SIGNAL SOLUTIONS

Sensor Systems, Software, Sleep

PiezoSleep 5

USER MANUAL

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PiezoSleep 5.0

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Contents

1. Introduction

The *PiezoSleep 5.0* data collection software, written and compiled in Qt, monitors and records high-throughput animal behavior-tracking experiments and outputs several files for classifying behavioral states.

When the experiment starts, the user creates the base file name, which will have distinguishable extensions and suffixes added. The piezoelectric sensors sample the basic pressure signal at 120 Hz and save it 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 to a file with the ***.FeatVec***/***.FeatVecfb*** extension. The ***FeatVec*** file is read using *SleepStats*, our data analysis program, to characterize sleep and wake behaviors over the duration of the experimental recording. The breath rate is estimated every 2 seconds during sleep states and stored in a file with the suffix and extension ***_br.Feat***/***_br.Featfb***. A measure of general activity is also computed in 2-second windows, which is stored in the file with the ***_at.Feat***/***_at.Featfb*** extension.

During the recording process, the *PiezoSleep* graphical user interface provides a variety of graphs, statistical summaries, and current sleep-wake states to enable real-time monitoring for data collection. This software supports the use of our data acquisition boxes (Calamari, Squid, and Giant Squid boxes), accommodating up to 80 cage recordings simultaneously. These boxes contain data acquisition (DAQ) modules and power distribution circuits for the sensor amplifiers. This software automatically detects all devices configured on your computer. It will run on a PC with a minimum of Windows 7 operating system (32- or 64-bit) and a USB port. During installation, drivers from National Instruments will be installed automatically 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 2GB or higher RAM.

B. Installation

1. To install the real-time sleep-wake monitoring software and support the National Instruments (NI) software, download and unzip the installation package via the instructions given with your purchase.

2. Transfer the file to the computer's hard drive, where data will be collected, and unzip the file. Once unzipped, the directory '*PiezoSleep*...' should have been created. Inside this directory, there is a volume labeled with an application file named ***setup***. Double-click 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 *PiezoSleep* shortcut will be created on the desktop. Double-clicking on the shortcut will launch the software program. It can also be accessed from the Start menu, under the *PiezoSleep* folder, or from the hard drive location it was saved to during the installation. To remove this software, open ***Programs*** under ***Control Panel*** from the PC menus and delete it.

4. After the software installation is complete, plug the data acquisition box into the PC USB port (and power it up if it requires external power). If this is the first time, the computer will install the driver and configure the power, which may take a few minutes.

2. Computer settings to change before running PiezoSleep

During a recording, it is important to ensure your computer does not power down the USB ports or reboot after an operating system update. Otherwise, the program will stop, and no future data collection will occur. Computer power-saving features for a PC are often turned on in the default system setup. These can cause longer recordings (greater than a few hours) to stop. Check the following settings to ensure the computer won't power down the USB ports, enter sleep mode, or reboot to install an update during an experiment recording.

1. Go to the ***Start*** menu and type *Device Manager* in the search bar (you may have to click the gear icon to get to the settings menu first to get the search panel). If you have an older operating system, you will see it listed under the ***Control Panel***. If you still don't see the option to open the *Device Manager*, try the keyboard shortcut ***Windows Key + R*** for the *Run Command* and type in the text box ***devmgmt.msc***. Then press *OK* or *Enter*. This will open the *Device Manager* window.

2. In *Device Manager*, click and expand the ***Universal Serial Bus controllers*** option to see a list of all USB ports available in the device. To reduce power consumption, the computer may be set to 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, uncheck ***Allow the computer to turn off this device to save power***, and press ***OK***.

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

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

6. Return to the ***Start*** menu and click *Settings* (gear icon), then select ***Power*** (or *Power & Sleep*) options. On older operating systems, it may be accessed from the ***Control Panel*** under ***System***

and Security. This option can also be accessed by pressing the *Windows key + X* keyboard shortcut and then selecting ***Power Options*** from the pop-up menu.

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***. On some desktops, this may not be an option, which implies it will not turn off or put the computer to sleep. The screen power-saving option will not impact the recording. If you are using a laptop, you will see the options for *plugged in* and *on battery*. Change the *plugged-in* setting to ***Never***. In case you don't see this option, you can type it in.

9. Click on ***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 batter***y. Change the *plugged-in* sleep after setting to ***Never***. In case you don't see this option, you can type it in.

11. Within the *Sleep* options, if an option exists to ***Allow hybrid sleep***, 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. On some older operating systems, the ***USB settings*** option exists (can be searched for in the settings option or the control panel) for the USB selective suspend setting. If so, set it to ***Disabled***. For laptops, disable the ***plugged-in*** options within the USB settings.

14. Go back to the *Start* menu and click on the Settings icon, then find or type in ***Windows Update Settings***. It may also be accessed from the *Control Panel* under ***System and Security*** on some of the older operating systems.

15. Under *Windows Update*, click on ***Change settings*** or ***Advanced options*** (may be at the bottom or in the right panel of the window) and turn off any auto-restart after update settings. Note that if you turn off auto updates, you should periodically manually check for updates on the computer and let it update when an experiment is not running.

3. Data Acquisition and Monitoring

A. Starting a Data Acquisition Session

Launch *PiezoSleep* from the desktop shortcut, the Start menu, or the location it was saved/installed on the hard drive. Once loaded, the main interface and *Recording Setup* window shown in Fig. 1 will appear.

NOTE: The color of the interface will depend on whether the theme on your computer is set to light or dark (or custom). All examples in this manual use the dark theme.

