Welcome to

HyperCAM Alpha

3Brain

User Guide

Essentials

Last updated 08/05/2024

Contents

1 Introduction

Welcome to the HyperCAM Alpha platform

and thank you for exploring science with us.

The HyperCAM Alpha platform is the most advanced system for functional cell-based assays using non-invasive biosignal processing microelectronics. HyperCAM Alpha was developed to work with our revolutionary CorePlate™ multiwells technology for the integration of high-density cell-electronic interfaces and real time signal processing in a multiwell format. HyperCAM Alpha allows for parallel monitoring and execution of your experiments, increases the overall data reliability and output, speeds up time-to-results and minimizes data storage requirements through data compression.

> "A cell-electronic interface that broadens the concept of label-free functional imaging beyond optical approaches."

This manual has been written to help you take advantage of all functionalities provided by your HyperCAM Alpha.

Please note: Before first use of your HyperCAM Alpha platform, make sure to have read and well understood in particular the section "Precautions" (section 3), which contains relevant handling information to preserve your CorePlate™ multiwells and HyperCAM Alpha from possible damages.

A biosignal processing microchip at the heart of our technology

Cell-electronic interface technologies measure electrical activity with high signal-to-noise ratio from electrogenic tissues including neuronal cultures, brain organoids, neuronal spheroids, acute brain slices or cardiac preparations.

The first basic cell-electronic interfaces like patch clamps or electrode arrays were developed in the early 70'. These electrodes are routed out to contact pads on the periphery of the interface area where analog discrete amplifiers are connected for signal digitization and processing on an electrophysiological device. The sterically occupancy of the discrete electronics, and the routing out of the electrodes to the periphery of the measurement device pose severe limits in the number and density of the electrodes and thus resolution and power of these basic cell-electronic interfaces.

> “Our bidirectional pixel array technology empowers researchers to communicate with cells directly through targeted sending and reading of biosignals in real-time.”

To overcome these limitations, 3Brain applied complementary metal–oxide–semiconductor technology (CMOS) in the development of our biosignal processing chips, building on knowledge from microelectronic devices (e.g., computer microprocessors) and light-imaging devices (e.g., camera sensors). Our bidirectional pixel array technology empowers researchers to communicate with cells directly through targeted sending and reading of biosignals in real-time. This microelectronic approach allows for circuit integration, thus reducing the dimensionality of electronic components as amplifiers and filter condensers. Furthermore, by implementing various multiplexing strategies we could reduce the number of wires needed to carry out the biosignals. Post-processing methods were also used to optimize the electrode performances. Our biosignal processing microchips are based on a proprietary Active Pixel Sensor array technology and feature a 64x64 electrode grid (in-pixel circuits, each pixel with a 25x25 µm2 sensing area), spaced at 35 µm, allowing for the rapid acquisition of biosignals at high resolution. In practice, this means that the real-time monitoring of electrical activity in hundreds of cells will generate up to 260Mbyte/s of recording data with high precision and accuracy to assist scientific inquiry. This incredible amount of information is further processed by our purpose-made acquisition software to generate movies of the electrical activity and to extract the relevant results.

To summarize, the HyperCAM Alpha platform is an all-in-one system which allows the conceptual equivalent of ‘non-invasive functional imaging’ of electroactive cells for the first time.

The CorePlate™ technology for parallelized assays

With the HyperCAM Alpha platform, 3Brain also introduces **CorePlate™**, our patented technology for cell-based assays which replaces standard plastic multiwells plates with intelligent devices.

CorePlate™ multiwells integrate thousands of sensing and actuating elements for each well providing massive data readout in a parallel form factor allowing to monitor network activity from several samples at the same time. CorePlate™ technology combines our many years’ experience on CMOS and functional imaging with novel concepts for on-chip signal pre-processing and compression to deliver experimental parallelization without giving up data quality.

The CorePlate™ powered 6-well plate integrates 4096 bidirectional electrodes for each chip in a well. Record up to 2304 electrodes at 10 kHz or 1024 electrodes at 20 kHz simultaneously per well. Different recording subsets are available to either maximize the recorded area or the spatial resolution, and to suit both cell cultures and brain tissues. Any of the 4096 electrodes per well, or combinations of multiple electrodes, can be used to release an electrical stimulation to your sample.

> “Drug screening and biomarker discovery was never so easy, even for first-time users.”

Our multi-site, non-invasive approach supports the development of kinetic assays, phenotypic or compound screening approaches or the monitoring of population activity of the biological sample with single cell resolution. Furthermore, the metallic electrodes allow for both sensing and actuation by releasing current pulses to stimulate cells in a targeted fashion, thus establishing a bi-directional interface to the cellular network.

All our products are developed and built with experimental scientists for experimental scientists.

With the assistance of our custom-build BrainWave software, performing, recording and analyzing experiments with the HyperCAM Alpha platform is simple and intuitive even for first-time users.

Doing great science is difficult.

We work hard to make your functional assays easy.

2

At a glance

Overview

The HyperCAM Alpha is an advanced measurement device utilizing **CorePlate™**. The system is able to manage 6-wells CorePlate™ and allows for incubator-like prolonged cell culture using an environmental chamber to control temperature and CO_2 concentration and monitor humidity.

The HyperCAM Alpha can also operate with biological models requiring an external fluidic system (e.g., brain slices) giving you maximum flexibility in your assays. A convenient LCD touch-screen will provide you all the main functionality to manage the HyperCAM Alpha and the USB 3.1 communication will allow fast and reliable data transfer to the host computer.

The HyperCAM Alpha has been optimized by our scientists to suit the need of researchers. No matter if your biological models are primarily neuronal cultures, human-derived stem cell preparations, brain organoids or spheroids, cardiac cells or brain tissues as slices and retinas, the optimized electronics of the HyperCAM Alpha will be able to detect both slow oscillations (field potentials) as well as spiking activity with unprecedented sensitivity.

The platform is also capable of releasing targeted electrical stimulation from each electrode, allowing for the development, utilization and analysis of evoked activity assays.

