



Beckman Coulter DU[®] 800 Spectrophotometer

Bergen County Technical Schools
Stem Cell Lab



**Beckman Coulter DU[®]800 Spectrophotometer Safety Sheet**

1. Samples should be handled according to good laboratory procedures and methods in order to prevent accidents.
2. Dispose of all waste solutions according to waste disposal procedures.
3. Do not leave any cuvettes in the instrument after finished using the instrument. An unknown sample left for the next user to clean up, whether it is water or a harmful chemical, could cause an accident in the lab.
4. Do not remove any panels or cords from the instrument to avoid electrical shock.
5. Cleaning the workstation around the instrument is necessary. The internal cuvette stage of the instrument may be cleaned with a lint-free cloth, but never attempt to clean any additional internal spaces of the instrument.
6. The instrument contains a Deuterium source bulb and a Tungsten Source bulb.
 - a. The bulbs should be turned on at least 20 minutes before use to allow them to warm up, and allow for the most accurate measurements.
 - b. These bulbs can get warm and have a finite life span. If not being used, the bulbs should be turned off and the instrument shut down.
7. Food and drinks should not be placed on or near the instrument.
8. Running unauthorized programs or changing preferences on the instrument's computer is not allowed. Laptops are provided for personal use and internet access. Do not plug memory sticks in the instrument's computer unless instructed to do so by the instructor.



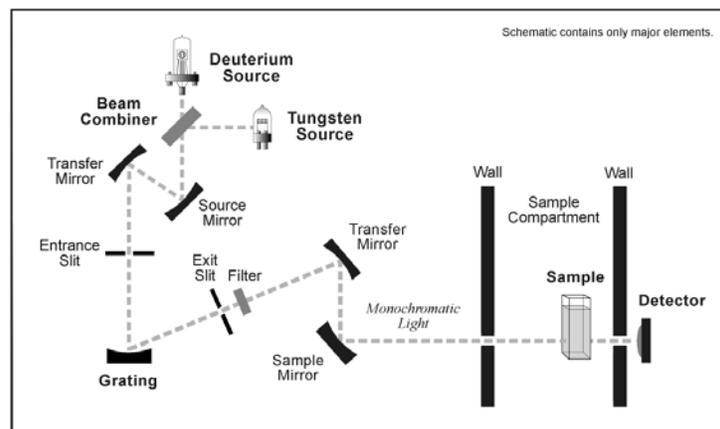
Beckman Coulter DU[®]800 Spectrophotometer Information Sheet

The Beckman Coulter DU[®]800 Spectrophotometer is a PC controlled system intended for use in quantitative and qualitative analysis of biological and chemical agents using spectrophotometric analysis of the UV and visible light regions of the electromagnetic spectrum. The instrument operates within the wavelength range of 190 to 1100nm, and has a bandwidth of $\leq 1.8\text{nm}$. These wavelengths are achieved by using two different source bulbs, a Deuterium bulb (for UV wavelengths) and a Tungsten bulb (for visible light wavelengths), to make up the focused micro-beam. The instrument design allows for small volume samples and precious samples to be analyzed easily.

All control of the instrument, data handling, and data reduction capabilities can be performed by using the systems software on the connected PC control system.