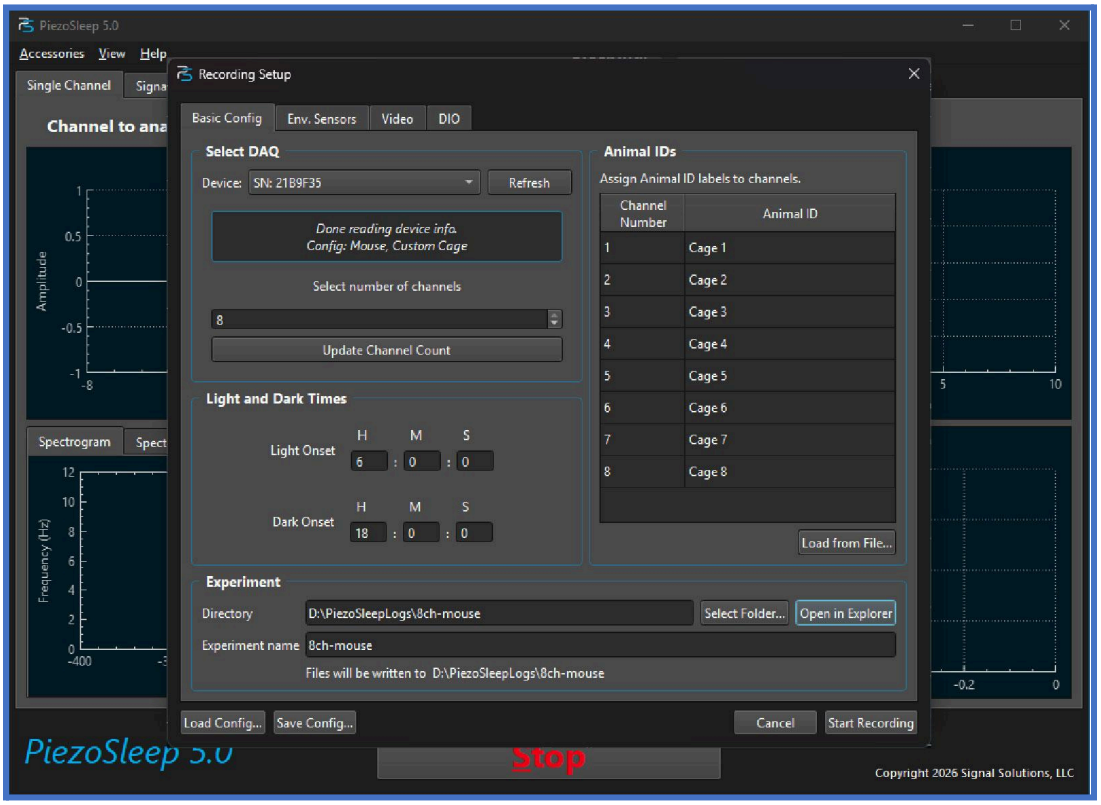

Figure 1: Main user interface (with select Recording Setup window)

Near the top of the *Recording Setup* window, you will see multiple tabs: Basic Config, Env. Sensors, Video, and DIO (Digital I/O). Each tab lets you edit/add specific features of the recording.

Basic Config

If a DAQ is already plugged into your computer before you start up *PiezoSleep*, the device should appear in the ***Select DAQ*** section. If multiple DAQs are connected to the computer (from other National Instruments systems running on the machine), the drop-down menu will list all connected DAQs. If any are missing, click the ***Refresh*** button to the right of the drop-down menu. In most cases, you will see only one device (unless nothing is plugged in). If multiple devices are 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 a note of this and select it for this and all future recordings.

Once a DAQ is selected, enter the number of **ACTIVE** channels. This ensures you record only the necessary channels. You can either type in the number or use the arrow keys on the right. The maximum number of channels is based on the DAQ's capabilities. If all channels are being used, enter the maximum number. If you use fewer channels than the maximum, enter the number of active channels to save storage space and help later analysis programs run more efficiently. For example, if only 4 animals are to be monitored (cages 1 to 4), then enter 4 as the number of active channels. In this case, connect the cages sequentially to the acquisition box channels, starting with channel 1 (the first cage is on channel 1, the next on channel 2, etc.). If a channel is skipped in the sequence, enter the highest channel number with a cage attached for recording. For example, if the cages are plugged into channels 1, 2, 5, and 6, then enter 6 for the number of active channels. In this case, Channels 3 and 4 will be recorded, but they will contain only noise and be marked as empty on the channel labels. This way, you can ignore them in future analyses or use them to observe the noise in the recording.

NOTE: If you are using a **Demo Unit**, there will be a section in the middle of the window (DAQ tab) showing the number of days (and hours) remaining to use it. Above the *Apply Selection* button is a button labeled ***Apply Unlock File***. Click this to select the ***.txt*** file needed to unlock your unit's full potential.

Once you enter the information needed in the ***Select DAQ*** section, you can assign animal ID labels to each channel (based on the number of channels you selected to record) in the ***Animal IDs*** table on the right. If you would like to use animal IDs from a previous recording, click the ***Load from File…*** button at the bottom right of the table.

In the ***Light and Dark Times*** section, click the text boxes to edit the light and dark onset times. Below that is the ***Experiment*** section. Click S***elect Folder…*** or ***Open in Explorer*** on the right to choose a directory and enter a name; this creates a folder for the experiment in that location. If an experiment with the same name already exists in the directory, a warning will pop up telling you to change the name or open the folder to move or delete the earlier recording.

Animal motion/pressure data will be saved for later processing or checking the signal quality of the recording. It is recommended to give this file 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 in the file name textbox, since *PiezoSleep* adds its own extensions, which 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). Our software *SleepStats*, which analyzes data after the experiment, uses the base name to locate 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.

At the bottom left of the window, you will see the ***Load Config…*** and ***Save Config…*** buttons. These buttons let you load or save any configurations to make it easier to set up future recordings. However, if the configuration does not match the number of selected channels for a new recording, you cannot use it.

The next two tabs are optional, so if you are not using an environmental sensor and/or a video license, you can click the ***Start Recording*** button at the bottom right of the window. The next two sections walk you through setting up both features when starting a recording.

Env. Sensors

If you are using an environmental sensor, you will be able to select and label it for your recording (more than one environmental sensor can be used). If the sensor(s) is not showing up, click the ***Rescan*** button near the bottom right (use this button if the sensor was not connected before opening PiezoSleep). More on this feature can be found later in the manual (see section titled ***Logging Environmental Sensor Data***).

