

Optimization of a Fiber Optic Freshwater DOM Sensing Device

By

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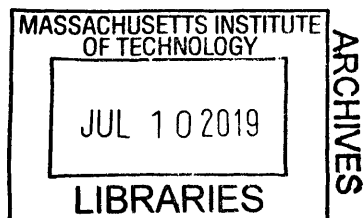
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Abstract

Fiber optical arrangements are useful for measurement of aquatic chemical species, especially in waters of high chemical concentrations. An optical fiber spectrofluorometer device known as the “Hammerhead” was developed in the Hemond lab in 2015 and improved in 2016 to measure concentrations of dissolved organic matter in water samples. The Hammerhead operates by detecting fluorescence, absorbance, and scattering of light within the water. However, inner shielding, particularly in high DOC concentrations, can lead to meaningless results. This project expands upon the earlier Hammerhead work by seeking the highest possible fluorescence signal from the Hammerhead. Experiments were run using a series of different geometric schemes for the Hammerhead optical fibers. Of the configurations tested, the narrowest spacing between excitation fiber and detector (0.076 inches from each fiber’s tip to the center of the chamber) produced optimal results. This new configuration shortens the light path length and largely avoids inner shielding effects. The new design was then compared with a traditional flow cell water measurement device known as the LEDIF, using both fluorescein and humic acid substances. Comparing the signals for both instruments indicates that the Hammerhead fiber optic scheme is superior in detecting fluorescence measurements at high DOC concentrations; it produces a nearly linear response for fluorescence, while inner shielding hinders LEDIF results. In addition, comparing the limit of detection for both instruments indicates that the Hammerhead performs better than the LEDIF even independent of inner shielding effects, producing a signal nearly 10 times greater than the LEDIF in low DOC concentrations.

Thesis Supervisor: Harold F. Hemond

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Finally, thank you to the various MIT faculty and staff members who taught me how to use laboratory equipment, order materials, and generally avoid any disasters during the course of this project.

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1. Introduction/Motivation

Of the many chemical species present in freshwater bodies, dissolved organic carbon (DOC) is arguably of critical importance. It plays a key role in the energy hierarchy of an ecosystem and can act as an indicator of changing environmental conditions. DOC is comprised of organic matter that has dissolved in water. This organic matter is formed by the decomposition of living organisms such as plants, animals, and bacteria. Chemically, DOC may be defined as humic acid ($C_9H_9NO_6$) or fulvic acid ($C_{14}H_{12}O_8$) (PubChem).

Organic carbon serves as a crucial food source for microbes living in freshwater ecosystems, and its availability to these organisms has far-reaching implications throughout the entire food chain. Moreover, the level of DOC transport in a freshwater body reveals features of the surrounding land: higher concentrations of DOC often indicate greater surface runoff, which is characteristic of barren or deforested areas (Gandois 197). It is important to be able to measure DOC concentrations because they provide clues about the health of an aquatic ecosystem.

There exist two main methods of measuring aquatic chemical species: electrode sensors and optical sensors. Electrode sensors use voltage potential or electrical current to estimate concentrations; examples include ion electrodes or pH probes (Senft-Grupp 20). Optical sensors use a chemical's response to light in order to estimate concentrations. Because organic compounds have a strong ability to emit and absorb light, optical measurement is preferable for DOC (20). These sensors quantify the levels of fluorescence, absorbance, and scattering occurring in a water sample; examples include LED excitation through a flow cell or fiber optics.

2. Optical Measurement Background

Optical measurement using a flow cell can be demonstrated by a device known as LEDIF (see Figure 1). In this instrument, water flows into a chamber that is illuminated by six Light Emitting Diodes (LEDs) of different wavelengths, as well as a broadband light source. A spectrometer on the opposite end of the chamber (at a distance of approximately 2 cm) detects the wavelengths traveling directly through the sample to obtain absorbance data, and detects wavelengths arising at right angles to the illumination to measure fluorescence and scattering (Ng et al., *A Multi-Platform Optical Sensor* 980). Absorbance and fluorescence by particles in the water indicate the concentration of humic acid (DOC), while scattering indicates the level of turbidity.

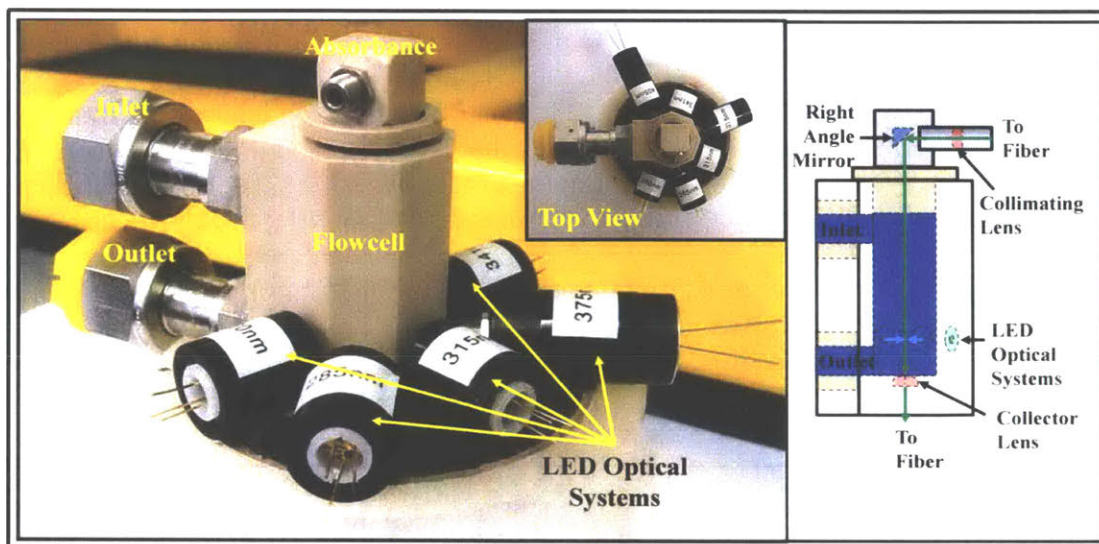


Figure 1: LEDIF Flow Cell (Source: Ng et al., 2012)

Optical measurement using fiber optics operates under the same principal: LED excitation of a water sample and spectrometer detection of the response. The difference with fiber optics lies in the size. A fiber optic device is designed for a much smaller scale by utilizing a millimeter-scale spacing between the light sources and the detector. The smaller dimensions allow for a more nearly linear response to DOC in water of high turbidity or high DOC content because it analyzes a smaller sample volume. This results in higher absorbance and fluorescence values by reducing the problem of “inner shielding.”

Inner shielding presents one of several challenges to optical measurement of aquatic chemical species. The inner shielding phenomenon occurs when the particles within the water sample absorb and block the light emitting from one another. In the case of DOC, humic acid molecules absorb the fluorescing light from surrounding molecules before the light reaches the detector, resulting in a weaker signal detected by the spectrometer. This problem becomes especially prevalent in water with higher DOC concentrations (Senft-Grupp 63). However, the smaller volume employed in fiber optic schemes can avoid these measurement inaccuracies. In a smaller volume, the path traveled by the light to the detector is shorter. By shortening the path length, the opportunity for light to be “self-absorbed” by other particles is reduced.

Another challenge to optical measurement of DOC occurs in eddy correlation studies. In this situation, two variables are measured: concentration as well as water velocity. If a sample volume is too small (and the fibers are too close together), the fibers may interfere with velocity measurements (Hu and Hemond 2).

Thus, the optimal fiber optic configuration may vary depending on the purpose of a study. This paper will analyze the performance of a fiber optic device designed to test humic acid concentration only.

3. Testing Methods and Results

The fiber optic device used in this project is nicknamed “the Hammerhead” due to its shape when fully constructed and deployed (not pictured in this paper). In this experiment, waters of varying humic acid content were sampled under several geometric configurations. The only measurement criteria considered is fluorescence; absorbance and scattering are disregarded in this paper.

The Hammerhead design uses three LED sources shining at three different angles into the sample chamber, all perpendicular to the detector (see Figure 2). In this project, all LEDs have a wavelength of 380 nm and are powered by a 20 milliamp current. The OceanOptics USB4000 spectrometer is used as a detector.

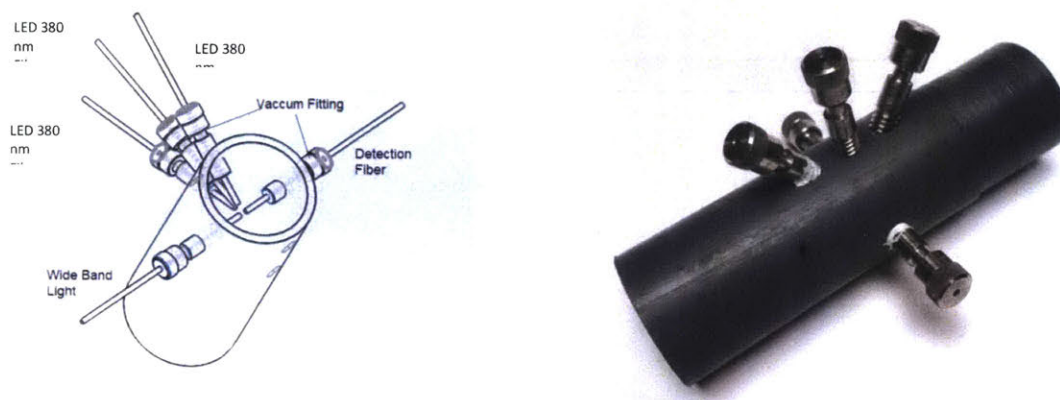


Figure 2: “Hammerhead” fiber optic scheme (Source: Xu, 2016)

3a. Fluorescein tests with three LEDs

In these initial experiments, only fluorescein was used as a test substance; no experiments were run using humic acid. This substitution ensures a high fluorescence response, allowing for clearer measurement observations. The following is the procedure for preparing the fluorescein concentrations:

1. Prepare 1 liter of 0.1 M sodium bicarbonate (NaHCO_3^-) by adding 8.4 grams of NaHCO_3^- to a container, then filling with 1 liter of deionized water. Stir well (2-3 minutes).
2. Prepare 1 liter of 2 ppm fluorescein stock solution by adding 400 milligrams of fluorescein powder to the 1 liter NaHCO_3^- . Stir well (2-3 minutes).
3. Dilute the 2 ppm fluorescein stock solution into desired concentrations using deionized water.

For these experiments, four different concentrations of fluorescein were tested: 1 ppb, 5 ppb, 10 ppb, and 20 ppb. Each concentration was tested in triplicate, with three “blank” samples of deionized water between each change in concentration.

The first set of experiments utilized all three LED sources, each of 380nm wavelength. Six different geometries were tested by varying the positions of the LED excitation fibers, as well as the detection fiber. Due to physical constraints on the angle of

the top LED excitation fiber, this fiber remained in the same position for all six tests (with the end tip of the fiber flush with the interior wall of the chamber). The positions of the other two excitation fibers were symmetrically varied for each set of tests. The six configurations are summarized in Table 1.

	Detector inserted 0 in. into chamber	Detector inserted 0.1 in. into chamber
Fibers inserted 0 in. into chamber	<p>Configuration 1</p>	<p>Configuration 4</p>
Fibers inserted 0.1 in. into chamber	<p>Configuration 2</p>	<p>Configuration 5</p>
Fibers inserted 0.3 in. into chamber	<p>Configuration 3</p>	<p>Configuration 6</p>

Table 1: Two-dimensional representation of the six fiber geometries tested. In reality, the detector is positioned at a 90 degree angle to the fibers, not directly across the chamber. The dark purple shapes represent the volume sampled by the detector.

The resulting fluorescence values were corrected for background noise. The results are summarized in in Figure 3. See Appendix 1 for graphs of the full spectrum for each sample under each configuration.

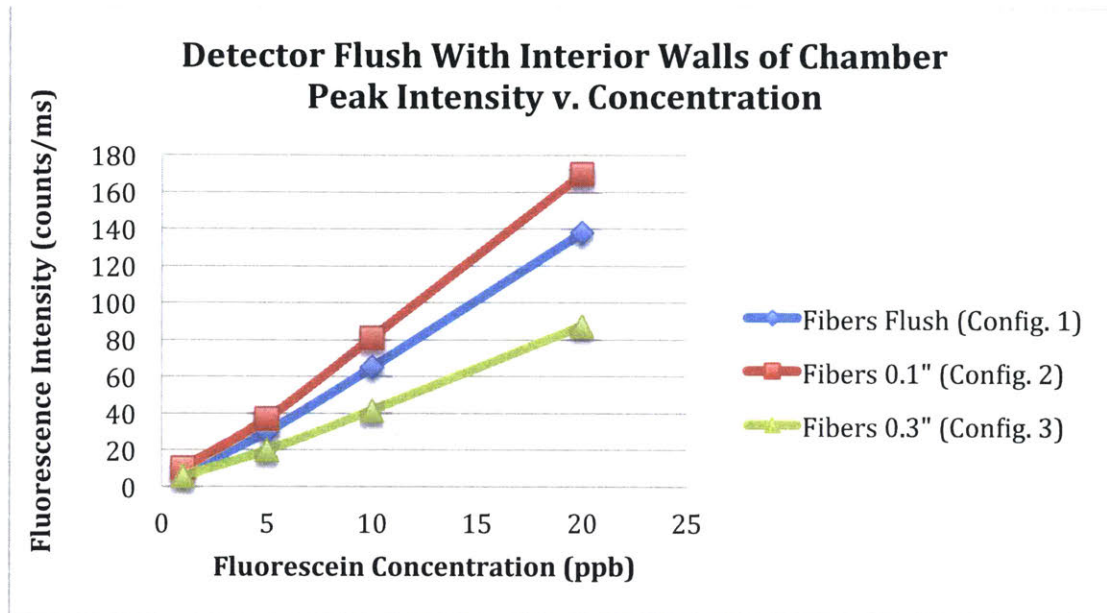


Figure 3a: Fluorescence intensity results for Configurations 1-3; the tip of the detector was not inserted beyond the interior walls of the chamber. Background spectrum has been subtracted from the intensity values in this graph.

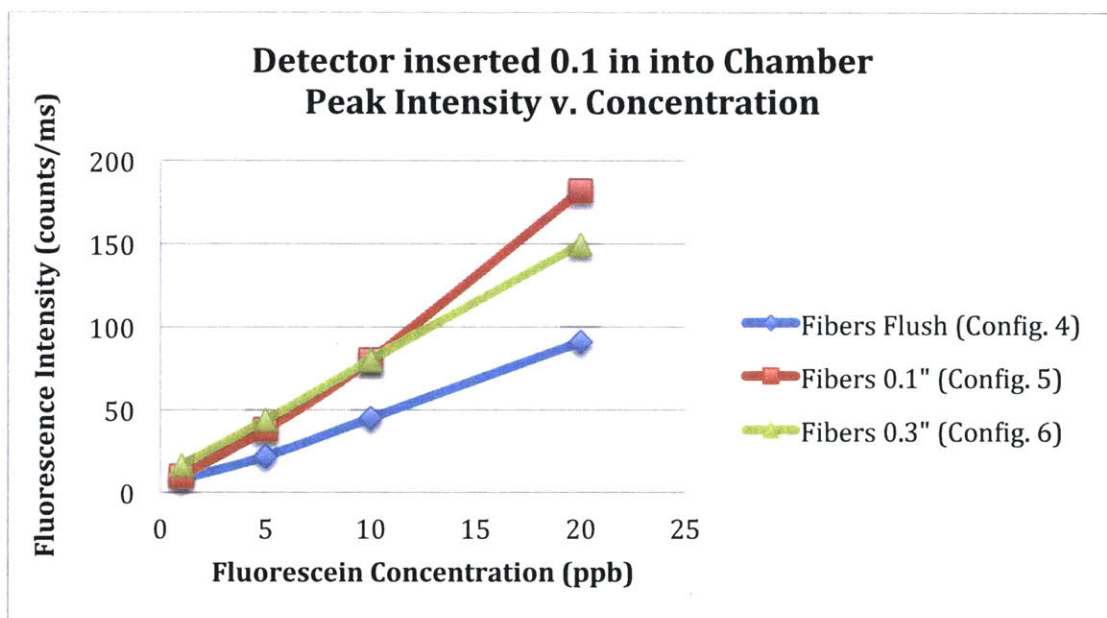


Figure 3b: Fluorescence intensity results for Configurations 4-6; the tip of the detector was inserted 0.1 inches into the interior walls of the chamber. Background spectrum has been subtracted from the intensity values in this graph.

The configurations displaying the highest fluorescence intensities are characterized by fibers inserted 0.1 inches into the detector (Configurations 2 and 5).

Furthermore, it is observed that slightly higher intensities are produced overall when the detector is inserted into the chamber as well. Thus, the next phase of the project focuses on narrowly tuning the spacing between the detector and the excitation fibers.

3b. Fluorescein tests with one LED

The second set of experiments utilized only the center LED source, at a 90-degree angle to the detector. The other two LEDs remained unlit; it may be assumed that the fluorescence response is additive, and results from a singular fiber can be multiplied to obtain projections for multiple LED excitation sources.

This time, four different geometries were tested (summarized in Table 2). Each spacing was achieved by inserting a drill bit of a known diameter into the chamber and aligning the centerline of each fiber on either side of the drill bit.

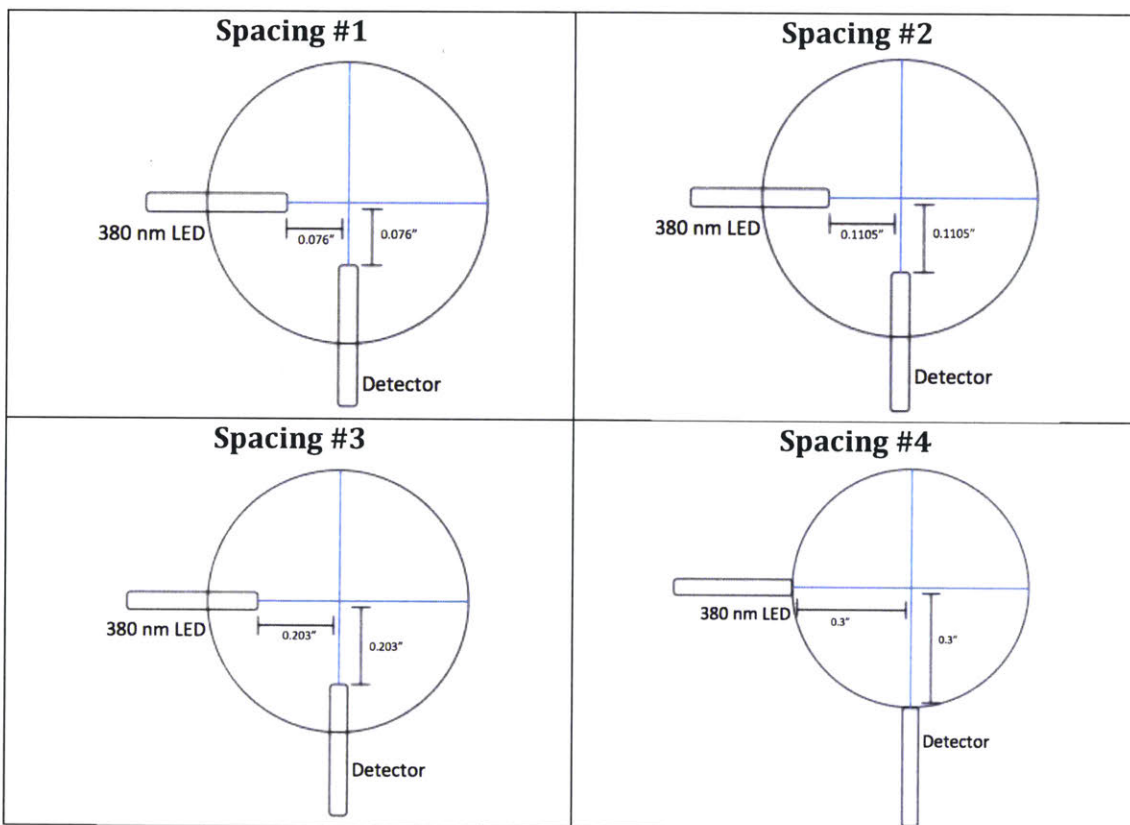


Table 2: Four fiber spacings tested.

The results of these tests demonstrated that closest spacing produced the highest fluorescence intensity readings (see Figure 4). See Appendix 2 for images of the spectra produced for all concentrations under each spacing.

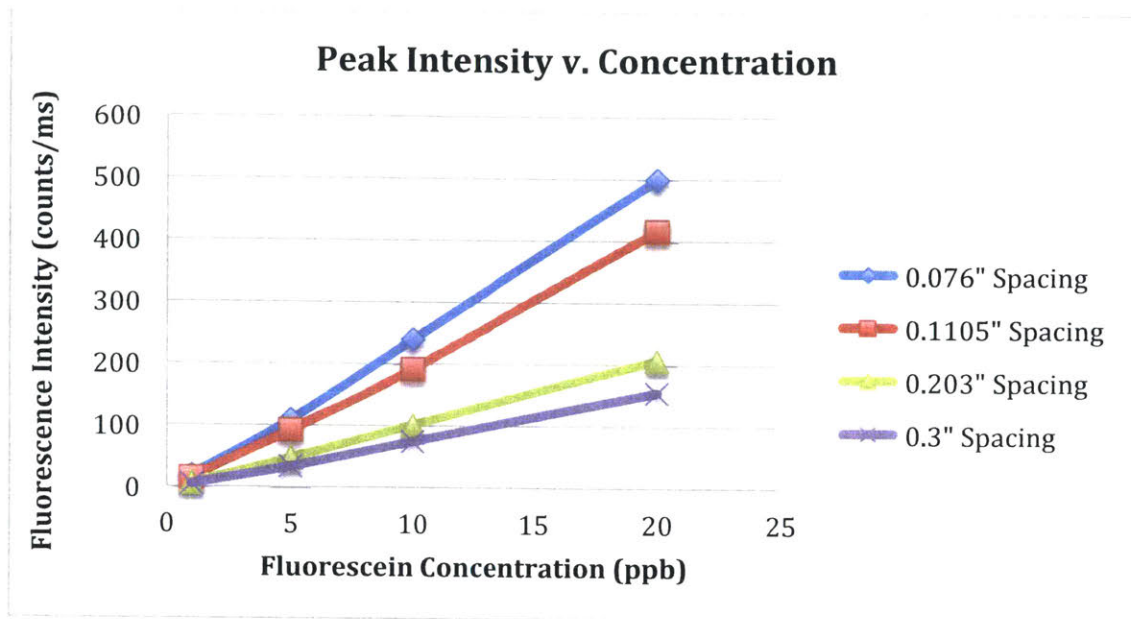


Figure 4: Fluorescence intensity results for the 4 spacings. The narrowest spacing between the fiber and the detector yielded the highest fluorescence intensity. Background spectrum has been subtracted from the intensity values in this graph.

The 0.076-inch spacing between the excitation and detector fibers serves as the optimal Hammerhead geometry to obtain a robust signal from the sample. Figure 5 displays photographs of this configuration, looking down into the chamber from the top.

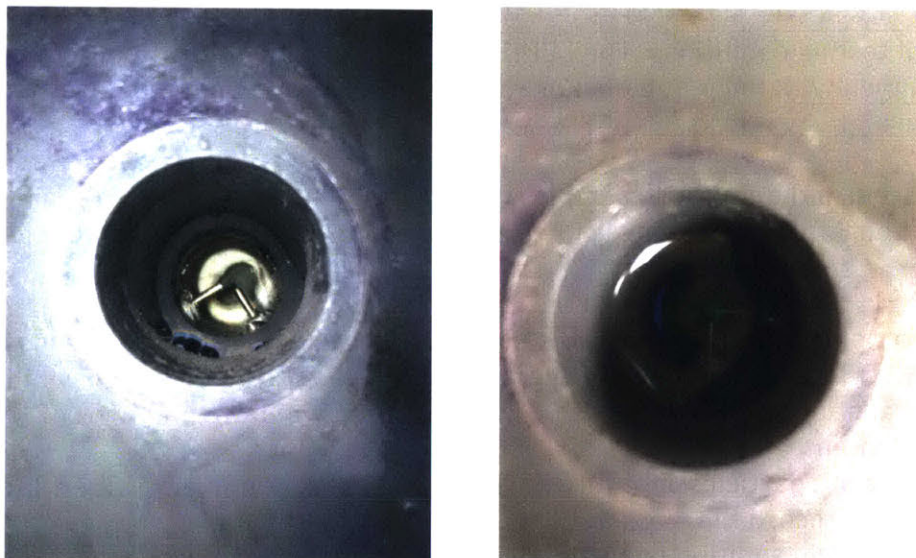


Figure 5: Optimal spacing between excitation and detection fibers (0.076 inches from the tip of each fiber to the center of the chamber). (Right image: empty chamber; Left image: excited sample)

The next stage of this project compares this optimal arrangement of the fiber optic device (Spacing #1) to a more traditional instrument of water quality measurement: the LEDIF flow cell.

4. Comparison to LEDIF Flow Cell

To compare the performance of the Hammerhead with the LEDIF, a number of confounding variables must be controlled. A summary of the instrument testing conditions is provided in Table 3.

	Hammerhead	LEDIF
Integration Time	80 ms	80 ms
LED Excitation Wavelength(s)	380 nm	375 nm 405 nm
LED Power Output (at 20 mA current)	1 mW	1 mW for 375 nm 6 mW for 405 nm
Optics	Fiber Optical	Lenses (flow cell)
Scans to Average	1	1
Number of trials	2	2 (1 with pump on, 1 with pump off)

Table 3: Instrument testing conditions. The LEDIF was run under two settings: first with its water pump on, then with the pump off. For each device, fluorescence intensities were taken as an average of the two tests.

Both fluorescein and humic acid samples were tested. Fluorescein was prepared as described in section 3a. The following is the procedure for preparing the humic acid concentrations:

1. Prepare 1 liter of 100 ppm humic acid stock solution by adding 100 milligrams of humic acid powder to a container (preferably brown glass to avoid the possibility of photodegradation), then filling with 1 liter deionized water.
2. Stir well (5-7 minutes).
3. Before filtering out particles, measure the dry weight of a 47 mm glass fiber filter (grade .691) and record the mass.
4. Filter the humic acid solution by using a vacuum filtration pump with the 47 mm glass fiber filter (grade .691).
 - a. If filtering takes too long and the filter gets clogged, pour out the contents of the container above the filter back into the humic acid container and replace the filter. Be sure to save all used filters.
5. When filtration is finished, pour the filtered humic acid into a new container.

6. Test the pH of the filtered humic acid using a pH probe. Titrate as needed with 0.1 M sodium bicarbonate (NaHCO_3) until the pH is around 7-10, to ensure more complete dissolution of the humic acid.
7. Stir well (7-10 minutes).
8. After the used glass fiber filter has dried, weigh its mass and subtract the pre-filtration mass to calculate how much humic acid powder was lost through filtration.
9. Use the lost mass of humic acid to calculate the actual concentration of humic acid stock solution – it will likely be less than 100 ppm.
 - a. In this case, 35.4 mg was removed through filtration, so the stock solution was 64.6 ppm humic acid.
10. Dilute the humic acid stock solution into desired concentrations using deionized water. Stir each concentration well (2-3 minutes).

For these experiments, six different concentrations of fluorescein were tested: 0.1 ppb, 0.2 ppb, 0.4 ppb, 0.8 ppb, 1 ppb, and 5 ppb. Five different concentrations of humic acid were tested: 1 ppm, 3 ppm, 10 ppm, 30 ppm, and 60 ppm.

Each concentration was tested in duplicate, with three “blank” samples of deionized water between each change in concentration. The results are shown in Figures 6 and 7. See Appendix 3 for images of the spectra produced for each fluorescein concentration and Appendix 4 for images of the spectra produced for each humic acid concentration.

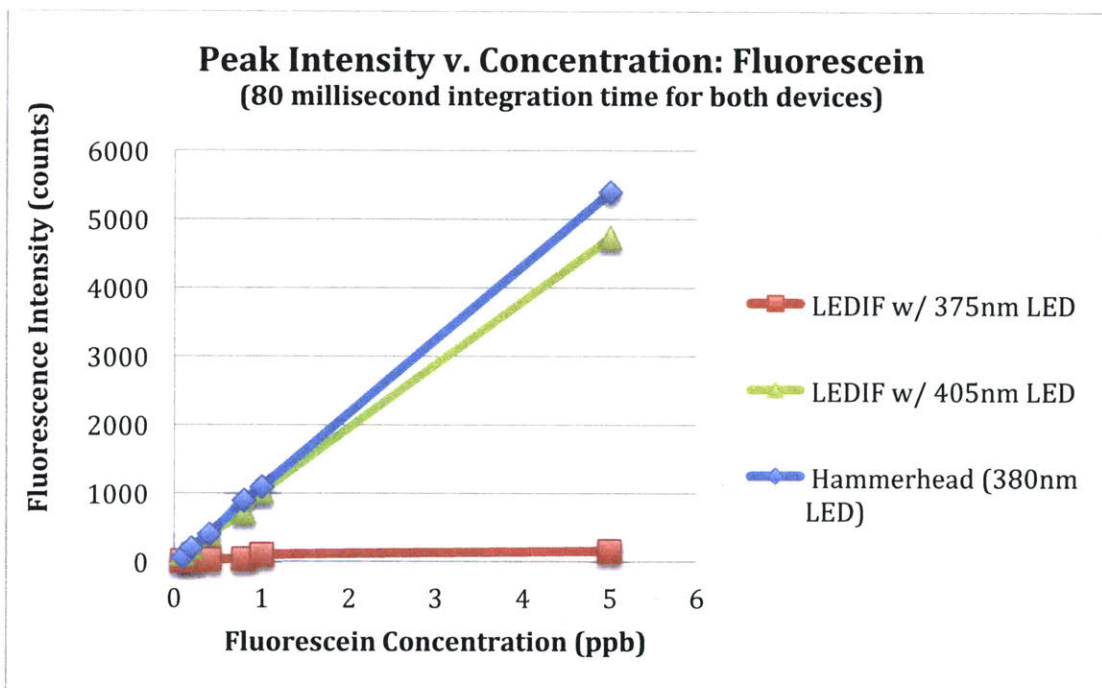


Figure 6: Fluorescein results for the Hammerhead and LEDIF. The Hammerhead exhibited greater sensitivity to the concentrations of fluorescein tested. Background spectrum has been subtracted from the intensity values in this graph.

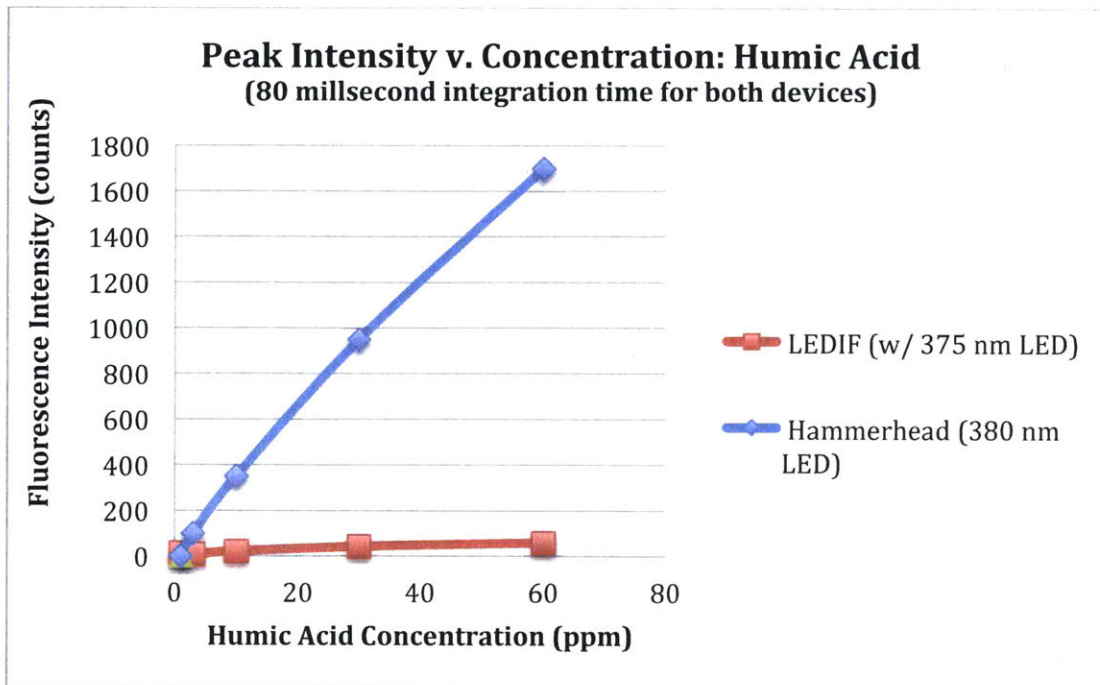


Figure 7: Humic acid results for the Hammerhead and LEDIF. The Hammerhead exhibited greater sensitivity to the concentrations of humic acid tested. Background spectrum has been subtracted from the intensity values in this graph.

For both fluorescein and humic acid, the Hammerhead exhibits greater sensitivity than does the LEDIF with 375 nm LED.

To further analyze the sensitivity of each instrument, the limit of detection was calculated for each of the testing methods: the Hammerhead (with 380 nm LED); the LEDIF with 375 nm; and the LEDIF with 405 nm. The limit of detection is defined as producing a signal at least three standard deviations above the background. See Table 4 for detection limit calculations and Figures 8 and 9 for results.

	Hammerhead	LEDIF w/ 375 nm: fluorescein trials	LEDIF w/ 375 nm: humic acid trials	LEDIF w/ 405 nm
Background average	16 counts	535 counts	555 counts	570 counts
Background standard deviation	54 counts	7 counts	7 counts	6.7 counts
3*(background standard deviation)	164 counts	21 counts	21 counts	20 counts
Limit of Detection	> 180 counts	> 556 counts	> 576 counts	> 590 counts

Table 4: Detection limit of each instrument

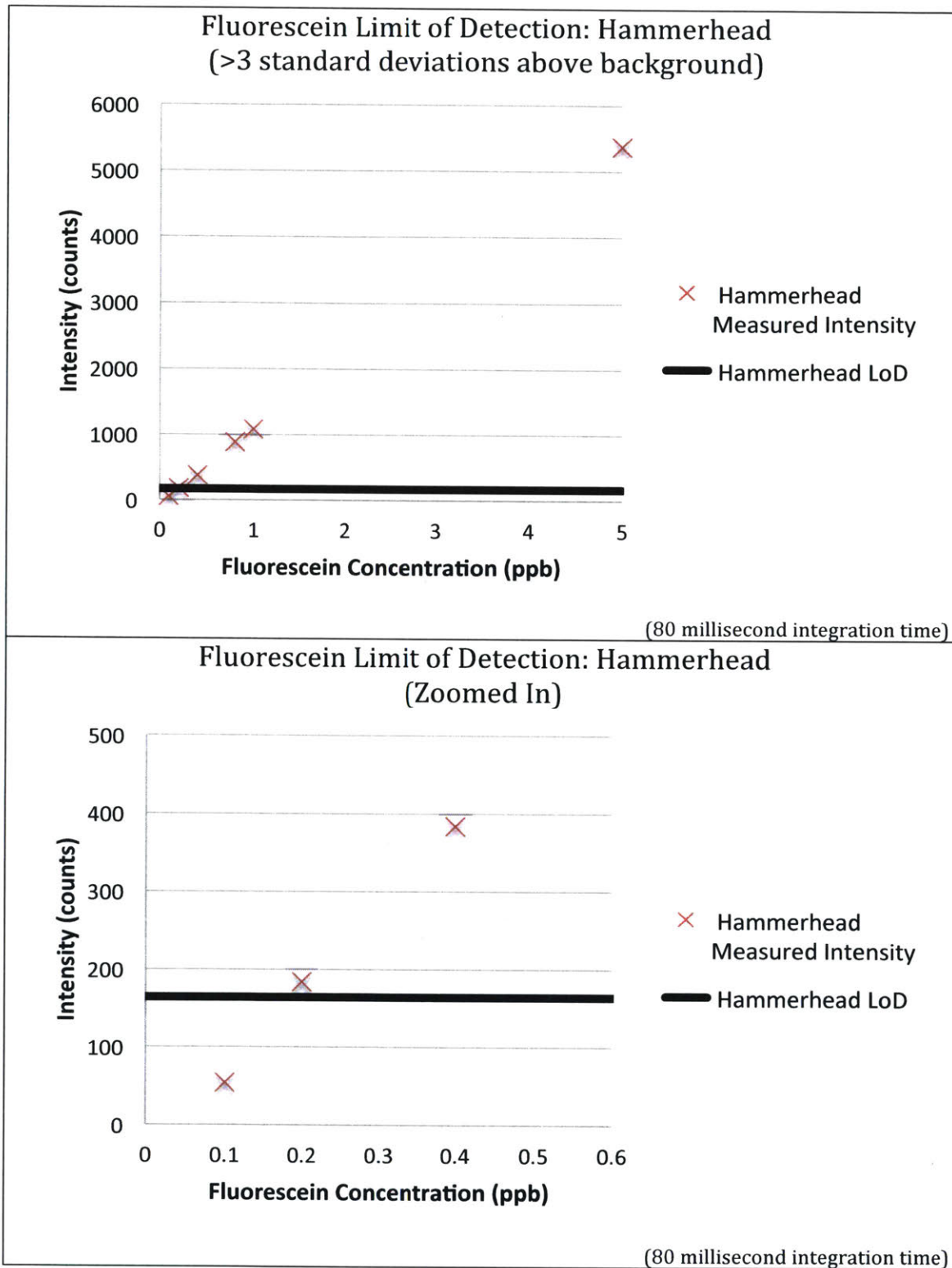


Figure 8a: Hammerhead limit of detection for fluorescein. Top: full view, bottom: zoomed in. Background has been subtracted from the intensity values in this graph.

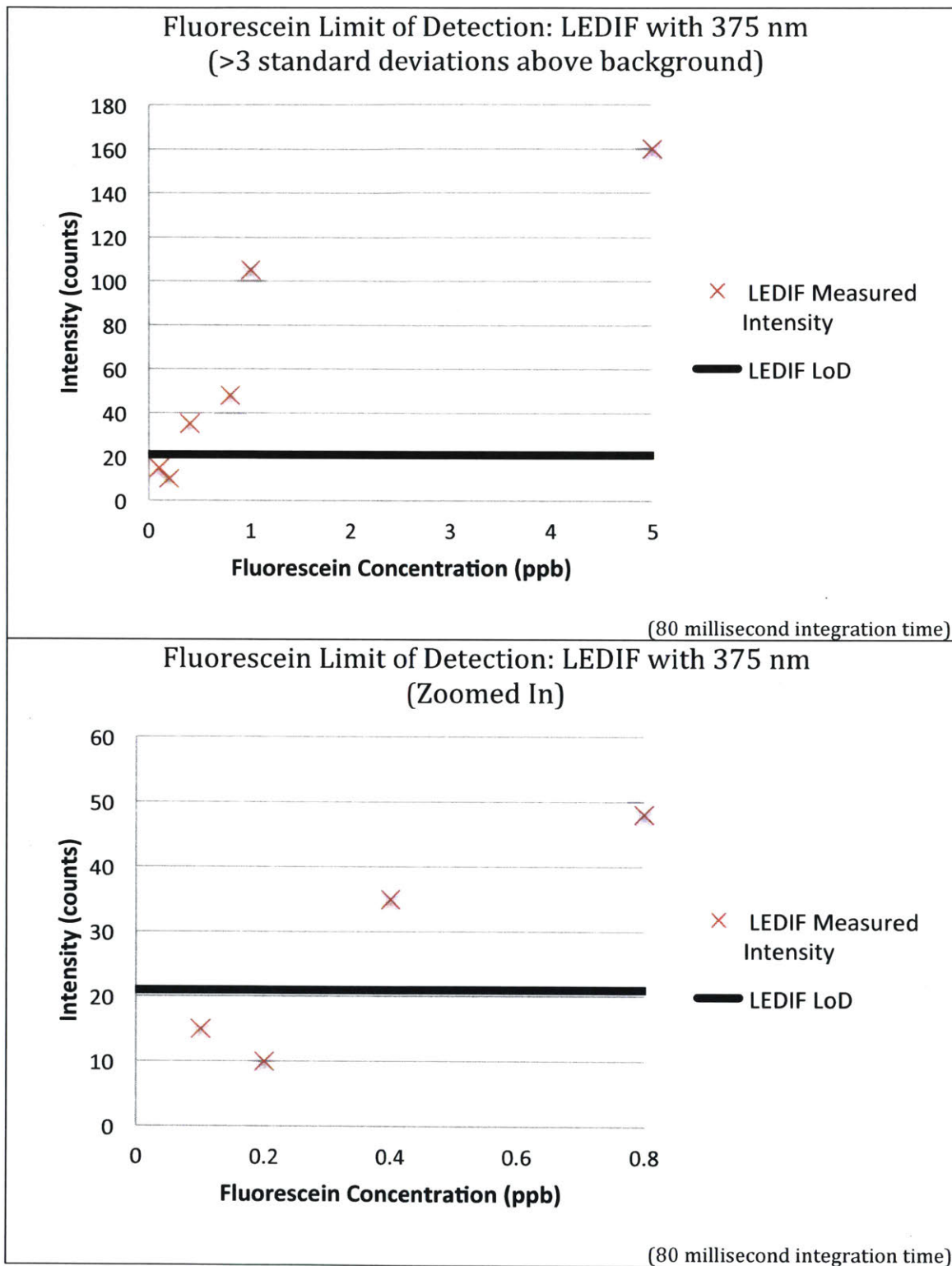


Figure 8b: LEDIF limit of detection for fluorescein with 375 nm. Top: full view, bottom: zoomed in. Background has been subtracted from the intensity values in this graph.

