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Measuring Optical Properties Using a Spectrophotometer

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October 24, 2018

1 Objective

The objective of this lab experiment was to measure the optical properties such as μ_a , μ_s' and g using a UV-3600 spectrophotometer. Multiple measurements were performed to obtain these parameters and the results were analyzed using Inverse Adding Doubling (IAD) program [1]. This was done to ultimately measure the concentrations of methylene blue and intralipid in an unknown solution.

2 Introduction

Spectrophotometers are instrument, which measure the wavelength distribution of light. They may be used to characterize the color of objects. When used in this manner, they give the percent reflectance or transmittance of the object relative to some reference. For most spectrophotometers used for color measurement this reference is a Perfect Reflecting Diffuser to which the white standardization tile may be traced. The output is a spectral curve, which is like a fingerprint of the color of the object. Spectrophotometers come in many sizes, shapes, geometries, and configurations such as single and dual beam configuration.

To obtain the optical properties three measurements are required:

1. Total transmittance: The collection of total light transmitted through a sample at all angles including light passing through the sample that is un-scattered and after scattering.
2. Diffuse reflectance: The collection of the portion of light reflected from a sample at all angles. It does not include the light passing directly through the sample.
3. Collimated transmittance: The collection of the portion of light transmitted through the sample that is un-scattered [1].

The above measurements are performed using an integrating sphere setup shown in Figure 1 below. The function of an integrating sphere is to spatially integrate radiant flux. It is usually a spherical shaped object with multiple ports on the sides that are used to attach samples and detectors. The internal walls of the sphere are coated using barium sulphate ($BaSO_4$), which is a highly reflective material. The selection of sphere coating or material can make a large difference in the radiance produced for a given sphere design. In using integrating spheres, it is important that the viewed radiance does not include a portion of the sphere surface directly irradiated by incident flux. This will introduce a false response. There is baffle placed between the entrance

port of the sphere and the detector port to avoid the straight path between the light source and the detector. Baffles coated with the same material as the integrating sphere wall block the view of incident flux which has not undergone at least two reflections from the sphere surface. The baffle is positioned to prevent first reflections from entering the field-of-view for the photodetector. The radiance of the sphere wall determines the total flux incident on a photodetector mounted at or near a port of the integrating sphere [1].

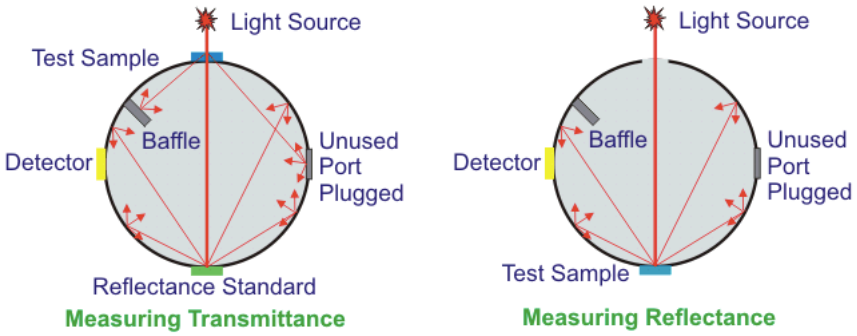

Figure 1. An integrating sphere setup for measuring transmittance and reflectance [2]

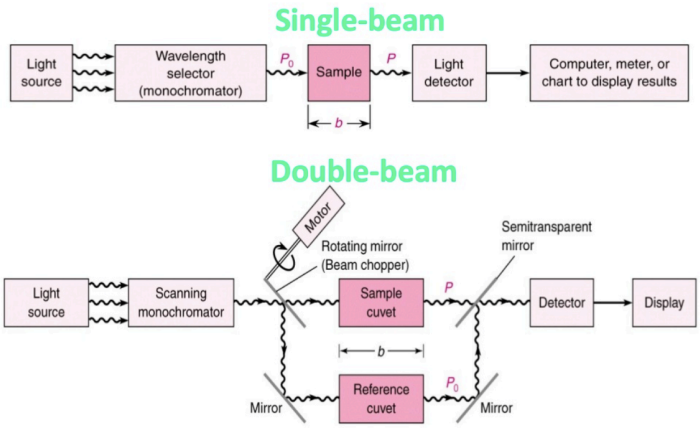

Figure 2. Schematic diagram of spectrophotometer for single and dual beam configuration [2]

In a single beam configuration, there is a single beam from the light source. A sample is placed on the opposite side of the light entrance port to collect the diffuse reflectance of the sample. The detector collects the maximum reflectance. Similarly the maximum transmittance is measured as well. The sample is placed in front of the sphere entrance port through which the light beam enters. The sphere collects all the light passing through the sample.

In a dual beam configuration, the beam from the light source is split in two. One beam illuminates the reference standard and the other illuminates the sample. As shown in Figure 3, two measurements are made and the reference beam is used to correct for system effects like lamp variation with wavelength or detector changes or anything that affects the light path. Since

using dual beam set up does not need to perform an extra measurement to normalize the acquired spectra, only two measurements are required [1].
For reflectance measurement the following are needed: $R\left(r_s^{direct}, r_s\right)$ and $R(0,0)$
For transmittance measurement the following are needed: $T\left(t_s^{direct}, r_s\right)$ and T_{dark})

To calculate the total transmittance and diffuse reflectance are as follows:

$$M_R = r_{std}\left(R\left(r_s^{direct}, r_s\right) - R(0,0)\right)$$

$$M_T = r_{std}\left(T\left(t_s^{direct}, r_s\right) - T_{dark}\right) \qquad \text{Equation 1. [1]}$$

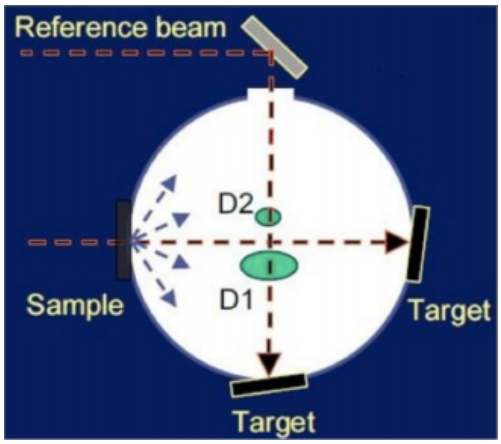

Figure 3. Dual beam integrating sphere setup [1]

Different substances absorb different wavelengths of light, and this can be used to help identify the substance, for example the presence of particular metal ions, or of particular functional groups in organic compounds. The amount of absorption is also dependent on the concentration of the substance if it is in solution. Measurement of the amount of absorption can be used to find concentrations of very dilute solutions. An absorption spectrophotometer measures the way that the light absorbed by a compound varies across the UV and visible spectrum [2].

