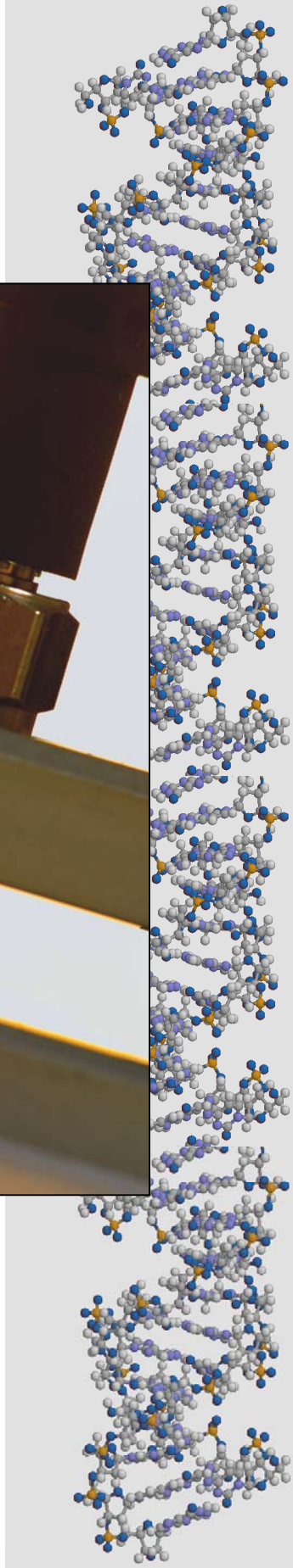
NanoDrop®

ND-1000 Spectrophotometer



V3.0.1 Users Manual

Table of Contents

1 Overview

1.1 Instrument Description

The NanoDrop® ND-1000 is a full-spectrum (220-750nm) spectrophotometer that measures 1 ul samples with high accuracy and reproducibility. It utilizes a patented sample retention technology that uses surface tension alone to hold the sample in place. This eliminates the need for cumbersome cuvettes and other sample containment devices and allows for clean up in seconds. In addition, the ND-1000 has the capability to measure highly concentrated samples without dilution (75X higher concentration than the samples measured by a standard cuvette spectrophotometer).

1.2 Operation

A 1 ul sample is pipetted onto the end of a fiber optic cable (the receiving fiber). A second fiber optic cable (the source fiber) is then brought into contact with the liquid sample causing the liquid to bridge the gap between the fiber optic ends. The gap is controlled to a 1mm path. A pulsed xenon flash lamp provides the light source and a spectrometer utilizing a linear CCD array is used to analyze the light after passing through the sample. The instrument is controlled by special software run from a PC, and the data is logged in an archive file on the PC.

1.3 Applications

UV/VIS spectrophotometry is simple for samples as small as 1 ul using the NanoDrop® ND-1000 Spectrophotometer. The small sample requirement and ease of use make the NanoDrop® ND-1000 Spectrophotometer ideally suited for measuring:

- Nucleic acid concentration and quality 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) up to 100 mg/ml (BSA)
- Bradford Assay analysis of protein
- BCA Assay analysis of protein
- Cell density measurements
- General UV-Vis spectrophotometry

2 Initial Set Up

2.1 Software

2.1.1 Computer Requirements

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

- Microsoft Windows 98, Millennium Edition, XP or 2000 operating system. **The operating software will not work with Windows 95 or Windows NT.**
- 233 MHz or higher processor
- CD ROM drive
- 32 MB or more of RAM
- 40 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)

2.1.2 Installation

WARNING: The system software must be loaded onto the PC before the USB cable is connected. Administrator access on the PC is required to install the software.

To properly install NanoDrop 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 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 "Install". Once the software menu is available, choose "Install Software" and follow the onscreen prompts. Restart your pc when prompted.

3. Remove warning label from back of the instrument and connect the USB cable to the PC and to the instrument.

4. There will be a delay of approx. 1 minute and then the Hardware Wizard should appear and indicate it has located new hardware and will install the software for it. If you are prompted whether or not to continue with the wizard, select "OK". Note Windows XP and 2000 operating systems may give the message: "The software you are installing for this hardware: OceanOptics USB2000- EEPROM Load has not passed Windows logo testing…" If you receive this message, select "continue anyway".

5. Your NanoDrop® ND-1000 Spectrophotometer should now be ready for operation. If the software does not start properly, you may need to configure the spectrometer. Refer to the "Troubleshooting" section if this occurs.

2.1.3 Configuring the System Font

The NanoDrop 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 "Displays 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 .

Other selections can be used, but may cause some text in the NanoDrop® software window to not fit well or may cause the function selection tabs across the top to become inaccessible.

2.1.4 Software Upgrades

NanoDrop Technologies makes periodic upgrades to the NanoDrop software. These upgrades are available for download at www.nanodrop.com.

2.2 Hardware

2.2.1 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 power supply can remain plugged into the NanoDrop® ND-1000 Spectrophotometer while the instrument is not in use. When the unit is in this "standby" mode, power consumption is ~1.5 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.

2.3 Registering Your Instrument

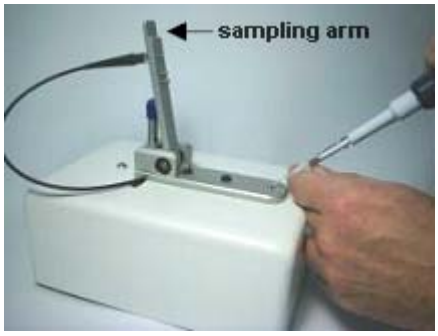
Please register your product! We periodically update our software and add new features free of charge. We would like to keep our user list updated so that we may alert you to these updates. All information supplied to NanoDrop Technologies is completely confidential. You can register at www.nanodrop.com

3 General Operation

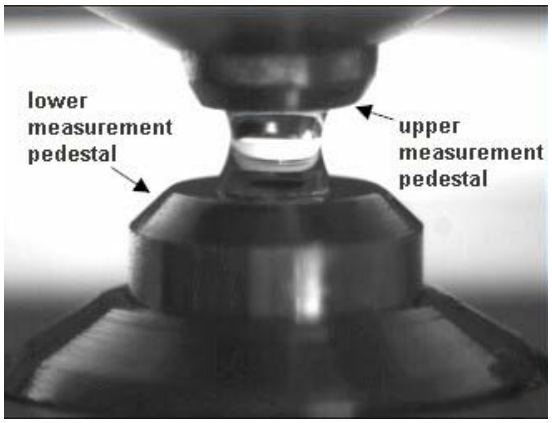
3.1 The Sample Retention System

3.1.1 Basic Use

The main steps for using the sample retention system are listed below.



1. With the sampling arm open, pipette the sample onto the lower measurement pedestal.



2. Close the sampling arm and initiate a spectral measurement using the operating software on the PC. The sample column is automatically drawn between the upper and lower measurement pedestals and the spectral measurement made.

3. When the measurement is complete, open the sampling arm and wipe the sample from both the upper and lower pedestals using a soft laboratory wipe. Simple wiping prevents sample carryover in successive measurements for samples varying by more than 1000 fold in concentration. See www.nanodrop.com for performance data on sample carryover

3.1.2 Cleaning the Sample Retention System

Upon completion of each sample measurement, wiping the sample from the upper and lower pedestals (as shown above) is sufficient to prevent sample carryover and avoid residue buildup.

However, after measuring a large number of samples, it may be necessary to clean the areas around the upper and lower pedestals thoroughly. This will prevent the wiping after each measurement from carrying previous samples onto the measurement pedestals and affecting low-level measurement. A final cleaning of all surfaces with de-ionized water is recommended prior to storing the instrument.

Decontamination of Measurement Pedestals

If decontamination is necessary, a 5.25% solution of sodium hypochlorite (bleach – freshly prepared) or other decontaminating solution 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 very resistant to most common laboratory solvents (see "Solvent Compatibility" appendix).

Special Cleaning Requirements for Proteins

Proteins and solutions containing surfactants can "un-condition" the measurement pedestal surfaces so that the liquid column does not form with 1ul samples. If this occurs, "buff" the measurement pedestal surfaces by rubbing each with a dry laboratory wipe 15-20 times. This will "re-condition" the surface allowing for the liquid sample column to form.

3.1.3 Sample Size Requirements

Although sample size is not critical, it is critical that the liquid column be formed so that the gap between the upper and lower measurement pedestals is bridged with sample, and the light path is completely covered by the sample.

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

- Aqueous solutions of nucleic acids: 1 ul
- Solutions containing significant amounts of dye: 2 ul
- Purified protein: 2 ul
- Bradford or BCA assay: 2ul
- Aqueous solution of cellular material: 1 ul

It is best to use a precision pipettor (0-2 microliters) with precision tips to assure that sufficient sample (1-2 microliters) is used. Lower precision pipettors (0-10 microliters and larger) are not as good for delivering 1 microliter to the measurement pedestal. If you are unsure about your sample characteristics or pipettor accuracy, a 2 ul sample is recommended.

3.1.4 Sample Carryover

Prevention of sample being retained on the ND-1000 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 data at www.nanodrop.com). This is possible since each measurement pedestal is in actuality a highly polished end of a fiber optic cable. There are no cracks or crevices for residual sample to reside in.

3.1.5 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 acid, such as resuspended nucleic acid preparations, are common examples known to the molecular biologist. Proteins are subject to denaturation, precipitation, and aggregation so they too have special handling requirements to ensure sample homogeneity.

3.1.6 Effect of Evaporation

Evaporation of the sample during measurement has a very small effect on results. Typically, this will account for 1-2% increase in sample concentration. This can be observed in the field by measuring the same sample successively over time.

3.1.7 Sample Recovery

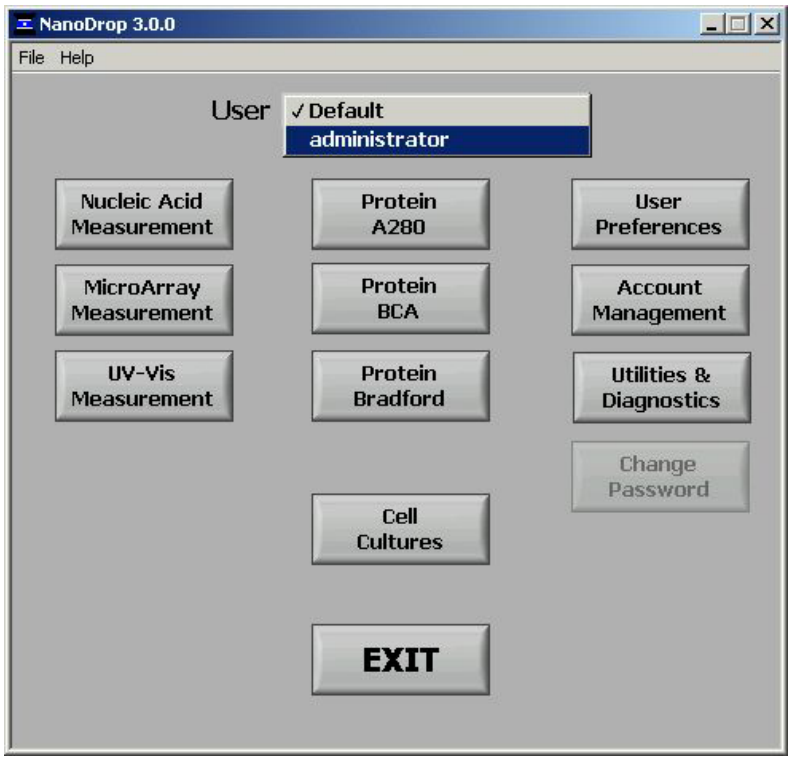
One of the advantages of the sample retention system is that the undiluted samples can actually be recovered from the upper and lower measurement pedestals by extracting with a pipette.

3.2 Software Architecture and Features

3.2.1 Main Menu

With the sampling arm in the down position, start the NanoDrop software by selecting the following path:

Start → Programs → NanoDrop → NanoDrop 3.0.1



3.2.2 Application Modules

This software has been tailored to meet the life scientist's needs. It includes the following application modules:

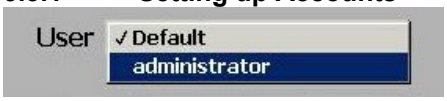
- **Nucleic Acid Measurement** – concentration and purity of nucleic acid
- **MicroArray Measurement** – dye incorporation concentration and purity of nucleic acid
- **General UV-Vis Measurement** – general UV-Vis measurements
- **Protein A280** – concentration and purity of purified protein
- **Protein BCA –** protein concentration using the BCA assay
- **Protein Bradford** – protein concentration using the Bradford assay
- **Cell Culture** – absorbance measurement of suspended cells

For a more detailed description of each module, refer to section 4.

3.3 Account Management

The Account Management module provides options for directing where specific data files are archived for users desiring to segregate their data into a specific folder. It allows existing NanoDrop® ND-1000 Spectrophotometer users, familiar with the existing archived data files, to operate as accustomed, enabling immediate access to and running of the software. The Account Management module is accessible to the Administrator only. The Change Password module can be accessed by any user having an authorized account ID.

3.3.1 Setting up Accounts

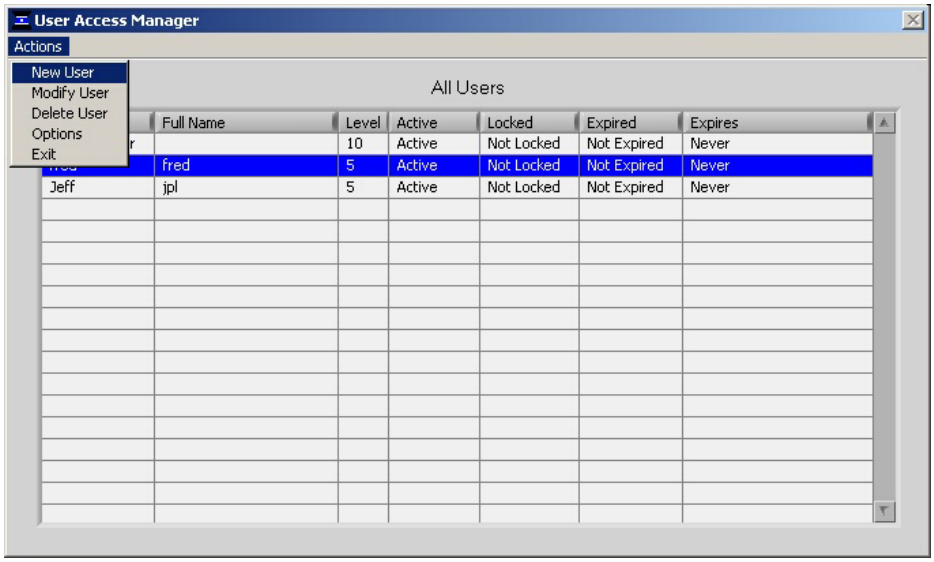


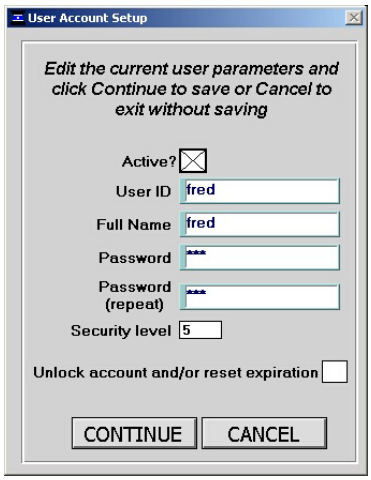
At the time of version 3.0.1 software installation, there are two specified users in the NanoDrop software. Others may be added (see Administrator below).

