Leaf Respiration - MA132 Project 1

Table of Contents

1 - Introduction

Green plants commonly undergo a process called photosynthesis, in which energy from the sun and carbon dioxide (CO_2) are consumed to form both oxygen gas (O_2) and food for the plant. The carbon dioxide is consumed by the plant through stomata, small openings in the plant itself. These stomatas are capable of opening, to allow CO_2 in, and closing, to prevent loss of water vapor.

The purpose of this project is to calculate a singular leaf's CO2 consumption rate, through a process of integration and other mathematical methods. With a standard method using chlorophyll fluorescence, the stomata of a leaf can be imaged as open or closed. Figure one depicts the process of determining which stomata are open or closed, with the bottom right box being the type of processed image used throughout the calculations (Figure 1).

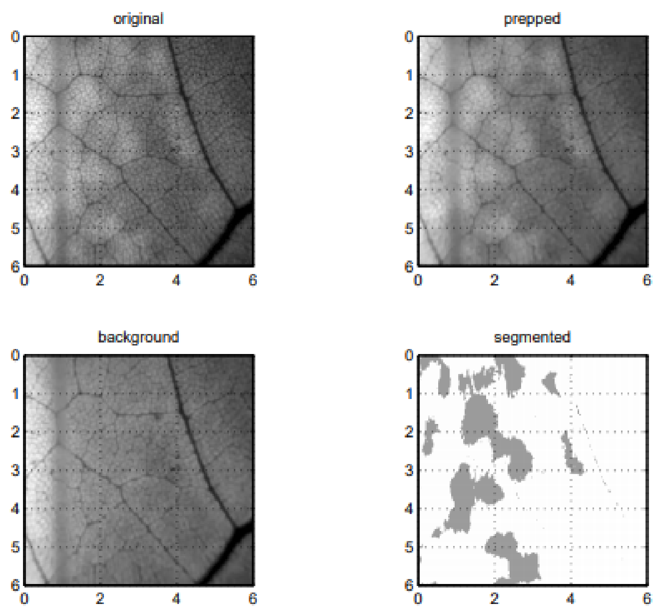

Figure 1: A visualization of the image processing used to determine open or closed stomata

The final calculations of this experiment show multiple different approximated rates of carbon dioxide consumption. More specifically, the value of the total carbon dioxide consumed by the leaf between Frame 100 and Frame 218 is approximated to be 272.0497 µmol.

2 Method- Calculating Area of Open Stomata

In the final processed images of the leaf, there are sections in which the stomata of the leaf are closed and sections where they are open. Because only open stomata play a role in the consumption of CO_2, the areas of these sections must be determined. As seen in Figure 2, the white area represents open stomata while the dark shaded area represents closed stomata.

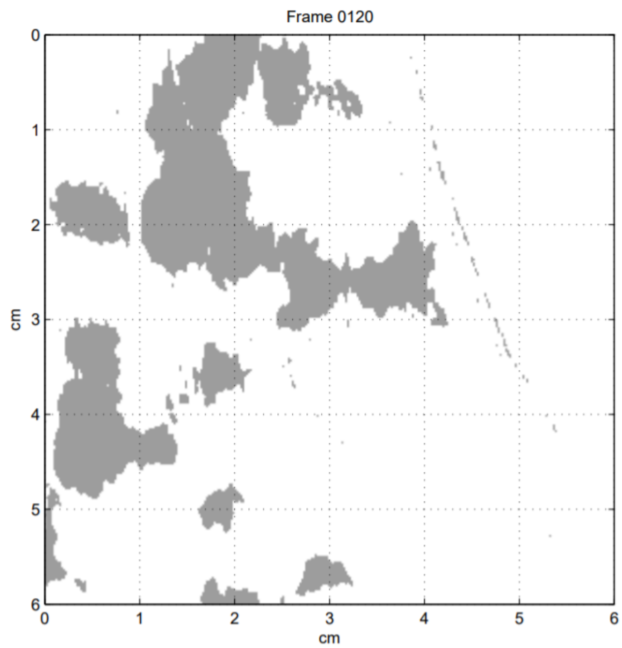

Figure 2: Frame 0120

Based on student number, one of seven different frames of the leaf were assigned to students. Frame 0120 is the individual frame assigned for this particular paper.

In order to calculate the area of the white section in the frame, the shaded area was approximated, and subtracted from the total area of the cell, $36cm^2$. In order to make the approximation more accurate, a grid was placed over the frame, separating each square of the frame into 16 smaller sections. Once the grid was placed, a combination of midpoint rule and trapezoidal rule were implemented to approximate the area of the shaded region (Figure 3).

