Supplement of:

A portable, open-source, low-cost incubation chamber for real-time characterization of soil respiration and microbial activity

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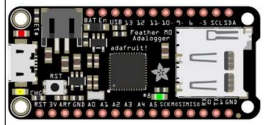
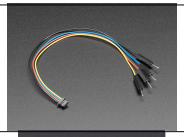
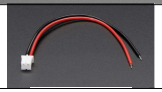
General notes:
The information provided below gives detailed instructions to build and operate the CO2/O2 incubation chamber. The instruction includes two sheets:
(1) 1st sheet: DIY building protocol (including all required components to build an incubation chamber and step-by-step instruction to assemble separate parts)
(2) 2nd sheet: User guide (How to embed the code to the datalogger and operate the incubation chamber)

Basically, an incubation chamber includes **a CO2/O2 sensing unit**- measuring CO2 and O2 concentration inside a closed soil-contained glass jar, and **a core unit**- collecting and logging CO2/O2 concentration data. The number of CO2/O2 sensing units (number of jars) can be customized up to 4 with the given hardware and embedded code provided in this study.

*Please consult with your institute's safety representative regarding soldering and any other related issue that can pose a fire or safety risk.

I. Components

1. Components of the core unit

No	Component	Quantity	Cost (USD)	Source of materials	Images	Link	Comments
1	Feather M0 Adalogger	1	19.95	Adafruit		https://www.adafruit.com/product/2796	
2	DS3231 RTC Precision Breakout with CR1220 battery	1	17.5	Adafruit		https://www.adafruit.com/product/3013	Provides accurate time for the data logger; can be purchased from other suppliers
3	Gravity: Digital 1-to-8 I2C Multiplexer	1	6.9	DFRobot		https://www.dfrobot.com/product-1780.html	Enables the connections of multiple CO2/O2 sensors to one data logger; Can be purchased from other suppliers
4	Monochrome 0.96" 128x64 OLED Graphic Display - STEMMA QT	1	17.5	Adafruit		https://www.adafruit.com/product/326	Can be purchased from other suppliers
5	Li-ion battery 3.7V 3500 MAH #18650		~5	Adafruit		https://www.adafruit.com/product/1781	Can be purchased from other suppliers
6	MicroSD memory card (8/16/32/64 GB SDHC)	1	~5-10	Adafruit		https://www.adafruit.com/product/1294	Can be purchased from other suppliers
7	22 AWG Wires 15cm long in colors white, green, red, black	2/color	~1	Local suppliers or online (e.g., Amazon)			
8	STEMMA QT / Qwiic JST SH 4-pin to Premium Male Headers Cable - 150mm Long	1	0.95	Adafruit		https://www.adafruit.com/product/4209	
9	JST 2-pin Extension Cable with On/Off Switch - JST PH2	1	2.95	Adafruit	 	https://www.adafruit.com/product/3064	For battery
10	Snap-action 5-Wire Block connector (12-24 AWG) - Pack of 3	4	4.95	Adafruit		https://www.adafruit.com/product/874	For connecting wires

11	M2.5 Standoffs (Spacer)	3	0.5	Local suppliers or online (e.g., Amazon)			Optional, for spacing different hardware components

2. Components of the CO2/O2 sensing unit

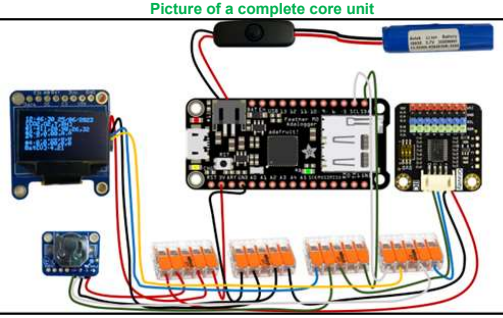
1	CO2 Humidity and Temperature Sensor -SCD30	1	61.79	DigiKey		https://www.digikey.co.il/he/products/detail/sensirion-ag/SCD30/8445334	Four sensors were used (240 USD); can be purchased from other suppliers
2	Gravity: Factory Calibrated Electrochemical Oxygen / O2 Sensor (0-25%Vol, I2C & UART)	1	84.9	DFRobot		https://www.dfrobot.com/product-2510.html	Four sensors were used (USD 340).
3	300 ml glass jar	1	~2	Local suppliers or online (e.g., Amazon)			Four jars were used (USD 8). We recommend using Mason jars instead of this type if better sealing is needed
4	PG7 Connector	1	0.5	Local suppliers or online (e.g., Amazon)			
5	Eight-wire cable 1.5 m	1	0.5	Local suppliers or online (e.g., Amazon)			

