Short Cycle Adaptation in the Circadian Rhythm of Arabidopsis

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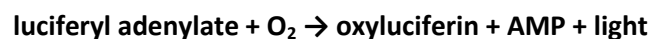
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**Abstract**

Bioillumination from a luciferase [+] transgenic mutant of Arabidopsis was used to assess protein production in plants illuminated by red LED light at decreasing cycle length to view adaptation. A charged coupled device was used to measure and determine the circadian rhythm by measuring the illumination output of the plants. The amount of illumination produced was found to be correlated with the length of the day/night cycle given to the plants indicating that the circadian rhythm is light dependant and capable of undergoing cycles shorter than natural.

**Introduction**

The use of bioillumination through the Luciferase Reporter System is commonly used to study gene expression and is outlined in "The Use of the Luciferase Reporter System for in Planta Gene Expression Studies" By Wessel Van Leeuwen of the Wageningen Univerisity. Bioillumination in Arabidopsis involves the introduction of the gene coding for luciferase production from Photinus pyralis, the North American firefly through the use of an agrobacterium. The gene is inserted into the chromosomal DNA of the Arabidopsis and expresses at levels that are correlated with expression levels of proteins on the same locus within the DNA. From this correlation the amount of luciferase can be taken to represent the amount of natural protein being produced at a given time. Bioluminescence works through the decarboxilation of a substrate luciferine that is coupled with the release of a photon of about 662 nm in wavelength. ATP is also required in this reaction but is assumed to be in excess in healthy growing plants. The reaction is as follows:

**luciferin + ATP → luciferyl adenylate + PP$_i$**

**luciferyl adenylate + O$_2$ → oxyluciferin + AMP + light**

Experiments to measure expression levels are set up such that the protein being produced is the limiting reagent in the reaction so that for every protein being produced a photon is released. Any

number of methods to measure this output can be used. In this case a charged coupled device will be used to measure photon output.

As discussed in "Real-Time Reporting of Circadian-Regulated Gene Expression by Luciferase Imaging in Plants and Mammalian Cells," by David K Welsh of the Kay laboratories at UCSD, protein expression in Arabidopsis is guided by a circadian rhythm that undergoes cycles in even in complete light conditions. In this experiment different lighting conditions were used to witness plant adaptation. 12 hour day/ 12 hour night cycles were used as a standard to view "natural" cycles and 6 hour day/6 hour night cycles were used to view adaptation.

