Measuring photometric accuracy using the double aperture method

Application Note

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Introduction

Photometric accuracy is critical for many applications of UV-Vis spectrophotometers. For example, when comparing measurements taken on different instruments, it is necessary to know the accuracy of each instrument before a true comparison can be made. Determining the photometric accuracy of a spectrophotometer has been traditionally performed by using solutions of high purity compounds prepared by the operator, or by measuring the absorbance of calibrated neutral density filters, issued by one of the national standards organizations. The former method is limited by the volumetric accuracy of the solution preparation and the measurement technique. The latter method relies upon the filters being kept clean, free from scratches and at a constant temperature. The transmittance of the filters can also change by as much as ±1% of the value over the first year. Typical uncertainties for both methods are shown in Table 1.

Table 1. The uncertainties associated with various methods of determining photometric accuracy

Method	Uncertainty (Abs)
Acidic potassium dichromate[1]	±0.004 (min)
NIST 930D filters[2]	±0.0022
NIST 2031 metal on quartz filters[3]	±0.004

The Double Aperture method, as discussed in this paper, was first used by Clarke at the National Physical Laboratory in England and later by Hawes[4]. It is used by the major national standards laboratories to measure the absolute accuracy of their reference spectrophotometers[5]. Requiring relatively simple apparatus, it has few of the limitations of the other methods.

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To illustrate the benefits of the Double Aperture method, it has been used to measure the absolute photometric accuracy of Cary spectrophotometers in this paper.

The linear resistor chains recommended by photomultiplier manufacturers for generating individual dynode voltage supplies can be shown, using the double aperture method, to produce significant non-linearity under the typical operating conditions used in UV-Vis spectrophotometers. The magnitude can be of order +0.002 Abs at 0.3 Abs and +0.004 Abs at 1 Abs.

The dynode supplies used in Cary spectrophotometers are not derived from linear resistor chains, but from systems that dramatically reduce the above errors. The magnitude of the improvement will be seen later.

This paper outlines the principles behind the method, and how it is used to generate a calibration curve for a spectrophotometer.

The principles of the method

A photometric transmittance scale is arbitrarily defined by setting the response with no light through the sample beam to be zero and the response with no sample in the beam to be 100%. If the response is linear, then the scale is absolutely accurate.

Absorbance measurements obtained by mathematical conversion from transmittance measurements on such a scale also have absolute accuracy. Scale errors in the transmittance can be converted to absorbance errors using the formula:

$$\Delta Abs = -\Delta T/2.3T.$$

There are several methods available for assessing photometric accuracy. Chemical standards are limited to accuracies of order 0.005 Abs. Calibrated neutral filters are available which are certified to be within 0.5-1% of their stated transmittance (0.0022 - 0.004 Abs) if used at the specified wavelengths, spectral bandwidths and within a degree or two of the defined temperature.

The Double Aperture method has no limitations on wavelength, SBW or temperature and can yield precisions about 2 orders of magnitude better than these uncertainties.

The equipment consists of a plate with two separated holes that can be separately covered or uncovered to produce, from the instrument's sample beam, two independent light fluxes onto the detector (refer to Figure 1).
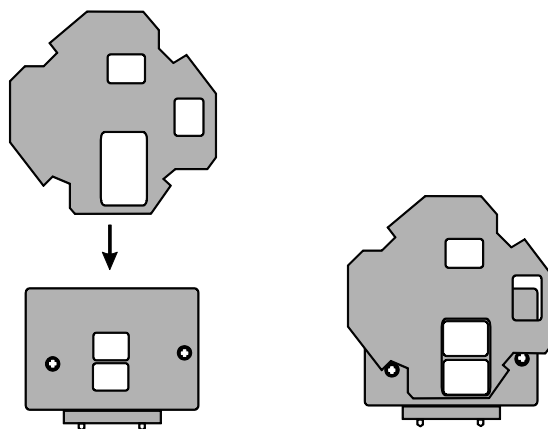

Figure 1. The equipment used for the double aperture method

If the detection and measurement system is linear, then the sum of the transmittance measurements of each single aperture should equal the transmittance measurement obtained with both apertures open.

