Experiment CM-6: Photosynthetic Gas Analysis using an Entire Plant

Equipment Required

PC or Mac computer

IXTA, USB cable, IXTA power supply

iWire-GA1 gas analyzer

A-CAL-200 calibration kit

AC-520 5.2 L closed chamber

A plant light or other light source (low heat output recommended)

Small plant that fits easily into the chamber (*Gerbera or Primula sp.*)

Desiccant moisture absorber (available from craft stores)

Set up the Metabolic Cart

1. Locate the large closed chamber, the gas analyzer, the gas analyzer power supply, two sensor output cables, a gas inlet filter, and gas sampling tubing.
2. Position the gas analyzer on the desktop, so that the analyzer can be connected to the data recording unit and the closed chamber at the same time.
3. Connect the gas analyzer to the TA using the small iWire cable. It should plug into the i1 port.

Figure CM-6-S1: The closed chamber to be used with a plant set inside.

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6. Place one gas sampling tube on the gas sampling port near one of the Luer-lock connectors on the chamber.
7. Place the filter on the sample in port. Attach the braided end of the gas sampling tube to the filter.
8. Place the second gas sampling tube on the outlet port of the gas analyzer and connect it to the other Luer-Lock connection on the closed chamber.
9. Stand the chamber upright so you can fit the plant into it.

Figure CM-6-S2: Closed chamber, iWire-GA1 and the IXTA showing the proper connections to measure O_2 and CO_2 exchange.

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Figure CM-6-S3: A Gerbera daisy used for the photosynthesis experiment.

Calibrating the Gas Analyzer

Before the iWire-GA1 gas analyzer is used, the following Two-Point Calibration procedure must be performed.

Calibration of the O_2 and CO_2 Channels

The outputs of the oxygen and carbon dioxide sensors of the GA-300 are voltages that are proportional to the concentrations of the gases being measured by the analyzer. To determine the volumes of oxygen consumed and carbon dioxide produced during metabolic testing, the voltage outputs of the sensors need to be converted, by the recording software, to the percentages of these gases in the inhaled and exhaled air.

To make this conversion, samples of two different concentrations of oxygen, and two different concentrations of carbon dioxide, will need to be put into the gas analyzer as the voltage outputs of each sensor are recorded.

One set of samples can be taken from room air, which contains 20.93% O_2 and 0.04% CO_2. The other set of samples can be taken from gas cylinders containing a combination of these two gases at different concentrations. Cylinders containing both oxygen and carbon dioxide are readily available from suppliers. Some of the most commonly used combinations contain:

- 12% O_2 and 5% CO_2, with the balance being N_2, or;
- 16% O_2 and 4% CO_2, with the balance being N_2.

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Record the Voltage Outputs of the Gas Sensors

1. Turn on the gas analyzer for at least 15 minutes before performing a calibration.
2. Prepare the equipment that will deliver any gas samples, other than room air, to the gas analyzer:
    - Clamp and secure any gas cylinders that will used to provide gas samples near the GA-300 gas analyzer.
    - Attach the regulator to the gas cylinder safely.
    - Attach a Luer-Lock connector to the outlet of the regulator that will allow the Calibration Kit for the GA to be connected to the regulator of the gas cylinder.
    - Open the primary and secondary valves of the regulator for a few seconds to purge the air from the regulator.
    - Close the secondary valve on the regulator to stop the flow of gas from the regulator. You will need the cylinder for the second sample of gas.
3. Measure the voltage outputs of the oxygen and carbon dioxide sensors when measuring a sample of room air.
4. Place the gas sampling tubing away from the users to prevent the sampling of exhaled air. Allow room air to be pumped through the gas analyzer for 10 seconds before recording the outputs of the sensors.
    - Type **Room Air** in the Mark box.
    - Click on the Record button. The recording should scroll across the screen.
    - While recording, click the mark button to mark the recording with information about the room air gas sample.
    - Record the outputs of the two gas sensors for about 15 seconds. The recording which should be like the first segment of data below.
    - Continue recording.
5. Measure the voltage outputs of the oxygen and carbon dioxide sensors when measuring a second sample of a gas mixture containing known concentrations of oxygen and carbon dioxide.
    - Open the secondary valve on the regulator of the cylinder providing the second gas sample. Adjust the flow rate to low.
    - While the gas sample is flowing from the regulator, connect the gas sample tubing of the Calibration Kit to the Luer-Lock connector on the output of the regulator.

