

SONOTRACK™

A sound idea!

Record, Analyze and Playback
ultrasounds of your animals
using the latest technology

Overview

SONOTRACK is a new and non-invasive measurement method to monitor and analyze ultrasonic sounds produced by (laboratory) animals. Unlike many other systems that are based on bat detectors, SONOTRACK uses special microphones and hardware that can pick up sounds in the frequency range of 15KHz to 100 kHz. The sounds picked up by the microphones are not only converted to audible signals but the “complete ultrasound signal” is also recorded in digital format.

The unique graphical presentations of the signals in the SONOTRACK software enables the user to analyze the sounds or "animal language" in much more detail than ever before. The innovative Ultrasound Vocalization (USV) detector enables automatic detection and counting of ultrasonic vocalizations of your animals. The batch processing function of the USV detector makes it possible to go through a large amount of measurements and provide you quickly the end points of your experiment.

It is this unique functionality, which can lead to a further refinement of your research, with the following advantages:

- New animal models involving the use of ultrasound as a measure for amongst pain, stress, depression, fear, anxiety, social interaction, sexual related behavior
- A decrease in the number of laboratory animals by shorter lead times and more consistent and reliable data
- Less discomfort, improvement/monitoring of animal welfare, and better living conditions for laboratory animals by using a non-invasive way of measuring.

How does it work?

In contrast to most of the other systems on the market SONOTRACK is a so-called full spectrum recording system. It means that it digitizes and records the whole ultrasound band from 15kHz to 100kHz. In contrast most of the other systems are based on bat detectors that have to be tuned to a specific frequency range and can only receive a small band that is approximately 15kHz wide. In applications involving rodents that vocalize over a broad frequency range it is obvious that these bat detectors systems are unsuitable.

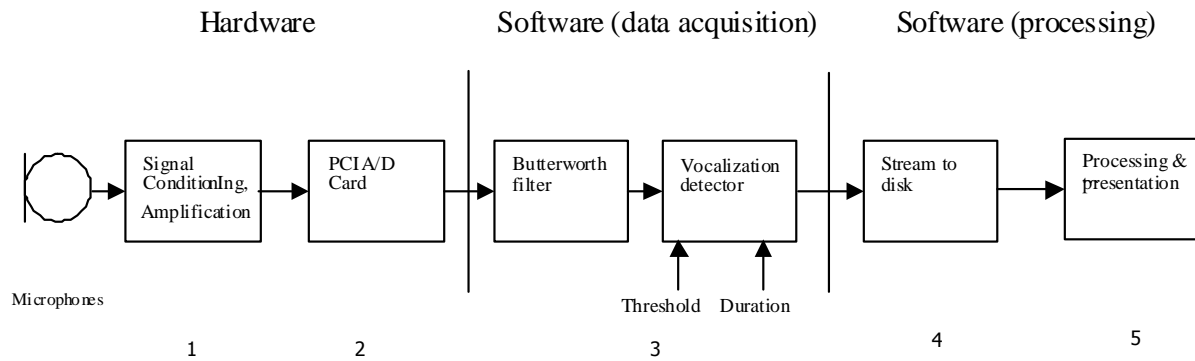
The full spectrum recording technique makes it also possible to present the recordings in all kind of graphical presentations such as Sonograms which reveal a lot of information about the actual call or vocalizations of your animals.

SONOTRACK and most other systems also have the capability to make ultrasounds audible for humans. There are several ways to convert the ultrasounds into audible frequencies. Commonly used techniques are frequency shifting (heterodyne method), frequency division and time expansion (often used in the more advanced digital systems).

The conversion technique used by the SONOTRACK system to make the ultrasounds audible for humans is based on the time expansion method.

To present detailed information about the recorded ultrasound signal, the following steps are applied:

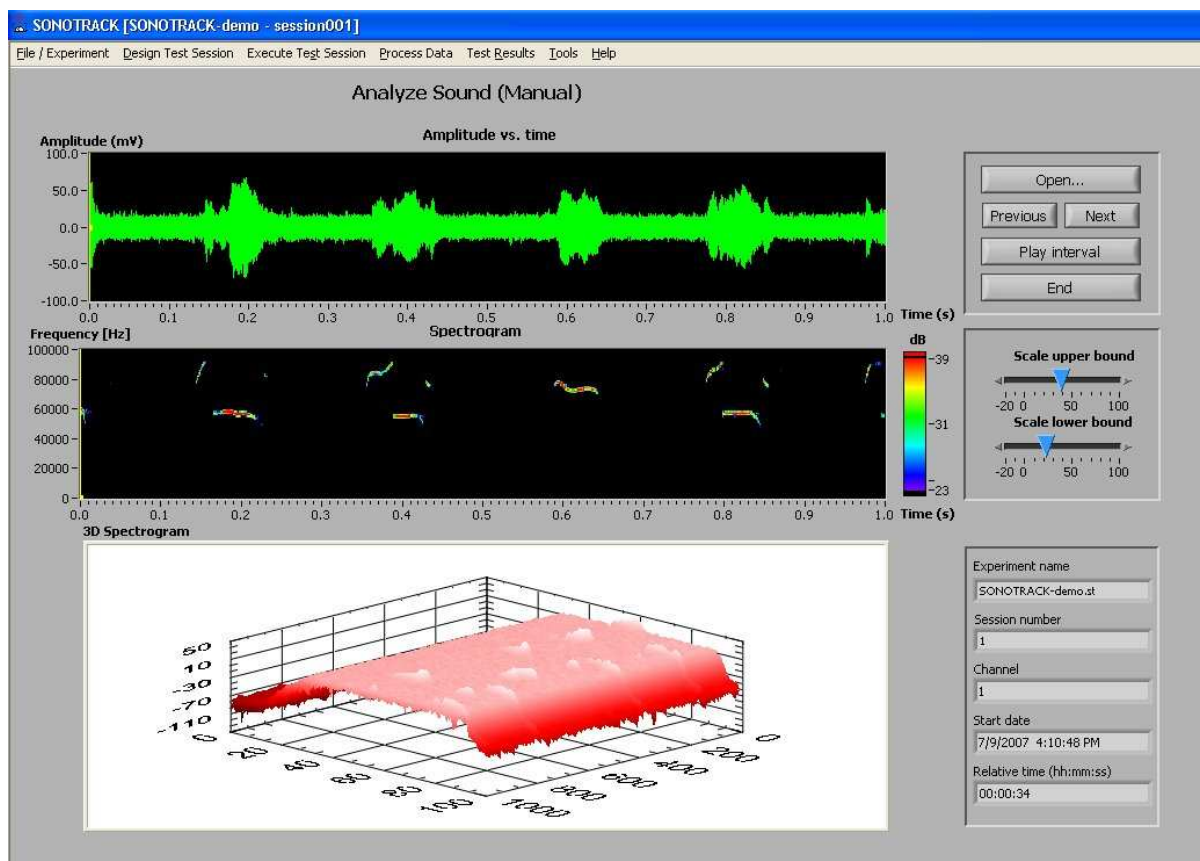
1. Amplification and analog filtering of the microphone signals
2. Analog to digital conversion of the amplified and filtered sound signals
3. Digital filtering of the sound signals (with user definable settings)
4. Storage of digital data on hard disk (only when signals are present)
5. Processing and presentation of the sounds signals in several domains.



