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Spin Dynamics Studies on the Green Fluorescent Protein

A Dissertation submitted in partial satisfaction of the

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by

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December 2003

The dissertation of Lea Fredrickson is approved.

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ABSTRACT

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Spin dynamics studies were performed on the Green Fluorescent Protein. Optical excitation with circularly polarized light was used to inject spin into GFP in an organic thin film and in solution. The spin polarization of the sample was determined by measuring the polarization of the visible light emitted from the sample during electron-hole recombination. A magnetic field was applied perpendicular to the spin injection direction to verify spin polarization. While evidence of spin polarization exists in the data presented, it has not been shown using field dependence measurements. Therefore the evidence is not conclusive. It is believed that these measurements could be successful with more study.

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I. Introduction

Current electronic devices rely on charge and ignore the quantum mechanical properties such as spin and effects such as tunneling. Computer processing speeds are limited by the speed of information travel. Circuits are designed to be smaller in order to reduce the distance the data must travel, therefore increasing the data processing speed. As devices become smaller, the ability to perform functions will be degraded due to quantum effects. Researchers are now working on the development of quantum computation for data storage and processing that would use the laws of quantum mechanics instead of being hindered by them. Incorporating the spin degree of freedom into semiconductor electronics will make computer processing more stable, faster, and allow devices to become smaller and consume less power. [1, 2]

Current research in quantum computation attempts to control electrons through quantum mechanical effects, using the charge and the spin of the electrons to process information. Many promising semiconductor materials are currently being used to develop computational components. One of the important first steps in selecting a material to use for quantum computation is determining if the spin states of the electrons within the material can be manipulated. If the spin can be controlled and placed into a particular state, the lifetime of that state is of critical importance. This lifetime determines whether information can be reliably processed in the material. One of the new areas within this field that is being explored is the interface between quantum computation and organic materials. My intention was to find a protein that could hold

spin information so that it might be used as a material for quantum computation. The protein that I worked with was the Green Fluorescent Protein. Inorganic quantum computation devices have many interesting applications, but if quantum computation could be developed in organic materials a whole new realm of possible applications could be explored. One could imagine organic systems with the ability to carry out computations or that could be integrated into semiconductor electronics.

II. Theory

The spin of electrons can be manipulated using circularly polarized light. To explain this process we use both the wave and the particle properties of light. The most general equation for a homogeneous plane wave traveling in the direction **k** is as follows.

$$E(\mathbf{x},t) = (e_1 E_1 + e_2 E_2) e^{(i\mathbf{k}\bullet\mathbf{x} - iwt)}$$

Where E_1 and E_2 are orthogonal to each other and to **k.** For circularly polarized light E_1 and E_2 have the same magnitude (E_0) and have a phase difference of 90 degrees.

$$E(\mathbf{x},t) = E_0 (e_1 \pm i e_2) e^{(i\mathbf{k}\bullet\mathbf{x} - iwt)}$$

The actual electric field amplitudes are the real part of $E(\mathbf{x},t)$. For a wave traveling in the z direction:

$$E_y(\mathbf{x},t) = E_0 \cos(kz - wt)$$

$$E_x(\mathbf{x},t) = \pm E_0 \sin(kz - wt)$$

The electric field vector rotates in a circular pattern with frequency w while its magnitude remains constant. If the electric field rotates in the counterclockwise direction (looking at it coming towards you) it is called left circularly polarized and has its angular momentum pointing positively along the direction of motion. For clockwise

rotation (right circularly polarized light) the angular momentum vector is opposite to the direction of motion. [3]

Quantum mechanics tells us that angular momentum is a quantized conserved quantity. A photon is a spin one particle and therefore has angular momentum plus or minus η, depending on its polarization. An electron has spin of plus or minus $1/2\,\eta$. Because of the conservation of spin, a spin up electron can only absorb a photon of spin $-\eta$, converting it into a spin down electron, while a spin down electron can only absorb a spin $+\eta$ photon converting it to a spin up electron. If an ensemble of electrons with random spin orientations is illuminated with light that is circularly polarized in only one direction (that is to say, photons with the same spin direction), then you should be able to populate spin in this ensemble of electrons. After the electrons absorb a photon they are at a higher energy and no longer in their ground state. As the electrons relax and return to the ground state they release photons of a specific direction of circular polarization, depending on their spin in the excited state, which can be measured. In this way the spin orientation of an ensemble of electrons can be controlled and measured. The increase of spins of a certain orientation is measured by the percent spin polarization and is defined as the intensity of one helicity of emitted light minus the other divided by their sum. This has been shown to work in a variety of materials in which electrons are allowed to undergo excitation. [1, 2, 4]

The Green Fluorescent Protein (GFP) from the jellyfish *Aequorea Victoria,* is commonly used in the biological sciences as a tag on cells and molecules because it

gives off visual light after being illuminated with UV light (under the right biological conditions it autoluminesces). These proteins are what enable the jellyfish from which it was originally derived to give emit light. Below is a photograph of the jellyfish *Aequorea Victoria*. In the first picture in Figure 1, the light that shows the jellyfish is not due to GFP luminescence but is reflected light from a source by the camera. The second picture in Figure 1 is an image of what the jellyfish really looks like when its green fluorescent proteins luminesce. Only a ring towards the outside of its body lights up, the animal appears invisible.

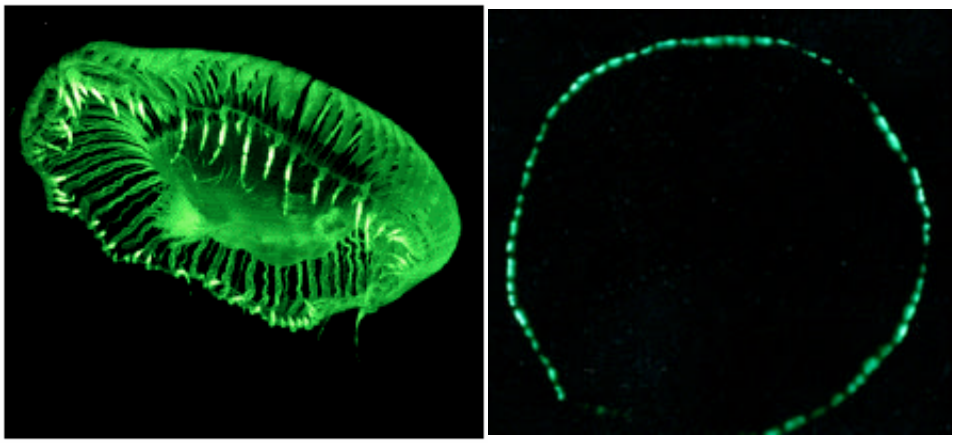

Figure 1. Two photographs of *Aequorea Victoria,* the first shows the structure of the jellyfish [5] and the other shows its luminescence [6].

