

## Automated Double Aperture Accessory

**For the Cary 1, 3, 100, 300, 4, 5, 400, 500, 500i, 4000, 5000, 6000i, Deep UV**

Installation Category II

Pollution Degree 2

Equipment Class I

### Table of Contents

- Introduction
- Theory
- Operation
  - Installation
  - Alignment
    - Visual alignment (for Cary 500i, 6000i and DeepUV)
    - Visual alignment (for Cary 1, 3, 100, 300, 4, 5, 4000 and 5000)
  - Reference beam attenuation
  - Validation and optimization software
- Instrument specifications

### Introduction

The Double Aperture method, first used by Clarke at the National Physical Laboratory in England, is used by the major national standards laboratories to measure the absolute accuracy of their reference spectrophotometers. It requires relatively simple apparatus and has few limitations compared to other methods. The Double Aperture accessory allows you to use this method to measure the absolute photometric accuracy of your Cary spectrophotometer.

When using the Automated Double Aperture (rather than the manual Double Aperture accessory) the adjustments to the double aperture plate are made by the software, rather than the user having to physically move the plate through its various positions.



**Agilent Technologies**

**NOTE**

The Double Aperture accessory can be used to measure photometric accuracy in the NIR on the Cary 500i and 6000i instruments.

**NOTE**

There is also a manual version of the Double Aperture accessory.

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### Theory

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 the 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.

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 transmittance can be converted to absorbance errors using the formula:

$$DAbs = -DT / 2.3T$$

There are several methods available for determining photometric accuracy. Chemical standards are limited to accuracies of order 0.005 Abs.

Calibrated neutral density filters are available, which are certified to be within 0.5–1% of their stated transmittance (0.002–0.004 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 SBW or temperature and can yield precisions about 2 orders better than these uncertainties. It is the method used by most standards laboratories.

In its basic form, 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.

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%T, then the difference between 50%T and the average of the two single aperture readings is the absolute scale error at 50% (refer to the following diagram).

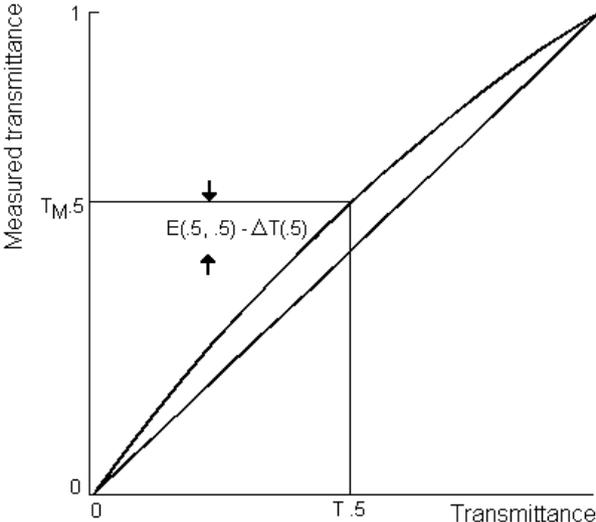


Figure 1. Transmittance scale error at 50%T

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  $T_m(x) = T(x) + DT$  (Equation 1)

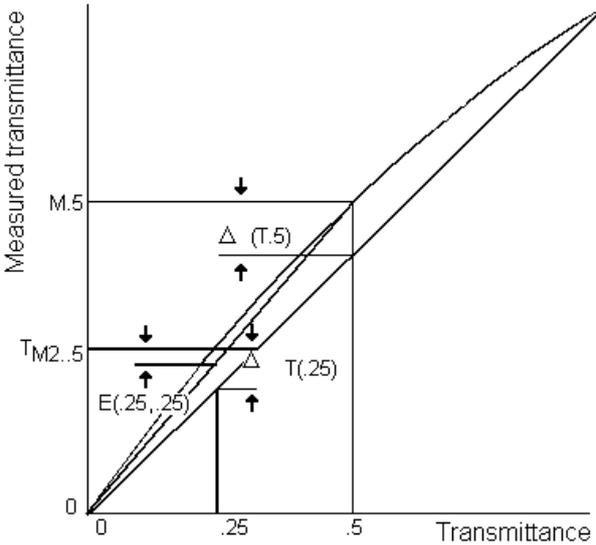


Figure 2. Transmittance scale error at 25%T

Where:

- $T_m(x)$  is the measured transmittance,
- $T(x)$  is the actual transmittance and
- $DT(x)$  is the scale error at transmittance  $x$ .

