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NanoDrop 8000 Spectrophotometer

V2.2 User Manual

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1. Overview

Instrument Description

The Thermo Scientific NanoDrop 8000 Spectrophotometer is a full-spectrum (220-750nm) instrument that accurately measures up to 8 individual 1 ul samples in one measurement cycle. The software allows the user to measure samples using either the full 8-position mode or a convenient single sample mode.

The NanoDrop 8000 utilizes the same patented sample retention technology employed on all NanoDrop™ instruments. The surface retention system holds the sample in place eliminating the need for cumbersome cuvettes and other sample containment devices. Clean-up is accomplished in seconds. In addition, the NanoDrop 8000 has the capability to measure highly concentrated samples without dilution (50X higher concentration than the samples measured by a standard cuvette spectrophotometer).

Applications

UV/VIS spectrophotometry is simple for samples as small as 1 ul using the NanoDrop 8000 Spectrophotometer. The small sample requirement and ease of use make the NanoDrop 8000 Spectrophotometer ideally suited for measuring:

- Nucleic acid concentration and purity of nucleic acid samples up to 3700 ng/ul (dsDNA) without dilution
- Fluorescent dye labeling density of nucleic acid microarray samples
- Purified protein analysis (A280 nm) up to 100 mg/ml (BSA)
- Expanded spectrum measurement and quantitation of fluorescent dye labeled proteins, conjugates, and metalloproteins
- Bradford Assay analysis of protein
- BCA Assay analysis of protein
- Lowry Assay analysis of protein
- Pierce Protein 660 nm analysis
- Cell density measurements
- General UV-Vis spectrophotometry
- Custom methods

Operation

Up to eight 1 ul samples are pipetted onto the sample pedestal using a low volume multi-channel pipettor.

Each position is actually the end of a fiber optic cable (the receiving fibers). A second set of fiber optic cables (the source fibers) are brought into contact with the liquid samples causing the liquid to bridge the gaps between the fiber optic ends. The pathlengths are automatically controlled to 1mm and 0.2 mm paths. Readings are acquired through sequential measurement across the 8 positions. A pulsed xenon flash lamp provides the light source and a spectrometer utilizing a linear CCD array is used to analyze the light that passes through the samples. The instrument is controlled by PC based software, and the data is logged in an archive file on the PC.

The NanoDrop 8000 is designed only for indoor use under the following conditions:

- Temperature: 40-100° F (4.4 - 37.8° C)
- Humidity: 10-90%

Safety

The NanoDrop 8000 is supplied with a 12V power supply. Use only the power supply provided with the instrument. The unit also comes with a grounded power cord. Plug this cord into a properly grounded outlet. Use of the instrument in a manner not specified by the manufacturer may impair the protection provided by the supplied power cord and power supply.

The power supply can remain plugged into the NanoDrop 8000 while the instrument is not in use. When the instrument is plugged in but not in use, the power consumption is ~5 W and the flash lamp is not energized. The instrument does not utilize a power switch. It is recommended that the instrument not be positioned in a way that makes it difficult to unplug the power supply from the unit or the wall.

WEEE Compliance

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EC. If compliance is required, the instrument is marked with the following symbol:

We have contracted with one or more recycling/disposal companies in each EU Member State, and this product should be disposed of or recycled through them. Further information regarding our compliance with these directives, the recyclers in your country, and information on our products which may assist the detection of substances subject to the RoHS Directive are available at www.thermo.com/WEEERoHS.

Patents

The sample retention technology used in the NanoDrop 8000 is covered under US patents 6,628,382 and 6,809,826. Other patents are pending.

2. Initial Set Up

Computer Requirements

The operating software will only run on an IBM compatible PC meeting the below criteria. No Mac versions of the software are currently available.

- Microsoft Windows 2000, XP, or Vista (32 bit) and Windows 7 (32 and 64 bit)
- Windows Vista has also been tested successfully with the software
- The operating software is not compatible with Windows NT, 95, 98 or ME
- 800 MHz or higher processor
- CD ROM drive
- 128 MB or more of RAM
- 100 MB of free hard disk space
- Open USB port (the instrument can only be connected via the USB port)
- Microsoft Excel or other spreadsheet program to manipulate archived data (optional)

Unless otherwise specified, all screen shots have been generated using the Windows XP operating software.

Software Installation

IMPORTANT - PLEASE READ BEFORE INSTALLING SOFTWARE!

Note 1: The system software must be loaded onto the PC before the USB cable is connected.

Note 2: Uninstall all previous versions of NanoDrop 8000 software using Add/Remove Programs BEFORE installing this version. Current saved User Preferences will be preserved during the uninstall process.

Note 3: Administrative privileges are required to install this software.

Note 4: All users of this software must have read and write access to the folder C:\NANODROP DATA\ and all of its subfolders.

When attaching the USB cable, please wait at least 30 seconds for the USB devices and internal drivers to be installed and recognized.

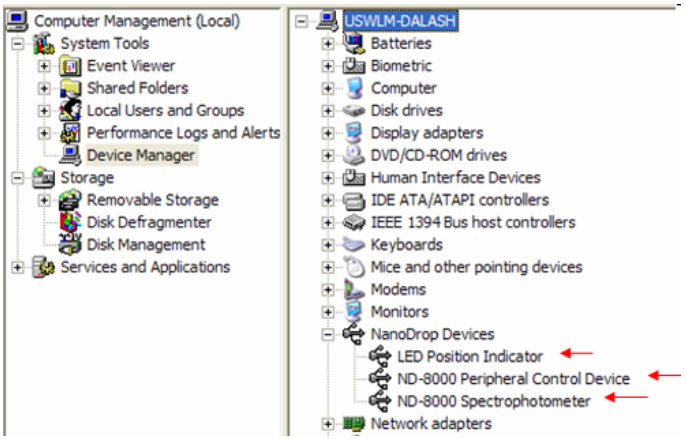
To properly install the operating software:

1. Close all programs and make sure that the USB cable is unplugged.

2. Insert the operating software CD in the CD drive of the PC. The software installation menu should appear automatically. If software menu does not appear, choose My Computer to view the contents of the CD. Double click on the file named **nd-8000…install.exe**.

3. After software installation, connect the USB cable to the instrument and the computer. The Found New Hardware Wizard should start.. Windows XP SP2 operating system will ask to allow it to search the internet for the proper software as shown - Select **No, not this time**. For other Windows operating systems, follow the prompts for automatic installation of the software.

4. Installation will require two cycles through the Found New Hardware Wizard, once for the NanoDrop 8000 Spectrophotometer and once for two devices internal to the instrument. For the NanoDrop 8000 to operate successfully a total of three USB devices need to install although only two cycles through the hardware wizard will be observed. To confirm installation, view the Windows Device Manager as shown below:



The NanoDrop 8000 Spectrophotometer should now be ready for operation. If the software does not start properly, refer to the Troubleshooting section for possible solutions.

Configuring the System Font
The software is designed to look best with the MS Sans Serif font, 8 point. To check that the system font is set to the proper selection:

1. Open the **Display Properties** by right clicking on the desktop and select Properties → Appearance. (Additional step for Windows XP: click on the **Advanced** button).
2. From item list select **icon**.
3. Select the **MS Sans Serif (western)** font and select **8 point** size.
4. Click **OK**.

Choosing an alternative font may result in some text being truncated in the operating software window.

Software Upgrades
Periodic upgrades are made to the operating software and are available for download. See our website for the latest available software version.

USB Flash Drive Port
Any standard PC USB flash drive may be used for exporting data. Note: When using the User Preferences Module to set-up a default automatic Export Report destination, keep in mind that the flash drive may not always be assigned the same removable device designation.

Cable Connections
To make measurements with the instrument, connect the USB cable to instrument and the PC, plug in the 12V power supply and connect to the power input at the back of the instrument.

Note: The NanoDrop 8000 Spectrophotometer is supplied with a 12V power supply. Use only the power supply provided with the kit. The unit also comes with a grounded power cord. Plug this cord ONLY into a properly grounded outlet. Use of the instrument in a manner not specified by the manufacturer may impair the protection provided by the supplied power cord and power supply.

The power supply can remain plugged into the NanoDrop 8000 Spectrophotometer while the instrument is not in use. When the instrument is plugged in but not in use, the power consumption is ~3 W and the flashlamp is not energized. Also, the instrument does not utilize

a power switch or give a visual indication of the operability of the 12V power supply.

Note: It is recommended that the instrument not be positioned in a way that makes it difficult to unplug the power supply from the unit or the wall.

Registering Your Instrument

Please register your product! We periodically update our software and add new features free of charge. We would like to keep our user list updated so that we may alert you to these updates. All information supplied is completely confidential. You can register your instrument on our website.

Lock Attachment Port

The NanoDrop 8000 is equipped with a lock slot that enables use of a standard locking cable typically used to secure a laptop PC.

3. General Operation

Loading samples when operating within the 8 Sample Mode.

Loading samples on position A when operating within the Single Sample Mode.

1) Position the instrument at an angle for optimal use of the pipette guide. With the sampling arm open, position the pipettor using the guide as shown above. Dispense the samples onto the lower measurement pedestal ensuring the samples “touch-off” (contact) the lower pedestal. If the tips splay or skew when touching the pedestal surfaces, please use an alternative brand or style with a more rigid tip structure.

2) Carefully withdraw the pipettor before releasing the pipettor’s dispensing mechanism. Visually verify all samples are correctly transferred to their respective pedestals. If some samples have not transferred adequately, it is recommended that the aliquots be wiped away with a lab wipe and fresh aliquots of all eight samples be reloaded to ensure consistent loading/measurement timing between samples.

3) Close the sampling arm and initiate a spectral measurement using the operating software on the PC. The sample columns are automatically drawn between the upper and lower measurement pedestals and the spectral measurement made.

4. When the measurement is complete, open the sampling arm and wipe the samples from both the upper and lower pedestals using a soft laboratory wipe.

Cleaning the Sample Retention System

Wiping the sample from both the upper and lower pedestals upon completion of each sample measurement is usually sufficient to prevent sample carryover and avoid residue buildup. Although generally not necessary, 2 ul water aliquots can be used to clean the measurement surfaces after particularly high concentration samples to ensure no residual sample is retained on either pedestal.

After measuring a large number of samples, it is recommended that the areas around the upper and lower pedestals be cleaned thoroughly by using a lab wipe dampened with dH_20. A final cleaning of all measurement surfaces with de-ionized water is also recommended after the user's last measurement. Note: Do not use a squirt bottle to apply de-ionized water.

Decontamination of Measurement Pedestals

If decontamination is necessary, a sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solution – freshly prepared), can be used to ensure that no biologically active material is present on the measurement pedestals. The metal fiber optic fittings are made from 303 stainless steel and are resistant to most common laboratory solvents (see "Solvent Compatibility" appendix).

It is recommended that a final cleaning using de-ionized water be made if using a sanitizing solution on the measurement surfaces to ensure any residual solution is removed. Note: Do not use a squirt bottle to apply bleach or de-ionized water.

Rapid Reconditioning of the Sample Retention System

The Bradford reagent as well as other buffers containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form well with 1ul samples. Use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement.

Sample Size Requirements

Although sample size is not critical, it is essential that the liquid column be formed so that the gap between the upper and lower measurement

pedestals is bridged with sample. Note: It is not necessary to have liquid on all 8 positions to make a measurement.

Field experience indicates that the following volumes are sufficient to ensure reproducibility:

- Aqueous solutions of nucleic acids: 1 ul
- Purified protein: 2 ul
- Bradford, BCA, Lowry or Pierce Protein 660 nm Protein Assay: 2 ul
- Microbial cell suspensions: 1-2 ul

It is best to use a calibrated precision pipettor (0-2 ul) with low retention precision tips to ensure that sufficient sample (1-2 ul) is used. Lower precision pipettors (0-10 ul and larger) are not as good at delivering 1 ul volumes to the measurement pedestal. If you are unsure about your sample surface tension characteristics or pipettor accuracy, a 2 ul sample is recommended.

Use an 8-channel pipettor with good fitting tips when loading multiple samples to minimize evaporation due to delays in sample loading. If the tips splay or skew when touching the pedestal surfaces, please use an alternative brand or style with a more rigid tip structure. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Sample Carryover

Prevention of sample being retained on the NanoDrop 8000 Spectrophotometer's measurement pedestals is easily addressed. Simple wiping of the upper and lower measurement pedestal with a dry laboratory wipe is highly effective in eliminating carryover for samples differing in concentration by as much as three orders of magnitude (see our website for NanoDrop 1000 carryover data).

Sample Homogeneity

Sampling from non-homogeneous solutions – particularly when using small volumes – can cause significant deviations in the data generated using all measurement technologies including spectrophotometry. Genomic DNA, lambda DNA and viscous solutions of other highly concentrated nucleic acids are common examples known to the molecular biologist. Proteins are subject to denaturation, precipitation, and aggregation and therefore may require special handling to ensure sample homogeneity.

Effect of Evaporation and Solvents

Evaporation of the sample during the measurement cycle usually has just a minimal effect on absorbance readings and may result in a 1-2% increase in sample concentration. This can be observed in the field by measuring the same sample successively over time. Highly volatile solvents, such as hexane, will likely evaporate before the measurement can be completed. Less volatile solvents such as DMSO can be used successfully.

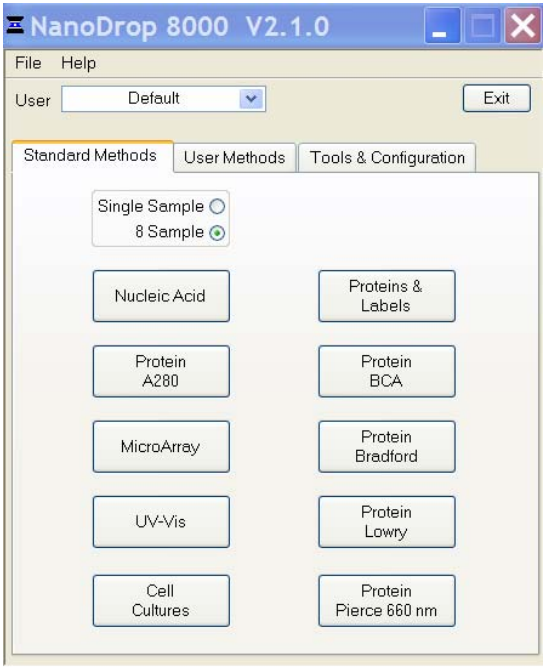
To minimize the effects of evaporation, t is recommended that an 8-channel, low volume pipettor be used to simultaneously dispense samples onto the measurement pedestals.

Sample Recovery

One of the advantages of the sample retention system is that samples can be recovered from the upper and lower measurement pedestals by extraction with a pipette.

Single vs 8 Sample Modes

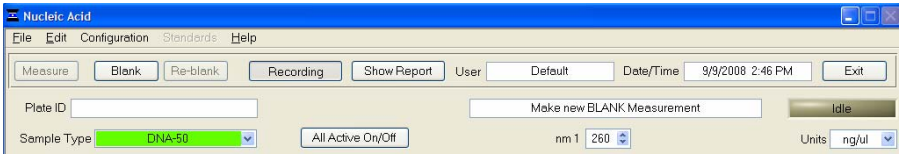
The NanoDrop 8000 may be used in either a Single Sample or an 8 Sample mode. The 8 sample mode will appear as the default each time the software is opened.



Functions Common to Single and 8 Sample Modes

Module Startup
When a software module is opened, the first message seen will indicate that the instrument motors are initializing. A second message will then appear with instructions to load water aliquots to initialize the spectrometer.

For best results, **ensure measurement pedestal surfaces are clean** and load 2 ul of water onto **each** lower measurement pedestal. Lower the arm and click OK. The message 'Please wait - Initializing Spectrometer' will then appear. When this message disappears, the instrument will be ready for use. All data taken will automatically be logged in the appropriate archive file. Note: It is only necessary to load water onto pedestal A when running the single sample mode.



Blank (F2)
Before making a sample measurement, a blank must be measured and stored. All eight positions are blanked with each blanking command when using the 8 sample mode. Only position A is blanked for the single position mode.

Note: When using the 8 Sample mode, the software initiates each blank and measurement cycle on the first position to be read. The user will, therefore, hear one less position increment than expected. After making an initial blank measurement, a straight line will appear on the individual graphs. Subsequent blanks will clear any sample spectrum and again display straight baselines.

Blanking Cycle
For the most consistent results, it is best to begin any measurement session with a blanking cycle.

1. Open the application software module.

2. Use an 8 channel pipettor to load an aliquot of the blank (the same buffer, or solvent the unknown samples are in) onto each of the lower measurement pedestals and then lower the sampling arm to the 'down' position.

3. Click on the **Blank** button. When the measurement is complete, wipe the buffer from all pedestals.

4. Select **All Active On** and analyze a fresh aliquot of the blanking solution on all pedestals using the **Measure** button (F1). The result should be 8 spectra with relatively flat baselines near zero.

5. Wipe the blank from all upper and lower measurement pedestal surfaces with a laboratory wipe and repeat the process until the spectrum varies no more than +/- 0.005 A (1 mm path) from the baseline. Note: The Nucleic Acid, A280 and Proteins & Labels modules display the absorbance values normalized to a 10 mm path so the effective variance should be +/- 0.05 abs from the baseline.

6. Reload the sample ID list before measuring samples if necessary by

using the Configuration drop down box.

See “Blanking and Absorbance Calculations” in the appendix for more information on blanking and absorbance calculations.

Measure (F1)
Each time a software module is opened (initiated), the Measure button is inactive as noted by its “grayed-out” appearance. A blank must first be measured before the Measure button will become active.

The Measure button is used to initiate the measurement sequence for all samples (non-blanks). It is activated by depressing the F1 key or clicking the Measure button. The Sample Position Illuminator allows the user to visualize the row of a standard 96-well micro titer plate that is to be sampled for measurement and corresponds to the sample status color coded guide on the screen. See the section on Sample ID file for more information about the Sample Position Illuminator. The entire measurement cycle takes approximately 20 seconds (less time if fewer than 8 positions are used.)

Re-blank (F3)
The Re-blanking option establishes a new reference (blank) that is used for the absorbance calculations of subsequent samples. The Re-blank is only applied to the specific samples selected and re-calculates the concentration for those samples respectively. Although a new spectrum will be displayed on graph and the previous samples data will be recalculated and saved in the archive file, the recalculated data will not be displayed in the current report.

See the “Blanking and Absorbance Calculations” appendix for more information on absorbance calculations.

Start Report / Recording
All data is automatically archived. The user can log measurement results in a active report table as the data is accumulating by using the Start Report / Recording feature. The default setting has the Recording feature activated for all modules. If Start Report is displayed, the accumulating data will still be archived but will not be shown in the active report.

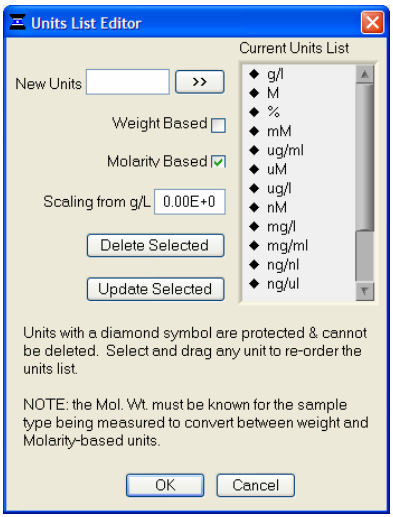
Show Report
Selecting this button will bring up the Report page, which is part of the integrated Data Viewer software. A full description of the features and options for the Report page can be found in the section on the Archived Data and Data Viewer.

User
This field displays the name of the user account in use.

User Guidance Display Box
This field displays either instructions or measurement status information as appropriate.

Units
A drop down box on the right side of the acquisition page allows the user to define what units to utilize when displaying and archiving calculated concentrations. Note: The data will be archived using the units chosen at the time of the measurement. Although the concentrations may change in the display as one selects different units from the drop-down, the archived values will not change.

From the drop down box- select Edit List to bring up the following pop-up box:



Enter in the name for the desired new units and include the appropriate weight based or molarity (M) scaling. For those modules (methods) for which a molecular weight value can be entered, the units selection accommodate both weight and molarity based scaling options. A molecular weight is required to convert between weight and molarity.

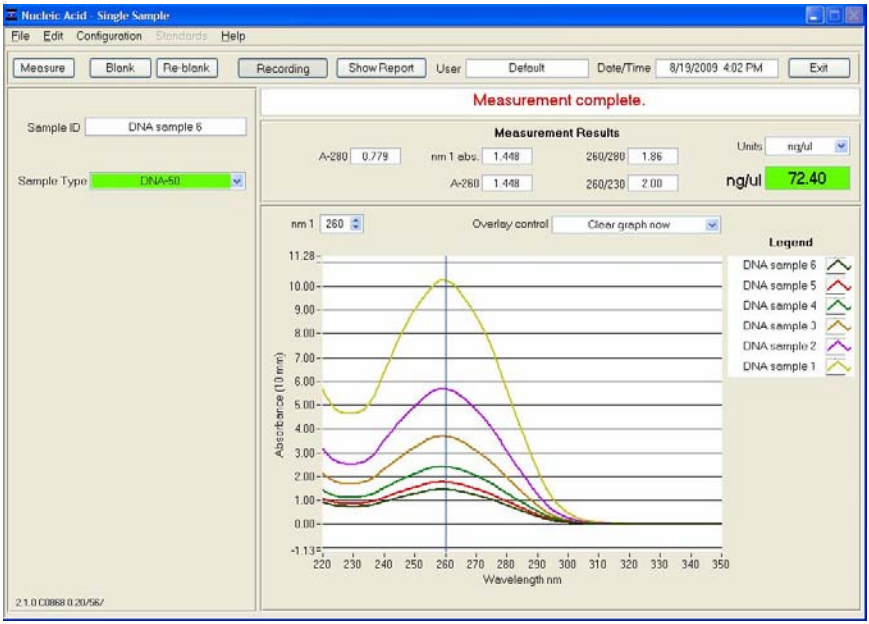
Sample ID

The Sample ID is highlighted for overtyping or barcode scanning. The user may input a sample ID that will be used to identify the measurement in a report print and in the archived data file. The sample ID entry is "key focused", meaning it is the default selection on the screen and should have a flashing text cursor when the instrument is waiting to make a new measurement. See the section on Sample ID List (plate) Format for additional information regarding the entry of sample IDs.

Exit

This command closes all application modules and supporting options. After clicking the Exit button, the user has 10 seconds to cancel the exit command. If no action is taken within 10 seconds, the exit command is carried out. Note: All measurement data is automatically saved to an archive file and requires no user action.

Functions Specific to the Single Sample Mode

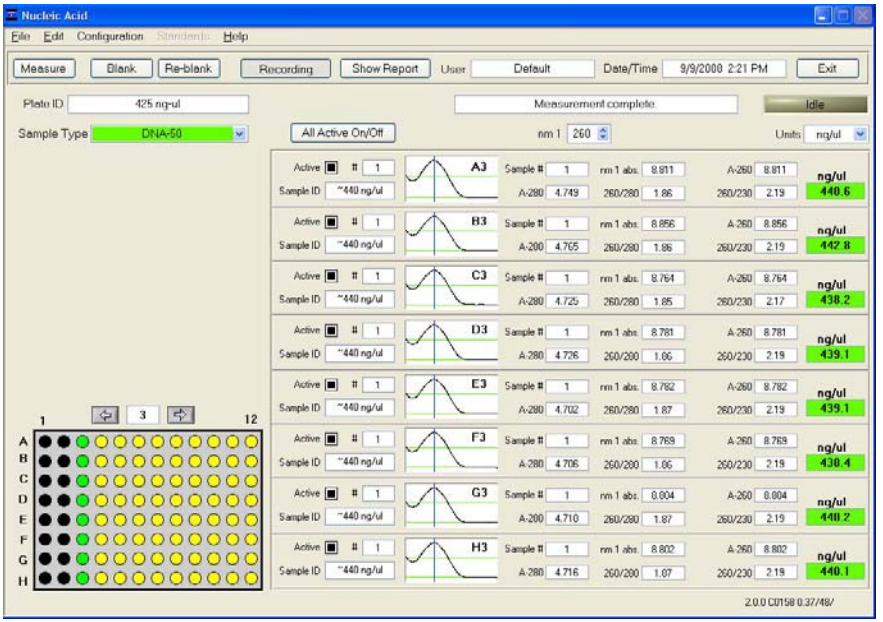


Load Next Sample ID (F7)
This button enables the user to automatically load the next sample ID when utilizing an imported list (.txt file) of sample names. Note: This button will only be displayed when a list of sample IDs has been loaded.

Legend
The most recent sample ID will be listed under the Legend heading on the right side of the screen. When the **Accumulate until clear** overlay control is selected, the most recent sample measured will be displayed at the top of the list. Note: A maximum of 12 sample IDs will be displayed in the list at any one time. When more than 12 samples are measured without clearing the spectrum- the earliest sample IDs will be removed from the legend list

Note: The Single Sample acquisition screen for each module is similar to the one displayed above. Screen shots of the single sample mode will not be presented for each application in section five.

Functions Specific to the 8 Sample Mode

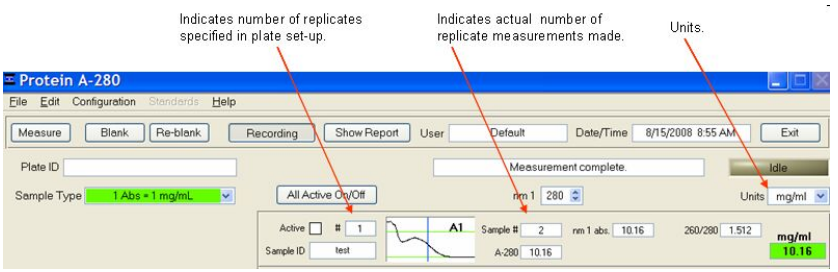


Sample Plate Map

The on-screen sample plate will be automatically populated if a Sample ID file is imported. Alternately- the user may manually type in a Sample ID or other identifying descriptor. The Sample Position Illuminator allows the user to visualize the row of a standard 96-well micro titer plate that is to be sampled for measurement. This lighted guide corresponds to the sample status color code displayed on the software screen and the pattern of illumination is determined by plate configuration at set up.