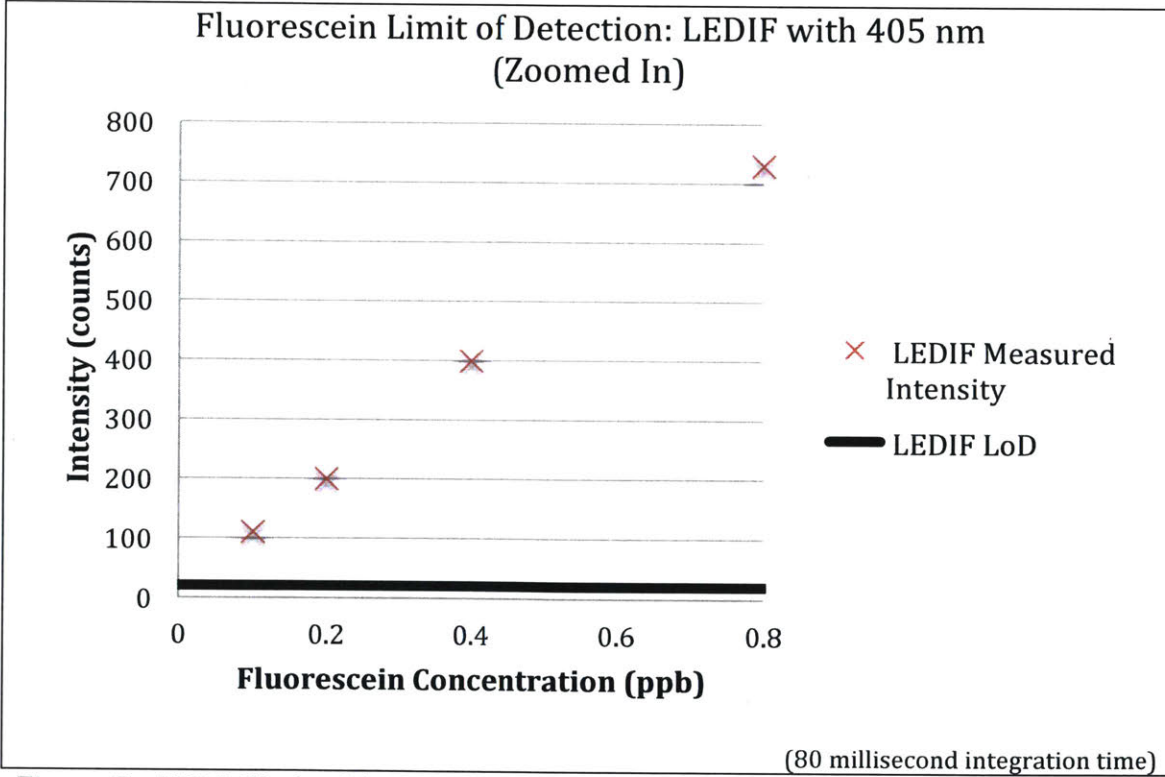
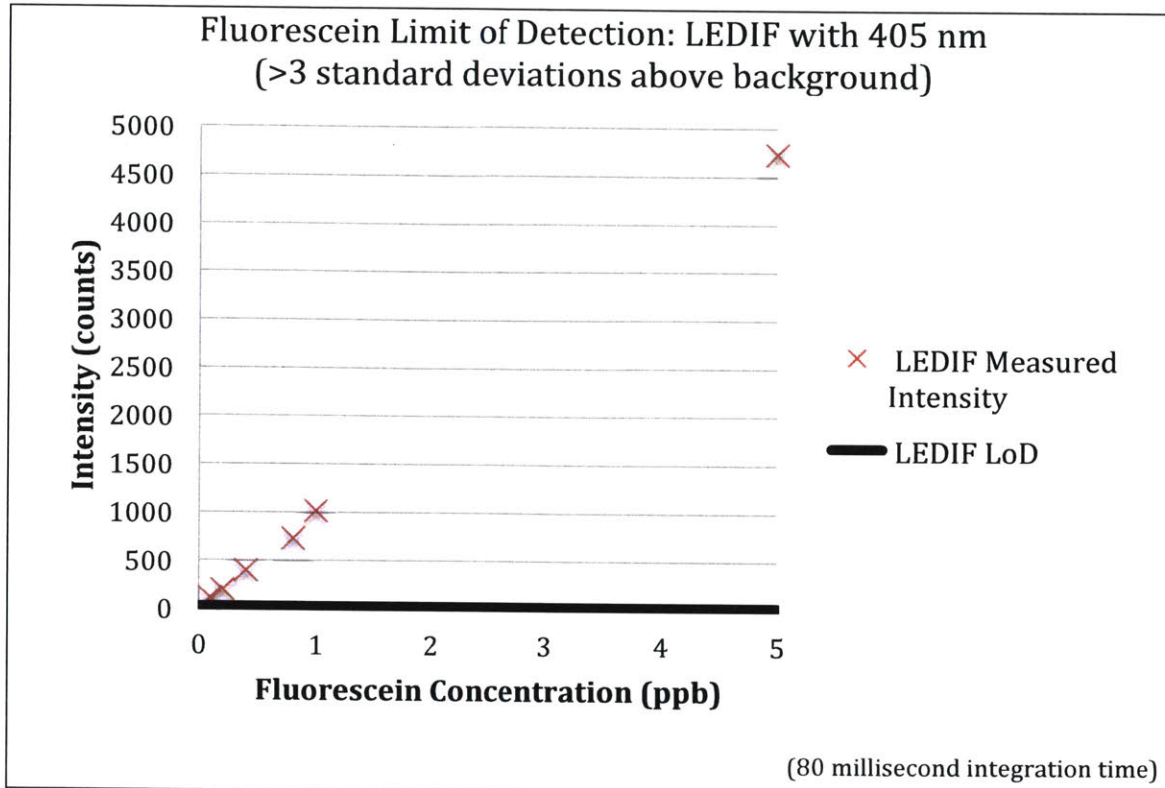


Figure 8c: LEDIF limit of detection for fluorescein with 405 nm. Top: full view, bottom: zoomed in. Background has been subtracted from the intensity values in this graph.

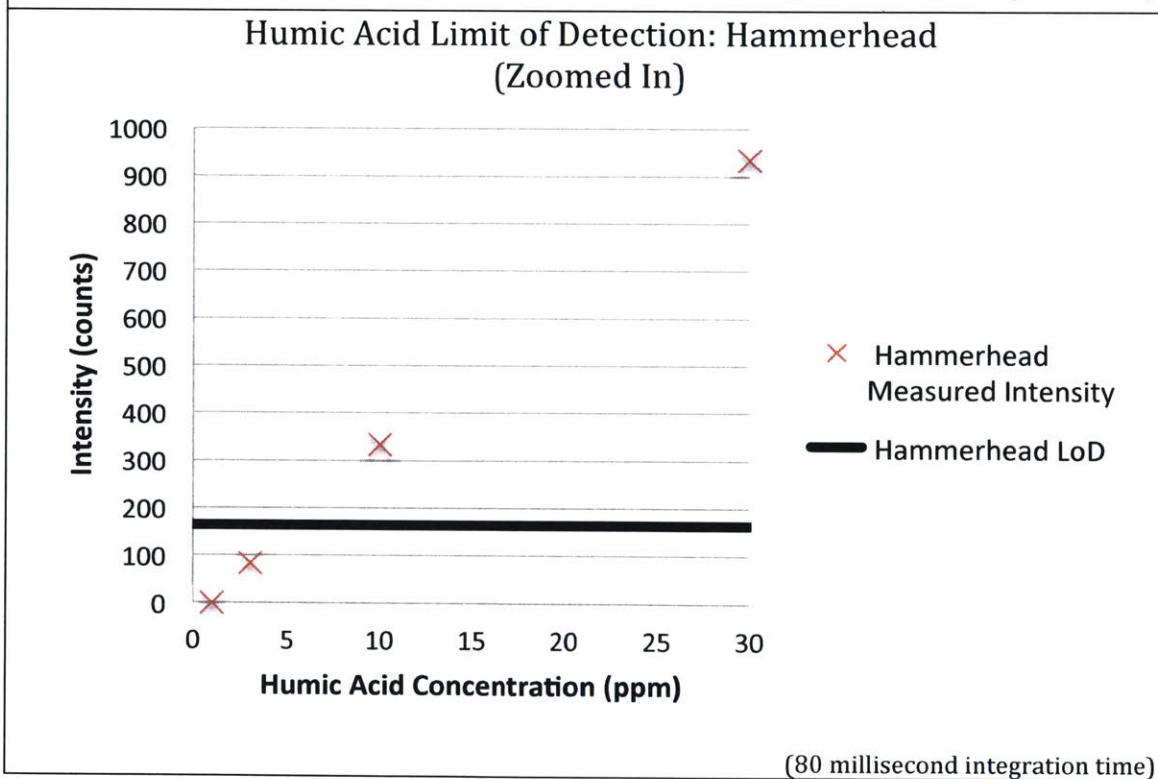
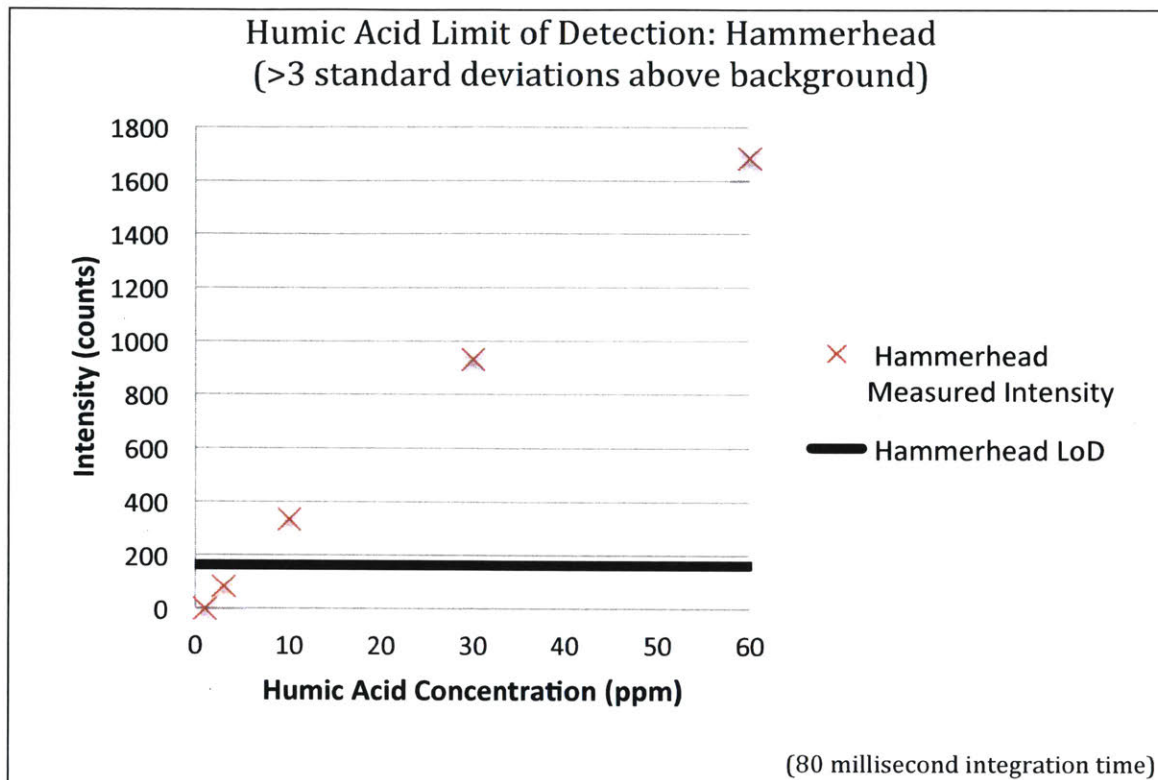


Figure 9a: Hammerhead limit of detection for humic acid. Top: full view, bottom: zoomed in. Background has been subtracted from the intensity values in this graph.

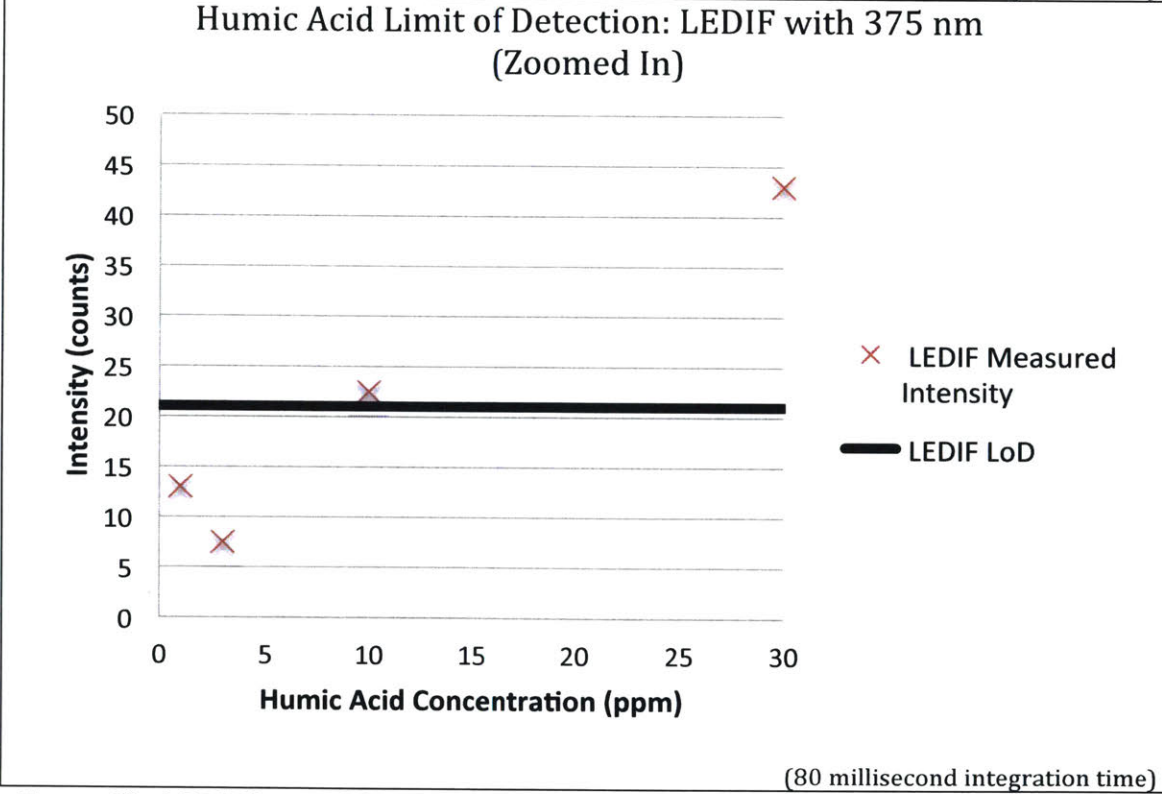
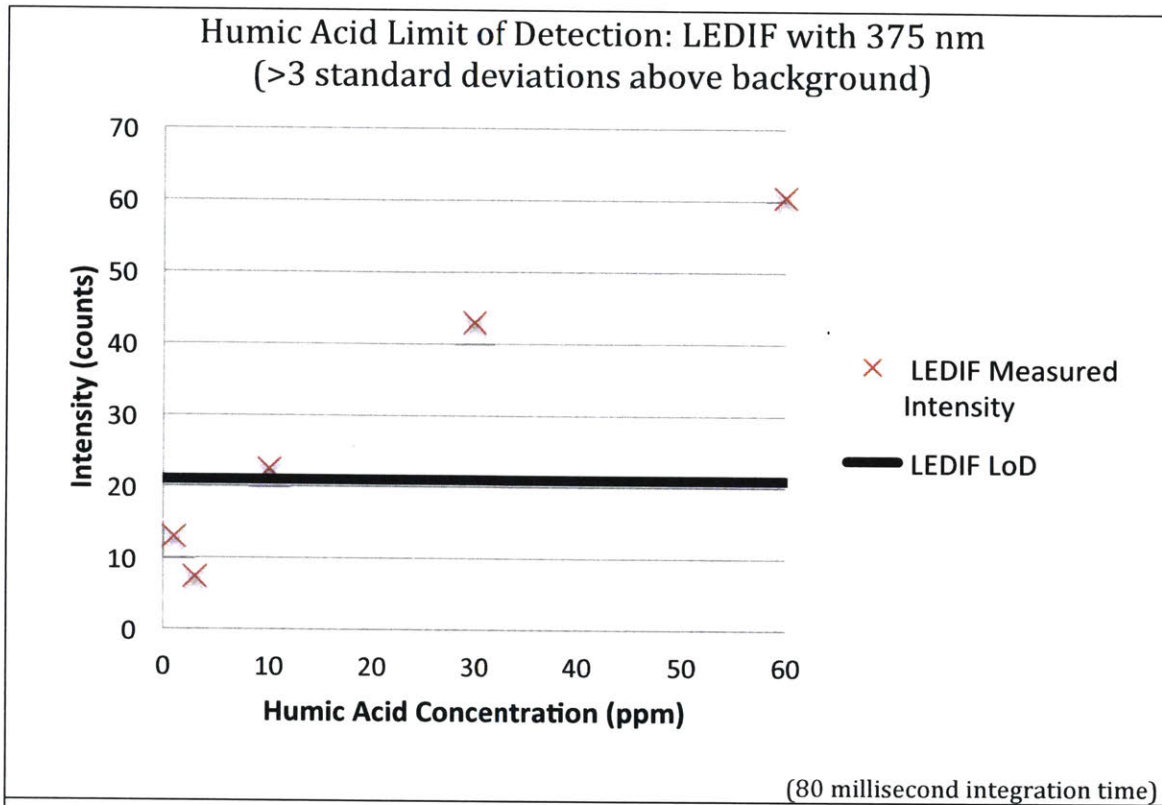


Figure 9b: LEDIF limit of detection for humic acid. Top: full view, bottom: zoomed in. Background has been subtracted from the intensity values in this graph.

Results for **fluorescein** indicate that the Hammerhead has a limit of detection around **0.2 ppb** (see Figure 8a). When tested with the 375 nm LED, the LEDIF has a limit of detection for fluorescein that is difficult to distinguish, but may be interpolated to be around **0.3 ppb** (Figure 8b). At low concentrations, the Hammerhead delivers a signal greater than the LEDIF (with 375 nm) by about a factor of 10. After subtracting background noise, the Hammerhead displays a signal of approximately 400 counts for 0.4 ppb fluorescein, while the LEDIF (at 375 nm) displays approximately 40 counts for 0.4 ppb fluorescein.

When tested with the 405 nm LED, the LEDIF has a limit of detection that is likely lower than 0.1 ppb, the lowest concentration tested (Figure 8c). Using 405 nm excitation, the LEDIF outperforms the Hammerhead at a fluorescein concentration of 0.4 ppb.

Results for **humic acid** indicate that the Hammerhead has a limit of detection around **5 ppm**, determined by interpolating the graph in Figure 9a. When tested with the 375 nm LED, the LEDIF has a limit of detection for humic acid that is difficult to distinguish, but may be interpolated to be around **9 ppm** (Figure 9b). In agreement with fluorescein results, at low concentrations of humic acid, the Hammerhead again outperforms the LEDIF by about a factor of 10. This data illustrates that the Hammerhead produces a stronger signal than the LEDIF flow cell, even at very low concentrations.

5. Conclusions

The greater sensitivity of the Hammerhead for the humic acid and fluorescein concentrations tested supports the hypothesis that a fiber optic scheme delivers a superior performance by utilizing short path lengths and close fiber spacing. Comparing the signals for both instruments indicates that the Hammerhead fiber optic scheme has the advantage in detecting fluorescence measurements at high DOC concentrations; it produces a nearly linear response for fluorescence. In contrast, the LEDIF delivers results impeded by inner shielding and exhibits a curve of decreasing slope for fluorescence versus DOC content, as seen in Figure 10. This information will be useful in future applications of the Hammerhead for in-situ measurements of DOC transport, particularly in environments of high DOC content such as peatlands. Moreover, the Hammerhead has a lower limit of detection, suggesting that it performs better than the LEDIF even independent of inner shielding effects, producing a signal nearly 10 times greater than the LEDIF in low DOC concentrations.

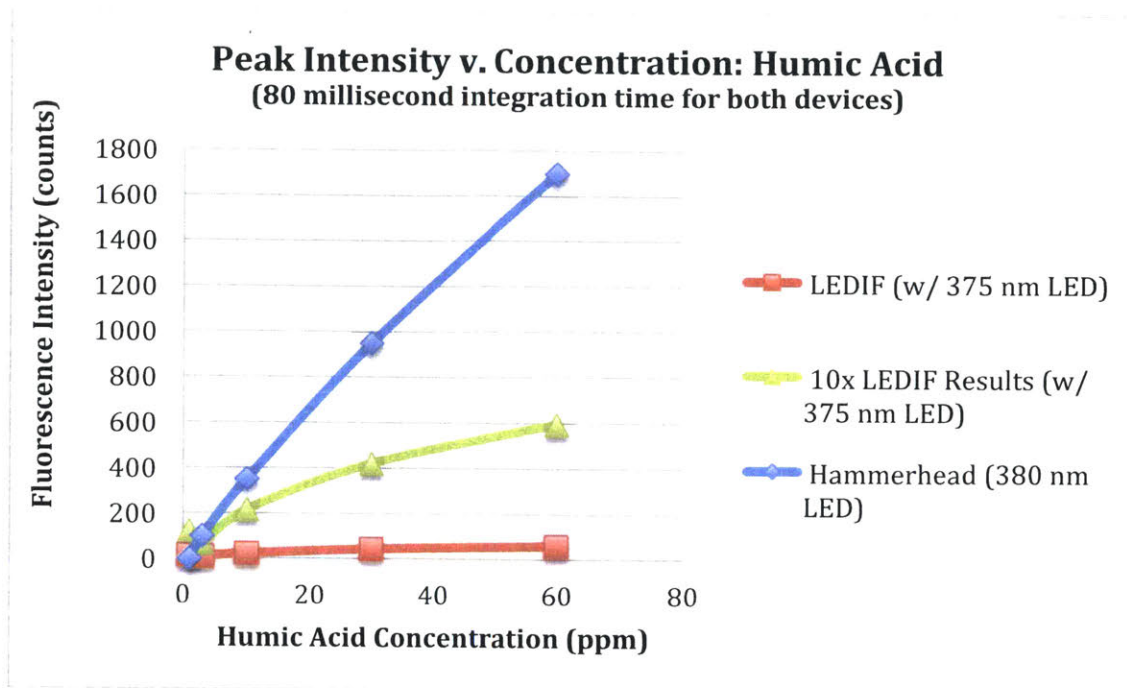


Figure 10: Magnified LEDIF versus Hammerhead humic acid results.

With its ability to accurately determine aquatic DOC levels, the Hammerhead holds important implications for water quality testing. As patterns of climate change continue, effects will be experienced regionally in surface water bodies. Land use changes, such as deforestation, as well as enhancement of the water cycle due to higher temperatures, will result in altered levels of DOC transport in many surface water bodies. Thus, quantification of DOC transport becomes increasingly important in order to determine the local effects of a globally changing climate.

6. Future Work

Moving forward with this project, it would probably first be helpful to compare the LEDIF and the Hammerhead using longer integration times. It would be interesting to see if, using a longer integration time, fluorescence signals would be higher above the limit of detection for each device.

It should be noted that as part of this project, brief experiments were run using a 1 second and 10 second integration time for the humic acid samples, but at a 1 second integration time, the signal was still very low for the LEDIF, and the Hammerhead became saturated at its excitation peak. In addition, the background noise from the Hammerhead was significant. A 10 second integration time produced a much larger signal from the LEDIF, but it saturated the Hammerhead at the fluorescence peak, so results could not be compared. Thus, the 80 millisecond integration time – the highest possible integration time without saturating either peak of the Hammerhead – was chosen. Future tests should use integration times between 80 milliseconds and 1 second.

In addition, tests should be run using all three LED excitation sources for the Hammerhead. The geometry of the current instrument makes this impossible because

the top fiber is confined to one position. Tests with a new Hammerhead model could provide greater flexibility for all three fibers to be simultaneously positioned very close to the detector. The Hammerhead's performance might prove even stronger with all three LEDs operating.

To provide a more comprehensive comparison between the Hammerhead and the LEDIF, it could also be beneficial to run experiments with the exact same excitation wavelength (i.e., 375 nm versus 375 nm, rather than the 375 nm versus 380 nm used in these trials). It would also be useful to test whether the greater sensitivity of the Hammerhead is maintained when different, higher, excitation wavelengths are used in each device.

Finally, it would be interesting to look at results for absorbance and scattering measured by the Hammerhead. The Hammerhead is equipped with a broadband light source for absorbance measurements; future experiments should incorporate this function as well as fluorescence.

7. References

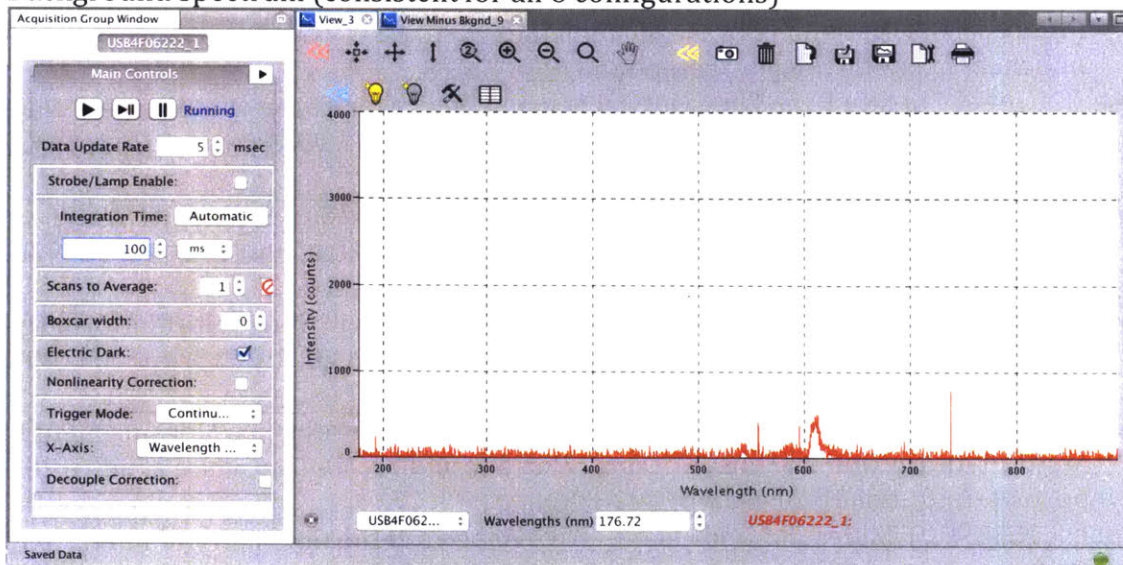
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Appendix 1 – Hammerhead Fluorescein Spectra for 6 Configurations in Table 1

Notes:

- The integration time for each sample may be seen in the box on the left-hand side of each image.
- Each integration time used was 100 milliseconds OR the highest possible for that sample without saturating the USB4000 detector.
- For reference, graph 1 depicts the background spectrum with an unlit chamber, which is consistent for all 6 configurations.
- Background spectrum has been subtracted in the graphs for each configuration (graphs 2-7).

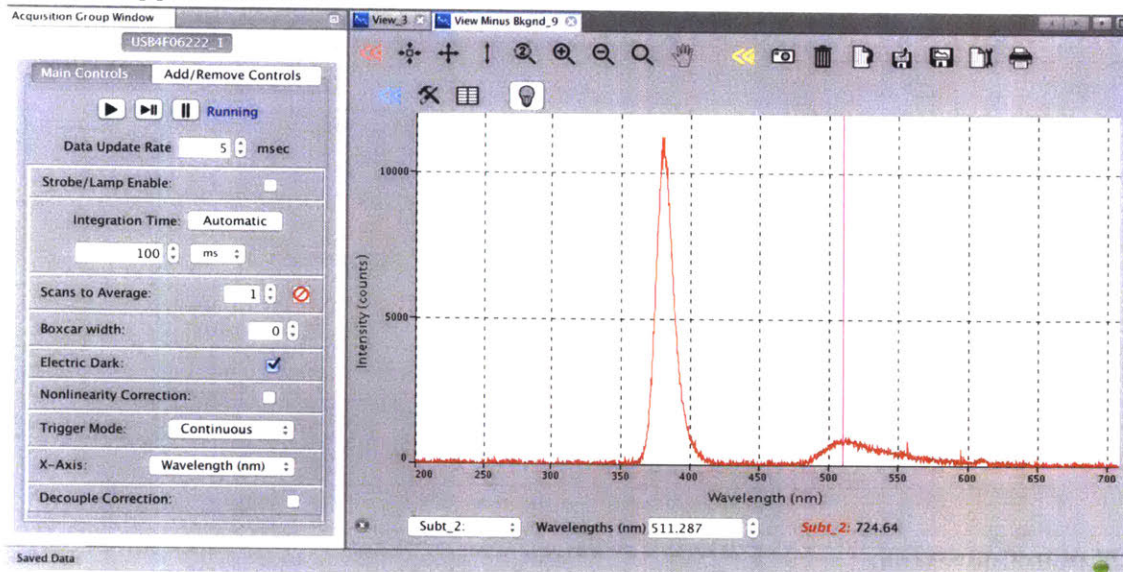
1. Background spectrum (consistent for all 6 configurations)



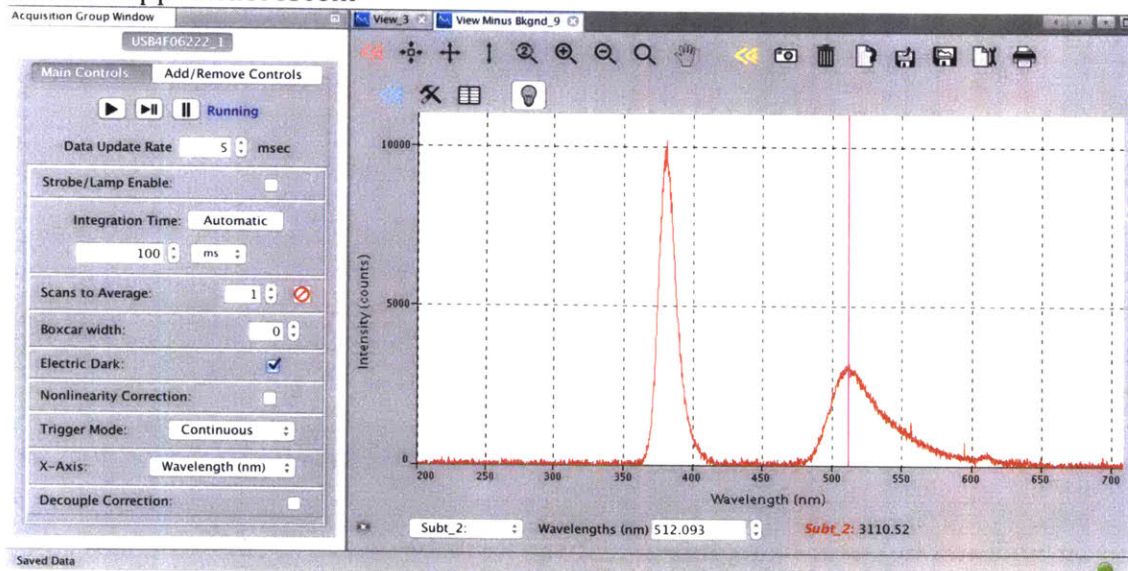
Background average: 16 counts

2. Configuration 1: detector and excitation fibers flush with chamber walls

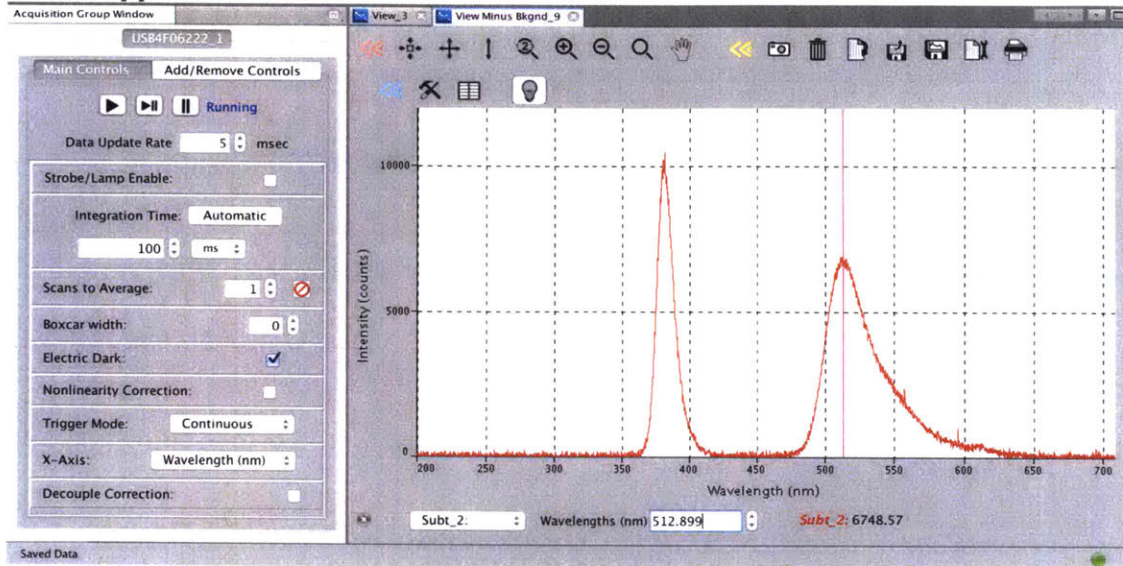
a. 0.001 ppm fluorescein



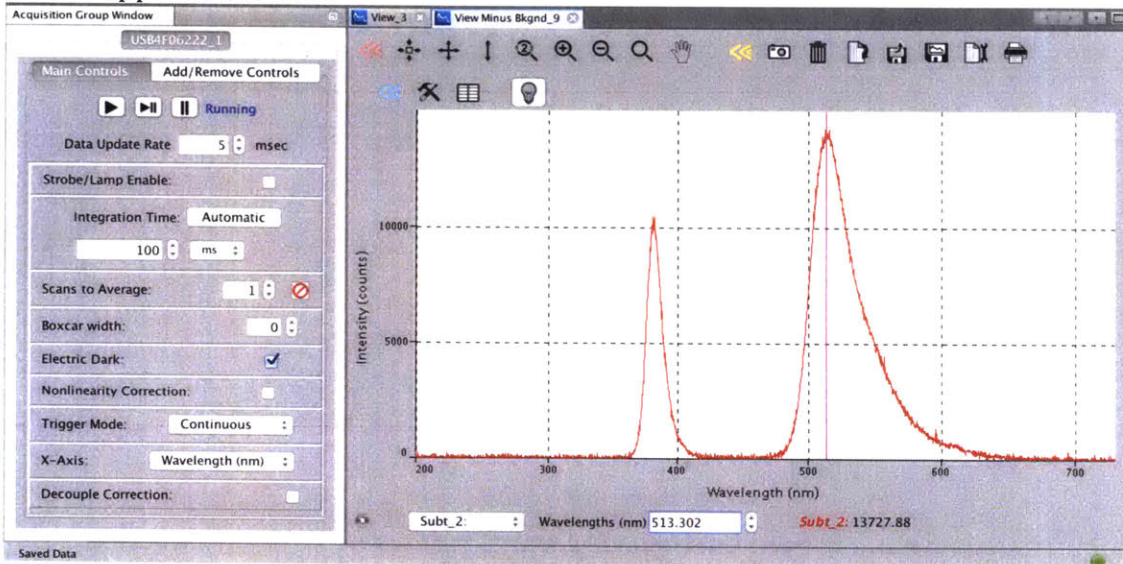
b. 0.005 ppm fluorescein



c. 0.01 ppm fluorescein

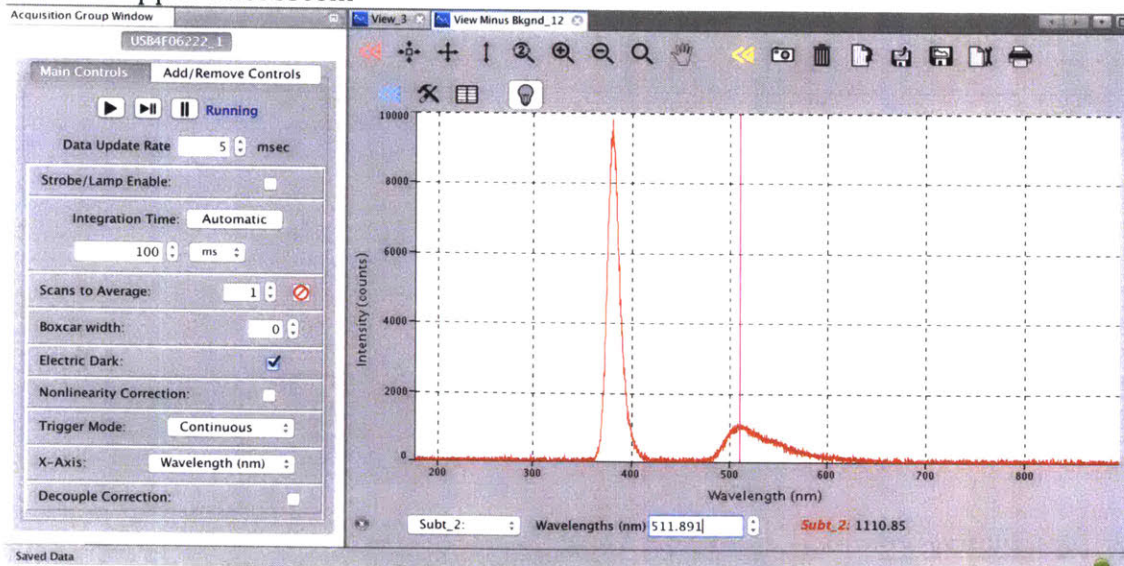


d. 0.02 ppm fluorescein

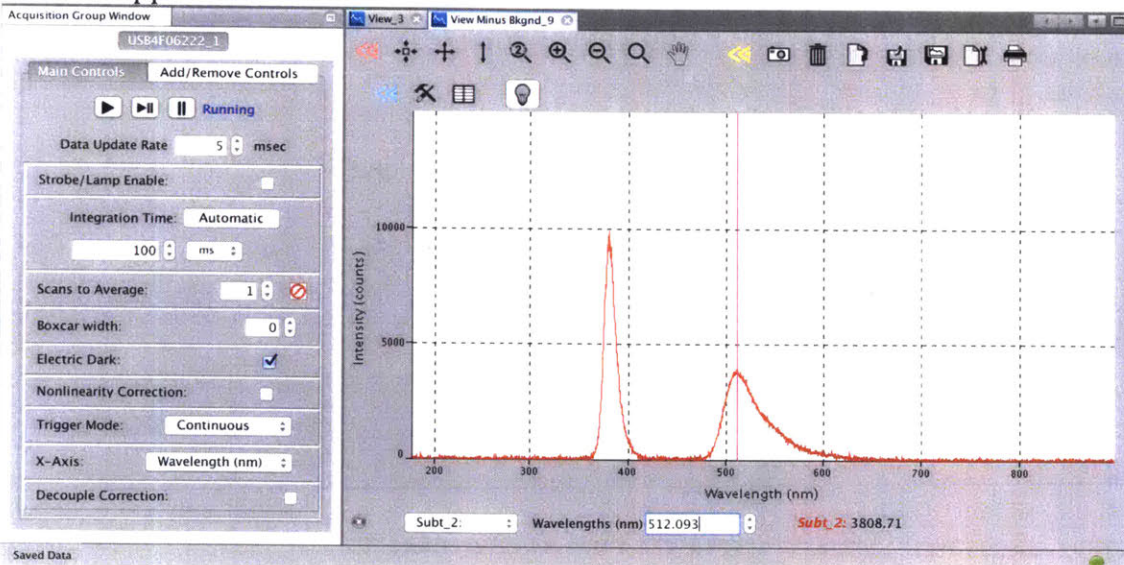


3. Configuration 2 (From Table 1): detector fiber flush with chamber walls, bottom 2 excitation fibers inserted 0.1 inches into chamber

