MultiShanksAnalysis

March 2015. Version: 0.79

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Changelog:

0.79 (Mar. 19, 2015) Fixed: The problem with plotting of mean events (due to out of range provoked by very last events at the end of the recording.

0.78 (Aug. 26, 2014) Fixed: Updated calculations of the confidence interval for the pdf of phase relations between signals (PLV related).

0.76 (Jul. 17, 2014) Added: Confidence interval for CCH of spike trains and events (all, paired, unpaired) in shank 1 and 2.

0.74 (Nov. 26, 2013) Added: Cross-correlation among spike trains and unpaired events.

0.72 (Apr. 12, 2013) Added: Confidence limits for triple correlation.

0.70 (Mar. 25, 2013) Added: Triple correlation between spike train and Events in two channels: Process->Triple Correlation.
Changed: The procedure of detection of Events has been updated.

0.68 (Feb. 5, 2013) Added: Distribution of amplitudes of events provoking spikes: Process->Spike-Event Amplitude Distribution.

0.66 (Jan. 17, 2013) Fixed: Legends for the two lower graphs plotted by pressing the button: Plot->Statistics of Correlated Events.

0.63 (Oct. 29, 2012) Added: Save results (probabilities) to a txt file.

0.61 (Oct. 26, 2012) Added: Double-Impact on neuronal firing.
Small fixes.

0.53 (Oct. 5, 2012) Added: Phase-Spike density plots.

0.52 (Oct. 4, 2012) Added: Import of spike trains from the 1–st (opened file) shank.
Added: CrossCorrelation Histograms of MicroEvents – Spike Trains

0.51 (Sept. 28, 2012) Added: Calculation of the maximum in the phase-locking histogram (appears inside the plot).

0.5 (Sept. 19, 2012) Added: Calculation of Phase Locking Value and histogram of phase differences.

0.43 (Sept. 7, 2012) Fixed: Sign of delay for cross-correlation. Now: signal #2 (green) in respect to signal #1 (blue).
Added: Mean unpaired events are triggered by each channel separately.
Added: Statistics of events' timings.
Changed: Scatter graph Duration difference vs Time difference (Statistics of Correlated Events). Now on x-axis the difference of time instants of events' beginnings (starts) is used instead of events' maximal activations.
Small fixes and improvements.

0.40 (August 2, 2012) Added option: Events, MaxShift

Added: Simple Statistics for events (Histograms of amplitudes and durations).
Added: Statistics of Correlated events. Histogram of time lags between events in different shanks, amplitudes, and durations.
Added: Manual rectification (deleting) of unpaired events.
Added: Mean waveforms of paired and unpaired events
Changed: Statistics of Correlated events. Now orthogonal regression is used.
Lots of small fixes and improvements.

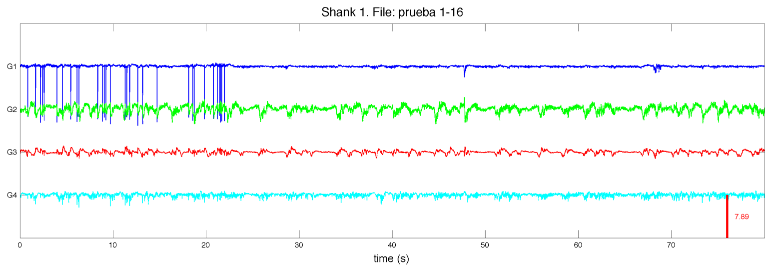
0.31 (July 3, 2012) Added option: Events, RandDuration
Small fixes in Detection of events

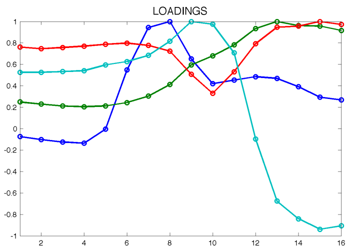
1. Preliminaries

This program performs the analysis of multi-shanks recordings. Data for each shank have to be stored in a separate mat file (provided by ICAofLFPs). The data in two files must be perfectly synchronized in time (i.e. the same initial point, length, and sampling rate). Important: the sampling rate must be multiple of 1 kHz (e.g. Fs = 1000, 2000, etc.). The data files must have LFP-generators already identified and one of them should be the Schaffer-generator (although you can select other generators, part of the program has been design with Schaffer in mind).

2. Basic operations with files

Step 1. Open 2 files with LFP-generators and select Schaffer (**Menu: File, Open File with LFP-generators**)



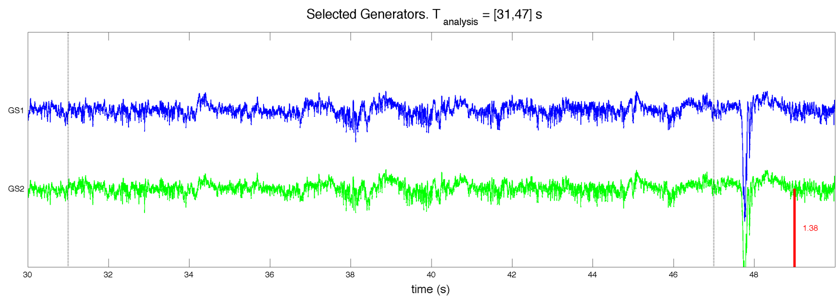


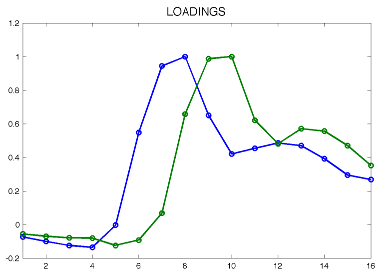
Note: At any point you can save your work in a temporary file (**Menu: File, SaveTMP**). Then you can load the work and continue working from that point.

Spike trains. If the first opened file has spike trains, these will be automatically imported. NOTE: Trains only from the 1-st file are imported, i.e. spike trains from the 2-nd file are ignored. TIP: Change the file's order to load spikes from the desired file.

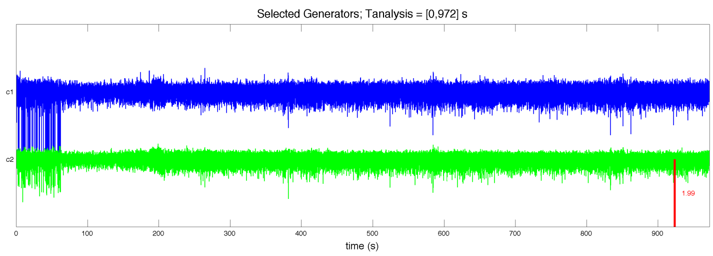
Step 2. Plot selected generators (**Menu: Plot, Selected Generators**)

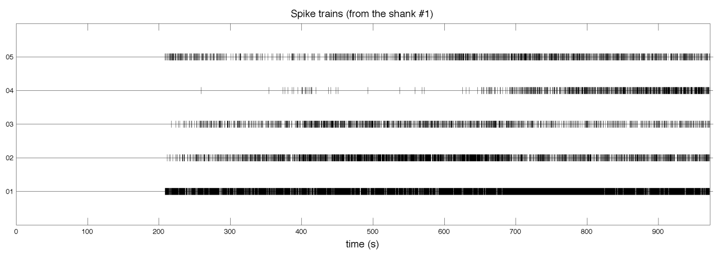
At this point you can move over selected generators and visually inspect them. Use the corresponding buttons and **Menu: Options, Plot Parameters.**





