MODEL AS-D1

DISSOLVED INORGANIC CARBON AND $\delta^{13}C$ ANALYZER

INSTRUCTION MANUAL

V2019.11

Apollo SciTech, LLC

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CAUTION!

1. **Please read this instruction manual before using the DIC-δ^{13}C Analyzer.**

2. **This product is designed for laboratory environments (land and onboard a ship).**

3. **Ensure the correct voltage is supplied to the instrument and the correct fuses are installed.**

This instrument requires a computer running Microsoft Windows to operate. We recommend that the computer is dedicated to the control of this instrument only. Connection to the Internet should be avoided. Virus protection software or Internet backdoor software (which constantly checks a website) may interfere with the execution of the analysis.

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A. UNPACKING AND INITIAL INSPECTION

A.1. Inspect the package

The Total Dissolved Inorganic Carbon (DIC) and isotopic CO_2 Analyzer (referred to as: "the DIC-$\delta^{13}C$ Analyzer" or "the Analyzer") was carefully packed in a sturdy carton box, with cushioning materials to withstand the shock during shipping. Inspect the exterior of the package for possible damage upon receipt. If damage to the exterior of the package is apparent, take photos and contact the shipping company immediately. The Analyzer is insured for its full price with the shipping company. Apollo SciTech LLC will not be liable for damage caused by the shipping carrier.

A.2. Unpack the package

Open the top of the package carefully and inspect for any signs of interior shipping damage. Please forward a report of any damages to Apollo SciTech LLC., in addition to contacting the shipping carrier.

When unpacking the instrument from the package, please ensure that the package contains all the items indicated on the Packing List. Please report any items on the Packing List which were not included in the shipment promptly to Apollo SciTech LLC.

B. INSTALLATION

B.1. Preparing to install your DIC-δ^{13}C Analyzer

For DIC-δ^{13}C analysis, you need a carrier gas, an acid solution and two waste solution containers.

B.1.1. Carrier gas: A compressed air is used as the carrier gas (not included with instrument). While compressed air in US has nearly zero CO_2 in it, a CO_2 scrubber such as soda lime or Ascarite II may be used to remove CO_2, and $Mg(ClO_4)_2$ is used to remove trace amount of H_2O in the air stream. A high-quality two-stage regulator capable of delivering a stable 16 psi pressure is required. For reference, 14.5 psi is about 1 atm. Fluctuations of the carrier gas pressure (when the tank pressure is very low) will reduce the accuracy of the analysis.

A PTFE filter should be installed to remove particles from the tank before going into the analyzer. Another PTFE filter is needed before the gas goes into the Picarro CO_2 analyzer to protect it from being contaminated by water or particle although internally Picarro has another inline filter. The PTFE filter may be replaced every year or as needed. A hydrophobic filter (0.20 μm pore size) is installed on the back of the Analyzer to filter out salt aerosols in the gas line. Inspect and replace it from time to time (every 500 runs or as needed).

B.1.2. Acid solution: A 1% H_3PO_4 acid + 7% NaCl solution (neither concentration needs to be precise) is used to convert all dissolved inorganic carbon to free CO_2. Take a 2.5-L reagent bottle, add 175 g of NaCl, add ~500 mL of DI water and shake it, then add 30 mL 85% concentrated H_3PO_4 to it. Now add DI water to the 2.5 L line and shake it.

B.1.3. Waste solution containers: Since the Analyzer discharges before and after each measurement, place a container, e.g., a bottle of 1 to 5 L, behind the Analyzer and run the waste tubing into the container. Put a 1-L container in front of the analyzer to collect the waste from port B (cf. Fig. 2 and section B.4), as the lines are cleaned before each analysis. Or run both waste tubes into the same waste solution container.

Note 1: It is recommended that the waste solution collector be positioned below the Analyzer's waste outlet, ideally on the floor below the Analyzer. Check the waste container periodically, and empty it as needed. Note that the waste solution is a relatively strong acid. Please discard it with sufficient dilution and follow local environmental protection regulations.

Note 2: The waste discharge valve inside the Analyzer is normally open. It is necessary to keep the waste discharge tubing placed inside the waste container. The connector marked "Waste" on the back panel of the Analyzer is also fitted with an internal shut-off valve; disconnecting the plug from the connector will stop the discharge. You may do so, or keep the end of tubing in a plastic bag without disconnecting the plug, when transporting the Analyzer to another location to avoid spilling acid during transport. Make sure that the waste tubing is reconnected and waste solution flow is open before use (see the end of section B.2 for more information).

B.2. Familiarizing yourself with the Analyzer

Your DIC-δ^{13}C Analyzer is composed of two main parts: the DIC analyzer (the front-end device) and the Picarro G2131-*i* Analyzer for Isotopic CO_2 (referred to as Picarro G2131-*i*; cf. Fig. 1). Inside the front-end device are the CO_2 gas extraction reactor, flow control devices, and the electronic control units. On the front panel is a power switch and a temperature indicator for the moisture reduction device.

Attached to the digital pump is a 12-port distribution valve with a 5- or 10-mL syringe, which is used to transfer acid and standard solution/sample for measurement. The digital pump is controlled through a computer by the original manufacturer's program. For more information about the digital pump please refer to the WinPump® User's Manual.

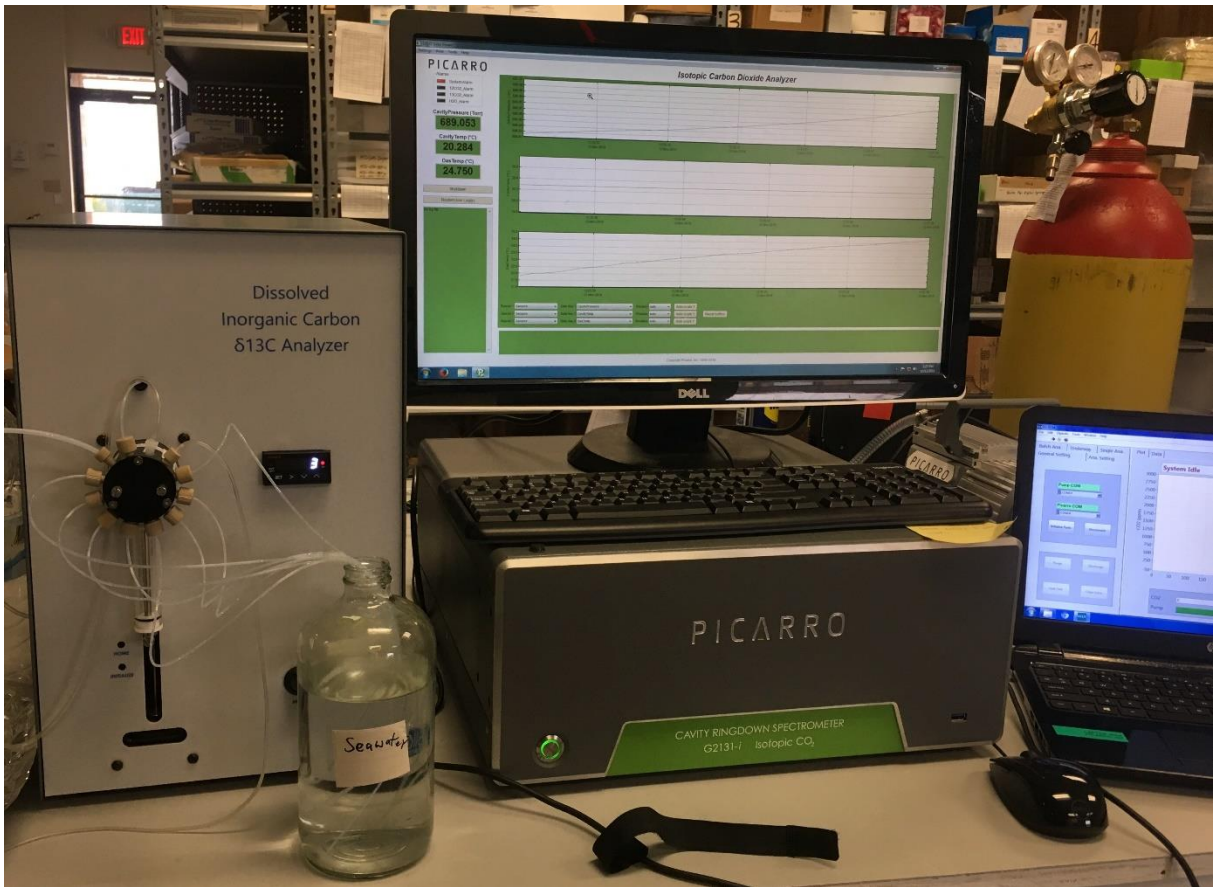

Figure 1. Front view of the DIC-δ^{13}C Analyzer model AS-D1 and Picarro G2132-*i*. For detailed information regarding Picarro G2132-*i*, please refer to its instruction manual.

B.3. Carrier gas connection

Install a high-quality regulator capable of delivering 16 psi (full scale is 30 – 60 psi) to a compressed air tank. Connect a piece of ¼" Tygon® tubing from the regulator to the "AIR IN" connector on the back of the Analyzer. Set the regulator outlet to 16 psi or slightly higher (< 17 psi) and check for air leakage. Fix any leakages. See B.1.1 for more information.

