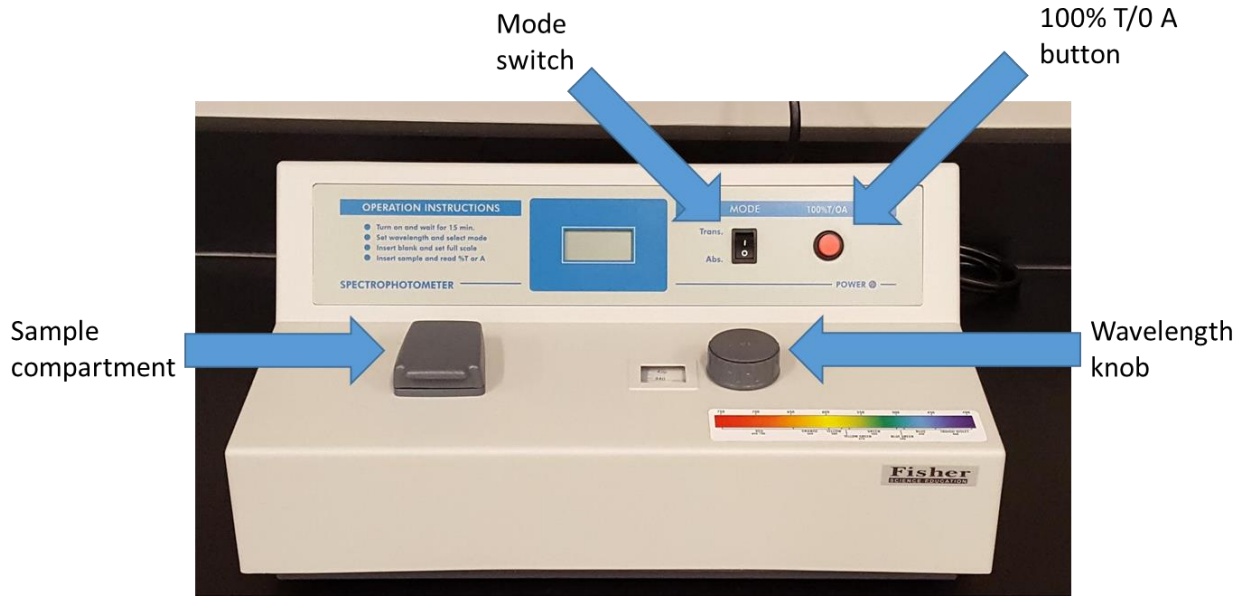
Spec 20 General Instructions

The first step in this process will be done before Gen Chem lab starts. Students should NOT do it themselves without explicit permission. If something seems wrong, ask your instructor.

Step 1: Turn on the spectrophotometer (switch on the back right), and allow the lamp to warm up for at least 15 minutes.



Collect background – This corrects for the absorption by the cuvette and solvent. It needs to be done for each set of samples. Background collection can be done in either transmittance or absorbance mode.

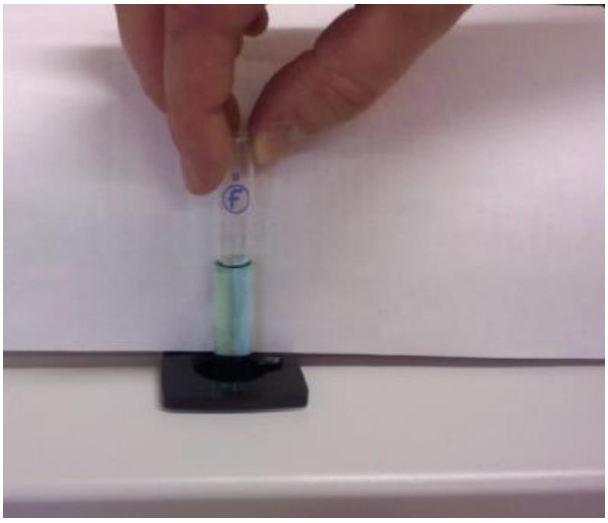
Step 2: Adjust the wavelength knob so that the desired wavelength appears in the window.

Step 3: Fill a blank cuvette no more than two thirds full with the solvent (often DI water). See picture below for appropriate level.

Step 4: Dry the outside of the cuvette with a Kim-wipe to remove moisture and smudges. Insert cuvette into the sample compartment. NEVER TWIST THE CUVETTE ONCE IT HAS BEEN PLACED! Close the lid.

Step 5: Press the 100% transmittance/ 0 absorbance button (red button on the right). Ensure that the display reads 100% when in percent transmittance mode or 0 when in absorbance mode.

The spectrophotometer is now zeroed and ready to collect data. If you are prepared to analyze your samples, steps 2-5 will not need to be repeated unless you step away from the spectrophotometer.



Data Collection.

Step 6: Fill a cuvette no more than two thirds full with your sample (see picture above). Wipe the outside of the cuvette with a Kim-wipe to remove moisture and smudges.

Step 7: Insert your sample. Do not twist the cuvette once it's been inserted. Close the lid .Be sure the mode is set to absorbance, and record the sample's absorbance.

Step 8: Repeat steps 6&7 for all samples.

Important notes

Do not turn the spectrophotometer off unless specifically instructed to do so. If it is switched off, allow 5 minutes before turning it back on. Then wait 15 minutes for the lamp to warm up.

Be careful not to get any liquid in the sample compartment. If a spill occurs, notify your instructor immediately.

The hardest part is often keeping track of which sample is which. NEVER LABEL CUVETTES. Instead, label the test tube rack.

Prepare all samples before you start measuring anything on the spectrophotometer.

If the absorbance is greater than 1 or the percent transmittance is negative, your sample is too concentrated for the instrument (or you didn't calibrate it properly (steps 2 – 5)). Ask your instructor how you should proceed.