Welcome to BioCAM X

User Guide
Essentials

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Introduction

Welcome to the BioCAM X platform and thank you for your purchase. The BioCAM X platform is among the most advanced systems for managing your experiments on the emerging generation of high-density CMOS-MEAs. This manual has been written to help you to take advantage of all functionalities provided by your BioCAM X. Be sure to read this manual thoroughly, and to keep it handy when using the BioCAM X platform.

Before any use of your BioCAM X platform, please read the "Precautions" on page 15, which contains relevant information to preserver your BioChips and BioCAM X from possible damages.

MEA technology

Among the different methodologies used for electrophysiological measures, metal microelectrodes integrated on-chip can provide multisite measures of extracellular signals with a high signal-to-noise ratio. In addition, by applying voltage or current stimuli to the same microelectrodes it is possible to depolarize cells or tissues, thus establishing bi-directional interfaces. As established over several decades of research, both sensing and actuating performances of microelectrodes can be applied to study a wide range of electrogenic cells and tissues, including neuronal and cardiac preparations.

Conventional microelectrode arrays (MEAs) are bio-sensing chips realized by means of thin-film technologies and do not integrate on-chip any microelectronic circuit. Therefore, conventional MEAs are passive devices made on silicon, glass or polymeric substrates. Each microelectrode can be made by different materials (e.g. Pt, IrOx, TiN) and it is individually wired on-chip and connected to an external amplifier and data acquisition (DAQ) instrument. Due to the need of this MEA technology of individually routing on-chip each electrode, space constraints and wiring encumbrance impede the realization of dense and large electrode arrays. Thus, for conventional MEAs the typical electrode pitch is in the range of 100 µm and the array includes from 60 up to 256 microelectrodes. In addition, given the distance between the electrodes and the off-chip amplifiers and the use of interconnecting wires, conventional MEAs are subjected to inductive coupling noise.

CMOS-MEA technology

The electrode density and array sizes can be increased by changing the technology used to realize microelectrode arrays (MEAs). High-density MEAs are realized with complementary metal–oxide–semiconductor technology (CMOS), as it is done for microelectronic devices (e.g., computer microprocessors) and light-imaging devices (e.g., camera sensor), and with post-processing methods to optimize the electrode performances.

Briefly, the CMOS technology allows to realize active electrode-pixels that integrate in small areas of a few square micrometers electrodes, amplifiers and signal conditioning circuits in-pixel, just underneath each electrode site. On-chip, additional amplification stages, multiplexing and high-speed addressing circuits are provided.

In particular, the circuit architecture of 3Brain's BioChips is based on the Active Pixel Sensor concepts, as commonly used for light imaging CMOS cameras, and was designed to allow full array recordings at

sufficiently high sampling frequency for each electrode.

In BioChips the local in-pixel buffers underneath each electrode allow to locally adapt signals from high impedance (electrode-side) to low impedance (wiring-side) to avoid the induction of coupling noise from electrical wiring before amplification. Additionally, the on-chip multiplexing and addressing circuits allow to minimize the number of wiring outputs even though the large number of electrodes that have to be simultaneously measured. Indeed, each electrode signal is not individually wired off-chip. Rather, signals of multiple electrodes are multiplexed at high frequency over a few number of wires. In this way, several thousands of electrodes can be recorded on only a few tens of output wires.

The in-pixel circuit implemented in BioChips allows recordings from a large signal bandwidth that includes field potentials and spikes. Instead of implementing an AC-coupling solution that would require large areas for the integration of adequate capacitors, the BioChips in-pixel circuit integrates an auto-zeroing circuit that is regularly calibrated to the electrode DC voltage and that subtracts this calibrated DC voltage from the electrode signal before the first amplification stage. This process is called calibration and it also allows to adapt the circuit performance to different experimental conditions. In particular, all CMOS devices are photosensitive and photo-generated charges give rise to different DC drifting times that can bring in-pixel amplifiers to saturate. BioChips mitigate these effects and hence allow experiments under light stimulation conditions (as required for instance for retina stimulation) by using higher calibration frequencies to keep the DC input signal to the working point of the amplifier and to avoid the saturation of the amplifier.

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At a glance

Overview

The BioCAM X platform is a high resolution electrophysiology system capable to perform in-vitro electrophysiological measures on electroactive cells and tissues. Even though, to some extents, the platform has some similarities with more conventional Microelectrode Array (MEA) systems, it is a radically new technology to perform extracellular measures. Differently than conventional MEA systems, the BioCAM X platform relies on active CMOS-MEA chips, which integrate thousands of miniaturized active electrodes over large areas. Combined to sub-millisecond temporal resolution, the dense integration of electrodes results in a spatial resolution that enables novel analysis, can increase the statistical significance of your experiments and opens new imaging approaches to investigate electroactive preparations.

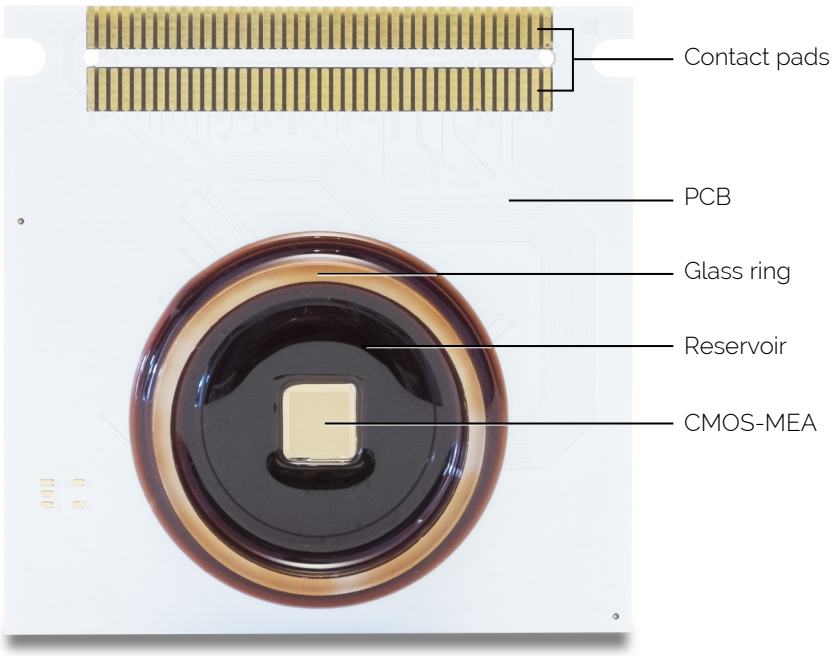
The 3Brain's BioCAM X platforms was tested so far for in-vitro electrophysiological experiments on neuronal cell cultures, brain slices, retina preparations and cardiac cultures. Electrophysiological signals ranging from slow field potentials to fast single cell spiking activity can be recorded simultaneously. Examples of recordings provided by different laboratories using the BioCam platforms are available on the 3Brain website and uploads of novel examples based on your research are warmly welcome.

Overall, the platform consists of three basic components: A) a multiuse BioChip cartridge available in different models and incorporating the CMOS-MEA silicon chip; B) the acquisition BioCAM X hardware that reads out electrophysiological signals from the BioChip; C) the host PC equipped with the acquisition board and the BrainWave X software to acquire, visualize, store and analyse experimental data recorded with the BioCAM X platform.

BioChip

The BioChip cartridge integrates on an electronic substrate the CMOS-MEA chip and a reservoir chamber to maintain cultures or tissues contacting the electrode array either under cell culture media or perfused media, respectively. The cartridge is 54 mm x 54 mm (2-1/8 in x 2-1/8 in) in size and is conceived to be placed in an incubator for cells or tissue cultures.

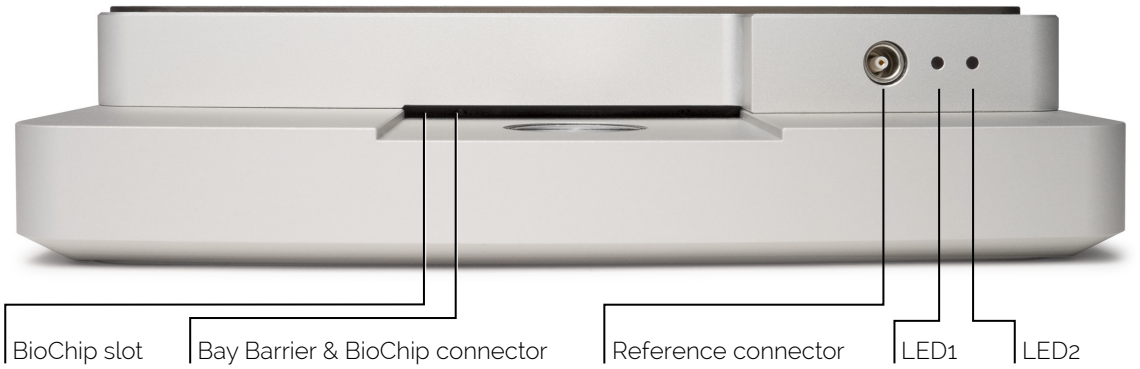
- *Contact pads*: gold-plated pads for connection between the BioChip and the BioCAM X. Properly cleaned and non-oxidized pads are needed for a stable connection.
- *PCB*: the printed circuit board substrate of the BioChip. Different colors are available and according to the layout of the CMOS-MEA.
- *Glass ring*: borosilicate glass ring defining the reservoir. External diameter 28mm (1-7/64 in), internal diameter 25mm (0-63/64 in), height 5mm (0-13/64 in).
- *Reservoir*: chamber for the bath solution used for the biological preparation. The volume of solution that can be contained is of about 2mL.
- *CMOS-MEA*: chip integrating thousands of active electrodes for high-resolution electrophysiological recordings from cells or tissues placed on its active area. Different application-specific MEA layouts according to the BioChip model are available.



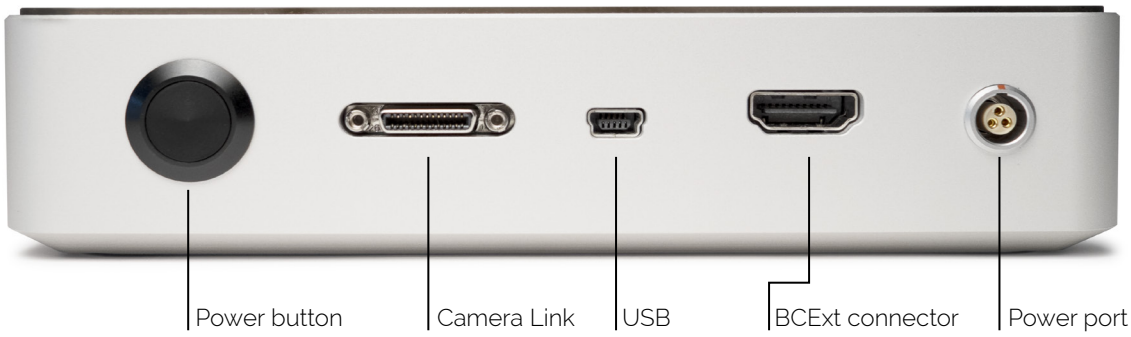
BioCAM X

The BioCAM X is the station where BioChips are plugged into for electrophysiological measures. This instrument is compatible with all BioChip types including also those with electrical stimulation capabilities. It contains all the electronics, real-time hardware and logic to control the CMOS-MEAs chips on the BioChip cartridges and to acquire and pre-process large-volume of electrophysiological data before sending it to the host PC.

