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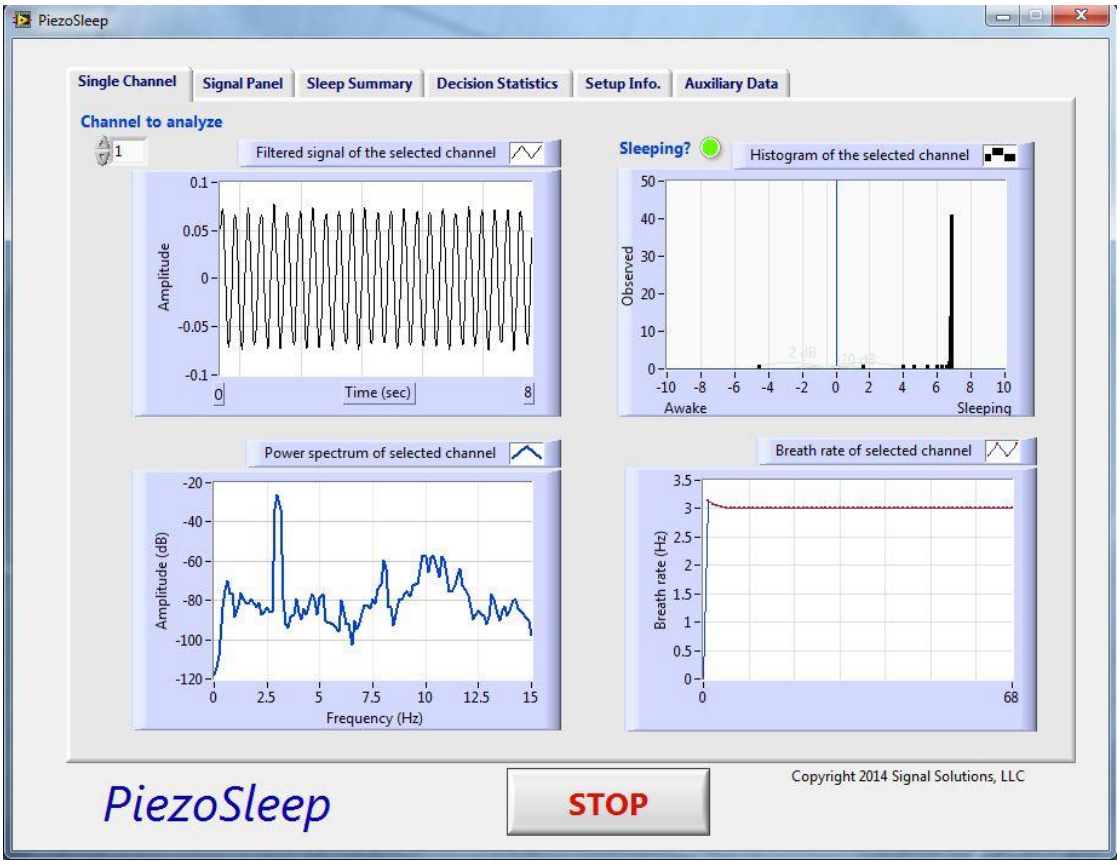
# Installation and User Manual for PiezoSleep Version 1

May 2014

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



# Installation and User Manual for PiezoSleep (Ver 1.0)

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May 2014

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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 mouse 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 a *.bin extension. Features associated with sleep and wake behaviors are extracted from the pressure signal and saved every 2 seconds in a file with the *.FeatVec 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. This file is also open by the analysis program for characterizing the sleeping breath rates. In addition to data related to the pressure signal, the program can also measure and save auxiliary data from a separate data acquisition module and saves to a file with the suffix and extension *_Aux.bin. This feature requires an additional analog-to-digital converter to input parameters such as ambient temperature, light intensity, humidity etc. 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 either PCI or USB NI data acquisition devices (NIDAQ) for simultaneous recording and monitoring of multiple channels. Examples of DAQ devices are (*PiezoSleep* is not limited to these only):

The NI-USB6009 DAQ records 1 to 8 input channels

The NI-PCI6220 DAQ records 1 to 16 input channels

The NI-PCI6224 DAQ records 1 to 32 input channels

The NI-USB6218 DAQ records 1 to 32 input channels

The NI-USB6229 DAQ records 1 to 32 input channels

The NI-PCI6225 DAQ records 1 to 40 input channels

The NI-USB6225 DAQ records 1 to 80 input channels

The software described in this document automatically detects all DAQ's configured on your PC. Software will run on a PC with Windows 7 or Windows XP operating systems (32 or 64 bit) and an available PCI slot (for NI-PCI DAQs) or a USB port (for the NI-USB DAQs). The installation of the *PiezoSleep* software automatically installs drivers for the NI DAQ, if they are not already installed. Updated drivers and the runtime engine

required to run *PiezoSleep* are freely available from National Instruments at http://www.ni.com/drivers/

## A. Installation

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

2. Transfer the file to the hard drive of the computer where data will be collected and unzip the file. Once unzipped, the directory *PiezoSleep_1* 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 2013, which is installed along with *PiezoSleep* and is present in the *National Instruments* folder under the *Program files(x86)* folder in *C* drive. 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. If you have a USB DAQ, plug it in after the software installation is complete. If you have a PCI card, turn off the computer now and install it. Turn the computer back on for the next step.

5. **If you are using two** USB DAQs to extend the number of channels collecting data, they need to be synchronized using external digital lines (called PFI lines) present on the DAQ. Physically connect the digital lines PFI-0 and PFI-1 of one DAQ to the PFI-0 and PFI-1 of the other DAQ, respectively. When launching the PiezoSleep software, the order of channel assignments to the DAQs can be selected.

# 2. Data acquisition and monitoring

## A. Starting a data acquisition session

A. 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. PiezoSleep software can simultaneously acquire piezo sensor data from two NI DAQs. This is useful if the number of animals to be monitored is larger than the maximum channels supported by a single DAQ. Select the number of DAQs you will be using to collect data in the dialog window.


