National Cancer Institute-Frederick, Frederick, MD BDP Biopharmaceutical Development Program	STANDARD OPERATING PROCEDURE	Effective Date MAY 28 2008	Procedure Number 22941
		Page 1 of 20	Revision 01

Title: Operation of the Beckman Coulter DU 800 Spectrophotometer

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Approvals/Date: [redacted]

SOP References: 21531	Supersedes: Revision 00

Purpose: This procedure describes the protocol used to operate the Beckman Coulter DU 800 Spectrophotometer for analysis of samples.

Scope: This procedure applies to Biopharmaceutical Quality Control personnel who will operate the Beckman Coulter DU 800 Spectrophotometer. This SOP covers the use of the Beckman DU 800 Series spectrophotometer for general assay methods, such as, fixed wavelength measurements and wavelength scanning measurements in Quality Control. This SOP does not include the operational procedures necessary to perform most kinetic-based (time course) measurements. Refer to the product-specific SOP (if available) for detailed spectrophotometric measurement information.

Contents:

[redacted]

1.0 Authority and Responsibility

1.1 The Director, Biopharmaceutical Quality Control (BQC) has the authority to define this procedure.

1.2 BQC is responsible for training on this procedure and documenting this training to Biopharmaceutical Quality Assurance (BQA).

1.3 BQC personnel are responsible for the performance of this procedure.

1.4 BQA is responsible for quality oversight of this operation.

2.0 Equipment and Materials

2.1 Quartz Cuvettes or Disposable Cuvettes, BDP PN 20070.

2.2 DirectQ water or approved BDP equivalent.

2.3 Calibrated pipettors.

2.4 Pipette tips, approved BDP PN.

2.5 Solvent

3.0 Preparation

Turn on the computer and log into Windows with your user password. Double click on the DU800 icon button on the left side of the screen. Install a cell holder in the sample compartment of the instrument. Configure the software for this particular holder in the **Transport/Holder** tab of the **Accessories** window. Typically a 6-position Multicell Holder is installed in the instrument, however, a Single Cell Holder can be installed and configured if needed.

Turn on the lamps of the spectrophotometer. The system includes a Visible Lamp (Tungsten) and a UV Lamp (Deuterium-Halogen). The visible lamp covers the range from 321 to 1100 nm while the UV lamp provides the energy for the wavelength range from 190 to 415 nm. Both lamps should be turned on. Click on the ***Visible*** icon button and the ***UV*** icon button at the bottom left of the screen.

Figure1 Lamp Icon Buttons

The visible lamp is turned on immediately while the UV lamp requires approximately 30 minutes warm up time before it can be used. The warm up period is indicated by the status message "Warming up UV lamp ..." located just above the icon button. The UV icon button flashes during the warm up period. When a lamp is turned on, the respective menu item is checked and the caption of the respective icon button turns red. After the UV lamp has

been warmed up, the message in the Status & Control Frame reads "UV Lamp On for x units" (the unit can be seconds, minutes, or hours). This provides additional information for the user in regard to the UV lamp warm up time.

4.0 Fixed Wavelength Procedure

The Fixed Wavelength mode is used to collect data in either Absorbance or transmittance mode, up to 12 wavelengths simultaneously. The reading at each wavelength can be multiplied by a user input factor to calculate a final result.

4.1 Calculations

The result is calculated using the equation:

Result = Reading x Factor

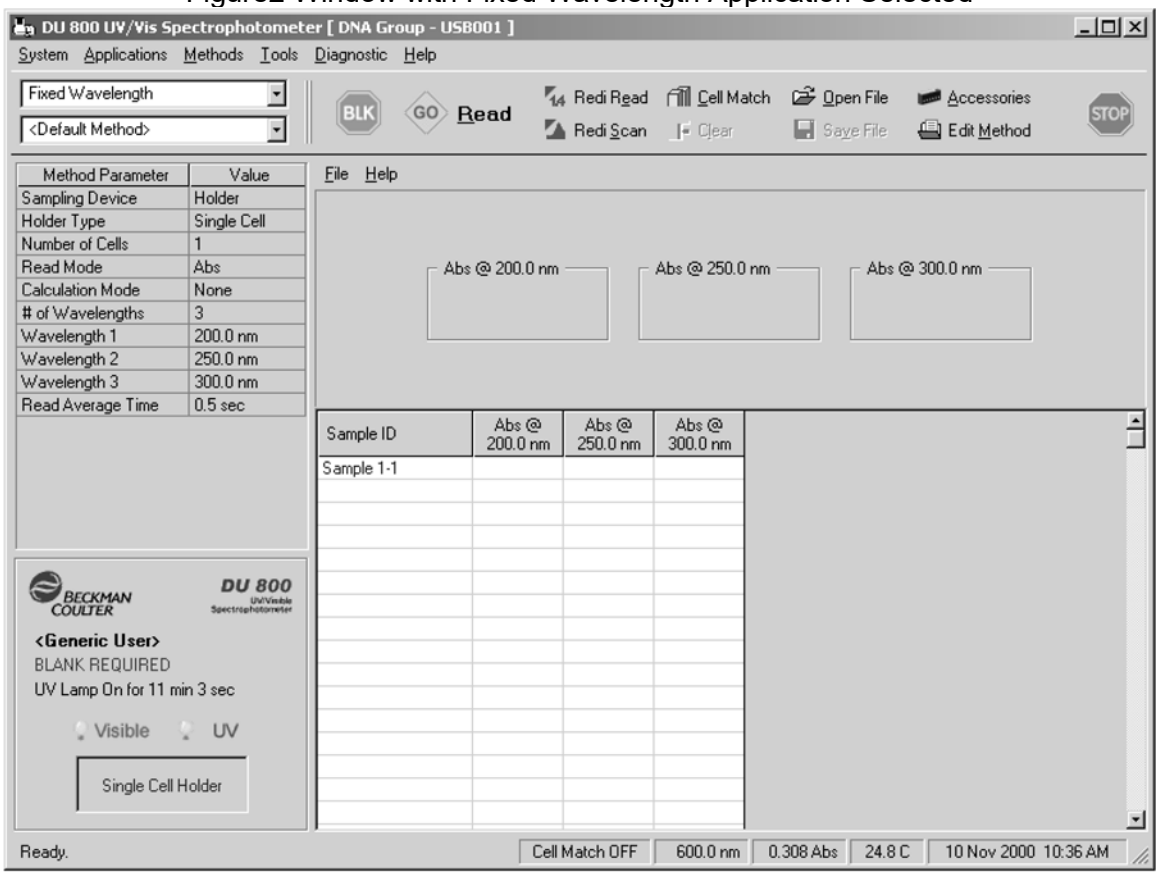
where the reading is in either absorbance or transmittance. The result is a concentration value if the reading is taken in absorbance.

