MED64

The most sensitive microelectrode array system for *in vitro* extracellular electrophysiology

User Guide

MED64-Quad II

ALPHA MED SCIENTIFIC

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Contents

1. MED64-Quad II introduction and components

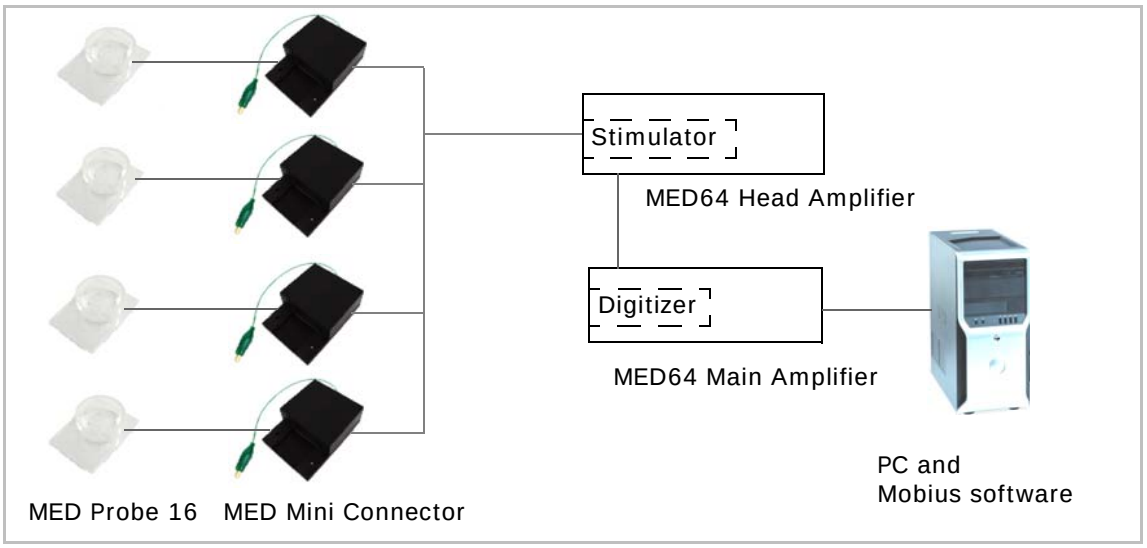
The MED64-Quad II System is a medium throughput system designed to record from 4 acute or cultured samples simultaneously. It affords the ability to acquire extracellular signals (field potentials) from 4 sets of MED Probe 16s (16 electrodes per a Probe), and deliver stimulation to any of the 16 electrodes in a Probe sequentially.

1-1. System configurations

1. MED64 Main Amplifier (MED-A64MD1)	1
2. MED64 Head Amplifier (MED-A64HE1S)	1
3. MED Mini Connector (MED-C04)	4
4. MED Probe 16 (MED-PG515A)	4
5. Acquisition PC	1
6. Mobius Software	1

Accessories:

1. MED Perfusion Cap (MED-KCAP01TU)	4	
2. MED ThermoBase (MED-CPB01)	2	
3. ThermoClamp™-2 controller	1	(Or ThermoClamp™-1 controller 2)
4. Peristaltic pump (4 channel)	2	



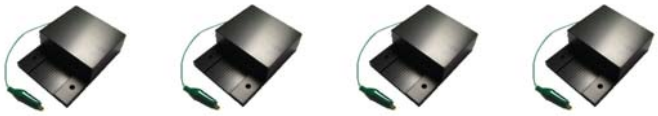
1-2. Components

Please have following items ready for your installation:

1. MED64 Main Amplifier [MED-A64MD1] (1)

2. MED64 Head Amplifier [MED-A64HE1S] (1)

3. MED Mini Connector [MED-C04] (4)



4. MED Probe 16 [MED-PG515A] (4)

5. Cables

1) 68pin cable (1) 2) BNC cable (2) 3) Stimulus control cable (1) 4) USB cable (1)

5) 20pin cable (4)

6) Power supply cord (2)

6. Acquisition PC

7. Power supply strip
(4 outlets for the MED64-Quad II / 8 outlets for the MED64-Quad II + perfusion system)

Accessories:

1. ThermoBase [MED-CPB01] (2)
A ThermoBase is provided with 2 of MED-C04 Connector loaded when they are purchased together.

2. Installation of the MED64-Quad II System

2-1. Installing the MED64-Quad II System

The MED64-Quad II System has several technical advantages due to the low-impedance platinum-black microelectrodes on the MED Probe 16 (typically 10 kohm at 1kHz for 50 μm electrodes). These include;

1. The system is more resistant to exogenous noise (e.g. hum noise).
2. Very low Johnson noise (baseline noise) as low as a few microvolts can be achieved.
3. The MED probe 16 / Mini Connector is physically separated from the amplifier (enabling long-term recordings in a humidified incubator). The connector cable is 2m long and engineered for
4. with low signal attenuation and low noise.

The MED64-Quad II System does NOT usually require a Faraday cage or vibration isolation table such as is used with a conventional electrophysiology rig. It is recommended that the MED64-Quad II System be **installed on a STABLE TABLE** such as a lab bench using aluminium foil. Some exceptions include when using the MED64 in conjunction with other equipment such as an incubator or microscope.

The following procedure is recommended when installing your MED64-Quad II System to identify the noise source if it possibly happens:

Step1: Install Amplifiers, MED Mini Connector, MED Probe 16, and PC ONLY.

Step2: Check the noise level.

(When using ThermoBase)

Step 3: Install the ThermoClamp controller, and then connect the ThermoBase to the ThermoClamp. Turn them on.

↓

Step 4: Check the noise level.

(When using perfusion) *Step 5: Install the perfusion system, start perfusion.*

↓

Step 6: Check the noise with perfusion.

CAUTION:

- Please read the product manual of all individual instruments before installation. Improper installation or operation may cause damage to the devices.

1. The MED64 Quad II System will be installed **on a STABLE table.** The Figure 2-1 (left) shows the MED64-Quad II System installed on a lab bench.

   Place the MED64 Main Amplifier (MED-A64MD1) on top of the Head amplifier (MED-A64HE1S). This location provides the MED Connector Cable (the cable connecting the Head amplifier and MED Connector) more stability. Make sure to follow this arrangement particularly when a laptop PC is used, otherwise noise could be generated.

2. Place the all for Mini Connectors on aluminum foil. (Figure 2-1, left)

3. Ground the aluminum foil to the SIGNAL GND in the Head Amplifier. (Figure 2-1, right)

4. When the MED64-Quad II System is used with perfusion accessories, place the peristaltic pump and ThermoClamp controller AWAY from the Mini Connectors and Connector Cables. Do NOT place such equipment on top of the aluminum foil. (Figure 2-2.)

    - Check the baseline noise level BEFORE connecting the ThermoBase or ThermoClamp. This will allow you to see the noise level without any peripheral items.

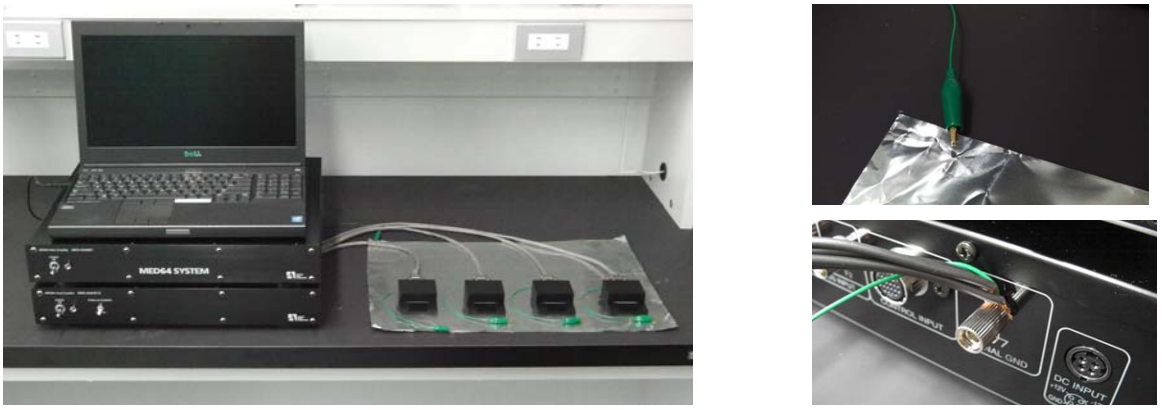


Figure 2-1. The MED64-Quad II System installed on a lab bench (left) and grounding the aluminum foil (right).

Figure 2-2. MED64 Quad II components and perfusion accessories placed on a lab bench.

CAUTION:

The aluminum foil under the MED Mini Connector MUST be grounded to the Head Amplifier. Please do so even if you see acceptable noise level without this grounding. Although the MED Connector is made of aluminum, its outer paint could prevent conductivity between the MED Connector and the aluminum foil. Without grounding the aluminum foil to the Head Amplifier, the system will be unstable, which could result in unexpected noise later.

5. Connect all equipment.

   (1) Connect the [INPUT] terminal of the MED64 Main Amplifier to the [OUTPUT] terminal of the MED64 Head Amplifier with the short 68 pin cable.

   (2) Connect [F1 STIMULUS OUTPUT] of the MED64 Main Amplifier to the [F1 STIMULUS INPUT] of the MED64 Head Amplifier with the BNC cable.

   (3) Connect [F2 STIMULUS OUTPUT] of the MED64 Main Amplifier to the [F2 STIMULUS INPUT] of the MED64 Head Amplifier with the BNC cable.

   (4) Connect the [CONTROL OUTPUT] of the MED64 Main Amplifier to the [CONTROL INPUT] of the MED64 Head Amplifier with a cable.

   (5) Connect the MED Mini Connectors to the terminals for [INPUT 1-16CH], [INPUT 17-32CH], [INPUT 33-46CH], and [INPUT-49-64CH]with the 20 pin MED Connector cables.

   - Leave the [INPUT] terminal (64ch terminal) FREE from any connection when the MED64-Quad II System is used.

   (6) Connect the ground wires that are attached onto the MED Connector Cable to the [SIGNAL GND] of the MED64 Head Amplifier.

   - Let the ground wires for all 4 Connectors go THROUGH the hole at the GND (Figure 2-3, top-right) to assure the grounding. If they do not fit together, use a short alligator clip wire (Figure 2-3, bottom left) to connect all cables (Figure 2-3, bottom right).

   - Make sure that the aluminum foil under the Mini Connectors is also grounded here.

Figure 2-3. Grounding the Connector cables to the GND terminal of the MED64 Head Amplifier.

   (7) Connect the [USB] terminal of the MED64 Main Amplifier to the [USB] port on a PC.

   (8) Connect DC power supply cables to both amplifiers.

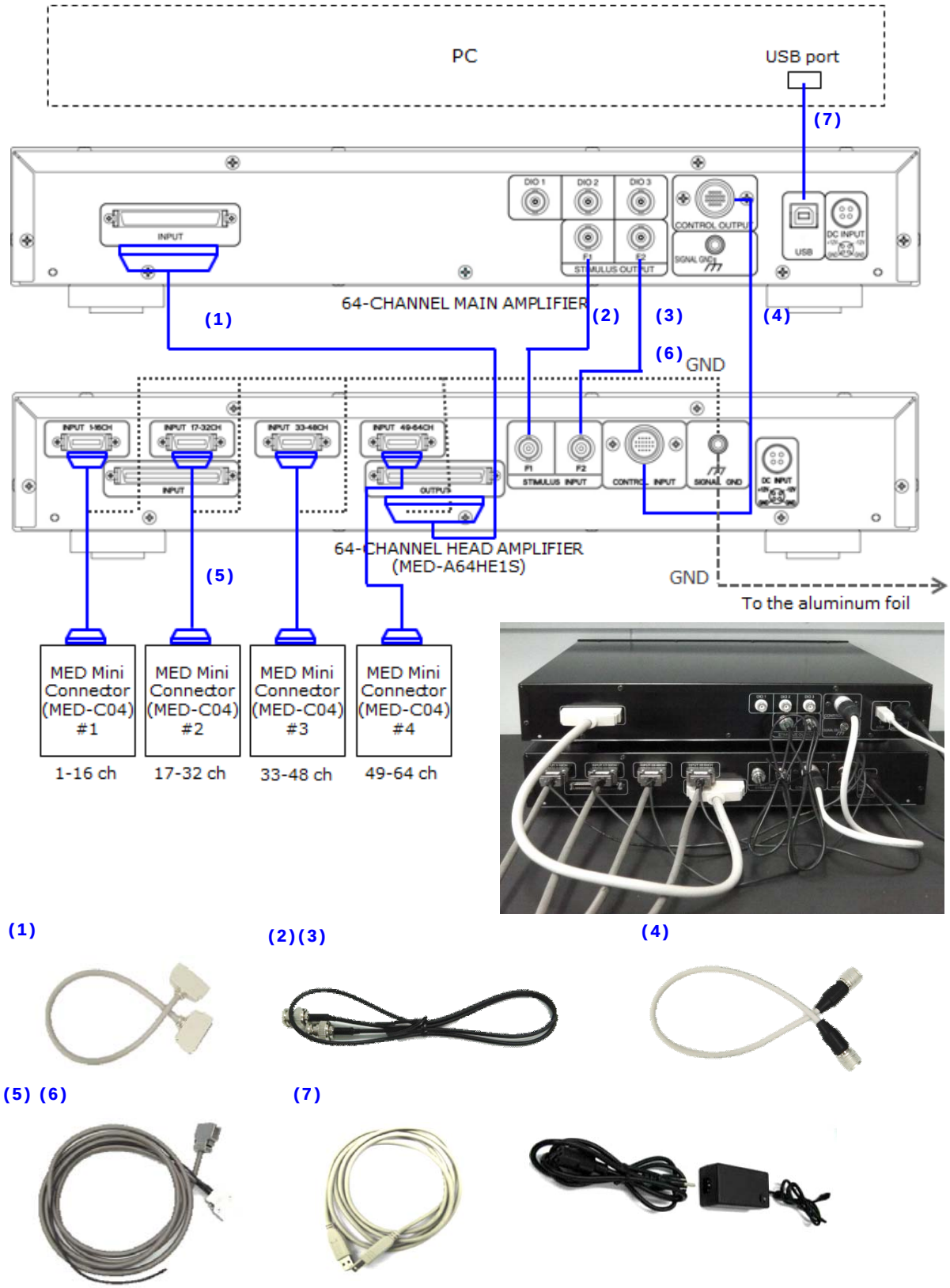


Figure 2-4. Cable connections for the MED64-Quad II System.

