The effects of red, blue, and green light wavelengths on the population growth of the photoautotrophic *Chlamydomonas reinhardtii*

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Bench 5 | L14

Abstract

The goal of this experiment is to see the photosynthetic efficiency of *Chlamydomonas reinhardtii* under different wavelengths of light, in relation to the types of lights they experience in nature. Cultures of *C. reinhardtii* were put under varying wavelengths including red, blue, green and fluorescent light to determine if the differing wavelengths influence their population growth. The cultures were first diluted, arranged in their specific light conditions with four replicates each, and placed in a temperature-controlled room of 17°C throughout a 15-day period. Cell counts were taken on days 3, 6, 7, 8, 10 and 15. Once all of the data was collected, a one-way ANOVA test was conducted which yielded a p-value of 0.003 indicating the results were statistically significant. Subsequently, a Tukey-Kramer post-hoc analysis was conducted on both data including and excluding the control group due to a skew in data. All experimental conditions were found statistically different than the control group but not from each other, however after removing the control group, the blue condition was statistically different than both the red and the green condition but the red and green were not statistically different from each other.

Introduction

Chlamydomonas reinhardtii is a unicellular flagellate in the genus of green algae, normally found in stagnant water and in freshwater or damp soil. It is used as a model organism in many

experiments due to its flagellar motility, dynamic chloroplasts, and the ease of manipulation of genetics. *C. reinhardtii* is also known to produce biopharmaceuticals and biofuel, as well as being a valuable research tool in making hydrogen (Tamburic et al., 2004).

C. reinhardtii is an important source of food at the base of the food chain for various aquatic species, specifically salmon. Therefore, insight into the growth of *C. reinhardtii* under certain light conditions could determine the fluctuations of salmon populations in different aquatic environments. (Kindle et al., 1990). Once salmon reach the full term of their life cycle and decompose, they will release nitrogen, which helps facilitate algae growth. Hence, salmon and *C. reinhardtii* have a mutualistic symbiotic relationship as both species benefit from the other. Salmon also help control algae blooms by feeding on algae, which have an adverse effect on the surrounding environment and can be toxic and harmful to the other organisms in the area.

One of the known features of the *C. reinhardtii* is its ion channels, which are activated by light. When illuminated, *C. reinhardtii* can grow photoautotrophically through photosynthesis, but can also grow in the dark when supplied with organic carbon (Blanket et al, 2013). Because photosynthetic efficiency varies with different wavelengths of light when under the same light intensity, in an effort to find the optimized growth conditions for *C. reinhardtii* to achieve maximum cell density, we will test whether changes in wavelength (red, green, and blue) will affect *C. reinhardtii* growth. This, in turn, will determine the available food source for the salmon in the surrounding environment.

Our null and alternative hypotheses are:

H_0: Different wavelengths of light will have no effect on the population growth of the *C. reinhardtii*.

H_A: Different wavelengths of light will have an effect on the population growth of *C. reinhardtii.*

An experiment conducted by Munzner and Voigt (1992) proposed that a delay in cell division was induced by blue light, but not by illumination with red or far-red light. It was found that *C. reinhardtii* experienced delayed division under blue light as compared to illumination under the red light, since they grew in size as biomass accumulated but multiplied slower than the smaller cells under the red light. Thus, we predict that *C. reinhardtii* will have a larger cell density count under longer wavelengths of light (red light) than under the blue light over time. In this experiment, we will test whether a difference in wavelength will affect cell density of the *C. reinhardtii* under constant light intensity and temperature conditions.

Methods and Materials

I. Initial *C. reinhardtii* stock preparation

Upon receiving the previously cultured *C. reinhardtii* stock, a sterile environment was created with a thorough wipe using ethanol on the surface counter, as well as an open ethanol flame throughout the entire process of transferring the stock. Once a sterile environment was created, an initial cell count was conducted by pipetting 10ul of the *C. reinhardtii* culture (thoroughly mixed by swirling the apparatus and pipetting up and down) and placing the amount between a

coverslip and haemocytometer, which was then looked at using a compound light microscope. The haemocytometer has two counting chambers with nine large 1mm squares on each (*Figure 1*), allowing for an easier way to determine the number of Chlamydomonas cells in a specific volume of fluid. The cell concentration was determined with this equation:

$$cell\ density/mL = (\#\ cells\ counted / \#\ of\ squares) * (Dilution\ factor\ of\ square\ size) * ((Correction\ for\ fixative)$$

