

# DPOAE User Guide

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RZ6 Processor



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Tucker-Davis Technologies  
11930 Research Circle  
Alachua, FL 32615 USA  
Phone: +1.386.462.9622  
Fax: +1.386.462.5365

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# DPOAE Measurements with the RZ6

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## Introduction

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This guide is primarily intended for researchers who are new to collecting DPOAEs using a TDT RZ6/BioSigRZ system. It brings together targeted information from our software and hardware manuals and goes beyond product specific details to answer common questions, including subject or test area considerations that might affect your testing. The information provided is based on our knowledge of the TDT system and years of experience helping our users. It's not intended to favor or dictate any particular experimental protocols, methods, or research goals.

We leave the science up to you, but we're here to support you on your way to successful experiment completion.

## Organization of the Guide

This guide begins with a general overview of DPOAEs; what they are, how they're used, and what type of response signal to expect. It discusses the various aspects of your experiment such as test subjects, your test area, and the hardware and software you will use.

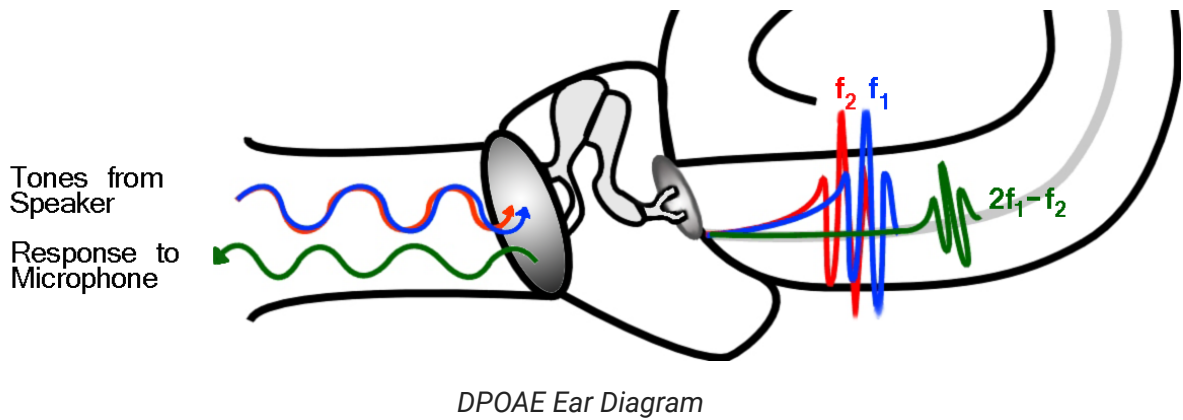
It includes detailed information about important tasks including:

- [Running the DPOAE](#)
- [Viewing and exporting test results](#)
- [System calibration](#)

## DPOAE Overview

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Distortion product otoacoustic emissions (DPOAE) are generated in the cochlea when the outer hair cells amplify and propagate particular sounds along the basilar membrane in response to two tones separated by a fixed ratio and played from separate sound sources (or speakers). DPOAE can be measured from the external auditory canal, making them an efficient screening and diagnostic tool in the study of hearing and hearing loss. Researchers who study DPOAE are interested in the energy or power of the Distortion Product as a function of the power of the sound sources.




The test stimulates the cochlea using two simultaneous continuous pure tones ( $F_1$  and  $F_2$ ) from separate sound sources and that differ by 20%, or you can say they have a frequency ratio (FR) of 1.2. As depicted in the DPOAE Ear Diagram above, the response from the cochlea is continuous during the stimulation. A microphone is enclosed with the speaker tubes in the ear canal. It records the sound pressure changes in the ear canal and streams a continuous voltage back to the recording system. The microphone detects all signals present in the ear canal. This includes both stimulation tones generated by the speakers, background noise, and any DPOAE response waveforms generated by the cochlea, all summed together.



Averaged DPOAE Response Waveforms

Standard DPOAEs measure the levels of the stimulation tones and the level of the cochlea response, which is one of many generated during stimulation. A harmonic is a frequency component of a signal that is an integral multiple of the signal's fundamental frequency. To separate out the energy of these unique frequencies (two tones and a harmonic cochlea response), the Time Domain signal is converted to a frequency domain signal that displays the amount of energy each frequency contributes to the acquired waveform. When we isolate the information we're looking for we can easily compare responses. The DPOAE measurements are repeated a number of times and averaged together into the final frequency domain plot for a desired stimulation level and frequency combination.

 **Note**

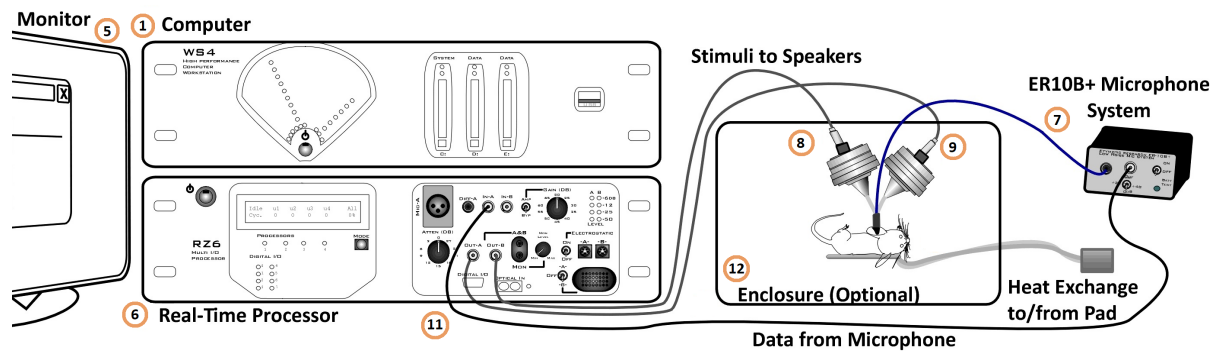
Values in BioSigRZ are inherently dBV. You can convert this in the Acquisition settings or, for a quick approximation of the dB SPL value, you can add 120. Also see [Converting dBV to dB SPL](#).

We know the F1 and F2 signals that we stimulate at, so we can easily find these on the x-axis and mark their y-axis levels on the plot. We also know the frequency that we expect the cochlea to respond at, so we can mark that level as well. Marking these frequencies and levels across various stimulation frequency combinations forms a DP-gram. Some researchers are also interested in varying the stimulation levels. As the stimulus levels are reduced the response is also reduced. The DP-gram shows the 'threshold' or the point where the DP can no longer be detected from the noise of the microphone.

## DPOAE Recordings

The TDT system for DPOAE recording features the RZ6 processor that brings together stimulus production and data collection on a single device and under a single clock for precise timing. The BioSigRZ system software for experiment control and data visualization is PC based and is more flexible than clinical systems while still easy to use. DPOAE recordings do not need a quiet environment. You can easily record DPOAE on a bench without sound attenuating chambers, but this is optional.


The diagram below shows a typical DPOAE system. Each part of the system will be discussed in more detail as you read through this guide.



Typical System Using TDT's RZ6 Processor and MF1 Speakers

What you'll need:

1. Windows computer (*WS4 shown*)
2. Optibit interface (*installed in computer*)
3. TDT Drivers (*software installed on computer*)
4. BioSigRZ (*software installed on computer*)
5. Computer monitor
6. Multi I/O Processor with optic port (RZ6-A-P1)
7. Etymotic microphone system (ER10B+)
8. Speaker (MF1)\*
9. Speaker (MF1)\*
10. Calibration microphone (PCB-378C01) (*not shown*)
11. Cable kit (BNC-ABR)
12. Sound attenuating subject enclosure (optional)
13. Heating pad (optional)
14. Subject
15. Anesthesia (*not shown*)

 **Note**

\* EC1 Electrostatic speakers may also be used for higher frequency studies.



# The Subject



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Planning or setting up your DPOAE experiment must begin with an understanding of the subject's normal hearing range and factors that might have an effect on its response or recording. DPOAEs have been measured for many species. In this guide, we'll focus on the three common species used in DPOAE experiments and screening protocols: mice and rats.

## Expected Hearing Range

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DPOAE tests are typically focused on the range in which the species is commonly known to hear well.

| Subject |   | Standard Testing Range | Standard Testing Range                    |
|---------|---|------------------------|---|
| Mice    |   | 4 kHz to 110 kHz       | 4 kHz to 48 kHz                           |
| Rats    |  | 2 kHz to 70 kHz        | 2 kHz to 32 kHz<br>(EC1: 4 kHz to 70 kHz) |

### Note

The hearing range of laboratory subjects may vary across different strains or colonies and can be affected by age, the environment, and anesthesia.

## Age-Related Effects on Hearing

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Many lab subjects experience hearing loss due to age. The rate of loss varies across species and strains. This effect has been observed as early as 12 weeks in mice.

## Anesthesia Considerations

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Two types of anesthesia protocols are typically used for DPOAE testing. Each has benefits and limitations. Check your IRB approved guidelines for dosages and protocol.

### **Xylazine(XYL) + Ketamine to start, Ketamine to Boost**

This aesthetic protocol uses an injectable combination of muscle relaxer and sedative. Typically, the subject is unconscious in a few minutes, begins to wake ~45 minutes and is completely awake at ~90 minutes after initial sedation. If a subject wakes early or a test runs longer than ~45 minutes, a booster of ketamine can be used to keep the subject anesthetized. Be sure to follow your approved IRB protocol.

### **Isoflurane (ISO)**

This aesthetic protocol uses a fast acting inhalant with a short duration. The subject is typically fully sedated within 4-5 minutes and wakes very quickly when the gas is removed.

 **Note**

While ISO affects the central nervous system it does not appear to affect the peripheral system, such as hair cells.

## Your Test Area

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DPOAES may be susceptible to ambient noise and rely heavily on good positioning of microphone and ear tubes. Shaking or other mechanical movement can dislodge these items, causing gaps to open in the signal path and degrading the signal quality. It is good practice to minimize noise in the test area and to ensure all conditions are carefully controlled during testing. Be sure to consider the elements detailed below in setting up your testing area.

## Enclosure Considerations

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A sound attenuating chamber and Faraday cage are not required for DPOAE testing, but can be used in excessively noisy environments.

### Note

Unlike ABR recordings, DPOAE recordings are not influenced by Electromagnetic noise.

## Heating Pads

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When the subject is sedated for testing, it generates less body heat and core temperature can drop. When the subject loses body heat, its hearing system is not as responsive which can artificially raise hearing thresholds (and reduce measured DP). A heating pad can be used to maintain the subject's temperature.

Below are two alternative types of heating pads used for this purpose:

**An Isothermal Pad** contains a phase change material that changes state near the subject's body temperature. It can be heated in a microwave and can typically hold a constant temperature for an hour or more.

Example: <https://www.braintreesci.com/temperature-measurement-control/deltaphase-isothermal-pads/deltaphase-isothermal-pads-8-x-8/>

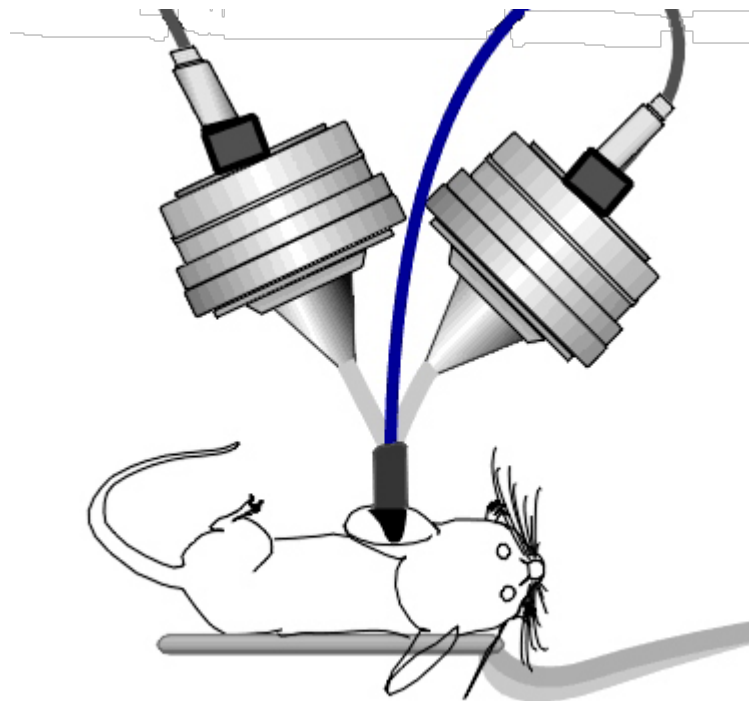
**A Warm Water Recirculator** moves warm fluid through a small subject heating pad to keep the subject's body temperatures stable. Because this method uses an external heating element

and pump to transfer water to the pad via non-ferrous tubes, the recordings will not be affected.