9. Insert the 3-pronged power plugs of the Main Amplifier, Head Amplifier, and PC into a SINGLE POWER STRIP, and connect the power strip in a wall socket **so that all equipment are grounded through the power strip** (Figure 2-5).

   Do NOT connect any other power plug to the power strip, or the same wall socket. Place the power strip, and all power supply unit and code AWAY from amplifiers, and MED Connector Cable. (Refer to page 8 for detailed.)

   - For the continental European model power plug (2-prolonged), use the power strip and wall socket which have ground terminals.
   - The power supply cord for ThermoClamp controller and peristaltic pump must be connected to the same power strip. **However, do NOT connect them for now to test the noise level without peripheral equipment**.

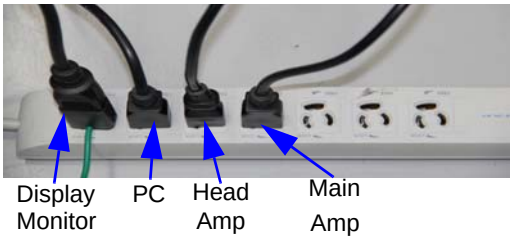


Figure 2-5. Power supply cord.

10. Place the all power supply units away from amplifiers, MED Connector, and the Connector Cable. Also, do NOT place any other power supplies close to the MED64 (refer to the Figure 2-6).

Figure 2-6. Examples of good and bad placing for power supply unit.

11. The MED Connector Cables (cable connecting the MED Mini Connector to the Head Amplifier) are very sensitive to noise as well as vibration. Place them AWAY from any power supply. Make sure that cables are placed flat on a table. **The best location for the cables is UNDER the amplifiers.**

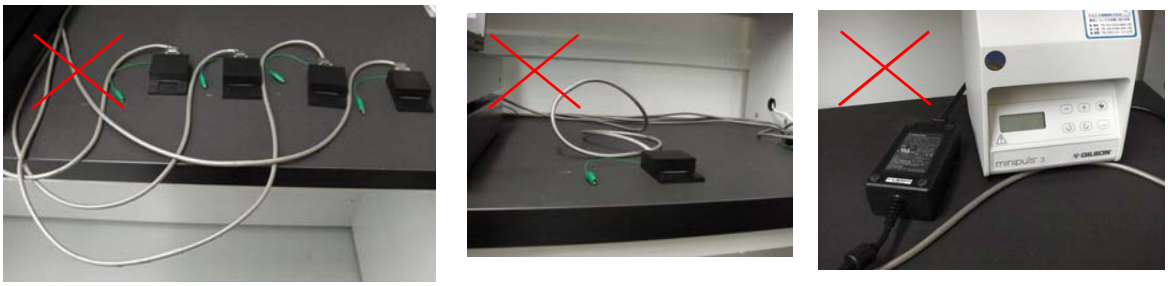


Figure 2-8. Bad examples for location of the Connector Cables. Cables are NOT placed flat on the table (left, middle) and touching the peristaltic pump (right).

12. The Figure 2-9 and 10 shows you MED64-Quad II System installed on a lab bench.

Figure 2-9. MED64-Quad II System installed on a lab bench.

Figure 2-10. MED64-Quad II System with perfusion accessories. (Perfusion accessories are not connected yet, and connections will be made in the following section.)

Orientation for the MED Mini Connector

There are two types of MED Mini Connector (MED-C04) and therefore two different orientation and channel assignments as seen in the Figure 2-11 and 2- 12 below.

1. Type B (MED-C04-B)

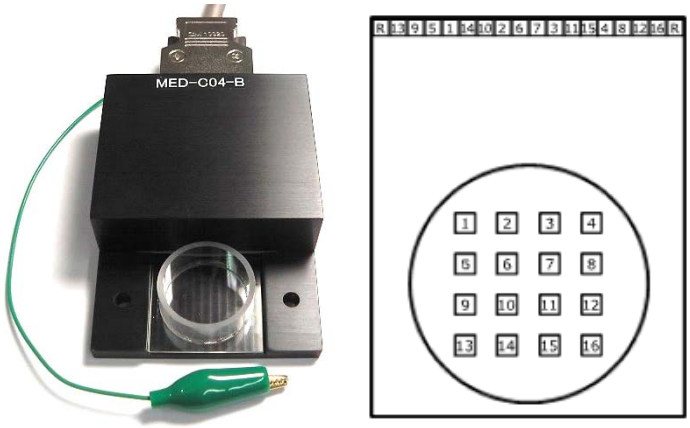


Figure 2-11. Type B Connector (left) and electrode orientation when the connector terminal is located on the top.

2. Type R (MED-C04-R)

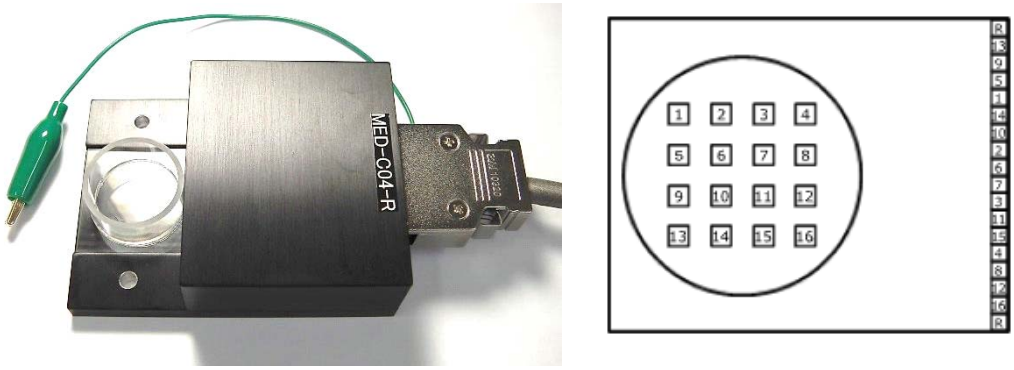


Figure 2-12. Type R Connector (left) and electrode orientation when the connector terminal is located on the right.

2-2. Running the MED64-Quad II System and checking the noise

1. Load the 4 MED Probe 16 filled with saline into the MED Mini Connector. Grab the chamber or edge of the bottom of the glass, and NOT the terminal (leads). Clean the terminal with kim-wipe before loading it (Figure 2-13).

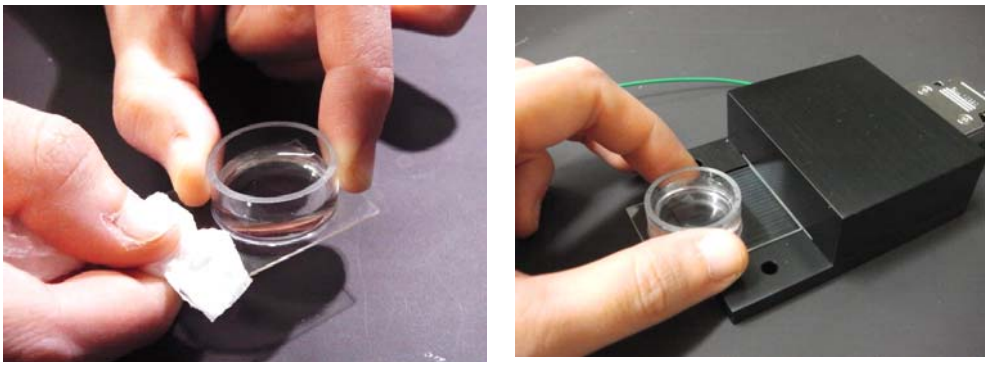


Figure 2-13. Loading the MED Probe 16 into the MED-C04 Connector.

CAUTION:

- Be extremely careful not spill the solution over the MED Mini Connector. Spilled liquid will cause rust at the contact pins and adversely affect conduction.

NOTE:

The test boards (accessory for the MED Mini Connector could be used for checking the noise level. However, it is still recommended that noise level be checked using new pre-coated MED Probes filled with saline. This recommendation is due to the test board's susceptibility to exogenous noise.

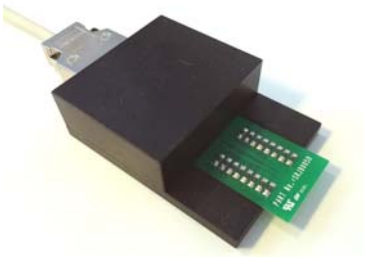


Figure 2-14. How to test the MED Mini Connector with the test board.

2. Turn on the amplifiers, PC and display monitor.

3. Start Mobius software by double-clicking the Mobius icon.

CAUTION:

- Do NOT run any other software while recording. It might cause the PC to stop while recording. Particularly be careful not to run software which runs in the background such as antivirus software.

- DO NOT connect to the internet while recording. It might cause the PC to stop while recording.

4. Select [Workflow] > [New] > [From Template]. Select either of [64MD1_1280x1024] or [64MD1_1920 x 1080] folder depending on the size of your display monitor. (Figure 2-15)

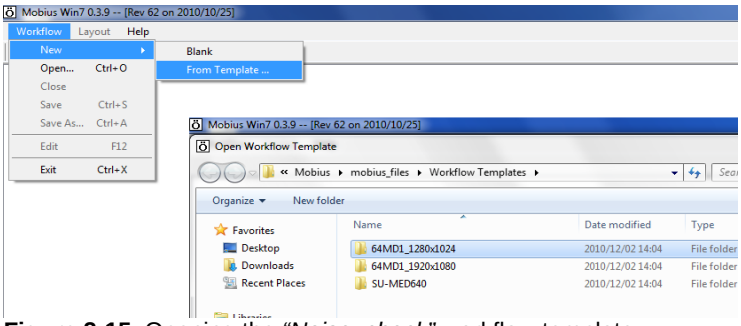


Figure 2-15. Opening the *"Noise_check"* workflow template.

5. Select [Basic Recording] folder, then open the *"Noise Check"* Workflow template (.moflo file). The default acquisition parameters are as follows. Please don't change these parameters for now to see whether system is working with acceptable noise level.

   * Input Range (mV) : 5.0
   * Low cut freq (Hz) : 1
   * High cut freq (Hz): 10000

NOTE:

Turn on both the Head/Main amplifier to open the "Noise Check" workflow template. Otherwise, the acquisition workflow will not work.

6. Click the [64th display] tab to open the 64-channels display (64 channel oscilloscope).

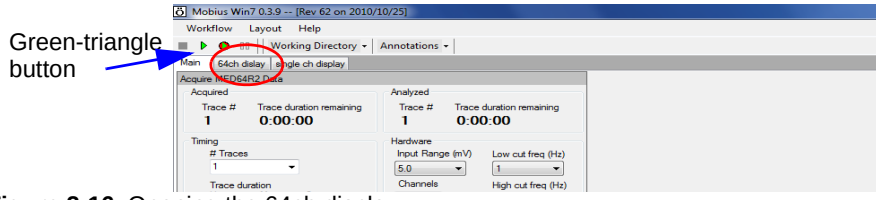


Figure 2-16. Opening the 64ch display.

7. Click the Green-triangle button to run Mobius without saving data.

8. The message "Please wait while amplifier is calibrated" will pop-up. Please wait for several seconds for the pop-up message to disappear. Mobius will start automatically after the calibration. Then you will be able to see the baseline signals of all 64 channels.

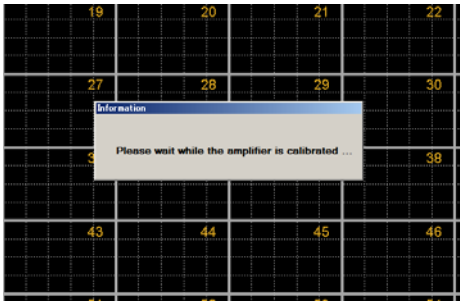


Figure 2-17. The pop-up message that appears when the MED64-Quad II System is run for the first time. Please wait for several seconds for the pop-up message to disappear.

9. The baseline noise level should be around a few micro volts at the scale of 25 μV/20 ms as seen in Figure 2-18. If a larger noise level is present, please minimize the noise according to the section on "Troubleshooting".