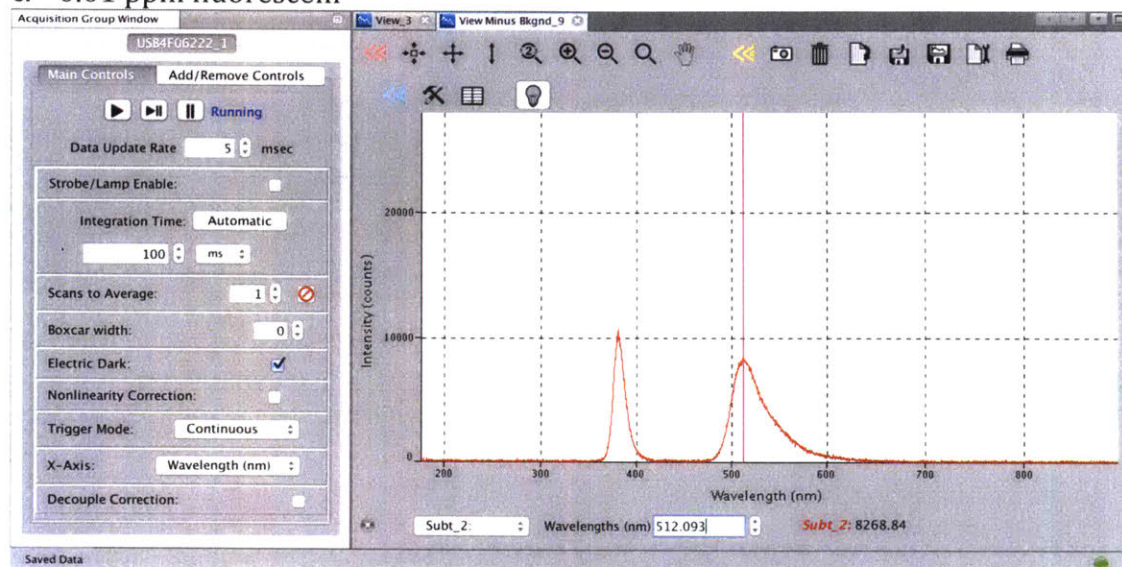
a. 0.001 ppm fluorescein



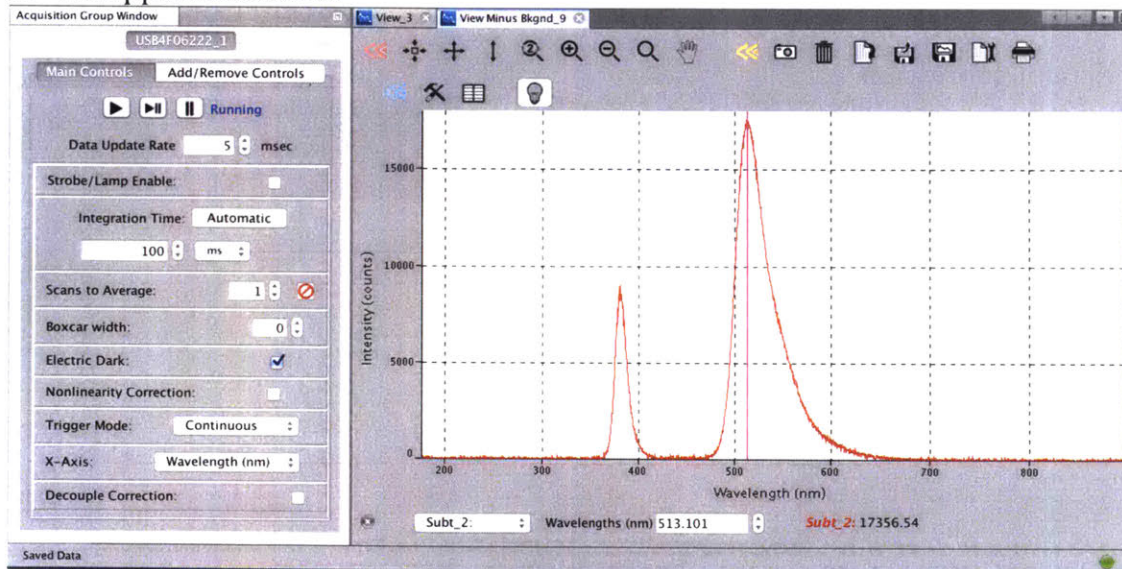
b. 0.005 ppm fluorescein



c. 0.01 ppm fluorescein

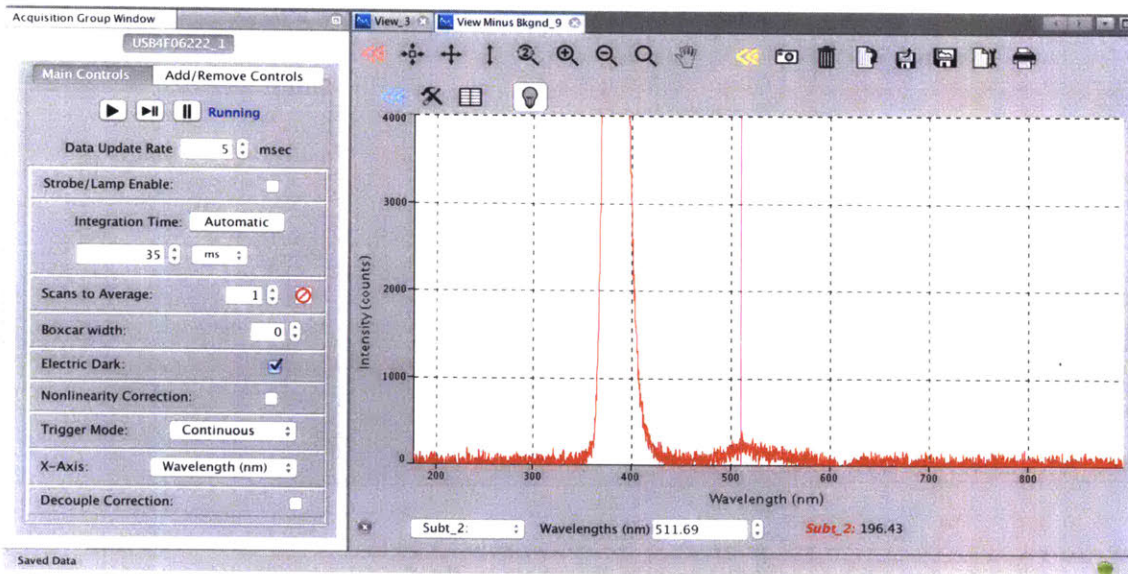
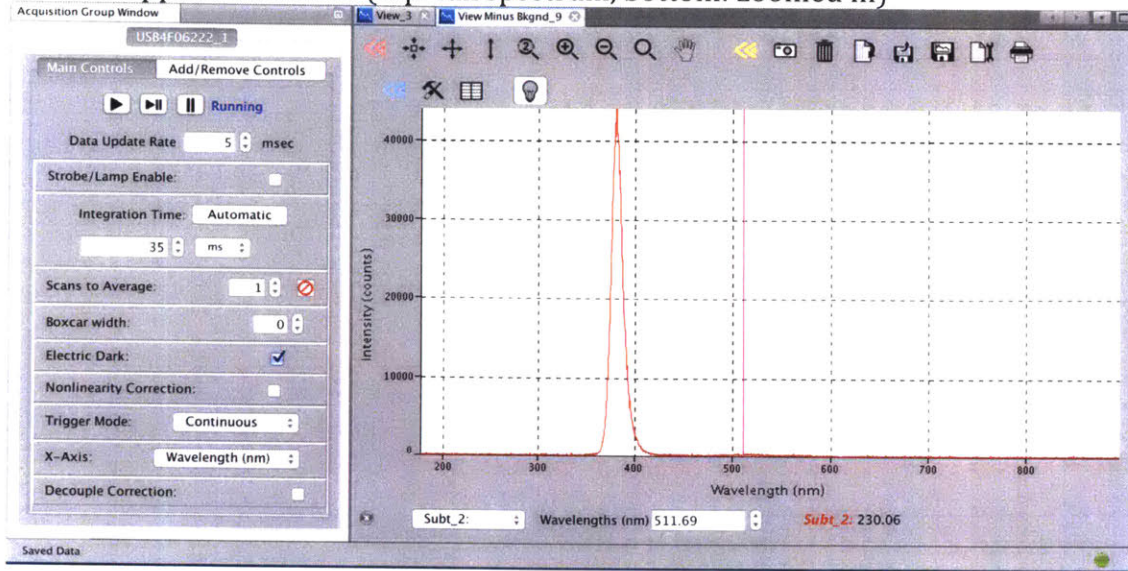


d. 0.02 ppm fluorescein

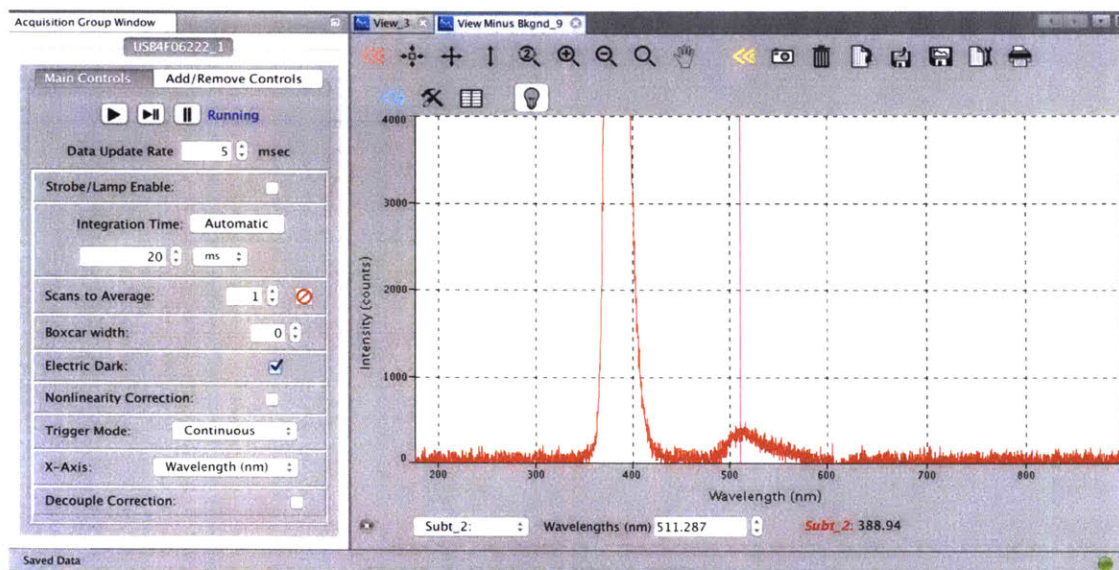
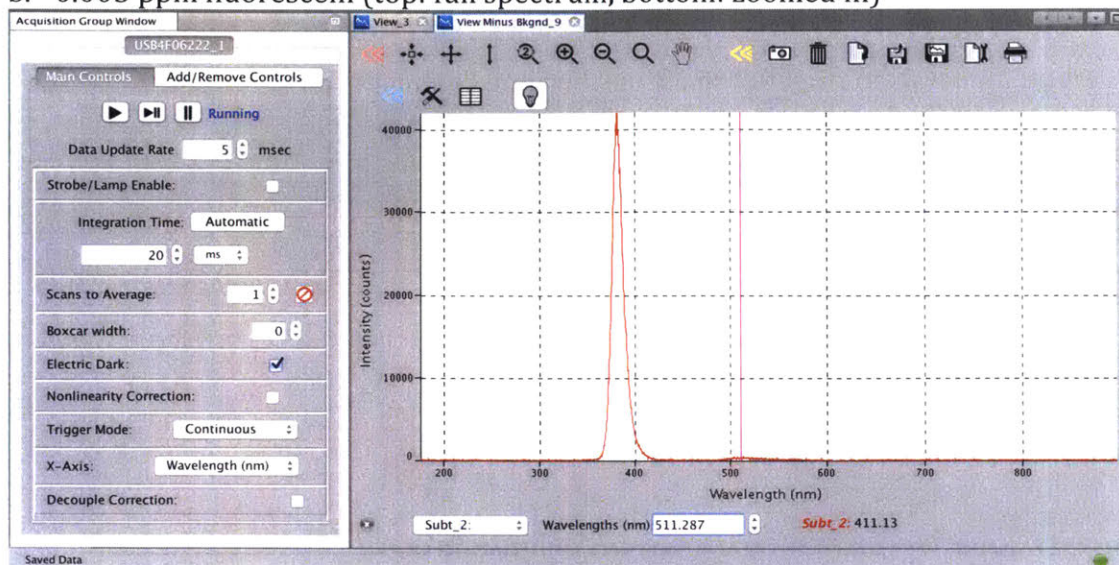


4. Configuration 3 (From Table 1): detector fiber flush with chamber walls, bottom 2 excitation fibers inserted 0.3 inches into chamber

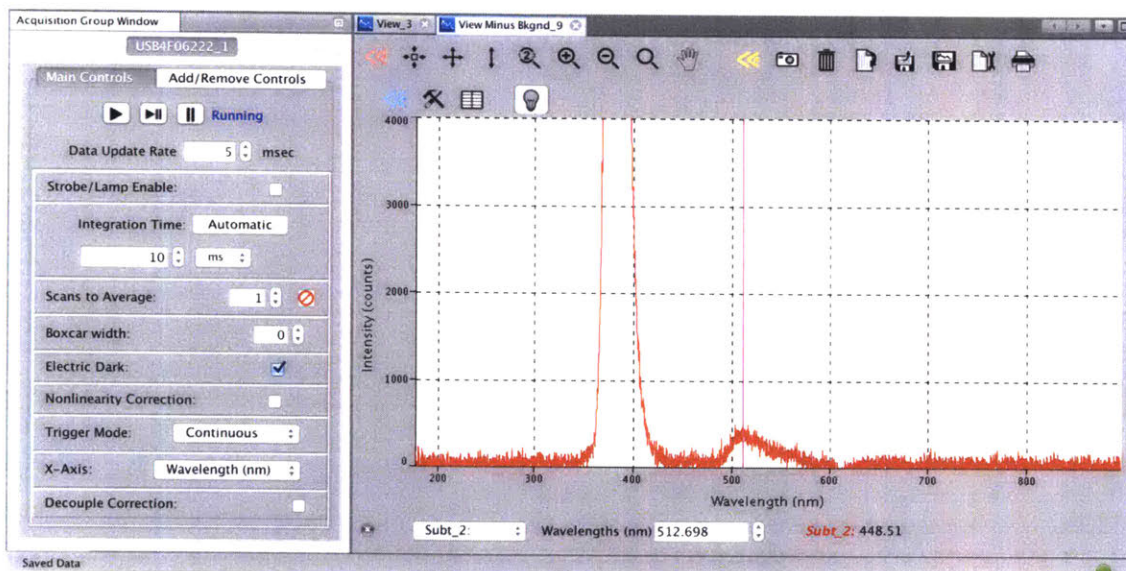
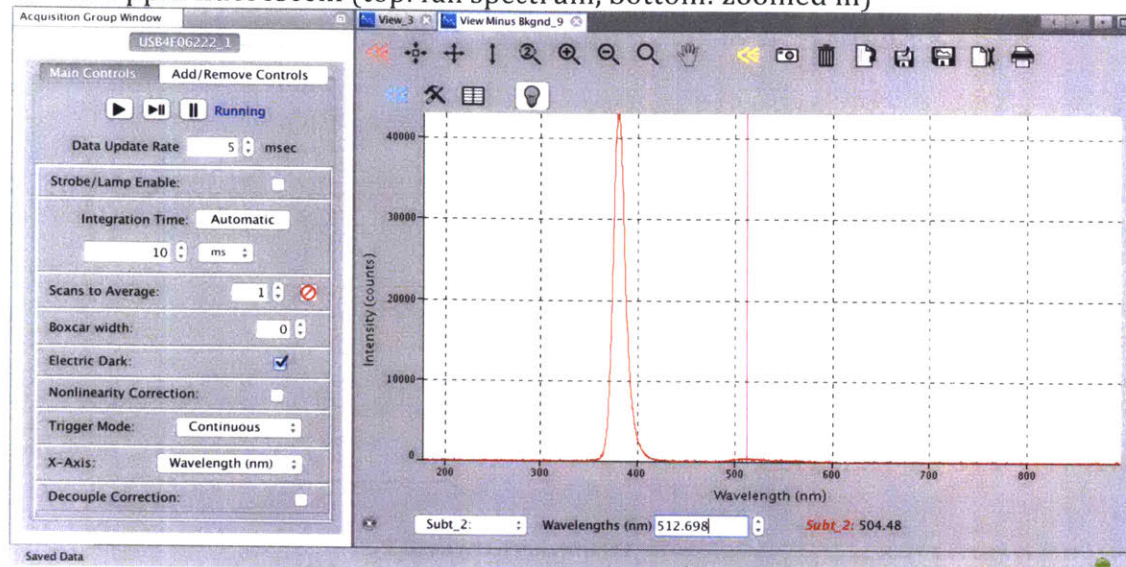
a. 0.001 ppm fluorescein (top: full spectrum, bottom: zoomed in)



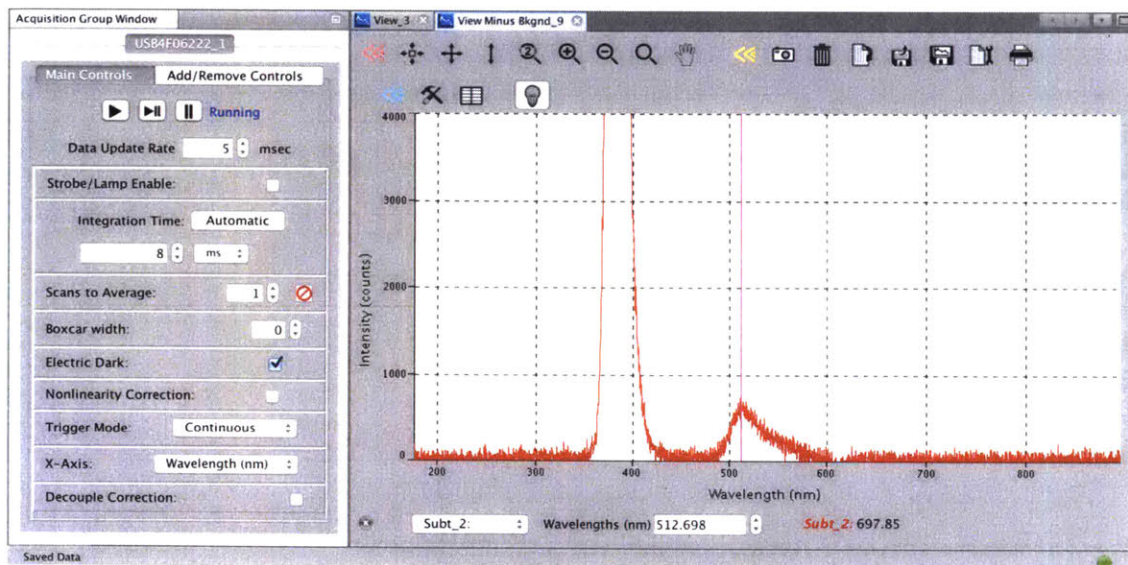
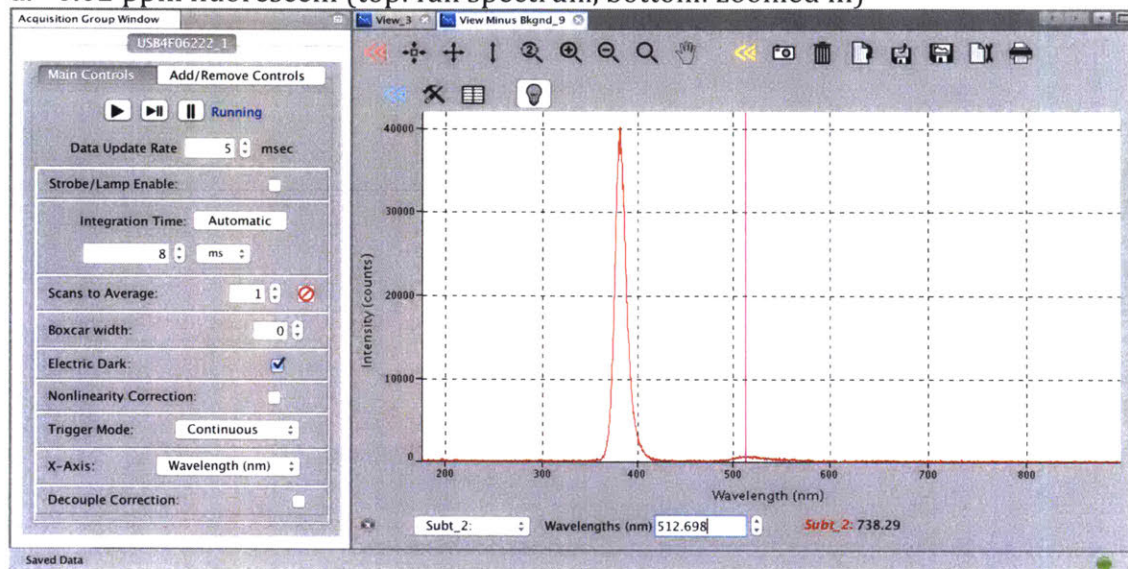
b. 0.005 ppm fluorescein (top: full spectrum, bottom: zoomed in)



c. 0.01 ppm fluorescein (top: full spectrum, bottom: zoomed in)

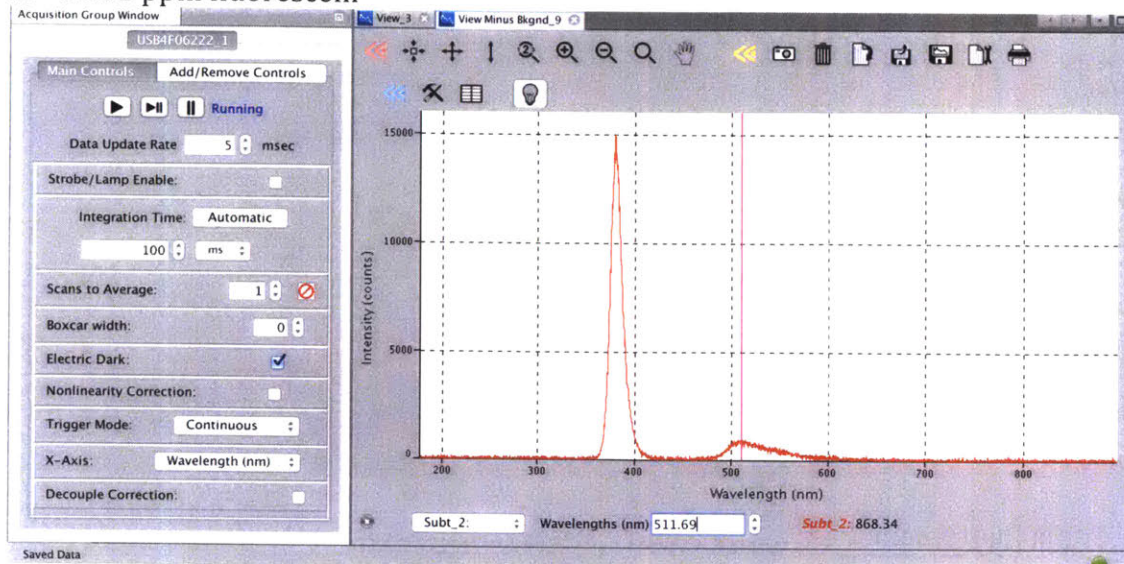


d. 0.02 ppm fluorescein (top: full spectrum, bottom: zoomed in)

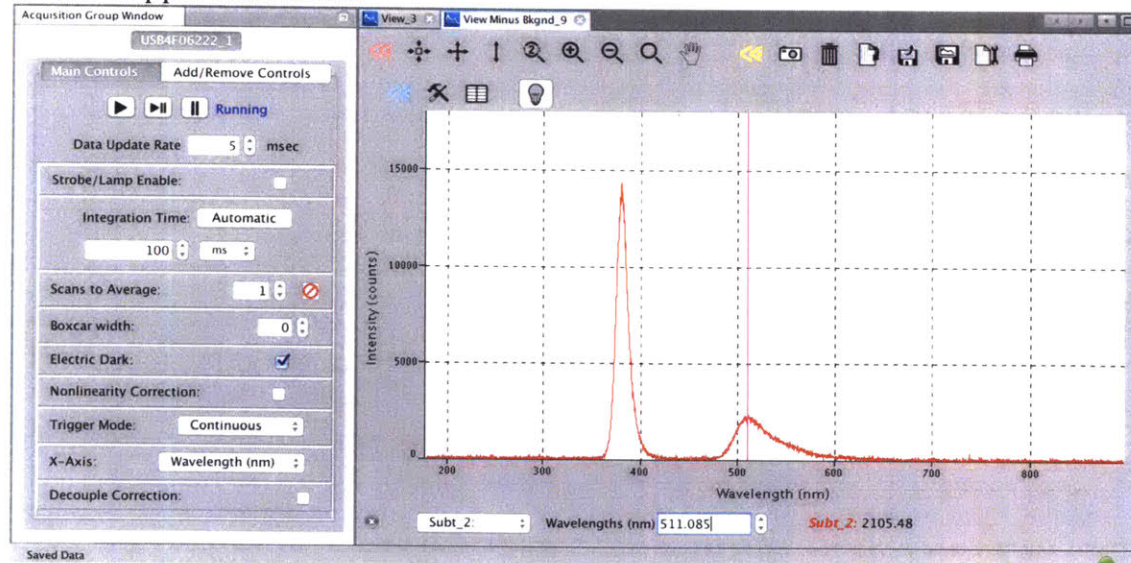


5. Configuration 4 (From Table 1): detector fiber inserted 0.1 inches into chamber, all excitation fibers flush with chamber walls

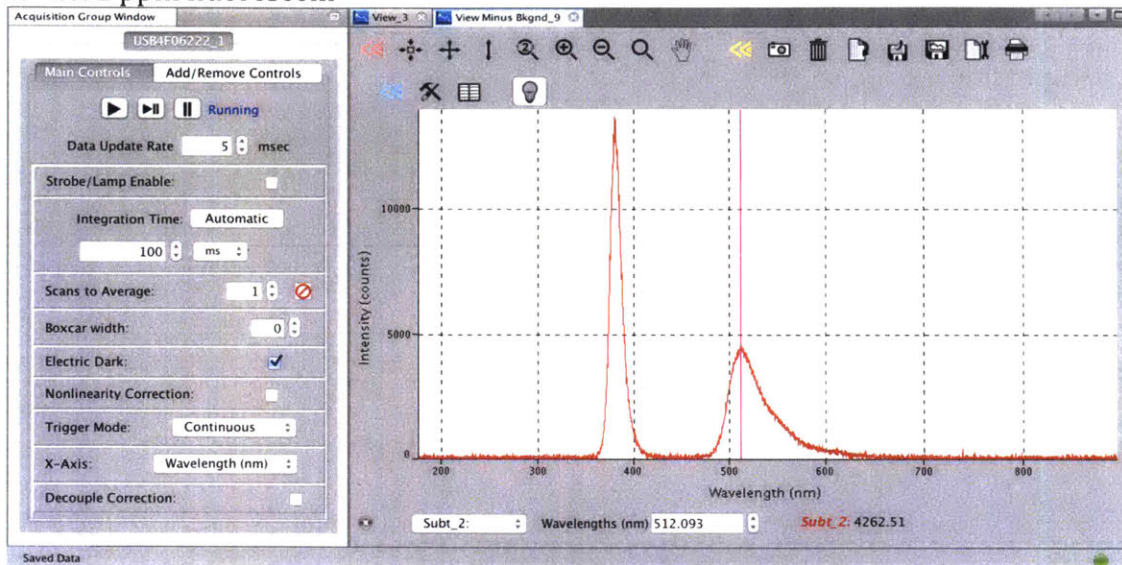
a. 0.001 ppm fluorescein



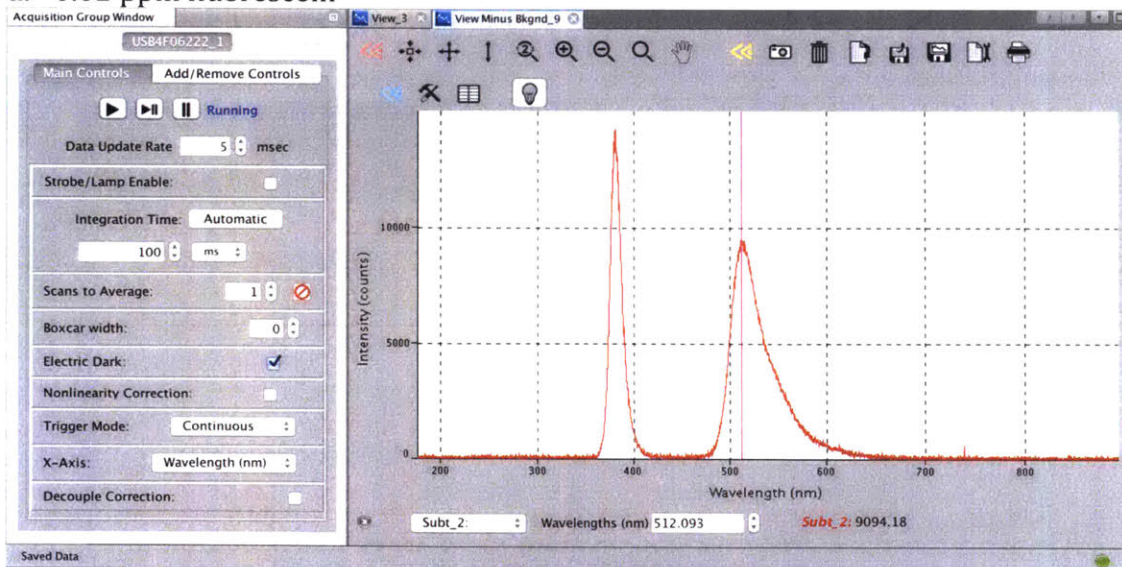
b. 0.005 ppm fluorescein



c. 0.01 ppm fluorescein

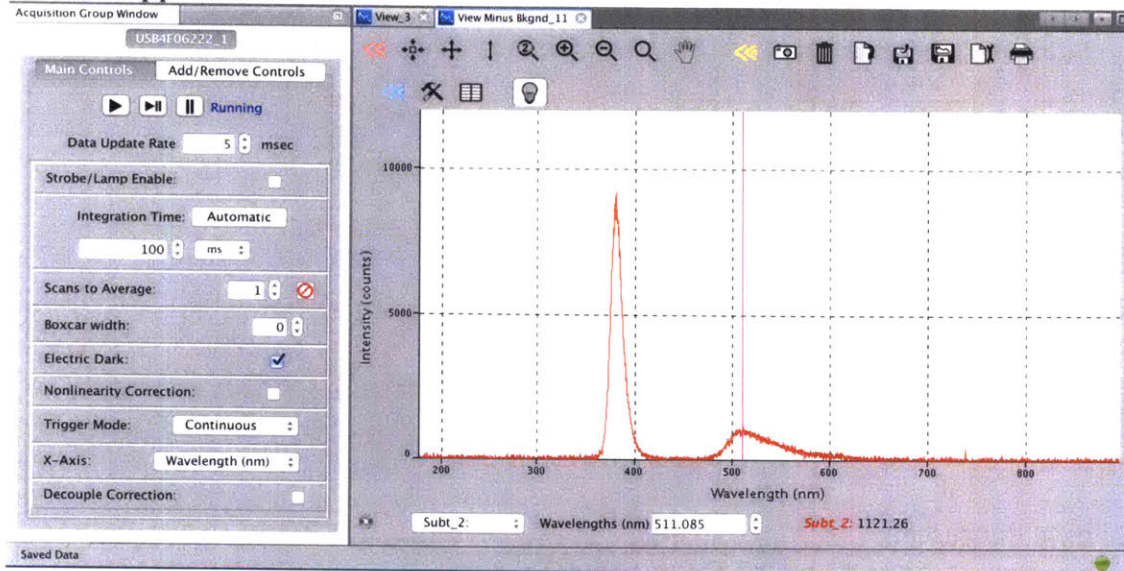


d. 0.02 ppm fluorescein

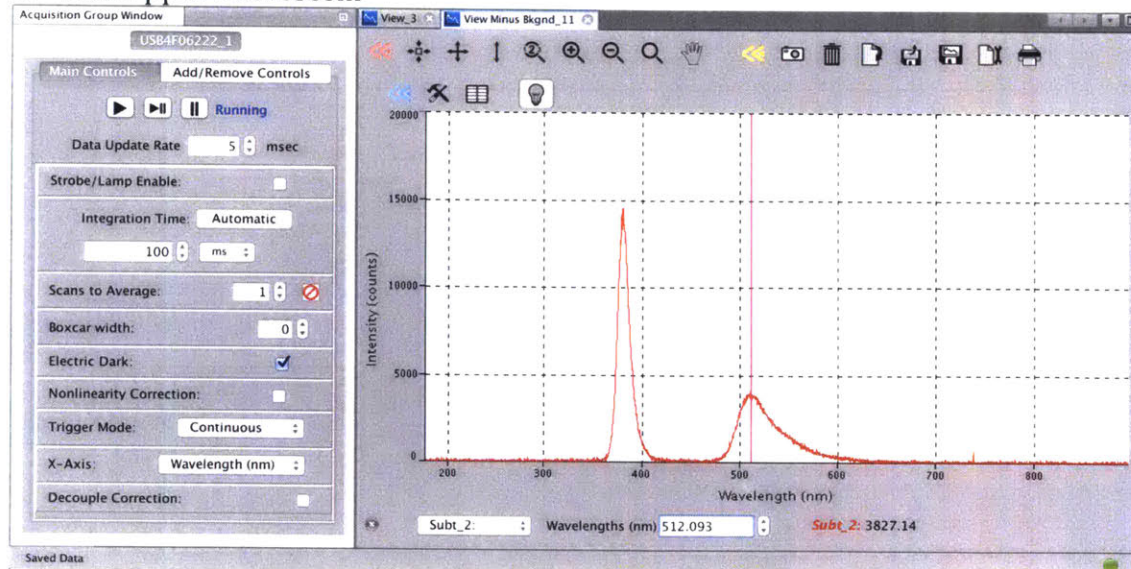


6. Configuration 5 (From Table 1): detector fiber inserted 0.1 inches into chamber, bottom 2 excitation fibers inserted 0.1 inches into chamber

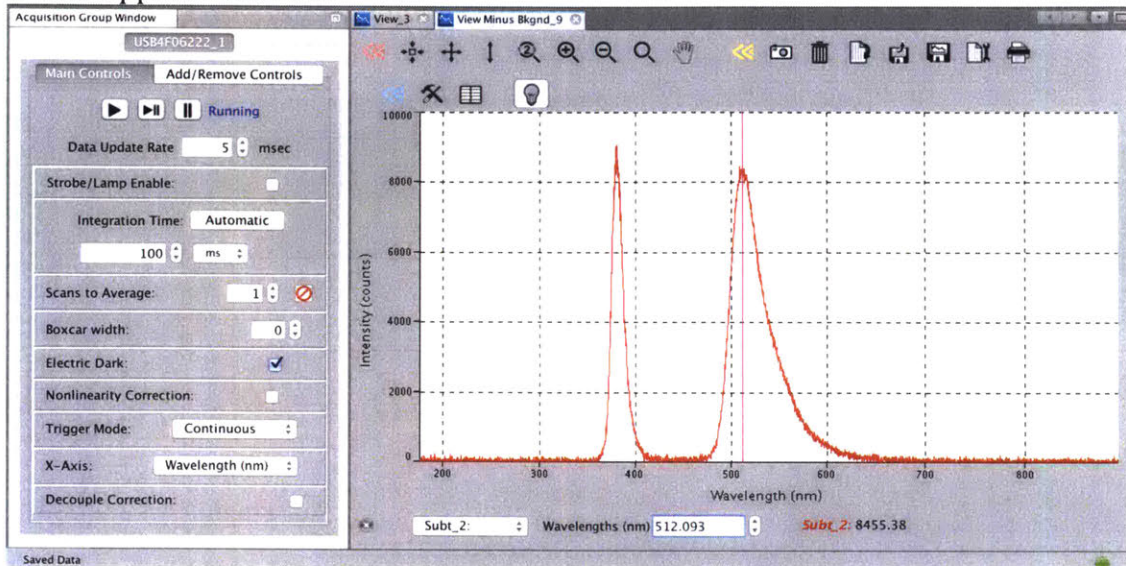
a. 0.001 ppm fluorescein



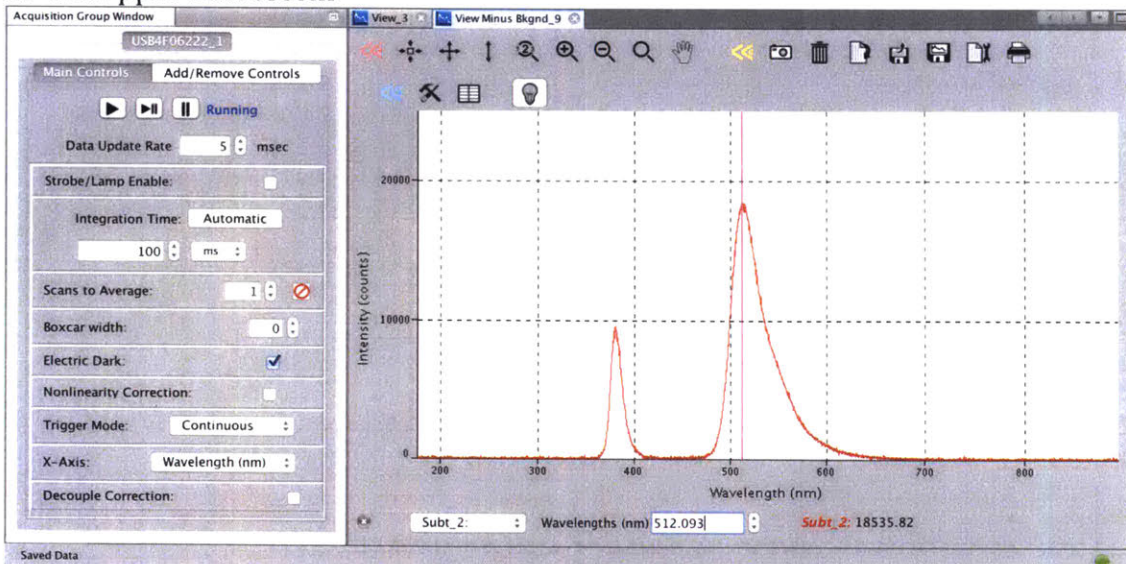
b. 0.005 ppm fluorescein



c. 0.01 ppm fluorescein

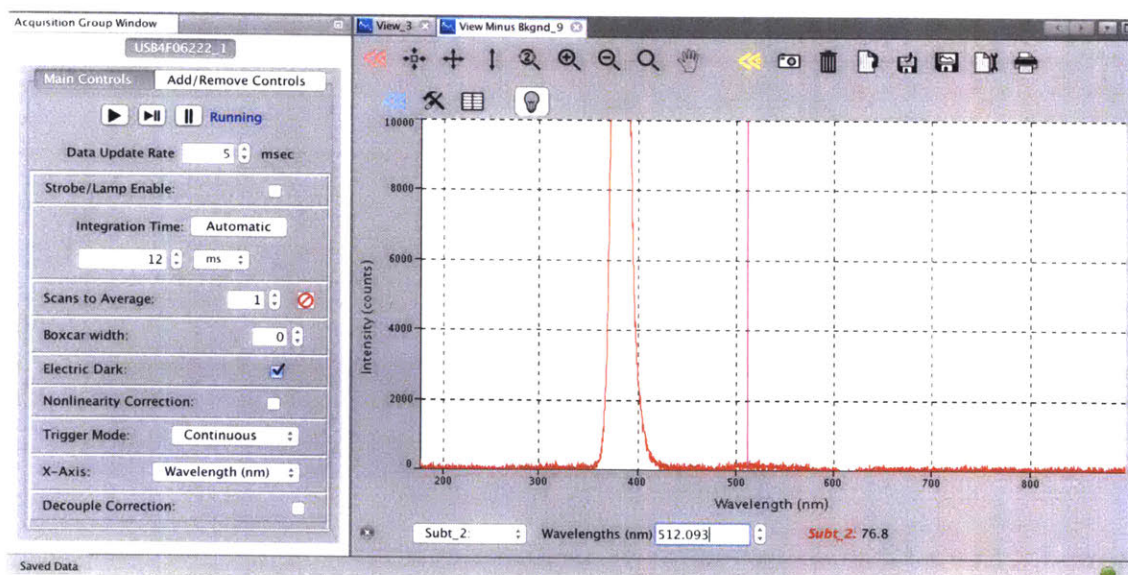
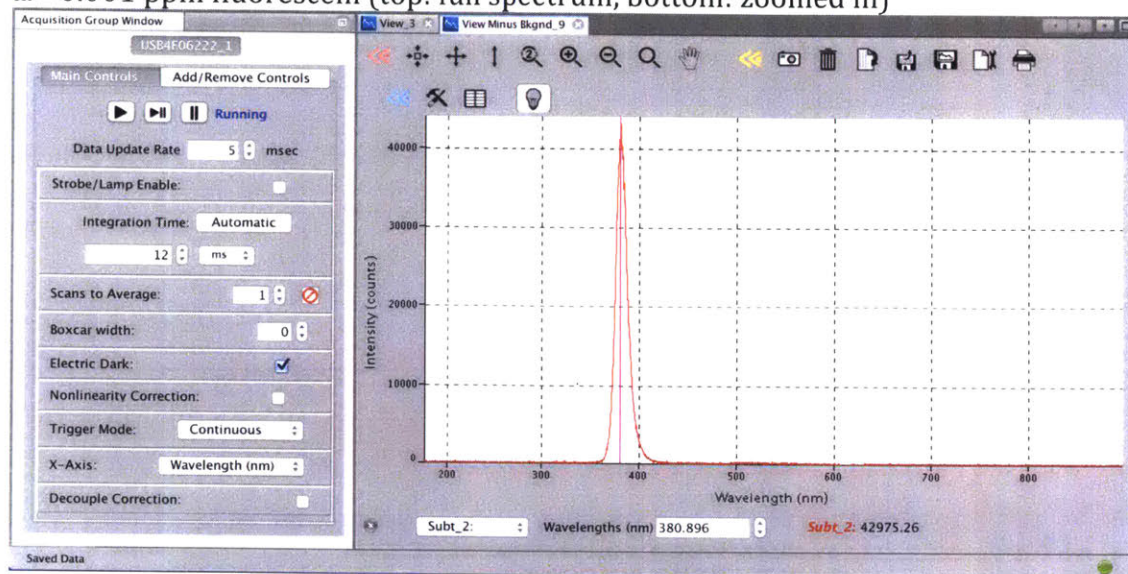


d. 0.02 ppm fluorescein

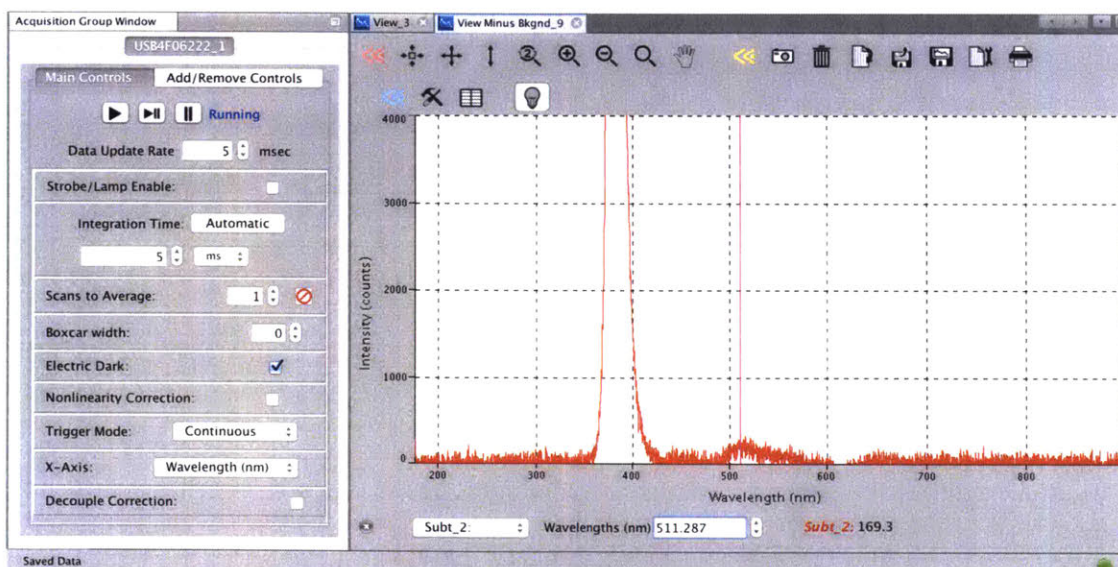
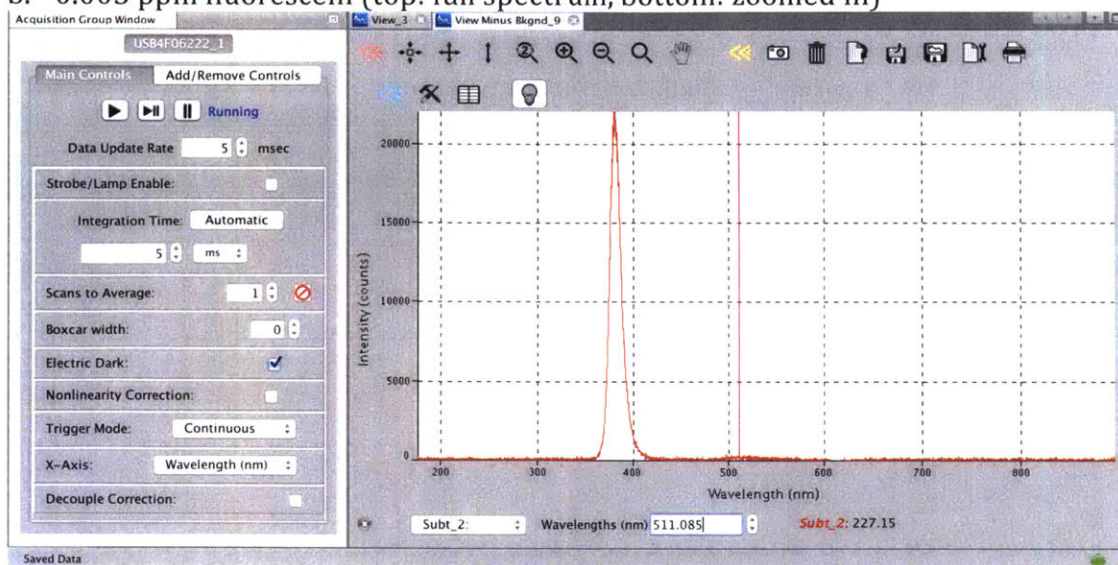


