MED64

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

Mobius Tutorial

AMPLIFIER TYPE
MED-A64MD1/MED-A64HE1

ALPHA MED SCIENTIFIC

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Chapter 1 Introduction

1-1. What is MED64 the Mobius?

MED64 Mobius is the data acquisition and analysis software for the MED64 Systems. It allows MED64 users to acquire signals and analyze each of the 64 channels during and after acquisition. Mobius consists of various moduleswhich correspond to task-specific control panels. You can design your own experimental protocol as your own "Workflow" by combining these modules using Mobius Editor, and then run those workflows both for acquisition and analysis. Pre-defined workflow templates are available so that you can run standard types of experiments immediately or design your own workflow easily by modifying them.

The goal of this Mobius Tutorial is to enable a quick, smooth start working with the Mobius software. Please refer to Mobius' on-line Help for detailed information about the software.

The following chart shows all the modules and their brief explanations. You can see which modules are included in the package you purchased.

Representative Mobius packages

1. Basic (MED-MS64MR10)
2. EPSP (MED-MS64MR11)
3. Spike Sorter (MED-MS64MR12)
4. Spike Sorter with stim (MED-MS64MR13)
5. Pro (MED-MS64MR14)
6. QT (MED-MS64MR21)
7. QT with Stim (MED-MS64MR22)
8. Extended (MED-MS64MR02)

Modules for Mobius

Modules	Module description
Acquire MED64R2 Data	Set acquisition parameters and acquires spontaneous activities
Acquire MED64R2 Data w/Stim	Programs stimulation protocols and acquires evoked responses
Acquire MED64R2 w/Pacing	Programs stimulation protocols and acquire evoked responses
Replay Raw Data File	Replays previously acquired data
Display All Channels	Displays data at all 64 channels
Display Single Channel	Displays data at a selected single channel
Export Raw Data	Exports the raw data to a binary or ASCII file
Extract EP Measures	Extracts evoked responses, computes and plots various waveform analysis (amplitude, slope, time, etc.)
Save Measures Data	Saves data measured with Extract EP (or Spike Measures) as a “csv” file
Compute Measure Averages	Computes measured averages and standard deviations for all experimental phases and plots the data
Save Measure Averages	Saves the Compute Measure Averages data as a “csv” file
Extract Spikes Advanced	Sets the thresholds and extracts the spikes which go over pre-determined thresholds
Cluster Spikes	Clusters the extracted spikes into group by shape similarity
Compute Spike Freqs	Builds a chart of spike frequency over time
Display Extracted Spikes (traces only)	Displays spikes’ extractions
Display Extracted Spikes (waveforms only)	Displays extracted spikes’ waveforms
Display Spike Frequencies	Display spike frequencies chart
Save Spikes	Saves extracted spikes / Time stamp as a “csv” file
Save Spike Freqs	Saves the spike frequency data as a “csv” file
Extract Spikes	Sets the thresholds and extracts the spikes which cross pre-determined thresholds (old version of Extract Spike Advanced)
Display Spike Measures	Display spikes’ extractions, extracted spikes’ waveforms, and spike frequency chart.
Extract Long Spikes	Detects and extracts the long spikes (>50ms) crossing pre-determined thresholds
Display Extracted Spikes	Displays the process of detecting spikes and extracted spikes waveforms
Compute Beats per Minute	Computes beats per minute
Display Beats per Minute	Graphs the beats per minute
Save Beats per Minute	Saves the computed beats per minute as a “csv” file
Compute Interspike Intervals	Builds a time chart of Inter-spike intervals (the time between each successive spikes)

Modules	Module description
Display Interspike Intervals	Displays the graph of Interspike Intervals
Save Interspike Intervals	Saves the computed inter spike intervals as a "csv" file
Extract Spike Measures	Computes and plots various extracted spike waveform analysis (amplitude, slope, time, etc) of extracted spikes
Display Results Table	Displays cumulative results from Extracted EP (Spike) Measures and Compute Measure Averages in a table
Extract Long Spikes (single channel)	Detects and extracts the long spikes (>50ms) crossing pre-determined thresholds at a selected channel
Display 4x4 channels	Display data at 16 (4x4) channels
Display 2x4 channels	Display data at 8 (2x4) channels
Display Remaining Time	Display the remaining time in a trace
Diaplay Micrograph	Open and display a graphic file
Filter Spike Data	Filters extracted spikes
Filter Raw Data	Filters raw data

Please note: Acquire MED64 Data / Acquire MED64 Data w/Stim are modules for SU-MED640 amplifier.

Availabilities for modules by packages

Modules / Package	Basic	EPSP	Spike Sorter	Spike sorter w/stim	Pro	QT	QT w/stim	Extended
Acquire MED64R2 Data	*	*	*	*	*	*	*	*
Acquire MED64R2 Data w/Stim		*		*	*		*	*
Acquire MED64R2 w/Pacing							*	*
Replay Raw Data File	*	*	*	*	*	*	*	*
Display All Channels	*	*	*	*	*	*	*	*
Display Single Channel	*	*	*	*	*	*	*	*
Export Raw Data		*	*	*	*	*	*	*
Extract EP Measures		*			*		*	*
Save Measures Data		*			*		*	*
Compute Measure Averages		*	*	*	*	*	*	*
Save Measure Averages		*	*	*	*	*	*	*
Extract Spikes Advanced			*	*	*			*
Cluster Spikes			*	*	*			*
Compute Spike Freqs			*	*	*			*
Display Extracted Spikes (traces only)			*	*	*			*
Display Extracted Spikes (waveforms only)			*	*	*			*
Display Spike Frequencies			*	*	*			*
Save Spikes			*	*	*	*	*	*
Save Spike Freqs			*	*	*			*
Extract Spikes			*	*	*			*
Display Spike Measures			*	*	*			*
Extract Long Spikes						*	*	*
Display Extracted Spikes						*	*	*
Compute Beats per Minute						*	*	*
Display Beats per Minute						*	*	*

Modules / Package	Basic	EPSP	Spike Sorter	Spike sorter w/stim	Pro	QT	QT w/stim	Extended
Save Beats per Minute						*	*	*
Compute Interspike Intervals						*	*	*
Display Interspike Intervals						*	*	*
Save Interspike Intervals						*	*	*
Extract Spike Measures						*	*	*
Display Results Table						*	*	*
Extract Long Spikes (single channel)						*	*	*
Display 4x4 channels	*	*	*	*	*	*	*	*
Display 2x4 channels	*	*	*	*	*	*	*	*
Display Micrograph	*	*	*	*	*	*	*	*
Display Remaining Time	*	*	*	*	*	*	*	*
Filter Spike Data						*	*	*
Filter Raw Data			*	*	*	*	*	*

1-2. Installation of Mobius and your acquisition PC

1-2.1. Installation of Mobius

The latest version of Mobius is available from the web site:
http://www.med64.com/products/trialsoftware.html
Mobius requires the amplifier-driver to be run for acquisition. Please download the driver from the same web site.

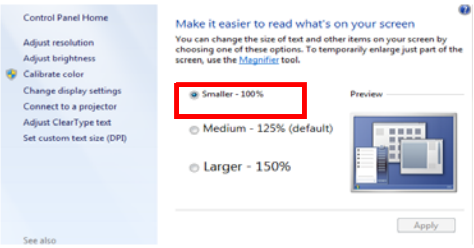
1-2.2. Settings for your acquisition PC

NOTE:

Ask your local distributor or support@med64.com for the latest requirements and specifications for your acquisition PC.

Display setting

- When a display monitor with a resolution of 1920 x 1080 is used, set the Windows 7 text size ([Control Panel] > [Appearance and Personalization] > [Display]) to “Smaller” (100%). Otherwise, some modules are not displayed correctly.



Make it easier to read what's on your screen

You can change the size of text and other items on your screen by choosing one of these options. To temporarily enlarge just part of the screen, use the Magnifier tool.

● Smaller - 100%

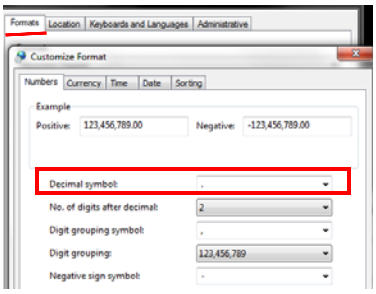
○ Medium - 125% (default)

○ Larger - 150%

Figure1-2.1. Display setting.

Language Setting

- Set the “Decimal symbol” to “period” (.). Mobius does not work properly with the “comma” (,) setting for the decimal symbol. Go to [Control panel] > [Clock, Language, and Regions]. Open the [Format], and change the “Decimal symbol” to “period” (.).



Positive: 123,456,789.00 Negative: -123,456,789.00

Decimal symbol: .
No. of digits after decimal: 2
Digit grouping symbol: ,
Digit grouping: 123,456,789
Negative sign symbol: -

Figure 1-2.2. Selecting. (period) for the “Decimal symbol”.

Following initial-settings, 1-4 (below) are recommended for stable recording. They are strongly recommended when long-term acquisitions (e.g., days) are performed.

1) Adding second hard drive

Save data to a drive where the operating system is NOT installed (e.g., D drive). For a desktop PC, use an internal second hard drive. For a laptop PC, use a hard drive with e-SATA (or USB3) connection.

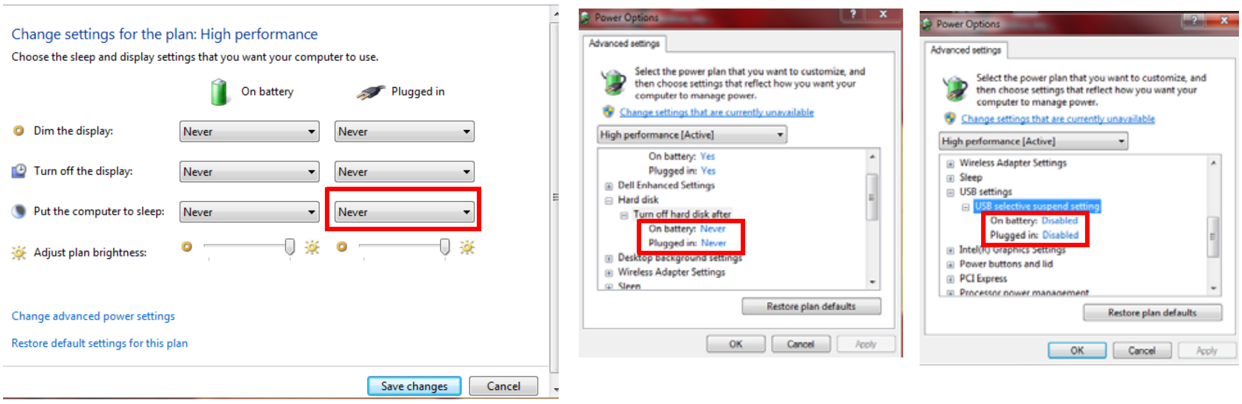
NOTE:

Mobius creates temporal "cache file" on the C drive whenever acquisition is made. This cache file is kept until the acquisition is stopped. (It is deleted automatically when acquisition is stopped.) Saving data to a second hard drive helps PC to avoid stopping due to insufficient disk space.

2) Setting for [Power Option]

Change the [Power Option] as following to achieve stable power supply. Unstable power supply could cause PC to stop due to "buffer overflow".

1. Go to [Control panel] > [Power Option].
2. Select [High performance], and then click the [Change Plan Setting].
3. Select "Never" for the [Computer to Sleep]. (Figure 1-2.3, left)
4. Click [Change advanced power settings].
5. Go to [Hard disk] > [Turn off hard disk after]. Select "Never" both on Plugged in and on Battery. (Figure 1-2.3. Middle)
6. Go to [USB settings] > [USB selective suspend setting]. Select "Disabled" in both options. (Figure 1-2.3, Right)
7. Click [OK] to save the changes.



Change settings for the plan: High performance

Choose the sleep and display settings that you want your computer to use.

	On battery	Plugged in
Dim the display:	Never	Never
Turn off the display:	Never	Never
Put the computer to sleep:	Never	Never

Select the power plan that you want to customize, and then choose settings that reflect how you want your computer to manage power.

High performance [Active]

Hard disk > Turn off hard disk after
On battery: Never
Plugged in: Never

USB settings > USB selective suspend setting
On battery: Disabled
Plugged in: Disabled

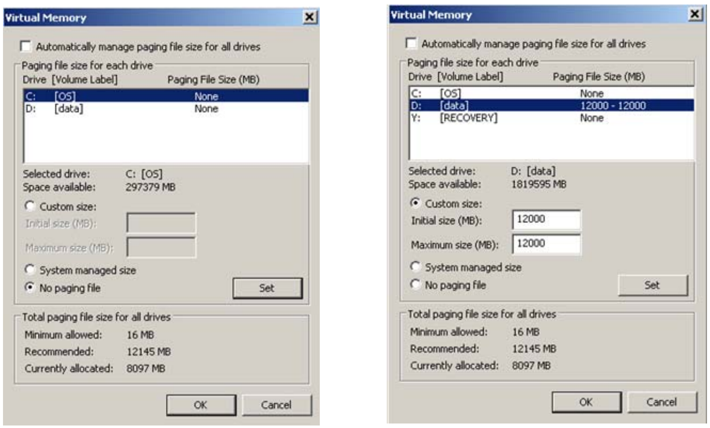
Figure 1-2.3. Setting for Power Option.

