EPL Cochlear Function Test Suite

Users' Manual

Version 1.0

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1. Introduction

The *Eaton-Peabody Laboratories Cochlear Function Test Suite* (CFTS) performs acoustic calibrations and basic assays of cochlear function in laboratory animals. The CFTS is a package of programs written in LabVIEW that control digital stimulus generation and data acquisition using National Instruments input/output (I/O) boards. The programs are already compiled and does not require a LabVIEW license to run. This means, however, that it cannot be changed. If a user wants to change the labeling or functionality, the underlying LabVIEW programs (called virtual instruments, or "VI's") can also be downloaded at: http://research.meei.harvard.edu/downloads/CFTS/EPL_CFTS_Full_Install_261.exe. Using these VI's requires the LabVIEW programming environment and a LabVIEW license (these can be obtained at http://www.ni.com/). The basic elements of LabVIEW are relatively easy to learn so that small changes (changing the labeling or adding an additional input) might be done by an experimenter who has little programming or engineering background.

The cochlear function tests and procedures are:

- **Acoustic Calibrations**
- **Auditory Brainstem Response (ABR) input-output functions**
- **Compound Action Potential (CAP) input-output functions**
- **CAP tuning curves**
- **Distortion Product Otoacoustic Emissions (DPOAE) input-output functions.**
- **Basic data-analysis routines such as creating equal-response contours (DPOAE "tuning curves") from multiple DPOAE level functions.**

System overview

Figure 1 illustrates the basic setup of the CFTS and is a useful point of reference throughout this user's guide. The animal in the diagram is fitted with an acoustic assembly that has two earphones whose outputs mix at the assembly tip, and a microphone coupled with a small probe tube that measures the sound pressure at the assembly tip. Auditory stimuli are generated digitally and amplified by external power amplifiers which drive the two earphones. The output of the probe-tube microphone is amplified and sent to an I/O board. The microphone output is used both to calibrate the acoustic outputs from the earphones and to record otoacoustic emissions. Figure 1 also shows leads attached to the animal at the vertex for recording ABRs and at the round window (RW) for recording CAPs. These signals are amplified and routed to the I/O boards.

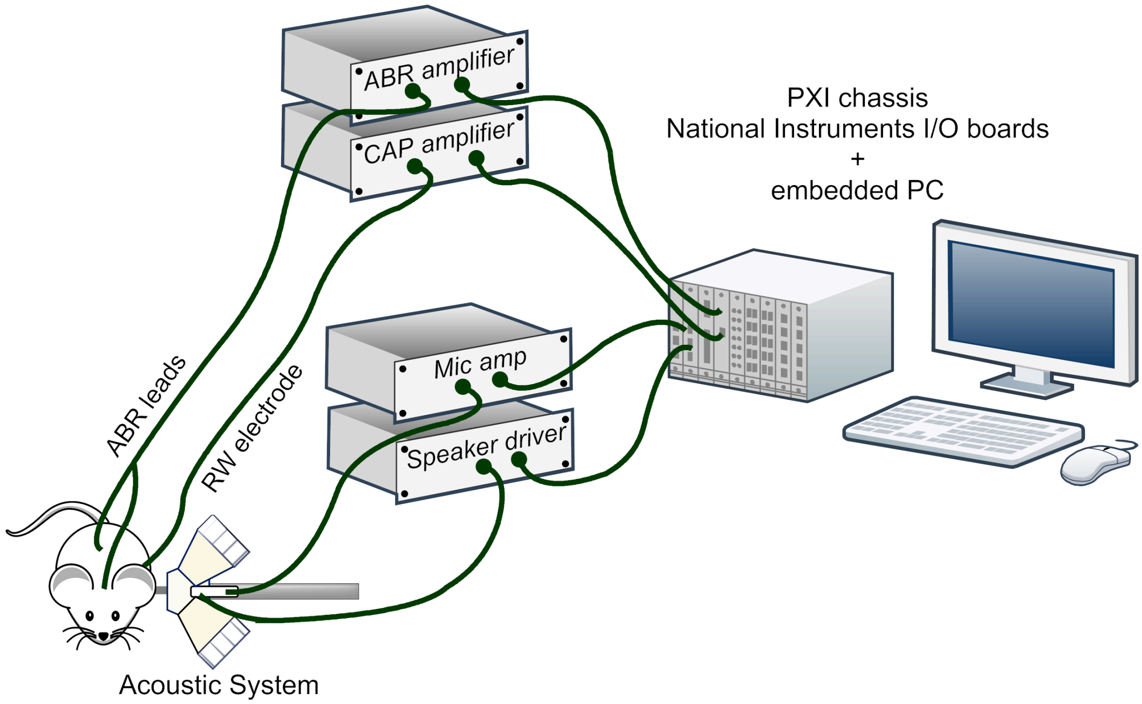


Figure 1: Schematic of the basic experimental setup.

Hardware considerations

The *Cochlear Function Test Suite* is designed specifically for use with a National Instruments PXI chassis. The chassis contains an embedded controller (a personal computer, PC) running windows, and one or more I/O boards. As many users are aware, in the “old days”, I/O boards for this type of application were typically inserted into expansion slots inside a PC. The PXI chassis is conceptually the same thing, only more powerful with respect to the number of boards that can be used, the power of the boards available, the ability to synchronize input and output across boards, and the quality of the grounds.

The CFTS is flexible regarding the number and type of I/O boards installed in the PXI chassis (see Section 3, Connection Manager). A minimal setup consists of one 24-bit I/O board (NI PXI-4461) and one 16-bit I/O board (e.g. NI PXI-6221). The high resolution of the 24-bit board makes it particularly well-suited for generating and acquiring acoustic signals (and requires the high-quality grounds of the PXI chassis to avoid spurious signals). The 16-bit board is typically used for acquiring signals of lower bandwidth and smaller dynamic range (e.g. ABR, CAP).

Obtaining and Installing the software

The software can be obtained at:
http://research.meei.harvard.edu/downloads/CFTS/EPL_CFTS_Full_Install_261.exe. After accepting the license agreement, you can download an executable file (32 MB) that allows you to install just the compiled program version, the LabVIEW programs (VI’s) on which the compiled program was based, or both.

2. Using the Cochlear Function Test Suite (CFTS)

Starting the Program

There are three ways to start the CFTS.

1. Ideally, during installation, a shortcut was created on the Windows desktop (the icon is shown on the left). Double click on the icon.

2. The CFTS can be started through the Windows Start menu:
Start>All Programs>EPL CFTS>Cochlear Function Test Suite

3. The software is installed in the following location:
`C:\Program Files\Cochlear Function Test Suite\EPL_CFTS.exe`
Double clicking on `EPL_CFTS.exe` will start the program.

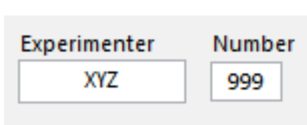
Starting the software brings up the CFTS main panel, shown in Fig. 2. Initially, the main panel shows three sections: Experiment Identification, Calibration, and Measurements. During an experiment, the natural flow carries the user from top to bottom of these sections. A fourth section at the bottom right, Advanced Options, has seldom used functions and is toggled on and off by CTRL-A. Before using the CFTS on an actual experiment, connections must be set up using the "Connections Manager" and the microphone + probe-tube must be calibrated using the "Probe Tube Sensitivity" subprograms.

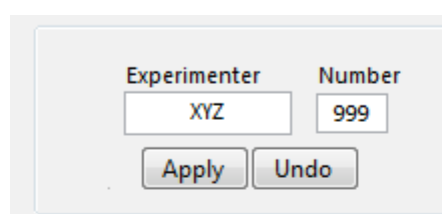
Experiment identification and Data Folder

Each experiment is identified by an experimenter ID (e.g. the experimenter's initials) and an experiment number. Both must be specified before doing anything else. After starting the CFTS, the "Experimenter" entry block is initially blank (see below left). Entering experimenter initials causes the "Apply" and "Undo" labels appear (see below right). Clicking on the Apply button creates (if this is a new experiment) or opens (if this is an existing experiment) the data folders determined by the experiment identification. For the example shown below, the data folder is:

`C:\Data\XYZ Data\XYZ999\`

When resuming an existing experiment (e.g. retesting an animal), the CFTS sees that the data folder already exists, and picks up the experiment where it left off.





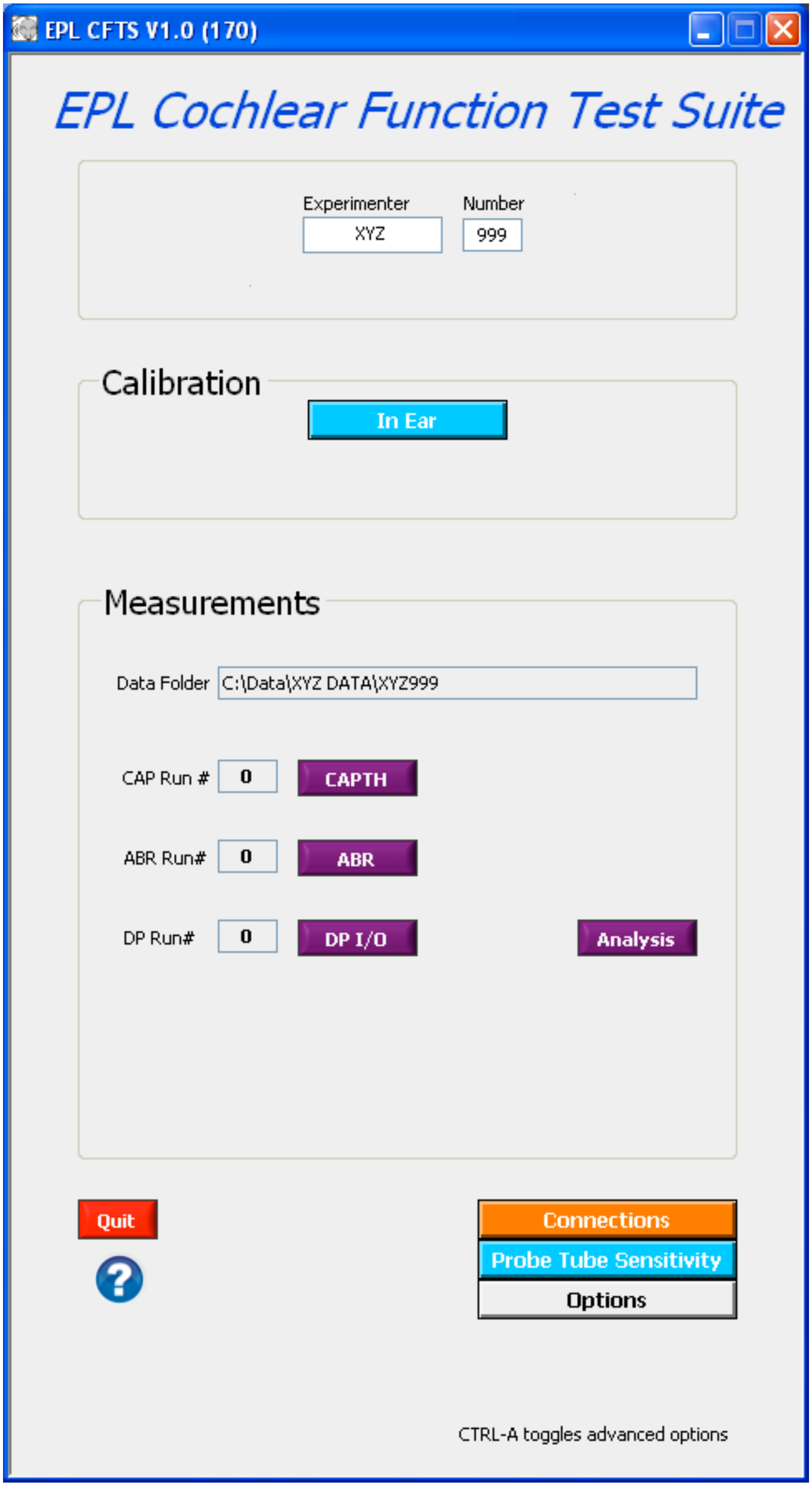


Figure 2: *Cochlear Function Test Suite* main panel.

