LTP-Director Manual

Imprint

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1 Introduction

1.1 About this Manual

This manual comprises all important information about the first installation of the software LTP-Director, and about the daily work with the program. It is assumed that you have already a basic understanding of technical and software terms. No special skills are required to read this manual.

If you are using the software and the device for the first time, please read the **important safety advice before installing the hardware** and **software**, where you will find important information about the installation and first steps.

The **printed manual** and **Help** are basically the same, so it is up to you which one you will use. The help offers you the advantage of scrolling through the text in a non-linear fashion, picking up all information you need, especially if you use the **Index**, and the **Search** function. If you are going to read larger text passages, however, you may prefer the printed manual.

The device and the software are part of an ongoing developmental process. Please understand that the provided documentation is not always up to date. The latest information can be found in the **Help**. Check also the Multi Channel Systems MCS GmbH web site (www.multichannelsystems.com) for downloading up-to-date manuals and help files.

2 Warnings

2.1 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.

2.2 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.

2.3 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.

2.4 Terms of Use for the program

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

2.5 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 Configuration

3.1 Hardware Configuration

For setting up an experiment with LTP-Director program you have three hardware configuration options:

- Using a **MEA2100-System** consisting of interface board and different configurations of headstages with integrated data acquisition, amplifier, and stimulus generator.
- Using a **data acquisition system** (USB-ME64- / 128- / 256-System or MC_Card), a **MEA1060-BC amplifier,** and a stimulus generator **STG** of the 2000 or 4000 series.
- Using an **USB-MEA32-STIM4-System** with integrated data acquisition, amplifier, and stimulus generator.

Certain hardware configurations provide the possibility to use the LTP-Director software with multiple instances on a single data acquisition computer to run more than one amplifier simultaneously, for example the MEA2100-System with two headstages, or an USB-ME256-System with four MEA1060-BC amplifiers. You can control each amplifier with one software instance completely independently, that means, you can run up to four independent experiments in parallel.

If no hardware is connected, the LTP-Director starts in simulation mode.

The following hardware configurations are supported and can be selected in the "Hardware Settings" of the LTP-Director:

Configu-ration	**Data Acquisition Device**	**Number of Amplifiers and Instances of LTP-Director**	**Amplifier**	**Stimulus Generator STG**
Simulation	-	-	-	-
MEA2100	MEA2100 Headstage	1 – 4	integrated amplifier	integrated STG
MEA60	MC_Card	1	MEA1060-BC	STG 2000 or 4000 series
ME64	USB-ME64-System	1	MEA1060-BC	STG 4002 / 4004 / 4008
ME128	USB-ME128-System	1 - 2	MEA1060-BC	STG4004 / 4008
ME256	USB-ME256-System	1 - 4	MEA1060-BC	STG 4008
ME32	USB-MEA32-STIM4	1 - 4	integrated amplifier	integrated STG

3.2 MEA2100-System

The **MEA2100-System** consists of two main devices: The interface board with integrated high-capacity digital signal processor DSP and the headstage equipped with amplifier, A / D converter and stimulus generator. It is possible to connect one headstage only or two headstages to one interface board. The **MEA2100-32-System** is a descendant of the MEA2100-System with all functions except of the real-time feedback via DSP.

Five different types of headstages are available for the **MEA2100-System**. A headstage for microelectrode arrays (MEAs) with 32 electrodes (MEA2100-HS32), a headstage for 60-electrode MEAs (MEA2100-HS60), and a headstage for MEAs with 120 electrodes (MEA2100-HS120). Additionally, headstages for two microelectrode arrays with 32 electrodes (MEA2100-HS2x32) and with 60 electrodes (MEA2100-HS2x60) are available.

If you are working with two headstages, each headstage can be operated independently by opening two instances of the LTP-Director software for each headstage separately. When using two headstages with two 60-electrode MEAs or two 32-electrode MEAs, please open four instances of LTP-Director software for independent control of each MEA.

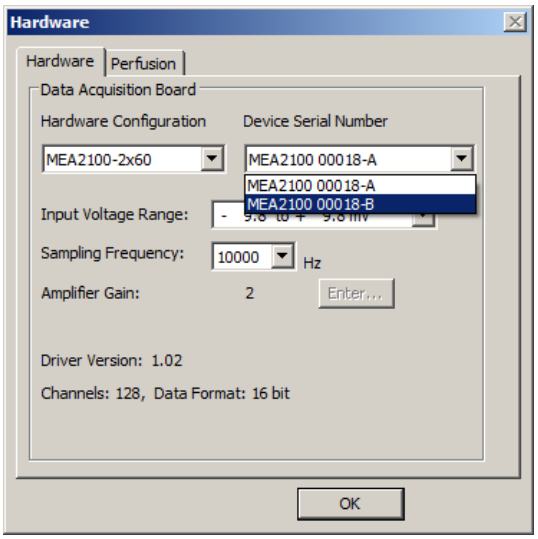
Software Settings

Using the MEA2100-System you are able to control up to two headstages with up to four MEAs with up to four instances of LTP-Director independently from a single computer. When starting more than one instance of LTP-Director in parallel, be aware that the MEA2100 headstage is still a single hardware component only. That is why the first instance of LTP-Director is acting like the master of all following instances for some hardware dependent settings. You can change the sampling frequency and the voltage input range in the hardware dialog of the main menu "Settings" in the first instance of LTP-Director only. The settings are saved in the "*.ini" file, and used for the following instances, respectively. The settings in “Hardware” tab are disabled in LTP-Director instances 2, 3 and 4. If you like to change hardware settings, please close all running instances of LTP-Director, and start the first instance again. All other settings can be adjusted for each amplifier independently in the assigned LTP-Director instance.

When connecting two headstages to the MEA2100-System, the first USB port is assigned to the MEA2100 with “A” in the serial number of the device and the second USB port is assigned to serial number “B”.

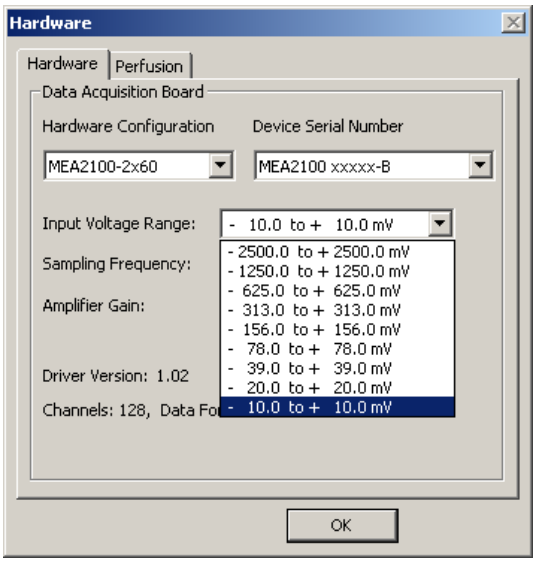
Using a headstage with 120-electrodes MEA (MEA2100-HS120), please open only one instance of LTP-Director. Using a headstage with two 32- or 60-electrodes MEAs (MEA2100-HS32 or MEA2100-S2x60), please open two instances of the software. The first instance will control the MEA on the left side of the headstage, when looking at the headstage with the articulation in the back. The second instance controls the MEA on the right side. Respectively when using two MEA2100-HS2x32 or MEA2100-HS2x60 headstages on headstage “B” the left MEA will be controlled by the third instance and the right MEA by the fourth instance of LTP-Director.

Click “Hardware” tab in main menu "Settings" in the first instance of LTP-Director and select the MEA2100. You can adjust the input voltage range and the sampling frequency for both connected headstages and each of the 60-electrodes MEA.



In MEA2100-Systems the “Amplifier Gain” is automatically set, depending on the version of the hardware.

Please select the “Input Voltage Range” and the “Sampling Frequency” according to the requirements of your experiment. For slice recordings, a sampling frequency of 10 kHz and an input voltage range of ± 10 mV is usually suitable.



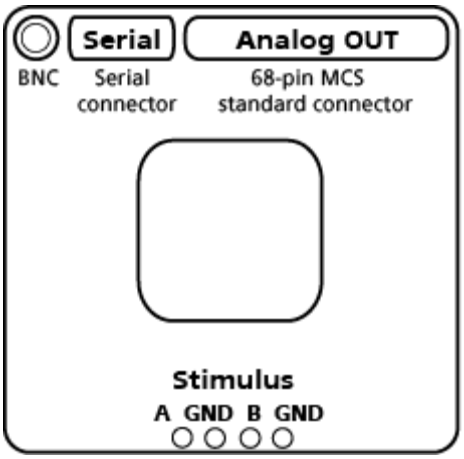
3.3 MEA1060-BC Amplifier

For LTP experiments it is recommended to use a **MEA1060-BC amplifier** with blanking circuit BC.A blanking signal (TTL pulse) transiently switches off the input stage of the amplifier during the stimulus, thus avoiding the stimulus artifacts on non-stimulating electrodes. Amplifier saturation is effectively prevented and the recovery time is greatly reduced. It is also possible to record from stimulating electrode shortly after stimulation. To set up and connect the MEA1060 amplifiers, please refer to the amplifier manuals.

Warning: If you want to use LTP-Director with a MEA1060-BC amplifier and a STG you need to connect the TTL outputs in a different way than while working with MC_Rack and MC_Stimulus. Please check your TTL connectors when switching between the two software packages.

Currently, the LTP-Director can control only voltage pulses. Please connect the MEA amplifier to the +V or -V outputs of the STG (2000 series) for voltage stimuli. Using a STG of the 4000 series you just have to connect it to the MEA amplifier.

The image schematically depicts the connectors of the MEA1060-BC amplifier. The shown nomenclature will be used later in the text.

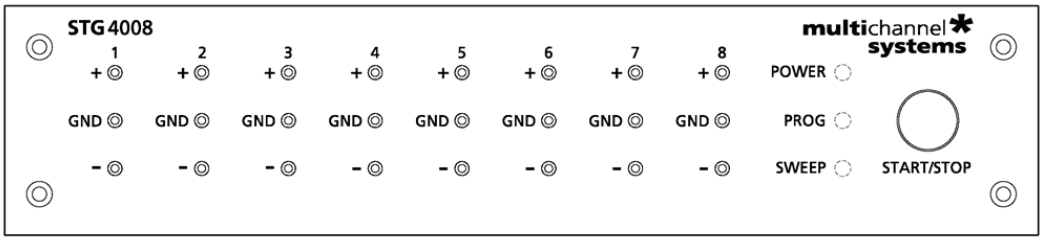


3.3.1 Stimulus Generator STG

Two series of stimulus generators are available from Multi Channel Systems: **STG** stimulus generators of the 2000 and 4000 series. They are general purpose stimulators designed to serve a very wide variety of applications. A stimulus generator is one of the requirements for using the software, because the LTP-Director displays triggered sweeps of 100 ms length. To work with the LTP-Director software, it is necessary to have a software controlled stimulus generator from MCS with USB connection. You can use the STG 2000 or the STG 4000 series. Please see chapter "Setting up an USB-ME256-System with multiple instances of LTP-Director".

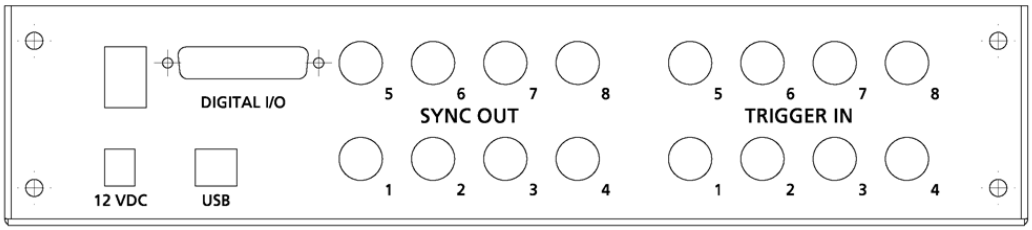
Currently, the LTP-Director can control only voltage pulses. Please connect the MEA amplifier to the +V or -V outputs of the STG (2000 series) for voltage stimuli. A stimulus generator of the 4000 series uses the same outputs for voltage and current.

The image schematically depicts the connectors of the STG. The shown nomenclature will be used later in the text.

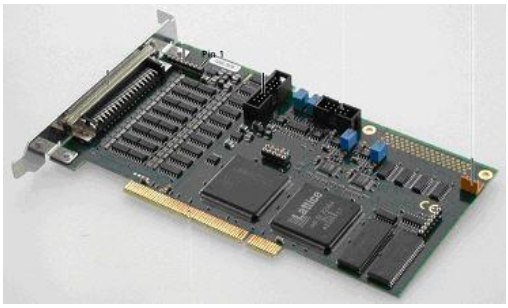


Front Panel

Rear Panel



3.3.2 Data Acquisition: MC_Card



The **MC_Card** is the data acquisition card of MEA- and ME-Systems from Multi Channel Systems MCS GmbH. Please see also the separate documentation of the systems and its components for more information about the setup and operation.

While working with LTP-Director and a MEA1060-BC amplifier and a STG, **you need a BNC connection between the Sync OUT 1 of the STG and Digital In Bit 0 of the MC_Card**: The LTP-Director software uses the STG to deliver a TTL-Pulse from Sync OUT 1 to the MC_Card, that starts recording 10 ms prior to the actual stimulus. A second TTL-Pulse must be delivered from Sync OUT 2 of the STG simultaneously to the stimulus, to trigger the blanking and to switch the stimulation electrodes. Hence, **the Sync OUT 2 of the STG must be connected to the BNC input of the BC amplifier**.

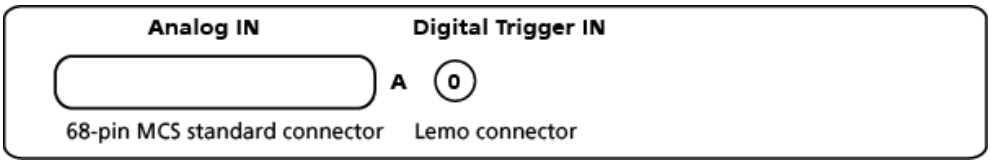
Warning: While using a MC_Card, either with 64 or 128 channels, you can use only a single instance of LTP-Director.

3.3.3 Data Acquisition: USB-ME64- / 128- / 256-System

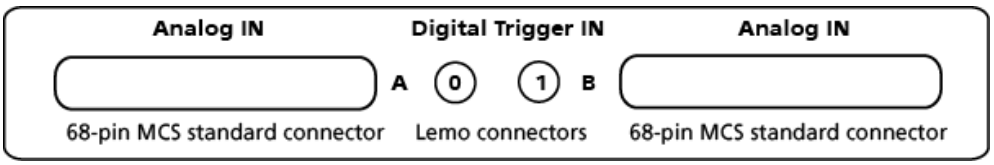
The USB-ME-Systems are stand-alone solutions for acquiring data from 64, 128 or 256 channels. The hardware replaces the internal data acquisition board MC_Card. The analog input signals are acquired and digitized by the USB-ME-Systems, and the digitized signals are transmitted to the connected computer via universal serial bus (High Speed USB 2.0). Thus, it is possible to use any computer for data processing.

The images schematically depict the connectors of the USB-ME-Data Acquisition devices. The shown nomenclature will be used later in the text.

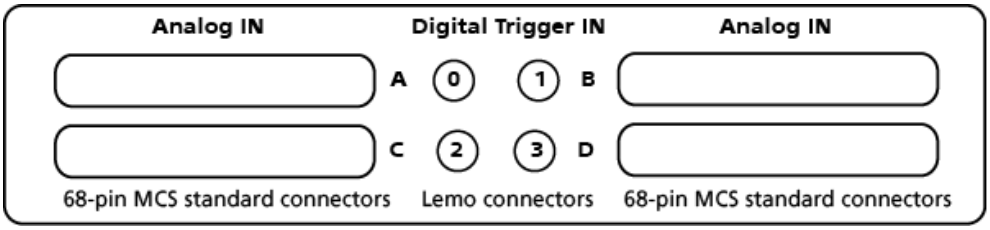
USB-ME64-System



USB-ME128-System

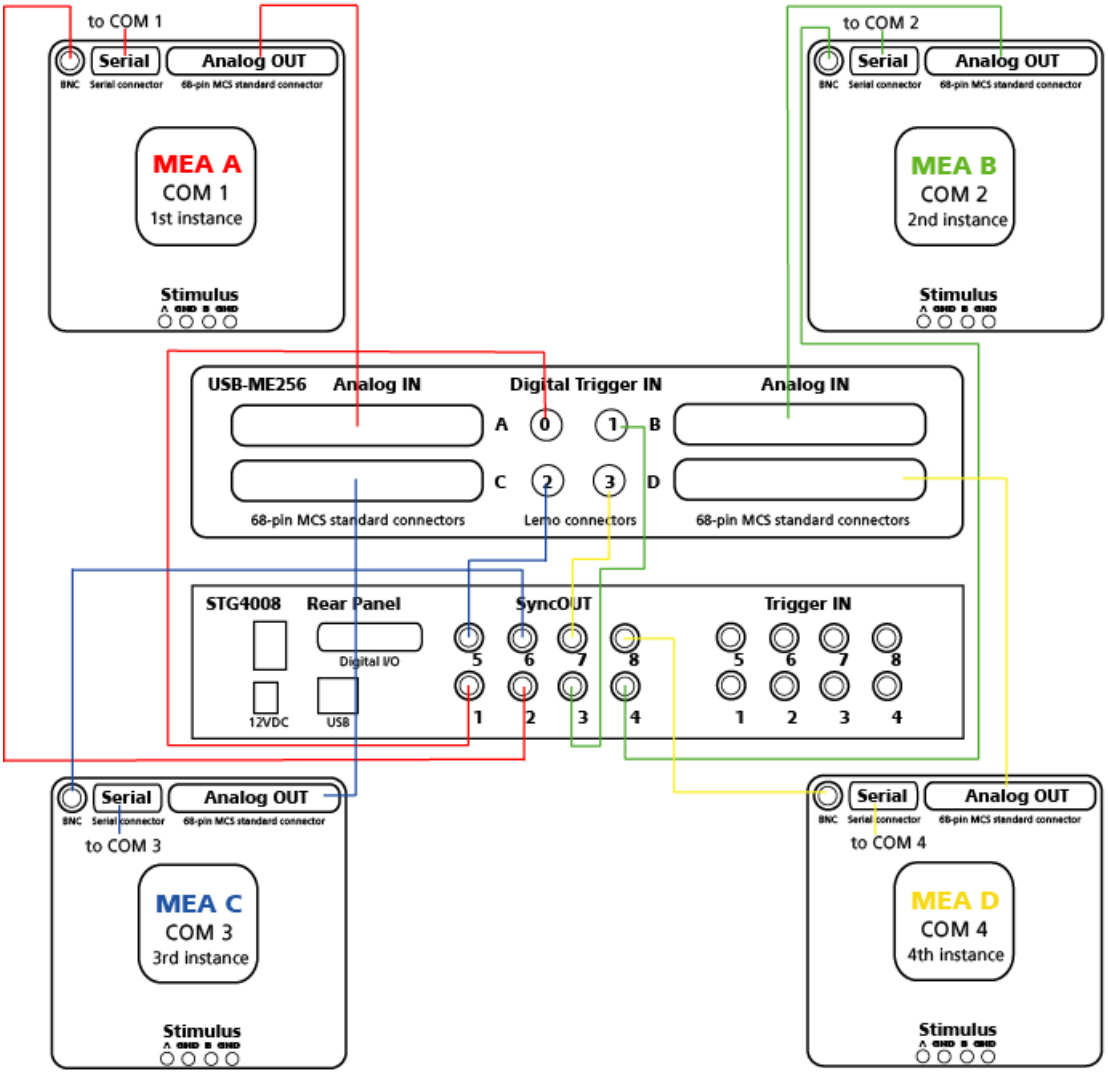


USB-ME256-System



Setting up an USB-ME256-System with multiple instances of LTP-Director

The scheme below shows the cable connections for setting up an USB-ME256 data acquisition device with four MEA1060-BC amplifiers and one STG4008. When using a USB-ME128 and only two amplifiers, the connections are similar. With the shown hardware configuration, you can then use multiple instances of LTP-Director on the same computer to control each amplifier as a completely independent unit. Please make sure to have four LTP-Director licenses to operate the USB-ME256 data acquisition device with four MEA1060-BC amplifiers. Please see also chapter "Installing the LTP-Director Software".



Important: The filter amplifier (FA), which must be located between the MEA1060-BC headstage and the USB-ME256 Analog In, is not shown in the scheme or the tables!

Important: After setting up the complete system with multiple instances of LTP-Director, please check the gain settings of each single instance! May be the gain setting (1100) is correct for the first instance, but incorrect for the following instances, when starting the system for the first time.

The following tables show the same information as the scheme.

Table: Connections for data transfer and amplifier control between USB-ME256 devices, MEA1060-BC amplifiers and data acquisition computer. Please note that each instance of the LTP Director **must** be assigned to a **specific data input**.

Device / Connector Type	Cable	Device / Connector Type
MEA1060-BC A / Analog OUT	68-pin MCS high grade	USB-ME256 / Analog IN A
MEA1060-BC B / Analog OUT	68-pin MCS high grade	USB-ME256 / Analog IN B
MEA1060-BC C / Analog OUT	68-pin MCS high grade	USB-ME256 / Analog IN C
MEA1060-BC D / Analog OUT	68-pin MCS high grade	USB-ME256 / Analog IN D
MEA1060-BC A / Serial	Serial	Serial to USB Converter / COM 1 + First Instance of LTP-Director
MEA1060-BC B / Serial	Serial	Serial to USB Converter / COM 2 + Second Instance of LTP-Director
MEA1060-BC C / Serial	Serial	Serial to USB Converter / COM 3 + Third Instance of LTP-Director
MEA1060-BC D / Serial	Serial	Serial to USB Converter / COM 4 + Forth Instance of LTP-Director
Computer / USB port	USB	Serial to USB Converter / USB port
Computer / USB port	USB	USB-ME256 / USB port

1. Connect the Analog IN A socket (68-pin MCS standard connector) of the USB-ME256 device to the Analog OUT socket (68-pin MCS standard connector) of the first MEA1060-BC amplifier via filter amplifier FA (not listed). This connection defines the MEA1060-BC amplifier as MEA A. The connection from Analog IN B of the USB-ME256 device to the Analog OUT of the next MEA1060-BC amplifier defines the amplifier as MEA B, and so on.

2. Connect the Serial sockets of the MEA1060-BC amplifiers (serial cables) via Serial to USB converter to the COM ports of the data acquisition computer. Please connect the MEA A amplifier to COM 1 of the computer, MEA B to COM 2, MEA C to COM 3, and MEA D to COM 4.

3. The COM ports of the computer define the instances of the LTP-Director:

   Important: Please make sure to connect MEA1060-BC amplifier A to COM 1 of the computer and start the first instance of LTP-Director. Connect MEA1060-BC amplifier B to COM 2 of the computer and start the second instance of LTP-Director. Connect MEA1060-BC amplifier C to COM 3 of the computer and start the third instance of LTP-Director. Connect MEA1060-BC amplifier C to COM 4 of the computer and start the fourth instance of LTP-Director.

Table: Connections for TTL signals between USB-ME256 device, MEA1060-BC amplifiers and stimulus generator STG.