Inverse Adding-Doubling (IAD) is a technique developed by Scott A. Prahl that uses adding-doubling to figure out the optical properties of slabs of material from the observed transmission and reflection. The goal of this IAD model is to generate fast and accurate estimates of light distributions. Such a model should generate internal fluence rates as well as the amount of light reflected or transmitted. The program typically used with the data files that contain the values of measured transmittance and reflectance M_R and M_T as a function of wavelength. The output files contain the calculated absorption and scattering coefficient values μ_a and μ_s' [3].

3 Materials

The main instrument used in this experiment is the Shimadzu UV-3600 spectrophotometer. Throughout this experiment, two different configurations of the spectrophotometer were used: Total Transmission (TT) and Diffuse Reflectance (DR). Figure 4 illustrates a picture of the spectrophotometer instrument.

Figure 4. Shimadzu UV-3600 Spectrophotometer

Aside from the spectrophotometer, two cuvettes were used, where one contained Phosphate-Buffered Saline (PBS) and the other contained a solution made from PBS, methylene blue, and intra-lipid, in unknown proportions. Figure 5 displays these two cuvettes.

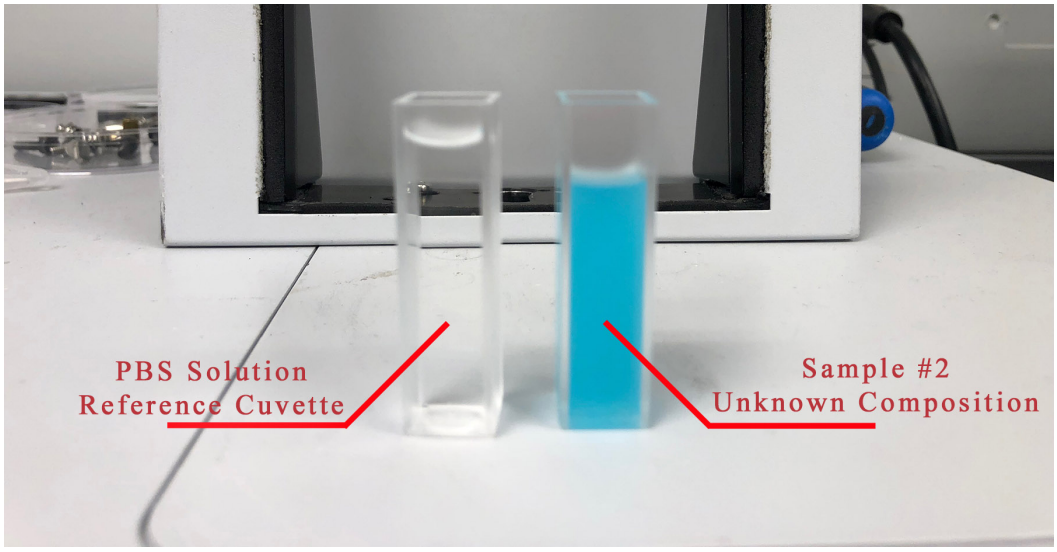

Figure 4. The Reference and Unknown Cuvett

4 Procedure

4.1 Data Collection

The experimenters were handed two cuvettes. One cuvette was filled with PBS solution and the other was filled with a mixture of PBS, methylene blue, and intralipid, in unknown proportions (referred to as sample #2 from now on) [1].

Before performing spectroscopy, the spectrophotometer was preliminarily setup. This pre-setup started with turning on the spectrophotometer and connecting it to the local computer. This was done by pressing the connect button on the front panel [1].

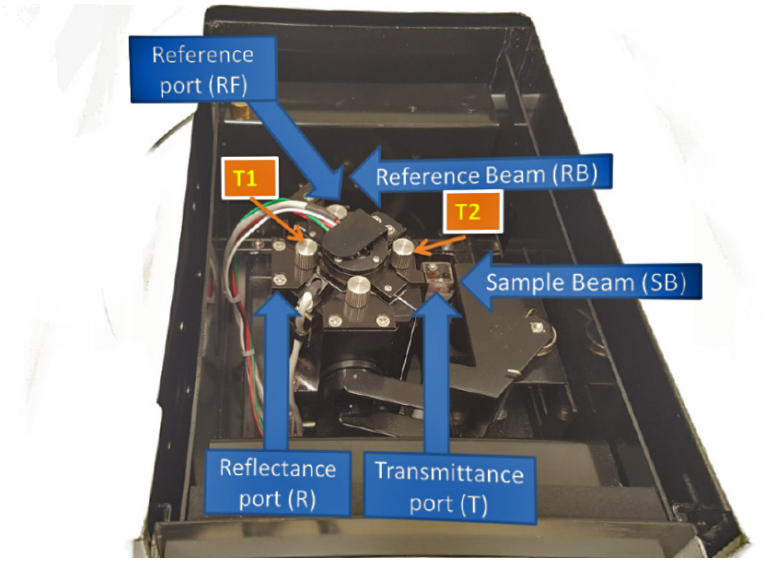

Figure 5. The Internals of UV-3600 Spectrophotometer's Chamber [1]

Thereafter, the spectrophotometer software was launched, and a baseline measurement was collected. Referring to Figure 5, this was done by leaving the transmittance port empty and capping the reflectance port with a reflection standard (Barium Sulphate). The wavelength range for this baseline was chosen to be from 400 to 800 nm, sampled every 5 nm. This menu is accessed by going into "Edit" and selecting the "Method…" option. The sampling setting was kept the same for the remainder of the experiment [1].

Then, the transmission and reflectance spectra of an unknown sample were collected. For the TT measurement, the PBS solution was placed into the reference port and sample #2 was placed into the transmittance port, while keeping the reflectance port capped with the reflectance standard. In the spectrophotometer software, the scanning mode was switched to "Transmittance", under the "Instrument Parameters" tab and the slit width was set to 8. The scanning interval and range was kept the same as the baseline scan (400 – 800 nm, every 5 nm). The "Scanning Speed" was set to "Medium". A scan was performed, which signifies the t_s^{direct}

values. Thereafter, the t_{dark} values were collected by taking sample #2 out and capping the transmittance port with the reflectance standard [1].

