Tilt Correction Algorithm

To Remedy Drifting Absorbance Spectra with 2 Known Intercepts

Tech Tip

Introduction

When running certain absorbance studies there may be environmental factors that affect the output of the light source, the transparency of some transmissive component, or some other optical inconsistency. These irregularities, when occurring in muted amounts, can often be corrected and the spectral output readjusted.

This is especially true if the sample has 2 known x-intercepts, or wavelengths where absorbance is *supposed to be* unchanging or independent of experimental variables. In this scenario the slope can be determined between these points and a linear correction may be applied to all observed wavelengths.

Example and Derivation

In the example shown below, we look at an aqueous titration of the common pH dye bromocresol green, which has an analytical base peak at 627nm (largest pH dependence), an isosbestic point at 510nm (pH-independent), and a large region in the NIR that remains pH independent (750nm was chosen).

OceanOptics

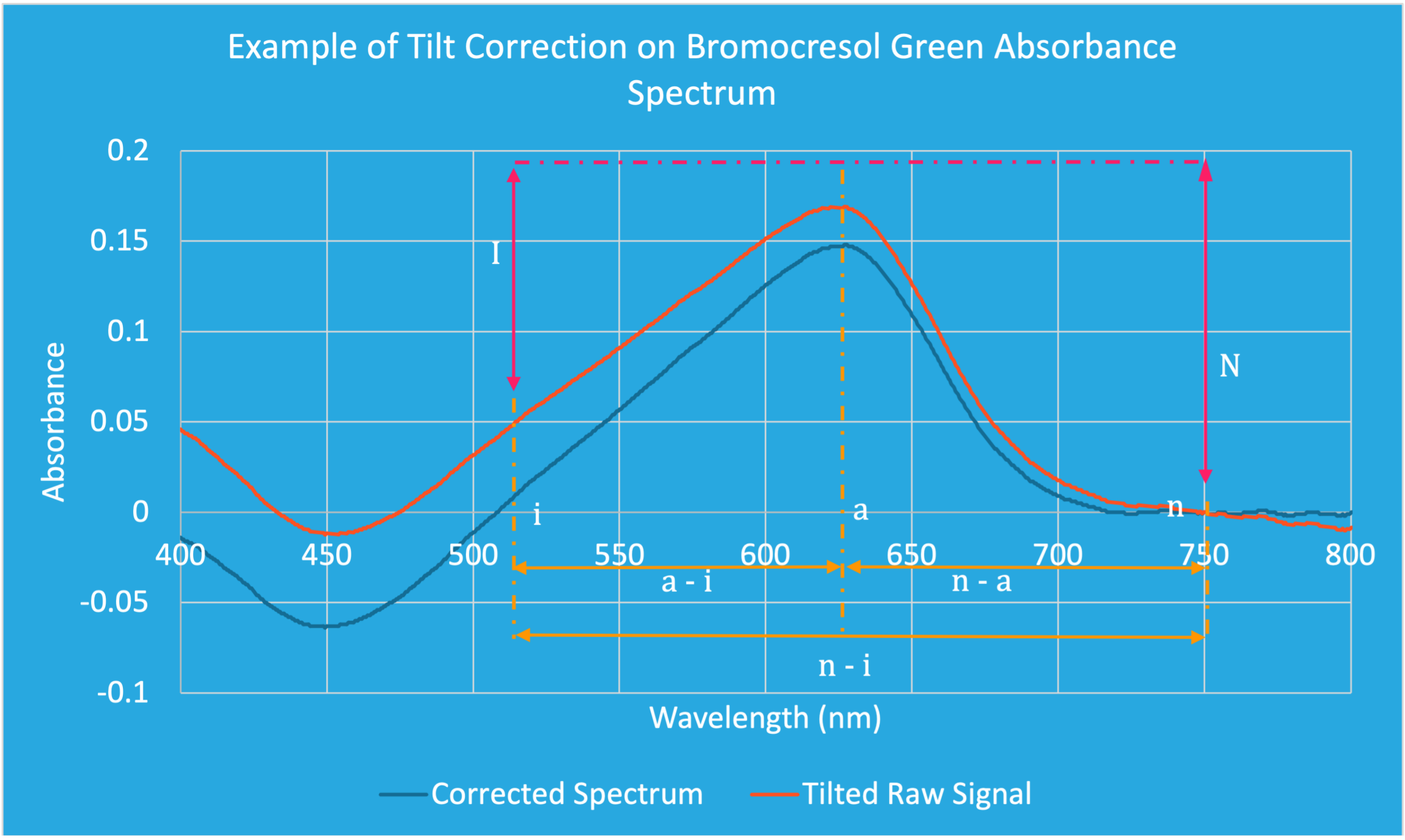

Figure 1

The blue plot is what the spectrum should look like, but we are seeing a tilt upwards in the lower wavelengths of the raw orange plot. We can set up the adjustment in simple *mx+b* terms:

$$A_{Corrected}(\lambda) = b + \lambda m$$

$$A_{\lambda,Corrected} = (A_{\lambda} - A_{750nm}) - (750-\lambda)\times\left(\frac{A_{510nm}-A_{750nm}}{750-510}\right)$$

A_{λ} = Absorbance at wavelength λ

a = Analytical Wavelength (nm)

n = NIR Baseline (nm)

i = Intermediate Baseline (nm)

$N = A_a - A_n$ *Single-Wavelength NIR Correction*

$I = A_a - A_i$ *Single-Wavelength VIS Correction*

$$A_{a,Corrected} = (A_a - A_n) - (n-a)\times\left(\frac{A_i - A_n}{n-i}\right)$$

$$N - I = (A_a - A_n) - (A_a - A_i) = A_i - A_n$$

$$A_{a,Corrected} = N - (n-a)\times\left(\frac{N-I}{n-i}\right)$$

$$A_{a,Corrected} = \frac{N(n-i)}{(n-i)} - \left(\frac{N(n-a)-I(n-a)}{n-i}\right)$$

$$A_{a,Corrected} = \left(\frac{N(n-i)-N(n-a)+I(n-a)}{n-i}\right)$$

$A_{a,Corrected} = \left(\frac{N(n-i)-N(n-a)+I(n-a)}{n-i}\right)$ *Simplified Tilt Equation*

Analysis

The most basic approach to baseline correction is subtracting out a single wavelength known to be a consistent x-intercept. However this cannot adjust for the tilting effect and can give misleadingly erroneous peak values. As you can see below, the orange and yellow plots each share *one* x-intercept with the blue plot, but do not have the other correct intercept and therefore give skewed analytical peak values.

