Getting started with Spike Recorder on PC/Mac/Linux

You can connect your SpikerBox to your computer using either the blue laptop cable, or the green smartphone cable.

How do I connect SpikerBox to computer using USB cable?

You can connect your:
- Neuron SpikerBox Pro
- Muscle SpikerBox Pro
- Plant SpikerBox
- Heart and Brain SpikerBox

to your laptop or computer with the provided micro USB Cable:

When you connect USB cable to your computer and turn the power switch ON on SpikerBox a new USB button will appear on the top of the Spike Recorder’s window. When you click on the button Spike Recorder will connect to SpikerBox and start acquiring data from it. By clicking on the same button again you will disconnect from SpikerBox and return to recording of default audio inputs on your computer.
How do I connect a SpikerBox to my computer using green smartphone cable?

It depends on your laptop. Most PC laptops have a separate headphone and line-in jack. You want to plug into the line-in (microphone) jack. If you have a Mac Laptop with two audio ports (like some MacBook Pro’s and older iBooks and MacBooks), you want to plug the green smartphone cable into the line-in port (has a weird symbol).

If you have a MacBook, MacBook Pro, or any computer with only 1 headphone port, then plug in the green smartphone cable into the headphone port. These laptops only have one audio jack and it is a combined mono mic input and headphone jack.

While all SpikerBoxes can be connected using this method, the following SpikerBoxes only support the Green Smartphone Cable:

- Muscle SpikerBox
- Neuron SpikerBox

And are connected like in the photo below. Note that the “Smartphone” end is going into the laptop. This is important as this cable is not reversible.
Configure Inputs

To set things up, you can use config screen (gear in upper left corner) to select the channels displayed as well as change the color of the waveform or add/remove channel from real-time view.

All the signal inputs through which you can acquire and record the signal will be listed out in the config screen. The items displayed is based on your computer’s audio configuration and type of the device connected to your computer.

By using color drop-down chooser, you can change the color of the channel’s waveform or turn off the channel by selecting black color.

On the same screen you can mute sound on your computer’s speaker during recording by clicking the selection box on the top of the config screen.
Also, in configuration you can enable real-time digital filtering of the signal that you are recording. In order to enable band-pass filter you can change cutoff frequencies using slider or by entering exact frequency for low and high cutoff frequency in boxes above slider. After you change cutoff frequencies new filter’s settings will be automatically applied to signal and frequencies outside selected band (marked green on slider) will be attenuated.

Beside band-pass filter you can enable Notch filter by ticking the checkbox at the bottom of filter section. Notch filters are made to strongly attenuate just one frequency. This is very useful for eliminating mains noise commonly present in electrophysiological signals (50Hz or 60Hz depending on country).
Arduino based devices

- Muscle SpikerShield,
- Muscle SpikerShield Pro,
- Heart & Brain SpikerShield

have additional dropdown chooser that enables you to set the number of channels the device will record. When you connect to those devices, initially, you will acquire only one channel at the sampling rate of 10 kilo-samples per second (10 000 samples per second).

If you change the number of channels using this drop-down list, Spike Recorder will instantly start recording multiple channels from device and it will accommodate the sampling rate of each channel so that it cumulatively does not breach the sampling limit of 10 kilo-samples per second. Therefore, if you select to use just one channel, the sampling rate of that channel will be 10 kilo-samples per second. But, if you select two channels, the sampling rate of both channels will be set to 5 kilo-samples per seconds (three channels will have the sampling rate of 3.3 ksamples/s, 4 channels 2.5 ksamples/s and so on).
Real-time view

By default, Spike Recorder will be in the real time view upon startup and will display the waveform of the signal recorded through your SpikerBox or SpikerShield.
By scrolling up and down, you can change the time scale of your signal presented at the right-hand bottom side of the window. We added great memory depth to Spike Recorder so that you can observe any aspect of the signal you want - from events as short as 0.1ms to long trends of up to dozens of seconds.

Sometimes, especially when recording a multichannel signal, it will be useful to change the vertical position of the signal axis. For this purpose, you can use the handle on the left-hand side of the axis. With a single click on the handle you can grab and move your axis vertically.

