

ULTRASONIC VOCALIZATION DETECTOR

ANL-937-1

SOF-937-1

USER'S MANUAL

DOC-043

Rev. 1.6

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CHAPTER 1 | INTRODUCTION

Empirical evidence suggests that ultrasonic vocalization (USV) can be used as a measure of motivational and emotional state (Knutson et al. 2002). For instance, rodent pups emit *distress* USVs as a consequence of maternal severance or hypothermia (Blumberg & Alberts, 1990; Hofer & Shair, 1993; Sokoloff & Blumberg, 2005) and maternal responsiveness is proportional to the number of calls mouse pups emit (D'Amato et al. 2005). Brudzynski & Pniak (2002) observed that mature laboratory rats emit USVs in anticipation of social contact. Furthermore, USVs are produced as a result of painful stimulation during fear conditioning (Antoniadis & McDonald, 1999; Lee et al, 2001) and as a result of morphine or ethanol withdrawal (Vivian & Miczek, 1991; Moy et al. 2000, respectively). Although the empirical evidence is somewhat inconclusive, one theory of motivation states that rat USVs can be divided between 2 different kilohertz bandwidths. Vocalizations ranging between 50-70 kHz represent positive affective states, but those in the 22 kHz range are characteristic of negative affective states (Knutson et al. 2002). Whether or not this theory continues to garner empirical support, the evidence is clear that USVs are good indicators of underlying motivational state.

USVs can be divided into four general classes: (1) distress calls, (2) long calls, (3) short calls, and (4) alarm calls. Neonatal rat pups emit *distress* USVs at the average frequency of 40 kHz (Sokoloff & Blumberg, 2005) and this frequency decreases with age. Mature rats produce long calls that are just above the audible range at 22 kHz (65-85 db), and these calls last for 300-4000 ms and have a narrow bandwidth of 1-6 kHz (Sales 1972). Long calls are associated with sexual behavior and aggression (Barfield et al 1979). Short calls (32-96 kHz, 30-65 ms) occur during high levels of physical activity, arousal, and social contact (Knutson et al. 1998). Finally, alarm calls (22 kHz) communicate the presence of a predator or are produced as the result of experiencing a painful event (Blanchard et al. 1991; Antoniadis & McDonald, 1999; respectively).

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Dependent Variables

The Med Associates, Inc. **ANL-937-1 Ultrasonic Vocalization (USV) Detector** is designed to capture the occurrence of ultrasonic vocalizations to provide quantitative data on a subject's motivational, emotional, or communicative state. The USV detector samples ultrasonic frequencies every 30 milliseconds and differentiates between two user-defined bandwidths.

MED-PC® can be used in conjunction with the USV detector to turn on the detector, and correlate events such as food or shock delivery with the USV data acquisition. See **Chapter 8, Using MED-PC® Inputs/Outputs** for details.

USV Occurrences

The USV data illustrate the onset and duration of vocalizations occurring within two user-defined frequency bands above an amplitude threshold. Refer to **Chapter 5, Capturing Ultrasonic Data**, for more information.

Sonogram

The Med USV Viewer data analysis utility included with the Med USV Detector produces a sonogram that graphs USV occurrences in kilohertz over time. Thus, the sonogram provides information on the duration and frequency of a USV, as well as relative changes in pitch. See **Chapter 7, The MED USV Viewer**, for more information on the sonogram analysis.

Spectrogram

The Med USV Viewer can also produce a spectrogram which is a three-dimensional surface plot that illustrates frequency, in kilohertz, by time and by amplitude in decibels. The spectrogram is particularly well suited to revealing ultrasonic artifacts in the testing environment, such as ultrasonic vibrations created by some fluorescent lamps or computer monitors. Once artifacts are recognized, the bandwidth can be adjusted to isolate USVs from other ultrasonic events. See **Chapter 6, Viewing The Spectrogram** and **Chapter 7, The MED USV Viewer** for details.

Cumulative Record

Data can be viewed as an event plot or in a cumulative fashion to represent the rate of USV occurrences. Each occurrence of an ultrasonic vocalization causes an increase on MED-PC®'s *SoftCR Pro*® cumulative plot. Med Associates provides the cumulative record as a method of estimating the rate of USV occurrences. Refer to **Chapter 8, Using MED-PC® Inputs/Outputs**, for further information on how to integrate MED-PC® with the USV Detector.

CHAPTER 2 | SOFTWARE INSTALLATION

Software Installation

If the computer was purchased as part of USV Detector system from Med Associates, Inc., the **SOF-937-1 USV Detector** software and driver installation was completed at the factory. If the computer was not purchased from Med Associates or was not a part of the system, follow the instructions below to install the drivers and software.

Before beginning the software installation, phone, fax or e-mail Med Associates with the registration information in order to receive the software installation password. This password will be necessary during the software installation process.

Insert the **SOF-937-1 Ultrasonic Vocalization Detector** software CD into the CD drive and at the welcome screen click **Install**. If the CD does not auto-start, navigate to the CD drive in Windows Explorer and double click the "autorun.exe" file and click Install at the welcome screen. Successful installation of each component will be indicated by a green check mark, a red X will indicate an unsuccessful installation. When prompted enter the user name, company name and software installation password. When the installation completes, click **Finish**.

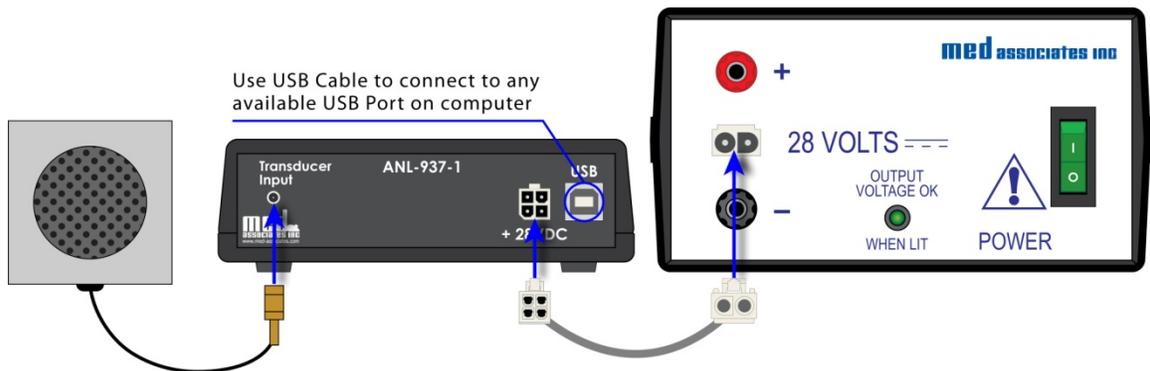
CHAPTER 3 | WIRING GUIDE

Stand-Alone Operation

NOTE: Do not connect or disconnect the unidirectional microphone when the ANL-937-1 is connected to 28-volt power. Doing so may cause damage to the detector.

Refer to Figure 3-1 when completing the following steps.

Figure 3-1 - Stand Alone Operation Connections



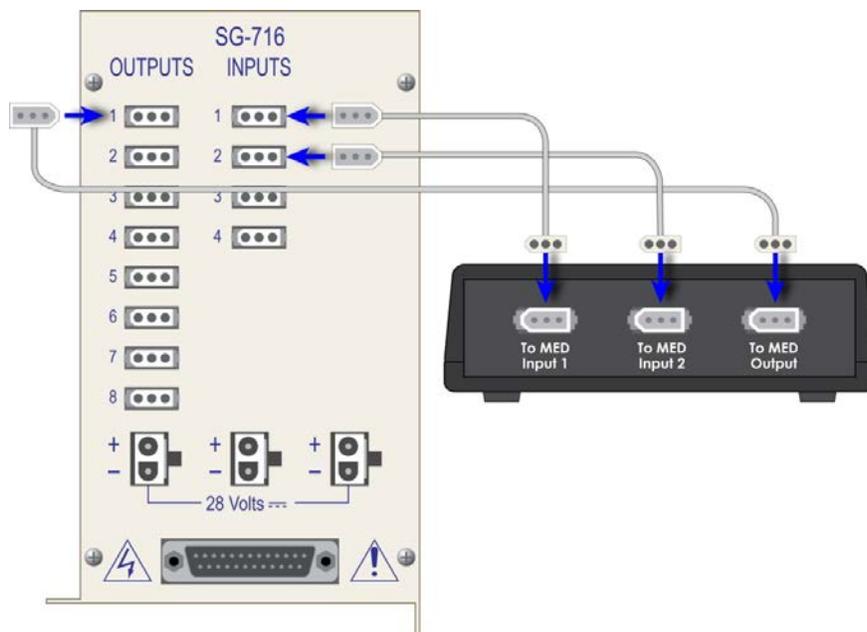
1. Connect the **USB** port on the ANL-937-1 USV Detector to any available USB port on the computer using the USB cable provided.
2. Connect the unidirectional microphone to the **Transducer Input** connector on the ANL-937-1 USV Detector.
3. Connect the **+28 VDC** connector on the ANL-937-1 USV Detector to the SG-501 power supply using the SG-937P-10 power cable.

MED-PC® Controlled Operation

NOTE: Do not connect or disconnect the unidirectional microphone when the ANL-937-1 is connected to the MED Inputs on the connection panel. The ANL-937-1 receives +28 VDC power from these connections. Doing so may cause damage to the microphone.

Follow the instructions in the **MED-PC® User's Guide, DOC-010**, for information on hardware and software installation and hardware configuration. All general information pertaining to translating and compiling the ANL_937.mpc program, and modifying the MSN code, can be found in the **MED-PC® Programmer's Manual, DOC-003**. Refer to Figure 3-2 when following the steps below.

Figure 3-2 – MED-PC® Connection Panel Connections



1. Connect the unidirectional microphone to the **Transducer Input** connector on the ANL-937-1 USV Detector, as shown in Figure 3-1.
2. Connect the **To MED Input 1** connector (Band 1) on the ANL-937-1 to Input 1 on the SG-716B Connection Panel using a 3-pin Molex cable.
3. Connect the **To MED Input 2** connector (Band 2) on the ANL-937-1 to Input 2 on the SG-716B Connection Panel using a 3-pin Molex cable.
4. Connect the **From MED Output** connector on the ANL-937-1 to Output 1 on the SG-716B Connection Panel using a 3-pin Molex cable.
5. For FFT Data collection, connect the **USB** port on the ANL-937-1 USV Detector to any available USB port on the PC using the USB cable provided.

CHAPTER 4 | DEVICE IDS AND OFFSETS

Both the Device IDs and offsets are factory preset, but if for any reason these values need to be changed or if a new ANL-937-1 is being added to the system, the following steps explain the procedure.

1. Ensure that none of the ANL-937-1 devices are connected to the USB port on the computer and open the USV Application software. The prompt shown in Figure 4-1 will appear. Connect the first device to the USB port. This device will automatically be assigned Device ID 1.

Figure 4-1 - Setup of the ANI-937-1



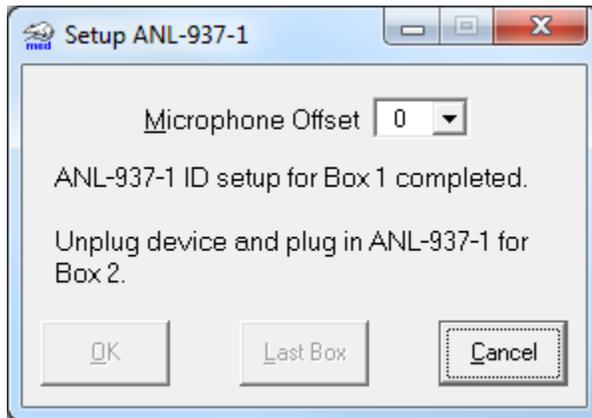
2. Once the device is connected and has been recognized by the software, the screen shown in Figure 4-2 will appear. Select the **Microphone Offset** from the pull down menu (This number is marked on each microphone). If there are additional devices to setup, select **OK**. If this is the only box to setup, select **Last Box**.

