SIGNAL SOLUTIONS

Sensor Systems, Software, Sleep

Signal Quality Analysis Tool

USER MANUAL

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Signal Quality Analysis Tool

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Contents

1. Introduction

The ***Signal Quality Analysis Tool*** creates a visualization of sensor data acquired with the PiezoSleep systems to determine signal quality and noise in the environment. The sleep-wake behavior classifier requires the signals from animal movement to exceed the signal levels from other vibrating sources (noise). The term *noise floor* refers to the level where noise dominates, and no reliable signal measurements can be made below this value. This tool creates graphs from which it can be determined if critical signals from the animal exceed the noise floor. This program creates summary reports useful for assessing the impact of the noise and identifying problems related to cage placements and other interfering activities in the environment.

Two types of tests can be run to assess the noise floor and determine signal quality. The first is an empty cage test where the PiezoSleep system records data for over 24 hours so that signals acquired are from non-animal activity (noise sources). The second is a mix of empty cages and those with animals. This test is more involved, but it provides an assessment of signal-to-noise powers, which may impact performance. This manual describes the functionality and configuration options for the program, along with examples.

Reports are saved as PDF files, which can be archived and compared to run tests periodically. These tests should be run especially when:

- The system is moved to a new environment
- Something in the environment changes
- Different types of animals are being monitored (*i.e.,* new disease model or lighter in weight)

2. Installation

This program requires a 64-bit Windows machine to use it. When downloading and running the installation script, the computer will need access to the internet to download library files from MathWorks (Natick, MA). After this, follow the prompts from the install script to complete the installation. Once installed, the program can be launched from the desktop icon (if the option for a desktop icon was selected during the installation process) or via the Windows Start Menu, which is identified by the program as ***SignalQualityAnalysisTool***.

3. Overview

After data has been collected from the PiezoSleep system under a test condition (all empty or a mix of empty and occupied cages), this program opens the pressure signal file (*bin(fb)* extension) and creates plots for assessing signal quality and identifying potential noise issues. Graphics are created and assembled in a report for assessment of room noise and sensitivity to animal activity.

Noise can be classified into two general categories. The first is persistent (or stationary) noise, which remains at a consistent average level over extended periods. This can arise from a variety of sources, such as motor vibrations, air flow on the cages, or ambient building vibrations from foot traffic or nearby construction. The power in these vibrations must be less than the signals generated by the animals. To characterize this type of noise, a Power Spectral Density (PSD) plot is generated to observe noise levels

in all the cages over a user-selected 3-hour interval. The PSD presents the average signal power as a function of frequency and should be averaged over intervals where the noise is relatively constant. The second type of noise is transient. This noise is typically seen as spikes that rise above the noise floor briefly and may not appear again for several minutes or more. This typically does not impact the performance of the sleep-wake decisions in a significant way. This type of noise can be caused by switches or valves turning on/off, occasional human handling, or closing doors. To observe this noise, the time series of the pressure waveforms are provided to show occurrences of transient noise. This type of noise can become a problem if it occurs often (several times a minute). In this case, the transient spike of energy can cause the platform to vibrate over time (like hitting a tuning fork) and interfere with the signal for extended periods of time. This noise can create more significant problems if the cages are not placed on a stable platform.

The third type of graphic produced in the report is a spectrogram over the selected 3-hour interval. This shows spectral power change over time as an image, where spectral powers are mapped into a range of colors to distinguish amplitudes. This graphic is useful when the cages are occupied, and the spectral power follows changes in the animal activity. This also shows how well the signal from respiration (thorax motions during periods of stillness) is distinguished from the noise floor. Examples of these plots and their interpretation are presented in this manual.

4. User Interface Options

After launching the program, the graphical user interface (GUI) shown in Fig. 1 appears. Operations are executed by selecting the items identified with the red numbers, which are described in this section.

1. **Menu**

    Clicking on this item presents options in the drop-down menu shown in Figure 2. ***Open File*** (Ctrl+O) generates a file navigation window with a filter to select files with signals for analysis. These files end with either a *binfb* or *bin* extension (mouse or rat system recordings). ***Close*** (Ctrl+X) closes the window and the program (same as clicking on the X in the upper right of the main window shown in Fig. 1b).
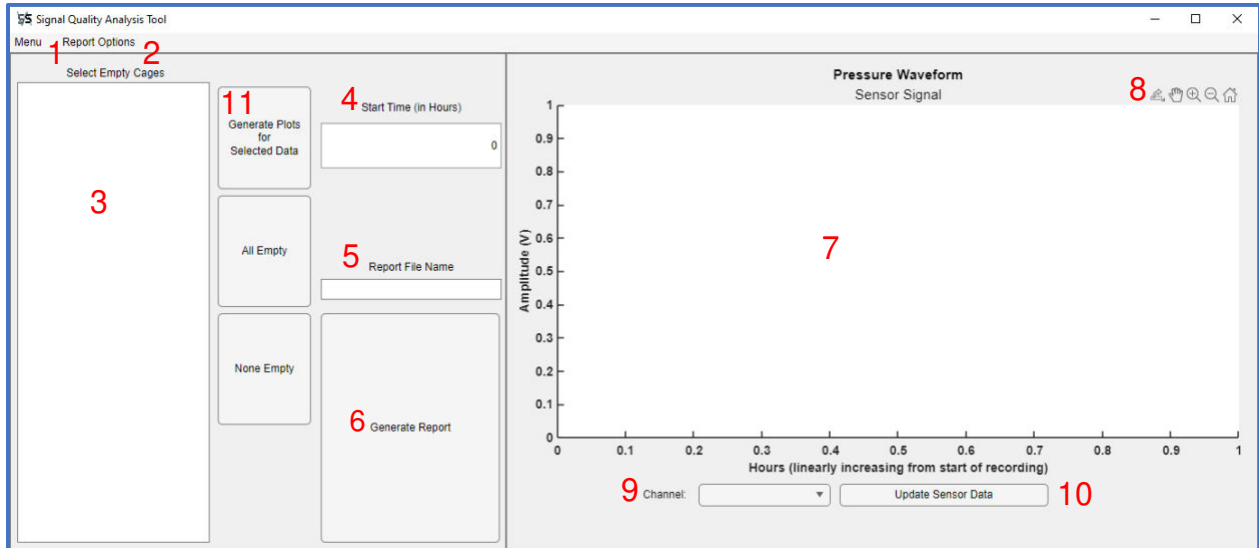
2. **Report Options**

    Clicking on this menu item presents options in the drop-down menu shown in Figure 3. ***Change Save Location*** generates a directory navigation window to select or create a folder where the reports will be saved. By default, the program creates/uses the folder called ***Reports*** in the same directory where the first *bin** file was opened in that session. Selecting multiple data files in the same session (without quitting the program) will result in all reports being stored in the same directory unless the folder is changed with this option.

   **Include Spectrograms** (default off)
    Click on this item to toggle the generation of the spectrograms for observing spectral changes caused by the changing states of a live animal or noise pattern changes over time.

Include Time Series (default on)
Click on this item to toggle the generation of the time-series waveform graphics for observing power level changes and transients. This option is on by default.