You will find zoom buttons next to the axis handle. With “+” and “-” buttons you can change the vertical scale of the signal and, by doing this, zoom in the details of the waveform.
As we have mentioned earlier, Spike Recorder has great memory depth. Therefore, if you think you have missed an interesting event, you can press the “Pause” button at any time at the bottom of the screen and scroll the signal waveform back in time. After you have found the event of interest, you can playback signal again by clicking on the play button.

To return back to real-time signal acquiring use the “Forward” button next to the “Play”/“Pause” button.

Recording

When you have set up your experiment and you are ready to record, simply click on the “Record” button (a round button with a red dot in the center) and Spike Recorder will automatically start saving signals to an audio file.

During the recording, you can mark events in your experiment by pressing keys from 0 to 9 on your keyboard. The event markers and their precise timings will be automatically saved to a separate text file in the same directory where your signal recordings had been saved. Spike Recorder will name events file with the same name as the signal recording file, except for the fact that the events file will have an additional “-events” suffix.
By using that name, coding Spike Recorder will be able to automatically load and present event markers on the waveform the next time you playback your experiment’s recording.

When you click for the second time on the “Recording” button, Spike Recorder will stop the recording signal and present you with a popup with the full path of the recorded file on your computer.

Thresholding

If you want to average neuron spikes or take a snapshot of a single spike, you can use threshold view. By clicking on the “Threshold” button, Spike Recorder will enter the threshold mode that will catch the snapshot of the signal every time the waveform crosses the threshold level.

There will be an additional slider on the top of the screen in the threshold mode. With this slider you can adjust the number of consecutive spikes that will be used for the calculation of the average spike. For example, if you select to average 25 consecutive spikes, Spike Recorder will always use the 25 most recently captured spikes for the calculation of the average spike. In extreme cases, when you set slider to the value of one, Spike Recorder will present only a raw signal of the most recent spike.
By using the handle on the right-hand side of the screen, you can adjust a threshold level that suits your needs in an experiment.

**Spike Analysis**

Once you have recorded and saved an experiment, you can open it for analysis. To open the experiment recording, click on the “Browse” button on the right-hand top side of the window. After the file browser has appeared, you can locate the audio file with the recorded signal and open it (audio files will have .wav file type extension). Spike Recorder will automatically load the other files created during the recording or the analysis of the signal (timestamps of events and sorted spike trains).

When you open a saved experiment, Spike Recorder will show one additional button that you can use to enter Spike Analysis view.
The Spike Analysis view will initially detect all the potential neuron spikes in the signal and mark them with a white dot. Detection algorithm is very sensitive and it will typically detect a lot of spike candidates that can represent a spike of the neuron we are interested in or the spikes of a distant neuron or even noise glitches. Therefore, in order to filter the spike set, you will need to adjust a valid peak amplitude interval using two handles on the right-hand side of the window. During the filtering, you can use the scrollbar at the bottom to check if the filtering interval is compliant with the rest of the signal.

For multichannel files you can use list on the right-hand side of the window to swap between the channels. Also, you can use “+” and “-“ sign in the same list to add new spike trains and remove existing ones.

When you are satisfied with the filtering results, you can click on the save button at the bottom-right corner of the screen to save all created spike trains.

In order to open statistical analysis graphs for created spike trains click on the arrow in bottom left corner of the window.
Statistical analysis graphs

Once you create spike trains for your recording Spike Recorder will automatically calculate four analysis of your spike trains:

- Average waveform graph
- Autocorrelogram
- Cross-correlogram
- Inter Spike Interval graph

You can access graphs in Spike Analysis window by clicking on the arrow button in the bottom-right corner of the screen.
Each analysis will be presented in separate tab. If you created multiple spike trains you will find multiple graphs in each analysis. Every graph corresponds to one spike train and its color matches to color of the spike train.

**Average waveform**
Average waveform graph displays waveform of average spike calculated by averaging all spikes in spike train centered around positive peak. Horizontal linear axis represents time in milliseconds centered around positive peak and vertical axis displays amplitude of average spike waveform in millivolts (mV) units. Beside average waveform (bright line in the middle of every graph) graph also displays standard deviation of waveform as a colored area around average waveform.
Inter Spike Interval graph

This graph is derived from single spike train. Horizontal axis represent time difference between two adjacent spikes in single spike train. For this graph we used logarithmic time scale to be able to show small inter spike intervals and greater inter spike intervals on the same graph. So, for example, -2 on horizontal axis represents $10^{-2}$ seconds which is equal to 0.01 seconds or 10ms.

