

PASCO Spectrometer



SCIENCE LEARNING CENTER

Why do we use spectrometers?

- Spectrometers are instruments that measure the **amount of light absorbed** by a substance in solution
- Why do we care about measuring light absorption?
It can help us answer questions like:
 - Is this water contaminated with organic compounds?
 - Which pigments contribute to the color of this leaf?
 - How much Red Dye #40 is in my sports drink?
 - How quickly does bleach reduce the concentration of dye molecules?
- We can use spectrometers to **measure the concentrations** of substances that are often sampled from the environment (e.g., contaminants in air, soil, and water).



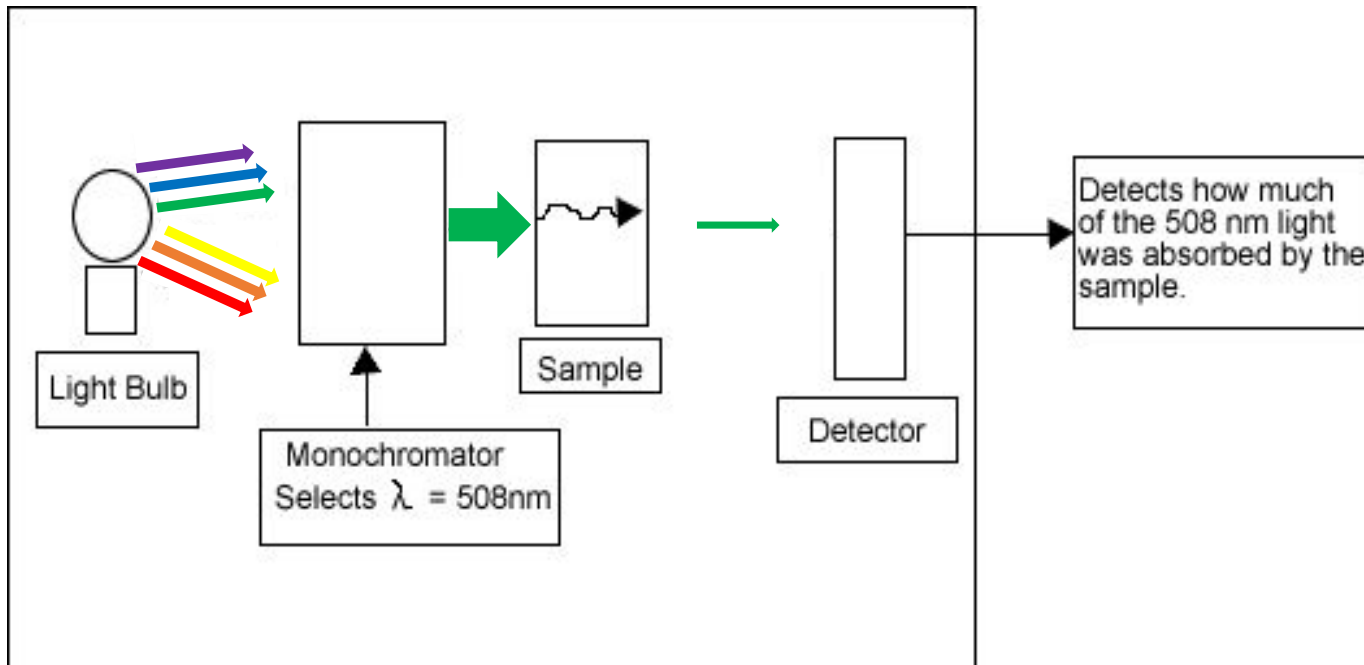
Goal of this Module

- Use the spectrometer to measure light absorbed for a given sample.



How does the spectrometer work?

- A lamp produces white light, then a monochromator selects one color (wavelength) of light to shine on the sample.
- The sample absorbs some of the light.
- The remaining light is transmitted to a detector, which measures the intensity of the light. (The intensity is decreased due to absorption.)



Calibration

- To get accurate measurements, we must calibrate the instrument to subtract two types of interference:
 - light from the environment (Calibrate Dark)
 - absorbance from other molecules in the solution (Calibrate Reference)

Cuvettes

- As seen below, you will need **two** cuvettes:
- one for your reference/blank
 - one for your sample solution



Rinsing Cuvettes

- If the cuvettes are not clean and dry, rinse them thoroughly **with the solution you will be measuring**.
- Several small rinses are preferred over one big rinse in order to coat the inside of the cuvette.

(Rinsing is not necessary for the post-test samples in the SLC.)

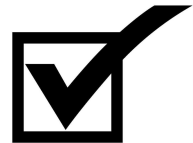


Filling Volume

- We need to fill the cuvette to make sure the light beam will pass through our solution, not air.
- Fill about $\frac{3}{4}$ of the cuvette. (At least 3 cm above the bottom.)

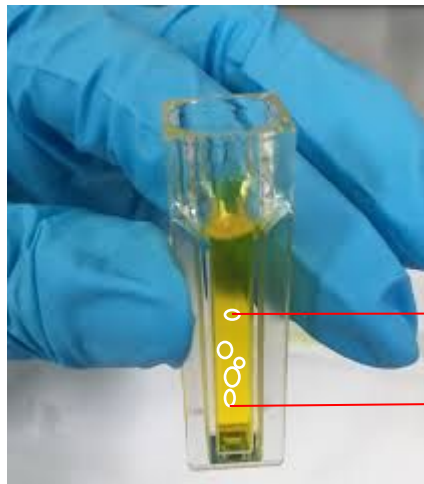


Sufficient
volume



Air Bubbles

- Air bubbles may cause errors.
- Before reading any sample (even a blank), you must remove all air bubbles.



Air
bubbles



Removing Air Bubbles

- To remove air bubbles, gently tap the bottom of the cuvette.
- If unsuccessful, cover the top of the cuvette with Parafilm and gently invert the cuvette several times as needed.



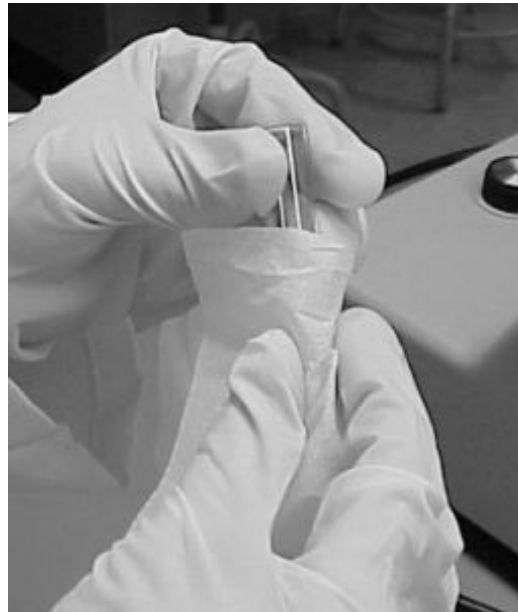
Kimwipes

- Clean the outside of the cuvette using **Kimwipes** before taking data.
- Fingerprints, liquid droplets, and smudges on the cuvette surface can give false light absorbance readings.



Cleaning with Kimwipes

- Dampen a Kimwipe with distilled water
- Clean the cuvette with the damp Kimwipe
- Then clean with a dry Kimwipe



Handling Cuvettes

- After you clean the cuvettes, only handle them above the fill line.
- **Don't touch the lower portion** of the cuvette, or you'll have to clean it again.