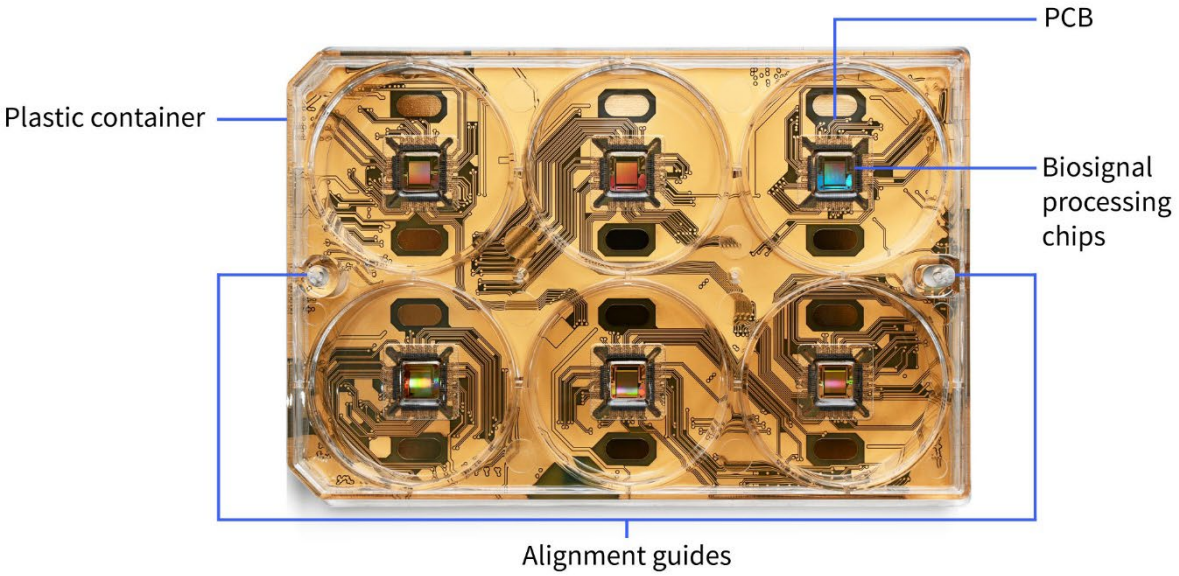
Overall, the platform consists of three basic components:

1) A **CorePlate™ powered 6-well plate** incorporating a Biosignal processing chip for each well;
2) The **HyperCAM Alpha acquisition hardware**, which reads out electrophysiological signals from the 6-well CorePlate™, and provides electrical stimulation through the electrodes.

3) A **high-performance host PC, pre-installed with BrainWave software** to acquire, visualize, store and analyze the data recorded by the HyperCAM Alpha.

6-well CorePlate™

The 6-well CorePlate™ has the same dimensions of a classical multiwell plate and follows the footprint dimensions of the standard set by the ANSI/SLAS Microplate Standards Advisory Committee for 96-well devices1. The six wells are arranged in a 3x2 configuration and each of them integrates a high resolution Biosignal processing chip in its center:



- **Biosignal processing chips**: integrates thousands of active electrodes for high-resolution electrophysiological recordings from cells or tissues placed on its active area. This chip is positioned in the center of each well.
- **Plastic container**: a biocompatible plastic frame protecting the underlying electronics and housing 6 separated wells to contain the bath solution used for the biological preparation. The volume of solution that can be contained for each well is about 13 mL. The internal diameter of each well is about 35 mm, with an internal height of about 14 mm.
- **Contact pads**: gold-plated pads for reliable connection between the 6-well CorePlate™ and the HyperCAM Alpha. Properly cleaned and non-oxidized pads are needed for a stable connection.
- **PCB**: the printed circuit board substrate housing the 6 HD-MEA chips. This multilayered component handles simultaneous acquisition and data transmission from all the chips to the HyperCAM Alpha.
- **Alignment guides**: two holes at the bottom of the PCB align the 6-well CorePlate™ with the connecting socket in the environmental chamber of the HyperCAM Alpha.
- **Plastic Lid**: fabricated with the same biocompatible plastic as the container, it has been designed to maximize CO_2 and oxygen exchange guaranteeing sterile conditions in the incubator and during the measurement with the HyperCAM Alpha. It can undergo the same sterilization process as the 6-well CorePlate™.

HyperCAM Alpha

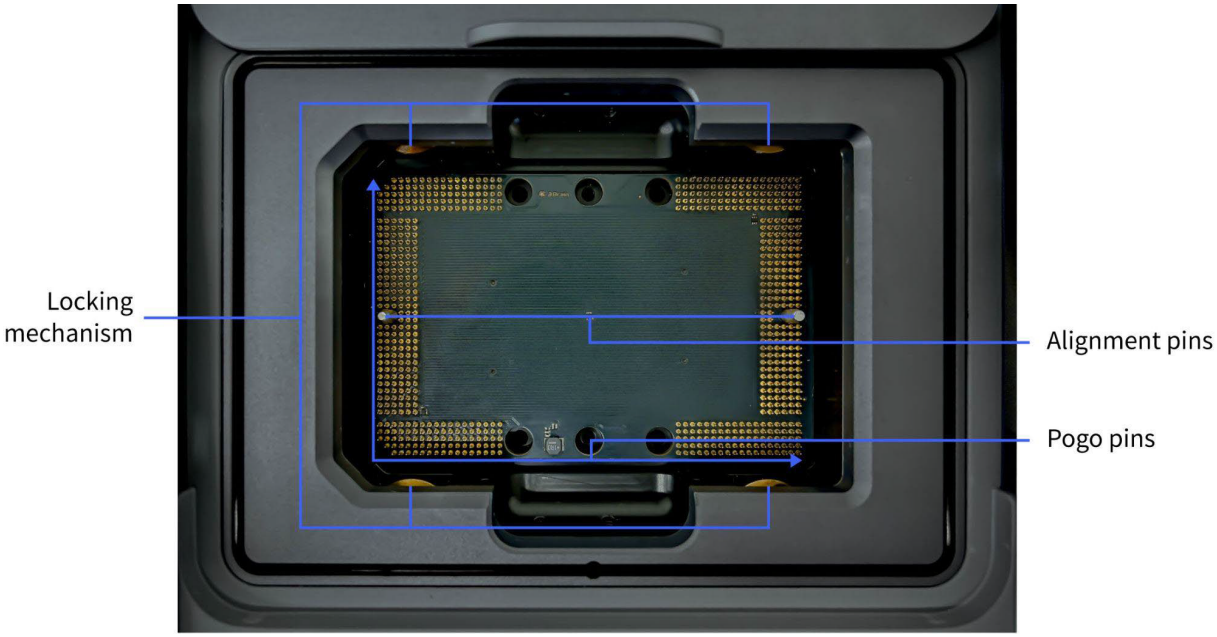
The HyperCAM Alpha is the station where the 6-well CorePlates™ are inserted for electrophysiological measurements. Its compact design integrates all the electronics, real-time hardware and logic to control the Biosignal processing chips of the 6-well CorePlate™. The HyperCAM Alpha acquires and pre-processes large volumes of electrophysiological data coming from the 6-well CorePlate™ before sending it to the host PC. The platform is also equipped with a mass flow controller that regulates gas concentrations in the environmental chamber and a heating system to maintain physiological cell culture conditions in the 6-well CorePlate™.