Advanced options

Some infrequently used options are hidden at the bottom of the main panel to prevent them from being changed inadvertently. Holding the control key and pressing the letter A makes these options appear. Pressing Control-A a second time hides them again. Specification of hardware "Connections" is described in Section 3. "Probe Tube Sensitivity" is discussed in Section 4. Other "Options" are described immediately below.

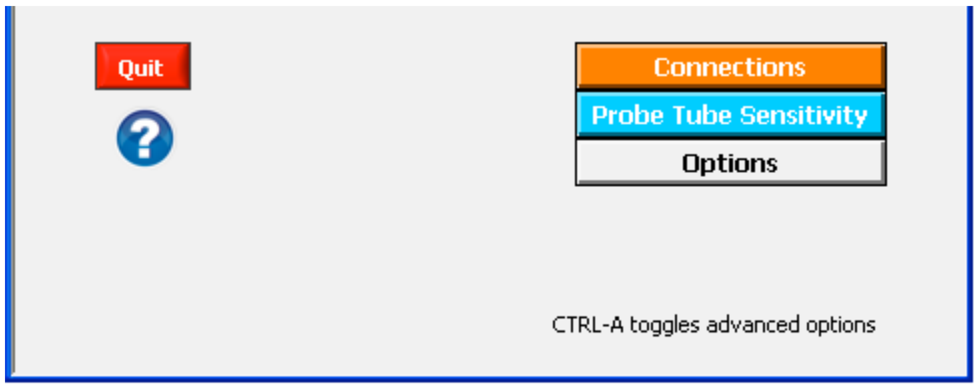
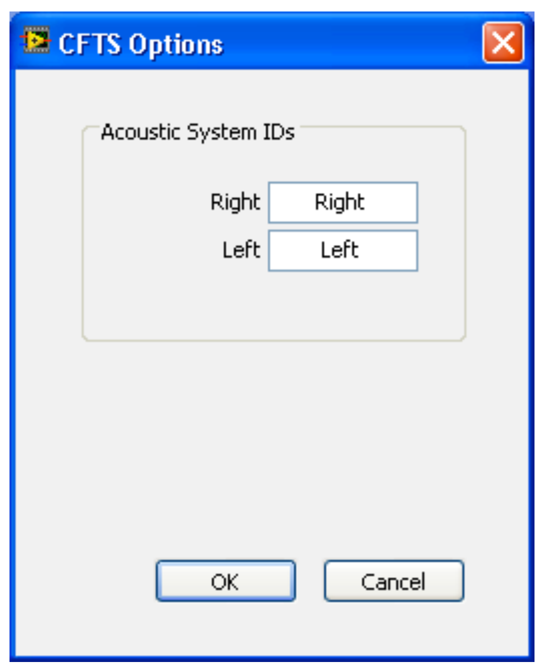


Figure 3. The lower part of the CFTS main panel toggled so it shows the advanced options.

Options: Acoustic system naming

Clicking the "Options" button in the main panel brings up the dialog box shown on the right. Here the user enters short names for the acoustic systems associated with each ear.



The default names are simply "Right" and "Left", which in the vast majority of cases will be sufficient and need not be changed, so long as each acoustic system is consistently paired with a certain ear.

On the other hand, if the user has several acoustic systems (perhaps shared among setups or used as backups), it may be more clear to enter serial numbers or some other device-specific identifier in this panel.

3. Connection Manager

One of the advantages of the *Cochlear Function Test Suite* is it its flexibility with respect to the I-O boards installed in the PXI chassis. The (small) price paid for this flexibility is the need to tell the CFTS where the various signals (e.g. those illustrated in Figure 1) are connected to the National Instruments boards. This should be a one-time procedure; there is no need to respecify the hardware configuration from experiment to experiment (unless the wiring set-up changes).

The connections are specified using the Connection Manager (Fig. 4), which is accessed under the Advanced Options, by clicking on the "Connections" button in the CFTS main panel. The Connection Manager automatically detects which I-O boards are installed in the chassis, so all that remains is to specify which signals are connected to which channels. The example in Figure 4 is how the Connection Manager appears after specifying the connections in a chassis containing one 24-bit board (in Slot 2) and one 16-bit board (in Slot 3).

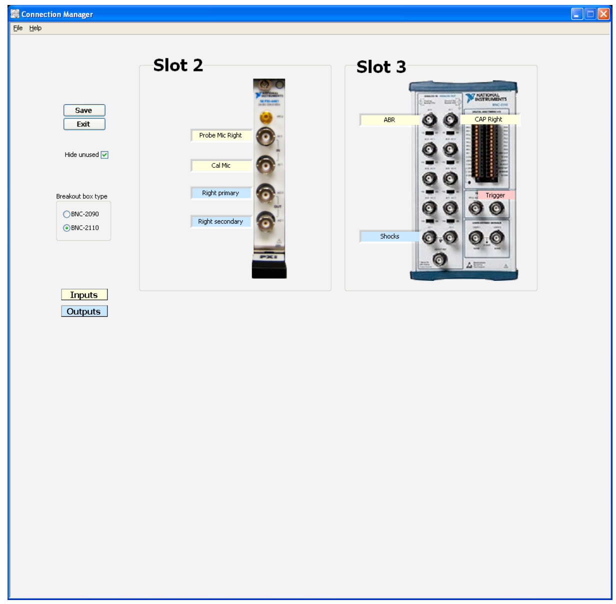


Figure 4. The Connection Manager Panel.

Figure 5 shows a closeup view of the main controls in the upper left portion of the Connection Manager panel. The functions of these controls are described in the following sections.

Specifying breakout box type

Two types of breakout box may be used with the NI 16-bit I-O boards. The BNC-2110 (Fig. 6) is a benchtop device, whereas the BNC-2090 is rack-mounted. Software performance is unaffected by breakout box type: it is entirely a matter of user preference which style to use. The "Breakout box type" control (Fig. 5) changes the Connection Manager display to show the breakout box type in use. (Note that the Connection Manager assumes all 16-bit boards use the same type.)

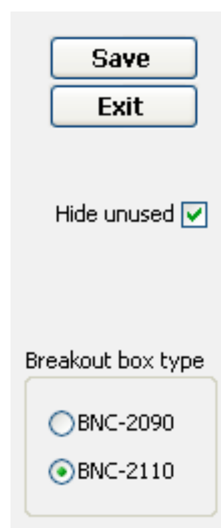


Figure 5. Closeup of upper left of Connection Manager panel.

Specifying connections

The Connection Manager shows a picture of the panel for each I-O board it finds (e.g. Fig. 4). Next to each connector on the panel is a label showing the corresponding signal. For clarity, the default behavior is to show labels only where a connection has been specified. To force labels to appear everywhere (as illustrated in Fig. 6) uncheck the “Hide unused” box on the left side of the window (Fig. 5).

Input channels are shown as light yellow and output channels as light blue.

Clicking on a label brings up a list of signals that can be connected to the corresponding channel. The example in Fig. 7 shows the list of input signals that appears when clicking on one of the yellow input labels.

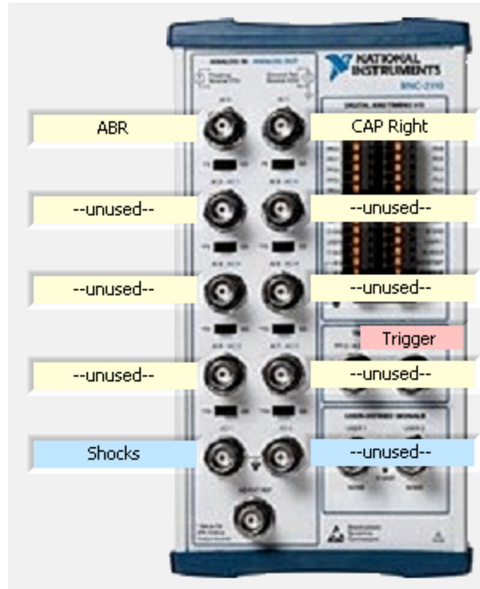


Figure 6. The Connections labels for the 16-bit board using the BNC-2110 breakout box.

How should it be connected?

Ultimately, it is entirely up to the user which signals are connected to which I-O channels. However, the CFTS will complain if a measurement is started and one or more of the inputs or outputs has not been specified. For example, attempting to measure an ABR when the “ABR” label does not appear next to any of the input channels in the Connection Manager will cause an error.

In choosing where to connect inputs, there are a couple of practical considerations. Audio signals (microphones and speakers) have large dynamic ranges and are best connected to 24-bit channels. Signals with lower bandwidths and dynamic ranges (e.g. evoked potentials) can be connected to 16-bit channels.

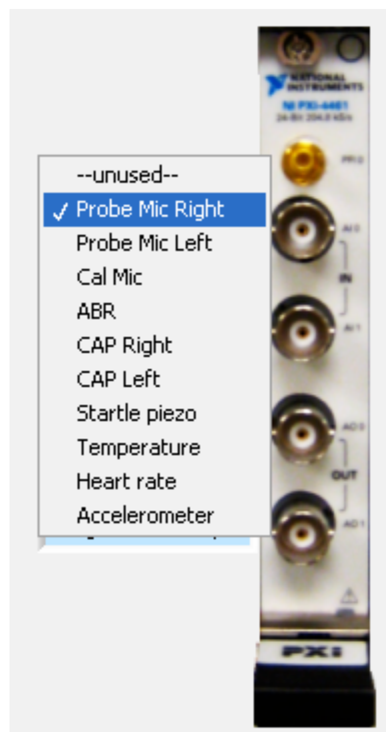


Figure 7. A popup shows input choices.

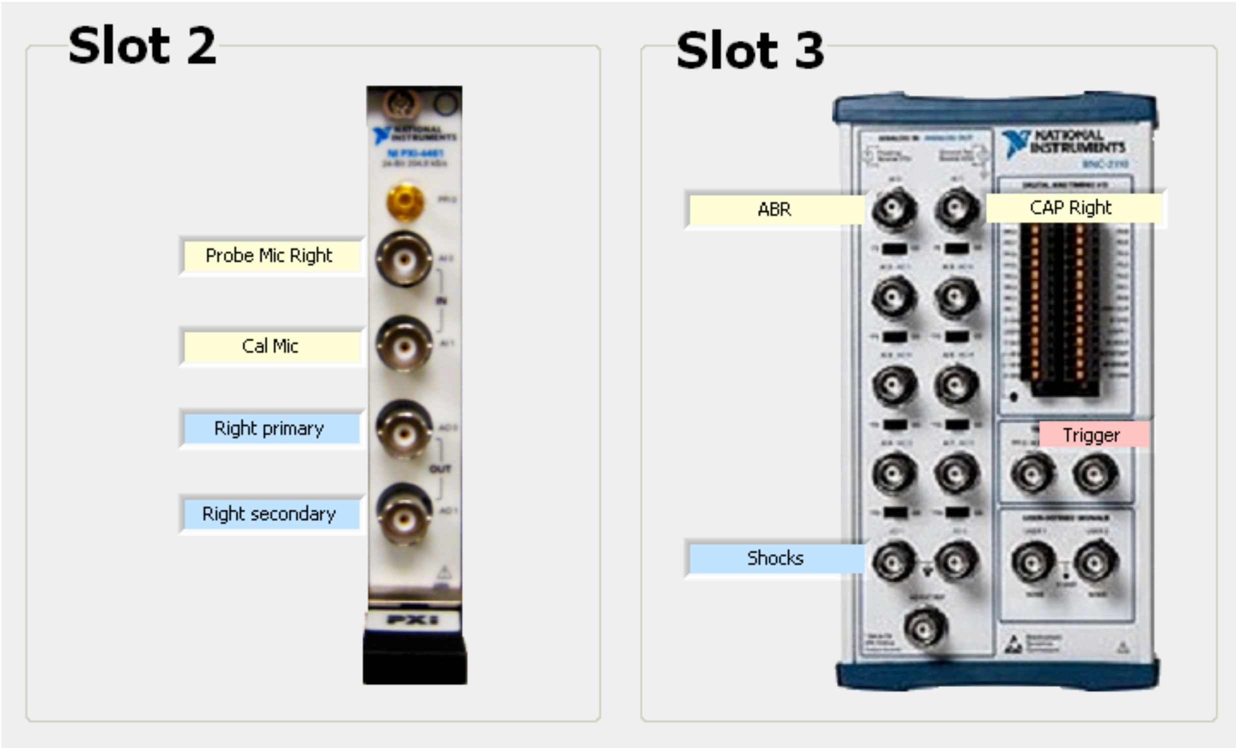


Figure 8. A typical configuration for using the CFTS in the right ear. The "Right primary" output produces the right-ear signal for the DPOAE primary tone (F1) and the sounds that evoke ABRs and CAPs . "Right secondary" produces the signal for the DPOAE secondary tone (F2). The Probe Mic, ABR and CAP inputs are used during experiments. The "Cal Mic" input is used only during calibration of the microphone + probe-tube (see Section 5).