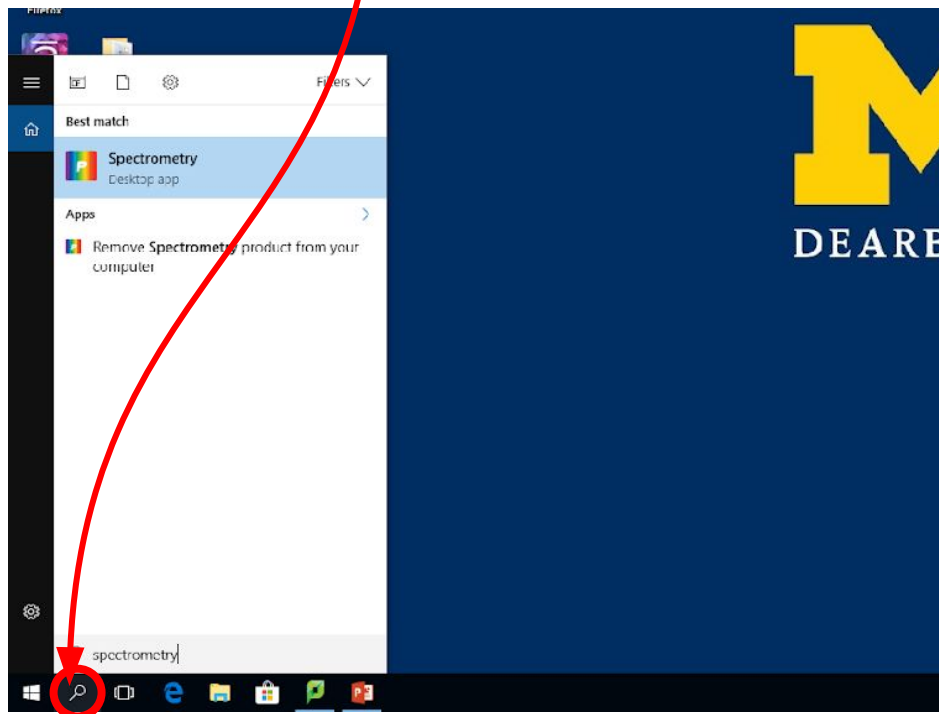
Inserting a Cuvette

- When inserting a cuvette into the chamber, gently push down until the base of the cuvette touches the bottom of the chamber.
- Pushing the cuvette too hard could damage the instrument.



Finding the Spectrometry Program

- Log in to the computer using your email / Canvas credentials
- Click on the magnifying glass on bottom left of the task bar.
- Type “spectrometry”, click on the icon, and the program will open.



Turning on the Instrument

- Make sure the unit is connected to the computer with the USB cord.
- **Turn on** the Pasco unit by pressing the power button until the LEDs blink in sequence



On vs Off

On

- 2nd light is flashing blue
- 3rd light is a constant green



Off

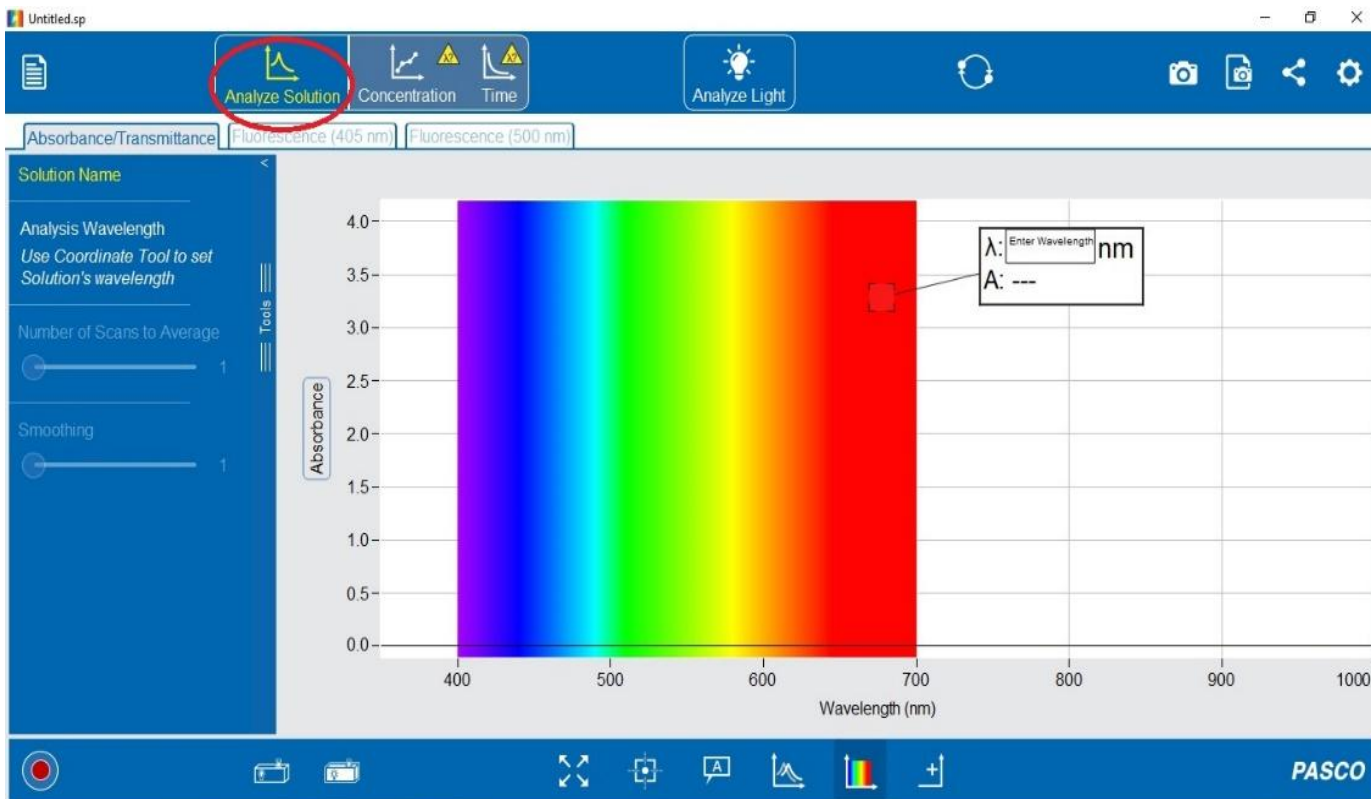
- Only 1st light is active (Green or Red)
- Program will **not detect** the unit



If the unit is **not detected**, make sure **only one copy** of the software is open.

Program Start-up

- When you first open the program, the default experiment type is “Analyze Solution”, shown in the top toolbar.



