Montgomery County Community College
340 DeKalb Pike
Blue Bell, PA

Document Number: QCB 11
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Effective Date: 10/24/2013
Page 1 of 9

SOP: Operation of NanoDrop 2000 Spectrophotometer

Approvals
Preparer: John Buford   Date: 24OCT13
Reviewer: Jason McMillan   Date: 24OCT13
Reviewer: Jack O'Neill   Date: 24OCT13

1. **Purpose**
   1.1. Measure the light absorbance of microliter (µL) samples using a NanoDrop 2000 spectrophotometer in order to analyze ultraviolet and visible light spectral signature and to quantify DNA and protein content based on spectral signature.
2. **Scope and Applicability**
   2.1. A spectrophotometer transmits known wavelengths of light through a sample and measure the amount of light absorbed by the sample. Nucleic acids (such as DNA) and proteins absorb light at the 260 nm and 280 nm respectively, so a spectrophotometer can be used to quantify the DNA and protein content of a sample. This SOP describes the general operation of the NanoDrop 2000 spectrophotometer and its use to measure DNA concentration, protein concentration, and ultraviolet-visible (UV-Vis) light absorption.
3. **Summary of Method**
   3.1. Connect and power up the spectrophotometer to a PC.
   3.2. Launch the NanoDrop 2000 software interface.
   3.3. Apply 2 µL blank solution onto the lower pedestal and initiate a blank measurement.
   3.4. Wipe the blank from both the upper and lower pedestals using a clean lint-free laboratory wipe.
   3.5. Apply 2 µL sample solution onto the lower pedestal and initiate a spectral measurement.
   3.6. Wipe the sample from both the upper and lower pedestals using a clean lint-free laboratory wipe.
4. **References**
   4.1. Thermo Scientific NanoDrop 2000/2000c Spectrophotometer V1.0 User Manual
5. **Definitions**

   Spectrophotometer   an instrument for measuring light intensity of known light wavelengths
   UV-Vis   Ultraviolet and visible light

6. **Precautions**
   6.1. The NanoDrop 2000 spectrophotometer contains no user serviceable parts; do not open.
7. **Responsibilities**
   7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
8. **Equipment and Materials**
   8.1. NanoDrop 2000 spectrophotometer
   8.2. 12V power supply

Montgomery County Community College
340 DeKalb Pike
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Document Number: QCB 11
Revision Number: 0
Effective Date: 10/24/2013
Page 2 of 9

SOP: Operation of NanoDrop 2000 Spectrophotometer

8.3. PC with NanoDrop 2000/2000c software interface installed

8.4. Precision micropipette (2 µL or 10 µL) and micropipette tips

8.5. Laboratory grade water (dH$_2$O)

8.6. Sample solution(s)

8.7. Blank solution

8.8. Lint-free laboratory wipes (e.g. Kimwipes)

9. Procedure

9.1. *Connecting and powering up the spectrophotometer*

    9.1.1.   Connect the USB cable to the NanoDrop 2000 spectrophotometer.

    9.1.2.   Plug in the 12V power supply and connect to the power input at the back of the spectrophotometer. (The power supply may remain plugged into the spectrophotometer while not in use.)

9.2. *Launching the NanoDrop 2000 software interface*

    9.2.1.   Double click the NanoDrop 2000 icon of the desktop.

    9.2.2.   Alternatively, select Start > All Programs > Thermo Scientific NanoDrop 2000 > NanoDrop 2000.

    9.2.3.   The Home window should appear, containing task options on the left pane (e.g. Home, My Data, Diagnostics) and application buttons on the right pane (e.g. Nucleic Acid, Protein A280, UV-Vis).

    9.2.4.   Selecting a task option or application replaces the Home window with a window for that specific task or application.

    9.2.5.   To return to the Home window from any other window, select the "Home" task option from the left pane.

9.3. *Basic use of the pedestal*

    9.3.1.   Raise the sampling arm.

    9.3.2.   Apply 2 µL solution onto the lower pedestal using a micropipette.

    9.3.3.   Lower the sampling arm.

    9.3.4.   Initiate a spectral measurement using the PC software interface.

    9.3.5.   When measurement is complete, raise the sampling arm and wipe the solution from both the upper and lower pedestals using a clean lint-free laboratory wipe.