(a)
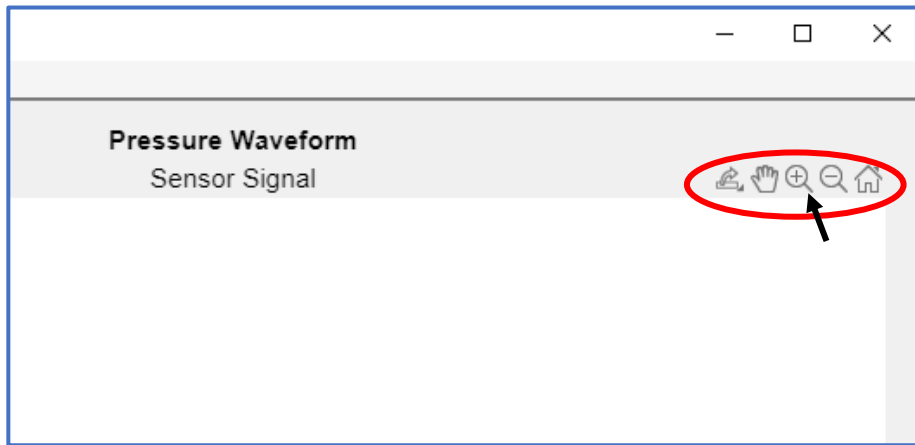
(b)

Figure 1: (a) Main graphical user interface (GUI) for loading new files (1), modifying report options (2), and viewing time series of pressure signals in plot area (7). (b) Hover the mouse over the plot area (8) to generate icons for manipulating plot limits.
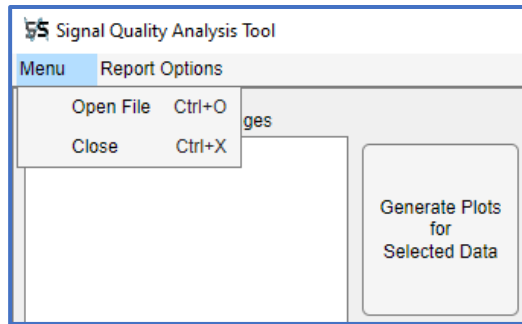
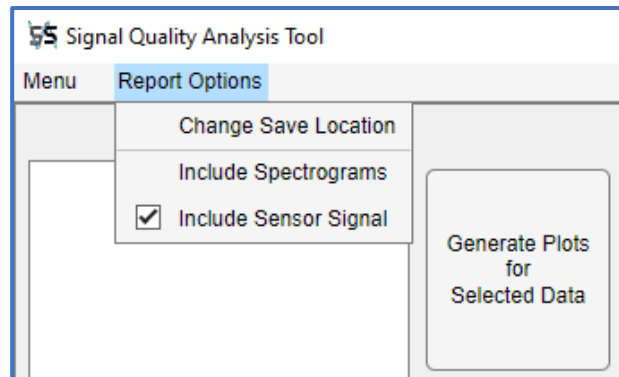Figure 2: Main menu to open the file and quit the program.

Figure 3: Report Options, sets location and content of the generated report.

3. **Select Empty Cages**

Once a file is opened, the main display will be populated with graphics and information about the recording as shown in Figure 4. The text display with the heading ***Select Empty Cages*** displays the names given to each channel at the time of the recording. Multiple cages can be selected by pressing the Ctrl key, or the Shift key while clicking on labels to select a group of channels. For convenience, the button to the right of this area can select predefined groups of cages. All cages selected will have an *empty* label on associate plots/graphs for identification and separated in the analysis from cages occupied by animals. For all occupied channels, the two highest energy, two lowest energy, and one median channel are computed and labeled to distinguish them. If you don't select these items, the plots in the report will still generate, but without the empty labels in the legends or the high and low rankings of the occupied cages.

4. **Start Time (in Hours from Start of Experiment)**

The text in this window initializes with the hour of the day (0 to 24 hours) that the recording started. It can be set to any time on the signal time axis (shown in area **7** of Fig. 4a). The purple-shaded area shows the time interval characterized in the generated report. If a starting hour is selected, such that the 3-hour window goes beyond the end of the recording, an error/warning message will appear, and you will need to reselect the starting time.

6

5. Report File Name

The text in this window is initialized to the base name of the recorded file. This can be edited to change the filename, and the generated PDF report will be saved with the base name. If multiple reports are to be generated from the same file (such as starting at different 3-hour segments over the recordings), then the report name should be changed to reflect this distinction, or the previous report will be overwritten. Note that the program will crash or give an error message if the file it is trying to write to is open. So, if you want an overwrite to occur, make sure the file is released by other programs.
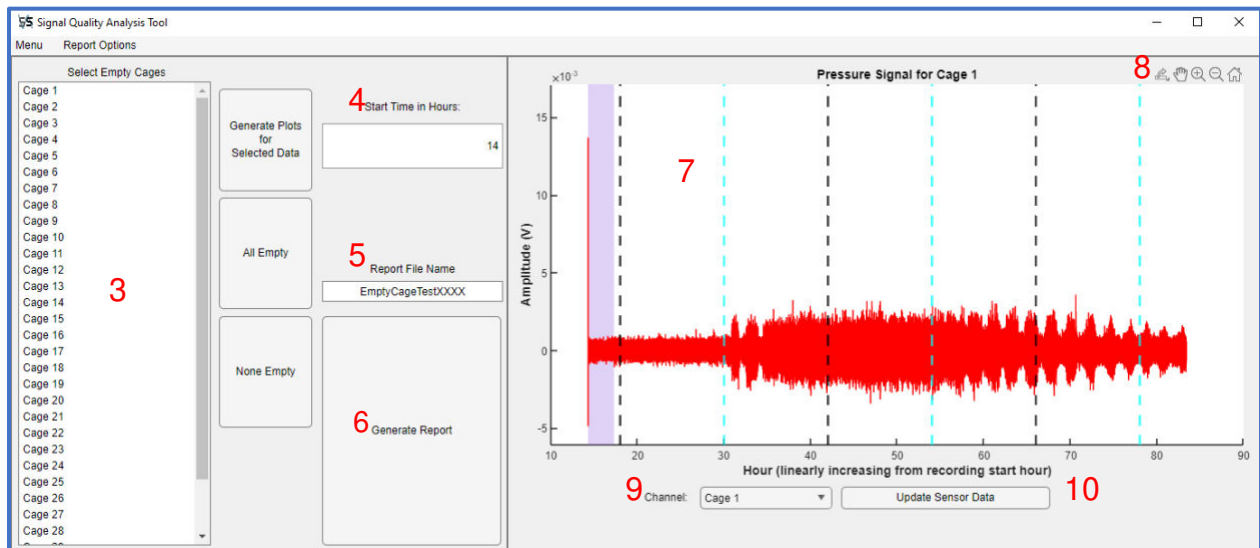

Figure 4: Example of GUI after opening a 32-channel recording of empty cages.