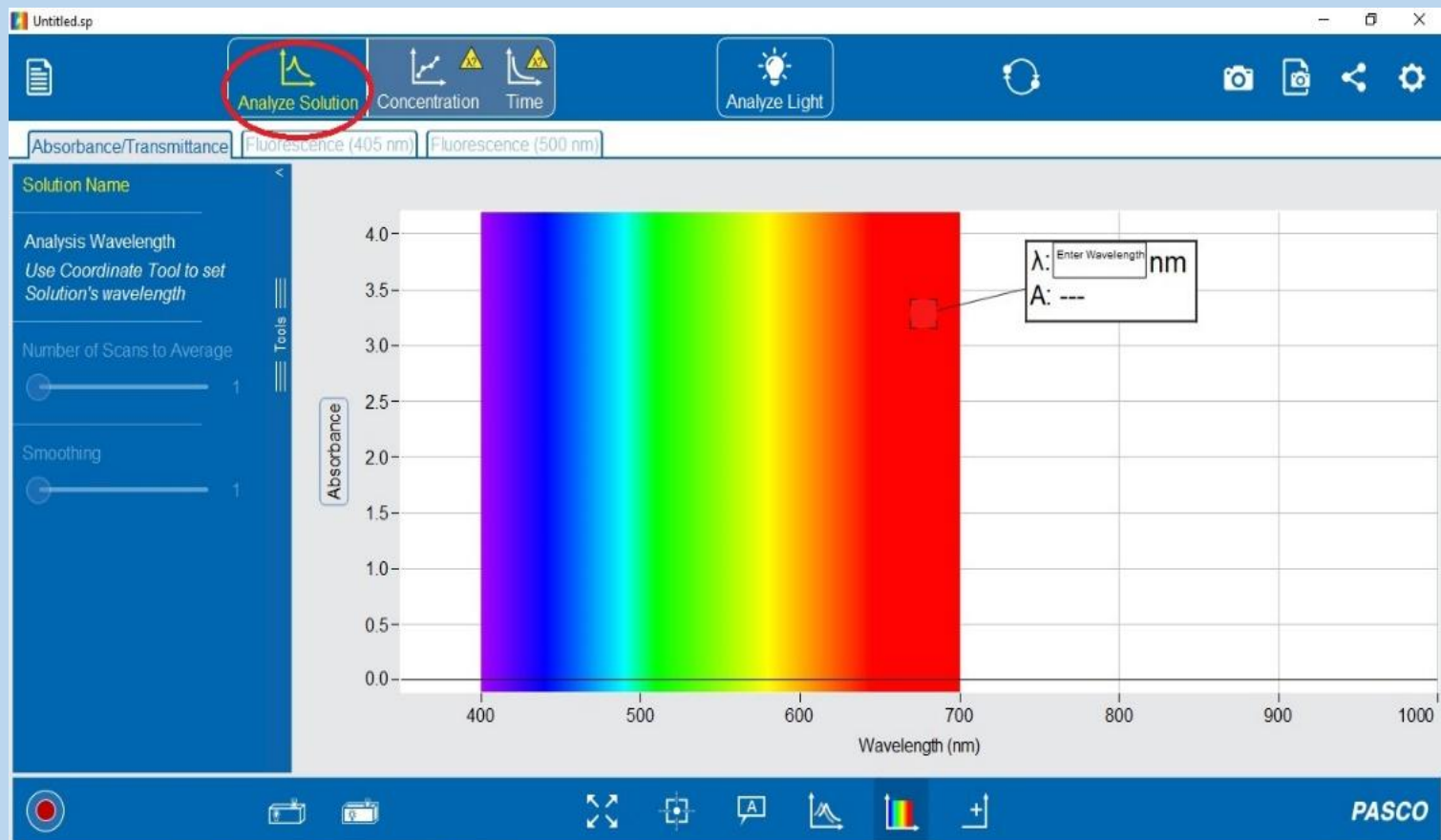
Two types of measurements

There are two types of measurements that can be done using the Pasco Spectrometer.

1. **Constant** absorbance over time
2. **Varying** absorbance over time

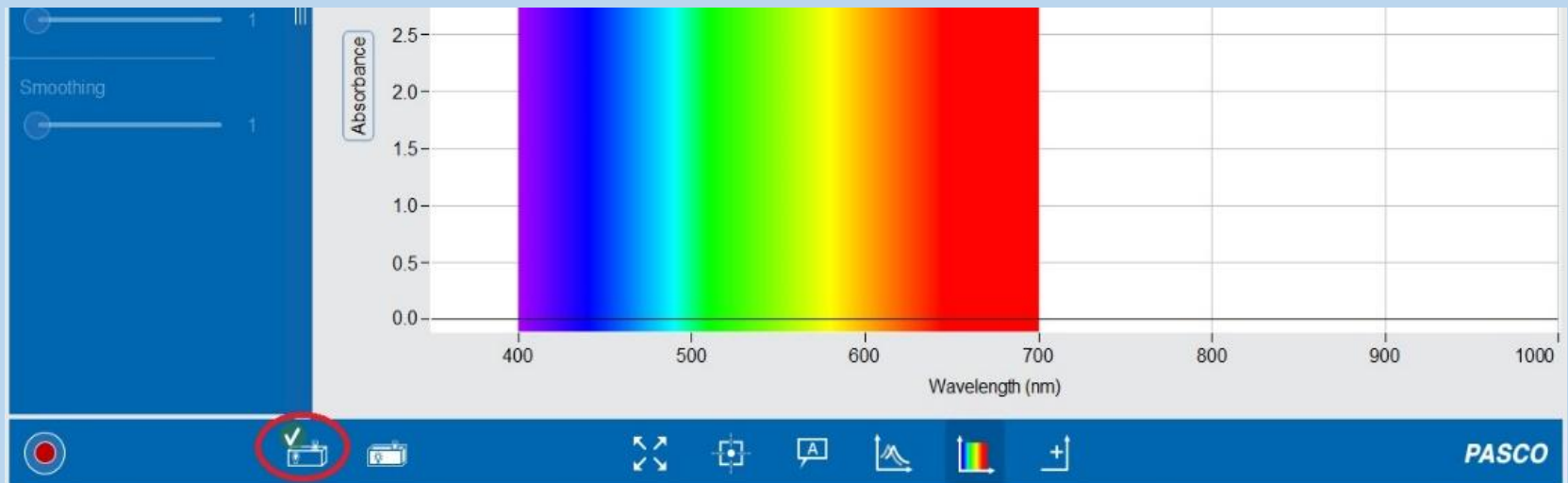
Constant Absorbance Over Time

- The software should be open, with “Analyze Solution” selected.



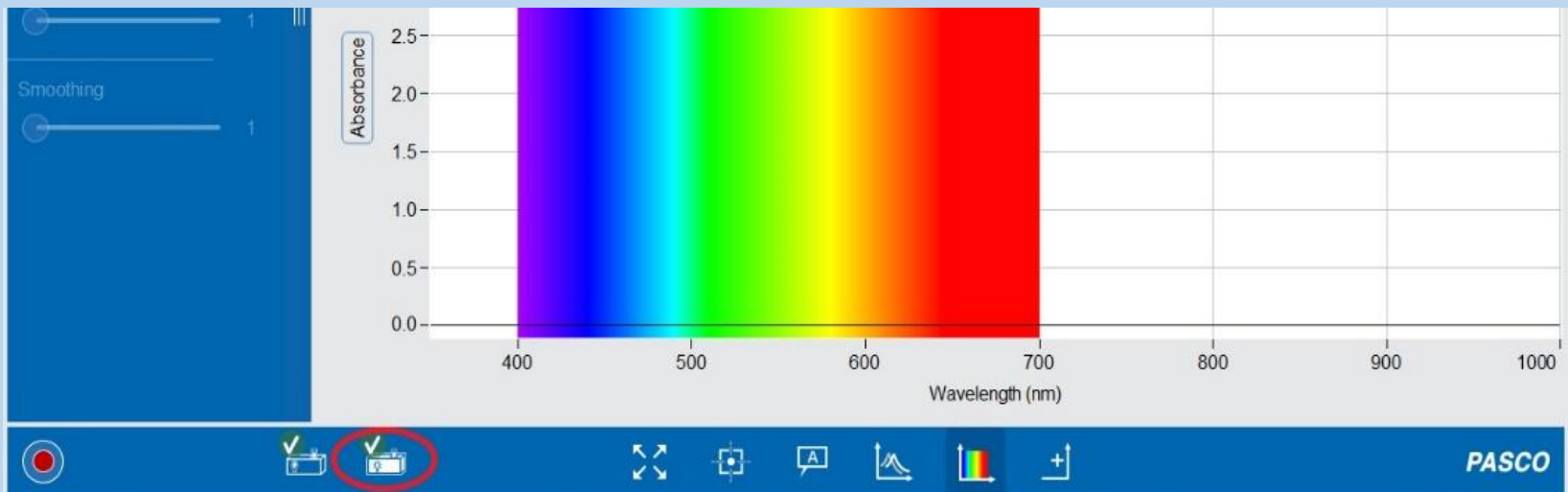
Calibrate Dark

- Insert the reference / blank cuvette into the sample chamber.
- Press the “Calibrate Dark” button, located near the bottom left of the screen. When finished, the symbol will have a check mark.
- This subtracts any stray light from the room.