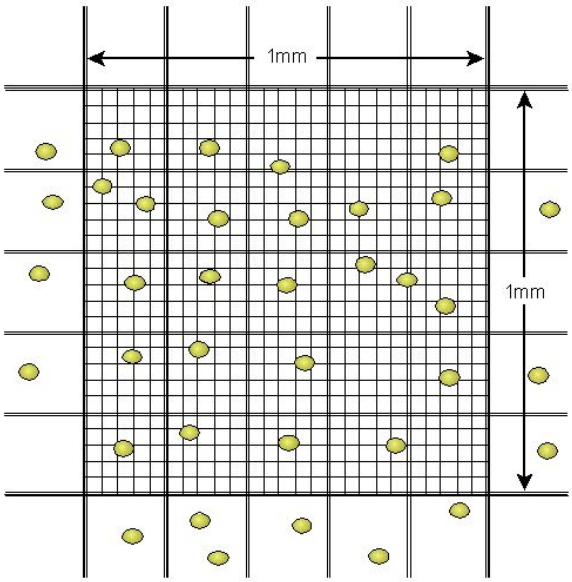

Figure 1. Haemocytometer 5x5 1mm (middle square) grid where initial cell counts were taken. This is only one square out of nine on the haemocytometer. The cell counts were 127, 101, and 111, making the average of the three counts 113. Therefore, our dilution factor is 1 x 10^{-4}. Since 10 uL IKI fixative was added to 100 uL of culture, we also have to multiply by 1.1 to correct it.

It was then calculated that if the desired volume of the diluted stock was 200mL, then 8.05mL of original *Chlamydomonas reinhardtii* stock and 191.95mL of the growth media was required.

Thus, the cell density (cells/mL) after correcting for the fixative added was $1.24x10^6$ cells/mL, making this the final concentration of the diluted stock.

II. Experimental Setup

Once the diluted stock was thoroughly mixed, 5mL was transferred into each of the 16x 6ml test tubes, resulting in 4 replicates for each of the red (~650nm), blue (~490nm), green (~560nm), and fluorescent (~400-700nm) light conditions. To test the different wavelengths, red , blue, and green acetate filters were used to cover the entire test tube, discluding the fluorescent light condition which was left completely uncovered. These test tubes were labelled by lab section, bench number, colour, and replicate number (ex. "L14 B5 R4"). Using a light meter, the light intensity was measured in lux for each condition with the resulting intensities of red at 31 lux, green at 60 lux, blue at 27 lux, and fluorescent light at 1650 lux. In a test tube rack, all the tubes were spread out evenly to minimize the effects of shadowing, which could affect the amount of light intensity each sample replicate is receiving. To ensure that the temperature remained constant, the test tubes were incubated in a temperature stable room of 17 degrees celsius.

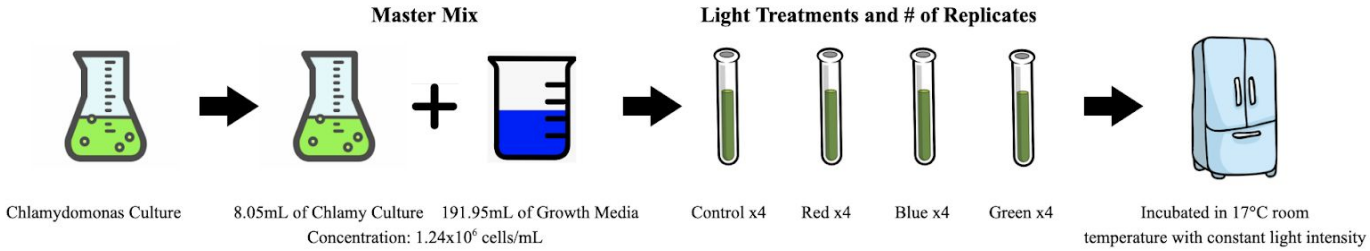

Figure 2. Experimental procedure for diluting the initial stock to desired concentration of $1.24x10^6$ cell/mL and transferring 5mL to 16 x 6mL test tubes. Placed in incubator of 17°C and under fluorescent light intensity of 1650 lux.