**Materials and Methods**
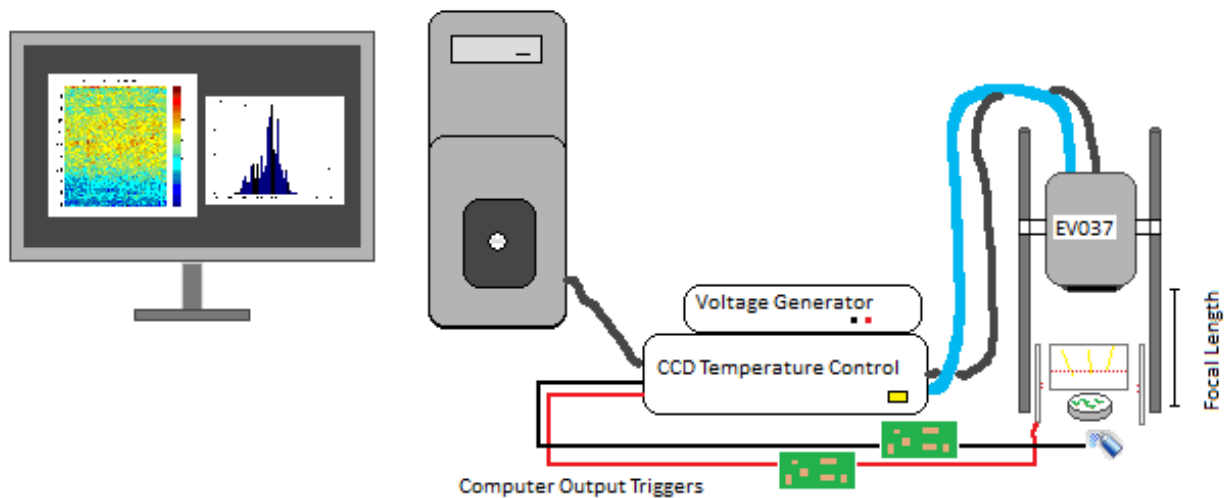


**Figure 1:** Experimental setup used to quantify the amount of bioluminescence from the plants.

Figure one shows the general setup used in this experiment. The experiment involves a EV037 CCD that is set to take images every two hours at a 20 minute exposure time. The CCD is cooled to its minimum temperature to reduce black body radiation which may increase noise in the data. Output triggers from the camera control both the luciferine delivery and lighting in the experiment. The output triggers are set such that 30 seconds before imaging a spray of ~.25 ml of .5mM luciferine is delivered to

the plants. Luciferine is kept in dark conditions suspended in 1% Triton X solution since the luciferine is light sensitive. Triton X serves as a weak detergent which disrupts the cell wall of the Arabidopsis allowing luciferine to be directly absorbed into the plant. The Arabidopsis were seeded in sterilized nutrient agar and grown for approximately three weeks before experiments were conducted. Lighting must remain off while imaging but otherwise lighting cycles using 40 red LED lights were used to provide day cycles of 12 and 6 hours. Resulting images are shown in results.

**Results and Data Analysis**

Data taken with no plants present showed pixels with non random/higher illumination indicating the presence of "hot spots," or in this case lines, in the CCD used for this experiment. As discussed in "Real-Time Reporting of Circadian-Regulated Gene Expression by Luciferase Imaging in Plants and Mammalian Cells" this is common for CCDs and can be removed through a number of data analysis methods as discussed later.
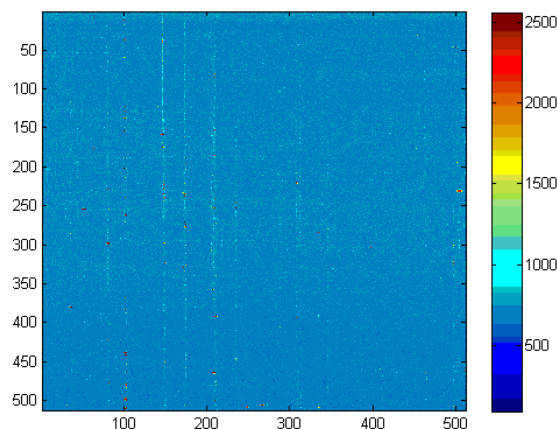


**Figure 2:** 20 minute exposure with no plant present. Note the consistent lines of hot spots along with the large variation in saturation displayed on the color bar.

Data was taken with Arabidopsis for three 4 days with 12 hour day 12 hour night cycles and the mean image is shown below.
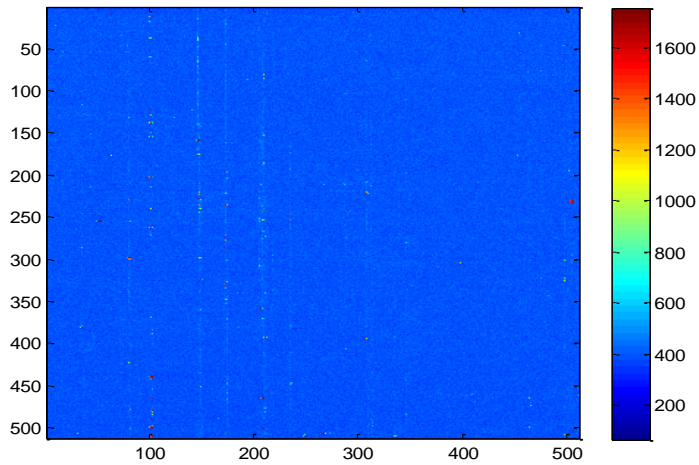
**Figure 3:** Shows the mean image of 4 days of measurements with a 12 hour cycle. Note that because the

hot spots force the color bar to be of a wide range it is impossible to see smaller variances within the

picture.