Trigger out

The software generates a TTL pulse at the beginning of each stimulus presentation, which may be used, for example, to trigger an oscilloscope. A red-shaded box in the Connection Manager indicates the BNC which provides this pulse. For the BNC-2090 breakout box only, it is necessary to connect a jumper wire between the PFI12 and USER1 terminals on the spring terminal block on the righthand side of the device (see BNC-2090 user manual for detailed instructions).

Saving the configuration

The configuration must be saved before exiting the Connection Manager by clicking the "Save" button in the upper left (Figs. 4,5). In practice, the configuration is not changed often, so the CFTS main panel button that chooses the configuration window is hidden to avoid inadvertent changes.

4. Acoustic calibration: Microphone and probe tube sensitivity

Overview

The microphone + probe-tube calibration relates the voltage out of the microphone and the sound pressure at the end of the probe tube, i.e. near the plane of the tympanic membrane. This measurement is made by measuring the voltage out of the assembly microphone while simultaneously measuring the sound pressure near the probe-tube tip with a reference microphone. The reference microphone, a Larson-Davis ¼" microphone (#377B10), has a very flat frequency response (for LD 2530: +/- 1 dB 20-50000 Hz, +/- 3 dB 20-100000 Hz) and a sensitivity that is determined by calibration with a pistonphone.

The microphone + probe-tube calibration is performed by holding the reference microphone a short distance from the end of the probe tube using a metal coupler (described in the EPL Acoustic System Assembly Manual). The microphone calibration should not be confused with an earphone calibration in which pressure is measured in an artificial ear whose volume (or impedance) is made to look like a real ear. In a microphone calibration, the sound pressure level in the cavity (or in open air – a cavity is not needed) is not important as long as the microphones are used within their linear range and their signal-to-noise ratio is adequate. The microphone calibration uses the ratio of the output of the acoustic-assembly microphone to the output of the reference microphone, so the SPL of the sound used for the calibration cancels out.

To do the measurement, a chirp (a brief sound that contains frequencies throughout the range to be calibrated) is produced by one of the earphones and the outputs of both microphones are simultaneously measured. From the results and the characteristics of the reference microphone, the system computes the ratio of the voltage out of the acoustic-assembly microphone to the SPL at the end of the probe tube. The frequency characteristics of the microphone + probe-tube combination comes almost entirely from the probe tube and this calibration is often called the "probe-tube calibration".

Probe-tube calibration panel

This probe-tube calibration procedure is accessed from the "Advanced options" section of the CFTS main panel. The Probe Tube Calibration panel is shown below (Fig. 9). Note that while this panel is active, the CFTS main panel is locked and cannot be used.

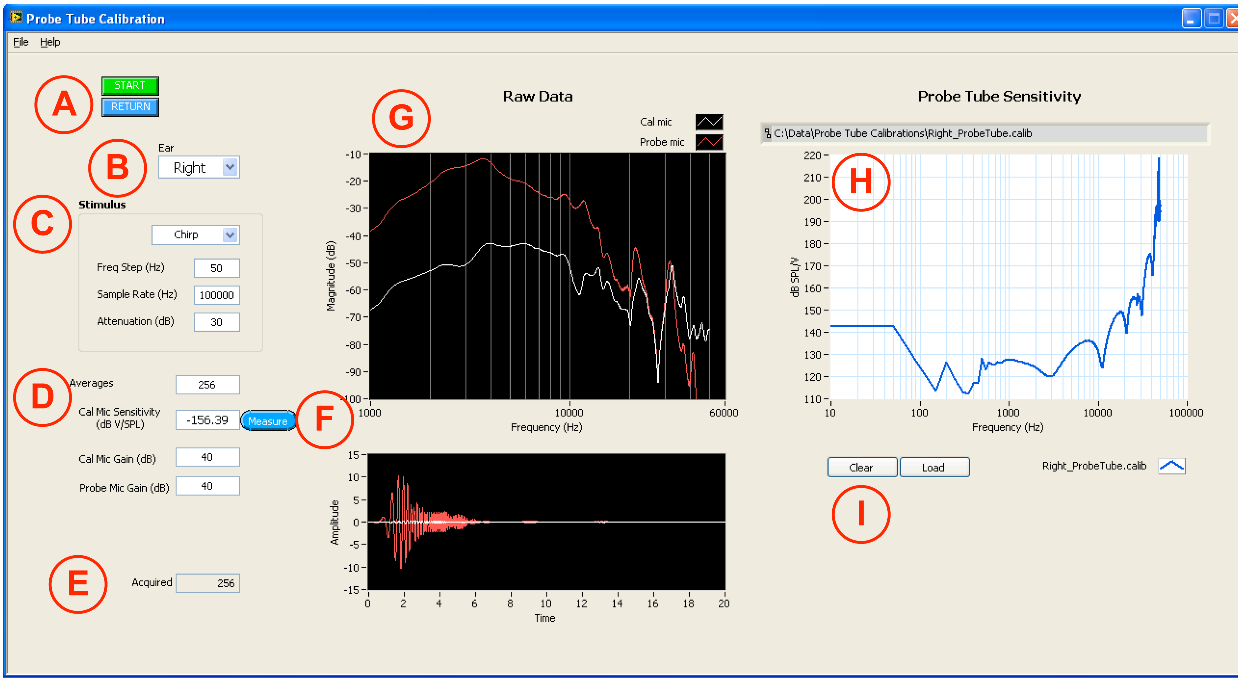


Figure 9. The Probe Tube Calibration panel.

The lettered items in Fig. 9 are:

A: Main controls. The green “Start” button begins a measurement. The blue “Return” button closes the window and returns to the CFTS main panel.

B: Ear whose acoustic system is to be calibrated.

C: Stimulus parameters (see below)

D: Response parameters (see below)

E: Displays number of averages acquired during a measurement.

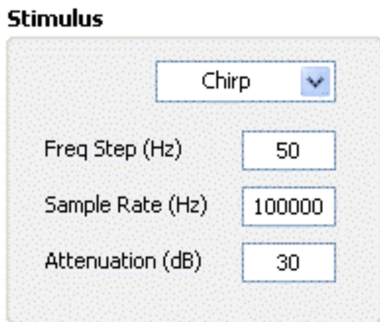
F: Opens panel to measure microphone sensitivity using a pistonphone (Section 5).

G: Running averages. Displays the average magnitude spectrum (top) and time-domain waveform (bottom) for the probe and cal microphones.

H: Probe tube calibration function (see below)

I: Buttons to “Clear” probe tube calibration display or “Load” previously measured data (useful for the purpose of comparison).

Stimulus parameters



→ Calibration stimulus waveform (always “Chirp”)

→ Frequency resolution of calibration

→ Calibration sampling rate

→ Attenuation of calibration stimulus from full scale

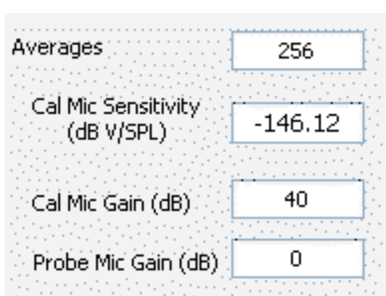
- The duration of the calibration stimulus (i.e. the chirp) is the inverse of the frequency resolution:

$$duration = \frac{1}{FreqStep(Hz)}$$

- The sampling rate must be at least twice the maximum frequency which the user intends to present to an animal during an experiment. A value 2.5 times the maximum frequency used is recommended.

- Attenuating the calibration stimulus prevents saturation of the microphone amplifiers. For the EPL acoustic systems and amplifiers, 30-40 dB attenuation is usually appropriate.

Response parameters



→ Number of averages to acquire

→ Sensitivity of calibrated microphone

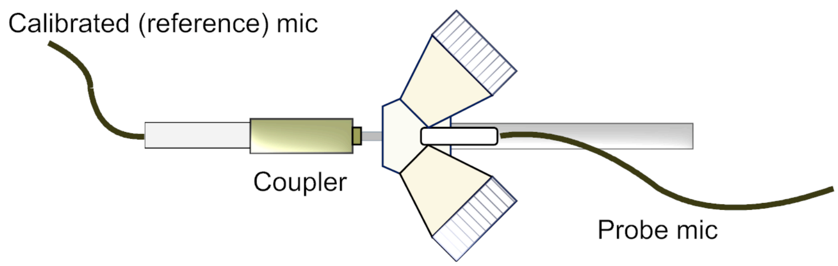
Enter gains of microphone amplifiers (see Fig. 1)

Probe tube calibration procedure

1. **Measure sensitivity of calibrated (reference) microphone**

The sensitivity of the calibrated microphone (i.e. the relationship between sound pressure level and the voltage measured by the microphone) must be accurately known. Typically, the sensitivity is measured using a pistonphone (see Section 5) immediately before performing the probe tube calibration. Alternatively, if an accurate value has been obtained by other means, that value can be entered in the “Cal Mic Sensitivity” control.

2. **Connect calibrated microphone to acoustic system**



The calibrated microphone is held in place very near to the output of the acoustic system using a coupler specially designed to prevent the acoustic system from actually contacting the diaphragm of the calibrated mic.

3. Verify parameter settings

Verify especially that the desired ear has been selected (Fig. 9B), and that the microphone gains have been entered correctly (Fig. 9D).

4. Press "Start"

Upon completion of the measurement, the probe tube calibration is automatically computed and its magnitude spectrum is displayed on the right hand side of the panel (Fig. 9H).

Data files

Data are stored in the folder

`C:\Data\Probe Tube Calibrations`

in text files whose names incorporate the acoustic system ID (see Section 2). For example,

`C:\Data\Probe Tube Calibrations\Right_ProbeTube.calib`

Existing probe tube calibrations are automatically backed up before saving a new measurement. These backups have the date and time they were obtained inserted into the file name, for example:

`C:\Data\Probe Tube Calibrations\Right_ProbeTube.16Jul10_103247.calib`

These backups may be useful for comparison purposes, but are not required and can be deleted at any time.

5. Acoustic calibration: reference microphone sensitivity

This section describes the procedure for measuring the sensitivity of the calibrated (reference) microphone used in the probe tube calibration (Section 4). The Microphone Sensitivity (Fig. 10) panel is accessed from the Probe Tube Calibration panel (Fig. 9F).