III. Count day procedures

Throughout the experiment, we made 6 counts in accordance to when the samples would hit an exponential phase of growth. This was noticed on count day 3 for the fluorescent light samples and decided to condense the number of times we counted during that phase.

For each count day, 16 eppendorf tubes were labelled with count number, red/blue/green/control, and replicate number (ex. 3 R1). Then, each sample test tube was thoroughly mixed with a micropipette and 100ul of each was placed into their corresponding eppendorf tubes. 10ul of IKI fixative was then added and mixed. Immediately after, the samples test tubes were brought back to the temperature-controlled room to maintain the intended temperature of 17°C. Cell count was then performed using 10ul of the sample and IKI mixtures pipetted onto the haemocytometer. With the haemocytometer, we went through each square aiming to hit a cell count of 100 or more and noted how many squares it took to reach that number (even if all 9 squares were counted and 100 cell counts weren't reached). As for the compound microscope, Kohler illumination is performed to ensure an even illumination of the samples and adjusting the microscope to phase 3 helped distinguish the organisms. 3 counts were taken of each replicate to obtain an average for every light wavelength. For each day of sample collection, there were 48 counts in total. Once all counts were made, we placed the eppendorf tubes in the fridge (~4°C) to ensure the samples were preserved. These steps were repeated for day 3, 6, 7, 8, 10, and 15. After counting cells using the haemocytometer, cell densities were calculated and adjusted for fixative added. A more visual representation of the procedures are shown in *Figure 3*.

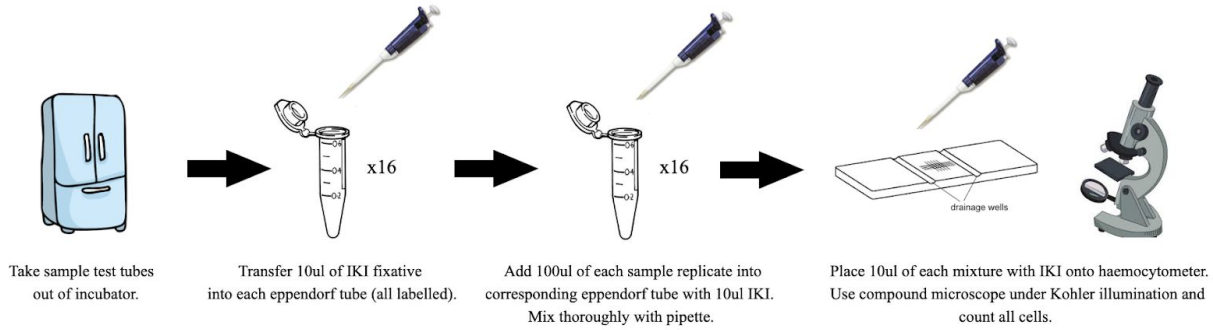

Figure 3. On the days of counting the samples, the procedure to follow is to take samples out of incubator, transfer 10ul of IKI fixative into 16 labelled eppendorf tubes, then 100ul of each sample into corresponding eppendorf tubes, and lastly pipetting 10ul of the mixture onto a haemocytometer. Compound microscope is under Kohler illumination.

Results

The averages of cell densities (cells/mL) were taken for each wavelength of light (red, blue, green, and control) and plotted on a graph using GraphPad Prism software (Figure 4). A log transformation was performed due to a skew in the control sample. After 2 weeks, it was found that the control group had reached its exponential growth phase, however, all 3 other experimental conditions had not. When comparing the experimental conditions, there was slightly greater total growth under blue light than under either green or red light. Figure 5 shows total growth of *C. reinhardtii* (final cell density - initial cell density). The greatest overall growth occurred under the control condition, followed by blue, then red light with almost no notable growth under the green light condition. There was an increase in variation of cell count as the experiment progressed.