Examples: <https://www.braintreesci.com/temperature-measurement-control/heat-therapy-pumps-pads/adroit-heat-therapy-pump/> and <https://www.braintreesci.com/temperature-measurement-control/heat-therapy-pumps-pads/gaymar-heating-cooling-t-pump-back-in-stock/>

## Speaker Placement

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To minimize the effects of distortion or interference, keep ear tubes short to minimize bending and prevent crimping. TDT recommends positioning the subject on it's side and mounting stimulus speakers directly above the subject's ear- allowing for tube lengths of 3 - 5 cm and repositioning or turning speakers as needed rather than bending ear tubes.

# The Test Equipment

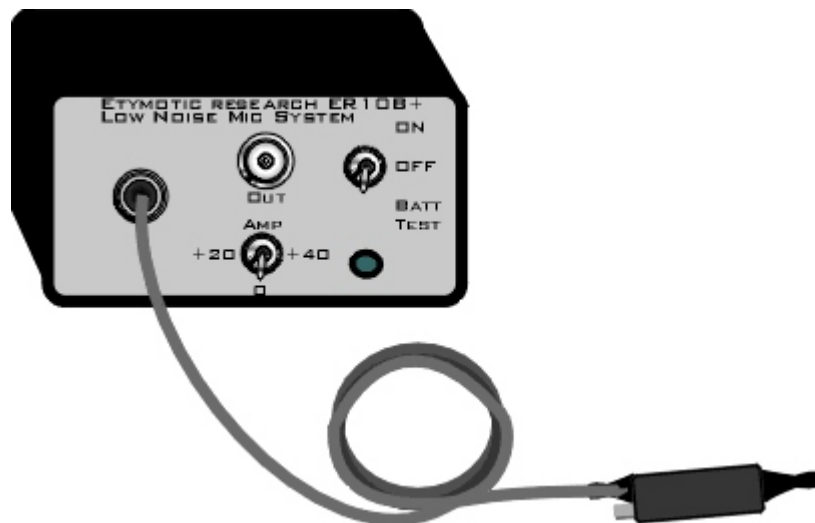
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The care used in setting up your test area must also be taken when selecting and setting up your test equipment. TDT's DPOAE system is part of a modular experiment platform. Each module in the system is designed for exceptional signal fidelity, precise timing, low noise, and ease of use.

The sub-sections below discuss the role each part of the system plays in running a DPOAE experiment and provides information on making the necessary connections.

## ER10B+

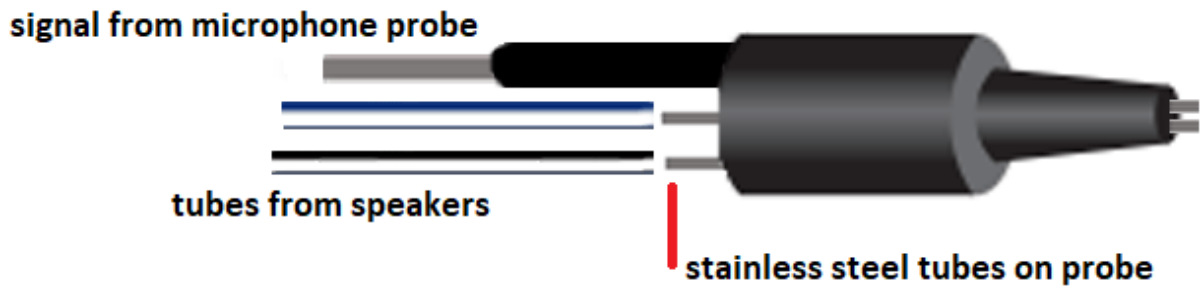
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The ER10B+ is a DPOAE microphone system manufactured by Etymotic Research. It can detect low intensity signals in the range of 0 Db SPL. It can detect signals out to 32 kHz, but is only calibrated to 15 kHz.

The provided cable and Ear Tip carry the response from the microphone into the system via a front panel BNC output.

The microphone tip also has two small tube-connectors to carry the stimuli to the subject's ear. Slide the closed-field speaker tubes over these small stainless steel tubes, ensuring a snug fit.

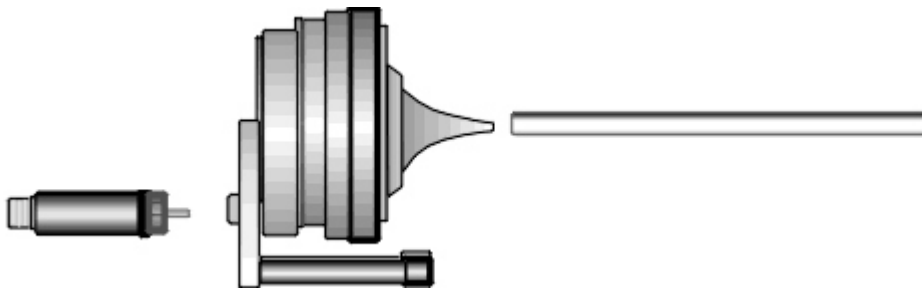


#### Note

Check Ear Tip for obstructions before using. It can become plugged with debris from the subject, reducing the measured DP.

See [Speaker Placement](#) for information on how to position the ear tip.

## Speakers



TDT's MF1 Multi-Field Magnetic Speakers are suitable for testing in the hearing range of mice and rats. They should be configured for **Closed Field** for DPOAE testing (see [Configuring the MF1 for Closed Field Operation](#)). Each MF1 comes with a tapered tube tip that mates with the provided  $\frac{1}{8}$ " outside diameter PVC tubing (10 cm included). Always use the shortest tube possible to avoid bending or crimping. You can cut the tube to the desired length, typically 3-5 cm. The tube should slide snugly into the tube tip. You can push firmly without fear of damaging the tube. If you have trouble inserting the tube, spray a small amount of silicone lightly on a cloth, then put the tip of the tube on the cloth to apply it.

#### Warning

Other lubricants (WD-40, grease sprays, or similar) should not be used as they can damage the O-rings surrounding the tube tip, or the tubes themselves.

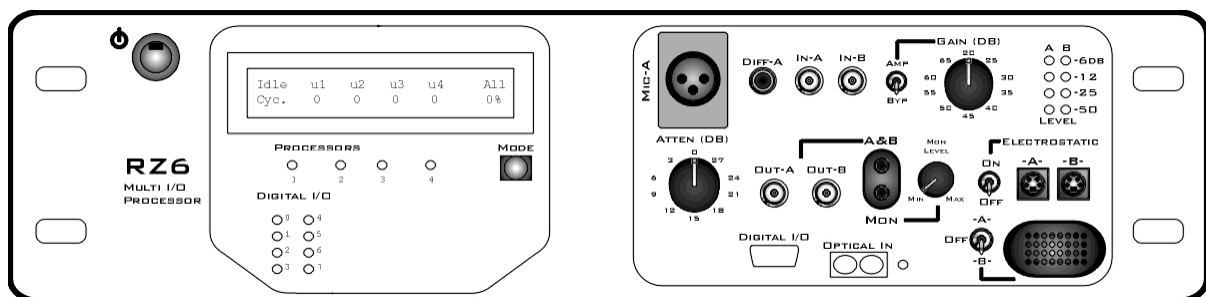
The MF1 comes with an inline filter (pictured to the left of speaker, above) that connects between the RCA cable and speaker input. This filter should always be in place when using the MF1 speakers in closed-field mode. In this mode, the MF1 has undesirably high, low-frequency output. This filter reduces the speaker output at low frequencies so that the output range can be equalized.

Each MF1 has a built-in 8-32 threaded hole for use with standard laboratory mounting hardware. Some mounting hardware is included with the MF1.

#### Note

The EC1, Closed-Field Electrostatic Speakers can be used for higher frequency studies.

## RZ6 Signal Processor



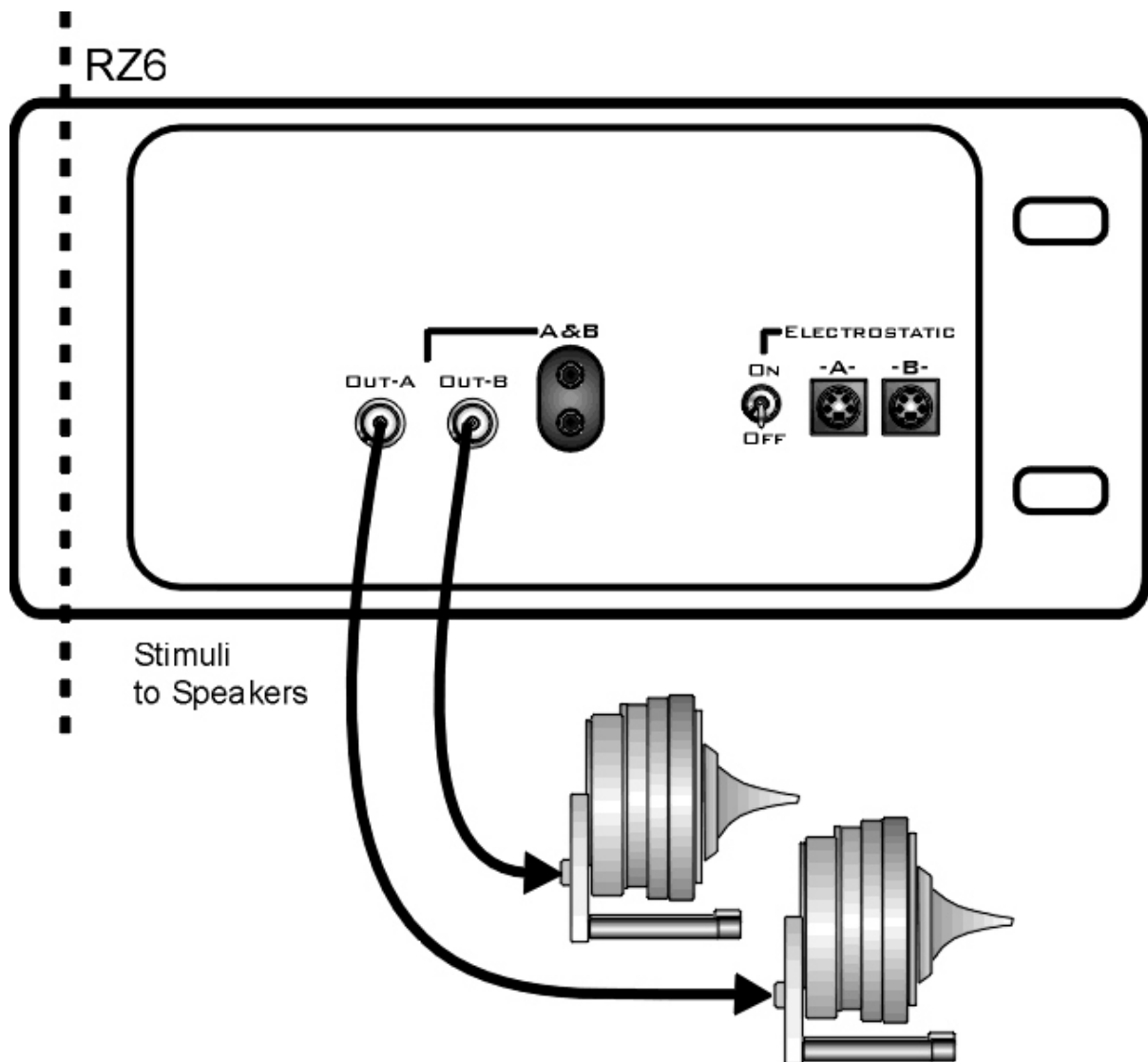
The RZ6 Multi I/O Processor provides the processing power for averaging signal inputs and the digital-to-analog converters and amplifiers for producing the stimuli. It also provides the precise timing essential to evoked response experiments. The power input and optical connection to the PC are located on the back. The input/output support (such as speaker drivers) and attenuation that is incorporated into the device are all accessed on the front panel.

## Stimulus Output

The RZ6 can generate a stimulus in the frequency range of DC - 88 kHz and is well suited for standard DPOAE testing.

