



USER MANUAL

Incub8-System

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Imprint

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General Information

Important Safety Advice



Warning: Make sure to read the following advice prior to installation or use of the device and the software. If you do not fulfill all requirements stated below, this may lead to malfunctions or breakage of connected hardware, or even fatal injuries.



Warning: Always obey the rules of local regulations and laws. Only qualified personnel should be allowed to perform laboratory work. Work according to good laboratory practice to obtain the best results and to minimize risks.



Warning: The device and the software are not intended for medical use and must not be used on humans. MCS assumes no responsibility in any case of contravention.

The product has been built to the state of the art and in accordance with recognized safety engineering rules. The device may only

- be used for its intended purpose.
- be used when in perfect condition.

Improper use could lead to serious, even fatal injuries to the user or third parties and damage to the device itself or other material damage.

Malfunctions which could impair safety should be rectified immediately.

Grounding

This product is grounded through the grounding conductor on the power cord. To avoid electric shock, the grounding conductor must be connected to earth.

Orient the Equipment Properly

Do not orient the equipment so that it is difficult to manage the disconnection device.

High Voltage

Electrical cords must be properly laid and installed. The length and quality of the cords must be in accordance with local provisions.

Only qualified technicians may work on the electrical system. It is essential that the accident prevention regulations and those of the employers' liability associations are observed.

Each time before starting up, make sure that the power supply agrees with the specifications of the product. Check the power cord for damage each time the site is changed. Damaged power cords should be replaced immediately and may never be reused. Check the leads for damage.

Damaged leads should be replaced immediately and may never be reused. Do not try to insert anything sharp or metallic into the vents or the case. Liquids may cause short circuits or other damage. Always keep the device and the power cords dry. Do not handle it with wet hands.

Requirements for the Installation

Make sure that the device is not exposed to direct sunlight. Do not place anything on top of the device, and do not place it on top of another heat producing device, so that the air can circulate freely.

Explanation of the Symbols used



Caution / Warning



DC, direct current

Guarantee and Liability

The general conditions of sale and delivery of Multi Channel Systems MCS GmbH always apply. They can be found online at:

<https://www.multichannelsystems.com/sites/multichannelsystems.com/files/documents/Terms%20and%20Conditions.pdf>

Multi Channel Systems MCS GmbH makes no guarantee as to the accuracy of any and all tests and data generated by the use of the device or the software. It is up to the user to use good laboratory practice to establish the validity of his / her findings.

Guarantee and liability claim in the event of injury or material damage are excluded when they are the result of one of the following:

- Improper use of the device.
- Improper installation, commissioning, operation, or maintenance of the device.
- Operating the device when the safety and protective devices are defective and/or inoperable.
- Non-observance of the instructions in the manual with regard to transport, storage, installation, commissioning, operation or maintenance of the device.
- Unauthorized structural alterations to the device.
- Unauthorized modifications to the system settings.
- Inadequate monitoring of device components subject to wear.
- Improperly executed and unauthorized repairs.
- Unauthorized opening of the device or its components.
- Catastrophic events due to the effect of foreign bodies or acts of God.

Operator's Obligations

The operator is obliged to allow only persons to work on the device, who

- are familiar with the safety at work and accident prevention regulations and have been instructed how to use the device;
- are professionally qualified or have specialist knowledge and training and have received instruction in the use of the device;
- have read and understood the chapter on safety and the warning instructions in this manual and confirmed this with their signature.

It must be monitored at regular intervals that the operating personnel are working safely. Personnel still undergoing training may only work on the device under the supervision of an experienced person.

Terms of Use for Incub8-System

You are free to use the software for its intended purpose. You agree that you will not decompile, reverse engineer, or otherwise attempt to discover the source code of the software.

Limitation of Liability

Multi Channel Systems MCS GmbH makes no guarantee as to the accuracy of any and all tests and data generated by use of the software. It is up to the user to use good laboratory practice to establish the validity of his findings.

To the maximum extent permitted by applicable law, in no event shall Multi Channel Systems MCS GmbH or its suppliers be liable for any special, incidental, indirect, or consequential damages whatsoever (including, without limitation, injuries, damages for data loss, loss of business profits, business interruption, loss of business information, or any other pecuniary loss) arising out of the use of or inability to use the program or the provision of or failure to provide Support Services, even if Multi Channel Systems MCS GmbH has been advised of the possibility of such damages.

FCC Rules

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules.

Warranty

In case of warranty, please contact the support team of Multi Channel Systems MCS GmbH. Please report all bugs, feature requests or other issues to:

<https://support.multichannelsystems.com/hc/en-us/articles/4413175249555-Warranty-Policy>

Welcome to the Incub8-System



The Incub8-System is a member of the Multi Channel Systems MCS GmbH MEA product family. Featuring 24- and 96-well plate format, it is the perfect tool for medium and high throughput electrophysiology.

The Incub8-System includes high-quality, low-noise amplifiers, and freely programmable stimulators. The data is sampled at up to 50 kHz per channel (simultaneously on all channels). Thus, the accuracy of your data is guaranteed, recording and analyzing the complex electrical activities.

The Incub8-System consists of four components:

- The system consists of a signal collector unit SCU-*in vitro* for up to four Incub8 headstages. The SCU also contains a connector for up to four outputs for optical LED stimulation (coming soon). The signal collector is connected via iX cable to the interface board IFB-C multiboot.
- The interface board IFB-C contains the USB interface to the data acquisition computer and to the Incub8 headstage. In addition it contains analog and digital in- and outputs for synchronization with other instruments.
- The Incub8 headstage includes 288 channel amplifiers, analog/digital conversion, integrated heating, integrated stimulator and voltage stimulation (selectable via software). Any electrode can be used as stimulation electrode. The sampling rate is up to 50 kHz per channel on all electrodes simultaneously.
- Three types of well plates are available: 24-well plates with 12 electrodes each and 96-well plates with 12 or 3 electrodes in each well. Additionally, there is at least one reference electrode integrated in each well. The well plates are made from opaque epoxy substrates with gold electrodes or from PET substrate with gold electrodes. In addition there are 24well glass MW-plates.
- For data acquisition and detailed analysis two software versions are available, Incub8-Screen and Incub8-Analyzer. Depending on your application, please run the software in "Cardio" or "Neuro" mode.

Setting Up and Connecting the Incub8

Hardware

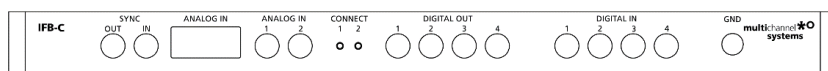
The Incub8-System consists of three devices, the interface board IFB-C, signal collector unit SCU, and the Incub8 headstage. The fourth component is the multiwell plate-

Interface Board IFB-C Multiboot



The multiboot interface board IFB-C facilitates operation of all MCS *in vitro* and *in vivo* headstages. This suite includes: Incub8 headstage, Multiwell-MEA-HS, MEA2100-HS, CMOS-MEA-HS, MEA2100-Beta-Screen-HS, W2100-HS and ME2100-HS. The modular solution suite design makes it easy to modify your lab equipment generally with modest hardware upgrade investments.

Front Panel



From left to the right:

Sync OUT / IN

Two or more interface boards IFB-C can be daisy-chained by use of the Sync Out / Sync In connectors. All daisy-chained interface boards run on the same clock – to allow perfectly synchronized recordings in large settings.

Analog Channels

Up to eight Analog In channels are available via 10-pin connector. Please read chapter “10-Pin Connector for Analog IN” in the Appendix for more information about the pin layout. The additional analog inputs are intended for recording additional information from external devices, for example, for recording patch clamp in parallel to the MEA recording.

Analog Channels 1 and 2

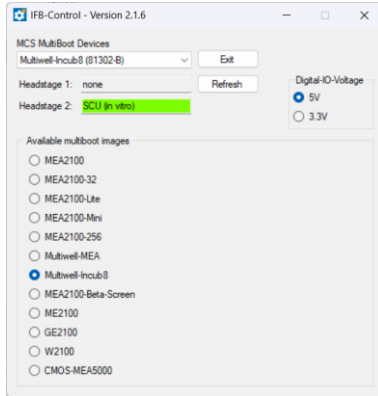
Two of these analog channels (Analog In 1 and 2) are separately available via Lemo connectors.

Two Status LEDs

The status LEDs indicate the link status of headstage HS 1 and / or HS 2. They light up when one or both headstages are connected to the IFB-C interface board via iX-industrial cable.

Digital IN / OUT

Four Digital IN and four Digital OUT bits are accessible via Lemo connector (DIG IN bit 0 to bit 3 and DIG OUT bit 0 to bit 3). The Digital OUT delivers TTL pulses with 3.3 V or 5 V. The voltage can be switched with the software IFB-Control, please read chapter "IFB-Control" in the Appendix.



If access to more bits the DIG IN / OUT channel is required, it is necessary to connect a Digital IN / OUT extension Di/o board with a 68-pin standard cable. This Di/o board is available as an optional accessory.

Ground

If an additional ground connection is needed, you can connect this plug with an external ground using a standard common jack (4 mm).

Rear Panel



From left to right:

Toggle Switch On / Off

Toggle switch for turning the device on and off. The Incub8-System is switched to status "ON" when the toggle switch is switched to the left. The device is switched "OFF" when the toggle switch is switched to the right. If the Incub8-System is "ON", and the device is connected to the power line, the "Power LED" on the front panel of the interface board should light up. If not, please check the power source and cabling.

Power 24 VDC

Connect the power supply unit here. This power supply powers both, the headstage and the interface board IFB-C of the Incub8-System. The device needs 24 V and 2.5 A / 60 W.

Ground

If an additional ground connection is needed, you can connect this plug with an external ground using a standard common jack (4 mm).

Digital IN / OUT

A Digital IN / OUT for 16 digital in- and output bits is available via Honda-PCS-XE68LFD connector. Please read chapter “Digital IN / OUT Connector” in the Appendix for more information about the pin layout of the connector. The Digital IN / OUT connection accepts or generates standard TTL signals. The Digital OUT delivers TTL pulses with 3.3 V or 5 V. The voltage can be switched between these two levels with the software IFB-Control, please read chapter “IFB-Control” in the Appendix.

TTL stands for Transistor-Transistor Logic. A TTL pulse is defined as a digital signal for communication between two devices. A voltage between 0 V and 0.8 V is considered as a logical state of 0 (LOW), and a voltage between 2 V and 3.3 V or 5 V means 1 (HIGH).

The Digital OUT allows generating a digital signal with up to 16 bits and read it out, for example, by using a Digital IN / OUT extension Di/o board. You can utilize this digital signal to control and synchronize other devices with the Incub8-System.

Bit 0 to 3 of the Digital OUT are separated and available as Lemo connector DIG OUT 0 to 3 on the front panel of the interface board. So, the Di/o extension is only necessary if more than four trigger inputs or outputs are needed.



Digital IN can be used to record additional information from external devices as a 16 bit encoded number. Digital IN is most often used to trigger recordings with a TTL signal. The 16 bit digital input channels is a stream of 16 bit values. The state of each bit (0 to 15) can be controlled separately. Standard TTL signals are accepted as input signals on digital inputs.

Warning: A voltage that is higher than +3.3 Volts or +5 Volts or lower than 0 Volts, that is, a negative voltage, applied to the digital input would destroy the electronics. Make sure that you apply only TTL pulses (0 to 3.3 V or 5 V) to the digital inputs.

Signal Collector Unit SCU-*in vitro*



The second element of the Incub8-System is the signal collector unit SCU-*in vitro* for up to four Incub8 headstages.

The SCU-*in vitro* also contains a connector to control an optical stimulation device (coming soon). Please check the MCS website once in a while. The control functions will be integrated into the Incub8-Screen software.

The signal collector unit and the interface board are connected via an iX cable, type B.

Connect the Incub8 headstage via the lemo cable to the signal collector unit SCU-*in vitro*. Up to four Incub8 headstages can be connected to one SCU-*in vitro*.

Up to two SCU-*in vitro* devices can be connected to one IFB-C multiboot interface board.

Important: It is not possible to connect a SCU-*in vivo*, designed for “*in vivo*” applications with an Incub8-headstage.

Incub8 Headstage



Connect up to eight Incub8 headstages via two signal collector units SCU-*in vitro* to one interface board IFB-C and one data acquisition computer.

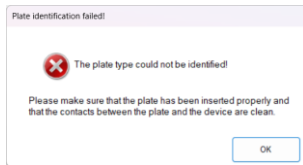


The front of the Incub8 headstage is equipped with its serial number on the right side and its explicit code on the left side . This way the headstage is characterized and cannot be mixed-up when using several headstages at once.



Slide the multiwell plate into the designated area of the Incub8 headstage. The well labelled “A1” needs to be inserted facing the upper right side . Move the multiwell plate in correct orientation exactly up to the limit stop: “Fully inserted when line is visible”.

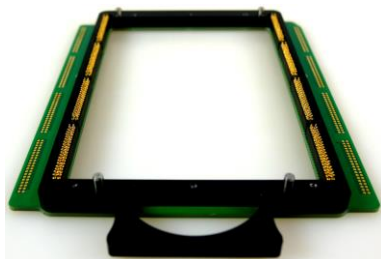
Close the device with the hinge joint. Be careful not to clamp your fingers!



If the well plate is not sufficient inserted, the following error message appears: “Plate identification failed”.

Connect the Incub8 headstage via the iX cable to the Signal Collector Unit and to the interface board IFB-C to input HS/SCU 1, when using only one to four Incub8 headstages. Ground the Incub8 headstage, if necessary. Please use the provided cable for grounding either the Incub8 headstage, the SCU or the interface board to an external ground.

Incub8-VariPlate Base



Add the four provided adapter cover inside the rim of the headstage (see pictures below) when using an Incub8-VariPlate Base (picture above). The Incub8-VariPlate Base enables the use of standard multiwell plates for your experiments without ID fit. Please read also the Incub8-VarioPlate Base datasheet in the appendix.



24-well and 96-well standard plates are available. Make sure to insert the plates in a correct orientation into the Incub8-VariPlate Base (please use the guide bolts on the VariPlate for the correct orientation of the multiwell plate).

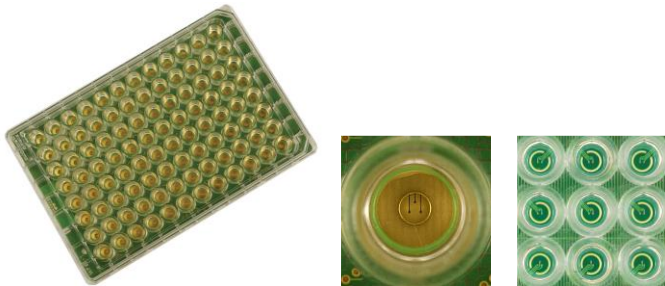
Important: Multiwell plates are not symmetrical!

(All types of multiwell plates have two holes on each of the short sides on the bottom, one pair wider apart, one pair closer together. They fit on corresponding bolts on the Incub8-VariPlate Base) Only if the well plate sits on those guide bolts and cannot be moved laterally, the hint joint of the Incub8 headstage closes properly.

Standard Multiwell Plates for Use with Incub8-VariPlate Base

Standard 96 Well Plates on Epoxy Base

96 well plate with 3 electrodes and 1 circular reference electrode in each well on Epoxy base:
96W700/100F-288.



96 well plates on Epoxy base are available with 3 gold electrodes per well. The electrode diameter is 100 μm , the distance between the electrodes 700 μm . They are perfect for neuronal cultures and the glass substrate facilitates visual check of cells under the microscope.

Standard 24 Well Plates on Epoxy Base

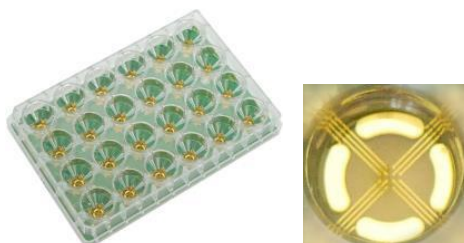


24 well plate with 12 electrodes and 4 reference electrodes in each well on epoxy base:
24W300/30F-288.

PEDOT coated gold electrodes on Epoxy base are available in 24 wells with 12 electrodes per well. The electrode diameter is 30 μm , the distance between the electrodes 300 μm . They are perfect for neuronal cultures and the glass substrate facilitates visual check of cells under the microscope. The PEDOT coated gold electrodes are ideally suited for recordings and stimulation. Every single electrode can be selected for stimulation.

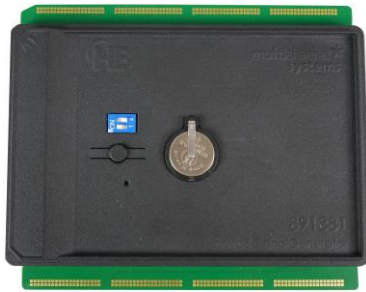
Standard 24 Well Plate on Glass Base

24 well plate with 12 electrodes and 4 reference electrodes in each well on glass base:
24W300/30G-288.



Please read also the respective data sheets for multiwell plates.

Incub8 Signal Generator



The Incub8 Signal Generator is a convenient tool adapted to the Incub8 device. Use the Incub8-SG instead of setting up an experiment with biological sample for training, controlling, and troubleshooting purposes. This reduces the number of experiments and saves consumables. Please see also the data sheet "Incub8-SG" in the appendix.

Incub8-Testplate

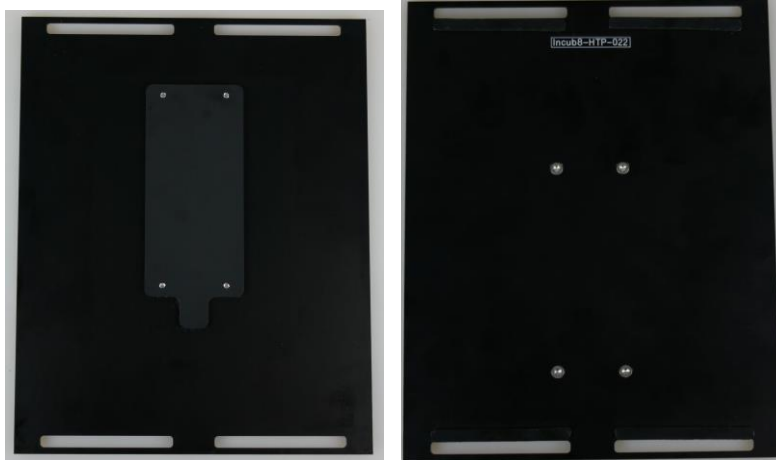


Use the Incub8-Testplate for testing the functionality of your Incub8-System. All 288 recording electrodes are internally connected to a resistor-capacitor pair. So, it can be used for testing the noise level of the electrodes of an Incub8-System for all 288 recording electrodes simultaneously.

The provided testplate simulates a multiwell plate with a resistor of 220 k Ω and a 1 nF capacitor between ground and electrode for each of the 288 electrodes. The impedance between signal source and contact pads averages 120 k Ω at 1 kHz.

Please read also the data sheet "Incub8-Testplate" in the appendix.

Incub8 AccuTemp Stabilizer



The Incub8 AccuTemp Stabilizer should be used in temperature sensitive experiments to make sure each of the wells of a 24-plate or 96-plate hold the same temperature. The Incub8 AccuTemp Stabilizer does not work actively but spreads the heat passively to all wells.

Please mount the Incub8 headstage exactly on the Incub8 AccuTemp Stabilizer, that the heightened area in the middle contacts the bottom of the inserted multiwell plate. This connection effects/leads? to a uniform temperature distribution to each well. Otherwise, it might be that the wells next to the rim(edge) of the headstage are heated more by the electronic elements included in the Incub8 headstage.

When using the Incub8-VariPlate Base, the small aluminum block in the center of the Incub8 AccuTemp Stabilizer has to be replaced with the thicker aluminum block that is part of the Incub8-VariPlate Base package (included). You have to remove and reattach the 4 screws with the screwdriver provided in the package.

If you use the Incub8-VariPlate Base together with the Incub8 AccuTemp Stabilizer, please do so in the following order:

- take the Incub8 headstage down from the Incub8 AccuTemp Stabilizer
- slide the Incub8-VariPlate Base, loaded with your prepared plate, into the Incub8 headstage
- replace the Incub8 headstage onto the Incub8 AccuTemp Stabilizer

Follow the same routing when removing your plate from the Incub8 headstage.



Important: Please use a separate incubator if you don't use the wake-up mode (page 53). Due to the nature of the Incub8 headstage electronics, the temperature of the Incub8 headstage(s) and the temperature of the incubator will rise over time. At MCS we've done tests with a Memmert ICO150 incubator and set the temperature 4°C less in order to maintain the adjusted temperature of 37°C inside the plate wells.

Please perform pre-tests with the Incub8 headstage(s) in your laboratory incubator in order to find the needed temperature for your experimental setup. Be aware that well volume and air impermeability of the incubator can also influence the temperature settings.

Software

Installing the Software

System Requirements

Software: The following Microsoft Windows[®] operating systems is required: 64 Bit Windows 11 or higher (English and German versions supported). Other language versions may lead to software errors.



Important: The Incub8-Screen and the Incub8-Analyzer software need both Windows 10(Windows 11) and higher (64 Bit) for data acquisition.

Important: Make sure to have a valid license for the Incub8-Screen and for the Incub8-Analyzer software!

Hardware: Incub8-System and data acquisition computer.



Important: It is strongly recommended to use a computer preconfigured from MCS for data acquisition!

Due to the amount of recorded data, a computer with low performance may lead to unpredictable problems; therefore, Multi Channel Systems recommends an up-to-date computer with SSD hard drive. Please contact MCS or your local retailer for more information on the recommended computer hardware specification. Be aware that Multi Channel Systems can not guarantee that everything will work properly if you configure your own computer.

Please note that there are sometimes hardware incompatibilities of the data acquisition system and computer components; or that an inappropriate computer power supply may lead to artefact signals.

It is not recommended to run any applications in the background when using the Incub8-Screen software. Please remove all applications from the Autostart folder.



Important: You need to have installed the latest data acquisition driver to operate the device, which is automatically installed with Incub8-Screen and Incub8-Analyzer software. The installation may be invalid if the data acquisition does not respond. Please contact Multi Channel Systems MCS GmbH or your local retailer in this case.

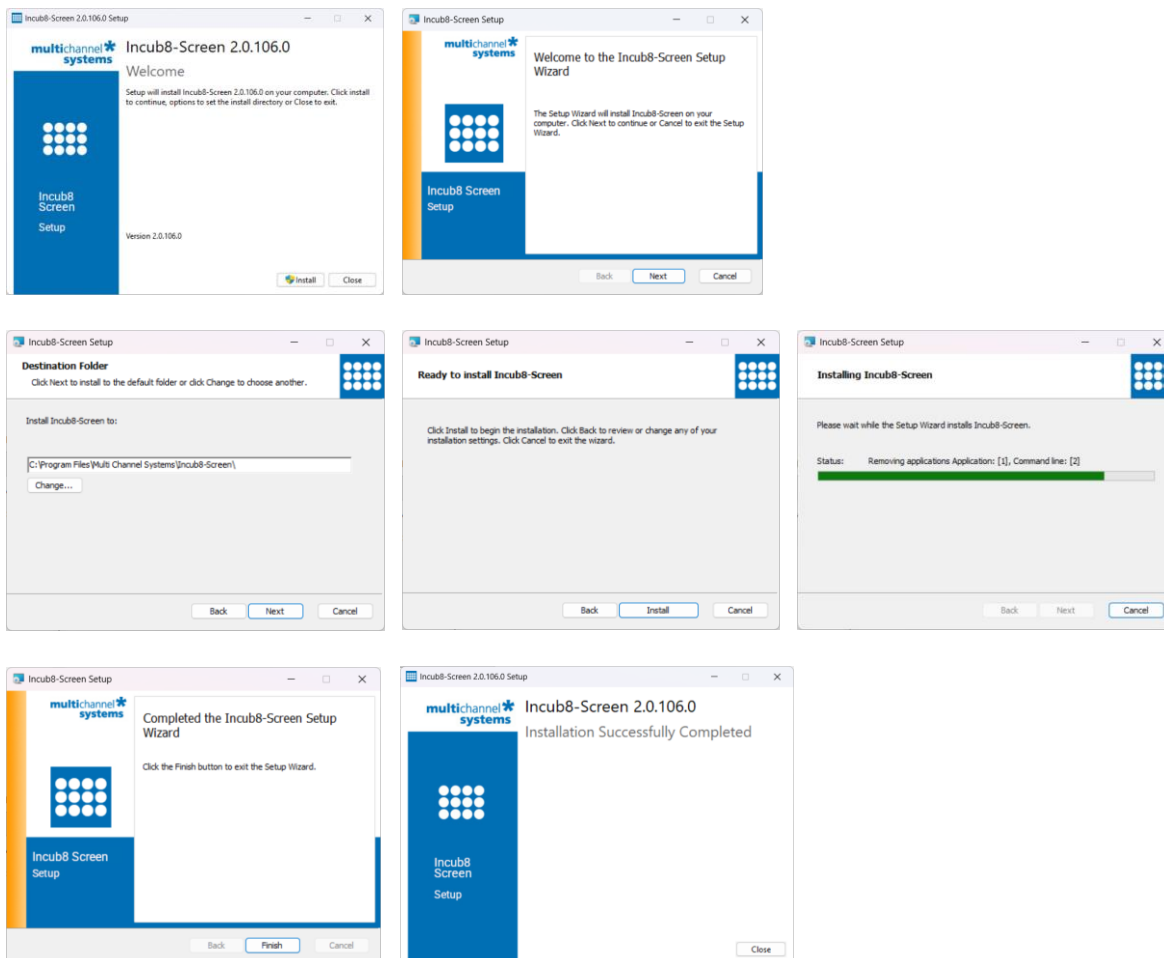
Important: Please make sure that you have full control over your computer as an administrator. Otherwise, it is possible that the installed software does not work properly.

Recommended Operating System Settings

The following automatic services of the Windows operating system interfere with the data storage on the hard drive (konsistent siehe oben und unten) and can lead to severe performance limits in Incub8-Screen. These routines were designed for use on office computers but are not very useful for a data acquisition computer.

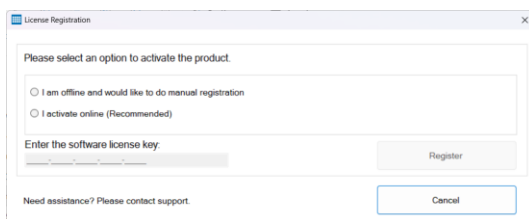
- Turn off "Screen Saver".
- Turn off "Windows Indexing Service". Only important for data hard drive.
- Manual management of automatic Windows Update.
- Power Options: Power scheme: Never turn off monitor, hard drive and system standby.
- It is not recommended to run any applications in the background when using Incub8-Screen software.
- Remove all applications from the "Autostart" folder.
- Be careful when using a virus scanner.

Start Incub8-Screen.exe file. The installation manager will guide your through the installation.



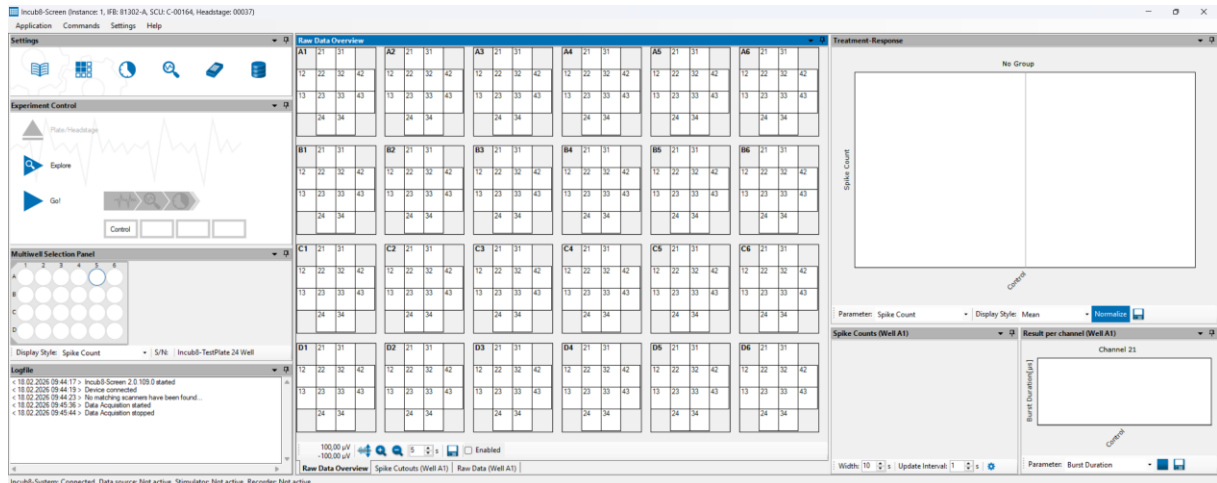
License Registration

You do not need a license for the Incub8-Screen software, if you like to test the software for getting an idea of how it works and what is possible. But soon as you start the data acquisition, the following dialog will appear. Please type the key into the window "Enter the software license key" and confirm the key with the button "Register".



The Incub8 software license is bound to the data acquisition computer. The license key will be saved on the computer.

Software for the Incub8-System

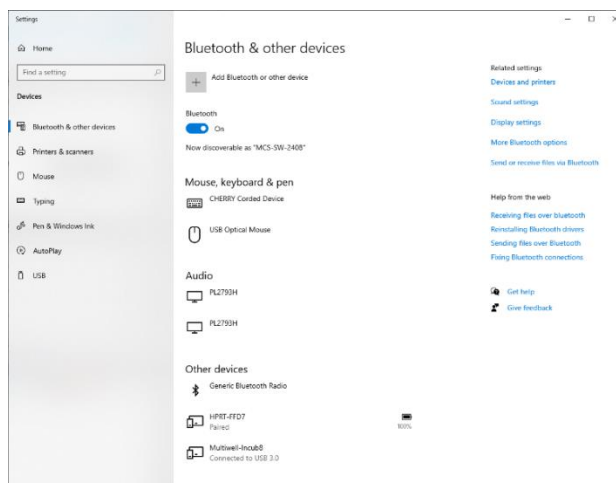


Before You Start

Before starting the Incub8-Screen software for the Incub8-System for the first time, please prepare the scanner.

Preparing the Scanner

Please connect the bluetooth adapter for the scanner to an USB port and open the “Settings” of the computer, “Bluetooth & other devices”. Activate “Bluetooth” and close the window again.

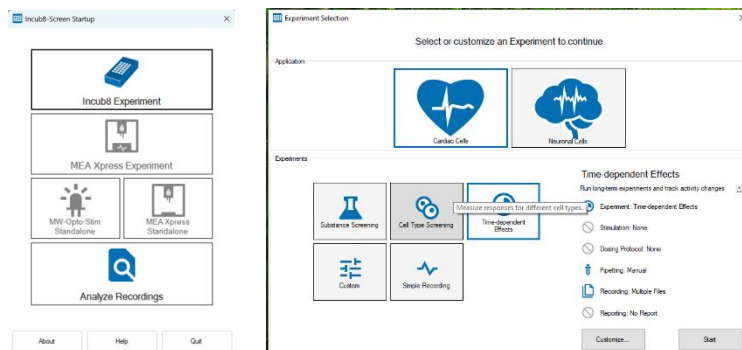


Activate the scanner by pushing the button on the right side of the device forward and press the upper button. The LED of the scanner starts to blink green



Please read chapter “Troubleshooting” if it is necessary to replace the scanner device. A new scanner has to be prepared in a special way for use with the Incub8-System.

Starting the Incub8-Screen Software



Open the Incub8-Screen software. The dialog “Incub8-Screen Startup” appears. Click “Incub8 Experiment”.

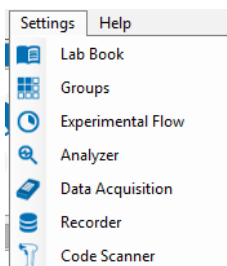
In the next dialog “Experiment Selection” you have to choose the “Application” and the “Experiment”. The Incub8 device is specially designed for “Time-dependent Effects” and long-term experiments.

Select the Scanner ID

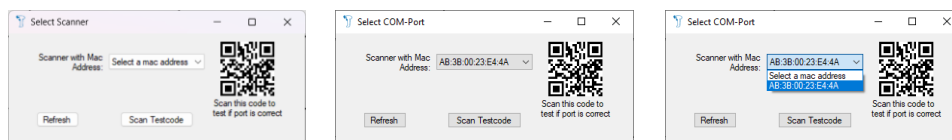


Switch the scanner on. Activate the scanner by pushing the button on the right side of the device forward and press the upper button. A green LED is blinking.

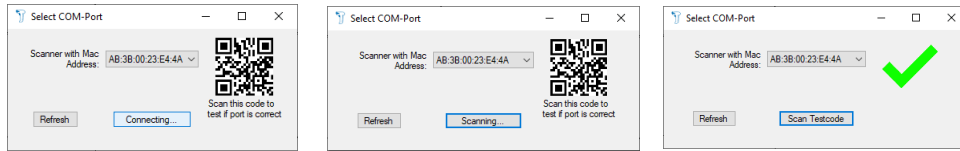
Open the “Settings” tab and select “Code Scanner”.



Select the scanner device in the drop down menu of the dialog “Select Scanner”.



Point with the scanner to the code in the dialog to connect scanner and computer “Connecting”. After connecting, the code will be scanned “Scanning”. If this process is successful, the scanner ID is saved.

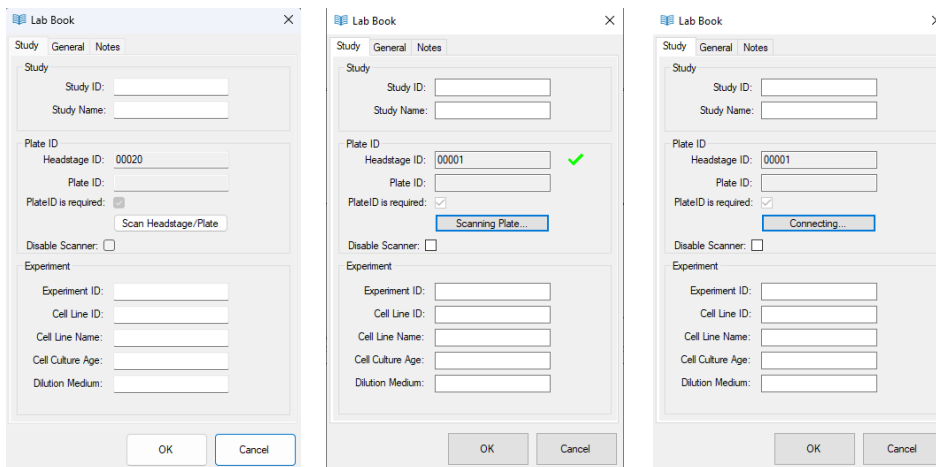


Select the Plate ID

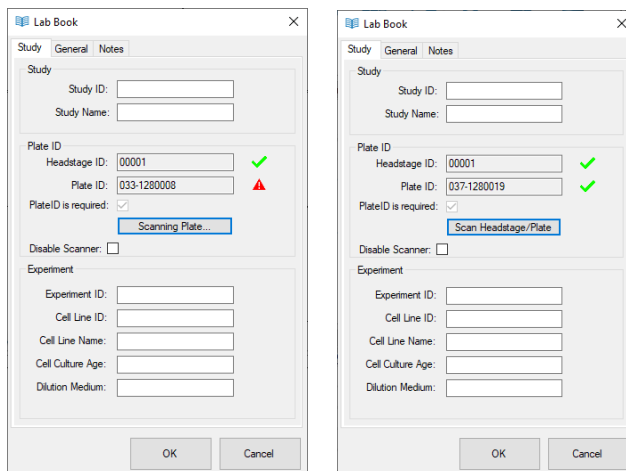
Open the “Lab Book”. Click “Scan the Headstage/Plate” in “Plate ID is required”. Point with the scanner directly to the Plate ID in front of the multiwell plate: “Scan Headstage Plate”. The ID of the well plate is scanned: “Scanning Plate”.

When the plate ID is scanned, the scanner and computer are connecting: “Connecting”.

Important: It is possible to work with more than one instance of “Incub8-Screen” in parallel on one computer. That is, why the announcement “Connected” will immediately change to “Disconnected” again, because the scanner is now available to process the next instance of Incub8-Screen.



After finishing the scanning process, the “Plate ID” is completed. The “Headstage ID” and the “Plate ID” are displayed and you see additionally two green check marks beside.



Incub8-Screen Software

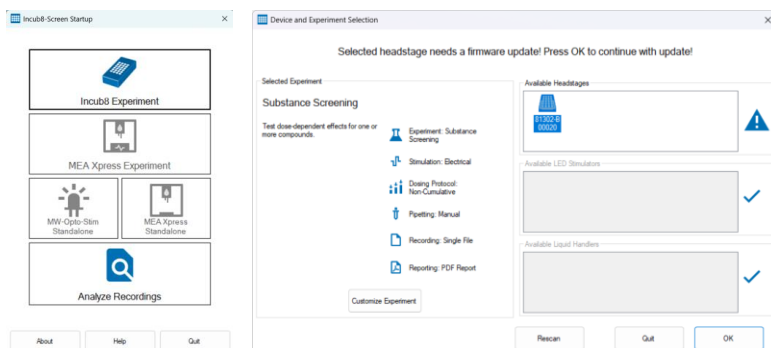
The software Incub8-Screen is developed for cardio and neuro applications and facilitates data acquisition "Incub8-Screen" and analysis "Incub8-Analyzer". The Incub8-Screen is used for online monitoring and recording. It controls the Incub8 headstage and SCU together with the IFB-C, as well as the MW24/96-Opto-Stim device, if connected.

To start the software, please click the icon or select it from the program menu.



If a firmware update is available and the Incub8-System is switched on, the firmware will be updated automatically.

The Incub8-Screen Startup



The "Incub8-Screen Startup" dialog for the selection of the operation mode of the Incub8-Screen software:

- "Incub8 Experiment": Record data from the Incub8 headstage.
- "MW-Opto-Stim Standalone": Control a MW24/96-Opto-Stim device without a connected Incub8 headstage.
- "Analyze Recordings": Analyze previous Incub8-Screen recordings either with the Incub8-Screen Replayer function or the Incub8-Analyzer software.

The advantage of this initial selection is the automatic adaptation of the software modules to your special experimental schedule. Parts of the software which are not needed will not be available, so the software is slenderly built and more intuitive. Nevertheless, there is the possibility to change the initial settings later on. Click "Help" to view this manual.

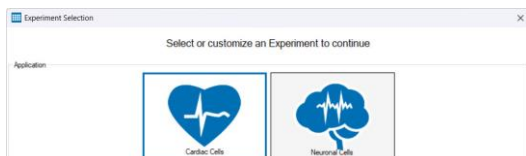
Click the "About" button to display the version number of the software. Click "Help" for online help.



Experiment Selection

Application

Cardio or Neuro Mode

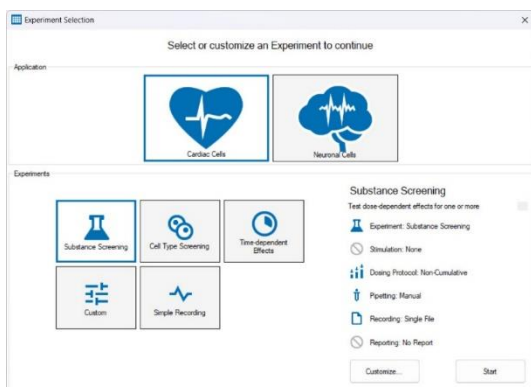


Determine the "Application": "Cardiac Cells" or "Neuronal Cells". Depending on this selection, the user interface and the analysis functions are optimized for experiments with either cardiac or neuronal cells.

After selecting "Incub8 Experiment", the first dialog "Experiment Selection" allows you to specify whether you want to run the software in "Cardio" or "Neuro" mode and the selection of which experimental workflow you want to work with. These choices are stored on your PC and will be used the next time you start the Incub8-Screen software. See section "Redesign of an Experiment" on how to change your selection from the main window of Incub8-Screen.

Experiments

The next step is to specify the type of experiment you are planning to run and to select the necessary software modules for it.



There are five different predefined experiments available: "Substance Screening", "Cell Type Screening", "Time-dependent Effects", "Custom" and "Simple Recording".

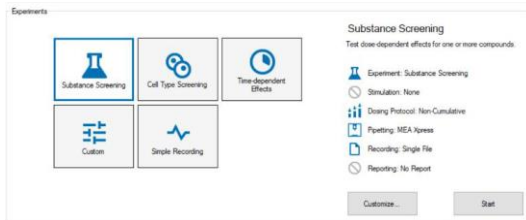
The first three options are intended to cover the typical use cases for the Incub8 software: Creating dose-response curves for test substances, measuring the activity of different cell types, and running long-term experiments to track activity changes over time, respectively. If none of these fit your needs, you may select the "Custom" experiment type.

The "Simple Recording" experiment type is most appropriate if you are planning to just record a single segment of data without stimulation. The selected experiment type has two effects on the software: First of all, some aspects of the user interface depend on the experiment type.

For example, in a "Substance Screening" experiment, you can group multiple wells together and assign them to the same "Compound". In "Cell Type Screening" mode, these well groups are termed "Cell Type" instead. These changes are mostly cosmetic, but they are intended to make working with the software more intuitive. Secondly, each experiment type comes with a default selection of software modules which impact the

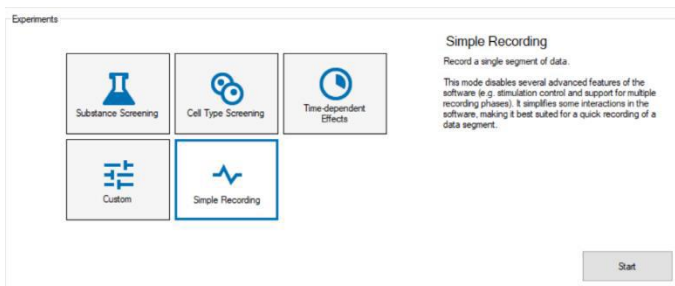
functionality of the software. The selected software modules can be fully customized for each experiment type, with the exception of the "Simple Recording" type, where only the most basic function is selected for each running software module.

Please see below for a description of each software module.



Select the experiment type on the left hand side. The default software modules for the selected experiment type are listed on the right, below a short description of the experiment type. You may customize the software modules by pressing the "Customize..." button to open the "Customize Experiment Settings" dialog. If you are satisfied with your selection, press the "Start" button to open the main display of Incub8-Screen.

Simple Recording

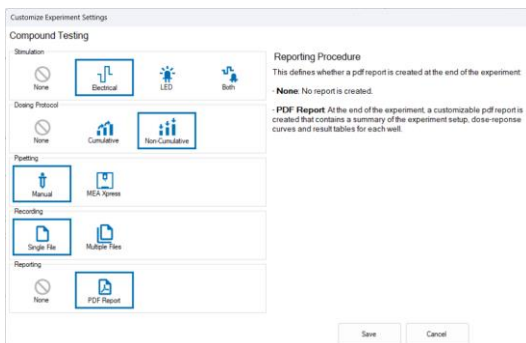


"Simple Recording" experiments are predefined to "Record a single segment of data".

Click "Start" button to open the main display of the Incub8-Screen software. Click "Customize" button to adapt your experiment with the "Customize Experiment Setting" dialog.

Click "Save" button to store your settings. The software will start in the defined mode until you change your settings. For changing, please click the "Application" menu, option "Define Experiment" to start up the "Experiment Selection" dialog again.

Customize Experiment Settings



There are five software modules in Incub8-Screen for which you can customize their functionality from this dialog.

Stimulation:

This defines if and what kind of stimulation is used during the experiment

None: Neither electrical nor LED stimulation is used.

Electrical: Voltage controlled electrical stimulation can be used.

LED: ____

Both: Voltage controlled electrical stimulation and LED stimulation can be used. This requires a connected MW24-opto-stim (for 24-well plates) or MW96-opto-stim (for 96-well plates) device.

Dosing Protocol:

The dosing protocol defines how the doses in the dilution series are applied to wells.