Now, the team proceeded to measure the DR data on sample #2. Sample #2 was placed in the reflectance port and the transmittance port was left open. The reference cuvette was also removed. On the spectrophotometer software, the scanning mode was changed to "Reflectance", under the "Measurements Parameters" tab. The scan was performed under a new dataset. Lastly, the dark reflectance R(0, 0) was measured by removing the cuvette from the reflectance port, and then performing a scan [1].

The software was then configured to output the measured reflectance M_R and the measured transmittance M_T. At this stage, the software automatically subtracted the dark measurements (t_{dark} and R(0, 0)) from the direct TT and DR values, as required by Equation 1. The output displayed the M_T and M_R values as percentages of sample transmittance and reflection. The data was saved as a text file, which contained the preliminary information necessary to perform IAD [1].

4.2 Data Analysis

The output data by the spectrophotometer software had an initial ".rtx" format, which was changed into a ".txt". The numbers under the columns of M_T and M_R were then modified by dividing them all by 100. This was done to convert those values from percentages to a ratio between 0-1. The resulting file was then reformatted back into a ".rtx" file. Then, the data file was passed onto the IAD algorithm. The input and output illustration of the IAD algorithm is shown in Figure 6.

Figure 6. IAD Algorithm Input and Output

As shown in Figure 6, the data files were passed onto the IAD algorithm and the resulting file contained the spectral values of μ_a and μ_s' of the sample. The results were later passed onto MATLAB to compute the concentrations of intralipid and methylene blue. Throughout the analysis, the resampling of the data was required to match the sampling intervals between the datasets. The further analysis of the data is elaborated in the "Results" section below.

5 Results

Figure 7 below represents the plotted output of μ_a and μ_s' values of the IAD output:

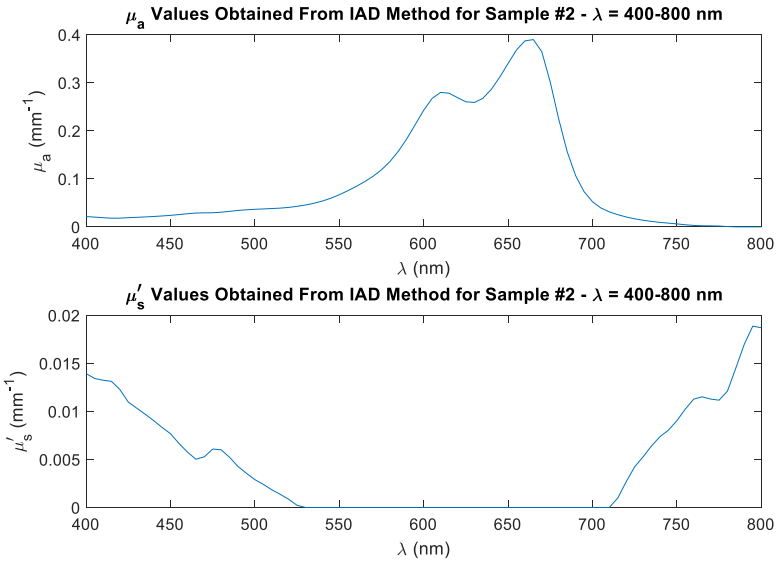

Figure 7. μ_a and μ_s' Spectral Plots of Sample #2

Now, in order to validate the output data and advance the calculation of the methylene blue concentration, the extinction coefficients of methylene blue were obtained from the document [4]. The extinction coefficient is a proportionality value that relates the absorbance coefficient (μ_a) of a sample to its molar concentration [4]. This idea is illustrated in the Equation 2 below. This is where ϵ is the extinction coefficient and C is the concentration in $mol.L^{-1}$.

$$\mu_a = \epsilon C \ln 10 \qquad Equation\ 2.[4]$$

Figure 8 illustrates the normalized plot of the methylene blue extinction coefficients overlaid on the normalized μ_a values obtained from IAD.

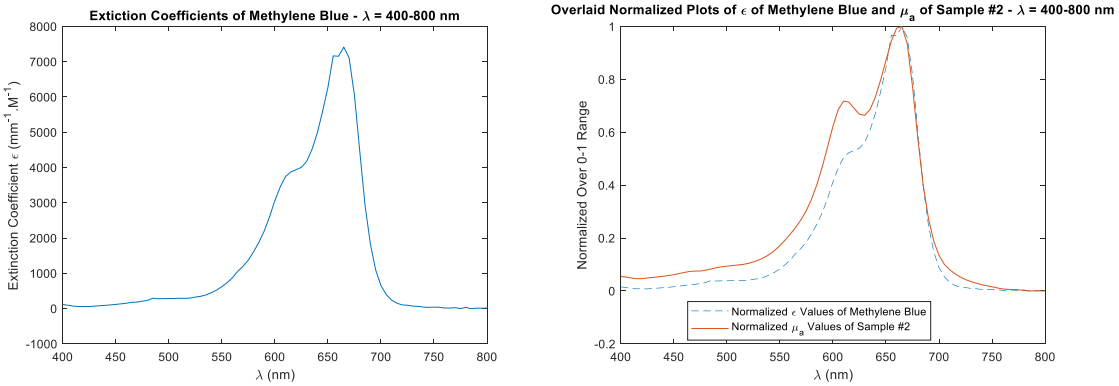

Figure 8. Overlaid Normalized Plots of Extinction Coefficients and Obtained μ_a Values

Figure 8 shows that the main peak of both mentioned plots line up. A high degree of correlation can be seen between 600 – 675 nm wavelengths. By rearranging Equation 2, one can compute the concentration of the methylene blue. In fact, a concentration value can be computed at every wavelength specified during the spectroscopy measurements. Figure 9 represents the computed methylene blue concentration within the sampled spectra. While computing this plot, the major assumption was that methylene blue is the theoretical sole contributor to the absorption.

