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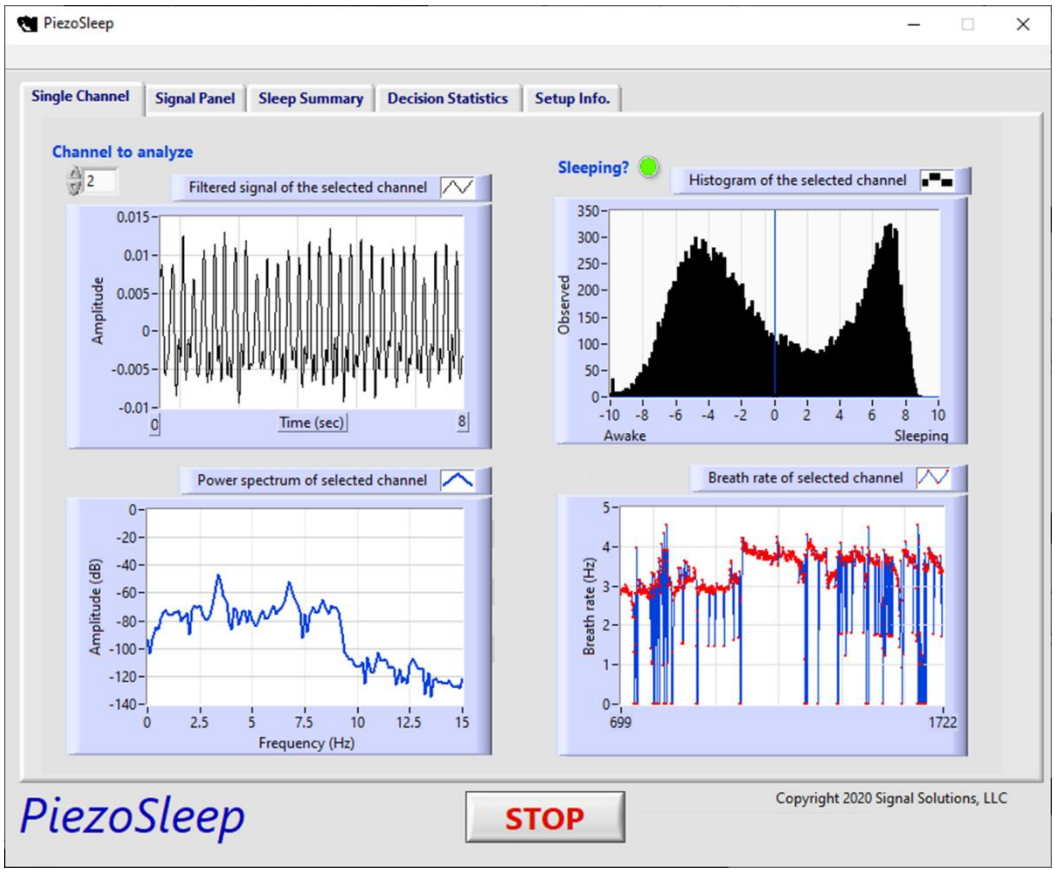
Installation and User Manual for PiezoSleep

Nov 2021

High-Throughput Pressure and Motion Recording Software with Real-Time Classification and Monitoring of Animal Sleep-Wake Behavior



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Installation and User Manual for PiezoSleep

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Nov 2021

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1. Introduction

The **PiezoSleep** software was written and compiled in LabVIEW (National Instruments, Austin, TX) to monitor and record high-throughput animal behavior tracking experiments. The program creates several files for classifying behavioral states. The base name for the files is provided by the user when the experiment starts up, and extensions and suffixes are added to distinguish the different files. The basic pressure signal from the piezoelectric sensors is sampled at 120 Hz and saved with either a *.bin or *.binfb extension (*.bin for rats, *.binfb for mice). Features associated with sleep and wake behaviors are extracted from the pressure signal and saved every 2 seconds in a file with the *.FeatVec/*.FeatVecfb extension. The FeatVec file is read in by an analysis program to characterize the sleep and wake behaviors over the duration of the experimental recording. The breath rate is estimated during sleep states every 2 seconds and stored in a file with a suffix and extension *_br.Feat/*_br.Featfb. A measure of general activity is also computed in 2-second windows, which is stored to the file with the *_at.Feat/*_at.Featfb extension. While the recording is in progress, the graphical user interface of **PiezoSleep** provides a variety of graphs, statistical summaries, and current sleep-wake states to monitor the data collection for real-time monitoring.

The *PiezoSleep* software supports the use of Signal Solutions data acquisition boxes (the Calimari, Squid, and Giant Squid boxes), recording up to 64 cages simultaneously. These boxes contain data acquisition (DAQ) modules as well as power distribution circuits for the sensor amplifiers.

The software described in this document automatically detects all device configured on your PC. Software will run on a PC with a minimum Windows 7 operating systems (32 or 64 bit) and a USB port. The installation of the *PiezoSleep* software automatically installs drivers from National Instruments to configure the computer to stream data through the USB port, if they are not already installed.

A. System requirements

Windows 7 or later with 6GB or higher RAM. See Section 4 to calculate file size when selecting Hard-Drive space.

B. Installation

1. To install the real-time sleep-wake monitoring software and supporting National

Instruments (NI) software, unzip the installation package provided with your purchase (either via mailed USB drive or download link).

2. Transfer the file to the hard drive of the computer to be used for data collection and unzip the file. Once unzipped, the directory '*PiezoSleep…'* should have been created. Inside this directory is one labeled volume and inside this is an application file named *setup*. Double click (execute) this file to start the installation process. Follow the instructions in the prompts to complete the installation.

3. Once the installation process is complete, a shortcut named *PiezoSleep* will be created on the desktop. Double clicking on the shortcut will launch the software program. The *PiezoSleep* program can be also accessed from the start menu, under the *PiezoSleep* folder or from the hard drive location it was saved to during the installation. The software requires the NI LabVIEW Run-Time Engine, which is installed along with *PiezoSleep* and is present in the *National Instruments* folder under the *Program files(x86)* folder. When removing this software, the *PiezoSleep* program can be identified in the *Program* option under the *Control Panel* from the PC menus and removed.

4. After the software installation is complete plug the data acquisition box into the PC USB port (and plug power supply in, if acquisition box requires external power). If this is the first time the device has been connected to the computer, the computer will start installing the driver (this may take a few minutes).

2. Computer settings to change before running PiezoSleep

Computer power saving program are often turned on in the default systems setup. These can cause longer recordings (greater than a few hours), to halt. Check the following setting to ensure the computer will not power down the USB ports, go into sleep mode, or reboot for an update during an experiment recording.

1. Go to '*Start menu*' and type '*Device Manager*' in the search bar. You will see it listed under the 'Control Panel'.

2. In the '*Device Manager*', click and expand the '*Universal Serial Bus controllers*' option to see a list of all USB ports available in the device. In order to reduce power consumption, the computer will turn off power to USB ports listed as '*Generic USB Hub*' and '*USB Root Hub*'.

3. Right click on one of these USB ports and select '*Properties*'. Go to the '*Power Management*' tab and **un-check** the option of '*Allow the computer to turn off this device to save power*' and press '*OK*'.

4. Repeat step 3 for EACH of the USB port listed as '*Generic USB Hub*' and '*USB Root Hub*'.

5. Close the '*Device Manager*' once you have finished step 3 for all USB ports.

6. Go back to the '*Start menu*' and type 'Power *options*'. It can also be accessed from the '*Control Panel*' under '*System and Security*'.

