UV-Vis-NIR Spectrophotometer Quick Start Guide

The following instructions are provided as a Quick Start Guide for powering up, running measurements, and shutting down the Lambda 950 UV-Vis Spectrophotometer.

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Introduction

Perkin Elmer's Lambda 950 is an ultra-high UV-Vis-NIR Spectrophotometer that has an operating range of 175 – 3000 nm with a tolerance of .1 nm. By measuring the deviance in light at specific wavelengths, the device can be used to conduct a spectroscopic analysis of sample materials. This spectrophotometer is primarily used for measuring the intensity of light absorbed or reflected off of a sample within the Ultraviolet to Near-Infrared range. In its leftmost compartment, the device can hold cuvettes in a front and back reference holder. In its rightmost compartment, the presence of an integrating sphere allows for high precision reflectance and scattered transmittance measurements on virtually any solid or liquid.
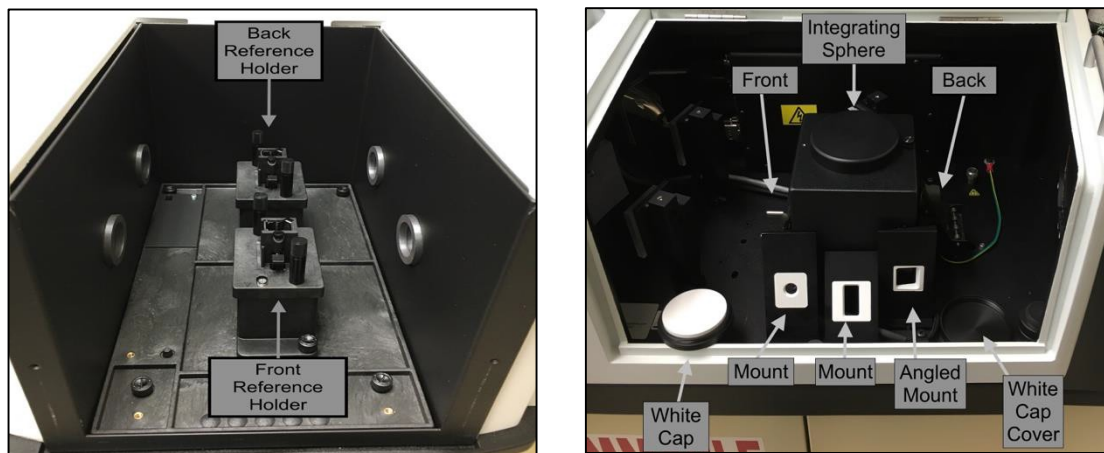

Figure 1: The physical layout of the Lambda 950 UV-Vis. The left image illustrates the reference holders in the left compartment. The right image shows the right compartment containing the integrating sphere for physical sample analysis along with various mounting equipment for light path dictation.

Instrument Setup

1. Log into the computer and turn on the Spectrophotometer. User
   Name: Student
   Password: P@ssword1
2. Check for any potential obstructions in the light path for both compartments pictured above in Figure 1.
3. Turn on the UV-Vis system using the green power toggle switch located on the top of the instrument housing in the back-right corner, and let the system stabilize for 20 minutes.
4. Open Perkin Elmer UV Win-Lab on the Desktop, and click OK if asked for password.
5. Double Click on *Scan –Lambda 950*, then *Data Collection* to adjust the measurement parameters
   a. Method Settings Scanning Range: Range of 3000 nm to 175 nm
   b. Method Settings Data Intervals/Speed: How much data to be received
   c. Cycles: Number of Scans/Interval
   d. Monochromator: Wavelength of pure light desired
   e. Slits: Adjust the slit size
   f. CBM: Adjusts the beam height to match different dimension samples.

