SPECTROPHOTOMETER USE

David B. Fankhauser, PhD

15 November 1982, rvsd 3 June '94, 9 July '95, 30 June '96, 3 July '97, 5 Sept '97, 28 June 02
http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Growth_Curve/Spectrophotometer.htm

The spectrophotometer is an instrument which measures the amount of light of a specific wavelength which passes through a medium. According to Beer's law, the amount of light absorbed by a medium is proportional to the concentration of the absorbing material or solute present. Thus colored solute concentrations may be determined in the lab by using a spectrophotometer to measure the absorbency of a solution at the wavelength (λ) of light which corresponds to the absorption maximum of the solute. Absorbency is indicated A_λ.

To learn to use the spectrophotometer, illustrate the instrument and label the following features which are important to its proper use. You should know the function and/or significance of each of these features before you use the instrument. Remember that the cuvettes in which the specimen is tested are carefully manufactured for their optical uniformity, are quite expensive, and should be handled and separately stored with care so that they do not get scratched. (Do not mix with standard test tubes.)

At the spectrophotometer, you should have two cuvettes in a plastic test tube rack, one for the blank (marked "B") and one for your sample (marked "S"), wipettes to polish them before insertion into the cuvette chamber, and your samples which you are testing.

TERMS TO LEARN AND/OR INCLUDE ON YOUR DIAGRAM:

power switch *and* zero adjust knob	read-out dial in absorbency and % transmittance: show numbers
blank adjust knob	cuvettes, marked B and S (blank and sample)
wavelength	cuvette chamber
wavelength selection knob	blank
absorbency	parallax error (error due to reading a dial from the side)
% transmittance (not used here)	Beer's Law

WARM-UP:

1. Plug in and turn on (left hand front dial). Allow about 30 minutes for warm up.

ZERO ADJUST:

2. With no cuvette in the chamber, a shutter cuts off all light from passing though the cuvette chamber. Under this condition therefore, the machine may be adjusted to read infinite absorbance (zero% transmittance) by rotating **zero adjust knob** (front left on Spectronic 20). *Do not touch this knob again during the rest of the following procedure.*

SELECT WAVELENGTH:

3. Select the desired wavelength of light at which absorbance will be determined by rotating **wavelength selection knob** (top right knob) until the desired wavelength in nanometers appears in the window. A nanometer (nm), formerly millimicron, equals 10^{-9} meter.

BLANK ADJUST:

4. Fill the B cuvette with the solvent used to dissolve specimen (often distilled water). Polish to clean, insert into the cuvette chamber, mark to front. Close chamber cover.
5. Rotate **blank adjust knob** (front right knob) to adjust absorbance to read zero.
6. Remove blank cuvette, place in plastic test tube rack.

READ SPECIMEN:

7. Pour the sample into the S cuvette, polish and insert into the chamber, mark to the front.
8. Read absorbance of dissolved specimen, record. (Note: scale reads from R to L.)
9. If you read additional specimens, you should confirm that the machine is still zeroed and blanked out, as in steps 2, 4 and 5 for all readings.

CLEAN UP:

10. Remove cuvette from machine, carefully wash and store spectrophotometer cuvettes keeping them separate from regular test tubes.