eProbe 5.8.9

Single Unit Recording Manual

ScienceBeam Co

eProbe

Version 5.8.9

Acquisition and analysis software
Oct 2016

Devices

◦Devices manufactured by **ScienceBeam** company are:

- **eWave**

- **eLab**

- **ePulse**

- **eMech**

- **eClamp**

- **electromodule**

◦**eProbe** software designed to work with above workstation devices.

- you can have access the eProbe manual for each device in separated part specified for each device.

eLab

eLab

eLab is all in one system that can record extracellular signals including:

- single unit Recording
- local field potential
- in vivo Brain Slice (LFP & single unit)
- ECOG–Electrocorticography

eLab Specification

◉Specification:

- Two Channel 12 bit digital to analog converter

- 8 analog input channels (24bits, sample rate: 50KS/S)

- 8 Digital I/O

- 4 channels bioamplifier for recording of EEG/LFP/ single unit/EOG/ECG/ECG/EMG

- 4 channel Pulse generator, 10µs pulse duration resolution

- Isolated constant current simulator (4mA/20mA)

- Optional mechanical stimulus controller

- Plug and Play (USB2 connector)

- Operating voltage: 12V DC

- 115 gr

eLab device

◉Power button:

◉There is no power button. Device turn on by connecting to computer through USB

◉LED status:

- **Constant red light** means ready for programming by manufacturer

- **Flashing red light** means out of charge

- **Fast flashing Green light** means device connected to computer and record signal properly

- **Slow flashing green light** (every 2 Sec) means device turned on but not connected to computer properly

USB/Charge

Digital input/output

LED status

Port A

eLab device

◉Port A

- EXG recording signal cables can be attached to port A or B

- Port A is the only active port for two / four channels device

- Port B is active for eight channel device (channel 5–8 of recording signal is throughout of port B)

◉USB / Charger

- To charge device, use 12 Volt adaptor via USB–B

- To connect device to computer, use this USB port

◉Digital input/ output

- It belongs to digital input/ output and manufacturer settings

- **Don't use it for EXG recording signal**

USB/Charge

Digital input/output

LED status

Port A

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1 Installation

Let's start

There is two folders in software package:
eProbe and Protocols

"

⊘ eProbe folder

- ◉ It consists of **eProbe.exe** and other files that is internally used by software.

- ➢ Run the **eProbe.exe** -> "eProbe is not activated" error → **you need to register:**
- ➢ **Help** menu → **About eProbe** → copy **eProbe Serial Number (12 digits)**
- ➢ Contact **ScienceBeam** via **phone** or **email** to receive **Product Key**→ paste it in the **Product Key** box in eProbe and click **Apply.**
- ◉ **unplug any extra USB drive or Hard drive or you might receive error even after registration!**

✉ **support@ScienceBeam.com**

eLiteSetting.txt
eProbe4.67.dll
∞ eProbe5.26.exe
☑ ∞ eProbe5.34.exe
eSpikeAnalysis.cfg
eSpikeTemplate.txt
eTraceAnalysis.cfg
eTraceTemplate.txt

About eProbe   —   □   ✕

eProbe Version 5.3.4.0

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www.ScienceBeam.com

eProbe Serial Number: 000000000000

Product Key [   ]

Apply

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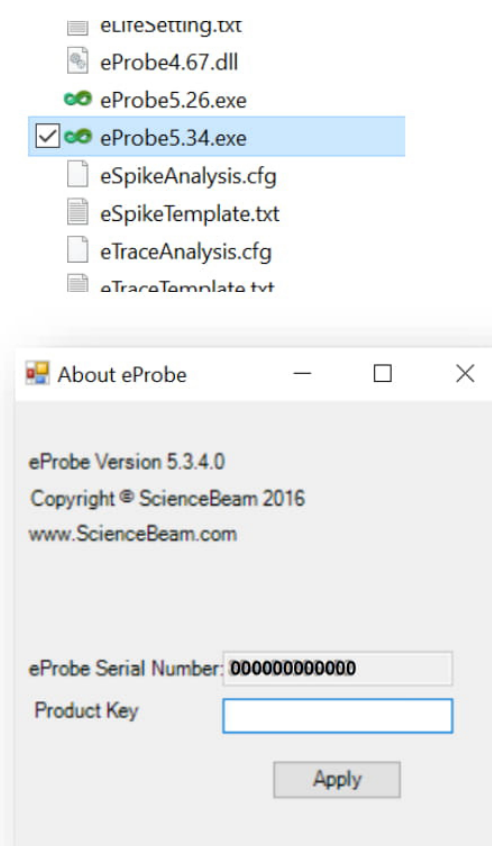
📌 Protocols folder

📁 Anim
📁 Games
📁 Image
📁 Movie
📁 Scene
📁 Video
📄 ADHD-b1.txt
📄 ADHD-SMR.txt

◉ The Protocols Folder consists of some folders and protocols.

◉ **Add every new protocol in protocol folder to work properly.**

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2 Start the program

Let's run

Run eProbe

●eProbe environment has four menu:

➢ **File**
- Open Template
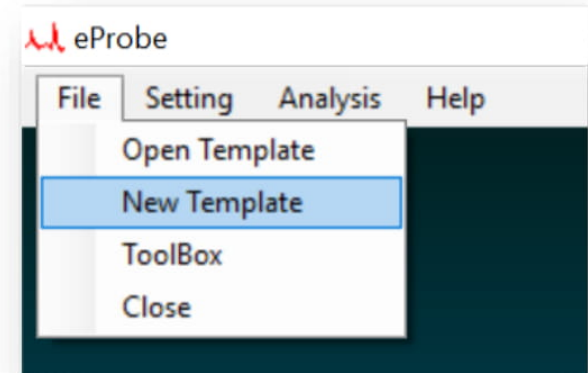- New Template
- ToolBox
- Close

➢ **Setting**

➢ **Analysis**
- eTrace
- eSpike

➢ **Help**
- About eProbe

eProbe

| File | Setting | Analysis | Help |

Open Template

New Template

ToolBox

Close

Template

Template is the environment you create in the eProbe by using ToolBox components for your study.

◉**New Template**

- To create new template by choosing a name for your template "single unit" and save it in Protocol folder

◉**Open Template**

- To open previously saved or sample template from Protocol folder
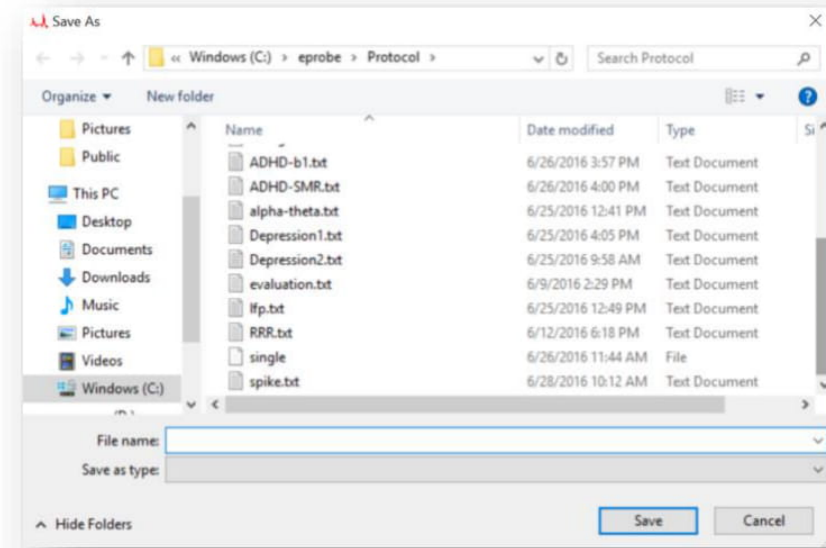
ToolBox

◉Here you can configure what you need in eProbe environment based on you're experiment design.

◉ToolBox includes:

- Bar
- Scope
- FFT
- Image
- Light
- Text

- Val
- ERP
- Game
- State
- Scene
- eSorter
- Content

◉You only need Scope & eSorter of ToolBox for single unit recording.

File   Setting   Analysis

Bar
Scope
FFT
Image
Light
Text
Val
ERP
Game
State
Scene
eSorter
Content
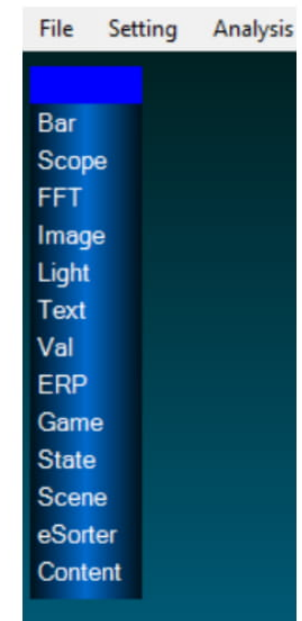
ToolBox

◉To **add** Scope or eSorter, just simply **click on it from ToolBox**(for example: Scope) and then click on the **available empty space** of program environment. You can also resize Scope or eSorter by dragging its corners.

