

# RISE Catalysis Center UV-Vis Manual

Version 1.0

*updated 31 May 2018*

## UV-Vis Setup Overview

RISE Catalysis Center is equipped with an Ocean Optics UV-Vis spectrometer housed inside of the wet glovebox. The UV-Vis setup consists of FLAME-S spectrometer with two 25 cm length fiber optic cables, a DH-MINI light source (high power Deuterium Tungsten Halogen lamps with shutter, 200-2000 nm range), cuvette holder, and USB extension feedthrough to a laptop computer for easy data collection (**Figure 1**). The diode array detector has best sensitivity in the range of 250-800 nm, and allows for collection of continuous real-time data, ideal for compound characterization and spectrophotometric titrations with air-sensitive samples.



**Figure 1.** UV-Vis setup in RISE Catalysis Center. Clockwise from the top left, this includes a DH-MINI light source, FLAME-S detector, two 25 cm fiber optics cables, and cuvette holder.

## General Procedures for Collecting Spectra

### *Assembling the UV-Vis Setup*

To avoid accidental bumping and spilling chemicals on the spectrometer, all setup components should be stored on the first shelf on the right-hand side of the box (**Figure 2**). The setup should be reassembled for each use and then returned to the shelf when done.

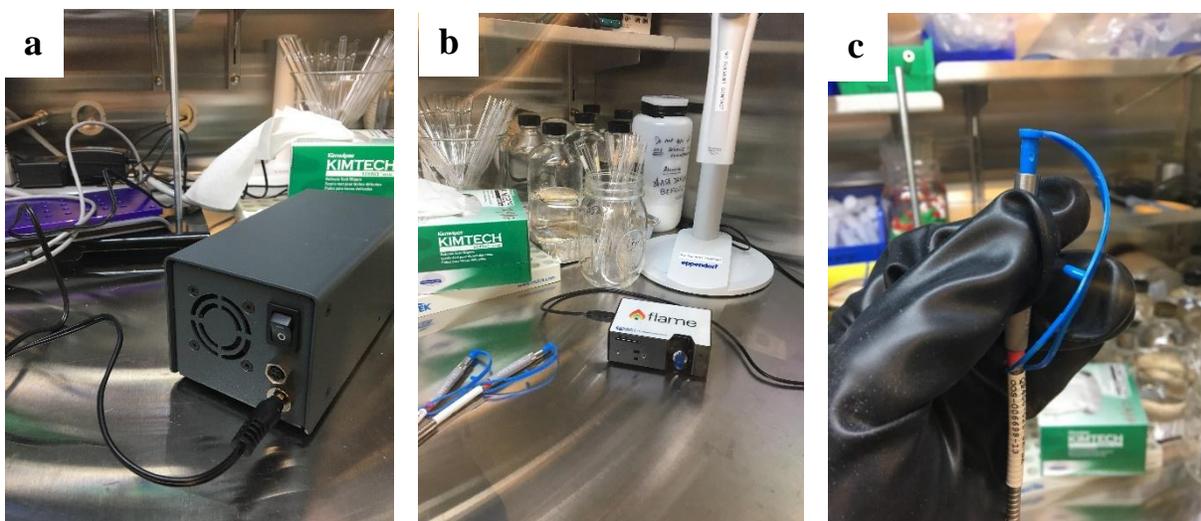


**Figure 2.** Proper storage arrangement of UV-Vis setup components.

To assemble the UV-Vis for sample measurement, follow the steps below:

1. Carefully take all system components off of the shelf and remove the cable port caps by gently screwing counter-clockwise.
2. Plug in the DH-MINI to the right-most adaptor on the power strip into the bottom port in the back of the light source (**Figure 3a**).
3. Plug in the spectrometer power source into the left side of the detector. The appropriate cable is kept along the right-most wall of the glovebox (**Figure 3b**).
4. Remove end caps from the fiber optic cables. Do this gently, by holding securely the ends of the cables and pulling straight up on the blue caps (**Figure 3c**).

**NOTE:** The fiber optics should never be bent more than their natural curvature. They can and will break if bent excessively!