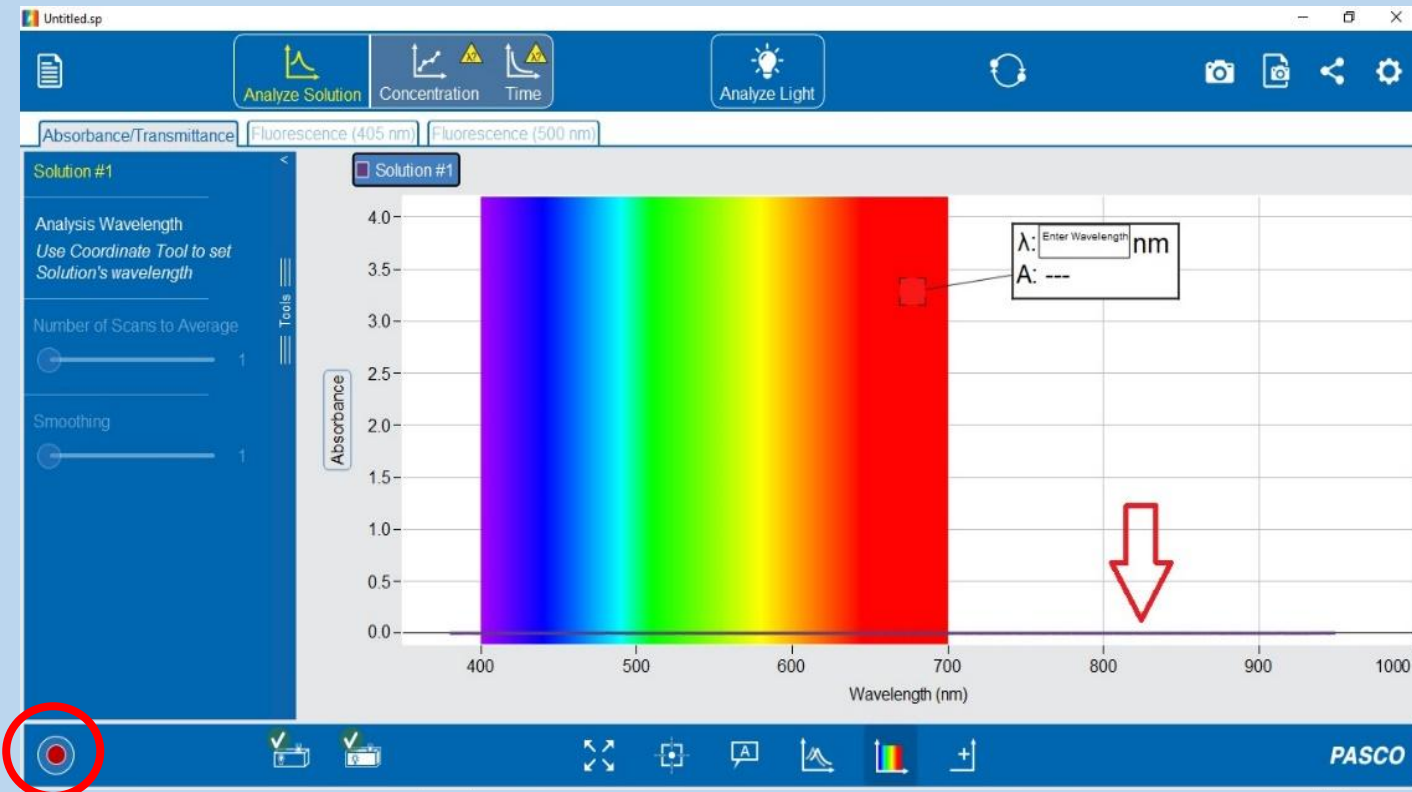
Calibrate Reference

- Wait ~ 1 minute for the instrument's lamp to warm up.
- Click the “Calibrate Reference” button, located next to the “Calibrate Dark” button.
- This subtracts any absorbance from the blank solution (due to solvent molecules, or anything that is not your substance of interest).



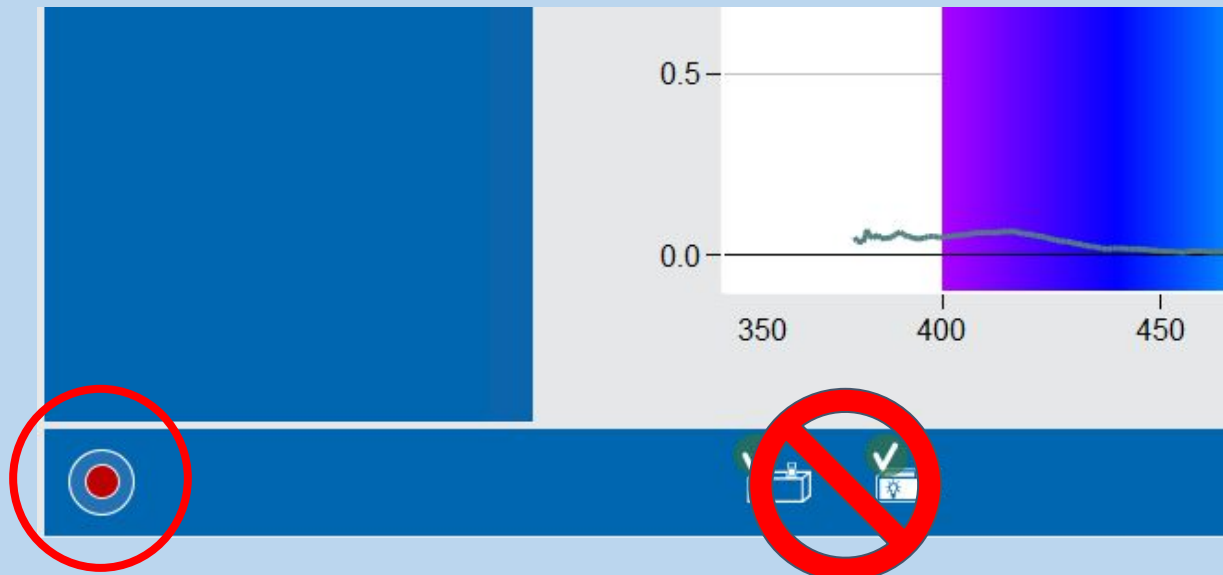
Checking the Calibration

- Click the “Record” button in the bottom left of the screen. The button will become a square while recording.
- Press the button again to stop recording. Because this is the blank solution, a flat line should appear at Absorption = 0.