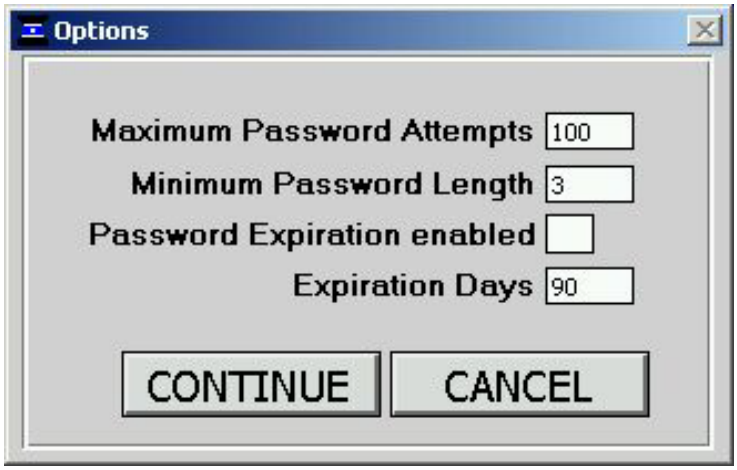
Administrator: the Administer has full access to all account settings and the highest security level in the NanoDrop software (level 10). At the time of software installation, only the Administrator can access the Account Management module. Through that software module, the Administrator can:

- Add New Users
- Modify a User
- Delete a User
- Set conditions for the accounts using the Options entry. The respective parameters within the Options menu are self-explanatory.

See examples of screens below:







Note: the initial Administrator's password is "nanodrop". It is strongly recommended that the Administrator change this password after initial account set up.

Default User: the Default setting enables any user to access all the active software measurement modules. It is not password protected. All data generated will be automatically archived into the "Default" folder within the NanoDrop data folder. Within the Default Folder are subfolders labeled with the respective name of the NanoDrop Application module used for measurement. Note: the Default user option is always functional even if all specific-user accounts, including the Administrator's, become locked.

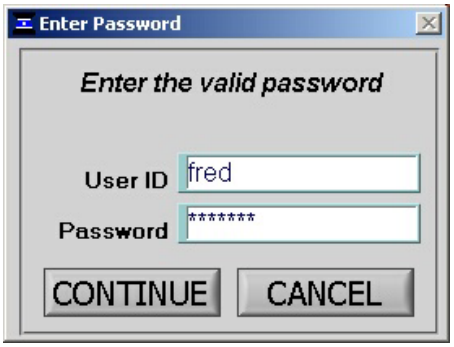
3.3.2 Account Levels

There are only two security levels in the current NanoDrop software: level 10 and everything else. Level 5 is the suggested level for a New User account. While the level can be set to 10, that isn't recommended since that would give another user full access to the Account Management module.

3.3.3 Account Log-in

1. The user may log into his account by clicking on the down arrow next to the User name on the main menu

2. This will bring up the enter password dialogue box below:



Note: The specific-user account ('fred' in the example above) will automatically revert back to the "Default" User after 4 hours.

If the user is using any of the Application modules, a screen indicating that the time is about to expire – with a 30-second countdown – will be displayed. If the user elects "CANCEL", the clock with re-set and the respective user-account and Application will remain active. If the time expires, the open Application module will close, returning to the 'Main Menu' and the "Default" user.

3.3.4 Account Log-out

The respective user's account will remain active until either the software 'System idle timeout' is exceeded or the user 'logs out'. The latter may be done by clicking on "Default" next to the User name on the main menu:

3.3.5 Account Lockout

User-specific accounts can become locked out in several ways, for example:

- 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) can be locked if the incorrect password entry described.

3.3.6 Password.log file

This file contains the respective User ID & password for all accounts. It is located within the Programs Folder under the NanoDrop 3.0.1 folder. It is strongly recommended that the Administrator make a copy of that file and store it in the same folder each time a New User account is added. If the Administrator's account becomes locked, the up-to-date copy of the password.log file can be renamed and used as the password.log file. Otherwise a blank password.log file can be obtained from the NanoDrop software CD or downloaded from www.nanodrop.com. In this case, all user accounts would need to be rebuilt by the administrator.

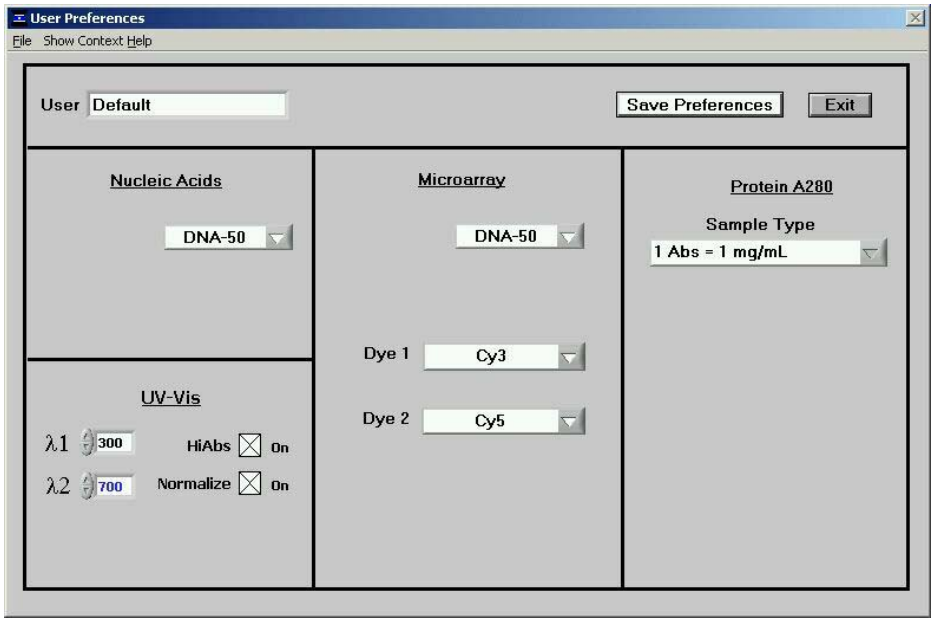
Note: If upgrading to V3.0.0 from V3.0.1, the "passwords.log" and "user preferences.log" files should be automatically copied to the proper directory for use with V3.0.1. If this occurs properly, then all the user accounts that were created with V3.0.0 will be present when running V3.0.1. If the accounts are not available, then "passwords.log" and "user preferences.log" files must be manually copied from the c:\program files\NanoDrop 3.0.0 folder to the c:\program files\NanoDrop 3.0.1 folder. Administrator privileges on the pc will be required for this.

3.4 User Preferences

Each respective user can select settings they most commonly use for four of the Application modules:

- Nucleic Acids
- MicroArray

- UV-Vis
- Protein A280



Once the settings are saved, they will be automatically opened when the respective user is logged-in to the NanoDrop software and any of the four Application modules are open.

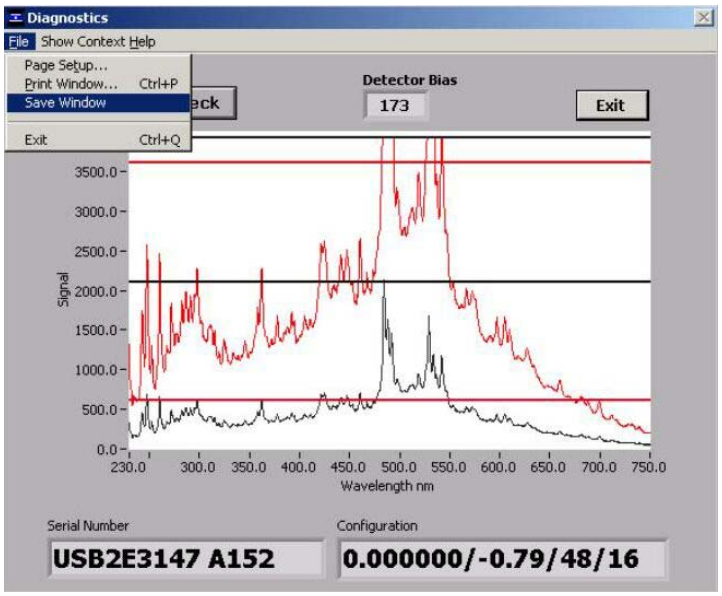
Note: user preferences are stored in a ".log" file. When upgrading to a newer version of the software, this file should be preserved. If after upgrading to a new software version the user preferences do not appear correctly, the .log file should be manually copied to the proper directory. See "Passwords.log" section for more detail.

3.5 Change Password

This module enables each user to change their respective password. Note: the Administrator, using the Options entry in the Account Management module, establishes whether User passwords will expire and if so, when (number of days).

3.6 Utilities and Diagnostics

This module is used to help troubleshoot operational problems with the instrument. By selecting "intensity check", a characteristic spectrum is displayed that gives clues to the performance of the instrument. For more information on using this module, refer to the "Troubleshooting" section of this manual.



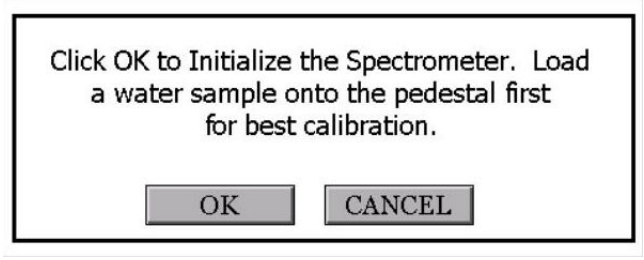
3.7

4 Applications Modules

4.1 Functions Common to Each Application Module

4.1.1 Module Startup

When the software starts, you should see this message:



For best results, **ensure measurement pedestal surfaces are clean** and load a water sample onto the lower measurement pedestal and then click "OK". After clicking OK, the messages **"Initializing Spectrometer- please wait**" and **"checking detector bias"** will appear. When these messages disappear, the instrument will be ready for use. All data taken will automatically be logged in the appropriate archive file.

4.1.2 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.

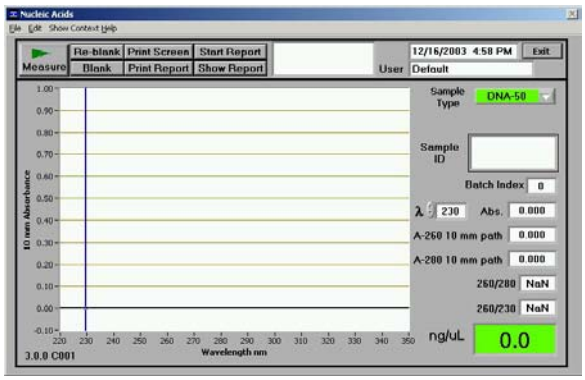
4.1.3 Measure (F1)

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.

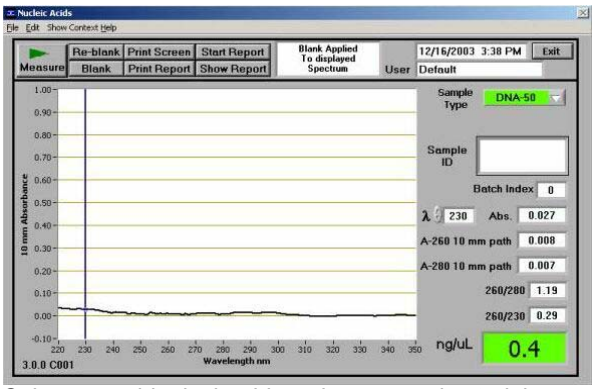
The "Measure" button is used to initiate the measurement sequence for all samples (non-Blanks). It is actuated by depressing the F1 key or clicking the "Measure" button. The entire measurement cycle takes approximately 10 seconds.

4.1.4 Blank (F3)

Before making a sample measurement, a blank must be measured and stored (see the Appendix "Blanking and Absorbance Calculations") for more details on absorbance calculations. After making an initial blank measurement, a straight line will appear on the screen, subsequent blanks will produce a nearly straight line that is very near zero (see images below).



Initial blank should produce a straight line



Subsequent blank should produce a nearly-straight curve that is very close to baseline

Blanking Cycle

For the most consistent results, it is best to begin any measurement session with a blanking cycle. This will assure the user that the instrument is working well and that any sample carryover from previous measurements is not a concern. To perform a blanking cycle, perform the following:

1. Load a blank sample (the buffer, solvent, or carrier liquid used with your samples) onto the lower measurement pedestal and lower the sampling arm into the "down" position.
2. Click on the "Blank" (F3) button in the Application module you have open.
3. Analyze a fresh replicate of the blanking solution as though it were a sample. This is done using the "Measure" button (F1). The result should be a spectrum that varies no more than 0.050 A (Nucleic Acids and Protein A280; 10mm path equivalent) or 0.005 A (all other Application modules; 1mm path) from the zero baseline.
4. Wipe the blank from both measurement pedestal surfaces with a laboratory wipe and repeat the process until the spectrum is within 0.005 A (1mm path).

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

4.1.5 Re-blank (F2)

The Re-blanking option (F2) establishes a new reference (blank) that is used for the absorbance calculations of subsequent samples. However, unlike the Blank (F3) function, the Re-blank feature recalculates the absorbance spectrum for the most recent sample and displays this on the screen. When the Re-blank function is used, the following message appears:

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

4.1.6 Print Screen (F4)

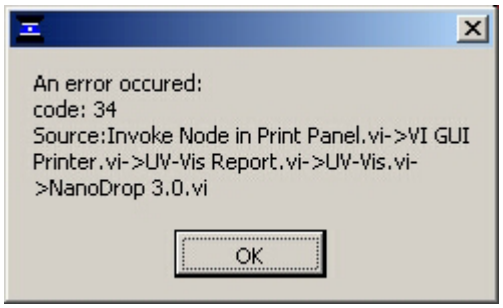
The "Print Screen" button will print a copy of the current operating screen to the default printer attached to the operating PC. (Note: The system is configured to work with the Dymo LabelWriter 330 printing on #30256 [2-5/16" X 4"] shipping labels, but can print on any printer connected to the PC.)