3) Setting for [Advanced system settings]

Increase the "virtual memory" to avoid overloading the PC due to memory shortage with following procedures.

Mobius usually uses up to 3Giga Bite to run. However, it could use more than 3GB when acquisition is made for long-term. Increasing the "virtual memory" helps the PC to keep running without stopping when long-term recording is performed.

1. Right-click the [Computer], and then select the [Properties].
2. Click the [Advanced system settings].
3. Select the [Advanced] tab, and click the [Settings] for "Performance". The [Performance Option] window pops up.
4. Select [Advanced] tab. Click [Change] for "Virtual memory". Uncheck the "Automatically manage paging file size for all drives" (Figure 1-2.4, left).
5. Select [No paging file] for the C drive. Select [Custom size] for the D drive, and then input 12000 for both [Initial Size] and [Maximum Size] (Figure 1-2.4. right). Click [Set], and finally [OK].



☐ Automatically manage paging file size for all drives

Paging file size for each drive

Drive [Volume Label]		Paging File Size (MB)
C:	[OS]	None
D:	[data]	None

Selected drive: C: [OS]
Space available: 297379 MB
○ Custom size:
Initial size (MB):
Maximum size (MB):
○ System managed size
● No paging file

Total paging file size for all drives
Minimum allowed: 16 MB
Recommended: 12145 MB
Currently allocated: 8097 MB

☐ Automatically manage paging file size for all drives

Paging file size for each drive

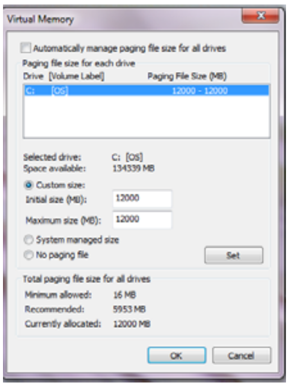
Drive [Volume Label]		Paging File Size (MB)
C:	[OS]	None
D:	[data]	12000 - 12000
Y:	[RECOVERY]	None

Selected drive: D: [data]
Space available: 1819595 MB
● Custom size:
Initial size (MB): 12000
Maximum size (MB): 12000
○ System managed size
○ No paging file

Total paging file size for all drives
Minimum allowed: 16 MB
Recommended: 12145 MB
Currently allocated: 8097 MB

Figure 1-2.4. Setting “Virtual memory” in the [Advanced system settings] for C drive (left) and D drive (right).

If the 2nd drive does not exist, select the [Custom size], and then input 12000 for[Initial Size] and [Maximum size] (Figure 1-2.5) Click [Set], and finally [OK] (Figure 1-2.5).



☐ Automatically manage paging file size for all drives

Paging file size for each drive

Drive [Volume Label]		Paging File Size (MB)
C:	[OS]	12000 - 12000

Selected drive: C: [OS]
Space available: 134339 MB
● Custom size:
Initial size (MB): 12000
Maximum size (MB): 12000
○ System managed size
○ No paging file

Total paging file size for all drives
Minimum allowed: 16 MB
Recommended: 5953 MB
Currently allocated: 12000 MB

Figure 1-2.5. Setting the “Virtual memory” for a single drive.

4) Setting for [Intel Rapid Technology]

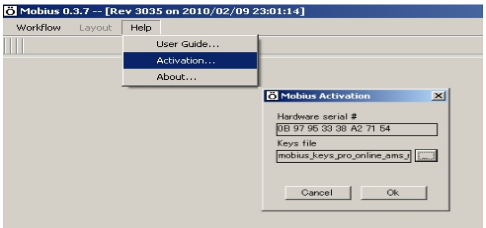
Change the setting for [Intel Rapid Technology] as following to achieve stable power supply to hard disc.

1. Go to [All programs] > Open the [Intel] folder.> Click [Intel Rapid Storage Technology].
2. Open the [Performance] tab. Select "Disable" for the [Link power management].

1-2.3. Activation

Mobius needs to be activated with your key-file.

1. Save the key-file in the [Program file] > [WitWerx] >[Mobius] folder.
2. Open Mobius. Click [Help] > [Activation].
3. You will see your Hardware serial # in the box. Click the box next to the Keyfile box to select your key-file. Click OK for activation.



Hardware serial #: 0B 97 95 33 38 A2 71 54

Keys file: mobius_keys_pro_online_ams_

Figure 1-2.6. Activating Mobius.

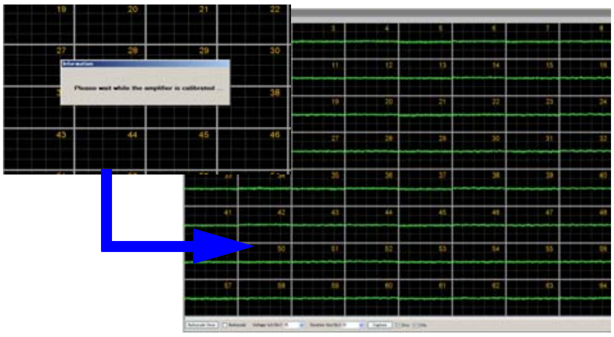
1-3. Important notice for the MED64 Mobius

Please read and follow the instructions below particularly during data acquisition.

General

- Connect your PC to the MED64 Head amplifier and Main amplifier and turn on both amplifiers to open [Acquire MED64R2 Data], [Acquire MED64R2 Data w/Stim], or [Acquire MED64R2 Data w/pacing] modules, or recording workflows including those modules.
- Make sure that your MED64PC's C drive has at least 50% free space.
- Make sure that the hard drive has enough free space when you start a long-term recording. Recording with all 64 channels for 1 hour creates a 9.216GB data file. (9.216GB is represented as 9,000,000KB, 8,739.06MB, or 8.58GB in the Windows.)
- Avoid saving data to the drive where the OS is installed (usually C drive). Save it to a separate hard drive, that is internal or eSATA-supported (See the page 6 in detail).
- When a recording workflow is run with Green or Green-Red button for the first time after it is opened (or made), Mobius needs several seconds for the calibration (as seen in the pop-up message). Acquisition starts automatically after the calibration.

  If you would like to start acquisition immediately after clicking the Green-Red button, run the workflow with the Green button for several seconds, and then stop it before starting your experiment. When the workflow is run the next time, it will not require the calibration and acquisition will start immediately.

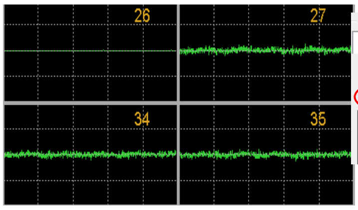


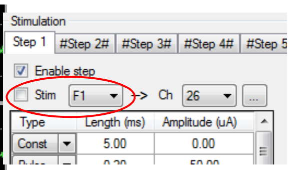
Please wait while the amplifier is calibrated ...

Figure 1-3.1. Initial calibration (left). Acquisition starts after the calibration (right).

- When running the [Acquire MED64R2 Data] module after running [Acquire MED64R2 Data w/Stim] or [Acquire MED64R2 w/Pacing], the channels previously used for stimulation are disabled (as seen in the Figure 1-3.2) and will not record. To activate these channels, one of two methods can be used:

  1) Open and run a NEW workflow including the [Acquire MED64R2 Data] module.
  2) Run the [Acquire MED64R2 Data w/Stim] module for more than one trace while disabling all stimulation options (uncheck all the stimulators, Figure 1-3.3), and then run [Acquire MED64R2] module.





☑ Enable step

☐ Stim F1 --> Ch 26

Type	Length (ms)	Amplitude (uA)
Const	5.00	0.00

Figure 1-3.2. (left) Example of disabled channel (ch26).
Figure 1-3.3. (right) Disabling the stimulator. Uncheck the“Stim” box for all stimulators, and then run workflow.

- When a display monitor with a resolution of 1920 x 1080 is used, set the Windows 7 text size ([Control Panel] > [Appearance and Personalization] > [Display]) to “Smaller” (100%). Otherwise, some modules are not displayed correctly.

- Set the “Decimal symbol” to “period” (.). Mobius does not work properly with the “comma” (,) setting for the decimal symbol. Go to [Control panel] > [Clock, Language, and Regions]. Open the [Format], and change the “Decimal symbol” to “period” (.). Or. select “English (US)” for the “Language”.

Acquire MED64R2 Data w/Stim

- Make sure that the STIMULUS CURRENT selection on the MED64 Head amplifier (MED-A64HE1) is set to [NORMAL] to deliver the stimulus current amplitude set in Mobius. Selecting [x2] doubles the output stimulus current amplitude set in Mobius.
(e.g. When 100 μA is selected by Mobius while [x2] is selected on the head amplifier, the output stimulus current is 200 μA)

- The maximum number of pulses for a [Step] in the "Stimulus waveform editor" (#12 in the Fig. 2-1.4, page 15) is **21**.

Replay Raw Data

- Opening a large data file can take several minutes. (e.g. Opening a 10GB file takes over 1 minute.)

- Mobius processes data in blocks of 500 msec. For replaying data, the Trace Time duration must be ≥501 msec.

Display All Channels

- For the data to be displayed properly in the Display All Channels module, you must set the Duration (ms/div) to 100 ms/div or greater. Mobius processes data in blocks of 500 msec and always displays 5 divisions of time in each display channel. Displaying less than 100 ms/div will result in cutting off some of the data display. For example, setting 20 ms/div results in a display of the first 100 msec of the 500 msec of data processes by Mobius (20 msec x 5 div). The other 400 msec of data will not be displayed and the next update to the display will again be the first 100 msec of the next 500 msec batch of data acquired. Conversely, for shorter duration applications (e.g., fEPSPs), shorter duration recordings are not a problem.

Extract Spikes Advanced / Extract Spikes / Extract Long Spikes

- These modules require powerful computation and need to be used carefully during acquisition. Mobius can appear to freeze when it extracts more spikes than it can analyze. This most often happens when non-biological signals cross the spike extraction thresholds on all channels at once. (e.g. unexpected noises cross the thresholds or the baseline fluctuates due to vibration.) Please keep in mind the following instructions when these workflows are used with acquisition.

- Avoid using these modules on-line (during signal acquisition) until you are familiar with your experiment(s) and biological assay(s). Particularly avoid using these modules for long-term recordings.

- Set the trace duration to less than 10 minutes and the trace interval for longer than the trace duration so that the extracting spikes can catch up with acquisition during the trace interval.

- For recording neuronal spikes, make sure to set the low-cut filter to 100 Hz in order to remove low-frequency noise.

- Make sure to PAUSE Mobius when drugs are applied.

- Minimize the number of channels for spike extraction.

Filter Raw Data

- Mobius acquires data in 500 msec blocks. Digital filtering can cause small noise spikes to appear at the end of a data block if the baseline is offset from 0 at the end of the data blocks. To eliminate this noise:

    - While recording neuronal spikes, set the Low-cut filter to 100 Hz.

    - While recording cardiac signals (low-frequency), avoid using the "Filter Raw Data" module. Instead, use "Filter Spike Data" so that the extracted beating signals (long spikes) are filtered.

Compute Measure Averages

- Since this module is always at the end of the analysis chain, it can slow down the display of incoming data. Thus, it is not recommended to use this module during acquisition. When it is used during acquisition:

    - Set the trace duration shorter than 10 minutes while the trace intervals for longer than the trace duration.

    - Minimize the number of recording channels.

1-4. Getting started

When Mobius is opened, you will see blank diaplsy. The basic concept for the Mobius software is **"you need to create and run your own "Workflows" both for acquisition and analysis.** You can create your Workflow by:

1. Building your own workflow using Mobius Editor.
2. Using the pre-defined workflow templates.

In this section, you will learn quickly:

1) How to use Mobius Editor to design your own workflow.
2) How to start data acquisition.
3) How to replay acquired data.
4) How to export raw data.

The following chapters will instruct you on how to start your experiments using some of workflow templates.