A built-in stereo power amplifier drives the stimulus signals through TDT's MF1 multi-function speakers via the BNCs labeled **Out-A** and **Out-B**.

Use RCA - BNC cables (included with the MF1 kit), or BNC to RCA adapters, to connect **OUT-A** and **OUT-B** to the RCA connectors on the speakers.

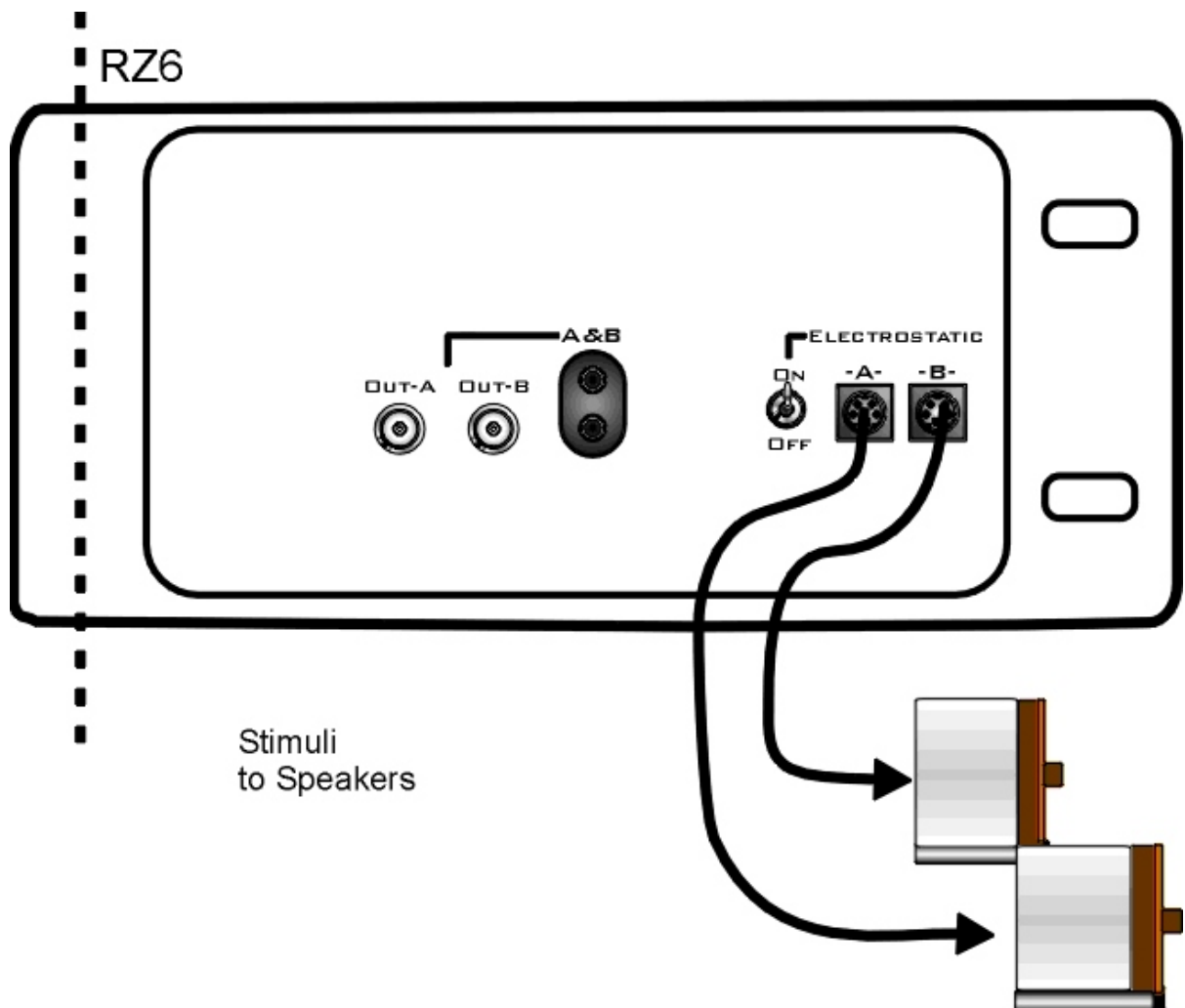


### Important

When using the MF1s, the **ON/OFF** switch next to the outputs marked Electrostatic must be in the **OFF** position to reduce noise.

If using EC1 speakers, the **ON/OFF** switch next to the outputs marked Electrostatic must be in the **ON** position.



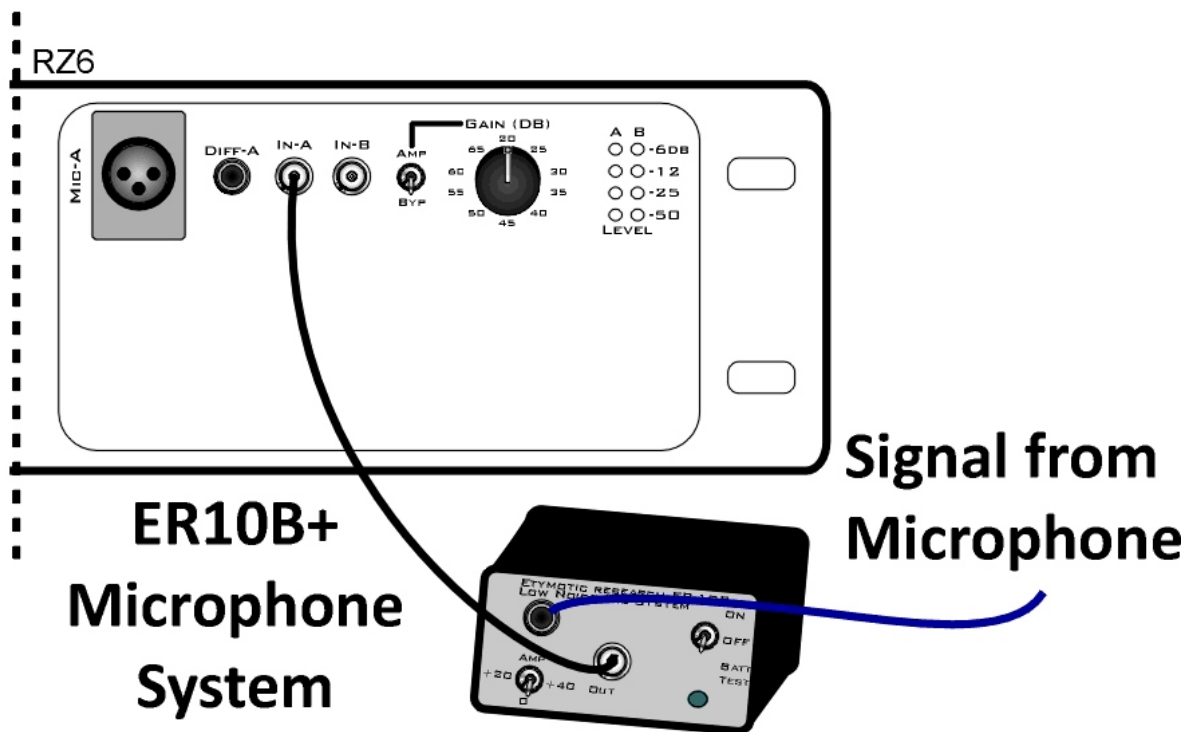


## Attenuation

Typically, stimulus attenuation is handled via software. The Atten knob on the front panel provides optional manual attenuation applied to the signal before it is output. This can be useful if the sound level is found to be systematically too high across all frequencies during speaker calibration. See [DPOAE Calibration](#).

## Mic Inputs

Connect the ER10B+ Out to the Microphone **In-A** BNC input connector. No gain is required for the signal, so the AMP/BYP switch must be set to **BYP**.



## PC and Interface

The RZ6 must be directly connected to a PC, which provides the user interface for the system. The PO5 or PO5e optical interface card is installed in the PC and connected to the RZ6 across fiber optic cables. This adds another layer of noise reduction, isolating the processor from the noisy PC environment.

The PC handles:

- Configuring the hardware and software.
- Communicating with the TDT hardware and transferring data across a fiber optic interface.
- Displaying and working with data during and after acquisition.

If you use TDT's WS4 computer, the optical interface and BioSigRZ software are preinstalled.

If you use your own PC, you'll need to install the interface card and BioSigRZ software yourself. Step-by-step instructions for installing software and drivers and setting up the processor are available in the [System 3 Install Guide](#) that came with your system. The installation guide also includes information for connecting the WS4 or your PC to the RZ6 Processor, including running a transfer test to ensure communication between them is working correctly.

 **Note**

A UZ3 (USB 3.0) interface is also available if you wish to use a laptop.

# BioSigRZ Software

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BioSigRZ software is streamlined and optimized for ABR and DPOAE screening. The software provides a simplified interface for selecting stimuli and configuring how the response will be recorded and processed.

## DPOAE

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Two tones are typically presented at multiple frequencies to target specific areas of the subject's hearing range.

## Out-of-the-Box Experiments

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When the software is installed, out-of-box an experiment file for DPOAEs is stored at:

```
C:\TDT\BioSigRZ\Configs\DPOAE
```

The default values used in the pre-configured experiment are set to values suited to typical experiments. Your protocol or circumstance may differ from these values but these will work as an example or a starting point and can be modified to fit the needs of your lab.

To open the DPOAE experiment configuration:

1. Launch **BioSigRZ**
2. Click the menu **File** → **Open Config. File**
3. Browse to and open the following file:

```
C:\TDT\BioSigRZ\Configs\DPOAE\Root_DPOAE.acf
```

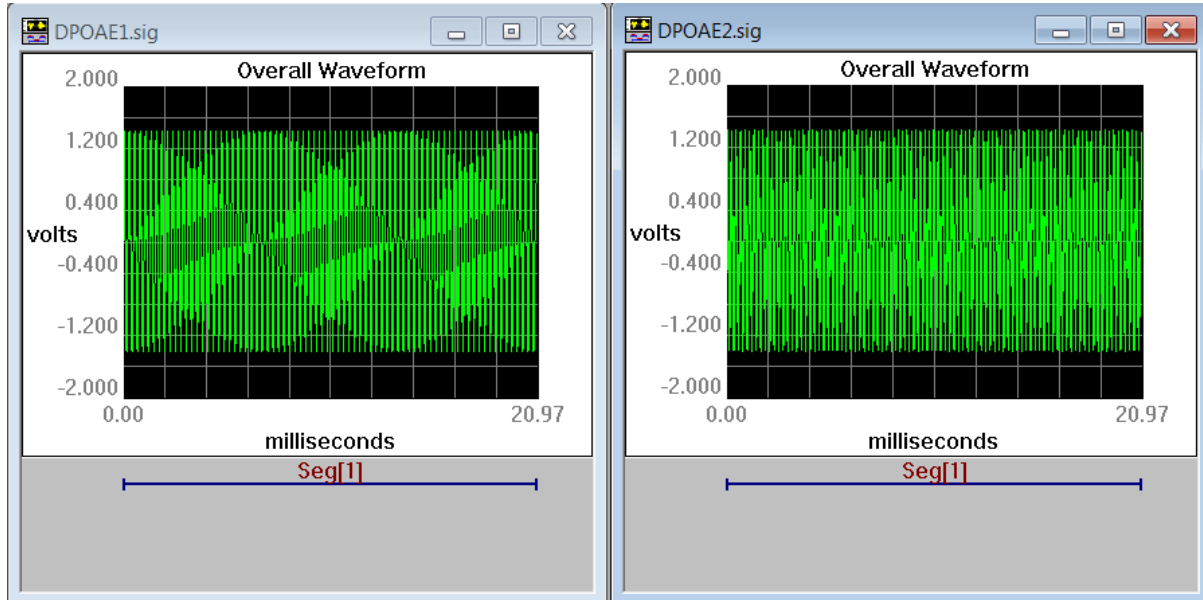
Two tones that are about 20% apart will be presented continuously. Distortion-product data will be collected every 20.971 milliseconds and averaged 512 times.

DPOAEs will be measured at the following center frequencies( $F_c$ ): 4000 Hz, 8000 Hz, 16000 Hz, and 32000 Hz.

The tone frequencies will be  $F_1$  and  $F_2$ , are calculated using the center frequency where  $F_1$  is  $F_c * 0.909$  and  $F_2$  is  $F_c * 1.09$ . This yields frequencies of primary 1, named Tone 1, and primary 2, named Tone 2, that are geometrically centered about  $F_c$ .