None: Choosing "None" means that no liquid handling will be done. Neither the Pipet nor the Wash-In phases will be available in the software.

Cumulative: Choosing the *cumulative* dosing protocol means that each well will receive the full dilution series, starting with the lowest concentration in the first pipet phase and increasing the concentration in each successive pipet step.

Non-Cumulative: Choosing the *non-cumulative* dosing protocol means that only a single dose will be applied to each well.

Pipetting:

This defines manual pipetting.

Manual: Liquid handling has to be performed manually. Between recording phases, a dialog window will display which concentrations are needed in which wells. The experiment will continue after a manual confirmation that pipetting is finished.

Recording:

This defines whether all recording phases are collected in a single file or split into multiple files.

Single File: The data for all recording phases will be stored in the same file. This makes file handling easier, because there is only a single file, but for long recordings especially the *.mwd files with raw data can get very large.

Multiple Files: The data from each recording phase is stored in its own file. This makes it possible to have smaller individual files and also to analyze only a subset of the recording phases in the Incub8-Analyzer.

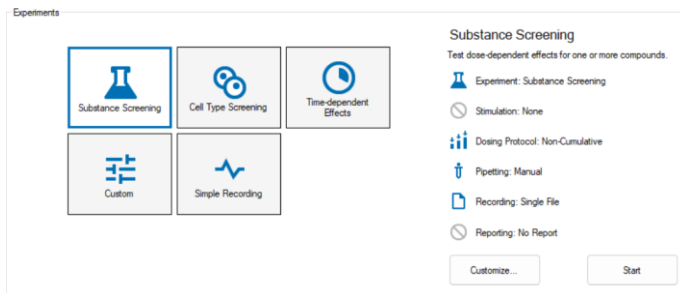
Reporting:

This defines whether a pdf report is created at the end of the experiment.

None: No report is created.

PDF Report: At the end of the experiment, a customizable pdf report is created that contains a summary of the experiment setup, dose-response curves, and result tables for each well.

Substance Screening



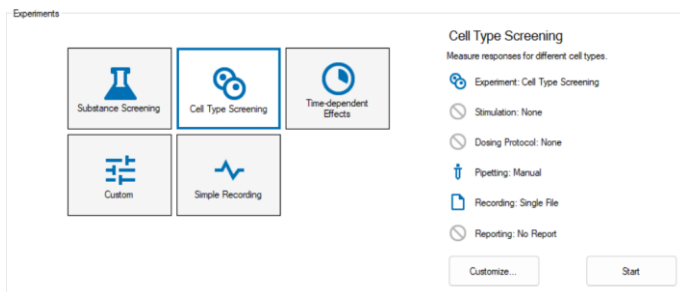
“Substance Screening” experiments are predefined to “Test dose-dependent effects for one or more compounds”.

Click “Start” button to open the main display of the Incub8-Screen software.

Click “Customize” button to adapt your experiment with the “Customize Experiment Setting” dialog.

Click “Save” button to store your settings. The software will start in the defined mode until you change your settings. For changing, please click the “Application” menu, option “Define Experiment” to start up the “Experiment Selection” dialog again.

Cell Type Screening

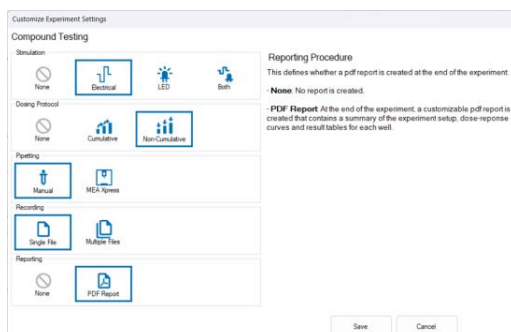


“Cell Type Screening” experiments are predefined to “Measure responses for different cell types”.

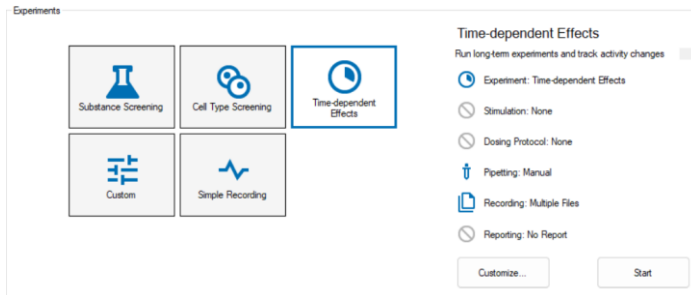
Click “Start” button to open the main display of the Incub8-Screen software.

Click “Customize” button to adapt your experiment with the “Customize Experiment Setting” dialog.

Click “Save” button to store your settings. The software will start in the defined mode until you change your settings. For changing, please click the “Application” menu, option “Define Experiment” to start up the “Experiment Selection” dialog again.



Time-dependent Effects

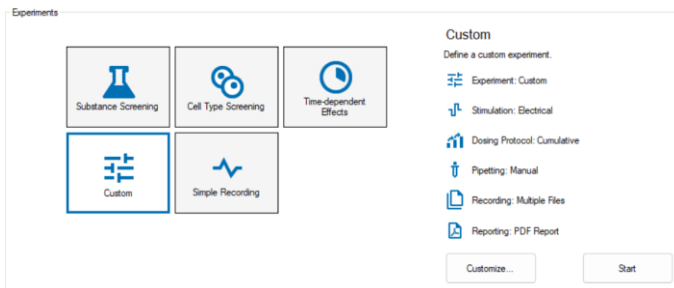


“Time-dependent Effects” experiments are predefined to “Run long-term experiments and track activity changes over time”.

Click “Start” button to open the main display of the Incub8-Screen software. Click “Customize” button to adapt your experiment with the “Customize Experiment Setting” dialog.

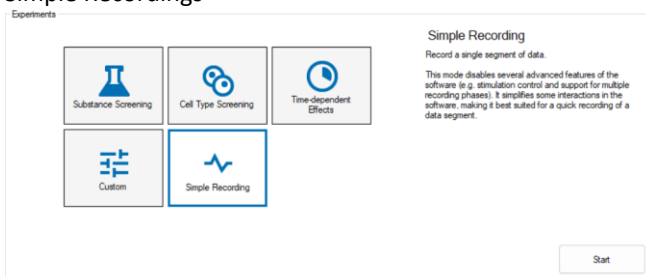
Click “Save” button to store your settings. The software will start in the defined mode until you change your settings. For changing, please click the “Application” menu, option “Define Experiment” to start up the “Experiment Selection” dialog again.

Custom



“Custom” experiments are predefined to “Define a custom experiment”.

Simple Recordings



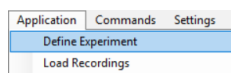
This mode disables several advanced features of the software (e.g. stimulation control and support for multiple recording phases). It simplifies some interactions in the software, making it best suited for a quick recording of a data segment.

Click “Start” button to open the main display of the Incub8-Screen software. Click “Customize” button to adapt your experiment with the “Customize Experiment Setting” dialog.

Click "Save" button to store your settings. The software will start in the defined mode until you change your settings. For changing, please click the "Application" menu, option "Define Experiment" to start up the "Experiment Selection" dialog again.

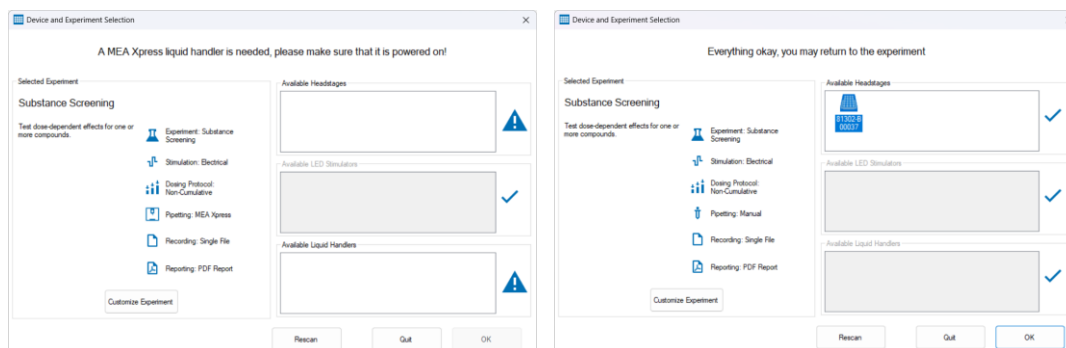
Redesign of an Experiment

You can change the experiment type and the selected software modules from the main window of Incub8-Screen: Please click on the menu "Application" the option "Define Experiment". The "Device and Experiment Selection" dialog opens again, and you can change your choices.



Missing Hardware in an Experiment

If some hardware is missing that is necessary for the selected experiment, the "Device and Experiment Selection" dialog is shown.



It gives you an overview of the available devices and highlights which devices are missing. You should either make sure that all necessary devices are connected or use the "Customize Experiment" button to modify your experiment so that it fits to the available hardware.

For example, if your experiment had been configured to use LED stimulation, but no MW-Opto-Stim device is connected, this dialog will notify you that no LED stimulator is available. If you were not planning to use LED stimulation in your current experiment, use the "Customize Experiment" button to select either "None" or "Electrical" for the stimulation module.

If you connect a device to your PC, it should be automatically shown in this dialog. If not, use the "Rescan" button to actively scan for connected devices.

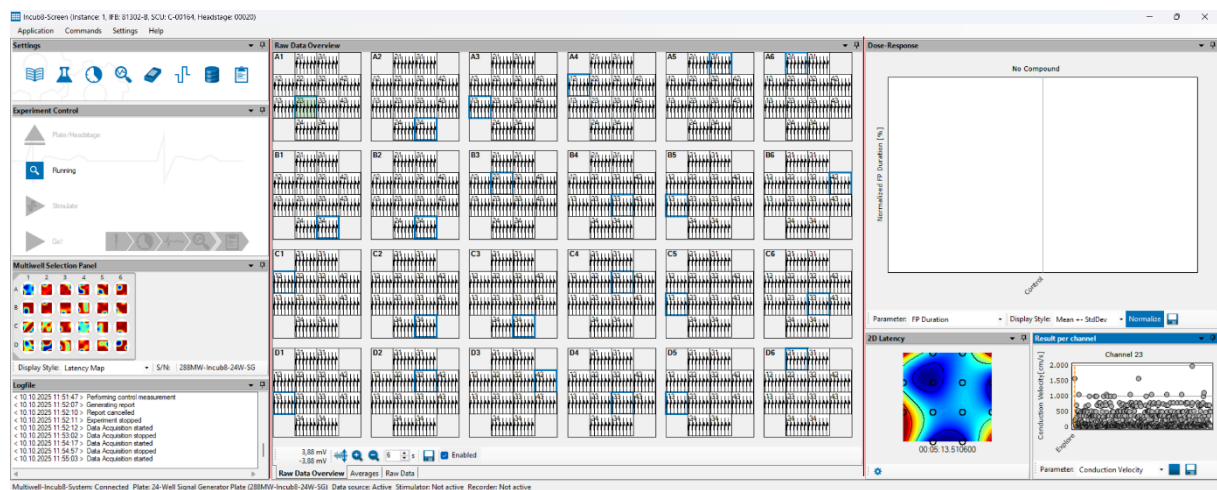
The main menu of Incub8-Screen opens after these selection processes by clicking "OK".

Incub8-Screen: Cardio Application



Main Menu

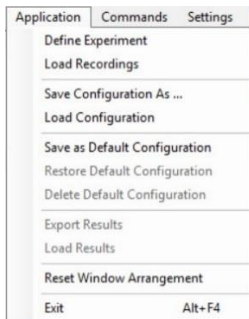
Main Menu of Incub8-Screen in “Cardio” mode:



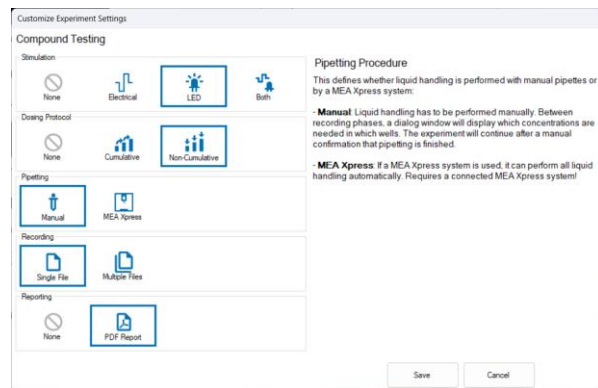
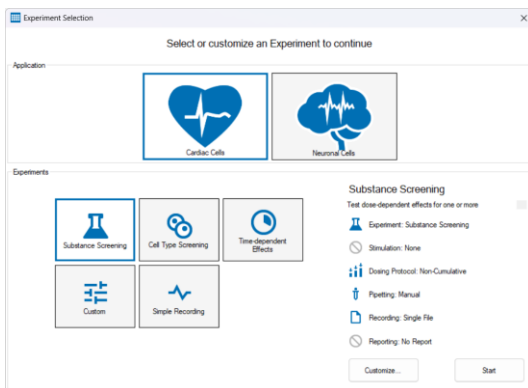
The main menu window is divided into three parallel sections: The left side contains control functions, the middle part displays data in overview and single view, and on the right side you see the current results. All windows are framed by the menu bar above and the footer below, which holds information about the status of the hardware and / or the file name.

Menu Bar

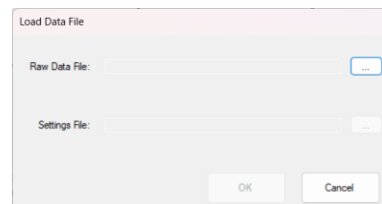
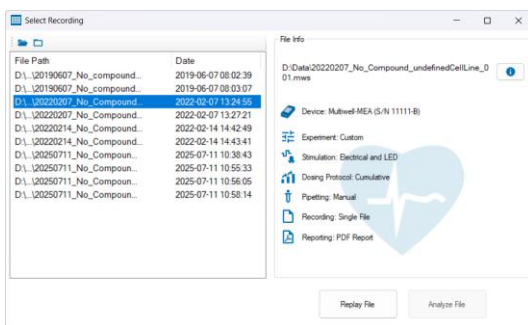
Application Menu



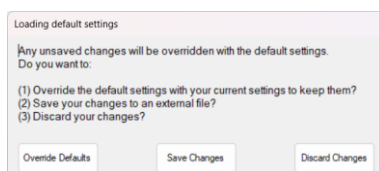
Menu to change the "Application" mode via first option "Define Experiment". The "Experiment Selection" dialog appears. Click the desired experiment type and start or the button "Customize" to customize experiment settings.



Menu to "Load Recordings" for replaying files.



Menu to "Save and Load Configurations" and to "Save, Restore and Delete Default Configurations".

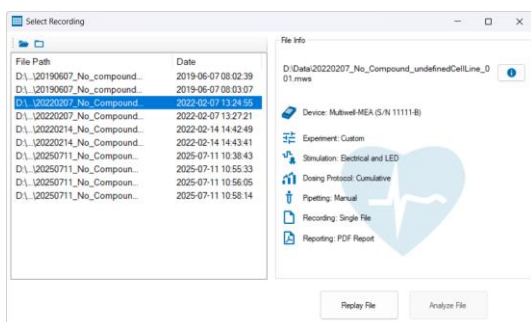


Menu to "Export Results" and "Load Results".

Menu to "Reset the Window Arrangements" restores the factory defaults of the docking status of the windows.

Menu to "Exit" the program.

Load Recordings

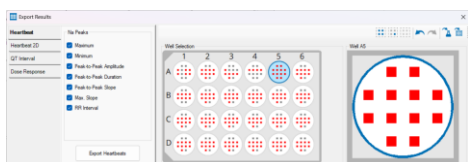


The "Load Recordings" menu allows you to select a recording and load it into either the Incub8-Screen Replayer or the Incub8-Analyzer.

Load Results

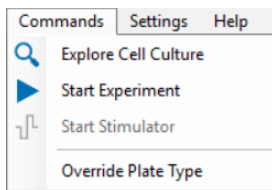
Immediately after starting the Incub8-Screen software before inserting a new well plate, it is possible to load a result file for further analysis. The main menu is empty except for the result section. Change the analysis parameter for a different analysis of the dose-response curve.

Export Results



To be free for further analysis of the results created with Incub8-Screen, the software offers the possibility to export results to ASCII format. Export the result file as a comma separated value file "*.csv" or as Excel "*.xlsx" format. Please read chapter "Result Files" later on.

Commands



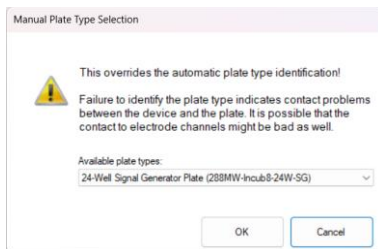
The options in the “Commands” menu depend on the hardware which is connected.

Menu to start and stop the exploring mode "Explore Cell Culture" or an "Experiment".

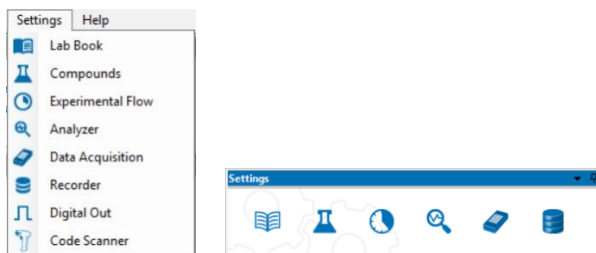
Menu to start and stop the electrical stimulation with "Start Stimulator".



Click "Override Plate Type" to open the plate type settings drop down menu in case the Incub8-System will not identify the plate type by itself. Select the currently used plate type and override the setting. If necessary, the well plate inserted in the headstage will automatically be moved into the respective position for recording.



Settings



This menu is also available as "Settings" dialog in the control section of the main menu.

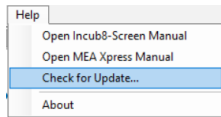
The options in the “Settings” menu depend on the selected tools and the hardware which is connected.



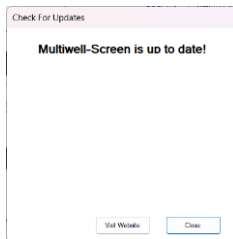
Examples: “Cell Type Screening” experiment with “Stimulation” and “Report”, “Time Dependent” experiments with “Stimulation and “Report” or without “Stimulation” and “Report”.

Help

Menu to view the online help and the "About" dialog and to check for updates.

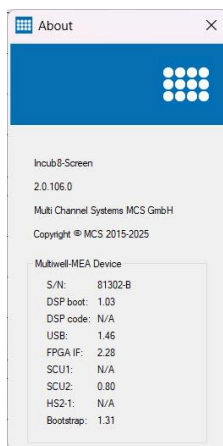


Enter the online help with the "Open Incub8-Screen" commands. The option "Check for Updates" provides fast access to the MCS web site.



If the Incub8-Screen software is not up to date, click the button "Visit Website" and download the newest software version.

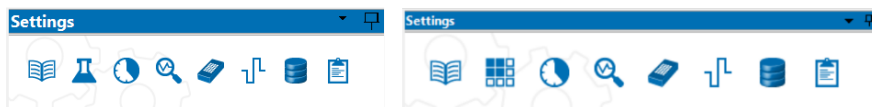
Click "About" to open the dialog. See the version number of the actual software and firmware information and about the Incub8-System device.



Control Section

Settings

First dialog of the "Control Section" is the "Settings" window.



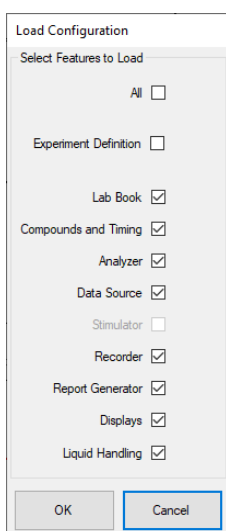
The icons displayed in this window vary depending on the experimental needs. Select the tools in the "Device and Experiment Selection" dialog, the desired tools are mirrored in the "Settings" window.

Settings Files

You can store default parameters for the Incub8-Screen software to have direct access to your preferred experimental settings each time you start the software. This covers not only display settings, but also analyzer settings, experiment settings, recorder settings and the content of all other dialogs accessible from the "Settings" section of the main window.

The default settings are specific for each plate layout and application mode, "Cardio" or "Neuro" and are loaded each time. A plate is inserted in the Incub8 headstage. To store the current configuration as default settings, select "Save as Default Configuration" in the "Application" menu. To revert the current configuration to the default configuration, select "Restore Default Configuration" from the "Application" menu. To delete the current default configuration and revert to the factory defaults, select "Delete Default Configuration" from the "Application" menu.

If a set of different experiments is run regularly, it can be beneficial to have a default configuration containing the common settings between the experiments, and a set of individual settings files, each storing the settings specific for a single experiment. The current configuration can be stored in an external configuration file in XML format by selecting "Save Configuration As..." from the "Application" menu. Pressing "Load Configuration" in the "Application" menu allows the loading of either such a settings file in XML format (*.xml) or a *.mws file which is generated when an experiment is started, storing the settings of this experiment.

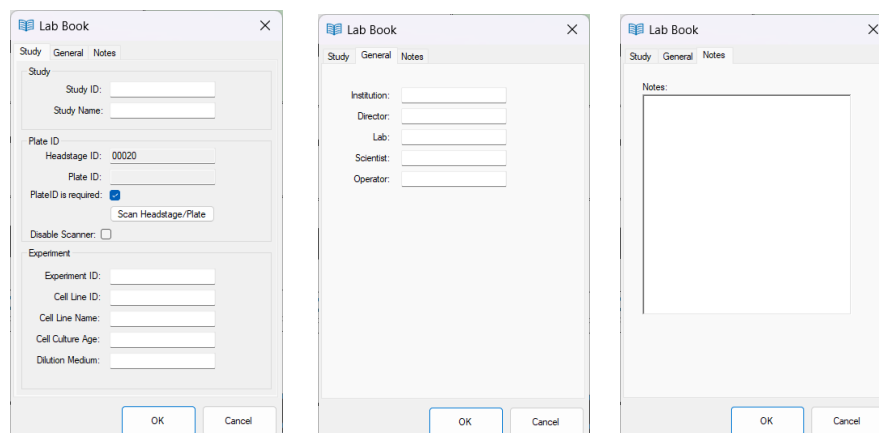
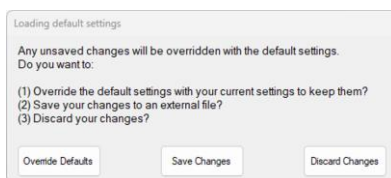


When loading a configuration, you can select which parts of the settings should be loaded. For example, if you want to keep your current "Analyzer" settings but load everything else from the settings file, disable the "Analyzer" check box in this dialog. Loading a configuration is only possible if neither an experiment nor an exploration is running.

The configured experiment type and the selected software modules might differ between the current configuration and the one in the configuration file. You can decide whether to override your current experiment type and software modules with the ones from the file, or not. To override them, activate the "Experiment Definition" check box in the "Load Configuration" dialog.

If settings have been modified with respect to the default settings and the software attempts to load the default settings again, either because the "Restore Default Configuration" entry in the "Application" menu has been selected, or because a plate has been inserted, the software prompts the user on how to proceed before overwriting the current configuration. This gives the user three options:

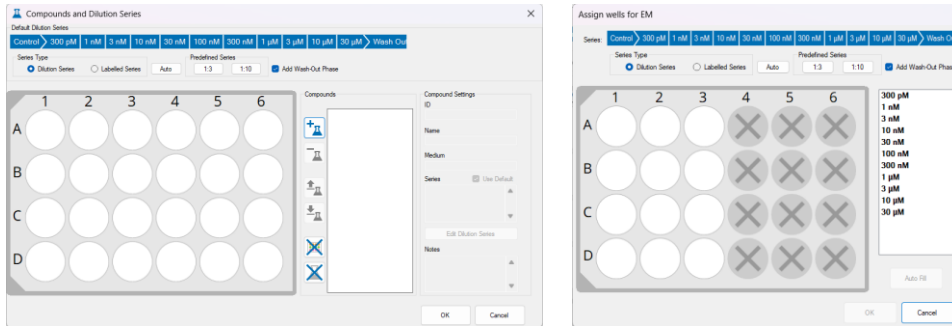
- Replace the default configuration with the current configuration by overwriting it.
- Save the current configuration in XML format.
- Discard the changes/current configuration.





Dialog with three tabs for general information about the experiment. Fill in data referring to your experiment in "Study" tab, "General" notes about the scientist and institution and free text in "Notes" tab.

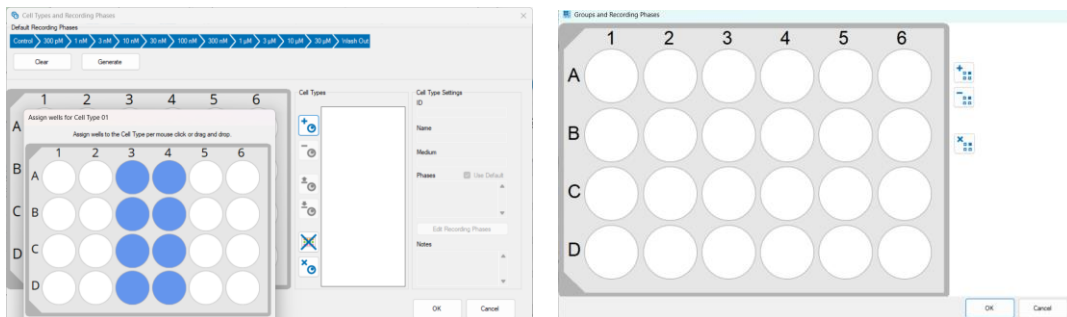
The "Lab Book Settings" will be stored in the "Multiwell" settings file *.mws.


This dialog allows you to define groups of wells that are treated the same way in your experiment. The look of this dialog and its functionality differ slightly depending on the selected experiment type:




“Compound Screening” (left screenshot),  is used to define the compounds and dilution series during the experiment. You may specify either dilution series with certain molarities for each compound or use freely assigned labels.

“Cell Type Screening” (right screenshot),  is used to define different cell types in the experiment. You can define a label for each recording phase of the experiment.

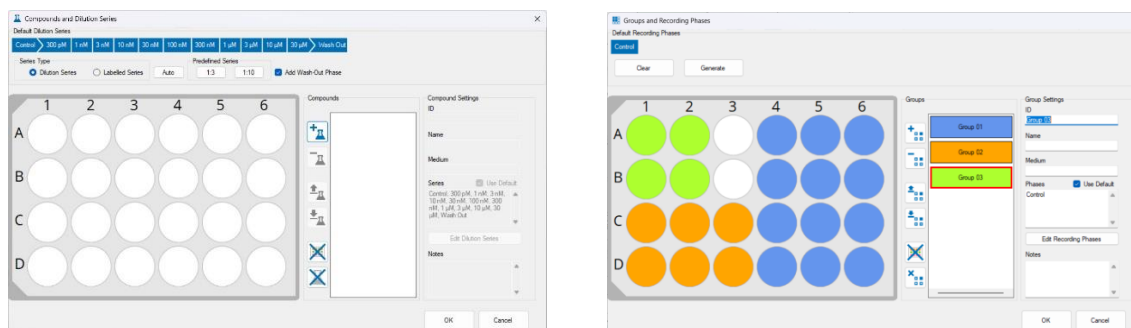


For **“Time-dependent Effects”** and **“Custom”** (left screenshot), the more general term "Group" is used, and the icon  shows a 3x3 set of rectangles. For “Custom” mode, the recording phases in each group can either be specified as a dilution series or as freely assigned labels. In “Time-dependent Effects” mode, only free labels are possible.

For **“Simple Recordings”** the icon  is the same 3 x 3 group of rectangles. However, the dialog is much simpler, it is only possible to create or delete groups of wells. Because only a single data segment is recorded in this mode, it is not necessary to define labels for the recording phases.

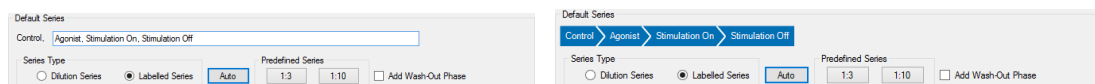
To avoid too much redundancy, the next dialog is shown here only for **“Compound Screening”** experiments, because its variants work very similar and only look slightly different.

Define „Compounds and Dilution Series“ in “Compound Screening” Experiments

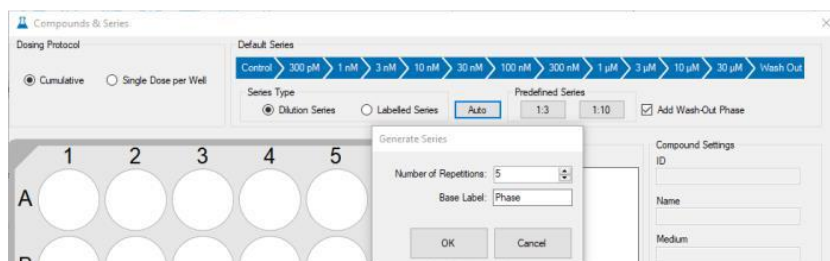


Default Dilution Series

Define a default dilution series for all compounds. Two predefined series of default dilutions are available, 1 : 3 and 1 : 10. "Add Wash-Out Phase" via check box, if necessary. It is not possible to change "Control", which always has to be the first phase of an experiment.



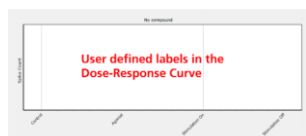
“Auto” generation of Dilution Series



Please click the button "Auto" if you want to generate series automatically. The "Generate Series" dialog appears. Select the "Number of Repetitions" from the up down box and assign a name to the created series in the "Base Label" window. This feature is useful for longterm studies, for example.

Series Type

Select the "Series Type" via radio button, "Dilution Series" or "Labeled Series". Please click the "Edit" button or click on the graphic to rename the labels. The user defined labels will also appear in the "Dose-Response Curve."

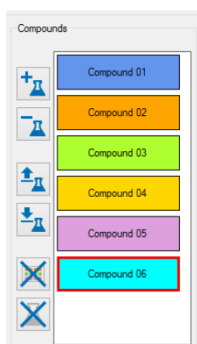








The “Dilution Series” have to be built from concentrations of the compound, for example “10 pM”, in following units: pM, nM, μ M and mM. The dilutions must be arranged in increasing order, separated by commas.

The “Labeled Series” with freely selected names should not contain a comma in the label, because again the compounds of a series have to be separated by commas.

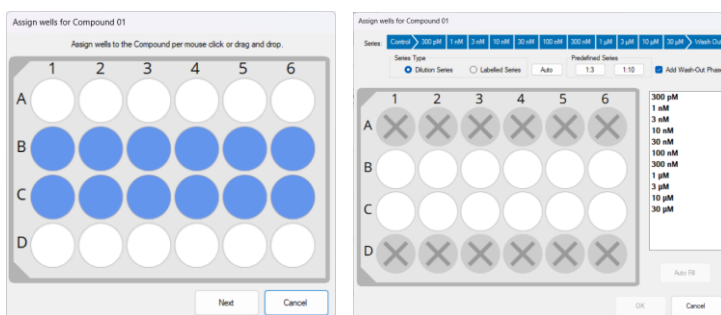
In both cases it is essential that a label is used only one time, duplicates of names are not allowed!

Compounds



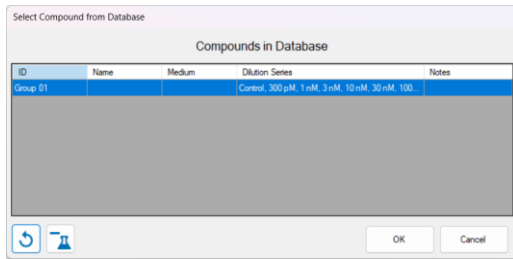
Add  up to 24 or 96 new compounds. Remove  compounds, delete all compounds  and clear the assignment for all compounds . Load a compound  from the compound database or save  a compound to the database. Click "OK" to proceed. The "Assign Wells for Compound X" dialog opens.


Assign Wells to Compounds

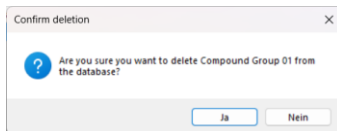


Please assign wells to the compound by drag and drop – das ist kein drag and drop “Click the desired concentration or label and assign it to a well”. Click "OK" to proceed.

As each well has to receive exactly one concentration in this protocol, assign concentrations to wells by clicking the concentration on the selection window (Autofill?), then selecting wells for these concentrations by clicking on the left well window. Pressing this button (Autofill?) assigns one or more columns of wells to each concentration so that all are distributed equally. Click "OK" to create the compound. For the "Single Dose per Well" protocol, the overview in the dialog will show the concentration assigned to each well.

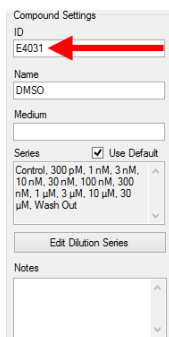


Delete a compound with the button "Delete Compound from Database" . Please confirm the removal of the entry in the "Confirm deletion" dialog.

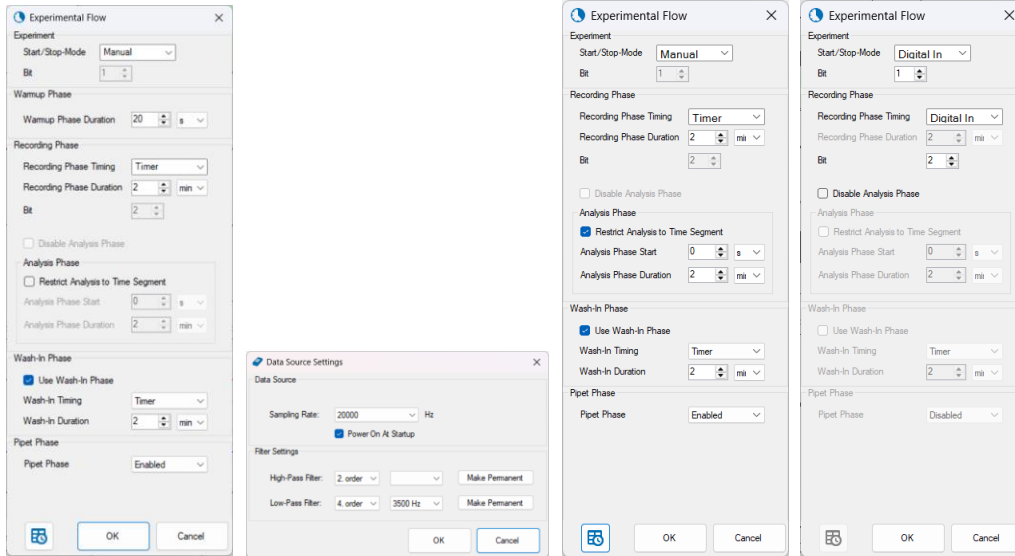


Conflicting IDs

If you try to load a compound from the database with the same ID as already open in the "Compounds" window of the "Compound & Dilution Series" dialog, the software points on this fact, which is not allowed and shows the "Conflicting compound ID's" dialog. Only the entries in the windows "Name", "Medium" and "Notes" are reloaded, but not the "ID" of the respective compound.



The user is asked to choose another compound ID manually in the "Compound Settings" window of the "Compound & Dilution Series" dialog.



The dialog „Experimental Flow“ is available in different appearances to meet your requirements.

Please define the “**Start/Stop Mode**” manual or digital in. Define the “**Bit**” in the “**Experiment**” section, if “**digital in**” is selected.

Define the “**Warm-up Phase Duration**” when using the “**Wake-up Mode**” (available under “**Data Acquisition Setup**”)

"Define the length of the "Recording" and "Wash-In" phases of the experiment. Within the "**Recording phase**", an "Analysis" phase can be optionally defined. This has the effect that all the data during the recording phase will be recorded, but the online data analysis will only run on the time window within the analysis phase. If no analysis phase is defined, the online analysis will run on the full recording phase.

Add a "**Wash-In Phase**" and a "**Pipet Phase**", if necessary.

Wake-up Mode

The settings in the „Experimental Flow“ dialog corresponds with the settings in the “**Data Acquisition Setup**” dialog. Please enable the checkbox “**Power on at Start-up**” to use the “**Wake-up**” mode.

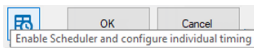
Important: Power down the Incub8-Screen software and start the software again, each time you enable or disable this checkbox.

For recording intervals, please define the “**Warm-up Phase Duration**” and the “**Recording Phase Duration**”. The initialization phase guarantees a warming up of the headstage, until stable recordings are possible. After recording the Incub8 headstage is switched off again.

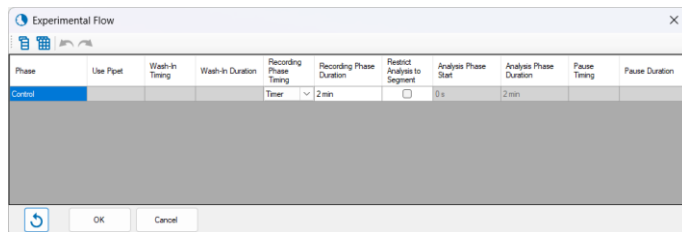
The “**Wake-up Mode**” gives the possibility to switch the headstage of the Incub8-System off during long term experiments. Switch the headstage off, for example, when using it in the incubator to prevent that additional heat from the headstage influences the well-adjusted temperature in the incubator.

In case you do not use the “**Wake-up Mode**” please lower the temperature in the incubator of about 3 to 5°C depending on the volume and the air impermeability of the incubator used.

Configuration of a Detailed Time Schedule

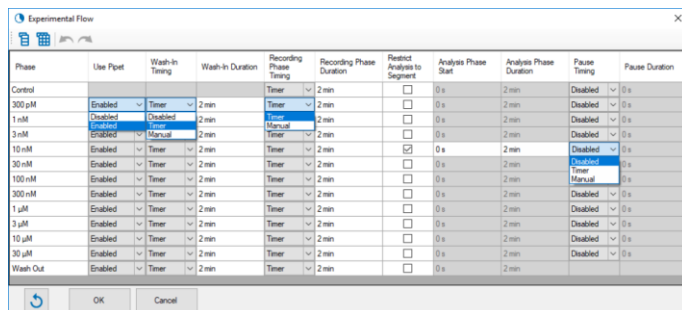



Please click the "Enable Schedule and configure individual timing" button in the "Experimental Flow" dialog to open the "Time Schedule" dialog for individual experiments. The schedule for the timing of each segment of the experiment is highly flexible: You can change the parameter for each concentration independent from others.




Please define the concentration series in the "Compound and Series" dialog first, otherwise the time schedule will not match with the number of phases set before. In this case, if an experiment is started, the following error message appears. After correcting the schedule, the experiment can be started.

Please define for each "Phase" of your experiment, whether you "Enable" or "Disable" the "Pipet" phase. Set the timing for the "Wash In", the "Recording", the "Analysis" and for the "Pause" phase to "Enable", "Timer" or "Manual". Define the "Duration" of these phases individually in seconds, minutes and hours.

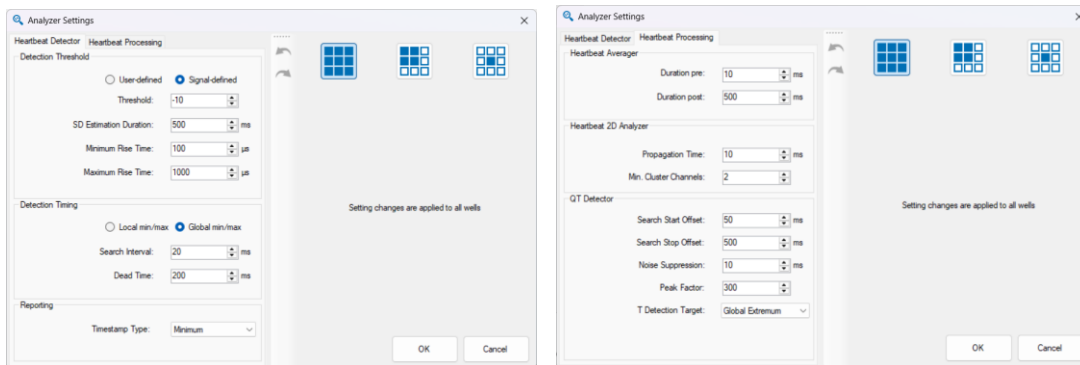


Use the "Copy Cell" button  to copy the contents of the current cell to all other cells in the same column.

Use the "Copy Row" button  to copy the current row to the complete time schedule.

Use the arrows to redo or undo last changes.

Please define first the level you like to work on: Should changes in the settings of the "Analyzer Settings" dialog affect all electrodes of the plate, all electrodes in a group, or the electrodes in one well only. The default is "Setting changes are applied to all wells". Please see chapter "Tutorial".

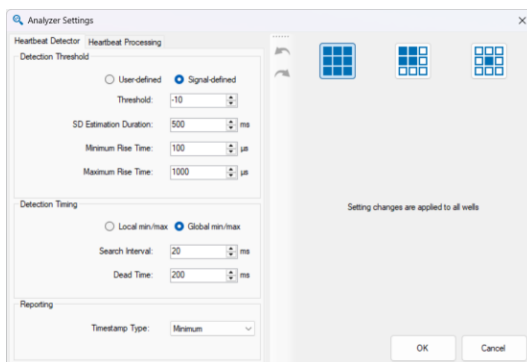


Tooltips are available and guide through the settings of the "Analyzer Settings" dialogs.

The "Analyzer Settings" dialog has two tabs for the configuration of the analyzer instruments: "Heartbeat Detector" and "Heartbeat Processing".

It is useful to configure the "Analyzer Settings" when the "Explore" mode is running. All changes are directly sent to the instruments, that is why you can directly see the effects on the data stream. Decide based on the running data, whether your actual settings fit or not.

Heartbeat Detector Tab






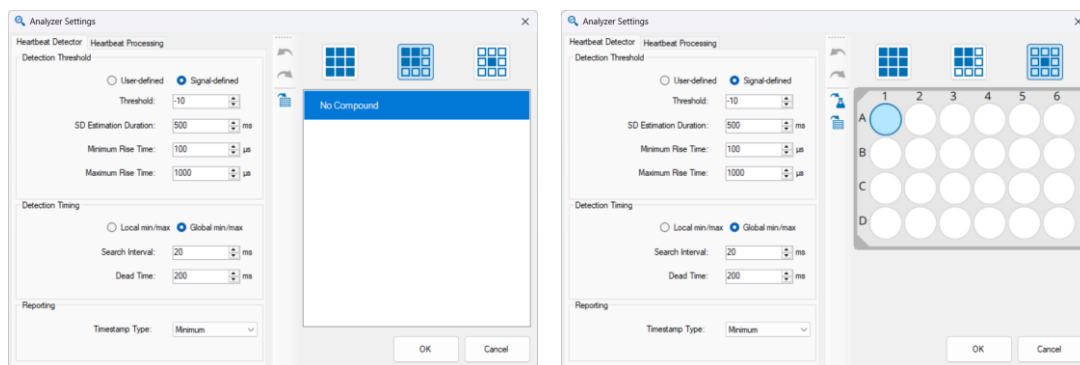
The left side of the dialog allows to define the parameters for detecting the waveform of a heartbeat / field potential.

In "Detection Threshold", please select "User-defined" or "Signal-defined" via radio button and "Threshold" with the numeric up-down boxes. Define the "SD Estimation Duration" in ms. Define the "Minimum Rise Time" and the "Maximum Rise Time" in μs .

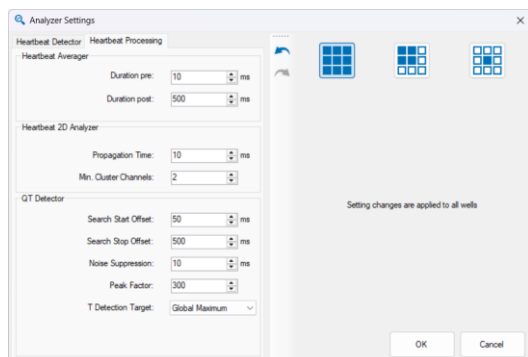
In "Detection Timing", select "Local min/max" or "Global min/max." "Global min/max" searches for the global minimum and maximum within the whole search interval. "Local min/max" extracts the first minimum from the second half of the search interval and the local maximum directly preceding this minimum. Define the "Search Interval" and the "Dead Time" in ms.