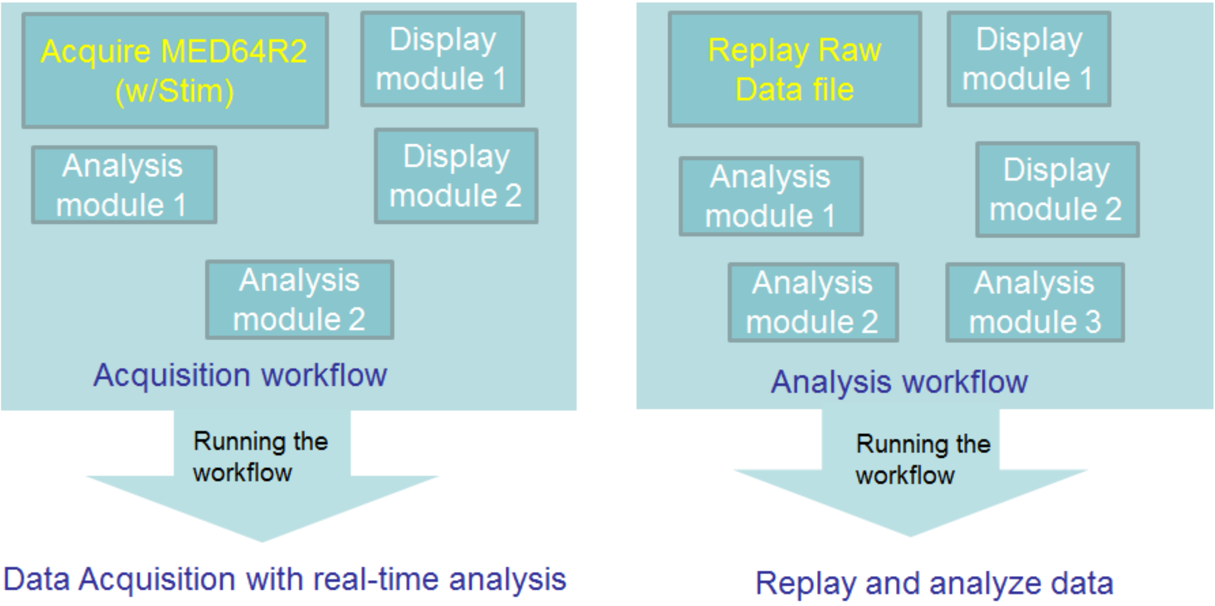


Figure 1-4.1. Basic concept for the Mobius software.

1-4.1. Building up your own workflow using Mobius Editor

This section will quickly introduce you how to use Mobius Editor.

1. Open Mobius. Select the [Workflow] > [New] > [Blank] to open the “Mobius Editor” window.

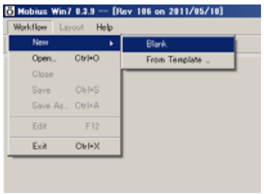


Figure 1-4.2. Opening the Editor.

2. This window shows a library of available task modules (task specific panels) on its right panel. Click on a module to see its description at the bottom of the screen.

   A workflow is created by building a list of task-specific modules on the left panel of the Editor. When a module is double-clicked, it is shifted to the left panel and the task-panel for the module appears on the screen.

   For acquisition, start by double clicking either the [Acquire MED64R2 Data] or the [Acquire MED64R2 Data w/Stim] module. For replaying or analyzing the acquired data, start with [Replay Raw Data File]. Other modules for display and analysis can follow either of these modules.

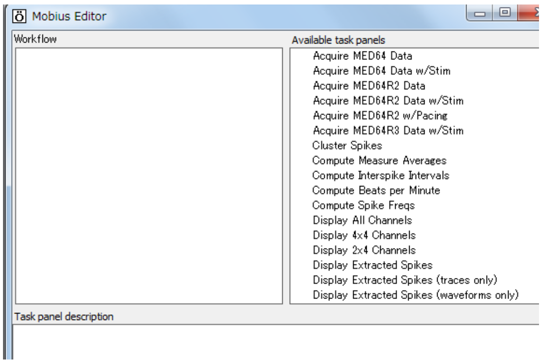


Figure 1-4.3. Mobius Editor.

- [Acquire MED64 Data] and [Acquire MED64 Data w/Stim] are acquisition modules for SU-MED640 amplifier. It can NOT be selected for your amplifier (MED-A64MD1)

Figure 1-4.3 shows the designed acquisition workflow (top-left), its Editor (middle), and the workflow structure (bottom).

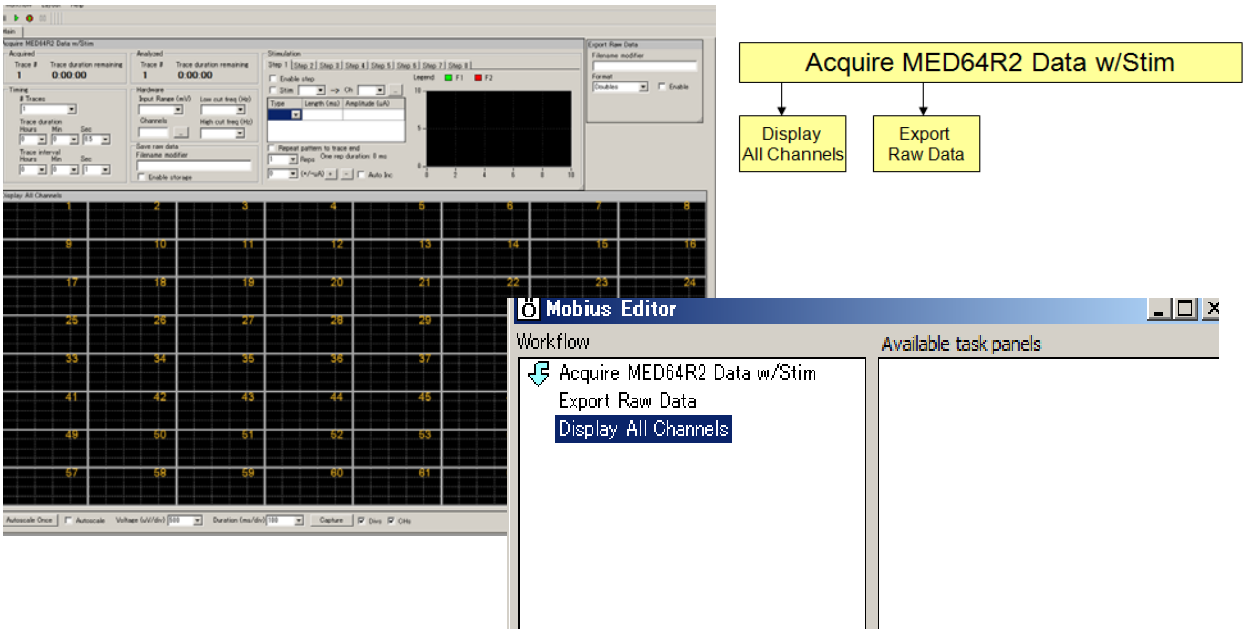


Figure 1-4.4. An example of a designed acquisition workflow. The modules on the Workflow box (left box) are now on the screen.

3. You will see arrows next to the modules on the left. Their meanings are described below;

   - Gray right pointing arrows: The modules with these arrows can be used AFTER the selected module (highlighted in dark blue) in the Workflow list. Double-clicking a module with this arrow next to it will insert it AFTER the selected module in the workflow.
   - Green right pointing arrows: The modules with these arrows are located AFTER the selected module (highlighted in dark blue).
   - Light blue downward pointing arrows: The modules with these arrows can be used BEFORE the selected module (highlighted in dark blue).
   - Gray downward pointing arrows: When these arrows appear next to a module name, they indicate that the output of this module can satisfy the input prerequisites of the currently selected module (highlighted in dark blue) in the Workflow list. Double clicking one of these will insert it BEFORE the selected step in the workflow.
   - Red steps: You may notice that some of module names are written in red text. This means that the step does not meet input prerequisites. Select the red step (click on it once) to see which modules can satisfy its needs (the ones with gray downward arrows next to them) and select the appropriate one to add to the Workflow box (double-click them). Or, right-click the red step and select [Delete] to remove this module from the Workflow box.

4. In many cases, you can not have all the desired control panels on a Tab (screen). You can create new tabs by selecting [Layout] > [Add Tab], then type a name for the new Tab. (Figure 1-4.5)

5. In order to have control panels on the new tab, 1) open the new tab by clicking the tab name, and 2) open the Mobius editor and edit modules on the new tab. You can go back and forth between these tabs during your experiments.

   The Figure 1-4.6 shows analysis and export modules are added to the workflow on a new Tab.

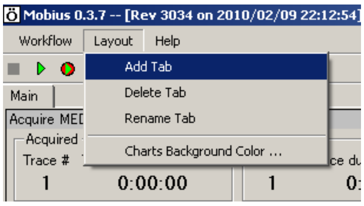


Figure 1-4.5. Creating a new tab.

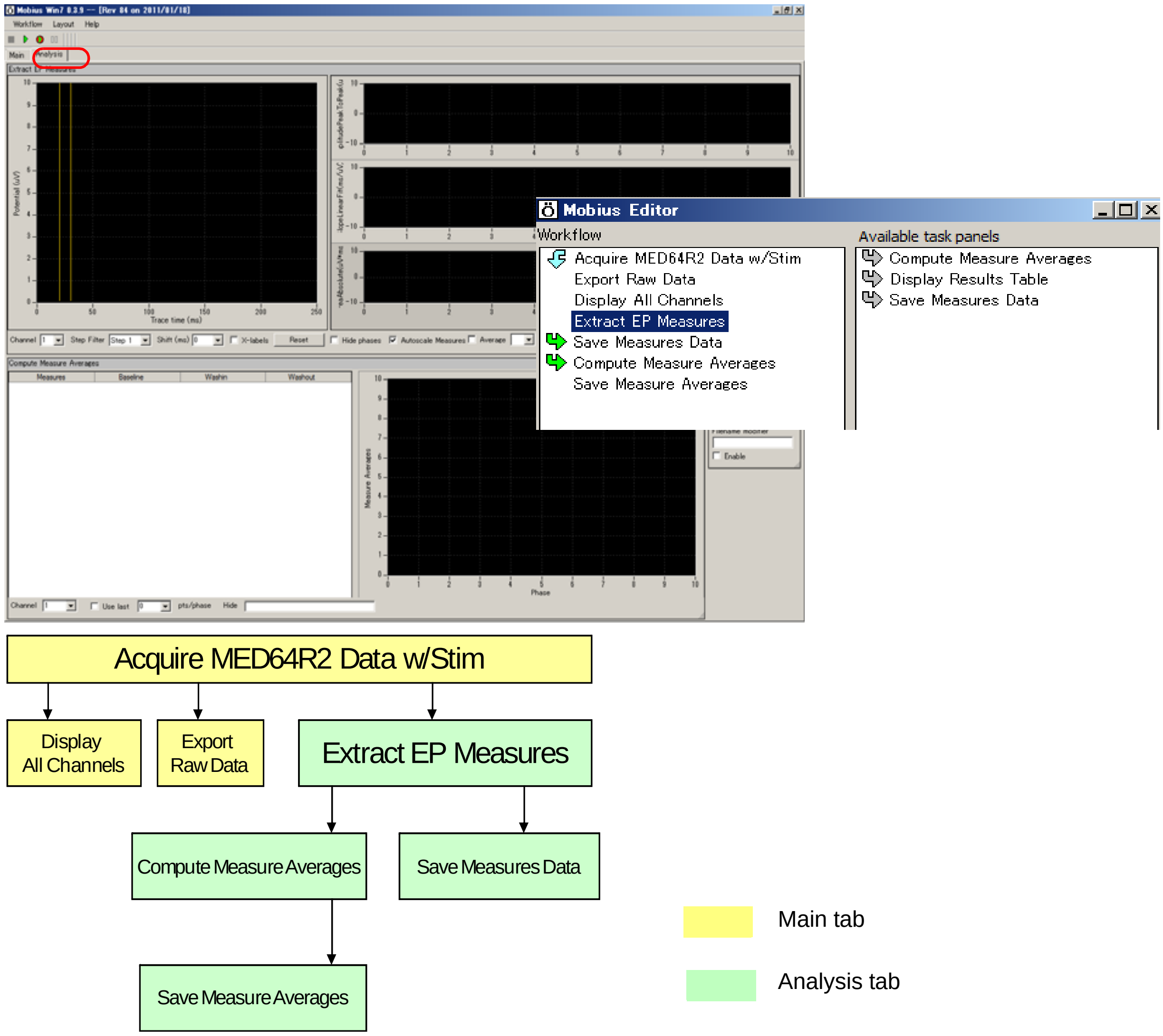


Figure1-4.6. An example of a workflow design. A new tab (Analysis) is created, then analysis and saving modules are added to this tab. The bottom chart shows the configuration of this workflow.

6. Save it as your own Workflow. (Select [Workflow] > [Save as]). Now you are ready to for acquisition, and start acquisition by clinking the Green or Green-Red button.

7. The Workflow can be opened by selecting [Workflow] > [Open].

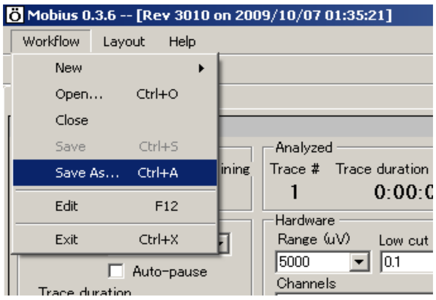


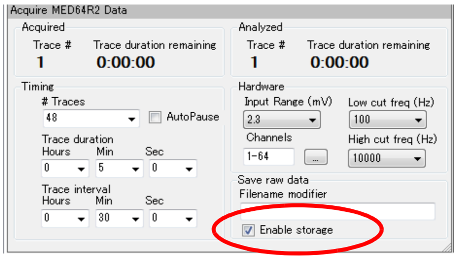
Figure 1-4.7. Opening a workflow

1-4.2. Data acquisition

Data acquisition is performed by running the acquisition workflow. Open your acquisition workflow by selecting [Workflow] > [Open]. Data acquisition starts by clicking either of Green or Green-Red button in acquisition workflow. For saving data:

1. Check the Enable storage check box, and then
2. Start acquisition with the Green-Red button.

Clicking the Green button starts acquisition WITHOUT saving data.