Block diagram of the SonoTrack Ultrasound recording and analysis system

The following types of data are presented and can be printed by the user:

- “Raw” recorded sound signal (amplitude against time)
- Spectrogram (frequency against time)
- Sonogram (frequency against time) in 2D and 3D graphs



Typical example of a recording showing the vocalizations of a mouse

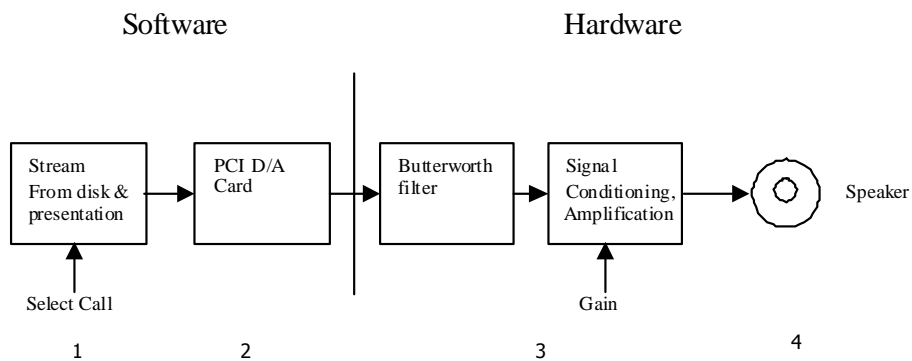
The user of SONOTRACK can also make the Ultrasounds audible for the human ear (using time expansion techniques).

- The sounds can be played from the SONOTRACK software or
- Converted to .wav files that can be played by external software or devices.

Playback of Ultrasounds (*optional*)

Playing back previously recorded ultrasounds or ultrasound Vocalizations from a library enable the researcher to generate a stimulus sound to the animal. The ability to reproduce specific vocalizations and sounds is useful in various applications involving research related to fear, aggression and several social responses such as defensive behaviours, submissive behaviours and sexually related behaviours.

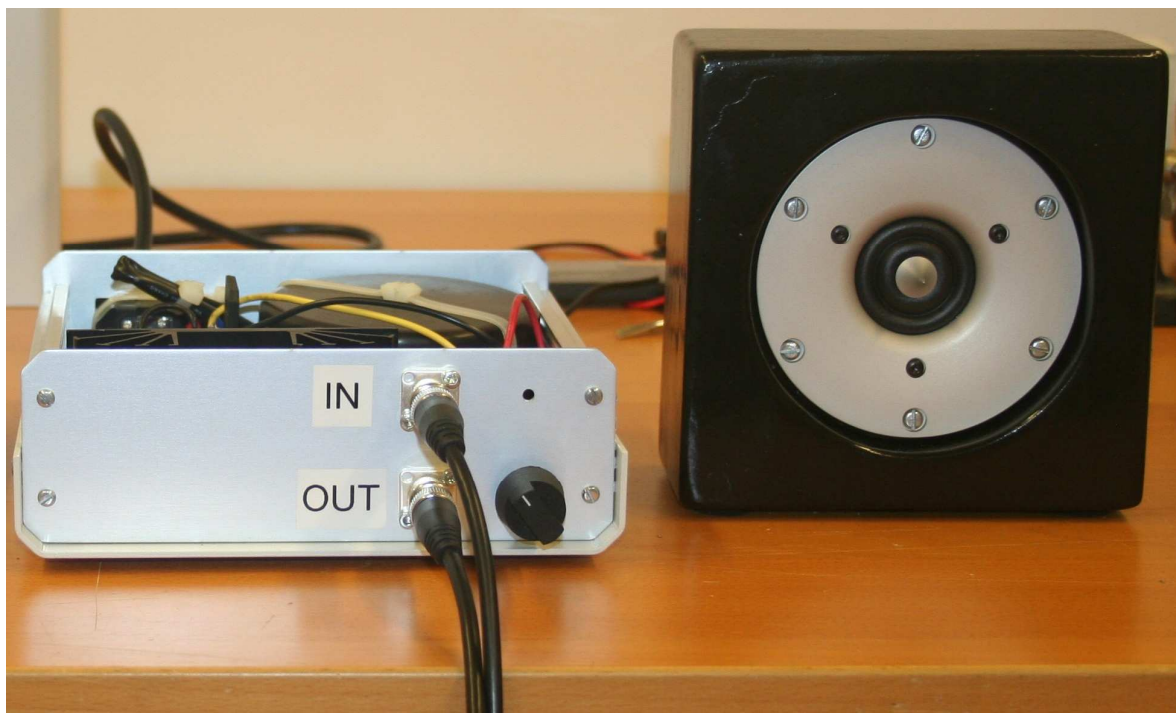
The SONOTRACK playback function is designed to reproduce the Ultrasound Vocalizations as accurate as possible. To do this a low noise 50W amplifier with a frequency range of 5 kHz to 200 kHz is coupled to an Ultrasound speaker that has an almost flat frequency response between 15 kHz and 100 kHz.



