

# MED64-Entry Manual



## Table of Contents

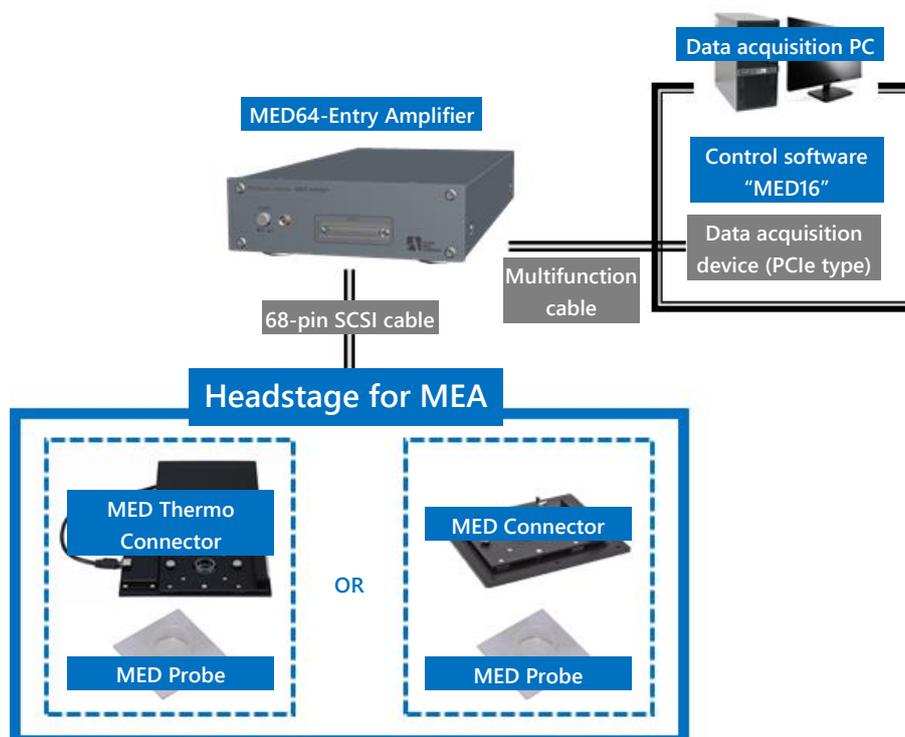
---

- 1. Introduction ..... 1
  
- 2. Configuration of the MED64-Entry ..... 1
  - 2.1. Part names and functions ..... 2
    - 2.1.1. MED64-Entry Amplifier ..... 2
    - 2.1.2. Accessories of the MED64-Entry Amplifier ..... 2
  
- 3. Setup of the MED64-Entry ..... 3
  - 3.1. Connection of the data acquisition device to the PC ..... 3
  - 3.2. Considerations for the position of devices ..... 4
  - 3.3. Power source ..... 4
  - 3.4. Connection to the MED64-Entry Amplifier ..... 5
    - 3.4.1. Part names and functions of the MED Connector ..... 5
    - 3.4.2. Connections of the MED Connector and the data acquisition device ..... 5
    - 3.4.3. Positioning of the terminal of the MED Connector ..... 6
    - 3.4.4. Electric shield with aluminum foil ..... 7
    - 3.4.5. Preparation for the noise check -attaching the MED Probe ..... 7
    - 3.4.6. Noise check ..... 8
    - 3.4.7. Maintenance of the MED Connector ..... 8
  
- 4. Data acquisition by the control software "MED16" ..... 9
  - 4.1. Setting the data acquisition schedule - RECORD subpanel ..... 10
  - 4.2. Creating the stimulation pattern - STIMULATION subpanel and STIMULATION PATTERN tabs ..... 11
  - 4.3. Set data file output - FILE OUTPUT subpanel ..... 13
  - 4.4. SIGNAL WAVEFORM panel ..... 14
  - 4.5. BASELINE STABILITY panel ..... 14
  - 4.6. Replaying data file output - function of REPLAY mode ..... 16
  
- 5. Abnormal noise ..... 17
  - 5.1. Noise check point ..... 17
  - 5.2. Noise relation to installation ..... 17
  - 5.3. Noise relating to perfusion ..... 19
  - 5.4. Identification of cause when a malfunction of the device is suspected ..... 20
  
- 6. Appendix ..... 21
  - 6.1. Specification ..... 21
    - 6.1.1. MED64-Entry Amplifier [MED-A16HM1] ..... 21
    - 6.1.3. MED Connector [MED-C03] and MED Thermo Connector [MED-CP04] ..... 22
    - 6.1.6 Data Acquisition PC System ..... 22
  - 6.2. Explanation of the technology ..... 23
    - 6.2.1. Signal acquisition by the MED64 System ..... 23
    - 6.2.2. Electric stimulation by the MED64 System - Applying current to the micro electrode array ..... 24
    - 6.2.3. Stimulus artifact and biphasic stimulation ..... 24
    - 6.2.4. Stimulus current value and electrolysis ..... 25
    - 6.2.5. About the stimulus interval ..... 26

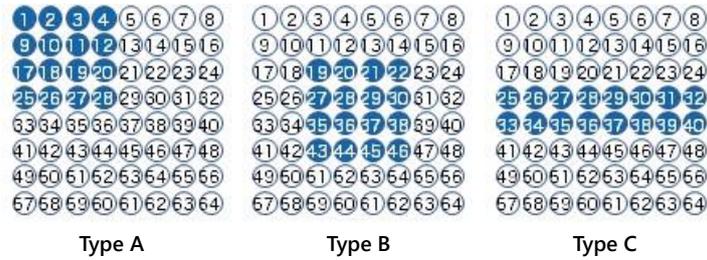
## 1. Introduction

- ◆ The MED64 system is a micro electrode array (MEA) system commercialized for the first time in the world in 1997. Since then, the MEA system has been widely used in the United States and the EU for basic research in the fields of the central nervous system and the cardiovascular system. Nowadays, its application to screening for drug discovery is rapidly spreading, thanks to its highly evaluated excellent operability and efficiency.
- ◆ The system use a MEA with 64 planar electrodes arrayed in a pattern (MED probe) on a glass substrate. Users can acquire extracellular potential simply by placing a tissue slice or by culturing cells on these electrodes. Current stimulation can be applied through any electrode selected by software. Unlike a glass capillary electrode, the MED64 system requires no troublesome operations to measure spontaneous activity or evoked response and no special training for electrophysiological experiment is necessary. The system allows for accurate and easy measurements even by unexperienced researchers.
- ◆ The electrodes are made of platinum black or carbon nanotube with impedance 7 to 10 kΩ, the lowest impedance among similar products on the market. The system is substantially free from external noise, thanks to its low impedance electrodes. It needs no special environment/facility for electrophysiological experiment, such as a shield box, and it can be placed on a laboratory desk for stable, daily measurement with no need for noise elimination.
- ◆ The MED64-Entry detects and amplifies extracellular potential using 16 out of 64 MEAs of the MED probe. The control software “MED16” is specifically designed for data acquisition and after an experiment, the data obtained can be analyzed in various ways by using the MED64 Offline toolkit.

## 2. Configuration of the MED64-Entry



The MED64-Entry Amplifier has a 68-pin SCSI cable terminal that can be connected to a MED Connector (MED-C03) or MED Thermo Connector (MED-CP04). The amplifier has a channel selector circuit inside and acquires signals from 16 out of 64 electrodes of the MED Probe in accordance with 1 of 3 patterns shown below (the pattern is defined at shipping).



The extracellular potential detected at the recording electrodes of the MED probe is amplified 100 times by the MED64-Entry amplifier and converted from an analog to a digital signal by a data acquisition device (National Instruments Corporation) with a built-in PC. The signal acquisition and the application of the stimulation current is controlled by dedicated control software.

## 2.1. Part names and functions

### 2.1.1. MED64-Entry Amplifier



- ❶ POWER ..... To switch ON/OFF of the power.
- ❷ INPUT ..... An analog input terminal to input signals from the MED connector. It is connected to a connector terminal via the 68-pin SCSI cable.
- ❸ SIGNAL GND ..... A ground terminal to connect a lead for grounding.
- ❹ PCIe-6343 ..... Output terminals to the data acquisition device (PCIe-6343, National Instruments Corporation). They are connected to a VDHCI terminals of the data acquisition device on the back of the data acquisition PC via multifunction cables (192061C-02, National Instruments Corporation). (0) is the output terminal for ch 1-8 (top VDHCI terminal) and (1) is the output terminal for ch 9-16 (bottom VDHCI terminal).
- ❺ DC INPUT ..... To connect to a power adaptor cord.

### 2.1.2. Accessories of the MED64-Entry Amplifier



### 2.1.3. Data Acquisition PC System



### 3. Setup of the MED64-Entry

Thanks to the excellent electrode impedance (impedance of 50  $\mu\text{m}$  electrode is 10 k $\Omega$  in frequency range 1 kHz) of the MED Probe, which is the sensor of the MED64-Entry, the MED64-Entry offers several technological advantages:

- 1) Less influence by external noise (hum noise, etc.)
- 2) Very low Johnson noise (baseline noise) at about several  $\mu\text{V}$
- 3) The MED Probe/Connector can be installed at a place physically distant from the amplifier (e.g., inside of an incubator with 100% humidity) via a 2 m connection cable, without being affected by noise or attenuation of the signal acquired.

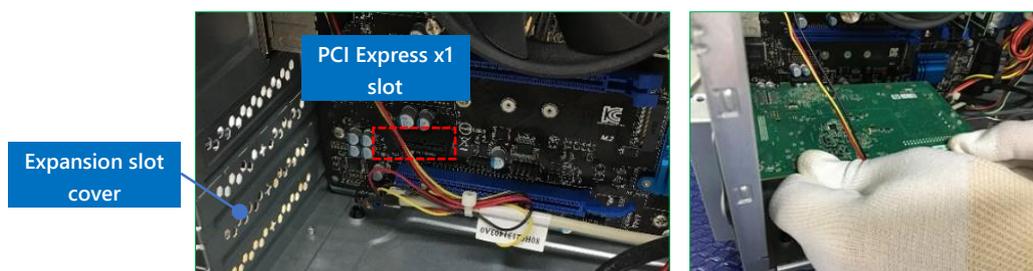
A faraday cage or vibration isolation table that is typically necessary for an electrophysiological experiment is not necessary for the MED64-Entry, but the MED64-Entry should be set up on a stable table without vibrations, such as a laboratory desk. A desk of about 100 cm width and 75 cm depth is necessary. For the user's convenience, a larger desk is preferable. The power adaptor connected to the amplifier, etc., serves as a magnetic field-derived noise source. When the MED64-Entry is set up on a desk against a wall, make space for the cord between the wall and the desk and place the adaptor on the floor in order to place the power adaptor at a distance from the amplifier and other components (the desk must not be fixed to the wall). Alternatively, a space is necessary to ensure sufficient distance between the MED64-Entry and the power adaptor.



#### 3.1. Connection of the data acquisition device to the PC

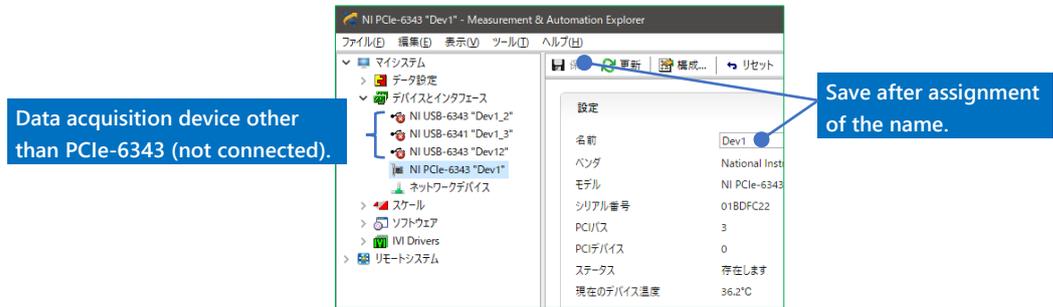
**Note:** For the MED64-Entry marketed in Japan, the process explained in this section is completed before shipment.

Open the housing of the PC, remove the extension slot cover on the back, and attach and fix with a screw the PCIe-6343 to the PCI Express x1 slot on the mother board.