Using a filter based on higher variance, helps remove unwanted pixels.
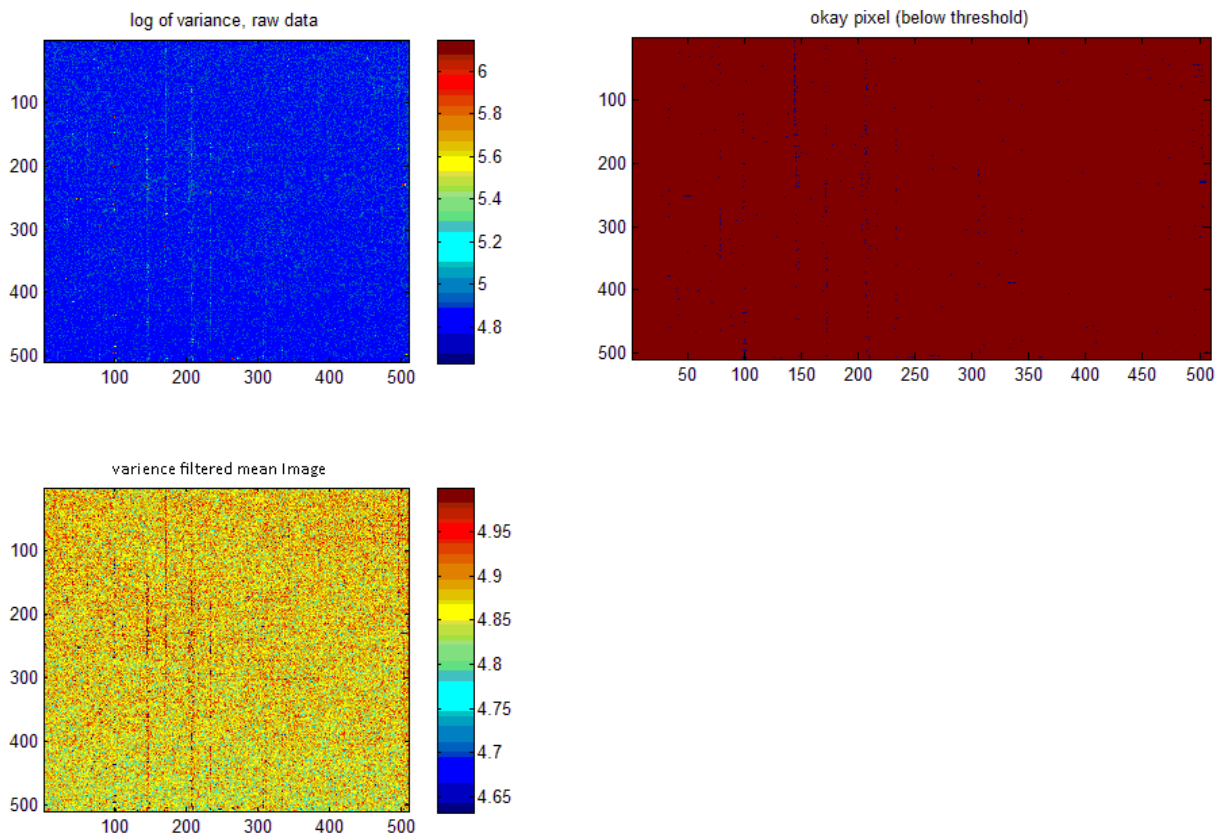
**Figure 4**: Variance filtering. Shows the log of the variance of each pixel which allows pixels that varies above a certain threshold to be removed. In this case the threshold of a variance of about 5 was used and pixels with a variance above this were replaced with the median value. Bottom shows the new mean image after the variance filter has been applied. Note the change in the scale of the colorbar such that a wider smaller range of variances is represented.