6. Generate Report

This button generates the report with the selected settings. Figure 5 shows an example indicating all cages empty and the analysis interval starting at hour 47. After clicking on the **Generate Report** button, a progress bar appears while the report is being generated, and it ends with a window showing the location where the report is saved. Once finished saving the report, it can be used again to generate another. To use a different bin file, simply go to **Open File** and select a different file. This will reset the report name to be the name of the new file opened, but the report location will not change unless the user changes it (see Fig. 3).
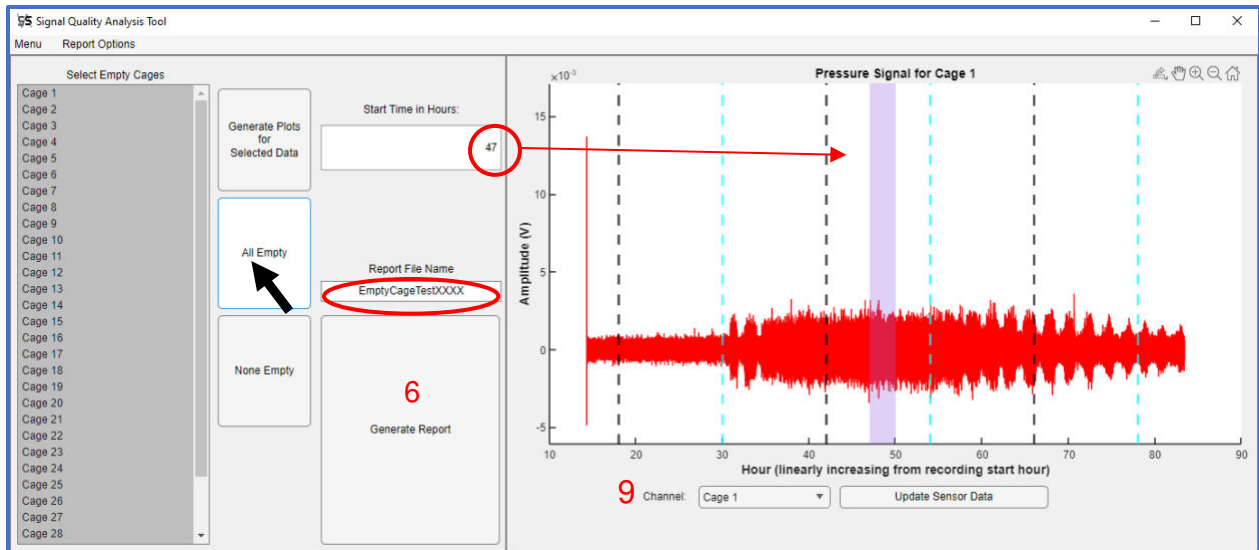
Figure 5: Example of selecting a 3-hour segment for analysis.

7. Pressure Waveform Plot Area

After a file is opened, the pressure waveform is plotted over the entire recording for the first channel, as shown in Fig. 5. The light and dark onset times entered in *PiezoSleep* at the time of the recording are superimposed as cyan (light onset) and black (dark onset) vertical broken lines. In Fig. 6, the list box (9) was used to select a different channel for viewing. Note the waveform in Fig. 6 has higher amplitudes with no significant changes observed over the entire recording. Whereas the channel shown in Fig. 5 shows an increase and changes in the noise floor level after hour 30. This display is useful to observe trends in the signal power/amplitude over the entire recording and guide the selection of an analysis window.
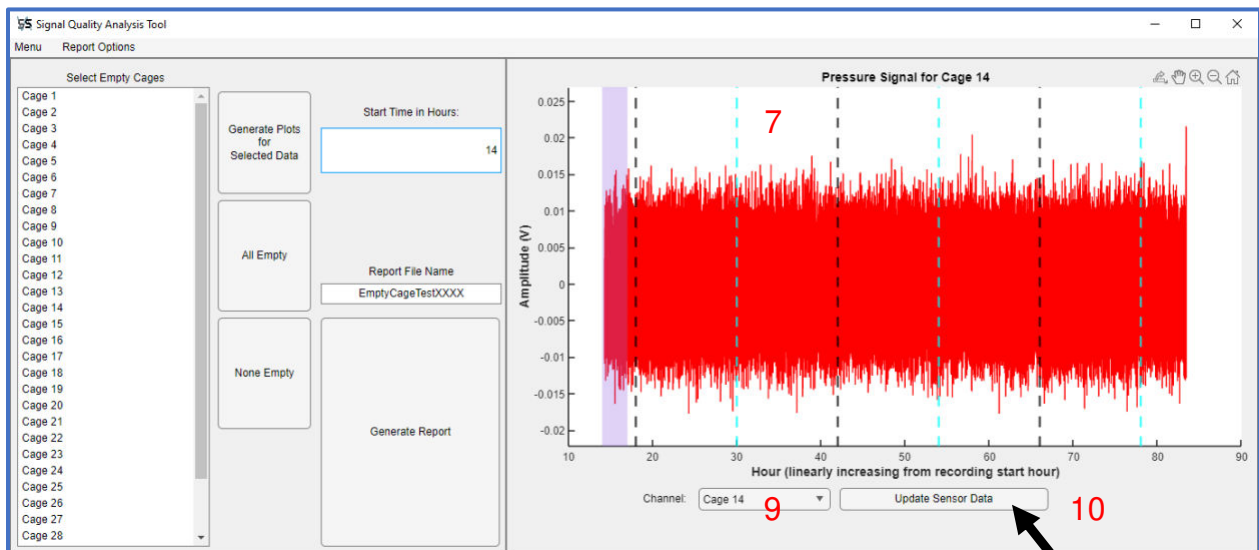

Figure 6: Example of viewing another pressure waveform in plot area 7.

8

8. **Graph Manipulation**

To view the waveforms in more detail, it is possible to zoom in on smaller sections of time and update the pressure sensor data resolution (using the button at 10). The original signal is subsampled when showing long time intervals since fine resolution cannot be observed on this scale. Clicking the *update sensor data* button after zooming in to a smaller time scale resamples the signal more densely to match the detail that can be displayed in the smaller interval. Figure 1b shows the menu for adjusting the image when the mouse is over the plot area. When zoom-in (+) is active, dragging the mouse over a smaller plot area will result in the axes being set to the area defined by the mouse drag. After this action, the resolution of the plot should be updated (10). Clicking on the *home* icon shown in Fig. 1b in the upper right of the plot area returns the plot to the original view. An example of this will be shown in the Plot Area Manipulation Section where a signal from an occupied cage is examined.

9. **Select Channel to display**

Use to change the channel displayed in the plot area.

10. **Update Plot Resolution**

This updates the sensor data plots after the axes have been changed through the zoom or pan actions to a resolution appropriate to the axis ranges.

11. **Generate Plots for Selected Data**

This button generates the PSD, time waveform, and spectrogram plots for the 3-hour interval currently selected on the current channel. These are the same plots that would go into the generated report. The advantages to using this option are that the plots are generated immediately, and they can be manipulated with the pan and zoom options. This is useful for examining a particular time/event in a cage, or in previewing the figures in the selected interval before generating the full report.

5. <u>Plot Area Manipulation</u>

This section presents an example of looking at signal patterns directly by manipulating the plot area. This is typically not needed for an assessment of environmental noise, since it is not practical for assessing the signal over large blocks of time. However, in some cases, it is useful for capturing transient signal dynamics or looking at higher resolutions of low amplitude signal patterns to confirm animal respiration regularity. The spectrogram plots are more practical for that since they can summarize the critical aspects of low-level signal behavior over larger time intervals (hours rather than seconds for observing the time signal).

The example shown in Fig. 7 opens a file from a test recording with empty and occupied cages. Note the default in Fig. 7a presents an empty cage signal with a very strong transient peak at the beginning of the recording, likely due to a power surge in the data acquisition box or human activity near the cage. As a result, the lower-level environment noise is not observed in sufficient detail to see if there are changes in the power levels. In this case, an area of the plot should be selected that avoids the transient at the

beginning and zooms in (both in x and y) on the lower-level signal. After moving the mouse onto the zoom area, the mouse cursor turns into a cross pattern and can be dragged over the region shown with the gray rectangle. The result will look like the pattern in Fig. 7b, except it is distorted by the reduced resolution for presentation in the bigger plot interval. By clicking the **Update Sensor Data** button shown in Fig. 7b, the plot will update to the best resolution for the given interval. The plot will be rescaled to fit the dynamic range of the signal in the zoomed-in area.