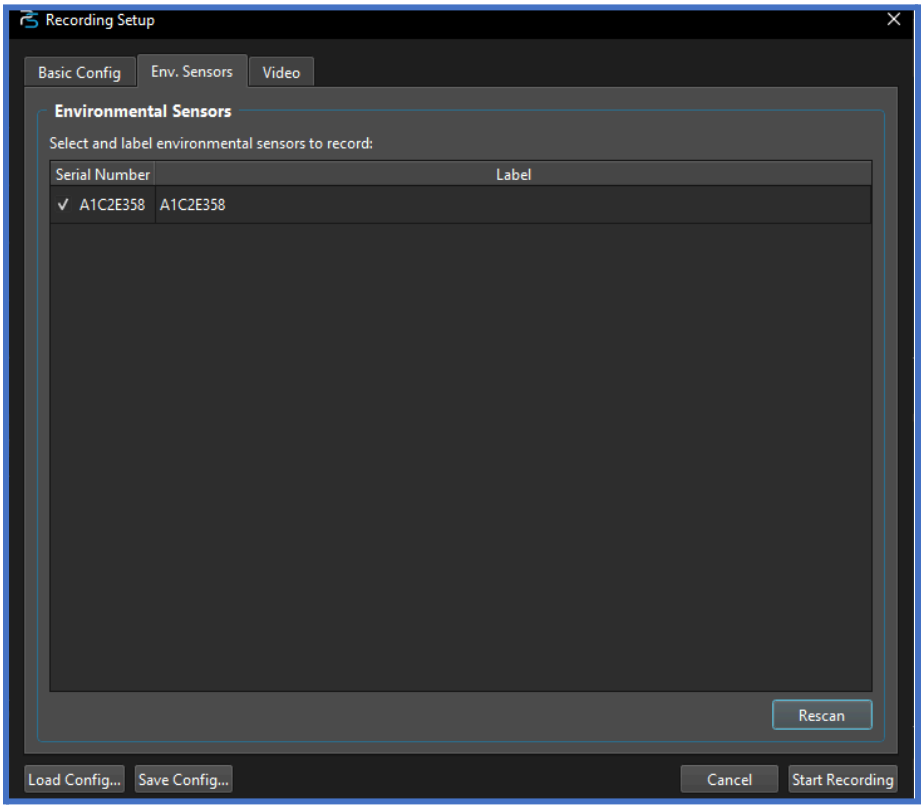

Figure 2: Environmental sensor tab

Video

If you have a video license for this software, this tab will allow you to select a video license file to enable camera recording. In this tab, you can adjust multiple camera settings: the assigned channel number, enable/disable the cameras, and select the resolution. If any cameras are not appearing, click the ***Rescan*** button near the bottom right (use this button if the camera was not connected before opening PiezoSleep). More on this feature can be found later in the manual (see section titled ***Synchronized Video Acquisition***).

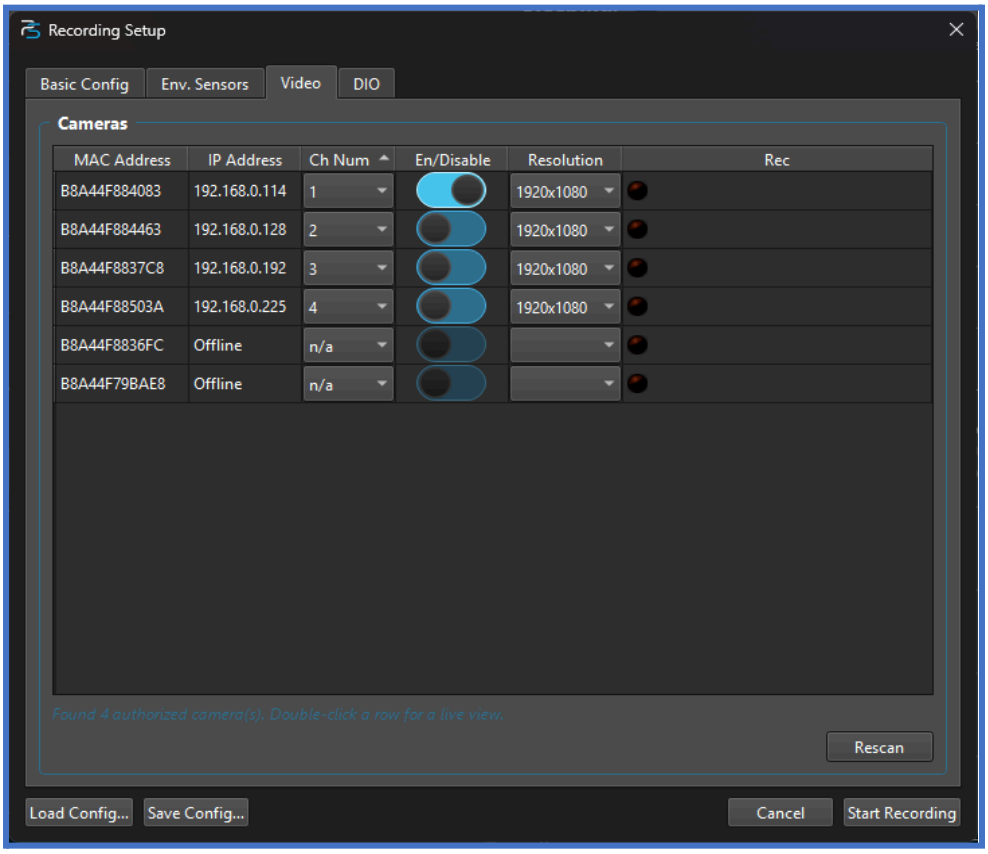

Figure 3: Video License tab

DIO (Digital I/O)

COMING SOON

After clicking the ***Start Recording*** button, the program will start recording and displaying data. Under each tab, the waveforms/ statistics will begin to appear shortly. When you are finished collecting data, click the ***STOP*** button at the bottom of the window.

B. 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. To view a particular channel, you can type the channel number into the ***Channel to Analyze*** text box or use the up and down arrows to the right of the text box.

This tab consists of four panels: the pressure signal (top-left), sleep decision statistic (top-right), power spectrogram/spectrum (bottom-left), and breath rate frequency (bottom-right) for a given channel, as shown in Figure 4. By default, the spectrogram is shown, but you can toggle between this and the spectrum. Additionally, hovering over ***View*** near the top-left of the interface reveals options to change the Y-axis scale and check/uncheck ***Show Activity***, as shown in Figure 6. By default, the y-axes of the pressure signal graphs are set to ***Autoscale***, but you can set them to -0.4 to 0.4 volts, -0.1 to 0.1 volts, or -0.04 to 0.04 volts (volts are proportional to pressure). This will also be applied to the ***Signal Panel*** tab. Meanwhile, enabling the ***Show Activity*** option (disabled by default) will switch the histogram to an actogram graph (same as the one shown in the ***Actogram*** tab) and the breath rate frequency graph (bottom-right) to an Activity graph, as shown in Figure 7.