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

B. If you are using a single DAQ, the window shown in Fig. 2 appears, containing a table listing all NI DAQ's currently plugged in and recognized by your PC. Select the DAQ you wish to use by clicking on its name inside the table cell and then press OK. A small window displaying your DAQ selection, as shown in Fig. 2B, will appear. Press OK to continue. In case of using two DAQs, A primary and secondary DAQ must be chosen. This affects how the sequence of channels is assigned in *PiezoSleep*. The program first prompts you to select the primary DAQ, as shown in Fig. 3A. The primary DAQ is the one connected to the first set of cages. For example, in Fig. 3A, USB-6225 is selected as the primary DAQ, i.e. cages 1 to 80 (maximum channels supported by USB-6225) should be connected to it. After selecting the primary DAQ, the program will prompt you to select the secondary DAQ, as shown in Fig. 3B. The cages numbered 81 (in case of USB-6225) and onwards should be connected to this DAQ. If you are using two DAQs of the same kind, the primary and secondary DAQs can be identified by their serial numbers, listed next to the device name in the table.

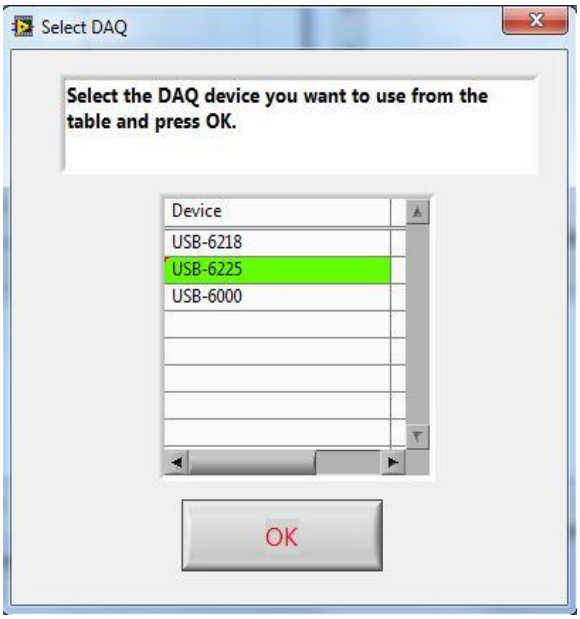
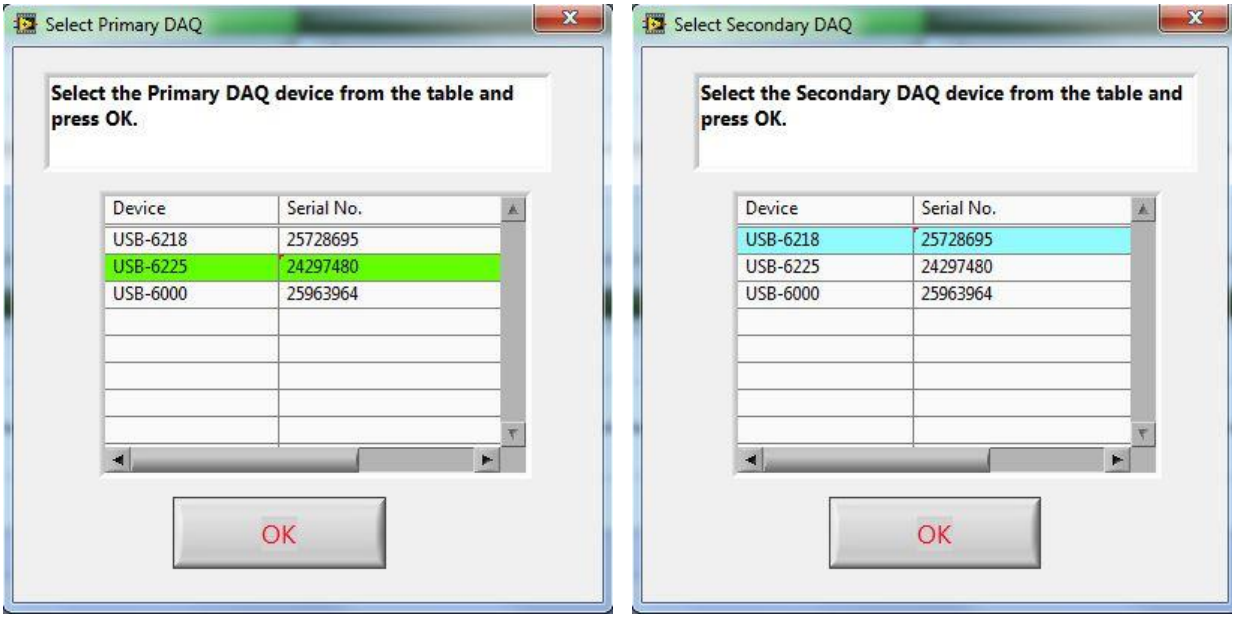



**Figure 2.** Prompt to select a single DAQ. Consists of a table listing all DAQs currently plugged in your PC. Click on a name inside the table and press OK.




(A) (B)

**Figure 3.** Prompt to select the (A) primary and (B) secondary DAQ. If you are using two DAQs of the same kind, the primary and secondary DAQs can be identified by their serial numbers, listed next to the device name in the table.

C. Next, the program prompts for the number of active channels with the dialog box shown in Fig. 4. 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 four animals are to be monitored (cages 1 to 4), then 4 should be entered as the number of active channels. In this case the mice should be placed sequentially in the cages (i.e. the first one in cage 1, the next in cage 2 …). If a cage is skipped in the sequence, then enter the number of channels corresponding to the highest cage number containing an animal. For example if mice are in cages 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.

D. After you enter the number of active channels, the dialog box shown in Fig. 5 appears, asking if you wish to import 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.


**Figure 4.** Prompt to enter number of sequential channels to be recorded.


**Figure 5.** Import request for spreadsheet.

E. After the spreadsheet prompt is complete (or dismissed), another dialog box will pop up as shown in Fig. 6. The dark-to-light and light-to-dark transition times can be entered in the designated area, where the times are in military format (0 to 24). These time values are stored in the data file headers and used in a later analysis program to compare sleep behavior during the light and dark periods. If light and dark onset times are not critical, then these can be left in their default settings and disregarded or changed in the analysis program. This entry will not affect the data acquisition. In this same window, the animal IDs can also be changed to meaningful labels if desired. These ID labels are also stored in the

header so subsequent analysis programs can identify each channel with these labels. When finished entering this information, click *OK* to proceed.