◉To have access to Scope or eSorter **settings**, simply do right click on Scope or eSorter already created on screen.
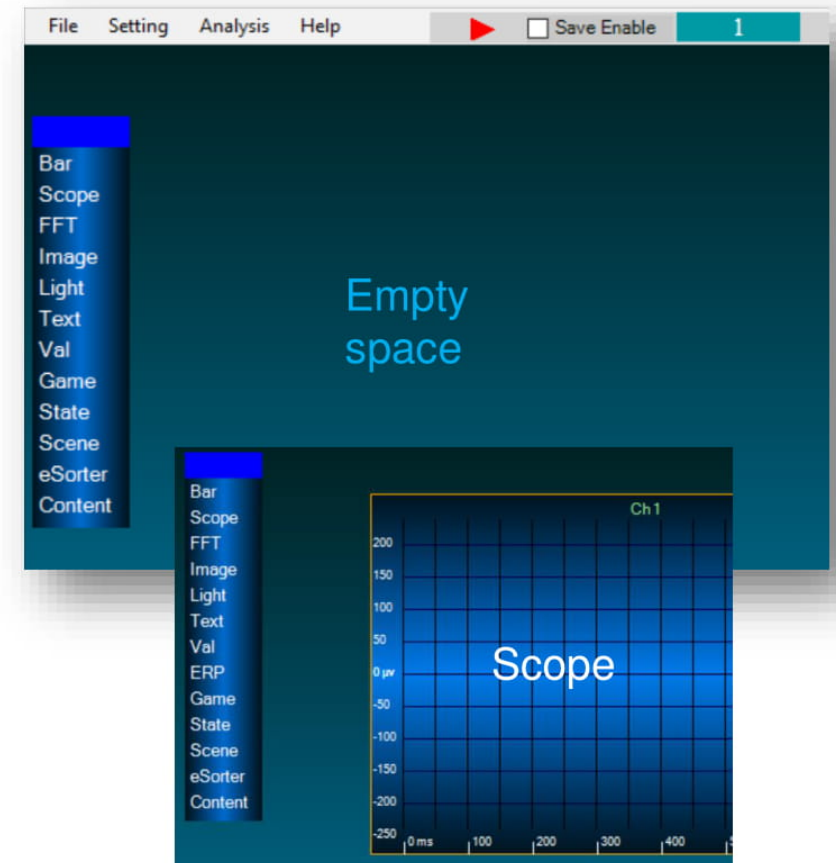
Single Unit Template

◉Now you added **Scope** & **eSorter** from **ToolBox** to the **available empty space** of program environment.

◉**eSorter panel** consisted of three windows, itself:

- Window discriminator
- Clustering Window
- PSTH
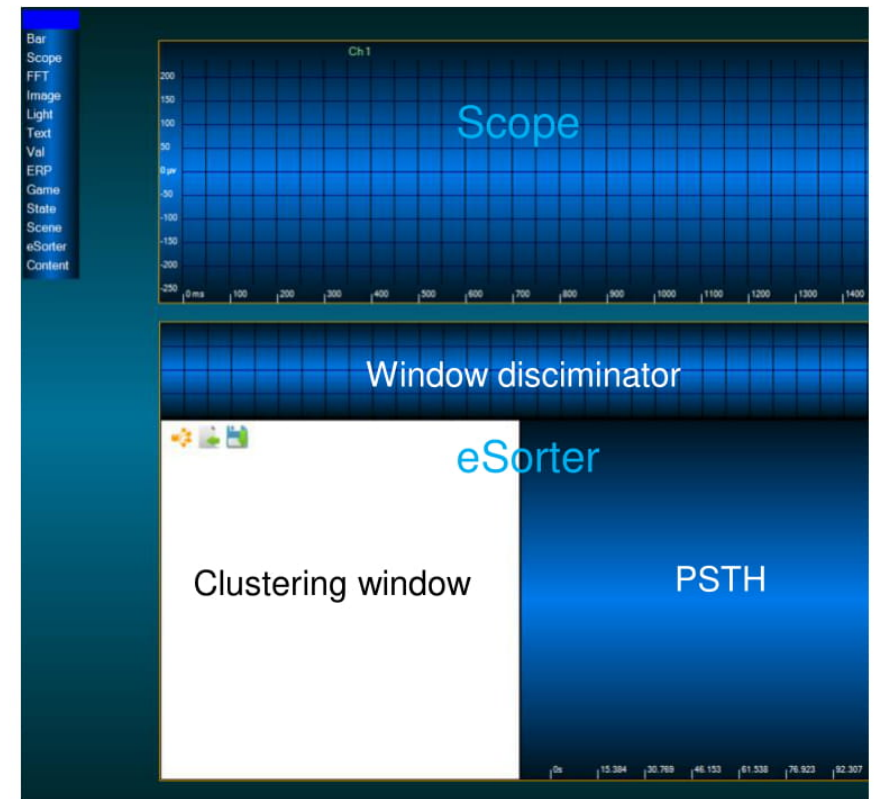
Setting menu

◉It consisted of:

- **eDevice:** eWave/eLab/ePulse/eMech/eClamp/Electromodule/WSI3108

- **Connection:** Offline/USB/WIFI/Bluetooth

- **File:** Record/Simulation/Stim Protocol/Make Stim Protocol

- **Event**

- **NetworkPort**

- **NetShare**

- **Stimulator:** Off/Normal/Inverted

- **Stim Current**

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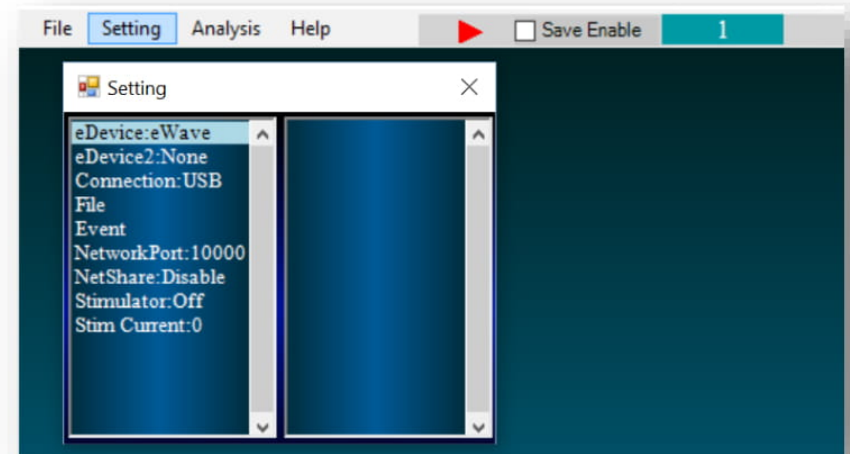
2 Connect to computer

Connect to computer

Choose your recording and stimulator device name:

- Choose **eLab-c** from **Setting menu/eDevice**

- Choose **ePulse-b** or **None** from **Setting menu/eDevice2**

Choose your connection type:

- **Offline – to work with previously saved data**

  - Choose **Offline** from **Setting** menu/**Connection** in eProbe software

- **USB cable**

  - Choose **USB** from **Setting** menu/**Connection** in eProbe software

➢ **No need to change other settings!**
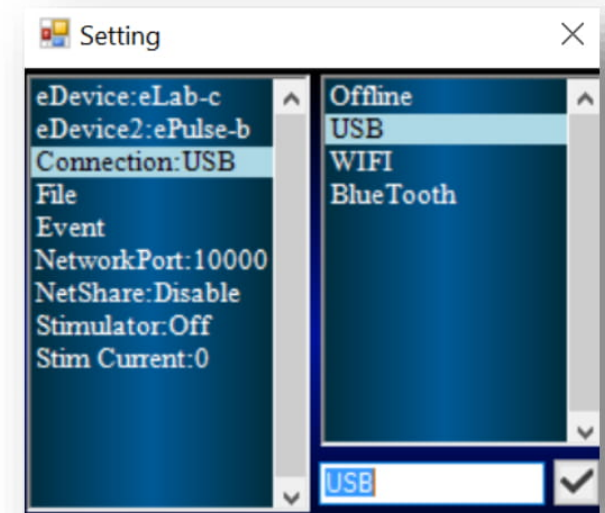
Run and Save experiment

◦To run your recording simply click on **red start button** ▶ to start recording.

◦By checking **Save Enable**, your data will be recorded in destination you will set.

• Also, from **Setting menu/ File/ Record File** , you're able to change the name & destination of data recording.

• You can access your saved data by **Setting/ File/ Simulation File**
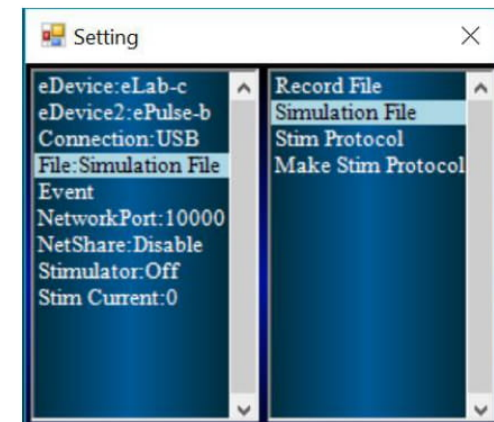
Scope

Introduction & settings

Scope

◉It displays the signal both in time and frequency domain.