The Level of the two primary tones will remain equal and will vary with each frequency from 80 dB SPL to 20 dB SPL in 10 dB decrements.

This DPOAE test is designed to measure the level of the "cubic difference tone," which is at  $2F_1 - F_2$ .

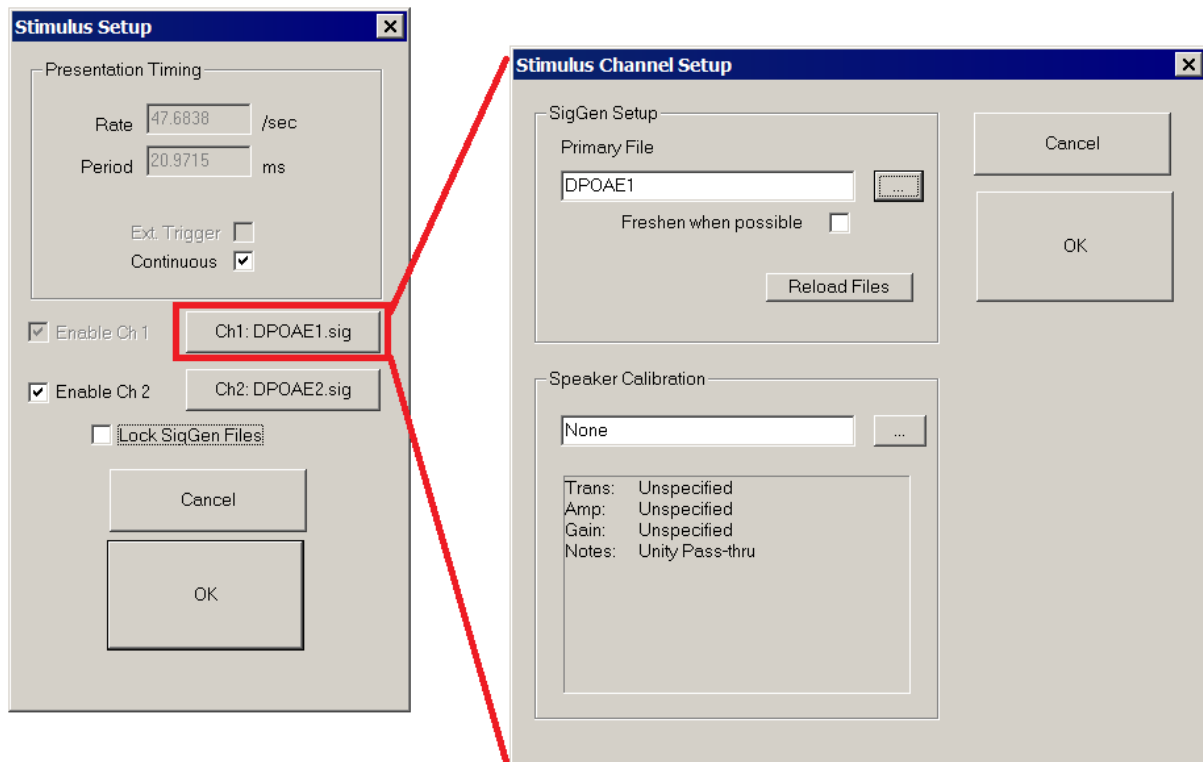


## The Stimulus Settings

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The default stimulus settings represent a typical configuration and should be fine to begin, but you may eventually want to make adjustments.

The stimulus setting dialog boxes can be opened by clicking **Setup** → **Stimulus**.



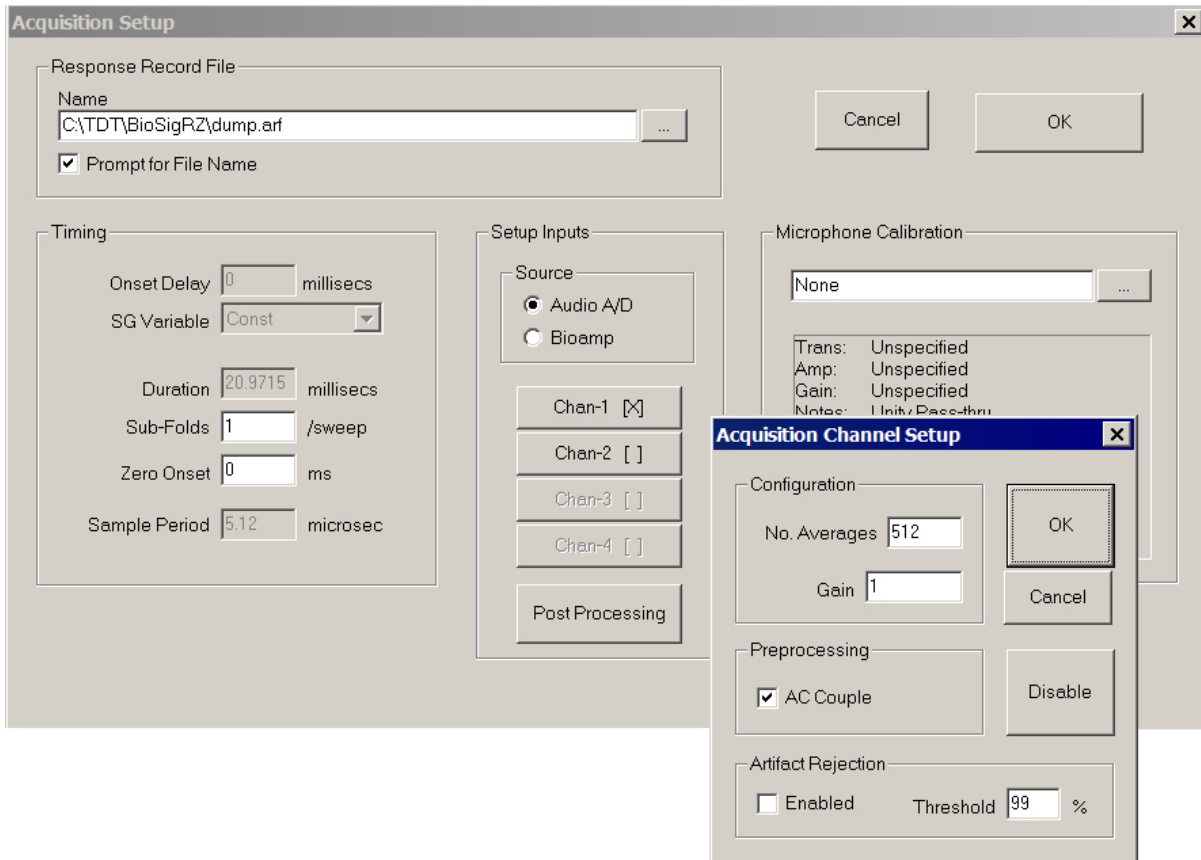
The setting notes below provide information about each setting.

| Setting             | Notes  |
|---------------------|--|
| Enable Ch2          | <b>Enabled</b>   |
| CH1                 | <b>DPOAE1.sig</b>  |
| CH2                 | <b>DPOAE2.sig</b><br>A complete list of signal parameters and variables from the .sig file are added to the BioSigRZ configuration file. During stimulus presentation, the variables change as a function of the SigGen Index (SGI), which is a set of variable conditions. The order of the presentation is called the stimulus schedule. You can access this schedule and choose to omit specific SGIs, if desired. See <a href="#">Modifying the Stimulus</a> for more information on modifying the standard protocol.  |
| Speaker Calibration | <b>Leave blank for now</b><br>All speakers have some variation in output across frequencies. When you use a speaker with a known frequency response, you can adjust the stimulus output for these known frequency characteristics. A USB drive with a speaker-specific calibration file is provided with your MF1 speaker. You will need to copy that file to the following directory: C:\TDT\BioSigRZ\TCF. After the file has been copied to this directory you will be able to select it from a list in the Stimulus Channel Setup. You can also make your own .tcf calibration file by following the instructions in <a href="#">BioSigRZ Manual</a><br><br>The first time you run a DPOAE, we recommend skipping this step and any other calibration until after you have been able to successfully record a response. If the system has not yet been fully tested, small mistakes in calibration can become more difficult to diagnose. It is important to make sure everything else is working before calibrating. |

## The Acquisition Settings

The default acquisition settings should be fine to begin, but you may eventually want to make adjustments.

The acquisition settings dialog boxes can be opened by clicking **Setup** → **Acquisition**.



The setting notes below provide information about each setting.

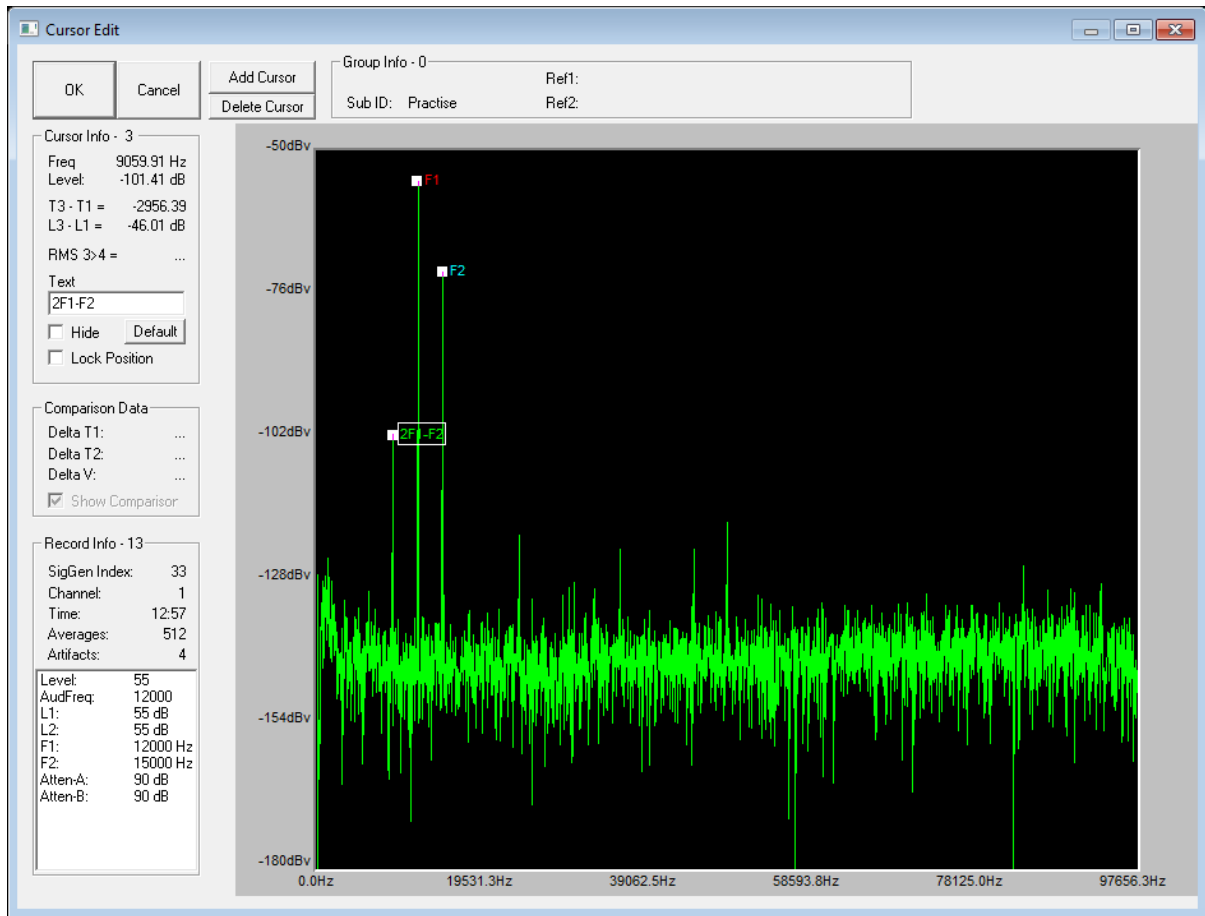
| Setting                | Notes  |
|------------------------|--|
| Response Record File   | <p>All the data collected and displayed in the History plot during the DPOAE test are stored in a response record file (.arf). By default the file will be saved at: C:\TDT\BioSigRZ\</p> <p>You can specify a name in the setup window or select the Prompt for File Name check box to be prompted for a name before each recording session. You will be prompted each time you click Begin.</p>  |
| Setup Inputs Source    | <b>Audio A/D</b>   |
| Number of Averages     | <p><b>128</b></p> <p>You need enough responses to get good signal quality and few enough to minimize the time to complete testing. For DPOAEs, the typical range is 64 - 128 averages.</p>   |
| Gain                   | <p><b>0.00001</b></p> <p>The ER10B+ microphone system can apply up to 40 dB gain amplification, however, in most cases no gain is needed when using the RZ6 for acquisition. Set the gain on the face of the ER10B+ microphone system to <b>0 dB</b>. Set the gain here to <b>0.00001</b> so the history plot units will match dB SPL. You must also set the Amp/Bypass switch on the RZ6 face to <b>BYP</b> (bypass). See <a href="#">Converting dBV to dB SPL</a> for related information.</p> |
| Microphone Calibration | <b>None</b>  |

## Auto Cursoring

The auto-cursor feature can place up to 10 cursors on each acquired trace as a mathematical function of a variable or at a specific frequency. BioSigRZ supports automatic feature finding to minimize the amount of manual cursor manipulation that has to be performed after data acquisition. Cursors are defined from the Setup menu, Auto Cursors command. Available choices include features such as peaks, valleys, zero-crossings, absolute positioning, and more. See the [BioSigRZ Manual](#) for more information on using Auto Cursor.

