Acquisition of High Quality Transmission Spectra of Ultra-Small Samples

Using the LAMBDA 950 UV/Vis/NIR and LAMBDA 850 UV/Vis Spectrophotometers

Introduction

The measurement of very small samples in transmission mode often poses problems. Large-scale losses of energy, scattered light and high backgrounds all impose severe performance requirements on UV/Vis and UV/Vis/NIR instruments. Using small beam apertures can lead to high background absorbance levels, often well over 2A.

The LAMBDA 850 and 950 spectrophotometers have a specified dynamic range to +/-8A. Even if 2A of dynamic range is sacrificed by aperturing, a wide dynamic range of 6A is still available.

Many small solids have non-uniform, non-parallel surfaces which can cause severe deviation and/or scatter of the transmitted beam. This kind of sample cannot be accurately measured with a normal instrument transmission detector.

Integrating spheres, on the other hand, are detectors designed to measure light at a broad angle (Figure 1). Because samples are placed directly at the transmission port of the integrating sphere, all light that is transmitted, refracted or scattered is collected and accurately measured.

Also, measurement of small samples requires that the instrument light beam is restricted to the size of the sample to ensure representative measurement. This is typically accomplished by adding either a beam aperture, which is easy to fix to the transmission port of the integrating sphere, or by adding a beam condenser accessory, which is installed in front of the sample.

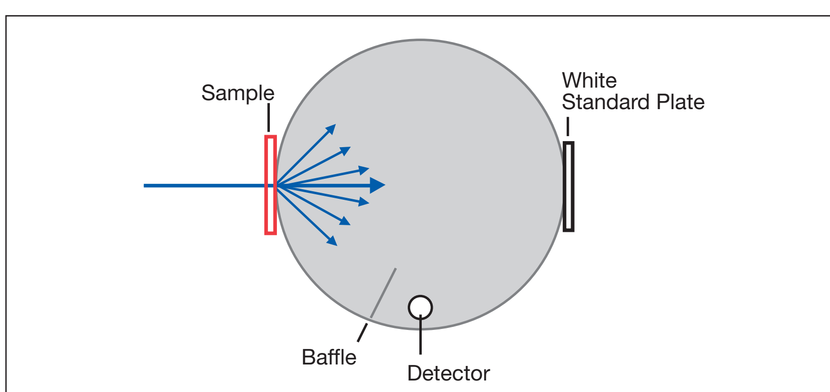

Figure 1. Integrating sphere for transmission measurements.

Beam Apertures or Condensers?

There are two common ways to reduce the beam size for measurement of small samples: **beam apertures** and **beam condensers.** Each has advantages and disadvantages (Table 1).

Beam apertures

These are small black anodized metal plates with small holes or slits cut into them. The size of the holes or slits can range from 1 mm to 5x5 mm in diameter. The apertures can either be purchased or custom made. PerkinElmer offers five apertures (Figure 2.) (Note: For the slit apertures, the 10 mm length can be reduced with black electrician's tape).

The custom made aperture material should be as thin as possible, yet stiff enough not to deform in use. Spraying the aperture with a matte black paint prevents unwanted reflections. The choice of aperture depends on the size of the sample which needs to be slightly larger than the hole or slit of the aperture. This ensures that stray light not actually transmitted through the sample will be blocked from entering the integrating sphere. The beam aperture should be mounted directly on the entrance port wall of the integrating sphere, centering the aperture to maximize the light beam intensity. To position the masks, the spectrophotometer is set to 0 (zero order), where bright white light is visible. Once the aperture is in place, a background correction is performed for 100 %T or 0A over the wavelength range.