If the first file has spike trains you can plot them **Menu: Plot, Spike Trains**



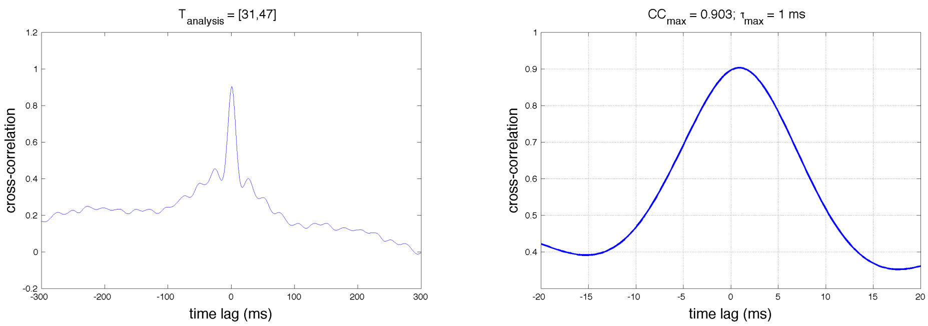


Step 3: Select time interval for the analysis. **Menu, Options, Analysis Time Interval.** You will see the selected interval in the title of the top figure. Important: the further analysis of the LFP-generators will be performed over THIS interval.

3. Data Analysis

3.1 Cross-Correlation of LFP-generators

Menu: Process, Evaluate Cross-Correlation

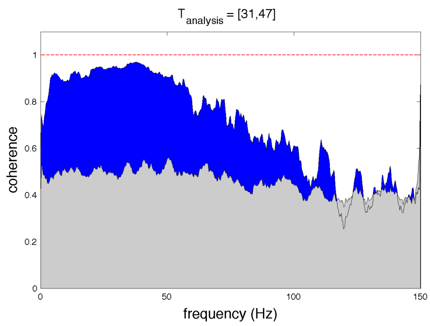


Right graph represents zoomed central part. In the title of the graphs you have the time interval used for cross-correlation, the maximal value of the cross-correlation and the corresponding time lag. The time lag is assumed from the 1st opened file to the 2nd.

You can select the maximal time lag. **Menu: Options, Cross-correlation**

3.2 Spectral Coherence between LFP-generators

Menu: Process, Evaluate Spectral Coherence



Blue area represents spectral coherence of the LFP-generators. It is bounded between [0, 1]. For example here we have high coherence in the interval [0, 60] Hz and then the coherence decays. Gray area shows the level of coherence by chance, i.e. statistically significant coherence is above this level. For example, coherence in the frequency band above 120 Hz is NOT statistically significant.

Options, Spectral Coherence,

Alpha – statistical level (usually 0.05 or 0.01).

Nsurrogates - number of surrogates to evaluate significance level. The more the better. Rise this as much as you computer allows (calcs take longer time).

nSmooth - the number of points for smoothing the figure (makes the curves more smooth). Play with this at your wish.

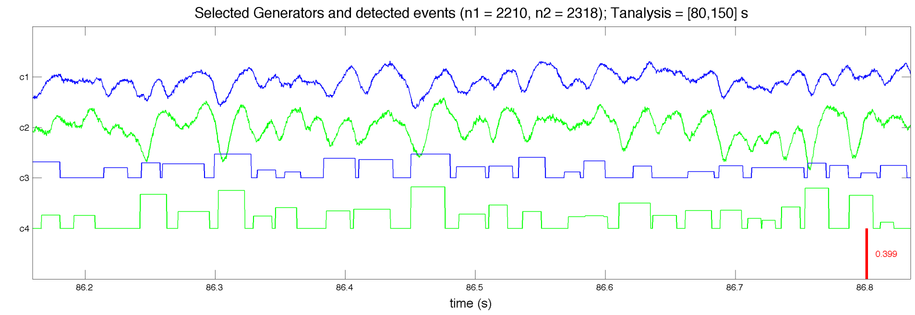
F - frequency interval to evaluate coherence (in Hz).

3.3 Micro LFP-events in the Schaffer-generators

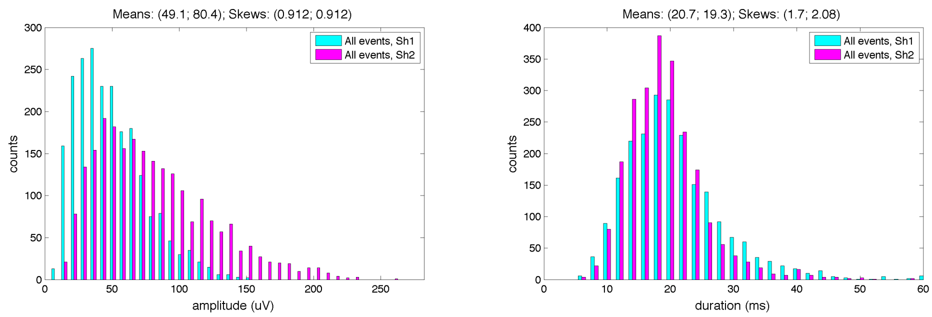
Note: that this part can be consciously applied to the Schaffer generator only.

3.3.1 Detection of Events

First set the time window for the analysis (**Menu: Options, Analysis time interval**). Then you can detect events in the time evolution of the LFP-generators (it provides initial positions, durations, and amplitudes of the events). **Menu: Process, Detect Micro Events**



In the figure you can see detected events (use Time menu buttons to scale and navigate in time). Their height and width reflect the amplitude and duration, respectively. In the graph title you will see the number of detected events in each shank. You can plot distributions of the events' amplitude and duration (**Menu: Plot, Simple Event's Statistics**).



In the figure title you will see the means for both shanks and the skewness of the histograms (skewness reflects the histogram's asymmetry).

Note: Starting from ver. 0.70 the procedure of detection of micro LFP-events has been updated. New algorithm is applicable for any Fs. It searches for maxima in a different way. Recalculate events. You can now also set the range of time scale for events (**Menu: Options, Events' detection**). The default is [4, 60] ms.

3.3.2 Detection of paired and unpaired events

Then you can find correlated (or paired) events, i.e. those that appear relatively synchronously and have similar durations (within some tolerance). **Menu: Process, Find Correlated Events.** Simultaneously the program detects uncorrelated (unpaired) events, i.e. without pair in other generator.

To set the tolerance use:

Options, Events, MinOverlap – Set the minimal overlapping of events in two Schaffer generators to consider them identical (simultaneous).

Options, Events, MaxShift – Set the maximal time lag between two events in two Schaffer generators to consider them identical (simultaneous).