The Green Fluorescent Protein folds into a cylindrical shape, which protects a central fluorophore (light emitting part of the protein) that is held rigidly within the center of the structure. This fluorophore is the part of the protein that emits light after excitation. GFP could be a good choice for optical spin dynamics studies because is very resistant to heat and pH, is already widely used in the biological sciences and is therefore very inexpensive and readily available, has a low toxicity to other organic materials, emits brightly in the visible and has been shown to have a fluorescence lifetime of 2.8ns at room temperature (Kneen M. et.al.,Swaminathan R. et.al.). Figure 2 is a diagram of the structure of GFP with a dramatization of the protein absorbing and emitting light. The cylindrical shape of the protein is modeled in this figure.

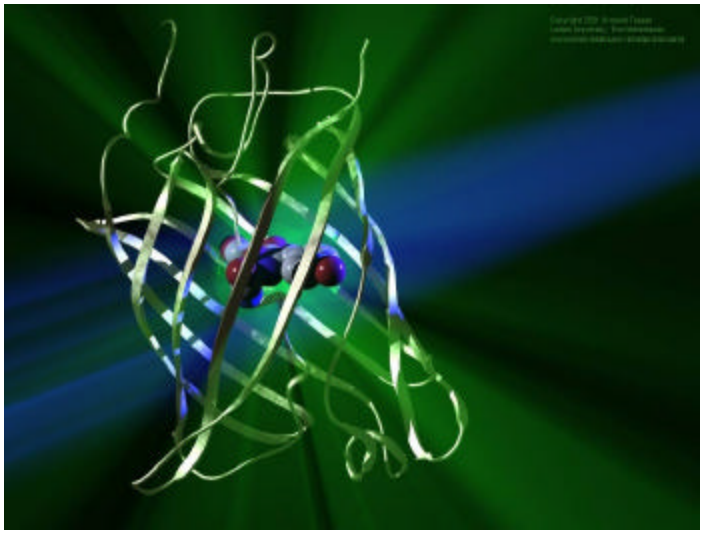

Figure 2. Representation of the structure of the Green Fluorescent Protein. [7]

The fluorophore in GFP (shown in Figure 3) originates from an internal Ser-Tyr-Gly sequence, which is post-translationally modified to a 4-(*p*-hydroxybenzylidene)-imidazolidin-5-one structure. The fluorophore itself is a p-hydroxybenzylidene-imidazolidone. It consists of residues of Ser65-*dehydro*Tyr66-Gly67 of the protein. [8]

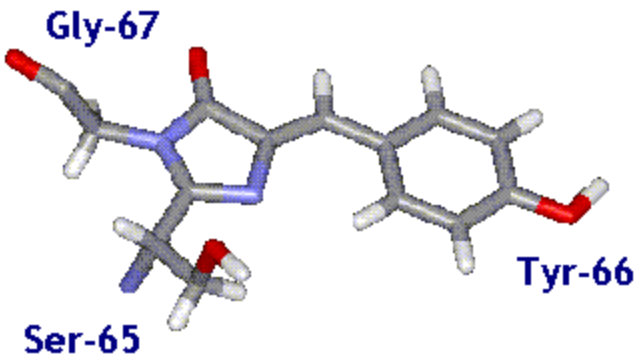

Figure 3. Structure of the fluorophore of GFP. [8]

III. Experimental Methods/Instrumentation

The Green Fluorescent Protein samples were sent to us from Professor George Rayfield at the University of Oregon. The GFP is diluted into an organic thin film that is dried. Some measurements were also done on a liquid form of the sample that was in solution.

The optical experimental apparatus was assembled on a Newport hydraulic vibration isolation optical table. Three different optical configurations were used in these studies. The first two have reflection geometry while the third is transmission geometry. Throughout the course of the measurements three different detectors were used; a Charge Coupled Device (CCD), a spectrometer, and Photo Multiplier Tube (PMT).

The cryostat used in these measurements is a Janis ST-300 model flow cryostat (diagram in Figure 4). It was cooled via a cryogenic transfer line using liquid Helium-4.

The sample mount was suspended between the poles of a GMW 3470 electromagnet, which provided the magnetic fields needed for some of the measurements. The laser used was a Coherent Mira 900. Data acquisition was done using programs written in Labview 6 and data analysis was performed in the program Origin 7.0.

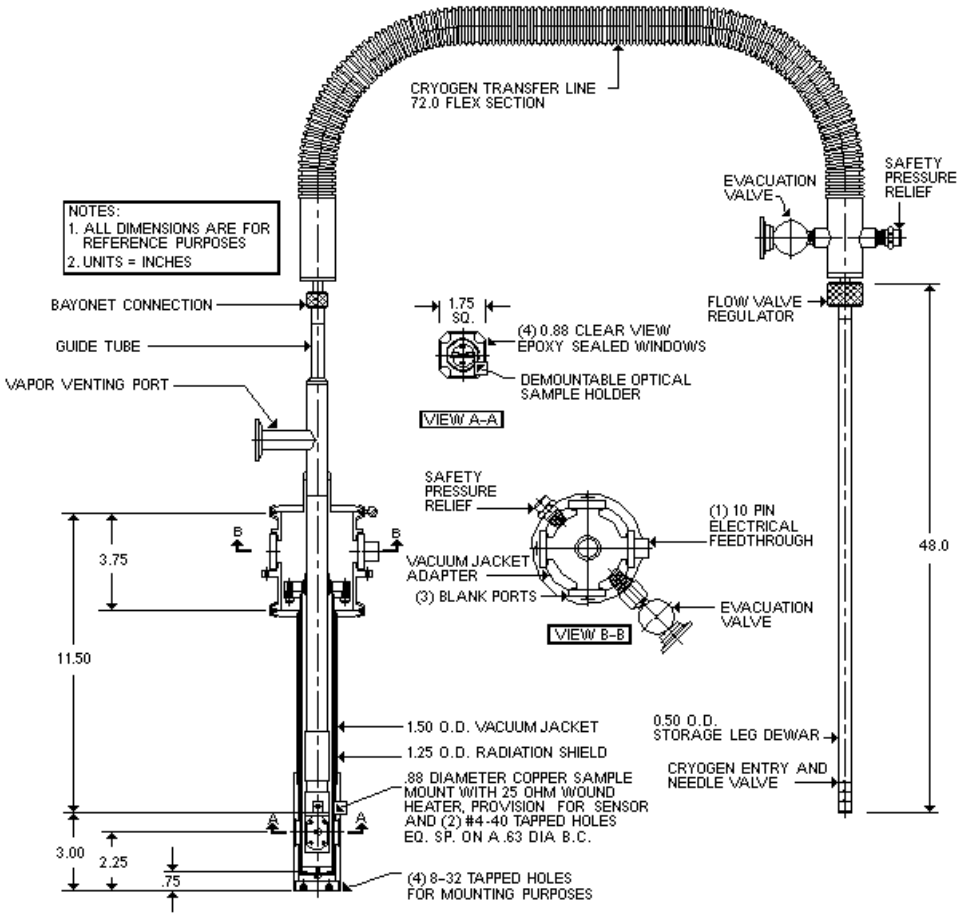

Figure 4. Diagram of the Janis ST-300 model flow cryostat used in the experiment.