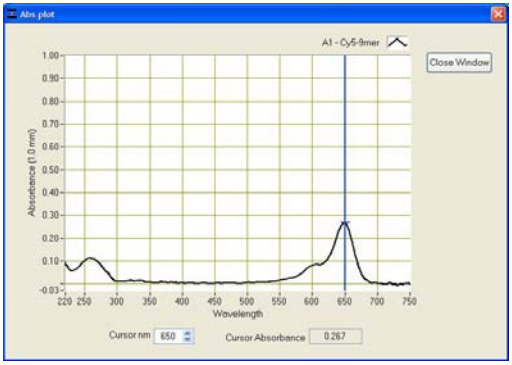
Status

The Status button will turn green during a measurement cycle.



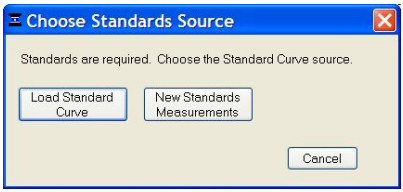
 # and Sample #
The # indicator to the left of the spectra indicates the number of replicates originally called for when sample names were entered in manually or by loading a plate file. The Sample # located to the right of the spectrum is activated when a sample measurement is being recorded. It indicates the replicate number of the last sample processed for a particular well and increments with each successive measurement.

Expanded Sample Spectrum View
The user may display an expanded view of a single spectrum by clicking on the spectrum of interest. This view will display one movable cursor with the respective nm and absorbance reported in the boxes at the bottom of the screen. Note: Data is archived as the measurement is made. Changing the cursor position after the data is archived will not change the data files. Moving the cursor via the expanded view will not change the selected wavelength positions on the main acquisition page.

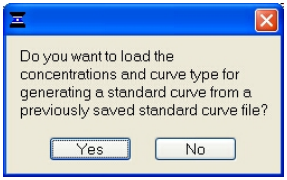


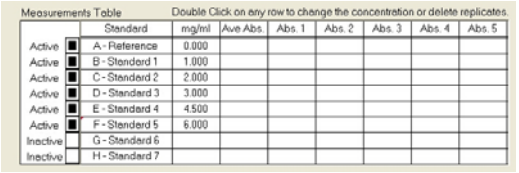
Standard Curves
A standard curve is required every time a BCA, Lowry, Bradford or Pierce 660 nm assay is run. Although curves can be saved and reloaded in the NanoDrop 8000 software, it is recommended that the user follow manufacturers' guidelines and generate new standard curves if appropriate. Both single and multi-point standard curve generation is incorporated into the software. A standard curve can be developed using a reference (assay reagent only – no protein) and a single replicate of one standard. There is no set order in which standards must be run. The multi-point standard curve generator allows a maximum of 5 replicates for each of 7 different standards.

The following box will appear after the module initialization is complete:



The user may load a previously saved standard curve or generate a new curve. Selecting the New Standards Measurements button will bring up the dialogue box on the left, below:





Clicking on the Yes button will allow the user to import just the Standard series without the respective measured values (See image on the right, above). This option is very useful when running a routine series of standards.

The Standards menu drop down may also be used to load a previously saved curve, generate a new standard curve, or view the current standard curve. See the respective protein assay section for additional details.

Additional features

User Manual
The User Manual is accessible from the Main Menu and from the Help menu in all of the application modules. It can also be accessed by selecting Start → Programs → NanoDrop →ND-8000 (version).

Print Window
A Print dialogue can be initiated from the File pull-down menu or by typing **Ctrl+P**.

Saving Current Screen as .JPG Image
The current screen can be saved as a .JPG image file by selecting **Save Window** from the File pull down menu.

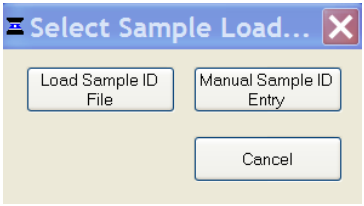
Escape Key (ESC)
The escape key is set to exit out of all screens. Hitting the **Escape** key twice will log the user out of an application module.

4. Sample ID Entry Options

Sample ID entry- Single Sample Mode

Selecting the Sample Loading Mode

After the instrument has completed the initialization process the following window will automatically display the options for plate setup:



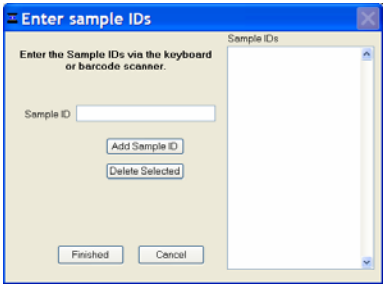
Load Sample ID File

The NanoDrop 8000 software enables the user to load a list of predefined sample IDs or names in either the 8 Sample or Single Sample mode.

The lists may be created in Excel or Notepad but all lists must be saved as a .txt file. It is recommended that the files be stored in the Plate Files folder at *C:\ND-8000 Data.* When creating a file, enter all sample names in a single file. Do not include a column header. A barcode reader can be used to scan in individual bar-coded sample names into the .txt file. The column format enables the user to predefine and load an unlimited number of sample IDs using just one file.

Manual Entry

This option opens the Enter Sample IDs window enabling the user to manually enter samples IDs as prompted by the following screen:



Alternatively, a sample name may be entered directly into the sample ID box on the acquisition screen prior to each measurement.

Barcode scanners may also be used to enter sample IDs. After each entry use either the Add sample ID button or the keyboard Enter key to add the sample to the Sample ID list on the right side of the box. Use the scroll feature to view sample IDs entered at the bottom of the list.

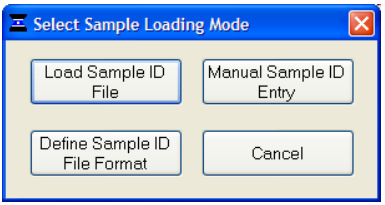
Configuration Options for Single Sample Mode
If the user cancels the Plate Setup Mode without loading a list file or manually entering in sample IDs, the entry operations may be accessed from the Configuration drop down on the main acquisition page. In addition, the drop down includes the following two options:

- **Sample ID Required** - If selected, the sample ID field must be populated prior to each measurement.

- **Prompt Close Data Viewer -** If selected, the user will be given the option of closing the Data Viewer when exiting the software module.

Sample ID entry- 8 Sample Mode

Selecting the Sample Loading Mode
After the instrument has completed the initialization process the following window will automatically display the options for plate setup:



Load Sample ID File
The NanoDrop 8000 offers several options for entering sample IDs. When making only a few measurements, it is easy to simply type in sample names prior to measurement or use the Manual Sample ID Entry. The NanoDrop 8000 software also enables the user to load a list of predefined sample IDs or names, which improves efficiency and reduces errors when measuring many samples. The Load Sample ID File option opens the Plate file folder enabling the user to select a list of pre-defined sample IDs.

The lists may be created in Excel or Notepad but all lists must be saved as a .txt file. It is recommended that the files be stored in the Plate Files

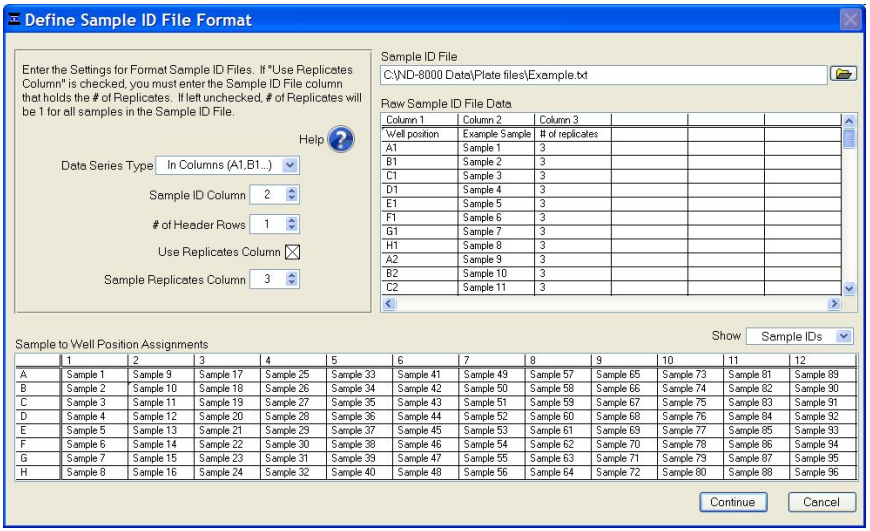
folder at *C:\ND-8000 Data.* When creating a file, enter all sample names in one column and the number of replicates desired for each sample in another column. A barcode reader can be used to scan in individual bar-coded sample names into the .txt file. The column format enables the user to predefine and load an unlimited number of sample IDs using just one plate file.

Note: Alternatively, sample names may be stored in an 8 x 12 array within a.txt file. The 8x12 format can only load one plate worth of sample ID's at a time (96 samples). As there is already a one to one direct correspondence with the on screen plate map when using this format, one does not need to use the Define Sample ID File Format button. Use the Load Sample ID File button to directly load 8 x 12 array sample ID information.

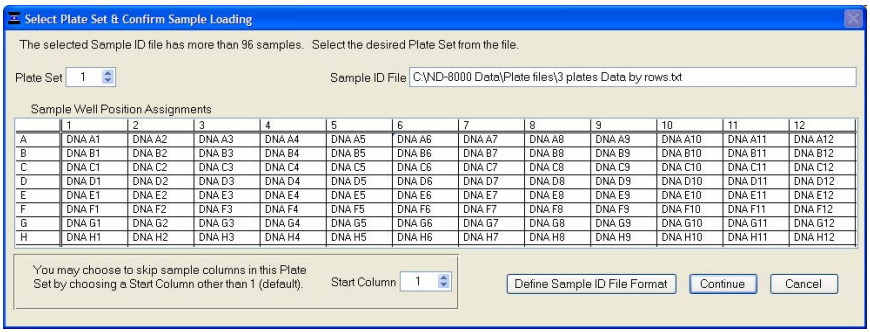
Define Sample ID File Format

With the exception of the 8 x 12 array format described above, before a .txt list file can be loaded, the Define Sample ID File Format feature should be used to correctly specify which orientation the sample IDs should correspond to well positions on the plate map on the software acquisition page. The user is first prompted to select a pre-existing plate file from the Plate file folder using the browser dialog box.

Use the Data Series Type drop down box to select the proper sequence (columns or rows) Sample names will either fill in and correspond to well positions in **Rows** (first 12 sample IDs will correspond to the A1 to A12 positions, the next 12 IDs to the B1 to B12 positions etc) or in **Columns** (first 8 sample names will correspond to the A1 to H1 positions, the next 8 names to the A2 to H2 positions etc). Each set of 96 samples are considered to be a "plate". Partial "plates" are allowable.



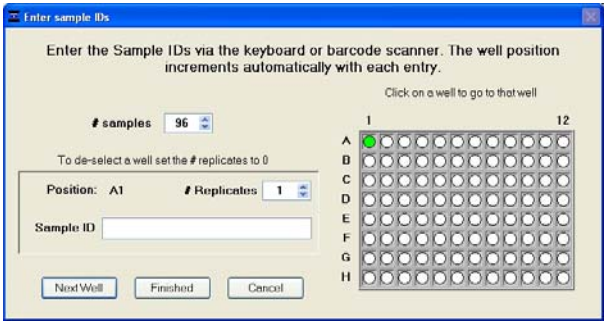
Selecting Continue after defining the format will load the plate file. If more than 96 sample IDs are in a list, the following pop-up window will appear.



The Plate Set selector will allow the user to review that each set of samples fills the screen map in the expected order. If there is a discrepancy- click on the Define Sample ID File Format button on the bottom right to return to the previous step.

Manual Entry

This option opens the Enter Sample IDs window enabling the user to manually enter samples IDs and number of replicates for each sample to be tested as prompted by the following screen:

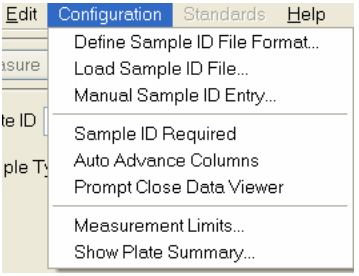


To move to the next well in a column, enter the sample name and then click on the **Next Well** button. To select a well in another column, highlight the well of interest, enter in the sample name and hit the keyboard **Enter** button.

Cancel
Closes the window without making any changes.

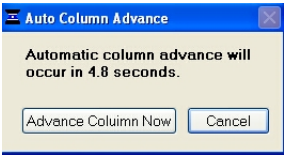
Note: If a Sample ID File format has been defined (see below), that format will be retained and applied to subsequent plates until the user selects a different format. For this reason, when loading a predefined list of sample names, (i.e. a plate file) it is crucial for a user to know whether the sample names should fill in the screen plate map by row (sets of 12) or by column (sets of 8).

Configuration Options for 8 Sample Mode
If the user cancels the Plate Setup Mode without loading a plate file, the above operations may also be accessed from the Configuration drop down on the main acquisition page. In addition, the Configuration drop down includes the following options:



- **Sample ID Required** - If selected, the sample ID field must be populated for each sample tested. Wells requiring a sample ID will appear red in the sample status color code (see below for description of this feature) and the following message will appear when the user attempts to make a sample measurement.

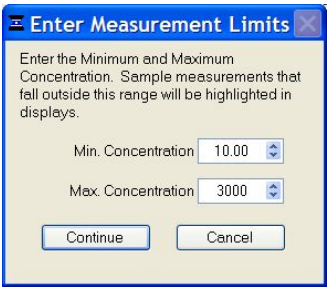
- **Auto Advance Columns** - If selected, there is a 5 second delay before the displayed spectra clear and the status code and Sample Position Illuminator (see below for description of these features) advance to the next column for sampling. This gives the user the option of canceling auto advance for that column. Auto advance will restart when the next column is measured. This feature can be set as a default function under the User Preferences application.



Note: All data is automatically archived and is accessible though the Data Viewer.

- **Prompt Close Data Viewer -** If selected, the user will be given the option of closing the Data Viewer when exiting the software module.

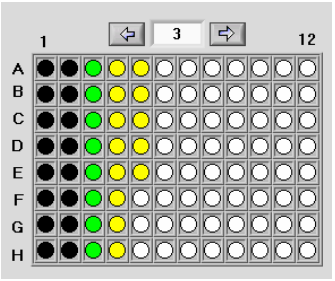
- **Measurement Limits** - Allows the user to set minimum and maximum concentration limits for samples to be measured. Additional information about setting measurement limits is included in each application module section.



- **Show Plate Summary** - Allows the user to review the results of the plate at any time during the measurement session. Once the entire plate has been read, this feature will allow the user the option of marking samples of interest for repeat testing

Sample Status Color Code

The 96 well plate schematic allows the user to monitor the position of the samples being measured. Black wells indicate that samples in the corresponding wells have been measured. Green wells indicate which positions are currently being measured while yellow represents well positions that are expected to be measured. Pre-loading a Sample ID file or manually entering in sample names will activate the respective wells. To select additional wells, use the Configuration drop down box and select Manual Plate Set-up. If Sample ID Required has been selected from the configuration drop down, any wells that do not have an ID assigned will appear red and a sample ID must be assigned before measurement can begin.



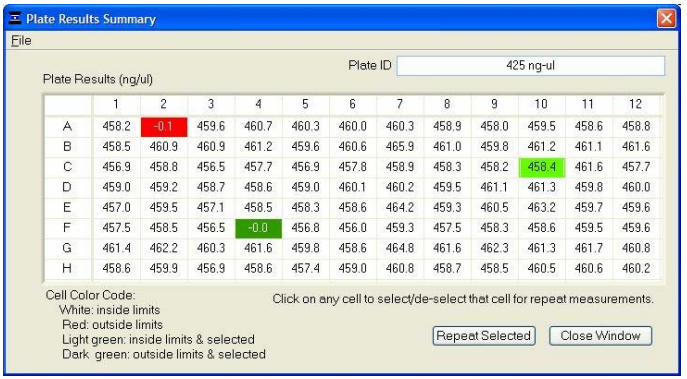
Sample Position Illuminator

This lighted guide allows the user to visualize the row of a standard 96-well micro titer plate that is to be sampled for measurement. The Sample Position Illuminator corresponds to the sample status color code displayed on the software screen and the pattern of illumination is determined by plate configuration at set up. If measurement limits have been defined, wells that are out of the defined range will flash on the

Sample Position Illuminator immediately after measurement. These positions will continue flashing until measurement of the current plate sample set is complete, the results have been reviewed and the Plate Results Summary window is closed.

Plate Review

The Plate Results Summary window will automatically appear if the auto advance column feature has been selected and measurement of the plate sample set is complete. Selecting Show Plate Summary from the configuration drop down menu will also open this window. From this window, sample wells of interest can be marked for repeat. If measurement limits were defined, sample measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator and the concentrations will appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.



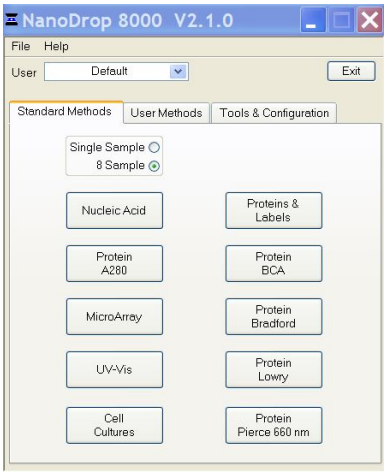
	1	2	3	4	5	6	7	8	9	10	11	12
A	458.2	-0.1	459.6	460.7	460.3	460.0	460.3	458.9	458.0	459.5	458.6	458.8
B	458.5	460.9	460.9	461.2	459.6	460.6	465.9	461.0	459.8	461.2	461.1	461.6
C	456.9	458.8	456.5	457.7	456.9	457.8	458.9	458.3	458.2	458.4	461.6	457.7
D	459.0	459.2	458.7	458.6	459.0	460.1	460.2	459.5	461.1	461.3	459.8	460.0
E	457.0	459.5	457.1	458.5	458.3	458.6	464.2	459.3	460.5	463.2	459.7	459.6
F	457.5	458.5	456.5	-0.0	456.8	456.0	459.3	457.5	458.3	458.6	459.5	459.6
G	461.4	462.2	460.3	461.6	459.8	458.6	464.8	461.6	462.3	461.3	461.7	460.8
H	458.6	459.9	456.9	458.6	457.4	459.0	460.8	458.7	458.5	460.5	460.6	460.2

5. Standard Methods

Software Architecture and Features

Main Menu

With the sampling arm in the down position, start the operating software by selecting the following path: *Start → Programs → NanoDrop → ND-8000 (version)* The software opens to display three tabs including Standard Methods, User Methods and Tools & Configuration.



Applications

The operating software has been tailored to meet the life scientist's needs. It includes the following pre-configured application modules:

- **Nucleic Acid** – concentration and purity of nucleic acid
- **Protein A280** – concentration and purity of purified protein
- **MicroArray** – dye incorporation concentration and purity of nucleic acid
- **UV-Vis** – general UV-Vis measurements
- **Cell Cultures** – absorbance (light scattering) measurement of suspended microbial cells
- **Proteins & Labels** – concentration of dye-labeled proteins, conjugates, and metalloproteins
- ***Protein BCA –** protein concentration using the BCA assay
- ***Protein Bradford** – protein concentration using the Bradford assay
- ***Protein Lowry –** protein concentration using the Modified Lowry assay
- ***Protein Pierce 660 nm-** protein concentration using the new 660 nm assay

Note: *Colorimetric applications are not available in the single sample mode and the associated menu buttons will be grayed out.

Nucleic Acids

Nucleic acid samples can be readily checked for concentration and quality using the NanoDrop 8000 Spectrophotometer. To measure nucleic acid samples, select the 'Nucleic Acid' application module on the Main Menu.

Sample Volume Requirements

Field experience has indicated that 1ul samples are sufficient to ensure accurate and reproducible results when measuring aqueous nucleic acid samples. However, if you are unsure about the surface tension properties of your sample or your pipettor accuracy, a 1.5-2 ul sample is recommended to ensure that the liquid sample column is formed and the light path is completely covered by sample.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Measurement Concentration Range

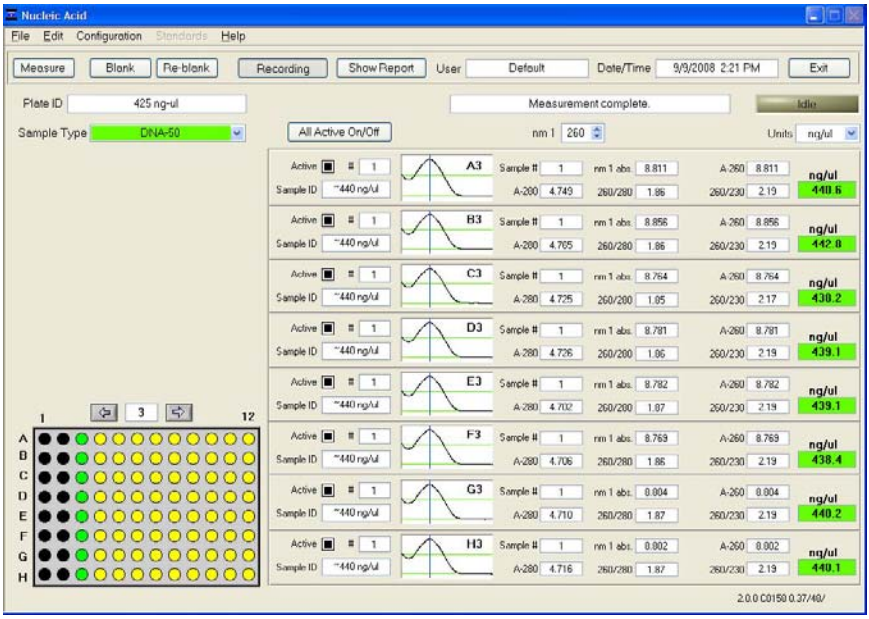
The NanoDrop 8000 Spectrophotometer will accurately measure dsDNA samples up to 3700 ng/ul without dilution. To do this, the instrument detects the high concentration and automatically utilizes the 0.2mm pathlength to calculate the absorbance. The table below lists the concentration range and typical reproducibility for nucleic acid measurements on the NanoDrop 8000:

Detection Limit (ng/ul)	Approx. Upper Limit (ng/ul)	Typical Reproducibility (minimum 96 replicates) (SD= ng/ul; CV= %)
2.5	3700 ng/ul (dsDNA) 3000 (RNA) 2400 (ssDNA)	sample range 2.5-100 ng/ul: ± 2.5 ng/ul sample range >100 ng/ul: ± 2.5%

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be set for nucleic acid measurements as defined by the absorbance at 260 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Nucleic acid measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary

has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features



- **Sample Type:** used to select the (color-keyed) type of nucleic acid being measured. The user can select 'DNA-50' for dsDNA, 'RNA-40' for RNA, 'ssDNA-33' for single-stranded DNA, Oligo Calc and 'Other' for other nucleic acids. The default is DNA-50.

  If 'Other' is selected, the user can select an analysis constant between 15-150. When navigating amongst the three general sample types within the Nucleic Acids module, the last constant value entered within the 'Constant' sample type will be retained. See the "Concentration Calculation (Beer's Law)" Appendix for more details on this calculation.

  Choosing the Oligo Calc option will allow the user to enter a defined oligo sequence. The molecular weight, molar extinction coefficient and the concentration factor specific to that sequence will be displayed. Sequences can be cut and pasted into the box from other sources. Use the browse button on the left of the Oligo box on the acquisition page to modify the sequence.

  See the Section below Unique Screen features for more details regarding the
  use of this feature as a stand alone Oligo Calculator.

- **nm 1 and nm 1 abs:** current value of the user-selectable wavelength cursor and corresponding 10 mm equivalent normalized absorbance at the respective wavelength absorbance. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.

- **A260:** absorbance of the sample at 260 nm represented as if measured with a conventional 10 mm path. Note: This is 10X the absorbance actually measured using the 1 mm path length and 50X the absorbance actually measured using the 0.2 mm path length.

- **A280:** sample absorbance at 280 nm is represented as if measured with a conventional 10 mm path. Note: This is 10X the absorbance actually measured using the 1 mm path length and 50X the absorbance actually measured using the 0.2 mm path length.

- **260/280:** ratio of sample absorbance at 260 nm and 280 nm. The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. See "260/280 Ratio" in the Troubleshooting section for more details on factors that can affect this ratio.

- **260/230:** ratio of sample absorbance at 260 nm and 230 nm. This is a secondary measure of nucleic acid purity. The 260/230 values for "pure" nucleic acid are often higher than the respective 260/280 values. They are commonly in the range of 1.8-2.2. If the ratio is appreciably lower, this may indicate the presence of co-purified contaminants.

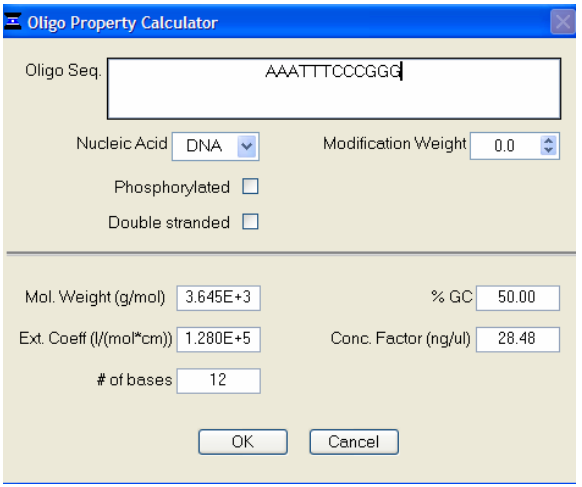
- **Concentration (ng/ul):** sample concentration based on the absorbance at 260 nm and the selected analysis constant. The calculated concentration is displayed in the units selected via the units drop down box. The units will default to ng/ul each time the software is opened. See the "Concentration Calculation (Beer's Law)" in the appendix for more details on this calculation.

- **Show Report:** formatted for 200 samples although the buffer size can be modified.

Oligo Calculator

The Oligo Calculator enables the user to type in a specific sequence and choose parameters associated with the oligo.

When the Oligo Calc sample type option is first selected the following box will appear:



The calculator allows the user to define the sequence and include relevant information such as whether the oligo is phosphorylated or double stranded. The information boxes in the bottom of the screen are automatically populated based about the sequence entered.

Spectrum Normalization

The baseline is automatically set to the absorbance value of the sample at 340 nm, which should be very nearly zero absorbance. All spectra are referenced off of this zero.