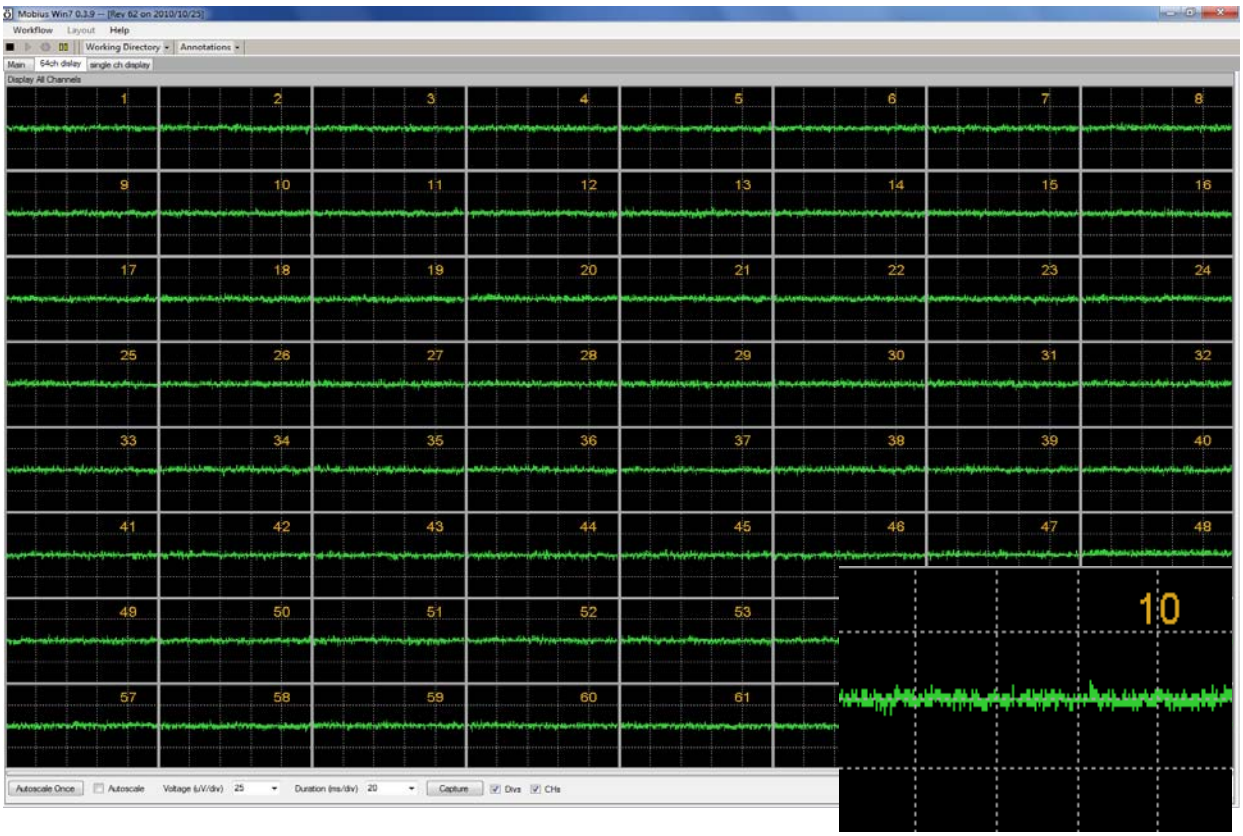


Figure 2-18. Appropriate noise level for the MED64-Quad II System (25 μV/ 20 msec).

2-3. Installing the ThermoBase

Once the MED-Quad II System is installed and appropriate noise is confirmed, install the ThermoBase to the ThermoClamp controller. One ThermoBase (where 2 MED Mini Connectors are loaded) connects to one ThermoClamp™-1 controller. (Or, Two ThermoBases are connected to one ThermoClamp™-2 controller.)

Figure 2-19. ThermoBase where two MED-C04 Connectors are installed (left) and safety plug (right).

1. Place a ThermoClamp AWAY from the MED Mini Connectors or Connector cables.
2. Connect the blue safety plug attached to the ThermoBase to the [Control TC] of the ThermoClamp.
3. Connect the white PS2 cable to the [Heater Power] of the ThermoClamp.
4. Connect the blue safety plug to the [Safety TC] of the ThermoClamp.
    - The MED ThermoBase does NOT work appropriately without the [Safety TC] connected with the safety plug.

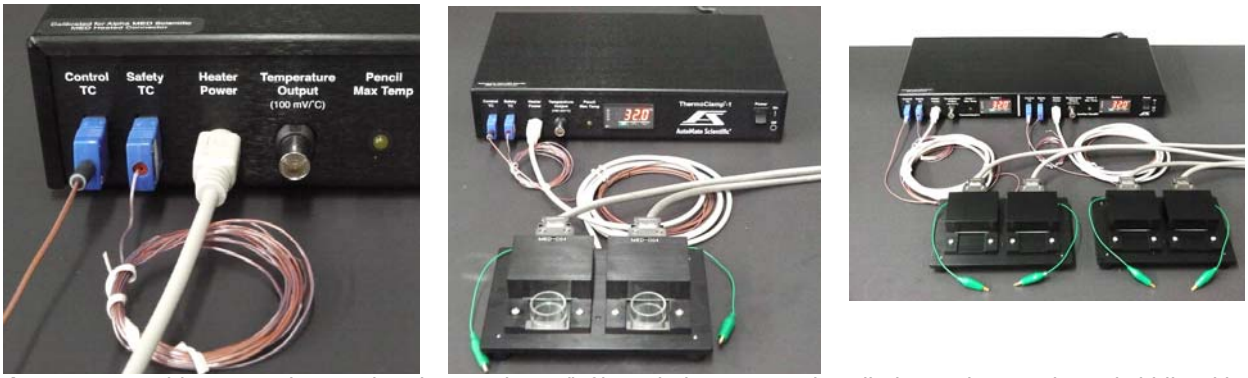


Figure 2-20. Cable connections to the ThermoClamp (left), and ThermoBase installed to a ThermoClamp (middle with the ThermoClamp-1, right with the ThermoClamp-2).

5. Connect the power cord of the ThermoClamp to the power strip where the amplifiers are connected.
6. Run the MED64-Quad II System and check the noise. The same noise level (seen in the Figure 2-18) should be achieved.

3. Installing the perfusion system for the MED64-Quad II

The Perfusion Cap (MED-KCAP01) is available for perfusion in the MED64-Quad II System. This section guides you through installation of the perfusion system for the MED64-Quad II using:

1. 4 sets of Perfusion Cap and Tube Kit (MED-KCAP01TU)
2. 2 sets of 4-channel peristaltic pump (Minipuls 3 manufactured by Giilson inc.)

CAUTION:

Please read and follow the instructions in the product manual for the ThermoClamp and Minipuls 3 (or your own peristaltic pump).

NOTE:

Install the perfusion system AFTER installing the MED64-Quad II System and achieving its adequate low noise.

3-1. Perfusion Cap and its accessories

Several small accessories to help your installation are included when the MED64-Quad II System is purchased together with “Perfusion Cap with Tubes” The Figure 3-4 shows 1 set of those accessories.

A: Perfusion Cap and Tube Kit (MED-KCAP01TU)

B: Dripper stand

C: Flask

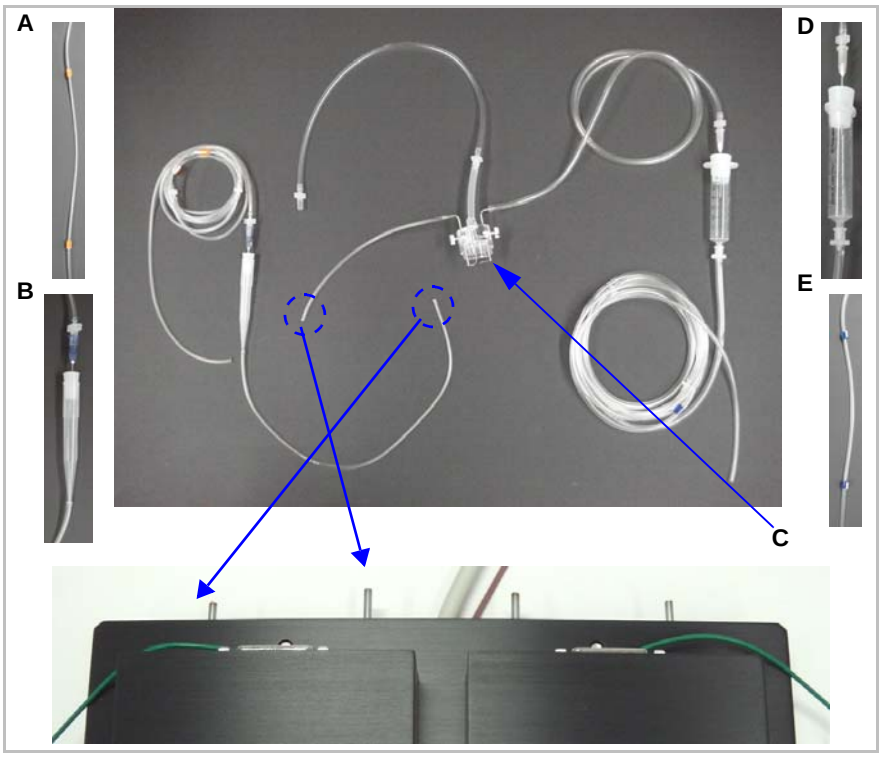
D: Extra drippers

E: Grounding wires

Figure 3-1. 1 set of perfusion Cap and Tube Kit (A) and accessories included in the new MED64-Quad II System.

Figure 3-2 shows the Perfusion Cap and Tube kit (MED-KCAP01TU). The MED64 ThermoBase has “Perfusion ports” for in-line heating. Connect the inlet tube to those ports so that solution so that solution is warmed up before sent to the MED Probe chamber.

The perfusion tubes in the Perfusion Cap and Tube kit (MED-KCAP01TU) are designed for for “Minipuls 3 manufactured by Gilson Inc. If another type of peristaltic pump is used, A and E in the Figure. 3-2 needs to be replaced by the ones for your peristaltic pump.



A: Inlet tube for the peristaltic pump.

B: Inlet dripper

C: Perfusion Cap (MED-KCAP01)

D: Outlet dripper

E: Outlet dripper for the peristaltic pump

Figure 3-2. Perfusion Cap and Tube Kit (top) and perfusion ports in the ThermoBase.

Figure. 3-3 shows the Perfusion Cap (MED-KCAP01). Be noted that **the inlet pipe has a hole in the bottom while the outlet pipe has a hole at the side** to minimize vibration of solution in the chamber during perfusion.

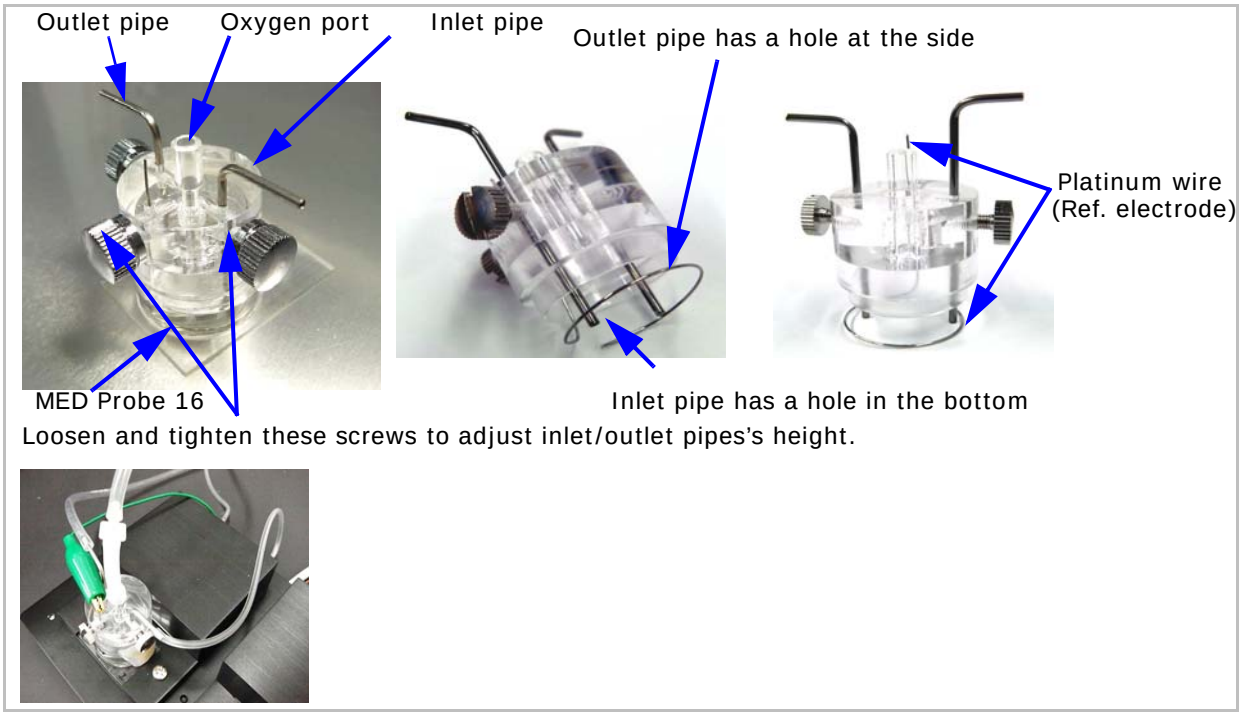


Figure 3-3. Perfusion Cap. Details (top) and placed onto the MED Probe 16 (bottom).

3-2. Installing the perfusion system

1. Place the Peristaltic pump **AWAY from the amplifiers, MED (Heated) Connector, and MED Connector Cable.**

2. Plug the power cables for the Peristaltic pumps into the same power strip as the MED64 Head/Main Amplifiers and ThermoClamps. Make sure that the power strip has a grounding terminal (3 pronged plug) and plug the power cord into a grounded wall socket so that all equipment is grounded to the earth.

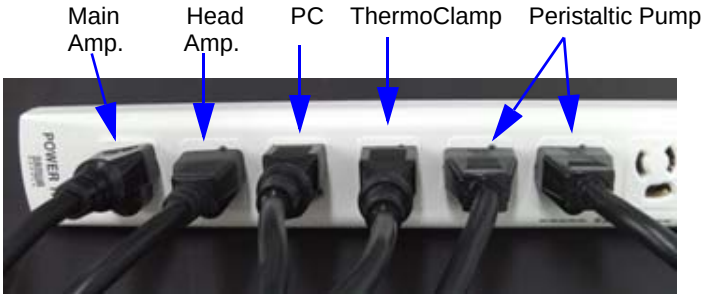


Figure 3-4. Grounding the peristaltic pump and ThermoClamp controller through a power supply cord.