Once invalid pixels have been removed a technique known has singular value decomposition (SVD) can was used to remove orthogonal modes which contain noise or do not contain any signal. This technique factorizes the data as matrices to pick out a number of orthogonal modes equal to the number of pictures taken and represents them along with their intensity over time. In this way shapes or patterns that vary together will remain in the same mode. The separation into the first 15 modes of highest intensity is shown in figure 5.
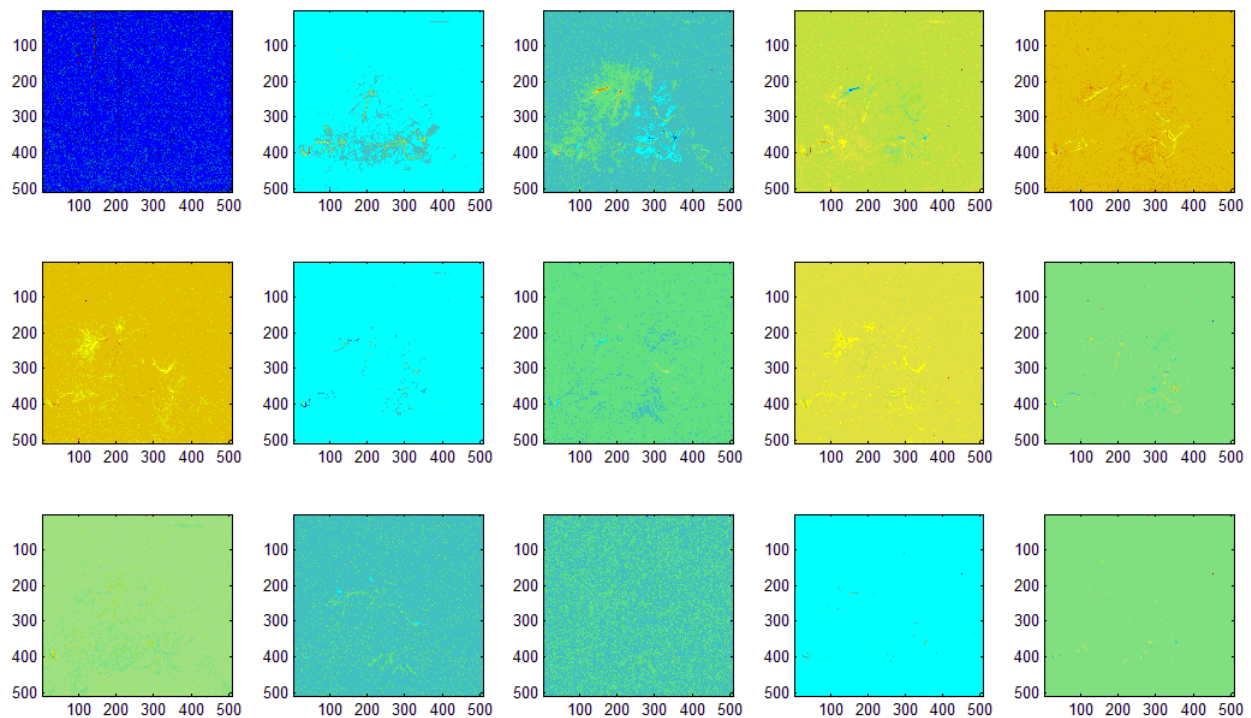


**Figure 5:** The first 15 modes of the first SVD decomposition. Note the similarities between the first mode and noise data and the presence of plant signal in modes 2 through 10.

