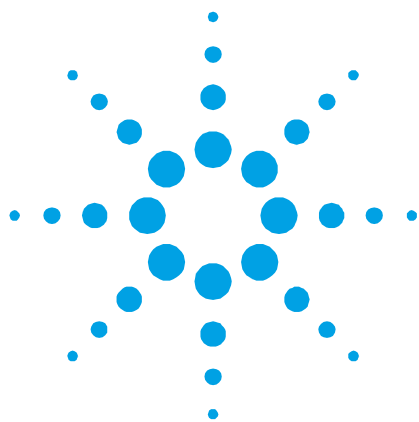




# Measuring baseline-corrected spectra on a Cary 60 UV-Vis

## Technical Overview

### Introduction

The advancement in electronics over the past decades has seen a significant change in design to UV-Vis spectrophotometers and spectrophotometric measuring techniques. Double-beam instruments, i.e., an instrument with a sample and a reference beam that both pass through the sample compartment, were essential to obtain accurate spectra. Any fluctuations in source intensity and the absorbance due to the cuvette and solvent were accounted for by placing the cuvette containing the solvent in the rear or reference beam. The sample was placed in the front beam in a matched cuvette. To match the geometry and mechanical characteristics of a cuvette to an acceptable tolerance can be quite costly.

The double-beam instruments of today do not require a reference cuvette in the rear beam. Software and electronics can now cope with recording and storing the reference signal in memory which can then be subtracted from the sample measurement. In instruments where two detectors are present, this rear beam now becomes redundant.

The design of the Agilent Cary 50 and Cary 60 UV-Vis spectrophotometers takes into account all these technological advancements. It has only one beam through the sample compartment, but two detectors, making it a dual-beam instrument. Any fluctuations in the source intensity is measured by the internal reference detector, which is inside the instrument and not accessible to the user. Only one cuvette is required, which also reduces the cost of having to purchase matched pairs.



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There are, in some cases, advantages in having a reference beam when a photomultiplier tube (PMT) is used as the detector. On double-beam instruments when measuring highly absorbing samples,  $>3.5$  Abs, an absorbing material is placed in the reference beam and is used to balance the signal levels in the sample and reference beams. This technique is called rear beam attenuation (RBA). A balanced beam allows the PMT detector to perform closer to its theoretical noise limit and the data quality for high absorbance samples is dramatically enhanced. A photomultiplier is a variable gain detector whereas a photodiode detector has a fixed gain. What this means is that the detection limit in a photodiode is fixed and does not benefit from rear beam attenuation, hence, no access, or attenuation of the Cary 50/60 reference beam is required.

Another example where using a reference beam is necessary is when measuring the absolute absorbance of a sample that is in a solvent matrix that is changing with time. The reference beam may also be used when measuring turbid samples. In this case, the solvent matrix is placed in the rear beam and the sample, within the matrix, in the front beam. However, the Cary 50 and Cary 60 can measure turbid solutions up to 3.3 Abs accurately, as the beam is focused near the sample detector, hence, collecting most of the scattered light. An alternative method is using an integrating sphere.

This paper explains, in a step-by-step procedure, how to measure baseline-corrected spectra using the Cary 60. It also provides the necessary mathematical proofs that show the results obtained for routine measurements in a dual-beam instrument are exactly the same as using a double-beam instrument with matching cuvettes.

## Discussion

The signal measured in a spectrophotometer are affected by the following variables.

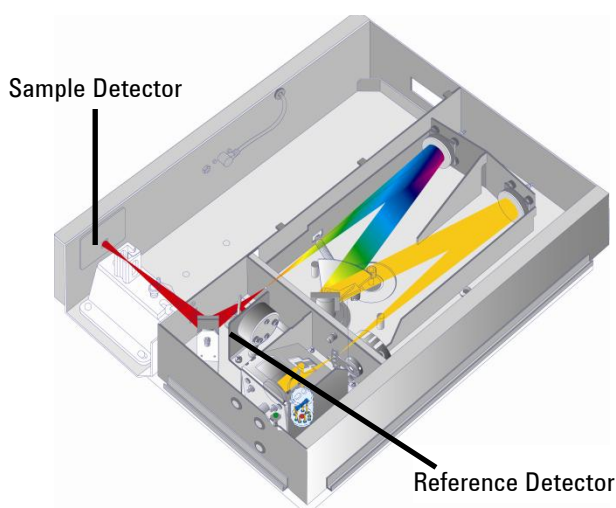
- (a) lamp brightness,  $B$
- (b) sample transmittance,  $T_{\text{sample}}$