7. You will see a list of power plans (e.g. Balanced or High performance) and a highlighted radio button will show you the plan currently used by the system. Click on '*Change plan settings*' in front of the currently used power plan.

8. Change the '*Put the computer to sleep*' option to '*Never*'. If you are using a laptop, you will see the options '*plugged in*' and '*on battery*'. Change the '*plugged in*' setting to '*Never*'. In case you don't see an option of never, you can type it in.

9. Click on the '*Change advanced power settings*'.

10. Expand the '*Sleep*' option. Change the '*Sleep after*' setting to '*Never*'. If you are using a laptop, you will see the '*Sleep after*' setting for '*plugged in*' and '*on battery*'. Change the '*plugged in*' sleep after setting to '*Never*'. In case you don't see an option of never, you can type it in.

11. Within the '*Sleep*' options, go to the '*Allow hybrid sleep*' and set it to '*Never*'. For laptops, set the '*plugged in*' option to '*Never*'.

12. Repeat step 11 for the '*Hibernate after*' option under '*Sleep*'. Remember that you can type in the word '*Never*' in these options.

13. Collapse (come out) the '*Sleep*' option and go to the '*USB settings*' option. Expand the '*USB selective suspend setting*' and set it to '*Disabled*'. For laptops, disable the '*plugged in*' options within the '*USB settings*'.

14. Go back to the '*Start menu*' and type 'Windows *Update*'. It can also be accessed from the '*Control Panel*' under '*System and Security*'.

15. Under '*Windows Update*', click on the '*Change settings*' option located on the left column of the window.

16. Click on the pull-down menu under '*Important updates*' and select the '*Never check for updates (not recommended)*' option. This will prevent the computer to re-start in the middle of a recording. It is recommended that you manually update Windows from

time-to-time. Manual update can be done by clicking on the '*Install updates*' option located under '*Windows Update*'.

3. Data acquisition and monitoring

A. Starting a data acquisition session

1) Launch *PiezoSleep* either from the desktop shortcut or from the start menu or from the location it was saved/installed on the hard drive. The windows shown in Fig. 1 appear after launching the program.

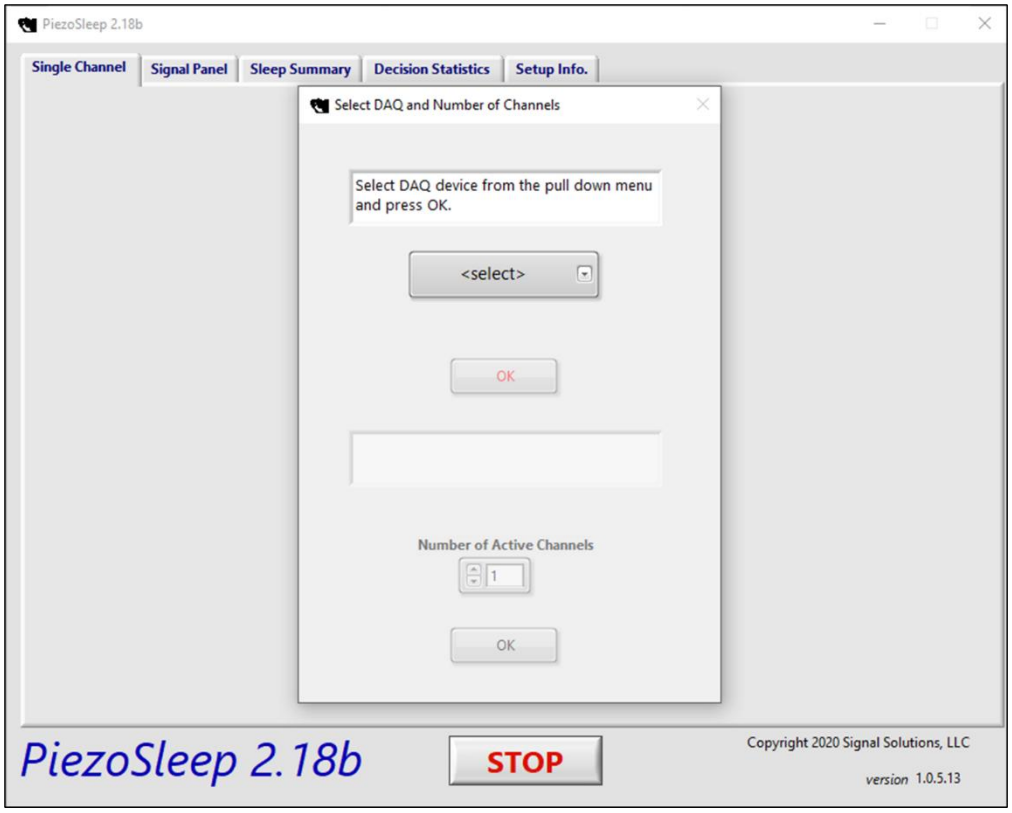


Figure 1. Starting windows for *PiezoSleep* program. From the dialog window, select the DAQs being used for the recording session.

2) If multiple DAQs are connected to the computer (from other National Instrument Systems that are running for the machine), after clicking on select, a drop-down menu will appear listing all the connected DAQs as shown in Fig. 2. In most cases, you will see only one device. If there are multiple devices associated with other data collection activities, then select the one associated with the *PiezoSleep* system. It is typically the newest device plugged into the system. If you are unsure, then temporarily unplug all other National Instrument USB devices to identify the

one associated with PiezoSleep. Make note of this and select for this and all future recordings. Once selected, press OK to move on to the next setting.

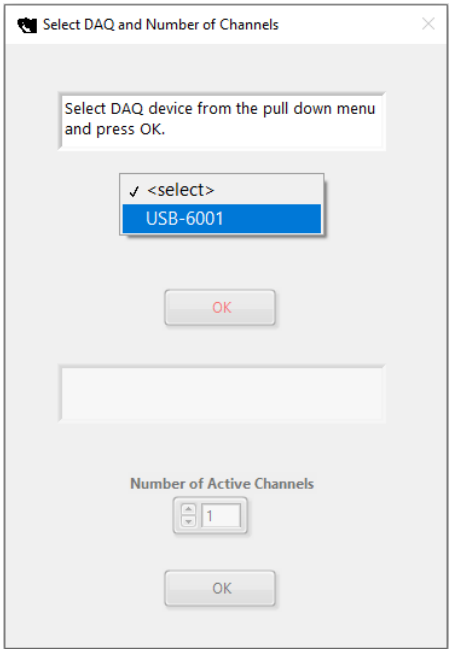


Figure 2. Prompt to select a DAQ. A listing of all DAQs currently plugged in your PC will appear in a dropdown menu. Click on a name inside the table and press OK.

3) Next, enter the number of active channels in the dialog box shown in Fig. 3. This is done so that only the necessary channels will be recorded. The maximum number of channels is based on the DAQ capabilities and is listed in the prompt. Type in the maximum number of channels if all cages are being used in the experiment. If fewer channels than the maximum will be used, then the number of active channels can be entered to save storage space and result in the later analysis programs running more efficiently. For example, if only 4 animals are to be monitored (cages 1 to 4), then 4 should be entered as the number of active channels. In this case the cages should be connected sequentially in the acquisition box channels, starting with channel 1. For example, the first cage in channel 1, the next in channel 2 …). If a channel is skipped in the sequence, then enter the number of channels corresponding to the highest channel with a cage for recordinging attached. For example, if cages are plugged into channels 1, 2, 5, and 6, then 6 should be entered for the number of active channels. Channels 3 and 4 in this case will be recorded, but they will contain only noise and should be marked as empty on the channel labels, so they can be ignored in future analyses or used to observe the noise in the recording.