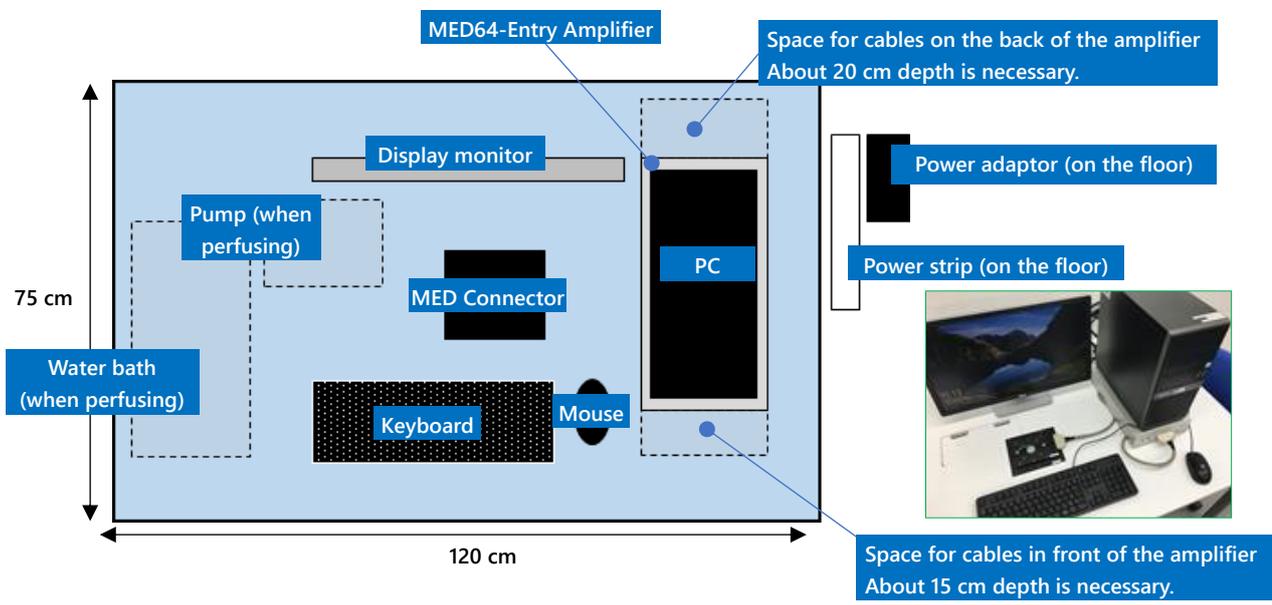
Run the NI-DAQ mx installer (ver. 16.0 or later) on the accessory DVD of PCIe-6343 to install NI MAX to the PC and connect the MED64-Entry amplifier to the PC using a multifunction cable.

Launch NI MAX to confirm the configuration of the device. If a data acquisition device other than the PCIe-6343 was previously connected to the PC, assign the name "Dev1" only to PCIe-6343 and assign other names to any other devices (on the control software, "Dev1" is used as the identification code of PCIe-6343).



### 3.2. Considerations for the position of devices

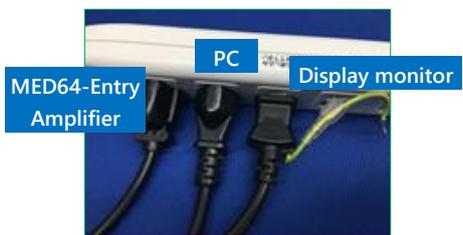
In order to ensure sufficient space for an experiment, place the PC on the MED64-Entry amplifier (not essential). Pay attention so as not to damage the housing of the MED64-Entry Amplifier with the legs of the PC. Place the display monitor next to the PC and the MED Connector in front of the monitor. Do not place the power adaptor or other electronic devices close to the connection terminal on the back of the amplifier.



Above is an image of the ideal positions of components. The user can perform an experiment smoothly when there is a sufficient space next to or at the back of the laboratory desk with a power strip on the floor. If no perfusion system is used, about 100 cm width is sufficient, otherwise a larger space is necessary.

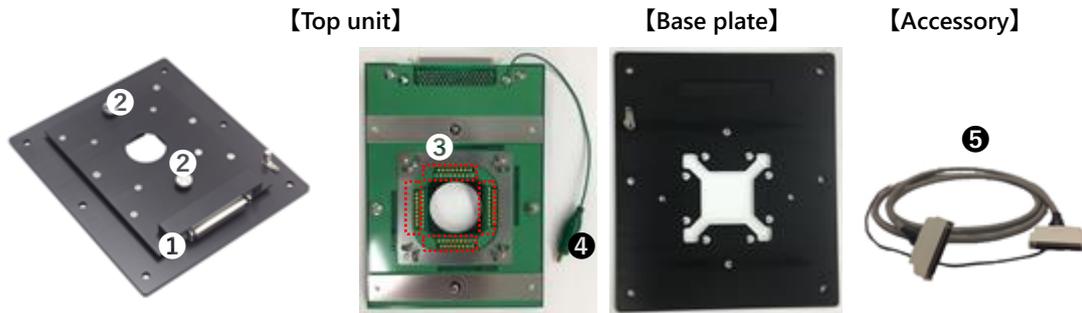
### 3.3. Power source

Three power sources are necessary for the MED64-Entry Amplifier and the Data Acquisition PC System (the desktop PC and the display monitor), and one additional power source is necessary to use the MED Thermo connector when it is used. Connect all the cords to one power strip connected to a wall outlet with the ground terminal (do not use an outlet on the desktop rack because it is often not grounded properly). Do not connect devices that are not necessary for the MED64-Entry to the power strip or to the wall outlet to which the MED64-Entry and other components are connected. Place the power cable and the power adaptor at a distance from the amplifier, the MED Connector and the 68-pin SCSI cable.



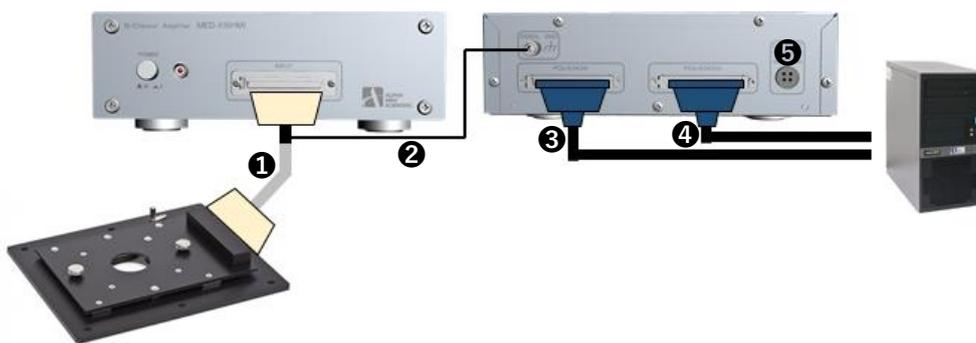
### 3.4. Connection to the MED64-Entry Amplifier

#### 3.4.1. Part names and functions of the MED Connector



- ❶ Output terminal ..... To connect to INPUT of the MED64-Entry Amplifier via the accessory, the 68-pin SCSI cable.
- ❷ Fixation screw ..... To fix the fitted top unit and the base plate with a MED Probe attached.
- ❸ Contact pin ..... To contact with the MED Probe terminal to read signals.
- ❹ Ground wire with an bagworm clip ..... To connect to a platinum wire with a perfusion cap when a perfusion system is used.
- ❺ 68-pin SCSI cable (2 m) ..... A conductive cloth tape and ground wire are wounded on the cable.

#### 3.4.2. Connections of the MED Connector and the data acquisition device



- ❶ Connect the output terminal of the MED Connector and the INPUT terminal of the MED64-Entry Amplifier, using the 68-pin SCSI cable.
- ❷ Connect the lead of the 68-pin SCSI cable to the SIGNAL GND terminal of the MED64-Entry Amplifier.

**Note1:** The 68-pin SCSI cable is wound with a conductive cloth tape and an uncoated lead is wound on it. If the other side of the lead is free, the conductive cloth tape may serve as an antenna that collects electric noise and may cause noise. Make sure that the other side of the lead is connected to the SIGNAL GND terminal of the MED64-Entry Amplifier (to use the conductive cloth tape as an electric shield).

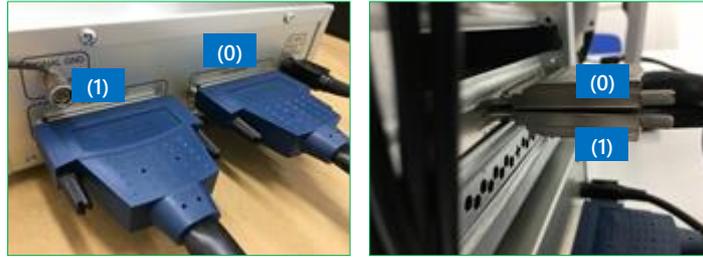
**Note2:** In order to prevent noise, use the SIGNAL GND terminal of the MED64-Entry Amplifier for single-point grounding when the peripheral devices are grounded.



Loosen the screw of the SIGNAL GND terminal, insert the ground wire into the hole (left panel), and fix with a screw (right panel).

- ❸ Connect the PCIe-6463 (1) terminal of the MED64-Entry Amplifier to the PCIe terminal (bottom) on the back of the PC, using the function cable.

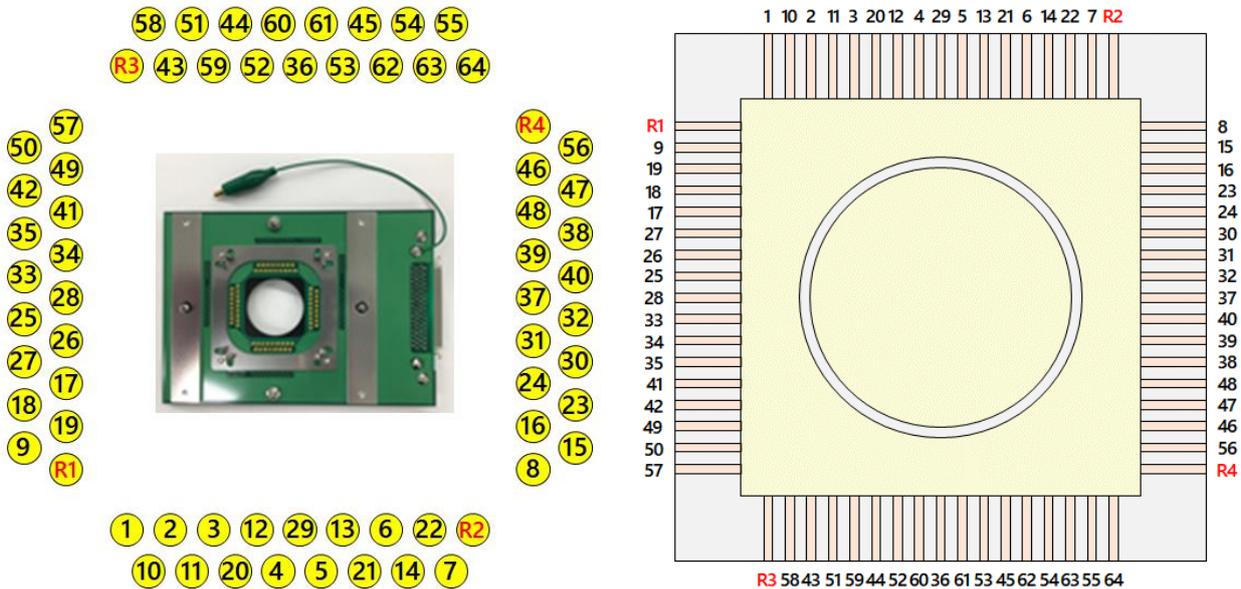
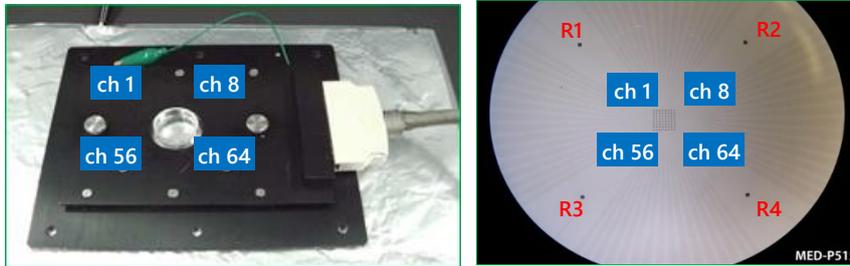
4 Connect the PCIe-6463 (0) terminal of the MED64-Entry Amplifier to the PCIe terminal (top) on the back of the PC, using the function cable.