Block diagram of the SonoTrack Ultrasound recording and analysis system

To playback Ultrasonic Vocalizations the following steps are applied:

1. Selection of a recorded measurement that contains Ultrasonic Vocalizations
2. Conversion of the digital data to analog data and presentation of the signal on the screen
3. Filtering and amplification of the analog signal
4. Playback of Vocalizations to the animal through a high performance ultrasound speaker



SONOTRACK 50W Ultrasound amplifier & Speaker

Benefits and unique features of SONOTRACK

The novel approach of SONOTRACK offers a number of unique features not offered by other systems for an affordable price.

- *Full spectrum analysis*

The SONOTRACK system enables real recording of the full ultrasound spectrum (up to 100 kHz).

Because of the fact that SONOTRACK not only records the on- and offset times in a limited frequency band (which is the case for most other systems based on bat detectors), the researcher gets much more in depth information about the ultrasonic vocalization behaviour of their animals.

By recording the full spectrum, all vocalizations over a broad frequency range are captured and are available for further analysis.

- *Multi channel continuous recording*

The SONOTRACK system can continuously record up to four channels (full bandwidth). If necessary the researcher can ask the system to record only those periods in which ultrasonic vocalisations exceed an user defined level. This can save a lot of hard disk space when recording multiple channels at the same time.

All recordings can be converted in audible sounds and can easily be exported in the commonly used “.wav” media format

- *High performance microphones and amplifiers*

The use of very sensitive microphones and low noise electronics enables SONOTRACK to detect and record low level ultrasonic vocalisations.

Back ground noise is effectively eliminated by the small opening angle of the applied microphones (12 degrees) and the band pass filter that removes all normal sounds below 15 kHz. The amplifiers that are used in the SONOTRACK system apply the latest components and technology that is available (2006) to get the lowest possible electronic noise.

The microphones and the associated electronics that are used in the SONOTRACK system have an extremely flat frequency response curve over the full spectrum enabling a proper and undistorted recording of all types of vocalizations.

To cover very large arenas (for example in open-field tests) it is also possible to couple microphones together which will then effectively work as one microphone.

- *Automatic Animal Vocalization detector*

The automatic vocalization detector in SONOTRACK makes it possible to process a large number of recordings without going through all the data yourself. The counter can be adjusted to your specific situation and can count in three user definable frequencies bands. In this way it is for example possible to count the vocalizations of a mother and a pup separately from the same recording. The vocalization detector efficiently removes background noise and can therefore also be used in rather noisy environments.

- *Various presentations of the ultrasound information*

In depth analysis of calls

The Ultrasonic vocalisations are presented in different “domains” using 2D and 3D graphs. Besides of presenting the raw ultrasound signal in the time domain, SONOTRACK offers the researcher amongst others frequency graphs, Spectrograms and Sonograms. All graphs can easily be copied to other Windows applications or directly be sent to a printer for further analysis and use.

Result summaries (statistical results)

Like with many measurement systems it is crucial to summarize the data such that research conclusions can be extracted in an easy and useful way.

The result summary generator enables you to combine the results from all your measurements in one overview and to enter an user definable bin size (in time) to present the results. In the result summary the number of calls per bin and for three user definable frequency ranges are presented. In addition parameters like minimum, maximum and average frequency and call duration are presented.

- *Experiment administration*

The SONOTRACK comes with an easy to use experiment administration that keeps track of your experimental parameters, such as the animal ID's, treatments and many other variables that are part of your experimental setup and conditions.

