Perkin Elmer Lambda 800 UV/Vis

Standard Operating Procedure

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1. Purpose

This standard operating procedure is intended as a summary of basic instrument operation, including safety, sample loading, instrument settings, cell cleaning, and sample disposal.

This SOP does not supersede the Perkin Elmer User Guides (hardware and software). The full user guides include theory, all cautions, and detailed explanations of parameters.

2. Overview

This SOP summarizes the steps to setup, acquire data, and export and process data using the Lambda 800 UV-Vis. Where specific examples are shown we default to transmission/cuvette operation.

3. Prerequisites

1. Use of the Perkin Elmer Lambda 800 UV/Vis requires:
2. Becoming a user of RSC at http://rsc.aux.eng.ufl.edu/
3. Requesting training through http://rsc.aux.eng.ufl.edu/ccb/resource.asp?id=130.
4. Reading the full instrument manual and this SOP.
5. Successfully completing hands on training under the supervision of RSC staff.

4. Safety

In room 239: Do not cross the yellow and black tape on the floor, this area is designated for mercury use. Should you need to enter this area, be sure to use the tacky floor mat before leaving to remove any traces of mercury.

Do not attempt to bypass any instrument safety locks.

Safety glasses should be worn whenever you or others are preparing liquid or powder samples or if the porosimeter is in operation. Safety glasses and gloves are supplied in room 239.

5. Procedure

5.1 Preparation:

5.1.1 Instrument Preparation

Turn on the UV-Vis system using the power toggle switch located on the top of the instrument housing in the back-right corner. System should be given approximately 30 minutes to stabilize before use, to allow lamps and detectors to stabilize before collecting data. You can turn on the system without being logged in to the TUMI station.

5.1.2 Instrument Purging

For best accuracy on measurements below 190nm and water vapor adsorption, the instrument should be purged with N_2. For analysis using N_2, users will need to make arrangements with RSC staff.

5.1.3 Sample Preparation: Cuvettes

Realize that for liquids or solids measured in transmission there are both absorbance and scattering phenomenon affecting the collected data. For best absorbance data the solution/sample should be transparent throughout the wavelength range. Recall, the maximum scattering occurs at flaws or particle sizes approximately ½ the wavelength being scanned.

DI water is supplied (via gray faucets) for dilution. Disposable polyethylene transfer pipets and kimwips are supplied in room 239 for use. Pipeters are available, but precision is not guaranteed as they are multi-user tools. Users are welcome to bring additional solvents/supplies for dilution; storage space in room 239 will be assessed on a case-by-case basis. See Section 7 for Waste Disposal.

5.1.4 Cuvette Selection

The RSC provides 4-sided polystyrene, 10mm path length cuvettes as well as 2-sided low volume (10mm path length) acrylic or polystyrene cuvettes. It is the user's responsibility to ensure chemical compatibility with the cells/cuvettes in use as well as wavelength range.
Polystyrene (PS): 340-800nm, Acrylic (PMMA): 280-800nm. (PS in rack look slightly blue, PMMA are clearer to slightly yellow)

- Cells should be held by the top edges to avoid fingerprints on the optical surfaces.
- Ensure there are no bubbles in the sample in the cell, bubbles will influence results.

5.1.5 Sample Preparation: Integrating Sphere

Integrating sphere samples need to be a minimum of 1-inch diameter to cover the sample port completely. No additional prep is required. Samples should not be loaded until after the alignment is complete. Contact RSC staff if you have powder samples or your sample does not meet the minimum dimensions.

5.1.6 System Startup

The desktop computer user UVV Administrator requires no password to log on. Open "PerkinElmer UV WinLab" on the Desktop (Figure 1). If you have just turned on the instrument, wait at least 1 minute before opening the UV WinLab software. Select Analyst from the user name selection dropdown (Figure 2).

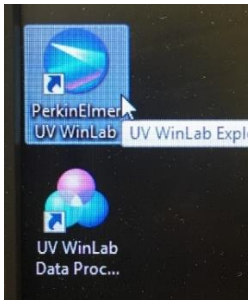

Figure 1: PerkinElmer UV WinLab program icon.

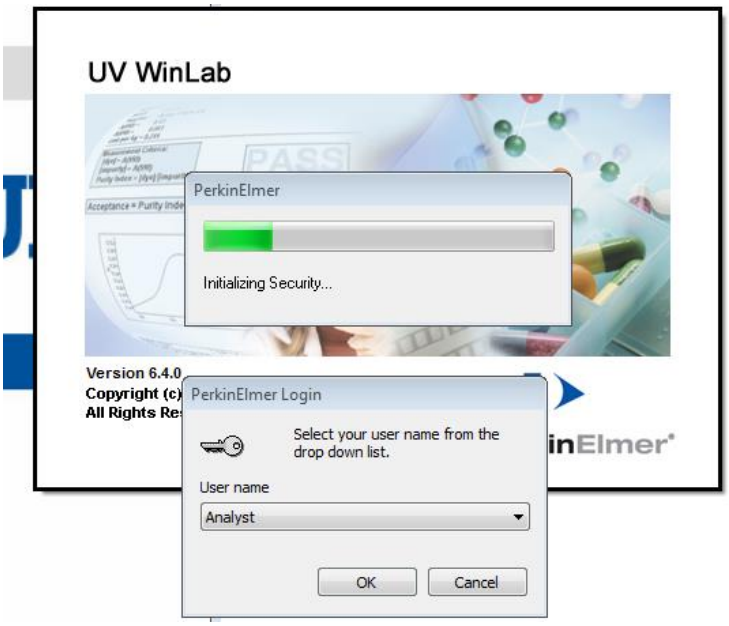

Figure 2: Startup of UV WinLab program.

