Cardio2D and Cardio2D+ Manual
Imprint

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1 Welcome to Cardio2D and Cardio2D+

The Cardio2D software consists of two main parts: The recording part is called Cardio2D program. It is for controlling the amplifier, the stimulator, and for scheduling and running the experiments.

The offline analyzer part is called Cardio2D+. The Cardio2D+ analyzer program is intended for reviewing and graphical representation of recorded experiments and for preparing the graphical results as far as possible for a publication.

Raw data is recorded in 24 bit resolution when using the MEA2100- or ME2100--Systems. The other data acquisition systems record raw data with 16 bit resolution.
2 Important Safety Advice

Warning: Make sure to read the following advice prior to install or to use 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: Obey always 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 best results and to minimize risks.

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 a 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.

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

Malfunctions which could impair safety should be rectified immediately.

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 mains 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. Keep the device and the power cords always 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. Never cover the vents, not even partially, so that the air can circulate freely. Otherwise, the device may overheat.
2.1 Guarantee and Liability

The General conditions of sale and delivery of Multi Channel Systems MCS GmbH always apply. The operator will receive these no later than on conclusion of the contract.

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 findings.

Guarantee and liability claims 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.

2.2 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.
2.3 Terms of Use

You are free to use the Cardio2D and Cardio2D+ programs for the intended purpose. You agree that you
will not decompile, reverse engineer, or otherwise attempt to discover the source code of the software.

2.4 Limitation of Liability

Multi Channel Systems MCS GmbH makes no guarantee as to the accuracy of any and all tests and
data generated by the use 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.
3 Software and Hardware Installation

3.1 Installing the Cardio2D Software

System Requirements

Software: One of the following Microsoft Windows ® operating systems is required: Windows 10, Windows 8.1 (English and German versions supported) with the NT file system. Other language versions may lead to software errors.

Hardware: Please read chapter "Hardware Acquisition Systems". One of the listed data acquisition hardware system (MEA2100-Systems, MEA2100-Mini-System, ME2100-System, USB-ME-Systems, USB-MEA256-System, USB-ME32-FAI-Systems, Portable ME-Systems or MC_Card) is recommended by MCS. Due to the amount of recorded data, a computer with low performance may lead to performance problems; therefore, Multi Channel Systems recommends an up-to-date computer. Please contact MCS or your local retailer for more information on recommended computer hardware specification. 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.

Important: You need to have installed the latest data acquisition driver to operate the device, which is automatically installed with Cardio2D. 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.

Recommended Operating System Settings

The following automatic services of the Windows operating system interfere with the data storage on the hard disk and can lead to severe performance limits in Cardio2D. These routines were designed for use on office computers, but are not very useful for a data acquisition computer.

- Deselect Windows Indexing Service for all local disks.
- Turn off Optimize hard disk when idle (automatic disk fragmentation).
- It is also not recommended to run any applications in the background when using Cardio2D.

Remove all applications from the Autostart folder.

Important: Please do not run MC_Rack software program from Multi Channel Systems MCS GmbH in parallel to Cardio2D software!

Please check the system requirements before you install the software. MCS cannot guarantee that the software works properly if these requirements are not fulfilled.

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.
Installing the Cardio2D Software
Doubleclick the Cardio2D.exe on the installation volume. The installation assistant will show up and guide you through the installation procedure. Follow the instructions of the installation assistant.

*Note: The hardware driver is installed (or updated) automatically.*

Installing the Cardio2D+ Analyzer Software
Doubleclick the Cardio2D+.exe on the installation volume. The installation assistant will show up and guide you through the installation procedure. Follow the instructions of the installation assistant.

You will again be asked for a path to put the log and data files. Cardio2D+ does not produce raw data files, result files only. Raw data files created by Cardio2D are never changed by Cardio2D+, but analyzed and saved as result files. It is advisable to use the same subfolders as used by the Cardio2D.
3.1.1 First Use of Cardio2D after Installation

Doubleclick the Cardio2D program icon.

You will be asked for a path to put the log and data files. The program will create subfolders for each of these file types. It is advisable to put all three subfolders in a common Cardio2D directory. These settings will be saved and do not have to be entered again. To change the settings later, please see main menu “Settings”, “File Location”.

![Location Settings dialog](image)

The “Data Acquisition Settings” dialog appears.

![Data Acquisition Settings dialog](image)

You have to select the data acquisition device, if more than one data acquisition is available. Select the "Sample Rate", the "Range" (when using the MC_Card only), and the gain as an intrinsic property of the connected amplifier. The "Resolution" and the number of "Electrode Channels" are displayed.

Select the check box “Digital Channel” if you are going to use the “External Trigger” feature.

The check boxes "Aux. Analog In" are only available if a system with interface board MCS-IFB is the data acquisition device. Select one or several of the check boxes "Aux. Analog In" if you are going to connect one or several devices which send analog signals. The analog signal data are collected in the raw data file and have to be exported via Multi Channel DataManager for analysis, currently it is not possible to view them in Cardio2D or Cardio2D+.

The combo box "ME2100-HS32 Ref." is only available, if a ME2100-HS32-System is the data acquisition device. Please use it for defining the reference electrode in this case.

Select the type of MEA or FlexMEA from the drop down menus. These entries can be saved with the command “Save Defaults”. If not saved, the dialog will appear each time Cardio2D is started. It is possible to change the default settings or to save several default files if you work with different configuration. The dialog is also available in the “Settings” menu in “Data Acquisition”.

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3.1.2 Data Acquisition Hardware

Caution: You have acquired a high performance data acquisition and analysis computer. Do not modify the system, do not install new hard- or software, or another operating system without asking MCS or your local retailer for advice. Cardio2D do not install virus scanners or firewalls because these programs are known to interfere with the data transfer to the hard disk. MCS cannot guarantee that a modified system is fully operational. Even data loss may occur.

Warning: The operating system settings of the data acquisition computer were preconfigured by MCS and should not be changed by the user. Changing these settings can lead to program instabilities and data loss.

Please read chapter “Data Acquisition Settings” in "First Use of Cardio2D after Installation".

Data Acquisition with the MEA2100- and ME2100-Systems:

Any type of the MEA2100-System is an all-in-one solution consisting of headstage and interface board with integrated amplification, data acquisition, online signal processing, and integrated stimulus generator. You can connect one or two headstages to the interface board. The MEA2100-System uses an USB 3.0 (USB 2.0 old version of interface board) connector per headstage to transfer digitized data to any computer.

Select one or several of the check boxes "Aux. Analog In" if you are going to connect one or several devices which send analog signals. The analog signal data are collected in the raw data file and have to be exported via Multi Channel DataManager for analysis, currently it is not possible to view them in Cardio2D or Cardio2D+.

Select the check box "Digital Channel" if you are going to use the “External Trigger” feature. Please read the respective MEA2100-System manual for detailed information.

When using a ME2100-H32 headstage, there is no fix electrode set as reference electrode. The reference electrode has to be assigned to a measurement electrode via the channel selection in the “Data Acquisition Settings” window. The selected electrode will be shown as not active in the MEA electrodes pane.

Note: The combined use of ME2100-HS32 and ME2100-µPA32 is not feasible. In such case, the headstage type on the lowermost connector index is taken as ‘master’ and all other types will be ignored.
Digital channels for MEA2100 (-Mini)- and ME2100-Systems

<table>
<thead>
<tr>
<th>Bit No #</th>
<th>Digital Event</th>
<th>Bit No #</th>
<th>Digital Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bit 0</td>
<td>DigIn 1</td>
<td>Bit 8</td>
<td>real time feedback 1</td>
</tr>
<tr>
<td>Bit 1</td>
<td>DigIn 2</td>
<td>Bit 9</td>
<td>real time feedback 2</td>
</tr>
<tr>
<td>Bit 2</td>
<td>DigIn 3</td>
<td>Bit 10</td>
<td>real time feedback 3</td>
</tr>
<tr>
<td>Bit 3</td>
<td>DigIn 4</td>
<td>Bit 11</td>
<td>real time feedback 4</td>
</tr>
<tr>
<td>Bit 4</td>
<td>Stimulus on STG Channel 1</td>
<td>Bit 12</td>
<td>real time feedback 5</td>
</tr>
<tr>
<td>Bit 5</td>
<td>Stimulus on STG Channel 2</td>
<td>Bit 13</td>
<td>real time feedback 6</td>
</tr>
<tr>
<td>Bit 6</td>
<td>Stimulus on STG Channel 3</td>
<td>Bit 14</td>
<td>real time feedback 7</td>
</tr>
<tr>
<td></td>
<td>(Zero for MEA2100-256-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and ME2100-HS32-Systems)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bit 7</td>
<td>Zero</td>
<td>Bit 15</td>
<td>real time feedback 8</td>
</tr>
</tbody>
</table>

Data Acquisition with the USB-ME-Systems

The external data acquisition board USB-ME256-System is a stand-alone solution for acquiring data from up to 256 channels from up to four MEAs. It can replace the internal computer hardware MC_Card. The analog input signals are acquired and digitized by the USB-ME256-System and the digital electrode signals are transmitted to the connected computer via universal serial bus (High Speed USB 2.0). Thus, it is possible to use any computer for data processing. Recorded data is graphed, analyzed, and reviewed with Cardio2D and Cardio2D+ program. The USB-ME256-System supports 256 channels with an amplification factor 1 that means no amplification, data acquisition only. You can connect, for example, up to four MEA1060 amplifiers via 68-pin MCS high grade cables.

Please select the check box “Digital Channel” if you are going to use the “External Trigger” feature.

The USB-ME128-System and the USB-ME64-System are in principle the same devices as the USB-ME256-System, but with 128 or 64 electrode channels respectively.