This experiment has auto-cursor pre-configured to mark F1, F2, and 2F1-F2 so they can be easily viewed and extracted later for analysis. After DPOAEs are acquired, you can add or edit cursors in the Edit Cursor window. This window is opened when you double click a trace (waveform) in the History or Worksheet plot.





# Running the Experiment

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After you feel comfortable with the system, you can begin to collect data. We recommend reading through the entire guide before using a live subject.

## Check Sedation

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If the subject is not fully sedated throughout testing, subject responses, such as muscle movements or distress, can interfere with DPOAE recordings. To avoid this, **always check the subject before beginning your experiment and monitor the subject for signs of wakefulness during testing.**

### Toe Pinch

For small lab subjects, a toe pinch is the most effective method to check for full sedation. Extend the rear leg and use your fingernails to firmly pinch toe or foot webbing. There should be no recoil. If there is any response, such as leg movement, the subject is not adequately anesthetized.

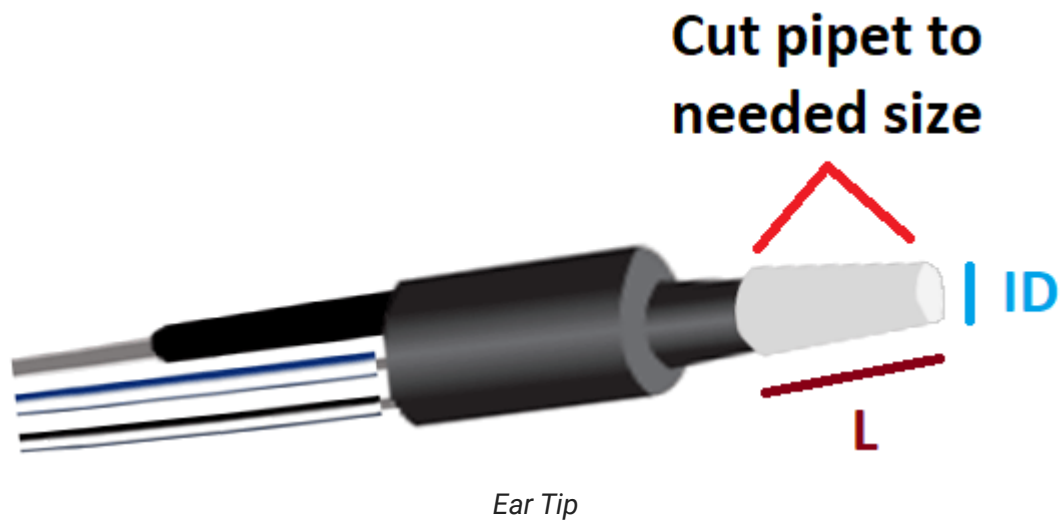
#### Note

Startling the subject with a loud clap or loud noise is not a rigorous enough way to check sedation. A subject may not respond to clapping even when only partially sedated. Also, a test subject with hearing loss may not respond regardless of sedation level.

### Check Integrity of the Signal Path

Good positioning of microphone and ear tubes is critical to getting a useful recording of the DPOAE response.

Check the positioning of the ER10B+'s Ear Tip, making sure there is good fit, with no gaps between the Ear Tip and the subject's ear. If the subject's ear is too small for good positioning, a pipet tip can be used to step down from the size of the Ear Tip into the subject's ear.



**Length (L)** should be as small as possible.

**Inner Diameter (ID)** should be as big as possible.

Check the seal of the clear tubes at the MF1 tube-tip and at the ER10B+ Ear Tip. The clear tube should slide snugly into the tube tip and snugly onto the Ear Tip's stainless steel input tubes.

Check the tubes to make sure they are as straight as possible and they are not crushed or crimped.

Make sure the in-line filters provided with the MF1 speakers are connected between each MF1 and the cable that connects it to the RZ6.

## Begin Experiment

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BioSigRZ is the user interface for DPOAE testing. It has three modes of operation: Idle, Running, and Averaging. Before you begin, it is in Idle mode. You can begin stimulus presentation or run the experiment using buttons on the left side of the BioSigRZ software main window. The options change depending on the mode. See below for more information about the operating modes.



To begin stimulus presentation:

1. Click the **Start** button. You may be prompted to specify a response record file where data will be stored.
2. If prompted, type a name in the **File Name** field and click **Open**

You are now in **Running Mode**.

The stimulus signal is presented using the first SigGen Index (SGI) in the stimulus schedule. No data is collected.

To begin data averaging:

1. Click the **Begin** button.
2. A Subject Information window opens. Information entered here is stored with the data in the response record file (.arf).
3. Enter any subject information and click **OK**.

You are now in **Averaging Mode**.

The first stimulus is presented and the DPOAE response data is acquired and included in the running average until the desired number of responses has been collected at that level. As averages are completed, the averaged DPOAE records will be appended to the History plot and the .arf data record file.

## Modifying the Stimulus Schedule

---

BioSigRZ provides several functions that allow you to override the current stimulus schedule and control data averaging in real-time.

**Advance** Current SGI is halted and running average is saved. Stimulus presentation advances to the next SGI in the stimulus schedule.

**Repeat** Current SGI is halted and running average is saved. Stimulus is presented again at the current SGI.

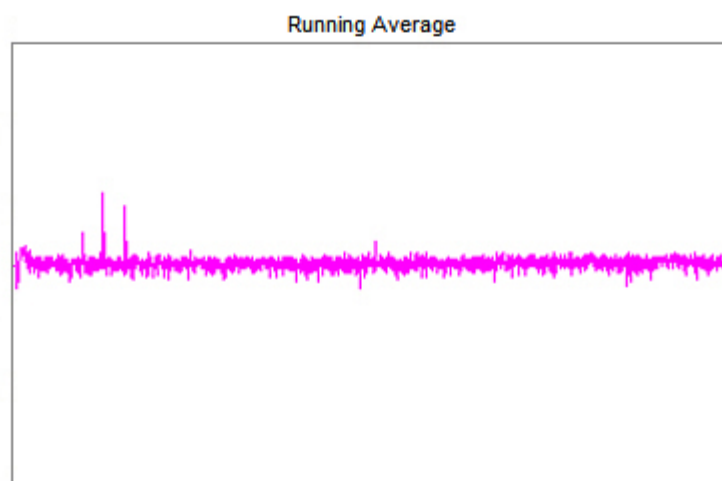
**Skip** Current SGI is halted and running average is discarded. Stimulus presentation is advanced to the next SGI in the stimulus schedule.

**Redo** Current SGI is halted and running average is discarded. Stimulus is presented again at the current SGI.

## Multi-Purpose Plot

---

During averaging, the Multi-Purpose Plot may be used to display a variety of signals. The plot is controlled through the use of the Plot Control Toolbar, available from the right-click menu. You can also control scaling using the toolbar. Consider using the options described below.



**Running Avg** This view displays the current running average in the frequency domain.

**Zooming** Click and drag from left to right over the area of interest to zoom in and change the scale of the frequency axis. Click and drag from right to left to zoom out.

## Termination

---

In BioSgRZ, control of termination of the signal presentation and acquisition is called boundary control. When a boundary is reached and the SGI stops incrementing, signal generation will continue for the current SGI, but no more response data will be collected.

You can manually terminate data averaging at any time by clicking the **End** button. This returns the system to **Running** mode.

You can halt stimulus presentation and return the system to **Idle** mode or run the experiment again by clicking the **Stop** button.

Click **Begin** to run the experiment again and enter the new subject information.

# Data and Export

Viewing data in the BioSigRZ plots and worksheet.

## History Plot

In averaging mode, BioSigRZ acquires data and calculates a running average. Data will be acquired until enough records have been gathered to compute the final averaged signal. At that time, the averaged signal will be appended to the end of the History Plot, the SGI will be incremented, a new stimulus signal will be generated according to the current stimulus parameters, and a new average will be computed. This process continues until the termination of the stimulus schedule is reached or until you manually end data averaging. Information about each SGI is displayed to the left of the history plot (pictured below). Right-clicking this data allows you to control what data is shown.



## The Worksheet

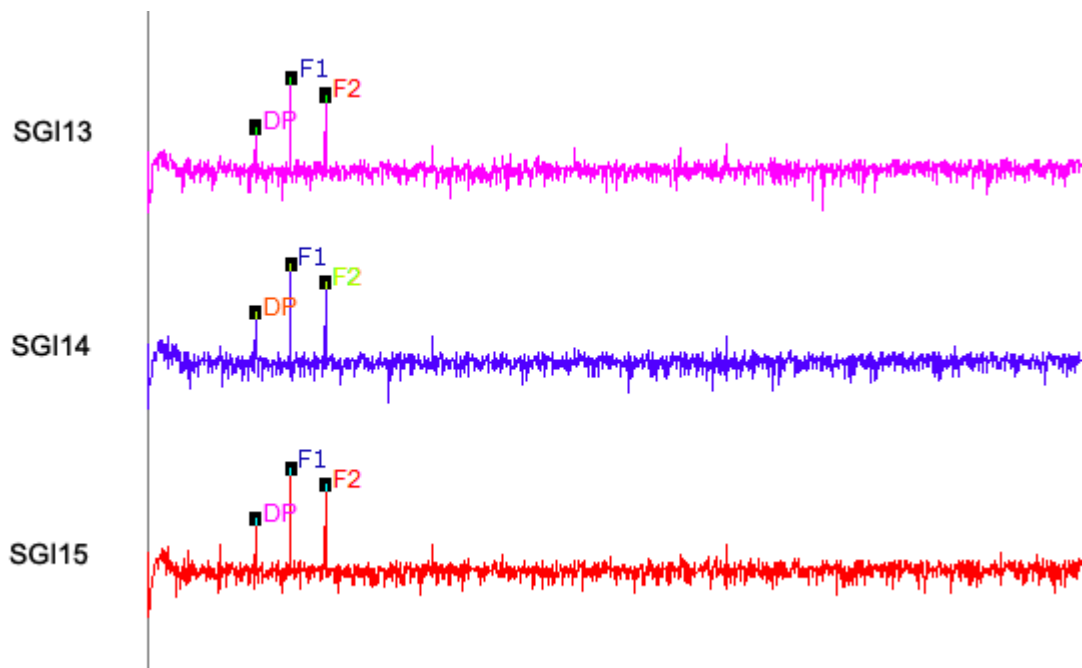
---

The worksheet provides you with several basic functions. It allows you to graphically organize acquired data and perform mathematical and visualization manipulations of the acquired data directly in BioSigRZ. The Worksheet is located to the right of the History plot. Waveforms can be copied from the Worksheet and pasted to other applications for report writing or exported by choosing to generate a report.