- *Lock button*: two-position button for locking (end position) or unlocking (mid position) the BioChips.
- *BioChip bay*: bay for inserting the BioChip and connecting it to the BioCAM X.
- *Magnetic plate*: ferromagnetic stainless steel to attach magnetic holders (e.g., for perfusion).
- *Peltier-Element*: active element to control the temperature of your preparation on BioChips



- *BioChip slot*: the slot through which the BioChip (contact pads side) is inserted into the BioCAM X for electrical interfacing.
- *Bay Barrier & BioChip connector*: immediately behind the BioChip slot, a protective movable Bay Barrier blocks accidental overflows of liquids that might get in touch and damage the internal electronics. Behind the Bay Barrier, the BioChip connector allows to connect the BioChip's contact pads to the internal circuitry. Electrical contacts are established only once the locking system is activated.
- *Reference connector*: connector for the pseudo-reference electrode that need to be placed in the bath solution of the BioChip cartridges.
- *LED1*: a solid blue LED indicates whether the BioCAM X is powered (LED ON) or not (LED OFF). A solid violet LED indicates a failure in BioCAM firmware. In addition, the LED1 blinks violet to indicate that the temperature control is ON.
- *LED2*: a solid green LED indicates whether the BioCAM X is acquiring signal (LED ON) or is in stand-by mode (LED OFF).
- *Power button*: push-button to power ON or OFF off the BioCAM X (the instrument need to be connected to a power line using the provided adaptor).



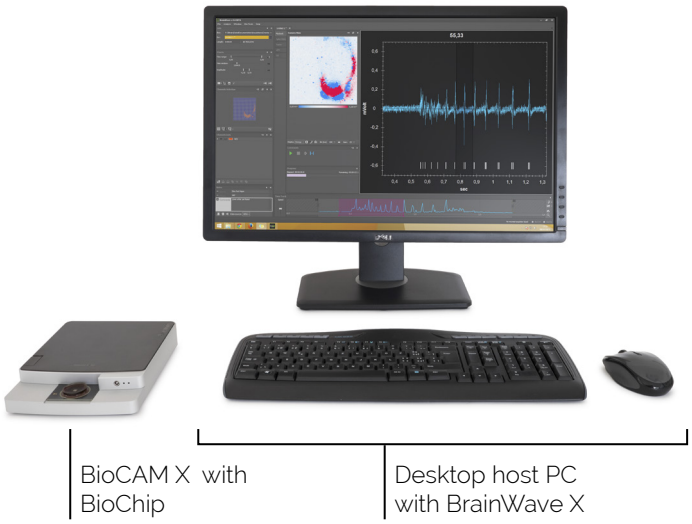
- *Camera-link*: SDR mini Camera Link connector to connect the BioCAM X to the acquisition board mounted in the host PC. Electrophysiological data and control signals are managed through this interface.
- *USB*: this port is used only for firmware updates of the BioCAM X. For normal use it is not needed to connect this interface.
- *BCExt connector*: plug the BCExtAdapter cable to access extended functionalities such us triggering and stimulation.
- *Power port*: plug for the power supply unit.

Host PC

Depending on your order the host PC can be a desktop PC or a laptop. The way the BioCAM X instrument is connected to the host PC changes according to these two cases.

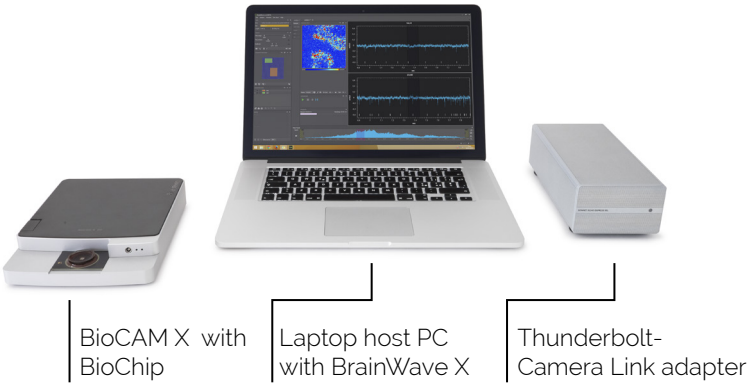
Desktop PC

When connected to a desktop workstation, the connection is direct between the BioCAM X and the host PC that is equipped with a Camera Link acquisition board. The typical configuration is as follow (tower desktop not shown).



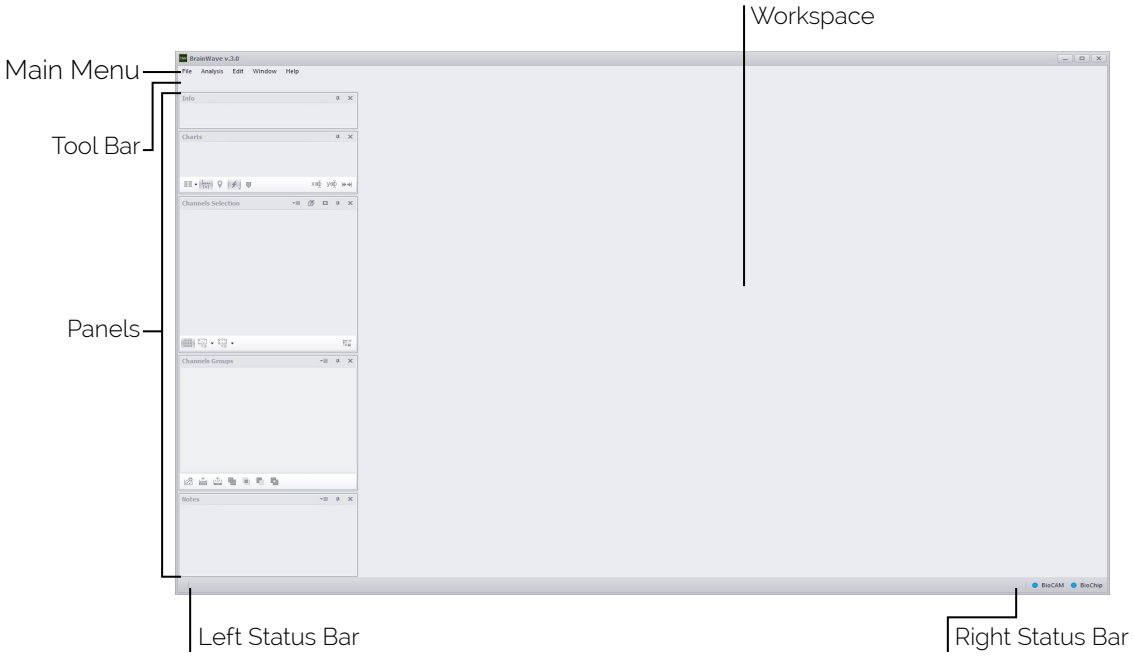
Laptop PC

When connected to a laptop PC, you need an adapter to connect the BioCAM X to the host PC. Such adapter is a Thunderbolt-Camera Link adapter box that encloses the Camera Link acquisition board. The typical configuration for a laptop hosting PC is illustrated here below.



BrainWave X

BrainWave X is a complete software application developed to exploit all the functionalities of your BioCAM X platform. It is equipped with advanced tools adapted to manage high-resolution electrophysiological data acquired from BioChips. When launched it appears as follows (without experiments ongoing).



- *Main Menu*: access main functions by clicking on the main menu.
- *Tool Bar*: displays tools provided by the data controls currently shown in the Workspace. Tools are refreshed according to the currently focused controls and for some controls can be missing.
- *Panels*: dockable panels that are automatically updated according to the currently selected experiment loaded into the Workspace. Panels allow you to modify the experiment's options and parameters and can be shown or hidden by choosing the Window menu on the Main Menu.
- *Left Status Bar*: notifies you about ongoing operations.
- *Workspace*: arena for visualizing through tabs online and offline experiments.
- *Right Status Bar*: notifies you about the status of the controlled devices. It comprises also LED icons for BioCAM and BioChip devices. If the LED icon is on (blue) the corresponding device is properly connected and recognized.

3 Precautions

BioCAM X handling and use

The BioCAM X instrument is made with a solid metal case and with optimized electronics that make the system robust to mechanical and vibrational noise. Even if it is recommended, the installation on an anti-vibration table is not mandatory.

Moreover, the instrument has been design to be shielded from external electrical environmental noise. However, to guarantee optimal recording performances simple precautions as listed here below should be followed:

- Position the BioCAM X far from potential strong noise sources, such as fridges, incubators etc… Indeed, if not well shielded these instruments might generate electromagnetic noise.
- Connect the BioCAM X to adequate power lines with low noise levels.
- Take care to avoid that electrical cables connected to the BioCAM X (in particular, the one for the power supply and for the Camera Link) do not pass near other power supplies.
- Check that the cable connectors are well inserted into the BioCAM X and the Host PC before operating the platform.

The system allows for the integration of a perfusion system as it is needed for maintaining tissues during electrophysiological measures. Even if precautions have been taken in designing the system to prevent liquids to enter in contact with the internal electronics, spill out of electrophysiological solution that might get into the BioChip slot should be strictly avoided. However, should this happen please follow these steps:

1. **Immediately switch OFF the BioCAM and remove the BioChip.**
2. Clean accurately the BioChip Bay of the BioCAM X with ethanol 96% and let it dry. Execute the same procedure for all the BioChip parts but for the BioChip reservoir and in particular for the BioChip connector pads.
3. **In case after the cleaning with ethanol, there are still doubts that some liquid could have been penetrated into the BioCAM X, do not switch on the system for at least one day, allowing the liquid to be completed dried and preserving internal circuitry from potential oxidative and short-circuit effects.**

Some precautions have to be also taken when manipulating the pseudo-reference, which from one side (connector) is connected to the BioCAM X and whose other end (platinum wire) needs to be placed into the bath solution inside the BioChips:

- Handle the reference with care by wearing gloves and avoid touching the platinum wire (i.e. the part of the reference that is in contact with the electrophysiological solution).
- **Clean regularly the reference to guarantee optimal recording performances.** The platinum wire of the reference should be rinsed with deionized water before starting and after an experimental session. Alternatively, for a heavier cleaning, the platinum wire only can be immersed in HCl 0.1M for about 1 minute and then abundantly rinsed with deionized water.
- **When the BioChip is inserted and the reference is positioned in the electrophysiological solution, the operation of switching ON or OFF the BioCAM X might cause electrical glitches that can potentially damage the circuitry of the CMOS-MEA chip.** To avoid this, it is a good practice to follow the operational sequence of plugging the BioChip, switching ON the BioCAM X and then insert the reference into the liquid. In an analogous way, remove the reference from the electrophysiological solution before switching OFF the BioCAM X and unplug the BioChip.

