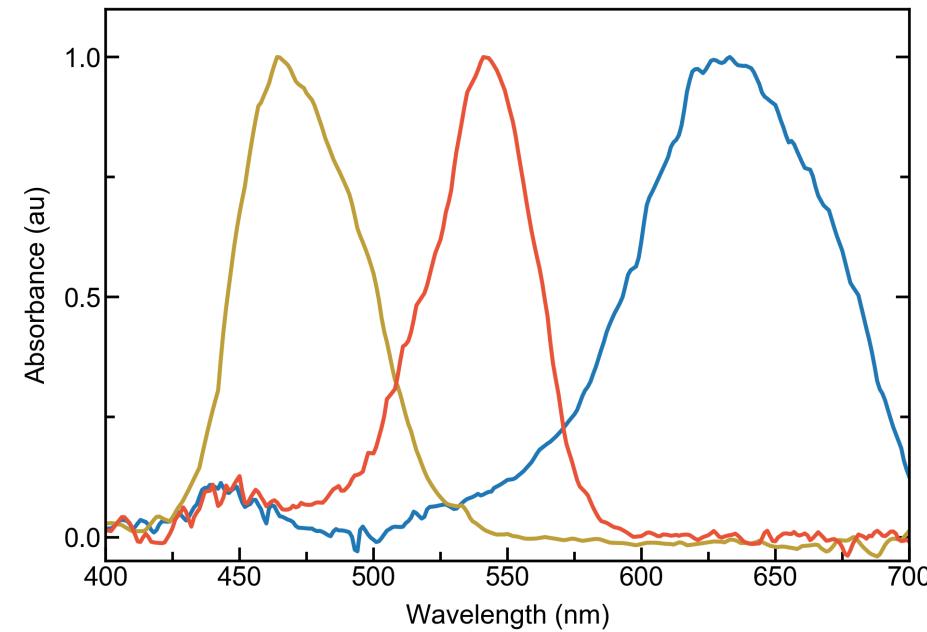


Collecting, Downloading, and Saving Data

Pt II of Introduction to Spectroscopy and Data Science: How many dyes are in a collection of colorful samples?



Part II: Collecting absorbance spectra

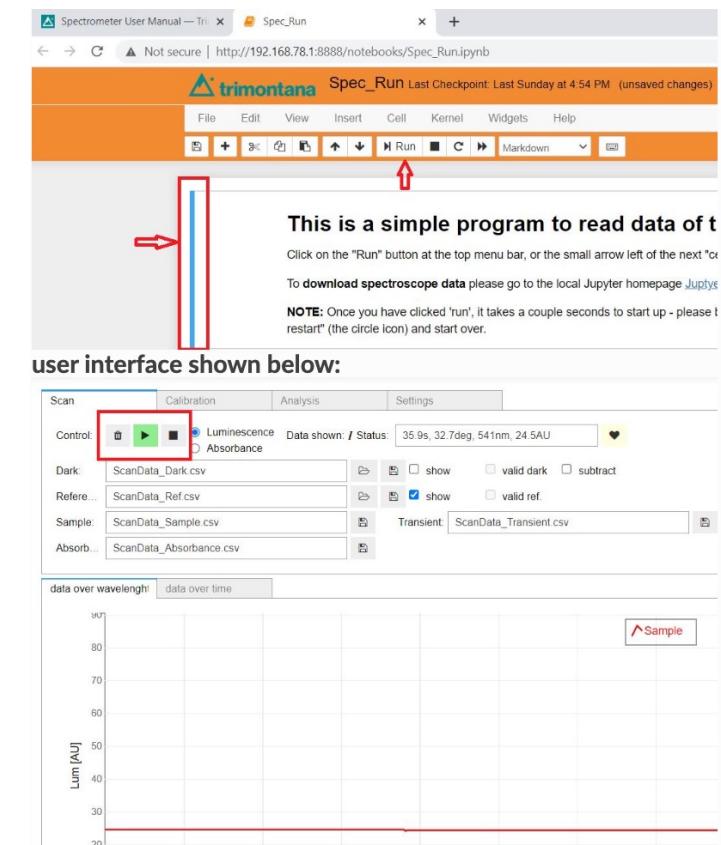
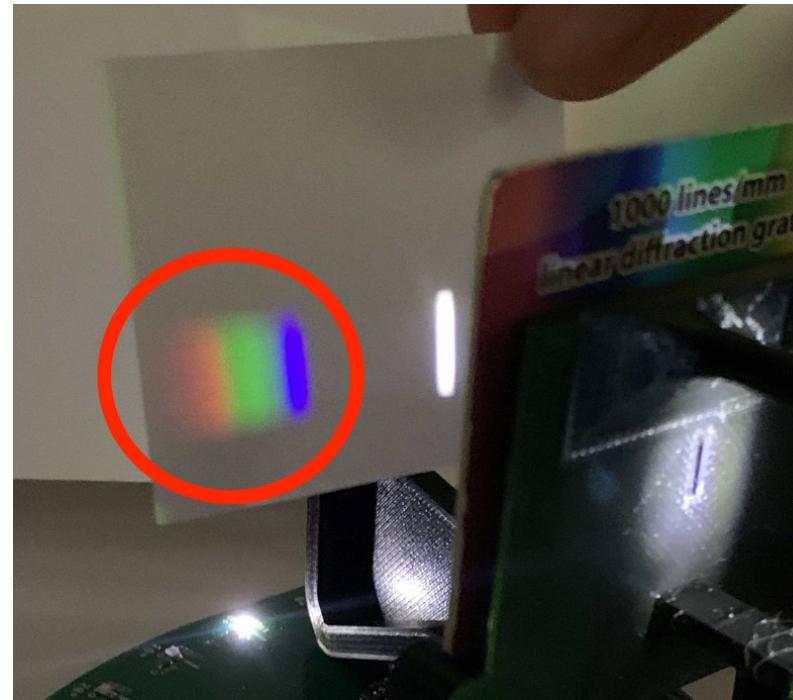
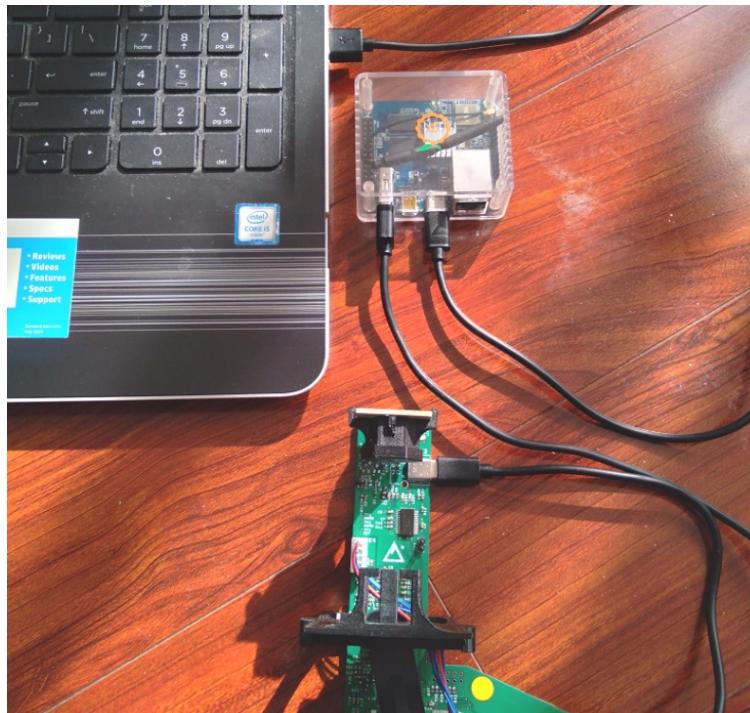
In this portion of the lab, you will collect absorbance spectroscopy data on food dyes

