Mark One Lifestyle, Inc.

# Cuvette Placement and Replacement for the StellarNet Spectrometer

**Data Collection**

- Date: 2015-04-21 and 2015-05-21
- Tickets: AS-293
- Git Repo: data.mkone.co/var/git/science/vessyl/as-293.git
- Git Branch: master

**Analysis**

- Ticket: VA-92
- Git Repo: git.mkone.co:vessyl-algorithms/algorithms.git
- Git Branch: va-92

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## Revision Control

| Revision Number | Revision Date | Notes | Owner |
|---|---|---|---|
| 1.0.0 | 2015-05-10 | First draft release | Ehson Ghandehari |
| 2.0.0 | 2015-05-15 | Second draft release | Ehson Ghandehari |
| 3.0.0 | 2015-05-22 | Third draft release | Ehson Ghandehari |

## Executive Summary


- The maximum deviance for cuvette replacement studies was 4%.

# Introduction

Mark One Lifestyle has one StellarNet Silver Nova spectrometer setup. This setup has a custom cuvette holder that is designed to control the position and alignment of the cuvette with respect to the fibre optic cables. The goal of this study was to characterize the variation in the spectrum between sequential removals and insertions of the cuvette in the holder. Low variation would indicate that the cuvette holder was able to maintain positional and alignment tolerances. These tolerances are important as it is known that the geometry of the optical fibre and cuvette will alter the measured spectrum.

# Methods

## Setup

The setup used in the data collection was the Red golden setup. The Red golden setup consisted of a light source (Ocean Optics DH-2000; serial number 005400821), an optical fibre (Thorlabs; M200L02S-UV), an optical switch (Ocean Optics; INLINE-TTLS ), a custom bifuricated fibre optic reflection probe (Thorlabs; 6 fibres from the optical switch to cuvette and 1 fibre from cuvette to the spectrometer; the length from the optical switch to the cuvette or the spectrometer to the cuvette is 1 m each; the fibre used in this reflectance probe is a Thorlabs (FG550UEC), a custom refurbished cuvette holder (Mark One Lifestyle Inc.), a quartz glass cuvette (Thorlabs; CV10Q3500F) with a custom aluminum mirrored surface, and a spectrometer (StellarNet; Silver Nova model, serial number 15040704). The light source was connected to the optical switch by M200L02S-UV fibre. The optical switch was then connected to the right side of cuvette holder by FG550UEC probe, and the left side of cuvette holder was connected to the spectrometer by the same probe. The spectrometer was placed in an environmental chamber (Espec BTL-433) at a temperature of 15 C and 70% relative humidity. The spectrometer responded to a wavelength range from 178.28 nm to 1121.7 nm. A linear silicon CCD array detector was able to distinguish between 2051 wavelengths.

## Data Collection

The data was collected under section 3.5.2 of the Wavelength Selection DOE. The cuvette was cleaned by rinsing it three times with distilled water using a squirt bottle. Then it was rinsed once with isopropyl alcohol (IPA) and blown dried with pressurized air. The clean and empty cuvette was placed in the holder and clamped into position. The mirrored surface was contralateral to the fibre cable. A series of 128 scans, 10 trials, was collected and stored. The cuvette was removed from the holder and then reinserted. Another trial was taken. This procedure was repeated for a total of ten trials.

The data was collected on red setup with one operator (Ehson).

## Data Analysis

The data was converted from XML format into tidy formatted comma separated values and all trials were concatenated together and compressed. This was done using a custom bash script that calls gsxmltidy.py and the output is concatenated. This output is then

compressed using gzip. These compress files were read into R version 3.1.2 (2014-10-31). Within R a four stage cleaning process was performed. All stages are optional and can be performed in any order. They include a conversion phase where the data is converted into the correct data type. This phase is often performed, in part, during the read operation. The transform phase is used to manipulate the values of the data and correct any errors. During the filtering phase, unneeded or erroneous data is removed from the data set. In the final phase the data is transformed into tidy format, if required.

After the cleaning process, the data is run through a series of checks. These checks are independent of the cleaning phase and they test the quality of the data and assumptions that are being made. These would include, but not limited to, checks to see if the data is the correct data type, if there are missing values, the correct number of levels exist in a factor, values are in range and to make sure that all data is present.

The light source produces several very large spikes in the spectrometer data. This causes saturation in some wavelengths and bleed over into the adjacent wavelengths. Therefore, if the reflectance data was clipped, reflectance measures greater than 58890, in any trial it was removed for all trials collected by a given operator. To remove the bleed over, wavelengths that were +/- 1 nm an either side were also removed.

The AS-293 data were loaded, cleaned and checked.

## Results

The operator performed 40 trials (two different days; about a month apart) and each trial consisted of 128 scans. All scans for an operator were combined and the mean, per wavelength, was plotted. The thickness of the line, at each wavelength, was plus or minus one standard deviation.

Figure 1, shows the mean reading collected during trials on an empty cuvette in the Red Setup. A different colour is used for each trial and a qualitative assessment shows that the signals recorded were in general agreement between placements. The graph showed a higher amount of variability in the 550-900 nm range. This can be seen by the fact that the samples to not overlay each other.

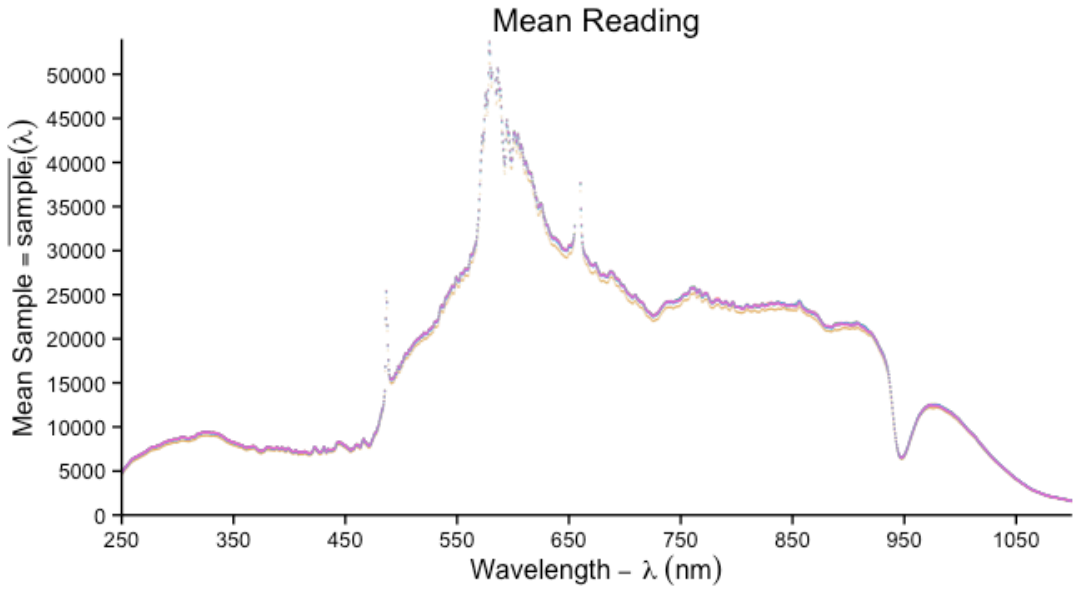


**Figure 1**: Mean readings of 10 trials/placements for the Red Setup. Each trial was conducted after removing and replacing the cuvette.