Digital channel for USB-ME-Systems

<table>
<thead>
<tr>
<th>Bit No #</th>
<th>Digital Event</th>
<th>Bit No #</th>
<th>Digital Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bit 0</td>
<td>DigIn 1</td>
<td>Bit 8</td>
<td>DigIn 9</td>
</tr>
<tr>
<td>Bit 1</td>
<td>DigIn 2</td>
<td>Bit 9</td>
<td>DigIn 10</td>
</tr>
<tr>
<td>Bit 2</td>
<td>DigIn 3</td>
<td>Bit 10</td>
<td>DigIn 11</td>
</tr>
<tr>
<td>Bit 3</td>
<td>DigIn 4</td>
<td>Bit 11</td>
<td>DigIn 12</td>
</tr>
<tr>
<td>Bit 4</td>
<td>DigIn 5</td>
<td>Bit 12</td>
<td>DigIn 13</td>
</tr>
<tr>
<td>Bit 5</td>
<td>DigIn 6</td>
<td>Bit 13</td>
<td>DigIn 14</td>
</tr>
<tr>
<td>Bit 6</td>
<td>DigIn 7</td>
<td>Bit 14</td>
<td>DigIn 15</td>
</tr>
<tr>
<td>Bit 7</td>
<td>DigIn 8</td>
<td>Bit 15</td>
<td>DigIn 16</td>
</tr>
</tbody>
</table>
Data Acquisition with the USB-MEA256-Systems

The USB-MEA256-System is a compact and portable stand-alone solution for MEA recordings with integrated amplification, data acquisition, and analog / digital conversion. The system acquires data from up to 252 electrode channels, four additional analog channels, and 16 digital IN / OUT channels. The digitized electrode data is transmitted to the connected computer via universal serial bus (High Speed USB 2.0). Thus, it is possible to use any computer as a data acquisition computer, also a laptop. Recorded data is graphed, analyzed, and reviewed with Cardio2D and Cardio2D+ program. The integrated filter amplifier supports 252 electrode channels. The gain of 1100 is fixed. The bandwidth of 1 Hz to 3 kHz is suitable for a broad range of applications.

Please select the check box “Digital Channel” if you are going to use the “External Trigger” feature.

Data Acquisition with the USB-ME32-FAI-Systems

The USB-ME32-FAI-System is a very compact and portable stand-alone solution. It features an integrated 32-channel filter amplifier and data acquisition. The digitally converted electrode signals are transmitted to the connected computer via universal serial bus (High Speed USB 2.0). As in other USB-ME-Systems, it is possible to use any computer as a data acquisition computer, also a laptop. The size of the complete system is small enough to fit into a laptop bag together with the computer. The system is for use with a 32-channel miniature amplifier MPA32I. You can directly connect the EcoFlexMEA36 to the 32-channel miniature preamplifier for recording with Cardio2D.

Please select the check box “Digital Channel” if you are going to use the “External Trigger” feature.

Data Acquisition with the MC_Card

The data acquisition computer with the with the internal data acquisition board MC_Card comes preinstalled and preconfigured by MCS for a flawless operation. You should contact your local retailer for assistance if you want to install additional hard- or software, or if you want to replace the computer, as incompatibilities of hardware components or software settings with the MC_Card or Cardio2D may occur.

Digital channel for USB-MEA256-, USB-ME32-FAI-, and MC_Card-Systems

<table>
<thead>
<tr>
<th>Bit No #</th>
<th>Digital Event</th>
<th>Bit No #</th>
<th>Digital Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bit 0</td>
<td>DigIn 1</td>
<td>Bit 8</td>
<td>DigIn 9</td>
</tr>
<tr>
<td>Bit 1</td>
<td>DigIn 2</td>
<td>Bit 9</td>
<td>DigIn 10</td>
</tr>
<tr>
<td>Bit 2</td>
<td>DigIn 3</td>
<td>Bit 10</td>
<td>DigIn 11</td>
</tr>
<tr>
<td>Bit 3</td>
<td>DigIn 4</td>
<td>Bit 11</td>
<td>DigIn 12</td>
</tr>
<tr>
<td>Bit 4</td>
<td>DigIn 5</td>
<td>Bit 12</td>
<td>DigIn 13</td>
</tr>
<tr>
<td>Bit 5</td>
<td>DigIn 6</td>
<td>Bit 13</td>
<td>DigIn 14</td>
</tr>
<tr>
<td>Bit 6</td>
<td>DigIn 7</td>
<td>Bit 14</td>
<td>DigIn 15</td>
</tr>
<tr>
<td>Bit 7</td>
<td>DigIn 8</td>
<td>Bit 15</td>
<td>DigIn 16</td>
</tr>
</tbody>
</table>
Overview: Possible Configuration of Data Acquisition System and Microelectrode Array MEA

**MEA2100-HS60, MEA2100-HS2x60, MEA2100-Mini-HS60, MEA2100-Lite-HS60**
- MEA_3D_8x8_100_12_40
- MEA_3D_8x8_100_12_50
- MEA_3D_8x8_100_12_80
- MEA_3D_8x8_100_12_100
- MEA_6Well_60MEA_200_30
- MEA_8x8_100_10
- MEA_8x8_200_10
- MEA_8x8_200_30
- MEA_8x8_1000_30
- MEA_6x10_500_10
- MEA_6x10_500_30
- MEA_10x6_100_30
- SquareMEA_8x8_200_50
- EcoMEA_8x8_700_100
- 60HDMEA30/10
- 60ThinMEA30/10
- Qwane MEA60_6x10_400_70
- Qwane MEA60_6x10_500_75
- Qwane MEA60_6x10_600_80

**MEA2100-HS120**
- MEA_12x12_200_30
- MEA_12x12_100_30
- MEA_12x12_30_10
- 120MEA1000-1500/30
- 120-3DMEA250/12/100

**MEA2100-HS256, USB-ME256**
- MEA_6Well_256MEA_200_30
- MEA_9Well_256MEA_200_30
- MEA_16x16_30_8
- MEA_16x16_60_10
- MEA_16x16_100_30
- MEA_16x16_200_30

**ME2100-HS32, ME2100-µPA32, USB-ME32**
- EcoFlexMEA_6x6_300_50
- FlexMEA_6x6_300_30
USB-ME64

- MEA_3D_8x8_100_12_40
- MEA_3D_8x8_100_12_50
- MEA_3D_8x8_100_12_80
- MEA_3D_8x8_100_12_100
- MEA_6Well_60MEA_200_30
- MEA_8x8_100_10
- MEA_8x8_200_10
- MEA_8x8_200_30
- MEA_8x8_1000_30
- MEA_6x10_500_10
- MEA_6x10_500_30
- MEA_10x6_100_30
- SquareMEA_8x8_200_50
- EcoMEA_8x8_700_100
- 60HDMEA30/10
- 60ThinMEA30/10
- Qwane_MEA60_6x10_400_70
- Qwane_MEA60_6x10_500_75
- Qwane_MEA60_6x10_600_80
- FlexMEA72_750_100
- FlexMEA_6x6_300_30  Note: It is possible to connect one or two FlexMEAs!
- EcoFlexMEA_6x6_300_50  Note: It is possible to connect one or two EcoFlexMEAs!

USB-ME128, USB-ME256

- MEA_3D_8x8_100_12_40
- MEA_3D_8x8_100_12_50
- MEA_3D_8x8_100_12_80
- MEA_3D_8x8_100_12_100
- MEA_6Well_60MEA_200_30
- MEA_8x8_100_10
- MEA_8x8_200_10
- MEA_8x8_200_30
- MEA_8x8_1000_30
- MEA_6x10_500_10
- MEA_6x10_500_30
- MEA_10x6_100_30
- SquareMEA_8x8_200_50
- EcoMEA_8x8_700_100
- 60HDMEA30/10
- 60ThinMEA30/10
- Qwane_MEA60_6x10_400_70
- Qwane_MEA60_6x10_500_75
- Qwane_MEA60_6x10_600_80
- FlexMEA72_750_100
- FlexMEA_6x6_300_30 Note: It is possible to connect one or two FlexMEAs per 68-pin MCS standard connector!
- EcoFlexMEA_6x6_300_50 Note: It is possible to connect one or two EcoFlexMEAs per 68-pin MCS standard connector!
- EcoFlexMEA128_12x12_2700_100

**MC_Card 64, MC_Card 128**

- MEA_3D_8x8_100_12_40
- MEA_3D_8x8_100_12_50
- MEA_3D_8x8_100_12_80
- MEA_3D_8x8_100_12_100
- MEA_6Well_60MEA_200_30
- MEA_8x8_100_10
- MEA_8x8_200_10
- MEA_8x8_200_30
- MEA_8x8_1000_30
- MEA_6x10_500_10
- MEA_6x10_500_30
- MEA_10x6_100_30
- SquareMEA_8x8_200_50
- EcoMEA_8x8_700_100
- 60HDMEA30/10
- 60ThinMEA30/10
- Qwane_MEA60_6x10_400_70
- Qwane_MEA60_6x10_500_75
- Qwane_MEA60_6x10_600_80
- FlexMEA72_750_100
- FlexMEA_6x6_300_30 Note: It is possible to connect one or two FlexMEAs!
- EcoFlexMEA_6x6_300_50 Note: It is possible to connect one or two EcoFlexMEAs!
4 The Cardio2D Program

The main window of Cardio2D consists of two in case of several connected MEAs of three window panes. The left “Control Window” is for controlling and documentation of the experiment. The bigger window on the right side, the “MEA Window” displays heartbeats in different characteristics in real-time monitoring. The “Summary” window is only available, if more than one MEA is connected and presents an overview of all connected MEAs. The green color highlights the MEA, which is displayed in the MEA tabbed page. The layout of the MEA is displayed respectively.

In the control view window, the experimental processing icons are located and the experimental settings can be entered and the experiment started and stopped. If more than one MEA is connected, each MEA is represented via a tabbed page in “MEA Window”.

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The "MEA Window" is divided into four single views. All of them display heartbeats in different characteristics. If more than one MEA is connected to the experimental setup, each MEA can be operated separately via the "MEA Tabbed Pages". Depending on the hardware up to nine MEAs (for nine wells of the 256-9-well MEA) are available. Select the respective MEA in the "Summary" window to display the data traces in the MEA window. If a MEA is disabled via check box in the "Summary" window, the corresponding MEA tabbed page will be removed from the display, and no data is recorded.