◉The **name of channel** or channels which stream data is shown above of scope (here shown as Ch 1)

- If the name of channel has green color it means that signal is unsaturated and natural

- If the name of channel has red color it means that signal is saturated and must be fixed
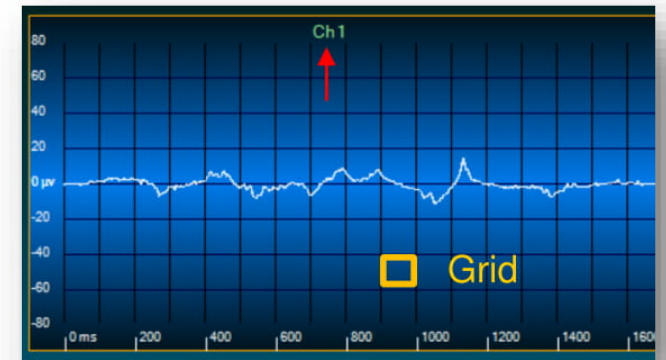
Scope setting

◉**Data Stream:** Channels or ports which stream data

◉You can choose more than one channel based on the numbers of channel your device supports

◉**Signal:** type of signal → Spike

◉Band

◉analyzeMode

◉ResultsText

◉**Theme:** Change the color of waves inside scope

Scope setting

◉**T/D:** Time scaling to optimize view

◉**V/D:** Amplitude scaling to optimize view

◉**Invert:** invert waves in scope

◉**Artifact:** removing artifact by giving value

◉**HPF:** eliminate the low frequency signals or noises

◉**LPF:** eliminate the high frequency signals or noises

➤ The range of single unit signal is 300–3K Hz

◉**Triger:** to trigger continuously or not

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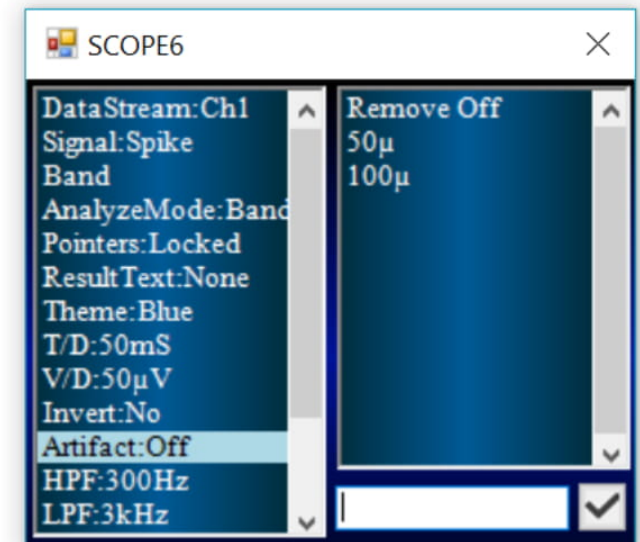
eSorter

Introduction & settings

eSorter

It designed for online & offline sorting and clustering of extracellular recoded action potentials.

eSorter panel consisted of three windows, itself:

- **Window discriminator:** amplitude window discriminator

- **Clustering Window:** separate action potential signals through 2D feature space clustering

- **PSTH:** Peri/Post Stimulus Time Histogram
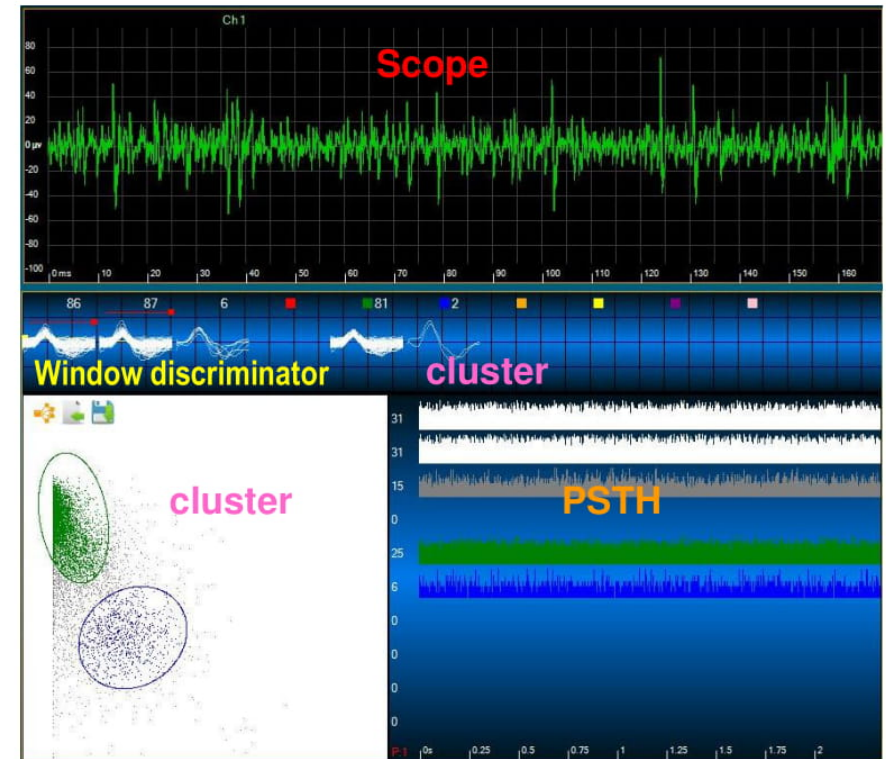
Window Discriminator

- Window discriminator give you the ability to define a windows by two leader line (upper & bottom threshold) to discriminate spikes by limiting the amplitude between these two leader line

- The most left cell display raw spikes and the other right cells display discriminated spikes you define by those two leader line.

- The number at the top of each cell is the count of spikes for that defined window

Raw Spike Numbers

Discriminated spikes

Raw spikes
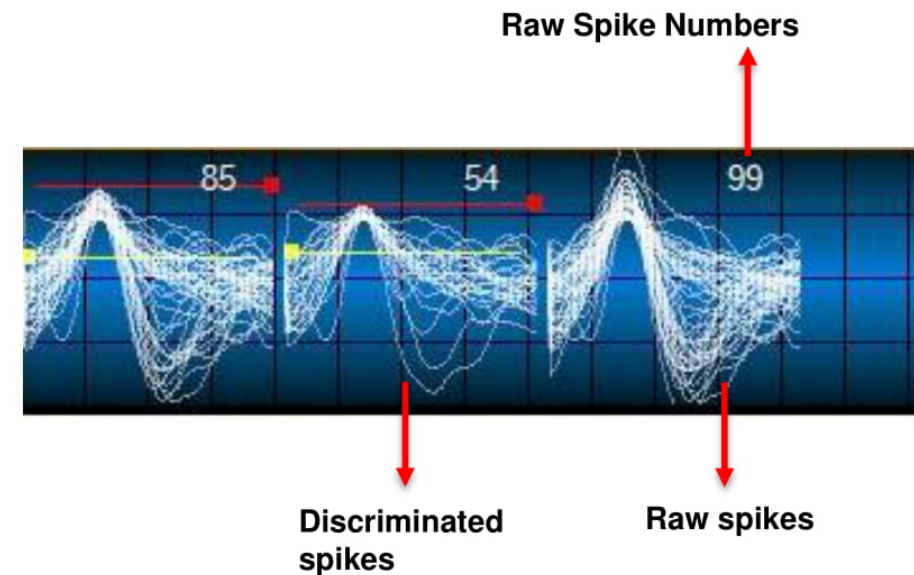
Window Discriminator

- You can change upper threshold by clicking on right side of red line which marked by a red square and drag it to desired upper limit of amplitude & change bottom threshold by clicking on the left side of yellow line which marked by a yellow square and drag it to desired lower limit of amplitude.

- In this way you will discriminate signals by limiting the amplitude between these two leader line or window.