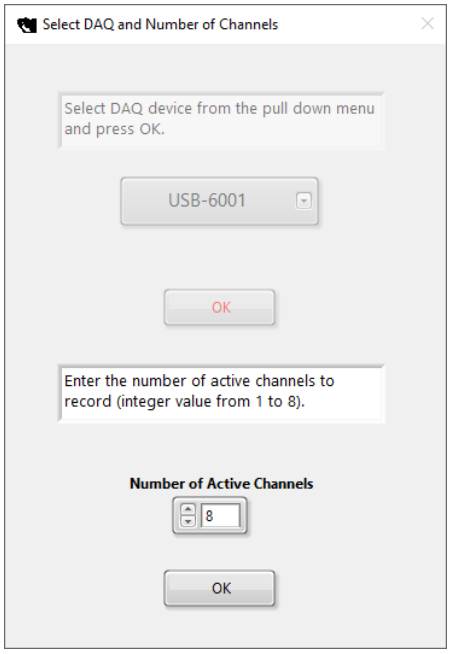


Figure 3. Prompt to enter the number of sequential channels to be recorded.

4) After you enter the number of active channels, the dialog box shown in Fig. 4 appears. The textboxes can be edited to select time for light and dark onset, and cage labels. Alternatively, animal ID names from an Excel spreadsheet. If you have a spreadsheet file (*.xls or *.xlsx) with the animal IDs, the format should be a series of names (text format) under column A, and the number of rows should be equal to the number of active channels selected. If you select *No*, then you have the option to enter IDs manually for each active channel or use the default channel labels. This information is stored in the file header to be used in subsequent analysis programs like *SleepStats*.

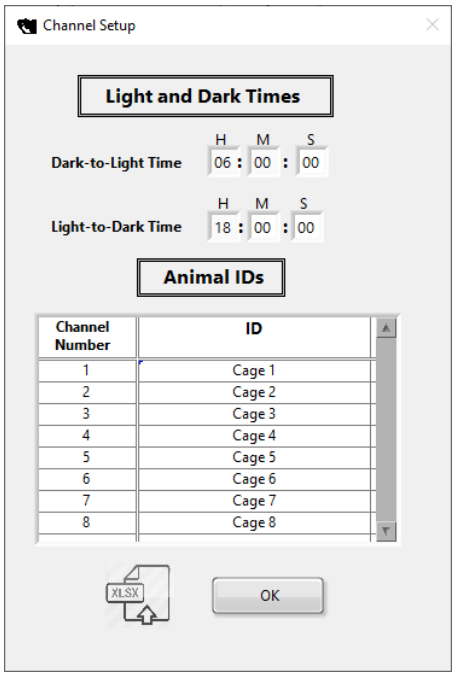


Figure 4. Select Light Dark times and cage labels or import a spreadsheet.

5) The program then prompts for a file name as shown in Fig. 5. Animal motion/pressure data will be saved to this file for later processing or checking the signal quality of the recording. It is recommended that this file be given a descriptive name that identifies the nature of the experiment and the date the experiment began. It is also recommended that you do NOT use a period in the file name or type in an extension on the file name textbox, since *PiezoSleep* adds its own extensions that are used in later programs (the *binfb* extension is given to the files with the unprocessed sensor data that are useful for reprocessing or updating when new algorithms are released). The software, *SleepStats*, which analyzes data after the experiment, uses the base name to look for other files from the experiment and opens them automatically. If they have different names and

extensions, the automatic loading will not work, and you will be prompted to search for them with a file/directory navigator.

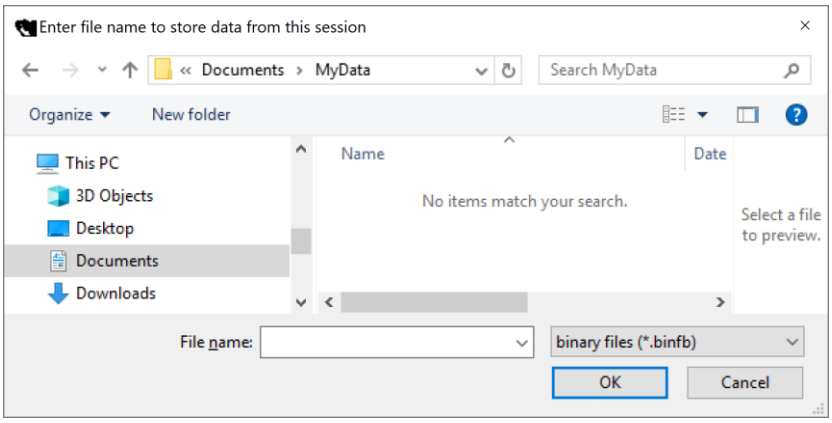


Figure 5. Prompt to enter a filename where data is to be stored during the experiment.

6) After selecting the filename and location, the program displays your selections as shown in Fig. 6. The display includes the DAQ device, number of active channels, file path, light and dark onset times, and animal ID names. It also contains a re-selection menu, which gives you the option to re-select the DAQ device, number of active channels, animal ID names, or filename and location. If no re-selection is needed, then select the last option and click *OK* to continue. Please note that if you re-select the DAQ device, you will also be prompted to re-select the number of active channels and the animal ID names. Similarly, if you re-select the number of active channels, you will also be prompted to re-select the animal ID names. It is recommended that the location of the stored files is noted for later retrieval and archiving.

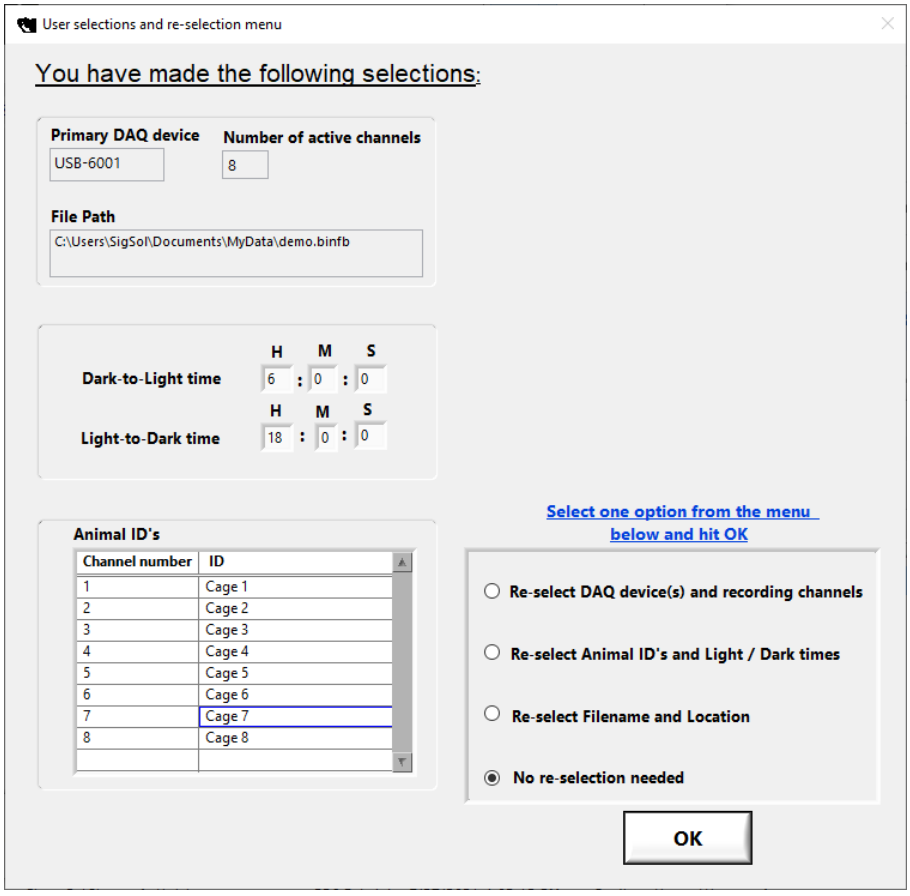


Figure 6. Prompt to check entries made and modify if incorrect information was entered, before the data collection process begins.