NOTE: Use of this mode to calculate concentration requires that the slope of the standard curve is constant and known, and that the y-intercept is zero. Concentration calculations, derived from a standard curve with multiple standards, are possible using the optional Single Component Analysis mode.

4.2 Procedure

For all applications, except Wavelength Scan I and II, take one or multiple single wavelength readings in one form or another (e.g., reading at 260.0 nm and 280.0 nm). Make sure that the **Fixed Wavelength** application is selected and that the current method is the **<Default Method>** by checking the drop-down list boxes in the Toolbar. If not, click on the down arrow of the drop-down list box and select the appropriate item. A window similar to the one shown below can be seen.

Figure2 Window with Fixed Wavelength Application Selected



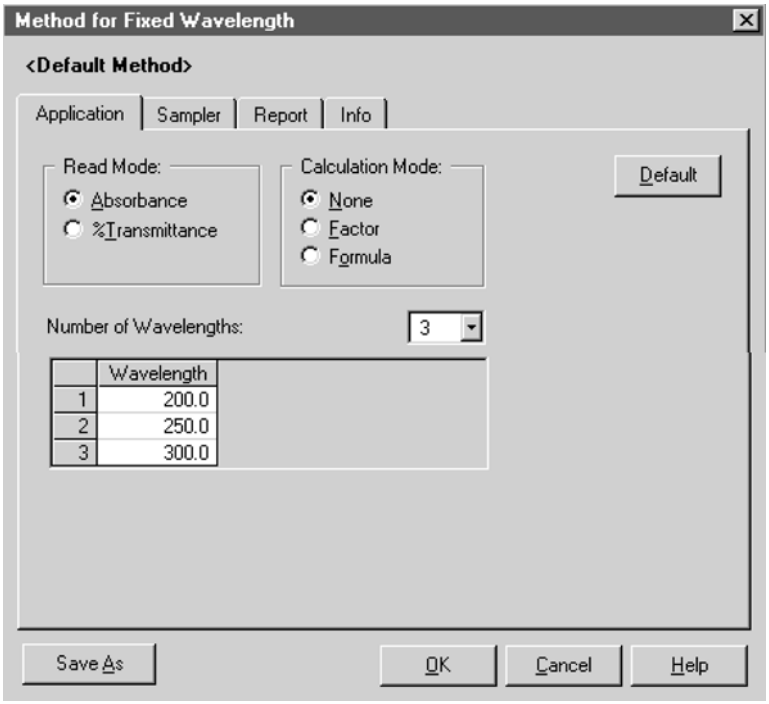
Using this screen as an example, a single wavelength reading at three wavelengths (200.0, 250.0, and 300.0 nm) can be taken. However, before taking the first reading, parameters can be changed and the Method can be edited by clicking on the **Method** window.

4.3 Editing a Method

The <Default Method> for Fixed Wavelength provides a set of pre-defined parameters. For example, there are 3 wavelengths, which are specified as 200.0, 250.0 and 300.0nm.

In order to change the parameters to fit your requirements click on the ***Create/Edit Method*** menu item or the ***Edit Method*** icon button. This brings up the window shown below with the default parameters for the Fixed Wavelength application.