BioChip handling and use

BioChips are low-power active electronic circuits and it is highly recommended to avoid the following operations that might result in a damaging of their electronics:

- Do not touch the contact pads of the BioChip. Electrostatic charges might damage the on-chip circuits and might result in damaging the device. Always manipulate the BioChip by holding it on the sides and ideally by using plastic gloves.
- Do not hold the BioChip by its glass ring to avoid detaching it.
- Do not touch the active area of the BioChips with any tools except those specifically allowed (see "Cleaning procedure for BioChips"). In particular, hard or semi-hard objects (e.g. metal or plastic tools) can irreparably damage the electrodes and the on-chip circuits.
- For cleaning or for sterilization avoid immersing the entire BioChip in water or ethanol. Prolonged immersion in water of the entire BioChip might cause oxidation of the contact pads. In ethanol, some parts of the BioChip might be deteriorated. As a general rule only the chamber can be wet while the rest of the chip should stay dry.
- Do not place the BioChips in autoclaves, ovens or under UV-light exposure for sterilization. These methods might deteriorate the CMOS devices, resulting in malfunctioning or in reduced lifetime. For the BioChip sterilization, please refer to the "Sterilization procedure for BioChips".
- Maintain the pH of the electrophysiological solutions used for neuronal cultures or brain tissues possibly at physiological conditions (7-7.5). Important changes in the pH of the solution might damage the electrodes.

During recordings (both cultures and brain tissues) users have to comply with the following precautions:

- Before contacting the BioChip into the BioCam, clean the metal pad contacts with a tissue paper soaked with ethanol 96% and let it dry for few seconds.
- Avoid liquids to spill out and strictly avoid liquids to enter in contact with the pads (the BioCAM system is equipped with a Bay Barrier to protect the connecting socket). In case users experience liquid spill out, follows instructions provided in the "BioCAM X handling and use" section.

In order to ensure a good quality of the recordings:

- Avoid using HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) in the solutions used for electrophysiological recordings, since we observed that it can interfere with the electrodes making the chip unstable and the recordings very noisy.

- BioChips are light-sensitive devices and their performances might be affected by a too high intensity direct exposure or by noisy light sources.

Cleaning procedure for BioChips

Normal cleaning

It is a good practice to clean the BioChips immediately after the experimental recordings both for cell cultures as well as brain tissues. First, rinse abundantly the BioChips with Double Deionized Water (DDW), then fill the chambers with a detergent as WPI-Enzol (WPI) or Terg-A-zyme (Alconox) and gently pipette for few seconds. Leave the detergent for few minutes (typically 3-5 min) and then pipette again.

After the use of detergent, rinse the BioChips abundantly with DDW, then leave the chamber filled with DDW for 1-2 minutes and repeat this operation 3-4 times in order to assure to wash out completely the detergent.

Intense cleaning

In case the chips are very dirty, users can mechanically remove cellular debris with a soft brush. Be aware that this operation can damage the sensing area of the chip, so it is suggested only if, after a normal cleaning procedure, the chips still present dirtiness and in any case it has to be performed very mildly and carefully.

Finishing and storage

Biochips can be dried by using a gentle flux of Nitrogen air (do not directly expose the recording area to the flux to avoid potential electrode damages). Alternatively let the BioChips dry on a bench by covering them with a plastic petri dish to avoid dust deposition on the recording area.

The Biochips area out of the chamber can be cleaned with a tissue soaked in ethanol 96%. It' a good practice to manipulate chips always wearing gloves.

Once the BioChips are dried they should be stored in a closed box in order to protect them from dust and dirtiness.

As a general rule, avoid sterilizing or using ethanol in the BioChip chamber without having preventively cleaned the chip from cellular debris of previous experiments. Alcohol can fix biological materials on the recording area of the chips, resulting in a degeneration of the signal recording performances.

Sterilization procedure for BioChips

As indicated in the previous section, BioChips cannot be put in autoclaves, ovens or under UV-light exposure for sterilization purpose. These procedures risk to deteriorate the chips resulting in not optimal recording performances and in a shorter lifetime.

If still not done, perform a cleaning procedure as indicated in the "Cleaning procedure for BioChips" previous section and adopt the following procedure (that has to be performed under a biological hood) to sterilize the chips:

1. Clean the area of the BioChips outside the glass ring (including the external part of the glass ring itself) by using a tissue soaked with ethanol 96% and put inside sterile plastic petri dishes.
2. Fill the reservoir completely with ethanol 70%. Also dip with the same solution the border of the glass ring (the tiny upward-oriented side of the ring). Hence, wait for 20 minutes.
3. Suck the ethanol with a pipette or with a vacuum pump always using sterile tips.
4. Fill 3/4 of the reservoir of the BioChips with sterile DDW, wait few seconds and suck the liquid always with sterile tips. Repeat these operations 4 times to ensure to wash out completely the ethanol. When removing sterile DDW for the last time, be sure to have completely dried the chamber. Aspirate the liquid on the recording area always by keeping the tip close to the glue/CMOS border but not touching the CMOS to avoid any risk to damage the electrodes.

4

Get started

Install the BioCAM X

Follow the below instruction to install the BioCAM X platform when you receive a pre-configured host PC from 3Brain. Instructions differs according to which type of hosting PC you have ordered. If you have not purchased a pre-configured host PC, please refer to section "Set up a non pre-configured host PC" before proceeding with the following instructions.

Desktop host PC

1. Plug the power supply cable into the power port located in the back of BioCAM X and then connect the power supply into an electrical outlet. Switch ON the BioCAM X by pressing the power button on the back. The BioCAM's LED1 should turn ON.
2. Turn on the PC, plug one end of the Camera Link cable into the acquisition board located in the back side of the PC and the other end of the Camera Link cable into the Camera Link port of the BioCAM X. Tighten the screws in the connectors on both sides to secure the Camera Link cable.
3. Launch BrainWave X software and verify that the BioCAM X is properly recognized by checking that the BioCAM LED icon in the BrainWave's Right Status Bar turns ON.

Laptop host PC

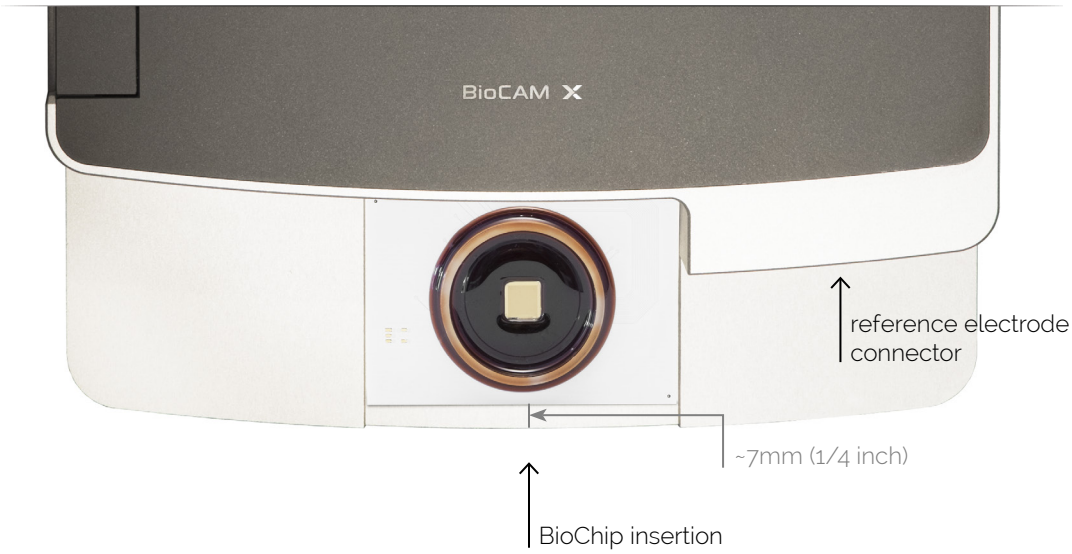
1. Plug the power supply cable into the power port located in the back of BioCAM X and then connect the power supply into an electrical outlet. Switch ON the BioCAM X by pressing the power button on the back. The BioCAM's LED1 should turn ON.
2. Plug the power supply cable into the power port located in the back of the Thunderbolt-Camera Link adapter box.
3. Connect one end of the Camera Link cable into the Thunderbolt-Camera Link adapter and the other end of the Camera Link cable into the Camera Link port of the BioCAM X. Tighten the screws in the connectors on both sides to secure the Camera Link cable.
4. Connect one end of the Thunderbolt cable into the Thunderbolt-Camera Link adapter and the other end into one free port in the laptop host PC.
5. Turn on the laptop PC. Please consider that according to the version of Windows and the drivers installed in your machine, Windows might not recognize the Thunderbolt-Camera Link adapter if this is connected after Windows is already running. Hence, in this case make sure to turn on the PC only once everything is properly connected as explained above. For such versions of Windows, if the connection with the BioCAM X is lost, a Windows restart might be required.
6. Launch BrainWave X software and verify that the BioCAM X is properly recognized by checking that the BioCAM LED icon in the BrainWave's Device Status Bar turns ON.

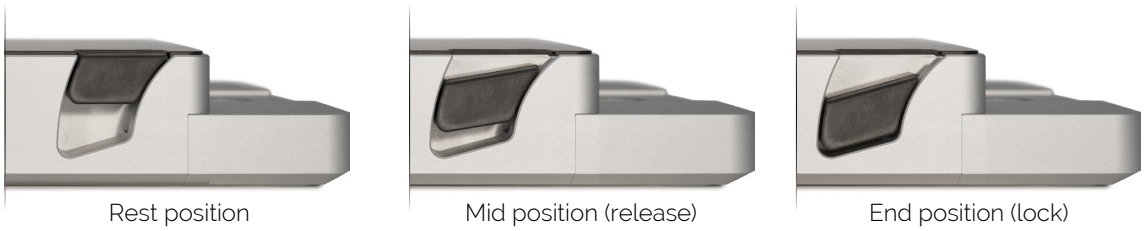
Plug the BioChip

BioChips are connected to the BioCAM X through the lock system, which is operated by a two-position button. When the button is pressed till its mid position the BioChip is released or the locking system is opened to allow to plug a BioChip into the BioCAM X. When the button is pressed till its end position the BioChip is locked and connected to the BioCAM X.