4.1.7 Start Report (F6)

The user can log up to 32 measurement results and print them to the desired printer. To initiate this feature, select the "Start Report" button. Once selected, this button will read "Recording" and will stay in this state until deselected. Once selected, a 'Sample Report' will automatically print to the Default printer when the maximum number of entries for that specific report has been reached. The 'Batch Index' number will automatically reset, the entries in the 'Sample Report' will be cleared with the next sample measured, and the respective parameters will begin to populate the next 'Sample Report'.

4.1.8 Print Report (F5)

Selecting the 'Print Report' (F5) button will print the existing 'Sample Report' on the default printer if there are any samples in the 'Sample Report'. It does not clear the 'Sample Report' contents. Note that the batch logging holds a maximum of 32 samples. A 'Sample Report' will automatically print to the Default printer when the maximum number of entries for that specific report has been reached. If the PC is not connected to a printer, you will see the following error message:



This is a non-fatal error and will not cause the software to shut down. (Note: The system is configured to work with the Dymo LabelWriter 330 printing on #30256 [2-5/16" X 4"] shipping labels, but can print on any printer connected to the PC.)

4.1.9 Show Report (F7)

At any time, the user can display the entries comprising the current 'Sample Report' by selecting the 'Show Report' button. Descriptive parameters specific for the individual Application modules are populated for each individual Sample ID.

There are three options within the 'Show Report' window:

- Save – allows the user to save the existing report.
- Print – prints current report to default printer.
- Exit – return to the specific Application module.

4.1.10 Sample ID

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. This makes the software barcode reader compatible.

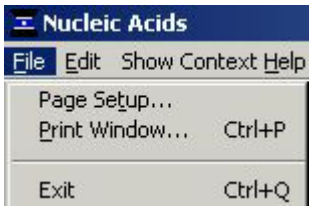
4.1.11 Batch Index

The 'Batch Index' indicator is activated when a 'Sample Report' is being recorded. It indicates the sample number of the last sample processed in the current batch and increments with each successive measurement until the 'Sample Report' is fully populated. The next sample measured,

after the current "Sample Report" is complete, will automatically reset the Batch Index number, clear the entries in the "Sample Report", and will begin to populate the next "Sample Report".

4.1.12 Printing via the Print Menu

A Print dialogue can be initiated from the "File" pull-down menu accessible within all modules.



The Print Window option and 'Ctrl P' enable selection of printers available to the respective user.

4.1.13 Exit

The command for closing all Applications and supporting options. After clicking the "Exit" button, the user has 10 seconds to cancel the exit command. If no action is taken after 10 seconds, the exit command is carried out. Note that all measurement data is automatically saved to an archive file and requires no user action.

4.1.14 Context Help

'Context Help' is enabled in the Main Menu, all function modules, and the Application modules. The help feature is enabled by choosing "Show Context Help" from the "Help" menu or by selecting "Ctrl H". Once enabled, placing the cursor on elements of the screen will automatically generate an explanation of that element. "Context Help" remains active until the user deselects it.

4.2 Nucleic Acid Measurement Module

Nucleic acid samples can be readily checked for concentration and quality using the NanoDrop® ND-1000 Spectrophotometer. To measure nucleic acid samples select the "Nucleic Acid" Application module.

4.2.1 Sample Volume Requirements

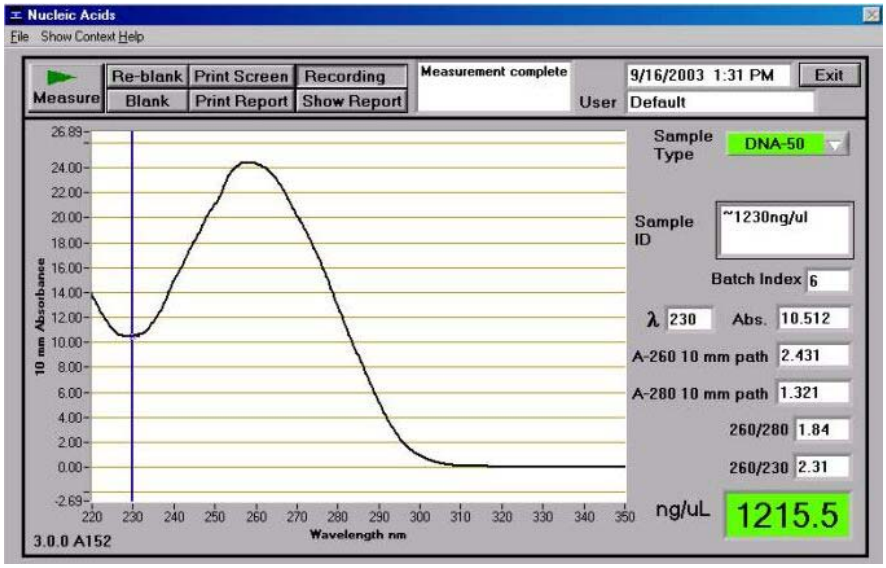
Field experience has indicated that 1ul samples are sufficient to ensure accurate and reproducible results when measuring aqueous nucleic acid samples. However, if you are unsure about your sample or your pipettor accuracy, a 1.5-2ul sample is recommended to ensure that the liquid sample column is formed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

4.2.2 Measurement Concentration Range

The NanoDrop® ND-1000 Spectrophotometer will accurately measure nucleic acid samples up to 3700 ng/ul without dilution. To do this, the instrument automatically detects the high concentration and utilizes the 0.2mm pathlength to calculate the absorbance.

Detection Limit (ng/ul)	Approximate Upper Limit (ng/ul)	Typical Reproducibility (minimum 5 replicates) (SD= ng/ul; CV= %)
1.5	3700 (dsDNA) 3000 (RNA) 2400 (ssDNA)	sample range 1.5-100 ng/ul: ±1.5 ng/ul sample range >100 ng/ul: ± 2%

4.2.3 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 or "Other" for other nucleic acids. The default is DNA-50. If "other" is selected, the user can select an analysis constant between 15-150. See the "Concentration Calculation (Beer's Law)" Appendix for more details on this calculation.

λ and **Abs:** the user selected wavelength and corresponding absorbance. The wavelength can be selected by moving the cursor or using the up/down arrows to the left of the wavelength box. 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 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 represented as if measured with a 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 and 280 nm. The ratio of absorbance at 260 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" section of the Troubleshooting section for more details on factors that can affect this ratio.

260/230: ratio of sample absorbance at 260 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.

ng/ul: sample concentration in ng/ul based on absorbance at 260 nm and the selected analysis constant.

4.2.4 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.

4.3 MicroArray Measurement Module

The capability to pre-select viable fluorescent-tagged hybridization probes for gene expression in microarrays can eliminate potentially flawed samples and improve research effectiveness. The NanoDrop software facilitates the measurement of DNA concentration and dye labeling effectiveness. The NanoDrop® ND-1000 Spectrophotometer measures the absorbance of the fluorescent dye, allowing detection at dye concentration as low as 0.2 picomole per microliter.

4.3.1 Sample Volume Requirements

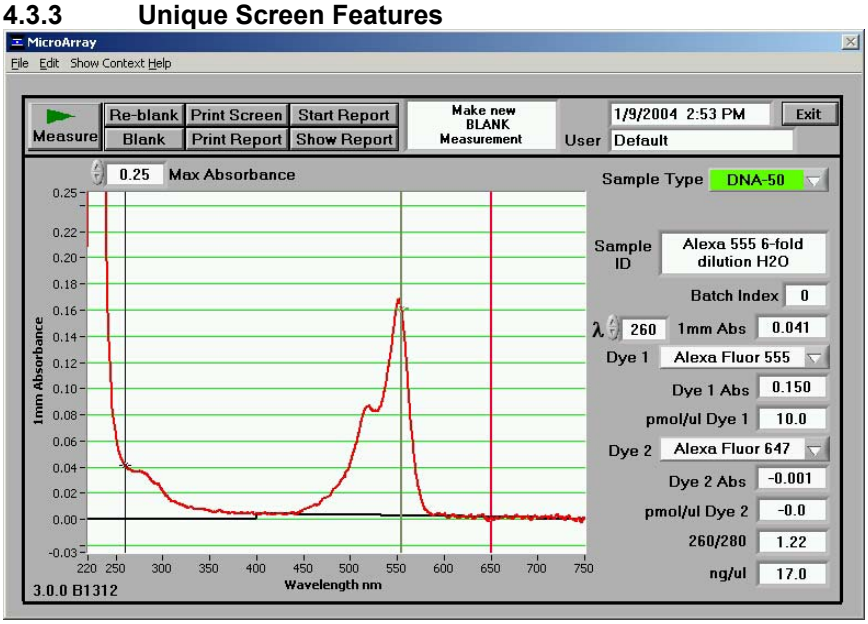
Field experience has indicated that 1 ul samples are sufficient to ensure accurate and reproducible results when measuring aqueous nucleic acid samples containing incorporated fluorescent dye. However, if you are unsure about your sample or your pipettor accuracy, a 1.5-2ul sample is recommended to ensure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

4.3.2 Measurement Concentration Range

The NanoDrop® ND-1000 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)	Approximate Upper Limit (pmol/ul)	Typical Reproducibility (minimum 5 replicates) (SD= pmol/ul; CV= %)
Cy3, Cy3.5, Alexa Fluor 555 and Alexa Fluor 660	0.20	100	sample range 0.20-4.0 pmol/ul: ± 0.20 pmol/ul sample range >4.0 pmol/ul: ± 2%
Cy5, Cy5.5 and Alexa Fluor 647	0.12	60	sample range 0.12-2.4 pmol/ul: ± 0.12 pmol/ul sample range >2.4 pmol/ul: ± 2%
Alexa Fluor 488 and Alexa Fluor 594	0.40	215	sample range 0.40-8.0 pmol/ul: ± 0.40 pmol/ul sample range >8.0 pmol/ul: ± 2%
Alexa Fluor 546	0.30	145	sample range 0.30-6.0 pmol/ul: ± 0.30 pmol/ul sample range >6.0 pmol/ul: ± 2%

4.3.3 Unique Screen Features



Max Absorbance: used to rescale the upper limit of the vertical axis.

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 or "Other" for other nucleic acids. The default is DNA-50. If "other" is selected, the user can select an analysis constant between 15-150. See the "Concentration Calculation (Beer's Law)" Appendix for more details on this calculation.

λ and **Abs:** the user selected wavelength (black cursor) and corresponding absorbance. The wavelength can be selected by dragging the black cursor or using the up/down arrows to the left of the wavelength box. Note: the user-selected wavelength and absorbance are not utilized in any calculations.

Dye 1: user selected dye

1mm Dye 1 Abs: measured absorbance of Dye 1

pmol/ul Dye1: concentration based upon Dye 1's extinction coefficient. See the "Concentration Calculation (Beer's Law)" Appendix for more details on this calculation.

Dye 2: user selected dye

1mm Dye 2 Abs: measured 1mm pathlength absorbance of Dye 2

pmol/ul Dye2: concentration based upon Dye 2's extinction coefficient. See the "Concentration Calculation (Beer's Law)" Appendix for more details on this calculation.

ng/ul: concentration of nucleic acids in the sample based on absorbance at 260 nm and the nucleic acid analysis constant and normalized at 340 nm.

260/280: ratio of sample absorbance at 260 and 280 nm. The ratio of absorbance at 260 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" section of the Troubleshooting section for more details on factors that can affect this ratio.

4.3.4 Baseline Calculation

The software will automatically calculate a baseline between 400 and 700 nm. The green vertical line on the screen represents the peak wavelength position for Dye 1, and the red vertical line represents the peak wavelength position for Dye 2.

4.3.5 Fluorescent Dye Selection

Eight additional fluorescent dyes have been added to the original Cy3 and Cy5 (cyanine) dyes. They can be selected using the scroll arrows or by highlighting the respective Dye 1 and Dye 2 boxes. The respective Absorbance (excitation) wavelength and extinction coefficient will automatically be utilized for measurement and subsequent concentration calculation of the respective dye.

Note: If upgrading to V3.0.1 from V3.0.0, the "dye list.log" file should be automatically copied to the proper directory for use with V3.0.1. If this occurs properly, then all the user defined dyes that were created with V3.0.0 will be present when running V3.0.1. If the user-defined dyes are not available, then the "dye list.log" file must be manually copied from the c:\program files\NanoDrop 3.0.0 folder to the c:\program files\NanoDrop 3.0.1 folder. Administrator privileges on the pc will be required for this.

4.4 UV-VIS Measurement Module

The "UV/VIS Absorbance" tab allows the NanoDrop® ND-1000 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.

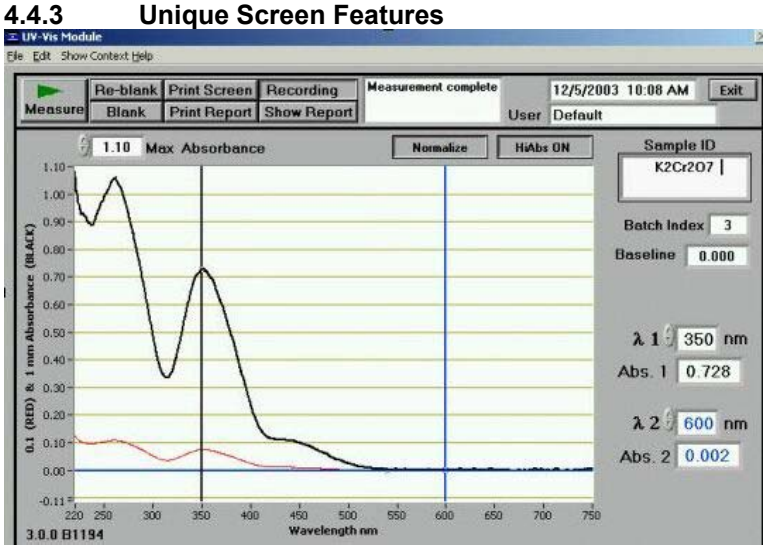
4.4.1 Sample Volume Requirements

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

4.4.2 Measurement Concentration Range

All ND-1000 spectrophotometers will measure absorbances up to the 10mm pathlength equivalent of 15 A. ND-1000s with serial number >500 or that have been field retrofitted, can utilize the short path length (0.2mm) which enables the 10mm pathlength equivalent of 75 A.