Figure 3 Method Window for Fixed Wavelength Before the Change

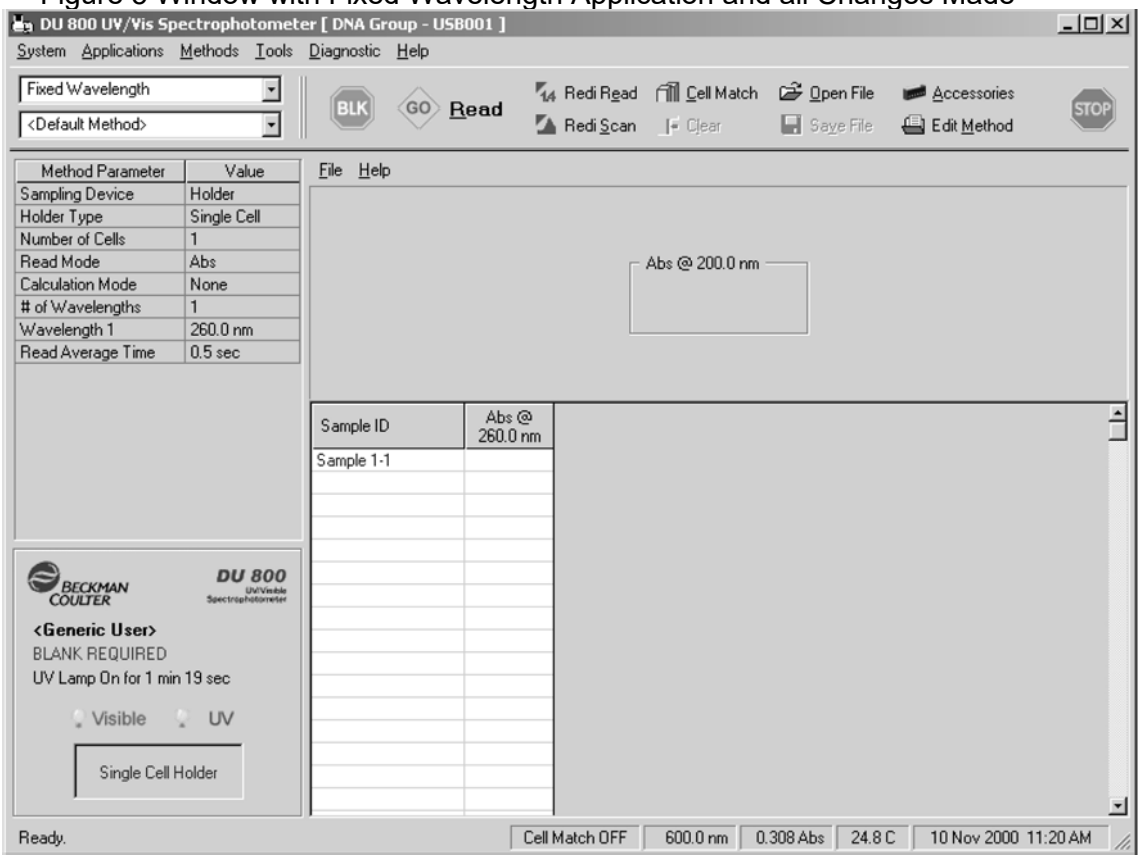


In order to change the number of wavelengths to 1 and the wavelength to 260.0 nm, as an example, first select 1 from the Number of Wavelengths drop-down list box. The grid with the wavelengths definition is now reduced to one (1) wavelength. Next, click on the cell in row 1, which shows 200.0 as the wavelength. Change the value to 260 and press Enter. The wavelength is now set to 260.0 nm and the method window should look like the one shown below.

Figure 4 Method Window for Fixed Wavelength After the Change

In this example, the modified default parameters are only temporarily used. Click on the **OK** button to confirm the changes. The selected parameters remain only valid until you leave the Fixed Wavelength application or select a new method. The modified default method parameters can be permanently saved under an appropriate method name by clicking on the **Save As** button. For a saved method, the next time you use the Fixed Wavelength application, select the saved method from the Method menu or the method drop-down list box and the saved parameters are loaded into the instrument software. The method parameters can be changed at a later time or saved under a different name. The Fixed Wavelength application display window, modified as above, should now look like the one shown in Figure 5.

Figure 5 Window with Fixed Wavelength Application and all Changes Made



The following three things have changed when the Method window is closed. First, the Method Parameter List has been updated. Notice that it only shows one wavelength with the entered wavelength value. Second, the data grid changed to only one column with the correct heading to reflect the current parameter settings. And third, the large-number display has only the field that is labeled with the respective unit and wavelength. The equipment is now ready to measure the samples using customized parameters. Before taking a reading of the first sample, blank the system on the selected wavelength.

4.4 Blanking

A blank is required when lamps are turned on or certain parameters change. In these cases, the instrument will remind the user with the message **BLANK REQUIRED**. Insert an empty cuvette or a cuvette with a blank solution in the cell holder at the measurement position and close the sample compartment.

Now click the **BLANK** button. The Status & Control Frame displays ***Blanking...*** during the blanking and ***Last blanked* at XX:XX** when it is complete. In this

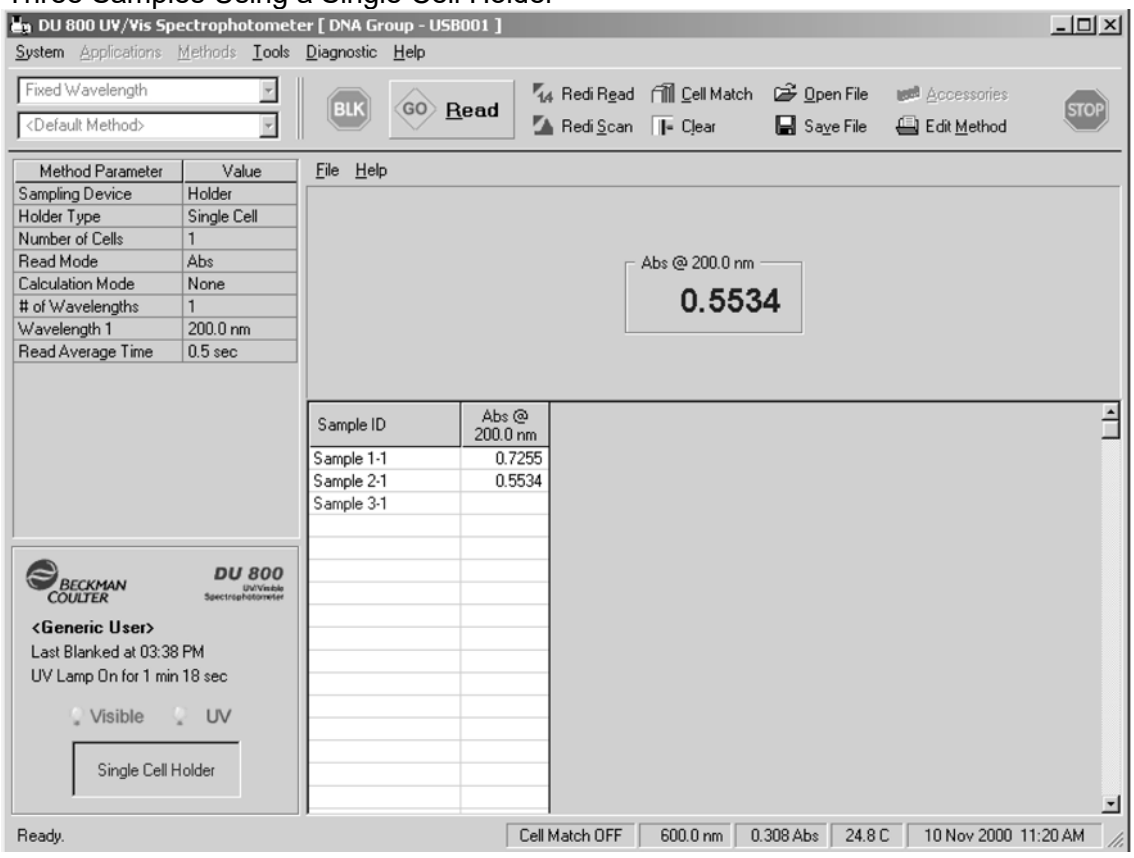
example, a single blank reading is taken at 260.0 nm. The system is now ready to provide accurate readings.