7. Configuration 6 (From Table 1): detector fiber inserted 0.1 inches into chamber, bottom 2 excitation fibers inserted 0.3 inches into chamber

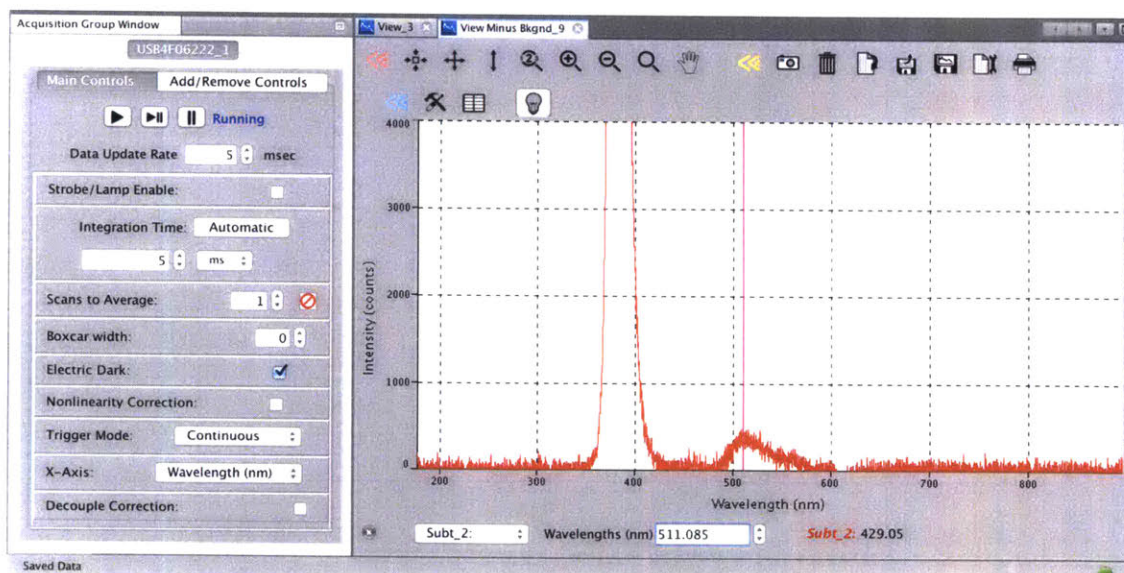
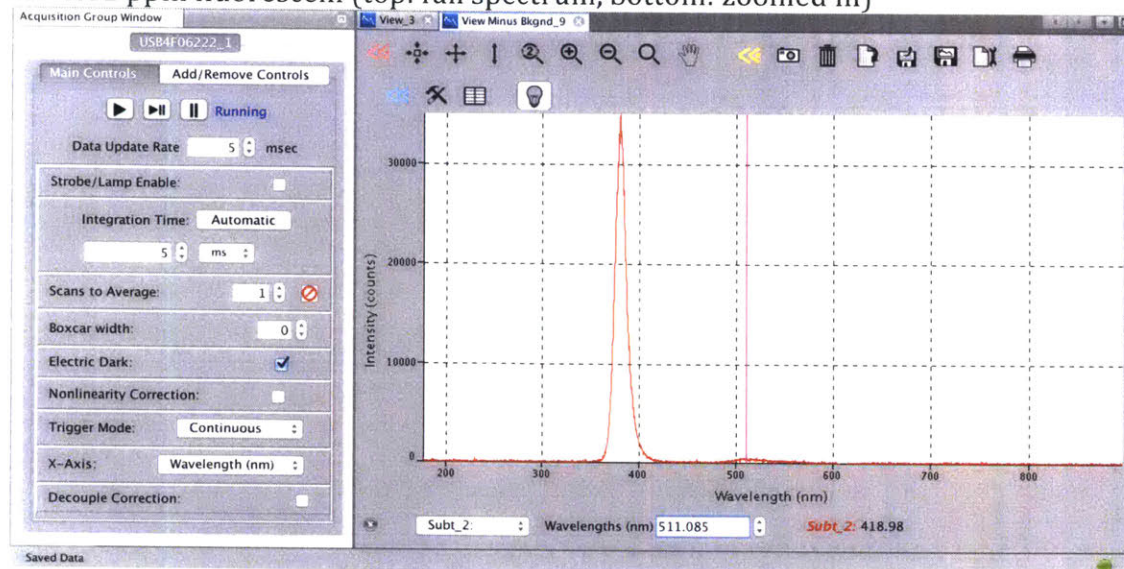
- a. 0.001 ppm fluorescein (top: full spectrum, bottom: zoomed in)



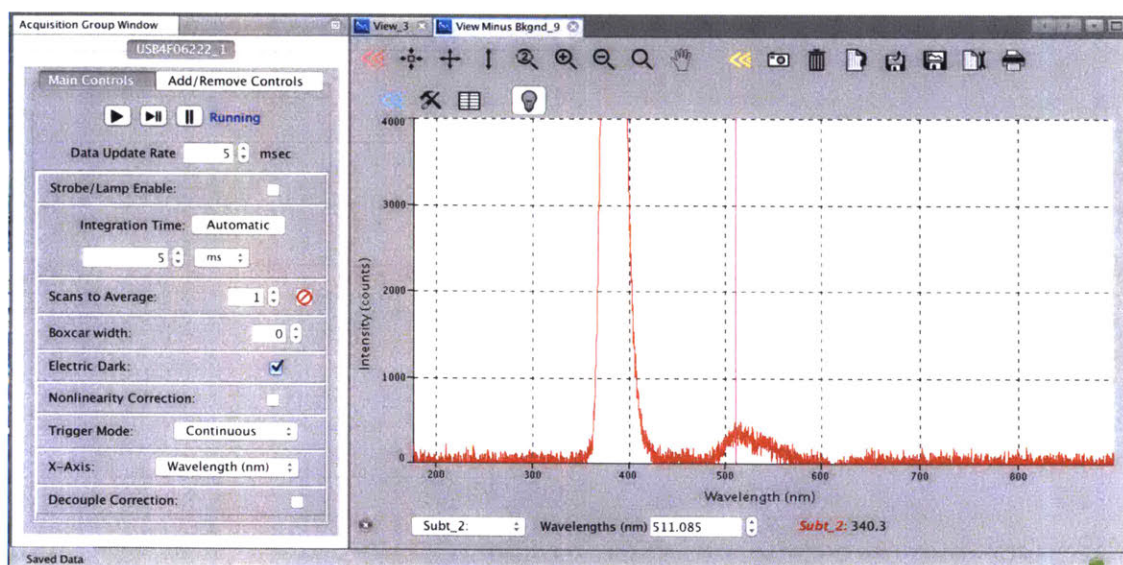
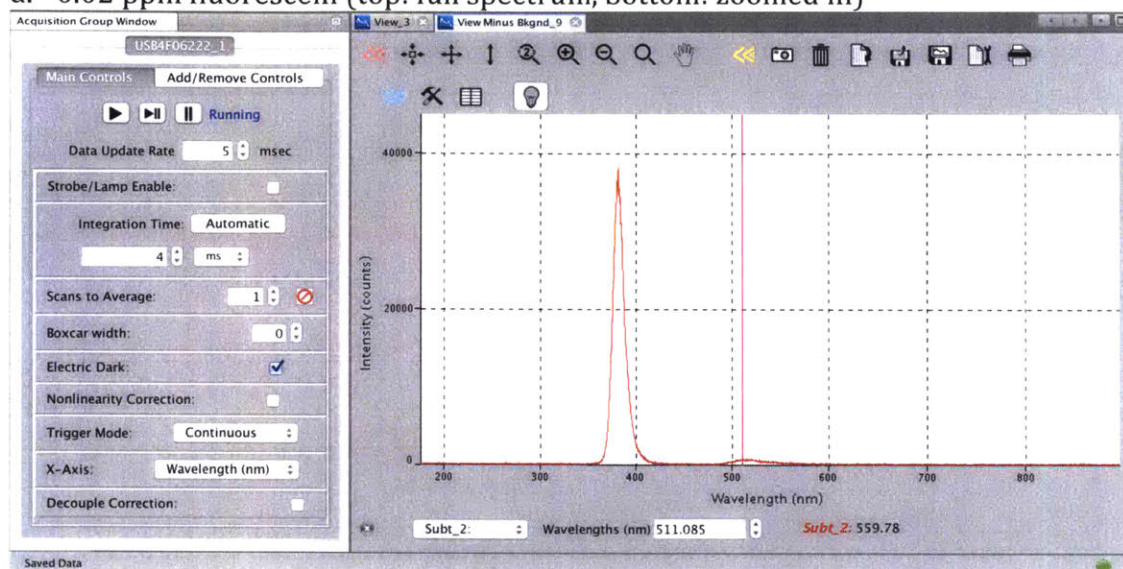
b. 0.005 ppm fluorescein (top: full spectrum, bottom: zoomed in)



c. 0.01 ppm fluorescein (top: full spectrum, bottom: zoomed in)



d. 0.02 ppm fluorescein (top: full spectrum, bottom: zoomed in)

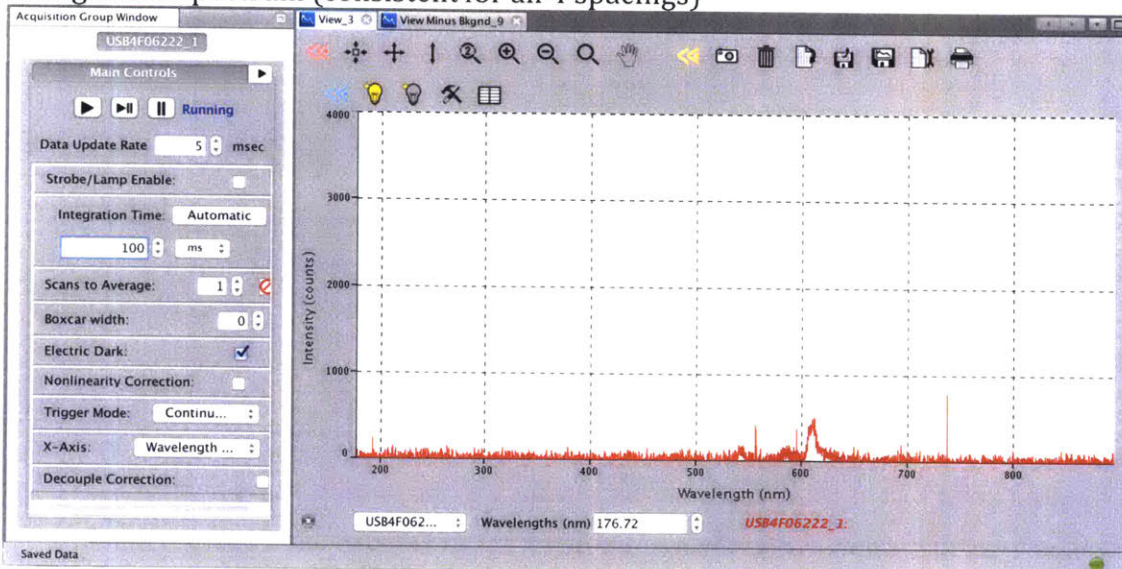


Appendix 2 – Hammerhead Fluorescein Spectra for 4 Spacings in Table 2

Notes:

- The integration time for each sample may be seen in the box on the left-hand side of each image.
- Each integration time used was 100 milliseconds OR the highest possible for that sample without saturating the USB4000 detector.
- For reference, graph 1 depicts the background spectrum with an unlit chamber, which is consistent for all 4 spacings.
- Background spectrum has been subtracted in the graphs for each spacing (graphs 2-5).

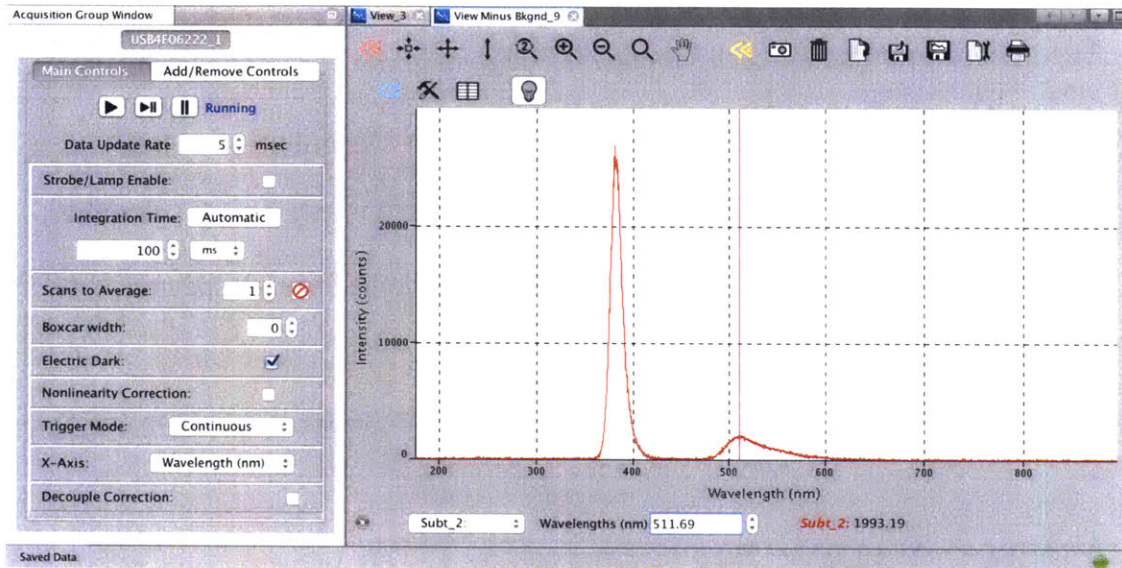
1. Background spectrum (consistent for all 4 spacings)



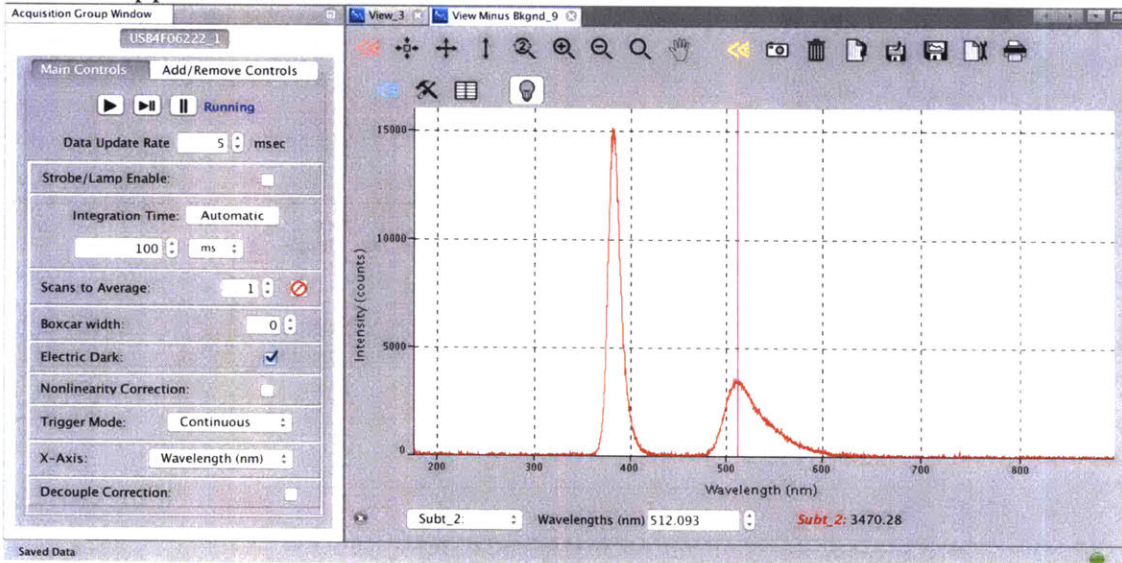
Background average: 16 counts

2. Spacing 1: 0.076 inches from tip of excitation fiber to center of chamber

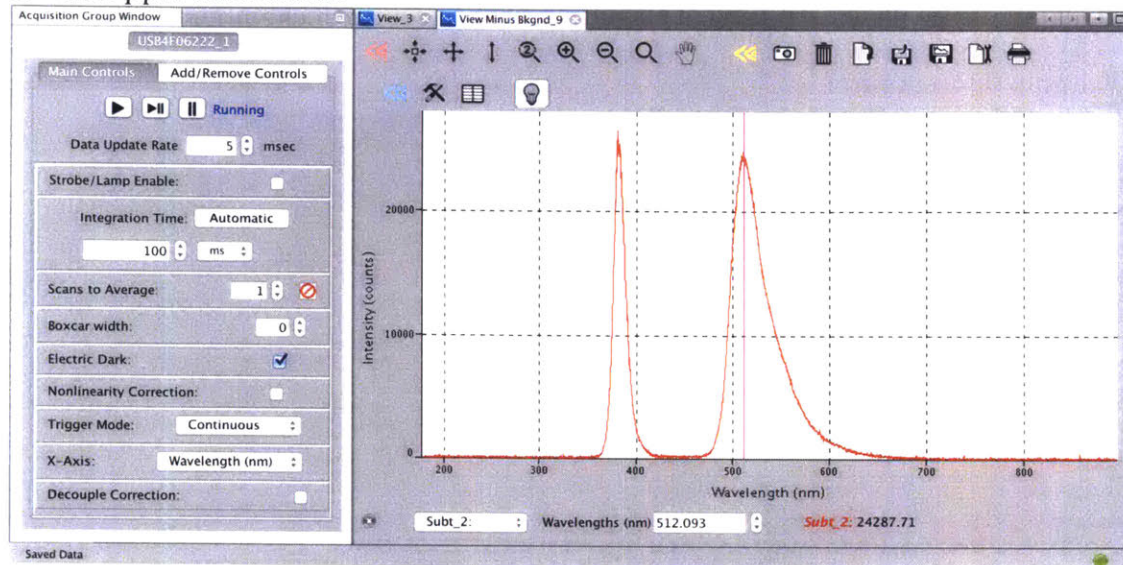
a. 0.001 ppm fluorescein



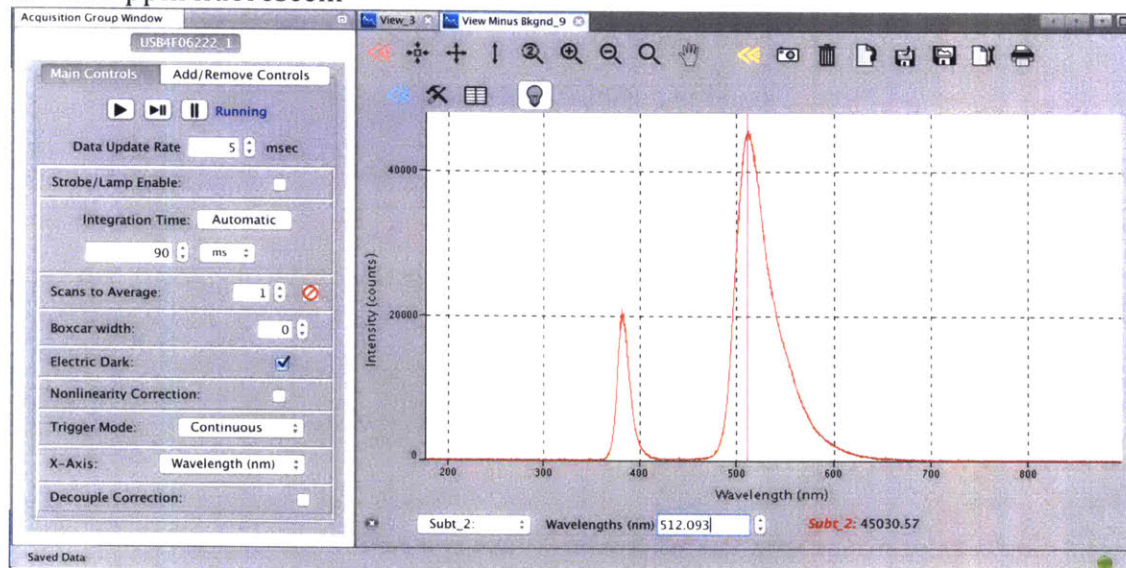
b. 0.005 ppm fluorescein



c. 0.01 ppm fluorescein

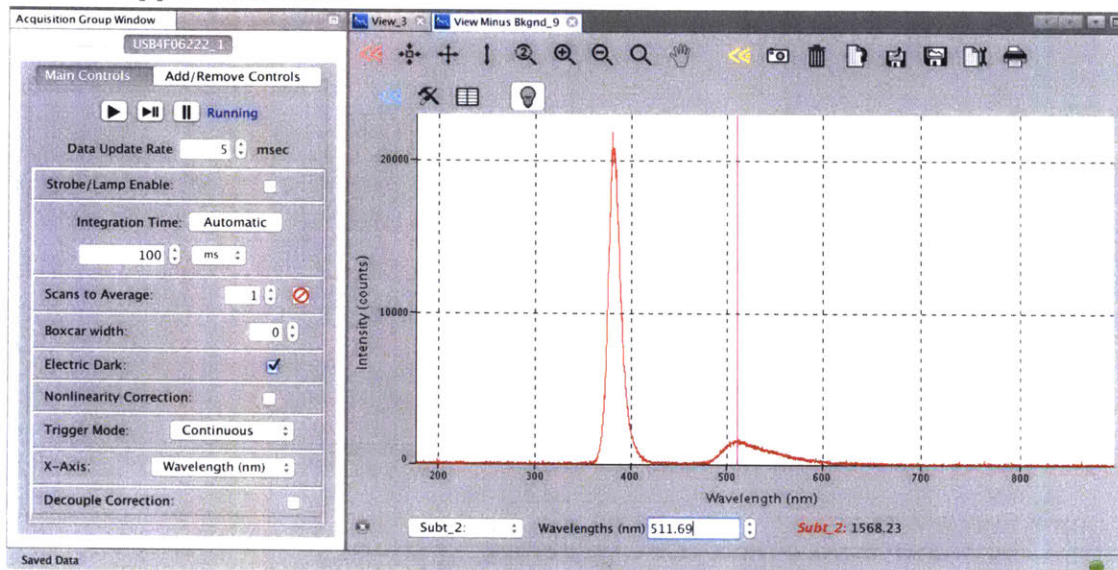


d. 0.02 ppm fluorescein

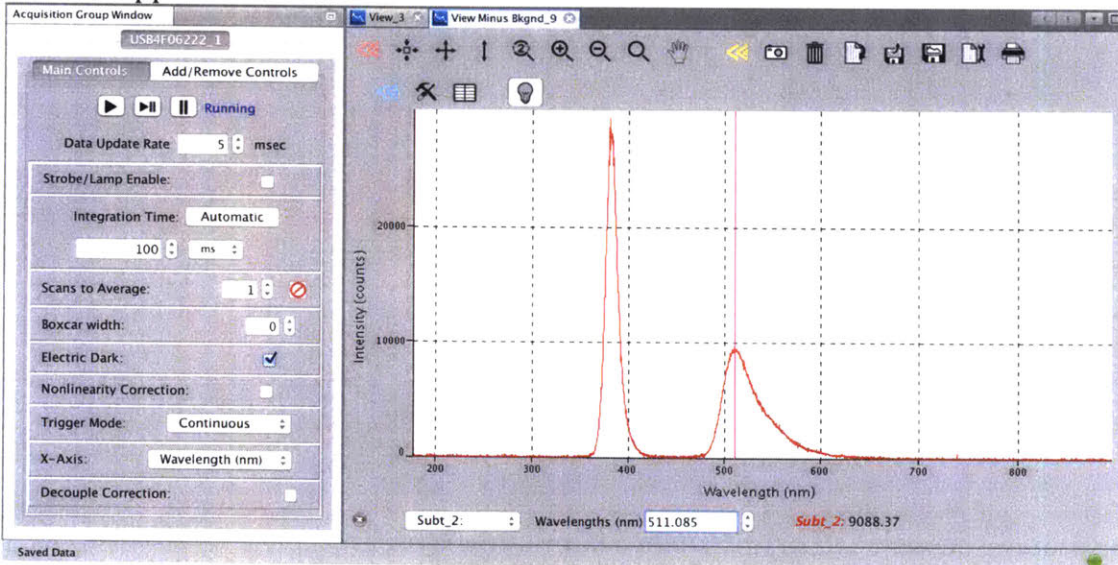


3. Spacing 2: 0.1105 inches from tip of excitation fiber to center of chamber

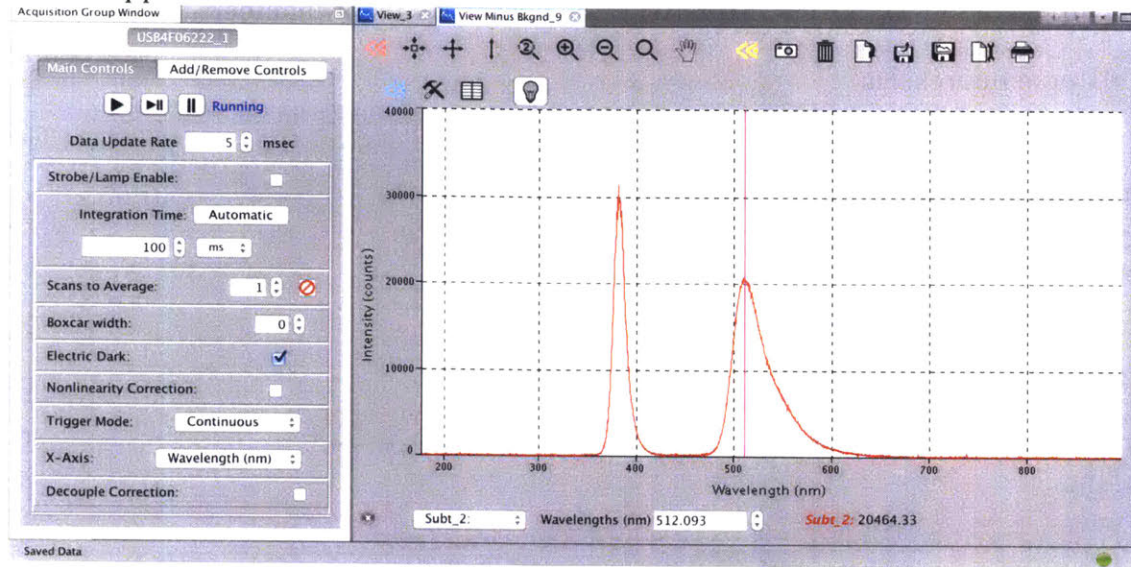
a. 0.001 ppm fluorescein



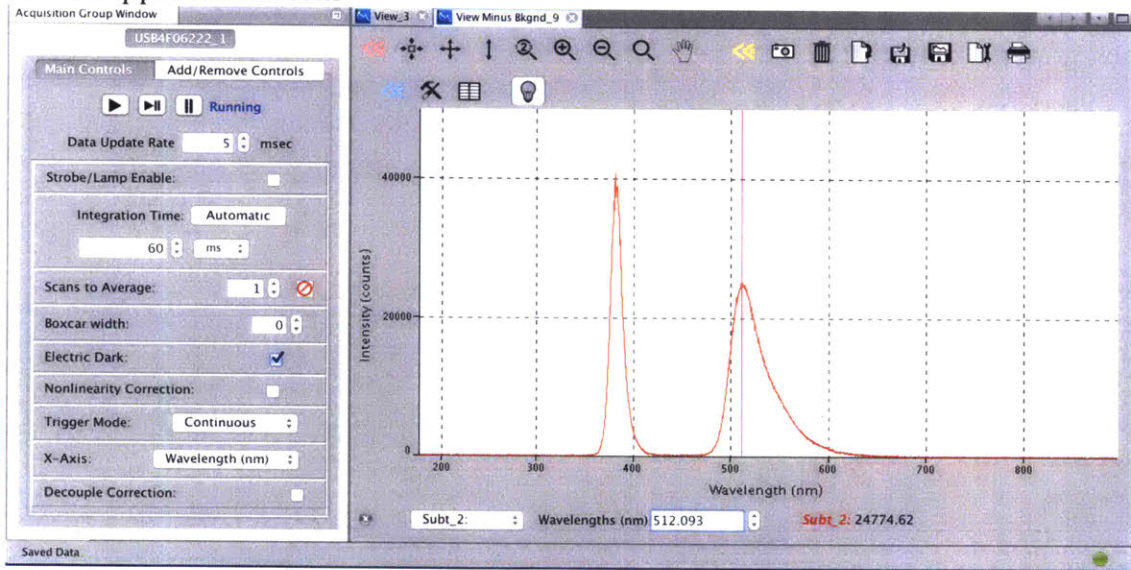
b. 0.005 ppm fluorescein



c. 0.01 ppm fluorescein

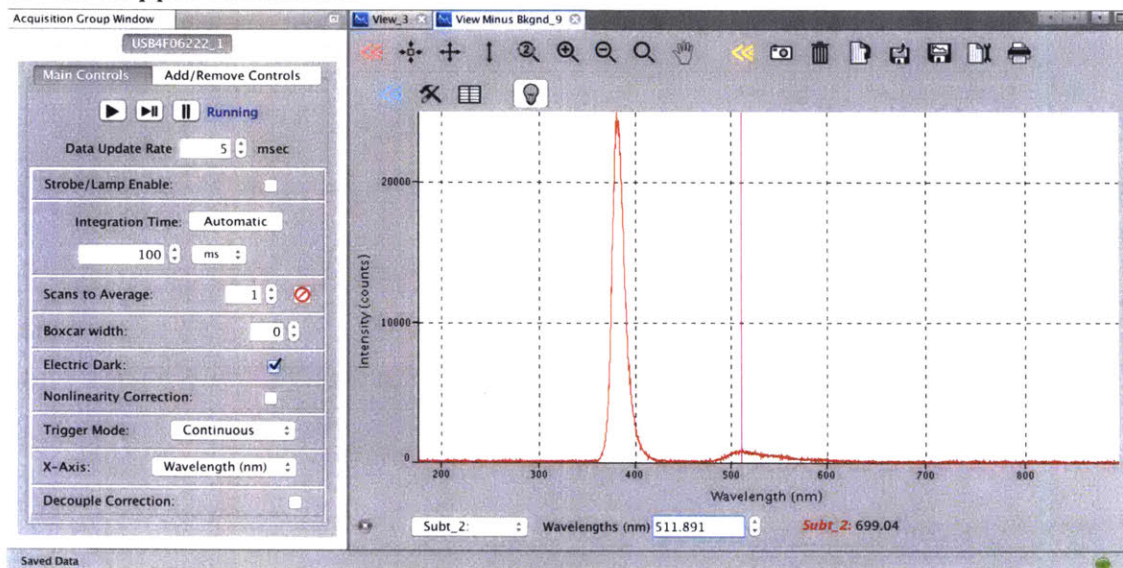


d. 0.02 ppm fluorescein

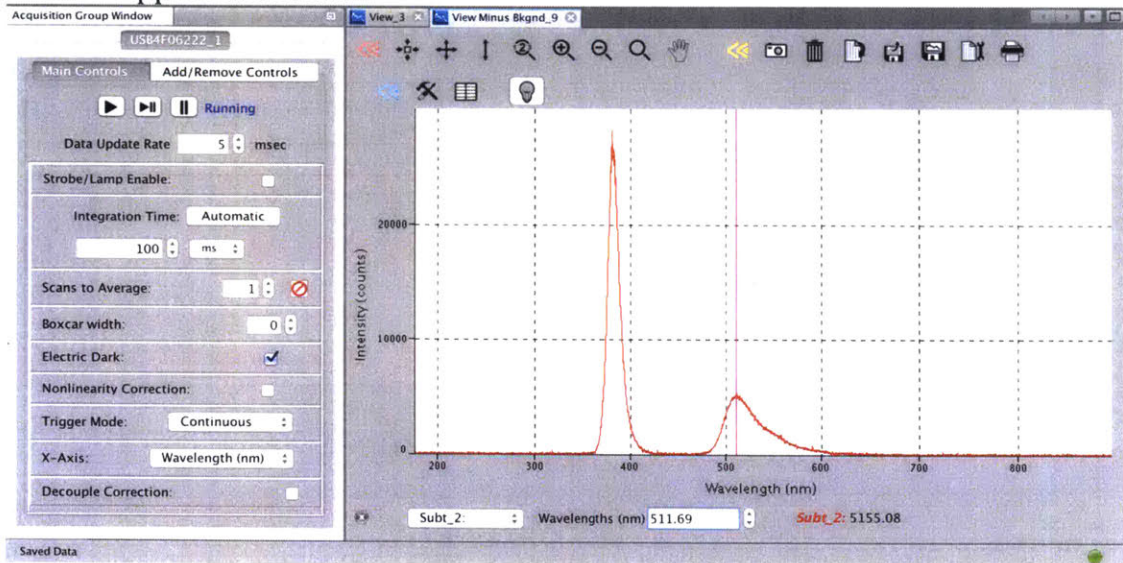


4. Spacing 3: 0.203 inches from tip of excitation fiber to center of chamber

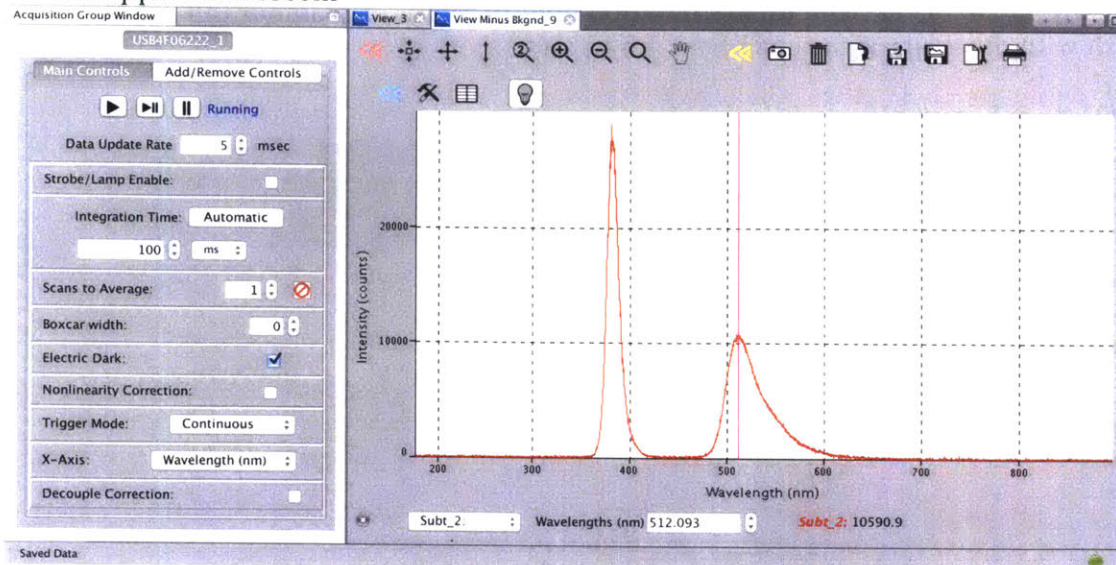
a. 0.001 ppm fluorescein



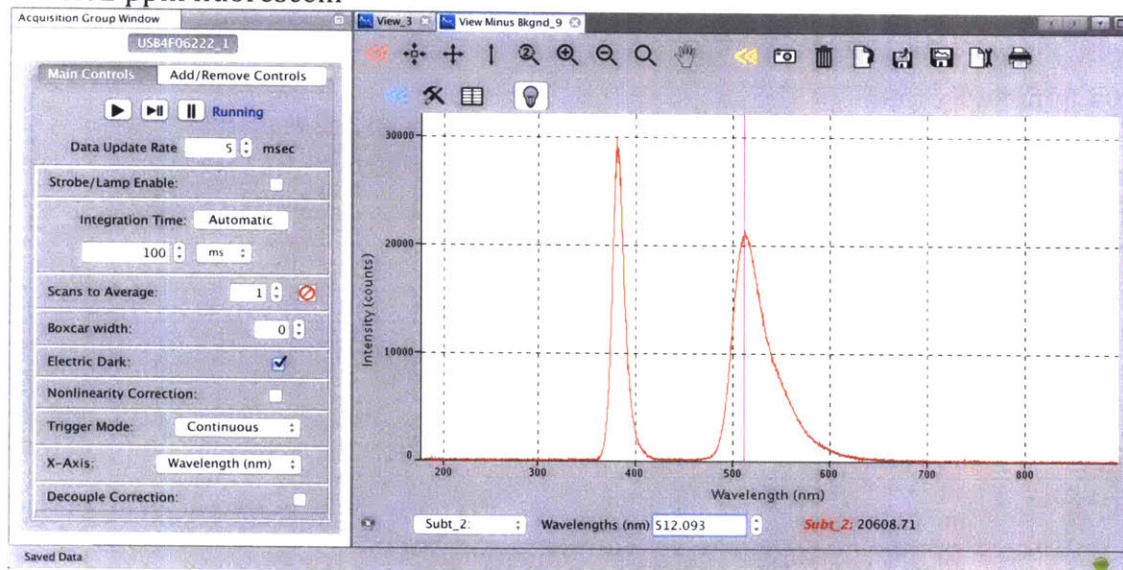
b. 0.005 ppm fluorescein



c. 0.01 ppm fluorescein

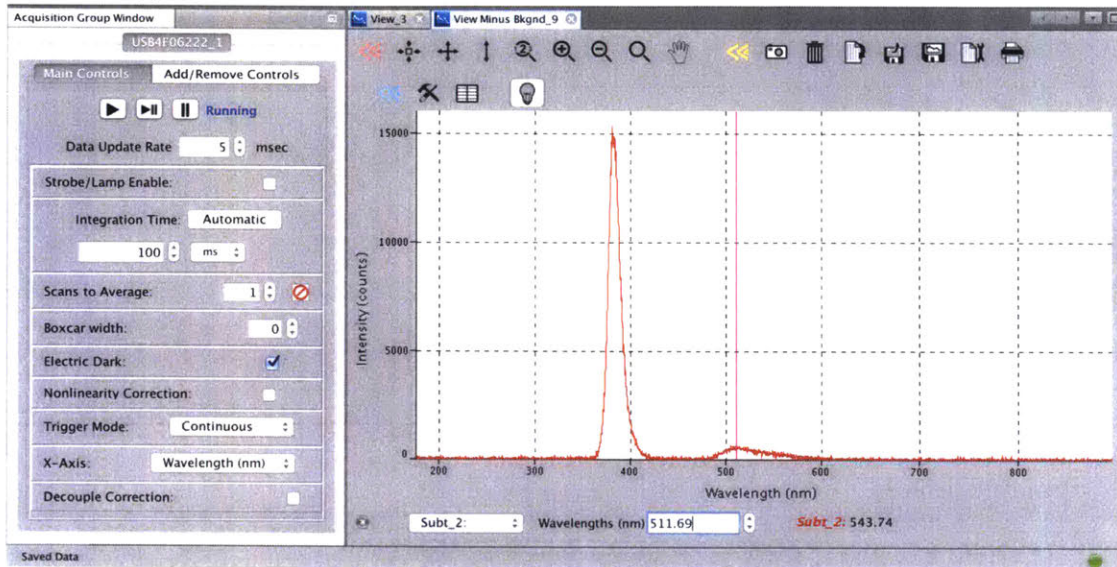


d. 0.02 ppm fluorescein

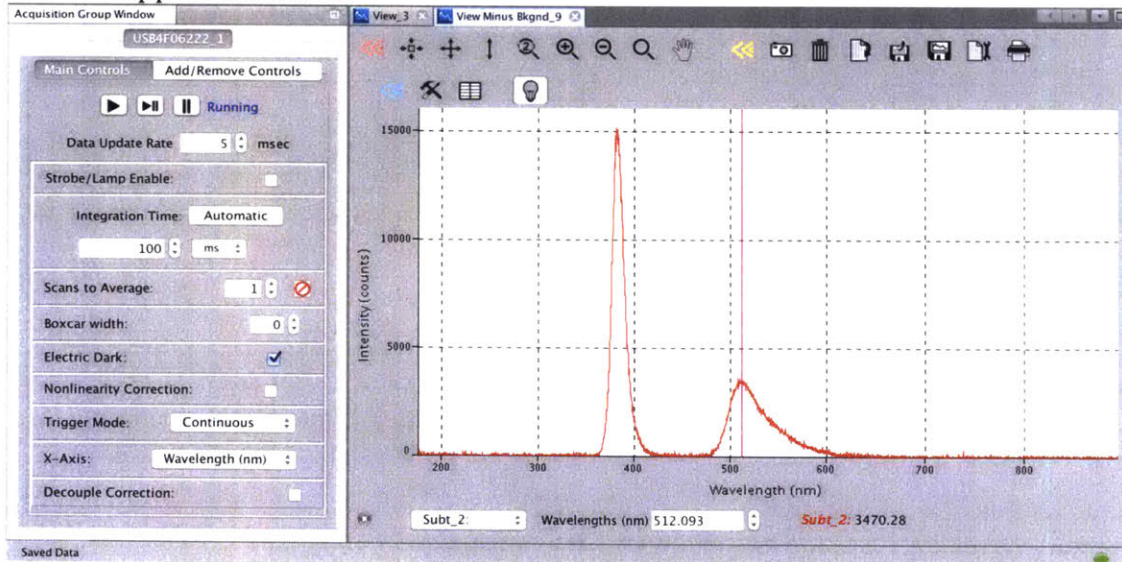


5. Spacing 4: 0.3 inches from tip of excitation fiber to center of chamber (all fibers flush with the walls of the chamber)