If the measurement with both apertures open is 100% then the average of the two approximately equal single aperture readings, less 50%, is the absolute scale error at 50% (refer to Figure 2).
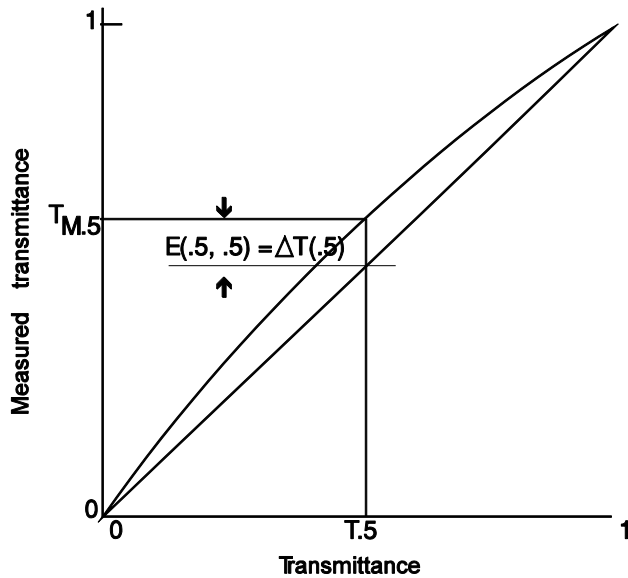
Figure 2. Transmittance scale error at 50%T

If the sample beam is now attenuated so the reading with both apertures open is 50%T, the absolute error at 25%T can be obtained from a similar set of measurements.

To show how this is derived, it is necessary to define some symbols, and develop some simple equations.

Let $Tm(x) = T(x) + \Delta T(x)$

Where $Tm(x)$ is the measured transmittance,

$T(x)$ is the actual transmittance and

$\Delta T(x)$ is the scale error at transmittance x.

For a pair of independent apertures that read $Tm(a)$ and $Tm(b)$ when measured separately and $Tm(a+b)$ when measured together, we can calculate

$$E(a,b) = (Tm(a) + Tm(b) - Tm(a+b)) / 2$$

Substituting for Tm from the equation above, this reduces to

$$E(a,b) = (\Delta T(a) + \Delta T(b) - \Delta T(a+b)) / 2$$

If $Tm(a+b)$ is 100%, $\Delta T(a+b)$ is zero, and if the two fluxes are adjusted to be equal, then
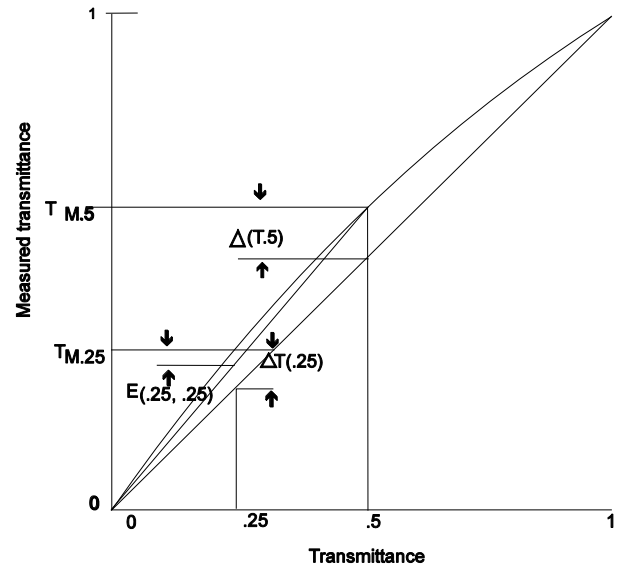
$$E(0.5,0.5) = \Delta T(0.5)$$

As seen in Figure 2.

Figure 3. Transmittance scale error at 25%T

When the reading with both apertures is attenuated to 50% we can measure

$$E(0.25,0.25) = (\Delta T(0.25) + \Delta T(0.25) - \Delta T(0.5)) / 2$$

$$= \Delta T(0.25) - 1/2\, \Delta T(0.5)$$

From which

$$\Delta T(0.25) = E(0.25,0.25) + 1/2\, \Delta T(0.5)$$

This can be seen graphically in Figure 3.

This process of attenuation and measurement can be continued to yield absolute error measurement at 12.5%, 6.25%. Conversion of transmittance errors to Absorbance errors yields a set of absolute absorbance errors at intervals of 0.3 Abs.