To plug the BioChip into the BioCAM X:

1. Insert the BioChip into the BioCAM X sliding it along the BioChip bay. The BioChip should enter into the BioCAM's BioChip slot till the distance between the BioChip end and the BioCAM X end is approximately 7mm (1/4 in). If the BioChip does not enter till the ending position, try to push the BioCAM X's lock button till its mid position to release the locking system and then try re-inserting the BioChip.
2. Push the Lock button in the BioCAM X to its end position to lock the BioChip to the BioCAM X connector. On BrainWave X software the BioChip LED icon in the Device Status Bar should turn ON.





3. To start using the BioChip, plug the electrode reference into the BioCAM X and place the platinum end of the electrode inside the BioChip's reservoir, which must be filled in with a physiological saline solution (or a simple saline solution when performing system testings).

To unplug the BioChip:

1. Press the BioCAM X's lock button down to its mid position till you hear a pop.
2. Slide out the BioChip by holding it aside its glass ring (pushing with two fingers on the BioChip PCB), hence avoiding any shear stress on the glass ring.

Set up a non pre-configured host PC

If your order included a pre-configured host PC you can skip this step. If you are going to configure your own PC, please follow this instructions before installing the BioCAM X.

1. Install the acquisition board driver by launching the installer and following the instructions. The acquisition board driver are provided to you by the customer support. If you have not received those, please contact customer support.
2. Get the latest version of BrainWave X by downloading it from http://www.3brain.com/downloads. Once downloaded, double-click on the installer and follow the instructions.
3. Start BrainWave X. A message requiring to update BrainWave X might be displayed. We strongly recommend to accept in order to keep your software running at its best. After the installation of updates, the software automatically restarts. Verify that in the BrainWave X's Right Status Bar there are no error message. If you see a message like "No detected acquisition board" it means that either the acquisition board is not installed properly in the PC or its driver are not installed. In the latest case, repeat step 1.
4. Follow the instruction in "Install the BioCAM X" to verify that all the components are properly installed.

Verify proper functioning

Once you have installed and turned on the BioCAM X, opened and updated BrainWave X on the host PC and positioned a BioChip together with the reference electrode (see above sections), you can easily verify the proper functioning of the system by the following check list:

1. Verify that both BioCAM LED and Biochip LED icons in the BrainWave's Right Status Bar are ON (see section "BrainWave X" on page 14).
2. On BrainWave go to File > Open Recorder. On the new opening window click on the Play button to start an acquisition and then select a few channels to open their graphs (see "Visualize live data" on page 29)and verify that data is acquired from the BioChip (if you have not placed a living sample on it, you should visualize noise traces in the range of +/- 100 µVolt)

If anything of the above went wrong, please contact the customer support.

5 Quick guide to your first use

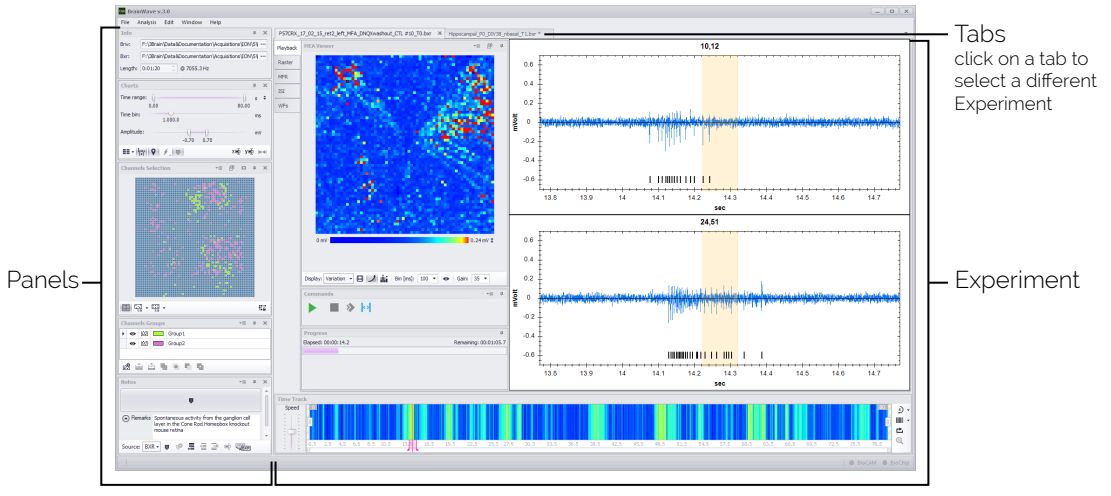
Basic concepts

Visualization of data

BrainWave X makes use of tools designed to manage high resolution data coming from CMOS-MEAs. This has been implemented by combining conventional approaches with innovative ones. In particular, since BioChips act as "cameras for extracellular electrophysiological potentials", several functionalities were introduced following similar approaches as used in multimedia.

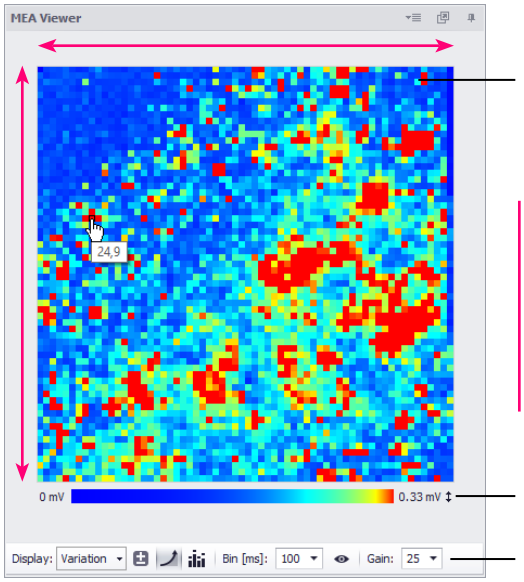
BrainWave X is an all-in-one software including tools for recording, visualizing, analyzing and exporting your data. Use of BrainWave X differs based on whether you are visualizing live data from your biological preparation or whether you are playing back data previously recorded. These two different scenario are referred as **online mode** and **offline mode** respectively.

The term **Experiment** can be used both for an online and an offline experiment. When a new Experiment is started (online) or open (offline), its data is loaded and displayed in the BrainWave X's Workspace area. More Experiments can be loaded during a single application session, in particular you can manage at the same time multiple offline Experiments and one single online Experiment. When multiple Experiments are open, they are organized in tabbed documents. When passing from an Experiment to another by clicking on its corresponding tab, BrainWave X updates all the panels with the Experiment's data.



MEA Viewer control

Electrodes integrated in the CMOS-MEA are referred in BrainWave as Channels (abbreviated Ch). A fundamental control to browse your data is the MEA Viewer



MEA Viewer
representation of the voltage values of the channels by a color map

Hover and click
to select channels. Move from top to down and from left to right to increase channel's row and column coordinates respectively. Hold Shift while clicking or drag an area to select multiple channels.

Color scale
for current settings

Settings
affecting the way the channels' voltage values are converted to colors

MEA Viewer displays the activity at the current time position by a false-color map. The map gives a representation of the activity by converting the channels' voltage values in your MEA to pixel colors. This is done according to the below color scale and settings. At a specific time location in your recorded Experiment the MEA Viewer gives an image representation of the activity. When instead you visualize live data acquired from the BioChip or when you playback recorded Experiments, the MEA Viewer refreshes continuously thus creating an activity movie.

Each square pixel in the MEA Viewer represents a channel in your MEA. Channels are identified by their row and column coordinates. By convention row coordinate comes first than column coordinate, hence for instance the channel [10, 5] is the channel at row 10 and column 5.

MEA Viewer allows you to select single or multiple channels. To select multiple channels you can either hold down Shift on your keyboard while clicking on channels or click and drag an area over the MEA Viewer.

Data formats

BrainWave X works with two main file types although it can export data in other formats. Those two own file types are BRW- and BXR-files. Both file types are using HDF5 hierarchical data format (hdfgroup.org). You can easily open them within most operating systems (including Windows, Linux and Mac OS) and most used data analysis environments, such as, among others, Python, Matlab, Scilab, Octave and R. You can find detailed documentation on both data files on 3brain.com/downloads#documentation.

BRW stands for Brainwave RaW data file and contains continuous or quasi-continuous data streamed from the BioCAM. BRW files, according to your acquisition settings, can contain for instance raw data recorded from all the BioChip MEA channels or a temporal subset of those data. In what follows you will learn to record plain raw data file.

BXR stands for Brainwave eXperiment Result data file and contains discrete data obtained by analyzing and processing the raw data. BXR files can for instance contain the timestamps of the detected spikes. In the following section you will learn to create a BXR-file starting from a BRW-file.

Conceptually, a BXR-file is created starting from a BRW-file, hence a BXR-file is associated and linked to its parent BRW-file (while it is not true that a BRW-file is linked to a BXR-file). BXR-file can be deleted and recreated starting from the BRW-file, while if a BRW-file is deleted the contained data is lost forever. However, since BRW-file are typically large files containing a huge amount of information, sometimes you might find useful to delete the BRW-file once the relevant results have been obtained and saved in a BXR-file.

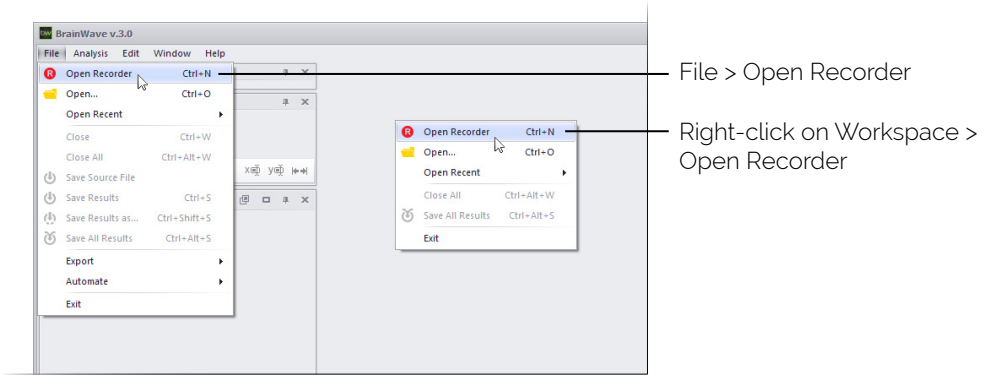
Online mode (Record mode)

Important notes

Before performing any recording it is worth to take note of how your PC is configured in order to store your data properly. If you have received a pre-configured host PC you need to identify proper drives to store data. These drives are either single high-performance drives (e.g., high-end HDD or SSD) or multiple drives (typically two) in striping configuration (also called RAID0). To locate these drives under Windows Explorer search for drives named RAID0 or LiveRec, respectively. When recording, you should always select these drives to avoid data loss. However, if you select a drive that is not fast enough and data loss is occurring you will be notified by BrainWave.

