

MED64 Mobius Quick-Start Guide

Alpha MED Sciences Co., Ltd.

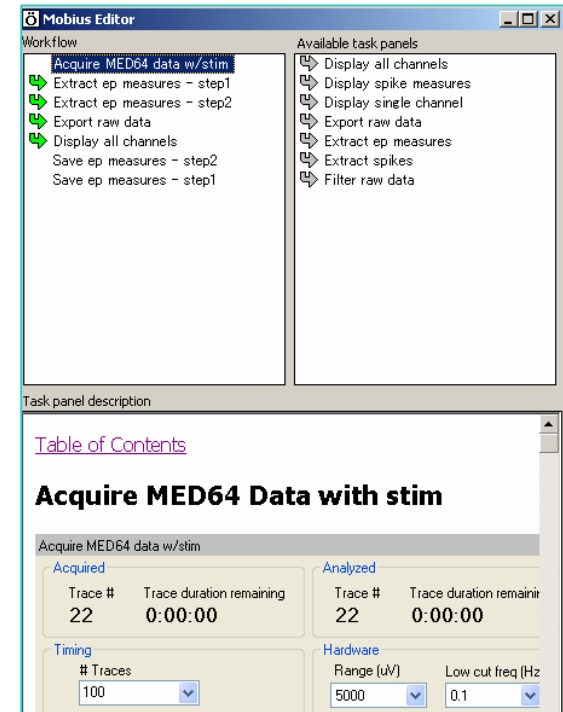
Introduction

The MED64 Mobius consists of various modules. You can create your own experimental protocols by combining these modules with Mobius Editor.

On the other hand, there are some pre-defined workflow templates available so that you can run standard types of experiments immediately.

The Mobius Quick-Start Guide introduces you how to start MED64 Mobius using these pre-defined templates.

Please refer to “Mobius User’s Guide” for detail instruction of Mobius.



The Configuration of the “Single Pulse Recording” Template

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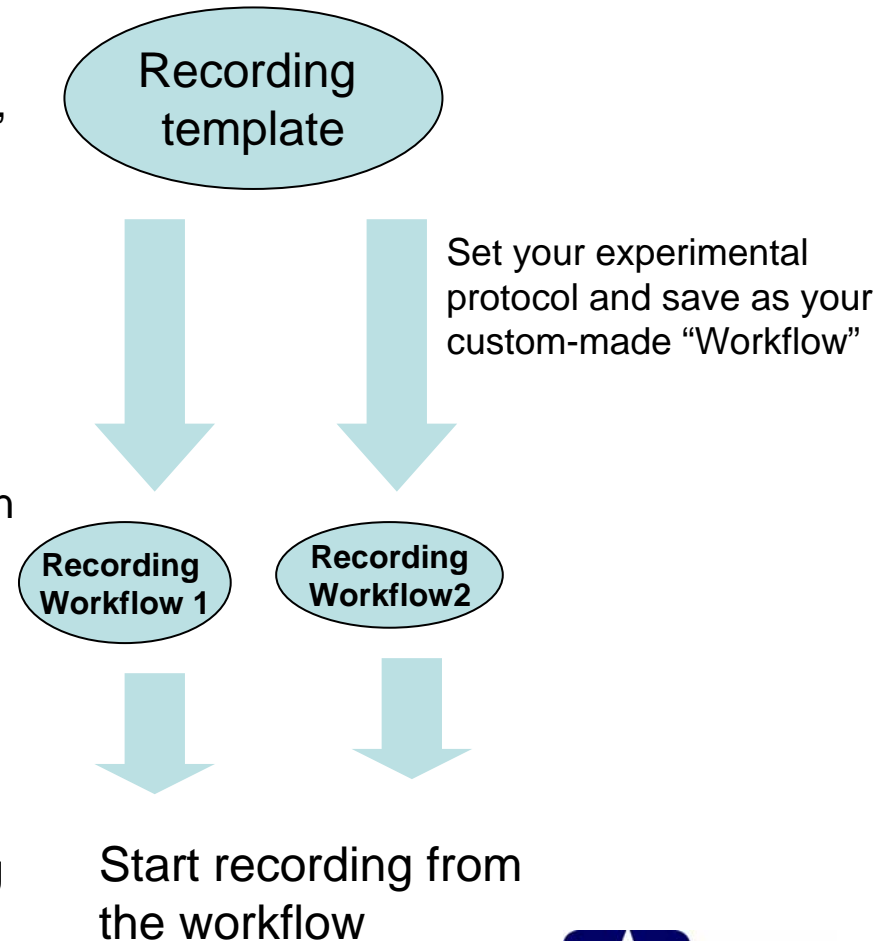
How to start Mobius

–On-line recording and analysis–

- Recording and on-line analysis are started from your self-designed “Recording Workflow”, which are made using “Recording Template” (pre-defined workflow) or “Mobius Editor”.
1. Open a “Recording template” and set your experimental and analysis protocol. Finally, save it as your own “Workflow”.
 - 1-. Or, Design your own recording Workflow by using “Mobius Editor”, then save it as your own “Workflow”.

Recording can not be done at templates.

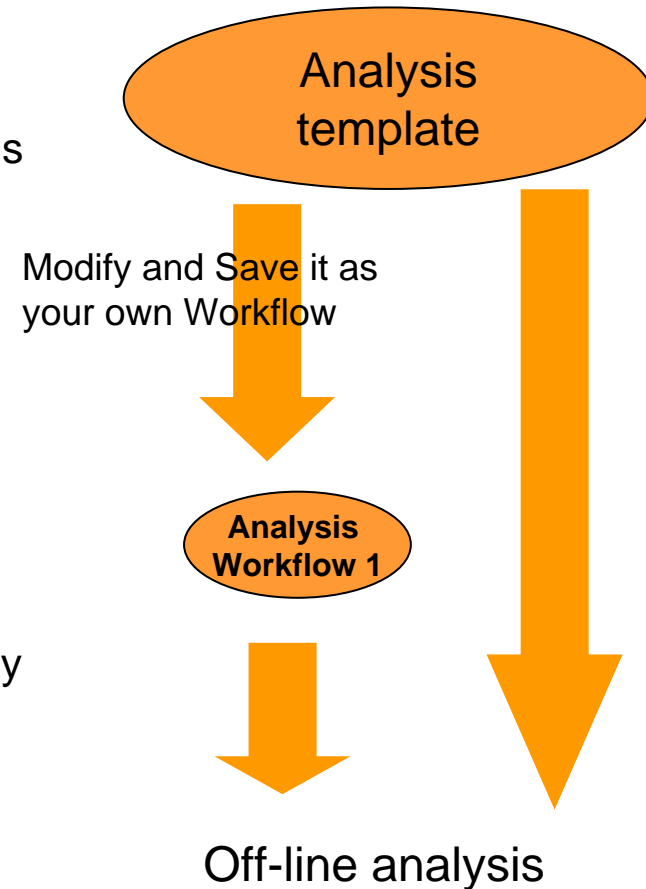
2. Start recording.
From the next time, your Workflow can be opened by [Workflow]-[Open]. Then, recording is started.



How to start Mobius

-Off-line analysis-

- The Mobius data is opened, replayed and analyzed off-line at your self-designed “Analysis Workflow”, which are made using “Analysis Template” or Mobius Editor.
1. Open an “Analysis Template” .
Save it as your own “Analysis Workflow” after your modification
 2. Or, Design your own “Analysis Workflow” by using “Mobius Editor”.
 3. Start replay.
From the next time, your Workflow can be opened by [Workflow]-[Open], then, analysis is started.
- * The data can be opened and replayed at the predefined “Analysis template” **However, exporting can NOT be done at Template.**



Mobius Templates

Following templates are available in Mobius

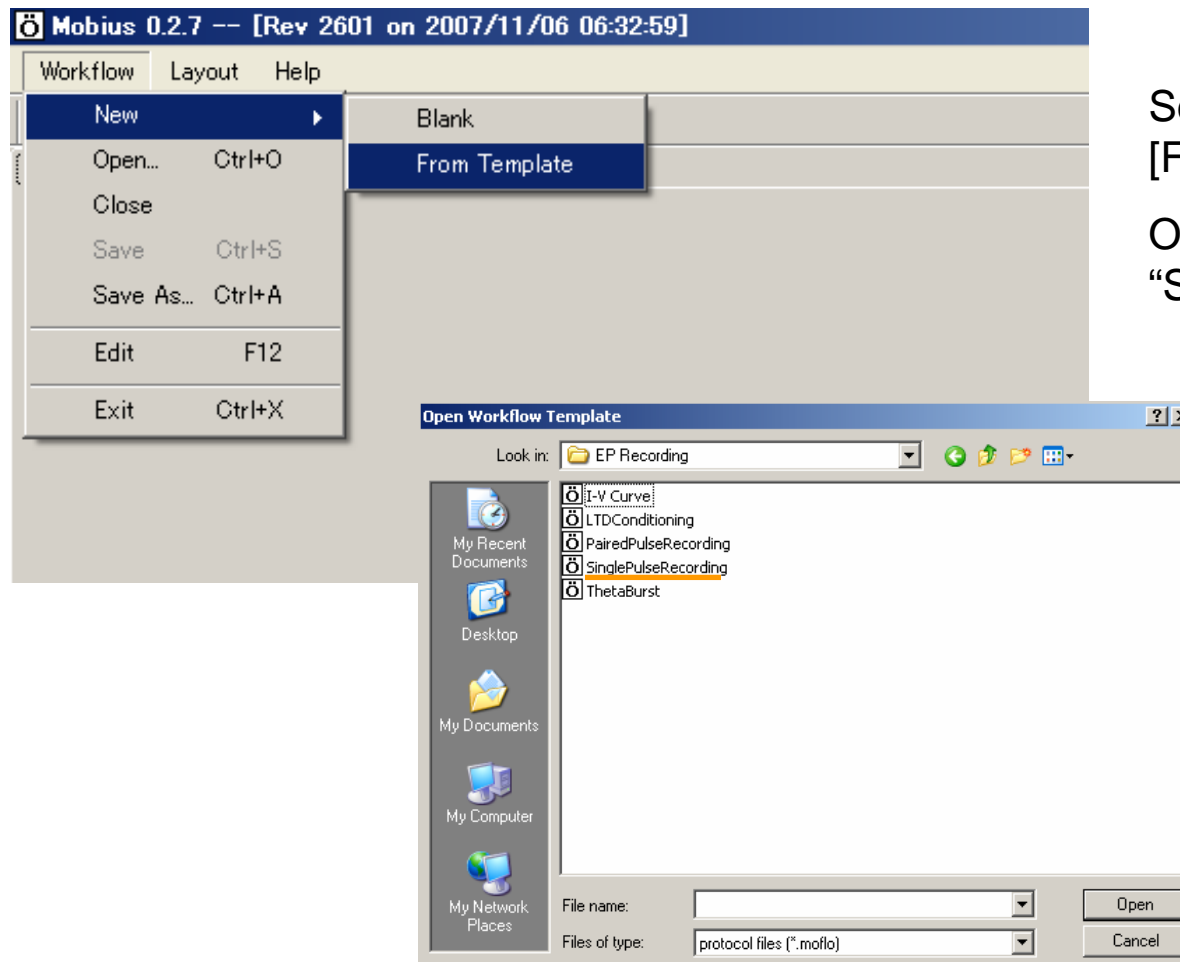
Folder	Folder	Template name	Packages						
			EP Measurement	Spike sorter	Spike sorter + filter row data module	Spike sorter with stim.	Spike sorter with stim + filter module	Mobius pro	Basic
EP Measurement and Analysis	EP Recording	1 Single Pulse Recording	0					0	
		2 Paired pulse Recording	0					0	
		3 Theta burst	0					0	
		4 I-V Curve	0					0	
		5 LTD Conditioning	0					0	
EP Measurement and Analysis	EP Analysis	4 Single Pulse Analysis	0					0	
		5 Paired pulse Analysis	0					0	
Spike Recording and Analysis	Spontaneous Recording	6 Spontaneous Recording		0	0	0	0	0	
		7 Recording_filter			0		0	0	
	Spike recording with stimulation	8 Recording with Stimulation				0	0	0	
		9 Recording_stim_filter					0	0	
	Spike Analysis	10 Spike Sorting		0	0	0	0	0	
11 Spike sorting_filter				0		0	0		
EP and Spike	EP and Spike Recording	12 Single Pulse Recording (EP and Spike)						0	
		13 Paired Pulse Recording (EP and Spike)						0	
	EP and Spike Analysis	14 Single Pulse Analysis (EP and Spike)						0	
		15 Paired Pulse Analysis (EP and Spike)						0	

Chapter 1

EP Measurement

EP measurement / single pulse recording

1. Open a recording template



Select [Work flow]-[New]-[From Template].

Open the template of "Single Pulse Recording".

EP measurement / single pulse recording

2. Framework of the Recording template

- ◆ “Single Pulse recording” (“Paired pulse recording”) consists of 2 screens.
- ◆ In the “Main” screen, experimental protocol is designed and signals acquired from 64 channels are displayed.
- ◆ In the “EPSP measures” analysis protocol is set. On-line analysis at selected channels are displayed.