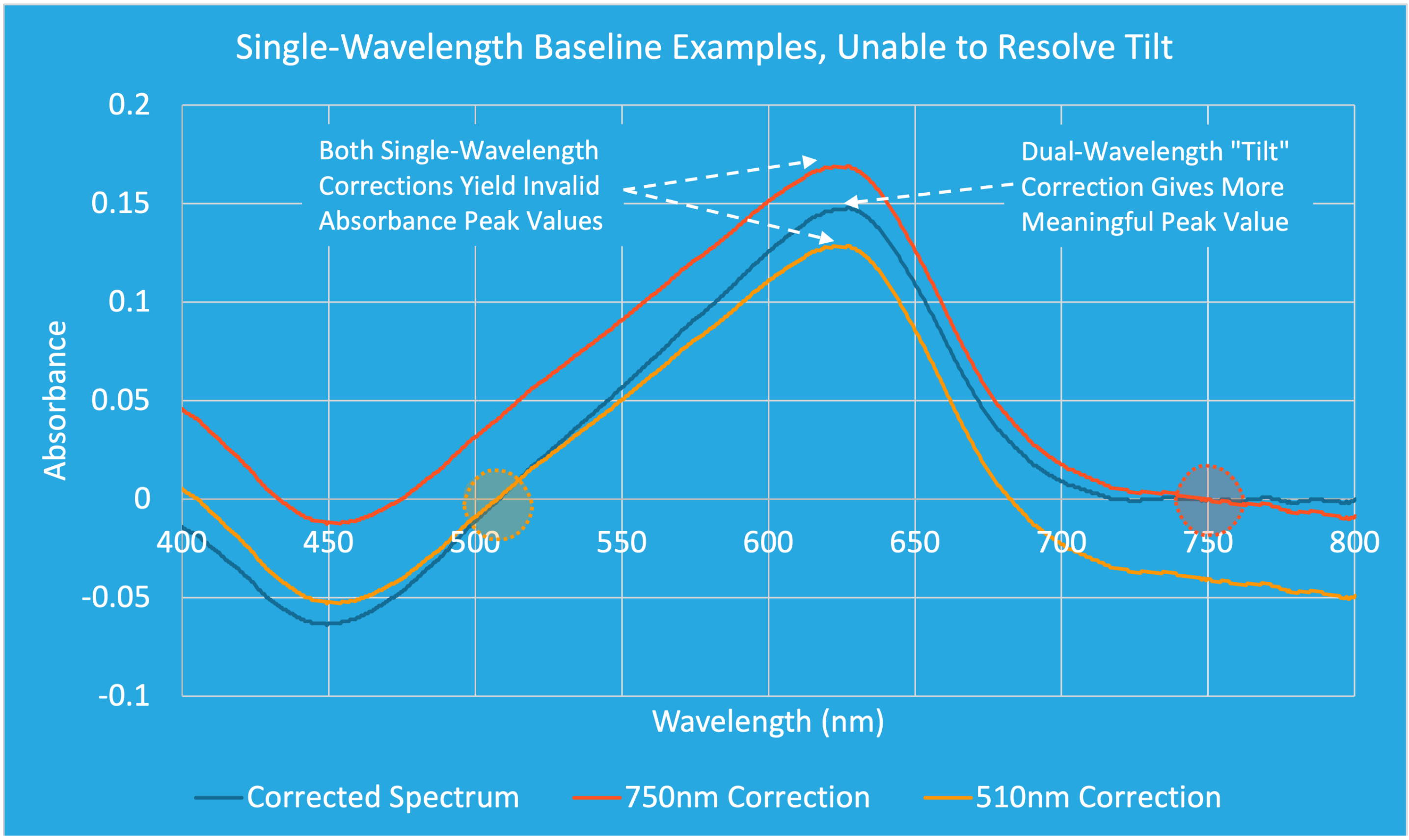

Figure 2

From the plot above, it looks as though the blue peak may be a mere average of the orange and yellow peak values since it sits right in the middle of those. The math for the two approaches actually looks quite similar, though as you can see what we are adding is a "weight of influence" for one baseline correction over the other based on the specific locations of the chosen wavelengths.

$$A_{a,Corrected/Averaged} = \frac{N+I}{2}$$

Basic Averaging of 2 Single-Wavelength Corrections

$$A_{a,Corrected/Tilted} = \frac{N(a-i)+I(n-a)}{n-i}$$

Simplified Tilt Equation

When looking at Figure 1 above as well as the simplified Tilt equation, we see that we are essentially creating a movable "slider" of position *a* within extremes *i* and *n*, which then adjusts the ratio of *a – i* to *n – a*.

Discussion

This linear derivation of a slope-based correction can yield more accurate absorbance values than typical single-wavelength subtractions. There are researchers who will even take the raw absorbance peak value without any correction employed, trusting that the discrete pixel value is valid enough on its own. This may be safe for self-contained systems with no moving optics, but any fiber optic assembly or setup with modular components should have some level of sanity check on the analytical values against at least one reference.

Furthermore, this mathematical method can be applied to monitoring key wavelengths over time. This can provide more meaningful values for process monitors or alarms that may see this type of optical drift over extended periods. The example below shows how using a single wavelength subtraction method causes the tracked analytical absorbance value to skew either high or low, but the Tilt Correction method holds the 627nm analytical value relatively steady.

