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in collaboration with



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1. Document History

Version	Date	
1.0	19-May-11	Initial document creation and first release

2. What you can do with BrainWave™

BrainWave™ (BW) is a software tool developed to acquire, store and analyze data provided by the high-density MEA system Biocam4096™. Raw data traces, simultaneously recorded from 4096 electrodes of the NeuroChip™ and sampled at about 8KHz/channel, are stored in .brw files. These files can successively be loaded by BW to visualize the recorded activity off-line. In addition, BW provides spike detection algorithm and first order analysis statistic such as Mean Firing Rate (MFR) and Inter Spike Interval (ISI) to provide to the user global parameters of the network activity. The analysis results are saved in a different file (.bxr) which is linked to the raw data allowing to conveniently move from the results to the raw data and vice versa.

3. Minimum platform requirements

BrainWave™ works under Windows XP®, Windows Vista® and Windows 7® at 32 or 64-bit configuration. The .net 4.0 Framework installation is a prerequisite. The minimum hardware requirements are:

- ◆ processor Intel 1.4GHz (or equivalent)
- ◆ at least 1 Gigabyte of RAM (2 Gigabytes suggested)
- ◆ minimum of 1 Gigabytes disk space available

4. How to install BrainWave™

For software installation launch the setup.exe and follow the instructions.

Important: For the software to work properly, set the “regional option” tab to “English”, in the “regional and language option” window in control panel!

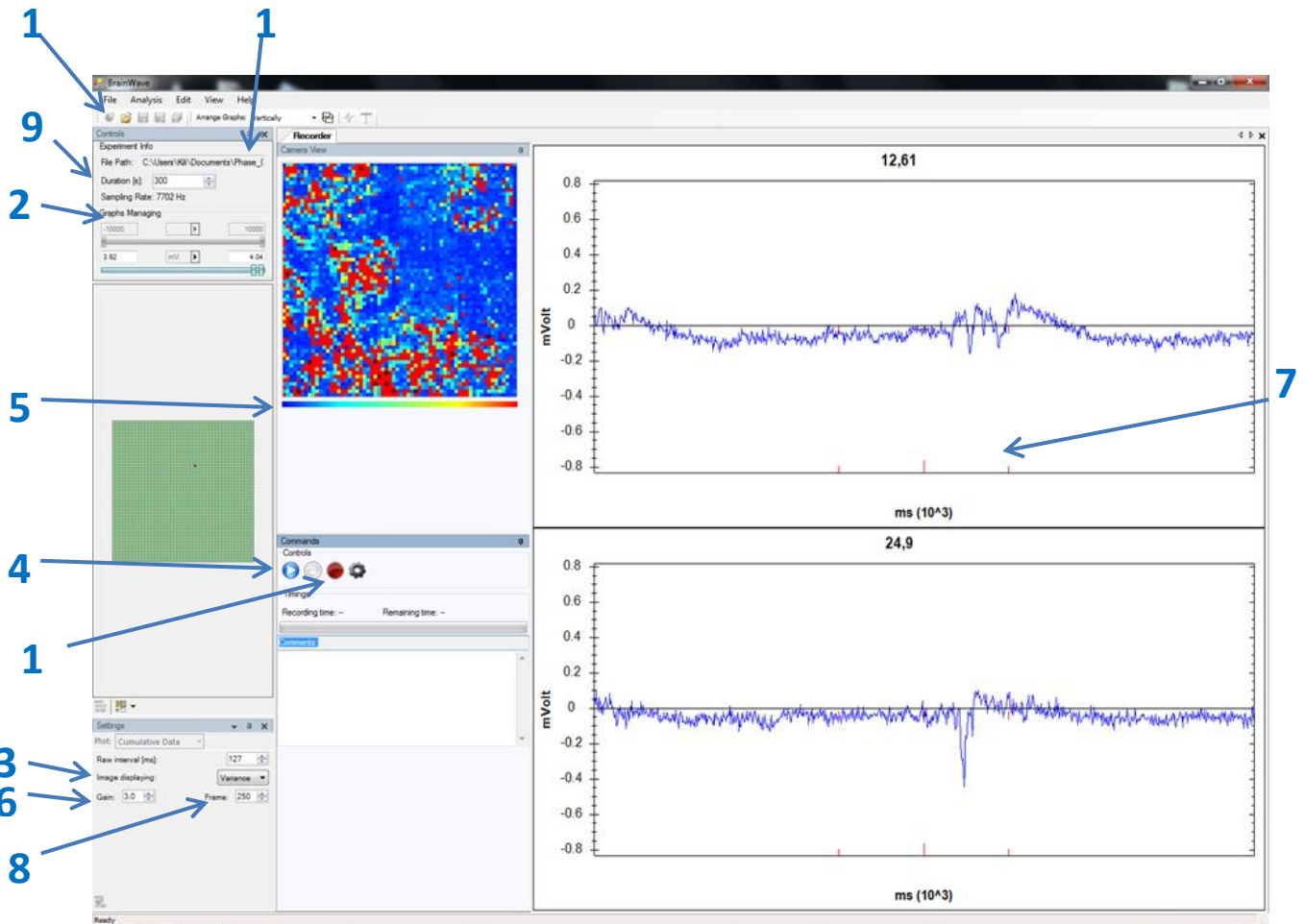
5. First step

5.1. Record a file

Initialization/Start procedure

1. Turn off the BioCAM.
2. Close BrainWave.

3. Plug in the test-chip and close the connector.
4. Turn on the BioCAM.
5. Launch BrainWave.



Click on the first icon from the left in the menu (1) to open the recording window. The right LED should turn on. That means the BioCAM sends data to the Computer.

System test

1. Make sure that the Amplitude Bar in the Graphs Managing Panel (2) is on full scale (you can double-click on the bar between the two limits or just move each limit to its absolute position).
[Typical setting: -0.7 - +0.7]
2. Set Image Displaying (3) to “Variance”.
3. Push the Play-Button in the Controls Panel (4) to start the acquisition.

Then, you should see the image of the array in blue (set colorbar (5) appropriately [typical setting: right click of the mouse with the pointer on the colorbar, set Exp scale]). Low noise should be visible on the image. Gain (6) shall be set between 1 and 5 [typical setting: 3.5]. Frame Rate must

not be changed for the full array recording. Click on a single Pixel on the 2D-Array to display a single channel's waveform. The interval of the crosshair (7) can be set with the number in the Frame Field (8) [typical setting: 250 frames (~ 30msec), above the raw interval is set to 250-500msec].