WARNING: Do not set the carrier gas pressure too low or too high. If the pressure is too low, it may not open the internal mass-flow-controller or deliver stable air flow at the desired flow rate. If the pressure is too high, it may burst connections inside the analyzer. A stable gas flow is critical. Inside the analyzer, a mass flow controller regulates the air flow to 60 mL/min.

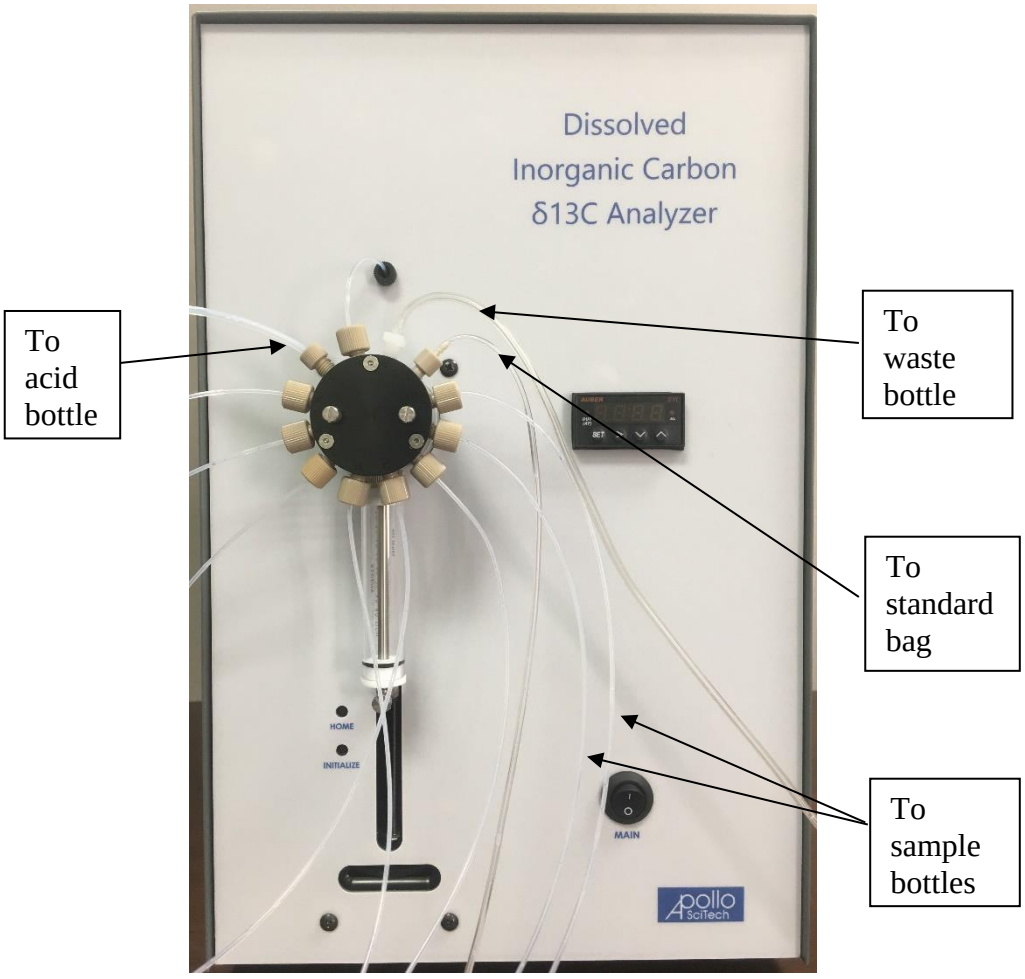

Figure 2. Front view of the AS-D1 analyzer with connection diagram

B.4. Acid solution and standard/sample solution supply

Place the acid solution (i.e., 1% H_3PO_4 + 7% NaCl) bottle to the left of the digital syringe. Connect a piece of 1/16" ID Tygon® tubing to port L of the digital pump, and place the tubing into the acid solution. Note that the end of the tubing should be completely submerged into the acid solution.

Connect a piece of 1/16" ID Tygon® tubing to port "B" of the digital pump, and place the tubing into a front waste bottle. This bottle doesn't need to be as large as the back waste bottle as the amount of discharge is small.

Attach 1/32" ID Tygon® tubes to port C – K of the digital syringe on the Analyzer. Connect port C to the standard solution bag and D – K to 8 different samples (see Fig. 2).

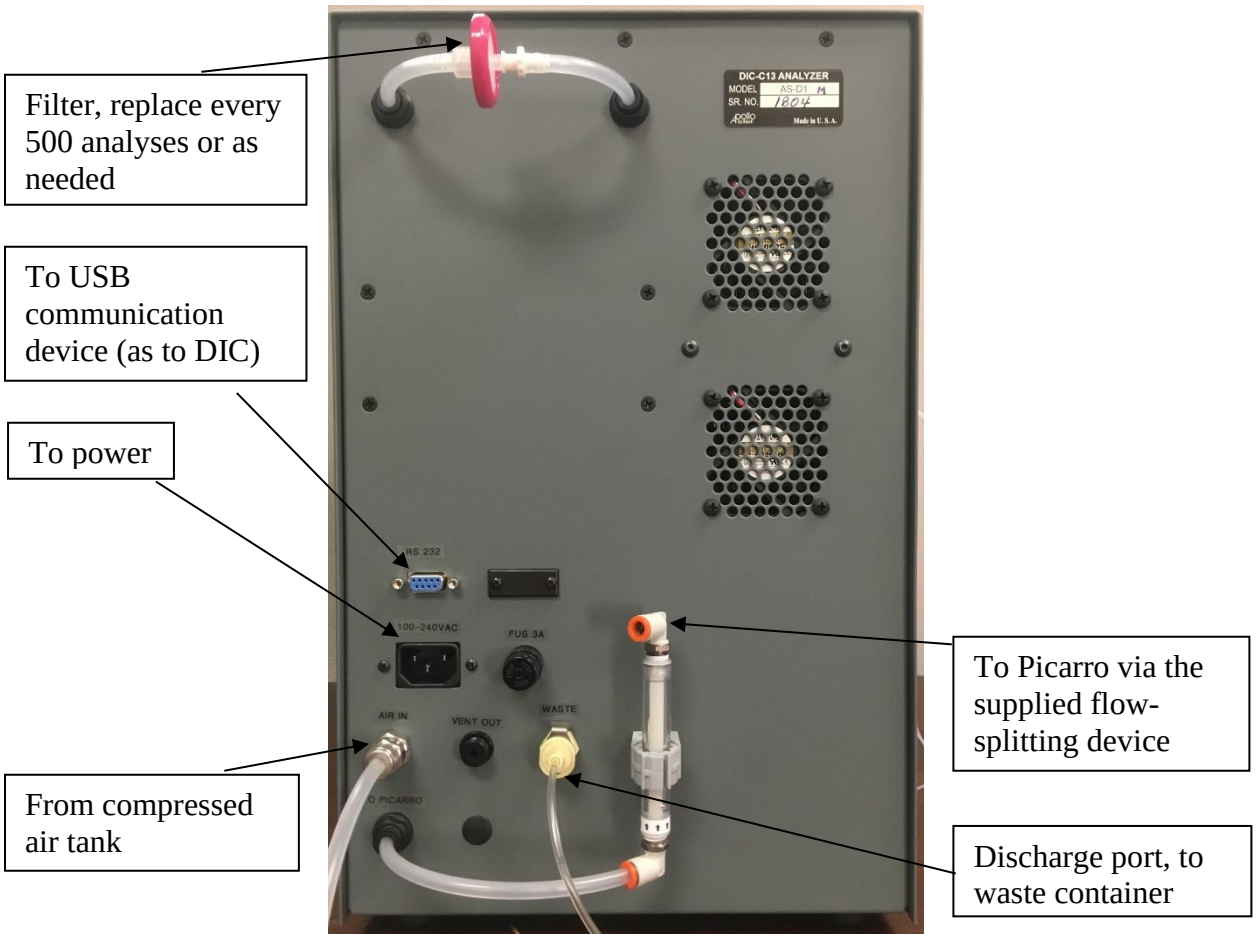

Figure 3. Back view of the AS-D1 analyzer with connection diagram (the exact position of each port may be different from this figure, in which case, refer to the labels on the analyzer)

When the sample volume is very limited, such as a total of a few mL of porewater, supplying the sample directly from a syringe via a piece of short tubing is recommended. Supplying water via a syringe may also be preferred if CO_2 degassing from a limited volume sample is a concern.

When plenty of the sample is available in a vial or bottle, withdrawing the sample via a tubing is the preferred method. The inlet of the tubing should be well below the liquid level to minimize the effect of CO_2 degassing to the headspace.

B.5. Computer connection

A Windows® operation system laptop computer is used to control the Analyzer. It is connected to the Analyzer via a USB port with a USB-serial converter (from USB in the computer to two RS232 serial ports, one connected to the Analyzer, the other one to Picarro G2131-*i*). If the user chooses to supply a computer, a similar laptop or desktop computer running the Windows® operation system is required.

We recommend that the computer is dedicated to the control of this instrument only. Connection to the Internet should be avoided. Virus protection software or Internet backdoor software (which constantly checks a website) may interfere with the execution of the analysis.

Refer to the Picarro manual and the digital pump manual for pin assignments.