Play (or Replay) **WITHOUT** recording

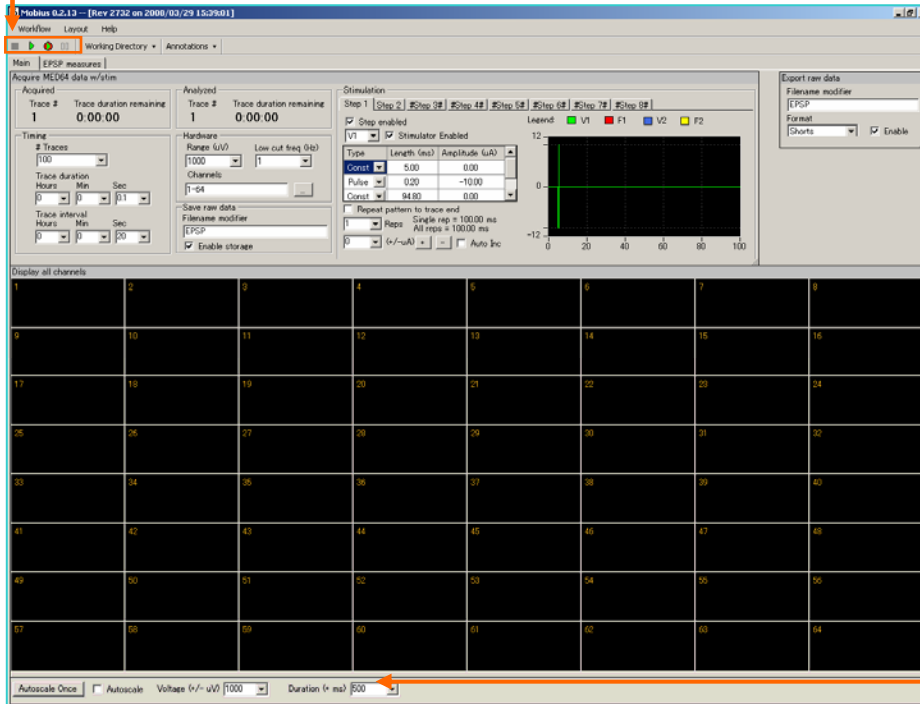


Play (or Replay) **WITH** recording

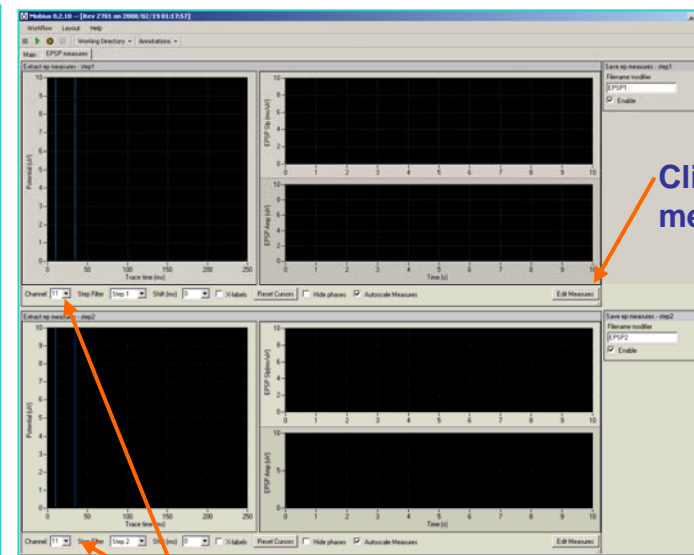
(At Analysis Workflow, exporting is executed by clicking this button.
Not by green button)

■ Stop recording (or replay)

|| Pause recording (or replay)



Main screen



EPSP measures screen

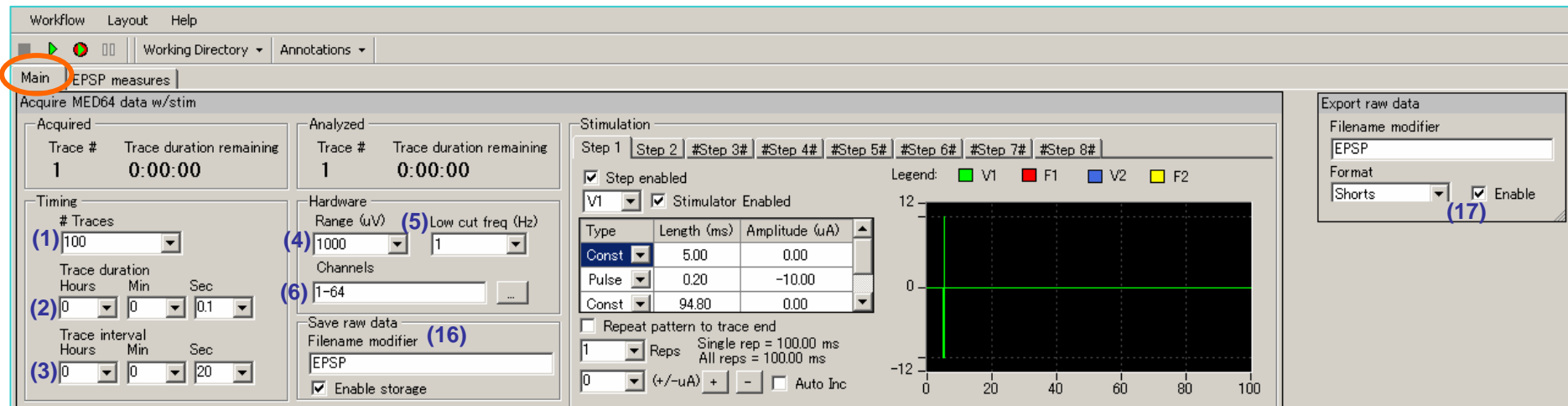
Click here to edit measurement

Channels to be monitored are selected

Change the scale for voltage and time

3. Design of experimental protocol (1)

◆ Design your experimental protocol in the control panel of the “Main” screen.



Recording parameter

- (1) Number of sweep (trace)
- (2) Sampling (Recording) time for each sweep (trace)
- (3) Interval between stimulations. (From the beginning of the one sweep to the beginning of the next. Refer to Figure.M-1 in the page 29) In the default , Stimulation programmed by Step1 and Step2 are applied alternatively at the interval of 20 seconds.
- (4) Signal range (The range of amplitude to be recorded)
- (5) Low cut filter. Selected from 0.1,1.0,10 and 100 Hz. If 0.1 is set, the signals which frequency are lower than 0.1 Hz are cut. Usually, 1.0 Hz is selected for the recording of evoked responses.
- (6) Channels to be recorded are selected here. (Click the square button to get the channel panel.) In the default, all channels are recorded.

Export

- (16) Raw data will be saved when the “Enable storage” is checked. Modify the file name by directly typing.
- (17) Raw data will be exported either by Double, Shorts, or ASCII when “Enable” is checked. File name can be modified by typing.



EP measurement / Single pulse Recording

4. Design of experimental protocol (2)

◆ Design your experimental protocol in the control panel of the “Main” screen.

The screenshot shows the MED64 control panel. The 'Stimulation' section is highlighted. It includes a table of steps:

Type	Length (ms)	Amplitude (uA)
Const	5.00	0.00
Pulse	0.20	-10.00
Const	94.80	0.00

Below the table, there are checkboxes for 'Repeat pattern to trace end', 'Reps', and 'Auto Inc'. A graph shows the stimulation protocol with a green vertical line at 0ms and a yellow horizontal arrow from 0ms to 100ms. A legend indicates V1 (green), F1 (red), V2 (blue), and F2 (yellow).

Stimulation protocol (1)

Each “Step” is independent, which allows you to program different type of stimulations in each Step. Stimulations which are programmed at the “enabled Step” will be applied by the number order of Step1-8 the interval of the “Trace duration”.

(7) When “Step enabled” is checked, the “Step” become valid. In the default, Step 3-8 are invalid while Step1 and 2 are valid and the stimulation programmed at Step1 and Step 2 are applied alternatively in the interval of 20 seconds.

(8) The stimulus output channel in the MED64 amplifier is selected here. V1 corresponds to [INT. V1] in the MED64 integrated amplifier. In the default, V1 is selected for Step 1 while V2 is selected for Step 2

*When the “Enabled” checkbox is unchecked, the stimulation is NOT applied. If you would like to change the stimulator to F1 from V1, uncheck the box for V1 (Figure. E4-1) , then check the box for F1.(Figure.E4-2)

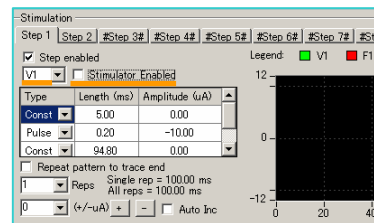


Figure.E4-1

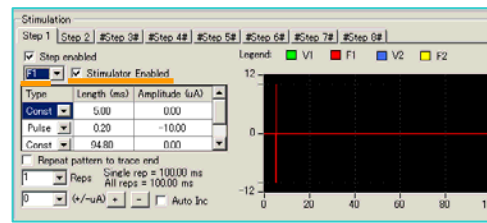


Figure.E4-2

5. Design of experimental protocol (3)

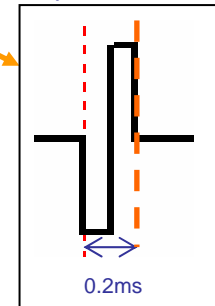
◆ Design your experimental protocol in the control panel of the “Main” screen.

Stimulation protocol (2)

- (9)-(11) Make the stimulus pattern using this chart. The numbers here can be changed by typing directly.
- (12) When this check box is checked, the set stimulation pattern will be repeated to the trace end. Useful for LTD conditioning.
- (13) Set the number of repeat for the designed stimulus pattern.
- (14) When the + button is clicked, the absolute value of the stimulus amplitude at “Pulse” is increment by the number which is set at the left box. (14*) For example, if number 2 is selected and + button is clicked once at the default, the stimulus amplitude will be changed to -12.00 uA. Useful to change the stimulus amplitude for multiple pulse stimulations (e.g. Thetaburst) in just one click.

The absolute number of stimulus amplitude will be decremented in the same procedure when - button is clicked.

- (15) When this check box is checked, stimulus current will be automatically incremented by the number of uA which is set at the left box (14)* with respect to each stimulation pulse independently in each Step. Useful for making IO curve. (Refer to the page 23, (4))



EP measurement / Single pulse Recording

6. Save as your experimental protocol

- ◆ Save your experimental protocol as your own “Recording Workflow” (Recording can not be started from Ttemplates.), then start recording. (Figure. EP-5)
- ◆ From the next time, open your own Recording Workflow to start recording. (Figure. EP-6)

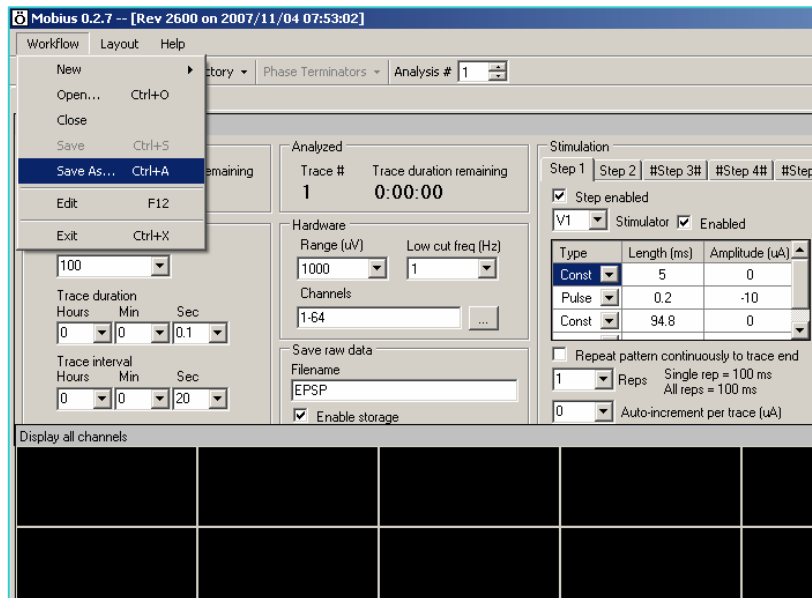


Figure.EP5: Select [Workflow]-[Save As]. Save your experimental protocol as your own “Workflow”. From the next time, the recording can be started from the Recording Workflow.