In "Reporting", please define the "Time Stamp Type" from the drop-down menu.

The right side of the dialog allows the assignment to the wells, the "Well Selection".  "Changes are applied to all wells" is the start screen. Click the "Changes are applied to the selected well group" or "Changes are applied to the selected well" button   and one of the following dialogs appears.



Heartbeat Processing Tab



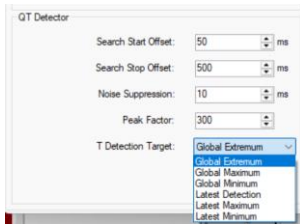
The "Heartbeat Processing" allows to define the "Heartbeat Averager", the "Heartbeat 2D Analyzer" and the "QT Detector".

The "Heartbeat Averager" averages the waveform of a signal in relation to each heartbeat in a time segment. Display several recordings of an electrode channel overlay and average the values. The pre and post time in ms before and after a heartbeat can be selected via up-down box.

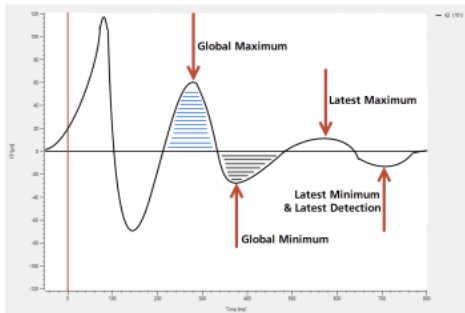
The "Heartbeat 2D Analyzer" calculates the latency between the different electrodes in one well. Define the time span in ms from the drop-down menu in which the "Propagation Time" should be calculated. The action potential runs over the heart cell culture or tissue or whole heart from the pacemaker to the peripheral regions of the syncytium. The excitation propagation is measured in high spatial resolution by the electrodes detecting cardiac field potentials and extracting local activation times. The propagation time is the longest possible period for passing of the excitation from the first detected excited electrode to the last detected excited electrode of one cluster. If the propagation time is too short, you miss data for the complete heartbeat. If the propagation time is too long, the next heartbeat starts and the results are incorrect.




The "Min Cluster Channels" gives the minimum number of channels at which heartbeats have to be detected to form a cluster. For example, if a syncytium covers all 12 electrodes of a well it could be 6 otherwise it should be less than 6. This function is displayed in the lower part of the "Result Section", the display "2D Latency" map.

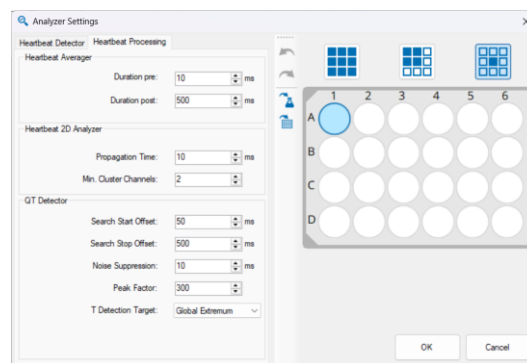
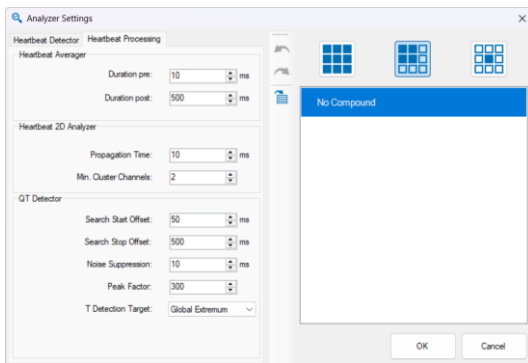
The "QT Detector" detects the QT-like interval in the averaged heartbeats. Define the parameters for the QT-like interval: The level of the offset, the "Search Start Offset" and the "Search Stop Offset" in ms and the "Noise Suppression" in ms from the up-down boxes. Select the "T Detection Target" from the drop-down menu.



The "T Detection Target" defines your preferred detection target. The "Global Extremum" describes the extremum over the greatest integral. "Maximum" or "Minimum" define the respective maxima or minima. Please see the following picture.



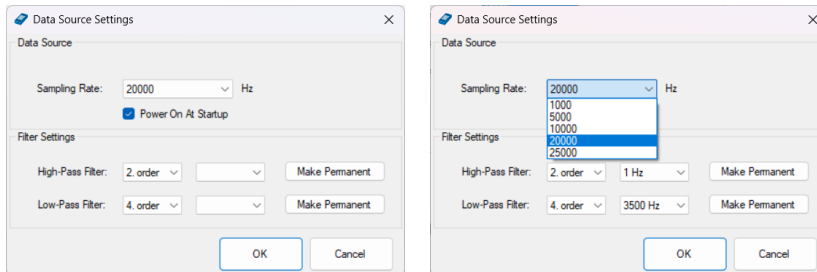
The right side of the dialog allows the assignment to the wells, the "Well Selection".  "Changes are applied to all wells" is the start screen. Click the "Changes are applied to the selected well group" or "Changes are applied to the selected well" button   and one of the following dialogs appears.





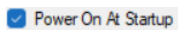
Data Acquisition

Click the "Data Acquisition" button to open the "Data Source Settings" dialog.



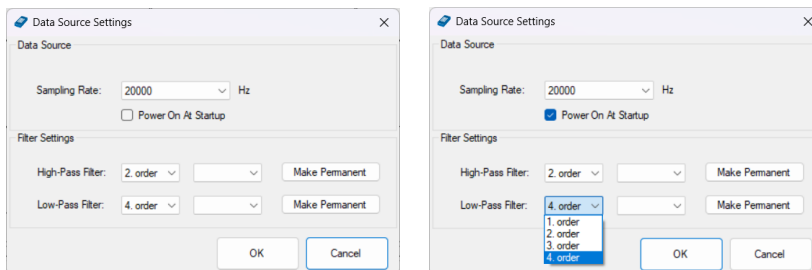
Data Source

Please select the "Sampling Rate" in Hz in the "Data Source" window from the drop-down menu.



Enable or disable the checkbox for the "Wake up Mode" "Power On At Startup". Each time when clicking this checkbox it is necessary to restart the software Incub8-Screen to apply the desired mode. Please read also chapter "Experimental Flow" for information about the "Wake-up Mode".

Filter Settings



Set the "High- and Low-Pass Filter" settings of the Incub8 device with the drop-down menus. Choose between 1st and 2nd order Butterworth filters for the "High-Pass Filters" and between 1st and 4th order for "Low-Pass Filters".

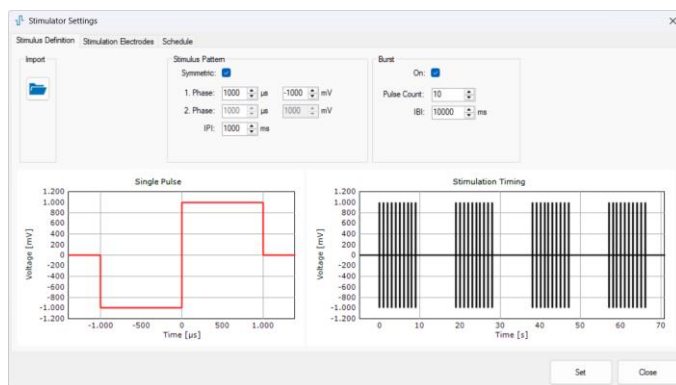
If the data acquisition is not active, press "Make Permanent" in order to write the filter settings to the device. This ensures that the same settings will be loaded after rebooting the Incub8 device.

The Incub8-Screen software controls electrical voltage stimulation in the range between -5000 mV and +5000 mV. Optical stimulation will be available in future.

Electrical Stimulation

Please select "Electrical" or "Both" in the "Stimulation" module otherwise the "Stimulation Settings" dialog is not available.

The headstage of the Incub8-System is equipped with an internal stimulator for voltage driven stimulation. Each electrode in each well can be used for stimulation via Incub8-Screen software. Currently, one stimulus pattern is available which can be inverted. Bipolar and monopolar stimulation is possible. For biphasic pulses, it is recommended to start with the negative part of the stimulation pulse.



The "Simulator Settings" dialog has three tabbed pages.

Stimulus Definition

"Import": Press the "Import" button to import a stimulation pattern from an ASCII file.

"Stimulus Pattern": Choose the shape of the stimulation pattern via check box "Symmetric". Modulate the amplitude of the rectangle pulse of "Phase 1" in mV and the duration in μs via up-down boxes. Modulate the amplitude of the rectangle pulse of "Phase 2" in mV and the duration in μs via up-down boxes when creating an asymmetric pulse. Define the inter stimulus interval "IPI" in ms via up-down box.

"Burst": Enable the check box "On" for burst stimulation. Define the number of pulses per burst, the "Pulse Count" and the "IBI", the inter burst interval in ms via up-down box.

The settings in the up-down boxes influence the complete stimulus pattern which is immediately displayed in the window below. On the left side you see the "Single Pulse", on the right side the "Stimulation Timing" of the bursts is shown. Download the stimulation parameter settings to the internal stimulus generator with the "Set" button.

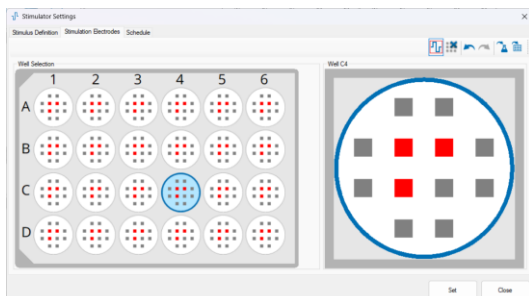
Import a Stimulation Pattern

It is possible to import user defined stimulation patterns from ASCII files. The ASCII file with stimulation pattern must have a determined format. The best way to get this required format is to create the stimulation pattern with the MC_Stimulus III software from Multi Channel Systems MCS.

Once imported, the stimulation pattern is shown in red, while the associated time course of the stimulation blanking and sync out signal is shown underneath it in blue. As for the simple biphasic pulses, this

stimulation pattern will be repeated continuously. Use the "Repeat Pause" numeric up-down box to define a pause duration between repeats. You can press the "Import" button to load a different ASCII pattern, or the "Discard" button to discard the imported pattern and return the definition of simple biphasic stimuli.

Stimulation Electrodes




Dialog to select the wells and the electrodes to which you want to apply the stimulus. In voltage controlled stimulation mode, you can assign more than one electrode per well to a stimulation pattern.

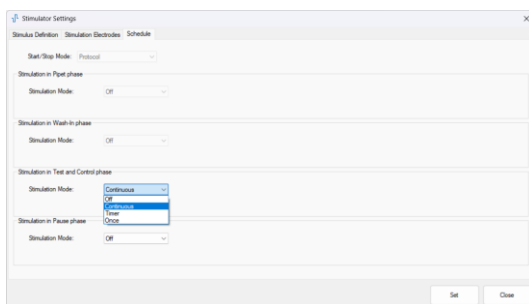
The defined stimulation pattern is indicated in red  and can be assigned to one or more of the electrodes, click on one of the icons and select the electrode you want to assign the pattern to.

Delete the electrode selection for the selected stimulator with the "Clear" button .

Undo or redo the last command with the arrow buttons. Apply the stimulation pattern to all wells with

the same compound or to all wells  of the plate. Download the settings to the internal stimulator with the "Set" button.

Schedule

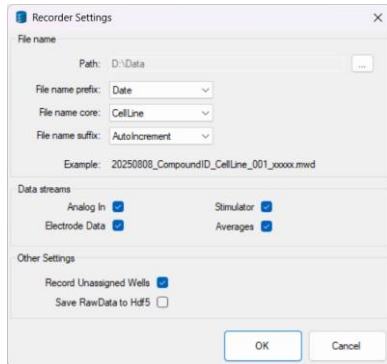


Start / Stop Mode

Define the start and stop mode from the drop down box. The stimulation is started or stopped manually or via protocol.

Define the "Stimulation Mode" in the respective experimental phases from the drop down menus in the "Pipet" phase, the "Wash-in" phase and in the "Test and Control" and for the "Pause" phase.

The options "Off", "Continuous", "Timer" or "Once" are available.



Dialog to define the "File name" and the "Path" where you want to store the files. Select a "File name prefix", a "File name core" and a "File name suffix" from the drop-down menu.

Select or deselect the "Data streams" you are going to record by clicking on the check boxes. Deselect data streams to reduce the size of the recorded files, but do not forget that the opportunities for offline analysis are restricted. For example, when deselecting the "Electrode Data", raw data is not recorded. Therefore, it is not possible to reload data in the "Replayer Mode" of Incub8-Screen or to display or to work on electrode raw data in the Incub8-Analyzer.

For each compound different files are created:

Extension *.mwd: Raw data files in binary format (analog, digital and stimulation)

Extension *.mwr: XML-files with analysis results and analysis settings.

Extension *.mws: XML-files with general settings of the experiment.

Optionally: Extension *.mwc: Cutouts ("Spike Cutouts" or "Heartbeat Averages") in binary format.

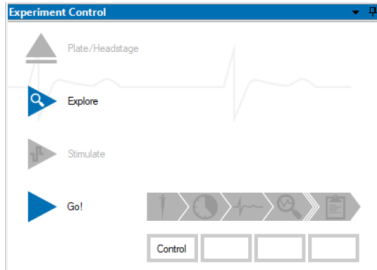
During an experiment, the names of all generated files are displayed in the status bar of the footer. Because of the huge amount of data, it is recommended to use a SSD data memory.

The check box "Record Unassigned Wells" in "Other Settings" is activated by default. Wells without group assignment will be recorded to avoid missing data in case of incorrect well assignment. Deselect the feature, if not necessary.

„Save Raw Data to Hdf5" option is a feature for processing multiwell data in third party programs.

Experiment Control

Second dialog of the "Control Section" is the "Experiment Control" window. The appearance is different for "Incub8 Experiment" or for "Incub8 Experiment with MEA Xpress". Please read the "MEA Xpress" manual for detailed information about the use of Incub8-Screen software and the liquid handler "MEA Xpress".



First step is running a test phase of the experiment without adding compounds and without recording. Use this "Explore" phase to adjust the "Analyzer Settings" to prepare the experimental phase. Click the

"Explore" button to start the "Explore" mode, and the symbol turns to

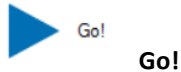


"Running".

Stop the "Explore" mode and the symbol turns to

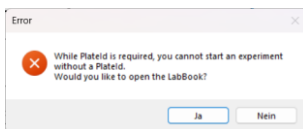


"Stop".



Go!

After checking the experimental parameters, start the experiment with "Go!". First, the software checks the "Plate ID".



Make sure that the "plate ID" is available, go back to the "LabBook". Click "Go!" again.

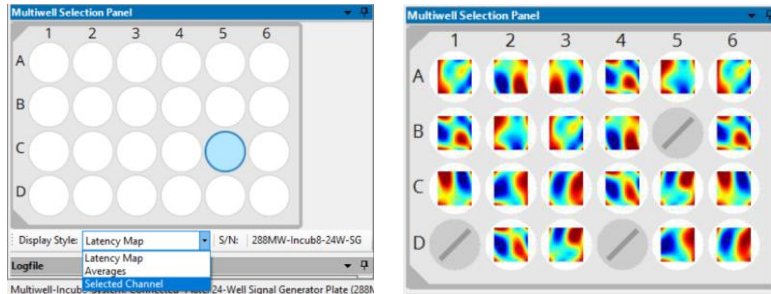
The different phases of the experiment will run automatically one after the other through the single wells of the plate. The process is stopped each time a new compound or another dilution of the compound has to be applied. Start the next phase automatically on "Timer" or manual by clicking on the respective phase icon, if "Manual" is selected in "Analyzer Settings".




The actual applied dilution and the phase of the experiment are highlighted in blue.

Multiwell Selection Panel

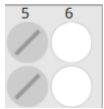
Third dialog of the "Control Section" is the "Multiwell Selection Panel". Please choose the "Display Style": In "Cardio" mode, the "Latency Map" is standard and will be displayed in each well. The serial number S/N and the type of the well plate is displayed beside.



This dialog allows the selection of the well from which data should be displayed. You can change your selection while the program is running, so you can view all wells to control the experiment without stopping in between.

Click on the desired well to select it. The selected well will be highlighted  in light blue. See the effect of your selection in the "Data Display Section" and in the "Result Section".

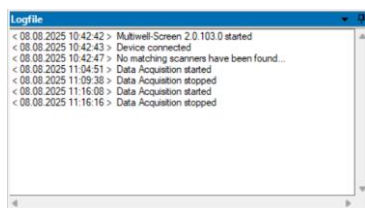
If no experiment is running, it is possible to exclude a well. Please click on the well with the right mouse button. To reselect it again, double-click the well with the right mouse.



Important: The electrode channels of a deselected well will neither be displayed nor recorded!

Logfile

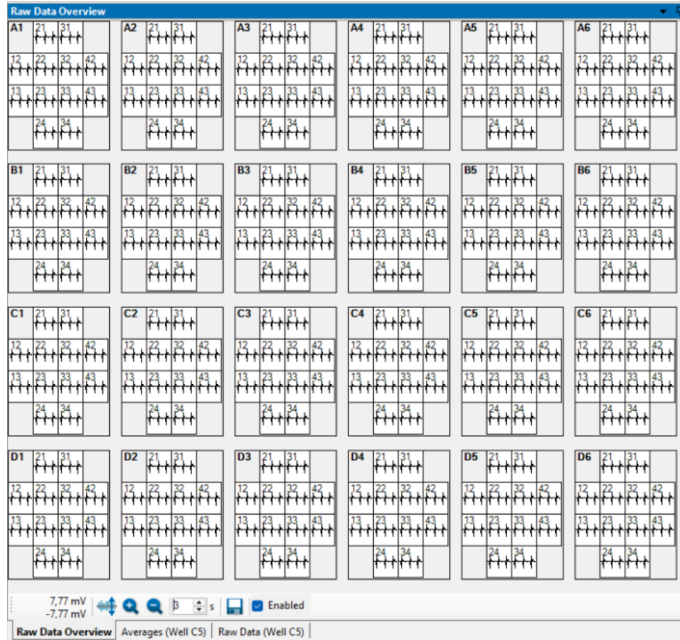
Last dialog of the "Control Section" is the "Logfile".



The "Logfile" documents the ongoing experimental proceedings online. The "Logfile" data is stored in the "*.mwr" results files.

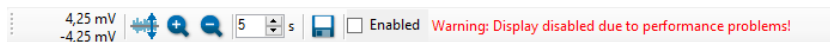
Data Display Section

Raw Data Overview



The "Raw Data Overview" window gives an overview of all wells of the actual inserted plate. The overview will automatically be adapted to 24 or 96 wells. Navigate from well to well by clicking on it.

By default, this display is initially disabled, because calculating the huge amount of data streams needs capacity and performance of the computer. Click the check box "Enable" in the footer of the window to see the data streams.

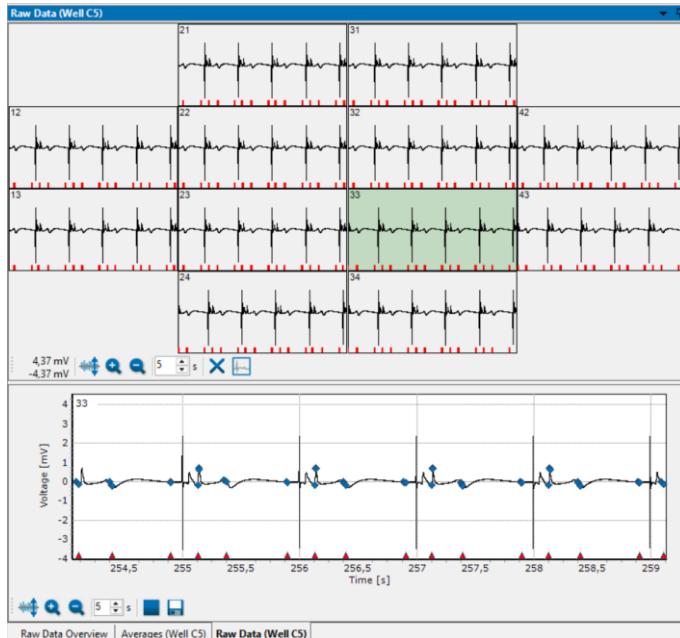


If the data acquisition computer is overloaded during an experiment, the overview display will be automatically disabled: "Warning: Display disabled due to performance problems!"


Export a picture of the "Overview Raw Data" window by clicking the button "Export to Image File". The file format is *.jpg, *.png or *.bmp.


Raw Data (Well X/Y)


The upper display shows the raw data of all electrodes in a well, so you have a good overview about the activity in the desired well. See the identification of the selected well in the blue header of the window.




The upper left electrode is selected by default. Jump between the electrodes without stopping the experiment, the selected electrode will be highlighted in green and viewed in detail in the "Single View". The red marker indicates the detection of a heartbeat or a spike in both displays.

Click the "Adjust to signal Min/Max" button . The scaling of the y-axis is set to the minimum and maximum of all samples in the well.

Clicking the button "Zoom In" cuts the scaling in a half and "Zoom Out" doubles the scaling .

Use the up-down box to scale the time x-axis in each window of the overview. Deselect electrodes by clicking the "Exclude Channel" button  first and then on the electrode. Double-click the electrode to select it again.

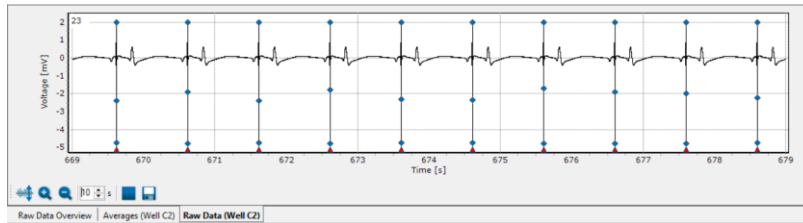
Important: Data of excluded electrodes will not be recorded and stored!

One channel of the well has to be defined for analysis and display of the "Dose-Response" curve. Define this electrode by clicking the "Select representative channel" button . The analyzed channel will be highlighted in green and framed in a blue frame.




Single View: Raw Data


The lower display shows the single view. The single view shows data of the selected electrode channel more detailed.



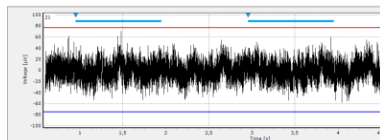
In this example, the raw data of the selected electrode No 23 is displayed. See the electrode ID in the left upper edge of the display. Each field potential is described by its data trace and two additional set points: The red triangle indicates the detection of a heartbeat, the blue diamonds mark the position of the maximum, the minimum and the maximum slope of the Na peak.

Click the "Adjust to signal Min/Max" button. The scaling of the y-axis is set to the minimum and maximum of all samples in the channel.

Zooming in cuts the scaling in a half and zooming out doubles the scaling . Additionally, you can zoom into a region of interest by moving the mouse from the left to the right while pressing the left mouse button. Move the mouse the other way round for zooming out. Please read chapter "General Software Features".

Use the up-down box to scale the time axis of the single view display. Start and stop the data display independently from the overview . In experimental mode the recording of the data will go on, even if the data display is stopped.

Stimulation Markers

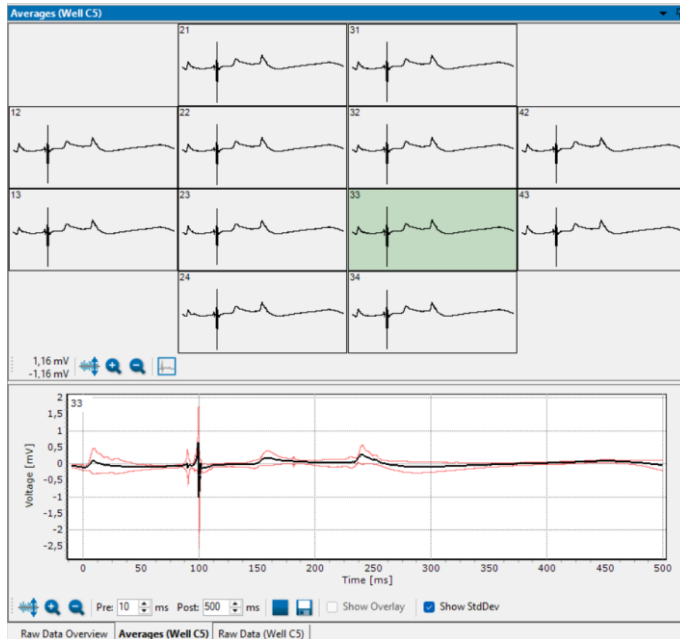


The display shows stimulation markers if stimulation was applied during the experiment. The start of a stimulus pattern is marked by a triangle in the upper part of the display.

Averages (Well X/Y)

The "Average" display is for "Cardio" mode only and like the "Raw Data" separated in the upper overview and the lower single view display.

In the "Overview" display, you see the mean values of the data segment around all detected heartbeats.



See the identification of the selected well in the header of the dialog. The upper left electrode is selected by default. Jump between the electrodes without stopping the experiment, the selected electrode will be highlighted in green and viewed in detail in the "Single View".

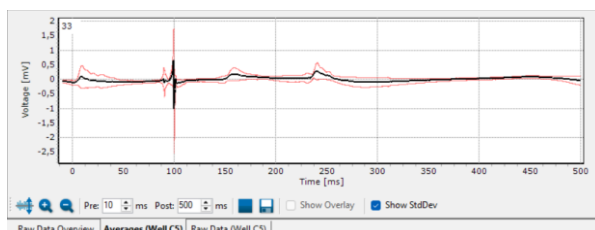
Click the "Adjust to signal Min/Max" button and read the current value beside. The scaling of the y-axis is set to the minimum and maximum of all samples in the well.

Clicking the button "Zoom In" cuts the scaling in a half and "Zoom Out" doubles the scaling.

One channel of the well has to be defined for analysis and display of the "Dose-Response" curve. Define this electrode by clicking the "Select representative channel" button. The channel analyzed will be highlighted in green and framed in a blue frame.

Single View: Averages

In the "Overview" display, you see the mean value of the data segment around all detected heartbeats. In the "Single View" you see the mean value of the data segment around all detected heartbeats in this channel (the black colored data trace) and additionally the standard deviation of each sample in red color.



In this example the average of the selected electrode No 33 is displayed. See the electrode ID in the left upper edge of the display.

Click the "Adjust to signal Min/Max" button. The scaling of the y-axis is set to the minimum and maximum of all samples in the channel.

Zooming in cuts the scaling in a half and zooming out doubles the scaling. Additionally, you can zoom into a region of interest by moving the mouse from the left to the right while pressing the left mouse button. Move the mouse the other way round for zooming out. Please read chapter "General Software Features".

Use the up-down box to scale the time axis of the single view display. Use the two up-down boxes to scale the time axis of the single view display via "Pre" time before the averages and the "Post" time. Start and stop the data display independently from the overview .

Select between checkbox "Show Overlay" or "Show Std Deviation" for detailed view to these parameters.

In experimental mode, the recording of the data will go on, even if the data display is stopped.

Result Section

View your current results in this section. Three types of result presentation are available in three windows: "Dose-Response", "2D Latency (Well X/Y)" and "Result per channel (Well X/Y)".

The upper display shows the "Dose-Response" dialog for each compound. The lower displays show the "2D Latency" map and the "Result per channel" on the base of the selected well.

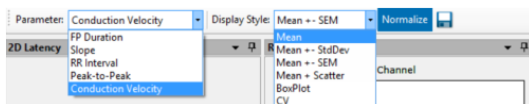
Dose-Response

The dose-response curve shows the variation of the physiological markers in relation to the concentration of the compound.

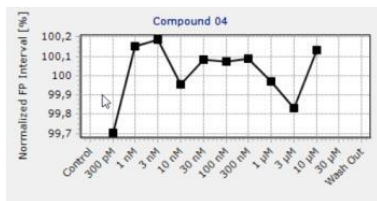
The "Dose-Response" dialog looks different in dependency of the settings. All available compounds are displayed. This example shows the field potential duration for the compounds 1 to 4.



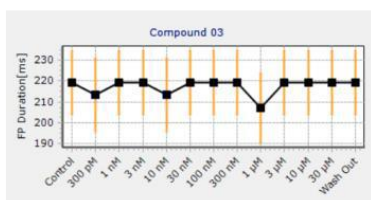
Use the drop-down menus to organize the dose-response curves.



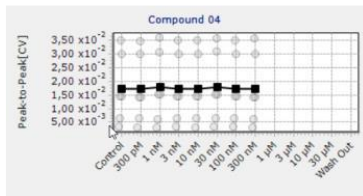
Choose the type of the curve from the "Parameter" drop-down menu: "FP Duration" describes the latency from one extremum of the T-component of the heartbeat to the next extremum. "Slope" is the maximal slope of the Na peak. The "RR Interval" is the time span between two heartbeats, measured from one maximum, that is the QRS-component to the next maximum. "Peak-to-Peak" describes the amplitude between the maximum and the minimum of a Na peak. The "Conduction Velocity" shows the velocity of the excitation, calculated between the first and the last detected electrode as a function of the time.



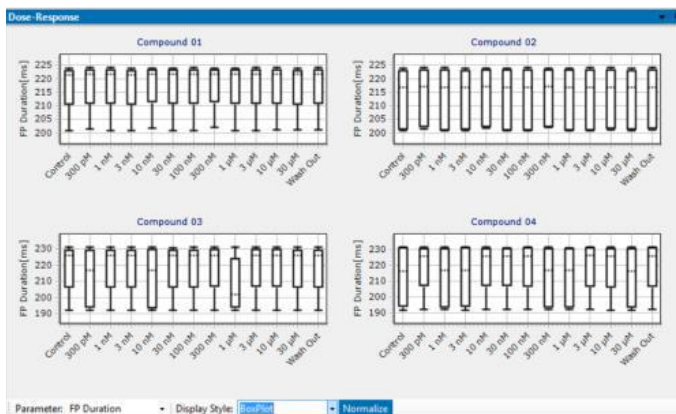
Choose the "Display Style": The "Mean" is calculated from the mean results of all wells of one compound. This simple display format does not show the variability of the wells.



The "Mean + StdDev" shows additionally ± 1 standard deviation of the mean and allows to estimate variability between the wells but does not show outlier values.



The "Mean + Scatter" plot shows additionally the mean the results of all concentrations of that well as own points. Click on the points to make the results of this well but other concentration available. Offers an easy way to find outliers and to correct them, if necessary, during offline analysis.

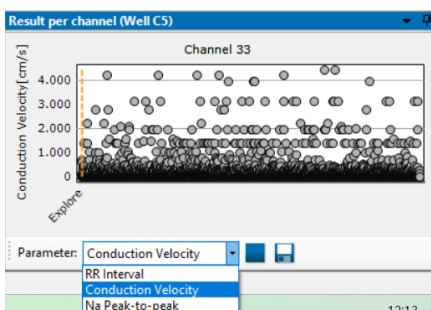


The "BoxPlot" shows the distribution of the results between the wells. You clearly see the wells which differ from the median.

The Coefficient of Variation "CV" is a degree for the variability of a distribution. The "CV" is calculated from mean divided by standard deviation and has no unit. You need at least two measurements to calculate the "CV", that is why it is useless for parameters like QT interval or spike count. The "CV" displayed in this plot is the mean of the "CV" of the concentrations in one single well.

See the variation of the physiological markers in the dose-response curve in absolute values "Raw Values" Raw Values or in "Normalize" Normalize mode. Normalized results are calculated in relation to the respective control section in %.

Result per Channel



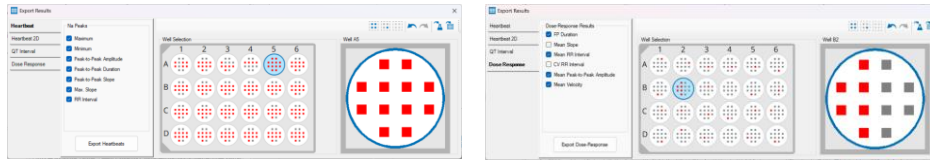
Have a single view of one channel of the selected well in the "Result per Channel" display. See the electrode ID in the middle over the display, the well ID in the header.

Define the "Parameter" via drop-down menu: "Na Peak-to-peak" or "RR-Interval" and "Conduction Velocity".

Start and stop the "Result per Channel" display independent from the compound overview ▶ ◀.

Zoom into accumulated data by moving the mouse from the left to the right while pressing the left mouse button. Move the mouse the other way round for zooming out. Please read chapter "General Software Features".

Result Files





When an experiment is finished, the analysis results can be exported by selecting "Export Results" from the "Application" menu in the header. This opens a dialog where the analysis parameters to be exported can be selected, as well as the channels.

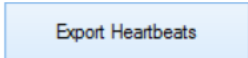
In "Cardio" mode, there are four tabbed pages from which you select the analysis parameters:

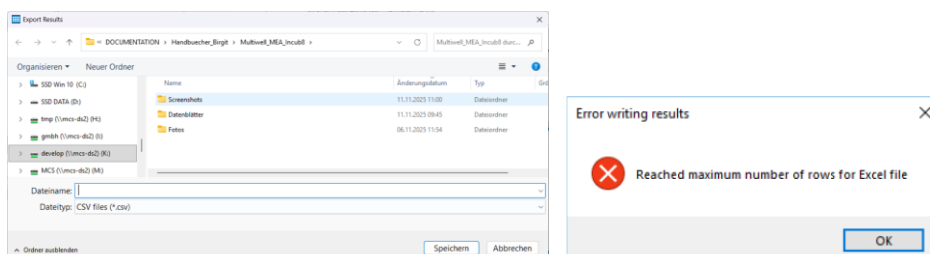
- Heartbeat
- Heartbeat 2D
- QT Interval
- Dose-Response

On each tab page, there are a number of parameters which can be selected or deselected. Clicking the "Export" button on one of the pages will export these parameters to a comma separated file "*.csv" or to an Excel "*.xlsx" file.

The tabs of the export dialogs are built analogical. On the left side of the dialog enable or disabled the check boxes to choose the parameters you are interested in. Select the designated electrode channel on the MEA electrode field and select the wells.

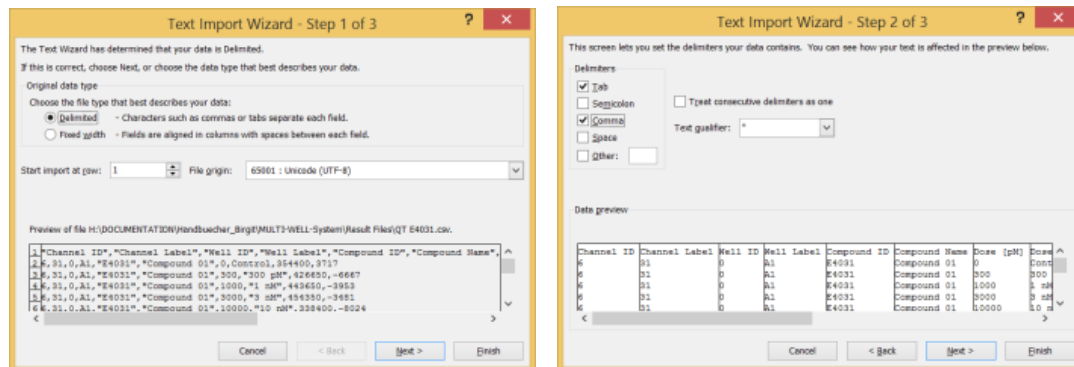
Use the "Select all Electrodes in the Well" buttons  to select or deselect all electrodes of a well, click on a single electrode to select or deselect one of them. Apply the stimulation pattern to all wells with the same compound or to all wells  of the plate.

Click the respective button  to export the file as comma separated value file "*.csv" without any limitations of data volume in one result file. Excel files "*.xlsx" are limited to 1048576 rows per file. If the limitation of data volume in Excel is reached, the following error message appears and the exported "*.xlsx" file will contain only 1048576 rows.



In order to import the generated comma separated value "*.csv" files into Excel, please use the "Text Import Wizard":

Comma separated files can also be opened, in Excel. Please import the file to Excel, do not double-click on the "*.csv" file to open it, otherwise the parameters are not separated in columns. Excel opens the "Text Import Wizard" dialog for the import of a "*.csv". Please click the option "Delimited" for the original data type and click "Next".



Select option "Comma" in "Delimiters" window. Click "Finish" to import the file.

The "*.csv" file is opened. Now you can use it for further analysis.

#	A	B	C	D	E	F	G	H	I	J
1	Channel ID	Channel Label	Well ID	Well Label	Compound ID	Compound Name	Dose [pM]	Dose Label	T Detection Timestamp [µs]	T Detection Amplitude [µV]
2	6	31	0	A1	E4031	Compound 01	0	Control	354400	3717
3	6	31	0	A1	E4031	Compound 01	300	300 pM	426050	6667
4	6	31	0	A1	E4031	Compound 01	1000	1 nM	443650	-3953
5	6	31	0	A1	E4031	Compound 01	3000	3 nM	454350	3481
6	6	31	0	A1	E4031	Compound 01	10000	10 nM	338400	-8024
7	4	22	0	A1	E4031	Compound 01	0	Control	350450	10225
8	4	22	0	A1	E4031	Compound 01	300	300 pM	380550	15517
9	4	22	0	A1	E4031	Compound 01	1000	1 nM	398950	14160

In "Neuro" mode, the spike time stamps and the spike bursts can be exported.

In the "Well Selection" part of the dialog, select a well by a left mouse button click. In the "Well" part of the dialog, you can select channels to export by clicking the grey squares. Selected channels are displayed in red. Deselecting a channel can be done by clicking it again. Pressing the "Select all" button will select all channels in the well, while pressing the "Deselect all" button will deselect all channels.

As in the "Analyzer" settings dialog, pressing the "Apply to compound" button will copy the channel selection of the current well to all wells with the same compound. Pressing the "Apply to all wells" button will copy the channel selection to all wells. The "Undo" button reverts the last action while the "Redo" button performs it again.

If no plate is inserted, results generated earlier during an experiment and stored in a file with analysis results with the extension "*.mwr" can be loaded by pressing "Load Results" in the "Application" menu. These results of a single compound are displayed in the dose-response plot and can be exported to a "Comma Separated File" with "*.csv" extension as above via the "Export Results" entry in the "Application" menu.

Incub8-Screen: Neuro Application

Tutorial for Neuro Experiments

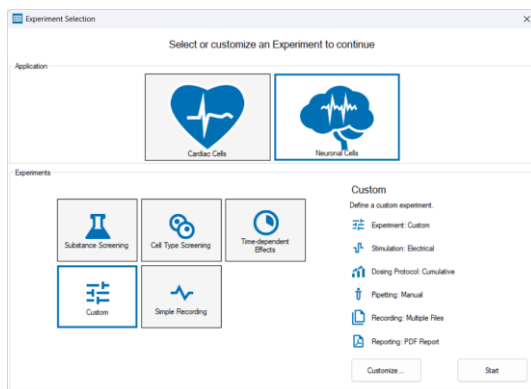
This tutorial explains step by step how to organize a neuro experiment with the Incub8-Screen software. The typical neuro experiment is arranged in different phases, but at first the experiment must be well prepared. Please read the tutorial step by step.

To start the software, please click the "Incub8-Screen" icon or select it from the program menu.



Neuro Mode

Please use option "Define Experiment" in the "Application" menu. The "Experiment Selection" dialog appears, and the first step is to define the type of experiment.



Click "Neuro Cells" in the "Application" window.



Depending on this selection, the user interface and the analysis functions are optimized for experiments with neuronal cells.

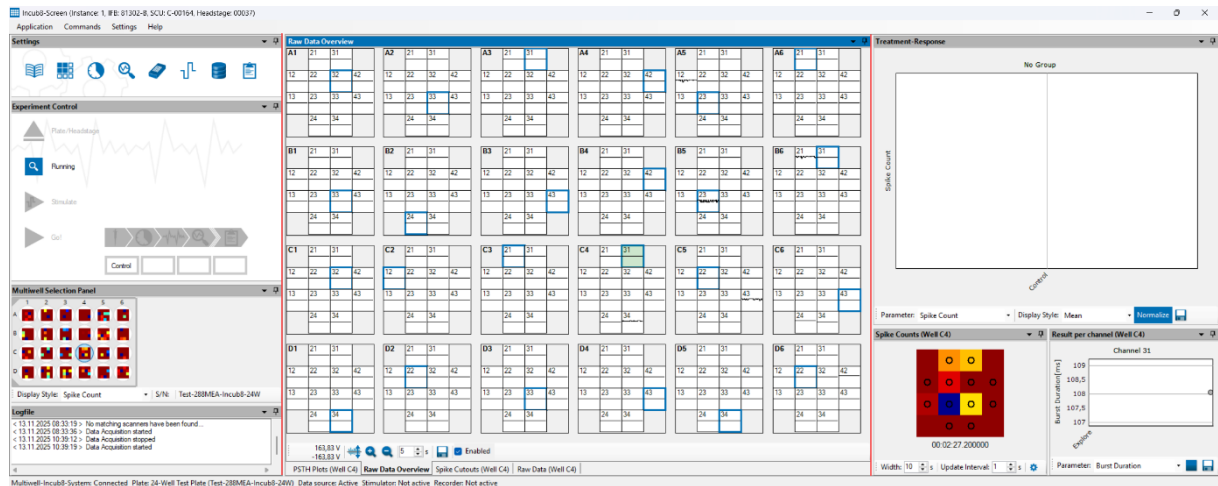
Experiments

The next step is to specify the type of experiment you are planning to run and to select the necessary software modules for it.

There are five different predefined experiments available: "Substance Screening", "Cell Type Screening", "Time-dependent Effects", "Custom", and "Simple Recording".

For settings in the "Experiments" window, please read chapter "Experiment Selection" above.

Main Window of Incub8-Screen "Neuro Mode"



The main window in "Neuro" mode is built analogously to the main window in "Cardio" mode. The left "Control Section" side consists of the same dialogs. The "Data Display Section" is different and has four tabbed pages: "Raw Data Overview", "Spike Cutouts" and "Raw Data". The "PSTH Plots" are only available, if the software module "Stimulation" is selected.

The "Result Section" on the right side holds additionally to the "Dose-Response" curve the neuro application specific dialog "Spike Counts" and "Result per Channel".

Most of the functions in "Neuro" mode are similar to them in "Cardio" mode. Please read chapter "Incub8-Screen: Cardio Application" for detailed information. This tutorial explains neuro specific functions only.

Control Section

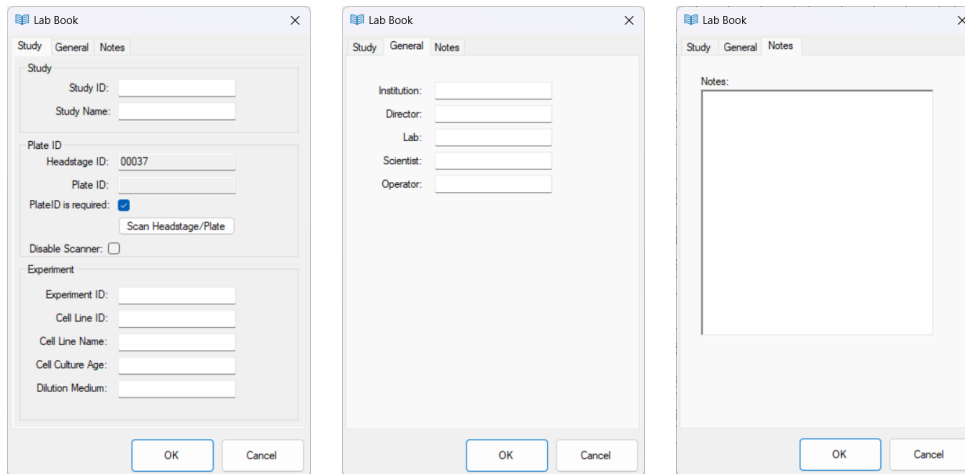
Configuration of the Experiment via "Settings" dialog.