Principle of Operation

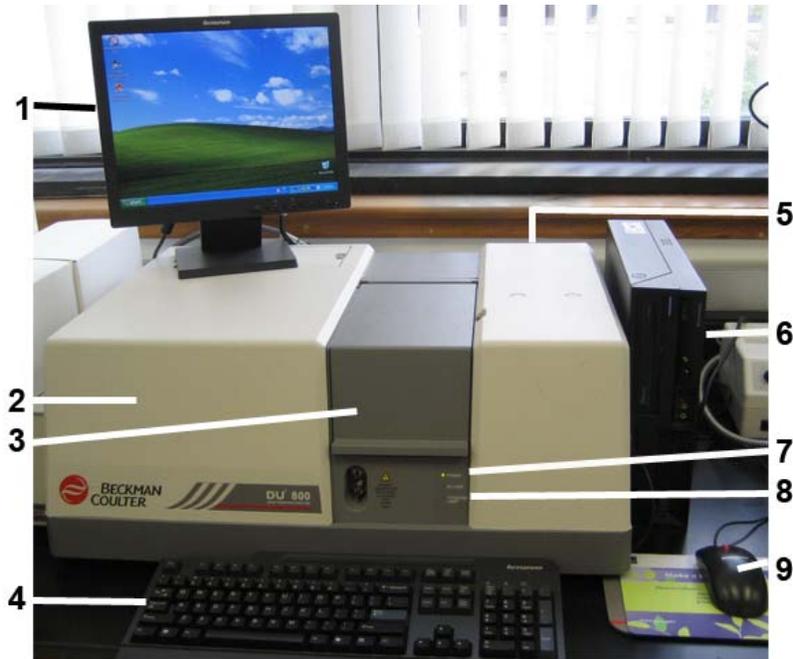
The spectrophotometer is a single beam instrument. Light from two sources, the Deuterium source and the Tungsten source, enter a monochromator, where it is dispersed by a concave holographic grating. Monochromatic light exits the monochromator and illuminates the samples. The amount of light that passes through the sample is measured by a single photodiode detector.





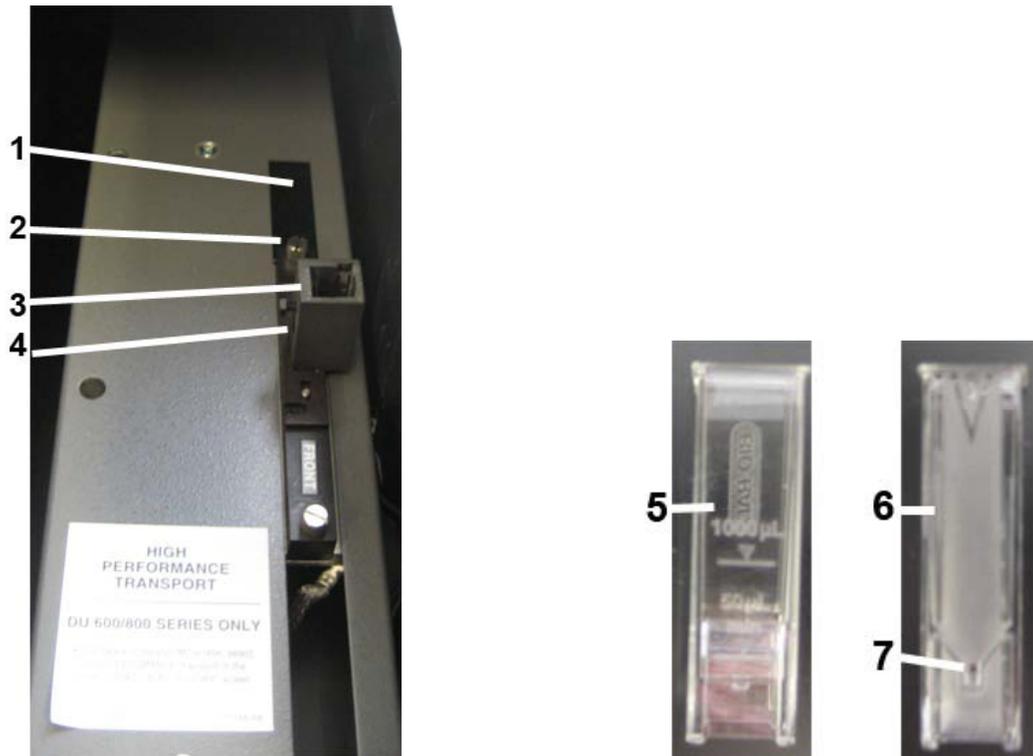
Beckman Coulter DU[®]800 Spectrophotometer Instrument Information Sheet