In case of a RAID0 configuration, bear in mind that this unit is backed by two physical hard disks that the OS operate in parallel to enhance speed. A RAID0 configuration should not be confused with the mirroring (RAID1) configuration that allows to copy your data on two physical hard disks thus protecting your data from loss due to hard disk failure. RAID0 configuration does not help protecting your data: a failure of any of the two underlying hard disks will cause a data loss. Hence you should always backup your important data. For details on RAID configurations check https://en.wikipedia.org/wiki/Standard_RAID_levels.

Before moving forward, follow all the steps detailed in "Install the BioCAM X" and "Plug the BioChip". Hence, make sure that the BioCAM and the BioChip are properly connected by checking that the corresponding LED icons in the BrainWave X's Right Status Bar are turned ON (blue icon).



Open recorder

Open the recorder by either

- Choosing File > Open Recorder, or
- Right-clicking on the Workspace > Open Recorder, or
- Pressing Ctrl+N

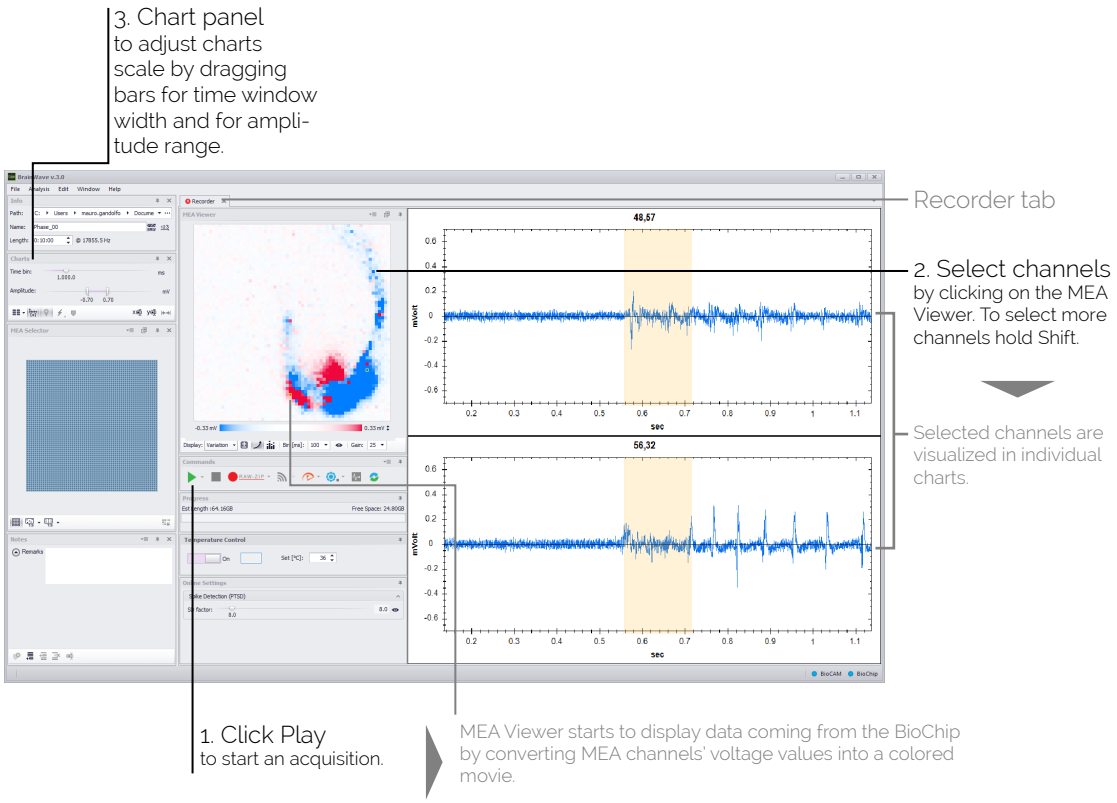
A new online Experiment opens as a tabbed document. Only one online Experiment can be open during an application session and it can be identified by the icon and the "Recorder" text on the document tab.

Visualize live data

To start an acquisition and visualizing data read out from the BioChip:

1. Click Play under the Commands panel to start the acquisition. The MEA Viewer starts refreshing and displaying data.
2. Click on the MEA Viewer to select individual channels or Shift-click to select more channels. For each selected channel a new chart is plotted on the right showing the voltage data being read out from that channel.
3. Use the Chart panel to adjust channels' charts. Two controls are available on the Chart panel for both the time (X) and amplitude (Y) axis scale values of the charts. The time control regulates the time window used by the charts. By selecting a given time window width (e.g., 500ms) the charts are refreshed by periods of the same time duration. The amplitude control allows to select the Minimum and Maximum values visible on the charts in the Y axis.

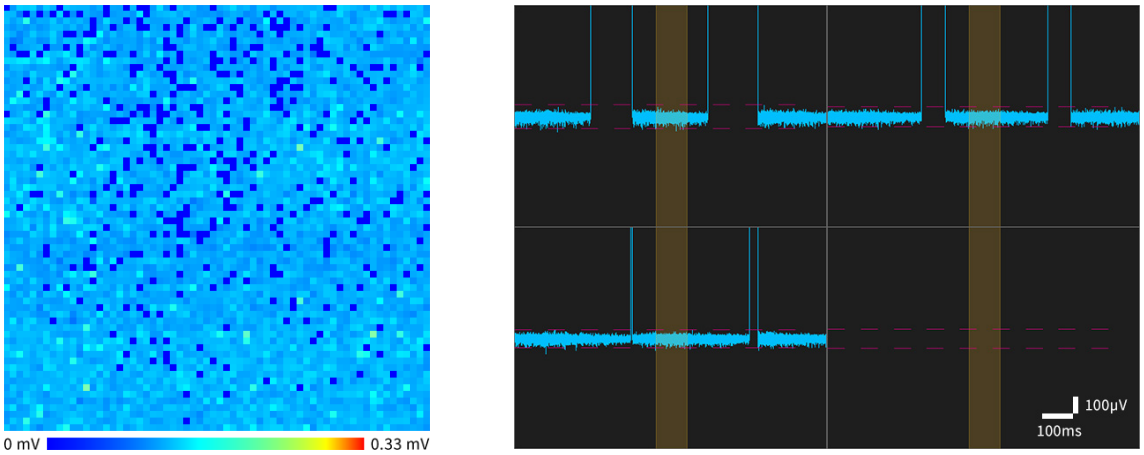
To stop the acquisition simply click on the Stop button under the Commands panel.



BioChip Calibration

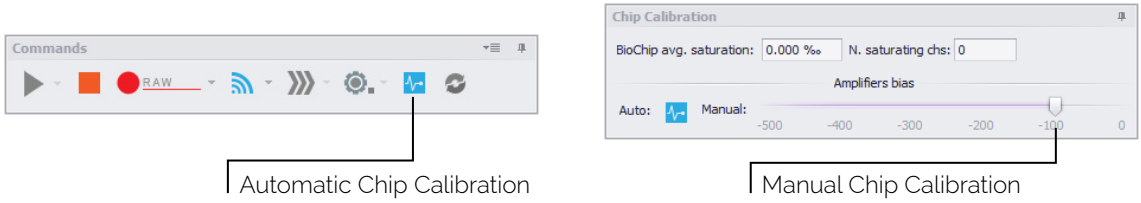
In case you experience several electrodes with short or prolonged periods of time where no signal is visible, you may suffer signal saturation problems. In this case, follow these steps before proceeding on with recording data.

A typical example of a chip with saturating behavior is illustrated in the image below. On the left, the MEA Viewer shows many deep blue colored pixels indicating not working channels while on the right typical raw data traces with short periods of saturation (top-left, top-right and bottom-left) or even with persistent saturation (bottom-right) are shown.



This behavior might be related to a wrong setting of the working point of the amplifiers that are integrated underneath the electrodes. In such case, follow the instructions below for a correct BioChip calibration; if the problem persists you can refer to the section "Electrodes with no-signal (saturation)".

BrainWave features two functions to perform BioChip calibration: an automatic and a manual procedure. The automatic calibration button is positioned in the Commands panel. To perform an automatic calibration you first need to start an acquisition (by clicking on the Play button) and then to click on the BioChip Calibration button. The procedure will take from few tens of seconds up to roughly a minute, depending on the BioChip conditions.

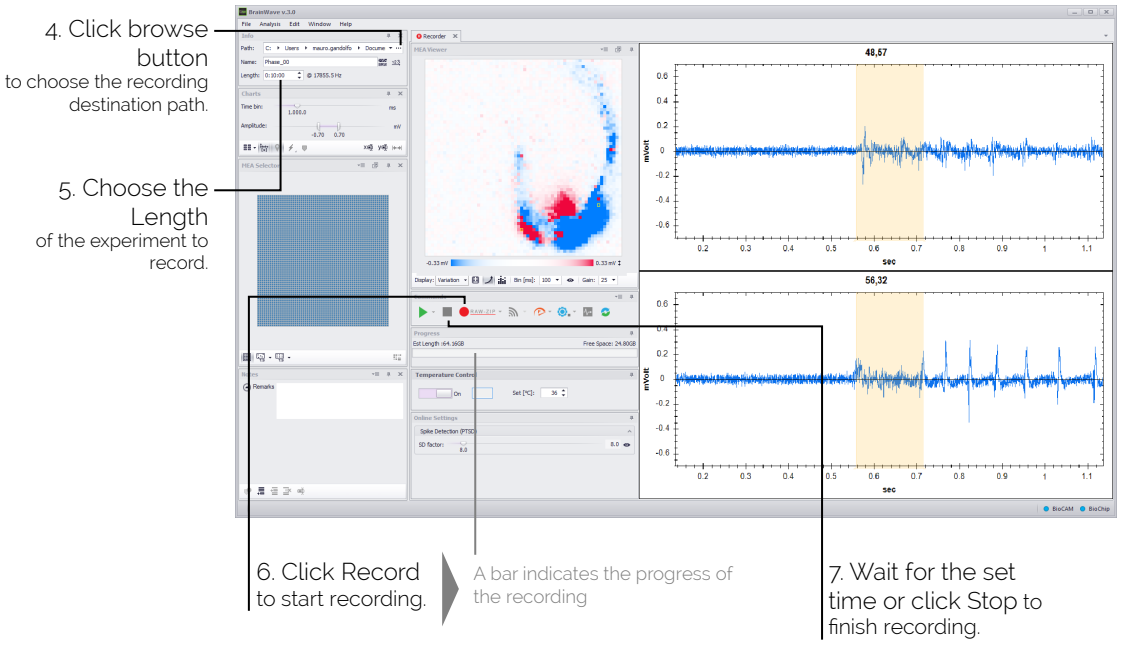


If automatic calibration is not satisfactory you can perform a manual calibration. To access this feature you need the Chip Calibration panel (if not opened, go to BrainWave menu: Window > Recorder > Chip Calibration). From the panel you can move the sliding bar to set a value for the amplifier bias that minimizes the saturation of the electrodes. Normally, value in the range of 0 to -100 allows to achieve good performances, but values out of this range might be needed too. Moving the bar toward higher negative values will eliminate saturation from those electrodes that oscillate between working condition to saturation (i.e. blinking deep blue pixels in the MEA Viewer control). If you set a too high negative value, some electrodes will start saturating permanently (i.e. fixed deep blue pixels in the MEA Viewer control). For a correct setting start from values close to 0 and move the bar by small steps towards the left until an optimal condition is found.