See Page 42 of the software user guide for detailed explanations of buttons and basic software functionality. The Software guide is available for reference C:Users/Public/Public Documents/PerkinElmer

5.2 System Alignment:

RSC staff will attempt to keep the module status (Cuvette or Integrating Sphere) updated on the tool status page. If you need the module changed please contact a staff member to assist with the change.

5.2.1 Cuvette Loading and Alignment

- Fill two cuvettes with your base solvent (reference), cap them and load them into the cuvette holders (shown in Figure 3), close the lid to the sample compartment.

- With plastic cuvettes, the face of the cuvette (side with the arrow) should be facing to the left, towards the direction of the beam, as you insert.

- Only stoppered cuvettes should be loaded into the machine. If a spill occurs, blot immediately to avoid liquid damage to interior coating. Do not rub or scrub the black coating, do not try to remove any existing spots.
- With glass or quartz cuvettes, ensure orientation is preserved between calibration and analysis.
- Sample cell windows are Silica, an optical component, do not touch the windows, and do not attempt to clean the windows. Should a splash or spill occur on or near a window, optical lens wipes must be used for cleaning.

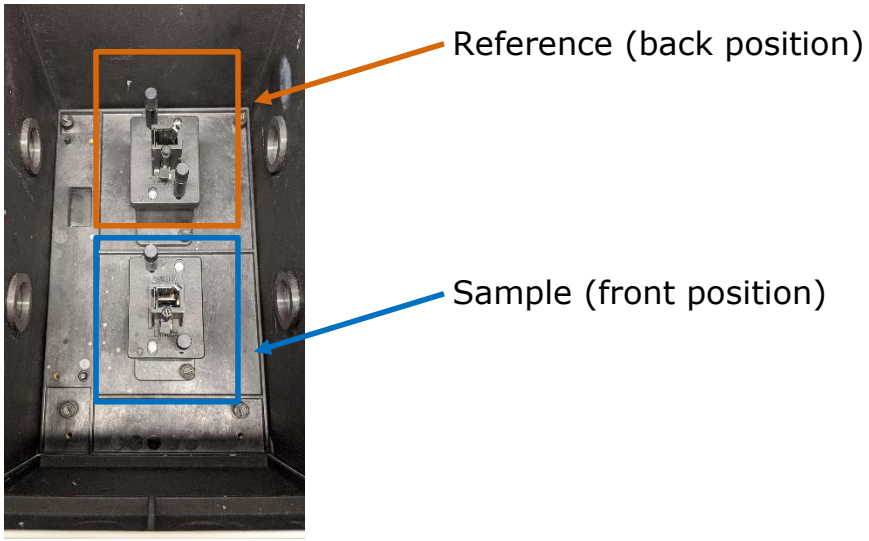

Figure 3: Interior view of the UV-Vis Cuvette Module.

Alignment

- Ensure proper beam alignment by using the *Align* icon in the top menu bar, shown in Figure 4.

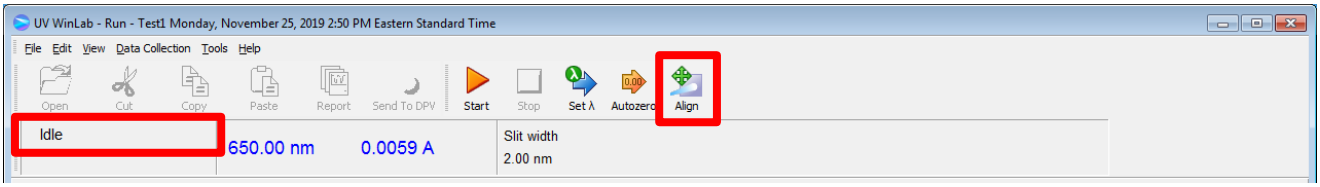

Figure 4: Align icon and tool status.

- To do this, select *Align*. The button will go gray and not allow you to select again until the alignment has finished and the beam is now ON.

- Wait until alignment has finished, machine is Idle, to check the location of the beam.
    - 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 5.
- Deselect the *Align* icon when finished.
- Do not remove cuvettes, they will be used again later.

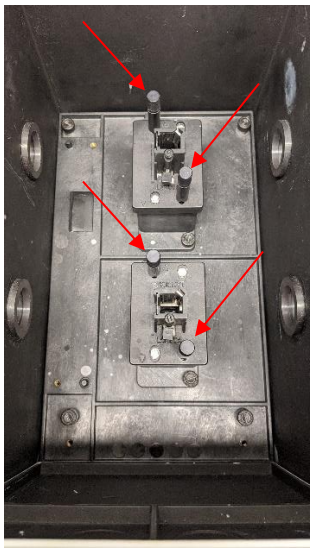

Figure 5: Cuvette alignment knobs.

5.2.2 Integrating Sphere Loading and Alignment

Figure 6 shows the Integrating Sphere and the sample and reference ports. Be sure to always wear gloves when handling the reflection standards. The cover over the sample reflection port is covered by a magnetic case, pull gently to access the port, and ensure the cover is replaced before beginning the analysis.

