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SMALL SAMPLE ISOTOPE MODULE

USER'S MANUAL

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INTRODUCTION

The Small Sample Isotope Module (SSIM) is an accessory designed to work with the Picarro G2101-*i*/G1101-*i* Isotopic CO_2 analyzers, G2132-*i* isotopic CH_4 analyzers, and the G2201-*i* CO_2 & CH_4 analyzers. When sample volumes are too small to be measured in a traditional "continuous flow" mode by the Picarro analyzer (generally requiring >100ml), the SSIM is an essential analyzer add-on, enabling such small-sample analysis. Combined, the analyzer and SSIM are capable of measuring very small (<20ml) gas samples and performing δ13C-CO2 / δ13C-CH4 and concentration analysis (CO_2, CH_4, H_2O concentrations of the gas are reported). The SSIM automatically prepares and introduces the gas sample(s) to the Picarro analyzer and reports the results, performing the requested number of replicate analyses of each sample and automatically corrects the measurement by also analyzing a user-supplied isotopic gas standard. The SSIM can optionally be paired with the Picarro 16-Port Distribution Manifold to enable it to automatically measure multiple samples (up to eight, including the gas standard).

PRINCIPLES OF OPERATION

Internally, the SSIM contains various valves and a sample loop. Prior to a measurement, the gas system is flushed with purge gas (CO_2 and CH_4 free "zero air") to remove traces of prior samples or atmospheric gas that would contaminate the measurement. Next, the purge gas is pumped out by an external vacuum pump bringing the tubing within the SSIM to a vacuum. At this point, user is prompted to manually open the sample container attached to the SSIM. Because the tubing in the SSIM is under vacuum, the sample expands (using ~20ml of sample) into sample loop where it is then introduced to Picarro's analyzer. For small or high concentration samples, there is an option to dilute with zero air. The analyzer's measurement cavity is open and continuously drawing in sample from the sample loop. With an external pressure sensor, it is possible to watch the sample loop pressure equilibrate with the cavity pressure as the analyzer takes in the sample. If the 16-Port Distribution Manifold is utilized, then the system performs the requested replicate measurements on each sample and then advances to the next sample inlet port on the 16-Port Distribution Manifold, continuing in this manner until all analyses are completed for each sample present. The SSIM can now skip or repeat standard measurements in addition to sample measurements.

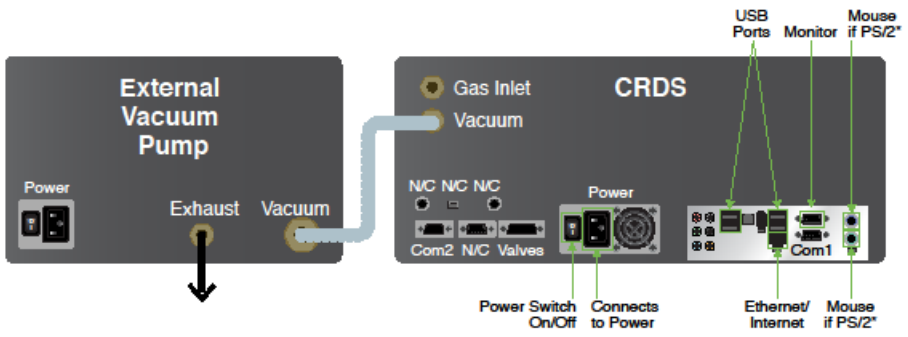
PRE-INSTALLATION REQUIREMENTS

Prior to setting up the SSIM, the user will need to complete the following steps:

1. A pressurized cylinder of dry, CO_2 and CH_4 free "zero air" complete with a pressure regulator so that gas can be supplied at ≥3 psi (0.2 bar) and <8 psi (~0.5 bar). It should have <1 ppm CO_2 and CH_4 and <10 ppm H_2O. This is generally what "zero air" or "ultra-high-purity" 99.9995% N_2 is specified to contain, for example.
2. Various lengths of 1/8" tubing (stainless steel recommended) with 1/8" Swagelok connections on each end.
3. A pressurized cylinder of calibrated CO_2 gas having a known concentration and known $\delta^{13}C$ value, complete with a pressure regulator so that gas can be supplied at ≥3 psi (0.2 bar) and <8 psi (~0.5 bar).
4. Sample container(s) (Mylar bags or fixed containers for example) which can be attached to the system via 1/8" Swagelok and which have manual valves so that they can be isolated from the system during purging. It is also possible to use an injection port if using a syringe.

	**Note: these manual valves on the sample bags/containers are a requirement.**

5. Appropriate wrenches for attachment of gas connections.
6. The basic gas analyzer setup (CRDS Analyzer & External Vacuum Pump) should be completed. The information can be found in the manual of the CRDS analyzer.



Basic Carbon Analyzer Setup

7. If using the 16-Port Distribution Manifold, find its manual to refer to while following the rest of the manual.

8. Prior to operation and especially if the SSIM is to be used on an analyzer that is also used with other valves for different applications, the standard Valve Sequencer in the Picarro GUI needs to disabled (and then re-enabled after use of the SSIM is finished if necessary). The user can do this through the “Setup Tool” in the “Utilities” Folder in the desktop. Set the “Valve Sequencer MPV” to “OFF.”

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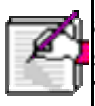
SSIM HARDWARE SETUP & INSTALLATION

Follow the steps described in this section to make the proper gas and electrical connections. All gas connections should be made with 1/8" Swagelok. There are two possible configurations – one with and one without the 16-Port Distribution Manifold. This section includes installation information for both of the configurations.