DU[®] 800 Spectrophotometer Overview



1. Instrument Display Monitor
2. Spectrophotometer Lamp Housing
3. Sample Holder Chamber Door
4. Keyboard
5. Power Switch (Rear right)
6. Instrument Computer
7. Power On/Off LED Light
8. UV and Visible Lamp On/Off LED Light
9. Mouse

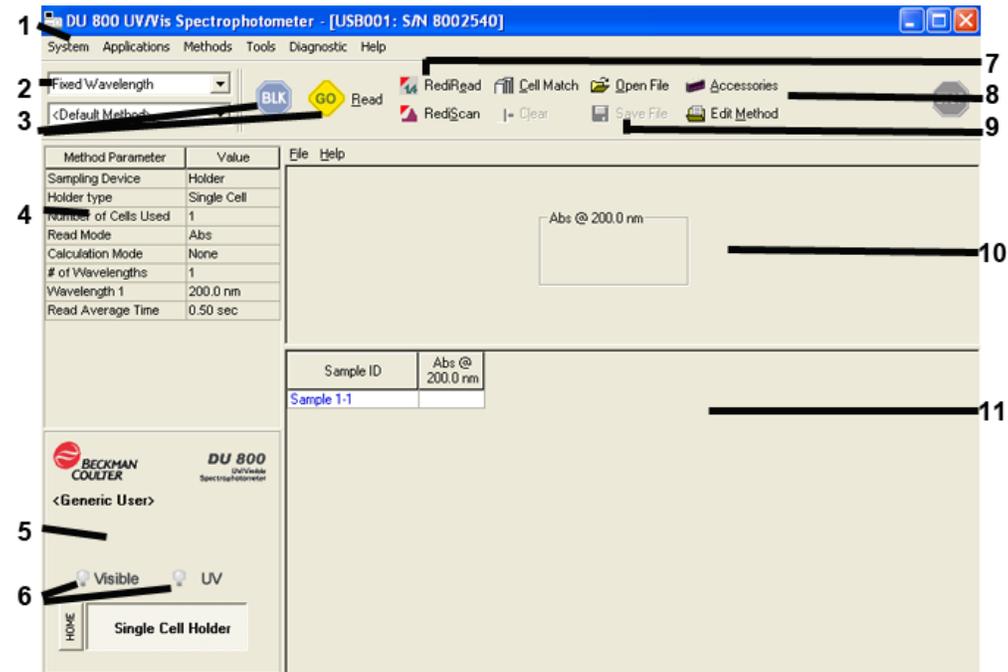
Sample Cuvette and Cuvette Carriage Overview



1. Cuvette Carriage Track
2. Cuvette Carriage Holder Screws
3. Cuvette Holder
4. Sample Reading Window
5. Disposable Cuvette (front view)
6. Disposable Cuvette (side view)
7. Cuvette Sample Reading Window

*The instructor also has additional cuvettes, including quartz cuvettes, which have special care instructions.

Software Window Overview



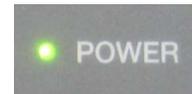
1. Software Toolbar
2. Method Selection Drop-down Menu
3. Block and Read/Go Buttons
4. Method Parameters Screen
5. UV Lamp Time On
6. Lamp On/Off Buttons
7. RediScan and RediRead Quick Read Buttons
8. Open/Edit Methods Buttons
9. Save File Button
10. Current Reading Display Window
11. Log of Readings Performed



Beckman Coulter DU[®]800 Spectrophotometer Quick Start Guide

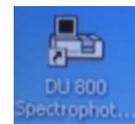
Starting Up the Instrument and Software

1. Turn the DU[®]800 on by flipping the **Power Switch** (located on rear right side of the instrument).



- a. The **Green Power LED Light** on the front of the instrument will turn on.