Troubleshooting

If you see single columns displayed in uniform colors (i.e. with no noise contribution) and different from the remaining array, contacts might be weak. Close and open the connector a few times and move the chip slightly back and forth when the connector is closed. If this does not help, try to clean the connectors on the chip with Ethanol or detergent. If, again, this does not help, the chip is probably partially broken. These columns will not work anymore, however the remaining columns can still work. Usually, columns that do not work (either because they are broken or because there might be a connector issue) appear in multiples of 4 and at a distance of 16, since four columns are multiplexed on one output channel and there are 16 output channels. If you open the connection between the Chip and the BioCAM you will have to re-start the program.

If you still have problem, try to clean gently the gold pads on the chip with a Q-tip or similar soaked with ethanol.

Once these initial tests to check for connectivity are terminated, you can proceed to recording.

Recording

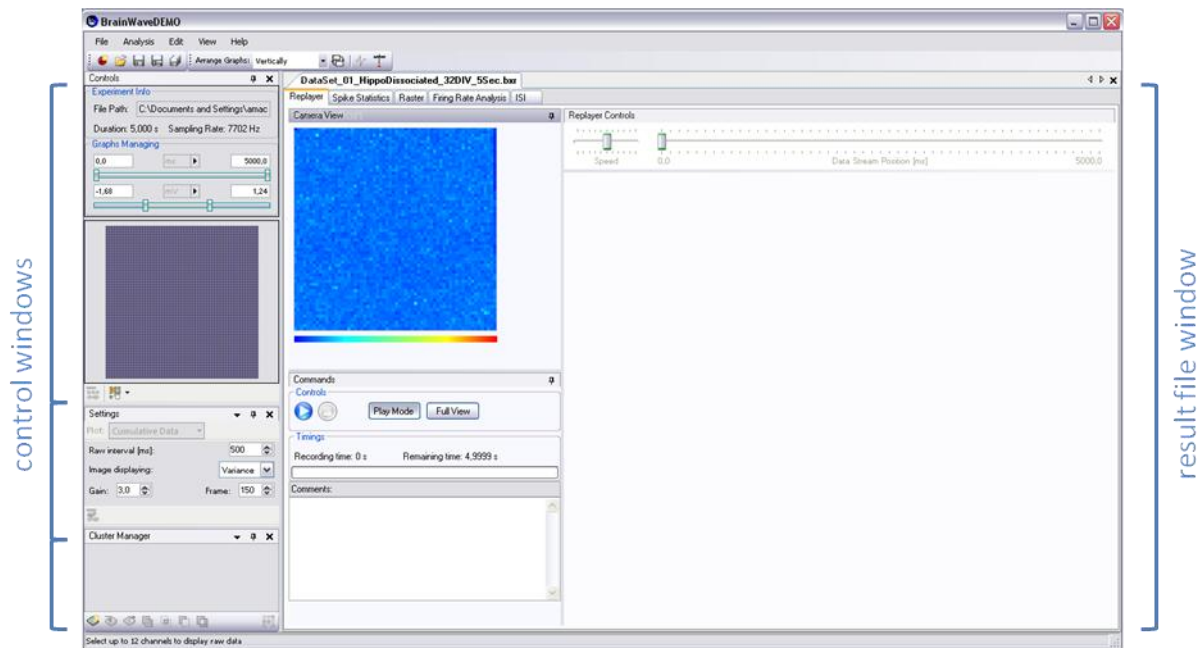
1. Set Duration (9).
2. Choose a file location and file name in the File Path of the Experiment Info Panel (10) [typical setting: select the hard drive with RAID0 configuration].
3. Press the Record Button (11).
4. You can select different pixels to display the waveforms of channels on-line, to visualize more channels at the same time, drag and drop on a selected area or *shift + click* on different channels. The maximum numbers of channels that can be visualized are generally set to 12. Select from the main menu Edit->Preferences, a "Preference Form" panel will appear, in "Graphs" set the max number of raw traces than click OK button. [typical setting: up to 20 traces while recording]
5. Push *Stop Button* to finish the recording.
6. You can open the recorded .bwr file in the 'file location' folder.
7. Proceed with instructions on "Replayer".

5.2. Load a file

BW can load both raw data files (.brw) and result files (.bwr). The first type allows viewing the voltage vs. time traces for each electrode, while the second type allows also exploring the detected activity (spike events), providing raster plot visualizations and statistics such as the Mean Firing

Rate (MFR) and the Inter Spike Interval (ISI). These statistics are available for each single channel or globally at network level.

Select *File->Open* in the main menu and load one of your .bwr result files or a file from the BrainWaveDemoFiles folder (nb: if you load the .brw file, results are not available). On the left side the three control windows appear: *Controls*, *Settings* and *Cluster Manager* (see General commands and controls). On the right side a tab linked to the loaded file is shown.



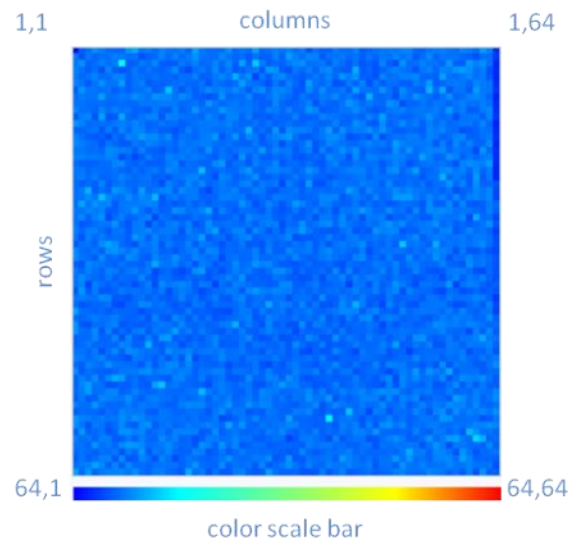
A set of sub-tabs for the loaded file are visible allowing the user to switch from raw data visualization to one of the available analysis results and vice versa. In the following paragraphs each tab is described in detail.

5.2.1. Replayer tab

This tab is made up of three sections: on the upper left corner a *Camera View* showing a 64 by 64 false color map representation of the voltage values recorded for each channel; on the bottom-left corner a *Commands* window with information and buttons to control the replaying of the raw data; on the upper right corner a *Replayer Controls* window containing other controls used during replaying; on the bottom right side a large panel to show voltage waveforms of individual channels.