Record live data

Complete the steps to "Visualize live data" and then:

4. Click the browse button on the Info panel to choose the path where to save your data. Be aware that to safely record from high resolution MEA you need high performance hard disks. Hence, browse to a file located either on a high-end disk (e.g., SSD; solid-state drive) or on a disk pool configured with RAID 0 (striping). If your order included a pre-configured host PC, the disk partition where you should record your file is typically labeled with LiveRec or RAID0. Once you chose the path, by default BrainWave X selects for you the file name, but you can modify this by typing in the Name field.
5. Choose the length of the experiment you want to record by bearing in mind that the experiment will last this duration or shorter but not longer.
6. Click the Record button in the Commands panel to start recording. In the Progress panel you can see the elapsed and remaining time of the recording.
7. Wait until the set experiment length or click the Stop button at any time to finish recording. The recorded file is a BRW-file located at the position you selected in step 4.



Offline mode (Playback mode)

The tutorial contained in this section is based on the demo file on dissociated neuronal cultures you can find on http://www.3brain.com/downloads. Download that file and then extract it to a known location by using 7-Zip or WinRAR.

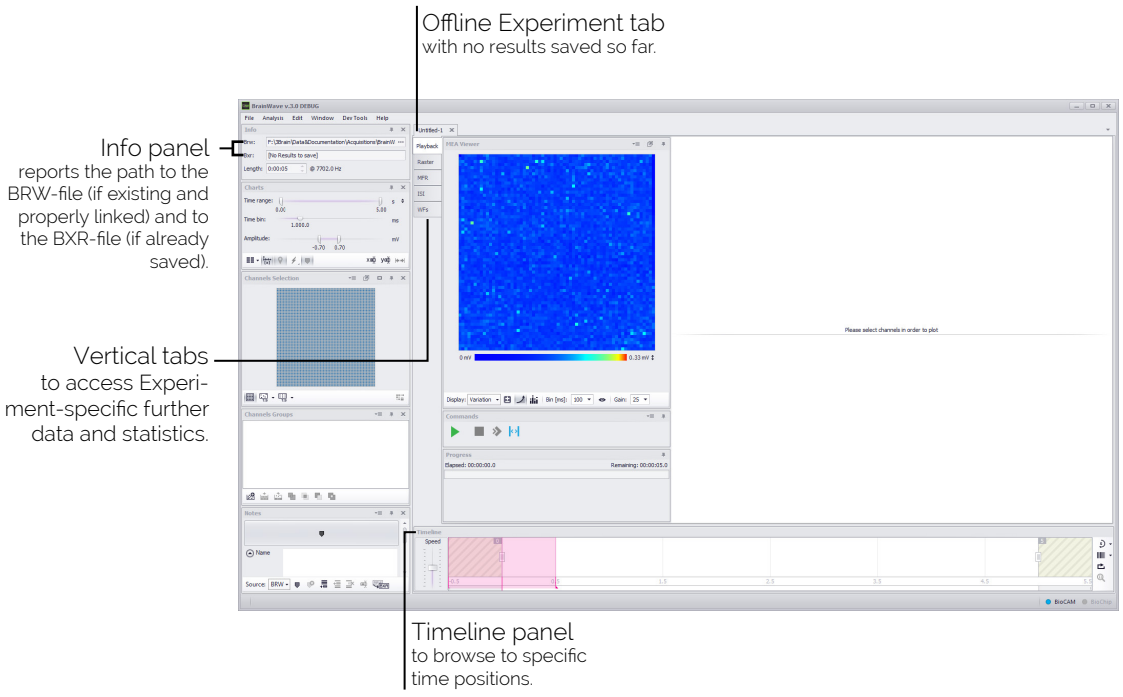
Open recorded data

Locate the demo BRW-file to open. Then to open it in BrainWave X do one of the following:

- Choose File > Open, or
- right-click on the Workspace > Open, or
- press Ctrl+O, or
- drag the file from Windows Explorer into BrainWave X, or
- double-click on the file in Windows Explorer.

A new offline Experiment opens as a tabbed document. The tab name is the name of the BXR-file associated to the BRW-file. Since you have open a BRW-file and no BXR-file has still been created the tab name will be in the form "Untitled-X", being X a progressive application session-wide number. Hence, if this is the first file you open in this session it will be named "Untitled-1".

The offline Experiment tab and the panels are similar to the online case, however some controls and functions change.



Quick analysis and save of results

As a first step, we perform a spike detection on the loaded data. Choose Analysis > Peak Detection from the Main Menu or alternatively press Ctrl+P. On the new opening window, simply leave the default parameters and click OK and then wait for the analysis to be accomplished.

Once finished, the BXR-field in the Info panel turns yellow to indicate that there are unsaved data. To save data do one of the following:

- Click on the icon right to the BXR field in the Info panel;
- Click File > Save
- Press Ctrl+S

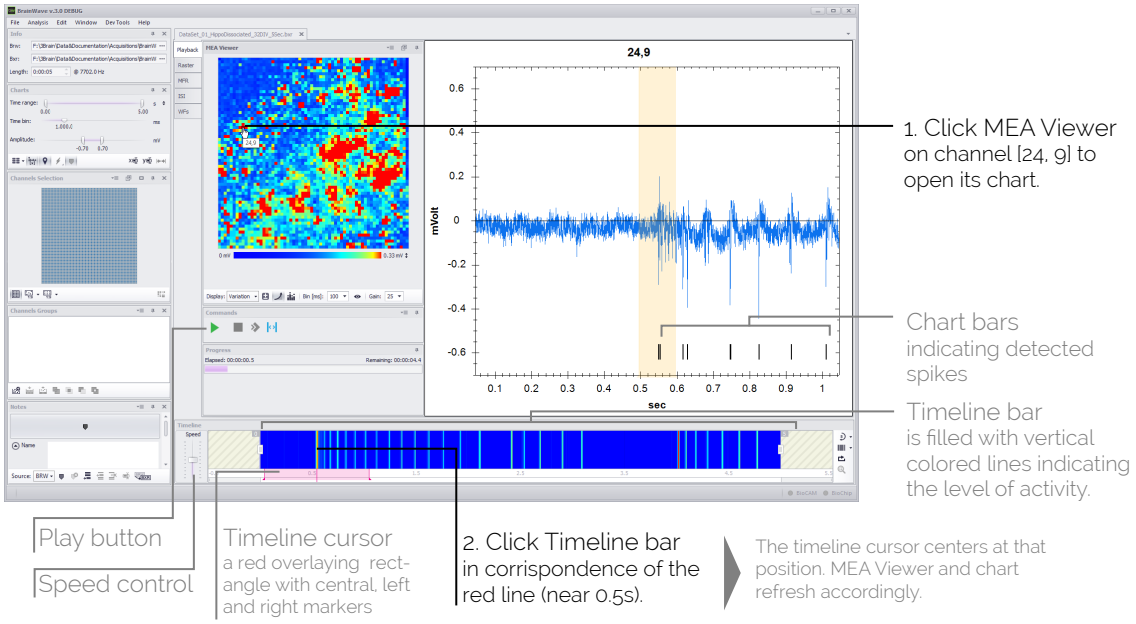
Once you have saved the data, the Experiment tab name indicates the BXR-file name and the BXR field in the Info panel updates too.

Visualize recorded data

As a consequence of the analysis, notice that the Timeline bar is now filled with vertical lines whose colors indicate the degree of the overall detected activity (averaged on the entire MEA) along the temporal axis. A blue-color indicates a relatively low activity, while a red-color a relatively high activity around that time position.

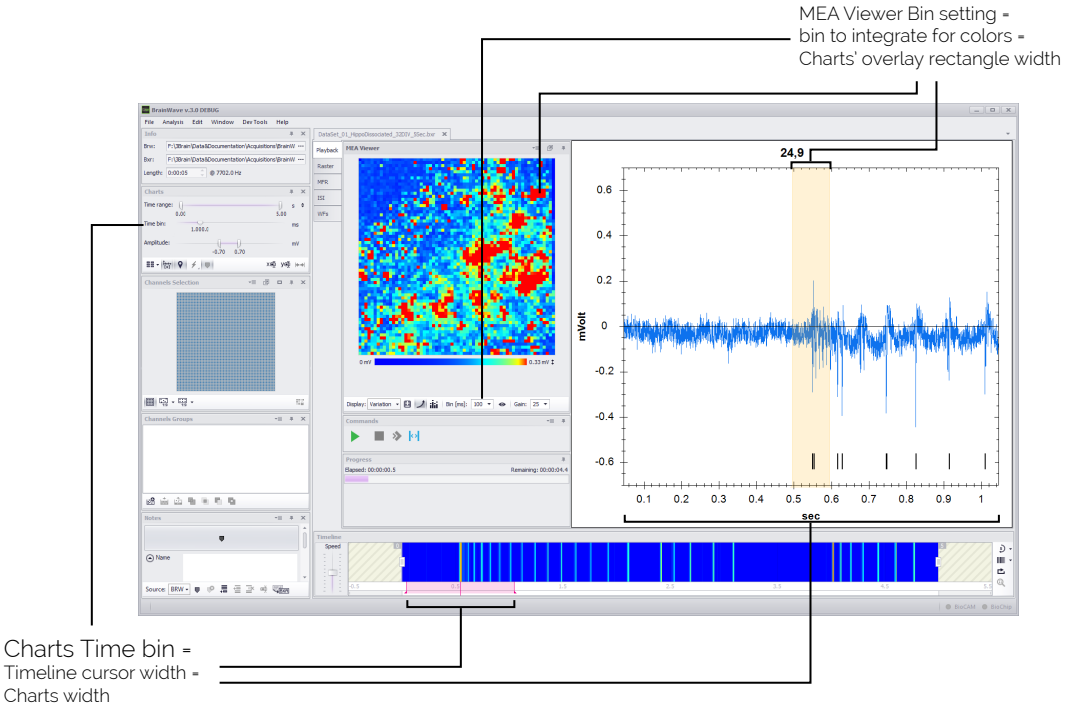
To browse your Experiment for signals:

1. Click the MEA Viewer on channel [24, 9] to open its chart
2. Click the Timeline bar in correspondence of the first red vertical line (near 0.5s). You will see the activity around the selected time position for the entire MEA in the MEA Viewer and the data for the channel [24, 9] in its chart.