Different types of MEAs are implemented. The design of the "MEA Window" is automatically adjusted to the type of the MEA, please see chapter "Data Acquisition Settings".

Recorded data traces are displayed in the "MEA Electrodes" pane. Any channel can be zoomed at by clicking on a channel in the MEA electrodes plate. The zoomed channel is displayed in the larger single channel view. The single channel view has three tabs, "FP" field potential, "HB" heartbeat and "Ave" average.

The heartbeat window shows either a diagram of the RR interval or of the frequency of the heartbeats, the heart rate. The current values of the RRI or the frequency are displayed above in red numbers.

The color map displays the propagation of a heartbeat from the pacemaker to the outer regions of the cardiac cell culture or tissue. The isochrones are indicated by black lines. The colors indicate the latency distribution over the MEA. Red color represents the pacemaker when using the color map "jet inverse".

The latency / velocity window shows the latency of the heartbeat in units of µs or the velocity in units of mm per ms. Both parameters are measured between the first and the last detected electrode in the range. The current absolute values are displayed in red letters above the plot.

Main Menu

The "File" menu allows to open or save templates, to restore and save defaults and to exit the program.

If experiments were designed for a certain purpose and you want to reuse the experimental setup, save the file as a template. You can reuse the template file as often as necessary. You can save as many templates as needed. You can also save local and global settings as a template.

Factory set defaults are the setting parameters, which are used when starting Cardio2D for the first time. Please enter your own defaults, for example, in "Data Acquisition Settings" dialog after installing the software. Click "Save Defaults" to save your settings as a default. This way your setting parameters will be present, each time you start Cardio2D. To restore default settings, please select "Restore Defaults".
**Experiment**

The main menu "Experiment" allows the selection between "Basic" or "Compound" experiments. The icon in the "Control Window" changes respectively.

**Tools**

In the "Tools" menu, the offset correction can be activated.

An offset correction may be necessary, if you observe disruptive voltage offset on the input channels. Click the check box "Correct Offset" and Cardio2D takes 100 ms of the recorded data in the moment when the check box is selected to calculate the DC offset. The mean of this 100 ms sweep is subtracted from the recorded data if the "Correct Offset" check box is activated. The offset correction works separately for each available channel. Click the "Update" button to update the calculation of the offset correction. This option is available when Cardio2D is running.
Settings

This menu allows to define the data acquisition device and to determine the path for the data and log files. Click “Data Acquisition” in the “Settings” menu. The “Data Acquisition” dialog opens.

The data acquisition device must be selected, if more than one data acquisition is available. Select the “Sample Rate”, the “Range” (when using the MC_Card only), and the gain as an intrinsic property of the connected amplifier. The “Gain”, the “Resolution” and the number of “Electrode Channels” are displayed.

Select the check box “Digital Channel” if you are going to use the “External Trigger” feature.

Select one or several of the check boxes “Aux. Analog In” if you are going to connect one or several devices which send analog signals. The analog signal data are collected in the raw data file and have to be exported via Multi Channel DataManager for analysis, currently it is not possible to view them in Cardio2D or Cardio2D+.

Select the type of MEA or FlexMEA.

Click “File Location” in the “Settings” menu. The “Location Settings” dialog opens.

Please define the file path for the raw data and the corresponding setting files, the result and the log files.
Depending on the selected data acquisition device it is possible to connect up to four MEA amplifiers, which can be operated independent from each other: That means you can select the settings for each MEA separately, but the starting and stopping of the experimental protocol is synchronous for all connected MEAs.

The design of the MEA tab is different depending on the type of the connected MEA.

Click "View Summary Page" to open the "Summary" window. The window presents an overview and all connected MEAs are visible. The green color code marks the MEA which is displayed in the MEA tabbed page. The layout of the MEA is displayed respectively.

You can uncouple the "Summary" window to move it to a different place on the monitor.

Click the "Map Settings" icon to open the "Summary Map Settings" dialog and start or stop monitoring the color map via "Start" and "Stop" icon.
In this example the “Summary” page shows 60-6wellMEAs with adapted color map.

Help

Select “About Cardio2D”.

The “Help” menu starts the Cardio2D online help, contains the dialog “About Cardio2D”, allows you to check for available software updates.

**Note:** The information of this dialog “About Cardio 2D” is necessary in case of support!
4.1 Viability

Before you start recording, a viability test is recommended. The data is not recorded.

Click **Viability**. The "Viability" icon changes into "Stop" icon, and the duration of the viability test is counted up in hours, minutes and seconds. Stop the viability test manually by clicking on "Stop" icon.

After the initial installation, a test of the amplifier is recommended. Use the provided model test probe. It simulates a MEA with a resistor of 220 kOhm and a 1 nF capacitor between bath and electrode. Use the Cardio2D program to record from the test model probe and to check the amplifier. You see the raw data streams of all available channels in the typical MEA layout grid. This also presents an opportunity for you to learn about the program before starting a real experiment.

In general, the viability test monitors whether the MEA electrodes are acceptable for recording, and the cell culture or tissue is fine. You can check the signal detection parameters, and focus on single channels. If the settings are too restrictive, the cardiac signal is not detected at all. Too broad settings will lead to false signal detection and results. The log file documents the experimental proceeding.

It is possible to change analysis parameters during an active viability test. The changes can be monitored in real-time. This option is not available during the recording, where the settings must be constant.
4.2 Lab Book

In this dialog you fill in basic data regarding your experiment. The "Lab Book Settings" will be stored in the settings file *.c2s.

You can set parameters on two levels, "Local" and "Global". Local settings will apply to the selected MEA, a single well or a multi well MEA, whereas global settings apply to all connected MEAs or wells on a multi well MEA. Local settings can be adjusted via the "Lab book" icon in the MEA window, global settings via the "Control View" icon for the two sub-folders "Study" and "General".

**Note:** Changes in global settings will overwrite the local settings.
4.3 Compound / Basic Experiments

The Cardio2D software allows two types of experiments to be selected via the main menu "Experiment". "Basic" experiment and "Compound" experiment. Both experiment types allow the control of the experiment procedure and external triggering, the compound experiment allows the additional setting of a series of compound application.

Experiments in Cardio2D are separated into "Recordings", each representing a different experiment condition. Recordings may optionally be broken further down into "Apply" and "Measurement" phases.

Apply phases are intended as intervals during which experiment conditions are adjusted, for example applying a compound, which could lead to signal artifacts that distort data analysis. In contrast, measurement phases are intervals with stable experiment conditions.

4.3.1 Basic Experiment

Click "Basic" in the "Control View", the "Basic Settings" window opens. The "Experiment Procedure" and "Phase Switching" as well as external triggering can be set.

![Image of Basic Settings window]

The „Experiment with Apply Phase“ allows the setting of a marker for the separation of apply phases from the actual measurement phases.

Note: In this setting data will not be recorded during the "Apply Phase".
This can be enabled via the „Record Apply Phase” setting. In these cases, after finishing or starting the next measurement via the “Basic” icon, an “Apply Phase” window opens. Adjust experiment conditions while this dialog is opened. An optional comment may be added before the “Apply Phase” is finished by clicking “Proceed to Measurement Phase”.

Experiment Phase Switching

Switching between phases in the recording of an experiment can be done manually when “Manual” is selected in the drop down menu. In this setting, every single phase must be started actively by clicking the "Basic" icon or "Start". The recording will start immediately after clicking the icon and must be stopped manually as well.
**Manual / Timer**

When "Manual/Timer" is selected, a "Measurement Phase Duration" must be defined and the measurement will run for a set time interval after a manual start.

![Basic Settings](image)

**External Trigger**

*Note: This feature is only available with MEA2100-Systems or USB-ME-64/128/256-Systems.*

Connect the peripheral device to the respective "Digital In" on the front panel of the interface board. The drop down menus in the “External Trigger” settings are now available. Assign the external trigger sequential to the "Digital In" bit 1, 2, 3, 4, rising or falling edge.

Define the "External Triggers" for the start and the end of the experiment, for the "Apply Phase Start" and the "Measurement Phase Start" as well. Please see the scheme below.

![Scheme](image)

The external trigger starts and stops the complete experiment, and the phase trigger starts the different apply and measurement phases. The Cardio2D software is in standby state until the first apply phase starts.
Measurement Count

A limited measurement count can be programmed by activating the "Measurement Count" check box. If selected, data acquisition and recording data will automatically be stopped after the last measurement is finished.

4.3.2 Compound Experiment

If the "Compound Experiment" is selected via the main menu "Experiment", the icon in the control window changes to "Compound".

In this dialog you can prepare a series of compound applications as well as set the "Experiment Procedure" and "External Trigger". The standard setting for "Series" can be edited by overwriting the concentration series displayed in the window to create a user-defined concentration series. A preset dilution factor is available for 1:10 and 1:3 dilution series, but any custom setting is possible if the syntax is accepted by the program.

For "Experiment Procedure", "Phase Switching" and "External Trigger" refer to section "Basic Experiment".
4.4 Experiment Log File

Each step of your experiment is documented linearly with time stamp. The log file is automatically saved as *.txt file when data is recorded, using the same directory and file name as for raw data files. Additionally, you can save the log to a user defined path by clicking on the "Save" icon. To clear the log file display, click the "Clear" icon.

4.5 Stimulation of MEA Electrodes with external Stimulator

The stimulation feature gives the possibility for voltage driven stimulation of any of the MEA electrodes for USB-ME- or USB-MEA-256-Systems without integrated stimulus generator.

Please connect a stimulus generator STG and to design the stimulation pulse.
You can connect either one stimulus generator STG with more than one channels, or several STGs to stimulate the different MEA amplifiers. Define either the channel number from the "Channel" drop down menu when using one STG, or the devices from the "Device" drop down menu when using several STGs.