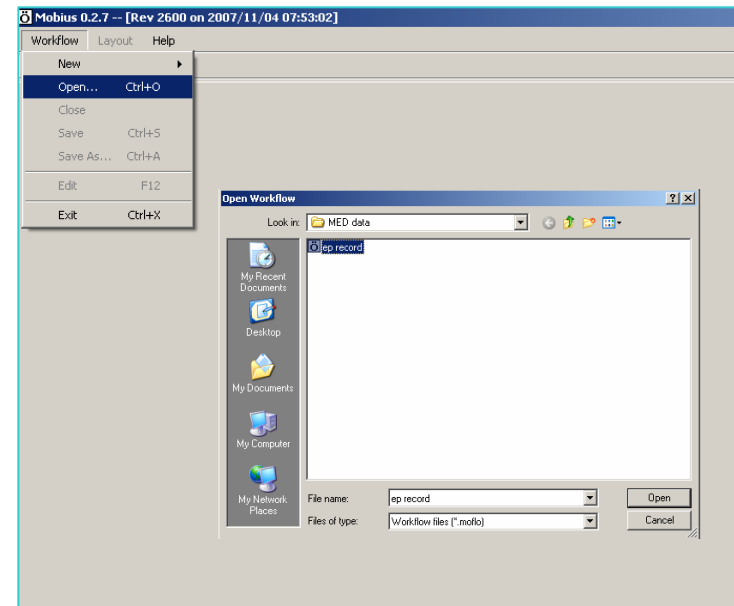


Figure.EP-6: From the next time, Open the workflow by selecting [Workflow]-[Open], then, start recording.

7. Set of Analysis protocol (1)

◆ Set analysis protocol in the “EP measurement” screen.

In “Single Pulse (Paired pulse) Recording (or Analysis)” Template, Slope linear fit (red) and Minimum amplitude (blue) are pre-set.

(1) Set the cursor for measurement by dragging with the mouse. The scale for the graph can be zoomed up by enclosing the area with the mouse while holding down the shift key. It can go back to the original scale by double clicking the graph. The scale can be also changed by changing the numbers of the largest scale. (Click the number and type.)

(2) The blue cursor for “Minimum amplitude” will appear when the chart for “Minimum amplitude” (blue) are clicked.

(3) Select channel for on-line analysis. It can be changed even during the recording.

(4) Select the Stimulation “Step”.

When Step1 is selected, the on-line analysis of the responses evoked by “Step1” stimulation is displayed.

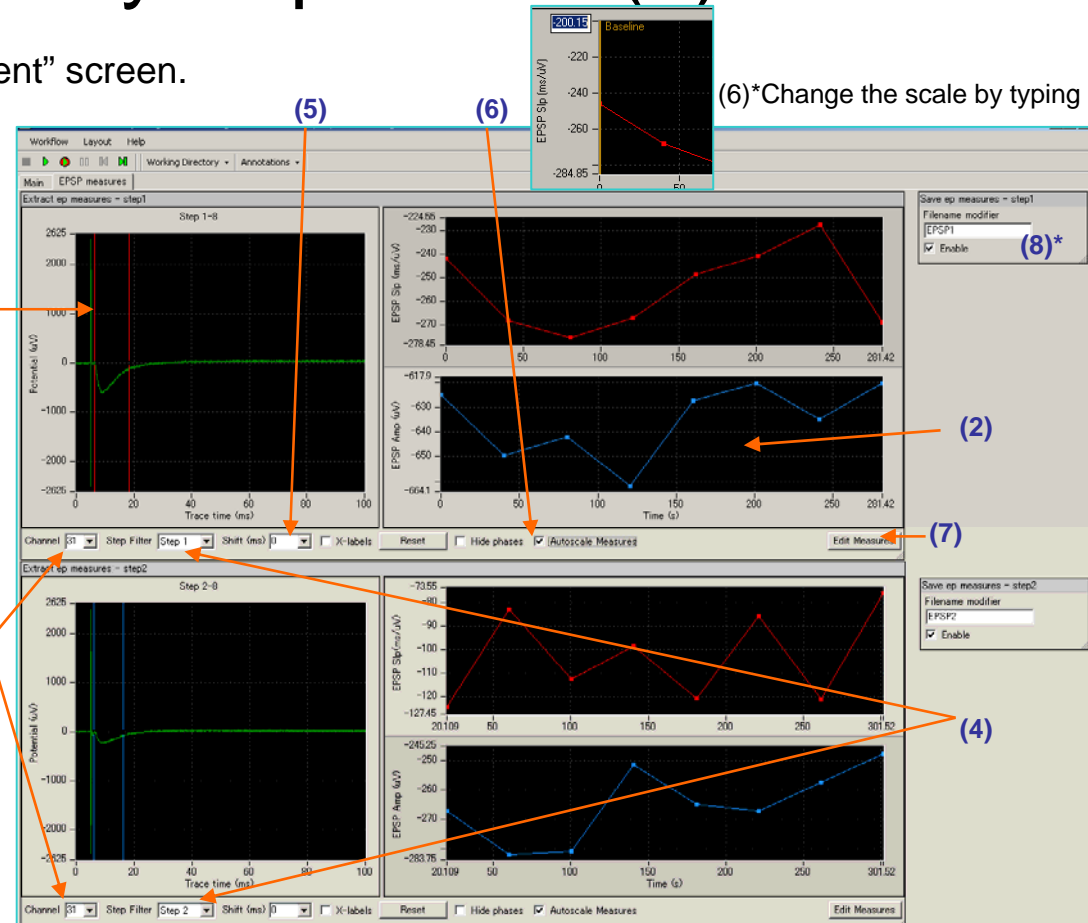
When Step2 is selected, the on-line analysis of the responses evoked by “Step2” stimulation is displayed.

In order to analyze Conductor data, select “ALL”

(5) It is used to measure paired pulse. Another cursor appears the set number of seconds after the first cursor. Another charts for the 2nd pulse will also appear. (Refer to the page 21.)

(6) When this check box is checked, the scales for the chart are changed automatically. The scale for the chart can be changed by right-click and typing the number. (6)* In order to fix the changed scale, uncheck this box.

(7) In order to edit measurement, click this button.



(8) The chart is exported by ASCII when the Enable check box is checked.

In this template, responses evoked by “Step1” stimulation and the one by “Step2” stimulation are exported to 2 separated ASCII files.



Single Pulse/Paired Pulse Recording and analysis

8. Set of Analysis Protocol (2)

◆ Add another measurements.

Click [Edit measures]. The measurement editor will appear. (Figure.E-1)

(1) Click “Add Measure”. Another line, cursor, chart and measurement will appear as seen in Figure E-2.

(2) Rename the type of measurement (“Custom Name”) by double-click and type. (Figure.E-2)

(3) Select measurement type. (Figure. E-2)

(4) Click [Hide Measures] to close it.

(5) In order to delete the measurement, right-click the “Custom Name”, then select [Delete].(Figure.E-3)

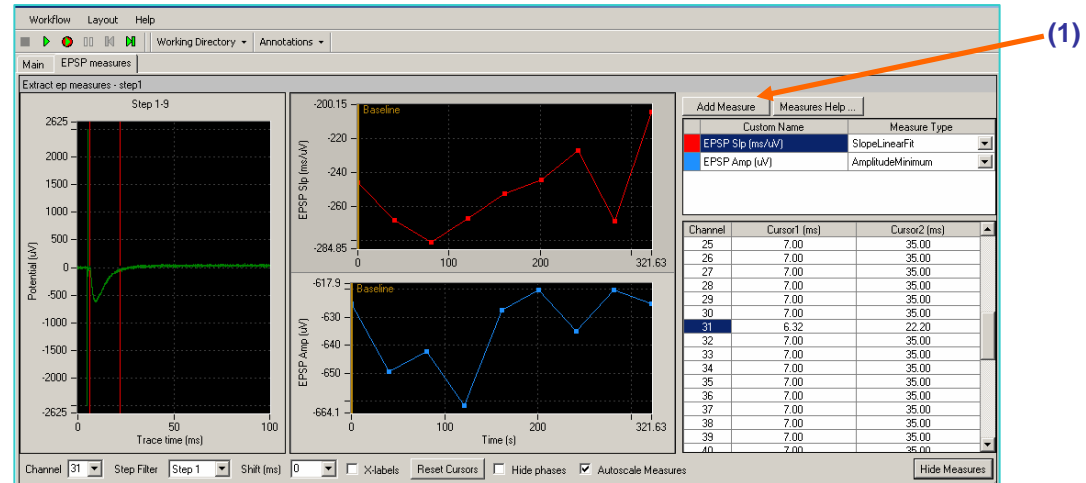


Figure.E-1

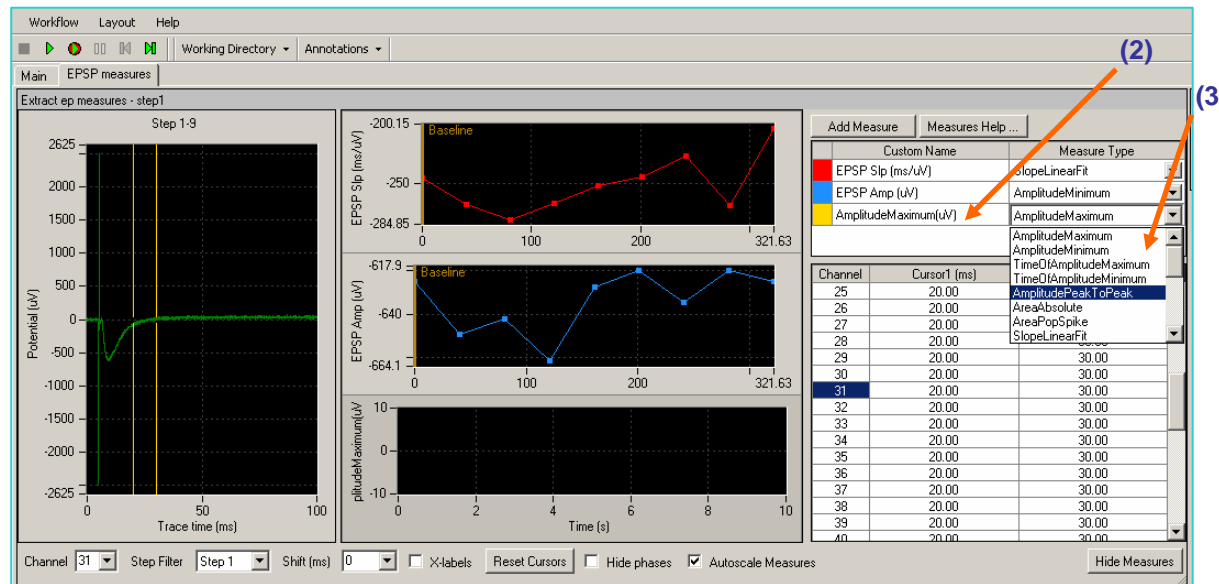


Figure.E-2

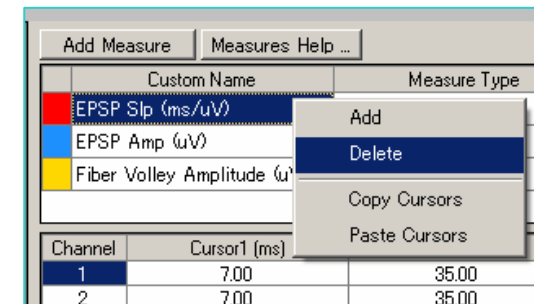


Figure.E-3

9. Increase the on-line analysis channels (1)

◆ Increase the number of the channels for on-line analysis by using Mobius Editor.

(1) Select [Workflow]-[Edit]. The “Mobius Editor” will appear. (Figure E-4).

(2) Click [Acquire MED64 data w/ stim] in the “Workflow” box, then, double-click [Extract ep measures] in the “Available task pannels” box . (Figure. E-4) The [Extract ep measures] are shifted to the box of “Workflow” and appears under the [Acquire MED64 data w/stim]. (Figure.E-5) Another chart appears on the screen.

(3) In order to export the new chart independently, click the [Save ep measures]. [Save ep measures] is shifted under the new [Extract ep measures] in the “Workflow” box and “Save ep measures” module appers. ((Figure. E-6)

(4) In order to delete the measurement, right-click the [Extracted ep measures], then select [Delete]. (Figure. E-7)

(5)Close the Mobius Editor

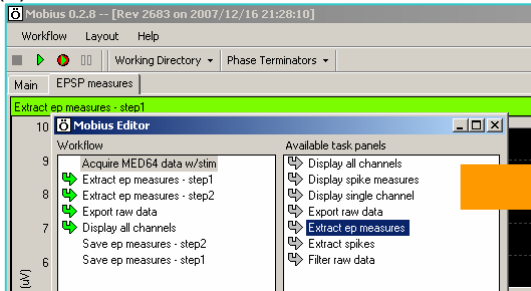


Figure.E-4

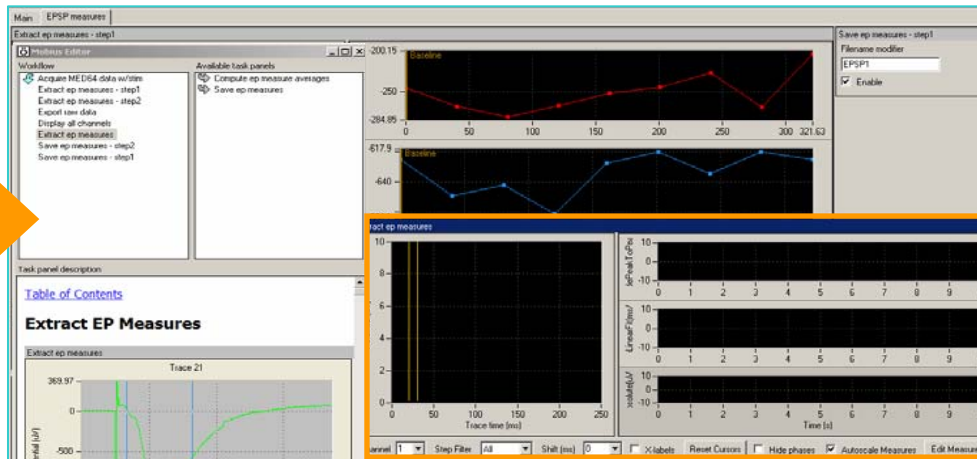


Figure.E-5

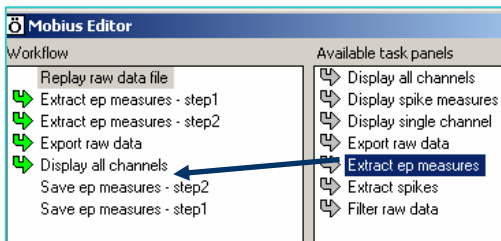


Figure.E-4*: In case of off-line analysis, [Acquire MED64 data/w/stim] is replaced by [Replay raw data file]. [Extract ep measures] will be shifted to under [Replay raw data].