3. Install the perfusion tubes. Figure 3-5 shows how a set of perfusion tube is installed.

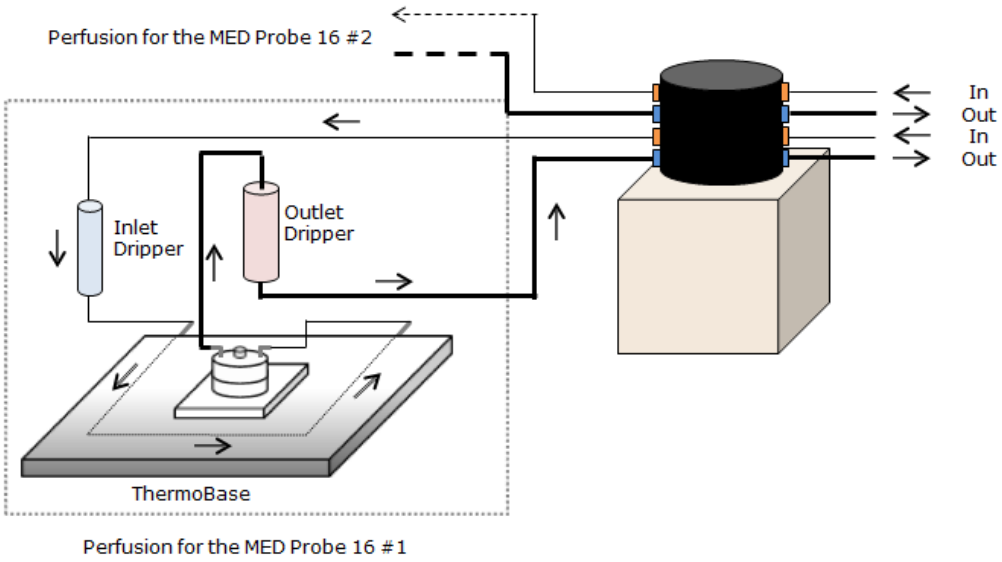
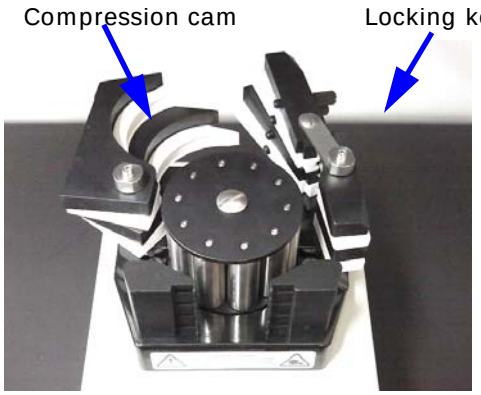
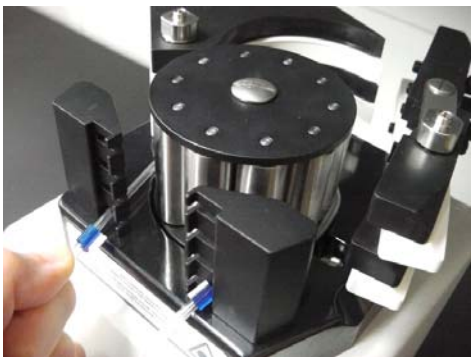


Figure 3-5. Perfusion Cap and Tube Kit installed in a peristaltic pump and MED Probe 16.

(1) Install the inlet/outlet tubes to the peristaltic pump. The orange represents inlet while the blue represents outlet. Loosen the bars of the peristaltic pump, hook the tube around the rollers, and then close the bars. Make sure that each tube goes under each bar (Refer to Figure 3-6).



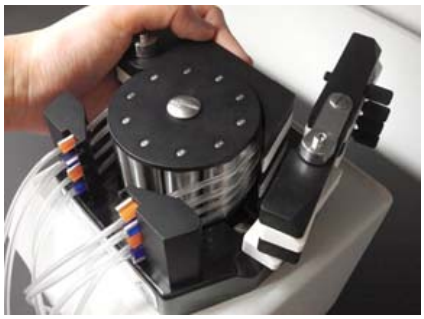
1. Open the Locking key and Compression cam.



2. Install the tube to the rollers.

3. Outlet(orange)/Inlet(blue) tubes installed to the Minipuls 3.

4. Make sure that all tubes are installed horizontally so that they sit under each compression cam.



4. Close the Compression cam and Locking key.

5. Installation completed.

6. Bad example: tubes are twisted.

7. Bad example: tubes are not installed horizontally.

Figure 3-6. Installing the perfusion tube to the Minipuls 3.

(2) Stand the inlet/outlet drippers vertically. For their installations, disconnect the tube in the top-joint temporally so that both drippers go through the ring in the dripper stand. (Figure 3-7) **Place them on the aluminum foil.**

NOTE:

The drippers function to prevent introduction of noise by breaking the solution flow within them. Make them to stand vertically so be sure that the solution is cut and bubbles are removed in them. .

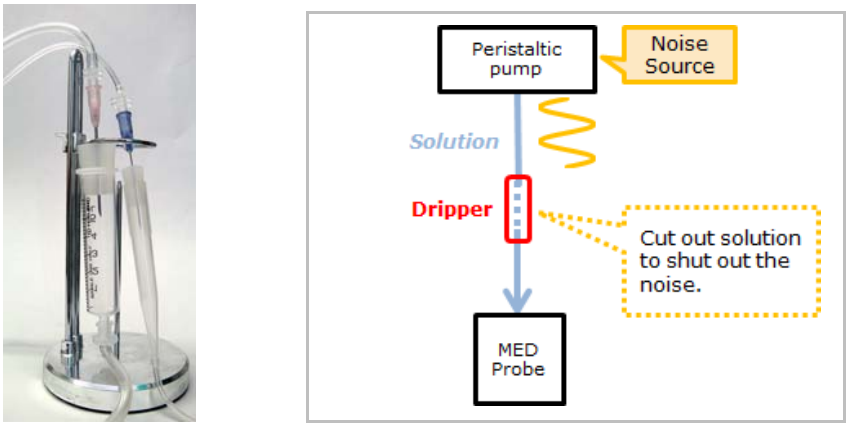


Figure 3-7. Inlet/Outlet drippers standing vertically within the dripper stand (left), and how they work to eliminate noise (right).

Figure 3-8. Installing the outlet dripper to the dripper stand.

3. Connect the oxygen ports of the Perfusion Cap to the port for mixed gas (O2:95%, CO2:5%). It is recommended that you humidify the gas using the flask containing water before sending to the MED Probe (Figure 3-9).

NOTE:

It is recommended to provide mixed gas from the oxygen port when the experiments are performed with the "interface" condition so that upper side of the slice is sufficiently oxygenated*. When the experiment is performed with "submerged" condition, these may not be necessary.* Humidifying *the mixed gas before sen*ding *to the MED Probe will maintain* proper humidity *inside of the the MED Probe chamber. The bubbles in the flasks could also indicate whether oxygen is supplied to all slices well enough.*

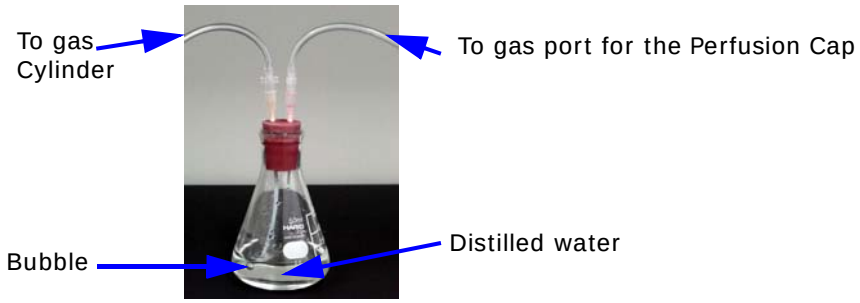


Figure 3-9. Moistening mixed gas before sent to the MED Probe.

4. Connect the green ground wires attached to the MED Mini Connector to the platinum wires (reference electrodes) for grounding. (Figure. 3-10)

NOTE:

The platinum wire functions as an additional reference electrode. The MED Probe has 4 reference electrodes embedded in the dish. However, the platinum wire decreases the total impedance of all reference electrodes in a MED Probe, that suppresses noise as well as the duration of stimulus artifact.

CAUTION:

Make sure to ground the platinum wire (reference electrode) to the MED Mini Connector using the alligator clip attached to it while acquiring data. Otherwise, large noise will be introduced.

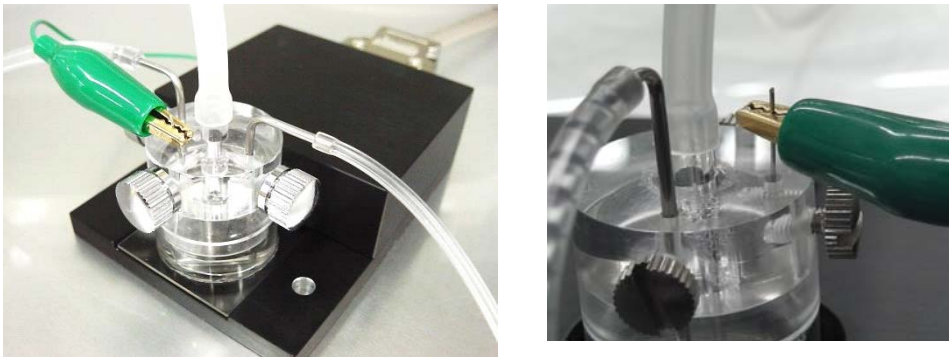


Figure 3-10. Grounding the ref. electrode (platinum wire).

Figure 3-11 shows how 1 set of perfusion cap and tubes installed.

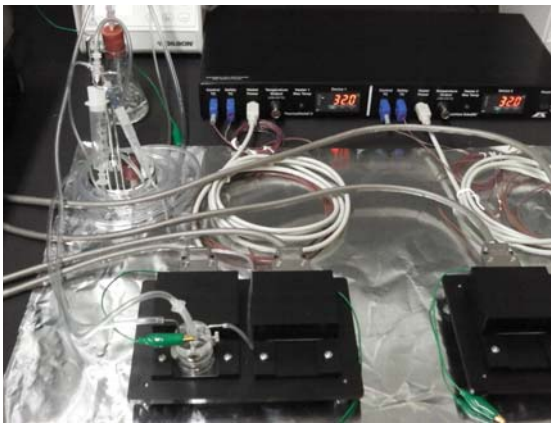


Figure 3-11.

5. Install perfusion tubes for other 3 Probes/Connectors. The Figure 3-12 shows the MED64-Quad II installed with perfusion system.

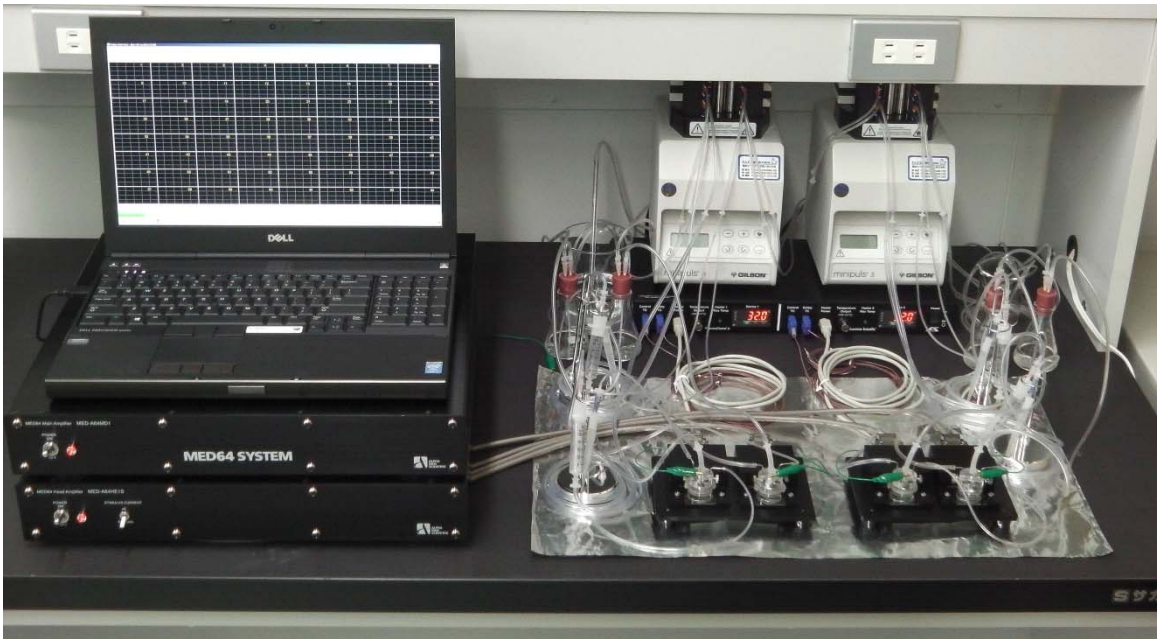


Figure 3-12. MED64 Quad II System with perfusion installed on a lab bench.

6. Adjust the height for the inlet/outlet pipes in the perfusion cap using the screw located at the sides of the Perfusion Cap. Adjust the height for the inlet pipe so that its bottom tip is slightly above the bottom glass of the MED Probe. Then, adjust the height for the outlet pipe in order to set the desired level of the solution in the MED Probe.

   For now, make the solution level high enough for all electrodes (including reference electrodes) to be submerged with solution for checking the noise level.

Figure 3-13. Location of the inlet and outlet pipes in the MED Probe. It is recommended to position the inlet pipe as low as possible to avoid fluctuation of the solution due to the water-drop.