The software automatically detects two available serial ports, e.g., COM5 and COM6. Click the drop-down menu and select one for each box in sequence.

B.6. Power supply connection

Check that all of the power switches are in the "OFF" position before connecting the wall outlet.

Figure 4. Communication connection at the back of the Picarro G2131-*i* analyzer.
*When you turn off Picarro G2131-*i*, make sure you also turn off the power switch on the back.
** Turn the vacuum pump on BEFORE Picarro G2131-*i* analyzer!

B.7. Computer program installation

Note that the supplied laptop computer has been installed with the control software and the USB-serial converter driver, and thus the following description only applies to users who supply their own computer.

Copy the entire folder in the memory stick to your computer desktop. Inside the folder AS-D1M v0.65 there is an executive file named AS-D1 X12. You can double-click it and run from there or send this file to the desktop and create a shortcut there.

C. DIC-$\delta^{13}C$ MEASUREMENT PRINCIPLE

AS-D1 allows simultaneous analysis of both DIC and $\delta^{13}C$ precisely in water sample.

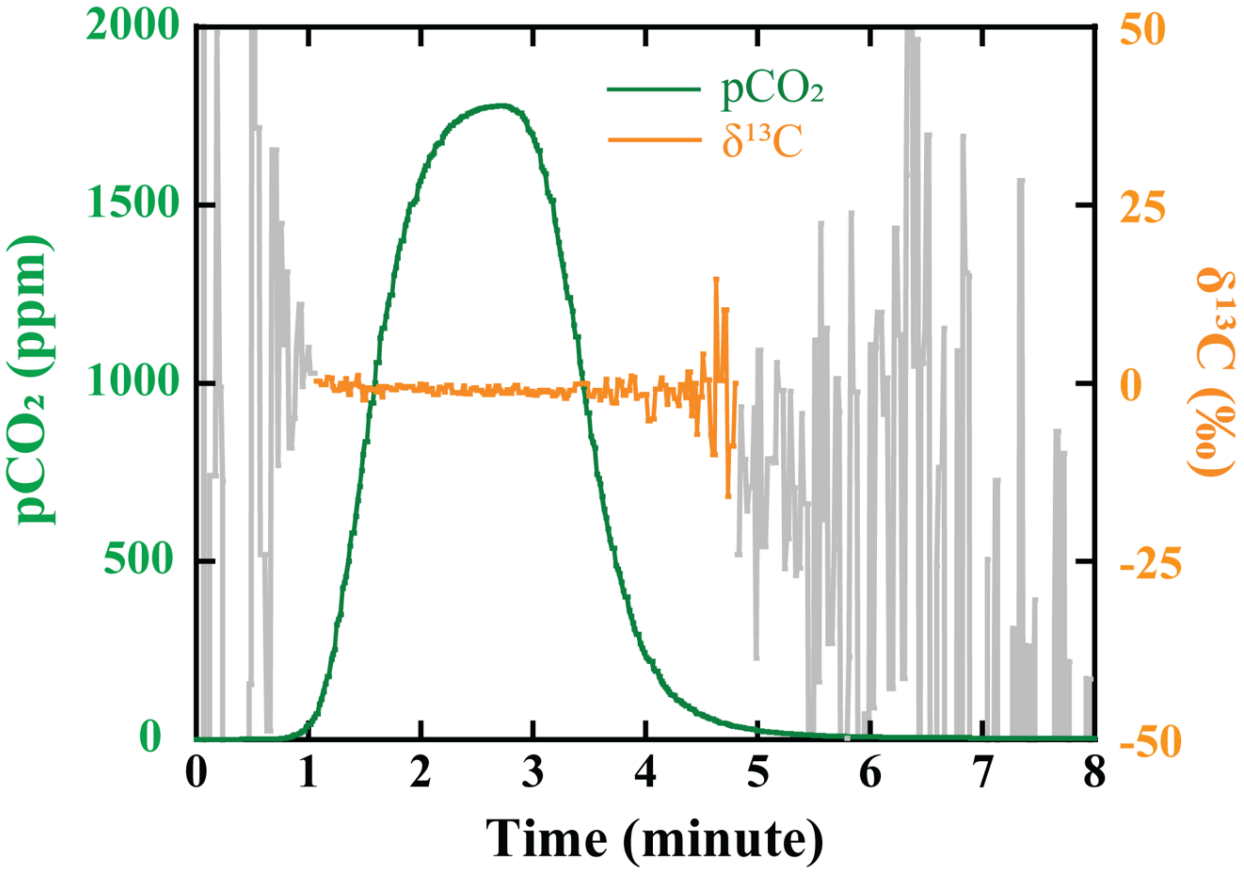

Figure 4. Illustration of the DIC and $\delta^{13}C$ signals.

The area under the pCO_2 curve is the precise DIC value after calibrated with the integrated area using a standard or a Certified DIC Reference Material (CRM). The $\delta^{13}C$ value of the sample is measured directly from the weighted averaged stable $\delta^{13}C$ values represented with pCO_2 > 350 ppm (user-definable). $\delta^{13}C$ measurements transition from very noisy readings in nearly CO_2-free carrier gas to stable readings in sample CO_2. The criterion to end the analysis is now based on a precision of 3 repeats of the DIC analysis (now set at 0.15% but is user adjustable).

D. OPERATION

⚠ ***Caution:*** *During every measurement cycle* ***the waste solution in the reactor is discharged both during the end of an analysis and at the beginning of the next analysis****. If it does not, stop running the program and check for waste discharge problems (such as blockages in the waste tubing). Do not run the Analyzer until the problem is solved!*

D.1. Warming up

It is preferred the **Picarro G2131-*i* Analyzer** is turned on the night before and is on all the time during a period of analysis (days or weeks). **Remember to always turn on the vacuum pump first**. Make sure a PTFE filter is installed before the inlet of the G2131-*i* despite the fact there are internal filters inside G2131-*i*. This will prevent water and particles from getting into the G2131-*i* and save you troubles and money.

Turn the two-stage regulator valves of the carrier gas (air) on with an output pressure of 16 psi. Make sure the gas exit valve in the back is open (Fig. 3, right, to the flow-splitting device). This valve should be open sufficiently to sustain a flow rate of 60 – 80 mL/min, which is regulated to a constant value (within ± 2 mL/min) by a mass flow controller inside the Analyzer. Note in a dust-free lab environment, you may leave this valve open all the time.

Turn the Analyzer's power switch on. Allow a warm up period of 60 min. The temperature display on the front panel of the Analyzer should show 3°C.

D.2. Settings of the G2131-*i*

<Refer to the User's Guide of **G2131-*i*** Analyzer for Isotopic CO_2>

D.3. Digital pump HOME position setting

When you receive the DIC Analyzer, the HOME position of the digital pump is already set at the correct position. Unless absolutely necessary, do not try to reset it.

If the zero volume position of the piston in the syringe has been changed and is more than 2 mm from the top, reset the HOME position (the newer version has only a very small gap of <0.5 mm). The procedure of resetting the digital pump HOME position is:

1. After powering up the DIC Analyzer, push the "INITIALIZE" button on the front panel of the digital pump.

Note: the piston position after you push the "INITIALIZE" button is ***NOT*** *the* ***"HOME"*** *position.*

2. Observe the top position of the piston inside the syringe while turning the thumb wheel on the lower level of the front panel to left until the piston is approximately 0.5 mm away from the top of the syringe. (or move the piston all the way to touch the top by turning the thumb wheel to the left, and then turn the wheel to right for about 1/16 of a turn)

   Note: If the piston is too close to the top of the syringe, the syringe may be damaged when pushed too hard by the piston.

3. Push the "HOME" key on the front panel of the digital pump to complete the HOME position setting.

WARNING: Whenever the thumbwheel is moved, whether the power is on or not, a new HOME position must be set to avoid possible damage to the digital pump's syringe.

D.4. Running the analytical program

Double-click the shortcut icon "δC13" on your computer's desktop to start. Note most of the steps below are already set in attached laptop.

Under the **General Setting Tab** on the left panel. Click the downward arrow on Pump COM and Picarro COM to bring out the drop-down menu, where you will find available serial port numbers. Choose accordingly and then click initialize to establish connection between the computer and the Analyzer (you should hear the pump moving noise).