4.4.3 Unique Screen Features



λ1/Abs1 and **λ2/Abs2**: current values of the user-selectable wavelength cursors and corresponding absorbances. The wavelengths can be set by dragging the cursor, using the up/down arrows or typing in the desired wavelength.

Baseline: the absorbance of the user selectable-baseline (horizontal) cursor. The user may drag this cursor to a new vertical position to create a new baseline. The absorbance value of the baseline is subtracted from the absorbance of the spectrum.

Max Absorbance: used to rescale the upper limit of the vertical axis.

Hi Abs: samples with high absorbance (up to 75 A equivalent @ 10 mm path) can be measured directly (applicable for instruments with serial number >500 only or that have been field-retrofitted). This capability is selected by choosing the "Hi Spec" button on the header bar. When this is selected, the absorbance is measured using the short path (0.2mm) and plotted as a red line as shown above. Sample ID label will be stored with sample data in the file folder "C:\NanoDrop Data\Username\UV-Vis HiAbs".

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

4.5 Protein A280 Module

Proteins, unlike nucleic acids, can exhibit considerable diversity. The A280 method is applicable to purified proteins exhibiting absorbance at 280nm. It requires no prior calibration 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 is the only other NanoDrop software module that displays and records 10 mm (1 cm) equivalent data on the screen and in the archived data file.

4.5.1 Sample Volume Requirements

Some proteins are hydrophobic and others hydrophilic giving rise to variable surface tension in the sample to be measured. Additionally the presence of surfactants or detergents in reagents, such as the Bradford reagent, can significantly alter surface tension. That occurrence can be overcome without

affecting the sample's absorbance by using a larger sample volume. **A 2 ul sample size is recommended for protein measurement**.

4.5.2 Special Cleaning Requirements for Proteins

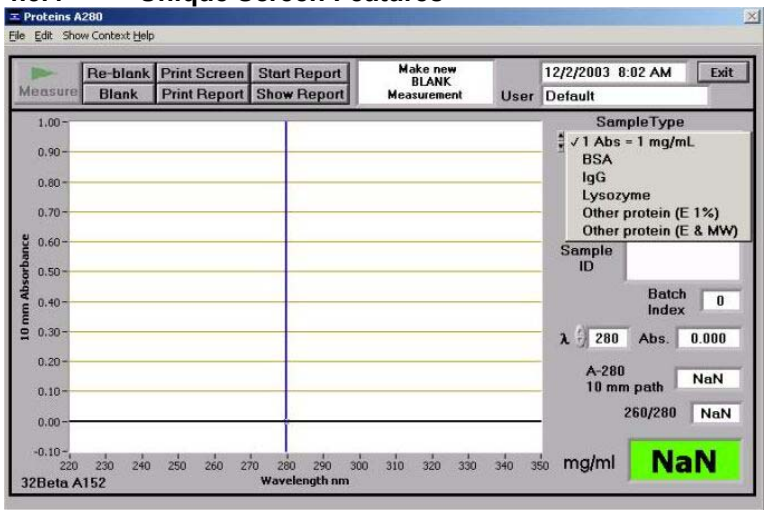
Proteins and solutions containing surfactants are known to "un-condition" the measurement pedestal surfaces so that the liquid column does not form. If this occurs, "buff" the measurement pedestal surfaces by rubbing each with a dry laboratory wipe 15-20 times. This will "re-condition" the surface allowing for the liquid sample column to form.

4.5.3 Measurement Concentration Range

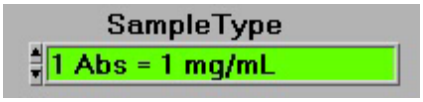
The NanoDrop® ND-1000 Spectrophotometer will accurately measure protein samples up to 100 mg/ml (BSA) without dilution. To do this, the instrument automatically detects the high concentration and utilizes the 0.2mm pathlength to calculate the absorbance. A table of concentration range and typical reproducibility is listed below.

Sample Type	Detection Limit	Approximate Upper Limit	Typical Reproducibility (minimum 5 replicates) (SD= mg/ml; CV= %)
Purified BSA	0.10 mg/ml	100 mg/ml	sample range 0.05-10 mg/ml: ± 0.10 mg/ml sample range >10mg/ml: ± 2%

4.5.4 Unique Screen Features



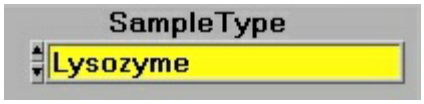
Sample Type: There are six sample types (options) available for purified protein analysis and concentration measurement. All of the options can be viewed by clicking the mouse while it is positioned within the Sample Type box. The sample type (color-keyed) can be selected by clicking on the preferred option or by scrolling through the selections using the up or down arrow keys located to the left of the sample type box. A description of each sample type is given below.



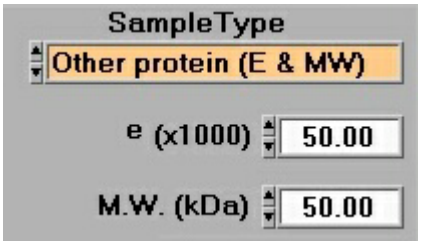
A general reference setting based on a 0.1% (1 mg/ml) protein solution producing an Absorbance at 280 nm of 1.0 A (where the pathlength is 10 mm or 1 cm).

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.

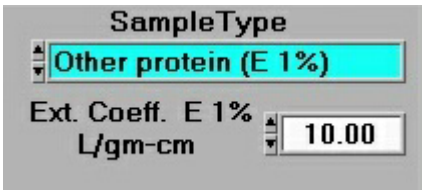
IgG reference. Unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (10 mg/ml) IgG solution.



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.

λ and A: current value of the user-selectable wavelength cursor and corresponding absorbance. The wavelength can be set by dragging the cursor, 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 and 280 nm

4.5.5 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.

4.6 Protein BCA Module

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 Standard Curve generation each time it is run, before unknown proteins can be measured. The resulting Cu-BCA chelate formed in the presence of protein is measured at its wavelength maximum of 562 nm. Pre-formulated reagents of BCA and $CuSO_4$, utilized in the assay, are available in kit form from numerous manufacturers. Follow their recommendations when mixing the respective reagents at the time the assay is to be performed.

4.6.1 Sample Volume Requirements

Some proteins are hydrophobic and others hydrophilic giving rise to variable surface tension in the sample to be measured. Additionally the presence of surfactants or detergents in reagents, such as the Bradford reagent, can significantly alter surface tension. That occurrence can be overcome without affecting the sample's absorbance by using a larger sample volume. **A 2ul sample size is recommended for protein measurement**.

4.6.2 Special Cleaning Requirements for Proteins

Proteins and solutions containing surfactants are known to "un-condition" the measurement pedestal surfaces so that the liquid column does not form. If this occurs, "buff" the measurement pedestal surfaces by rubbing each with a dry laboratory wipe 15-20 times. This will "re-condition" the surface allowing for the liquid sample column to form.

4.6.3 Measurement Concentration Range

On the NanoDrop® ND-1000 Spectrophotometer, the regular BCA assay can run from ~0.20 mg/ml up to 8.0 mg/ml. A mini-BCA assay covers an approximate range of 0.01 – 0.20 mg/ml.

Assay Type	Approximate Lower Limit (mg/ml)	Approximate Upper Limit (mg/ml)	Typical Reproducibility (minimum 5 replicates) (SD= mg/ml; CV= %)
Regular BCA	0.2	8.0	± 2% (over entire range)
Mini BCA	0.01	0.20	± 0.01 mg/ml (over entire range)

4.6.4 BCA Kits, Protocols, and Sample Preparation

Commercial BCA Protein kit manufacturers typically outline procedures for two different protein concentration ranges:

- A regular assay – using a 20:1 reagent / sample volume ratio. To accurately prepare Standards, we suggest using a minimum sample volume of 4 ul in 80 ul of BCA reagent (larger sample volume is preferable).
- A mini assay – using a 1:1 reagent / sample volume ratio. To prepare sufficient volume of these 1:1 mixtures, we suggest using a minimum of 10 ul of sample and 10 ul of BCA reagent in a PCR tube . Using the same pipettor for both volumes will eliminate any pipette-to-pipette accuracy differences. **Note**: If you run the assay at 60°C, doubling the volumes may afford greater insurance against skewed results from evaporation / condensation within the sealed reaction tube.

In addition to the kit reagents, protein standards (BSA) for generating a Standard Curve are also provided for the BCA method by the manufacturer. 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 (mg/ml) range of interest. Note: since the ND-1000 can measure higher protein concentrations, you may need to supply your own protein standards at higher concentrations than provided by the manufacturer.

4.6.5 Unique Screen Features

View Standard Curve (F8): selecting this button allows the user to view the Standard Curve at any time.

Sample Type: choices are Reference, Standards 1-5, and Sample. The software will guide you to measure your Reference, then at least one Standard before allowing measurement of samples.

Replicate #: counter for tracking replicate number during Reference and Standard measurement.

Reset Window (F11): clears all replicates of all standards.

Reset This Std (F12): clears all replicates of the selected standard.

Absorbance at 562nm: the Cu-BCA complex's absorbance at 562 nm.

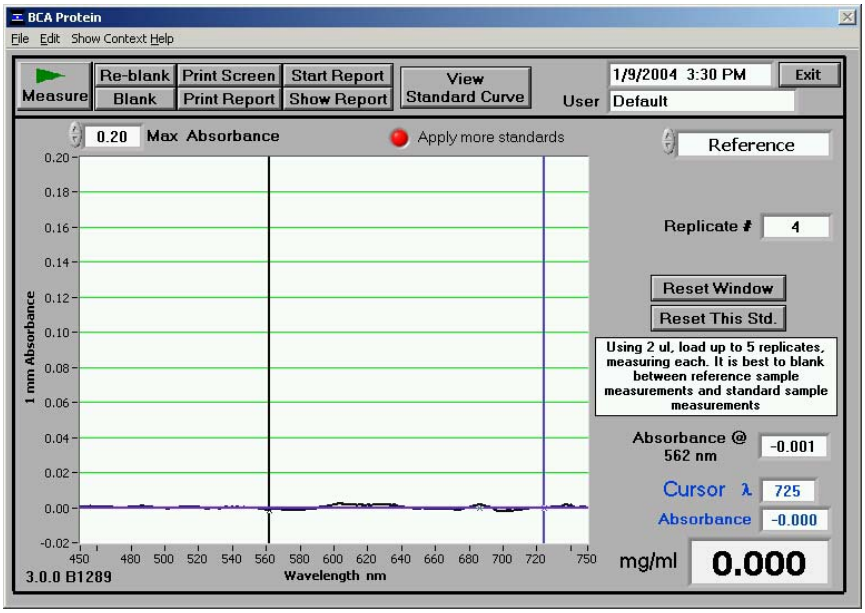
Cursor λ and Absorbance: this feature allows the user to adjust the cursor wavelength and view the corresponding absorbance. The cursor wavelength can only be selected by dragging the cursor. Note: the user-selected wavelength and absorbance are not utilized in any calculations.

mg/ml: the concentration of the sample (unknown).

4.6.6 Making BCA Measurements

In keeping with kit manufacturers' guidelines, generation of a Standard Curve is required every time the BCA Assay is run. Single or Multi-point standard curve generation is 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 allows a maximum of five (5) replicates for each of five (5) different Standards. There is no set order in which Standards must be run.

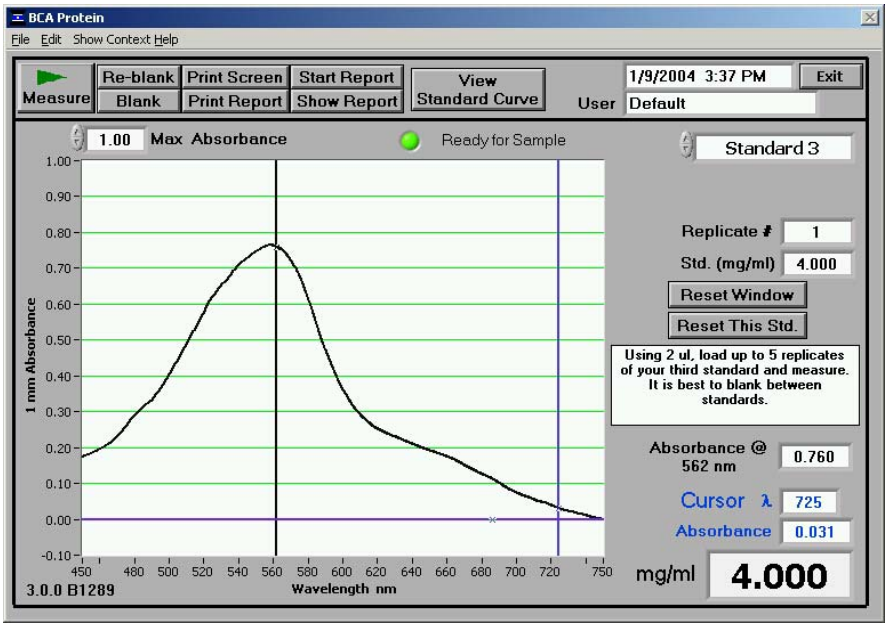
Using NanoDrop version 3.0 software, there are only three (3) general procedural steps to unknown protein concentration measurement. The requisite order, including generating the standard curve, is as follows:



Step 1: Measure the "Reference" (BCA reagent – a 'zero' Standard).

The software will not allow measurement of Samples until at least 1 Standard and Reference – or 2 Standards – are measured.