The first optical configuration is shown in Figure 5. The signal is modulated with an optical chopper and a linear polarizer is used to make the polarization in the laser beam

uniform. A Soleil-Babinet compensate is then used either as a quarter or a three quarters wave plate to turn the linearly polarized light into circularly polarized light. This circularly polarized light passes through a window on the flow cryostat and illuminates the sample. The light emitted by the sample is collimated and the resulting beam is reduced in size. This light then passes through a variable wave plate that is set to act as a quarter or three quarters wave plate to turn the elliptically polarized light emitted by the sample into linearly polarized light. The light then enters a linear polarizer where only one component of the light is allowed to pass through, corresponding to only light emitted of a certain helicity. The light then enters a spectrometer where its intensity as a function of wavelength is measured. The sample is between the poles of the electromagnet such that a local magnetic field can be applied in the horizontal direction with respect to the page.

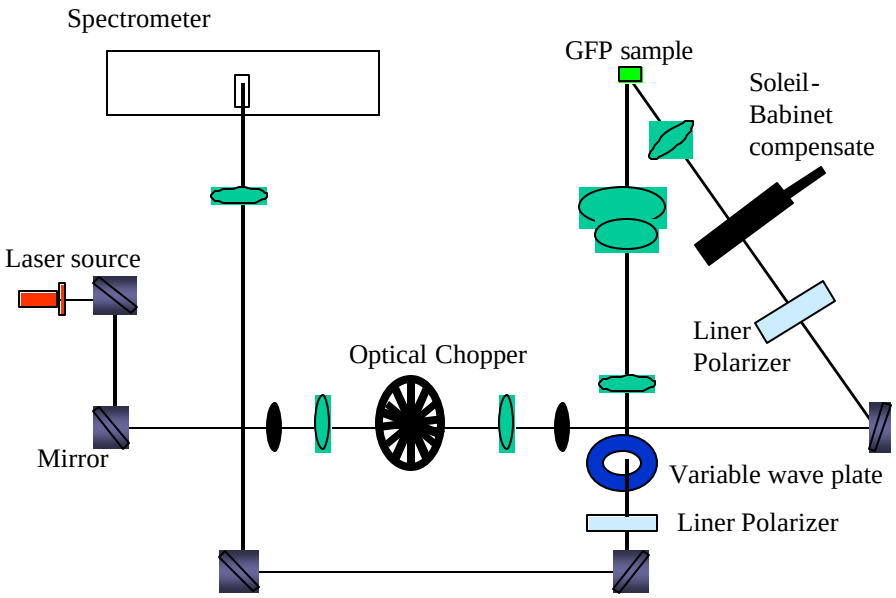

Figure 5. Optical configuration with reflection geometry and a spectrometer as the detector.

The second optical configuration used is shown in Figure 6. It also has reflection geometry, but now the collected light is focused into an optical fiber so that a variety of detectors can be used. Polarizing beam splitting cubes are used in place of the linear polarizers, though they serve the same purpose. The variable wave plate is moved into the excitation path and a Photo-Electric Modulator (PEM) is used in the collection path. This is advantageous because the PEM modulates the signal at 40 kHz, which can be measured using a lock-in amplifier to filter out a lot of noise or it can be used in conjunction with an optical chopper set to a different frequency and a second lock-in amplifier to remove even more noise from the signal. A filter was also placed in the

collection path to remove the UV light that is used to excite the sample while letting through the visible light emitted by the sample itself.

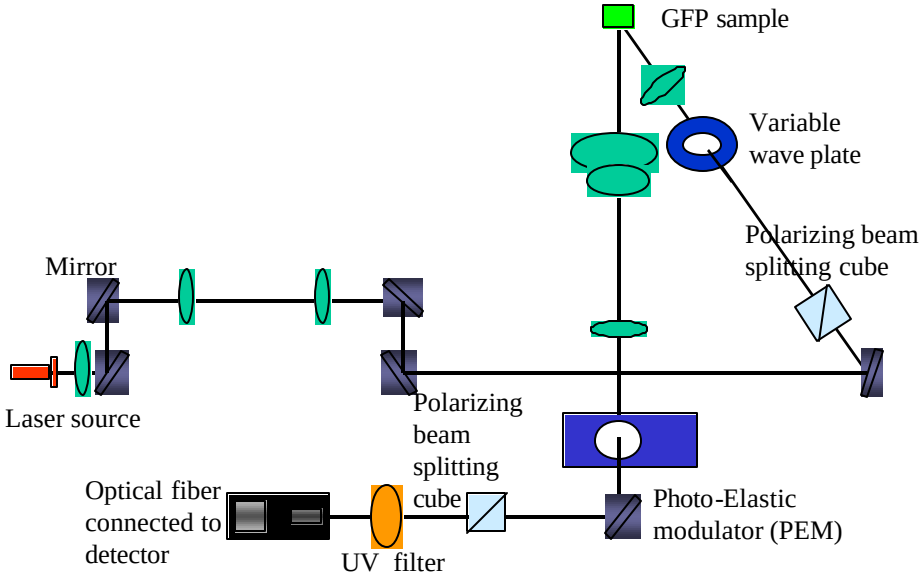

Figure 6. The second optical configuration with reflection geometry, a Photo-Elastic Modulator, UV filter and the ability to use a variety of detectors.

In Figure 7 the final optical configuration is shown. A hole was drilled into the sample stick of the flow cryostat to allow the emitted light to pass through. This configuration allows the direction of spin injection to be perpendicular to the applied field and removes the possibility of light reflecting off the sample stick (which would reverse its polarization) and exciting the sample. A filter wheel is also shown in the Figure to control light intensity on excitation.