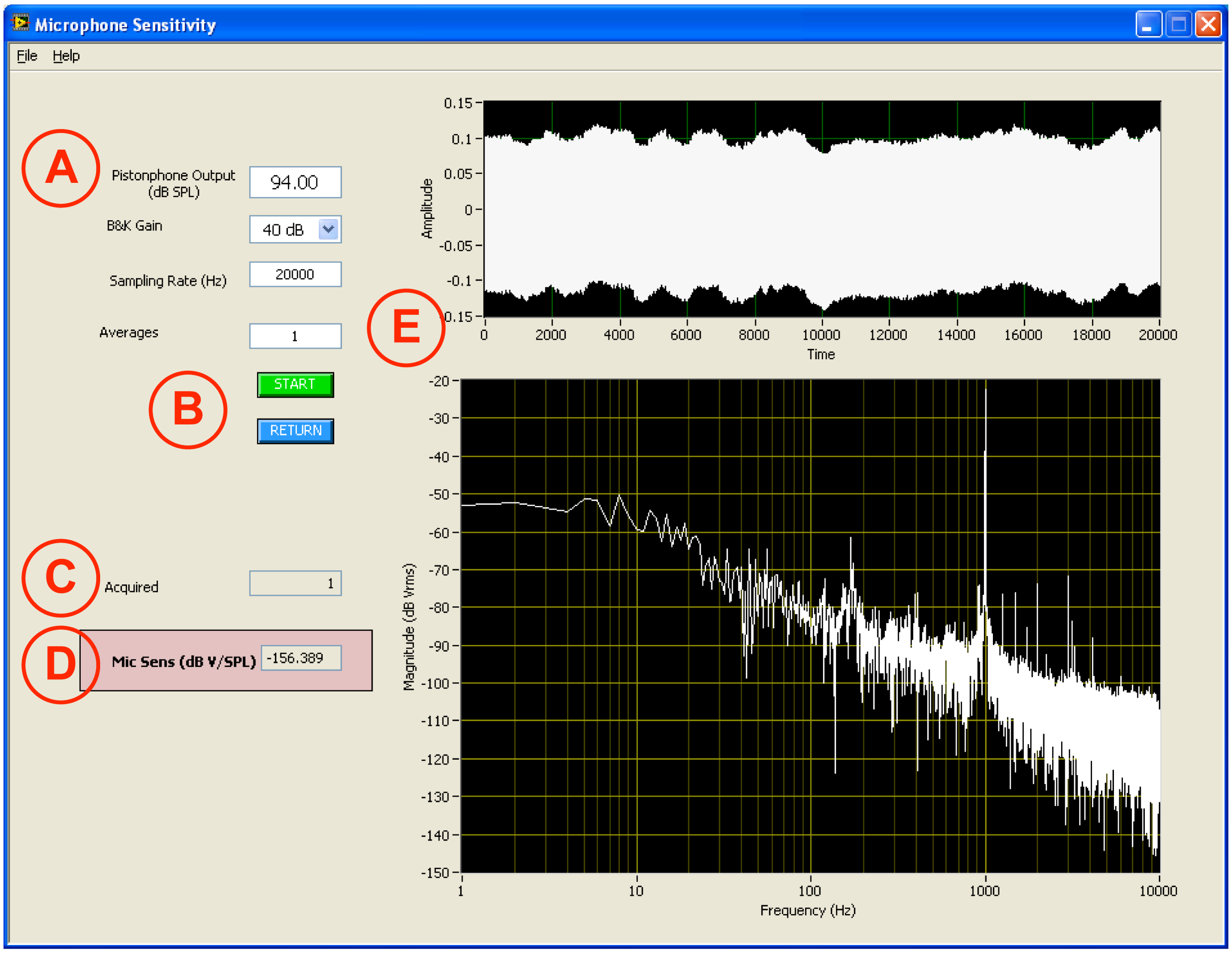


Figure 10: Microphone Sensitivity panel.

A: Measurement parameters (see below)

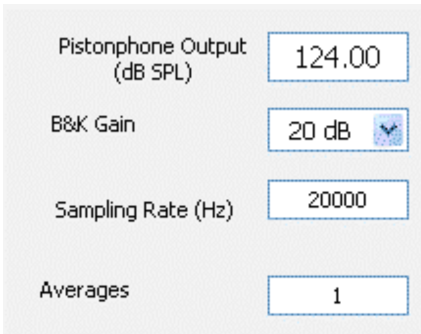
B: Main controls. The green “Start” button begins a measurement. The blue “Return” button closes the window and returns to the CFTS main panel.

C: Displays number of averages currently acquired during ongoing measurement.

D: Measured microphone sensitivity (dB V/SPL)

E: Top, time domain waveform recorded by microphone. Bottom, corresponding power spectrum.

Measurement parameters



→ Pistonphone level (dB SPL)

→ Gain of microphone amplifier (Fig. 1)

→ Calibration sampling rate

→ Number of spectrum averages to acquire

There is no need to specify the frequency of the pistonphone tone because it is automatically detected from the measured power spectrum. Since the signal/noise of the measurement is very high, it is only necessary to average one response

Sensitivity measurement procedure

1. Insert calibrated microphone into pistonphone.

2. Verify that the pistonphone is turned on.

3. Press “Start”.

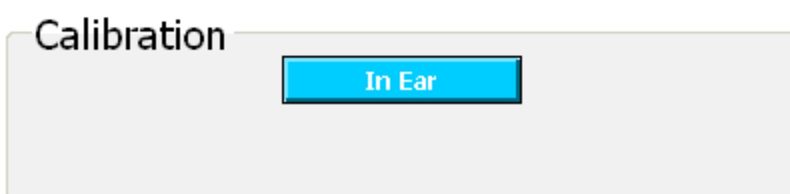
Upon completion of the measurement, the computed microphone sensitivity is displayed in the lower left (Fig. 10D). Press “Return” to close the panel and return to the Probe Tube Calibration, where the “Microphone Sensitivity” box will be updated with the just-measured value.

6. Acoustic calibration: in-ear

Overview

The in-ear calibration relates the voltage applied to the earphone to the sound pressure produced at the output of the acoustic system (near the tympanic membrane of the animal). To do the measurement, a chirp (a brief sound that contains frequencies throughout the range to be calibrated) is produced by one earphone while the voltage output of the probe tube microphone is simultaneously measured. Combining this measurement with the probe tube calibration (Section 4), the system computes the ratio of the SPL at the end of the probe tube to the voltage applied to the earphone. The procedure must be performed separately for each earphone used in the experiment.

In-ear calibration panel



The In-ear Calibration panel (Fig. 11) is accessed from the CFTS main panel. Note that while this panel is active, the CFTS main panel is locked and cannot be used.

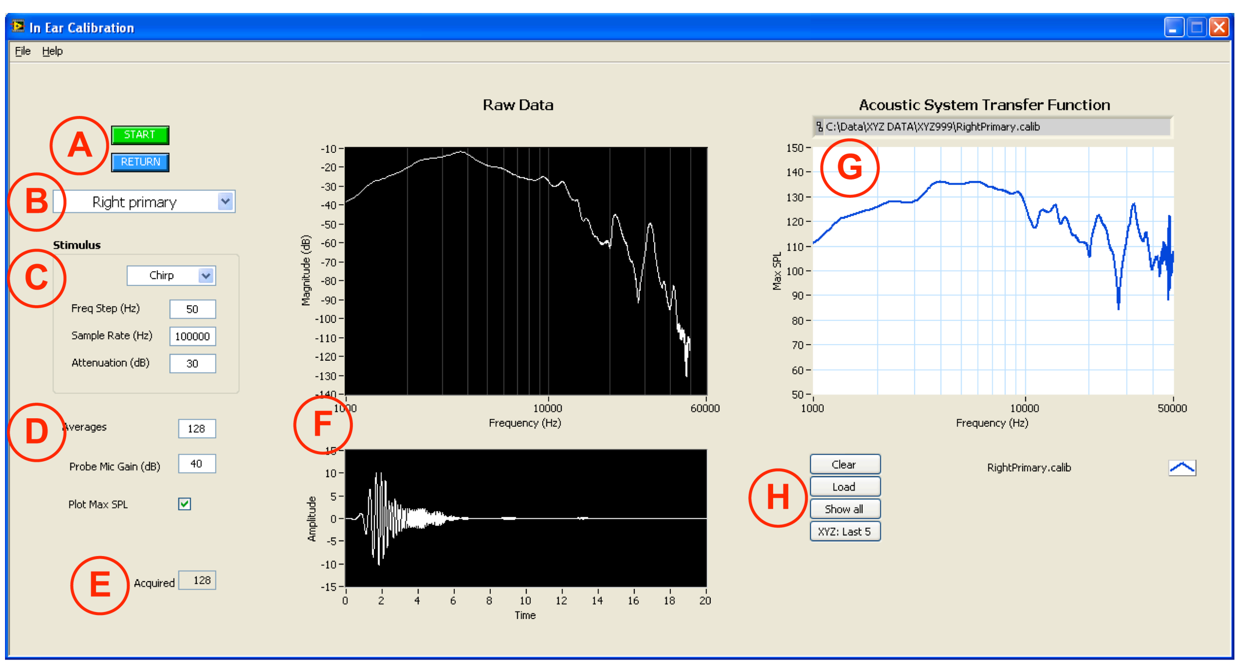


Figure 11. The In-Ear Acoustic Calibration Window.

A: Main controls. The green “Start” button begins a measurement. The blue “Return” button closes the window and returns to the CFTS main panel.

B: Earphone to be calibrated.

C: Stimulus parameters (see below)

D: Response parameters (see below)

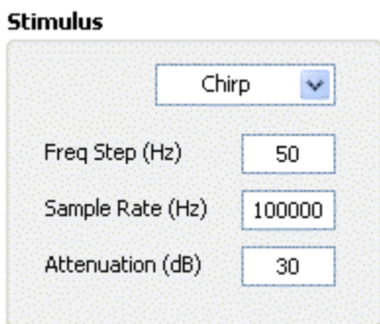
E: Displays number of averages currently acquired during an ongoing measurement.

F: Running averages. Displays the average magnitude spectrum (top) and time-domain waveform (bottom) for the probe microphone.

G: In-ear calibration (see below)

H: Buttons for manipulating in-ear calibration plot.

Stimulus parameters



→ Calibration stimulus waveform (always “Chirp”)

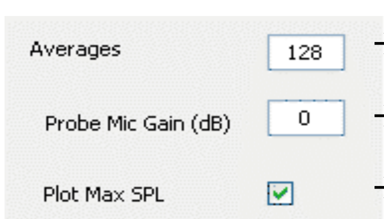
→ Frequency resolution of calibration

→ Calibration sampling rate

→ Attenuation of calibration stimulus from full scale

- The sampling rate must be at least twice the maximum frequency which the user intends to present to an animal during an experiment. A value 2.5 times the maximum frequency used is recommended.
- Attenuating the calibration stimulus prevents acoustic trauma and/or saturation of the microphone amplifiers. For the EPL acoustic systems and amplifiers, 30-40 dB attenuation is usually appropriate.

Response parameters



→ Number of averages to acquire

→ Gain of probe tube mic amplifiers (see Fig. 1)

→ Specifies how to scale the in-ear calibration plot (see below)

In-ear calibration procedure

This procedure assumes that the acoustic system has already had its probe tube calibration performed (Section 4).

1. Secure acoustic system near animal's ear canal

2. Verify that the probe mic amplifier gain is set correctly (Fig. 11D)

3. Select earphone to be calibrated (Fig. 11B)

4. Press "Start"

Upon completion of the measurement, the in-ear calibration is automatically computed and its magnitude spectrum is displayed on the right side of the panel (Fig. 11G).

5. Repeat steps 3-4 for each earphone

Plotting options

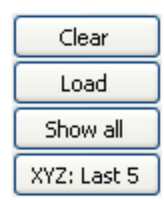
In-ear calibration magnitude scale

- If "Plot Max SPL" is checked (Fig. 11D), the in-ear calibration is scaled to show the maximum achievable SPL as a function of frequency, assuming pure tone stimulation.

- If unchecked, the in-ear calibration is plotted as a (normalized) transfer function (dB SPL/V) which can be interpreted as plotting the SPL produced by a 1-Vrms tone at each frequency. This scale provides better intuition about the sound pressure produced by broadband stimuli such as noise or clicks.

Other display options

Data shown on the in-ear calibration plot are controlled with the four buttons shown on the right (see Fig. 11H).



- "Clear" removes all accumulated data from the plot.

- "Load" brings up a dialog box allowing the user to select a calibration data file which is then added to the plot.

- "Show all" shows the in-ear calibrations (if measured) for every earphone (Right Primary, Right Secondary, Left Primary, Left Secondary) in the current data folder.

- "XYZ: Last 5" compares the last 5 in-ear calibrations performed by the current experimenter. (Note that "XYZ" is replaced with the current experimenter ID.) For example, suppose the experimenter is "XYZ", the current experiment number is 999, and “Right Primary” has just been calibrated. Pressing the “XYZ: Last 5” button will cause the system to search for Right Primary in-ear calibrations in the folders “XYZ994”, “XYZ995”, “XYZ996”, “XYZ997”, and “XYZ998”, and overplot them with the current measurement.