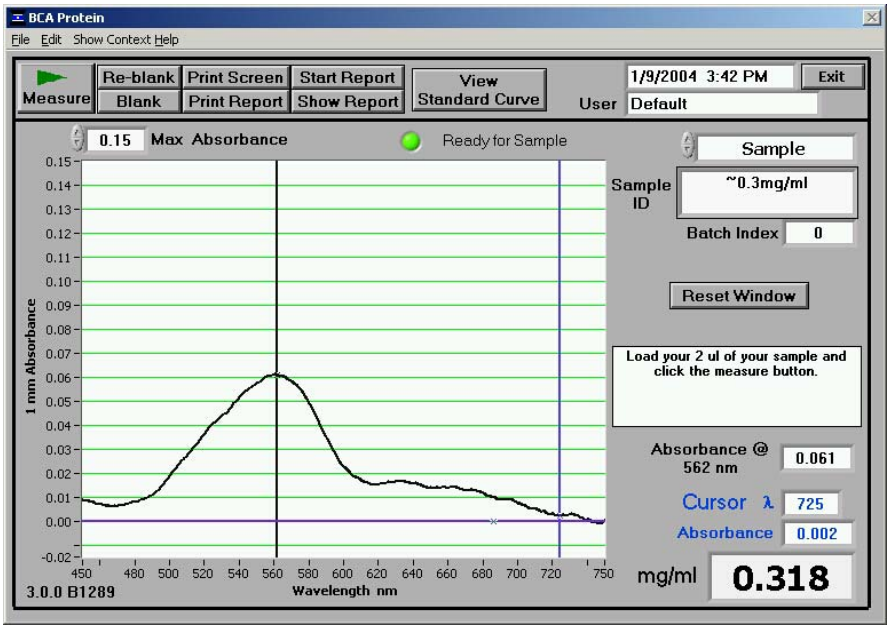
Note: software will guide the user with instructions in the large text box on the right side of the screen. The red light indicates the standard curve is incomplete and not yet ready for sample measurements.



Step 2: Measure Standards

Up to 5 replicates of each standard can be measured. The software will not allow measurement of Samples until at least 1 Standard and Reference – or 2 Standards – are measured.

If erroneous or non-representative readings are encountered for a specific standard, all replicates of that standard are cleared by selecting "Reset this Std" (F12). Additionally, all Standards can be deleted at once using the "Reset This Window" (F11) button.

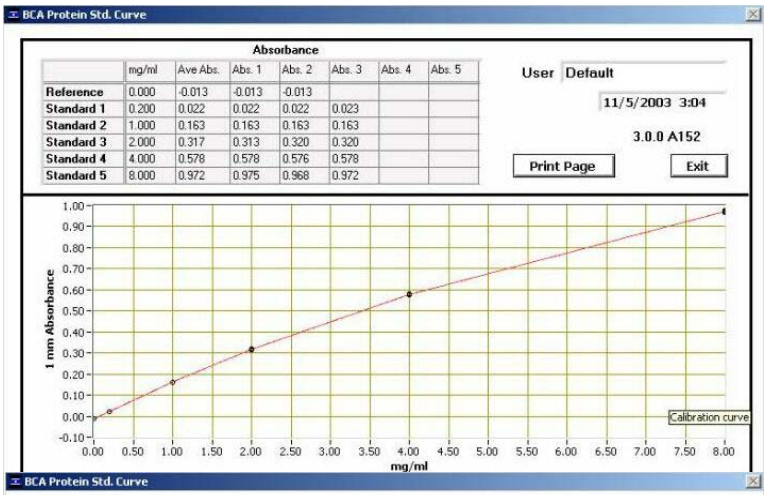


Step 3: Measure Samples

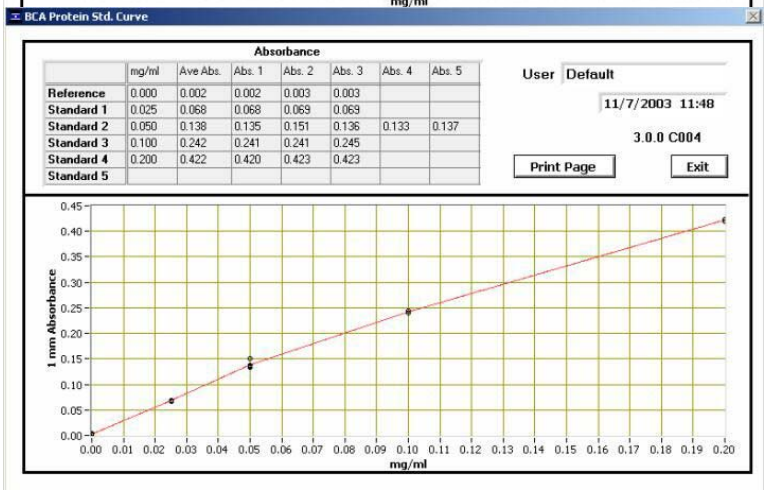
Once a minimum Standard curve has been established, the **red** indicator light will turn **green**, allowing the user to either start measuring samples. Sample concentrations are calculated using linear interpolation (point-to-point) between the two Standards flanking the unknown Sample. [Note: In order to obtain a concentration value (mg/ml) the sample (unknown) must fall within the limits of the Standard curve.

4.6.7 Standard Curve Features

Unlike the Bradford Protein assay, the BCA Protein Assay (562 nm) is much more linear throughout the assay's range and has approximately 10-fold more signal available. A representative standard curve is shown. The Standard Curve can be printed but not saved for later use since its utility is limited to the specific time period in which it and the respective unknowns are assayed.



Regular BCA Standard Curve: 0.2 – 8.0 mg/ml



mini-BCA Standard Curve: 0.01 – 0.20 mg/ml

4.6.8 Exiting the BCA Module

Be sure not to Exit the BCA software module until you've processed all the unknowns to be assayed! The Standard Curve is automatically deleted when the software is closed and cannot be recalled.

4.7 Protein Bradford Module

The Bradford Assay is a second alternative method commonly utilized 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 requires Standard Curve generation each time the assay is run, before unknown proteins can be measured.

The Bradford uses the protein-induced Absorbance shift of Coomassie Blue dye to 595 nm as a measure of protein concentration. 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 & Samples (unknowns) ensuring they are subjected to the identical conditions and timing throughout the assay.

4.7.1 Sample Volume Requirement

Some proteins are hydrophobic and others hydrophilic giving rise to variable surface tension in the sample to be measured. Additionally the presence of surfactants or detergents in reagents, such as the Bradford reagent, can significantly alter surface tension. That occurrence can be overcome without

affecting the sample's absorbance by using a larger sample volume. **A 2 ul sample size is recommended for protein measurement**.

4.7.2 Special Cleaning Requirements for Proteins

Proteins and solutions containing surfactants are known to "un-condition" the measurement pedestal surfaces so that the liquid column does not form. If this occurs, "buff" the measurement pedestal surfaces by rubbing each with a dry laboratory wipe 15-20 times. This will "re-condition" the surface allowing for the liquid sample column to form.

4.7.3 Measurement Concentration Range

On the NanoDrop® ND-1000 Spectrophotometer using the regular Bradford assay, unknown protein concentrations from ~100ug/ml up to several thousand micrograms/ml (ug/ml) can be determined. The best linearity is in the 100 – 1000ug/ml range. A mini-Bradford assay covers the approximate range of 15 to 125ug/ml.

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.

Assay Type	Approximate Lower Limit (ug/ml)	Approximate Upper Limit (ug/ml)	Typical Reproducibility (minimum 5 replicates) (SD= ug/ml; CV= %)
Regular Bradford	100 ug/ml	8000 ug/ml	sample range 100-500 ug/ml: ± 25 ug/ml sample range 500-8000 ug/ml: ± 5%
Mini Bradford	15 ug/ml	125 ug/ml	sample range 15-50 ug/ml: ± 4 ug/ml sample range 50-125 ug/ml: ± 5%

4.7.4 Bradford Kits, Protocols, and Sample Preparation

Commercial Bradford Protein kit manufacturers typically outline procedures for two different concentration ranges:

- A regular assay – using a 50:1 reagent / sample volume ratio. To accurately prepare Standards, we suggest using a minimum sample volume of 4 ul in 200 ul of Bradford reagent (larger sample volume is preferable).
- A mini assay– using a 1:1 reagent / sample volume ratio. To prepare sufficient volume of these 1:1 mixtures, we suggest using a minimum of 10 ul of sample and 10 ul of Bradford reagent in a PCR tube. Using the same pipettor for both volumes will eliminate any pipette-to-pipette accuracy differences.

In addition to the kit reagents, protein standards (e.g., BSA) for generating a Standard Curve are provided by the manufacturer. Follow the manufacturer's recommendation using Standard (BSA) dilutions that cover the analytical (ug/ml) range of interest. [Note: since the ND-1000 Spectrophotometer can measure higher protein concentrations, you may need to supply your own protein standards at higher concentrations than provided by the manufacturer].

4.7.5 Unique Screen Features

View Standard Curve (F8): selecting this button allows the user to view the Standard Curve at any time.

Sample Type: choices are Reference, Standards 1-5, and Sample. The software will guide you to measure your Reference, then at least one Standard before allowing measurement of samples.

Replicate #: counter for tracking replicate number during Reference and Standard measurement.

Reset Window (F11): clears all replicates of all standards.

Reset This Std (F12): clears all replicates of the selected standard.

Absorbance at 595nm: protein-dye complex's absorbance at 595 nm.

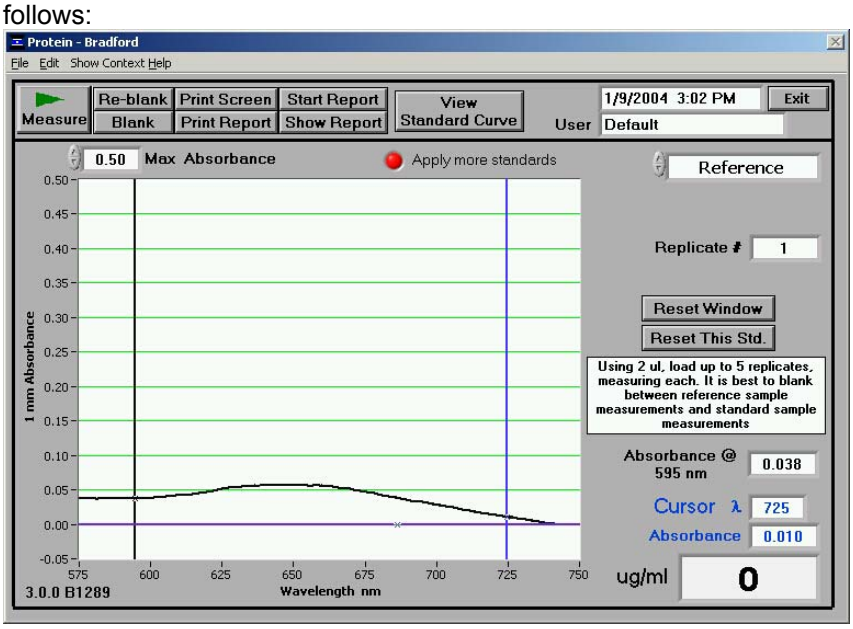
Cursor λ and Absorbance: this feature allows the user to adjust the cursor wavelength and view the corresponding absorbance. The cursor wavelength can only be selected by dragging the cursor. Note: the user-selected wavelength and absorbance are not utilized in any calculations.

ug/ml: concentration of the sample (unknown).

4.7.6 Making Bradford Protein Measurements

In keeping with kit manufacturers' guidelines, a Standard Curve is required every time the Bradford Assay is run. Single or Multi-point calibration is incorporated into the software. An operable Standard Curve can be developed using a single replicate of the Reference (Bradford reagent only – no protein) and a single replicate of one Standard. The multi-point calibration allows a maximum of five (5) replicates for each of five (5) different Standards. There is no set order in which Standards must be run.

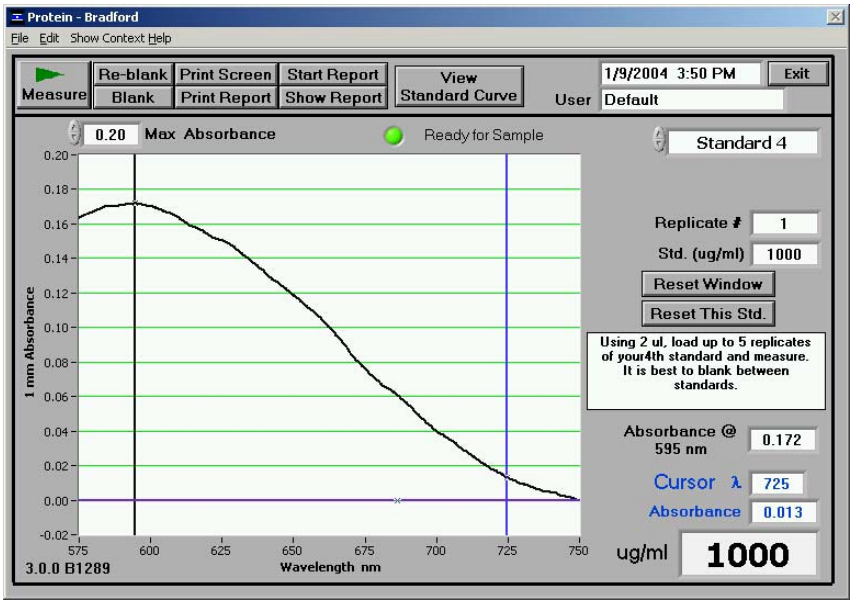
Using NanoDrop version 3.0.1 software, there are only three (3) general procedural steps to unknown protein concentration measurement. The requisite order, including generating the standard curve, is as follows:



Step 1: Measure the "Reference" (Bradford reagent – a 'zero' Standard).

The software will not allow measurement of Samples until at least 1 Standard and Reference – or 2 Standards – are measured.

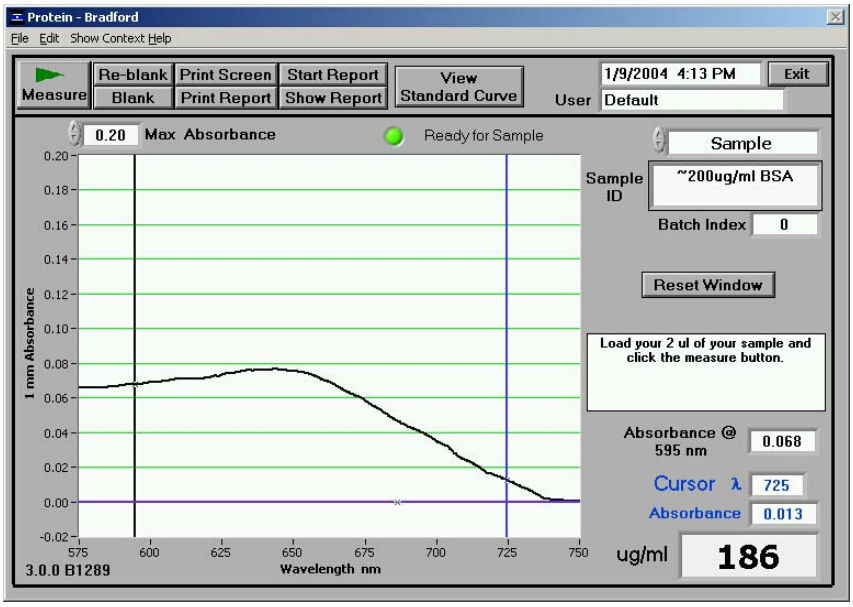
Note: software will guide the user with instructions in the large text box on the right side of the screen. The red light indicates the standard curve is incomplete and not yet ready for sample measurements.