g. CBD: Beam correction for instrument polarization in birefringent samples.
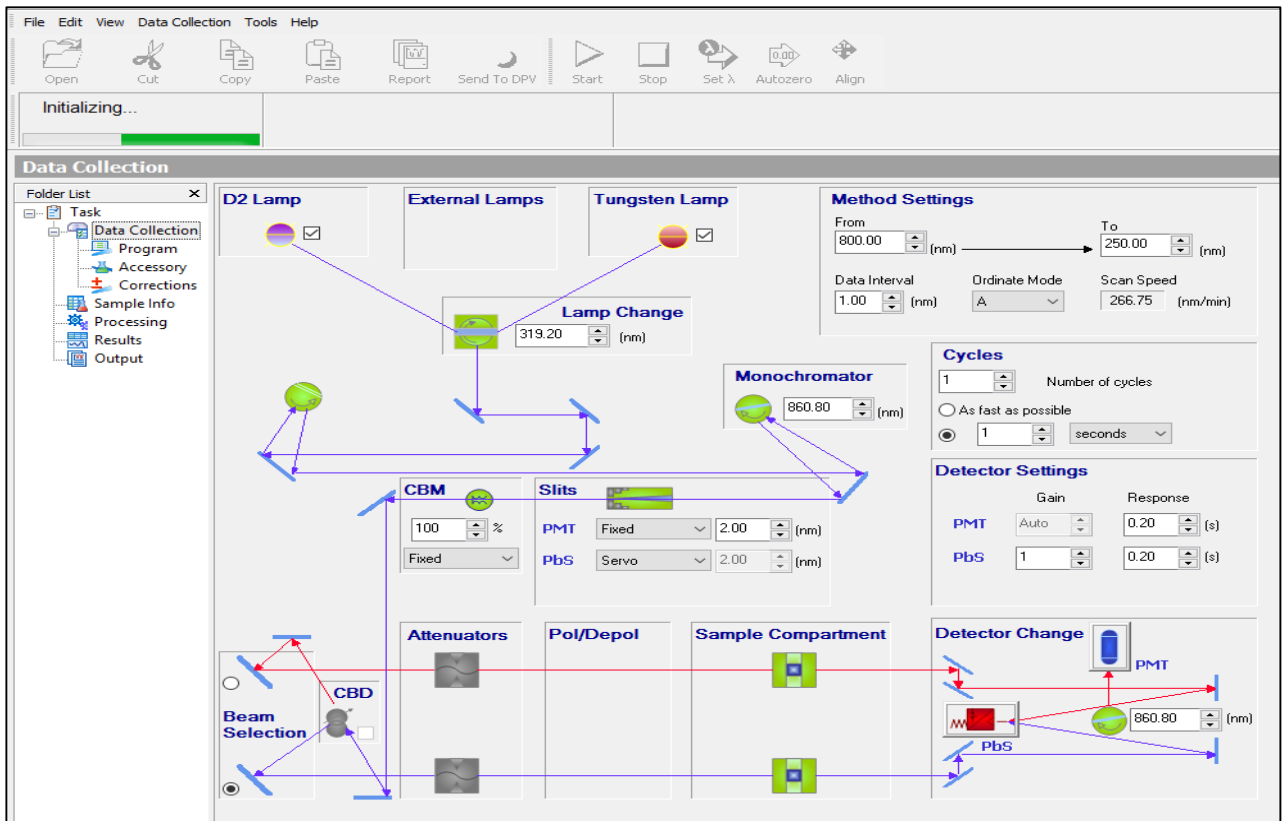h. Attenuators: Adjust when measuring on high absorbing samples.

Figure 2: The menu for setting the parameters for operations on the Lambda 950.
Some settings may not be available for modification in all menus.

Reflectance Setup

The Reflectance method is a technique used for non-invasive analysis of a samples surface through its spectral response.

Reflected light off of samples can give an insightful look into the composition of materials.

1. Install the Angled Sample Mount in the back location shown in Figure 1.
2. Mount the Spectrolon coated White Cap behind the Angled Sample Mount and secure it using the spring clamp with the angled wedge pressing against the back of the White Cap as shown in Figure 3.
3. Ensure proper beam alignment by using the *Align* icon in the top menu bar.
   a. To do this, select the *Align* icon and observe the spot location as shown in Fig 2.
   b. Deselect the *Align* icon when finished.
4. After running a baseline scan with the Spectrolon coated White Cap, this cap will be replacing your sample for analysis.
5. Proceed to the section labeled *Running a Scan* to complete the baseline analysis before loading the sample.
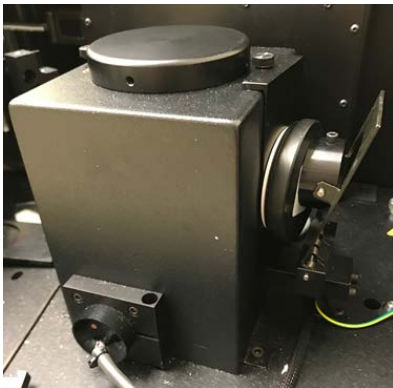

Figure 3: Securing the White Cap against the Angled Sample Mount.