Protein A280

Proteins, unlike nucleic acids, can exhibit considerable diversity. The A280 method is applicable to purified proteins exhibiting absorbance at 280 nm. It does not require generation of a standard curve and is ready for quantitation of protein samples at startup. This module displays the UV spectrum, measures the protein's absorbance at 280 nm (A280) and calculates the concentration (mg/ml). Like the Nucleic Acid module, it automatically switches to the 0.2 mm pathlength at very high concentrations of protein. Also analogous to the Nucleic Acid module, the Protein A280 module displays and records 10 mm (1 cm) equivalent data on the screen and in the archived data file.

Sample Volume Requirements

Some proteins are hydrophobic and others hydrophilic giving rise to variable surface tension properties in the sample to be measured. Additionally the presence of surfactants or detergents in reagents, such as the Bradford reagent, can significantly alter the surface tension resulting in difficulty forming adequate columns for measurement. The column formation issue can be overcome without affecting the sample's absorbance by using a larger sample volume. A 2 ul sample size is recommended for protein measurements.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Pedestal Reconditioning

Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form properly. If this occurs, "buff" the measurement pedestal surfaces by rubbing each measurement surface aggressively with a dry laboratory wipe 30-40 times. This will "re-condition" the surface allowing the liquid sample column to form.

Alternatively, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about the PR-1 kit may be found on our website.

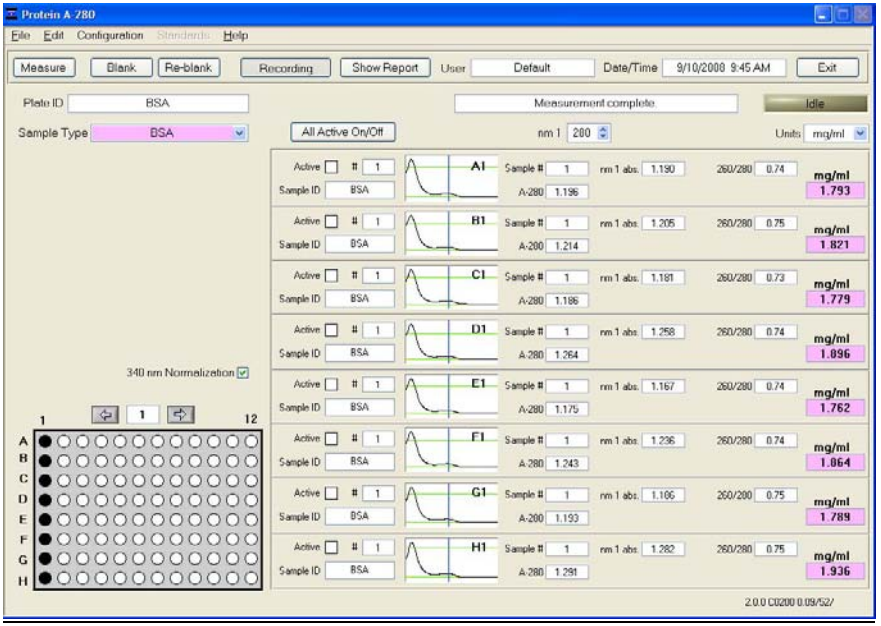
Measurement Concentration Range

The NanoDrop 8000 Spectrophotometer will accurately measure protein samples up to 100 mg/ml (BSA) without dilution. To do this, the instrument detects the high concentration and automatically utilizes the 0.2 mm pathlength to calculate the absorbance. The table below lists the concentration range and typical reproducibility for purified BSA measurements on the NanoDrop 8000:

Sample Type	**Detection Limit**	**Approx. Upper Limit**	**Typical Reproducibility (minimum 96 replicates) (SD= mg/ml; CV= %)**
Purified BSA	0.15 mg/ml	100 mg/ml	sample range 0.15-5 mg/ml: ± 0.15 mg/ml sample range >5 mg/ml: ± 2.5%

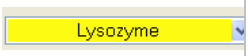
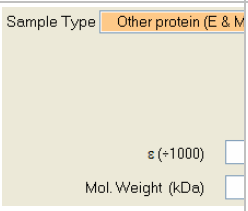
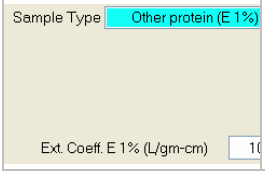
By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be set for protein measurements as defined by the absorbance at 280 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Protein measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features



- **Sample Type:** There are six sample types (options) available for purified protein analysis and concentration measurement. All of the options can be viewed by clicking the mouse while it is positioned within the 'Sample Type' box. The sample type (color-keyed) can be selected by clicking on the preferred option or by scrolling through the selections using the up or down arrow keys located to the left of the sample type box. Note: Concentrations for all eight samples will be calculated using the same mass extinction coefficient as determined by the Sample Type selection. A description of each sample type is given below.

1 Abs = 1 mg/mL	A general reference setting based on a 0.1% (1 mg/ml) protein solution producing an Absorbance at 280 nm of 1.0 A (where the pathlength is 10 mm or 1 cm).
BSA	Bovine Serum Albumin reference. Unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 6.7 at 280 nm for a 1% (10 mg/ml) BSA solution.
IgG	IgG reference. Unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (10 mg/ml) IgG solution.

	Lysozyme reference. Unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 26.4 at 280 nm for a 1% (10 mg/ml) Lysozyme solution.
	User-entered values for molar extinction coefficient (M^{-1} cm^{-1}) and molecular weight (MW) in kilo Daltons for their respective protein reference. Maximum value for e is 999 X 1000 and maximum value for M.W. is 9999 X 1000.
	User-entered mass extinction coefficient (L $gm^{-1}cm^{-1}$) for a 10 mg/ml (1%) solution of the respective reference protein.

- **nm 1 and nm 1 abs**: current value of the user-selectable wavelength cursor and corresponding 10mm equivalent normalized absorbance at the respective wavelength absorbance. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.

- **A280 10-mm Path**: 10 mm-equivalent absorbance at 280 nm for the protein sample measured.

- **A260/280**: ratio of sample absorbance at 260 nm and 280 nm.

- **Concentration (mg/ml):** sample concentration based on the absorbance at 280 nm and the selected analysis constant. The calculated concentration is displayed in the units selected via the units drop down box. The calculated concentration is displayed in the units selected via the units drop down box. The units will default to mg/ml each time the software is opened. See the “Concentration Calculation (Beer’s Law)” in the appendix for more details on this calculation.

- **Show Report:** formatted for 200 samples although the buffer size can be modified.

Spectrum Normalization

The baseline is automatically set to the absorbance value of the sample at 340 nm, which should be very nearly zero absorbance. All spectra are referenced off of this zero unless the user elects to turn off the normalization.

MicroArray

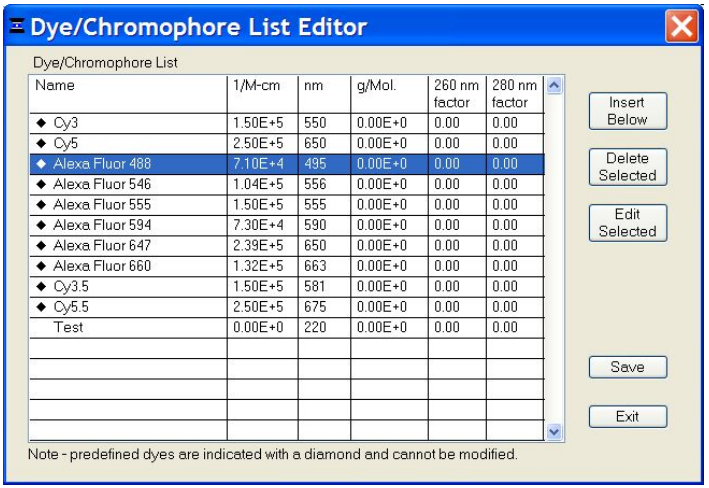
The capability to pre-select viable fluorescent-tagged hybridization probes for gene expression in micro-arrays can eliminate potentially flawed samples and improve research effectiveness. The NanoDrop 8000 Spectrophotometer measures the absorbance of up to 2 fluorescent dyes, allowing detection at dye concentrations as low as 0.2 picomoles per microliter.

Fluorescent Dye Selection

There are currently a number of fluorescent dyes that are hard-coded for use with the MicroArray module (see table below). Users can also enter & save fluorescent dyes not coded within the NanoDrop 8000 software using the Dye/Chromophore Editor button found in the main menu.

Dyes can be selected using the scroll arrows or by highlighting the Dye box. The respective absorbance wavelength, extinction coefficient, and 260 nm and 280 nm % corrections will be automatically utilized for measurement and concentration calculation. The default setting for Dye 1 is Cy3 and Dye 2 is Cy5. See User Preferences under the Tools & Configuration main page tab to designate alternative dyes as the defaults.

Note: Please refer to the dye manufacturer for the appropriate correction factors for user entered dyes.



Sample Volume Requirements

Field experience has indicated that 1 ul samples are sufficient to ensure accurate and reproducible results when measuring aqueous nucleic acid samples containing incorporated fluorescent dyes. However, if you are unsure about the surface tension properties of your sample or your pipettor accuracy, a 1.5-2 ul sample is recommended to ensure that the liquid sample column is formed and the light path is completely covered by sample.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Measurement Concentration Range

The NanoDrop 8000 Spectrophotometer will accurately measure fluorescent-dye and nucleic acid concentrations up to 100 pmols/ul (Cy3) and 750 ng/ul (DNA) respectively without dilution. A table of sample concentration ranges is listed below.

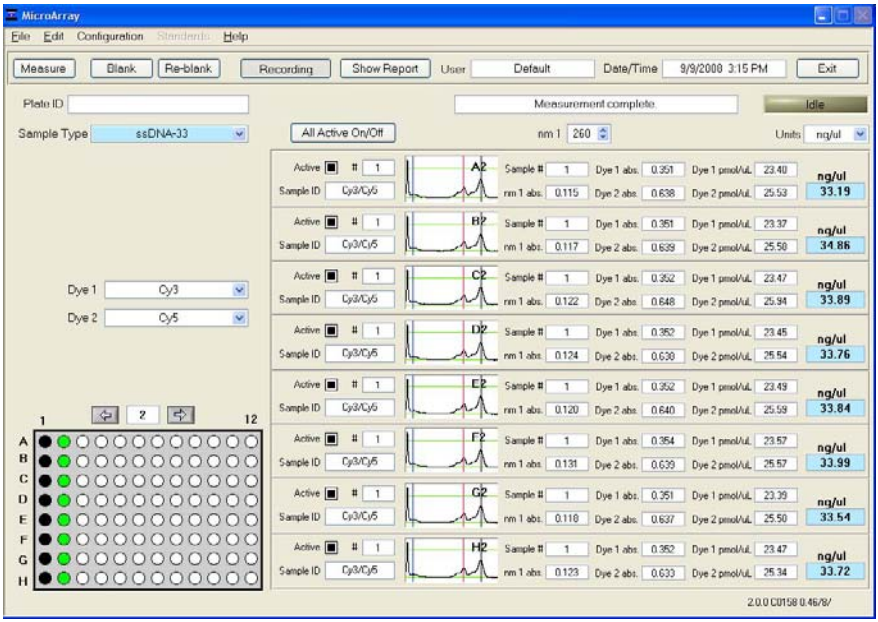
Sample Type	Detection Limit (pmol/ul)	Approx. Upper Limit (pmol/ul)	Typical Reproducibility (minimum 48 replicates) (SD= pmol/ul; CV= %)
Cy3, Cy3.5, Alexa Fluor 555 and Alexa Fluor 660	0.25	100	sample range 0.25-4 pmol/ul: ±. 0.25 sample range >4.0 pmol/ul: ± 2.5%
Cy5, Cy5.5 and Alexa Fluor 647	0.17	60	sample range 0.17-2.4 pmol/ul: ± 0.17 sample range >2.4 pmol/ul: ± 2.5%
Alexa Fluor 488 and Alexa Fluor 594	0.50	215	sample range 0.50-8.0 pmol/ul: ±. 0.50 sample range >8.0 pmol/ul: ± 2.5%
Alexa Fluor 546	0.42	145	sample range 0.42-6.0 pmol/ul: ±. 0.42 sample range >6.0 pmol/ul: ± 2.5%

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be set for the nucleic acid component as defined by the absorbance at 260 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Nucleic acid measurements that are outside of the defined range will be indicated by a flashing light on the

Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed.

Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features



- **Sample Type:** used to select the (color-keyed) type of nucleic acid being measured. The user can select 'DNA-50' for dsDNA, 'RNA-40' for RNA, ssDNA-33' for single-stranded DNA, or 'Other' for other nucleic acids. The default is ssDNA-33. If 'Other' is selected, the user can select an analysis constant between 15-150. When navigating amongst the three (3) general sample types within the MicroArray module, the last value of the constant entered within the Constant Sample Type will be retained. See "Concentration Calculation (Beer's Law)" in the appendix for more details on this calculation.

- **nm 1 and nm 1 abs:** current values of the user-selectable wavelength cursor and corresponding absorbance value for a 1 mm pathlength. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.

- **Dye 1:** user selected dye.

- **Dye 2:** user selected dye.

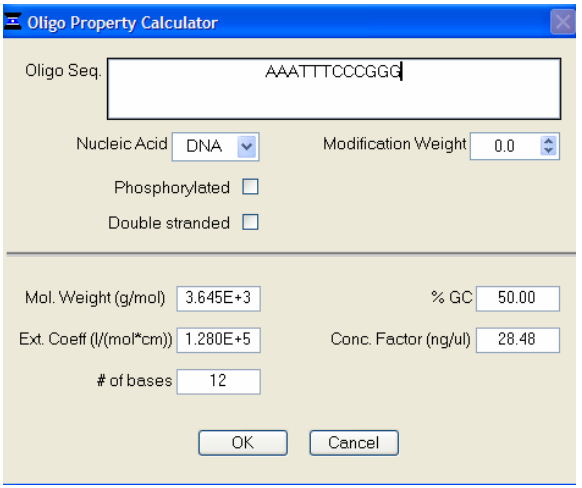
- **Dye 1 (or 2) pmol/ul:** concentration based upon selected dye's extinction coefficient. See "Concentration Calculation (Beer's Law)" in the appendix for more details on this calculation.

- **Concentration (ng/ul):** sample concentration based on the absorbance at 260 nm and the selected analysis constant. The calculated concentration is displayed in the units selected via the units drop down box. The calculated concentration is displayed in the units selected via the units drop down box. The units will default to ng/ul each time the software is opened. See the "Concentration Calculation (Beer's Law)" in the appendix for more details on this calculation.

- **Show Report:** formatted for 200 samples although the buffer size can be modified.

Oligo Calculator
The Oligo Calculator enables the user to type in a specific sequence and choose parameters associated with the oligo sequence.

When the Oligo Calc sample type option is first selected the following box will appear:



The calculator allows the user to define the sequence and include relevant information such as whether the oligo is phosphorylated or double stranded. The information boxes in the bottom of the screen automatically populated based about the sequence entered.

Baseline Calculation & Normalization

The software normalizes the visual spectrum display for all readings at 750 nm. Normalization for the fluorescent dyes is based upon a software determined slope between 400 and 750 nm for dye concentration calculations.

UV-VIS

The 'UV/VIS Absorbance' module allows the NanoDrop 8000 Spectrophotometer to function as a conventional spectrophotometer. Sample absorbance is displayed on the screen from 220 nm to 750 nm and cursors permit the measurement of individual peaks.

Sample Volume Requirements

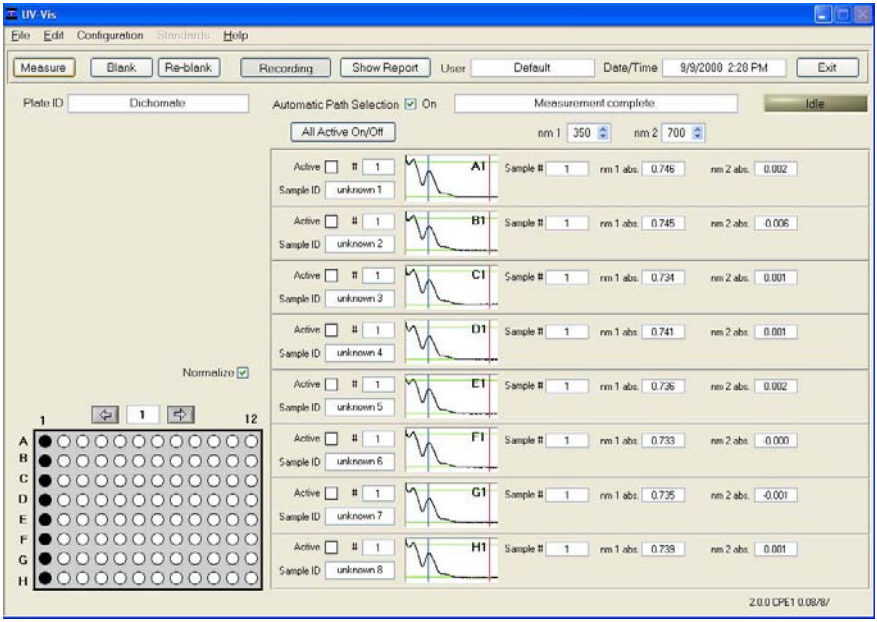
Field experience has indicated that 1 ul samples are sufficient to ensure accurate and reproducible results when measuring most aqueous samples. However, if you are unsure about the surface tension properties of your sample or your pipettor accuracy, a 1.5-2 ul sample is recommended to ensure that the liquid sample column is formed and the light path is completely covered by sample.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Measurement Concentration Range

The NanoDrop 8000 Spectrophotometer is configured to measure absorbance up to the 1 mm pathlength equivalent of 7.5 A when utilizing the 0.2 mm path via the automatic path selection feature. By selecting Measurement Limits from the configuration drop down menu, minimum and maximum absorbance limits can be set for the shorter of the two user selectable wavelengths. These limits cannot be set as a default and must be defined each time the application module is opened. Sample absorbances that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The absorbances will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing

Unique Screen Features



- **Automatic Path Selection:** If selected, the software will automatically switch from using the 1.0 mm pathlength to the 0.2 mm path when the absorbance value first reaches 1.25 for EITHER of the two wavelengths indicated with the two cursors.

  Note: It is important to pre-select a cursor position for the wavelength of interest before the measurement is taken. If the cursors are set for wavelengths with minimal absorbance, the 0.2 mm pathlength will not be utilized.

  Note: When the 0.2 mm pathlength is utilized, the data will be archived and displayed normalized to the 1.0 mm pathlength. The feature can be turned off using the UV/Vis tab in the Users Preferences module.

- **nm 1/abs1** and **nm 2/abs2:** current values of the user-selectable wavelength cursors and corresponding absorbencies for a 1 mm pathlength. The wavelengths can be set by using the up/down arrows or typing in the desired wavelength. The default wavelengths are 300 nm and 700 nm.

- **Normalize:** a user-selectable feature in this module**.** If selected, the software will automatically normalize the spectrum based on the lowest absorbance value in the range 400-700 nm.

- **Show Report:** formatted for 200 samples although the buffer size can be modified.

Proteins & Labels

This software module can be used to determine protein concentration (280 nm) as well as fluorescent dye concentration (protein array conjugates), or to measure the purity of metalloproteins (such as hemoglobin) using wavelength ratios.

Fluorescent Dye Selection

There are currently ten fluorescent dyes that are hard-coded for use with the Proteins and Labels module (see table below). Users can also enter & save fluorescent dyes not coded within the NanoDrop 8000 software using the 'Dye/Chromophore Editor' button found under the Tools & Configuration button.

The NanoDrop 8000 Spectrophotometer measures the absorbance of up to 2 fluorescent dyes, allowing detection at dye concentrations as low as 0.2 uM.

The respective absorbance wavelength, extinction coefficient, and 280 nm % corrections will be automatically utilized for measurement and concentration calculation. The default setting is Cy3. In addition to the fluorescent dyes available from the drop-down menu, an option on the main acquisition page entitled 'None' is also available. Selecting 'None' disables the respective calculations & numeric displays corresponding to a dye.

Note: Please refer to the dye manufacturer for the appropriate correction factors for user entered dyes.

Sample Volume Requirements
Additionally the presence of surfactants or detergents in reagents, such as the Bradford reagent, can significantly alter the surface tension resulting in difficulty forming and/or maintaining adequate columns for measurement. The column formation issue can be overcome without affecting the sample's absorbance by using a larger sample volume. A 2 ul sample size is recommended for protein measurements.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Pedestal Reconditioning
Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not from properly. If this occurs, "buff" the measurement pedestal surfaces by rubbing each measurement surface aggressively with a dry laboratory wipe 30-40 times. This will "re-condition" the surface allowing the liquid sample column to form.

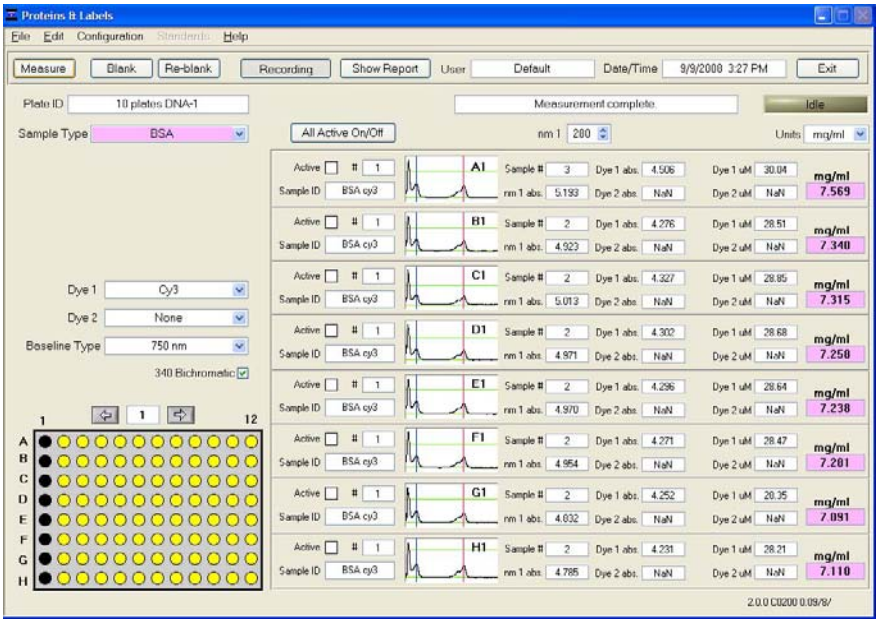
Alternatively, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about the PR-1 kit may be found at on our website.

Measurement Concentration Range
The NanoDrop 8000 Spectrophotometer will accurately measure protein samples up to 20 mg/ml (BSA) without dilution. A table of concentration range and typical reproducibility is listed below:

Sample Type	Lower Detection Limit	Approx. Upper Limit	Typical Reproducibility (minimum 5 replicates) (SD= mg/ml; CV= %)
Purified BSA	0.15 mg/ml	20 mg/ml	sample range 0.15-5 mg/ml: ± 0.15 mg/ml sample range >10mg/ml: ± 2.5 %
Cy3	0.25 uM	100 uM	sample range 0.25-4 pmol/ul: ± 0.25 sample range >4.0 pmol/ul: ± 2.5 %

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be defined for the protein component in mg/ml as defined by the absorbance at 280 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Protein measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features



- **Sample Type:** The same six sample types (options) listed in the Protein A280 section are available for purified Proteins & Labels analysis and concentration measurement. All of the options can be viewed by clicking the mouse while it is positioned within the sample type box. The sample type (color-keyed) can be selected by clicking on the preferred option or by scrolling through the selections using the up or down arrow keys located to the left of the sample type box. See the Protein A280 section for a detailed description of each sample type.

- **nm 1 and nm 1 abs:** current value of the user-selectable wavelength cursor and corresponding 10mm equivalent normalized absorbance at the respective wavelength absorbance. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.

- **Dye 1:** user selected dye.

- **Dye 2:** user selected dye.

- **Dye 1 (or 2) abs:** normalized 10 mm equivalent absorbance of selected Dye.

- Dyes can be selected using the scroll arrows or by highlighting the Dye box. The respective absorbance wavelength, extinction coefficient, and 260 nm and 280 nm % corrections will be automatically utilized for measurement and concentration calculation. The default setting for Dye 1 is Cy3 and Dye 2 is Cy5. See User Preferences under the Tools & Configuration main page tab to designate alternative dyes as the defaults.

- **Concentration (mg/ml):** sample concentration based on the absorbance at 280 nm and the selected analysis constant. The calculated concentration is displayed in the units selected via the units drop down box. The calculated concentration is displayed in the units selected via the units drop down box. The units will default to mg/ml each time the software is opened.

- **Show Report:** formatted for 200 samples although the buffer size can be modified.

Baseline Type

This application module has two user-selectable “Baseline Type” options. The default setting is set to normalize the display spectrum at 750 nm. Alternatively, the 400-750 Slope Baseline Type will normalize the display at 750 nm and accommodate any linear baseline offset across the 400 to 750 nm range. The 280 nm absorbance value is normalized at 340 nm unless the user elects to turn off the normalization.

Protein BCA

The BCA (Bicinchoninic Acid) Protein Assay is an alternative method for determining protein concentration. It is often used for more dilute protein solutions and/or in the presence of components that also have significant UV (280 nm) absorbance. Unlike the Protein A280 method, the BCA Assay requires that a standard curve be generated before unknown protein concentrations can be determined. The resulting Cu-BCA chelate formed in the presence of protein is measured at its wavelength maximum of 562 nm and normalized at 750 nm. Pre-formulated reagents of BCA and CuSO4, utilized in the assay, are available in kit form from numerous manufacturers. Follow the respective manufacturer's recommendations for all standards and samples (unknowns).