Figure 4-2 - Selecting the Device Offset



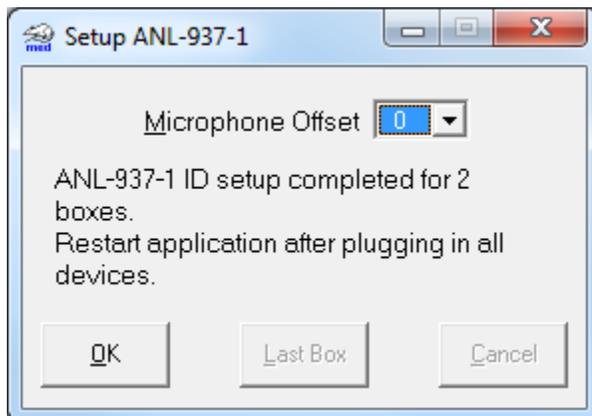
3. If **OK** was selected from the step above, the screen shown in Figure 4-3 will appear. Follow the prompt by disconnecting Device ID 1 from the USB port and plug in the next ANL-937-1. This device will automatically be assigned Device ID 2.

Figure 4-3 - Setup for Second ANL-937-1



4. Follow steps 1-3 for each additional ANL-937-1 and select **Last Box** when the offset has been selected for the final device. The screen shown in Figure 4-4 will appear. Select **OK** and connect all of the ANL-937-1 devices that have been setup. When the application is restarted, the Device ID tabs will be displayed, as shown in Figure 5-1.

Figure 4-4 - ANL-937-1 Setup Complete



CHAPTER 5 | CAPTURING ULTRASONIC DATA

Upon launching the MED USV application the screen in Figure 5-1 will appear if the ANL-937-1 is running current firmware. ANL-937-1s running older firmware will have a display as presented in Figure 5-2 where the **All FFT Data** and **Maximum Band Amplitude** options are grayed out.

Enter the desired values in the **Low (kHz)**, **High (kHz)** and **Threshold (dB)** fields. In Figure 5-1, “Band 1” will capture ultrasonic vocalizations occurring between 20 and 50 kHz, if they occur at 40 dB or higher. “Band 2” will capture vocalizations occurring between 50 and 70 kHz at 40 dB or higher. In this example a vocalization of 50 dB at 50 kHz will fall within the parameters set for both bands.

Once the values for bands 1 and 2 and the threshold are set, set the **Save FFT Data** option, the **Threshold Criteria** option, and the **Enable MED Input** option. Then click the **Update** button to apply the settings.

The MED USV software can be used with up to 16 devices. To configure each device independently, select the device to configure by clicking on the Device ID tab. To configure all devices with the same settings, select **Configure Parameters > Set All**, make a selection and then click the **Update** button.

NOTE: Acceptable band frequencies range from 20–100 kHz. The amplitude threshold limits are between 10-150 dB. Bands 1 and 2 may overlap.

After clicking **Update**, click the **Browse** button to supply a data file name and save to location. Then click **Start** to begin data acquisition.

Figure 5-1 - USV Application Display

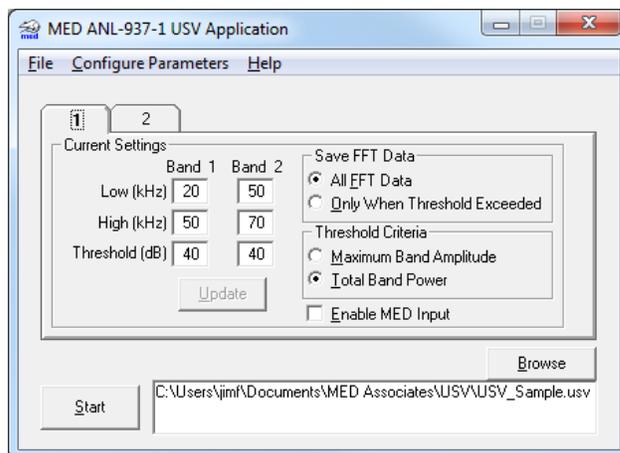
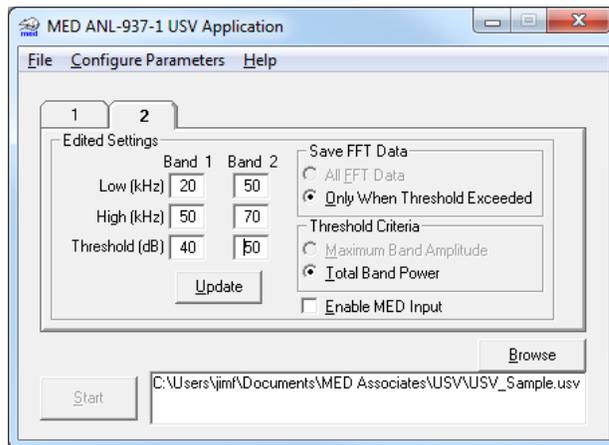


Figure 5-2 - USV Application Display With Disabled Items



Menu Options

- **File Menu**
 - **Exit:** Exits the MED USV application.
- **Configure Parameters Menu**
 - **Set All:**
 - **Med Input:**
 - **Disable:** The MED Input will be disabled for all devices.
 - **Enable:** The MED Input will be enabled for all devices.

NOTE: Enable MED Input should only be selected if the ANL-937-1 is being used in conjunction with the optional MED-PC® software. Refer to **Chapter 8, Using MED-PC® Inputs/Outputs**, for more information.

- **FFT Data:**
 - **All FFT Data:** All FFT Data will be saved.
 - **Only When Threshold Exceeded:** FFT data will only be saved when the threshold is exceeded.
- **Threshold Criteria:**
 - **Maximum Band Amplitude:** The dB threshold is calculated using the maximum band amplitude.
 - **Total Band Power:** The dB threshold is calculated using the total band power.
- **Same As:** Sets the same parameters for all connected devices based on the currently selected device. The **Update** button must be clicked on each device, or the **Configure Parameters > Update All** menu option must be selected to apply the settings to the hardware.
- **Update All:** Parameter settings will be sent to all devices with an enabled **Update** button and valid band settings. Any invalid settings will cause a warning message to appear, and the **Update All** command will not be completed. Correct any invalid settings and select **Update All** again.

- **Reset All:** The software will reset the settings on all connected ANL-937-1s to the default values and update the Device ID tabs. This selection should be made whenever a new ANL-937-1 is connected to the computer, or if a Device ID tab is not appearing.
- **Help Menu**
 - **ANL-937-1 Manual:** Opens the .pdf version of this manual.
 - **About...:** Provides the Ultrasonic Vocalization Detector software version information.

Data Collection Options

- **Low (kHz):** Vocalizations at frequencies below this cut-off value will be disregarded by the software. The valid frequency range is between 20 kHz and 100 kHz.
- **High (kHz):** Vocalizations at frequencies above this cut-off value will be disregarded by the software. The valid frequency range is between 20 kHz and 100 kHz.
- **Threshold:** Vocalizations must occur at an amplitude greater than this value in order to be recorded by the software. The valid range is between 10 dB and 150 dB.
- **Band 1:** The first frequency range of interest.
- **Band 2:** The second frequency range of interest.
- **Update:** After making changes to the options click the **Update** button to apply the changes to the hardware. If any settings are invalid, an error message indicating which setting is incorrect will appear.
- **Save FFT Data:**
 - **All FFT Data:** Records all FFT data for the experiment duration.
 - **Only When Threshold Exceeded:** Only records FFT data when the amplitude threshold has been exceeded.
- **Threshold Criteria:**
 - **Maximum Band Amplitude:** The dB threshold is calculated using the maximum band amplitude.
 - **Total Band Power:** The dB threshold is calculated using the total band power.
- **Enable MED Input:** Check the **Enable MED Input** box to facilitate the starting and stopping of the ANL-937-1 from within a MED-PC® protocol.

NOTE: Select Enable MED Input only if the ANL-937-1 is being used in conjunction with the optional MED-PC® software. Refer to **Chapter 8, Using MED-PC® Inputs/Outputs**, for more information.

- **Browse:** Click the **Browse** button to set the data file name and save to location. The data file information must be set to enable the **Start** button.
- **Start:** Click **Start** to begin data collection.
- **Stop:** Click **Stop** at any point after clicking **Start** to discontinue data collection.

NOTE: Data will be automatically saved as a “*.bin” file. For instructions on how to access the *.bin file, refer to **Chapter 6, Viewing the Spectrogram** and **Chapter 7, The MED USV Viewer**.

CHAPTER 6 | VIEWING THE SPECTROGRAM

The *.bin files created during USV data acquisition can be accessed using the provided macros for Microsoft Excel® or with the **Med USV Viewer** (see **Chapter 7, The MED USV Viewer**). The macros include a graphing utility that creates the spectrogram.

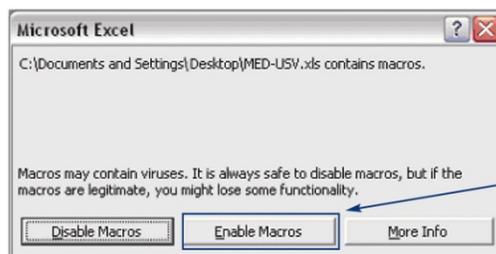
Open Microsoft Excel® and select **File > Open**, and browse to **C:\Program Files\Med Associates\Ultrasonic Vocalization** or **C:\Program Files(x86)\Med Associates\Ultrasonic Vocalization** depending on your operating system. If using Microsoft Excel® 2000 or 2003, select **MED-USV_2000_v2.xls** and click **Open**. If using Microsoft Excel® 2007 or higher, select **MED-USV_2007_v2.xls** and click **Open**.

Opening a Macro

Microsoft Excel® 2000 or 2003

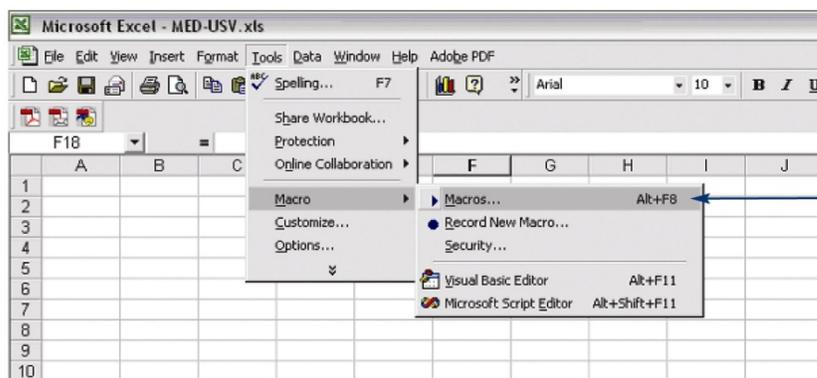
After opening MED-USV_2000_v2.xls, the screen shown below should appear. Click **Enable Macros**. If this screen does not appear, select **Tools > Macro > Security**, and set the security level to “medium.”