### To place History Plot records into the Worksheet:

1. Select the desired record(s).
2. Click and drag the selected record(s) from the History Plot to the Worksheet.

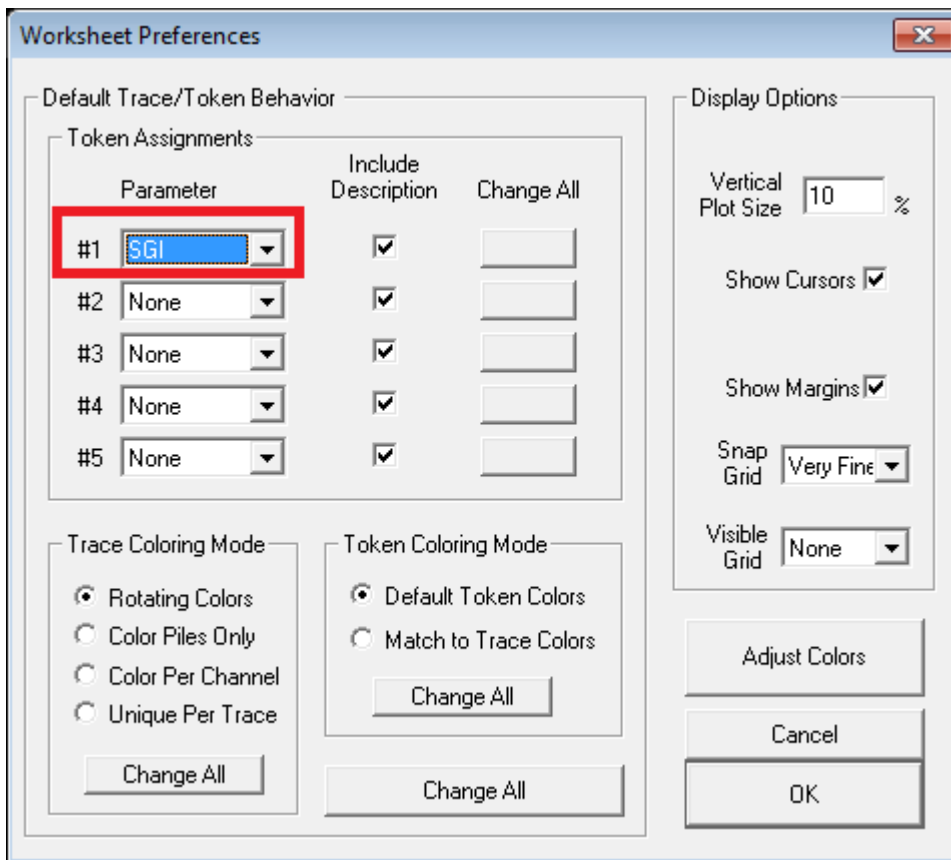
The selected History Plot record(s) will be copied to the Worksheet.



### To change the token (#1) that appears to the left of the trace:

1. Right-click the worksheet.
2. The Worksheet Preferences dialog is displayed. Select the desired parameter for **Token #1** from the drop-down parameter list.





Changes made here, will be applied to records added to the worksheet after this change is made. To change existing tokens, click the **Change All** button to the left.

Other tokens can also be changed in this dialog.

3. Click **OK**, to apply the change.

For more information on using the worksheet, see the *Using the Worksheet* section of the [BioSigRZ Manual](#).

## Export Data

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BioSigRZ has many onboard tools for viewing and working with responses. Records may be exported to a CSV (spreadsheet) file or an ASCII text file (.txt file) or you can choose to do further analysis in an environment such as Excel. We can provide support for exporting data to the desired format, but we leave further analysis up to you so that you can do it your way.

### To export to CSV:

1. Select the desired records in the History plot or worksheet.
2. On the **Menu** bar, click the corresponding menu (**History** or **Worksheet**) and click **Export to CSV** file.

The settings shown are the typical/recommended export settings, including cursors, for DPOAEs. If you wish to further customize the export, click the check boxes or radio buttons to toggle on/off the desired options.

To export the data points that constitute each trace, check the box next to **Data Samples**. These data points can be used by MATLAB or Excel to recreate the exact trace shown in BioSigRZ

Type a report title in the **Title** field.

3. Click the **Generate Report** button.
4. When prompted, specify a file name and location to store the export file and click the **Save** button.
5. When you are returned to the CSV File Export dialog box, you can change options and generate additional report exports or click **Done** to exit.

Selecting Trace Data check boxes will also export the data needed to recreate the entire trace as seen in BioSigRZ.

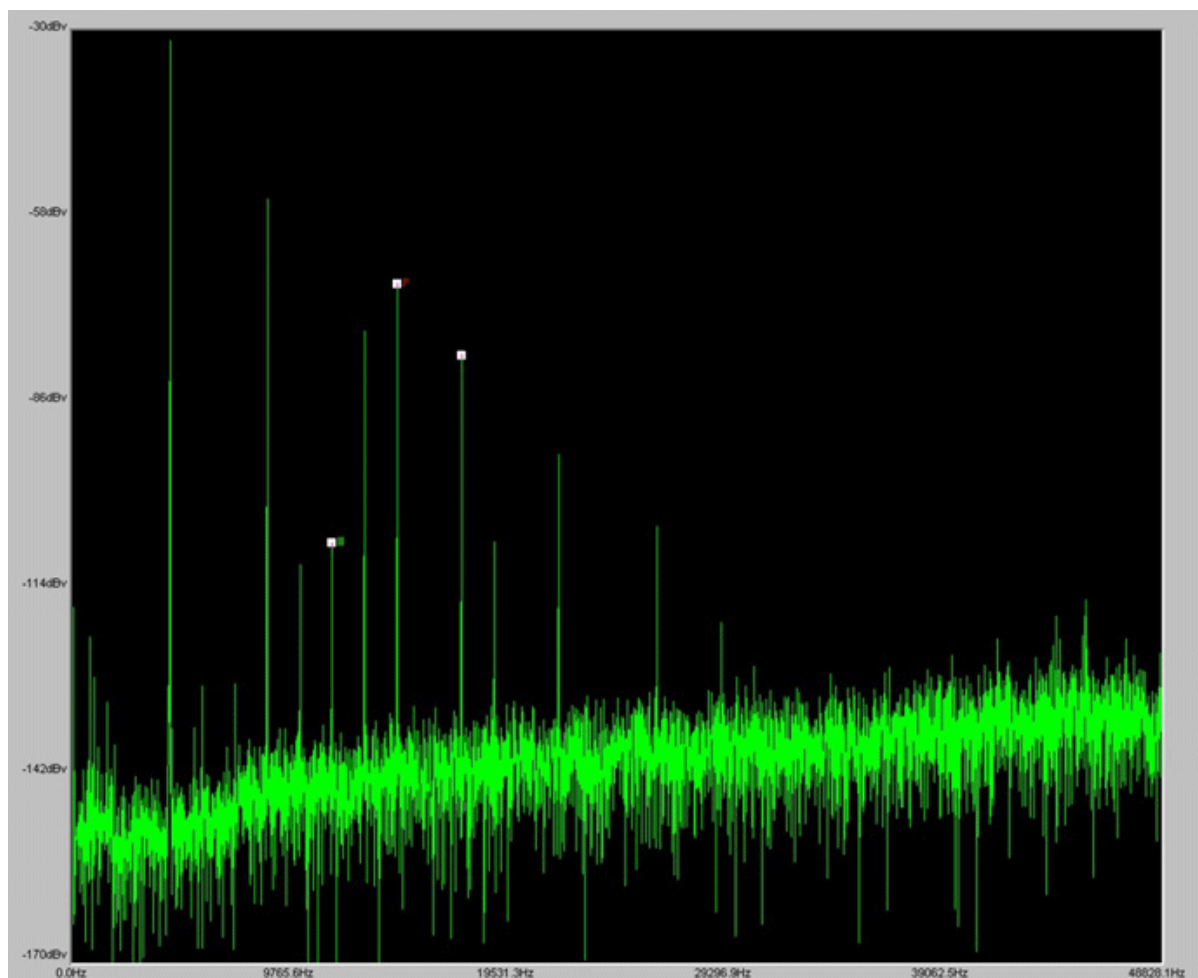
See the [BioSigRZ Manual](#). for further information on data viewing tools and export features.

# Troubleshooting

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## Diagnosing Noise Issues

---



*Noise DPOAE Response with Harmonics*

The image above shows a noisy DPOAE recording that contains harmonics (frequency components of a signal that is an integral multiple of the signal's fundamental frequency) that could easily be mistaken for the DPOAE response. If a frequency component is present when no living subject is present, it is not the DPOAE response.

Here are a few common sources of noise:

1. Something near the recording area is causing noise.

The nearby area should be as free of mechanical vibration sources as possible. Common sources are motors and fans, refrigerators, HVAC systems, elevators, refuse pumps, centrifuges.

2. A tube is obstructed or there is a gap in the connections.
3. Length of tube is too short or too long.
4. Improper seal between ear and ER10B+.
5. Battery is low, use ER10B+ Battery Check tool to verify.

## Troubleshooting On-The-Fly

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If a problem occurs during an experiment, the BioSigRZ Multi-Plot window provides several views useful for on-the-fly troubleshooting. Here are a few common problems that can be identified and resolved quickly so you can resume your experiment.

The EEG view in allows you to monitor recordings during testing.

### **Random jumps in signal level**

Cause: An animal that is awake or not fully sedated

You can also view a running average of the responses.

### **Missing F1 or F2 peak**

Cause: A speaker problem. Check the speaker by listening during stimulation.

### **Missing F1 and F2 peaks**

Cause: A problem with the RZ6 or ER10B+.

### **No DP at any SGI**

Cause: A hearing impaired animal. Check proper fit of Ear Tip and tubes.

Cause: A deaf animal. Try the test again on with a normal hearing animal.

Cause: An obstruction in the ER10B+ Ear Tip.

### Noisy signal

Cause: A noisy environment (fans, etc.) or low ER10B+ battery. Check battery.

## Getting Help

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If you are unable to diagnose the noise problem, contact TDT for assistance at [support@tdt.com](mailto:support@tdt.com) or +1.386.462.9622.

When you contact us be prepared to answer questions such as, what frequencies you're testing, what subject and anesthetic you're using, and when the system was last calibrated. We may also ask you for data, screen-shots, or photos of your experimental setup. We may also ask you to participate in a remote desktop session, which requires an Internet connection to your experiment PC running BioSigRZ software.

# Calibration

---

All speakers have some variation in output across frequencies. When you use a speaker with a known frequency response in BioSigRZ, you can adjust the stimulus output for these known frequency characteristics by applying a Calibration File (.tcf) in the Stimulus Setup. You can create custom files specifically for your speakers and setup, using BioSigRZ's Calibration tools.

During calibration, BioSigRZ performs a sweep over the frequency range of interest and generates a speaker response curve along with a correction curve that would be required to flatten the response. An FIR filter is calculated from the correction curve and stored in the contents of the calibration file. When you load the calibration file, that filter is loaded onto the RZ6 so that any tones presented through the RZ6 will be calibrated to have a flat response across all frequencies.

The first time you run a DPOAE, we recommend skipping calibration until after you have been able to successfully record a DPOAE response. If the system has not yet been fully tested, using a custom calibration file can make problems more difficult to diagnose.

When you're ready to tackle calibration, you can review the [BioSigRZ Manual](#). It covers a standard single channel speaker calibration. The remainder of this section covers important details you'll need for calibrating your DPOAE setup.

## DPOAE Calibration

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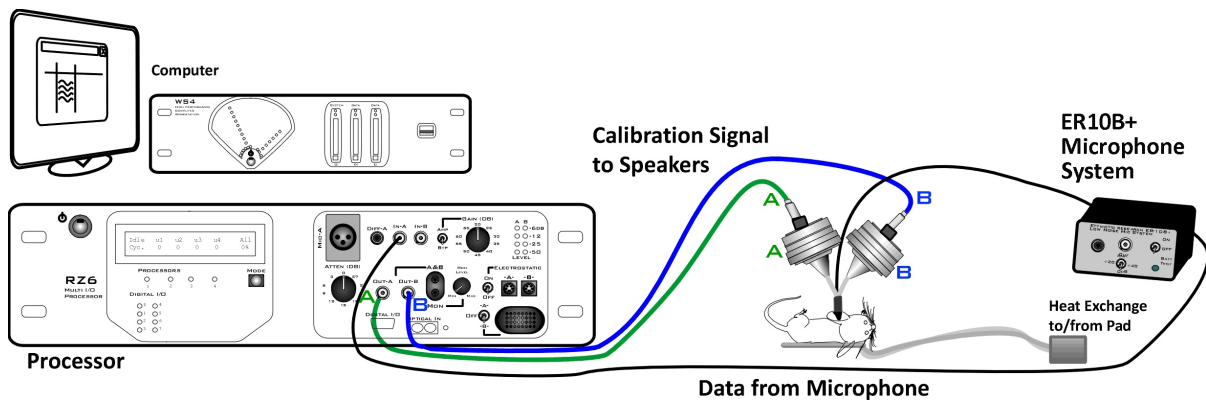
DPOAE testing differs from other hearing tests in two key ways that affect calibration: it always uses two channels and it's always closed-field.