4.5 Reading

Insert a cuvette containing a sample into the Single Cell Holder (or the appropriate position(s) of the Multi-Position Cell Holder) and close the sample compartment. The *Sample ID* for the current sample (Sample 1-1) is a default and is shown in blue. With a Multi-Position Cell Holder, there would be a set of default Sample ID's (e.g., from Sample 1-1 to Sample 1-6). You can modify those *Sample ID's* that are shown in blue by clicking on the respective cell and changing the text, followed by the Enter key. This must be done before the measurement of the sample(s) and allows the user to customize *Sample ID's* for the entire set. After a sample reading is complete, the respective *Sample ID* turns black and the cell is locked.

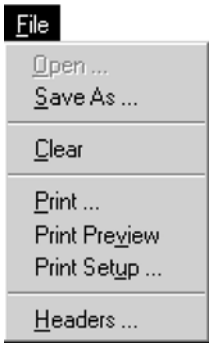
Now click on the **READ** button to take a reading. After the reading has been taken, the result is reported in the data grid and the system is now ready to process the next sample (or sample set). Open the sample compartment, replace the cell with the next sample (or sample set when using a multi-position cell holder), and then close the sample compartment to read the next sample.
The window should now look like the one shown below in Figure 6.

Figure 6 Window with Fixed Wavelength Application and Measurement Taken From Three Samples Using a Single Cell Holder



After the readings have been taken of all samples, the acquired data can be printed and/or saved. To complete the current run, select another application or method, or proceed with something else, the data must be *Saved* and/or *Cleared*.

4.6 Print and Save



Print - To print the acquired data, select ***Print*** from the File menu in the Applications Frame.

Save - To save the acquired data, click on the ***Save File*** icon button. Once the *Save File* dialog box appears, select "Quality Control on **'bdpmaster' (I:)** from the drop down box in order to save data on the network. Click on the **QC Public Folder**. Data is saved in the **Du800 Data Folder** using an appropriate filename. Typically the data is saved using the QC Test Request number, for example, QC26511. Click on the **OK** button to save the data or **Cancel** to abort.
A new Fixed Wavelength task or another application can now be started.