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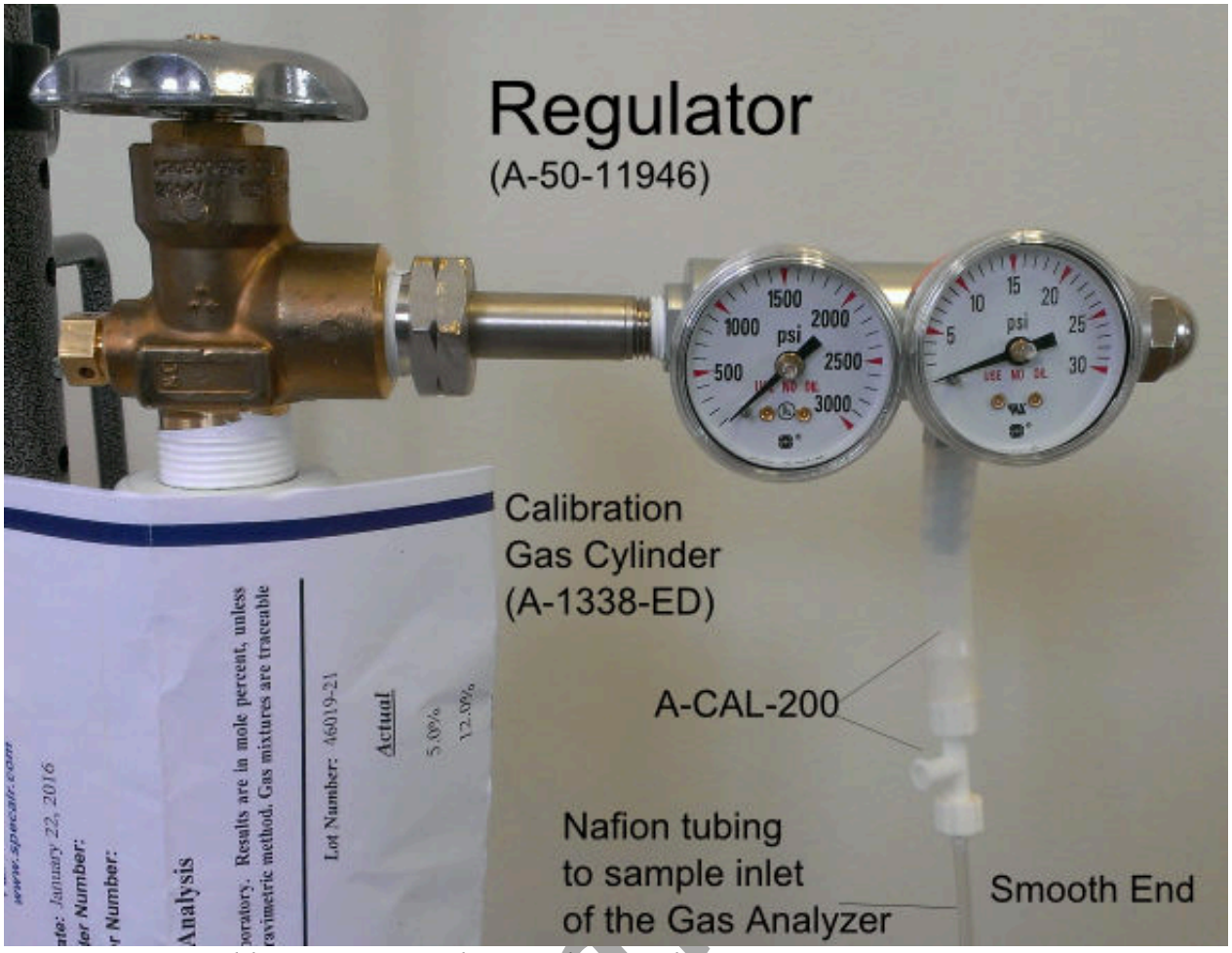

Figure CM-6-S6: Calibration Kit, regulator and gas tank.

- Connect the outlet from the Calibration Kit syringe to the sample in port on gas analyzer.

Note: *The gas sample will automatically be pulled into the GA. Make sure the air flow is set low, too high a flow may damage the CO2 and O2 sensors.*

- Type **Gas Calibration** in the Mark box.
- Click on the Record button. The recording should scroll across the screen.
- While recording, click the mark button to mark the recording with information about the second gas sample.

7. Once the recordings of the gas concentrations reach a steady level, record for another ten seconds. This can take up to two minutes.
8. Click the Stop button.

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9. Select Save As in the File menu, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder. Designate the file type as *.iwxdata. Click on the Save button to save the data file.

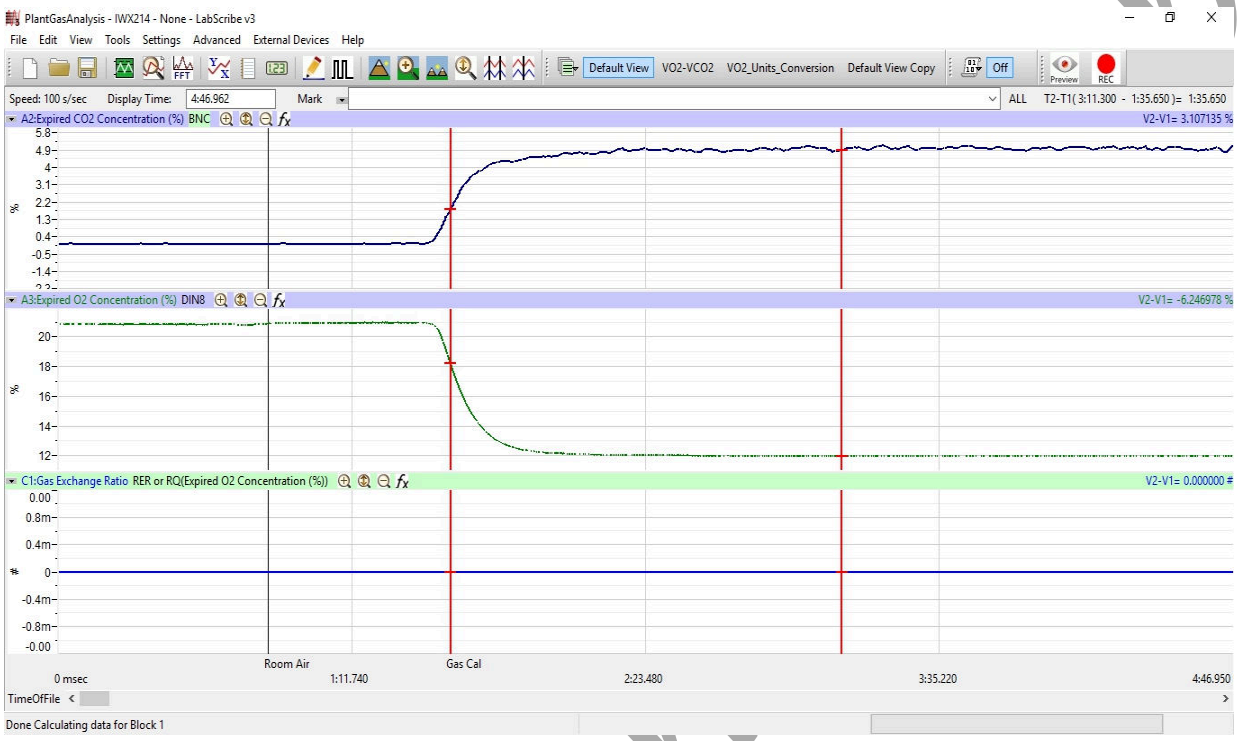

Figure CM-6-S7: The voltage outputs of the two sensors in the GA-300 gas analyzer, carbon dioxide on the top and oxygen in the center.