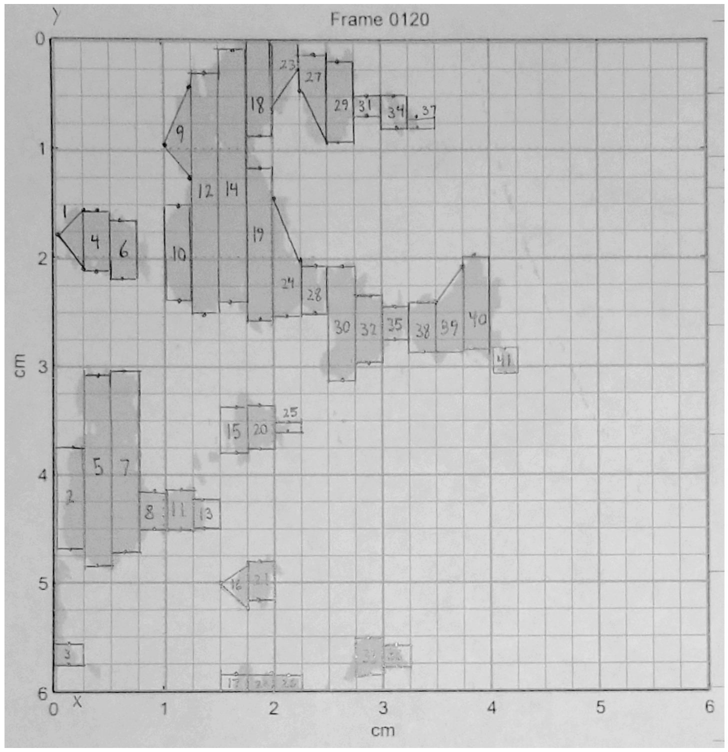

Figure 3: Hand drawn model of trapezoids and rectangles used for area approximation

As seen in Figure 3, each shaded area along the x axis was broken down into basic polygons using midpoint and trapezoidal rules, allowing for basic area calculations. When using the midpoint rule, upper and lower bounds of the rectangle (i.e, height) are determined by the position on the y axis between two values of x. The width of the rectangle is determined by a value of Δx. The general formula of the midpoint rule is as follows in equation 1:

$$M_n = \Delta x[f(\overline{x_1}) + f(\overline{x_2})...+f(\overline{x_n})] \tag{1}$$

W here M_n = Approximate Area
Where n = Number of Subintervals
Where Δx = Length of Each Subinterval

By using equation 1, and adding together the area of each individual polygon, the found area of the shaded region was 89.05 cells. Because the original grid had been manipulated, this number must be divided by sixteen, resulting in 5.566cm^2 for the shaded area. When subtracted from the total 36cm^2 of the frame, the area of open stomata is equal to 30.434cm^2.

3 Method - Aggregated Data

3.1 - Sorting the Data

Because the area of open stomata needed to be evaluated for seven different frames, and because the methods used to calculate area were only approximations, there was a large amount of data for each frame. Over 419 seperate area approximations were made collectively for the seven different frames.

Because the data was not sorted in any way, a format needed to be developed to sort the different values. In order to do this, ‘If, Then’ functions can be employed in microsoft excel. By using the following formula, seven separate columns were made, with each stating only the values that belonged to the desired frame.

$$= if(A = Frame\ Number,\ B) \tag{2}$$

Where A = Column for Frame Number
Where *Frame Number* = Value of Frame Number
Where B = Area of Corresponding Frame

By using this technique, the area values of each column were shown as false, unless the frame number was equal to the desired frame number for that column (Figure 4).

	A	B	C	D	E	F	G	H	I
1	**Frame Number**	**Area**	**Frame 100**	**Frame 120**	**Frame 140**	**Frame 160**	**Frame 180**	**Frame 200**	**Frame 218**
2	160	21.6344	FALSE	FALSE	FALSE	21.6344	FALSE	FALSE	FALSE
3	100	26.825	26.825	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
4	180	29.5	FALSE	FALSE	FALSE	FALSE	29.5	FALSE	FALSE
5	140	28.62	FALSE	FALSE	28.62	FALSE	FALSE	FALSE	FALSE
6	140	28.92	FALSE	FALSE	28.92	FALSE	FALSE	FALSE	FALSE
7	120	30.5	FALSE	30.5	FALSE	FALSE	FALSE	FALSE	FALSE

Figure 4 - A table of the first seven sorted values of the data set

3.2 - Removing Outliers and Finding Averages

Once the mass of data was sorted into each individual frame, an average needed to be calculated that did not include statistical outliers. The first step in the process of removing outliers was to find the median area of each frame. Once the median was found, all numbers within 20% of it, above or below, were considered valid. Anything not within this range was considered to be an outlier and eliminated from the average calculations. All of these values, including the medians, upper and lower bounds of the ranges, and unedited averages can be seen in Figure 5.