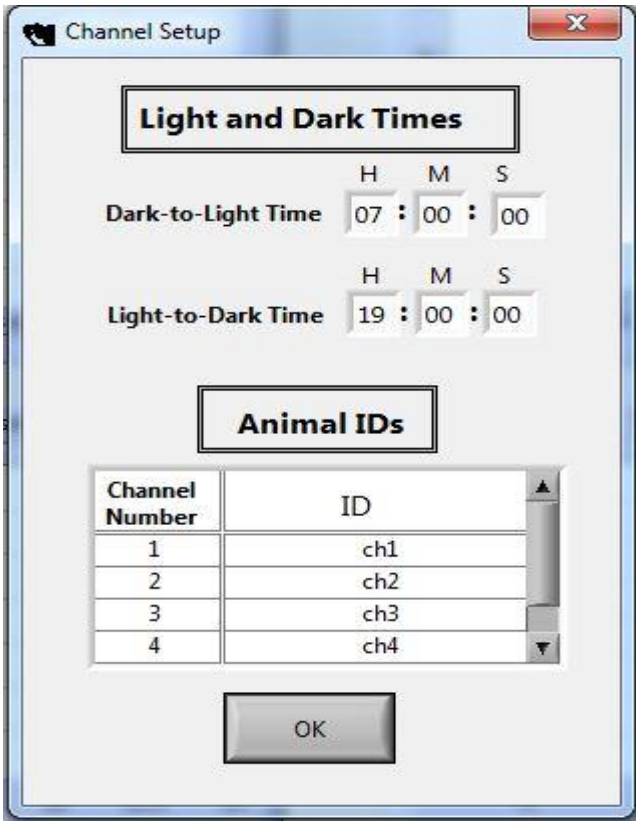



**Figure 6.** Prompt to enter labels for recorded channels and the light and dark transition times used during the experiment.

F. Next, the program prompts for a file name as shown in Fig. 7. Animal motion/pressure data will be saved to this file for later processing. If a file of the same name already exists in the location, the program will prompt you with an option to overwrite the existing file, as shown in Fig. 8. It is recommended that this file be given a descriptive name that identifies the nature of the experiment or mice and the date the experiment began. It is also recommended that NO extension be given to the file name, since *PiezoSleep* adds its own extensions that are used in later programs (the *bin* extension is given to the files with the raw sensor data). The software that analyzes data after the experiment uses the base name to look for processed files 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.

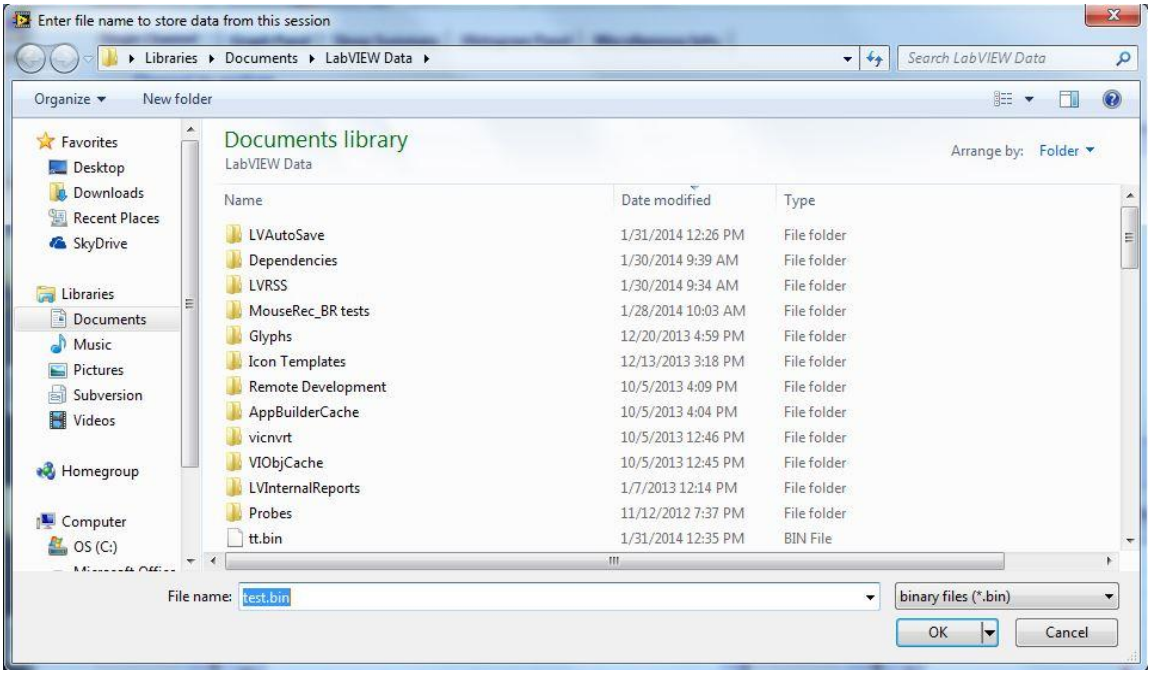



**Figure 7.** Prompt to enter a filename where data is to be stored during the experiment.

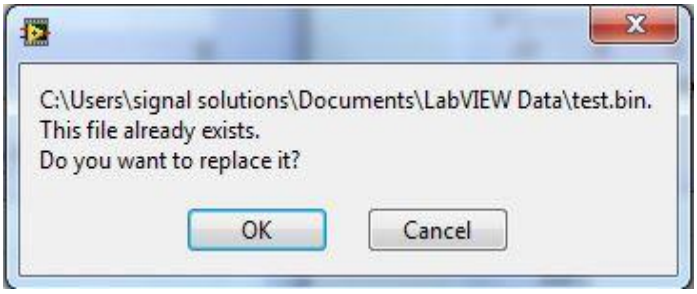



**Figure 8.** Overwrite existing file?

G. After selecting the filename and location, the program displays your selections as shown in Fig. 9. The display includes the DAQ device name (primary and/or secondary), 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.