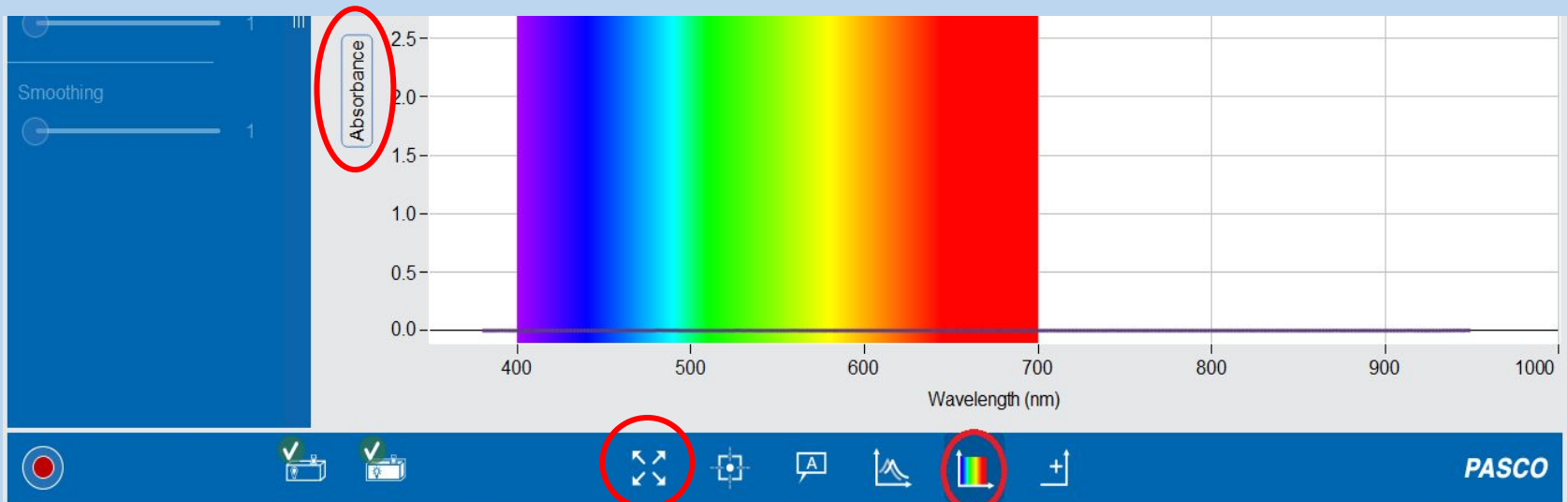
Taking Data

- Remove the reference / blank cuvette from the sample chamber.
- Insert the sample cuvette with your solution to be analyzed.
- **Do NOT** click the calibration buttons or you will zero out your signal.
- **Record** the spectrum for a few seconds.
- Stop recording.



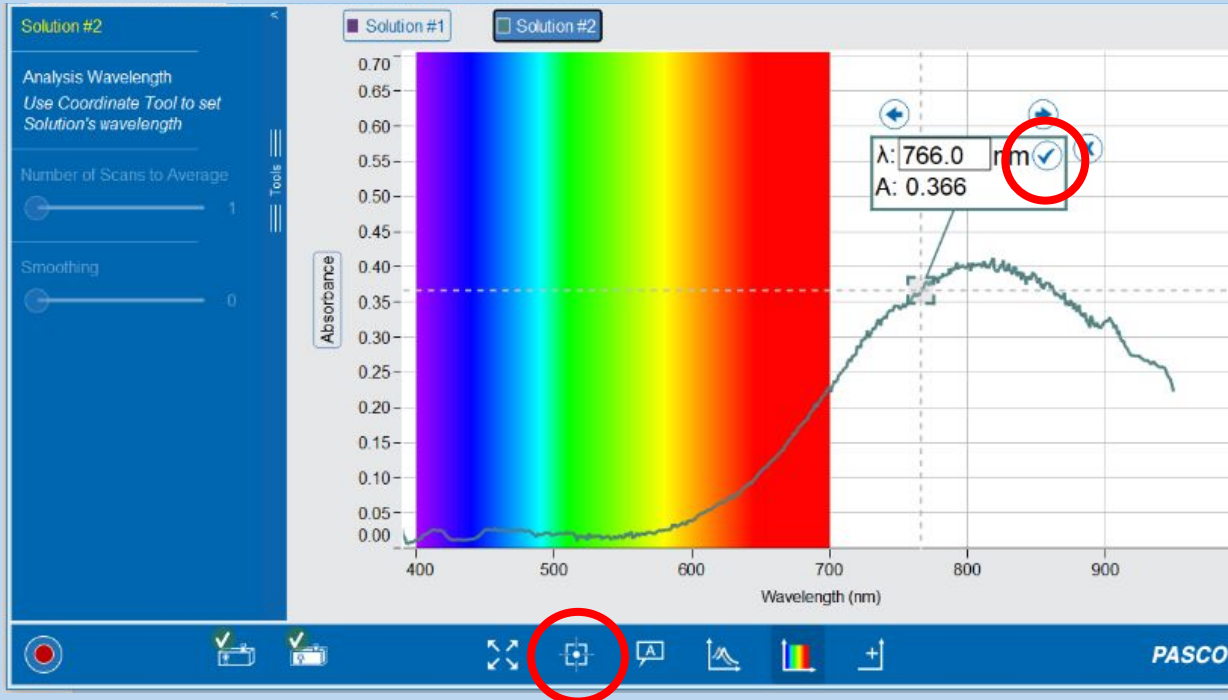
Viewing the Spectrum

- The rainbow background can be toggled on and off by clicking the “Color Spectrum Background” button on the bottom toolbar.
- To adjust the axes, use the “Scale to Fit” option, or click and drag on either axis between the values.
- Absorbance can be changed to Transmission by clicking on the “Absorbance” axis label.



Choosing a Specific Wavelength

- Click “Coordinate Tool” on the bottom toolbar.
- Drag the crosshairs or type a number to select the desired wavelength. This is usually the **highest point** on the curve.
- To select this wavelength for further analysis, click the blue check mark next to the wavelength.