(c) cell transmittance,  $T_{\text{cell}}$

(d) solvent transmittance,  $T_{\text{sol}}$

Therefore, to measure the signal due solely to the sample (b), the other contributions must be determined. This is achieved in the Cary 60 as follows.

A beam splitter divides the beam in two, with one beam passing through the cuvette to the sample detector and the other internal beam hitting a reference detector (Figure 1).



**Figure 1.** Ray trace diagram of Cary 60 showing Reference and Sample detectors

With no cell in the beam, the signal at the sample detector,  $I_0$ , and the signal at the reference detector,  $I_{\text{ref}}$ , are both proportional to the lamp brightness as shown by Equations 1 and 2, where  $k_1$  and  $k_2$  are constants.

$$I_0 = k_1 \times B \quad \text{Equation 1}$$

$$I_{\text{ref}} = k_2 \times B \quad \text{Equation 2}$$

The transmittance of the solvent and the cell are accounted for by performing a blank. The solvent used to prepare the sample is placed in a cuvette and the signal at the sample detector measured (Equation 3).

$$I_{\text{blank}} = I_0 \times T_{\text{cell}} \times T_{\text{sol}} \quad \text{Equation 3}$$

The transmittance of the blank is given by Equation 4, where normalizing to the reference signal,  $I_{ref}$ , takes into account any fluctuations in lamp brightness. It is worth noting that single-beam spectrophotometers, ie instruments having no reference detector and only one beam through the sample compartment, cannot correct for lamp intensity fluctuations.

$$T_{blank} = \frac{I_{blank}}{I_{ref}} = \beta \quad \text{Equation 4}$$

The operation of "zero", ie., performing a blank, is to determine the value of  $\beta$ , which is then used to correct subsequent measurements. Therefore, with the blank in the sample compartment, the corrected transmittance is given by Equation 5.

$$T_{blank,corrected} = \frac{T_{blank}}{\beta} = 1 \quad \text{Equation 5}$$

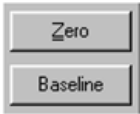
If the sample is placed in the cell, the signal measured at the sample detector is given by Equation 6 and the corrected signal by Equation 7.

$$I_{sample} = I_0 \times T_{cell} \times T_{sol} \times T_{sample} \quad \text{Equation 6}$$

$$\begin{aligned} T_{sample,corrected} &= \frac{I_{sample}}{I_{ref} \times \beta} \\ &= \frac{I_0 \times T_{cell} \times T_{sol} \times T_{sample}}{I_{ref} \times \frac{I_0 \times T_{cell} \times T_{sol}}{I_{ref}}} \\ &= T_{sample} \end{aligned} \quad \text{Equation 7}$$

A "Baseline" measurement is similar to a "zero" measurement in which a "baseline" measures a "zero" correction for each wavelength in a scan.

Figure 2 shows the ease at which a corrected spectrum can be collected using the WinUV software on the Cary 60.

1.  Place cuvette with solvent only in cell holder.
2.  Press Zero or Baseline to record the blank.
3. Remove and empty contents of cuvette. Wash cuvette with deionised water.
4.  Rinse cuvette with sample 2-3 times and fill cuvette 2/3 full with sample solution.
5.  Place cuvette in cell holder.
6.  **Start** PRESS  **Read**

**Figure 2.** Step by step procedure to obtain baseline-corrected spectra

Evaluations of all equations are performed in the software and the corrected sample signal is displayed in absorbance, Equation 8.

$$Abs_{sample} = -\log_{10} T_{sample} \quad \text{Equation 8}$$

## Conclusion

The Cary 60 can be used to measure baseline-corrected spectra, obtaining data quality similar to, or better than, any double-beam instrument.

Although the instrument design and measurement technique between a double-beam or dual-beam instrument may differ, the result for routine measurements is the same.

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