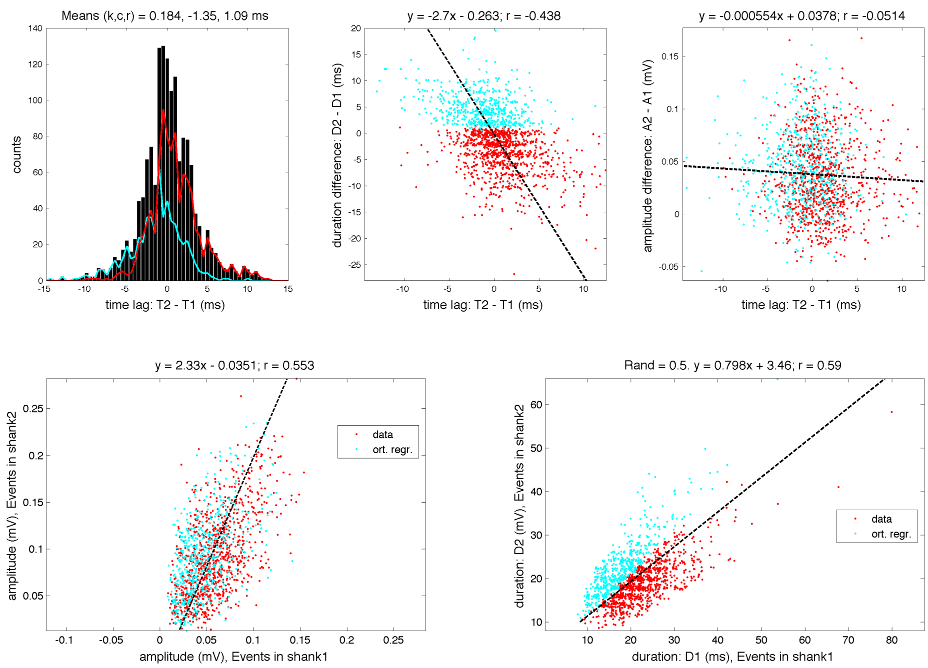
The overlapping index is given by:

$$\Omega = 2\frac{\Delta_{overlap}}{d_1 + d_2}$$

$\Delta_{overlap}$ is the overlapping time interval of two nearest in time events, d_1, d_2 are the durations of the events. Then events with $\Omega > MinOverlap$ are considered simultaneous.

3.3.3 Statistics of correlated events

Once the detection of paired/unpaired events has been finished you will see a graph with statistics for paired events (you can plot later by **Menu: Plot, Statistics of Correlated Events**)



We divided all pairs into two subsets according with the relative durations of events in each pair D1 and D2: cyan (D2 > D1) and red (D1 > D2).

1st subplot shows the histogram (in black) of the time lags between all events in the Shank 2 relative to the Shank 1: T2 – T1 (T1, T2 are time instants of maximal activations during paired events). Cyan and Red curves show distribution of the time lags for cyan and red pairs, respectively. In the graph title you will see the means for all, cyan, and red pairs.

2nd plot shows relation between time lags T2 – T1 and the differences between durations D2 – D1. In the title you will see equation of the best fit (black dashed line) and the correlation coefficient.

3rd graph shows relation between time lags and differences between amplitudes

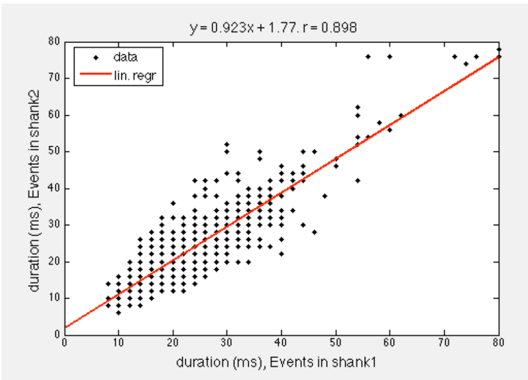
4th and 5th graphs show inter pair amplitude and duration relations, respectively.

Note: Starting from the version 0.4 the orthogonal regression (errors in both variables) is used instead of the linear one. For better visualization the statistical plots also have been changed to “axis equal”.

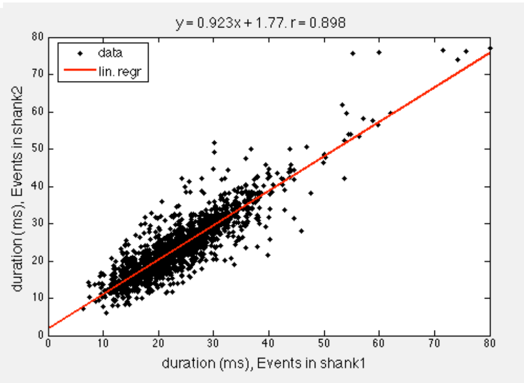
Note: Starting from the version 0.66 the legends of the two lower graphs is corrected.

Note on plotting durations: Since the duration of micro-events is detected with the precision of up to 1ms, you usually get greedy figure (see figure below, left). To improve the figure quality you can randomize the data (the factor for randomization is selected in **Menu: Options, Events, RandDurations**, default value 0.3, 0 means no randomization). The figure to the right is done with RandDurations = 0.5.