Video instructions for spectrometer operation (optional):

<https://vimeo.com/661075622>

Password: Trimontana

Check the connection between spectrometer and computer

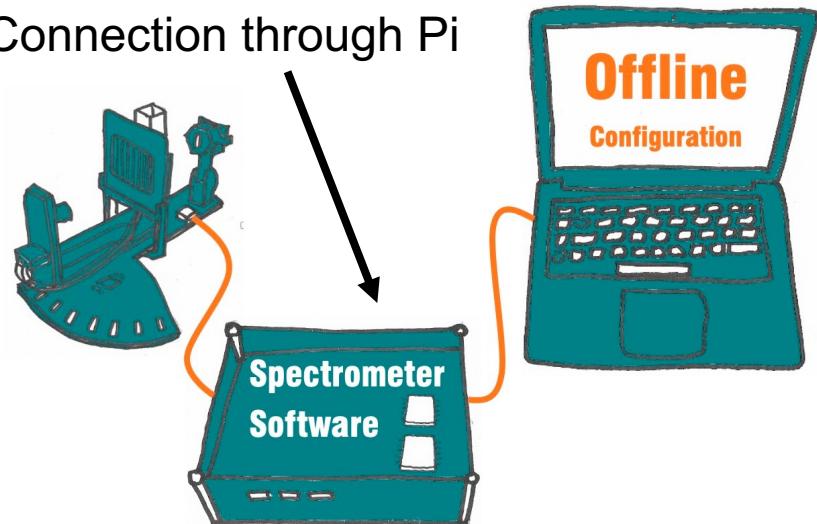


1.) Follow the steps in your spectrometer building instructions to connect your spectrometer and check the connection, in either (1) online mode or (2) offline mode. Instructions can be found in the corresponding PDF/PowerPoint slides or online via the Trimontana website: <https://docs.trimontana.tech/index.html>

Calibrate your spectrometer

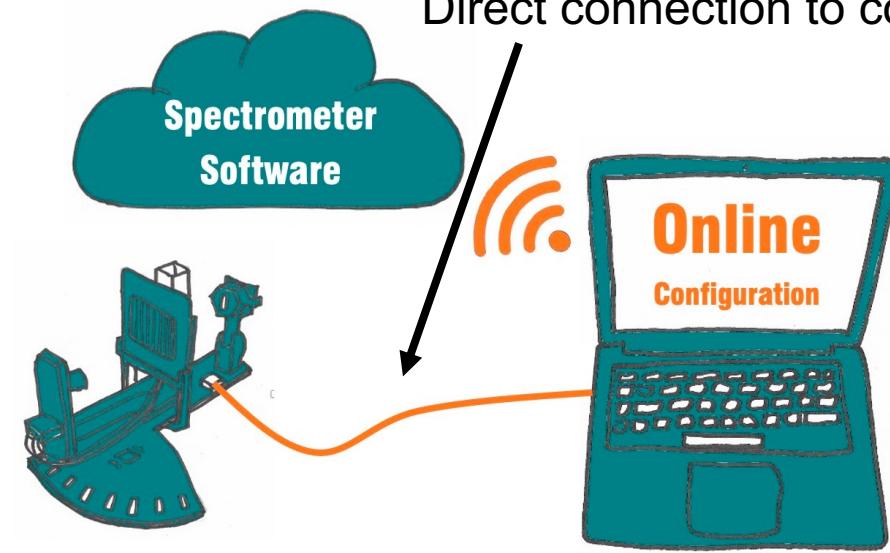
2.) Follow the instructions on the Trimontana website to calibrate your spectrometer