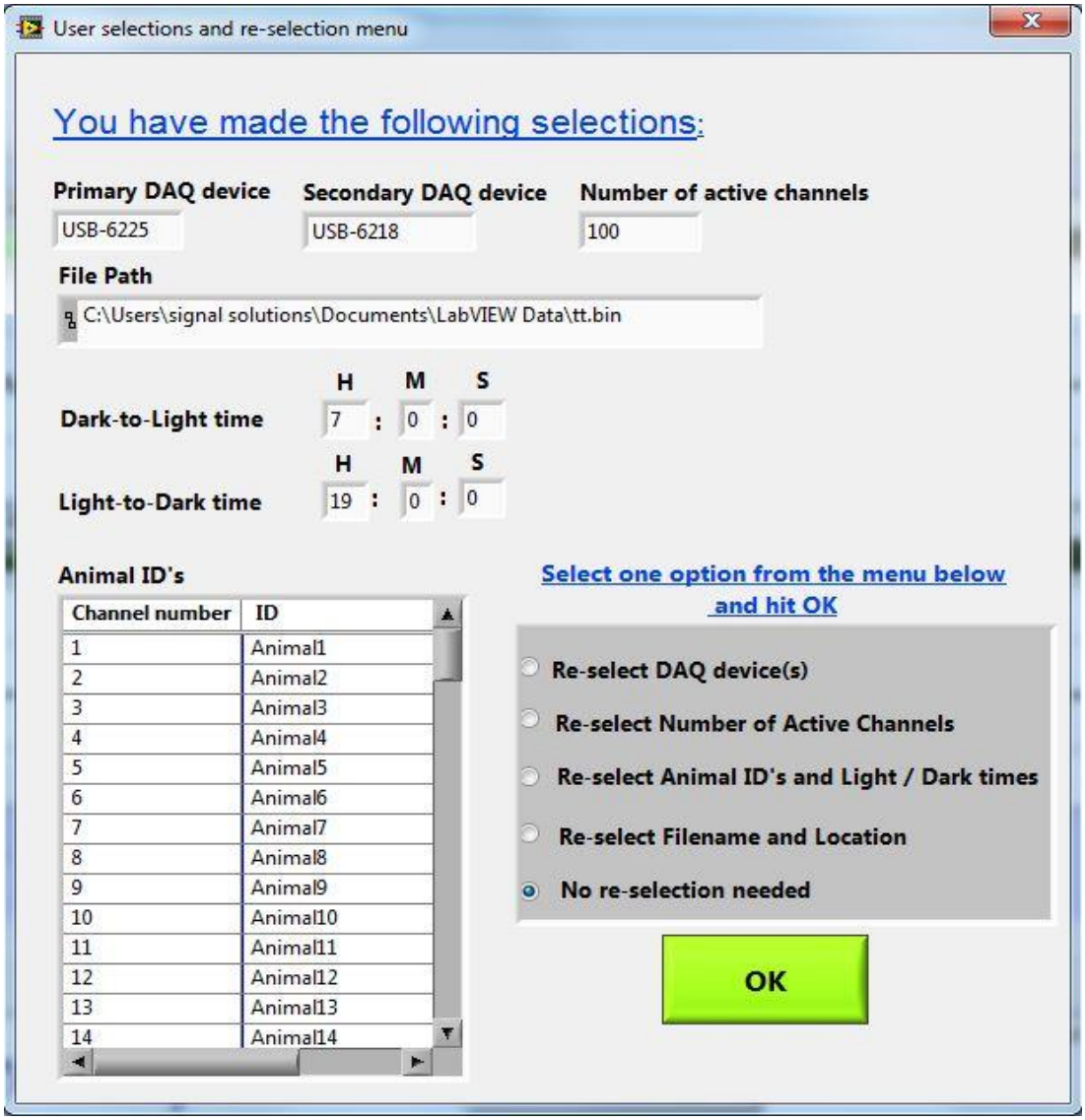



**Figure 9.** Prompt displaying user selections and the re-selection options.

H. Next, the program prompts for the use an auxiliary DAQ, as shown in Fig. 10. This DAQ can be used to record auxiliary data such as room temperature, light intensity, humidity etc. If you select *NO*, the program starts the data acquisition. Steps *I* and *J*, given below, contain instructions for setting up the auxiliary DAQ. If you are not using an auxiliary DAQ, jump to step *K* for further instructions.

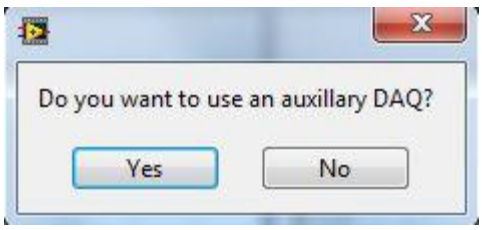



**Figure 10.** Prompt to select the use of an auxiliary DAQ.

I. The auxiliary DAQ is set up in a way similar to the primary DAQ. The program prompts you to first select the DAQ device, followed by the number of active channels, and then the channel IDs. Figure 11 shows these prompts sequentially.