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

$$E(a,b) = (T_m(a) + T_m(b) - T_m(a+b)) / 2 \text{ (Equation 2)}$$

Substituting for  $T_m$  from equation (1), this reduces to:

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

If  $T_m(a+b)$  is 100%,  $DT(a+b)$  is zero, and if the two fluxes are adjusted to be equal, then:

$$E(.5,.5) = DT(.5)$$

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

$$E(.25,.25) = (DT(.25) + DT(.25) - DT(.5)) / 2 = DT(.25) - 1/2 DT(.5)$$

from which:

$$DT(.25) = E(.25,.25) + 1/2 DT(.5)$$

This process of attenuation and measurement can be continued to yield absolute error measurement at 12.5% and 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 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.

## Operation

The Double Aperture accessory consists of two holders, one for the reference beam and one for the sample beam. Both holders fit onto a cell holder base.

The holder for the sample beam has a motorized accessory on a horizontal plate, which must be placed in the appropriate position for the instrument model and wavelength you are measuring (see Figure 3).

The holder for the reference beam is designed to hold microscope slides, which attenuate the beam when the wavelength is in the visible region. If you are going to perform the measurements in the UV region, you will need to use the V-shaped attenuator supplied with the Double Aperture accessory.

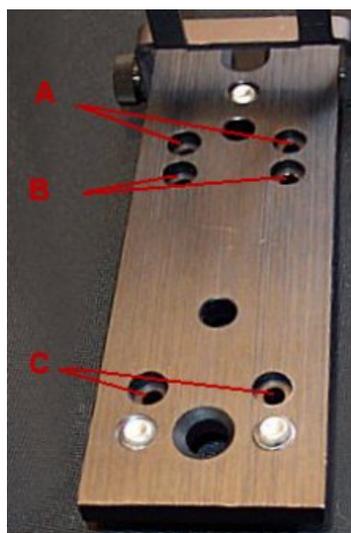
## Installation

The Automated Double Aperture accessory includes:

Item	Description
Automated Double Aperture accessory	With double aperture plate attached
Double aperture plate	To fit the reference port
Thumb screw	
Hex screw	
1 x 'V'-shaped slide	
3 x glass slides	

### To install the Automated Double Aperture accessory:

- 1 Remove the holder with the double aperture plate from the packing box.



**Figure 3.** Double aperture plate showing positions for the different instrument models. A) Cary 1, 3, 100, 300. B) Cary 4, 5, 400, 500, 4000, 5000. C) Cary 500i, 6000i, Deep UV

- 2 Slide back the sample compartment lid and remove the front panel.
- 3 Install the cell holder bases in the instrument, one at the sample beam, the other at the reference beam. Refer to the instrument user's guide for instructions on how to do this.

#### NOTE

If you are using an old cell base in a Cary 4000, 5000 or 6000i, you must first remove the lock-down plate.

- 4 Place the Automated Double Aperture accessory on the standard cell holder in the instrument.
- 5 Use the two posts on the cell holder to position the accessory.
- 6 Use either the thumb or hex screw to secure the accessory onto the holder.
- 7 Connect the Automated Double Aperture accessory to the instrument via the 9-way connector port located at the rear of the Cary 4/5/400/500/500i/4000/5000/6000i/DeepUV instrument or the sample compartment floor of the Cary 1/3/100/300 instrument.
- 8 Replace the front panel.
- 9 Close the sample compartment lid.
- 10 Turn on the instrument and let it warm up for at least an hour.

## Alignment

The alignment of the Automated Double Aperture accessory has two stages: the first is a rough alignment made visually; the second stage involves fine-tuning and uses the Validate software.

The Cary WinUV software contains a suite of software tools to check the performance of your instrument. Double-click the Validate icon in the Cary folder to start the application. Refer to the Validate Help for details on how to use this application.