SONOTRACK vs. other ultrasound analysis systems

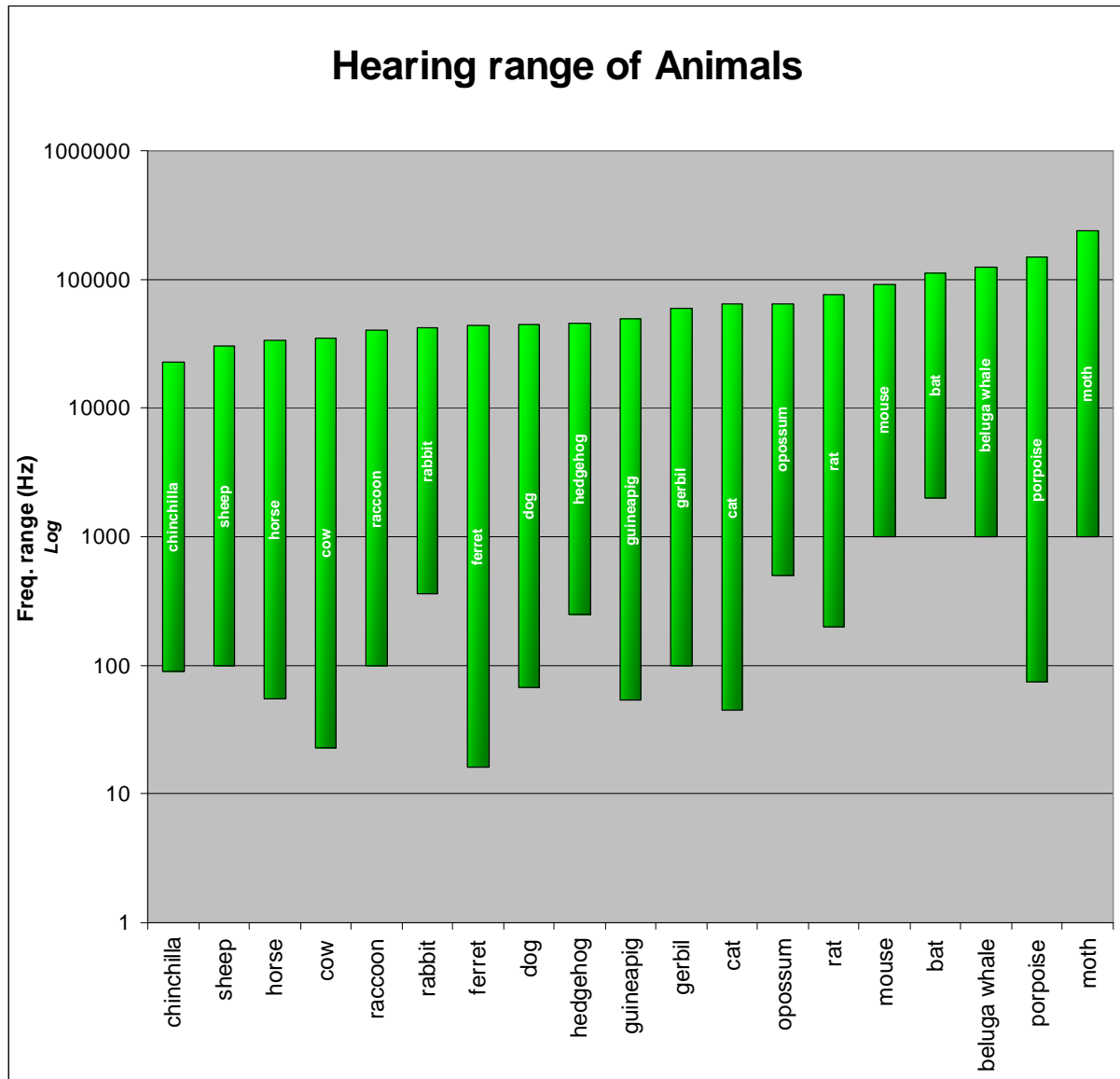
	Bat Detector with tuning	Bat Detector with freq. divider	Ultrasonic Microphone & A/D card (<i>home made</i>)	SONOTRACK
Methods of ultrasound measurement and analysis	Frequency shifting & peak detection	Frequency division & peak detection	Time expansion & storage of signal	Time expansion & storage of signal
Full spectrum analysis	No	No	Depends on software	Yes
User has to know and select desired frequency range	Yes (tuning of bat detectors)	Yes (tuning of bat detectors)	No	No
Number of channels	Mostly 1 channel	Mostly 1 or 4 channels	Depends on A/D card (mostly 1 or 2 channels)	Up to 4 channels
Storage and playback of sound signal	Only with analog tape recorder	Only with analog tape recorder	Yes Digital storage	Yes Digital storage
Conversion to Media player compatible .wav files	No	No	Depends on software	Yes
Presentation of recorded sound signal				
- Signal against time	-	-	+	+
- Frequency plot	-	-	+	+
- Spectrogram	-	-	±	+
- Sonogram	-	-	±	+
Application characteristics				
- Different species	Yes	Yes	Yes	Yes
- Multiple cages	With multiple bat detectors	With multiple bat detectors	Depends on setup	Yes
- Mother and pups at the same time	No	When call freq. both in range	Yes	Yes
Measurement specifics				
- Back ground noise reduction	Limited	Limited	Depends on microphones	Good
- Measurement coverage	Depends on nr. of detectors	Depends on nr. of detectors	Depends on microphones	Excellent
- Dynamic range	Limited	Limited	Depends on microphones and A/D res.	High
Automatic USV Call detector	No	No	No	Yes
Playback of Ultrasound Vocalizations	No	No	Depends on equipment	Yes, optional
Cost / functionality ratio	Poor	Poor	Depends on software	Excellent

‘-’ = not capable, ‘±’ = potential capability, ‘+’ = capable

Applications

SONOTRACK can be advantageous for a variety of users and in several types of research.

Ultrasonic vocalization (USV) are sounds within the frequency range of 20 kHz to 100 kHz. This frequency range is above the human range of hearing. However, it is well known that bats, marine mammals, rodents, nocturnal primates and insects produce and hear USV as part of their behaviour.



USV is not only used for navigation but for communication too. There is evidence that USV is used as a sensitive indicator of subtle emotional and motivational changes in a number of species. Therefore, USV produced by animals in various conditions could be explored as a non-invasive method for registering the welfare status of the animal.

Rat Vocalizations as currently known

Rat	Juvenile and Adult		Pups
Frequencies	22-kHz vocalizations'		40 kHz vocalizations
Bandwidth	18-32 kHz		30-65 kHz
duration of calls	1 to 6 kHz		
Sound Pressure Level	300-4000 ms		
	65 to 85 dB		
Affective state	negative*		positive
Behavioral situation	aversive		nonaversive
	exposure to predators	sexual behaviors	seperation from mother or litter
	inescapable pain	male agonistic behaviors during fighting	
	response to startling noises	juvenile play	
	intermale agression	manual tactile stimulation ('tickling') by experimenters	
	social defeat		
	distressing events		
	response to stimuli associated with distressing experiences		
Behaviors exhibited			
	tense	locomotor activity (approaching another rat, rearing, exploring)	licking or search
	motionless couching ('freezing')		retrieval behavior
	pronounced breathing		

* The exception of the 22kHz call reflecting negative affect is when male rats emit a vocalization with energy around 22kHz after copulation (a behavior not normally considered aversive)

Mouse Vocalizations as currently known

Mice	Adults		Pups
	male	female	
Frequency range	30-110 kHz		above 35 kHz (2 categories)
			group1 group2
			around 70 kHz around 100 kHz intercall interval 200 ms
Housing environment	diversity and complexity of vocalizations		
single gender lab cages		Less	
enriched cages (socially and environmentally)		More	
Behavioral situation	nonaggressive interactions**		distressing
	male mice encounter female mice	when pups removed from nest	isolated from mother
	male mice encounter female mice	female-female interactions	exposed to cold
	urinary phermones	when alone	
		when having pups and litter is removed	
Syllable types* :			
frequency modulated			
down-sweeps	exposed to female phermones		
u-shaped		when pups removed from nest	
up-sweeps	exposed to female phermones		
constant frequencies			
hump-shaped	exposed to female phermones		

* Syllable is defined as an unit of sound seperated by a silent period before another sound and is based on the following acoustic parameters: starting frequency, ending frequency, frequency with peak energy, frequency modulation, and duration.