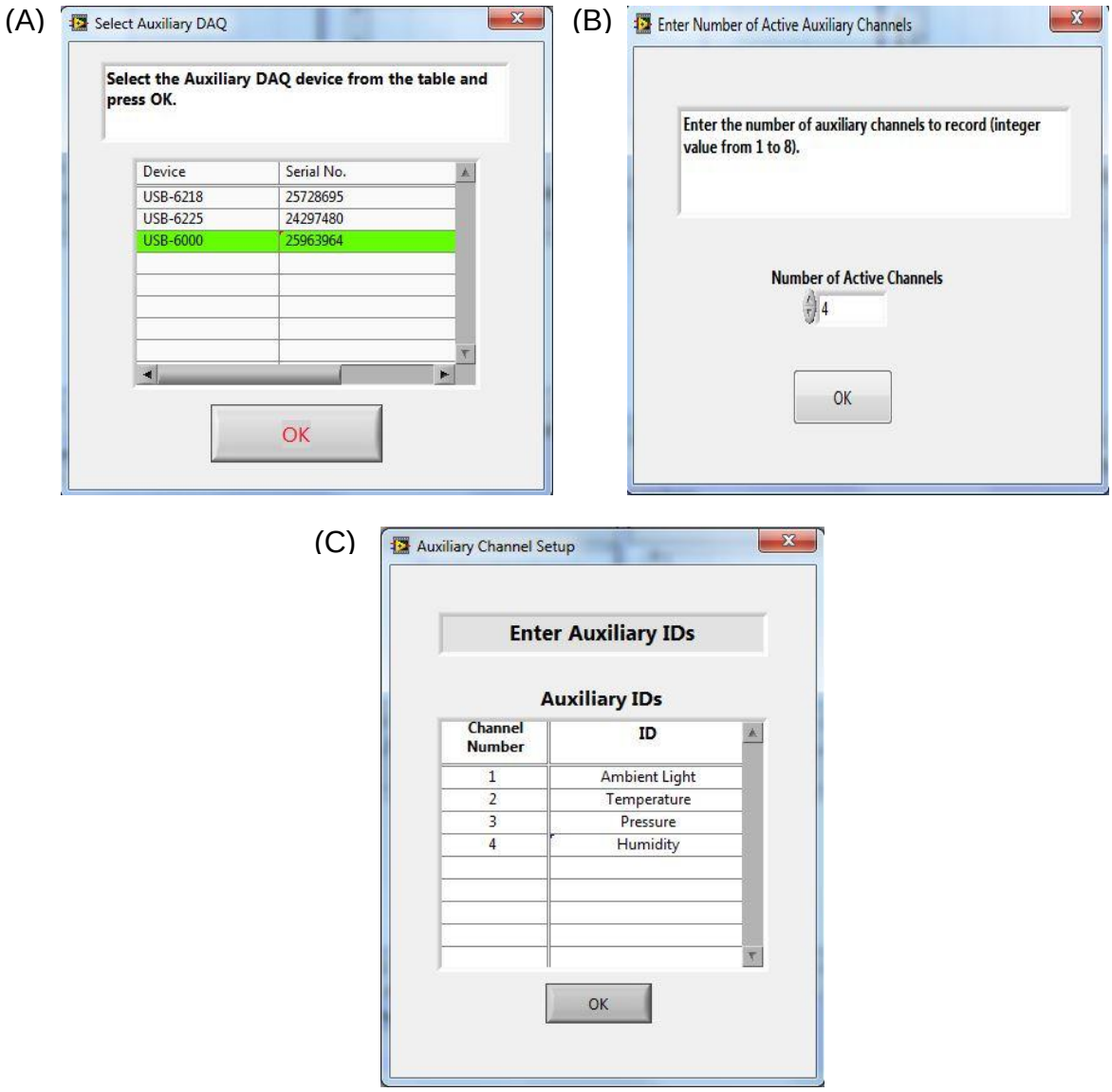



**Figure 11.** Steps to set up an auxiliary DAQ. (A) Select the DAQ device. (B) Enter the number of active channels. (C) Enter the auxiliary channel IDs.

J. Next, the program displays your auxiliary selections and a re-selection menu, as shown in Fig. 12. If needed, you can re-select the auxiliary DAQ device, number of active channels, or channel IDs. If no re-selection is needed, then select the last option and click *OK* to start data acquisition.

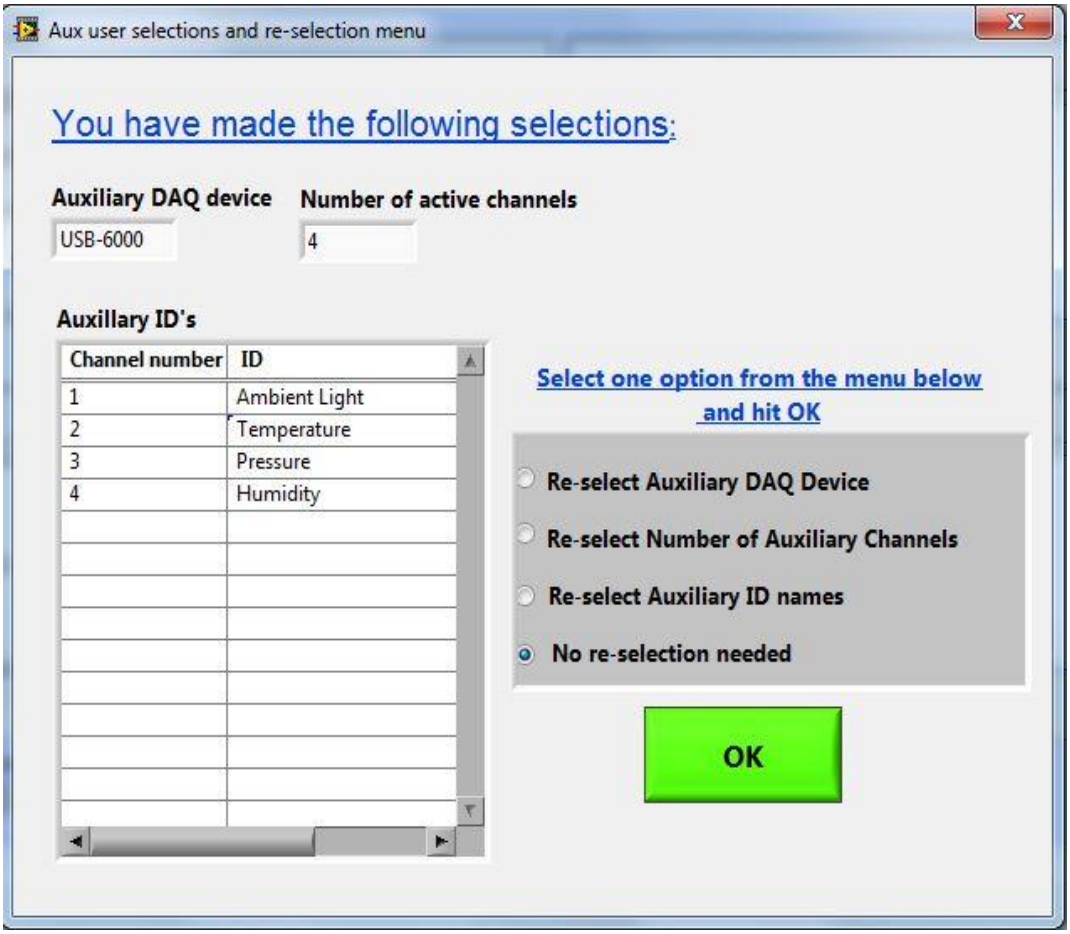



**Figure 12.** Prompt displaying user selections and the re-selection options for the auxiliary DAQ.

K. 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.

L. When data collection is finished, click the *STOP* button located in the bottom of the window. A new test can then be started by clicking the arrow button,  located on the top left corner, or the program can be terminated by clicking the *X* in the upper right corner to close 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. As shown in Fig. 13, 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* 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. 13 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 of 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 decision statistics 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. 13, 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.

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. The breath rate plot in Fig. 13 shows that the breathing frequency of the animal was around 3 Hz.