Based on one's configuration, either follow Section 1 (SSIM - Picarro Analyzer Setup) or Section 2 (SSIM - Picarro Analyzer - 16 Port Distribution Manifold Setup)



Note: It is imperative that all gas connections be free of leaks in order to achieve proper measurement of sample and ensure performance of the system. For more details on ensuring leak-free connections, see Troubleshooting section at the end of this document. All gas connections should be made with 1/8" Swagelok (stainless steel tubing recommended).

SECTION 1: SSIM – CRDS ANALYZER SETUP

	**Note: This module enables very precise concentration of small samples. For the best precision measurements, the analyzer should be allowed to run for at least 2 hrs prior to making all connections.**

If the SSIM is used alone with the analyzer, the required hardware setup steps are:

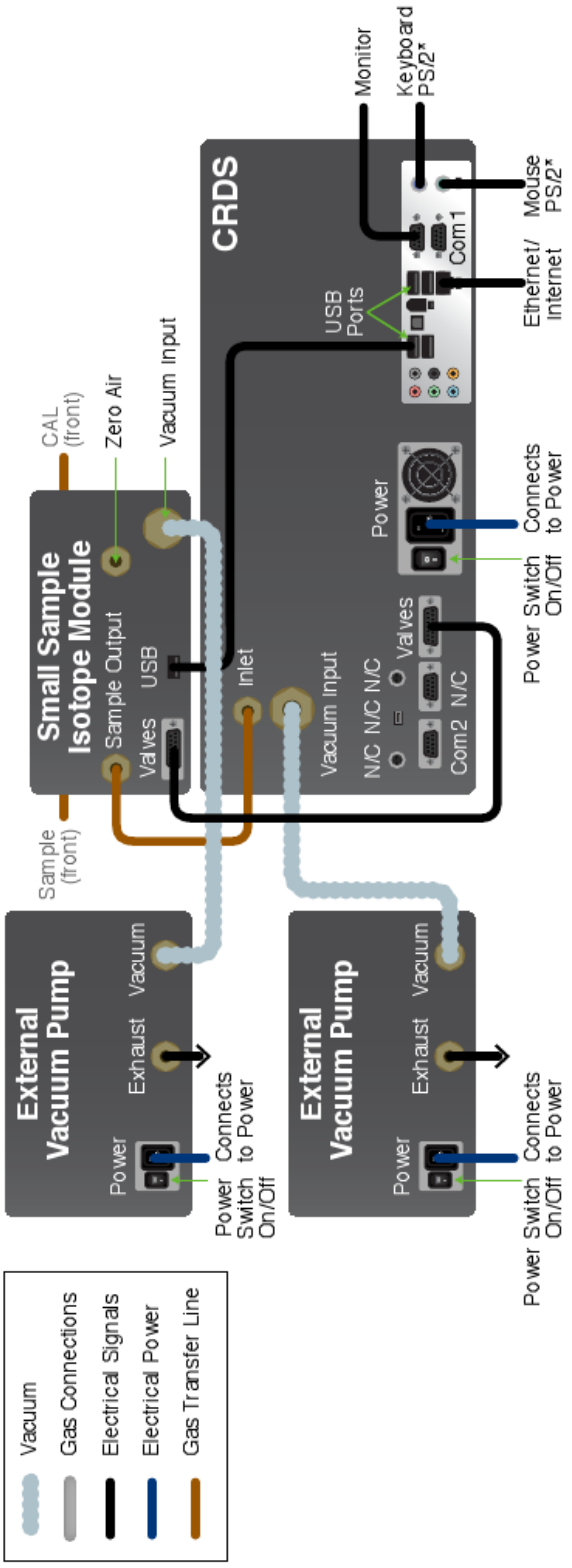
1. Before starting, make sure all the "Pre-installation Requirements" have been met.
2. Position SSIM near the analyzer's inlet connection to minimize the dead space of gas line connecting the SSIM **"Sample outlet"** (located on SSIM's rear side) and the analyzer gas **"Inlet."** Prepare tubing to connect (but **DO NOT yet connect SSIM to analyzer**) these connections. A short length of 1/8" stainless tubing having 1/8" Swagelok connections on both ends should be used. Picarro supplies the tubing that attaches from the Sample outlet of SSIM to the analyzer's gas inlet.

	**Note: it is not necessary to shut down the analyzer during the installation of the SSIM, but it is imperative that gas pressures from the high-pressure cylinders be regulated to ≥3 psi (0.2 bar) and <8 psi (~0.5 bar) prior to connecting the SSIM and the analyzer.**

3. Connect the external vacuum pump to the **"Vacuum" port** on the SSIM with the supplied vacuum line. Connect the pump to line power but do not switch on until just before sample analysis begins.
4. Attach the purge gas supply (zero air as mentioned on Step 1 of Pre-Installation Requirements) to the back **"Zero Air"** connection of the SSIM and supply gas at optimally ≥3 psi (0.2 bar) and <8 psi (~0.5 bar).
5. Attach the sample bag or container to the **"Sample" port** on the front of the SSIM using a very small length of 1/8" tubing.

	**Note: it is required that the sample containers have manual valves to isolate it during the purging of the SSIM.**

6. Connect the SSIM to the analyzer as described in step 1.
7. Make the cable connections between the SSIM and the analyzer: "**Valves"** (back of the SSIM) – "**Valves"** (back of the CRDS). **USB** (back of the SSIM) - **USB** (back of the CRDS).
8. Turn on the external vacuum pump.



SSIM - CRDS ANALYZER SETUP

SECTION 2: SSIM-16 PORT DISTRIBUTION MANIFOLD- CRDS ANALYZER SETUP

Note: This module enables very precise concentration of small samples. For the best precision measurements, the analyzer should be allowed to run for at least 2 hrs prior to all the connections being made.

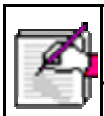
If the SSIM is used with the 16-Port Distribution Manifold and the analyzer, the required hardware setup steps are the following.