**In contrast to rats, adult mice do not produce ultrasonic vocalizations during aversive situations. Also ultrasonic vocalizations in mice have not been shown to indicate negative or positive affect. Therefore, the function of ultrasonic vocalizations in

A few applications areas are for example:

- laboratory animal welfare research
- pain research
- toxicology / safety pharmacology
- behavioural pharmacology
- Transgenic animal research

Laboratory animal welfare research

Animal welfare is difficult to quantify with other systems or by visual observation. The use of USV to measure the welfare status of an animal can therefore be advantageous in a number of situations, such as:

- recognition of discomfort during animal experiments (enabling corrective actions at an early stage)
- monitoring of animals with respect to housing conditions
- monitoring of animals during transport
- monitoring of animals during acclimatization
- quantification of animal discomfort or well-being
- reduction of animals in laboratory experiments by taking effective counter measures in case of animal discomfort

Pain Research

In pain research it is very important to have a quantitative measure (parameter) of the animal's well being or discomfort. It is also important to recognize the pain at an early stage, preferably before a behavioural change occurs. Examples where SONOTRACK could be used are:

- recognition of discomfort in a very early stage of an experiment, where up pain relief can be administered
- measurement (monitoring) of the effectiveness of the pain relief
- determining dose response curves for pain related drugs

Toxicology

The monitoring of USV with SONOTRACK could also be used to find possible harmful side effects of new compounds or substances by detecting the occurrence of "negative" or "positive" sounds produced by the laboratory animals.

Behavioral pharmacology

There is clear evidence that in rodents USV is directly related to their wellbeing or emotional state. USV can therefore be a valuable tool for use in behavioral pharmacology related to amongst others:

- Anxiety
- Stress
- Depression
- Fear

USV can also be used to monitor the interaction behaviour between the animals, such as social interaction between male and female, group behavior or a mother and her pups.

Transgenic Animal research

Today, many laboratories are working with genetically modified mice or rats. Often, the effects on behavior are not known or difficult to predict. USV could be an interesting add on to behavioural research batteries by measuring a completely different parameter. SONOTRACK can be used to monitor the animals over a long period of time where normal observation would not be possible or not very time efficient .

SONOTRACK operation

Measuring and analysing ultrasounds has never been so easy. SONOTRACK is a turn key system that has an intuitive user interface and provides the researcher instantly with all relevant details of the (ultra)sounds picked up by the SONOTRACK microphone units.

Typically an experiment using SONOTRACK will consist of the following steps:

- Definition of the experiment
Step 1: Definition of your experiment
- Preparing a measurement
Step 2: Definition of your test session (measurement)
Step 3: Preparing a measurement (threshold level, low & high cut-off frequency)
- Data collection
Step 4: Actual collection of data (immediate or delayed start)
- Data Processing
Step 5: Conversion of data in usable file formats and file sizes
- Generating and viewing the test results
Step 7: Displaying of graphs (frequency plots, spectrograms, sonograms etc.)
Step 8: Exporting and printing the results

Defining the experiment

Each SONOTRACK experiments starts with the definition of your experiment, which is done only one time. The SONOTRACK software enables the user to enter a number of relevant details that identify the experiment, like

- Experiment name
- Experiment description
- Animals (strain, sex, age, etc.)
- Treatments
- Environment

Defining a test session (measurement)

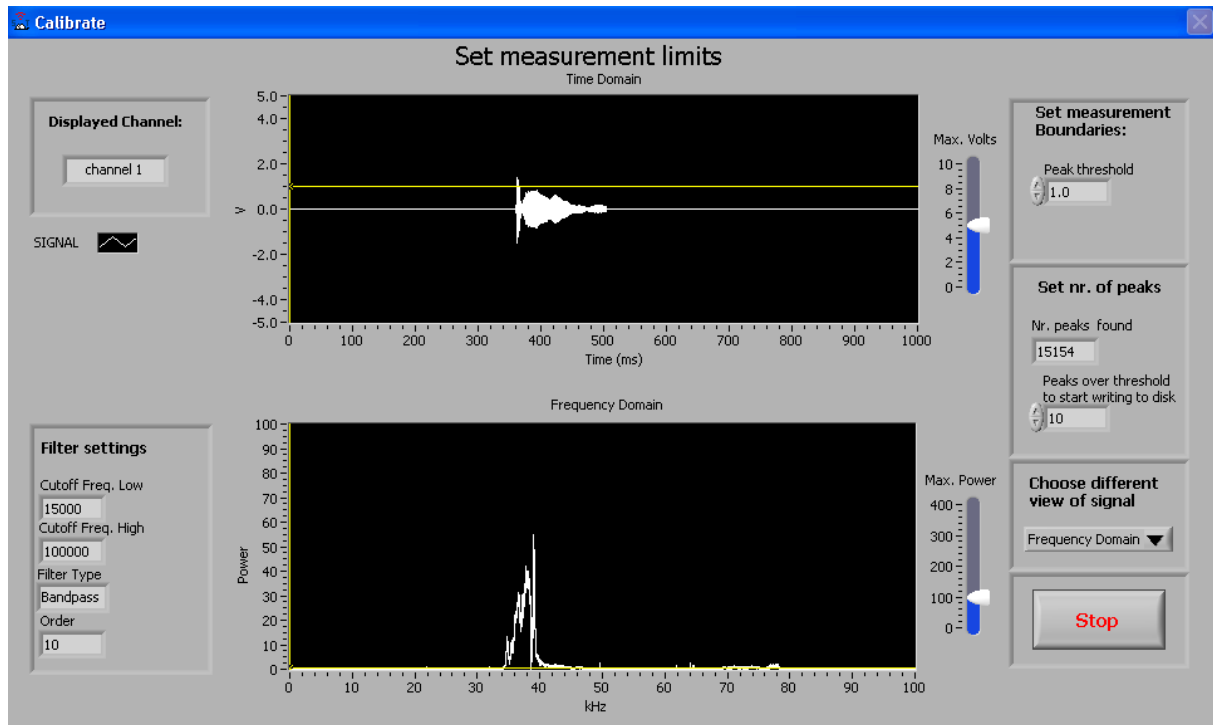
Within an experiment the user can perform a number of so called test sessions or measurements. SONOTRACK enables you to run up to 1000 measurements in an experiment and a test session can vary from 1 second to a week. Before starting a test session the researcher enters a number of details related to the measurement such as:

- Start date / Start time
- Duration of experiment
- Animal ID's
- Animal Treatment codes (if applicable)
- Etc.