### Stimulus Pulse

In the "Stimulus" menu you design the stimulus pulse.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Symmetric</td>
<td>✓</td>
</tr>
<tr>
<td>1. Phase</td>
<td>500 µs</td>
</tr>
<tr>
<td>2. Phase</td>
<td>500 µs</td>
</tr>
<tr>
<td>IPI</td>
<td>899 ms</td>
</tr>
</tbody>
</table>

The pulse shape of a stimulus pulse is usually biphasic symmetric rectangular. The parameters are the negative or positive sign of the first pulse phase, the duration of each phase, and the amplitude of the pulse. Click the check box "Symmetric", and the pulse will automatically be symmetric and biphasic. To design the pulse shapes, please use the numeric updown controls "Phase 1" and "Phase 2". The "IPI", the inter pulse interval, is the time frame between two pulses in sequence.

The red line indicates the stimulus pulse, the blue line indicates the TTL blanking pulse.
Blanking Pulse

The "Sync Out" menu is for the blanking feature if an amplifier with blanking circuit is connected.

<table>
<thead>
<tr>
<th>Sync Out</th>
</tr>
</thead>
</table>
| On       | ✔️
| Extension| 300 µs |

When using the blanking feature, please enable the "Sync Out" check box. You have to define the time of "Extension". The TTL pulse for the blanking signal starts synchronal to the stimulus pulse, but it should last longer to avoid stimulus artefacts. This time interval is called extension time. The extension time varies between 0 and 1000 µs, the default is 300 µs.

In some cases it may be necessary to see the stimulus artefacts. Please deselect the check box "Sync Out" to switch off the Sync Out signal. Additional please select blanking feature in the MEA_Select program, please see chapter "MEA_Select".

<table>
<thead>
<tr>
<th>Blanking</th>
</tr>
</thead>
<tbody>
<tr>
<td>✔️ Blanking</td>
</tr>
</tbody>
</table>

Click the Start icon.

If your experimental schedule will always need the same pulse paradigm, please store the settings in "File" menu "Save Defaults". This way the settings appear in a new file, and you do not have to type in them again.

Stimulus Generator STG

It is recommended to use a stimulus generator STG of the 2000 or 4000 series from Multi Channel Systems MCS GmbH.

**Important:** When working, for example, with four amplifiers with blanking circuit BC, you have to connect a STG4004 or a STG2004. The TTL blanking signal and the stimulation pulse must be synchronized, that is, you have to connect the stimulus pulse of channel 1 on MEA amplifier 1 to the TTL blanking pulse of Sync Out 1 of the STG, channel 2 to Sync Out 2, channel 3 to Sync Out 3 and channel 4 to Sync Out 4 respectively.

When using an USB-ME256 device for data acquisition you can connect to up to four MEA amplifiers. You define the stimulation settings for each of the connected MEA amplifier independent from each other, and you can start and stop the stimulation independent from each other.
**Amplifier with or without Blanking Circuit BC**

The blanking feature is used to reduce stimulus artefacts in MEA recordings. A TTL pulse triggers both, the stimulation and the blanking. During the blanking signal both stimuli are connected to the stimulation electrode(s), while all recording electrodes are disconnected from the amplifier. The last signal value on all electrode channels before the blanking pulse will be saved and then kept until the channels are reconnected to the amplifier. This avoids the saturation of the amplifier during stimulation.

Amplifiers without “Blanking Circuit” do not offer this adjustment and the stimulus and stimulus artefacts will be visible and recorded.

**MEA_Select**

MEA_Select is a software program for controlling the blanking circuit of the MEA amplifier. With MEA_Select it is possible to select a chosen MEA electrode to stimulate. Start MEA_Select parallel to Cardio2D.

For more information about MEA_Select software or MEA amplifiers with “Blanking Circuit” please refer to the respective chapters in the amplifier manuals “MEA1060-Inv-BC_Manual” or “MEA1060-Up-BC_Manual”, available on the MCS web site.
4.6 Stimulation with the MEA2100- or ME2100-Systems

When working with Cardio2D and any type of MEA2100- or ME2100-Systems with integrated stimulus generator STG the "Stimulate" dialog provides the possibility of stimulating via two or three (depending on the connected hardware) different stimulation patterns on user defined electrodes.

The dialog mirrors the layout of the currently used MEAs, in this case a MEA2100-2x60-System with two slots for two 60-electrode MEAs. Use the tool bar thereunder to define the stimulation sites.

Tool Bar

The tool bar in the stimulate dialog is divided into three sections: A position indicator, controls for single stimulus patterns, and controls that affect all three stimulus patterns.

The position indicator helps to navigate through the MEA electrodes. It shows the position of the mouse on the left MEA1 or right MEA2. The numbering of MEA electrodes in the 8 x 8 grid follows the standard numbering scheme for square grids: The first digit is the column number, and the second digit is the row number. For example, electrode 23 is positioned in the second column and the third row.

Three or two (when using a ME2100- or a MEA2100-256-System) separate stimulus patterns can be defined, displayed in green, blue, red.

The location of the selected stimulus can be defined by clicking on electrodes in the electrode panel or by drawing rectangles over the desired area. Each stimulation electrode accepts one stimulus pattern at a time. It is not possible to overlap patterns but to overwrite them with a new selection or to clear the sites.

Note: When using a ME2100-HS32, internal and external stimulation can be used simultaneously. The external stimulation electrode can be activated via the check box above the electrode panel. The signal will be emitted via the connector on the side of each connected headstage.
To remove the currently selected stimulus from all sites, please use the "Clear selected stimulus sites" icon. All created sites of the selected pattern will be removed. To remove stimuli from single electrodes only, cancel the current stimulus pattern selection first by clicking on the "Select Stimulus" icon again, then click on the electrode to be removed.

The "Define Stimulus" icon will open a new dialog enabling the definition of the stimulus pattern as explained on the following pages. To start stimulation with the currently selected pattern only, click the "Start Stimulus" icon.

To clear all sites, click the "Clear all stimulus sites" icon. Start all stimulus patterns simultaneously via the "Start" icon.

Stimulation of electrodes produces stimulation artefacts, which can be eliminated. To select the blanking artefact suppression, please click the "Blanking" icon. "Blanking" will blank all recording electrodes on a MEA while a stimulus pattern is applied to any electrode of the same MEA. Choosing the "Dedicated Electrode" icon all selected electrodes will be defined to be dedicated stimulation electrodes. These electrodes will be permanently isolated from the amplifier, hence recording from these electrodes will be stopped.

Note: It is not possible to select electrode 15 for stimulation, because this electrode is connected to the internal reference.
Stimulation Pattern

To define the stimulation pattern, please select a stimulus, green, blue or red and open the "Define Stimulus" dialog via icon . All settings will correspond with the selected stimulus pattern.

The "Define Stimulus" dialog is divided in an upper and a lower section. The upper part includes the icons for the provided stimulus patterns, such as flat line, rectangle pulse, ramp or sine waves or biological signals, which can be imported into the Cardio2D software.

The lower section of the dialog shows the "Complete Stimulus Pattern" and a tool bar. The settings in the tool bar influence the complete sequence of the stimulus patterns which are immediately displayed in the window for the "Complete Stimulus Pattern".

The white menu bar "Building a Stimulus Pattern" in the middle of the dialog is for creating the desired stimulus pattern from the provided default stimulus patterns, called "Primitives". Each "Primitive" pattern can be modulated via individual "Parameter Settings" and the modulation will be immediately displayed in the "Single Pattern" view and in the view for the "Complete Stimulus Pattern".
Building Stimulation Patterns

To select a defined stimulus pattern, move the primitive icon via drag and drop into the white field in the middle of the dialog for creating a user defined stimulation pattern. Add as many stimuli as needed. It is possible to change the sequence of the primitives by drag and drop. To delete one of the patterns, just drop it into the bin.

Set Marker Signal

Additionally, it is possible to set a marker signal. Open the "Set Marker Signal" dialog by clicking the check box "Set Marker" in the window for parameter settings.

Click the check box "Set Marker" and click "Repeat" if you want to repeat the marker signal at each cycle of the stimulation pattern. Adjust the "Offset" in µs and the "Duration" in µs of the marker signal via the up down boxes.
Modulation of the provided Stimulation Patterns

Initially the provided stimulation patterns are default. To adjust a primitive to your requirements, please click onto the icon, which will be highlighted in pale blue. In the upper part of the dialog the setting parameters for this pattern appears on the left and a picture of the actual shape of the pattern on the right. Immediately after changing a pattern, the modulation is visible in the “Single Pattern” view and in the “Complete Stimulus Pattern” view. Save the stimulus pattern as default by clicking the “Save Primitive as Default” icon.

Most of the parameters must be adjusted with up down boxes. Click into the up down box and move the wheel of the mouse for quick adjustments in wide steps. Use the arrows for fine tuning. The modulation is immediately displayed in the single pattern and in the complete pattern window. Overwrite the letter in the up down box, the modulation will be displayed after confirming the value with “Enter” or after clicking into another box. This allows a fine adjustment to the shape of a pulse. Additionally, it is possible to modulate the shape, when repeating pulses in relation to each other.

Stimulus Pattern: Flat Line

The first provided primitive is a flat line stimulus. To adjust the duration, please use the respective up down boxes: Define the value of the pattern in the “Amplitude (mV)” up down box. This pattern can also be used as pause or non-stimulation element between two other patterns, when setting the amplitude to zero volt.
Stimulus Pattern: Sine Wave

Modulate the amplitude "PP Amp (mV)", the period "Period (µs)", the shift "Shift" and the phase "Phase (°)" of the sine wave pattern via up down boxes. Setting the number of cycles to more than one enables the inter signal interval "ISI (µs)" up down box. Additionally, the "Arrow" button will be activated.