Acquire MED64R2 Data

Acquired
Trace # 1
Trace duration remaining 0:00:00

Analyzed
Trace # 1
Trace duration remaining 0:00:00

Timing
Traces: 48
☐ AutoPause
Trace duration: Hours 0, Min 5, Sec 0
Trace interval: Hours 0, Min 30, Sec 0

Hardware
Input Range (mV): 2.3
Low cut freq (Hz): 100
Channels: 1-64
High cut freq (Hz): 10000

Save raw data
Filename modifier:
☑ Enable storage

Figure 1-4.8. Checking the Enable storage.

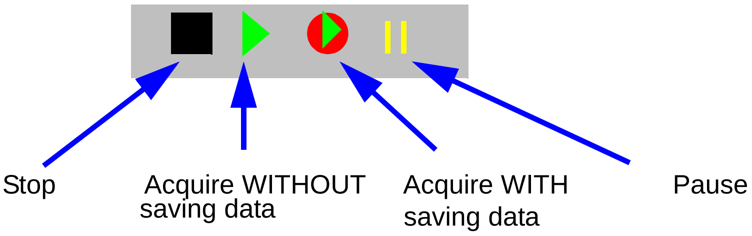
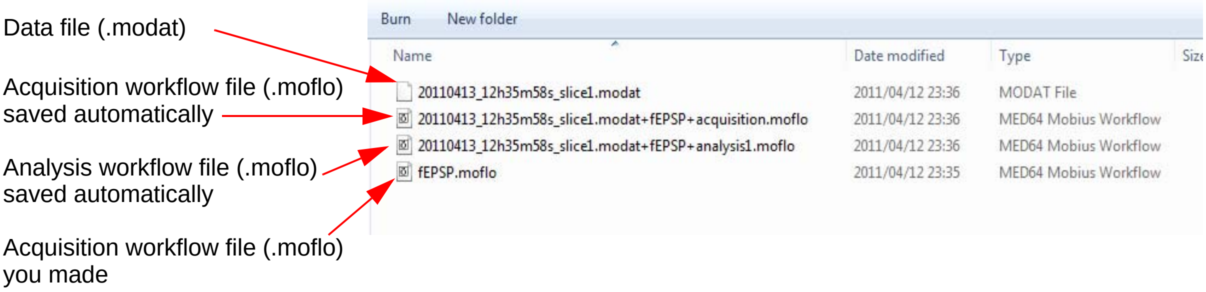


Figure 1-4.9. How the execution button works for acquisitions.

Mobius requires to save the acquisition workflow you made for saving the data file. Save your acquisition workflow if it is not made yet (See #6 in the page 14) in a folder. Acquisition with saving data (clicking the Green-Red button) generates following files automatically at the same folder where the acquisition workflow (.moflo) file is saved:

- Data file (.modat)
- Analysis workflow file(.moflo) including analysis modules you selected for your recording
- Acquisition workflow file (.moflo)



Name	Date modified	Type
20110413_12h35m58s_slice1.modat	2011/04/12 23:36	MODAT File
20110413_12h35m58s_slice1.modat+fEPSP+acquisition.moflo	2011/04/12 23:36	MED64 Mobius Workflow
20110413_12h35m58s_slice1.modat+fEPSP+analysis1.moflo	2011/04/12 23:36	MED64 Mobius Workflow
fEPSP.moflo	2011/04/12 23:35	MED64 Mobius Workflow

Data file (.modat)

Acquisition workflow file (.moflo) saved automatically

Analysis workflow file (.moflo) saved automatically

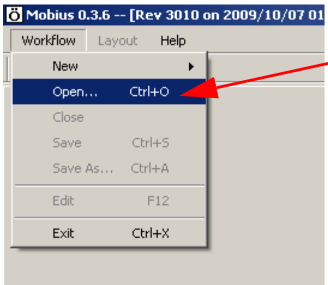
Acquisition workflow file (.moflo) you made

Figure 1-4.10. Data and Workflow files generated in a folder after acquisition (with GREEN-RED button).

1-4.2. Opening and replaying acquired data

Acquired data (.modat file) can be opened with the [Replay Raw Data File] module. Analysis is performed in “analysis workflow” that contains this module. The quickest way to open the data file is using the analysis workflow that was generated automatically when data was saved.

1. Open an analysis workflow.



This Open menu is to open a workflow file (.moflo), NOT data file (.modat)

Figure 1-4.11. Opening an analysis workflow.

2. Click the box next to the Filename, and select the data file. When the file name appears on the Filename box (right in the Figure 1-4.12), the data is ready to be replayed.

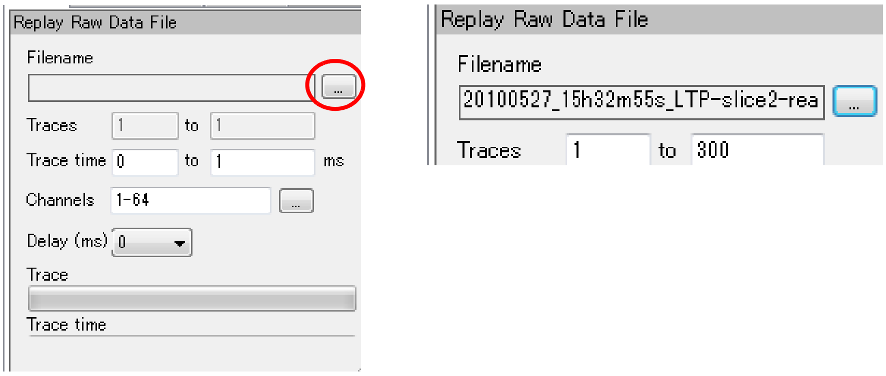


Figure 1-4.12 Opening data file.

3. Data is replayed when the Green button is clicked. (Clicking the Green-Red button activates saving/exporting enabled (See page 18)).

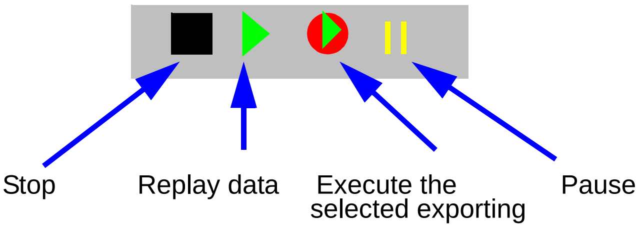
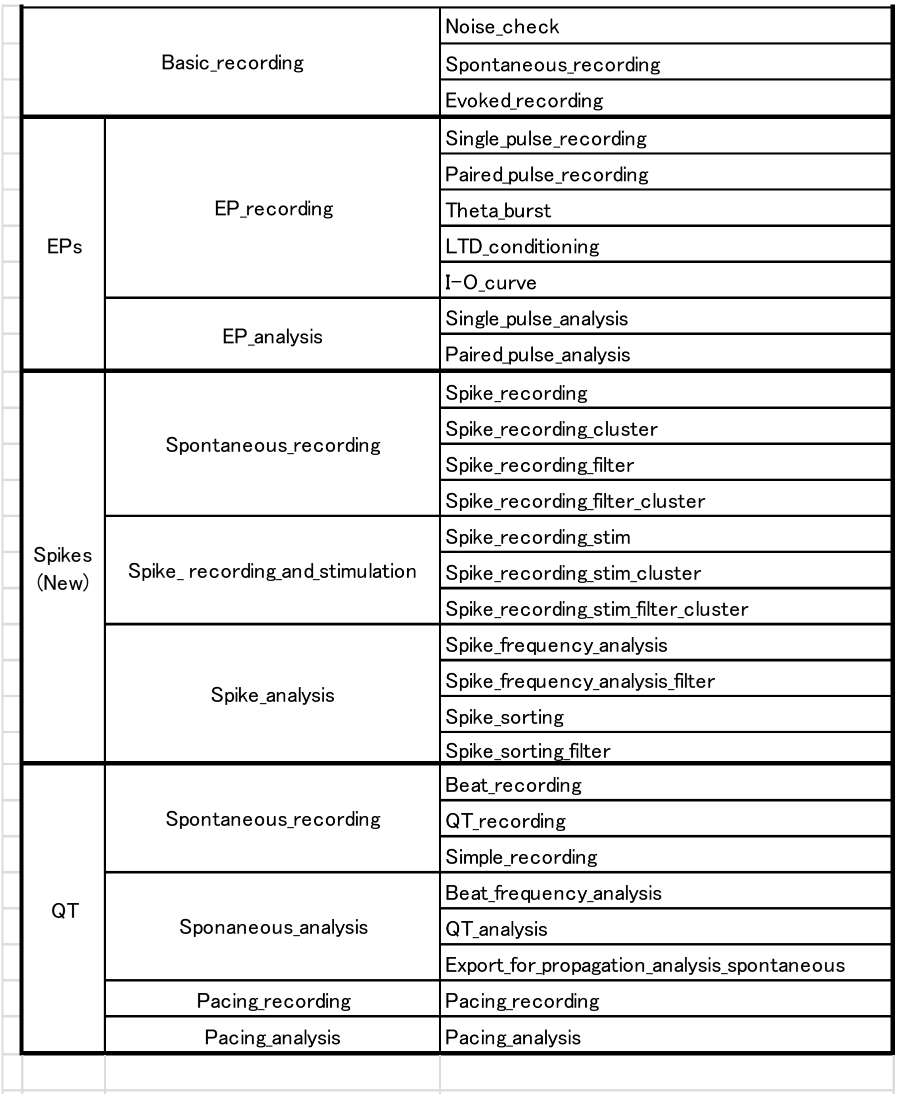


Figure 1-4.13. The way the execution buttons works for data replay and analysis.

1-4.3. Starting with Workflow Templates

The easiest way to get started with Mobius is to use the predefined workflow templates. You can start your experiments using these workflow templates right away or design your own workflow by editing these templates. The following chapters will instruct you on how to start your experiment using these workflow templates.



Basic_recording		Noise_check
		Spontaneous_recording
		Evoked_recording
EPs	EP_recording	Single_pulse_recording
		Paired_pulse_recording
		Theta_burst
		LTD_conditioning
		I-O_curve
	EP_analysis	Single_pulse_analysis
		Paired_pulse_analysis
Spikes (New)	Spontaneous_recording	Spike_recording
		Spike_recording_cluster
		Spike_recording_filter
		Spike_recording_filter_cluster
	Spike_ recording_and_stimulation	Spike_recording_stim
		Spike_recording_stim_cluster
		Spike_recording_stim_filter_cluster
	Spike_analysis	Spike_frequency_analysis
		Spike_frequency_analysis_filter
		Spike_sorting
		Spike_sorting_filter
QT	Spontaneous_recording	Beat_recording
		QT_recording
		Simple_recording
	Sponaneous_analysis	Beat_frequency_analysis
		QT_analysis
		Export_for_propagation_analysis_spontaneous
	Pacing_recording	Pacing_recording
	Pacing_analysis	Pacing_analysis

Figure 1-4-14. Available workflow templates.

1. Click [Workflow] > [New] > [Templates]. Select either of [64MD1_1280x1024] or [64MD_1920x1080] folder depending on the size of your display monitor, and then select desired template.

2. You can change parameters and save the template as your own workflow to go directly to your experiment

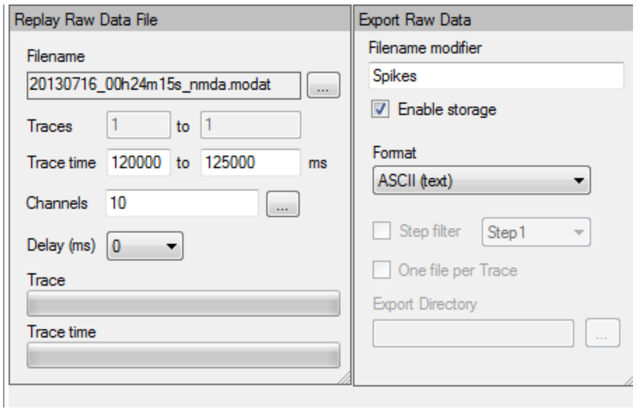
3. Or, modify the templates with Mobius Editor. Select [Workflow] > [Edit] to open the Mobius Editor.

1-5. Exporting raw data

Raw data can be exported using Mobius [Export Raw Data] as either:

1) Binary shorts (2 byte integer values).
2) Binary doubles (8 byte floating point values).
3) ASCII text in “CSV” format.
4) Mobius native data format (.modat).