II. Building protocol

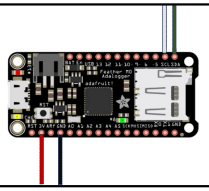
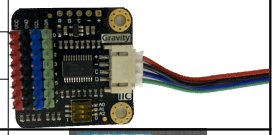
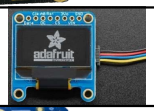
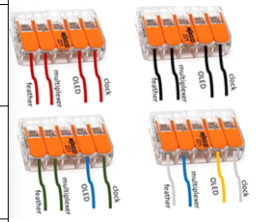
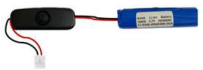
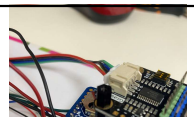
*In this guide we elaborate on the main building steps.
If additional knowledge is needed, we recommend reading the product page of each component.
For example, for the DS3231 RTC go to https://www.adafruit.com/product/3013 and read the complete guide and tutorials.

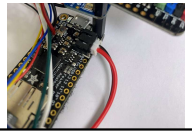
1. Building the core unit

Picture of a complete core unit



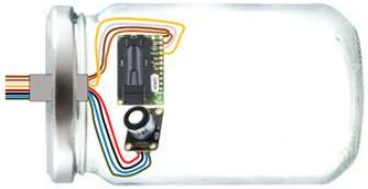
Step	What	How	images

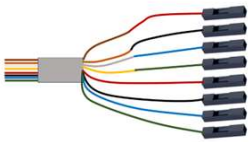
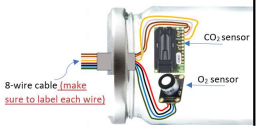
1	**Preparing the feather M0 Adalogger**	Solder 4 single wires, 15 cm long, in colors: white, green, red, black to the feather in order: White to SCL port Green to SDA port Red to 3V port Black to GND port Insert the microSD card into the Adalogger.	
2	**Preparing the multiplexer.**	Use a 4-pin cable (red, black, blue, green), cut and take the 4P-connector (the white part). At the cut end, remove 2-3mm of the plastic isolator to expose internal metal wires, and coat the four exposed wires with tin. Plug the 4P connector to the port on the multiplexer.	
3	**Preparing the OLED.**	Plug the connector of the STEMMA QT 4pin cable into the port on the	
4	**Preparing the RTC**	Solder 4 single wires, 15 cm long, in colors white, green, red, black to the clock in order: •white to SCL port •green to SDA port •red to VIN port •black to GND port Connect the CR1220 battery to the RTC.	
5	**Unite into a datalogger unit.**	Connect wires from the feather M0 datalogger, multiplexer, OLED, and RTC using 4 connector blocks: •Block 1 accommodates following wires: red from the feather red from the multiplexer red from OLED red from the RTC •Block 2 accommodates following wires: black from the feather black from the multiplexer black from the OLED black from the RTC •Block 3 accommodates following wires: green from the feather green from the multiplexer blue from the OLED green from the RTC •Block 4 accommodates following wires: white from the feather blue from the multiplexer yellow from the OLED white from the RTC	
6	**Connecting a battery with an on/off switch.**	Connect the li-ion battery with an on/off switch: ○ Slide a shrinking sleeve over both of the battery's wires. ○ Cut the female connector off the JST PH 2pin cable. ○ Solder the battery's black wire first to the switch's black cable and shrink the sleeve using a hot air blower, then do the same for the red wire (*** be careful not to connect the battery's exposed wires – electric short)	
		For better maintenance and organization: •Use spacers to fix the OLED, RTC, and the multiplexers to the feather M0 datalogger. •Arrange all the cables in a way to minimize tension on the cables.	