NOTE:

It is fairly well established that larger and more stable signals are observed in slices maintained under the "interface" conditions compared to "submerged" condition.

7. Turn on the peristaltic pump. If air bubbles are seen regularly in the outlet tube (Figure 3-14), it is a good sign that solution stays at the same level. On the other hand, irregular bubbles with variable size (Figure 3-15) indicates that solution-level is irregular and is an indication of an instable base-lines.

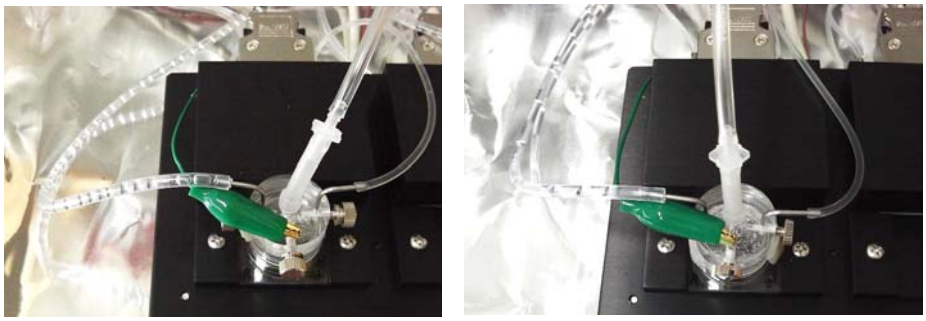


Figure 3-14. Bubbles with regular intervals, which indicates stability in solution level.
Figure 3-15. Bubbles with irregular intervals.

8. Run the MED64-Quad II System and check the noise level while perfusing solution. Try to achieve the same noise-level as Figure 2-17 (page13) with perfusion.

9. If perfusion noise appears, connect the inlet pipe to the ref. electrode (platinum wire) with a short alligator clip.

Figure 3-16. Grounding the inlet pipe to the ref. electrode.

CAUTION:

Grounding the inlet pipe to the ref.electrdoe (platinum wire) forms "grounding loop". Make sure to use a short alligator to connect them. Otherwise, large noises could be introduced.

A tip for operating MED64-Quad II with perfusion

Sharing a peristaltic pump for 2 (or 4) Probes makes your slice mounting onto Probe more difficult. A slice mounted onto a Probe needs to be perfused immediately while perfusion needs to be stopped during mounting the next slice.

This dilemma could be solved by using previously used Probes as temporary storages for perfusion caps. Move the Perfusion Cap to the used-Probes when the 2nd (or later) slice is mounted.

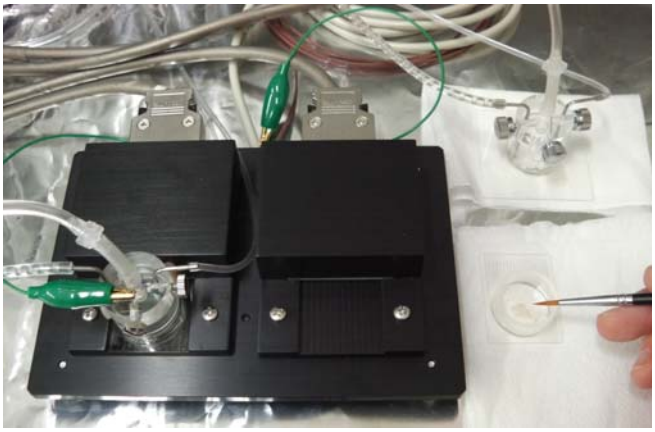


Figure 3-17. The 2nd slice is mounted without stopping perfusion to the 1st slice.

4. Instructions for use

4-1. Running the MED64-Quad II System

The MED64-Quad II can be run using Mobius' acquisition and analysis modules using the same procedure as the MED64-Basic System. Please refer to Mobius Tutorial for operation of the Mobius software. This section describes the unique feature of the MED64-Quad II System.

CAUTION:

Avoid performing your experiments without MED Probe loaded as that will introduce noise. Load the test board or MED Probe 16 filled with saline to the MED Mini Connector when experiments are performed with fewer slices than 4.

Display 8x8 channel

Mobius' [Display 8x8 channel] module allows you to see signals independently per Probe/Connector. Workflow templates including this display module are available from the web site.

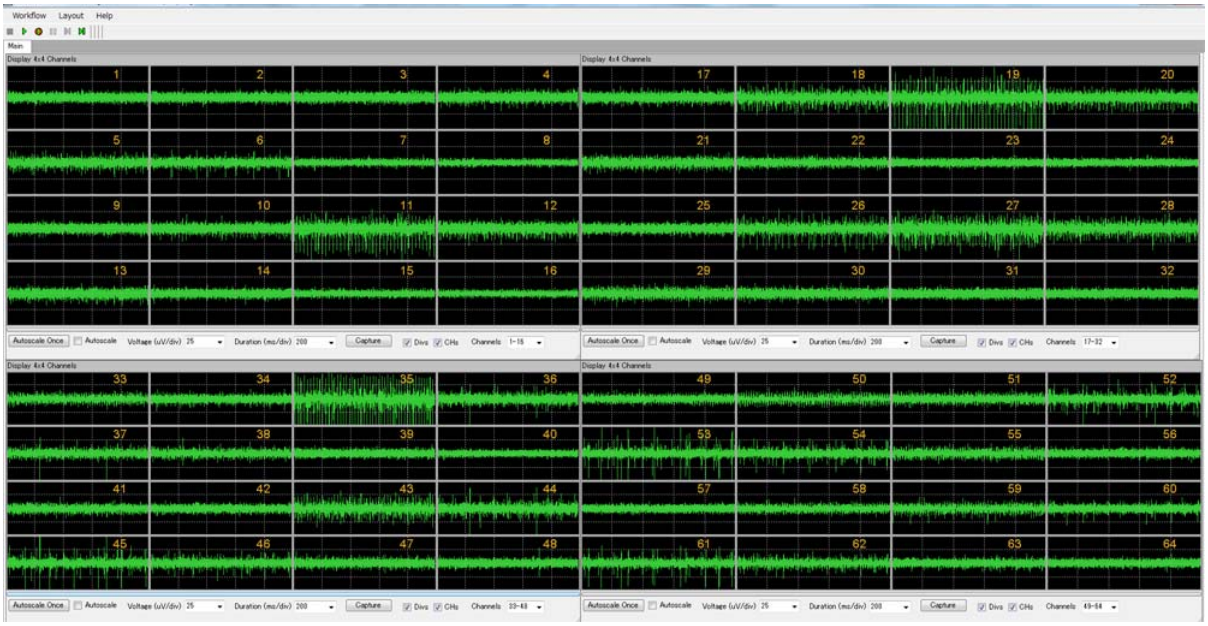


Figure 4-1. Spontaneous signals displayed at 4 "Display 8x8 channel" module.

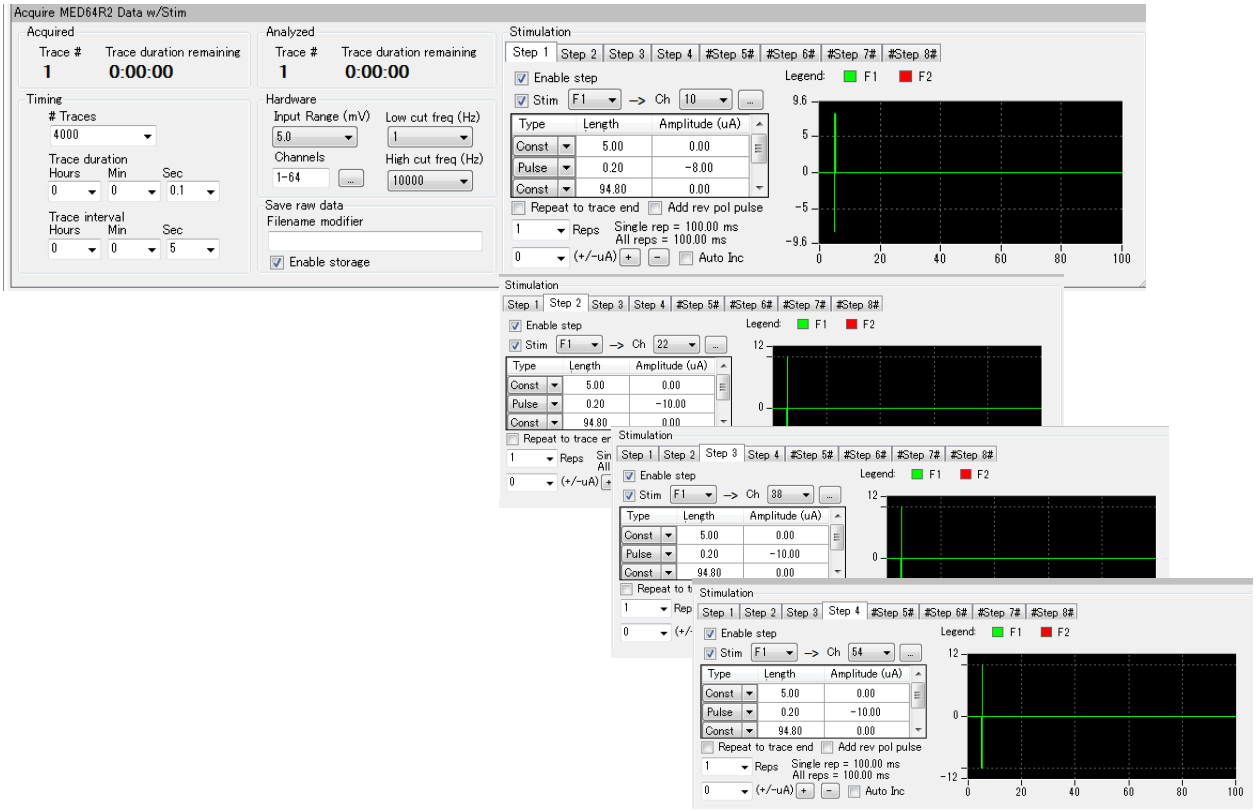
Recording with stimulation

It is NOT recommended to stimulate multiple samples/probes simultaneously due to leakage of stimulus artifacts over adjacent samples/probes. **Stimulate each sample / probe sequentially with interval longer than 5 seconds.** This can be done with following parameters set in the [Acquire MED64R2 w/stim]:

1. Enable Step 1-4.
2. Select single stimulator (e.g. F1) for each Step.
3. Set the trace interval for longer than 5 seconds.

CAUTION:

Do NOT set the Trance Interval to shorter than 5 seconds. The Amplifiers require at least 5 seconds to refurbish themselves.



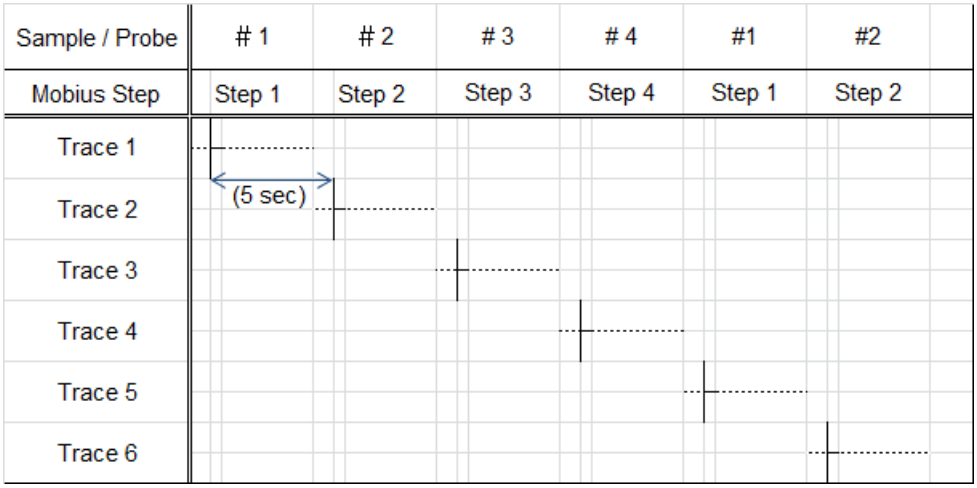


Figure 4-2. An example of stimulus and recording of the MED64-Quad II System. Stimulation is delivered in Step 1-4 (corresponding to Connector/Probe 1-4) with intervals of 5 secondes.

4-2. Running the MED64-Quad II System with perfusion

It can be challenging to control perfusion for 4 slices, you may have to make compromises in the beginning by starting with fewer slices. Below are some tips and suggestions for running the MED64-Quad II with perfusion.

- Make sure that the Inlet/Outlet drippers are standing vertically and placed on top of the aluminum foil grounded to the GND of the Head Amplifier.
- Verify that Gas is delivered sufficiently to all slices.
- For acquisition with low-noise: make sure that the ref. electrodes (platinum wires):
    - Do NOT touch the Inlet/Outlet Pipes.
    - Sit in the bottom of the MED Probe and NOT moving.
    - Are completely covered by the solution.
- Be careful not to overflow solution onto the MED Mini Connectors. Particularly, pay extra attention when starting perfusion. (Solution could become higher for a moment before the suction starts.)
- Position the Inlet Pipe as low as the top of the solution. (Dripping can fluctuate solution in the MED Probe chamber, which may introduce noise.) On the other hand, be careful not to scratch the bottom of MED Probe with the Inlet/Outlet Pipes to avoid damaging the insulation layer or leads.

Maintenance for the perfusion tubes

It is important to preserve the integrity of your perfusion tubing for smooth perfusion and stable recording. It is highly recommended that you following maintenance procedures for your perfusion tubing.

