

UV/Vis/NIR Spectroscopy

Authors:

Frank Padera

PerkinElmer, Inc.
Oak Brook, IL 60522 USA

Christopher Lynch

PerkinElmer, Inc.
Shelton, CT

High Absorbance Scanning with the PerkinElmer LAMBDA 850 UV/Vis and LAMBDA 950/1050 UV/Vis/NIR Spectrophotometers

Introduction

In materials research there is sometimes a need to scan high absorbance samples such as laser protection lenses, optical filters, and polarization materials. Such sample types often need to be measured across the whole UV, Vis, and NIR ranges of the electromagnetic spectrum.

Accurate high absorbance scanning requires a high performance UV/Vis/NIR spectrophotometer with double grating and double monochromator. The stray light level of a spectrophotometer is key to high absorbance scanning and is determined by the quality of the instrument gratings.

Table 1. Equivalent absorbance (A) for a given percent transmission value (%T).

| %T | Absorbance |
|----------|------------|
| 100 | 0 |
| 10 | 1.0 |
| 1.0 | 2.0 |
| 0.1 | 3.0 |
| 0.01 | 4.0 |
| 0.001 | 5.0 |
| 0.0001 | 6.0 |
| 0.00001 | 7.0 |
| 0.000001 | 8.0 |

For high absorbance scanning, the user will need to correlate the stray light specifications of the spectrophotometer to the required sample absorbance levels. For example, if a sample needs to be verified at 6 A, and the spectrophotometer stray light specification is at 0.001 (5 A), the experiment will yield poor results as the stray light levels exceed the target range of the sample.

This application note describes the procedure for high absorbance scanning with the reference grade PerkinElmer LAMBDA™ 850 UV/Vis and LAMBDA 950/1050 UV/Vis/NIR double monochromator spectrophotometers.

For full technical data on the LAMBDA 1050 and LAMBDA 950 UV/Vis/NIR and the LAMBDA 850 UV/Vis spectrophotometers, see specification sheets. The LAMBDA 1050 with its InGaAs detector includes 1%, 0.1% attenuation making very high absorbance measurements across the full range of the instrument simple. The LAMBDA 850 and 950 include 10%, 1% internal attenuation capability as standard.

Experimental considerations

The success of scanning high absorbance materials depends on a number of factors.

Available energy

As lamps age, they produce less energy, and the dynamic range of the instrument will be reduced. Make sure the lamps have recently been replaced.

Age of instrument

As a spectrophotometer ages, the optics surfaces collect display oxidation, and/or dust and grime that will cause light beam scatter and reduced dynamic range.

Wavelength range

At the extremes of the wavelength scale, energy is typically lower and a lower OD range can be expected.

Slow scan speeds (high integration times) are required

1-2 second integration times are recommended. Scanning speed in the NIR is improved with an InGaAs detector reducing the time it takes to generate data for high absorbance measurements.

Baseline correction at 0 %T is required

Use of wider bandpasses is recommended

Allowing the highest energy throughput for a given measurement is always best practice. However, using higher resolution for a measurement than the sample type or specification required only adds to the time necessary to generate data with no value added to the measurement.

Supplemental reference beam attenuation may be required

Attaining absorbance levels in excess of 8 A may require additional magnetic screens.

The standard instrument detector is required

For high absorbance measurements, the optical detector unit of the basic spectrophotometer is recommended in most cases.

Reference beam attenuation

For high absorbance measurements, reference beam attenuation is almost always needed. Additional attenuation screens can be added if a particular response is needed.

Reference attenuation

To improve the accuracy and signal-to-noise ratio of spectra measured at high absorbance (> 5 A), reference beam attenuation is recommended. When measuring high absorbance samples, the photomultiplier voltage for a UV/Vis measurement needs to be set very high in order to differentiate between the very low light levels on the sample beam (i.e., 0.0001 %T), and a blast of energy (i.e., 100 %T) on the reference beam. Reference beam attenuation involves placing a screen or neutral density filter in the reference beam before scanning the sample. By better equalizing the energy between the sample and reference beams, the photomultiplier voltage is lowered and the signal-to-noise ratio of the spectrum is improved.

When an attenuator (or 'screen') is placed in the reference beam, the absorbance reading will be reduced by exactly the absorbance amount of the screen. For example, if a reading of 6 A is observed, and a 1 %T (2 A) screen is placed into the reference beam, the observed absorbance will now be 4 A. More importantly, the noise level of the spectrophotometer will be reduced to the approximate level seen at 4 A rather than at 6 A.

Reference beam attenuators are typically perforated neutral density screens or neutral density filters, and are available in a variety of sizes and shapes. Multiple reference beam attenuators can be used to produce a specific level of attenuation in the reference beam. Note that screens should not be adhered together – one screen should be placed on the left side window, and the other on the right side window. A representation of reference beam attenuation is shown in Figure 1.