It is imperative that the mask is not moved during the background process or scanning of analytical data. If the mask is moved, the instrument baseline will have to be re-measured. Once the baseline is acquired, the

	Apertures	Condensers
Advantages	Low cost	Use full beam energy
	Full wavelength range	Beam spot tunable
	Many sizes available	No mask required
		Small spot measurement of large samples possible
Disadvantages	Attenuate energy	More expensive
	Sample positioning critical	Lens material may limit wavelength range
	Measuring small spots on large samples difficult	

Table 1. Beam apertures vs. condensers

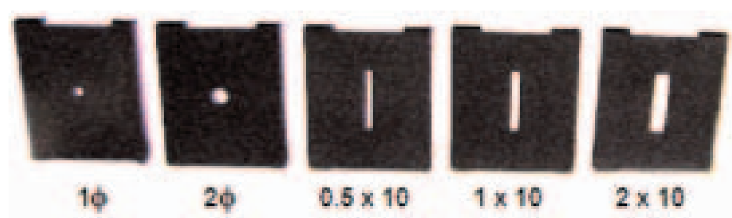

Figure 2. Beam apertures (sizes in mm).

sample is placed at the hole or slit of the aperture for measurement.

A convenient way to adhere very small samples is with a small amount of thin double-sided film tape which is layered around the aperture hole or slit. Samples can then be securely placed using tweezers directly on the aperture opening for the measurement.

Note that the smallest aperture masks (1 mm in diameter) can attenuate the sample beam to 2A or greater. It is usually helpful to use reference beam attenuation in these instances to improve the signal-to-noise of the spectra.

The Lambda 950 and 850 spectrophotometers have motorized reference beam attenuators, which can be inserted and removed automatically via software. Reference attenuators act to balance the energy

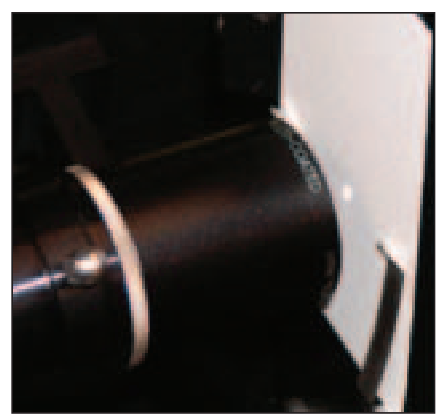

Figure 3. 1 mm beam spot using the condenser.

of the reference beam so it more closely matches the energy level of the sample beam. In doing so, photomultiplier gain is lowered, and noise levels are reduced.

Beam condensers

Another way to reduce the beam size in the measurement of small samples is to use a **beam condenser**.

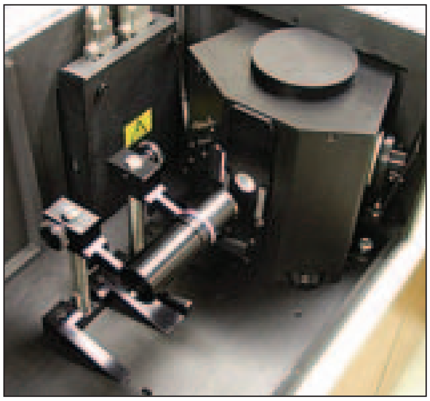

Figure 4. Beam condenser fitted in the cradle holder.

This accessory reduces the normal beam size to a sharply focused point of light. Beam condensers can have different focal lengths producing different spot sizes on the sample. The beam condenser is positioned in front of the sample and focused to optimize the spot size of the light beam to 1 mm or less. (Figure 3).

A background correction is performed, and the sample inserted and measured. If the sample is larger than the beam spot, no masking is required.

The advantage of a using a beam condenser rather than a beam aperture is that the full energy of the original beam is retained and focused through the sample, often without the need for any further masking. The signal-to-noise ratio of data obtained with a beam condenser will therefore be much higher than that obtained with a beam aperture.

A disadvantage is that the lens composition may limit the wavelength range of the measurement (for example, glass lenses cannot be used below 340 nm).

PerkinElmer's beam condenser (part number PELA9048) contains a wheel with three lenses of different focal lengths. This can be used with the 150 mm integrating sphere accessory. A custom beam condenser consisting of a Meade multi-coated lens and a long path cradle type holder (part number PELA1005) is shown in Figure 4.