Connection through Pi



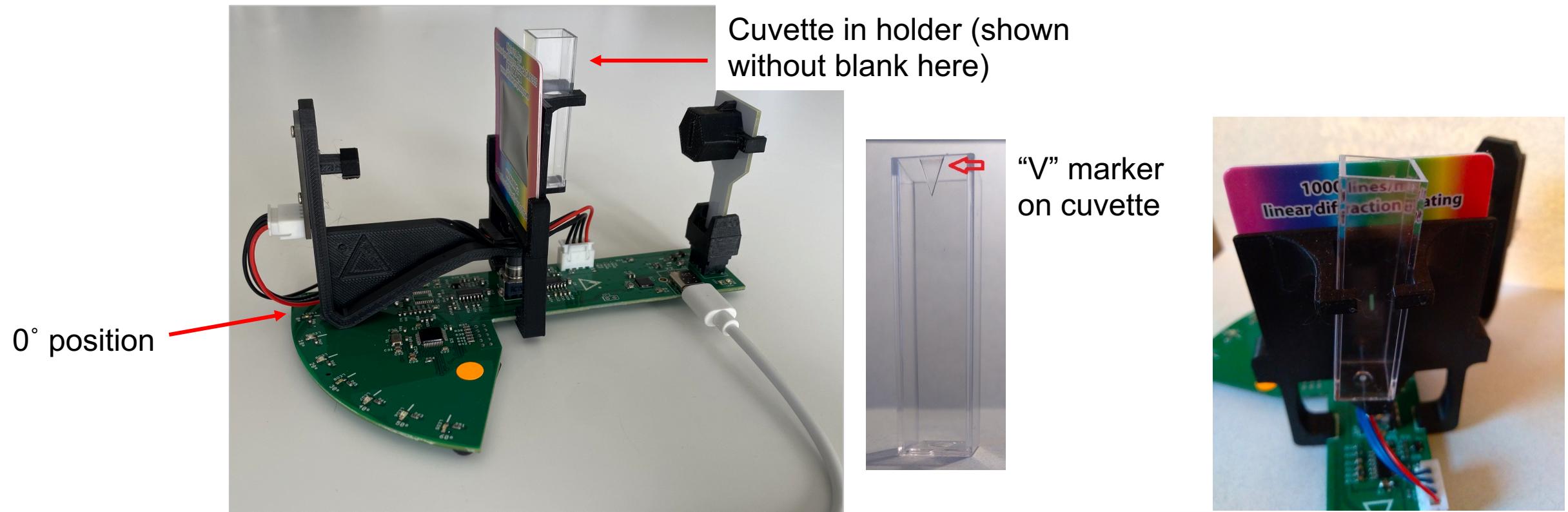
For **OFFLINE** operation through the
Raspberry Pi:
<https://docs.trimontana.tech/tutorial/offline.html#wavelength-calibration>

Direct connection to computer



For **ONLINE** operation through the
JupyterHub:
<https://docs.trimontana.tech/tutorial/online.html#wavelength-calibration>

Preparing blank/reference sample



3.) Once you have confirmed your spectrometer is connected and calibrated, position your detector/swivel arm at the 0° position. Fill your cuvette with your blank solution (2 mL of water), and place your cuvette in the cuvette holder with the marker on the cuvette facing towards the light source. Make sure you do not have any bubbles in solution.

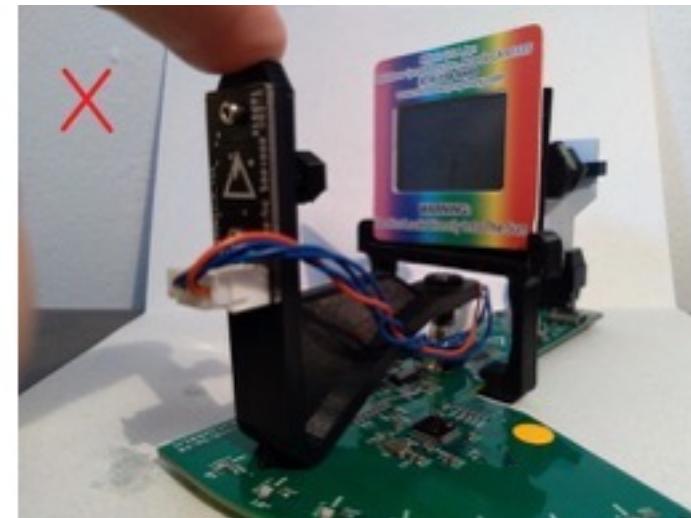
**Make sure you place the cuvette properly into the cuvette holder. It should be as close to vertical as possible, and it should not tilt forward or backward. In the picture shown here, the cuvette is tilted. The tilt will affect data quality.

Tips for data collection

More tips for your next steps:

In the next steps, you'll raster the swivel arm to collect the light intensity at each angle, which will be used to produce your absorbance spectrum. Keep tips (a) and (b) in mind when doing so.

Correct technique:
rotating swivel arm
without putting any
upward or downward
force on it