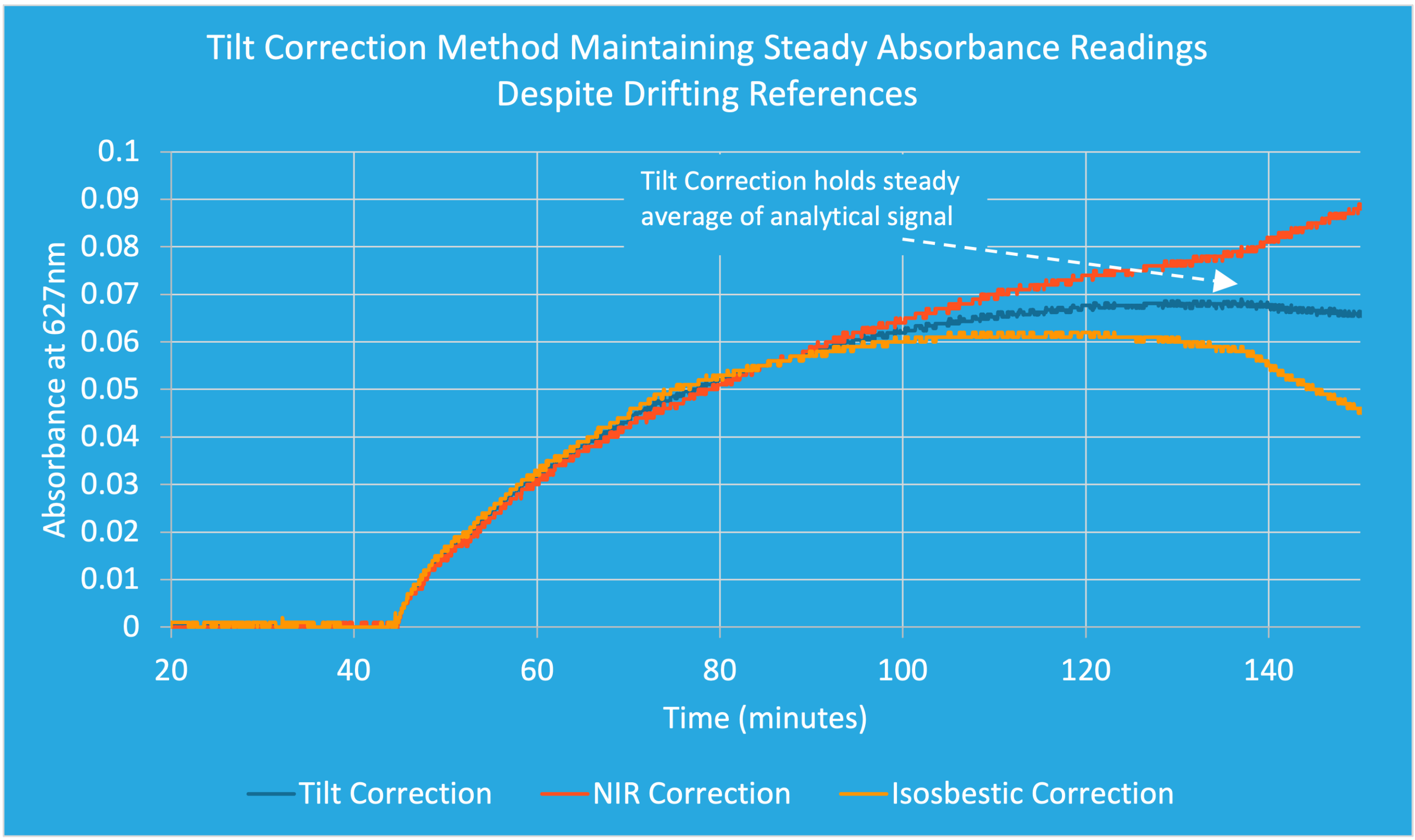

Figure 3

This numerical method has much value as well as limitations. If the user is wrong about one or both of their baseline wavelength selections, the resulting absorbance values may be even less valid than if no correction method was used. The example shown here used a very well-known pH dye with a sharply-defined isosbestic point and an obvious region of pH independence. Of course there is also some limit to what level of distortion this can correct; if film growth, discoloration, or some other influencing factor becomes too extreme this method will eventually lose validity. Nonetheless the approach may also be used for similar optical effects seen when working in transmittance and reflectance modes, and may be applicable to setups using cuvettes, dip-probes, flow-cells, microtiter well plates, reflection probes, and any number of potential accessories.