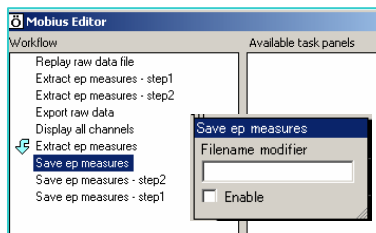


Figure.E-6

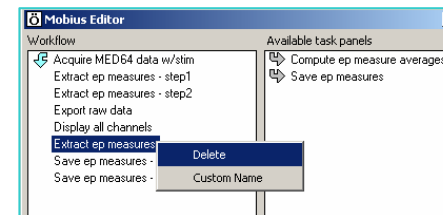


Figure.E-7



EP measurement / Single pulse Recording and Analysis, Use Mobius Editor

10. Increase the on-line analysis channels (2)

◆ Increase the number of the channels for on-line analysis in another tab by using Mobius Editor

3. Double click [Extract ep measures]

2. Open the “Mobius Editor” in the new tab.

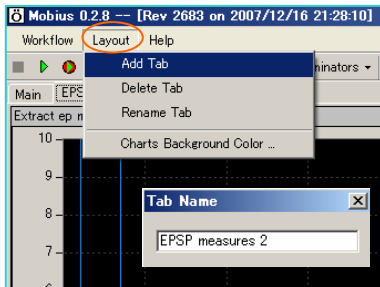


Figure.E-7

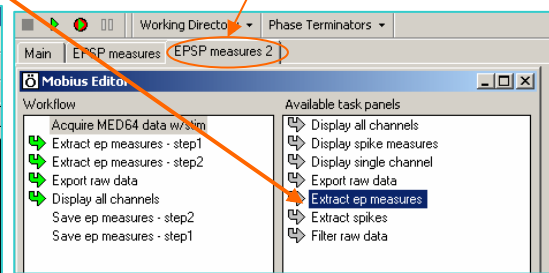


Figure.E-8

(1) Select [Layout]-[Add tab]. Type a name in the “Tab Name” pop-up as seen in Figure. E-7. Another tab will appear.

(2) Open the new tab by clicking the title. Open the “Mobius Editor” by selecting [Workflow]-[Editor] in the new tab. (Figure.E-8).

(3) Click the [Acquire MED64 data w/stim] in the “Workflow” box (left), then, double click [Extract ep measures] in the “Available task panels” box (right).

(4) [Extract ep measures] is shifted to the box of “Workflow” and appears under the [Acquire MED64 data w/stim]. Another “EPSP measurement” appear in the new tab.(Figure.E-9)

(5) Add “Save ep measures” by double click the “Save ep measures” in the “Available task panels box if needed. (Refer to page 16)

(6) The number of “EPSP measurement” can be increased with the same procedure.

(7) Close “Mobius Editor”, then adjust the screen. (Figure.E-10)

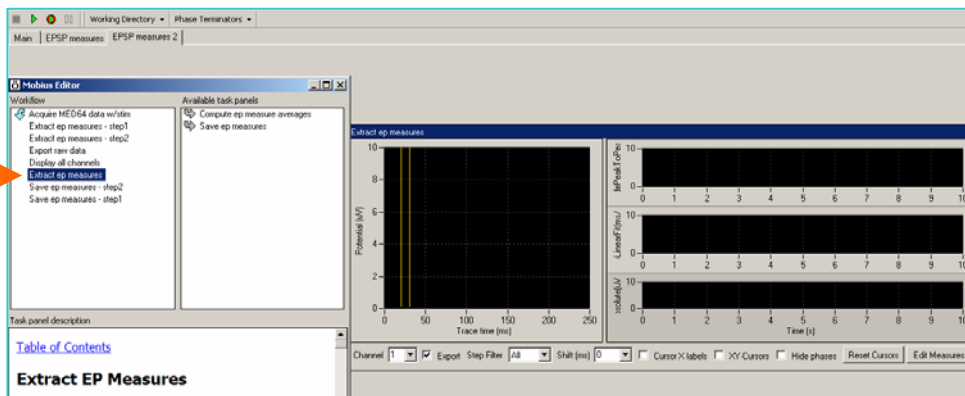


Figure.E-9

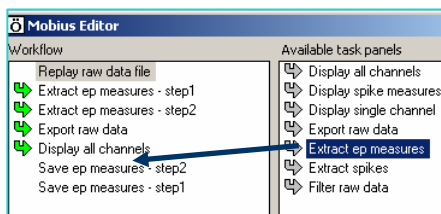


Figure.E-8*: In case of **off-line analysis**, [Acquire MED64 data w/stim] is replaced by [Replay raw data file]. [Extract ep measures] will be shifted to under [Replay raw data].

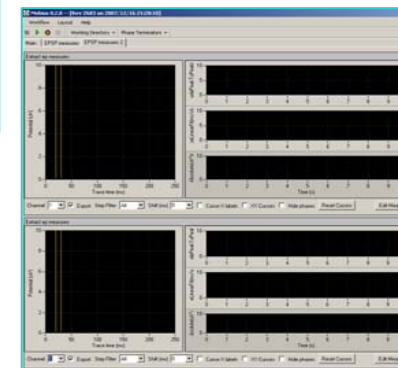


Figure.E-10



EP measurement / Single pulse Recording and analysis, Use Mobius Editor

11. Measure and graph the Average and SD (1)

◆ Measure and make the graph of the Average and Standard Deviation by using Mobius Editor

(1). Make the new tab for "Average". (Select [Layout]-[Add tab]. Type the name in the "Tab Name" pop-up. Refer to the page 17 (1))

(2). Open the new tab by clicking the title. Open the "Mobius Editor" in the new tab. (Select [Workflow]-[Editor])

(3) Click "Extract ep measures-Step1" in the "Workflow" box, then double click [Compute ep measure averages] in the "Available task panels" box. (Figure.E-11)

(4) [Compute ep measure averages] is shifted to the box of "Workflow" and appears under the [Extract ep measurements]. The chart and graph appears. (Figure. E-12)

(5) Double click the [Save ep measure averages] in the "Available task panels" box. It will be shifted to the "Workflow" box, under the [Compute ep measure average] and the task panel will appear. (Figure. E-13) The carts will be exported by ASCII.

(6) Now, the average and SD of the measures set in the "Extract ep measurement-step 1" will be computed and graphed. Please set the one for "Extract ep measurement-Step2" with the same procedure of (3)-(5). (Another Compute ep measures average will be shifted to under "Extract ep measres-Step2". (Figure.E-14)

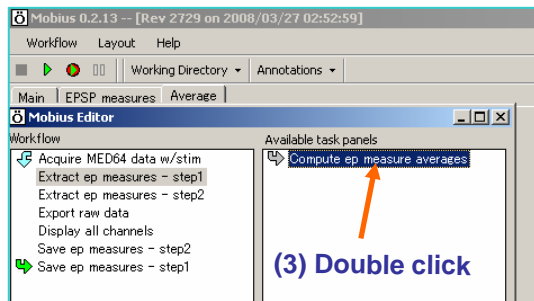


Figure.E-11

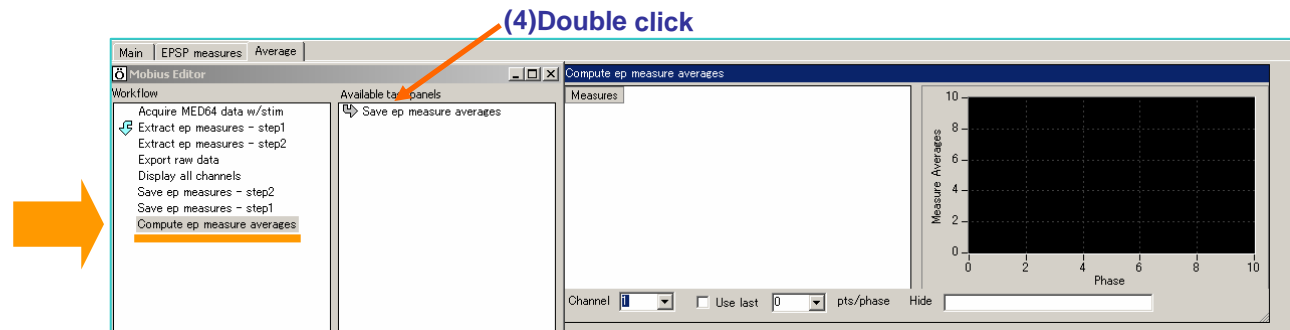


Figure.E-12

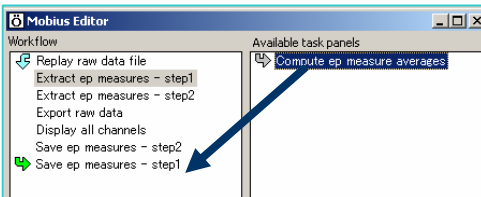


Figure.E-11*: In case of **off-line analysis**, [Acquire MED64 data/w/stim] is replaced by [Replay raw data file]. [Compute ep measures averate] will be shifted to under [Extracted measures]

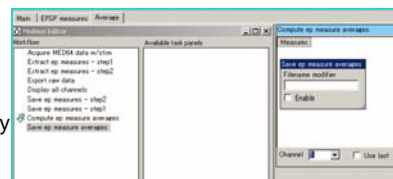


Figure.E-13

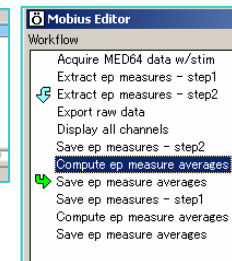


Figure.E-14



EP measurement / Single pulse Recording and analysis, use Mobius Editor

12. Measure and graph the Average and SD (2)

◆ Measure and make the graph of the average and Standard Deviation for the process of baseline, drug1, and washout.

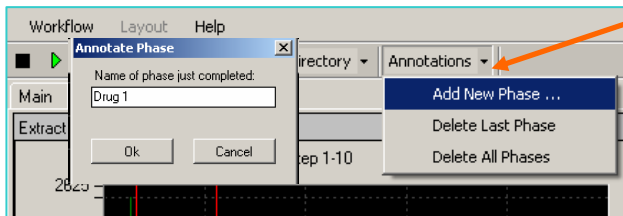


Figure.E-15



Figure.E-16

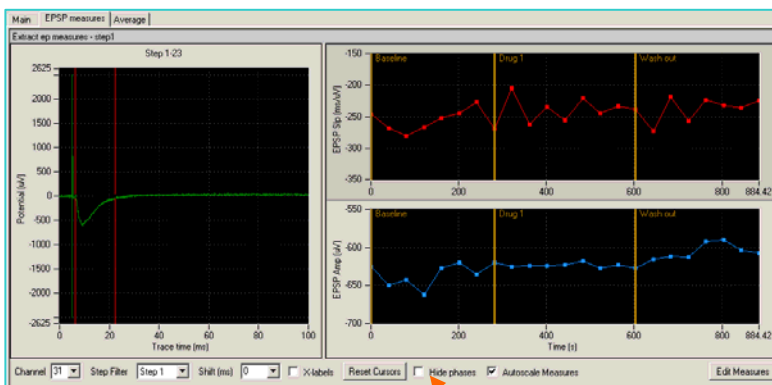


Figure.E-17

(1) NOT checked.

- (1) Make sure that [Hide phase] is NOT checked. When the software is run, the first phase is recognized as “base line” automatically.
- (2) When the recording for baseline is completed, pause the recording by click yellow || button. (Do NOT “stop” the recording.) Select [Annotations]-[Add New Phase]. Pop-up of “Annotate Phase” appears. Type the name. (e.g. Drug 1), then click [OK]. (Figure.E-15)
- (3) Re-start experiment. The brown bars appear and “Drug1 “ phase starts as seen in Figure.E-16
- (4) When the drug applications is completed, PAUSE the recording. (Do NOT “stop” the recording.) Create the new phase for the “Wash out” according to the above instruction.
- (5) Restart the experiment for Wash out.
- (6) At the completion of the experiment, the EP measurement charts with indication of each phase (Figure. E-17), and the chart and graph of Average and Standard deviation by each phase (Figure. E-18) are completed.