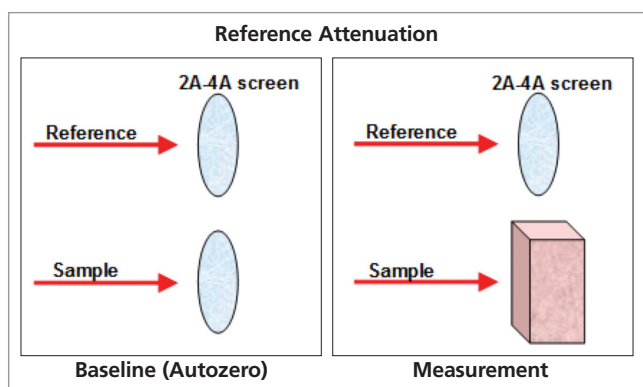


Figure 1. A representation of the principle of reference beam attenuation. A screen is added to the reference beam path, and an autozero performed. For the measurement, the screen is left in place in the reference beam, and the sample is placed in the sample beam and scanned.

Recommended levels of reference beam attenuation

To understand how much attenuation is being applied refer to the attenuators levels in absorbance units:

10 %T = 1 A

1 %T = 2 A

0.1 %T = 3 A

0.01 %T = 4 A

The recommended beginning levels of attenuation for sample absorbance levels on the LAMBDA instruments are shown. Note, the LAMBDA 1050 has built-in attenuation up to 0.1% making measurements 7-8 A possible with no additional attenuation. The LAMBDA 950/850 typically require additional attenuation to be used above 7 A as stated. The PerkinElmer attenuator kit (Part No. L1160560) includes five screens ranging from 32% down to 1%.

0-4 A (no attenuation needed)

5-6 A (1 %T built-in reference beam attenuator)

6-7 A (1 %T built-in reference beam attenuator, plus additional 1-1.5 A screen – PerkinElmer kit 6 %T screen)

7-8 A (1 %T built-in reference beam attenuator, plus additional 2 A screen – PerkinElmer kit 1 %T screen).

If some non-linearity and spectral artifacts are observed, reduce the level of attenuation slightly by using a less opaque screen selection.

Creating a reference beam attenuation scan method on the LAMBDA spectrophotometers

A scan method will need to be created which incorporates the essential principles described above.

Click on **Data Collection**. Enter your scan range. Enter the data interval. A value of 1 or 2 nm is suggested. For a 1 nm data interval, the approximate maximum scan speed is 30 nm/min. For a 2 second integration time and for a 2 nm data interval, the approximate maximum scan speed is 60 nm/min. The ordinate mode can be A or %T and all optical setup parameters such as slit, detector gain, integration time and attenuation can be set.

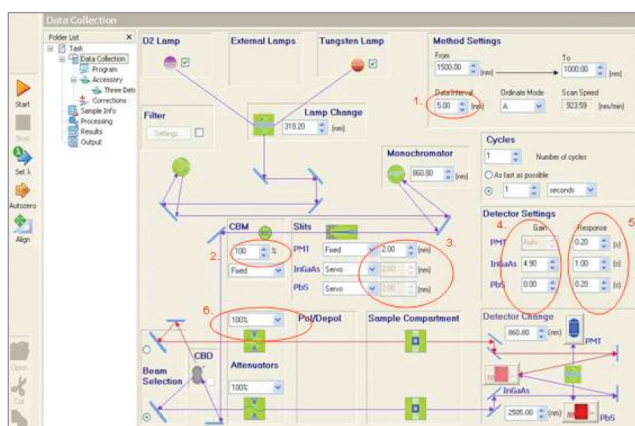


Figure 2. Data Collection Window.

In particular, make sure that:

The data interval is set to 1.00 or 2.00 (the larger the data interval, the faster the scan speeds allowed).

The Common Beam Mask (CBM) is set to 100%.

The UV/Vis slit is set to 2 or 4 nm.

The NIR detector sensitivity is set to 1 for PbS or 6-10 for InGaAs as a start.

The integration times for the UV/Vis and the NIR ranges are set to 1.00 seconds.

The reference beam attenuator is set to 1 %T.

Click on the **Corrections** menu. Make sure the box is checked for 0 %T, as shown.

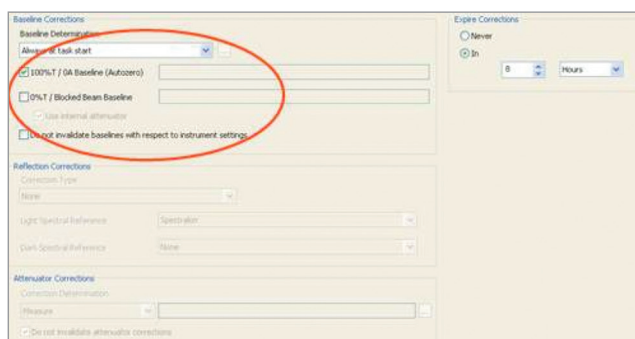


Figure 3. Corrections Display Window.