2. Start the instrument software by double clicking the **DU800 Spectrophotometer Icon** on the *Desktop*.

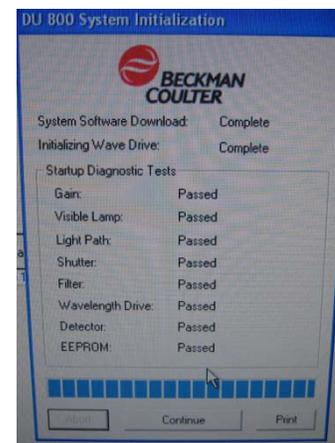


- a. If the icon is not on the desktop, go to:

Start → Programs → Beckman Coulter → DU800 Spectrophotometer



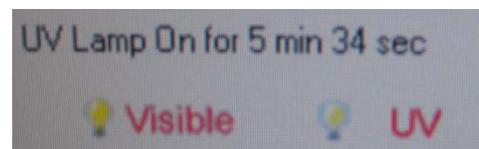
3. When the software window appears, allow the instrument to download system software updates, initialize the wave drive, and perform all of the startup diagnostic tasks.



- a. Once all of these are completed, the words **Complete** or **Passed** will appear next to each task.

- b. If all items have passed the diagnostic check, click the **Continue Button** at the bottom of the screen.

4. Turn on the two lamps by pressing the **Visible** and **UV Buttons** in the bottom left corner of the software screen. The Red Lamp LED lights on the front of the instrument will turn on.



- a. This will turn on the tungsten lamp for visible



wavelengths, and the deuterium lamp for UV wavelengths.

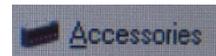
Note: The lamps must warm up for at least 20 minutes before use. Plan accordingly. Do not perform any readings until the timer shows the UV lamp has been on at least 20 minutes.

Note: The lamps have a limited lifespan. If not in use, turn the lamps off to conserve their usable life.

5. Once the lamps have warmed up, follow the protocol for one or more of the following types of reads to analyze a sample.

Placing a Cuvette in the Sample Carriage

- The sample carriage in the instrument at most times is a single sample holder. Ask the instructor to change the carriage if one with multiple holders is needed for an assay.
 - If the type of carriage is changed, click the **Accessories Button** on the main screen and select the type of carriage that was installed.
- Each cuvette has two sets of identical sides. One side has a small, square window at the bottom that is used to read the sample, and the other side cannot be used to read the sample (see Instrument Information Sheet for image of the two cuvette sides). Always hold the cuvette at the top on the sides that cannot be read.
 - When placing a sample in the instrument, load it in the carriage with the window sides facing left and right. The light source will pass through the sample from the left side of the instrument to the right.



- Prior to each run, a cuvette with a blanking agent (water, the medium used, etc.) must be placed in the sample holder and blanked in order to remove all “background noise” from the readings.

Note: Make sure to close the lid before blanking or taking a reading

- Once the blanking is complete, the sample can be placed in the carriage for analysis.

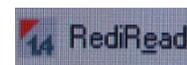
Note: Readings acquired using any of the following methods can be printed by going to **File → **Print** in the *Toolbar* found within the data window.**



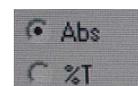
Setting up and Performing a RediRead

The **RediRead Function** is used to quickly analyze a sample at a single wavelength, without having to setup an entire method for the run. Results and the method cannot be saved in this setting.

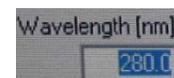
1. Click the **RediRead Button** on the screen.



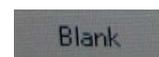
2. In the pop-up window that appears, select either **Absorbance** or **%T** as the read type.



3. Enter the **Wavelength** to analyze the sample at in the wavelength (nm) window.

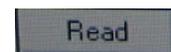


4. Place a **Blanker Cuvette** in the holder and press the **Blank Button** to blank the sample.



- a. The reading should show up as 0.00 for the reading.

5. Place the sample in the holder and press the **Read Button**.



- a. The screen will display the sample ID, wavelength at which it was read, and the reading (in absorbance).

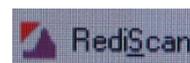
- i. Repeat steps 2 – 5 as needed.