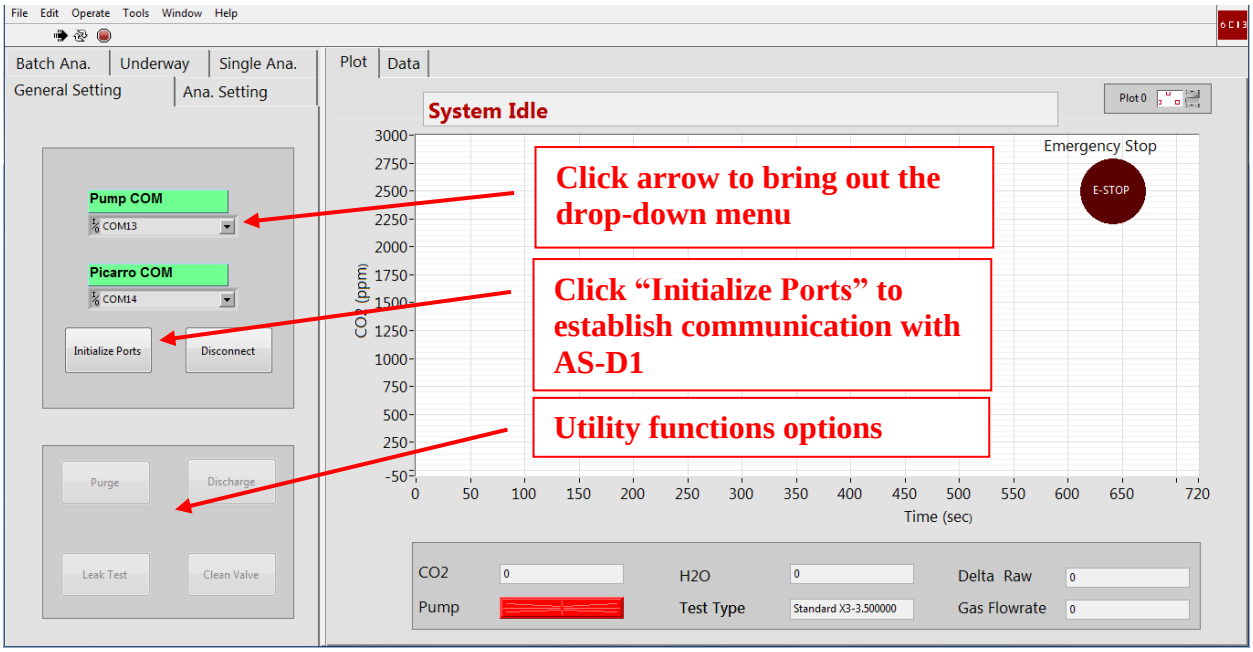

Figure 5. C13 program interface

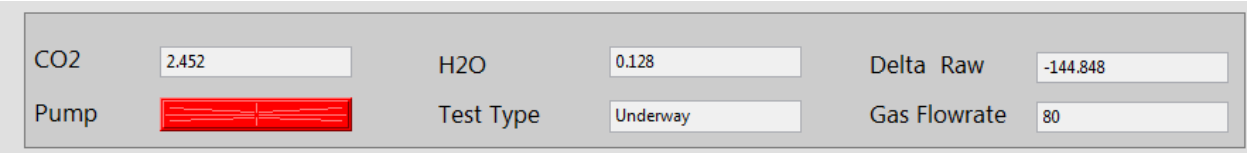

Figure 6. C13 program parameter setup box (*the red bar indicates the pump is busy; do not click any button when the pump is busy*)

Once the communication is established, CO_2, H_2O, and gas flow rate reading will start streaming in and display on the status panel on the right panel. Together with Picarro data streaming, you will also find pump status and other information of the analyzer.

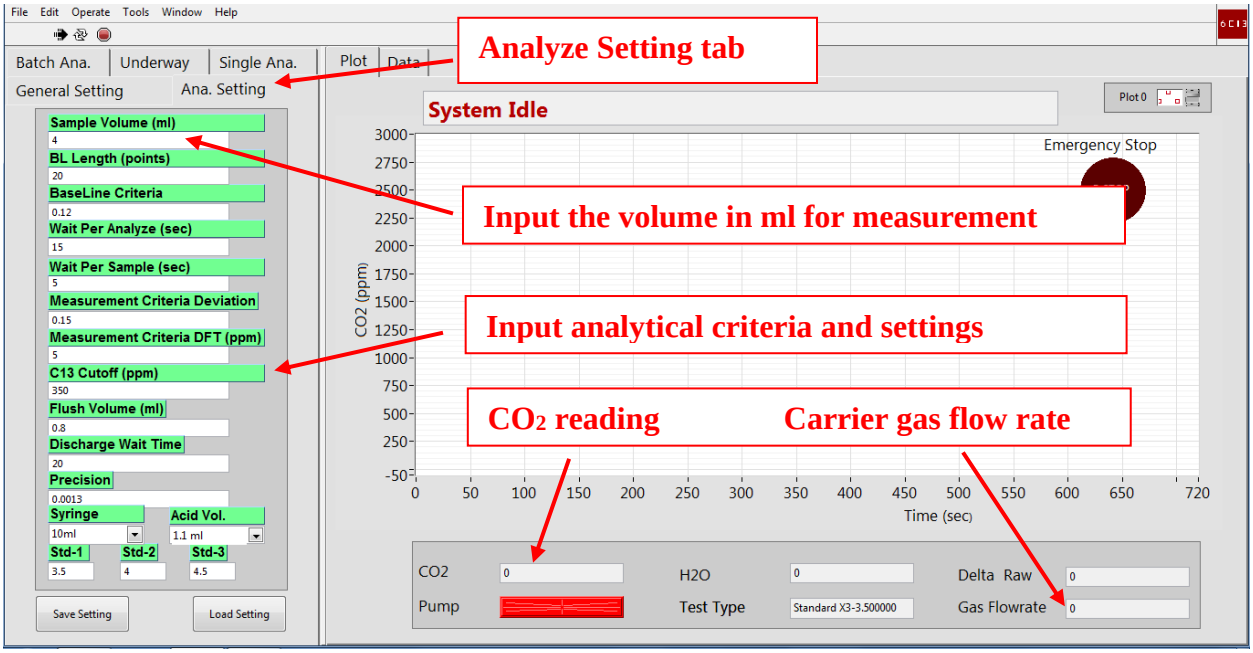

Figure 7. DIC program communication created and sampling volume box.

Click **Analyze Setting** tab to setup basic analytical criteria and settings.
1. **Sample Volume** determines the volume of the sample in both batch test and underway test. Recommended Value (RV) = 4 – 5 mL. Single test has its own independent volume setting.

2. **BL length (pt)** dictates how many points of CO_2 reading should be averaged as baseline for the subsequent area integration. RV = 20.

3. **BaseLine Criteria** dictates how stable the CO_2 reading should be, before the sample is injected, e.g., 0.12 means 20 readings of CO_2 should reach a standard deviation of 0.12 before the sample is injected. (This criterion is currently disabled with an internal fixed setting.)

4. **Wait Per Analyze** determines how many seconds is needed between each repeat. During the waiting time, the carrier gas bypasses the reactor and is directed into the CO_2 analyzer, which helps with stabilizing the baseline reading for the next measurement. RV = 15.

5. Wait Per Sample determines how many seconds is needed between each new sample. RV = 5.

6. **Measurement Criteria Deviation** determines how a measurement is deemed successfully completed by standard deviation. Once a standard deviation of 10 reading falls under the specified value (e.g., 0.15 ppm), the measurement is deemed successfully finished, and result recorded. Note: either Deviation or DFT (baseline difference, see next paragraph) is triggered, the measurement will be finished and recorded. The Deviation is a preferred mode. We recommend 0.15 as the termination condition. With this criterion the baseline difference (BL Diff) is typically 8 – 9 ppm. You may also try a higher deviation of 0.2 to save analytical time or a lower deviation of 0.12 to have a more stringent condition.

7. **Measurement Criteria DFT (ppm)** determines how a measurement is deemed successfully completed by the difference of the two baseline values. Once the average of 10 readings falls between the baseline and the sum of the baseline and the specified value, the measurement is deemed successfully finished, and result recorded. Normally a difference of 5 ppm between baseline after or baseline before is good enough.

8. **Flush Volume**. At the beginning of each sample measurement session, the specified volume of sample will be drawn to flush the sample tubing and valve path. 0.6 mL is set now. If you have a very long sample tubing (for example at an underway mode), you may consider use a larger Flush Volume of 2 mL.

9. **SD-1, SD-2, SD-3** determines a set of volumes of CRM to be used for standardization. A $NaHCO_3$ solution (~ 2 mM) with known $\delta^{13}C$ value can be calibrated against CRM and serve as the DIC standard. By using 3 volumes, we can use one standard of known DIC concentration to create a DIC calibration line. A recommended standard series is 3.5, 4.0, and 4.5 mL for a sample volume of 4 mL. The calibration line can be extrapolated to sample concentrations of at least 50% – 200% of the standard line range. Otherwise, users can prepare different concentration range of their standard.

10. A recommended setup is shown in Figure 8. Once the setup is done, click the Save Setting button, all settings in the **General Setting** and **Ana. Setting** tabs will be saved and reloaded each time the application is opened.

11. **Gas flow rate**: This rate is not adjustable in the software. Internally, Picarro G2131-*i* has a flow of about 28 – 30 mL/min. We set the mass flow rate to the reactor at 60 mL/min. Users should not adjust the flow rate until they have enough experience with the analysis and have consulted the manufacturer. The flow rate can be adjusted via a knob on the mass flow controller inside the front-end device (turn clockwise to higher rate).