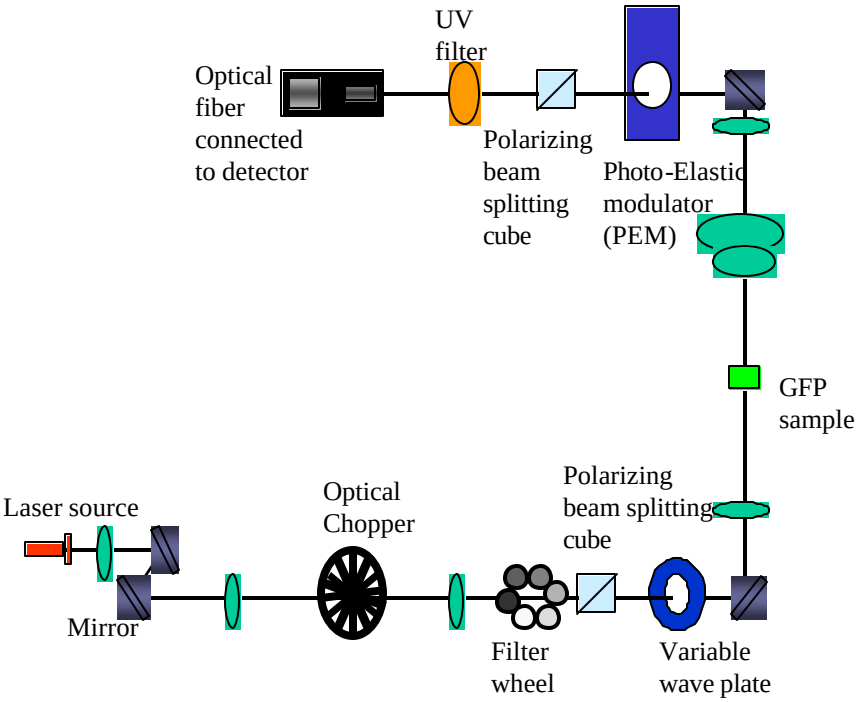

Figure 7. Optical configuration with transmission geometry.

IV. Results/Discussion

The first important measurement to perform when using a sample for optical excitation is an absorption spectra measurement to verify the wavelength at which the sample (in this case GFP) absorbs maximally. The data shown in Figure 8 is from this measurement. The absorption data was taken at room temperature using a Perkin-Elmer UV-VIS spectrometer, Lambda 20. The sample was mounted to a glass slide by a dot of Ross rubber cement on one corner of the sample.

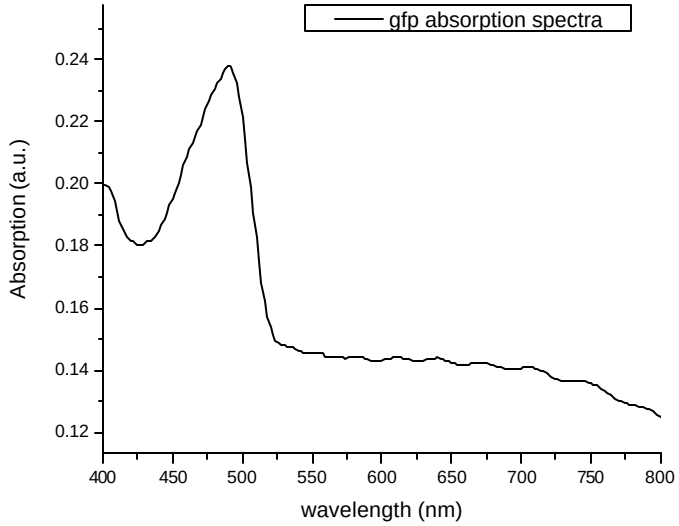

Figure 8. Absorption spectra for the Green Fluorescent Protein sample.

From the data it is apparent that the sample absorbs at a wavelength of 490 nm. From comparison with papers on GFP, this measurement confirms that the type of GFP that comprises my sample is S65TGFP, which has one main absorption peak at 490 nm and an emission peak between 508 and 515 nm. Figure 9 shows absorbance spectra for some common GFP mutants.

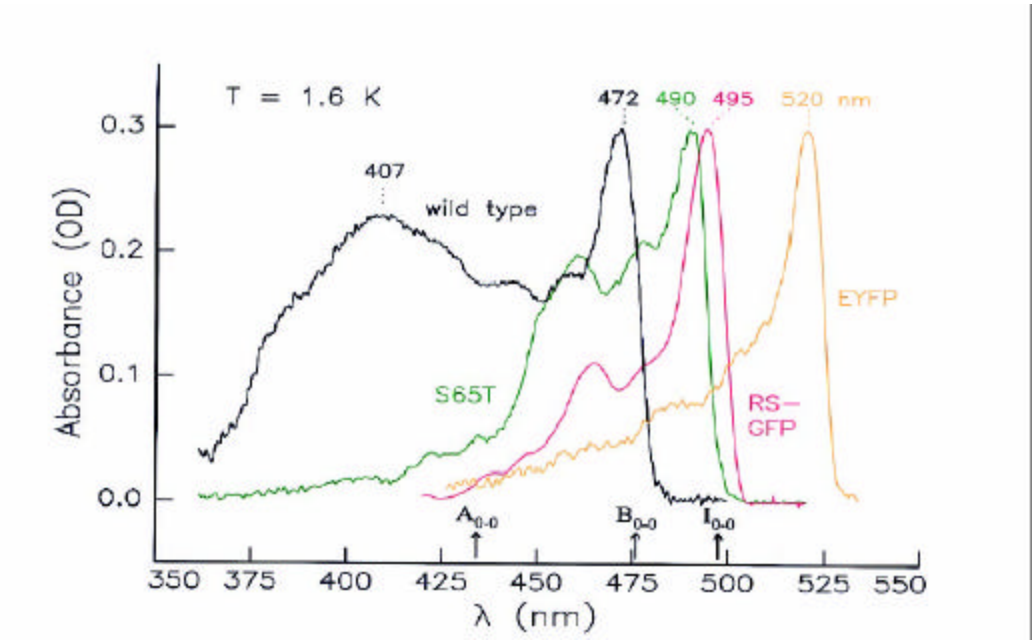

Figure 9. Absorbance spectra for a variety of GFP mutants. [9]

The next step was to do a Photo-Luminescence (PL) measurement to see if the sample does indeed emit around 511 nm and is therefore S65TGFP.

The first measurements were performed using the reflection geometry in Figure 4 with the polarization optics removed. The sample emits light isotropically, but this geometry collects only the light that is emitted towards the front of the sample. To perform this measurement the sample was excited (at room temperature) with 405 nm light and then the emission was measured with the spectrometer to see at which wavelengths the sample emits light. The sample was mounted to the sample stick of the flow cryostat using Ross brand rubber cement. Below are the results from this measurement. The emission peak is at 515 nm.