- **Power button**: push-button to power ON or OFF the HyperCAM Alpha (*the instrument needs to be connected to a power line using a power cord on the rear of the system and the master switch (refer to rear image of Alpha) needs to be set to the ON position*).
- **Sliding hatch**: allows closing of the 6-well CorePlate™ environmental chamber to control temperature and maintain constant CO_2 and humidity levels. The sliding hatch can be maintained open to guarantee accessibility to the 6-well CorePlate™ if the experimental setup doesn't necessitate CO_2 and humidity control and requires external components (e.g a fluidic system for brain slices).

The hatch can be controlled by a button on the LCD touchscreen, manually or by the BrainWave software.

Do not insert fingers or objects into the moving parts while operating the sliding hatch.

- **Environmental chamber**: fits perfectly the 6-well CorePlate™ shape and allows only one insertion direction to avoid user mistakes. The chamber is water-proof to prevent accidental spill out from damaging the underlying electronics. However, if a spill happens, the procedure detailed at section P.16 should be followed. The bottom of the chamber is equipped with a heating system that monitors the temperature measured by on-chip sensors on the 6-well CorePlate™. A set of gas inlets distributed homogeneously around the chamber are connected with an internal mass flow controller that allows adjustments to airflows with desired CO_2 concentration.

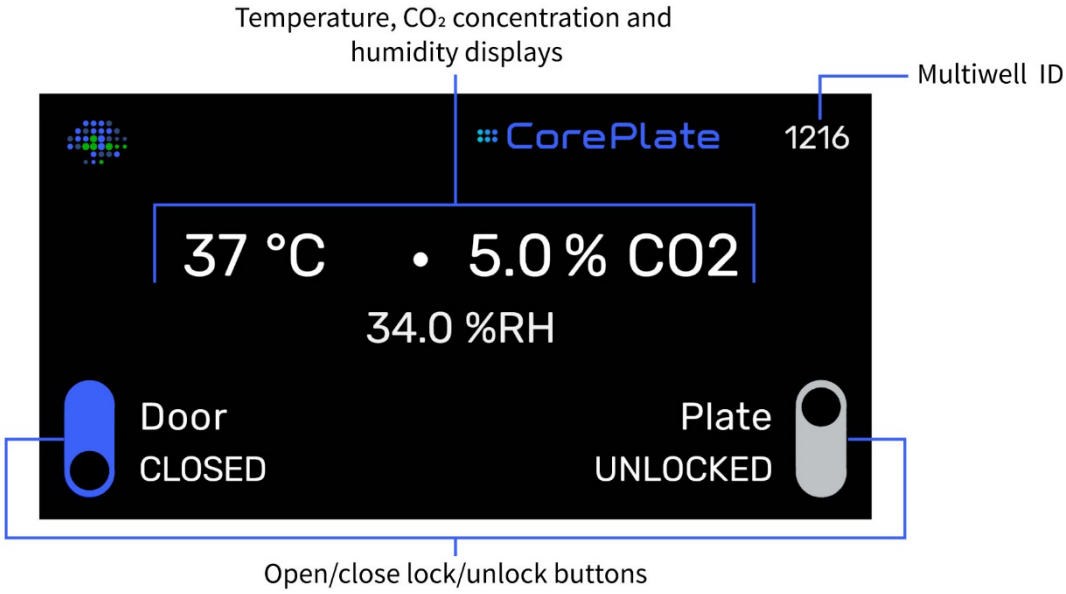


  - **Pogo pins**: these gold spring connectors on the HyperCAM Alpha guarantee a uniform contact with the gold pads on the back of the 6-well CorePlate™. Tested for being fully working after more than 5.000 test cycles without any signs of wear or tear, they maintain stable contact and long-term usability with the 6-well CorePlates™.
    Please note: The contact pads of the 6-well CorePlates™ require proper cleaning for optimal working conditions.
  - **Alignment pins**: paired with the holes on the back of the 6-well CorePlate™, these guides will allow a perfect alignment of the pogo pins with the contact pads of the 6-well CorePlates™.
  - **Locking mechanism**: four rotating metallic wings will lock and push down the 6-well CorePlate™ to the base of the environmental chamber. This uniform push has been set to guarantee enough pressure to

Do not insert fingers or objects into the moving parts while operating the sliding hatch.

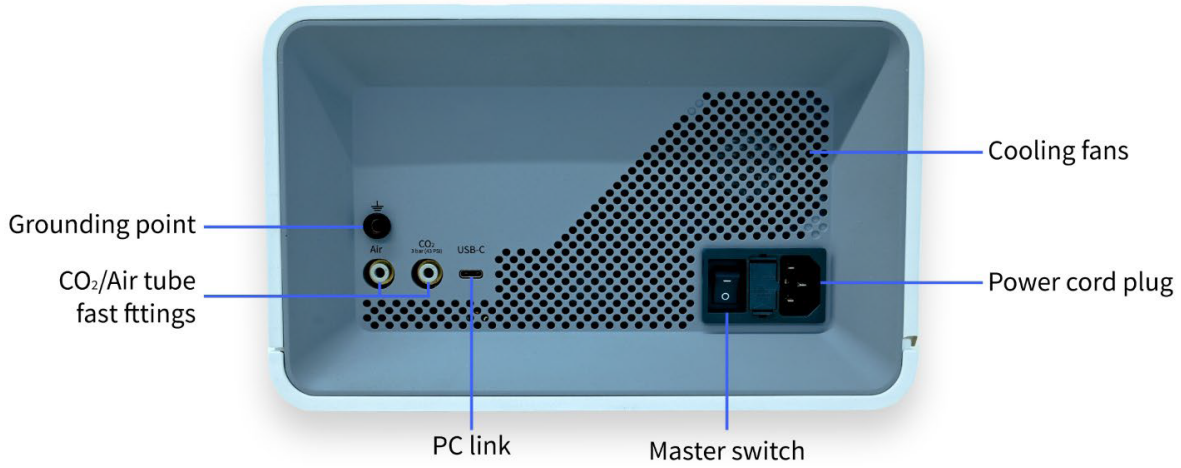
contact all the pogo pins simultaneously. The opening and closing of the locking mechanism are controlled by a button on the LCD touchscreen or through BrainWave.