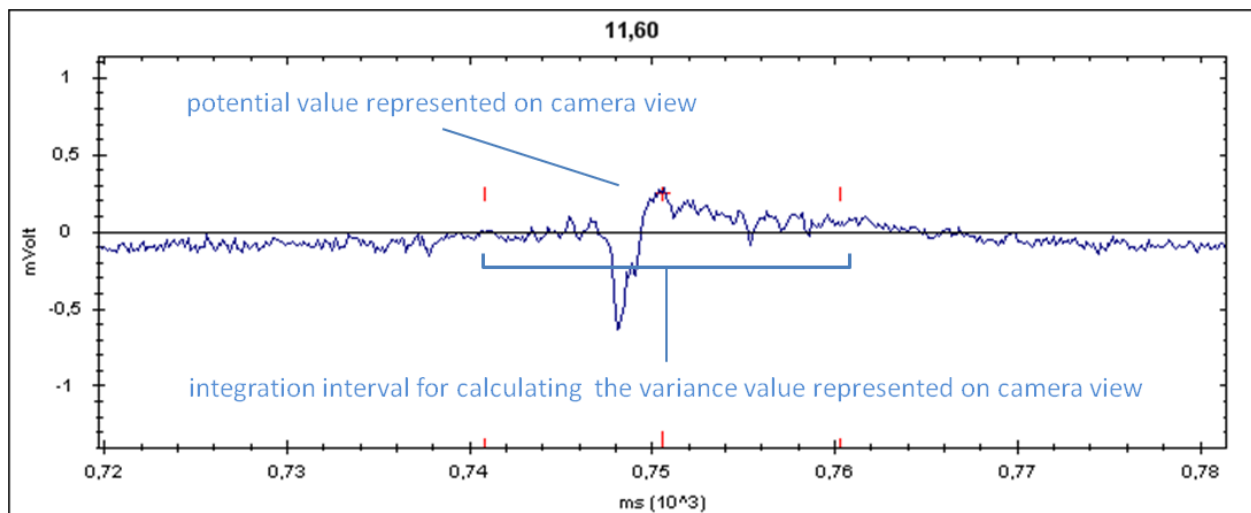
Two possible color visualization modes (selectable by the *Settings* window) are available for the *Camera View*.

- ◆ **Variance:** this mode is the visualization of the difference between the max and min voltage values within the moving time window. This window can be set using the *Frame* control in the *Settings* window
- ◆ **Potential:** this second mode is the absolute voltage potential. The minimum and maximum values of the color scale bar correspond to the minimum and maximum voltage value in the voltage range track bar in the *Controls* window under *Graphs Managing*

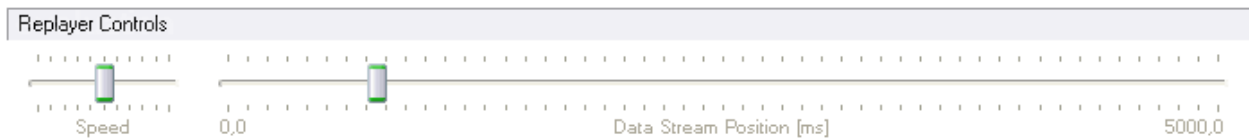


By moving the cursor over the color map or over the 64 by 64 grid shown in the *Controls* window, a tool tip showing the coordinate of the channel corresponding to cursor location will appear. A context menu is available by clicking the right mouse button on the color scale bar under the *Camera View* representation. By using this menu, the user can change the color scale to exponential or can change the colors used for representing voltage values.

By selecting a channel on the color map or on the 64 by 64 grid shown in the *Controls* window, the voltage versus time plot of the corresponding channel appears in the right panel. To open more than one channel just drag an area or hold the *Shift* key during the selection. A context menu for short-cut selection/de-selection of channels is available by clicking the right button of the mouse. The red marker on the raw data trace represents the voltage value plotted in the color map when *Image displayed* is on *Potential* visualization mode. The two red lines at the right and left of the red marker delimit the moving/sliding window within which the variation visualization is calculated.



The “Play” and “Stop” buttons in the *Commands* window allow starting and stopping of the streaming visualization. Under the *Replayer Control* window, the user can set the speed of streaming by moving the speed bar or jump from one point to another of the recording by scrolling the temporal bar (*Data Stream Position* bar).



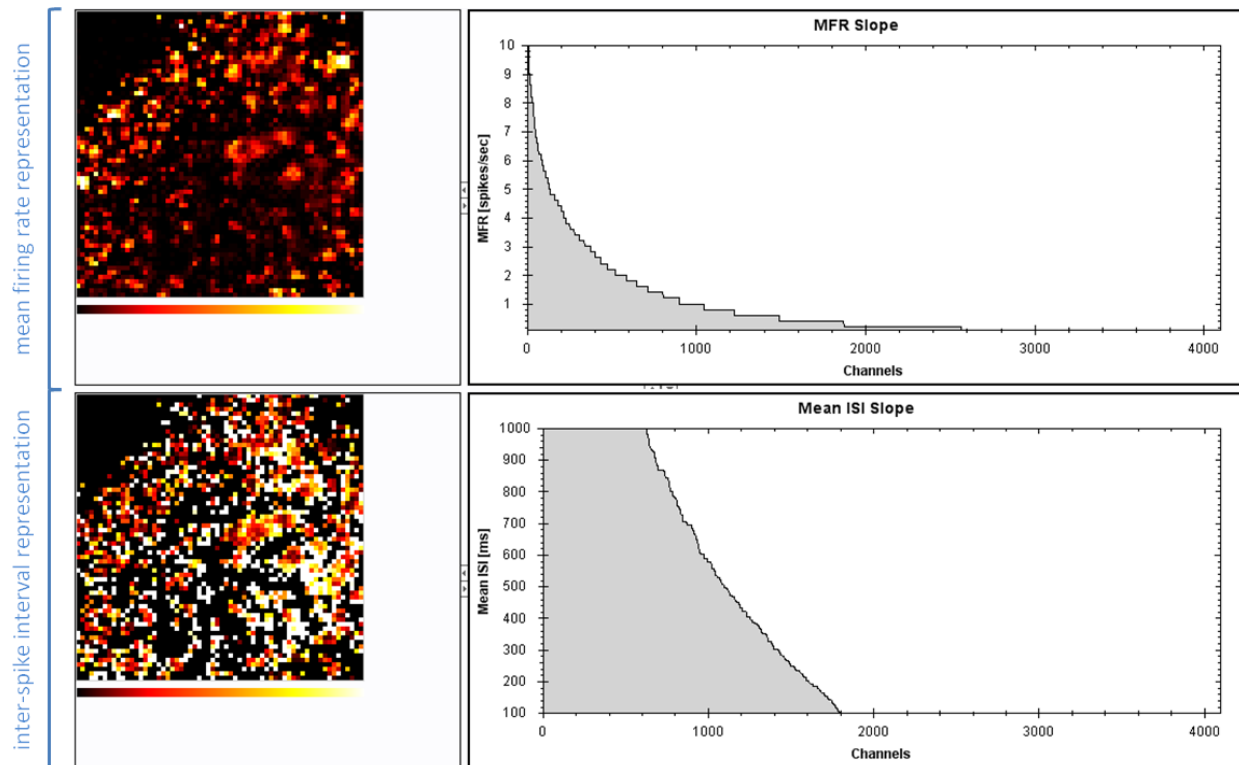
When *Replay* is stopped the user can move forward or backward just by selecting the temporal bar and using the “left” or “right arrow” keys. In this configuration data are visualized by a small moving time window of the recorded signal (the user can adjust the time window size by changing the *Raw Interval* under the *Settings* window). To visualize bigger portions of the signals, the user has to switch from the *Play Mode* to the *Full View* visualization by clicking the corresponding button under the *Commands* window. In the “Full View” mode no playback is allowed and the plotted raw data time interval corresponds to the interval set in the temporal range track bar in the *Controls* window under *Graph Managing*. In this modality all sampled points are visualized allowing fine zooming that can be used for signal shape recognition, for example. By clicking the “Play Mode” button, the user can return to the streaming visualization.