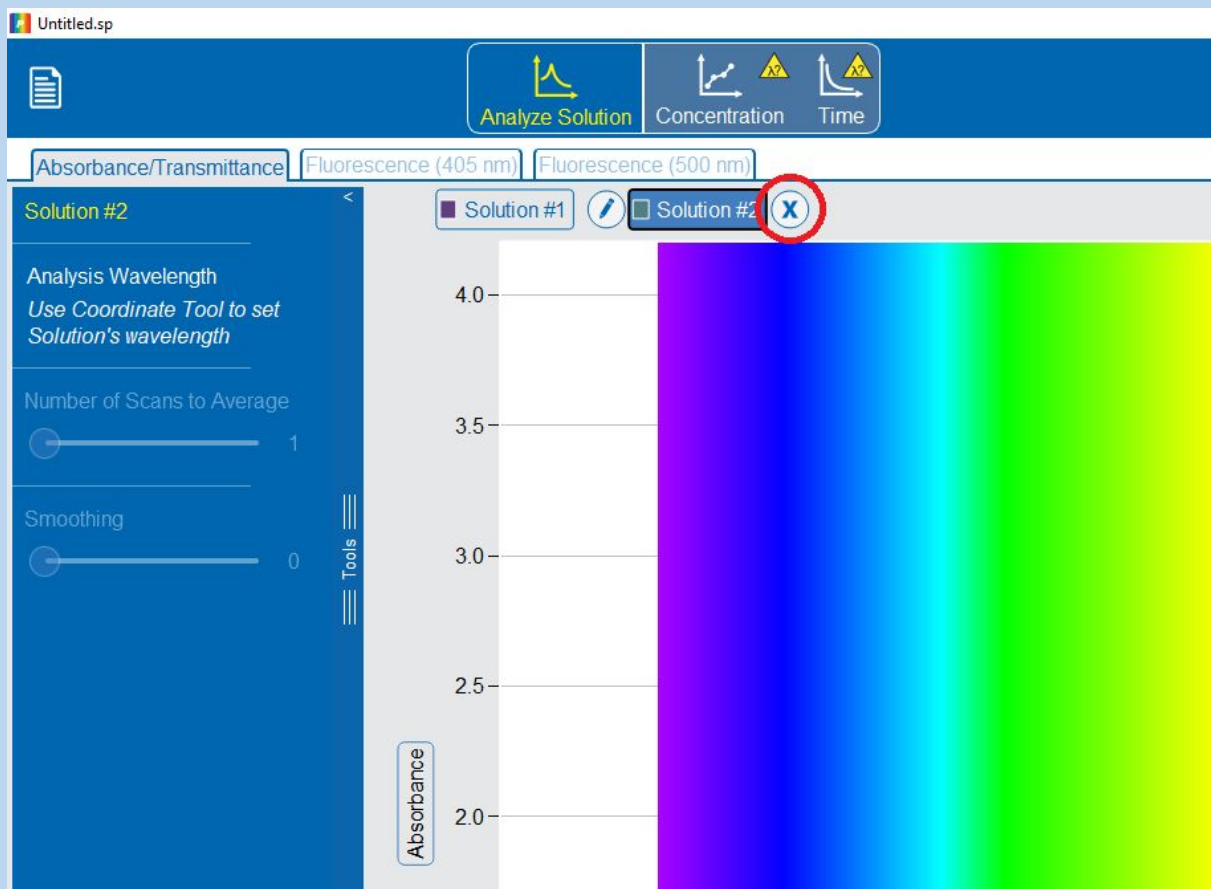
Saving Data

- The camera icon will take a snapshot that you can save as a .png file.
- To save values for all points along the graph, the data can be exported as an Excel file.
 - Click the “Share” button in the top toolbar on the right. This will pull down an option to export data.



Deleting Data

- To discard data, select the data set at the top left and click the “X”.

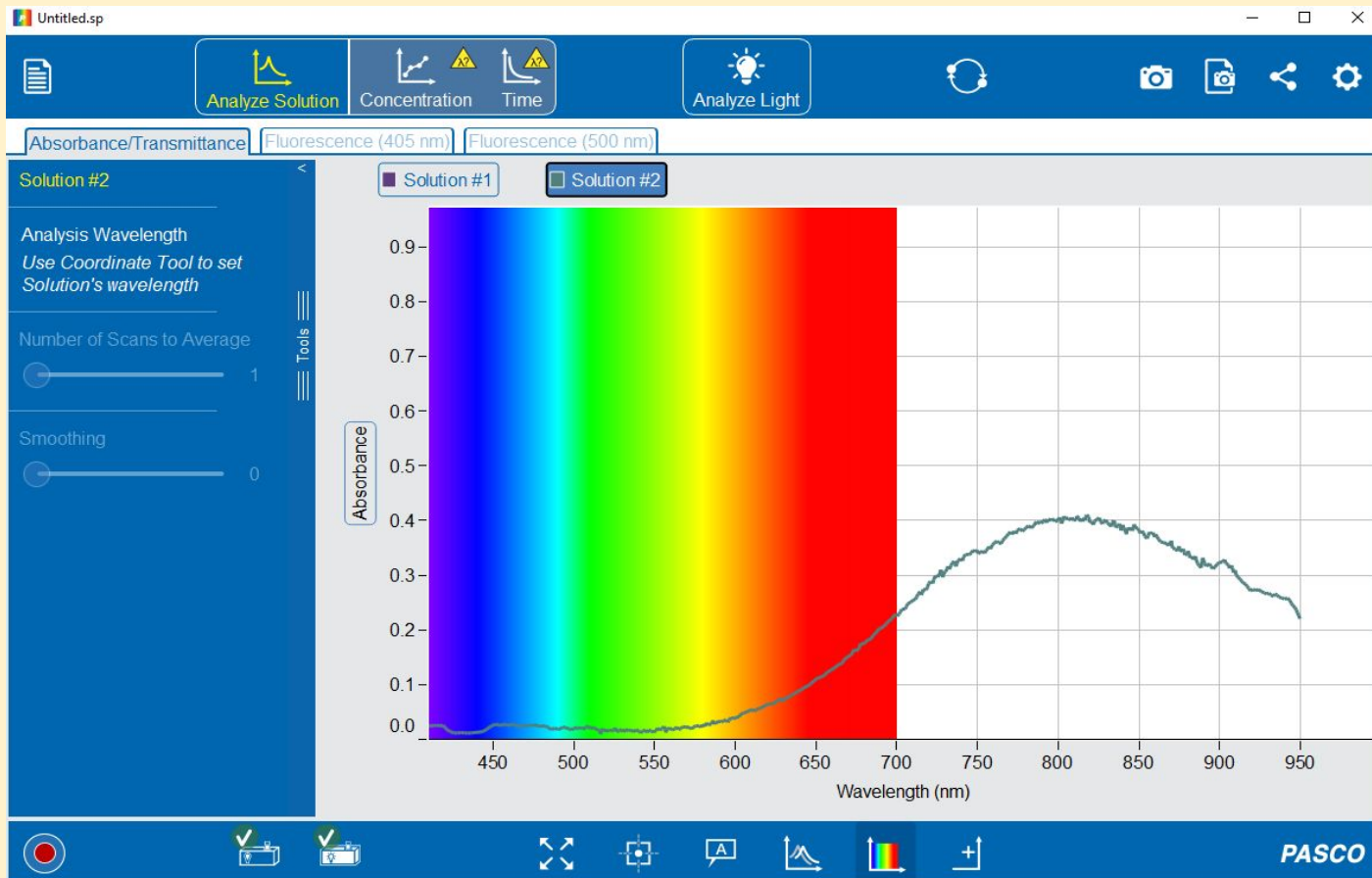


Moving from
constant absorbance over time
to
varying absorbance over time

- We've been analyzing a solution whose absorbance doesn't change.
- Now, we will analyze a solution whose absorbance changes over time.