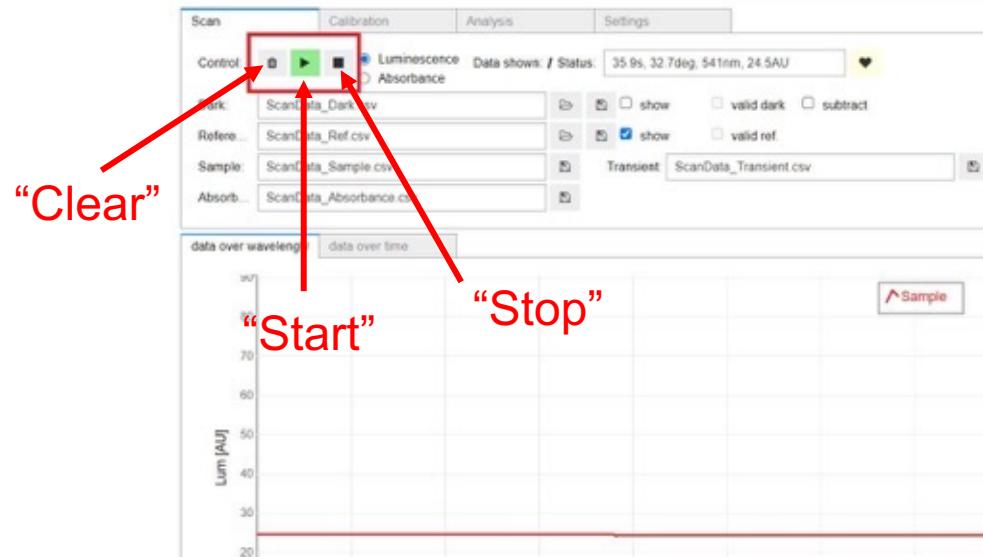


Incorrect technique:
pushing down on
swivel arm when
rotating

(a) When you move the swivel arm to scan, make sure that you do not press down or push up on the arm and distort the alignment of the detector. In the photos shown here, the left image depicts a correct way of scanning. The right image depicts an incorrect way of scanning.

(b) Make sure to scan the entire range of angles at the same speed. If you scan at an inconsistent speed, you may see noise or spikes in the data.

Collect reference spectrum



4.) When you are ready to begin data acquisition, press "Stop" (black square) then "Clear" (trash can icon).



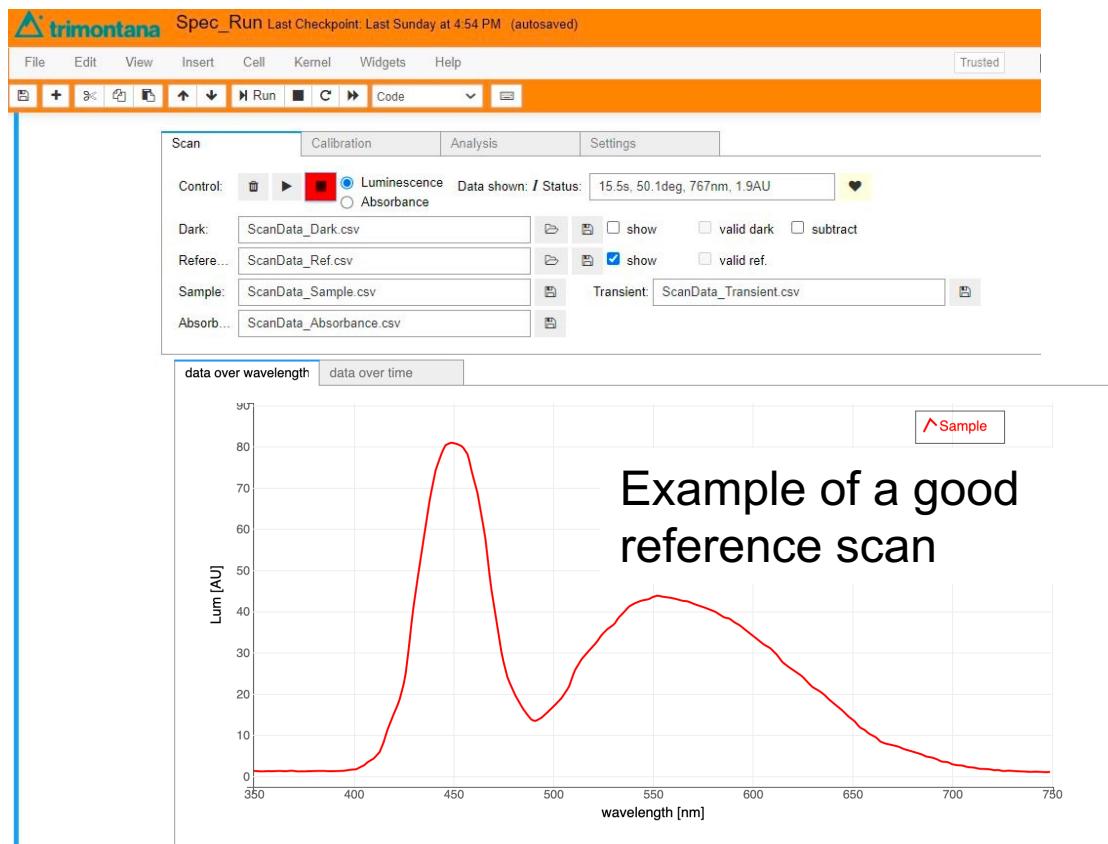
****Do not forget to select absorbance before starting data collection**



5.) Press "Start" (forward arrow) in the user interface, then use your hand to slowly and smoothly sweep the swivel arm from the 0° position (left) to the 60° position (right).