Because the two stimulus tones (F1 and F2) are presented on separate channels, with two different speakers, you will need two calibration files (.tcf), one for each of the two stimulus speakers.

The closed-field nature of DPOAEs makes it even more important that you use calibration files that are not only specific to your speakers, but specific to your setup. Tube length, curve, and position in the ear can all affect the frequency characteristics of the delivered stimulus. TDT recommends in-ear calibration, using your experiment setup, to get the best possible results. An added benefit of this type of calibration is that it is a good test of the fit and seal of the tubes and their positioning in the ear.

## Hardware Setup

For in-ear calibration, you must have everything set-up as you will for your experiment. That is, your subject is sedated and the microphone is in the subject's ear.



*In-Ear DPOAE Calibration Setup*

The Amp/BYP switch on the front of the RZ6 will be set to **BYP** in most cases. In BYP (bypass) mode, the Gain knob is not used and will not affect the signal.

Because you'll be creating and using two TCF files and it is critical to use the correct file for each speaker when you begin testing, TDT recommends labeling the speakers and each connection point (as pictured above, using 'A' and 'B' with colored stickers, polish, or paint) to identify the speaker. Whatever labels you use can also be appended to the TCF file names to distinguish between them.

## Creating the Calibration Files in BioSigRZ

When you're ready to run your calibrations, follow the instructions in *Creating a Custom Calibration (.tcf) File* in the [BioSigRZ Manual](#), with the following changes.

In the Calibration window under Microphone Calibration, select the **ER10B\_50mV.tcf** file.

The A/B switch in the Probe area allows you to turn off one of the speakers while calibrating the other. The first time you run through the calibration, set this switch to 'A'. Speaker 'B' will be turned off, temporarily. When you're satisfied with the new calibration file you've made, be sure to save it, using "A" in the name.

Next, change the switch to 'B'. Do NOT change hardware connections. Speaker 'A' will be turned off temporarily, so that speaker 'B' can be calibrated, with no changes to your setup. Run the calibration again, and when you're satisfied with the new calibration file you've made, save it with "B" in the name.

When you have created your two calibration files (.tcf), you can close the Calibration window. Open the Setup and Stimulus menu and assign the newly created speaker-specific TCF files for channels one and two.

You can use the calibration verification utility (also described in *Testing the Calibration (TCF) File*, in the [BioSigRZ Manual](#)).

### Important

To ensure accurate calibration, give the microphone 10 minutes to warm-up before testing.

## How Often to Calibrate

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For DPOAE recordings, TDT recommends in-ear calibration for each subject. Please note that the ER10B+ is not a calibration microphone.

## Testing Speakers and Microphones

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A simple RPvdsEx test circuit can be used to ensure your speakers and microphones are working. The test circuit, `SpeakerMicTest.rcx`, is part of the [BioSigRZExamples.zip](#) zipped example folder, which can be downloaded from the TDT website.

### Speaker Test

If you suspect that your speakers may not be functioning correctly, download and extract the zipped files to:

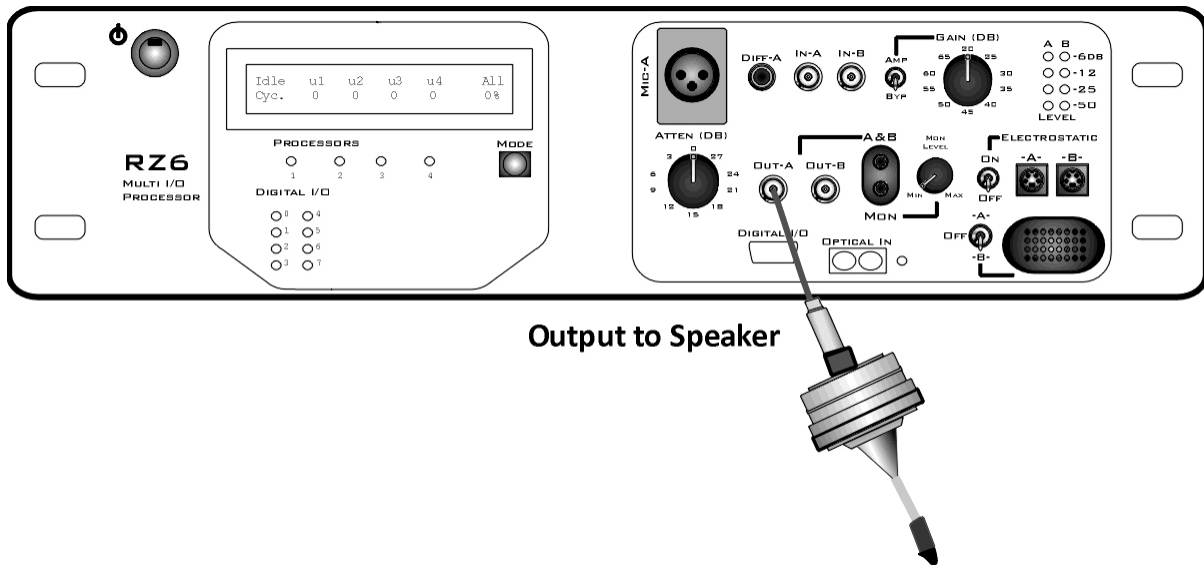
```
C:\TDT\BioSigRZ\RCX
```

Then double-click the `SpeakerMicTest.rcx` file to launch RPvdsEx and open the file.



To use this circuit there are a few steps required for setup:

1. Connect your speaker to Out-A.



RZ6 with MF1 Speaker

To test your speakers:



Click the **Compile, Load, Run** button on the toolbar. You should be able to hear a tone from the speaker.



Click the **Stop** button to stop the test.

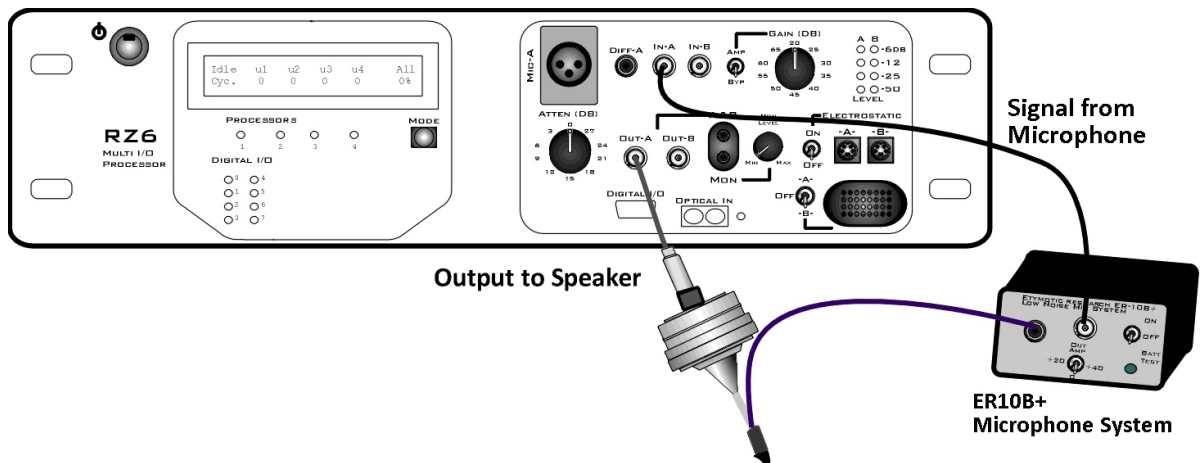
Repeat with second speaker.

## Microphone Test

After you've confirmed at least one speaker is working, connect the calibration microphone and run the circuit again.

### Important

To ensure accurate calibration, give the microphone 10 minutes to warm-up before testing.



*RZ6 with MF1 Speaker and ER10B+ Microphone*

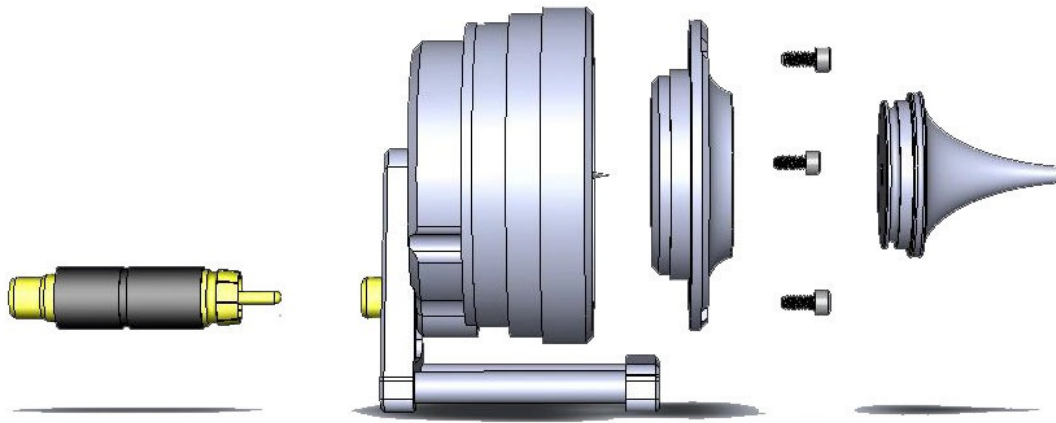
**To use this circuit there are a few steps required for setup:**

To use this circuit there are a few steps required for setup:

1. Connect your DPOAE Microphone to the In-A port on the RZ6.
2. Ensure your Amp/BYP switch on the front of the RZ6 is set to Bypass.

You should be able to see a sine wave in the graph in RPvdsEx. You may need to adjust the scale using the green arrows on the sides of the graph. Click the Stop button again to stop the test.

# Configuring MF1 for Closed Field



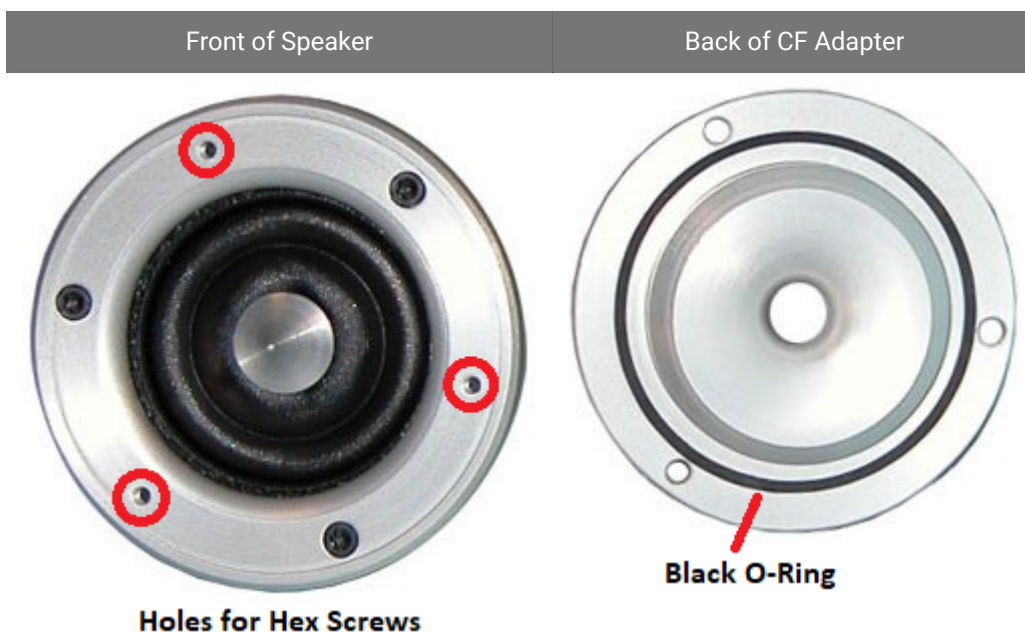
*MF1 Closed Field Speaker Assembly Diagram*

## Important

Closed Field Adapter, Tip, and CF Line Filter used only for Closed Field Configuration!

### To configuring the MF1 for closed field operation:

1. Ensure Black O-ring is in place on back of CF Adapter, as shown.



2. Attach the CF Adapter to the front of the speaker using three of the provided  $\frac{1}{4}$  x 4-40 hex screws.



3. Ensure the blue O-ring is in place at the base of the desired tip, as shown.

Insert the tip into the groove on the CF adapter. Ensure the tip is bottomed in its socket. Gently insert the tube into the narrow end of the tip.