Click on the **Sample Info** menu and enter your sample information, as shown.



Figure 4. Graph Properties section show circled.

Next, click on the **Graph Properties** button (circled) and for an ordinate of A, enter a scale of 8 for the top and 0 for the bottom (for an ordinate of %T, enter 0.01 for the top, and 0 for the bottom). Uncheck the Autorange on new data option.

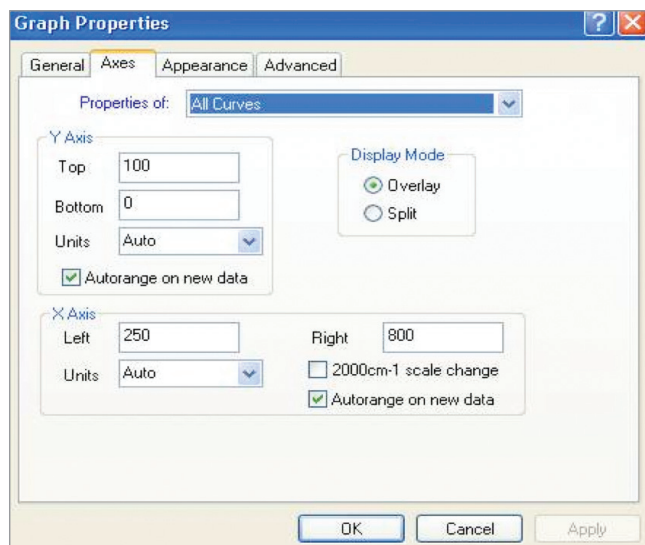


Figure 5. Graph Properties selections available.

Click on **File**, select **Save Setting**, the **As New Method**. Call the method **Scan – High Absorbance**.

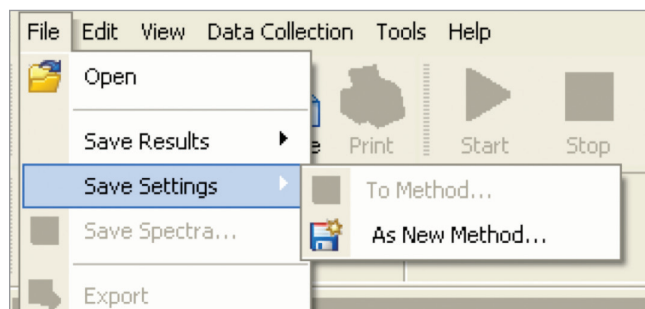


Figure 6. Method information can be stored.

Before running the method, add any additional attenuator(s) to the reference beam. For measuring the highest absorbance levels, the goal is to provide total reference beam attenuation between 3.5 A and 4 A. Since the motorized reference beam attenuators have already been set to 1 %T in the method just saved to disk, an additional 1 %T - 4 %T reference attenuation screen is required.

Attach the 1 %T or 4 %T screen to the left side window of the back reference beam.

The method is now ready to be run. Click on the **Start** button on the top toolbar.

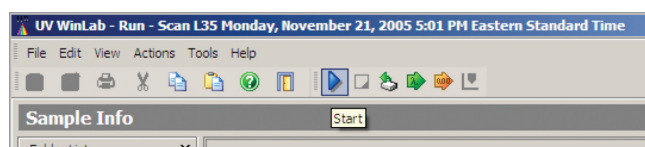


Figure 7. Click the Start icon to scan sample.

You will be prompted to perform a **Background Correction**. Click **OK**.

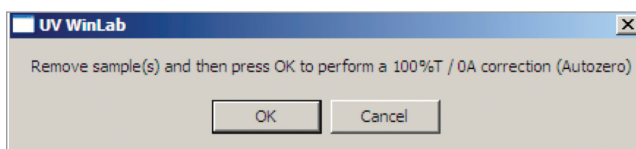


Figure 8. System will request action to be taken.

Do not lift the cover during this process. Remember, both a light (100 %T) and a dark (0 %T) correction is being performed.

When the background is completed, you will be prompted next to run your sample. Insert the sample at the sample holder position. If it is a solid sample such as a filter, make sure the sample is placed normal to the beam, and is not angled in any way. Click OK to start the scan.

If the screen is autozeroed, the data will automatically be corrected for the amount of the reference attenuation. Shown in Figures 9 and 10 is an example of the benefits of using reference beam attenuation. Didymium filters of increasing absorbance were measured, with and without reference beam attenuation. With attenuation, the peak at 525 nm can be accurately quantified.