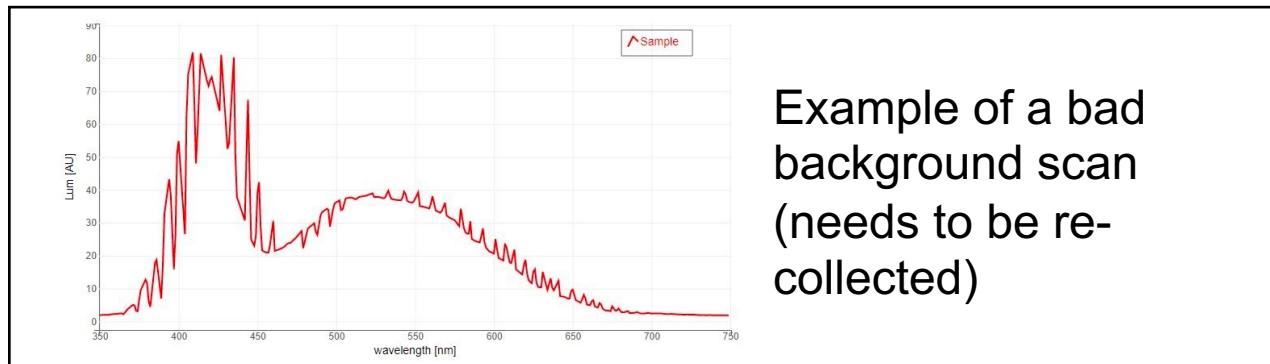
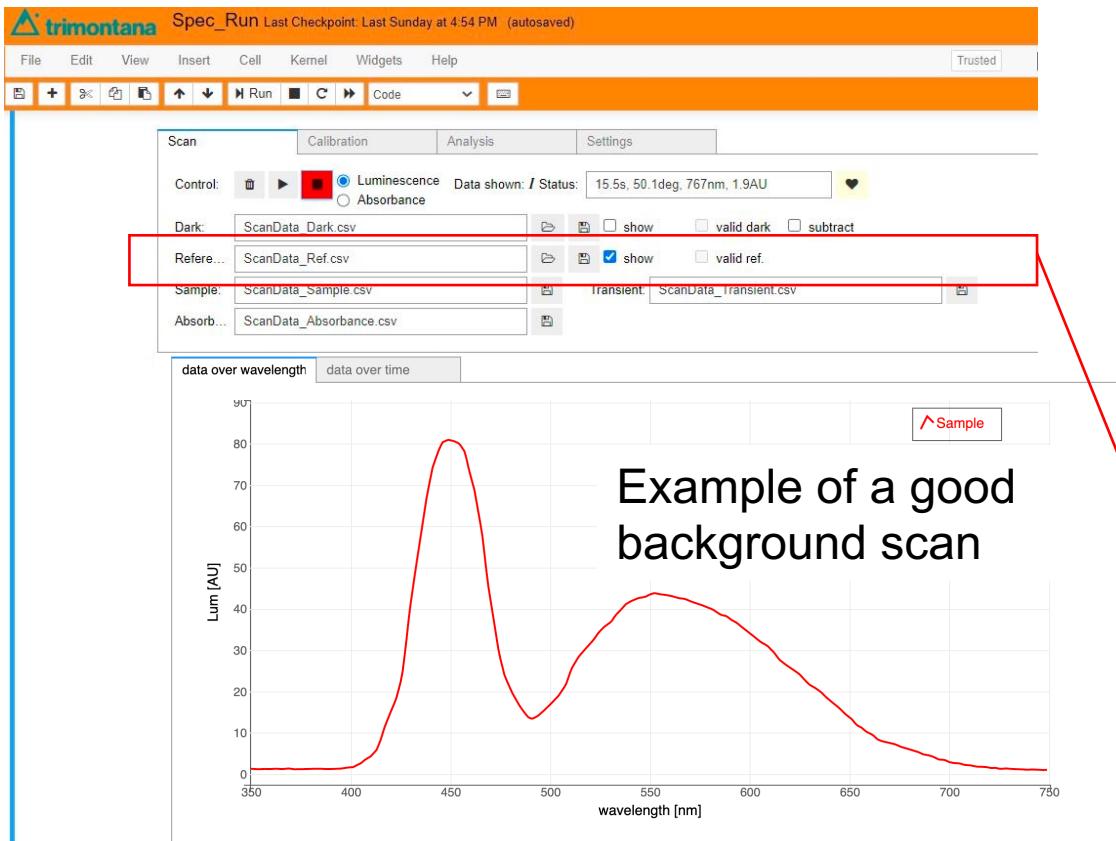
6.) When you are done scanning from 0° to 60°, press "Stop" in the user interface to stop data collection. If you do not press "Stop" the program will keep running and write over your spectrum bringing back a flat line.

Inspecting reference spectrum



- 7.) After pressing stop, your reference scan will show up in red and be labeled “Sample” in the Trimontana Jupyter notebook for data collection. Light intensity for the highest peak should be between 70 and 85 Lum/AU, and the second peak should sit around 35-45 Lum/AU. Ask your instructor how to change the intensity if it is not.

Inspecting and saving reference spectrum



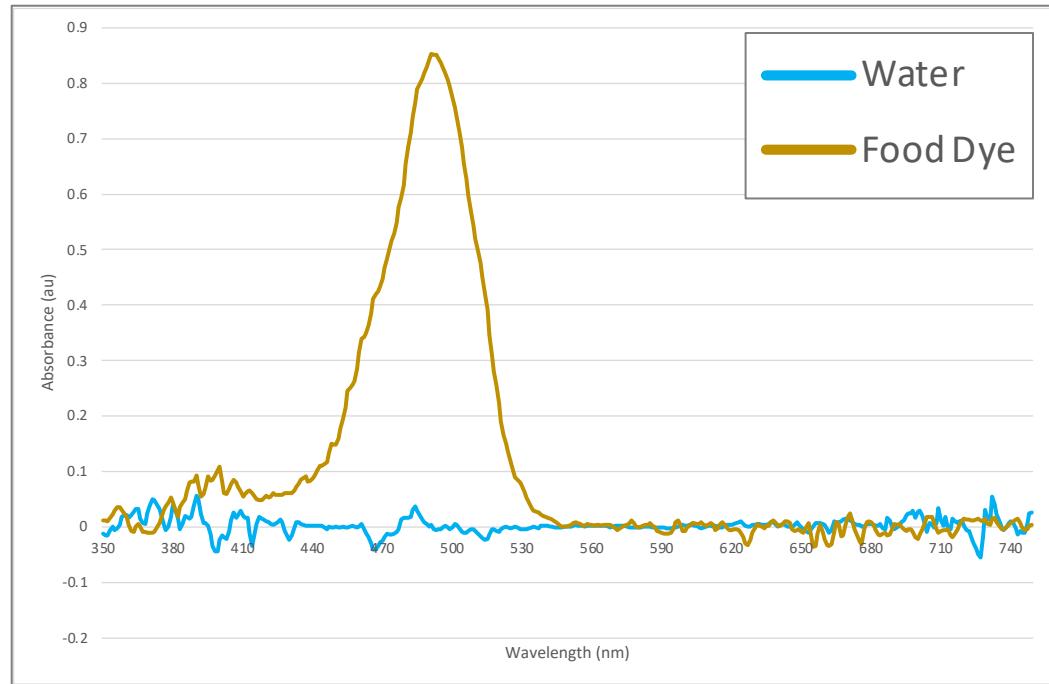
Example of a bad background scan (needs to be re-collected)