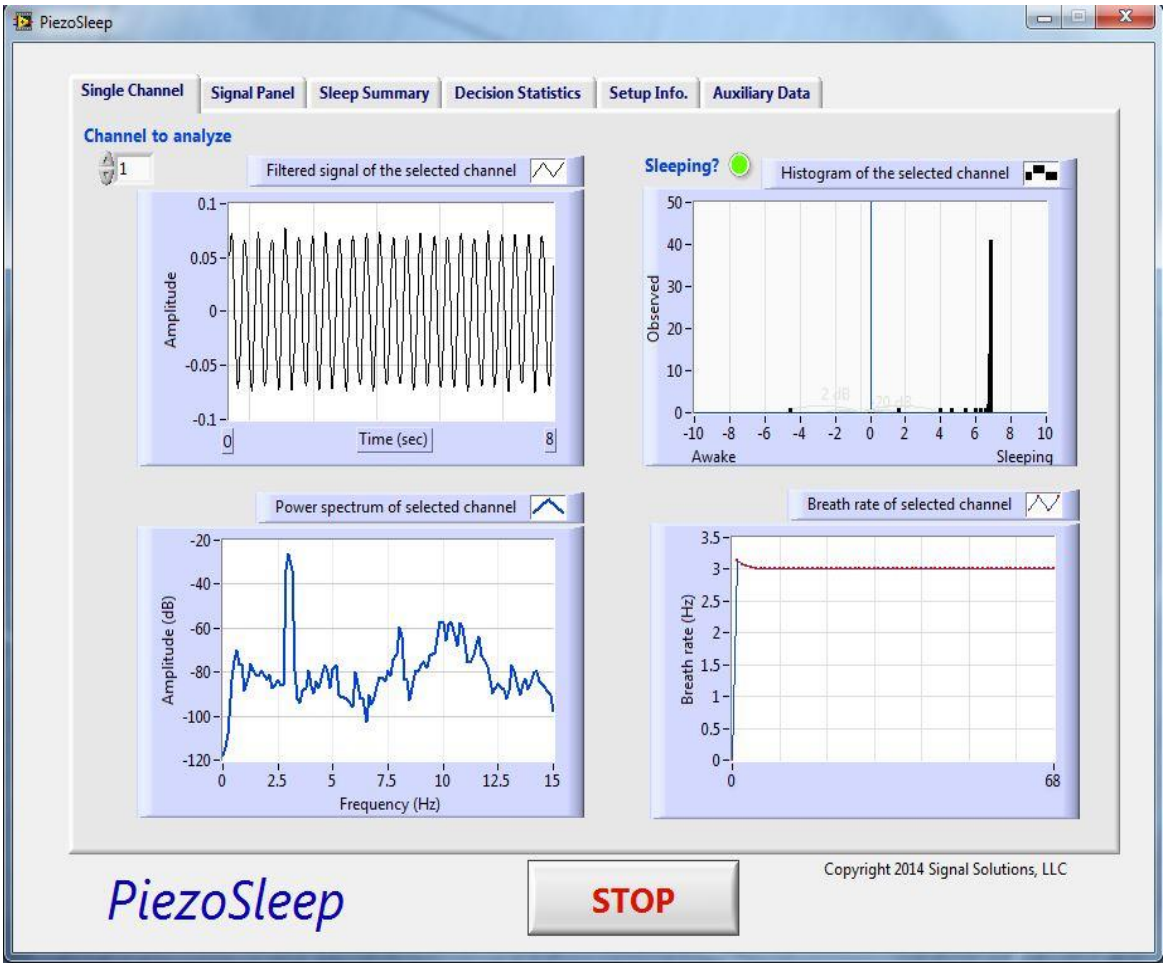


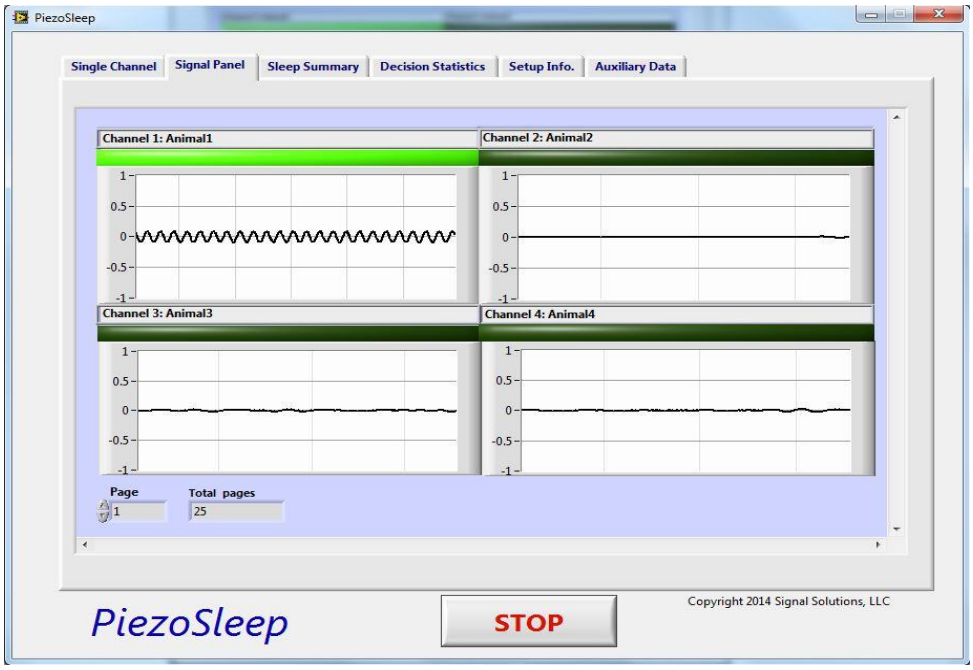

**Figure 13.** *Single Channel* tab showing the recorded signal (top left), sleep decision statistic (top right), power spectrum (bottom left), and the breath rate frequency (bottom right) for an individual channel.

## 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. The Signal *Panel* will appear, as shown in Fig. 14, 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 -1 to 1. 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 second delay between what is happening in the cage and what appears on the screen.

(A)




(B)