The mean value, within a wavelength, was computed across all trials. Then all readings were subtracted from the mean value for its respective wavelength. This centred the data and is referred to as the deviance. Figure 2 shows the 2604800 data points that were recorded and their deviance. A separate colour was used to represent each placement. If the placement had no effect on the measurement then the data should appear as a uniformly distributed cloud with no apparent pattern to the colour. However, it is apparent that at lease one trial, shown in black, was quite different from the others in 500-900 nm wavelength range. The shape of the data is not uniformly distributed. This may be the result of noise scaling because of the difference in the signal's amplitude, as can be seen in Figure 1. To examine the readings in more detail it was necessary to reduce the variance within a trial, by computing the mean measurement across all scans for each trial. These trial means then had the global means, within a wavelength, subtracted. This in effect centered the mean values within a trial. Figure 3, shows the mean deviance for each trial in a different color.

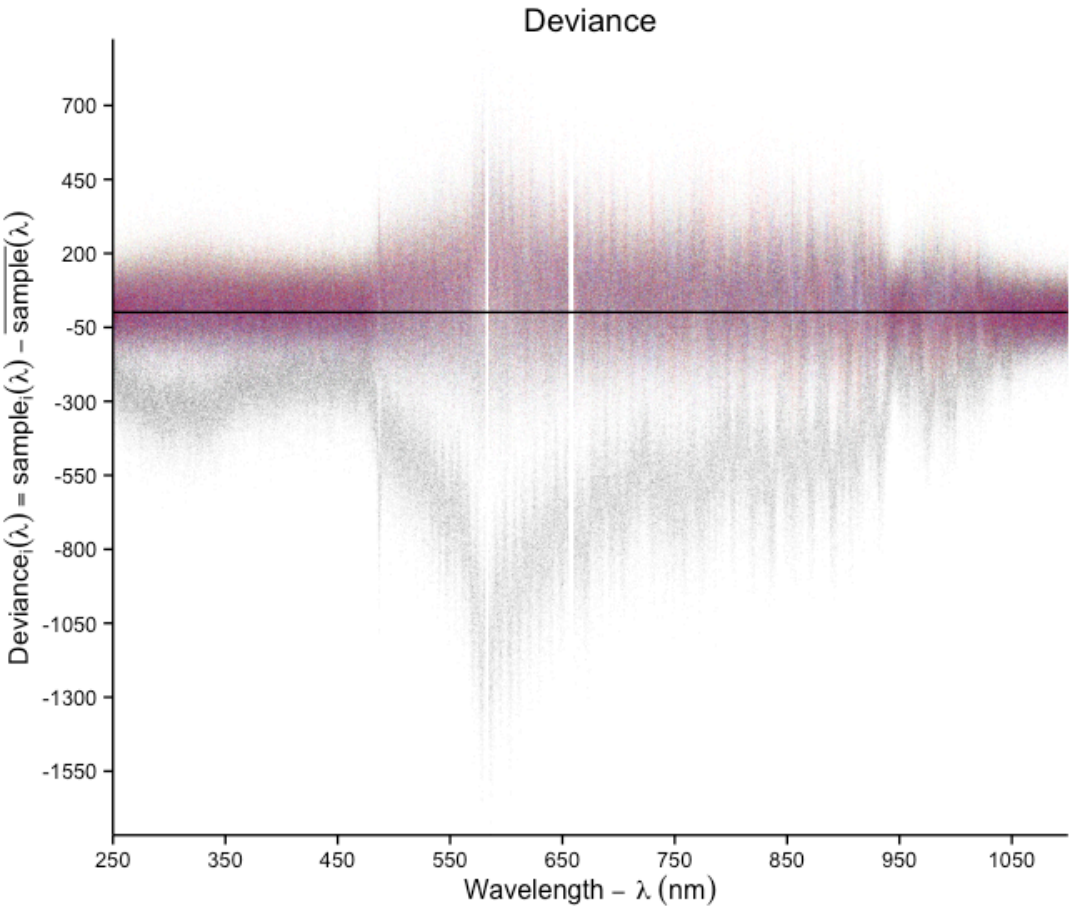


**Figure 2**: Deviance from the overall mean for each reading. The each trail/placement is a different trial.

There were 10 trials, but only 2 patterns are easily discernible. The figure clearly shows that there is overlap of the trials and this demonstrates a high degree of repeatability in placement at least most of the time. Figure 3 also shows that the spread of the mean data points is much smaller then the deviance seen in the raw sample date, Figure 2. The one exception to this is the black trial which shows a greater amount of deviance than the other trials.

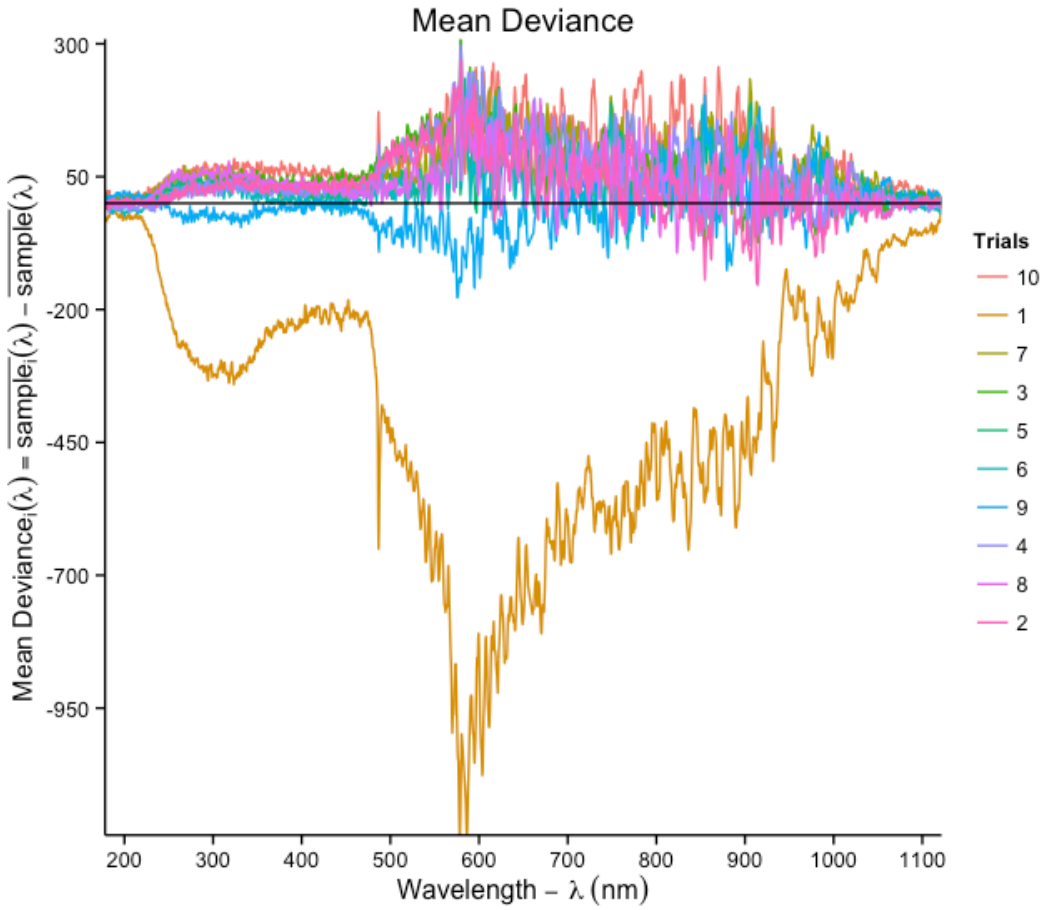


**Figure 3**: The mean deviance for each position/trial. There is some overlap in the trials, thus demonstrating placement repeatability, but it is clear that there is also variance between placements.