Please read chapter "Control Section" in "Incub8-Screen" "Cardio Mode" for detailed information.

Lab Book

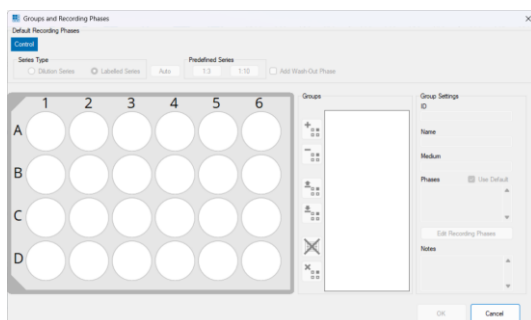
Start the configuration of your experiment with the first "Settings" command "Lab Book".

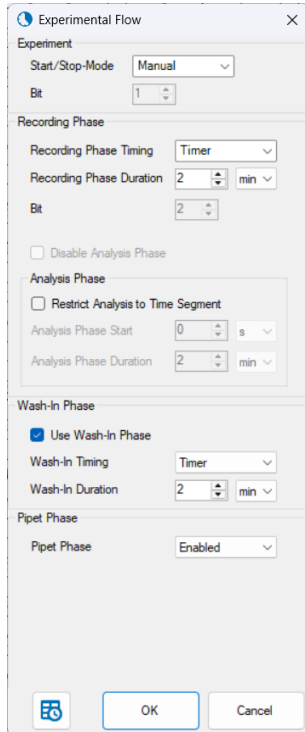


Type in all information you need for this experiment in the "Study", "General" and "Notes" tabbed pages.

Compounds

Define the "Groups and Recording Phases". Please read chapter "Compounds" in "Cardio Application" for detailed information.

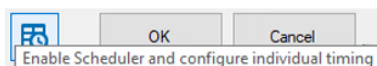




Define the "Experimental Flow" for neuro experiments. The dialog „Experimental Flow“ is available in different appearances to meet the requirements of your experiment in dependency of the settings. Please read chapter “Experimental Flow” in “Cardio Mode” also for detailed information.

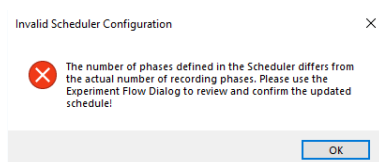
"Define the length of the "Recording" and "Wash-In" phases of the experiment. Within the "Recording" phase, an "Analysis" phase can be optionally defined. This has the effect that all the data during the recording phase will be recorded, but the online data analysis will only run on the time window within the analysis phase. If no analysis phase is defined, the online analysis will run on the full recording phase. Add a "Wash-In" phase, if necessary.

Configuration of a “Detailed Time Schedule” for Experiments



Please click the “Enable Schedule and configure individual timing” button in the “Experimental Flow” dialog to open the "Time Schedule" dialog for individual experiments. The schedule for the timing of each segment of the experiment is highly flexible: You can change the parameter for each concentration independently from others.

Please define the concentration series in the "Compound and Series" dialog first, otherwise the time schedule will not match with the number of phases set before. In this case, if an experiment is started, the following error message appears. Please correct the schedule before the experiment can start.



Please define for each "Phase" of your experiment, whether you "Enable" or "Disable" the "Pipet" phase.

Set the timing for the "Wash In", the "Recording", the "Analysis" and for the "Pause" phase to "Enable", "Timer" or "Manual". Define the "Duration" of these phases individually in seconds, minutes and hours.

Phase	Use Pipet	Wash-In Timing	Wash-In Duration	Recording Phase Timing	Recording Phase Duration	Restrict Analysis to Segment	Analysis Phase Start	Analysis Phase Duration	Pause Timing	Pause Duration
Control				Timer	2 min	<input type="checkbox"/>	0 s	2 min	Disabled	0 s
300 µM	Enabled	Timer	2 min	Timer	2 min	<input type="checkbox"/>	0 s	2 min	Disabled	0 s
1 nM	Disabled	Disabled	2 min	Timer	2 min	<input type="checkbox"/>	0 s	2 min	Disabled	0 s
3 nM	Enabled	Manual	2 min	Manual	2 min	<input type="checkbox"/>	0 s	2 min	Disabled	0 s
10 nM	Enabled	Timer	2 min	Timer	2 min	<input checked="" type="checkbox"/>	0 s	2 min	Disabled	0 s
30 nM	Enabled	Timer	2 min	Timer	2 min	<input type="checkbox"/>	0 s	2 min	Timer	0 s
100 nM	Enabled	Timer	2 min	Timer	2 min	<input type="checkbox"/>	0 s	2 min	Manual	0 s
300 nM	Enabled	Timer	2 min	Timer	2 min	<input type="checkbox"/>	0 s	2 min	Disabled	0 s
1 µM	Enabled	Timer	2 min	Timer	2 min	<input type="checkbox"/>	0 s	2 min	Disabled	0 s
3 µM	Enabled	Timer	2 min	Timer	2 min	<input type="checkbox"/>	0 s	2 min	Disabled	0 s
10 µM	Enabled	Timer	2 min	Timer	2 min	<input type="checkbox"/>	0 s	2 min	Disabled	0 s
30 µM	Enabled	Timer	2 min	Timer	2 min	<input type="checkbox"/>	0 s	2 min	Disabled	0 s
Wash Out	Enabled	Timer	2 min	Timer	2 min	<input type="checkbox"/>	0 s	2 min	Disabled	0 s

Use the "Copy Column" button to copy the contents of the current cell to all other cells in the same column. Use the "Copy Row" button to copy the current row to the complete time schedule. Use the arrows to redo or undo last changes.

Analyzer Settings

Analyzer Settings for "Spike Detector" and "Spike Processing".

Please read the chapter "Spike Detection" in the Appendix for more information about spike detection. Tooltips are available and guide through the "Analyzer" dialogs.

The 'Analyzer Settings' dialog box is shown with the 'Spike Processing' tab selected. It features a 4x6 grid of wells on the right side, with the well at row B, column 2 highlighted in blue. The left side contains several configuration sections:

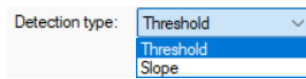
- Detection type:** Threshold
- Edge Selection:** Rising Edge , Falling Edge
- Automatic Threshold Estimation:**
 - Number of Segments: 1
 - Baseline Duration: 1000 ms
 - Rising Edge: 5.0 Std. Dev.
 - Falling Edge: -5.0 Std. Dev.
 - Buttons: Estimate, Estimate For All
- Timing:** Dead Time: 3000 µs
- Cutouts:** Pre Trigger: 1000 µs, Post Trigger: 2000 µs

Buttons for 'OK' and 'Cancel' are located at the bottom right of the dialog.

Define the "Analyzer Settings" in the "Spike Detector" and "Spike Processing" tab. It is useful to configure the "Analyzer Settings" when the "Explore" mode is running. All changes are directly sent to the instruments, that's why you can directly see the effects in the data stream. Decide on the basis of the running data, whether your settings fit or not.

Spike Detector

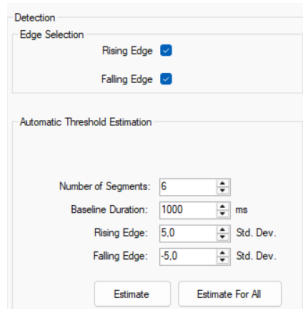
The "Spike Detector" tab allows to set the "Detection type" from the drop-down menu. In dependency of the detection type, the dialog offers different options.



Detection Type: Threshold

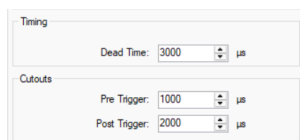
Define the "Rising Edge" or "Falling Edge" in the "Edge Selection" window.

In the "Automatic Threshold Estimation" window, you can select the standard deviation for the rising and falling edge from the numeric up-down boxes. Apply these estimations to the currently selected well by pressing the button "Estimate" or to all wells with the button "Estimate for all wells".



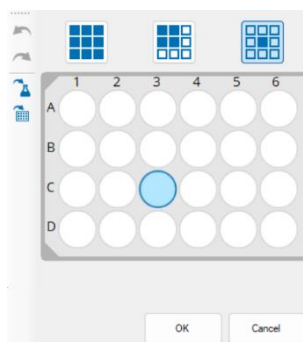
Use the up-down box "Number of Segments" to define, how many individual segments are used to estimate the thresholds. The duration of each segment is defined by the "Baseline Duration". The threshold is computed as the minimum standard deviation over all segments, multiplied by either the "Rising Edge" or "Falling Edge" factor. Using multiple short segments for the computation of the standard deviation instead of one long segment reduces the risk that the standard deviation is strongly influenced by spike activity.

Select the "Dead Time" in μs in the "timer" window.



When using "Cutouts", please define the "Pre Trigger" and "Post Trigger" in μs from the numeric up-down boxes.

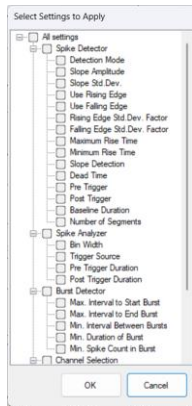
On the right side of the dialog, the well plate is displayed for the "Well Selection". As usual, the currently selected well is highlighted with a blue circle.



Click "Redo" and "Undo". Use the arrow buttons for "Undo last changes" and "Redo last changes".



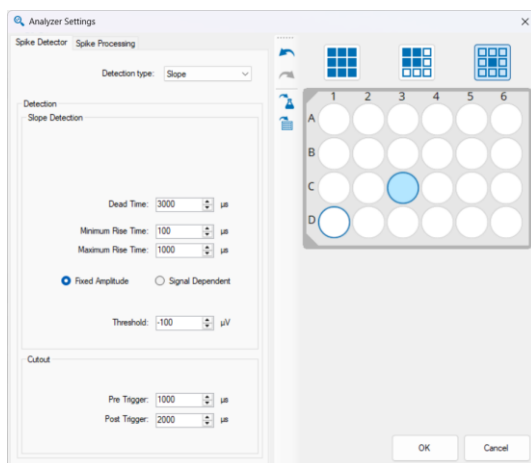
Click "Apply Changes to all Wells with the same Compound" or "Apply Changes to all Wells" and the following dialog appears "Select Settings to Apply".



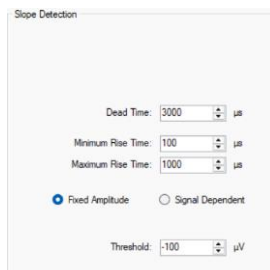
Select the settings you want to apply via check boxes. The settings will be immediately applied to all wells of the currently selected compound.

Detection Type: Slope

Changing the type of the detection for the spike detector to "Slope" the left side of the dialog will change respectively.

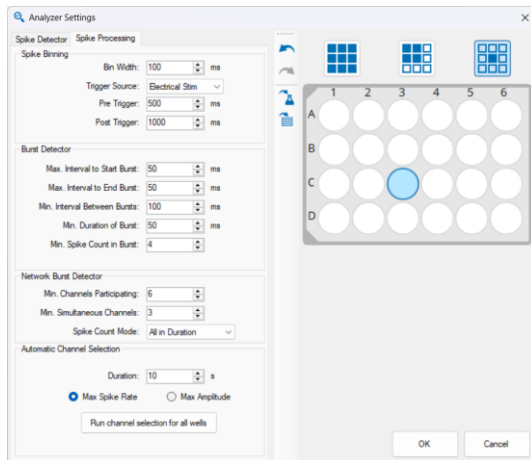


In this case, you have to define the following parameters for „Slope Detection".



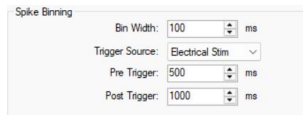
“Dead Time”, “Minimum and Maximum Rise Time” and “Threshold” via up-down menu and “Fixed or Signal dependent Amplitude” via radio box.

Spike Processing



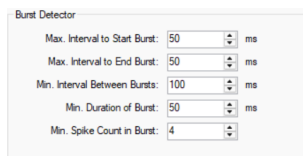
The "Spike Processing" tabbed page to adjust the "Spike Binning", the "Burst Detector", the "Network Burst Detector" and the "Automatic Channel Selection".

Spike Binning



The "Spike Binning" window is only available, if the software module "Stimulation" is selected. Use the up-down boxes in the "Spike Binning" window to define the time duration of the "Bin Width" in ms together with the "Pre Trigger" and "Post Trigger". The "Trigger Source" has to be "Electrical Stimulation".

Burst Detection



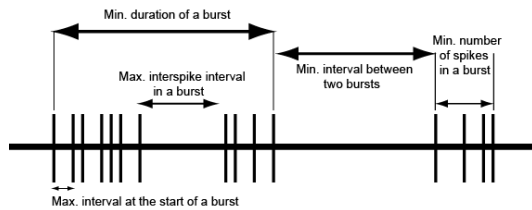
The "Burst Detection" option allows the detection and analysis of spike bursts. The spikes have to be detected first. The quality of the burst analysis therefore depends on the accuracy of the spike detection.

The MaxInterval Method for detecting bursts

Find all bursts using the following algorithm:

Scan the spike train until an interspike interval is found that is less than or equal to Max. Interval. While the interspike intervals are less than Max. End Interval, they are included in the burst. If the interspike interval is more than Max. End Interval, the burst ends. Merge all the bursts that are less than Min. Interval between Bursts apart. Remove the bursts that have duration less than Min. Duration of Burst or have fewer spikes than Min. Number of Spikes.

The detection parameters for bursts are shown in the following schema.



Parameter for burst detection:

Max. interval to start bursts: maximum interspike interval to start the burst.

Max. interval to end a burst: maximum interspike interval to end the burst.

Min. interval between bursts: minimum interspike interval between two bursts.

Min. duration of burst: minimum burst duration.

Min. number of spikes in burst: minimum number of spikes in a burst.

(Reference: Legendy C.R. and Salzman M. (1985): Bursts and recurrences of bursts in the spike trains of spontaneously active striate cortex neurons. J. Neurophysiology, 53(4) : 926-939).

Network Burst Detector

Network Burst Detector

Min. Channels Participating:

Min. Simultaneous Channels:

Spike Count Mode:

The "Network Burst Detector" tool identifies time regions with simultaneously occurring bursting activity on multiple channels in a well. A "Network Burst" is characterized as a sequence of temporally overlapping bursts detected by the "Burst Detection" tool on single channels.

To qualify as a "Network Burst"

(a) the number of distinct bursting channels in the sequence needs to be at least the number defined for "Min. Channels Participating" and

(b) at some point during the sequence, there need to be at least "Min. Simultaneous Channels" channels that are bursting at the same time.

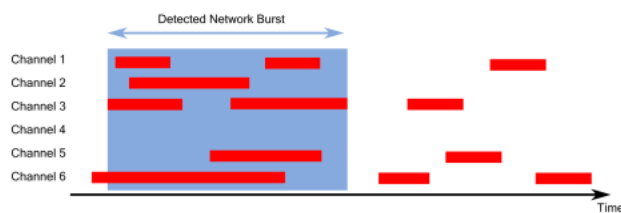


Figure: Network Burst_1

A toy example is shown in figure "Network Burst_1" above:

For 6 channels in a well, the time regions are marked in red where the "Burst Detector" has detected a burst for each of these channels. These burst time regions are used as input for the "Network Burst Detector", which is configured with "Min. Channels Participating" = 4 and "Min. Simultaneous Channels" = 3.

A single "Network Burst" is detected for the time region marked in blue, because during that time region there is overlap for the bursting activity on channels 1, 2, 3, 5 and 6, which means that the "Min. Channels participating" criterion is fulfilled. In addition, there are multiple times where four of these channels are bursting simultaneously, so the "Min. Simultaneous Channels" criterion is fulfilled as well.

The first part of the bursting activity of channel 6 is not part of the "Network Burst", because a "Network Burst" starts at the time point where at least two channels are bursting simultaneously. It ends with the last bursting channel in the temporally overlapping sequence of bursts.

The second half of the figure displays another sequence of temporally overlapping bursts on channels 1, 3, 5 and 6. Although this sequence also fulfills the "Min. Channels Participating" criterion, there is no time point where more than two of these channels are bursting simultaneously, violating the "Min. Simultaneous Channels" criterion, so this time region is not recognized as a "Network Burst".

It is possible that a sequence of temporally overlapping bursts actually contains multiple separate "Network Bursts", for example because there is a single channel with almost continuous bursting activity. An example for this is shown in the next figure "Network Burst_2" where channel 6 has continuous bursting activity.

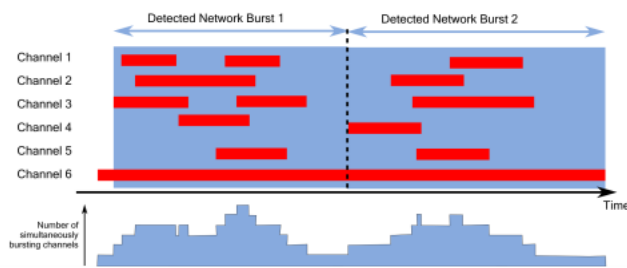


Figure: Network Burst_2

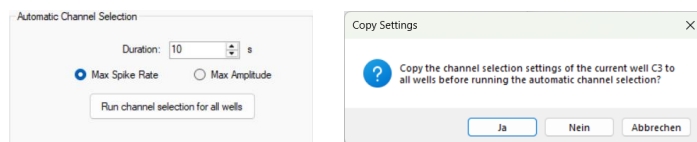
In such a case, the sequence of overlapping bursts is split into two (or more) subsequences. The time points of this splitting procedure are determined by looking at the number of simultaneously bursting channels, drawn in the lower portion of the figure, and finding "valleys" in this graph.

The third parameter for the "Network Burst Detector", "Spike Count Mode", determines which spikes are counted as part of the "Network Burst". This has an effect when computing the spike rate and the percentage of spikes in a "Network Burst". If this parameter is set to "All in Duration", all spikes in the duration of the "Network Burst" are counted for all channels in the well.

For example, if any spikes were detected for channel 4 in figure "Network Burst_1" during the "Network Burst", these would be counted towards the spike count of the "Network Burst", although channel 4 never participated with any bursting activity to the "Network Burst". If, on the other hand, the "Spike Count Mode" is set to "Participating Bursts", only spikes within bursts on the individual channels within the "Network Burst", for example the time regions marked in red in figure "Network Burst_1" and "Network Burst_2" are counted towards the spike count.

(References: Wagenaar D., DeMarse T.B. and Potter S.M. (2005): MeaBench: A toolset for multielectrode data acquisition and online analysis. Conference Proceedings. 2nd International IEEE EMBS Conference on Neural Engineering, 2005. Mendis G.D.C, Morrisroe E., Petrou S. and Halgamuge, S. K. (2016): Use of adaptive network burst detection methods for multielectrode array data and the generation of artificial spike patterns for method evaluation. J. Neural Eng. 13 (2016) 026009 (19pp))

Automatic Channel Selection



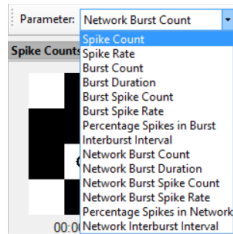
This tool selects the representative channel for the calculation of the dose-response curve via "Max Spike Rate" or via "Max Amplitude". Define the time span for the calculation interval with the up-down box "Duration" in seconds. Apply the selection to all wells by pressing the button "Run channel selection for all wells".

Click button "Run channel selection to all wells" and the "Copy Settings" dialog will appear.

Select all "Burst Detection" and "Network Burst Detection" parameters that can be selected in the "Dose Response Display" window and in the "Multiwell Selection Panel".

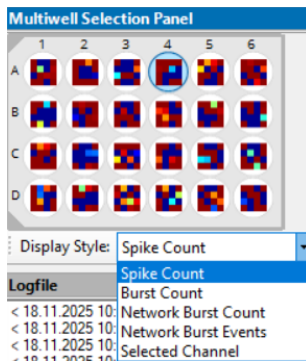
Crosslink to Treatment Response Display

All "Burst Detection" and "Network Burst Detection" parameters can be selected in the "Treatment Response Display" window.

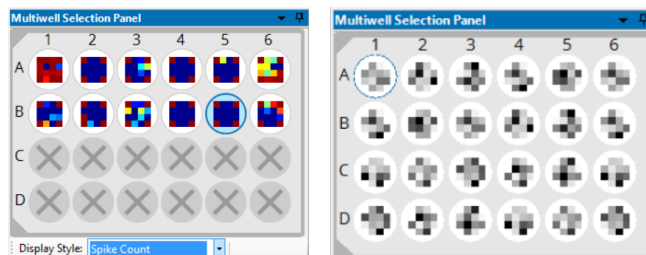


Crosslink to the Multiwell Selection Panel

The difference to the "Control Section" in "Cardio" mode is the function of the display in the "Multiwell Selection Panel". In "Cardio" mode you have an excitation map, in "Neuro" mode you have five opportunities to be selected from the drop-down menu "Display Style".

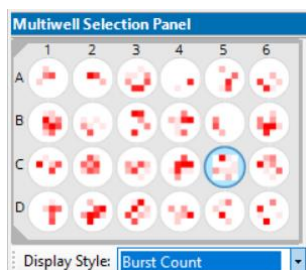


Spike Count



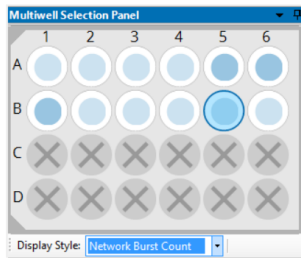
"Spike Count": The channel activity is displayed in terms of spike count per channel in a moving window. The higher the spike count rates, the darker the color of the channel in the 2D map. Change the color of the map in the "Spike Count" display in the "Map Settings" dialog.

Burst Count



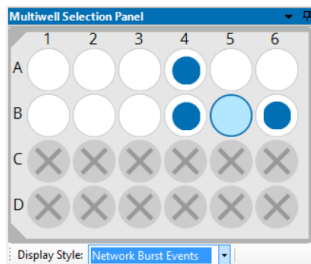
"Burst Count": The channel activity is displayed in terms of burst count per channel in a moving window. The higher the burst count rates, the darker the color of the channel in the 2D map.

Network Burst Count



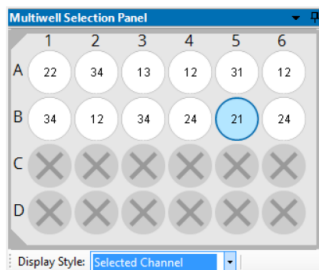
"Network Burst Count": The channel activity is displayed in terms of network burst count per well in a moving window. The higher the network burst count rates, the darker the blue color of the circle in the 2D map.

Network Burst Event



"Network Burst Events": The channel activity is displayed in terms of network burst events per well in a moving window. If a network burst event is detected, a circle in blue color is flashing in that well of the 2D map.

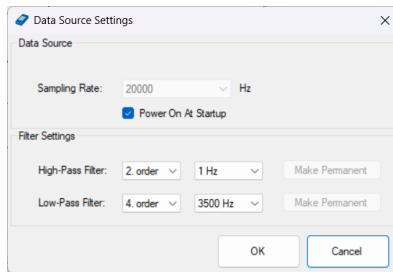
Selected Channel



"Selected Channel": Click "Selected Channel" to show the number of channels which are automatically selected via spike detection. Change the selection manually by clicking onto a different electrode in the data display.



Data Acquisition



Please define the parameter in the “Data Source settings” dialog.

Data Source

Please select the "Sampling Rate" in Hz in the "Data Source" window from the drop-down menu.

Enable the checkbox “Power On at Startup” to initialize the “Wake-up Mode, if necessary. The Incub8-Screen software has to be restarted to apply the desired mode.

Wake-up Mode

The settings in the „Experimental Flow“ dialog corresponds with the settings in the “Data Source Settings” dialog. Please enable the checkbox “Power on at Start-up” to use the “Wake-up” mode.

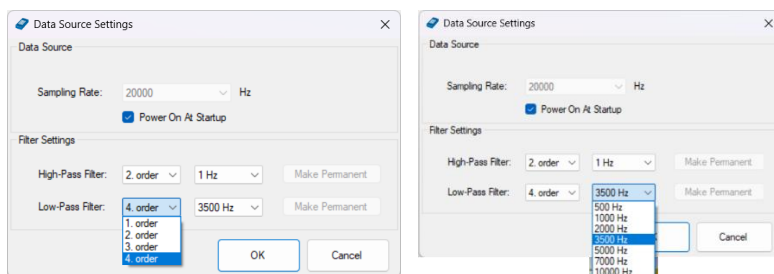
Important: Power down the Incub8-Screen software and start the software again, each time you enable or disable this checkbox.

For recording intervals, please define the “Warm-up Phase Duration” and the “Recording Phase Duration”. The initialization phase guarantees a warming up of the headstage, until stable recordings are possible. After recording the Incub8 headstage is switched off again.

The “Wake-up Mode” gives the possibility to switch the headstage of the Incub8-System off during long term experiments. Switch the headstage off, for example, when using it in the incubator to prevent that additional heat from the headstage influences the well-adjusted temperature in the incubator.

In case you do not use the “Wake-up Mode” please lower the temperature in the incubator of about 3 to 4 °C in dependency of the volume and the air impermeability of the incubator used.

Filter Settings



Set the "High- and Low-Pass Filter" settings of the Incub8 device with the drop-down menus. Choose between 1st and 2nd order Butterworth filters for the "High-Pass Filters" and between 1st and 4th order for "Low-Pass Filters".

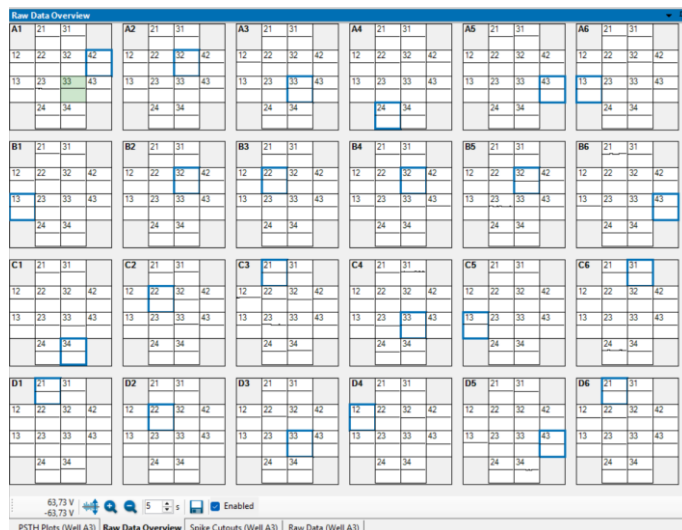
If the data acquisition is not active, press "Make Permanent" in order to write the filter settings to the device. This ensures that the same settings will be loaded after rebooting the Incub8 device.

Data Display Section

View your current data in this section. In "Neuro" mode, there are four displays in the tabbed pages: "Raw Data Overview", the "PSTH Plots" Peri-stimulus time histogram, visible only if the software module "Stimulation" is selected, the "Spike Cutouts" and "Raw Data".

The upper display shows an overview of all wells in the "Raw Data Overview" window or of the electrodes of one well that was selected in the "Multiwell Selection Panel" dialog in the left "Control Section". The lower display shows the single view of one electrode.


Raw Data Overview





The "Raw Data Overview" window gives an overview of all wells of the plate. The overview will automatically be adapted to 24 or 96 wells. Navigate from well to well by clicking on it.

By default this display is initially disabled, because calculating the huge amount of data streams needs capacity and performance of the computer. Click the check box "Enable" in the footer of the window to see the streams.



If the data acquisition computer is overloaded during an experiment, the overview display will be automatically disabled: "Warning: Display disabled due to performance problems!" Export a picture of the "Overview Raw Data" window by clicking the button "Export to Image File" . The file format is *.jpg, *.png or *.bmp.

Click the "Adjust to signal Min/Max" button  and read the current value beside. The scaling of the y-axis is set to the minimum and maximum of all samples in the well.

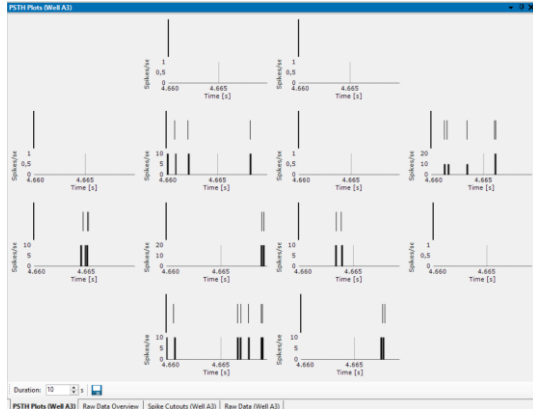
Clicking the button "Zoom In" cuts the scaling in a half and "Zoom Out" doubles the scaling .

Define the time span displayed in the overview in seconds via up-down box  5 s.


PSTH Plots

The PSTH plot is visible only if the software module "Stimulation" is selected.

Peri-stimulus time (PST) histograms display the distribution of spike times relative to the onset of each stimulus presentation. They are often the most useful way to show temporal discharge patterns.



The neuronal response due to a single stimulation consists of one component, which is causally determined by the pulse and of several components, which are not caused by the stimulation, spontaneous activity, for example. If all components of the response have similar amplitudes as the stimulus induced component, it is not possible to see this component. That is, why the Peri-Stimulus-Time-Histogram (PSTH) is calculated. The histogram is the arithmetic mean over several identical stimulations and shows the time point, which is directly correlated to the stimulus "Incub8-Screen Software".

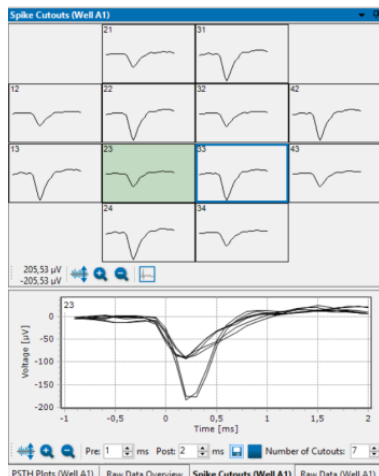
The screen is permanently updated. Data which cannot be displayed for lack of space are available for the calculation of the histogram anyway. Save an image of the screen by clicking the "Export"  icon.

If a PSTH plot is plotted during stimulation, a blue bar in the PSTH plot indicates the time stamp of the stimulus.

Spike Cutouts

The "Spike Cutouts" window is separated in the upper overview and the lower single view display.

In the "Overview" display, you see the mean values of the data segment around all spike cutouts.



The neuronal response due to a single stimulation consists of one component, which is causally determined by the pulse and of several components, which are not caused by the stimulation, spontaneous activity, for example. If all components of the response have similar amplitudes as the stimulus induced component, it is not possible to see this component. That is, why the Peri-Stimulus-Time-Histogram (PSTH) is calculated.

The histogram is the arithmetic mean over several identical stimulations and shows the time point, which is directly correlated to the stimulus.


The screen is permanently updated. Data which cannot be displayed for lack of space are available for the calculation of the histogram anyway. Save an image of the screen by clicking the "Export" icon.


Spike Cutout Overview



The "Spike Cutouts" window is separated in the upper overview and the lower single view display.

In the "Overview" display, you see the mean values of the data segment around all spike cutouts.

The "Spike Cutouts" display is specific for Neuro mode. Functions and tools are similar to the "Averages" display in Cardio mode.

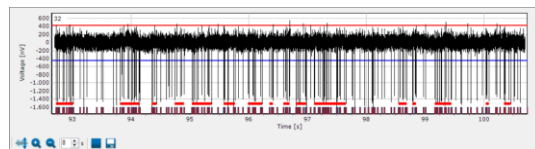
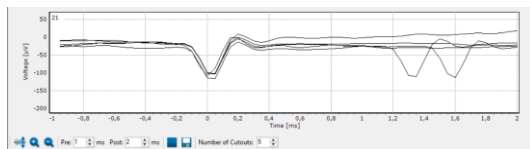
Click the "Adjust to signal Min/Max" button  and read the current value beside. The scaling of the y-axis is set to the minimum and maximum of all samples in the well.

Clicking the button "Zoom In" cuts the scaling in a half and "Zoom Out" doubles the scaling .

One channel of the well has to be defined for analysis and display of the "Dose-Response" curve. Define this electrode by clicking the "Select representative channel" button . The channel analyzed in the single view will be highlighted in green and the channel with the blue frame will be used for the result analysis. The screen is permanently updated. Data which cannot be displayed for lack of space are available for the calculation of the histogram anyway. Save an image of the screen by clicking the "Export"  icon.

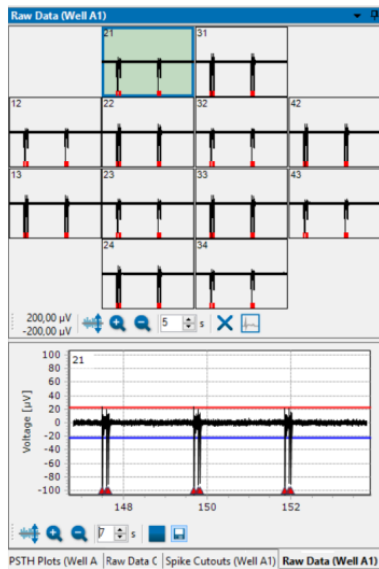
Spike Cutout Single View

The single view shows an overlay plot of the last cutouts of the selected channel. Define the "Time" scale in the single view with the "Pre" and "Post" up-down boxes in ms. Select the number of the plotted cutouts via "Number of Cutouts" up-down box.





Raw Data


The "Overview" in the "Raw Data" display shows the raw data of all electrodes in the well, so you have a good overview of the activity in the desired well.




See the identification of the selected well in the header of the dialog. The upper left electrode is selected by default. Jump between the electrodes without stopping the experiment, the selected electrode will be highlighted in green and viewed in detail in the "Single View". The vertical red marker indicates the detection of a heartbeat or a spike in both displays. A horizontal red bar above the spike markers indicates the detection and duration of a spike burst.

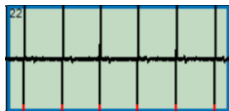
Click the "Adjust to signal Min/Max"  button and read the current value beside. The scaling of the y-axis is set to the minimum and maximum of all samples in the well.

Clicking the button "Zoom In" cuts the scaling in a half and "Zoom Out" doubles the scaling .

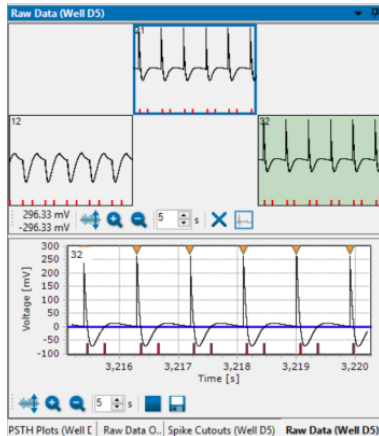
Use the up-down box to scale the time x-axis in each window of the overview. Deselect electrodes by clicking the "Exclude Channel" button  first and then on the electrode. Click the electrode to select it again.

Important: Data of excluded electrodes will not be recorded and stored!

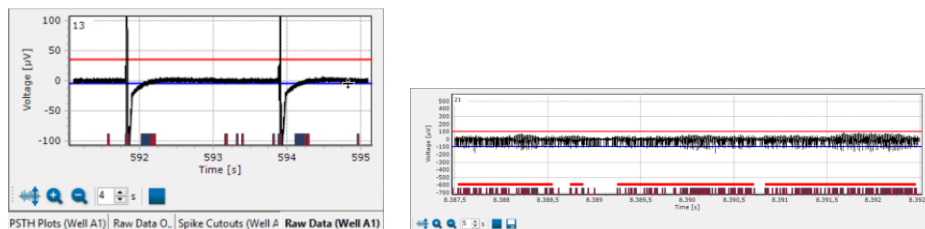
One channel of the well has to be defined for analysis and display of the "Dose-Response" curve. Define this electrode by clicking the "Select representative channel" button . The analyzed channel will be highlighted in green and framed in a blue frame.



The following screenshot shows "Raw Data" display with the time stamps of stimulation indicated by the orange triangle in the "Single View".





Raw Data Single View




The lower display shows the single view of one electrode of the "Raw Data" display. The single view shows data of the selected electrode channel more detailed.

In this example, the raw data of the selected electrode No 13 is displayed. See the electrode ID in the left upper edge of the display. Set the threshold for the spike detection individually for each electrode. The detection events are displayed in red for the upper detection point and blue for the lower detection point on the "Time" axis.

Click the "Adjust to signal Min/Max" button . The scaling of the y-axis is set to the minimum and maximum of all samples in the channel.

Zooming in cuts the scaling in a half and zooming out doubles the scaling . Additionally, you can zoom into a region of interest by moving the mouse from the left to the right while pressing the left mouse button. Move the mouse the other way round for zooming out. Please read chapter "General Software Features".

Use the up-down box to scale the time axis of the single view display. Start and stop the data display  independent from the overview. In experimental mode the recording of the data will go on, even if the data display is stopped.

Result Section

View your current results in this section. Three types of result presentation are available in three dialogs: "Treatment Response" and "Spike Count" and "Results per Channel (Well X/Y)".

The upper display shows the "Treatment Response" dialog for each compound. The lower displays show the "Spike Count" map on the base of the selected well and the "Result per Channel (Well X/Y)" display on base of one channel of the selected well.

Treatment Response

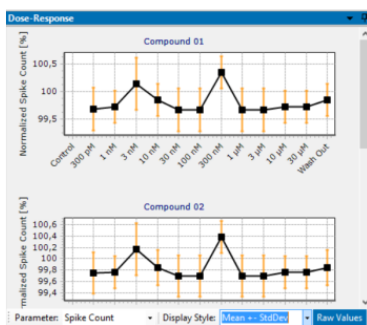
The treatment response curve shows the variation of the physiological markers in relation to the concentration of the compound. Please read more about this theme in chapter "Theoretical Background of Dose-Response Curve Calculation".

The "Treatment Response" dialog looks different depending on the settings. All available compounds are displayed. This screenshot shows the "Normalized" "Mean" value of the only available parameter "Spike Count" for the compounds 1 and 2.

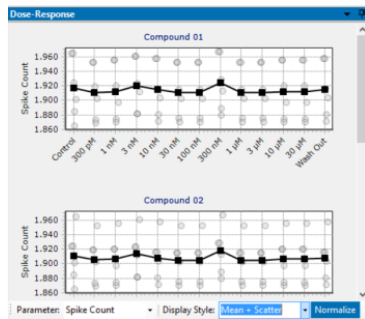


Choose the "Display Style": The "Mean" is calculated from the mean results of all wells of one compound. This simple display format does not show the variability of the wells.

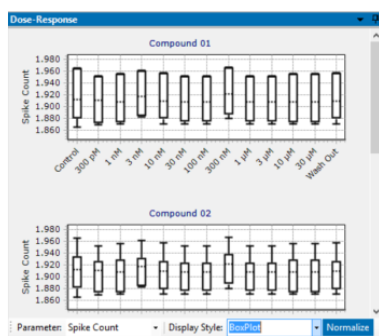
See the variation of the physiological markers in the dose-response curve in absolute values "Raw Values" or in "Normalize" mode. Normalized results are calculated in relation to the respective control section in %.



The "Mean + StdDev" shows additionally ± 1 standard deviation of the mean and allows to estimate variability between the wells but does not show runaway values.



The "Mean + Scatter" plot shows additionally to the mean the results of all concentrations of that well as own points. Click on the points to make the results of this well but other concentration available. This display format allows simply to find runaways to correct them, if necessary during offline analysis.

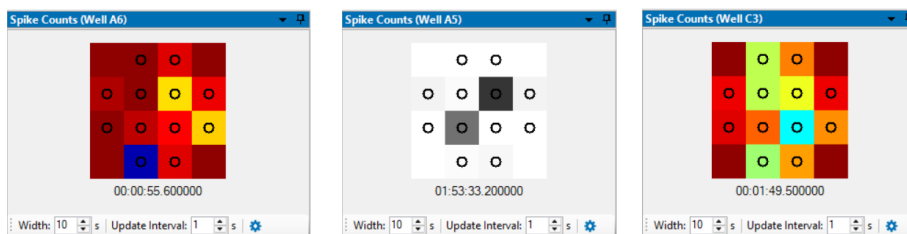


The "BoxPlot" shows the distribution of the results between the wells. You clearly see the wells which differ from the median.

The Coefficient of Variation "CV" is a degree for the variability of a distribution. The "CV" is calculated from mean divided by standard deviation and has no unit. You need at least two measurements to calculate the "CV", that is why it is useless for parameters like spike count or QT interval in "Cardio" mode.

Additionally, to the "Treatment Response" curve, the windows "Spike Counts" and "Result per Channel" are available in "Neuro" mode.

Spike Counts

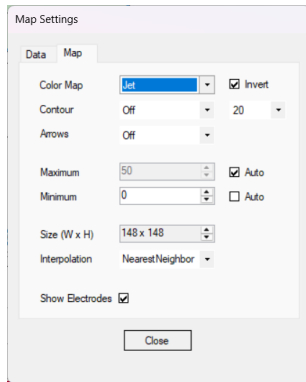


The channel activity is displayed in terms of spike count in a moving window. The higher the spike count, the darker the color of the channel in the 2D map. Spike counts are not interpolated between channels.

Below the map the current timestamp is displayed in hour, minute, second, and millisecond.

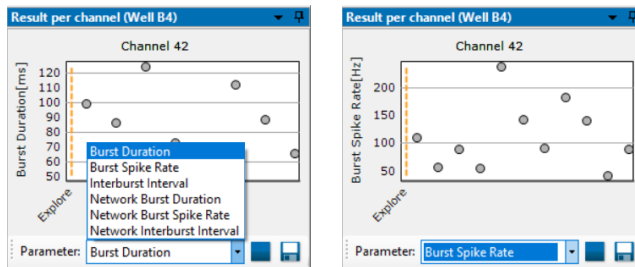
Modify the value in the "Width" numeric-up-down control to adjust the time range in which the spike counts are computed. The value given in the "Update Interval" numeric-up-down control specifies the update rate of the spike count maps. The mapping of spike counts to color can be adjusted in the "Map Tab" of the "Map

Settings Dialog" with the parameters "Minimum" (represented by white color) and "Maximum" (represented by black color). Values outside this minimum-maximum range will be displayed either as white or black.



Click the icon to adjust the map parameters in the "Map Settings" dialog. Please read the respective chapter in "Incub8-Screen Software". Change the color of the map for a better view.

Results per Channel

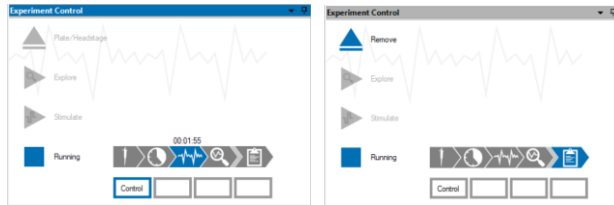


Display the results of one channel of a respective well in the "Result per Channel" view. Please select the parameter which should be shown from the drop-down menu.

Start and stop the display via "Start / Stop" button and save data as comma separated file "*.csv".

Experimental Process


See the "Experiment Control" window in the "Control Section" on the left side of the main menu. This dialog schedules your experiment.



Prepare the well plate you want to use carefully. Insert the well plate in correct orientation in the Incub8 headstage. Before you can start an experiment, please fix the well plate in the incub8 headstage. Use the Incub8-VariPlate Base to work with standard well plates.

It is useful to configure the "Analyzer Settings" when the "Explore" mode is running. All changes are directly sent to the instruments, that is why you can directly see the effects in the data stream. Decide on the basis of the running data, whether your actual settings fit or not.

Changes of "Analyzer Settings" effect all electrodes in one well. The only exception is the "Spike Detector" in the "Raw Data Display" in the "Neuro" modus, the threshold settings can be changed per channel. Please see "Raw Data Section" on in this chapter.

After passing all these preparation steps you can now start your experiment. Click the "Go"  button.

During the experiment, the phases are repeated in dependency of the number of compounds. All experiment phases are run automatically. When the experiment is finished, you can save the "Report" file as *.PDF.