No randomization

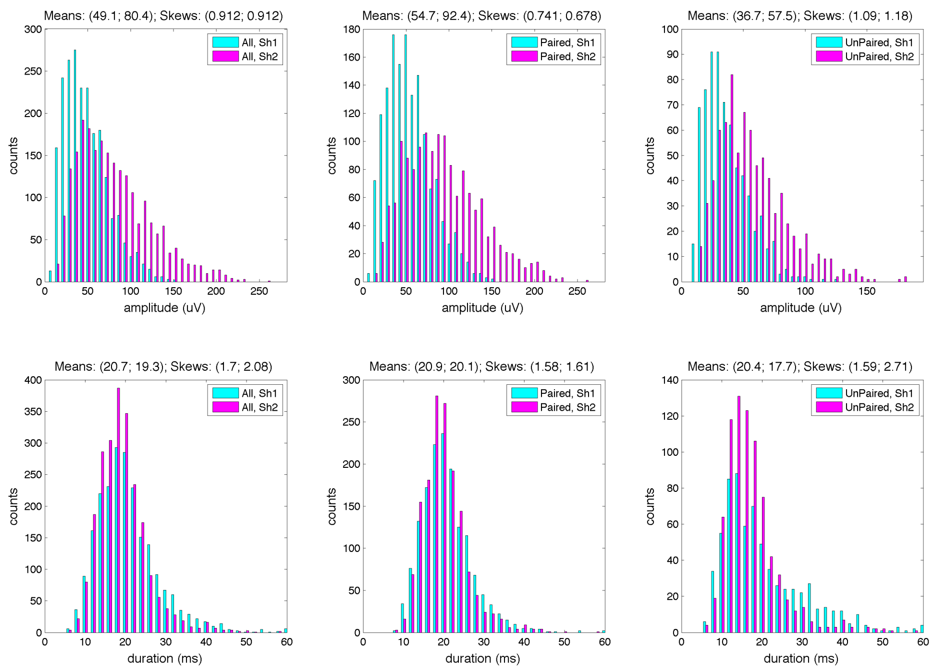


With Randomization



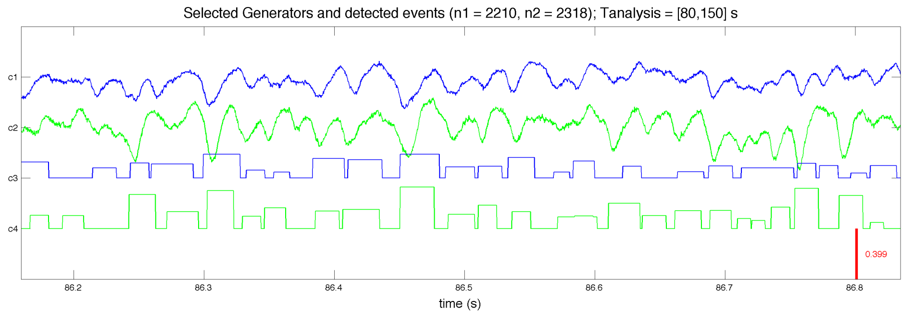
3.3.4 Simple statistics

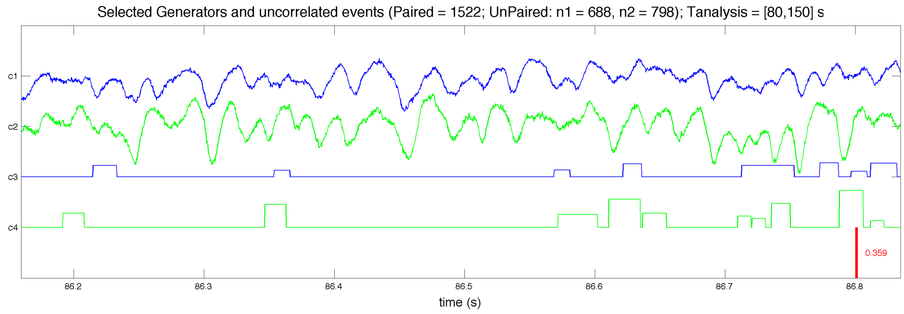
The simple statistics discussed above is also evaluated over paired and unpaired events (if those are available). **Menu: Plot, Simple Event's Statistics**



3.3.5 Plotting of uncorrelated (unpaired) events

You can plot all found events **Menu: Plot, Detected events** (Cntrl+D) or events without pairs **Menu: Plot, Uncorrelated Events** (Cntrl + U).

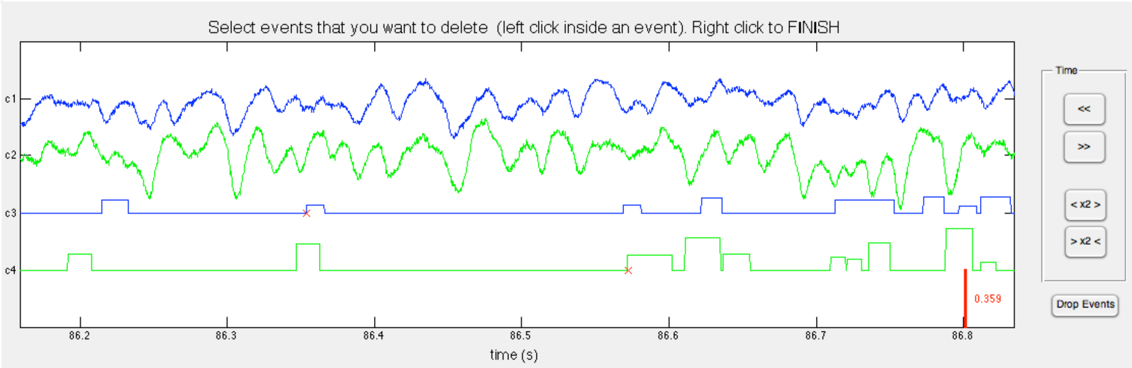




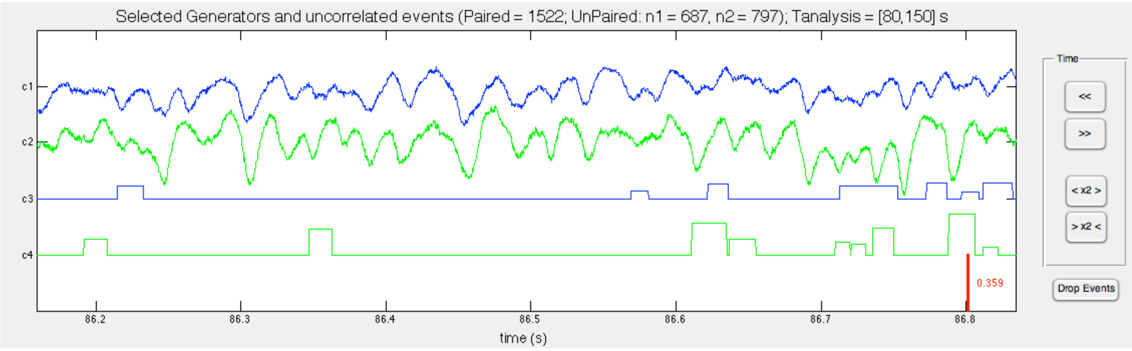
In the graph titles you will see the numbers of all detected events in each shank (Detected Events) or the numbers of pairs and unpaired events in each shank (Uncorrelated events).

3.3.6 Manual deleting of unpaired events

If you are not satisfied with automatic detection of unpaired events you can rectify the results manually by deleting some of them. First plot unpaired events (**Menu: Plot, Uncorrelated Events**). You will see a button called "**Drop Events**". Press it and then a cursor will appear. Left-click inside those events you wish to drop.

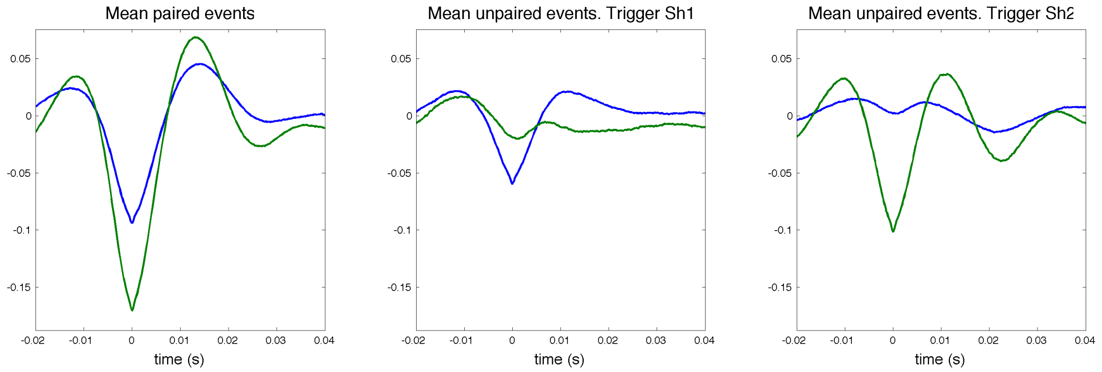


The selected events will be marked by red "x". To finish press right mouse button, the selected events will be deleted.