Device / Connector Type	Cable	Device / Connector Type
STG 4008 / Sync OUT 1	BNC - Lemo	USB-ME256 / Digital Trigger IN 0
STG 4008 / Sync OUT 2	BNC - BNC	MEA1060-BC A / BNC
STG 4008 / Sync OUT 3	BNC - Lemo	USB-ME256 / Digital Trigger IN 1
STG 4008 / Sync OUT 4	BNC - BNC	MEA1060-BC B / BNC
STG 4008 / Sync OUT 5	BNC - Lemo	USB-ME256 / Digital Trigger IN 2
STG 4008 / Sync OUT 6	BNC - BNC	MEA1060-BC C / BNC
STG 4008 / Sync OUT 7	BNC - Lemo	USB-ME256 / Digital Trigger IN 3
STG 4008 / Sync OUT 8	BNC - BNC	MEA1060-BC D / BNC

4. Connect the Digital Trigger IN sockets (lemo sockets) of the USB-ME256 device to the Sync OUT (BNC sockets) of the STG (Lemo - BNC cable). Digital Trigger IN 0 of the USB-ME256 device must be connected to Sync OUT 1 of the STG, Digital Trigger IN 1 to Sync OUT 3, Digital Trigger IN 2 to Sync OUT 5, and Digital Trigger IN 3 to Sync OUT 7.

5. Connect the BNC sockets of the MEA1060-BC amplifiers to the Sync OUT (BNC sockets) of the STG (BNC - BNC cables). Please connect the BNC socket of the MEA1060-BC A amplifier to the Sync OUT 2 of the STG, MEA B amplifier to the Sync OUT 4, MEA C to Sync OUT 6, and MEA D to Sync OUT 8.

Table: Connections for stimulation pulses between MEA1060-BC amplifier and stimulus generator STG for stimulating MEA electrodes on pathway 1 and 2 with LTP-Director. Please connect additional grounding cables (2 mm plugs cable black) to the STG Stimulus GND connectors.

Device / Connector Type	Cable	Device / Connector Type
STG / Stimulus OUT 1 +	2 mm plugs cable blue	MEA1060-BC A / Stimulus A
STG / Stimulus OUT 2 +	2 mm plugs cable red	MEA1060-BC A / Stimulus B
STG / Stimulus OUT 3 +	2 mm plugs cable blue	MEA1060-BC B / Stimulus A
STG / Stimulus OUT 4 +	2 mm plugs cable red	MEA1060-BC B / Stimulus B
STG / Stimulus OUT 5 +	2 mm plugs cable blue	MEA1060-BC C / Stimulus A
STG / Stimulus OUT 6 +	2 mm plugs cable red	MEA1060-BC C / Stimulus B
STG / Stimulus OUT 7 +	2 mm plugs cable blue	MEA1060-BC D / Stimulus A
STG / Stimulus OUT 8 +	2 mm plugs cable red	MEA1060-BC D / Stimulus B

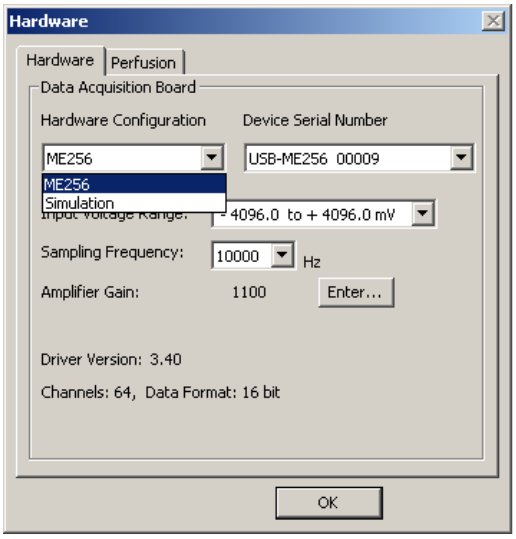
6. Connect the Stimulus A and B plugs (2 mm) of the MEA1060-BC amplifiers to the Stimulus OUT plugs (2 mm) on the front panel of the STG. Additionally you have to connect the Ground GND plugs (2 mm) of the MEA1060-BC amplifiers to the STG (not listed). Please connect the Stimulus A plug of MEA A amplifier to Stimulus OUT 1 of the STG, and Stimulus B of MEA A to Stimulus OUT 2 of the STG. Connect the Stimulus A plug of MEA B amplifier to Stimulus OUT 3 of the STG, and Stimulus B of MEA B to Stimulus OUT 4 of the STG. Connect the Stimulus A plug of MEA C amplifier to Stimulus OUT 5 of the STG, and Stimulus B of MEA C to Stimulus OUT 6 of the STG. Connect the Stimulus A plug of MEA D amplifier to Stimulus OUT 7 of the STG, and Stimulus B of MEA D to Stimulus OUT 8 of the STG.

Note: It is not possible to connect, for example, two STG4002 for running two instances of LTP-Director with an USB-ME128 device. Respectively, it is not possible to connect more than one STG for running four instances of LTP-Director with an USB-ME256 device.

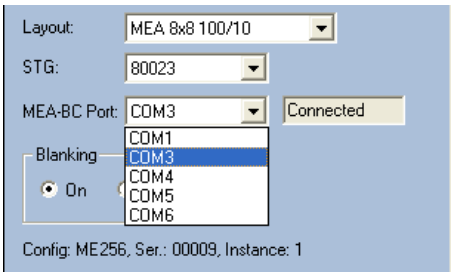
Software Settings

With the USB-ME256-System you are able to control up to four MEA1060-BC amplifiers with up to four instances of LTP-Director independently from a single computer. When starting more than one instance of LTP-Director in parallel, be aware that the USB-ME256 device is still a single hardware component only. That is why the first instance of LTP-Director is acting like the master of all following instances for some hardware dependent settings. You can change the sampling frequency and the voltage input range in the hardware dialog of the main menu "Settings" in the first instance of LTP-Director only. The settings are saved in the "*.ini" file, and used for the following instances, respectively. The settings in Hardware tab are disabled in LTP-Director instances 2, 3 and 4. If you like to change hardware settings, please close all running instances of LTP-Director, and start the first instance again. All other settings can be adjusted for each amplifier independently in the assigned LTP-Director instance.

Click Hardware tab in main menu "Settings" in the first instance of LTP-Director and select the ME256. You can adjust the input voltage range and the sampling frequency for all connected amplifiers.

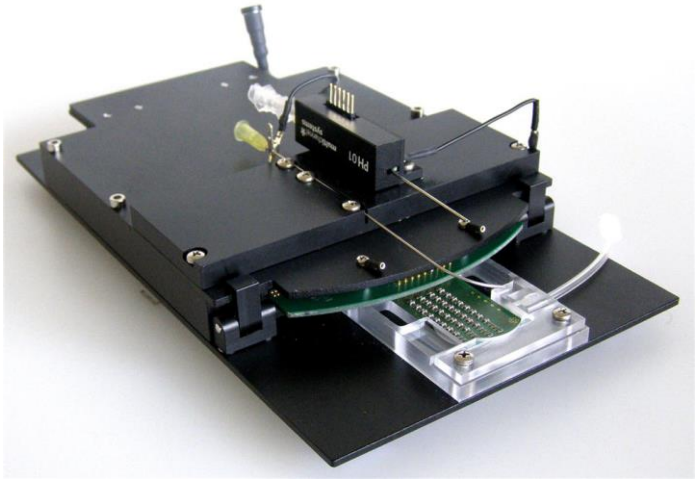


In the Control Window of the Test Mode, the hardware configuration and the number of the instance are displayed. Select the correct computer COM port for that instance (see table), the correct STG4008, and the MEA layout from the drop down menus.



Important: After setting up the complete system with multiple instances of LTP-Director, please check the gain settings of each single instance! May be the gain setting (1100) is correct for the first instance, but incorrect for the following instances, when starting the system for the first time.

3.4 USB-MEA32-STIM4-System



The USB-MEA32-STIM4 is a compact and portable stand-alone solution for extracellular recordings with integrated data acquisition, amplification, analog / digital conversion, and a four-channel current stimulus generator. Perfusion heating and the possibility to apply suction through the perforated MEAs are also included. The system acquires data from up to 32 electrode 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 data acquisition computer, also a laptop.

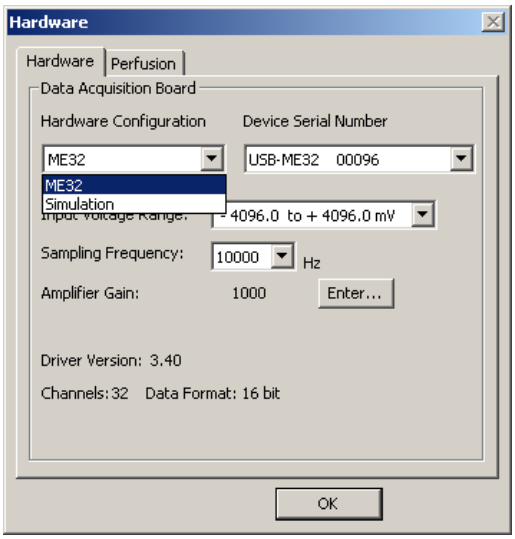
Setting up an USB-MEA32-STIM4-System

Up to four systems can be controlled independently from a single computer, just connect the USB-MEA32-STIM4 devices to the USB ports of the data acquisition computer.

Note: It is not recommended to use an USB hub for connecting several USB-MEA32-STIM4 devices to the USB ports of the data acquisition computer.

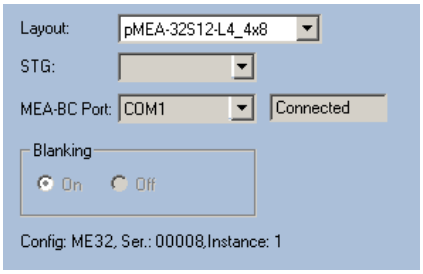
Software Settings

Start up to four instances of LTP-Director and configure the settings independent of each other. Click Hardware tab in main menu "Settings".



You can select all hardware settings in the hardware dialog for each instance separately. The serial number defines which device is controlled by which LTP-Director instance. The LTP-Director software assigns the settings of the different instances to the connected device. Thus, you can run completely independent experiments with up to four USB-MEA32-STIM4 amplifiers.

In the Control Window of the Test Mode the hardware configuration and the number of the current instance are displayed. Select the correct MEA layout from the drop down menus. When working with USB-MEA32-STIM4-Systems, the drop down menus for the selection of the STG and the MEA-BC Port have no function and are disabled because of the internal amplifier and the internal stimulus generator.



The LTP-Director software supports typically voltage driven stimulation. With USB-MEA32-STIM4-System the current driven stimulation of the integrated four-channel current stimulator is supported as well.

Important: After setting up the complete system with multiple instances of LTP-Director, please check the gain settings of each single instance! May be the gain setting (1000) is correct for the first instance, but incorrect for the following instances, when starting the system for the first time.

3.5 Installing the Software

The data acquisition computer with the MC_Card comes preinstalled and preconfigured by Multi Channel Systems MCS GmbH 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 LTP-Director may occur.

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. Especially 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.

When operating a MEA2100-System, an USB-ME64- / 128- / 256-System or an USB-MEA32-STIM4-System you can connect the devices to any data acquisition computer. Make sure that the recommended system requirements are fulfilled.

3.5.1 System requirements

Software: One of the following Microsoft Windows ® operating systems is required: **Windows 7** (32 or 64 Bit), **XP**, or **Vista** (**English** and **German** versions supported) with the NT file system. Other language versions may lead to software errors.

Hardware: (Not required for LTP-Analyzer or demo mode)

Recommended data acquisition systems: **MEA2100-System, MEA2100-32-System, USB-MEA32-STIM4-System, USB-ME64- / 128- / 256-Systems,** and **MC_Card.**

If no hardware is present, LTP-Director opens in demo mode.

A computer with low performance may lead to performance limits more often; therefore, MCS recommends an up-to-date computer. Please note that there are sometimes hardware incompatibilities of the data acquisition system and computer components; or that an inappropriate computer power supply may lead to artifact signals. Please contact your local retailer for more information on recommended computer hardware.

Important: You need to have installed the latest driver to operate the data acquisition system, which is automatically installed with LTP-Director. The installation may be invalid if the data acquisition system does not respond. Please contact Multi Channel Systems or your local retailer in this case.

3.5.2 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. These routines were designed for use on office computers, but are not very useful for a data acquisition computer.

- Turn off Screensaver.
- Turn off Windows Indexing Service for all local disks.
- Turn off Windows System Restore.
- Turn off Optimize hard disk when idle (automatic disk fragmentation).
- Manual management of automatic Windows Update.
- Power Options: Power scheme: Never turn off monitor, hard disk and system standby.
- It is also not recommended to run any applications in the background when using LTP-Director.
- Remove all applications from the Autostart folder.
- Be careful when using a Virus Scanner. These programs are known to disturb LTP-Director, and even data loss may occur.

  When using the MEA2100-System or an USB-ME-System or an USB-MEA-System it is recommended to connect a high performance computer with a separate hard disc for program files and data storage. The provided possibility to use up to 256 channels with a sample rate of up to 40 kHz needs high memory capacity. Please remove data and defragment the hard disc regularly to ensure optimal performance. The computer display must have a resolution of 960 pixel in y-direction.

3.5.3 Installing the LTP-Director Software

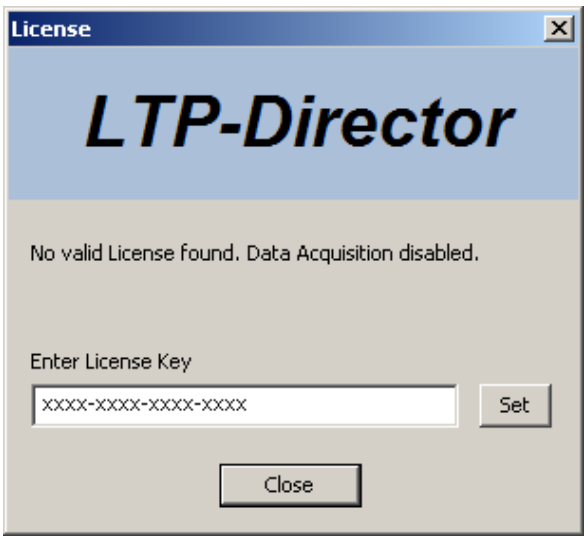
Important: Please make sure that you have full control over your computer as an administrator for software installation. Otherwise, it is possible that the installed software does not work properly. For using the software it is not necessary to have administrator control.

1. Double-click the **Setup**.exe on the installation volume. The installation assistant will show up and guide you through the installation procedure.

2. Follow the instructions of the installation assistant. The MC_Card driver, the USB-ME device drivers, the STG driver and LTP-Director are installed (or updated) automatically.

Make sure to have a valid license for the LTP-Director software! Please ask license@multichannelsystems.com.

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



The LTP-Director software license is bound to one dedicated hardware device. The license key will be saved on the device. The license key does not unlock the software – as usual – but the hardware. This way it is possible to connect the device to different data acquisition computers.

If you connect, for example, four USB-MEA32-STIM4 devices to one data acquisition computer you need to have four license keys, for each USB-MEA32-STIM4 device a separate key. Start the first instance of LTP-Director and connect the first USB-MEA32-STIM4 device via port settings from the drop down menu. Type in the first license key. Start the second LTP-Director instance and connect the second USB-MEA32-STIM4 via port settings. Type in the second license key. Repeat these steps for the third and the fourth LTP-Director instance and connect the third and the fourth USB-MEA32-STIM4 device.

If you connect, for example, an USB-ME256 data acquisition you need only one key which will be saved on the device. But you have to buy one, two or four LTP-Director licenses to operate the USB-ME256 data acquisition device with one, two or four MEA amplifiers and one, two or four LTP-Director instances.

If you connect, for example, two MEA2100 headstages via interface board to the data acquisition computer you need to have two license keys, for each MEA2100 headstage a separate key. Start the first instance of LTP-Director and connect the first MEA2100 headstage via port settings from the drop down menu. Type in the first license key. Start the second LTP-Director instance and connect the second MEA2100 via port settings. If you connect two MEA2100 headstages with 2 x 60 electrodes, you need to have a double license per headstage for operating four instances of LTP-Director. Please contact Multi Channel Systems MCS GmbH. The staff will be glad to help you.

3.5.4 Installing the LTP-Analyzer Software

1. Double-click the **Setup**.exe on the installation volume.
   The installation assistant will show up and guide you through the installation procedure.

2. Follow the instructions of the installation assistant.

4 Welcome to the LTP-Director

4.1 Welcome to LTP-Director and LTP-Analyzer Software

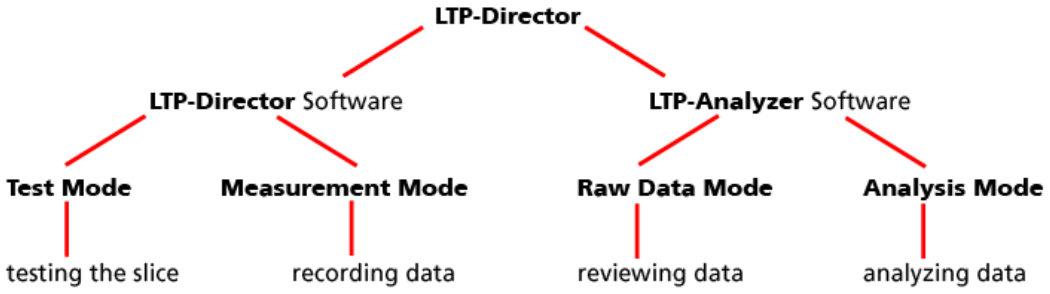
The LTP-Director is an easy to use software package to do standardize LTP and LTD recordings in hippocampal acute slices. The LTP-Director can control MEA amplifiers and stimulus generators from Multi Channel Systems MCS GmbH from within one software.

Currently, the LTP-Director can control only voltage stimulation pulses.

The software package for long term potentiation and long term depression experiments consists of two separate programs. The **LTP-Director** is for online recording of data, the **LTP-Analyzer** is for offline analysis of data prior generated with the LTP-Director.

Because the experimental setup for LTP (long term potentiation) or LTD (long term depression) is often similar, the idea was to develop an easy to use software, which can do the data acquisition and data analysis for a standardized form of experiment. Most of the functions are self-explanatory, if you are familiar with LTP experiments.

LTP-Director and LTP-Analyzer are structured in two main windows each: Test mode (testing the slice), measurement mode (recording data), raw data mode (reviewing recorded data), and analysis mode (analyzing recorded data).



4.2 About LTP-Director Program

The first part of the software package is the **LTP-Director Program** for online data recording. The software will always display and record triggered sweeps of 100 ms length.

For setting up and running an experiment, the LTP-Director has two modes with two main windows: **Test Mode** and **Measurement Mode**.

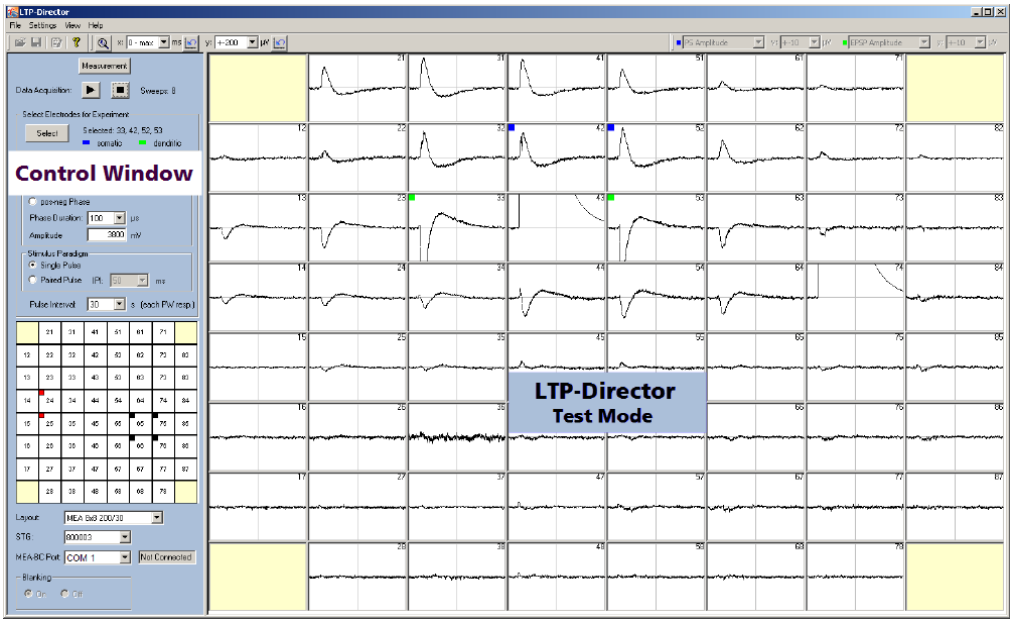
If there is no data acquisition hardware connected, the software starts in **Demo Mode** for simulation purposes. In demo mode the LTP-Director runs a simulation file to show how the program is working. It has no effect to change the parameters due to the fixed simulation file in the background.

4.3 Test Mode

Start the LTP-Director. The program will start in **Test Mode**.

In test mode you have the possibility to select the best stimulation electrodes and to adjust the stimulation parameters. On the left window pane of the main window there is the blue control window for setting up the experimental conditions.

On the right window pane you see the data display for the electrode field. You can define two independent stimulation pathways, and select up to four electrodes to be analyzed online in measurement mode. In test mode, no data is recorded and saved. See chapter "Data Display Test Mode" for further information.



4.4 About the LTP-Analyzer Program

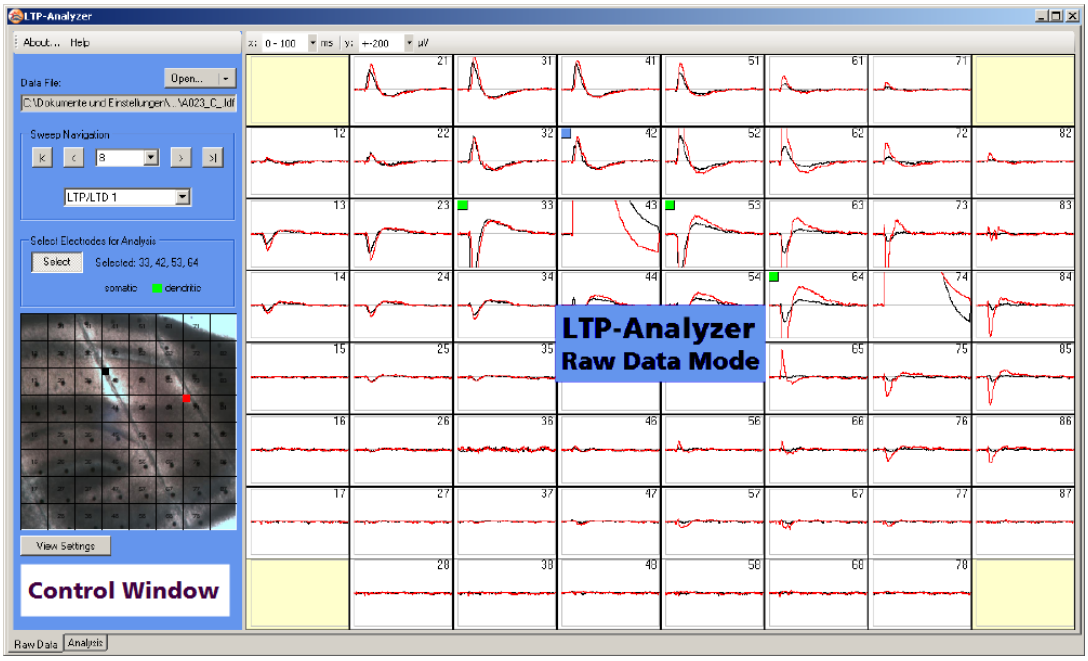
The second part of the software package is the **LTP-Analyzer** for offline analysis of data, generated in measurement mode of LTP-Director.

Parallel to the software concept of LTP-Director, the LTP-Analyzer software also works in two modes and with similar designed dialogs and data displays.

The two modes of LTP-Analyzer are the **Raw Data Mode** for reviewing raw data of all electrodes, and the **Analysis Mode** for analyzing data of selected electrodes.