Transmission Setup

The Transmission method is a technique that studies light that has been transmitted through a sample. Interrelated to absorption, transmission spectroscopy can be used for determining the presence and/or concentration of a target material.

1. Remove all mounting shims from the rear of the Integrating Sphere.
2. Mount the Spectrolon coated White Cap behind the Integrating Sphere and secure it using the spring clamp as shown in Figure 3.
3. Ensure proper beam alignment by using the *Align* icon in the top menu bar.
   a. To do this, select the *Align* icon and observe the spot location as shown in Figure 2.
   b. Deselect the *Align* icon when finished.

Notice: A baseline scan without a sample in the beam path must be completed before a sample is loaded for transmission analysis. Please proceed to the section labeled *Running a Scan* to complete the baseline correction before mounting your sample.

Cuvette Setup

Cuvettes are primarily used to analyze liquid samples within the spectrophotometer. They are typically made from plastic, glass, or quartz. Plastic and glass cuvettes are useful for taking measurements in the visible light wavelength. Quartz cuvettes are used in the UV-NIR range.

Figure 4: The front reference holder with the cuvette adjustment knobs.

1. Remove all mounting shims from the rear of the Integrating Sphere.
2. Mount the Spectrolon coated White Cap behind the Integrating Sphere and secure it using the spring clamp with the flat face pressing against the back of the White Cap as shown in Figure 3.
3. Fill both cuvettes with the buffer solution and load them into the cuvette holders.
4. Ensure proper beam alignment by using the *Align* icon in the top menu bar.
    a. To do this, select *Align* and observe the spot location as shown in Figure 1.
    b. You may find it helpful to place a piece of paper behind the cuvette to observe the light passing through the cuvette. Adjust the cuvette's position using the adjustment knobs shown in Figure 4.
    c. Deselect the *Align* icon when finished.
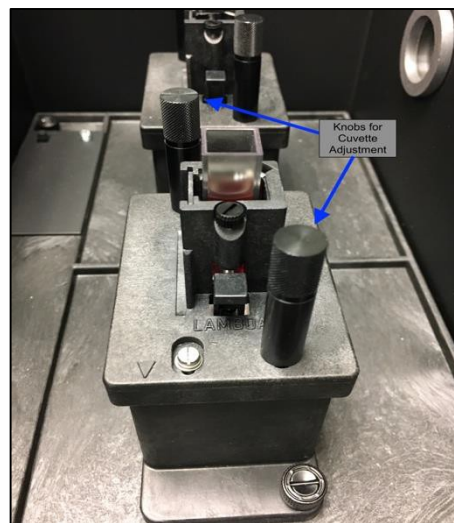5. Proceed to the section labeled *Running a Scan* to complete the baseline analysis before loading the sample.

Notice: It can be helpful to attenuate the reference beam path in high absorbance materials so that the reference signal does not swamp the desired signal for analysis. Note the attenuator setting in Figure 2

1) Enter the *Data Collection* tab in the software.
2) Set the attenuator on the reference beam path to 10%

Running a Scan

This section shows the user how to acquire data regardless of the current scanning method chosen. The first scan that the system will perform is a baseline correction scan. This scan is performed without a sample loaded (except in the case of cuvettes where the buffer solution should be added to cuvettes in both the sample and reference beam paths).

1. Navigate to the *Sample Info* tab and enter the total number of samples that you with to analyze along with sample names – the number and names of samples can be changed even after sample analysis has been started.
2. Ensure that the sample compartments are fully closed and select the *Start* icon in the top menu bar to start collecting the baseline scan and allow it to finish.
3. When prompted load your sample into the front reference holder or integrating sphere for analysis (for a cuvette replace the empty cuvette in the front reference holder with one filled with your sample while leaving an empty cuvette in the back reference holder).
4. Ensure that the sample compartments are fully closed and continue to start scanning.
5. Repeat and exchange the samples (when using cuvettes, only load samples in the front reference holder) after each scan until the analysis is complete.

Analyzing and Exporting the Data

This section shows the user how to analyze, format, and export their data after using the UV-Vis.