    9.3.6.   Lower the sampling arm.

    9.3.7.   Leave the sampling arm in the down position between uses.

9.4. *Pedestal cleaning*

    9.4.1.   Use dH$_2$O to clean the measurement surfaces before the first sample measurement and after the last sample measurement.

    9.4.2.   Raise the sampling arm.

    9.4.3.   Apply 3-5 µL dH$_2$O onto the lower pedestal using a micropipette.

    9.4.4.   Lower the sampling arm.

    9.4.5.   Let sit for approximately 2-3 minutes.

    9.4.6.   Raise the sampling arm and wipe the dH$_2$O from both the upper and lower pedestals using a clean lint-free laboratory wipe.

    9.4.7.   Lower the sampling arm.

    9.4.8.   Generally, it is not necessary to clean with dH$_2$O between samples.

9.5. *Performing an intensity check*

SOP: Operation of NanoDrop 2000 Spectrophotometer

9.5.1.   At regular intervals (weekly or monthly), confirm that the internal spectrometer is functioning as expected.

9.5.2.   Select the "Diagnostic" task from the Home window.

9.5.3.   Select the "Intensity Check" task.

9.5.4.   Ensure that the sampling arm is down.

9.5.5.   Click "Measure".

9.5.6.   If yellow triangles are displayed in the top left of the spectral display, clean the pedestal and then repeat the intensity check.

9.5.7.   Return to the Home window by selecting the "Home" task option from the left pane.

9.6. *Measuring DNA concentration*

9.6.1.   Select the "Nucleic Acid" application from the Home window.

9.6.2.   If the wavelength verification window appears, ensure that the sampling arm is down and click OK.

9.6.3.   Select File > New Workbook, enter a filename and directory, then click Save.

9.6.3.1.(Optional) Select the type of sample to be measured from the "Type" drop-down list (the default is DNA-50).

9.6.4.   Select the "Add to report" checkbox to include measurements in the current report.

9.6.5.   Select the "Overlay spectra" checkbox if more than one sample is to be measured and displayed on the same spectral graph.

9.6.6.   Establish a blank using the appropriate blank solution:

9.6.6.1.Apply 2 µL blank solution onto the lower pedestal per *9.3 Basic use of the pedestal*.

9.6.6.2.Click the "Blank" button.

9.6.6.3. When the blank measurement is complete, wipe the blank solution from both the upper and lower pedestals using a clean lint-free laboratory wipe per *9.3 Basic use of the pedestal*.

9.6.7.   Measure each sample:

9.6.7.1.Enter a sample ID in the "Sample ID" field.

9.6.7.2.Apply 2 µL sample solution onto the lower pedestal per *9.3 Basic use of the pedestal*.

9.6.7.3.Click the "Measure" button.

9.6.7.4. When the sample measurement is complete, wipe the sample solution from both the upper and lower pedestals per *9.3 Basic use of the pedestal*.

9.6.7.5.Repeat with the next sample solution if more than one sample is to be measured and displayed on the same spectral graph.

9.6.8.   Click the "Print" button to print the spectral display. Alternatively, select the Reports task option to format and print a report.

9.6.9.   Save the data and return to the Home window by selecting File > Close Workbook and go Home.

9.6.10. After the last sample of the day, clean the pedestal per *9.4 Pedestal cleaning*.

SOP: Operation of NanoDrop 2000 Spectrophotometer

9.7. *Measuring protein concentration*

    9.7.1.   Select the "Protein A280" application from the Home window.

    9.7.2.   If the wavelength verification window appears, ensure that the sampling arm is down and click OK.

    9.7.3.   Select File > New Workbook, enter a filename and directory, then click Save.

        9.7.3.1.(Optional) Select the type of sample to be measured from the "Type" drop-down list (the default is 1 Abs = 1 mg/mL).

    9.7.4.   Select the "Add to report" checkbox to include measurements in the current report.

    9.7.5.   Select the "Overlay spectra" checkbox if more than one sample is to be measured and displayed on the same spectral graph.

    9.7.6.   Establish a blank using the appropriate blank solution:

        9.7.6.1.Apply 2 µL blank solution onto the lower pedestal per *9.3 Basic use of the pedestal*.

        9.7.6.2.Click the "Blank" button.

        9.7.6.3. When the blank measurement is complete, wipe the blank solution from both the upper and lower pedestals using a clean lint-free laboratory wipe per *9.3 Basic use of the pedestal*.

    9.7.7.   Measure each sample:

        9.7.7.1.Enter a sample ID in the "Sample ID" field.