5 Connect the power adaptor to the MED64-Entry Amplifier.

### 3.4.3. Positioning of the terminal of the MED Connector

Place the MED Connector with its output terminal and the 68 pin SCSI cable on the right side. In this position, the left top of the MED Probe is assigned to ch 1 and the right bottom is assigned to ch 64. The orientation of the MED Probe is important because the electrode number of the MED Probe is defined by the contact pins of the MED Connector.



Correspondence between the contact pin of the MED Connector and the electrode number: correspondence from the contact surface side with the output terminal on the right (left panel) and correspondence with the MED probe terminal (right panel). R indicates the reference electrode.



29	5	21	6	22	7	50	49	41	35	33	28	26	27	18	19	8	15	23	24	31	32	40	39	48	47	56	64	63	54	45	53	36	60
4	13	12	14	20	57	3	42	11	34	2	25	10	17	1	9	R2	16	R4	30	R1	37	R3	38	58	46	43	55	51	62	59	61	44	52

Correspondence between the MED Connector output terminal and the electrode number. R indicates the reference electrode.

### 3.4.4. Electric shield with aluminum foil

If a noise check reveals that external electric noise is affecting the MED Connector, the noise may be avoided by placing a sheet of aluminum foil under the MED Connector and using a lead to connect the aluminum foil sheet and the SIGNAL GND terminal of the MED64-Entry Amplifier for grounding.



A sheet of aluminum foil serves as an electric shield by grounding. Magnetic field-derived noise cannot be prevented by an electric shield.

**Note:** Although the MED Connector is made from aluminum, its surface is coated and insulated from the aluminum foil. Make sure that the aluminum foil is grounded to the SIGNAL GND terminal of the MED64-Entry Amplifier when a sheet of aluminum foil is placed under the MED Connector, even if the baseline noise level is within an acceptable range. Without grounding, the aluminum foil serves as an antenna to collect electric noise and may generate unexpected noise.



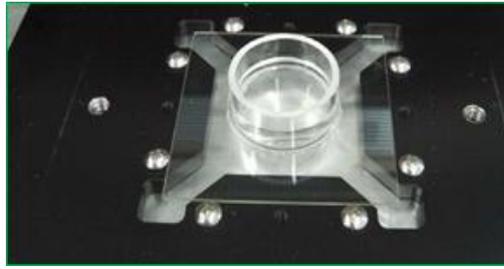
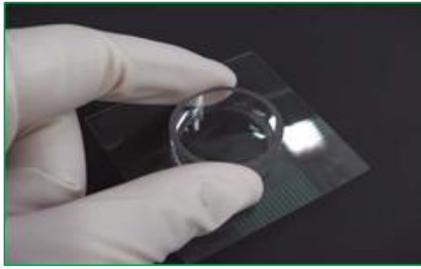
MED64-Entry after setup. In this example, the PC is placed on the right side.

### 3.4.5. Preparation for the noise check – attaching the MED Probe

Loosen the fixation screw of the MED Connector to remove the top unit.



On the base plate, place the MED Probe filled with saline, such as aCSF or PBS holding the ring chamber of the MED probe or the end of the glass substrate. Pay attention so as not to touch the terminals. If any waterdrop, medium or smudge is on the terminal, wipe the terminal with a Kim wipe before setting the MED Probe. Subsequently, place the top unit on the MED Probe and fix with a fixation screw.

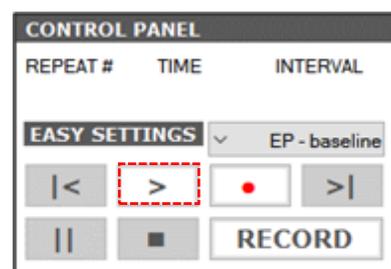
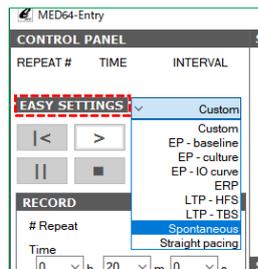


Note1: Loosen/tighten both screws little by little at the same time. When the screws are sufficiently loosened/tightened, they can be completely loosened/tightened one by one. If only one screw is completely tightened/loosened without tightening/loosening the other screw, the second screw cannot be inserted perpendicular to the hole.

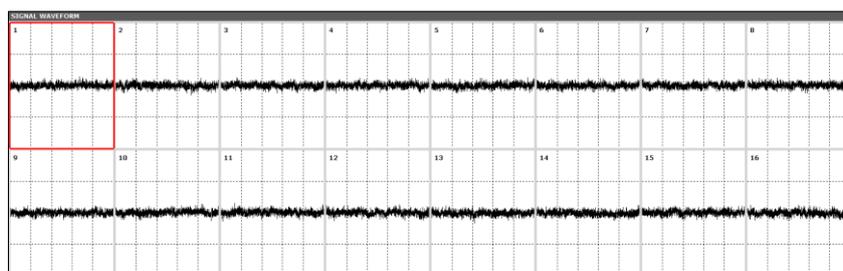
Note2: Do not touch the contact pin of the MED Connector with bare hands. If sebum of the hand attaches to the pin, contact impedance between the contact pin and the terminal of the MED Probe will increase and may cause noise. Pay attention so as not to spill liquid (saline, medium etc.) on the MED Probe after it is set. Prevent liquids from coming into contact with the contact pin.

### 3.4.6. Noise check

Double-click the MED16 icon on the desktop to launch the control software "MED16". Select Spontaneous from EASY SETTINGS and click the replay button.



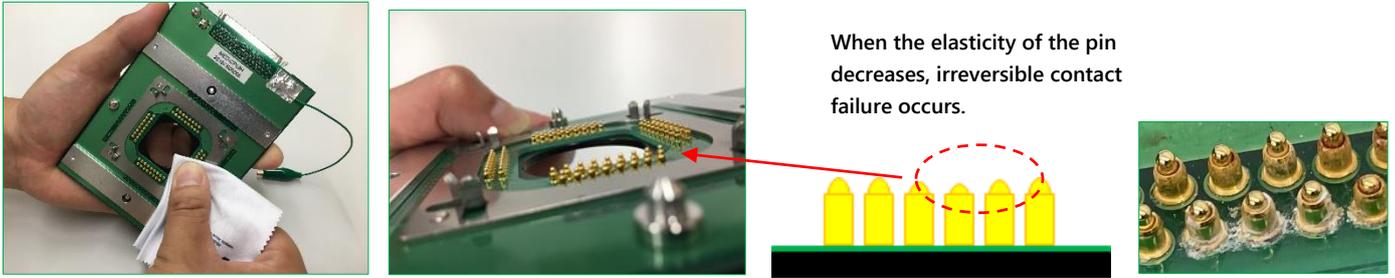
Data acquisition will start 5 seconds later and the baseline noise will be displayed. The ideal baseline noise level is about  $\pm 8 \mu\text{V}$  peak-to-peak. When baseline noise shows a normal pattern, the setup ends. For the details of operating the MED16 refer to p. 9 "Data acquisition by the control software" Refer to the relevant application note for the preparation of specimens used for the experiment.



Normal baseline noise.

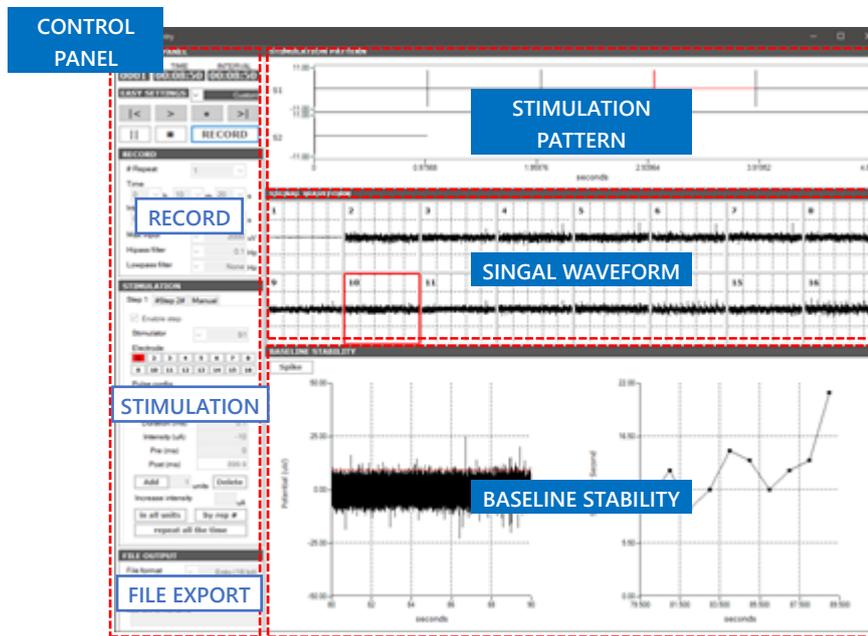
### 3.4.7. Maintenance of the MED Connector

A printed circuit board onto which 68 contact pins contacting the terminal of the MED probe are soldered is fixed by screws to the top unit of the MED Connector. Spring-loaded pins are used as contact pins and keeping the surface of the pins clean is important for the maintenance of the MED Connector. If any abnormal noise is found at a particular electrode and noise persists on that electrode even when the orientation of the MED Probe is changed, the noise may be caused by deformation (crushing) due to the wear of the contact pins associated with everyday use or dust or sebum on the contact pins. In the latter case, the noise may be reduced by wiping the contact pin by pushing the eyeglass cleaner cloth on the pin. Do not use ethanol because the contact pin is filled with lubricant for the spring. The printed circuit board needs to be replaced (paid repair) in the case of malfunction due to wear of the contact pin associated with everyday use or malfunction due to salt (perfusion solution or media) attached on the pin.



#### 4. Data acquisition by the control software "MED16"

Turn on the power of the amplifier, the PC and the display monitor. Launch the control software by double clicking the MED16 icon on the desktop. The software has two modes; RECORD mode to acquire data and REPLAY mode to replay the data file output already. The MED16 starts in the mode that was used in the last session. At the first use, it starts in RECORD mode.



Main window of data acquisition in RECORD mode

The Main window of the RECORD mode has 4 panels.

**CONTROL PANEL** ..... To set conditions for data acquisition. It has the sub-panels of RECORD, STIMULATION and FILE OUTPUT.

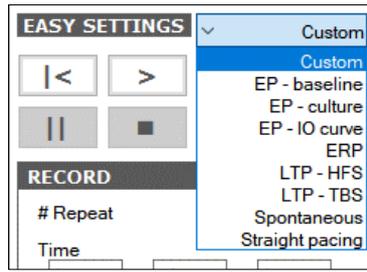
**STIMULATION PATTERN** ..... To display the stimulation pattern set in the CONTROL PANEL.

**SIGNAL WAVEFORM** ..... To display the signal waveforms of all 16 electrodes during data acquisition.

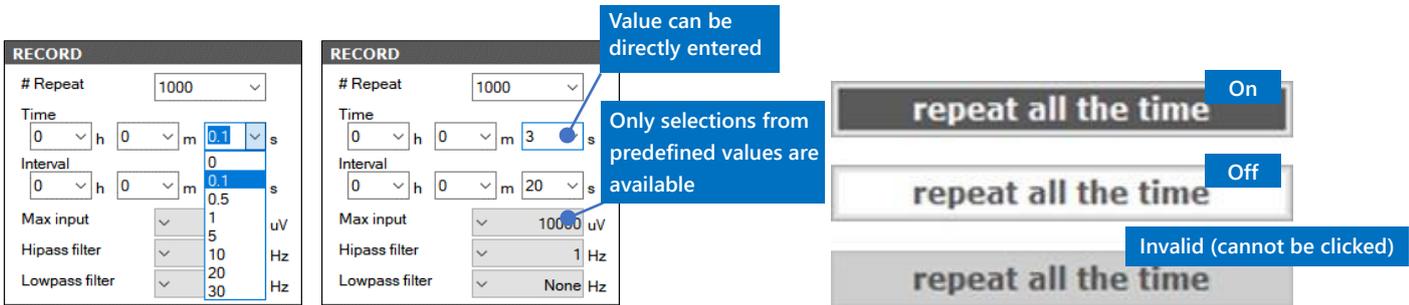
**BASELINE STABILITY** ..... To display an enlarged view of the signal waveform of 1 electrode selected on the SIGNAL WAVEFORM panel. From the signal waveform, it calculates a measure that indicates the stability of the activity or response of the biological specimen, and it displays it as a time chart. BASELINE STABILITY has three modes; Spike, EP and ISI. In Spike mode, the calculation is based on the frequency of spike detection; in EP mode, the calculation is based on amplitude value of the evoked potential; and in ISI mode, the calculation is based on the interval of the spike detection. None of the modes is intended for data analysis, but the calculation in each mode provides information to help determine the timing of experimental manipulation, such as the application of electric stimulation or the administration of a drug.