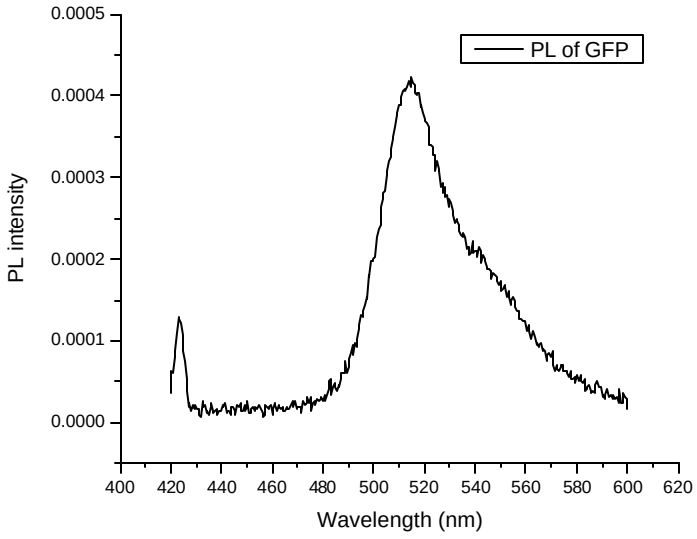

Figure 10. Emission spectra for GFP sample at room temperature.

The polarization optics were then put back into the light path (as in Figure 5) and the polarization of the light emission was measured. This measurement is similar to the PL measurement, except it is performed twice, once with the variable wave plate in the detection path set as a ¾ wave plate and once set as a ¼ wave plate. The difference between these two measurements is divided by their sum, and the result represents the spin polarization of the PL. This measurement describes the percent difference of spin up and spin down electrons excited in the sample. The data is shown in Figure 11. Either there was no spin polarization to detect or the experiment was not sensitive enough.

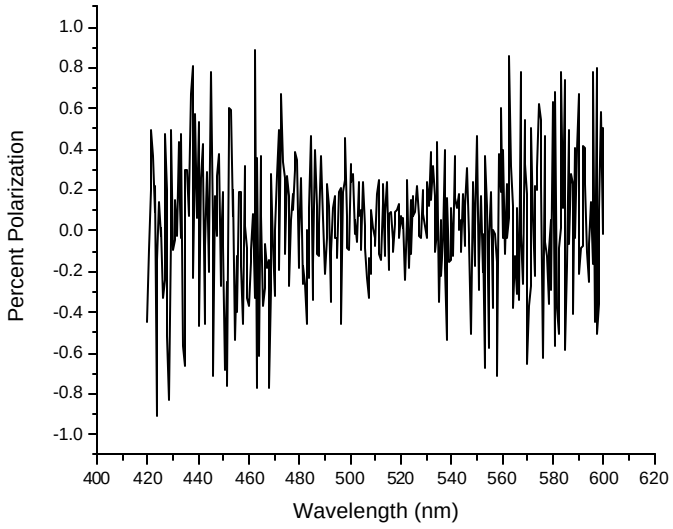

Figure 11. Percent spin polarization.

The CCD was also used as the detector at sample temperatures of 60 Kelvin and 5 Kelvin, but no polarization was detectable. The setup was then changed as in Figure 7. For the PL and Polarization data in Figures 12 and 13 respectively, the PMT measured the excitation wavelength to be 439 nm. The laser was set to 860 nm. The laser light passes through a doubling crystal, which is supposed to change the 860 nm light to 430 nm. The best signal to noise ratio was achieved with the PMT settings as follows, high voltage at 800V and the gain on the amplifier at 1nA/V. The power of the light hitting sample is 7.3 microwatts and the temperature is 5 Kelvin. The polarization measurement in Figure 13 is not normalized and shows only noise.

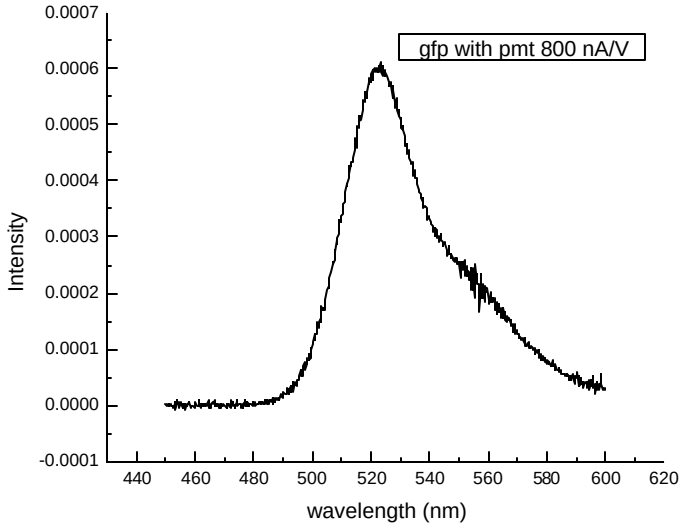

Figure 12. PL of GFP using PMT at a gain of 800 nA/V.

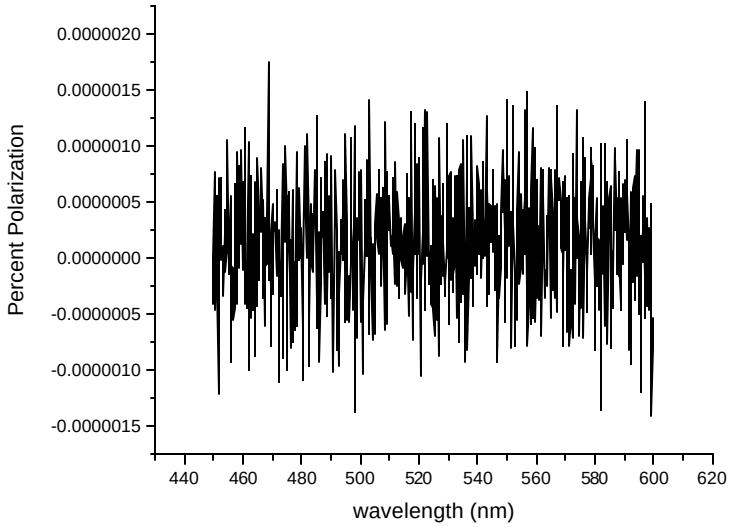

Figure 13. Polarization of GFP using PMT at a gain of 800 na/V.

In an attempt to improve the signal to noise ratio, a change was made in the collection path. The polarizing cube beam splitter was moved after the mirror to guard against the mirror changing the polarization of the signal. Figure 14 shows the PL after this change. The laser power hitting the sample is 670microwatts at a wavelength of 430 nm. The temperature is 5 Kelvin. The signal in the PL looks better but the polarization measurement taken directly after (Figure 15) showed only noise.