- **Heating system**: balances the heat created by the processing chips in the environmental chamber to maintain physiological cell culture conditions together with the cooling fans. Temperature is monitored by sensors on the 6-well CorePlate™ with an accuracy of +/- 0.5°C.
- **Gas exchange:** The environmental chamber is flooded with a customizable gas mixture creating a pressurized environment that maintains a constant CO_2 concentration in the chamber with the help of a mass flow controller. An infrared CO_2 sensor regulates how the mass flow controller will mix air and CO_2 to maintain the desired CO_2 concentration. HEPA filters can be applied externally to the air supply tube to filter dust and other contaminants.



- **LCD touchscreen:** this convenient screen summarizes all environmental control parameters and is fitted with buttons to operate the HyperCAM Alpha
  - **Multiwell ID:** a unique ID identifying the 6-well CorePlate™, which is read by the HyperCAM Alpha and displayed on the LCD and on BrainWave.
  - **Open/close lock/unlock buttons:** press to open or close the hatch and to lock or unlock the 6-well CorePlate™ in the environmental chamber.

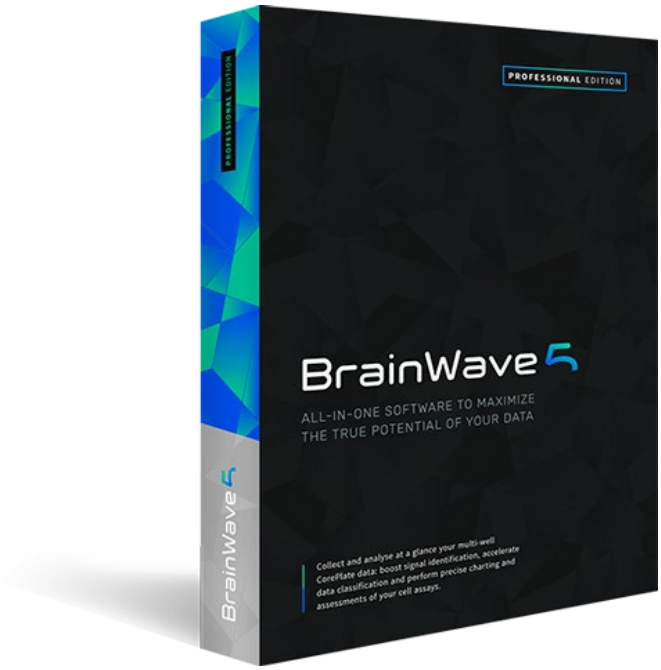
- **Temperature, CO_2 concentration and humidity displays:** the three parameters can be monitored in real time for optimal experimental conditions. By pushing on the CO_2 display it will be possible to set the desired concentration (range 0%-5%, automatically set to physiological 5%). Temperature is not actively regulated but is set to maintain physiological culture (range 34,5-37,5 °C).



- **Cooling fans:** release excess heat generated from the processing core of HyperCAM Alpha.
- **PC link:** the UCB-C provides a connection for streaming the electrophysiological data from the HyperCAM Alpha to the host PC. It is also used by BrainWave to provide HyperCAM Alpha setting commands and firmware updates and to read information from the 6-well CorePlate™. **Only USB 3.1 supporting at least 10 Gbps should be used.**
- **Power cord plug:** plug for the power supply cable.
- **Master switch:** the general switch to power ON/OFF all the electronic and mechanical components of the HyperCAM Alpha.
- **CO_2/Air tube fast fittings:** tube connectors to plug two lines, one for the air (left) and one for the CO_2 (right). The CO_2 line requires a gas supply pressure between 0.8 bar and 1.5 bar relative to atmospheric pressure. **It is crucial not to exceed 3 bar above atmospheric pressure to prevent damage to the internal mass flow controller of the HyperCAM Alpha.** For the air line, atmospheric pressure is sufficient, and the maximum pressure should not surpass 0.5 bar relative to atmospheric pressure. Additionally, **it is essential to incorporate a standard gas inlet HEPA in the air line** to safeguard against the ingress of dust and other contaminants.

⚠ **Warning :** Always use a regulator approved for the specific gas with high- and low-pressure gauges.

BrainWave



BrainWave is our custom made an **all-in-one software** application developed to exploit all the functionalities of your HyperCAM Alpha platform (Alpha requires BrainWave 5.0 or higher). It is equipped with advanced graphic and AI-driven computational tools to manage the electrophysiological data acquired from the 6-well CorePlate™, comprising real time visualization of all the wells simultaneously, online analysis and offline reprocessing to extract statistics and make publication-ready figures.

Brainwave software is preinstalled on your PC, for guidance please refer to the BrainWave user manual.

3 Precautions

HyperCAM Alpha handling and use

The Hypercam Alpha electronic and mechanical components are encapsulated in a plastic case with a solid metallic core that makes the system robust to mechanical and vibrational noise. Even if it is recommended, setting up the HyperCAM Alpha on an anti-vibration table is not mandatory.

Moreover, the instrument has been designed to be shielded from external electrical environmental noise. However, for certain types of applications (e.g. reading slow electrophysiological oscillations) and if the environment is affected by electromagnetic radiation,the system should be operated inside a Faraday Cage (see “Troubleshooting” Section 5, Scenario S7).

In any case and to guarantee optimal recording performances these simple precautions should be followed:

- Position the HyperCAM Alpha far from potential strong noise sources, such as fridges, incubators etc… Indeed, if not well shielded these instruments might generate strong electromagnetic noise.
- Connect the HyperCAM Alpha and the host PC to adequate power lines (110 or 230 V) with low noise levels. It is recommended to plug the system, the PC case and the PC monitor to a single power strip that is then plugged to a wall socket (110 or 230V).
- Take care in avoiding that the electrical cables connected to the HyperCAM Alpha (in particular, the one for the power supply and for the PC Link) do not pass near power supply cables used for other instruments.
- Check that the cable connectors are well inserted into the HyperCAM Alpha and the host PC before operating the platform. To avoid potential shortcuts, turn OFF the main switch on the rear side of the system whenever you have to plug and unplug the power cord of the HyperCAM Alpha.

To avoid potential shortcuts, turn OFF the main switch on the rear side of the system whenever you have to plug and unplug the power cord of the HyperCAM Alpha.

Leave adequate space around the system to guarantee good ventilation, in particular next to the cooling fan on the rear. Position the system on a flat rigid table to provide airflow at the bottom of the system. **Important: Do not keep the HyperCAM Alpha close to other instruments that can generate heat; the system cannot work properly with an environmental temperature higher than 25°C (77°F).**

The HyperCAM Alpha allows for the integration of a perfusion system as it is needed for maintaining tissues during electrophysiological measures. Liquid spill outs of electrophysiological solutions should be strictly avoided. The environmental chamber was designed to be water-proof to prevent liquids from entering into contact with the internal electronics, but when a current is running, liquids can still oxidize on top the golden pogo pin connectors, forming residues.