Sample Volume Requirements

The presence of surfactants or detergents in reagents can significantly alter the surface tension, resulting in difficulty forming and/or maintaining adequate columns for measurement. The column formation issue can be overcome without affecting the sample's absorbance by using a larger sample volume. **A 2 ul sample size is recommended for protein measurements.**

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Pedestal Reconditioning

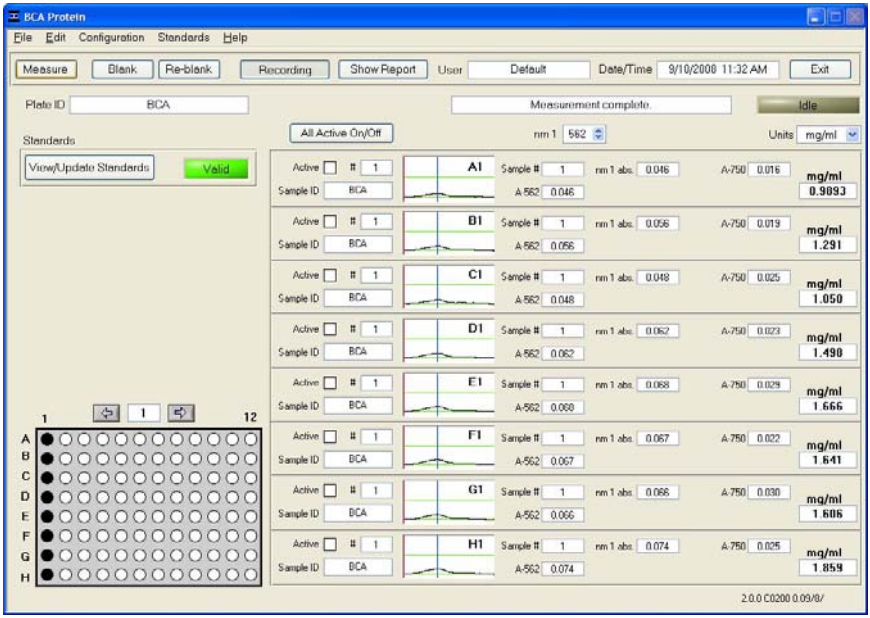
Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form properly. If this occurs, "buff" the measurement pedestal surfaces by rubbing each measurement surface aggressively with a dry laboratory wipe 30-40 times. This will "re-condition" the surface allowing the liquid sample column to form.

Alternatively, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about the PR-1 kit may be found on our website.

Measurement Concentration Range
When using a 20:1 reagent to sample volume dilution, the BCA assay concentration range of detection is ~0.20 mg/ml to 8.0 mg/ml on the NanoDrop 8000. Using a 1:1 reagent to sample volume dilution the concentration range of detection is 0.01 mg/ml – 0.20 mg/ml.

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be set for protein (mg/ml) measured with this method at 562 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Protein measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features



- **A 562nm:** the Cu-BCA complex's absorbance at 562 nm for the 1 mm pathlength.

- **nm 1 and nm 1 abs**: current value of the user-selectable wavelength cursor and corresponding absorbance value for a 1 mm

pathlength. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.

- **mg/mL:** the concentration of the sample (unknown). The calculated concentration is displayed in the units selected via the units drop down box.

- **Show Report:** formatted for 200 samples although the buffer size can be modified.

BCA Assay Sample Preparation

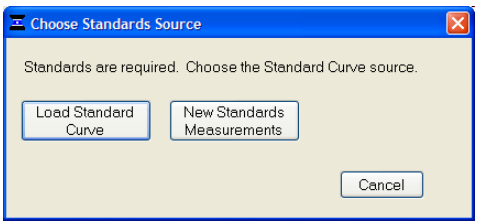
Follow the manufacturer's protocol for the assay including recommended incubation times and temperature.

In addition to the kit reagents, protein standards (BSA) for generating a standard curve for the BCA method are often provided by the manufacturer. Use the respective standard (e.g., BSA) and dilutions that cover the analytical range (mg/ml) of interest. Note: Since the NanoDrop 8000 can measure higher protein concentrations than a cuvette based spectrophotometer, you may need to supply your own protein standards at higher concentrations than routinely provided by the manufacturer.

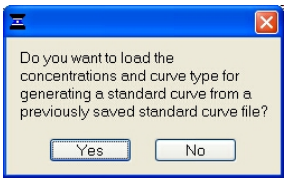
Making BCA Measurements

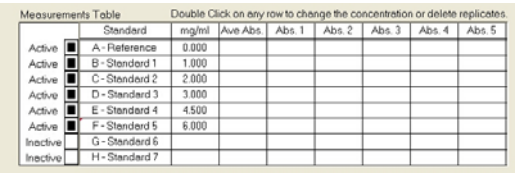
A standard curve is required every time the BCA assay is run. Although curves can be saved and reloaded in the NanoDrop 8000 Spectrophotometer software, it is recommended that the user follow manufacturers' guidelines and generate fresh standard curves for each assay. Both single and multi-point standard curve generation are incorporated into the software. A standard curve can be developed using a reference (BCA reagent only – no protein) and a single replicate of one standard. The multi-point standard curve generator displays a maximum of 5 replicates for each of 7 different standards. There is no set order in which standards must be run.

The following box will appear after the module initialization is complete:



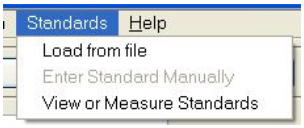
The user may load a previously saved standard curve or generate a new curve. Selecting the New Standards Measurements button will bring up the dialogue box on the left, below:





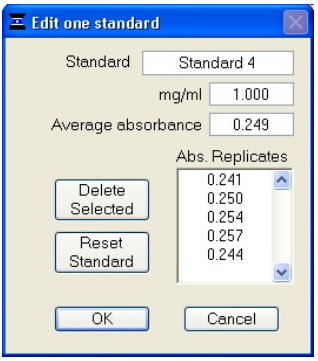
Clicking on the Yes button will allow the user to import just the Standard series without the respective measured values (See image on the right, above). This option is very useful when running a routine series of standards. Selecting 'No" enables the user to enter new concentrations values for standards 1-7. The reference should remain set at 0.00.

The Standards menu drop down may also be used to load a previously saved curve, generate a new standard curve, or view the current standard curve.



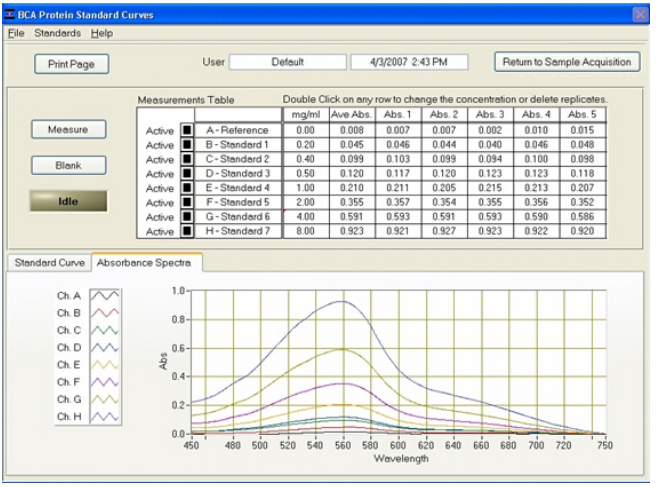
Then follow the steps below to either generate or modify the curve as needed:

- **Enter the concentration for each standard.**
  The user may either click on the Active/ Inactive box to the left of each standard or double click any where in the row of a particular standard to bring up the Edit Standard dialog box to enter in the concentrations for each standard. This box is also used to delete a single absorbance replicate value or reset the entire standard.



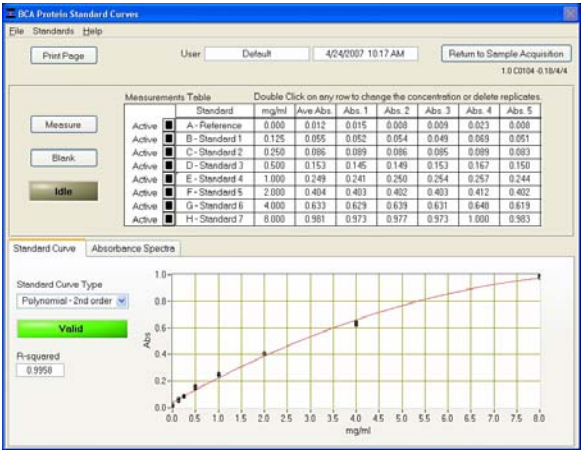
Alternatively, previously saved standard curves and standard curve concentration series may be loaded using the Standards menu bar drop-down options.

- **Measure Standards**
  Up to 5 replicates of each standard will be displayed. The software will not allow measurement of samples until a minimum of either a reference and 1 standard or 2 standards – are measured. Polynomial curve fitting requires more standard points depending on the polynomial degree selected.

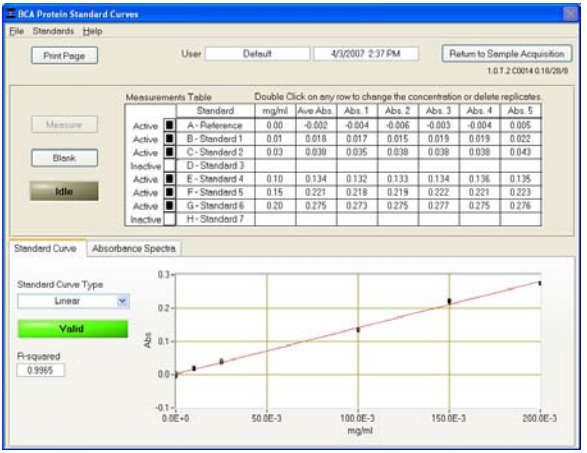


- **Measure Samples**
  Once a minimum standard curve has been established, the standard curve indicator will change from gray (invalid) to green (valid), allowing the user to start measuring samples. The designation of "Valid" only indicates that the minimum requirement for a 2-point curve has been met. Sample concentrations are calculated by using

linear interpolation (point-to-point) between the two standards flanking the unknown sample or by using polynomial fitting. Note: In order to obtain a concentration value (mg/ml) the sample (unknown) must fall within the limits of the standard curve.



BCA Standard Curve using a 20:1 reagent to sample volume: 0.2 – 8.0 mg/ml



BCA Standard Curve using a 1:1 reagent to sample volume: 0.01– 0.20 mg/ml

Exiting the BCA Module

It is recommended that you process all of the unknowns to be assayed before exiting the BCA software module.

Protein Lowry

The Modified Lowry Protein Assay is an alternative method for determining protein concentration based on the widely used and cited Lowry procedure for protein quantitation. Like the BCA and Bradford Assays, the Modified Lowry Assay requires that a standard curve be generated before unknown protein concentrations can be determined. The Modified Lowry procedure involves reaction of protein with cupric sulfate in an alkaline solution, resulting in the formation of tetradentate copper-protein complexes. The Folin-Ciocalteu Reagent is effectively reduced in proportion to the chelated copper-complexes resulting in a water-soluble blue product that is measured at 650 nm and normalized at 405 nm. Pre-formulated reagents, utilized in the assay, are available in kit form from numerous manufacturers. Follow the respective manufacturer's recommendations for all standards and samples (unknowns).

Sample Volume Requirements

The presence of surfactants or detergents in reagents can significantly alter the surface tension resulting in difficulty forming and/or maintaining adequate columns for measurement. The column formation issue can be overcome without affecting the sample's absorbance by using a larger sample volume. A 2 ul sample size is recommended for protein measurements.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

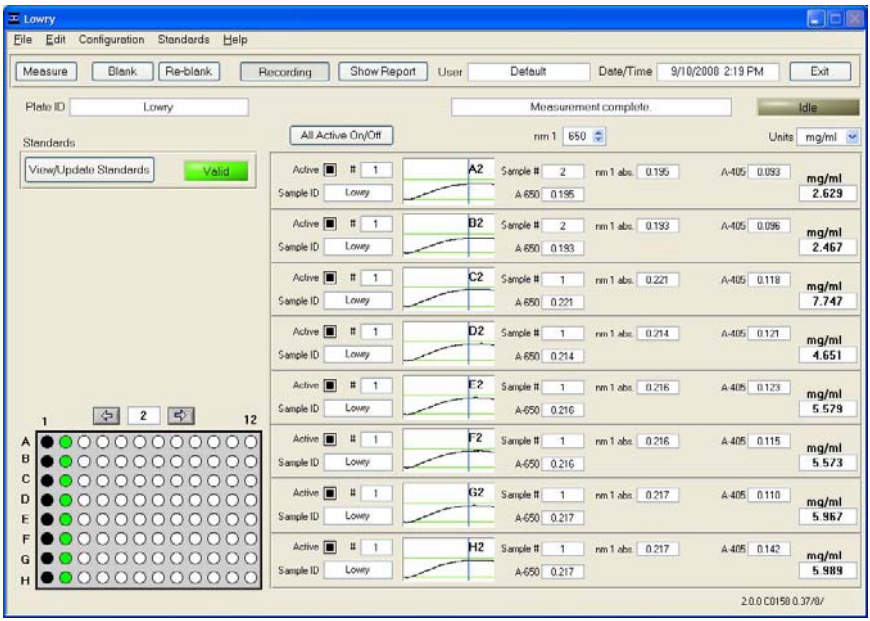
Pedestal Reconditioning

Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not from properly. If this occurs, "buff" the measurement pedestal surfaces by rubbing each measurement surface aggressively with a dry laboratory wipe 30-40 times. This will "re-condition" the surface allowing the liquid sample column to form. Alternatively, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about the PR-1 kit may be found on our website.

Measurement Concentration Range
The Modified Lowry assay concentration range of detection is ~0.20 mg/ml to 8.0 mg/ml (BSA) on the NanoDrop 8000.

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be defined for protein (mg/ml) measured with this method at 650 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Protein measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features



- **A 650nm:** the Cu-complex's absorbance at 650 nm for the 1 mm pathlength.

- **nm 1 and nm 1 abs:** current value of the user-selectable wavelength cursor and corresponding absorbance value for a 1 mm pathlength. The wavelength can be set by using the up/down arrows

or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.

- **mg/mL:** the concentration of the sample. The calculated concentration is displayed in the units selected via the units drop down box.

- **Show Report:** formatted for 200 samples although the buffer size can be modified.

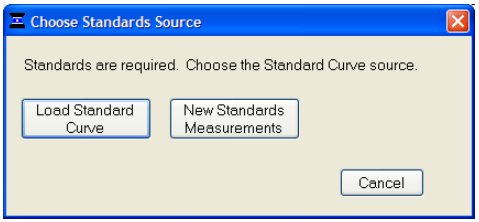
Modified Lowry Assay Sample Preparation
Follow the manufacturer's protocol for the assay including recommended incubation times and temperature.

In addition to the kit reagents, protein standards (BSA) for generating a standard curve for the Modified Lowry method are often provided by the manufacturer. Use the respective standard (e.g., BSA) and dilutions that cover the analytical range (mg/ml) of interest. Note: Since the NanoDrop 8000 can measure higher protein concentrations than a cuvette based spectrophotometer, you may need to supply your own protein standards at higher concentrations than routinely provided by the manufacturer.

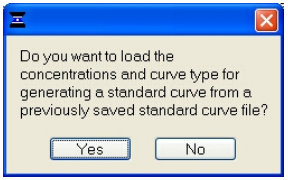
Making Lowry Measurements
A standard curve is required every time the Modified Lowry assay is run. Although curves can be saved and reloaded in the NanoDrop 8000 Spectrophotometer software, it is recommended that the user follow manufacturers' guidelines and generate fresh standard curves for each assay.

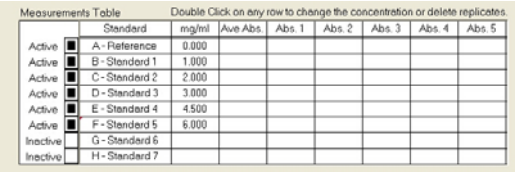
Both single and multi-point standard curve generation is incorporated into the software. A standard curve can be developed using a reference (Modified Lowry reagent only – no protein) and a single replicate of one standard. The multi-point standard curve generator displays a maximum of 5 replicates for each of 7 different standards. There is no set order in which standards must be run.

The following box will appear after the module initialization is complete:



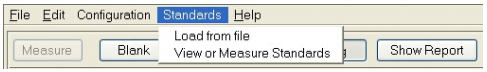
The user may load a previously saved standard curve or generate a new curve. Selecting the New Standards Measurements button will bring up the dialogue box on the left, below:





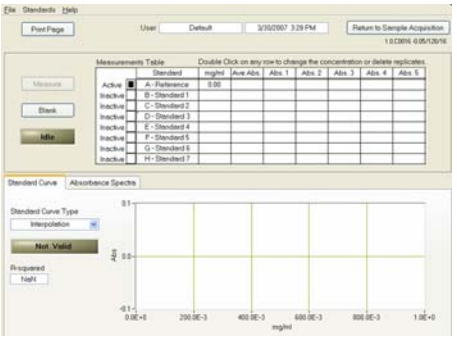
Clicking on the Yes button will allow the user to import just the Standard series without the respective measured values (See image on the right, above). This option is very useful when running a routine series of standards. Selecting 'No" enables the user to enter new concentrations values for standards 1-7. The reference should remain set at 0.00.

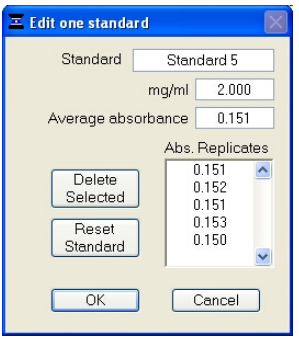
The Standards menu drop down may also be used to load a previously saved curve, generate a new standard curve, or view the current standard curve.



Follow the steps below to either generate or modify the curve as needed:

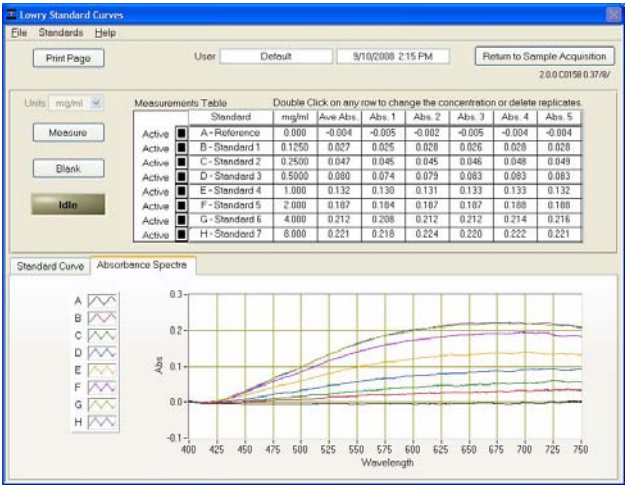
- **Enter the concentration for each standard.**
  The user may either click on the Active/ Inactive box to the left of each standard or double click any where in the row of a particular standard to bring up the Edit Standard dialog box to enter in the concentrations for each standard. This box is also used to delete a single absorbance replicate value or reset the entire standard.





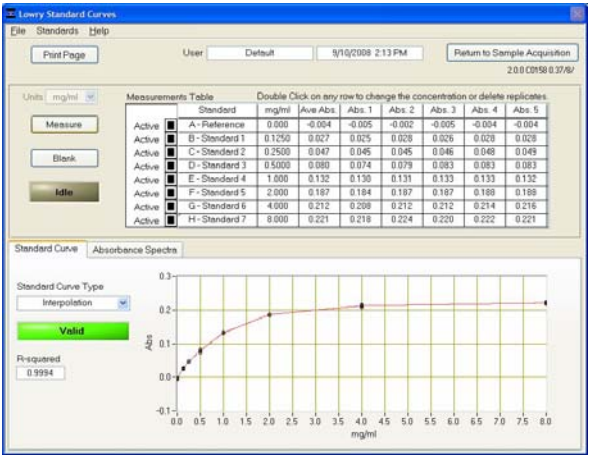
Alternatively, previously saved standard curves and standard curve concentration series may be loaded using the Standards menu bar drop-down options.

- **Measure Standards**
  Up to 5 replicates of each standard can be measured. The software will not allow measurement of samples until a minimum of either a reference and 1 standard or 2 standards – are measured. Polynomial curve fitting requires more standard points depending on the polynomial degree selected.



- **Measure Samples**
  Once a minimum standard curve has been established, the standard curve indicator will change from gray (invalid) to green (valid), allowing the user to start measuring samples. The designation of "Valid" only indicates that the minimum requirement for a 2-point curve has been met. Sample concentrations are calculated by using linear interpolation (point-to-point) between the two standards

flanking the unknown sample or by using polynomial fitting. Note: In order to obtain a concentration value (mg/ml) the sample (unknown) must fall within the limits of the standard curve.



Modified Lowry Standard Curve: 0.2 – 4.0 mg/ml

Exiting the Lowry Module

It is recommended that you process all of the unknowns before exiting the Lowry software module.

Protein Bradford

The Bradford Assay is a commonly used method for determining protein concentration. It is often used for more dilute protein solutions where lower detection sensitivity is needed and/or in the presence of components that also have significant UV (280 nm) absorbance. Like the BCA method, the Bradford method r requires that a standard curve be generated before unknown protein concentrations can be determined.

The Bradford uses the protein-induced Absorbance shift of Coomassie Blue dye to 595 nm as a measure of protein concentration. The bound protein-dye complex is measured at 595 nm and normalized at 750 nm. A single stabilized reagent mixture containing Coomassie Blue dye, alcohol, and surfactant in kit form is available from numerous manufacturers. Follow the respective manufacturer's recommendations for all standards and samples (unknowns).

Sample Volume Requirement

The presence of surfactants or detergents in reagents, such as the Bradford reagent, can significantly alter the surface tension resulting in difficulty forming and/or maintaining adequate columns for measurement. The column formation issue can be overcome without affecting the sample's absorbance by using a larger sample volume. **A 2 ul sample size is recommended for protein measurements.**

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Pedestal Reconditioning

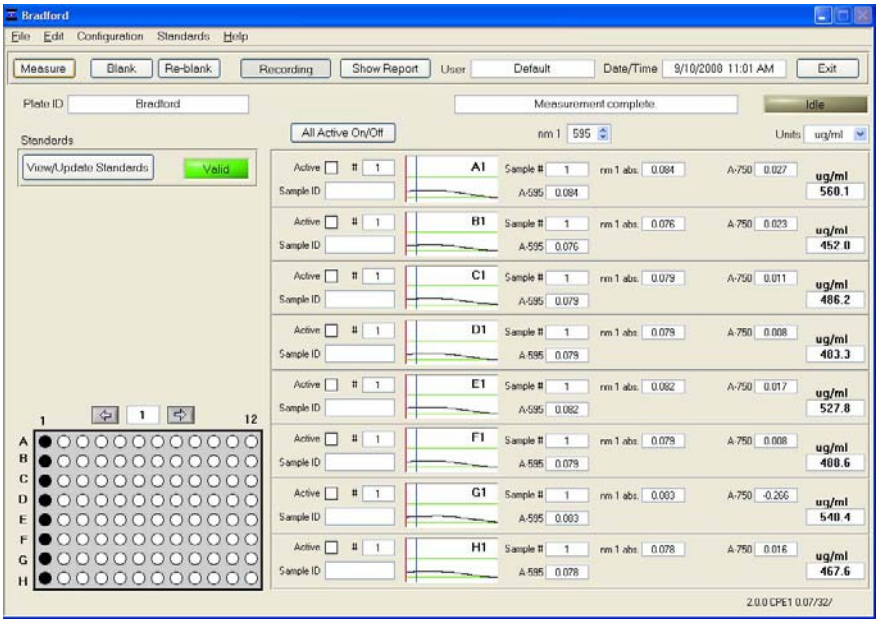
Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not from properly. If this occurs, "buff" the measurement pedestal surfaces by rubbing each measurement surface aggressively with a dry laboratory wipe 30-40 times. This will "re-condition" the surface allowing the liquid sample column to form. Alternatively, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about the PR-1 kit may be found on our website.

Measurement Concentration Range
Using the regular Bradford assay the concentration range of detection is ~100 ug/ml up to 8000 ug/ml on the NanoDrop 8000. The best linearity is in the 100ug/ml – 1000 ug/ml range. The concentration range for the mini Bradford assay is 15 ug/ml to 125 ug/ml.

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be set for protein (mg/ml) measured with this method at 595 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Protein measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Coomassie dye-dye and Coomassie dye-protein aggregates are frequently encountered in Coomassie dye-based protein assays. With time, particulate can be observed, which can cause significant fluctuations in Absorbance readings. It is also important to note the total analyte (protein-dye) signal at 595nm is limited to ~ 0.150 A as a result of the 1.0mm pathlength of the instrument, the Bradford (Coomassie dye) reagent concentration, and the acidic pH. Making measurements in triplicate of standards and samples (unknowns) is good practice, particularly with the limited assay signal obtained with the Bradford Assay.

Unique Screen Features



- **A595 nm:** protein-dye complex's absorbance at 595 nm at the 1mm pathlength.

- **nm 1 and nm 1 abs:** current value of the user-selectable wavelength cursor and corresponding absorbance value for a 1 mm pathlength. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.

- **ug/mL:** the concentration of the sample. The calculated concentration is displayed in the units selected via the units drop down box.

- **Show Report:** formatted for 200 samples although the buffer size can be modified.

Bradford Assay Sample Preparation

Follow the manufacturer's protocol for the assay including recommended incubation times and temperature. Allowing the reaction to incubate for a longer than suggested time frame increases the potential for interfering aggregates. Higher protein concentration may increase the possibility of dye or dye-protein aggregates contributing to interfering light scattering.

In addition to the kit reagents, protein standards (BSA) for generating a standard curve for the Bradford method are often provided by the manufacturer. Use the respective standard (e.g., BSA) and dilutions that cover the analytical range (mg/ml) of interest. Note: Since the NanoDrop 8000 can measure higher protein concentrations than a cuvette based spectrophotometer, you may need to supply your own protein standards at higher concentrations than routinely provided by the manufacturer.

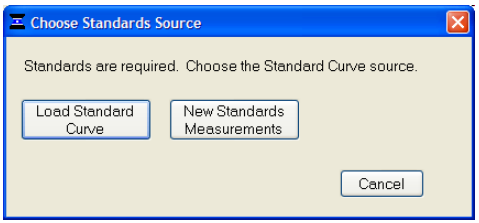
Variation Between Different Kits

When comparing Coomassie G-250 and the Coomassie Plus Protein Assay kit from Pierce, preliminary results show the Coomassie Plus Protein Assay produces better reproducibility amongst triplicate readings than the regular G-250 stain when reading the samples directly after the optimal incubation time.