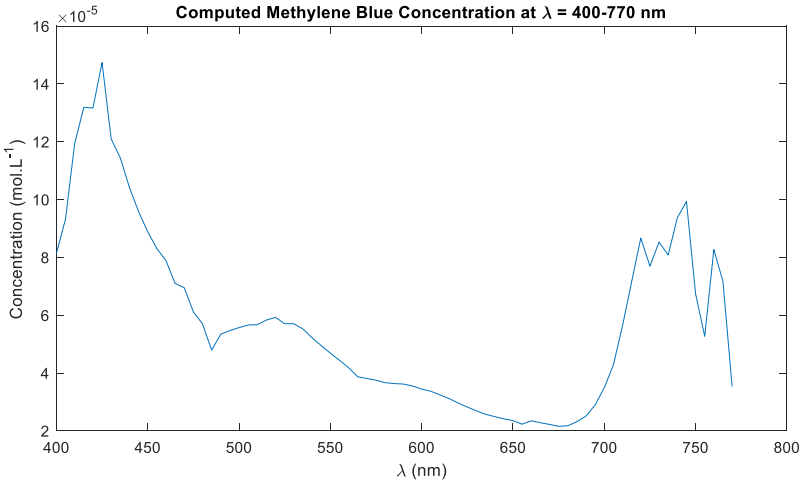

Figure 9. Computed Methylene Blue Concentrations Within the Sampled Spectra

Two concluded values were extracted from the plot above. The reason behind this selection is elaborated in the "Discussion" section. These values are summarized in Table 1. The results were reported as mean plus and minus the average deviation from the mean. The average deviation from the mean is shown in Equation 3 shown below:

$$Average\ Deviation\ From\ Mean = \sum_{n=1:N}^{i} \frac{|x_i - \bar{x}|}{N} \qquad Equation\ 3.$$

Table 1. Evaluated Concentration of Methylene Blue in Sample #2

Wavelength Range (nm)	Concentration (μM)
600 – 675	26.72 ± 3.70
400 – 800	57.62 ± 31.95

Now, in order to evaluate the intralipid concentration, one has to assume that the intralipid is the sole contributor to the scattering, thus the μ_s'. In order to find the intralipid concentration, one has to know the trend of spectral change of μ_s' with variations in the intralipid concentration. [5] was used to obtain the reference data necessary. Pixel measurements were used from the PDF document to recreate the numerical plots on MATLAB. Figure 10 contains this information sampled at three different wavelengths.

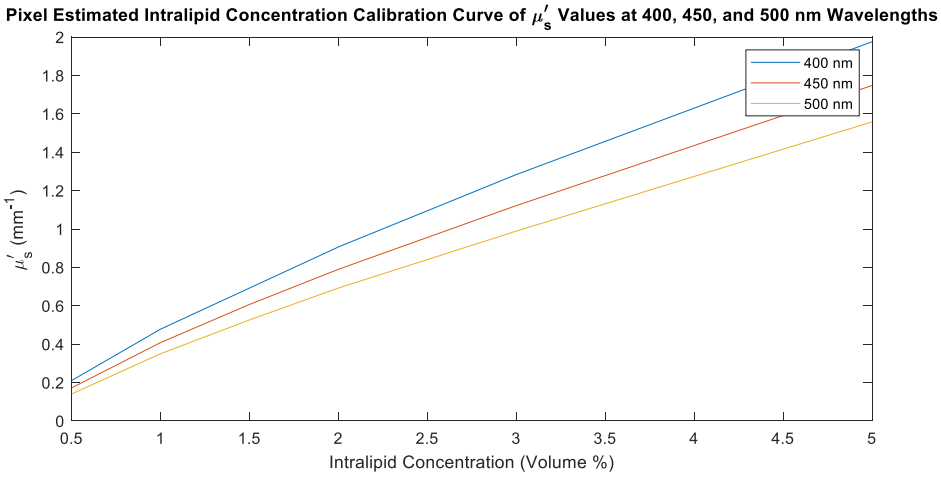

Figure 10. Diffuse Scattering Coefficients at Different Concentrations of Intralipid at 400, 450, 500 nm Wavelengths, Obtained From Pixel Measurements from [5]

Now, to further analyze the data, one has to plot the obtained μ_s' values and the reference spectra of the μ_s' values of different concentrations of intralipid.

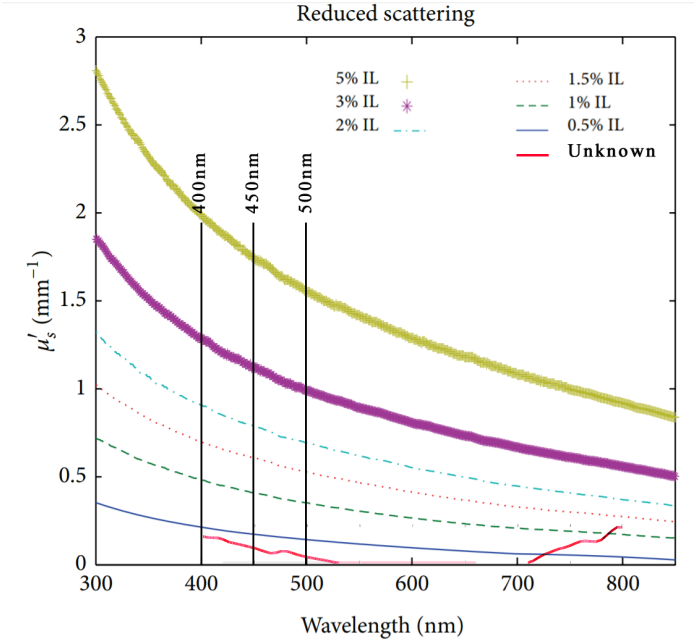

Figure 11. Obtained μ_s' Values Plotted Against the Reference μ_s' Values of Intralipid at Various Concentrations [5]

As seen in Figure 11, our obtained μ_s' values are below the reference μ_s' values of various intralipid concentrations. From the plot trends, one can see that the μ_s' spectra are shifted upwards with increasing concentration. Thus, by observing our μ_s' values, one can conclude that the Intralipid concentration in sample #2 is below 0.5%. Thus, in order to resolve the concentration, one has to extrapolate. The extrapolation was done at 400, 450, and 500 nm Wavelengths and the obtained concentrations were averaged together. Table 2 illustrates this result [5].

Table 2. Evaluated Intralipid Concentrations

Wavelength (nm)	Calculated Intralipid Concentration (Volume %)
400	0.1740
450	0.0937
500	0.0113
Average ± Deviation from Mean:	0.093 ± 0.0545

6 Discussion

Referring to Figure 8, one can initially approve the somewhat validity of the obtained data. This can be seen from the similar plot shapes of the methylene blue extinction coefficients and the μ_a values of sample #2. The two plots were normalized between 0 and 1 in order to make them comparable in terms of scale and allow for the comparison of their shape. The shapes suggest similarities. From this, a conservative assumption was made to think of methylene blue as the sole contributor to the absorption. This is not completely true because theoretically the PBS and the intralipid also contribute to the absorption and ultimately to μ_a. This reason may explain the differences in the shapes of the extinction coefficient spectra and the collected μ_a spectra. Though, this assumption will significantly simplify the estimation of the concentration.

