Spectrophotometric quantitation via Nanodrop[1]

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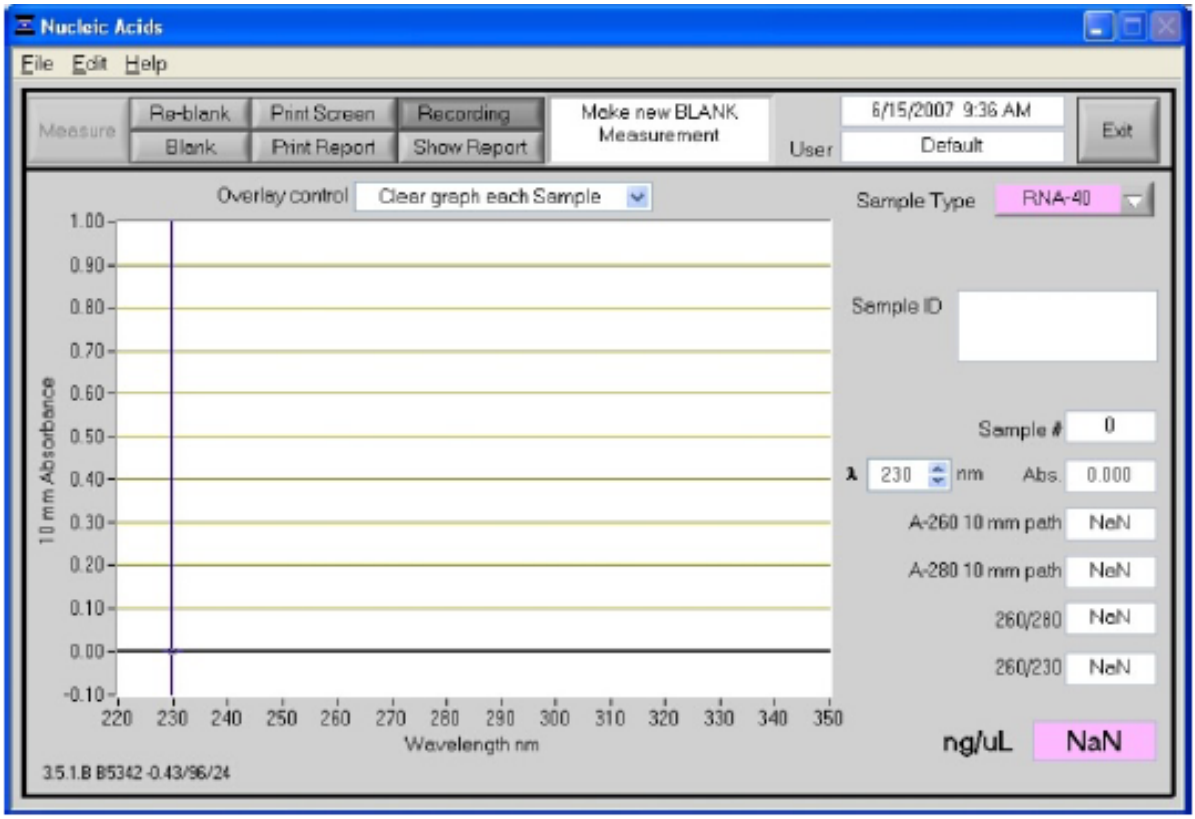
Measure (F1)

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. (See Blanking and Absorbance Calculations on page 3.)

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.

Blank (F3)

Before making a sample measurement, a blank must be measured and stored. After you have made an initial blank measurement, a straight line will appear on the screen; subsequent blanks will clear any sample spectrum and display a straight line, as shown in the following image:

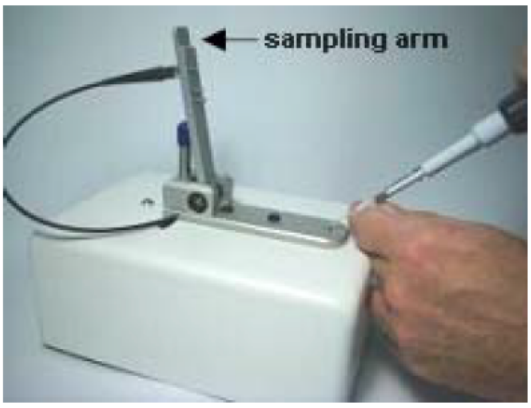


[1] Information and instructions taken from Thermo Scienfic's NanoDrop 1000 Spectrophotometer V3.8 User's Manual, http://www.nanodrop.com/library/nd-1000-v3.7-users-manual-8.5x11.pdf, accessed 1/14/13.

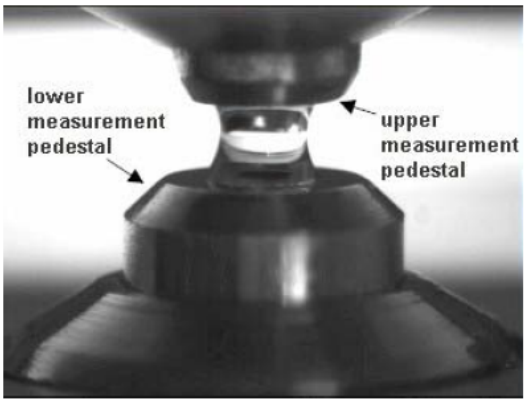
For the most consistent results, it is best to begin any measurement session with a blanking cycle. This will assure the user that the instrument is working properly and that the pedestal is clean. Follow the steps below to perform a blanking cycle:

1. Load a 1 µL 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.

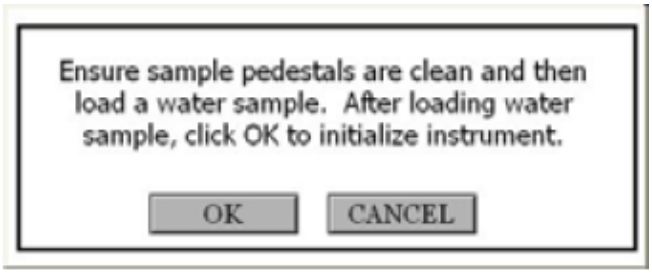
With the sampling arm open, pipette the sample onto the lower measurement pedestal.



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.



When the software starts, you should see this message:



For best results, ensure measurement pedestal surfaces are clean, load a water sample onto the lower measurement pedestal and then click 'OK'. The message "Initializing Spectrometer- please wait" will appear. When this message disappears, the instrument will be ready for use. All data taken will automatically be logged in the appropriate archive file.

2. Click on the Blank (F3) button.

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 the Nanodrop website (www.nanodrop.com) for performance data on sample carryover.

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

4. Analyze an aliquot of the blanking solution as though it were a sample. This is done using the "Measure" button (F1). The result should be a spectrum with a relatively flat baseline. Wipe the blank from both measurement and pedestal surfaces and repeat the process until the spectrum is flat.

5. Analyze 1 µL of your sample with the "Measure" button.

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 respectively, 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.

Blanking and Absorbance Calculations

When the NanoDrop 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.

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).

Nucleic Acids

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

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

Where

c is the nucleic acid concentration in ng/microliter,

A is the absorbance in AU,

e is the wavelength-dependent extinction coefficient in ng-cm/microliter and

b is the path length in cm.

The generally accepted extinction coefficients for nucleic acids are:

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

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