accurate path lengths designed as fixed anchor points. To guarantee the preciseness of the two path lengths (0.67 and 0.07 mm) the sealed pedestal is designed with high precision metal parts (indicated by red lines in figure 1 & 2). The pedestal

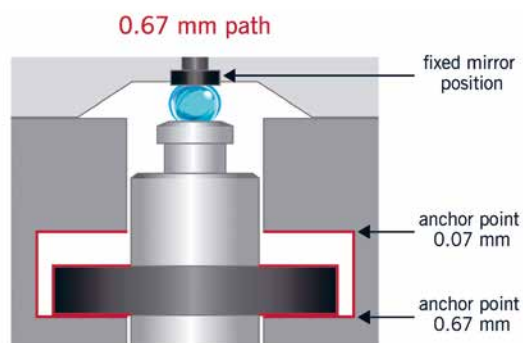


Figure 1: Sealed mechanical setup with pedestal position anchor point 1 (path length 0.67 mm/virtual dilution factor 15).

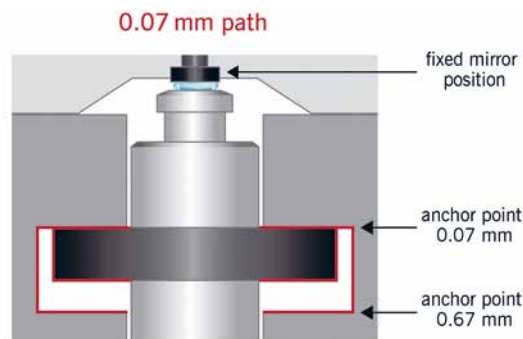


Figure 2: Sealed mechanical setup with pedestal position anchor point 2 (path length 0.07 mm/virtual dilution factor 140).

position at anchor point 1 (Figure 1) assures the path length setting for the 0.67 mm path and at anchor point 2 (Figure 2) for the 0.07 mm path.

Depending on the sample absorbance the instrument automatically chooses the right path length for achieving best measurement results in the Nucleic Acid and Protein UV method. In all other methods, the path length has to be chosen manually.

As the pedestal can only move between the two defined path lengths the NanoPhotometer® is recalibration free, the dimensions will not change over the entire lifetime.

Other small volume instruments are working with stepper motor based technologies for path length adjustment. These systems are likely to cause a drift from the correct path length positioning over time. It is recommended for such technologies to check the path length setting within certain time intervals set by the manufacturer.

Because of its optical geometry and the high performance detection system of the NanoPhotometer® the linearity is excellent across the entire range. Please refer to Technical Note #4 Nucleic Acids Performance Data and #5 Protein UV Performance Data for further details and specific performance data.

### Conclusion

The unique True Path Technology™ is the key component of the NanoPhotometer® for precise path length setting. It provides accurate and linear results over the lifetime of the instrument without any need for recalibration.