1. Open an acquired data in analysis workflow including the [Export Raw Data].
2. Check the “Enable storage” checkbox in the [Export Raw Data] module. (File name can be modified in the Filename modifier box.)
3. Select the data format.
4. Select channels, Trace No, trace time for the Saving/Exporting in the [Replay Raw Data] module. Channels shown with green will be enabled.



Replay Raw Data File

Filename: 20130716_00h24m15s_nmda.modat

Traces: 1 to 1

Trace time: 120000 to 125000 ms

Channels: 10

Delay (ms): 0

Trace

Trace time

Export Raw Data

Filename modifier: Spikes

☑ Enable storage

Format: ASCII (text)

☐ Step filter: Step1

☐ One file per Trace

Export Directory

Figure 1-5.1. Exporting raw data. Raw data (waveforms) for channel 10 at 120000-125000 sec will be exported as an “ASCII (text)” file in this example.

5. After setting all parameters, save the analysis workflow as your own (Figure 1-5.2).

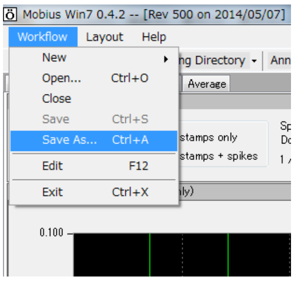
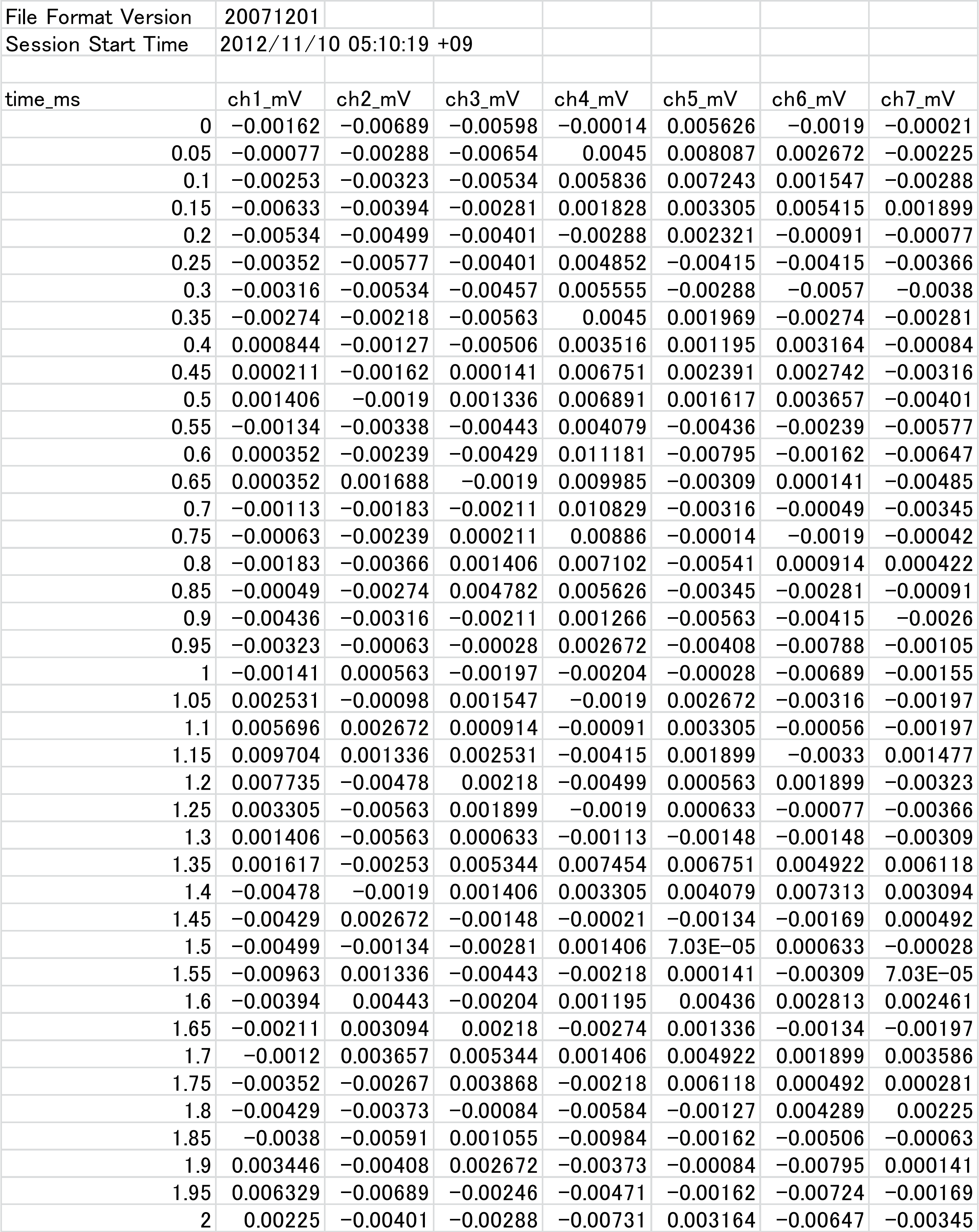


Figure 1-5.2. Saving the analysis workflow as your own.

6. Run the analysis workflow with the Green-Red button. The exported data will be saved in the same folder where the analysis workflow is saved.



File Format Version	20071201						
Session Start Time	2012/11/10 05:10:19 +09						
time_ms	ch1_mV	ch2_mV	ch3_mV	ch4_mV	ch5_mV	ch6_mV	ch7_mV
0	−0.00162	−0.00689	−0.00598	−0.00014	0.005626	−0.0019	−0.00021
0.05	−0.00077	−0.00288	−0.00654	0.0045	0.008087	0.002672	−0.00225
0.1	−0.00253	−0.00323	−0.00534	0.005836	0.007243	0.001547	−0.00288
0.15	−0.00633	−0.00394	−0.00281	0.001828	0.003305	0.005415	0.001899
0.2	−0.00534	−0.00499	−0.00401	−0.00288	0.002321	−0.00091	−0.00077
0.25	−0.00352	−0.00577	−0.00401	0.004852	−0.00415	−0.00415	−0.00366
0.3	−0.00316	−0.00534	−0.00457	0.005555	−0.00288	−0.0057	−0.0038
0.35	−0.00274	−0.00218	−0.00563	0.0045	0.001969	−0.00274	−0.00281
0.4	0.000844	−0.00127	−0.00506	0.003516	0.001195	0.003164	−0.00084
0.45	0.000211	−0.00162	0.000141	0.006751	0.002391	0.002742	−0.00316
0.5	0.001406	−0.0019	0.001336	0.006891	0.001617	0.003657	−0.00401
0.55	−0.00134	−0.00338	−0.00443	0.004079	−0.00436	−0.00239	−0.00577
0.6	0.000352	−0.00239	−0.00429	0.011181	−0.00795	−0.00162	−0.00647
0.65	0.000352	0.001688	−0.0019	0.009985	−0.00309	0.000141	−0.00485
0.7	−0.00113	−0.00183	−0.00211	0.010829	−0.00316	−0.00049	−0.00345
0.75	−0.00063	−0.00239	0.000211	0.00886	−0.00014	−0.0019	−0.00042
0.8	−0.00183	−0.00366	0.001406	0.007102	−0.00541	0.000914	0.000422
0.85	−0.00049	−0.00274	0.004782	0.005626	−0.00345	−0.00281	−0.00091
0.9	−0.00436	−0.00316	−0.00211	0.001266	−0.00563	−0.00415	−0.0026
0.95	−0.00323	−0.00063	−0.00028	0.002672	−0.00408	−0.00788	−0.00105
1	−0.00141	0.000563	−0.00197	−0.00204	−0.00028	−0.00689	−0.00155
1.05	0.002531	−0.00098	0.001547	−0.0019	0.002672	−0.00316	−0.00197
1.1	0.005696	0.002672	0.000914	−0.00091	0.003305	−0.00056	−0.00197
1.15	0.009704	0.001336	0.002531	−0.00415	0.001899	−0.0033	0.001477
1.2	0.007735	−0.00478	0.00218	−0.00499	0.000563	0.001899	−0.00323
1.25	0.003305	−0.00563	0.001899	−0.0019	0.000633	−0.00077	−0.00366
1.3	0.001406	−0.00563	0.000633	−0.00113	−0.00148	−0.00148	−0.00309
1.35	0.001617	−0.00253	0.005344	0.007454	0.006751	0.004922	0.006118
1.4	−0.00478	−0.0019	0.001406	0.003305	0.004079	0.007313	0.003094
1.45	−0.00429	0.002672	−0.00148	−0.00021	−0.00134	−0.00169	0.000492
1.5	−0.00499	−0.00134	−0.00281	0.001406	7.03E−05	0.000633	−0.00028
1.55	−0.00963	0.001336	−0.00443	−0.00218	0.000141	−0.00309	7.03E−05
1.6	−0.00394	0.00443	−0.00204	0.001195	0.00436	0.002813	0.002461
1.65	−0.00211	0.003094	0.00218	−0.00274	0.001336	−0.00134	−0.00197
1.7	−0.0012	0.003657	0.005344	0.001406	0.004922	0.001899	0.003586
1.75	−0.00352	−0.00267	0.003868	−0.00218	0.006118	0.000492	0.000281
1.8	−0.00429	−0.00373	−0.00084	−0.00584	−0.00127	0.004289	0.00225
1.85	−0.0038	−0.00591	0.001055	−0.00984	−0.00162	−0.00506	−0.00063
1.9	0.003446	−0.00408	0.002672	−0.00373	−0.00084	−0.00795	0.000141
1.95	0.006329	−0.00689	−0.00246	−0.00471	−0.00162	−0.00724	−0.00169
2	0.00225	−0.00401	−0.00288	−0.00731	0.003164	−0.00647	−0.00345

Figure 1-5.3. example of raw data exported with ASCII displayed in Excel.

Chapter 2 fEPSPs Recording

This chapter describes how to record and analyze fEPSPs using Mobius workflow templates. In this chapter, you will learn about

1. Recording fEPSPs from hippocampal acute slices.
2. Long Term Potentiation (LTP).
3. Drug testing.
4. Replay and analysis of the acquired data.
5. Dat output.

2-1. Recording of fEPSPs

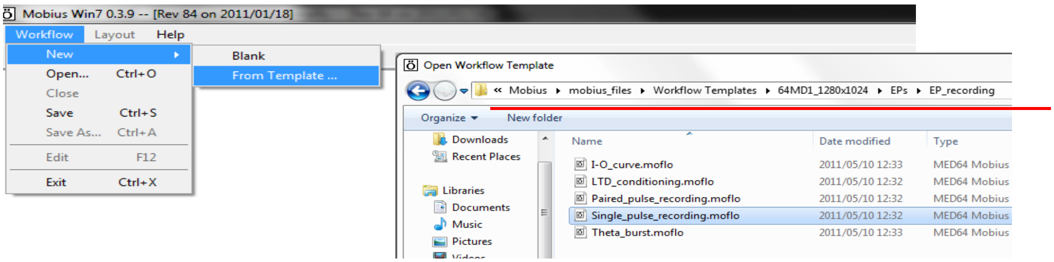
The goal of this section is that you be able to record fEPSP signals while measuring their amplitude and slope using *"Single_ pulse_recording"* workflow template.

Opening the "*Single_pulse_recording*" workflow template.

1. Click [Workflow] > [New] >[From Template]. Select either of [64MD1_1280x1024] or [64MD1_1920x1080] folder, depending on the size of your display monitor.

2. Select [EPs] > [EP_recording] folders. Select the *"Single_pulse_recording"* workflow template.

NOTICE:
In order to open the recording workflows, connect your PC to the MED64 amplifiers and turn on both amplifiers.



Name	Date modified	Type
I-O_curve.moflo	2011/05/10 12:33	MED64 Mobius
LTD_conditioning.moflo	2011/05/10 12:32	MED64 Mobius
Paired_pulse_recording.moflo	2011/05/10 12:32	MED64 Mobius
Single_pulse_recording.moflo	2011/05/10 12:32	MED64 Mobius
Theta_burst.moflo	2011/05/10 12:33	MED64 Mobius

Figure 2-1.1. Opening the workflow template.

2-1.1. Overview of the *“Single_ pulse_recording”* workflow template

The *"Single_ pulse_recording"* workflow template consists of the following modules located in the “Main” and “EPSP measures” Tabs.

Tab	Modules
Main	Acquire MED64R2 Data w/Stim / Display All Channels / Export Raw Data
EPSP measures	Extract EP Measures (2) / Save Meausres Data (2)

Evoked signals are acquired with the [Acquire MED64R2 Data w/Stim] module. The acquired signals are sent to the [Extract EP Measures] module, where several types of amplitude, slope, and time are measured and graphed. Raw data can be exported to binary or ASCII ("CSV" formatted text file).The measurement chart can be saved as a "CSV" formatted text file.