Data files

Data are stored as text files in the current data folder. The filename incorporates the earphone, for example

```
C:\Data\XYZ DATA\XYZ999\RightPrimary.calib
```

Existing in-ear calibrations are automatically backed up before saving a new measurement. These backups have the date and time they were obtained inserted into the file name, for example:

```
C:\Data\XYZ DATA\XYZ999\RightPrimary.16Jul10_103247.calib
```

These backups may be useful for comparison purposes, but are not required and can be deleted at any time.

7. ABR/CAP input-output functions

The ABR/CAP module of the Cochlear Function Test Suite records evoked potentials as a function of sound pressure level (SPL) in response to either tone pips or clicks.

This test is accessed from the Measurements section of the CFTS main panel by pressing the "ABR" button. The ABR/CAP I-O panel is shown below. Note that while the ABR/CAP I-O panel is active, the CFTS main panel is locked and cannot be used.

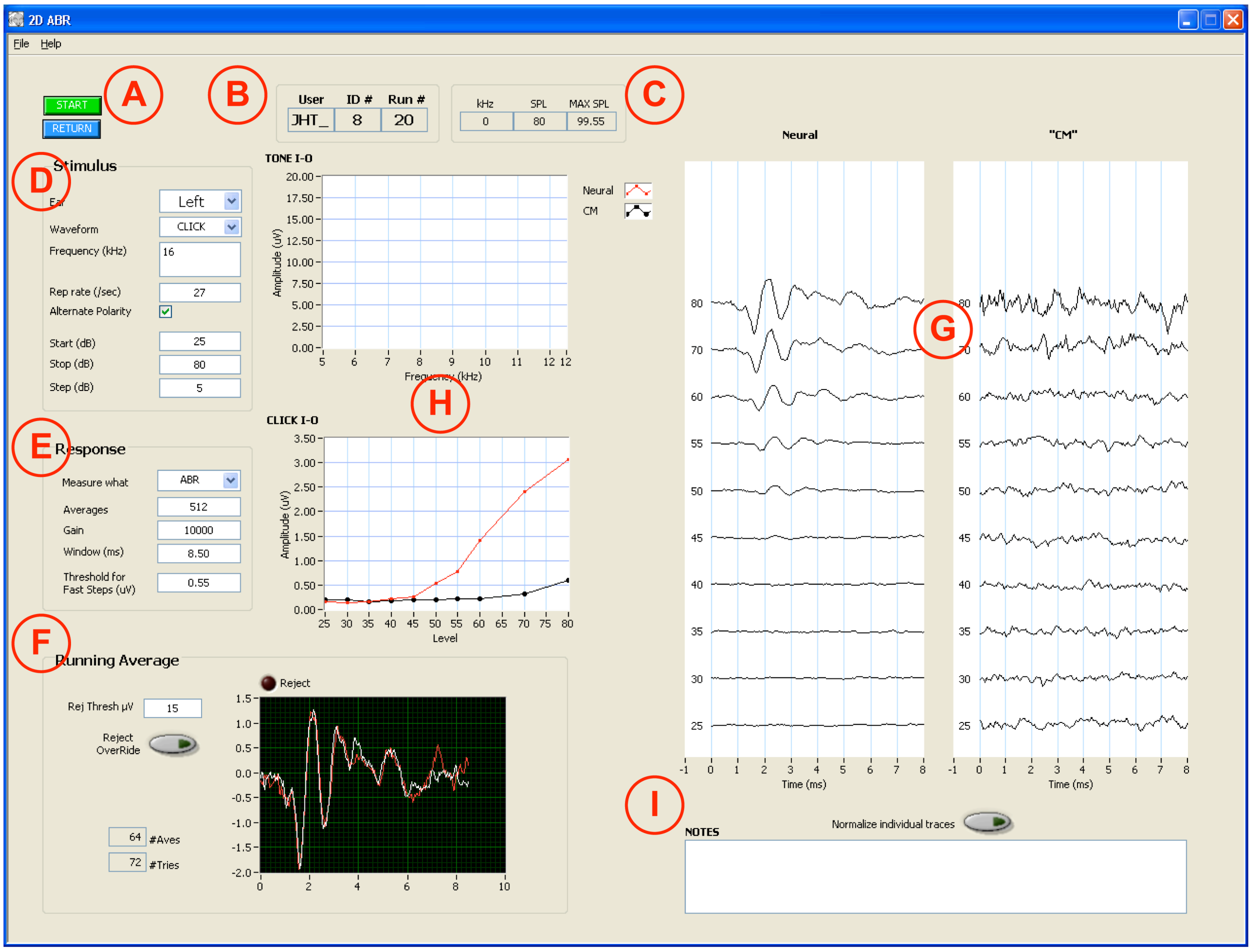


Figure 12: The ABR/CAP Controller front panel.

ABR/CAP I-O Controller

The lettered items in Fig. 12 are:

A: Main controls. The green “Start” button begins a measurement. The blue “Return” button closes the window and returns to the CFTS main panel.

B: “Bookkeeping” information. The experiment user ID and number are displayed here, along with the most recent run number.

C: Sound Pressure Level. During a measurement, the SPL of the stimulus is updated and displayed here. The maximum achievable SPL at the measurement frequency is also shown.

D: Stimulus parameters (see below)

E: Response parameters (see below)

F: Running average. Displays the average waveform for the currently-presented SPL, and provides controls for artifact rejection.

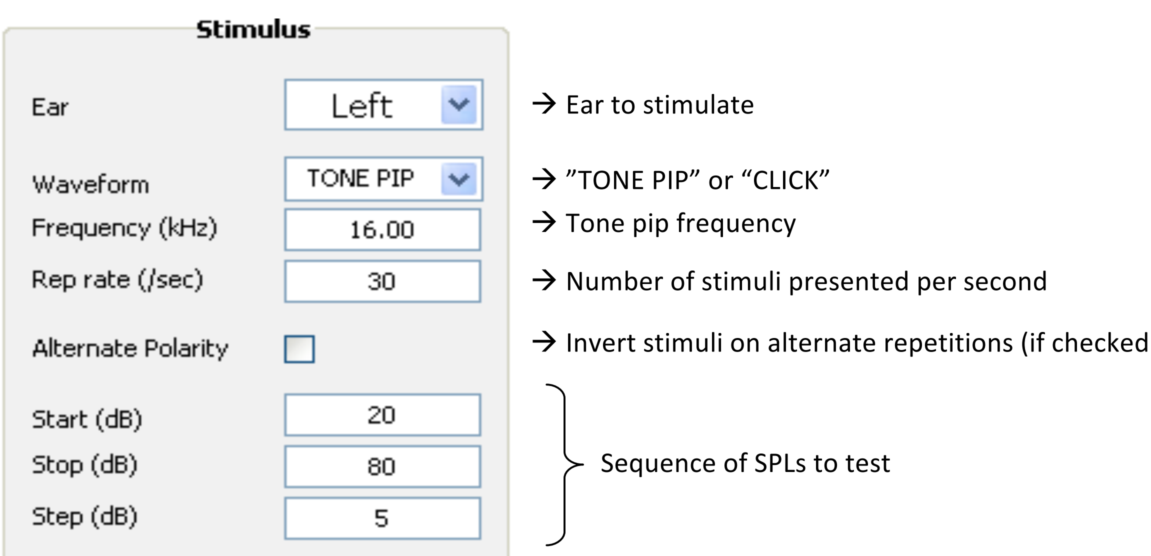
G: Stacked waveform displays (see below)

H: Displays of tone (top) or click (bottom) amplitudes as functions of sound level.

I: Experimenter’s notes. Text typed in this box is stored in the data file upon completion of the measurement. If the notes are changed after the data have been saved, the user is asked whether or not they wish to reinsert the notes into the file. The box is cleared when a new measurement is started.

Stimulus parameters

The specification of stimulus parameters is done in the panel shown below (D in Fig. 12). If the maximum SPL requested is greater than that which the acoustic system can deliver, the measurement will simply stop after reaching the maximum deliverable sound level.

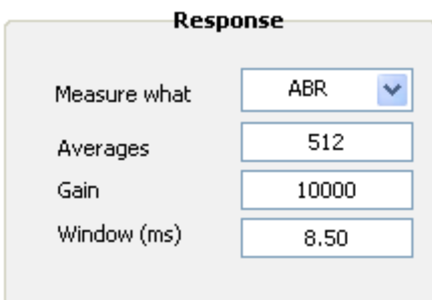


Alternating stimulus polarity

Inverting the stimulus polarity on alternate repetitions makes it possible to isolate the neural component of the evoked response because the neural component does not invert when stimulus polarity is inverted but the cochlear microphonic (CM) component does invert. The traces from alternate responses are stored in separate buffers. The traces shown in the "Neural" column are the average of the responses in the positive and negative polarity buffers. The traces in the "CM" column are the average of the responses in the positive polarity buffer minus the responses in the negative polarity buffer.

Response parameters

Selection of response parameters is shown below (E in Fig. 12). Selecting "ABR" or "CAP" ensures that the correct input source is recorded and that the data files are named appropriately. Beyond that, the ABR/CAP I-O program functions exactly the same for both measurement types.



→ "ABR" or "CAP"

→ Number of averages to acquire (*excluding* rejections)

→ Gain of amplifier (see Fig. 1)

→ Duration of response acquired on each repetition, beginning at stimulus onset

Fixed parameters

A few additional parameters have fixed values. These are shown in the following table:

Parameter	Value
Output sampling rate	100 kHz
Input sampling rate	20 kHz
Tone pip duration	5 ms
Tone pip ramp length	0.5 ms

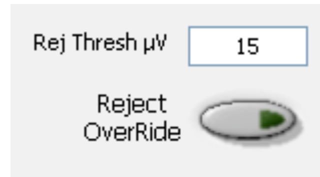
Running a measurement

Start a measurement by pressing the green "Start" button in the upper left (Fig. 12A). The "Start" button disappears while the measurement is being made and reappears when it is finished. The stimulus and response parameters cannot be changed once the measurement has begun.

The black-background grid in the lower-left of the window (Fig. 12F) shows the Running Average which resets for each SPL in the sequence. Two traces are shown, the red one corresponds to the odd-numbered repetitions, and the green one to the even-numbered repetitions. When

"Alternate polarity" has been selected, these are the responses to positive and negative polarity stimuli, respectively.

Artifact rejection



The running average section of the display contains controls for rejecting artifacts (e.g. electro-cardiogram (ecg) or myogenic potentials). Responses whose peak-to-peak amplitudes exceed the specified threshold are rejected. The software can be forced to accept all waveforms by turning on the "Reject OverRide" button. Both of these controls can be changed during the acquisition of an average.

Stopping a measurement

A measurement can be stopped at any time by pressing the red "Stop" button that appears in place of the Start button. When a measurement is stopped, the user is asked whether or not to save the data.

Online analysis

The stacked waveform and I-O function graphs (Fig. 12G, H) are updated as each SPL in the sequence is acquired. The stacked waveforms are divided into "Neural" and "CM" responses as described in "Alternating stimulus polarity".

The I-O function graphs (Fig. 12H) plot evoked potential amplitude (maximum absolute deviation from the mean) as a function of SPL. "Neural" and "CM" responses are quantified separately. Tone-evoked and click-evoked responses are shown in separate graphs. The data in each graph are retained until another measurement using that stimulus type is performed.

Data files and run numbers

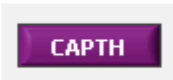
Data are stored in text files whose names incorporate the measurement type (i.e. "ABR" or "CAP"), the experiment number, and the run number. For example,

```
C:\Data\XYZ Data\XYZ999\ABR-999-14
```

where the hypothetical run number is 14. Note that the data file has no extension. Independent run numbers are kept for ABR and CAP measurements.

8. CAP Audiogram

The CAP Audiogram program measures CAP thresholds as a function of pure tone frequency.