**Figure 3.** (a) Connection port of DH-MINI to power strip. (b) Connection of USB cable adaptor to spectrometer. (c) When removing the blue end cap from the fiber optic cables, hold the cable ends securely to avoid accidentally bending the cables.

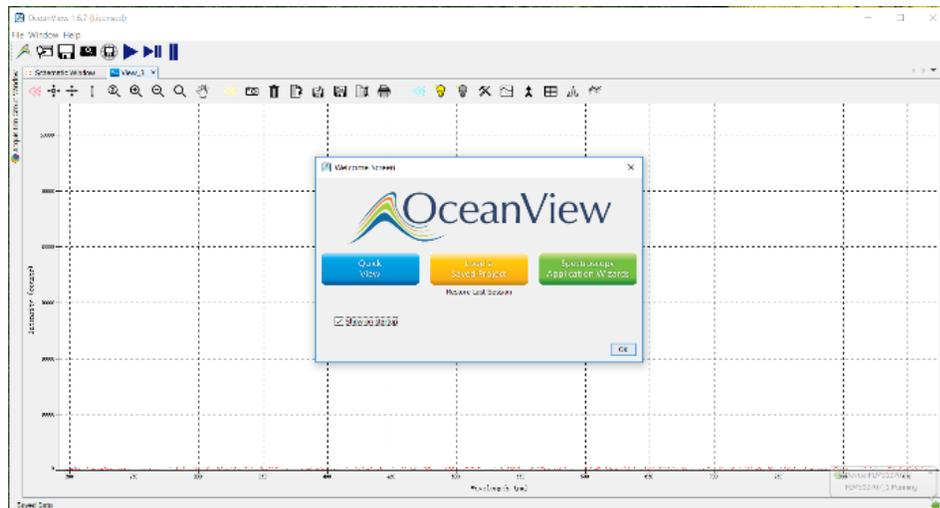
5. Connect the fiber optic cables between the cuvette holder and the light source, and then between the cuvette holder and FLAME-S detector as shown below (**Figure 4**). When connecting the fiber optic cables to each component, the cable ends should easily slide into connecting ports. Do not force the cables in if there is any resistance as this poses a risk of damaging the cable ends. After inserting the cable into the port, secure the connection by screwing the cable end clockwise. Once assembled, keep the UV-Vis setup close to the front of the box within the labelled UV-Vis area.



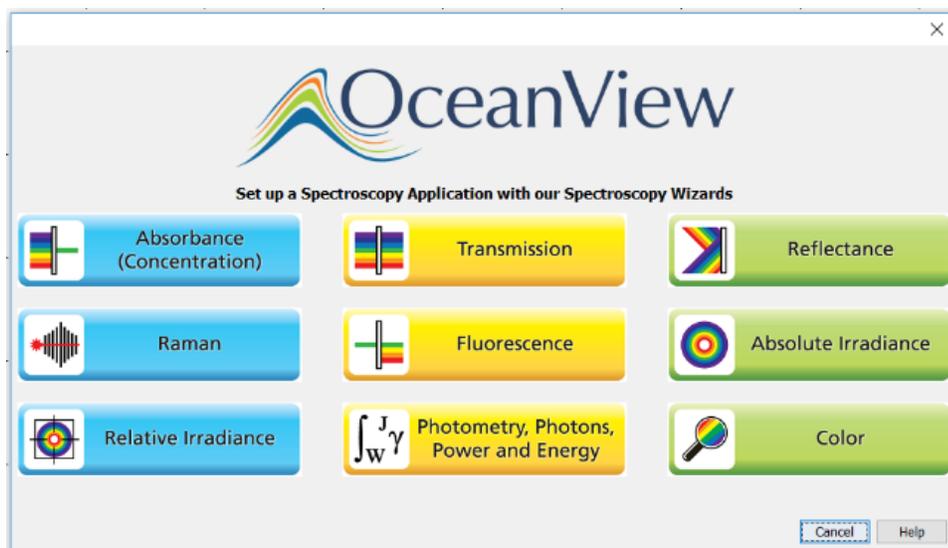
**Figure 4.** Assembled UV-Vis setup with light source, fiber optic cables, cuvette holder, and detector.