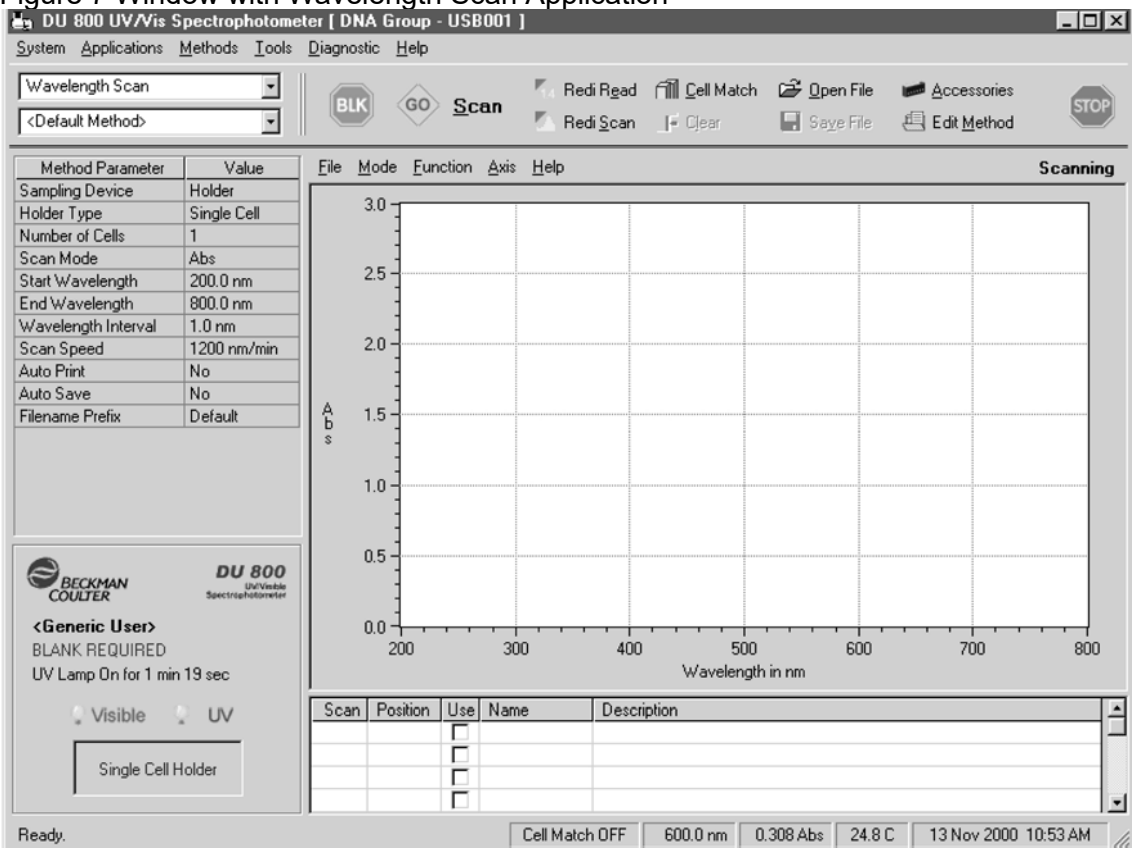
5.0 Wavelength Scan Procedure

Wavelength scans can be collected in either absorbance or transmittance mode. Acquired scan data are stored and may be used for various manipulations and calculations.

5.1 Procedure

In comparison to all other applications, the Wavelength Scan application (I or II) takes scans, which are represented by a continuous wavelength range (e.g., readings from 200.0 nm to 800.0 nm in 1.0 nm intervals). Make sure that the **Wavelength Scan** application is selected and that the current method is the **<Default Method>** by checking the drop-down list boxes in the Toolbar.
If not, click on the down arrow of the drop-down list box and select the appropriate item. A window similar to the one shown below can be seen.

Figure 7 Window with Wavelength Scan Application



Using this screen as an example, a wavelength scans from 200.0 to 800.0 nm at 1200 nm/min can now be performed. However, before taking the first reading the parameters can be changed and the Method can be edited by clicking on the **Method window.**

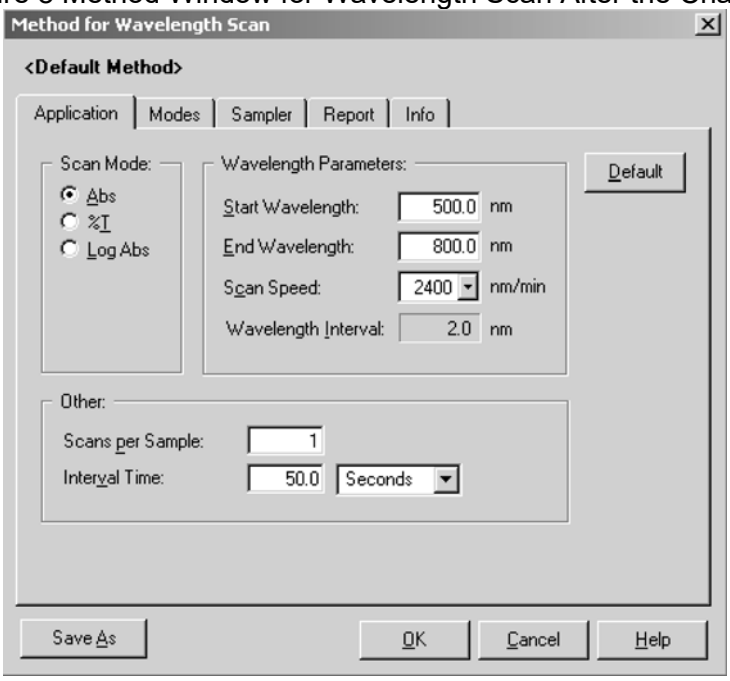
5.2 Editing a Method

Editing a Method

The <Default Method> for Wavelength Scan provides a set of pre-defined parameters. For example, the wavelength range is specified from 200.0 to 800.0 nm and the scan speed at 1200 nm/min. In order to change these parameter to fit test requirements, click on the **Create/Edit Method** menu item or the **Edit Method** icon button. This will bring up the Method window with the default parameters for the Wavelength Scan application. To change the Start Wavelength to 500.0 nm, leave the End Wavelength at 800.0 nm, and then set the Scan Speed to 2400 nm/min. This new scan speed allows us to scan faster but at the expense of the scan resolution. Instead of a 1.0 nm interval at 1200 nm/min, the data acquisition for a scan at a speed of 2400 nm/min is performed with a 2.0 nm interval.

First, change the Start Wavelength to 500.0 nm. Then change the scan speed to 2400nm/min. Select 2400 from the Scan Speed drop-down list box. The Interval value will be recalculated and should now show 2.0 nm as in the following window.

Figure 8 Method Window for Wavelength Scan After the Change



In this example, the modified default parameters are temporarily being used. To do so, confirm the changes by clicking on the **OK** button. The selected parameters remain only valid until you leave the Wavelength Scan application or select a new method. The modified default method parameters can be saved permanently under an appropriate method name by clicking on the **Save As** button. For a saved method, the next time the Wavelength Scan application is to be used, select the saved method from the **Method** menu or the **Method drop-down** list box and the saved individual parameters are ready to be used. The method parameters can be changed at a later time or saved under a different name. The Wavelength Scan application is now set up in a way to fit the test requirements by modifying the method, in this case the <Default Method>. The current parameter settings are reflected in the Method Parameters Frame.

Figure 9 Method Parameters Frame After the Change

Method Parameter	Value
Sampling Device	Holder
Holder Type	Single Cell
Number of Cells	1
Scan Mode	Abs
Start Wavelength	500.0 nm
End Wavelength	800.0 nm
Wavelength Interval	2.0 nm
Scan Speed	2400 nm/min
Auto Print	No
Auto Save	No
Filename Prefix	Default

The Method Parameters Frame has been updated and the Method window was closed. Notice that it now shows the Start Wavelength with 500.0 nm, the Wavelength Interval with 2.0 nm, and the Scan Speed with 2400 nm/min. The samples are now ready to be scanned using the customized parameters. Before taking a scan of the first sample, the system needs to be blanked at the selected wavelength range.

