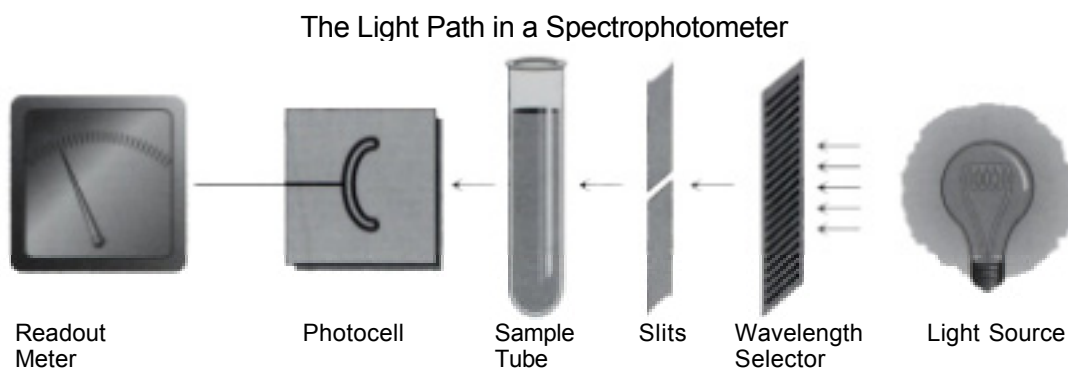


## The Spectrophotometer

Molecules can absorb or transmit electromagnetic radiation. White light is the combination of all wavelengths of the visible spectrum. The different colors we see are determined by how light waves are absorbed and transmitted by objects or solutions.

A spectrophotometer can be used to measure the amount of light absorbed or transmitted by molecules in a solution. When a wavelength of light is transmitted through a solution, the light energy absorbed, called absorbance ( $A$ ), is directly proportional to the ability of the solute molecules to absorb light of that wavelength, as well as the concentration of the solution and the length of the light path from its source (typically 1 cm in a spectrophotometer) to the point where the percentage of light energy transmitted or absorbed is measured by a phototube.



Most spectrophotometers in biology use UV or visible light. The wavelength that will be maximally absorbed is typically selected. After the light passes through the test solution, the light energy that strikes the phototube is expressed as the ratio of transmitted light  $I_T$  (the light that passes through the sample) to incident light  $I_0$  (the intensity of light at the source before it enters the sample). The light received at the phototube is measured as percent transmittance ( $T$ ) or as the log of its inverse, absorbance ( $A$ ). We measure absorbance in our laboratory exercises.

$$\%T \text{ (percent transmittance)} = \frac{I_T}{I_0} \times 100$$

$$A \text{ (absorbance)} = \log \frac{I_0}{I_T}$$

Bellevue College's Biology department uses the Unico Spectrophotometer, Model 2100. Instructions for using the Unico Spectrophotometer 2100 are provided below.

The Bellevue College Chemistry department has Thermo Scientific Spectronic 20D+ spectrophotometers that are sometimes used in Biology. Students in Biology 211 A, fall 2009 provided the set of instructions for using the Thermo Scientific Spectronic 20D+ below.

## Procedure for using the Unico Spectrophotometer, Model 2100

1. Set up the instrument away from direct sunlight, and any strong magnetic or electrical fields.
2. Select 115V on the voltage selector.
3. Plug in the instrument.
4. Turn on the instrument and allow it to warm up for 15 minutes.
5. The instrument performs self-calibrations when the power is turned on. Inform your instructor, if error codes appear.
6. Press **Mode** until the red light for **T** (%transmittance) or **A** (absorbance) is on.
7. Choose the wavelength by pressing the up or down arrows.
8. The readout will display **BLA**, this means that a reference is necessary.
9. Make a blank reference by filling a clean cuvette half full with distilled water or designated control substance.
10. Wipe all sides of the cuvette with Kim Wipes, and hold the cuvette by the frosted (grooved) sides.
11. Fit the blank cuvette into the slot closest to you on the 4-cell stage. Be sure the frosted sides of the cuvette are not in the light path.
12. Push the 4-cell stage in so the cuvette is in the light path.
13. Press the **0ABS/100%T** button.
14. Fill your sample (test) cuvette(s) half full with the sample solution and wipe the sides with Kim Wipes.
15. Put the sample cuvette(s) in the sample compartments.
16. Close the lid.
17. Read and record your data.
18. Move the stage to read and record your other samples.
19. To test the samples at other wavelengths, repeat steps 7-18.

### Special Note

- The instrument must warm-up for 15 minutes for proper functioning.



Front Panel

- |                                 |                       |
|---------------------------------|-----------------------|
| 1. Digital Readout              | 6. Enter (ENT) Button |
| 2. Wavelength Display           | 7. 0A/100%T Button    |
| 3. Mode Indicator               | 8. Mode Button        |
| 4. Wavelength Control Button    | 9. Print Button       |
| 5. Concentration/Factor Buttons |                       |

## Procedure for using the Thermo Scientific Spectronic 20D+ Spectrophotometer

1. Plug in and turn on the spectrophotometer.
2. Use the **wavelength knob** to select the desired wavelength
  - a. Increase wavelength by turning to the left
  - b. Decrease wavelength by turning to the right.
3. Use the **Mode** button to set the spectrophotometer to the desired reading (*Absorption*, *Transmission* or *Concentration*). The light to the left indicates which reading is being selected.
4. Calibrate the spectrophotometer
  - a. Fill a cuvette (provided) with distilled water or solvent (depending on the exercise) to just below the bottom of the vertical line on the cuvette.
  - b. Wipe the cuvette to ensure there are no fingerprints or liquid drops on the tube.
  - c. Insert the cuvette into designated area of the spectrophotometer (see illustration), wait for a "click" sound, and close the lid.
  - d. Use the knob labeled **100%T/OA** to calibrate the spectrophotometer
    - i. Turn the knob to the right to increase
    - ii. Turn the knob to the left to decrease.
  - e. The spectrophotometer should read "0" when calibrated.
5. Data Collection
  - a. Fill your sample (test) cuvette(s) half full with the sample solution and wipe the sides with Kim Wipes.
  - b. Put the sample cuvette(s) in the sample compartments.
  - c. Close the lid.
  - d. Read and record your data.
6. Move the stage to read and record your other samples.

Mode : changes reading between Transmittance, Absorbance, and Concentration