Step 2: Measure Standards

Up to 5 replicates of each standard can be measured. The software will not allow measurement of Samples until at least 1 Standard and Reference – or 2 Standards – are measured.

If erroneous or non-representative readings are encountered for a specific standard, all replicates of that standard are cleared by selecting "Reset this Std" (F12). Additionally, all Standards can be deleted at once using the "Reset Window" (F11) button.

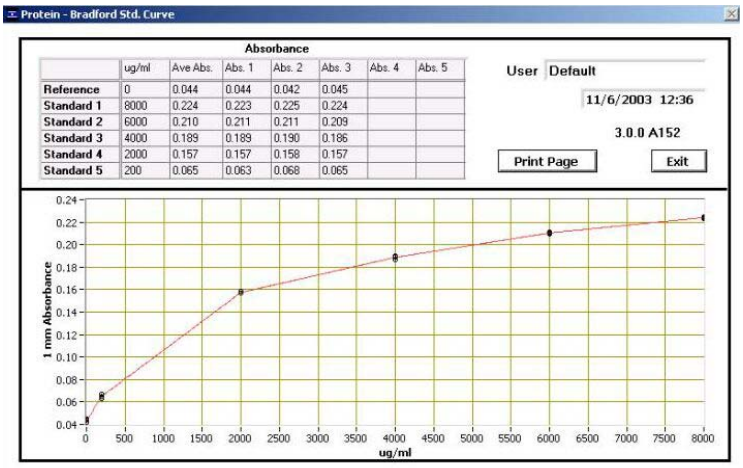


Step 3: Measure Sample

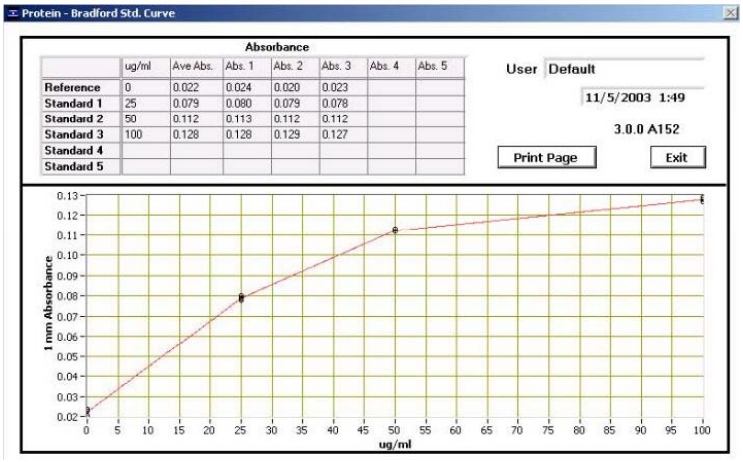
Once a minimum Standard curve has been established, the **red** indicator light will turn **green**, allowing the user to either start measuring samples. Sample concentrations are calculated using linear interpolation (point-to-point) between the two Standards flanking the unknown Sample. [Note: In order to obtain a concentration value (ug/ml) the sample (unknown) must fall within the limits of the Standard curve.

4.7.7 Standard Curve Features

Selecting this button allows the user to view the Standard Curve at any time. The respective Standard Curve can be printed but not saved for later use since its utility is limited to the specific time period in which it and the respective unknowns are assayed.



Regular Bradford curve covers 200-8000 ug/ml. Note the linear range is 100-1000 ug/ml



A mini-Bradford assay covers an approximate range of 15-100 ug/ml.

4.7.8 Exiting the Bradford Module

Be sure not to Exit the Bradford software module until you've processed all the unknowns to be assayed! The Standard Curve is automatically deleted when the software is closed and cannot be recalled.

4.8 Cell Cultures Module

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 differences amongst the optical systems of the numerous spectrophotometer designs.

The "Cell Cultures" module displays the sample spectrum from 250 nm to 700 nm. One cursor is fixed at the frequently used wavelength for monitoring cell suspensions (600nm) while the second cursor can be set to the wavelength of interest.

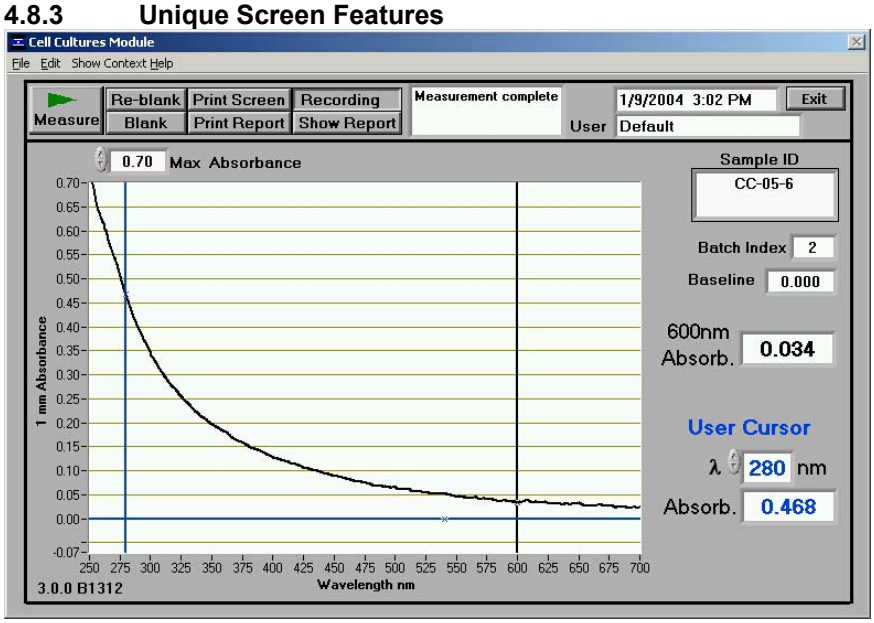
4.8.1 Sample Size Requirements

Field experience has indicated that 1 ul samples are sufficient to ensure accurate and reproducible results when measuring aqueous samples. However, if you are unsure about your sample composition 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 completely covered by sample.

4.8.2 Cell suspension Concentrations

Due to its shorter pathlength, the ND-1000 can measure absorbances that are 10-fold higher than those measurable on a standard cuvette spectrophotometer. This makes it possible to directly monitor concentrated cell suspensions. Since the entire spectrum is displayed, diluted samples exhibiting very low 'Absorbance' at 600 nm can be monitored at lower wavelengths, for example 280 nm.

4.8.3 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.

λ and Absorb.: current value of the user-selectable wavelength cursor and corresponding absorbance. The wavelength can be set by dragging the cursor, using the up/down arrows or typing in the desired wavelength. Note: the user-selected wavelength and absorbance are not utilized in any calculations.

Baseline: the absorbance of the user selectable-baseline (horizontal) cursor. The user may drag this cursor to a new vertical position to create a new baseline. The absorbance value of the baseline is subtracted from the absorbance of the spectrum.

Max Absorbance: used to rescale the upper limit of the vertical axis.

4.8.4 Sample Homogeneity

The user must be sure to homogeneously suspend the cells when sampling for 'absorbance' measurement and read the sample immediately to avoid significant cell settling. Vigorous mixing may be required particularly when measuring concentrates.

5 Archived Data

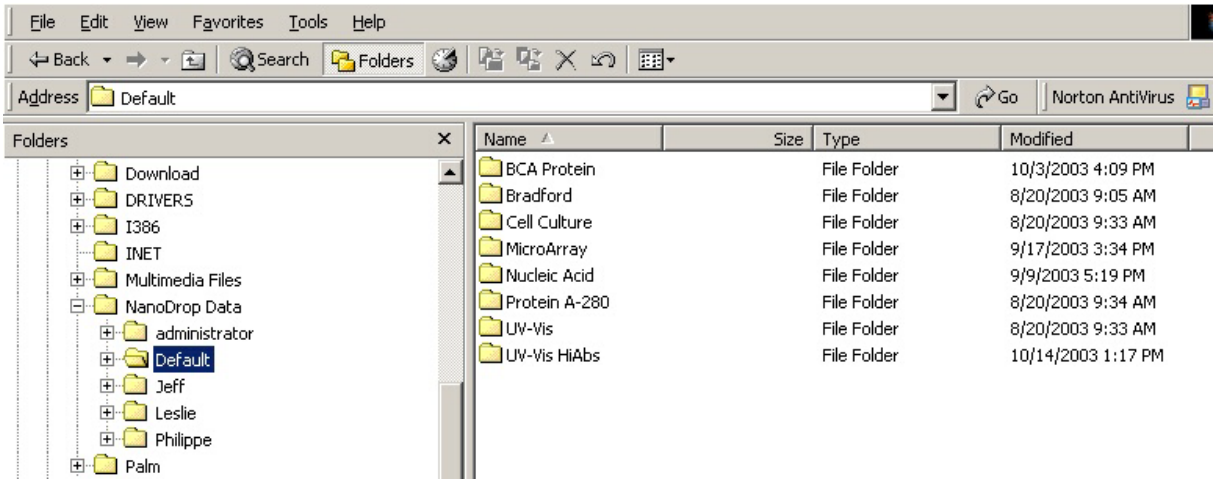
Sample data from all Application modules is automatically stored in archive files. The data in these files can then be manipulated by MS Excel or other spreadsheet program.

5.1.1 Data Storage Hierarchy

The hierarchy for archive files is as follows:

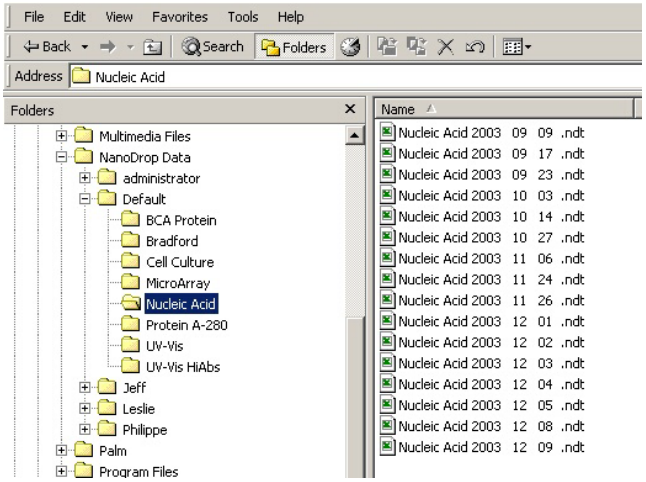
C:\NanoDrop Data → *Username* → Application Module (BCA Protein, Bradford, Cell Culture, MicroArray, Nucleic Acid, Protein A-280, UV-Vis or UV-Vis HiAbs)

All archived data files are stored within an Application Module folder that is within the User folder as shown below.



5.1.2 Archive File Creation

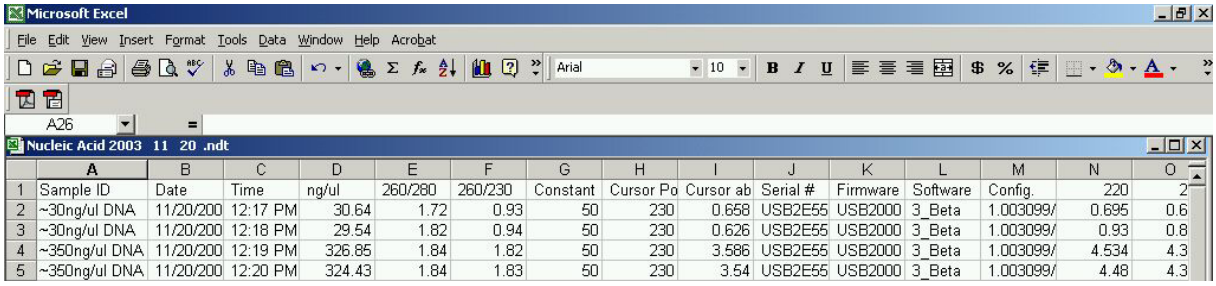
Every time an Application module is started, an application-specific archive file is created for the user that is logged in. All measurements made by the user (in that Application Module) for a given calendar day are stored in a single archive file. These files bear the name of the respective Application module with the date appended. For example, an archived file entitled "Nucleic Acid 2003 12 03.ndt" corresponds to Nucleic Acid data from the software session that began on December 3, 2003. A unique file extension (.ndt) has been given to these files to enable automatic startup with MS Excel or other spreadsheet format (see section below). Examples of nucleic acid files are shown below.



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.

5.1.3 Archive File Format

The files are in tab-delimited format and should open in Microsoft Excel or an equivalent spreadsheet program. Once the data has been opened in Excel, the result should be similar to that below. The first row is a header that identifies the data in the columns. The columns beginning with "N" and extending to the right are the absorbances at the wavelengths shown in the header row.

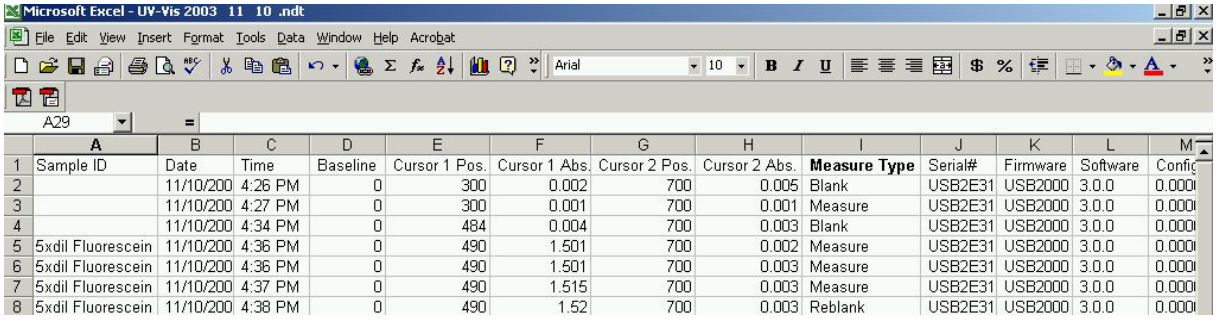


	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Sample ID	Date	Time	ng/ul	260/280	260/230	Constant	Cursor Po	Cursor ab	Serial #	Firmware	Software	Config.	220	2
2	~30ng/ul DNA	11/20/200	12:17 PM	30.64	1.72	0.93	50	230	0.658	USB2E55	USB2000	3_Beta	1.003099/	0.695	0.6
3	~30ng/ul DNA	11/20/200	12:18 PM	29.54	1.82	0.94	50	230	0.626	USB2E55	USB2000	3_Beta	1.003099/	0.93	0.8
4	~350ng/ul DNA	11/20/200	12:19 PM	326.85	1.84	1.82	50	230	3.586	USB2E55	USB2000	3_Beta	1.003099/	4.534	4.3
5	~350ng/ul DNA	11/20/200	12:20 PM	324.43	1.84	1.83	50	230	3.54	USB2E55	USB2000	3_Beta	1.003099/	4.48	4.3

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.