Preliminary Phase

Control Phase

Pipetting of the first compound

Wash in

Test

Analysis

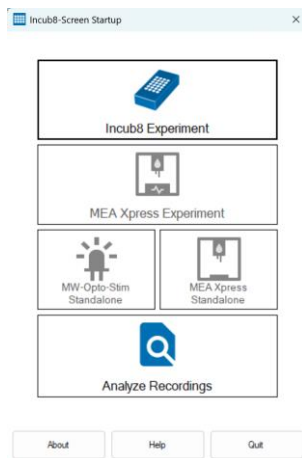
Pipetting of the next dilution of the compound

Wash in

Test

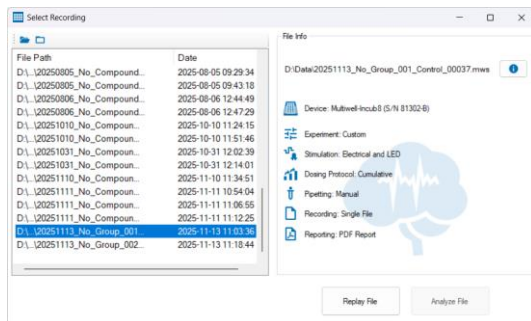
Analysis


Analyze Recordings



The “Analyze Recordings” mode gives you the opportunity to re-run the analysis on your recorded Incub8-Screen data files in order to optimize the analysis parameters.

If you open the software for the first time, it may prompt you to select an experiment type. This selection has no further effect on the "Analyze Recordings" mode.



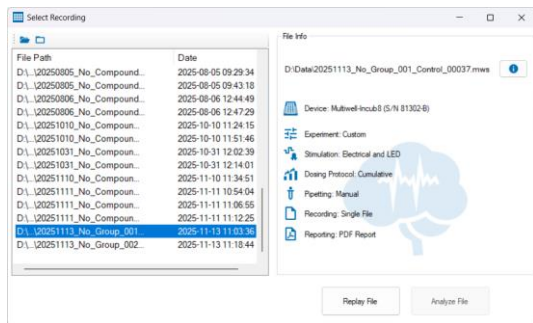
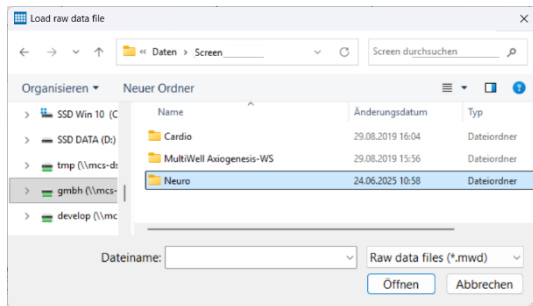
The “Select Recording” dialog lists all recordings in the default recording folder for Incub8-Screen. This folder can be changed in the “Recorder Settings” dialog. If a recording is selected in the list on the left hand side, the file name, experiment type and the selected software modules are shown on the right hand side. You can use the button  to open an overview of all parameters of the experiment in the “Experiment Info” dialog.



The “Experiment Info” dialog holds five tabs: Lab Book, Compound, Timing, Stimulation and Data Source.

Use the "Select File..." and "Select Folder..." dialog to show either a single file or all Incub8-Screen recordings in a folder in the file list.

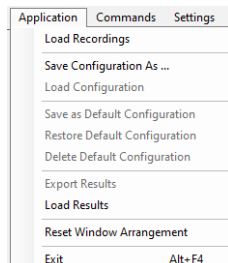
If you use the "Select File..." button, for example, the "Select Multiwell File to Open" dialog appears.



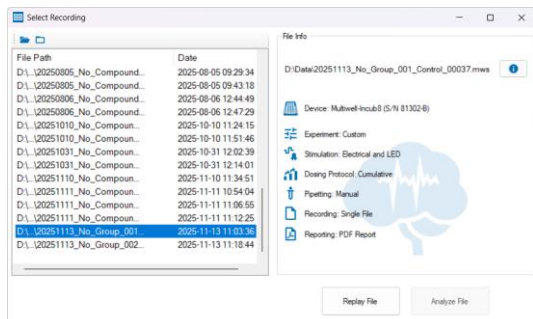
After selection, all parameters available in this file are displayed in the "Select Recording" dialog.

Click the button "Replay File" for replaying the selected file in the Incub8-Screen program. "Analyze File" opens the file directly in the Incub8-Analyzer software.

Application

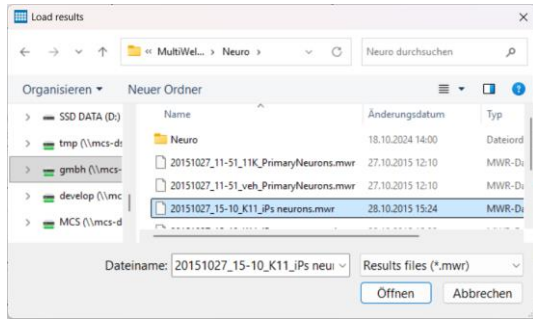


Click "Load Recordings" to select Incub8-Screen files directly for the Replayer "Replay File" button or click the "Analyze File" button for the "Analyzer".

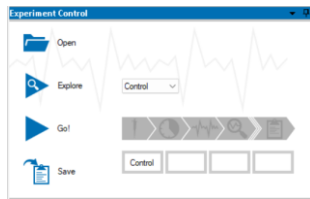


"Save Configuration As..." in the "Save Settings" dialog or in "Application".

“Load Results”

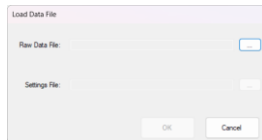



The user interface “Experiment Control” and corresponding commands in the main menu will change.




Load Data File

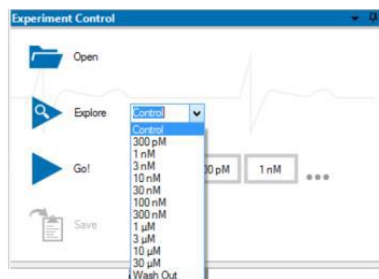
Please select a raw data file first. Load raw data files using the “Open” or “Load Replayer File” commands.



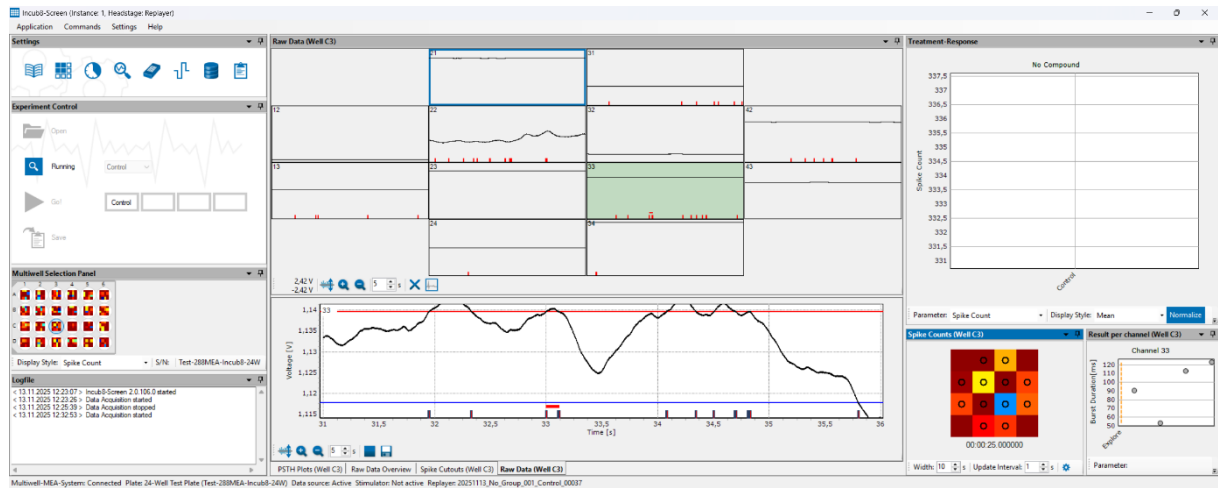
In the “Load Data File” dialog, press the upper "Open" button  to load a raw data file with the extension "*.mwd".

If the folder containing the selected raw data file also contains a matching settings file with the extension "*.mws", it will be automatically selected. This should be the case as long as all files generated in an experiment are kept together in the same location. Otherwise, or if you want to load a different settings file, press the lower "Open" button  to select a settings file with the extension "*.mws" or "*.xml" file. With the exception of the analyzer settings, the contents of the settings file have to match the settings that were used during data acquisition. Pressing the “OK” button will load the associated files or an error message appears if the settings file does not match the data file.

The name of the file is displayed in the status bar of the footer.



Explore Mode



In "Explore" mode, the recorded data is displayed and analyzed in its original speed. Click on the "Explore" icon in order to start or stop exploring. The data recorded during the test phase of this concentration will be played after pressing the "Explore" button. Please use the drop-down menu to the right of the "Explore" button to select one of the substance doses contained in the data file, for example 300 pM. Just like in recording mode, the explore function of the replayer enables you to modify analysis. You can directly see the effects on incoming raw data in real-time.

The screenshot above shows the main menu of the "Replayer Mode" replaying a file recorded in "Neuro" application.

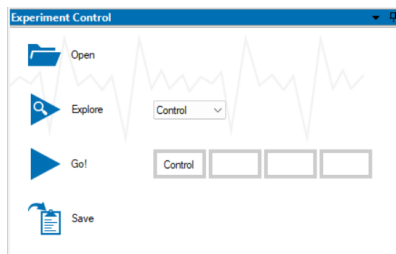
Use the "Explore" mode to select a representative channel for each well, which will later be used for dose response results. This selection will be used for all doses. Keep the "Explore" mode running for at least 10 seconds in order to automatically select representative channels.


Experiment Mode: Restart the Analysis of a complete File

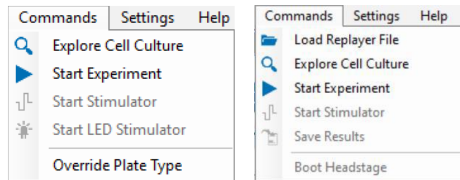
Restart the analysis of the complete file using the „Go!“ command. The analysis runs through, one concentration after the other until the experiment is stopped with the "Wash Out" phase.

Running a complete analysis will be performed as quickly as possible. In order to improve performance, raw data will not be displayed in this mode either in overview nor in single view. Please switch to "Averages" display to observe data. Additionally, the "2D Latency" maps in "Cardio" application and "Spike Count" maps in "Neuro" application are disabled.

Save Results



After an experiment is finished and you are satisfied with the analysis results, press the "Save results after replaying" button  or use the main menu "Command" option to generate a report sheet and store the result files on the hard disk.



Using the "Save Results" command the Incub8-Screen replayer data files results (*.mwr), settings (*.mws) and cutout (*.mwc) will be saved to the directory defined in the recorder settings. File names are automatically created according to the recorder settings. No existing file will be overwritten, file names will automatically be modified with an increasing number. The file dialog, which opens after clicking the "Save" icon, enables you to save a new report file. Please select location and name for the new file. If you are not going to save a new report file, just close the dialog.

Incub8-Analyzer Software

Introduction

The "Incub8-Analyzer" software is developed to analyze data, produced with the "Incub8-Screen" software and facilitates the detailed analysis of the generated data. The analyzer is built-on two application modes almost in the same manner as the "Incub8-Screen" software, in Cardio and Neuro Mode. Appropriate tools allow the analysis of the incoming files and an output of significant results on base of graphs or data files.

System Requirements

One of the following Microsoft Windows[®] operating systems is required: (64 Bit) Windows 11 or higher (English and German versions supported) with the NT file system. Other language versions may lead to software errors.

Main Features

The main features of the Incub8-Analyzer software are:

- Merging of results from different experiments.
- Exploration of the raw data.
- Selection of time segments for analysis.
- Spectral filtering (high-pass, low-pass, notch).
- Generation of a dose-response curves.
- Export of results from each analysis in form of a graph or a file.
- Report generation.

Cardio Mode

- Heartbeat detection and averaging.
- 2D latency analysis.
- Manual and automatic analysis of the field potential duration (QT-related interval analysis).

Neuro Mode

- Spike detection.
- Burst detection.
- Network burst detection.
- Export of spike cutouts.

Input Files

Please use following files for the detailed analysis, created with the Incub8-Screen or Incub8-Analyzer software.

- Raw data files with the extension "*.mwd". Multiwell raw data files include raw data of the electrodes, auxiliary channels and digital-in in binary format.
- Result data files with the extension "*.mwr". The result XML-files include analysis settings and analysis results. Open "*.mwr" files directly from the Windows Explorer via double-click in Incub8-Analyzer.
- Settings files with the extension "*.mws". The settings XML-files contain the settings of the experiments.
- Cutout files with the extension "*.mwc" are optional. Cutouts files contain spike cutouts or heartbeat averages in binary format.
- Analysis files with the extension "*.mwa". Open "*.mwa" files directly from the Windows Explorer via double-click in Incub8-Analyzer.

Additionally, you can import files with the extension "*.c2r", generated with the Cardio2D software for individual analysis or for merging with multiwell files, generated with Incub8-Screen software. The Cardio2D files have to fulfill specific requirements: They have to be generated in Cardio2D "Basic" mode with an Incub8-System, a 60-6wellMEA, a 256-6wellMEA or a 256-9wellMEA and need to have a dilution series, which starts with a control phase.

It is possible to reanalysis an analysis file generated with the Incub8-Analyzer software.

Due to the huge amount of data, from up to 96 well with a sampling rate of up to 50 kHz, Multi Channel Systems MCS GmbH recommends a SSD drive for a faster access to the data.

Output Files

Analysis files with the extension "*.mwa" and cutout files with the extension ".mwc". The cutout files can be exported to other data formats with the Multi Channel DataManager.

Comma separated value files with the extension "*.csv", which can be processed with common software, respectively.

Graphs or images with the extension "*.bmp", "*.jpg" or "*.png" from each display window. Report Sheet files in "*.PDF" format.

Report Sheet



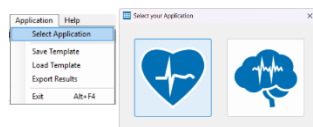
Save Analysis

Please click the "Save Analysis" button to save the current state of the analysis as an *.mwa file. You may also generate a detailed PDF report with the analysis results.

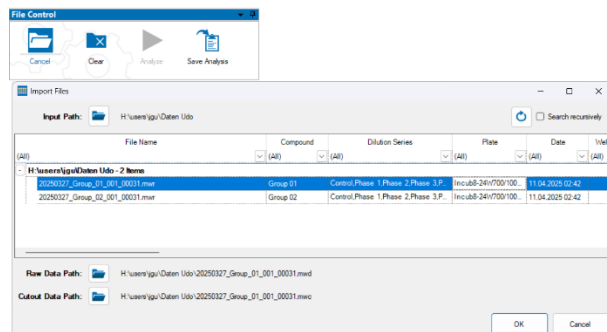
Find detailed information about the "Neuro" and "Cardio" mode in the following chapters. Software features which are important for both modes are explained in chapter "General Software Features".

Start Working

To start working with the Incub8-Analyzer software, please select your application mode, and all following functions are adapted to "Cardio" or "Neuro" mode.



After that, please import a file and set the analysis parameter step by step. After analyzing export your results and generate a report sheet.



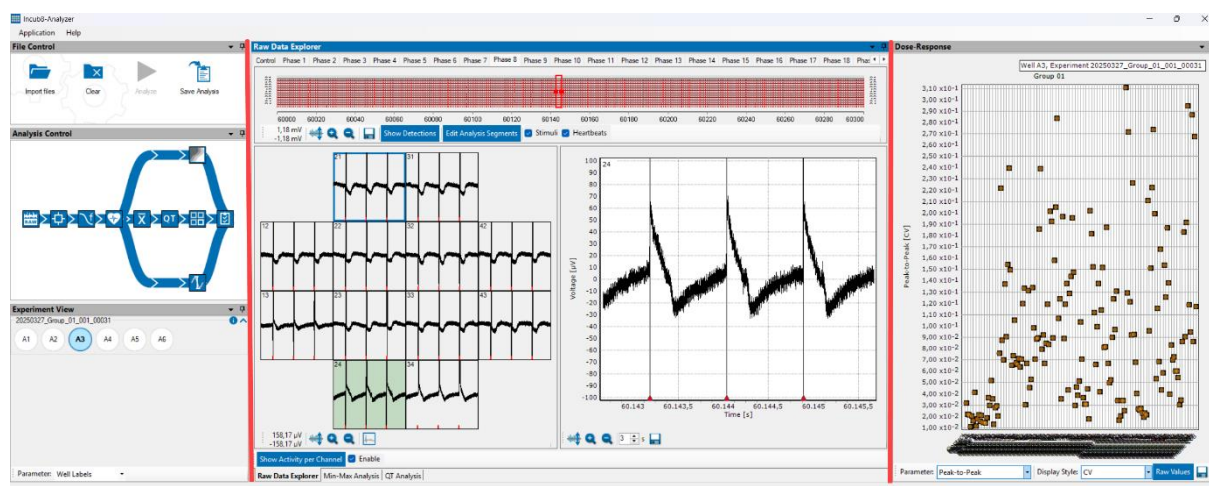
Incub8-Analyzer: Cardio Mode

The Incub8-System was developed on the base of the established MEA technology to meet the need for higher throughput electrophysiology.

Use the system in "Cardio Mode" for a broad field of applications. For electrophysiological recordings from cardiac cell cultures, for stem cell derived cell types and for cardio myocytes, for example in order to measure pharmacological effects on the heartbeats and the QT potential.

Please find detailed information about the Incub8-Analyzer in "Cardio Mode" in the following chapters.

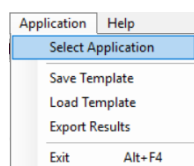
Main Menu



The main window of the "Incub8-Analyzer" software is divided in three windows with a menu bar above. The control window on the left includes the "File Control" window, the "Analysis Control" and the "Experiment View" window. In the data display of the center part you can decide between three tabs, the "Raw Data Explorer", the "Min-Max Analysis" or the "QT Analysis". On the right side, you see the window for the "Dose-Response" curve.

Menu Bar

Application



Select Application "Cardio" or "Neuro" mode.



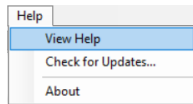
Use the "Save Template" function to select a well and store the analysis parameters of this well to a template file with the extension *.mwt.

With the "Load Template" function, such a template file can be loaded later on and the analysis parameters stored in it will be applied to all wells of all experiments currently opened in the Incub8-Analyzer.

Click "Export Results" to open the export dialog. Please read chapter "Export" for more information later on.

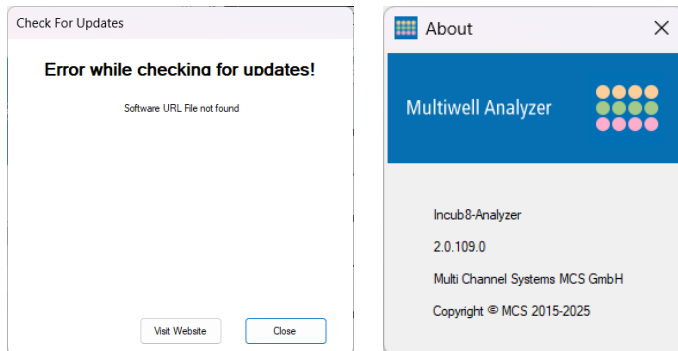
Menu to "Exit" the Incub8-Analyzer program.

Help



Enter the online help with the "View Help" command.

The option "Check for Updates" provides fast access to the MCS web site.



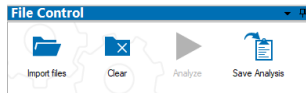
If the Incub8-Analyzer software is not up to date, click the button "Visit Website" and download the newest software version.



Click "About" to open the dialog. The dialog shows basic information about the Incub8-Analyzer software version.

Note: Please keep in mind that the information of this dialog is necessary in case of support!

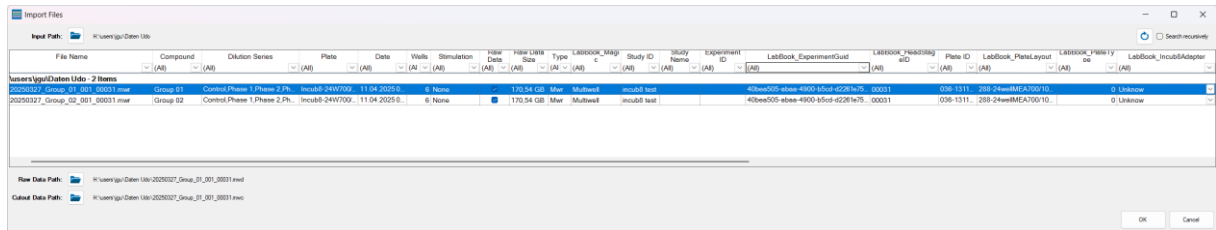
Control Window

File Control Window



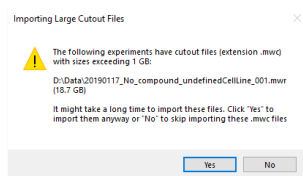
Click "Import File"  to open the import dialog. Click the icon "Clear"  to clear the loaded files.

Import Files



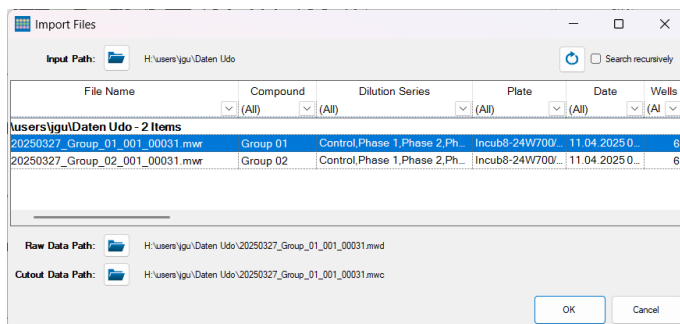
The Incub8-Analyzer needs at least the raw data file "*.mwd" or the cutout file "*.mwc" for analysis. To open a result file "*.mwr" or an analysis file "*.mwa" file, the corresponding cutout file "*.mwc" or settings file "*.mws" file has to be available. If a file is not automatically detected, please add it manually.


Import of large Cutout File



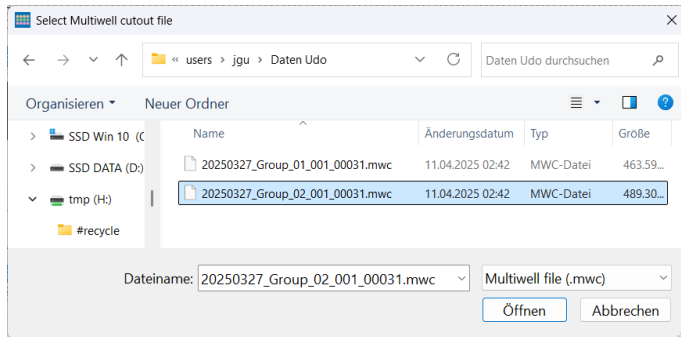
An import of a cutout „*.mwc“ file, larger than 1 GB data volume, possibly needs a long time. That is why you have to confirm the import of large files via the “Import Large Cutout Files” dialog.

Data Path

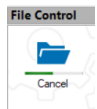


Please define the data path for the "Input Path" in the headline of the dialog. "Reload the files from the input path"  or click the check box "Search recursively", if necessary.

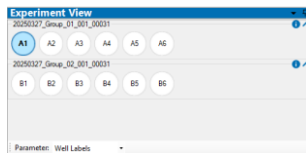
Click "Input Path" to find the experiment files recorded by Incub8-Screen or analysis files created by the Incub8-Analyzer software. The list of available files will show all files that are created in the current mode. Define the paths, where to load raw data and cutout files, the "Raw Data Path" and the "Cutout Data Path" in the footer. If any file is not found automatically, its path is marked in red. Please search the missing file manually by clicking on the respective data path icon to open, for example, the "Select Multiwell cutout file" dialog.



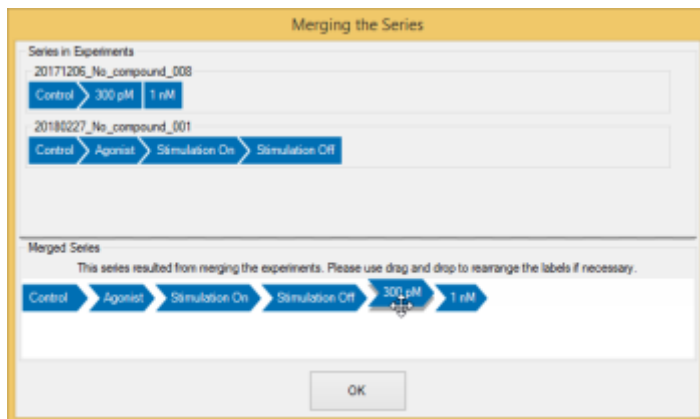
Merging Files



It is possible to import more than one experiment file at a time in order to merge the results. The files are sorted one below the other, you can see them in the "Experiment View".



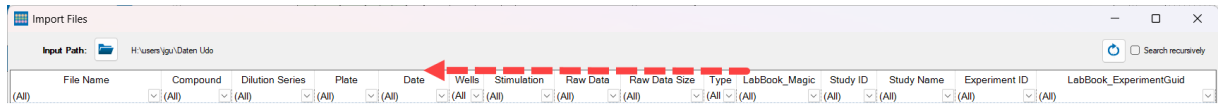
When merging files generated with the Incub8-Screen software which have different dilution series, the "Merging the Series" dialog opens.



The Incub8-Analyzer software tries to organize the different series of files which should be merged in a reasonable order. If the arrangement is not optimal, please reorganize the sequence manually. Click onto one of the labels and move it via drag and drop to the desired position. It is not possible to move the "Control" label, this phase must always be the first phase of the experiment.

File Information

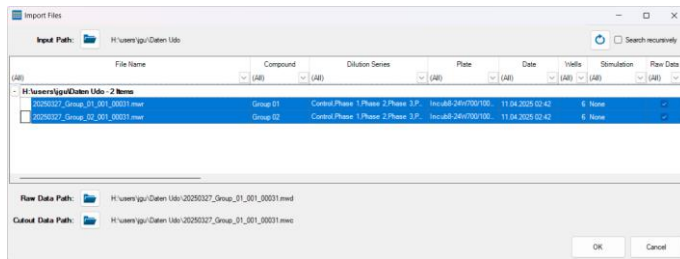
Find important information about the offered files directly in the "Import Files" dialog: File Name, Compound, Dilution Series, Plate, Date, Well (total number of wells in use) Raw Data (available or not), Raw Data Size, Type (Cardio or Neuro), Study ID, Study Name, Experiment ID, Plate ID, Cell Line ID, Cell Line Name, Cell Culture Age, Dilution Medium, Institution, Director, Lab, Scientist, Operator, Notes.



Please sort the columns for your needs via drag and drop.

Hide or expand the files in a subfolder by clicking on the + plus or - minus symbols on the left side of a row.

Use the filter function in the header of the "Import File" dialog to search for appropriate files. Each item contains the filter function "All", that means, no filter and "Empty" to search for missing entries. Select the search parameter from the drop down list. When using more than one parameter, the file selection is displayed with the "AND gating" principle.

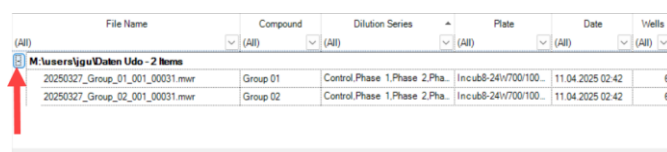


Select one file for analysis and open it via double click or with the "OK" button. Select more files via drag and drop and open them with the "OK" button.

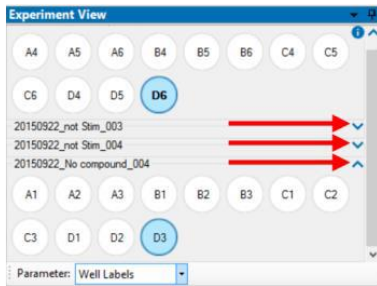


When opening a subfolder via + "Plus" symbol, information about its content is displayed: "Nested Experiments, 2 items", for example, if two raw data files had been analyzed together and stored in a "*.mwa" file, or if an experiment has been recorded in Incub8-Screen with the setting "Recording: Multiple Files" and files containing data from recording phases of the experiment have been found.

You find the note "merged" in an "Incub8-Analyzer" file with the extension "*.mwa" if the compounds in the analyzed raw data files had been different, find "multiple" if the type of the multiwell plates used in that files had been different.



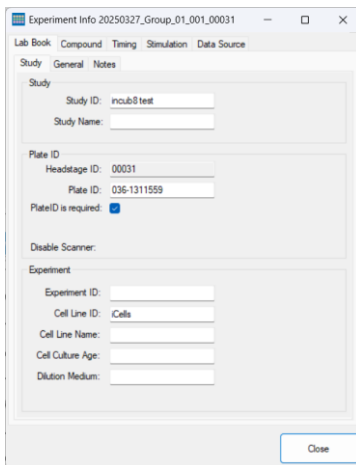
Experiment View



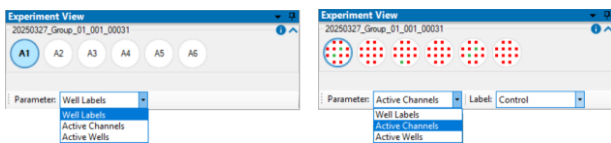
See the imported raw data file(s) in the "Experiment View". Import more than one file to merge the data.



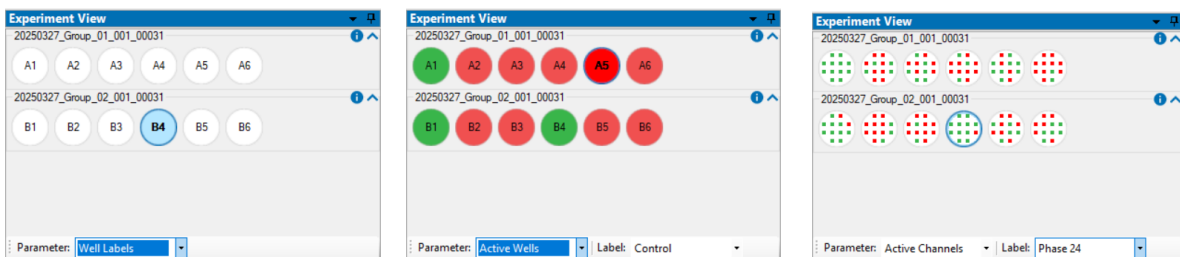
Use the blue arrow to expand or retract the display of the available wells in that file. Click onto a well to display the data in the "Data Display" beside. The selected well is highlighted in blue or with a blue frame.



Click the blue "i" to get detailed information about the file to be analyzed. The dialog has four tabbed pages: "Lab Book", "Compound", "Timing" and "Stimulation". This information should work as aid memoire, you are not allowed to change entries in the original file with the exception of the lab book which can be completed.



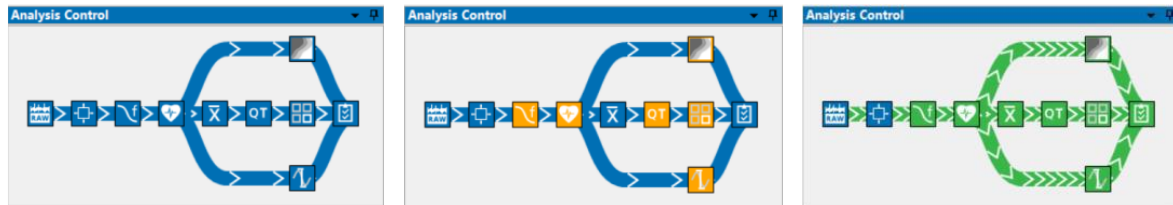
Select the "Parameter" from the drop down menu. Display "Well Labels", "Active Wells" or "Active Channels" and choose the "Label", if available.



Select parameter "Active Wells" and the active wells are color coded in green, inactive wells appear in red. Use the drop down box "Dose" to switch from dose to dose.

The display format "Active Channels" shows the active and inactive channels, color coded in green and red and the designated channel for the dose-response curve, additionally framed in blue. Again, you can switch from dose to dose.

Analysis Control Window



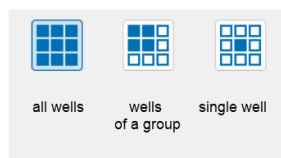
Control the state of the analysis in the flow process chart of the "Analysis Control" window. Squares symbolize the raw data source, analysis instruments and result storage. Their colors indicate the current state of the analysis: Blue instruments are idle, while orange instruments indicate that their analysis settings have been modified but are not yet applied in an analysis. If an analysis is running the data flow from the data source to the result, storage is indicated in green and instruments participating in the analysis are also shown in green. The base instrument of the analysis will start in blue and gradually fill up in green to indicate the progress of the analysis. The tool tip on this instrument also displays the progress as a percentage.

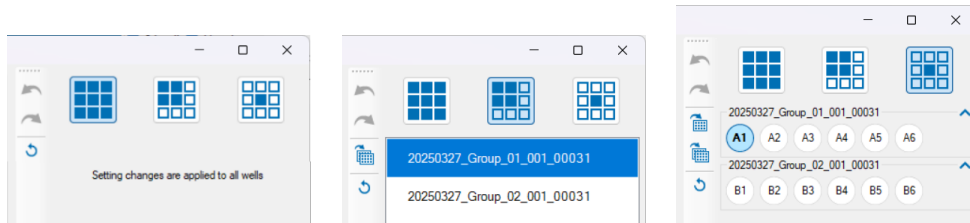
Click onto the respective buttons in the diagram to open the dialogs for fast interactions. Alternatively adapt the settings parameter for the "Time Segment" via "Edit Analysis Segment" button [Edit Analysis Segments](#) in the "Raw Data Explorer" tab.

Example: Suppose you want to analyze the data with modified settings for the spectral filter and the heartbeat detection. If you click on their buttons in the chart, perform the appropriate modifications in the dialogs and click on "Save Settings" in the dialog and the spectral filter and the heartbeat detector will be highlighted in orange: The settings have been modified, but the analysis applying these settings has not yet been performed. Clicking on any instrument and selecting "Run Analysis" will start the analysis with the new settings. The time segment settings have not been changed so this instrument stays inactive (blue). However, the spectral filter has been modified, so it is the base instrument for this analysis and its update from blue to green shows the progress of the analysis. Because the filtering has an effect on any subsequent instrument, the spike detector (also with modified settings) and the burst detector need to be active (green) as well. After the analysis is finished, all instruments are idle (blue) again, because all modifications have been handled during the analysis. If the analysis was stopped prematurely, some modifications have not yet been applied, so some instruments remain modified (orange). They will be handled when the next analysis is started. Click on the respective buttons in the diagram to open the dialogs for fast interactions during analysis.

Working Level

Most of the tools need a definition on the level you like to work on: Should changes in the settings of the respective tool dialog affect all electrodes of the plate, all electrodes in a group, or the electrodes in one well only. The default is "Setting changes are applied to all wells". Please see also chapter "Tutorial".







Time Segment

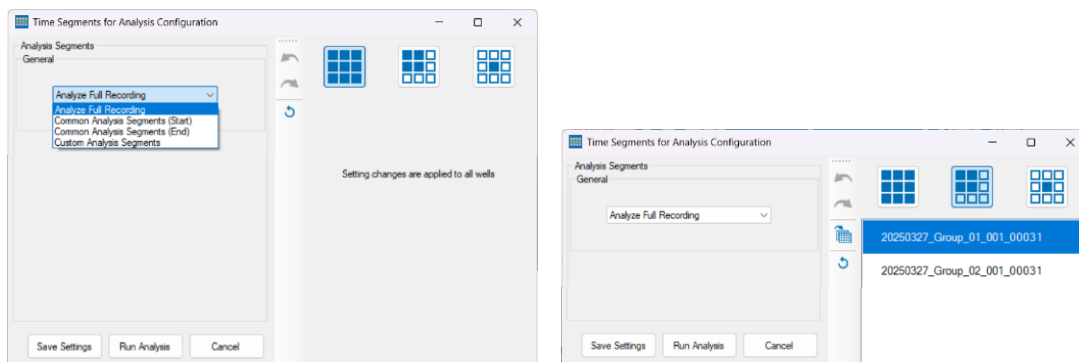
Adapt the "Time Segment" settings to define the time span to which analyze parameters should be applied. There are two ways to do so, via the "Time Segment Selection" icon in the "Analysis Control" flow chart or via "Edit Analysis Segment" in the "Raw Data Explorer".

Click the icon "Time Segment Selection" in the flow chart.

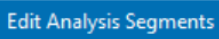
Based on the "Raw Data" , the definition of the "Time Segment Selection"  is the first step.

Analyze Full Recording

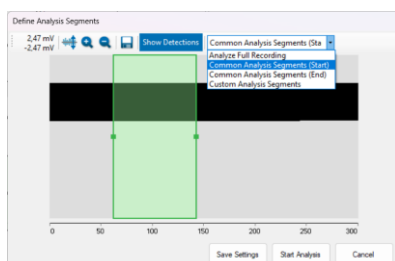
The first option of the menu is "Analyze Full Recording". Analysis parameters are applied to the complete duration of the recorded file. This is indicated by the green frame all over the time display, when clicking the blue button "Edit Analysis Segments".



Select the first option from the drop down menu "Analyze Full Recording" on the left side and select the working level on the right side.

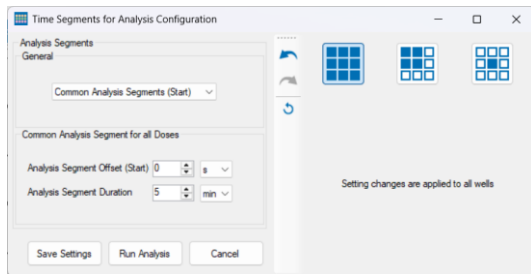
Alternative: Click the button "Edit Analysis Segment"  in the "Raw Data Explorer" tab of the "Data Display" window to open a window with the same functionality. The drop down menu of this dialog is identical to the drop down menu in the "Time Segment for the Analysis Configuration" dialog.

The dialog allows to define time frames for analysis graphically. You can see data as raw data in "Show Raw Data" mode or heartbeat detections only in "Show Detection" mode.

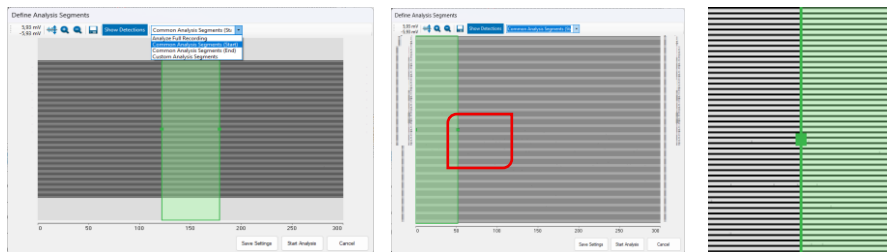


Define the time frame for analysis individual for each well or compound on the well plate.

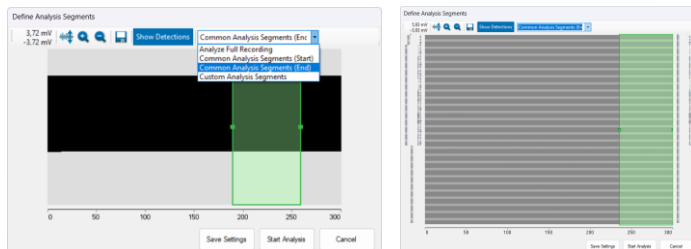
Common Analysis Segments



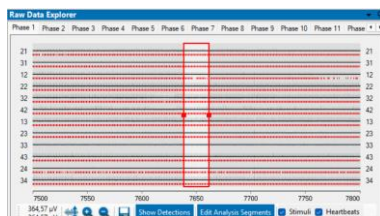
Select the option "Common Analysis Segments (Start)" or "Common Analysis Segments (End)" either in the "Time Segment for the Analysis Configuration" dialog or in the "Define Analysis Segments" dialog. Define the start and the duration of the analysis for all doses via up-down boxes in seconds on the one hand or by drag and drop on the other hand. Please click on the dark green point in the green frame and keep the mouse button pressed to modify the borders of the frame. Click into the green frame to move the complete time frame over the display.



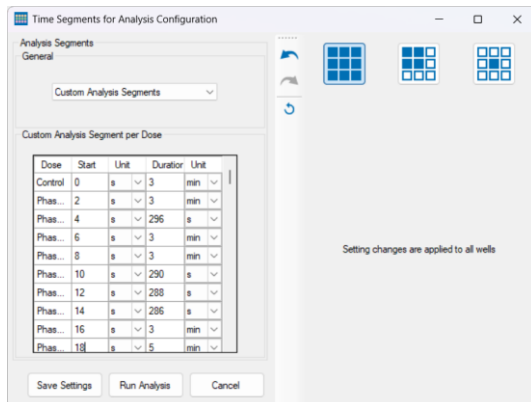
Analysis parameters are applied to the beginning of the experiment with the option "Common Analysis Segments (Start)". Use the option "Common Analysis Segments (End)" to couple the analysis parameter to the end of the experiment.



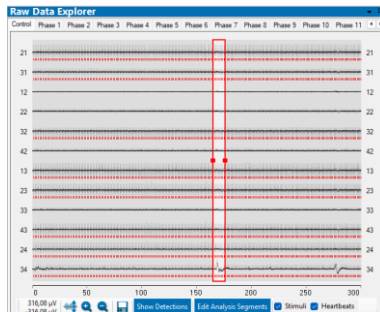
The data display in the "Raw Data Explorer" is adapted respectively. Again, the time frame for analysis is highlighted in green. The red frame is for display settings only and indicates the period of time shown in the "Raw Data Explorer" windows below, in the over- and single view.



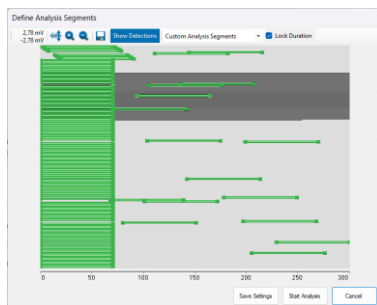
Custom Analysis Segments



Select the option "Custom Analysis Segments" and define the time stamp of the start of the analysis for each dose separately. The duration of the time segment is fix for all doses if the check box "Lock Duration" is active. Deselect the check box to define the duration of the analysis segments variable by expanding or reducing the green areas. Move the contact point in front of the green area or at the end. Please see the red arrows in the screenshot.

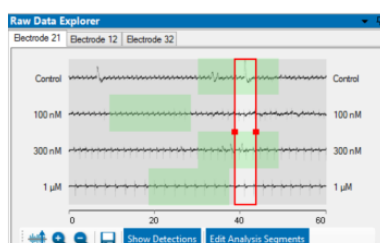


Click "Edit Analysis Segment" in the "Raw Data Window".



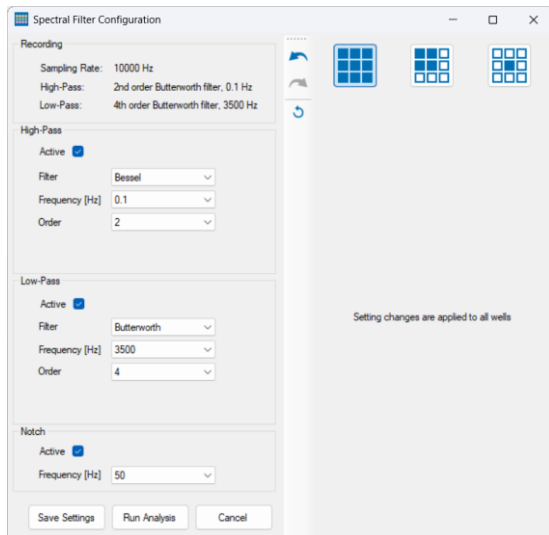
Alternatively select "Custom Analysis Segment per Dose" and fill in the table in the "Time Segment for the Analysis Configuration" dialog.