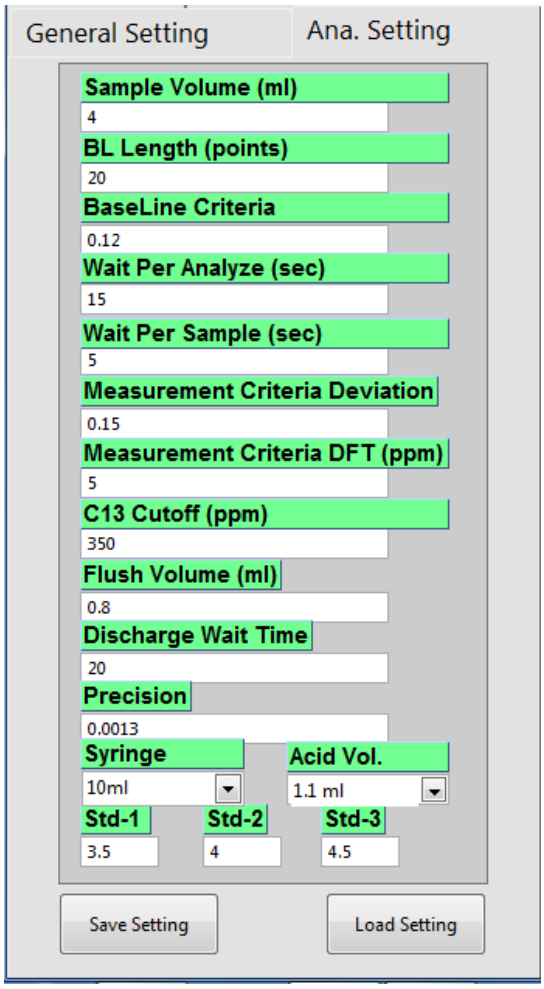

Figure 8. Recommended setup for test setting. We recommend two sets of standards: for small sample sizes, use 2.6, 3.0, and 3.4 mL, and for medium sample sizes, use 3.5, 4.0, and 4.5mL. Larger volumes of the standard such as 4.5, 5, and 5.5 mL are recommended for larger sample sizes if a 10 mL syringe is used. Samples of up to 8 mL will take longer to measure and do not necessarily provide analytical benefit.

D.5. Analytical procedure

This function is designed for test and learning purposes. For real sample analyses please use the tab **Batch Ana.** (cf. section D.8**)**.

Place the tubing from port C of the digital pump into a standard solution or water sample, or connect the tubing to a glass syringe containing a standard solution or sample. Click **Single Test** then click sample volume cell to input a volume of the sample/standard to be measured. See Fig. 7. The value of the previous measurement is shown in the cell. The allowable range of the volume is from 0.5 to 4 mL (preferred range is from 2 to 3.5 mL) for a 5-mL syringe with a 0.9 ml acid selected. A volume of 3.0 mL is recommended for seawater samples. For a 10 mL syringe and 1.1 mL acid, use 4.0 mL or slightly larger sample volume (though one can go up to 8 mL).

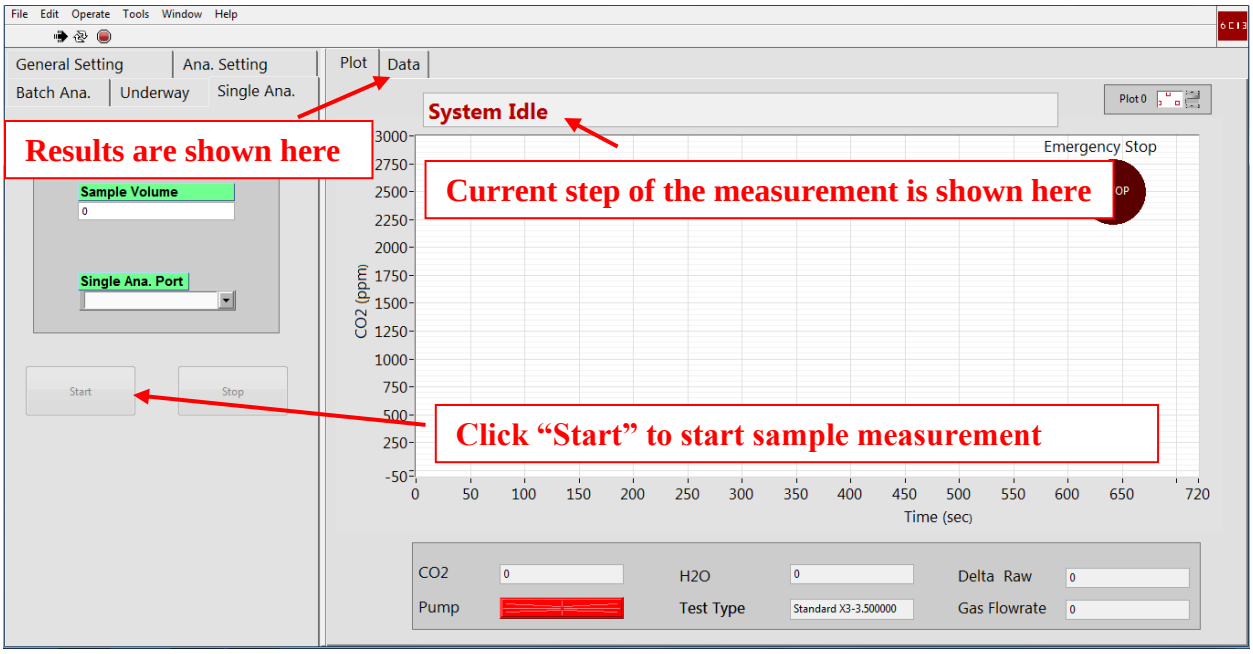

Figure 9. Single sample measurement

Click the **Start** button on the left panel to start measurement (Fig. 9). After pumping the reagent and sample solutions, the Analyzer will start to integrate the CO_2 produced over time. Once the integration has started, the plot window on the right hand side will start drawing the curve of the CO_2 reading from Picarro G2131-*i* until the integration is completed. The result is shown in the **Data** tab, with the integration area and other info (Fig. 10).

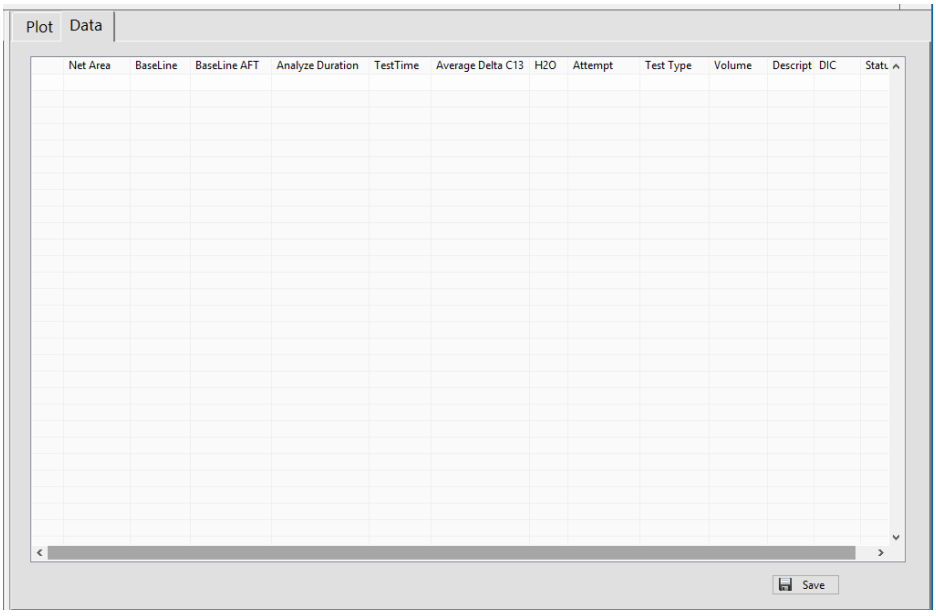

Figure 10. Data table

After flushing, the repeated analyses usually agree within ± 0.1%. Following each measurement, record the result (peak area) and the volume measured.

Tip: Waiting for similar intervals (or idle times) between measurements, e.g., 20 seconds, will provide more precise results.

D.6. Standardization

Connect a standard solution of known DIC concentration to port C of the digital pump. Then create the standard line in an excel file and calculate the final sample DIC in the excel file, after calculating the average net integration area for three volumes of standard based on three repeats of each volume.

You may run the standard as a sample, using different volumes of a primary standard you purchased or a secondary standard you prepared. Solution volumes between 2 and 5 mL are recommended (such as 2.6, 3.0 and 3.4 mL or 3.5, 4.0 and 4.5 mL, depending on the expected DIC concentration range of your samples and the size of your syringe). If you are analyzing open ocean seawater with a limited DIC range, then a tight range is recommended.

After all standardization volumes are completed, use a linear regression software program to determine the relationship between net integration area and the DIC content by converting each volume of standard solution into total DIC content. This can be performed conveniently on a spreadsheet program such as MS Excel® (as illustrated in Fig. 11).