7) The program now starts recording and displaying data. Under each tab, the waveforms / statistics will begin to appear in a few seconds, the details of which are explained in the following paragraphs.

8) When data collection is finished, click the *STOP* button located in the bottom of the window or by clicking the X in the upper right corner of the window.

B. Ensuring Sensor Connectivity

After selections have been made, data collection begins. While data is collected, the acquisition system will prompt the user if a sensor short is detected or if a discrepancy is found between the number of channels being recorded vs the number of sensors connected.

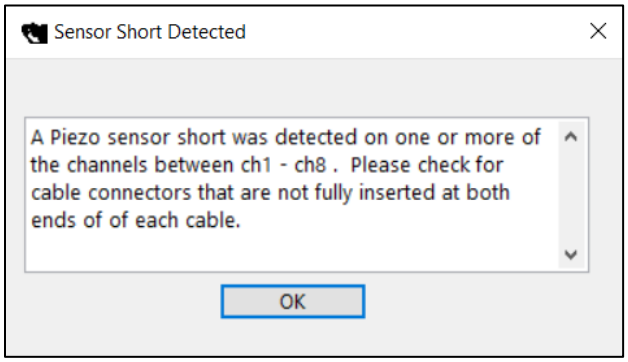


Figure 7A. Prompt alerting the customer if a sensor short is detected. This prompt will continue to pop-up until there are no shorts detected.

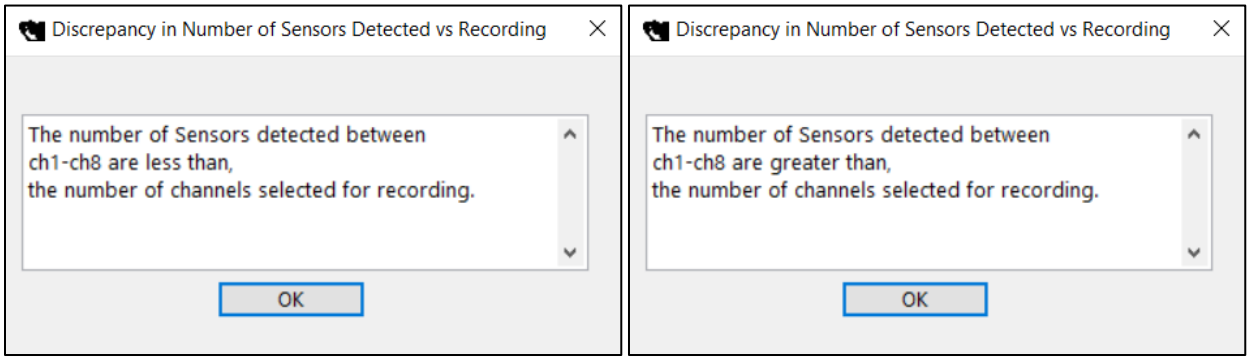


Figure 7(B & C). Prompt alerting the customer if a discrepancy is found between the number of sensors detected does not equal the number of channels being recorded. This discrepancy is only tested when a change in the number of connected sensors is detected.

If any of these messages occur, check the cable connections. You can check the signal panel (described in Section D) to look at these channels to see if any have no signal (flatline and not responsive to touch) pattern. Then check the cable on both ends to make sure that they are fully plugged in or water has not come into contact with a sensor. If you can see signals on all the flagged channels, then this could be just a false alarm. You can close the window and ignore it.

C. Selecting and monitoring a channel signal (Single Channel tab)

The *Single Channel* tab displays the most recent 8 seconds of the recorded signal along with additional graphical descriptions. As shown in Fig. 8, the tab consists of four panels, displaying the pressure signal (top left), sleep decision statistic (top right), power spectrum (bottom left), and the breath rate frequency (bottom right) for a given channel. To view a particular channel, the channel number can be typed into the *Channel to analyze the text* box or incremented up and down with the arrows to the left of the text box. To facilitate real-time observation, the *PiezoSleep* program computes and updates the power spectrum, breath rate, and the sleep decision statistics every 2 seconds.

The power spectrum shows the signal's power distribution over a range of frequencies. The power spectrum example in Fig. 8 shows one dominant peak at around 3 Hz. The amplitude on the y-axis is expressed in the logarithmic unit decibels (dB).

The sleep decision statistic comprises a histogram plot showing the sleep-wake distribution for the selected channel. The sleep-wake state (with about a 4 second lag from real time) is shown via a green LED, located just above the panel, which lights up to indicate sleep. Detection is based on comparing the real-time decision statistic to a sleep threshold, which by default is zero and is depicted on the histogram by a blue vertical line. Larger positive decision statistics indicate a greater likelihood of sleep, while negative decision statistics indicate a greater likelihood of wake. In Fig. 8, the green LED is lit, indicating a current sleep state and the histogram shows a larger concentration on the positive side, indicating that the animal has been asleep for some time. **NOTE: the real-time statistics and classification are about 5 to 10% less accurate than the decision analysis program *SleepStats*. *SleepStats* performs a more extensive calibration than is possible in real-time, which is done for recording 24 hours or more.** Therefore, all assignments of mouse sleep behavior should be made using *SleepStats* after the recording is complete. The purpose of the real-time monitoring is to check on potential outlying behavior, sick mice, or bad connections while the experiment is running and potential corrections can be made.

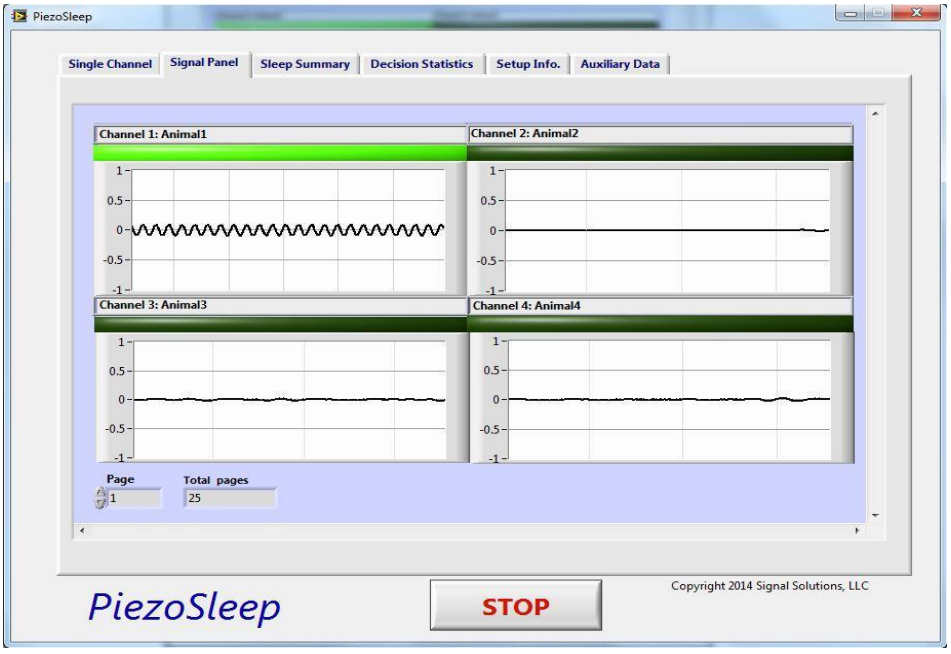
The breath rate plot displays the most recent 32 minutes of data, updating itself once every 2 seconds. For the first 32 minutes after starting the program, the breath rate plot will accumulate data and from there on plot the most recent 32 minutes of data. If the signal is too irregular to estimate an average breath rate, then a zero is output to the plot. The plot in Fig. 8 shows that most of the signals were irregular (typically of the active animal state) with some periods of where the breathing frequency of the animal was around 4 to 5 Hz.