1. Position the SSIM near the analyzer's inlet connection to minimize the volume of the gas line connecting the SSIM **"Sample" outlet** (located on SSIM's rear side) and the **"Inlet" port** back of the CRDS analyzer. Prepare tubing to connect (but **DO NOT yet connect SSIM to analyzer**) these connections. It should be a short length of 1/8" stainless tubing having 1/8" Swagelok connections on both ends.

Note: it is not necessary to shut down the analyzer during installation of the SSIM, but it is imperative that gas pressures from the high-pressure cylinders be regulated to ≥3 psi (0.2 bar) and <8 psi (~0.5 bar) prior to connecting the SSIM and analyzer.

The easiest way to make all connections requires that the 16-port distribution module be placed on top of CRDS analyzer first. Position the SSIM to the right side of the 16 port manifold, and make sure it faces the front of the CRDS analyzer. This will allow use of the supplied sample delivery tubing provided by Picarro. The external pump may be positioned adjacent to the SSIM on top of the 16 port.

2. Connect the external vacuum pump to the **"Vacuum" port** on the SSIM with the supplied vacuum line. Connect the pump to line power but do not switch on until just before sample analysis begins.
3. Connect line power, the hand pad, and the **"COM MPV"** connection on the Distribution Manifold to the CRDS Analyzer's **"COMP"** port with the supplied cables.

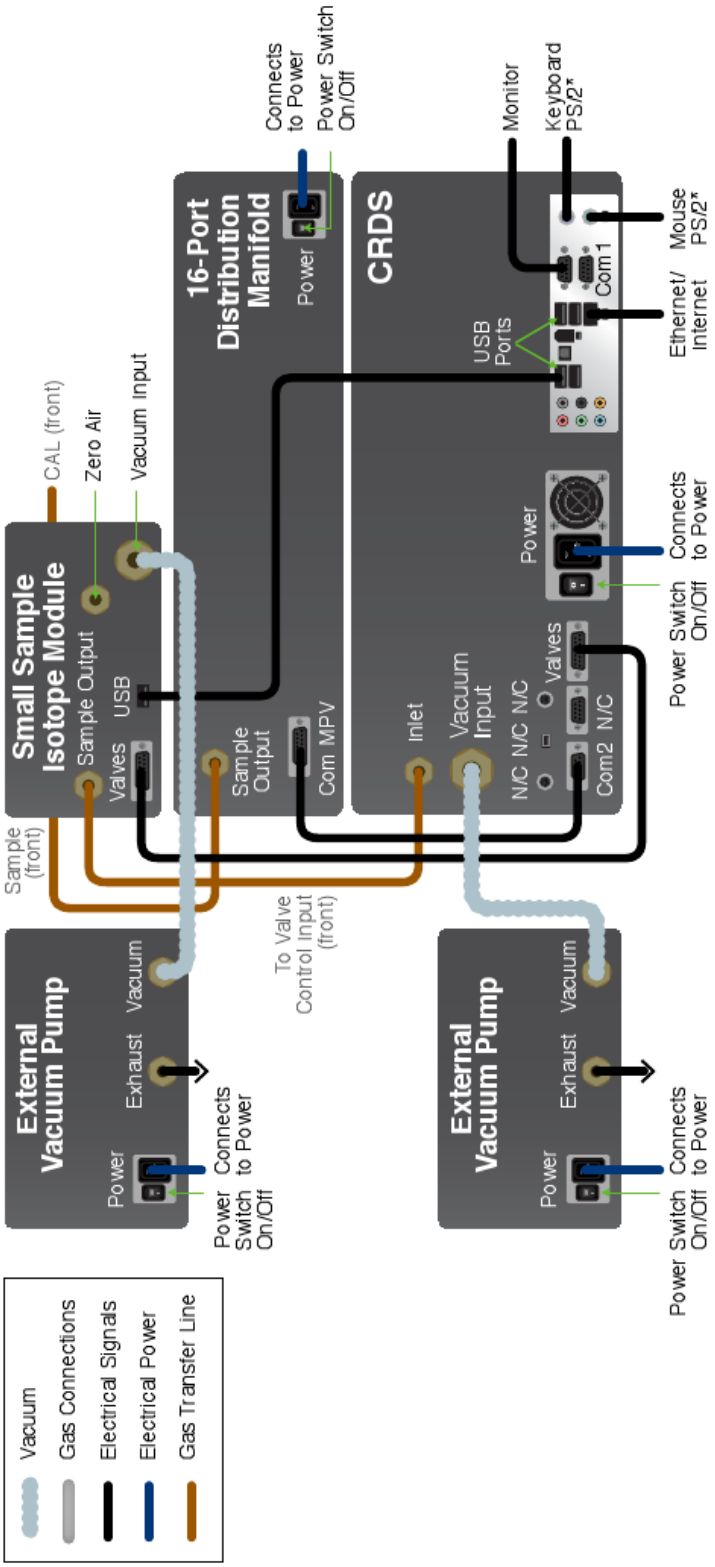
	**Note: for full details about the Distribution Manifold, see the User's Guide for this accessory.**

4. Connect the **"Sample" output** from the rear of the Distribution Manifold to the **"Sample"** input located the front of the SSIM (Tubing provided).
5. Attach the zero air supply to the back **"Zero Air"** connection of the SSIM and supply gas at optimally **≥3 psi (0.2 bar) and <8 psi (~0.5 bar).**
6. Attach the calibration gas standard supply to the **"Cal**" input of the SSIM (front side of the SSIM). Supply gas at optimally **≥3 psi (0.2 bar) and <8 psi (~0.5 bar).**
7. Attach sample containers to only **EVEN-NUMBERED** "Sample" ports on the front of the Distribution Manifold using as minimal as possible length of 1/8" tubing