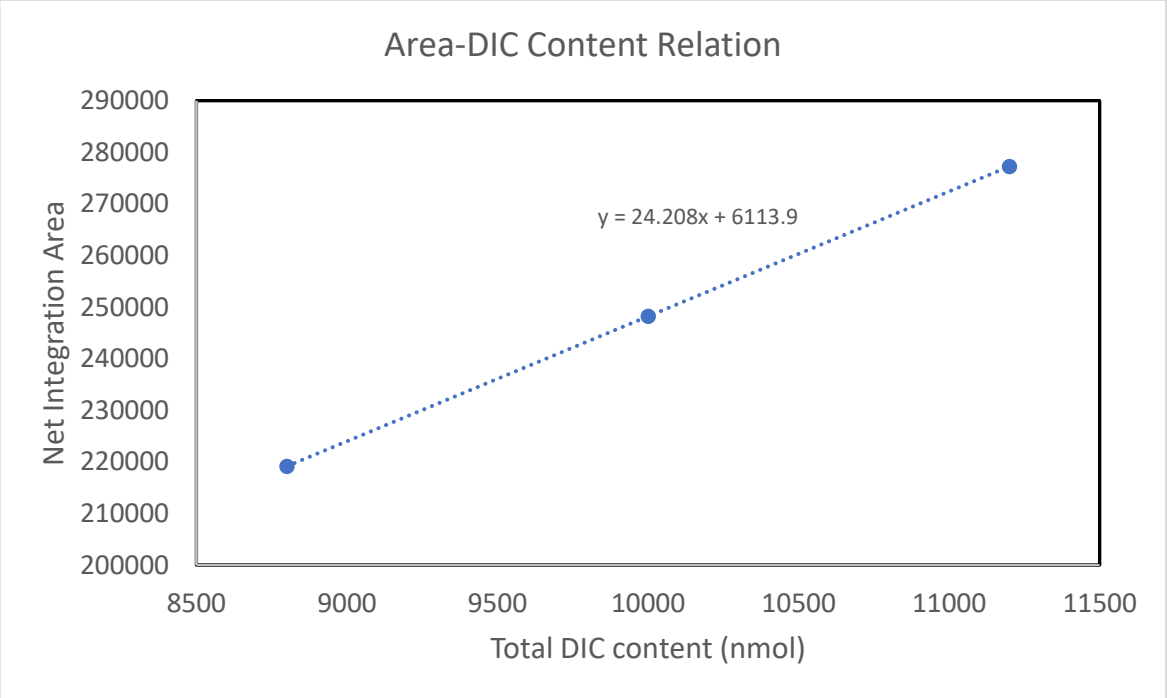

Figure 11. Net integration area-total DIC content relationship

Example Calculation:
DIC of standard: 2000 μM
Sample volume: 5 mL
Measured Integration Area: 218723
Regression equation: Integration Area = 24.208 * Total DIC content – 6113.9
Re-arranging the regression equation:

$$Total\ DIC\ content = \frac{Integration\ Ara + 6113.9}{24.208} = \frac{218723 + 6113.9}{24.208} = 9287.7\ (nmol)$$

DIC = $\frac{9287.7\ nmol}{5\ mL}$ = 1857.5 μmol/L = 1857.5 μM

You may want to keep your standard in a gas-tighten bag. Instrument will drift slightly. Therefore, you should correct the drift using standard runs before and after you run a set of samples. In this automated analyzer, a set of standards is followed by 8 samples and then another set of standards).

For the analysis of seawater samples, a standard solution of Certified Reference Material (CRM) is recommended. The CRM is prepared by Dr. A. Dickson of the Scripps Institution of Oceanography, La Jolla, CA 92093, U.S.A. (http://andrew.ucsd.edu/adickson/co2qc/). For low DIC freshwater samples, a very small volume of the Dickson CRM may be used, or you should prepare a low DIC solution and use it after calibrating against the CRM. For high DIC (> 5 mM) sediment, porewater or groundwater samples, you may prepare custom DIC standards. Such a standard may be prepared from reagent grade Na_2CO_3 after baking at 285°C for two hours or you may prepare your standard with $NaHCO_3$ and calibrated it with CRM. It is recommended that any self-prepared DIC standard solutions be standardized against the CRM.

For DIC-$\delta^{13}C$, we suggest two types of standards.

1. Acquire a bottle of $NaHCO_3$ solid. Send some to a stable isotope lab with high credit to measure its $\delta^{13}C$ with isotope ratio mass spectrometry (IRMS) method. Prepare your standard with DIC concentration similar to that of seawater, fill a few bottles and use these bottles of artificial seawater as a standard. You may bubble DI water with N_2 to drive out dissolved CO_2 gas before using it to prepare the standard.
2. Prepare your standard with DIC concentrations similar to that of seawater and bubble this water with room air for 3 days (its $\delta^{13}C$ value will be nearly stable) before filling it in 250 mL borosilicate reagent bottles. Prepare 30 bottles. Send 3 bottles to a stable isotope lab for analysis. Use other bottles as the standard.

D.7. Sample measurement

Port D – K are reserved for samples. In the software program, select the desired volume for the measurement (4 mL is recommended). Then click the "Start Test" button (Fig. 9) to start sample measurement. The program will record the results (Net Area, Peak, etc.) and repeat until a consistent result is achieved.

Note that the results will also be saved to a file. Click the tab "Data" to open the result sheet (Fig. 9), which you can export to either Excel file or text file.

Calculate the DIC content according to the linear regression function obtained during standardization. This can be performed on a spreadsheet program such as MS Excel®. Alternatively, convert the final value directly into concentration units (μM or μmol/L). Again, this is automated in the Analyzer. Note it is preferred to run samples using the batch analysis (cf. D.8.)

Record the room temperature of each measurement (sample or standard). This allows calculation of the sample density and conversion of units between μmol/L and μmol/kg, according to this equation:

Density = $(999.842594 + 0.06793952*T - 0.00909529*T^2 + 0.0001001685*T^3 - 0.000001120083*T^4 + 0.000000006536332*T^5 + (0.824493-0.0040899*T + 0.000076438*T^2 - 0.00000082467*T^3 + 0.0000000053875*T^4)*S + (-0.00572466 + 0.00010227*T - 0.0000016546*T^2)*S^{1.5} + 0.00048314*S^2)/1000$
(check : at T=25°C and S=35, density = 1.02334)

Running samples with high surfactants: Some users may analyze samples with high surfactant content (such as soil pore fluid). Foams/bubbles may form during the analysis. Such bubbles may go through the gas line and into Picarro G2131-*i* and damage it. While the instrument was not designed for this type of analysis, we have recognized the need to analyze such samples and have added filters to stop the bubbles from entering Picarro G2131-*i*. Great care must be exercised in analyzing high surfactant samples. If significant amounts of foams or bubbles are noticed inside the filter or in the tube before the filter, do not repeat the measurement of the same sample. Instead, flush the contaminated tubing (and dry it with gas flow) and replace the filter.

D.8. Batch process for standard and sample measurement

There is a batch process function in the program for sample measurements. We recommend users to use this function to do all the analyses. Click tab **Batch Analysis** (Fig. 12) to perform batch measurement.