5.3 Blanking

A blank is required when lamps are turned on or certain parameters change. In these cases, the instrument will remind the user with the message **BLANK REQUIRED**. Insert an empty cuvette or a cuvette with a blank solution in the cell holder at the measurement position and close the sample compartment. Now click the **BLANK** icon button. The Status & Control Frame displays *Blanking*...when a blank is taken and *Last blanked* at 11:13 AM when it is completed. In this example, a blank scan is taken from 500.0 nm to 800.0 nm with an interval of 2.0 nm at a scan speed of 2400 nm/min. The system is now ready to provide accurate scans.

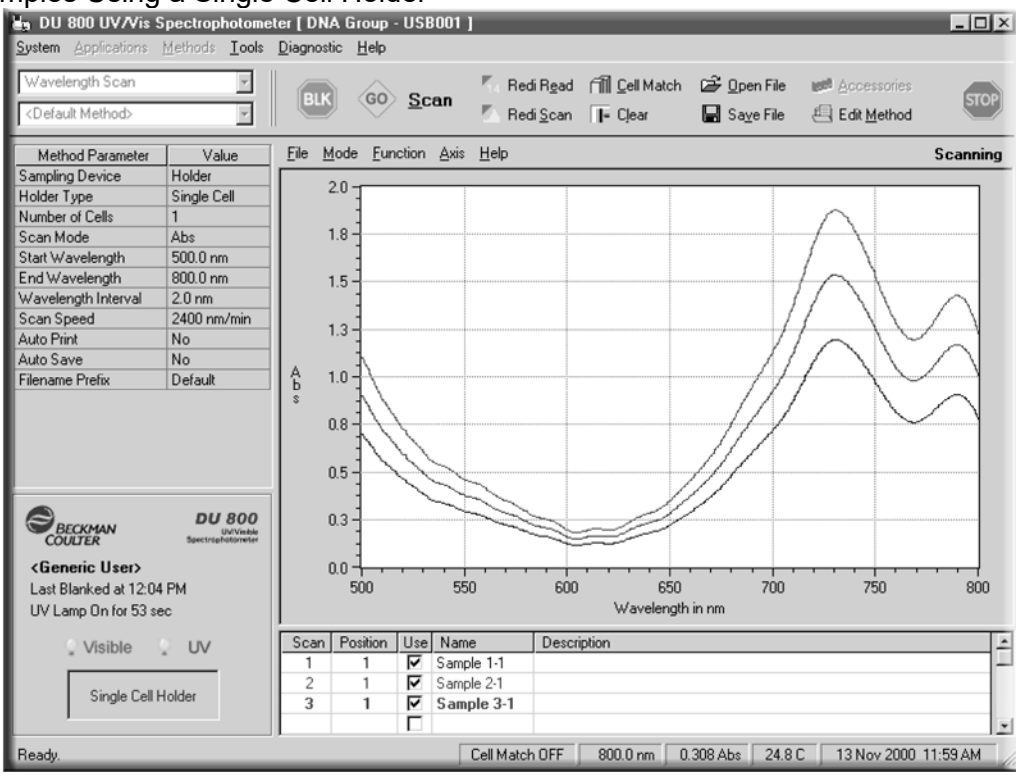
5.4 Scanning

Insert a sample into the Single Cell Holder (or the appropriate position(s) of the Multi-Position Cell Holder) and close the sample compartment.

The grid below the scan window will be automatically populated when the scans are taken. The **Use check box** allows the user to click and select if the scan should be used and displayed or not. By default, each acquired scan will be used and displayed.

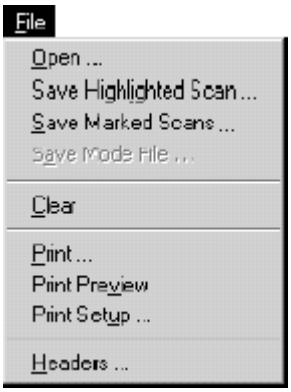
Now click on the **SCAN** button to take a scan. The window will show how the readings for each wavelength within the range are taken in real time. If the Dynamic Autoscaling item in the Axis menu is checked, the y-axis will be automatically resized during the scanning process. If not, the Autoscale Y item can be selected in the Axis menu to autoscale the y-axis manually when the scan is finished.
Open the sample compartment, replace the cell with the next sample (or sample set when using a multi-position cell holder), and then close the sample compartment to read the next sample. The window should now look like the one shown below.

Figure 10 Window with Wavelength Scan Application and Scans Taken From Three Samples Using a Single Cell Holder



After the scans of all samples have been taken, the scans can be printed and/or saved. Execute ***Save*** and/or ***Clear*** to complete the current run, select another application or method, or proceed with something else.

5.5 Print and Save



Print - To print the acquired data, select ***Print*** from the File menu in the Applications Frame.

Save - To save the acquired data, select ***Save As*** from the File menu in the Applications Frame or click on the ***Save File*** icon button. Once the *Save File* dialog box appears, select "Quality Control on **'bdpmaster' (I:)** from the drop down box in order to save data on the network. Click on the **QC Public Folder**. Data is saved in the **Du800 Data Folder** using an appropriate filename. Typically the data is saved using the QC Test Request number, for example, QC26511. Click on the **OK** button to save the data. Another *Wavelength Scan* task or another application can now be selected.