8.) Pay close attention to your blank/background scan, which will appear in RED on the absorbance graph in the Trimontana Jupyter notebook. If there are any sharp spikes or peaks that deviate from your reference background spectra (included with your Trimontana spectrometers), you will need to start over and collect your data again. (Discussion Q: why do you think this might happen, and why is it important?)

Gray check will appear when reference/background spectrum has been saved

9.) If your background spectrum looks good, save your reference/background spectrum by pressing the "Save" icon (a floppy disc).

Collect water test spectrum



10.) Use the same 2 mL of water to collect an absorbance spectrum. This scan should not show any absorbance peaks if you collected your data correctly, apart from normal noise associated with the spectrometer (with a significant amount of noise from 400 - 500 nm. An example of a [water spectrum is shown above in blue](#) (plotted using Excel), along with an example of a [food dye spectrum](#) (which you'll collect in the next steps) [in yellow/brown](#).

Save data

Sample: ScanData_Sample.csv

Absorb... ScanData_Absorbance_group1.csv



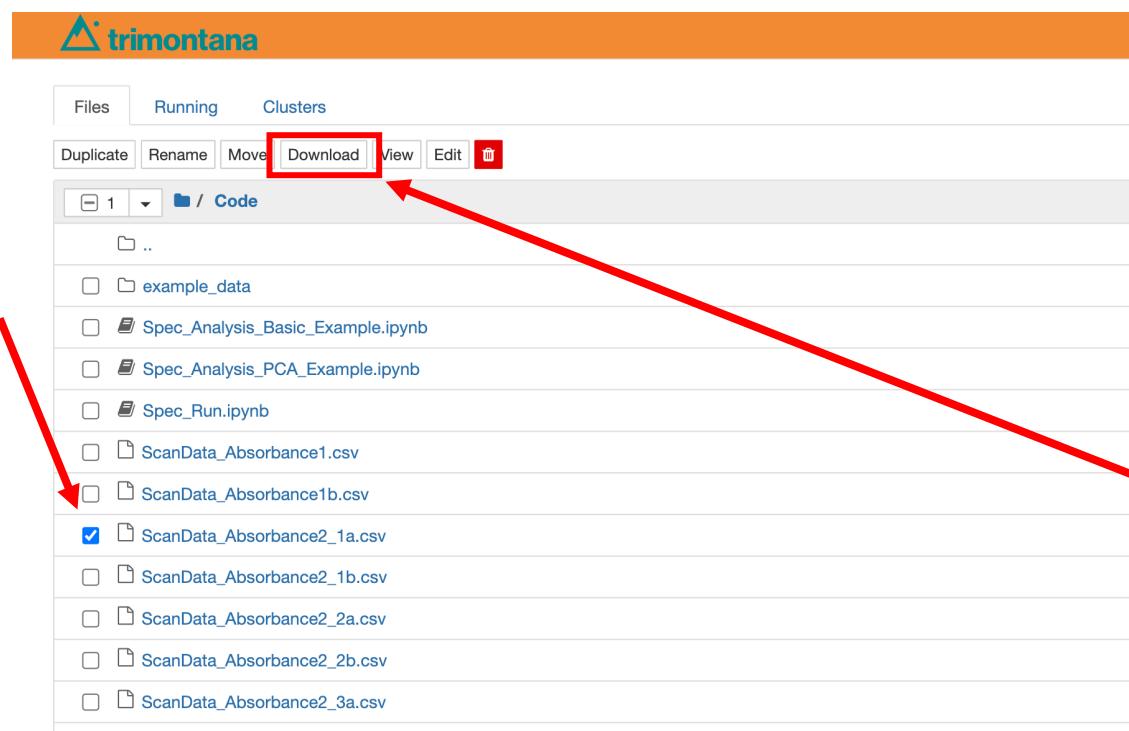
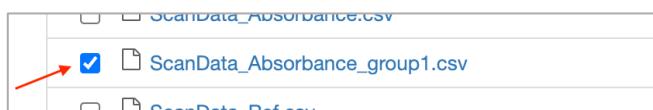
11.) Save your absorbance data in the data collection window. If your instructor does not give you other instructions, pick one name for your group and keep this name for the entire lab. You'll need to download your spectrum before collecting new data to avoid overwriting it (e.g., ScanData_Absorbance_group1.csv).

Download water test spectrum

12.) Download your absorbance data by opening the following URL:

- Offline operation through Raspberry Pi: 192.168.78.1:8888/tree
- Online operation through JupyterHub in the “Code” folder: <http://hub.trimontana.tech>

13.) Select your group's absorbance data.