A more interesting plot is when there is an animal in the cage, and areas of low activity are observed relative to the noise floor. In that case, zooming in on a smaller interval (on the order of about a minute or less) will often show a quasi-periodic signal from the respiration (during sleep) or more random during rest. Figure 8 illustrates this. The channel view is switched to Channel 12 (labeled R012), shown in Figure 8a. The zoom-in mode is selected, and the mouse is dragged over the axis area shown in Fig. 8b, where contiguous periods of high and low activity/sleep can be observed. Now, a smaller area can be selected that shows little variation in amplitude (most likely stillness), and this results in an image where a quasi-periodic pattern from the respiration is observed (from thorax pressing on the cage floor) in Figure 8c. This also shows a signal from stirring or shifting position for a few seconds (higher more irregular amplitudes around hour 33.914) and then a resting period right after (low amplitude with more irregular patterns).
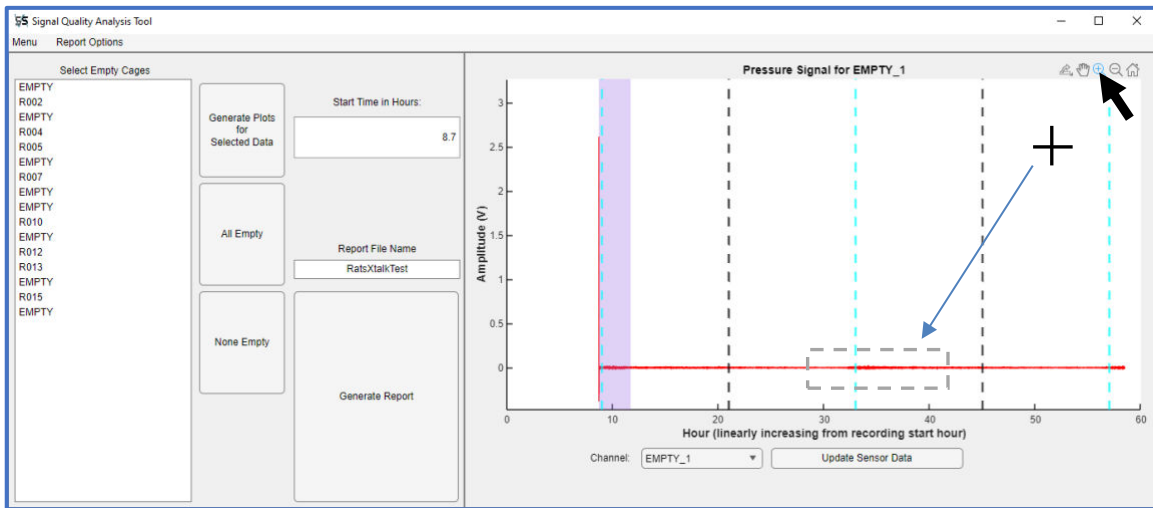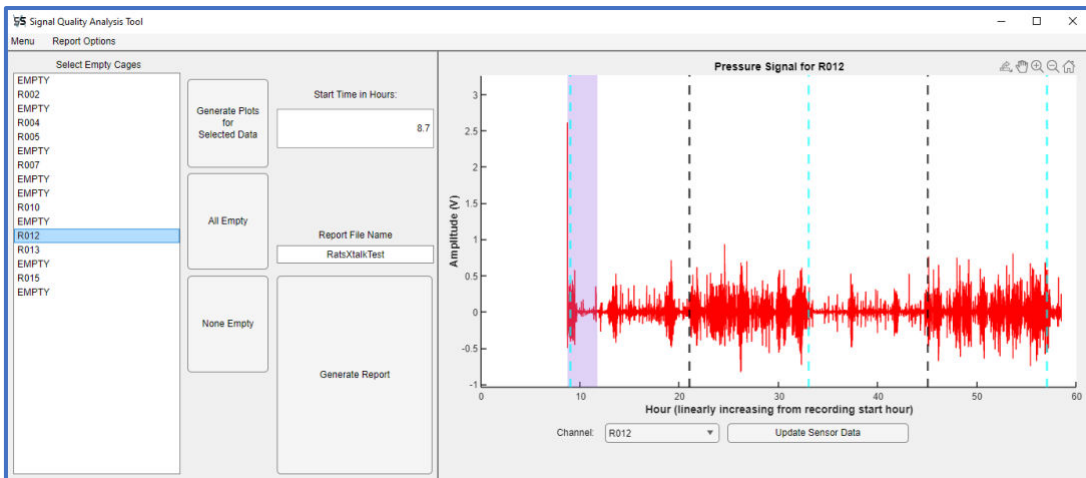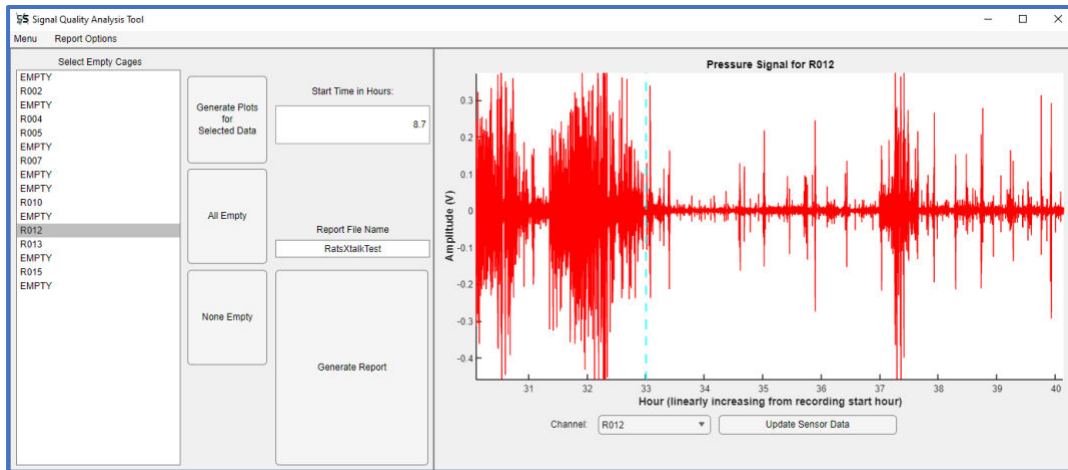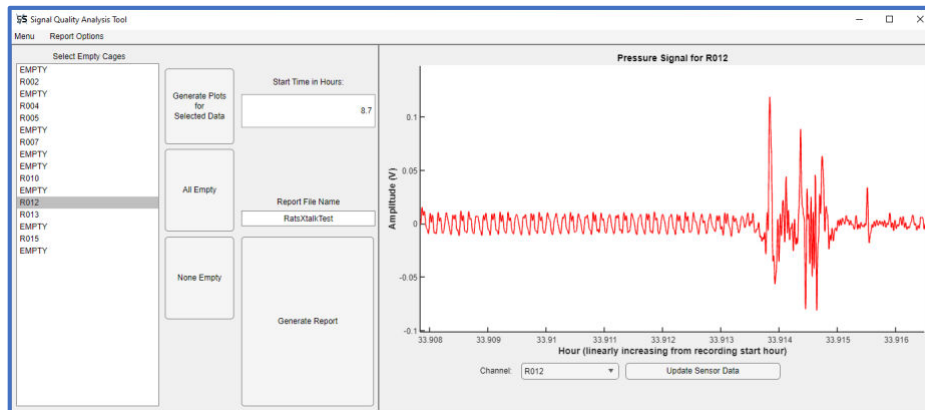