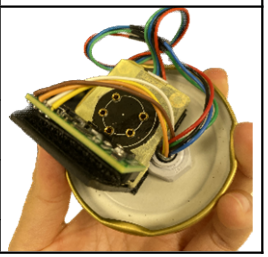
7	**Completing the data logger unit.**	•Put the unit into a comfortable box (round 30x30 cm) that you can open and access parts, including easy access for taking out the microSD when needed. •Use Velcro tape to fix the battery, on/off switch and M0 feather datalogger to the logger system container / box.	

2. Building the CO2/O2 sensing unit

Picture of a complete CO2/O2 sensing unit

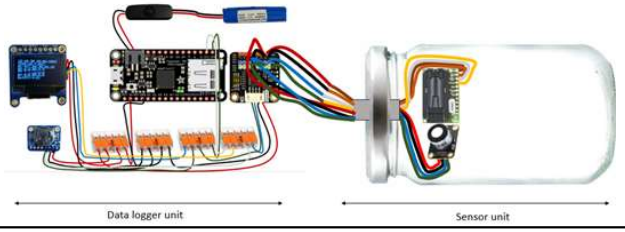


Step	What	How	images
1	**Preparing the glass jar**	Drill a 12 mm hole on a flat surface of the lid, away as you can from the center. Make sure you don't drill over any decorative ridges or channels of the lid (see pic). Fit a PG7 plug to the hole with the flatter end positioned towards the jar (see pic)	
2	**Preparing cables**	• Using one eight-wire cable, 1-1.5m long, remove 10-15cm of the plastic isolator at each end to expose the 8 wires inside. In our example colors of 8 wires used: yellow, red, orange, blue, black, brown, white, green. •One end of the cable is to connect to the multiplexer (*multiplexer end*), while the other end is to connect to CO2/O2 sensors (*sensor end*).	
3	**Preparing the multiplexer end**	•Remove 2-3mm of the plastic isolator from each end of the 8 wires and coat them with tin, and put shrinking sleeves over them. •Put the 8-wire cable through the PG7 of the jar lid and tighten the PG7. •Cut (or reuse) two 4pin cables and take the black female Dupont connector halves. Expose and tin coat the wire edges. •Solder two 4pin cables with 8 wires in order: <u>First 4-pin cable:</u> Red to brown Black to orange Blue to white Green to yellow <u>Second 4-pin cable:</u> Red to red Black to black Blue to blue Green to green	
		To connect the 8-wire cable to sensors: •Solder 4 small wires with colors: yellow, white, orange, brown to the corresponding ports on the CO2 sensor (SCD30) in order: Brown to VIN Orange to GND White to SCL Yellow to SDA	

4	**Preparing the sensor end**	•Use the remaining 4 wires with colors: red, black, blue, green for the O2 sensor. oSlide a shrinking sleeve over each of the 4 wires oTake a 4pin cable and cut it in half. Take the white 4P-connector and cut half of its wire away to shorten it. oExpose, tin coat and solder each wire from the 4-pin cable to the 4 wires from the 8-wire cable: Red with red Black with black Blue with blue Green with green. oPlug the white 4P connector to the port on the O2 sensor.	
5	**Prepare a housing for CO2 and O2 sensors**	•Use an L-shape plastic frame for housing – cut a hole through the L arm that will stick to the jar lid, so that the PG7 wouldn't push the housing out. If needs be, add a piece of plastic foam sheet to create the needed space. •Fix the O2 sensor to one of the L arms using tape (make a hole in the tape for both parts of the sensor to connect). •Use Velcro to fix the SCD30 to the second L arm with the sensors' ports and light visible. •Fix the housing under the lid with Velcro tape.	

3. Connecting the CO2/O2 unit to the core unit

Picture of a complete CO2/O2 incubation chamber



Step 1 Plug the female black Dupont connectors of CO_2 to port 0 on the multiplexer and O_2 into port 1.
Step 2 Repeat these stages (section 2 and 3) four times if four chambers are needed .

Picture of a system with four incubation chambers