After setting the condition for data acquisition in the RECORD or STIMULATION (only for experiments using electric stimulation) subpanel on the CONTROL PANEL, start the data acquisition by executing the software using the control button described below.

This software has 9 formats predefined for data acquisition assuming a standard experiment and EASY SETTINGS can call up one of these formats (refer to p. 12 "Menus in EASY SETTINGS" for details). When the software starts, Custom is called up from these nine pre-defined conditions.



Each panel has fields in the form of a text box or pull-down menu to define the condition. From the gray pull-down menu in the figure below, users can only select one condition from the predefined values, while from the white pull-down menu, users can directly enter any numerical value (the central panel in the figure below). The click button changes its color according to whether on, off or invalid (the right panel in the figure below).



After defining the condition on the CONTROL PANEL, data acquisition is controlled by the control button on the upper part of the CONTROL PANEL. The detailed conditions set on each panel are explained in the sections below.

**Control button**

- |< Replay one previous repeat. This button is not used for a record.
- > Acquire data without outputting the data file.
- Acquire data while outputting the data file (record).
- >| Replay one repeat forward. This button is not used for a record.
- || Pause data acquisition between repeats.
- Stop data acquisition.

Displays time elapsed during data acquisition.

Calls up predefined condition for data acquisition.

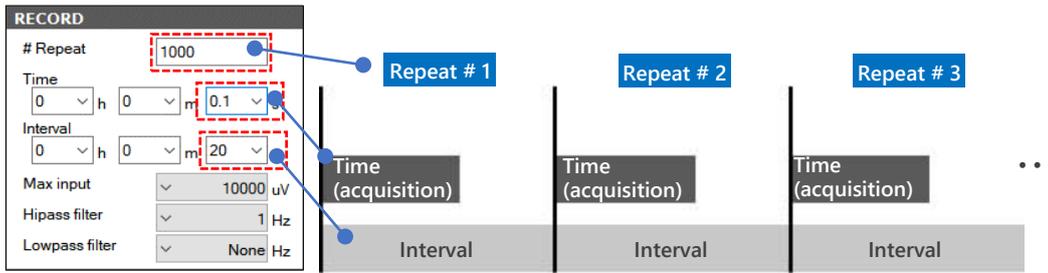
Displays current mode. Click to switch mode.

**Note1:** Do not start other software during the data acquisition because it may place an unexpected load on OS and cause it to freeze or to force a shutdown. Especially, you need to pay attention to software running background, such as anti-virus software. Do not connect to the Internet, because it may place an unexpected load due to the communication and cause it to freeze or to force a shutdown.

**Note2:** In this document, data acquisition with data file output is called "record."

#### 4.1. Setting the data acquisition schedule – RECORD subpanel

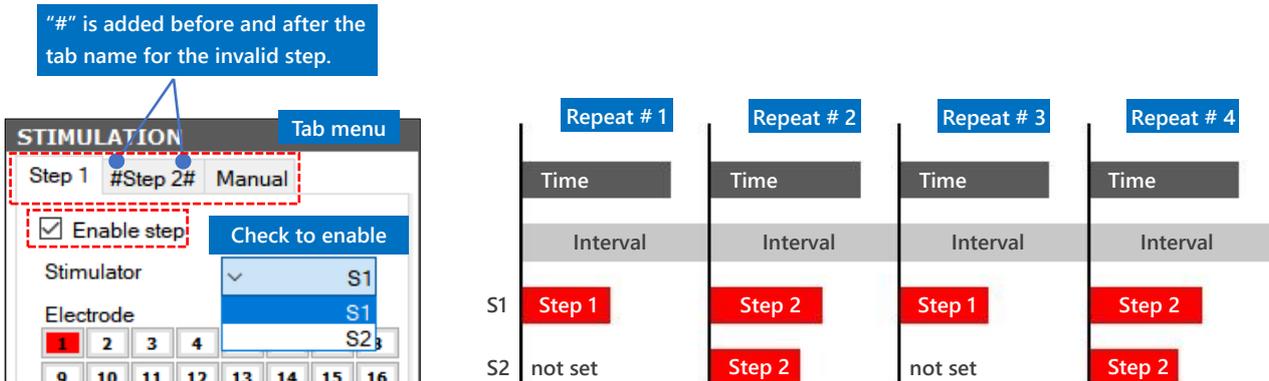
The data acquisition schedule consists of 1 or more data acquisition sessions (called "repeat" in this software) set arbitrarily by the user on the RECORD subpanel. In the example shown below, a session (repeat) consisting of 0.1 s data acquisition and 19.9 s waiting time is repeated 1,000 times. The minimum increment for Time (data acquisition) is 0.1 s and for Interval (repeat) is 1 s. The value of Interval must be at least 0.1 s higher than the value of Time.



The maximum input voltage is entered to Max input. When a signal exceeding the set value is input during recording, the set value is acquired as the saturated value. Digitizer resolution is 16 bit in the MED64-Entry, which follows that the resolution of the voltage value is a value obtained by dividing Max input by  $2^{16}$ . Set an appropriate value according to the magnitude of extracellular potential obtained from the biological specimen. High pass filter or Lowpass filter is not applied to the data file output. Instead, they are applied to the displayed raw waveform in OSCILLOSCOPE or RAW WAVEFORM - SELECTED ELECTRODE during data acquisition.

**4.2. Creating the stimulation pattern – STIMULATION subpanel and STIMULATION PATTERN tabs**

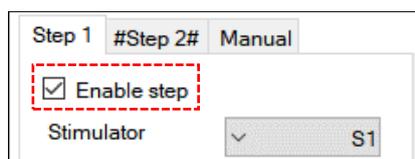
The STIMULATION subpanel has 3 tab windows; Step 1, Step 2 and Manual. When either Step 1 or 2 is enabled and the stimulation pattern is set in that tab, the pattern is applied to all repeats. If both Step 1 and 2 are enabled and stimulation pattern is set, the pattern is applied alternatively for each repeat. For repeat 1, stimulation pattern of Step 1 is applied; for repeat 2, stimulation pattern of Step 2 is applied; for repeat 3, stimulation pattern of Step 1 is applied, and so on (see the right panel of the figure below). Because the MED64-Entry Amplifier has 2 terminals (S1 and S2) for stimulation output, the stimulation pattern is applied to up to 2 electrodes in one Step.



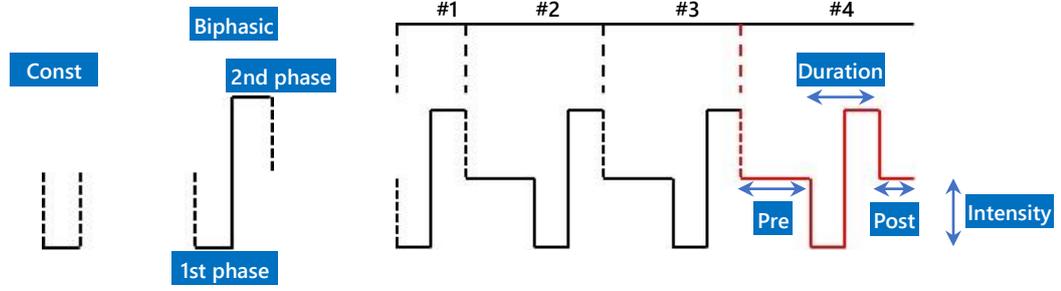
Schematic drawing of data acquisition and stimulation pattern. In this example, a stimulus is applied to 1 electrode in Step 1 and a different stimulus is applied to the other 2 electrodes in Step 2.

**Note:** The length of the stimulation pattern must not exceed the length of Time.

The Manual tab has a Start Stim button enabled only during the pause between repeats. When the Start Stim button is clicked, the stimulation set in the Manual tab can be applied manually at any time. When the stimulation is applied during the recording of data, the data file for that interval is output as a separate file.



The minimum unit composing a stimulation pattern is a biphasic pulse or a monophasic pulse. A stimulation pattern is composed by coupling 1 or more stimulation pulse set at the Pulse config.



In the Pulse type, Biphasic (biphasic pulse) or Const (monophasic pulse) is selected. Usually, select Biphasic. In Duration, the pulse width is specified in 0.1 s increments. When Biphasic is selected, 50% of the specified value is applied to each phase. In Intensity, a positive or negative integer is specified. When Biphasic is selected, the designated value is assigned to the first phase and the value with an inverted sign is assigned to the second phase. In Pre and Post, the waiting time before and after a pulse is specified in 0.1 s increments. For the reason why a biphasic pulse is selected, refer to p. 24 “6.2.3 Stimulus artifact and biphasic stimulation.”

**Note:** In order to return the output current of the stimulation pulse to the 0 level, be sure to set the Post value for the last pulse of the stimulation pattern.

The Pulse config represents 1 pulse range of a stimulation pattern. A stimulation pattern is composed by clicking the buttons shown below to add, delete or repeat 1 pulse range to the whole stimulation pattern.

**Menus in EASY SETTINGS**

Custom ..... When the software is closed while Custom is selected, all the defined data acquisition condition can be stored and called up at the subsequent launching of the software. This menu is provided for the user’s convenience and users can repeatedly use the specific data acquisition condition defined by themselves without having to define it again.

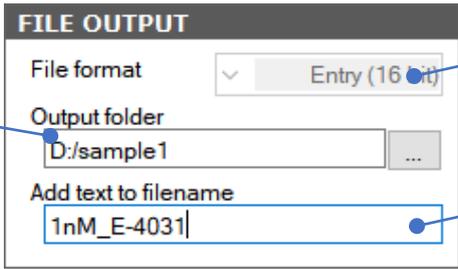
**Note:** In menus other than Custom, the condition defined by the user cannot be stored. Default values are called up every time the software is launched.

EP - baseline ..... This menu is provided for recording the evoked response (the evoked potentials) in a brain slice. A session consisting of 0.1 s data acquisition and 19.9 s waiting time is repeated 1,000 times. Max input is set at 10,000 uV and the data in bandwidths 1 to 10,000 Hz are shown. For example, for an acute hippocampus slice, it is in good condition if amplitude  $\geq$ -300 uV is obtained by a default stimulation pulse (0.2 ms width, -10 uA) (an estimated value).

- EP - culture ..... This menu is provided for recording the evoked response (the spike) in a dissociated neural culture (cultured neural network). A session consisting of 0.1 s data acquisition and 4.9 s waiting time is repeated 100 times. Max input is set at 10,000 uV. A low frequency component may be generated at baseline by LFP (local field potential) caused by spontaneous activity, and filtering is applied to bandwidths 100 to 10,000 Hz to allow for the easy observation of a spike. Whether a spike is evoked or not by a default stimulation pulse (0.1 ms width, -20 uA) at any electrode in a constant delay time from stimulation artifact is observed while switching the stimulation electrode. When no evoked spike is observed at any stimulation electrode, repeat the same procedure with the stimulation intensity increased (e.g., -30 uA).
- EP - IO curve ..... In this menu, the input-output relationship (the input-output curve of the evoked potentials relative to the stimulation intensity) is recorded in a brain slice. The data acquisition condition is almost the same as the EP-baseline, but the stimulation intensity is increased by 1 uA for every 1 repeat because by rep # it is enabled. The number of repeats is set to 90 because the max stimulation intensity is 100 uA. The data acquisition is stopped manually when a saturation response is obtained.
- ERP ..... This menu is provided for recording the effective refractory period by premature stimulation in a dissociated cardiomyocytes. Electric stimulation with width 0.6 ms, intensity -10 uA and basic cycle length (interval between pulse) 1,000 ms is applied 10 times, followed by the 11th electric stimulation with a shorter cycle length of 900 ms.
- LTP - HFS ..... This menu is provided for LTP induction by tetanus stimulation. The data acquisition condition is almost the same as the EP - baseline, but tetanus stimulation with 10 ms interval (100 Hz) for 1 s (100 stimulation) is set in the Manual tab. The default stimulation intensity is -20 uA. To change the value to a value defined based on the result of the input-output relationship, enter the difference between the default value and the new value in Increase intensity, and click on the in all units button to increase/decrease the stimulation intensity for the stimulation patterns as a whole.
- LTP - TBS ..... This menu is provided for LTP induction by  $\theta$  burst stimulation. The data acquisition condition is almost the same as the EP - baseline, but 4 pulse stimulation of 100 Hz is set at 200 ms (5 Hz) interval for 2 s (10 times) in the Manual tab. The default stimulation intensity is -20 uA. To change the value to a value defined based on the result of the input-output relationship, enter the difference between the default value and the new value in Increase intensity, and click on the in all units button to increase/decrease the stimulation intensity for the stimulation patterns as a whole.
- Spontaneous ..... Data in the bandwidths 1 to 10,000 Hz is acquired with Max input 10,000 uV. Data for continuous 20 m interval is acquired with no stimulation.
- Straight pacing ..... This menu is provided for recording the paced responses in a fixed cycle for a cardiac culture. Repeat all the time is enabled and pacing is applied continuously at stimulation width 0.6 ms, stimulation intensity -10 uA, and cycle length (interval between pulses) 1,000 ms. To change the cycle length, edit the value in Post.
- Note: When any menu is called up from EASY SETTINGS and switched to Custom, the Custom takes over the acquisition condition of that menu.**