The data display in the "Raw Data Explorer" is adapted respectively. Please select "Show Activity per Concentration" **Show Activity per Concentration** display. Again, the time frame for analysis is highlighted in green. The red frame indicates the period of time shown in the "Raw Data Explorer" windows below, the over- and single view.



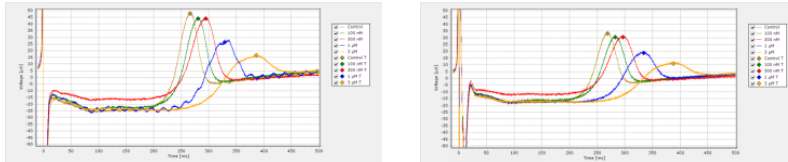
Spectral Filter

After defining the time frame for analysis, the data stream is applied to the "Spectral Filter" .



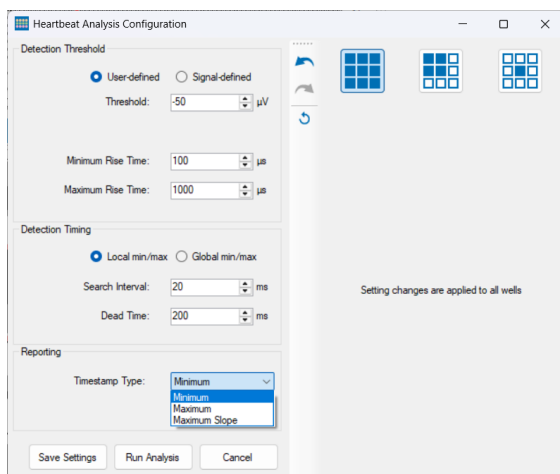
Select the filter settings, if necessary. Select the filter type via check box and choose between "Bessel", "Butterworth" or "Chebyshev" for high-pass and low-pass. Define the filter "Frequency" in Hz from the drop down menus. Choose the "Order" of the high and low pass filter. The "Notch" filter is helpful to remove noise.

Example: Without Notch filter on the left, with Notch filter on the right screenshot.



Heartbeat Analysis

Then, the filtered data are processed via the "Heartbeat Detection" tool. Click onto the icon to open the "Heartbeat Analysis Configuration" dialog.



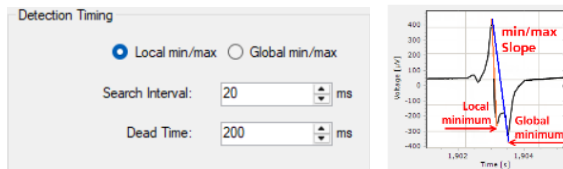
Detection Threshold



The following parameters characterize the heartbeat, which should be detected. The heartbeat detection can be done either via "User defined" threshold values in μV or based on exceeding a defined number of standard deviations of the noise level "Signal defined". Using the signal dependent threshold detection, the waveform detection is more flexible.

Define the "Threshold" of the amplitude for the detection in μV . The value of the threshold is positive regarding the rising edge of the waveform, the value is negative regarding the falling edge. Define the time frame for the estimation of the signal "SD Estimation Duration" in ms. Use the up-down boxes for the "Minimum Rise Time" and "Maximum Rise Time" in μs . If the rise time is chosen very short, the slope of the peak is very steep. If the rise time is longer, the slope will be smooth.

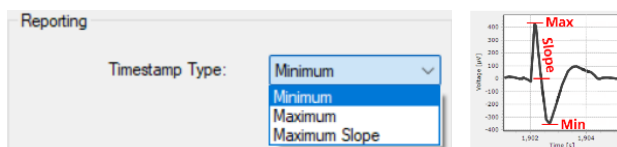
Detection Timing



Select via radio button the type of the minimum or maximum: "Local" or "Global". These options refer to one single field potential signal: "Local" means the first detected minimum or maximum, "global" means the deepest minimum or highest maximum in the field potential within the min./max. "Search Interval" in ms. Please see the picture.


The detection "Dead Time" in ms defines the time in which no new detection is performed.

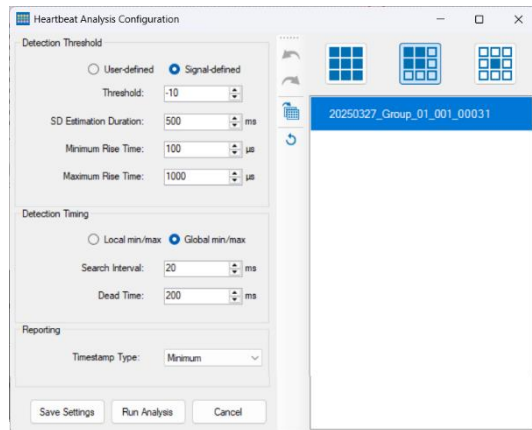
Reporting



Choose where to set the time stamp from the "Time Stamp Type" drop down box.

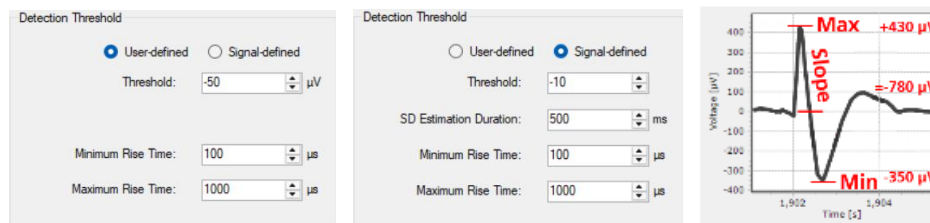
Heartbeat Analysis

Then, the filtered data are processed via the "Heartbeat Detection" tool. Click onto the icon  to open the "Heartbeat Analysis Configuration" dialog.



The "Heartbeat Averager" in the Incub8-Screen software averages the waveform of a signal in relation to each heartbeat in a time segment. The display shows several recordings of an electrode channel in overlay and average the values. The pre and post time in ms before "Duration pre" and after "Duration post" a heartbeat can be selected via up-down box. Modify the pre and post time in the Incub8-Analyzer software to run an analysis.

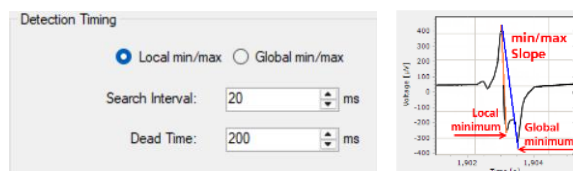
Detection Threshold



The following parameters characterize the heartbeat, which should be detected. The heartbeat detection can be done either via "User defined" threshold values in μV or based on exceeding a defined number of standard deviations of the noise level "Signal defined". Using the signal dependent threshold detection, the waveform detection is more flexible.

Define the "Threshold" of the amplitude for the detection in μV . The value of the threshold is positive regarding the rising edge of the waveform, the value is negative regarding the falling edge. Define the time frame for the estimation of the signal "SD Estimation Duration" in ms. Use the up-down boxes for the "Minimum Rise Time" and "Maximum Rise Time" in μs . If the rise time is chosen very short, the slope of the peak is very steep, if the rise time is longer, the slope will be smooth.

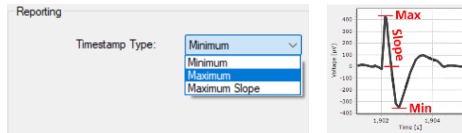
Detection Timing



Select via radio button the type of the minimum or maximum: "Local" or "Global". These options refer to one single field potential signal: "Local" means the first detected minimum or maximum, "global" means the deepest minimum or highest maximum in the field potential within the min./max. "Search Interval"


in ms. Please see the picture. The detection "Dead Time" in ms defines the time in which no new detection is performed.

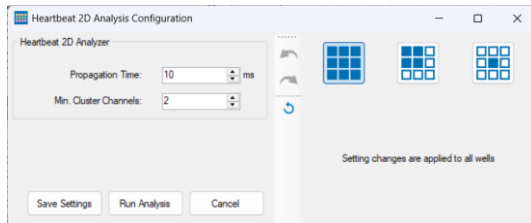
Reporting



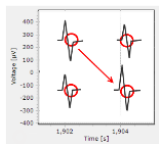
Choose where to set the time stamp from the "Time Stamp Type" drop down box.

Heartbeat 2D Latency Analysis

Click the "Heartbeat 2D Latency Analysis" icon  to open the "Heartbeat 2D Analysis Configuration" dialog.



In this dialog you can define the time span from the drop down menu in which the "Propagation Time" should be calculated.

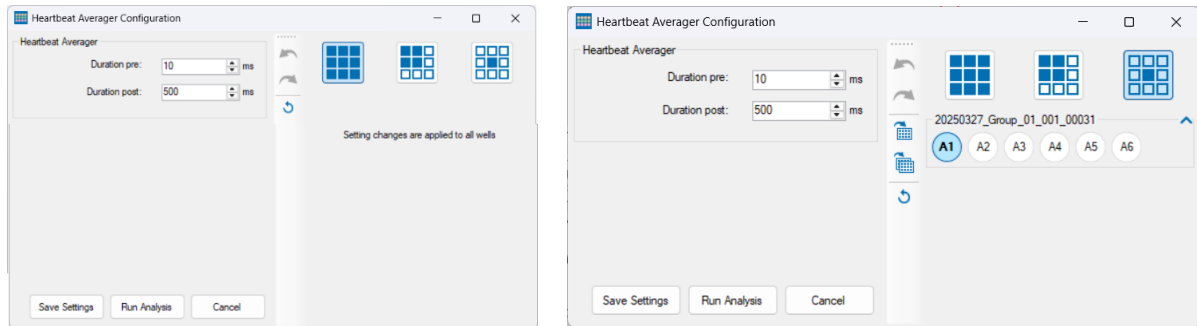


The action potential runs over the heart cell culture or tissue or whole heart from the pacemaker to the peripheral regions of the syncytium. The excitation propagation is measured in spatial resolution by the electrodes detecting cardiac field potentials and extracting local activation times. The propagation time is the longest possible period for passing the excitation from the first detected excited cell to the last detected excited cell of one cluster. If the propagation time is too long, the next heartbeat starts, and the results are incorrect. So, the appropriate time has to be tested empirically, depending on the experiment.

The "Min Cluster Channels" gives the minimum number of channels to form a cluster. For example, if a syncytium covers all 12 electrodes of a well from a 24-well plate it could be 10, otherwise it should be less than 10. For 96-well plates with three electrodes per well, it must be 2 at least.

Heartbeat Averager Analysis

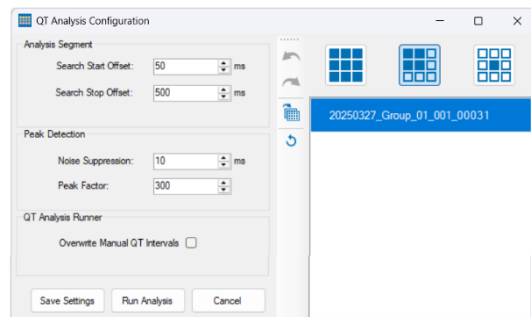
Click the "Heartbeat Averager Analysis" icon to open the "Heartbeat Averager Configuration" dialog.



The "Heartbeat Averager" in the Incub8-Screen software averages the waveform of a signal in relation to each heartbeat in a time segment. The display shows several recordings of an electrode channel in overlay and average the values. The pre and post time in ms before "Duration pre" and after "Duration post" a heartbeat can be selected via up-down box. Modify the pre and post time in the Incub8-Analyzer software to run an analysis.

QT Latency

Click the "QT Latency" icon to open the "QT Latency Configuration" dialog.



In Incub8-Screen software the "QT Detector" detects the QT-like interval in the averaged heartbeats.

Important: We are aware the technical term "QT-Analysis" is not correct for the analysis described here, because data are not recorded from an electrocardiogram of a whole heart but from cell tissues or cell cultures. Nevertheless, the term is used synonymously.

Theoretical background: The field potential duration corresponds to the action potential duration, which can be correlated to a QT-like Interval in an electrocardiogram. It is measured from minimum of the Na⁺ peak to the maximum / minimum of the IKr current peak. Please read also chapter "Theoretical Background".

Analysis Segment

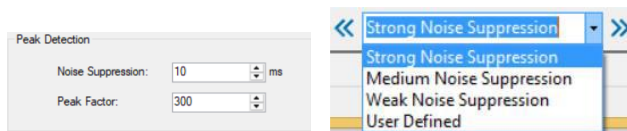
Define the parameters for the QT-like interval in Incub8-Analyzer software: In "Analysis Segment" window, please modify the "Search Start Offset" and the "Search Stop Offset" in ms.

Note: The averaging interval must be at least as large as the QT Analysis interval.

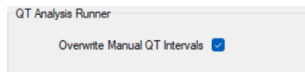


Peak Detection


In the "Peak Detection" window, select the "Noise Suppression" in ms and the "Peak Factor" from the up-down boxes. Think about the settings in the "Single View" window, please read chapter "Single View" in "QT-Analyzer Window".



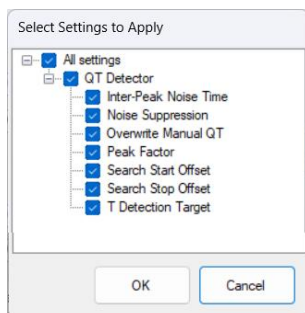
QT-Analysis Runner




The check box "Overwrite Manual QT Intervals" defines whether the manually modified "T" detection points in the current well will be overwritten by the automatic analysis.

Click the "Apply settings to all wells of that compound" button  and the following dialog appears.

The settings will be applied to all wells, all compounds and all plates. Please read the chapter above to decide which option you want to apply.




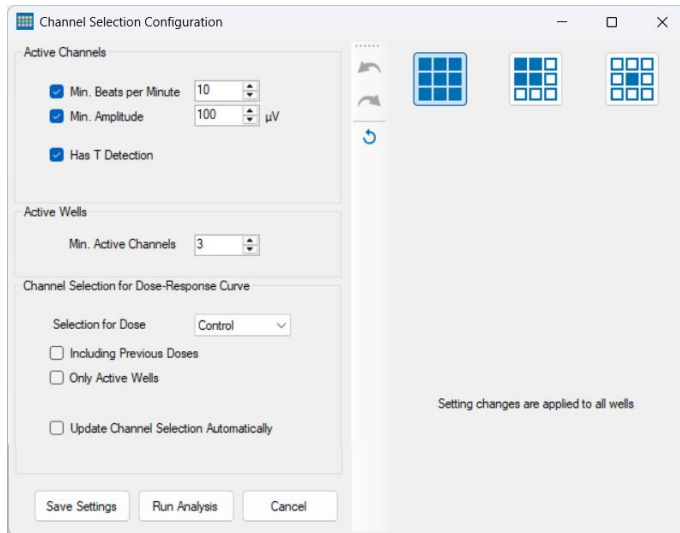
Select the settings you want to apply via check boxes. The settings will be immediately applied to all wells of the currently selected compound.

Click the "Apply changes to all wells" button  and the same dialog appears. Select the settings you want to apply via check boxes. The settings will be immediately applied to all wells.

Use the arrow buttons for "Undo last changes" and "Redo last changes" and for the "Reset".

Channel Selection

Click the "Channel Selection" icon  to open the "Channel Selection Configuration" dialog.



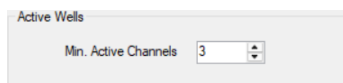
The "Channel Selection" is a tool to estimate and define the activity of a channel or a well.

Active Channels



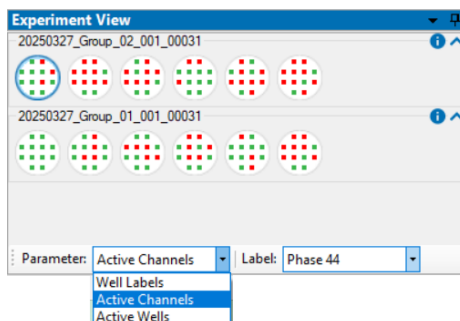
Define the parameter for channel activity, the minimum heartbeat rate "Min Beats per Minute" and the "Min Amplitude" in μV from the up-down boxes. Click the check box "Has T Detection" if this parameter should be considered for the activity definition.

Active Wells

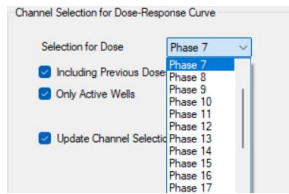


Define the minimum number of active channels for the definition of an active well from the "Min. Active Channels" up-down box.

Visualize the active channels or wells via "Parameter" drop-down menu in the "Experiment View".



Channel Selection for Dose-Response Curve



Select the respective channels for the analysis via dose-response curve. Please select the dose from the drop-down menu first. "Include the Previous Dose" if necessary, by clicking the check box. Use "Only Active Wells" if desired and "Update Channel Selection Automatically" via check boxes.

This menu defines, which channel in a well is selected as the "representative channel", for example, the one included in the dose-response curve. Only channels that fulfill the criteria defined under "Active Channels" in the "Channel Selection Configuration" dialog can be selected as the representative channel. If no channel fulfills the criteria, no representative channel is selected for this well and it is not used for the dose-response curve.

Because the set of active channels can change between concentrations, use the drop-down menu "Selection for Dose" in the "Channel Selection Configuration" dialog to define, which set of active channels should be used. For example, if "Selection for Dose" is set to "10 nM", the representative channel is selected from the set of channels that are active for the 10 nM concentration.

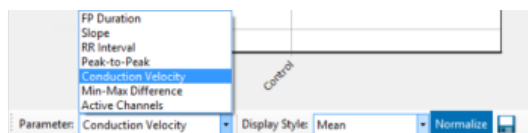
If the check box "Including Previous Doses" is enabled, the representative channel will be selected from the set of channels that are active for all concentrations up to the one selected in the "Selection for Dose" drop-down menu.

For example, if "Selection for Dose" is set to "10 nM", a channel has to fulfill the criteria set for "Active Channels" for both, the control condition and the 10 nM concentration in order to be eligible to become the representative channel. In order to make sure that representative channels are active for all concentrations in the experiment, set "Selection for Dose" to the last dose in the experiment and check the "Including Previous Doses" check box.

If the "Only Active Wells" check box is enabled, wells with a low number of active channels can be excluded from the dose-response curve. If a well is not active for the concentration defined in "Selection for Dose" (or all concentrations up to and including this dose if "Including Previous Doses" is checked), no representative channel is selected even if active channels exist. The threshold number of active channels per well is defined in the "Active Wells" menu.

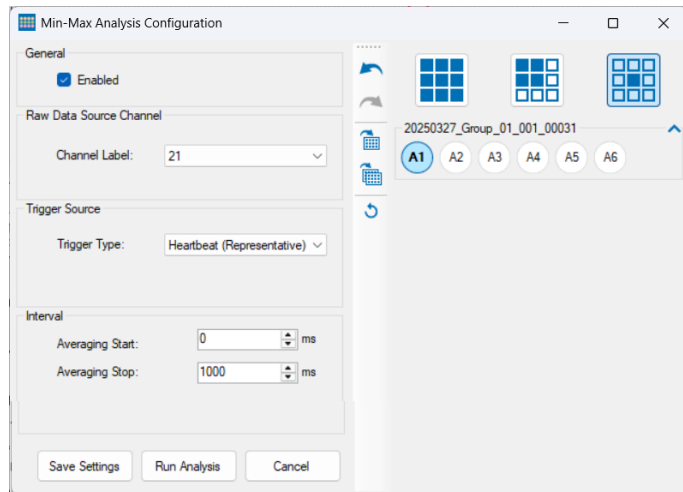
If the "Update Channel Selection Automatically" check box is enabled, the channel selection process will run after each analysis and may select a different channel. If it is disabled, the channel selection will not be automatically updated. This can be helpful if a specific representative channel should be kept.

The parameter "Active Channels" per well is available in the display of the dose-response curve and in the report sheet.



Minimum-Maximum Detection

Click the “Min-Max Detection” icon to open the “Min-Max Analysis Configuration” dialog.



The “Min-Max Analysis” is a tool that extracts a cutout of raw data relative to each heartbeat, averages the cutouts and detects the minimum and maximum in the averaged cutout. This tool does not operate on all channels in each well. Instead, you have to select one channel as the source of raw data and one channel as the source of the heartbeats, which can be the same channel.

To enable the tool for a well, enable the check box in the "General" section of the dialog. Select the number of the channel which delivers the raw data from the drop down menu in the “Raw Data Source Channel” section.

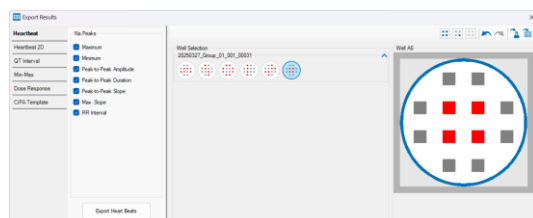
For the channel providing the heartbeats, you may either select "Heartbeats (Representative)" or "Heartbeats (Manual)" in the "Trigger Source" section. Selecting "Heartbeats (Manual)" lets you define a fixed channel in the well as the heartbeat source. Selecting "Heartbeats (Representative)" will use the representative channel of the well as the heartbeat source. This means that if the representative channel changes, either because it has been changed manually or because the “Channel Selection” tool has chosen a new one, the “Min-Max” tool will have to reanalyze this well.

Define the start and stop timestamp of the cutout relative to the heartbeat timestamp in the "Interval" section. The "Averaging Start" value defines the start timestamp of the cutout. A negative value indicates a start of the cutout before the trigger timestamp.

Save the settings to arm the system with the “Save Settings” button or run the analysis by clicking “Run Analysis” button.

Export Result

Click the “Export Result” icon to open the “Export Result” dialog.



This "Export" feature is analogous to the feature in the "Multiwell Screen" software.


Choose the tab and the parameters you want to export via check box on the left side. Select the electrodes and the wells by clicking on them on the right side of the dialog.


There are six tabbed pages providing analysis parameters:

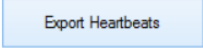
- Heartbeat
- Heartbeat 2D
- QT-related Interval
- Dose-Response
- Min-Max
- CiPA Template

On each of the pages, there are a number of parameters which can be selected or deselected via check box. Clicking the "Export" button on one of the pages will export these parameters to a "comma separated value" ".csv" file or to an Excel ".xlsx" file for further processing.

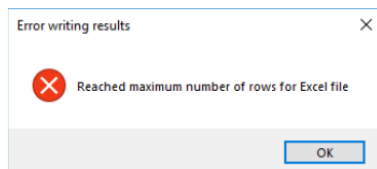
The tabs of the export dialogs are built analogously. On the left side of the dialog enable or disabled the check boxes to choose the parameters you are interested in. Select the designated electrode channel on the electrode field and select the wells.

Use the "Select all Electrodes in the Well" button  to select all electrodes of a well for the export. Click the "Select Representative Channel in the Well" button to export the representative electrode only or deselect all channels with the "Deselect all Electrodes in the Well" button.

Apply the settings to all wells  with the same compound or to all wells of the plate.

Click the respective button  to export the file as comma separated file ".csv" without any limitations of data volume in one result file.

Excel files ".xlsx" are limited to 1048576 rows per file. If the limitation of data volume is reached in Excel, the following error message appears and the exported ".xlsx" file will contain only 1048576 rows.



Heartbeat Tab

Select the parameter you want to export via check box. Click the "Export Heartbeat" button to save the data as comma separated value file.



Heartbeat 2D Tab

Select the parameter you want to export via check box. Click the "Export 2D Heartbeat" button to save the data as comma separated value file.



QT Interval Data Tab

Select the parameter you want to export via check box. Click the "Export QT Interval Data" button to save the data as comma separated value file.



Min-Max Difference Tab

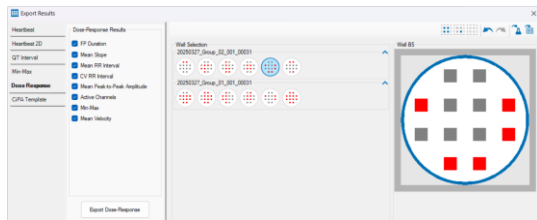
Select the parameter you want to export via check box. Click the "Export Min-Max" button to save the data as comma separated value file. Additionally, it is possible to export the Min-Max detection points or the data traces separately.



Choosing the "Aggregated" option will export the Min-Max detection points, their amplitude difference and the number of averaged samples. Choosing the "Averaged Trace" option will export the averaged traces.

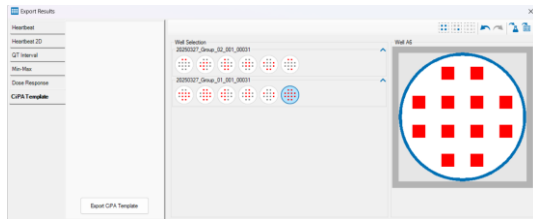
Dose-Response Tab

Select the parameter you want to export via check box. Click the "Export Dose-Response" button to save the data as comma separated value file.

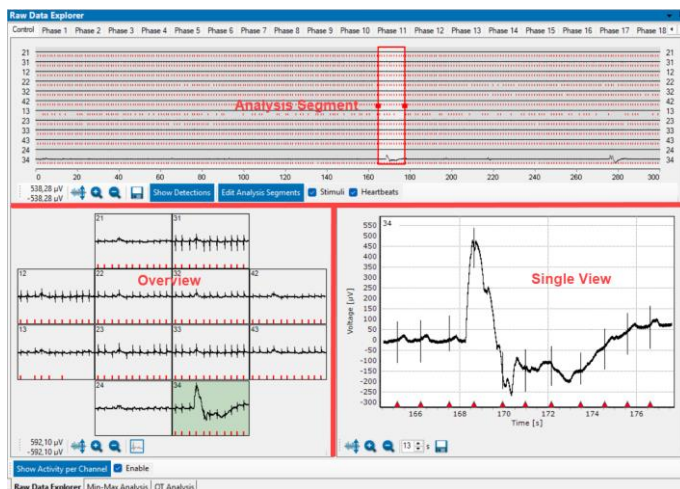


CiPA Template

Click the "Export CiPA Template" button to save the data as comma separated value file.



Data Display Window



The "Data Display" window has three tabbed pages "Raw Data Explorer", "Min-Max Analysis" and "QT Analysis".

Raw Data Explorer

The "Raw Data Explorer" tab is divided in three smaller windows, the "Analysis Segment" view of all data in the file above, the "Overview" includes all electrodes of the selected well and the detailed "Single View" of one selected electrode.

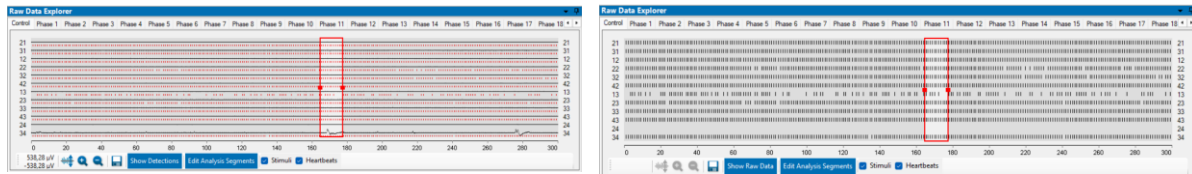
The raw data window shows the data in overview on the left side and in single view on the right side. To see all channels of the well, as shown on the left screenshot, please click the button "Show Activity per Channel" [Show Activity per Channel](#).

Click the button "Activity per Concentration" [Show Activity per Concentration](#) to display all concentrations of the selected well in overview as shown in the right screenshot.

Click the check box "Enable" to "Show Activity per Channel" or to "Show Activity per Concentration". If more than one concentration is available, you can switch between respective tabs. Please read chapter "General Software Features" for the operation of this window. To save computer performance, please disable the check box "Enable" and the raw data display window is empty. Nevertheless, data are analyzed and saved.

Analysis Segment

See the overview of the complete data file in the window above. Select the output mode "Show Raw Data" or "Show Detections".



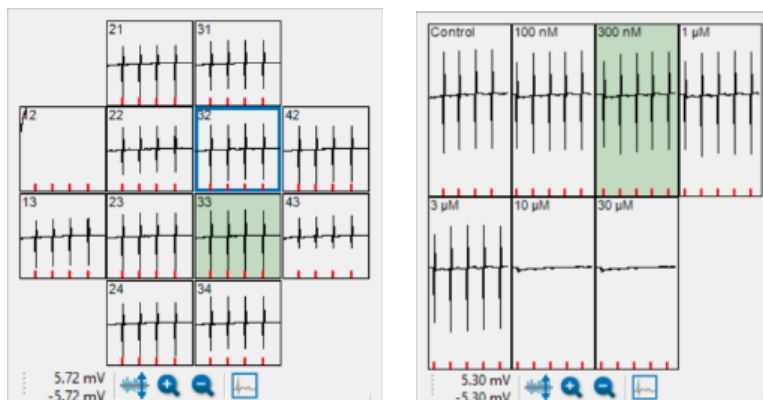
Move the red frame through the data file to select the region of interest. It is possible to enlarge the frame to enlarge also the time span shown in the displays. Alternatively, you can use the up-down box in the "Single View" to modify the time span. The variation of the time span is immediately adapted in all three displays. See data as raw data in "Show Raw Data" mode or spike detections only in "Show Detection" mode.


Use the "Export" button to export this graph in "*.bmp", "*.jpg" or "*.png" format.


Click the "Edit Analysis Segments" button to open the "Define Analysis Segment" dialog. The dialog allows to define time frames for analysis graphically. Again, you can see data as raw data in "Show Raw Data" mode or heartbeat detections only in "Show Detection" mode. Please read chapter „Time Segment" for detailed information.


Overview

In the "Overview" display you see all electrodes of the well, selected in "Experiment View". Click on one of the electrode channels for the "Single View". The electrode will be highlighted in green. Jump between the electrodes for a narrow view. On the left screenshot, you see the activity per channel, on the right screenshot the activity per concentration. Each electrode channel or each concentration is labeled for exact correlation.

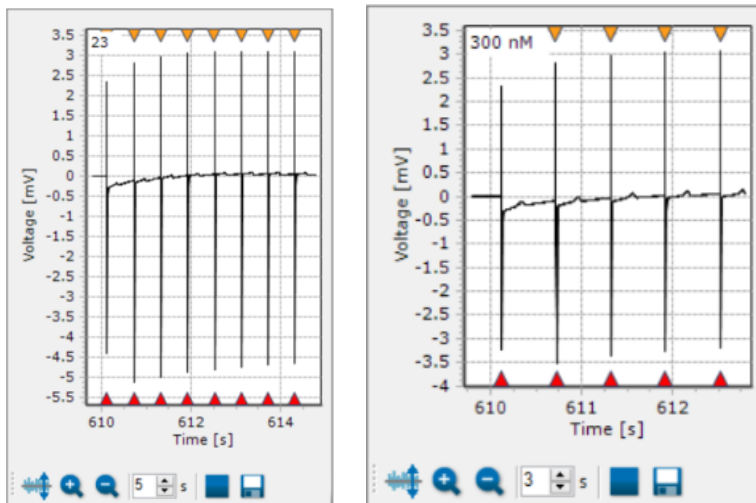


Click the "Adjust to signal Min/Max" button  and read the current value beside. The scaling of the y-axis


is set to the minimum and maximum of all samples in the well. Clicking the button  "Zoom In" cuts the scaling in a half and "Zoom Out" doubles the scaling. One channel of the well has to be defined for analysis and display of the "Dose-Response" curve. Define this electrode by clicking the "Select representative channel"


button . The channel framed in blue is then representative for the dose-response curve.


Single View



The channel analyzed in the single view is highlighted in green in the overview. The red triangles indicate heartbeat detection points, the yellow triangles indicate electrical stimulation pulses at that time. The beginning of LED stimulation pulses is marked with a triangle and the duration with a horizontal line. Both have the same color as the stimulating LED.

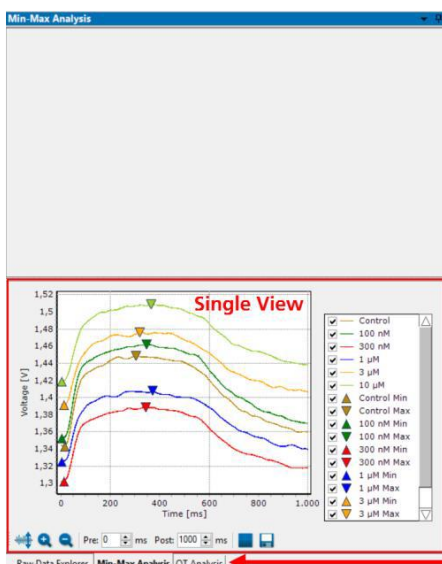
Click the "Adjust to signal Min/Max" button  and read the current value beside. The scaling of the y-axis

is set to the minimum and maximum of all samples in the well. Clicking the button  "Zoom In" cuts the scaling in a half and "Zoom Out" doubles the scaling. Adjust the time scale in seconds via up-down box.

Start, stop and save  the single view separately. Create a comma separated value file or an image file.

Min-Max Analysis Window

The "Data Display" window has three tabbed pages "Raw Data Explorer", "Min-Max Analysis" and "QT Analysis". Please see the red arrow at the bottom of the screenshot.



In this window an "Overview" display is not necessary, because there is one Min-Max Analysis per well only. The selected electrode is shown in the detailed "Single View". See the "Min-Max Analysis" of each dilution of the experiment color coded as overlay plot. The minimum of a track is indicated by a triangle with its tip up, the maximum of a track is indicated by a triangle with its tip down.


In the legend you see the color coded assignment of the dilutions of the compound. Show or hide a track or both triangles of a track by selecting or deselecting the respective check box. If the check box of one of the triangle markers is deselected, the min-max distance for this dose will be removed from the analysis and the triangle markers will be hidden in the display. Checking one of the triangle marker check boxes will show the markers in the display and include the min-max distance in the analysis. To change a maximum or a minimum manually, please move the respective triangle via drag and drop.

Important: In the "Report" sheet manually modified "Min or Max" detection points will be marked in red.

Extract from the Report Sheet


Dose	Heart Beat Count	Peak-to-Peak [µV]	Slope [V/s]	RR Interval [ms]	FP Duration [ms]	Min-Max Difference [µV]	Active Channels
Control	33	2107.505	-6.023	1749.819	657.3	50.059	14
100 nM	34	2139.894	-6.147	1703.636	631.5	47.392	14
300 nM	34	2074.398	-6.074	1700.464	623.4	18.525	13
1 µM	34	1916.344	-5.987	1694.161	609.2	47.183	12
3 µM	36	1584.938	-5.176	1599.92	587.1	31.618	14
10 µM	35	1615.19	-5.441	1624.65	585	42.872	13

If the check box of a trace is deselected, the track is hidden in the display but will be included in the analysis

anyway. Click the "Adjust to signal Min/Max" button  and read the current value beside. The scaling of the y-axis is set to the minimum and maximum of all samples in the well. Clicking the button "Zoom In"

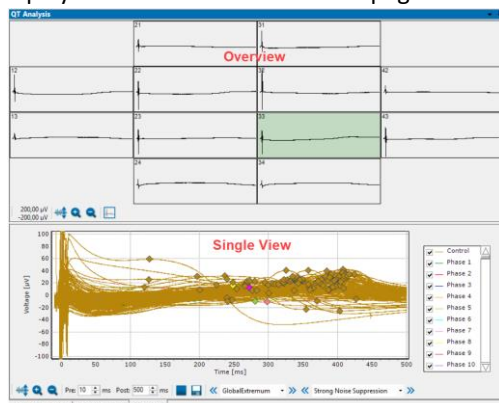


cuts the scaling in a half and "Zoom Out" doubles the scaling.

Adjust the "Pre" and "Post" time in ms via up-down boxes. Start, stop and save  the single view separately. You may save the displayed data either as a *.csv file, or you can export it as an image in *.png, *.bmp or *.jpg format.

QT-Analyzer Window



The "Data Display" window has three tabbed pages "Raw Data Explorer", "Min-Max Analysis" and "QT




Analysis".

Overview

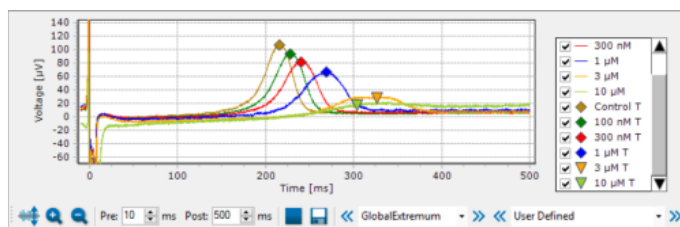
In the "Overview" display, you see the averaged value of the data segment around all detected heartbeats. Select one electrode channel for the "Single View". The electrode will be highlighted in green. Jump between the electrodes for a narrow view. The averages of the available data traces from different dilutions are displayed in overlay in the "Single View" window below.

Click the "Adjust to signal Min/Max" button  and read the current value beside. The scaling of the y-axis is set to the minimum and maximum of all samples in the well. Clicking the button "Zoom In"  cuts the scaling in a half and "Zoom Out" doubles the scaling.

One channel of the well has to be defined for analysis and display of the "Dose-Response" curve. Define this electrode by clicking the "Select representative channel" button . The channel framed in blue is then representative for the dose-response curve.

Single View



Select one of the electrode channels for the "Single View". The single view displays all measured concentrations in an overlay plot. For a better discrimination the compound concentrations are color coded. The detection point of the automatic T-detection is indicated by a diamond. When moving one of the detection points manually, the diamond turns into a triangle.



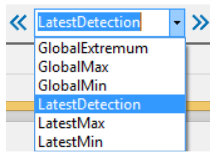
In the legend you see the color coded assignment of the dilutions of the compound. A diamond indicates a "T" peak found by the automatic analysis of the software, while a triangle indicates a manually modified detection point. If no peak is found, you have the possibility to add a detection point manually. Select the check box beside the triangular symbol and the symbol will appear in the right edge of the "Single View" window. Drag the symbol with your mouse and move the triangle to the point of your detection.


For the representative channel of the well, only the "T" detection points with a checked check box are included in the computation of the dose-response curve. Deselecting the check box besides a "T" detection point will remove it from the analysis and hide it in the display, while checking it will show the marker in the display and it will be included in the analysis. Show or hide a track or a symbol by selecting or deselecting the respective check box.

Important: In the "Report" sheet manually modified "T" detection points will be marked in red.

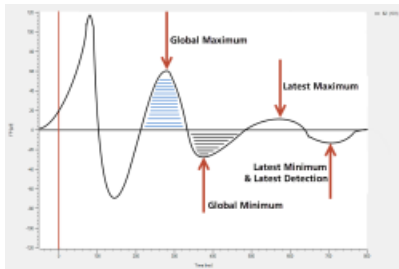
Click the "Adjust to signal Min/Max" button  and read the current value beside. The scaling of the y-axis is set to the minimum and maximum of all samples in the well. Clicking the button  "Zoom In" cuts the scaling in a half and "Zoom Out" doubles the scaling. Adjust the "Pre" and "Post" time in ms via up-down boxes. Start, stop and save the single view separately.

Detection

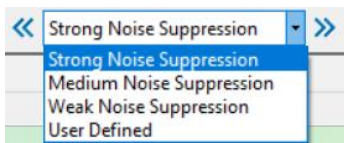



The "QT Detector" detects the QT-like interval in the averaged heartbeats. Define the parameters for the QT-like interval from the drop-down menu or click on the blue double arrows  beside the drop-down menu to change the setting.

The "T Detection Target" defines your preferred detection target. The "Global Extremum" describes the extremum over the greatest integral. "Maximum" or "Minimum" define the respective maxima or minima. Please see the following picture.



Noise Suppression

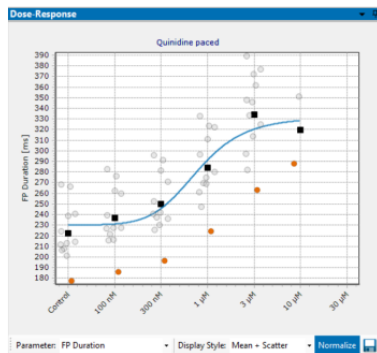


Maybe the recorded data are overlapped with noise and the "T-Detection" is not possible. Please use the algorithm of the "Noise Suppression" for a successful detection. Define the parameters from the drop-down menu or click on the blue double arrows  beside the drop-down menu to change the setting.

	Noise Suppression	Peak Factor
Strong Noise Suppression	10 ms	300
Medium Noise Suppression	2 ms	300
Weak Noise Suppression	2 ms	100
User Defined	All other combinations of parameter.	

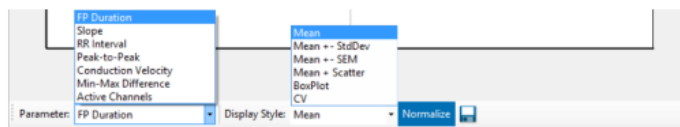
Dose-Response Window

The "Dose-Response" window includes the dose-response curve that shows the variation of the physiological markers in relation to the concentration of the compound.



The window on the right side of the main menu of the Incub8-Analyzer software shows the "Dose-Response" curve, calculated from the averages of the merged files.

Select the "Parameter" and the "Display Style" from the drop-down menus. Decide whether you want to see "Raw Values" or "Normalized" data. Click the "Export" icon to export and save the data.



Choose the type of the curve from the "Parameter" drop-down menu: "FP Duration" is the "QT-related Interval" and describes the latency from one extremum of the T-component of the heartbeat to the next extremum. "Slope" is the maximal slope of the Na peak. The "RR Interval" is the time span between two heartbeats, measured from the detection time stamp of one QRS-component to the next. "Peak-to-Peak" describes the amplitude between the maximum and the minimum of a Na peak. The "Conduction Velocity" shows the velocity of the 2D propagation of the field potential across the MEA. The "Min-Max Difference" the difference between minimum and maximum. Define the "Active Channel" in the "Channels Selection Configuration" dialog.

Choose the "Display Style": The "Mean" is calculated from the mean results of all wells of one compound. This simple display format does not show the variability of the wells.

The "Mean + StdDev" shows additionally ± 1 standard deviation of the mean and allows to estimate variability between the wells but does not show outlier values. Similarly, the "Mean + SEM" shows additionally ± 1 standard error of the mean.

The "Mean + Scatter" plot shows additionally the mean the results of all concentrations of that well as individual points. Click on a point to highlight the results of this well. This display format allows to identify outliers easily to correct them, if necessary during offline analysis.

Clicking on the orange dots in the "Mean and Scatter" display style gives the possibility to jump directly to the corresponding curve in the "Single View" of the "Raw Data Explorer" or the "QT Analysis" window. Control the data and correct them, if necessary. After correction the dose-response curve will be adapted immediately.

The "BoxPlot" shows the distribution of the results between the wells. You clearly see the wells which differ from the median.

The Coefficient of Variation "CV" is a degree for the variability of a distribution. The "CV" is calculated from mean divided by standard deviation and has no unit. You need at least two measurements to calculate the "CV", that is why it is useless for a parameter like the QT interval as based on averaged data. The "CV" displayed in this plot is the mean of the "CV" of the concentrations in one single well.

See the variation of the physiological markers in the dose-response curve in absolute values **Raw Values** "Raw Values" or in "Normalize" mode. Normalized results are calculated in relation to the respective control section in %.

Toolbar

Start the "Analyze" and "Save Analysis".

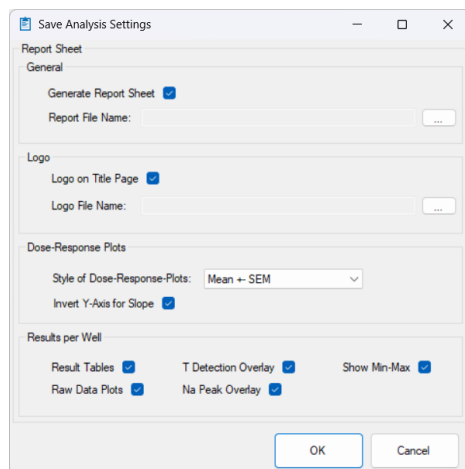


Click the "Analyze" button to start the analysis. Save the results after analysis with "Save Analysis".

Save Analysis



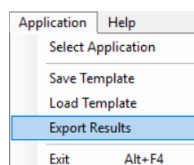
Click the "Save Analysis" button to open the "Save Analysis Settings" dialog.