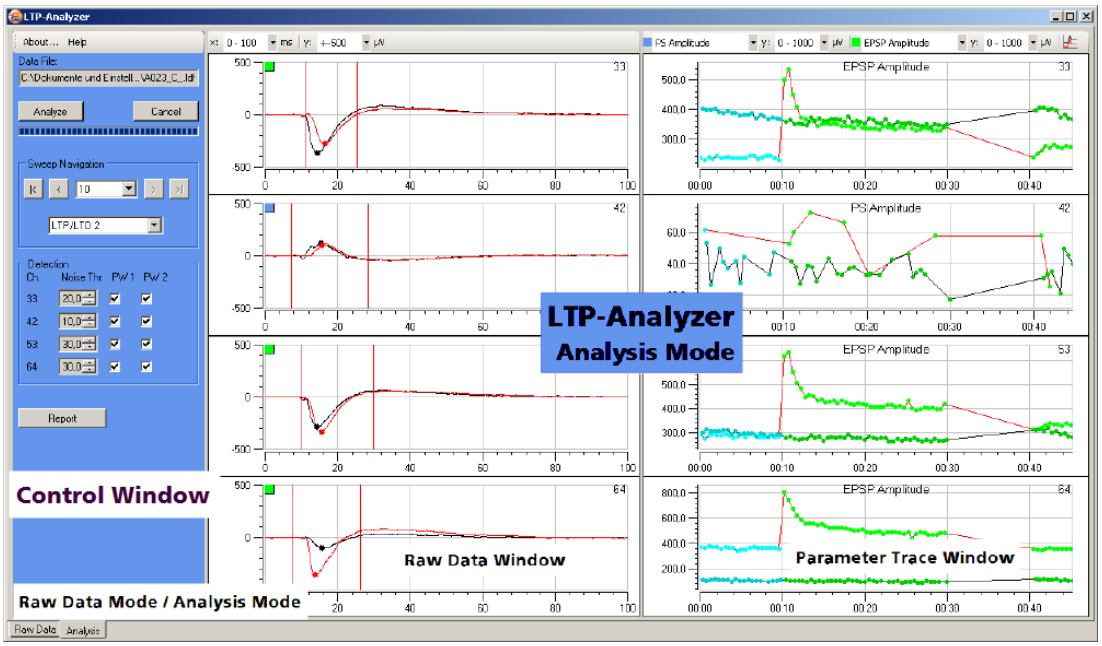
4.4.1 Raw Data Mode

In the same way as in the LTP-Director, up to four electrodes can be selected in **LTP-Analyzer Raw Data Mode**. The electrode raw data can be reviewed sweep by sweep. It is also possible to switch between the different phases of the experiment. Please see chapter "Data Display Raw Data Mode" for detailed information.



4.4.2 Analysis Mode

All recorded parameters are available for analysis in the **Analysis Mode** of the **LTP-Analyzer**. You are able to rerun the analysis with new detection parameters or with different analysis electrodes as often as necessary.

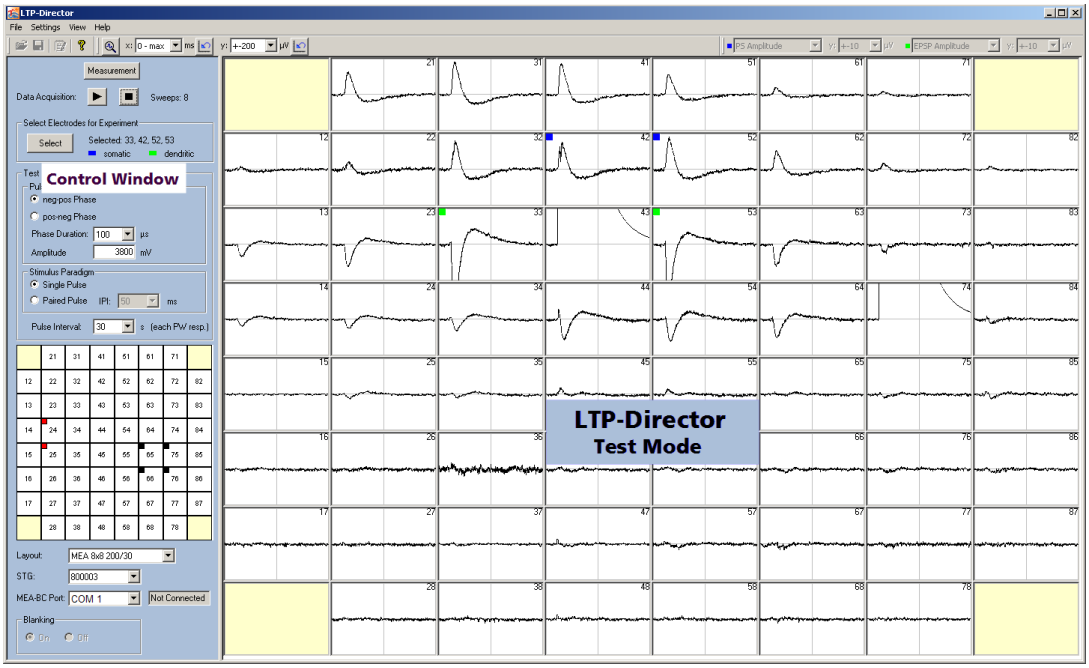


The data display shows red and black data traces in overlay plots depending on the stimulation pathway they are generated with. In the parameter plots data belonging to different stimulation pathways and different experimental phases is color coded. See chapter "Data Display Analysis Mode" for more information.

5 LTP-Director

5.1 Preparing an Experiment in Test Mode

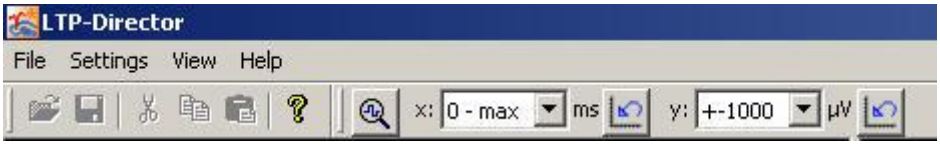
5.1.1 Data Display Test Mode



On the left window pane of the main window there is the blue control window for setting up the experimental conditions. On the right window pane you see the data display for the electrode field. The data display will change when you select a different MEA layout.

In test mode you can define the stimulation electrodes for one or two independent pathways and select up to four electrodes for online analysis. You can define maximal two stimulation pathways and maximal four electrodes, which are analyzed online in measurement mode.

5.1.2 Toolbar



This is the common toolbar for the main windows of LTP-Director in test and measurement mode.

File menu and Templates

Experimental settings can be saved in template files. Template files have the extension "*.let". To open a template file, please click the icon "File Open" or click on the menu. You will have three options: "Exit", "Save Template", and "Open Template". In test mode the options "Save Template" and "Open Template" are not available.

In measurement mode all options are enabled. Templates are used to save and load the software settings to or from a file. It can be useful to save your experimental settings, including the contents of the lab book (Please see chapter "Lab book") which can be reloaded for later experiments without having to enter all the relevant data again manually. The current settings can be saved via "Save Template" in file menu or via "Save" button on the toolbar. When a template is loaded, the current settings are overridden with the values from the file.
A template can only be saved or loaded when the software is idle, for example, when no dialog is open, and the experiment is not running. Template files do not contain recorded data.

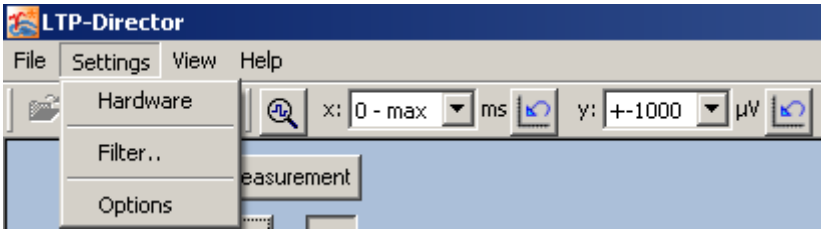
Saving data files

For saving the experimental data, LTP-Director uses two different file formats. For each electrode a separate file will be generated with file extension "*.lrf" (**L**TP **r**aw data **f**ile). However, for each set of files belonging together, there is a XML file that contains additional information, for example, the content of the lab book, experimental settings etc., and serves as a kind of archive file for the individual electrode data files. This file has the extension "*.ldf" (**L**TP **d**ata **f**ile). Both types of files will by default be stored together in one folder and belong together as a data set.

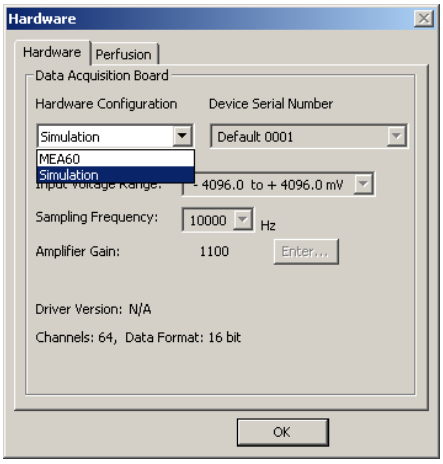
An additional text file will be generated, if you type comments in the dialog "Experiment Comment" under "View" in measurement mode. This text file has the extension "*.txt".

Important: When moving or copying LTP-Director files, please always move or copy all "*. lrf","*. ldf" and "*.txt" files - belonging together - in one common folder.

Settings

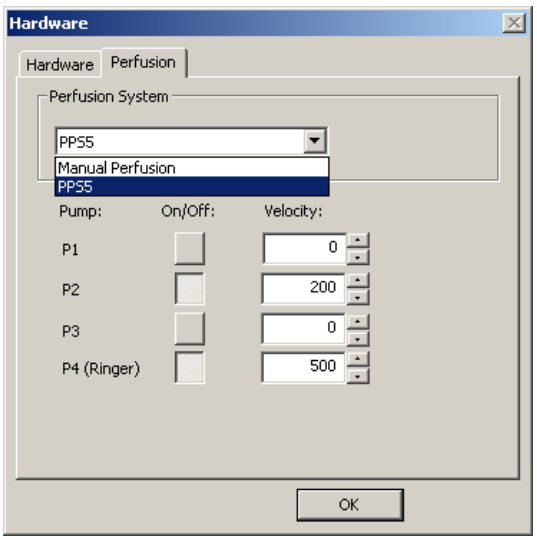


In the settings menu, the hardware configuration, the filter parameters, and several options can be defined. To define the hardware settings, click the "**Hardware**" tab under "Settings".



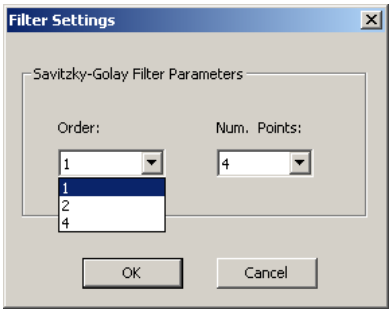
In the "Hardware Configuration" drop down menu you can switch between Simulation and all data acquisition systems connected to your computer. The window beneath shows the serial number of the selected device. In this dialog you also specify the "Input Voltage Range", the "Sampling Frequency", and factor of the "Amplifier Gain".

Click "**Perfusion**" tab under "Hardware" in "Settings".

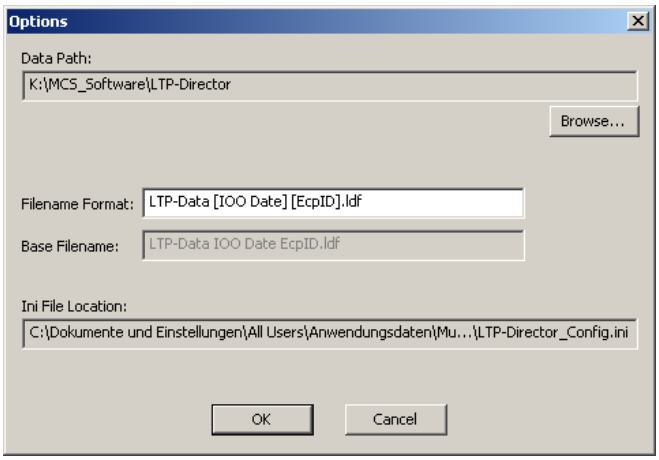


The "Perfusion" option gives the possibility to define, whether you apply the drugs manually or you are using an automated system for drug application, for example the PPS5-System. For automated application, please read chapter "Automated Perfusion System".

To select the parameters of the Savitzky-Golay filter used for signal detection, please click "**Filter**" under "Settings".



To define a file name and a file path, click "**Options**" under "Settings".



If you do not want to keep the default settings, browse through you folders and define the "Data Path".

You can choose **any** file name, or optional generate the file name automatically based on the information in the Lab Book (please see chapter "Lab Book"). You use one or more of the following placeholders in squared brackets to generate a file name:

- [ISO Date] Date taken from your system clock
- [ExpID] Experimental ID taken from the Lab Book
- [Director] Directors' initials taken from the Lab Book
- [Scientist] Scientists' initials taken from the Lab Book
- [Technician] Technicians' initials ID taken from the Lab Book

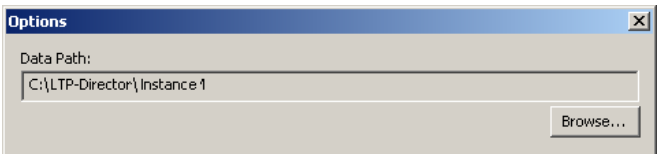
These characters create the "Base Filename", too. If additional experiments are started without modifying the file name, the old file will not be overwritten. Instead, a new file will be generated with the same name plus the addition 0001, 0002 and so on.

Hint: It is useful to characterize the "Base Filename" by date first, because in this case the files are listed in order of appearance in windows explorer.

The "*.ini" configuration file "Ini File Location" is displayed. This is fixed for the program and cannot be changed.

Warning: Please do not modify the "*.ini" configuration file or the location of the file!

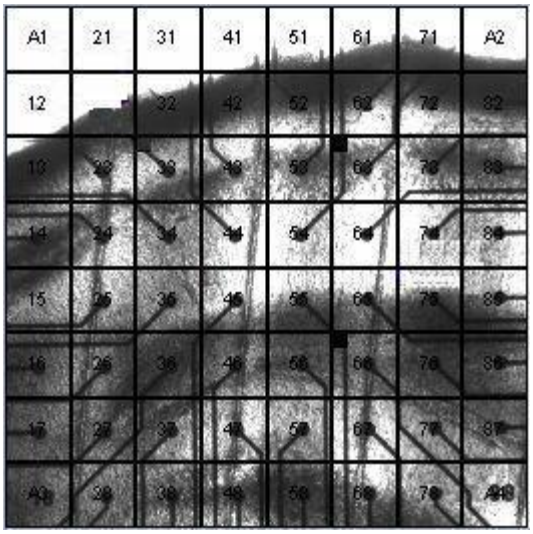
When using more than one instance of LTP-Director, you can choose different data paths for each instance, or you can use one data path only. The number of the instance is displayed.



5.1.3 View

Click "**View**" in the main menu toolbar.

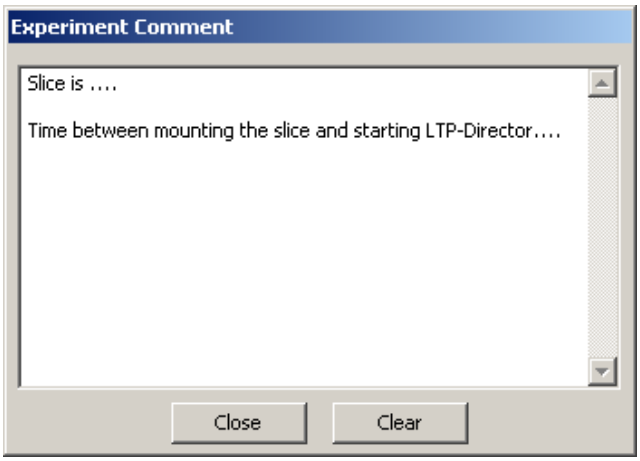
It is possible to load a photo as background picture into the electrode field of the "Test Mode Control Window". You have the direct optical control of the position of your stimulation and recording electrodes.



Open the image file with the "Load Image" command from the "View" menu. The image must be correct orientated! To arrange the picture in relation to the electrodes please click on the exact position of an electrode within the image. A text window will come up, where you need to type in the number of that electrode. You have to do this for two electrodes, which should be far apart (B3 and L10, for example). With this information, the image is cut and aligned to the electrode window. It will also be saved with the data and turn up in the report sheet.

To discard the image use "Unload Image".

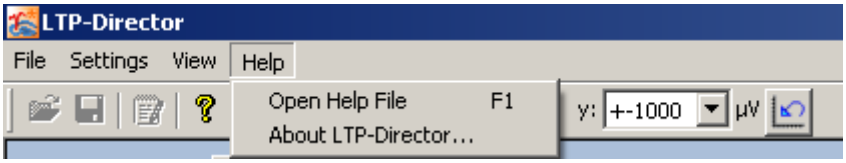
Click "**Experiment Comment**".



The option "Experiment Comment" opens a dialog to type in comments concerning the experiment in test mode and in measurement mode. The comments will be stored in the comment file with the extension "*.txt". You can keep the dialog open while displaying other dialogs and you can switch between test mode and measurement mode. It is possible to close the dialog and open it again later to complete your comments. With the command "Clear" all comments are deleted.

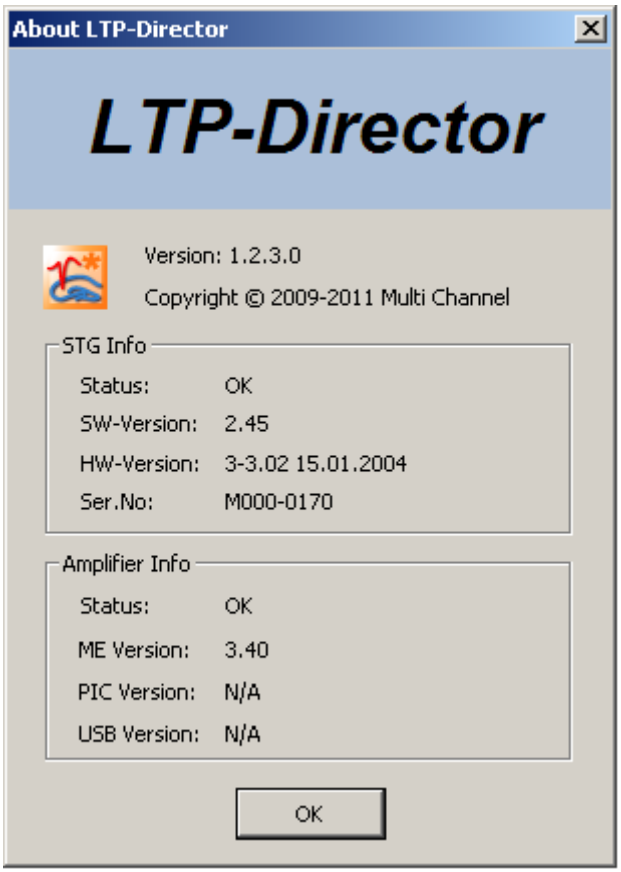
5.1.4 Help

Click "**Help**" in the main menu toolbar.



Click "**Open Help File**" to open the online help.

Click "**About LTP-Director**".



The dialog shows basic information about the LTP-Director software and about the connected devices, the stimulus generator STG, and the MEA amplifier.

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

5.1.5 Drop Down Menus

The drop down menus in the main menu toolbar belong to the data display. They are one of the possibilities to change the adjustments of the x- and y-axis. For zooming and scrolling the axes via mouse, please refer to chapter "General LTP-Director Software Features".

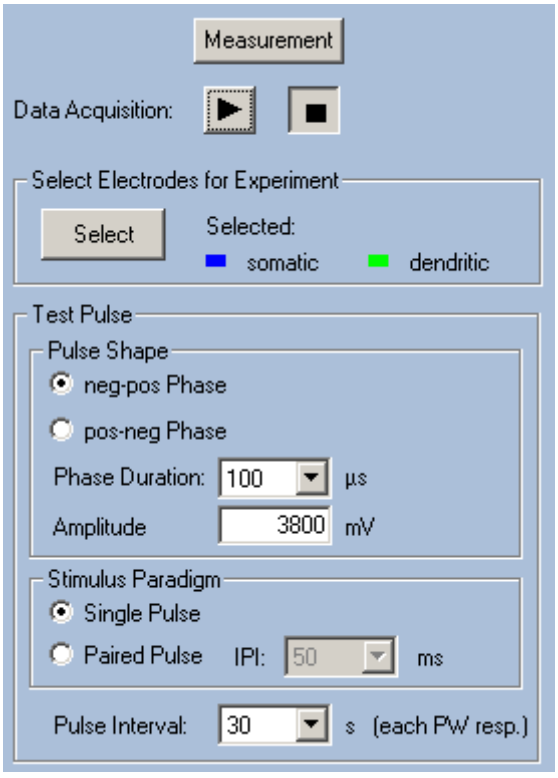
Icon "Axes Default": Resets all axes of all channels to the default. This is 10 to 40 ms for the x-axis and -2000 to +2000 µV for the y-axis.

With the drop down menus you can adjust the axes separately. The x-axis has the unit millisecond, the y-axis has the unit microvolt. The command "Zoom to fit" for the x-axis and "Zoom to fit" for the y-axis shows all available data in that window.

The drop down menus in the right part of the main menu toolbar (in this screenshot: PS Amplitude) are disabled. They belong to the recording display. Please read chapter "Data Display Measurement Mode".

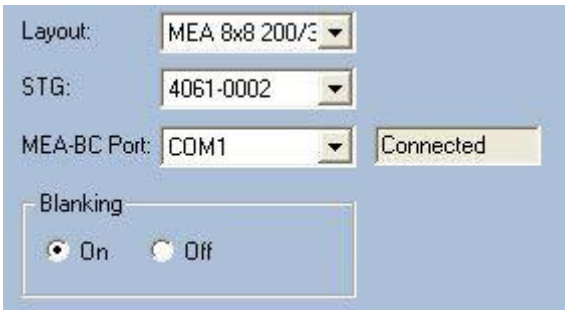
5.1.6 Control Window Test Mode

Control Window Test Mode: Control window for the experimental protocol in test mode.

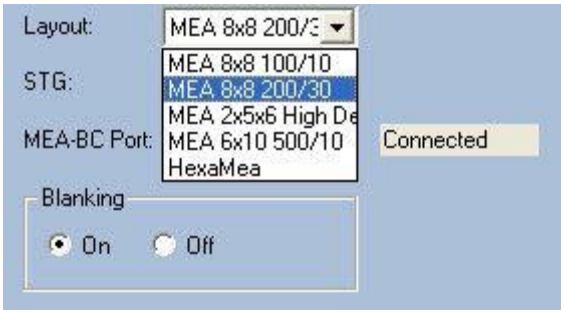


The blue window pane on the left side of the main window in **Test Mode** includes the command buttons for setting up, controlling, and running a preliminary experiment in test mode. In test mode, it is possible to test several possible stimulation electrodes and stimulation parameters before running the actual experiment. During test mode, the raw data is displayed but not recorded.

Setting up the Hardware



Recording and stimulation hardware connected to your computer will be detected automatically. If you have more than one stimulator, you can select one of them with the drop down menus. Here you also find the menus to specify the layout of the MEA chip and control the blanking.