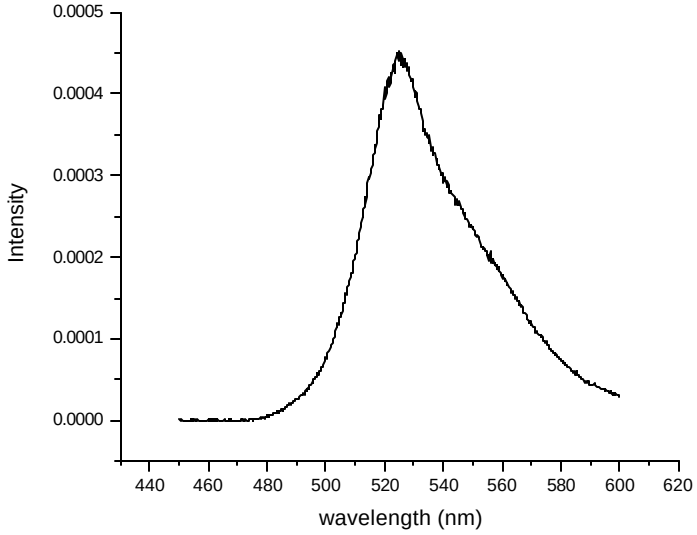

Figure 14. PL after change of polarizing cube position at 5 K.

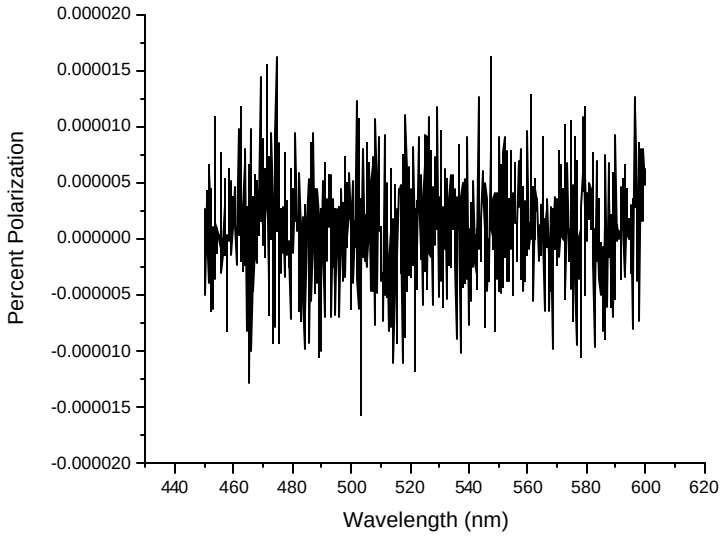

Figure 15. Polarization of GFP using one lock-in amplifier at 5 K.

To gain sensitivity a cascade lock-in amplifier technique was implemented. The signal is modulated at two different frequencies. The PEM modulates the signal at 40 kHz and an optical chopper modulates at 990Hz. The first lock-in amplifier receives the data from the PMT and picks out the signal that is modulated at 40 kHz and then sends the resultant signal to the second lock-in amplifier, which looks for a signal within this data that is modulated at 990Hz. In this way this method filters out noise better than using just one lock-in amplifier. The best signal to noise was attained with a time constant of 160μs for the first lock-in amplifier and 500ms for the second. The data acquired using this method is shown in Figure 16. There is a significant improvement in the signal, as structure can be seen. The data is not normalized.

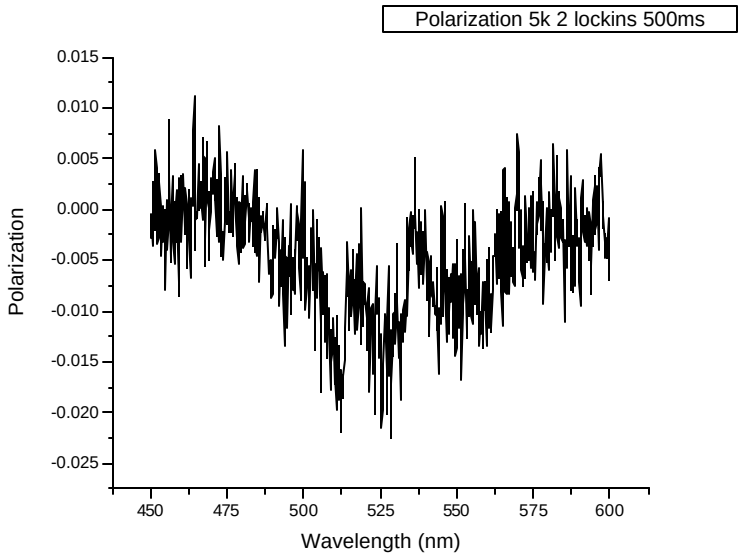

Figure 16. Polarization with cascading lock-in amplifier technique.

The polarization was then measured at different magnetic fields. Polarization measurements were carried out at three distinct fields, one at 0 Gauss, one at 620 Gauss and 1227 Gauss. The laser power hitting the sample was 680μW and the temperature was 5 Kelvin. Data was taken with two lock-in amplifiers, one modulated by the chopper (990Hz) and the other by the PEM (40 kHz). The results of these measurements appear in Figure 17. The data is not normalized because not enough information was collected to normalize it.

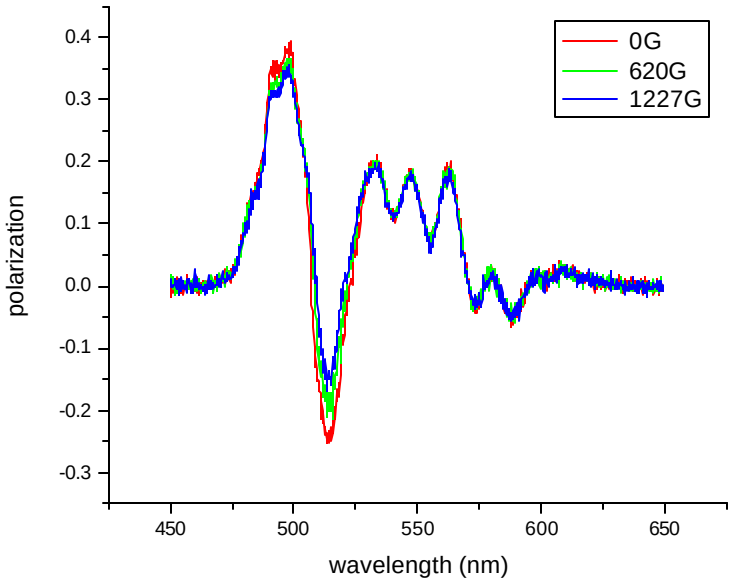

Figure 17. Non-normalized polarization curves at different external magnetic fields.