#### 4.3. Set data file output – FILE OUTPUT subpanel

When data acquisition is executed by the record button, a data file is generated after the data acquisition. The folder to output and the file name are specified in the field shown in the figure below:



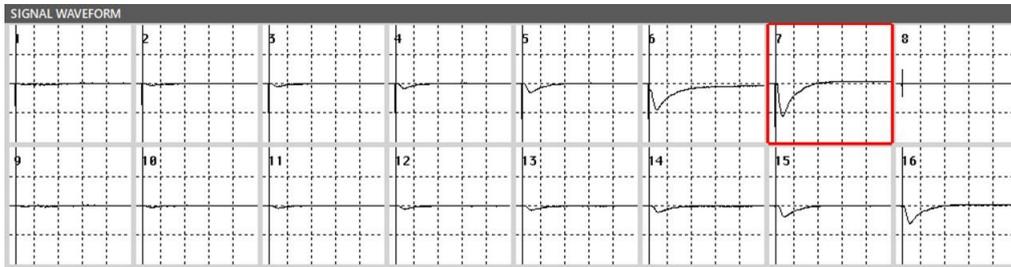
The screenshot shows the FILE OUTPUT subpanel with the following fields and callouts:

- File format:** A dropdown menu set to "Entry (16 bit)". Callout: "The format of the data file to be output is specified. The format cannot be specified for recording (it can be specified for replay only)."
- Output folder:** A text field containing "D:/sample1" with a browse button (...). Callout: "Must be specified. If no folder is specified before data acquisition, a prompt window appears."
- Add text to filename:** A text field containing "1nM\_E-4031". Callout: "Optional item. Text to be added to the file name is entered."

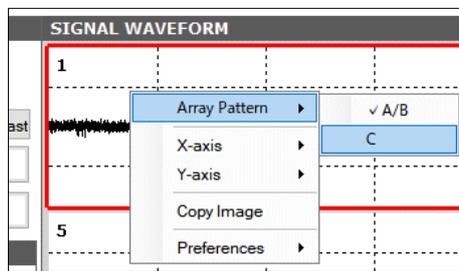
In RECORD mode, the output format of the data file cannot be specified and the file is output in a unique .endat format. The file name is automatically assigned in the format "Year Month Date\_####h##m##s(\_added text).endat."

#### 4.4. SIGNAL WAVEFORM panel

The signal waveform (raw data) is depicted for all 16 electrodes. Select one electrode (in the red frame) by left clicking on the chart of the electrode to show the waveform for that electrode on the BASELINE STABILITY panel.



Right clicking on the panel calls up the menu to switch the display of the electrode array pattern or the scope of the display of the axis common to the electrode charts.

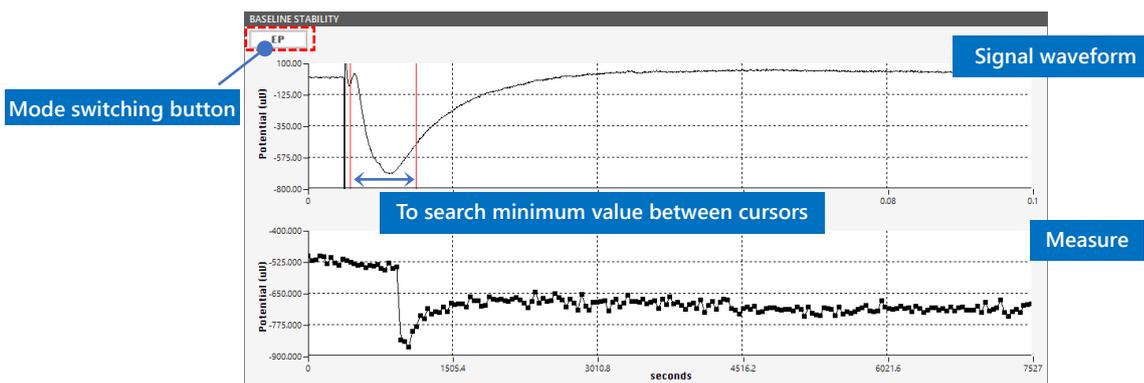


Each electrode chart is divided by auxiliary lines into 5 divisions on the X axis and 4 divisions on the Y axis. The values specified on the right-click menu X-axis and Y axis indicate the display time (ms/div) and the voltage (uV/div) per division. The voltage specified is a positive/negative value with 0 uV as the center. By selecting On in Autoscale, the Y axis is automatically adjusted in 2.5 s increments for each electrode, based on the maximum voltage value. Copy Image copies the panel image onto the clipboard.

In Preferences, Line Color customizes the color of data points, Background Color customizes the background color of the chart and Grid Color customizes the color of the auxiliary lines. By selecting Default, the changes are returned to the default values.

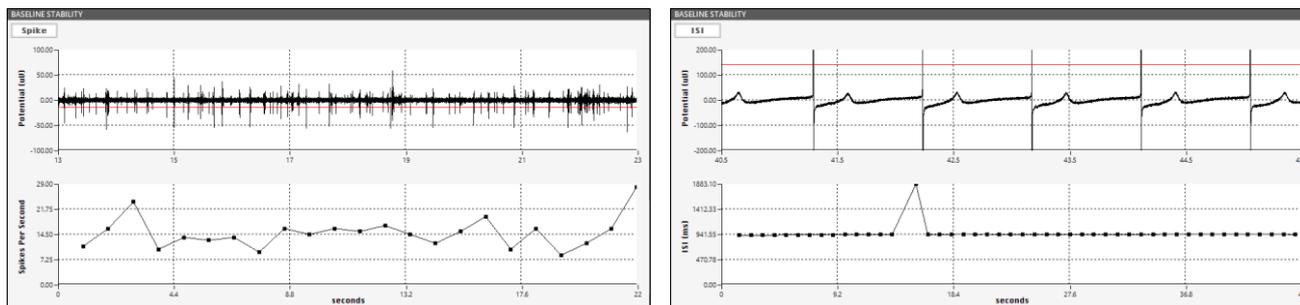
#### 4.5. BASELINE STABILITY panel

The stability of the activity of the biological specimen is shown on the monitor. There are 3 modes to select—EP, SPIKE and ISI— each of which has a different measure as the indicator of activity. The upper panel shows an enlarged view of the signal waveform of the electrode selected in the SIGNAL WAVEFORM panel, and the range to calculate the measure is specified by the cursor. The lower panel shows a time chart of the measure calculated.



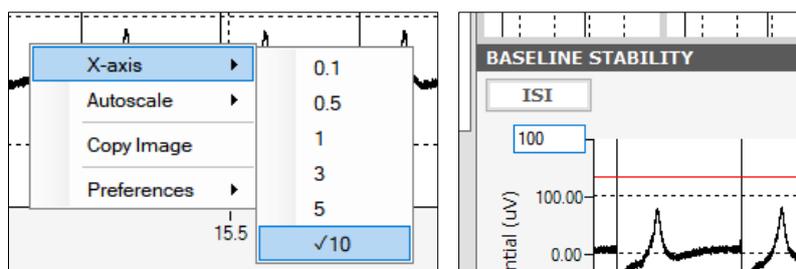
BASELINE STABILITY panel in EP mode

In EP mode, two perpendicular cursors appear on the signal waveform chart. Use drag & drop to change the position on the X-axis and calculate the minimum value of the raw data between the two perpendicular cursors to obtain the indicator of stability of activity.



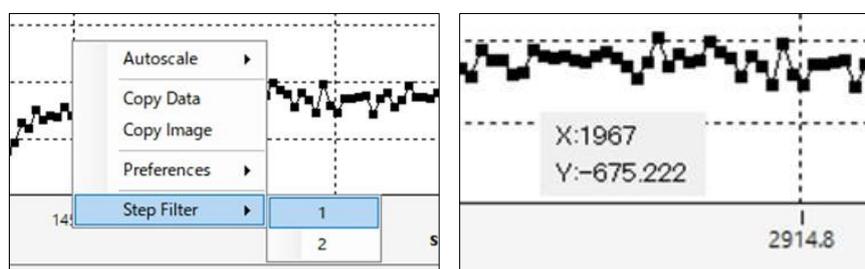
SPIKE mode (left) and ISI mode (right) of BASELINE STABILITY panel.

In SPIKE mode or ISI mode, one horizontal cursor (threshold) appears on the raw data chart. Use drag & drop to change the position on the Y-axis, to count the frequency of the signal crossing with the threshold (SPIKE), or to calculate the interval between crossings (ISI: inter-spike interval). The target of the measure is the cross point for rising when the threshold is on the positive side, and the cross point for falling when the threshold is on the negative side.



The same as in the SIGNAL WAVEFORM panel, a menu to change the scope to display on an axis appears by right clicking on the signal waveform chart. The value specified on the X-axis indicates the maximum value (s) on the X-axis. For the Y-axis, click the axis label of the extreme value to change the scope of display by direct editing.

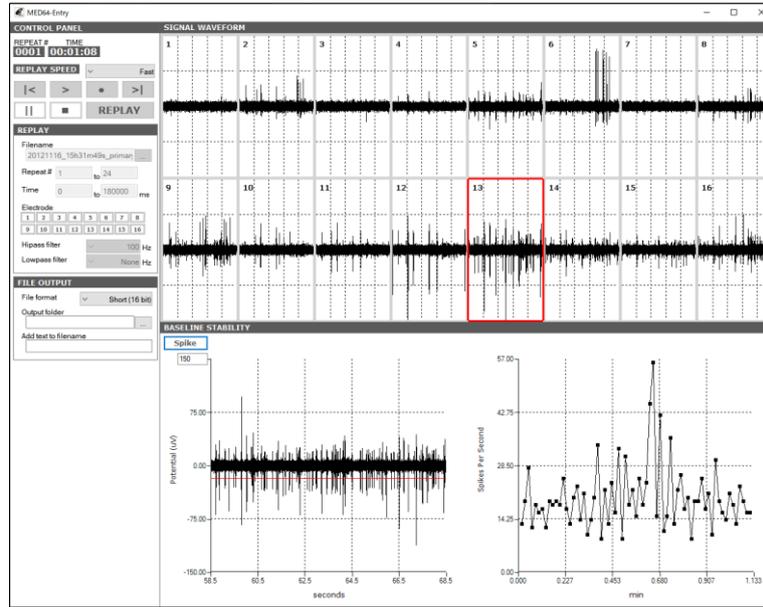
Other menus are the same as those available in the SIGNAL WAVEFORM panel.



The measure chart is similar to the signal waveform chart. Step Filter shows only the evoked response at either step as a time chart, for data acquisition with Step 1 and 2 enabled. Copy Data stores the numerical data of the time chart on a clipboard. Moving the mouse cursor over each plot of the chart shows the numerical information of that plot point.

### 4.6. Replaying data file output – function of replay mode

This software has a replay mode to check a recorded data file.



Main window of REPLAY mode.

As shown in the figure above, the screen looks almost same as that of RECORD mode, except for some differences in the CONTROL PANEL. The data file to be replayed, the scope of replay and the number of electrodes are specified on the REPLAY subpanel. To convert and output a data file in an endat format to a binary format or csv format, specify accordingly on the FILE OUTPUT subpanel.