Figure 8. *Single Channel* tab showing the recorded signal (top left), sleep decision statistic (top right), power spectrum (bottom left), and the breath rate frequency (bottom right) for an individual channel.

D. Monitoring sensor signals for all active channels (Signal Panel tab)

To simultaneously view sensor signals for the active channels, click the Signal *Panel* tab. The Signal *Panel* will appear, as shown in Fig. 9, and display 4 active channel signals over the last 8 seconds. If there are more than 4 active channels, the *Page* text box can be incremented to page through the rest of the channels. The animal IDs with their corresponding channel number, are displayed over each plot. The thin green bar (virtual LED) above each plot on the Signal *Panel* is the sleep indicator. A lit indicator implies detected sleep, as seen for channel 1 in the figure. The y-axes of the graphs on the Signal *Panel* are fixed from -0.4 to 0.4 volts (volts are proportional to pressure). These graphs by default do not auto-scale the signal like the filtered signal plot on the *Single Channel* panel so relative amplitudes can be observed. The primary purpose of these graphs is to view signals from all cages quickly to see if there are problems (broken connections, amplifiers not plugged in, dead mice, etc.). However, by right clicking on the y-axes of these graphs, you can turn on the auto-scale to see more detail for weaker signals. To go to a fixed scale, set to manual scale, and directly click on the y-axis numbers (maximum and minimum) and type over to set the limits. There is a 2 to 4 second second delay between what is happening in the cage and what appears on the screen.

(A)



(B)

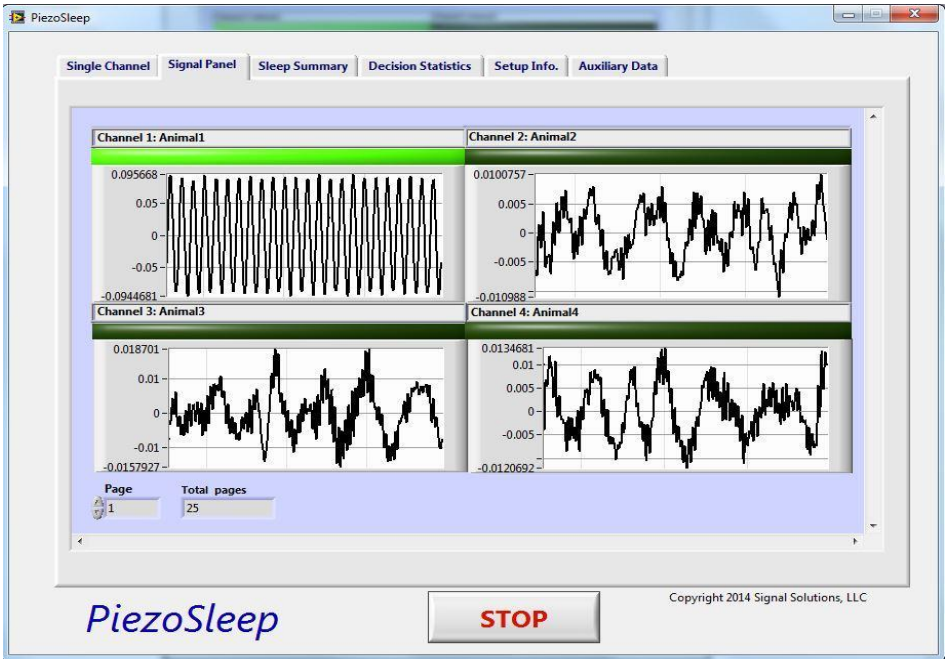


Figure 9. *Signal Panel* tab displaying piezoelectric sensor signals for 4 active channels. The *Page* box is used to switch to other active channels when more than 4 channels are active. (A) Y-axis fixed (default) and (B) y-axis switched to auto-scale, shown with a simulated sleep signal plugged in channel 1.

E. Monitoring percent sleep for all active channels (Sleep Summary tab)

The percentage sleep for each channel can be observed at any point during the recording in the *Sleep Summary* panel shown in Fig. 10. The percentage is based on the sleep threshold and the histogram formed from the accumulating data. The example shown below has one simulated sleep signal plugged into channel 1 with detected sleep percentage greater than 99% and the rest are noise only channels with sleep percentages all less than 8%. Outliers can be identified here early in the experiment and can be followed up with direct observation of the animal or checking the function of the sensor (e.g. ensure cable is plugged in). Again, *SleepStats* will provide more accurate numbers after the experiment has been completed.

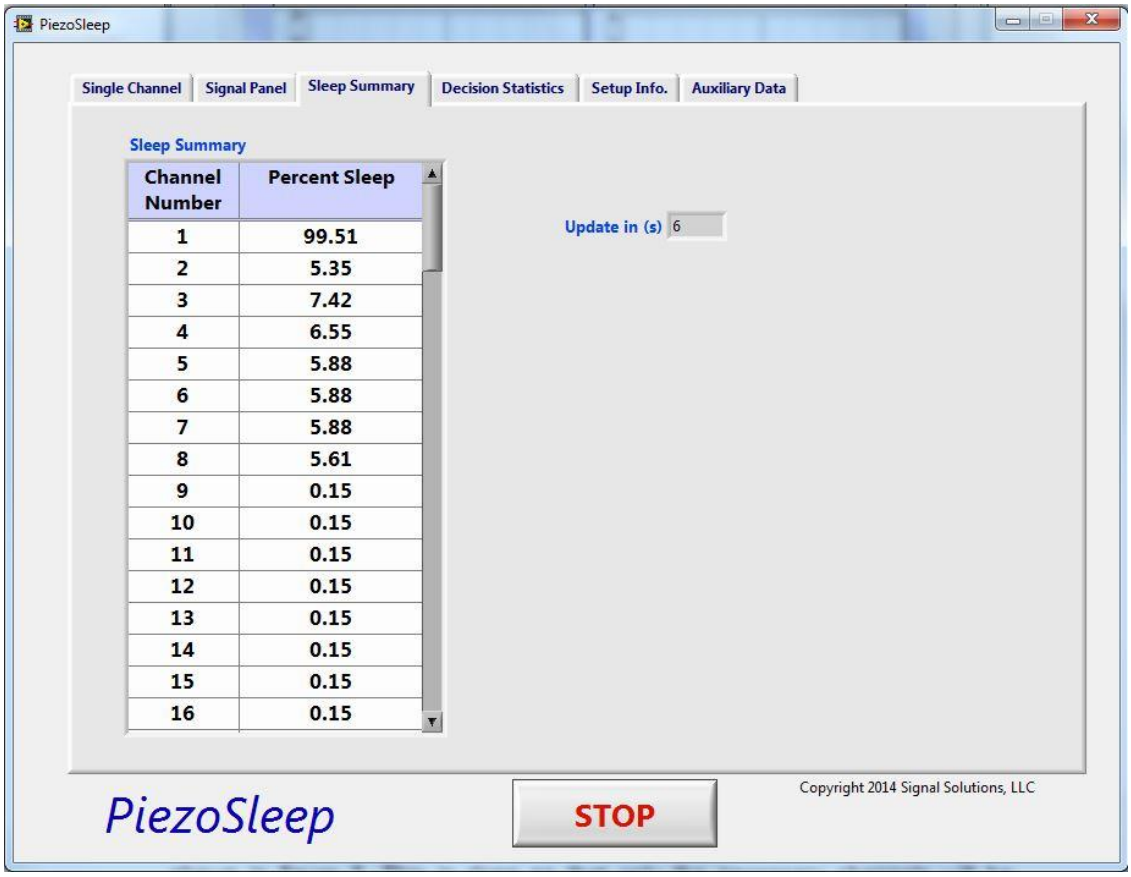


Figure 10. Sleep percentage summary.