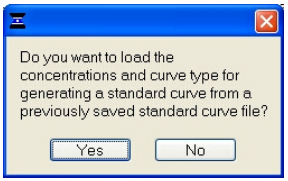
Making Bradford Protein Measurements

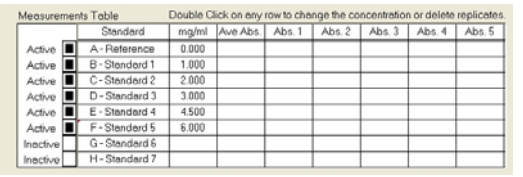
A standard curve is required every time the Bradford assay is run. Although curves can be saved and reloaded in the NanoDrop 8000 Spectrophotometer software, it is recommended that the user follow manufacturers' guidelines and generate fresh standard curves for each assay. Both single and multi-point standard curve generation is incorporated into the software. A standard curve can be developed using a reference (Bradford reagent only – no protein) and a single replicate of one standard. There is no set order in which standards must be run. The multi-point standard curve generator displays a maximum of 5 replicates for each of 7 different standards.

The following box will appear after the module initialization is complete:

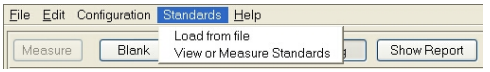


The user may load a previously saved standard curve or generate a new curve. Selecting the New Standards Measurements button will bring up the dialogue box on the left, below:



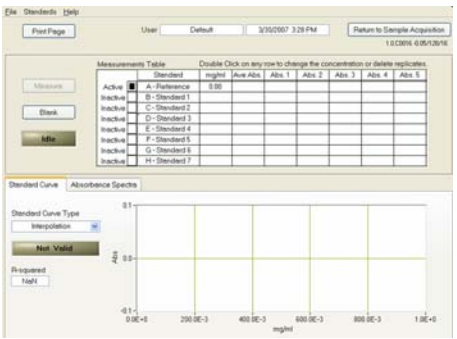


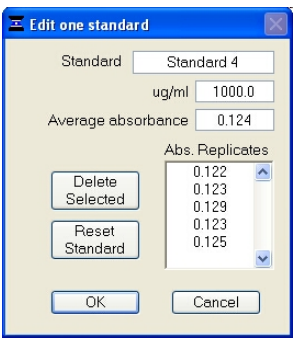
Clicking on the Yes button will allow the user to import just the Standard series without the respective measured values (See image on the right, above). This option is very useful when running a routine series of standards. Selecting 'No' enables the user to enter new concentrations values for standards 1-7. The reference should remain set at 0.00. The Standards menu drop down may also be used to load a previously saved curve, generate a new standard curve, or view the current standard curve.



Then follow the steps below to either generate or modify the curve as needed:

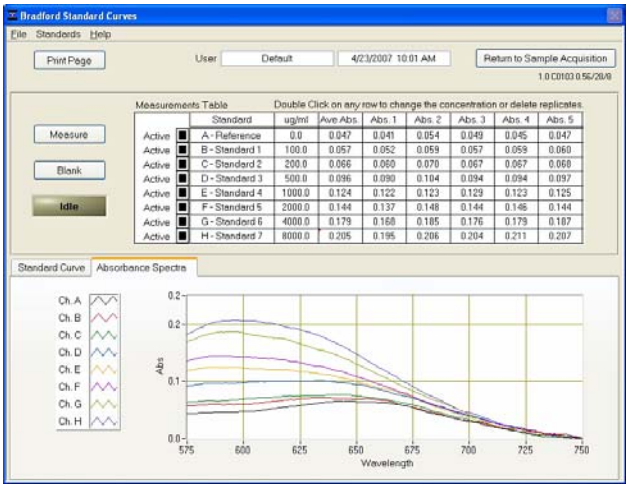
- **Enter the concentration for each standard.**
  The user may either click on the Active/ Inactive box to the left of each standard or double click any where in the row of a particular standard to bring up the Edit Standard dialog box to enter in the concentrations for each standard. This box is also used to delete a single absorbance replicate value or reset the entire standard.



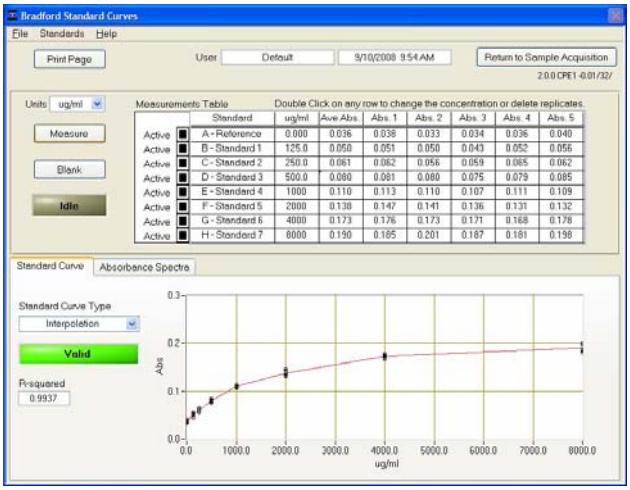


Alternatively, previously saved standard curves and standard curve concentration series may be loaded using the Standards menu bar drop-down options.

- **Measure Standards**
  Up to 5 replicates of each standard can be measured. The software will not allow measurement of samples until a minimum of either a reference and 1 standard or 2 standards – are measured. Polynomial curve fitting requires more standard points depending on the polynomial degree selected.



- **Measure Samples**
  Once a minimum standard curve has been established, the standard curve indicator will change from gray (invalid) to green (valid), allowing the user to start measuring samples. The designation of "Valid" only indicates that the minimum requirement for a 2-point curve has been met. Sample concentrations are calculated by using linear interpolation (point-to-point) between the two standards flanking the unknown sample or by using polynomial fitting. Note: In order to obtain a concentration value (mg/ml) the sample (unknown) must fall within the limits of the standard curve.



Regular Bradford curve using a 50:1 reagent to sample volume covers the range of 100-8000 ug/ml. Note the linear range is 100-1000 ug/ml

A Bradford assay using a 1:1 reagent to sample volume covers an approximate range of 15-100 ug/ml

Exiting the Bradford Module

It is recommended that you process all of the unknowns to be assayed before exiting the Bradford software module.

Protein Pierce 660 nm

The Thermo Scientific Pierce 660 nm Protein Assay reagent is a ready-to-use formulation that offers rapid, accurate and reproducible colorimetric detection of minute amounts of protein in solution. The reagent is ideal for measuring total protein concentration in samples containing both reducing agents and detergents.

Sample Volume Requirement
The presence of surfactants or detergents in reagents, such as the Bradford reagent, can significantly alter the surface tension resulting in difficulty forming and/or maintaining adequate columns for measurement. The column formation issue can be overcome without affecting the sample's absorbance by using a larger sample volume. **A 2 ul sample size is recommended for protein measurements.**

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Measurement Concentration Range
The assay has a linear range for BSA of 50-2000 ug/ml using a reagent to sample ratio of 15:1.

Assay Type	Approx. Lower Limit	Approx. Upper Limit	Typical Reproducibility (minimum 5 replicates) (SD= mg/ml; CV= %)
15:1	50 ug/ml	2000 ug/ml	± 5% (over entire range)

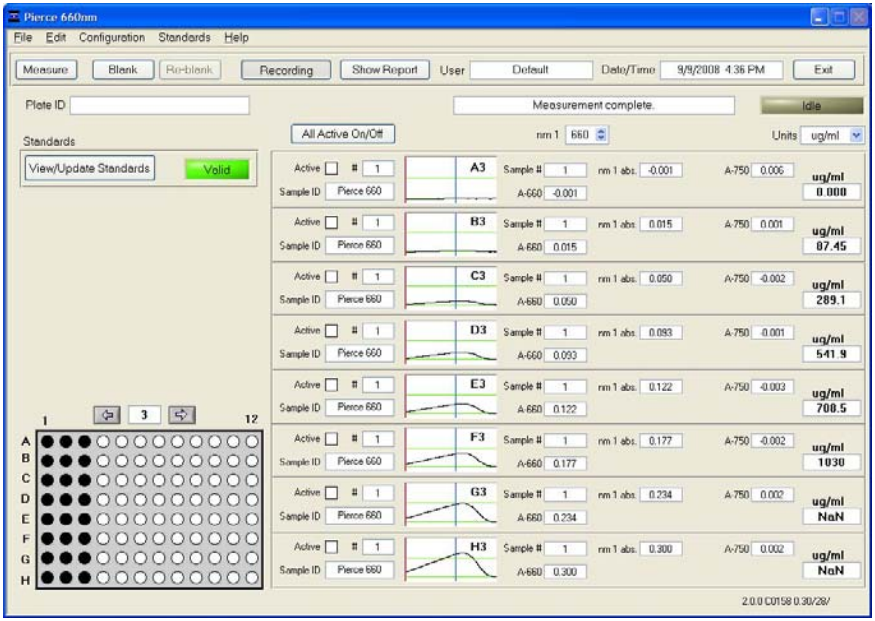
To accurately prepare standards, we suggest using a minimum sample volume of 4 ul in 60 ul of the Pierce 660 nm reagent (larger sample volume is preferable).

Follow the manufacturer's protocol for the assay including recommended incubation times and temperature. Additionally, use the respective standard (e.g. BSA) and dilutions that cover the analytical range (mg/ml) of interest.

Note: Since the NanoDrop 8000 can measure higher protein concentrations than traditional cuvette-based spectrophotometers, you may need to supply your own protein standards at higher concentrations than provided by the manufacturer.

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be set for protein (mg/ml) measured with this method at 660 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Protein measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features



- **A660 nm:** protein-dye complex's absorbance at 660 nm at the 1mm pathlength.

- **nm 1 and nm 1 abs:** current value of the user-selectable wavelength cursor and corresponding absorbance value for a 1 mm pathlength. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.

- **ug/mL:** the concentration of the sample (unknown)

- **Show Report:** formatted for 200 samples although the buffer size can be modified.

Making Pierce 660 nm Protein Measurements

A standard curve is required by the software every time the Pierce Protein 660 nm assay is run. Although curves can be saved and reloaded in the NanoDrop 8000 Spectrophotometer software, it is recommended that the user follow manufacturers' guidelines regarding the use of saved curves when running this assay. Additionally, a standard curve 'set-up' may be reloaded. This feature will recall the respective standard series used in a previously saved standard curve. Both single and multi-point standard curve generation is incorporated into the software.

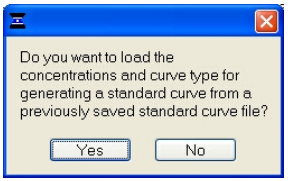
A standard curve can be developed using a reference (reagent only – no protein) and a single replicate of one standard. The multi-point standard curve generator allows a maximum of five replicates for up to seven different standards. There is no set order in which standards must be run.

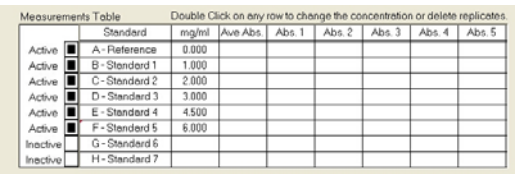
A blank must be measured before the standard curve may be generated. It is advisable to use use the dye reagent without any protein added as both the Blank and the "0" reference sample.

Note: This is unlike the other colorimetric assays on the NanoDrop 8000 where it is recommended that water be used as the blank.

The following box will appear after the module initialization is complete:

The user may load a previously saved standard curve or generate a new curve. Selecting the New Standards Measurements button will bring up the dialogue box on the left, below:





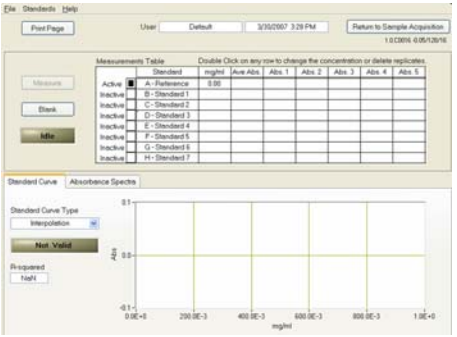
Clicking on the Yes button will allow the user to import just the Standard series without the respective measured values (See image on the right, above). This option is very useful when running a routine series of standards. Selecting 'No' enables the user to enter new concentrations values for standards 1-7. The reference should remain set at 0.00.

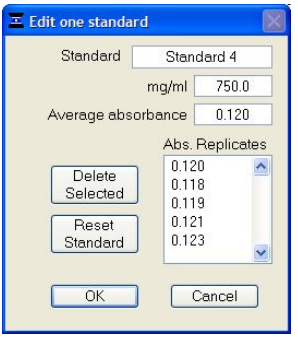
The Enter Standards Manually button allows the user to type in a predefined set of concentration and absorbance values that are supplied by the reagent manufacturer.

The Standards menu drop down may also be used to load a previously saved curve, generate a new standard curve, view the current standard curve or manually enter in standard curve values.

Follow the steps below to either generate or modify the curve as needed:

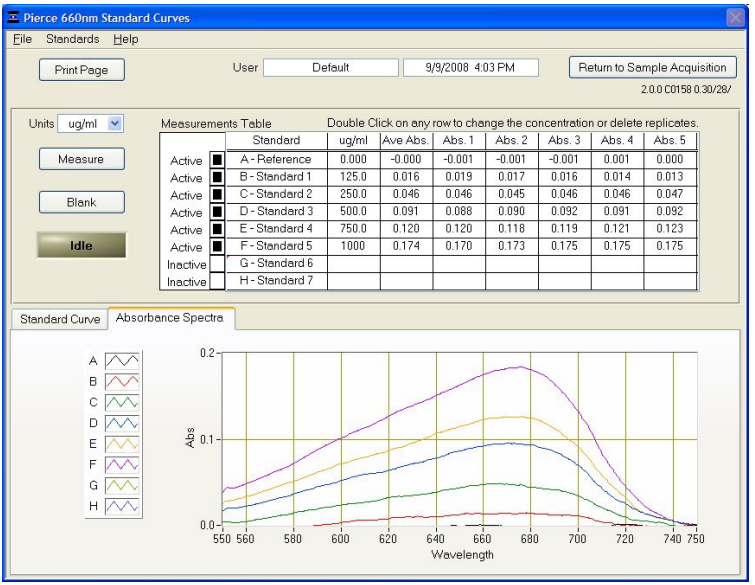
- **Enter the concentration for each standard.**
  The user may either click on the Active/ Inactive box to the left of each standard or double click any where in the row of a particular standard to bring up the Edit Standard dialog box to enter in the concentrations for each standard. This box is also used to delete a single absorbance replicate value or reset the entire standard.





Alternatively, previously saved standard curves and standard curve concentration series may be loaded using the Standards menu bar drop-down options.

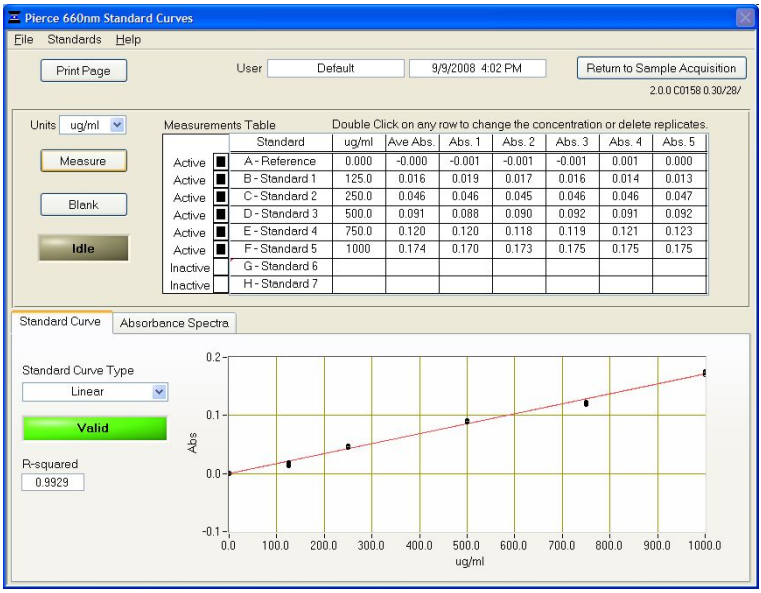
- **Measure Standards**
  Up to 5 replicates of each standard can be measured. The software will not allow measurement of samples until a minimum of either a reference and 1 standard or 2 standards – are measured. Polynomial curve fitting requires more standard points depending on the polynomial degree selected.



		Standard	ug/ml	Ave Abs.	Abs. 1	Abs. 2	Abs. 3	Abs. 4	Abs. 5
Active	■	A - Reference	0.000	-0.000	-0.001	-0.001	-0.001	0.001	0.000
Active	■	B - Standard 1	125.0	0.016	0.019	0.017	0.016	0.014	0.013
Active	■	C - Standard 2	250.0	0.046	0.046	0.045	0.046	0.046	0.047
Active	■	D - Standard 3	500.0	0.091	0.088	0.090	0.092	0.091	0.092
Active	■	E - Standard 4	750.0	0.120	0.120	0.118	0.119	0.121	0.123
Active	■	F - Standard 5	1000	0.174	0.170	0.173	0.175	0.175	0.175
Inactive		G - Standard 6							
Inactive		H - Standard 7							

- **Measure Samples**

Once a minimum standard curve has been established, the standard curve indicator will change from gray (invalid) to green (valid), allowing the user to start measuring samples. The designation of “Valid” only indicates that the minimum requirement for a 2-point curve has been met. Sample concentrations are calculated by using linear interpolation (point-to-point) between the two standards flanking the unknown sample or by using polynomial fitting. Note: In order to obtain a concentration value (mg/ml) the sample (unknown) must fall within the limits of the standard curve.



A Pierce 660 nm assay using a 15:1 reagent to sample volume covers an approximate range of 50-2000 ug/ml

Exiting the Pierce 660 nm Module

It is recommended that you process all of the unknowns to be assayed before exiting the software module.

Cell Cultures

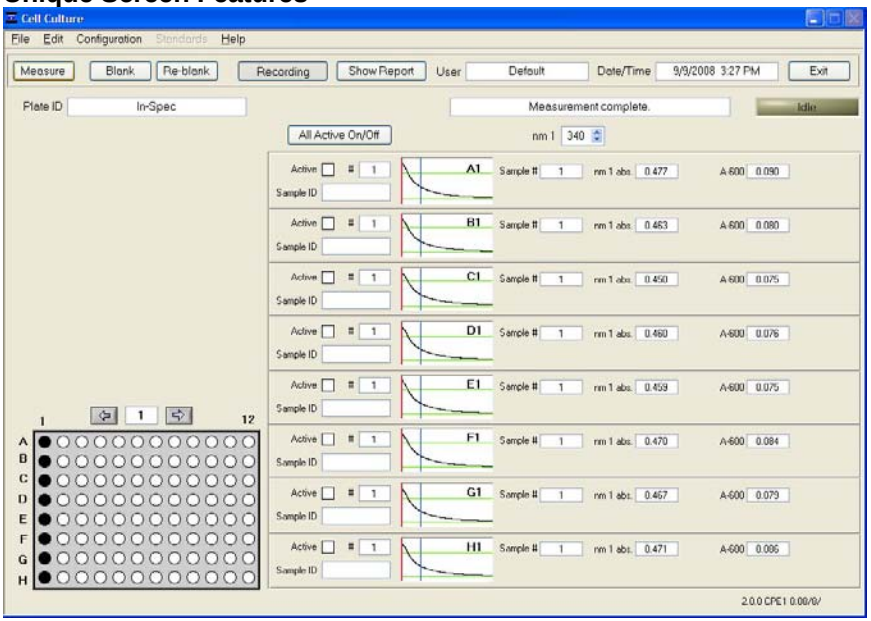
Using an absorbance spectrophotometer to monitor light scattered by non-absorbing suspended cells is common practice in life science laboratories. Such applications, more than any other, accentuate the differences between the optical systems of the numerous spectrophotometer designs.

Note: The most distinct difference between the NanoDrop 8000 Spectrophotometer “absorbance” values for cell microbial cultures and those observed using classical cuvette based systems will be attributable to the shorter pathlength (1 mm vs. 1 cm.) Values may not be exactly 10 fold different as readings are dependent on both the optics of a specific spectrophotometer as well as the cell type in suspension.

The Cell Cultures module displays the sample spectrum from 250 nm to 700 nm. The software will display the absorbance data for the frequently used wavelength for monitoring cell suspensions (600nm) in addition to displaying the value for a second wavelength of interest.

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum absorbance limits can be set for the user selectable wavelength (cursor position). These limits cannot be set as a default and must be defined each time the application module is opened. Sample absorbances that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The absorbance values will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features



- **600nm Absorbance:** current value of the absorbance at the λ1 cursor with the baseline absorbance subtracted. Note: The actual 1 mm absorbance is displayed.

- **nm 1:** current value of the user-selectable wavelength cursor and corresponding absorbance. The wavelength can be set by using the up/down arrows or typing in the desired wavelength.

- **Show Report:** formatted for 200 samples although the buffer size can be modified.

Sample Size Requirements

Field experience has indicated that 1ul samples are sufficient to ensure accurate and reproducible results when measuring aqueous samples. However, if you are unsure about the surface tension properties of your sample or your pipettor accuracy, a 1.5-2 ul sample is recommended to ensure that the liquid sample column is formed and the light path is completely covered by sample.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Cell Suspension Concentrations
Due to its shorter pathlength, the NanoDrop 8000 can measure absorbances that are 10-fold higher than those measurable on a standard cuvette spectrophotometer. It is important to note that the absorbance reported is that for the 1 mm path and will be approximately 10 fold lower than absorbance values reported when samples are measured with a traditional cuvette-based spectrophotometer. Since the entire spectrum is displayed, diluted samples exhibiting very low 'Absorbance' at 600 nm can be monitored at lower wavelengths, for example 300 nm.

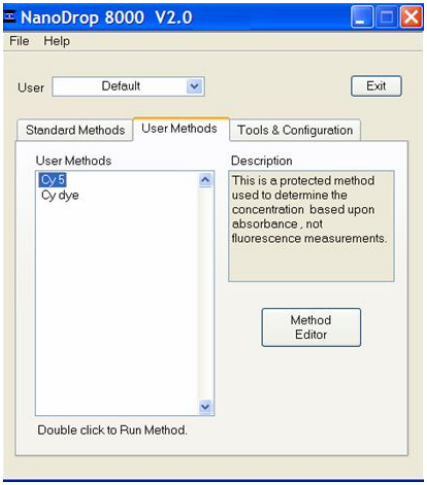
Sample Homogeneity
The user must be sure to homogeneously suspend the cells when sampling for "absorbance" measurements and read the sample immediately to avoid significant cell settling. Vigorous mixing may be required particularly when measuring concentrated samples.

Decontamination of Measurement Pedestals
If decontamination is necessary, a sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solution – freshly prepared), can be used to ensure that no biologically active material is present on the measurement pedestals. The metal fiber optic fittings are made from 303 stainless steel and are resistant to most common laboratory solvents (see "Solvent Compatibility" appendix.) A final cleaning of all surfaces with de-ionized water is also recommended after the user's last measurement. Note: Do not use a squirt bottle to apply bleach or de-ionized water. Routine use of ethanol or isopropanol for cleaning is not recommended.

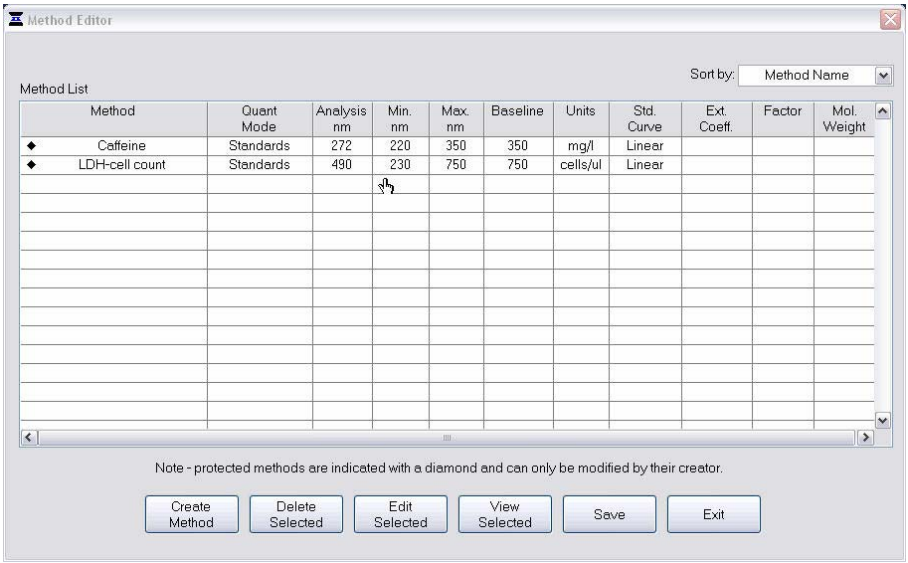
6. User Methods

Users Methods are customized absorbance analysis methods that allow the scientist to create, save and edit a set of parameters for both unique and routine measurements. All user defined methods except for those requiring standard curves, are accessible for both 8 Sample and Single Sample operating modes.

In the left hand box are the current available user configurable methods. Highlighting a method will display whatever descriptive text is associated with the method in the description box to the right.



Method Editor
The Method Editor is used to Create, Delete, Edit, and View and Save methods.
Use the button on the bottom right to access the following screen:



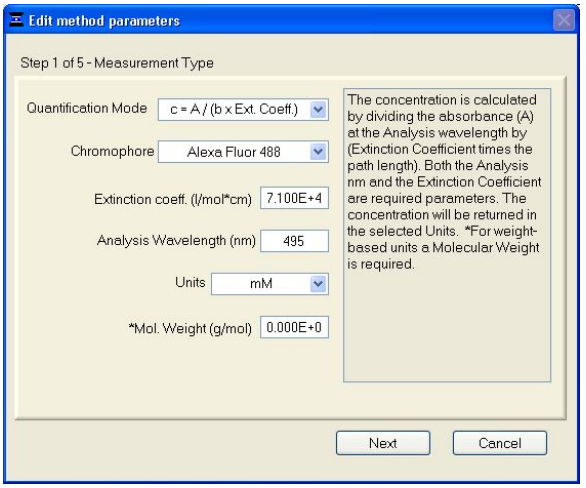
Note: Diamonds indicate predefined methods which cannot be modified.