Method and Results

Small gemstones present a challenge for accurate transmission measurement. In addition to their small size (1 mm to 2 mm), gemstones have multi-faceted surfaces and a high refractive index. A number of natural white diamonds, a natural yellow diamond, and a synthetic blue sapphire were obtained for testing. Figure 5 shows the relative size of the stones compared to a pen point. Both the beam aperture and the beam condenser configurations were used. The 1 mm beam aperture mask was used, as illustrated in Figure 6.

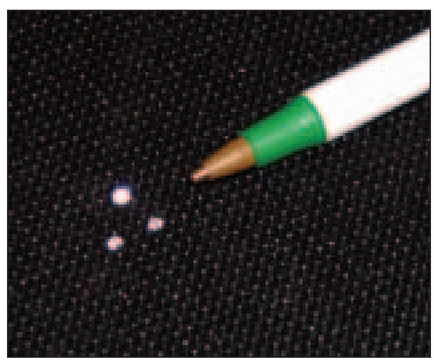

Figure 5. Small diamonds (approximately 1- 2 mm diameter).

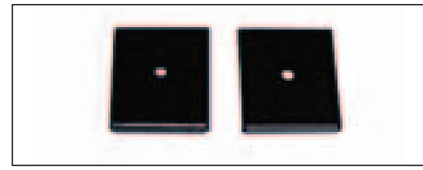

Figure 6. Small diamonds on a mm apertures.

After fitting the aperture mask to the entrance port of the integrating sphere, the background absorbance was observed to be above 2A. A 1%T reference beam attenuator was selected and used for all measurements with the apertures. Spectra were scanned between 34 nm and 800 nm at 250 nm/min, with a 2 nm slit width, 0.2 second integration time. The corrected baseline with the 1 mm aperture and 1%T reference beam attenuator is shown in Figure 7. The corrected baseline using the beam condenser is shown in Figure 8. Because a beam condenser delivers most of the original energy from the sample beam, the noise levels using this accessory are much lower. However, even using a 1 mm beam aperture, the noise level is less than +/- 0.002A over much of the spectrum.

The spectrum of a white natural diamond is shown in Figure 9, overlaid with the spectrum of a natural yellow diamond. The

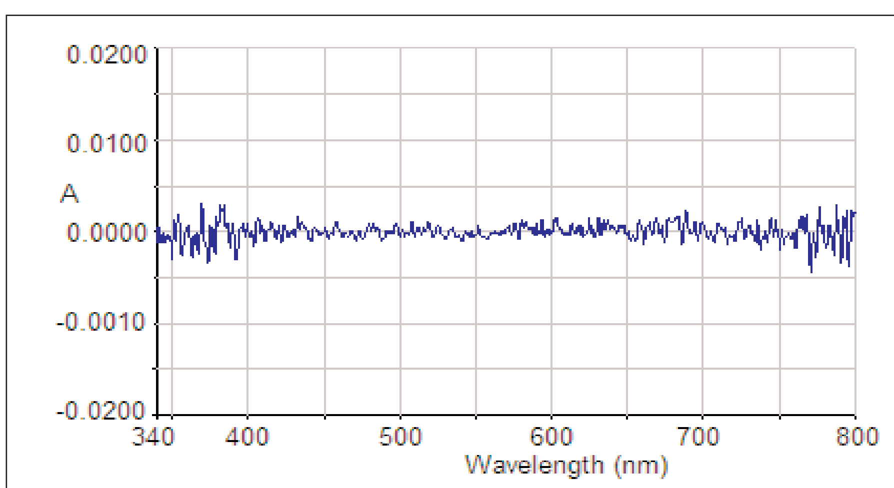

Figure 7. Baseline using a 1mm aperture and 1% T Reference Beam Attenuator.

spectra were normalized at 800 nm. The significant absorption bands observed with the yellow diamond are normal, and are caused by trace impurities of nitrogen.

A synthetic medium blue sapphire gemstone was measured under the same instrumental conditions, using a beam condenser which produced an approximately 1 mm beam spot on the sample. Sapphire is the blue variety of corundum, an aluminum oxide mineral (ruby is the red form of corundum). Sample beam attenuation was not necessary in this case, as the beam spot was smaller than the sample. The absorbance spectrum of synthetic blue sapphire is shown in Figure 10.