If there is a liquid spill into the chamber, please proceed to the following steps:

- Immediately unlock and remove the 6-well CorePlate™ and turn OFF the main switch of the HyperCAM Alpha.
- Accurately clean the environmental chamber of the HyperCAM Alpha with ethanol >96% and let it dry. In particular, clean the pogo pin connectors to be sure to remove saline solution and eventual oxidation residuals. Use the same procedure to clean the 6-well CorePlate™, keeping particular attention to the contact pads on the bottom of the plate. Make sure that all the external surfaces of the multiwell are well dried and clean.
- After cleaning and drying, the machine is ready to use again. However, if there are doubts that some liquid could have penetrated into the HyperCAM Alpha, not just the chamber, do not switch ON the system for at least one day, allowing the liquid to be completely dried and preserving internal circuitry from potential oxidative and short-circuit effects.

6-well CorePlate™ handling and use

6-well CorePlate™ are low-power active electronic circuits. As a general rule only the wells can be wet while the rest of the plate should stay dry. It is highly recommended to avoid the following operations that might cause damage to their electronics:

- Do not touch the contact pads on the back of the device and do not place the device in direct contact with a metallic worktop. **Use a plastic tray below the 6-well** CorePlate™ **when in the incubator or under the hood**. Electrostatic charges might damage the on-chip circuits and might result in damaging the device. Always handle the 6-well CorePlate™ by holding it on the lateral sides and ideally by using plastic gloves.
- Do not touch the active area of the CMOS chips integrated in the 6-well CorePlate™ with any tools except those specifically allowed (see “Cleaning procedure for 6-well CorePlate™” (section 3.3). 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, do not insert the entire 6-well CorePlate™ in water or ethanol. Immersion in water might cause oxidation of the contact pads and liquid entering in between the wells in contact with the internal electronics. In ethanol, some parts of the 6-well CorePlate™ might have deteriorated.
- **Do not place the 6-well CorePlate™ in autoclaves**. For the 6-well CorePlate™ sterilization, please refer to the “Sterilization procedure for 6-wellCorePlate™” (section 3.4).
- Maintain the pH of the electrophysiological solutions used for neuronal cultures or brain tissues, possibly at physiological conditions (pH 7-7.5). Strong changes in the pH of the solution might damage the electrodes.

During recordings for both cultured cells or acute tissue measurements users have to comply with the following precautions:

- Before inserting the 6-well CorePlate™ into the HyperCAM Alpha, clean the metal contact pads on the bottom of the device with a tissue paper soaked with ethanol 96% and let it dry for a few seconds.
- Avoid liquids from spilling out and strictly avoid liquids from entering into contact with the pads on the bottom of the plate. In case users experience liquid spill out, follow instructions provided in the “HyperCAM Alpha handling and use” (section 3.1).

In order to ensure a good quality of the recordings please remember that 6-well CorePlates™ are light-sensitive devices and their performances might be affected by high light intensity direct exposure or by noisy light sources, see “Troubleshooting” (section 5 Scenario S2).

Cleaning procedure for 6-well CorePlate™

6-well CorePlate™ are warranted as single-use plates for cell culturing and they are shipped already cleaned, sterilized and ready to be used, so you don't have to perform any cleaning operation before use.

However, depending on the experimental setup, it may be needed to clean the plate before use. During each step of the cleaning procedure, please Make sure the pipette tip (or any other object) does not directly touch or scratch the embedded microchip surface. To perform standard cleaning, please follow the indications below:

- Rinse each well of the 6-well CorePlate™ abundantly with Double Deionized Water (ddH2O)
- Fill each well with a detergent such as WPI-Enzol (WPI) or Terg-A-zyme (Alconox) and gently pipette for a few seconds.
- Leave the detergent for a few minutes (typically 3-5 min) and then pipette again.
- Rinse each well of the 6-well CorePlate™ abundantly with ddH2O, then leave each well filled with ddH2O for 1-2 minutes and repeat this operation 3-4 times in order to be assured of washing out the detergent completely.

While cleaning the 6-well CorePlate™, it is highly recommended not to wet the contact pads under the plate.

Intense cleaning

In case the chips of the 6-well CorePlate™ are very dirty, you can mechanically remove residues with a soft brush. Be aware that this operation can damage the sensing area of the chip. This step is only suggested if, after a normal cleaning procedure, the chip is not clean. Any brushing has to be performed very mildly and carefully.

Finishing and storage

6-well CorePlate™ can be dried by using a gentle flux of Nitrogen air (do not expose the recording area to an intense flux to avoid potential electrode damages). Alternatively, let the 6-well CorePlate™ dry on a bench. Make sure to put the plate cover with the lid slightly open to avoid dust deposition inside the wells.

The area out of the wells of the 6-well CorePlate™ can be cleaned with a tissue soaked in ethanol >96%. It's a good practice to always wear gloves while handling the plate.

Once the 6-well CorePlates™ are dried they should be stored closed (with the lid on) in a box in order to protect them from dust and other contaminants.

Sterilization procedure for 6-well CorePlate™

As already indicated in the previous section, the 6-well CorePlate™ are single-use plates for cell culturing and they are shipped already cleaned and ready-to-use. However, in case you need to sterilize them, please follow one of the procedures below. Make sure to not directly touch or scratch the embedded microchip surface.

1) UV-light sterilization:
    - Expose the 6-well CorePlate™ with the lid open to UVC-light for 45 min.
    - Ensure that the light is well illuminating all the 6 wells in a homogeneous way, preferably the light should come from the top to guarantee a strong and direct UV-illumination of each well.

    Exposure time can vary depending on the effective UV-light intensity and power. The same procedure applies to sterilize the lid of the 6-well CorePlate™.

2) EtOH Sterilization:
    - Fill each well of the 6-well CorePlate™ completely with ethanol 70% up to the border. Also dip the area in between the wells with EtOH. Be sure all the edges are wet with EtOH.
    - Wait for 25 minutes.
    - Suck the ethanol with a pipette or with a vacuum pump, always using sterile tips.
    - Fill 3/4 of each well of the 6-well CorePlate™ with sterile ddH2O, wait a few seconds and suck the liquid with sterile tips. Repeat these operations 3 times in order to ensure the complete washing out of ethanol. When removing sterile ddH2O for the last time, be sure to have completely dried the well. Aspirate the liquid on the recording area by always keeping the tip close to the well/chip border but not touching the chip to avoid any risk in damaging the electrodes.