3.3.7 Mean waveforms of paired and unpaired events

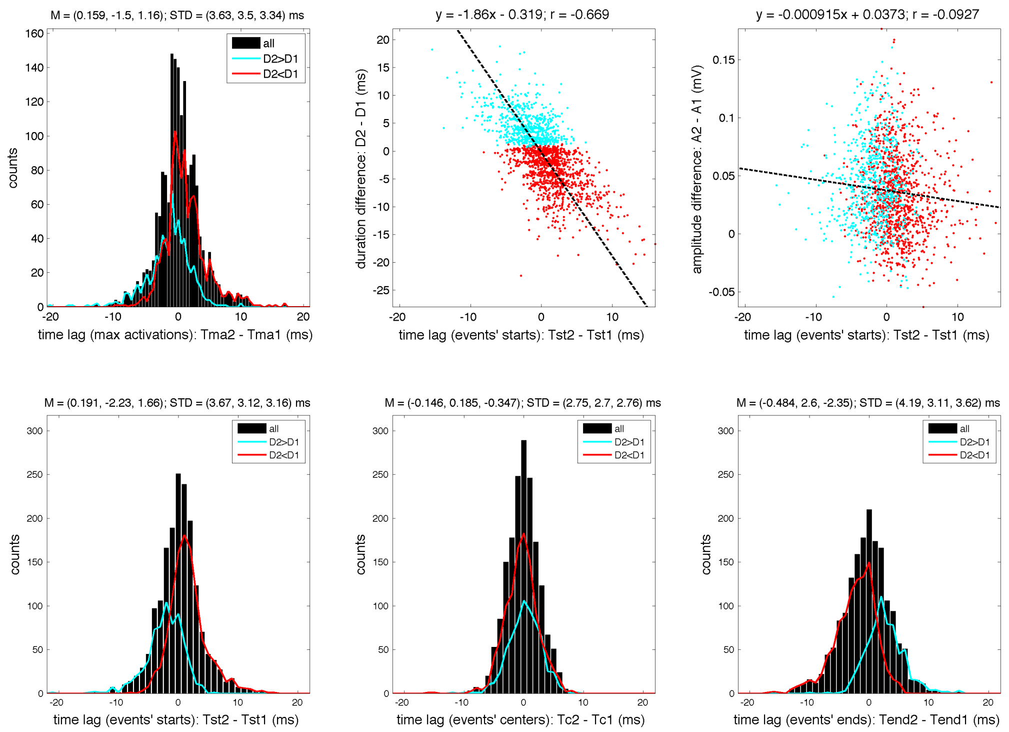
You can plot mean waveforms averaged over paired and unpaired events **Menu, Plot, Mean Events**



Second graph shows mean waveforms triggered by unpaired events in Shank 1, whereas 3rd by events in Shank 2.

3.3.8 Statistics of relative timings of paired events

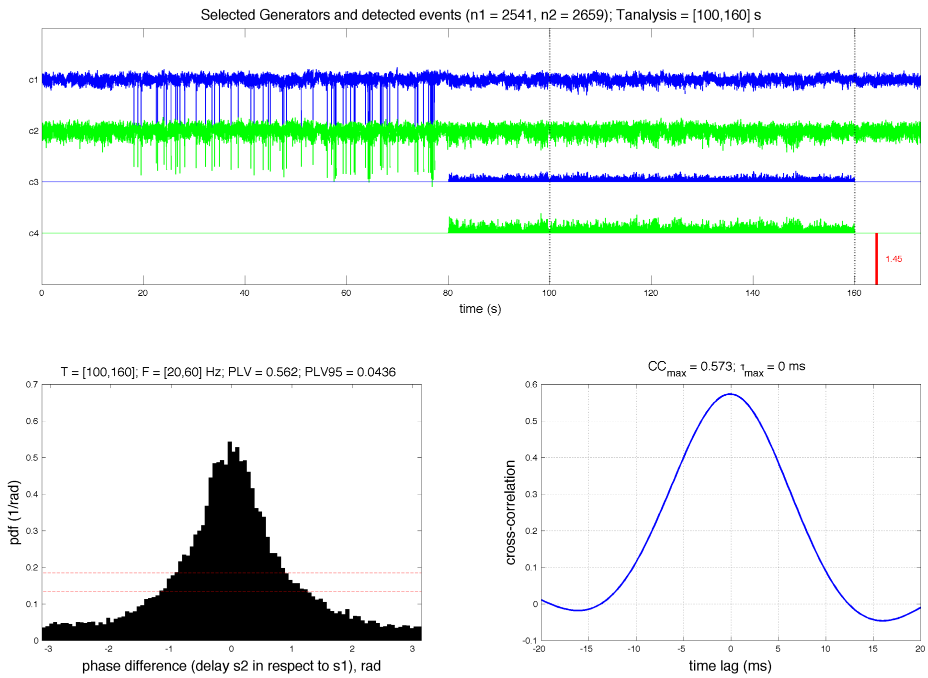
You can plot statistics of relative timings (start, center, end) of paired events **Menu, Plot, Statistics of Events' Timings**



In the bottom row we have histograms of time lags T2 – T1 for events' beginnings (left), for events' centers (middle), and for events' ends (right).

3.4 Calculation of Phase Locking Value and the Histogram of phase differences

You can evaluate the so-called Phase Locking Value between two LFP-generators **Menu, Process, Phase Locking Value**



It is based on the calculation of phase difference between two signals:

$$P = \left| \frac{1}{N} \sum_{k=1}^{N} e^{i\Delta\varphi_k} \right|$$

$P \in [0,1]$. PLV attains the value 1 if and only if the signals are phase-locked (synchronized), whereas $P \to 0$ if the signals are independent.

After calculations you get a figure with the probability density function (histogram) of the phase differences between two signals. If there is statistically significant peak (like in the example), then there is a functional coupling between phases of the signals. Its position (displacement from zero) marks delay between signals (in the example delay = 0, i.e. the signals are in-phase). Positive phase difference corresponds to positive delay of the second signal in respect to the first one. The phase difference π (i.e. a peak at the extremes of the phase interval) corresponds to anti-phase oscillations. Red dashed lines mark the region of statistical significance (values outside are statistically significant). In the graph's title you have the value of PLV and the statistical limit for it (PLV95). Values above the limit are significant.

The PLV (and histogram) is computed in certain frequency band, which can be set in **Menu: Options, Phase Locking Value, FreqRange.** For example in the graph F = [20,60] Hz, which is default. There you can also set other parameters like: the confidence tolerance, **alpha**, the number of bins for the histogram of phase differences, **nBins**, and the number of surrogates to test statistical significance, **nSurr.**