## Collecting Data

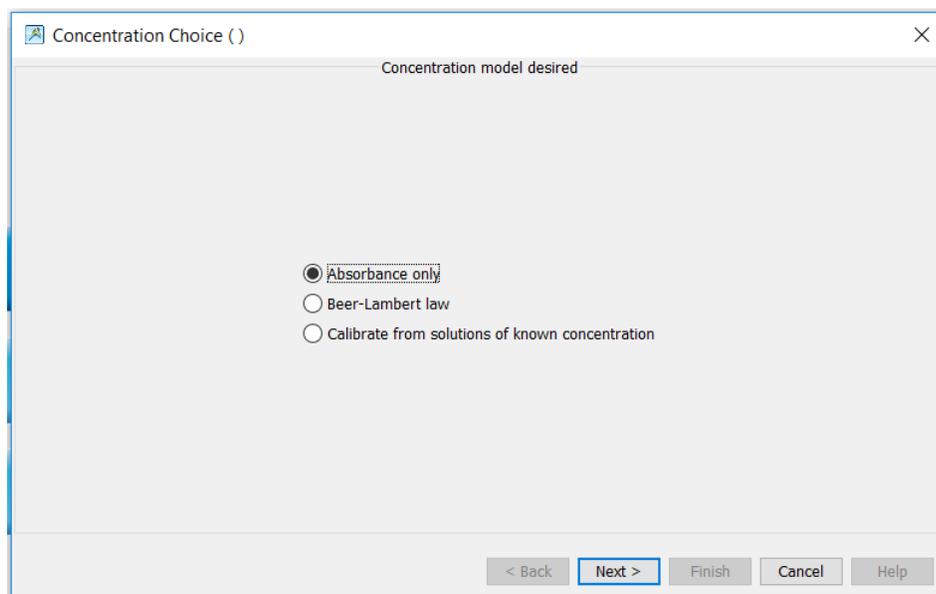
1. Turn ON the light source by flipping the main switch on backside of the DH-MINI. This should turn on a green power light and orange light next to the TTL mode button.
3. Turn OFF the TTL mode (extinguish = manual mode) by pressing the TTL mode button. This will turn off the orange light next to this button.
4. Turn ON the deuterium and halogen lamps by pressing on their respective buttons. Once on, a green light will turn on next to both lamp buttons.
5. Wait 5-10 minutes for the lamps to stabilize before beginning data acquisition.
7. Turn on the UV-Vis laptop computer and double-click on the Ocean View icon to open the software and connect to the spectrometer.
8. The software window will look as shown in **Figures 5-7**. Select “Spectroscopy Application Wizard” and then “Absorbance (Concentration)” followed by “Absorbance only”.



**Figure 5.** Opening window of Ocean Optics Software program.



**Figure 6.** Select Absorbance (Concentration) to collect UV-Vis absorption spectra.



**Figure 7.** For regular absorption spectra data, select “Absorbance only” and then begin setting parameters for data acquisition.

9. Place a cuvette of blank solvent in the cuvette holder.

10. Turn ON the Shutter button (indicated by a green light) to open the shutter and allow light to pass through the cuvette.

11. In the settings box, you will see the absorbance profile of the solvent blank. Adjust the integration time setting to have the maximum intensity below the saturation limit (blue line shown). The intensity should ideally be 70-80% of the way to the blue line.

12. To adjust the signal to noise ratio of your data, you can adjust the number of scans averaged and boxcar width. For very clean data, it is recommended that you average 5-10 scans. The boxcar width will improve signal to noise at values greater than 0 but will distort spectra and eliminate fine features if too large (see Appendix). When done adjusting parameters, click the “Next” button.

13. On the next screen, take a reference background spectrum by clicking on the yellow lightbulb icon.

14. Next, collect a dark background by turning OFF the Shutter button and then clicking on the gray lightbulb icon.

15. Place sample in cuvette into cuvette holder, and then turn ON the Shutter button to open the shutter on the light source. Press the “Finish” button. The software will then open two tabs, a first showing the reference background spectrum and a second labeled “Absorption” that shows the calibrated background-subtracted data.