**REPLAY**

Filename:

Repeat #:  to

Time:  to  ms

Electrode:  1  2  3  4  5  6  7  8  
 9  10  11  12  13  14  15  16

Hipass filter:  Hz

Lowpass filter:  Hz

**FILE OUTPUT**

File format:

Output folder:

Add text to filename:

Click to select a data file to replay. When the file name appears in the Filename field, the file can be replayed using the control button.

When the data file name appears in Filename field, replay the data file using the control button. To convert the data file format to be output, execute the software using the record button.

**Control button**

- < Replay one previous repeat. This button is not used for a record.
- > Replay data without outputting the data file.
- Replay data while outputting the data file (record).
- >| Replay one repeat forward. This button is not used for a record.
- || Pause replaying data between repeats.
- Stop replaying data.

Displays time elapsed during replaying data.

Calls up predefined speed for replaying data.

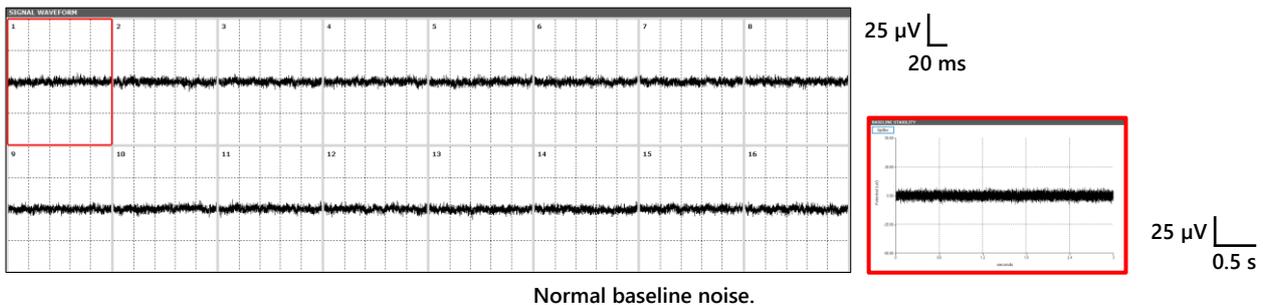
Displays current mode. Click to switch mode.

The MED16 has limited functions for data analysis in order to reduce the load during operations. For data analysis, consider using the MED64 Offline Toolkit or the separately sold MED64 Burstscoope and MED64 Peakmap that are compatible with the endat format. Refer to the product manuals of each product for further details.

## 5. Abnormal noise

### 5.1. Noise check point

- The amplitude of normal baseline noise has about  $\pm 8 \mu\text{V}$  peak-peak value in bandwidth between Low cut freq 1 Hz and High cut freq 10,000 Hz for signal acquisition. Visually check whether the baseline noise is flat on the display with 20 ms/div on the horizontal axis and a display 500 ms/div on the horizontal axis.

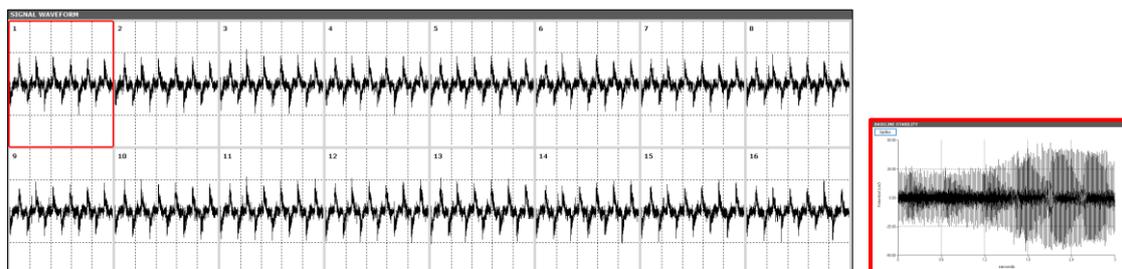


- For electrodes with noise, check whether noise occurs in a particular subset of electrodes or in all 16 electrodes and whether there is any bias in the noise.
- For the shape of noise, set the horizontal axis at 20 ms/div and check whether the noise is a sinusoidal-like noise repeated 5 or 6 times or a high-frequency noise disproportionately observed in particular electrodes.
- There are two types of extrinsic noise; noise that can be shielded by an electric shield regardless of distance, and noise for which an electric shield is not effective but can be reduced by moving the noise source to a distant position. The latter noise may be caused by a magnetic field generated by a device connected to the power cable (power adaptor, perfusion pump, and water bath, etc.). Move the responsible device to a position where the noise disappears or unplug the power cable of that device when MED64-Entry is being used.

**Note:** To contact our help desk, select Copy Image by right clicking on SIGNAL WAVEFORM, paste the image of 16 electrode on an image editor such as paint, store in jpg or tif format, and send it to the help desk as an e-mail attachment. Do not use bmp format because it has a large file size. Prepare images with 20 ms/div and 500 ms/div horizontal axis and 25  $\mu\text{V}$ /div vertical axis, or a vertical axis automatically adjusted by Autoscale.

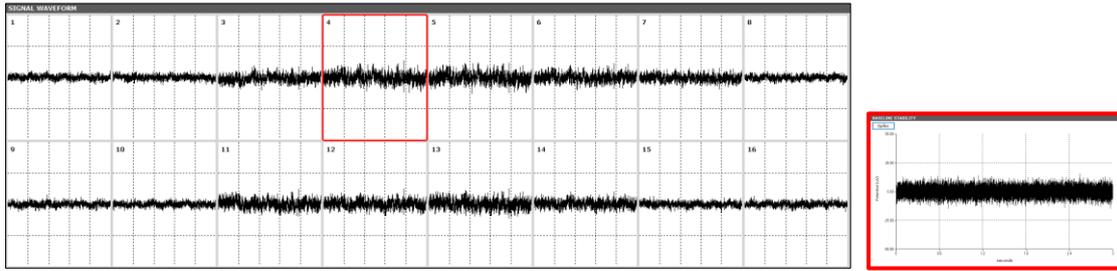
### 5.2. Noise relating to installation

- A fixation screw of the MED connector is loose.



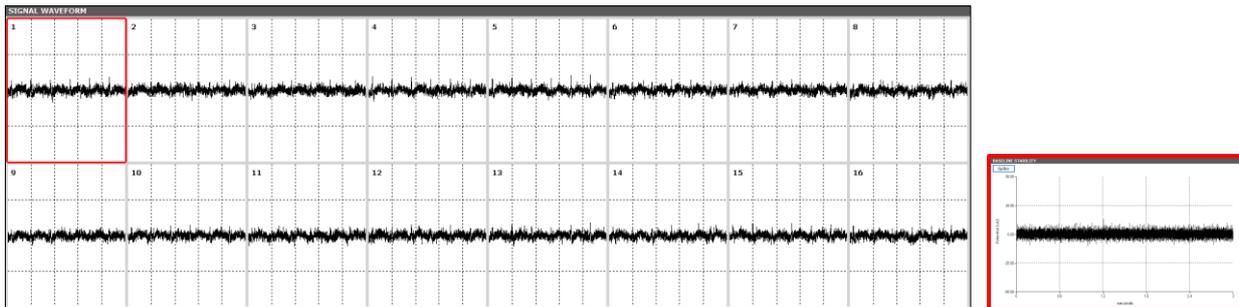
An example of noise when a fixation screw is loose. Noise is likely to occur in all 16 electrodes.

- Poor contact between the SCSI cable and the analog input terminal.



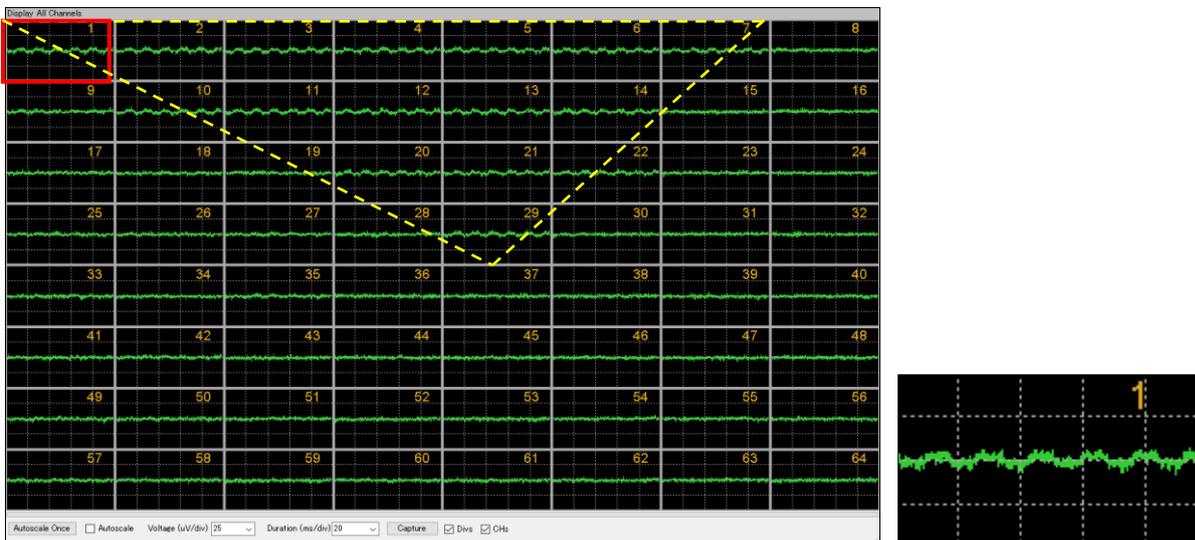
An example of noise when the contact of the SCSI cable is poor.

- The power adaptor or other electronic device (incubator, etc.) is placed close (about 30 to 100 cm) to the terminal of the MED64-Entry Amplifier or the 68 pin SCSI cable.



Magnetic field noise caused by a nearby power adaptor. Beard-shaped characteristic noise. Interference (an effect of the magnetic field) occurs in particular electrodes.

- A heater circuit or electromagnetic valve of the incubator operates and a magnetic field is generated inside the incubator.

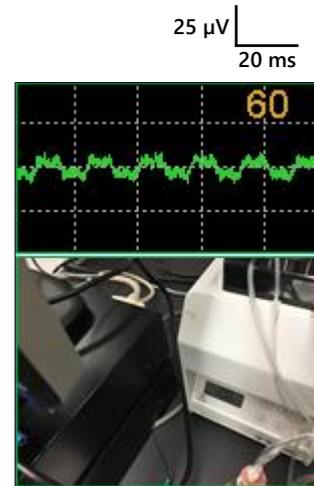
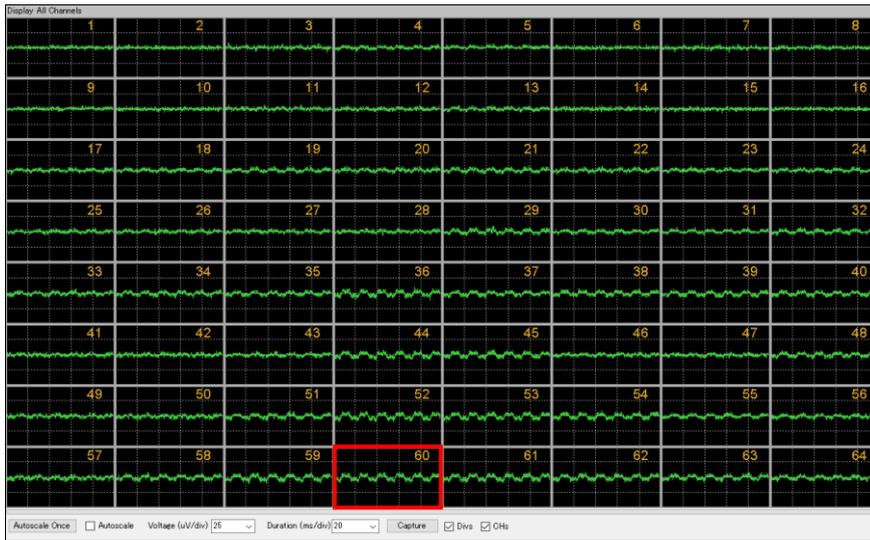


An example of magnetic field noise caused by an incubator. Interference occurs in particular electrodes, the magnitude of effect depends on the position in the incubator, and the noise disappears when the temperature is stabilized (an example screen of the MED64-Basic to clearly show the disproportionate effect).