	**Note: all ODD-NUMBERED ports on the Distribution Manifold must be capped with 1/8" Swagelok caps. (These odd-numbered ports are used in the purge operation). IF SAMPLES ARE CONNECTED TO ODD-NUMBERED PORTS, THEY WILL BE EXHAUSTED THROUGH THE VACUUM PUMP AND WILL BE LOST!!! It is required that the sample containers have manual valves to isolate them during the SSIM's purge operation.**
	**Note: Remember to tighten the caps already present on the exhaust ports.**

8. Connect the SSIM to the analyzer as described in step 1. (Tubing supplied)
9. Make the cable connections between the SSIM and the analyzer: **Valves** (back of the SSIM) - **Valves** (back of the CRDS). **USB** (back of the SSIM) - **USB** (back of the CRDS).
10. Turn on the external vacuum pump.
11. Turn on the Distribution Manifold.

	**Note: if the valve in the Distribution Manifold is not recognized by the software, it may be necessary to re-start both the analyzer "GUI" software as well as the SSIM Coordinator program.**



SSIM - CRDS ANALYZER – 16 PORT DISTRIBUTION MANIFOLD SETUP

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OPERATION AND SOFTWARE – TAKING A MEASUREMENT

Once all connections have been made, and samples have been attached to the system, it is appropriate to begin analysis by launching the Coordinator program and following the steps below.

Based on one's configuration, either see section 1 (SSIM - CRDS Analyzer Setup) or section 2 (SSIM - 16 port distribution manifold Setup - CRDS Analyzer).

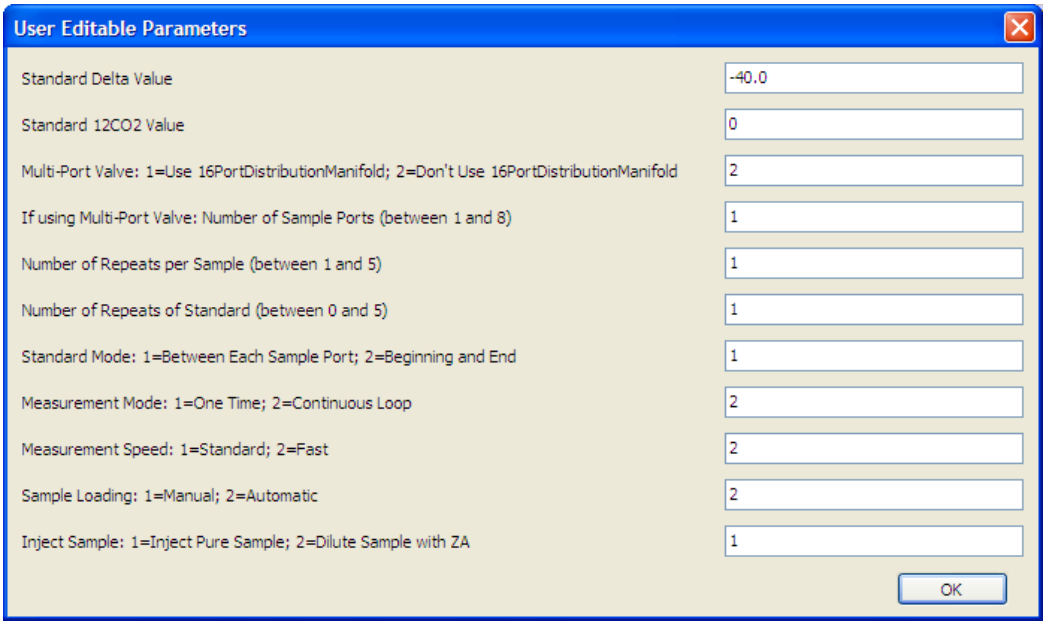
SECTION 1: SSIM - CRDS ANALYZER SETUP

If using the SSIM alone with the analyzer, follow the steps below:

1. Locate the "Coordinator Launcher" program on the desktop and double click it. A screen will appear, allowing the user to choose "**Discrete Sample iCO2,**" "**Discrete Sample iCO2 with HP CH4,**" or "**Discrete Sample Dual**" depending on which instrument the user is using. Choose the appropriate coordinator mode, and then click "Launch" to continue. In the "User Editable Parameters" window (next step) there will be an option to proclaim if the user is using the 16-Port Distribution Manifold (MPV) or not.
    - **Discrete Sample iCO2:** To make discrete iCO2 measurement.
    - **Discrete Sample iCO2 with HP CH4:** To make discrete iCO2 measurement and high precision measurement of CH4 concentration.
    - **Discrete Sample Dual:** To measure iCO2 and iCH4.

Picarro Coordinator Launcher

2. Upon clicking **"Launch,"** the "User Editable Parameters" screen will appear. Enter the appropriate values based on the description below. Once finished click "OK" to continue.



User Editable Parameters Window

- **Standard Delta Value:** Enter the actual calibrated value of standard ($\delta^{13}C$ in per mil units)
- **Standard 12C02 Value:** Enter the actual calibrated value of standard ($^{12}CO_2$ in per mil units)
- **Multi-Port Value:** Specify whether using the 16 Port Distribution Manifold. Enter 2 if no, 1 if yes. Enter 2.
- **If using Multi-Port Value: Number of Sample Ports:** Specify the number of sample ports of the 16 Distribution Manifolds the user will be measuring from. The number of samples can range from 1 to 8. When not using the 16 Port Distribution Manifold, enter "1".
- **Number of Repeats per Sample:** Specify the number of times the user wants to measure each sample. The number can be as little as 1 and as great as 5.
- **Number of Repeats of Standard:** Specify the number of times the user wants to measure each standard. The number can be as little as 0 and as great as 5.
- **Standard Mode:** 1 if wanting to measure standard between the measurement of each sample port, 2 if wanting to measure standard just in the beginning and at the end.
- **Measurement Mode:** 1 if samples will be measured one time, 2 if they will be measured in a loop.
- **Measurement Duration:** 1 if wanting standard measurement (12 minutes/measurement), 2 if wanting fast measurement (8 minutes/measurement)**.** The times do not include the 3 minute purge and pump cycle to clean the SSIM and analyzer between measurements.