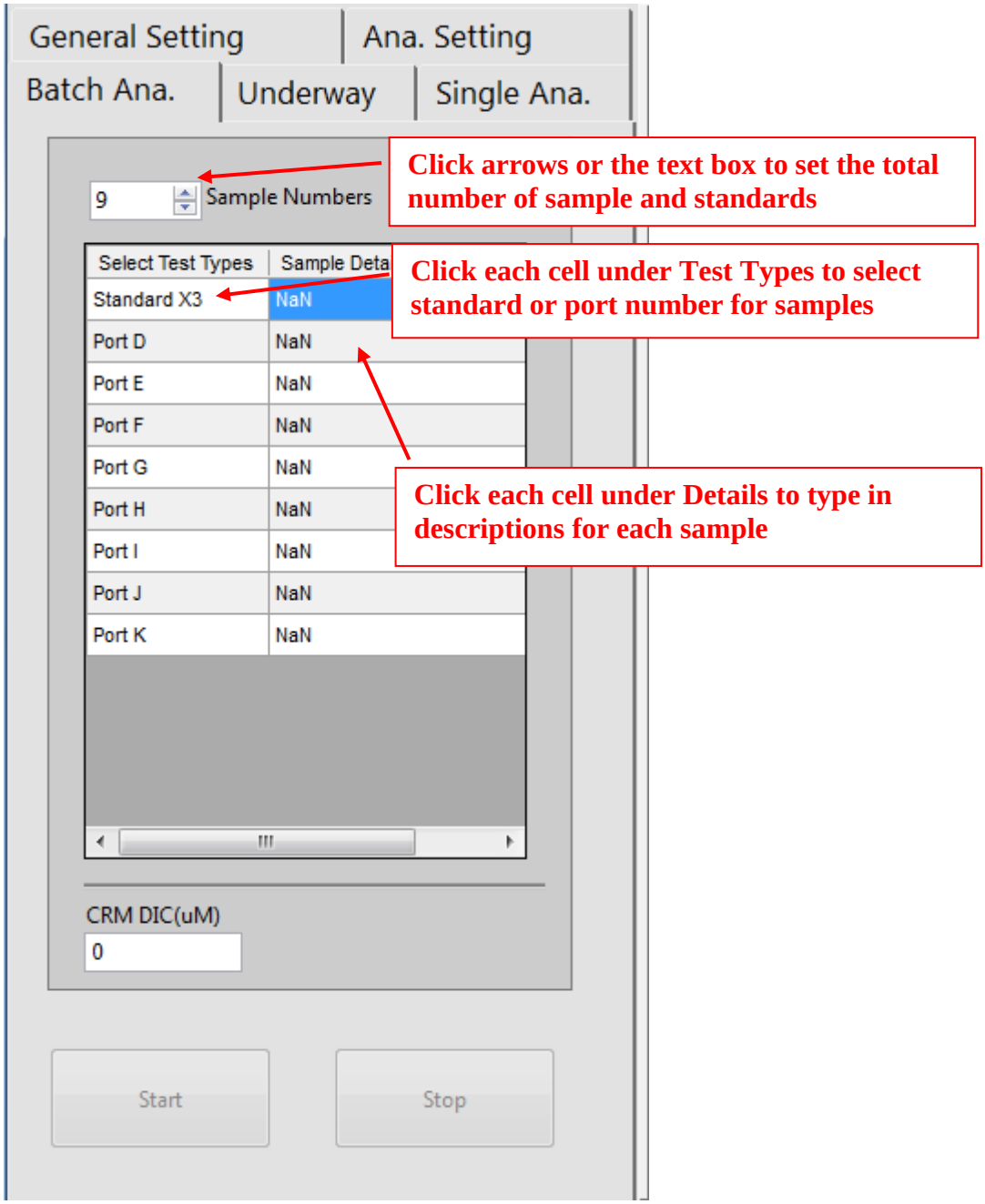

Figure 12. Batch analysis

Under the Batch Ana. tab, you will find a view grid for the batch test orders. Each row represents a sample in the batch. For each sample, the analyzer performs up to 5 runs, and if 3 out of 5 runs has a standard deviation within 0.12% (listed as 0.0012 in the Ana. Settings tab and is user definable), the sample is deemed valid. If the first 5 runs fail to fall within a standard deviation within 0.12%, the program makes a second attempt. Depending on your needs, you may change the precision to values 0.001 – 0.002. The default batch list starts with standard 3X (3 volumes of the standards), which is connected to port C. We recommend that you store your standard in a gas-tight bag. Then the list includes 8 samples on port D, E, F, G, H, I, J, and K. Then the list

cycles from Standard X3 to port K. Another Standard X3 is recommended after a group of samples to facilitate the two-standard-correction for instrument drift with time. You may also choose to insert 1X or 3X standards at any point.

Click on Sample Numbers to add more rows to the grid. Click on Test Type option boxes to bring out the drop-down menu to select a Standard X3 or a sample port if desired.

Click on Sample Details to type in descriptions of each standard or sample.

Click the button "Start" to start the measurement. The program will automatically perform the batch analysis order.

Note 1: Many users may find it easier to simply treat the standard as unknown samples (again use 3 volumes). At the beginning and end of analysis (or a day), run a standard curve with 3 volumes. Then, insert the standard as an unknown every 4 to 8 samples using only one volume (the same as the samples). Then do the calculation in a spreadsheet program. This is convenient for a few reasons. As the instrument will drift slightly with time, one cannot apply the same standard curve over a day, thus adjustment is needed. Also, one will have to convert the molarity (μmol/L) to molality (μmol/kg) using sample salinity and room temperature, so it is necessary to use a spreadsheet program.

Note 2: Air bubbles may form inside the sample syringe on top of the piston or attached to the ceiling/top of the syringe). Small air bubbles of < 1mm in size do not seem to be a problem as they stay there stable most time. If you see large bubbles (3 – 4 mm), this is often the reason of large noises in the data as bubbles change size with time and with room temperature. First check if the digital syringe is not tightened. Please tighten it by turning the syringe clockwise using two fingers. Do not overtighten it. If the syringe is tight, check if the tube connectors are tight (if not, use fingers to tighten them. Do not overtighten them.). One may flush bubbles out by tapping the (bottom of the) syringe. Lift the acid tubing to air (or disconnect) for a few seconds to create an air space in the syringe, which will eliminate all air bubbles.

Air bubbles may also form even if there is no leakage. This problem of bubble formation occurs most with river/estuarine samples (cold and fresh water is rich in dissolved air) inside a warm room. **We strongly recommend putting bottles of samples and standard in a cold water bath** (~16°C). The best result is expected with this approach even with seawater analysis as a precisely known temperature allows a precise density for the conversion from molarity to molality.

If the syringe is leaking, then it needs to be replaced.

Procedures to remove the syringe: 1) unscrew the screw on the bottom of the syringe using a screwdriver, 2) hold the top of the syringe and unscrew it from the valve.

Procedures to reinstall the syringe: 1) Screw the syringe to the valve. 2) install the screw on the bottom of the syringe. Note that the lower screw may not be at the exact height as the screw hole on the pump. In that case, do not force it in, but push the Initialize button on the pump and the

then turn the thumb wheel to move the hole to a proper position. After installing the screw. Reset the home position by turning the thumb wheel all the way to the left and turn right for 1/16 of a turn. Then push the Home button to finish the procedure.

D.9 Underway Process for Sample Measurement

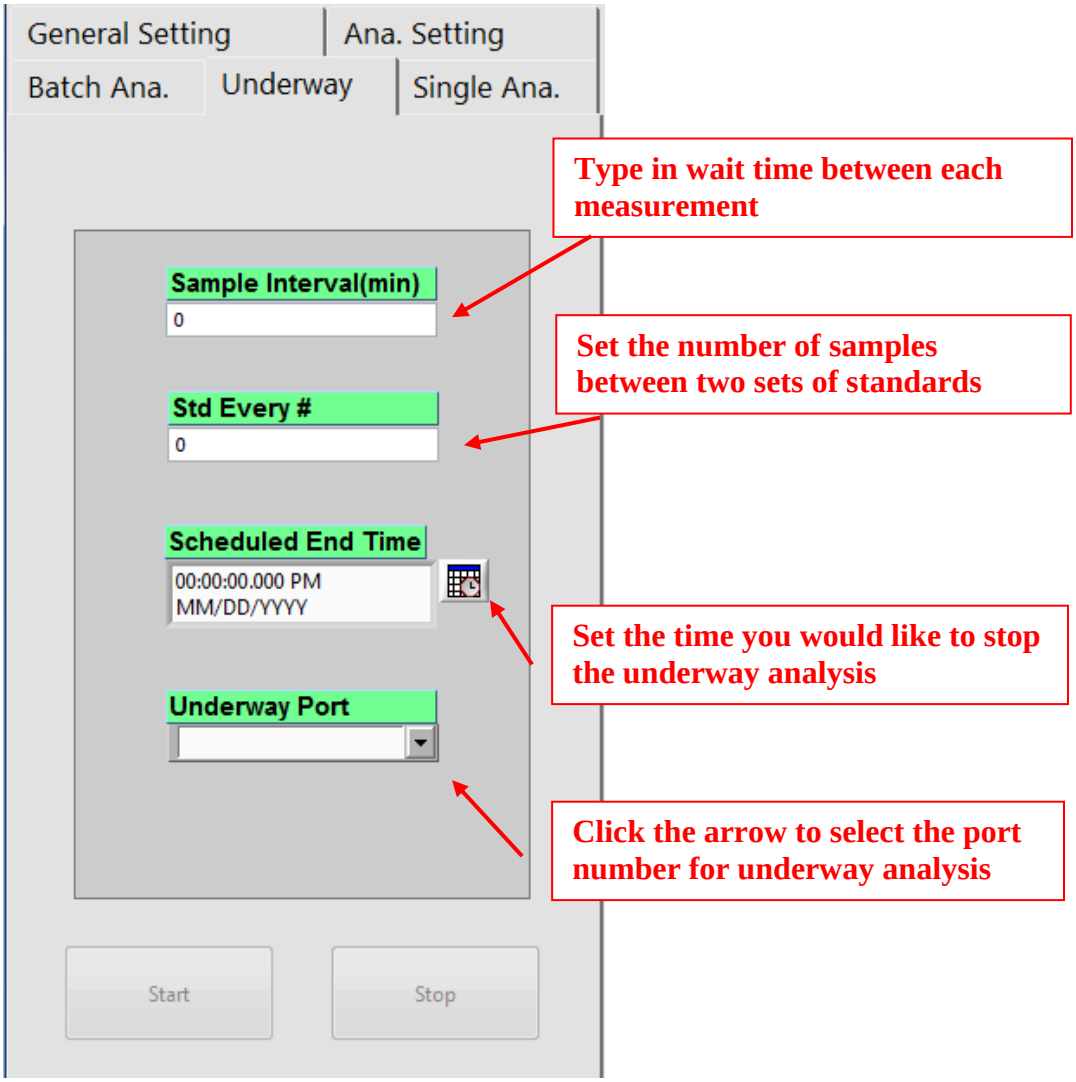

Figure 13. Underway process

This function is still under development is not fully tested. The underway process is best suited for on board (or at the dockside) measurement for a continuous period of time. **Sample Interval (min)** controls the wait time between each underway sample. **Std Every #** controls how many samples are analyzed before a set of standards is measured. **Scheduled End Time** controls when the underway process should stop. **Underway Port** controls from which port the program takes underway samples.