Create New Method

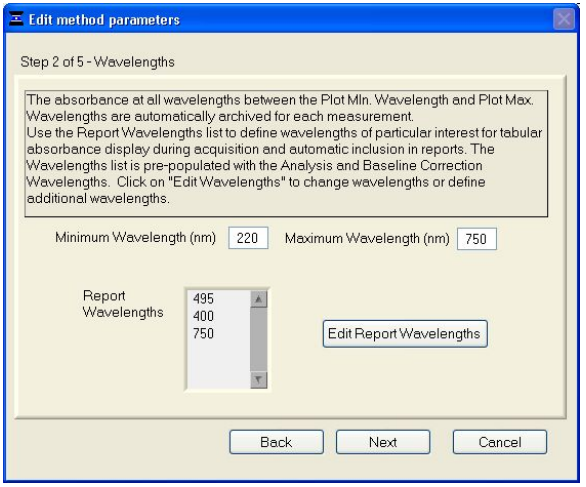
A wizard style series of new windows will appear that will guide the user through the creation of a new method. The first window is entitled Measurement Type and is used to select the method of calculating the sample concentration. The options allow the user to choose between using Beers Law (standard or modified to utilize “constants” instead of extinction coefficient) or standard curves as the means of calculating concentration. The “Oligo Required” option will use the calculated extinction coefficient of the entered oligo sequence in calculating concentration.

A fifth option of ‘none’ enables the creation of methods that report absorbances at selected wavelengths without calculating concentrations.

A series of secondary drop-down options and required parameter boxes are displayed as appropriate based upon the Quantification Mode selected. Note: Methods requiring standard curves are not available when operating under the Single Sample mode. The following screen shots illustrate the steps involved with setting up a custom method using a potential Alexa 488 dye method as an example.

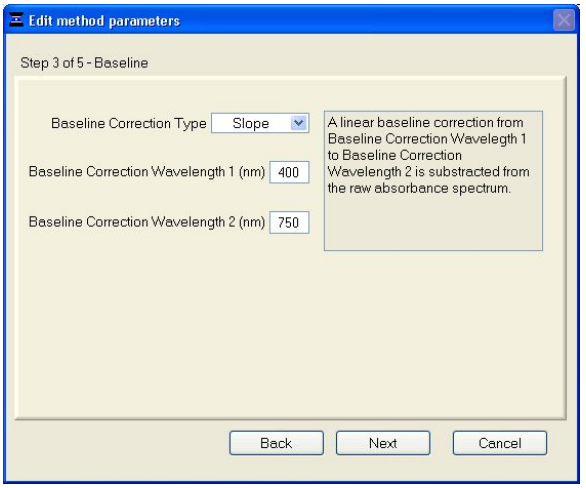


Selecting the Next button will bring up the Wavelengths screen:

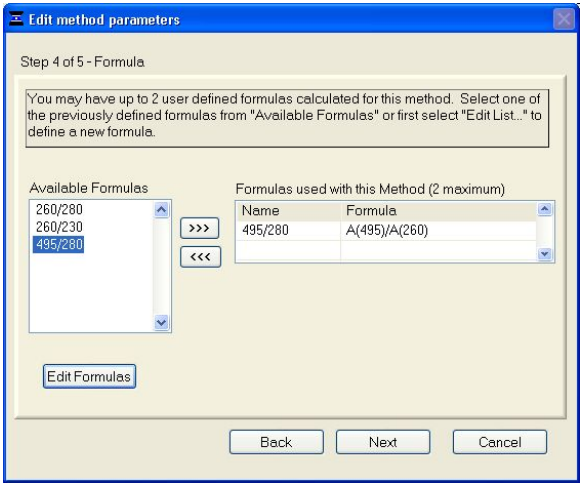


Use this screen to select the wavelengths of interest as well as the minimum and maximum wavelengths to display on the screen. Up to four wavelengths may be included as Report Wavelengths.

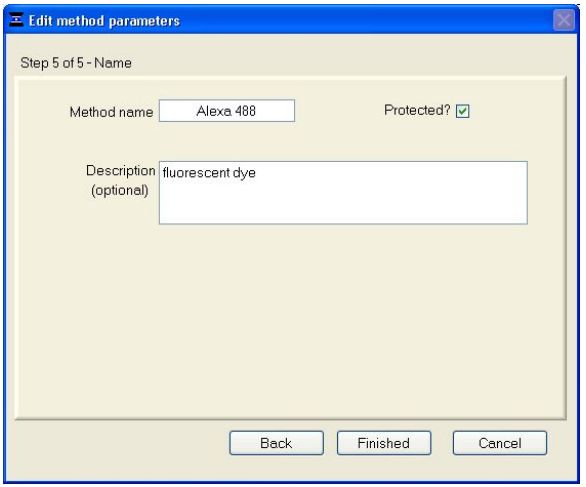
The third window is used to define the baseline correction type.



Step 4 allows the option of including results of up to 2 user defined formulas in the display and archived data. The formulas are not used in the calculation of the sample concentration.



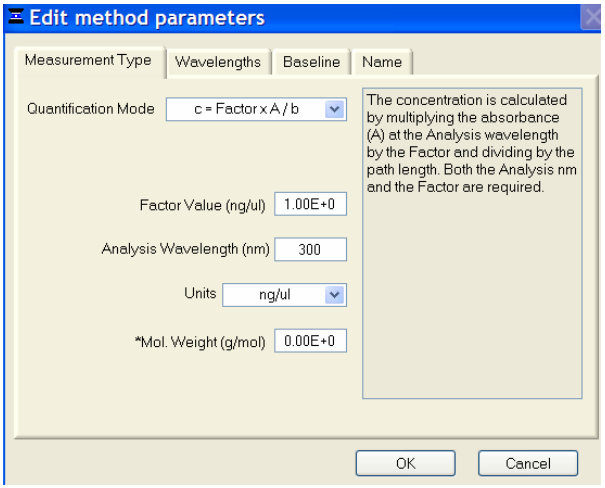
The final page of the wizard allows the user to name and describe the new method.



Note: A method that is protected can only be modified when accessed under the user account from which it was created. It is recommended that a method be created from a password protected user account rather than the Default user account to ensure that methods are not inadvertently modified.

Edit Selected Method

Predefined methods with a black diamond next to them are protected and cannot be edited. User defined methods with a black diamond by only be edited the user hat created. All other methods may be edited at any time by highlighting the method name and hitting the Edit Selected button. All four wizard pages are accessible as tabbed pages when editing a method.

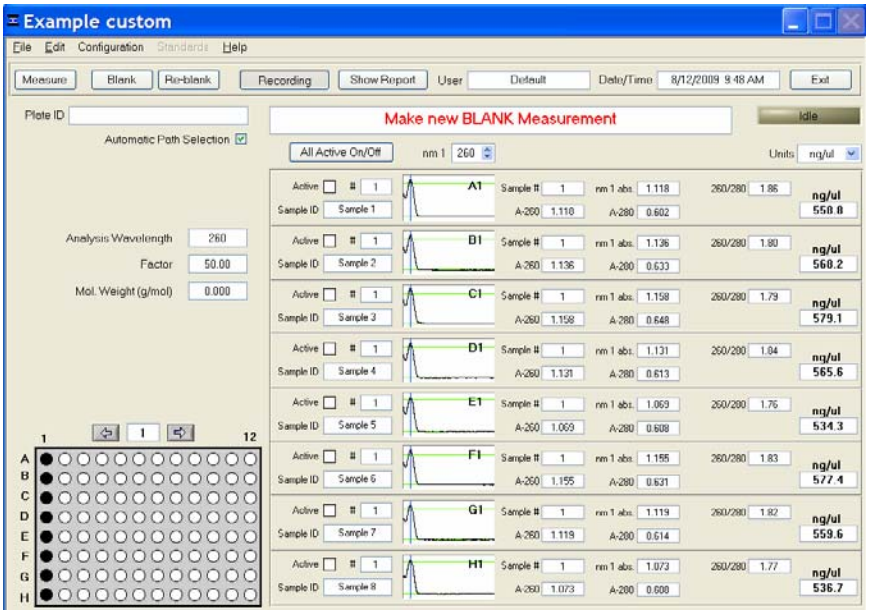


The user may move to the appropriate window by using the top tabs and edit only the parameters of interest. The user may also edit a method from within the acquisition module by using the Edit drop down box. Note: When editing is complete, the changes must be saved in order to be implemented.

View Selected

This button allows the user to review the parameters of a method but will not save any changes.

User Method Acquisition Screen



The analysis wavelength and associated factors will be displayed on the acquisition screen when operating in either the Single or 8 Sample mode. The results of each additional formula will be displayed on the acquisition page when generated in the single sample mode but will not be displayed on the data 8 Sample mode. The additional formula results will be available in the data report and archived data for both operation modes.

Automatic Path Selection: If selected, the software will automatically switch from using the 1.0 mm pathlength to the 0.2 mm path when the absorbance value of the specified analysis wavelength reaches a threshold of 1.25.

7. Tools & Configuration

Archived Data

Sample data from all application modules is automatically stored in archive files and can be opened by either the integrated Data Viewer software program or spreadsheet programs such as MS Excel.

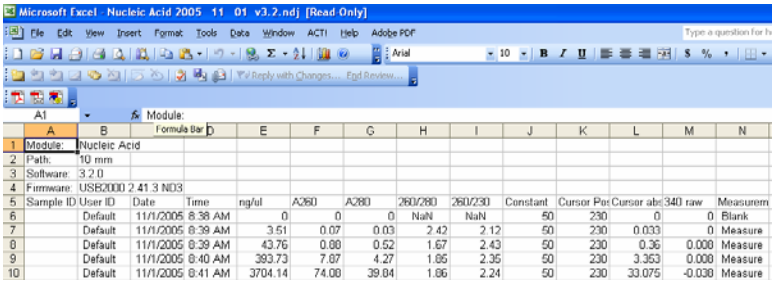
Archive File Creation

Every time an application module is started, an application-specific archive file is created for the user that is logged in. All measurements made by the user (in that application module) for a given calendar day are stored in a single archive file. These files bear the name of the respective application module with the date appended. For example, an archived file entitled "Nucleic Acid 2007 03 21.nd8" corresponds to Nucleic Acid data from the software session that began on March 21, 2007. A unique file extension (.nd8) has been given to these files to enable automatic startup with the Data Viewer (see the description of Data Viewer later in this section).

The data may be edited and/or reformatted and stored under names of the user's choice. The spectrum can be re-plotted from the wavelength data if needed for further analysis.

Absorbance data shown in archive files are represented as they are displayed on the screen. For Nucleic Acids, Protein A280, and Protein and Labels application modules, data is stored based on a 1.0 cm (10.0 mm) path. For MicroArray, UV-Vis, BCA Protein, Bradford, Lowry and Cell Culture application modules the data is normalized to a 1.0 mm (0.1 cm) path.

For data from all modules, a column entitled 'Measurement Type' is included. For each measurement, this column will contain 'Measure', 'Blank', or 'Reblank'. If the value is 'Measure', then the values in that row are from a normal measurement that has utilized the stored blank value. If the value is 'Blank', it indicates that the measurement is the initial blank recorded. If the value is 'Reblank', it is the re-analysis of the previous measurement with a new blank.



Data Storage Hierarchy
The hierarchy for archived files is as follows:
C:\ND-8000 Data → User name → Application Module (BCA Protein, Lowry, Bradford, Cell Culture, MicroArray, Nucleic Acid, Protein A-280, Proteins & Labels, UV-Vis).

All archived data files are stored in an application module folder that is within the User folder as shown below:

User-Defined Data File Export Location
In addition to the primary data storage, users may elect to export their data as anto an additional location. This option can be chosen under the Data Export Exporting tab in User Preferences from within the Tools & Configuration tab. Select the Automatic Data Export feature by enabling the On box. Select the data export destination using the icon next to the Data Export Folder dialog window. Save the alternative path by clicking on the 'Save and Exit' button before exiting the User Preferences window.

All data are written to the archive file immediately upon completion of the measurement. Inadvertent software or PC shutdowns should not affect the archive file.

Data Viewer

Data Viewer is a versatile data reporting software program incorporated into the operating software that offers the user the ability to customize

report structures, import stored data and re-plot data from previously generated data. Using the Data Viewer is the most expedient method to review data. This feature may be accessed during measurement sessions from the Show Report function found within each method module. It may also be accessed from the Main Menu page. A NanoDrop 8000 Spectrophotometer does not need to be connected to the PC to use the Data Viewer module.

Data Viewer Features
The Data Viewer is composed of two or three pages in a tabular form consisting of Plots, Reports and Standard Curves (where utilized). The user may access any page by clicking on the tabs.

The software opens to the Report page whether accessed through the Main Menu or Show Report. Note: Recording rather than Start Report must be selected in order to access the Data Viewer via Show Report.

Tool Bar Features common to all three pages include:

- **File:** Allows the user to define the page set up for printing out the spectra, the report and the standard curve. This drop down also allows the user to save the window as a jpg.

- **Configuration:** Options controlled by this tool bar function include 'Auto Scale', 'Include graph in printout' and 'Include standards in printout'.

- **Data:** Includes options to import data, rename samples and delete sample data. Note: After deleting all samples, it is important to exit out of the Data Viewer module and re-enter if importing data for a different application type.

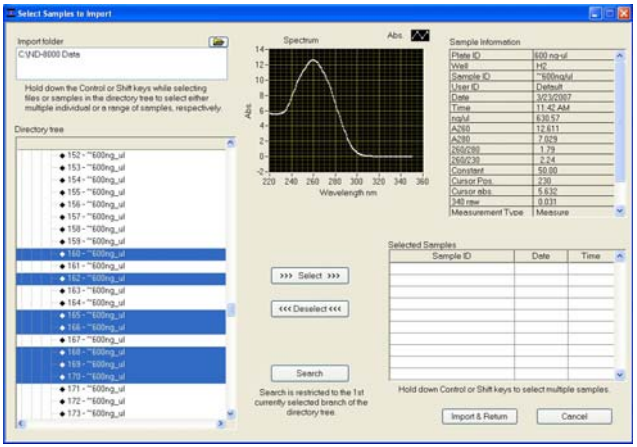
- **Reports:** This tool bar function allows the user to select columns of interest to be included in a report. See following section on Reports Page for details on additional drop down box options.

- **Print Window:** The current Plot, Report, or Standards screen may be printed by selecting 'Print Window' or 'CTL +P'.

- **Save Window:** Saves files as .JPGs.

Import Page

To select samples for viewing, select 'Import Samples' from the Data menu bar drop down options from either the Plots or Report pages within the Data Viewer software. This will bring up a new window with an Import Folder box and a Directory Tree as shown below.



Features include:

- **Import folder:** Used to select folder where data is to be imported from. Folder selection must be at the level of user or higher--may not select an application or method folder within a user in this activity box.

- **Directory tree:** Used to select specific data to be imported. Clicking on the square to the left of each file name will provide further detail to each level. Users may choose to select either individual samples within a file or the entire file. All import selections must be of the same application or method type.

- **Select or Deselect:** Used to move the highlighted sample choices to or from the Selected Samples box. Note: The software defaults to a buffer size of 1000 samples.

- **Search:** Function allows the user to locate specific data by searching through sample ID names.

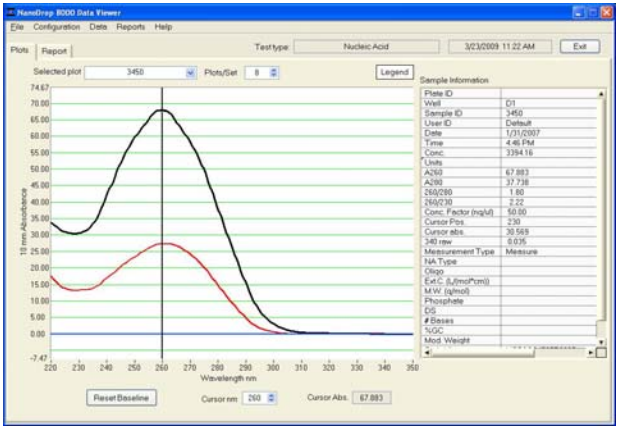
- **Sample Information and Spectrum:** Are populated with the information associated with the most recently highlighted sample.

- **Import and Return:** Uses selected sample data to populate Plots and Reports windows. Note: Holding down the shift or control PC

function keys will allow the user to select multiple samples and/or files for importing. The keys can also be used to deselect multiple samples.

Plots Page

The Plots page displays selected sample spectra.



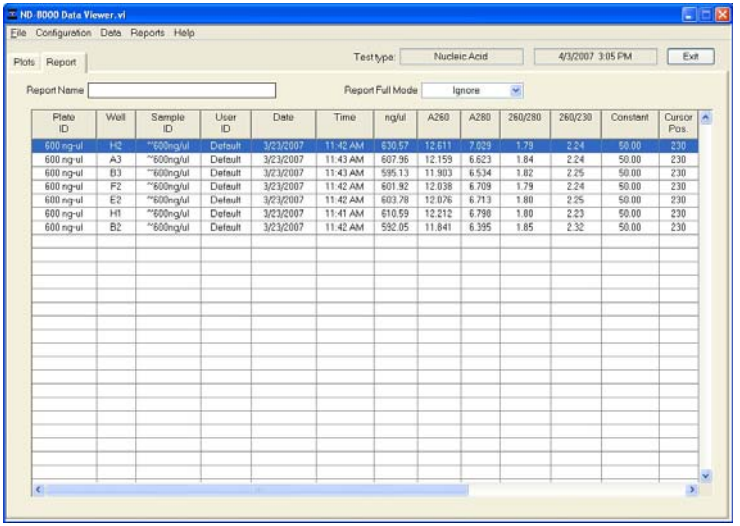
Features include:

- **Test Type:** Auto fills in module name.
- **Date:** Auto fills in date and time of report.
- **Selected Plot:** There are two methods of selecting or highlighting individual sample data. The user may simply move the cursor over the plot of interest and click or use the 'Selected Plot' drop down box (which will also display the legend). The selected sample will show up as a bold plot line.
- **Plots/Sets:** Users may select the maximum number of individual plots (up to 20) graphed per page. Since a report can hold data for many samples and a graph page is limited to 20 plots, additional sample spectra are displayed on new pages. Each page is then referred to as a set.
- **Legend:** Positioning the cursor over the legend box will bring up a visual display matching the sample name to a plot color. The user is not able to select or highlight a sample from the legend.
- **Sample information:** Automatically populates with data associated with selected sample. Data displayed is appropriate for data type chosen. Note: Information is based on data collected at the time the sample was measured and is not modified by a change in cursor position on the Data Viewer real time display.

- **Movable x and y axis:** Available for all data types. If the cursor is out of view in either direction- rescale the axis by typing over one of the outer limit numbers. The cursor absorbance information displayed at the bottom of the page is determined by the position of the movable cursors. The movable X determines the baseline from which the peak of the Y position is calculated. Reset Baseline will reposition the x axis back to zero.

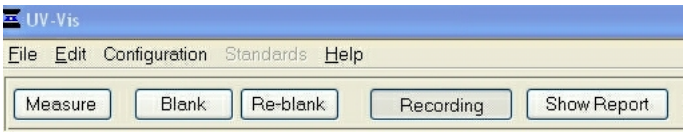
Report Page

The Report page displays the data for selected samples in a table format. The user may modify column configurations for each method type and save multiple customized formats.



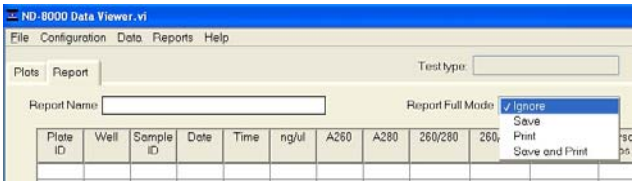
Start Report / Recording

All data is automatically archived. The user can log measurement results in an active report table as the data is accumulating by using the Start Report / Recording feature in the acquisition module. The default setting has the Recording feature activated for all modules. If **Start Report** is displayed, the accumulating data will still be archived but will not be shown in the active report.



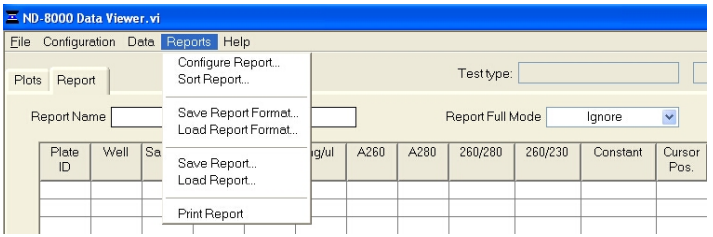
Show Report

Selecting this button will bring up the Report page. Hitting the Exit button at the top right will exit back to the acquisition module. The data will continue to populate the report after exiting.

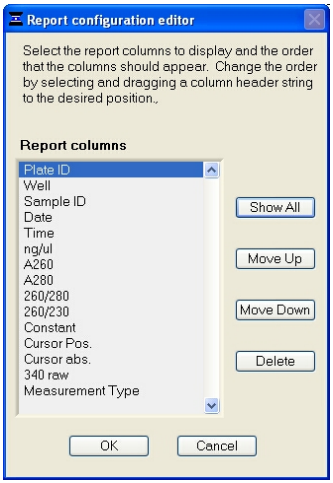


All data is stored in the archive file at *c:\ND-8000 Data* and may be exported to an alternate location. To open these reports, go to the *C:\ND-8000 Data\Reports* folder and right click on the file of interest.

Some key options useful for the Report page are accessible through the Report tool bar drop down.



Choosing the **Configure Report** option brings up the following box:



Selecting **OK** will return the user to the Report page displaying only the columns of interest.

Additional options include:

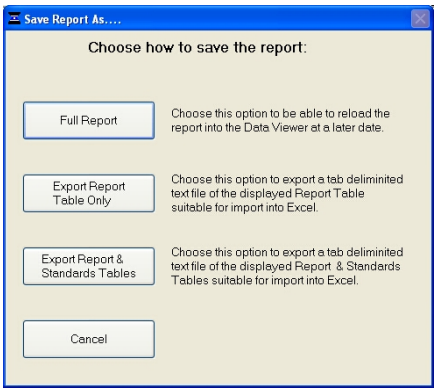
- **Sort:** Allows users to sort data by column (example-by date or sample name) and by either ascending or descending order.

- **Save Report Format:** Saves the current report format as an .nr8 file for retrieval and future use. To designate a saved report format as the default format, exit to the Main Menu, choose Users Preferences and click **Reports**. Use the Select Default Report Format to see the list of saved formats available for the specific method type.

- **Load Report Format:** Allows saved report formats to be loaded either before or after data is imported.

- **Print Report:** Will print out only the Report page by default. Users may choose whether or not to print out the standards or plots pages by selecting these options under the Configuration drop down on the tool bar.

- **Save Report and Load Report:** There are several options for this feature as seen in the following window:



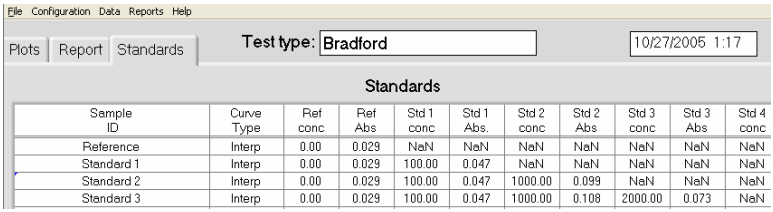
Using the Full Report option will allow the user to use the Data Viewer to reload the report at a later date. The saved report may be recalled using the pull down Load Report. If using the Load Report feature- the report will be displayed with the default column configuration. Note: Access the User Preferences module on the main menu to modify and save preferred default configurations. Reports are saved in an .nr8 format. The user may select specific default report configurations for each pre-defined method. Only one default report formula is available for all user defined methods. Use the drop down Load Report Format to utilize a different saved configuration when running a custom method.

The other two options are meant for reports that are expected to be opened in Excel type spreadsheets. To open these reports, go to the *C:\ND-8000 Data\Reports* folder and right click on the file of interest.

Additional features of the Report page:

- **Method:** Automatically populated with data method type.
- **Date and Time:** Automatically populated when report is generated.
- **Report Name:** User defined designation for the current report
- **Report Full mode:** Drop down box defining options for managing reports.
  The user may elect to Print, Save, or Print and Save a report at any time by using the Report Full Mode drop down box shown below. The default setting of 1000 samples per report may be modified by highlighting the box and typing in the desired number. Choosing 'Ignore' from the drop-down will allow the user to include an unlimited number of samples in a report.
- **Max Report Size:** Default number is set at 1000.

Standards Page
The Standards page will display the actual reference standards applied to each particular sample at the time of measurement. Note: This page is only available for software modules utilizing a Standard Curve.


File Configuration Data Reports Help

Plots | Report | Standards

Test type: Bradford

10/27/2005 1:17

Standards

Sample ID	Curve Type	Ref conc	Ref Abs	Std 1 conc	Std 1 Abs.	Std 2 conc	Std 2 Abs	Std 3 conc	Std 3 Abs	Std 4 conc
Reference	Interp	0.00	0.029	NaN	NaN	NaN	NaN	NaN	NaN	NaN
Standard 1	Interp	0.00	0.029	100.00	0.047	NaN	NaN	NaN	NaN	NaN
Standard 2	Interp	0.00	0.029	100.00	0.047	1000.00	0.099	NaN	NaN	NaN
Standard 3	Interp	0.00	0.029	100.00	0.047	1000.00	0.108	2000.00	0.073	NaN

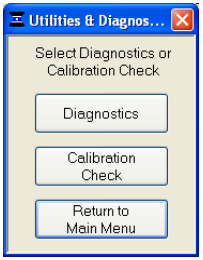
Opening Archived Data with Spreadsheet Programs
The archived files are in tab-delimited format and can be opened in Microsoft Excel or an equivalent spreadsheet program. To open these reports, go to the *C:\ND-8000 Data\Reports* folder and right click on the file of interest.

Note: Save and rename files before making any changes if opened with Excel type of programs to ensure that the original archived data is available for importing using the Data Viewer function.

Diagnostics and Utilities

Calibration Check

The Calibration Check is found within the Utilities and Diagnostics module and is accessed through the Main Menu. It is used to confirm that both pathlengths are within calibration specifications.



A CF-8 kit is required to run the calibration check procedure. The kit includes 8-well PCR strip tubes and two ampoules of an aqueous potassium dichromate ($K_2Cr_2O_7$) solution (CF-1) for use in confirming calibration of NanoDrop 8000 Spectrophotometers

The Thermo Scientific NanoDrop 8000 Calibration Check diagnostic utilizes five measurements on each of the 8 positions to verify the pathlength calibration. It is important to pay careful attention to technique when performing this procedure, as a single outlier caused by user error can result in a failed calibration check on one or more pedestals.

Calibration Check Best Practices

- Do not open the vial of CF-1 until ready to perform the calibration check.
- Position the instrument at an angle that will allow for optimal use of the pipette guide.
- Ensure the NanoDrop 8000 is not situated near an air vent or an exhaust fan from a nearby instrument.