When using more than one cycle you can click onto the arrow symbol for additional options: A highlighted parameter field appears to modulate the amplitude and the period of the sine waves in relation to each other. The displayed example shows an increase of the amplitude by 23 mV for each following sine wave. Choosing a negative value will decrease the amplitude in comparison to the wave before. Analogous you can adjust the value of the period in µs in relation to the wave sequences. The window "Single Pattern" shows the sine waves in an overlay plot. The lower window "Complete Stimulation Pattern" shows the combined sequence of all stimulus pulses.
Stimulus Pattern: Rectangle

Modulate the amplitude "Amplitude (mV)" of the ascending and the descend part of the pulse and the duration "Duration (µs)". Setting the number of cycles to more than one enables the inter stimulus interval "ISI (µs)" up down box. Additionally, the "Arrow" button will be activated.

When using more than one cycle you can click onto the arrow symbol for additional options: A highlighted parameter field appears to modulate the amplitude and the duration of the rectangle pulses in relation to each other, again independently for both parts of the pulse and for the duration between the ascending and descend arm. The window "Single Pattern" shows the rectangle pulse in an overlay plot. The lower window "Complete Stimulation Pattern" shows the combined sequence of all stimulus pulses.
Stimulus Pattern: Ramp

Modulate the amplitude "Amplitude (mV)" and the duration "Duration (µs)" of the ramp pulse via up down boxes. To adjust the steepness of the arms of the pulse, please use the three up down boxes for the ascending part, the plateau and the descend arm independent from each other. Setting the number of cycles to more than one enables the inter stimulus interval "ISI (µs)" up down box. Additionally, the "Arrow" button will be activated.

When using more than one cycle you can click onto the arrow symbol for additional options: A highlighted parameter field appears to modulate the amplitude and the duration of the ramp pulse in relationship to each other. The window "Single Pattern" shows the ramp pulse in an overlay plot. The lower window "Complete Stimulation Pattern" shows the combined sequence of all stimulus pulses.
Stimulus Pattern: Biological Pulse

If a user defined biological signal is used as stimulus, files can be imported via the "Import" button. The imported file must mandatorily have the format of "Timestamp" in µs followed by "Voltage Value" in µV as displayed in the example below:

<table>
<thead>
<tr>
<th>Time stamp</th>
<th>Voltage Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>45147</td>
</tr>
<tr>
<td>300</td>
<td>19072</td>
</tr>
<tr>
<td>500</td>
<td>21903</td>
</tr>
<tr>
<td>700</td>
<td>0</td>
</tr>
<tr>
<td>800</td>
<td>11026</td>
</tr>
<tr>
<td>1000</td>
<td>21307</td>
</tr>
<tr>
<td>1200</td>
<td>18178</td>
</tr>
<tr>
<td>1500</td>
<td>-21158</td>
</tr>
<tr>
<td>1600</td>
<td>-9387</td>
</tr>
<tr>
<td>1800</td>
<td>17880</td>
</tr>
</tbody>
</table>

The unit for the time stamp is µs and for the voltage value µV. The units are not part of the file. Cardio2D software accepts integers only and commas, tabulators or spaces to separate the time stamp and the voltage value. Please remove a possible header and use a new line for each integer pair. Save the file with the extension "*.dat" or use a comma separated file "*.csv". Adapt the amplitude of the signal in percent (%) via up down box. Setting the number of cycles to more than one enables the inter stimulus interval “ISI (µs)” up down box. Additionally, the "Arrow" button will be activated.
When using more than one cycle you can click onto the arrow symbol ➡️ for one additional option: A highlighted parameter field appears to modulate the “Amplitude (%)” in relationship to each circle of the signal. The window “Single Pattern” shows the imported pulse as overlay plot. The lower window “Complete Stimulation Pattern” shows the combined sequence of all stimulus pulses.

**Stimulus Pattern: Brackets**

```
{}
```

“Place Brackets” are not a waveform, but a means to repeat parts of the complete stimulation pattern. If the bracket icon is placed in the stimulus pattern and a stimulus pattern is dragged onto the second bracket, it will be placed between the brackets and thus be repeated.

Pairs of brackets can be used in series or as brackets within brackets.

The sequence will be executed by the same rules as a mathematical operation. Click on each pair of brackets to select the number of cycles for the complete sequence of patterns placed within the brackets. The “Bracket” pattern is fix in the sequence of the stimulus patterns. To change the sequence of the stimulus pattern, the set must be discarded and created again.
The settings in this tool bar influence the complete sequence of the stimulus patterns. Modulate the "Amplitude (%)" and the "Offset (mV)" of the complete pattern via up down boxes.

Clicking the “Loop Stimulus” button enables you to infinitely repeat the defined stimulus pattern.

Load a previous created stimulus pattern, save or delete the pattern. When using marker signals, you have to define which one of the Digital Out ports should show the corresponding TTL signals. Select one of the marker ports from the "Digital Out" drop down menu and connect a TTL signal source to the port.

Download the complete stimulus pattern to the internal MEA2100 stimulator by clicking the icon “Stimulus to the stimulator.”
4.7 Analyzer Settings

In this dialog, the parameters for detecting the waveform of a field potential, please see also "Automated Waveform Detection" and "Detection Parameter", the averaging and signal propagation can be designed. The "Analyzer Settings" dialog shows the "Heartbeat (HB) Detection", "HB Averager" and "2D".

The analyzer settings can set parameters on a local or global setting. Local settings will apply to the selected MEA or a single well on a multi well MEA, whereas global settings apply to all connected MEAs or wells on a multi well MEA.

Note: Differences between local or global settings are indicated by orange background color of the respective input field. Changes in global settings will overwrite local settings.

The first parameter for detection is the "Rise Time". Select the minimum and the maximum of the rise time from the drop down menus. The range involves 100 µs to 2 ms for the minimum rise time, and 200 µs to 5 ms for the maximum rise time.

The rise time refers to the time required for a signal to change from a specified low value to a specified high value. 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. In a heartbeat, the sodium current is detected by the rapid change in the field potential, indicated by the rise time.

For the "Amplitude", a fixed amplitude for detection or a signal dependent amplitude can be selected. The "Fixed Amplitude" involves a voltage range from -10 µV up to -1 mV. Using the "Signal Dependent" amplitude, the waveform detection is more flexible: The detection routine detects the amplitude in relation to the background noise. The amplitude is calculated by the standard deviation of the signal times the chosen factor. It is recommended to use a signal dependent amplitude.
This illustration shows how the Cardio2D routine detects the fast sodium peak by the three parameters field potential change, minimum and maximum rise time. The software scans the recorded data points and checks whether following data points meet the required criteria. For example, when the waveform detection routine arrives at the data point marked by the red line, the following data points meet the amplitude criterion (shaded in gray). The signal reaches this field potential change in the preset range of the rise time (shaded in green). The data point, at which both the FP change and the rise time criteria are met is marked with a red dot. If the signal were smaller than the minimum FP change or faster or slower, it would not be detected. The signal needs to cross the bottom border of the green shaded area.

Any signal component that matches the criteria will be detected. The FP change parameter is the main parameter that should discriminate biological signals from noise. The rise time parameter ensures that biological or artefact signals that meet the FP change criterion but have a shorter or longer rise time than the sodium peak, are not detected.

The following parameters characterize the detected field potential. "Detection Deadtime" defines the time after a previous successful detection during which no detection is performed. This prevents double-detection of a field potential by falsely interpreting potential changes following a rapid sodium current as a separate FP.

The drop down menu "Min/Max Type" defines the type of the minimum or maximum to be detected in a single field potential. "Local" defines the extrema that are closest to the detection timestamp to be selected, whereas "Global" will lead to the lowest minimum / highest maximum reached within the "Min/Max Search" interval. The graph displays the changes in slope for local and global Min/Max Type settings.

The first local minimum will give the maximal slope of the action potential, whereas the global minimum / maximum will detect the absolute minimal / maximal values.
Select the time interval for detection of the minimum or maximum of the field potential "Min/Max Search" from the drop down menu (range from ±1 ms up to ±200 ms).

The "HB timestamp" parameter defines to which attribute of detected field potentials the heartbeat timestamps should refer. It can be set either to the maximum, to the minimum or to the point with the maximal slope of the field potential. This timestamp is visualized in the plot with a black dot and saved in the raw data file.

The "HB Averager" tab allows to display averaged heartbeat cutouts of an electrode and in overlay for several recordings. In this dialog the time before and after a heartbeat can be selected via up down box. Enable the check box "On" to display and save the averaged data traces.

It is possible to display several recordings of an electrode channel in overlay and to average the values. In this dialog the pre and post time before and after a heartbeat can be selected via up down box. Enable the check box "On" to display and save the averaged data traces. If you do not need the average function, disable the check box to save computer performance and disk space.

In the 2D tab the time span for the signal propagation time calculation can be defined via the drop down menu in a range of 1 to 200 ms.
The action potential spreads 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 MEA 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, data for the complete heartbeat will be missed. If the propagation time is too long, the next heartbeat starts, and the results are incorrect. Therefore, the appropriate time must be tested empirically, depending on the experiment.

The "Min Cluster Channels" defines the minimum number of channels at which heartbeats must be detected to form a cluster. For a 60 MEA and a syncytium covering all 60 electrodes, 10 out of 60 channels should be considered for a cluster.

4.8 Recording Data

Start

Click **Start** to start recording. The "Start" icon changes into "Stop" icon, and the duration of recording is counted up in seconds. Stop recording manually by clicking on "Stop" icon.

When using the "External Trigger" feature, the program will go into standby and wait for a start trigger condition to be detected.

Cardio2D generates five types of files:

The "Raw Data" file (*.c2d), the "Settings" file (*.c2s), the "Result" file (*.c2r) and the "Log" file (*.log) will always be created. A fifth "Averager" file (*.c2a) will be created if the "HB Averager" has been activated during the recording.

All files share the same file name, which can be defined in the “Lab Book” dialog.

It is mandatory to save the raw data file and the associated settings file in the same directory. Raw data files can be opened and analyzed in the analyzer software Cardio2D+ only in combination with the appropriate settings file. The same rule applies to result and average files.
4.9 MEA Window

The "MEA Window" is divided into four windowpanes, all showing heartbeats in different characteristics. The settings made in the "MEA Window" influence all four smaller windows respectively. The window changes depending on the connected hardware. If a multiwell MEA is used, each of the wells is displayed and controlled separately in window tabs, accordingly. It is possible to switch between the different MEA window tabs to set different setting parameters in the four views.