When calculating the methylene blue concentration, a concentration value was obtained for every sampled μ_a across the sampled spectra. A proposition was then made in order to enhance the certainty of the final answer. The proposition entailed to only focus on the concentration values obtained between the 600 – 675 nm wavelengths. Looking at Figure 8, one can see that the methylene blue extinction coefficients peak around the mentioned wavelength range. It is justified that due to this peak, one can relatively achieve the highest signal-to-noise ratio. Figure 12 illustrates another justification to truncate the methylene blue concentration between the mentioned wavelengths.

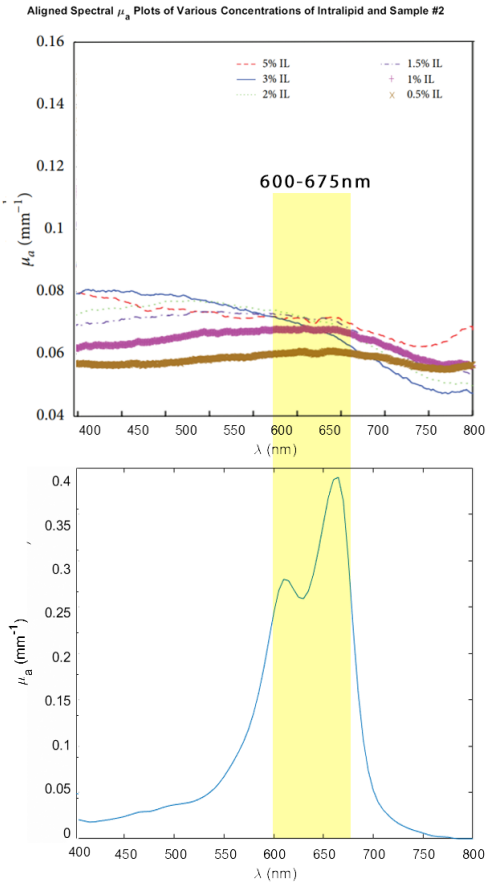

Figure 12. Aligned Extinction Spectra of Methylene Blue and the Absorption Spectra of Intralipid [5]

As seen, the intralipid also has relatively less contribution to the μ_a at the mentioned wavelengths. The last justification is from Figure 9 where the concentration values are relatively similar between 600 – 675 nm wavelengths. Though, the full spectrum (400 – 800 nm) averaged concentration was also computed which resulted in a relatively uncertain range of concentrations.

The reason behind picking the 400, 450, and 500 nm wavelengths to extrapolate the intralipid concentration from the measured μ_s' from sample #2, is the presence of measured signal at that range. Referring to Figure 11, at the 500 – 700 nm range, the measured μ_s' fall below the sensitivity level, thus rendering the data useless. At the 700 – 800 nm wavelength range, the measured μ_s' values appear again but the reference μ_s' values fall in terms of magnitude. Thus, a proposition was made to perform the extrapolation of the intralipid concentration at the 400, 450, 500 nm wavelengths. The reason behind choosing three wavelengths was to allow for the calculation of the average deviation from the mean.

One major assumption was made upon calculating the intralipid concentration, which entails the intralipid being the sole contributor to the scattering, and ultimately contributing to μ_s'. Extrapolating the concentration values might not be accurate due to the intrinsic nature of extrapolation itself. Extrapolation's ability to predict is only supported from one side of existing known data.

8 Conclusion

In this experiment, the concentrations of Intralipid and methylene blue were measured using a spectrophotometer. This was done by measuring TT and DR values from 400 – 800 nm spectral range, every 5 nm. The IAD algorithm was used to post-process the spectrophotometer output data (M_T and M_R) to obtain μ_a and μ_s' spectrum of sample #2, an unknown sample of PBS, methylene blue, and intralipid.

Two variations of the methylene blue concentration were evaluated. In one variation, the spectrum of interest was limited to 600 – 675 nm wavelengths. This methylene blue concentration was evaluated to be 26.72 ± 3.70 µM. The full spectrum (400 – 800 nm) concentration of methylene blue was evaluated to be 57.62 ± 31.95 µM.

The intralipid concentration was evaluated by extrapolating from the reference intralipid μ_s' spectral variations at 400, 450, and 500 nm wavelengths and averaging them together. This value was evaluated to be 0.093 ± 0.0545 % volume.

Some sources of error are perceived to be not having enough integration time, human error (finger print contamination on cuvettes), as well as the conservative assumptions mentioned earlier. A future improvement to this experiment may include scanning with longer integration times, thoroughly cleaning the cuvette and working with gloves, and implementing better data analysis methods in order to include all of the constituents of the unknown solution as sources of scattering and absorption. As well, obtaining μ_s' spectral variations of intralipid at very low concentrations may also be effective in bettering the accuracy of the final results.

References

[1] A. Douplik. “Measuring optical properties using a spectrophotometer”. Department of Physics, 2018

[2] Labsphere. “Integrating Sphere Theory and Application”. Retrieved on October 21, 2018 from: https://www.labsphere.com/site/assets/files/2551/integrating_sphere_theory_apps_tech_guide.pdf

[3] S. A. Prahl, van Gemert, Martin J. C and A. J. Welch, "Determining the optical properties of turbid media by using the adding–doubling method," *Applied Optics,* vol. 32, *(4),* pp. 559, 1993.

[4] S. A. Prahl. “Methylene Blue Spectra”. Retrieved October 24, 2018, from: https://omlc.org/spectra/mb/

[5] H. Assadi, R. Karshafian and A. Douplik, "Optical Scattering Properties of Intralipid Phantom in Presence of Encapsulated Microbubbles," *International Journal of Photoenergy*, vol. 2014, pp. 1-9, 2014.