- Aliquot 60 uL of CF-1 into each of the 8 tubes in the PCR strip. A volume of 60 ul in each PCR tube is required to reduce the effects of evaporation and potential fluid concentration.

- Use a small-volume (0.5-10 uL) multi-channel pipettor and well fitting low retention tips. If using an electronic pipettor do not draw up large volumes to dispense multiple aliquots. The CF-1 reserve stored in the tips may begin to concentrate, affecting the quality of results. Draw and dispense individual 1.5 uL aliquots for each measurement cycle.

- Ensure that adequate volumes of CF-1 are being pipetted onto the center of each pedestal. If tips are not fitted properly on each channel of the multi-channel pipettor, individual channels may not properly draw up, or dispense an adequate volume onto each pedestal. Columns that are too thin, or shifted off to one side may not completely cover the fiber-optic center of each pedestal.

- Always use fresh aliquots for each replicate set of measurements.

Required Materials

- CF-8 Calibration Kit (Cat # CHEM-CF-8)
- low volume, 8-channel pipettor with the appropriate tips
- dH_20
- low lint lab wipes

Preparation

Follow the cleaning and reconditioning steps prior to beginning the calibration check procedure:

1. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.

2. Apply a very thin, even layer of PR-1 to the surface of the upper and lower pedestals.

3. Wait 30 seconds for the PR-1 to dry.

4. Fold a clean, dry laboratory wipe into quarters and remove the PR-1 by aggressively rubbing the surface of the upper and lower pedestals until all compound residue is removed

Note: The black appearance of the removed residue is normal and is not indicative of surface dirt.

5. Ensure the measurement pedestals are clean and that a 1.5 ul water sample "beads" up on each of the lower pedestals. Repeat reconditioning procedure if a water sample droplet flattens out and covers the surface of any pedestal.

NOote: CF-1 is supplied in two single use vials and both ampoules must be used within 30 minutes of opening. Prolonged exposure to the environment may cause a significant concentration change.

Calibration Check Procedure

1. Position the instrument at an angle that will allow for optimal use of the tip guide.

2. Select the Calibration Check: Tools & Configuration Tab > Utilities & Diagnostics>Calibration Check

3. Enter the Target Absorbance of the CF-1 in the pop-up box "Target absorbance @350 nm, 1 mm pathlength."

   Note: The target absorbance is located on the ampoule label and is lot specific.

4. Add 60 ul of dH_20 to each well of one of the 8-well strips provided.

5. Use an 8-channel pipettor to simultaneously pipette 1.5uL of water to each pedestal position, lower the arm and click the **Blank** button. At the end of the measurement cycle, use a lab wipe to remove the water aliquots from the upper and lower pedestals and change tips.

6. Thoroughly mix CF-1 Calibration Fluid by vigorously shaking ampoule and ensure all solution is collected in the bottom portion of the vial.

7. Carefully break the neck of ampoule to open the CF-1 Calibration Fluid and aliquot 60 ul into each of well of the second 8-well strip.

8. **Use an 8-channel pipettor** to simultaneously pipette 1.5uL of CF-1 fluid onto each pedestal, lower the arm, and click the **Measure** button.

9. At the end of each measurement cycle, use a lab wipe to remove the CF-1 aliquots from the upper and lower pedestals.

10. **Change pipette tips**, then repeat step 8. Measure a total of 5 separate sets of replicates.

Calibration Check Results

After the 5th measurement, the results will be displayed on-screen.

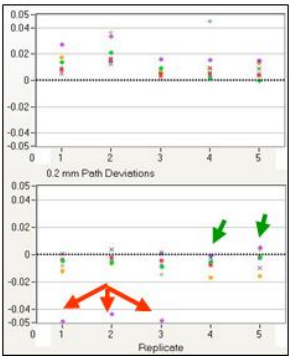
When the instrument passes the check procedure, a pop up box will indicate that the instrument works within specifications and the results will be saved as both a JPG and a .TXT file in the *ND-8000 Data\Calib check* folder on the local drive.

If the calibration check results in a failure or conditional pass, please review each series of data points looking at trends associated with individual positions. Choose the "Take No Action" option to review the data before exiting the diagnostic module. Refer to the Common Technique Issues section below for troubleshooting information.

If the instrument needs to be recalibrated, contact your local distributor or Technical Support for assistance.

Common Technique Issues

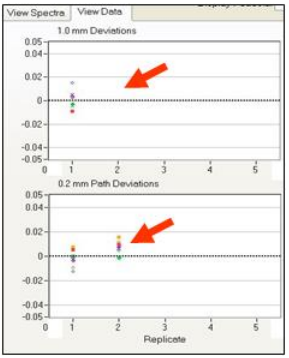
Improper Blank
When individual positions appear to have very low absorbance values as shown by the red arrows in the figure below, it often indicates an improper blank was measured. The green arrows in the same figure illustrate the difference in measurements after a new blank was measured.



When this occurs, reset the calibration check and ensure all pedestals are clean before making a new blank measurement.

Multiple Measurements with Same Aliquot

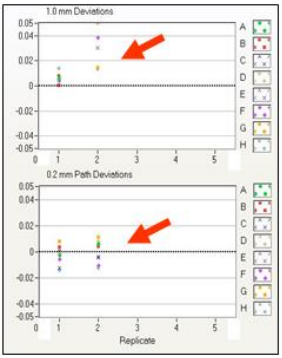
Measuring the same aliquots for subsequent measurements often results in most or all of the 1.0 mm data points moving off the reported scale. In the image below- note that the 1.0 mm data is off the scale while the 0.2 mm data for replicate 2 is within the accepted deviation.



When this occurs, reset the calibration check and ensure fresh aliquots are used for each replicate.

Using Single Channel Pipettor

Loading samples with a single channel pipettor often results in most or all of the 1.0 mm measurements being reported as more concentrated than would be expected.



When this occurs, reset the calibration check. Use an 8 channel pipettor with fresh tips for each measurement.

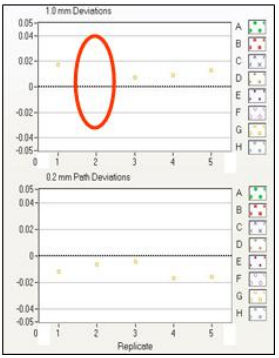
Multiple Aliquots Using Same Pipet Tips

Using the same pipet tips for subsequent measurements often results in one or more of the 1.0 mm measurements being reported as more concentrated than would be expected. (See image above.)

When this occurs, reset the calibration check. Use an n 8 channel pipettor with fresh tips for each measurement.

Insufficient Volumes

When just one or two samples do not show 1.0 mm data points, but do show 0.2 mm path deviation data as illustrated below, the full aliquot may not have been delivered to the pedestal resulting in a broken column.

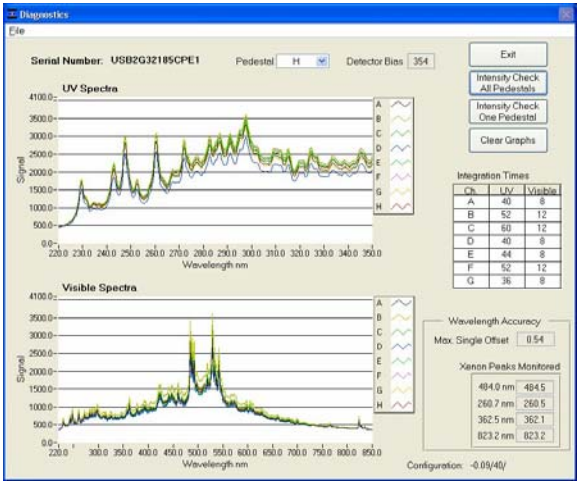


When this occurs, reset the calibration check and ensure sufficient volumes are used for each replicate.

Reviewing Previous Calibration Check Results
The calibration check module included in NanoDrop 8000 software versions 2.2 and above enables the user to review absorbance values for each replicate for all 8 positions as well as by individual positions. This feature is useful when troubleshooting calibration checks that result in conditional passes and/or failures. The latest software version is avaialble for download at www.nanodrop.com. Previous results may be reviewed at any time by using the "Load Cal Check File" feature avaialble under the File drop down menu.

Additional information
Cleaning instructions, the calibration check procedure as well as a table of calibration check tolerances may be accessed via the menu bar Help drop-down feature.

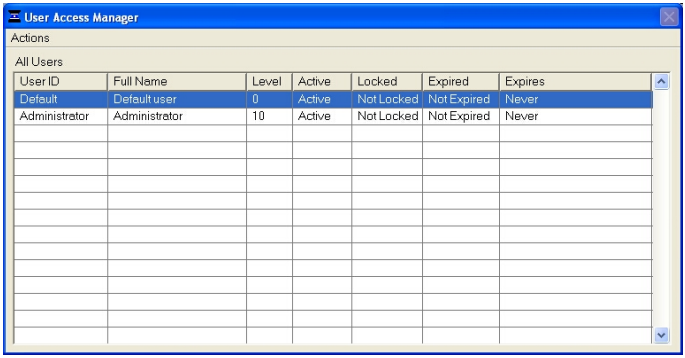
Intensity Check
The Intensity check is used for troubleshooting purposes. The below image is representative of a typical spectra. Refer to the chapter on Troubleshooting for additional information.



Account Management

The Account Management module provides options for directing where specific data files are archived, allowing users to segregate their data into

personal folders. The Account Management module is accessible to the administrator only.



Account Types
There are three types of user accounts:

Level 10- this is the highest security setting and all level 10 users can add new users, modify a user, delete a user and set password options. At the time of software installation, the only level 10 account is Administrator whose initial password is “nanodrop”. It is strongly recommended that the password be changed after initial account set up. Any user can be set to a level 10 access, although this is not recommended (see Level 5 below). Note: The administrator (or the last level 10 user) account may not be deleted.

Level 5- this is the security setting recommended for an ordinary user account. An account with this access will be password protected and will be able to select specific user preferences. Also, all data generated will be automatically archived in to the user’s account in *c:\ND-8000 Data* (and the user specified location if that preference is selected).

Default (level 0 security) - this access level is reserved for the Default account only. This account enables any user without an account to access all the active software measurement modules. Although it is not password protected, user preferences can be set for this account. All data generated will be automatically archived into the Default folder within the *c:\ND-8000 Data* folder. Note: For laboratories requiring that every user have a unique user-account, the administrator may disable the default user account.

Account Log-in/Log-out and Time Out

The user's account will remain active until 1) a user logs out of his/her account by using the pull down menu to select either Default or another user name or 2) the user closes the software.

A user account may also be logged out automatically if the software "System Idle Timeout" is exceeded. After 4 hours of inactivity the software account will automatically revert back to the Default user. A screen will appear indicating that the time is about to expire, with a 30-second countdown. If the user elects **CANCEL**, the clock with reset and the user account and application module will remain active for another 4 hours. If the time expires, the open application module will close, returning to the Main Menu and the Default user.

Account Lockout

User-specific accounts can become locked out in several ways as noted below:

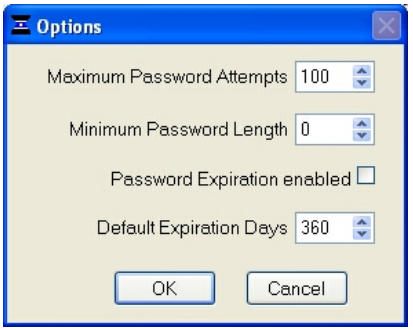
- Failure to change password within the allotted time
- Incorrectly entering the password 99 consecutive times
- The administrator locks a specific account

Only the administrator (level 10) can unlock a locked account. This is done by using the **Modify User** entry in the Account Management module. Note: All accounts (even the administrator) may be locked if the incorrect password entry occurs as described above.

Change Password

This module enables each user having an authorized account ID to change their respective password.

Note: The administrator, using the Options or the Modify User entries in the Account Management module, establishes whether individual user passwords will expire and, if so, after how many days.



Passwords.log file

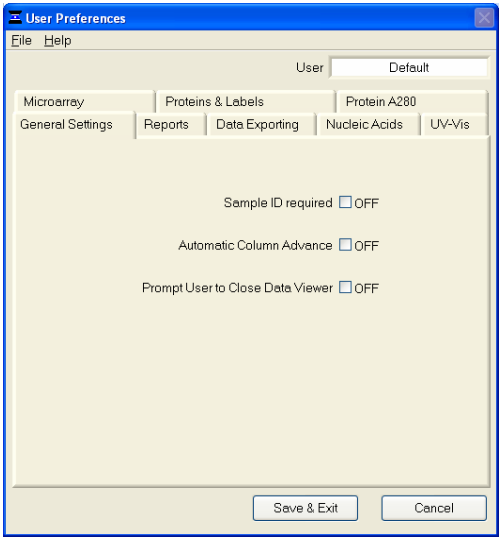
This file contains the User ID & password for all accounts and is readable only by the software. It can be found in the *c:\ND-8000 Data\log files* folder. It is strongly recommended that each time a new user account is added or a password is changed, the administrator make a **copy** of the updated file and store it in the *c:\ND-8000 Data\log files* folder. If the administrator's account becomes locked, the up-to-date copy can be renamed and used as the password.log file.

User Preferences

Each user has the option to configure a number of settings in the various application modules. The user preferences options for each application module are self explanatory and include options applicable for that module.

Some key features include:
The General Settings tab allows the user to either select or deselect as default settings the options of:

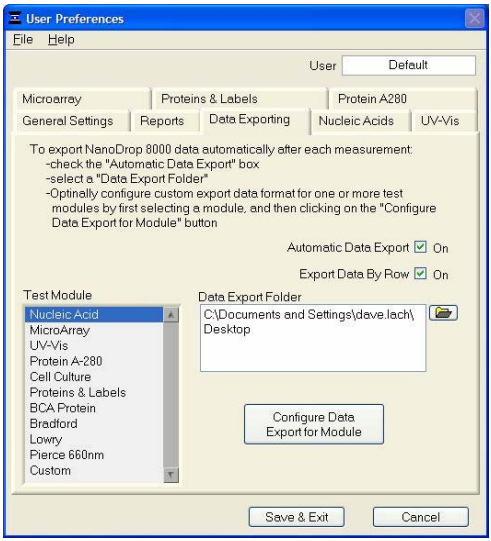
- Requiring sample ID's before a measurement is taken
- Auto advancement to the next set of samples (i.e. move to the next column on the 96 well visual)
- Requiring the user to confirm that they want the Data Viewer to close when closing out of an acquisition module



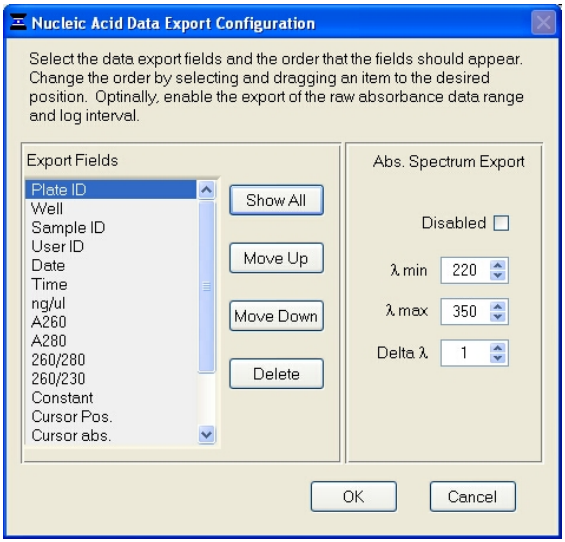
Data Exporting
In addition to the primary data storage of archive files at *c:\ND-8000 Data*, users may elect to export their data to an additional location. This option can be chosen under the 'Data Exporting' tab by selecting the 'Automatic Data Export' box and then choosing the file path by clicking on the file folder icon under 'Data Export Folder'. The archived files may

be opened with Excel by using the right click option of the mouse to open the .nd8 file. Note: It is important to save the file with a new name before making changes with Excel.

The Export Data by Row option is applicable to the 8 sample operation mode only. When selected, data will be sorted in reports by plate well rows (A1 to A12, then B1 to B12) rather than by columns (A1 to H1, then A2 to H2).

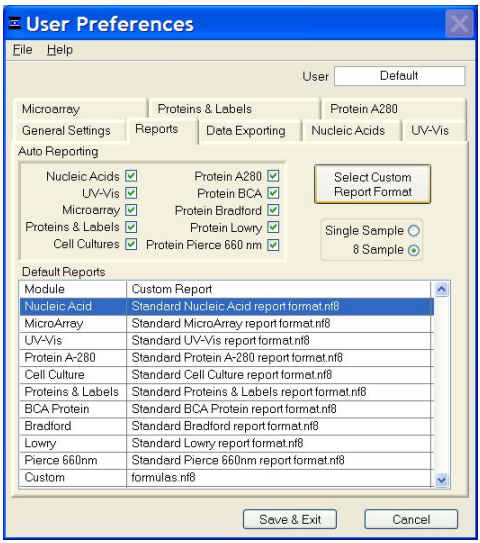


Selecting the **Configure Data Export for Module** button will bring up the following pop-up box:



This box is used to select the particular data columns of interest when exporting data. Save the designated path by clicking on the Save & Exit button before exiting the User Preferences module.

Note: The user may select specific default report configurations for each pre-defined method. As seen in the image below, only one default report formula is available for all user defined methods. Use the drop down "Load Report Format" to utilize a different saved configuration when running a custom method.



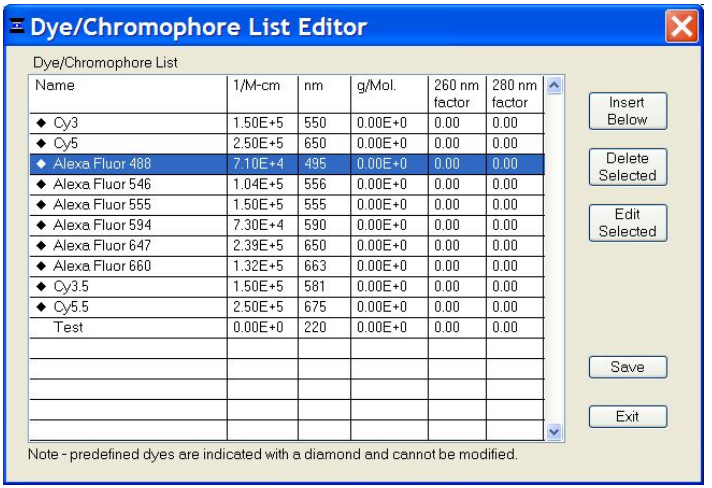
Auto Reporting
Users may choose to select the Auto Reporting option for any of the application modules. The auto reporting option allows data to automatically be saved to the report for all samples. Users may choose this option under the Reports tab by selecting the corresponding box next to the modules listed under Auto Reporting. Save the auto reporting functions by clicking on the Save & Exit button before exiting the User Preferences window.

Note: User preferences are stored in a '.log' file. When upgrading to a newer version of the software, this file should be preserved. If the user preferences do not appear correctly after upgrading to a new software version, the .log file should be manually copied to the proper directory. This file contains the User ID & password for all accounts and is readable only by the software. It can be found in the *c:\ND-8000 Data\log files* folder. It is strongly recommended that each time a new user account is added or a password is changed, the administrator make a **copy** of the updated file and store it in the *c:\ND-8000 Data\log files* folder. If the administrator's account becomes locked, the up-to-date copy can be renamed and used as the password.log file.

Dye/Chromophore Editor

The Dye/Chromophore Editor gives the user the ability to add additional dyes or chromophores to the list of predefined fluorescent dyes available for use with the MicroArray and Proteins & Labels modules. Predefined dye methods are indicated by a diamond and cannot be modified.

Absorbance contribution at 260nm and 280nm from the respective dye can be corrected by entering the appropriate decimal correction in the respective field when adding a new dye to the list. Please refer to the dye manufacturer for the appropriate correction factors for user-entered dyes.



8. Troubleshooting

Error Codes

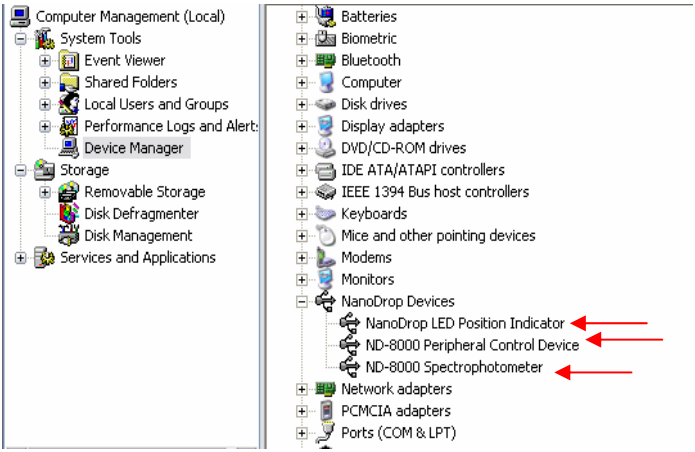
Instrument Not Found

This error might appear upon software startup and usually indicates that either the power supply or the USB cable is not properly connected or the software is not loaded properly. To troubleshoot, do the following:

1. Check that the power supply is connected to the instrument. Confirm that the instrument is getting power by observing that light can be seen through the USB opening on the rear of the instrument.

2. Confirm that the USB cable is connected to both the PC and the instrument. Note: There are internal USB drivers in addition to the USB cable. When attaching the USB cable, please wait at least 30 seconds for the multiple USB devices to be installed and recognized before opening the software.

3. If the cable is connected properly but the Instrument recognition error persists, open the Windows Device Manager by either right clicking on the **My Computer** icon on the desk top or selecting *Start →My Computer (right click) → Manage → Device Manager → NanoDrop Devices.*

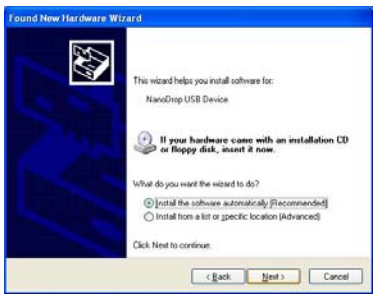


4. If an unknown device, a yellow exclamation point or a question mark appears next to one of three expected NanoDrop devices, manually uninstall the device by right clicking and selecting **Uninstall** from the options displayed.

5. Disconnect the USB cable first and then disconnect the power supply from the NanoDrop 8000.

6. Reconnect the power supply, wait 5 seconds and then reconnect the USB cable. At this point you may or may not see the Found New Hardware Wizard. If the wizard appears, follow the prompts for automatic installation of the software. Windows XP SP2 operating system will ask to allow it to search the internet for the proper software as shown - Select **No, not this time**.

Intro Page: Windows XP- SP2



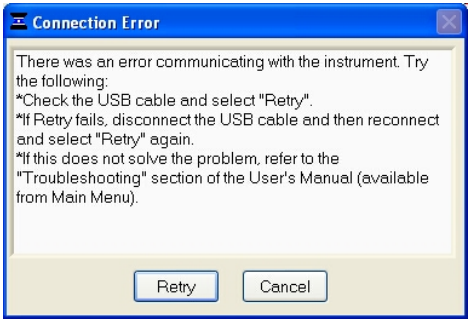
Other Windows Operating Systems

Note: If the Found New Hardware Wizard appears, installation will require two cycles through the Wizard, once for the spectrometer

and once for the peripheral control devices that are internal to the instrument.

7. Install the instrument on another PC to rule out a faulty USB hub/port on the original PC. Windows Device Manager on the 2nd PC as described in step 3 above. If the instrument is still not recognized, then the instrument may need service. Contact your local distributor or Technical Support for assistance.

Connection Error

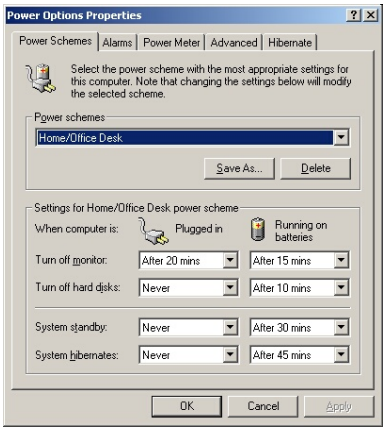


This error occurs whenever the USB connection is disrupted while operating a software module. Ensure that the USB cable is firmly inserted into the instrument and the computer then select 'Retry'. In most cases this will correct the problem. When attaching the USB cable, please wait at least 30 seconds for the USB devices and internal drivers to be installed and recognized.

Some additional possible causes for the error message and solutions are listed below:

Power management scheme on the PC:
If your PC is automatically going into standby or hibernate mode, the USB communication will be lost whenever it occurs and 'Retry' will NOT reconnect the instrument. If this occurs, the USB cable will need to be disconnected/reconnected before selecting 'Retry'.

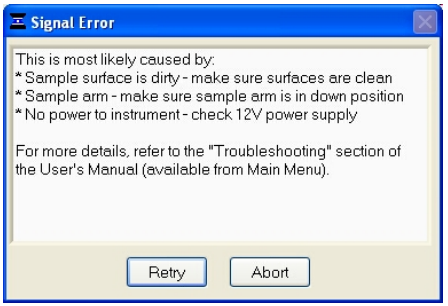
You can confirm that the power management settings are correct by opening the Power Options Properties page by choosing *Start → Control Panel → Power Options*. The 'System Standby' and 'System Hibernate' should be set to 'never' for the 'Plugged In' column.



Defective USB Port on PC

If your instrument operates properly most of the time, but the Connection Error appears intermittently, it could be caused by the USB port on the PC. If this occurs, install the software and operate on another PC. If the error does not occur on the second PC, it may be necessary to replace the USB card on the original PC.