(a)
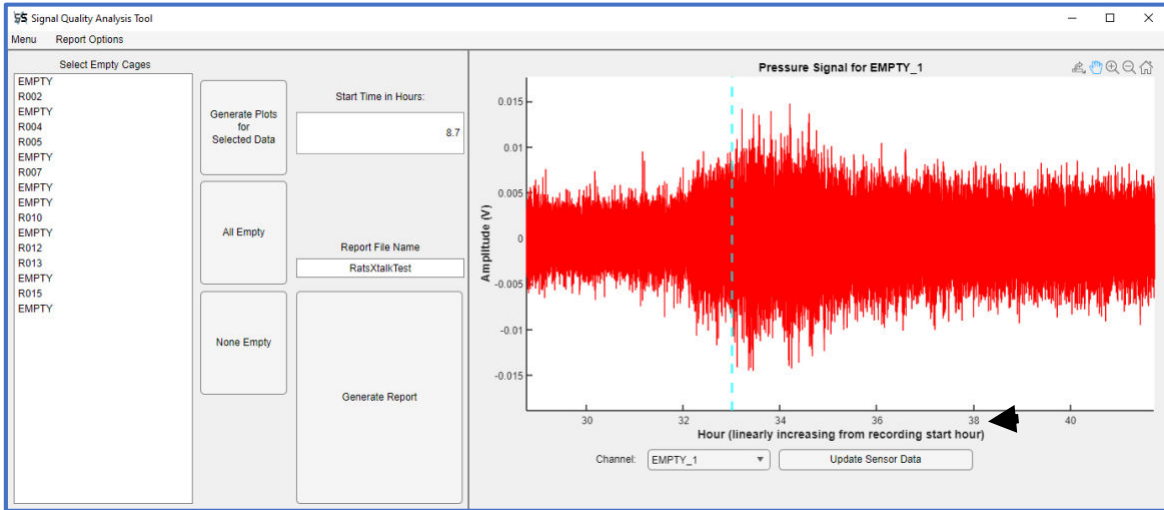
(b)

Figure 7: Example of zooming in to observe finer resolution. (a) Initial screen, select the zoom-in (+) operation and drag the mouse over a smaller region to observe. (b) Zoomed in on the plot after clicking the update button to increase resolution and rescale plot limits.

(a)

(b)

(c)

Figure 8: Example of examining pressure signal from the occupied cage. (a) Initial view (b) Intermediate zoom resolution showing periods with various levels of activity (c) detailed resolution with respiration patterns observed before and after a small movement during a sleep period.

6. Report contents

For a selected 3-hour analysis window, the report content options are as follows:

1. Power Spectral Density (PSD) plots, showing the averaged signal power distribution over the frequency axis from all the channels in the recording.
2. Spectrogram images show changes in spectral power distributions over time for each cage.
3. Pressure Data Time waveform showing the time series for each cage.

This section explains the meaning of each of these characterizations and describes what to look for when predicting signal quality from the empty and mixed cage test recordings. Examples are provided to illustrate typical patterns seen in these types of recordings.

The Power Spectral Density (PSD)

The PSD shows average signal power distributed over frequency. The PSD is estimated by averaging the spectral magnitudes from smaller time segments (~8 seconds) from the full 3-hour segment. The averaging process over this interval reveals persistent signal and noise patterns from the environment that occur over the 3-hour period. Any short-time events are typically averaged out and have a minimal impact on the final plot (unless they are very large relative to the other short-time segments in the selected interval). For an empty cage test, only environment noise is present, and the ideal PSD would be flat over the spectrum and low level (less than -50 dB). Peaks in the PSD indicate resonant noise, which can come from sources like motors or fan vibrations. In addition, the platform that the cages rest on can also shake/vibrate in response to the impact of foot traffic or machine activity. This will often show up as peaks in the PSD if they occur often. The plastic cages can have resonant frequencies and vibrate when in direct airflow from a vent. When animals are in the cage and the 3-hour period covers mostly active states, the PSD should be relatively flat and at a higher power level than the empty cage measurements. The spike-like pressure signals from locomotion and random motions typically have flat spectra; however, these motions can create resonances if their motions cause the cage to rock or the platform/racks to vibrate.

The examples below illustrate using the PSD to observe noise and sensitivity to animal motion. Figure 9 shows PSDs for 2 empty cage tests. Figure 9a shows all cages at about the same noise level and well below -50 dB. This is an example of a quiet environment where the cage sensors pick up low-amplitude vibrations from the room. There are resonances around 4 Hz. Resonances are almost always present in the noise floor since all mechanical structures have frequencies that they will tend to vibrate (shake/rock) at, but these should all be less than -50 dB for good signal quality. Also, note the power increases as the frequencies approach 0.5 Hz. This is typical since lower frequencies tend to travel better through the common structures the cages are placed on. Figure 9b shows a case of a noisier environment. In this case, the cages were in shelved enclosures that had ventilation fans attached to the structure. There are bigger variations in noise power from cage to cage, and the spectra tilts toward the higher frequencies. This is likely due to the fan motor vibrations. Cage 22 goes above -50 dB and should be checked for instabilities, or in the path of airflow, or proximity to machinery. Ways to reduce noise will be listed in the last sections where the empty and mix cage tests are described in more detail.
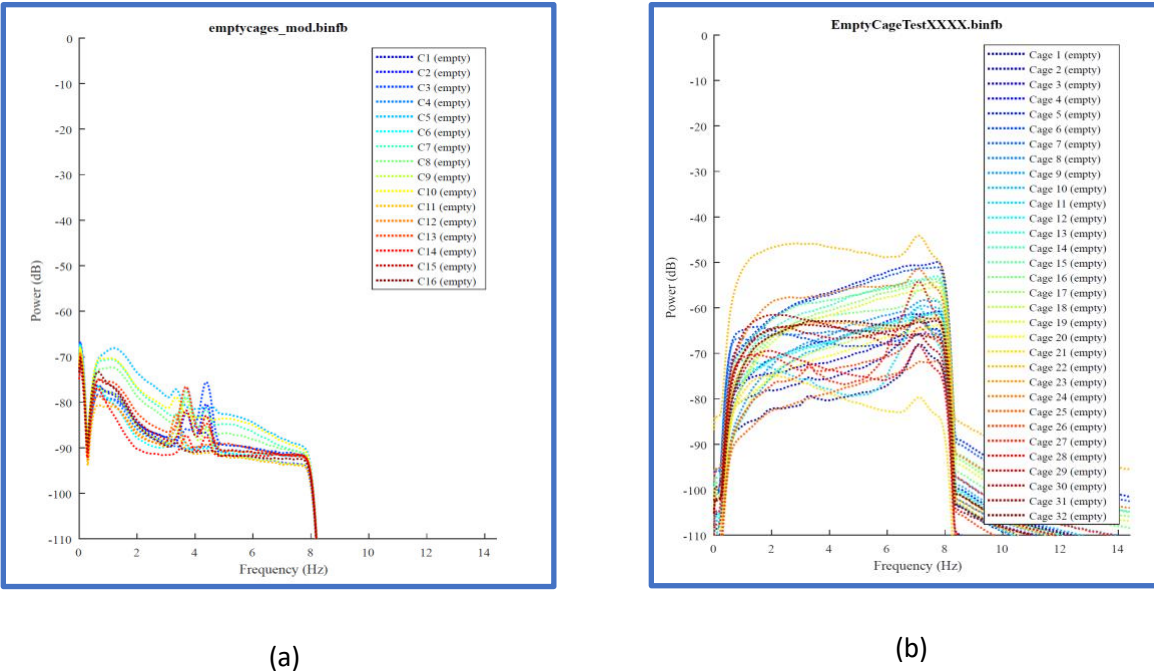
Figure 9: Examples of PSD for empty cages. (a) a very quiet environment all noise levels well below -50 dB. Resonance around 4 Hz, too small to impact signals from animal movements. (b) a moderately noisy environment (fans were operating on cages in enclosed shelves), but signals were still below 50 dB, except for Cage 22.