Convert the Units on Gas Concentration Channels

1. Use the Display Time icons to adjust the Display Time of the Main window to show the complete calibration data on the Main window at the same time. The required data can also be selected by:
    - Placing the cursors on either side of data required.
    - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the entire segment of data to the width of the Main window.
2. Click the 2-Cursor icon so that two cursors appear on the Main window. Place one cursor on the section of data recorded when gas analyzer was collecting a sample of room air and the second cursor on the section of data recorded when the second sample was collected.

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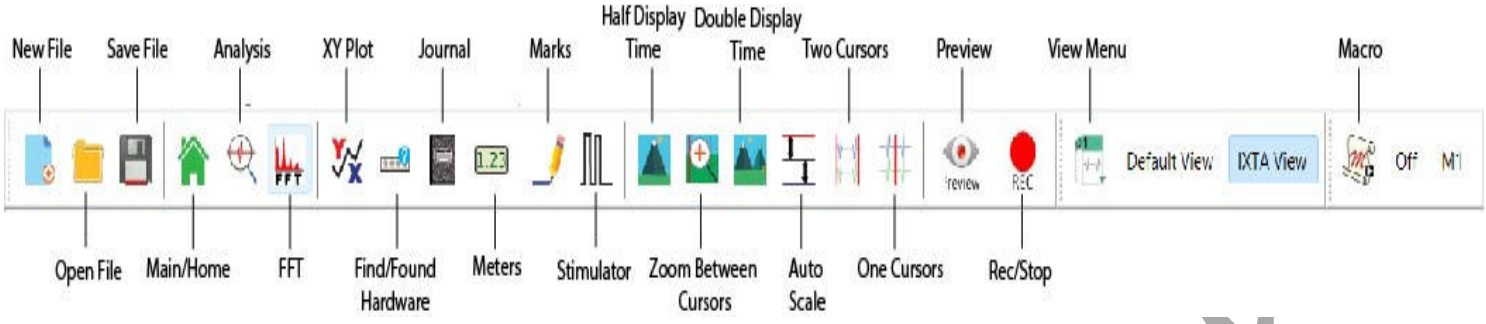

Figure CM-6-S8: The LabScribe toolbar.

3. Convert the voltages at the positions of the cursors to concentrations using the Advanced Units Conversion dialog window.

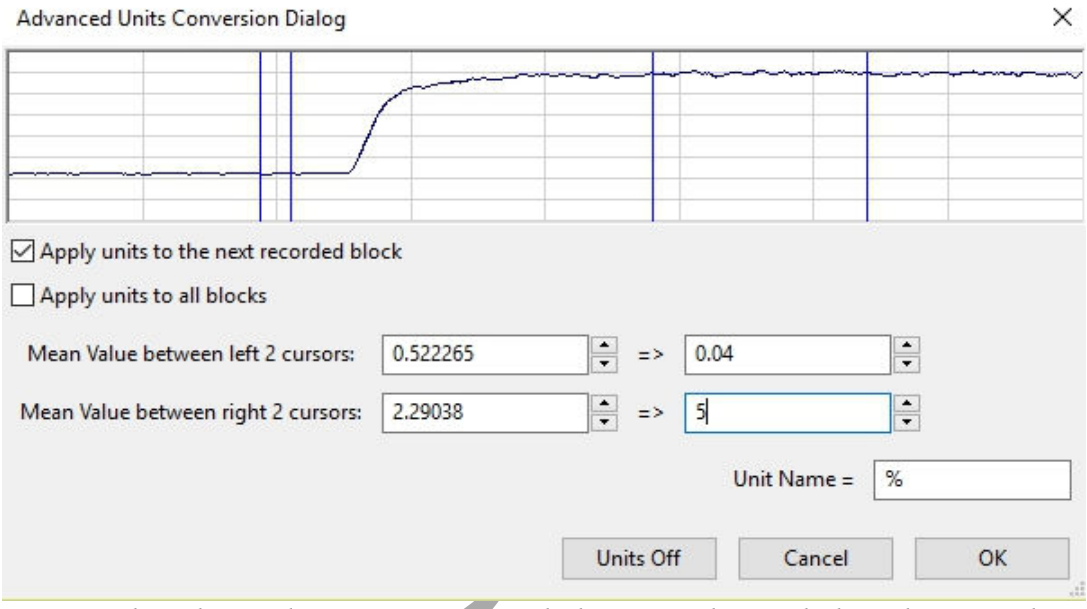

Figure CM-6-S9: The Advanced Units Conversion dialogue window with the voltages at the cursors set to equal the concentrations used in calibration.

4. To convert the voltages on the CO2 Concentration (%) channel, click on the arrow to the left of the channel title to open the channel menu. Select Units from the channel menu, and select Advanced from the Units submenu.
5. On the Advanced Units Conversion window:
    - Move 2 cursors to the area recorded using room air.
    - Move the other 2 cursors to the area recorded using the gas from the calibration kit syringe.
    - Put a check mark in the box next to Apply units to the next recorded blocks.