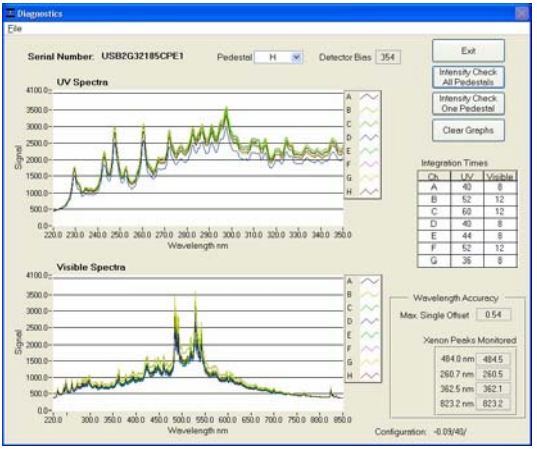
Signal Error



This error occurs because no light or not enough light is reaching the detector. If the troubleshooting steps outlined in the message do not fix the problem, perform an Intensity Check:

- Open the Utilities and Diagnostics module from the main menu.
- With the sampling arm down, select **OK** to initialize the spectrometer and then select **Intensity Check**. You will see two panels of 8 spectra each and a bias value greater than 65 as

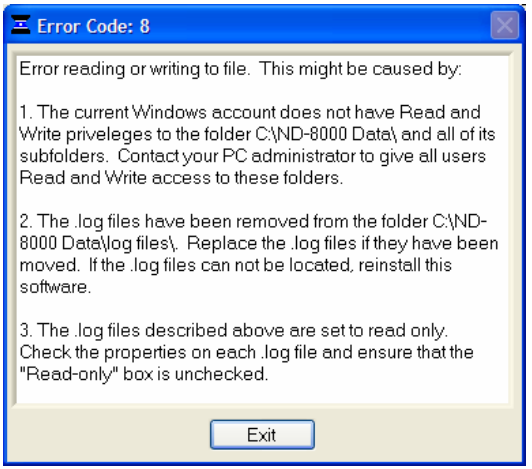
shown below. This indicates that the USB communication is normal, the power supply is operational and the flashlamp is functioning.



- If no spectra appear in the image, confirm that the power supply is firmly connected to the instrument and the plug is connected to a working outlet. Next, confirm that the power supply is operating properly. To do this, connect the leads of a volt-ohmmeter to the outlet of the supply. The voltage should be 12-20 Vdc, center positive.

If none of the troubleshooting steps above solves the problem, contact your local distributor or Technical Support for assistance.

Error Code 8



This error code is most likely to occur if the user does not have read and write access to the folder *c:\ND-8000 Data* or one of its subfolders. See your PC administrator to make sure that all users of the operating software have the appropriate Windows access level.

Error Code 8013

The software installation process installs two USB drivers. The above error message indicates that the motor UBS driver was not properly installed. Follow the instructions given above for the' Instrument Not Found error'. When attaching the USB cable, please wait at least 30 seconds for the USB devices and internal drivers to be installed and recognized.

Report Format Error

This error occurs when the user does not have “Write” access to one of the report format files located at c:*ND-8000 Data*\custom report formats or the file has been moved from this folder. This error is similar to Error Code 8 (see above). Contact your PC administrator to give all users Read and Write access to this folder. Replace the files if they have been removed. If the file cannot be located, reinstall the software.

Other Software Error Messages

- **Source Error…**
  This error indicates that there is insufficient light getting through to make good absorbance measurements. Check that the sampling arm is in the down position and the power is connected.

- **No Active Samples for Measurement…**
  This error indicates that there are not any sample positions currently selected for measurement. The user may click either ‘All Active’ or select individual positions for the next measurement.

- **Error 8005**
  This error occurs when trying to load a plate file that is not in a .txt format.

- **Error 9000**
  This error occurs when the passwords.log file is missing or corrupt. Reinstall the operating software and "overwrite" the existing copy when prompted." A new copy of the passwords.log file should appear in the C:\ND-8000 Data\Log Files folder.

- **Error 9003**
  This error indicates that the monitor resolution is below the 1024x768 required. Check the computer settings. Be sure that the Start menu tool bar is set to the bottom and not along the side.

- **Low Detector Bias…**
  This occurs when the software has identified a problem with the detector. Contact your local distributor or Technical Support if you encounter this error.

Driver X Configuration Failed- You Must Manually Edit the Registry

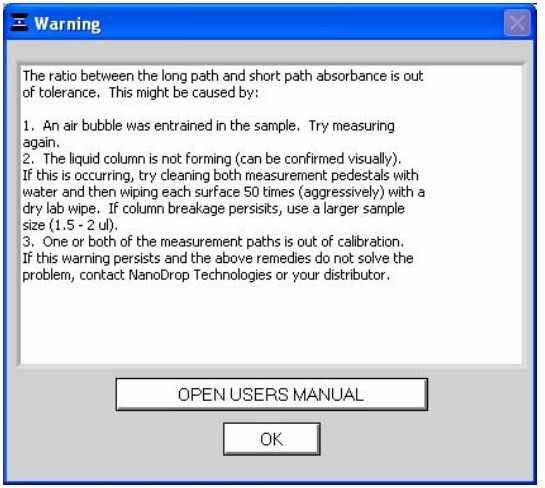
This error message (or others with similar wording) occurs when attempting to install the operating software on a computer running

Windows 2000 or XP. It occurs because the user does not have the necessary authorization to install the software. Contact your system administrator if this occurs.

Insufficient Memory…

This error message (or others with similar wording) occurs when attempting to install the operating software on a computer that does not have at least 100 MB of free hard disk space.

Liquid Column Breakage



This warning occurs when a possible problem with the column is detected. The software compares the long path and short path absorbances and issues a warning to the user if the short path is not 20% of the long path absorbance, within a tolerance. The most common explanation is that the column is not forming properly due to the pedestal being “unconditioned”.

When a pedestal becomes unconditioned, sample droplets applied to the bottom pedestal will 'flatten-out' and cover the entire pedestal surface rather than 'bead up'. Buffers containing detergents and various other reagents may cause the pedestal surfaces to become unconditioned. We have noted that routine use of the Bradford reagent may result in difficulty forming columns with 1 ul samples.

Pedestal Reconditioning

Use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement.

1. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.,

2. Apply a very thin, even layer of PR-1 to the surface of the upper and lower pedestals

3. Wait 30 seconds for the PR-1 to dry

4. Fold a clean, dry laboratory wipe into quarters and remove the PR-1 by aggressively rubbing the surface of the upper and lower pedestals until all compound residue is removed. Note: The black appearance of the removed residue is normal.

The reconditioning process is complete once the laboratory wipe shows no more black residue. To check the effectiveness of the reconditioning, load a 1 ul aliquot of dH_20 onto the lower measurement pedestals and visually verify that the water “beads” up.

As an alternative to using the PR-1 Kit, the pedestals may be reconditioned us follows:

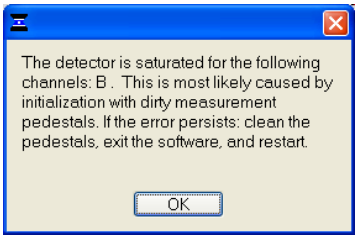
1. Fold a clean dry lab wipe over several times to increase its thickness.

2. Press the lab wipe firmly down on the lower pedestals and "buff/rub" very aggressively at least 50 times - the lab wipe will rip during this procedure and will have to be refolded several times throughout the procedure. The upper pedestals may also be buffed but care should be taken not to put too much force on the upper arm.

To check the effectiveness of the reconditioning, load a 1 ul aliquot of dH_20 onto the lower measurement pedestals and visually verify that the water “beads” up.

If the warning persists and the user visually confirms that the liquid column is forming, contact your local distributor or Technical Support for assistance.

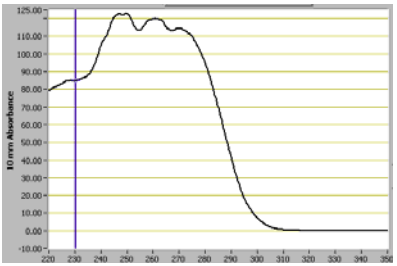
Saturated Detector



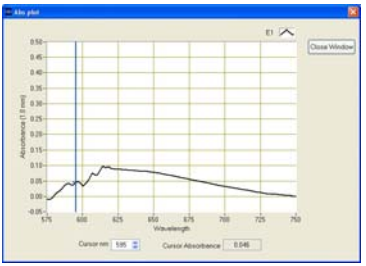
This error message can occur when the software calculates high integration times for particular positions during initialization. This is most likely due to the presence of an air bubble or a dried sample left on the measurement surface. Cleaning both the top and bottom pedestals with de-ionized water and exiting out of the software module to the main menu should alleviate the problem. It is not necessary to close the software completely as each module is re-initialized when it is opened. If the error persists, contact your local distributor or Technical Support for assistance.

Unusual Spectrum

A sample that exhibits jagged “cuts” out of the spectrum, but an otherwise normal shape, may be the result of detector saturation. This can be caused by the software selecting too high of an integration time due to a dirty sample pedestal upon startup. Try cleaning lower and upper sample pedestals thoroughly and restarting the software. For reference, examples of spectra generated with a saturated detector are shown below.



Detector saturation- nucleic acid measurement



Detector saturation- Bradford measurement

A spectrum that is very “un-smooth” or “ragged” can be caused by insufficient light intensity reaching the spectrometer. If you suspect that this is occurring, contact your local distributor or Technical Support for assistance.

Sample Accuracy and Reproducibility

If you are obtaining results that seem inaccurate or not reproducible, it could be the result of sample or aliquot non-homogeneity or liquid column breakage. It may be helpful to try the following to ensure representative results:

- **Make sure the sample surfaces are clean before starting the software module**
  A dirty sample pedestal on startup can cause erroneous absorbance readings (even negative values) and signal saturation. It is always a good practice to clean the sample surfaces with de-ionized water to remove any dried sample that might be present. Note: Do not use a squirt bottle to apply de-ionized water.

- **Use a 1.5-2 ul sample size**
  Inaccurate results can occur when the liquid sample column is not completely formed during a measurement. While making a measurement, visually confirm that the liquid column is formed. If necessary, try 1.5-2 ul samples to ensure the column is formed. Proteins and solutions containing surfactants are known to "un-condition" the measurement pedestal surfaces so that the liquid column does not form properly. Use the NanoDrop Pedestal Reconditioning Compound PR-1 as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. See **Column Breakage** in this section for further details.

- **Heat DNA samples to 55 °C and vortex before measurement**
  Due to the small volumes required by the NanoDrop 8000, it is extremely important to ensure that the sample being measured is homogeneous. Field experience has shown that samples containing large molecules such as genomic or lambda DNA are particularly susceptible to this phenomenon. Note: Larger volumes used by cuvette-based spectrophotometers will minimize or mask the effect of sample non-homogeneity.

- **Perform a Blanking Cycle**
  This will confirm that the instrument is working well and that any sample carryover from previous measurements is not a concern. To run a blanking cycle, perform the following:

  1. Open the application software module.

2. Load an aliquot of the blank (the same buffer, or solvent the unknown samples are in) onto each of the lower measurement pedestals and then lower the sampling arm into the 'down' position.

3. Click on the Blank button. When the measurement is complete, wipe off the buffer from all pedestals.

4. Select "All Active On" and analyze a fresh aliquot of the blanking solution on all pedestals using the 'Measure' button (F1). The result should be 8 spectra with relatively flat baselines near zero.

5. Wipe the blank from all upper and lower measurement pedestal surfaces with a laboratory wipe and repeat the process until the spectrum varies no more than +/- 0.005 A (1 mm path) from the baseline. Note: The Nucleic Acid, A280 and Proteins & Labels modules display the absorbance values normalized to a 10 mm path so the effective variance should be +/- 0.05 abs from the baseline.

6. Reload the plate list file before measuring samples.

- **Confirm that reference (blank) solution and solvent are the same material**
  Buffers often absorb in the UV range and therefore it is critical to blank the instrument with exactly the same material that the sample is suspended in.

- **Confirm that your sample is not too dilute**
  Measuring samples at or near the detection limit will result in measurements that can vary a significant amount. Refer to the table of concentration ranges provided within the respective application module section of the manual for lower detection limits.

- **Confirm instrument accuracy with CF-1**
  CF-1 is a concentrated potassium dichromate calibration standard and is manufactured exclusively for use with NanoDrop instruments and available from Thermo Fisher Scientific and its distributors. It is a good practice to check the instrument's performance every six months with fresh CF-1.

260/280 Ratio

Many researchers encounter a consistent 260/280 ratio change when switching from a standard cuvette spectrophotometer to the NanoDrop 8000 Spectrophotometer. The three main causes for this are listed below:

- **Change in sample acidity**
  Small changes in solution pH will cause the 260:280 to vary[**]. Acidic solutions will under-represent the 260:280 ratio by 0.2-0.3, while a basic solution will over-represent the ratio by 0.2-0.3. If comparing the NanoDrop 8000 Spectrophotometer to other spectrophotometers, it is important to ensure that the pH and ionic strength of an undiluted sample measured on the NanoDrop 8000 is at the same as the diluted sample measured on the second spectrophotometer.

  [**] William W. Wilfinger, Karol Mackey, and Piotr Chomczynski, Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity: *BioTechniques 22:474-481 (March 1997)*

- **Wavelength accuracy of the spectrophotometers**
  Although the absorbance of a nucleic acid at 260nm is generally on a plateau, the absorbance curve at 280nm is quite steeply sloped. A slight shift in wavelength accuracy will have a large effect on 260:280 ratios. For example, a +/- 1 nm shift in wavelength accuracy will result in a +/- 0.2 change in the 260:280 ratio. Since many spectrophotometers claim a 1 nm accuracy specification, it is possible to see as much as a 0.4 difference in the 260:280 ratio when measuring the same nucleic acid sample on two spectrophotometers that are both within wavelength accuracy specification.

- **Nucleotide mix in your sample**
  The five nucleotides that comprise DNA and RNA exhibit widely varying 260:280 ratios[***]. The following represent the 260:280 ratios estimated for each nucleotide if measured independently:
  Guanine: 1.15
  Adenine: 4.50
  Cytosine: 1.51
  Uracil: 4.00
  Thymine: 1.47

  The resultant 260:280 ratio for the nucleic acid being studied will be approximately equal to the weighted average of the 260:280 ratios for the four nucleotides present. It is important to note that the generally accepted ratios of 1.8 and 2.0 for DNA and RNA are

"rules of thumb". The actual ratio will depend on the composition of the nucleic acid. Note: RNA will typically have a higher 260:280 ratio due to the higher ratio of Uracil compared to that of Thymine.

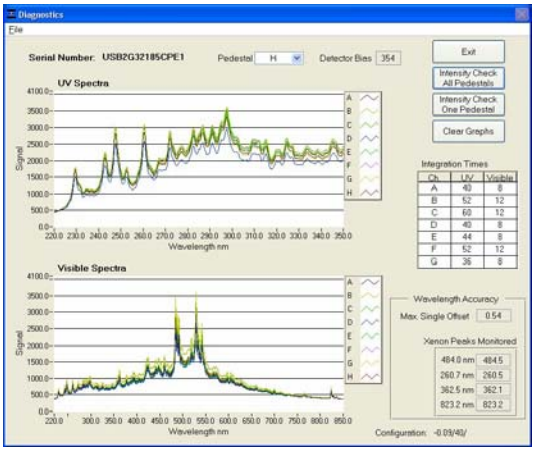
*** Leninger, A. L. *Biochemistry*, 2nd ed., Worth Publishers, New York, 1975

Technical Support

If, after referring to the above troubleshooting tips, you are unable to resolve your problem, please contact your local distributor or Technical Support for assistance. The following information will be very helpful:

- **Serial Number of the instrument**
  The number is located on the bottom of the unit.

- **JPG image of Utilities and Diagnostics module**
  To get this, open this module and select OK to initialize the module. Select Intensity Check. Once the spectrum has been created, choose File → Save Window as shown below. Save to your hard drive and email as an attachment to your local distributor or to Technical Support.



- **Application Module Screen Captures**
  Screen captures of the actual spectrum as seen on your PC are of great use in diagnosing problems. Making a screen capture is quite easy. When in an application module, press Alt+Print Screen. This copies the highlighted screen window to the PC's

clipboard. Next, paste this screen capture into MS Word, MS Paint (this program usually comes standard with the PC and can usually be found in the Start → Accessories menu), or other graphics programs. Save this as a .jpg or .doc file and send as email attachment to your local distributor or to Technical Support.

- **Data Archive Files**
  If you have questions about your data, please send the archive file containing the suspect data as an email attachment to your local distributor or to Technical Support. The archived file can be found at *C:\ND-8000 Data → User name → Application Module* (BCA Protein, Bradford, Cell Culture, Protein Lowry, Proteins and Labels, MicroArray, Nucleic Acid, Protein A-280, UV-Vis)

9. Maintenance and Warranty

Cleaning

The primary maintenance requirement of the NanoDrop 8000 Spectrophotometer is to keep the measurement pedestal surfaces clean. Upon completion of each sample measurement, wipe the sample from the upper and lower pedestals to prevent sample carryover and avoid residue buildup. A final cleaning of all surfaces with de-ionized water is also recommended after the user's last measurement. Note: Do not use a squirt bottle to apply bleach or de-ionized water.

1. Apply 5 ul of dH_20 onto each bottom pedestal.

2. Lower the upper pedestal arm to form a liquid column; let it sit for approximately 2-3 minutes.

3. Wipe away the water form each upper and lower pedestal with a clean lab wipe.

Note: Typically dH_20 is sufficient for removal of samples that have dried on the optical pedestals. There are a few cases (i.e. dried proteins) that may require a more rigorous cleaning protocol. For these cases, we recommend that 0.5M HCl with 5 ul of dH_20 to remove any residual HCl.

Pedestal Reconditioning

Use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement.

1. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.,

2. Apply a very thin, even layer of PR-1 to the surface of the upper and lower pedestals

3. Wait 30 seconds for the PR-1 to dry

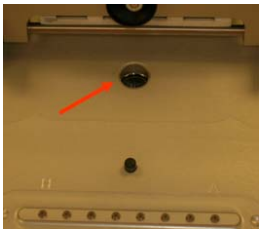
4. Fold a clean, dry laboratory wipe into quarters and remove the PR-1 by aggressively rubbing the surface of the upper and lower pedestals until all compound residue is removed. Note: The black appearance of the removed residue is normal.

The reconditioning process is complete once the laboratory wipe shows

no more black residue. To check the effectiveness of the reconditioning, load a 1 ul aliquot of dH_20 onto the lower measurement pedestals and visually verify that the water "beads" up.

Cleaning of Light Source Window

The Xenon flash lamp window must be kept free of debris and obstruction. The window may be cleaned with a water-dampened laboratory wipe. Note: Do **not** use organic solvents such as acetone to clean the window.



Decontamination of Measurement Pedestals

If decontamination is necessary, a sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solution – freshly prepared), can be used to ensure that no biologically active material is present on the measurement pedestals. The metal fiber optic fittings are made from 303 stainless steel and are resistant to most common laboratory solvents (see "Solvent Compatibility" appendix). A final cleaning of all surfaces with de-ionized water is also recommended after the user's last measurement. Note: Do not use a squirt bottle to apply bleach or de-ionized water.

Rapid Reconditioning of the Sample Retention System

The Bradford reagent as well as other buffers containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form well with 1ul samples. Use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement.

Calibration

Pathlength (Accuracy) Calibration Check

It is good practice to verify the pathlength accuracy of the instrument every six months using the CF-8 Calibration Fluid Kit. Refer to the calibration check instructions under the Diagnostics & Utilities heading in section 7 for additional information.

Wavelength

Each time the software is started, the wavelengths are auto-calibrated based on known peaks in the xenon lamp spectra. No calibration is required by the user.

Warranty

All NanoDrop spectrophotometers and accessories manufactured by Thermo Fisher Scientific are warranted against manufacturing defects in parts and labor for a period of one year. Additional one, two, and three year service plan are available. Additional information may be found on our website.

Parts That Require Replacement

In general, the xenon flash lamp is the only part that will need to be replaced. The lamp has a lifespan of at least 30,000 measurements. When the flash lamp fails, the light output will become very erratic or stop altogether. Contact Technical Support or your local distributor is you suspect your lamp may need replacing.

10. Appendices

Instrument Specifications

- Sample Size: 1microliter
- Sample Number: up to 8
- Path Length: 1 mm (with auto-ranging to 0.2 mm)
- Light Source: Xenon flash lamp
- Detector Type: 2048-element linear silicon CCD array
- Wavelength Range: 220-750 nm
- Wavelength Accuracy: 1 nm
- Wavelength Resolution: 3 nm (FWHM at Hg 546 nm)
- Absorbance Precision: 0.003 absorbance (1mm path)
- Absorbance Accuracy: 3% (at 0.74 absorbance at 350 nm)
- Absorbance Range: 0.02-75 (10 mm equivalent absorbance)
- Detection Limit: 2 ng/microliter (dsDNA)
- Maximum Concentration: 3700 ng/microliter (dsDNA)
- Measurement Cycle Time: 20 seconds
- Dimensions (footprint): 24 x 32 cm
- Weight: 3.5 kg
- Sample Pedestals Material of Construction: 303 stainless steel and quartz fiber
- Operating Voltage: 12 Vdc
- Operating Power Consumption: 30 W
- Standby Power Consumption: 3 W
- UL/CSA and CE approval: all units

- Included in system: software, compatible with Windows 2000, XP, Vista (32 bit and Windows 7 (32 bit and 64 bit)

Blanking and Absorbance Calculations

When the NanoDrop 8000 Spectrophotometer is “blanked”, a spectrum is taken of a reference material (blank) and stored in memory as an array of light intensities by wavelength. When a measurement of a sample is taken, the intensity of light that has transmitted through the sample is recorded. The sample intensities along with the blank intensities are used to calculate the sample absorbance according to the following equation:

$$\text{Absorbance} = -\log\left(\text{Intensity}_{\text{sample}}/\text{Intensity}_{\text{blank}}\right)$$

Thus, the measured light intensity of both the sample and of the blank are required to calculate the absorbance at a given wavelength.

Concentration Calculation (Beer's Law)

General

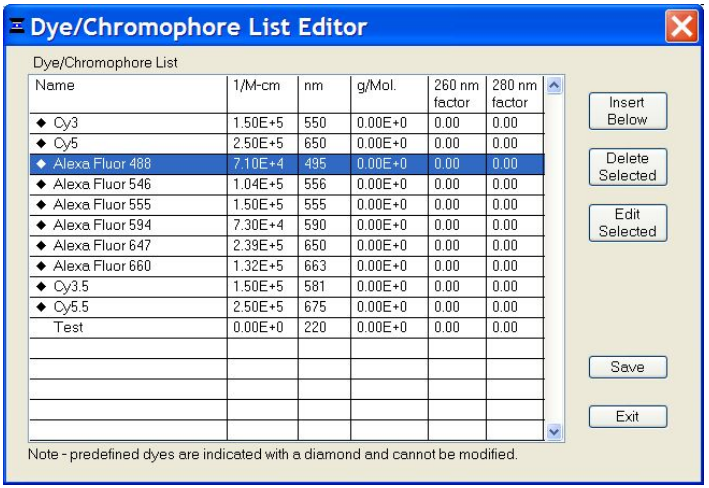
The Beer-Lambert equation is used to correlate the calculated absorbance with concentration:

$$A = E * b * c$$

Where **A** is the absorbance represented in absorbance units (A), **E** is the wavelength-dependent molar absorptivity coefficient (or extinction coefficient) with units of liter/mol-cm, **b** is the path length in cm, and **c** is the analyte concentration in moles/liter or molarity (M).

Fluorescent Dyes

The software uses the general form of the Beer-Lambert equation to calculate fluorescent dye concentrations in the MicroArray module. The table of extinction coefficients for each dye is below:



Name	1/M-cm	nm	g/Mol.	260 nm factor	280 nm factor
◆ Cy3	1.50E+5	550	0.00E+0	0.00	0.00
◆ Cy5	2.50E+5	650	0.00E+0	0.00	0.00
◆ Alexa Fluor 488	7.10E+4	495	0.00E+0	0.00	0.00
◆ Alexa Fluor 546	1.04E+5	556	0.00E+0	0.00	0.00
◆ Alexa Fluor 555	1.50E+5	555	0.00E+0	0.00	0.00
◆ Alexa Fluor 594	7.30E+4	590	0.00E+0	0.00	0.00
◆ Alexa Fluor 647	2.39E+5	650	0.00E+0	0.00	0.00
◆ Alexa Fluor 660	1.32E+5	663	0.00E+0	0.00	0.00
◆ Cy3.5	1.50E+5	581	0.00E+0	0.00	0.00
◆ Cy5.5	2.50E+5	675	0.00E+0	0.00	0.00
Test	0.00E+0	220	0.00E+0	0.00	0.00

Nucleic Acids

For nucleic acid quantification, the Beer-Lambert equation is modified to use an extinction coefficient with units of ng-cm/ml. Using this extinction coefficient gives a manipulated equation:

$$c = (A * e)/b$$

Where c is the nucleic acid concentration in ng/microliter, A is the absorbance in AU, e is the wavelength-dependent extinction coefficient in ng-cm/microliter and b is the path length in cm.

The generally accepted extinction coefficients for nucleic acids are:

- Double-stranded DNA: 50 ng-cm/ul
- Single-stranded DNA: 33 ng-cm/ul
- RNA: 40 ng-cm/ul

For the NanoDrop 8000 Spectrophotometer, path lengths of 1.0 mm and 0.2 mm are used compared to a standard spectrophotometer using a 10.0 mm path. Thus, the NanoDrop 8000 Spectrophotometer is capable of measuring samples that are 50 times more concentrated than can be measured in a standard spectrophotometer.

Note: Absorbance data shown in archive files are represented as displayed on the software screen. For Nucleic Acid, Protein A280 and Proteins and Labels modules, data are normalized to a 1.0 cm (10.0 mm) path. For MicroArray,
UV-Vis, Protein BCA, Protein Bradford, Protein Lowry and Cell Culture modules the data are normalized to a 0.1 cm (1.0 mm) path.

Solvent Compatibility
The NanoDrop 8000 Spectrophotometer is compatible with most solvents typically used in life science laboratories. These include: methanol, ethanol, n-propanol, isopropanol, butanol, acetone, ether, chloroform, carbon tetrachloride, DMSO, DMF, acetonitrile, THF, toluene, hexane, benzene, sodium hydroxide, sodium hypochlorite (bleach), dilute HCl, dilute HNO3, dilute acetic acid.

All forms of Hydrofluoric Acid (HF) are incompatible, as the fluoride ion will
dissolve the quartz fiber optic cable.

Decontamination of Measurement & Optical Surfaces

If decontamination is necessary, a sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solution – freshly prepared), can be used to ensure that no biologically active material is present on the measurement pedestals. The metal fiber optic fittings are made from 303 stainless steel and are resistant to most common laboratory solvents (see “Solvent Compatibility” appendix). A final cleaning of all surfaces with de-ionized water is also recommended after the user’s last measurement. Note: Do **not** use a squirt bottle to apply bleach or de-ionized water.