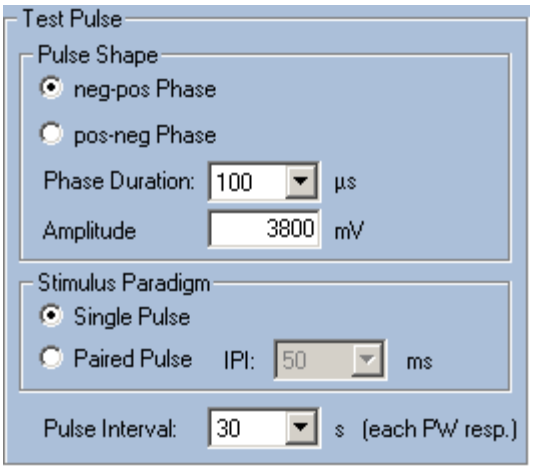
From the drop down menu "**Layout**", please choose the correct layout for your MEA chip inside the MEA amplifier. The data display will change accordingly. While working with only one STG, there is no need to set the drop down menu in "**STG**", but you always have to set the COM port for the MEA amplifier in "**MEA-BC-Port**" drop down menu. If you are working with multiple devices parallel, you have to specify the COM ports to which the actual STG and MEA amplifier are connected. The "MEA-BC-Port" drop down menu shows all available COM ports of the data acquisition computer. Please choose the port, to which the MEA amplifier is connected. The "STG" drop down menu shows all available STGs, please select the actual used device. The window beneath will display the status. Now you can activate or deactivate the blanking.

A stimulus generator is one of the requirements for applying the software, because the LTP-Director cannot display spontaneous data, but only triggered sweeps of 100 ms length. That is, why "STG" has to be correctly connected, too. There is an error message, if STG or MEA amplifiers are not accessible from data acquisition computer.

For common experiments using a MEA1060-BC amplifier with blanking circuit, the function "**Blanking**" may be selected or not. Using a MEA1060 amplifier without blanking circuit, the "Blanking" option is disabled.

Important: It is possible to switch the blanking mode while the data acquisition is running!

Test Pulse Parameter

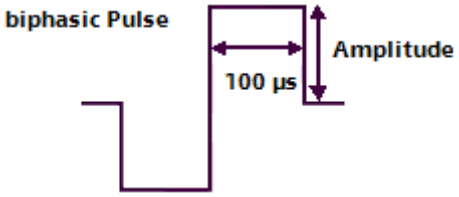


Next step in setting up an experiment is to define the pulse parameters for your stimulation test pulse. You can determine the shape of the pulse, the stimulus paradigm and the pulse interval. The stimulation test pulse is for testing the response of the slices and for selecting the stimulation electrodes at each slice.

Note: It is not possible to change pulse parameter while the stimulation is running!

Pulse Shape

The pulse shape of a stimulus pulse is 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.



The phase duration refers to one phase of a biphasic pulse, for example100 µs, as shown in the picture above. The total length of the pulse will be double the phase duration.

For MEA electrodes, it is recommended to use biphasic negative / positive pulses.

The amplitude of the stimulus test pulse has to be an integral number in a range between 0 and 8000 mV. Out of these range an error message appears. Usually, pulse amplitudes between 500 and 3500 mV are enough to elicit responses in a hippocampal slice.

Stimulus Paradigm

Apart from single pulses, it is also possible to apply paired pulses to check for short term plasticity.

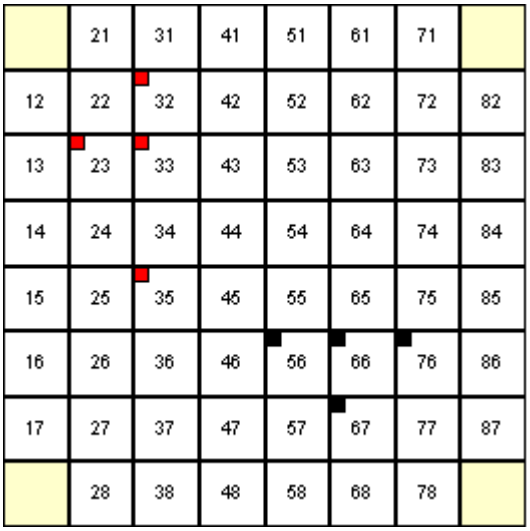
Using a paired pulse, you have to define the "**IPI = inter pulse interva**l" from a drop down menu in units of milliseconds.

According to the stimulus paradigm the analysis parameter will change: Using single pulse, you have one region of interest (ROI), using paired pulse paradigm, you will have two regions of interest.

Pulse Interval

The pulse interval lasts between 0.5 and 180 seconds. This interval refers to one stimulation pathway. If you are using two stimulation pathways, pathway S2 will be delivered time delayed after pathway S1. The time delay between a stimulus on pathway S1 and S2 will be half of the selected pulse interval. Example: Selected a pulse interval of 1 min and two pathways. There will be one stimulus every 30 s alternately on pathway one and two.

Selecting the Stimulation Electrodes



You can select stimulation electrodes for one or two independent stimulation pathways (S1 and S2). Please use the white electrode field in the blue Control Window Test Mode.

The LTP-Director can deliver two independent stimulation pathways to different electrodes. Up to four electrodes can be selected for each pathway. The electrodes of stimulation pathway S1 are labeled with small red squares in the upper left corner of the electrode symbol in the electrode grid. Electrodes belonging to stimulation pathway S2 are labeled in black. Selected pulse parameters apply to both pathways. All selected electrodes per pathway are active at the same time.

To select an electrode for stimulation pathway S1, just click on the electrode you want to select, it will be marked in red. To select an electrode for S2, hold the Shift key and click on the electrode, it will be marked in black. To deselect an electrode, click on the electrode symbol again or press Shift key and click again. LTP-Director does not allow to select more than four electrodes for one pathway.

In the data display the data traces belonging to one pathway are labeled in the corresponding color, in red (S1) or black (S2).

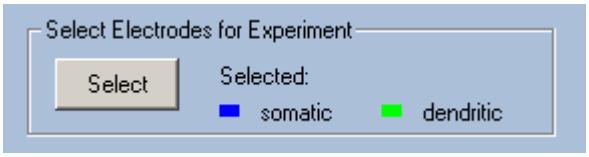
The signals of these two stimulation pathways S1 and S2 are delivered alternately. The pulse interval refers to one pathway. So, if the pulse interval is 60 s, for example, there will be a stimulus pulse from all stimulating electrodes of pathway S1, after 30 s a stimulus pulse from all stimulating electrodes of pathway S2. After another 30 s again stimulus pulse from S1 and so on.

Important: It is possible to change the stimulation electrodes while the data acquisition is running!

In "Test Mode" both stimulation pathways are set with the same stimulation pulse parameters. Later on in "Test pulse" tab in "Preparation" of "Measurement Mode" it is possible to set up the amplitude of the stimulation pathways independent of each other.

If you select the same electrodes for stimulating and recording, and you do not use the "Blanking Feature" you will get a warning message when starting "Measurement" in test mode.

Selecting Electrodes for Online Analysis



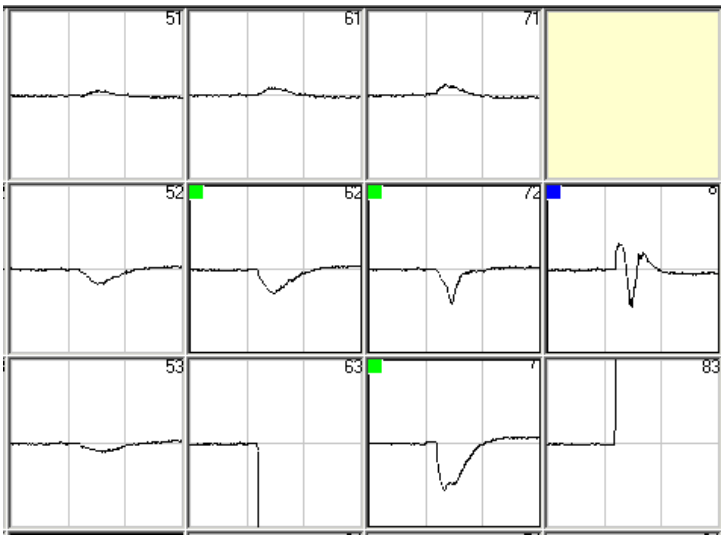
To select electrodes for online analysis during the experiment, please click the "**Select**" button. Now it is possible to select up to four electrodes that will be analyzed online. However, data from all electrodes will be recorded, and can be analyzed offline with LTP-Analyzer.

In the electrode data display of test mode you can label electrodes according to whether they show either somatic signals (PS = population spikes) or dendritic signals (EPSP = excitatory postsynaptic potential).

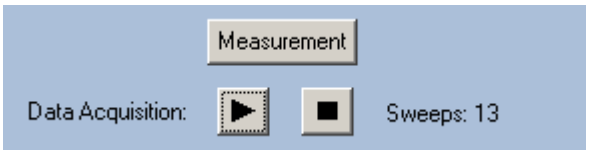
To label an electrode as somatic, just click it. The electrode will be marked by a blue square in the upper left corner of the channel view. To label an electrode as dendritic, hold the Shift key and click it. The electrode will be labeled in green. If you try to select more than four electrodes, LTP-Director will deselect the first selected electrodes.

The selected electrodes will be analyzed online, but again, data from all electrodes will be recorded. To deselect an electrode, click or Shift click it.

In the following picture, you see a sweep from stimulation pathway S2 (traces are black). Electrodes 62, 73 and 73 are labeled as dendritic, electrode 82 is labeled as somatic.



Test Stimulation



After pressing the start button besides "Data Acquisition", the preliminary experiment is running. Stimuli with the user defined parameters will be delivered continuously on one or both stimulation pathways. The sweeps are counted until you stop the recording manually by pressing the stop button. In "Test Mode" data is displayed but not recorded.

Important: Changing the stimulation electrodes or the electrodes selected for online analysis is only possible in test mode!

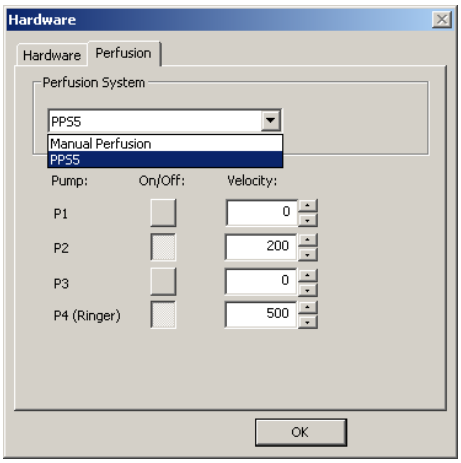
After selecting stimulation and analysis electrodes, press the "Measurement" button to switch to "Measurement Mode". Please read chapter "Data Display Measurement Mode".

5.1.7 Automated Perfusion System

The PPS5 is a perfusion system with five peristaltic pumps and optical fluid control from Multi Channel Systems MCS GmbH which can be automatically controlled by the LTP-Director.

Test Mode

Click "Hardware" under "Settings" and select the "Perfusion" tab. Select "PPS5" from the drop down menu for the manually control of the automated perfusion.



The four peristaltic pumps P1 to P4 from the left side of the device control the inlet tubes, independent from each other. They are connected to the compound bottles. The fifth peristaltic pump on the right side of the device controls the outlet tube, connected to the waste bottle. This pump works additionally as soon as one of the inlet pumps is activated and is connected to a bubble detector. The outlet pump runs as fast as all other active pumps together multiplied by 1.5 to guarantee that overflow is not possible. You can apply three compounds of your choice. It is recommended to have the perfusion dilution used as standard in the experiment, for example Ringer's solution, as forth compound. When starting more than one inlet peristaltic pump you can mix compounds in any desired ratio by the velocity of the influx.

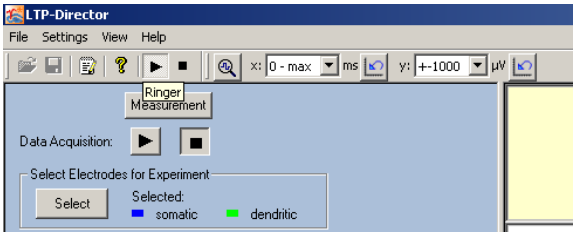
The bubble detector connected to the outlet pump prevents overflow. A light barrier detects the changes of fluid to air in the outlet tube and measures whether this event takes place all ten seconds or not. The LED indicates the fluid flow: The LED is permanently on without tube, when the fluid flow is not correct it flashes fast, with correct flow the LED flashes slow. Please pay attention to the LED.

Please use the dialog in the "Hardware" "Perfusion" tab for manual control of the PPS5 device. For example, you can use it to eliminate the air in the tubes before starting an experiment.

Select the velocity of fluid transport via up down menus in the dialog window. The upper limit is 5000 and the unit is roughly microliters per minute, so the maximum is 5 ml/min.

Click the button "On/Off" to start the peristaltic pump, click the button again to stop the pump. It is possible to change the velocity via up down menu or by writing into the window while the pump is running. When starting any of the inlet pumps, the outlet pump is automatically activated, and the speed is always adjusted to the total of all other pumps times 1.5.

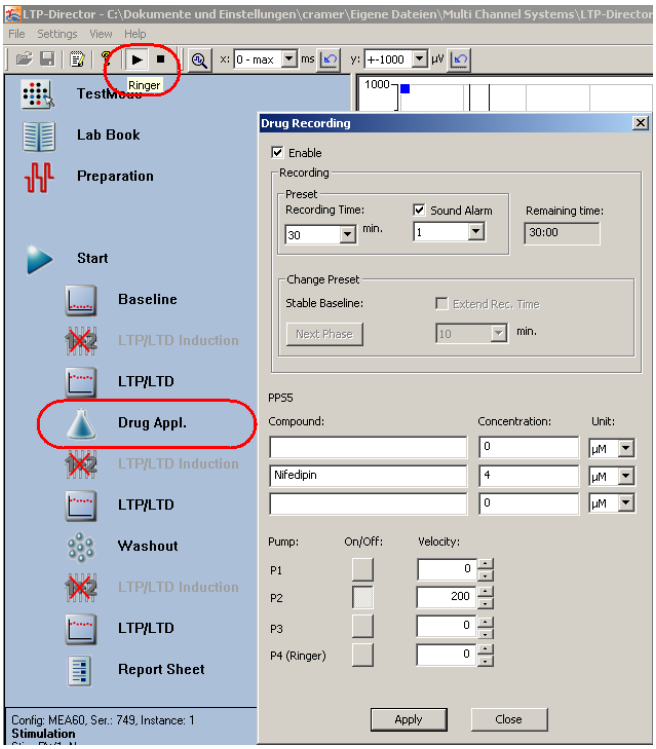
Important: When changing from Test Mode in Measurement Mode the pumps are running as defined in the "Perfusion" dialog, until the first valid experimental phase starts. Then pump P1 to P3 are automatically stopped, pump 4 (P4 Ringer) continuous running. In the drug application phase you can set new parameters for pump P1 to P4 in the "Drug Recording" dialog, which are applied in this phase and the following phases until wash out phase is reached. During wash out phase and all following phases only pump 4 (P4 Ringer) is working, that means only Ringer, for example, is applied but no compound any longer.



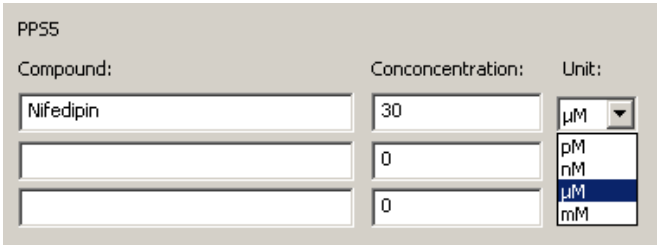
In the main control panel there is a "Start" and "Stop" button for the perfusion. By pressing the "Start" button, **only** pump 4 (P4 Ringer) is activated. The status of pumps P1 to P3 is unaffected. The "Stop" button stops all pumps. These buttons are available in Test Mode and in Measurement Mode.

Measurement Mode

Click icon "Drug Application". The "Drug Recording" dialog appears.



In this dialog you can label the three compounds of your choice in pump P1 to P3 and their concentration for the report sheet. This information has no influence on the pump speed.



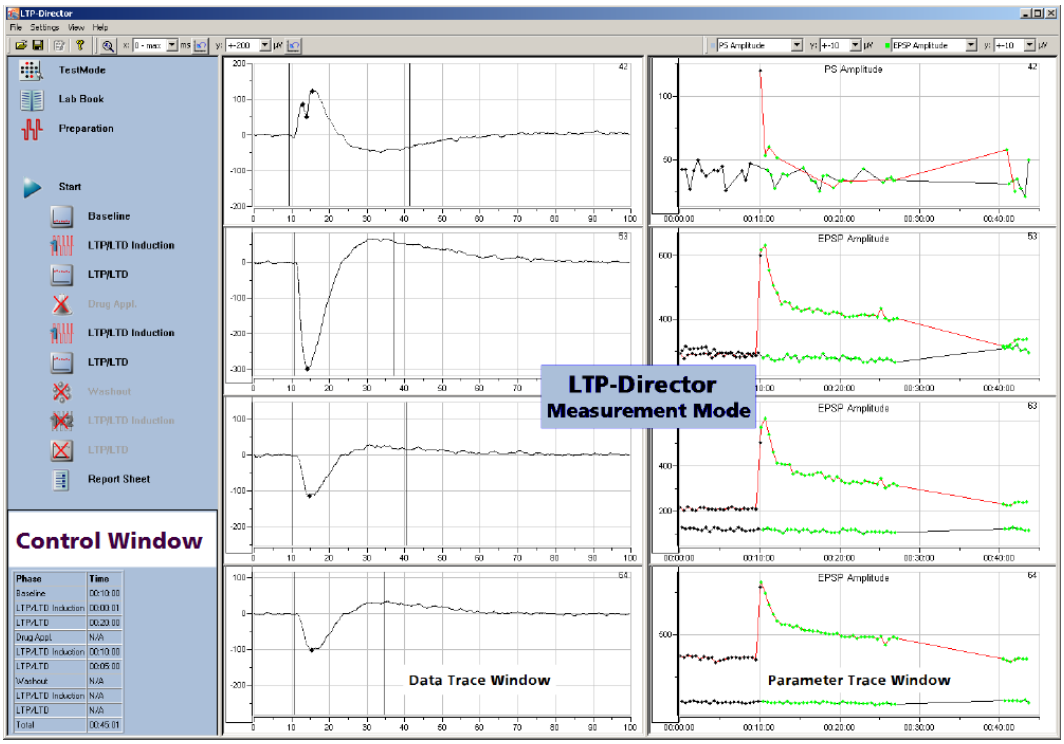
For the drug application phase, you can define the pump speed for all four pumps in the same way as described before. These settings will be valid during the drug application phase and the following phases until the "Washout" phase starts. During washout and all other experimental phases, pump P4 (Ringer) for Ringer perfusion is running only, no compound is applied any longer.

5.2 Running an Experiment in Measurement Mode

5.2.1 Data Display Measurement Mode

In contrast to the "Test Mode", the function of the "**Measurement Mode"** is the design of the actual experiment and the recording of data. Data and analysis parameters are displayed. An experiment can consist of different phases, represented as icons. By activating or deactivating individual phases, it is possible to design the desired experimental layout. Measurement mode also includes an online documentation and an automatic generation of a report sheet at the end of the experiment.

Measurement Display



The design of the main menu in "**Measurement Mode"** is similar to that of the test mode. On the left side there is the blue **Control Window**, besides the **Data Display** window. The data display is separated into smaller windows.

Hint: To handle the multiple display features, for example, scrolling and zooming, please read chapter "General LTP-Director Software Features".

Main Menu Toolbar of Measurement Mode

The main menu toolbar of measurement mode includes the same functions as in Test Mode. Please read chapter "Data Display Test Mode".

Data Display Measurement Mode

The data of the electrodes, selected in test mode is displayed on the left, and the analyzed parameter on the right. Data traces belonging to stimulation pathway S1 are shown in red, data traces belonging to stimulation pathway S2 are shown in black.

The data traces are marked with dots (in red or black). These dots indicate all maxima and minima, detected inside the region of interest. The region of interest ROI is defined between two lines. You can adjust the ROI by moving the borders with the mouse. Please read also chapter "Signal Detection".

It is possible to zoom in on any data or analysis window by double clicking the window.

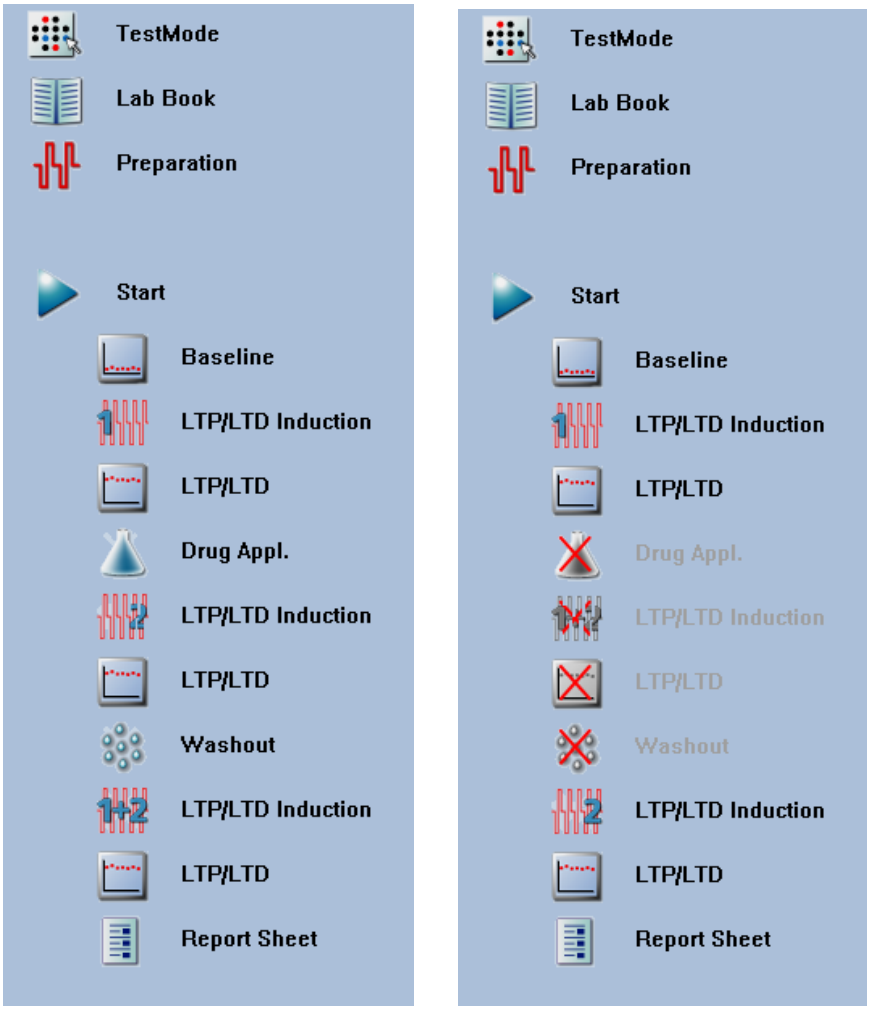
Hint: To handle the multiple display features, for example, scrolling and zooming, please read chapter "General LTP-Director Software Features".

On the right part of this display you see the parameter display for the online analysis related to the four selected electrodes. The drops down menus for changing analysis parameter are enabled now. Only one parameter is displayed at a time, but all available parameters are analyzed simultaneously. Hence, it is possible to switch between the different parameters during recording. The displayed analysis parameter can be selected individually for electrodes marked as somatic (blue) or dendritic (green), respectively.

In the parameter display, the stimulation pathway is color coded by the color of the line connecting the data points (red or black). The color of the data points codes different experimental phases (Please refer to chapter “Data Display Raw Data Mode”).