The fibre cable that accepts the reflected light filters light where the angle of the light is within a Cone of Acceptance. That is, there is a cone that starts at the fibre and forms a full angle of 25.4 degrees. Light that does not travel within that cone, it is filtered out and not passed to the spectrometer. The cuvette holder is designed to be nearly orthogonal to the light source and transmission cables. Since the contralateral surface of the cuvette is mirrored such that light is reflected back. If the cuvette is tilted therefore the mirrored surface is also tilted. Any tilt in the mirror will reduce the amount of light reflected directly back into the the transmission fibre via the Cone of Acceptance.

Comparing Figures 1 and 3 show that the deviance may be proportional to the amplitude of the signal. Figure 4, is the mean deviance of each trial that has been normalized to the mean amplitude of the signal, reported as percentage, as shown in Equation 1:

$$Normalized\ Deviance_i\ (\lambda)(\%) = \frac{\overline{Sample_i}(\lambda) - \overline{Sample}(\lambda)}{\overline{Sample}(\lambda)} \times 100 \qquad Equation.\ 1$$

If there was no dependence on the position or wavelength, the data should be scattered along the line Normalized Deviance = 0. Figure 4 shows that nine of the trials do this within a plus or minus one percent margin of error. Only the black (first trial) showed a maximum error margin of 4% in the extreme sides of the spectrum. The first trial (gold color) was noticably off from the other nine trials.

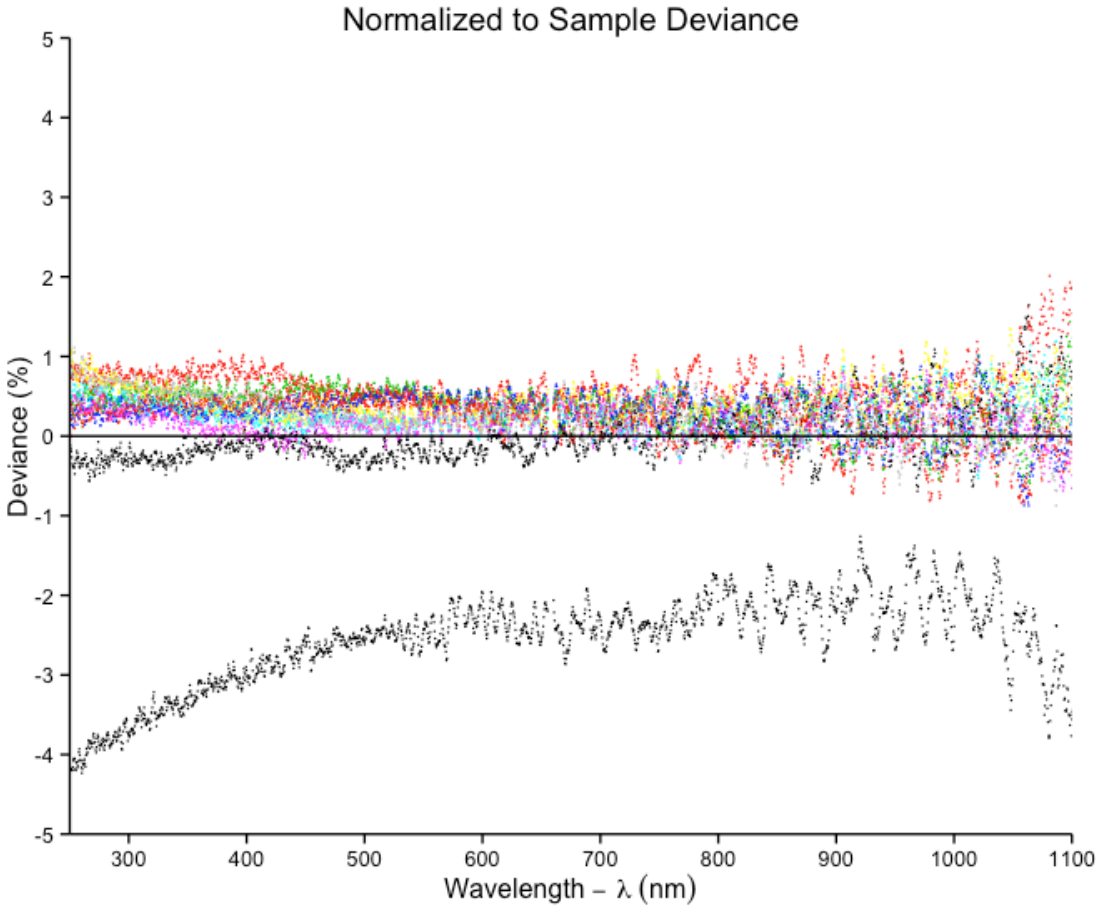


**Figure 4**: Sample data normolized to the sample mean.

There is a possibility that the light source has a time drift. In order to eliminate this possible source of variation, the raw data for each trial was normalized to its white measurement by Equation 2:

$$R\ (\%) = \frac{W_i^{IT}(\lambda) - \overline{D}^{IT}(\lambda)}{\overline{W}^{IT}(\lambda) - \overline{D}^{IT}(\lambda)} \times 100 \qquad Equation.\ 2$$

Where, $W_i$ is individual white scans, $\overline{D}$ is the average dark signal, and $\overline{W}$ is the average white signal. IT in this whole experiment was 250 ms.

As shown in Figure 5, after white normalization, the maximum cuvette replacement deviance was still 4-5% all along the spectrum.