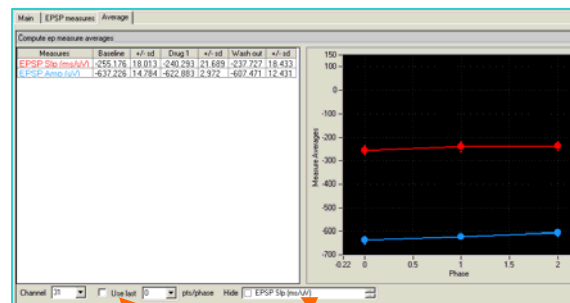


Figure.E-18 (7)



(8)

(7) If this box is unchecked, the average and SD for all plots are computed. If it is checked and the number of 5, for example, is selected, the last 5 plots at each phase is computed

(8) If it is checked, the chart and graph for the selected measure will be hidden.



13. Off-line Analysis

- ◆ The data file are opened and replayed from either of “Analysis Template” or your self-designed “Analysis Workflow”.
- ◆ Same analysis protocols to the on-line analysis including ones introduced 5 to 13 can be done off-line.
- ◆ Exporting can be done only at your self-made Analysis Workflow. Exporting is executed when the data is replayed with  button. (NOT by  button)

How to open a “Analysis Template”

*Select [Workflow]-[Template]. Select “Single pulse analysis” (Paired pulse analysis)

(Design your own analysis protocol, then save as your own “Analysis Workflow” (Select [Workflow]-[Save as]. From the next time, data file can be opened from the “Analysis Workflow”.)

How to open a “Workflow”

*Select [Workflow]-[Open]. Select a “Workflow”. (Figure. E-17)

How to open data file

*Click the box next to the file name. Then, select a file. (Figure.E-18)

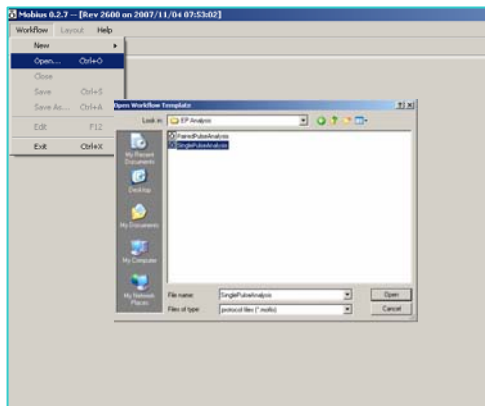


Figure.E-17

Click this button to open the data file.

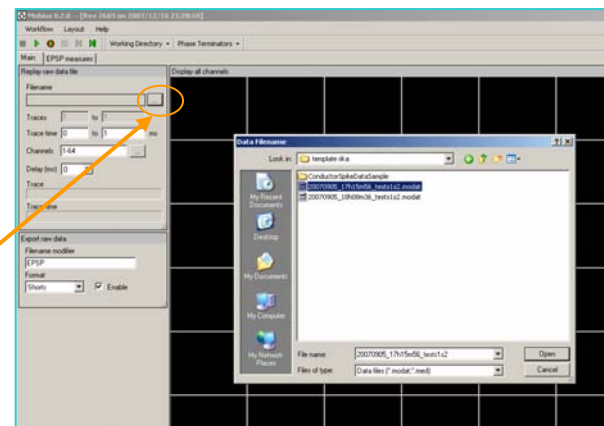
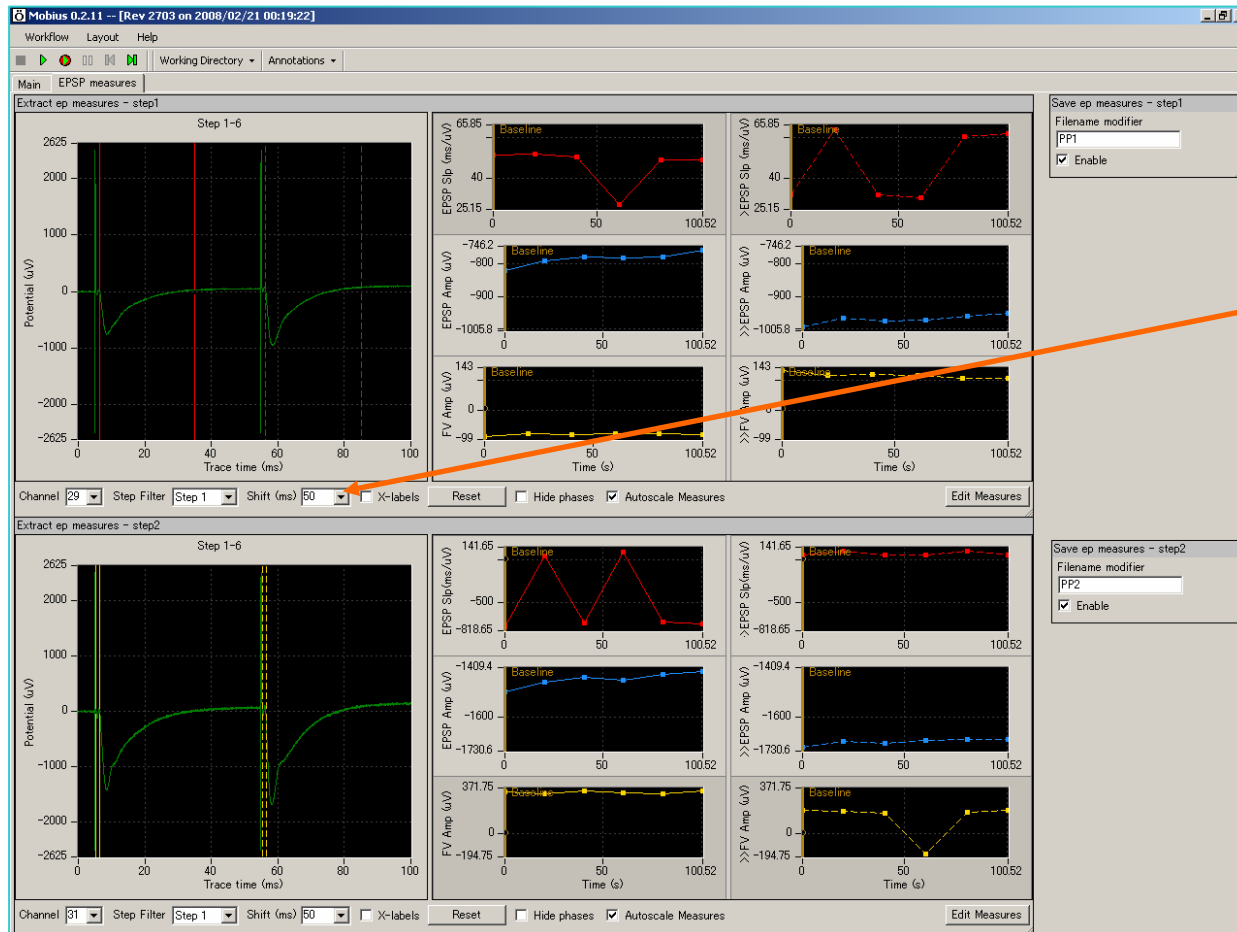


Figure.E-18

Paired Pulse Recording and Analysis

14. Paired pulse Recording and Analysis

- ◆ In the Paired Pulse Recording (Analysis) templates, 2sets of cursors and charts are displayed.



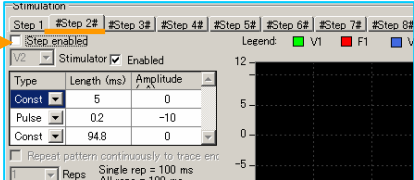
Set the interval of 1st and 2nd pulse.

EP measurement

15. LTP by Mobius -1-

There are several ways for LTP experiment using Mobis. One example is introduced here.

◆ 1. Select Pathway

(1)  Figure. L-1

Step1: EXPERIMENTAL stimulation

Step2: Check the "Step Enables" CONTROL stimulation

Figure. L-3

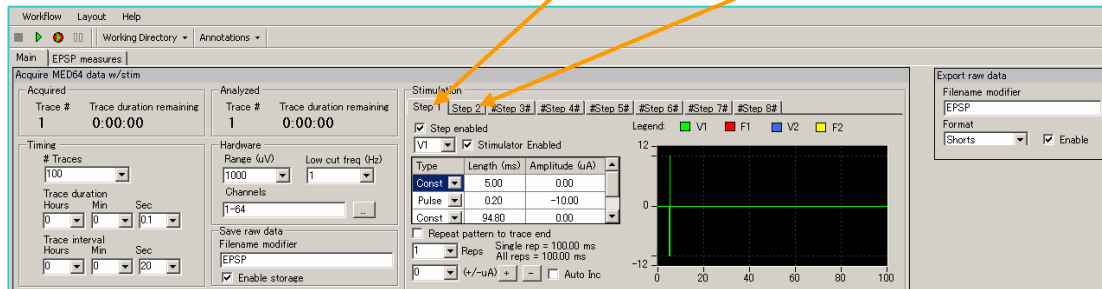



Figure. L-2: Look for the stimulation points while changing the stimulation points. In this example, channels are selected as follows:
(EXP: Ch30, CONTROL:Ch32, On-line analysis Ch.31)

- (1) Open the "Single Pulse Recording" Template. Uncheck the "Step enabled" at Step 2 so that only Step1 stimulation is available. (Figure.L-1)
- (2) Click Play (green triangle) button and apply single pulse stimulation from V1stimulus output channel. Look for the best stimulation points for CONTROL and EXPERIMENTAL and a channel for on-line analysis by using the stimulation plug and vernier dial of the MED64 amplifier. (Figure. L-2)
*You might want to have shorter "Trace interval" in this process for your convenience.
- (3) Check the "Step Enabled" at Step 2 again. In this example, "Step1" is used for "EXPERIMENTAL" while "Step2" is used for "CONTROL". (Figure. L-3)
- (4) In the "EP measurement" screen, change the number of channels for on-line analysis to the one you selected.
- (5) Set the cursors both for Slope Liner fit and Minimum amplitude.
- (6) Save it as your "LTP" Workflow. Leave it open this Workflow, which will be used in the next step.

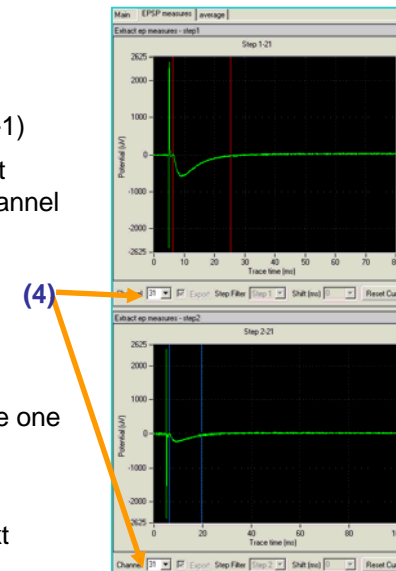


Figure. L-4: The ch 31 is selected for on-line analysis.

Set the cursor for the measurements by dragging.



Single Recording and Analysis

16. LTP by Mobius -2-

◆2. Making IO curve

- (1) At the workflow which was used in the 1"Select Pathway step", change the enabled stimulator to F1 from V1 and F2 from V2. (Please refer to page 11 (8)) **Change the stimulus plug at the MED amplifier to F1 and F2 from V1 and V2**
- (2) Type the appropriate number for the first value to make IO curve for stimulus current.
- (3) Set the appropriate number for "Auto-increment", then, check "Auto Inc" button.

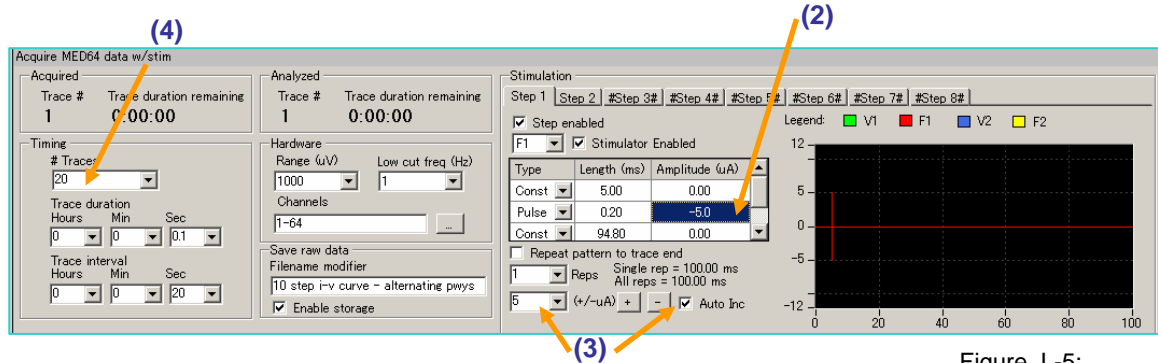


Figure. L-5:

- (4) Set the appropriate "Number of Trace". The stimulus current will be incremented by the number of uA which is set at "Auto-increment" in each Step.