Figure 6-1 - Enable the Excel® Macro



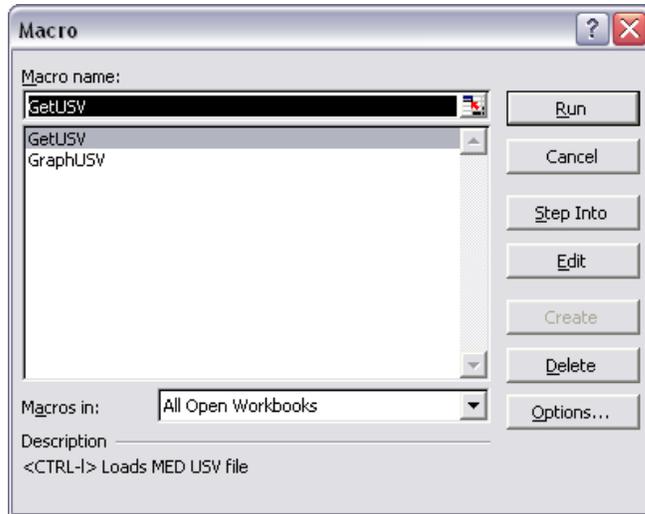
Once the Excel® workbook is open, select **Tools > Macro > Macros....**

Figure 6-2 - Select the Macro Function



Select the macro named **GetUSV**, and click **Run**. Then select the *.bin data file of interest.

Figure 6-3 - Using the "GetUSV" Macro



Microsoft Excel® 2007 or higher

Open **MED-USV_2007_v2.xls** in Excel® the screen Figure 6-4 will appear. Depending on the security settings, a security warning may appear, as shown below. If it does, click **Options...** and the screen shown in Figure 6-5 will appear. Click **Enable this content**, then click **OK**.

Figure 6-4 – Excel Security Warning

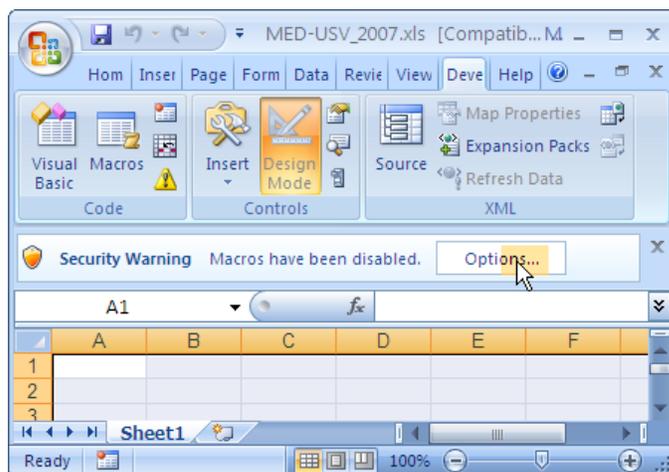


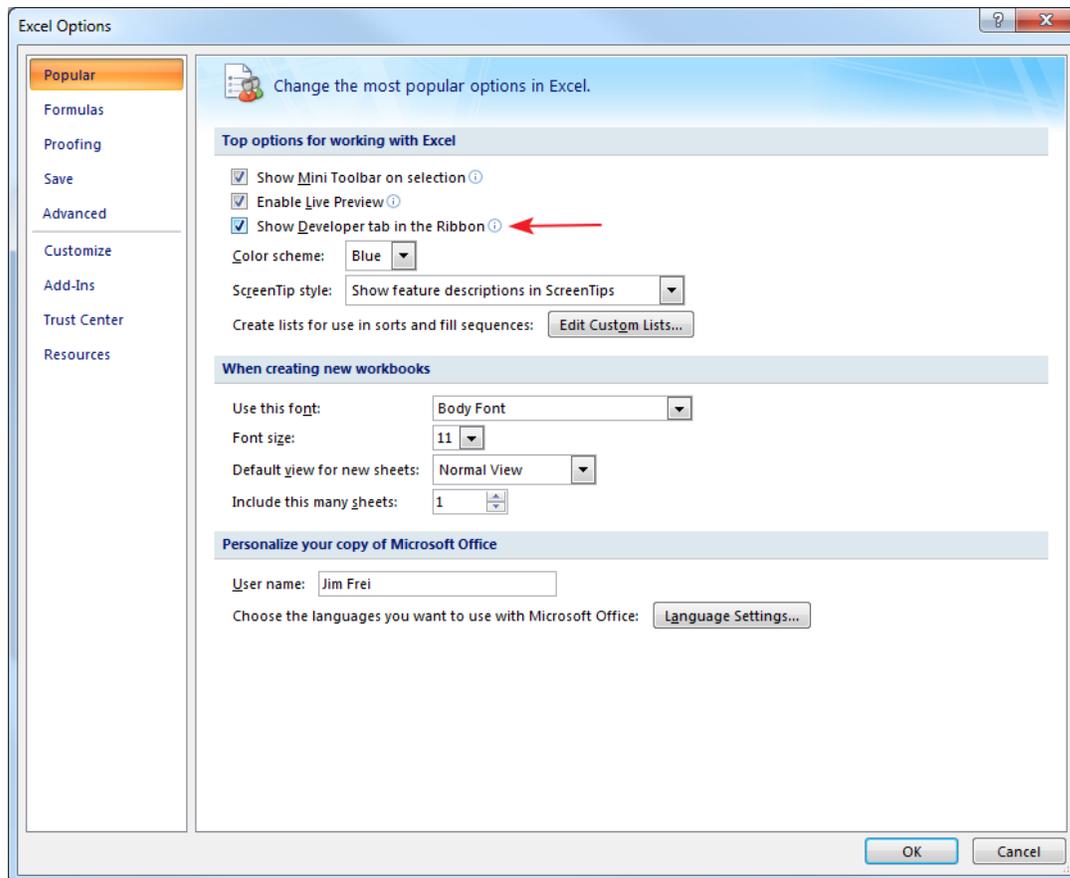
Figure 6-5 – Excel 2007 Security Alert



The menu options that allow the user to run and record macros appear on the Developer tab, which is not visible by default. In order to view the Developer tab, click on the Office Button, and then click on the **Excel Options** button. The screen shown in Figure 6-6 will appear.

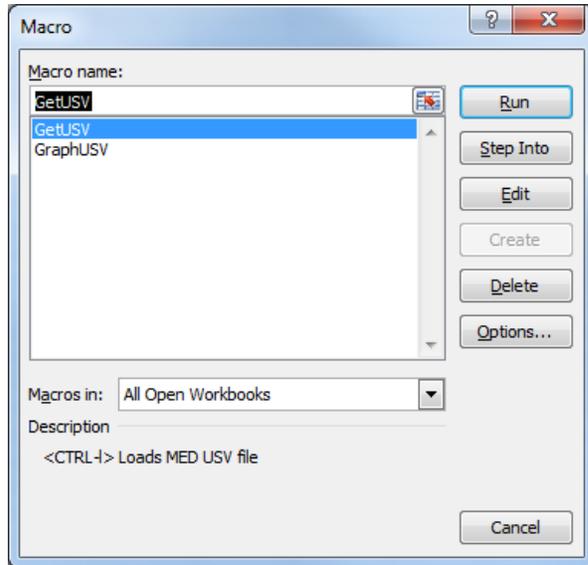
Enable **Show Developer tab in the Ribbon** and click **OK**. The Developer tab should now appear on the ribbon.

Figure 6-6 – Excel 2007 Options Screen



Click the **Macros** button on the Developer tab and the screen in Figure 6-7 will appear. Highlight the macro named **GetUSV** and click **Run**. Select the *.bin data file of interest and click **Open**.

Figure 6-7 – Excel 2007 Macro Menu



If the screen in Figure 6-7 does not appear, click on the **Macro Security** button in the Code group (Figure 6-8) and select **Disable all macros with notification** (Figure 6-9) and click **OK**.

Figure 6-8 - Macro Security Button

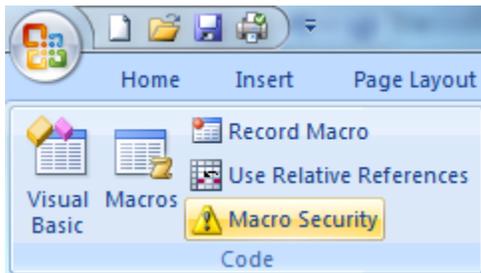
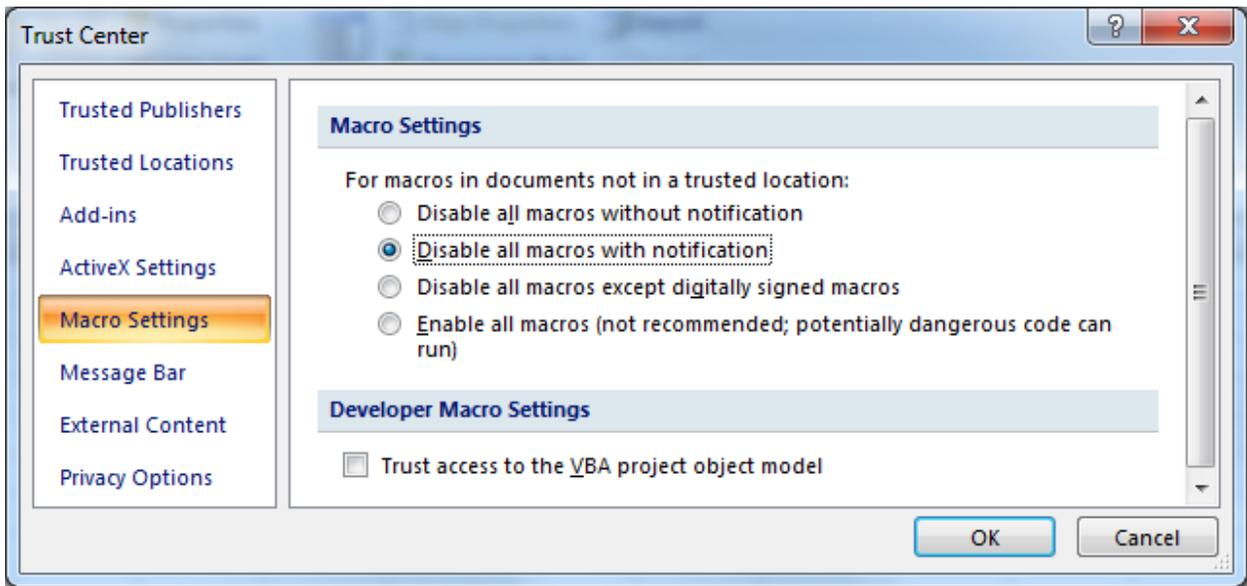