The voltage scale, the *Camera View* color map visualization and the time interval displayed in the raw data plots can be set in the *Controls* and *Settings* windows respectively. Close-up of the raw data traces can be easily done just by dragging a selection box around the interested area on the plot. A context menu for zooming operation is available by clicking the right button of the mouse.

5.2.2. Spike Statistics tab

This tab is a summary of the computed analysis in terms of firing rate. On the top left a color map represents the Mean Firing Rate (MFR), i.e. the number of spike/sec, calculated on the whole recording for each channel. On the right, a graph provides the distribution of the MFR ordering channels from the most spiking to the least spiking one. On the bottom left a table with the statistic for each channel showing the number of detected spikes, the MFR and the Inter Spike Interval (ISI) i.e. the mean latency between two consecutive spikes belonging to the same channel.

The right panel is dedicated to the overall statistics. The “In-Range Channels” represents a set of channels whose firing rate is between a minimum and a maximum firing threshold selectable by the *Settings* window. In this way it is possible to eliminate noisy channels (i.e. channels with a high firing rate not biologically plausible) or the too few active ones. The overall statistics also provides statistics for the selected cluster (see *Cluster Manager* window). By changing the representation mode from “Mean Firing Rate” to “Inter-Spike Interval” in *Settings* window, the color map and graph commute representing ISI statistic.



5.2.3. Raster tab

The raster tab allows visualizing the detected events for each channel vs. time. User can select the desired channels simply by clicking them on the channel layout map in *Controls* window (use the *Shift* key for multiple selections). In *Settings* window user can change the type of visualization. The available options for Plot type are:

- ◆ “Cumulative Data”: all the selected channels are visualized in a unique graph
- ◆ “Per-Channel Data”: each channel is plotted in a separate graph
- ◆ “Per-Cluster Data”: a raster plot for each cluster is visualized (to create clusters see *Cluster Manager* window)

By using drag-and-drop on the raster plot it is possible to zoom on a specific temporal window. The temporal range track bar in *Controls* window is automatically updated. By moving the cursor on the graphs a tool tip shows the coordinate of the channel pointed by the cursors.

5.2.4. Firing Rate Analysis tab

This tab allows visualizing the calculated firing rate (i.e. the number of detected events per user-defined time bin) for each channel vs. time. User can select the desired channels simply by clicking them in the channel layout map in *Controls* window (use the *Shift* key for multiple selections). In *Settings* window user can change the type of visualization. The available options are:

- ◆ “Cumulative Data”: all the selected channels are used to yield cumulative firing rate values visualized in a single graph
- ◆ “Per-Channel Data”: each channel is plotted in a separate graph
- ◆ “Per-Cluster Data”: a cumulative firing rate plot for each cluster is visualized (to create clusters see *Cluster Manager* window)

By drag and drop on the raster plot it is possible to zoom on a specific temporal window. The temporal range track bar in *Controls* window is automatically updated. By moving the cursor on the graphs a tool tip shows the coordinate of the channel pointed by the cursors. The firing rate is calculated by integrating the number of detected events in a certain temporal window, this interval can be changed in *Settings* window.

5.2.5. ISI tab

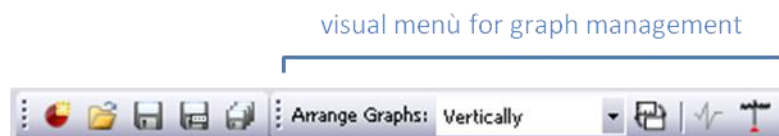
This tab allows visualizing the calculated Inter Spike Interval (ISI), i.e. the latency probability between a detected event and the following on the same channel. User can select the desired channels simply by clicking them in the channel layout map in *Controls* window (use the *Shift* key for multiple selections). In *Settings* window user can change the type of visualization. The available options are:

- ◆ “Cumulative Data”: all the selected channels are used to calculate cumulative ISI values that are visualized in a single graph
- ◆ “Per-Channel Data”: each channel is plotted in a separate graph
- ◆ “Per-Cluster Data”: a cumulative ISI plot for each cluster is visualized (to create clusters see *Cluster Manager* window)

By moving the cursor on the graphs a tool tip shows the coordinate of the channel pointed by the cursors. The ISI probability is reported in the distribution plot by integrating the number of latencies for a temporal bin that can be set in the *Settings* window. Moreover, the user can choose the maximum ISI value to be considered to plot the graph and the type of scale to use, either linear or semi-logarithmic.

6. General commands and controls

BrainWave™ provides a set of commands and controls “general purpose” that allow the user to change visualization settings or to manage analysis parameters specific to each tab. When a file is loaded (both .brw and .bxr), in the main window user can select graph arrangement and exclude/include scale visualization by using the visual menu on the top tool strip. When the *Replayer* tab is selected the “Show spike event” button allows visualizing the voltage raw traces superimposed with the detected events.