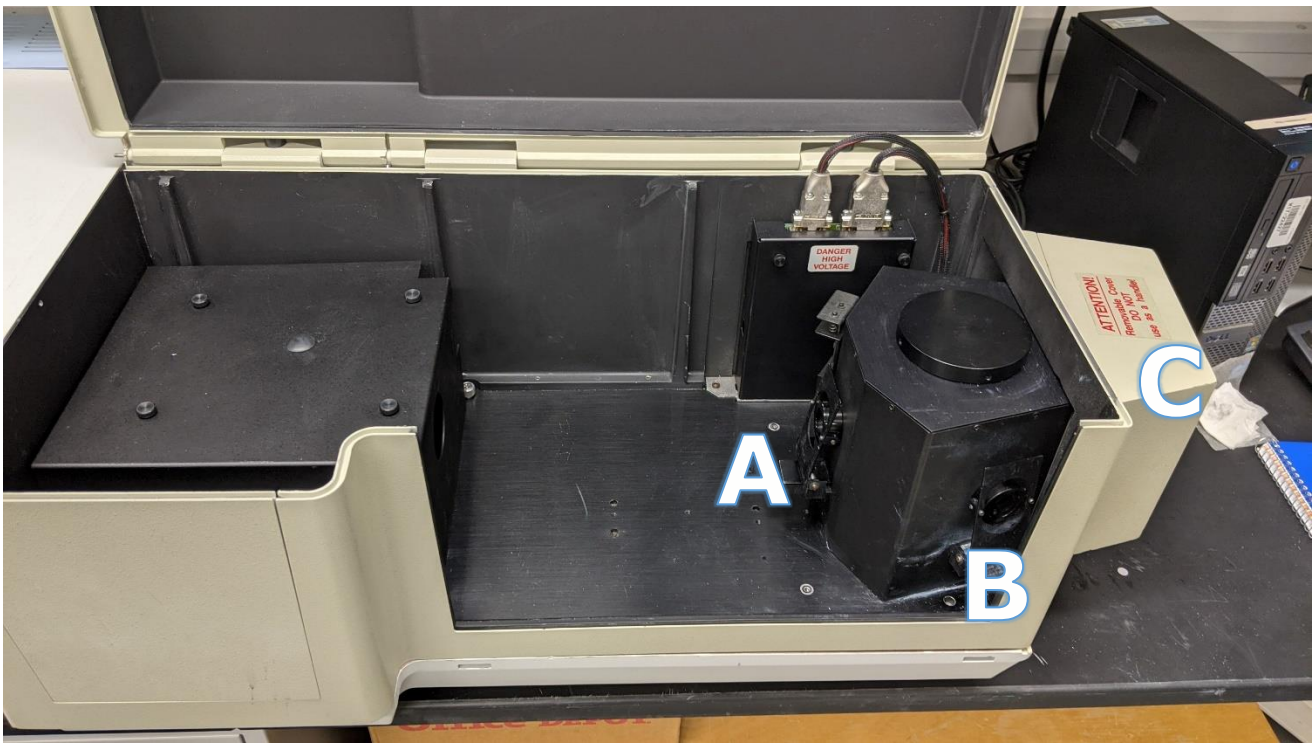

Figure 6: Integrating Sphere Configuration A) Transmission sample holder, B) Reflection standard holder, C) Reflection sample holder.

The sample holders are spring secured holders. Gently pull back the top of the metal frame, slide the sample into position, and secure by slowly lowering the frame. The central O piece should adjust to secure the sample flush against the port. For alignment, you will want a piece of paper covering the transmission port and the reflection standard port.

Alignment

- Ensure proper beam alignment by using the *Align* icon in the top menu bar (Figure 4).
- To do this, select *Align*. The button will go gray and not allow you to select again until the alignment has finished and the beam is now ON.
- Wait until alignment has finished, machine is Idle, to check the location of the beam.
    - You will want to check the beam position by placing a piece of white paper into the spring sample holder in the transmission position, and the reflection standard position.
    - Check that the beam is a rectangular shape and not being cut off by the sample holder in both the sample beam path and the reference beam path.

- Alignment of the integrating sphere should only be done by RSC staff. If you determine the beam is out of alignment, please notify staff for assistance.
- Deselect the *Align* icon when you have confirmed the proper beam position to turn off the beam.
- Load your baseline correction standards into the ports, you should be using 2 pure white samples for this baseline, one loaded into the reflection standard port, and the second into the sample reflection port.
    - Remember to wear gloves when handling the reflection standards. The standards are located in the cabinet above the tool.

5.3 Definitions:

A **Method** is analogous to a "procedure". A Method defines the wavelength range, the slit width, and other instrument parameters.
A **Task** is an experiment run using a Method.

For example, you might take a series of spectra, changing the polarity of the samples each time. Or, you might have a series of spectra, each of a different concentration of analyte. You use the same Method, but store the data as a Task.