To facilitate real-time observation, this program computes and updates the power spectrum, breath rate, and sleep decision statistics every 2 seconds. The power spectrum shows the signal's power distribution over a range of frequencies. The amplitude on the y-axis is expressed in the logarithmic unit decibels (dB). The sleep decision statistic displays a histogram plot showing the sleep-wake distribution for the selected channel. Detection is based on comparing the real-time decision statistic to a sleep threshold, which is zero by default and shown on the

histogram as a blue vertical line. Larger positive decision statistics indicate a greater likelihood of sleep, while negative decision statistics indicate a greater likelihood of wake.

NOTE: The real-time statistics and classification are about 5 to 10% less accurate than the decision analysis program *SleepStats*, which performs more extensive calibration than is possible in real time. This is done for recordings of 24 hours or more. Therefore, make all assignments of mouse sleep behavior using *SleepStats* after the recording is complete. The purpose of real-time monitoring is to detect potential outlying behavior, sick mice, or bad connections while the experiment is running so that potential corrections can be made.
The breath rate plot displays the most recent 32 minutes of data, updating every 2 seconds. For the first 32 minutes after starting the program, the breath rate plot accumulates data and, from then on, plots 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.

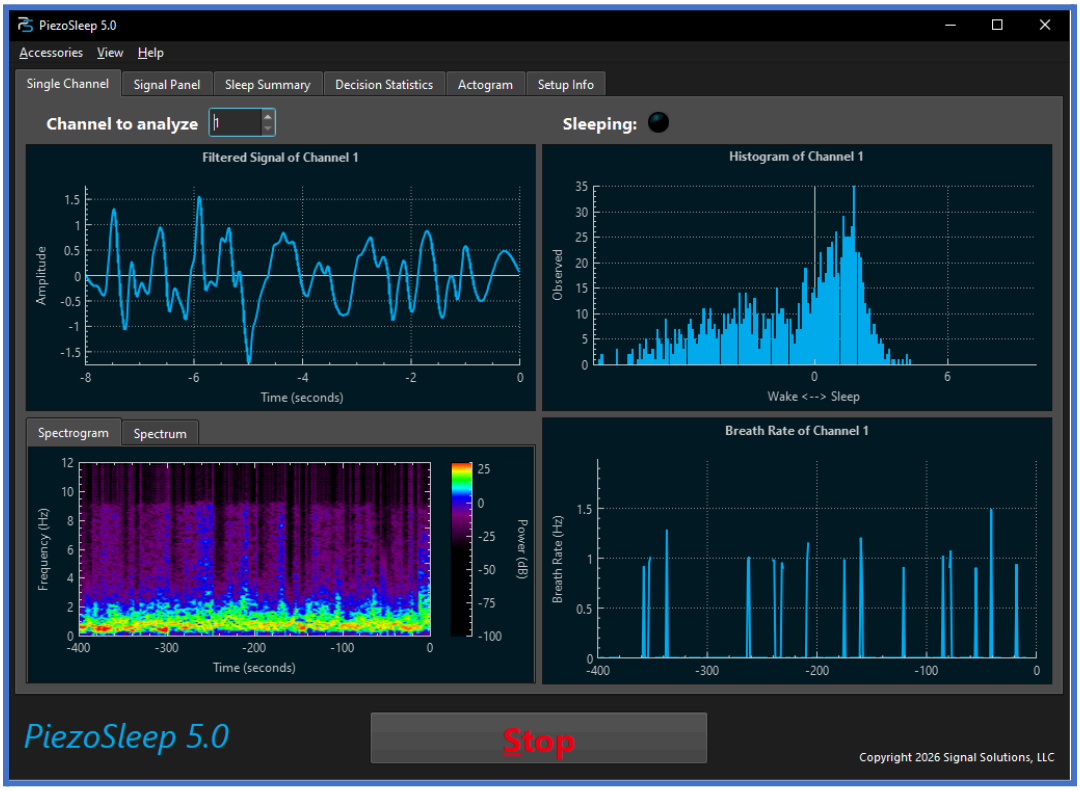

Figure 4: Single Channel tab

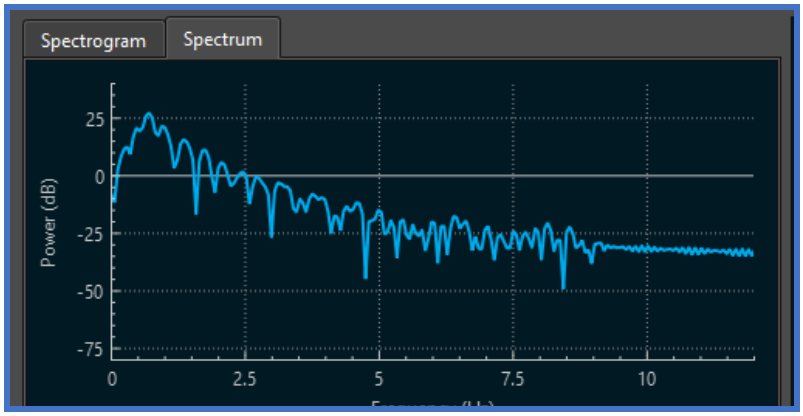

Figure 5: Spectrum

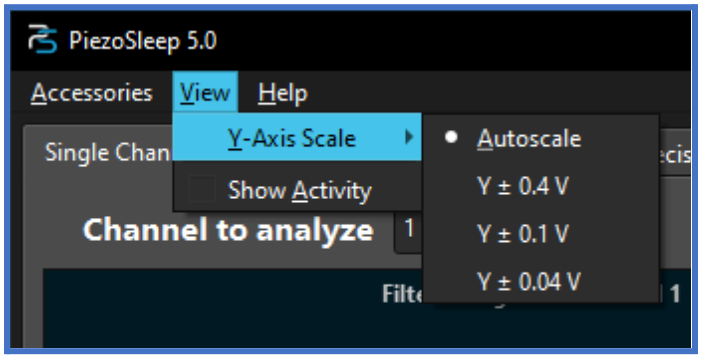

Figure 6: View menu

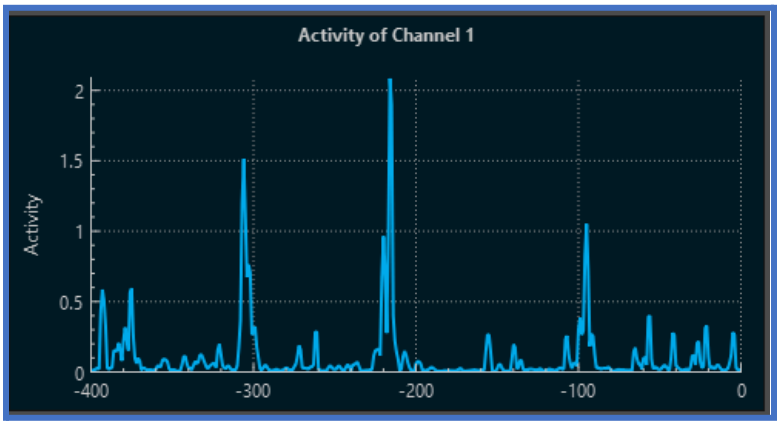

Figure 7: Show Activity (activity graph in bottom right)