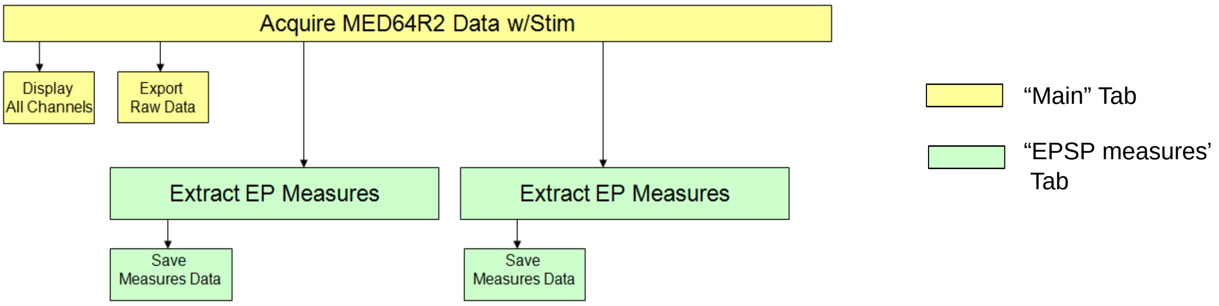


Figure 2-1.2. Module configuration for the *“Single_pulse_recording”* workflow template.

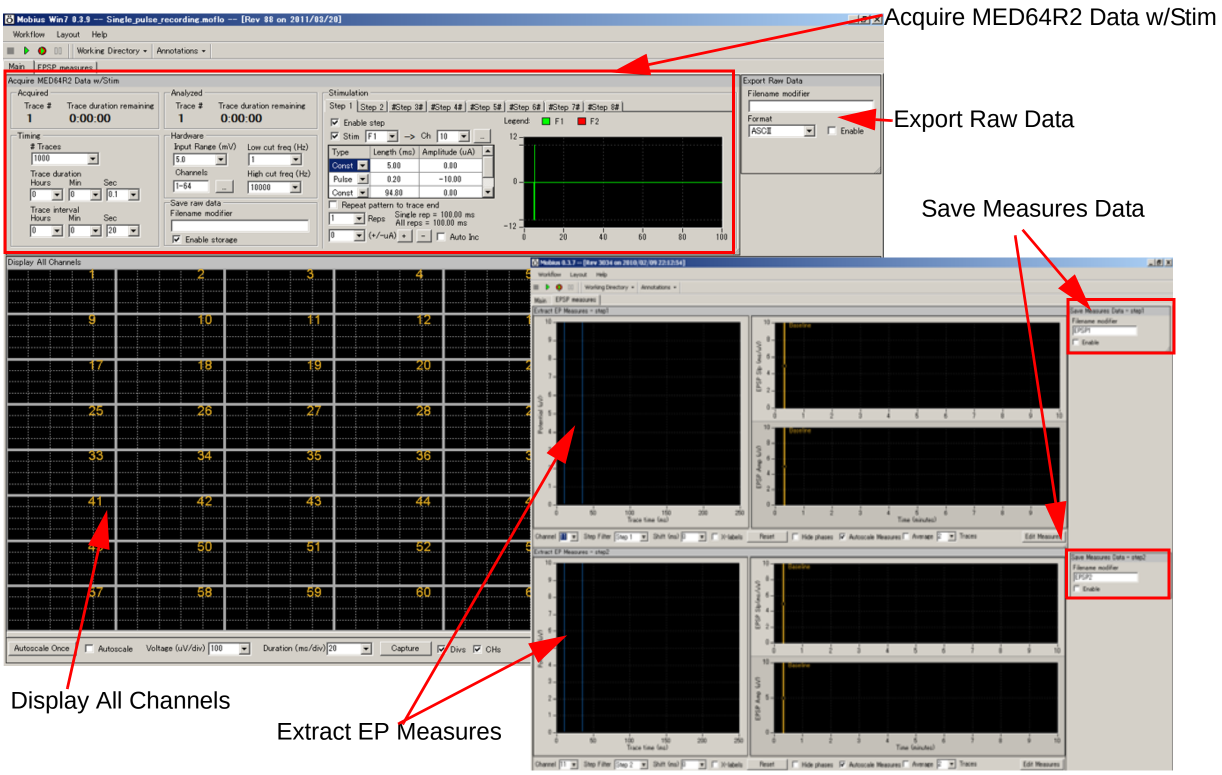
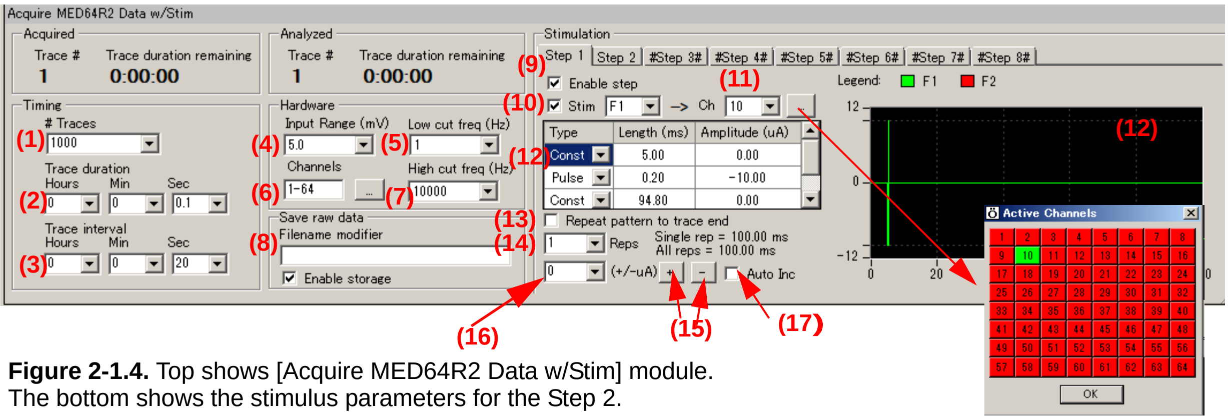


Figure 2-1.3. *“Single_pulse_recording*” workflow template. “Main” tab (left), “EPSP measures” tab (right).

2-1.2. Overview of the [Acquire MED64R2 Data w/Stim] module

The acquisition and stimulation parameters are set in this module.

NOTICE:
Connect your PC to the MED64 amplifiers and turn on both amplifiers to open this module.



Acquire MED64R2 Data w/Stim

Acquired: Trace # 1, Trace duration remaining 0:00:00

Analyzed: Trace # 1, Trace duration remaining 0:00:00

Timing: # Traces 1000; Trace duration Hours 0, Min 0, Sec 0.1; Trace interval Hours 0, Min 0, Sec 20

Hardware: Input Range (mV) 5.0; Low cut freq (Hz) 1; Channels 1-64; High cut freq (Hz) 10000

Save raw data: Filename modifier; ☑ Enable storage

Stimulation: Step 1 | Step 2 | #Step 3# | #Step 4# | #Step 5# | #Step 6# | #Step 7# | #Step 8#

☑ Enable step

☑ Stim F1 -> Ch 10

Legend: 🟩 F1 🟥 F2

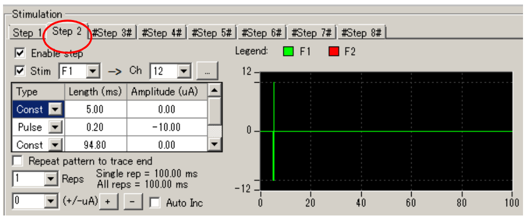
Type	Length (ms)	Amplitude (uA)
Const	5.00	0.00
Pulse	0.20	-10.00
Const	94.80	0.00

☐ Repeat pattern to trace end

1 Reps Single rep = 100.00 ms All reps = 100.00 ms

0 (+/-uA) + - ☐ Auto Inc

Figure 2-1.4. Top shows [Acquire MED64R2 Data w/Stim] module. The bottom shows the stimulus parameters for the Step 2.



Stimulation: Step 1 | Step 2 | #Step 3# | #Step 4# | #Step 5# | #Step 6# | #Step 7# | #Step 8#

☑ Enable step

☑ Stim F1 -> Ch 12

Legend: 🟩 F1 🟥 F2

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

☐ Repeat pattern to trace end

1 Reps Single rep = 100.00 ms All reps = 100.00 ms

0 (+/-uA) + - ☐ Auto Inc

Acquisition

1. # Traces: Number of Traces (Sweeps).

2. Trace duration: Sampling (Recording) time for each trace.

3. Trace interval:
Interval between traces. (From the beginning of the first trace to the beginning of the next trace)

4. Input Range (mV):
Maximum input signals level. This setting should be adjusted according to the signal amplitude to achieve the maximal dynamic range. Select 2.3 mV, 2.9 mV, 5.0 mV, 12.5 mV, or 25 mV.
5 mV is recommended for recording of fEPSPs.

5. Low cut freq (Hz):
High pass filter. Select 0.1 Hz, 1.0 Hz, 10 Hz, or 100 Hz. If 1.0 Hz is selected, signals with frequencies lower than 1 Hz are filtered out. Usually, 1.0 Hz is recommended for recording of fEPSP.

6. Channels:
Select the channels for recording here. When the square box is clicked, the channel selector pops up. The enabled recording channels are colored green.

7. High cut freq (Hz):
Low pass filter. Select 1000 Hz, 2500 Hz, 5000 Hz, 7500 Hz, or 10000 Hz. If 5000Hz is selected, signals with frequencies higher than 5000 Hz are filter out. Usually, 10000 Hz is good enough for recording of fEPSP. Values lower than 2500 Hz is NOT recommended for recording of fEPSPs.

8. Save raw data:
When Mobius is started by clicking the Green-Red recording button while the [Enable storage] is checked, the raw data is saved. Enter a descriptive file name of your choice.

Stimulation

Each Step is independent and can be programmed with different types of stimulation protocols. Enabled steps will be applied in order (1 to 8) at the intervals set in the Trace Interval.

9. “Enable step” check box:
   When the box to the left of [Enable step] is checked, the Step becomes valid. The default settings have Step1 and Step 2 enabled, and Steps 3-8 disabled (shown with # next to each Step). Step1 and Step 2 are applied alternately at 20 second intervals.

10. Stimulator selector and check box:
    Select the MED64 amplifier’s built-in stimulator (F1 or F2) here. Checking the box on the right of “Stim” activates the selected stimulator. The default settings have F1 enabled both for Step 1 and Step 2. Stimulation is applied alternately at 20 second intervals.

    F1 and F2 can be enabled at the same Step to stimulate 2 channels simultaneously.

11. Stimulus channel selector:
    Select stimulus channel here. The stimulus channel can be changed either by the drop-down or the pop-up which appears by clicking the square box. The green shows the stimulus channel.

    Mobius needs to be stopped (with black button) or paused (with yellow button) for the stimulus channel to be changed.

    - Stimulus channel can be changed without stopping or pausing by using the pop-up stimulus channel selector. However, the changes are activated on the 2nd trace after the new channel is selected by software since Mobius uses the next trace to reset itself.

12. Stimulus waveform editor:
    Make your stimulus protocol here by selecting either [Const] or [Pulse] and typing in the desired Length and Amplitude. Refer to Figure 2-1.5 for a schematic representation of a stimulus protocol example.

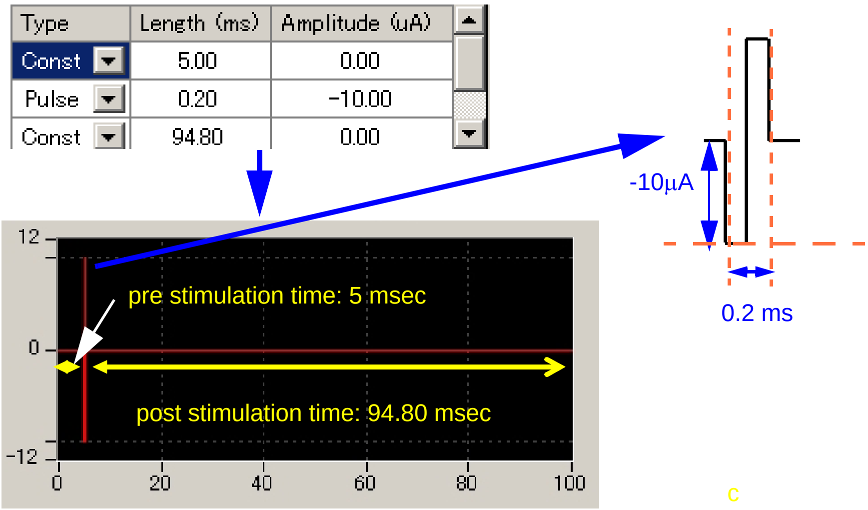
    - Make sure that the STIMULUS CURRENT selection on the MED64 Head amplifier (MED-A64HE1) is set to [NORMAL] to deliver the stimulus current amplitude set in Mobius. Selecting [x2] doubles the output stimulus current amplitude set in Mobius.

      (e.g. When 10 µA is selected by Mobius with [x2] selected, the output stimulus current is 20 µA. When -10 µA is set in the Mobius with NORMAL selected, the output stimulus current amplitude is 10 µA)

    - The maximum stimulus amplitude set by Mobius is 100 µA. Set the STIMULUS CURRENT selector for x2 when stimulus amplitude with greater than 100 µA is applied.