Appendix

The below is the MATLAB Code:

```
%Loading the Data and Downsampling it to Have Loggings at Every 5 nm
a = xlsread('sam.xlsx', 'A4:G404');
lam = downsample(a(:, 1), 5); ua = downsample(a(:, 6), 5); us = downsample(a(:,
7), 5);
clear a;

% Plotting What We Have
figure(1); subplot(2, 1, 1); plot(lam, ua);
xlabel('\lambda (nm)'); ylabel('\mu_{a} (mm^-^1)');
title('\mu_{a} Values Obtained From IAD Method for Sample #2 - \lambda = 400-
800 nm');
subplot(2, 1, 2); plot(lam, us);
xlabel('\lambda (nm)'); ylabel('\mu_{s}^\prime (mm^-^1)');
title('\mu_{s}^\prime Values Obtained From IAD Method for Sample #2 - \lambda =
400-800 nm');

% %Loading Extinction Coefficients and Sample-wise Aligning the Wavelength it
out dataset
a = load('ext_co_mb.txt');
extmb = downsample(interp(a(101:end, 2), 2), 5)./10; %Unit Conversion from cm-1
to mm-1
clear a;
figure(2); subplot(1, 2, 1); plot(lam, extmb);
xlabel('\lambda (nm)'); ylabel('Extinction Coefficient \epsilon (mm^-^1.M^-
^1)');
title('Extiction Coefficients of Methylene Blue - \lambda = 400-800 nm');
subplot(1, 2, 2); plot(lam, extmb./max(extmb), '--', lam, ua./max(ua));
legend('Normalized \epsilon Values of Methylene Blue', 'Normalized \mu_{a}
Values of Sample #2');
xlabel('\lambda (nm)'); ylabel('Normalized Over 0-1 Range');
title('Overlaid Normalized Plots of \epsilon of Methylene Blue and \mu_{a} of
Sample #2 - \lambda = 400-800 nm');

%Calculating Concentration
con_mb = (ua./log(10))./extmb;
%Taking out Outliers
con_mb = con_mb(1:75);
%Plotting
figure(3);
plot(lam(1:75), con_mb);
xlabel('\lambda (nm)'); ylabel('Concentration (mol.L^-^1)');
title('Computed Methylene Blue Concentration at \lambda = 400-770 nm');


%Truncated Estimation
concentration = mean(con_mb(41:56)) * 1e6; %MicroMolar
ad = mean(abs((con_mb(41:56).*1e6) - concentration));
concentration = [concentration ad]; clear ad;

%Full-Spectrum Estimation
concentration_fs = mean(con_mb) * 1e6; %MicroMolar
ad_fs = mean(abs((con_mb.*1e6) - concentration));
concentration_fs = [concentration_fs ad_fs]; clear ad_fs;

%mu_s estimation and Intralipid
a = xlsread('us_Pixel_Measurements.xlsx');
%Normalizing Knowing 2 (mm^-1) is at 568.45 Pixels relative to X-axis
a = (a./568.45)*2;
```

```
il_con = [0.5 1 1.5 2 3 5];
figure(4); plot(il_con, a(1, 2:7), il_con, a(2, 2:7), il_con, a(3, 2:7));
title('Pixel Estimated Intralipid Concentration Calibration Curve of
\mu_{s}^\prime Values at 400, 450, and 500 nm Wavelengths');
xlabel('Intralipid Concentration (Volume %)'); ylabel('\mu_{s}^\prime (mm^-
^1)');
legend('400 nm', '450 nm', '500 nm');

%Doing Regression and Then Extrapolate
m = polyfit(il_con, a(1, 2:7), 1);
ilcon400 = ((a(1, 1) - m(2))/m(1));
m = polyfit(il_con, a(2, 2:7), 1);
ilcon450 = ((a(2, 1) - m(2))/m(1));
m = polyfit(il_con, a(3, 2:7), 1);
ilcon500 = ((a(3, 1) - m(2))/m(1)); clear m;
%Placing in an Array
il_con = [ilcon400 ilcon450 ilcon500]; clear ilcon400 ilcon450 ilcon500;
%Plotting:
figure(5); plot([400 450 500], il_con);
title('Estimated Intralipid Concentration in Sample #2 at 400, 450, and 500 nm
Wavelengths');
xlabel('\lambda (nm)'); ylabel('%Volume Concentration');
%Calculating Mean and Absolute Deviation From Mean
il_concen = mean(il_con);
ad_il = mean(abs(il_con - il_concen));
```