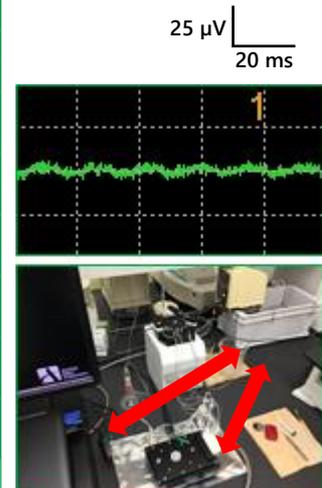
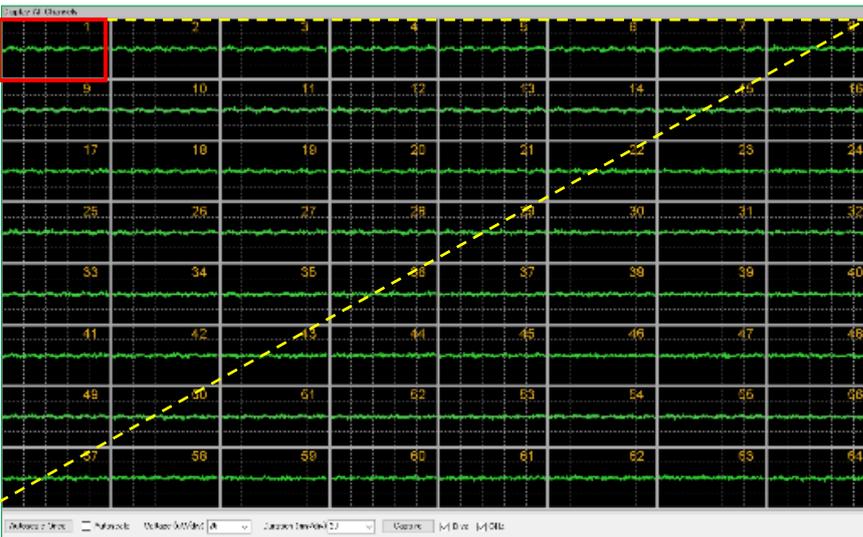
- A power cable of an electronic device not relating to the MED64 system is connected to the power strip, and the noise disappears when the device is disconnected.

### 5.3. Noise relating to perfusion

- A perfusion pump or its power adaptor is placed close to the amplifier, the MED Connector, or the 68 pin SCSI cable.



An example of magnetic field noise caused by a nearby perfusion pump. Interference occurs in particular electrodes (an example screen of the MED64-Basic to clearly show the disproportionate effect).



An example of magnetic field noise caused by a thermostat bath placed close to the MED64 system. A relatively large area is affected (an example screen of the MED64-Basic to clearly show the disproportionate effect).

- The electrodes' surfaces of the MED Probe or platinum wire is not completely immersed in perfusion solution. Some parts are not exposed to the atmosphere.
- The platinum wire is not gripped by a ground wire with a bagworm clip.
- The platinum wire is not stably fixed with a fixation screw (it is prone to staggering).
- A spike-like noise occurs at several seconds interval. A flat baseline noise is obtained when the peristaltic pump is turned off.
- \* The inside of the dropper is completely hydrophilic with a lot of water dropping. It is likely that the inside insulation is destroyed every time the perfusion solution drops, and the noise from the pump is conducted. Connect the inlet pipe and the platinum wire using a ground wire with a bagworm clip. When the noise disappears, the inlet dropper is identified as the cause (this procedure cannot be used for the outlet dropper because an air layer is mixed).
- \* Check whether the tip of the inlet pipe is too close the platinum wire to not putting hydraulic pressure when the perfusion solution drops.
- \* Check whether air entrainment occurs in the inlet dropper and bubbles enter the MED Probe chamber periodically to compress the platinum wire.

#### 5.4. Identification of cause when a malfunction of the device is suspected

---

If the noise persists in a particular electrode even after setting an electric shield, moving the possible noise source or unplugging the power cable, a malfunction may have occurred in the device. Identify the cause by the procedures below:

##### Procedure 1: MED Probe

Turn the orientation of the MED Probe by 90° and connect it to the MED Connector. Check whether the electrode where the noise is generated moves to a position according to the orientation of the MED probe (e.g., whether the position where the noise occurs moves from Electrode 1 to Electrode 8). If the position moves in line with the orientation of the MED probe, the coating of the relevant electrode may have come off or the insulation layer on the ITO lead pattern to the electrode may have been damaged (if it is detected in the first time use within 6 months after buying, it has an early failure and will be replaced with a new one). If the position does not move, proceed to Procedure 2 and the subsequent procedure.

##### Procedure 2: poor contact of cable or lead

If poor contact is detected in the output terminal of the MED Connector or the input/output terminal of the MED64-Entry Amplifier, disconnect the SCSI cable completely and connect it again. Check whether the lead or alligator clip connected to the GND terminal has come off or not.

##### Procedure 3: MED Connector

Check whether poor contact with the MED Probe terminal occurs or not because of sebum or dust on the contact pin. Wipe the contact pin by pushing it with an eyeglass cleaner cloth and attach it the MED Probe again. If the noise persists, visually check whether the levels of some contact pins are lower than those of other contact pins. Contact pins are filled with oil. If the oil comes off due to age deterioration and a pin becomes less elastic, the pin stays in a depressed position. This causes unreversible poor contact between the pin and the MED Probe terminal, which necessitates repair by replacement of the substrate. If the noise disappears after replacement with a normal MED connector, the cause is identified as wear of the contact pin.

## 6. Appendix

### 6.1. Specification

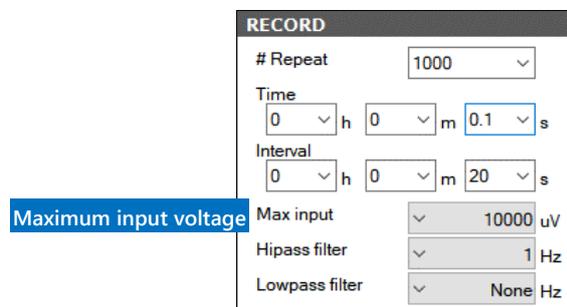
#### 6.1.1. MED64-Entry Amplifier [MED-A16HM1]

General Information	
Power	DC ±12V
Size	3.3 kg
Weight	W215 x D347 x H74 mm
Input Terminal	SCSI 68 pin
Output Terminal	D-sub 68 pin x 2
Amplifier	
Number of Channels	16
Gain	×100
Bandwidth	0.1 Hz - 10 kHz
Input Impedance	100 MΩ
PCIe-6343 Digitizer	
Resolution ※	16 bit
Sampling Rate	20 kHz
Power Unit	
Input	AC 100 - 240 V (50 / 60 Hz)
Output	DC ±12V

#### \* Correlation between gain and voltage resolution

The resolution of signals acquired is selected by changing "Max input (maximum input voltage)" of the MED16. The maximum input voltage selected represents the upper limit of the amplitude of the potential acquired. When 2000 μV is selected for example, a signal exceeding 2000 μV is acquired as a 2000 μV signal. The low level maximum input voltage means high resolution. The resolution of amplitude obtained by the maximum input voltage is shown in the table below.

Maximum Input Voltage	Resolution
2000 μV	0.061035 μV
10000 μV	0.305176 μV



Setting of maximum input voltage and high/low pass filter on the RECORD panel.

**6.1.3. MED Connector [MED-C03] and MED Thermo Connector [MED-CP04]**

MED Connector	
MED Probe Securing Mechanism	Screw down
Material	Aluminum, Gold plate for contact pins
Contact Resistance	< 30 mΩ (With MED Probe attached)
Weight	480 g
Size	W174 x D150 x H21 mm

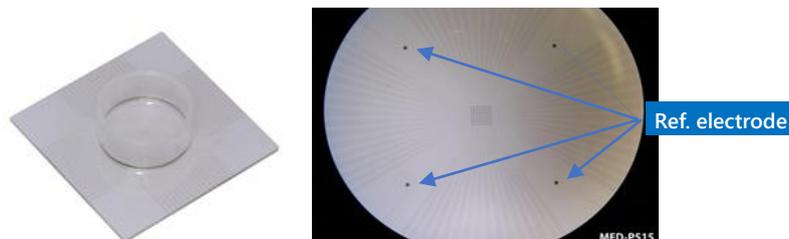
MED Thermo Connector	
MED Probe Securing Mechanism	Screw down
Material	Aluminum, Gold plate for contact pins
Contact Resistance	< 30 mΩ (With MED Probe attached)
Weight	Connector unit: 510 g Thermal controller: 357 g
Size	Connector unit: W200 x D112 x H21 mm Thermal controller: W153 x D115 x H50 mm
Temperature Accuracy	< ±1°C
Range of verified < ±1°C	With perfusion: 32°C (Ta=25°C) Without perfusion: 37°C (Ta=25°C)
Heater	Transistor

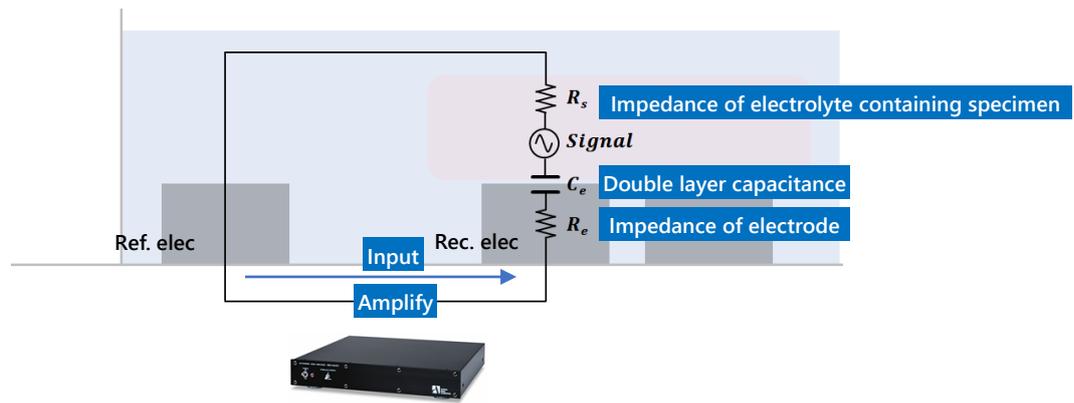
**6.1.4. Data Acquisition PC System (recommended specification, any PC model with equivalent performance can be used.)**

Desktop PC	
Operating System	Windows 10 Pro 64bit
CPU	Intel Core i7-6700K (4 Cores/4.00 GHz/8 MB Cache) * The latest high performance model when buying
RAM	16 GB (8 GB×2)
1st Disk Drive (for OS)	256 or over GB SSD
2nd Disk Drive (for Data Storage)	1 or over TB HDD
Display Monitor	
Resolution	1920 x 1080

**6.2. Explanation of the technology**
**6.2.1. Signal acquisition by the MED64 system**

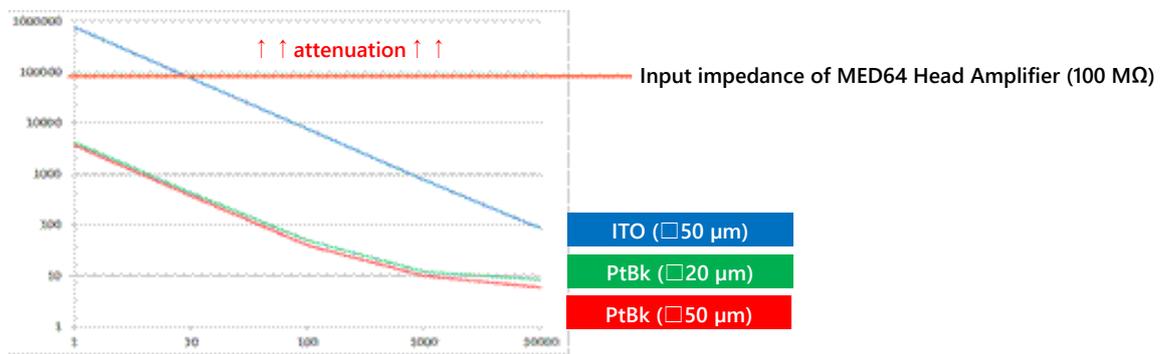
In the MED Probe, 64 recording electrodes and 4 (or 8, 16) reference electrodes are arrayed in a pattern. The MED64 System acquires the potential between the recording electrodes and the reference electrodes. The figure below illustrates an equivalent circuit between the recording electrodes and the reference electrodes.