The prominent "MEA Electrode" view displays electrode activity of all electrodes in the layout and allows real-time monitoring of field potential data. The electrode channels are arranged in a grid according to the MEA layout. The channel identifier refers to the columns and rows of the MEA layout grid. The drop down menus at the bottom of the window are for scaling the axes. The electrode highlighted in green is the electrode which is displayed in the "Single channel view".

The "Single Channel View" displays a user defined single channel to have a closer view. The "FP" field potential, the "HB" heartbeat and the "Ave" average can be displayed.

The FP plot shows the field potential (µV) over time (s). To scale the axes, use the drop down menus. The range for the field potential is ±1 µV to ±5000 µV, the time can vary between 1 and 60 seconds. Reset the axes by the "Reset" icon. The detected heartbeats are marked with black dots. The detection timestamp can be set to minimum or maximum or maximum slope in the "HB Timestamp" drop down menu in the "Analyzer" dialog. If the single channel view is switched between channels during a measurement, the switch will be indicated by a red marker, displaying the channel number in the single view at that time.
The HB tab shows a diagram of the inter beat intervals (RRI) or beating frequency (HeartBeat), depending on the selection in the drop down menu.

In whole heart experiments the RR interval is defining one complete heartbeat from one ventricular depolarization to the next. 1/RR is the beating frequency of the heart. Any modulation of the beating frequency is an indicator for possible arrhythmia. In cell cultures ECG terminology does not apply. However, the depolarization driven by rapid sodium channels causes an as distinctive peak as the QRS complex in the ECG. Analysis can be performed in strict analogy to the ECG analysis in respect of signal propagation patterns and conduction velocity.

If "Heart Rate" is selected, the rate of the heartbeats is calculated from the reciprocal value of the RR interval. The last actual value of the RRI (ms) or of the heart rate (Hz) is displayed in red above the plot.

The recordings of one user defined electrode channel are displayed in a color coded overlay plot if the average tab “Ave” is selected. Each recording is indicated by a different color. The number of the recording is displayed beside the plot and the number of averaged segments are denoted in the brackets behind.

Specify the length of the averaged segments in “Analyzer Settings” “HB Average” tab. The data displayed is not saved automatically.

The "Latency" window shows the latency of the excitation, calculated between the first and the last detected electrode as a function of the time. The actual value of the latency is displayed above the plot in red letters. The different recording phases are displayed color coded in overlay.
The "Latency" window can be switched to "Velocity" window via drop down menu. The "Velocity" window shows the velocity of the excitation, calculated between the first and the last detected electrode as a function of the time. The actual value of the velocity is displayed above the plot in red letters. The different recording phases are displayed color coded in overlay.

When defining a propagation path in the color map window, the latency or velocity is calculated via the propagation path. The velocity is added up from point to point, that means the result is not zero when defining a circular path. Start a viability test and define the propagation path. Do not change the path later during the experiment. See also chapter "Propagation Path" in "Color Map".

The "Color Map" displays the propagation of each heartbeat across the MEA in false color. The electrodes on the MEA are symbolized by circles. Black circles indicate the detection of a heartbeat on the electrode in the currently displayed cluster, white circles indicate no detection of a heartbeat. The color map is completed by interpolation of data. The timestamp of the first heartbeat detection on a single electrode (latency = 0 µs) is displayed below the map.
In the display above one well of a 256-9well MEA with a 6 x 5 electrode grid is displayed.

The "Color Map Toolbar" contains the icons to copy the color map to the clipboard, to save a picture of the color map in "*.png" format and to start or stop and pause the real-time monitoring.

**Note: Stopping the real-time monitoring saves computer workload!**

Setting a propagation path is possible via the "Propagation Path" icon.

The "Map Settings" allows to define the settings for the color map and opens the tab "Map" in the "Map Settings" dialog.
The optics of the color map can be selected. The usual color map for cardiac topics is “Jet Invers”, but various color settings can be selected. To remove the colors from the map, click “Off” and only isochrones will remain.

The color for the isochrones can be set via the “Contour” drop down menu, which also allows to remove the isochrones. The direction of the propagation of each heartbeat across the electrode field can be displayed by arrows, which are normalized vectors, calculated from the gradient of the field potential.

The “Maximum” and “Minimum” inputs set the scale of the color map and define the time frame, in which the excitation pattern is displayed in the color map. The widest selectable time frame for the color map spans from 0 µs in the minimum to 10000 ms in the maximum. If the Auto feature is enabled, the Cardio2D program automatically finds the minimum and maximum of each heartbeat and adapts the scaling of the color map. It is recommended to use the auto scaling function. The size of the color map can be modified as well.

Interpolation is a mathematical method to calculate missing information in-between two points of a line. The bilinear interpolation uses four points of the nearest vicinity to calculate the interpolated point. The bicubic method uses eight points to calculate the interpolated point. The interpolation method of the “nearest neighbor” uses the mean value of four or five points to calculate the interpolated point.
If the “Show Electrodes” feature is activated, the electrodes are displayed as circles in the color map.

In the “Data” tab of the “Map Settings”, the interpolation can be defined. Here it refers to the data generating the map. Default setting is the interpolation method “Nearest Neighbor” but “Missing Data” or “Missing Channel” can also be chosen for interpolation.

The heartbeat time stamp can be added to the color map using the option “Show Time Stamp”. Different settings are offered for the actual time stamp, which is displayed below the color map. Generally, a time stamp in milliseconds (ms) will be sufficient for displaying heartbeats, for potential propagation the time stamp option hours, minutes, seconds, and microseconds can be used.

A movie of the propagation can be recorded via the “Record Movie” icon. The dialog “Movie Recorder” appears, where the frame rate and file name and location can be selected. The movie will be recorded in "*.avi" format. Recording the color map movie starts as soon as "Start" is acknowledged.
4.10 General Software Features

Tracking Axis

For tracking the axis manually, click on the axis and the mouse pointer will change into a hand. To scroll the axes, please move the mouse with the hand pointer. For zooming the axes, press the CTRL key and keep it pressed while moving the mouse or the wheel of the mouse.

The mouse pointer turns into a double arrow symbol.

A right mouse click on the axes gives four options:

- "Edit",
- "Tracking Enabled",
- "Update Rescue Values",
- "Zoom to Fit".

With the command "Tracking enabled" you can move the axis by moving the mouse or by moving the mouse wheel in horizontal direction on the x-axis, and in vertical direction on the y-axis.

Edit

Click "Edit" and the "Plot Axis Editor" appears. You can adjust general and specific parameters for your plot layout.
Click the “Plot” icon. The following toolbar appears.

<table>
<thead>
<tr>
<th>Icon</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>🔄</td>
<td>Tracking</td>
</tr>
<tr>
<td>🔷</td>
<td>Scroll axes</td>
</tr>
<tr>
<td>🔼</td>
<td>Zoom axes</td>
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<tr>
<td>🔻</td>
<td>Zoom in and zoom out</td>
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<tr>
<td>💡</td>
<td>Select</td>
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<tr>
<td>💡</td>
<td>Zoom box</td>
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<tr>
<td>🔍</td>
<td>Data cursor</td>
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<td>🔍</td>
<td>Edit</td>
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<td>🔍</td>
<td>Copy to Clipboard</td>
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<td>Print</td>
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<td>Preview</td>
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Click “Edit” icon. The following dialog appears. Now it is possible to format the plot.
Main Window: Overview

The main window of Cardio2D+ consists of two windowpanes. The left "Control Window" and the right "MEA Window" with the "Field Potential", "ROI Selection", "Heartbeat", "HB Averager", and the "QT-related Analysis" tabs. Depending on the selected tab, the window shows varying displays of the data. Please refer to the respective chapters. "The "Field Potential Analyzer" visualizes the recordings on the MEA and the analysis of the "Field Potential Duration" is also called "QT-related Interval"."
5.1 Control Window

5.1.1 Recording Control

The control window displays the currently opened raw data and/or result file name(s).

The "Recording Control" displays information about the recorded experiment in a table. To select a recording phase, click on the desired row. Data from the selected electrode (see chapter "Channel Control" below) and selected recording phase will be displayed in the "Single Channel" view.

<table>
<thead>
<tr>
<th>ID</th>
<th>Type</th>
<th>Date</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>T</td>
<td>0 pM</td>
<td>60 s</td>
</tr>
<tr>
<td>1</td>
<td>T</td>
<td>100 nM</td>
<td>60 s</td>
</tr>
<tr>
<td>2</td>
<td>T</td>
<td>1 pM</td>
<td>60 s</td>
</tr>
<tr>
<td>3</td>
<td>T</td>
<td>10 pM</td>
<td>60 s</td>
</tr>
<tr>
<td>4</td>
<td>T</td>
<td>100 pM</td>
<td>60 s</td>
</tr>
</tbody>
</table>

The "Recording Control" window displays information about the recorded experiment in a table. To select a recording phase, click on the desired row. Data from the selected electrode, please see chapter "Channel Control" below and the selected recording phase will be displayed in the single channel view.

5.1.2 Channel Control

The "Channel Control" window shows the MEA electrodes. You can zoom to any electrode by clicking on the electrode. The zoomed channel is displayed in the larger "Single Channel View".

It is possible to define a cluster for cases of cluster formation. Please see also the "2D" tab in "Tools" "Analyze" dialog.

Mark the electrodes belonging to a cluster by using the "Add Channel" icon to select channels. Deselect the other electrodes by clicking the "Remove Channels" icon.

Run the analysis and save the results for each cluster separately.
5.1.3 Settings File

The "Settings File" window displays all information about the currently analyzed Cardio2D result or raw data file in a data tree. It is not possible to change the information in a Cardio2D raw data file other than by re-analyzing it with new parameters and saving the results in a new Cardio2D result file.
5.2 Main Menu

File

The “File” menu allows to open or save result files, to open raw data files and to export results to ASCII format.