Spike Numbers of window

Upper threshold

Bottom threshold

Discriminated spikes

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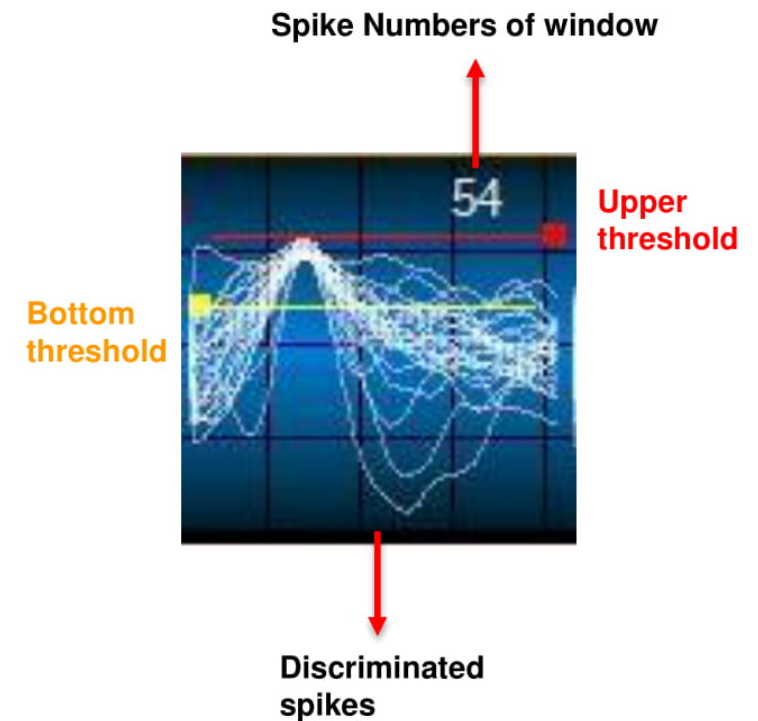
Clustering window

- Clustering Window itself has two panel, one for visualization of cells in a 2D feature display; and the other for visualization of spikes

- It separate action potential signals through 2D feature space clustering and visualize spikes in Spike visualization panel.

Reset   Open cluster   Save cluster

Spike visualization panel

Cell feature panel

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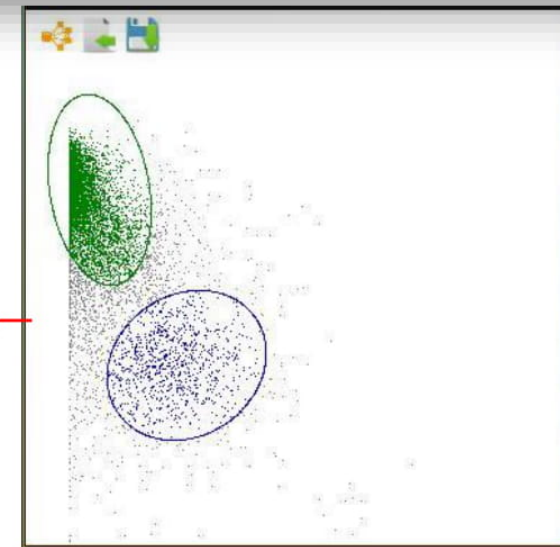
Automatic Clustering window

- In **auto** mode of sorting, automatically spikes will be sorted and will be shown on **Spike visualization panel.**
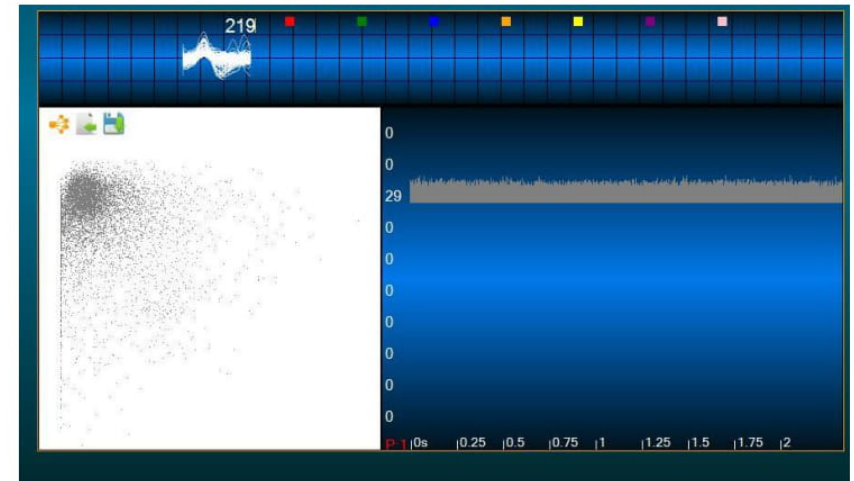
Manual Clustering window

- In **manual** mode of sorting, first you select a cell specified by colorful squares above, in **Spike visualization panel** and then in **cell feature panel** draw an oval around your desired cluster of neurons. Software transform your drawing oval to the best situated oval and will shown it's content on Spike visualization panel.

- Color of each oval in **cell feature panel** is matched with square color of it's cell in **Spike visualization panel**

- To **delete** cluster you made, just select that cell and press delete button.

Spike visualization panel

cell

Draw an oval

Best situated oval

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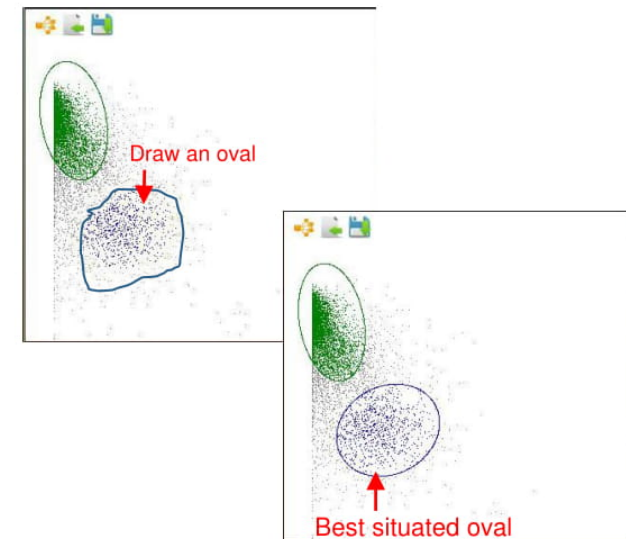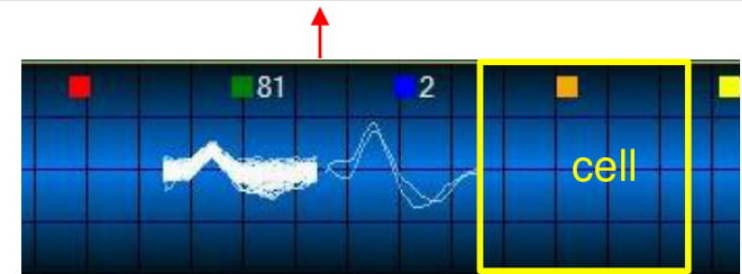
Watch neuron manual clustering from ScienceBeam channel:
https://youtu.be/6muWfWvmyA4

PSTH window

- **PSTH: P**eri/**P**ost **S**timulus **T**ime **H**istogram
- In this window
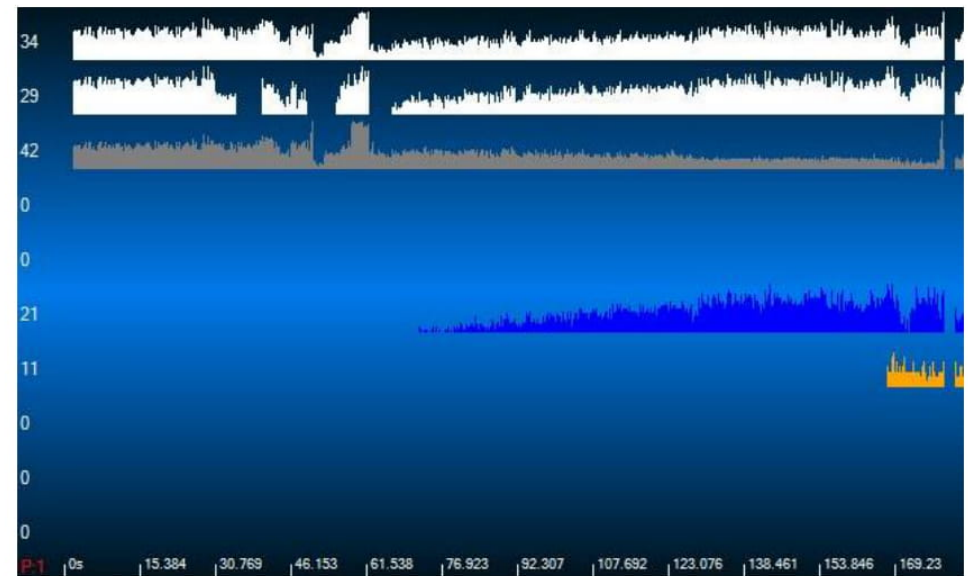
eSorter setting

◉**DataStream:** Channels or ports which stream data

◉**V/D:** Amplitude scaling to optimize view

◉**Histogram Type:** PSTH/ ISIH

◉**Audio:** Set this item on raw to hear raw signal or cluster number (1-7) to hear the sound of that cluster activity.