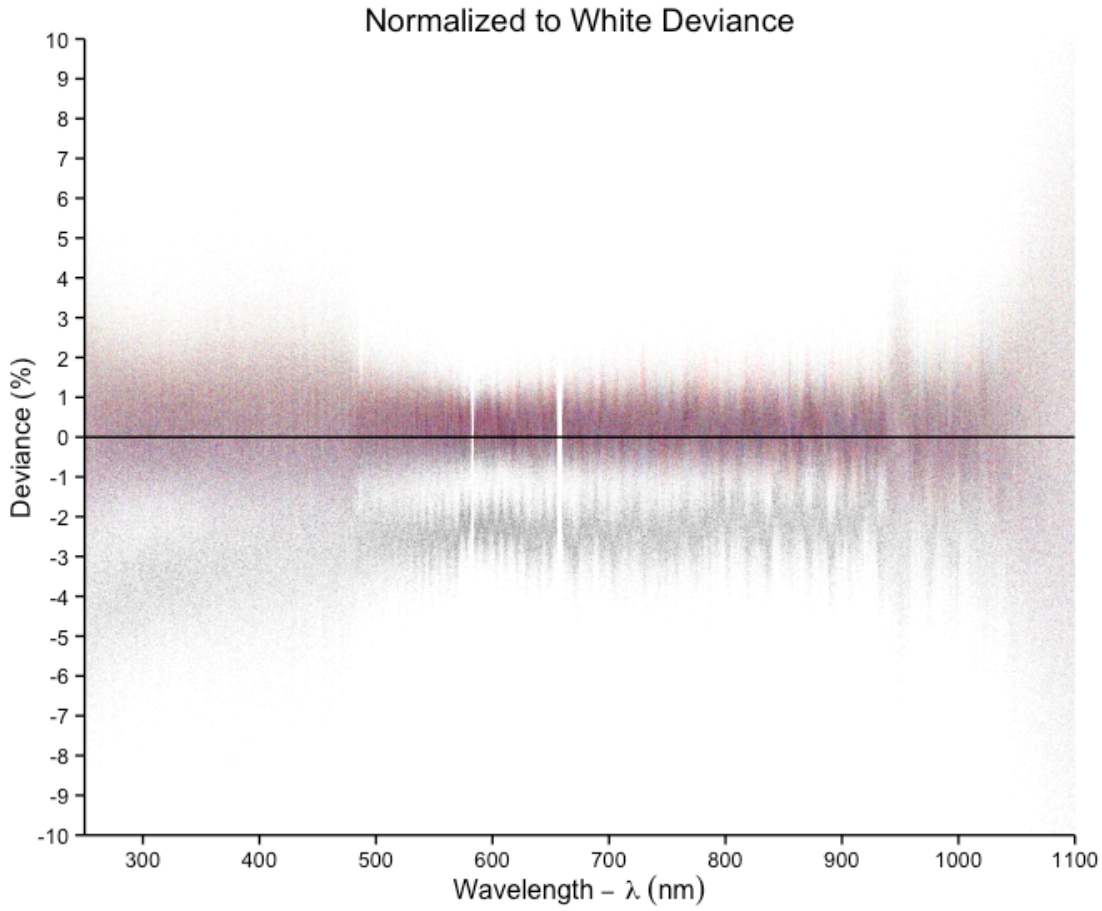


**Figure 5**: Cuvette replacement error after normilized raw data to white signal.

One can argue that the black trial, Figure 4, was an outlier. As examples, the black trial could be caused by operator error (not properly closing the cuvette holder, or replacing cuvette in the wrong direction; aluminium coating touching the light probe) or system error (voltage fluctuation changing lighsource output). As futher investigation, 20 more cuvette replacement trials were collected. Figures 6-10 are the recreation of Figures 1-5, with new 20 sets of data. It should be noted that this measurement was completed about a month after the first data collection. The Halogen light intensity had been reduced in this time period. However, the measurement was still completed in 250 ms integration time

(consistent with prior data collection procedure). The low signal recorded in Figure 6, compare to Figure 1, was caused by lower Halogen light intensity.

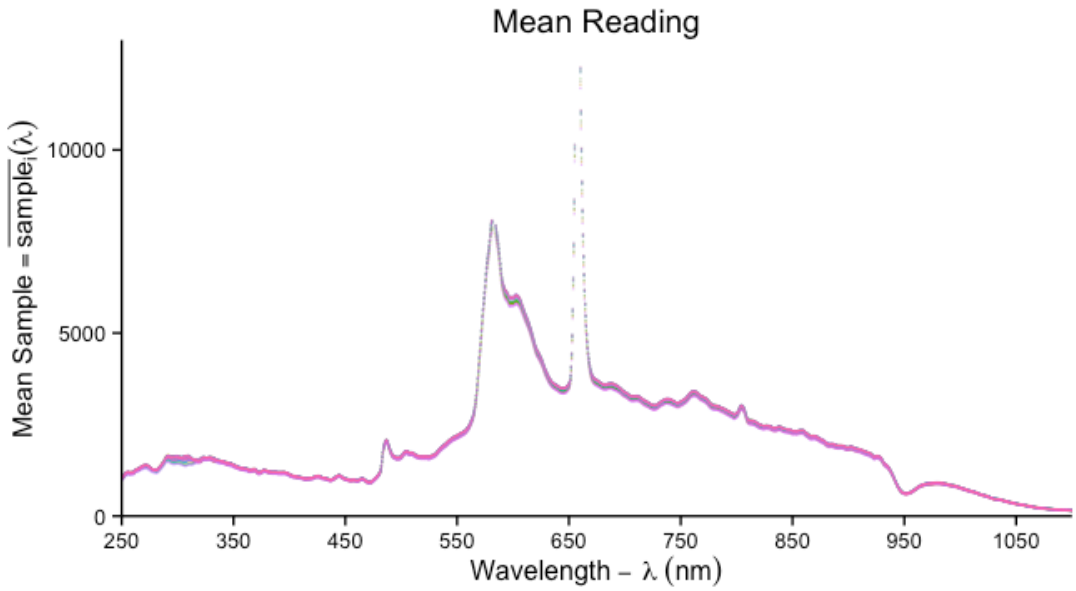


**Figure 6**: Mean readings of 20 trials/placements for the Red Setup. Each trial was conducted after removing and replacing the cuvette.