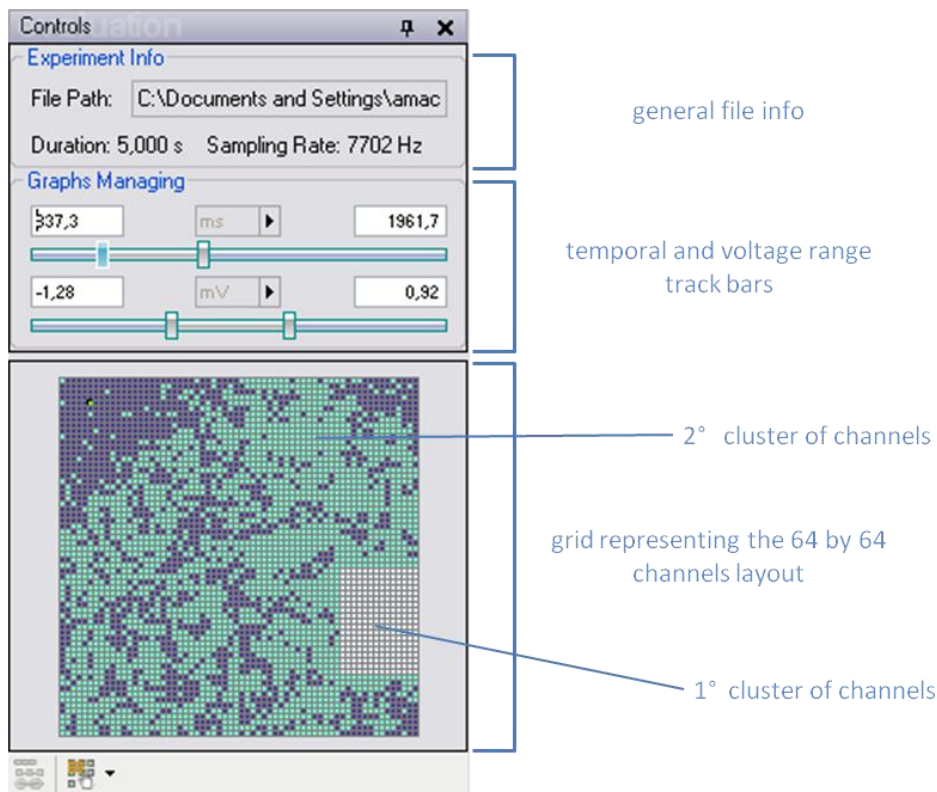


In the following section the three main managing windows (selectable from the main menu in *View->...*) are discussed in details.

6.1. Controls window

It contains two main sections, the first regarding general information about the experiment (*Experiment Info* panel): the user can read the loaded file path, the duration and the sampling

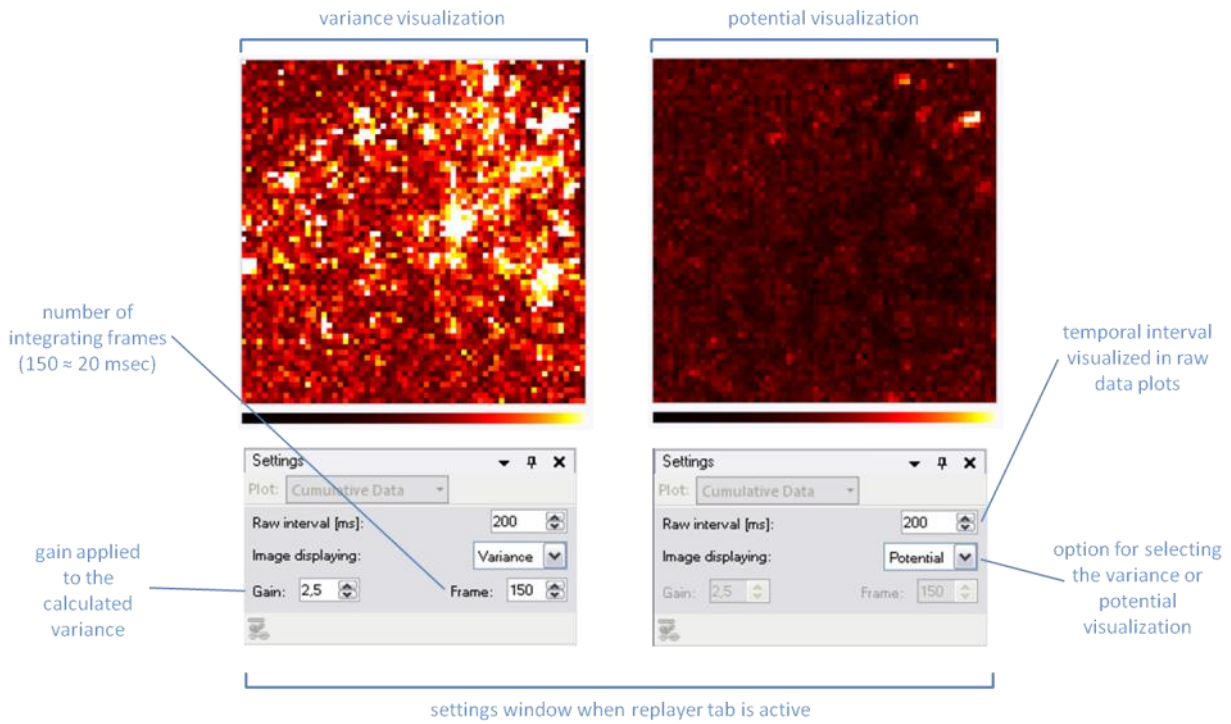
frequency at which the recording has been acquired. The second is the *Graphs Managing* panel where the temporal and the voltage scale can be set by using the range track bars. Channels can be selected independently on the active result tab, by clicking on the 64 by 64 layout representation. Channels belonging to a cluster are colored with the cluster color itself (see *Cluster Manager* window).



6.2. Settings window

All the parameters concerning visualization, which are specific for a certain result tab (*Spike Statistics*, *Raster*, etc.), are collected in this window. When the *Replayer* tab is active it is possible to define the visualization time interval for the raw data plots, and the color visualization mode for the *Camera View* (see *Replayer*). In *Spike Statistics* tab the user can select the Mean Firing Rate or the Inter Spike Interval visualization and apply the low and high threshold values to set the range of active channels.