SONOTRACK enables the users to start at a later time when the researcher is not present (delayed start).

Preparing a measurement

Before starting the actual measurement the user has to prepare the equipment by setting a number of hardware related parameters, such as the threshold level for recording, the low and high cut-off frequency to eliminate background noise and to record only when there are vocalizations. The user is supported by the software to find the best possible settings. The user can also accept the default settings in which case the SONOTRACK will be adjusted to the best possible performance.



Setting of threshold and frequency range

Data collection

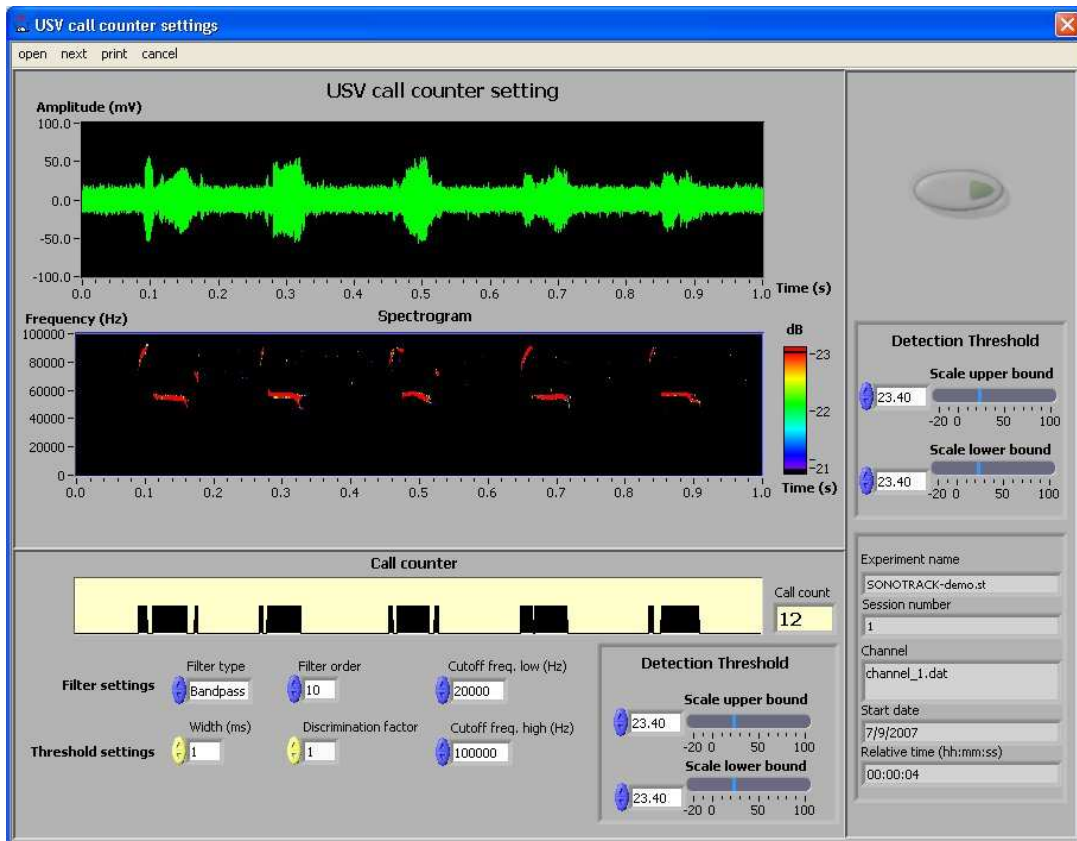
The data collection can be started after a user-defined wait cycle or instantly. In case of a delayed start the user has to enter the desired start time during the preparation of a test session.

When collecting data collection SONOTRACK will show a minimum of information. This done to minimize the processor load and enable collection of four independent channels at the same time. During the data collection, the user can stop the storage of data to hard disk without interruption of the measurement. In addition the user is able to adjust the peak over threshold value if the background noise in the environment should change for some reason and to enable fine tuning.