An alternative method of extending the calibration curve from 0.3 Abs to higher Abs is to use the method of 'Addition of Filters'. This method used on its own, is capable of measuring relative linearity errors in an Abs scale but is not capable of yielding absolute errors. When coupled with the Double Aperture method, absolute errors can be measured.

The basis of the method is to measure the absorbance of two filters individually and with the two filters in series.

If the filters are uniform and surface inter reflections are eliminated by tilting the filters slightly, then the difference between the series measurement and the sum of individual measurements is the additional error at the summed absorbance. If the error in the individual measurements is known then the error at the summed absorbance can be calculated as the sum of the individual measurement errors plus the additional error.

If the initial pair of filters are 0.3 Abs, their measurements can be corrected by the Double Aperture method. The error at 0.6 Abs is then twice the Double Aperture error plus the additional error. The series can be extended up or down the Abs scale by a suitable choice of filters. Note that the precise value of the filters need not be known—only the correction that needs to be applied to the measured value.

Results

For a Cary 4E or 5E, the photomultiplier yields error curves as in Figure 4. The error bars give an indication of the precision obtained.

The general shape of this curve, i.e. becoming horizontal at high numbers, is typical for all operating conditions. The parameter affecting the magnitude of the error is the photomultiplier gain or voltage. If this is fixed, as in 'Fixed Energy' or 'Autoslit' mode, the error function becomes independent of wavelength, as shown in Figure 5.
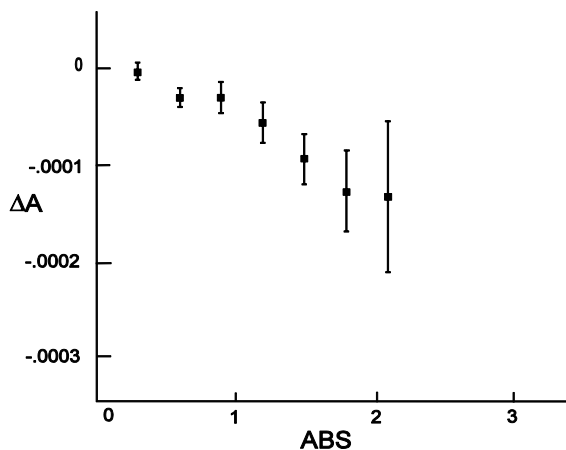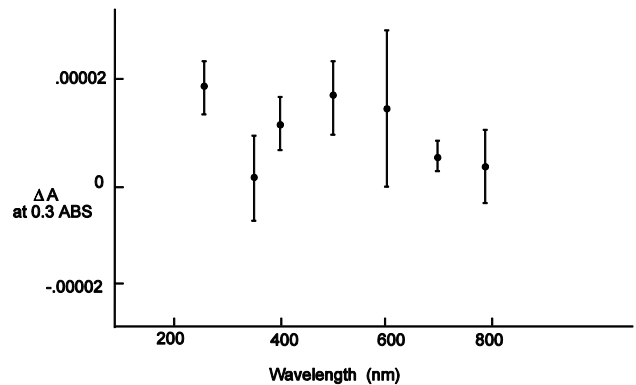

Figure 5. Cary 4E/5E ΔAbs at 0.3 Abs vs wavelength for energy 20

The mean error at 0.3 Abs for wavelength 590 nm and 3 nm SBW for a population of Cary 4E and 5E instruments was 0.000001, with a standard deviation of 0.000065.

The mean error at 0.3 Abs for wavelength 590 nm and 3 nm SBW for a population of Cary 1 and 3 instruments was 0.000002, with a standard deviation of 0.00018.

Conclusion

The errors measured using the Double Aperture technique were approximately an order of magnitude smaller than the uncertainties associated with chemical standards or calibrated filters. Given the ease of use and lack of susceptability to temperature and ageing effects, the double aperture method should be the method of choice for determining the photometric accuracy of UV-Vis spectrophotometers.

Figure 4. Cary 4E/5E ΔAbs vs Abs at 500 nm, 2 nm SBW, 3 sec SAT

References

1. Burgess C., Knowles A., Standards in absorption spectrometry, UV spectrometry group, Chapman and Hill, London, **1981.**

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