**Input impedance necessary to avoid attenuation of the signal**

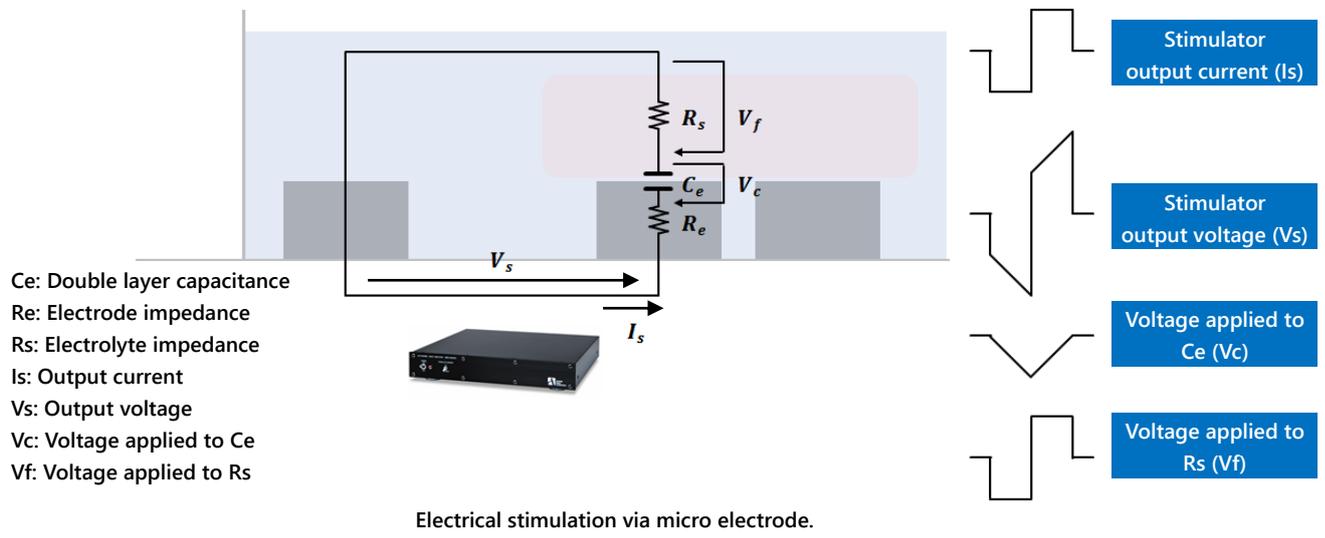
One of the unique characteristics of a MEA is an "electrical double layer capacitance formed between the interface of the electrodes and the electrolyte. An electrical double layer capacitance increases impedance in the low frequency area of the electrode (see the figure below). When the electrode impedance is higher than the input impedance of the amplifier, attenuation occurs in the signal acquired. The platinum black electrode on the MED probe has very low impedance (at signal frequency 1 kHz, 10 kΩ for 50 μm×50 μm electrode, and 15 kΩ for 20 μm x 20 μm electrode), and impedance is low in the low frequency area. Therefore, the MED64 System has sufficiently high input impedance of 100 MΩ and a low frequency signal can be acquired without attenuation, such as the evoked potential of a brain slice or the pacemaker potential of a gastrointestinal tract muscle layer tissue. When 1 Hz signal is acquired at an ITO electrode without a coating, the signal is attenuated because its electrode impedance is higher than 100 MΩ (blue line in the figure below). On the other hand, a platinum black electrode can acquire the signal without attenuation because its impedance is lower than 100 MΩ.



Impedance relationship between electrode and MED64 Head Amplifier.

**6.2.2. Electric stimulation by the MED64 system – Applying current to the micro electrode array**

The MED64 head amplifier has a 2-channel current drive stimulator that can apply electric stimulation to any of the 64 electrodes. The figure below illustrates an equivalent circuit of electric stimulation by the MED64 system. The stimulation current flows from the electrode selected to the reference electrode, and then it is stored in the electrical double layer capacity on the electrode surface and changes the electrode potential. This change in potential changes the extracellular potential and induces depolarization of the cell membrane. As shown on the right side of the figure below, the extracellular potential ( $V_f$ ) changes according to output current ( $I_s$ ).



### 6.2.3. Stimulus artifact and biphasic stimulation

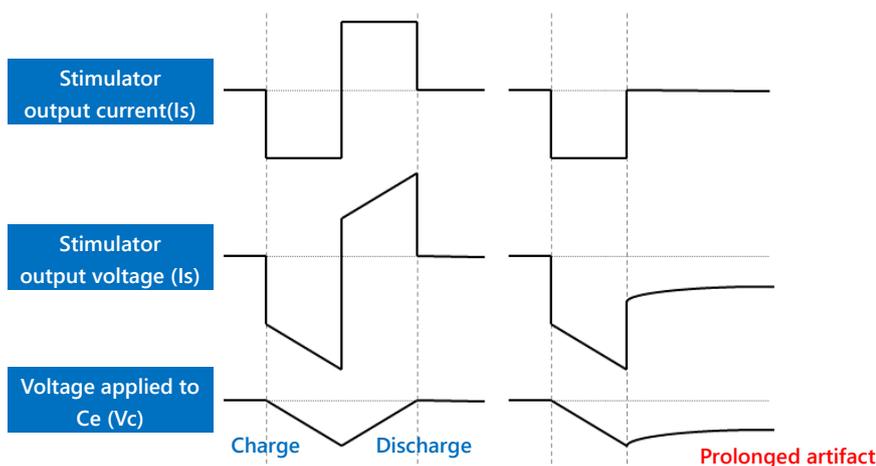
Because of cross-talk between electrodes, the stimulation pulse waveform is received as a “stimulation artifact” by all recording electrodes. A stimulation artifact remains after the end of the stimulation pulse and interferes with the signal waveform from the biological specimen. Mainly the factors listed below influence this process.

1. Typically, the amplitude of the stimulation artifact exceeds the maximum input voltage of the MED64-Entry Amplifier and causes tentative saturation of input. Time is necessary to recover from this condition. This time lag increases in proportion to the stimulator output voltage ( $V_s$ ).
2. At the electrode, the time necessary for discharge is longer than that for charge. This time lag increases in proportion to voltage  $C_e$  ( $V_c$ ).

$V_s$  and  $V_c$  are low because of the low impedance (high capacitance) of the electrode. The MED64 system should be operated properly to minimize the duration of the stimulation artifact. Follow the instructions described below to minimize the width of the stimulation artifact.

1. Apply a current drive biphasic pulse stimulation consisting of a negative and positive pulse with the same width. When the biphasic pulse stimulation is applied, charge and discharge at the electrode occur quickly. On the other hand, when a monophasic pulse stimulation is applied, discharge takes longer than charge at the electrode, which markedly prolongs the stimulation artifact and damages the electrode.

**Note:** For the MED64 system, a current drive biphasic stimulation consisting of a negative and positive pulse of the same width is recommended. A monophasic pulse stimulation damages the electrodes.



Biphasic pulse stimulation and monophasic pulse stimulation.

2. A platinum wire is used as additional reference electrodes. When a platinum wire is gripped with a ground wire with a bagworm clip of the MED Connector, the platinum wire functions as an additional reference electrode. The reference electrodes of the MED Probe are positioned at 4 points, and this additional reference electrode of the platinum wire further reduce the total impedance of the reference electrodes and reduce the noise from the perfusion pump and the stimulation artifact.

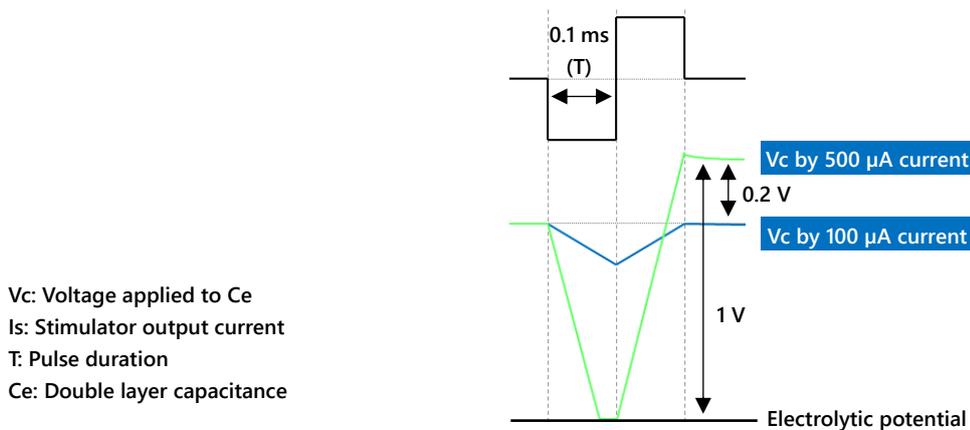
**Note:** Make sure that the platinum wire is fully immersed in the perfusion solution (electrolyte) to conduct with the reference electrodes of the MED Probe, and that it is gripped by a ground wire with a bagworm clip. If the platinum wire is open, it serves as antennas that collect electric noise and cause noise.



Reducing a stimulation artifact by a platinum wire. Examples without (left panel) and with (right panel) a MED perfusion cap

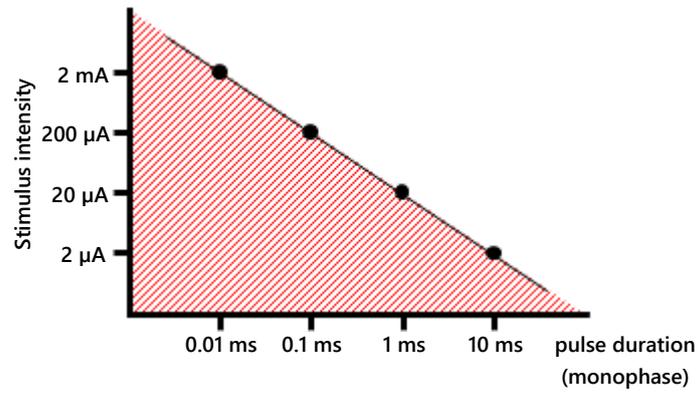
**6.2.4. Stimulus intensity and electrolysis**

As described in the above section, the stimulation current is charged in the electrical double layer capacity and causes voltage  $V_c$  (see figure below). When the voltage  $V_c$  exceeds 1 V, the electrodes induce electrolysis and discharge  $H_2$ . This causes a marked decrease in stimulation efficiency and a marked expansion of the stimulation artifact, and it damages cells and the electrodes. Therefore, it is essential to prevent a decomposition voltage by stimulation current ( $I_s$ ).



$V_c$  is defined as  $V_c = I_s \times T / C_e$ . A 50 x 50 µm electrode of the MED Probe has a 50,000 pF capacitance ( $C_e$ ) (22,000 pF for 20x20 µm electrode). Up to 0.2 ms width (1 phase 0.1 ms) is acceptable for a 200 µA stimulation current.

**Note:** Do not apply a stimulation intensity and pulse duration width spanning to outside of the shaded area in the figure below. The numerical values in the graph are based on a "single pulse stimulation." When a multiple pulse stimulation is applied at a short inter-stimulation interval of less than 1 s, reduce the stimulation intensity and pulse duration to 80% of the value shown in the graph.



**6.2.5. About the stimulus interval**

The stimulus intensity and stimulation electrode are set in the STIMULATION panel of the MED16 (for details, refer to p. 11 "4.2 Creating stimulation pattern – the STIMULATION subpanel and STIMULATION PATTERN tabs). Please note that in the amplifier, about 5 seconds is necessary to cancel mute at the stimulation electrode (recovery of baseline noise). Therefore, define an interval of not less than 5 seconds between traces.

This document is subject to change without notice. Reproduction or reprinting of this document, in whole or in part, without the permission of the copyright holder, Alpha MED Scientific Inc., is prohibited. Although every possible care has been taken in preparing this document, the authors assume no responsibility for any errors or omissions in the descriptions in this document, or for any damages that may result from these errors or from the programs or source code described in this document. In no event shall the publisher or authors be liable for any direct or indirect damages resulting from the use of this document.

© 2022 Alpha MED Scientific Inc. ★ All rights reserved.

Version: 1.00; 2022.5.13

■ Manufacturing

Alpha MED Scientific, Inc.

Saito Bio-Incubator 209, 7-7-15, Saito-asagi, Ibaraki, Osaka, 567-0085, Japan

E-mail: [support@med64.com](mailto:support@med64.com)

Website: <https://alphamedsci.com/english>

■ Sales & Technical Support

SCREEN Holdings Co., Ltd. Bioscience & Pharmaceuticals

Furukawa-cho 322, Hazukashi, Fushimi-ku, Kyoto 612-8486, Japan

Website: <https://screen-cell3imager.com/english>