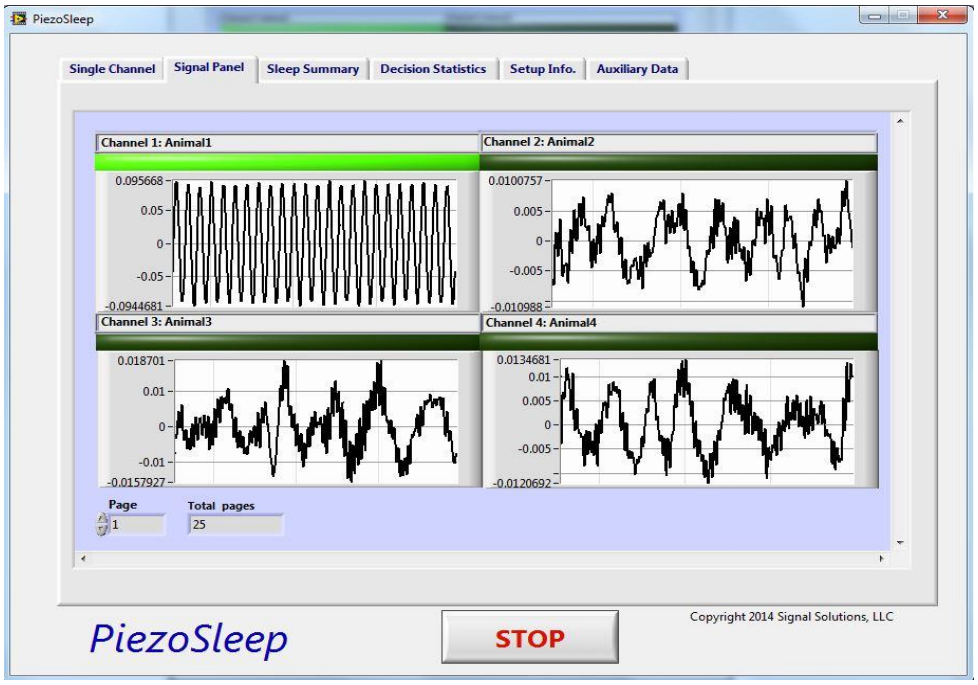



**Figure 14.** *Signal Panel* tab displaying piezoelectric sensor signals for 4 active channels. The *Page* box is used to switch to other active channels when more than 4 channels are active. (A) Y-axis fixed (default) and (B) y-axis switched to auto-scale, shown with a simulated sleep signal plugged in channel 1.

## D. 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. 15. 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).

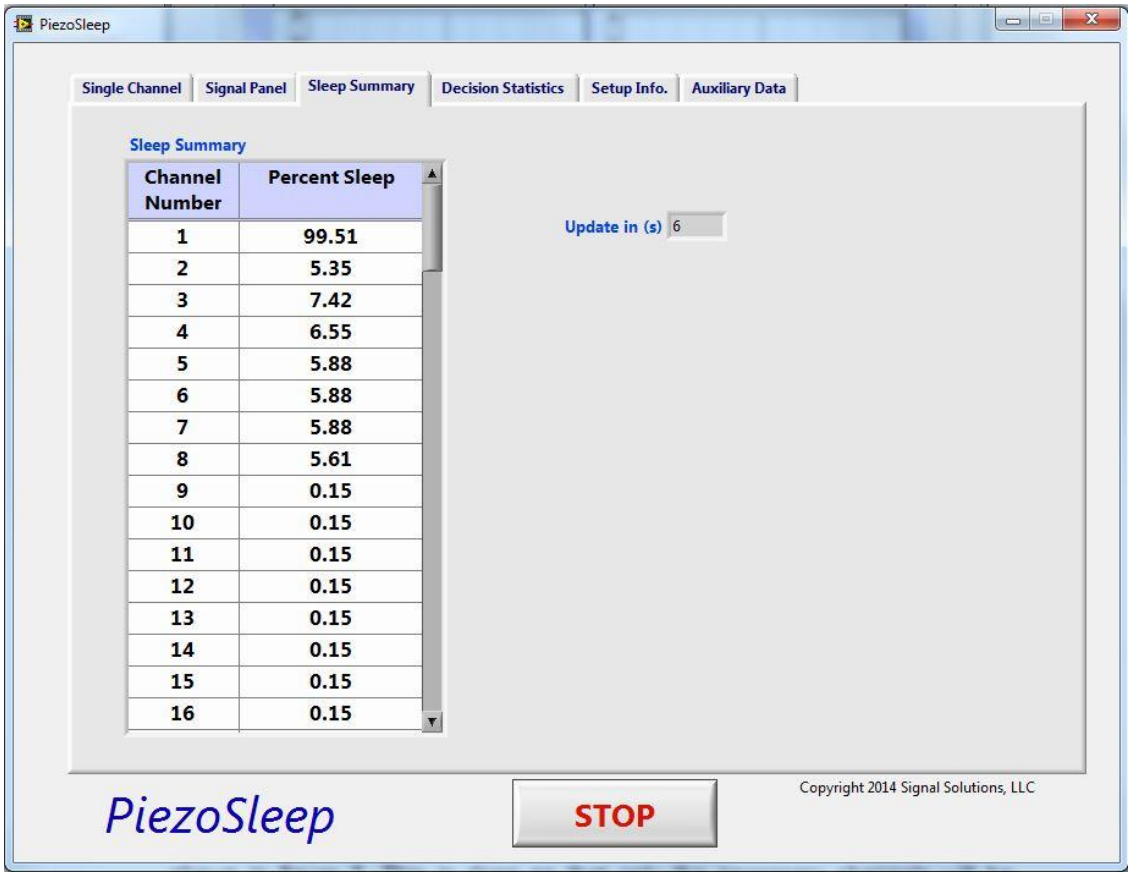



**Figure 15.** Sleep percentage summary.

## E. 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. 16. 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 animal, a clear bimodal distribution should appear in the histograms as a result of mapping sleep signal dynamics into positive values and wake signal dynamics into 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)
- 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 8% 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 in the **MouseRecDataTools** toolbox.