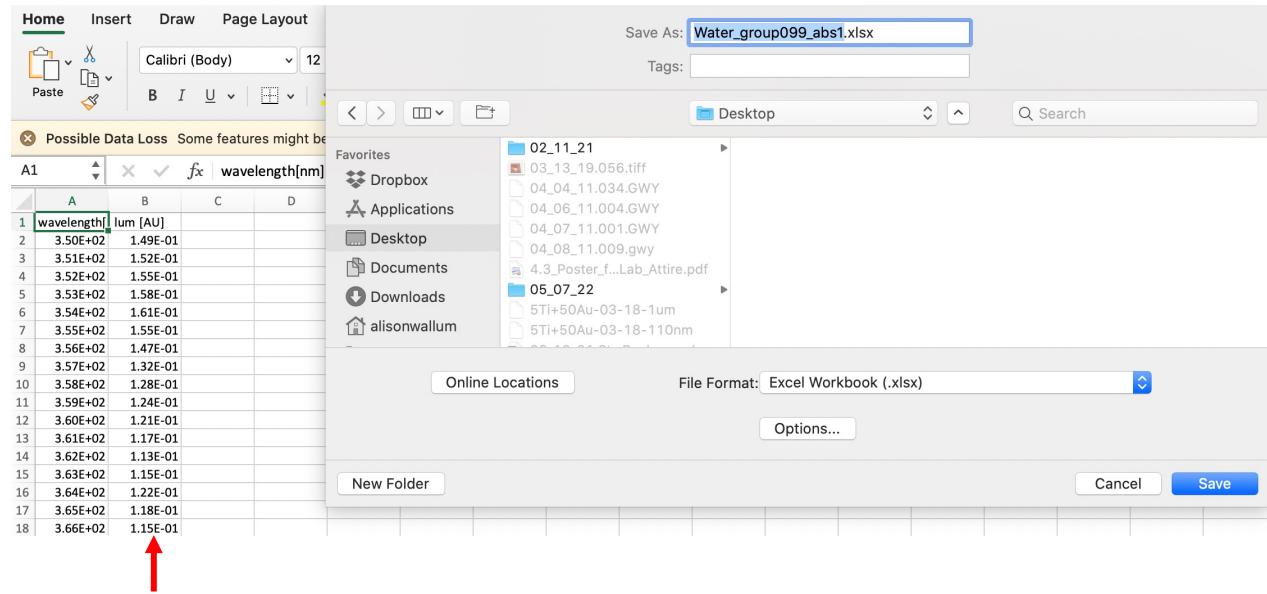


14.) Finally, press the download button at the top of the window.



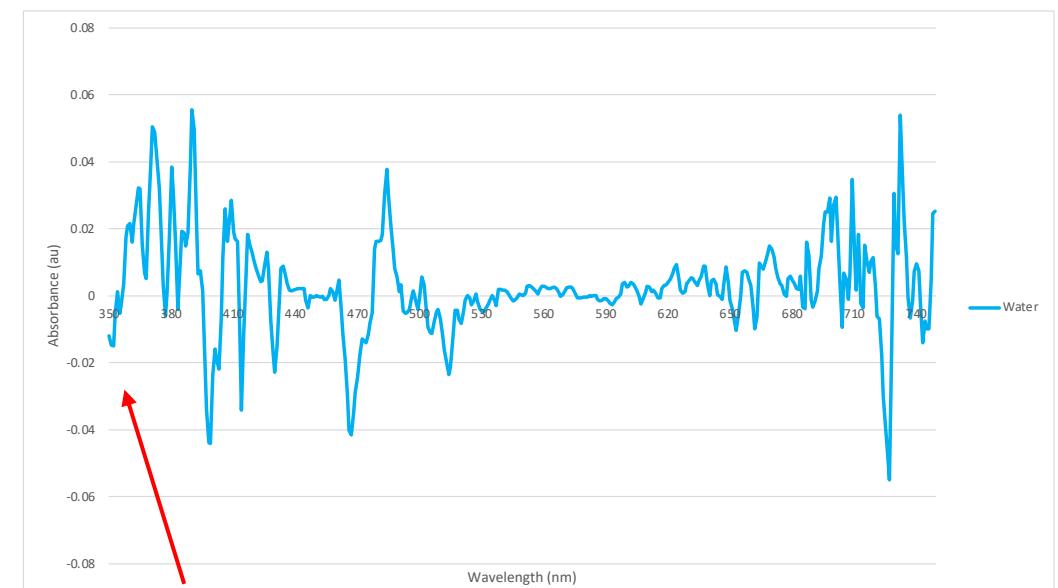
Plot water test spectrum

Save data



Values in “lum [au]” column will be absorbance values if absorbance was selected when collecting data (slide 7)

Plot data
(don't worry about making this graph look nice, it's just a visual tool to make sure you collected data correctly)