◉**Sorting Mode:** Manual/ Auto

◉**Theme:** Change the color of eSorter panel

◉**Cluster Number:** number of shown cluster

◉**Active Protocol:** Auto/ 1-8

ESORTER8

DataStream:Ch1
V/D:500μV
Histogram Type:PST
Audio:Mute
Sorting Mode:Manu
Theme:Blue
ClusterNumbers:8
Active Protocol:Aut
BinSize:1
PSTH Offset:0
PSTH Duration:2k
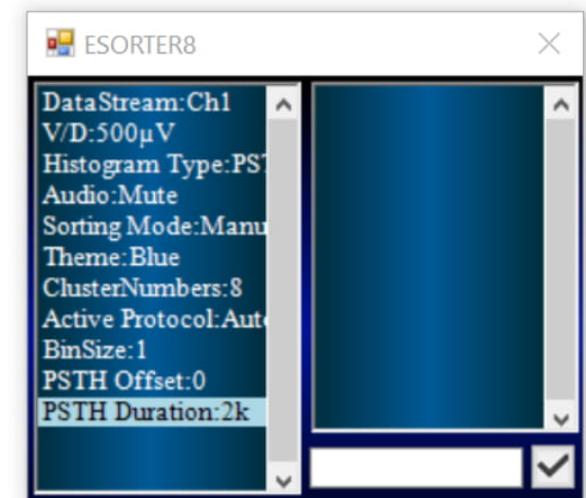
eSorter setting

◉**BinSize:** Use this item to set a bin size for PSTH. Bin size will plot in the X-axis. Through choosing bin size, users could define the **time resolution** of PSTH demonstration. Bin size could have a value from 1ms to several minutes.

◉**PSTH Offset:** Define the beginning of PSTH

◉**PSTH Duration:** Define the duration of PSTH from Offset time

PSTH Offset   **PSTH duration**
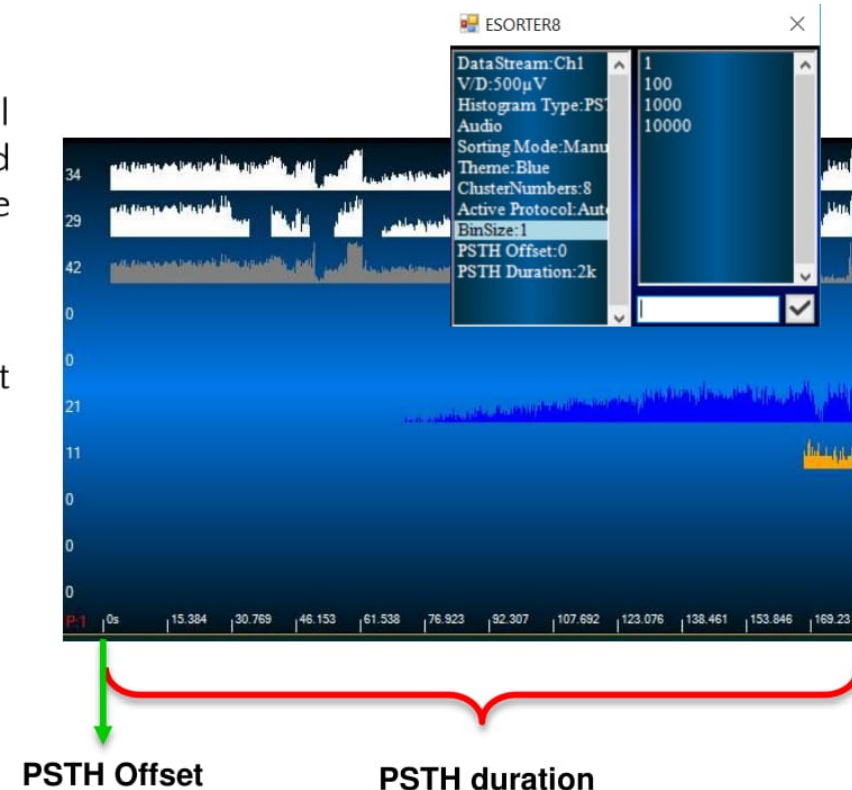
Bin Size

- Each bin is a division of time and appears as a column in the PSTH. The altitude of each column shows frequency of spikes in that bin size.

- The optimal bin size (assuming an underlying Poisson point process) Δ is a minimizer of the formula, $(2k-v)/\Delta^2$, where k and v are mean and variance of k_i (number of spikes).

- If you chose small bin size, you will have high–resolution of spike frequency in time. However, if you chose a big bin size, time resolution will decrease although frequency values will increase.

use a wide bin size when the spontaneous activity of the unit is low or when the spiking activity in response to the stimulus is low!
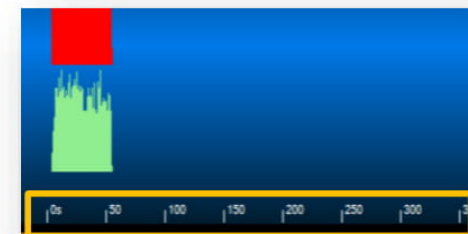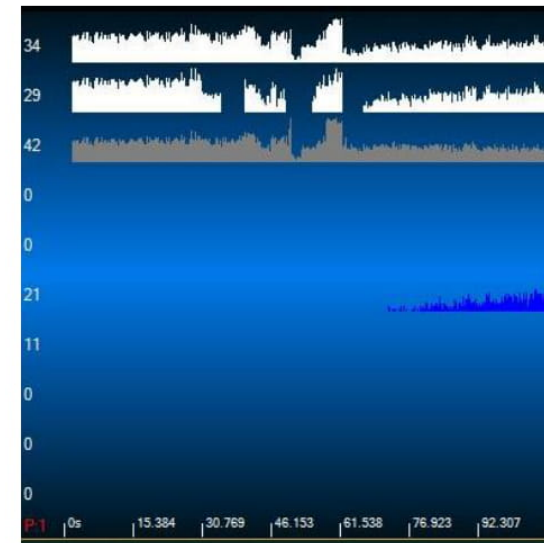
PSTH

- **PSTH: P**eri/**P**ost **S**timulus **T**ime **H**istogram – It used to visualize the rate and timing of neuronal spike discharges in relation to an external stimulus or event.

- The prefix *peri,* for *through,* is typically used in the case of periodic stimuli, in which case the PSTH show neuron firing times wrapped to one cycle of the stimulus.

- The prefix *post* is used when the PSTH shows the timing of neuron firings in response to a stimulus event or onset.
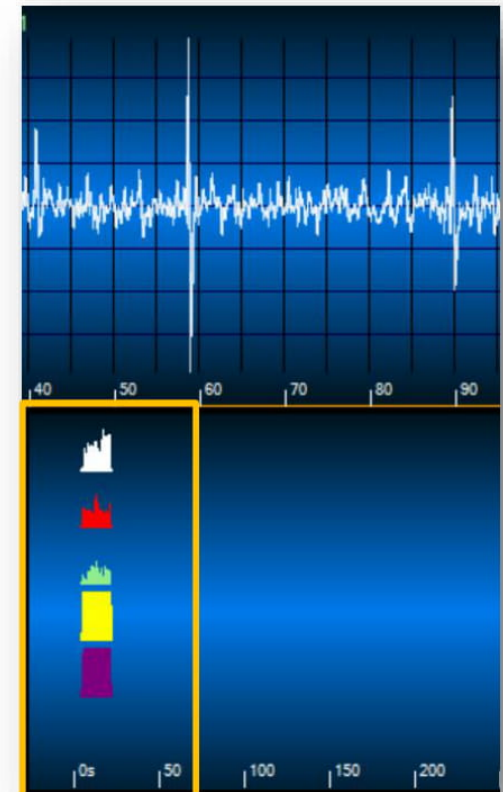
Stimulation protocol

Run experiment with stimulation

⊙In single unit recording, you also need to design stimulation protocol and apply it on the cell.

- **Design** stimulation protocol from **Setting menu/ File/ Make Stim Protocol**

- **Open** stimulation protocol from **Setting menu/ File/ Stim Protocol**

- **Activate** stimulation from **Setting menu/ Stimulator** by changing **Off → Normal** or **Invert**

- **Define amount** of stimulation current from **Setting menu/ Stim Current** by giving a **value** to it

- To run your applied stimulation simply click on **red start button** ▶ to start recording.

Setting ×

eDevice:eLab-c
eDevice2:ePulse-b
Connection:USB
File:Simulation File
Event
NetworkPort:10000
NetShare:Disable
Stimulator:Normal
Stim Current:500m

Current value

0.5 ✓

File   Setting   Analysis   Help   ▶   ☐ Save Enable   1

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Make Stimulation Protocol

- Stimulation Parameters
- Recording Parameters
- Preview
- Mixers

Stimulation Parameters

Pattern (1 .. 4)

Pattern1 ▾

• **Pattern (1 .. 4):** make four different patterns of stimulation. you can mix these patterns to produce a complex protocol by using **Mixers**.

• **T1 (Delay):** Latency between starting the recording time and applying the **first** stimulation pulse of each train

• **T2 (Pulse Duration):** Duration of a single stimulation pulse.

• **T3 (Pulse Cycle):** Duration from starting a single pulse to starting the next single pulse.