### Visual alignment for Cary 500i, 6000i and DeepUV

#### To perform a visual alignment:

- 1 Click the Windows **Start** button, then **Programs, Cary WinUV** and **Validate**.
- 2 With nothing in the beams, close the sample compartment lid and choose **Go to** from the **Commands** menu.
- 3 Enter 0.0 nm in the Wavelength field and click **OK**.
- 4 Swing the screen down so that it falls into the light path.
- 5 Look at the beam image on the screen to observe where the beam is falling. The beam should fall so that half of it is on the top half of the screen and half on the bottom.
- 6 If the beam is out-of-line, undo the screws holding the cell holder in position and remove the cell holder and accessory from the sample compartment.
- 7 Loosen the hex screw (or thumbscrew).
- 8 Use the hex ball driver to adjust the three screws holding the accessory onto the cell holder. Adjusting these screws will alter the height of the accessory.
- 9 Reposition the cell base and accessory in the instrument, and check the beam image is again on the screen.
- 10 Repeat Steps 5 to 9 until the sample beam strikes the target as describes in Step 5.

### Visual alignment for the Cary 1, 3, 100, 300, 4, 5, 4000 and 5000

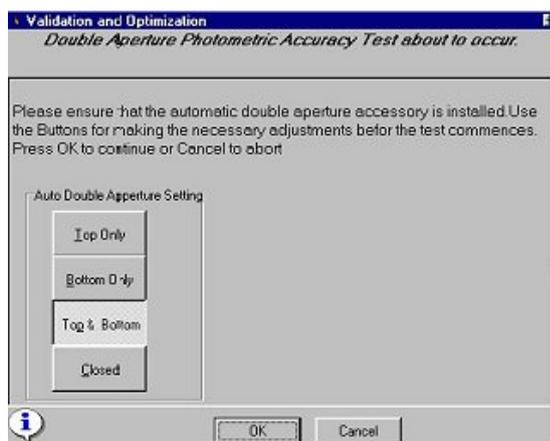
#### To perform a visual alignment:

- 1 In the Validate application, click the **Tests** button.
- 2 Select **Enable All Tests**, and from the list on the right side of this dialog box select **Photo Accuracy – Double Aperture** from the list.
- 3 Ensure that the **Double Aperture** check box is selected.

#### NOTE

There are detailed instructions on the Photometric Accuracy test available in the Help. If you are only performing the Double Aperture test, make sure that all other tests are not selected. Also, ensure that the Double Aperture check box is selected.

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- 4 Click **OK**.
  - 5 Click the **Setup** button. On the **Analyze** tab, select the **Auto Double Aperture** check box. The following dialog box will appear, advising you that the Double Aperture Photometric Accuracy test is about to commence.



**Figure 4.** This dialog box appears to assist you in aligning the accessory

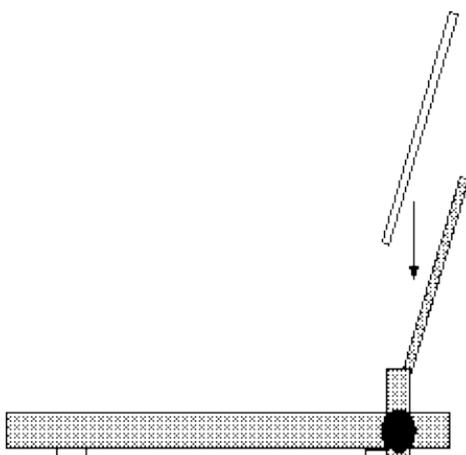
- 6** Click the **Closed** button under Auto Double Aperture Settings'. This will rotate the aperture disk to the closed position.
- 7** Click **Go To** on the **Commands** menu or press F4 on the keyboard, then select the **Zero Order** check box.
- 8** Look into the viewing port and check that the beam is centered on the marked target behind the aperture disk. If the beam is not centered, use a hexagonal ball driver to adjust the three height adjustment screws on the cell holder base in the sample beam until the image is centered.
- 9** Click the **Top Only** button. Look through the viewing port and check that only the top port of the accessory is open. This enables you confirm that the accessory is correctly rotating.
- 10** Click the **Start** button again. Click the **Top and Bottom** button in the Auto Double Aperture field.
- 11** Look into the viewing port and check that both the top and bottom apertures are open.
- 12** Click **Cancel** and go to zero order. To do this, click **Go To** on the **Commands** menu or press F4 on the keyboard, then select the **Zero Order** check box.
- 13** Check that the beam is not clipped. It may help to hold a piece of white paper in front of the apertures, so that you can clearly see the image. You can use the aperture mask to draw the apertures on the paper first. You may need to read just the three height adjustment screws on the cell holder base to ensure that the beam is unimpeded.