Plotting the intensities of these modes shows the relative impact each mode has on the final image. We would expect to see much higher intensity for the first mode as it clearly dominates the original mean image and a decrease in intensity after mode 10 because the loss of any signal from plants after that mode.
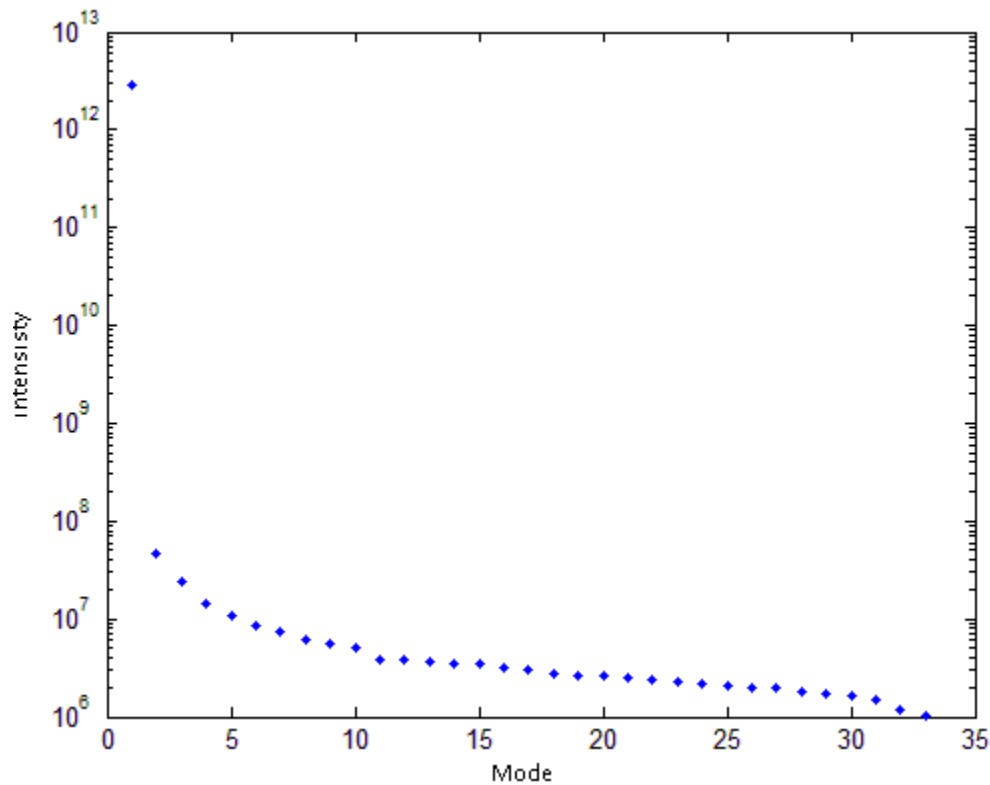


**Figure 6:** SVD mode intensity. Note the drastic drop after the first mode and the slight drop after mode 10. This indicates that the first mode is of the greatest impact on the final mean image and is clearly nose data as shown by figure 5. A drop from mode 10 to mode 11 is indicative of the loss of the signal from that mode on.

Based on the SVD images in figure 5 and the intensity images in figure 6 a final image will be the selection of modes 2-10. Recombining images 2-10 and reverting them back to normal notation results in a mean image as shown in figure 7.
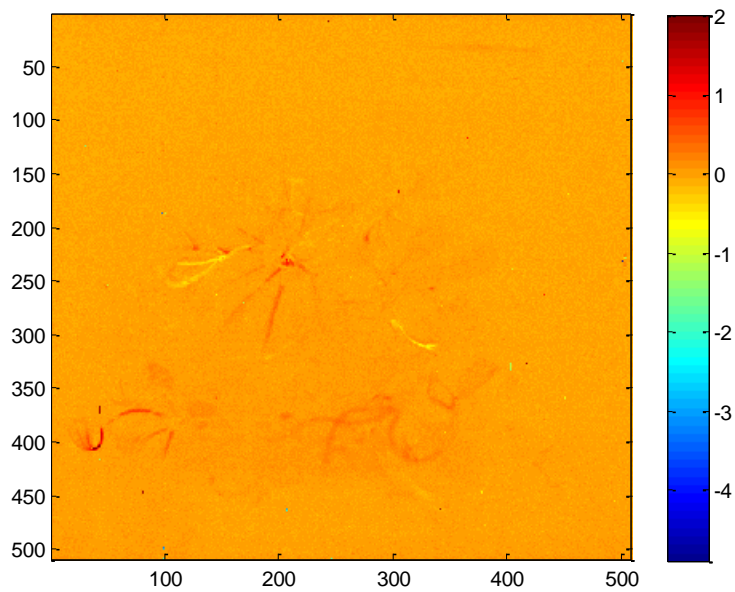
**Figure 7:** Mean image after SDV analysis and variance filtering. Note the presence of only a few large

plants and their stems.