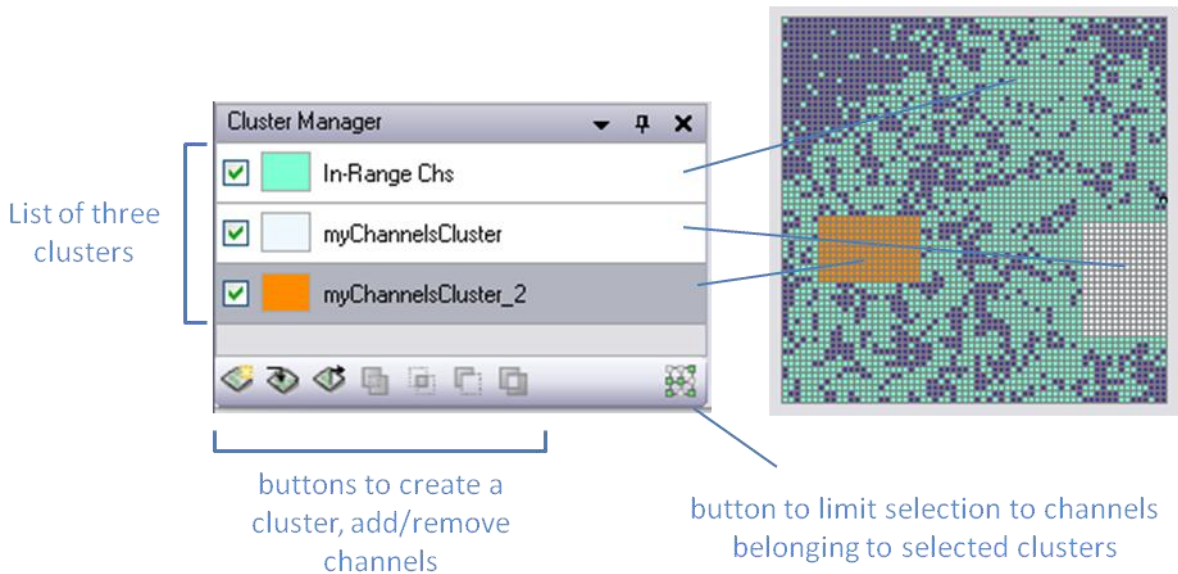
By clicking the *down arrow* on the top right and selecting “New List from In-range Chs...” a cluster containing the channels, whose firing rate is in the range between Low and High Threshold, is created. Visualization settings specific for each analysis are available for *Raster*, *Firing Rate Analysis* and *ISI* tab.



settings window when replayer tab is active

6.3. Cluster Manager window

This window contains a list of all the clusters created by the user. User can create clusters simply by selecting the desired channels and clicking the “Create new cluster” button. Hence, a “Create new Cluster” window appears; the user has to add a name, choose the identifying color and select the “add Selected Channels” option. When a cluster is created its statistics appear in the *Overall Statistic* panel in the *Spike Statistic* tab. A cluster can be deleted by selecting it and clicking the “Canc” key.



To select channels that belong to the clusters only, user has to click on the “limit selection” button in the bottom right of the window. This allows visualizing only the channels belonging to the checked clusters in the list.

7. Spike detection analysis

To perform spike detection analysis by varying the parameters, click on analysis in the main menu and select the “peak detection” option. A *Peak Detection* window appears where user can set the following three main parameters:

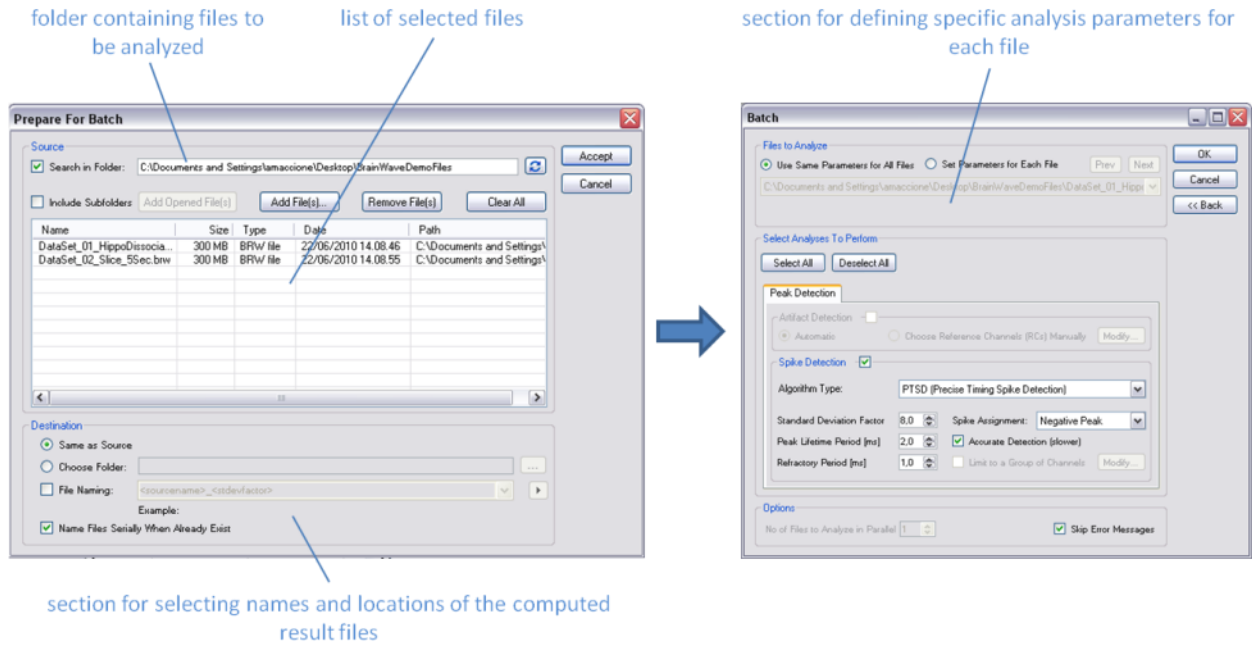
- ◆ “Standard deviation factor”: the number of times the signal has to overcome the StdDev of the basal noise to be considered as an event
- ◆ “Peak Lifetime Period”: the maximum duration of an event expressed in milliseconds
- ◆ “Refractory Period”: the minimum distance between two detected events expressed in milliseconds

Then user can define whether to assign the timestamp of the detected event either to the positive or to the negative peak or the higher of both. The user can check the option for an accurate detection, by which the StdDev of the noise is constantly updated while the raw data is scanned for spike events.

The analysis can be performed on the entire array or can be restricted to a subset of channels by clicking on the “Limit to a Group of channels” checkbox and selecting the area of interest by multiple click or drag and drop (a selection via cluster definition can also be done).

7.1. Automate batch

BrainWave™ provides a batch to automatically analyze multiple files. This feature allows the unsupervised analysis of large dataset; files are computed in parallel taking advantage of PC multi-core architecture. To start a batch, click on *File->Automate->Batch* in the main menu. The *Prepare For Batch* window appears, the user has to select the folder containing the .brw files to be analyzed by double clicking the “Search in Folder” box. A list of the files appears, click on “Accept” button. The *Batch* window appears where the user can set the parameters for detecting events as described in the previous paragraph. Click the “OK” button to start analysis.