    - The maximum number of stimulus pulses to be programmed here at a Step is **21**.

    - Do NOT select [Ramp] for the stimulation with the MED64. Only bi-phasic stimulation is recommended.



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

Figure 2-1.5. Example of a stimulus protocol and itsschematic diagram.

13. Repeat pattern to trace end check box:
    When this box is checked, the stimulus protocol programed at the Stimulus waveform editor (12) will be repeated to the end of the trace. Useful for LTD conditioning.

14. Reps selector:
    The stimulus protocol set at the “Stimulus waveform editor” (12) will be repeated the number of times set here. Useful for multiple pulse stimulation such as LTP conditioning.
    - Repeat of the stimulus waveforms which durations are longer than 1 minute is not recommended.

15. Stimulus current amplitude increment button:
    When the + button is clicked, the absolute value of the stimulus current amplitude for the [Pulse] is incremented by the value set it the left box. (16*) For example, if 2 is set in the box (16) and the + button is clicked once, the stimulus amplitude will be changed to -12.00 µA from -10.00 µA. This is useful for changing the stimulus amplitude for multiple pulse stimulations (e.g. Theta burst) with just one click. The absolute value of the stimulus amplitude can be decreased sequentially using the same procedure when - button is clicked.

    - The stimulus current amplitude can be changed even during recording by using this button. However, the changes are activated on the 2nd trace after the change since Mobius uses the next trace to reset itself.

16. See #15

17. Auto Inc check box:
    When this box is checked, the stimulus current amplitude will automatically increase adding the value (in µA) set in the left box (16) to each preceding stimulation pulse. This is useful for making I/O curves. Please refer to section 2-2.3.Input/Output curve (page 35) for details.

In the default setting, Step1 (F1) and Step 2(F1) run alternating every 20 seconds in this workflow.

2-1.3. Running workflow

The workflow is run and stopped (paused) with the execution buttons.

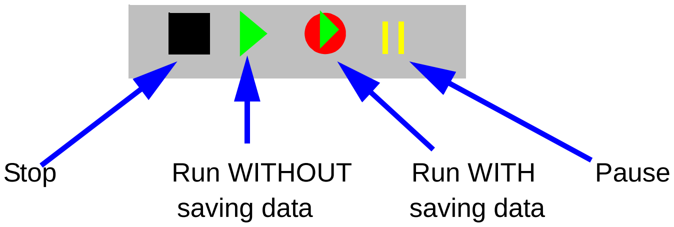
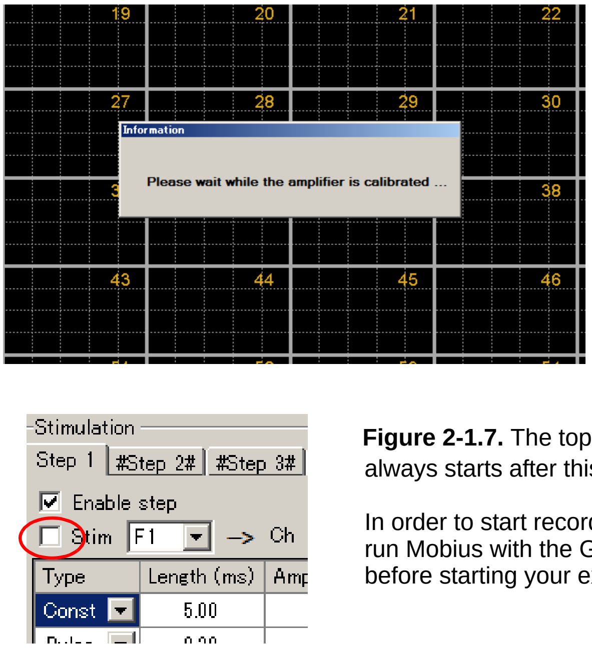


Figure 2-1.6. Execution buttons.

2-1.4. Before starting your experiment

When a recording workflow is run with Green or Green-Red button for the first time after it is opened (or made), Mobius needs several seconds for its calibration (as seen in the pop-up message). Acquisition starts automatically after the calibration.

If you would like to start acquisition immediately after clicking the Green-Red button, run the workflow with the green button for a few traces with both stimulators un-enabled, and then stop before starting your experiment. When the workflow is run the next time, Mobius does not require calibration but acquisition will start immediately.



Information

Please wait while the amplifier is calibrated ...

☑ Enable step

☐ Stim F1 –> Ch

Type	Length (ms)	Amp
Const	5.00	

Figure 2-1.7. The top shows pop-up mesage for the calibration. Acquisition always starts after this message disappears.

In order to start recording immediately after clicking the Green-Red button, run Mobius with the Green button for a few traces with stimulator un-enabled before starting your experimen. (bottom figure.)

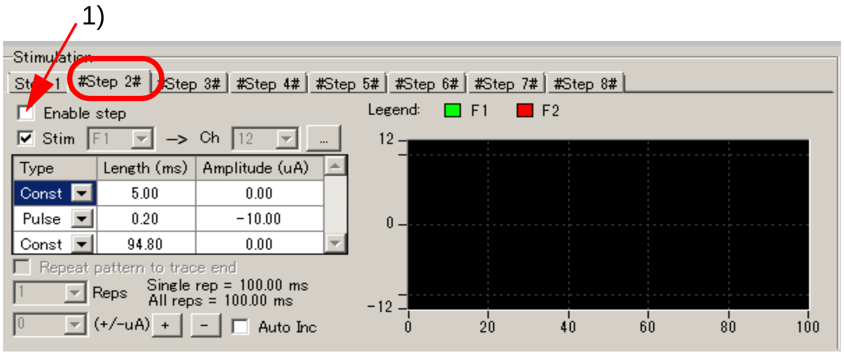
2-1.5. Recording fEPSPs with single pulse stimulation

Now you will set the parameters for single pulse stimulation at a single channel and record fEPSPs.

1. [Step1] and [Step2] are enabled in the *"Single_pulse_recording"* workflow template. Disable [Step2] so that stimulation is applied only through the channel selected in the [Step1].

    1) Click the [Step2] tab and uncheck [Step enabled].

    2) You will see that the # mark appears next to [Step2], indicating that this Step is disabled. (Refer to Figure 2-1.8)



☐ Enable step

☑ Stim F1 –> Ch 12

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

☐ Repeat pattern to trace end

1 Reps Single rep = 100.00 ms All reps = 100.00 ms

0 (+/-uA) + - ☐ Auto Inc

Figure 2-1.8. Disabling [Step2].

2. Click on the tab for [Step1] so that parameters for the Step1 appear.

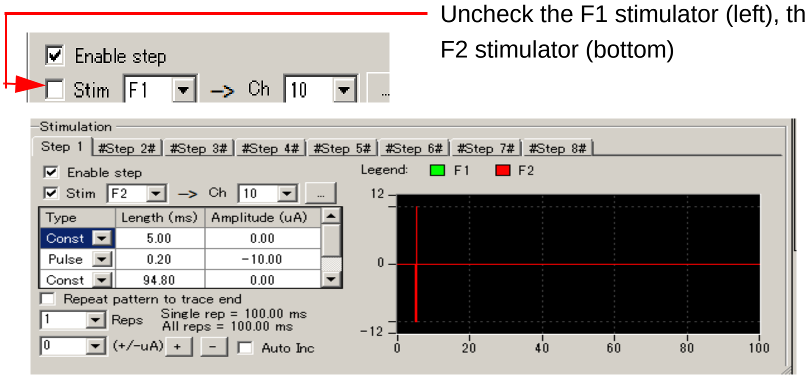
How do I change the stimulator at a given Step?

F1 is enabled for Step 1 in the default settings. Unless stimulation is applied through 2 channels simultaneously, F1 can be used for all Steps. However, if you would like to use another stimulator (for example, F2 instead of F1, change the stimulator following the procedure below:

1) Uncheck [Stimulator Enabled] for F1 by clicking the check box.

2) Select F2 at the stimulator selector and check [Stim] for F2. Now F2 stimulator is enabled instead of F1 (Figure 2-1.9)

- Check both F1 and F2 to stimulate through 2 channels simultaneously.



Uncheck the F1 stimulator (left), then check F2 stimulator (bottom)

☑ Enable step

☐ Stim F1 –> Ch 10

☑ Enable step

☑ Stim F2 –> Ch 10

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

☐ Repeat pattern to trace end

1 Reps Single rep = 100.00 ms All reps = 100.00 ms

0 (+/-uA) + - ☐ Auto Inc

Figure 2-1.9. Changing the stimulator to F2 from F1.

3. Make sure that the STIMULUS CURRENT selector on the MED-A64HE1 amplifier is set to [NORMAL].

    - Selecting of [x2] doubles the output stimulus current.
      (e.g. when -10 μA is selected in the Mobius with x2 selected, the output stimulus current is -20 μA.)

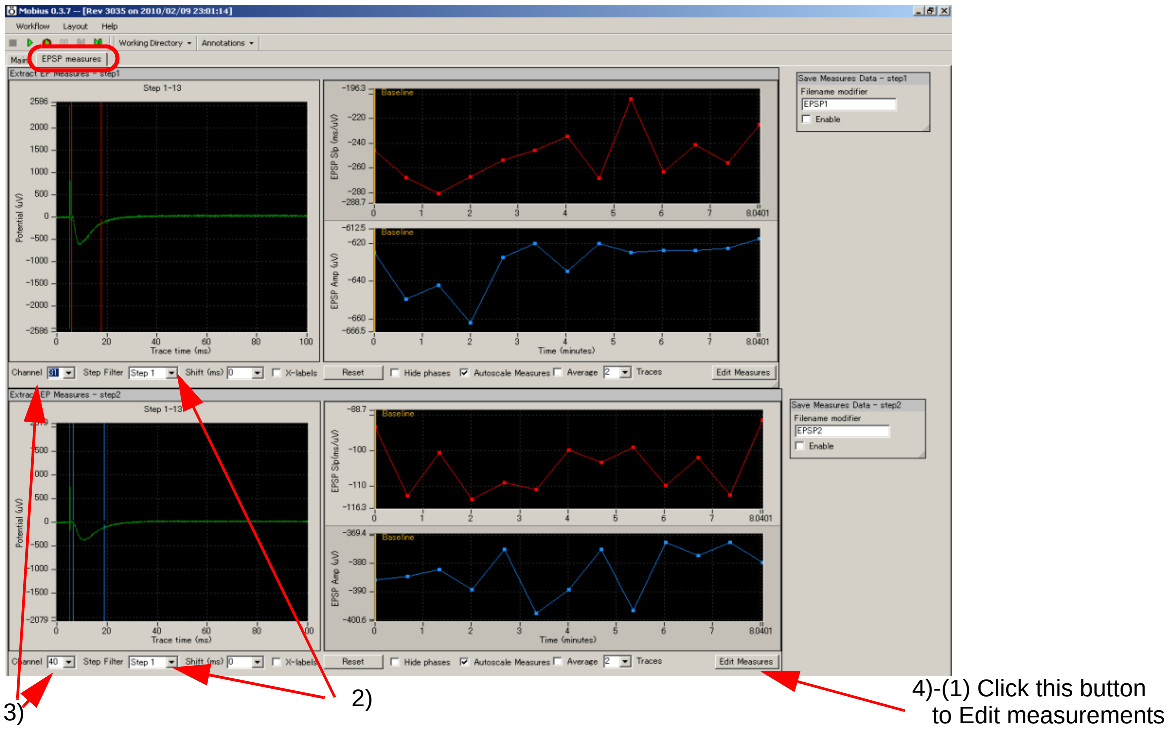
4. Start Mobius with the Green button (Run WITHOUT saving data). Find the best channel for stimulation by looking at the 64 channel display and changing the stimulation channel. You can also change the amplitude during this procedure for optimal stimulation.

    - Mobius needs to be stopped or paused to change stimulus channels or/and amplitude.

    - The stimulus channels and/or amplitude can be changed without stop or pause by using the “stimulus channel selector” and/or “the stimulus current amplitude increment button.” However, the changes are activated on the second traces after new channel/amplitude is set. (Mobius uses the next trace to reset itself.)

5. When you find the optimal stimulation channel and amplitude, set the protocol for on-line analysis as follows: (Refer to Figure 2-1.10)

    1) Open the "EPSP measures” tab.
    2) Set the [Step Filter] to Step1 for both measures.
    3) Select the channels you want to monitor analysis on-line during your recording.
        - Channels can be changed even during recording (or running) by scrolling or typing the numbers directly.



4)-(1) Click this button
to Edit measurements

Figure 2.1-10. fEPSP measurements.

4) The default settings have "Slope 1040 LinerFit" (red cursor) and "Amplitude Minimum" (blue cursor) selected for measurement. Change the measurement menu if necessary. (Refer to Figure 2-1.11)

- Mobius must be stopped to change the measurements.
- Analysis can be done later off-line.

(1) Click [Edit Measures], and then select the measurement (analysis) you want at the [Measure Type] column. (The name can be customized by typing it directly into the [Custom Name] column.)