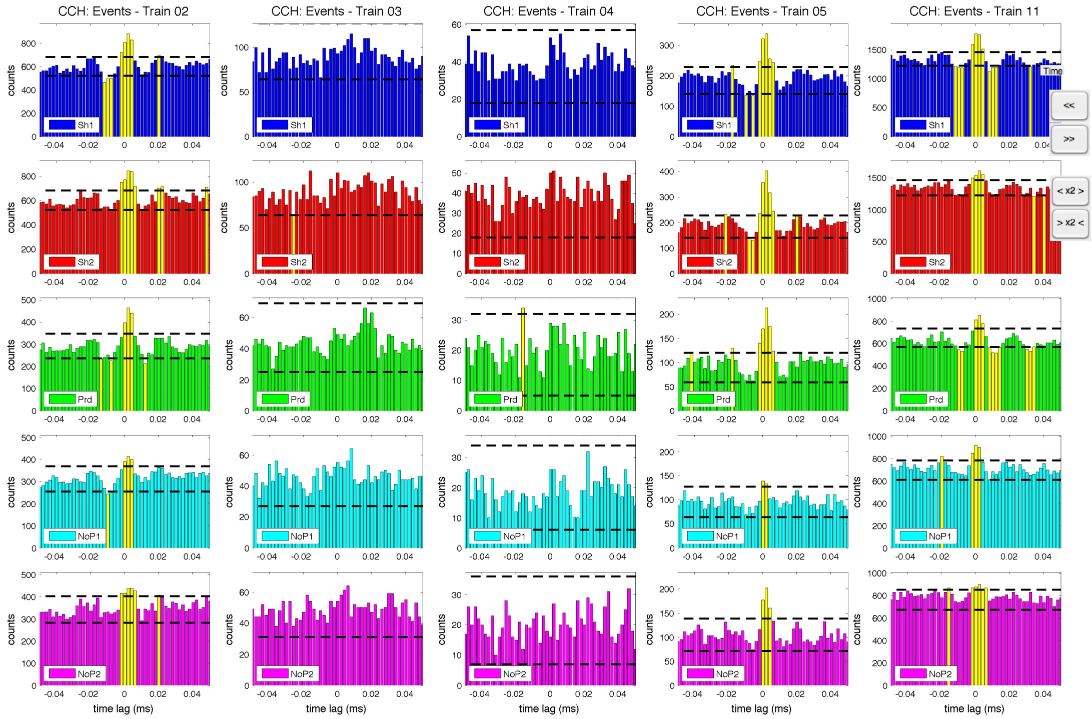
Starting from v. 0.78 the calculation of the confidence interval has been updated. The Bonferroni correction is used now.

4 Working with units (spike trains)

Starting from Ver. 0.52 the program automatically loads spike trains (see Sec. 2).

4.1 Cross-Correlation of MicroEvents – Spike trains

To correlate Events with Spike trains: **Menu, Process, Evaluate Events – Spikes Correlations**



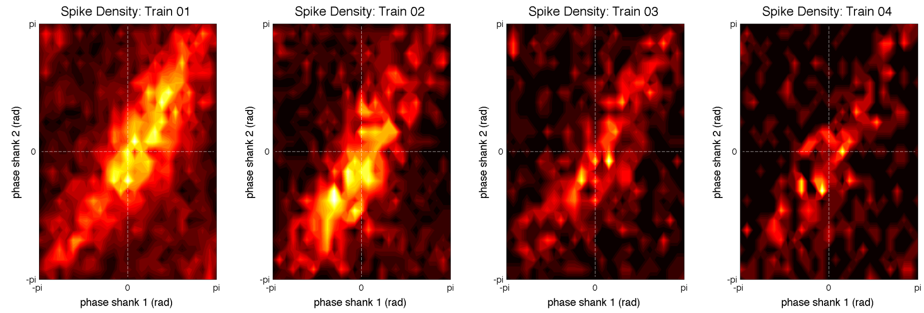
Starting from ver. 0.74 this function produces five graphs (blue, red, green, cyan, and magenta) for each spike train. NOTE: histograms for up to 5 spike trains can be visualized (i.e. no figures will be plotted for spike trains starting from 6). The graphs correspond to Cross-Correlation Histograms (CCHs) for a given spike train and: 1) Events in Shank 1 (blue), 2) Events in Shank 2 (red), 3) Paired Events in Shank 1 and 2 (green), 4) Unpaired events in Shank 1 (cyan), and 5) Unpaired events in Schank 2 (magenta). The last three graphs (green, cyan, and magenta) are plotted if paired events have been previously detected. Note: a) In CCHs microEvents are used as a trigger, thus time the lags are given for spikes relative to microEvents; b) Time instants for paired events are evaluated as a mean of the corresponding events in Shank 1 and 2; c) Some single events in Shank 2 can be paired simultaneously with two events in Shank 1.

Starting from ver. 0.76 this function also provides the significance interval. Correlations above (or below) critical values are statistically significant and the corresponding bars are colored in yellow. The test uses for Ho random trains with the Poisson distribution and by default applies the Holm-Bonferroni correction.

To change the number of bins in the histograms, the interval of time lags, the significance level or the type of correction: **Menu, Options, Corr Events – Spikes.** Then you have to recalculate the cross-correlations.

4.2 2-Dimensional Phase-Spike Density Plots

To study the phase locking of spikes to LFP-generators press: **Menu, Process, Evaluate Phase–Spike Locking** (don't forget to set the analysis time interval).



The obtained figures show 2-dimensional spike density relative to phases of LFP-generators in Shank 1 and Shank 2 for each spike train. NOTE: up to 12 figures can be visualized (i.e. no figures will be plotted for spike trains starting from 13).

Available parameters (**Menu, Options, Phase–Spike Locking**). **nBins** sets the number of bins (grid) in the graphs; **FreqRange** sets the frequency range used for filtering before calculation of phases of the LFP-generators; **nColors** sets the number of contour levels (colors) used for plotting (this can be changed between plotting without recalculation **Menu, Plot, Phase-Spike Locking**).

5 Saving figures

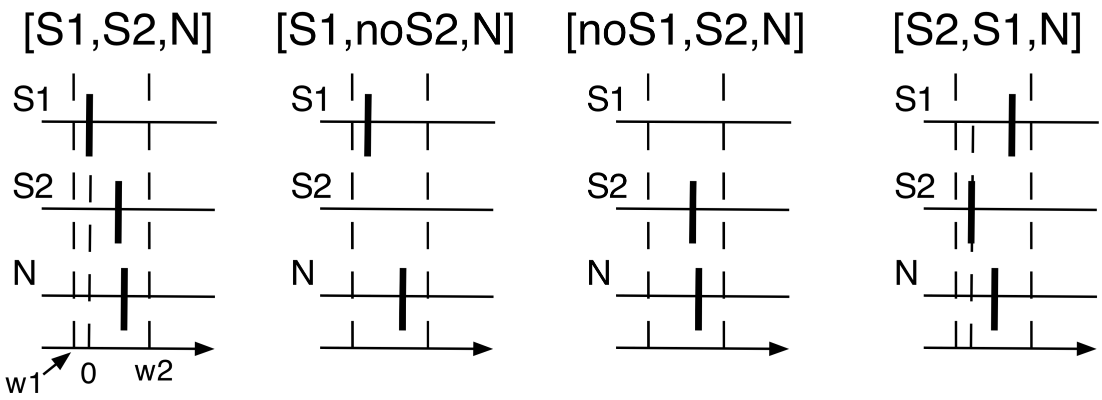
At any point you can save the drawings (current figures shown in the program window) either to PNG (raster graphics, for preliminary analysis) or to EPS (vector graphics, for preparing final figures). **Menu: File, Save Figure to.**

6 Double Impact of two LFP-generators on neuronal firing

Let $S_1 = \{t_i\}_{i=1}^{n_1}$ and $S_2 = \{t_i\}_{i=1}^{n_2}$ be two point processes representing MicroLFPs of two LFP-generators recorded over the time interval T. Besides we consider a spike train $Q = \{t_i\}_{i=1}^{n_3}$ in the same time interval. This situation, for example, appears when dealing with two previously separated inputs (lateral and medial) from the enthorinal cortex to the hippocampus. Then the spike train may correspond to firing of a granule cell.