**NOTE:** The Dempsey lab has found that Ocean Optics FLAME UV-Vis spectrometers are very sensitive to movement of the cuvette. To minimize any baseline shifting during spectrophotometric titrations, do not move the cuvette once it has been placed in the cuvette holder. If running a titration, carefully add aliquots directly into the cuvette in the holder and then mix by pipette directly in the cuvette in between saving traces.

16. Data can be saved by a number of methods. Two simple methods include:

a) Individual traces can be saved by clicking on the  icon to copy the data onto the clipboard. This data can then be saved by opening a notepad and pasting the data there.

b) During a titration experiment, it can be convenient to save all traces in an organized folder. To do so, press the  icon to set up file saving settings and file location. Once these settings have been selected, traces can be saved by pressing the  icon.

### *Taking Apart the UV-Vis After Use*

1. Unscrew and gently remove fiber optic cables connecting light source and detector to the cuvette holder. Replace blue caps on ends of the cables.

2. Screw protective caps back onto the ports of the light source, detector, and cuvette holder. The blue protective caps go on the light source and detector, and the red caps on the ports of the cuvette holder.

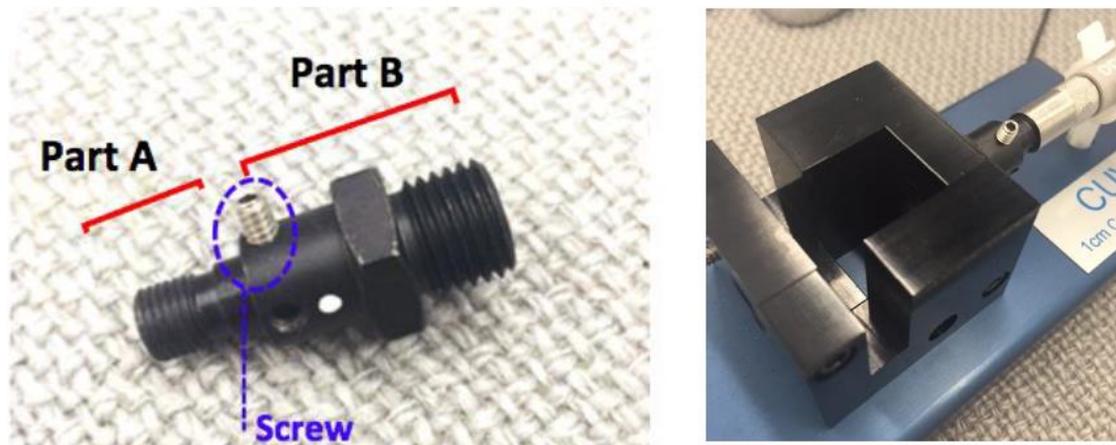
3. Return setup components to the first shelf on the right-hand side of the glovebox. Take care not to bump or drop the light source as this will break the lamps. Place the cables across or in front of the other components so that they do not bend.

## System Maintenance and Repairs

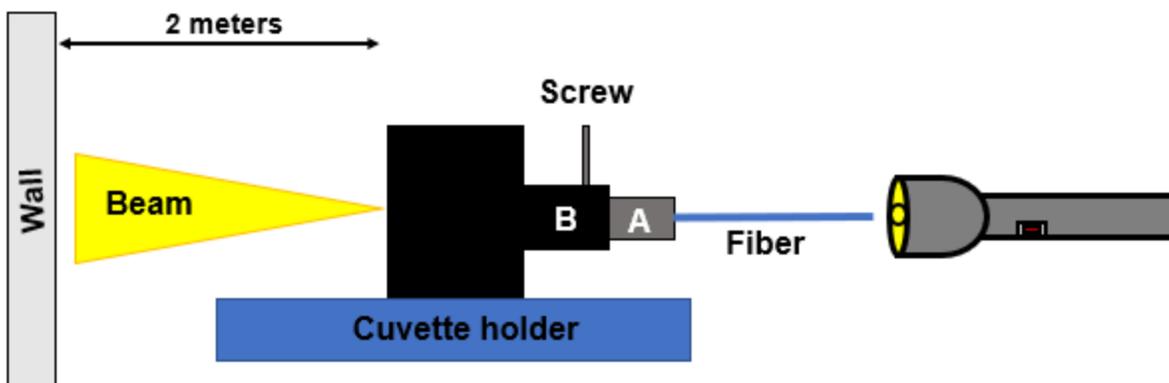
### Calibrating Collimating Lens of Cuvette Holder