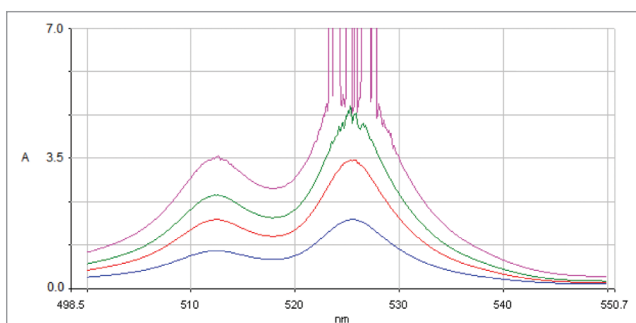


Figure 9. Didymium filters scanned on the PerkinElmer LAMBDA 950 without reference beam attenuation (note – in this example faster scan speeds were used to promote noise).

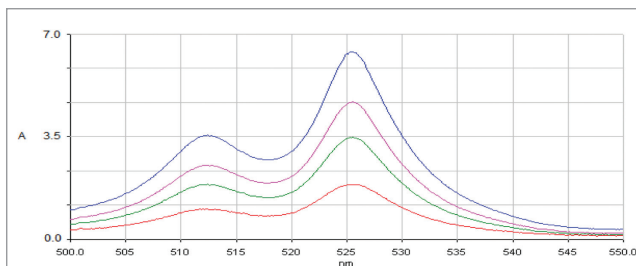


Figure 10. Didymium filters scanned on the PerkinElmer LAMBDA 950 with reference beam attenuation.

The LAMBDA 1050 with its improved performance in the NIR range can provide high absorbance NIR measurements not possible with a conventional Lead Sulfide (PbS) detector alone. Typically a spectrophotometer equipped with just a PbS detector scanning at very long integration times on the order of 6-10 seconds per data point can achieve approximately 6A in the NIR range.

The addition of an InGaAs detector in the NIR region will greatly improve the measurement of high absorbance materials and will allow less demanding samples to be scanned at much higher speeds with better signal-to-noise performance. Figure 11 below shows a scan of a highly absorbing safety lens in the NIR region taken with the LAMBDA 1050 using an InGaAs detector. The system utilized servo slit mode and 0.1% reference beam attenuation in the method setup.

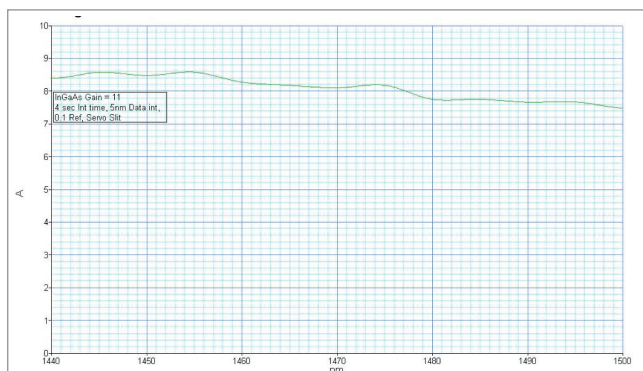


Figure 11. The spectra of a safety lens with very high absorbance in the NIR region measured with the InGaAs detector on a LAMBDA 1050.

Pitfalls to watch for

If the sample is not reaching the optical density expected, consider a number of factors:

Measuring absorbance between 6A and 8A demands a clear sample. The smallest haze or scatter present in the sample will degrade the optical density.

Ideally, the sample should have perfectly parallel surfaces. If a sample has some curvature, position it at its flattest spot.

Any beam displacement on the detector can cause the optical density to be degraded. If possible, do not measure at the edges of the sample, where beam aberrations can occur.

Increasing the level of reference attenuation does NOT increase optical density of a sample.

Increasing reference attenuation lowers the noise level and increases precision of the measurement. It is, however, possible to over-attenuate the reference beam energy and prevent enough light to calculate a reference signal. Generally, it is not recommended to exceed a total attenuation of 4 A, but where circumstances require, the results should be carefully validated.

Conclusion

Reference beam attenuation is a simple technique that can be incorporated into a spectroscopic method to extend the dynamic range for high absorbance samples. Although reference beam attenuation is a principle that can be used to improve the performance of any spectrophotometer the resulting benefit is limited by the stray light specification of the instrument being used.

Note:

Combining the reference beam attenuation principle with the ultra-low stray light levels of the LAMBDA 850/950/1050 double grating, double monochromator spectrophotometers gives accurate measurement to 8 absorbance (or higher). The high sensitivity InGaAs detector used on the LAMBDA 1050 improves the photodynamic range needed for high absorbance measurements in the NIR region and can be configured in minutes to optimize the full UV/Vis/NIR range for analyses typically encountered by the optical filter and safety glass industries.