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- Notice that the voltages from the positions of the cursors are automatically entered into the mean value equations: Mean Value between left 2 cursors and Mean Value between right 2 cursors.
- Enter the two concentrations of carbon dioxide measured from the two samples in the corresponding boxes on the right side of these conversion equations.
- If using room air - the concentration of CO2 = 0.04
- The second gas concentration will be the one from the gas cylinder.
- Enter the name of the units, %, in box below the concentrations.
- Click on the OK button in the lower right corner of the window to activate the units conversion.

6. Repeat Steps 4 and 5 on the O2 Concentration (%) channel.
    - Room air = 20.9
    - Second gas concentration will be the one from the gas cylinder.
7. Click on the Save button.
8. Disconnect the Calibration kit from the gas analyzer and connect the two gas sampling tubes to the Luer-lock connectors on the top of the chamber.

Before Starting

1. Read the procedures for the experiment completely before beginning the experiment. Have a good understanding of how to perform the experiment before making recordings.

Note: *Place a 1 inch layer of desiccant in the bottom of the chamber prior to beginning the experiment.*

2. Place the plant, in its pot, in the closed chamber. Carefully close the lid but do not seal it at this time. Make sure not to have any leaves caught in the lid.
3. Fill the closed chamber with air from the calibration tank using the calibration syringe.
    - Connect the calibration syringe to the chamber and slowly allow the gas to move into the chamber.
    - Try to ensure that the chamber is full of gas – it is approximately 5 liters.

Note: *The carbon dioxide in the chamber must start out at a greater percentage than room air.*

4. Seal the lid by pressing the center button, making sure no leaves are caught.
5. Disconnect the calibration syringe from the chamber and quickly connect the chamber to the gas analyzer as shown in the previous figures.

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Experiment CM-6: Photosynthetic Gas Analysis using an Entire Plant

Before Starting – note this is stated in the Set Up procedure as well

1. Read the procedures for the experiment completely before beginning the experiment. Have a good understanding of how to perform the experiment before making recordings.

Note: *Place a 1 inch layer of desiccant in the bottom of the chamber prior to beginning the experiment.*

2. Place the plant, in its pot, in the closed chamber. Carefully close the lid but do not seal it at this time. Make sure not to have any leaves caught in the lid.
3. Fill the closed chamber with air from the calibration tank using the calibration syringe.
    - Connect the calibration syringe to the chamber and slowly allow the gas to move into the chamber.
    - Try to ensure that the chamber is full of gas – it is approximately 5 liters.

Note: *The carbon dioxide in the chamber must start out at a greater percentage than room air.*

4. Seal the lid by pressing the center button, making sure no leaves are caught.
5. Disconnect the calibration syringe from the chamber and quickly connect the chamber to the gas analyzer as shown in the previous figures.

Exercise 1: Determining the Gas Exchange in a Plant undergoing active Photosynthesis

Aim: To determine the rate of oxygen production and carbon dioxide utilization in a photosynthesizing organism.

Approximate Time: At least 60 minutes

Oxygen production is a result of photosynthetic electron transport through both photosystems. This experiment will be performed with light shining on the plant.

Procedure

Note: *Angle the plant light so that it is shining as directly on the leaves of the plant as possible. Make sure the light is close enough to shine brightly on the leaves, but far enough away so as to not heat up the chamber. It is important for the chamber to remain as cool as possible to prevent moisture build up.*

1. Place the chamber with the plant in the light shining from the plant light source.
2. Type **PlantActive** in the Mark box. Click the Record button and the click the mark button.

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3. Quickly observe the starting values of gas concentration in the CO_2 and O_2 channels. This will be located in the right hand margin of each of the channels (Value =). Note these beginning gas concentrations.
4. Record the gas exchange of the plant in the light for at least an hour or until there is at least a 2% change in both the CO_2 and O_2 concentrations from the baseline noted at the start of the experiment.
5. Once a 2% change is noted, click Stop to halt the recording.
6. Select Save in the File menu.