5.4 Setting up the Method:

To begin, you will need to create and save a method, or open and edit an existing method. For a quick and simple scan you can open the Scan method with the shortcut on the far left toolbar. Otherwise follow the directions below to create or edit the analysis type you need:

5.4.1 Create New Method

- Click File -> New -> Method (Figure 7)
- Select <High performance UV/VIS NIR Instrument>, click <Next> (Figure 8)
- Select <Lambda 800>, click <Next>
- Select a method type and click <Next>
    - Scan- scanning spectra
    - Timedrive- measurements over time (kinetics)
    - Wavelength quant- see manual
    - Scanning quant- see manual
    - Wavelength program- see manual
    - Polarization scan- see manual
- Select accessories: Leave blank, click <Next>
- Check the "Edit on completion" box, click <**Save/Finish**> (Figure 9)

- Save to your user folder in the given format <NAME_Sample Name> (e.g., Peterson_SiN on Silicon). Please do not save in the general methods folder. If space needs to be made on the computer, methods whose purpose or ownership is unclear will be deleted first.

Once saved, your method will open in a new window.

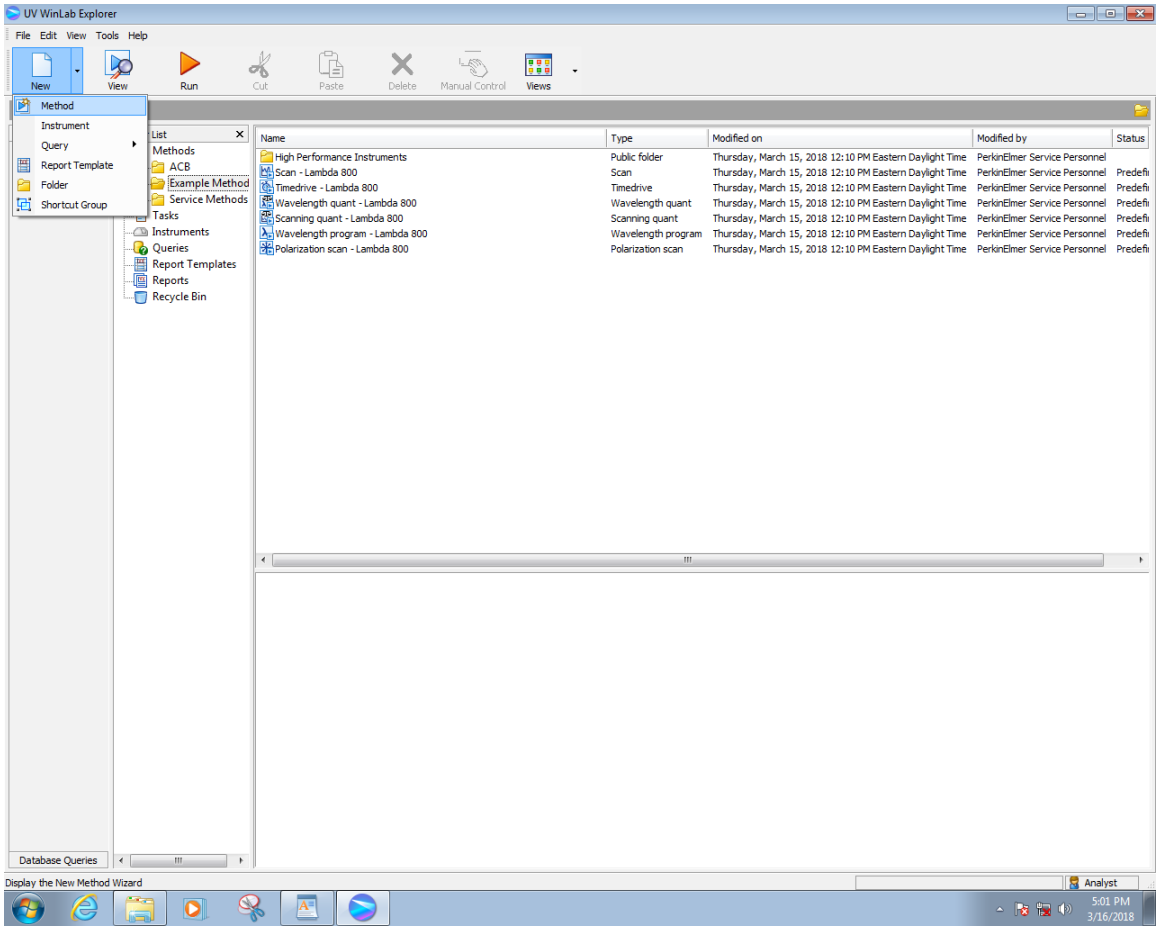

Figure 7: Creating new method.