        9.7.7.2.Apply 2 µL sample solution onto the lower pedestal per *9.3 Basic use of the pedestal*.

        9.7.7.3.Click the "Measure" button.

        9.7.7.4. When the sample measurement is complete, wipe the sample solution from both the upper and lower pedestals per *9.3 Basic use of the pedestal*.

        9.7.7.5.Repeat with the next sample solution if more than one sample is to be measured and displayed on the same spectral graph.

    9.7.8.   Click the "Print" button to print the spectral display. Alternatively, select the Reports task option to format and print a report.

    9.7.9.   Save the data and return to the Home window by selecting File > Close Workbook and go Home.

    9.7.10. After the last sample of the day, clean the pedestal per *9.4 Pedestal cleaning*.
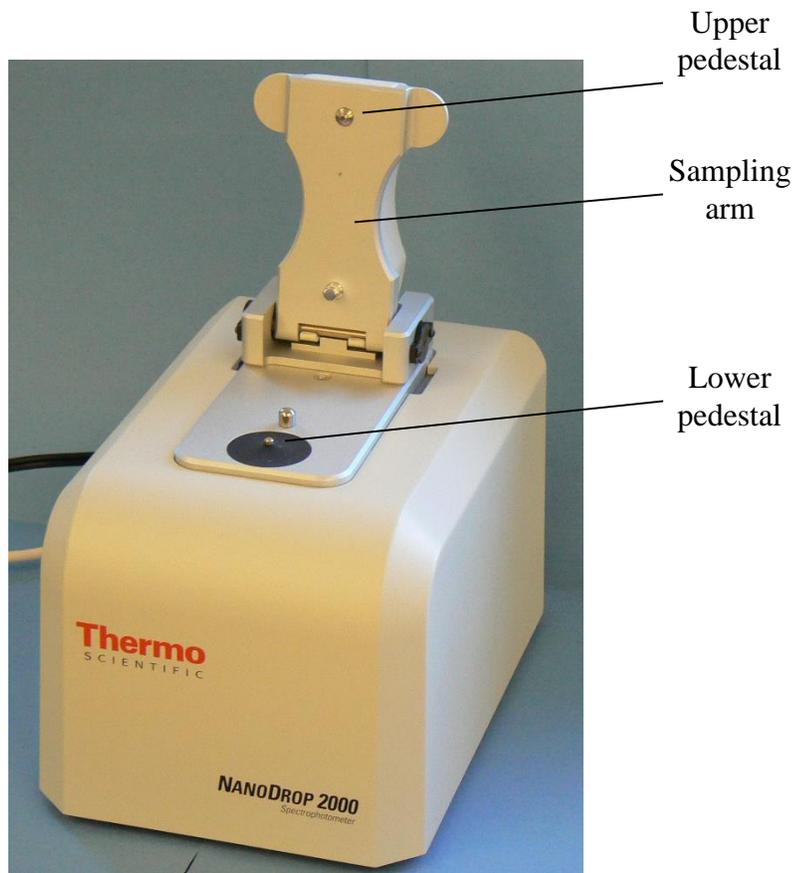
9.8. *Measuring UV-Vis absorbance*

    9.8.1.   Select the "UV-Vis" application from the Home window.

    9.8.2.   If the wavelength verification window appears, ensure that the sampling arm is down and click OK.

    9.8.3.   Select File > New Workbook, enter a filename and directory, then click Save.

    9.8.4.   Enter up to 40 wavelengths of interest in the "nm – Add wavelength(s)" list:

        9.8.4.1.Select the first row, enter a wavelength (in nm), then press the Entry key.

        9.8.4.2.Continue entering values until all wavelengths of interest have been added.

        9.8.4.3.A wavelength can be cleared by selecting the wavelength, then clicking the "Clear Wavelength" button.

    9.8.5.   Select the "Add to report" checkbox to include measurements in the current report.

SOP: Operation of NanoDrop 2000 Spectrophotometer

9.8.6. Select the "Overlay spectra" checkbox if more than one sample is to be measured and displayed on the same spectral graph.

9.8.7. Establish a blank using the appropriate blank solution:

    9.8.7.1. Apply 2 µL blank solution onto the lower pedestal per *9.3 Basic use of the pedestal*.

    9.8.7.2. Click the "Blank" button.

    9.8.7.3. When the blank measurement is complete, wipe the blank solution from both the upper and lower pedestals using a clean lint-free laboratory wipe per *9.3 Basic use of the pedestal*.