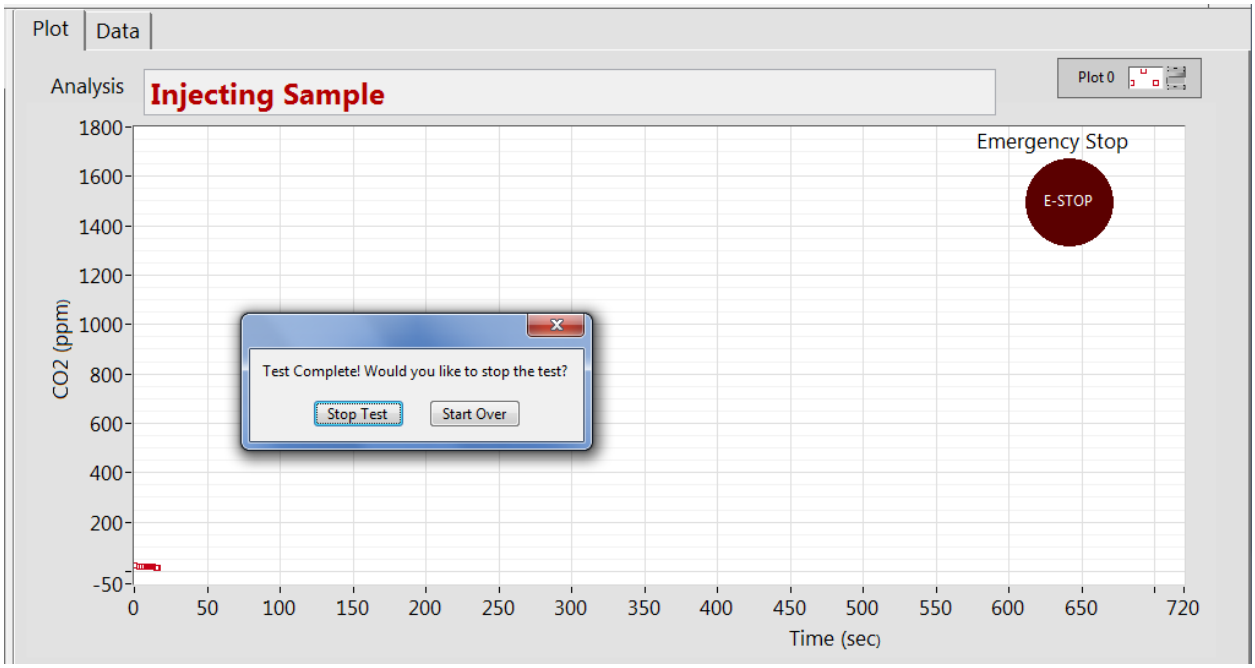

Figure 14. Pop-up dialog

When the test is completed, a pop up window will appear and the user may decide to perform the same task again or set the Analyzer to idle.

D.10 Ending the measurement and cleaning the analyzer

Reminder: Before shutting down the instrument, put the reagent/acid inlet into DI water and do the following:

Once the measurement is finished, put both the acid and all sample tubes (including the standard tube) into the DI water bottle. Then in the General Setting tab, click the button Purge to flush the entire acid path and syringe. Remove the acid tube from DI water and leave it in air, click Purge again.

Then put all tubes in DI water and click the button Clean valve. It will draw liquid of 1/10 of the syringe's total volume (1 for a 10 mL syringe or 0.5 for a 5 mL syringe) from each port of C, D, E, F, G, H, I, J, K, and L and discharge it via port B to the waste. It will repeat these 3 times. Then repeat this procedure with all tubes in air.

D. 11 Data saving and retrieving

Click the "Save" button at the bottom right corner of the "Data" tab (Fig. 10) to save the data to your desired folder before closing the software.

If the software was closed before you were able to save the data, back data files can be found in folder AS-D1M V0.65 > data > E > Personal > Backup > Apollo Scitech > ASC5-6 Project > Local File > AS-D1M V0.65 beta.vi. The text files are named by the date and time that they were created, before the measurements took place.

E. SPECIFICATIONS

Power supply: 100 – 240V AC (universal power, auto-switch)
Carrier gas required: Compressed air, ~16 psi exiting pressure
Repeatability: ± 0.1% at DIC concentrations ~ 2000 μmol/L
Repeatability: ± 0.05 – 0.1 in $\delta^{13}C$
Sample volume: 2 – 8 mL
Time required: ~11 minutes per measurement
Computer requirement: 80486 or up
Computer operating system: Windows XP®, 7, 8 or 10
Communication: RS232C or USB (a USB-RS232 converter is included)
LI-COR setup
- **Baud rate:** 9600
- **Data Bits:** 8
- **Stop Bits:** 1
- **Parity:** None

Environment Required: Indoor
Temperature: Room temperature (steady at 15 – 28°C) preferred. Temperature fluctuations will affect the precision of the measurement.
Humidity: Up to 85%
Positioning: Upright use. No angle greater than ± 15 degrees from vertical is allowed.
Dimensions: 16 ½" × 10 ¼" × 14 ¾" (H × W × D)

F. MAINTENANCE

F.1. Picarro G2131-*i* Analyzer for Isotopic CO_2 maintenance

⚠ ***Caution:*** *Disconnect the AC power supply before opening the cover. The AC power is high voltage which is hazardous!*

Contact the vendor

G. TROUBLESHOOTING

G.1. Common problems

The DIC Analyzer was completely checked during and after manufacturing and assembly operations. It was carefully packed to prevent damage during shipment. If the Analyzer is not functioning properly, check for the following common problems first:

Are all cables connected correctly?

Are there any gas and/or liquid leaks?

Does your power supply outlet match the selected value?

Were the cable connector pin-outs matched properly?

G.2. Troubleshooting guide

G.2.1. Power supply problems

1. Check the power supply cord to ensure it is connected properly.
2. Check the fuses, and replace any fuses that are burned out. If the DIC Analyzer's main power switch works but the LI-COR does not get power when switched on, check the LI-COR fuse.
3. Verify that the power supply setting matches the supply outlet voltage.

   Note: If the voltage supplied to the instrument is greater than the selected value on the power cord switch, the Analyzer could be damaged.

G.2.2. Picarro G2131-*i* Analyzer for Isotopic CO_2 problems

Refer to the its instruction manual.

G.2.3. Software problems

G.2.3.1. DIC Program does not run

Check that your computer is an IBM/Windows compatible, 80486 or better system.

If the DIC program displays "Disk is full" and then stops, check your hard disk and ensure that there is enough free space to save the measurement record.

G.2.3.2. No communication between the computer and the Analyzer

1. Check the communication cables (RS232 cables). There are two cables required for communication between the DIC Analyzer and the computer. One is for the main DIC system, and the other is for the **Picarro G2131-*i* Analyzer for Isotopic CO_2**. Use the cables that come with the Analyzer. If the "D" connector plugs do not fit your computer, use standard connector converters to match your computer's communication ports. Note that all the pin-outs should have good contact.
2. Check that the correct communication port numbers have been selected in the DIC program. The default setup is COM1 for the DIC system and COM2 for the **Picarro G2131-*i* Analyzer for Isotopic CO_2**.
3. Check the communication parameters. The program uses the following parameters:
   Baud rate: 9600
   Data bits: 8
   Stop bits: 1
   Parity: None
   Make sure the Baud rate of the **Picarro G2131-*i* Analyzer for Isotopic CO_2** has been set to the same value as the DIC program. Refer to the user's guide of **Picarro G2131-*i* Analyzer for Isotopic CO_2** for instructions to set the Baud rate.

G.2.4. Cooling system problems

1. Make sure the ambient room temperature is not too high.
2. Check the cooling fans to see if they are blocked by foreign objects or dust.

G.2.5. Measurement stability problems

G.2.5.1. Carrier gas flow rate not stable

1. Check the air supply pressure. Confirm that the pressure is ~16 psi (~1.1 atm) or slightly higher (<17 psi). If the pressure is too low, it will not open the mass-flow-controller's valve. If the pressure is set too high, it will burst fittings within the system.
2. Make sure the regulator valve for the compressed air is open enough to supply the flow rate of 60 mL/min.
3. The pressure of air should be stable with no fluctuation.

G.2.5.2. Solution problems

1. Check that the reagent/acid tubing is immersed into the solution and that no air bubbles are trapped inside the tubing.
2. Ensure that the sample solution is homogeneous- mix or stir if obvious separation is visible.
3. Samples with suspended particulates should be avoided since they may block the tubing.

G.2.5.3. Digital pump problems

1. If you accidentally pressed the "Home" button on the front panel of the digital pump and set the syringe to an incorrect home position, reset the home position. Follow the "WinPump® Software User's Manual" to set home for the digital pump, and also see section D.3 of this manual.
2. Check that the programs stored in the pump are not corrupted. Re-install the programs if necessary (see Section B.7).

H. WARRANTY

Apollo SciTech offers a one year full warranty on the DIC-δ^{13}C Analyzer against defects in parts and workmanship from the date of purchase. Apollo SciTech's sole obligation under this warranty shall be to repair or replace any part of the instrument which Apollo SciTech's examination discloses to have been defective in material or workmanship without charge and only under the following conditions:

1. The defects are reported to Apollo SciTech in writing within one year after the shipping date of the instrument.
2. The instrument has not been maintained, repaired, or altered by anyone who was not approved by Apollo SciTech.
3. The instrument was used in a normal, proper, and ordinary manner and has not been abused, altered, misused, neglected, involved in an accident or damaged by act of God or other casualty.
4. The purchaser packs and ships or delivers the instrument to Apollo SciTech within 20 days after Apollo SciTech has received the written notice of the defect.
5. No-charge repair parts may be sent at Apollo SciTech's sole discretion to the purchaser for installation by purchaser.
6. Apollo SciTech's liability is limited to repair or replace any part of the instrument without charge if Apollo SciTech's examination discloses that part to have been defective in material or workmanship.

This warranty defines the obligation of Apollo SciTech and no other warranties expressed or implied are recognized.