Try to move to other spatial positions in the MEA by selecting other channels in the MEA Viewer (in particular red channels) and to other time positions by clicking or dragging on the Timeline bar. To move along the time axis, you can also click Play (Stop) in order to automatically playback (stop) the data stream or alternatively you can use the Left and Right Arrow keys on the keyboard. When Play is on or you use the arrow keys, the speed at which data is refreshed is given by the Speed control under the Timeline panel. Speed can also be adjusted with the Up and Down Arrow keys.

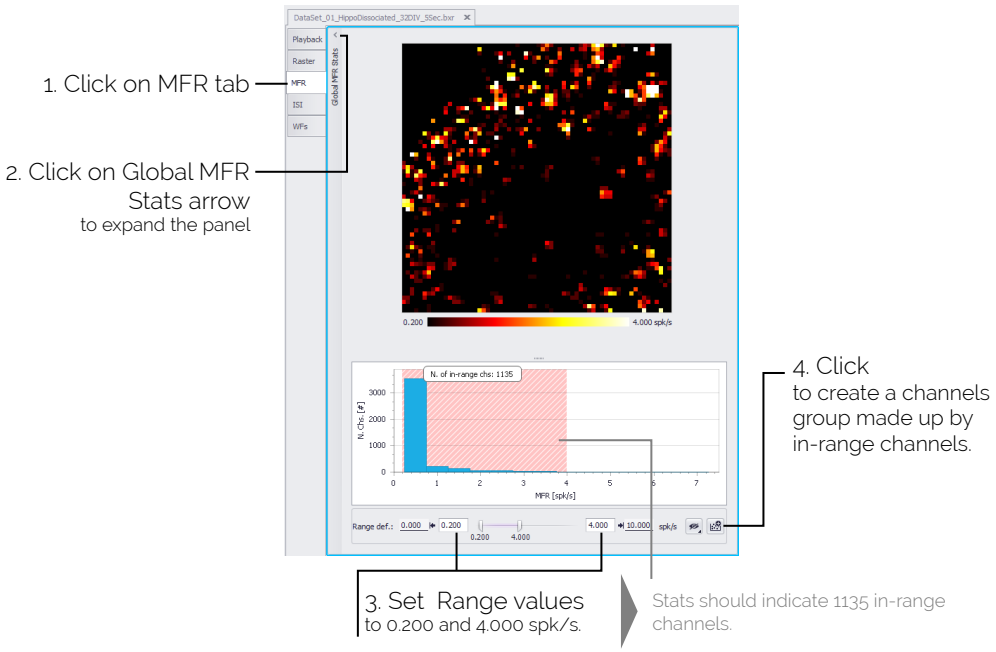
Understanding main controls affecting graphs



Create a group of relevant channels

You can create groups of channels according to your selection or a given statistical information. Here we create a group considering channels' global mea firing rate (MFR) values. Global MFR is computed for each MEA channel as the number of detected spikes divided by the length of the recording (spk/s). To create a group of most relevant and active channels:

1. Click on the MFR tab under your Experiment document.
2. Click on the arrow aside the Global MFR panel to expand the panel. The new enlarged panel contains a colored map representing the activity on the entire MEA as global MFR. For each channel the global MFR value is converted into a color according to the below color scale.
3. Set the Range definition left and right values to 0.200 and 4.000 spk/s respectively. In this way, we consider only those channels whose global MFR ranges between such values. The number of in-range channels should be 1135 with such settings.
4. Click on the button to create a new channel group and then in the new opening window click OK.



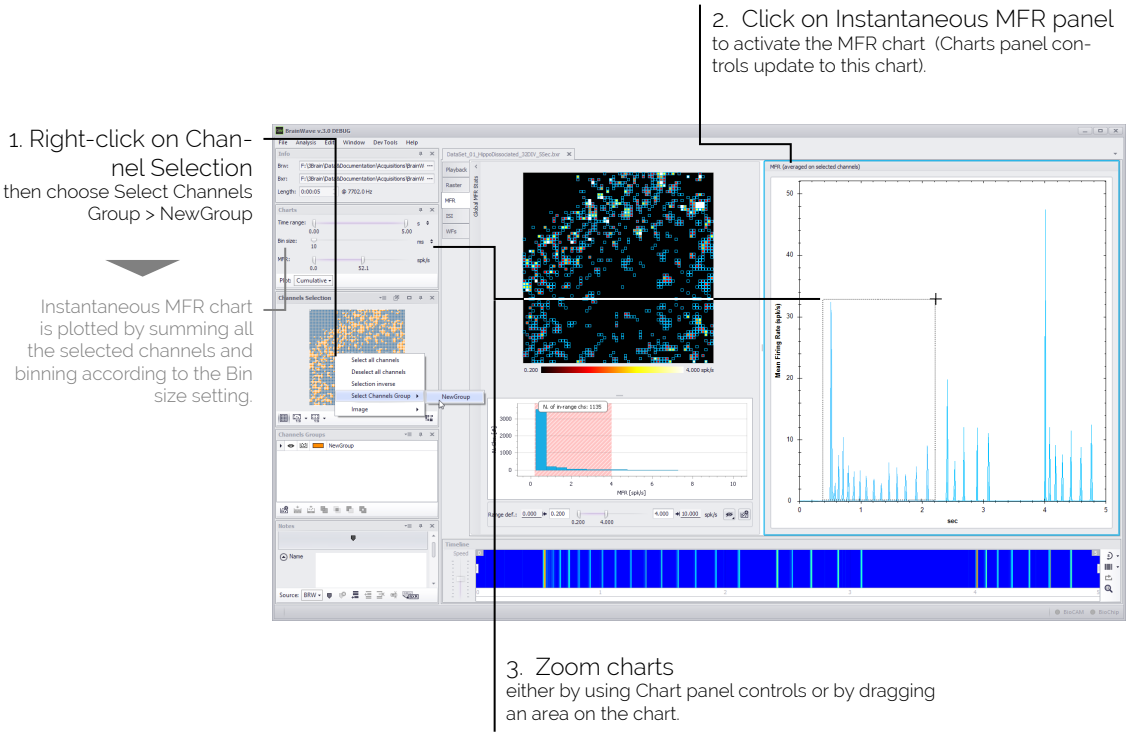
Visualize Instantaneous MFR

In addition to the Global MFR it might be useful to observe the instantaneous MFR, which gives the trend of the MFR computing it on successive small temporal bins. To plot the Time MFR:

1. Right-click on Channel Selection panel then choose Select Channels Group > NewGroup to select all the channels belonging to the newly created channels group. An instantaneous MFR chart is plotted by summing up together all the selected channels and binning according to the Bin size setting in the Chart panel.

To zoom the MFR chart:

2. Click on the Instantaneous MFR panel to activate the MFR chart. In this way the Charts panel updates to show the controls corresponding to this chart.
3. Zoom the MFR chart either by using Chart panel controls or by dragging an area of interest on the chart. If you have dragged an area on the chart, you can un-zoom with right-click on the chart and choosing Un-Zoom.



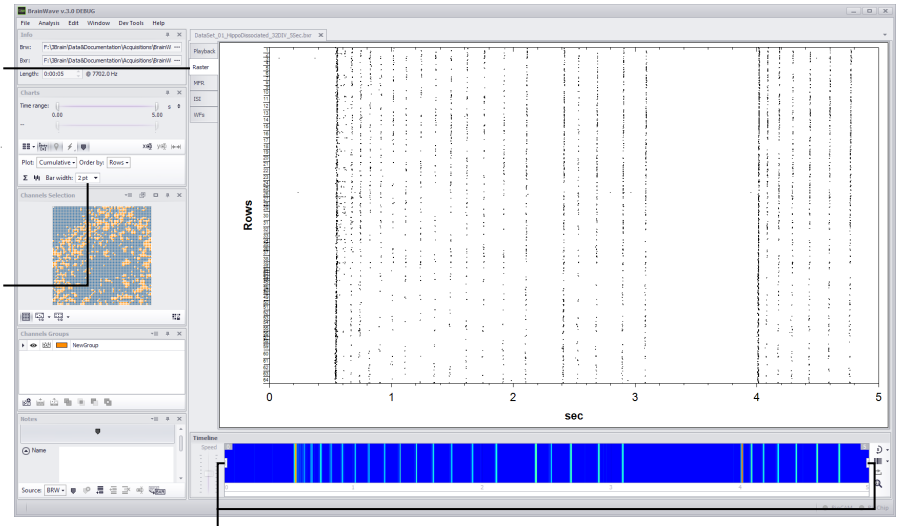
Visualize Raster

The time raster is another common tool used to observe activity on your data.

1. Click on Raster tab

A time raster chart is plotted.

2. Increase Bar width
for the raster to 2pt in order to have a more defined raster.



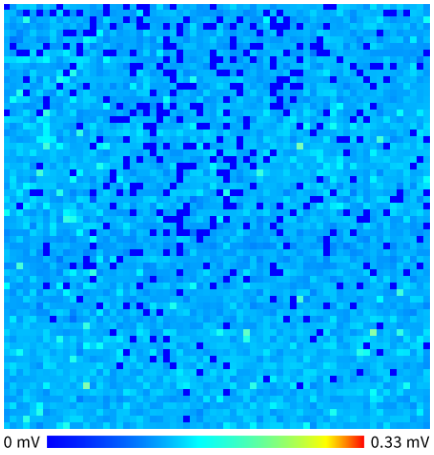
3. Zoom on time axis
by using the Timeline bar control and moving the aside handlers.