F. Monitoring decision statistics for all active channels (Decision Statistics tab)

To simultaneously view the sleep-wake decision statistics for the active channels, click the *Decision Statistics* tab, which shows histograms for 4 active channels at a time, as illustrated in Fig. 11. The animal IDs with their corresponding channel number, are displayed over each plot. If there are more than 4 active channels, the *Page* text box can be incremented to view results from the rest of the channels. This provides a quick way to examine the data collection and sleep-wake behavior over the experiment. Unusual behaviors or data collection problems can be identified while the experiment is running, which can be addressed by direct observation of the animal. For example, after running the program for 24 hours with a **normal/control** animal, a bimodal distribution should appear in the histograms from mapping sleep signal dynamics toward positive values and wake signal dynamics toward negative values. If the histogram is not bimodal, it could be the result of noise or weak signals, for which there are a number of causes:

- an electrically or mechanically noisy environment
- poor animal contact with the piezoelectric sensor on the floor of the cage (sometimes cause by too much bedding or the cage shield not being properly seated on the sensor platform)
- faulty amplifier or sensor
- unusual animal behavior not observed in the training phase of the classifier (i.e. animal is ill, dead, or has a very unusual respiratory pattern during sleep)

The bimodal pattern in the histogram below is exploited by adaptive thresholding, used in later analysis programs, which seeks a minimum point between the clusters (modes) of decision statistics. Real-time analysis is limited in that it uses the same threshold over all channels and can vary by as much as 10% from the later analysis using an adaptive threshold. The primary purpose of real-time monitoring in the acquisition program is to alert those monitoring the experiment. If problems arise, they can be verified (by direct observation of the animal) or corrected during the experiment. More accurate sleep and wake parameters are generated with the *Sleepstats* program. More than one panel (group of 4) can be examined simultaneously by undocking the current tab using the arrow in the upper right of the plot panel.

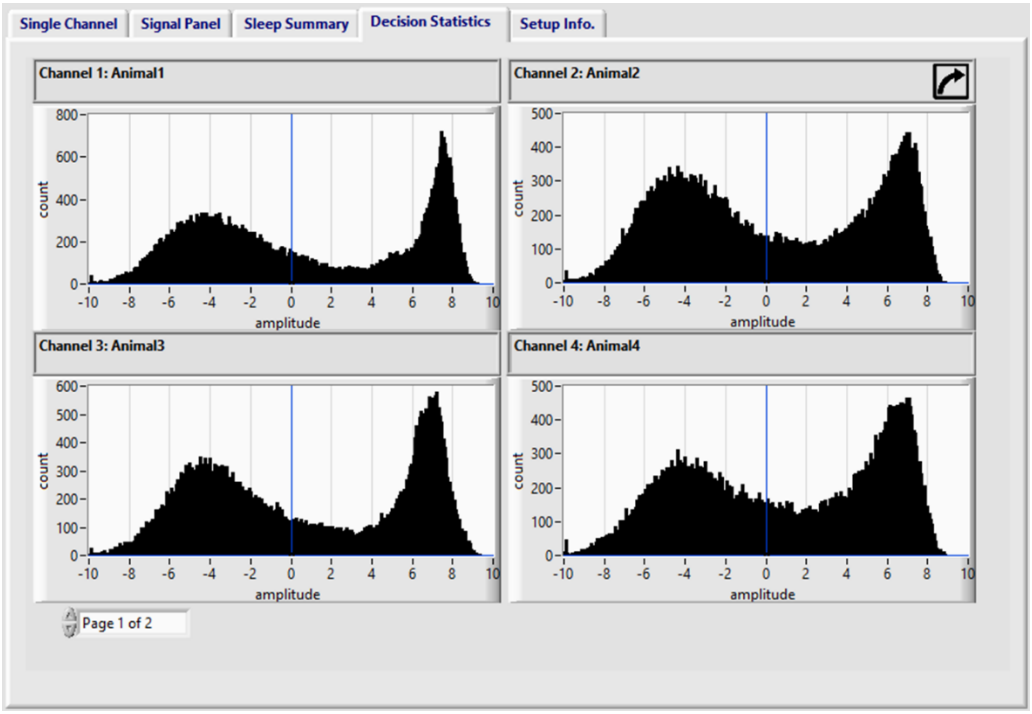


Figure 11. *Decision Statistics* tab showing histograms of 4 channels simultaneously. The *Page* box can be used to switch to other active channels if more than 4 channels are active. The undock arrow in the upper right corner displays the current tab in an independent window, so other panels can be observed simultaneously.

G. Current file and setup information display (Setup Info tab)

After data collection has begun, current information and settings will be displayed on the *Setup Info* tab, as shown in Fig. 12. The display includes: Start Time, Number of active channels, Primary and Secondary DAQ selections and their channel information, pseudo LED showing Auxiliary DAQ usage, File Path, and Light and Dark transition times. These selections were made by the user at the start of the data acquisition session. The start time is stored in the data file header, so it is saved with the data to create an absolute time axis for computing statistics related to the light and dark periods, as well as confirming the date the experiment began.