The IAD Algorithm output is listed below:

```
# Inverse Adding-Doubling 3-9-10 (3 Apr 2013)
#
#                 Beam diameter =    7.1 mm
#              Sample thickness =  10.000 mm
#            Top slide thickness =   2.000 mm
#         Bottom slide thickness =   2.000 mm
#       Sample index of refraction =  1.3470
#     Top slide index of refraction =  1.5138
#   Bottom slide index of refraction =  1.5138
#
#   Fraction unscattered refl. in M_R =   100.0 %
#  Fraction unscattered trans. in M_T =   100.0 %
#
# Reflection sphere
#                 sphere diameter =    60.0 mm
#            sample port diameter =    18.7 mm
#           entrance port diameter =    16.7 mm
#           detector port diameter =    13.1 mm
#               wall reflectance =    98.0 %
#            standard reflectance =    98.0 %
#            detector reflectance =     0.0 %
#
# Transmission sphere
#                 sphere diameter =    60.0 mm
#            sample port diameter =    16.7 mm
#           entrance port diameter =     0.0 mm
#           detector port diameter =    13.1 mm
#               wall reflectance =    98.0 %
#           standard transmittance =   100.0 %
#            detector reflectance =     0.0 %
#
# M_R and M_T were measured using the substitution (single-beam) method.
# Single sphere corrections were used with light incident at 0 degrees from the normal.
# The inverse routine adapted to the input data.
#
#
#            AD quadrature points =   8
#          AD tolerance for success =   0.00010
#     MC tolerance for mu_a and mu_s' =   0.010 %
#  Photons used to estimate lost light =   100000
#
#              Measured      M_R         Measured      M_T         Estimated     Estimated     Estimated
##wave          M_R          fit           M_T          fit          mu_a          mu_s'          g
# [nm]          [---]        [---]         [---]        [---]        1/mm          1/mm          [---]
 400.0        8.819e-002   8.818e-002   2.400e-001   2.400e-001   5.274e-002   1.115e-001   0.000e+000   # *
 405.0        8.821e-002   8.822e-002   2.455e-001   2.455e-001   5.147e-002   1.102e-001   0.000e+000   # *
 410.0        8.844e-002   8.844e-002   2.507e-001   2.507e-001   5.020e-002   1.092e-001   0.000e+000   # *
 415.0        8.805e-002   8.805e-002   2.555e-001   2.555e-001   4.944e-002   1.075e-001   0.000e+000   # *
 420.0        8.756e-002   8.756e-002   2.594e-001   2.594e-001   4.899e-002   1.060e-001   0.000e+000   # *
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425.0	8.685e-002	8.684e-002	2.626e-001	2.626e-001	4.887e-002	1.043e-001	0.000e+000	# *
430.0	8.576e-002	8.576e-002	2.650e-001	2.650e-001	4.920e-002	1.023e-001	0.000e+000	# *
435.0	8.504e-002	8.504e-002	2.671e-001	2.671e-001	4.942e-002	1.009e-001	0.000e+000	# *
440.0	8.409e-002	8.409e-002	2.693e-001	2.693e-001	4.979e-002	9.908e-002	0.000e+000	# *
445.0	8.317e-002	8.317e-002	2.712e-001	2.712e-001	5.022e-002	9.738e-002	0.000e+000	# *
450.0	8.214e-002	8.214e-002	2.728e-001	2.728e-001	5.080e-002	9.560e-002	0.000e+000	# *
455.0	8.115e-002	8.115e-002	2.742e-001	2.742e-001	5.145e-002	9.390e-002	0.000e+000	# *
460.0	7.980e-002	7.979e-002	2.753e-001	2.753e-001	5.244e-002	9.176e-002	0.000e+000	# *
465.0	7.886e-002	7.886e-002	2.765e-001	2.765e-001	5.316e-002	9.010e-002	0.000e+000	# *
470.0	7.782e-002	7.783e-002	2.776e-001	2.776e-001	5.398e-002	8.832e-002	0.000e+000	# *
475.0	7.728e-002	7.728e-002	2.799e-001	2.799e-001	5.421e-002	8.694e-002	0.000e+000	# *
480.0	7.628e-002	7.629e-002	2.811e-001	2.811e-001	5.490e-002	8.528e-002	0.000e+000	# *
485.0	7.518e-002	7.518e-002	2.821e-001	2.821e-001	5.576e-002	8.351e-002	0.000e+000	# *
490.0	7.438e-002	7.438e-002	2.828e-001	2.828e-001	5.649e-002	8.211e-002	0.000e+000	# *
495.0	7.359e-002	7.359e-002	2.844e-001	2.844e-001	5.701e-002	8.060e-002	0.000e+000	# *
500.0	7.293e-002	7.293e-002	2.864e-001	2.864e-001	5.733e-002	7.921e-002	0.000e+000	# *
505.0	7.252e-002	7.253e-002	2.888e-001	2.888e-001	5.734e-002	7.807e-002	0.000e+000	# *
510.0	7.203e-002	7.203e-002	2.918e-001	2.917e-001	5.731e-002	7.676e-002	0.000e+000	# *
515.0	7.166e-002	7.167e-002	2.941e-001	2.941e-001	5.725e-002	7.578e-002	0.000e+000	# *
520.0	7.094e-002	7.094e-002	2.957e-001	2.956e-001	5.766e-002	7.443e-002	0.000e+000	# *
525.0	7.024e-002	7.024e-002	2.957e-001	2.957e-001	5.838e-002	7.336e-002	0.000e+000	# *
530.0	6.882e-002	6.882e-002	2.949e-001	2.949e-001	5.999e-002	7.137e-002	0.000e+000	# *
535.0	6.739e-002	6.739e-002	2.917e-001	2.917e-001	6.249e-002	6.945e-002	0.000e+000	# *
540.0	6.553e-002	6.552e-002	2.868e-001	2.868e-001	6.576e-002	6.717e-002	0.000e+000	# *
545.0	6.362e-002	6.362e-002	2.794e-001	2.794e-001	6.980e-002	6.508e-002	0.000e+000	# *
550.0	6.130e-002	6.130e-002	2.707e-001	2.707e-001	7.485e-002	6.238e-002	0.000e+000	# *
555.0	5.899e-002	5.899e-002	2.605e-001	2.604e-001	8.063e-002	5.965e-002	0.000e+000	# *
560.0	5.679e-002	5.679e-002	2.494e-001	2.494e-001	8.682e-002	5.706e-002	0.000e+000	# *
565.0	5.464e-002	5.464e-002	2.374e-001	2.374e-001	9.361e-002	5.445e-002	0.000e+000	# *
570.0	5.239e-002	5.240e-002	2.249e-001	2.249e-001	1.013e-001	5.140e-002	0.000e+000	# *
575.0	5.009e-002	5.009e-002	2.102e-001	2.103e-001	1.104e-001	4.819e-002	0.000e+000	# *
580.0	4.750e-002	4.750e-002	1.925e-001	1.925e-001	1.223e-001	4.423e-002	0.000e+000	# *
585.0	4.522e-002	4.522e-002	1.723e-001	1.723e-001	1.360e-001	4.083e-002	0.000e+000	# *
590.0	4.275e-002	4.275e-002	1.492e-001	1.492e-001	1.537e-001	3.661e-002	0.000e+000	# *