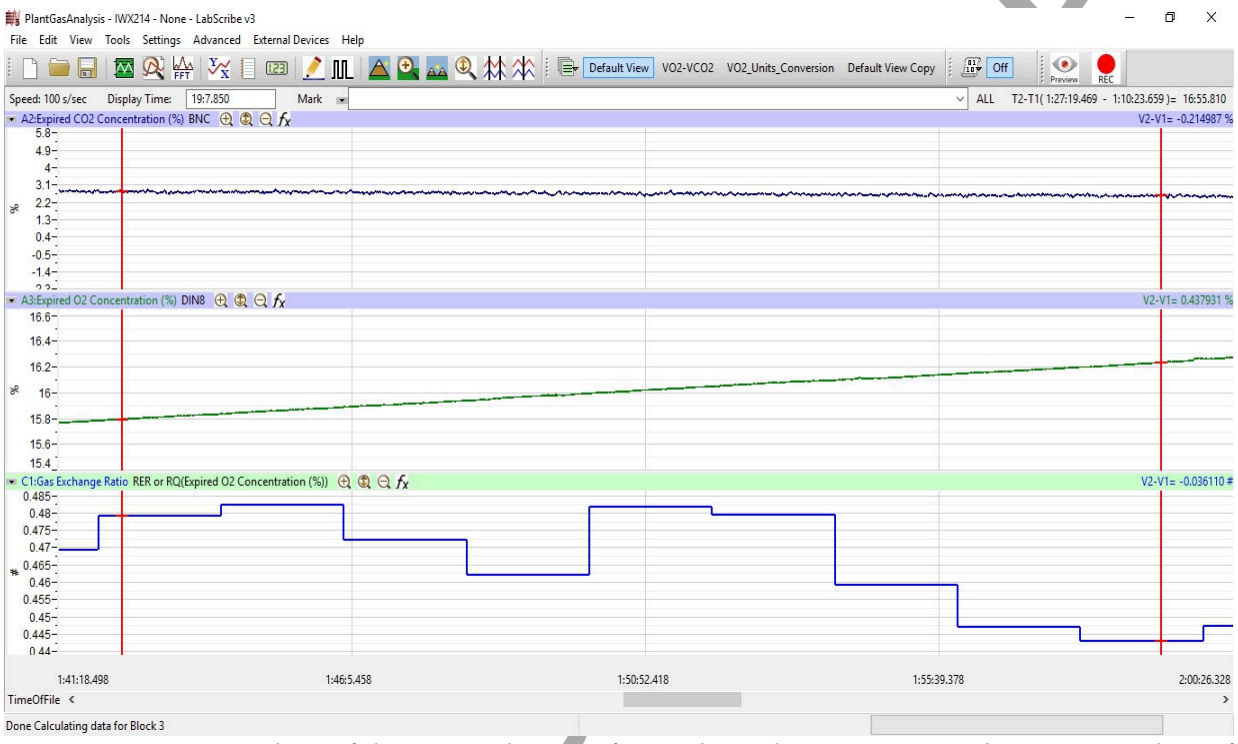

Figure CM-6-L1: Recording of the gas exchange of a Gerbera daisy as seen in the Main Window of LabScribe. The carbon dioxide, oxygen and gas exchange channels are visible.

Data Analysis

Gas Exchange Ratio Channel Set Up

1. Display approximately 20 minutes of the data recording in the Main window. Use the Display Time icons to adjust the Display Time of the Main window to show the data on the Main window. Data may be selected by:
    - Clicking the Double display Time button on the LabScribe toolbar to approximately 20 minutes in the Main window, or;
    - Choose Edit, Preferences from the toolbar. Change the display time to 1200 seconds. Click OK.

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2. Select and display a section of data that shows the change in either the CO_2 or O_2 concentration at 1%. This can be done by:
    - Clicking on and dragging the cursors to either side of the data, and looking at the V2-V1 value in the upper right corner of either the CO2 Concentration or O2 Concentration channel.
    - When the V2-V1 value is equal to 1%, click the Zoom between Cursors button on the LabScribe toolbar to expand this section of data to the width of the Main window.
    - Look at the T2-T1 value in the upper right to determine the time it took for the gas concentration to change by 1%.
3. Click on RER Expired CO2 (or O2) Concentration (%) on the Gas Exchange Ratio channel. Choose Setup Function from the drop down list. This will open the Calculation Dialog window .

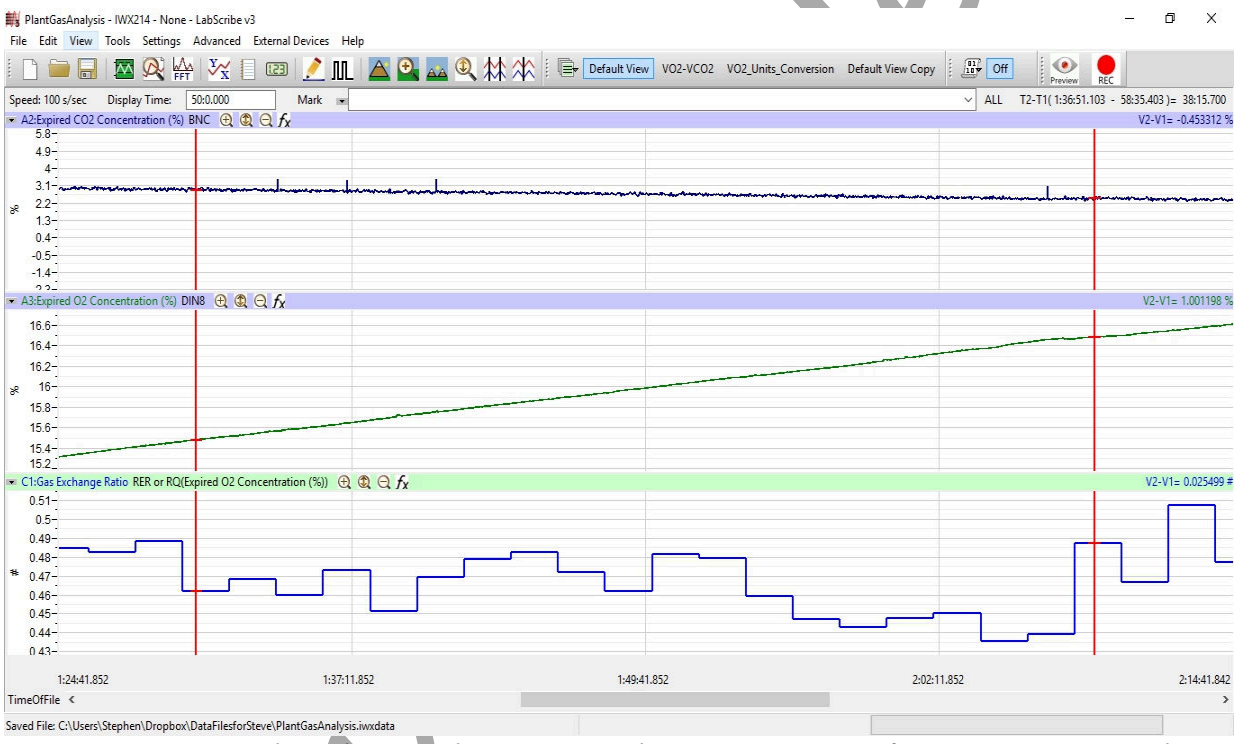

Figure CM-6-L2: Recording showing the cursors in the correct position for measuring a 1% change on either the CO_2 or O_2 channels.