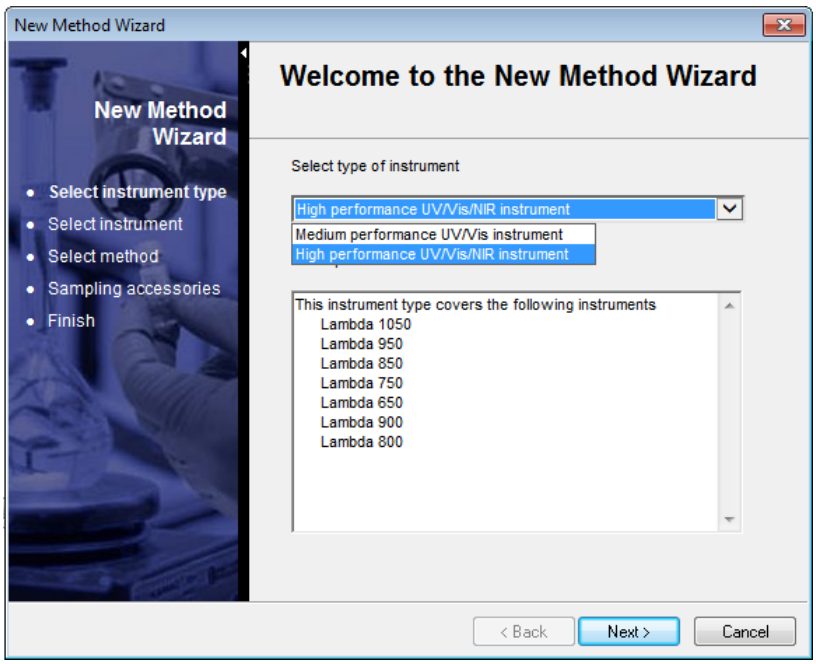

Figure 8: Selection of instrument during new method.

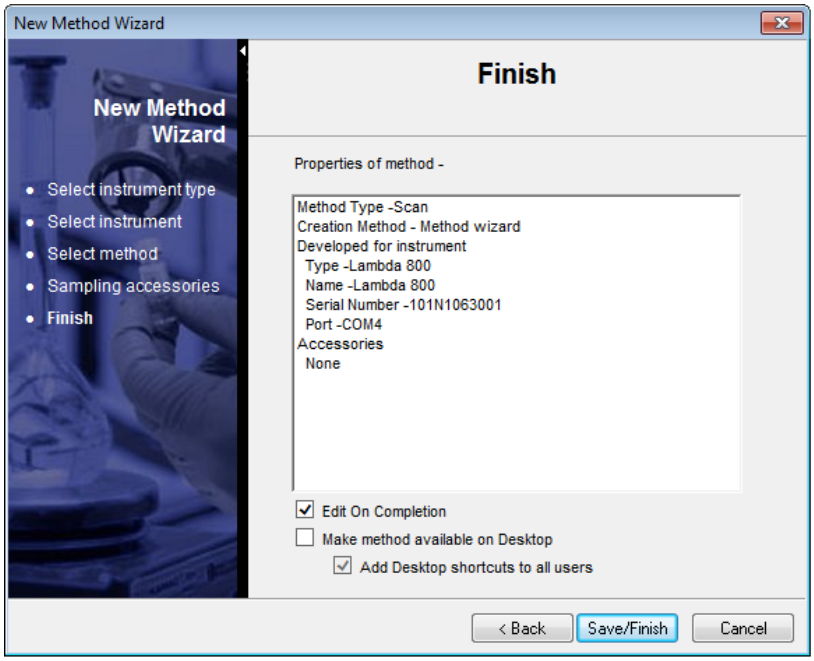

Figure 9: Finalizing new method properties.

5.4.2 Open Saved Method

- Navigate to existing method from the WinLab explorer window
- Single click to display method file metadata
- Double click to open saved method

5.4.3 Open an Example Method

- In WinLab explorer data tree (folder list) Methods> RSC
  - The RSC folder contains templates for the example methods outlined in the Software guide (page 103 of Software guide).
    - Note: The following example methods had a scan range outside the limitations of the machine and were adjusted to the maximum 900 nm wavelength.
    - Camouflage Using Integrating Sphere
    - Measurement of Glass and Architectural Materials to EN410
    - ASTM E 892-87 Terrestrial Solar Irradiance
- The manual contains suggested settings and descriptions to use for the example methods.

If editing a previously used Method, use the **Save As** option to save as a new method and not overwrite any existing methods.

5.4.4 Analysis Setup

The setup is the same for scans using the cuvette holders or the integrating sphere, and despite the illustrations not changing, scans using the integrating sphere will run correctly following this setup.

Select *Data Collection* under folders list to adjust the measurement parameters shown in Figure 10.

a. Method Settings: Scanning Range: Scans from higher to lower wavelength
   - Available wavelength: 175 – 900 nm

b. Method Settings: Scanning Intervals/Speed: How much data to be received
   - Data interval range: 0.01 – 10 nm
   - Ordinate mode options: A (absorbance), %T (transmittance), E1 (energy from sample beam), E2 (energy from reference beam), %R (reflectance)

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

The *Program* page is an "expert mode" that allows you to create a custom setup of the instrument. The following settings are available for programming: UV/Vis detector response, UV slit width, sample beam attenuator, and reference beam attenuator. The respective items must be set to "Programmed" on the *Data*

Collection page to define settings on the *Program* page. See the Software guide (page 417) for additional information.