Figure 10 shows examples of recordings from cages where some were empty and some occupied. This test is good for showing how sensitive the sensors are to the animals in the cages relative to the background noise. In the case of Fig. 10a, there is at least a 20 dB difference between occupied and empty cages. This should be at least 10 dB to expect consistent performance from the system. Figure 10b shows PSD plots from another case of a mixed test. While the 2 empty channels look low, the last channel (cage label e6) shows the noise floor of the electronic system without the sensor properly plugged in. In general, before each recording, it is recommended to run the system in PiezoSleep briefly and touch each cage while looking at the signal monitor to make sure it responds. If not, the cable and connection need to be checked. If all is plugged in securely and it still does not respond, a component like a sensor, cable, or amplifier in that channel may need to be replaced/repaired. The signal levels in occupied cages can vary due to the level and density of animal activity in the interval examined.

The PSD is the simplest test to understand and draw conclusions from. It is produced in every report (no option to turn off). The other 2 signal characterizations are optional and can be selected with the program option window. They are useful for identifying important patterns from animal movements or noise disturbances that exist for short periods of time (*i.e.*, not persistent over the 3-hour analysis time window). In most cases, the PSD report should be all that is needed for the empty cage tests. For the mixed tests, the weakest signal from the rodents should be above the noise floor. The PSD plots for occupied signals are dominated by the active states in the analysis window. It is difficult to find a 3-hour interval where the animal is just sleeping in all the cages to make the PSD useful for assessing signal and noise levels. The weakest signal detected by the system is the movement of the thorax during respiration and stillness during rest. During sleep periods, the low-level signals become regular (quasi-

periodic) and there are no other movements of the body. In these intervals, the spectrogram is useful to confirm the system is detecting respiration patterns.
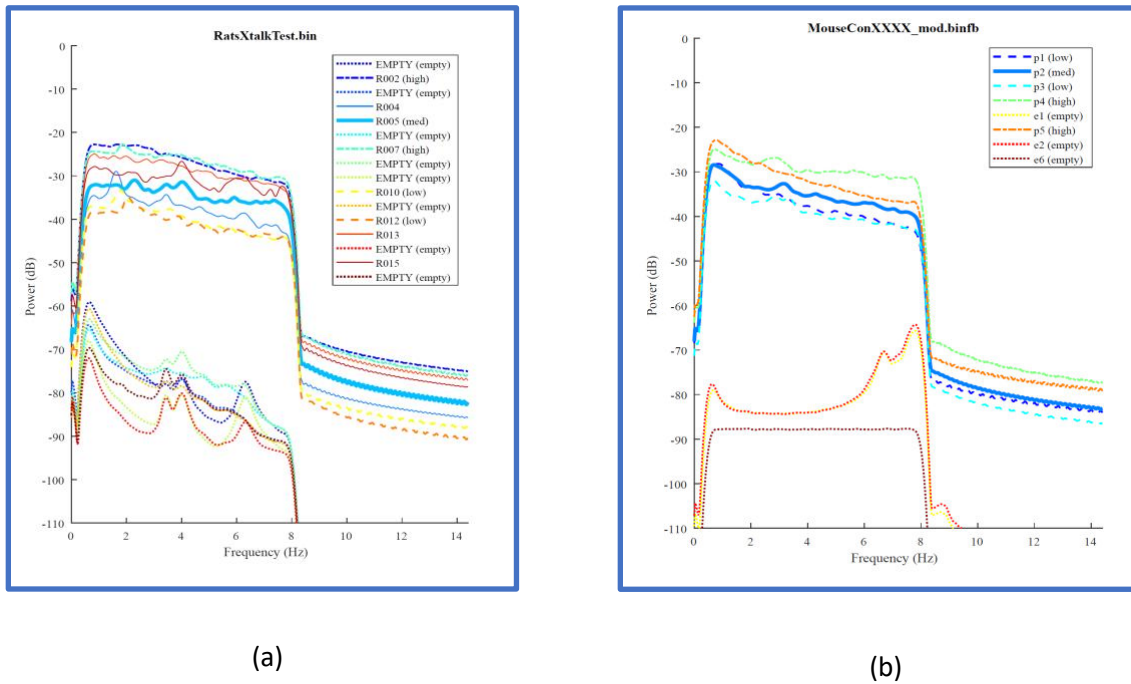

(a)

(b)

Figure 10: Examples of PSD for a mixed set of occupied and empty cages. (a) The empty labeled cages indicate a quiet environment with all noise levels below -50 dB. Resonances around 4 and 6 Hz, all well below animal signal levels. (b) The 2 empty cages are also below the -50 dB line; one cage shows a resonance around 7 and 8 Hz. The flat spectrum is the result of a cable not fully plugged in (no signal).

The Spectrogram

The spectrogram is an image that shows how the spectrum changes over time. This is different from the PSD, which averaged all the spectral magnitudes from a shorter time (on the order of seconds). So, if there are important changes in the spectrum over time, they will be lost or distorted through the averaging process. For the spectrogram, the x-axis is time, and the y-axis is frequency in Hz. The amplitude of the spectrum is mapped into a range of colors based on an amplitude-to-color mapping indicated in the color bar to the right of the image. For an empty cage test, this ideally should have a uniform appearance over time. This graph is useful for identifying intermitted noise problems, where vibrations are not persistent over the entire analysis period. Changes seen over the time axis of the spectrogram in an empty cage test could be caused by a heating and air conditioning system going on and off, or a machine turning on for limited periods of time. In the case of an occupied cage, it typically shows periods of activity and inactivity. For a good signal during rest periods (low energy over the frequency spectrum), a 1 to 5 Hz band can typically be seen resulting from the animal's thorax pressure during respiration. If this is observed, then the cage environment is good for tracking the sleep and wake patterns of these animals. The amplitudes in these spectrograms are clipped and scaled to better bring out what is happening in the lower power portions of the analysis window.