4. Setup the Gas Exchange calculations:
    - Choose the appropriate RER Type by clicking on the down arrow and choosing Closed Small Animal Chamber.
    - Check the O2 and CO2 channel information so that they are being calculated from the correct channels.
    - Change the Time(s) to average to 120 seconds

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- Change the Delta Time (min) to be the T2-T1 value noted in Step 2. This is the time it took for the either the CO_2 or O_2 concentration in the plant chamber to change by 1%.

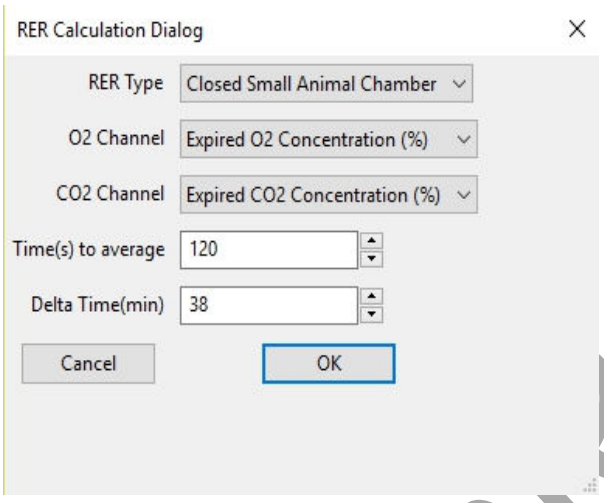

Figure CM-6-L3: RER Calculation dialog window showing 120 seconds as the Time(s) to average and the Delta Time (min) for the 1% change in gas concentration.

5. Click OK.
6. The recording on the main window will now have a histogram on the Gas Exchange Ratio channel.

Calculating the Gas Exchange Ratio

1. After setting up the Gas Exchange Ration calculation, use the Display Time icons to adjust the Display Time of the Main window to show at least 20 minutes of data. Data may be selected by:
   - Clicking the Double Display Time button on the LabScribe toolbar to until the recording shown is approximately 20 minutes, or;
   - Choose Edit, Preferences from the toolbar. Change the display time to 1200 minutes. Click OK.
2. Click on the Analysis window icon in the toolbar or select Analysis from the Windows menu to transfer the data displayed in the Main window to the Analysis window.
3. Look at the Function Table that is above the CO2 Concentration channel displayed in the Analysis window. The functions, Mean and T2-T1, should appear in the table.
4. Place the cursors so that the T2-T1 value is 600 seconds. In the 10 minutes of recording, the mean gas exchange ratio can be determined by looking at the Mean function above the Gas Exchange Ratio channel.

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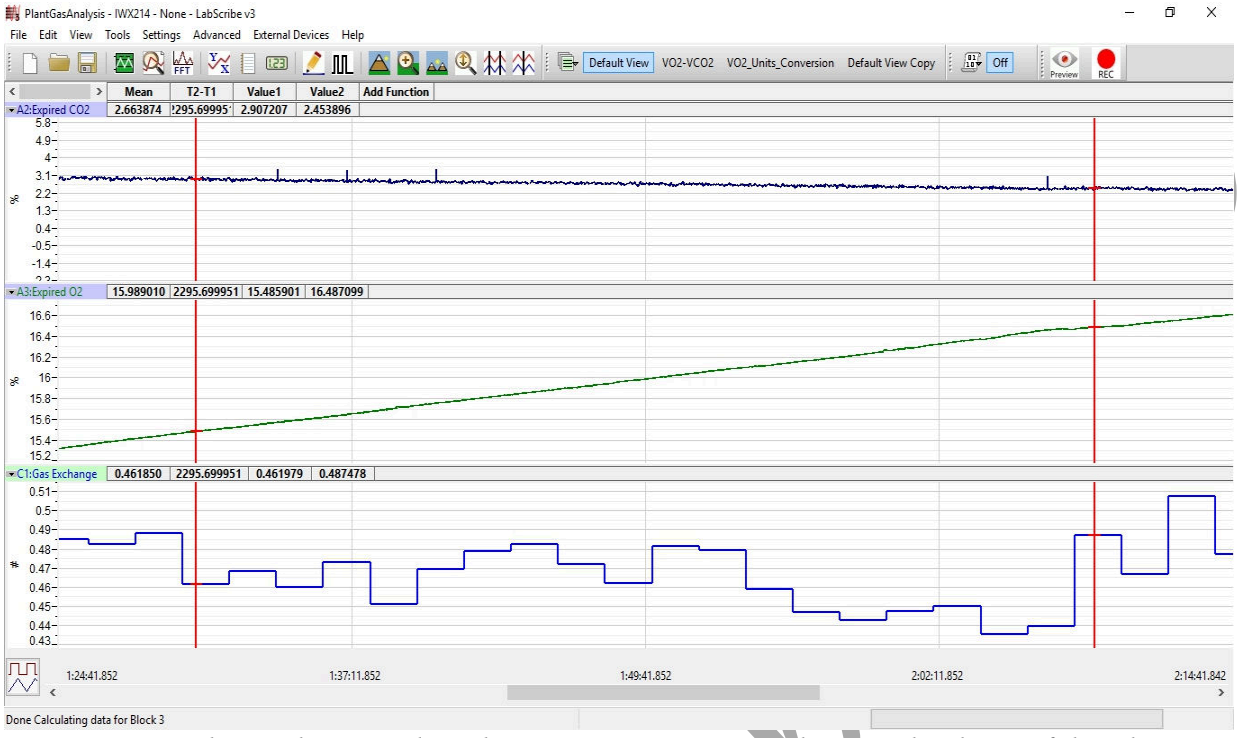

Figure CM-6-L4: The Analysis window showing Mean, T2-T1, Value 1 and Value 2 of the Plant Gas recording.