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

To simultaneously view sensor signals for the active channels, click the ***Signal Panel*** tab. This tab, shown in Fig. 8, displays 4 active channel signals over the last 8 seconds. If there are more than 4 active channels, use the ***Page*** text box to page through the other channels. The animal IDs and their corresponding channel number are displayed over each plot.

The blue circle at the top left of each plot on the *Signal Panel* is the sleep indicator. A lit indicator implies detected sleep, as seen for channel 1 in the figure. Like the filtered signal plot on the *Single Channel* panel, the y-axes of these graphs are set to the option you selected from the ***View*** menu (Figure 6). The primary purpose of these graphs is to view signals from all cages to look for problems (broken connections, amplifiers not plugged in, dead mice, etc.).

Figure 8: Signal panel

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

The percentage of sleep for each channel can be observed at any point during the recording in the ***Sleep Summary*** panel shown in Figure 9. Like the *Signal Panel*, a blue circle (lit indicator) appears on any channel where sleep is detected. The percentage is based on the sleep threshold and the histogram formed from the data. Outliers can be identified early in the experiment and followed up with direct observation of the animal or checking the sensor's function (e.g., ensuring the cable is plugged in). Again, *SleepStats* will provide more accurate numbers once the experiment is complete.

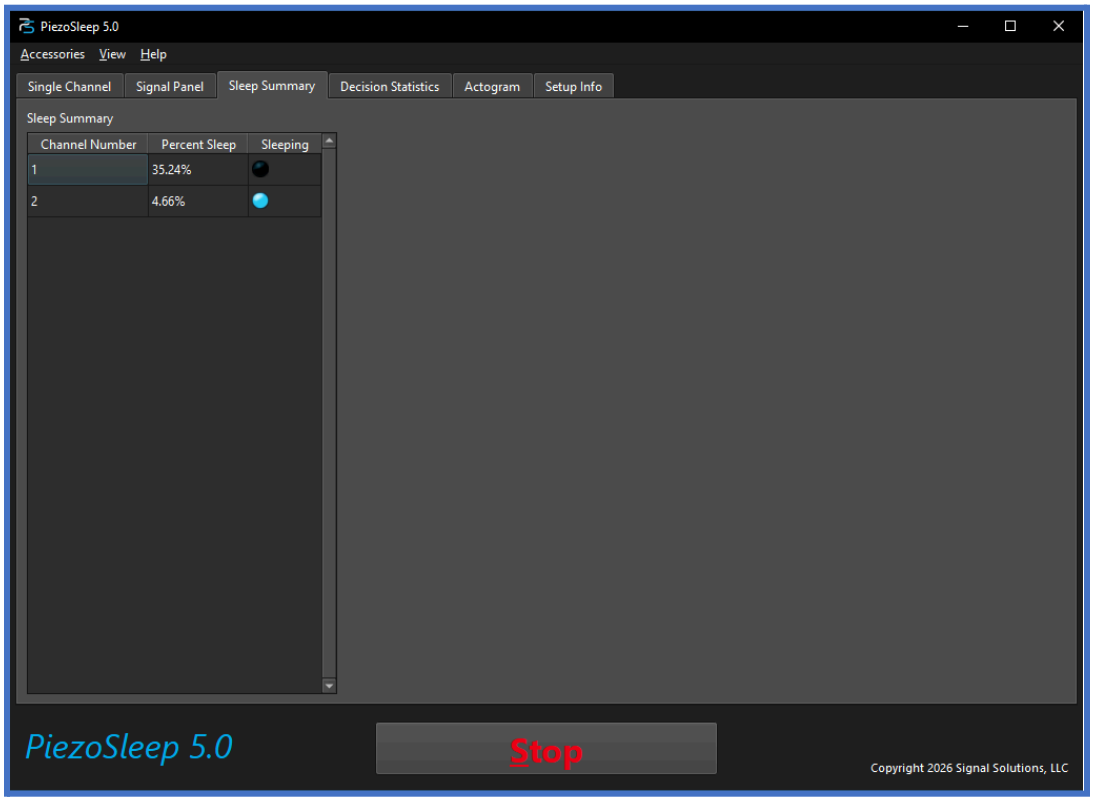

Figure 9: Sleep Summary

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

To simultaneously view 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 Figure 10. The animal IDs and their corresponding channel number are displayed over each plot. If there are more than 4 active channels, use the ***Page*** text box 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 during the experiment, 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 several causes:

- An electrically or mechanically noisy environment
- Poor animal contact with the piezoelectric sensor on the floor of the cage (sometimes caused by too much bedding or the cage shield not being properly seated on the sensor platform)
- Faulty 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 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 because it uses the same threshold over all channels and can vary by as much as 10% from 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.