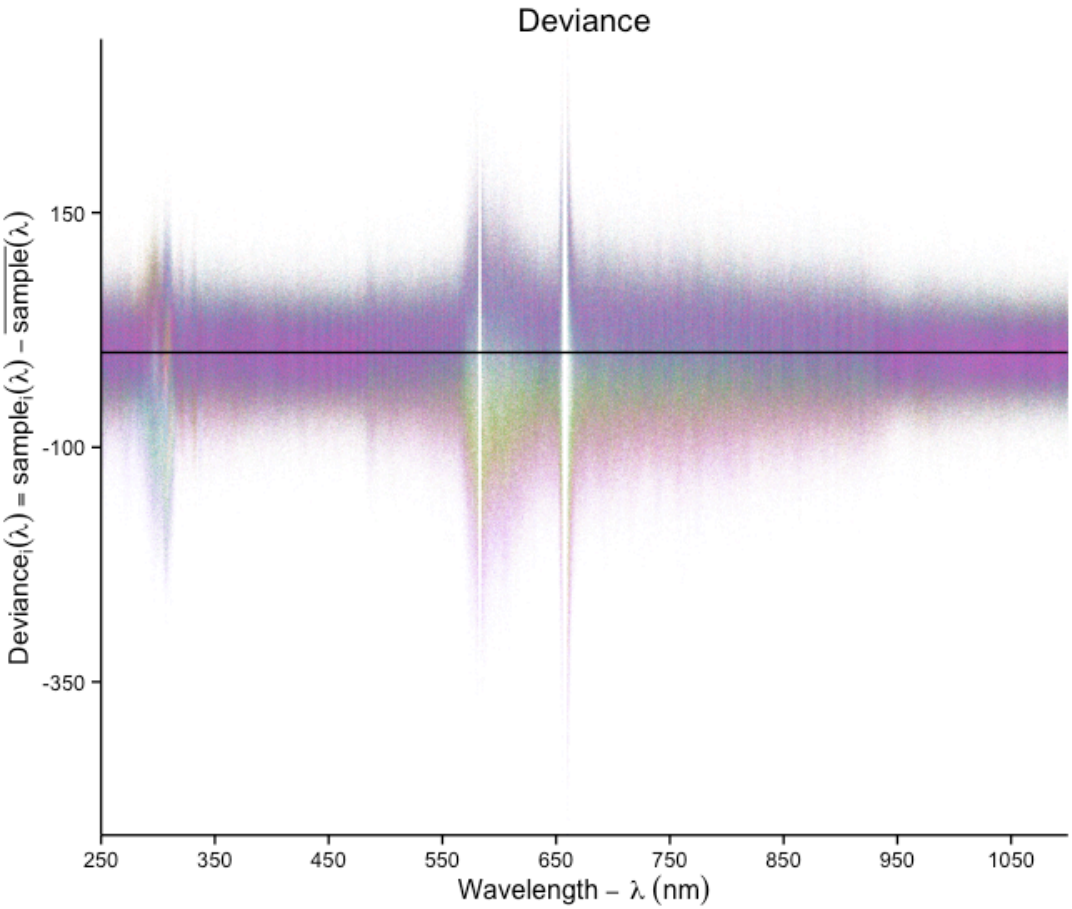


**Figure 7**: Deviance from the overall mean for each reading. The each trail/placement is a different trial.

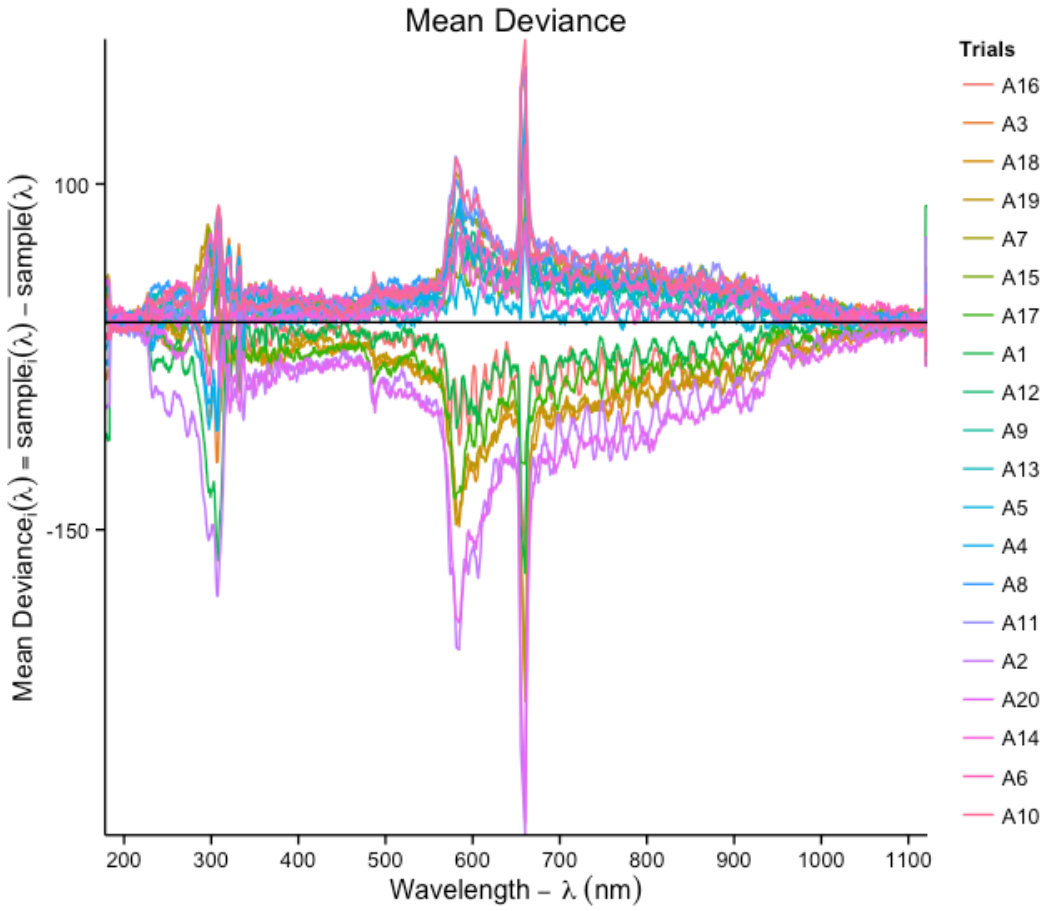


**Figure 8**: The mean deviance for each position/trial. There is some overlap in the trials, thus demonstrating placement repeatability, but it is clear that there is also variance between placements.