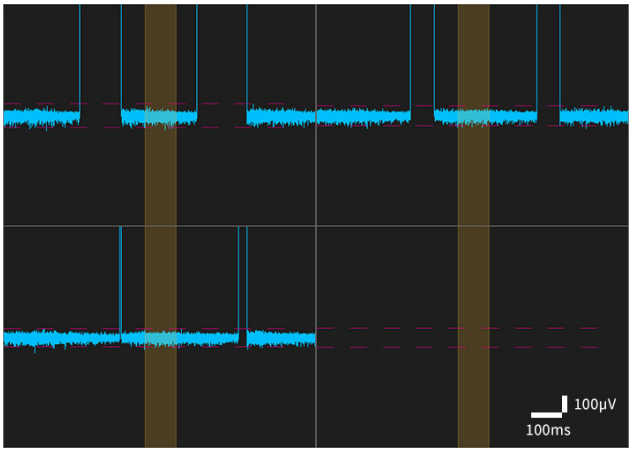
6 Troubleshooting

Electrodes with no-signal (saturation)

Symptoms

Few electrodes in the array have either persistent or short periods with no visible signal. This effect is overall called saturation and in this section we will refer to its two possible subtypes: persistent saturation (i.e. a channel is always saturated and no signal is displayed) and jumping saturation (i.e. raw signal is affected by short periods of saturation typically of tens to hundreds of milliseconds).





In the MEA Viewer control (left image) in BrainWave saturating electrodes show a lowest fixed color scale value (persistent saturation) or they blink toward the lowest scale value. The lowest color scale value is typically deep blue but may differ according to the chosen color scale. By displaying the raw traces of the single electrodes it is possible to see either "jumping" signals disappearing for short periods of time (right image: top-left, top-right and bottom-left traces) or no signals (right image: bottom-right trace).

Causes

Saturation arises (i) when the input signal amplitude exceeds the operational range of the amplifiers integrated inside the BioChip's CMOS or (ii) when the amplifiers working range is not properly set. In the first case, this could be due to invalid signal input, for instance; in the second case a wrong calibration of the BioChip could be the cause.

Possible scenarios and remedies

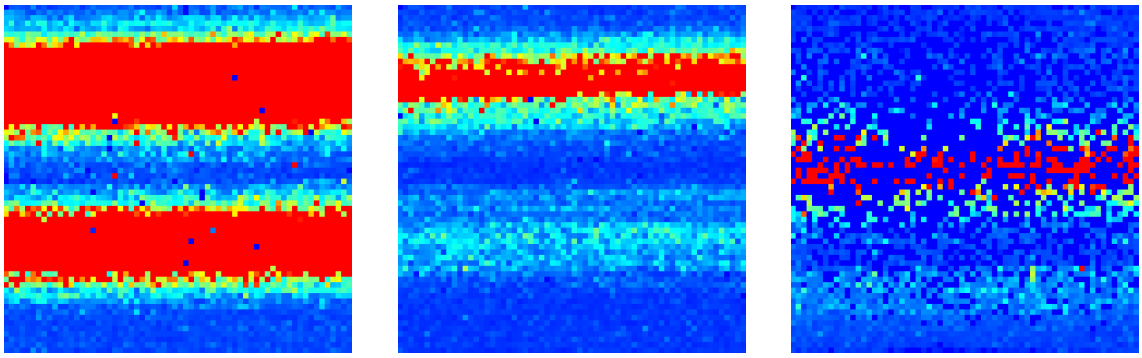
#	Scenario	Remedy
S1	Electrodes on the BioChip are insulated by tiny bubbles that form when adding a saline solution because of the hydrophobicity of the CMOS surface. Insulated electrodes exhibit persistent saturation because the input signal is missing. This scenario arises only when trying a new BioChip or a BioChip that has not been used for a long period. A 2x or 4x magnification lenses are enough to observe these bubbles.	Use a plastic pipette with a small tip to flow liquid on the CMOS surface in order to remove the bubbles. While doing so, be very careful not to touch the CMOS and to avoid electrodes damages.

#	Scenario	Remedy
S2	CMOS is exposed to too high an environmental light source and it is not properly configured to work in such conditions. In such scenario several electrodes typically exhibit jumping saturation.	Turn off the light source or cover the BioChip with a cap (as those that can be provided together with the BioCAM X system). If you cannot perform this operations, or if you need light on the BioChip (e.g. for light stimulation purposes) configure BrainWave to make the BioChip working in such conditions. If the problem is not solved, check following scenarios and remedies.
S3	Pseudo-reference electrode is dirty or it has not been cleaned for a relatively long period. Proteins and impurities can attach with time to the reference electrode and degrade the reference performances, causing few or many channels to exhibit persistent saturation.	Check section "BioCAM X handling and use" how to clean the pseudo-reference electrode.
S4	BioChip is not properly calibrated. In such scenario some channels might show persistent saturation while others suffer from jumping saturation.	Check section "BioChip Calibration" to calibrate the chip.
S5	BioChip is either damaged or worn out.	If no one of the above remedies worked you may need to change the BioChip.

BioChip not properly initialized

Symptoms

On the MEA Viewer control in BrainWave, large red horizontal stripes move up or down across the entire array.



Causes

BioChip are monolithic CMOS-MEA chips integrating active semiconductor components (i.e. transistors) underneath and around the active area (i.e. the area exposing the sensing surface of the electrodes). Some of these active components need to be correctly biased by sending configuration settings from BrainWave to the BioCam and the BioChip before starting the read out of the data. This operation is performed when starting the acquisition in BrainWave (e.g.by clicking Play or Record buttons). If the connection to the BioChip is not in place or it is compromised, the initialization of the chip will fail.

Possible scenarios and remedies

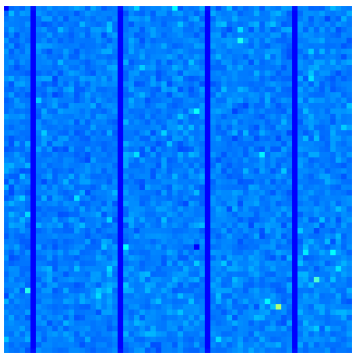
#	Scenario	Remedy
S6	BioCAM locking system (see section "Plug the BioChip") was in the unlocked state when acquisition was initiated.	Stop the acquisition, make sure that the BioChip is properly inserted till the end of its bay and push the Lock button of the BioCAM X to its end position (see section "Plug the BioChip" for more details). Make sure that the BioChip LED icon on the BrainWave's Right Status Bar (see section "BrainWave X") is on, signalling that the BioChip is properly inserted and locked. Restart the acquisition. If the BioChip LED icon on BrainWave does not turn on, see following scenarios.

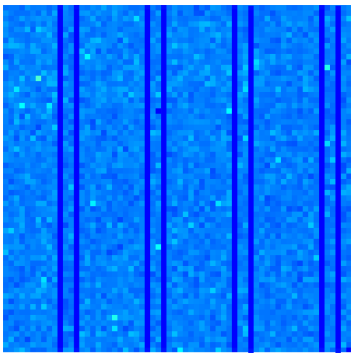
#	Scenario	Remedy
S7	BioChip contact pad is dirty or damaged.	Extract the BioChip from the BioCAM and clean all the gold contact pads on the BioChip by using a tissue soaked with pure ethanol (>96%). In case you observe some dirt on the pads you can also try to remove it by using a soft rubbe. Be careful not to damage or remove the gold layer covering the electrical contacts. Then, clean again with pure ethanol and let it dry for at least one minute. If cleaning has no effect, try one or more different BioChips to verify the problem does not lie in the BioCAM. If also other BioChips exhibit the same problem, go to next scenario.
S8	BioCAM X bridge module, which is responsible to connect the BioChip to the BioCAM, is dirty or damaged.	Take one BioCAM Contact Cleaner and follow these instructions to try to clean the BioCAM X bridge module: 1. Soak the cleaner on the marked area with pure ethanol (>96%) 2. Unlock the BioCAM locking system 3. Insert the cleaner, making sure it is inserted till the very end of the BioChip bay 4. Lock the BioCAM locking system 5. Slowly and carefully remove the cleaner moving it in parallel to the BioCAM main axis (zigzagging can damage the contact springs) while the locking system is locked down. 6. Repeat points 2 to 5 three times or until the BioCAM X bridge module is clean If cleaning has no effect, please contact customer support.

Not working columns

Symptoms

Groups of 4 columns do not work. This typically happens on 4 columns but might also involve 8 or 12 columns and in any case a multiple of 4 columns (columns are grouped by 4 in the multiplexing logic). If you see only part of a column or a single column in a non-working conditions, then the BioChip is inherently damaged at that location. In most cases it is still possible to record from the remaining working electrodes.





Causes

One or more of the lines that go from the BioChip to the BioCAM X exhibit a faulty connection. This issue typically occurs between the BioChip contact pad(s) and the BioCAM X bridge module integrated inside the mechanical locking system.

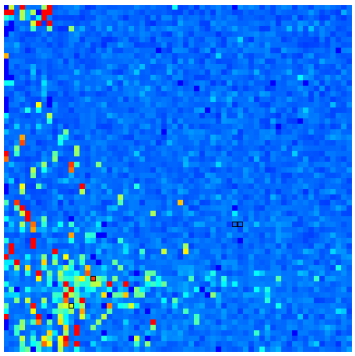
Possible scenarios and remedies

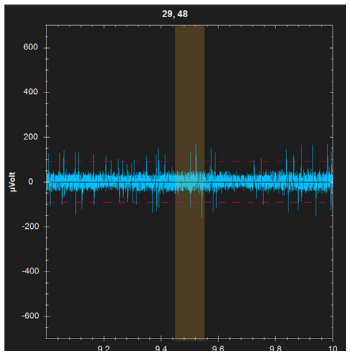
Check scenarios "S7" and "S8" in previous sections.

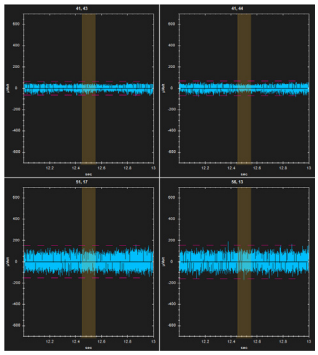
BioChip with dirty surface

Symptoms

This scenario can occur when testing a BioChip with no biological preparation. The chip might not have been properly cleaned or has not been used for a long period. In this situation, several electrodes on the array could exhibit either higher level of noise or signals with fake peaks. In Brainwave, you typically see several spots (single electrodes) with red values on the overall array (left image) . Single electrode raw traces can have either high noise levels (centre image: bottom traces are more noisy than average traces on top) or peaks at a sustained pace (right image). Most of the peaks have a shape quite different from that of a real spike, but some might still look similar.







Causes

BioChip CMOS surface is dirty, which makes some electrodes unstable.

Possible scenarios and remedies

#	Scenario	Remedy
S9	BioChip electrodes are dirty of debris and/or more likely of dust particles.	While the chip is connected and is filled with saline solution, start acquiring and visualizing incoming data with BrainWave to have a feed-back on whether the problem is solved. As a first step, try pipetting with a small plastic pipette by placing the tip roughly at 45° and very close to the CMOS central area. **Be very careful not to touch the CMOS with the tip**. If this does not help, you can also try to use a soft brush directly on the CMOS surface by gently brushing repeatedly until all the instable electrodes get recovered.