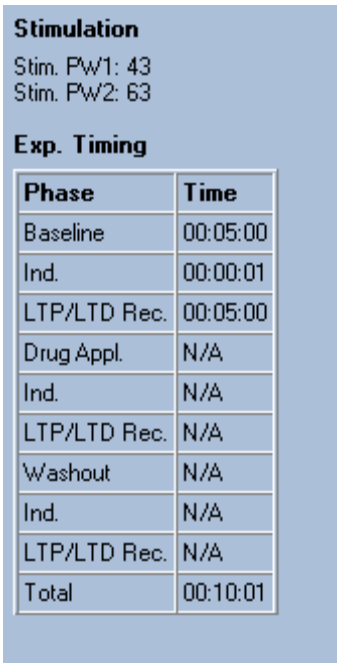
5.2.2 Control Window Measurement Mode

Control Window Measurement Mode: Click "**Measurement**" in **Test Mode**.



The control window in measurement mode includes the online documentation (Lab Book), the control for the stimulation and detection parameters, and the experimental layout. Different modules, like baseline recording, LTP / LTD induction, drug application, and wash out can be selected or deselected to define the course of the experiment. Data recording will start as soon as you press the "Start" button. It is possible to change, add or remove modules while the experiment is running.

Table for overview:



Stimulation

Stim. PW1: 43
Stim. PW2: 63

Exp. Timing

Phase	Time
Baseline	00:05:00
Ind.	00:00:01
LTP/LTD Rec.	00:05:00
Drug Appl.	N/A
Ind.	N/A
LTP/LTD Rec.	N/A
Washout	N/A
Ind.	N/A
LTP/LTD Rec.	N/A
Total	00:10:01

The table below the modules for experimental proceedings gives an overview about the stimulation electrodes, the experimental timing of each phase, and about the experimental time in total. When starting more than one instance of LTP-Director the number of the currently used instance is additionally displayed.

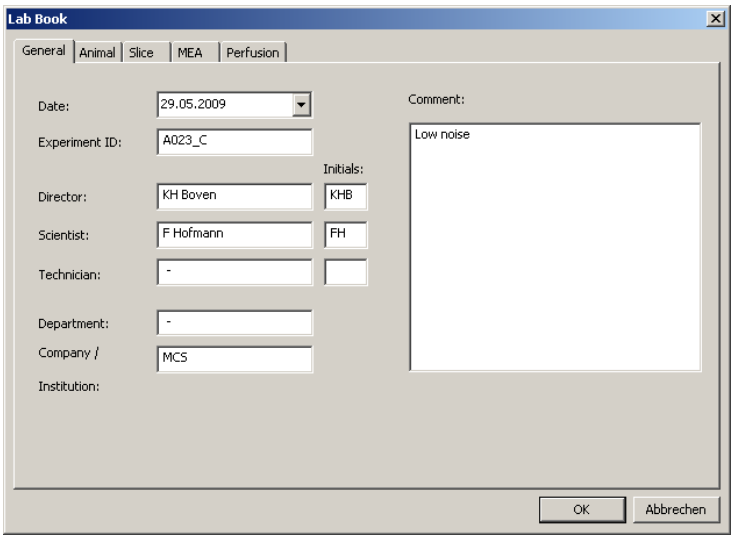
5.2.3 Test Mode

It is not possible to change stimulation electrodes or analysis electrodes in measurement mode. If you want to change these, you have to go back to test mode. Click the icon "**Test Mode**". The display will change accordingly to the main window of test mode, and you are able to do the desired settings.

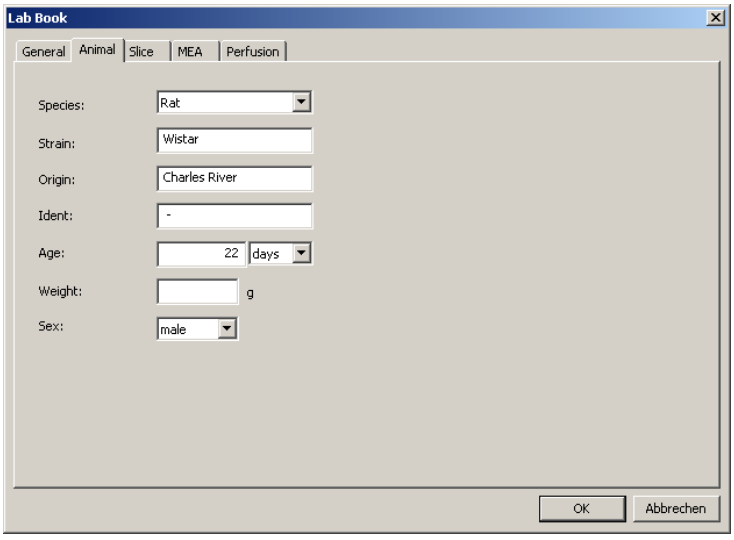
5.2.4 Lab Book

The lab book is the online documentation for the experiment, which will be saved together with the recorded data, and visualized in the Report Sheet. All entries in the laboratory book from "**General**", "**Animal**", "**Slice**", "**MEA**" and "**Perfusion**" tab will be stored in the XML file with the extension "*.ldf". As most of the entries in the lab book will often be the same for each experiment, they can be saved in a template file (see "File Menu and Templates").

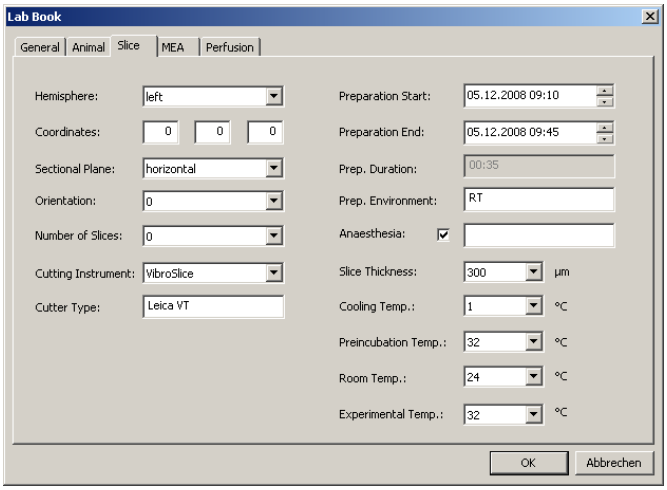
Click the icon "**Lab Book**" for entering the following dialog.



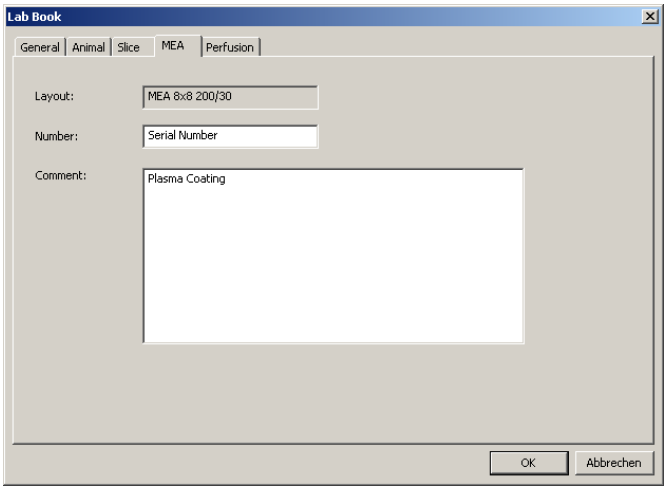
In "**General**" tab of lab book you can enter general information about the experiment and the people involved. There is also a free text field available for additional comments. The data is automatically updated based on your system clock. The date, the experiment ID and the initials of the director, scientist and technician may appear as a part of an automatically generated file name. Please see chapter "**Options**" in "Settings" in main window toolbar.



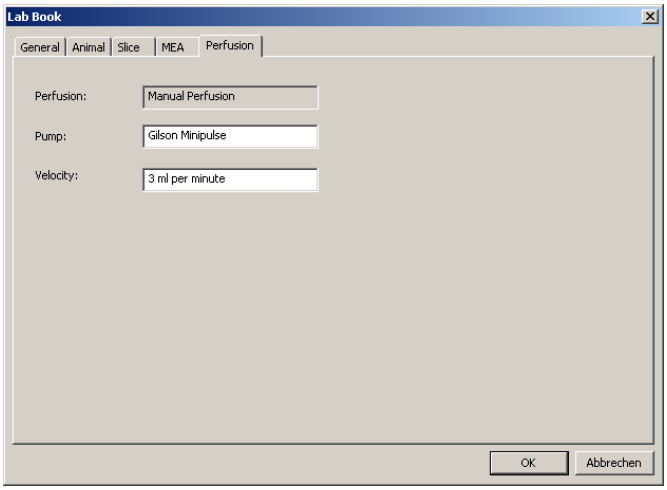
In "**Animal**" tab of the lab book you store information about the laboratory animal used in this experiment.



In "**Slice**" tab of the lab book you store information about the preparation procedure.



In "**MEA**" tab of the lab book you store information about the MEA, and you can type in comments about the pretreatment of the MEA, for example.

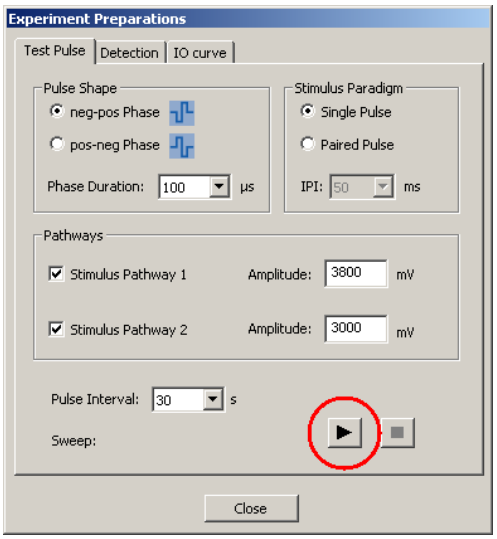


In "**Perfusion**" tab of the lab book you store information about the perfusion pump and perfusion velocity. The information given in these tabs of the lab book are summarized - among others - in the "Report Sheet" of the LTP-Director.

5.2.5 Preparation

The settings possible in preparation dialog allow changes in stimulation and detection parameters. It is also possible to run automatic IO (Input / Output) curves, and to deliver test pulses without recording data.

Click the icon **"Preparation"** to enter the "**Test Pulse**" tab.



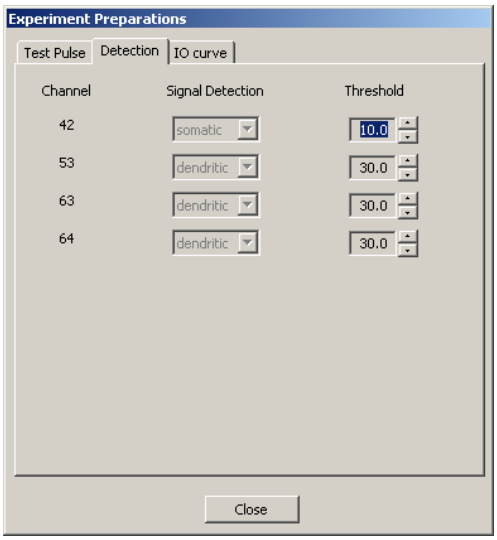
With the help of this "**Test Pulse**" dialog - similar to the control window of test mode - it is possible to adjust the stimulation parameters for the recording. You can change the "**Pulse Shape**" and the "**Stimulus Paradigm**" for both stimulation "**Pathways**" S1 and S2 individually. It is recommended to use one of the stimulation pathways for LTP / LTD Induction, and the other one as internal reference.

Again the "**Pulse Interval**" can be chosen from a drop down menu. This parameter will also apply to the pulses in the IO curve.

Hint: For test purposes, a short pulse interval can be chosen. For the actual experiment, it is advisable to use a longer pulse interval.

Pressing the play button in the test pulse tab will **not** result in data recording.

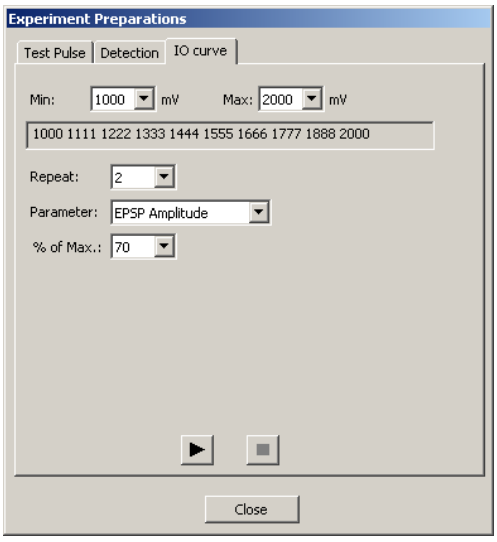
Click the **"Detection"** tab.



The "**Detection**" tab displays the channels of the four electrodes, selected in test mode for online analysis. Each electrode is labeled as somatic or dendritic, as you defined it in the test mode. In "**Threshold**" you set the detection level for local maxima or minima for each electrode as a multiple of the noise level. Local maxima or minima will be shown as dots in the data trace.

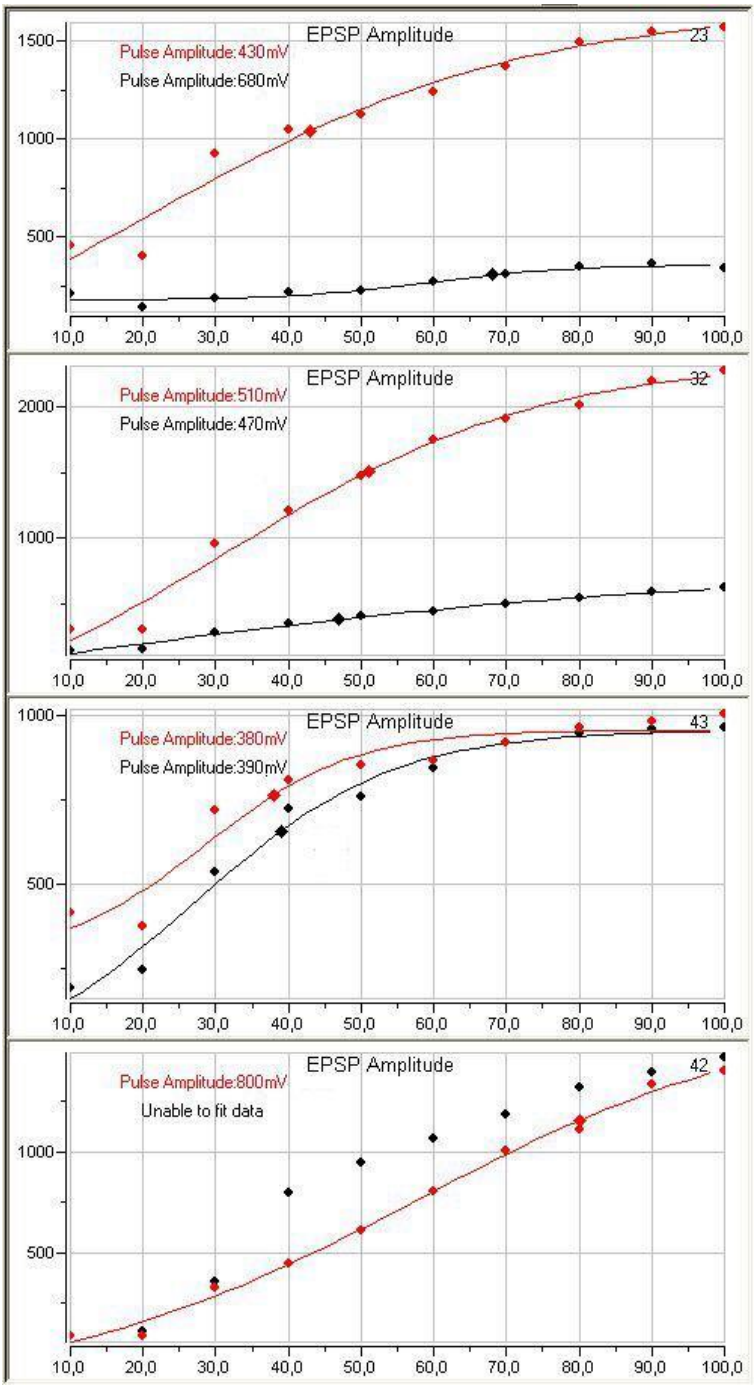
So, if you have, for example, a bit of artefact left that gets detected as a local minimum or maximum, increase the threshold level. If you do not detect a local maximum or minimum in a small signal, decrease the threshold level. A threshold of 30 times the noise for EPSPs (dendritic) and about 20 times the noise for population spikes (somatic) is a good value to start with.

Click the **"IO curve"** tab.



In the "**IO curve**" tab, there is the option to run an IO curve with stimuli of increasing strength on either one or both pathways to determine the optimal stimulation strength individually for either stimulation pathway.

The IO curve is performed by applying 10 amplitude values in equal distance from the given minimum and maximum amplitude values in millivolt. For example: Minimum amplitude 50 mV, maximum amplitude 500 mV, resulting amplitudes: 50 100 150 200 250 300 350 400 450 500.

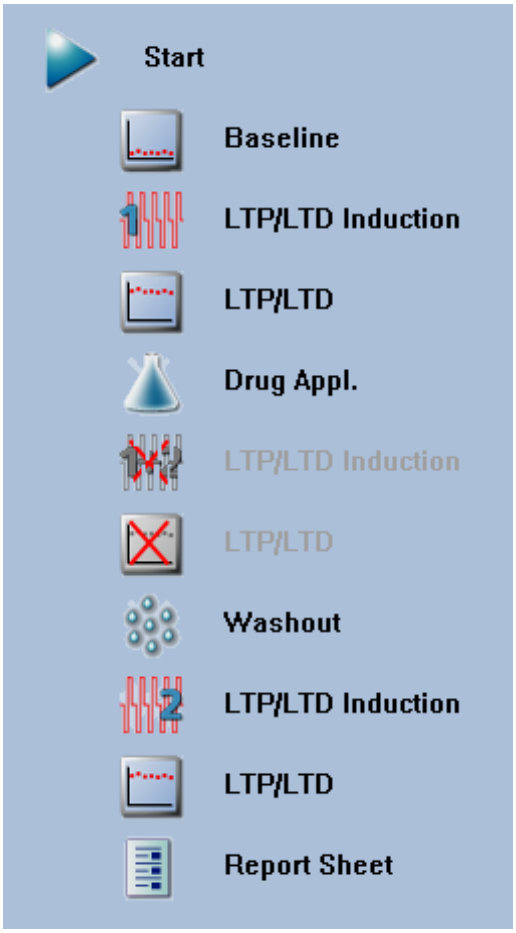


Select one parameter for analysis from the drop down menu. The IO curve can be repeated up to three times, the results are averaged.

Click ▶ to start the IO curve. If possible, a sigmoid fit curve is automatically calculated. If not, there is the error message: "Unable to fit data". The stimulation strength resulting in 70 % or 30 % of the maximum response is displayed. As usual, stimulation pathway 1 is shown in red and stimulation pathway 2 is shown in black.

This data is not recorded, but you can export the IO curve parameter results as ASCII file or image capture.

5.2.6 Experimental Protocol in Measurement Mode

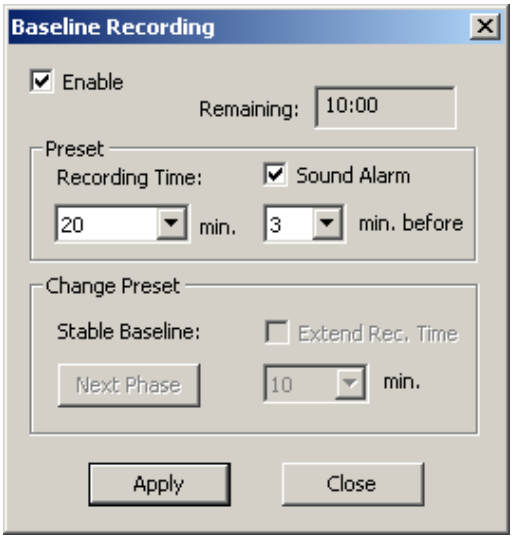


Once the stimulation parameters and the electrodes to be analyzed in measurement mode are selected, the outline of the experiment must be defined. Please remember, if you press the "**Start**" button, data is recorded. If you just want to test the stimulation, please use the "**Preparation**" menu.

Different experimental phases can be selected or deselected to define the course of the experiment. Clicking the corresponding icon opens a dialog with the settings for each phase. Each dialog contains an enable / disable option. If a phase is disabled, the icon appears grey and crossed out. The user is free to choose any sequence of experimental phases. The **Report Sheet** will be generated automatically at the end of the experiment.

Baseline

Click the icon "**Baseline**". As with all experimental phases, there is the possibility to enable or disable this phase by checking the "Enable" box.



During the "Baseline" phase of the running experiment, the user defined stimulation paradigm is applied for a chosen period of time. This phase is often used as reference for the following phases with LTP / LTD Induction and / or drug application.

The duration of the baseline recording is selected from the "Recording Time" drop down menu. When the experiment is running, in the combo box "**Remaining**" the time for that phase is counted down. With "**Sound Alarm**" an acoustic alarm can be set off shortly before the end of the phase.

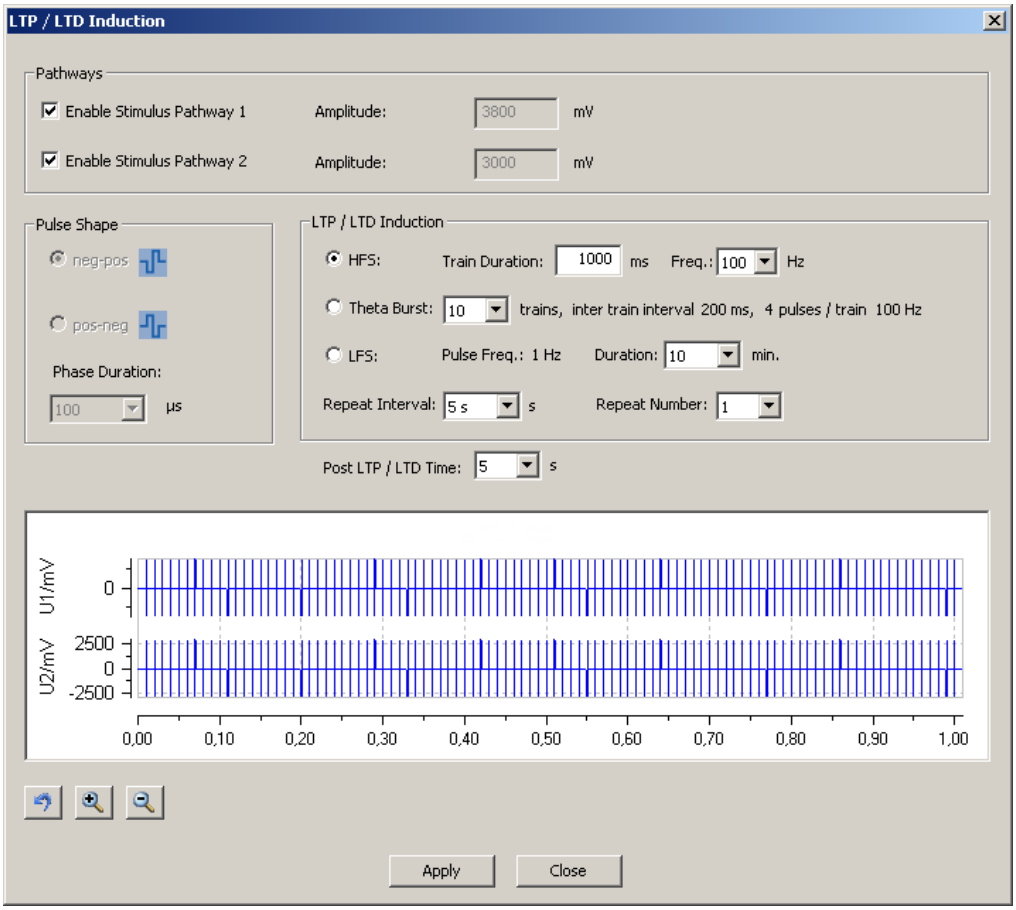
To extent this phase, click the "**Extend Recording Time**" marker. Now it is possible to increase the duration of that phase while experiment is running.