Sample ID	Abs @ 200.0 nm
Sample 1:1	

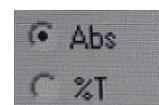
Setting up and Performing a RediScan

The **RediScan Function** is used to quickly analyze a sample over a range of wavelengths, without having to setup an entire method for the run. Results and the method cannot be saved in this setting.

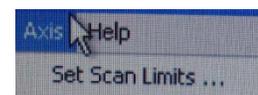
1. Click the **RediScan Button** on the screen.



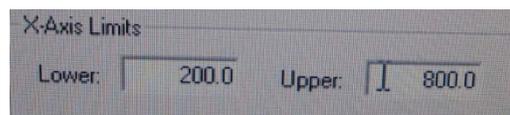
2. In the pop-up window that appears, select either **Absorbance** or **%T** as the read type.



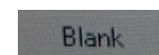
3. On the *Toolbar*, select **Axis** → **Set Scan Limits** to enter the range of wavelengths to scan. Click **OK**.



- a. The lowest wavelength is entered in X-axis Lower, and the highest wavelength is entered in X-axis Upper.

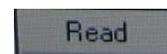


4. Place a **Blanker Cuvette** in the holder and press the **Blank Button** to blank the sample.

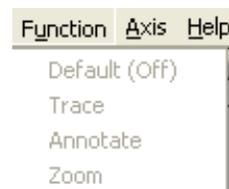


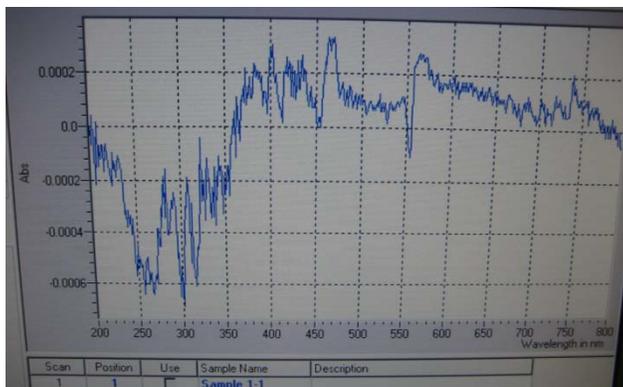
- a. The reading should show up as 0.00 for all wavelengths in the graph.

5. Place the sample in the holder and press the **Read Button**.



- a. The screen will display a graph of the absorbance at all the wavelengths the sample was scanned at. The user can determine the absorbance at a specific wavelength by going to **Function** → **Trace** in the data set *Toolbar*, and then moving the mouse over the wavelength of interest.





- i. Repeat steps 2 – 5 as needed.

Note: For each of the following protocols, a method can be created and saved for later use, and data can be saved or exported.

Setting up and Performing a Fixed Wavelength Read

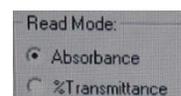
1. From the dropdown menu on the top left of the screen, select **Fixed Wavelength**.



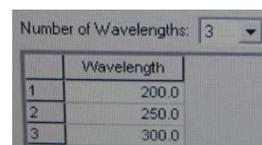
2. To create a method for the fixed wavelength, click the **Edit Method Button**.



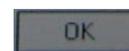
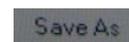
3. On the pop-up screen, enter:



- a. The **Type of Read** (absorbance or %T).
- b. The **Number of Set Wavelengths** at which to read the sample.
- c. The **Actual Wavelengths** for each of those reads.

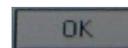


- i. The method can be saved in the system memory for later use by clicking **Save As** on the screen, entering in a method name, and clicking **OK**.



- ii. A method that has been created can be recalled and used again by selecting it from the drop-down menu below where fixed wavelength was selected.

d. Clicking **OK** will return the user to the main screen.



4. Place a **Blanker Cuvette** in the holder and press the **Blank Button** to blank the sample.



a. The reading should show up as 0.00 for the reading.