    In general, this sterilization procedure is not preferable, as it cannot guarantee the same level of sterilization compared to UV-light. EtOH sterilization can also be done in combination with UV-light sterilization. The same procedure applies to sterilize the lid of the 6-well CorePlate™.

4

Getting started

Preparing for a new experiment

Installing BrainWave

The Brainwave software is already installed in the acquisition PC of the HyperCAM Alpha. However, if there are issues, Brainwave software can always download and installed by logging in to your private area from this link: [https://shop.3brain.com/#/].

You will also find a quick tutorial for the first use of our BrainWave software.

Setting up the HyperCAM Alpha

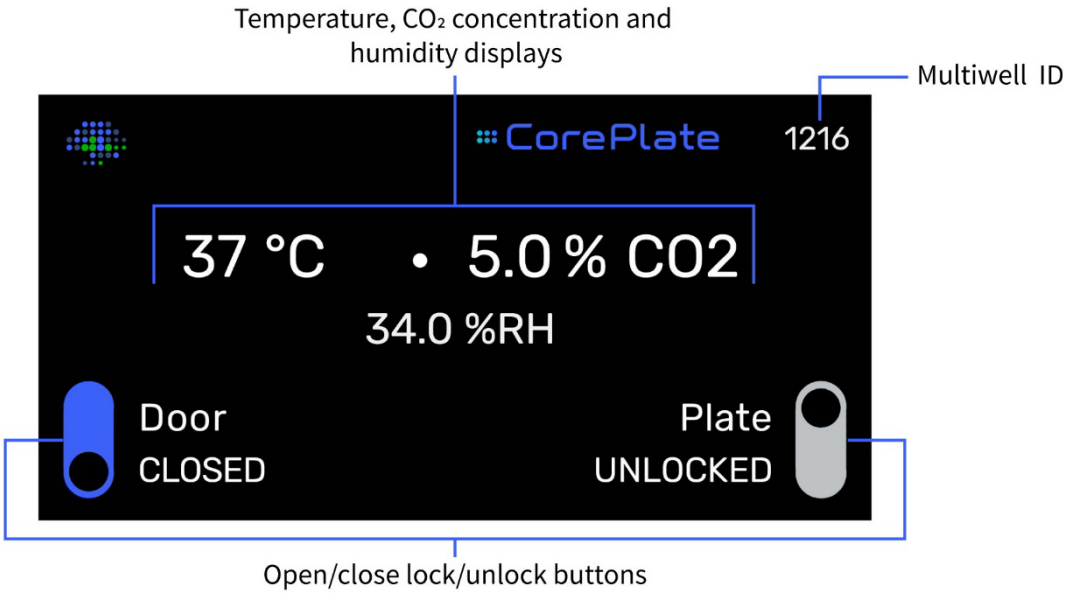
1) Connect a CO_2 line to the right tube fast fitting, positioned on the back of the HyperCAM Alpha. Open the CO_2 line regulating the pressure between 0.8-1.5 bar. **Please make sure to not exceed 3 bar for incoming gas pressure** (as written on on the inlet) to avoid damaging the internal mass flow controller of the HyperCAM Alpha. When the HyperCAM Alpha is not in use keep the CO_2 line closed.

2) Insert the power cord supply cable into the power cord plug at the rear of HyperCAM Alpha and then connect the power cord into an electrical outlet. Switch ON the master switch found aside the power cord plug and then push the power button located beside the LCD panel at the top of the system. After a few seconds the LCD panel will light up and the “loading” window will appear. During this phase the sliding hatch will move to the open position. When the “loading” window disappears, the LCD will show temperature, humidity and CO_2 parameters. The system is ready to be used.

3) Turn on the acquisition PC, use the USB Type-C (v.3.1) cable provided with the platform to connect the USB Type-C port on the back of the HyperCAM Alpha with one of the USB Type-C ports of the computer. **In case you need to replace the cable, please make sure to use only USB 3.1 supporting at least 10 Gbps**.

Plug in the 6-well CorePlate™

The 6-well CorePlate™ is connected to the HyperCAM Alpha through a locking system consisting of four rotating metal wings that push the plate on the metal pogo pins of the environmental chamber guaranteeing a stable electrical connection.



1) Insert the 6-well CorePlate™ into the HyperCAM Alpha environmental chamber. Two metal pins guide the plate insertion while the geometry of the chamber fits the multiwell shape and allows only one insertion direction. Ensure that the plate is horizontal and parallel with the bottom of the environmental chamber. If the 6-well CorePlate™ is properly inserted the serial number (Muliwell ID) of the plate will appear on the LCD screen.
2) Push the lock button on the LCD screen, the four metallic wings will hook the sides of the plate and push it downwards. If the 6-well CorePlate™ is properly locked and recognized, its number can be read by BrainWave (please refer to the BrainWave user manual).
3) Push the close button to move the sliding hatch in the closed position. Activate the mass flow control through BrainWave to control the CO_2 concentration. **Note: the mass flow controller stops when the sliding hatch opens. Every time the sliding hatch closes the mass flow controller has to be activated**.

Start the host PC, BrainWave and the recording of your data

Switch on the PC. In case there are any updates, BrainWave will notify you and they should be installed. Once you have installed and turned on the HyperCAM Alpha, you can easily verify the proper functioning of the system by the following checklist:

1) Verify that both HyperCAM Alpha and the 6-well CorePlate™ are properly recognized by BrainWave (please refer to the BrainWave user manual).
2) Start acquiring data on BrainWave and verify that electrode signals are within expected ranges (please refer to the BrainWave used manual).

If anything of the above went wrong, please check “Troubleshooting” (section 5). If the problem is still not solved, contact the customer support.

Power down after an experiment

Stop recording with BrainWave

Before unplugging the 6-well CorePlate, make sure to stop any acquisition that may be running on BrainWave (please refer to BrainWave user manual).

Unplug the 6-well CorePlate™

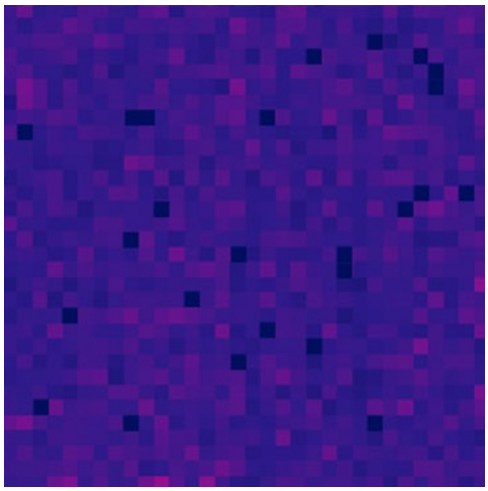
1) Press the unlock button on the LCD screen, the four metallic wings will release the 6-well CorePlate™. Once released, the number of the plate is no longer displayed in BrainWave while it will still be displayed on the LCD of the HyperCAM Alpha.
2) Press the open button on the LCD screen, the sliding hatch will move in the open position. You can now remove the 6-well CorePlate™.