For Y axis application calculates interspike intervals for all adjacent spikes and sort them in bins. Namely, height of one bin represents number of occasions we encountered given interspike interval in signal.
**Autocorrelogram**

Autocorrelogram graph is very similar to ISI graph and is also calculated on single spike train. The horizontal linear axis represent time difference between any two different spikes (note that ISI is calculated only for consecutive spikes). Unit on the time scale is second. Vertical axis represents absolute number of spike pairs we measured in spike train. Therefore height of one histogram’s bin represents number of occasions we encountered given time difference between two spikes in signal.

![Autocorrelogram Graph](image)

**Cross-correlogram**

Cross-correlation is used to discover hidden relation between two neurons. Therefore it is calculated using information from two spike trains. When you select crosscorrelation tab first thing you should do is to select spike train in the list at the right-hand side of the screen. After you select spike train Spike Recorder will calculate cross-correlation between selected spike train and all other spike trains. Similarly to previous graphs horizontal axis represents time difference between two spikes given in seconds. On vertical axis height of one histogram’s bin represents number of occasions we encountered given time difference between two spikes from different spikes trains. Note that if cross-correlation is calculated between two same spike trains resulting graph is equal to autocorrelation graph.

![Cross-correlogram Graph](image)
Signal measurement

During the playback of the recorder signal, you can pause it at any time and inspect an interesting part of the signal in more details. You can hold the right-click button (press two fingers on trackpad on Mac OSX) and drag the mouse cursor to select the interval of the signal. Spike Recorder will show you the time length of the interval in milliseconds and calculate RMS (Root mean square) of the selected portion of the signal. If you have added spike trains to your recording Spike Recorder will display number of spikes and frequency of spikes in selected interval for all added spike trains.
Updating firmware on SpikerBox Pro

**Note:** Update of firmware for the SpikerBox Pro works only on the Windows operating systems

In order to update functionality of your SpikerBox Pro you can choose to update firmware on device through Config screen.

Each time you start Spike Recorder the application will download information about available updates for SpikerBox Pro from BackyardBrains website. If your device has available new updates for firmware you will be presented with additional dropdown chooser in Config menu. Using the dropdown you can choose new version of firmware that you want to upload to SpikerBox Pro.

In order to update firmware you must first turn OFF power switch on SpikerBox Pro and then click on button on right-hand side of firmware dropdown chooser (see image below).
When update of firmware starts Spike Recorder will display a screen that shows progress of firmware update. Please do not unplug the SpikerBox Pro from USB during firmware update. When Spike Recorder upload new firmware to device SpikerBox Pro will disconnect from Spike Recorder and software will display screen with message "Firmware update Successfully".

After successful update of firmware you can immediately connect again to SpikerBox Pro and continue using device with updated functionalities.

At any time you can choose old firmware from dropdown chooser and downgrade to previous functionality of SpikerBox Pro.

If you lose internet connection or accidentally unplug USB cable during update of firmware Spike Recorder will display error message on the screen. If you see error message you can try to upload firmware again following procedure below:

- Unplug SpikerBox from computer,
- Turn OFF SpikerBox
● make sure that you have internet connection on computer
● restart Spike Recorder
● plug in SpikerBox Pro again to USB
● connect to SpikerBox from Spike Recorder
● choose firmware in Config screen
● start firmware upload again

Updating firmware on Arduino based products

Products:
● Muscle SpikerShield,
● Muscle SpikerShield Pro,
● Heart & Brain SpikerShield,
● Plant SpikerBox

are all based on Arduino platform and you can update firmware for the device using Arduino IDE software. You can find latest code for each device on it’s product page on BackyardBrains website. You can find a detailed explanation on how to install the Arduino IDE software and upload sketch to the Arduino board on the arduino.cc website.

**Note:** It is important to understand that Spike Recorder and Arduino Software are using the same serial port and USB cable to communicate with Arduino board. Because of this, you cannot connect to Arduino board from Spike Recorder and Arduino IDE software at the same time. Therefore, the safest thing to do is to simply close Spike Recorder when you are updating code on the device and to close Arduino IDE software when you start using Spike Recorder.