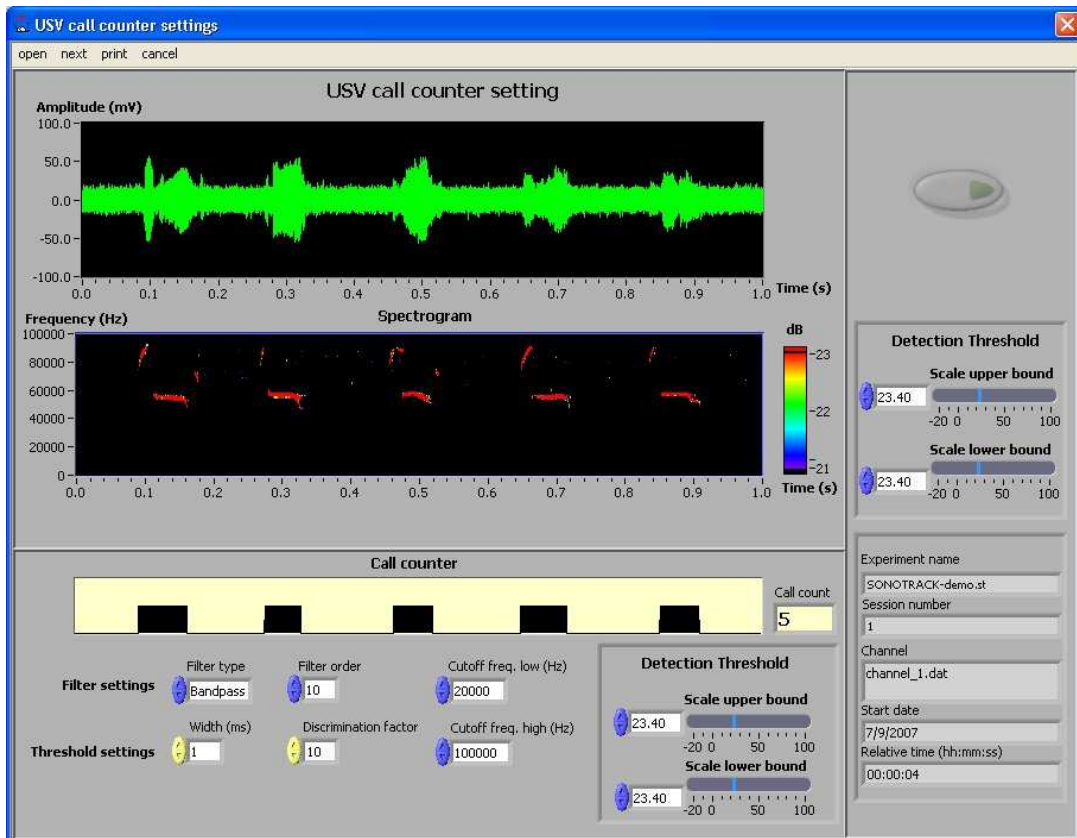
SONOTRACK will automatically end the data collection when the end of the test period has been reached.

Automatic Vocalization detector

After data collection the SONOTRACK software is capable to recognize the animal vocalizations automatically in the recorded data. The researcher can adjust the Vocalization detector based on its specific preferences for counting the calls. In the examples below the counter is set to count all syllables of calls separately or as one call.



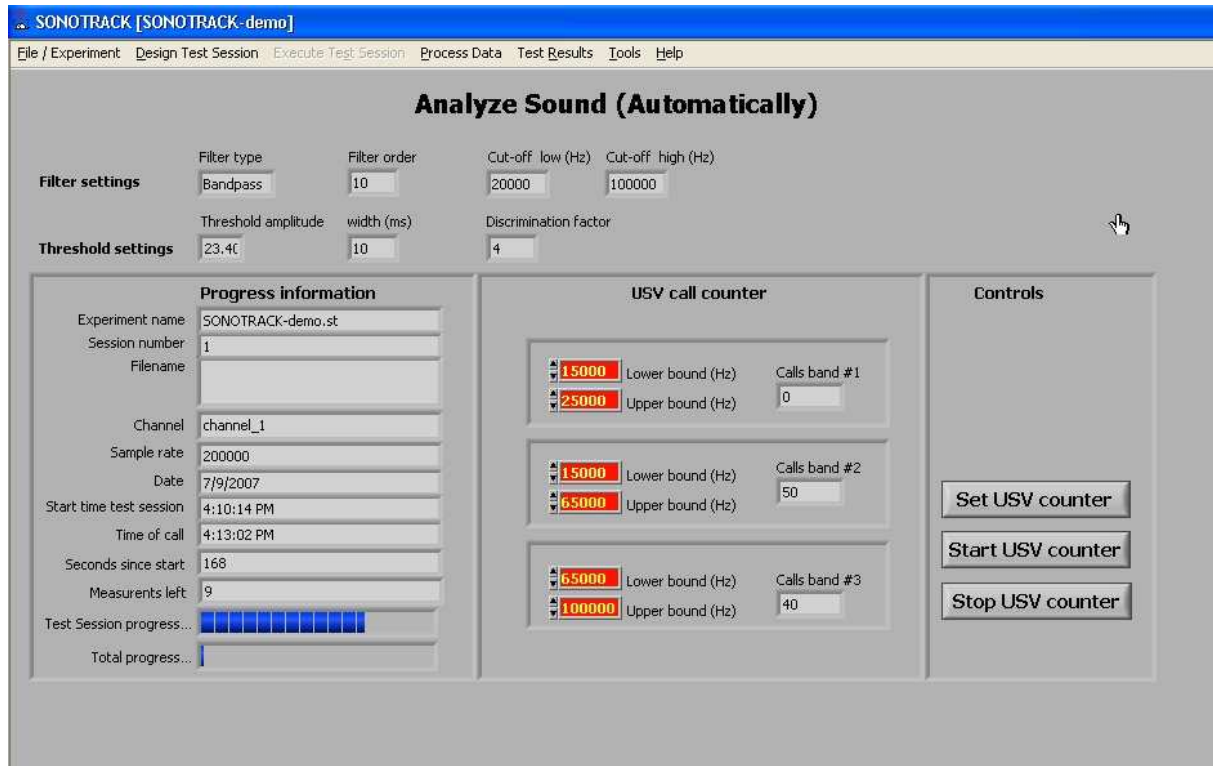
Example 1: Setting of Automatic Vocalization counter to count all calls separately



Example 2: Setting of Automatic Vocalization counter to count different syllables as one call)

The call counter has also frequency filters and call duration filters that can be used to skip or count specific vocalizations.

After setting the call counter, all measurements can be processed at once, saving a lot of time for the researcher. The automatic Vocalization counter will process all test sessions and recorded channels and will provide detailed as well as summarized data. The user can select three frequency bands in which the vocalizations are counted separately. These user defined frequency bands can be overlapping in frequency (see screen below).