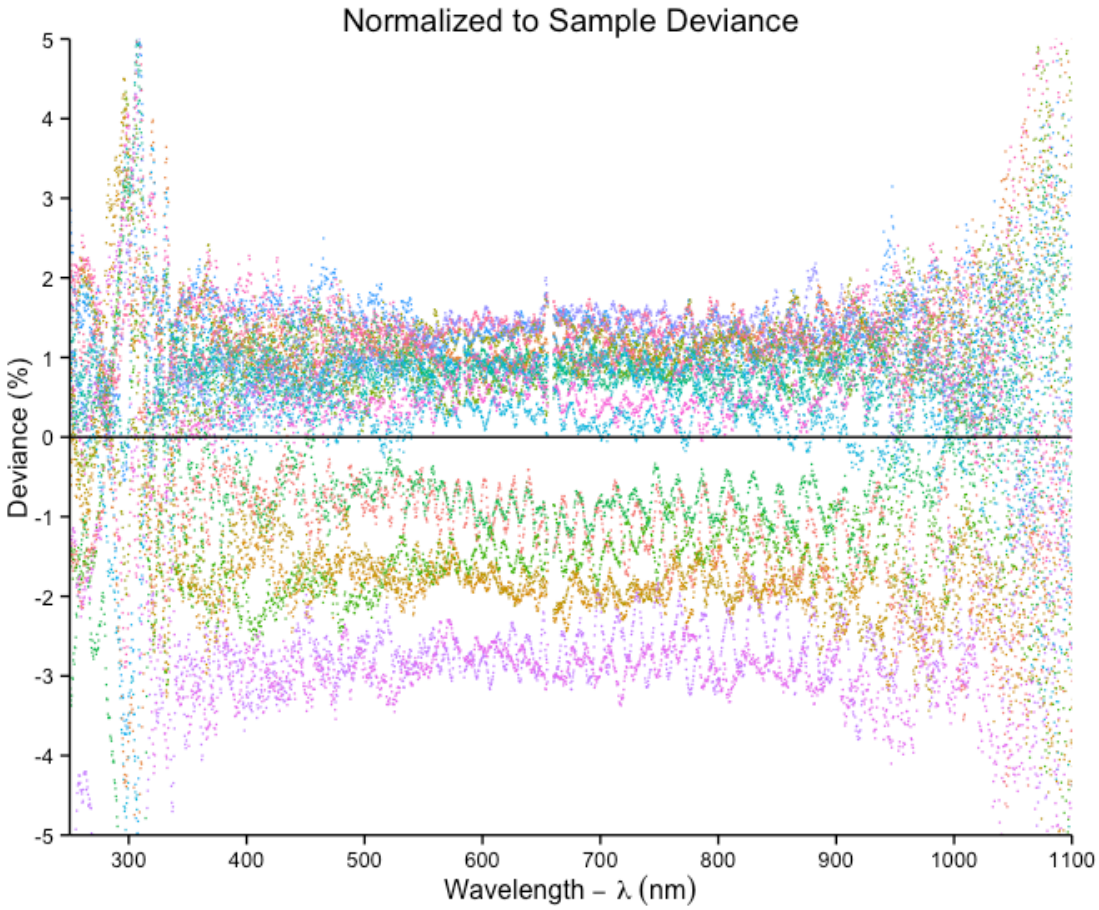


**Figure 9**: Sample data normolized to the sample mean.

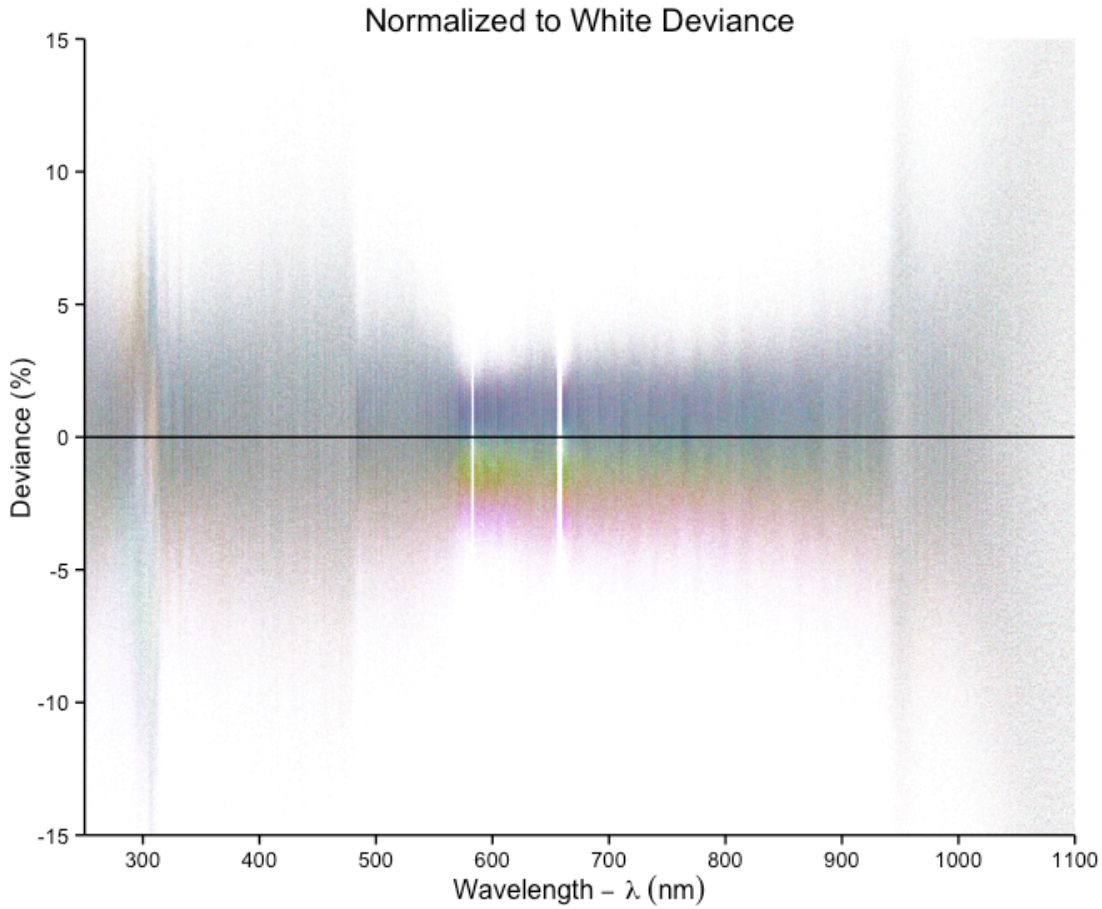


**Figure 10**: Cuvette replacement error after normilized raw data to white signal.

Figures 5-10 proved that the 4% error caused by cuvette replacement was repeatable. In other words, the 4% error observed in Figure 4 was not influenced mainly by an outlier. To further investigate the possible sources of error, the customized Markone cuvette holder was replaced by a Thorlabs CVH100 cuvette holder. Another 10 trials were collected with Thorlabs cuvette holder. Figures 11-15 are recreation of Figures 1-5, using the new 10 trial dataset.

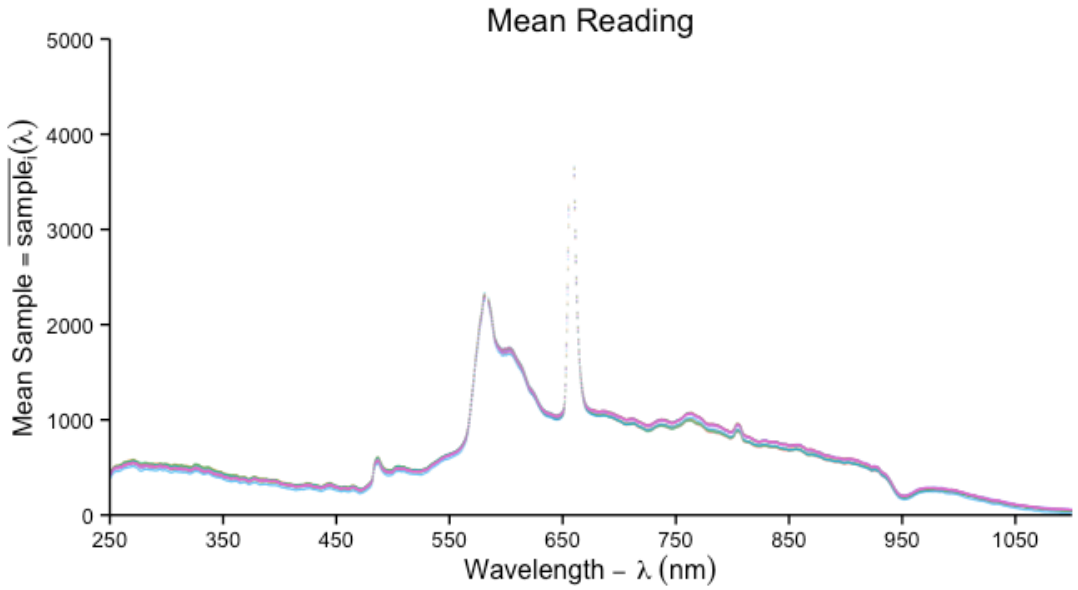


**Figure 11**: Mean readings of 10 trials/placements for the Red Setup. Each trial was conducted after removing and replacing the cuvette. The Thorlabs cuvette holder was used.