9.8.8. Measure each sample:

    9.8.8.1. Enter a sample ID in the "Sample ID" field.

    9.8.8.2. Apply 2 µL sample solution onto the lower pedestal per *9.3 Basic use of the pedestal*.

    9.8.8.3. Click the "Measure" button.

    9.8.8.4. When the sample measurement is complete, wipe the sample solution from both the upper and lower pedestals per *9.3 Basic use of the pedestal*.

    9.8.8.5. Repeat with the next sample solution if more than one sample is to be measured and displayed on the same spectral graph.

9.8.9. Click the "Print" button to print the spectral display. Alternatively, select the Reports task option to format and print a report.

9.8.10. Save the data and return to the Home window by selecting File > Close Workbook and go Home.

9.8.11. After the last sample of the day, clean the pedestal per *9.4 Pedestal cleaning*.

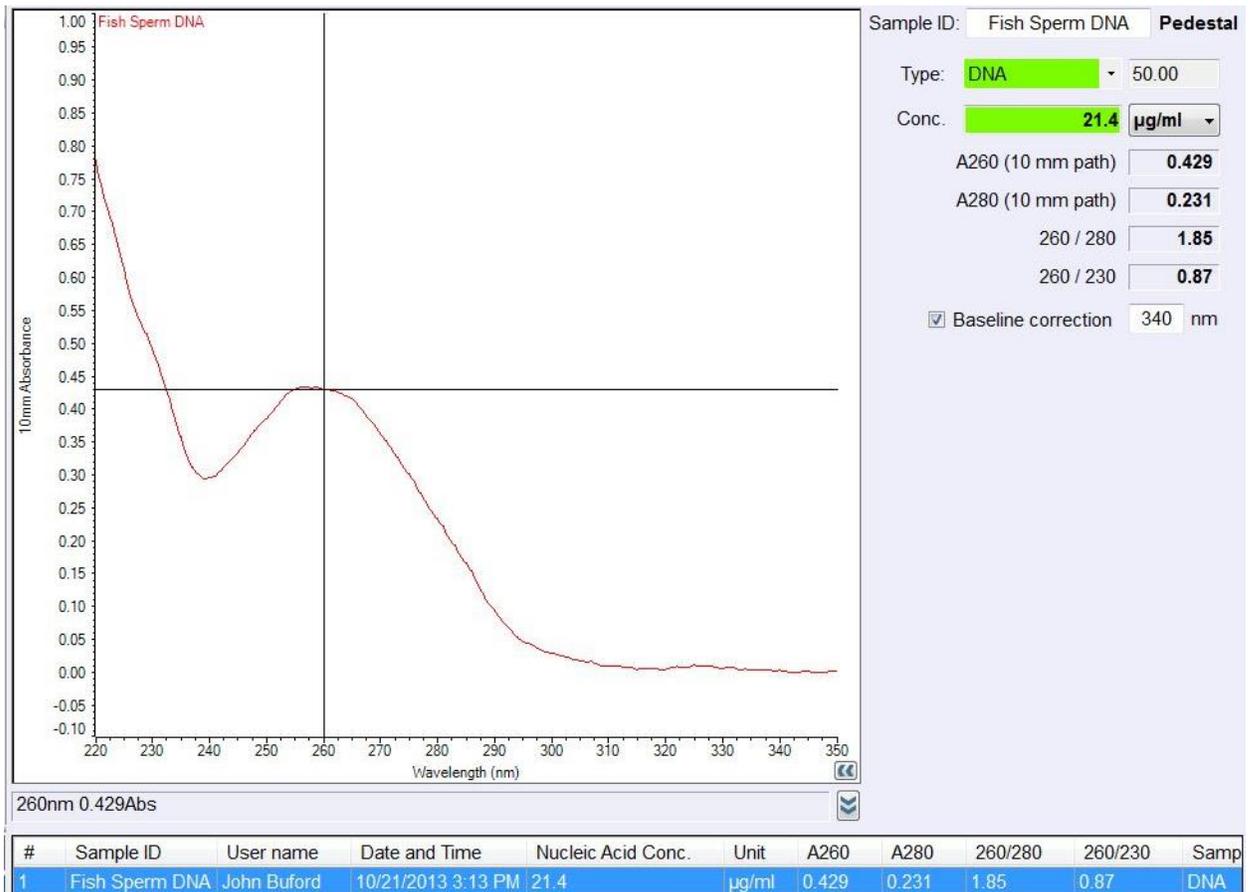# SOP: Operation of NanoDrop 2000 Spectrophotometer