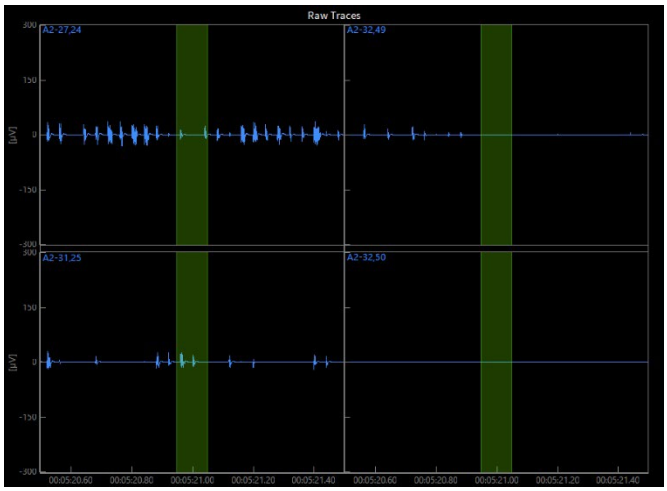
Turn off the HyperCAM Alpha (optional)

5 Troubleshooting

Identifying problems

Electrodes with a not-stable or zero noise bandwidth (saturation)





Symptoms

Some electrodes in one or more wells show a noise bandwidth that is not constant, the signal oscillates from large to small values or is fixed to a zero value. This effect is overall called saturation, in case of an electrode with no signal the corresponding pixel in the raw signal map will have a fixed full-scale color (left image), while in case the signal is oscillating the raw traces (right image) will present a not stable peak to peak value over time.

Causes

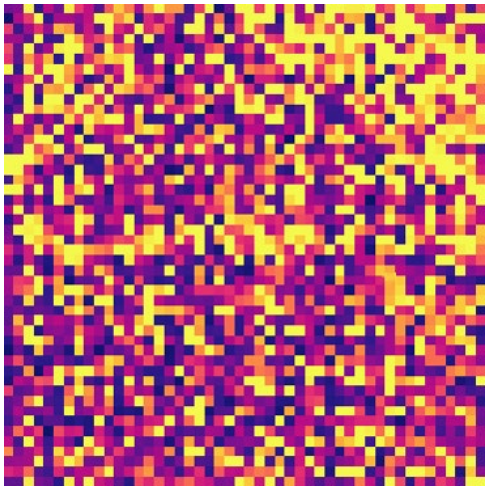
Saturation arises when the input signal amplitude exceeds the operational range of the amplifiers integrated in the chips of the 6-well CorePlate™.

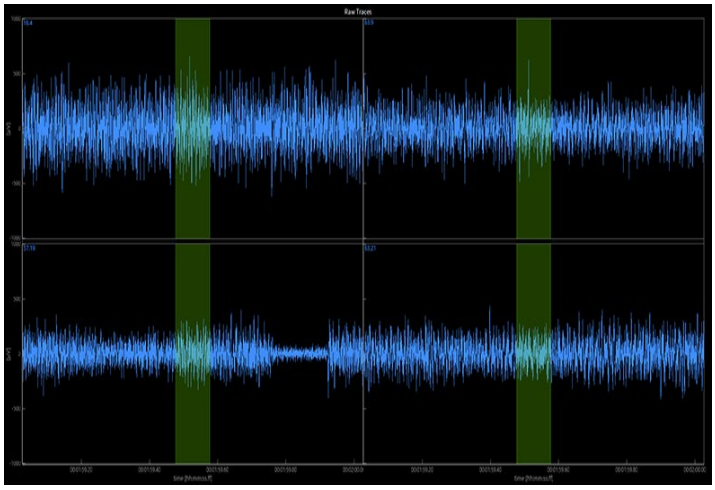
Possible Scenarios and remedies

#	Scenario	Remedy
S 1	Electrodes in the chips of the 6-well CorePlate™ are insulated by tiny bubbles that form when adding a saline solution because of the hydrophobicity of the chip surface. This scenario can arise when trying a new, not precoated 6-well CorePlate™ or a 6-well CorePlate™ that has not	Use a plastic pipette with a small tip to flow liquid on the chip surface in order to remove the bubbles. While doing so, be very careful not to touch the chip and to avoid electrode damages. Alternatively empty the chip's reservoirs, put a drop of pure EtOH on the recording surface

	been used for a long period. A 2x or 4x magnification lens is generally enough to observe these bubbles.	for a few seconds, rinse abundantly with water and put back in the solution.
S 2	The chips of the 6-well CorePlate™ are exposed to a strong environmental light source and the HyperCAM Alpha is not properly configured to work in such conditions.	Turn off the light source or move the HyperCAM Alpha away from the direct light exposure. If you cannot perform these operations, or if you need light on the HyperCAM Alpha (e.g. for light stimulation purposes) , configure the chip calibration setting in the BrainWave 5 software to make the chips of the 6-well CorePlate™ work in such conditions.
S 3	One or more chips of the 6-well CorePlate™ are either damaged or worn out.	If none of the above remedies worked, you may need to change the 6-well CorePlate™.