From these measurements one would expect to see decreasing polarization at increasing field due to the Hanle effect, where the spins presses perpendicular to the applied field. This causes any polarized electrons to rotate, decreasing the measured polarization. At higher fields, the spins presses faster, and therefore less polarization is measured. Although it appears that the polarization decreases with increasing field, this data is not normalized so it is impossible to tell whether the decrease is due to a change in polarization or a change in intensity. The periodic oscillations in the data caused a lot of confusion and the cause was searched for extensively.

One test to see if the polarization data really represents spin injection is to take two polarization measurements, illuminating the sample with circularly polarized light of a

different helicity each time. This should populate spin in the opposite direction in one measurement as opposed to the other. Therefore, if the polarization subtraction is performed in the same order both times, the percent polarization with one helicity should be negative of the other (assuming that the intensity of the excitation is the same for both helicities). The normalized polarization data for this type of measurement is shown in Figure 18. The black curve is the percent polarization of the sample when illuminated with linearly polarized laser light that passes through a quarter wave plate, which gives it a polarization of a certain helicity. In the red curve that same light passes through a three quarters wave plate, which gives it a polarization of the opposite helicity. As is evident in the graph, the data does flip sign under a change of excitation helicity. While this does not prove polarization, it is suggestive of its existence. This data was taken at 5K using a laser power of 190 μW incident on the sample. The scale of the polarization axis is greater than one because the intensity of the PL used for normalization was measured on a different lock-in amplifier that had different amplification settings.

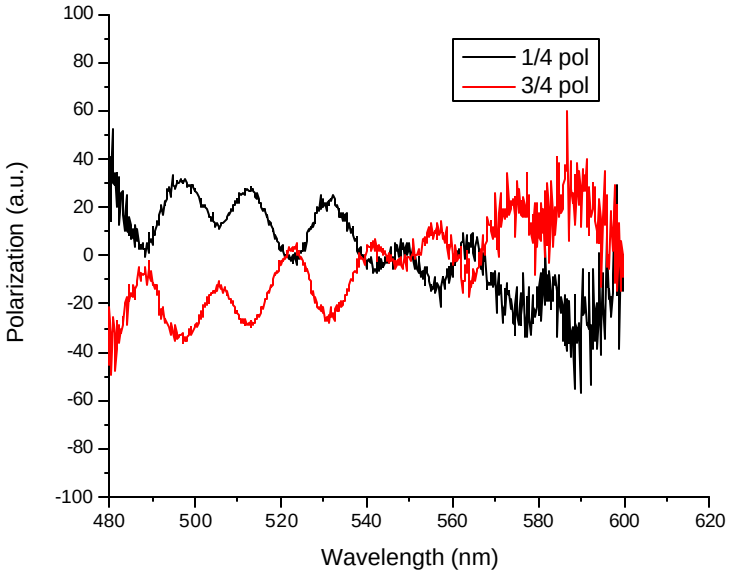

Figure 18. Percent polarization curves for different excitation helicities.

There was some concern that the periodic oscillations could be caused by some thin film effects within the sample. To test this, a liquid form of the sample in solution was measured and compared to the organic thin film data. The liquid solution was placed in a thin glass rectangular container. The results of this test are in Figure 19. The measurements were taken at room temperature (the 5 Kelvin data show was taken the day before and is there for comparison) with a laser power of 550μW. The scale is large for the same reason as Figure 18.

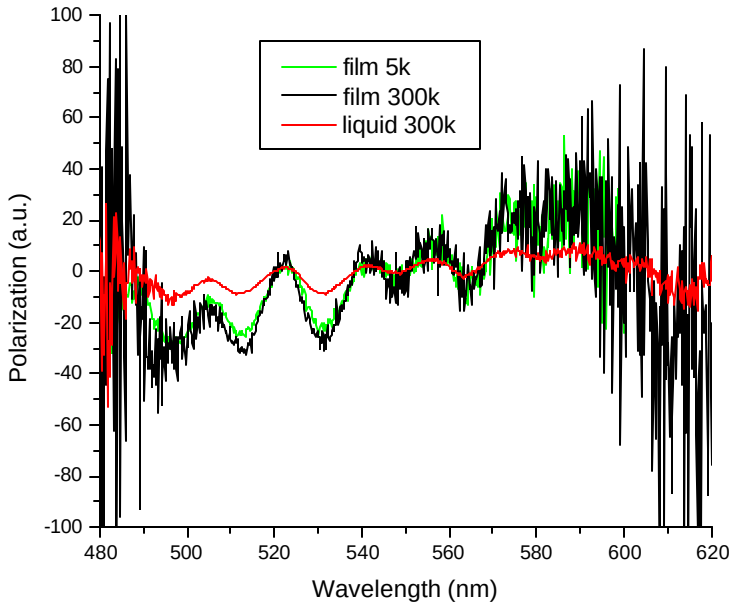

Figure 19. Polarization of liquid sample vs. thin film.

The periodic oscillations were still present in the liquid GFP data. While the signal to noise was better for the liquid sample (the liquid container was thicker than the film therefore much more material was excited optically), there also appeared to be less polarization present in the data. If this data really does represent polarization, then there should be less polarization in the liquid sample because the individual proteins in solution have more freedom to rotate than in a dry thin film. If this rotation time at room temperature is not long enough to be irrelevant when compared to the relaxation time of the polarized electrons, then this causes spin decoherence and the emitted light has a lower percent polarization.

The cause of the periodic oscillations was finally discovered. The UV mirror used in the collection path had a reflectivity that changed in a periodic way with respect to wavelength. After the mirror was removed the measurement in Figure 18 was repeated. The results are shown in Figure 20. Laser power is 1.4mW and temperature is 5 K.

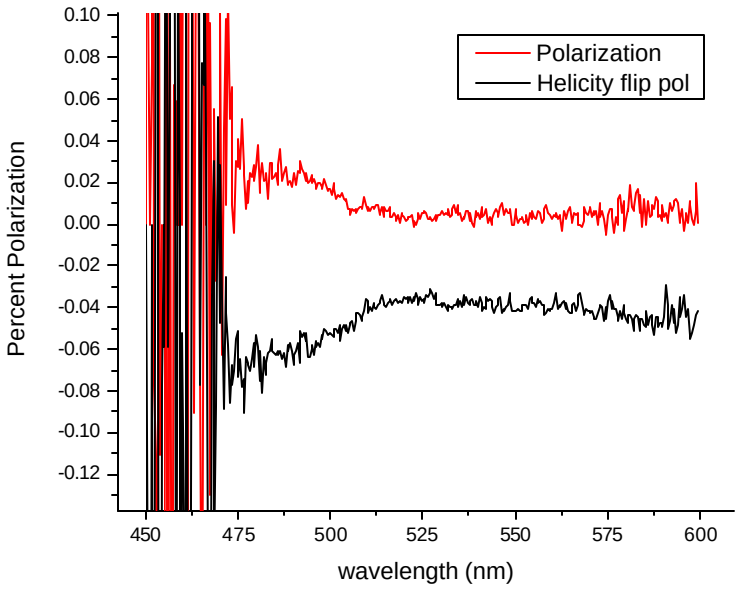

Figure 20. Helicity comparison of polarization with mirror removed.