From this a single plant can be selected and the mean intensity over time can be plotted as

shown in Figure 8.
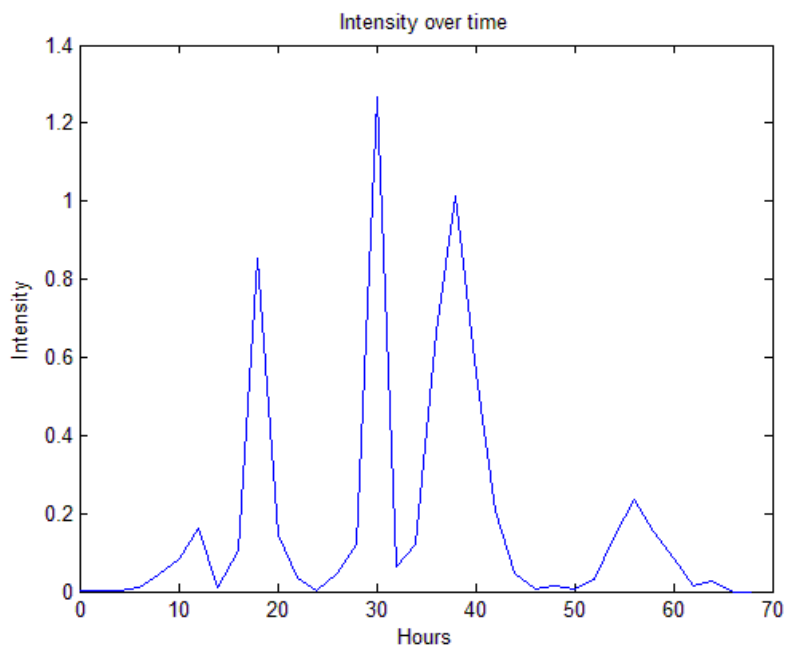
**Figure 8:** Circadian rhythm for a 12 hour day 12 hour night cycle.

Spectral analysis using Cronux allows multiple tapering windows to be used to correct for data that does not continue in both directions however in this case using only one window gives the best results. The frequency we are trying to pull out of this data set is large relative to the amount of data that was taken and therefore trying to resolve frequency out with a larger bandwidth results in large error and loss of signal. Figure 9 shows this Fourier transform conducted using cronux representing the different frequencies represented in the data and their intensities.
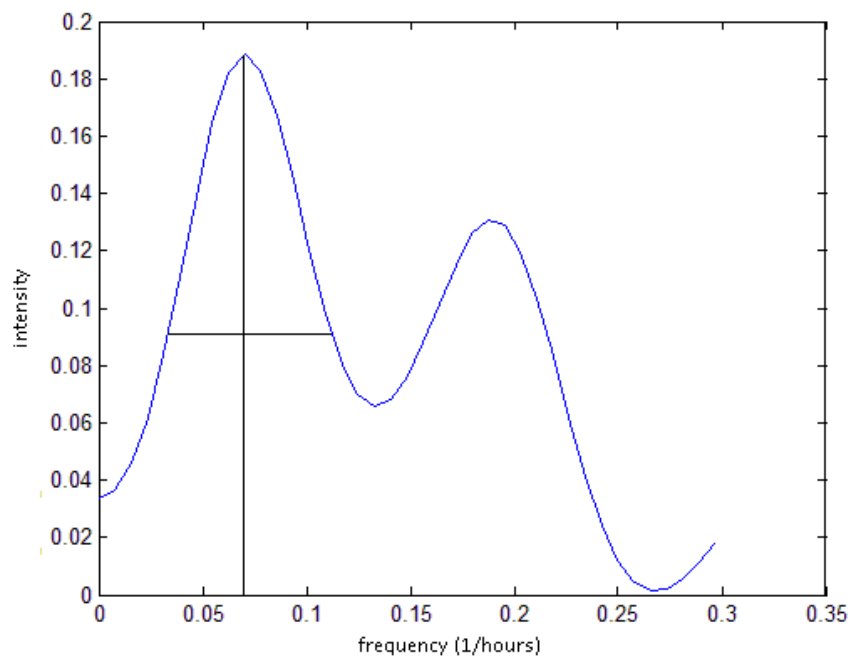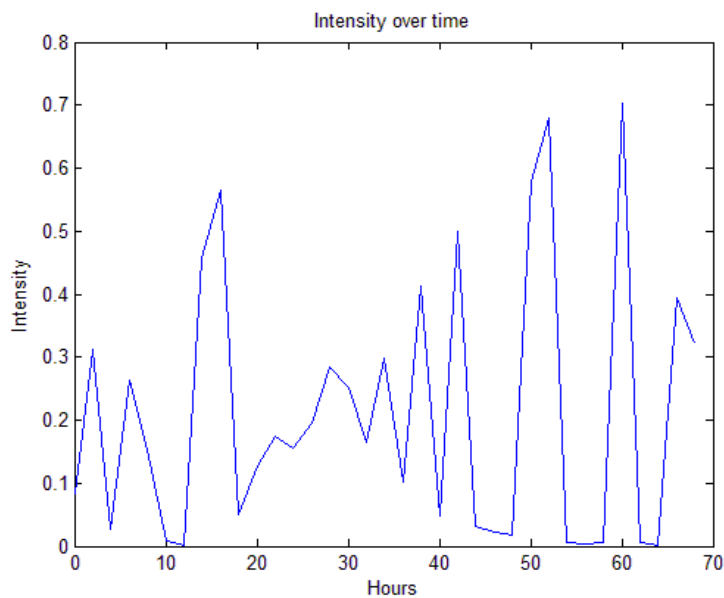