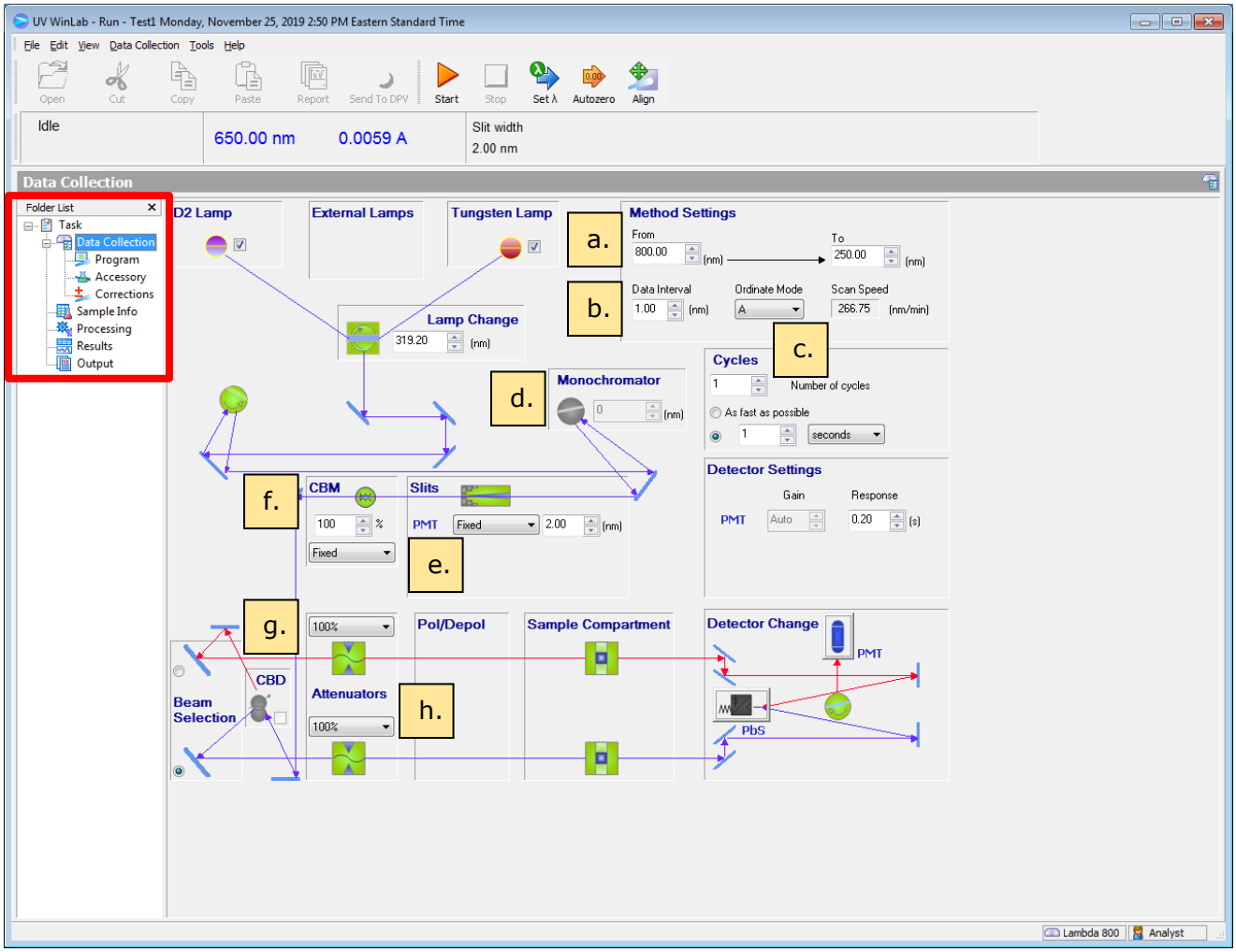

Figure 10: Data Collection tab of WinLab, this is used to designate system settings and to perform alignment.

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

If the settings specified in a method do not match the current instrument configuration, an error message will appear when opening the method as shown in Figure 11. Click <Edit Settings> to modify the method for use; the settings that need to be adjusted will be highlighted in red in the *Data Collection* and *Program* tabs (Figure 12).

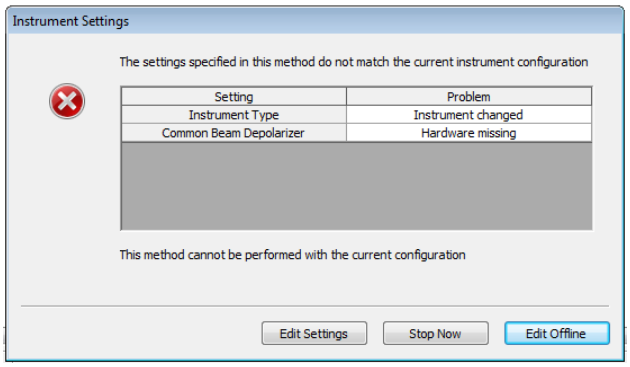

Figure 11: Error message for incorrect instrument settings.

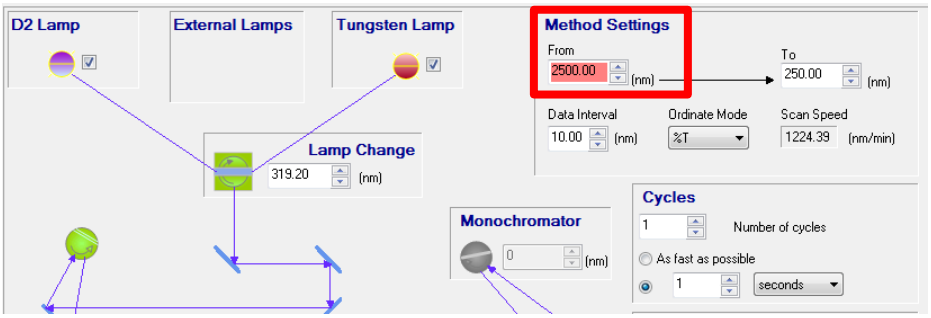

Figure 12: Highlighted setting that does not match the instrument configuration.