Examples of spectrograms from empty and occupied cages are shown in Figure 11. The only empty cage example is shown in Figure 11a. Note the uniform appearance over time relative to the other spectrograms. The frequency range between 6.5 and 8 Hz shows a resonance that changes power levels over time, but not its frequency position. This consistent frequency location is characteristic of the cage and platform's machine vibrations or mechanical resonances. This could result from nearby foot traffic or the ventilation system causing the cages/platform to shake. This would appear in the PSD as peaks around 6.5 and 7.7 Hz. Quasi-periodic motions from breathing typically generate spectral peaks between 1 and 4 Hz that are best seen in extended periods of low activity. Breathing rhythms generate peaks at frequencies corresponding to their rate – the number of breaths per second. The frequency corresponding to this rate is sometimes referred to as the fundamental frequency. A periodic signal that is not a simple sinusoid, will also have peaks at integer multiples of that frequency (harmonic frequencies). These fundamental and harmonic patterns are seen in the low-activity portions of Figs. 11b-11d. The yellow blocks streaking in the vertical direction are periods of activity (locomotion, grooming, head movements, rearing …). For typical animals, the patterns in Figs. 11b-11d should be seen to confirm good signal quality. The peak intensity/height may change over time depending on the posture (thorax contact with cage floor) as well as the level of consistency of breathing interval over the short time segments used to generate the spectrogram.
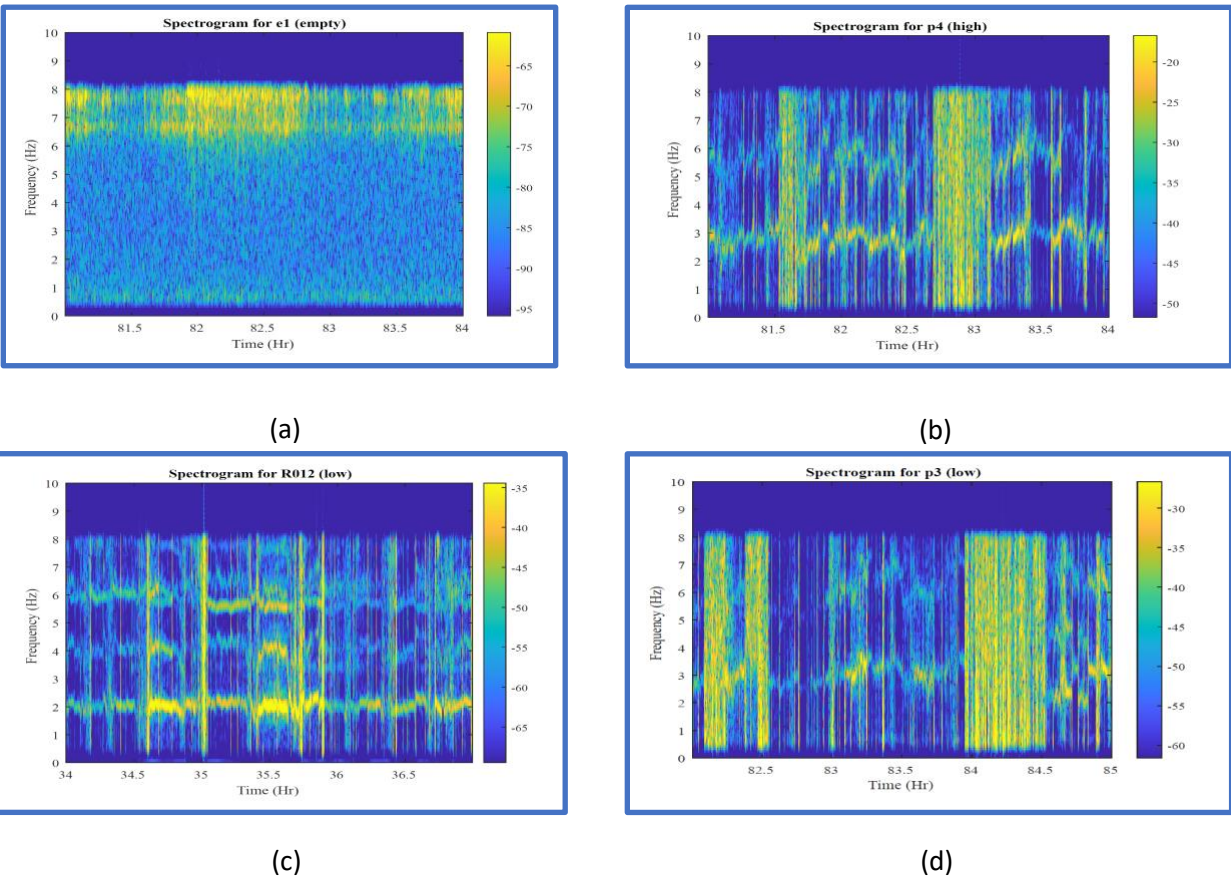
(a)

(b)

(c)

(d)

Figure 11: Spectrogram examples: (a) Empty cage (b) Mouse in a cage with fundamental spectral peaks seen between 2.5 and 3.5 Hz in periods of low activity (c) Rat in a cage with fundamental spectral peaks around 2 Hz seen in periods of low activity. (d) Mouse in a cage with spectral peaks seen near 3 Hz over most of the low activity regions.

The Pressure Data Time Waveform (Time Series)

The waveform plots show variations in pressure on the cage floor. The 3-hour segment cannot display details of signals that change on the order of seconds, the resolution is too low to observe these signal dynamics. However, these plots show the average range of signal amplitudes over the block of time, which effectively presents the envelope of the signal. These plots are also useful for identifying the occurrences of transient spikes that exceed this envelope. These plots complement the PSD, in that the PSD will typically average out the transients. If the transients are excessive over the period or there is one very large one, it could impact the PSD and give a misleading result by masking out the information in the noise floor. Isolated transients typically occur from slamming doors, nearby/heavy foot traffic, flipping switches, or cage/platform handling. Isolated/sparse transients will not significantly impact the performance of the sleep-wake detection. Occupied cages should show many transients as caused by the mouse alternating between active and resting states.

Figure 12 shows typical plots from empty and occupied cages. Figures 12a and b show a low-level envelope from the ambient noise vibrations in the room with very mild transient peaks (not noticeably

greater than the envelope outline). The peak signal levels are mostly below 5 mV. These would be examples of setups based on their consistency at a low level. However, the relative noise power/level is better assessed in the PSD plots. Figure 12c shows an example of another good setup, however, there are a few transient spikes that exceed the envelope levels that are around 5 mV. These spikes are 2 to 5 times greater than the envelope, but there are very few of them so they will not impact the sleep-wake decisions. Figure 12d shows a typical plot from a cage that is occupied. The periods of activity are observed and often 100 times greater than the noise floor. The patterns in the low-level signals between the transient signal bursts, corresponding to active states, are not well observed in these plots because both the time and amplitudes axis are too large to provide the needed resolutions to see actual levels. However, the spectrogram plots are better scaled to bring out these lower levels to identify the critical respiration patterns that occur often during periods of low activity and sleep.

The primary usefulness of these plots is to examine transients that occur in the 3-hour analysis period. These should occur sparsely across the analysis period if at all. If more than 20 spikes are observed per hour, the environment should be checked for what may be causing those patterns (valves actuating or heavy machines in nearby rooms turning on and off, or fans attached to the shelving system that frequently turn on and off). These plots are also useful to confirm the relative signal level observed in the PSD. For multiple cage systems, the color codes on the PSD may be hard to distinguish. If there is ambiguity as to which cage had the higher noise level, these plots can be used to confirm the cage noise powers. Finally, if there are 1 or 2 very large spikes (20 times greater than the noise floor envelope or more), this can dominate the PSD computation and result in a spectrum that is flatter and higher power level than the true noise floor. In this case, it is best to select a different 3-hour segment for characterizing the noise floor.

(a)   (b)

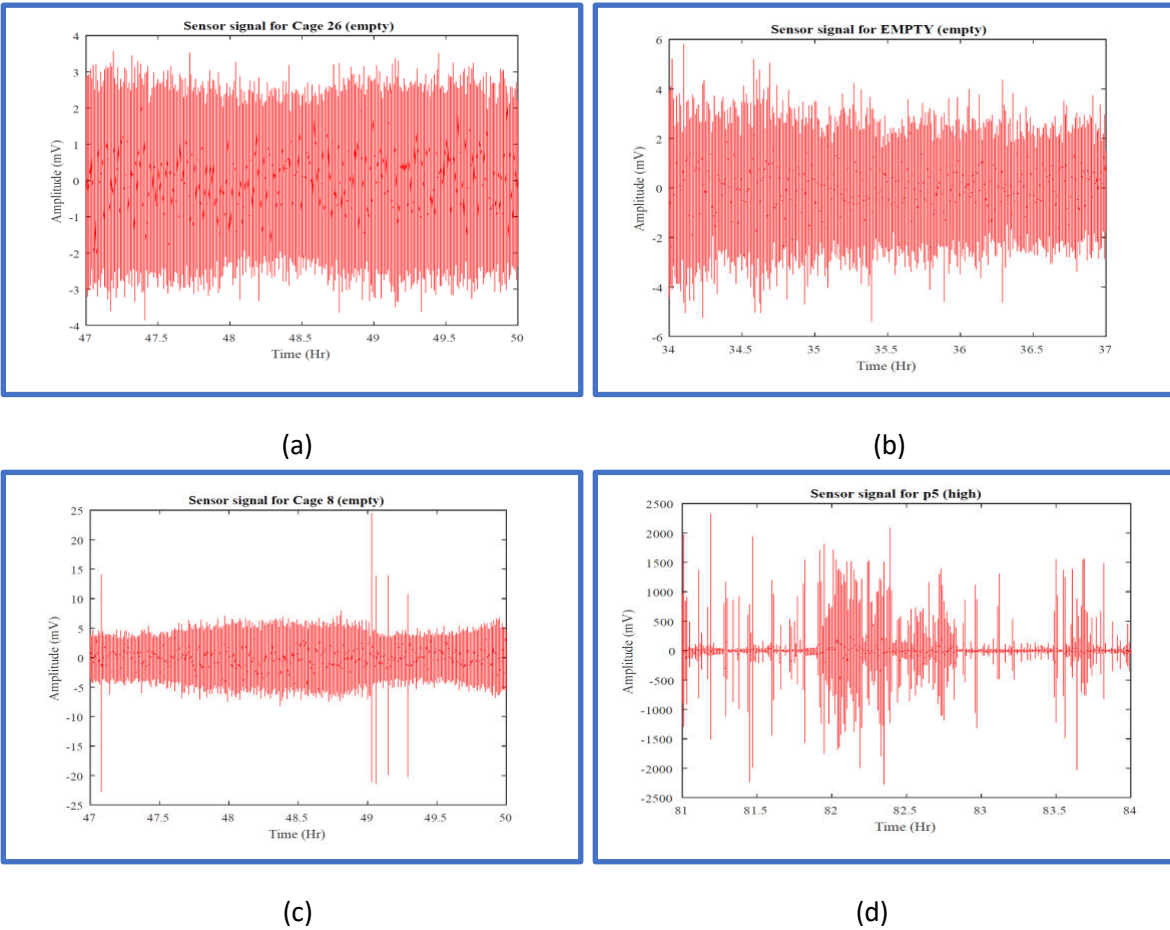(c)   (d)