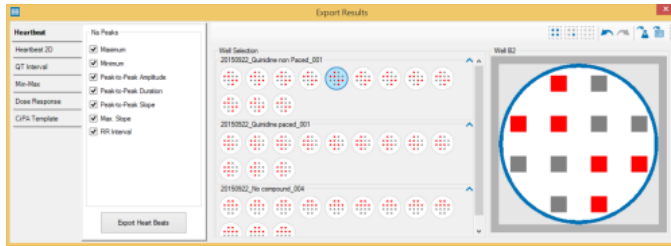


Define the settings for the report sheet: "General", "Logo", "Dose Response Plots" and "Results per Well". Click "OK" to save the current state of the analysis to a *.mwa file and (optional) to a PDF report.

Export

This "Export" feature is analogous to the feature in the "Incub8-Screen" software. Click the option "Export Results" in the "Application" menu to open the "Export Results" dialog.





Choose the tab and the parameters you want to export via check box on the left side. Select the electrodes and the wells by clicking on them on the right side of the dialog.

There are six tabbed pages providing analysis parameters:

- Heartbeat
- Heartbeat 2D
- QT-related Interval
- Dose-Response
- Min-Max
- CIPA Template

On each of the pages, there are a number of parameters which can be selected or deselected via check box. Clicking the "Export" button on one of the pages will export these parameters to a "comma separated value" "*.csv" file or to an Excel "*.xlsx" file for further processing.

The tabs of the export dialogs are build analogously. On the left side of the dialog enable or disabled the check boxes to choose the parameters you are interested in. Select the designated electrode channel on the electrode field and select the wells.



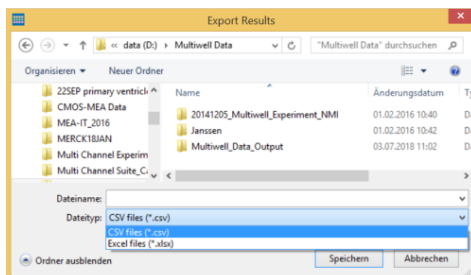
Use the "Select all Electrodes in the Well" button to select all electrodes of a well for the export. Click the "Select Representative Channel in the Well" button to export the representative electrode only or deselect all channels with the "Deselect all Electrodes in the Well" button.

Apply the settings to all wells with the same compound or to all wells of the plate

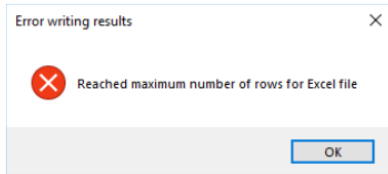


Export Heartbeats

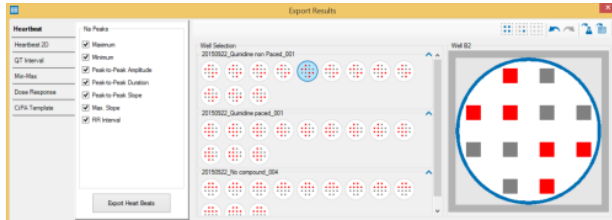
Click the respective button to export the file as comma separated file "*.csv" without any limitations of data volume in one result file.



Excel files "*.xlsx" are limited to 1048576 rows per file. If the limitation of data volume is reached in Excel, the following error message appears and the exported "*.xlsx" file will contain only 1048576 rows.

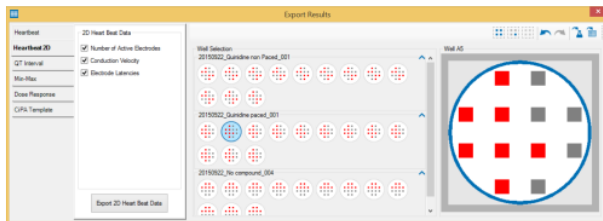


Heartbeat Tab



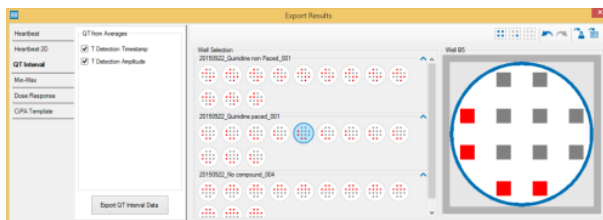
Select the parameter you want to export via check box. Click the "Export Heartbeat" button to save the data as comma separated value file.

Heartbeat 2D Tab



Select the parameter you want to export via check box. Click the "Export 2D Heartbeat" button to save the data as comma separated value file.

QT Interval Data Tab



Select the parameter you want to export via check box. Click the "Export QT Interval Data" button to save the data as comma separated value file.

Min-Max Difference Tab



Select the parameter you want to export via check box. Click the "Export Min-Max" button to save the data as comma separated value file. Additionally, it is possible to export the Min-Max detection points or the data traces separately.

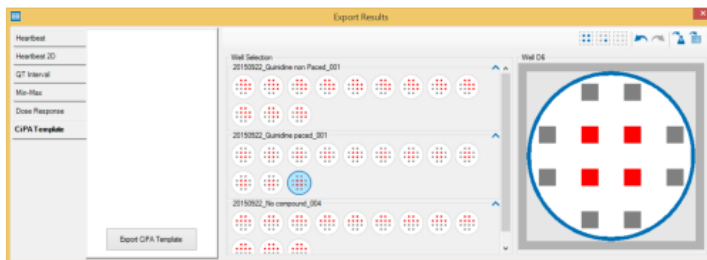
Choosing the "Aggregated" option will export the Min-Max detection points, their amplitude difference and the number of averaged samples. Choosing the "Averaged Trace" option will export the averaged traces.

Dose-Response Tab



Select the parameter you want to export via check box. Click the "Export Dose-Response" button to save the data as comma separated value file.

CiPA Template

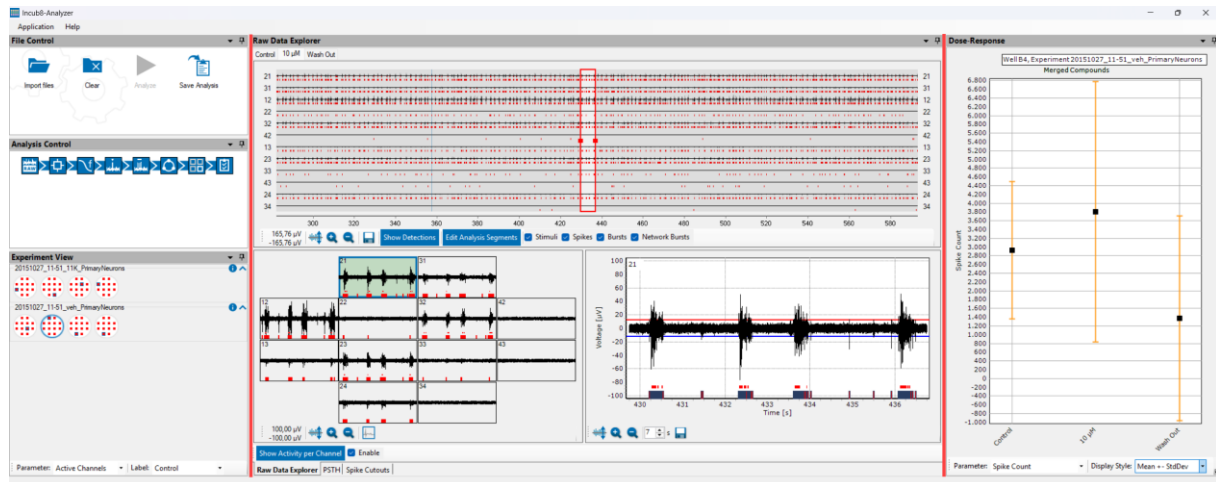


Click the "Export CiPA Template" button to save the data as comma separated value file.

Incub8-Analyzer: Neuro Mode

The "Incub8-Analyzer" software is developed to analyze data, produced with the "Incub8-Screen" software. The analyzer is built on two application modes almost in the same manner as the "Incub8-Screen" software: "Cardio" and "Neuro Mode". Find information about the "Neuro Mode" in the following chapters.

Main Menu

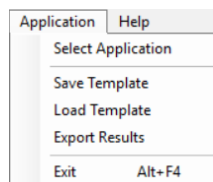


The main window of the "Incub8-Analyzer" software is divided in three smaller windows with a menu bar above. The "Control Window" on the left side, the "Data Display" window in the center and on the right side you see the window for the "Dose-Response" curves.

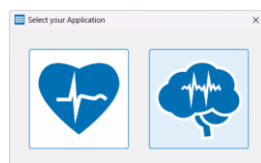
Use the "Neuro Mode" analysis software for the reanalysis of raw data files under modified conditions, with parameter settings well per well independent from each other.

Menu Bar

Application



Select Application "Cardio" or "Neuro" mode.



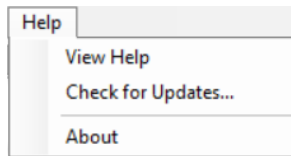
Use the "Save Template" function to select a well and store the analysis parameters of this well to a template file with the extension *.mwt.

With the "Load Template" function, such a template file can be loaded later on and the analysis parameters stored in it will be applied to all wells of all experiments currently opened in the Incub8-Analyzer.

Click "Export Results" to open the export dialog. Please read chapter "Export" for more information later on.

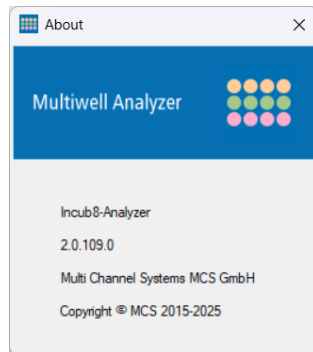
Menu to "Exit" the Incub8-Analyzer program.

Help Menu



Enter the online help with the "View Help" command.

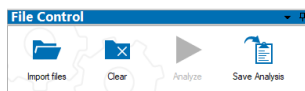
The option "Check for Updates" provides fast access to the MCS web site.



If the Incub8-Analyzer software is not up to date, click the button "Visit Web site" and download of the newest software version.

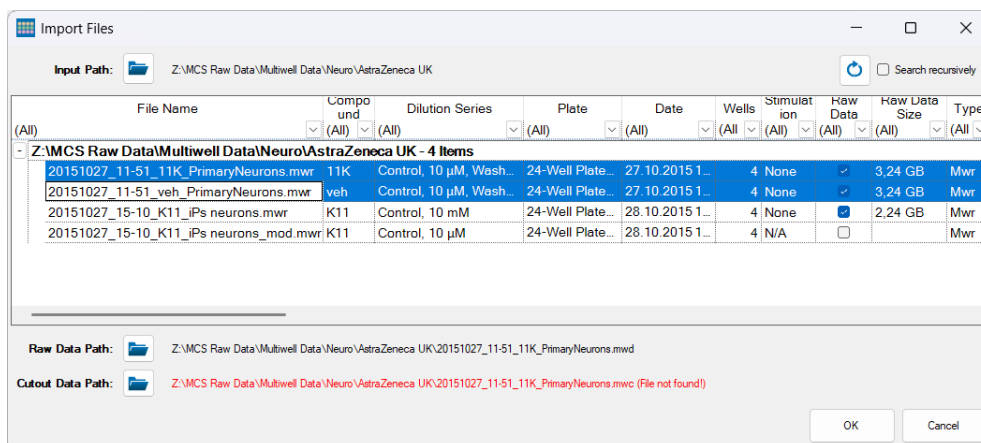
Click "About" to open the dialog. Find information about the software version of the "Incub8-Analyzer".

Control Window



File Control

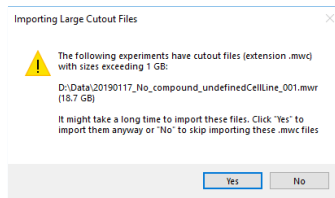
Click "Import File" to open the import dialog. Please read also chapter "Control Window" in "Incub8-Analyzer: Cardio Mode".



The Incub8-Analyzer needs at least the raw data file "*.mwd" or the cutout file "*.mwc" for analysis.

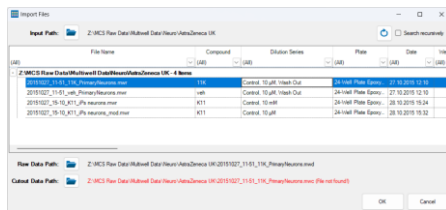
To open a result file "*.mwr" or an analysis file "*.mwa" file, the corresponding cutout file "*.mwc" or settings file "*.mws" file has to be available. If a file is not automatically detected, please add it manually.

Import of large Cutout Files



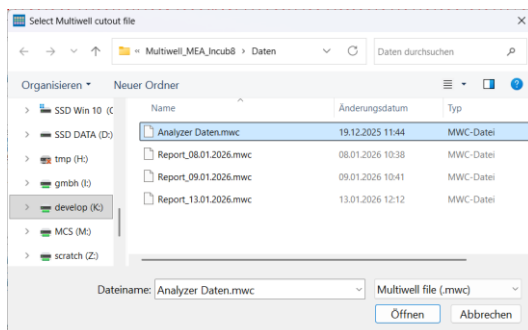
An import of a cutout „*.mwc” file, larger than 1 GB data volume possibly needs a long time. That is why you have to confirm the import of large files via the “Import Large Cutout Files” dialog.

Data Path



Please define the data path for the "Input Path" in the headline of the dialog. "Reload the files from the input path" or click the check box "Search recursively", if necessary. Click "Input Path" to find the experiment files recorded by Incub8-Screen or analysis files created by the Incub8-Analyzer software. The list of available files will show all files that are created in the current mode, for example only Neuro files in "Neuro Mode".

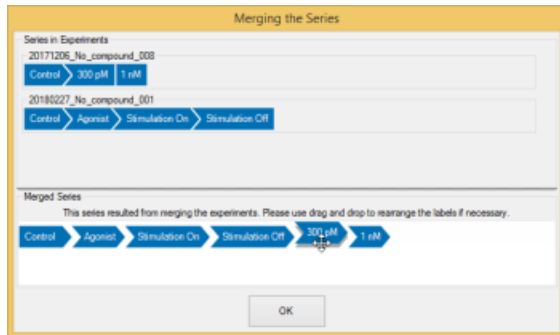
Define the paths, where to load raw data and cutout files, the "Raw Data Path" and the "Cutout Data Path" in the footer. If any file is not found automatically, its path is marked in red. Please search the missing file manually by clicking on the respective data path icon to open, for example, the "Select Multiwell cutout file" dialog.



Merging Files

It is possible to import more than one experiment file at a time in order to merge the results. The files are sorted one below the other, you can see them in the "Experiments" view.

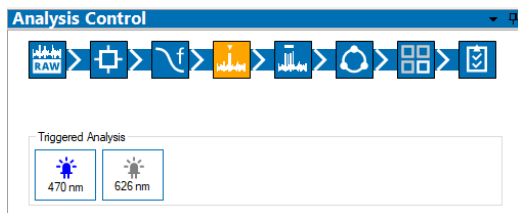
When merging files generated with the Incub8-Screen software which have different dilution series, the "Merging the Series" dialog opens.



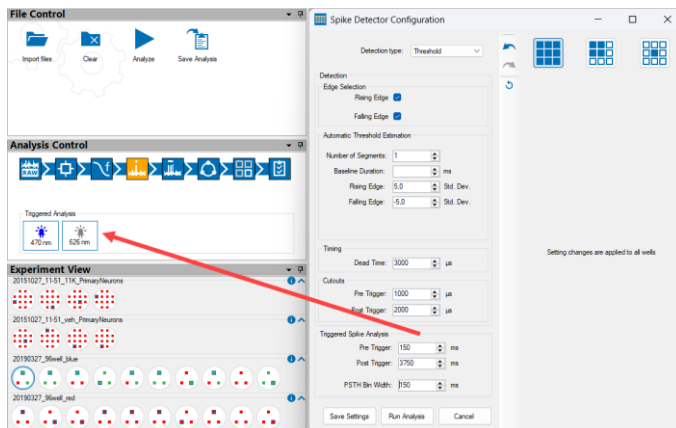
The Incub8-Analyzer software tries to organize the different series of the files which should be merged in a reasonable order. If the arrangement is not optimal, please reorganize the sequence manually. Click onto one of the labels and move it via drag and drop to the desired position. It is not possible to move the “Control” label, this phase must always be the first phase of the experiment.

File Information: Please read also chapter "Control Window" in "Incub8-Analyzer: Cardio Mode".

Analysis Control

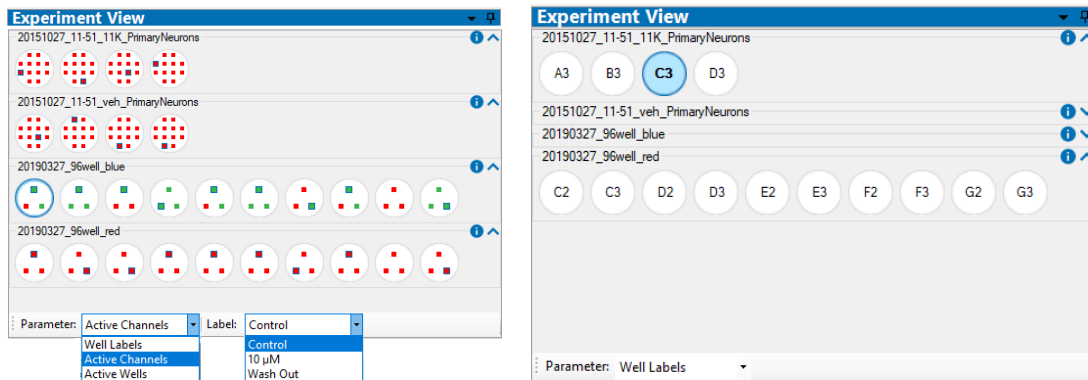


After files with triggers have been imported and triggered analysis has been activated, the “Triggered Analysis” section in the “Analysis Control” shows the available trigger type(s). Click one of the trigger types to prepare the triggered analysis.



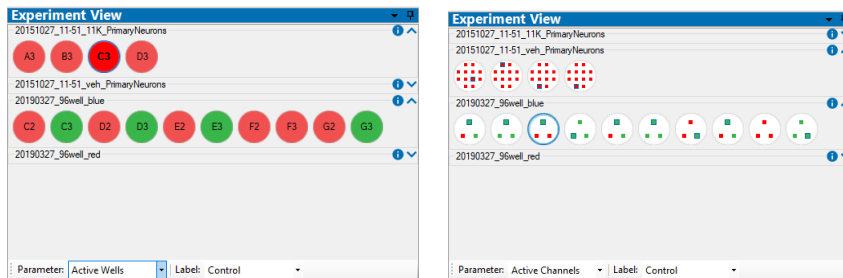
Once a trigger has been activated, use the “Spike Detector Configuration” to set “Pre- and Post Trigger” intervals and “PSTH Bin Widths” for the triggered analysis. As usual, this can be set on a per-well basis. Then, use the “Analyze” button in the “File Control” dialog to start the triggered analysis.

Experiment View



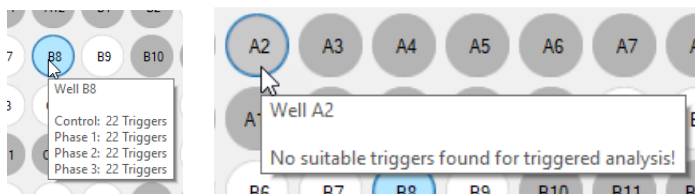
See the imported raw data file(s) in the "Experiment View". Import more than one file to merge the data.

Use the arrows to expand or retract the display of the available wells in that file. Click onto a well to display the data in the "Data Display" beside. The selected well is highlighted in blue or with a blue frame.



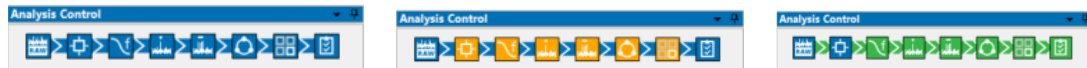
Select the "Parameter" from the drop down menu. Display "Well Labels", "Active Wells" or "Active Channels" and choose the "Dose", if available.

In case of a triggered analysis, after the analysis has finished, all wells that are part of the analysis are shown with white background in the "Experiment View", while wells that do not contain the correct trigger and are thus not part of the analysis are shown with grey background.



Tooltips are available for the wells included in the analysis: The duration of the different phases during the experiment is displayed in wells which are not triggered. The numbers of the triggers are displayed in triggered wells.

Analysis Control



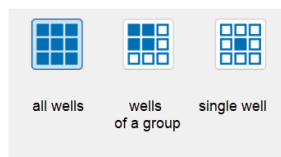
See the state of the analysis in the flow process chart of the "Analysis Control" window. Squares symbolize the raw data source, analysis instruments and result storage. Their colors indicate the current state of the analysis: Blue instruments are idle, while orange instruments indicate that their analysis settings have been modified but not yet applied in an analysis. If an analysis is running the data flow from the data source to the result storage is indicated in green and instruments participating in the analysis are also shown in green. The base instrument of the analysis will start in blue and gradually fill up in green to indicate the progress of the analysis. The tool tip on this instrument also displays the progress as a percentage.

Click onto the respective buttons in the diagram to open the dialogs for fast interactions during analysis.

Example: Suppose you want to analyze the data with modified settings for the spectral filter and the spike detector. If you click on their buttons in the chart, perform the appropriate modifications in the dialogs and click on "Save Settings" in the dialog, both the spike detector and the spectral filter will be highlighted in orange: Their settings have been modified, but the analysis applying these settings has not yet been performed. Clicking on any instrument and selecting "Start Analysis" will start the analysis with the new settings. The time segment settings have not been changed, so this instrument stays inactive (blue). However, the spectral filter has been modified, so it is the base instrument for this analysis and its update from blue to green shows the progress of the analysis. Because the filtering has an effect on any subsequent instrument, the spike detector (also with modified settings) and the burst detector need to be active (green) as well. After the analysis is finished, all instruments are idle (blue) again, because all modifications have been handled during the analysis. If the analysis was stopped prematurely, some modifications have not yet been applied, so some instruments remain modified (orange). They will be handled when the next analysis is started.

Click onto the respective buttons in the diagram to open the dialogs for fast interactions during analysis.

Working Level




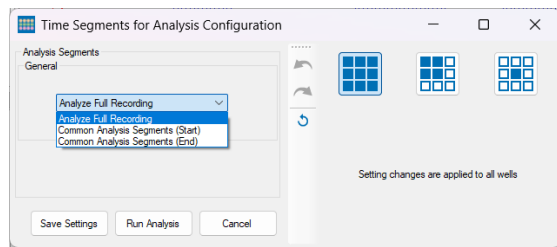
Most of the tools need a definition on the level, you like to work on: Should changes in the settings of the respective tool dialog affect all electrodes of the plate, all electrodes in a group, or the electrodes in one well only. The default is "Setting changes are applied to all wells". Please see also chapter "Tutorial".

Time Segment

Adapt the "Time Segment" settings to define the time span to which analyze parameters should be applied. There are two ways to do so, via "Time Segment Selection" icon in the "Analysis Control" flow chart or via "Edit Analysis Segment" in the "Raw Data Explorer".

Click the icon "Time Segment Selection" in the flow chart.

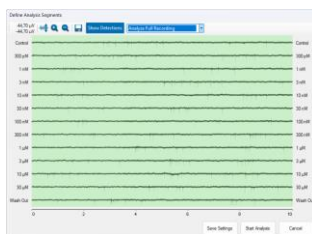
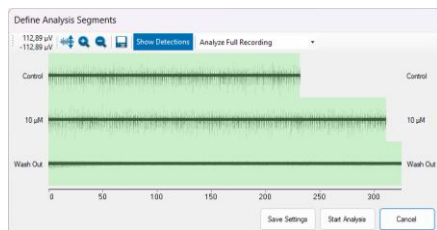
Based on the "Raw Data" , the definition of the "Time Segment Selection" is the first step.



Select the first option from the drop down menu "Analyze Full Recording" on the left side and select the well to apply the analysis to a working level on the right side.

Alternative: Click the button "Edit Analysis Segment" in the "Raw Data Explorer" tab of the "Data Display" window to open a window with the same functionality. The drop down menu this dialog is identical to the drop down menu in the "Time Segment for the Analysis Configuration" dialog.

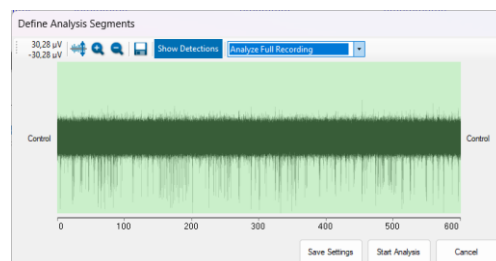
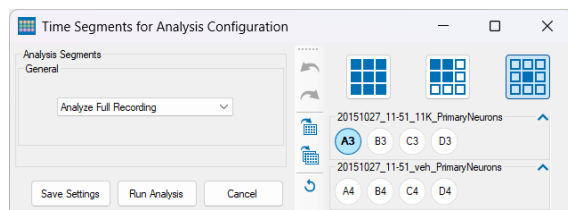
The dialog allows to define time frames for analysis graphically.



Define the time frame for analysis individual for each well or compound on the well plate.

Analyze Full Recording

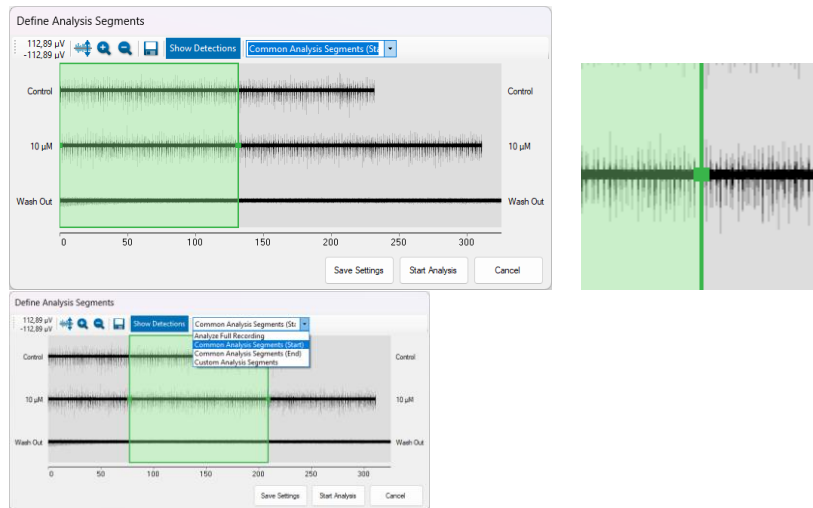
First option of the menu is "Analyze Full Recording". Analysis parameters are applied to the complete duration of the recorded file. This is indicated by the green frame all over the time display.



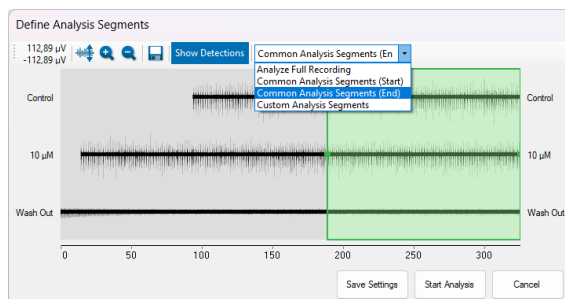
Common Analysis Segments

Select the option "Common Analysis Segments (Start)" or "Common Analysis Segments (End)" either in the "Time Segment for the Analysis Configuration" dialog or in the "Define Analysis Segments" dialog. Define the start and the duration of the analysis for all doses via up-down boxes in seconds on the one hand or by drag

and drop on the other hand. Please click on the dark green point in the green frame and keep the mouse button pressed to modify the borders of the frame. Click into the green frame to move the complete time frame over the display.



Analysis parameter are applied to the beginning of the experiment with option "Common Analysis Segments (Start)". Use the option "Common Analysis Segments (End)" to couple the analysis parameter to the end of the experiment.

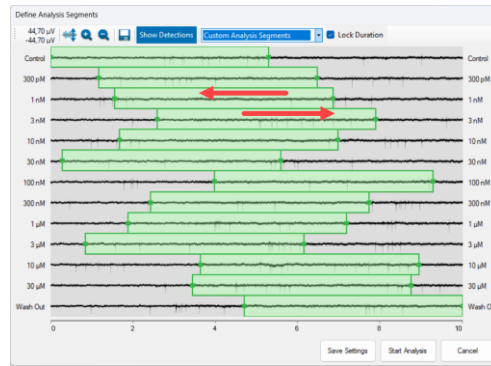
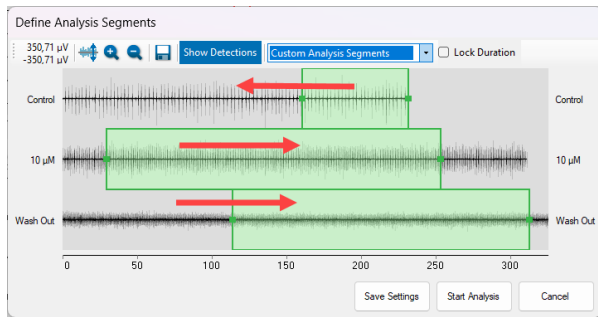


When starting the analysis, the data display in the "Raw Data Explorer" is adapted respectively. Again, the time frame for analysis is highlighted in green. The red frame is for display settings only and indicates the period of time shown in the "Raw Data Explorer" windows below, in the over- and single view.



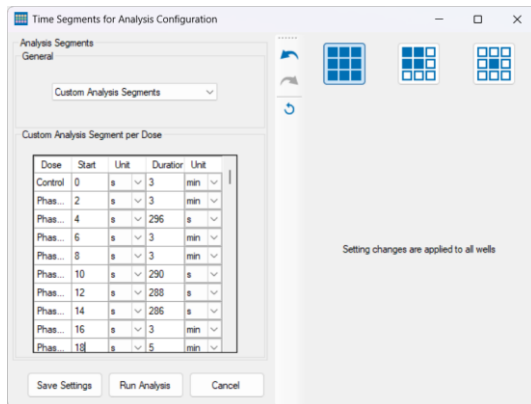
Custom Analysis Segments

Select the option "Custom Analysis Segments" and define the time stamp of the start of the analysis for each dose separately. The duration of the time segment is fix for all doses if the check box "Lock Duration" is active. Unabled the check box to define the duration of the analysis segments variable by expanding or reducing the green areas. Move the contact point via drop and drag in front of the green area or at the end. Please see the red arrows in the screenshot.

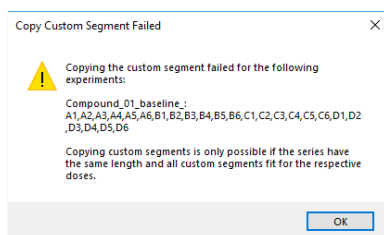


Select "Custom Analysis Segments" and fill in the table "Analysis per Dose" in the "Time Segment for the Analysis Configuration" dialog. Or move the green time frames in the "Define Analysis Segments" dialog to separate dose per dose to select the interesting time spans for analysis.

Alternatively select "Custom Analysis Segment per Dose" and fill in the table in the "Time Segment for the Analysis Configuration" dialog.



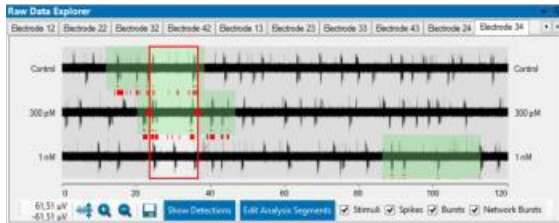
Copy of Custom Analysis Segments



The copy of custom analysis segments from one experiment to another experiment is only possible if the phases of both experiments match with each other.

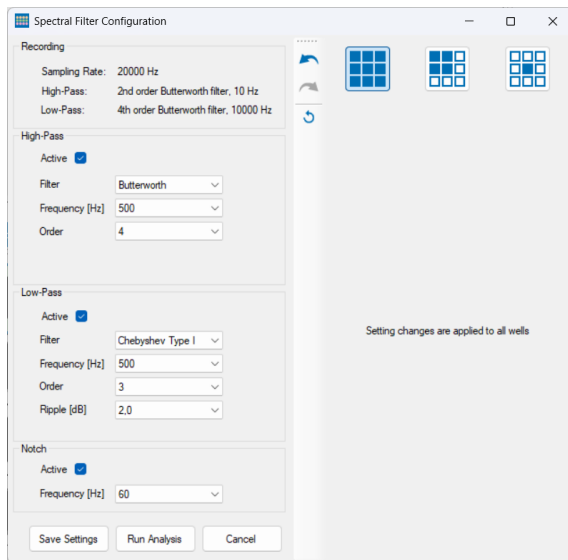
If not, the following error message appears: A list of experiment phases is displayed which do not match and the hint "Copying custom segments is only possible if the series have the same length and all custom segments fit to the respective doses".

The data display in the "Raw Data Explorer" is adapted respectively. Please select "Show Activity per Concentration" display [Show Activity per Concentration](#). Again, the time frame for analysis is highlighted in green. The red frame indicates the period of time shown in the "Raw Data Explorer" windows below, the over- and single view.



Spectral Filter

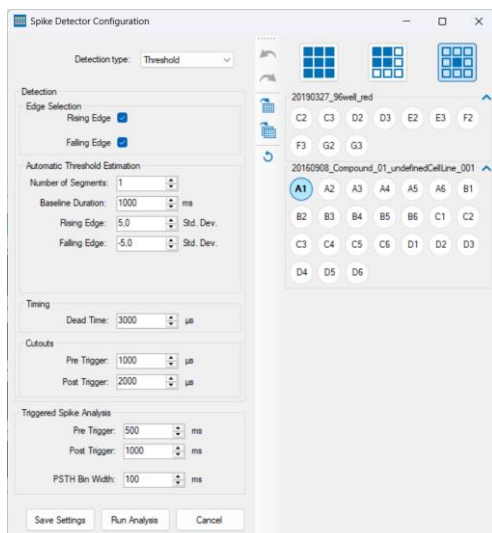
After that, the data stream is processed by the "Spectral Filter".



Select the filter settings, if necessary. Choose between "Bessel", "Butterworth" or "Chebyshev" for high-pass and low-pass. The "Notch" filter is helpful to remove noise.

Spike Detection

. Click the button to open the "Spike Detector Configuration" dialog.

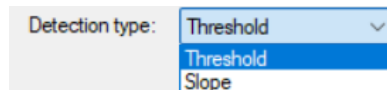


Spike Detector Configuration

The dialog is divided in two parts. Define the spike detection parameters on the left side and see the available raw data file(s) on the right side. Apply the settings from the left side to the wells on the right side with the tool bar in between.

On the right side of the dialog the well plate of the raw data files are displayed for the "Well Selection". As usual, the currently selected well is highlighted with a blue circle.

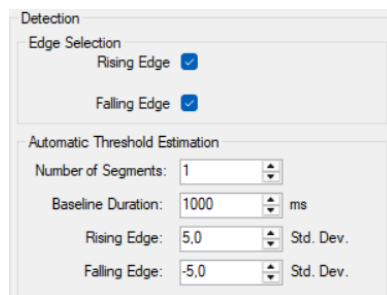
Detection Parameter



Please set the "Detection type" from the drop-down menu first. In dependency of the detection type the dialog offers different options.

Detection Type: Threshold

Detection



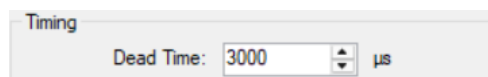
Define the "Rising Edge" or "Falling Edge" in the "Edge Selection" window.

Automatic Threshold Estimation

In the "Automatic Threshold Estimation" window you can select the standard deviation for the rising and falling edge from the numeric up-down boxes.

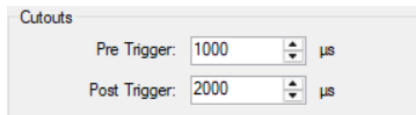
Use the up-down box "Number of Segments" to define, how many individual segments are used to estimate the thresholds. The duration of each segment is defined by the "Baseline Duration". The threshold is computed as the minimum standard deviation over all segments, multiplied by either the "Rising Edge" or "Falling Edge" factor. Using multiple short segments for the computation of the standard deviation instead of one long segment reduces the risk that the standard deviation is strongly influenced by spike activity.

Timing



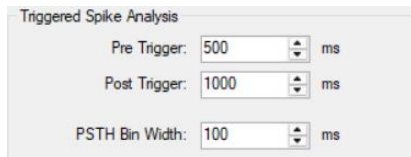
Select the "Dead Time" in μs in the "Timing" window.

Cutouts




When using "Cutouts", please define the "Pre Trigger" and "Post Trigger" in μs from the numeric up-down boxes.

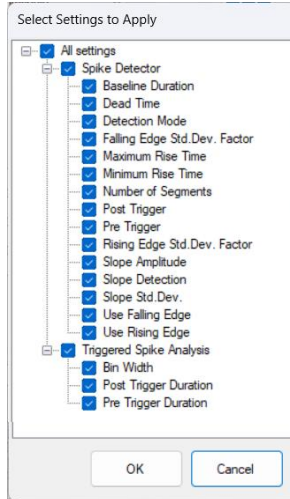
Spike Triggered Analysis




After files with triggers have been imported and the triggered analysis has been activated, the "Triggered Spike Analysis" section is available. Please set the "Pre- and Post Trigger" from the up-down boxes in ms and the "PSTH Bin Widths" for the triggered analysis. As usual, this can be set on a per-well basis.


Click the button "Save Settings" to confirm your selections. The analysis in the "Analysis Control" window is now armed which is indicated with the orange color of the "Spike Detection" icon. When clicking on the icon, the dialog appears again. Click the button "Run Analysis" to start the analysis and the processing of the data stream is indicated in green. All modified wells are included. Clicking "Cancel" stops the analysis.

Click the "Apply changes to all wells of the same compound"  button and the following dialog appears "Select Settings to Apply".



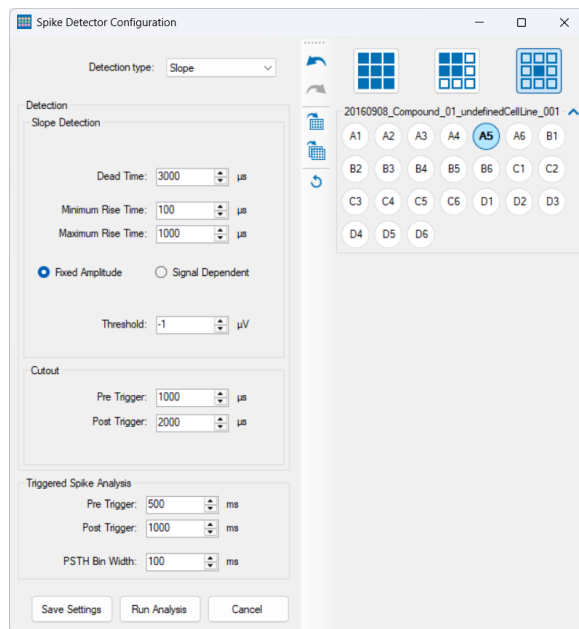
Select the settings you want to apply via check boxes. The settings will be immediately applied to all wells of the currently selected compound.

Click the "Apply changes to all wells"  button and the same dialog appears. Select the settings you want to apply via check boxes. The settings will be immediately applied to all wells.

Use the arrow buttons for "Undo last changes" and "Redo last changes" and the "Reset"  button to reset the settings to default.

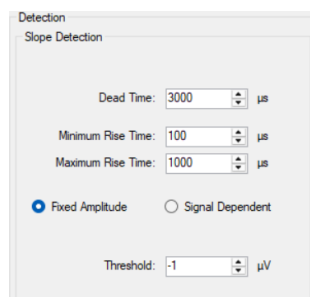
Detection Type: Slope

Changing the type of detection for the spike detector to "Slope" the left side of the dialog will change respectively.



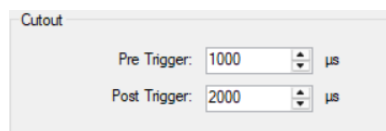
In this case you have to define the parameters for the "Slope Detection".

Detection



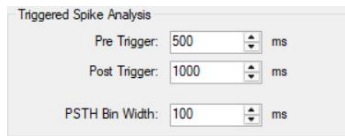
Select the "Dead Time", the "Minimum Rise Time" and the "Maximum Rise Time" in µs from the up-down boxes in the "Detection" window. Decide to use the "Fixed Amplitude" or the "Signal Dependent" amplitude. For the fixed amplitude you have to define the "Baseline Duration" in ms and the "Threshold" in µV. The signal dependent amplitude needs the standard deviation of the threshold.

Cutout



When using "Cutouts", please define the "Pre Trigger" and "Post Trigger" in µs from the numeric up-down boxes.

Triggered Spike Analysis



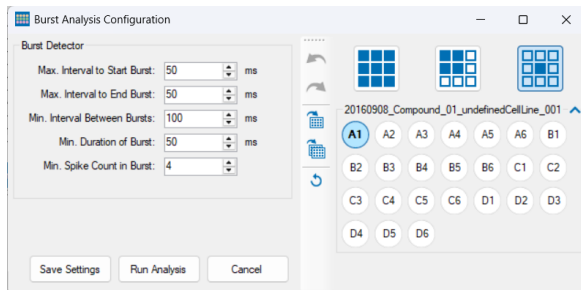
After files with triggers have been imported and the triggered analysis has been activated, the “Triggered Spike Analysis” section is available. Please set the “Pre- and Post Trigger” from the up-down boxes in ms and the “PSTH Bin Widths” for the triggered analysis. As usual, this can be set on a per-well basis. Click the button "Save Settings" to confirm your selections. The analysis in the "Analysis Control" window is now armed which is indicated with the orange color of the "Spike Detection" icon. When clicking on the icon, the dialog appears again. Click the button "Run Analysis" to start the analysis and the processing of the data stream is indicated in green. All modified wells are included. Clicking "Cancel" stops the analysis.

Burst Detection Configuration



After spike detection the data are processed by the "Burst Detection" tool. Click the button to open the "Burst Detector Configuration" dialog.

This dialog is like the "Spike Detection Configuration" divided in two parts. Define the burst detection parameters on the left side and see the available raw data file(s) and wells on the right side. Apply the settings from the left side to the wells on the right side with the tool bar in between.



Burst Detection

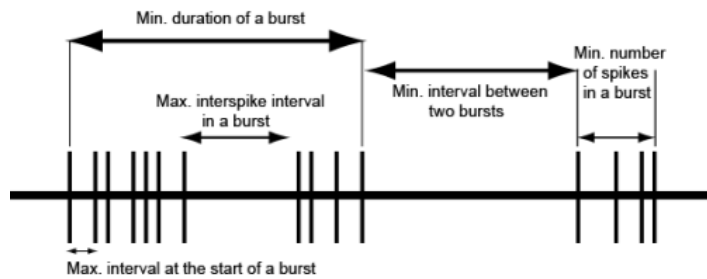
The “Burst Detection Configuration” option allows the detection and analysis of spike bursts. The spikes had been detected first. The quality of the burst analysis therefore depends on the accuracy of the spike detection.

The MaxInterval Method for detecting bursts

Find all bursts using the following algorithm:

- Scan the spike train until an interspike interval is found that is less than or equal to Max. Interval.
- While the interspike intervals are less than Max. End Interval, they are included in the burst.
- If the interspike interval is more than Max. End Interval, the burst ends.
- Merge all the bursts that are less than Min. Interval between Bursts apart.
- Remove the bursts that have duration less than Min. Duration of Burst or have fewer spikes than Min. Number of Spikes.

The detection parameters for bursts are shown in the following schema.




Parameter for burst detection:

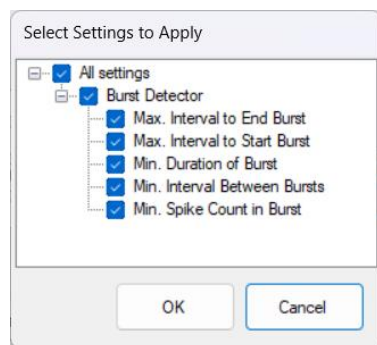
- Max. interval to start bursts: maximum interspike interval to start the burst.
- Max. interval to end a burst: maximum interspike interval to end the burst.
- Min. interval between bursts: minimum interspike interval between two bursts.
- Min. duration of burst: minimum burst duration.
- Min. number of spikes in burst: minimum number of spikes in a burst.