5. Place the sample in the holder and press the **Read Button**.



a. The screen will display the sample ID (which can be changed by clicking on it), the wavelength at which it was read, and the reading (in absorbance).

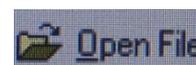
Sample ID	Abs @ 200.0 nm
Sample 1-1	

i. Repeat steps 2 – 5 as needed.

6. To save data from the reads, click the **Save File Button**, and choose where to save the file, enter a file name, and click **OK**.



a. This data can be reopened in the software at a later time by clicking the **Open File Button**.



7. To export data for use in Excel, select **Tools** → **Data Export**, and save the file as a **.csv** file.

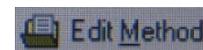


Setting up and Performing a Wavelength Scan Read

1. From the dropdown menu on the top left of the screen, select **Wavelength Scan**.

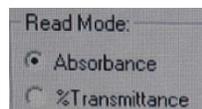


2. To create a method for the wavelength scan, click the **Edit Method Button**.

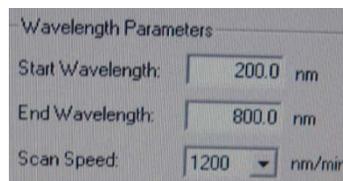


3. On the pop-up screen, enter:

a. The **Type of Read** (absorbance or %T).

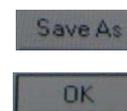


b. The **Start and End Wavelengths** of the scan.

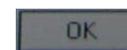


c. The **Scan Speed**.

i. The method can be saved in the system memory for later use by clicking **Save As** on the screen, entering in a method name, and clicking **OK**.



ii. A method that has been created can be recalled and used again by selecting it from the drop-down menu below where fixed wavelength was selected.



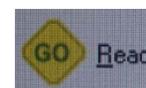
d. Clicking **OK** will return the user to the main screen.

4. Place a **Blanker Cuvette** in the holder and press the **Blank Button** to blank the sample.

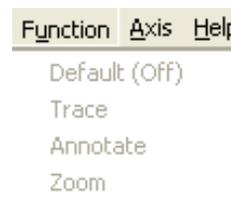


a. The reading should show up as 0.00 for the reading.

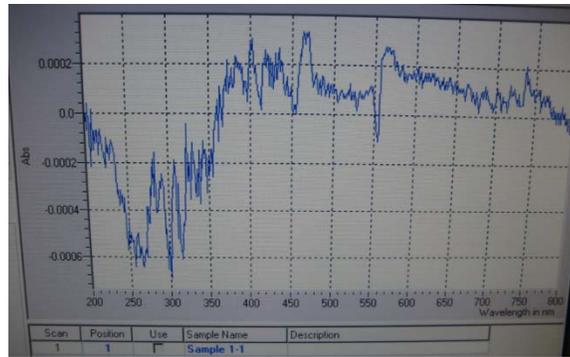
5. Place the sample in the holder and press the **Read Button**.



b. The screen will display a graph of the absorbance at all the wavelengths the sample was scanned at. The user can determine the absorbance at a specific wavelength by going to **Function** → **Trace** in the data set *Toolbar*, and then moving the mouse over the wavelength of interest.



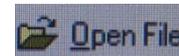
i. Repeat steps 2 – 5 as needed.



6. To save data from the reads, click the **Save File Button**, and choose where to save the file, enter a file name, and click **OK**.



- a. This data can be reopened in the software at a later time by clicking the **Open File Button**.



7. To export data for use in Excel, select **Tools** → **Data Export**, and save the file as a **.csv** file.



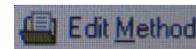
Setting up and Performing a Kinetics/Time Read

This method can be used to look at a change in absorbance in a single sample over time, with readings being taken at set time intervals.

1. From the dropdown menu on the top left of the screen, select **Kinetics / Time**.



2. To create a method for the fixed wavelength scan, click the **Edit Method Button**.



3. On the pop-up screen, enter:

- a. The **Analytical** and **Background Wavelength**.
- b. The **Time Intervals** at which to read the sample.
- c. The **Total Time** for the entire read.