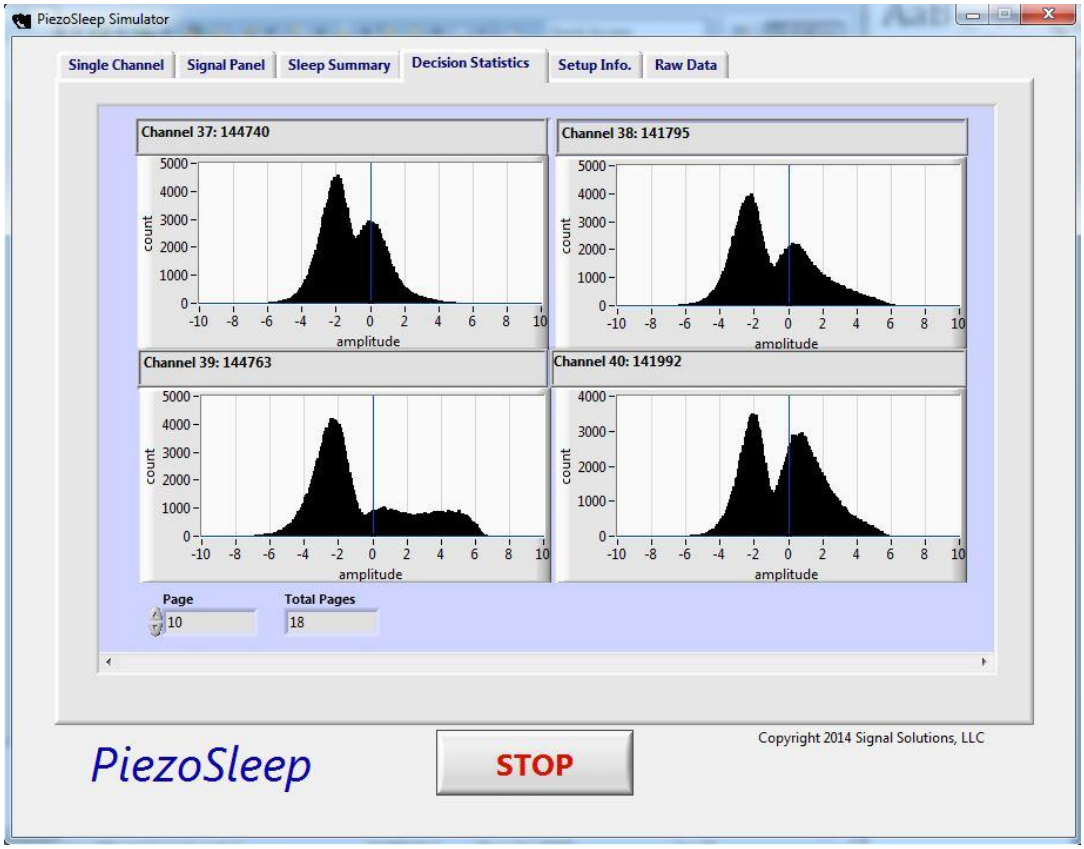



**Figure 16.** *Decision Statistics* tab showing histograms of 4 channels simultaneously. The *Page* box can be used to switch to other active channels if more than 4 channels are active.

## F. 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 figure 17. 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.

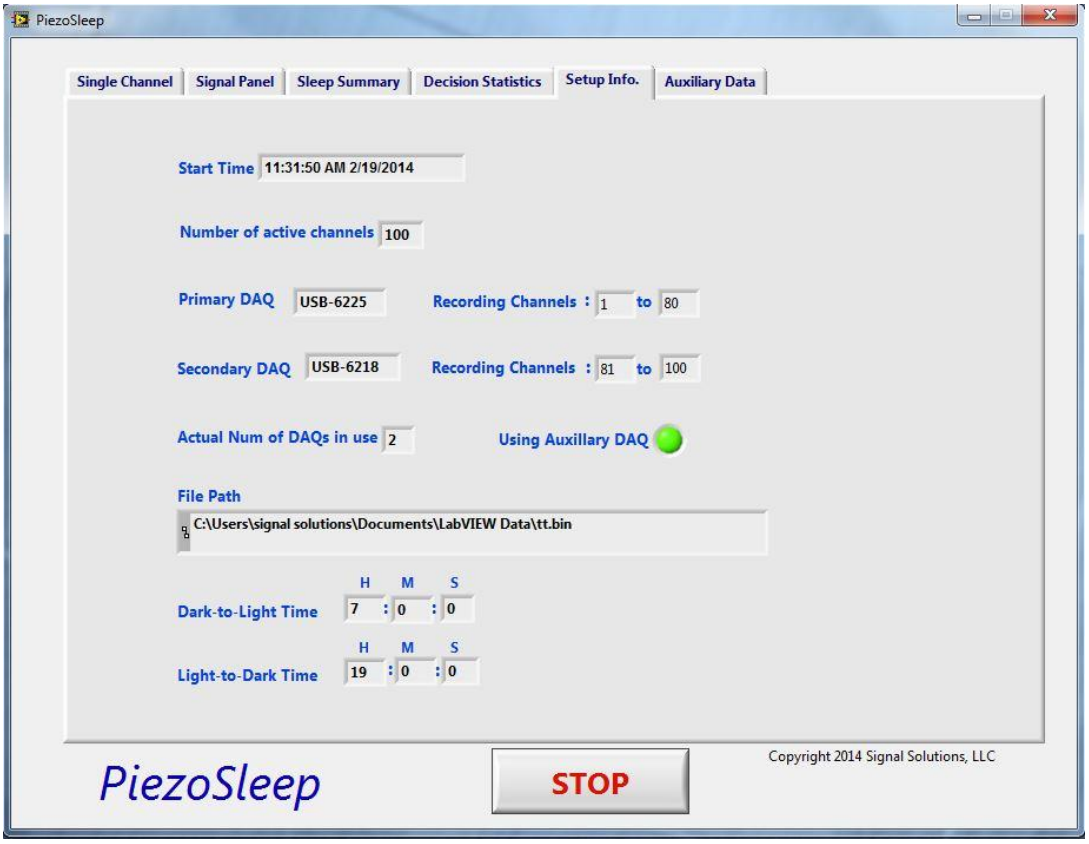



**Figure 17.** *Setup Info* tab.

## G. Monitoring signals from auxiliary sensors (Auxiliary Data tab)

The *Auxiliary Data* tab simultaneously displays 4 signals from auxiliary sensors, as shown in Fig. 18. If there are more than 4 active channels, the *Page* text box can be incremented to page through the rest of the auxiliary channels. The auxiliary IDs with their corresponding channel number, are displayed over each plot. Auxiliary data tab provides a quick way to notice ambient disruptions, such as in temperature or humidity control, as these can have a pronounced effect on the sleep-wake characteristics.


**Figure 18.** *Auxiliary Data* tab example showing 4 pseudo auxiliary channels. Note that the output on y-axis, seen here, is in terms of voltage and is not calibrated to its respective units.

## 3. File Information

*PiezoSleep* saves three piezo data files and one auxiliary data file (if selected for use). The file with a base name (provided at the beginning of the data acquisition session) and the *bin* extension, stores the raw data from the piezo sensors. The file with the *Feat* extension and '*_br*' padded to the base name stores the breath rates for all channels. The file with the *FeatVec* extension stores the feature vector used to calculate the sleep statistics. The file with the *bin* extension and '*_Aux*' padded to the base name stores the auxiliary data for all channels. The *FeatVec* file is used by the analysis programs in the **MouseRecDataTools** toolbox developed by Signal Solutions LLC. These 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 sufficient 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 *Feat* and *FeatVec* file are considerably smaller in size as compared to the piezo *bin* file. The *Feat* file consumes space of about 0.34 MB/day and the *FeatVec* file consumes roughly 1.4 MB/day for each channel as compared to the 20.74 MB/day for the *bin* 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.