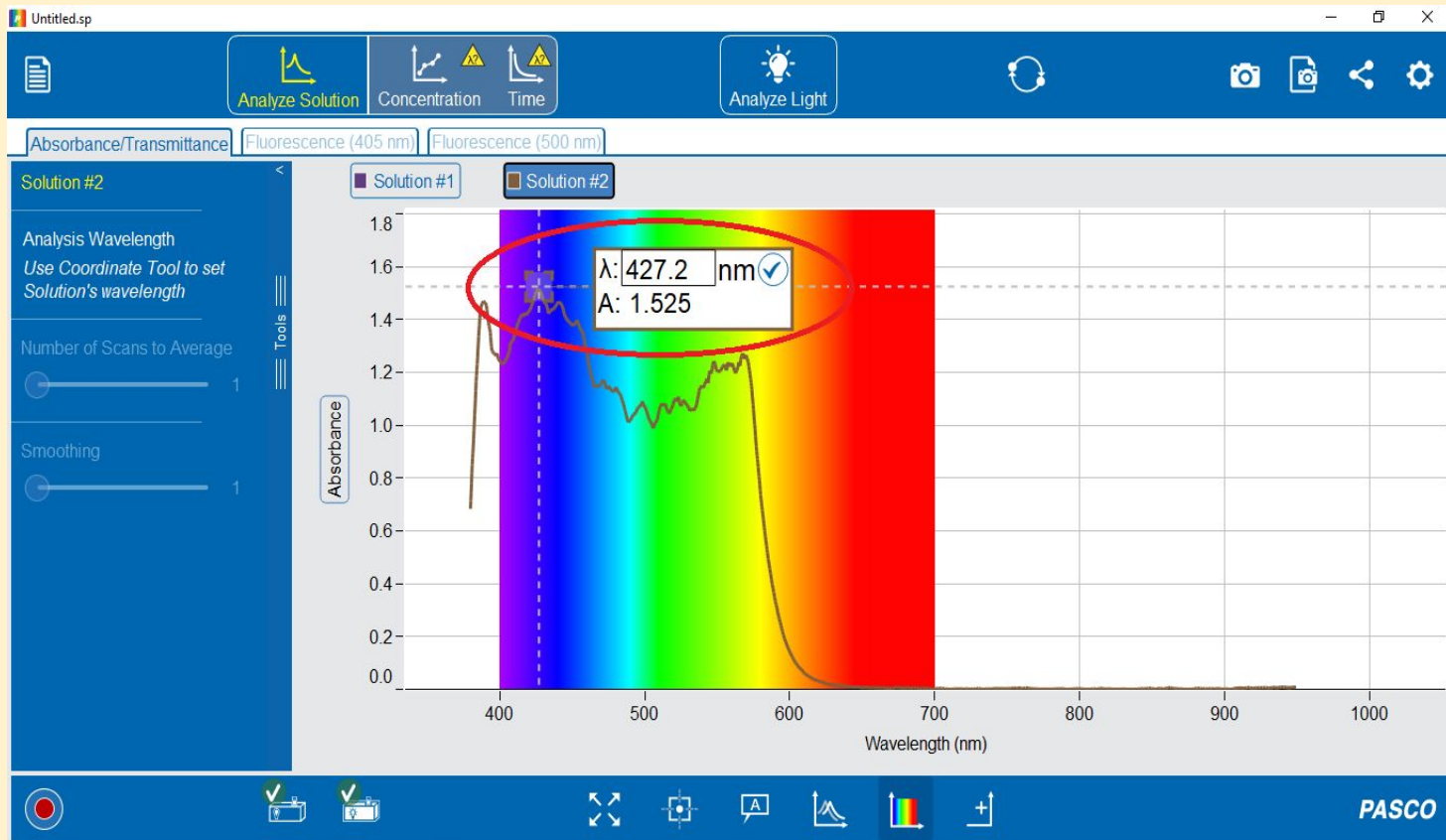
Absorbance that Varies over Time

- Follow the previous steps to calibrate and record a spectrum.
- You must choose a specific wavelength to observe.



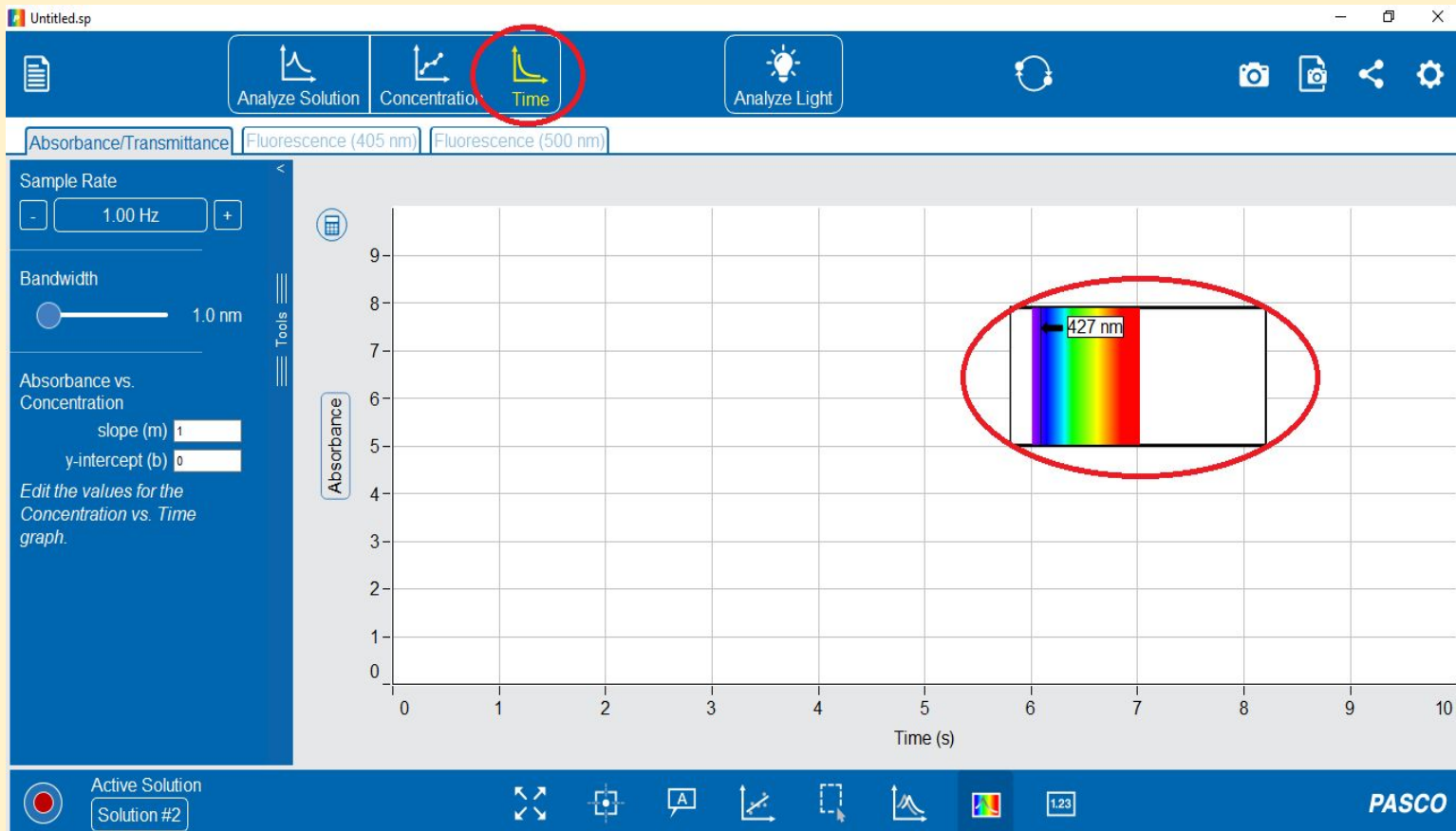
Choosing a Specific Wavelength

- Use the coordinate tool to select the desired wavelength.
- Click the check mark to use this wavelength for the time analysis.