6-well CorePlate™ chips with dirty or hydrophobic surface





Symptoms

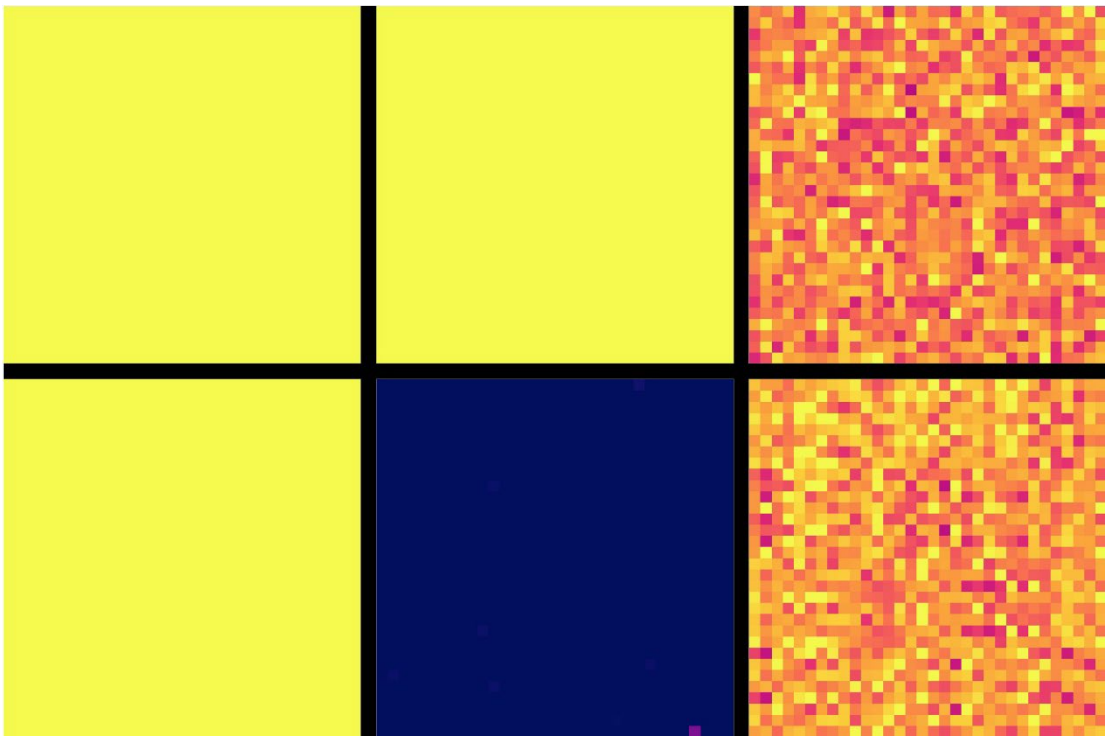
This scenario can occur when testing a 6-well CorePlate™ with no biological preparation (e.g., before an experiment with brain slices). The chips might not have been properly cleaned or have not been used for a long period resulting in a hydrophobic surface. In this situation, electrodes in one or more wells could exhibit higher levels of noise. In BrainWave 5, the raw signal map shows several pixels (single electrodes) with voltage values higher than the average (left image). Single electrode raw traces typically have higher noise levels compared to normal working electrodes (right image).

Causes

The chip surfaces of the 6-well CorePlate™ are dirty or too hydrophobic and tiny bubbles remain trapped on the electrode surface (similarly to scenario S1 in the previous section). In both cases the electrodes show an unstable behavior.

#	Scenario	Remedy
S4	Check Scenario S1	Check Scenario S1
S5	Electrodes in the chips of the 6-well CorePlate™ are dirty of debris and/or more likely of dust particles. This scenario arises only when trying an already used 6-well CorePlate™ that has not been properly cleaned or stored. A 2x or 4x magnification lens is generally enough to observe dirtiness or cellular debris.	Follow the instructions of section 3.3 “Cleaning procedure for 6-well CorePlate™”.

50 Hz noise oscillations



Symptoms

One or more columns in a chip or entire chips of the 6-well CorePlate™ are not working. In case of not working columns the corresponding electrodes in the raw signal map will have a fixed full-scale color (above image), in case of a fully not working chip the entire raw signal map will have a fixed full-scale color

Causes

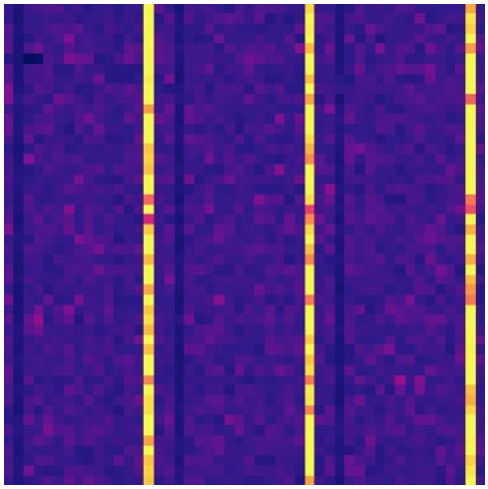
The HyperCAM Alpha has been designed with proper shielding to protect the electronics from environmental noise such as the electrical field generated by the power lines. However, very strong noise sources can still affect the system.

Possible Scenarios and remedies

#	Scenario	Remedy
S 6	The 6-well CorePlate™ shows 50 Hz noise disturbances during an experiment with cell culture or a biological model required to collect high frequency spike activity.	Move the HyperCAM Alpha far from potential noise sources (e.g., fridge, biological hood etc...). If this operation doesn't solve the problem, increase the high pass filter setting to 300-350 Hz on BrainWave 5.
S 7	The 6-well CorePlate™ shows 50 Hz noise disturbances during an experiment with brain tissue or a	Move the HyperCAM Alpha far from potential noise sources (e.g., fridge, biological hood etc...).

	biological model required to collect low frequency field potentials.	If this operation doesn't solve the problem, the HyperCAM Alpha has to be operated inside a faraday cage.

Not working columns or not working chips



Symptoms

One or more columns in a chip or entire chips of the 6-well CorePlate™ are not working. In case of not working columns the corresponding electrodes in the raw signal map will have a fixed full-scale color (above image), in casof a fully not working chip the entire raw signal map will have a fixed full-scale color.

Causes

One or more of the lines that go from the 6-well CorePlate™ to the HyperCAM Alpha exhibit a faulty connection. This issue typically occurs between the 6-well CorePlate™ contact pad(s) in the back and the pogo pins inside the environmental chamber of the HyperCAM Alpha.

Possible Scenarios and remedies

#	Scenario	Remedy
S 8	One or more columns in a chip or entire chips of the 6-well CorePlate™ are not working due to faulty connection.	Extract the 6-well CorePlate™ from the HyperCAM Alpha and clean all the gold contact pads on the back of the 6-well CorePlate™ by using a tissue soaked with pure ethanol (>96%). If cleaning has no effect go to the next scenario
S 9	One or more chips of the 6-well CorePlate™ are either damaged or worn out.	If the above remedy didn't work you may need to change the 6-well CorePlate™ or there could be a problem with the HyperCAM Alpha. Try one or more different 6-well CorePlate™ to verify the problem does not lie in the

		HyperCAM Alpha. If other 6-well CorePlate™ exhibits the same problem, contact 3Brain's customer support.

Contact information

If there are any further questions which you would like to personally contact us for assistance, please feel free to contact us through the following link:

https://www.3brain.com/contact

For urgent communications contact:

Phone: +41-813227086

Email: support@3brain.com