Cardio2D generates four types of files that can be reviewed in Cardio2D+: The Raw Data file (*.c2d), the Settings file (*.c2s), the Result file (*.c2r), and the Averager file (*.c2a) if the HB Averager had been activated during the recording. It is mandatory to save the raw data file and the associated settings file in the same directory. The same rule applies to result and averages files.

When opening a raw data file, an associated results file (and, if applicable, averages file) will automatically also be loaded.

It is not possible to change the data of a raw data file recorded with Cardio2D. A raw data file can be re-analyzed with Cardio2D+ Analyzer as often as necessary and the new information can be saved in a result file. When a result file is re-analyzed, no data will be overwritten but a backup file of the old result data is generated (*__backup[number].c2r). For the export of results see chapter "Export of Results".
The “Tools” menu opens the “Analyze Raw Data” dialog. This dialog is similar to the “Analyzer Settings” in the Cardio2D Software. Please see chapter “Analyzer Settings”.

Additionally, the Cardio2D+ program allows the re-analyzing with different settings for the “HB Detection” and “Average” as well as the 2D and QT-related analysis. A new result file will be generated.

In addition to the Cardio2D recording program, the Cardio2D+ Analyzer program allows a QT-related analysis. QT-related analysis is possible only if the heartbeat average is enabled. QT-related analysis will always be performed on the interval from the heartbeat time stamp to the last data point of the average heartbeat. The parameter “Detection Deadtime” defines a time window after the heartbeat time stamp within which no analysis will be performed, to avoid detections on artefacts originating from the sodium peak. Please see chapter “Export of Results”.
Click the "Help" menu to use the online help and to show the "About Cardio2D+" dialog with information about software version. Click "Check for Updates" to see whether your software version is up to date or not.
5.3  Cardio2D+ Field Potential Analyzer

The "Field Potential Analyzer" visualizes the recordings on the MEA.

The "Field Potential" tab is divided in four separate windows, the "Single Channel View", the "MEA Electrode Field", the "Channel Overlay" and the "Color Map".

Single Channel View

The single channel view displays the field potentials of one recording phase selected in the "Recording Control" and of the MEA electrode selected in the "Channel Control".

With the "Zoom Box" icon a region of the data trace can be enlarged, and the default settings can be restored via the "Reset Axes" icon.

With the plot menu it is possible to modify several parameters. The plot menu is accessed by the "Edit Plot" icon.

See also chapter "General Software Features".
The two green bars in the single channel view represent the region of interest ROI, which directly defines the time axes of the displays in the "MEA Electrodes" window and the "Overlay Plot" window. The whole ROI can be moved via drag and drop. By clicking on the middle of one bar near to the zero line the mouse pointer will turn into an arrow and the single bar can be moved, effectively changing the duration of the ROI. The displays in the "MEA Electrodes" window and in the "Overlay Plot" window are adapted to these changes immediately.

**MEA Electrode Field**

Recorded data traces are displayed in the MEA Electrode Field. The y-axis resolution can be adapted via the drop down menu. The "MEA Electrode Field" corresponds with the "Overlay Plot2 window. Toggle electrode selection for the overlay by clicking in any of the electrode displays. The data traces of the MEA electrodes, selected in the MEA electrode field and highlighted in green, are displayed in the overlay plot window in different colors.

**Channel Overlay**

If more than one MEA electrode is selected in the MEA Electrode Field, they are displayed in an overlay plot in the "Channel Overlay" window. The y-axis resolution can be adapted via drop down menu. The red bar marks a timestamp on the currently shown excitation patterns of the "Color Map".

To move the timestamp, select "Snapshot" to move the timestamp bar by hand or step-by-step with the icons "Step Backward" and "Step Forward".

To create and play a movie of the excitation pattern in the "Color Map", switch to the "Movie Mode" via the "Create Movie" icon.
A second red bar appears to limit the time span for the movie. A click on the selected region activates the bars and these can be moved separately. Additionally, the complete region can be moved. Click "Start" to begin playing the movie. A blue arrow in the Channel Overlay displays the progress of the color map movie, and it is possible to pause and stop the movie.

The step size in µs allows adjusting speed and duration of a movie.

---

**Color Map**

The color map of the "Field Potential Analyzer" displays the field potentials (µV) on the MEA electrodes at a timestamp, which is defined in the channel overlay display. It shows the excitation of the cardiac cell culture or tissue. The colors indicate the field potential differences over the MEA at a given timestamp.

The "Color Map" in the Cardio2D+ Analyzer is similar to the recording program Cardio2D. See chapter "Color Map" for map settings, "Movie Recorder" and "Interpolation".
5.4 Cardio2D+ ROI Selection

Via "Region of Interest ROI" one or several parts of the recording sequence can be discarded, which then will be excluded from the analysis. The data in the ROI will not be included in the analysis. It is possible to discard a complete recording sequence by deselecting the respective check box.

The "ROI Selection" tabbed page lists the single recording sequences from one electrode. Select the electrode in the "Channel Control" field of the "Control Window". The y-axis for the recording sequence can be scaled via the drop down box, a region can be zoomed at and reset via the icons at the window bottom.
Single Channel View

To set a "Region of Interest" to eliminate, click into the desired recording sequence and draw a red box by keeping the left mouse button pressed. A click on the selected region activates the bars and these can be moved separately. Additionally, the complete region can be moved. To delete the ROI, click with the right mouse button into the red box and confirm the command "Remove Marker".
5.5 Cardio2D+ Heartbeat

Click "Heartbeat" tab. In the "Heartbeat Analyzer" tab, a single heartbeat can be analyzed at a given time and the latency and velocity is displayed.

The "Field Potential" tab is divided in four separate windows: The "Single channel view", the "MEA Electrode", the "Latency" and the "Color map".

Single Channel View

The single channel view displays the amplitude and the beating rate of all heartbeats on a single electrode, selected in the "Channel Control" during one recording phase, selected in the "Recording Control".

Amplitude and timestamp of each single electrode heartbeat detection are symbolized by black bars.
The “MEA Electrode” window displays the field potential of the electrode selected in the color map. The time resolution can be adjusted from 0 to 200 ms in the control box. Via the plot menu it is possible to modify several plot parameters.

Latency / Velocity

This diagram shows the latency (ms) of the excitation in color coded overlay for each recording. The latency window can be switched to “Velocity” via drop down menu, which shows the velocity of the excitation in cm/s.

Clicking on any data point in the plot can be used to browse through 2D heartbeats. See the following chapter “Color Map” for details.
Color Map

The color map of the "Heartbeat Analyzer" displays the excitation propagation on the MEA as latencies (ms). It summarizes cardiac activity during a short interval. The colors indicate the time lag between heartbeat detections on different electrodes.

There are several options to browse through 2D heartbeats:

- A click anywhere in the “Single Channel View” will select the closest 2D heartbeat, indicated by a blue arrow.
- Each 2D heartbeat and the related color map can be analyzed step by step via the "Previous" and "Next" icons in the “Single Channel View”.
- Using the "Play" feature will run through all heartbeats and can be stopped at the desired position.
- A click on any data point in the plot of the "Latency / Velocity" window will select the respective heartbeat and also switch the selected recording phase if necessary.

The "Color Map" in the "Cardio2D+ Analyzer" is identical to component used in the recording program "Cardio2D". See chapter "MEA Window" "Color Map" for map settings, "Movie Recorder" and "Interpolation".
5.6 Cardio2D+ Heartbeat Averager

Click the "HB Averager" tab to show three separate windows: The "MEA Electrode Field", the "Recording Overlay" and the "Channel Overlay" window.

Important: Please pay attention to the "Analyze" dialog. To use the heartbeat average tool, the check box in the "HB Averager" tab of the "Analyze" dialog in main menu "Tools" must be enabled.
MEA Electrode Field

Recorded data traces are displayed in the "MEA Electrode Field". The y-axis can be scaled via the drop down menu. The electrode field shows the averaged heartbeats of all channels of the currently selected recording. The data traces selected in the "MEA Electrode Field" are displayed in the "Channel Overlay" window in different colors.

Recording Overlay

![Recording Overlay](image)

In the "Recording Overlay" window, the field potentials of several recordings on one single channel are displayed color coded in an overlay plot. The channel is selected via the "Channel Control" on the left side of the "HB Averager" tab. The number of averaged heartbeats is displayed in brackets. The y-axis can be scaled via the right drop down menu. The recording or apply phases, or all phases can be selected for display via the left drop down menu.

The Sodium-peak of the heartbeats can be zoomed via the “Na-peak” icon.

Channel Overlay

![Channel Overlay](image)

In the "Channel Overlay" window the averaged heartbeats of multiple channels are displayed in an overlay plot, the number of heartbeats averaged is displayed in brackets. The channels are selected in the "MEA Electrode Field" and the recording is selected in the "Recording Control".
5.7 Cardio2D+ QT Analysis - Field Potential Duration

The analysis of the "Field Potential Duration" is also called "QT-related interval". The field potential duration corresponds with the action potential duration. This can be correlated to a QT-like interval in an electrocardiogram, which is measured from the minimum of the Na+ peak to the minimum of the IKr current peak.

In Cardio2D, the QT-like interval is measured from the heartbeat timestamp (min, max, or max slope of the sodium peak) to an extremum within the post HB interval as defined in the "HB Averager" settings. The T-related timestamp can be modified subsequently.

The "QT Analysis" tabbed page shows the QT-related intervals for the different recordings and a dose response curve. The channel of interest is selected in the "Channel Control" window.

Note: To use the QT-related Analysis tool, the check box in the "HB Averager" tab of the "Analyze" dialog in main menu "Tools" and the respective check box in the "QT-related Analysis" tab must be enabled.
Select the "Detection Deadtime" in ms in the first box to define the time span in which no detection is performed. Detecting extreme values for the QT-related analysis is achieved by analyzing the slope of the signal.