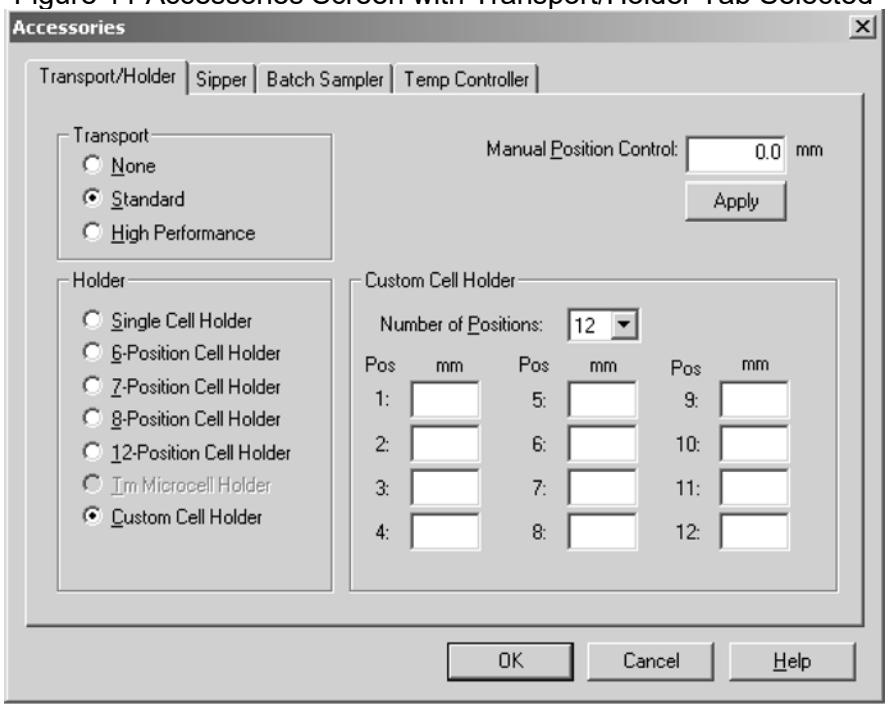
6.0 Accessories

The Accessories item allows access to the setup window for the system accessories, which includes Transport/Holder and Temp Controller. The installed accessories must be defined for the system to function properly. After making any hardware changes to the DU 800, such as changing the cell holder the appropriate tab of the accessories, the window must be updated.

6.1 **Transport/Holder** - Two types of Transports are available, Standard Transport and High Performance Transport. The Standard Transport is for ambient and water temperature-controlled cell holders and all types of cuvettes. The High Performance Transport is required for Peltier temperature-controlled cell holders. This transport contains a fan to remove excess heat and provides additional accuracy in positioning microcells. Depending on the mode, the software automatically detects the presence and type of the Transport. The proper selection is made automatically in the Transport/Holder tab. The user must still select the Holder that is mounted on the transport.

On-Line Mode: Transport is detected automatically; user cannot select it.
Off-Line Mode: Transport is set to "None"; user cannot select it.

Figure 11 Accessories Screen with Transport/Holder Tab Selected



The current Cell Holder must be defined by the user. Based on the Transport, the compatible cell holder items are enabled and the installed cell holder can be selected. For example, a multi-position cell holder can only be selected if a transport is installed or has been selected. The Tm Microcell Holder can only be selected if the High Performance Transport is installed and selected.

With the selection of the Custom Cell Holder, the custom cell holder frame becomes visible and the position for each cell location can be defined in millimeters. A custom cell holder can have a maximum of 12 positions. The cell locations must be within the range of 0 to 112 mm.

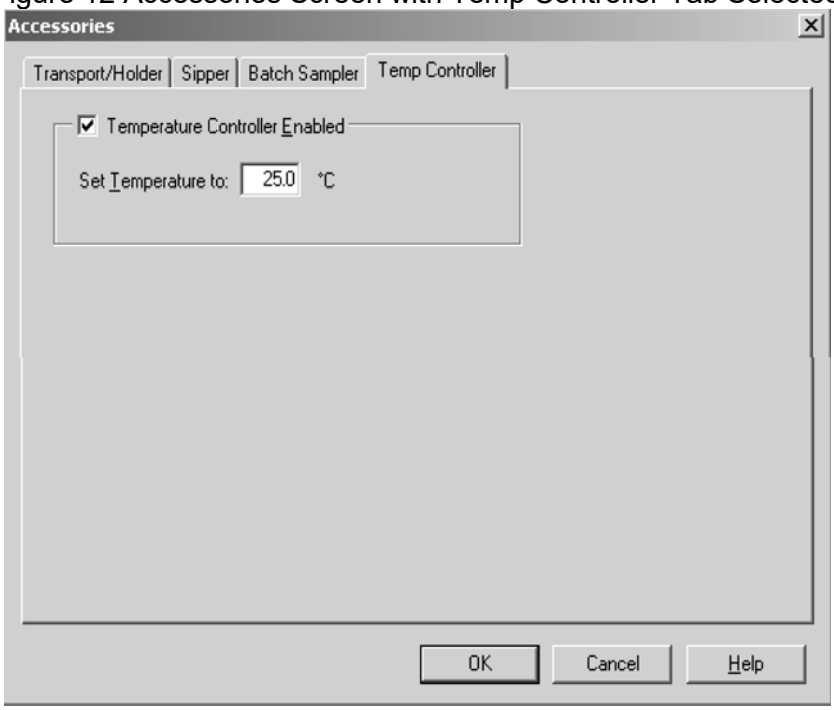
The Manual Position Control determines the cell position in mm. The Enter key or the Apply button will execute the command and move the transport to the specified position. The input range is from 0 to 112 mm.

NOTE: Selecting Cuvette Types

Often the sample volume available for spectrophotometric study is less than 250 μL, necessitating the use of 8 mm high Micro-Cuvettes that have a fluid capacity range of 50-140 μL. At least 50 μL of solution must be used to obtain an accurate reading when utilizing Micro-Cuvettes. A separate Micro-Cuvette carriage must be installed and properly aligned with the light source prior to the use of Micro-Cuvettes.