**Figure 9:** Fourier transform using Cronux of the circadian rhythm for 12 hour day/night cycles. Note the peak at a frequency of about .07 which indicates a dominant period of about 14 hours. At half maximum this value varies from about .03 to about .11 which indicates that there is a large error in this number but also that this number roughly correlates with a 12 hour cycle within error.

Similar analysis working with 6 hour day cycle yielded a final mean image shown in figure 10.
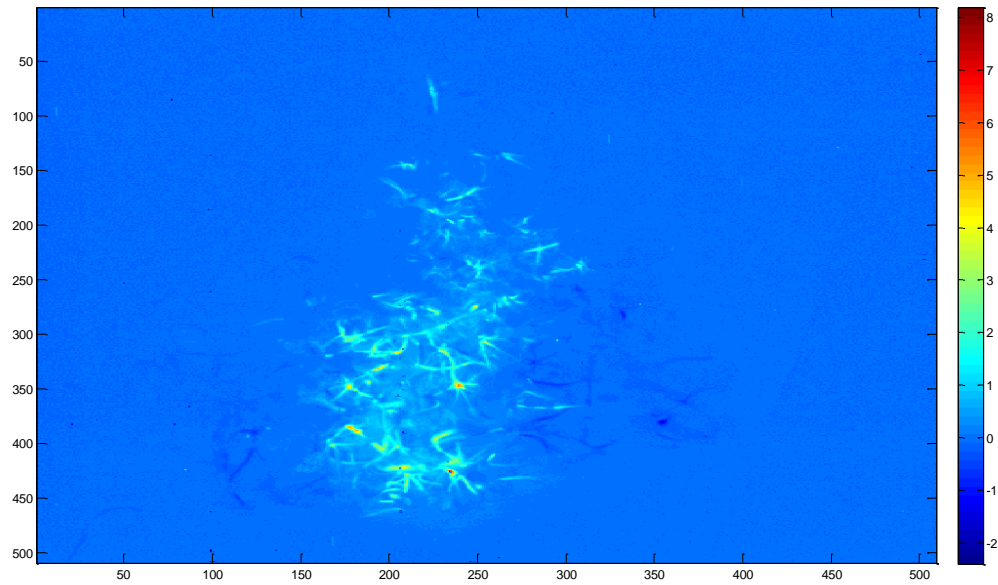
**Figure 10:** Mean image from a 6 hour day cycle after variance filtering and SVD analysis. It must be

noted that the plant samples used for this experiment were much smaller and in larger number than for

the 12 hour cycles. This resulted in difficulty of picking out a single plant but rather a region of plants

that varied uniformly.

From there, the intensity over time can be plotted as before and is shown in figure 11.