The CAP Tuning Curve control panel is opened from the Measurements section of CFTS main panel using the button labeled “CAPTH”. Note that the CFTS main panel is locked and cannot be used while the CAP Tuning Curve panel is open.

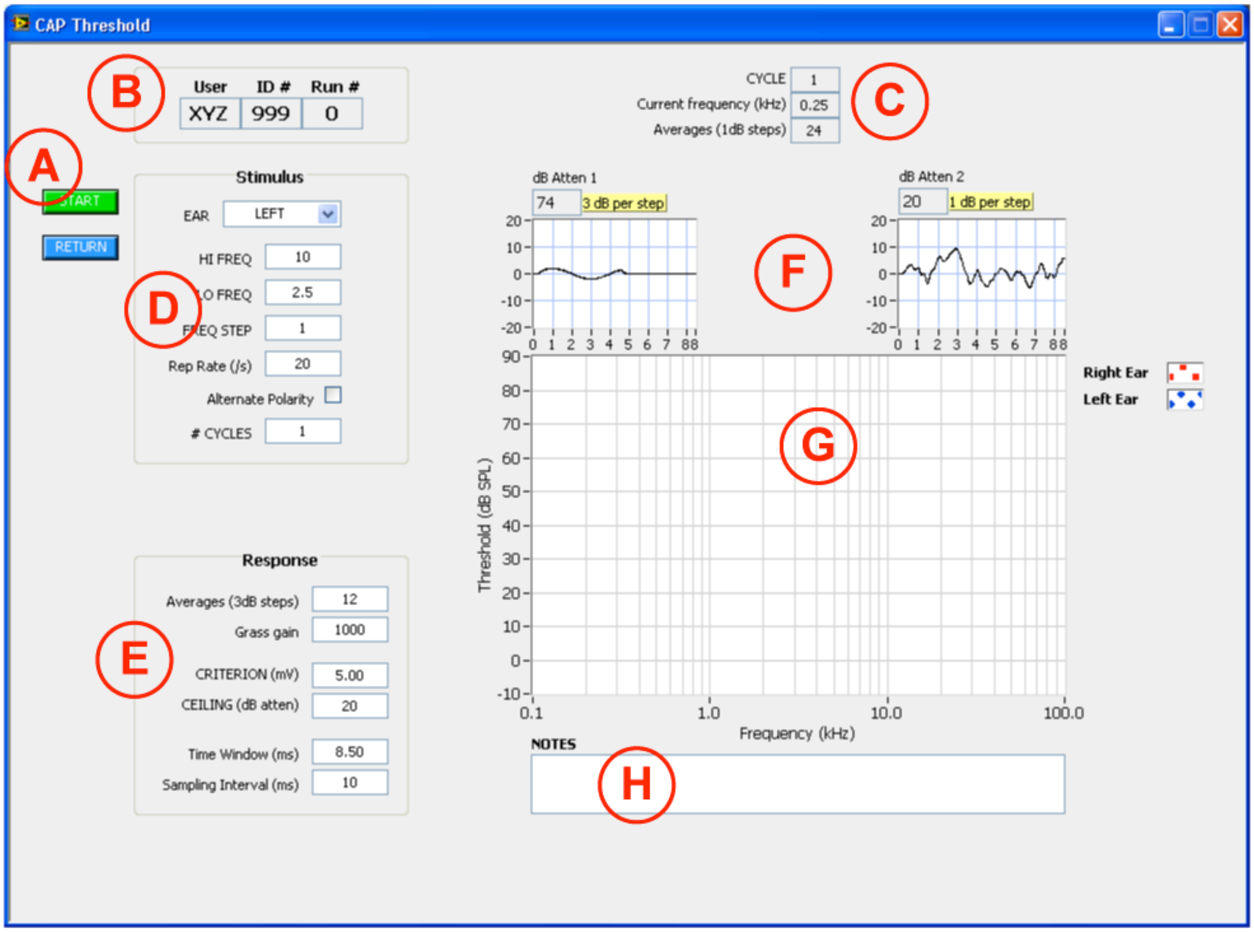


Figure 13: The CAP Threshold front panel.

A: Main controls. The green “Start” button begins a measurement. The blue “Return” button closes the window and returns to the CFTS main panel.

B: “Bookkeeping” information. The experiment user ID and number are displayed here, along with the most recent run number.

C: Measurement status. “CYCLE” displays the current number of criterion crosses (see *How does it work?)* “Current frequency” is the tone frequency for which threshold is currently being measured. “Averages (1dB steps)” displays the number of averages that are acquired for the fine resolution portion of the procedure (see *How does it work?)*

D: Stimulus parameters (see below)

E: Response parameters (see below)

F: CAP waveform averages (see Online Analysis below)

G: CAP tuning curve display

H: Experimenter's notes. Text typed in this box is stored in the data file upon completion of the measurement. If the notes are changed after the data have been saved, the user is asked whether or not they wish to reinsert the notes into the file. The box is cleared when a new measurement is started.

How does it work?

In order to understand the function of the stimulus and response parameters, it is useful to consider the basics of how a CAP tuning curve is measured. This is schematized in Fig. 14.

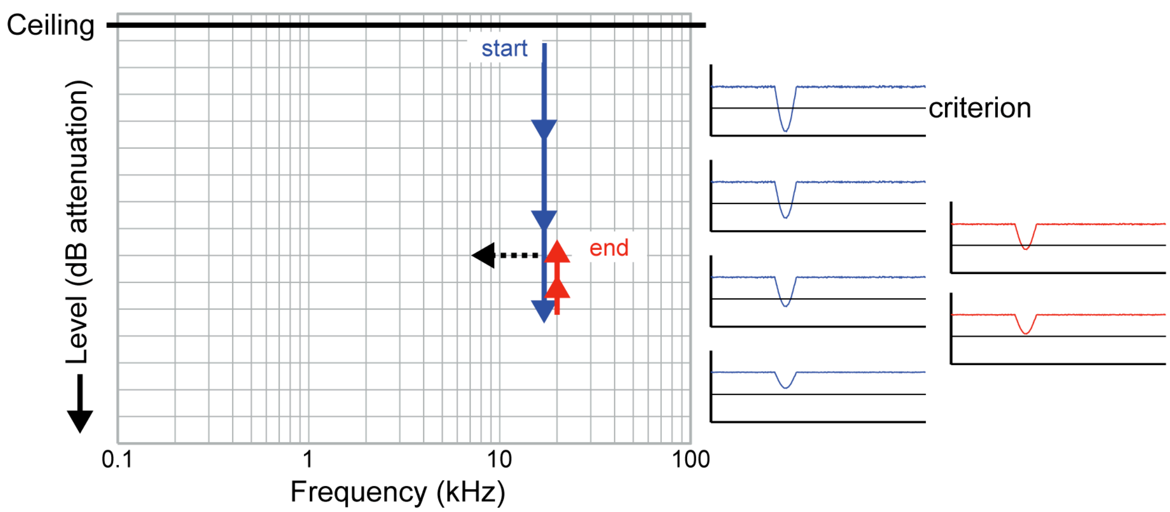
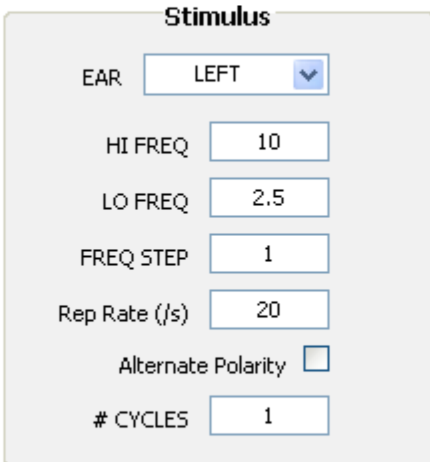


Figure 14: Cartoon showing how the CAP threshold seeking program works (see text).

The tuning curve begins by measuring threshold at the highest frequency specified (~20 kHz in the example). CAP averages are measured repeatedly, with the sound level decreased in 3-dB steps after each average (blue arrows on graph and corresponding blue CAP waveform traces at right) until the CAP amplitude drops below a **criterion** value. After that, the sound level is increased in 1-dB steps (red arrows and waveform traces – offset for clarity) until the CAP amplitude again crosses the criterion line (i.e. is greater than the criterion). This second crossing of the criterion determines the threshold. Note that the frequency doesn't change while obtaining a threshold. This threshold is used as the starting point in finding the threshold at the next lower frequency (black dotted line). To avoid causing damage, the sound level is never allowed to become higher than the value indicated by "**Ceiling**" which is specified in attenuation from the maximum acoustic output at that frequency.

Stimulus parameters

When “BOTH” ears are selected, the threshold is measured in the right ear and then the left ear at each frequency (i.e. the two tuning curve measurements are *interleaved*).



→ “LEFT”, “RIGHT” or “BOTH”

Sequence of frequencies (kHz) on which to measure the tuning curve. FREQ STEP is in octaves.

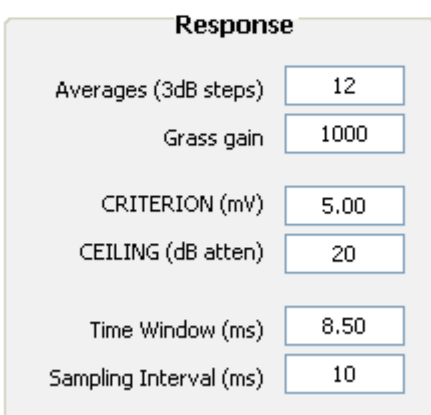
→ Number of stimuli presented per second

→ Invert stimuli on alternate repetitions (normally checked).

→ # of crosses of criterion line.

Response parameters

The number of averages at each 1-dB step of the threshold seeking procedure is twice the number of averages at each 3-dB step.
The CRITERION is an absolute value; the polarity of the response does not matter.



→ Number of averages in CAP at each 3-dB level step

→ Gain of CAP amplifier (See Fig. 1)

→ CAP magnitude at threshold (See *How does it work?)*

→ Minimum stimulus attenuation (See *How does it work?)*

→ Length of response acquired on each repetition

→ CAP input sampling rate

Fixed parameters

A few additional parameters have fixed values. These are shown in the following table (the Sampling Rate is set by the Sampling Interval specified above):

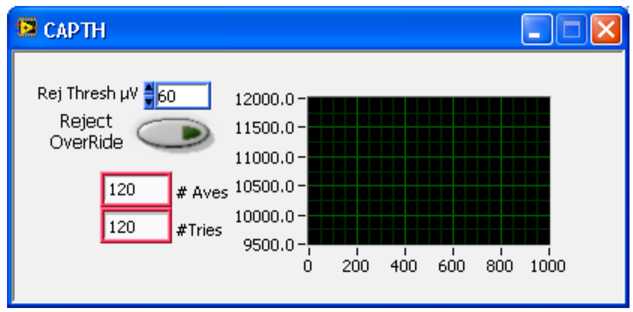
Parameter	**Value**
Output sampling rate	= Input sampling rate
Sampling rate	xxx kHz
Tone pip duration	5 ms

Tone pip ramp length	0.5 ms

Running a measurement

Start a measurement by pressing the green "Start" button in the upper left (Fig. 13A). The stimulus and response parameters cannot be adjusted once the measurement has begun.

A dialog box appears that shows the running CAP average and provides artifact rejection controls. The running average resets for each level in the tracking procedure. Two traces are shown, one corresponding to the odd-numbered repetitions, and the other to the even-numbered repetitions. When "Alternate polarity" has been selected these correspond to positive and negative polarity stimuli, respectively.



Artifact rejection

The running average display contains controls for rejecting artifacts (e.g. large responses due to ECG or myogenic potentials). Responses whose peak-to-peak amplitudes exceed the specified threshold are rejected. The software can be forced to accept all waveforms by turning on the "Reject OverRide" button. Both of these controls can be changed during an average.

Stopping a measurement

A measurement can be stopped at any time by pressing the red "Stop" button that appears in place of the Start button (Fig. 13A). When the measurement is stopped, the user is asked whether or not to save the data.