• **N1 (Train):** Number of pulses in a *trial period*.

• **T4 (Trial Period):** Duration from starting a trial period to starting the next one.

• **N2 (Trial Numbers):** Number of repeating a desired trial.

T1 (Delay)		T2 (Pulse Duration)		T3 (Pulse Cycle)		N1 (Train)	T4 (Trial Period)		N2 (Trial Numbers)
0	µs	0	µs	0	µs	0	0	µs	0

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Example

◉A train of **20 single pulses at 100 Hz**, each single pulse has **200μs duration**.

◉stimulation pattern in 100 Hz (100 pulses/second): **pulse cycle** must be 10ms (1000ms/100pulse).

• Through the pulse cycle you could establish frequency of a train and vice versa.

◉N1 will explain how many pulses you want to have in a train.

◉According to T1, T3 and N1 you must write a value for T4.

Preview

Stimulation Parameters

Pattern (1 .. 4)	T1 (Delay)		T2 (Pulse Duration)		T3 (Pulse Cycle)		N1 (Train)	T4 (Trial Period)		N2 (Trial Numbers)
Pattern1	0	μs	200	μs	10000	μs	20	200000	μs	1

Recording Parameters

◉Usually, following the stimulation, you have an electrophysiological response and you want to save it. Record your data using the below menu:

◉**Recording time:** It is part of trial period, which you wish to save it on the computer.

◉**Trial period** and **Trial Numbers** are the same as described in stimulation parameters.

◉Recording time must not be longer than the trial period (could be lesser or equal).

◉check the sync box to equalize the values of Trial period and Trial Numbers in both stimulation and recording parameters.

Preview of Stimulation Protocol

◉According to T1, T3 and N1 you must write a value for T4!

Mixers

Simple mixer

⊚You can mix your stimulation protocol here.

⊚**Electrical Stimulator:** mix your defined electrical pattern here by choosing **patterns** and **inputs** and also **fixed current**.

⊚**Mechanical:** it is mechanical mixer that you can choose **patterns** and **inputs** and also **Voltage** and **Rise time**

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Advanced mixer

⊙You have access to more advanced settings for mixer here

Analysis

Analysis

◉**eTrace :** It designed for offline analysis of data collected through eTrace Experiments (local field potentials) such as evoked fEPSP and PS, EEG, EMG, ….

◉It analyzing all basic properties of synaptic potentials (Slope, Peak Amplitude, Latency, Area, PopSpike Amplitude, …) and also EEG, EMG, phase and frequency of signals.

◉**eSpike:** It developed as an offline analyzing program for collected data through eSpike Experiments (neuronal unit activity) such as single or multi unit activity .

eSpike
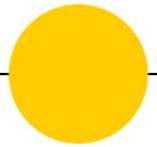
Graph window

Data window

eSpike Analysis   single.bin

File

72
64
56
48
40
32
24
16
8
0
(S) 0   10   20   30   40   50   60   70   80   90   100   110

A   B

Scale 5   Spike Channel: 3   ☐ Grids off   Comment
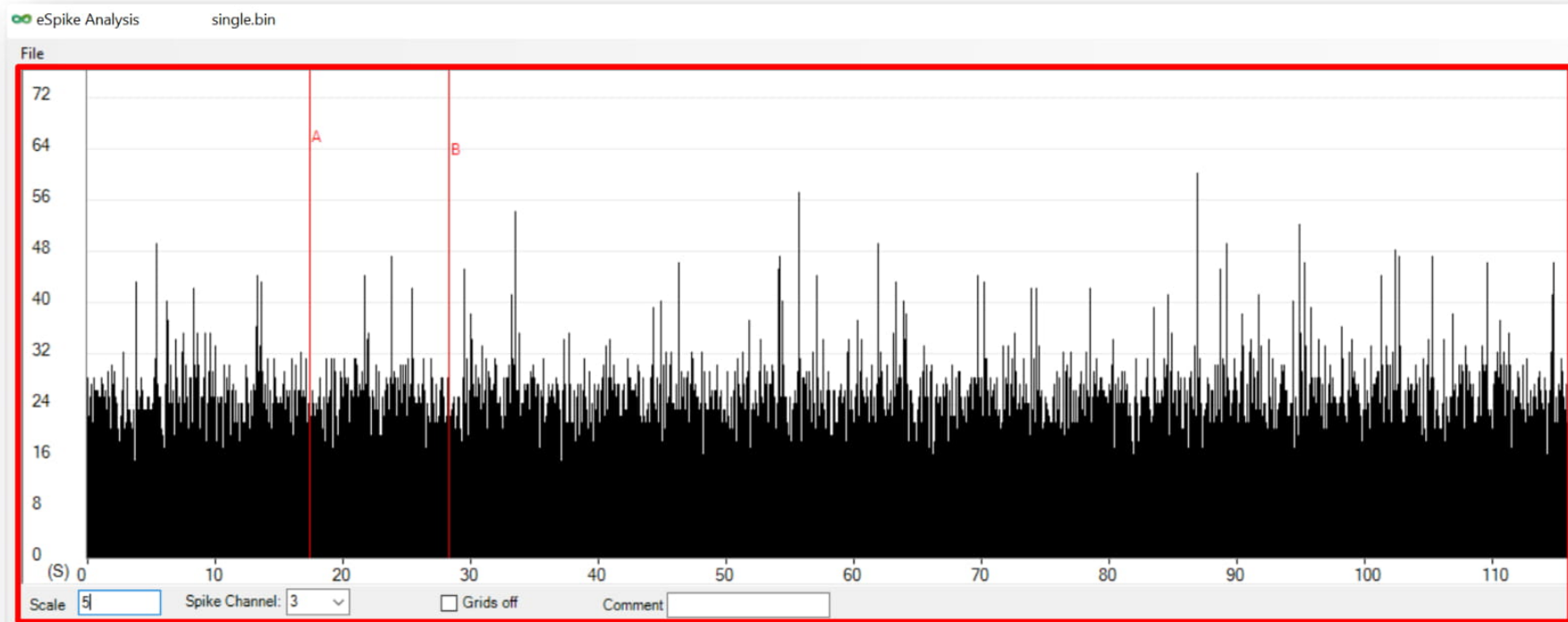
Anlyze Mode

RATE HISTOGRAM
RATE HISTOGRAM2
ISIH
MULTI
SPINAL

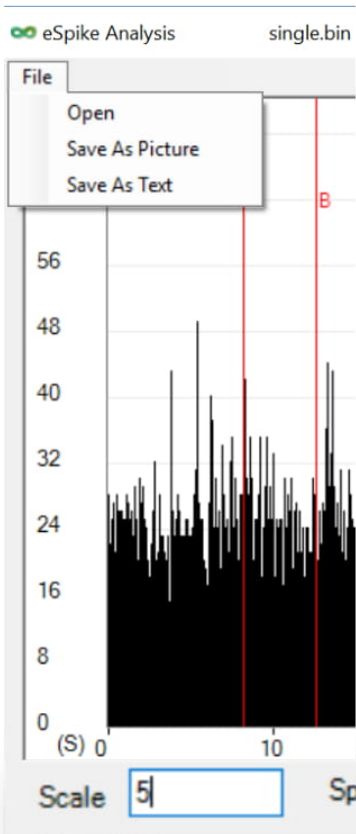Parameters

Time From
Trial From
Trial To
BinSize
A
B

28300

>>

Recorded Parameters:
  Trial period(ms): 0 Recording Time(ms): 36000000 Trial Numbers: 1
RATE HISTOGRAM Analysis:
Trial From: 1 To:1 BinSize: 100 TimeFrom: 0 To: 36000000

TOTAL: 2854
AVERAGE: 26.183
SD: 4.74
SE: .454
MAX: 47
MIN: 17
FREQ: 261.835

eSpike – graph window

- ◉You can **open** the data you need to analyze by **Analysis** –> **eSpike** –> **File** –> **Open File**

- ◉You also can **save** your data as TXT file (**text** format) or as a BMP (**picture** format) file.

- ◉At the bottom of graph window you can see a bar with this elements:

  - **Scale:** you can change the scale number to optimize the graph view

  - **Spike Channel:** choose which channel you want to analyze

  - **Grid off:** If you check the box, grids will be hidden

  - **Comment:** You can write any comment here

eSpike- data windows

◉At the data window below the graph window, you can see these boxes:

- **Analyze Mode**

- **Parameters**

- **Results**

◉ You can see the trial period, recording time, trial numbers in result window despite which analyze mode you choose.