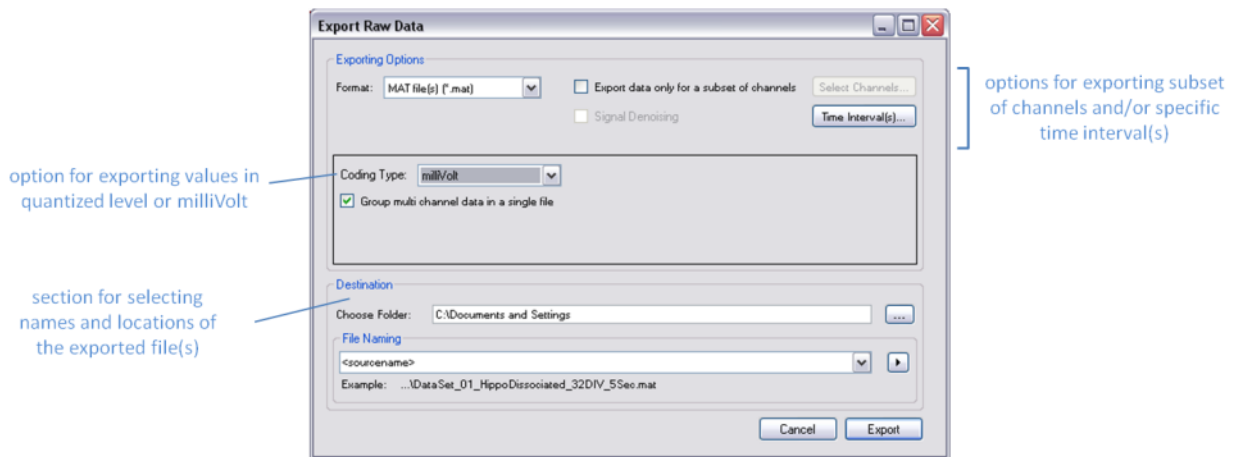
8. Exporting in MATLAB®

Both raw data and result files can be exported in different formats. This allows the compatibility with other analysis software, leaving the user free to develop “ad hoc” algorithms in other languages. In this version, files can only be exported in .mat files (MATLAB® see www.mathworks.com).

8.1. Raw data

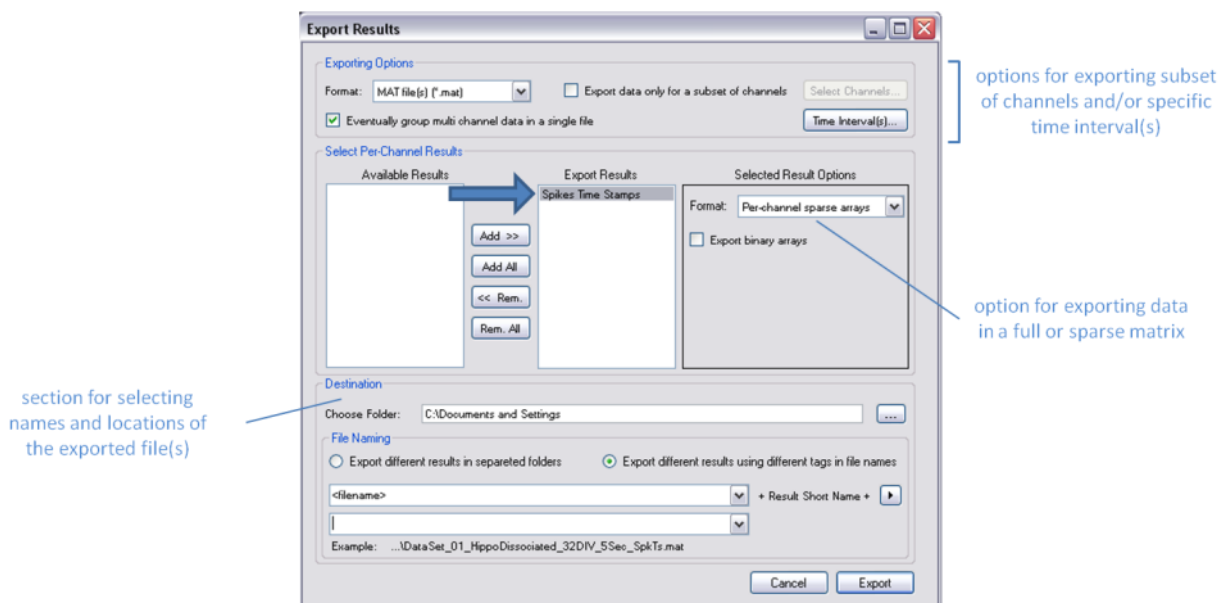
Load the file that has to be exported in MATLAB. Click on *File->Export->RawData*. The *Export Raw Data* window appears, by which the user can select the name of the file(s) and the folder where to store it. The export can be performed on the entire channel array or can be restricted to a subset of channels by selecting the “Export data only for a subset of channels” and selecting the area of interest by multiple click or drag and drop (a selection via cluster definition can also be done). Also the exporting time interval can be set by clicking on “Time Interval(s)...” button and choosing one or more temporal windows.

Channels can be exported in separate files or grouped into a single file by selecting the “Group multi channel data in a single file” checkbox. Raw data are saved in an array for each channel of quantized levels or MilliVolt values depending on the “Coding Type” selected option. Each element of the array represents a sample (sampling frequency 7702Hz).



8.2. Spike trains

Load the file to be exported in MATLAB. Click on *File->Export->Results* in the main menu. The *Export Results* window appears, by which the user can select the name of the file(s) and the folder where store it in the *Destination* section. The export can be performed on the entire channels array or can be restricted to a subset of channels by selecting the “Export data only for a subset of channels” and selecting the area of interest by multiple click or drag and drop (a selection via cluster definition can also be done). Also the export time interval can be set by clicking on “Time Interval(s)...” button and choosing one or more temporal windows. The user has to select the “Spike Time Stamps” option in the *Available Results* section and click on “Add >>” button. The “Spike Time Stamps” option moves to the *Export Results* section, by clicking on it, the user can select whether to export the results to a sparse matrix or to a full matrix by setting the “Format” option in the *Selected Result Options* section.