The "Slope Calculation" of each data point is achieved by linear regression. This linear regression is performed within a time interval around each data point. Increasing the interval will help to reduce noise on the slope.

Select the "Slope Calculation" in ms in the second box to define the time interval, in which the slope of one point of the signal is calculated. Please read chapter "Automated Waveform Detection" in "Analyzer Setting". This feature works like a filter, since the remaining noise is reduced. The resulting slope graph is then analyzed for peaks. To reduce further noise, only peaks with an integral above a certain threshold are considered. The extremum with the greatest amplitude is finally taken as T-like component.

**QT-related Interval**

The QT-related intervals of the selected electrode are listed sequential by concentration, starting with the smallest amount of compound (0 µM), which is by default the reference. The molarity of the compound and the number of detected and averaged heartbeats are displayed in the green rectangle.

The blue bar indicates the end of the calculated QT-like interval. If the level is changed manually, the bar turns red. The duration of the QT-related interval in milliseconds is given beside the bar.
The curve is available in "Normalized" or "Absolute" form on a logarithmic x-axis. In "Normalized" plots the reference is not integrated in the plot. In "Absolute" plots the standard reference of 0 µM is set to 1 µM and displayed in the logarithmic scale.

To deselect a recording for the calculation of the dose response curve, click the green rectangle.

**Data Export to ASCII**

Click the "Plot" icon. Via the plot menu it is possible to modify several parameters. The plot menu is accessed by the "Edit Plot" icon.

See also chapter "General Software Features".

The data displayed in the different windows can be exported to ASCII format by clicking the icon "Save".
5.8 Export of Results

The result files can be exported to ASCII format for further analysis with user defined software via the main menu “File” and “Export”. The export file is organized as a comma separated value file “*.csv”.

The dialog has five tabs, “Heartbeat”, “Heartbeat 2D”, “Averaged Heartbeat”, “Dose-Response” and “Dose-Response 2D”. Select the recording of interest, the designated electrode channel, and the analysis parameters for export in the different tabs and initiate via the “Export” button. In the header of the comma separated value file, all exported parameters are listed. The square brackets show the respective units.
5.9 Troubleshooting

Most problems occur under specific circumstances. In most cases, it is only a minor problem that can be easily avoided or solved. If the problem persists, please contact your local retailer. The highly qualified staff will be glad to help you. Please inform your local retailer if problems not mentioned in this document occur. This helps us to help other users and it helps MCS to optimize the instrument and the documentation.

Please report all issues to Support@multichannelsystems.com. We can only fix things we know about.

Please pay attention to the safety and service information in chapter "Important Safety Advice".

Multi Channel Systems MCS GmbH has put all effort into making the product fully stable and reliable, but like all high performance products, it has to be handled with care.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Solution</th>
</tr>
</thead>
</table>
| Installation or update of Cardio2D/Cardio2D+ breaks. | Please make sure the Microsoft .NET framework 4.6.2 is installed on your computer.  
6 Theoretical Background

Cardiac Action Potential

Over the last 30 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 Cardio2D. 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-like Interval in an electrocardiogram. It is measured from minimum of the Na+ peak to the maximum / minimum of the IKr current peak.

Electrocardiogram

7 Technical Support

Please read the "Troubleshooting" part of the user "Manual" or "Help" first. Most problems are caused by minor handling errors. Contact your local retailer immediately if the cause of the trouble remains unclear. Please understand that information on your hardware and software configuration is necessary to analyze and finally solve the problem you encounter.

If you have any question or if any problem occurs that is not mentioned in this documentation, please contact your local retailer. The highly qualified stuff will be glad to help you.

Please keep information on the following at hand

- Description of the error (the error message text or any other useful information) and of the context in which the error occurred. The more information on the actual situation you can provide, the easier it is to track the problem.
- The serial number of the device. You will find it on the bottom side of the main unit, and the amplifiers.
- The software and hardware version you are currently using. On the "Help" menu, click "About". The displayed dialog box shows the version numbers.
- The operating system and service pack number on the connected computer.
- The hardware configuration (microprocessor, frequency, main memory, hard disk) of the connected computer. This information is especially important if you have modified the computer or installed new hard- or software recently.

MEA Assignment

MCS data acquisition hardware and Cardio2D software enables you to work with multiple MEAs in parallel. In a regular setup, each connector of the data acquisition main unit (for example MEA2100-IFB, USB-ME-System, USB-MEA or MC_Card) collects data from a single MEA. In this case the MEAs are named corresponding to the number of the connector on the data acquisition main unit they are attached to. Some data acquisition systems however allow to attach two MEAs per connector:

1. The MEA2100-HS2x60 supports two 60 channel MEAs in parallel on a single MEA2100-IFB headstage connector.
2. Using the signal collector SC2x32 allows the operation of two FlexMEA36 per 68-pin MCS standard connector on MC_Card and USB-ME-Systems with 64, 128 or 256 channels.

MEA2100-System

The MEAs are labeled according to the number of the headstage and their position on the headstage in a constant order. Place the MEA2100 headstage(s) with the articulation facing backwards. When working with two MEA2100 headstages connected to the MEA2100 interface board, the left headstage is defined to be headstage 1 and the right headstage to be headstage 2. The MEAs are then numbered from left to right with increasing numbers. As an example, please see the picture above with two MEA2100-HS2x60.
**USB-ME-System or MC_Card**

Please see the datasheet SC2x32

<table>
<thead>
<tr>
<th>Signal Collector SC2x32</th>
<th>68-pin MCS standard Connector 1</th>
<th>68-pin MCS standard Connector 2</th>
<th>68-pin MCS standard Connector 3</th>
<th>68-pin MCS standard Connector 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connector 1</td>
<td>MEA_1</td>
<td>MEA_3</td>
<td>MEA_5</td>
<td>MEA_7</td>
</tr>
<tr>
<td>Connector 2</td>
<td>MEA_2</td>
<td>MEA_4</td>
<td>MEA_6</td>
<td>MEA_8</td>
</tr>
</tbody>
</table>
Datasheet Signal Collector SC2x32

SC2x32

Use and Operation

The SC2x32 is a signal collector for up to two MPA32I preamplifiers, connected via 37-pin D-SUB connectors. The 2 x 32 input channels are collected, and can be connected to the following amplifier via 68-pin MCS high grade cable. If only one MPA32I is connected, please connect a termination plug to the unused input to prevent it from picking up noise.

The SC2x32 includes a 0.08 Hz high pass filter for removing DC offsets.

Channel Layout

Note:

When using two EcoFlexMEA36 together with the software Cardio2D, please connect the first EcoFlexMEA, labelled MEA 1 in Cardio2D to channel 1 - 32 and the second EcoFlexMEA, labelled MEA 2 in Cardio2D to channel 33 - 64.

68-pin MCS-standard output
**Input**  
D-SUB 37 Connectors

<table>
<thead>
<tr>
<th>Pin</th>
<th>Description</th>
<th>Pin</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GND (ground)</td>
<td>29</td>
<td>Channel 18</td>
</tr>
<tr>
<td>20</td>
<td>REFERENCE INPUT</td>
<td>11</td>
<td>Channel 19</td>
</tr>
<tr>
<td>2</td>
<td>Channel 1</td>
<td>30</td>
<td>Channel 20</td>
</tr>
<tr>
<td>21</td>
<td>Channel 2</td>
<td>12</td>
<td>Channel 21</td>
</tr>
<tr>
<td>3</td>
<td>Channel 3</td>
<td>31</td>
<td>Channel 22</td>
</tr>
<tr>
<td>22</td>
<td>Channel 4</td>
<td>13</td>
<td>Channel 23</td>
</tr>
<tr>
<td>4</td>
<td>Channel 5</td>
<td>32</td>
<td>Channel 24</td>
</tr>
<tr>
<td>23</td>
<td>Channel 6</td>
<td>14</td>
<td>Channel 25</td>
</tr>
<tr>
<td>5</td>
<td>Channel 7</td>
<td>33</td>
<td>Channel 26</td>
</tr>
<tr>
<td>24</td>
<td>Channel 8</td>
<td>15</td>
<td>Channel 27</td>
</tr>
<tr>
<td>6</td>
<td>Channel 9</td>
<td>34</td>
<td>Channel 28</td>
</tr>
<tr>
<td>25</td>
<td>Channel 10</td>
<td>16</td>
<td>Channel 29</td>
</tr>
<tr>
<td>7</td>
<td>Channel 11</td>
<td>35</td>
<td>Channel 30</td>
</tr>
<tr>
<td>26</td>
<td>Channel 12</td>
<td>17</td>
<td>Channel 31</td>
</tr>
<tr>
<td>8</td>
<td>Channel 13</td>
<td>36</td>
<td>Channel 32</td>
</tr>
<tr>
<td>27</td>
<td>Channel 14</td>
<td>18</td>
<td>GND (ground)</td>
</tr>
<tr>
<td>9</td>
<td>Channel 15</td>
<td>37</td>
<td>Positive supply voltage</td>
</tr>
<tr>
<td>28</td>
<td>Channel 16</td>
<td>19</td>
<td>Negative supply voltage</td>
</tr>
<tr>
<td>10</td>
<td>Channel 17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Output**  
68-pin MCS-standard connector

<table>
<thead>
<tr>
<th>Pin</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GND (ground)</td>
</tr>
<tr>
<td>2</td>
<td>REFERENCE OUTPUT</td>
</tr>
<tr>
<td>3 - 66</td>
<td>Channel 1 - 64</td>
</tr>
<tr>
<td>67</td>
<td>Positive supply voltage</td>
</tr>
<tr>
<td>68</td>
<td>Negative supply voltage</td>
</tr>
</tbody>
</table>
8 Contact Information

Local retailer
Please see the list of official MCS distributors on the MCS web site.

Newsletter
If you have subscribed to the Newsletter, you will be automatically informed about new software releases, upcoming events, and other news on the product line. You can subscribe to the list on the contact form of the MCS web site.

www.multichannelsystems.com
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