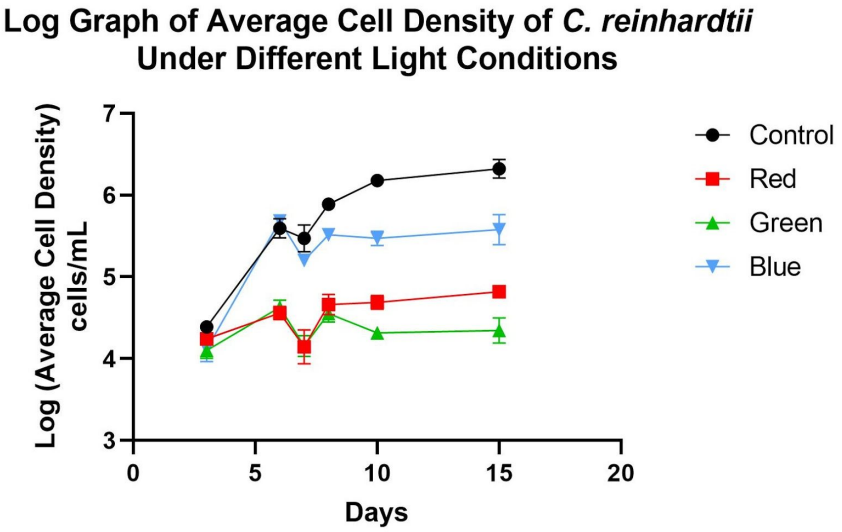

Figure 4. Log graph of the average cell density of C. reinhardtii under different light conditions over a 15 day time interval. Error bars represent the 95% confidence intervals.

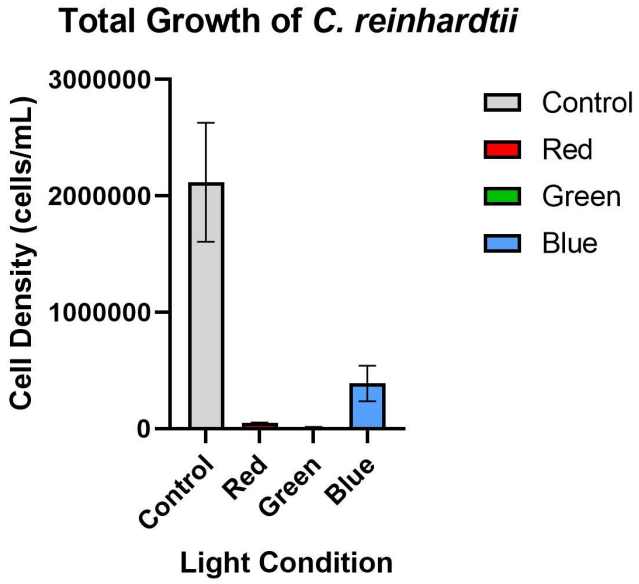

Figure 5. Total growth (final cell density - initial cell density) of C. reinhardtii under different light conditions. Initial cell density was taken on November 1, 2019 and final cell density was taken on November 13, 2019. Error bars represent the 95% confidence interval. One way ANOVA test

yielded a p-value of 0.0001 and 0.003 (with and without control, respectively).

Two one-way ANOVA statistical analyses were conducted on the total growth of *C. reinhardtii*, one including the control condition and one without due to the skew of exponential growth found in the control. Further analysis was done through subsequent Tukey-Kramer on each ANOVA test that compared each pair conditions. Both ANOVA tests produced a p-value of less than 0.05 (p-value = 0.0001 with control and p-value = 0.003 without control). When comparing the differences between conditions using the Tukey-Kramer test, all of the means compared against the control condition were found to be statistically different. All tests comparing the experimental conditions (red vs. green, red vs. blue, green vs. blue) were not statistically different. When the Tukey-Kramer test conducted without the control, all of the means compared the blue condition were found to be statistically different whereas the test comparing the red and green conditions was not.

Discussion

A one-way ANOVA test showed that there is a significant difference between the four groups. A subsequent Tukey-Kramer test determined that the control group was statistically different from the three experimental groups. When examining the total growth of all groups, the control group displays significantly higher growth than the treatment groups as it was the only group to reach the exponential growth phase. We suspect this is due to a large difference in light intensity between the control group and experimental groups. A study conducted by Bonente et al. (2011) showed that *C. reinhardtii* growth rate is positively correlated with light intensity. The control group's light intensity was measured to be 1650 lux compared to 31, 60 and 27 lux for the red,

green and blue groups respectively. We believe that this difference in light intensity may have skewed our results; however, the light intensities of the three experimental groups are similar and still allow for meaningful comparisons to be made between them. For the purpose of better understanding the effect of light wavelength on cell growth decided to perform a second set of statistical tests without the control group data.