- Rinse (perfuse) the tubing with distilled/Milli-Q water for at least 10 minutes **after every experiment.**
- Release the “tubes for peristaltic pump” release from the peristaltic pump after each experiment. Failing to do so will reduce the life of your tubing.
- Replace the tubing/drippers with new ones often. Old tubing can impede solution from flowing smoothly and can cause perfusion noise. Below are recommended replacement times for your tubing when several experiments are conducted each day:
    - Tubing for peristaltic pump: 2-3 weeks
    - Inlet/outlet drippers: 1 month
    - Other tubing: 2-3 months

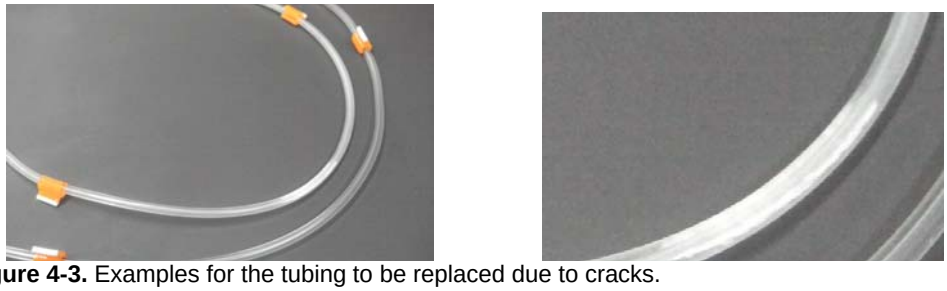


Figure 4-3. Examples for the tubing to be replaced due to cracks.

Note:

Connecting a lure fitting to new profusion tubing forcibly can damage the tip. Making the tube-tip wider using a pipet-tip before connecting the tubes to the lure fitting will make the connection easier (Figure 4-4).

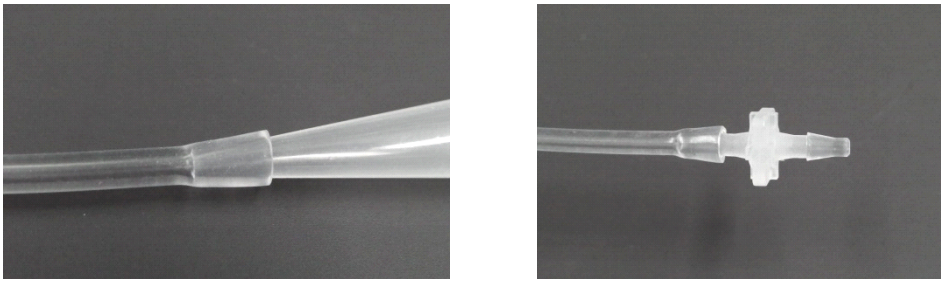


Figure 4-4. Making the tube-tip wider first (left), and then connect it to the lure fitting (right).

Specifications of the perfusion tubes for the MED-KCAPTU

Inlet tubes

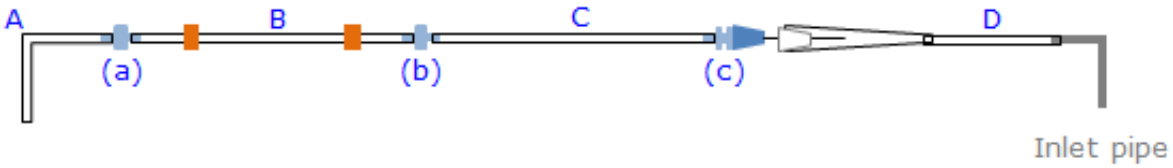


Figure 4-5. Inlet pipe and tubes

		I.D/O.D (mm)	Length (cm)	Cat #
Tube	A,C	0.79/2.38	50	ACF00001 (E-3603, Tygon S3™)
Tube	B	0.89 (I.D) (Peristaltic tube)	-	F117937 (Gilson®)
Tube	D	0.79/2.38	30	ACF00001 (E-3603, Tygon S3™)
Tube fitting	(a), (b)	For 2.5mm ID, lure fitting	VFI116	
Tube fitting	(c)	For 1.5mm ID, lure fitting	VRS106	

Table 1. Specifications for the Inlet tubes.

Outlet tubes

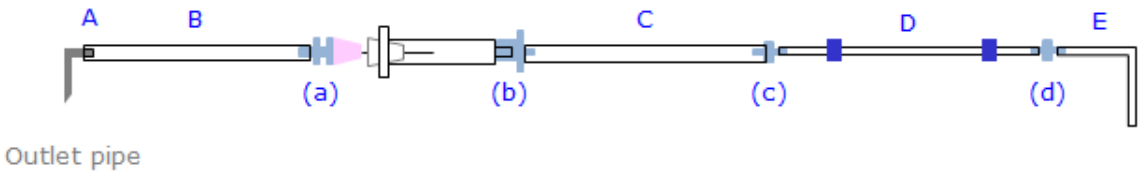


Figure 4-6. Outlet pipe and tubes

		I.D/O.D (mm)	Length (cm)	Cat #
Tube	A	0.79/2.38 (Adjust the outlet pipe into B tube)	About 0.5	ACF00001 (E-3603, Tygon S3™)
Tube	B	2.38/3.97	90	ACF00004 (E-3603, Tygon S3™)
Tube	C	1.59/4.76	85	ACF00003 (E-3603, Tygon S3™)
Tube	D	1.65 (I.D) (Peristaltic tube)	-	FF17943 (Gilson®)
Tube	E	1.59/3.18	100	ACF00002 (E-3603, Tygon S3™)
Tube fitting	(a)	For 2.5mm ID, lure fitting	VRS206	
Tube fitting	(b)	For 1.5mm ID, lure fitting	VRF106	
Tube fitting	(c), (d)	For 1.5 mm ID	VFI116	

Table 2. Specifications for the outlet tubes.

Inlet/outlet drippers

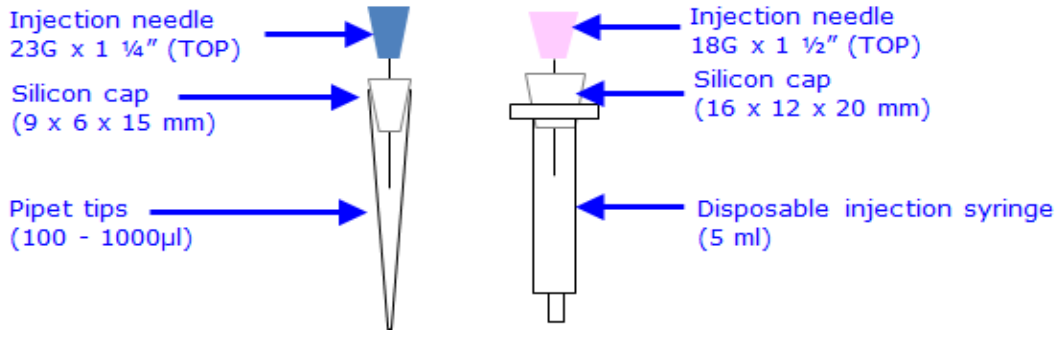


Figure 4-7. Specifications for Inlet/outlet drippers.

5. Trouble shooting

It is always recommended to check the noise with saline (without biological samples) before starting your experiments. When noises appear during your experiment, identify “the source for the noise” using following procedure, and then trouble-shoot.

1. Stop perfusion, and then run the system.

   If the noises are gone, the noises came from perfusion. Most likely, solution was connected somewhere in the perfusion tubes. Please check whether both inlet/outlet drippers are standing vertically and placed on top of aluminum foil. If the noise is still present, possible solutions are:

   - Replace the inlet/outlet drippers.
   - Replace all perfusion tubing.
   - Clean inlet/outlet pipes and platinum wire with ethanol.

2. Swap the MED Probe 16 with the “test board”, and then run the system.

   If the noise is gone, the noise is likely coming from the Probe. Swap the Probe with another one, and then double-check the noise.

   Note:

   The test board is less sensitive to exogenous noise than electrodes embedded on the MED Probe. Make sure to double-check the noise level with another Probe even if the noise is gone with the testing board.

   If the noise is still there, they probably comes from another factors such as environment, connector, or amplifier. Possible solutions are:

   - Move any equipment that generates electric or magnetic field (e.g. power supply, ThermoC-lamp, Peristaltic pump, power-supply-extension cord) AWAY from the amplifiers, connectors, and connector cables.
   - Check whether all hardware are grounded through a single power supply cord.
   - Verify that all connectors are placed on aluminum foil that is grounded to the GND of the Head amplifier.

3. If noise appears at just specific channels (not at all 16 channels per a connector) with a good quality of probe or the test board, the noise is likely due to poor contacts on the contact pins in the MED Mini Connector. To confirm this, swap the connector that has noises with another one.

   - If noises move to the same channel assigned to the other connector (e.g. Ch1 to Ch16 by swapping the connector 1 and 2), the noise comes from the contact pins. Clean the contact pins. (See the Product manual for the MED Mini Connector for cleaning the contact pins.)
   - If noise remains at the same channels after swapping of connectors, the noise is likely coming from amplifiers, cable, or/and PC. Disconnect the cable at the side of amplifier, and then re-connect it. If noise still remains, contact your local distributor or support@med64.com.

6. Mobius Tutorial - Long Term Potentiation (LTP) experiment-

This section describes how to perform an LTP experiment with 4 slices using the MED64-Quad II System. The method involves recording baseline activities and LTP with one workflow, whereas conditioning stimuli are applied with another. The following procedures are detailed in this section.

1. Selecting the stimulation channels for all 4 slices.
2. Identifying the appropriate stimulus current amplitude with an I/O curve for all 4 slices.
3. Making the LTP workflow.
4. Making Theta burst workflow.
5. Perform LTP.

6-1. Available workflows

Following workflows are available for LTP experiments from 4 slices. Download them from the web site.

1. *"Single_pulse_recording_QuadII"*
2. *"Theta_burst_QuadII"*

Both workflows have the **Step 1-4 enabled** and are expected to have following assignments:

1. Step 1: Channel 1-16 / Slice (Connector) #1
2. Step 2: Channel 17-32 / Slice (Connector) #2
3. Step 3: Channel 33-48 / Slice (Connector) #3
4. Step 4: Channel 49-64 / Slice (Connector) #4

It will allow you to monitor the waveform analysis at one channel per slice by selecting different Step at the Step filter in the [Extract EP Measures] modules.

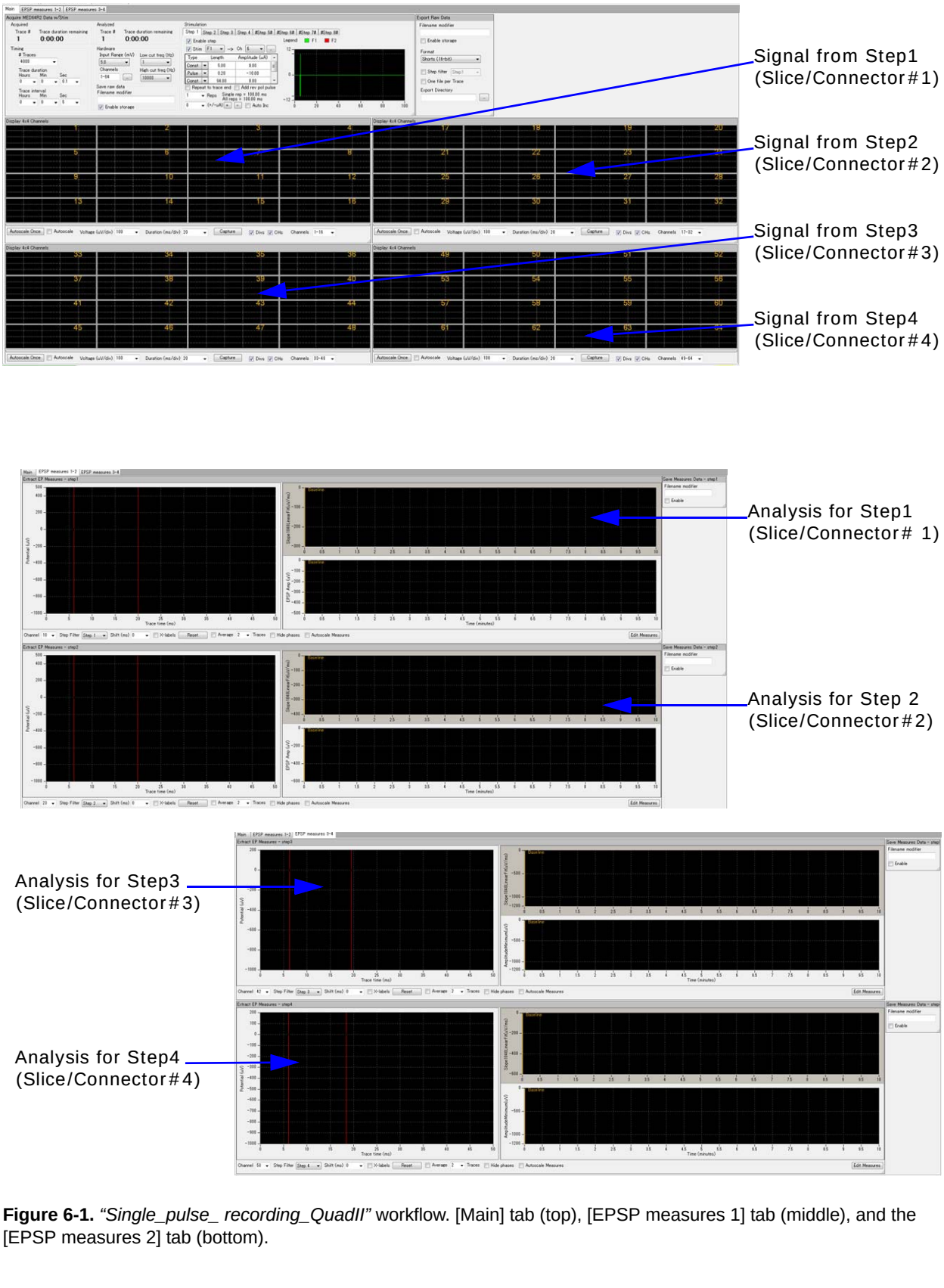


Figure 6-1. *"Single_pulse_ recording_QuadII"* workflow. [Main] tab (top), [EPSP measures 1] tab (middle), and the [EPSP measures 2] tab (bottom).