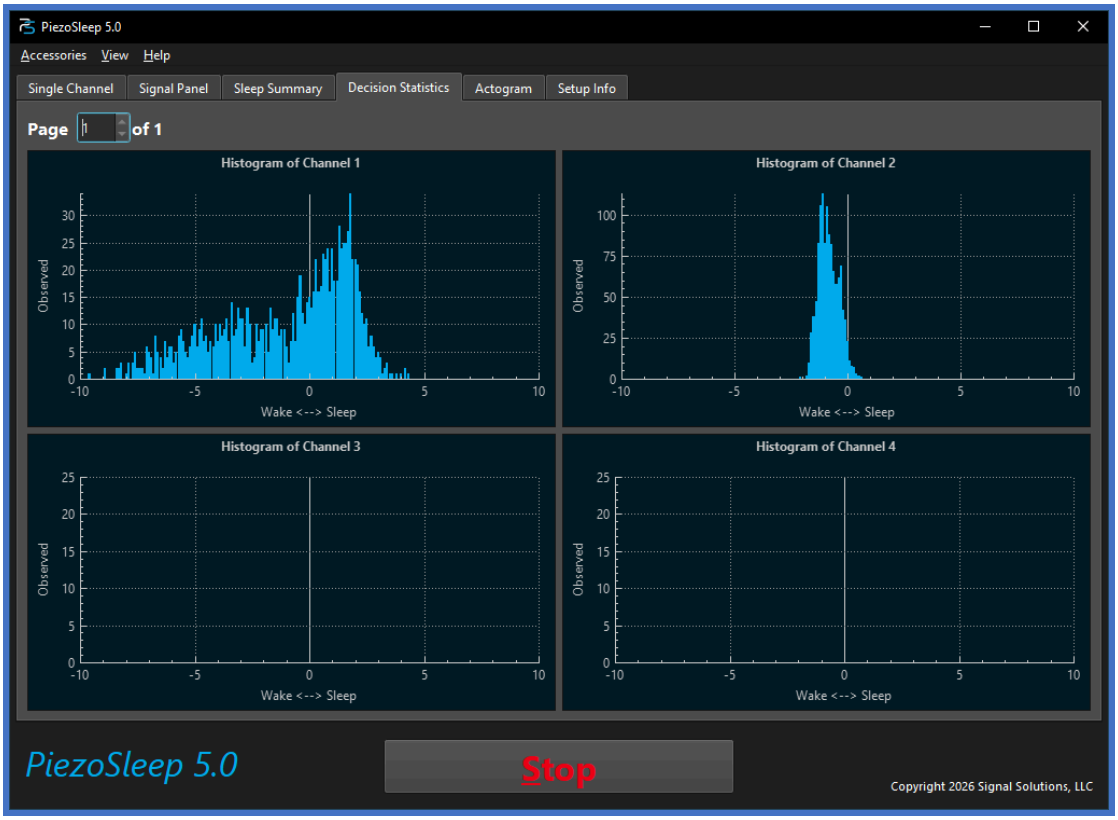

Figure 10: Decision Statistics

F. Actogram

To simultaneously view activity and rest patterns over multiple days for the active channels, click the ***Actogram*** tab, which shows actograms for 4 active channels at a time, as illustrated in Figure 11. As mentioned before, this is the same graph that replaces the histogram on the ***Single Channel*** tab when enabling the ***Show Activity*** option (hover over ***View*** near the top-left of the interface). The x-axis shows the hour of day (yesterday and present day), and the y-axis shows activity over rest.

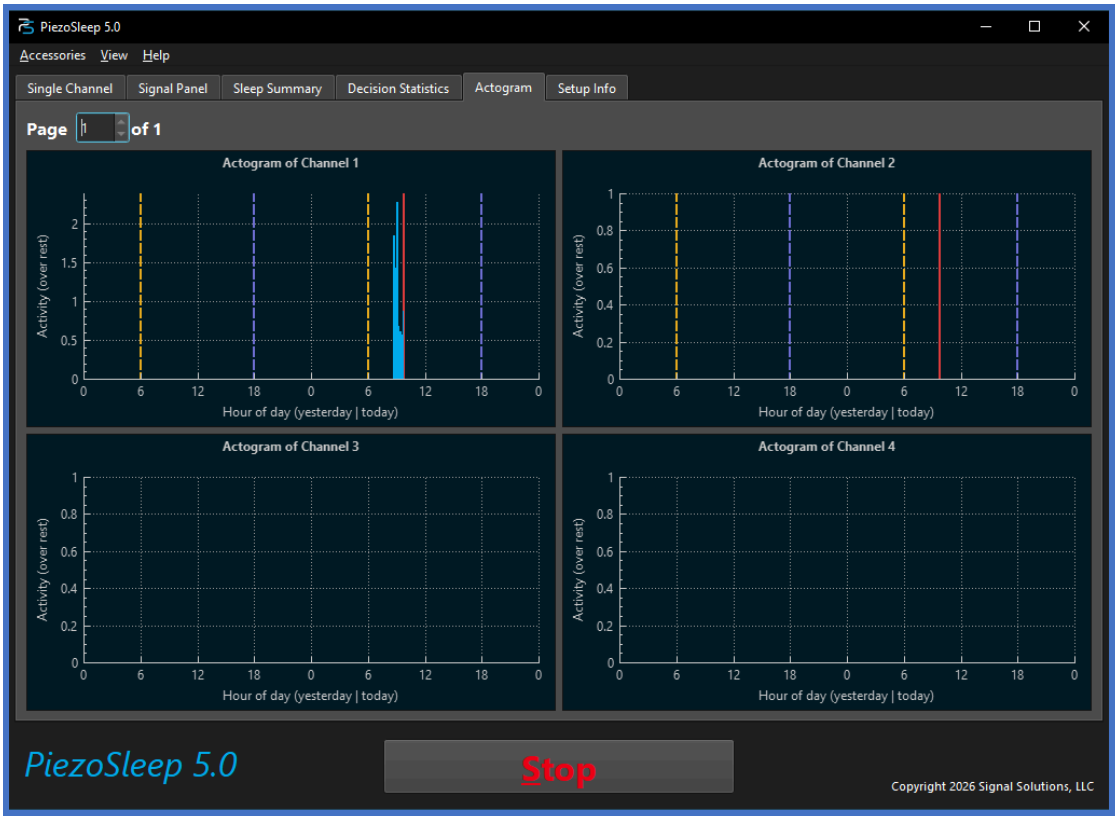

Figure 11: Actogram Tab

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

On the left of the ***Setup Info*** tab, the current information and settings are displayed (made at the start of the experiment): Start Time, Number of active channels, Primary and Secondary DAQ selections and their channel information, File Location, and Light and Dark Cycle times (shown in Figure 12). 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. This also confirms the date the experiment began.

FOR CALAMARI ONLY: On the right of the tab is the Markers table. The ***Mark*** button on the Calamari DAQ lets you mark certain events during the experiment. The table shows the time you hit the button, along with a label you can edit to make it easier to remember what

happened at that specific time. All marked events will be saved to a CSV file in the same location you chose to save the recording file.