10. Attachments

Upper pedestal

Sampling arm

Lower pedestal

Figure 1. NanoDrop 2000 Spectrophotometer

Montgomery County Community College
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Document Number: QCB 11
Revision Number: 0
Effective Date: 10/24/2013
Page 7 of 9

SOP: Operation of NanoDrop 2000 Spectrophotometer

Figure 2. Applying solution onto the lower pedestal

Montgomery County Community College
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Document Number: QCB 11
Revision Number: 0
Effective Date: 10/24/2013
Page 8 of 9

SOP: Operation of NanoDrop 2000 Spectrophotometer

Figure 3. DNA Concentration Spectral Graph
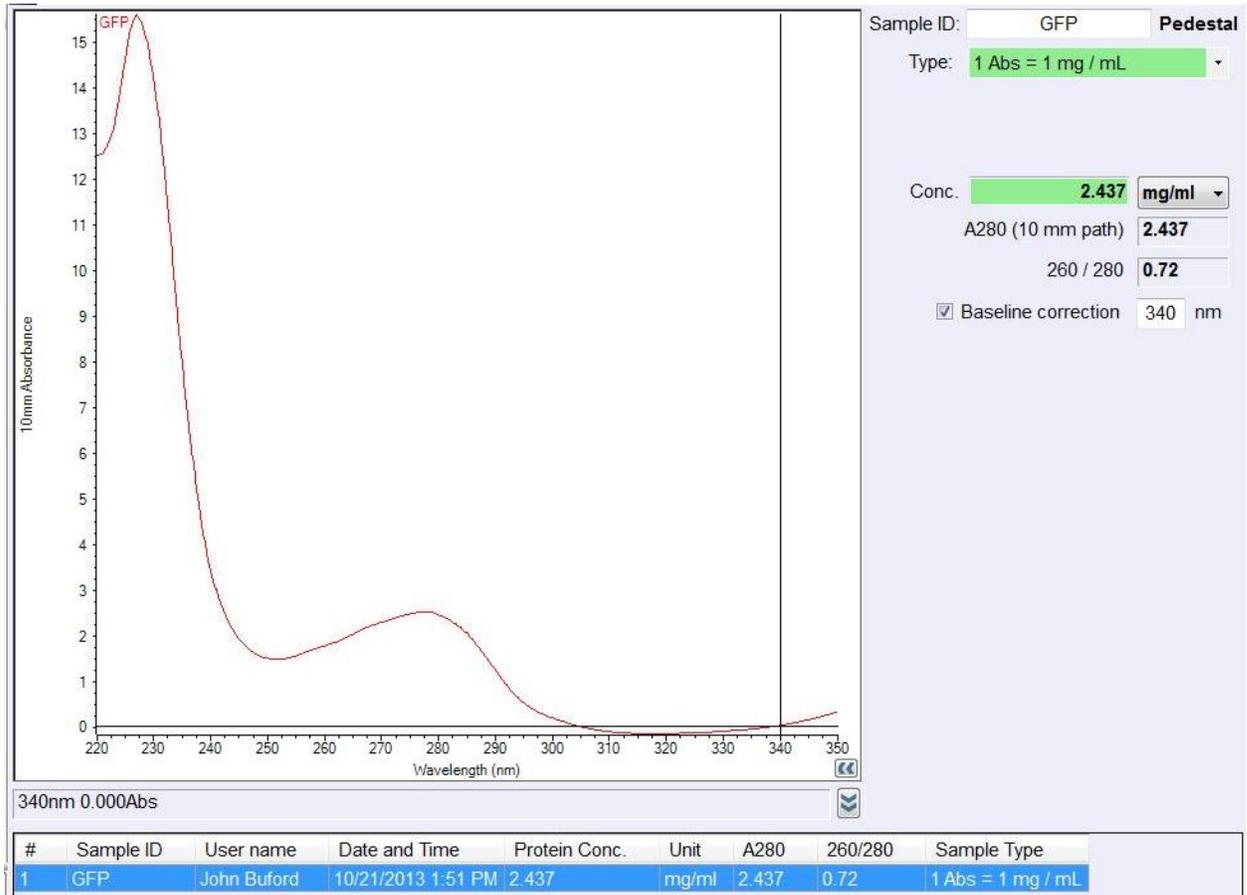
Figure 4. Protein Concentration Spectral Graph