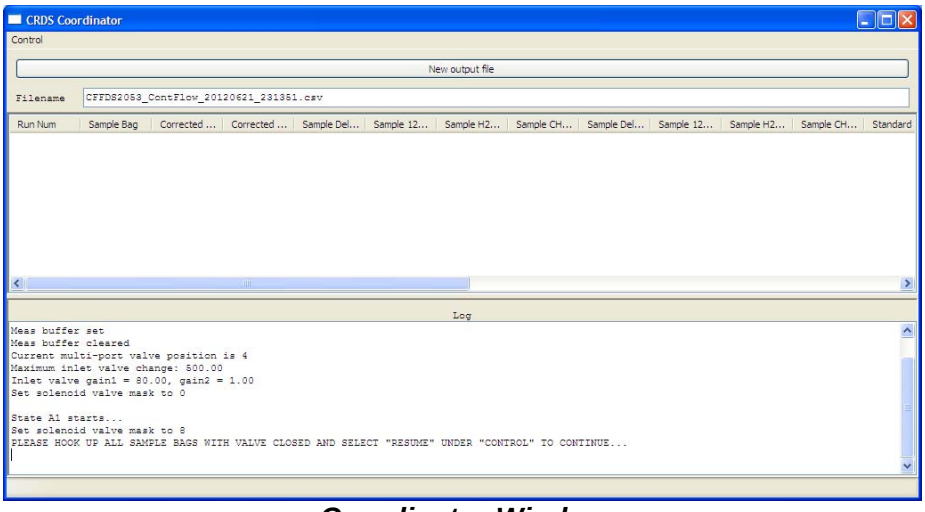
- **Sample Loading:** 1 if loading each sample manually, 2 if loading sample automatically.
- **Sample Dilution:** 1 if injecting pure sample, 2 if injecting sample is to be diluted with zero air.

3. The Coordinator window will appear. At any time during analysis, a new comma-delimited (CSV) file can be created and is saved to **C:\Isotopedata.** A file is automatically started each time the Coordinator is started. Various messages about the state of the system are displayed in the bottom window and prompts to the user are also noted there in capital letters. The upper portion of the coordinator window shows the various parameters measured during analysis, and is identical to the data that is saved in the CSV file.

	**Note: If the user ever desires to close the Coordinator, the red "X" can be used to simply close the window which will terminate the program. Allow the program to terminate itself (it could take nearly one minute depending on the state of the SSIM and analyzer). The Coordinator, if allowed to close itself properly, will return the SSIM and analyzer to a safe state.)**

4. After the SSIM has completed the purge and pump steps, it will prompt the user to attach a sample container with manual valve closed.

	**Important: make sure the manual valve (connected to the sample bag) is closed or the sample will be lost!**



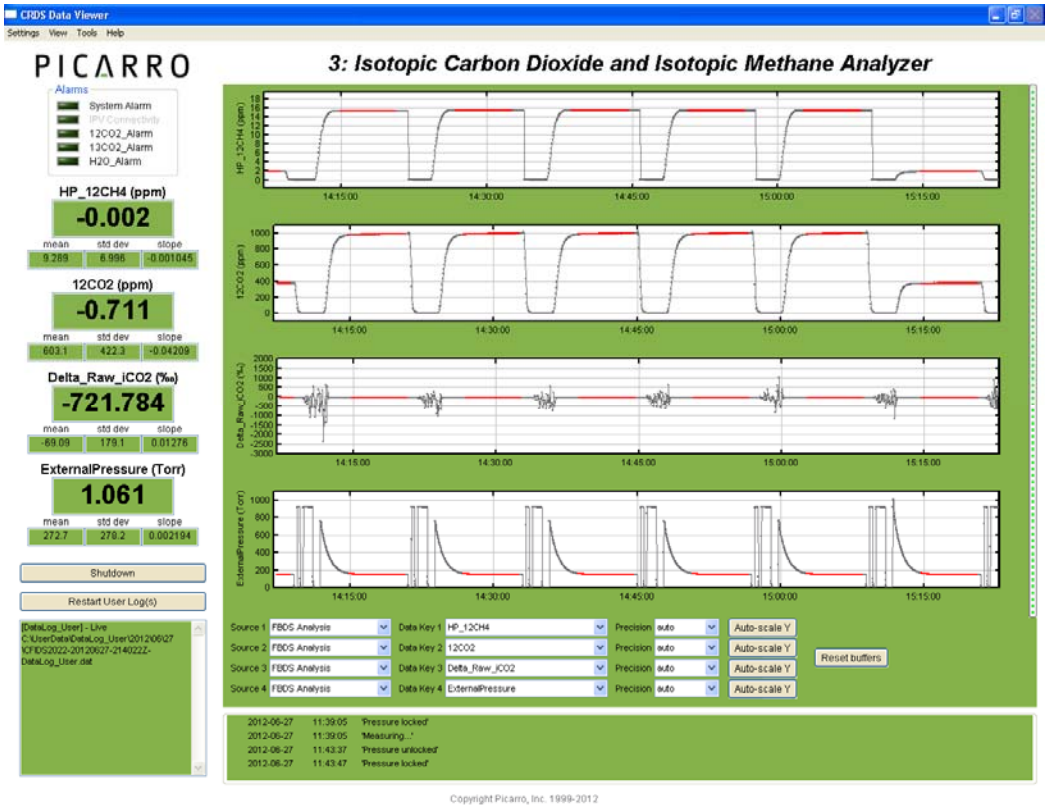
Coordinator Window

5. The SSIM will then initiate another pump and purge cycle. Once complete, coordinator will prompt user to open manual valve (connected to the sample bag) and then “Resume” under the “Control” menu.