Note1 : absorbance data shown in archive files are represented as they are displayed on the screen. For Nucleic Acids and Protein A280 Application modules, data are normalized to a 1.0 cm (10.0 mm) path. For MicroArray, UV-Vis, BCA Protein, Bradford, and Cell Culture Application modules the data is normalized to a 0.1 cm (1.0 mm) path. For high absorbance UV-Vis samples, data are normalized to a 0.1 mm path.

Note 2: for the UV-Vis and Hi-Abs archive data, a column entitled "Measure Type" is displayed. 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. For example in the image below, row 8 contains the absorbances for the 'Reblanked' sample "5x dil Fluorescein" utilizing the new blank, while row 7 is the initial absorbances of the same sample utilizing the original blank value.


Microsoft Excel - UV-Vis 2003 11 10 .ndt

File Edit View Insert Format Tools Data Window Help Acrobat

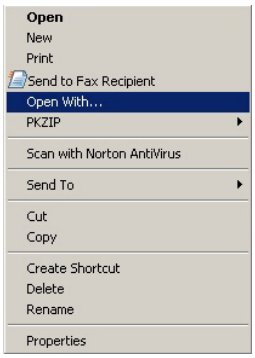
A29 =

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Sample ID	Date	Time	Baseline	Cursor 1 Pos.	Cursor 1 Abs.	Cursor 2 Pos.	Cursor 2 Abs.	**Measure Type**	Serial#	Firmware	Software	Confic
2		11/10/200	4:26 PM	0	300	0.002	700	0.005	Blank	USB2E31	USB2000	3.0.0	0.000
3		11/10/200	4:27 PM	0	300	0.001	700	0.001	Measure	USB2E31	USB2000	3.0.0	0.000
4		11/10/200	4:34 PM	0	484	0.004	700	0.003	Blank	USB2E31	USB2000	3.0.0	0.000
5	5xdil Fluorescein	11/10/200	4:36 PM	0	490	1.501	700	0.002	Measure	USB2E31	USB2000	3.0.0	0.000
6	5xdil Fluorescein	11/10/200	4:36 PM	0	490	1.501	700	0.003	Measure	USB2E31	USB2000	3.0.0	0.000
7	5xdil Fluorescein	11/10/200	4:37 PM	0	490	1.515	700	0.003	Measure	USB2E31	USB2000	3.0.0	0.000
8	5xdil Fluorescein	11/10/200	4:38 PM	0	490	1.52	700	0.003	Reblank	USB2E31	USB2000	3.0.0	0.000

5.1.4 Automatically Opening Archive Files with MS Excel

To set up your PC to automatically open archive files with MS Excel, the .ndt extension must first be "associated" with MS Excel. To do this, perform the following:

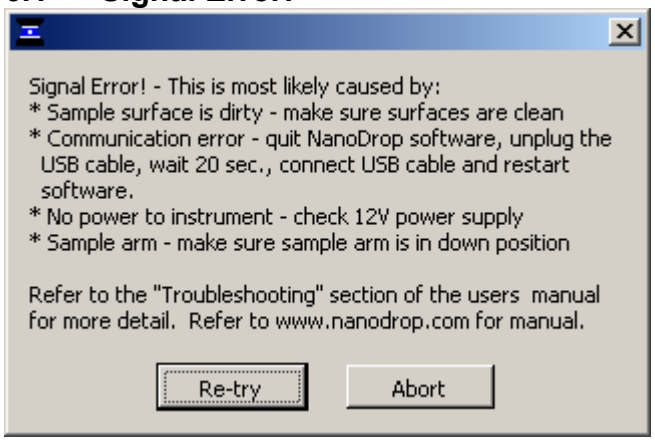
1. Go to the C:\NanoDrop Data\Default directory and find any .ndt file. Right click on the file to bring up the menu at right. Select "Open with…"



2. Choose MS Excel or other spreadsheet program and select the check box at bottom called "Always use this program to open these files". Select OK. From now on, double-clicking a .ndt file will open the file in Excel.

6 Troubleshooting

6.1 Signal Error!

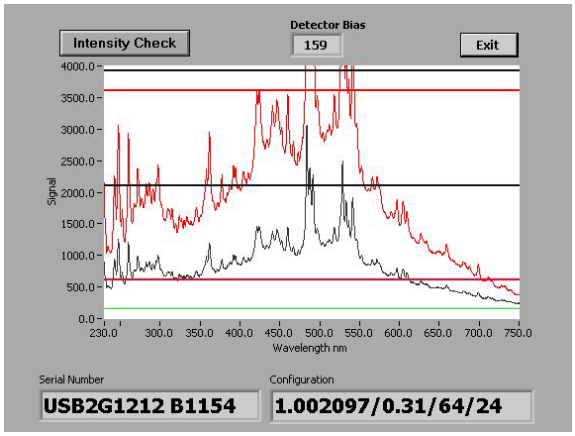


This is the most common software error encountered and usually occurs when starting. It occurs because no light or not enough light is reaching the spectrometer, or because the USB communication has not been established or has been lost. Try the following troubleshooting steps to diagnose and fix the problem:

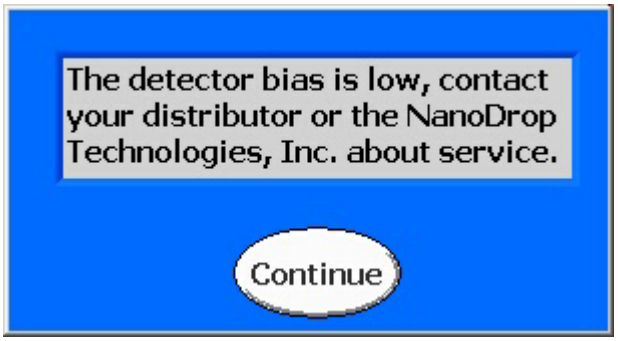
1. **Confirm that the sampling arm is in the down position**.

2. **Clean the Measurement Pedestal Surfaces**. A sample may have dried on one or both of the sample pedestals and is now absorbing the light. Clean both of the sample pedestals gently but vigorously with de-ionized water and a laboratory wipe.

3. **Confirming/Resetting the USB Communication**. With the sampling arm down, open the Utilities and Diagnostics Module. Select OK to initialize the spectrometer and then select "Intensity Check". The USB communication is functioning properly if screen is similar to the one below left. The bias value should be greater than 65 counts.



Proper Operation



No USB Communication

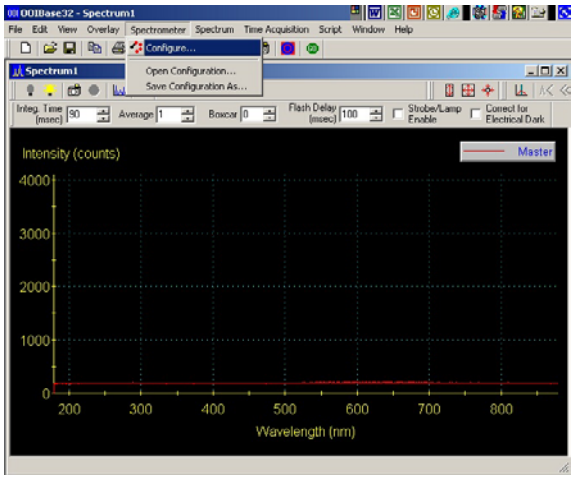
If the Bias is less than 65 counts or you receive a Low Bias error (above right), this indicates no USB communication between the PC and instrument. Try the following possible remedies to correct the problem:

- Confirm that the USB cable is connected to both the PC and instrument

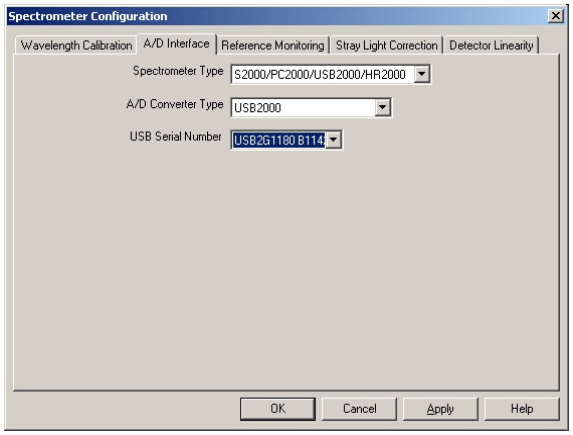
- Close the NanoDrop software, unplug the USB cable, wait 20 seconds, reconnect the USB cable and restart the software
- Restart the PC
- Reset the spectrometer configuration (see step 4 below)
- If steps above do not correct the problem, uninstall the NanoDrop software and the OOIBase32 software and restart the PC. Then reinstall the NanoDrop software (refer to section 2.1.2).

4. **Reset the Spectrometer Configuration**. Occasionally the software driver used to control the USB communication must be reset manually. To do this, perform the steps below.

   1. Start the OOIBase32 software: *Start → Programs → Ocean Optics → OOIBase32 → OOIBase32*. If you received the message "This appears to be the first time…", choose "default" and select "OK". Select the following file: c:\program files\ocean optics\ooibase32\default.spec. Once the software starts, select the "Spectrometer" menu and select "Configure" from the dropdown menu.



   2. In the Spectrometer Configuration window, select the "A/D interface" tab and choose the settings as shown below right. Even if the USB Serial Number slot is already filled with the proper serial number, use the pull-down menu to select the serial number. This will enable the "Apply" button at the bottom of the screen. Select "Apply" and then select "OK". The spectrometer should now be configured properly. Shut down the OOIBase32 software and restart the PC.



   If no USB serial number is available as a choice in the dropdown menu, close the OOIBase32 software and restart the PC. Then open OOIBase32 again and configure the A/D interface again as outlined above.

5. **Confirm Power Supply Operation**. To confirm that the 12V power supply is working properly, connect the leads of a volt-ohmmeter to the outlet of the supply. The voltage should be 12-20 Vdc, center positive.

6. **Confirm that Light is Reaching the Sample**. Open the Utilities and Diagnostics module of the software. Select OK to initialize the spectrometer. You should see a detector bias value greater than 65. This indicates that the PC and instrument are communicating properly. With the sampling arm down, select "Intensity Check". This should generate an output similar to the image in step 3. If no output is present, the flashlamp may have failed. If there is a spectrum present, this indicates that the flashlamp is operating. In this case, one of the fiber optic cables might be broken, reducing the amount of light that reaches the spectrometer.

7. **Confirm Spectrometer Operation**. Open the Utilities and Diagnostics module of the software. Select OK to initialize the spectrometer. You should see a detector bias value greater than 65.

This indicates that the PC and instrument are communicating properly. To confirm that the spectrometer is functioning, open the sampling arm and shine a flashlight directly into the lower measurement pedestal or place very near a fluorescent light. If the spectrometer is operating properly, the detector bias value will increase significantly.

8. If none of the troubleshooting steps above solve the problem, refer to the "Technical Service" section for getting help from your distributor or NanoDrop Technologies.

6.2 Other Software Error Messages

6.2.1 Error USB2000- Unable to find device with serial.

This message appears when communication has been lost between the PC and instrument. This usually occurs due to inadvertent disconnection of the USB cable. In most cases, reconnecting the USB cable will re-establish the connection. With Windows 98 and ME, it may be necessary to restart the computer to re-establish the connection.

This message will also appear if the software was installed while the instrument was connected to the PC via the USB cable. In this case, perform the procedure in the "ISR Not Installed" section of "Troubleshooting" to remedy this.

6.2.2 Can't find file OOIDRV.INI…

This error occurs when trying to install the software without administrator privileges. Contact your system administrator to install the software.

6.2.3 An Error Occurred: Code: 7 Source: Open file in…

This error occurs when any of the five ".log" files ("dye list.log", "passwords.log", "user preferences.log", "protein methods.log" and "photo methods.log") has been removed from the folder where the program files were installed (default is Program Files\NanoDrop 3.0.1). These files must be present in this folder in order for the NanoDrop software to operate.

6.2.4 An Error Occurred: Code: 8 Source: Open file in…

This error occurs when access to any of the five ".log" files ("dye list.log", "passwords.log", "user preferences.log", "protein methods.log" and "photo methods.log") has been denied. This usually occurs when a user without administrator access runs the software. If this is the case, contact your system administrator to "share" the program files were installed (default is Program Files\NanoDrop 3.0.1).

This error can also occur if the ".log" files are set to "read-only". Check this by going to the NanoDrop 3.0.1 folder and right click on the "dye list.log" file. Deselect the "read-only" box if it was selected. Repeat this for all of the .log files.

6.2.5 Source Error

This error indicates that there is insufficient light getting through to make good absorbance measurements, or the USB connection has been lost. Check that sampling arm is in the down position and the power is connected.

If the USB connection has been lost, the "Low Detector Bias" error will also appear. Perform the steps outlined in the "Signal Error" section of "Troubleshooting".

6.2.6 Low Detector Bias

This occurs when the software has detected a problem with the spectrometer or the USB connection is lost while a measurement is being made.

If this message appears simultaneously with the "Source Error" message, the USB connection has probably been lost. To troubleshoot, perform the steps outlined in the "Signal Error" section of "Troubleshooting".

6.2.7 Error 7 Occurred at New File……

This occurs when the "C:\NanoDrop Data" file folder has been removed from the C: drive, or it has been corrupted. If this message appears, click on the stop button in the error window. Close the NanoDrop software. Open Windows Explorer and create a new folder on the C: drive. Name the folder "C:\NanoDrop Data" (case sensitive) and close Windows Explorer. Click on the arrow in the far upper left of the NanoDrop software. Restart the NanoDrop software.