When pressing the "**Next Phase**" button, the program jumps to the next experimental phase, regardless of the remaining time of the current phase. After the phase duration is over, the next phase will start.For any changes to take effect, they have to be confirmed with the command "**Apply**".

LTP / LTD Induction

Click the "**LTP / LTD Induction**" icon. This icon is available in three versions. The number on the pulse train symbol indicates, which stimulation pathway is used for the LTP / LTD Induction. 1 means stimulation pathway S1, 2 means stimulation pathway S2, and 1+2 means both stimulation pathways.

The LTP / LTD Induction phase defines the high or low frequency stimulation that induces either long term potentiation LTP or long term depression LTD.



"**Pathways**": The stimulus can be delivered on stimulation pathway S1, or on S2, or on both stimulation pathways. Usually, LTP or LTD is induced just on one of the both pathways, and the other one is used as an internal control. LTP can be induced either by a high frequency stimulation or by a theta burst. LTD is induced by low frequency stimulation. You are free to change the stimulation pathway for each LTP / LTD Induction phase of the experiment.

Three different "LTP / LTD Induction" protocols can be selected:

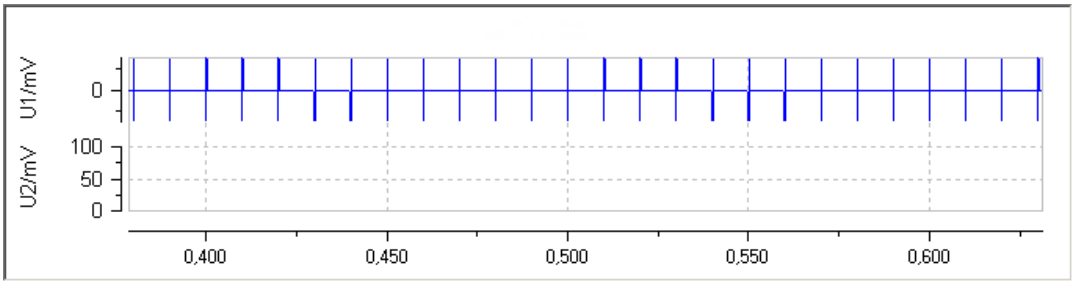
"**HFS**" (high frequency stimulation): A pulse with stimuli in equal time intervals is applied. The pulse duration "Train Duration" in ms, and the frequency "Freq." in Hz can be selected.

"**Theta Burst**" (high frequency pulse trains): An adjustable number of pulse trains is applied. The trains have an inter train interval of 200 ms. There are four pulses each train with the high frequency of 100 Hz per train.

"**LFS**" (low frequency stimulation): Low frequency stimulation with 1 Hz stimuli for a selected duration.

"**Post LTP / LTD Time**" in a range of 0 to 120 seconds the user can select a time span for recovery of the hippocampus slice after high frequency stimulation before the next phase starts.

A graphical display of the actually selected induction protocol for both pathways is depicted in the dialog window. Any changes made to the induction paradigm will be shown only after having been confirmed with the "Apply" button.



The settings in "**Pulse Shape**" are fix in this dialog. You are able to change them in "**Preparation**" menu.

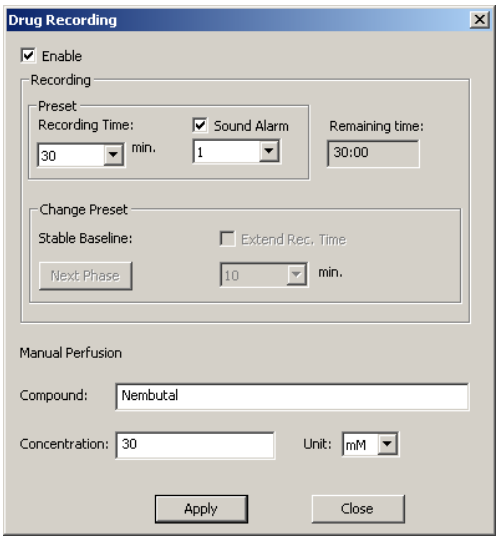
LTP / LTD

Click icon **LTP / LTD**. This "**LTP Recording**" dialog is identical to the "Baseline" dialog, please see above.

Drug Application

Click icon "**Drug Application**".

If your experimental outline includes the application of a drug, you can apply the compound in this phase of the experiment. If an automated application system is used, the drug application starts automatically. If the drug is applied manually, the program stops at the beginning of the drug phase and prompts the user to apply the drug.



In "**Drug Recording**" dialog you have the same "Recording" functions as in Baseline and LTP / LTD: You set "**Recording Time**", the "**Sound Alarm**", and the "**Remaining Time**" is counted down during running experiment. You can extent the drug recording time by "**Extent. Rec. Time**" or you can jump to the "**Next Phase**".

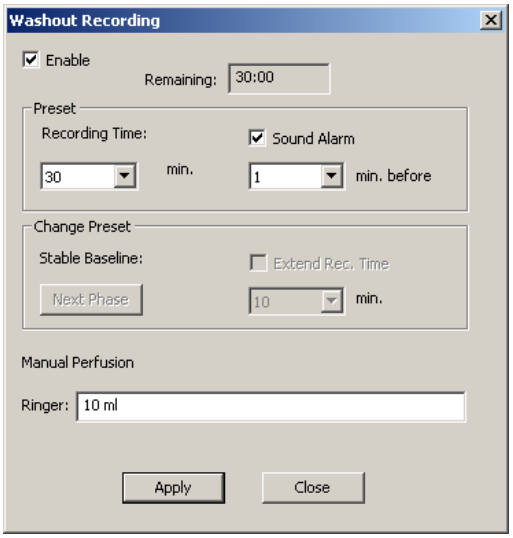
Additionally, there are the options "**Compound**" and "**Concentration**" to note the name and concentration of the drug. The "**Unit**" of the drug must be chosen from the drop down menu. This information will show up in the lab book.

All settings are confirmed with the command "**Apply**".

Washout

Click icon "**Washout**".

This phase is for removing the drug from the slice, usually done by applying standard ringer solution. Analog to Drug Phase, if an automated application system is used, the washout phase starts automatically. Otherwise, the program stops at the beginning of the washout phase and prompts the user to apply ringer.



In "**Washout Recording**" dialog again you have the typical "Recording" functions: You set "**Recording Time**" and "**Sound Alarm**". The "**Remaining Time**" of this phase is counted down. You can extent the drug phase recording time by clicking "**Extent Rec. Time**" during running experiment, or you can jump to the "**Next Phase**".

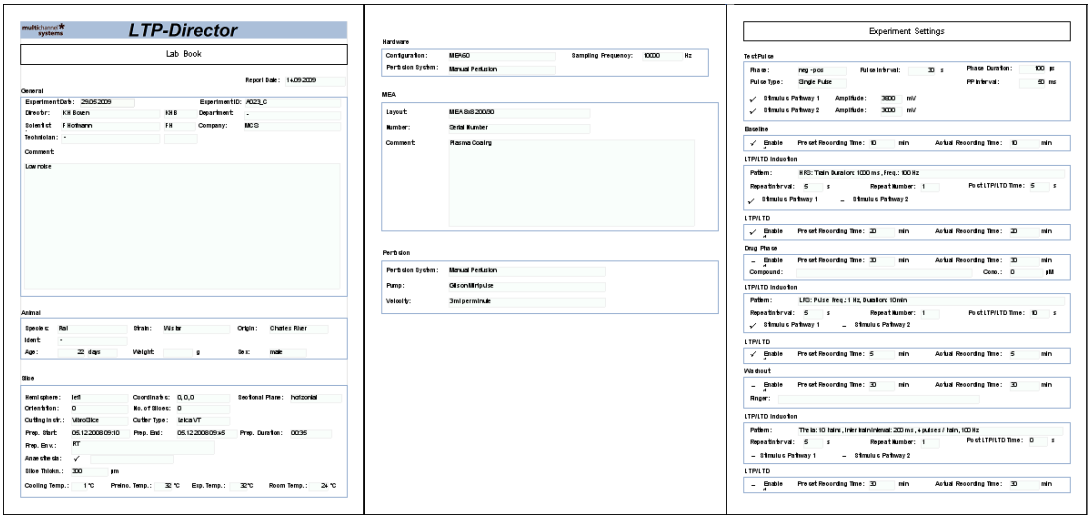
In the "**Ringer**" box you may write comments about the ringer solution.

All settings are downloaded with the command "**Apply**".

5.2.7 Report Sheet

Click the icon "**Report Sheet**".

The LTP-Director will generate a complete protocol of the experiment, including the entries of the lab book, the chronological sequence and the experimental settings for each phase of the experiment. The report prepares all background information necessary for analyzing the data with LTP-Analyzer.



5.2.8 Recording Check List

1. Close MC_Rack, MC_Stimulus and MEA_Select, if necessary.
2. Open LTP-Director.
3. Test Mode:
    - Select Hardware (Settings / Hardware).
    - Select electrode layout, MEA BC-Port and STG, if necessary.
    - Select Data Path and Filename (Settings / Options).
    - Load slice image, fit the image to the electrode grid.
    - Adjust stimulation parameters.
    - Select stimulation electrodes for pathways S1 and S2.
    - Select up to four dendritic and / or somatic electrodes for online analysis.
    - Switch to Measurement Mode.
4. Measurement Mode:
    - Optional: Open template.
    - Fill in Lab Book.
    - Run IO Curve.
    - Optional: Save IO curves as pictures or ASCII files.
    - Adjust stimulation parameters.
    - Adjust detection levels.
    - Select / Deselect experimental phases.
    - Adjust phase parameters.
    - Recheck stimulation pulse interval. (For testing, usually intervals of 5 or 10 s are used. For the real experiment, this might be too fast.)
    - Recheck file name and file path.
    - Optional: Open Experiment Comment window to make notes during the experiment.
    - Press Start.

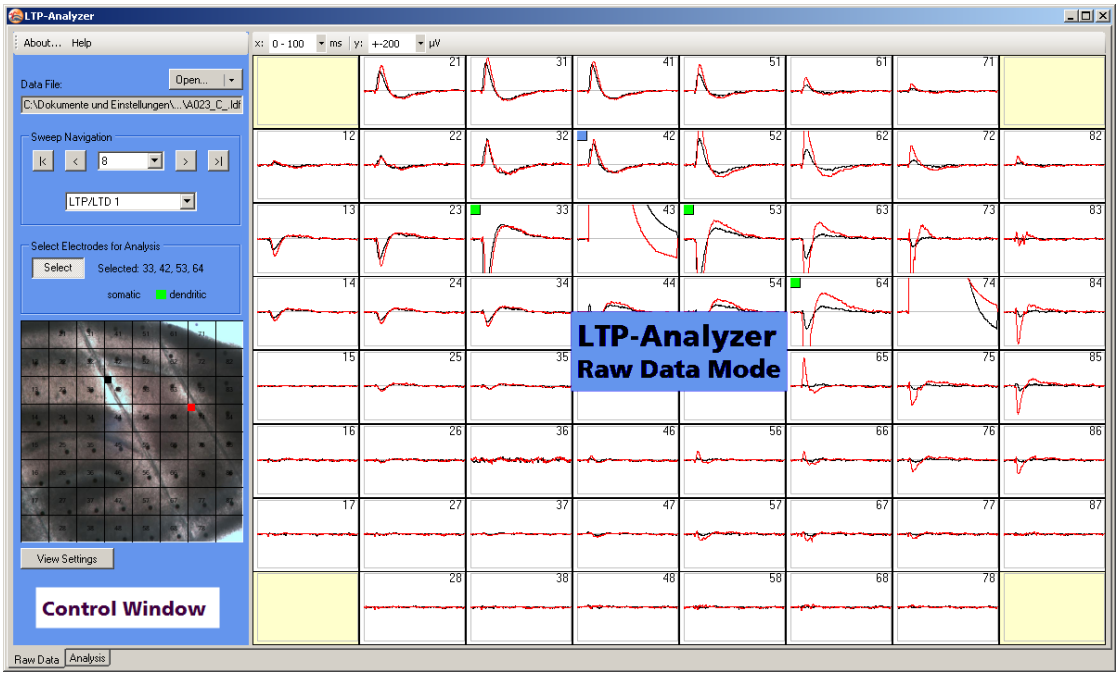
6 LTP-Analyzer

6.1 Replaying an Experiment in Raw Data Mode

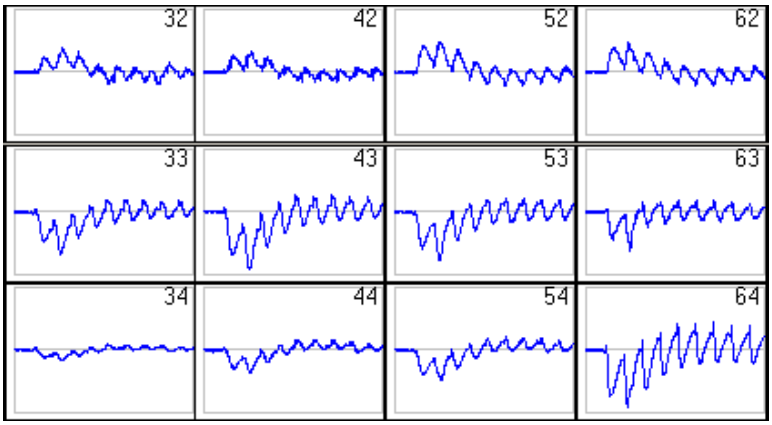
6.1.1 Data Display Raw Data Mode

The **LTP-Analyzer** is used to replay and analyze the data files generated by LTP-Director. Similar in design to the LTP-Director, LTP-Analyzer has two modes: Raw data mode is for displaying raw data, and analyzer mode is for analyzing data from selected electrodes.

The main window of the "**Raw Data Mode**" is designed similar to "Test Mode" in LTP-Director: On the left side the blue control window, and on the right side the raw data display with overlay traces in red and black, encoding the signals generated by stimulation pathway S1 (red) or S2 (black). Blue raw data traces are generated during the LTP / LTD induction phases. You can toggle between the two windows using the tabs at the bottom left side.

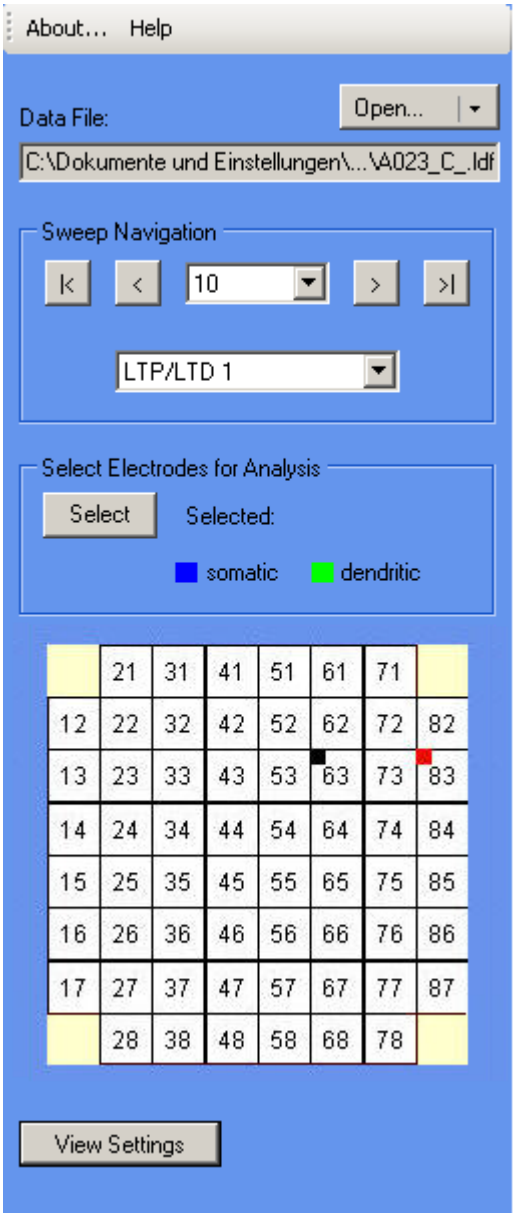


Example of blue data traces during LTP / LTD induction.



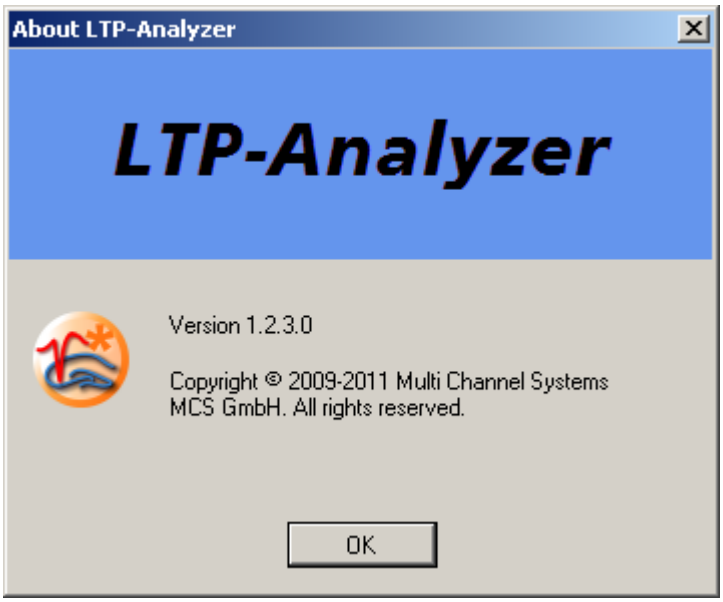
6.1.2 Control Window Raw Data Mode

Control Window Raw Data Mode: Control window for the experimental protocol in raw data mode.



About

Click on "**About**" in the header of the control window raw data mode to see information about the LTP-Analyzer software version.

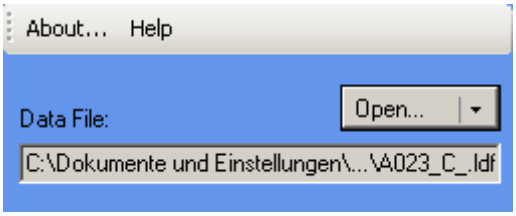


Help

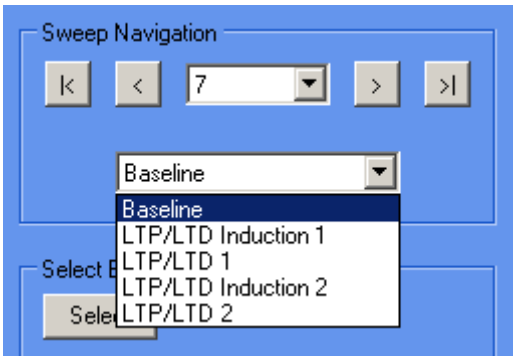
Clicking the option "**Help**" will open the online help file.

Open

Click "**Open**" to open a file browser and select one of the "*.ldf" files, generated by LTP-Director. Alternatively, you can open a drop down menu with recently opened files. The file name and file path of the opened files will be shown.



Sweep Navigation

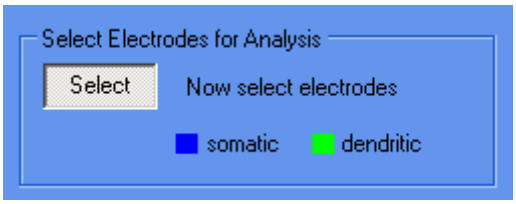


With "**Sweep Navigation**" it is possible to look at every individual sweep. The > button selects the next sweep; the >| button selects the last sweep of the experimental phase.

To select an experimental phase, use the phase navigation drop down menu. Data from the LTP Induction phase is displayed in blue. Data from all other phases is shown as an overlay of both pathways in red (pathway S1) and black (pathway S2) for each sweep.

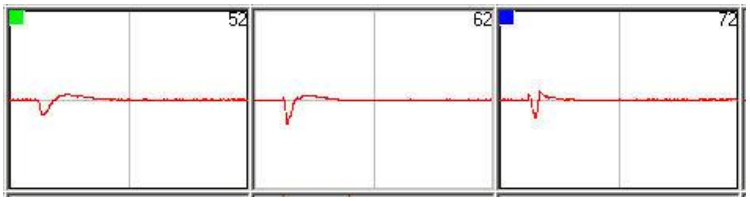
Select Electrodes for Analysis

Click "**Select**" to define electrodes with somatic or dendritic signals in the raw data display.

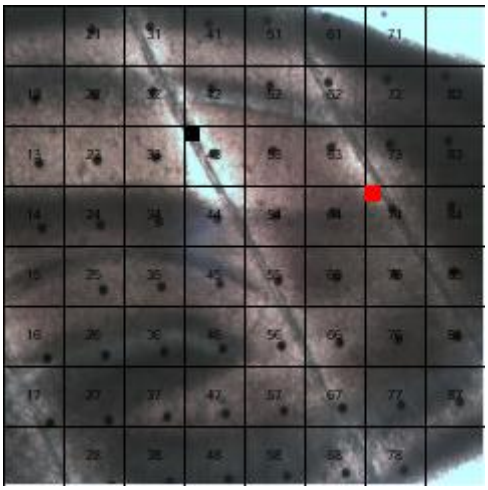


Similar to LTP-Director, you can select up to four electrodes for analysis, and label them as dendritic or somatic according to whether they show either somatic signals (PS = population spikes) or dendritic signals (EPSP = excitatory postsynaptic potential). To label an electrode as somatic, just click it. The electrode will be marked with a small blue square in the upper left corner. To label an electrode as dendritic, hold the Shift key and click it. The electrode will be labeled in green. To remove a selection, click on the blue or green square again. For comparison, please read chapter "Control Window Test Mode".

The data of selected electrodes will be displayed and analyzed in the analysis mode (see chapter "Analysis Mode").



Stimulation Electrodes

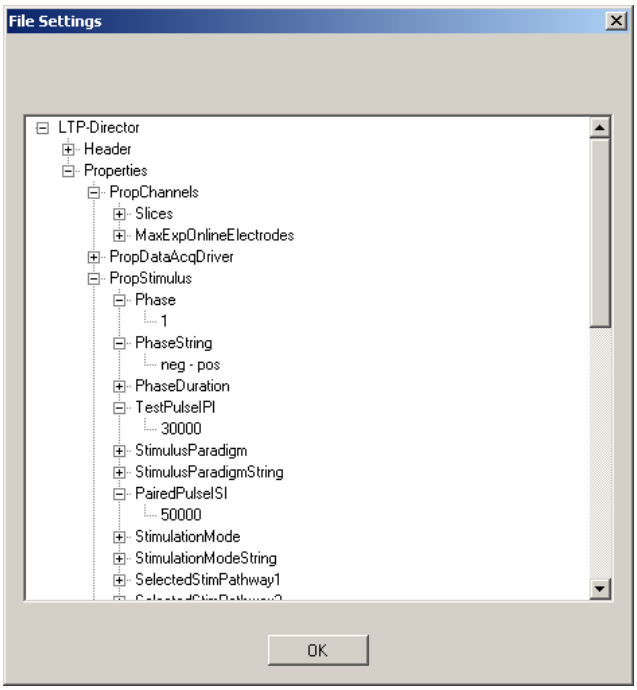


The stimulation electrodes that were used in the experiment are labeled in red (S1) and black (S2). If there has been an image in the back of the stimulation electrode field in LTP-Director, the image appears in LTP-Analyzer respectively.