6-2. Selecting the stimulation channels

Open the *"Single_pulse_recording_QuadII"* workflow. Determine a stimulation channel for LTP induction per slice with following procedure.

1. Disenable the Step2-4.
2. **Select any channel out of 1-16 for stimulation** and run Mobius with the Green button.
3. Stimulate several different channels, and look for 1) the best stimulus channel and the 2) a channel which analysis will be monitored for the Slice#1.

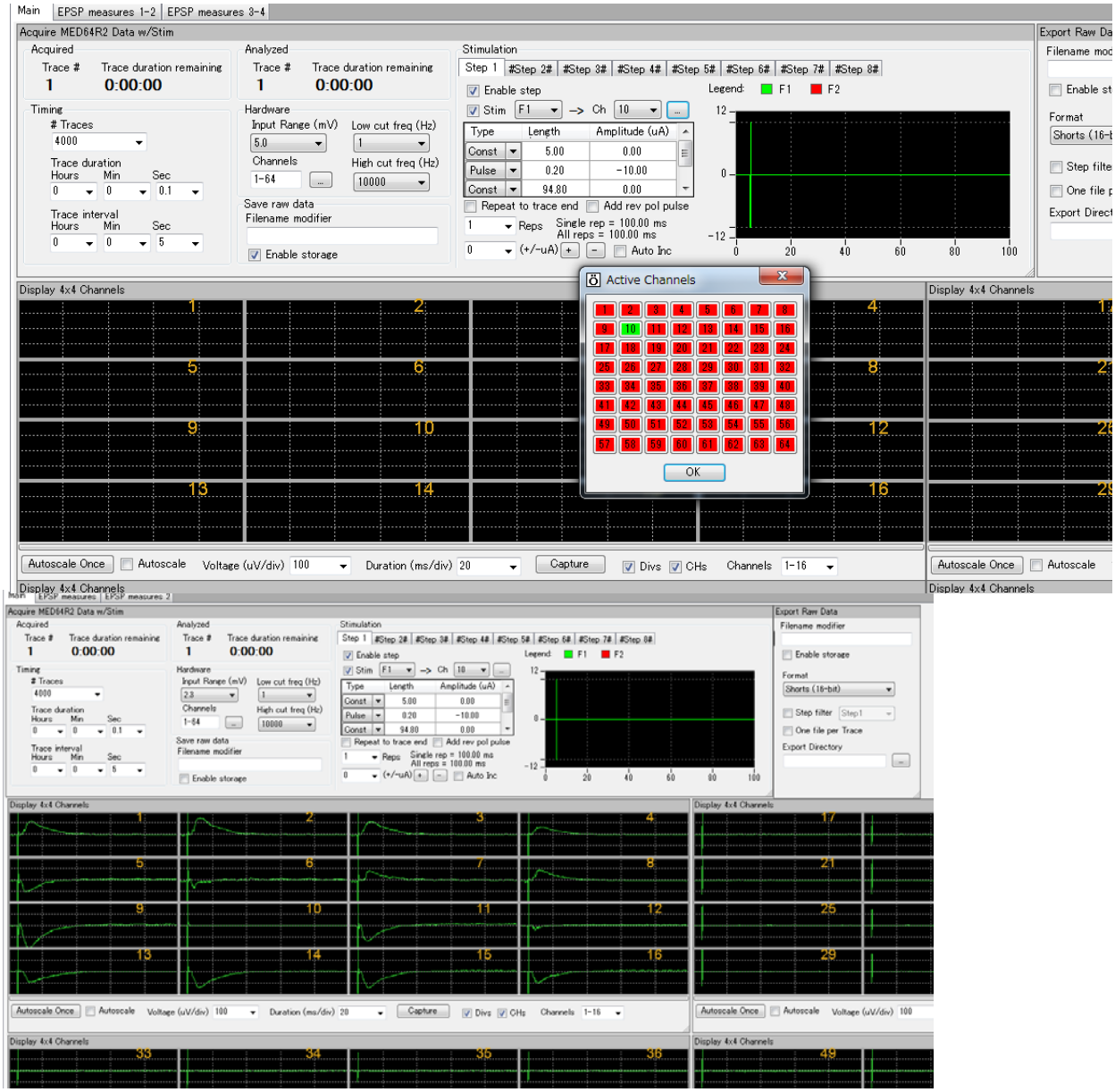


Figure 6-2. Looking for the best stimulus channel. Channel 10 is selected for stimulation (top) and stimulation is applied once by clicking the Green button (bottom). fEPSP signals are displayed at the channel 1-9,11-16.

4. Open the [EPSP measures 1-2] tab. The upper chart (where the Step1 is selected) shows analysis for the Slice#1.

5. Set the cursor both for Slope 10-40% (red) and Amplitude Minimum (blue) by dragging the cursor in the left waveform chart. Change the analysis menu according to your desire. (Refer to page xxxx in the Mobius Tutorial.)

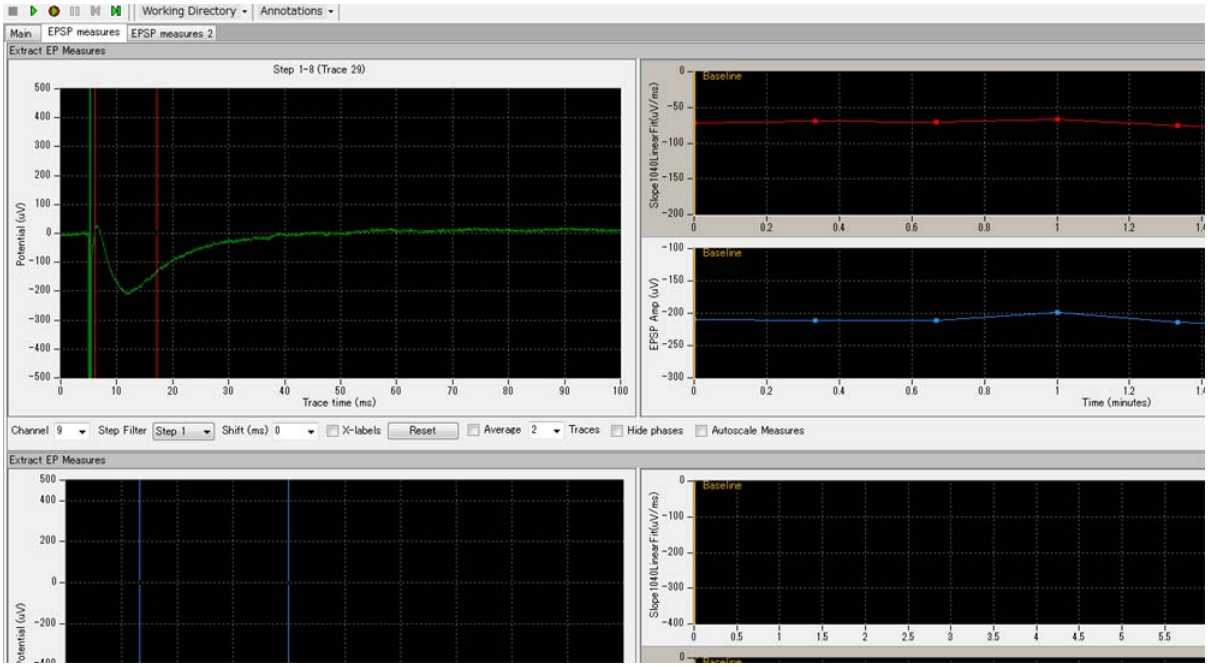


Figure 6-3. The [EPSP measures 1-2] tab. Set the cursor for both analyses (red and blue in the right graph) by dragging the cursor in the left waveform chart.

6. Now, both stimulus and monitored channel for the Step 1/Slice are selected, and analysis parameters are set. You will go to setting parameters for Step2/ Slice#2.

7. Disable Step1, and then enable Step2.

8. Select any channel out of 17-32 for stimulation and run Mobius with the Green button.

9. Stimulate several different channels, and look for the best stimulus channel to Slice#2.

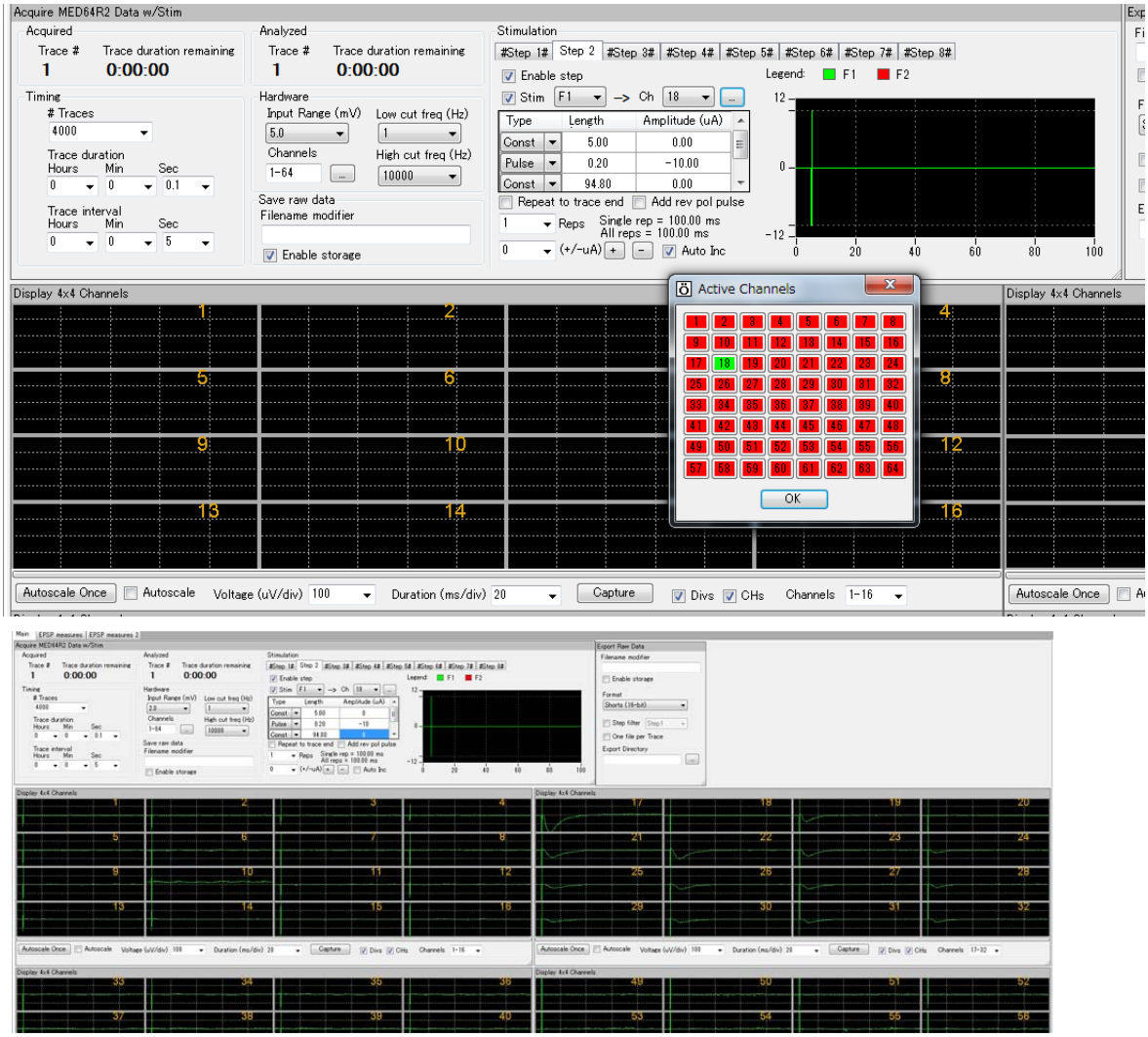


Figure 6-4. Looking for the best stimulation channel for the Slice 2 using the Step 2.

10. Open the [EPSP measures] tab. The bottom chart (where the Step2 is selected) shows analysis for the Slice 2. Set the cursor both for Slope 10-40% (red) and Amplitude Minimum (blue) by dragging the cursor in the left waveform chart. Change the analysis menu if you want to.

11. Identify the best stimulus and monitored channel and their analysis parameters for Slice#3 (Step3) and Slice#4 (Step4) with the same procedure.

6-3. Identifying the stimulus intensities for your LTP experiments using the I/O curve

This procedure is performed to determine the appropriate stimulus current amplitude for your LTP experiment. You will identify it by making an Input/Output curve for all 4 slices one by one. The slope or/and amplitude are usually selected as the output parameters. A current amplitude eliciting 30% to 50% of the maximal amplitude is typically selected.

1. Stay with the "*"Single_pulse_recording_QuadII"* workflow, where stimulus channels were selected and analysis parameters were set.
2. Enable the Step 1 ONLY while disenable the Step2-4.
3. Select the stimulus channel determined with the previous process.
4. Click [Auto inc] button. Type an appropriate number for the Auto incrementation (e.g. 5 μA).
5. Type a number for the stimulus current amplitude you would like to start the I/O curve with (e.g. 5 μA).
6. Change the Trace interval to 20 second (or interval you would like to have).
7. Make sure that F2 stimulators are dis-enabled at all Steps.