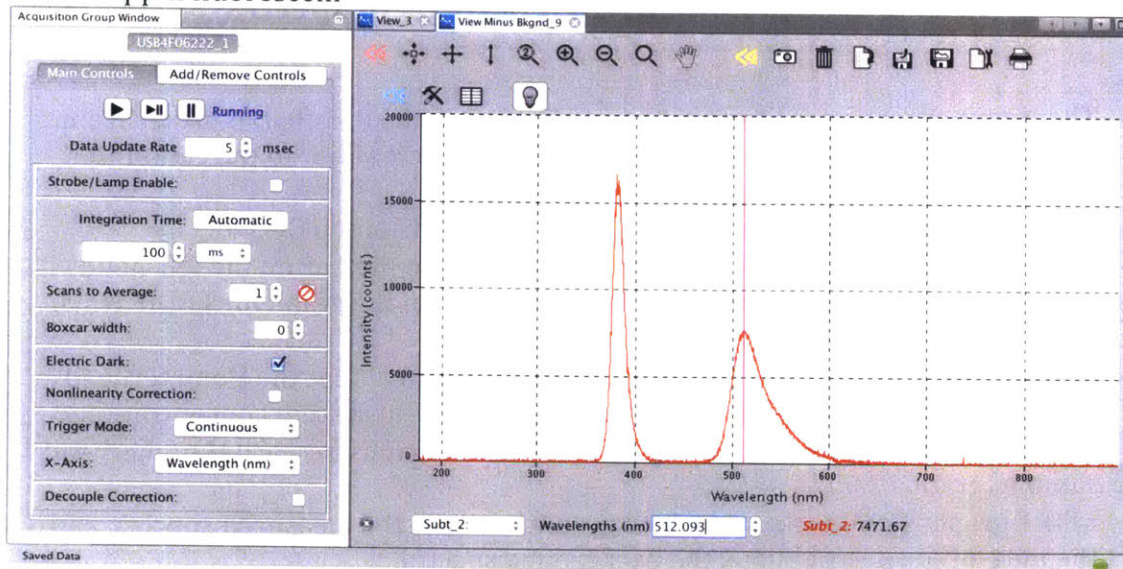
a. 0.001 ppm fluorescein



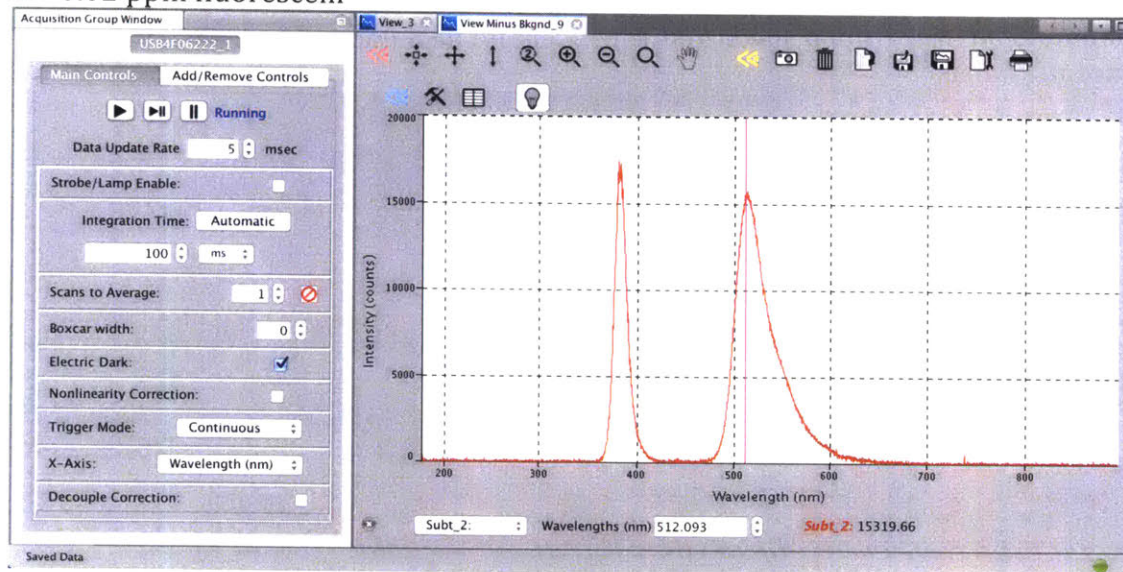
b. 0.005 ppm fluorescein



c. 0.01 ppm fluorescein



d. 0.02 ppm fluorescein

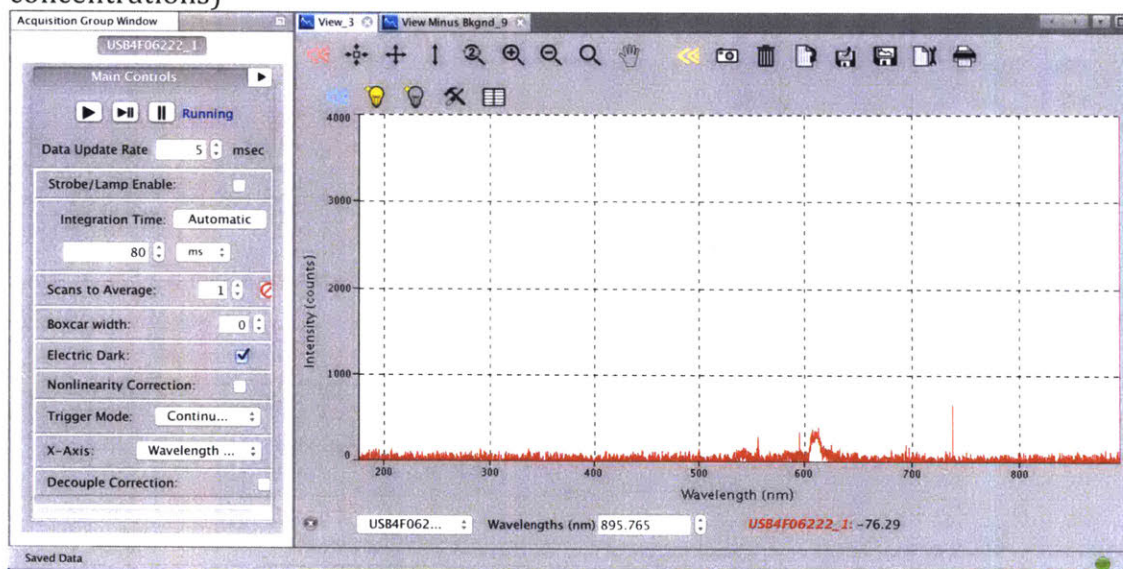


Appendix 3 – Hammerhead and LEDIF Fluorescein Spectra

Notes:

- The Hammerhead uses Spacing #1 from Table 2 in the text (0.076 inches from the tip of the excitation fiber and the tip of the detector fiber to the center of the chamber)
- The LEDIF graphs shown are for LEDIF trials with its pump ON.
- The integration time for each sample may be seen in the box on the left-hand side of each image for Hammerhead tests and within the graph for LEDIF tests.
- Each integration time used was 80 milliseconds.
- For reference, graph 1 depicts the background spectrum for the Hammerhead with an unlit chamber, which is consistent for all 6 fluorescein concentrations.
- Background spectrum has been subtracted in the graphs for each Hammerhead sample (graphs 2-7).
- LEDIF graphs (2-7) include the background spectra, the measured spectra, and the measured spectra minus the background spectra.

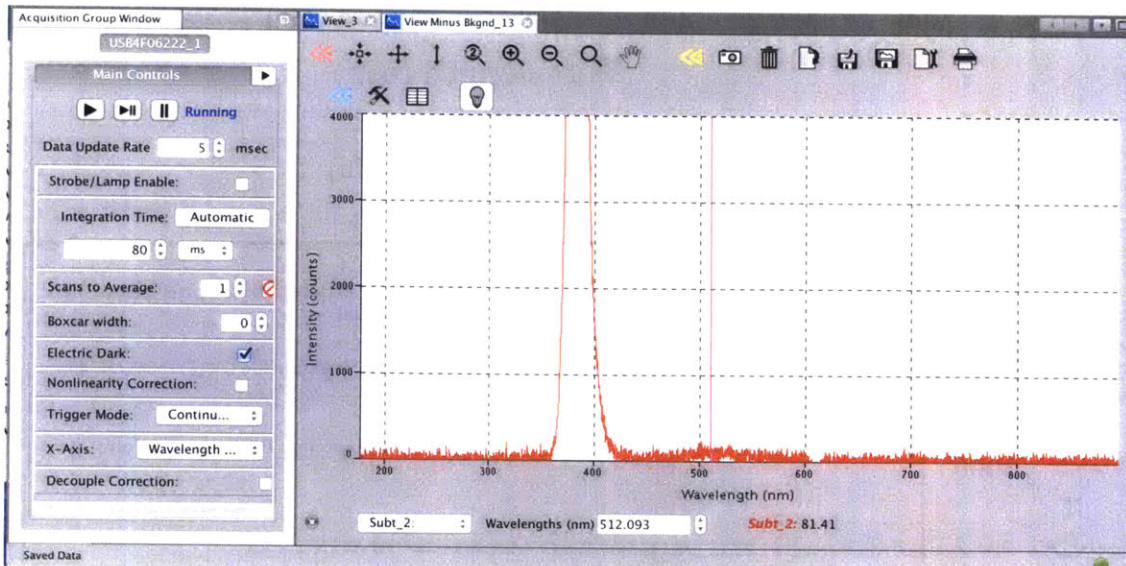
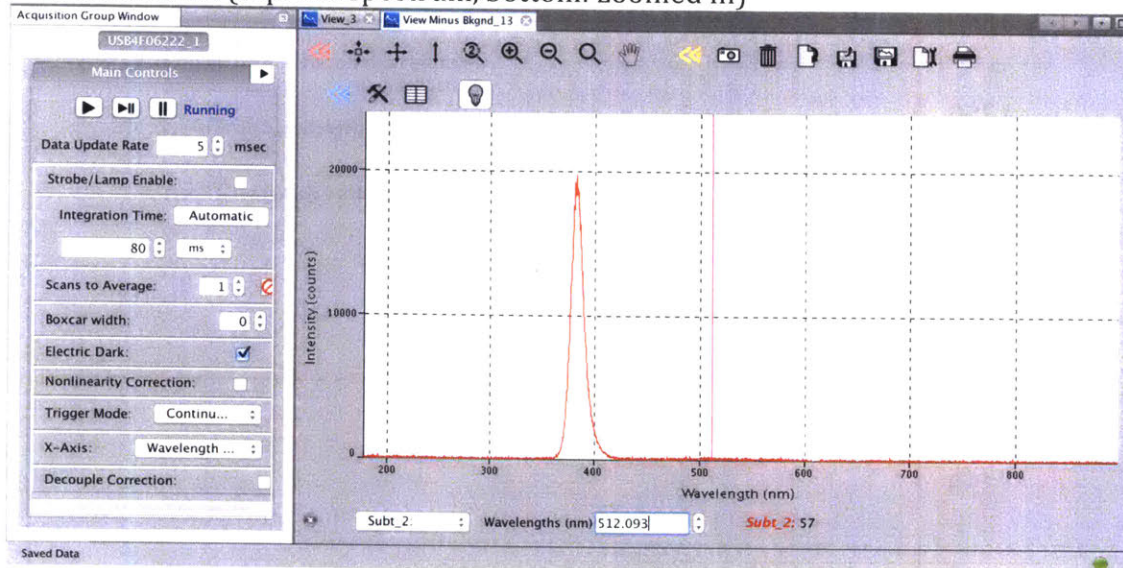
1. Hammerhead background spectrum (consistent for all 6 fluorescein concentrations)



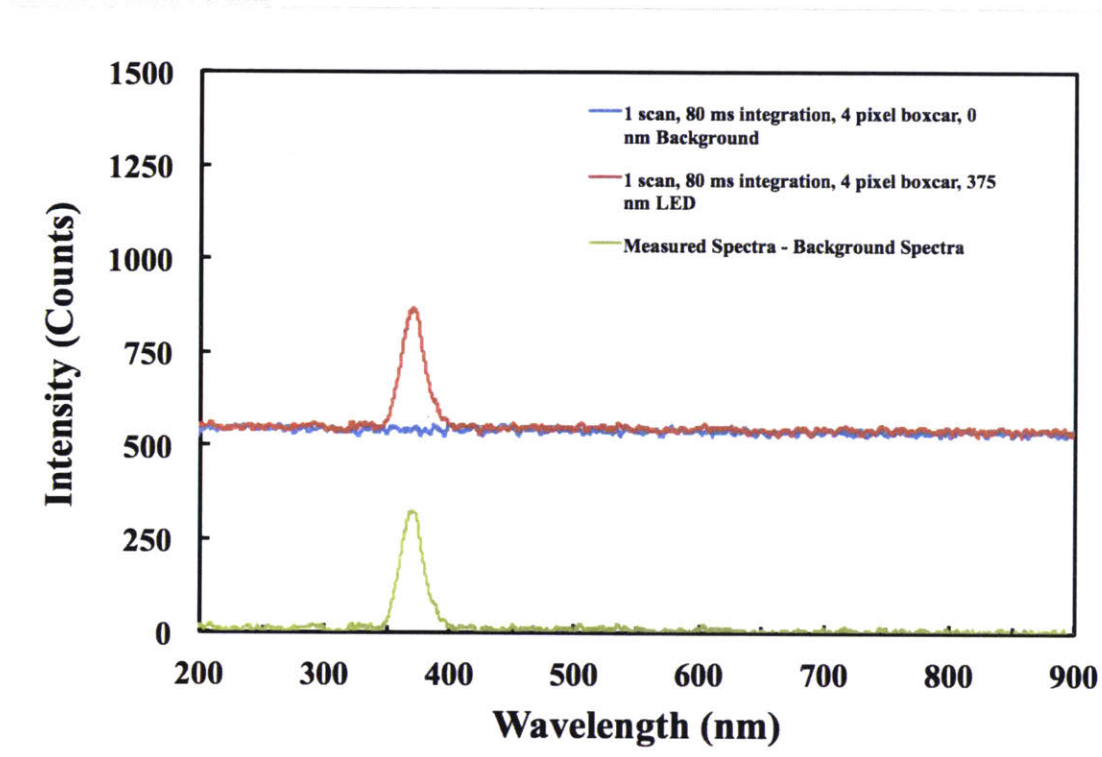
Background average: 16 counts

2. 0.1 ppb fluorescein

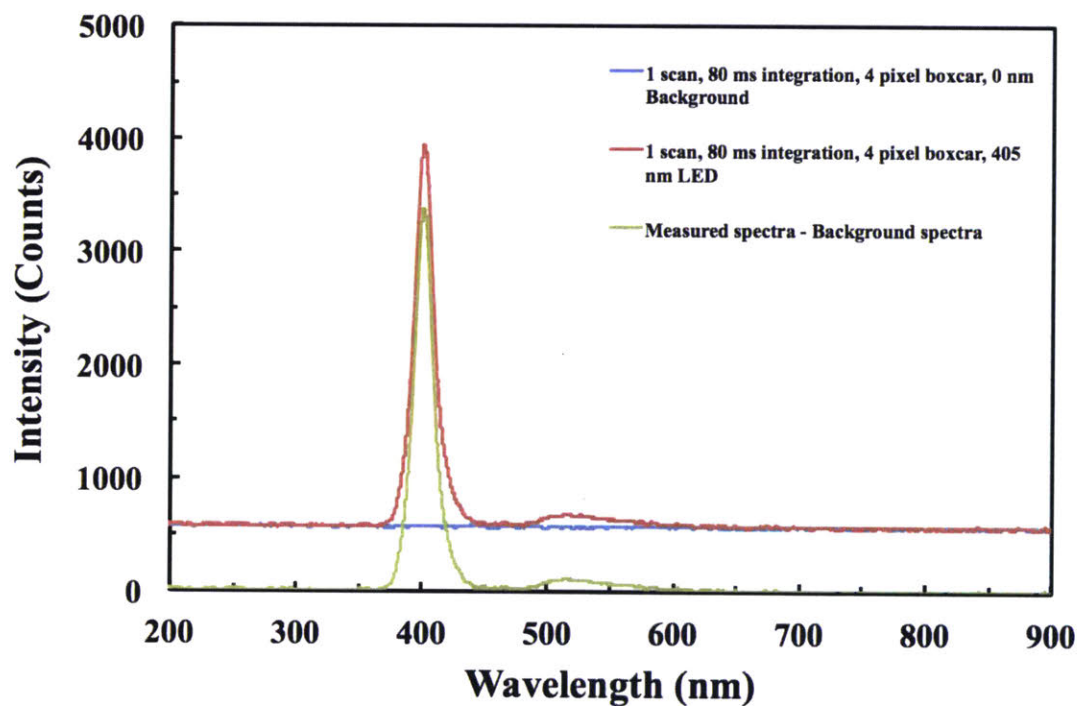
a. Hammerhead (top: full spectrum, bottom: zoomed in)

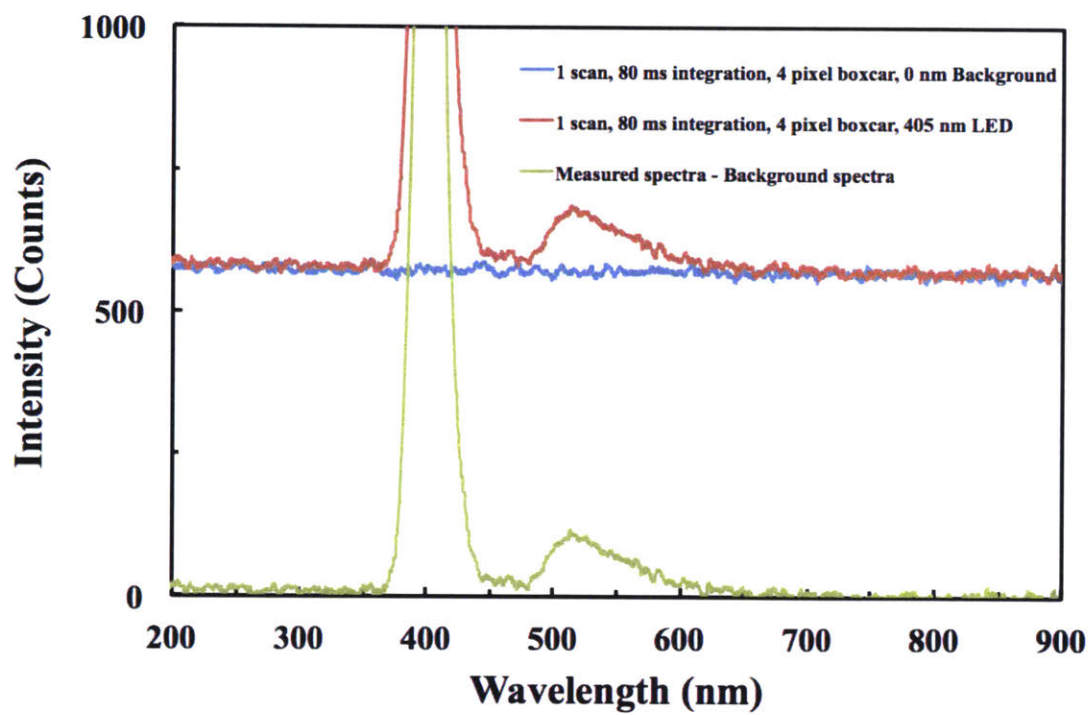


b. LEDIF with 375 nm



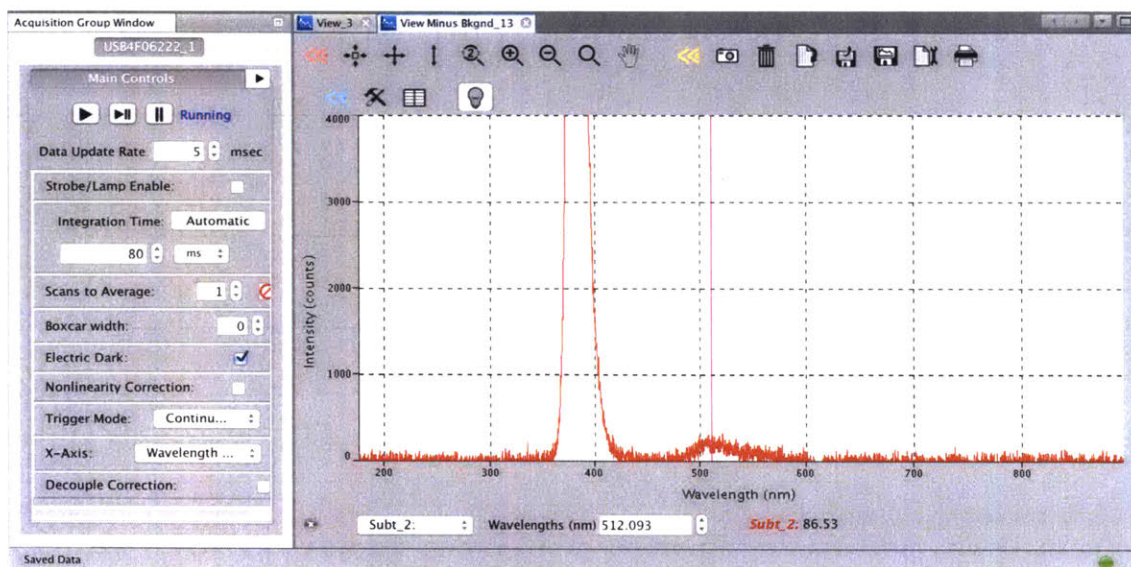
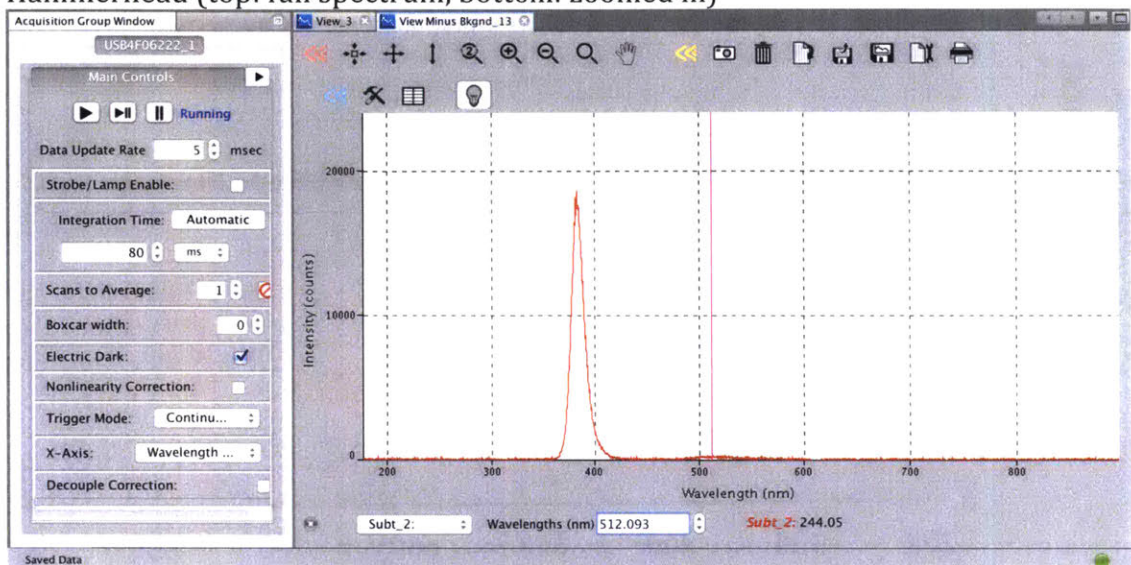
c. LEDIF with 405 nm (top: full spectrum, bottom: zoomed in)



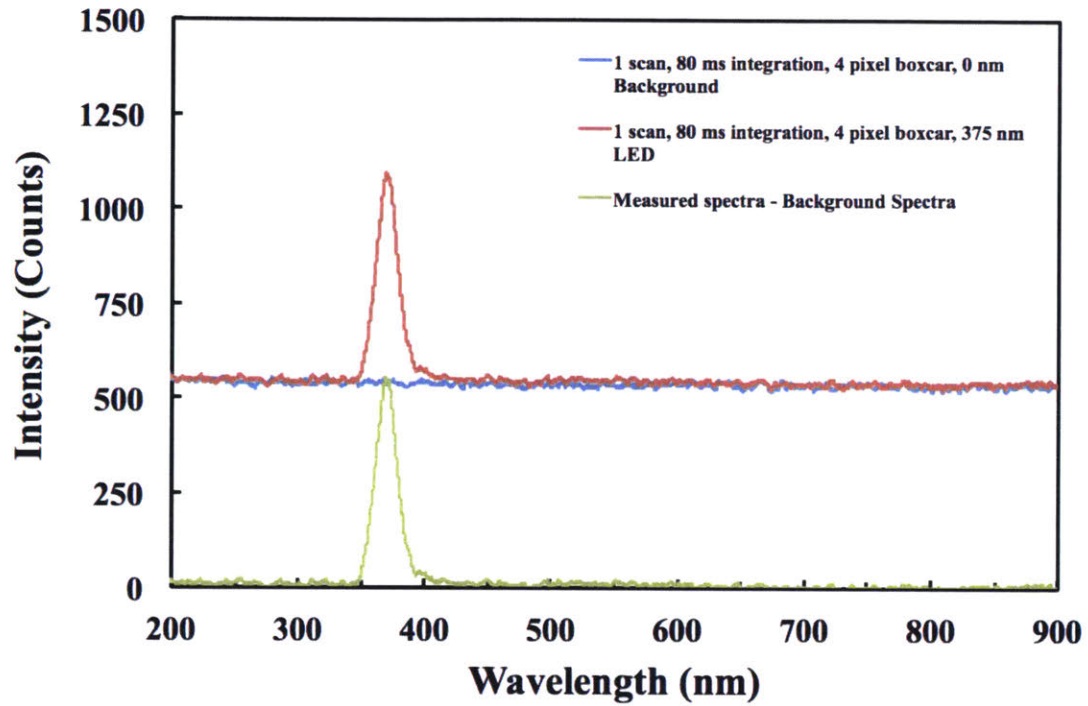


3. 0.2 ppb fluorescein

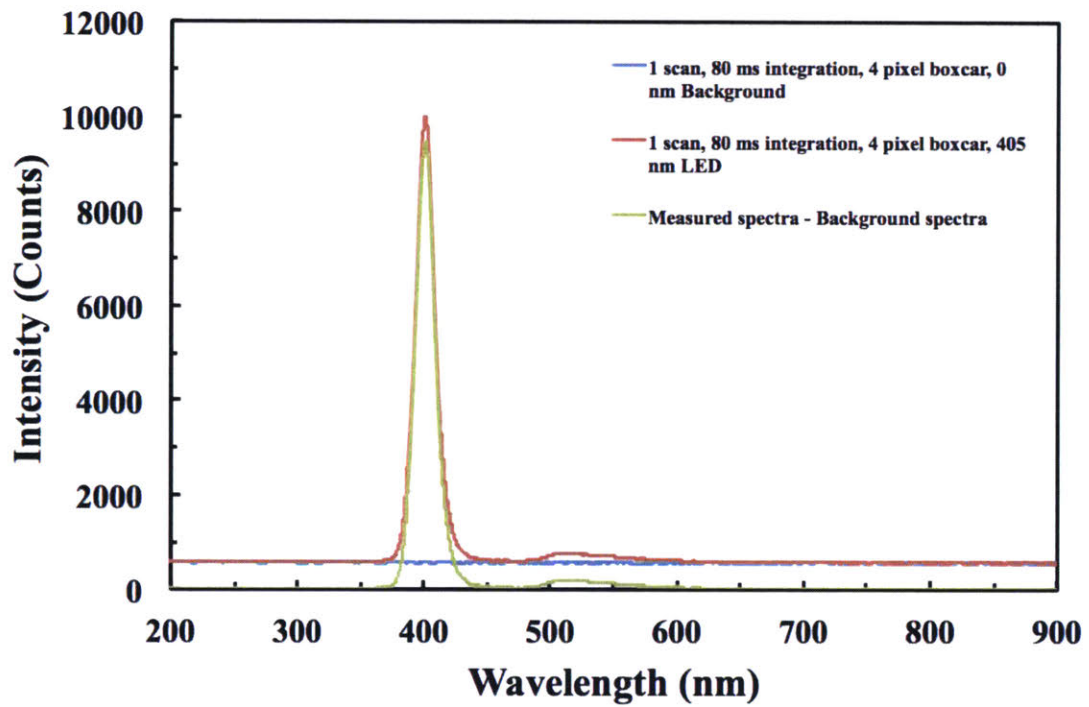
a. Hammerhead (top: full spectrum, bottom: zoomed in)

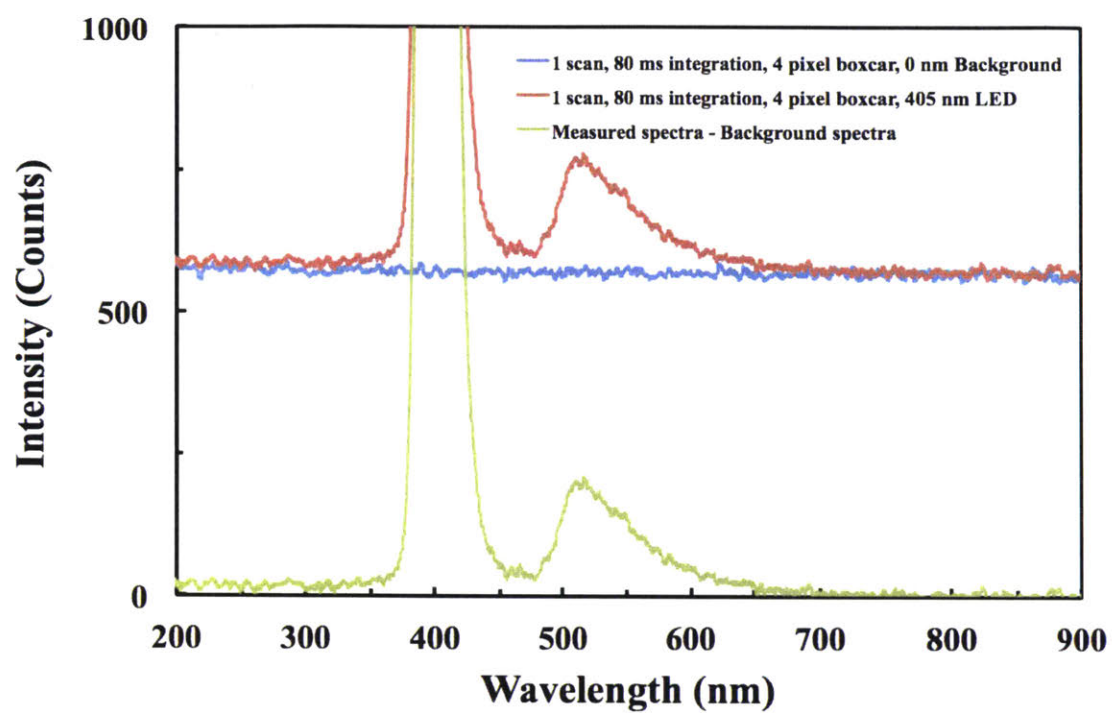


b. LEDIF with 375 nm



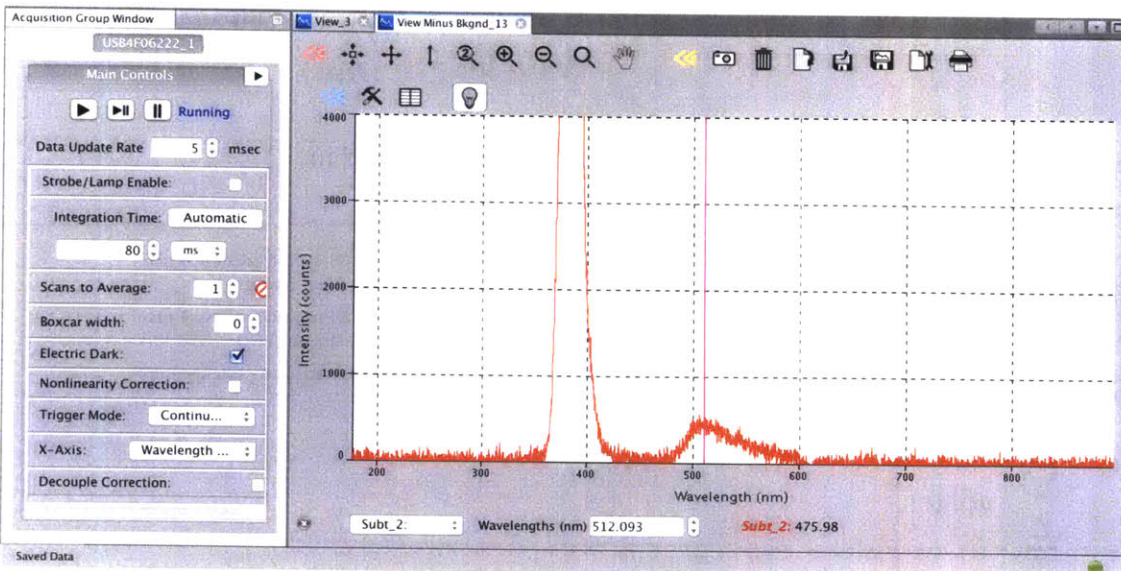
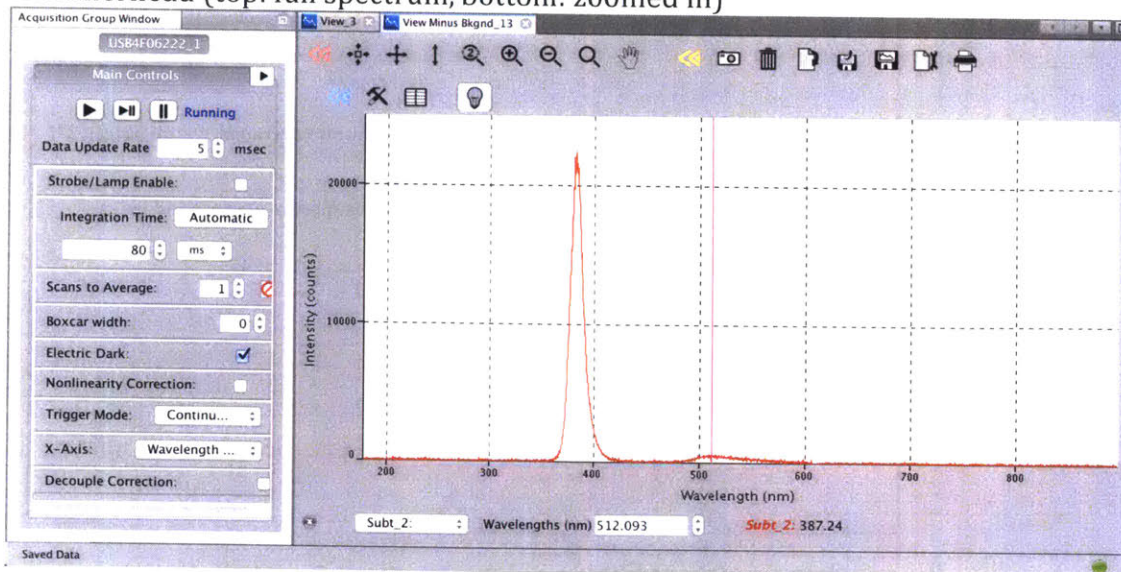
c. LEDIF with 405 nm (top: full spectrum, bottom: zoomed in)