Conclusion

The experimental data and procedures presented in this application note demonstrate the acquisition of high quality transmission spectra of ultra-small samples, approaching 1 mm in diameter.

The PerkinElmer LAMBDA 950 and 850 spectrophotometers, fitted with either beam apertures or beam condensers, are ideal instruments for analyzing these difficult samples, because of their large dynamic range and built-in reference beam attenuation systems. The 150mm integrating sphere accessory is adaptable for the measurement of small samples, either using an aperture mask or a beam condenser lens.

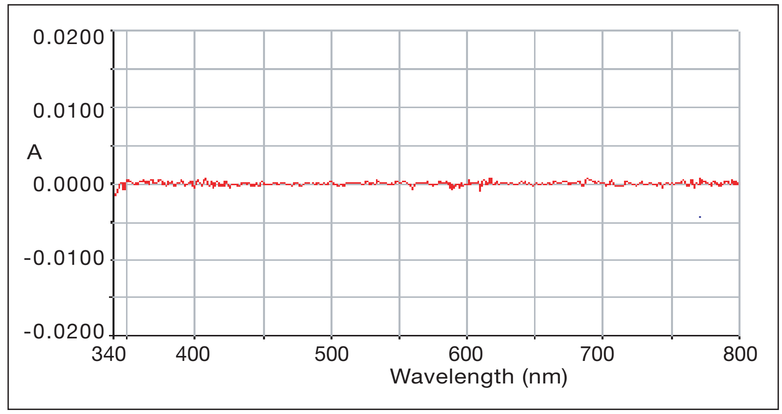

Figure 8. Baseline using a beam condenser.

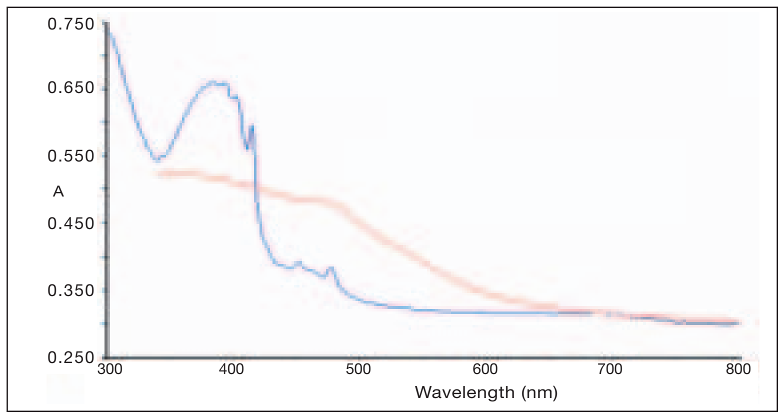

Figure 9. Absorption spectra of a natural white diamond (red trace) and a natural yellow diamond (blue trace).

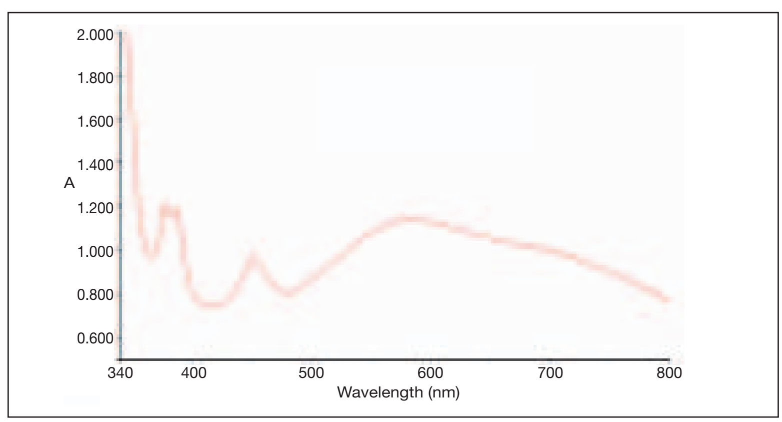

Figure 10. Absorbance spectrum of synthetic blue sapphire.

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