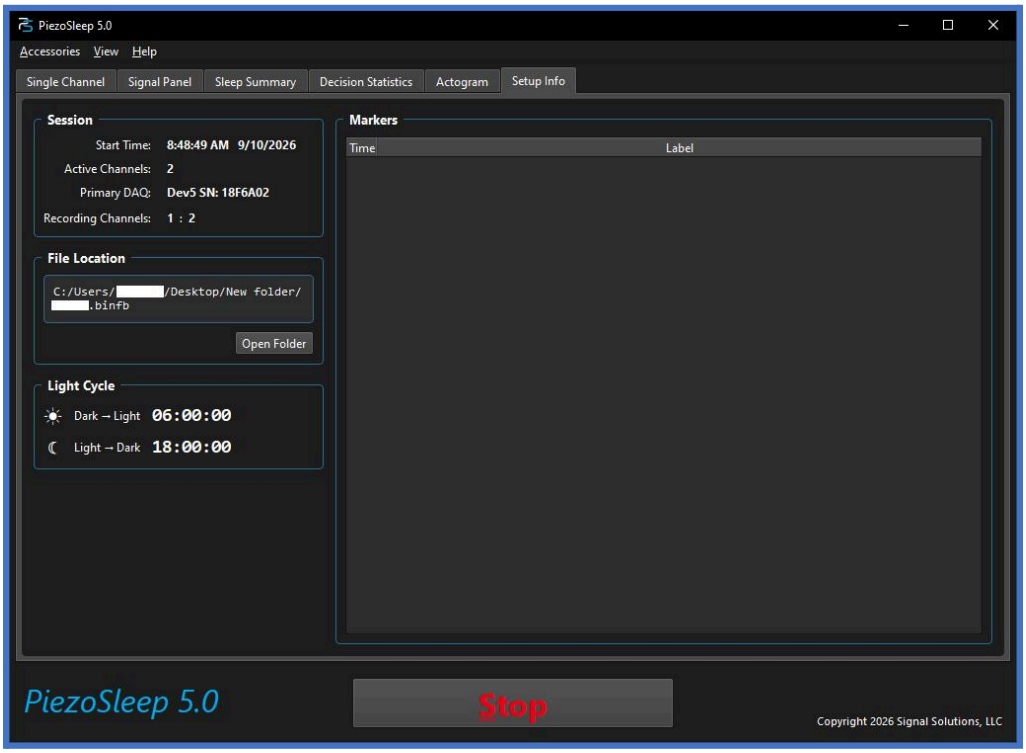

Figure 12: Setup Info

H. Logging Environmental Sensor Data

After clicking the ***Start Recording*** button, a separate datalogger window for this sensor will appear, as shown in Figure 14. This window consists of four panels: Lux (top-left), Temperature (top-right), Atm Pressure (bottom-left), and Relative Humidity (bottom-right). You can minimize or close the window, and acquisition will continue in the background. If you accidentally close the window, you can reopen it by going to the *Accessories* menu and clicking ***Environmental Sensors***, as shown in Figure 13.

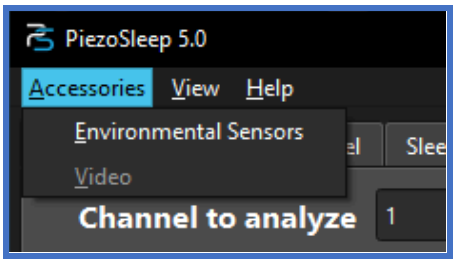

Figure 13: Environmental sensor in Accessories menu

Figure 14: Environmental sensor window

If environmental sensor data is not currently being logged, click on ***Environmental Sensors*** in the ***Accessories*** menu (Figure 13) to open the settings window shown in Figure 14. If there are no sensors shown and the device is connected via USB, click the ***Rescan Devices*** button. Once the device appears, the device label can be modified to tailor it to the experiment. Once the settings are complete, click ***Begin Logging***. If an environmental sensor disconnects at any point during the experiment, the program will notify you. Once reconnected, the recording will resume.

When you click the ***Configure…*** button, near the top right as shown in Figure 14, a window similar to the one in Figure 15 will pop up. This will allow you to add, remove, or edit the label of any environmental sensor used in your recording (the ***Begin Logging*** button is replaced with a button labeled ***Apply Changes***).

Hovering over the ***Display Units*** menu near the top-left of the interface will present two submenus: *Temperature* and *Pressure* (Figure 16). ***Temperature*** lets you change the y-axis of the temperature graph (top right) to either Fahrenheit or Celsius. ***Pressure*** lets you change the y-axis of the Atm pressure graph (bottom left) to inches of mercury (inHg), hectopascals (hPa), or millimeters of mercury (mmHg).

When you close the main *PiezoSleep* window at the end of the experiment, the environmental sensor acquisition will stop automatically. The data is stored in a binary file with a ***.env*** extension in the same directory as the ***.binfb*** file.