6. If the user is in the manual injection mode and not using the 16 Port Distribution Manifold, the coordinator will prompt the user to attach the sample bag with valves closed and then opened before each sample measurement.

7. The analysis of the sample will begin (duration of measurement is 12/8 minutes depending on the measurement option chosen – standard or fast). The gas concentration and isotope ratio data will look similar to the figure below where a standard was measured, followed by a waiting period (where the analyzer simply measures zero air before the user prompts the software to begin the next measurement), then followed by a sample, and finally another measurement of the standard.

	**Note: it is normal to have alternating “Pressure High/Pressure Low” warnings in the GUI Status Log since the analyzer is constantly adjusting the pressure during the discontinuous gas flow that occurs as a result of the SSIM’s purging and gas delivery steps. If these warnings persist and are accompanied by a persistent “System Alarm” indicator, this could indicate a problem and it is advisable to contact Picarro.**



The GUI above is for a G2201-i analyzer (measures iCO2 & iCH4). Depending on the type of the CRDS analyzer being used, the look of the main GUI will vary slightly

8. The Coordinator will continue to prompt the user for samples until the Coordinator program is terminated.

SECTION 2: SSIM–16 PORT DISTRIBUTION MANIFOLD–CRDS ANALYZER SETUP

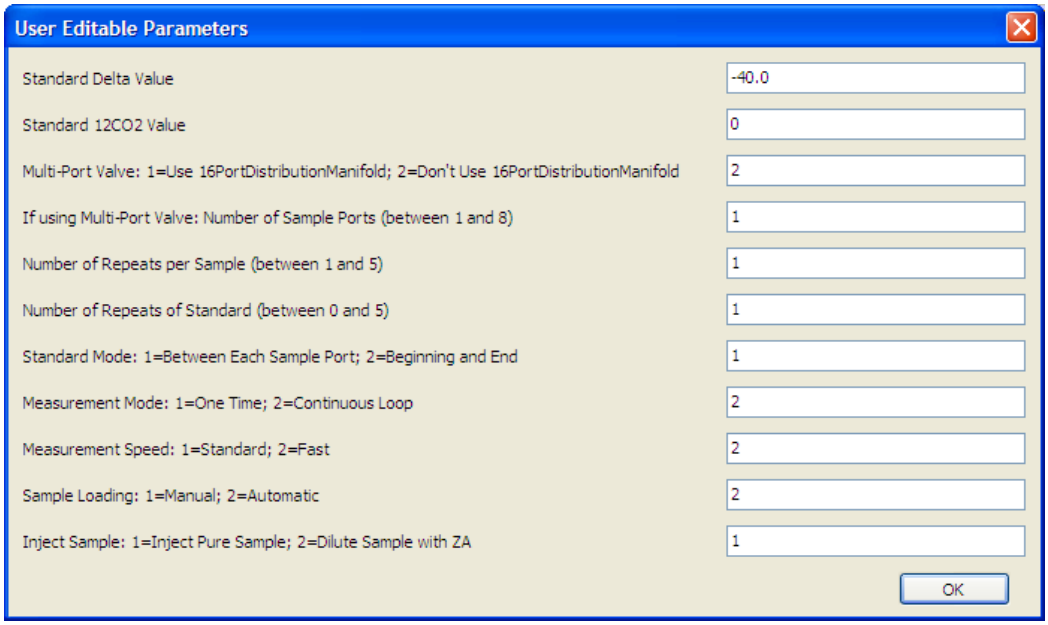
If the SSIM is used with the 16-Port Distribution Manifold and the analyzer, follow these steps:

1. Locate the "Coordinator Launcher" program on the desktop and double click it. A screen will appear allowing the user to choose "**Discrete Sample iCO2,**" "**Discrete Sample iCO2 with HP CH4,**" or "**Discrete Sample Dual.**" Choose the appropriate coordinator mode, and then click "Launch" to continue. In the "User Editable Parameters" window (next step), there will be an option to proclaim if the user is using the 16-Port Distribution Module (MPV) or not.
    - **Discrete Sample iCO2:** To make discrete iCO2 measurement.
    - **Discrete Sample iCO2 with HP CH4:** To make discrete iCO2 measurement and high precision measurement of CH4.
    - **Discrete Sample Dual:** To measure iCO2 and iCH4.

Picarro Coordinator Launcher

2. Once the user clicks "Launch," the "User Editable Parameters" screen will appear. Enter the appropriate values to continue.
    - **Standard Delta Value:** Enter the actual calibrated value of standard ($\delta^{13}C$ in per mil units)
    - **Standard 12C02 Value:** Enter the actual calibrated value of standard ($^{12}CO_2$ in per mil units)
    - **Multi-Port Value:** Specify whether using the 16 Port Distribution Manifold. Enter 2 if no, 1 if yes. Enter 1.
    - **If using Multi-Port Value: Number of Sample Ports:** Specify the number of sample ports of the 16 Distribution Manifold the user will be measuring from. The number of samples can range from 1 to 8.