Wavelengths:

Analytical Wavelength: nm

Background Wavelength: nm

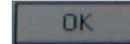
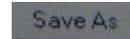
Kinetic:

Samples:

Interval Time: Seconds

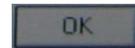
Total Time: Seconds

- i. The method can be saved in the system memory for later use by clicking **Save As** on the screen, entering in a method name, and clicking **OK**.



- ii. A method that has been created can be recalled and used again by selecting it from the drop-down menu below where fixed wavelength was selected.

- d. Clicking **OK** will return the user to the main screen.



4. Place a **Blanker Cuvette** in the holder and press the **Blank Button** to blank the sample.

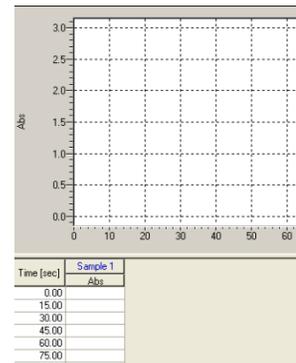


- a. The reading should show up as 0.00 for the reading.

5. Place the sample in the holder and press the **Read Button**.



- a. The screen will display the sample name (which can be changed by clicking on it), and the reading associated with a specific time interval. In addition, a chart will show the change in absorbance over the course of the read.

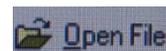


- i. Repeat steps 2 – 5 as needed.

6. To save data from the reads, click the **Save File Button**, and choose where to save the file, enter a file name, and click **OK**.



- a. This data can be reopened in the software at a later time by clicking the **Open File Button**.



7. To export data for use in Excel, select **Tools** → **Data Export**, and save the file as a **.csv** file.



Setting up and Performing a Nucleic Acid Analysis

This method is used to analyze the DNA content of a sample based on a comparison of readings at 260nm and 280nm. Method settings cannot be changed for this method.

1. From the dropdown menu on the top left of the screen, select **Nucleic Acid Analysis**.



2. Place a **Blanker Cuvette** in the holder and press the **Blank Button** to blank the sample.



- a. The reading should show up as 0.00 for the reading.

3. Place the sample in the holder and press the **Read Button**.

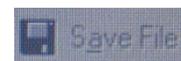


- a. The screen will display the sample name (which can be changed by clicking on it), the absorbance at 260nm, the absorbance at 280nm, the ratio of 260/280nm, and the ratio of 280/260nm.

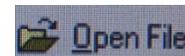
Sample ID	Abs @ 260.0 nm	Abs @ 280.0 nm	Ratio 260/280	Ratio 280/260
Sample 1-1				

- i. Repeat steps 2 – 3 as needed.

4. To save data from the reads, click the **Save File Button**, and choose where to save the file, enter a file name, and click **OK**.



- a. This data can be reopened in the software at a later time by clicking the **Open File Button**.



5. To export data for use in Excel, select **Tools**→ **Data Export**, and save the file as a **.csv** file.



Shutting Down the Instrument and Software

When finished using the spectrophotometer:

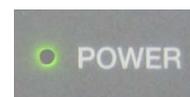
1. Click the **UV** and **Visible Buttons** at the bottom left of the screen to turn off the tungsten and deuterium lamps. The **Lamp LED Lights** on the instrument will turn off.



- a. This step can be done if the user is not finished, but will not be using the instrument for a period of time.
 - i. When turned back on, the user only needs to wait 5 minutes for the lamps to warm back up, instead of 20 minutes.
2. Close the *Software Main Screen* by clicking the **(X) Button** at the top right corner of the screen.
 3. **Log Off** the computer.
 4. Shut the instrument power off by flipping the **Power Switch** (located on the bottom right side of the back of the instrument).



- a. The **Green Power LED Light** on the front of the instrument will turn off.



5. **Make sure the station is neat before leaving the instrument!**