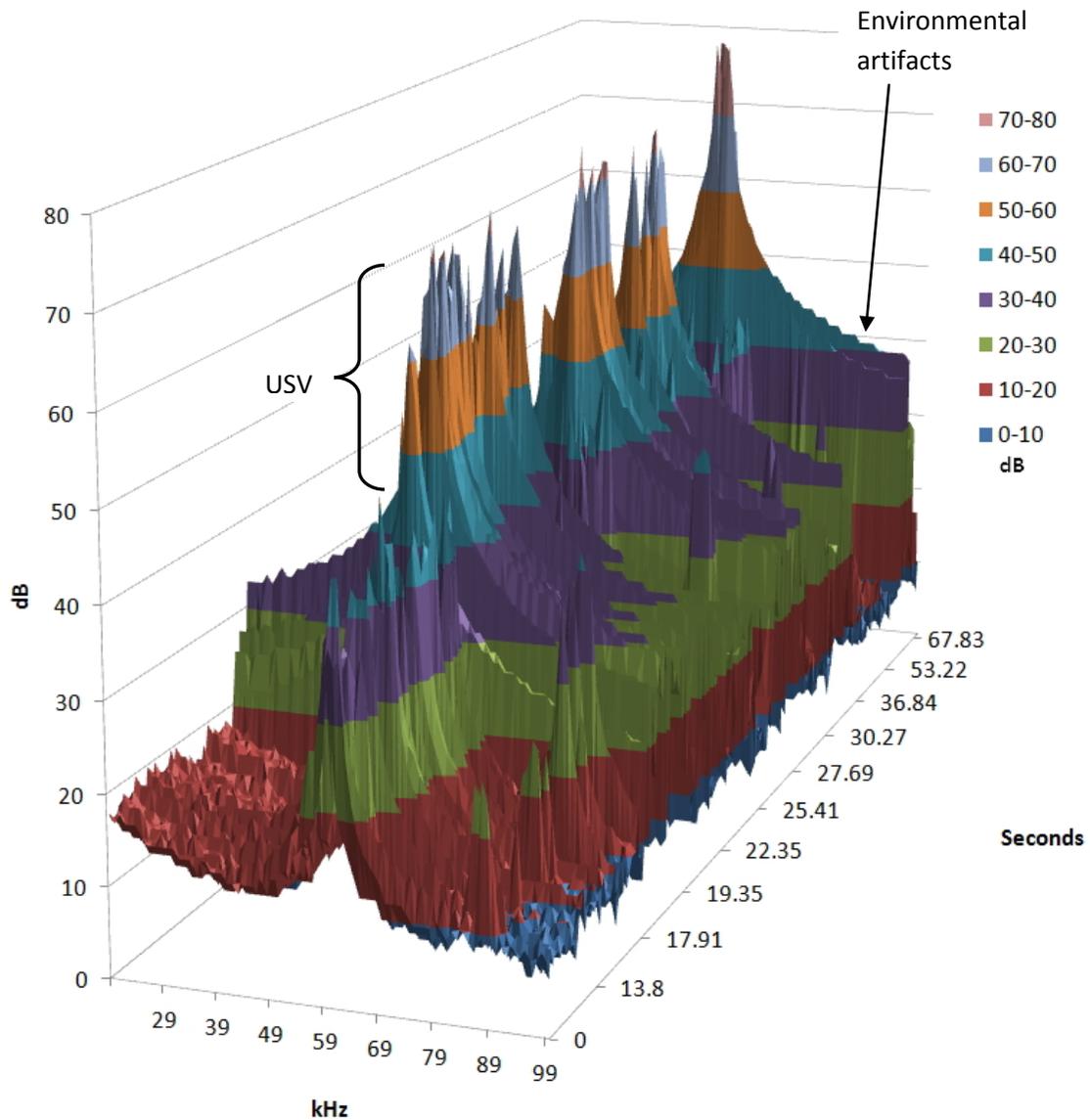
Figure 6-9 - Macro Settings Screen



The Spectrogram Macro "GetUSV"

Upon opening a *.bin data file, the spectrogram will automatically appear as an Excel® Workbook. Each spectrogram is viewed as a "chart" worksheet, and the corresponding raw data are listed on the subsequent spreadsheet.

Figure 6-10 – Sample Spectrogram Analysis Using GetUSV Macro



NOTE: The spectrogram macro utilizes the “surface plot” from Microsoft Excel® that is limited to 255 continuous cells; therefore, the spectrogram macro is limited to the first 7.5 continuous seconds of an experimental session. To view ultrasonic vocalizations that may occur after 7.5 seconds, use the “GraphUSV” macro described below.

Using the “GraphUSV” Macro

The *.xls worksheet contains the data from which the spectrogram was created. To view a specific portion of the data as a spectrogram, select the portion of cells in column D (Figure 6-11) that correspond to the interval that is of interest.

The “GraphUSV” macro can also be used to view ultrasonic events that occur after the first 7.5 continuous seconds of data acquisition (see the **NOTE** above). Because of the limitations imposed by Microsoft Excel®, only 255 total Excel® cells can be plotted in any single spectrogram. Open “GraphUSV” in the same manner as described in the **Opening a Macro** section of this manual.

Figure 6-11 - Use “GraphUSV” to View a Portion of the *.xls Data

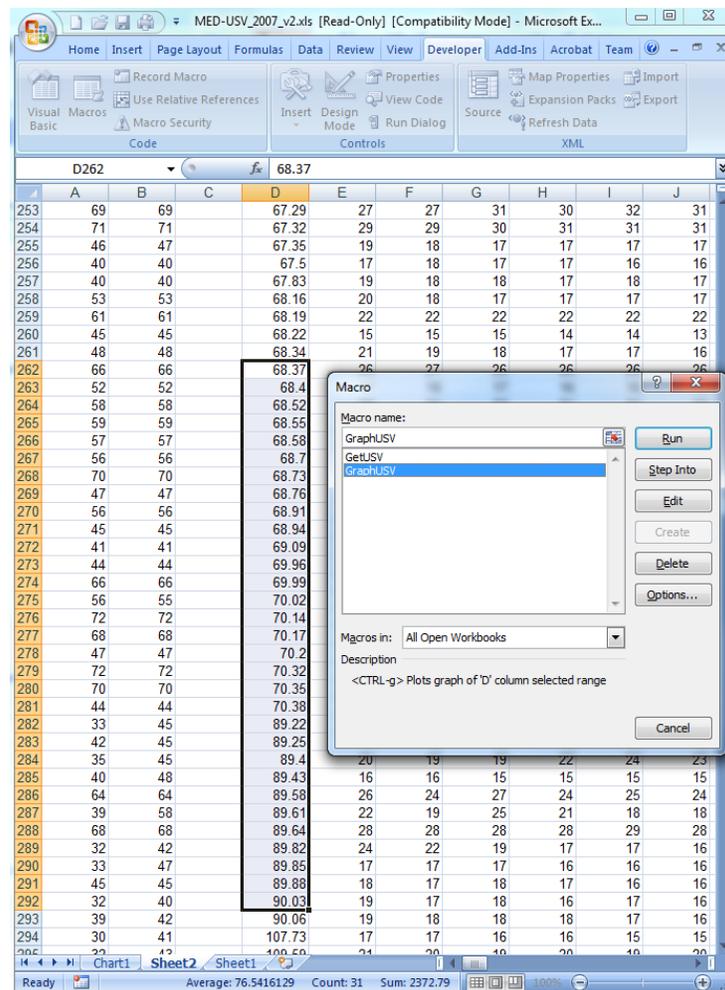
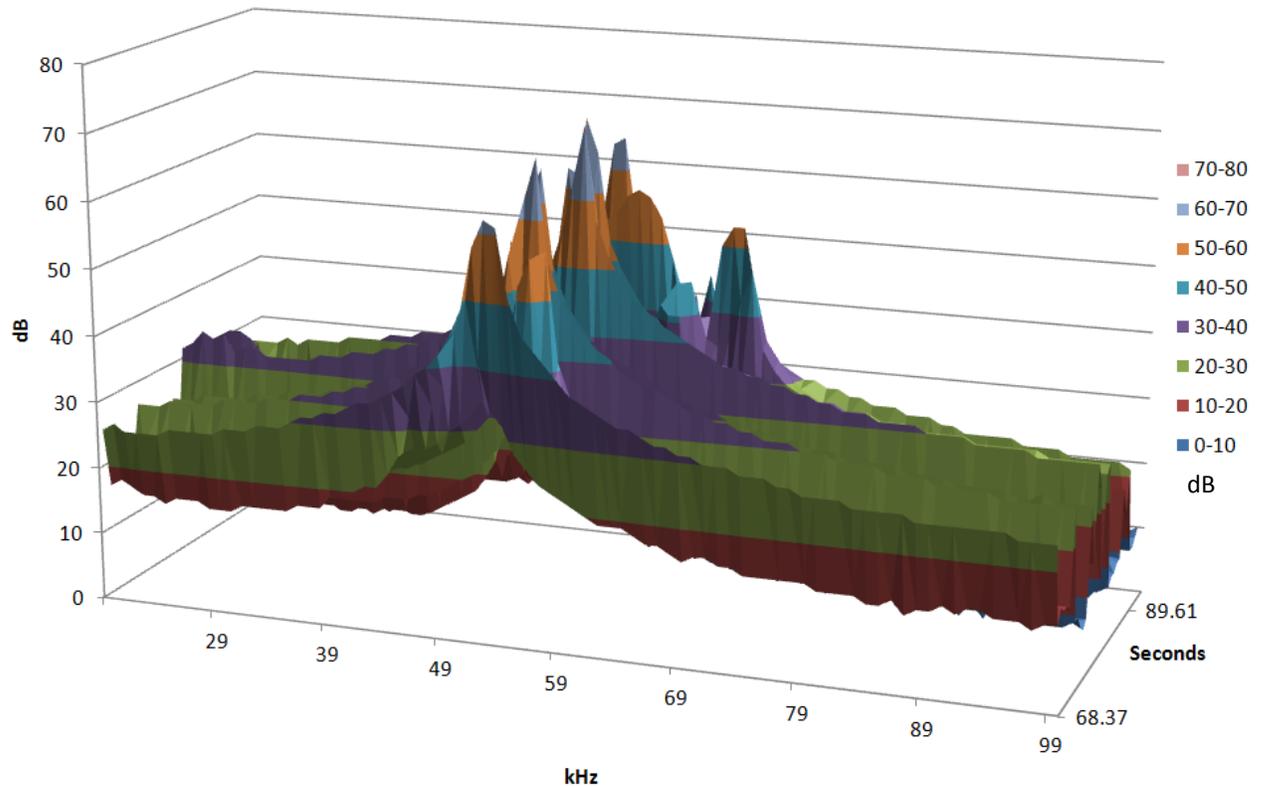


Figure 6-12 - The Spectrogram Resulting From the "GraphUSV" Macro



Comparison of Data Options

The Ultrasonic Vocalization Detector software allows the user to set the threshold value based on the Maximum Band Amplitude (MBA) or the Total Band Power (TBP) as well as selecting whether to record all FFT data or to only record FFT data while the threshold is met or exceeded. The following figures were generated with sample data files that were not produced with live subjects, but were produced in an attempt to illustrate the differences between the data options.

Figure 6-13 provides an example of sample data collected using a 40dB threshold based on the MBA, and all FFT data was collected. In Figure 6-14 the FFT data is recorded only when the 40 dB threshold is met or exceeded. One can view the “noise” before and between the ultrasonic vocalizations in Figure 6-13 and not in Figure 6-14.

Selecting to record all FFT data or only recording FFT data at or above the threshold using the TBP value produces similar results.