Analyze Mode

⊙**Rate Histogram:** Use this mode for continuous recording without electrical stimulation (for example to investigate the effect of drug X)

⊙**ISIH:** It gives you an InterSpike nterval Histogram

⊙**Multi:** Use this mode to check the response number of neurons

⊙**Spinal:** you can evaluate the impact of every neuronal fiber specially in the pain research
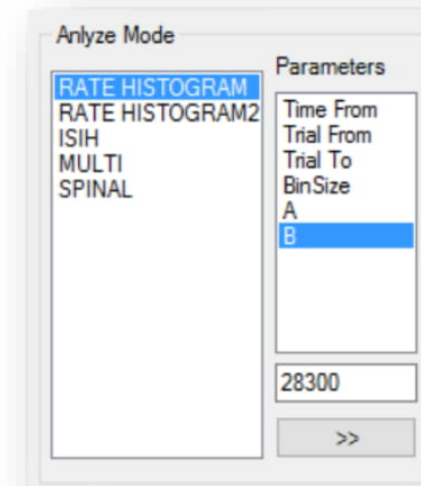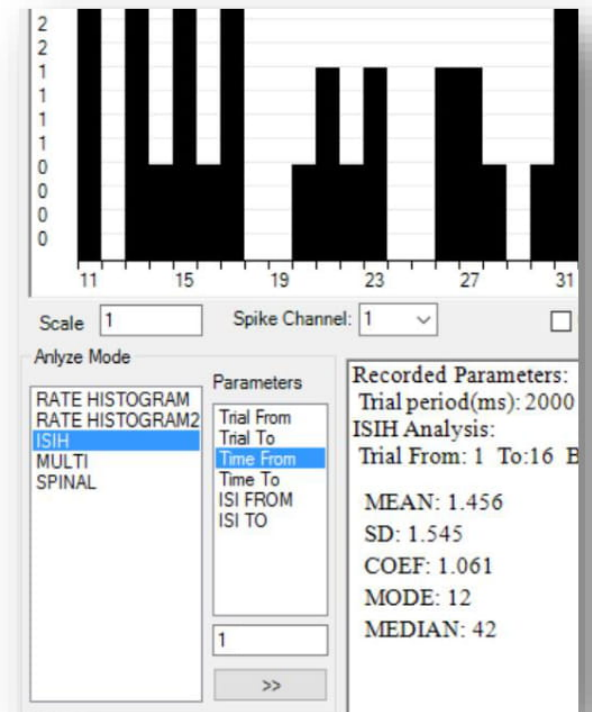
Rate histogram

◉In the parameters box, set appropriate value for Bin size

◉Choose from which trial, it begun to analyze and to which trial, it ends by locating desired trial number

◉Set the appropriate value for point A and B to limit the analyze between A and B

◉Recorded parameters for this analyze mode are:

- **TOTAL:** total number of spikes

- **AVERAGE**

- **SD** (Standard Deviation)

- **SE** (Standard Error)

- **MAX** (Maximum)

- **MIN** (Minimum)

- **FREQ** (Frequency)
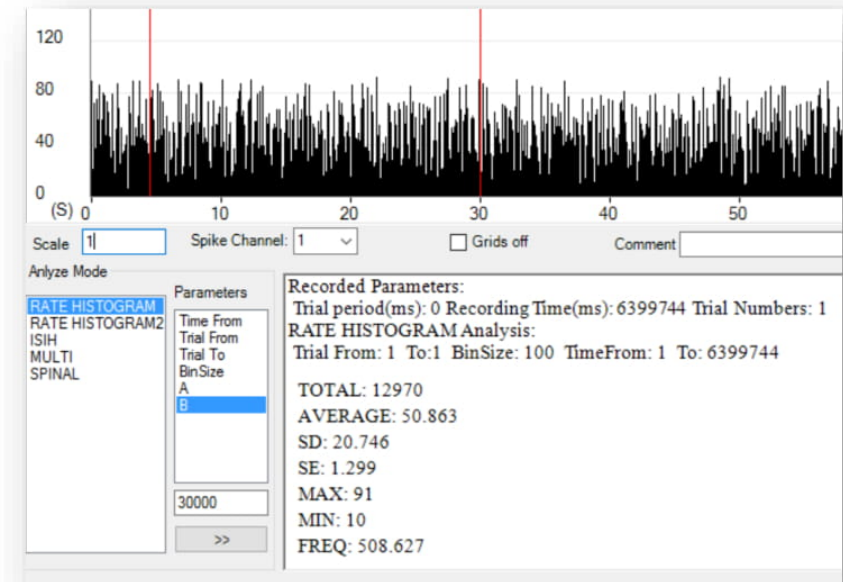
ISIH

◉Choose from which trial **(Trial from)**, it begun to analyze and to which trial **(Trial to)**, it ends by locating desired trial number

◉Set the appropriate value for **ISI FROM-TO** to limit the ISI Histogram between those values.

◉Recorded parameters for this analyze mode are:

- **MEAN**

- **SD** (Standard Deviation)

- **COEF** (Coefficient)

- **MODE**

- **MEDIA**

MULTI

◉In the parameters box, set appropriate value for Bin size

◉You get the result which is SUM of neuronal responses in result box
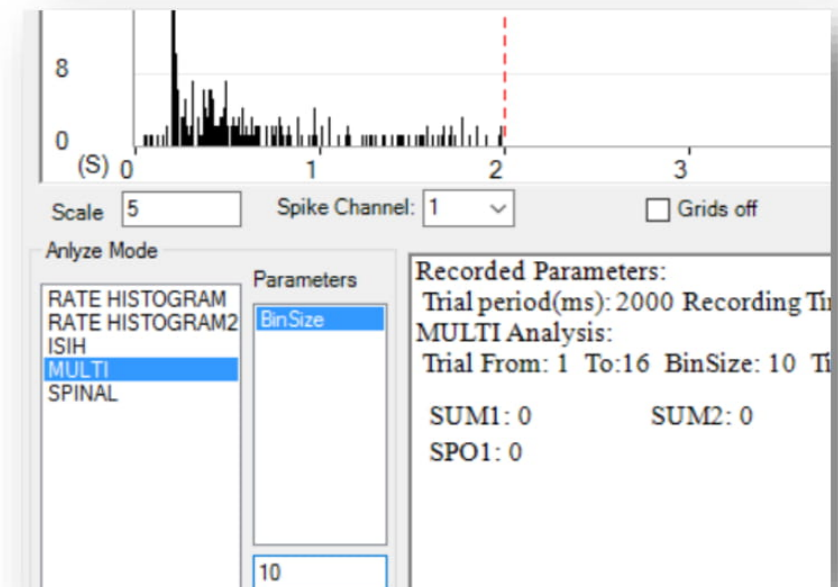
SPINAL

◉In the parameters box, set appropriate value for Bin size

◉Choose from which trial, it begun to analyze and to which trial, it ends by locating desired trial number

◉You get the result which is Total neuronal fibers and also the number of every fiber type (C Fiber) and also PostDischarge in the result box.
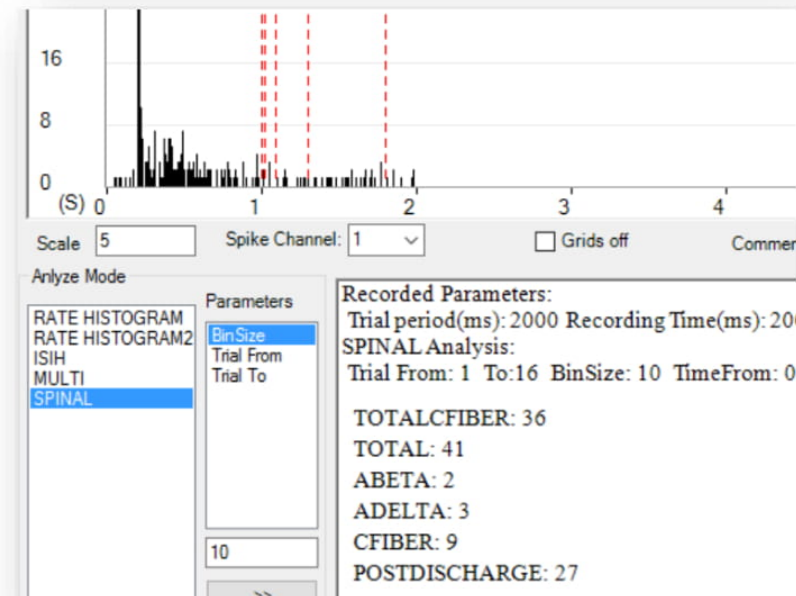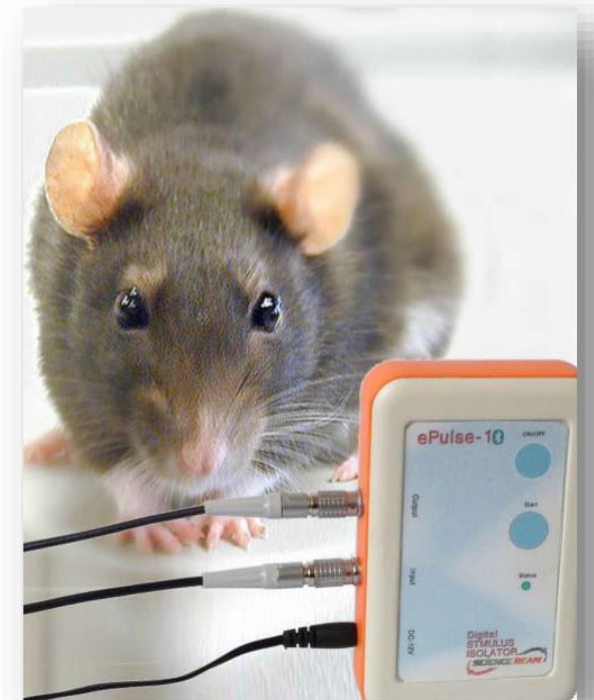
ePulse

ePulse

• ePulse is a Wireless Stimulus Isolator which is used for deep brain stimulation