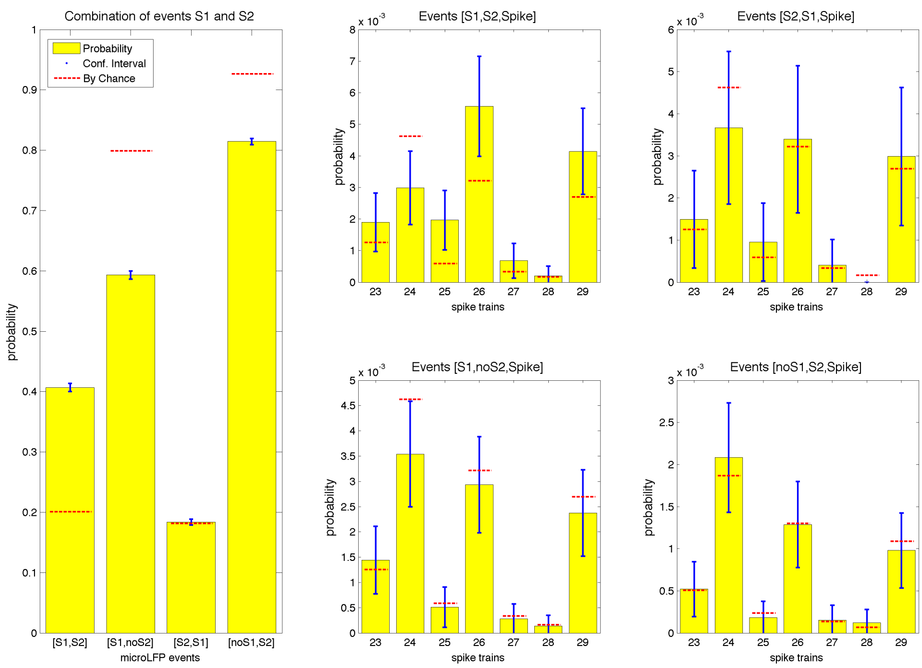
Menue, Events-Spike Double Impact

S1 – micro Event in the Lateral Cortex
S2 – micro Event in the Medial Cortex
[S1,S2] – sequence of micro Events (lateral and then medial)
[S1,noS2] – singular micro Event (lateral and then no medial)
[S2,S1] – sequence of micro Event (medial and then lateral)



Note: Results may strongly depend on the time window.

Time window relative to S1: w = [3,8] ms



We observe that:

- Medial event follows lateral event with probability significantly higher than by chance
- Medial event without preceding lateral event appears with probability significantly lower than by chance
- Trains 23, 27, and 28 have no functional connection with LPP and MPP generators
- Train 24 **is inhibited** (fire with lower probability) by a double LPP-MPP event, while showing no effect in other cases (single event in either LPP or MPP generator)
- Trains 25, 26, and 29 **are excited** (fire with higher probability) by a double LPP-MPP event, while showing no effect in other cases (single event in either LPP or MPP generator)

7 Distribution of amplitudes of events provoking spikes

We consider two channels with mLFP events and a spike train. We assume that events provoke spikes. Then the hypothesis may be “events with higher amplitude provoke more spikes”. To study this hypothesis we search for spikes falling into a time window after each event.

Then the events that are followed by a spike (red in the figure) are separated into a class of events “provoking spikes”. Finally we plot the amplitude histogram of this class.

We also do the same for paired events in two channels. Then the amplitude histogram is done over the mean amplitude, i.e. for each pair we evaluate $a_m = (a_1 + a_2)/2$.

To test the statistical significance we check the null hypothesis that spikes in the trains are random events and hence they fall into time windows (blue in the figure) by chance. The probability of single spike to fall into one of the time windows is given by

$$p = \frac{N_{ev}(A)W}{T}$$

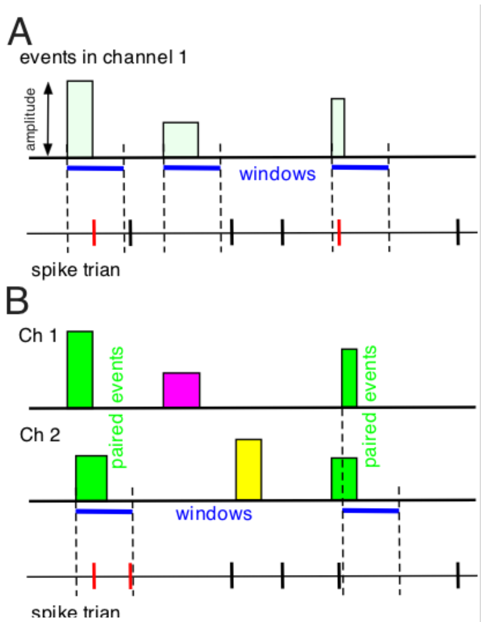
where $N_{ev}(A)$ is the total number of mLFP events in the time interval T of a given amplitude and W is the length of each window (given that the windows do not overlap). Then the random variable X describing the number of “good” events follows the binomial distribution:

$$X \sim B(M, p)$$

where M is the number of spike in the train. Since $M \gg 1$ we have $X \sim N(Mp, \sqrt{Mp(1-p)})$. Hence given the significance level α we can calculate the threshold to reject the null hypothesis:

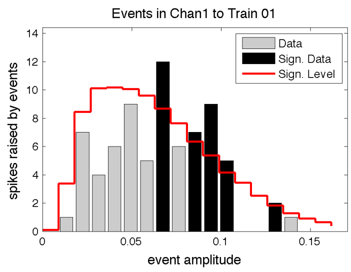
$$X_{thr}(A) = Mp + \lambda_{1-\alpha}\sqrt{Mp(1-p)}$$

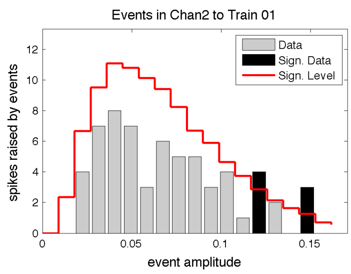
If a bar in the histogram for given amplitude exceeds the threshold then we can reject the null hypothesis, i.e. we can say that events with this amplitude provoke spikes (statistically coupled to spikes).

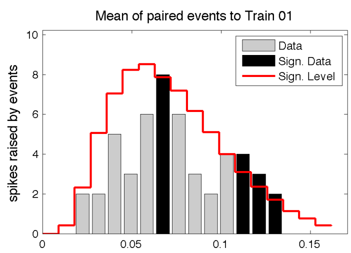


This figure illustrates an example of the analysis **Process->Spike-EventAmplitude Distribution.**

First two subplots show histograms (grey bars) of the amplitude of events coupled to spikes (each graph for one channel). The second graph corresponds to paired events in two channels (mean amplitude is shown). Red stairs-line marks the threshold level for rejecting the null hypothesis. Black bars exceed the threshold and hence







