

Baseline Correction

Technical Note 119

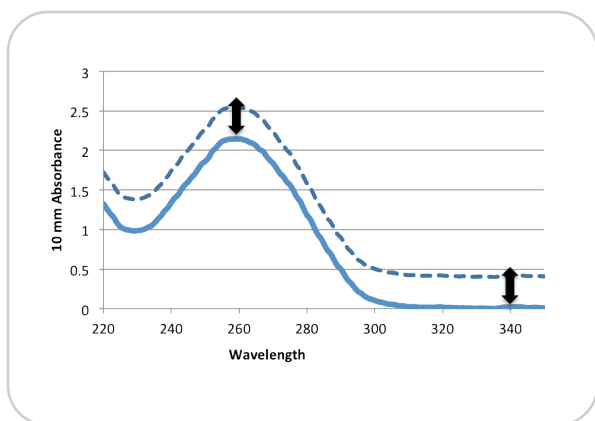
Introduction

The DeNovix® DS-11 spectrophotometer uses an automatic single-point correction for most applications. The correction subtracts the absorbance value at a specific wavelength from all wavelengths across the sample spectrum. The correction is used to account for the effect of instrument noise and light-scattering particulates in the sample that can cause an offset in the overall sample absorbance.

This technical note describes how baseline corrections are handled in various DS-11 apps and provides helpful hints on selecting baseline wavelengths for custom dyes and methods.

Potential Baseline Offset

Figure 1 below illustrates how an incorrect absorbance reading can be obtained from a non-baseline corrected sample.



The dotted line represents a sample exhibiting a significant shift from the baseline at 340 nm. The solid line represents the same sample that has been baseline corrected. For this example, the reported absorbance at 260 nm and therefore the calculated concentration would be about 20% higher than expected.

Baseline Wavelength Selection

The optimal wavelength to use for baseline correction is a wavelength at which there is no absorbance attributed to the sample buffer or the molecule of interest. Most methods for standard absorbance based assays found in the literature will include the wavelength used as for baseline correction. A general recommendation would be to use 340 nm for methods that use UV only wavelength ranges (190 nm to 350 nm) and 750 nm for methods using ranges that extend to 750 nm or greater.

Assessing a Baseline Correction Wavelength

1. Ensure that both the top and bottom measurement surfaces are clean.
2. Launch the **UV-Vis** app, and deselect the 220 to 750 nm range to enable the 190 to 840 range.
3. Access the Baseline Correction option from the Overflow menu (three vertical dots icon in upper right of screen) and select the **None** option.
4. Make a **Blank** measurement using 1 μL of dH_2O . Remove with a dry lab wipe.
5. Pipette a fresh 1 μL of dH_2O aliquot onto the surface and tap the **Measure** button.
 - The spectra should result in a relatively flat line along the baseline. If there is significant absorbance in any region, clean the surfaces and repeat steps 4 and 5.
6. Pipette 1 μL of the sample buffer onto the surface and tap the **Measure** button.
7. Ensure that the buffer does not have significant absorbance at the wavelength to be used for the correction.
8. Pipette 1 μL of the sample onto the surface and tap the **Measure** button.
9. Ensure that the sample does not have significant absorbance at the wavelength to be used for the correction.



Nucleic Acids and Protein Apps

Baseline correction for nucleic acid and protein applications have been traditionally performed at 320 nm. However, with the advent of microvolume spectrophotometers capable of reading highly concentrated samples, the correction is now routinely done using 340 nm. The DeNovix® dsDNA, ssDNA, RNA, Protein A280 and the Peptide apps all use 340 nm as the default baseline correction wavelength. Users may change the selection by using the Baseline Correction feature accessed from the app Run screen Overflow menu.

Microarray and Labeled Proteins Apps

The software anchors the visual spectrum displayed in both the Microarray and Labeled Protein apps using the baseline at 750 nm for all measurements. A 340 nm bichromatic normalization is applied for the nucleic acid or protein analysis wavelengths (260 nm or 280 nm respectively,) and a sloping baseline between 400 and 750 nm is applied to the dye-specific analysis wavelength dye concentration calculations.

When adding a new dye, it is important to empirically determine the appropriate baseline correction wavelength. User-defined options for Microarray and Labeled Protein apps are enabled between 750 and 840 nm. Consider using a baseline correction at 800 nm or greater for dyes with absorbance maxima greater than 700 nm.

The new selection will be used for both the visual display and the sloping dye baseline. Once a baseline correction wavelength is selected, it is applied to all subsequent measurements and is not dye specific. When changing dyes, always confirm that the proper baseline correction is applied.

The wavelength currently in use is displayed in small print at the bottom the Run screen.

Colorimetrics App

The default baseline correction wavelengths are preconfigured for each of the included assays types and may not be changed.

UV-Vis App

The default baseline correction wavelength for the UV-Vis app is 750 nm although it is suggested that the optimal wavelength be empirically determined for each sample type. Use the Baseline Correction feature accessed from the Overflow menu to change selections.

Custom Method Apps

A baseline correction wavelength is required for the Standard Curve app methods but not for Formula Methods. The optimal baseline correction wavelength should be empirically determined for each method and should take into account the sample type and reagents to be measured. A general recommendation would be to use 340 nm for UV only wavelength ranges and 750 nm for methods including the Vis wavelength ranges.

OD600 App

Microbial cell culture OD 600 values are measurements of light scattering. The DS-11 software does not use a baseline normalization unless specified as a user selection.

Kinetics App (DS-11+)

A baseline correction wavelength is required for all Kinetics app methods. As in all custom methods, the optimal baseline correction wavelength should be empirically determined.

DeNovix, Inc.

3411 Silverside Road - Hanby Building
Wilmington, DE 19810 USA

Phone: +1.302.442.6911
Email: info@denovix.com
www.denovix.com