	Frame Number						
	100	120	140	160	180	200	218
Median	28.57	28.75	28.29	27.55	27.87	29.53	34.00
Lower Limit (Median -20%)	22.86	23.00	22.63	22.04	22.29	23.62	27.20
Upper Limit (Median +20%)	34.28	34.50	33.95	33.06	33.44	35.44	40.80
Average (Including Outliers)	36.91	27.31	25.32	26.08	26.71	28.66	29.87
Average (Excluding Outliers)	28.53	28.79	28.36	27.76	28.27	29.13	33.65

Figure 5 - Table of All Important Statistical Evaluations

The results of Figure 5 prove the necessity of removing outliers to find the average area of open stomata. In the second to last row, there are examples of the average areas calculated with statistical outliers included. They are less accurate, and in one case, entirely impossible. For Frame 100, the non-accurate average is higher than physically possible, as the maximum area of the frame was $36cm^2$. The values in the bottom row are far more statistically accurate averages of the open stomata area.

3.3 - Unit Conversion

In order to give a final result that is in the units $\frac{\mu mol}{m^2 s}$, some of the current units in use must be converted to others. The area units, cm^2, must be placed in terms of m^2 and the frame numbers must be expressed as units of time. These conversions can be viewed in Figure 6.

Frame Number	Time (s)	Area (cm^2)	Area (m^2)
100	0	28.53	0.002853
120	800	28.79	0.002879
140	1600	28.36	0.002836
160	2400	27.76	0.002776
180	3200	28.27	0.002827
200	4000	29.13	0.002913
218	4720	33.65	0.003365

Figure 6 - Unit Conversions

The reasoning behind the time values for each frame number is that each frame was taken 40 seconds apart. By setting t=0 at frame 100, it would take 800 seconds to reach frame 120 (20 frames x 40 seconds). This logic continues for each subsequent frame used. Also, because the area must be converted to meters squared, the area in centimeters squared must be divided by 10,000 (100^2).

4 Method - Estimating CO2 Consumption

4.1 - Finding CO_2 Consumption Rate

As defined by the project description, the model for the rate of CO_2 uptake of the leaf is

$$U(t) = P \times A(t) \tag{3}$$

Where *U(t)* = Rate of CO_2 Uptake at a Given Time
Where *P* = Constant of Photosynthetic Activity = $20\mu mol * s^{-1}$
Where *A(t)* = Area of Open Stomata at a Given time

Based on Equation 3, the different rates of CO_2 consumption can be determined at the different times of the experiment. When calculated, the results are as follows

Time (s)	*U(t)* ($\mu mol * s^{-1}$)
0	0.0571
800	0.0576
1600	0.0567
2400	0.0555
3200	0.0565
4000	0.0583
4720	0.0673

Figure 7 - Values of *U(t)*

The next step after finding the values of U(t) at different times is to plot the two values against each other. The resulting scatter plot is the following.

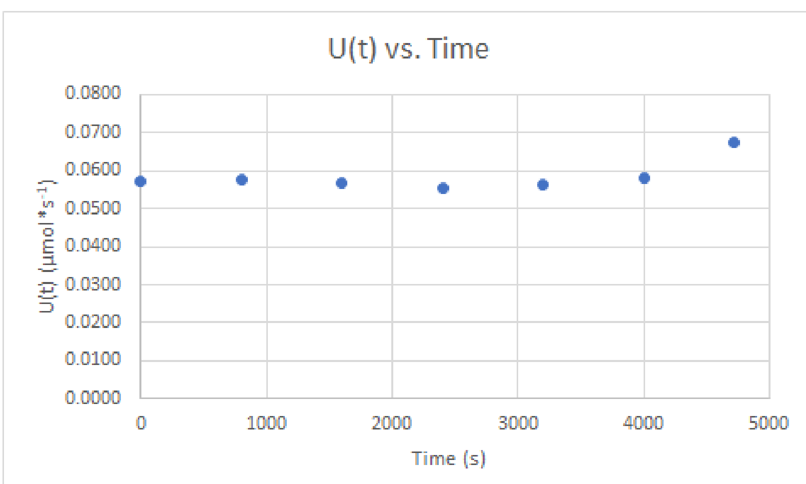

Figure 8 - Scatter Plot of *U(t)* vs. Time

4.2 - Finding Total CO_2 Consumed by the Leaf

While the different rates of consumption at different values of time have been found, a definitive approximation of the CO2 consumed during the experiment has not yet been calculated. Under normal circumstances, with a known function that represents rate of consumption, an integral could be used, with the lower bound set to zero and the final bound set to the total time. This would show the exact value of carbon dioxide consumed by the leaf over a certain period of time. However, because there is no defined function, and only data points, area approximation must once again be used.