**Figure 11:** Circadian rhythm of the illumination of the Arabidopsis from 6 hour day 6 hour night cycles.

Lastly cronux can again be used to determine the dominant frequency of this rhythm.
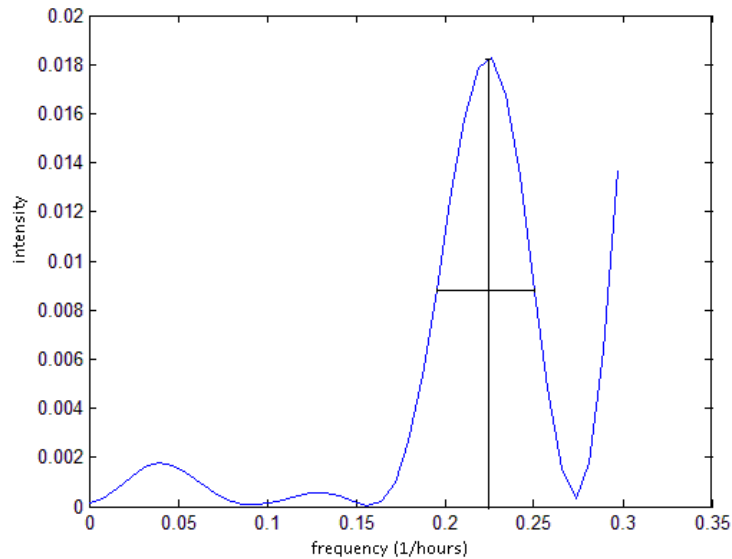


**Figure 12:** Fourier transform using cronux of the 6 hour day 6 hour night circadian rhythm cycle. Note

the predominant peak at around .23 which indicates a period of about 5 hours which roughly correlates

with the 6 hour time interval of the lighting. At half maximum this value varies from $.23 \pm .02$ which

indicates a large error in this value likely due to small sample size.

**Discussion, Sources of error, and further experimentation**

Large error in frequency measurements may be due to many factors. It is assumed that

Arabidopsis undergoing their normal cycle will require time to adjust to new cycles which is roughly seen

in intensity plots. Ideally, a section of data after adjustment would be used for frequency measurement.

However, since this experiment was only conducted for a short time such a measurement does not yield

meaningful results.

In both cases the circadian rhythm from the plants indicated a period that is roughly correlated

with the length of the day cycle. This indicates that the plants are capable of adapting to shorter day

cycles. Protein levels seem to peak during the day during lighting conditions and drop during the night. However, this data suggests that there is a peak both the beginning and towards the end of the day cycles twice as frequent as expected. This may be due to many factors. The "day" cycle is not faded in but rather is an instant transition from non illumination, total darkness, to total light conditions. This may for some reason affect the way in which the plants react to the lights. Also only red light was used which may affect the health of the plant. Long exposure times result in large sections of time, 20 minutes every two hours, during which lighting is turned off resulting in a short "night". This lighting may affect the plants protein production. This also means that imaging cannot be taken more often than about once every two hours or it will further disrupt the time cycle.

As discussed in "Real-Time Reporting of Circadian-Regulated Gene Expression by Luciferase Imaging in Plants and Mammalian Cells," plants normally used are seedlings of around two weeks of age. In this experiment plants much older and in the end stages of life were used. This may have affected their ability to adapt to shorter cycles or different cycles from which they were exposed earlier in life. Despite this the use of bioillumination to study protein expression has shown that the level of protein expression is governed by light exposure. The ability to adapt to shorter cycles of light may be indicative the ability to adapt to conditions in which the plants are shaded at certain or most times of the day.

A number of experiments can be conducted to further these results. An intermediate cycle can be measured such as 8 hours or a lengthened day cycle can be measured.  Minimal lighting conditions can be assessed or randomized day lengths can be used. Any condition in which the Arabidopsis would be forced to adapt in some way can teach us something about its biology. An experimental setup that allows for longer data collection is needed. This will allow frequency measurements to be more accurate and more meaningful. It will also allow one to analyze the correlation between the lighting frequency and the circadian rhythm to see if these become more correlated over time.

**References**

▶ **Welsh, D.K., et al. "Real-Time Reporting of Circadian-Regulated Gene Expression by Luciferase Imaging in Plants and Mammalian Cells"** *. Methods in Enzymol* **393:269-88. (2005)**

▶ **Leeuwen V. W, Hagendoorn JM, Ruttink T, Poecke V R, Van Der Plas L HW, Krol V R Alexander "The use of the Luciferase Reporter System in Planta Gene Expression Studies" Plant Molecular Biology reporter 18 143A-143t, (2000)**