- 4 channel Pulse generator
- Professional mixer in designing stimulus pattern

Specification

Electrical stimulator

Mode	Constant current, unipolar, isolated
Number of channel	Optional, 1 or 2
Current range	0–4 mA or 0–20 mA (optional)
Current resolution	1 μA or 5 μA (optional)
Output waveform	DC or current pulse
Current control	Yes, software control by 12 bit DAC
Current amplitude error	3 LSB (maximum)
Polarity inversion	Yes, software control by relay
Output switch	Yes, software control by relay
Output voltage compliance	150 V
Current rise time and delay	5 μs, typical (1KΩ load)
Current fall time and delay	5 μs, typical (1KΩ load)
Isolation type	Optical
Isolation voltage	2500 V
Isolation resistance	$10^{12}\ \Omega$

Specification

	Experiment protocols	Single trial, multi trial, single protocol, multi protocol
	Stimulation timing pattern	4
	Pattern parameters	Delay, pulse duration, pulse cycle, pulse numbers, trial period, trial number
Pulse generator	Timing pattern resolution	10 µs
	Mixers	2Ch internal stimulator, 2Ch mechanical stimulator, 2Ch digital outs
	Mixer inputs	Pattern1, pattern2, pattern3, pattern4, digital input1, digital input2

Make Stimulation Protocol

- Stimulation Parameters
- Recording Parameters
- Preview
- Mixers

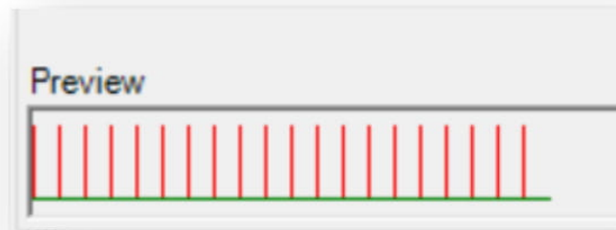# Stimulation Parameters

Pattern (1 .. 4)

Pattern1 ▾

• **Pattern (1 .. 4):** make four different patterns of stimulation. you can mix these patterns to produce a complex protocol by using **Mixers**.

• **T1 (Delay):** Latency between starting the recording time and applying the **first** stimulation pulse of each train

• **T2 (Pulse Duration):** Duration of a single stimulation pulse.

• **T3 (Pulse Cycle):** Duration from starting a single pulse to starting the next single pulse.

• **N1 (Train):** Number of pulses in a *trial period*.

• **T4 (Trial Period):** Duration from starting a trial period to starting the next one.

• **N2 (Trial Numbers):** Number of repeating a desired trial.

T1 (Delay)		T2 (Pulse Duration)		T3 (Pulse Cycle)		N1 (Train)	T4 (Trial Period)		N2 (Trial Numbers)
0	µs	0	µs	0	µs	0	0	µs	0

Example

⊙A train of **20 single pulses at 100 Hz**, each single pulse has **200µs duration**.

⊙stimulation pattern in 100 Hz (100 pulses/second): **pulse cycle** must be 10ms (1000ms/100pulse).

• Through the pulse cycle you could establish frequency of a train and vice versa.

⊙N1 will explain how many pulses you want to have in a train.

⊙According to T1, T3 and N1 you must write a value for T4.

Preview

Stimulation Parameters

Pattern (1 .. 4)	T1 (Delay)		T2 (Pulse Duration)		T3 (Pulse Cycle)		N1 (Train)	T4 (Trial Period)		N2 (Trial Numbers)
Pattern1	0	µs	200	µs	10000	µs	20	200000	µs	1

70

Recording Parameters

◉Usually, following the stimulation, you have an electrophysiological response and you want to save it. Record your data using the below menu:

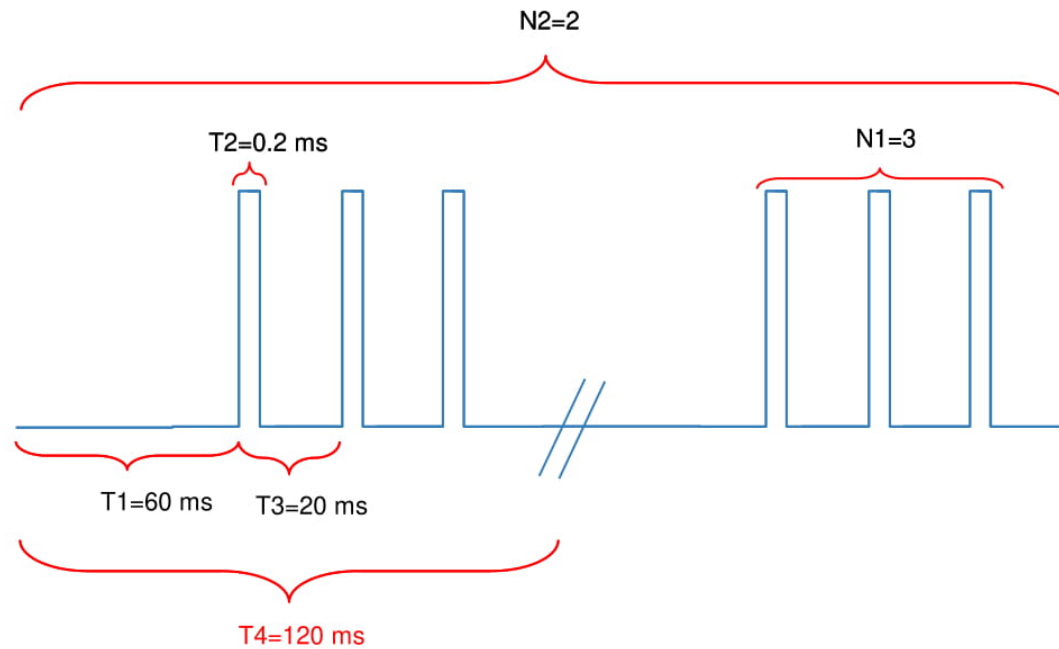◉**Recording time:** It is part of trial period, which you wish to save it on the computer.

◉**Trial period** and **Trial Numbers** are the same as described in stimulation parameters.

◉Recording time must not be longer than the trial period (could be lesser or equal).

◉check the sync box to equalize the values of Trial period and Trial Numbers in both stimulation and recording parameters.

Preview of Stimulation Protocol

⊙According to T1, T3 and N1 you must write a value for T4!

Mixers

Simple mixer

◉You can mix your stimulation protocol here.

◉**Electrical Stimulator:** mix your defined electrical pattern here by choosing **patterns** and **inputs** and also **fixed current**.

◉**Mechanical:** it is mechanical mixer that you can choose **patterns** and **inputs** and also **Voltage** and **Rise time**
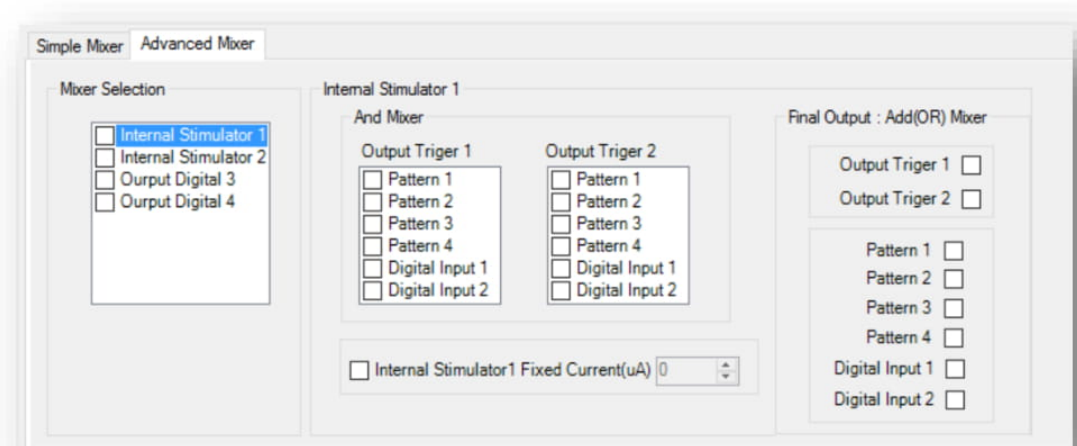
Advanced mixer

- You have access to more advanced settings for mixer here
- You have access to **AND/ OR** feature to apply it on your stimulus design.

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