Montgomery County Community College
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Document Number: QCB 11
Revision Number: 0
Effective Date: 10/24/2013
Page 9 of 9

SOP: Operation of NanoDrop 2000 Spectrophotometer
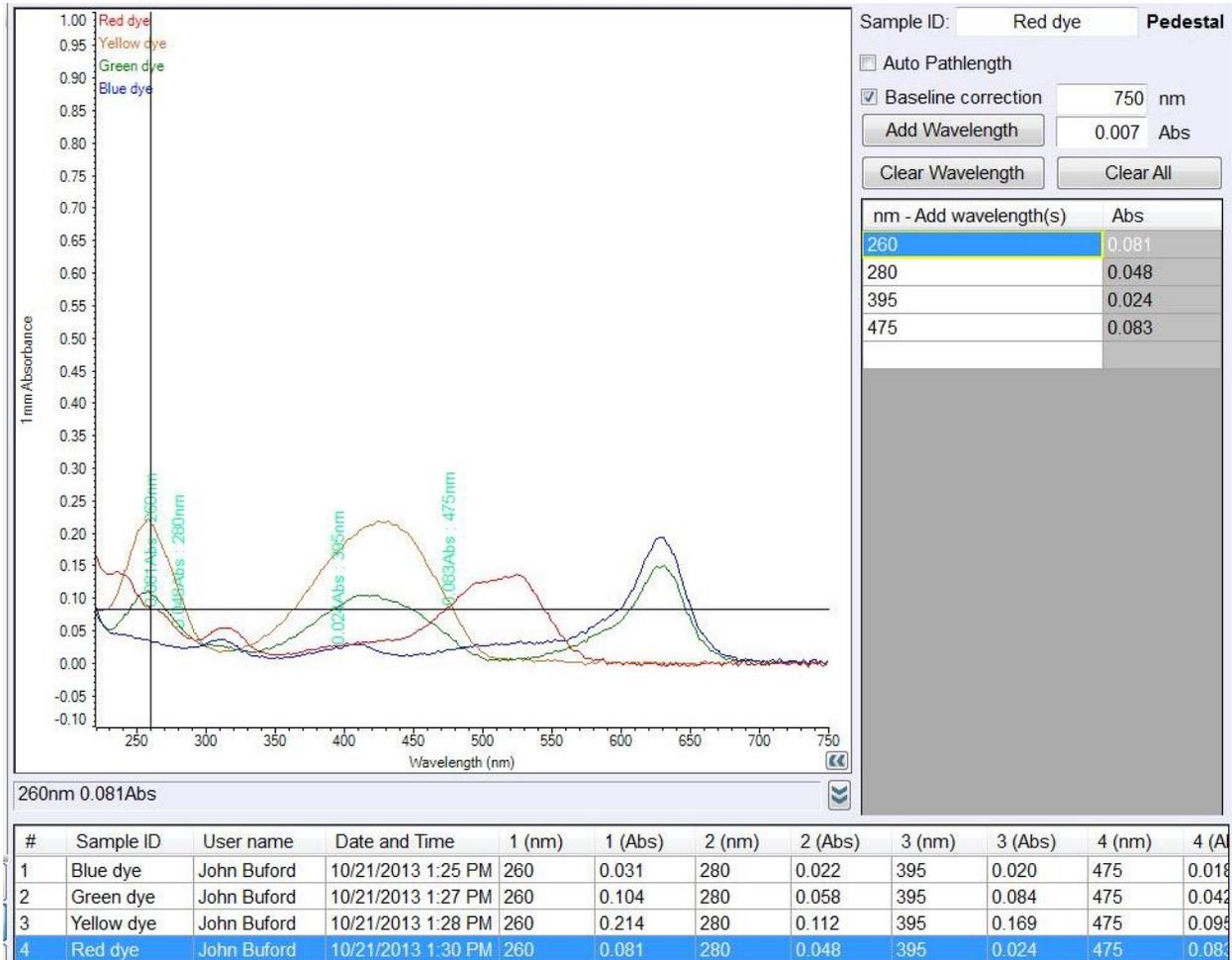
Figure 5. UV-Vis Spectral Graph

11. History

Revision Number	Effective Date	Preparer	Description of Change
0	10/24/2013	John Buford	Initial release