View Settings

Click "**View Settings**" to review all available setting of the actual used LTP-Director file.

The information is organized in a tree view.

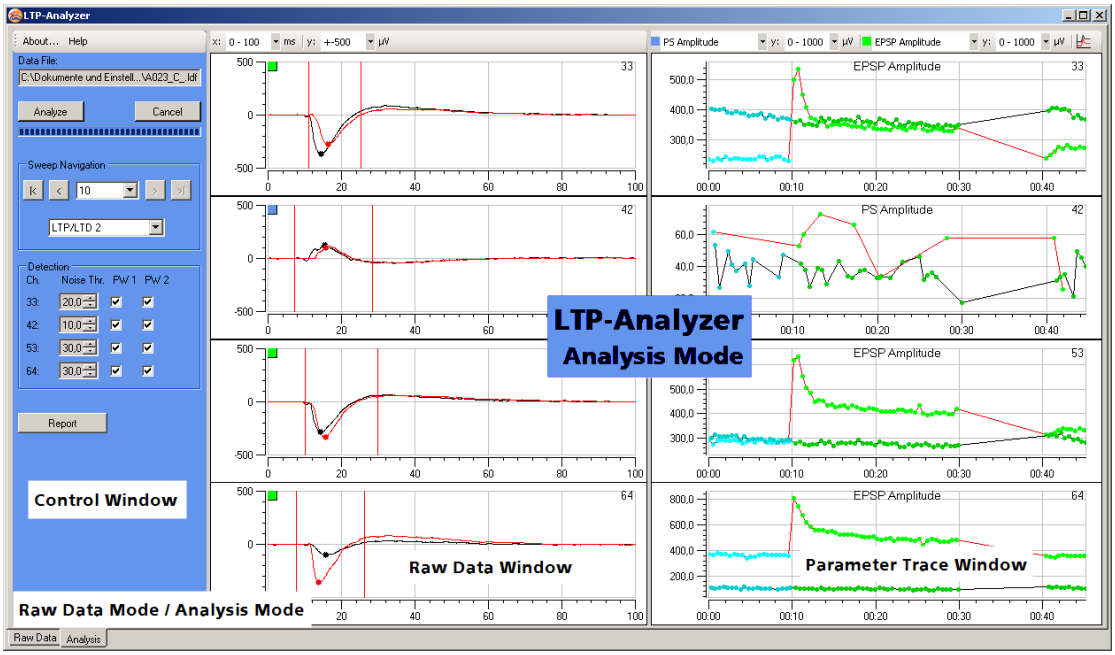


6.2 Analyzing an Experiment in Analysis Mode

6.2.1 Data Display Analysis Mode

On the bottom left corner of your screen, you can toggle between "Raw Data" and "Analysis" mode.

The design of the main window of the **Analysis Mode** of LTP-Analyzer is similar to that known from the measurement mode of LTP-Director: On the left side, there is the blue control window, besides the data display. The data display is separated into smaller windows. You can observe the data traces of up to four - in raw data mode selected - electrodes and you see parallel the online analysis of the selected parameter of these electrodes.



Hint: For information about the multiple display features like scrolling and zooming, please read chapter "General LTP-Director Software Features".

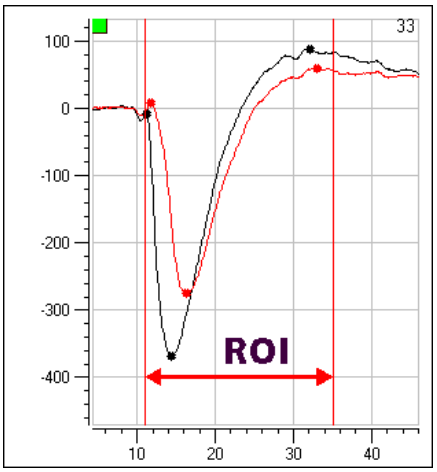
Data Trace Window

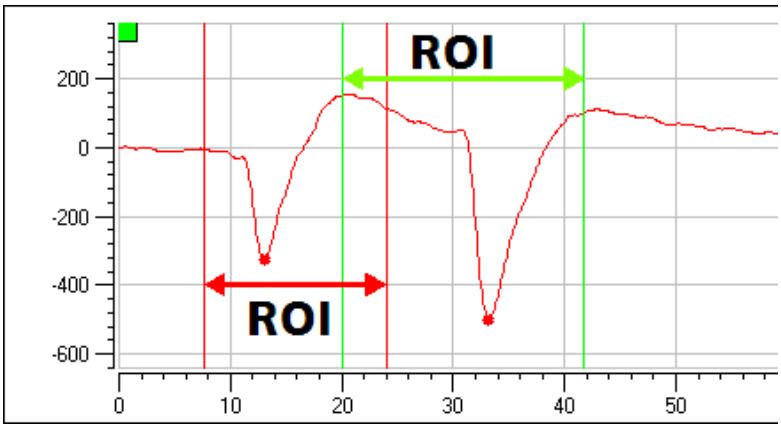
On the left part of the display you see the data traces of the four - in raw data mode selected - electrodes one below the other. Data traces belonging to stimulation pathway S1 are shown in red, data traces belonging to stimulation pathway S2 are black. Both data traces are displayed in overlay. Data traces of the LTP / LTD Induction phases are blue.

The data traces are marked with dots (in red or black). These dots indicate all maxima and minima, detected inside the ROI (region of interest). The ROI is defined between two lines. You can adjust the ROI by dragging the lines with the mouse. Please read also chapter "Signal Detection".

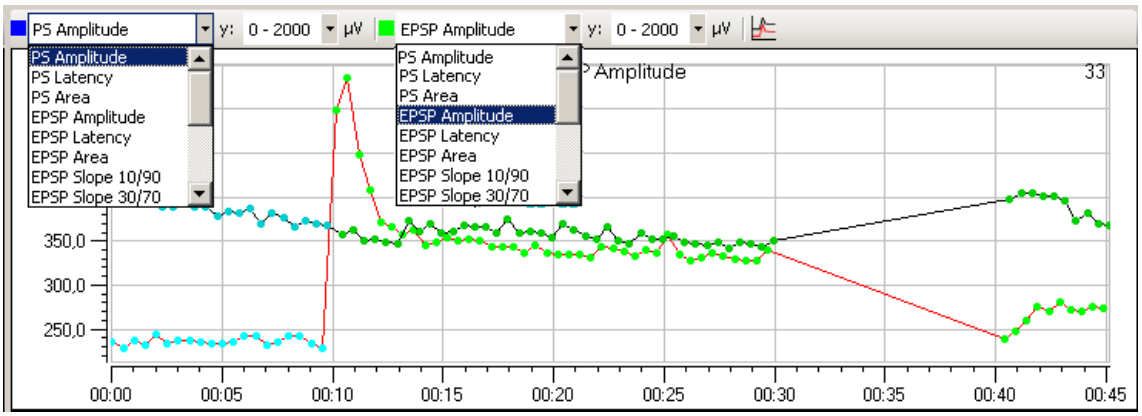
Enhance a data trace window in analysis mode by double clicking it. Click on to show a crosshair cursor. The crosshair can be used to determine the exact x- and y-values of any given point in the data trace. Click on to export the actual visible trace as ASCII file. Click on the drop down menus to scale the axes.

When single pulses have been used only one ROI is active. When a paired pulse paradigm was used, there are two ROIs, which may overlap each other.

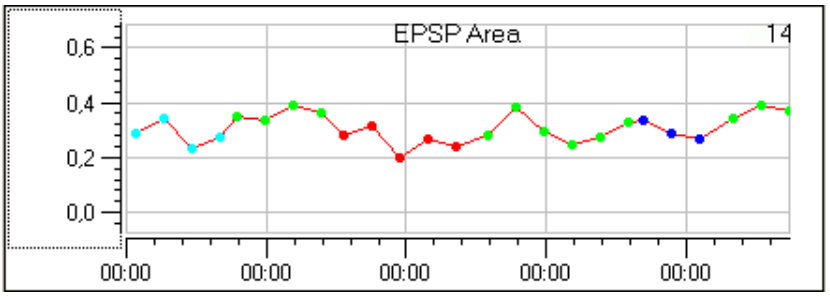




Parameter Trace Window

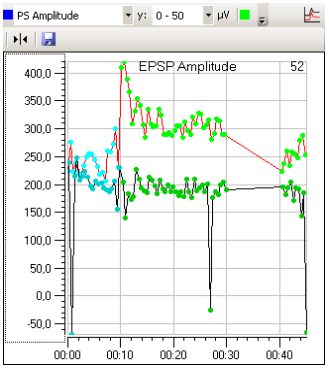


On the right part of the main window you see the parameter display for the online analysis related to the four selected electrodes. All available parameters are analyzed. The drop down menus in the toolbar can be used to select the parameter to be displayed. Different parameters can be displayed for somatic and dendritic electrodes.



The colors of the data points encode the different phases of the experiment. The color of the connecting lines encodes the stimulation pathway S1 and S2 in red and black as usual. If you click on one of the data points in the parameter window, the corresponding data trace will be shown in the data trace window. Additionally you see in "**Sweep Navigation**" in the control window the number of that sweep and the appropriate phase of the experiment.

Enhance a parameter trace window in analysis mode by double clicking it. Click on to show a crosshair cursor. The crosshair can be used to determine the exact x- and y-values of any given point in the data trace. Click on to export the actual visible trace as ASCII file. Click on the drop down menus to select the displayed parameter.



Hint: To handle the multiple display features for scrolling and zooming, for example, please read chapter "General LTP-Director Software Features".

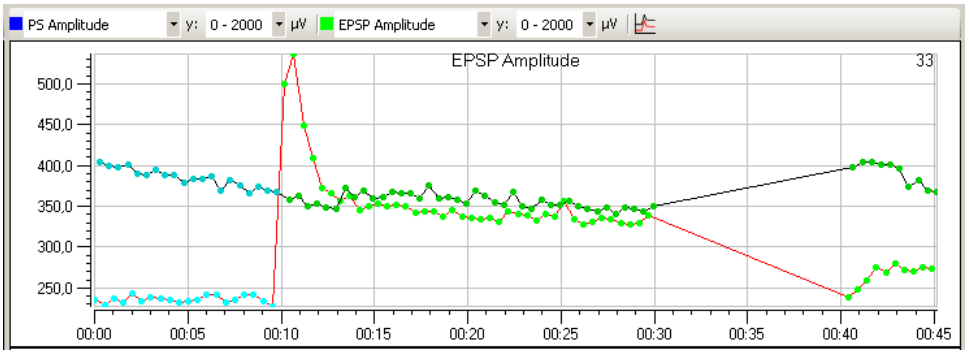
Option: Relative Values

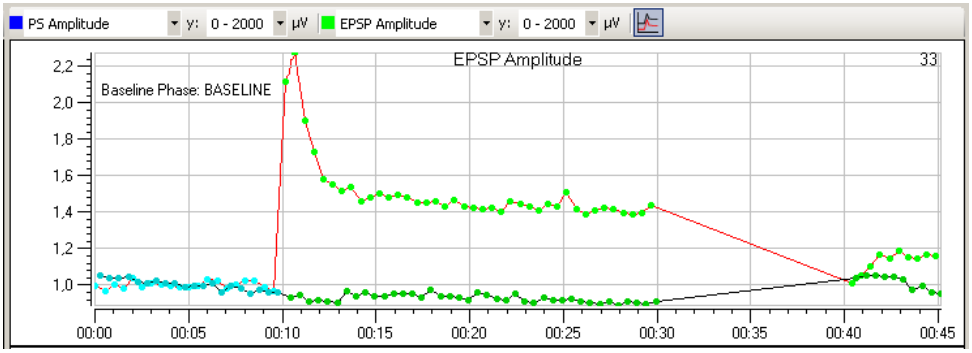
To compare data from different experiments, absolute values from individual experiments are usually normalized on the baseline of the respective experiment. This can be done automatically in LTP-Analyzer before exporting the data as ASCII file.

After the analysis is finished, click the icon "**Show relative values**" in "**Analyzer Mode**".

The analyzed data values are recalculated relative to the first phase of your experiment, "Baseline", "Drug Application" or "Washout". Which phase is used as baseline is displayed in the data trace window. If none of these three phases was used as first phase in the recording, no relative values can be calculated.

All absolute values of the underlying baseline are averaged, and all data points are calculated relative to this average. The result is plotted in the parameter trace window instead of the absolute values. This option is only available after a successful analysis.

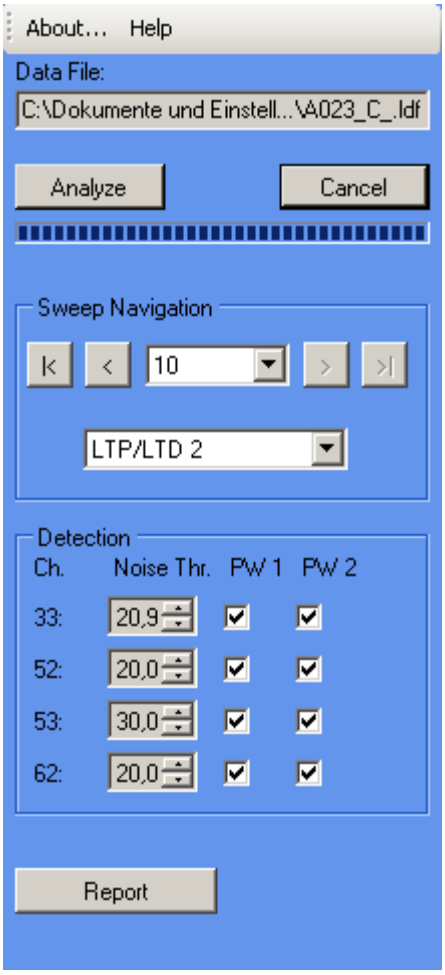




Relative data can be exported as ASCII file and directly averaged over several experiments, for example, in Excel or Origin.

6.2.2 Control Window Analysis Mode

In the control window it is possible to navigate between experimental phases and sweeps, and to adjust the detection threshold for the individual electrodes. Data can be analyzed sweep by sweep, using the arrow buttons, or the whole data file can be analyzed at once with the "**Analyze**" button.

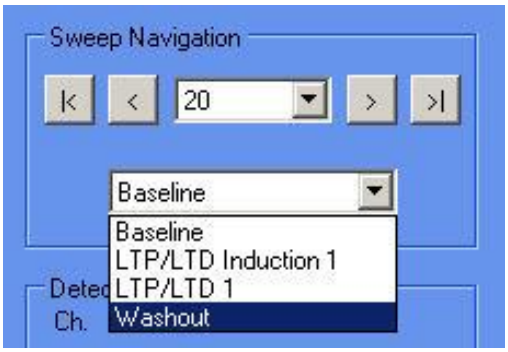


The blue window pane on the left side of the main window in analysis mode includes the command buttons for analyzing the recorded data sweep for sweep with sweep navigation or in running progression with "**Analyze**" button. You are able to switch between the phases of the experiment, and you can change the detection parameters for the up to four selected electrodes. When the analysis has finished you are able to create a "Report" sheet.

Analysis

After adjusting the detection levels (see paragraph "Detection" below) and selecting a parameter to analyze (see the drop down menus in "Parameter Display"), press the "**Analyze**" button. One parameter is displayed at a time, but all parameters are analyzed. The LTP-Analyzer will analyze the complete data file. The progress of analysis is shown in the status bar below. To stop the analysis, click "**Cancel**".

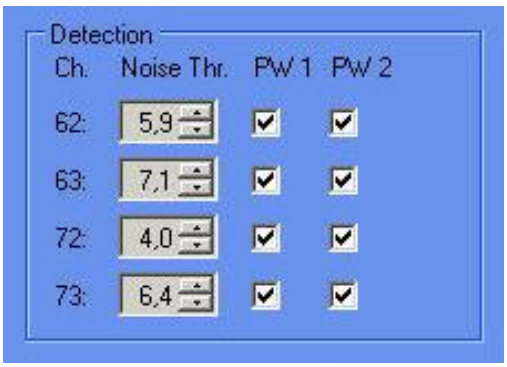
Sweep Navigation



Sweep navigation is identical to raw data mode. You can select the designated sweep from the drop down menu, or you can navigate by clicking the icons besides the drop down menu. The > button selects the next sweep, the >| button selects the last sweep of the experimental phase.

To select an experimental phase, use the phase navigation drop down menu. Data from the LTP Induction phase is displayed in blue. Data from all other phases is shown as an overlay of both pathways in red (pathway S1) and black (pathway S2) for each sweep.

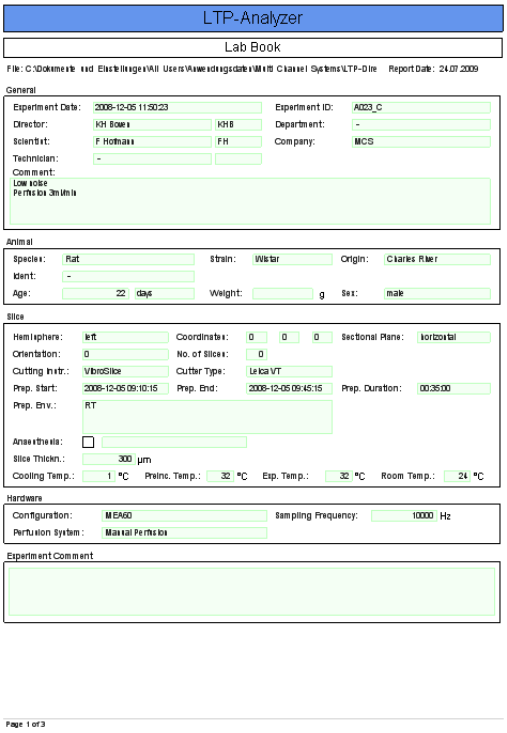
Detection



The "**Detection**" levels are given in multitudes of the noise level. It is possible to adjust the detection levels to improve the analysis results. For each electrode, stimulation pathway S1 and S2 can be selected or deselected. After changing detection levels or the region of interest (ROI) it is necessary to rerun the analysis to refresh the Parameter Display.

Report

Click the icon "**Report**" to create a report sheet with all relevant information about the lab book and the experimental settings of the actual analyzed file.



LTP-Analyzer

Lab Book

File: C:\Dokumente und Einstellungen\All Users\Anwendungsdaten\Multi Channel Systems\LTP-Dire Report Date: 24.07.2009

General

Experiment Date:	2008-12-05 11:50:23		Experiment ID:	A023_C	
Director:	KH Boven	KHB	Department:	-	
Scientist:	F Hofmann	FH	Company:	MCS	
Technician:	-				

Comment:
Low noise
Perfusion 3ml/min

Animal

Species:	Rat		Strain:	Wistar	Origin: Charles River
Ident:	-				
Age:	22 days		Weight:	g	Sex: male

Slice

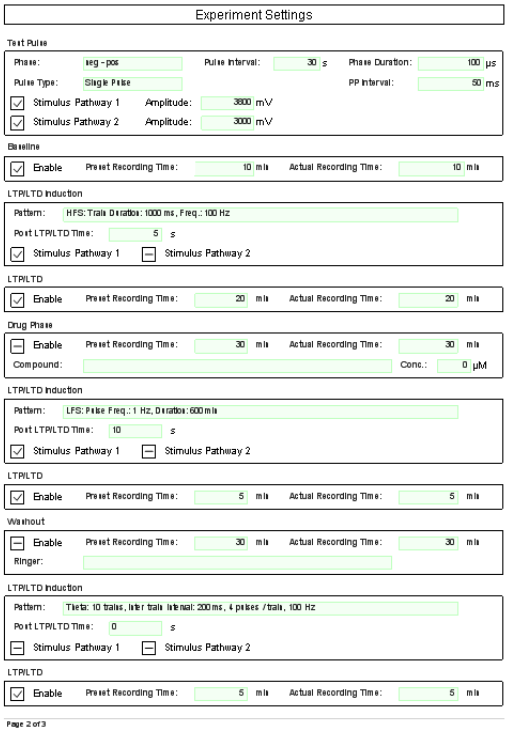
Hemisphere:	left		Coordinates:	0 0 0	Sectional Plane: horizontal
Orientation:	0		No. of Slices:	0	
Cutting Instr.:	VibroSlice		Cutter Type:	Leica VT	
Prep. Start:	2008-12-05 09:10:15		Prep. End:	2008-12-05 09:45:15	Prep. Duration: 00:35:00
Prep. Env.:	RT				
Anaesthesia:					
Slice Thickn.:	300 µm				
Cooling Temp.:	1 °C	Preinc. Temp.:	32 °C	Exp. Temp.:	32 °C Room Temp.: 24 °C

Hardware

Configuration:	MEA60	Sampling Frequency:	10000 Hz
Pertusion System:	Manual Perfusion		

Experiment Comment

Page 1 of 3



Experiment Settings

Test Pulse

Phase:	neg - pos	Pulse Interval:	30 s	Phase Duration:	100 µs
Pulse Type:	Single Pulse			PP Interval:	50 ms
☑ Stimulus Pathway 1	Amplitude:	3800 mV			
☑ Stimulus Pathway 2	Amplitude:	3000 mV			

Baseline

☑ Enable	Preset Recording Time:	10 min	Actual Recording Time: 10 min

LTP/LTD Induction

Pattern:	HFS: Train Duration: 1000 ms, Freq.: 100 Hz
Post LTP/LTD Time:	5 s
☑ Stimulus Pathway 1	☐ Stimulus Pathway 2

LTP/LTD

☑ Enable	Preset Recording Time:	20 min	Actual Recording Time: 20 min

Drug Phase

☐ Enable	Preset Recording Time:	30 min	Actual Recording Time: 30 min
Compound:			Conc.: 0 µM

LTP/LTD Induction

Pattern:	LFS: Pulse Freq.: 1 Hz, Duration: 600 min
Post LTP/LTD Time:	10 s
☑ Stimulus Pathway 1	☐ Stimulus Pathway 2

LTP/LTD

☑ Enable	Preset Recording Time:	5 min	Actual Recording Time: 5 min

Washout

☐ Enable	Preset Recording Time:	30 min	Actual Recording Time: 30 min
Ringer:			

LTP/LTD Induction

Pattern:	Theta: 10 trains, Inter train interval: 200 ms, 4 pulses / train, 100 Hz
Post LTP/LTD Time:	0 s
☐ Stimulus Pathway 1	☐ Stimulus Pathway 2

LTP/LTD

☑ Enable	Preset Recording Time:	5 min	Actual Recording Time: 5 min

Page 2 of 3

7 General LTP-Director Software Features

7.1 Display Toolbar for Single Channel View

Double-click on one channel of the data display. The window will be enhanced and you will find several options for dealing with the data. Several tools for zooming and scrolling the data as well as exporting the graph are provided on the toolbar of the enhanced online display.

Enable scrolling	Scroll the axes by clicking and dragging with your mouse or by pressing keys on your keyboard.
Enable zooming	Zoom the axes by clicking and dragging with your mouse or by pressing keys on your keyboard.
Zoom in	Zoom all axes in.
Zoom out	Zoom all axes out.
Zoom box	Zoom in a particular region of the display. The box can start at any point, but must be dragged to the lower right to actually cause a zoom.
Show/hide data cursors	Show a vertical cursor / crosshair. The cursor shows the x- and y-values for the trace line of the assigned channel. By right clicking on the cursor, a context menu is displayed where you can change the channel assignment.
ASCII export	Exports display contents in ASCII file format.
Snapshot	Exports display contents in graphic file format.