*In this example, stimulation is applied through F1 and F2 alternatively. The stimulus current at Step 1 starts with 5 uA and incremented by 5 uA such as 10, 15, 20 uA, then stopped with 50 uA. (10 traces for Step1)

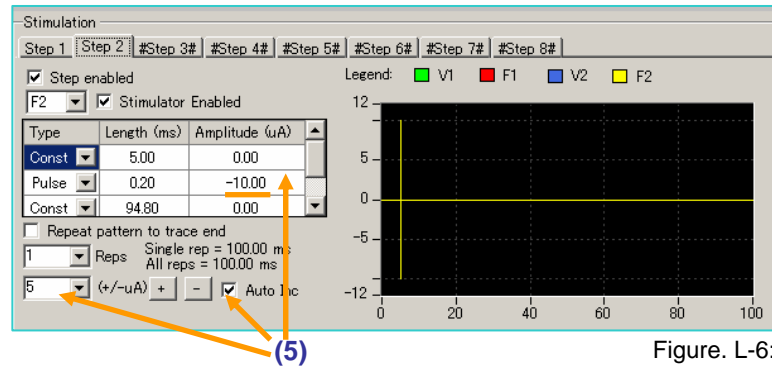


Figure. L-6:

- (5) Set the parameter for "Stimulus current", "Auto-increment" at Step 2. (Figure. L-6)
- (6) Save it as another Workflow such as "I-O curve".
- (7) Run Mobius and make IO curve. (Figure. L-7)
- (8) Calculate the amplitude of the 30%-50% for both Steps and determine the stimulus current both for Step1 and Step2.
- (9) Close this Workflow.

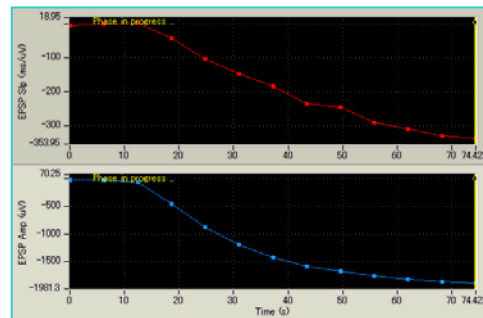


Figure. L-7:

Single Recording and Analysis

17. LTP by Mobius -3-

◆ 3. Baseline

(1) Open the “LTP workflow” which you saved in the “Select Pathway” step.

(2) Change the “enabled stimulator to F1 from V1 and F2 from V2. (Please refer to the page 11 (8).)

(3) Set the stimulus current you calculated in the “IO curve” step for “EXPERIMENTAL” (Step1)

(4) Set the stimulus current you calculated in the “IO curve” step for “CONTROL” (Step2)

(5) Change the number of [Trace] to be appropriate one.


Both of “Baseline” and “LTP” will be conducted in this workflow. Please set the number long enough to cover these processes..

(e.g. “300 time with the interval of 20 sec”: 6000 seconds (100 min.) for whole experiment. 20 min for baseline, 60 min for experiment, 20 min for extra)

(6) Overwrite save this workflow as your own “LTP” Workflow.

(6) Click  button to start recording.

(5) When the baseline recording is completed, PAUSE the experiment by clicking yellow  button. Leave this Workflow open.

Do NOT stop experiment by clicking black square  button. If it is done, the experiment is stopped and Baseline and LTP can not be recorded in a data.

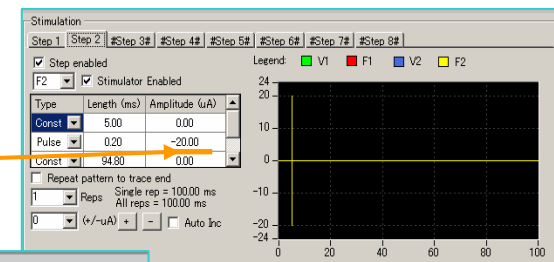
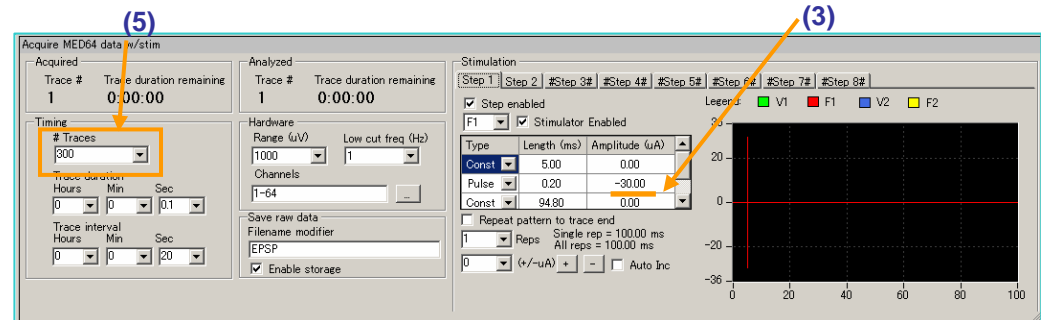


Figure. L-8

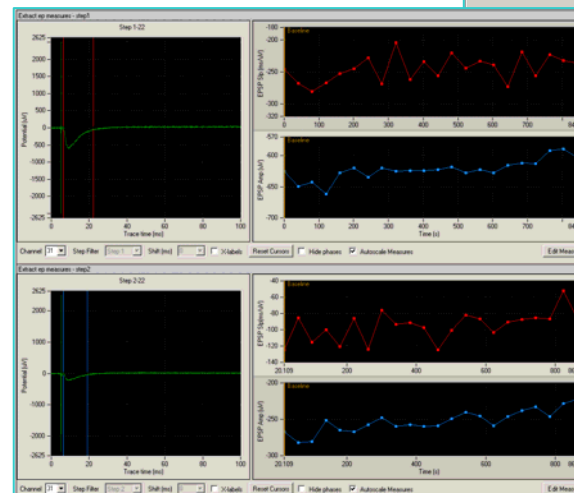


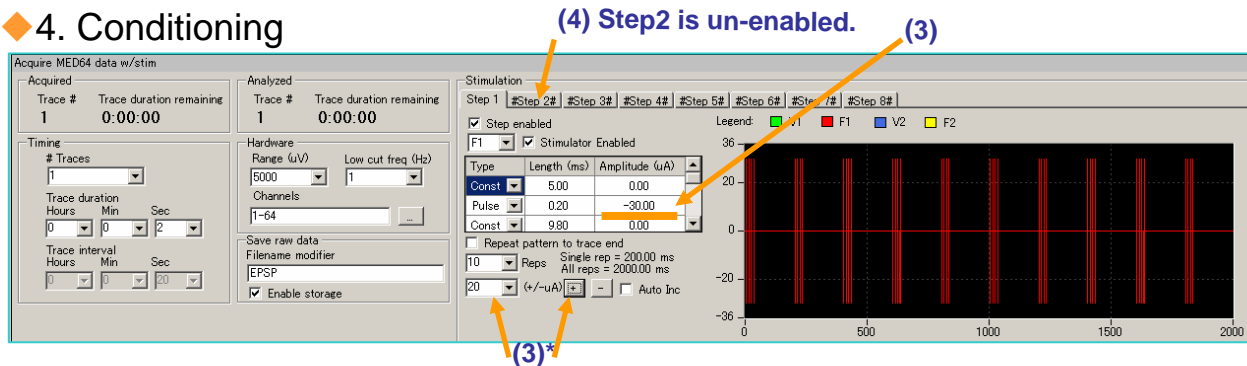
Figure. L-9



Single Recording and Analysis

18. LTP with Mobius -4-

◆ 4. Conditioning



- (1) Open the "Thetaburst" Template.
- (2) Un-enable V1 stimulator and enable F1 stimulator.
(Refer to the page 11 (8).)

(3) Change the stimulus amplitude to the value used in the "Baseline" by using + or - button.

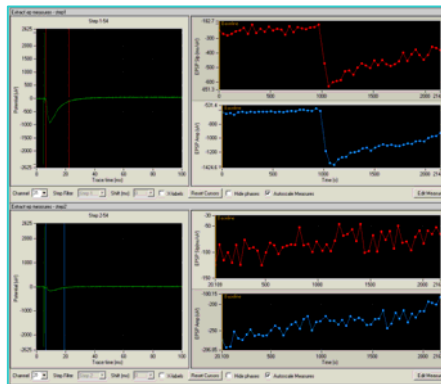
In this sample, 20 uA was set and + button was clicked once (3)*. The stimulus amplitude for all 4 pulse was changed to -30.00uA from -10.00 uA (default.)

(4) Make sure the Step2 is un-enabled.

(5) Click the green button once to apply Thetaburst. (With the green button, recording is NOT done.)

(6) Close the Workflow

◆ 5. LTP



(1) Go back to the LTP workflow where Baseline was recorded and paused.

(2) Click the  button to restart recording.

Do NOT click green button.

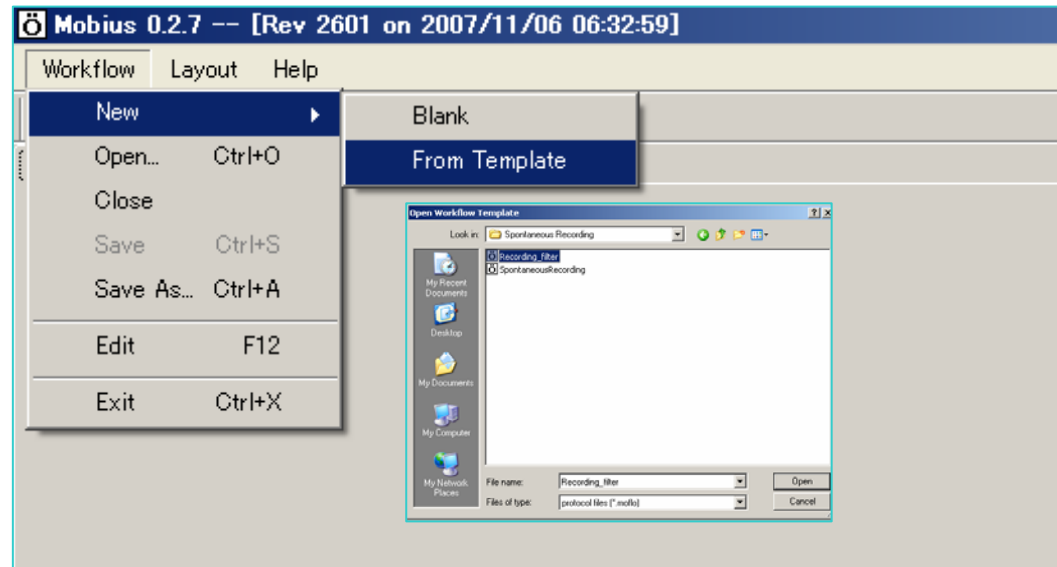
Do NOT click stop button until the experiment is completed.

Chapter 2

Spike Sorter

Spike Recording

1. Open Templates



Select [Work flow]-[New]-[From Template]. Open a template:

1. Spontaneous Recording: “Spontaneous Recording”
2. Spontaneous Recording with filter : “Recording_filter”
3. Recording with stimulation: “Recording_stim”
4. Recording with stim and filter: “Recording_stim_filter”

Spike Sorter

2. Framework of Spike Sorter templates

- ◆ “Spontaneous Recording “ consists of the 2 screens, while “Recording_filter” template consists of 3 screens.
- ◆ In the “Main” screen, experimental protocol is set and raw data acquired from 64 channels are displayed.
- ◆ In the “Spike” screen, sorting protocol is set. Acquired data, extracted spike, and chart of spike frequency in the selected channel is displayed.
- ◆ In the “Filter” screen, raw data (top) and filtered data (bottom) are displayed.

- ▶ **Play (or Replay) WITHOUT recording**
- ▶ **Play (or Replay) WITH recording**
(At Analysis Workflow, exporting is executed by clicking this button. Not by green button)

- **Stop recording (or replay)**
- || **Pause recording (or replay)**

The screenshot displays the Spike Sorter software interface. The **Main screen** (left) shows acquisition settings like 'Acquired' and 'Trace duration remaining' (0:00:00), and hardware parameters like 'Range (mV)' (1000) and 'Channels' (1-64). The **Spike tab** (center) features a table of 'Extract spikes' with columns for Channel, Threshold, Peak, and Post, and three plots: 'Acquired Data', 'Extracted Spikes', and 'Spike Frequency'. The **Filter tab** (right) shows 'Filter on data' and 'Display length channel: original' plots. Arrows point to the 'Channel' dropdown in the Spike tab and the 'Autocalc' buttons at the bottom of the Main screen.