Figure 6-13 – MBA, 40 dB Threshold, All FFT Data Recorded

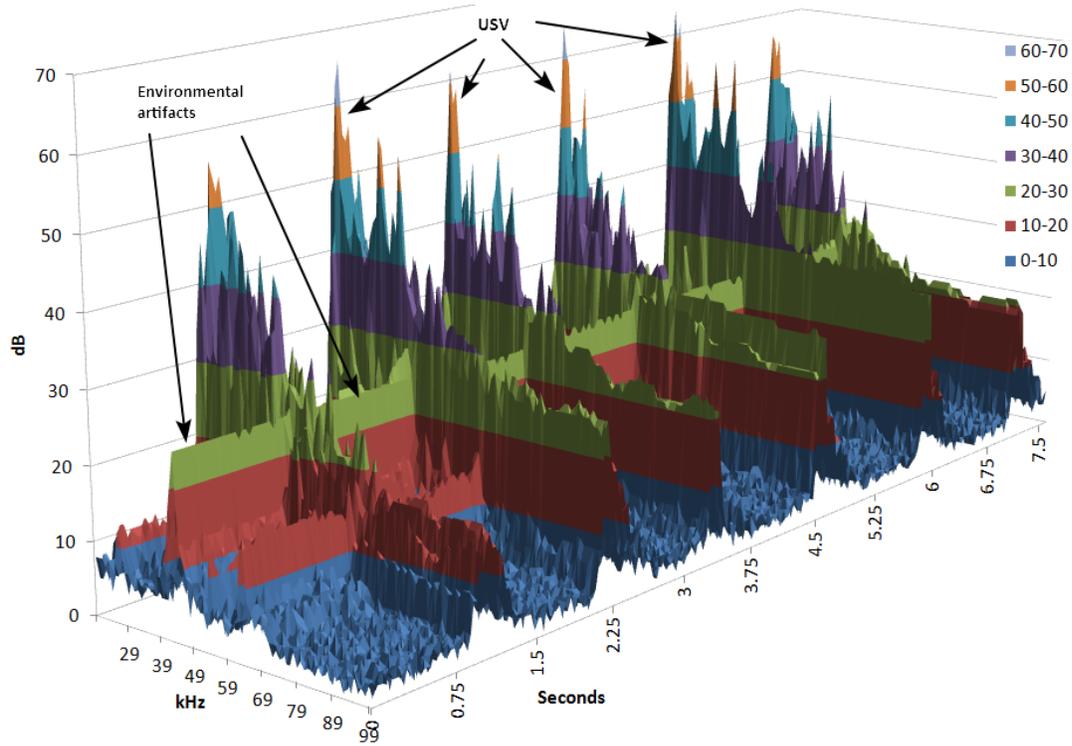
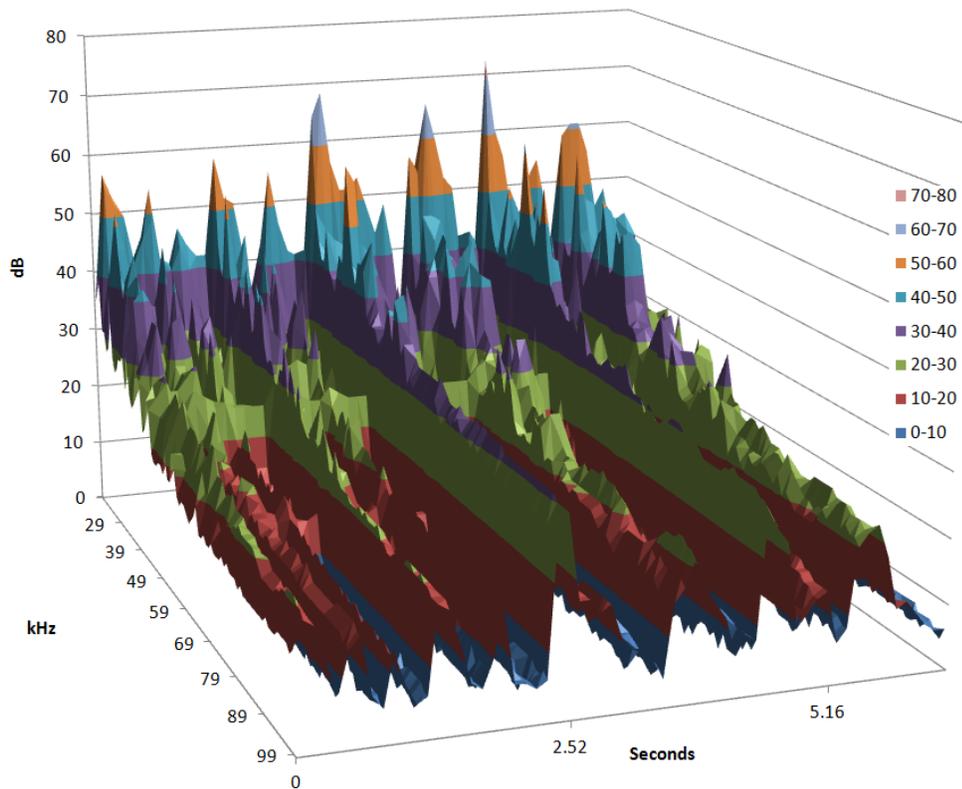


Figure 6-14 - MBA, 40 dB Threshold, FFT Data Only Recorded At Or Above Threshold



The *.xls Data File

View the Excel® raw data created from the *.bin file by selecting the spreadsheet corresponding to a specific spectrogram chart. Figure 6-15 illustrates a portion of a sample data file that is created when a spectrogram is created. Refer to the explanation following Figure 6-15.

Figure 6-15 - Sample .xlsx Data File

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	#1-C:\Users\Sample USV\A7 pup-4_01.bin													
2	1/12/2016 11:03													
3	20	60	40											
4	50	90	40											
5														
6					20	21	22	23	24	25	26	27	28	29
7				0	18	17	18	17	17	17	16	15	15	15
8				0.03	17	17	16	16	16	16	15	14	14	14
9				0.15	17	18	17	16	16	15	15	15	15	16
10				0.18	17	17	16	15	15	15	15	15	14	14
11				3.45	18	19	18	18	18	17	16	16	16	16

- 1.) Data file name.
- 2.) Date and time data file was recorded.
- 3.) Band 1 frequency range and amplitude threshold.
- 4.) Band 2 frequency range and amplitude threshold.
- 5.) Band 1 power (dB) during the vocalization. This is based on the maximum band amplitude or the total band power.
- 6.) Band 2 power (dB) during the vocalization. This is based on the maximum band amplitude or the total band power.
- 7.) Time at which the vocalization occurred in seconds from the start of data acquisition.
- 8.) Row 6 is the entire frequency range from 20 kHz to 100 kHz. The values under each frequency are the amplitudes at which the frequency occurred during that particular vocalization.

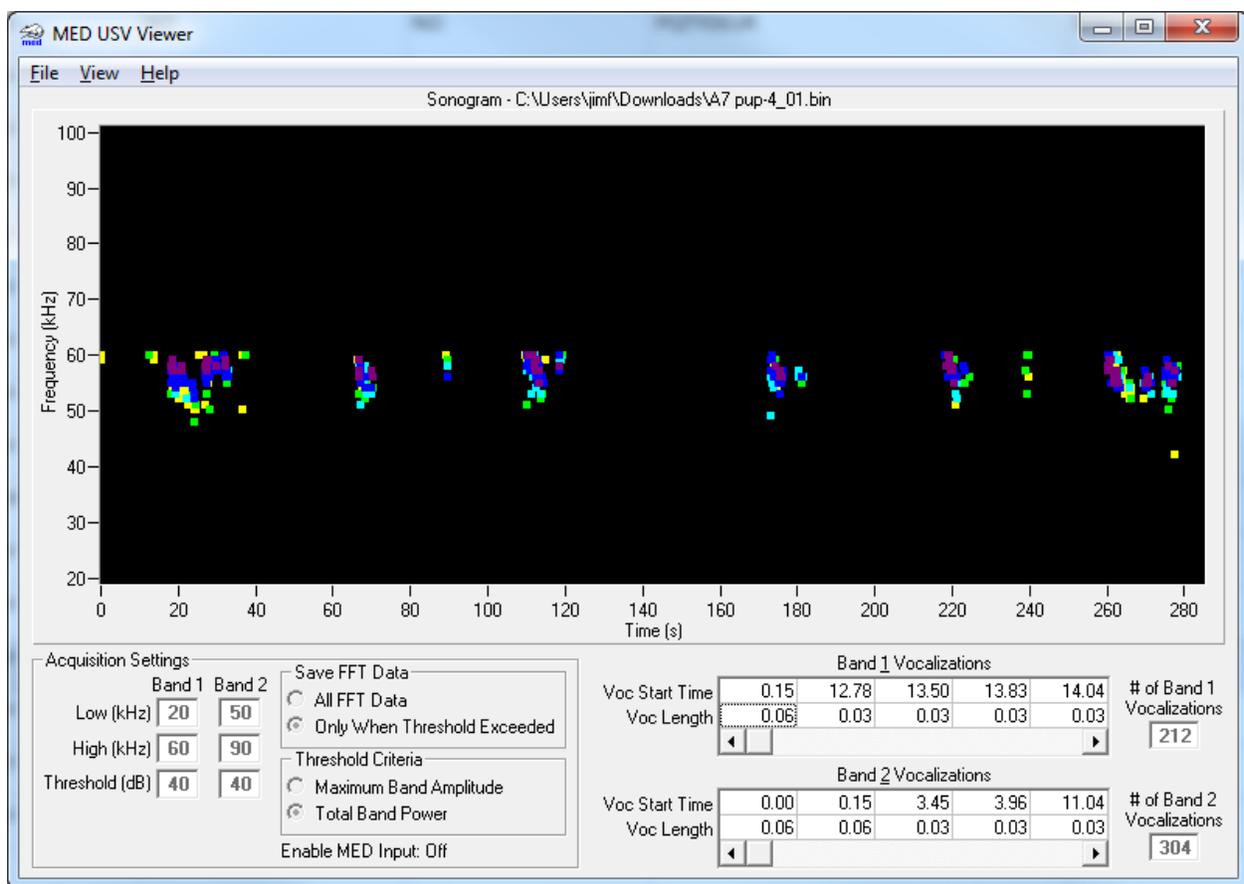
CHAPTER 7 | THE MED USV VIEWER

The MED USV Viewer may be used to produce a spectrogram or a sonogram to view the quantity and decibel level of USVs in each user-defined bandwidth. Any *.bin data file created by the ANL-937-1 can be examined with the USV Viewer.

Opening a *.bin file with the USV Viewer

After opening the MED USV Viewer click the **View** menu and select **Sonogram** or **Spectrogram** to view either display. Then click **File > Open File**, navigate to and select the *.bin file to be used and click **Open**. Figure 7-1 is a sample sonogram. Click **View** and select the other view at any time to switch views. Figure 7-3 is the spectrogram of the same sample data used in Figure 7-1.

Figure 7-1 - Sonogram of Sample Data



While viewing a sonogram click **View > Scroll Forward**, or press F8, to scroll forward through the timeline. Click **View > Scroll Back**, or press F7, to scroll backward through the timeline.

NOTE: The sonogram in the MED USV Viewer plots only the maximum kHz value for each band for any given time point; therefore, two data points (band 1 & 2) may exist at any given time.