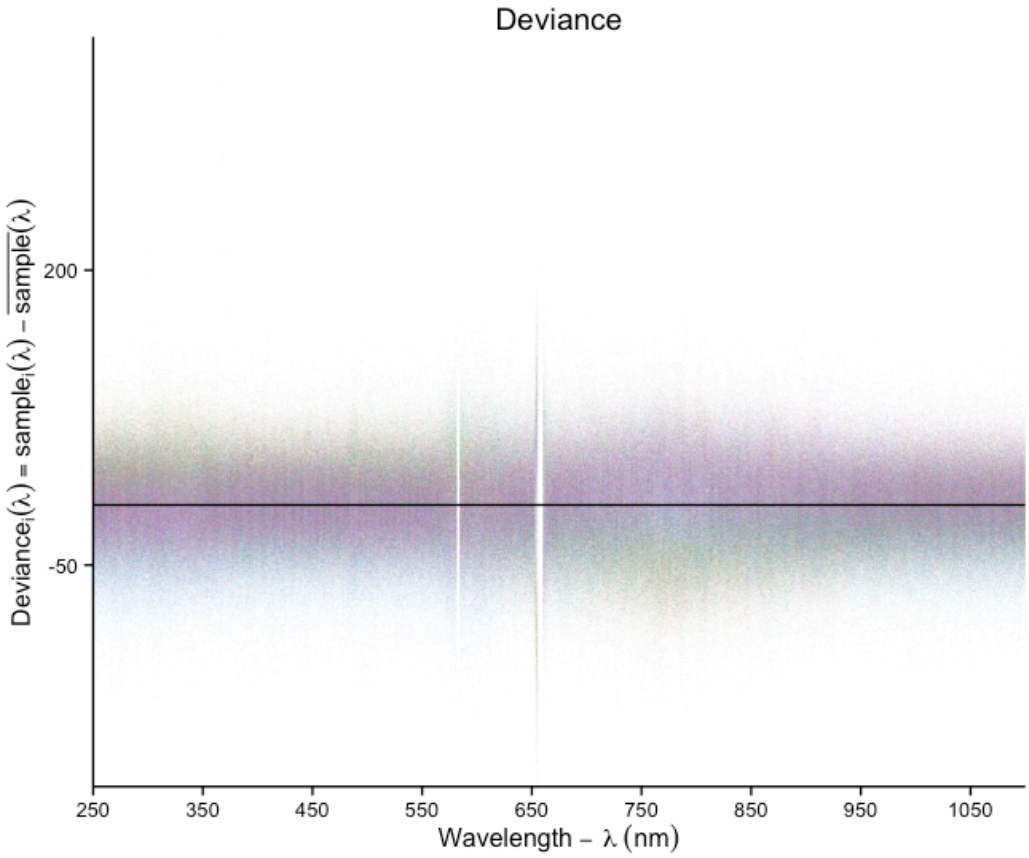


**Figure 12**: Deviance from the overall mean for each reading. The each trail/placement is a different trial. The Thorlabs cuvette holder was used.

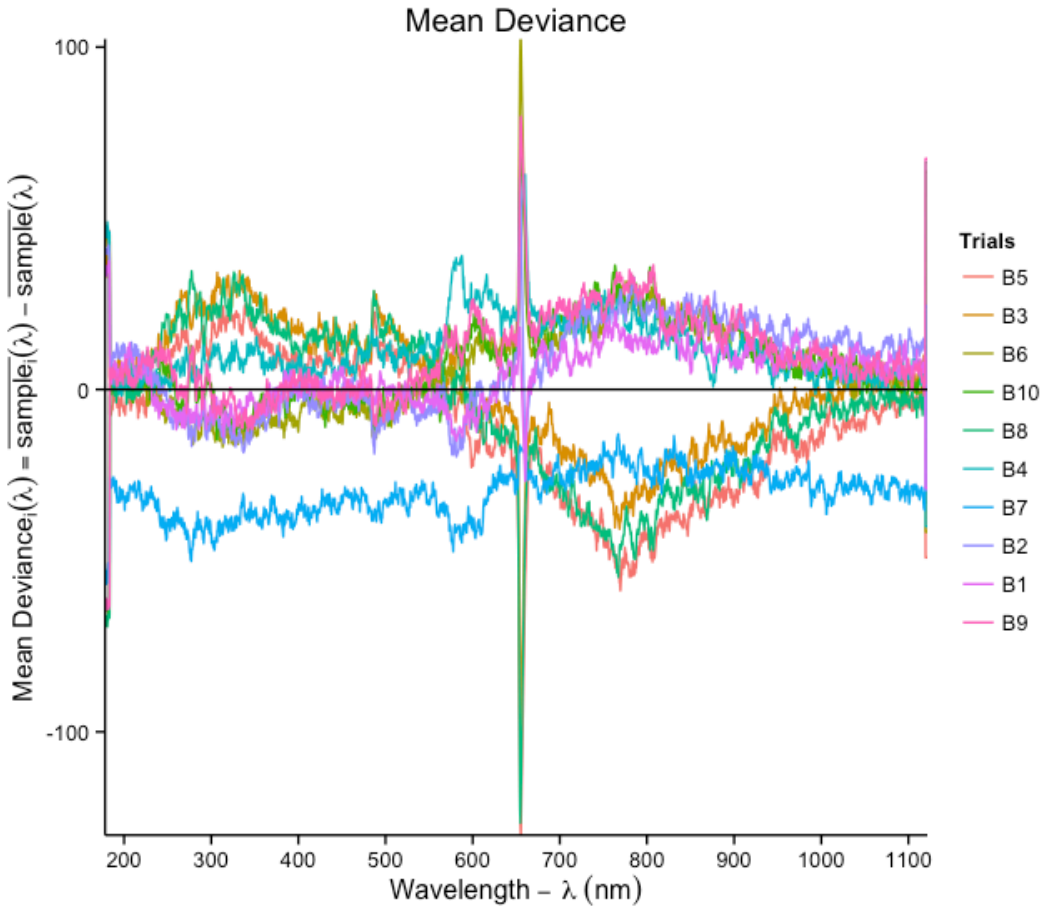


**Figure 13**: The mean deviance for each position/trial. It is clear that there is variance between placements. The Thorlabs cuvette holder was used.