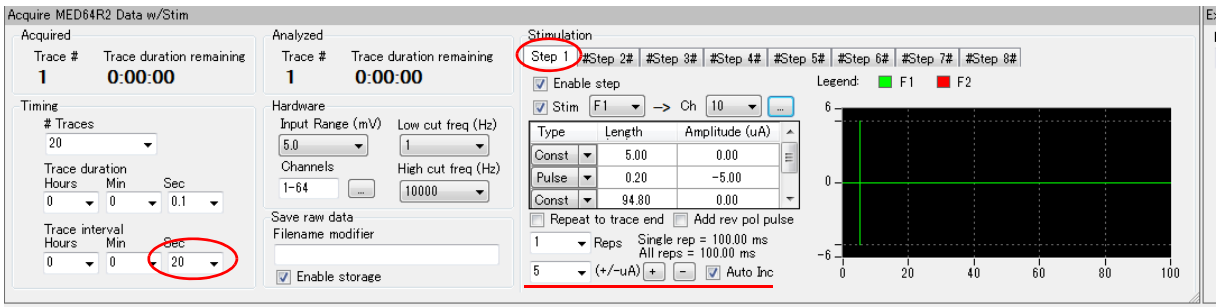


Figure 6-5. Acquisition workflow to make I/O curve for Slice 1(Setp1). The stimulation will be applied ONLY to slice 1 (Step 1) with the interval of 20 seconds and stimulus current amplitude will be incremented by 5 μA.

8. Open the [EPSP measures 1-2] tab. Make sure that the top chart has analysis menu you want with cursors set to the right position.
9. Save the workflow as your custom I/O curve workflow.
10. Click the Green-Red button to start the recording. (Click on the Green button if you would not like to save data).
11. You will see the stimulus current amplitude is incremented by every trace (e.g. 5, 10, 15, 20------ μA), and amplitude/slope will increase.
12. When the amplitude/slope is saturated, stop Mobius with the black button.
13. Determine the 30% (or 50%) for the maximal (saturating) stimulus amplitude. The following example shows a protocol in which the stimulation started at 5 μA, and then increased by 5 μA in every

subsequent trace. The responses saturated at the 12th trace (60 μA) at approximately -1900 μV. The 30% and 50% of -1,900 μV are 633 μV and 950 μV respectively. The stimulus current amplitude eliciting 30% of the maximal response is 20 μA and the stimulus current amplitude eliciting 50% of the maximal response is 25 μA.

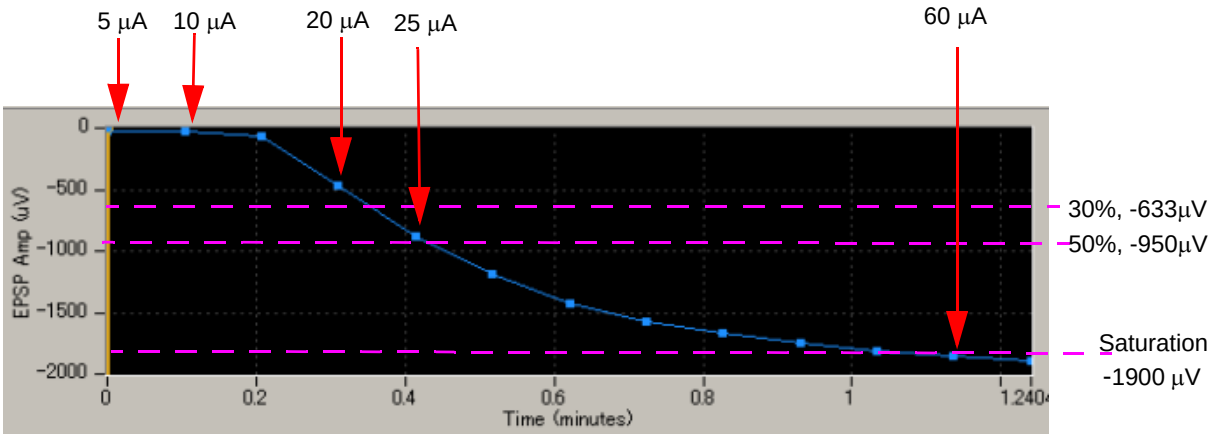


Figure 6-6. Determination of the stimulus current amplitude eliciting 30% and 50% of the maximal response.

14. Type in the number you determined for the [Pulse] at the Step 1.

15. Disable Step1

16. Enable Step2 ONLY. Determine the stimulus current amplitude for the Slice#2 using the same procedure as 1-15.

17. Determine the stimulus current amplitudes for the Slice#3-4 with the same procedure.

6-4. Making an LTP workflow

You now have the *"Single_pulse_recording_Quad II"* workflow ready, where:

- Stimulus channels and intensity are selected for 4 all slices.
- All analysis parameters and locations for cursors are set.

Customize this workflow for your LTP experiment.

1. Activate ALL Step1-4.

2. UN-CHECK the [Auto inc] button.

3. Set the [#Traces] for the one which is large enough for both baseline and LTP recorded for 4 slices.

4. Set the [Trace interval] for 5 seconds (or any larger number you want)

   - 5 for the [Trace interval] in this workflow means each slice will be stimulated every 20 seconds.
   - Do NOT select the number smaller than 5 for the Trace Interval.

5. Save it as your own LTP workflow (Click [Workflow] > [Save as], and type file name) and leave it open.

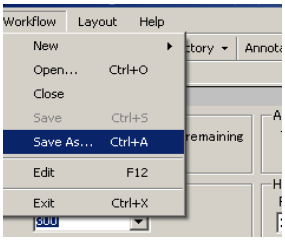


Figure 6-7. Saving the workflow.

6-5. Making a workflow for LTP conditioning stimuli (e.g. Theta burst)

In an LTP experiment, you will need to apply a conditioning stimulus such as a theta burst right after recording the baseline activity. The best way to do so is to have the 2 workflows, for baseline/LTP recording and conditioning stimulus, open and ready on your acquisition PC. In this section, you will make a workflow for the theta burst stimulation using the workflow template.

1. Open the *"Theta_burst_QuadII"* workflow.
2. Change the stimulus amplitude at the [Pulse] to the value determined in the previous step for all Step 1-4.
    - Use the +/- button to change the amplitude. For example, in order to change to -20 μA from -10 μA, select 10 μA, then click the + button once (Figure 6-8) so that amplitudes for all 4 "pulses" are changed to -20 μA at a time. If you directly type the number at the [Pulse] boxes, you must type it in 4 times for all [Pulse] boxes.
3. Change the stimulus channel to that determined for all Step 1-4.
4. Make sure Step 1-4 are activated while the Steps 5-8 are NOT.
5. Make sure F2 stimulators are NOT enabled at all Steps.
6. Save as your own Theta Burst workflow

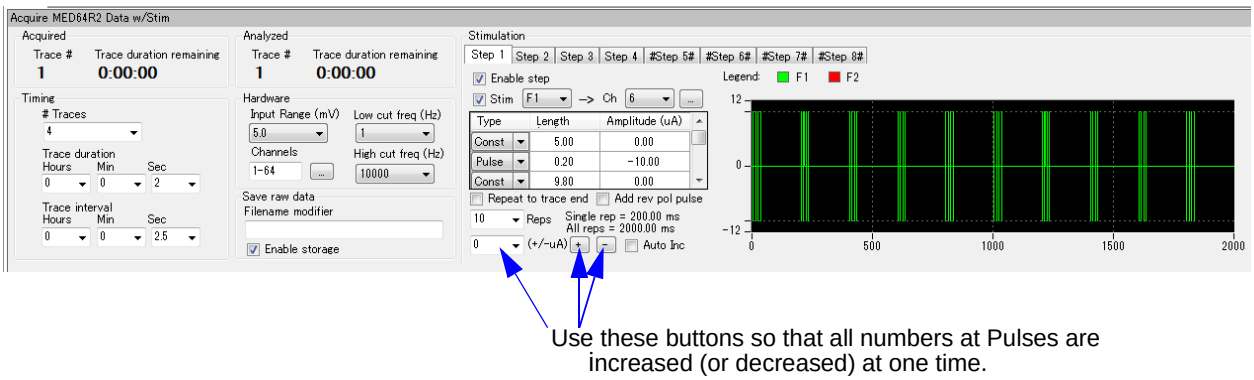


Figure 6-8. Example of a theta burst conditioning stimulus protocol.

7. Uncheck the F1 Stimulator for all Steps, and then run this workflow with the GREEN button once, and then stop.

   - When a recording workflow is run for the first time, Mobius needs several seconds for its calibration before it starts acquisition. This prevents you from applying conditioning stimuli quickly during your LTP experiment.

     Running this workflow with stimulator (F1.2) disabled will help you to apply conditioning stimuli right after the baseline recording at your LTP experiment.

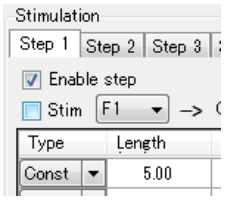


Figure 6-9.
Disable the stimulator.

8. Check the F1 Stimulator for all Steps again. Leave it open on your MED64 PC.

6-6. Perform LTP experiment

Now you have 2 workflows (LTP workflow and Theta Burst workflow) open on your MED64 PC, and are ready to start recording.

1. Activate the LTP workflow by clicking the title bar on the LTP workflow. Make sure that [Enable storage] is checked. Make sure that the STIMULUS CURRENT selector on the MED-A64HE1S amplifier is set for [NORMAL]. Start your experiment by clicking the GREEN-RED button.

2. After you finish recording baseline activity, PAUSE the experiment with the YELLOW button.
   DO NOT stop the recording with the Black button. The experiment will be stopped. Baseline and LTP data can NOT be stored in the same data file.

3. Quickly activate your theta burst workflow by clicking the title bar on the theta burst workflow. Apply the theta burst by clicking the Green button once. (or Green-red button if you would like to save this data.)

4. When the theta burst stimulus protocol is finished, quickly go back to the LTP workflow and restart your experiment by clicking the GREEN-RED button.

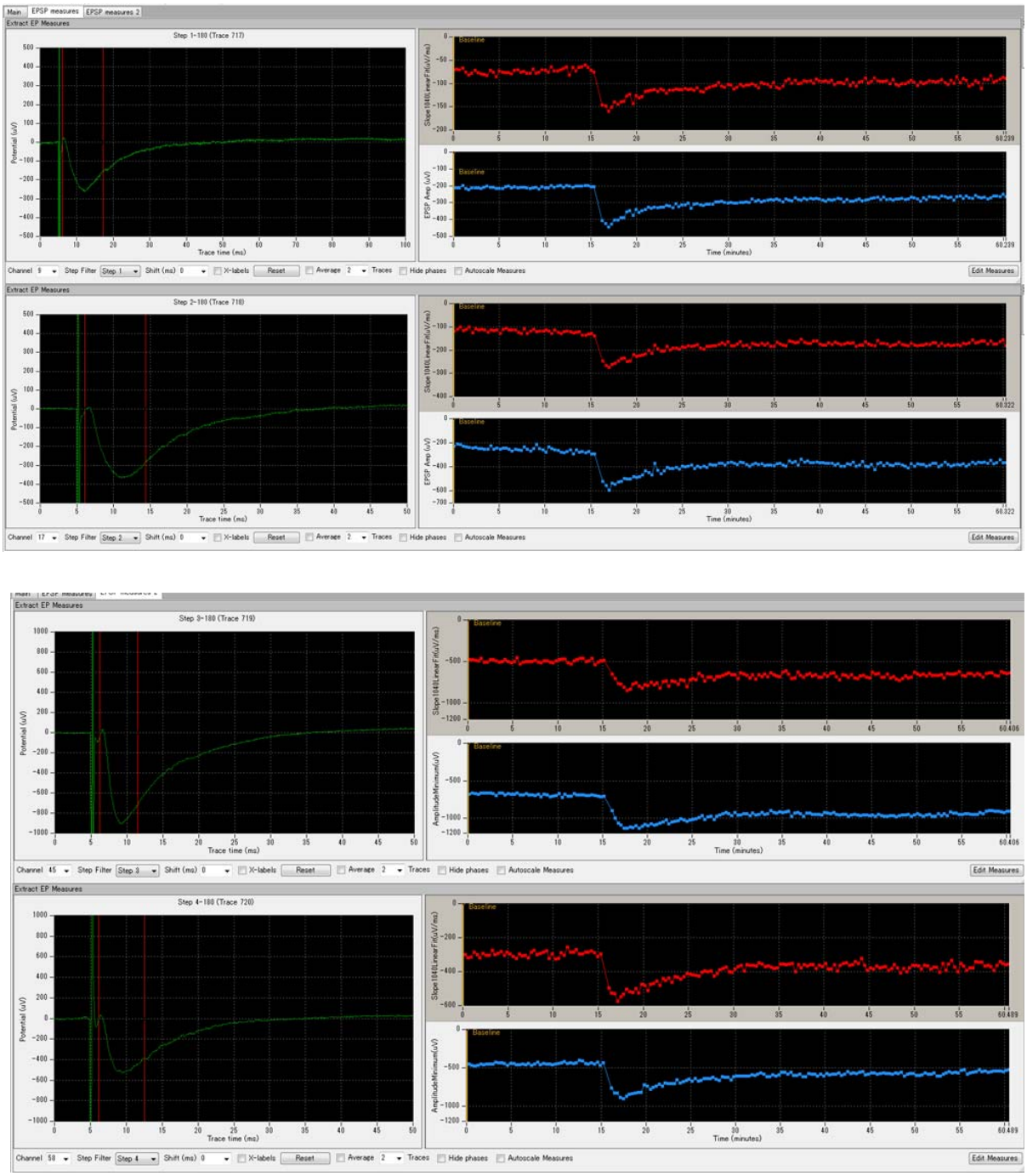


Figure 6-10. LTP experiments with 4 slices.

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