To measure accurate and clean data, the light entering and exiting a sample by means of a fiber/collimating lens assembly must be well collimated. The following may be done if the data starts becoming noisy to adjust the focus of light collected by the spectrometer:

1. Find a dark room or area to do the maintenance.
2. Take the whole set of collimating lens and tighten Part B on one side of the cuvette holder with an allen wrench (**Figure 8**).
3. Connect the illumination fiber on Part A of the set (**Figure 8**).
4. Set up the holder perpendicular to a flat wall with 2 meters of space between the setup and the wall. Dim the room lights.
5. Using a flashlight on the other end of the fiber, let the light go through the lens (**Figure 9**)
6. Loosen the set screw that holds the barrel of the collimating lens in place and slide the barrel until the spot of light focused on the wall has crisp edges. You are looking for the sharpest and cleanest image possible. Tighten the set screw.
7. Remove from the holder and set up the second collimating lens to repeat the procedure.



**Figure 8.** (Left) Collimating lens Parts A, B, and set screw. (Right) Collimating lens tightened into cuvette holder with attached illumination fiber.



**Figure 9.** Schematic of arrangement for collimating lens calibration.

### *Bringing the UV-Vis Setup Out/Into the Glovebox*

To avoid damage of the lamps in the DH-MINI light source, it is preferable to not take the UV-Vis setup outside of and back into the glovebox repeatedly. If, for any reason, the setup must be taken out of and back into the glovebox, please use the following procedure:

Gently place items into the large antechamber. Apply a partial nitrogen flow (open valve 1/2 of the way open) followed by opening the vacuum valve 1/4 of the way. Keep the antechamber under this simultaneous loop of nitrogen purge and vacuum for 30 minutes. To maintain a suitable glovebox pressure while applying a continuous stream of N<sub>2</sub> to the antechamber, the wet side glovebox pressure should be increased every minute or so using the pressure-control foot pedals.

Do not apply full vacuum to the antechamber during this process, as this poses a risk of bursting the lamps! If you are only bringing the cuvette holder in and out of the glovebox, it is fine to use the small antechamber under full vacuum.

## **Safety Notes and Common Practices**

Because the glovebox is a shared space, the UV-Vis should be stored on the shelf when not actively in use. In storage, the protective caps should always be on the ends of the fiber optics cables and each of the connecting ports on the light source, cuvette holder, and detector.

To ensure long-term high-quality data from the spectrometer, do not get any chemicals or solvents on any component of the UV-Vis setup. If there is an accidental spill, please wipe up immediately with a Kimwipe!

If you have a sample that is especially light sensitive, there is a sample cover you can put over it in the cuvette holder. Because it is not used regularly, this cover is kept in a drawer of UV-Vis supplies in the Catalysis Center.

## Useful References

Parts of this user manual were taken and adapted from the Dempsey Group UV-Vis and Fluorometer manuals, as well as adapted from information on the Ocean Optics website:

[https://oceanoptics.com/product/flame-spectrometer/?\\_stock\\_status=instock](https://oceanoptics.com/product/flame-spectrometer/?_stock_status=instock)