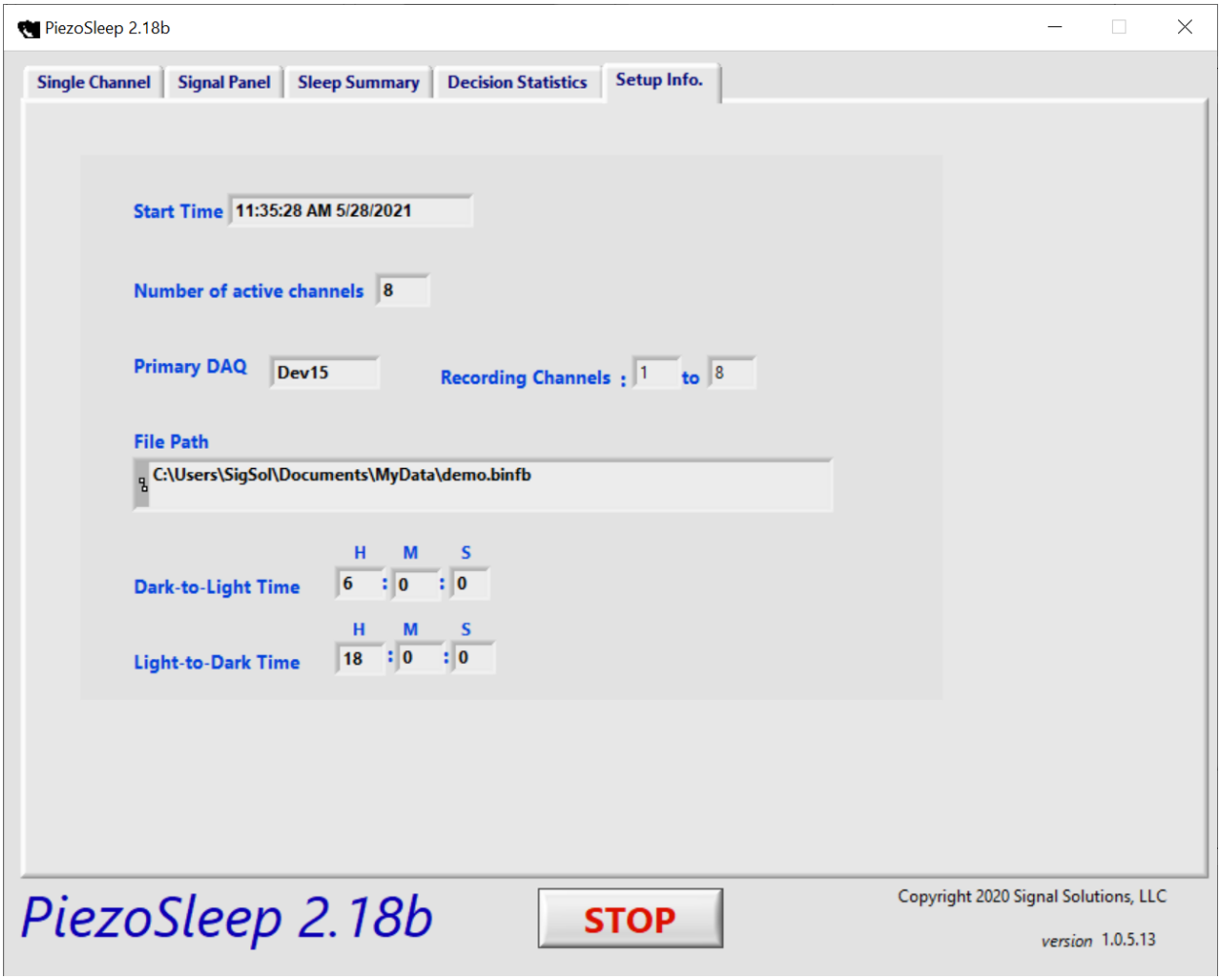


Figure 12. Check on setup parameters.

H. Logging Environmental Sensor Data

If an Environmental sensor accessory is attached via USB, the user can enable logging this data through the *Accessories* menu.

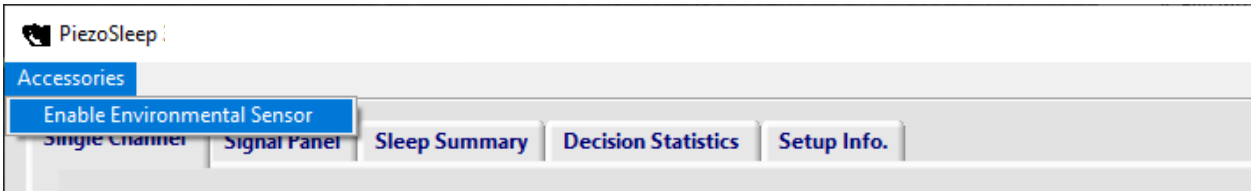


Figure 13. Accessories menu showing access to the Environmental Sensor

Enabling the Environmental Sensor will open the settings window shown below in Fig.14. If there are no sensors shown and you have the device connected to the USB, click the *Rescan Devices* button. Once the device is shown, the device label can be modified to tailor it to the experiment.

Under the *Other Settings* menu, there is an option to *Disable LED*. The LED being referred to here is a physical LED on the sensor that blinks each time the device is sampled. Disabling the LED will prevent it from illuminating.

Once the settings are complete, clicking the *Begin Logging* button will open the Datalogger window.

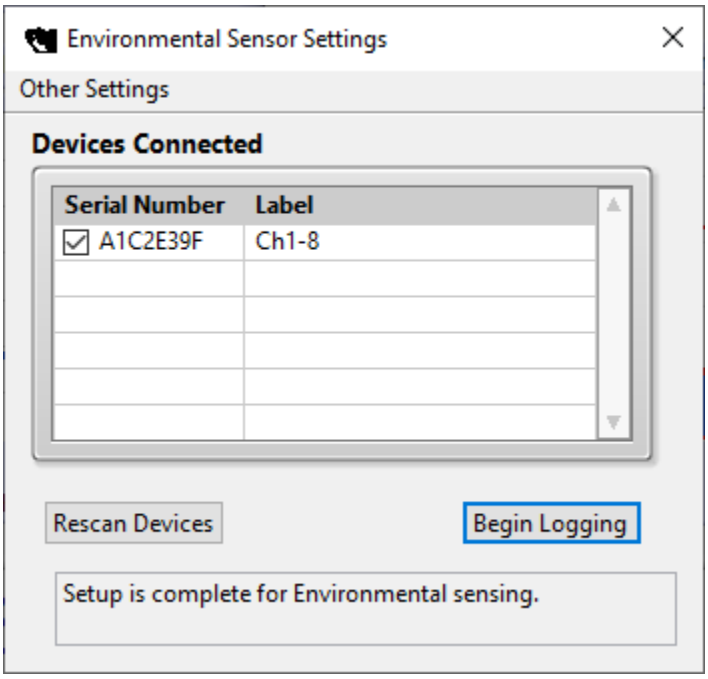


Figure 14. Environmental Sensor Settings Window

After clicking the *Begin Logging* button in the settings window, the Datalogger window opens and environmental sensor data acquisition begins. The Datalogger window can be minimized and acquisition will continue to run in the background.