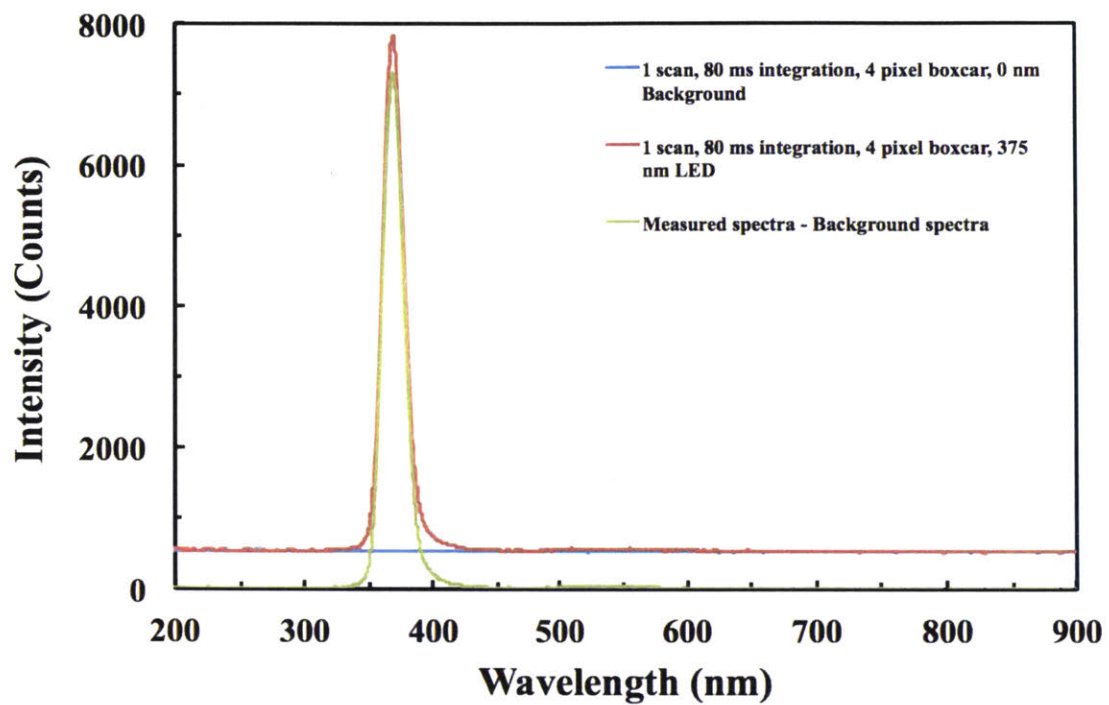


4. 0.4 ppb fluorescein

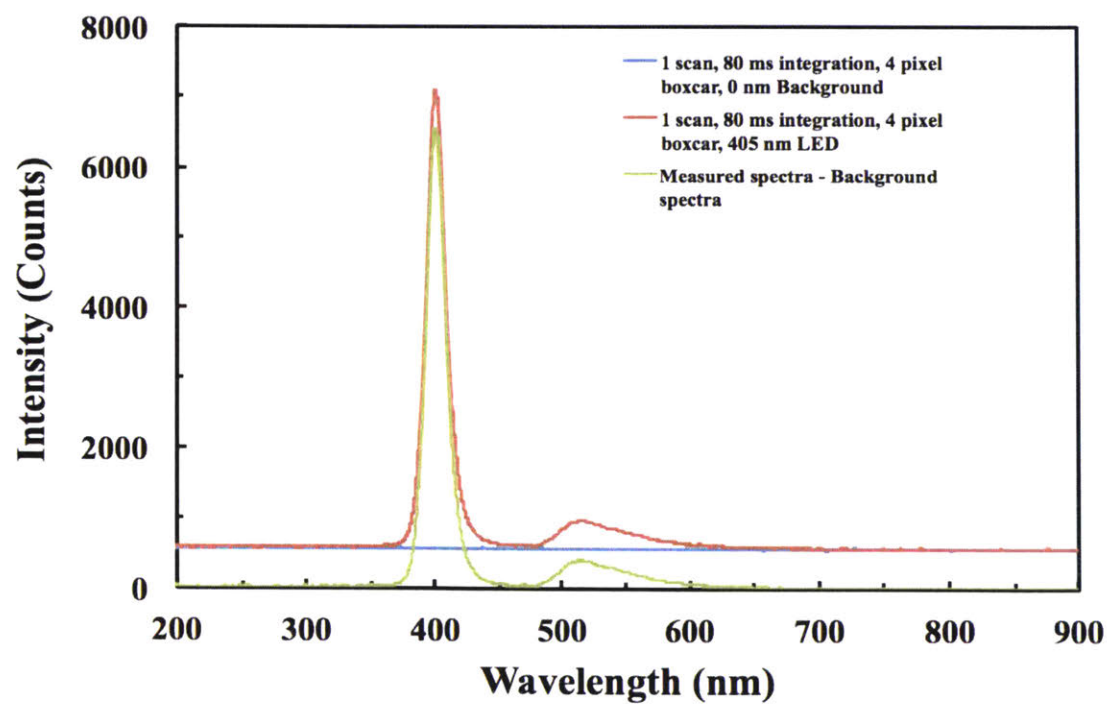
a. Hammerhead (top: full spectrum, bottom: zoomed in)

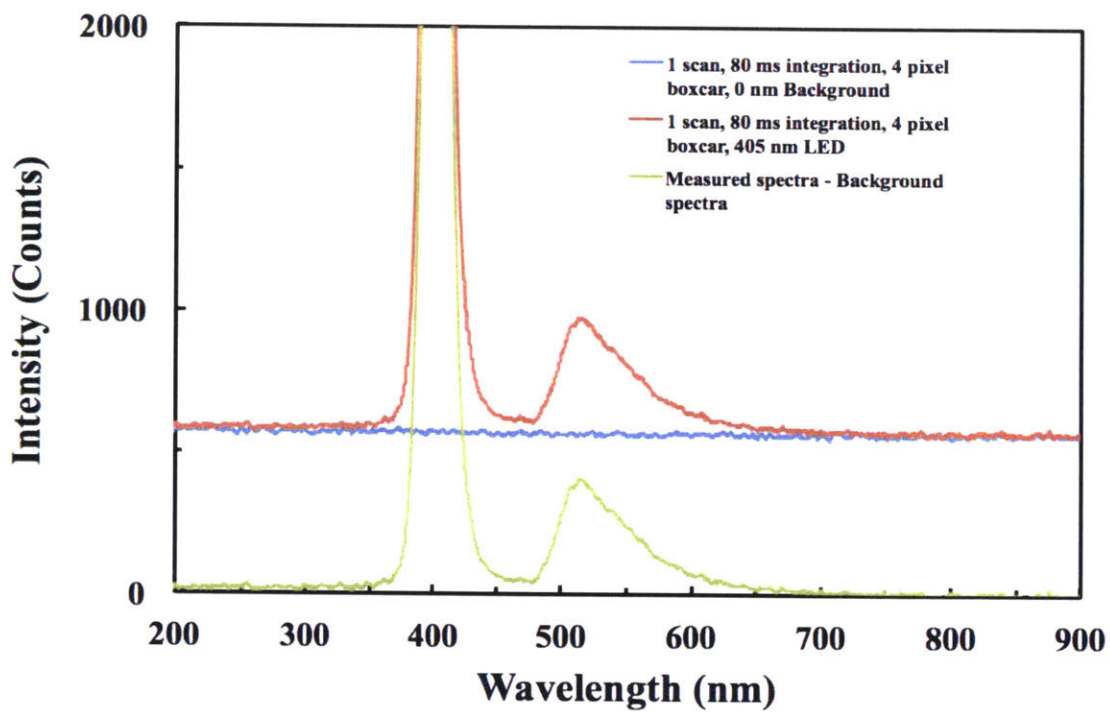


b. LEDIF with 375 nm



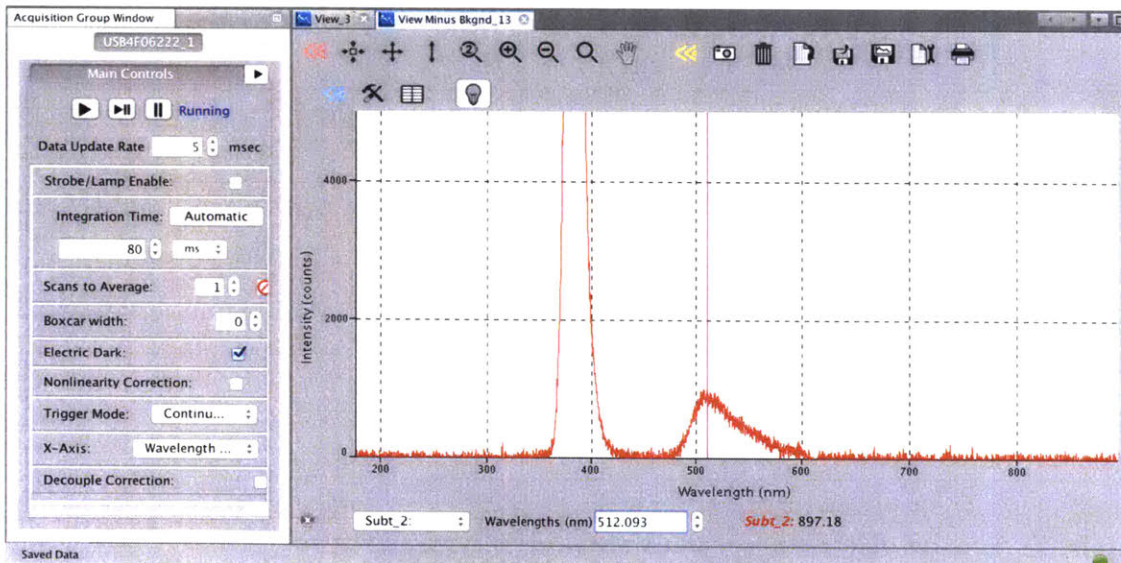
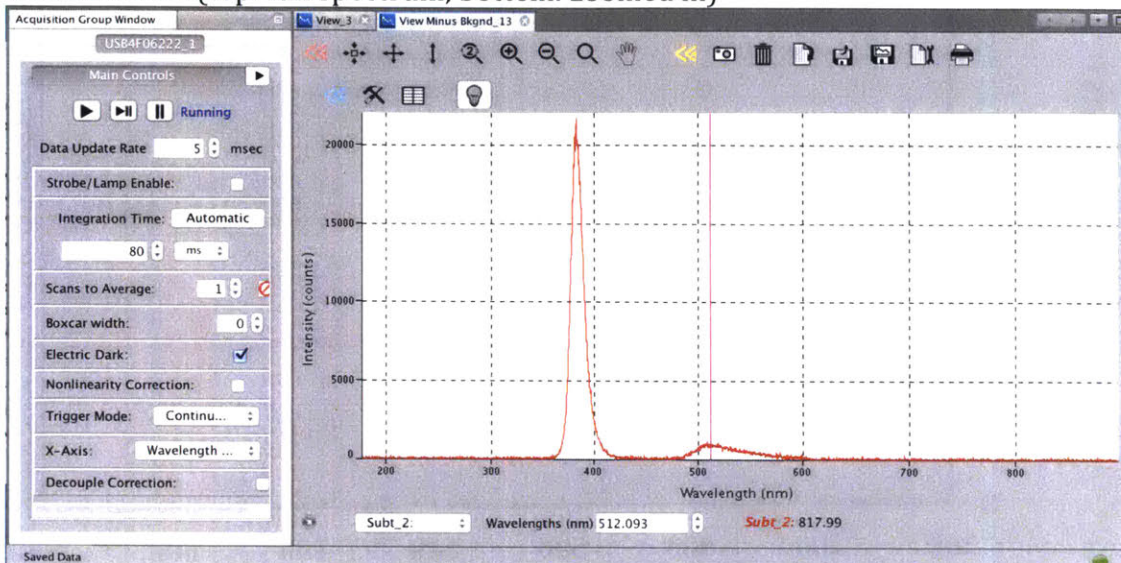
c. LEDIF with 405 nm (top: full spectrum, bottom: zoomed in)



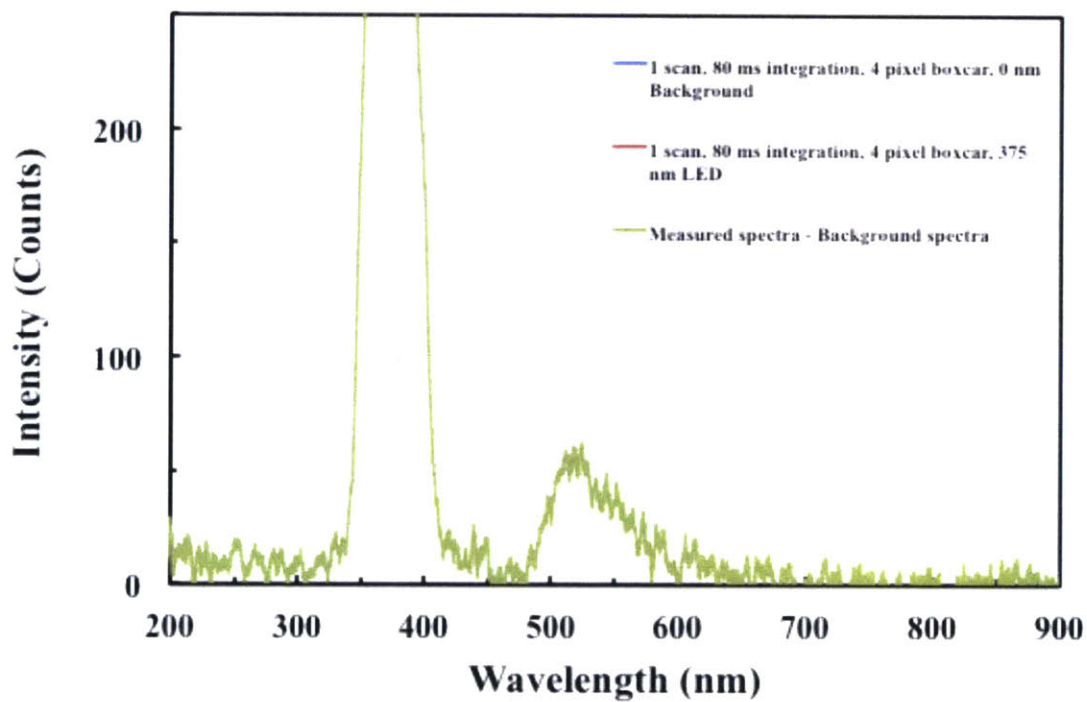
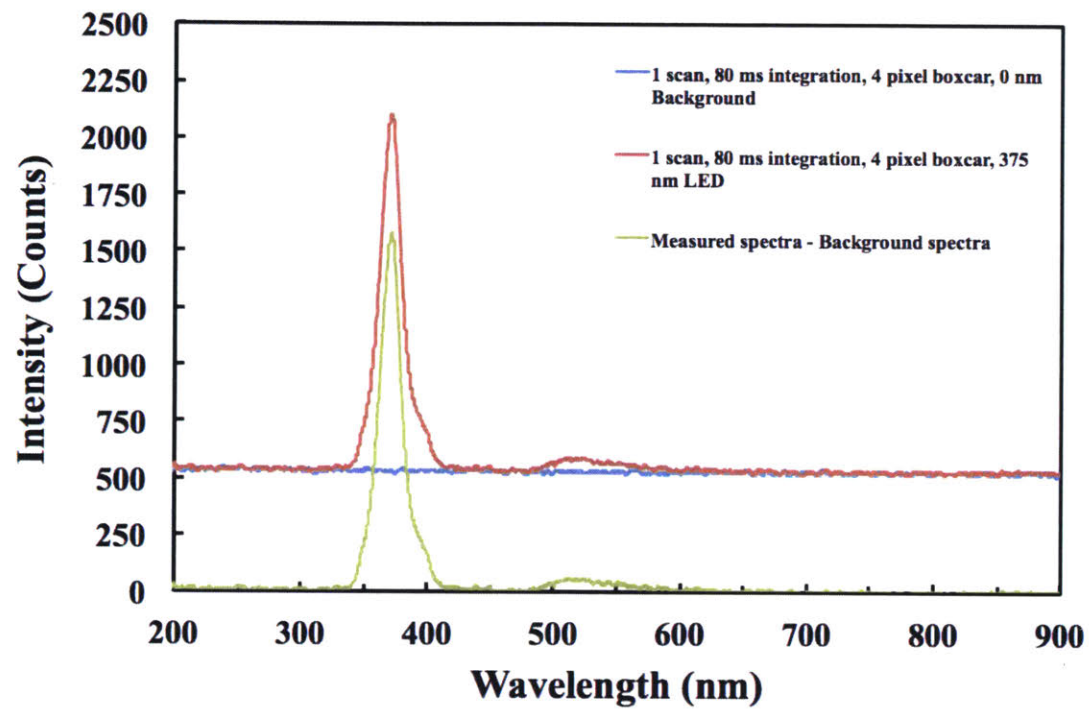


5. 0.8 ppb fluorescein

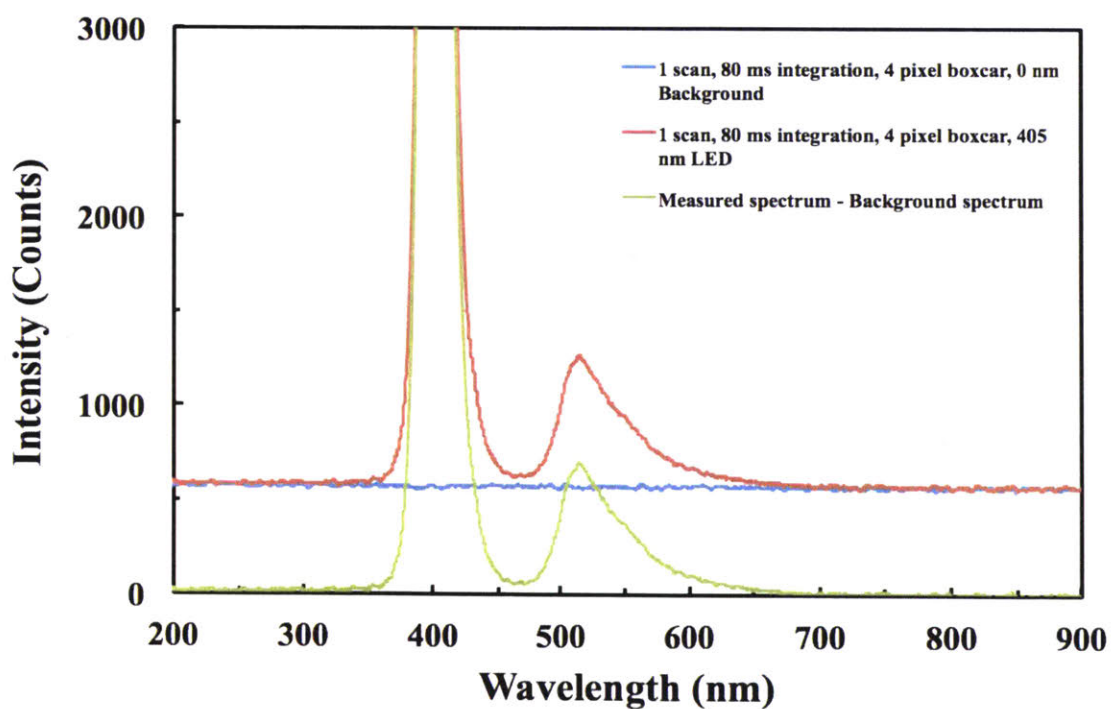
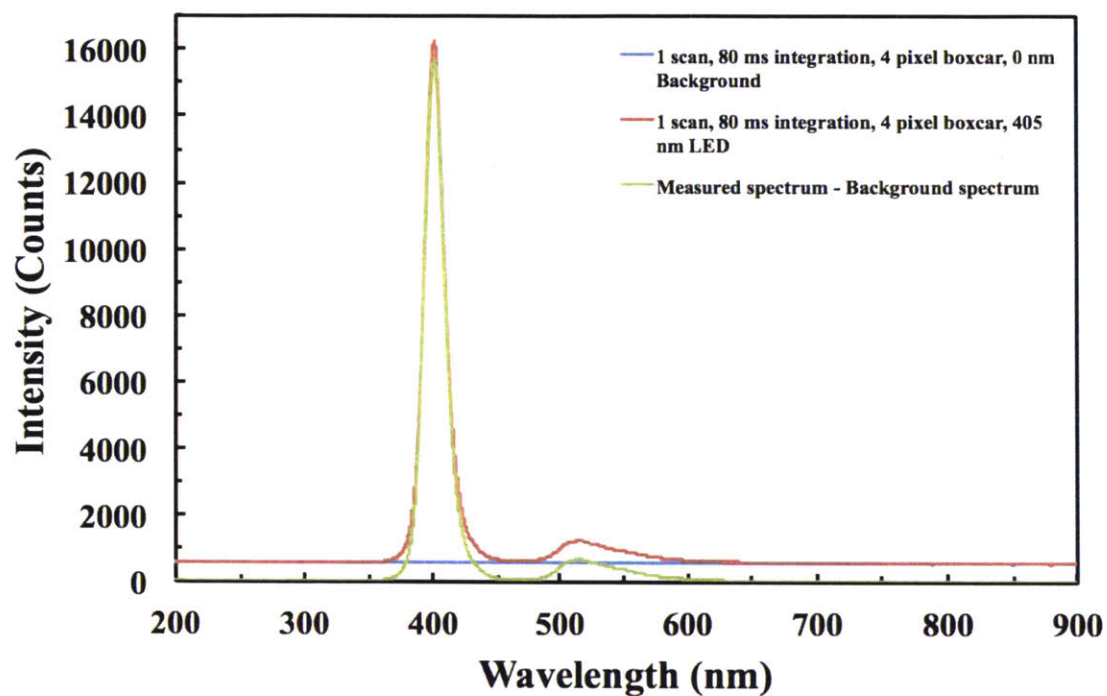
a. Hammerhead (top: full spectrum, bottom: zoomed in)



b. LEDIF with 375 nm (top: full spectrum, bottom: zoomed in)

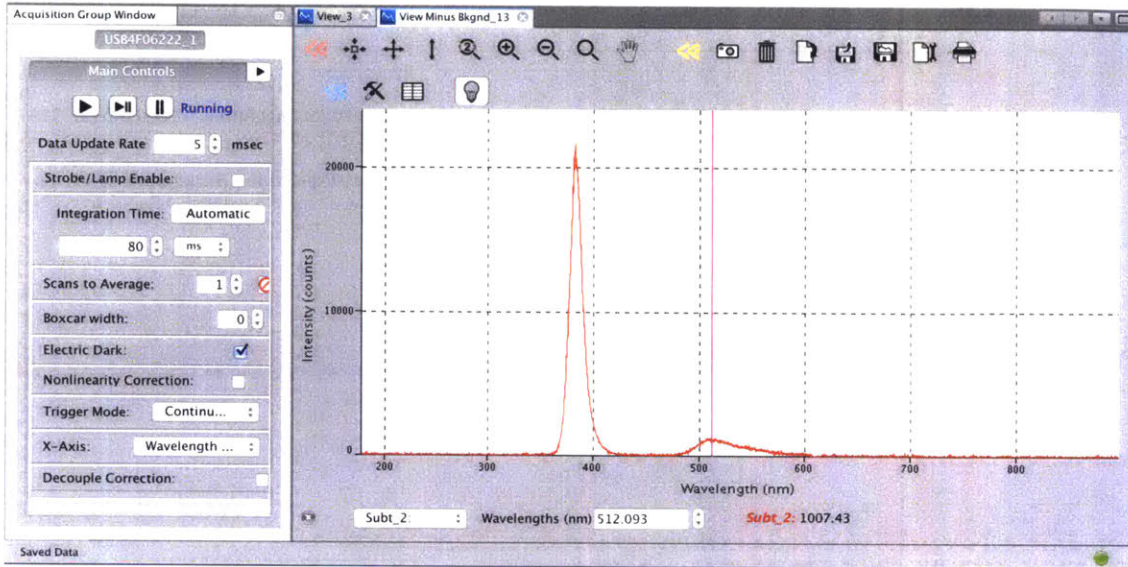


c. LEDIF with 405 nm (top: full spectrum, bottom: zoomed in)

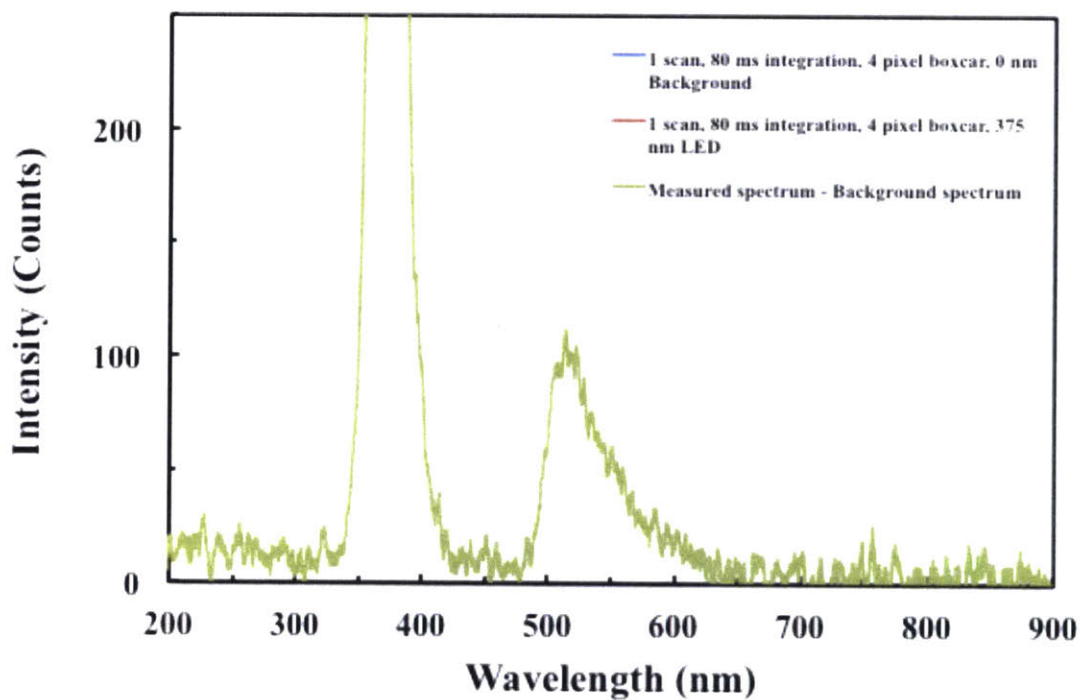
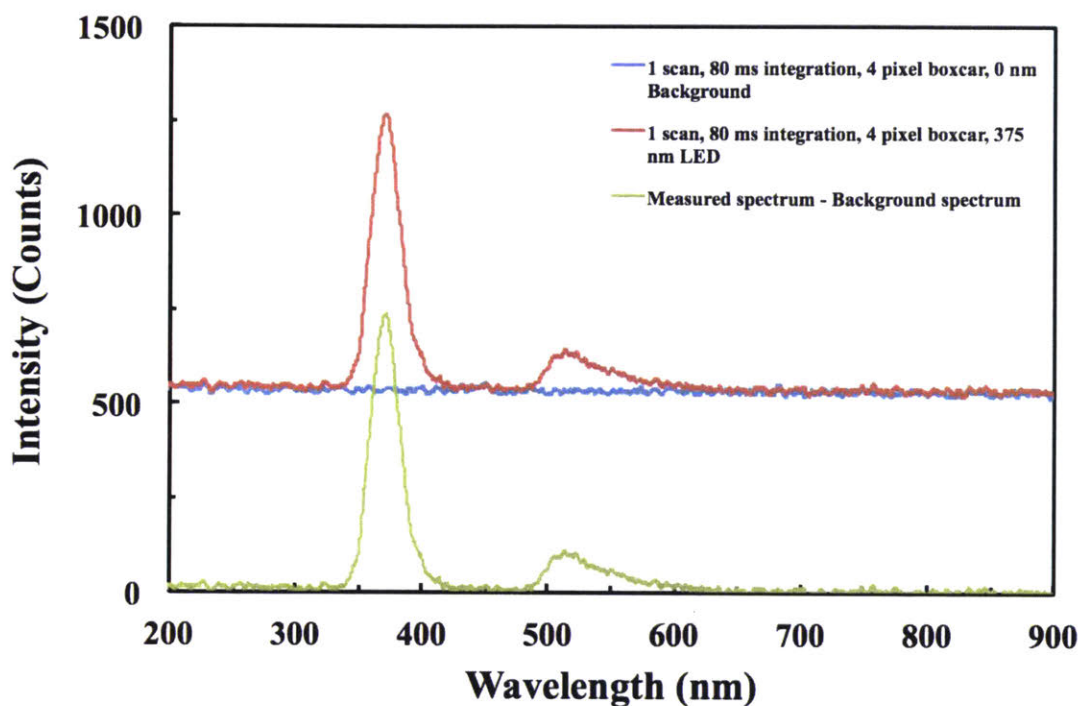


6. 1 ppb fluorescein

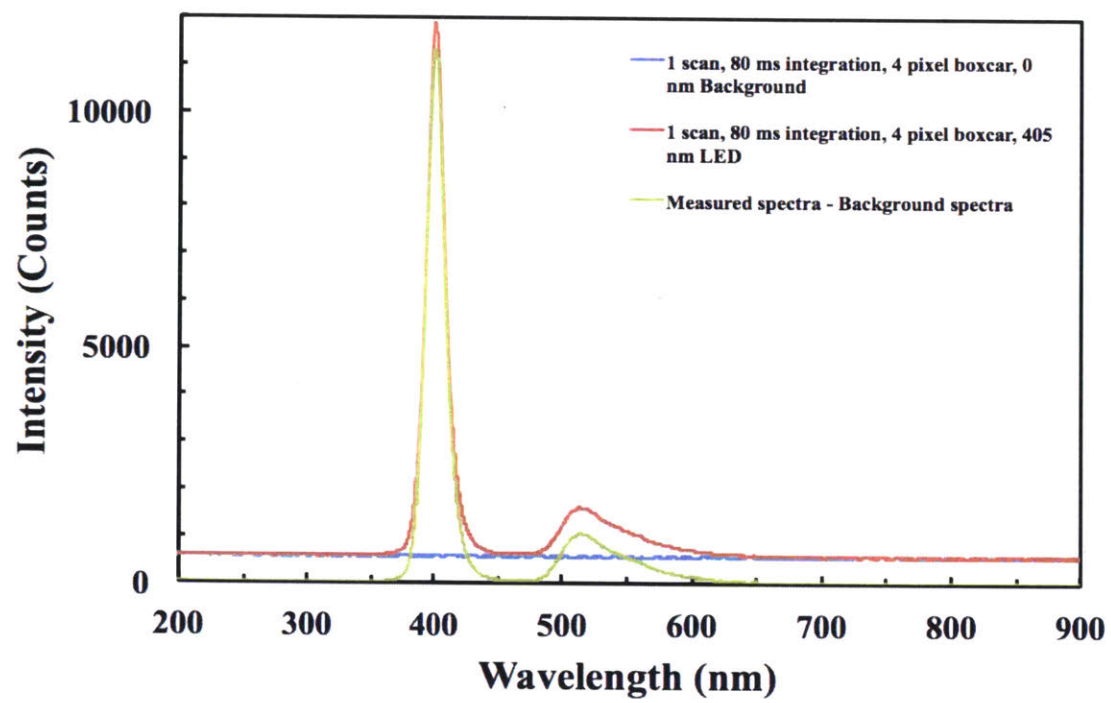
a. Hammerhead



b. LEDIF with 375 nm (top: full spectrum, bottom: zoomed in)

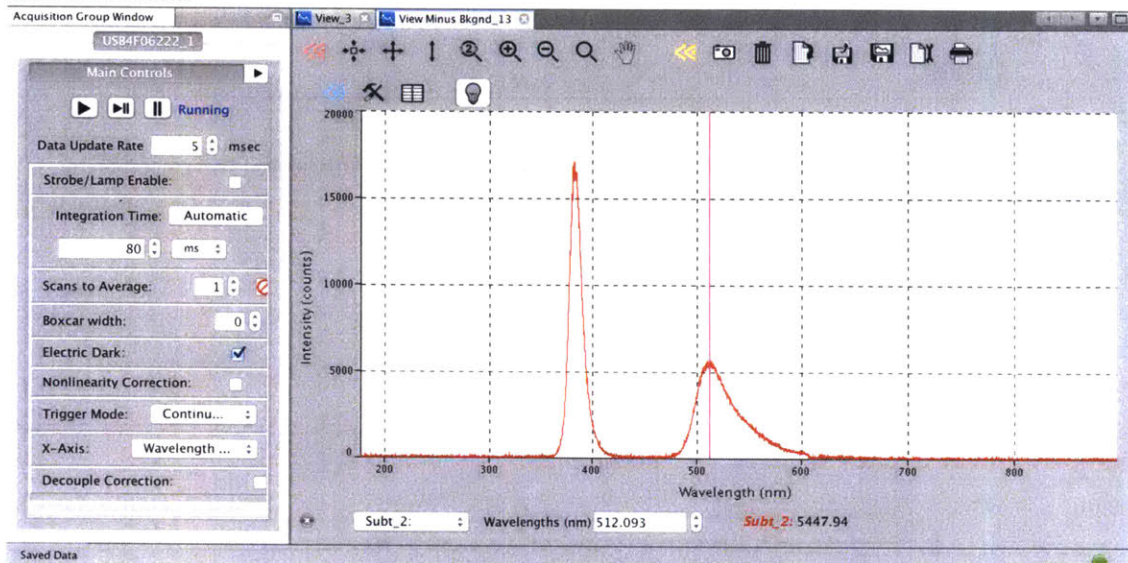


c. LEDIF with 405 nm

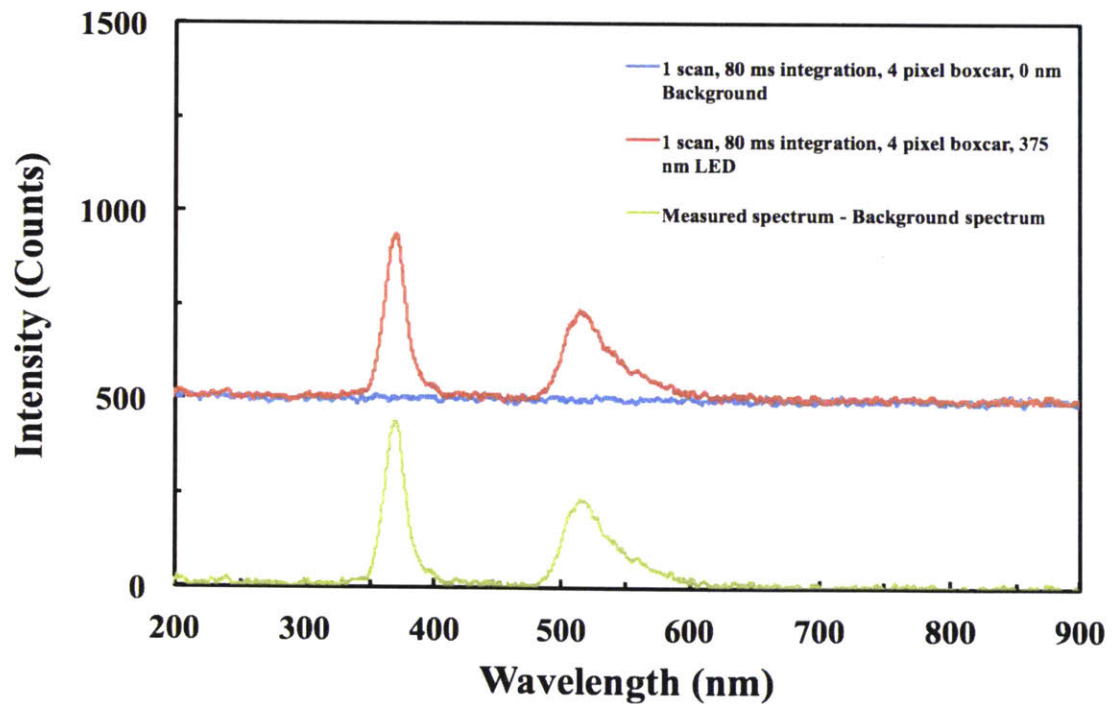


7. 5 ppb fluorescein

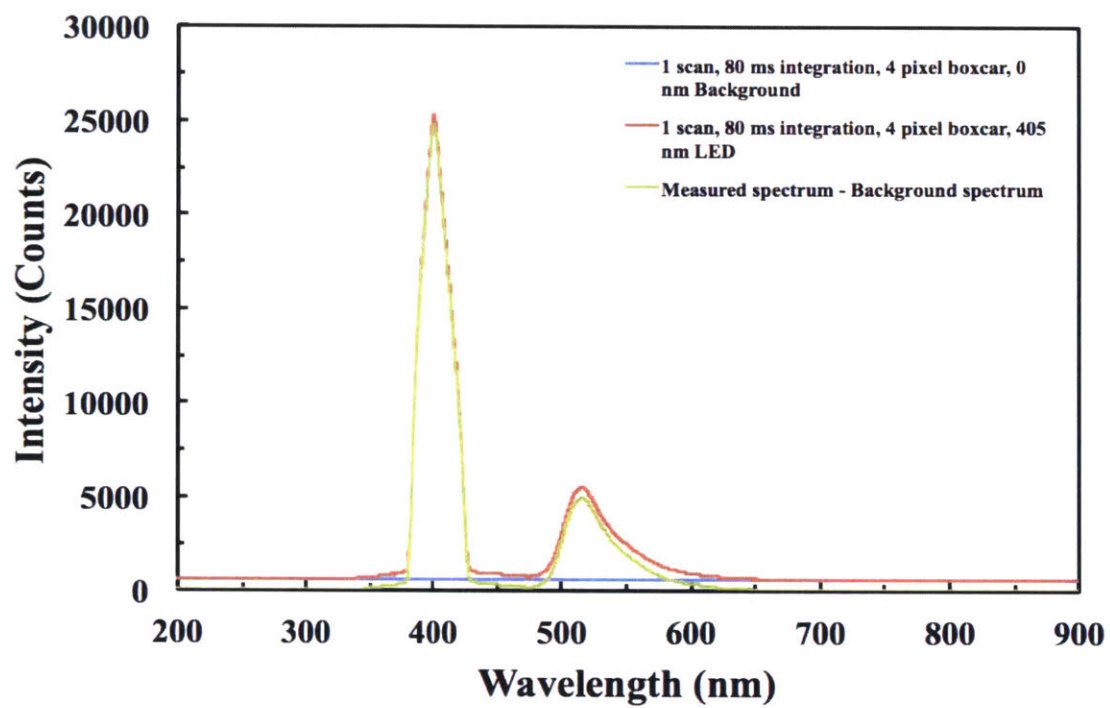
a. Hammerhead



b. LEDIF with 375 nm



c. LEDIF with 405 nm

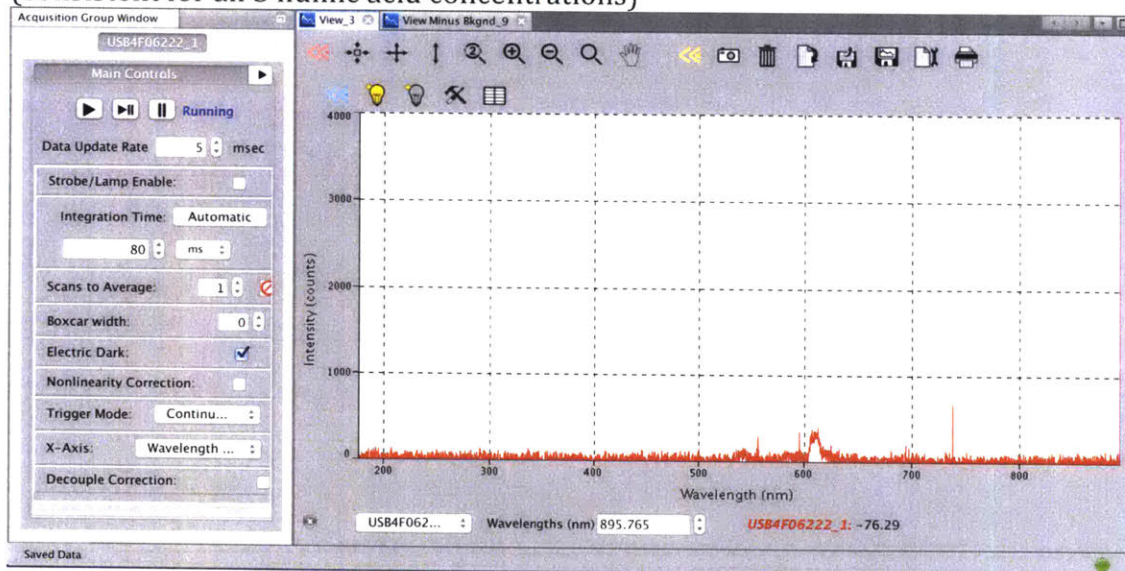


Appendix 4 – Hammerhead and LEDIF Humic Acid Spectra

Notes:

- The Hammerhead uses Spacing #1 from Table 2 in the text (0.076 inches from the tip of the excitation fiber and the tip of the detector fiber to the center of the chamber)
- The LEDIF graphs shown are for LEDIF trials with its pump ON.
- The integration time for each sample may be seen in the box on the left-hand side of each image for Hammerhead tests, and within the graph for LEDIF tests.
- Each integration time used was 80 milliseconds.
- For reference, graph 1 depicts the background spectrum for the Hammerhead with an unlit chamber, which is consistent for all 5 humic acid concentrations.
- Background spectrum has been subtracted in the graphs for each Hammerhead sample.
- LEDIF graphs depict the background spectra, the measured spectra, and the measured spectra minus the background spectra.