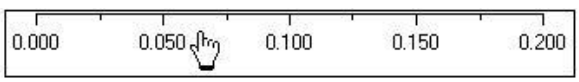
7.1.1 Scrolling and Zooming

Scrolling and zooming with the mouse wheel

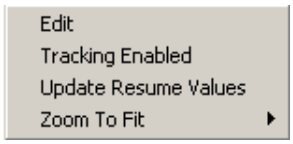
1. Click an axis to select it. This is indicated by a tiny black line around the selected axis.
2. Use the mouse wheel to scroll the axis.
   - OR -
   Keep pressing the left mouse and move the mouse to scroll the axis.
3. Press the CTRL key at the same time for zooming the axis.

Scrolling with the mouse or keyboard

1. Click to enable the scrolling.
2. Click the axis you like to scroll. The mouse pointer becomes a hand. The unit of this axis will appear as a tool tip.



3. Click on the right mouse button. Click "Tracking Enabled".



While holding down the left mouse button, move the mouse pointer to the left and right, or up and down.
- OR –
You can use the keyboard: Press LEFT ARROW or DOWN ARROW to move the selected axis to the left (down), RIGHT ARROW or UP ARROW to move the selected axis to the right (up).
- OR –
Use the PAGE UP and PAGE DOWN keys for a faster scrolling (larger steps): PAGE DOWN to move to the left (down), PAGE UP to move to the right (up).

Zooming with the mouse or keyboard

1. Click to enable the zooming.

2. Click the axis you like to zoom.

   The mouse pointer becomes a double-headed arrow.

3. While holding down the mouse button, move the mouse up/right to zoom the axis in, and move the mouse down / left to zoom the axis out.
   - OR –
   You can use the keyboard: Press LEFT ARROW or DOWN ARROW to zoom the axis out, RIGHT ARROW or UP ARROW to zoom the axis in.
   - OR –
   Use the PAGE UP and PAGE DOWN keys for a faster scrolling (larger steps): PAGE DOWN to zoom out, PAGE UP to zoom in.

Zooming with the zoom box

The zoom box allows the user to outline a region in the display for zooming into. The box can start at any point, but must be dragged to the lower right to actually cause a zoom. The zoom action will affect all axes.

1. Click to enable the zoom box.

2. Move the mouse pointer over the display.

   The mouse pointer becomes an arrow with a little box.

3. Click any point in the display and move the mouse pointer to the lower right. The zooming box appears around the outlined region.

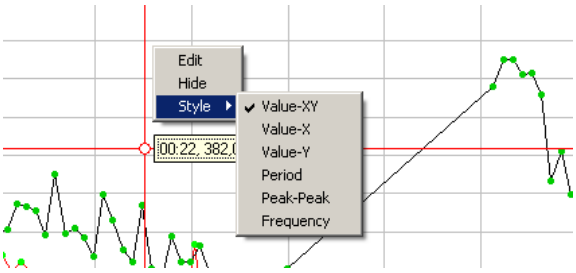
4. Release the mouse button for zooming into the selected region.

Zooming with the Zoom In and Zoom Out buttons

1. Click to zoom in all axes or click to zoom out all axes.
2. If you want to zoom only the x- or y-axis, click the respective axis first (the selected axis will be highlighted with a dotted line) and then click the appropriate zoom button.

Showing / hiding the data cursor labels

Click to show a vertical cursor or a crosshair.



The cursor shows the x- and y- values for the trace line of the assigned channel. By right mouse click on the cursor, a context menu is displayed where you can change the channel assignment.

ASCII Export of Graphics

Click to save the displayed data as a tab-delimited ASCII file (text without formatting). The format is tab-delimited ASCII, so that the file can easily be loaded into other applications. ASCII export is available for all plot types.

You can open the exported files with an editor or with your custom analyzing software, for example, Excel or Origin.

Image Capture

You can take a screen shot of the all display types. On the display toolbar, click the Snapshot button to take a screen shot of the displayed data.

The pictures are screen shots, which means, the resolution of the output file resembles the screen resolution, that is, 72 dpi. If you then print the image with a 300 dpi resolution, the size of the image will be reduced accordingly. Therefore, you should expand the display as much as possible to obtain a big image with a good printing quality. The higher resolution you need, the bigger has to be the display.

If you select the Enhanced Meta File format, a vector graphic is generated. You can then use a standard vector graphics program such as Adobe Illustrator or Coreldraw to scale and edit the graphics without quality loss.

The following image file formats are supported.

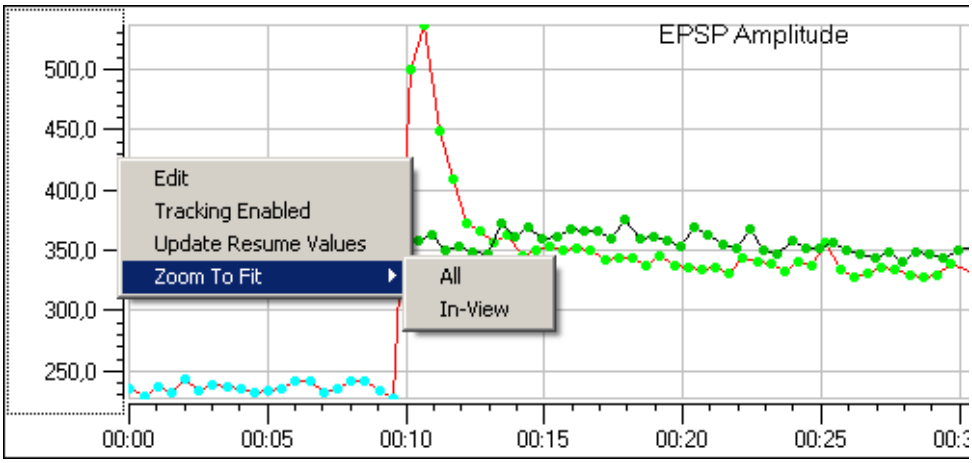
· (*.emf), Enhanced Meta File format

· (*.bmp), Bitmap

· (*.jpg), JPEG

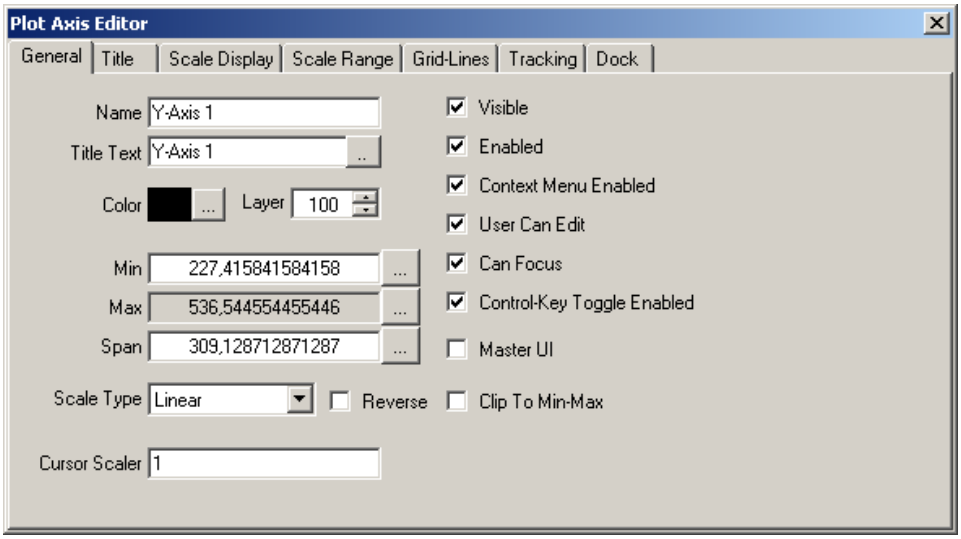
Plot Axes Editor

If you click on an axis with the right mouse button, for example, in the parameter window of the LTP-Analyzer, following menu appears.



You have four options: "Tracking Enabled", "Update Resume Values", "Zoom To Fit" and "Edit".

Click "Edit".



With the "Plot Axis Editor" you can adapt the format of the graph, for example, for special requirements of a publication.

8 Signal Detection

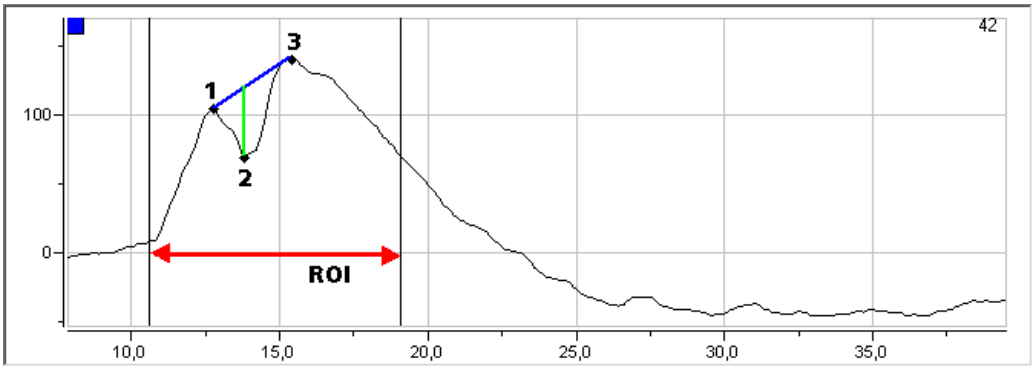
8.1 Signal Detection

All input signals are filtered by a Savitzky Golay filter. The Savitzky Golay method essentially performs a local polynomial regression on a series of values to determine the smoothed value for each point. The purpose is to smooth a noisy signal without greatly distorting the signal. Please set the parameters of the Savitzky Golay filter in main menu "Settings", "Filter".

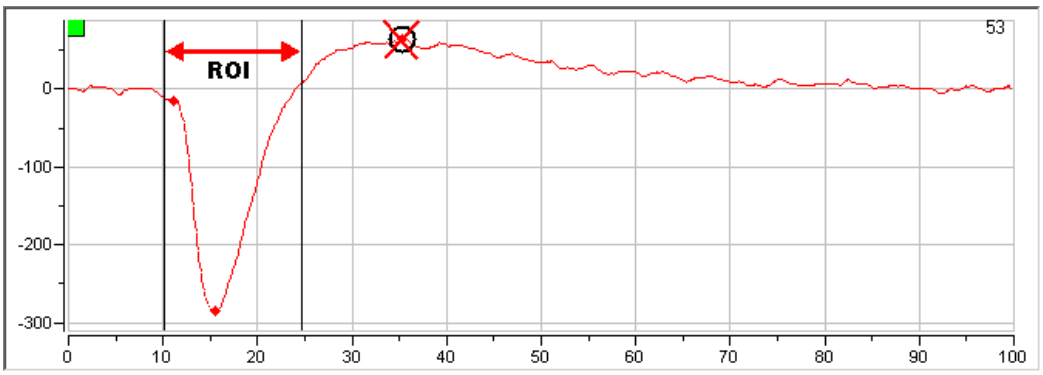
To analyze the data of each sweep it is necessary to detect the evoked response of the slice. In order to separate the signal from the stimulation artifact, the detection is performed within a user adjustable region of interest (ROI).

The detection algorithm finds the signal via detection of local minima and maxima and applies a three point pattern to detect population spikes (PS) from the soma of a neuron, and excitatory post synaptic spikes (EPSP) from the dendrite of a neuron. The detected maxima and minima are shown as dots in the signal trace. It is important for the signal detection to work properly with the ROI. The region of interest has to be set left and right of the response signal, otherwise the three point pattern can not be used.

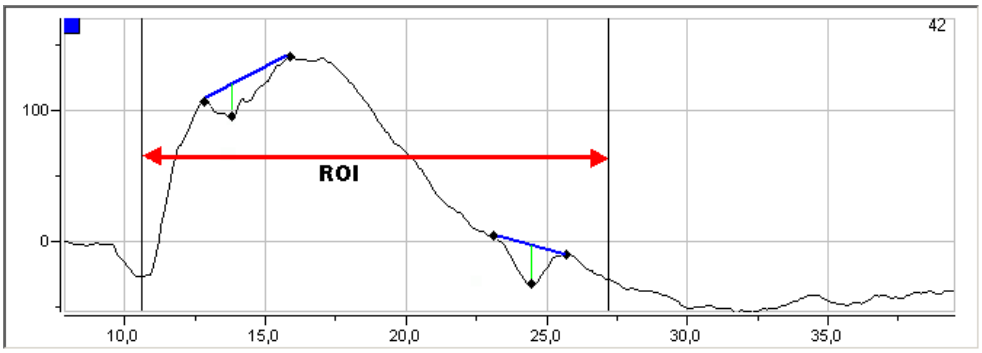
The picture shows a typical signal of a somatic evoked response. The ROI settings are correct for detecting the pop spike (PS) via three point pattern.



In this example the ROI settings are not correct for spike detection via three point pattern. The right ROI cursor is moved too much left, and the third maximum peak of the dendritic EPSP is not detected anymore.

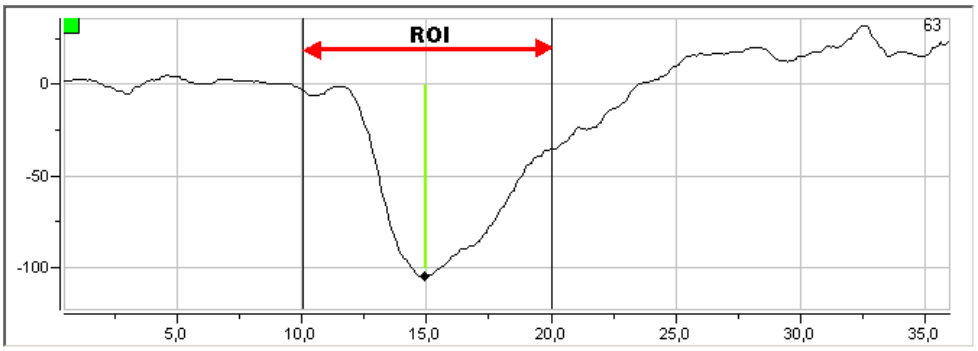


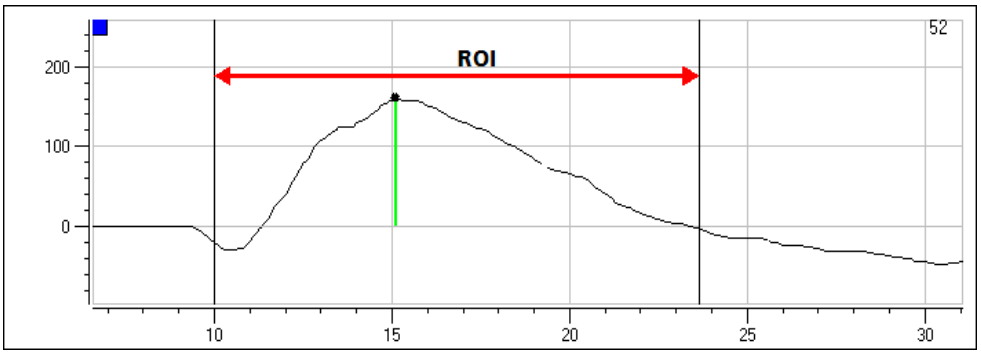
If the three point pattern matches more than once for the signal, the other matches are marked in the signal trace as well. For the analysis of the signal parameters the pattern with the maximum amplitude is used for calculation.



EPSP or PS Amplitude

For calculation of the amplitude either somatic or dendritic, the distance between baseline and absolute maximum or minimum point is the background.



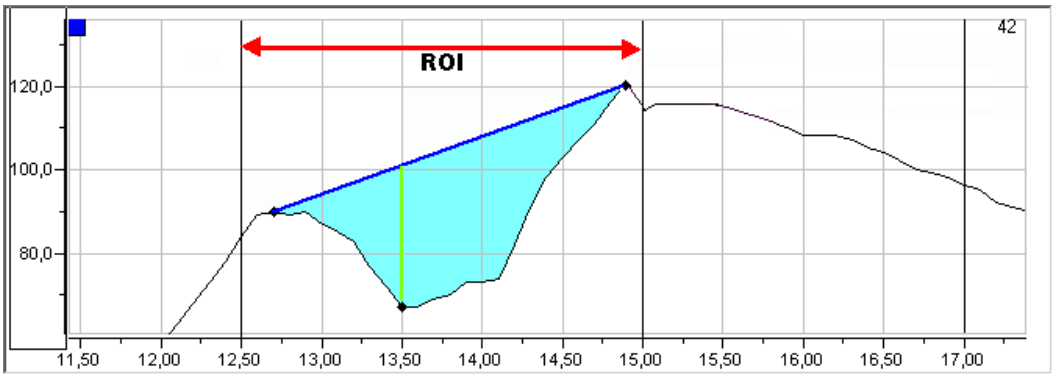


EPSP or PS Latency

The latency of a detected EPSP or PS spike is the time distance from the event of the stimulus to the middle extremum point. EPSP Latency and PS Latency values are in units of ms.

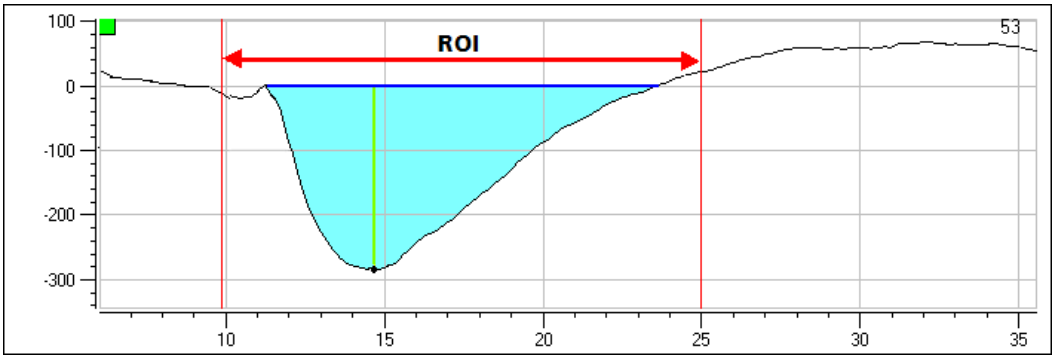
PS Area

The PS Area is calculated as area between the signal maximum and the straight line connecting the left and right extremum points. The first value counted is first value of the trace below the baseline. The last value is taken either the first value above the baseline or a fixed limit. The left and right borders are the left and right extremum points. The PS Area value is in units of µV * ms (10^{-9} Vs).



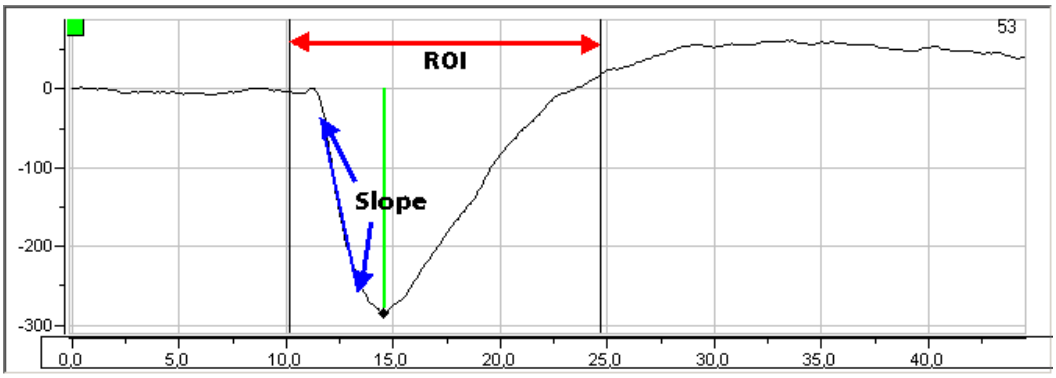
EPSP Area

The EPSP Area is the area between the data trace and the baseline. The first value counted is first value of the trace below the baseline. The last value is taken either the first value above the baseline or a fixed limit.



EPSP Slope

The slope is calculated by using a linear regression line of the data points in the 10 % to 90 % (or 30 % to 70 %) interval of the first EPSP amplitude (stretching from extremum to baseline) in the region of interest. The slope of the resulting straight line is extracted as the EPSP slope value. The EPSP Slope value is in units of V/s.



Corresponding Parameter for Paired Pulse Pattern

It is possible to analyze and display the parameters explained above for paired pulses respectively. The principle of signal detection is not modified. As usual PS = population spikes are somatic signals, and EPSP = excitatory postsynaptic potential are dendritic signals.

PP Ratio PS Amplitude

PP Ratio PS Latency

PP Ratio PS Area

PP Ratio EPSP Amplitude

PP Ratio EPSP Latency

PP Ratio EPSP Area

PP Ratio EPSP Slope 10/90

PP Ratio EPSP Slope 30/70

9 Troubleshooting

9.1 About Troubleshooting

The following hints are provided to solve special problems that have been reported by users. Most problems occur seldom and only under specific circumstances. Please check the mentioned possible causes carefully when you have any trouble with the product. In most cases, it is only a minor problem that can be easily avoided or solved.

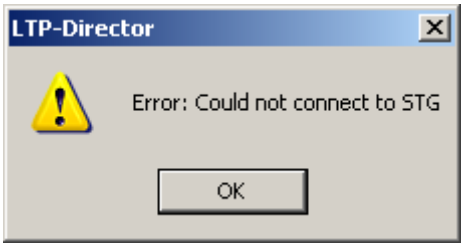
If the problem persists, please contact your local retailer. The highly qualified staff will be glad to help you. Please inform your local retailer also, if other problems not mentioned here occur, even if you have solved the problem on your own. This helps other users, and it helps MCS to optimize the instrument and the documentation.

9.2 Error Messages

LTP-Director: Data Display Test Mode

If you start the LTP-Director in Test Mode, the software tries to connect the MEA1060 amplifier. Please select the correct COM port from the drop down menu "MEA-BC Port" in the Control Window for connecting the MEA amplifier.

LTP-Director: Data Display Test Mode



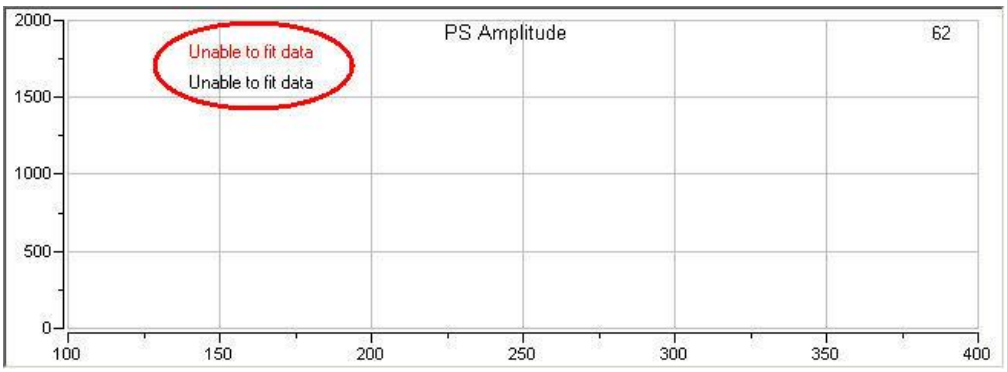
If you start the LTP-Director in Test Mode, the software tries to connect the STG. Please select the correct STG from the drop down menu "STG" in the Control Window for connecting the STG.

LTP-Director: Data Display Test Mode

The amplitude of the stimulus test pulse has to be an integral number in a range between 0 and 8000 mV. Please select an appropriate value.

LTP-Director: Data Display Measurement Mode

Parameter Window



When running an IO curve under "**Preparation**" in measurement mode, the program will try to fit the data to a sigmoidal function. If the data is not sigmoidal, you will receive the error message "Unable to fit data" shown above. Please try to change the minimal and maximal stimulation strength to circumvent the problem.

10 Appendix

10.1 Contact Information

Local retailer

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

User forum

The Multi Channel Systems User Forum provides the opportunity for you to exchange your experience or thoughts with other users worldwide. You can access the forum via the Multi Channel Systems web site.

Mailing list

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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