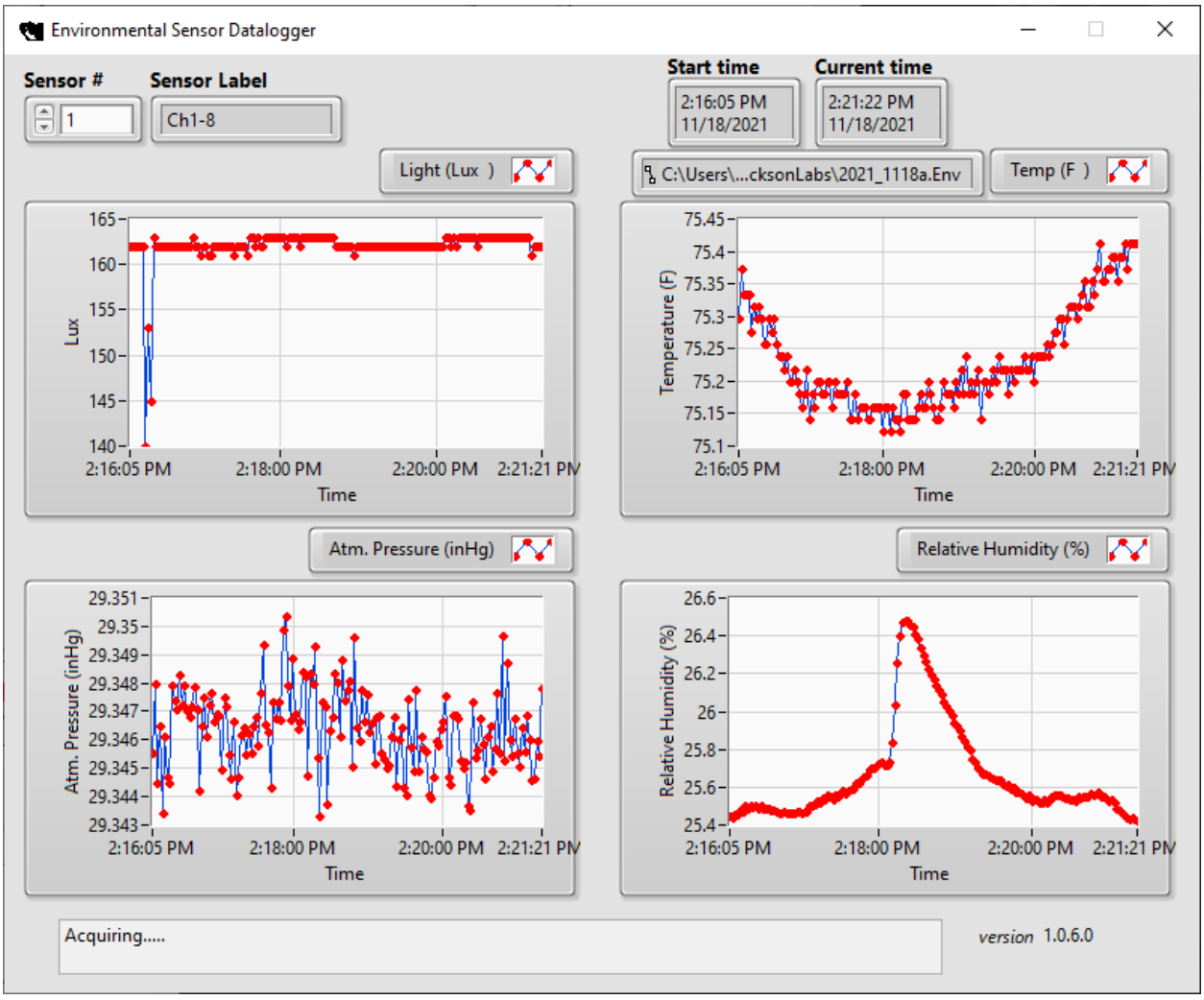


Figure 15. Environmental Sensor Datalogger Window

At the end of the experiment, when the main PiezoSleep window is closed, the environmental sensor acquisition will be stopped automatically.

4. File Information

PiezoSleep saves three piezo data files. The file with a base name (provided at the beginning of the data acquisition session) and the *bin*/*binfb* extension, stores the raw data from the piezo sensors. The file with the *Feat*/*Featbf* extension and '*_br*' padded to the base name stores the breath rates for all channels. The *Feat*/Featfb file with '_at' padded

to the end stores a measure of general activity for all channels. The file with the *FeatVec/FeatVecfb* extension stores the feature vectors used to calculate the sleep statistics. These files are used by the analysis program *SleepStats* developed by Signal Solutions LLC. Files are updated to the disk at least once every 2 seconds during the data acquisition session. Therefore, if the system crashes due to a power failure, data up to the point when the computer system failed are saved.

The program samples the piezoelectric signals at 120 Hz and 16 bits (2 bytes) per sample per channel. A 16-channel recording will write 332 MB of data per day. If disk storage is limited, it may be necessary to periodically stop data collection, remove existing data files, and restart the recording with a new file. This, however, complicates file and data management. It is best to have a computer with enough hard disk space and removable media to transport the large files to other systems for archiving and analysis.

The size of the raw piezo data file can be estimated in terms of the number of days and active channels by the following formula:

$$B = \frac{(2*24*60^2*120)*d*C}{10^6} \approx 20.74\, dC,$$

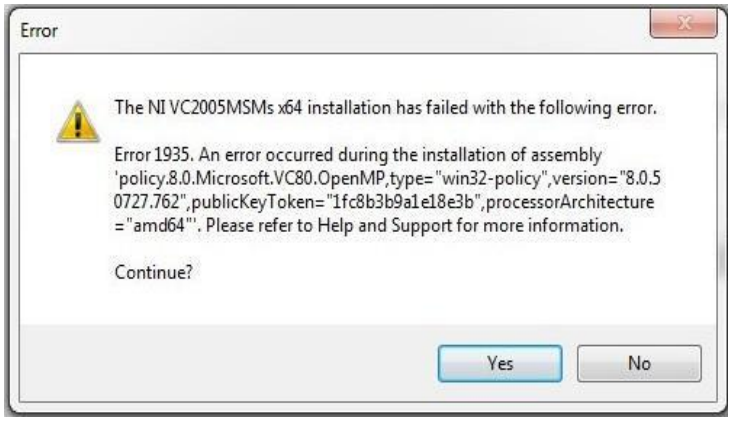
where *d* is the number of days, *C* is the number of active channels, and *B* is the file size in megabytes (MB).

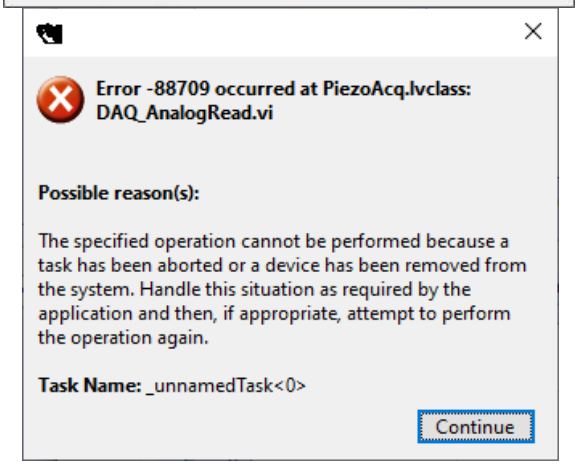
The *Featfb* and *FeatVecfb* file are considerably smaller in size as compared to the piezo *binfb* file. The *Featfb* file consumes space at about 0.34 MB/day and the *FeatVecfb* file consumes roughly 1.4 MB/day for each channel as compared to the 20.74 MB/day for the *binfb* file.

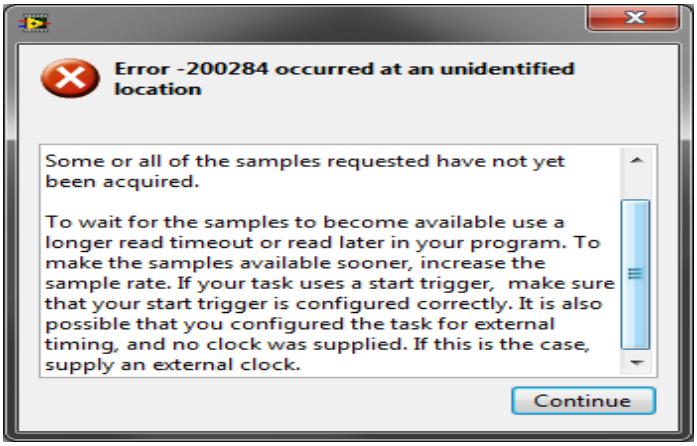
The auxiliary data is sampled at 120 Hz and 36 bits (4 bytes) per sample per channel. The size of the auxiliary data file (B_1), estimated in terms of the number of days (d) and active channels (C_1) is given by: $B_1 \approx 41.48\, dC_1$. A 4-channel recording will write 166 MB of auxiliary data per day.

5. Troubleshooting

Troubleshooting errors during PiezoSleep installation or operation

Error	Reason / Solution
	This error generally occurs when the software is being run from a user account different than the one it was installed using. Please switch to the user account used during installation of the 'PiezoSleep' software and try running the software again.
	This error generally occurs due to the following two reasons: 1) Windows Update is running or has some updates pending. 2) The anti-virus software in your system is blocking the installation of "NI VC2005MSMs" because it sees it as a threat. Please do the following: 1) Turn off the anti-virus software and firewall temporarily. 2) Go to the system's 'Control Panel' and check if there is any 'Windows Update' running or pending. If that's the case, finish updating and then re-start your system and try the installation again.





This error generally occurs when the USB port on your computer, which powers the DAQ, loses power (i.e. shuts down). Please refer to section on Computer Setting of the manual and make the appropriate changes.