Online analysis

The online analysis produces the main tuning curve display and two different CAP waveform traces. The waveform trace on the left (Fig. 13F) is the most recent CAP acquired during the 3-dB segment of the tracking procedure. The corresponding level (in dB attenuation) is shown above the waveform. Similarly, the waveform on the right displays the most recent CAP for the 1-dB segment of the procedure.

The tuning curve displayed on the main graph (Fig. 13G) is the threshold found at each frequency. Although the tracking is performed by varying the level in dB attenuation, the threshold level is plotted in the more useful "dB SPL".

Data files and run numbers

Data are stored in text files whose names incorporate the experiment number, and the run number. For example, C:\Data\XYZ Data\XYZ999\CAP-999-15

where the hypothetical run number is 15. The data file has no extension.

9. DPOAE input-output functions

The DPOAE input-output (I-O) test presents tones at frequencies F1 (the primary tone) and F2 (the secondary tone) and measures distortion product otoacoustic emissions (DPOAEs) at the frequency 2F1-F2. The program runs DPOAE level functions at multiple frequencies from which DPOAE "tuning curves" can be obtained.

The DPOAE I-O program is opened from the Measurements section of CFTS main panel using the button labeled "DP I/O". Note that the CFTS main panel is locked and cannot be used while the DPOAE I-O panel is open.

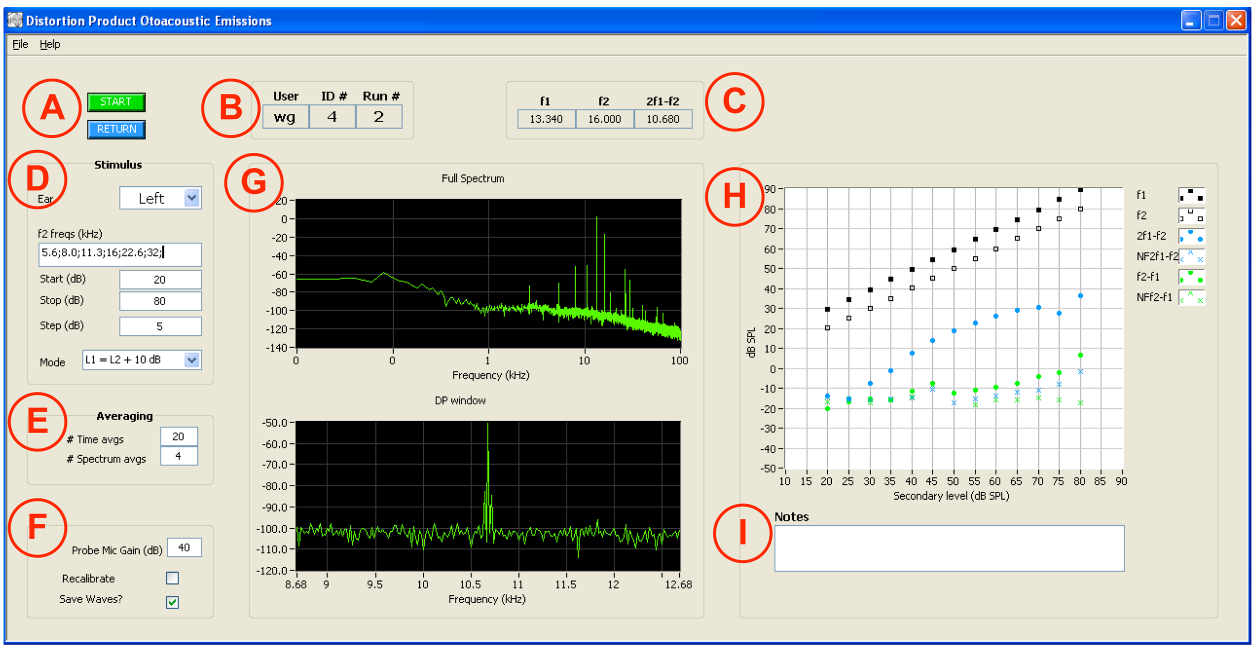


Figure 15: The DPOAE controller front panel.

A: Main controls. The green "Start" button begins a measurement. The blue "Return" button closes the window and returns to the CFTS main panel.

B: "Bookkeeping" information. The experiment user ID and number are displayed here, along with the most recent run number.

C: Measurement status. Displays values of current primary and secondary frequencies (F1 and F2), as well as their combination 2F1-F2.

D: Stimulus parameters (see below)

E: Averaging parameters (see below)

F: Other options (see below)

G: Spectrum display. The top graph shows the full spectrum. The bottom shows a small range centered on the 2F1-F2 frequency.

H: Displays the measured SPL of F1, F2, 2F1-F2, 2F1-F2 noise floor, F2-F1, and F2-F1 noise floor as functions of the F2 sound level.

I: Experimenter's notes. Text typed in this box is stored in the data file upon completion of the measurement. If the notes are changed after the data have been saved, the user is asked whether or not they wish to reinsert the notes into the file. The box is cleared when a new measurement is started.

Stimulus parameters

→ Ear to stimulate

→ List of *secondary* tone frequencies (F2's), separated by semicolons. The last semicolon is optional.

Sequence of *secondary* SPLs to test

→ Primary level: "L1 = L2" or "L1 = L2 + 10 dB"

The *primary* tone frequency is determined by the secondary frequency:

$$f_1 = \frac{f_2}{1.2}$$

The frequency values are coerced so that an integer number of periods of the primary and secondary tones fit into one 50-ms sampling window (see *Averaging parameters*). Frequency values are thus constrained to be integer multiples of 20 Hz.

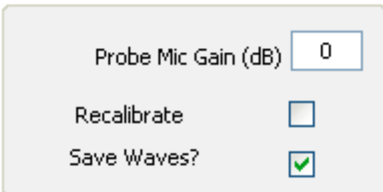
A complete level series is measured for one frequency at a time. At each frequency step, if the maximum SPL specified is greater than that which the acoustic system can deliver, the measurement will simply stop after reaching the "ceiling", then continue on to the next frequency in the list.

Averaging parameters

For each frequency and level in the test, the DPOAE is measured by averaging a number of 50-ms probe microphone waveform samples. This averaging is broken up into the time- and frequency-domains.

In the example shown, 32 waveform samples are acquired divided into 4 groups of 8. For each group, the (time-domain) average of 8 waveforms is obtained and from each waveform average a frequency spectrum (FFT) is computed. The frequency spectra of the 4 groups are averaged to obtain the final DPOAE spectrum. Increasing the number of time averages increases the signal-to-noise ratio (by the square-root of N) but produces traces with large variances. Increasing the number of spectral averages does not improve the S/N ratio but reduces the variance of the result.

Other options



→ Gain of the amplifier connected to the probe microphone (Fig. 1)

→ Recalibrate the acoustic system before each frequency step

→ Save DPOAE waveforms to disk

If the “Recalibrate” option is checked, the maximum SPL is quickly remeasured for just the primary and secondary frequencies, immediately before beginning each level series. This is particularly useful when the coupling between the acoustic system and the ear canal is not well-sealed, and the calibration drifts with time. Nothing is displayed during this quick recalibration.

Fixed parameters

A few additional parameters have fixed values. These are shown in the following table:

Parameter	**Value**
Output sampling rate	200 kHz
Input sampling rate	200 kHz
Response window	50 ms
f1	f2 / 1.2

Running a measurement

Start a measurement by pressing the green “Start” button in the upper left (Fig. 15A). The stimulus and response parameters cannot be adjusted once the measurement has begun.

Stopping a measurement

A measurement can be stopped at any time by pressing the red “Stop” button that appears in place of the Start button. When a measurement is stopped, the user is asked whether or not to save the data.

Online analysis

The magnitude spectra (Fig. 15G) are refreshed as the response to each F2/L2 combination is acquired. The spectrum in the bottom “DP window” is identical to that in the top “Full spectrum” window, but scaled to highlight the 2F2-F1 component.

The input-output function (Fig. 15H) displays the measured SPL of F1, F2, 2F1-F2, 2F1-F2 noise floor, F2-F1, and F2-F1 noise floor as functions of the F2 sound level. The plot is cleared each time F2 changes (beginning a new L2 series).

The 2F1-F2 and F2-F1 noise floors are computed by averaging the measured SPLs for the two frequencies on either side of 2F1-F2 and F2-F1, respectively.

Offline analysis

Upon completion of a measurement, the data are automatically displayed as a “DP audiogram” using the DP OAE offline analysis program (Section 10).

Data files and run numbers

Data are stored in text files whose names incorporate the experiment number, and the run number. For example,

```
C:\Data\XYZ Data\XYZ999\DP-999-15
```

where the hypothetical run number is 15. The data file has no extension.

10. DPOAE Offline Analysis

The DPOAE Offline Analysis computes DPOAE isoresponse contours (or “audiograms”) from DPOAE input-output data (Section 9). The DPOAE Offline Analysis (Fig. 16) is accessed using the “Analysis” button the CFTS main panel. It also runs automatically upon completion of a DPOAE I-O measurement.

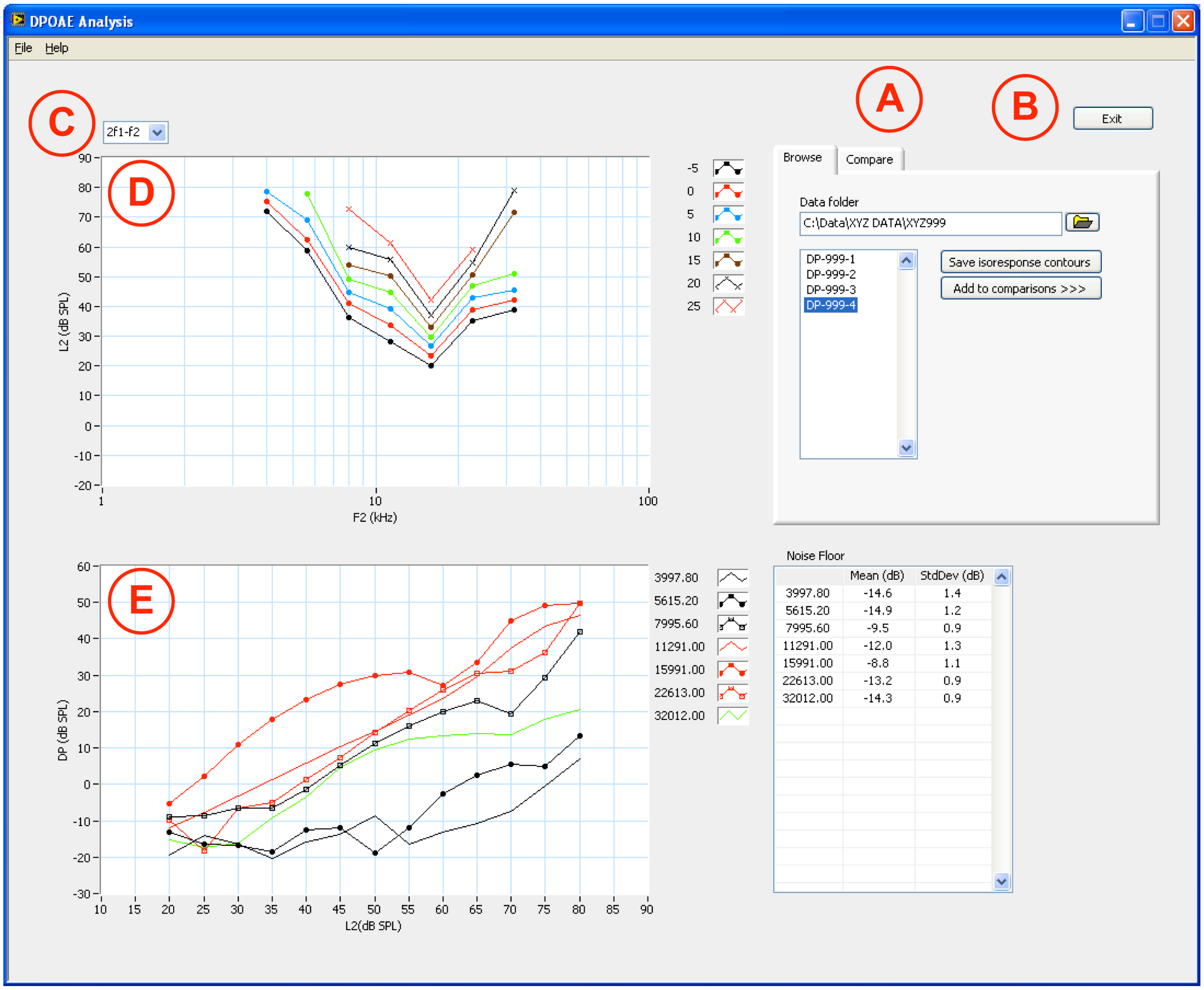


Figure 16: DPOAE Offline Analysis panel

A: Tabs for browsing data folders and comparing data files (see below).