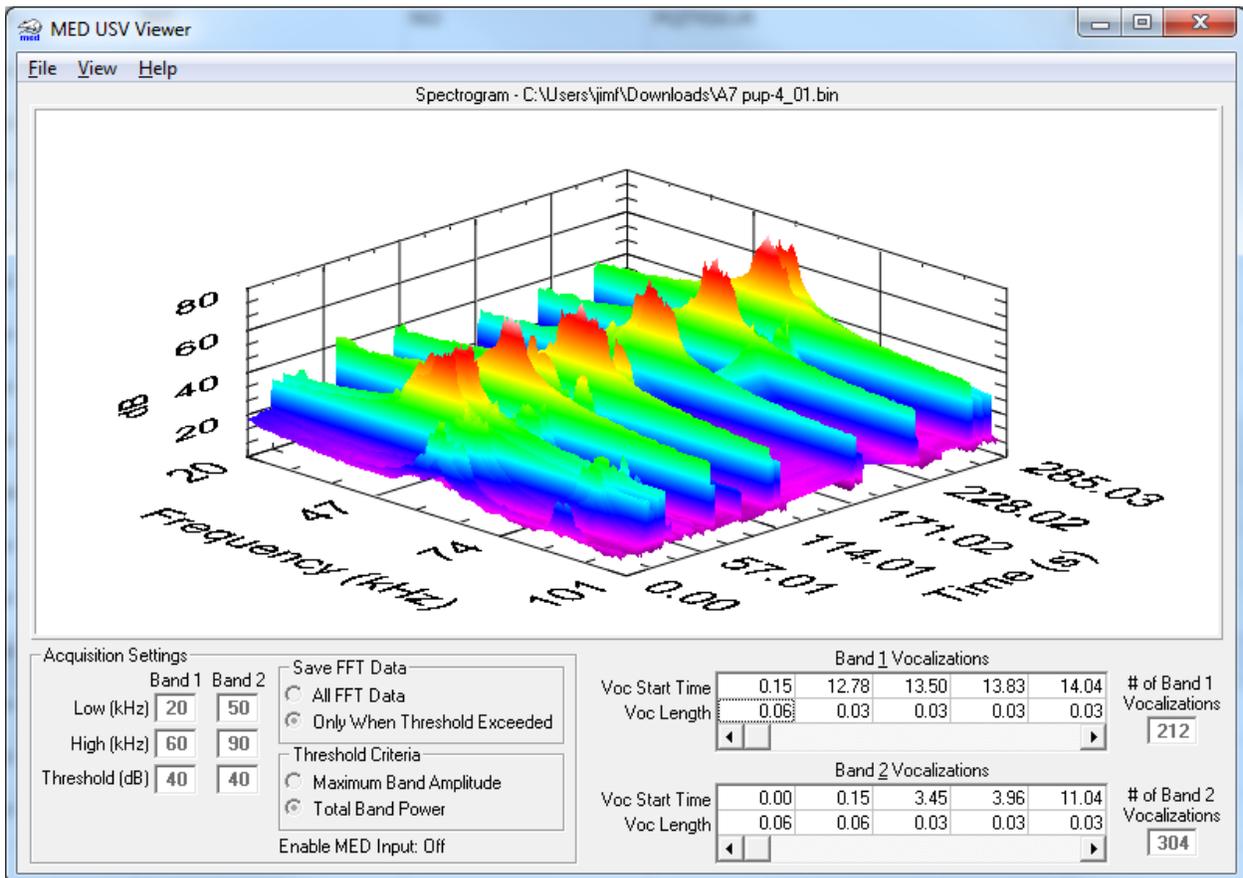
The number of USVs for each band, as well as the onset time and length of each USV, appear in the

lower right portion of the display, see Figure 7-2 . Clicking on a vocalization start time or length value will zoom the sonogram in on that data point.

Figure 7-2 - Onset, Duration and Total Number USVs per Band

Band 1 Vocalizations						
Voc Start Time	0.15	12.78	13.50	13.83	14.04	# of Band 1 Vocalizations
Voc Length	0.06	0.03	0.03	0.03	0.03	
Band 2 Vocalizations						
Voc Start Time	0.00	0.15	3.45	3.96	11.04	# of Band 2 Vocalizations
Voc Length	0.06	0.06	0.03	0.03	0.03	

Figure 7-3 - Spectrogram of Sample Data



While viewing a spectrogram the mouse wheel may be used to zoom in or out on the spectrogram. Left click and hold the mouse button and move the mouse to rotate the spectrogram around each axis. Click **View > Restore to Max**, or press **Ctrl + R**, to restore the spectrogram to the default view.

Editing Viewer Parameters

Click **File > Edit Parameters** to adjust the properties of the USV Viewer sonogram or spectrogram. The adjustable parameters depend on whether a sonogram or spectrogram is being viewed, see Figure 7-4 and Figure 7-5.

The Y-axis minimum and maximum values are 19 kHz and 101 kHz respectively. To modify the fields, use the up/down arrows to the right of each field, or place the cursor in each field and type a numeric value. Change the colors corresponding to each decibel range by clicking that decibel range. When all modifications have been made, press **OK**.

Figure 7-4 – Adjustable Sonogram Display Parameters

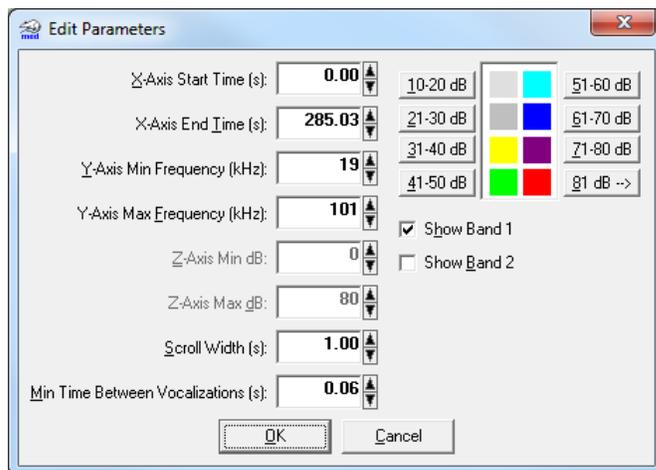
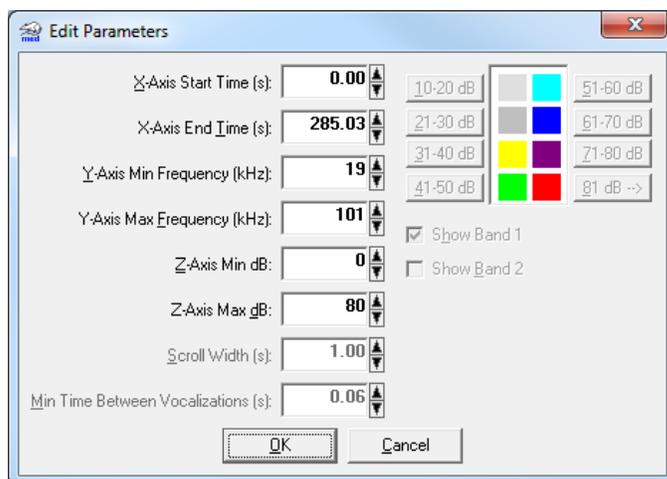


Figure 7-5 - Adjustable Spectrogram Display Parameters



Printing a Sonogram or Spectrogram

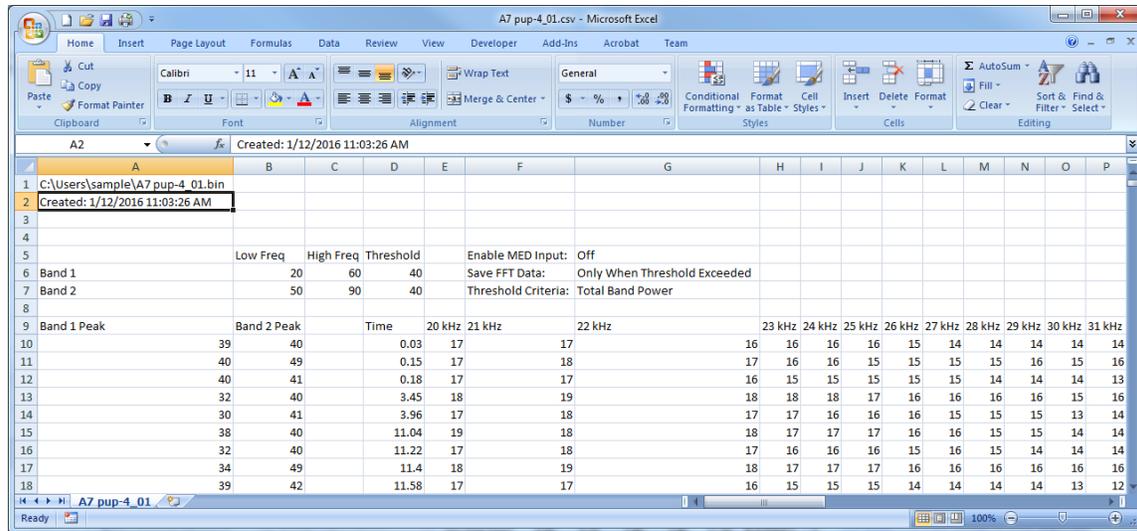
Sonograms and spectrograms may be printed by clicking **File > Print**, selecting which item is to be printed, selecting a printer and clicking **Print**. Click **Cancel** to cancel the printing operation.

Exporting Data to Microsoft Excel®

To export data for use in Microsoft Excel®, click **File > Export to CSV File** and in the “Save USV -> CSV File” window supply a file name and save to location.

To open the .csv data file in Excel® click the open file icon and in the “Open” window set the file type drop down to “All Files (*.*)”, select the *.csv file and click **Open**. See Figure 7-6.

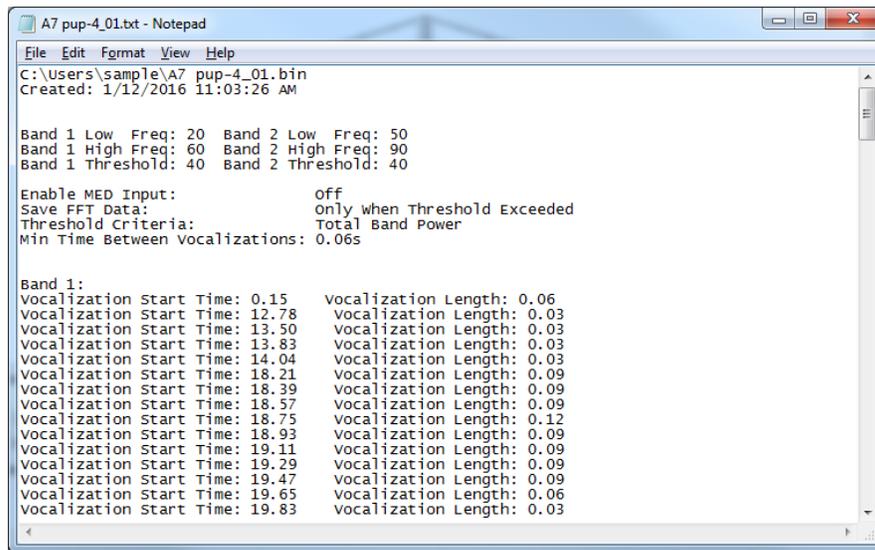
Figure 7-6 - Excel® Spreadsheet From *.csv Data File



NOTE: The export function in the USV Viewer creates a data file that does not differ from the file created by the GetUSV Macro.

Exporting Data To A Text File

To create a *.txt data file that may be opened in Notepad, WordPad, or any word processing program with the USV Viewer, click **File > Save As**. In the “Save File As” window supply a file name and save to location and click **Save**. See Figure 7-7.