Select *Sample Info* under folders list to enter the number of samples you have for measurement as shown in Figure 13.

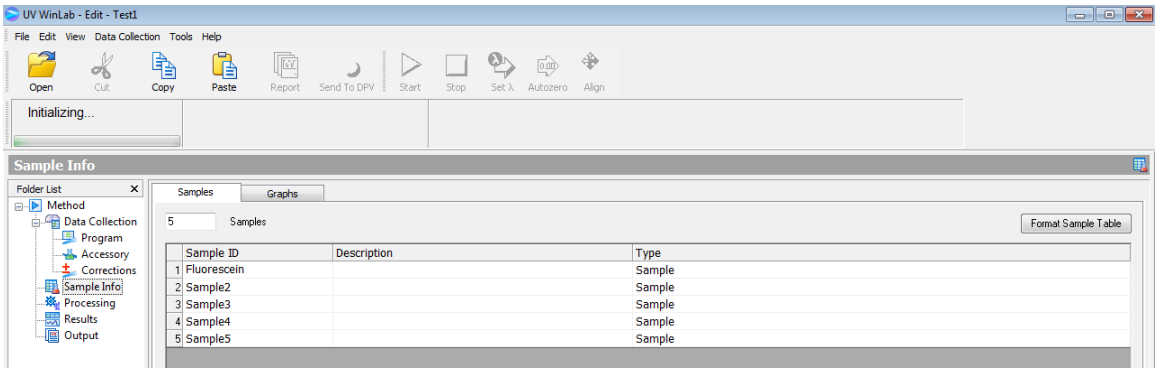

Figure 13: Sample info table.

Next, go to *Output* under folders list and select <Print to file> and <Output to file>. Click the setup option for <Data Export>. You will now get a pop-up page <Export Data> as shown in Figure 14; select all boxes of interest. If applicable, change the <Spectrum Export File (Raw)> and <Spectrum Export File (Processed)> from .SP to .ASC.

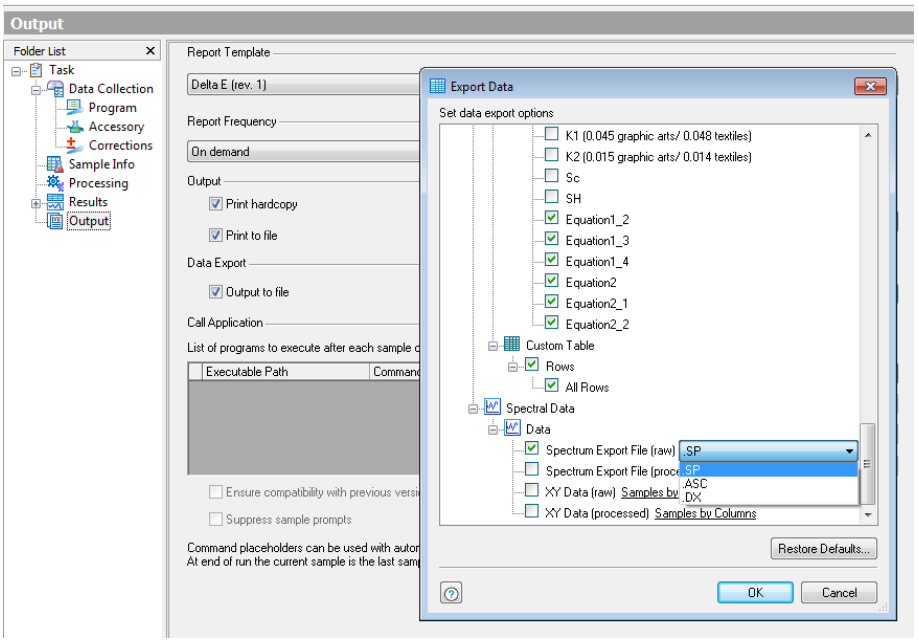

Figure 14: Export data window.

Now click the setup option for <Output> and browse for your destination folder (Figure 15), typically Computer/USER's Documents/<Folder with your name>.

The software is now ready for analysis.