B: Exit button

C: Distortion product to be analyzed (either 2F1-F2 or F2-F1)

D: Isoresponse contours (audiograms)

E: Input-output functions

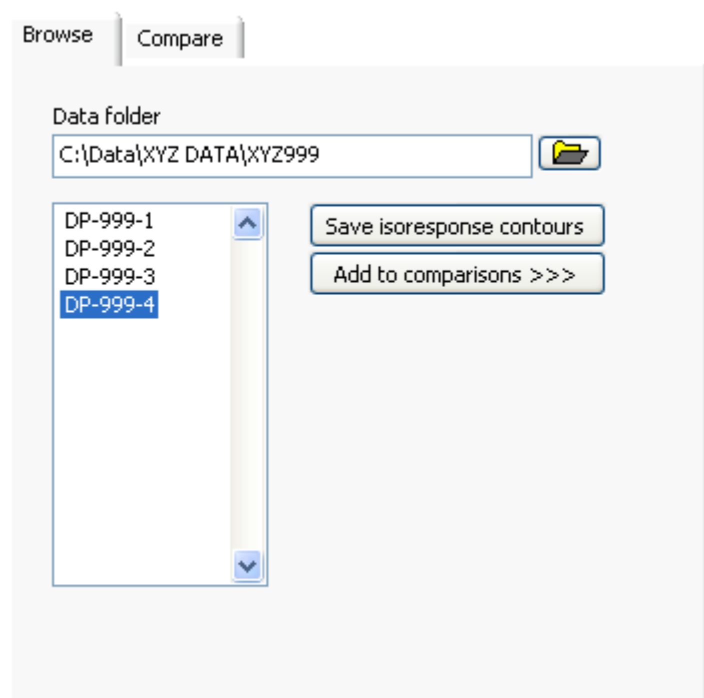
Browse data

The figure below shows a closer view of the upper right of the Offline Analysis panel (Fig. 16A), with the "Browse" tab selected. Select a data folder by typing its name in the box, or by pressing the "open folder" icon to choose a folder using the standard dialog box. Once a folder is selected, the DPOAE data files contained in that folder are listed as shown. Click on a file name to display it.

When the Offline Analysis program is invoked automatically upon completion of a DPOAE input-output measurement, the data folder is automatically set to the current data folder, and the most recent measurement is automatically selected.

The function of the "Save isoreponse contours" button is described below under *DP isoresponse contours (audiograms)*.

The function of the "Add to comparisons >>>" button is described below under *Compare data*.



DPOAE I-O functions

The DPOAE input-output display (Fig. 16E) plots the measured SPL of the distortion product as a function of the F2 level. Select the desired distortion product frequency (2F1-F2 or F2-F1) using the drop-down menu in the top left of the panel (Fig. 16C). An input-output function is shown for each value of F2, listed on the legend immediately to the right.

The table to the far right lists the mean and standard deviation of the noise floor for each input-output function. (For a given F2, the mean and standard deviation are taken across L2.)

DPOAE isoresponse contours (audiograms)

The DPOAE isoreponse contours (Fig. 16D) are computed by interpolating each input-output function to find the value of L2 that produces a criterion DP magnitude (e.g. -5 dB SPL). These L2 values are then plotted as a function of F2. A family of such isoreponse contours is created by varying the response criterion, indicated in the legend to the right.

The family of isoreponse contours can be saved to a text file by pressing the “Save isoresponse contours” button in the Browse tab (Fig. 16A). The file name will be the same as the original DPOAE I-O file name, but with “DP” replaced by “IsoDP”. For example, the isoresponse contours for

`C:\Data\XYZ Data\XYZ999\DP-999-15`

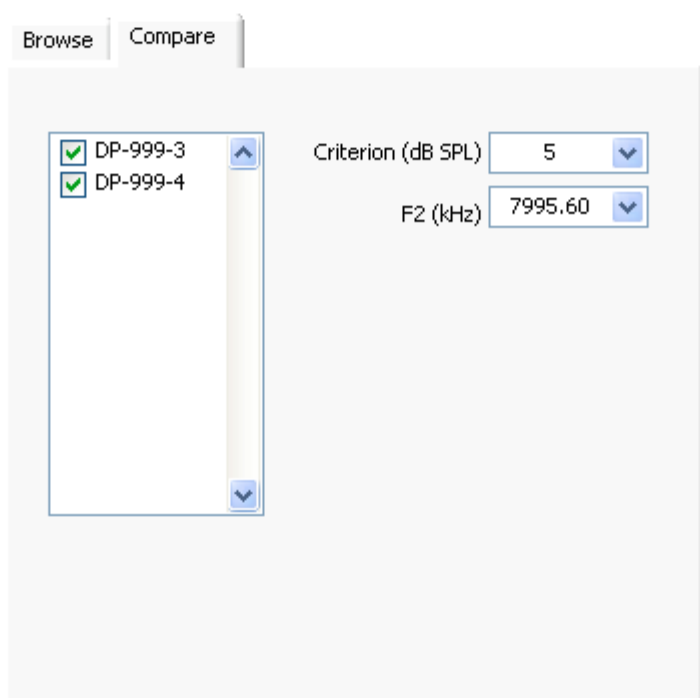
are saved as

`C:\Data\XYZ Data\XYZ999\IsoDP-999-15`

A message appears confirming that the data have been saved.

Compare data

It is sometimes useful to compare DPOAE measurements, either across animals or over time for a single animal. The “Compare” tab (Fig. 16A) facilitates such comparisons. A closer view of the



“Compare” tab is shown below.

The left side of the tab contains a list of data files to compare. Pressing the “Add to comparisons >>>” button in the “Browse” tab adds the currently selected file to the comparison list and automatically brings the “Compare” tab to the front.

Only data files having a check mark next to the name are plotted in the graphs (Fig. 16D,E). The check mark is toggled on and off by double-clicking on the file name. Files can be permanently removed from the list by clicking on a name to select it, then pressing the “Delete” key.

When the “Compare” tab is active, DPOAE input-output functions (Fig. 16E) are compared across data files for *one* value of F2 and for *one* response criterion. The value of F2 is selected using the drop-down menu on the right side of the “Compare” tab. The response criterion is selected using the drop-down menu on the right side of the “Compare” tab. Data file names are indicated in the legend to the right of the plot.

Appendix: Calibration details

This appendix gives a brief description of the acoustic calibration procedures in mathematical terms. Figure 17 is a schematic of the acoustic assembly which indicates the important voltages and sound pressures. These quantities are most conveniently expressed as complex-valued frequency-domain functions (in practice, these are the FFTs of the time-domain signals recorded at those points).

For simplicity, only one earphone speaker is shown (and represented mathematically by the generic subscript S). In practice, of course, this may be either of the two earphones ("primary" or "secondary") attached to the acoustic assembly.

A voltage $V_S(f)$ is applied to the earphone speaker producing a sound pressure $SPL_{PT}(f)$ at the output of the acoustic assembly (i.e. at the opening of the probe tube, PT). The sound travels down the probe tube (distorting its spectrum) and impinges on the probe tube microphone, producing the voltage $V_{PTM}(f)$. When performing a probe tube calibration (Section 4), the sound pressure at the probe tube opening is simultaneously measured using a second microphone, called the reference or "calibrated" microphone. The output voltage of this microphone is labeled $V_{CM}(f)$.

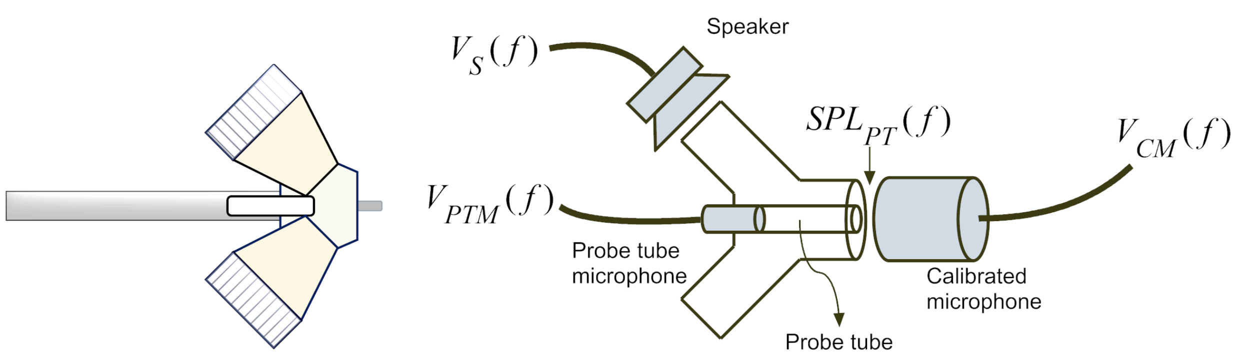


Figure 17: Schematic of acoustic assembly showing variables relavent to acoustic calibration procedures. For clarity only one earphone speaker is shown. See text for detailed description.

Big picture

The ultimate goal is to know the relationship between the voltage applied to the speaker and the resulting sound pressure at the output of the acoustic assembly, when the acoustic assembly is in place near the ear canal of an experimental animal. This relationship is the in-ear calibration

$$Cal_{InEar}(f) = \frac{SPL_{PT}(f)}{V_S(f)} \tag{A1}$$

The in-ear calibration is measured through the probe tube using the probe tube microphone, and thus it necessary to first measure the (frequency-dependent) sensitivity of the probe tube and probe tube microphone system (Section 4).

Probe tube calibration

The probe tube calibration relates the pressure at the probe tube opening to the voltage recorded on the probe tube microphone

$$Cal_{PT}(f) = \frac{SPL_{PT}(f)}{V_{PTM}(f)} \tag{A2}$$

To measure the probe tube calibration, a reference or "calibrated" microphone is positioned at the output of the acoustic assembly. This microphone is termed the "calibrated" microphone because its sensitivity, $Sens_{CM}$, is measured directly using a pistonphone (Section 5). This sensitivity is assumed to be independent of frequency and a scalar quantity. The CFTS convention is to express the calibrated microphone sensitivity with dimensions of V/SPL.

A chirp (a brief sound that contains frequencies throughout the range to be calibrated) is produced by one of the earphones and the outputs of both microphones are simultaneously measured. The SPL at the probe tube opening can be computed from the calibrated microphone voltage and sensitivity

$$SPL_{PT}(f) = \frac{V_{CM}(f)}{Sens_{CM}} \tag{A3}$$

Substituting A3 into A2

$$Cal_{PT}(f) = \frac{V_{CM}(f)}{V_{PTM}(f)} \cdot \frac{1}{Sens_{CM}} \tag{A4}$$

This is the computation performed by the Probe Tube Calibration program (Section 4). Note that the actual SPL produced at the probe tube opening by the chirp stimulus factors out of the computation.

In-ear calibration

To measure the in-ear calibration (Section 6), a chirp stimulus is produced by the earphone to be calibrated and the voltage on the probe tube microphone is recorded. The sound pressure at the probe tube opening can be computed from the probe tube microphone voltage and the probe tube calibration function by rearrangment of A2

$$SPL_{PT}(f) = V_{PTM}(f)\, Cal_{PT}(f) \tag{A5}$$

Substituting A5 into A1

$$Cal_{InEar}\left(f\right)=\frac{V_{PTM}\left(f\right)}{V_S\left(f\right)}Cal_{PT}\left(f\right) \tag{A6}$$

Equation A6 is the computation performed by the In-ear Calibration program, where $V_S(f)$ is the FFT of the calibrating chirp stimulus.