*Video tutorial* - <https://www.youtube.com/watch?v=6C6H4DkOc04>

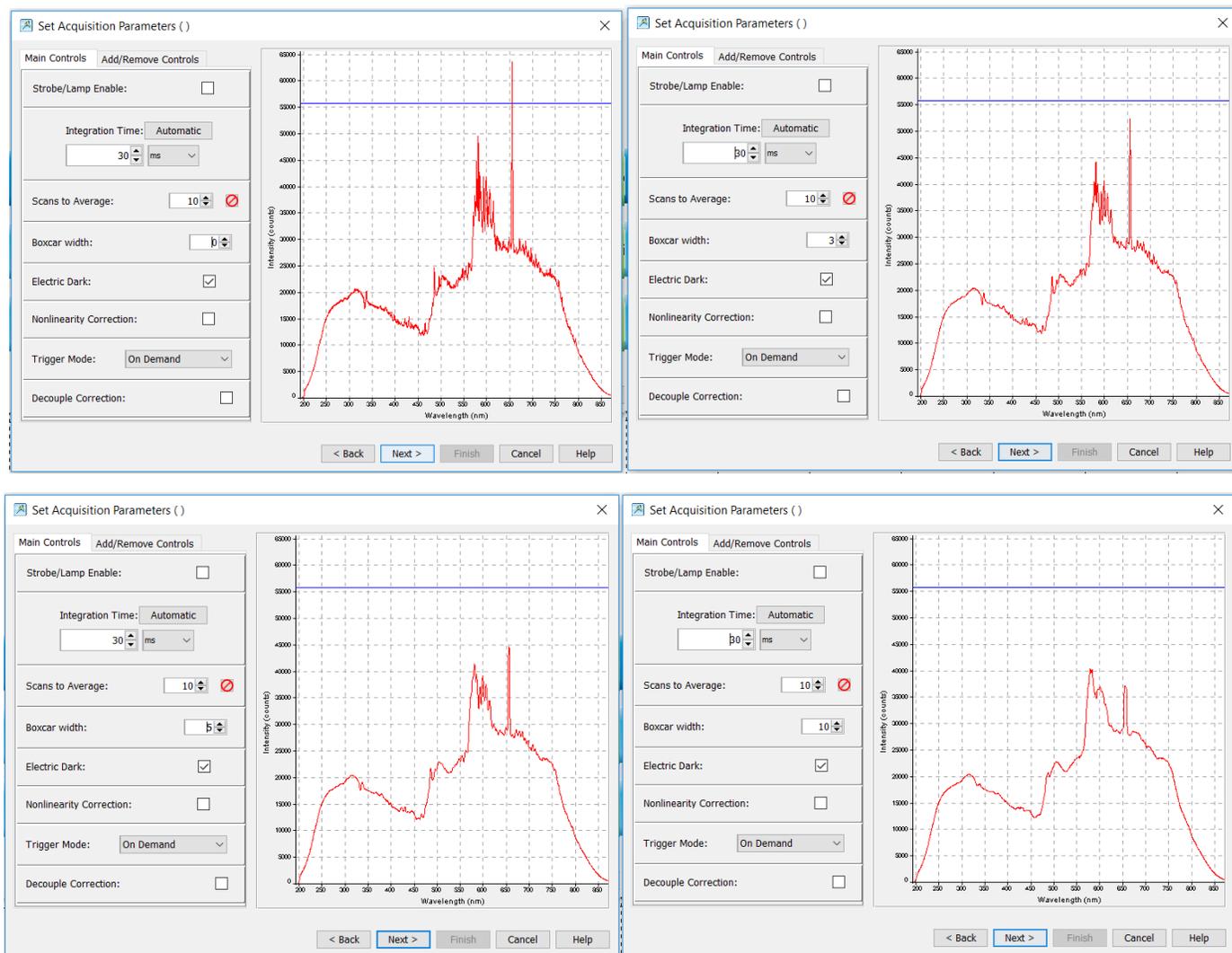
Reusch, William H. 1999. Introduction to Spectroscopy. Michigan State University  
<https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/UV-Vis/spectrum.htm#uv1>

If there are any issues with the UV-Vis spectrometer or data collection, please contact Carolyn Hartley (Dempsey group) at [hartleyc@live.unc.edu](mailto:hartleyc@live.unc.edu)

## Appendix:

### Boxcar Width Parameter Effects

Changes in signal to noise and fine structure of spectra by changing boxcar width. Appropriate boxcar width should be assessed for each new sample.



**Figure A1.** Clockwise from top left: Boxcar width of 0, 3, 5, and 10.