Labels and annotations:

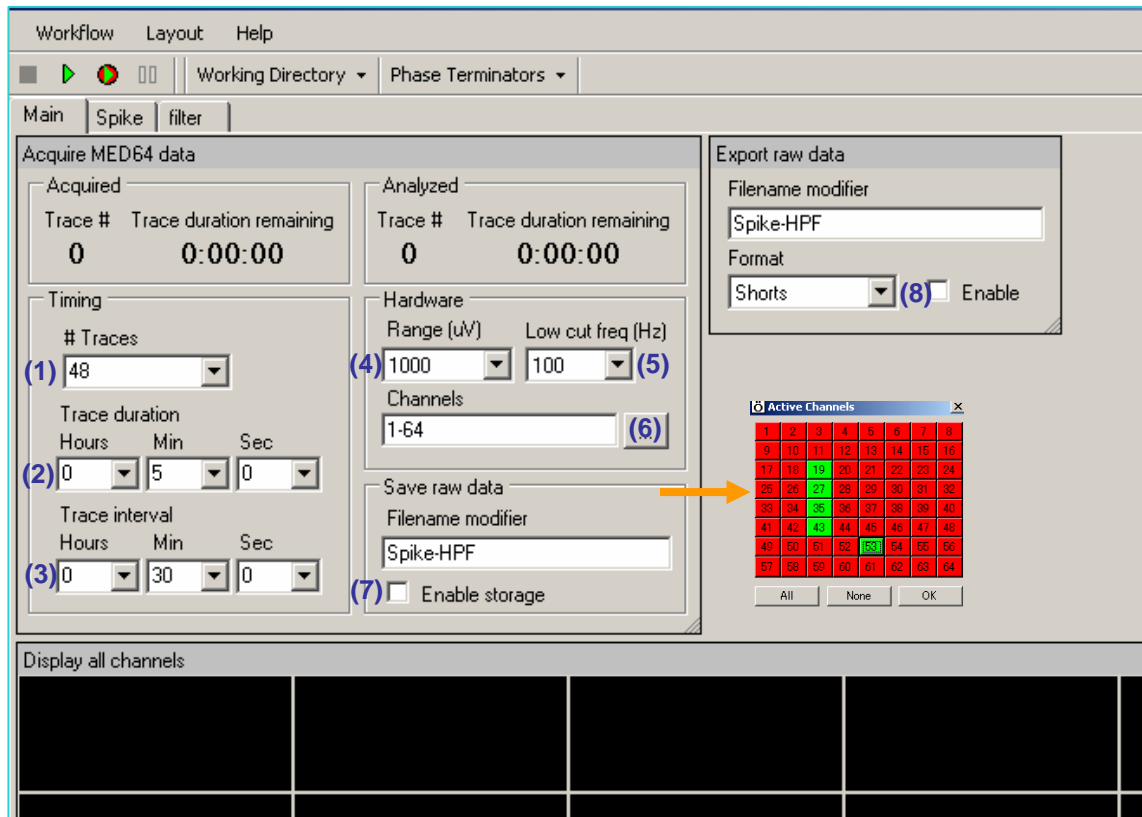
- Main screen**: Points to the leftmost window.
- Spike tab**: Points to the center window.
- Filter tab**: Points to the rightmost window.
- Channels to be monitored are selected**: Points to the 'Channel' dropdown in the Spike tab.
- Change the scale for voltage and time**: Points to the 'Autocalc' buttons at the bottom of the Main screen.



Spike Sorter

3. Design of experimental protocol

◆ Design your experimental protocol in the control panel of “Main” screen.



(7) If the [Enable storage] box is checked, the raw data for the selected channel is saved. In the default, the raw data is NOT saved.

(8) If the [Enable] box is checked, the raw data for the selected channels is exported by either format of shorts, double, or ASCII. In the default, the raw data is NOT exported

(1) Number of traces (Number of sampling)

(2) Sampling time (Recording time)

(3) Interval between each trace (sampling).

Beginning from the first trace to the beginning of the next trace.

*In the default, recording is done for 5 minutes every 30 minutes.

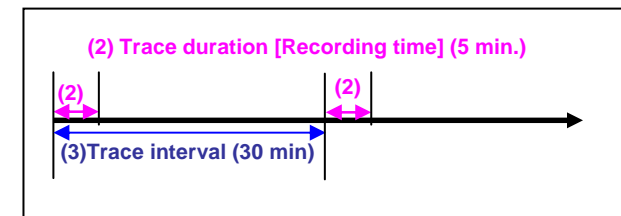


Figure. M-1

(4) Signal range (The limitation of amplitude to be recorded)

(5) Low cut filter: Select from 0.1, 1, 10, 100 Hz. If 1.0 is selected, the signals which frequency is lowered than 1.0 Hz is cut. Usually 100Hz is selected for spike recording.

(6) Click this box and select the recording channels. (Green for recording, Red for NOT recording)



Spike Sorter

4. Save as your experimental protocol

- ◆ Save the experimental protocol as your “Recording Workflow” (Recording does not start from the templates.) (Figure.S-1)
- ◆ From the next time, open your own workflow to do recording. (Figure.S-2)

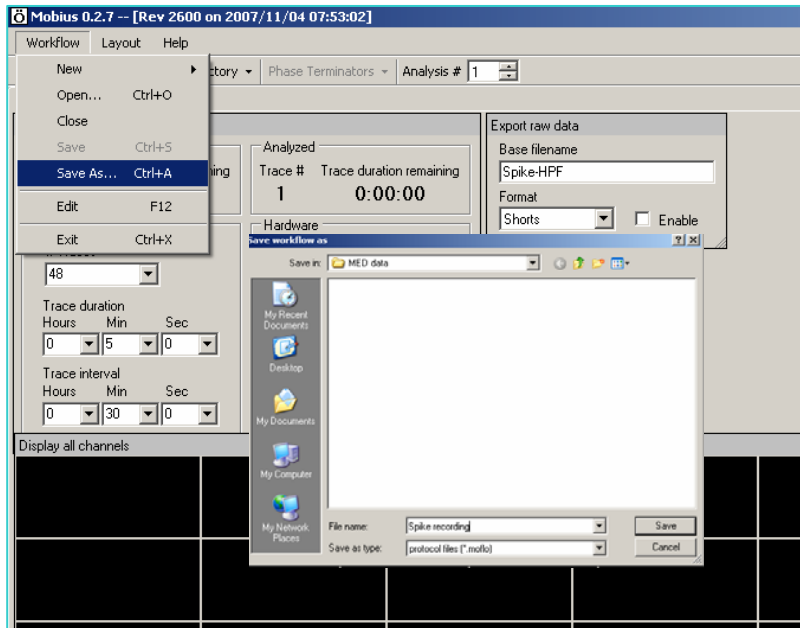


Figure.S-1: Select [Workflow]-[Save as]. Save the experimental protocol as your “Recording Workflow”. From the next time, the recording can be started from this workflow.

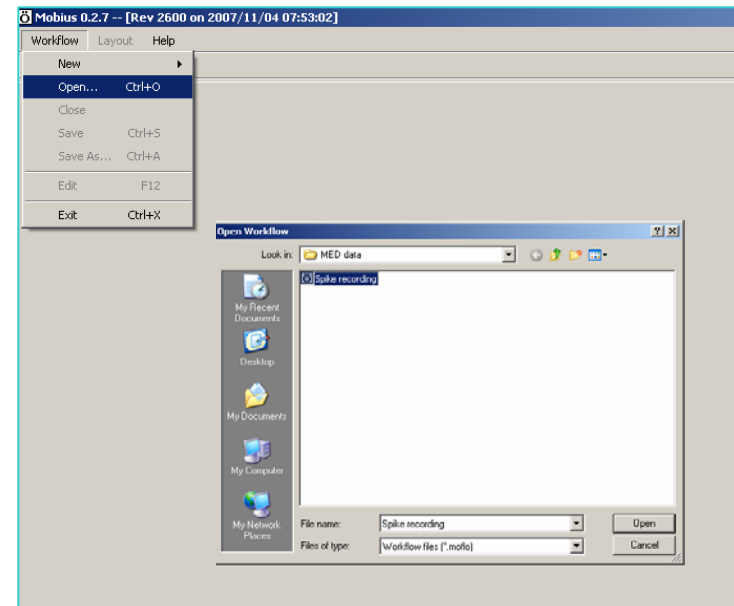
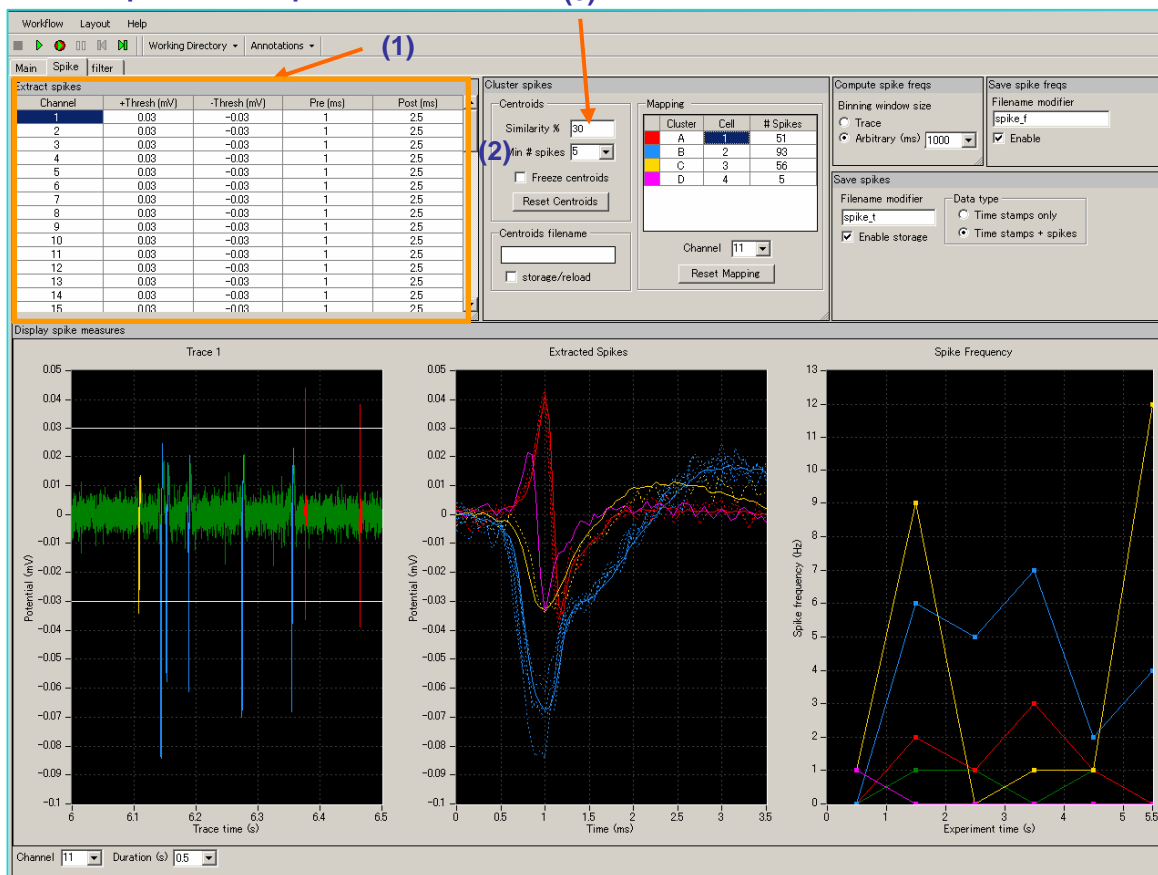


Figure.S-2: Select [Workflow]-[Open]. Open the workflow and start your experiment.

Spike Sorter

5. Set of Spike Sorting protocol (1)

The purpose of clustering spikes by Mobius is to discover and separate the signals of multiple cells recorded on a single channel based on the shapes of their spikes. (3)



(1) Set the thresholds both for positive and negative and pre (post) time to extract spikes. A number can be applied to all channels by right-click and select [Apply to all]

(2) Set the minimum number for the cluster.

A “valid” cluster must have a user determined minimum number of spikes defining its centroid (center of mass vector.) Set this minimum number for the cluster here.

As the number of processed spikes are beyond this number, clusters emerge.

The higher the minimum # of spikes is the greater the significance of valid clusters.

(Their centroids are plotted as the solid lines in the chart of “Extracted Spikes.”)