Using the Auto Find Feature

Note: *Using the Auto Find feature offered in LabScribe allows the user to get mean values from an entire recording without having to manually move the cursors from place to place.*

1. While in the Analysis window, choose the channel data from which the mean values are to be found.
    - On the LabScribe toolbar, click the down arrow to the right of Default or IXTA View.
    - Choose the View to have the Auto Find feature calculate from: CO2 Concentration, O2 Concentration or Gas Exchange Ratio.
    - For ease of analyzing the data, it is best to choose only one View at a time.
2. After you have chosen the View for Auto Find to work on, click Tools, Auto Find from the menu bar. A new window will open as shown.
3. Set the New Cursor1 Position to Old Cursor1 from the drop down box. Change the number of seconds to 100.
4. Set the New Cursor2 Position to New Cursor1 from the drop down box. Change the number of seconds to 10.
5. Set the Repeat Until to Block Limits.
6. Click Find.

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Note: *This will set the software to average 10 times every 100 seconds to give the user a wide sampling of data.*

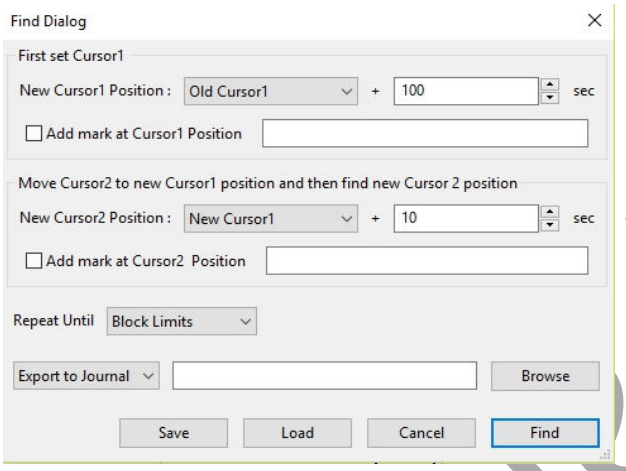

Figure CM-6-L5: Auto Find SetUp window.

7. The values of the parameters in the Function Table for the specific View chosen will be automatically recorded in the Journal.

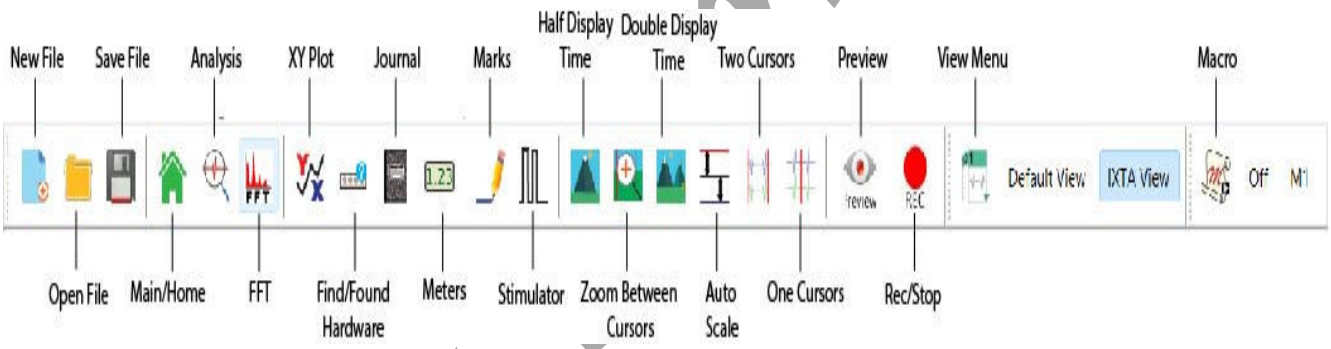

Figure CM-6-L6: The LabScribe toolbar.

8. Click the Journal icon to open the on-line notebook included with the LabScribe software.
9. Note that the data set to be found has been entered automatically into the Journal.
10. Choosing a different View and repeating the Auto Find set up, will add data to the Journal from whichever View has been chosen.
11. Data from the Journal can be exported or copied to Excel (or other program) to graph the mean changes of either the CO_2 or O_2 concentrations over the time of the recording.
    - Open the math program you wish to use.
    - Click and highlight the data in the Journal, choose Copy.

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- Paste the data set into the appropriate program.

Additional Experiments

Other experiments can be performed by students for comparing:

- A plant a room temperature to a plant at either high or low temperature -this can be accomplished by wrapping the chamber in a cold pack or a hot pack depending on the experiment chosen.
- A plant contained in one concentration of CO_2 to a plant in a higher concentration of CO_2. Adjust the concentration of CO_2 in the chamber by filling it with a higher concentration of carbon dioxide at the beginning of the experiment.
- A plant with the light shining on the leaves to a plant in the dark. Wrap the chamber in dark paper to prevent light from entering.