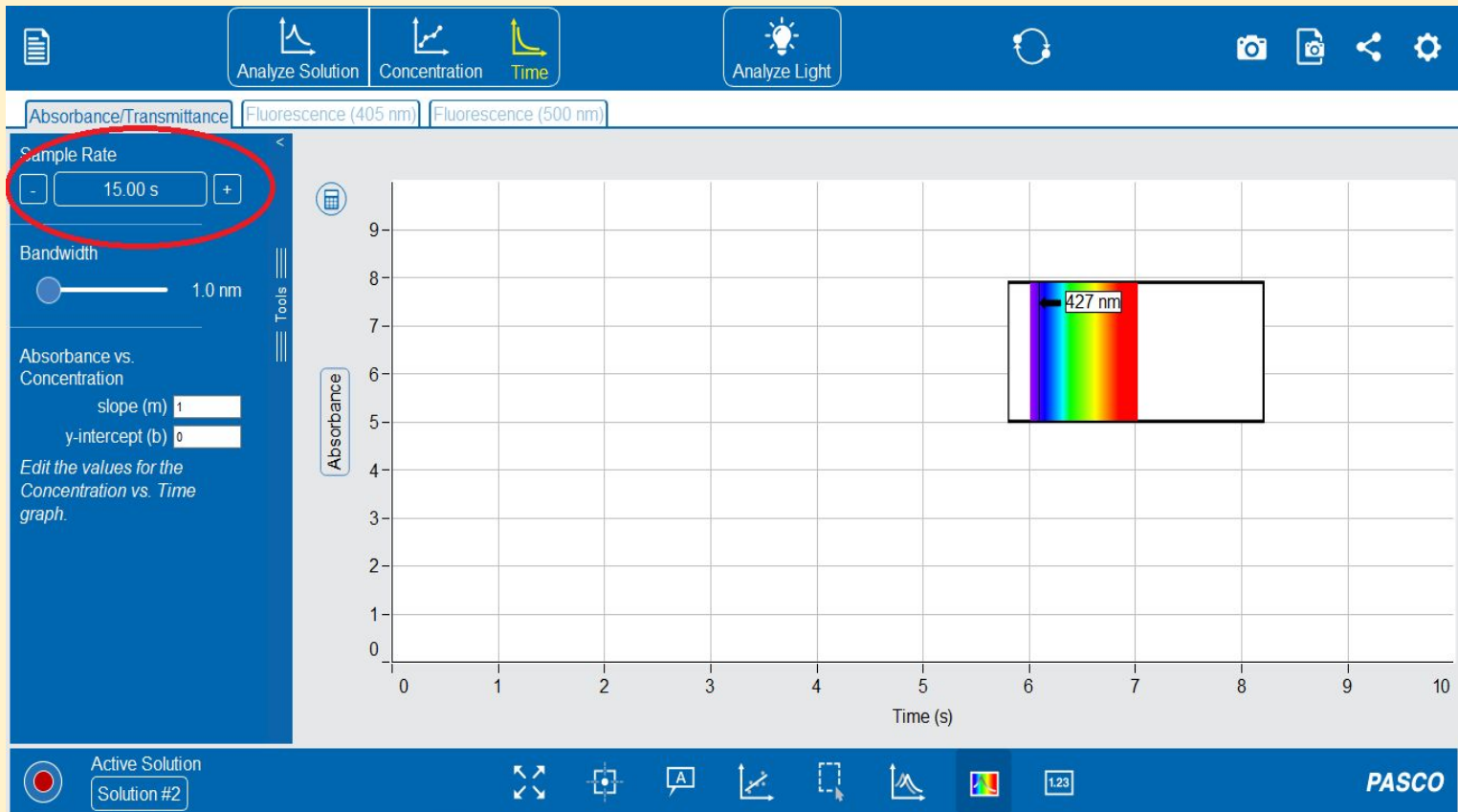
Time Analysis

- Click the “Time” button on the top toolbar.
- Notice the small window displays the wavelength to be monitored.



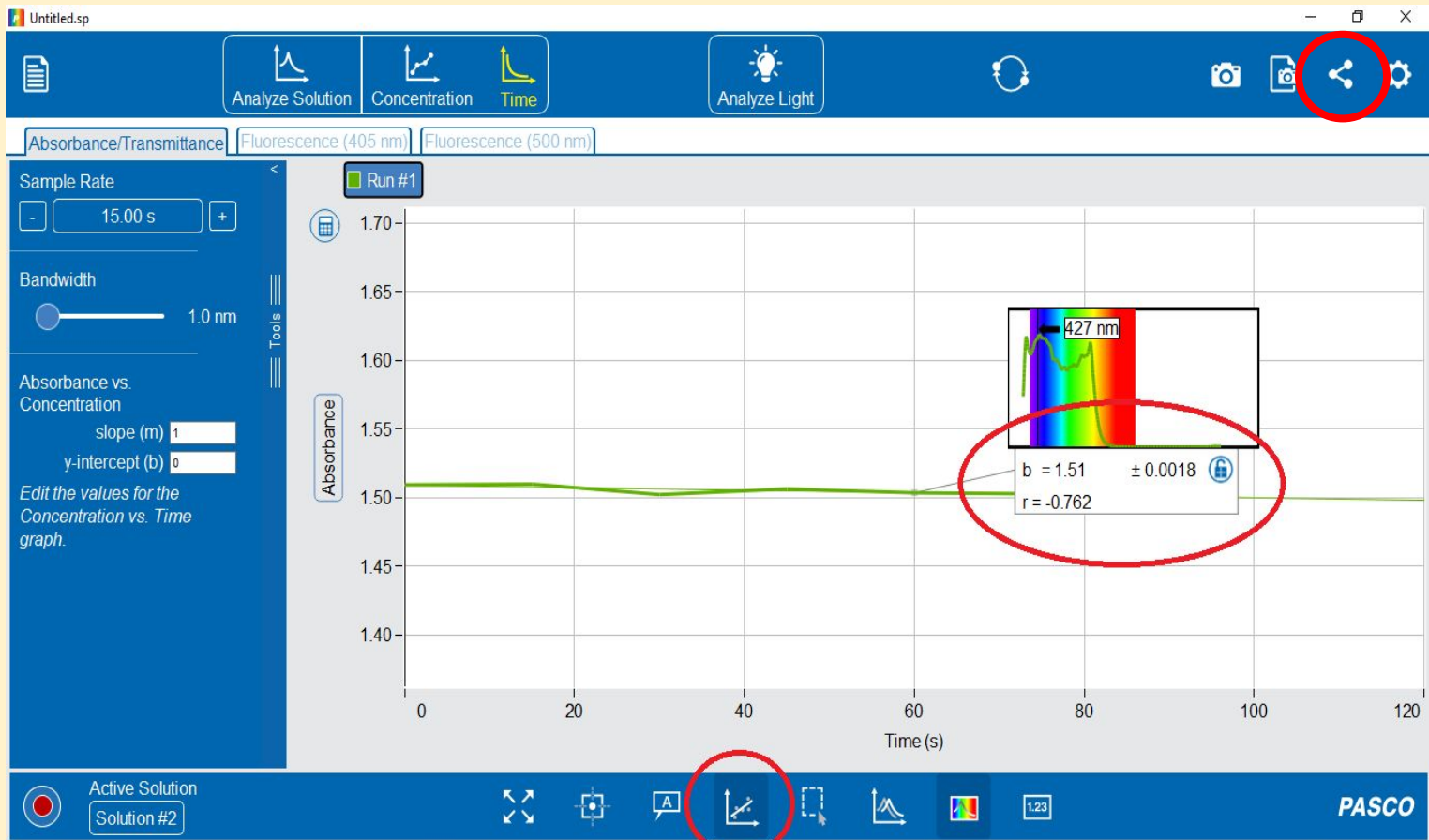
Taking Data

- “Sample Rate” determines how often readings will be taken. Click the plus or minus sign to adjust the time between readings.
- Click **Record** to begin collecting data.



Analyzing and Saving Data

- You can fit your data using “Show linear fit” in the bottom toolbar.
- To export the data for further analysis, click the “share” button.



Practice

(Do not write on here)

Record the letter of the sample solution that you have:

Unknown sample _____

1. Determine the Absorbance (A) of the sample at 590 nm:

A = _____

2. Determine the Absorbance (A) of the sample at 630 nm:

A = _____

3. Take snapshots of your absorbance spectrum with and without the rainbow background

Post-test

- When you feel confident in your ability to use the Spectrometer, ask the Science Learning Center staff for a post-test.
- You will measure the absorbance of an unknown sample.