(3) Set Similarity:

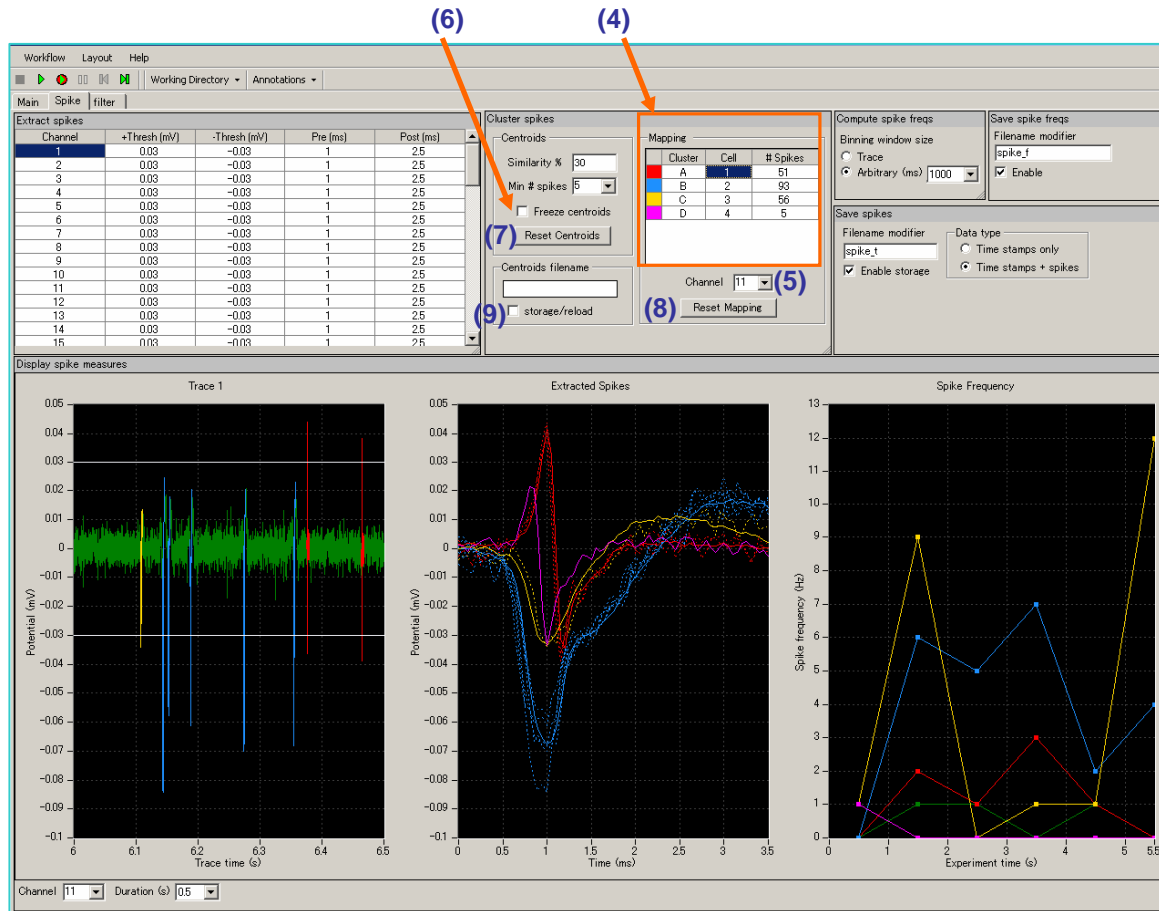
Centroids represent a kind of “average spike shape” for a set of similarly shaped spikes. Sometimes you will get more clusters than you think can possibly represent real cell, or too few. You can adjust this by tuning the [Similarity %] setting.

Higher values reduce the number of clusters found.



Spike Sorter

6. Set of Spike Sorting protocol (2)



(4) The mapping table lets you see a list of the clusters that have been discovered per selected channels, and map them to cells. Each is assigned a color in the first column, a cluster label in the second column (Letters), and a cell number in the third column. It also has the column of labeled number of spikes.

You might have some cluster that you think represent the same cell. You can merge them by double clicking and typing the same cell number in the “Cell” column. (Figure.S-1)

You can also eliminate clusters (e.g. noise spikes) by deleting the “Cell number”. (Right click the number, then select [Delete]). (Figure. S-2)

(5) The mapping of the channel which is selected here is displayed.

(6) Once you have a valid set of clusters that you believe present different cells, you can freeze them by checking the “Freeze centroids” check box. When this check box is checked, incoming spikes will not alter the definitions of the existing clusters (their centroids), nor cause new clusters to form.

(7) Click on this button to reset the centroids and start the clustering process over again.

(8) This button simply resets the cell mapping, so the modified mapping such as by “merge” or “delete” will be returned to the original one.

Cluster	Cell	# Spikes
A	1	162
B	1	235
C	3	123
D	4	25
E	5	7
F	6	17

Figure.S-1: Merge cells

Cluster	Cell	# Spikes
A	1	162
B	1	235
C	3	123
D	4	25
E	5	7
F	6	17

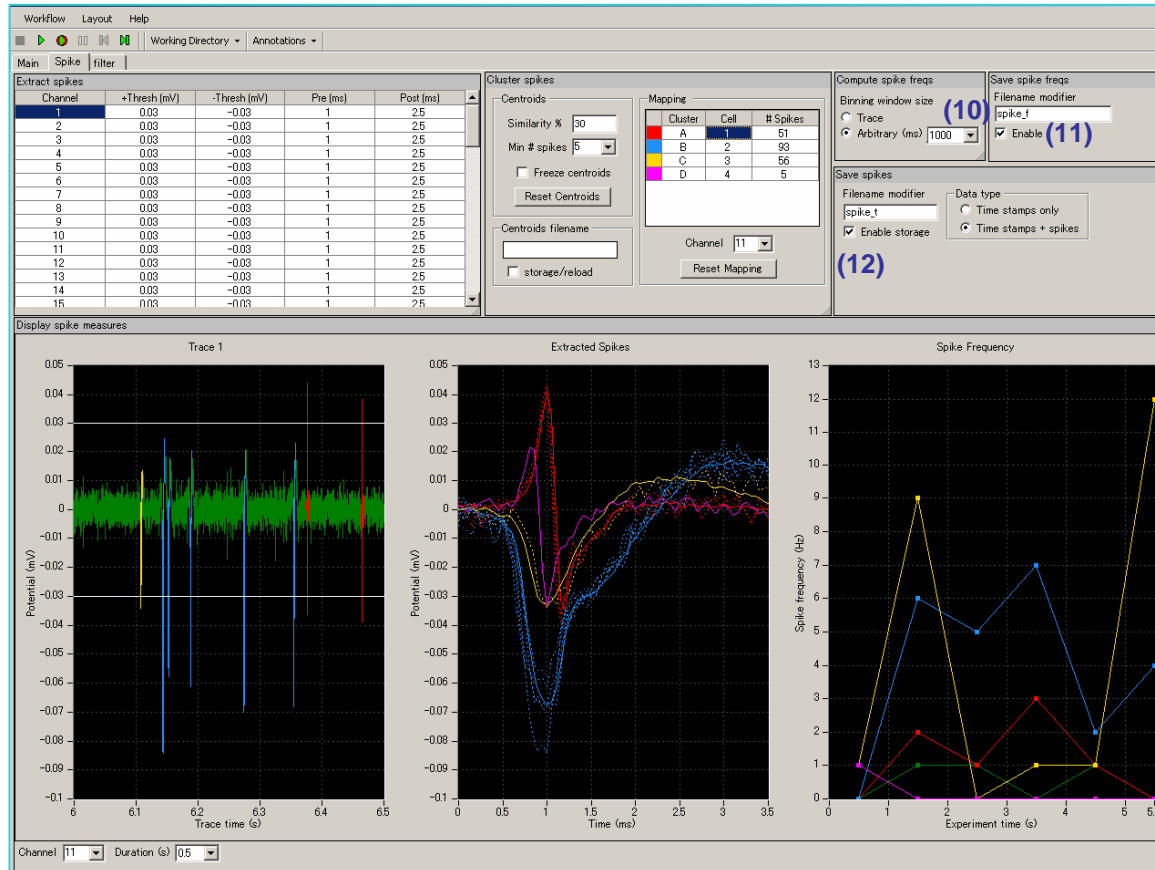
Figure.S-2: Eliminate cell

(9) When this box is checked, centroid vectors that defines the clusters are exported by ASCII. This file includes cluster centroids for all clusters as well as those that don't have enough member spikes yet to pass the validity threshold.



Spike Sorter


7. Set of Spike Sorting protocol (3)



(10) Select the binning window size to compute Spike Frequency. The chart for Spike frequency is displayed in the right-bottom.

(11) When this box is checked, the chart of the spike frequency is exported. Modify the file name by typing.

(12) "Time stamp only" or "Time stamp and extracted spikes" is exported when this box is checked.

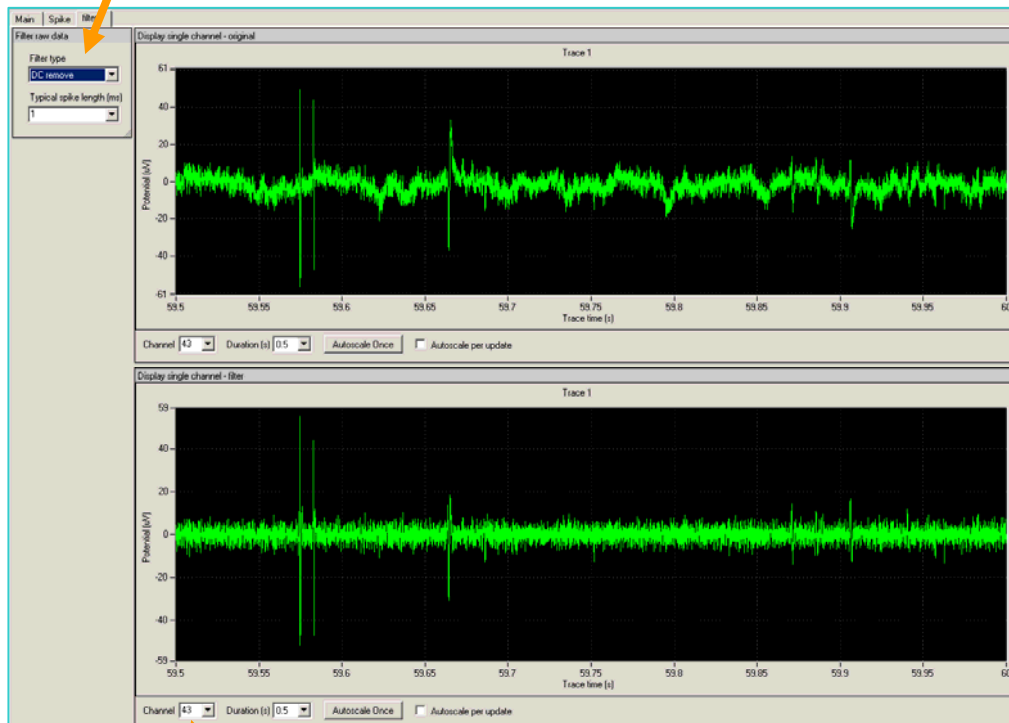
*All exports including (9)-(12) are executed when the data is played (or replayed) with red and green  button. NOT by green button.

(13) Channels for on-line sorting can be changed here even during recording or replay.

(13)

8. Set of Filtering Protocol

Select the type of Filter and set the number to be used for filtering.





Raw data

Filtered data

Channels can be changed during recording.

* Both templates of “Recording_filter” and “Recording_stim_filter” are designed so that the filtered data will be clustered. Please select “NONE” if you would like to sort the original raw data.

9. Off-line Analysis

- ◆ Data file can be opened and replayed from either of “Analysis Template” or your self-designed analysis “workflow”.
- ◆ Exporting can be done only at your self-made Workflow. Exporting is executed when the data is replayed with  button. (NOT by  button)
- ◆ Same analysis protocols to on-line analysis can be done off-line at Analysis template or workflow.

How to open a “Analysis Template”

*Select [Workflow]-[Template]. Open the “Spike Analysis” folder, then select either of “Spike Sorting” or “Spike Sorting _filter” template.

(Design your own analysis protocol, then save as your own “Analysis Workflow” (Select [Workflow]-[Save as]. In and after next time, data file can be opened from the “Analysis Workflow” .)

How to open “Workflow”

*Select [Workflow]-[Open]. Select a “Workflow”. (Figure. S-3)

How to open the data file

*Click the box next to the file name. Then, select the file. (Figure.S-4)

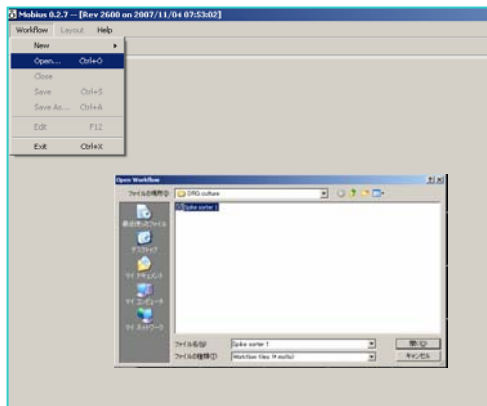


Figure.E-3

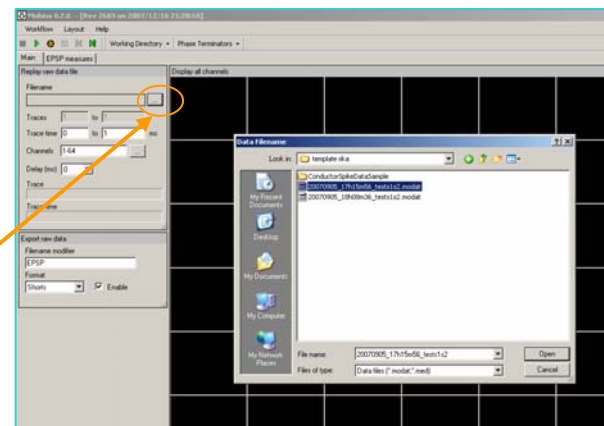


Figure.s-4

Analysis of Conductor data

There are some limitations for analysis of Conductor data. Only raw data where all 64 channels are recorded can be opened by Mobius.

- ◆ Following data can not be opened by Mobius
 - Resaved data used “Save as” function
 - Raw data where only selected channels (not all 64 channels) were recorded.
 - Some data which were recorded using more than 2 loop depth.

- ◆ Performer data can NOT be opened by Mobius.