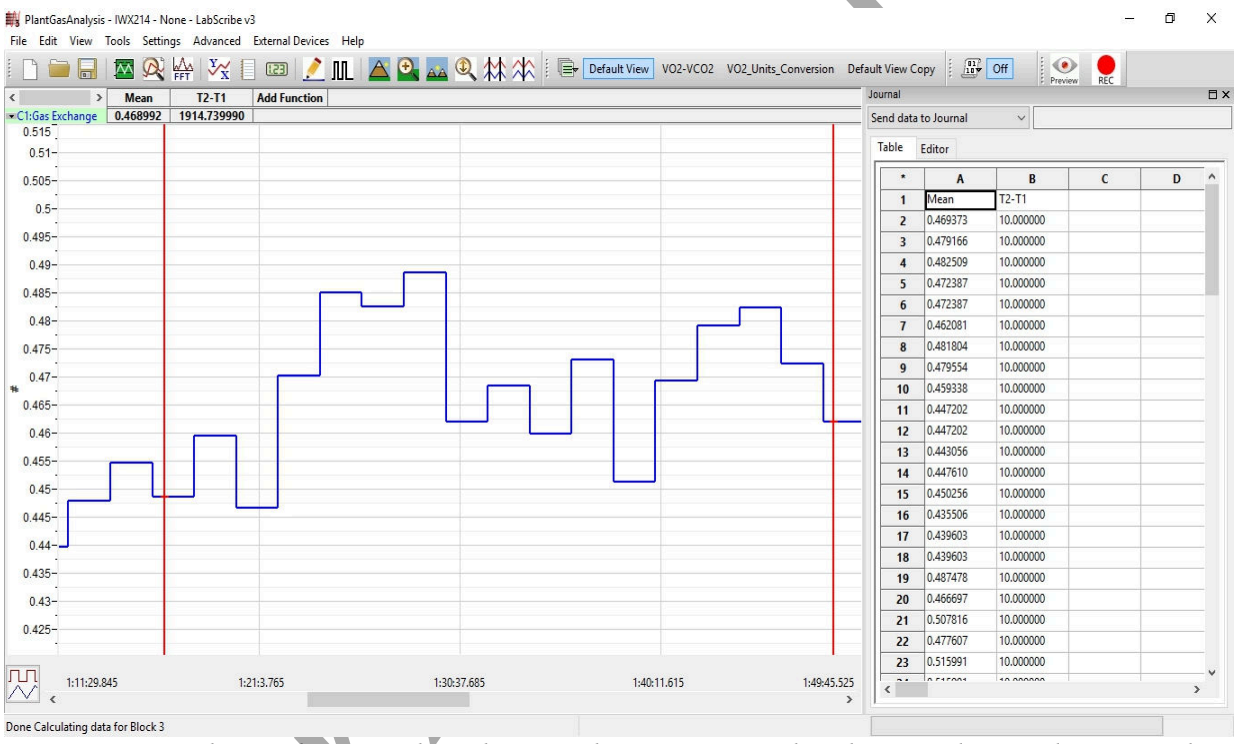

Figure CM-6-L7: The Analysis window showing the open Journal with mean data and T2-T1 values from the Gas Exchange Ratio channel.

Data Displayed using Excel as an example

After recording for 2 hours with a *Gerbera* daisy in the chamber, data was collected, Auto Find was used, and then the data was copied to Excel. The results are illustrated below.

Work le Lab

	A	B	C
2	Expired C(	4.526792	0.004722
3	Expired C(	4.531514	-0.08095
4	Expired C(	4.450567	-0.05602
5	Expired C(	4.394552	-0.08663
6	Expired C(	4.307919	-0.04232
7	Expired C(	4.265596	-0.05482
8	Expired C(	4.210778	-0.04302
9	Expired C(	4.167762	-0.00921
10	Expired C(	4.158551	-0.04918
11	Expired C(	4.109367	-0.0355
12	Expired C(	4.073862	-0.06275
13	Expired C(	4.011114	-0.03303
14	Expired C(	3.978088	-0.00931
15	Expired C(	3.96878	-0.04602
16	Expired C(	3.922765	-0.04204
17	Expired C(	3.880728	-0.00153
18	Expired C(	3.879203	-0.04142
19	Expired C(	3.837778	-0.0412
20	Expired C(	3.796582	0.003501
21	Expired C(	3.800083	-0.06011
22	Expired C(	3.739968	-0.01473
23	Expired C(	3.725236	-0.01663
24	Expired C(	3.708607	-0.05227
25	Expired C(	3.656339	-0.0153

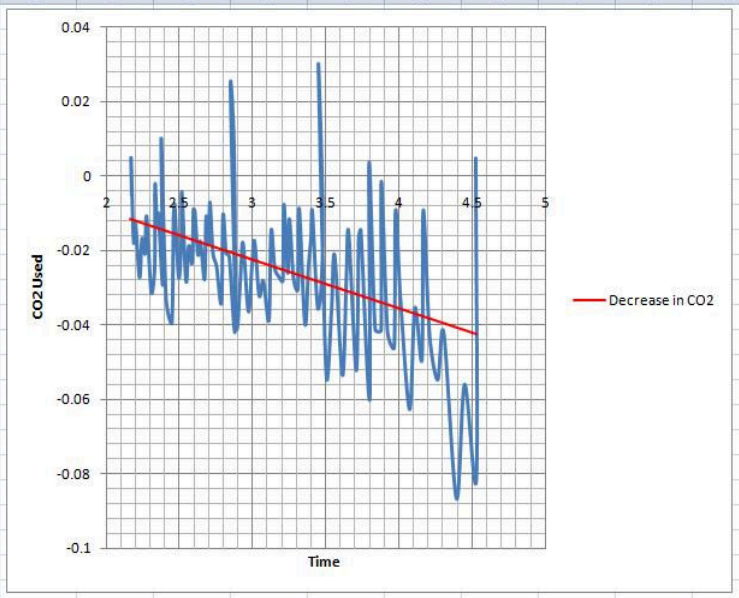

Figure CM-6-L8: Example of data set for change in mean CO_2 concentration over time, graphed in Excel with a trend line showing the decrease in CO_2.

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