A second ANOVA test performed on just the experimental groups produced a different ($P = 0.003$) but still significant P-value. The subsequent Tukey-Kramer test determined that the blue light treatment condition was statistically different from the green light and red light groups. Thus, we reject the null hypothesis that light wavelength has no effect on the growth of *C. reinhardtii* and lend support to the alternative hypothesis that light wavelength does affect the growth of *C. reinhardtii*. However, the results disagree with the initial prediction that C. reinhardtii population growth would be greatest under red light. We discovered that blue light conditions yielded the highest total growth by a significant margin over red and green light conditions. This unexpected result goes against the findings of Munzner et al. (1992) which suggest that continuous blue light delays cell division, thus resulting in a lower growth rate, while the opposite is true for red light.

The results of this experiment were inconsistent with those of similar experiments performed on *C. reinhardtii.* However, it has been discovered that blue light enhances the activity of carbonic anhydrase (CA) - an enzyme that plays an important role in the photosynthesis of green algae in

carbon dioxide limiting environments (Dionisio et al., 1989). CA was found to be part of a mechanism that delivers CO2 to Rubisco during photosynthesis. (Gee et al., 2017)
Another theory is that due to the short wavelength of blue light, it was able to deliver more energy to the organisms than red or green light. Photosynthetic organisms are affected by both brightness and wavelength, therefore the difference in wavelength may have been enough to demonstrate a difference in growth rate between the three experimental groups. (Johkan et al., 2010)

Due to lab constraints, the cultures were grown at a suboptimal temperature of 17° C. *C. reinhardtii* optimal growth temperature is 20°C – 25°C. This resulted in slower growth of all groups and may have prevented the experimental groups from reaching the exponential phase within the duration of the experiment. Had the experimental groups reached the level of growth expected, we may have observed different overall trends and a more definitive result. Correcting for this in the future would allow for increased validity and power of conclusions in future studies. Another limitation was the small sample size. More replicates would increase the reliability of the results.

In addition to the aforementioned limitations there were some sources of error that should be accounted for in future experimental designs. The main potential source of error is that we did not control for the light intensity. In the future all groups should have similar light intensity that is at least of the same order of magnitude. To achieve this, a better control group design would have been to wrap the control test tubes in layers of cheesecloth until the lux reading reaches a

similar level to the other three groups. There may also have been discrepancies in counting, as some members may have been more lenient with their conditions for including a cell in their count. This could be avoided by having one person do all the counting, or by having multiple people count the same sample and then taking an average. Finally, the cultures may not have been mixed well enough before a sample was taken for counting, therefore the cells were unevenly distributed. This may explain why the cell count on November 5 was lower than November 4 for all groups but increased again on November 6, as seen in Figure 4.

During the cell counts, varying sizes of cells were present and made it difficult to decide which ones to count or not. Our main goal was to count the bright green coloured cells and ignore the deformed or small ones out of the counts. However, we must consider the factor of the abnormal shapes and sizes of the particles and take that into account as a limitation in our experiment.

Conclusion

Based on the results of this study, we are able to reject the null hypothesis that the population growth of *C. reinhardtii* does not change under different wavelengths of light. However, due to a skew in the light intensity of the control condition, wavelength appeared to negatively affect the growth of *C. reinhardtii.* Our results found that *C. reinhardtii* grew best under blue light conditions contrary to existing literature, but this could have been due to an increase in energy obtained from the shorter, blue wavelengths to compensate for lower light intensity in experimental conditions. Future studies should attempt to limit the sources of error and test the optimal growth conditions between different light intensities and wavelengths of light.

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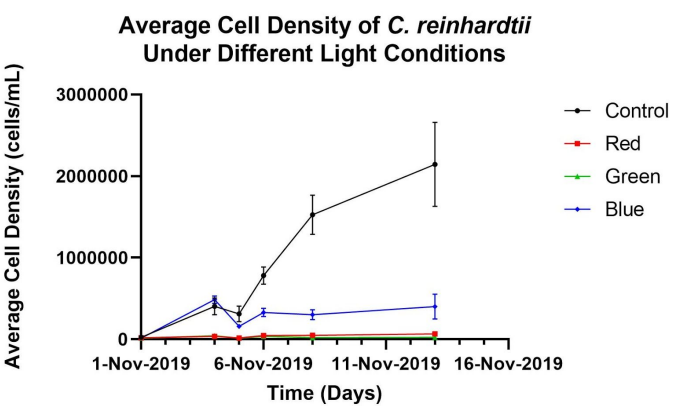
Appendix