- **Number of Repeats per Sample:** Specify the number of times the user wants to measure each sample. The number can be as little as 1 and as great as 5.
- **Number of Repeats of Standard:** Specify the number of times the user wants to measure each standard. The number can be as little as 0 and as great as 5.
- **Standard Mode:** 1 if wanting to measure standard between the measurement of each sample port, 2 if wanting to measure standard just in the beginning and at the end.
- **Measurement Mode:** 1 if samples will be measured one time, 2 if they will be measured in a loop.
- **Measurement Speed:** 1 if wanting standard measurement (12 minutes/measurement), 2 if wanting fast measurement (8 minutes/measurement). The times do not include the 3 minute purge and pump cycle to clean the SSIM and analyzer between measurements.
- **Sample Loading:** 1 if loading each sample manually, 2 if loading sample automatically.
- **Inject Sample:** 1 if injecting pure sample, 2 if injecting sample diluted with zero air.



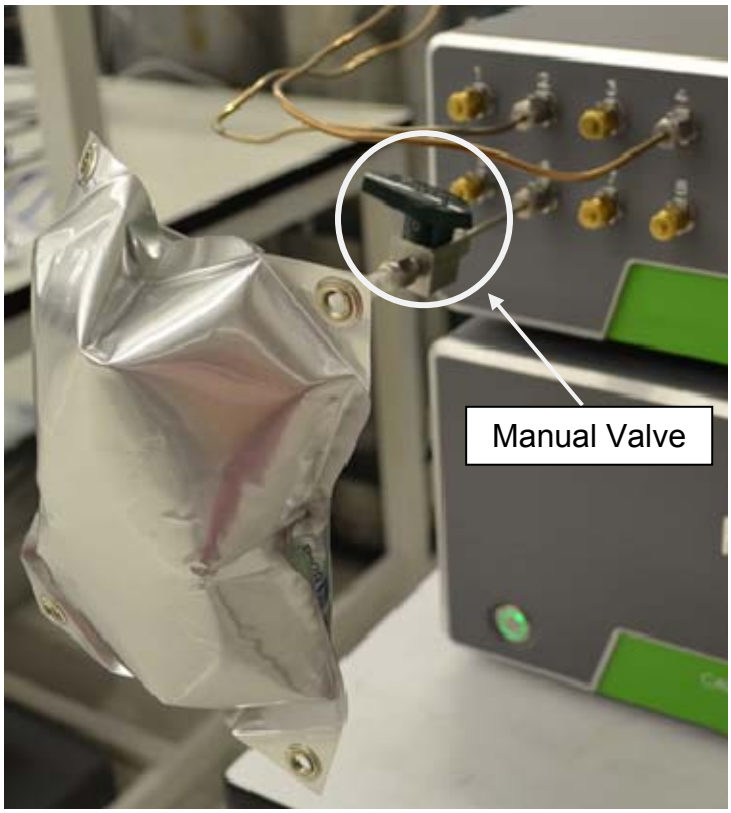
User Editable Parameters Window

3. The Coordinator window will appear. At any time during analysis, a new comma-delimited (CSV) file can be created and saved to C:\Isotopedata. A file is automatically started each time the Coordinator is started. Various messages about the state of the system are displayed in the bottom window and prompts to the user are also noted there

in capital letters. The upper portion of the coordinator shows the various parameters measured during analysis, and is identical to the data that is saved in the CSV file.

	Note: If the user ever desires to close the Coordinator, the red "X" can be used to simply close the window which will terminate the program. Allow the program to terminate itself (it could take nearly one minute depending on the state of the SSIM and analyzer). The Coordinator, if allowed to close itself properly, will return the SSIM and analyzer to a safe state.

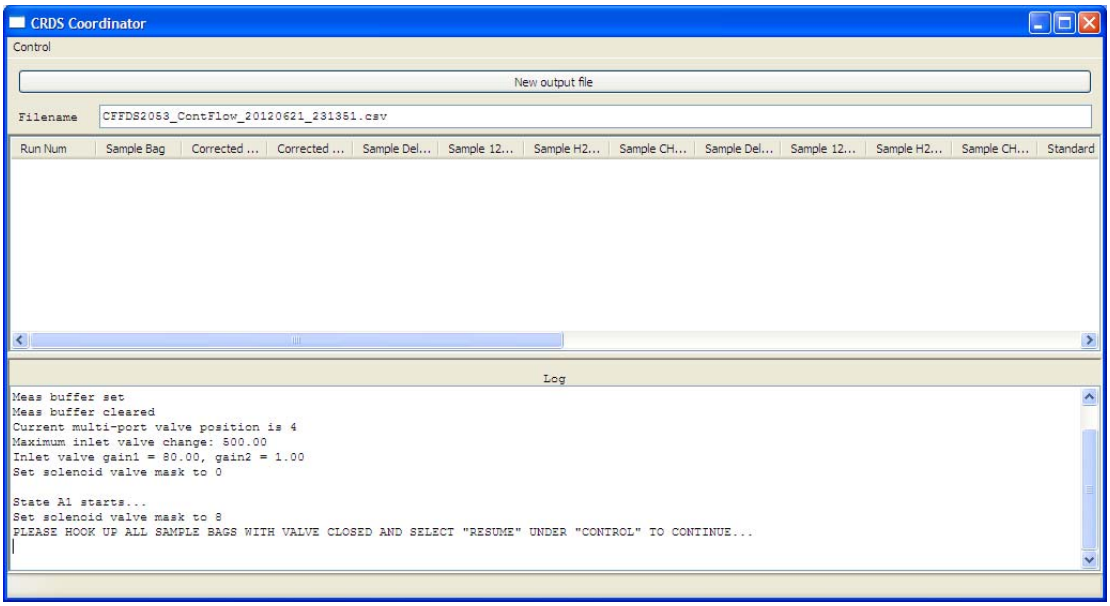
4. After the SSIM has completed the purge and pump steps, it will prompt the user to attach all sample container(s) with the manual valve(s) closed.



Example of a sample bag connected to the 16-port manifold via a manual valve

	Important: make sure the manual valves are closed or the samples will be lost!