4. Attach the CF filter to the provided RCA cable.



*CF Line Filter*

**Important**

For Closed Field Configuration Only

5. Attach a BNC to RCA adapter to the BNC amplifier port of your source device.



*Back of Speaker (shown with included stand)*

6. Connect the MF1 to the amplifier using the RCA cable (with CF filter attached).

For more information on the MF1 Speaker, see the [System 3 Manual](#).

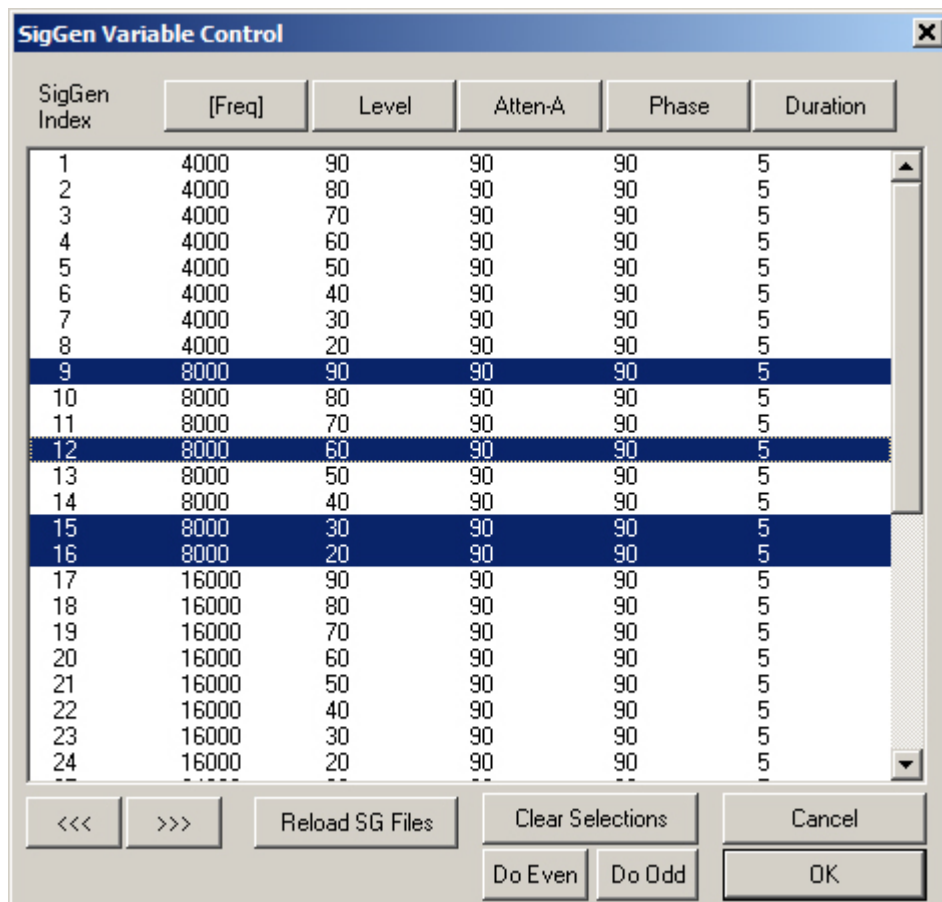
## Modifying the Stimulus

When the stimulus is defined, a complete list of signal parameters and variables are added to the BioSigRZ file. During stimulus presentation, the values of the variables change as a function of the SGI. This is called the stimulus schedule.

You may wish to present a subset of the current stimulus schedule. Both the stimulus schedule and the variable definitions can be modified in BioSigRZ. To do so, display the stimulus schedule (as below) then select the SGIs to be included in the subset for presentation.

### To view the current stimulus schedule:

- Select **Modify Schedule** from the **Operations** menu.
- Or select **SigGen Variables** from the **Setup** menu.
- Or click the **Modify Schedule** button.



The **SigGen Variable Control** dialog displays the current stimulus schedule. Variable values are shown for each SGI. Select SGIs to include in signal presentation by using standard Windows

techniques, such as click, SHIFT-click, and CTRL-click. You can also choose to 'Do Odd' or 'Do Even', using buttons in the dialog box. When no SGIs are selected, BioSigRZ will present the entire stimulus schedule. Otherwise, BioSigRZ will present only the selected SGIs.

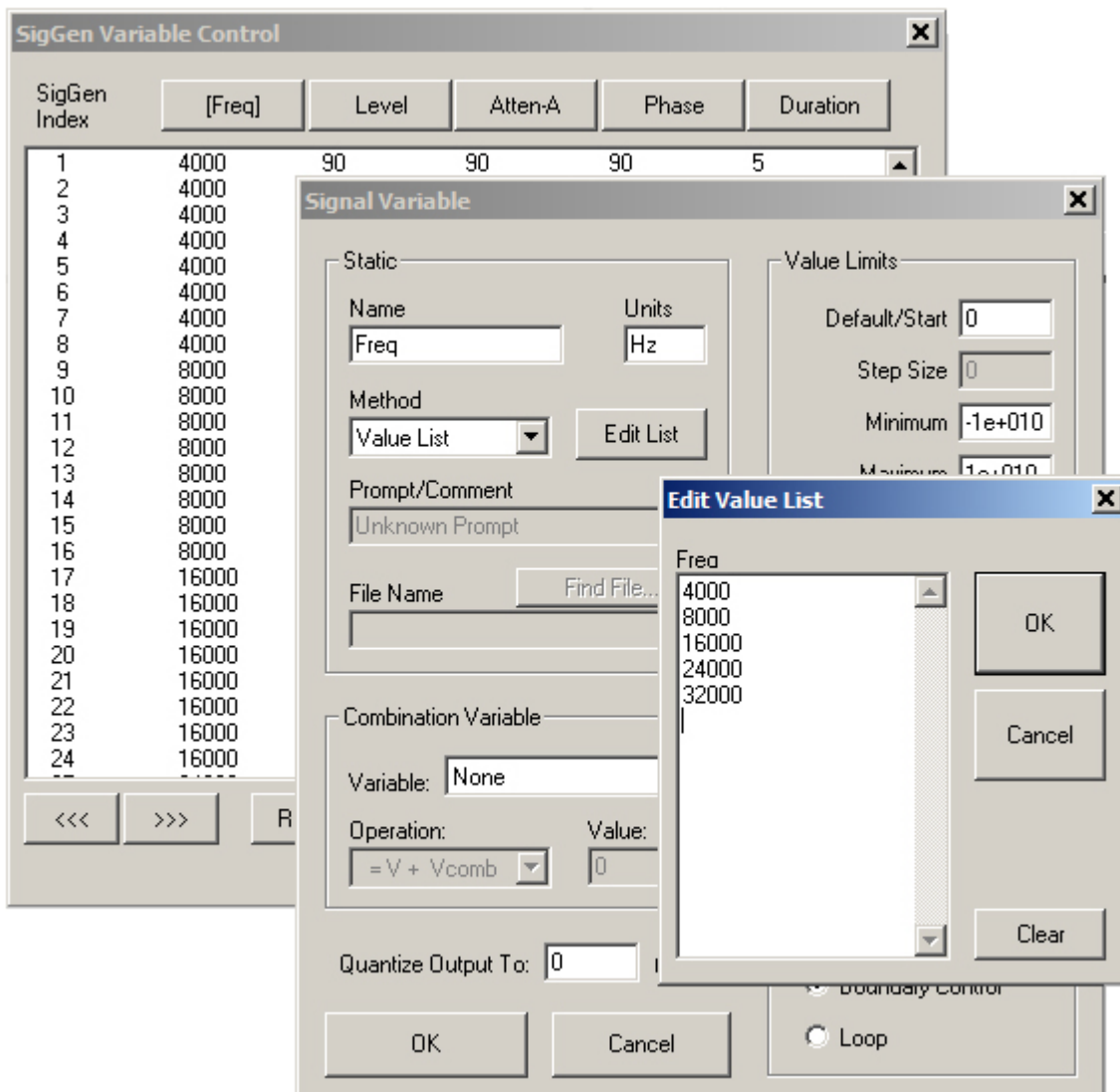
## Modifying Variables

The variables can also be modified to meet your needs.

### To modify a variable:

1. Click the *variable button* for the desired variable, such as **Freq**.
2. In the *Signal Variable* dialog box, make any changes then click **OK** to confirm and return to the *SigGen Variable Control*.

Different variables may have different editable options. For example, the variable frequency for a tone stimulus may be configured by entering a Value List (shown below).



 **Important**

The changes will only be saved in the configuration file, if the **Lock SigGen Files** check box is selected in the **Stimulus Setup** dialog box. If this check box is not selected, any changes to variables or SGI selections for presentation will be lost.

Also, if you select the **Reload Files** button in the Stimulus Channel Setup window, the changes will be overwritten in favor of the values in the .sig file.

The schedule tool was designed for quick edits you may want to make while testing or setting up a new paradigm. For permanent edits to your stimulus, TDT recommends using SigGenRZ (included with BioSigRZ installations) to modify the stimulus files (.sig) directly.

## Customizing the Stimulus

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SigGenRZ is a signal design software that enables you to create stimuli in a dialog box driven environment. Most importantly, it allows most settings to be used as variables for your experiments. All of the signals used in the BioSigRZ out-of-the-box experiment configurations have been created in SigGenRZ. It is also a helpful tool for modifying aspects of the stimulus that are not editable from within BioSigRZ.

**To modify a file directly in the SigGenRZ software you will need to follow these basic steps:**

1. Open the stimulus file (.sig) in SigGenRZ.
2. Make changes in the file.
3. Save the file or save the file with a new name.
4. Open the desired BioSigRZ configuration file (.acf).
5. Load or reload the stimulus file.
6. Save the BioSigRZ configuration file.



## Opening the File

The stimulus files (.sig) for the BioSigRZ out-of-the-box experiment configuration are stored at:

```
C:/TDT/BioSigRZ/SIG/
```

For DPOAEs there are two files: **DPOAE1.sig** and **DPOAE2.sig**.

It is generally a good idea to leave the original files unchanged and in place, but you can still use and modify these files then rename the file.

### To open the signal file you wish to modify:

1. Click the **Start** button on the Windows taskbar, point to **Programs**, point to **TDT Sys 3**, and click **SigGenRZ**.
2. Select **Open** from the **File** menu.
3. Click the desired file name.
4. Click **OK**.

### To save the file with a new name:

1. Select the signal by clicking anywhere in the signal window.
2. Choose **Save As** from the **File** menu.
3. The **Save As** dialog box will be displayed.
4. Type a new name for the signal and click **OK**.

SigGenRZ builds a signal from mini-signals called segments. The standard DPOAE stimuli have only one segment. Some settings affect the entire signal while others affect only a segment.

The most common changes to the signal settings include *Level*, *Frequency*, *F1*, and *F2*. These can be found in the **Signal Parameters** dialog box.

### To open the Signal Parameters dialog box:

- Click Signal from the Modify menu.

The most common changes to the segment parameters include: *Gate Type*, *Gate Time* and *Duration*. Parameter settings that define the segment can be modified in the **Edit Signal Segments** window.

**To access the Edit Signal Segment window:**

- Select **Modify** → **Segment** menu option in the main window.
- **Or** double-click the segment marker in the signal window.

## Converting dBV to dB SPL

---

The RZ6 Processor is a  $\pm 10$  V device and so levels described in BioSigRZ are inherently interpreted as dB Volts. When the microphone, which converts mechanical energy to a voltage, is known; you can use the gain settings in the software to convert the dB Volts to display values in dB SPL.

For example, assume you have a peak of -97.48 dBV you'd like to convert. You can find the linear voltage with  $V = 10^{(\text{dBV}/20)}$  and then compare this to the Etymotic microphone response at 93.9 dB (.05) to get a dB value relative to 93.9, and then add 93.9. So the resulting equation is:

$$20 * \log([10^{(\text{dBV}/20)}] / .05) + 93.9$$

For -97.48 dBV the output would be:

$$20 * \log([10^{(-97.48/20)}] / .05) + 93.9 = 22.44 \text{ dB}$$

The [Acquisition Settings](#) section discusses where to apply this type of conversion.

### Tip

Notice that the starting dBV value +120 = 22.52, which is a close approximation to the actual value of 22.44 dB. So, when you are viewing a plot that uses dBV, you can use +120 to reach a quick approximation of the dB SPL.