Figure 12: Pressure data time waveforms where all plot y-axes are auto-scaled (a) Typical signal from an empty cage in a quiet and consistent environment. (b) Signal from quiet environment some low-level transient peak above the envelope. (c) Signal from a quiet environment with a few strong transients near the beginning and between hours 49 and 49.5. (d) Signals from occupied cages show a bust of activity (clusters of strong transients) and periods of rest/sleep.

7. Empty Cage Test

Before using the system for animal recordings for the first time, or if the cage system is moved to a new environment, an empty cage test is highly recommended to ensure the ambient vibration in the room will not interfere with the sleep and wake classifications. The empty cage test is a recording of all empty cages for at least 24 hours in the facility where the animal recording will occur. While the systems sent out from Signals Solutions have been assembled and tested at our facilities to ensure all components are working properly, changes between packing and unpacking may have occurred, as well as connections between components during setup not being made properly. The signals acquired during the empty cage test ensure all electronics are working (*i.e.,* ambient noise signals from the environment are detected), and the noise levels are within an acceptable range.

19

For the empty cage test, follow these steps:

1. Set up all cages with sensors connected to the data acquisition box, then connect it to the PC. It is best to have the cages completely assembled with lids and water bottles and make sure the cages are stable on the platforms (no rocking or bouncing).
2. Start up PiezoSleep for a short recording to ensure all sensors are working.
3. Once recording has started, view the first 4 cage signals in the *Signal Panel* tab while gently tapping on each cage to ensure the signal responds to the touch (a 2 to 3-second delay exists between the signal display and activity in the cage). Repeat this for all cages in the system. If there is no response for a particular cage, double-check the connectors between the cages. Make sure plugs are inserted all the way into the jacks. If there is still no response, contact Signal Solutions for support.
4. If all cages showed a signal response to the touch, then end the recording (can discard recorded files in this case).
5. Start recording again and let it run for at least 24 hours. It is best to do this on a typical day with normal activity in the facility. The 24-hour period captures signals from activities and machine noises that occur over the course of the day in a building (ventilation, people traffic, etc.).
6. When the recording is done, move the *bin*(*fb*) file to the computer with this noise floor analysis program on it and perform the noise test.
7. Open the *Signal Quality Analysis* program and load in the pressure signal recorded from the empty cage test. It is best to first look through signals from all cages, using area 7 shown in Fig. 4, and using the channel/cage selection box (9). From this, determine 3-hour intervals to select. It is recommended to select an interval during the peak activity time in the building and another during the low activity period. So, 2 reports should be generated.
8. The PSD plot is always generated, so the waveform plots can also be helpful if trying to identify cages with outlying power values (if they exist).

Assessments and Actions

If the PSD ensemble looks like those in Fig. 9a, where all noise floor PSDs are clustered closer together and lower than -50dB, then there is good cage placement and PiezoSleep should work as expected. The following highlights issues where adjustments are needed:

1. If there are cage PSDs that exceed -50 dB, attempts should be made to lower overall noise floor power. This can be done by identifying noise sources and moving cages further away or isolating them from the vibrations/interaction from the source.
2. If a cage(s) PSD level is a part of the cluster, as for cage 22 in Fig. 9b, the placement of this cage should be checked. Even if all the noise power is under -50dB, it is best to have a uniform noise exposure for each cage. The same would hold for a cage that is well below the cluster of all the other cage powers. A typical cause of this is cages in the air path of a vent or fan, more exposed to human activity, or on a less stable part of the platform. This can be addressed by moving the cages to a place more like the others, blocking the direct airflow on the cage, and putting more weight on the platform to reduce/change the resonant vibrations of the platform. Other issues to check may include debris between the cages and the sensors.
3. If there is a high resonance (strong narrow peak in PSD above -50dB) on many of the cages, the shelf or platform is typically the problem. If placed on shelves, the shelves should be as stiff as

possible to limit the vibrations generated from foot traffic and machine activity. For long shelves with no support between endpoints, there is typically more noise for cages at the center, where vibrations have the highest amplitudes. If a better platform cannot be used, adding weight to the shelves with heavy padding or metal/cement blocks placed near the center can help dampen the vibrations.

4. If there are still odd patterns that you cannot interpret or solve, or have questions on, please send the report and a picture of the cages in the environment to Signal Solutions for help.

8. Mixed Cage Test

For a more thorough assessment of signal quality for the intended experiments, a test where cages alternate between occupied, and empty can be run. The idea behind this is that the empty cages are picking up noise/interference only, whereas the cages adjacent to them are receiving similar noise plus the signal from animal movement. This provides an assessment of the signal-to-noise power. With the spectrogram graphics, the ability to distinguish respiration signals from noise can be assessed for the animals tested.

The setup for the mixed cage tests should be the same as that for the empty cage test for steps 1 through 4. Then:

1. Place animals in cages leaving an empty cage next to an occupied cage. In many cases, this would be animals in the odd-numbered cages and empties in the even-numbered cages, or vise vera.
2. Same as steps 5 through 7 in the empty cage test.
3. In addition to the PSD plots, select the spectrograms in the report generations. The time series would be optional in this case.

Assessments and Actions

If the PSD set looks like those in Fig. 10a, where all empty cage PSDs are clustered closer together and lower than 10dB from the occupied cages, then PiezoSleep should work as expected in separating active from inactive states. In addition, the spectrograms should show some respiration detection for a good assessment of sleep (ability to separate sleep from resting low activity states), as illustrated in Figs. 11b through d. It is fine for this to disappear over segments, especially if the animal is sick and spending more time in a resting state; however, at some point, this band should become visible to have confidence these signals are being picked up. The following highlights issues where some adjustments are needed:

1. If significantly higher noise levels are seen in the empty cages (above -50db), there may be an issue with interference between cages. In this case, separate the cages further from each other.
2. If there is an occupied cage with significantly higher or lower power than the other cages, this could be due to variations in animal activity, or the cage not being placed on the sensors in a stable manner. Another 3-hour segment can be selected where there is more dense activity for the lower-powered occupied cage to see if this makes a difference.

3. If there are still odd patterns that you cannot interpret or solve, or have questions about, please send the report and a picture of the cages in the environment to Signal Solutions for help.