Figure 7-7 - Sample Text Data File in Microsoft Notepad

```
A7 pup-4_01.txt - Notepad
File Edit Format View Help
C:\Users\sample\A7 pup-4_01.bin
Created: 1/12/2016 11:03:26 AM

Band 1 Low Freq: 20 Band 2 Low Freq: 50
Band 1 High Freq: 60 Band 2 High Freq: 90
Band 1 Threshold: 40 Band 2 Threshold: 40

Enable MED Input: Off
Save FFT Data: Only when Threshold Exceeded
Threshold Criteria: Total Band Power
Min Time Between Vocalizations: 0.06s

Band 1:
Vocalization Start Time: 0.15 Vocalization Length: 0.06
Vocalization Start Time: 12.78 Vocalization Length: 0.03
Vocalization Start Time: 13.50 Vocalization Length: 0.03
Vocalization Start Time: 13.83 Vocalization Length: 0.03
Vocalization Start Time: 14.04 Vocalization Length: 0.03
Vocalization Start Time: 18.21 Vocalization Length: 0.09
Vocalization Start Time: 18.39 Vocalization Length: 0.09
Vocalization Start Time: 18.57 Vocalization Length: 0.09
Vocalization Start Time: 18.75 Vocalization Length: 0.12
Vocalization Start Time: 18.93 Vocalization Length: 0.09
Vocalization Start Time: 19.11 Vocalization Length: 0.09
Vocalization Start Time: 19.29 Vocalization Length: 0.09
Vocalization Start Time: 19.47 Vocalization Length: 0.09
Vocalization Start Time: 19.65 Vocalization Length: 0.06
Vocalization Start Time: 19.83 Vocalization Length: 0.03
```

The time of the vocalization onset and the duration of each vocalization are listed underneath each “band” heading. The data reflect only those vocalizations that occurred above the user-defined decibel threshold and within each band range.

Help Menu

The help menu provides access to the MED USV Viewer manual and software version information.

CHAPTER 8 | USING MED-PC® INPUTS/OUTPUTS

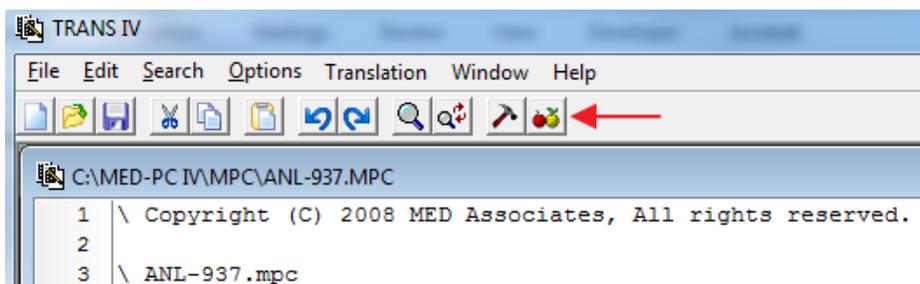
Follow the wiring instructions listed in the **MED-PC® Controlled Operation** section of this manual to connect the ANL-937-1 USV Detector to the MED-PC® inputs and outputs. MED-PC® can automatically control the start and stop of USV data acquisition to correspond to stimulus events within an operant chamber.

One benefit of using the USV detector in conjunction with MED-PC® is that data can be viewed via the Med Associates SoftCR (SOF-721) or SoftCR Pro (SOF-722) Cumulative Recorder applications. The MedState Notation application titled **ANL-937.mpc** can be used to do this.

Running the ANL-937.mpc Program in MED-PC®

Programs written in MedState Notation must be compiled in Trans IV before they may be executed in MED-PC®. To compile a program, open Trans IV and click **File > Open**, select the ANL-937.mpc file in the Open file window and click **Open**. Now click the Make icon, see Figure 8-1, and Trans IV will compile the program for use in MED-PC®. If any problems are encountered during this process, refer to the on-screen help menu or the **MED-PC® User's Manual, DOC-010**, or contact Med Associates, Inc. for assistance.

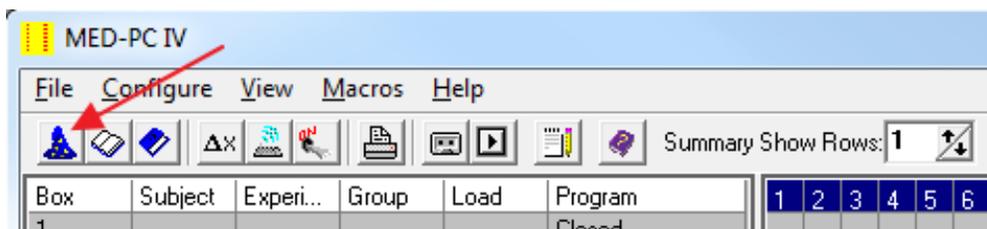
Figure 8-1 - Trans IV Make Icon



After ANL-937.mpc is compiled open the USV detector application and set the data acquisition parameters, refer to Figure 5-1. **Be certain that “Enable MED Input” is checked.** Click **Browse**, and in the “Experiment Name” window supply a file name and save to location, then click **Save**. Click the **Update** button to send the parameters to the ANL-937-1 and enable the **Start** button.

Open MED-PC® and use the Experiment Loading Wizard to select the box(es) to load, adjust any program parameters and issue the start command. If the wizard does not automatically open, click the wizard icon on the MED-PC® tool bar, see Figure 8-2.

Figure 8-2 – Program Wizard Icon In the MED-PC® Tool Bar



Sample Data File Created by ANL-937.mpc

The ANL-937.mpc program creates a *.txt data file that can be viewed in any word processing program or spreadsheet. An example of a MED-PC® data file appears below with comments explaining the different sections and data values.

File: D:\Program Files\MED Associates\MED-PC.new\DATA\!2005-07-07.Ss1

Start Date: 07/07/10	}	Subject & session identifying information
End Date: 07/07/10		
Subject: 1		
Experiment: 0		
Group: 0		
Box: 1		
Start Time: 9:29:00		
End Time: 9:32:22		
MSN: ANL_937		
B: 0.000		
E: 8.000	Total band 1 vocalizations above dB threshold value	
G: 6.000	Total band 2 vocalizations above dB threshold value	
H: 0.000	Not Used	
I: 44.000	Subscript that codes for the IRT Array C (for SoftCR)	
J: 16.000	Subscript for the Vocal Band 1, see Array D	
K: 12.000	Subscript for the Vocal Band 2, see Array E	
L: 0.000	Not Used	
M: 0.000	Not Used	
N: 0.000	Not Used	
O: 0.000	Not Used	
P: 0.000	Not Used	
Q: 0.000	Not Used	
R: 0.000	Not Used	
S: 180.010	Elapsed Time in Session	
T: 1720.000	Elapsed time in 0.01 second increments for SoftCR	
U: 0.000	Not Used	
V: 0.000	Not Used	
W: 0.000	Not Used	
X: 0.000	Not Used	
Y: 0.000	Counter for Vocal Band 1 Above Threshold	
Z: 0.000	Counter for Vocal Band 2 Above Threshold	

Array A includes the independent variables defined during the load wizard.

A:	Acclimation time(seconds)	Session Length(minutes)	Using SoftCR?(Yes=1 No=0)
0:	3000.000	3.000	1.000

Array C is used by SoftCR Pro and contains codes for event pens and the cumulative recorder.

C:	0:	3149.100	0.600	580.110	0.610	116.500	116.510
	6:	108.100	0.600	38.500	55.110	0.610	71.510
	12:	179.100	0.600	2.110	0.610	410.500	295.510
	18:	2410.100	0.600	188.110	0.610	352.500	10.510
	24:	189.100	0.600	259.500	297.100	0.600	316.500
	30:	501.110	0.610	2666.510	922.100	0.600	328.500
	36:	1079.100	0.600	546.110	0.610	518.510	581.500
	42:	1720.300	0.310				

Array D lists Band 1 On/Off Times.

D:	0:	31.490	38.450	40.690	41.070	44.120	48.240
	6:	75.290	80.690	82.680	85.270	88.240	91.400
	12:	132.290	135.570	146.360	162.810		

Array F lists Band 2 On/Off Times.

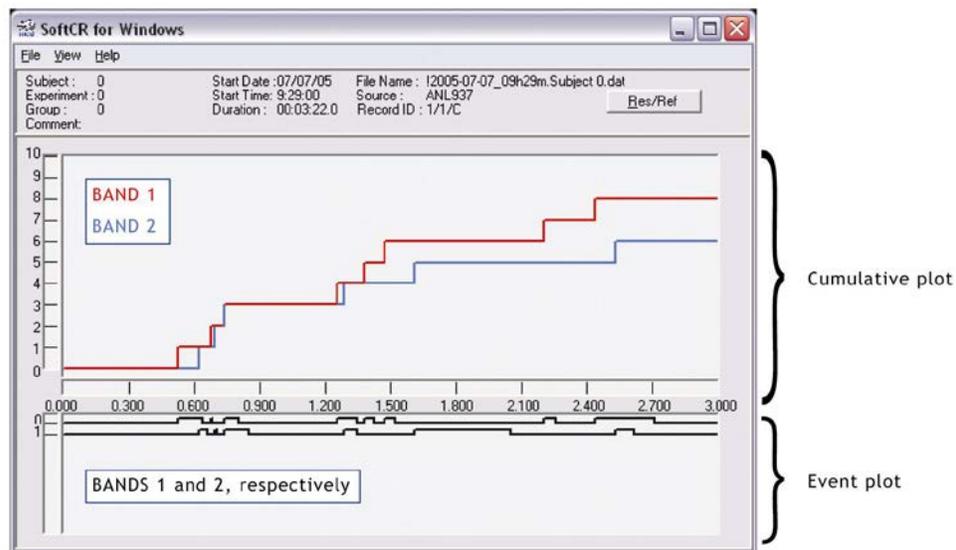
F:

0:	37.290	39.610	41.620	42.330	44.140	51.190
6:	77.170	80.790	96.410	123.070	151.820	157.000

The SoftCR & SoftCR Pro Report

The SoftCR or SoftCR Pro Cumulative Recorder can be used to graphically plot the data obtained from the ANL-937.mpc application. Refer to the **SOF-721 SoftCR, DOC-034**, or **SOF-722 SoftCR Pro, DOC-204**, users' manuals for instruction on how to use these optional data utilities. Graphing parameters can be modified and the file can be saved as a *.pdf.