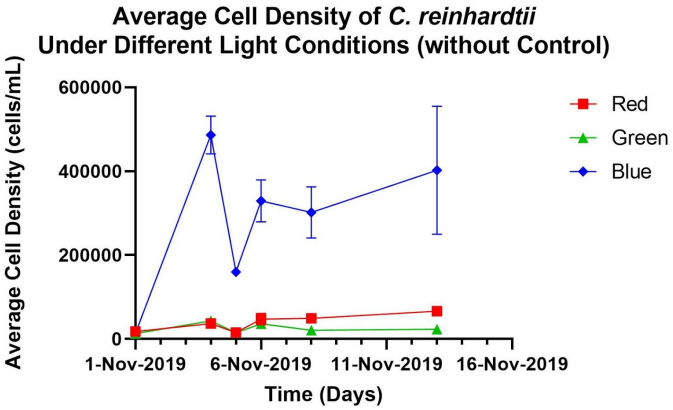
Equations

$$cell\ density/mL\ =\ (\#\ cells\ counted\ /\ \#\ of\ squares) * (Dilution\ factor\ of\ square\ size)\ *\ (Correction\ for\ fixative)$$

Graphs

Prior to applying log function





Raw Data

Total Cells/mL (corrected for fixative added) - Control												
Replicate	**1**			**2**			**3**			**4**		
Count #	1	2	3	1	2	3	1	2	3	1	2	3
Nov. 1	3.85E+04	2.64E+04	2.97E+04	2.42E+04	2.31E+04	1.65E+04	1.98E+04	1.76E+04	2.75E+04	2.86E+04	2.20E+04	2.42E+04

Nov. 4	3.33E+05	1.91E+05	3.11E+05	4.00E+05	2.53E+05	4.95E+05	6.22E+05	4.29E+05	5.28E+05	3.63E+05	4.29E+05	4.95E+05
Nov. 5	1.99E+05	2.01E+05	1.02E+05	3.58E+05	3.63E+05	3.38E+05	3.19E+05	3.92E+05	3.80E+05	3.44E+05	4.11E+05	3.19E+05
Nov. 6	7.10E+05	6.05E+05	6.55E+05	6.99E+05	7.32E+05	8.47E+05	1.32E+06	4.99E+05	5.83E+05	7.81E+05	8.36E+05	1.11E+06
Nov. 8	1.21E+06	1.51E+06	9.90E+05	1.63E+06	2.02E+06	1.74E+06	1.11E+06	1.52E+06	1.71E+06	1.67E+06	1.71E+06	1.50E+06
Nov. 13	2.71E+06	2.73E+06	2.59E+06	2.48E+06	2.02E+06	1.84E+06	1.22E+06	1.73E+06	1.40E+06	2.42E+06	2.34E+06	2.24E+06
Nov. 14	3.30E+06	2.81E+06	3.71E+06	7.15E+06	6.27E+06	6.60E+06	1.69E+07	1.11E+07	8.20E+06	4.95E+06	2.75E+06	2.81E+06

Total Cells/mL (corrected for fixative added) - Red												
Replicate	**1**			**2**			**3**			**4**		
Count #	1	2	3	1	2	3	1	2	3	1	2	3
Nov. 1	1.87E+04	1.54E+04	9.90E+03	1.10E+04	1.76E+04	2.64E+04	2.75E+04	1.54E+04	1.43E+04	1.32E+04	2.20E+04	1.87E+04
Nov. 4	2.09E	2.97E	3.19E	4.40E	3.30E	4.51E	3.63E	3.41E	3.96E	3.41E	3.74E	5.17E