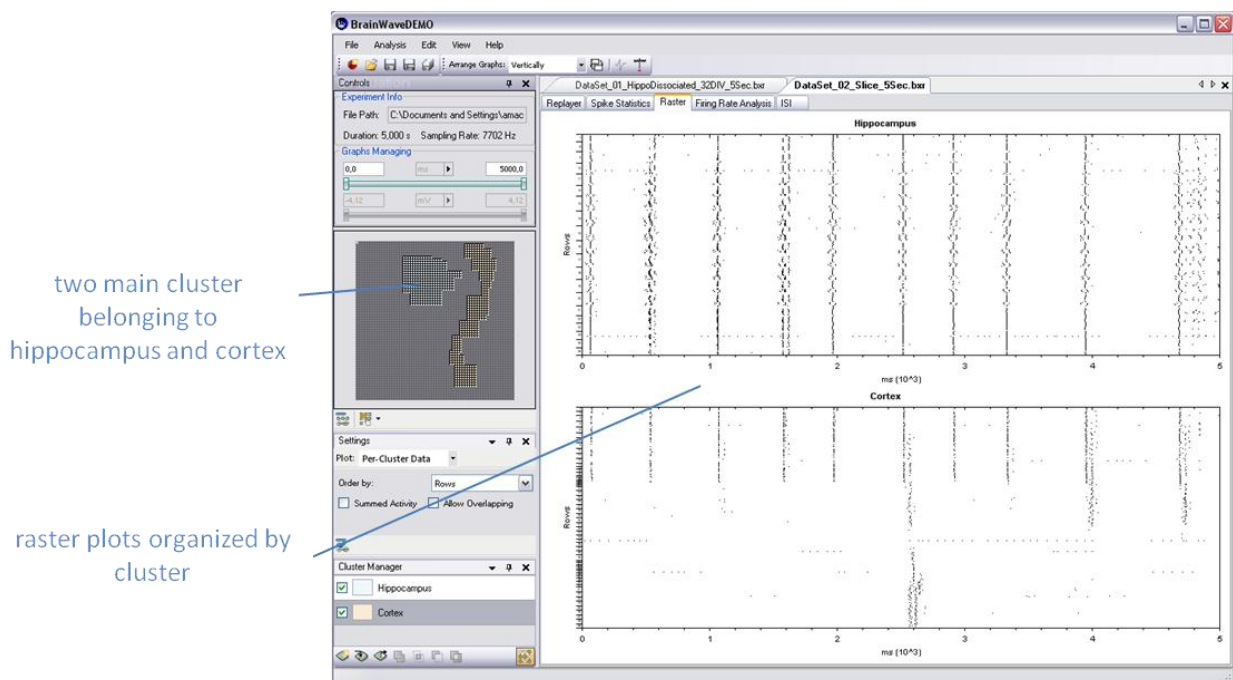
The generated .mat files are organized in rows, one for each channel. Each row is a full or sparse array (depending on the selected option) where every value represents a sample (sampling frequency 7702Hz). The array values are zero where no events are detected, while when an event is detected the sample value is the peak amplitude expressed in MilliVolts or 1 (if “Export binary arrays” option is selected).

9. Tips and tricks

Simultaneous recording of 4096 channels at about 8 kHz is an important technological progress in the “MEA world” but introduces some issues in terms of analysis regarding the amount of collected data. For example, 5 minutes raw data recording is equivalent to a file of about 18 Gigabytes. Therefore analysis and results visualization have been designed in order to help users in data mining and fast extraction of the searched results. Here some tips and tricks are described to help the user in his work.

9.1. Spike statistics tab as your starting point

We suggest having a global idea of your analysis by looking at the MFR channel distribution on your experiment in the *Spike Statistics* tab. This immediately gives an idea of how activity is distributed, and by setting low and high threshold visualization, it is possible to create a cluster from the *Settings* window only containing the channels of interest. Then, from the main cluster of valid channels it is possible to create a series of sub-clusters that can be visualized separately in the *Raster* tab. In any case keep the selection of channels always *limited to the checked cluster* (see cluster manager paragraph) to avoid the selection of undesired channels.

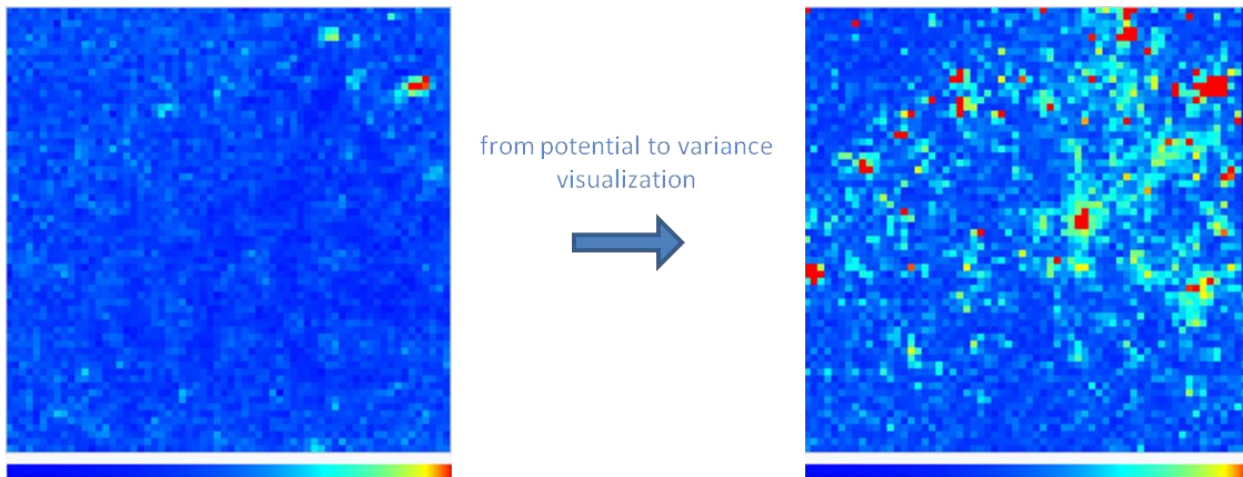


9.2. Organizing activity in cluster

The segregation of channels into clusters can be very useful especially when the biological model presents spatially well defined regions of cell population. For example this is the case of slices where it is easy to recognize precise areas of activity like the CA1, CA3 and dentate gyrus in the hippocampus. In this condition the overall statistics can be really insignificant due to the highly variable behaviors of the brain regions. Dividing into clusters and looking at the statistics for each of them can be an approach to better understand electrical activity dynamics among regions.

9.3. Fast recognition of activity

In *Replayer* tab the global visualization of the activity can be very useful in finding neuronal signaling. We suggest always working in variance mode (see *Settings* window) because it computes a sort of integration of the signal within a moving temporal window.



In order to better highlight the activity, we suggest setting the scale color bar in the exponential mode and setting the number of integration frames to at least at 300-350 (about 400 msec). In this way the software provides a rough visualization that can help in finding the desired activity. Then after having temporally localized it and in order to better appreciate the spatial propagation of the signal, decrease the number of integration frames to 15 (about 2 msec), or move to potential mode color map visualization.

9.4. From raster to raw data visualization

Looking for activity by using the raw data replayer is not always trivial in fast localization of signaling both at temporal scale and spatial distribution. We suggest visualizing the raster plot of the main active channels. Then after localization of the desired activity, zoom on it by drag and drop directly on the raster. In this way the temporal range track bar in *Controls* window is set on the temporal close-up selected in the raster plot. Moving to the *Replayer* tab, the playback is limited to the selected temporal interval to avoid wasting time in finding the right instance of activity.

10. Contact Details

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