

## Operating Instructions for Agilent 8453 UV-Visible Spectrometer

### Turning on the Instrument

1. Press the power button on the bottom left side of the instrument.
2. Turn on and/or log on to the computer (user name and password are given on the computer).
3. Click on the prism icon on the computer screen titled Instrument 1 online to start the software. When the window asks for a user name and password, hit cancel.
4. On the software, the lamp pictures should be red and yellow, respectively. If not, the lamps are not turned on. To do so, click each lightbulb and select "Lamp On".
5. The instrument should warm up at least 10 minutes prior to collecting data.

### Collecting Spectra Using Standard Mode

This is the default working mode of the spectrometer and should be useful for most data collection needs.

1. Set the parameters for your data collection by clicking "Setup" next to the drop-down box which should be set to "Fixed Wavelengths".
  - a. In the Use Wavelengths box(es), you can set the values for the peaks you would like the instrument to automatically measure. For quantitation of protein concentration, you would type "280" into the first box. Note that after the data is collected, you can click "Setup" again and change this value. The data on the screen will update accordingly.
  - b. Set the method you would like to use for background correction. Most common is single reference wavelength. In this case, enter a value for where you would like the spectrum to be "zeroed" at. Typically, 800 nm is used.
  - c. All other parameters can be left alone.
2. Insert the cuvette into the cuvette holder with the windows facing the front and back of the instrument.
3. Push the lever on the left side on the sample holder down to lock the cuvette in place.
4. To collect a background scan, click "Blank".
5. After a background scan has been collected, you can collect sample spectra by clicking "Sample" once you have inserted your sample into the cuvette holder.
  - a. You can collect more than one spectrum (e.g. multiple samples) by simply clicking "Sample" again after inserting a new sample. The computer will display all spectra collected.
6. To save your data, go to "File" and select "Save Samples As..." This will save all of the data on the screen. To save a single spectrum, click on the spectrum itself and then under "File" select "Save...Selected Spectrum As..."
7. Data can be exported to other file formats by clicking on a single spectrum and going to "File" and then "Export Selected Spectrum As..."