As defined in Equation 1, the midpoint rule will be used on the data shown in Figure 8. A visual representation of the subintervals can be shown in Figure 9:

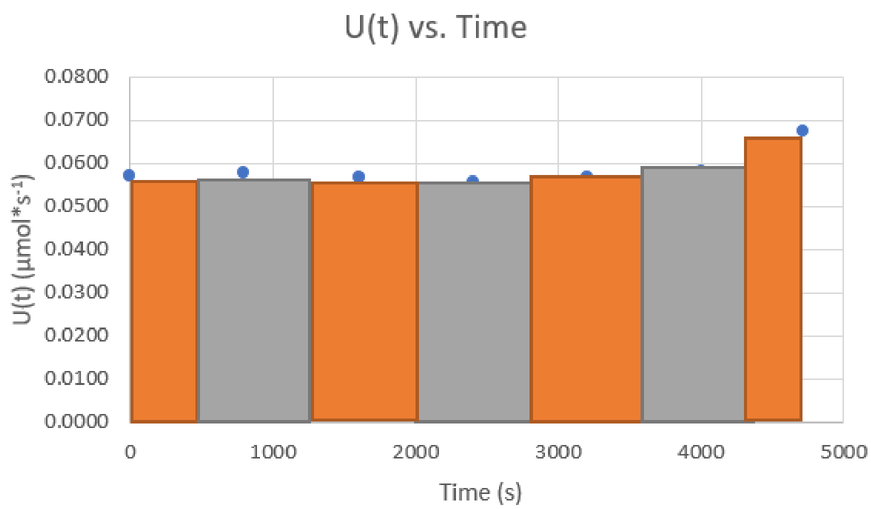

Figure 9 - Representation of Subintervals for Midpoint rule

It should be noted however, that because each of the data points are being used as the midpoints of the subintervals, some of the Δx values must be edited. For example, the subinterval on the far left has a Δx value of 400s, the far right subinterval has a Δx value of 320s, while all other subintervals have a Δx value of 800s.

When fully calculated, the final value of CO2 consumed from Frame 100 to Frame 218 is equal to:

$$272.0497 \ \mu\text{mol}$$

5 - Results and Discussion

The final results of this paper were fairly varied. There are seven separate rates of carbon dioxide consumption, which can each be seen in Figure 7. The final approximation for the carbon dioxide consumed between Frame 100 and Frame 218 was concluded to be 272.0497 μmol.

The calculations of this particular leaf's carbon dioxide consumption were primarily approximations. And although not perfectly accurate, are based on hundreds of different approximations and methods. In an imperfect model such as this, sources of error will always be found. Aside from calculation mistakes, a source of error could potentially be confusion between open and closed stomata regions. If the results of this paper were to be calculated using the shaded regions instead of the white, a completely different, and incorrect, answer would be found.

For future iterations of this experiment and paper, more data frames would help to provide a more accurate approximation. Area approximation heavily relies on the value of Δx. With a smaller value, which would be created given more frames, the approximation would use far more subintervals. This would provide a better approximation. But, as with all approximations, there is a limit to the degree of accuracy available. A perfect area calculation would use an integral with an infinite number of subintervals. Because it is physically impossible to provide infinite frames, there is a limit to the approximations accuracy.

While the methods used in this experiment resulted in a fairly accurate approximation, they take a substantial amount of time to complete. A far simpler, but less accurate method would be to view each of the 36 separate boxes created by the grid in Figure 2. By simply counting each box as either filled, or not filled, a general area approximation could be made that would be able to multiply by the constant P (as seen in Equation 3). This would result in a single rate for carbon dioxide consumption. Performing this task with other frames and forming a quick riemann sum provides less accurate, but far faster results. When performing this method, the conclusions of this paper are within a range of the result of the simpler method that proves them to be fairly accurate.

6- Conclusion

The methods used here were subject to inaccuracies. There are always trade-offs between accuracy and speed. Increasing the number of intervals will increase the accuracy, but it is impossible to continue into infinity. Therefore, inaccuracies are an essential component of approximations and must be reckoned with in all mathematical models.

7 - Bibliography

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