Figure 8-3 – SoftCR Cumulative Record of Ultrasonic Events



The ANL_937.mpc Program

The entire program for integrating the USV Detector with MED-PC® is listed below. This information is included in the manual as a reference. Please make a backup copy of ANL-937.mpc so that if modifications are made to the code, an unadulterated copy will be available.

```
\ Copyright (C) 2008 MED Associates, All rights reserved.
\ ANL-937.mpc
\
\ This example program demonstrates how to turn on the recording for the ANL-937
\ microphone device and how to record the responses from the device.
\
\ The Array's D and F contain the absolute times from when the program started
\ that each event happened. For example Array Element D(0) contains the first
\ absolute time that the Band 1 Vocal went above its Threshold value. Array
\ Element D(1) contains the first absolute time that the Band 1 Vocal fell below
\ its Threshold value. Array Element D(2) contains the second absolute time
\ that the Band 1 Vocal went above its Threshold value. Array Element D(3)
\ contains the second absolute time that the Band 1 Vocal fell below its
\ Threshold value. Etcetera.
\
\ The Variables E and G contain the total number of times that each Vocal Band
\ went above their respective Threshold values.
```

```

\
\ The Array C contains the same information as the Array's D and F and the
\ Variables E and G, but it is recorded using the SoftCR data format. SoftCR is
\ an external program from MED-PC IV that can graph the data contained in Array C
\ so that it may be more easily understood. If SoftCR data is not desired, then
\ just set the value in Array Element A(2) [ A(^SoftCR) ] to 0.
\
\ SoftCR data is recorded in Relative Mode with a 10ms Resolution.
\
\ The Input and Output constants listed below should be changed to match the
\ existing hardware configuration. The two Inputs need to be in Level Mode.
\ Level Mode is set by selecting the appropriate Jumper or Switch (depending on
\ the Input Card version) on the Input Card.

\ Inputs
^VocalBand1 = 1 \ Level Mode
^VocalBand2 = 2 \ Level Mode

\ Outputs
^StartRec = 1

\ A() = Control Variables with Assigned Aliases as Defined
Var_Alias Acclimation Time (sec)          = A(0) \ Default = 60 seconds
Var_Alias Session Length (min)           = A(1) \ Default = 60 minutes
Var_Alias SoftCR Data Array (Yes=1 No=0) = A(2) \ Default = Yes

\ Constants for Array A()
^Acclim = 0
^Session = 1
^SoftCR = 2

\ List Data Variables Here
\ C() = IRT Array and Event Pen Code for Soft Cum. Recorder

\ D() = Array of Band 1 On/Off Times
\ D(0) = Time When Vocalization Went Above Band 1 Threshold
\ D(1) = Time When Vocalization Went Below Band 1 Threshold
\ D(2) = Time When Vocalization Went Above Band 1 Threshold
\ D(3) = Time When Vocalization Went Below Band 1 Threshold
\
\ ...
\ D(998) = Time When Vocalization Went Above Band 1 Threshold
\ D(999) = Time When Vocalization Went Below Band 1 Threshold
\ E = Total Band 1 Vocalizations

\ F() = Array of Band 2 On/Off Times
\ F(0) = Time When Vocalization Went Above Band 2 Threshold
\ F(1) = Time When Vocalization Went Below Band 2 Threshold
\ F(2) = Time When Vocalization Went Above Band 2 Threshold
\ F(3) = Time When Vocalization Went Below Band 2 Threshold
\
\ ...
\ F(998) = Time When Vocalization Went Above Band 2 Threshold
\ F(999) = Time When Vocalization Went Below Band 2 Threshold
\ G = Total Band 2 Vocalizations

\ List Working Variables Here
\ I = Subscript for the IRT Array C
\ J = Subscript for the Vocal Band 1 Array D
\ K = Subscript for the Vocal Band 2 Array E
\ S = Elapsed Time in Session
\ T = Elapsed Time in 0.01 sec Increments for SoftCR Data
\ Y = Counter for Vocal Band 1 Above Threshold
\ Z = Counter for Vocal Band 2 Above Threshold

DIM A = 2
DIM C = 10000
DIM D = 1000

```

DIM F = 1000

```
\ Z-Pulses Used in this Program
^StartRec = 1 \ Z1 = Start Recording
^VocallOn = 2 \ Z2 = Signal Vocal 1 Above Threshold
^VocallOff = 3 \ Z3 = Signal Vocal 1 Below Threshold
^Vocal2On = 4 \ Z4 = Signal Vocal 2 Above Threshold
^Vocal2Off = 5 \ Z5 = Signal Vocal 2 Below Threshold
^EndSess = 32 \ Z32 = End of Session Signal
```

DISKOLUMNS = 6

```
\*****
\
\ ANL-937 Schedule
\ S1 - Set Default Values
\ Acclimation Time (60 seconds)
\ Session Length (60 minutes)
\ SoftCR Activation (1-Yes)
\*****
S.S.1,
S1,
  0.001": SET A(^Acclim) = 60, A(^Session) = 60, A(^SoftCR) = 1 ---> S2

S2,
  \ First Statement: Wait for START signal.
  \
  \ Second Statement: Update screen display with default values
  \ for control variables. This will show any changes made via
  \ the "Configure | Change Variables" Window prior to START.
  #START: CLEAR 1,60; SET A(^Acclim) = A(^Acclim) * 1" ---> S3
  1": SHOW 1,Acclim Time,A(^Acclim), 2,Session Length,A(^Session), 3,SoftCR Code,A(^SoftCR) --->
SX

S3,
  \ Time Acclimation Period.
  \ Turn on Recording Output and Signal
  \ that Recording has Started.
  A(^Acclim)#T: SHOW 2,Total Band 1,E, 3,Total Band 2,G;
  ON ^StartRec; Z^StartRec ---> S4

S4,
  1': ---> SX

\*****
\ RECORD RESPONSES FOR BAND 1
\*****
S.S.3,
S1,
  \ Wait for Acclimation Period to End.
  #Z^StartRec: ---> S2

S2,
  \ Record the Absolute Time that the Vocal
  \ Band 1 went above its Threshold and the
  \ Total Number of Responses on Vocal Band 1.
  #Z^EndSess: ---> S4
  #Z^VocallOn: SET D(J) = S, J = J + 1, D(J) = -987.987;
  ADD E; SHOW 2,Total Band 1,E ---> S3

S3,
  \ Record the Absolute Time that the Vocal
  \ Band 1 went below its Threshold.
  #Z^EndSess: ---> S4
  #Z^VocallOff: SET D(J) = S, J = J + 1, D(J) = -987.987 ---> S2

S4,
  \ Holding State at End of Session
  1': ---> SX

\*****
\ RECORD RESPONSES FOR BAND 2
\*****
S.S.5,
S1,
  \ Wait for Acclimation Period to End.
  #Z^StartRec: ---> S2
```

```

S2,      \ Record the Absolute Time that the Vocal
          \ Band 2 went above its Threshold and the
          \ Total Number of Responses on Vocal Band 2.
#Z^EndSess: ---> S4
#Z^Vocal2On: SET F(K) = S, K = K + 1, F(K) = -987.987;
            ADD G; SHOW 3,Total Band 2,G ---> S3

S3,      \ Record the Absolute Time that the Vocal
          \ Band 2 went below its Threshold.
#Z^EndSess: ---> S4
#Z^Vocal2Off: SET F(K) = S, K = K + 1, F(K) = -987.987 ---> S2

S4,      \ Holding State at End of Session
1': ---> SX

\*****
\ VOCAL BAND 1 THRESHOLD - 20ms ABOVE, 20ms BELOW
\*****
S.S.7,   \ Vocal Band 1 Threshold - 20ms Above, 20ms Below.
S1,      \ Inputs in Level Mode generate an Input "count" on each
          \ Interrupt. With a 10ms system Resolution 2 counts
          \ will be reached in 20ms. The Z^Vocal1On pulse is used
          \ to signal that it is above its Threshold. The second
          \ statement resets the counter every 20 ms so that false
          \ readings less than 20ms will not constitute a response.
#R^VocalBand1: ADD Y; IF Y >= 2 [@AboveThreshold, @FalseReading]
               @Above: Z^Vocal1On; SET Y = 0 ---> S2
               @False: ---> SX
0.02": SET Y = 0 ---> S1

S2,      \ As long as the Vocal 1 level is above its Threshold
          \ the second statement causes a re-entry to this State.
          \ This resets the the internal 20ms Timer so it never
          \ times out. When the level falls below the Threshold
          \ in Band 1 for 20ms the Timer times out and a
          \ Z^Vocal1Off pulse signals the release of the event.
0.02": Z^Vocal1Off ---> S1
#R^VocalBand1: ---> S2

\*****
\ VOCAL BAND 2 THRESHOLD - 20ms ABOVE, 20ms BELOW
\*****
S.S.9,
S1,
#R^VocalBand2: ADD Z; IF Z >= 2 [@AboveThreshold, @FalseReading]
               @Above: Z^Vocal2On; SET Z = 0 ---> S2
               @False: ---> SX
0.02": SET Z = 0 ---> S1

S2,
0.02": Z^Vocal2Off ---> S1
#R^VocalBand2: ---> S2

\*****
\ INCREMENT TIME T FOR IRT AND EVENT ARRAY
\*****
S.S.11,
S1,
#START: IF A(^SoftCR) > 0 [] ---> S2

S2,
0.01": ADD T ---> SX
#Z32: ---> S1

\*****
\ ADD A STEP 0 ELEMENT TO ARRAY C FOR EACH VOCAL BAND 1 RESPONSE
\*****
S.S.13,

```

```

S1,
  #Z^StartRec: IF A(^SoftCR) > 0 [] ---> S2

S2,      \ Vocal 1 Response above its Threshold
#Z^EndSess: ---> S1
#Z^Vocal1On: SET C(I) = T + 0.10, T = 0; ADD I;
            IF I = 10000 [@True, @False]
                @True: ---> S1
                @False: SET C(I) = -987.987 ---> SX

\*****
\ ADD A STEP 1 ELEMENT TO ARRAY C FOR EACH VOCAL BAND 2 RESPONSE
\*****
S.S.15,
S1,
  #Z^StartRec: IF A(^SoftCR) > 0 [] ---> S2

S2,      \ Vocal 1 Response above its Threshold
#Z^EndSess: ---> S1
#Z^Vocal2On: SET C(I) = T + 0.11, T = 0; ADD I;
            IF I = 10000 [@True, @False]
                @True: ---> S1
                @False: SET C(I) = -987.987 ---> SX

\*****
\ RAISE THE EVENT 0 ELEMENT IN ARRAY C FOR THE LENGTH OF TIME
\      THAT VOCAL BAND 1 IS ABOVE ITS THRESHOLD
\*****
S.S.17,
S1,
  #Z^StartRec: IF A(^SoftCR) > 0 [] ---> S2

S2,      \ Raise the Event 0 Element
#Z^EndSess: ---> S1
#Z^Vocal1On: SET C(I) = T + 0.60, T = 0, I = I + 1;
            IF I >= 10000 [@True, @False]
                @True: ---> S1
                @False: SET C(I) = -987.987 ---> S3

S3,      \ Lower the Event 0 Element
#Z^EndSess: ---> S1
#Z^Vocal1Off: SET C(I) = T + 0.50, T = 0, I = I + 1;
            IF I >= 10000 [@True, @False]
                @True: ---> S1
                @False: SET C(I) = -987.987 ---> S2

\*****
\ RAISE THE EVENT 1 ELEMENT IN ARRAY C FOR THE LENGTH OF TIME
\      THAT VOCAL BAND 2 IS ABOVE ITS THRESHOLD
\*****
S.S.19,
S1,
  #Z^StartRec: IF A(^SoftCR) > 0 [@ActivateSoftCR, @NoSoftCR]
                @ActSoftCR: ---> S2
                @NoSoftCR: SET C(0) = -987.987 ---> SX \ Seal Array

S2,      \ Raise the Event 1 Element
#Z^EndSess: ---> S1
#Z^Vocal2On: SET C(I) = T + 0.61, T = 0, I = I + 1;
            IF I >= 10000 [@True, @False]
                @True: ---> S1
                @False: SET C(I) = -987.987 ---> S3

S3,      \ Lower the Event 1 Element
#Z^EndSess: ---> S1
#Z^Vocal2Off: SET C(I) = T + 0.51, T = 0, I = I + 1;
            IF I >= 10000 [@True, @False]
                @True: ---> S1
                @False: SET C(I) = -987.987 ---> S2

```

```
\*****  
\          SESSION CLOCK  
\*****  
S.S.21,  
S1,  
  #START: SHOW 1,Session,S/60 ---> S2  
  
S2,  
  0.01": SET S = S + 0.01; SHOW 1,Session,S/60;  
    IF S/60 >= A(^Session) [@EndSession, @ContinueTiming]  
      @End: SET C(I) = T + 0.30, C(I+1) = 0.31;  
        SET I = I + 2, C(I) = -987.987;  
        OFF ^StartRec; Z^EndSess ---> S3  
        @Cont: ---> SX  
  
S3,    \ Wait for Screen Update and end with  
      \ STOPABORTFLUSH for Automatic Data Saving  
2": ---> STOPABORTFLUSH
```

Appendix A | CONTACT INFORMATION

Please contact Med Associates, Inc. for information regarding any of our products.

Visit our website at www.med-associates.com for contact information.

For technical questions, please call 802-527-2343 or email support@med-associates.com.