6.2 **Temperature Controller** - The Peltier Temperature Controller is used in conjunction with the High Performance Transport and a Peltier Temperature-Controlled Cell Holder. It cannot operate if these two accessories are not installed. To control the sample temperature with the Peltier Temperature Controller, the High Performance Transport is required to remove the excess heat from the cell holder. The Peltier method of temperature control allows both heating and cooling of the sample, hence the ability to cool below ambient temperature. If use of the Peltier Temperature Controller is desired, make sure the Temperature Controller Enabled box in the Temp Controller tab of the Accessories window is checked. See figure below.

Figure 12 Accessories Screen with Temp Controller Tab Selected



Manual Control - The temperature controller is enabled by entering the desired temperature and checking the Temperature Controller Enabled box. The input range is from 10ºC to 90ºC. The range that is actually suitable for a 6-Position Cell Holder is from 20ºC to 40ºC. Clicking the **OK** button will activate the temperature controller. If the temperature controller does not activate, the following warning message will be displayed: “The Peltier Temperature Controller could not be activated. Please verify that the Temperature Controller, High Performance Transport, and the Cell Holder are connected properly and that the controller is powered on”.

Automatic Control - With Kinetics/Time, Enzyme Mechanism, and Experimental Tm Analysis applications, the temperature is controlled directly from the respective application. Temperature control will be initiated within these applications if the Temperature Controller Enabled box is checked and if all the requirements are met. The temperature range that is actually controlled depends on the cell holder. The

Tm Microcell Holder has a much wider temperature range than other cell holders and significantly different control dynamics.

NOTE: Notice that the glass (silica or quartz) of the cuvettes provides insulation between the samples contained in the cell and the cell holder, which is heating/cooling the sample. A moderate amount of time (~3 minutes) is required to heat the sample after the cell holder has equilibrated at the desired temperature. The temperature controller is turned on using the switch on the back. When turned on, the POWER indicator light on the front of the controller is illuminated continuously. When the temperature controller is activated, the OPERATING indicator light on the front of the controller blinks and the cooling fan, located under the transport, turns on (High Performance Transport only). The fan remains on for about 5 minutes after the temperature controller is disabled to remove the remaining heat from the cell holder. Peltier temperature-controlled cell holders are plugged into the temperature connector on the left sample compartment wall.

7.0 Documentation

All spectrophotometer use is recorded using the equipment logbook. Refer to ***SOP 21531 Equipment/Facility Logs***. The raw spectrophotometric data (Attachment I) should be printed out and attached to the BQC test request.

8.0 Maintenance

8.1 To clean full-size quartz cuvettes, use the vacuum washer with an initial wash of deionized water or WFI followed by a drying rinse of >95% methanol. Avoid the use of acetone since it will render quartz opaque over time. For micro cuvettes, use a squirt bottle to wash cuvette with deionized water followed by a liberal dry rinse with methanol. Remove exterior finger oils and salts from quartz cuvettes using lens paper or paper certified to have a low grit content such as Kim wipes. DO NOT wash Acrylic and plastic cuvettes using organic solutions such as methanol and acetone. If necessary, remove lint and dust particles from the surface of plastic cuvettes with a piece of lens paper and avoid excessive contact with the cuvette surface.

8.2 No routine operator maintenance for the Beckman DU 600 series is required. The *exterior* of the instrument case may be cleaned using a mild detergent, do not attempt to clean the cuvette carriage while the carriage is installed in the instrument. The instrument optical lens may be cleaned of lint and dust, if necessary, by using compressed air or nitrogen with the nozzle held at least 4 cm away from the lens to avoid oil contamination of the lens. Annual servicing and performance certification is performed under contract by Beckman Coulter and includes:

1. Replacement of the UV and visible light lamps when necessary
2. Lamp and monochromator alignment
3. Other instrument adjustments or repairs as necessary

Record all activity in the equipment logbook. For emergency service call: Beckman Coulter Service (800) 742-2345 and refer to the system ID number located on the front of the instrument. For the BDP-QC instrument, the number is 482789.

9.0 References

9.1 Beckman Coulter DU Series 800 Operating Instructions Manual, 2002, Beckman Coulter, Inc.

9.2 Beckman Coulter Auto 6-Sampler Accessories Manual 514525A, 1990, Beckman Coulter, Inc.

9.3 Beckman Coulter Software Validation Pac DU Series 800, Beckman Coulter, Inc.

9.4 ***SOP 21531, Equipment/Facility Logs***

ATTACHMENT I

Sample Spectrophotometer Printout

Fixed Wavelength

Print Date: November 22, 2005 3:36 PM

Instrument Name:	DU 800 Spectrophotometer
Serial Number:	8001525
Software Version:	2.0, Build 83 2.0.081
Method Name:	A280
Department:	QC
Operator:	[redacted]
Product Name:	BSA
Lot Number:	LX0000
Filename:	H:\Quality Control\QC_Public\Du 800 Data\sampleA280.dux
Acquired:	September 14, 2005 3:47 PM
Read Mode:	Abs
Calculation Mode:	None
Number of Wavelengths:	1
#	Wavelength
1	280

Sample ID	280.0 nm Abs
PBS Buffer	0.0001
BSA 1mg/mL	0.6698
BSA 1mg/mL	0.6667
BSA 1mg/mL	0.6678
Sample 1	0.2603
Sample 2	0.2600
Sample 3	0.2604
BSA 1mg/mL	0.6680
BSA 1mg/mL	0.6665
BSA 1mg/mL	0.6673

DU 800 System and Applications Software Page: 1