	+04	+04	+04	+04	+04	+04	+04	+04	+04	+04	+04	+04
Nov. 5	2.64E +04	1.54E +04	1.21E +04	1.87E +04	2.09E +04	2.20E +04	5.50E +03	7.70E +03	7.70E +03	1.32E +04	1.43E +04	1.76E +04
Nov. 6	2.42E +04	3.85E +04	3.41E +04	4.07E +04	4.51E +04	4.29E +04	7.92E +04	7.04E +04	4.51E +04	5.72E +04	4.73E +04	4.18E +04
Nov. 8	4.07E +04	3.63E +04	4.18E +04	4.51E +04	3.96E +04	6.16E +04	5.94E +04	6.82E +04	6.05E +04	3.63E +04	5.94E +04	4.40E +04
Nov. 13	5.94E +04	6.27E +04	4.73E +04	8.47E +04	6.60E +04	6.38E +04	6.49E +04	5.94E +04	7.70E +04	7.81E +04	6.71E +04	6.27E +04
Nov. 14	4.73E +04	3.52E +04	5.28E +04	2.42E +04	3.30E +04	3.96E +04	3.85E +04	4.18E +04	5.06E +04	4.84E +04	2.86E +04	4.73E +04

Total Cells/mL (corrected for fixative added) - Green												
Replicate	**1**			**2**			**3**			**4**		
Count #	1	2	3	1	2	3	1	2	3	1	2	3
Nov. 1	7.70E +03	1.43E +04	7.70E +03	1.54E +04	1.54E +04	1.32E +04	1.98E +04	1.65E +04	1.10E +04	1.32E +04	9.90E +03	9.90E +03
Nov. 4	6.27E +04	5.50E +04	4.95E +04	4.84E +04	2.20E +04	3.08E +04	4.07E +04	4.51E +04	3.96E +04	4.40E +04	3.52E +04	4.73E +04
Nov. 5	1.21E	1.54E	5.50E	1.21E	1.65E	1.21E	1.65E	1.76E	3.08E	1.54E	1.10E	1.21E

	+04	+04	+03	+04	+04	+04	+04	+04	+04	+04	+04	+04
Nov. 6	4.40E +04	3.19E +04	3.85E +04	3.08E +04	3.08E +04	3.85E +04	3.52E +04	2.31E +04	2.20E +04	4.29E +04	5.17E +04	4.73E +04
Nov. 8	0.00E +00	0.00E +00	0.00E +00	0.00E +00	0.00E +00	0.00E +00	0.00E +00	0.00E +00	0.00E +00	0.00E +00	0.00E +00	0.00E +00
Nov. 13	2.42E +04	1.76E +04	2.86E +04	2.42E +04	1.87E +04	2.75E +04	2.64E +04	3.85E +04	3.08E +04	1.43E +04	1.54E +04	1.10E +04
Nov. 14	2.64E +04	3.52E +04	4.62E +04	2.42E +04	1.87E +04	2.97E +04	2.86E +04	1.87E +04	1.10E +04	1.76E +04	1.21E +04	1.76E +04

Total Cells/mL (corrected for fixative added) - Blue												
Replicate	**1**			**2**			**3**			**4**		
Count #	1	2	3	1	2	3	1	2	3	1	2	3
Nov. 1	1.87E +04	1.98E +04	1.43E +04	1.43E +04	2.64E +04	1.32E +04	7.70E +03	7.70E +03	1.54E +04	7.70E +03	6.60E +03	1.32E +04
Nov. 4	5.28E +05	5.50E +05	3.52E +05	3.96E +05	4.07E +05	5.28E +05	4.29E +05	6.49E +05	5.72E +05	5.06E +05	5.94E +05	3.19E +05
Nov. 5	1.87E	1.98E	1.32E	1.54E	1.87E	1.32E	1.43E	8.80E	2.09E	1.21E	1.98E	1.65E

	+05	+05	+05	+05	+05	+05	+05	+04	+05	+05	+05	+05
Nov. 6	2.53E +05	1.98E +05	3.30E +05	3.63E +05	3.41E +05	2.97E +05	3.52E +05	3.52E +05	4.29E +05	3.19E +05	3.74E +05	3.41E +05
Nov. 8	1.76E +05	2.53E +05	3.74E +05	2.31E +05	2.64E +05	2.09E +05	4.73E +05	3.52E +05	2.75E +05	3.52E +05	3.41E +05	3.19E +05
Nov. 13	3.08E +05	4.73E +05	2.75E +05	4.62E +05	4.84E +05	4.73E +05	2.86E +05	1.76E +05	1.87E +05	4.73E +05	7.26E +05	5.06E +05
Nov. 14	3.30E +05	2.97E +05	3.96E +05	2.20E +05	3.74E +05	3.30E +05	2.20E +05	2.31E +05	1.98E +05	5.06E +05	5.83E +05	4.40E +05