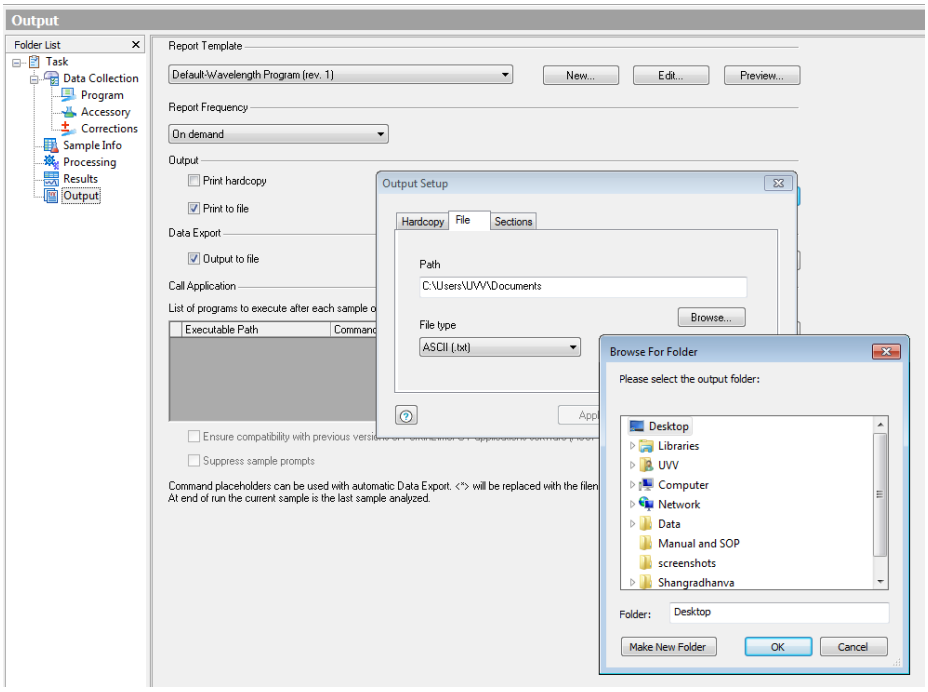

Figure 15: Output setup window for saving results.

5.5 Analysis:

When ready to continue, select the orange **Start** button at the top of the screen.

- WinLab prompts you to remove samples to perform a 100%T or 0%A correction. **NOTE:** DO NOT REMOVE THE REFERENCE CUVETTES OR REFLECTION STANDARDS.
  You are correcting here for any mis-match in your reference and sample cuvettes. This background scan is recorded and then used as the baseline for all subsequent measurements using this method.
- Click ok to perform the autozero.
- When prompted, remove the cuvette in the sample position (or standard in the reflection position), and place your sample in the appropriate sample position, and click OK.
- To collect more than one spectrum with this method, click Sample Info in the Folder List
  a) Change the number of samples, rename samples with meaningful names
  b) When you click the Start, the software will automatically prompt you to change samples until all samples in the table have been run. The background is usually only re-scanned when you change scan parameters.

6. Analyzing and Exporting the Data

1. Format the graph to your specifications using the Format icons in the *Results* tab.
2. Click on *Output* in the Folders List to customize and print and set the report parameters.
3. Click on *Report* (middle-top of the screen) to save it as an RTF file.

7. Waste Disposal

Waste disposal will be assessed on a case-by-case basis. Samples that are non-hazardous can be disposed of in either of the sinks in room 239. Users that have hazardous samples must notify staff prior to equipment usage so that staff can arrange for proper disposal.

8. System Shutdown

1. In the *Data Collection* tab, uncheck the D2 Lamp and Tungsten Lamp before closing the program (shown in Figure 16).
2. Exit out of the data collection and analysis software programs. Be sure all occurrences are closed.
3. Remove all samples and reference standards. Place reference standards in the box and return to the cabinet above the tool.
4. Turn off the power to the spectrophotometer using the toggle switch.
5. Put the computer in sleep mode.
6. Log off the instrument in the TUMI.

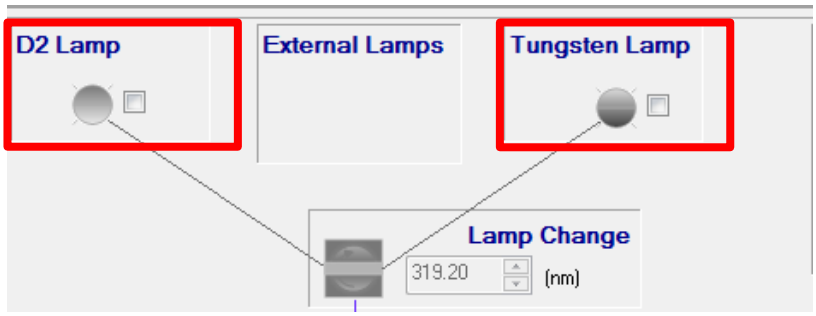

Figure 16: Unchecked D2 Lamp and Tungsten Lamp on Data Collection tab.

9. References

1. PerkinElmer. (2013). UV WinLab Software User's Guide. Waltham, MA.

10. Negligence

It is expected from all authorized users of this instrument to follow appropriate and safe operational procedures in the manipulation of components and operation of the system. Oversight or negligence in the operation and use of this instrument will lead to charges for repairs or replacement of damaged parts and components.

What is negligence – Lambda 800 UV/VIS
(NRF Room 239)
Includes but not limited to:

1. Shutdown without turning off external lamps (D2 and tungsten) first.
2. Chemical incompatibility between sample and cuvette.
3. Improper disposal of hazardous samples.

If you have any questions or concerns regarding the statements above, please contact a member of the RSC staff for clarification.

11. Quick Checklist

1. In room 239, do not cross yellow and black tape on floor, this area is designated for mercury use.
2. Turn on power to UV/Vis to allow lamps to stabilize before use.
3. Login and start WinLab software.
4. Add reference cuvettes (cuvette module) or reflection standards (integrating sphere module).
    a. Remember to wear gloves when handling the reflection standards.
5. Perform alignment.
6. Open saved method or create new.
7. Adjust *Data Collection* parameters.
8. Enter *Sample Info*.
9. Select *Output* to select data to export and destination folder.
10. Select *Start* to run analysis.
    a. Software will prompt you before performing correction for baseline, make sure references are loaded.
    b. Software will then prompt you for each sample.
11. Save results.
12. Uncheck D2 and Tungsten lamps before closing program.
13. Exit program.
14. Remove samples and place reflection standards back in cabinet.
    a. Remember to wear gloves when handling the reflection standards.
15. Turn off power.
16. Logoff TUMI.