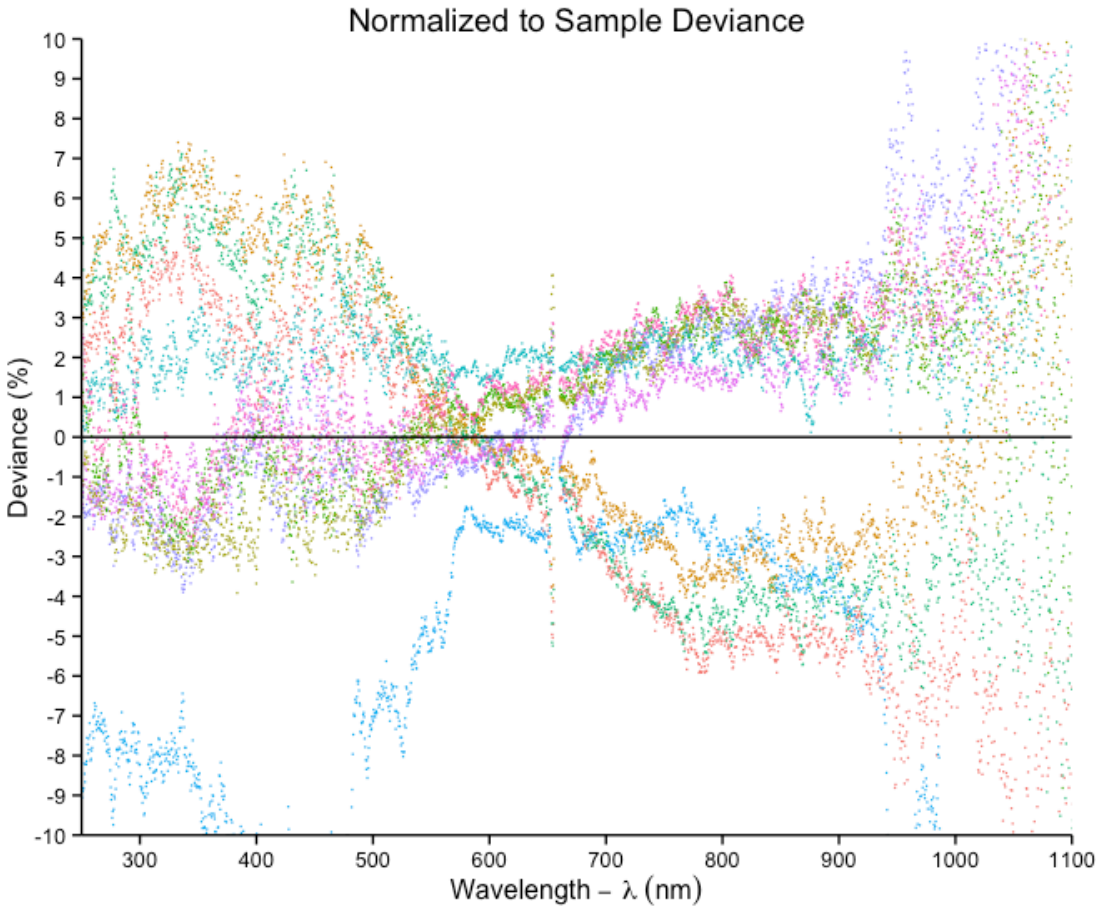


**Figure 14**: Sample data normolized to the sample mean. The Thorlabs cuvette holder was used.

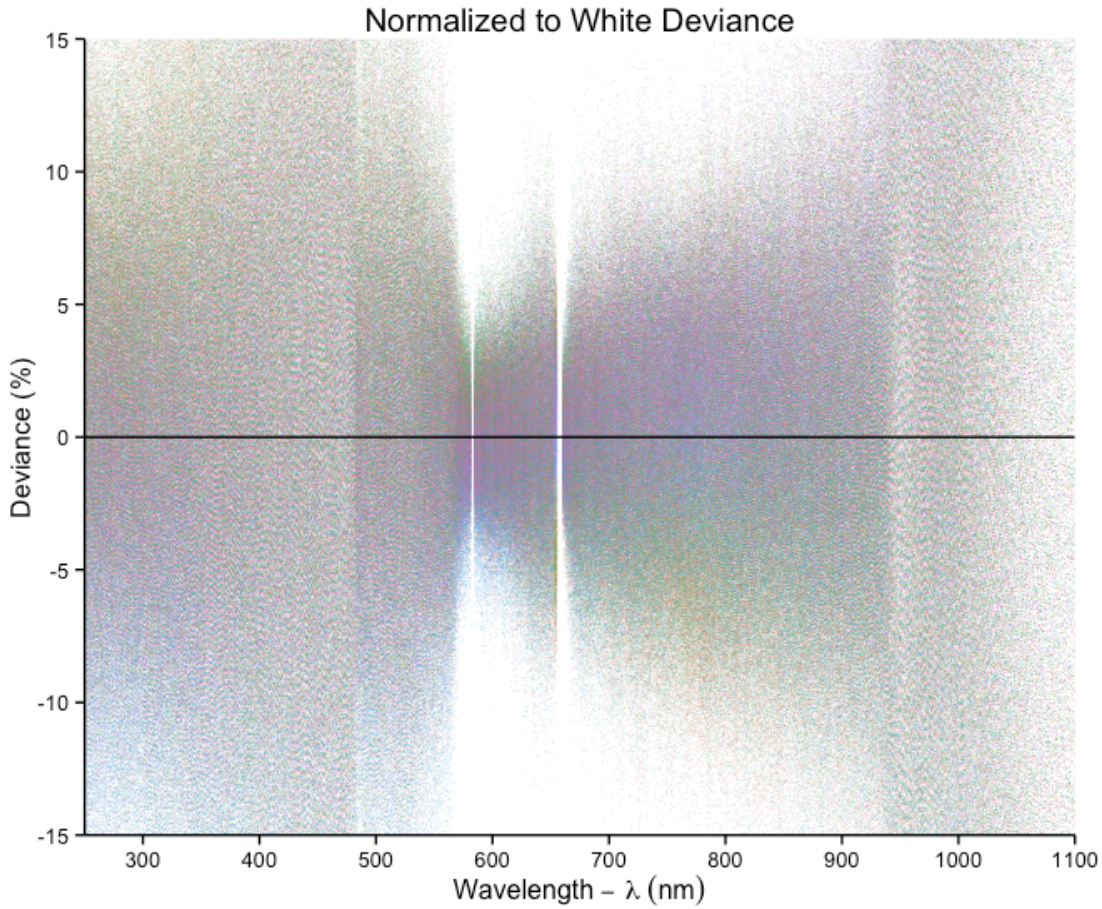


**Figure 15**: Cuvette replacement error after normilized raw data to white signal. The Thorlabs cuvette holder was used.

Figures 11-15 proved that using Thorlabs cuvette holder did not reduce the cuvette replacement error.

## Conclusion

The Red Setup showed normalized deviance error, using the first 10 trials, typically in the range of +/- 1 % but a single trial was as high as 4%. This setup also demonstrated that the deviance error is linearly proportional to the amplitude of the signal. The white normalization of raw data did not reduce the marginal error, and the maximum deviance for cuvette replacement was measured to be 4%. The first trial was observed to be off from the rest of the trials. Since this trial might have been caused be an operator error, a new set of 20 trials was collected. A cuvette replacement error of 4% was still observed. Replacing the customized cuvette holder with a Thorlabs cuvette holder also did not reduce the cuvette replacement error.