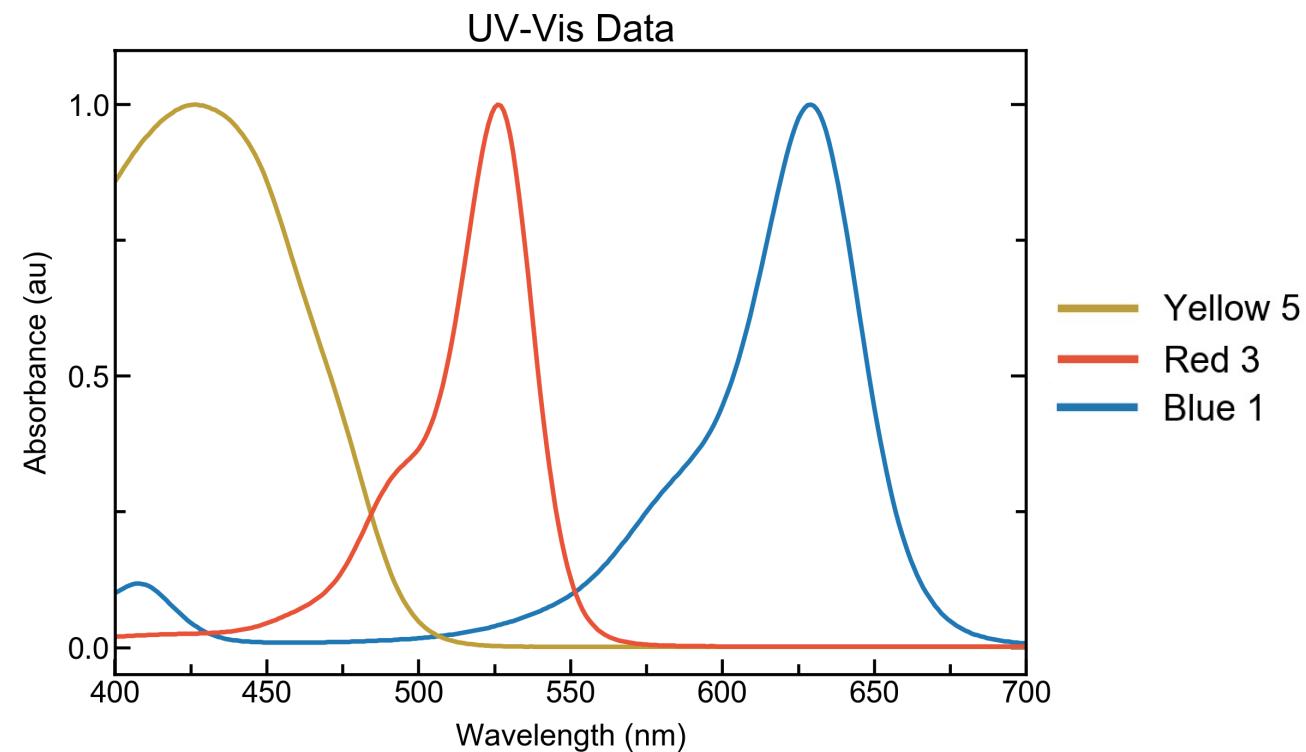
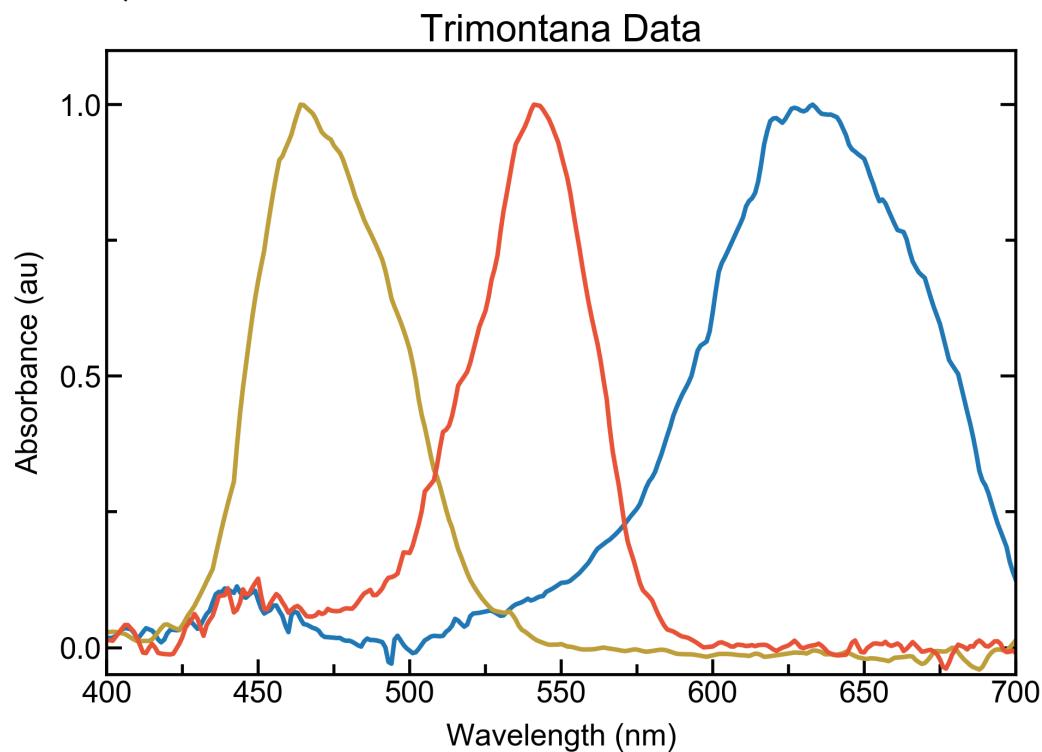
Absorbance axis units are small for water, as the features in the data below only correspond to noise

15.) Save your absorbance data as an Excel file. If time allows, you can quickly plot your absorbance data to make sure there aren't any peaks (remember, this scan should not show any absorbance peaks if you collected your data correctly, apart from normal noise associated with the spectrometer).

****If you need more help plotting your data, please refer to the Excel tips document**

Collect sample spectrum

16.) Repeat steps 10 – 15 (slides 10 – 12) with a food dye sample instead of water (you should have ~3 to choose from). Do not recollect your water reference spectrum unless you have bumped/moved your diffraction grating. You should see a spectrum close to the sample spectra shown below (also shown with a comparison to UV-vis data).



Discussion questions

- (1) What purpose does each of the following components in your spectrometer serve, and how do they work together to give you an absorbance measurement?
 - a. The light source
 - b. The slit and diffraction grating
 - c. The swivel arm/detector
- (2) How does our software determine an absorbance value from the information it receives from the spectrometer? How might scattering affect this measurement?
- (3) List 2-3 steps involved in collecting data (e.g., steps in the sample prep, operating the spectrometer, etc.). What aspects of these steps do you think are the most important for collecting consistent data? Where might you introduce any errors in your measurements?