### Fine alignment

#### To perform fine-alignment:

- 1** When the accessory is aligned, place the accessory back on the cell holder and secure the accessory using the thumb or hex screw.
- 2** Close the sample compartment lid.
- 3** Repeat Steps 1 to 5 in the above section.
- 4** Click the **Start** button and click **Top and Bottom** in the Auto Double Aperture field.
- 5** Note the %T reading (at the top left of the screen).
- 6** Click **Top** aperture, then adjust the height screws on the cell holder base until the %Trans reading on the screen is half ( $\pm 1\%$ ) of the reading noted in Step 5.
- 7** Click **Bottom** aperture and adjust the height screws again until the %Trans reading is half ( $\pm 1\%$ ) of the reading with both apertures open (the reading noted in Step 5).

### Reference beam attenuation



**Figure 4.** Place a microscope slide on the holder in the reference beam

#### **If you are performing measurements in the *visible* region:**

- 1 Install the attenuator holder supplied with the Double Aperture accessory in the reference beam onto the cell holder base at the back of the sample compartment.
- 2 Use the thumb or hex screw to secure the attenuator holder.
- 3 Place one of the microscope slides on the holder rotate the aperture mask so that both apertures are open.
- 4 Close the sample compartment lid and note the %Trans reading. If it is not  $100\%T \pm 1\%$ , adjust the angle and/or number of the microscope slide(s) until this is achieved. You can use more than one glass slide.
- 5 Click **Go To** on the **Commands** menu and enter the wavelength you are going to use for the measurement.

#### **If you are performing measurements in the *UV* region:**

- 1 Drive the instrument to 0 nm.
- 2 Open the sample compartment lid and install the attenuator holder supplied with the Double Aperture accessory in the reference beam.
- 3 Place the V-shaped attenuator on the holder.
- 4 The image should fall on the V-shaped attenuator. Slide the attenuator across the beam until the V shape clips both the top and bottom of the beam.
- 5 Close the sample compartment lid and set the instrument to the wavelength you are going to use.
- 6 Note the %Trans reading. If it is not  $100\%T \pm 1\%$ , adjust the position of the V-shaped attenuator until this is achieved.

### **Validation and optimization software**

The Cary WinUV software contains a suite of software tools to check the performance of your instrument. Double-click the Validate icon in the Cary folder to start the software.

Refer to the Validate Help for details on its use.

## Instrument specifications

Using the Double Aperture technique, the specifications for photometric accuracy for the Cary spectrophotometers are:

### **Cary 1E, 100, 3E and 300**

- $\pm 0.0006$  Abs at 0.3 Abs (50%T), 190–900 nm, 2 second SAT
- $\pm 0.0012$  Abs at 1 Abs (10%T), 190–900 nm, 2 second SAT

### **Cary 4E, 400, 5E, 500 and 500i**

- $\pm 0.0003$  Abs at 0.3 Abs (50%T), 190–900 nm, 2 second SAT, PMT detector
- $\pm 0.0006$  Abs at 1 Abs (10%T), 190–900 nm, 2 second SAT, PMT detector

### **Cary 4000, 5000 and 6000i**

- $\pm 0.0003$  Abs at 0.3 Abs (50%T), 190–900 nm, 2 second SAT, PMT detector
- $\pm 0.0006$  Abs at 1 Abs (10%T), 190–900 nm, 2 second SAT, PMT detector

This information is subject to change without notice.



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