1. Format the graph to your specifications using the Format icons in the *Results* tab.
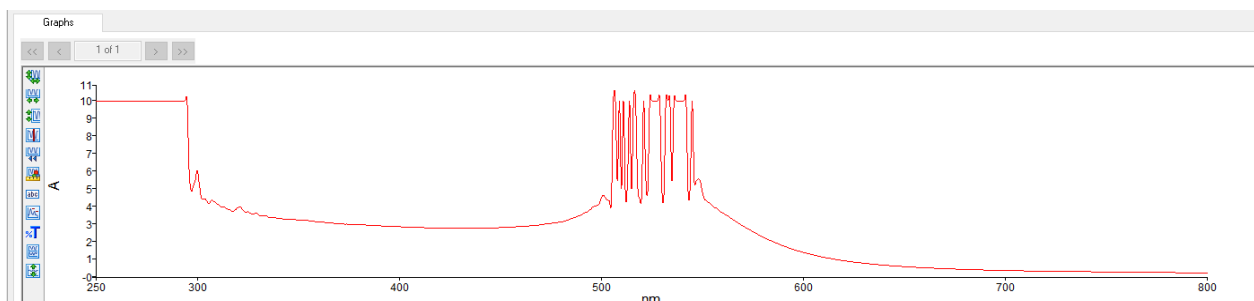

Figure 5: An example of data sampled by the UV-Vis between 250 to 800 nm.

2. Click on *Output* to customize and print and set the report parameters as in Figure 7.
3. Click on *Report* (middle-top of the screen) to print the report or save it as a file.
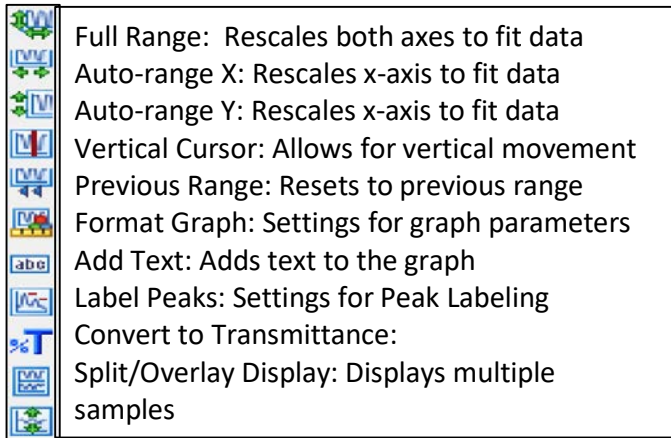
	Full Range: Rescales both axes to fit data
	Auto-range X: Rescales x-axis to fit data
	Auto-range Y: Rescales x-axis to fit data
	Vertical Cursor: Allows for vertical movement
	Previous Range: Resets to previous range
	Format Graph: Settings for graph parameters
	Add Text: Adds text to the graph
	Label Peaks: Settings for Peak Labeling
	Convert to Transmittance:
	Split/Overlay Display: Displays multiple samples

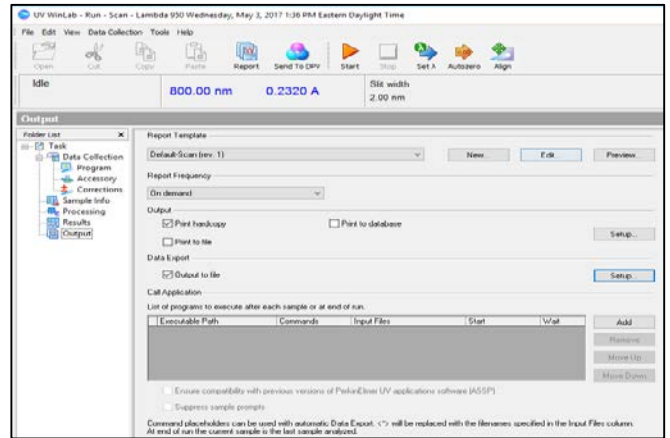Figure 6: The graph customization menu on the left side of the graph.

Figure 7: The output file report customization page.

Finishing Up

1. Exit out of the data collection and analysis software programs.
2. Remove all samples.
3. Remove and cover the Spectrolon coated white cap.
4. Turn off the power to the spectrophotometer using the toggle switch
5. Log out of the computer.

Notice: For problems or training information, visit labs.jhu.edu or explore Perkin Elmer's operation manual at goo.gl/ioh8rJ