5. The SSIM will then initiate another pump and purge cycle for all selected input ports and will prompt the user to open the manual valve(s). Then select “Resume” under the “Control” menu.



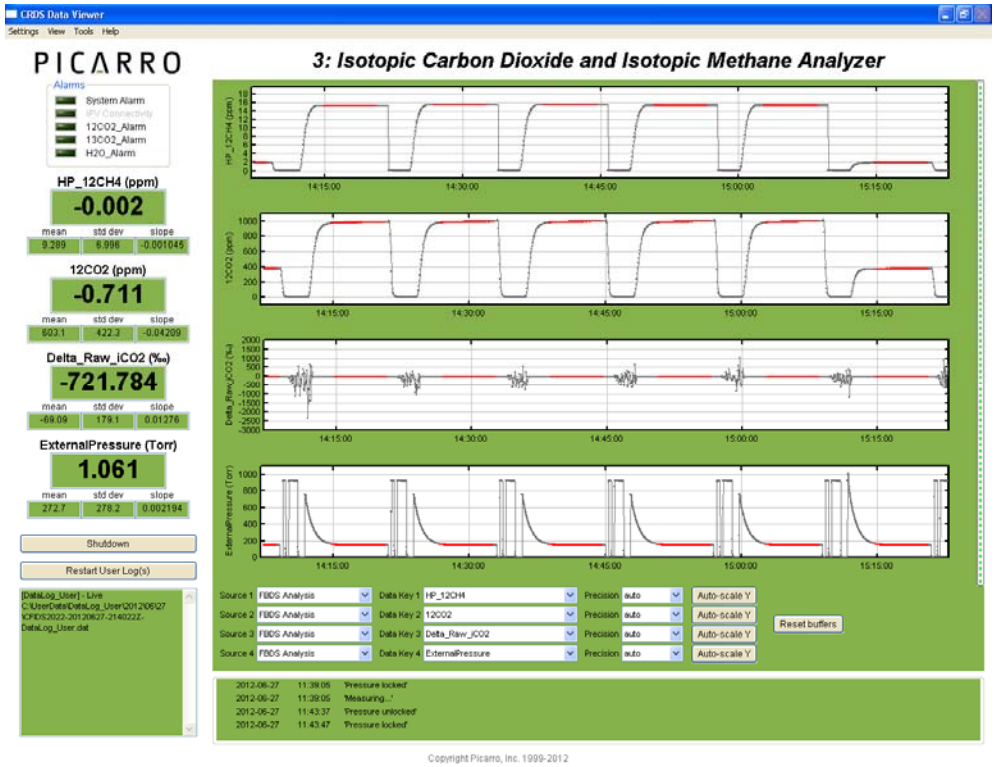
CRDS Coordinator Window

6. The sample from the first sample port will expand into the sample loop of the SSIM. There the sample will make its way to the analyzer, where measurements will be taken automatically. At this point, no further user input is necessary to analyze sample(s) from sample container(s).

7. The analysis of the samples will begin (duration of measurement is 12/8 minutes depending on the measurement option chosen – standard or fast).

	Note: it is normal to have alternating “Pressure High/Pressure Low” warnings in the GUI Status Log since the analyzer is constantly adjusting the pressure during the discontinuous gas flow that occurs as a result of the SSIM’s purging and gas delivery steps. If these warnings persist and are accompanied by a persistent “System Alarm” indicator, this could indicate a problem and it is advisable to contact Picarro.

8. The Coordinator will measure samples until it finishes the job (if the user has selected the Measurement Mode to be “One Time”) or it will continue to run until the Coordinator program is terminated (if “Continuous Loop” was selected).



The GUI above is for a G2201-i analyzer (measures iCO2 & iCH4). Depending on the type of the CRDS analyzer being used, the look of the main GUI will vary slightly

USE-CASE RECOMMENDATIONS AND TROUBLESHOOTING

LEAK TIGHTNESS:

It is critical that there be no leaks in the plumbing connections associated with the SSIM and analyzer. It is possible to use the analyzer itself as a diagnostic to find leaks. The purge (zero air) should be nearly dry, as should the calibration standards. By looking at the analyzer's water vapor concentration measurement during the time that it is not measuring samples (it will be drawing gas from the Zero Air" port of the SSIM) a leak in the plumbing associated with the zero air supply or SSIM-to-analyzer connecting will be evidenced by a high water level (significantly higher than ~50ppm for example). A similar check can be used for the calibration gas – during a calibration measurement, the water should be indicating near zero if there is no leak. For leaks in the sample container connections, this can be more elusive depending on the water content of the sample, but can be diagnosed by filling the sample containers with dry gas.

One way to run dry gas through the SSIM as if the dry gas were a sample is to run "Sample Inject" in "2: Run sample diluted in ZA (Zero Air)." Do not add any sample. The SSIM will be evacuated, and then it will ask for the sample to be connected and the manual valve opened. Connect a sample, but do not open the manual valve. Just select resume when asked to open the manual valve. Then the SSIM will fill the empty sample volume with zero air, and introduce this to the analyzer. High H2O levels in these samples indicate that there is a leak in the connections.

ELECTRICAL CONNECTIONS:

If the valve control cables are not appropriately attached, this may cause unexplained errors in the Coordinator program and the cabling should be checked and the analyzer and/or Coordinator software should be restarted if necessary. If the message appears that the multi-position (rotary) valve is not correctly recognized, consult the user's guide for the Distribution Manifold or contact Picarro.

PUMP LIFETIME:

The vacuum pump has a rated lifetime of 10,000 hours and has an hour meter attached to it so this can be checked if necessary. An ageing pump will have the same symptom as a plumbing leak – the Coordinator program may time out.