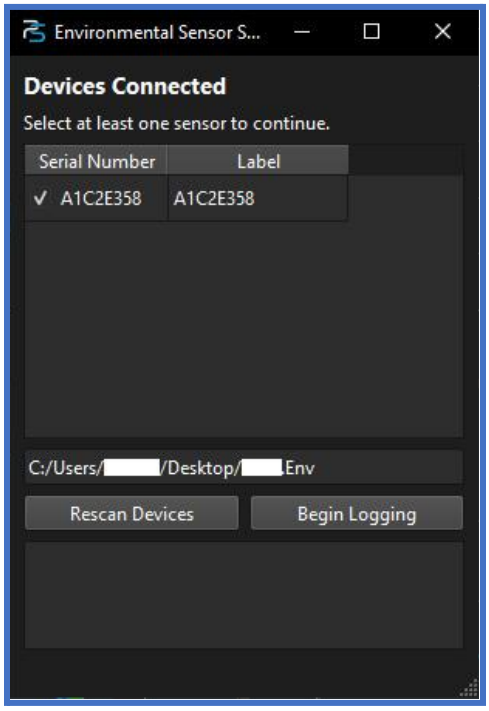

Figure 15: Environmental sensor settings

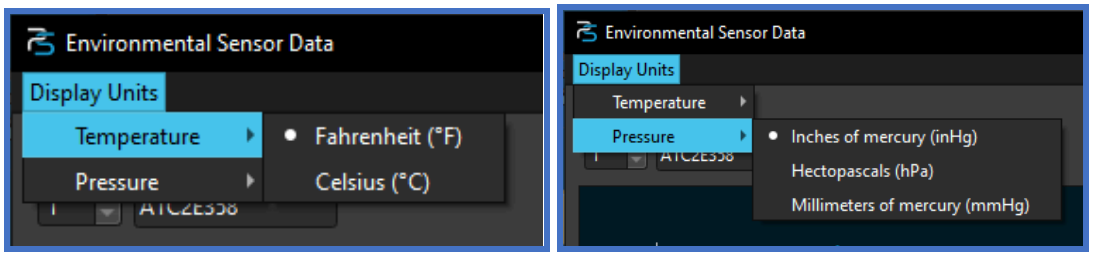

Figure 16: Temperature (left) and Pressure (right) settings

I. Synchronized Video Acquisition

COMING SOON

J. DIO (Digtial I/O)

COMING SOON

K. Help Menu

In the menu bar, there is a section labeled ***Help***. When you hover over this item, you will see multiple options, as shown in Figure 17.

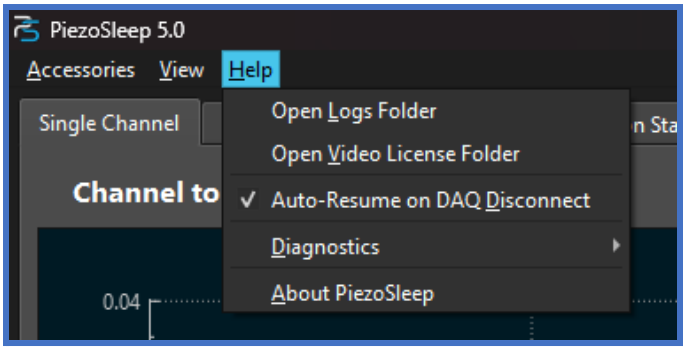

Figure 17: Help menu

Open Logs Folder

If you are sending logs for support, this option provides an easy way to open the logs folder and find which files to send. When you click it, a window will pop up to select the desired folder.

Open Video License Folder

When you obtain the license file for the optional video component for this software, you can use it to open the folder where you need to put the file. When you click it, a window will pop up to select the desired folder.

Auto-Resume on DAQ Disconnect

If the DAQ disconnects during the experiment, one of two windows will pop up notifying you, as shown in Figures 18 and 19. Enabling this feature automatically pauses the recording and resumes once you plug it back in. A new file will be automatically created for the resumed segment; however, you can also stop the recording. If you disable this feature, the recording will automatically stop.

NOTE: Enabling/disabling this will require you to restart PiezoSleep for the change to take effect. A message will pop up reminding you to do so.

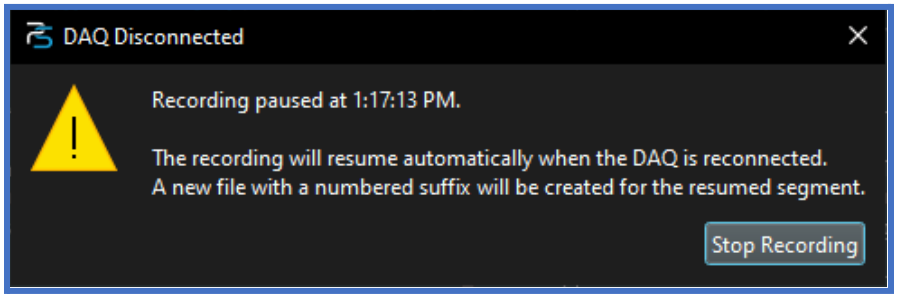

Figure 18: Auto-Resume enabled

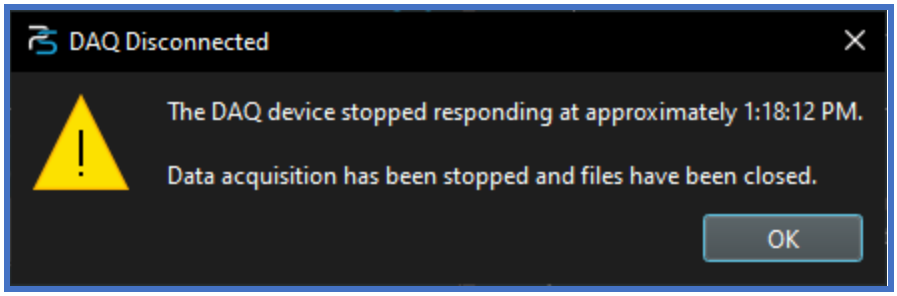

Figure 19: Auto-Resume disabled

Diagnostics

When you hover over *Diagnostics*, you will see the ***Sensor Detection*** option. This option uses signal analysis to estimate a good connection and animal presence over the last 20 seconds. In other words, enable this feature to have *PiezoSleep* check for disconnected or shorted sensor connections. Once enabled, the acquisition system will prompt the user if it detects a sensor short or a discrepancy is found between the number of channels being recorded and the number of sensors detected, as shown in Figure 20.

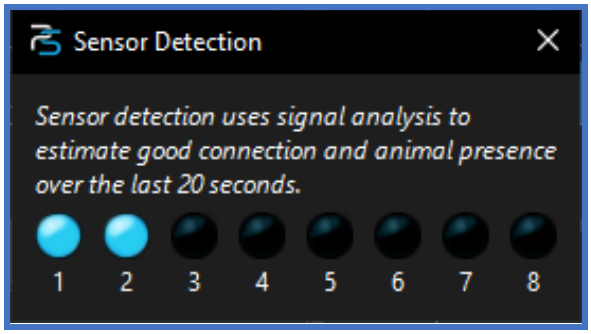

Figure 20: Sensor Detection

About PiezoSleep

When you click this option, a window will pop up with the software version number, a link to our website, and a link to our support ticket page. If you have any questions about this software or our products, use the link to submit a ticket.

4. File Information

PiezoSleep saves four piezo data files by default. 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/Featfb*** 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 computer system crashes due to a power failure, data up to that point is 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 stop data collection periodically, 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:

$$B = \frac{(2*24*602*120)*d*c}{10^6} \approx 20.74 \text{ dC}$$

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* files are considerably smaller in size 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, compared to 20.74 MB/day for the *binfb* file.

5. Troubleshooting

COMING SOON