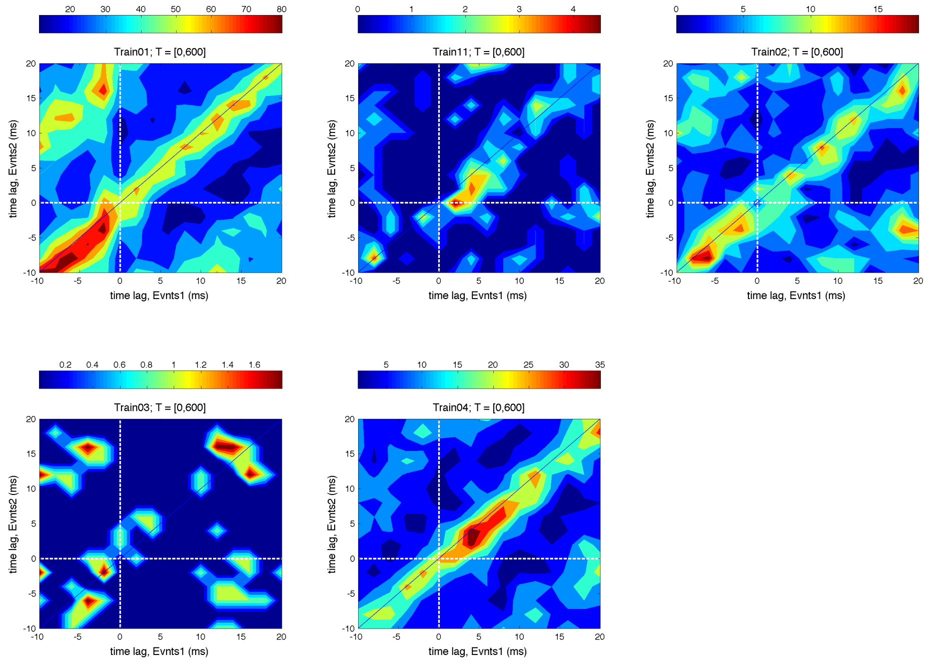
correspond to amplitudes of events that provoke spikes (with probability higher than by chance).

The following parameters **Options->Spike-EventAmplitude** can be adjusted:
nBins = 20 (the number of bins in the histograms)
tWind = [0, 10] (the length in ms of the time window, blue in the figure)
alpha = 0.05 (the significance level)

Note that the results are sensible to the selected parameters. Try to use for example: **tWind = [0, 5], alpha = 0.01.**

8 Triple correlation of events in two channels with spike train

We consider two channels with mLFP events and a spike train. Then triple coincidences (events in channels 1 and 2, and spike) are searched for. To invoke the processing use: **Menu: Process, Triple Correlation.**



Spikes are considered as trigger. Then on the x and y axis you have time lags of events in channel 1 and 2, respectively. Positive (negative) time lag means that the beginning of a coincident event in the corresponding channel occurs after (before) spike. Color represents the number of triple events. In the graph's title you see the title of the spike train and the time interval used for the analysis. For example in the figure spikes in Train 01 follow events in the channels 1 and 2 with a lag [2,10] ms. Moreover, we can observe that the red island appears below the bisector line, which means that the coincident events in the channel 2 have higher (negative) delay, i.e. they appear earlier than those in the channel 1. The sequence is the following:

Event Chan 2 -> Event Chan 1 -> Spike

For processing you can define the following options (available from **Menu: Options, Triple Correlation**):
BinSize -> Default 2 ms (the bin for 3D density plot)
MaxLags -> Default [-20, 30] ms (the limits for time lags)

Also don't forget to set the appropriate **Analysis Time Interval** (default [0, inf], i.e. complete file).

Confidence interval (ver. 0.72): Let X be the number of events A in time window Δ. Then X is a Poisson random variable. The probability to have at least one event is given by

$$P_A(X \geq 1) = 1 - P_A(X = 0) = 1 - e^{\lambda} = 1 - \exp\left(-\frac{N\Delta}{T}\right)$$

where N is the number of events occurred in time T. Then assuming that events A and B are uncorrelated we can write the joint probability to find at least one event A and one event B:

$$P_{AB} = P_A P_B = \left(1 - \exp\left[-\frac{N_A \Delta}{T}\right]\right)\left(1 - \exp\left[-\frac{N_B \Delta}{T}\right]\right)$$

Let Y be the number of joint events A and B, i.e. at least one event of each type in a window triggered by a spike C. Assuming that $N_C \gg 1$ but the mean spike frequency is low enough we can write:

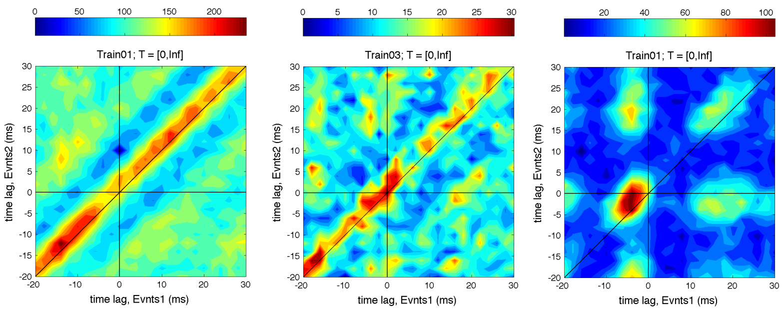
$$Y \sim \mathcal{B}(N_C, P_{AB}) \approx \mathcal{N}\left(P_{AB} N_C, \left(N_C P_{AB}(1 - P_{AB})\right)^{\frac{1}{2}}\right)$$

The by introducing $Z = \frac{Y - P_{AB} N_C}{\left(N_C P_{AB}(1 - P_{AB})\right)^{\frac{1}{2}}}$ we have $Z \sim \mathcal{N}(0,1)$. Finally the confidence threshold is given by:

$$Y_{th} = P_{AB} N_C + Z_{1-\alpha}\left(N_C P_{AB}(1 - P_{AB})\right)^{\frac{1}{2}}$$

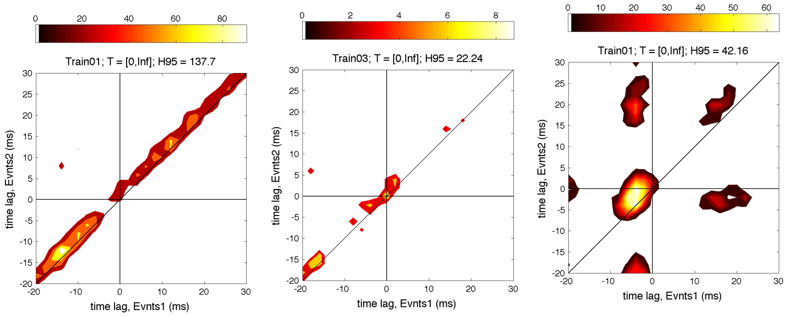
You can plot triple correlations either with or without statistical significance level. To change between plots either check or uncheck the option: **Menu: Options, Triple Correlation, Significant**

This figure shows triple correlations for 3 neurons without setting the significance level:



The same as before, but now with significance level taken into account. Note that the color gradient corresponds to the number of counts in the histogram with subtracted level of significance (the value H95 in each graph), i.e.

$$H_{sign} = H - H_{95}.$$


Train01; T = [0,Inf]; H95 = 137.7
time lag, Evnts1 (ms)
time lag, Evnts2 (ms)
Train03; T = [0,Inf]; H95 = 22.24
time lag, Evnts1 (ms)
time lag, Evnts2 (ms)
Train01; T = [0,Inf]; H95 = 42.16
time lag, Evnts1 (ms)
time lag, Evnts2 (ms)