595.0	4.063e-002	4.063e-002	1.266e-001	1.266e-001	1.733e-001	3.256e-002	0.000e+000	# *
600.0	3.909e-002	3.909e-002	1.071e-001	1.071e-001	1.926e-001	2.943e-002	0.000e+000	# *
605.0	3.797e-002	3.797e-002	9.300e-002	9.300e-002	2.089e-001	2.674e-002	0.000e+000	# *
610.0	3.729e-002	3.729e-002	8.579e-002	8.580e-002	2.187e-001	2.467e-002	0.000e+000	# *
615.0	3.729e-002	3.702e-002	8.548e-002	1.051e-001	2.190e-001	2.473e-002	0.000e+000	# *
620.0	3.752e-002	3.752e-002	8.843e-002	8.843e-002	2.151e-001	2.538e-002	0.000e+000	# *
625.0	3.759e-002	3.759e-002	9.122e-002	9.122e-002	2.120e-001	2.528e-002	0.000e+000	# *
630.0	3.762e-002	3.791e-002	8.981e-002	7.224e-002	2.133e-001	2.562e-002	0.000e+000	# *
635.0	3.720e-002	3.720e-002	8.349e-002	8.348e-002	2.214e-001	2.460e-002	0.000e+000	# *
640.0	3.648e-002	3.648e-002	7.298e-002	7.299e-002	2.365e-001	2.257e-002	0.000e+000	# *
645.0	3.589e-002	3.588e-002	6.105e-002	6.105e-002	2.555e-001	2.119e-002	0.000e+000	# *
650.0	3.553e-002	3.553e-002	5.053e-002	5.052e-002	2.747e-001	2.077e-002	0.000e+000	# *
655.0	3.461e-002	3.460e-002	4.178e-002	4.178e-002	2.974e-001	1.651e-002	0.000e+000	# *
660.0	3.468e-002	3.468e-002	3.696e-002	3.696e-002	3.086e-001	1.771e-002	0.000e+000	# *
665.0	3.520e-002	3.521e-002	3.645e-002	3.645e-002	3.070e-001	2.116e-002	0.000e+000	# *
670.0	3.597e-002	3.598e-002	4.292e-002	4.292e-002	2.877e-001	2.468e-002	0.000e+000	# *
675.0	3.695e-002	3.695e-002	6.543e-002	6.543e-002	2.444e-001	2.607e-002	0.000e+000	# *
680.0	3.927e-002	3.928e-002	1.105e-001	1.105e-001	1.892e-001	2.968e-002	0.000e+000	# *
685.0	4.347e-002	4.347e-002	1.779e-001	1.779e-001	1.377e-001	3.484e-002	0.000e+000	# *
690.0	4.926e-002	4.926e-002	2.520e-001	2.519e-001	9.896e-002	4.024e-002	0.000e+000	# *
695.0	5.562e-002	5.562e-002	3.197e-001	3.197e-001	7.209e-002	4.470e-002	0.000e+000	# *
700.0	6.163e-002	6.163e-002	3.710e-001	3.710e-001	5.468e-002	4.855e-002	0.000e+000	# *
705.0	6.605e-002	6.605e-002	4.068e-001	4.068e-001	4.413e-002	5.079e-002	0.000e+000	# *
710.0	6.965e-002	6.965e-002	4.301e-001	4.301e-001	3.727e-002	5.284e-002	0.000e+000	# *
715.0	7.177e-002	7.177e-002	4.467e-001	4.467e-001	3.309e-002	5.363e-002	0.000e+000	# *
720.0	7.378e-002	7.377e-002	4.586e-001	4.586e-001	2.983e-002	5.464e-002	0.000e+000	# *
725.0	7.528e-002	7.528e-002	4.686e-001	4.687e-001	2.740e-002	5.520e-002	0.000e+000	# *
730.0	7.635e-002	7.635e-002	4.760e-001	4.760e-001	2.574e-002	5.549e-002	0.000e+000	# *
735.0	7.748e-002	7.748e-002	4.832e-001	4.832e-001	2.388e-002	5.601e-002	0.000e+000	# *
740.0	7.846e-002	7.847e-002	4.900e-001	4.900e-001	2.253e-002	5.614e-002	0.000e+000	# *
745.0	7.949e-002	7.949e-002	4.946e-001	4.946e-001	2.116e-002	5.679e-002	0.000e+000	# *
750.0	8.068e-002	8.068e-002	5.002e-001	5.002e-001	1.992e-002	5.717e-002	0.000e+000	# *
755.0	8.117e-002	8.117e-002	5.044e-001	5.044e-001	1.904e-002	5.727e-002	0.000e+000	# *
760.0	8.181e-002	8.182e-002	5.102e-001	5.102e-001	1.783e-002	5.744e-002	0.000e+000	# *
765.0	8.280e-002	8.280e-002	5.141e-001	5.141e-001	1.671e-002	5.807e-002	0.000e+000	# *
770.0	8.316e-002	8.316e-002	5.171e-001	5.171e-001	1.613e-002	5.809e-002	0.000e+000	# *
775.0	8.364e-002	8.363e-002	5.225e-001	5.225e-001	1.536e-002	5.780e-002	0.000e+000	# *
780.0	8.408e-002	8.408e-002	5.258e-001	5.258e-001	1.473e-002	5.784e-002	0.000e+000	# *
785.0	8.461e-002	8.462e-002	5.292e-001	5.292e-001	1.393e-002	5.811e-002	0.000e+000	# *
790.0	8.494e-002	8.494e-002	5.338e-001	5.338e-001	1.331e-002	5.781e-002	0.000e+000	# *
795.0	8.577e-002	8.577e-002	5.376e-001	5.376e-001	1.247e-002	5.809e-002	0.000e+000	# *
800.0	8.597e-002	8.597e-002	5.401e-001	5.400e-001	1.204e-002	5.804e-002	0.000e+000	# *

The below represents the used extinction coefficients of methylene blue.

400	1143
402	1085
404	990
406	899
408	777
410	693
412	687
414	616
416	575

418	543	558	8080	698	7947
420	598	560	8724	700	6511
422	629	562	9381	702	5331
424	565	564	10172	704	4303
426	589	566	10892	706	3670
428	662	568	11499	708	2866
430	711	570	11981	710	2406
432	652	572	12527	712	2103
434	769	574	13318	714	1750
436	783	576	14130	716	1389
438	861	578	14954	718	1297
440	900	580	16093	720	1028
442	943	582	16927	722	926
444	1010	584	18402	724	948
446	1041	586	19208	726	868
448	1090	588	20767	728	620
450	1169	590	21983	730	702
452	1253	592	23962	732	526
454	1303	594	25365	734	607
456	1405	596	26811	736	519
458	1556	598	28807	738	294
460	1500	600	30580	740	432
462	1637	602	32239	742	233
464	1791	604	33577	744	298
466	1735	606	35201	746	365
468	1854	608	36035	748	433
470	1825	610	37418	750	394
472	2037	612	38121	752	217
474	2022	614	38626	754	391
476	2173	616	38847	756	296
478	2254	618	39148	758	331
480	2324	620	39384	760	154
482	2429	622	39694	762	73
484	2631	624	39847	764	278
486	3055	626	40321	766	-2
488	2548	628	40968	768	246
490	2773	630	41725	770	253
492	2744	632	42960	772	-50
494	2730	634	44176	774	5
496	2911	636	46129	776	-2
498	2902	638	47803	778	221
500	2847	640	49754	780	311
502	2927	642	51895	782	63
504	2858	644	54622	784	-23
506	2889	646	57263	786	-6
508	2911	648	60272	788	156
510	2919	650	62654	790	93
512	2892	652	66008	792	40
514	2916	654	68935	794	223
516	2901	656	73230	796	-73
518	2977	658	70273	798	50
520	2965	660	71547	800	121
522	2998	662	73004		
524	3142	664	74028		
526	3319	666	74014		
528	3319	668	73044		
530	3461	670	71089		
532	3677	672	67725		
534	3832	674	63368		
536	3951	676	57423		
538	4344	678	50928		
540	4468	680	44792		
542	4833	682	38591		
544	5093	684	31941		
546	5409	686	27209		
548	5843	688	22710		
550	6189	690	18448		
552	6720	692	14973		
554	7094	694	12227		
556	7613	696	9865		