A change in helicity of the excitation still reversed the sign of the polarization. The absolute values of the amplitudes of the curves are different which could be due to a difference in intensity of the excitation helicities. Laser power is 1.4mW and temperature is 5 K.

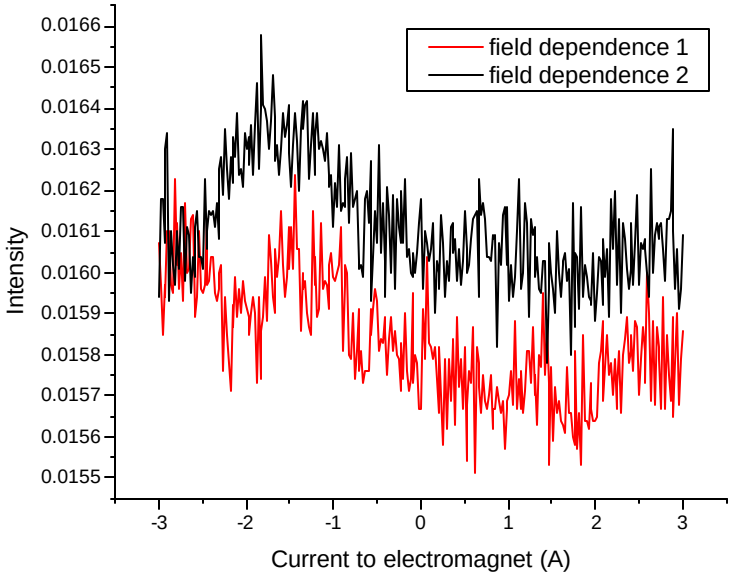

Figure 21. Field dependence of polarization at a wavelength of 495 nm.

An external magnetic field of magnitude 1200 Gauss was applied to see what effect it would have on the polarization. Figure 22 shows that polarization decreased with an applied field. The laser power in these measurements was 1.4mW and the temperature was 5 K.

This data was not reproduced, and might be due to a change in intensity instead of actual polarization. There is some evidence of spin polarization, but it is not very convincing for the following reason. Accurate magnetic field dependence measurement is the best evidence for spin polarization. This measurement did not yield meaningful data; therefore one cannot rule out the possibility that the percent polarization curves were due to something other that spin polarization.

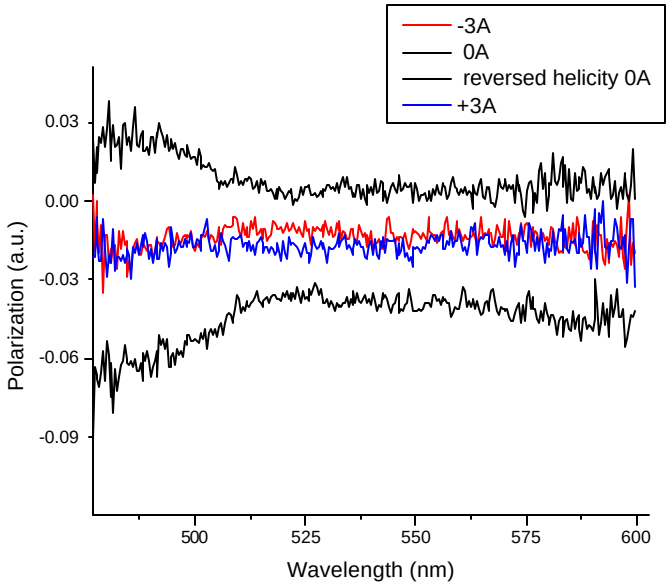

Figure 22. Decrease in polarization at 1220 Gauss.

Figure 23 shows the intensities of the left and right circularly polarized light measured at 1200 Gauss magnetic field on the same graph for comparison. This was a repeatable measurement, and it does appear that there is a difference in the intensities of the polarizations measured. This data suggests that there was spin polarization.

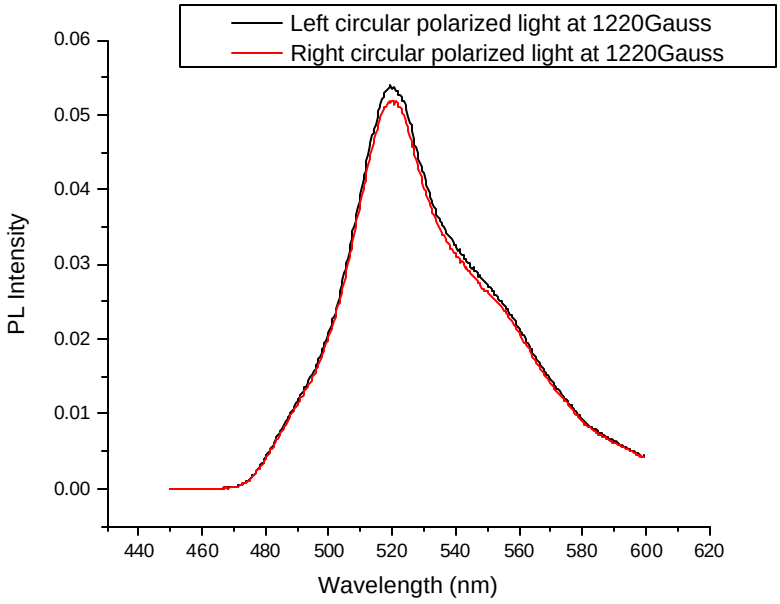

Figure 23. Difference between left and right circularly polarized light at 1200 Gauss.

V. Conclusion

In the results from the experiments on spin dynamics on the Green Fluorescent Protein there is some evidence to suggest polarization, but it is not conclusive. Field dependence data that shows evidence of the Hanle effect is needed to say that spin polarization exists. I believe that these measurements should work. A.W. Knight et. al. measured the polarization of GFP emission and used it to distinguish the light emitted by GFP from that by fluorescein, which emits light at a similar wavelength. Others have measured a fluorescence lifetime of 2.8ns for S65TGFP. [8] I believe that these

measurements could be improved with better control over laser light intensity, more attention to detail in the measurements and possibly being able to apply higher magnetic fields. With more careful study I believe that one can show spin polarization in GFP.

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