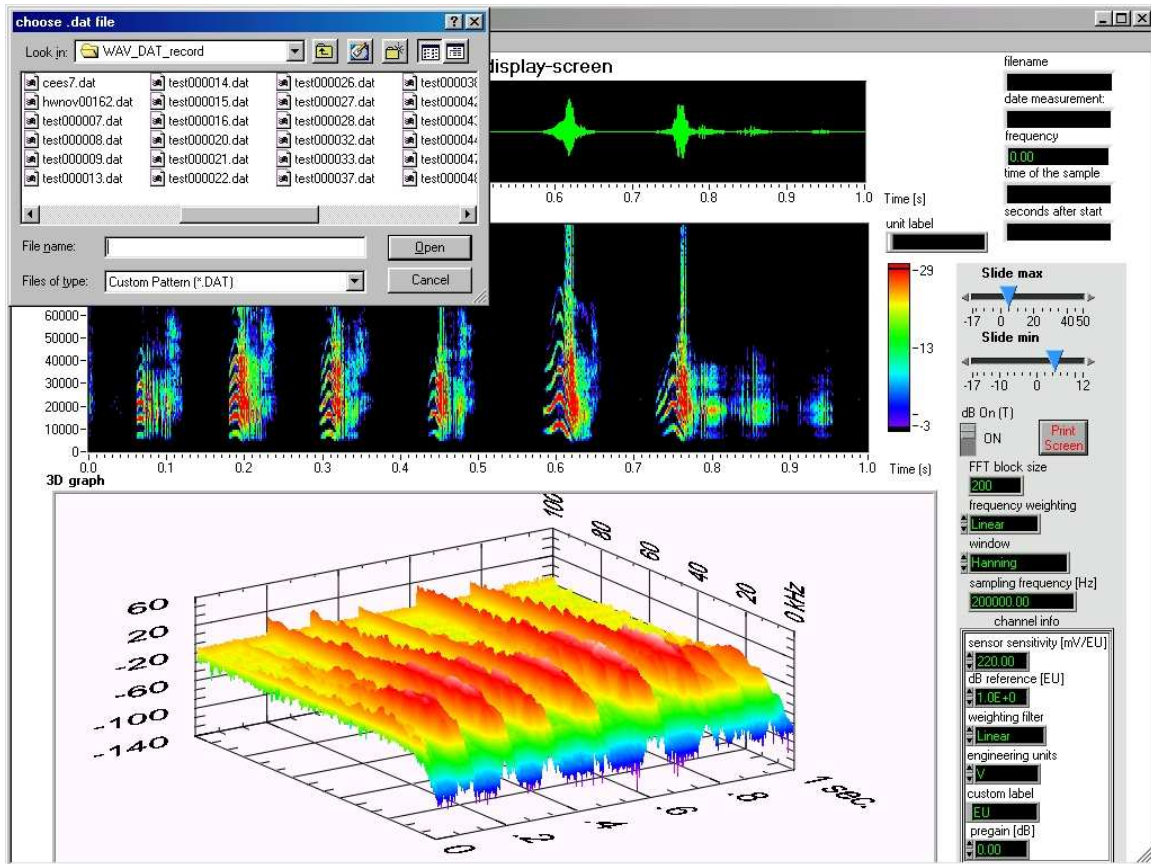


Example of Automatic Vocalization detector (batch processing screen)

Generating and viewing test results

The SONOTRACK software offers a wide range of possibilities to visualize the recorded sound signals, such as raw data display (amplitude against time), spectrogram (frequency against time, 2D sonogram (frequency against amplitude), 3D sonogram (frequency and amplitude against time).

All graphs can be customized by adjusting type and setting of filters and characteristics of the graph. After selecting the interesting parts of the data and selection of the required graphs, the information can easily be exported to other Windows packages or directly send to a printer.



Example of Marmoset Ultrasound calls

Configurations and Accessories

SONOTRACK is available in several configurations. The SONOTRACK configuration can be tailored to your requirements by selecting:

- the number of channels,
- upgradeable with or without A/D card replacement
- Playback functionality

Configuration	No. of channels	Upgradeable w/o A/D card replacement
Ultrasound 1	1	No
Ultrasound 1U	1	Yes
Ultrasound 4	4	N/A
Playback function	1	N/A

All SONOTRACK configurations consist of one or more microphones (including microphone cables), the SONOTRACK control unit (with one or more ultrasound amplifiers), an A/D converter Card for the PC, and the SONOTRACK software.

- The SONOTRACK microphone is a wide spectrum microphone that is provided in a metal housing that can be installed on a tripod or mounted on a cage using the screw connections.
- The SONOTRACK control unit contains up to 4 ultra low-noise amplifiers that are using the latest electronics that combine a high dynamic range with a very low noise.
- The *A/D converter card* (National Instruments M-series) can handle up to 4 channels, dependent on the configuration. Configuration 1U and 4 use a A/D converter card that can handle up to 4 channels. Although 1U is a single channel version, upgrading to 4 channels can be done at a later stage without replacing the A/D card.
- The SONOTRACK *software* will be delivered on a CD-ROM and is designed for use on PC running the Windows-XP or Windows Vista operating system. Other Windows platforms are possible but are not advised and supported by Metris. The computer and operating system are not included in the package

The Playback functionality consists of a high quality Ultrasound speaker (including cables), an Ultrasound amplifier of 50W, an D/A convertor Card for the PC and the SONOTRACK playback software upgrade.

- The SONOTRACK Ultrasound speaker can provide a maximum continuous power of 50W over the full ultrasound spectrum.
- The SONOTRACK amplifier contains a wideband low-noise 50W amplifier that has a flat frequency response curve between 5 kHz and 200 kHz. The amplifier gain can be adjusted by the researcher with an external knob on the SONOTRACK amplifier. The power supply is included in the amplifier unit.
- The D/A convertor card (National Instruments M-series) converts one digital signal into an analog signal and sends it to the SONOTRACK amplifier.
- The SONOTRACK playback software is integrated with the other SONOTRACK software and is enabled by Metris when the customer orders the playback functionality.

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Ordering Information

Prices

For details on prices, product part numbers and ordering requirements refer to the most recent pricelist or contact the Metris marketing and sales department or your local distributor for a recent pricelist or a quotation.



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