6.2.8 ISR Not Installed

This error is most likely caused by installing the software while the USB cable was connected to both the PC and instrument. This will cause the OOIBase32 USB driver to not be properly linked. To remedy this, you must remove the OOI USB driver, uninstall the operating software and then reinstall it. Follow these steps to accomplish this:

1. Plug the USB cable into the instrument and the PC.
2. Select the following path to bring up the System Properties screen: ***Start→ Settings → Control Panel → System***.
3. Once at the System Properties screen: For Windows 2000 and XP select the ***Hardware*** tab and then click on ***Device Manager***; For Windows 98 and ME select the ***Device Manager*** tab.
4. At the bottom of the screen of the Device Manager page, click on the "+" that is to the left of the "Universal Serial Bus controllers" line.
5. Find the "Ocean Optics USB2000- EEPROM Load" device driver, highlight it and then delete it.
6. Unplug the USB cable from the instrument.
7. Uninstall the "OOIBase32" software. To do this, select the following path and follow the onscreen instructions: ***Start→ Settings → Control Panel → Add/Remove Programs.***
8. Restart computer.
9. Reinstall the NanoDrop software.
10. Restart computer again.
11. After computer has rebooted, reconnect the USB cable to the instrument and wait 1 minute.
12. Start NanoDrop software. System should operate normally.

6.2.9 Can't Find LabView RunTime Engine

This error message likely means that one or more of the software components have been removed or corrupted. If this occurs, reinstall the NanoDrop software using the installation CD or downloaded from www.nanodrop.com.

6.2.10 EZUSB.SYS Cannot Be Found

If this error message appears, do the following (depending on your operating system):

Windows 2000: type **C:\WINNT** in the file path text box. If the file **ooi_usb.inf** cannot be found, type **C:\WINNT\INF** in the file path text box.

Windows 98/ME: type **C:\WINDOWS\SYSTEM** in the file path text box. If the file **ooi_usb.inf** cannot be found, type **C:\WINDOWS\INF** in the file path text box.

Windows XP: type **C:\WINDOWS** in the file path text box. If the file **ooi_usb.inf** cannot be found, type **C:\WINDOWS\INF** in the file path text box.

This should allow the installation to complete successfully.

6.2.11 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 on the computer. Contact your system administrator if this occurs.

6.2.12 Error 8 Occurred at Open File

This error message occurs when the user attempts to take a measurement while the data file is open. Close the data file and you should be able to continue taking samples normally.

6.2.13 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 40MB of free hard disk space.

6.2.14 Error- Code 31

This error appears when attempting to print when a printer is not attached to the PC. It is non-fatal and will not cause the software to shut down.

6.3 Hardware

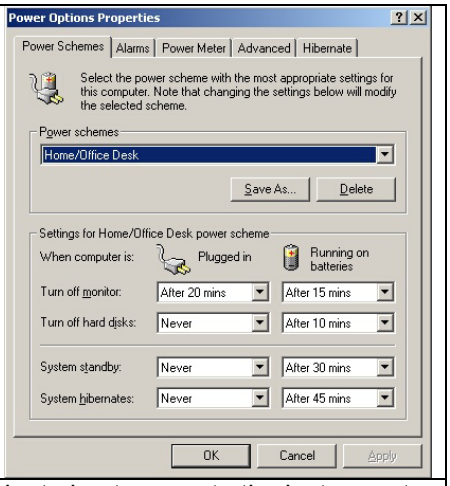
6.3.1 "Chattering" Solenoid

This phenomenon is characterized by a very rapid chattering, not a distinctive clicking sound. If this occurs, it is caused by a malfunctioning component on the circuit board. If this occurs, contact your local distributor or NanoDrop Technologies for service.

6.3.2 Intermittent USB Connection Failure

Unusual performance that occurs intermittently is very hard to diagnose. In some cases, this can be caused by a faulty USB board in the PC. If your instrument is exhibiting strange performance that is not addressed by any of the other troubleshooting tips, it would be helpful to install the software and operate the instrument on another computer to see if the behavior is duplicated. A list of known USB problems is listed below:

Possible Cause	Possible Solution
Static Electricity from the user	Use a grounding wrist strap

PC has gone into "standby" or "hibernate" mode	Change the PC's power management properties so that it does not go to Standby or Hibernate mode when plugged in. To reach the power management screen choose Start → Control Panel → Power Options. Change the System Standby and System Hibernate to "never" for the "Plugged In" column as shown at right. 
Faulty USB card on PC	Confirm that the USB is faulty by trying to operate the instrument on a different PC that is known to have properly functioning USB ports. If necessary, replace the USB card on the PC.

6.4 Data Concerns

6.4.1 Sample Accuracy and Reproducibility

Most of the occurrences of strange data are a result of sample preparation
All of the causes listed below can potentially cause apparent problems with data:

Possible Cause	Explanation and Possible Solution
Sample is not homogeneous	Due to the small volumes required by the ND-1000, 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 that the larger volumes used by cuvette-based spectrophotometers will minimize or mask the effect of sample non-homogeneity.
Liquid sample column breakage	Very strange 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 2 ul samples to ensure the column is formed. Also, proteins and solutions containing surfactants are known to "un-condition" the measurement pedestal surfaces so that the liquid column does not form. If this occurs, "buff" the measurement pedestal surfaces by rubbing each with a dry laboratory wipe 15-20 times. This will "re-condition" the surface allowing for the liquid sample column to form.
Blanking with a sample instead of the reference solution	When this occurs, a "non-blank" light intensity array will be used in place of the normal blank. This can cause negative absorbances to be calculated. If this occurs, blank again with the reference solution and measurements should then be normal
Last sample measured has dried on lower measurement pedestal and then a blank was made without cleaning the pedestal first.	In this case the dried sample resolubilizes when a water blank is pipetted onto the lower measurement pedestal, which creates the same effect of blanking with a sample. Make sure both measurement pedestals are clean and blank again with reference solution.

Sample is too dilute	Measuring samples at or near the detection limit will result in measurements that can vary a significant amount. Refer to the "Measurement Concentration Range" of the Application Module that you are using for the applicable measurement range.
Broken fiber optic cable	If the upper (black) fiber optic cable is broken, you can get widely varying results. If you suspect that this is occurring, refer to the "Technical Service" section for instructions on how to contact NanoDrop Technologies and what information must be sent to allow for diagnosing the problem. If the fiber optic cable is broken, it must be replaced.
USB connection drops out causing solenoid failure during measurement	Close NanoDrop software, unplug USB cable, wait 20 seconds, reconnect cable, wait 1 minute and restart software. With Windows 98 & ME PCs, you will need to restart the PC.

6.4.2 260/280 Ratio

Many researchers encounter a consistent 260/280 ratio change when switching from a standard cuvette spectrophotometer to the NanoDrop® ND-1000 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[**]. An acidic solution 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® ND-1000 Spectrophotometer to other spectrophotometers, it is important to ensure that the pH of an undiluted sample measured on the NanoDrop® ND-1000 Spectrophotometer is at the same pH 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 the 260:280 ratio. 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:

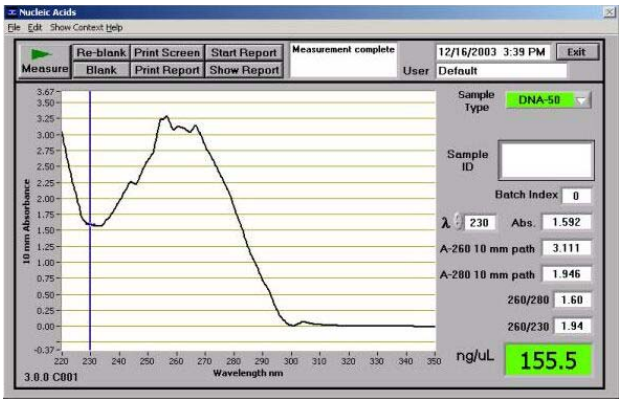
Guanine:	1.15
Adenine:	4.50
Cytosine:	1.51
Uracil:	4.00
Thymine:	1.47

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.

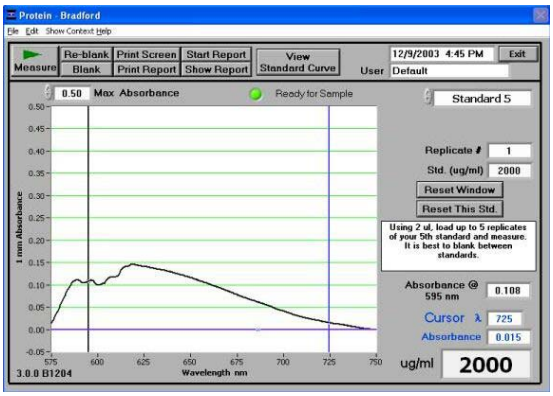
[***] **Leninger, A. L.** *Biochemistry*, 2nd ed., Worth Publishers, New York, 1975

6.4.3 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 both 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



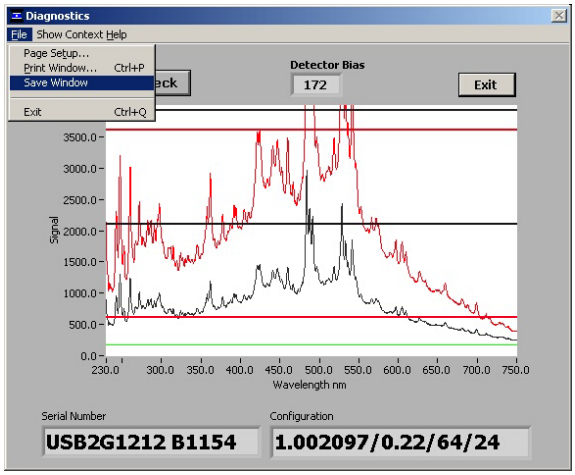
Detector saturation- Bradford measurement

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, refer to the "Technical Service" section for instructions on how to contact NanoDrop Technologies and what information must be sent to allow for diagnosing the problem.

6.5 Technical Service

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

1. 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 distributor or to info@nanodrop.com.



2. Application Module Screen Captures
Screen captures of the actual spectrum as seen on your PC 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 program. Save this as a .jpg or .doc file and send as email attachment to your distributor or to info@nanodrop.com.

3. 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 distributor or to info@nanodrop.com.

7 Maintenance and Warranty

7.1 Cleaning

The primary maintenance requirement of the NanoDrop® ND-1000 Spectrophotometer is keeping 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. It is also recommended that both measurement pedestals be cleaned with de-ionized water upon completion of a testing series. No other regular maintenance is required

7.2

7.3 Parts That Require Replacement

In general, the only part that should periodically require replacement is the flash lamp. The flash lamp should last a minimum of 30K measurements. There is no way to test the flash lamp to confirm its remaining life. When the flash lamp fails, the light output will become very erratic or stop altogether.

7.4 Warranty

All spectrophotometers and accessories manufactured by NanoDrop Technologies are warranted against manufacturing defects in parts and labor for a period of one year. A one-year warranty extension may also be purchased.

7.5 Recalibration

7.5.1 Wavelength

This is auto-calibrated each time the software is started. No calibration is required by the user.

7.5.2 Pathlength (Accuracy)

Generally, this is not required as field experience has shown that the path does not change appreciably even after several years of heavy use. However, it is a good idea to check the calibration every six months using CF-1 calibration fluid. A calibration check procedure is available from the Download section at www.nanodrop.com.

8 NanoDrop Technologies Contact Information

Shipping Address:
100 W. Rockland Rd, Suite I
Montchanin, DE 19710
USA
Voice: 302-984-0334
Fax: 302-984-0336

Mailing Address:
PO Box 415
Rockland, DE 19732
USA
Voice: 302-984-0334
Fax: 302-984-0336

Email: info@nanodrop.com

Website: http://www.nanodrop.com

Appendices

8.1 A. Instrument Specifications

- Long Path Length: 1mm
- Short Path Length (for high concentration measurement): 0.2mm
- Sample Size Requirement: 1-2 ul
- Light Source: pulsed Xenon flash lamp
- Detector: 2048-element linear silicon CCD array
- Wavelength Range: 220-750 nm
- Wavelength Accuracy: 1 nm
- Wavelength Resolution: 3 nm (FWHM at Hg 546 m)
- Absorbance Precision: 0.003 absorbance
- Absorbance Accuracy: 2% (at 0.76 absorbance at 257 nm)
- Absorbance Range: 0.02-75 (10mm equivalent absorbance)
- Measurement Cycle Time: 10 seconds
- Dimensions: 20 cm X 15 cm x 12 cm
- Sample Pedestal Material of Construction: 303 Stainless Steel
- Fiber Optic Cable Material of Construction: quartz
- Operating Voltage: 12 Vdc
- Operating Power Consumption: 6W
- Standby Power Consumption: 1.5W

8.2 B. Blanking and Absorbance Calculations

When the NanoDrop® ND-1000 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:

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

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

8.3 C. 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 (Microarray Measurement)

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

Dye Type	Extinction Coefficient (liter/mol-cm)	Measurement Wavelength (nm)
Cy3	150000	550
Cy5	250000	650
Alexa Fluor 488	71000	495
Alexa Fluor 546	104000	556
Alexa Fluor 555	150000	555
Alexa Fluor 594	73000	590
Alexa Fluor 647	239000	650
Alexa Fluor 660	132000	663
Cy3.5	150000	581
Cy5.5	250000	675

Nucleic Acids

For nucleic acid quantification, the Beer-Lambert equation is manipulated to give:

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

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
- Single-stranded DNA: 33
- RNA: 40

For the NanoDrop® ND-1000 Spectrophotometer, a path of 1.0 mm and 0.2 mm are used compared to a standard spectrophotometer using a 10.0 mm path. Thus, the NanoDrop® ND-1000 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 both Nucleic Acid and Protein A280 samples, data are normalized to a 1.0 cm (10.0 mm) path. For MicroArray, UV-Vis, BCA, Bradford, and Cell Culture samples the data are normalized to a 0.1 cm (1.0 mm) path. For high absorbance UV-Vis samples, data are normalized to a 0.1mm path.

8.4 D. Sample Retention System Solvent Compatibility

The NanoDrop® ND-1000 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.

8.5 E. Setting Up a Dymo 330 Label Writer Printer for Proper Operation

To set DYMO default label to print with ND-1000:

1. Open Control Panel, then Printers and Faxes
2. Right click on Dymo printer then select Properties
3. Under General tab, choose "Printing Preferences" then "Advanced". Then set the following:
   - **Paper Size:** 30256
   - **Halftoning:** Super Cell
   - **Print Quality:** Barcodes and Graphics.

4. Click "OK" to close this window then click "Apply"
5. Next, click on the "Advanced" tab. Ensure that "Enable advanced printing features" box is checked. Then click on "Printing Defaults" then "Advanced". Then set the following:
   - **Paper Size:** 30256
   - **Halftoning:** Super Cell
   - **Print Quality:** Barcodes and Graphics.
6. Click "OK" to close this window then click "Apply"
7. Last, click on the "Device Settings" tab and ensure 30256 Shipping Label is selected as the "Default" label.