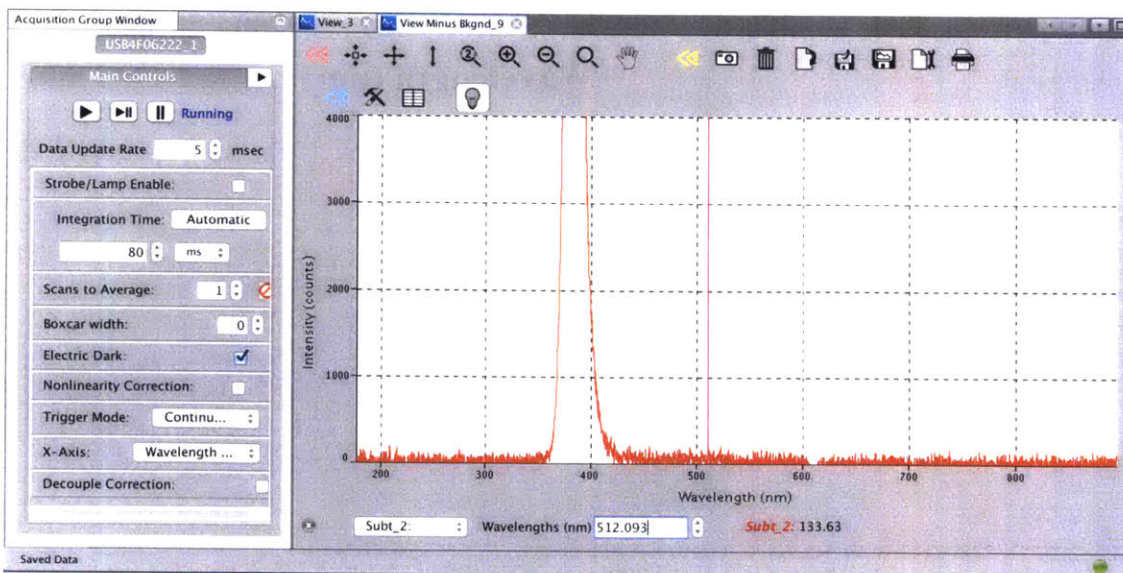
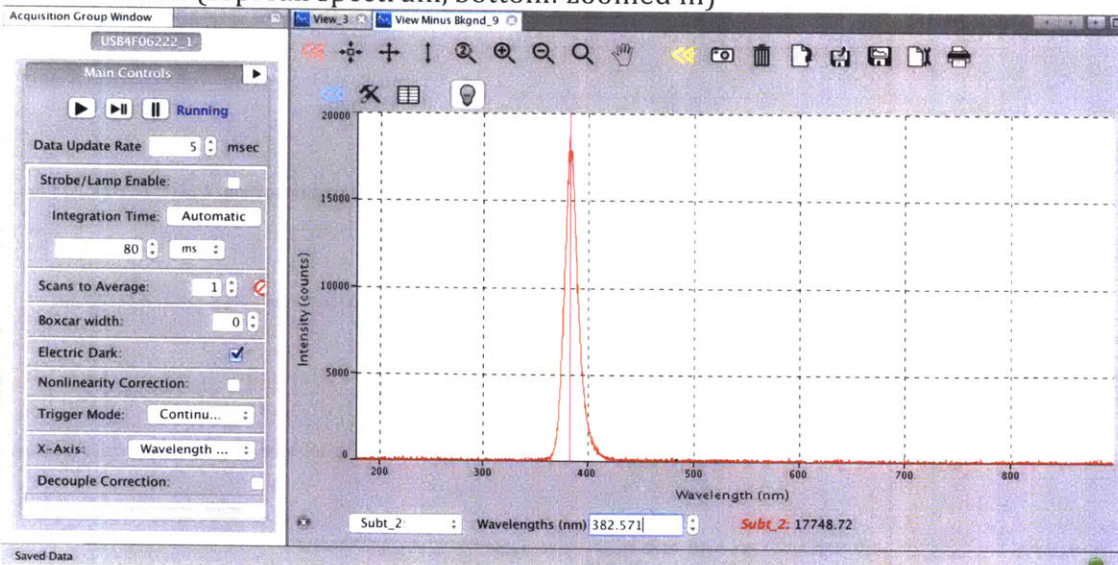
1. Hammerhead background spectrum (consistent for all 5 humic acid concentrations)



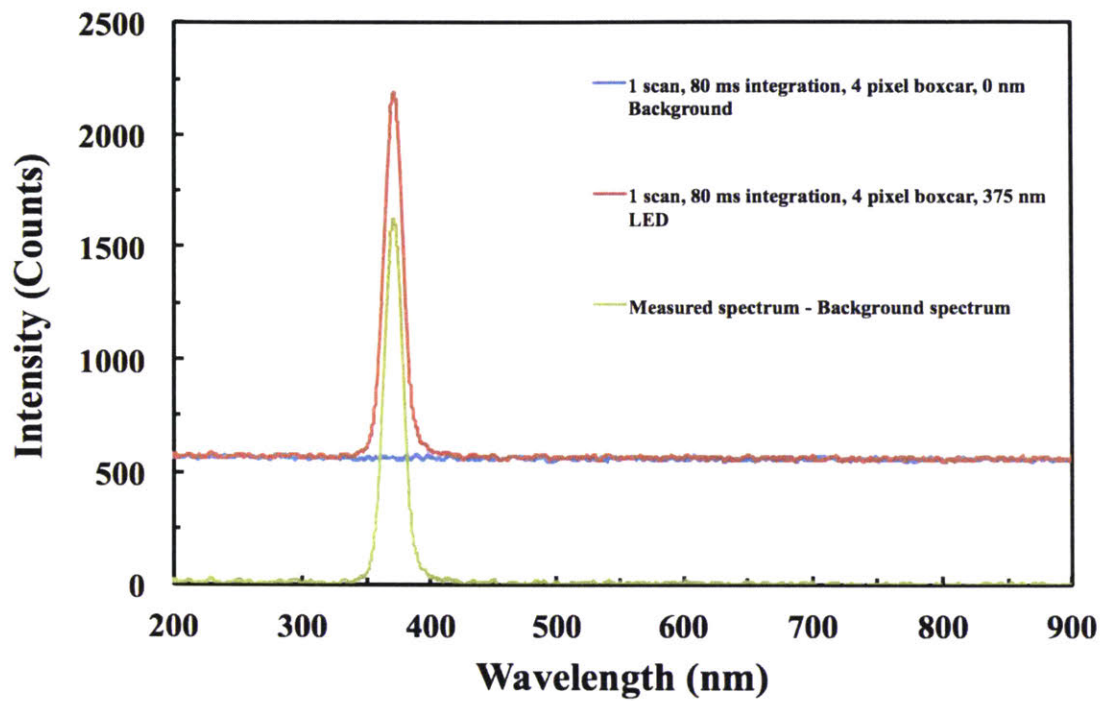
Background average: 16 counts

2. 1 ppm humic acid

a. Hammerhead (top: full spectrum, bottom: zoomed in)

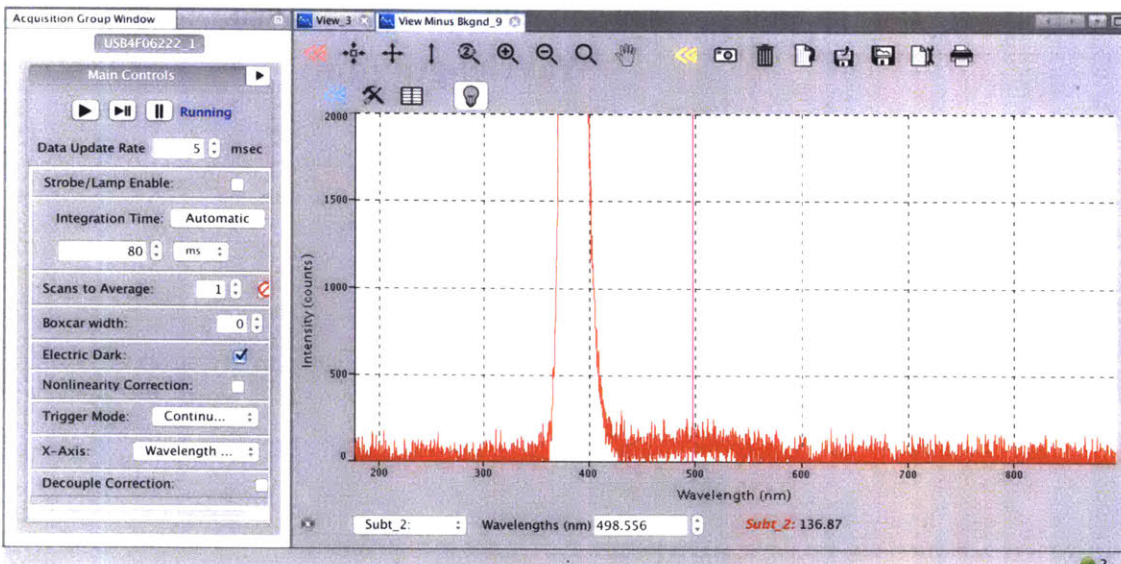
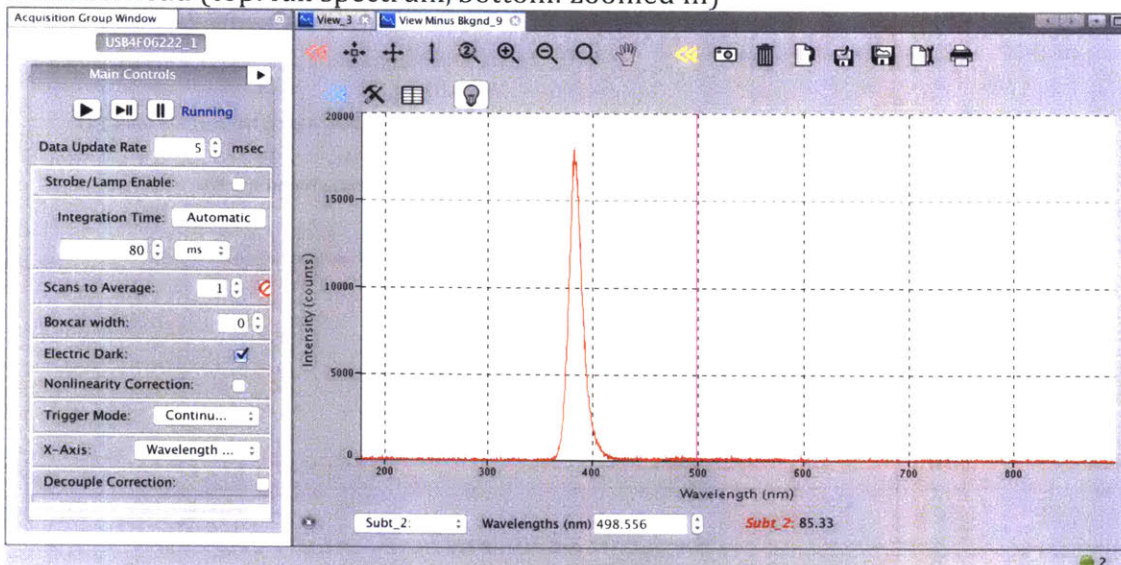


b. LEDIF with 375 nm

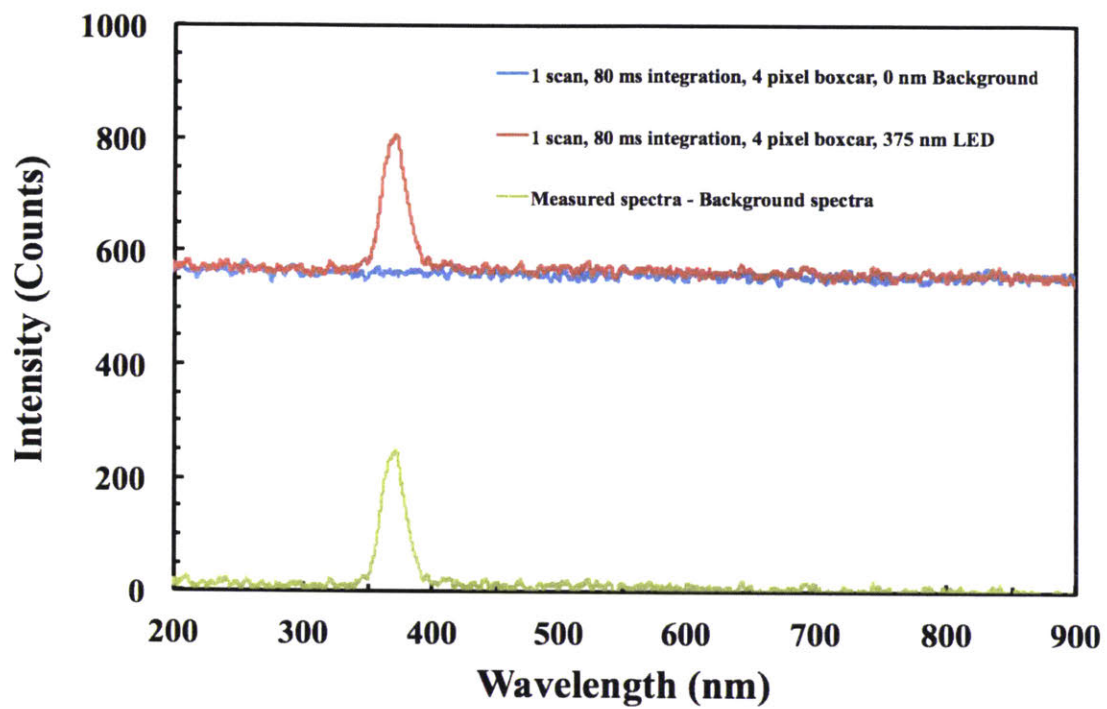


3. 3 ppm humic acid

a. Hammerhead (top: full spectrum, bottom: zoomed in)

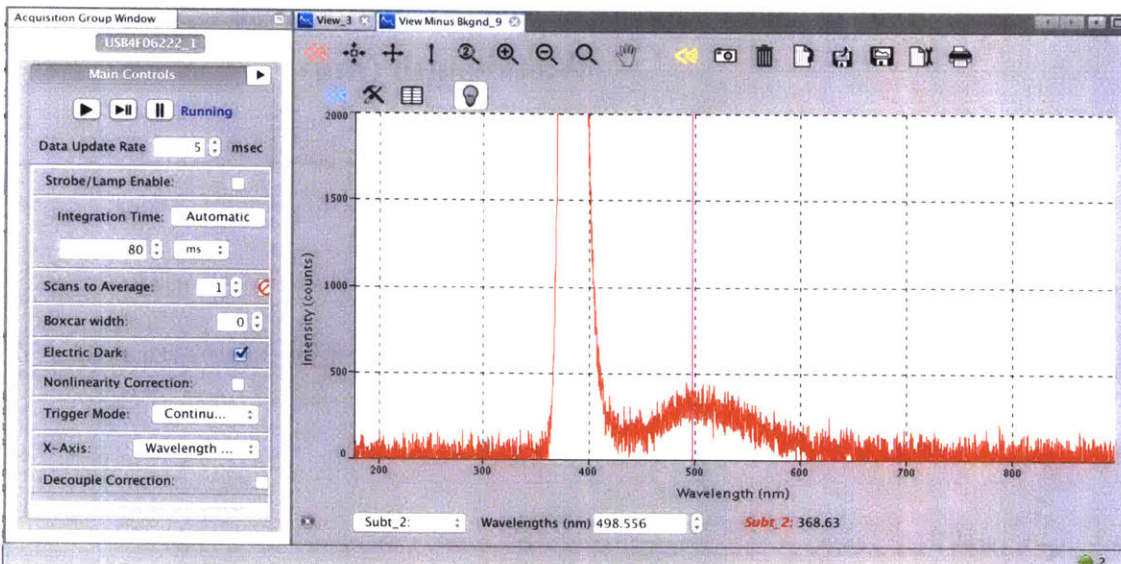
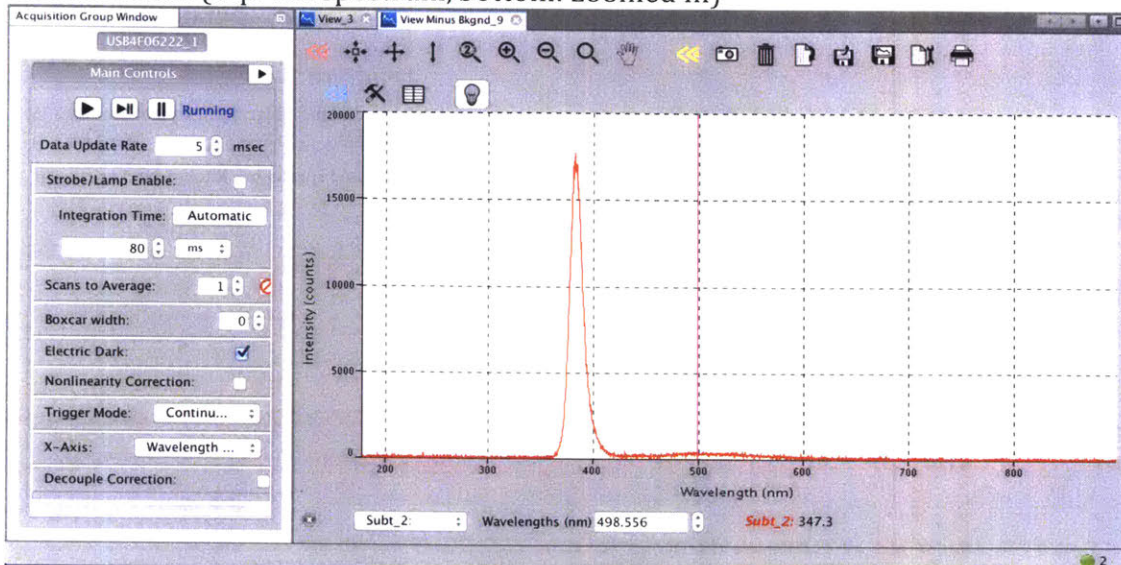


b. LEDIF with 375 nm

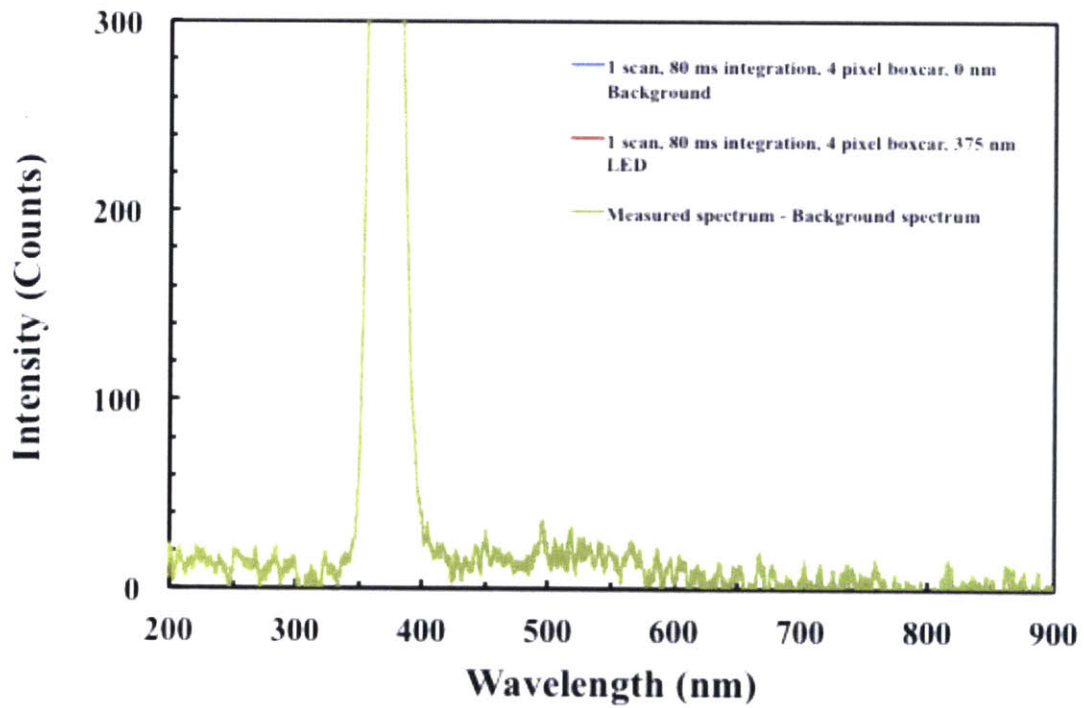
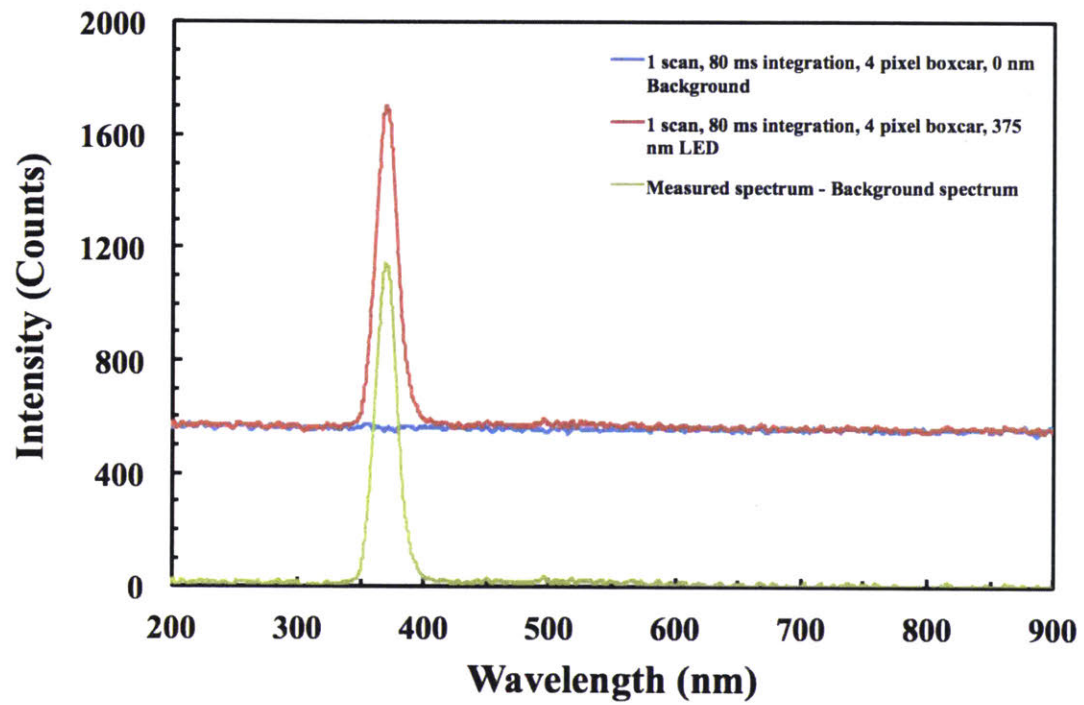


4. 10 ppm humic acid

a. Hammerhead (top: full spectrum, bottom: zoomed in)

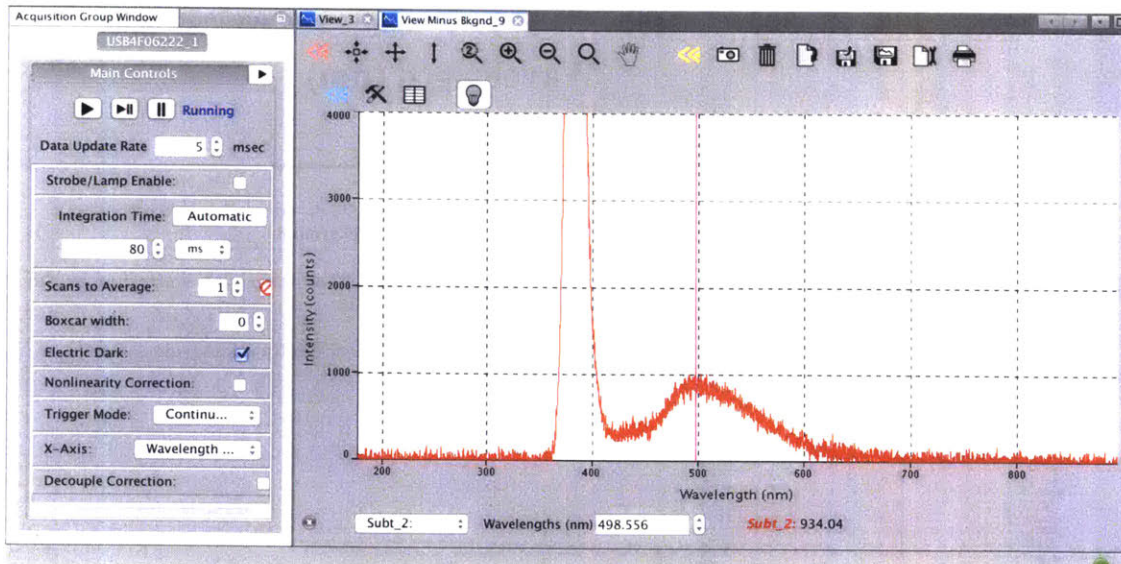
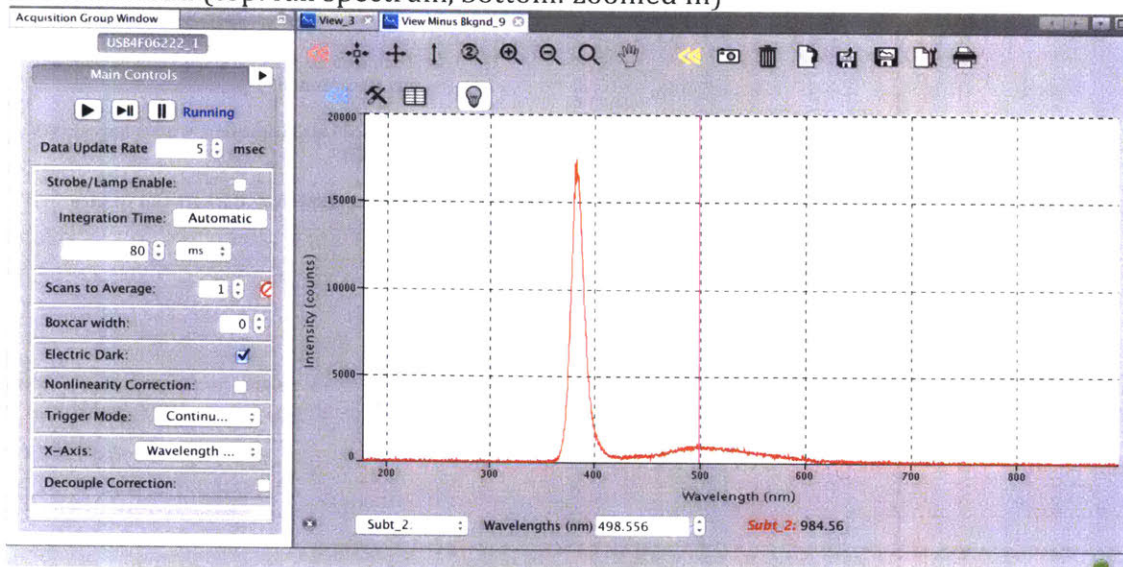


b. LEDIF with 375 nm (top: full spectrum, bottom: zoomed in)

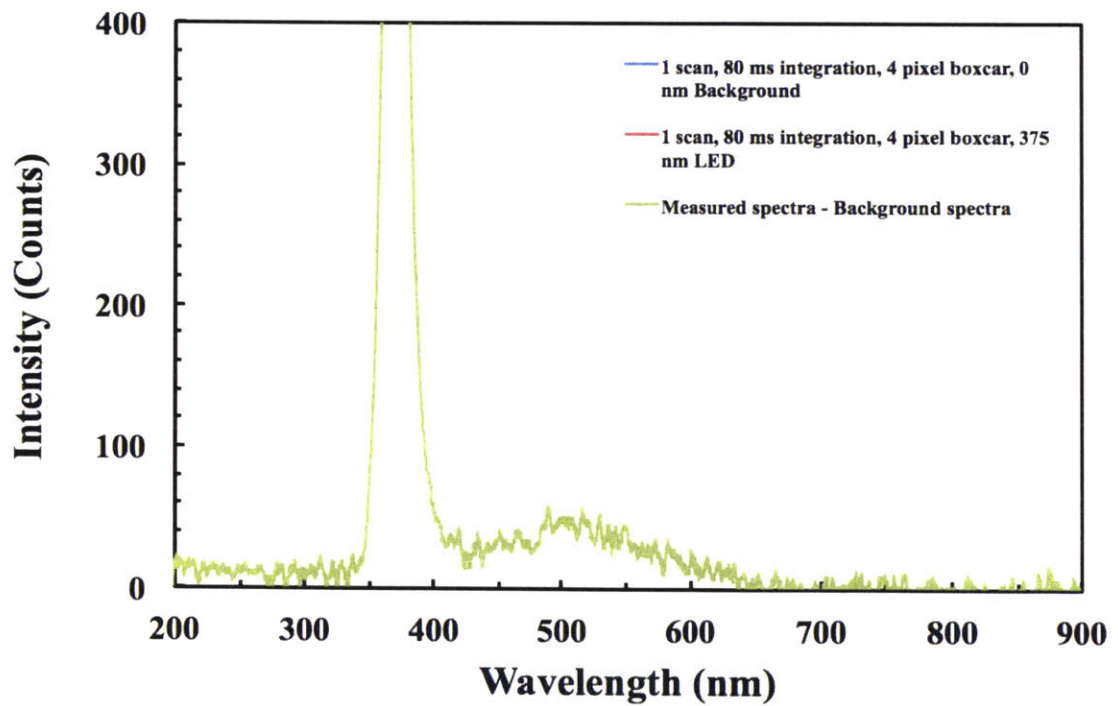
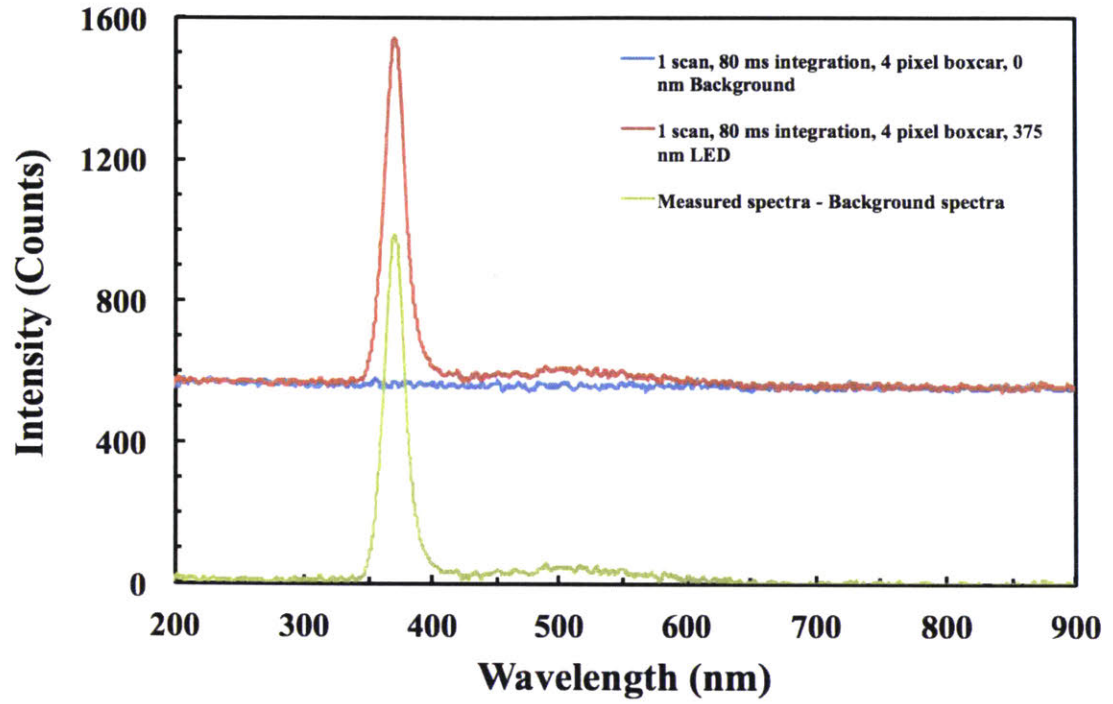


5. 30 ppm humic acid

a. Hammerhead (top: full spectrum, bottom: zoomed in)

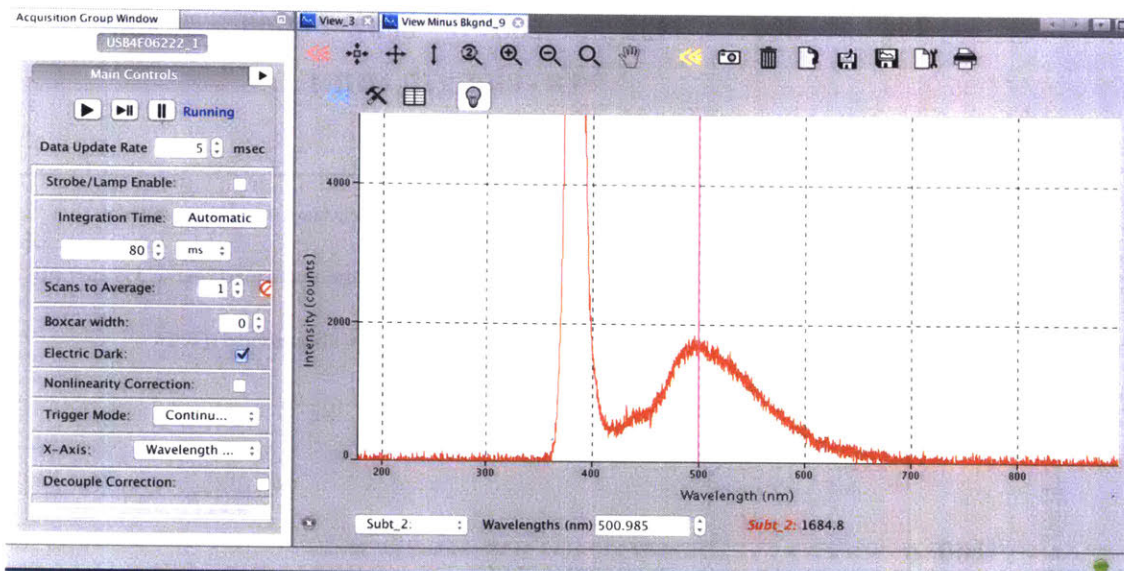
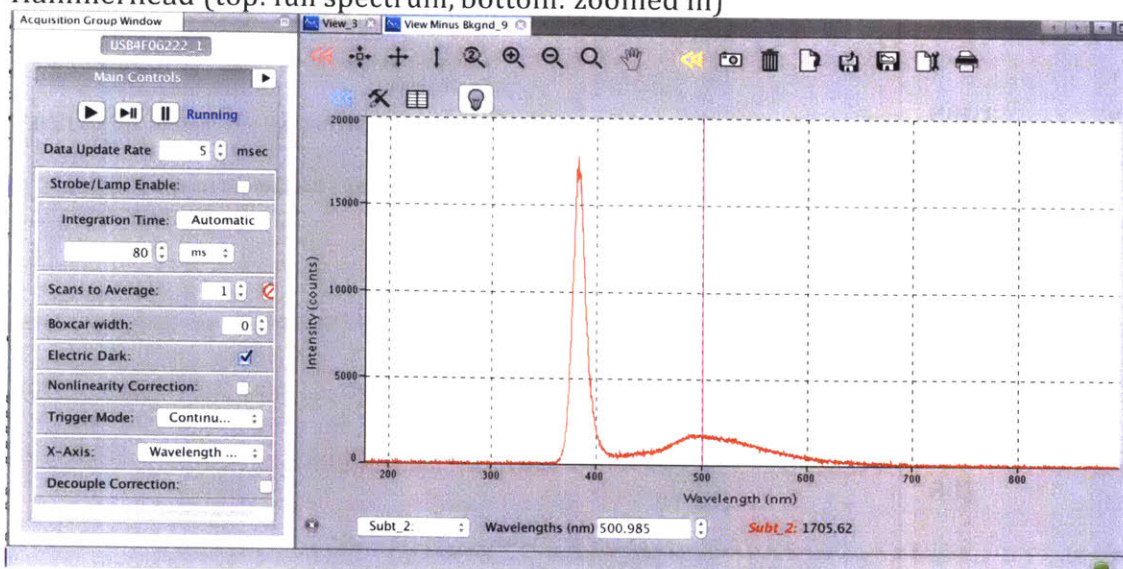


b. LEDIF with 375 nm (top: full spectrum, bottom: zoomed in)

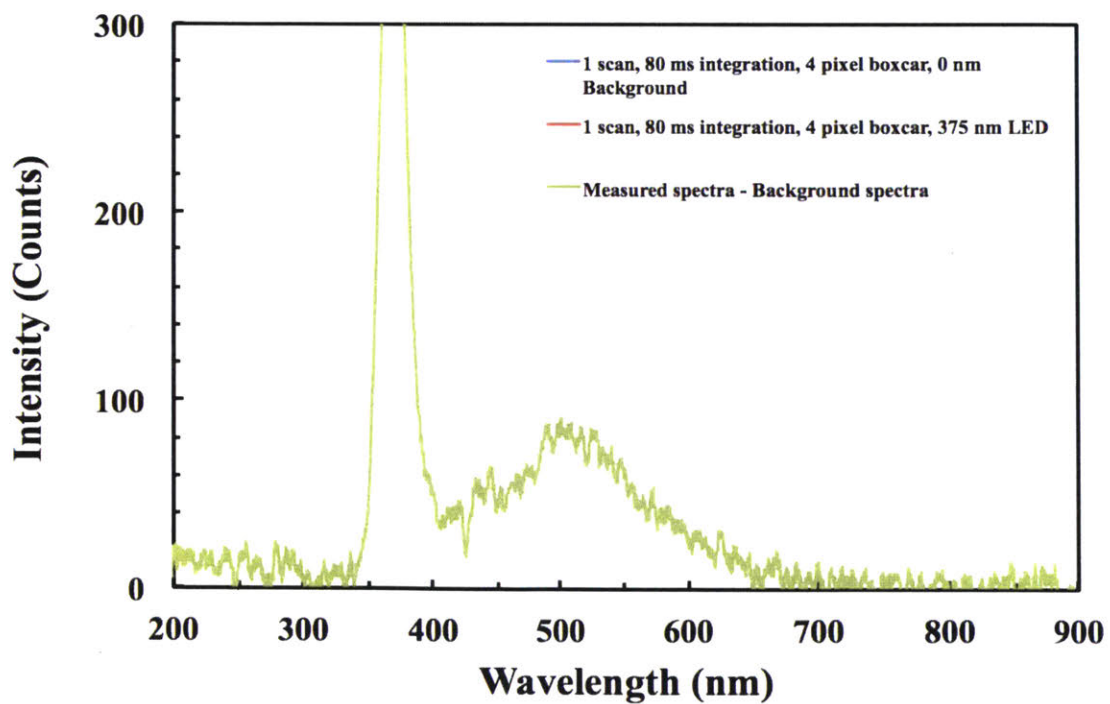
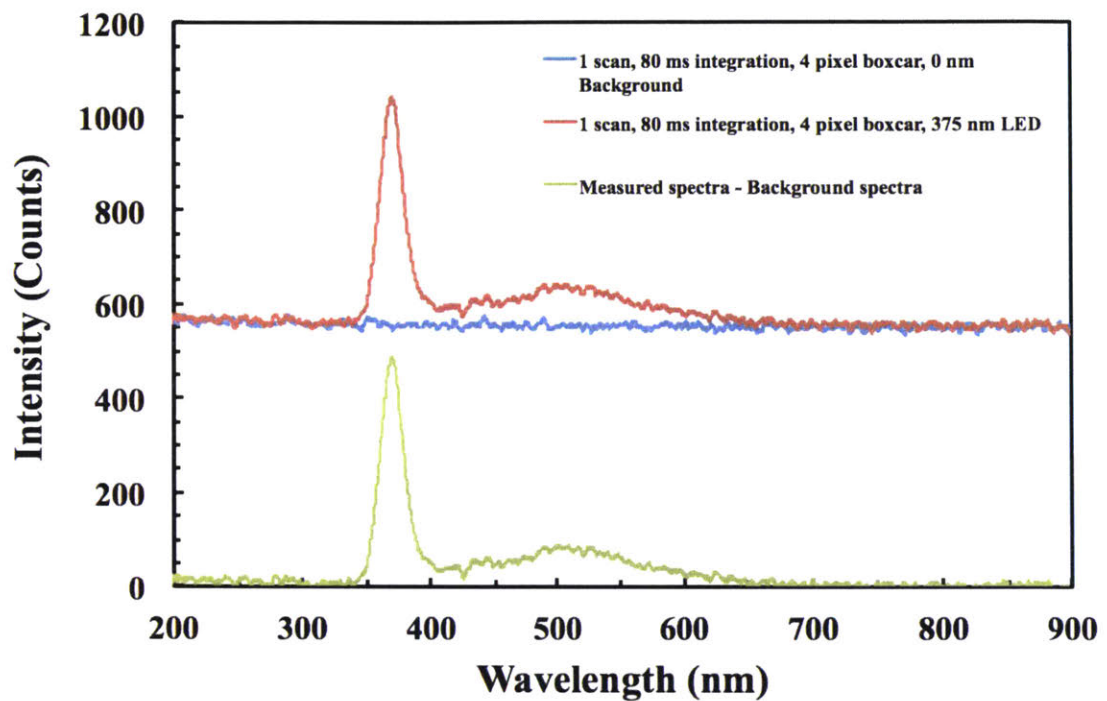


6. 60 ppm humic acid

a. Hammerhead (top: full spectrum, bottom: zoomed in)



b. LEDIF with 375 nm (top: full spectrum, bottom: zoomed in)

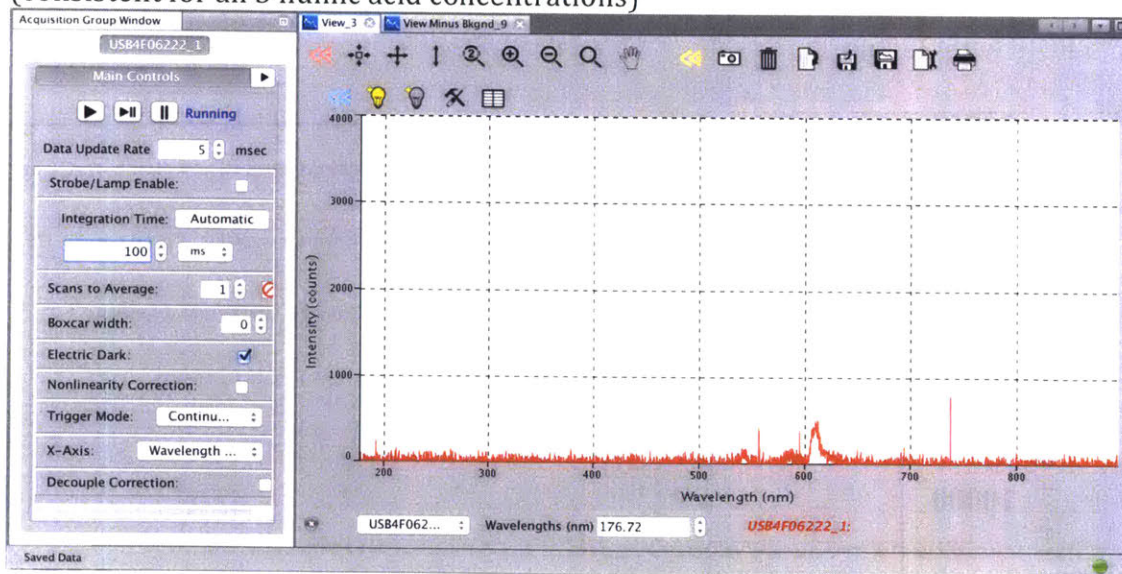


Appendix 5 – Hammerhead and LEDIF Humic Acid Spectra at Longer Integration Times

Notes:

- The Hammerhead uses Spacing #1 from Table 2 in the text (0.076 inches from the tip of the excitation fiber and the tip of the detector fiber to the center of the chamber)
- The LEDIF graphs shown are for LEDIF trials with its pump ON.
- The integration time for each sample may be seen in the box on the left-hand side of each image for Hammerhead tests, and within the graph for LEDIF tests.
 - The Hammerhead uses a 1 second integration time
 - The LEDIF uses a 10 second integration time
- For reference, graph 1 depicts the background spectrum for the Hammerhead with an unlit chamber, which is consistent for all 5 humic acid concentrations.
- Background spectrum has been subtracted in the graphs for each Hammerhead sample.
- LEDIF graphs depict the background spectra, the measured spectra, and the measured spectra minus the background spectra.