(Reference: Legendy C.R. and Salzman M. (1985): Bursts and recurrences of bursts in the spike trains of spontaneously active striate cortex neurons. J. Neurophysiology, 53(4) : 926-939).


Click the button "Save Settings" to confirm your selections. The analysis in the "Analysis Control" window is now armed, which is indicated with the orange color of the "Burst Detection" icon. When clicking on the icon, the analysis starts and the data stream is green.


Click the button "Run Analysis" to start the analysis.

Click the "Apply changes to all wells of the same compound"  button and the following dialog appears "Select Settings to Apply".



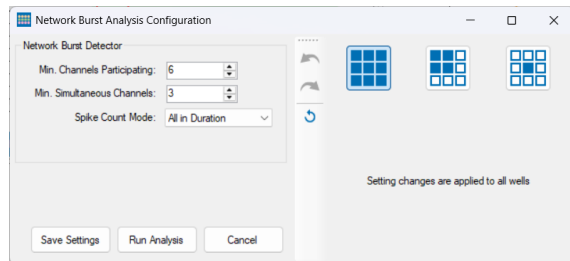
Select the settings you want to apply via check boxes. The settings will be immediately applied to all wells of the currently selected compound.

Click the "Apply changes to all wells"  button and the same dialog appears. Select the settings you want to apply via check boxes. The settings will be immediately applied to all wells.

Use the arrow buttons for "Undo last changes" and "Redo last changes" and the "Reset"  button to reset the settings.

Network Burst Detector Configuration

After spike and burst detection, the data can be processed by the "Network Burst Detection" tool. Click the button to open the "Network Burst Detector Configuration" dialog.



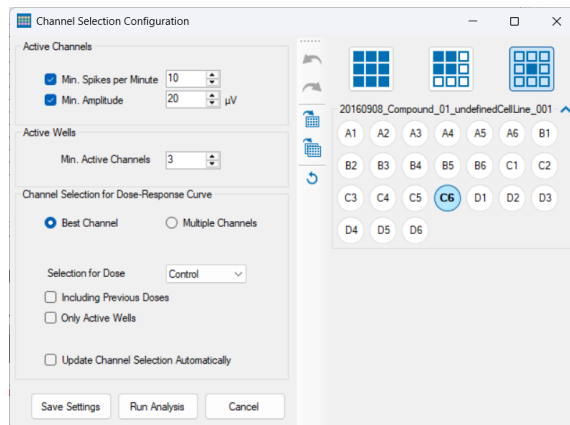
This dialog is like the "Spike Detection Configuration" divided in two parts. Define the network burst detection parameters on the left side and see the available raw data file(s) and wells on the right side. Apply the settings from the left side to the wells on the right side with the tool bar in between.

The "Network Burst Detection Configuration" option allows the detection and analysis of bursts which occur on more than one electrode channels in a well in synchrony. The spikes had been detected first. The quality of the network burst analysis therefore depends on the accuracy of the spike detection.

Please define the minimum number of participating channels "Min. Channels Participating" and the minimum number of simultaneously bursting channels "Min. Simultaneous Channels" from the up-down menus. Define the "Spike Count Mode", which means to count "All Spikes in Duration" or the number of spikes in "Participating Bursts".

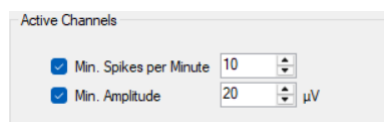
Channel Selection

Click the "Channel Selection" icon to open the "Channel Selection Configuration" dialog.



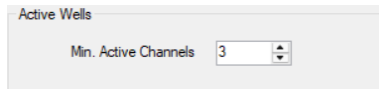
The "Channel Selection" is a tool to estimate and define the activity of a channel or a well.

Active Channels



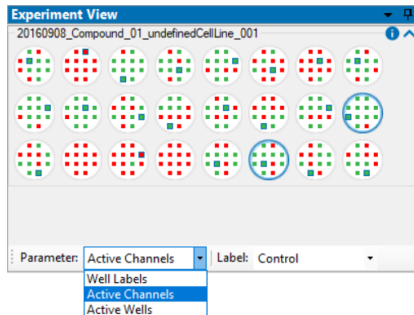
Define the parameter for channel activity via check box. Define the minimum spike rate "Min Spikes per Minute" and the "Min Amplitude" in μV from the up-down boxes.

Active Wells

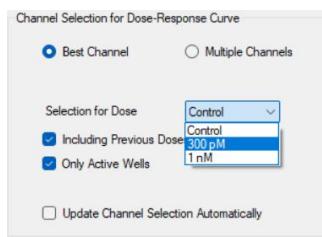


Define the minimum number of active channels for the definition of an active well from the "Min. Active Channels" up-down box.

Visualize the active channels or wells via "Parameter" drop-down menu in the "Experiment View".



Channel Selection for Dose-Response Curve



In "Neuro" mode it is possible to select the "Best Channel" or "Multiple Channels" for the analysis via dose-response curve.

If multiple channels are selected as representative, the value for the well in the dose-response curve is the average over all representative channel of the well.

This menu defines, which channel in a well is selected as the "representative channel", i.e. the one included in the dose-response curve. Only channels that fulfill the criteria defined under "Active Channels" in the "Channel Selection Configuration" dialog can be selected as the representative channel. If no channel fulfills the criteria, no representative channel is selected for this well and it is not used for the dose-response curve.

Because the set of active channels can change between concentrations, use the drop-down menu "Selection for Dose" in the "Channel Selection Configuration" dialog to define, which set of active channels should be used. For example, if "Selection for Dose" is set to "10 nM", the representative channel is selected from the set of channels that are active for the 10 nM concentration.

If the check box "Including Previous Doses" is enabled, the representative channel will be selected from the set of channels that are active for all concentrations up to the one selected in the "Selection for Dose" drop-down menu.

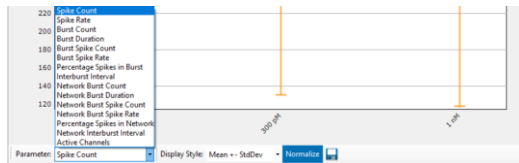
For example, if "Selection for Dose" is set to "10 nM", a channel has to fulfill the criteria set for "Active Channels" for both, the control condition and the 10 nM concentration in order to be eligible to become the representative channel. In order to make sure, that representative channels are active for all concentrations in the experiment, set "Selection for Dose" to the last dose in the experiment and check the "Including Previous Doses" check box.

If the "Only Active Wells" check box is enabled, wells with a low number of active channels can be excluded from the dose-response curve. If a well is not active for the concentration defined in "Selection for Dose" (or all concentrations up to and including this dose if "Including Previous Doses" is checked), no representative

channel is selected even if active channels exist. The threshold number of active channels per well is defined in the "Active Wells" menu.

If the "Update Channel Selection Automatically" check box is enabled, the channel selection process will run after each analysis and may select a different channel. If it is disabled, the channel selection will not be automatically updated. This can be helpful if a specific representative channel should be kept.

The parameter "Active Channels" per well is available in the display of the dose-response curve and in the report sheet.



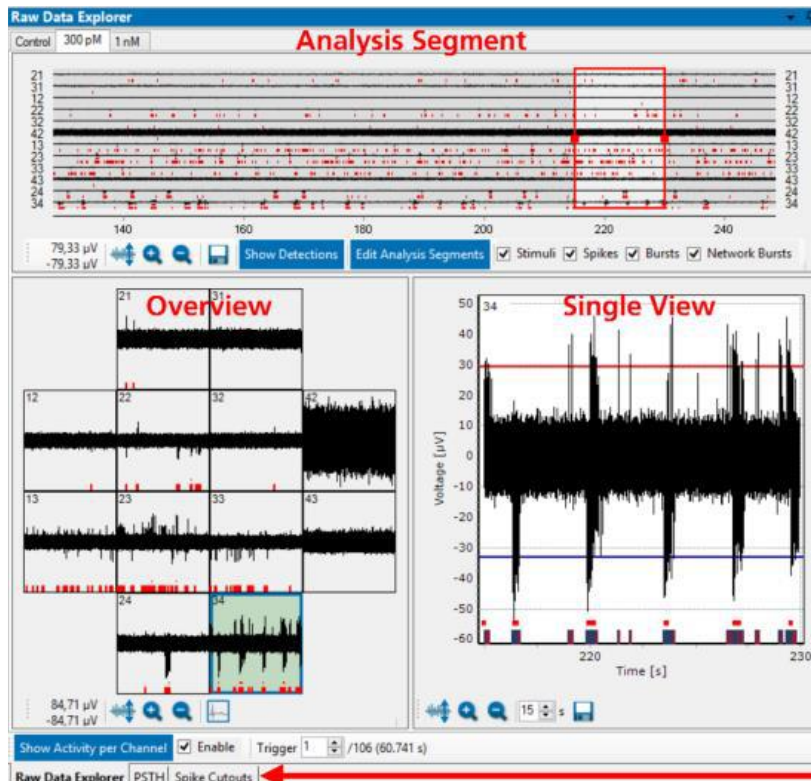
Report

Data Display Window

Please read chapter "General Software Features" for the operation of this window.

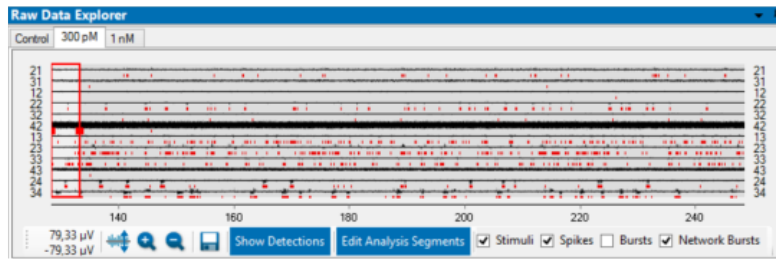
The "Data Display" window has two tabbed pages "Raw Data Explorer" and "Spike Counts". Please see the arrow in the screenshot. The "Raw Data Explorer" tab is divided in three smaller windows.

Raw Data Explorer

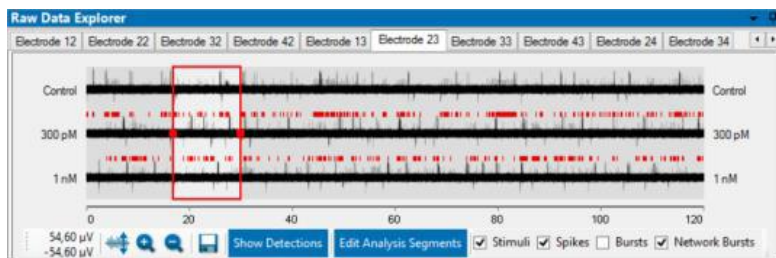


See the complete data file in the window above. Select the display format "Show Activity per Channel" or "Show Activity per Concentration".

Analysis Segment



Select the mode of the "Raw Data Explorer" window and the "Analysis Segment" display will be adapted: "Show Activity per Channel" or "Show Activity per Concentration".



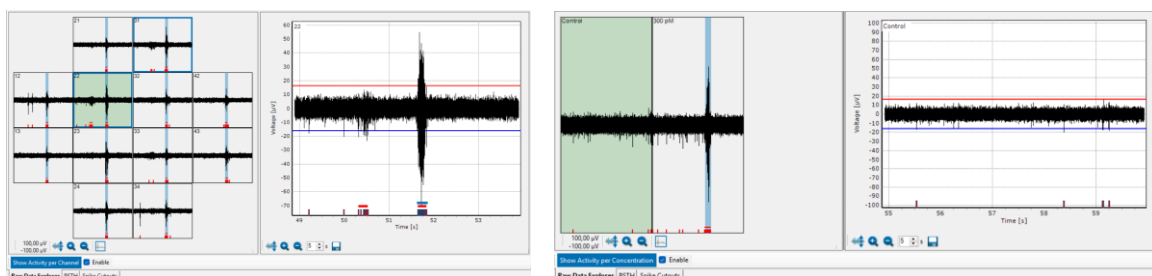
Move the red frame through the data file to select the region of interest. It is possible to enlarge the frame to enlarge also the time span shown in the displays. Alternatively, you can use the up-down box in the "Single View" to modify the time span. The variation of the time span is immediately adapted in all three displays. See data as raw data in "Show Raw Data" mode or spike detections only in "Show Detection" mode.

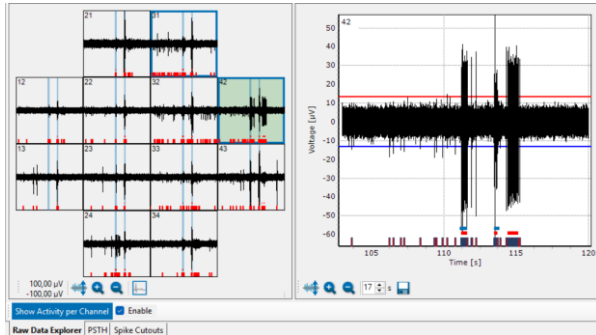
Use the "Export" button to export this graph in an overview in "*.bmp", "*.jpg" or "*.png" format.

Click the "Edit Analysis Segments" button to open the "Define Analysis Segment" dialog. The dialog allows to define time frames for analysis graphically. Again, you can see data as raw data in "Show Raw Data" mode or spike detections only in "Show Detection" mode. Please read chapter "Time Segment" above for more information.

Raw Data Window

The raw data window shows the data of the available electrodes in overview on the left side and in single view on the right side. Choose either to display the "Activity per Channel" or the "Activity per Concentration".





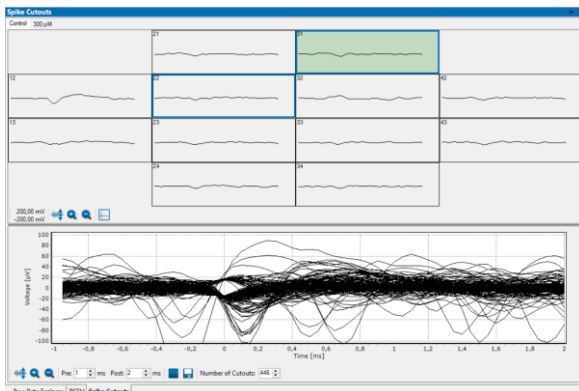
One or more channels of the well have to be defined for analysis and display of the "Dose-Response" curve. Define this electrode by clicking the "Select representative channel" button. The channel framed in blue is then representative for the dose-response curve.

Pressing the "Show Activity per Channel" button switches to a view where each tab page represents a single channel (as opposed to a single dose) and the raw data traces shown are the raw data for the different concentrations of this channel (as opposed to the raw data of the different channels for the same dose). Clicking the "Show Activity per Concentration" button switches back to the first view.

If a triggered analysis has been run, the "Show Activity per Channel" display offers the possibility to select the "Trigger" via up-down box. Using the trigger control jumps to the respective trigger time and shows the raw data.

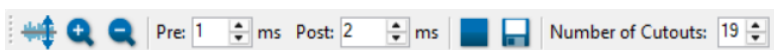
To save computer performance, please deselect the check box "Enable" and the raw data display window is empty. Nevertheless, data are analyzed and saved.

Spike Count Window

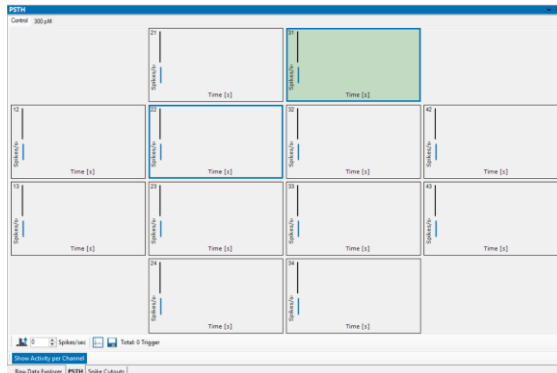


The "Spike Counts" window is divided in the "Overview" with all available electrodes above and the "Single View" below. Each concentration has its own tab page. Per default, only up to 500 spike cutouts are displayed. If more spikes are detected for a channel, only the first 500 cutouts are shown, but more can be displayed by increasing the number in the "Number of Cutouts" numeric-up-down.

If a triggered analysis has been run, you can select the trigger value via the "Trigger" up-down box. Changing the trigger value displays the spike cutouts associated with this particular trigger. One channel of the well has to be defined for analysis and display of the "Dose-Response" curve. Define this electrode by clicking the "Select representative channel" button. The channel framed in blue is then representative for the dose-response curve.



PSTH Window



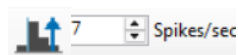
The PSTH is only available, if the software module “Stimulation” is selected.

The neuronal response due to a single stimulation consists of one component, which is causally determined by the pulse and of several components, which are not caused by the stimulation, spontaneous activity, for example. If all components of the response have similar amplitudes as the stimulus induced component, it is not possible to see this component. That is, why the Peri-Stimulus-Time-Histogram (PSTH) is calculated.

The histogram shows the mean spike rate over several identical stimulation pulses relative to the onset of the stimulation pulse. Please read also chapter “Raw Data Window” in "Incub8-Screen Software":


The "PSTH" window is available in two displays: "Show Activity per Channel" and “Show Activity per Concentration”. Click onto the button to toggle the screen presentation. PSTHs can be found for each channel. Both displays have the same tool bar.

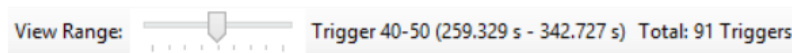
Use the icon to auto-scale the y-axis of the histogram so that it extends to the maximum spike rate on all channels.



Use the “Spikes/sec” up-down box to scale the histogram by yourself.

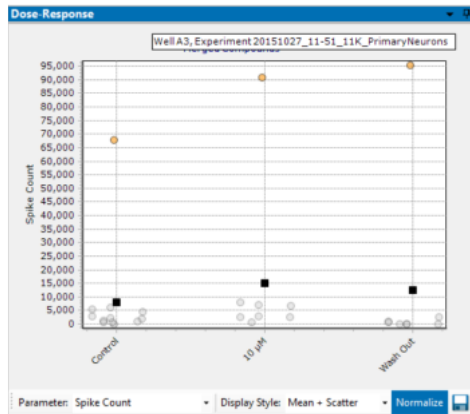


Use the “Select representative channel” button  or “Export” the data to “*.csv” or Excel “*.xlsx” format.

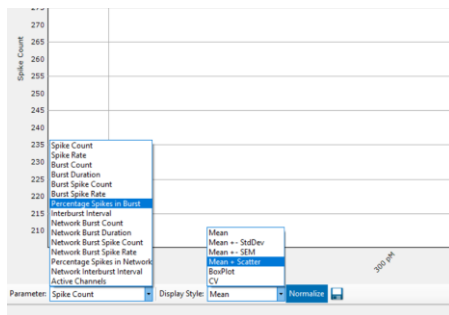


A track bar controls for which triggers the raster plots are shown if there is not enough vertical space to show all. The number of included triggers and the time span on the display are written beside. Read the “Total” number of “Triggers” included in the complete plot on the right side of the tool bar.

Dose-Response Curve



The window on the right side of the main menu of the Incub8-Analyzer Neuro Mode software shows the "Dose-Response" curve calculated from the averages of the merged files, generated accumulative result point per result point.



Select the "Parameter" and the "Display Style" from the drop-down menus. Decide whether you want to see "Raw Values" or "Normalized" data. Click the "Export" icon to export and save the data as bitmap image "*.bmp" as "*.csv" file.

Select the "Parameter" type: "Spike Count, Spike Rate, Burst Count and Burst Duration, Burst Spike Count, Burst Spike Rate, Percentage Spikes in Burst and Interburst Interval". Define the best or multiple "Active Channel" in the "Channel Selection Configuration" dialog.

Choose the "Display Style": The "Mean" is calculated from the mean results of all wells of one compound. This simple display format does not show the variability of the wells.

The "Mean + StdDev" shows additionally ± 1 standard deviation of the mean and allows to estimate variability between the wells but does not show outlier values.

Similarly, the "Mean + SEM" shows additionally ± 1 standard error of the mean.

The "Mean + Scatter" plot shows additionally to the mean the results of all concentrations of that well as individual points. Click on a point to highlight the results of this well. This display format allows to identify outliers easily to correct them, if necessary, during offline analysis.

Clicking on the orange dots in the "Mean and Scatter" display style gives the possibility to jump directly to the corresponding curve in the "Single View" of the "Raw Data Explorer" or the "Spike Cutouts" window. Control the data and correct them if necessary. After correction the dose-response curve will be adapted immediately.

The "BoxPlot" shows the distribution of the results between the wells. You clearly see the wells which differ from the median.

The Coefficient of Variation "CV" is a degree for the variability of a distribution. The "CV" is calculated from mean divided by standard deviation and has no unit. You need at least two measurements to calculate the

"CV", that is why it is useless for a parameter like the spike count as based on averages. The "CV" displayed in this plot is the mean of the "CV" of the concentrations in one single well.

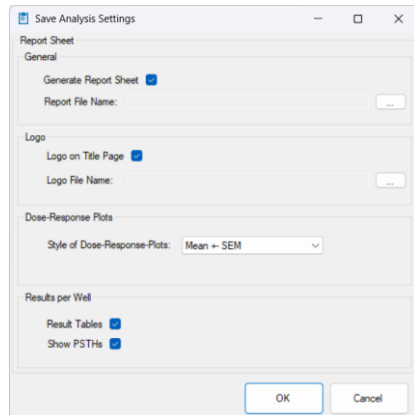
See the variation of the physiological markers in the dose-response curve in absolute values "Raw Values" **Raw Values** or in "Normalize" **Normalize** mode. Normalized results are calculated in relation to the respective control section in %.

Toolbar

"Analyze" and "Save Analysis".




Click the "Analyze" button to start the analysis. Save the results after analysis with "Save Analysis".

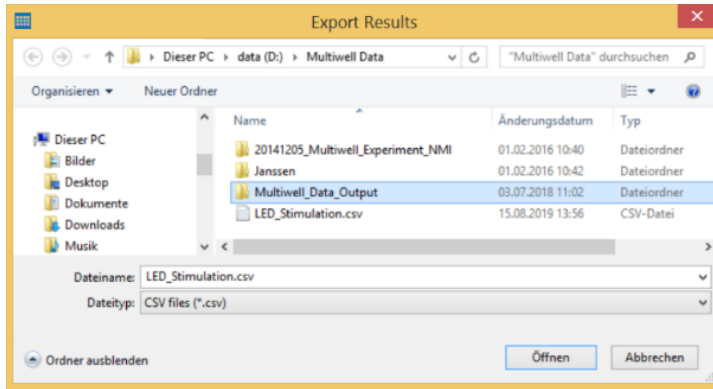


Enable the "Save Analysis Settings" check box and define the "Report File Name" and path. If you like to have a logo on the front page, please enable the check box "Logo on Title Page" and determine where to find it. Select the style of the dose-response plots from the drop-down menu. Enable the check box "Result Table" to generate "Results per Well. Click "OK" to save the current state of the analysis to a *.mwa file and (optional) to a PDF report.

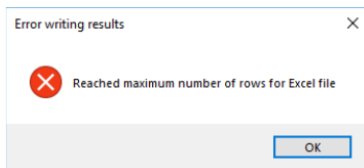
Export Results



Click the "Results" button  to open the "Export Results" dialog. The dialog has four tabbed pages "Timestamp", "Burst", "Network Burst" and "Dose Response". On each of the pages, there are a number of parameters which can be selected or deselected via check box. Clicking the "Export" button on one of the pages will export these parameters to a "comma separated value" "*.csv" file or to an Excel "*.xlsx" file for further processing.

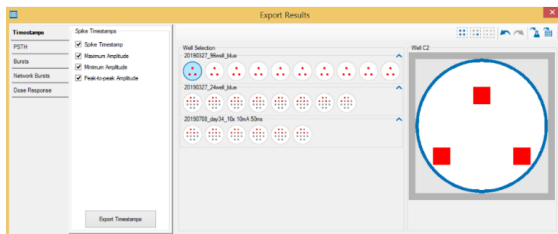


Click the respective button to export the file as comma separated file "*.csv" without any limitations of data volume in one export file.

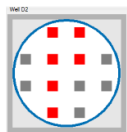


Excel files "*.xlsx" are limited to 1048576 rows per file. If the limitation of data volume is reached in Excel, the following error message appears and the exported "*.xlsx" file will contain only 1048576 rows.

Selection Window



On the "Selection" side of the dialog you see the result files which open and one enhanced well are to select the electrode for export. Please use the toolbar above. To select electrode channels, please click on the electrode. It's color will turn to red. Deselect an electrode by clicking it again.



Toolbar




Use the toolbar to transfer the electrode channel selection to all available wells.



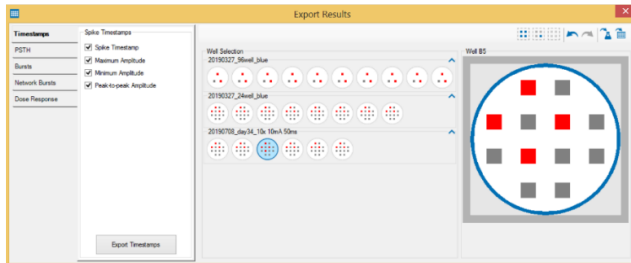
Use the "Select all Electrodes in the Well" button to select all electrodes of a well for the export. Click the "Select Representative Channel in the Well" button to export the representative electrode only or deselect all channels with the "Deselect all Electrodes in the Well" button.



Use the arrow buttons for "Undo" or "Redo" the last action.

Click the "Apply to Compound" button to transfer the electrode selection of the currently open well to all wells with the same compound. For a transfer to all wells, please click the "Apply to All"  button.

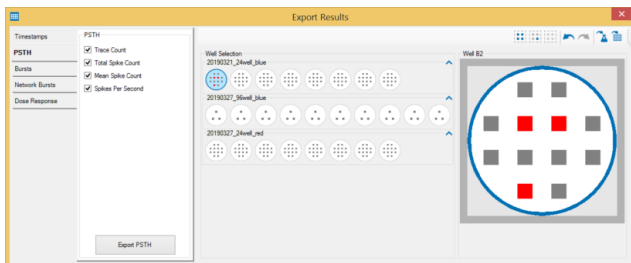
Timestamps Export Parameter



Please select the desired timestamp export parameter via check box. Export "Timestamps", "Maximum Amplitude", "Minimum Amplitude" and "Peak to Peak Amplitude".

Press the "Export Timestamp" and find the place to store the analysis result file for further processes in comma separated value format "*.csv" or in "*.xlsx" Excel format.

PSTH Export Parameter

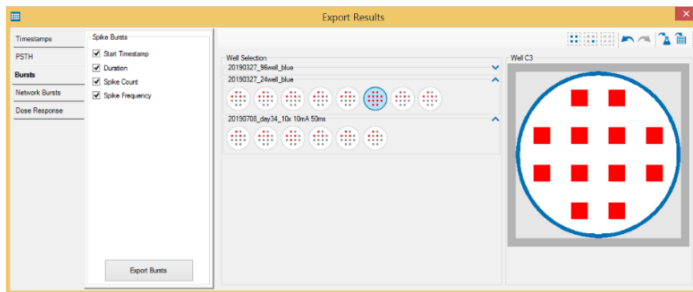


The functionality of the tabbed page "PSTH" is the same as in "Timestamp". Please select the desired PSTH export parameter via check box: "Trace Count", "Total Spike Count", "Mean Spike Count", and "Spikes per Second".

The PSTH Export is only available after a triggered analysis has been performed. This will export the histograms, not the content of the raster plots shown in the PSTHs. Use the "Timestamps" export to export the spike timestamps shown in the raster plots."

Press the "Export PSTH" to store the analysis result file for further processes in comma separated value format "*.csv" or in "*.xlsx" Excel format.

Burst Export Parameter

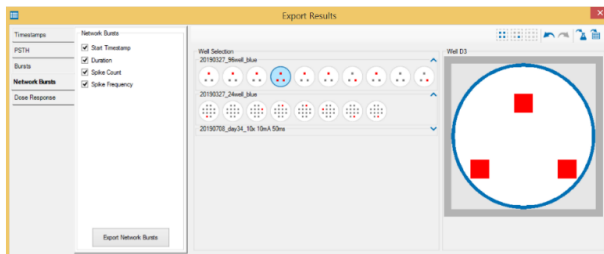


Click the "Bursts Detection Results" button to open the same dialog with the "Bursts" tab open.

The functionality of the tabbed page "Bursts" is the same as in "Timestamp". Please select the desired burst export parameter via check box: "Start Timestamp", "Duration", "Spike Count" and "Spike Frequency".

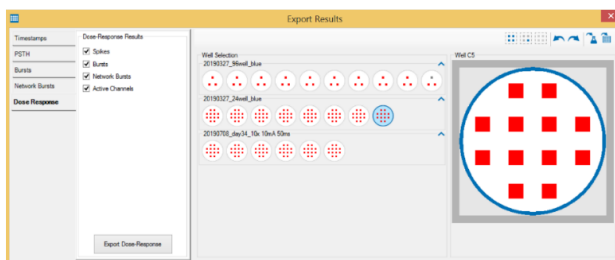
Press the "Export Burst" to store the analysis result file for further processes in comma separated value format "*.csv" or in "*.xlsx" Excel format.

Network Burst Export Parameter



The functionality of the tabbed page "Network Bursts" is the same as in "Burst". Please select the desired burst export parameter via check box: "Start Timestamp", "Duration", "Spike Count" and "Spike Frequency".

Dose-Response Export Parameter



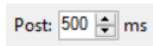
The functionality of the tabbed page "Dose-Response" is the same as in "Timestamp" and "Burst". Please select the desired dose-response export parameter via check box: "Spikes", "Bursts" and "Network Bursts", and "Active Channels".

Press the "Export Dose-Response" button to store the analysis result file for further processes in comma separated value format "*.csv" or in "*.xlsx" Excel format.

General Software Features

The chapter "General Software Features" describes some of the Incub8-Screen and Incub8-Analyzer software features on base of examples.

Numeric Up-Down Box



Adjust a value in the numeric up-down box either by clicking on the arrow buttons or by clicking into the window and moving the mouse wheel. Turn the wheel forward and the level increases, turn the wheel backward, and the value decreases in fast steps. Use the arrow buttons for fine tuning the adjustment. The third possibility is a replacement of the number in the window by overwriting it, if the value is predefined, for example.

Zoom In and Zoom Out

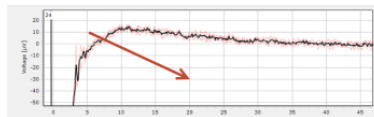
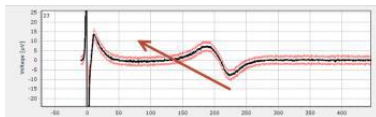
Zoom Buttons



Click the "Adjust to signal Min/Max" button. The scaling of the y-axis is set to the minimum and maximum of all visible samples in the channel.



Click the "Zoom" buttons. Zooming in cuts the scaling of the respective axis in a half and zooming out doubles the scaling.



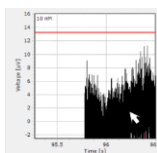
Zoom by Mouse-click

Additionally to the zoom buttons you can freely zoom into a region of interest by moving the cursor inside a display from the left to the right while pressing the left mouse button. Move the cursor from the right to the left for zooming out.

Alternatively, you can draw a rectangle with the left mouse cursor in the display to zoom into a region of interest.

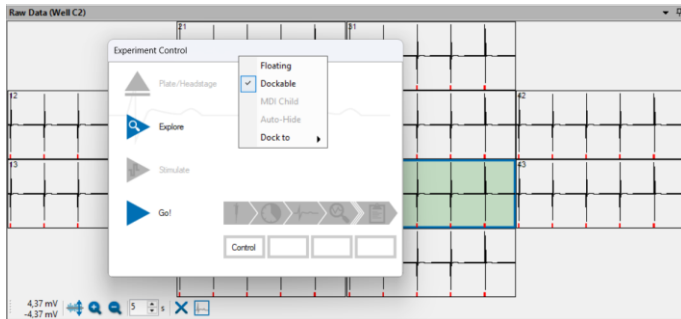
Moving the Display Presentation

Click onto the display with the right mouse cursor button and keep the button pressed to move the data content of the display in any direction.



Display Floating

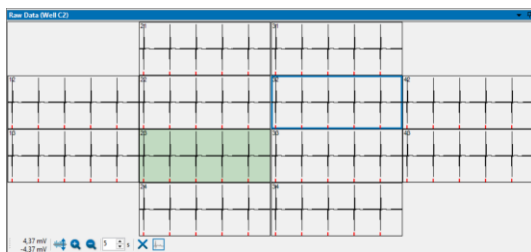
Design your own display with the floating feature. Uncouple a dialog display of your choice and place it wherever you want.



Double-click the floating window in the header line to send it back to its default location.

Selecting Representative Channels

Click one of the channels of the well to select it and display a magnification of that channel in the single view. The selected channel is highlighted in green.

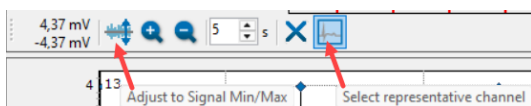


To create the "Dose-Response" curve one channel has to be selected. It should be a representative channel which mirrors the activity in the well. Please click the „Select representative channel“ button first. The channel will be framed blue. The actual selected channel and the representative channel may overlap, but they do not have to overlap.

Select a different well and define the representative channel. This way it is possible to collect data for the dose-response curve.



Tooltips



Tooltips are available in most of the toolbars, please see the example above

APPENDIX

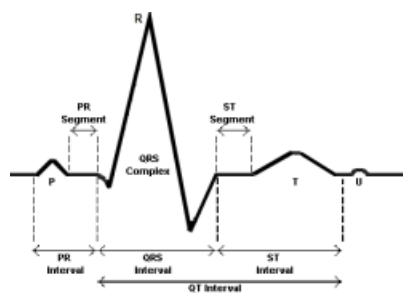
Theoretical Background

Cardiac Action Potential

Over the last 40 years, non-invasive extracellular recording has developed into a widely used standard method. The semi-permeable lipid bilayer cell membrane separates different ion concentrations (charges) on the inner and outer side of the membrane. Conventional methods measure the membrane potential that results from the electrochemical gradient directly with an intracellular electrode. When ion channels are opened due to chemical or electrical stimulation, the corresponding ions are moving along their electrochemical gradient. In other words, the resistance of the membrane is lowered, resulting in an inward or outward flow of ions, measured as a transmembrane current. The extracellular space is conductive as well, and though the resistance is very low, it is not zero. According to Ohm's law ($U=R*I$), the extracellular current results in a small voltage that can be measured with extracellular electrodes. Extracellular signals are smaller than transmembrane potentials, depending on the distance of the signal source to the electrode. Extracellular signal amplitudes decrease with increasing distance of the signal source to the electrode. Therefore, a close interface between electrode and cell membrane is very important for a high signal-to noise ratio.

The transmembrane current and the extracellular field potential follow the same time course. The field potential is roughly equal to the first negative derivative of the transmembrane potential, thus revealing an information that is comparable to conventional methods. This has been shown for different types of signals derived from neuronal preparations as well as cardiac preparations. This convenient method is used by Incub8-Screen. Simultaneous recordings of action potentials (with intracellular electrodes) and field potentials (with extracellular electrodes) have shown that there is a linear relationship between the rise time of the cardiac action potential (AP) and field potential (FP) as well as between AP and FP duration.

The contribution of different ionic transmembrane currents can be identified in the shape of the FP waveform as well, as shown in the following picture. The correlation between the waveform components and the ion channel activities was shown by using ion channel blockers or depleting the medium of the respective ions. You can clearly see the rapid component of the depolarizing sodium current and the slow calcium current. The delayed rectifying K^+ current (IKr) is represented either by a positive or negative peak. The polarity of the peak depends on several parameters, for example, the proximity of the cell layer to the measuring electrode, and cannot be predicted, which does not matter for this assay. The field potential duration corresponds to the action potential duration, which can be correlated to a QT-related interval in an electrocardiogram. It is measured from minimum of the Na^+ peak to the maximum / minimum of the IKr current peak.



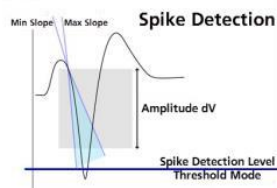
(Reference: "Estimation of Action Potential Changes from Field Potential Recordings in Multicellular Mouse Cardiac Myocyte Cultures", Marcel D. Halbach et al., Cell Physiol Biochem 2003;13:271284).

Spike Detection

The "Spike Detector" is used for extracting spikes from the electrode raw data. There are two ways of detecting and extracting spikes with Incub8-Screen software. One method uses a threshold, the other recognizes the shape that is, the amplitude and slope of a spike curve. Both methods regard either the rise or the fall of a curve, depending on your settings. Waveforms that meet the requirements defined by either of the two methods are cut out from the raw data. The size of the cutout, that is, the time length before and after the spike detection event, can be chosen by the user. It is also possible to extract only the timestamps.

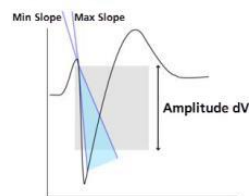
The "Threshold" method, the spike detection level is especially useful if the overall signal is steady, and the spikes appear approximately on the same height. If you have local field potentials or a high noise level, you will miss spikes or detect noise signals with the "Threshold" method. In this case, the "Slope" method is more appropriate.

The following illustrations show how the two methods work.

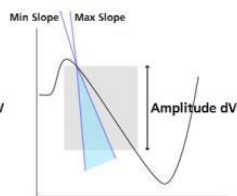


Not Detected Waveforms

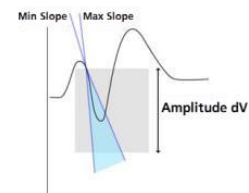
Slope too high



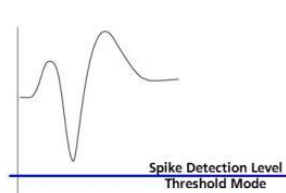
Slope too low



Amplitude too small



Voltage threshold not reached



Cleaning and Maintenance



Warning: Make sure no liquid is sucked into the device! This can lead to irreversible damage.

Cleaning of the System

Always unplug the Incub8-System prior to cleaning. To clean the exterior surfaces, use a lint-free cloth to remove loose dust. For more efficient cleaning, use a soft cloth dampened (not soaked) with an aqueous solution of 75 % isopropyl alcohol, or a mild detergent.

For cleaning the multiwell plates, please read the respective datasheet.

<http://www.multichannelsystems.com/>

Sterilization



Warning: Do not autoclave or sterilize the Incub8 headstage by high heat (above 70 °C) or vapor. Vapor can lead to corrosion of the electronic modules.








Incub8 headstages can be sterilized with standard methods that are not based on high heat or vapor, for example, with 70 % ethanol (only wet wipe the headstage – no submersion), UV-light, or by thermal sterilization in an oven at 56 °C with an incubation time of 8 hours.

Preparing a new Scanner

In case of trouble with the scanner or if the scanner must be replaced, the device has to be initialized via the following sequence of scanner codes, please see the picture below.

It is necessary to connect the USB bluetooth to the computer and switch the scanner on. Scan one code after the other, starting from top to bottom, working one step after the other.

Initial Setup:

Step	FunctionName	As Text (differs if scanned with german/US Keyboard)	As Code
1	Enter Setup (LEDs should glow blue)	-EnterSet-	
2	Reset to Factory defaults	-(SetFun00(-	
3	Enter Setup (LEDs should glow blue)	-EnterSet-	
4	Set to Bluetooth Interface (GATT-Mode from BCST-43)	-(BLE?GATT(-	
5	Do not add enter as suffix	-(SetFun74(-	
6	Disable AutoSleep	-(SetFun05(-	
7	Save and Exit	-(ExitSave(-	
8	Scan TestCode in Multiwell Screen		

Spilled Liquid

Spilled liquid can damage or even destroy the electronics of the Incub8 headstage. Please be careful when filling the well plate with culture medium. Please take very good care that flooding of the Incub8 headstage is efficiently prevented.

Checking the Incub8 Headstage

Please check the Incub8 headstage each time before use:

Insert the “Test Plate” prior to each experiment. Check the Incub8 headstage and the complete system.

You should clean the contact pads of the test plate carefully from time to time with a soft tissue and alcohol or isopropanol. Test the contact of the well plate to the headstage in dependency of the angle of the hinge joint.

Test the contact via VariPlate Base and well plate and hinge joint.

Noise on Single Electrodes in a Well

The noise level on single electrodes can be significantly higher than expected or you might see artifact signals.

May be the electrode in the well or the contact pad for this electrode is defective. Please clean the contact pads of the well plate.

Use the test plate to test the Incub8 headstage again.

Appendix

Technical Specifications

General Characteristics	
Operating temperature	10 °C - 50 °C / 50 °F - 122 °F
Storage temperature	0 °C - 50 °C / 32 °F - 122 °F
Relative humidity	10 % to 95 %, non-condensing
Pollution degree	1
Overvoltage category	II
Headstage	
Well plate contacting unit	manual
Dimensions	200 mm x 160 mm x 28 mm (w/o connector)
Weight	1000 g
Integrated Amplifier	
Number of analog recording channels	288
Data resolution	24 bit
DC - Input voltage range	± 70 mV
Bandwidth	0.1 Hz to 10 kHz, modifiable via software; default 1 Hz to 3.5 kHz
Sampling frequency per channel	25 kHz or lower, software controlled
Integrated Stimulus Generator	
Voltage stimulation	max. ± 5 V
Stimulation pattern: Pulse or burst	Stimulation sites freely selectable
Power Supply Unit MPU 30	
Voltage range	100 - 240 VAC
Output voltage	24 VDC
Max. power	65 W
Frequency	50 / 60 Hz
Mark of conformity	CE
European standard	EN61010

Signal Collector Unit SCU	
Dimension (W x D x H)	250 mm x 83 mm x 25 mm
Weight	300 g
4 Inputs for headstages (HS1 to HS 4)	Lemo connector, EPG.0B.307.HLN
1 Connector with 4 analog outputs for LED driver	Lemo connector, EPG.0B.304.HLN
Voltage output of each analog output	0 - 5 V
2 Signal Collector Unit SCU to IFB Interface Board connectors	External power over iX-industrial cable, type B

Interface Board IFB-C Multiboot	
Dimensions (W x D x H)	250 mm x 83 mm x 25 mm
Weight	300 g
Front Panel	
2 Sync IN and Sync OUT	Connectors for IFB-C, connection in a chain: Lemo connector, EPL.00.304 NLN
1 8-Channel Analog IN	10-pin connector, DIL10Header-100mil
2 Analog Inputs, Channel 1 and Channel 2	Lemo connector, EPL.00.250 NTN
Signal input range for analog channels	±10 V voltage input range @ 24 bit ADC
2 LEDs	LED light is on, while link to headstage is working
4 Digital Inputs	Lemo connector, EPL 00250 NTN
4 Digital Outputs	Lemo connector, EPL 00250 NTN
1 Ground	Common jack 4 mm, banana plug
Rear Panel	
I / O	Switch On / Off
1 Power supply	MPU 30, PWR DC 0.65 x 2.75 mm
1 Ground	Common jack 4 mm, banana plug
1 16 bit Digital In / Out	68-pin MCS standard connector, Honda-PCS-XE68LFD
2 Auxiliary channels (Not in use!)	Lemo connector, EPL.00.250 NTN
1 Audio output	Stereo jack 3.5 mm

1 JTAG DSP	14-pin JTAG connector, DIL14Header-100mil-angeled
2 IFB-C to SCU connectors	External power over iX-industrial cable, type B
2 USB-C ports	USB-C A and USB-C B
Software	
Windows® Operating System: Version 11 or higher	
Incub8-Screen	
Incub8-Analyzer	
Multi Channel DataManager	

Contact Information

Local retailer

Please see the list of official MCS distributors on the [MCS web site](#).

multichannelsystems.com