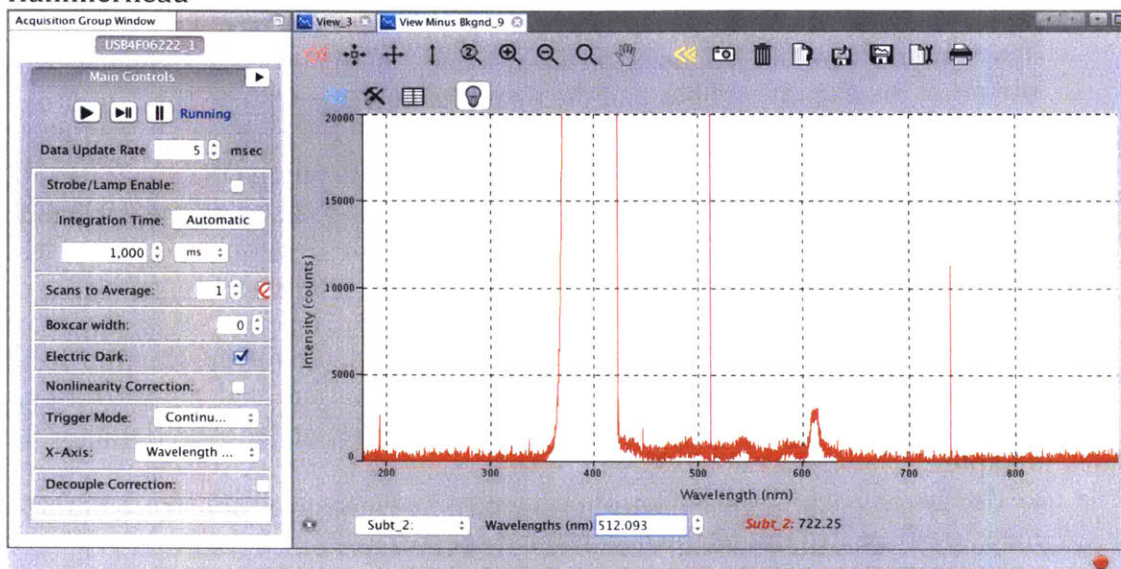
1. Hammerhead background spectrum (consistent for all 5 humic acid concentrations)



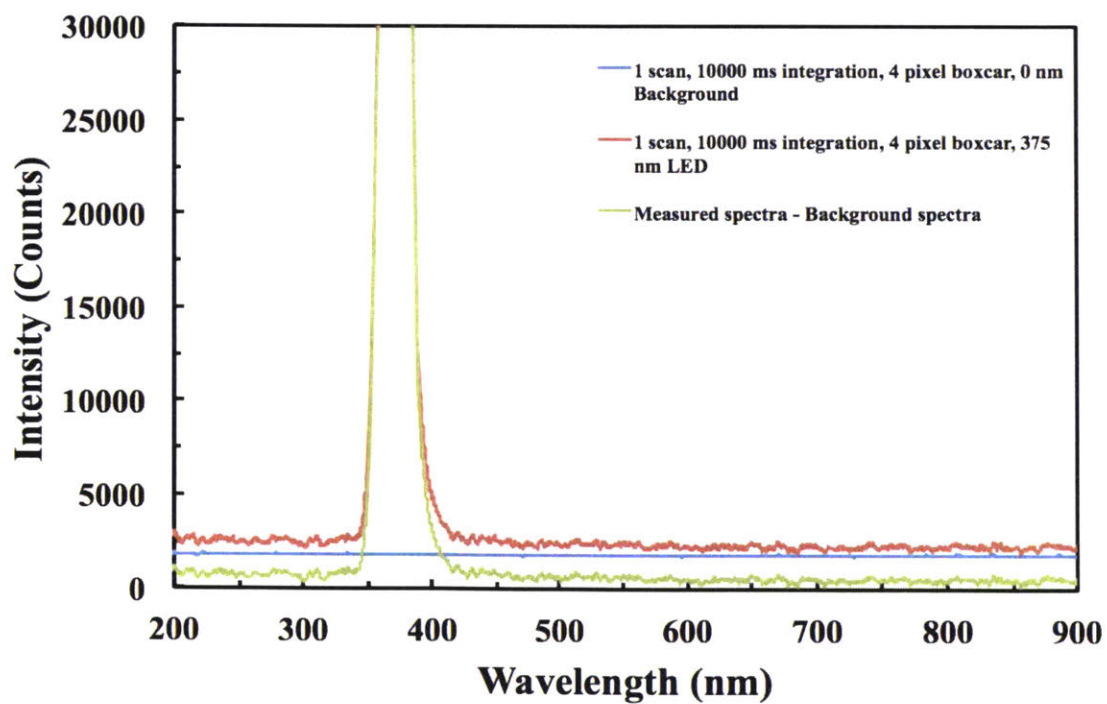
Background average: 16 counts

2. 1 ppm humic acid

a. Hammerhead

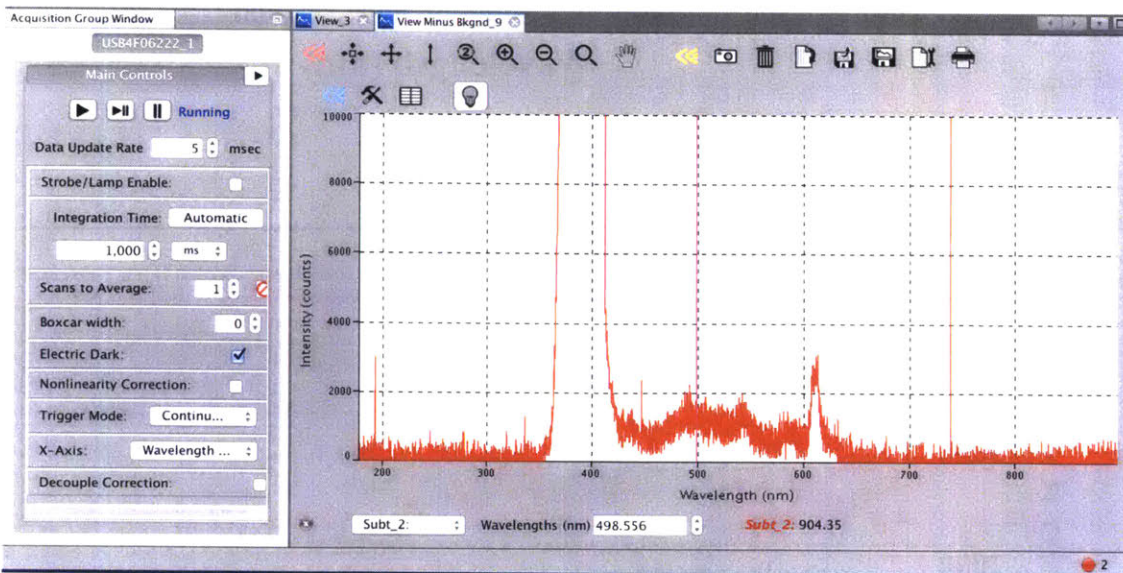
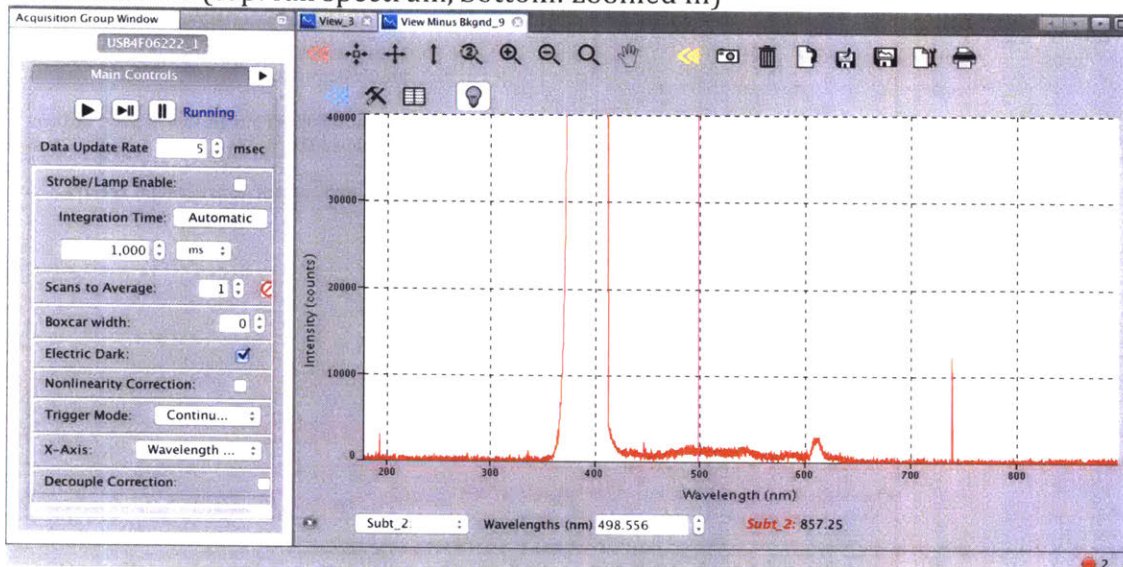


b. LEDIF with 375 nm

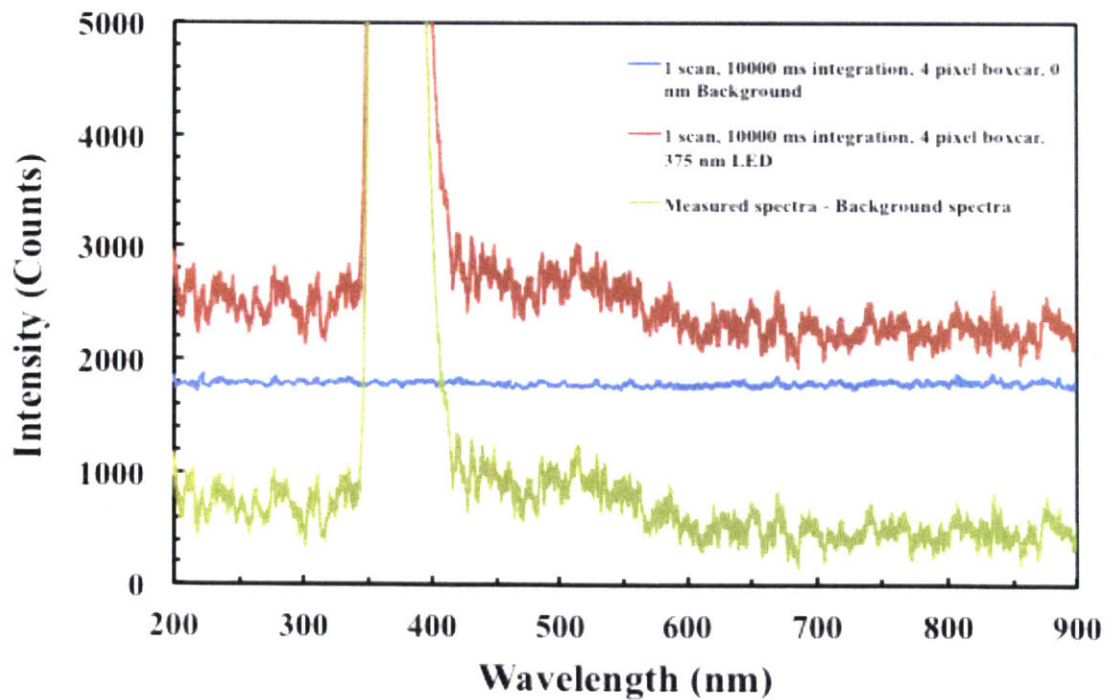
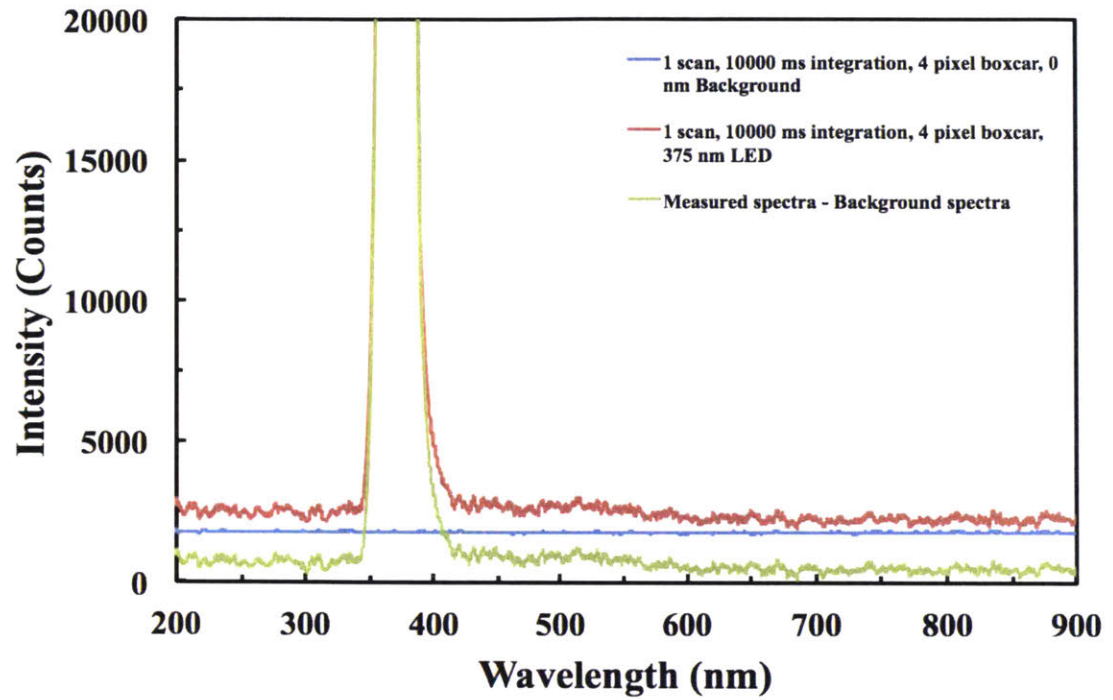


3. 3 ppm humic acid

a. Hammerhead (top: full spectrum, bottom: zoomed in)

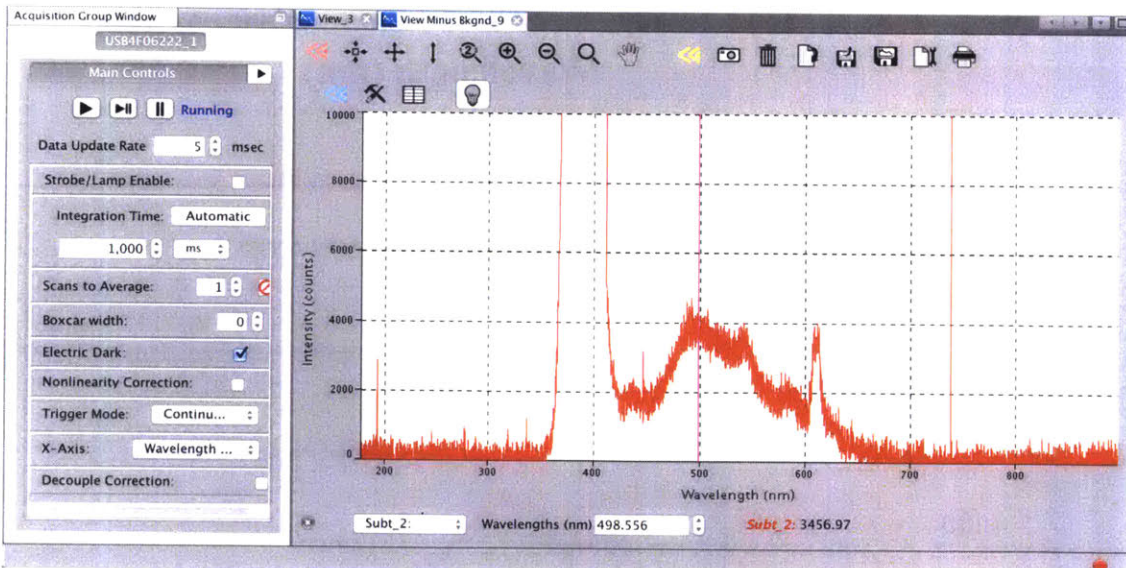
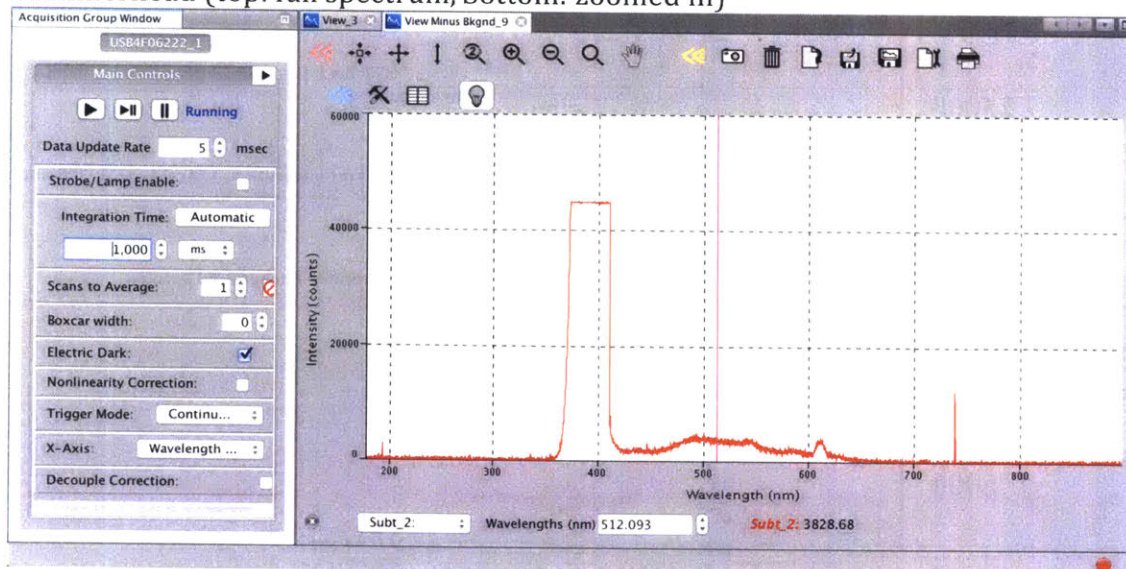


b. LEDIF with 375 nm (top: full spectrum, bottom: zoomed in)

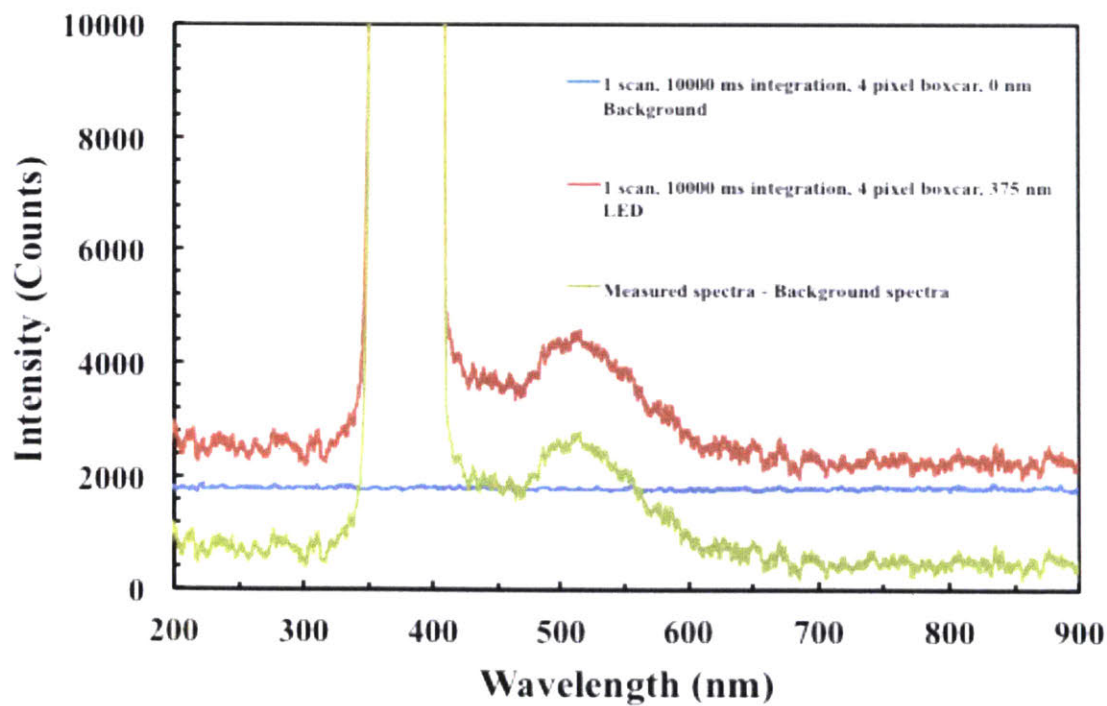
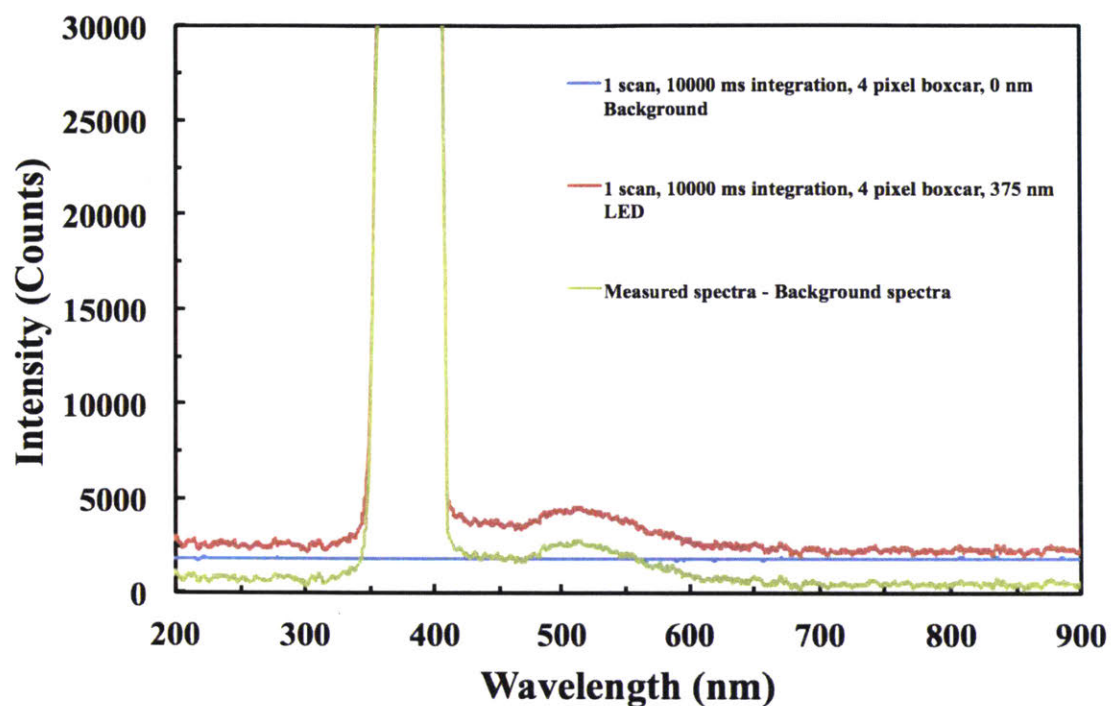


4. 10 ppm humic acid

a. Hammerhead (top: full spectrum, bottom: zoomed in)

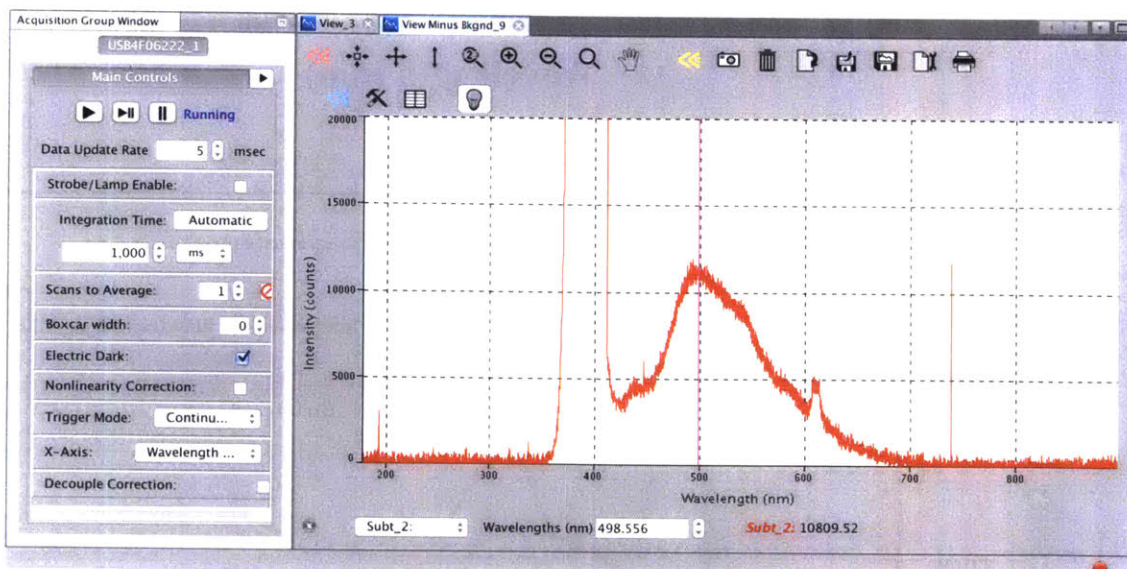
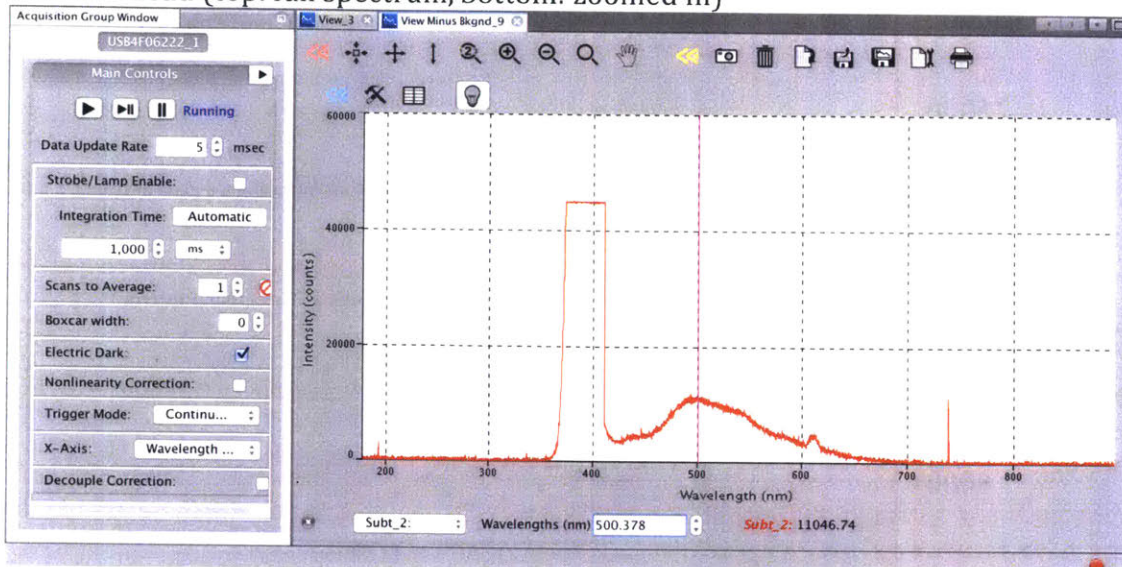


b. LEDIF with 375 nm (top: full spectrum, bottom: zoomed in)

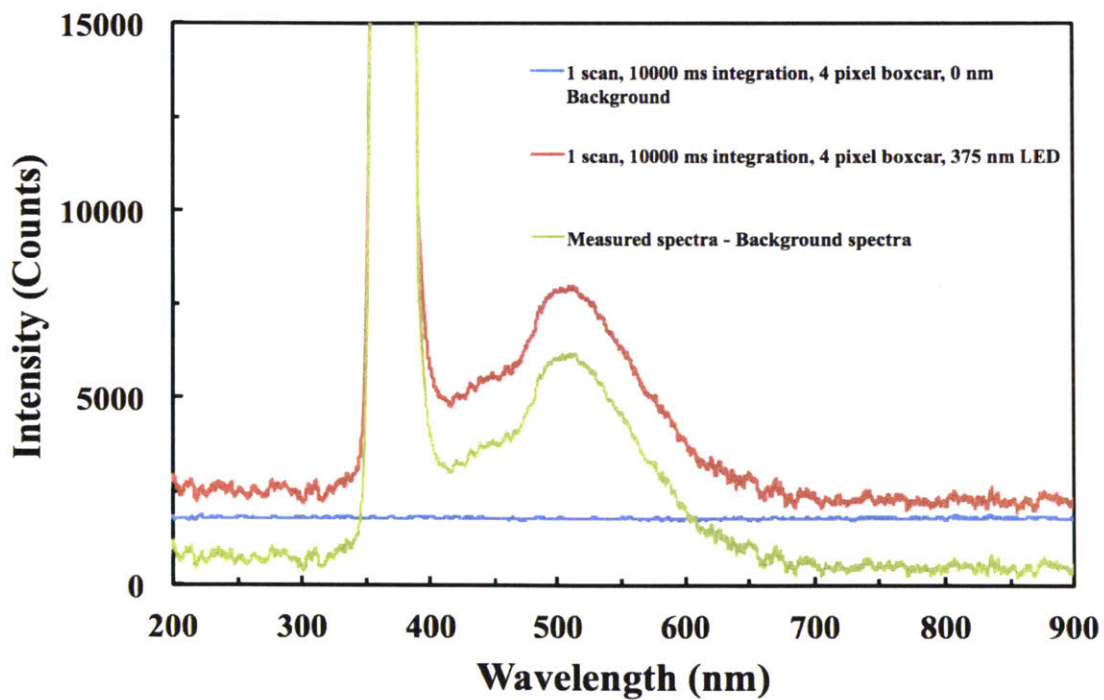
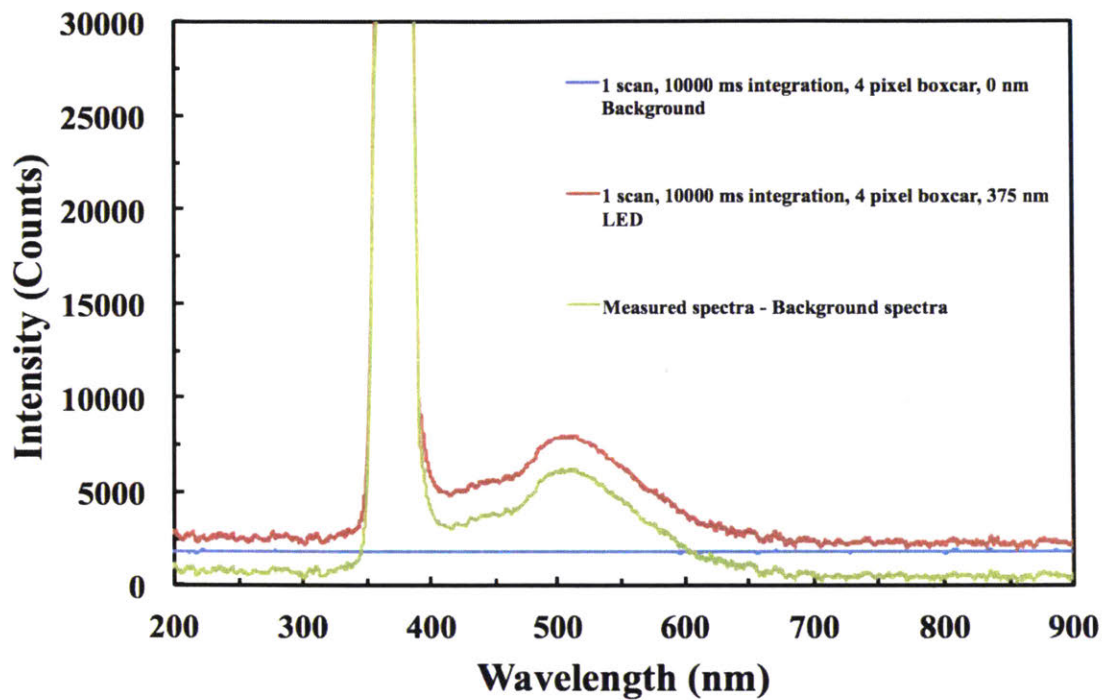


5. 30 ppm humic acid

a. Hammerhead (top: full spectrum, bottom: zoomed in)

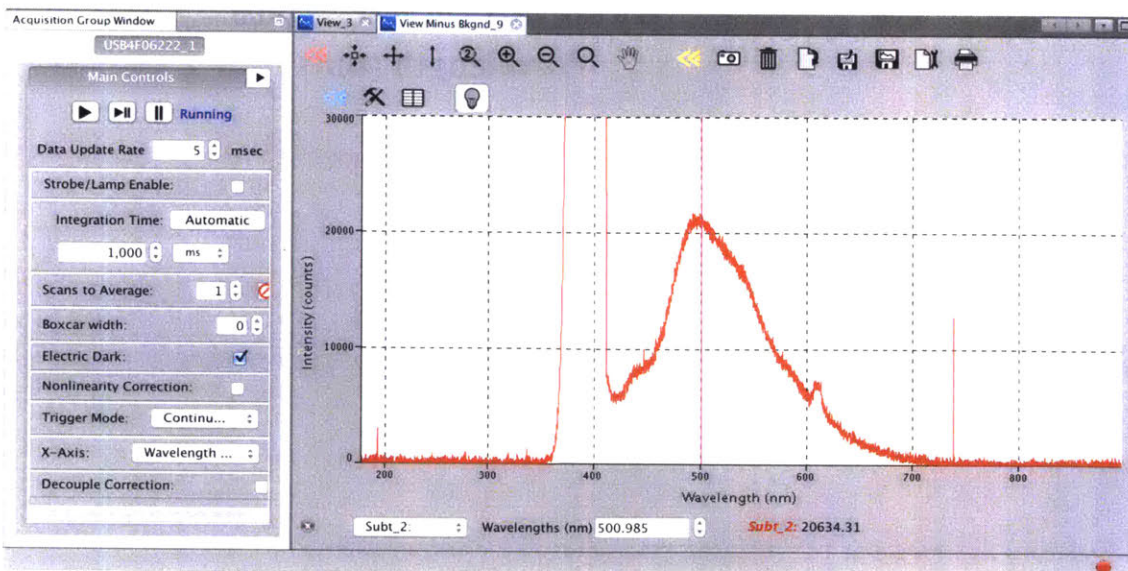
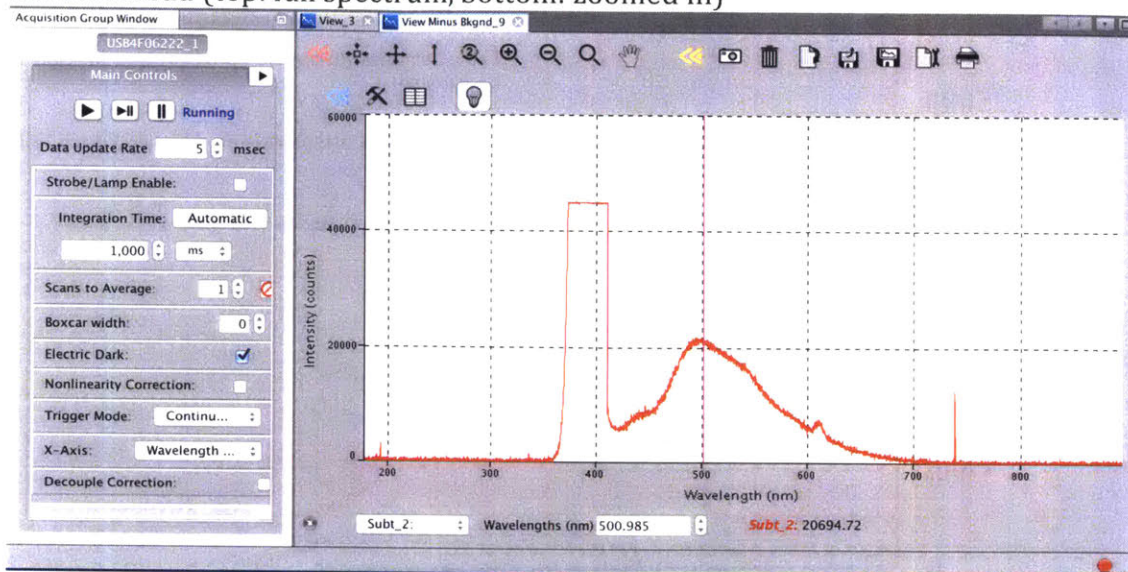


b. LEDIF with 375 nm (top: full spectrum, bottom: zoomed in)



6. 60 ppm humic acid

a. Hammerhead (top: full spectrum, bottom: zoomed in)



b. LEDIF with 375 nm