  - Please click [Measures Help] for detailed explanations for the measurements.

(2) Click [Add Measures] to perform more analysis.

(3) To delete a measurement, right click the custom name and select the [Delete].

(4) Close this editor by clicking the [Hide] button.

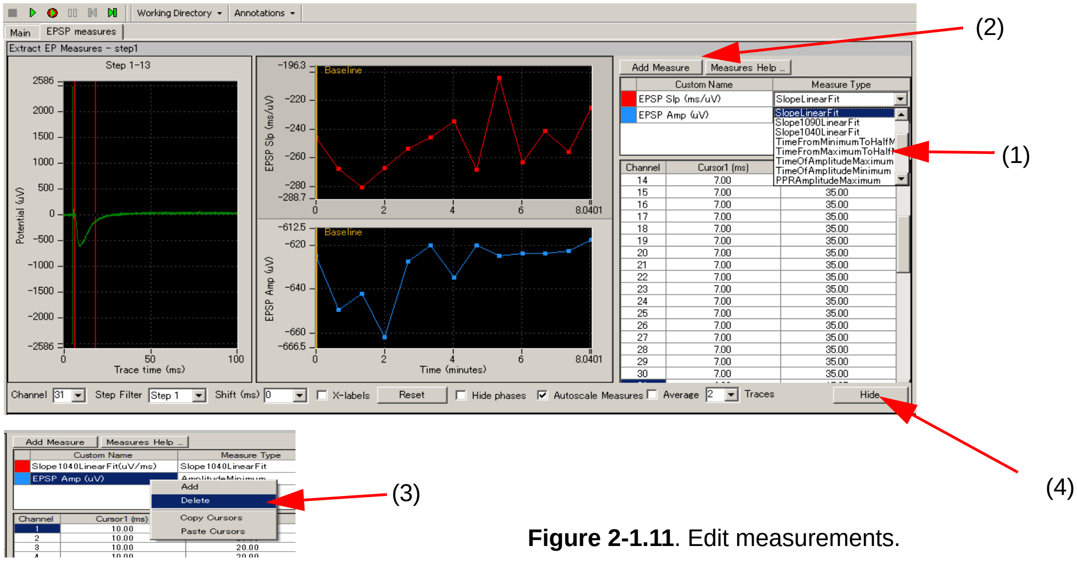


Figure 2-1.11. Edit measurements.

5) Set the cursors by clicking on them and dragging them to the desired position.

- For detailed information on analysis, please refer to section2-4.3. Analysis of fEPSP signals on page 45.

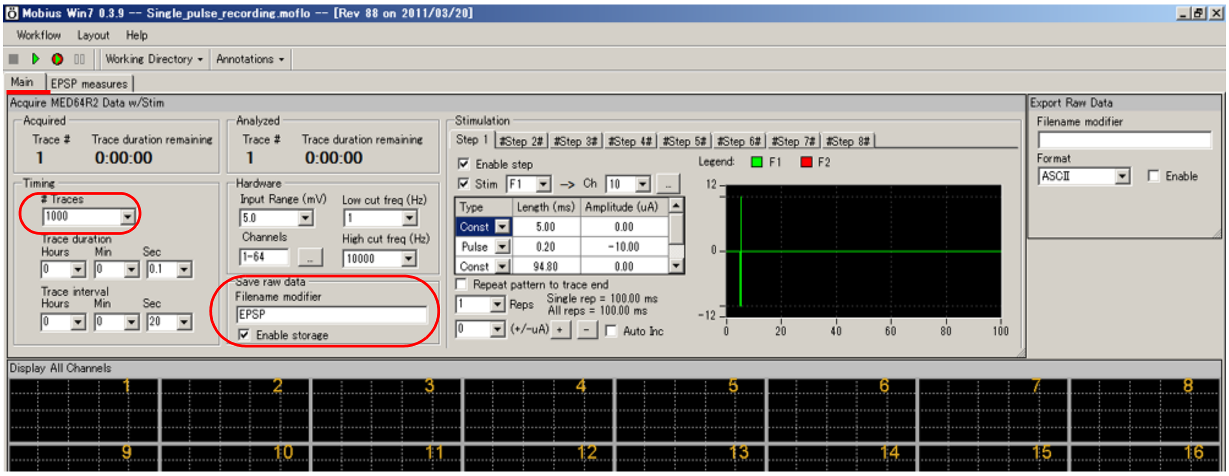
How do I adjust the graph scale to zoom in on the waveforms?

1) Left-clicking and dragging while pressing the shift button.

2) Changing the maximum (and/or minimum) numbers for both X (time) and Y (amplitude) axis. Click, then, type in the new value(s) for the maximum and/or minimum.

- Double-clicking anywhere on the chart to return to the original axis range.

6. Make sure that [Enable storage] is checked in the [Save Raw Data] (“Main” tab) to save the data. (The file name can be modified.) Set the # of traces.



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Acquire MED64R2 Data w/Stim

Acquired: Trace # 1, Trace duration remaining 0:00:00

Analyzed: Trace # 1, Trace duration remaining 0:00:00

Timing: # Traces 1000

Trace duration: Hours 0, Min 0, Sec 0.1

Trace interval: Hours 0, Min 0, Sec 20

Hardware: Input Range (mV) 5.0, Low cut freq (Hz) 1, Channels 1-64, High cut freq (Hz) 10000

Save raw data: Filename modifier EPSP

☑ Enable storage

Stimulation: Step 1

☑ Enable step

☑ Stim F1 -> Ch 10

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

☐ Repeat pattern to trace end

1 Reps, Single rep = 100.00 ms, All reps = 100.00 ms

0 (+/-uA) ☐ Auto Inc

Export Raw Data: Filename modifier (blank), Format ASCII, ☐ Enable

Figure 2-1.12. Workflow for single pulse recording stimulating at 1 channel. Only the F1 stimulator is enabled for [Step1]. [Step2] is diabled.

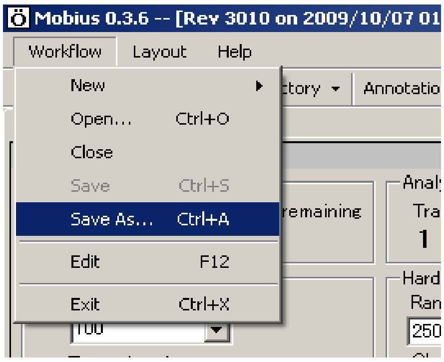


Figure 2-1.13.

7. Save this workflow as your Recording Workflow by clicking [Workflow] > [Save As]. (Figure 2-1.13)

8. Start Mobius with the GREEN-RED button.

   - Mobius will require you to save the workflow as your own if you try to start Mobius with the Green-Red button without having saved it already.

   - The data file (.modat file) and analysis workflow including all analysis modules selected for the recording workflow (.moflo file) and [Replay Raw Data] module will be saved in the same folder.

   (Refer to Figure 1-4.10 on page 17 for files generated after recording.)

2-2. Long Term Potentiation (LTP)

This section describes how to perform an LTP experiment. This method involves recording baseline activity and LTP with one workflow, whereas conditioning stimuli is applied with another. The following procedures are detailed in this chapter.

1. Selecting the stimulation channels for experimental and control pathways.
2. Identifying the appropriate stimulus current amplitude for both pathways with an I/O curve.
3. Making the LTP workflow.
4. Making Theta burst workflow.
5. Recording LTP.

1. Selecting the stimulation channels

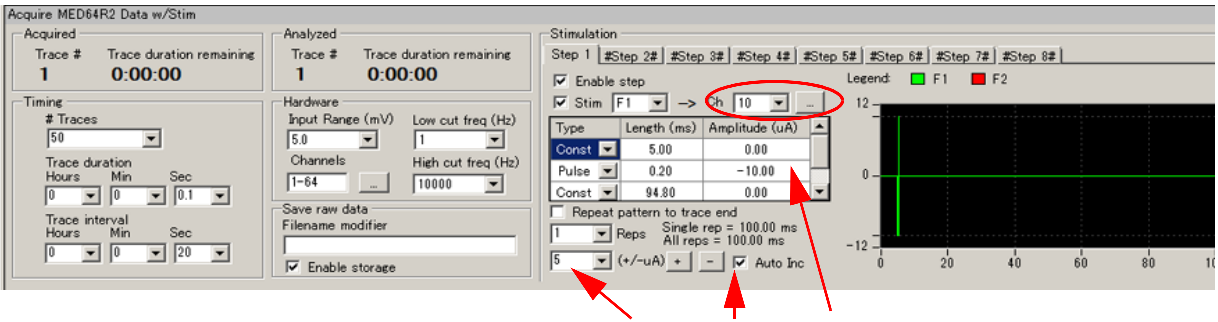
Open the *"Single_pulse_recording"* workflow template. Determine the 2 different stimulation channels for experimental pathway and control pathways, and the desired channel for on-line monitoring by running the workflow without recording using the Green button. (Please refer to section 2-1.5. Recording of fEPSPs" on pages 29-32)

2. Input/Output (I/O) curve

This procedure is carried out to determine the appropriate stimulus current amplitude for recording fEPSPs. 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. For LTP recording, the appropriate stimulus amplitudes need to be identified with I/O curves for the control and experimental pathways.

1. Open the *"IO_curve"* workflow template. In the default setting, (1)-10 µA is selected for the initial stimulus current amplitude, (2) the [Auto Inc] button is checked, and (3)5 µA is set for the increment value. The current amplitude will increase automatically by 5 µA in subsequent trace until the end of the recording (e.g. 10, 15, 20------. 105 µA).

   Modify the stimulus current amplitude, length, and increments depending on your signals and slice viability. Change the stimulus channel to that identified for experimental pathway (or control pathway).



Acquire MED64R2 Data w/Stim

Acquired
Trace # 1
Trace duration remaining 0:00:00

Analyzed
Trace # 1
Trace duration remaining 0:00:00

Timing
Traces 50
Trace duration Hours 0 Min 0 Sec 0.1
Trace interval Hours 0 Min 0 Sec 20

Hardware
Input Range (mV) 5.0
Low cut freq (Hz) 1
Channels 1-64
High cut freq (Hz) 10000

Save raw data
Filename modifier
☑ Enable storage

Stimulation
Step 1
☑ Enable step
☑ Stim F1 -> Ch 10

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

☐ Repeat pattern to trace end
1 Reps
Single rep = 100.00 ms
All reps = 100.00 ms
5 (+/-uA)
☑ Auto Inc

Figure 2-2.1. *"IO_curve"* workflow template.

2. Open the "EPSP measures" tab. Change the measurement to analyze if necessary, and then set the cursors. (Refer to section 2-4.3. Analysis of fEPSP signals on page 45) Change the channels to those you selected for on-line monitoring.

3. Save the workflow as your custom I/O curve workflow. Click the Green-Red button to start the recording. (Click on the Green button if you would not like to save data).

4. 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.

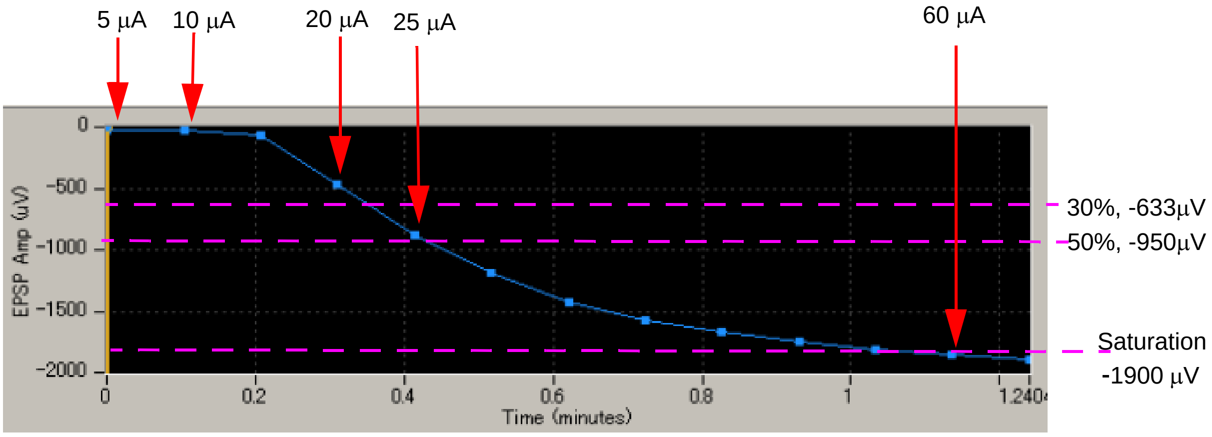


Figure 2-2.2. Determination of the stimulus current amplitude eliciting 30% and 50% of the maximal response.

5. Determine the stimulus amplitude for the experimental and control pathways.
   - You can do test both pathways simultaneously if Step2 is enabled. In this case, stimulus amplitude increases by 5 µA independently for each Step.

6. After you identify the stimulus current for both pathways, close this workflow.

3. Making an LTP workflow

In this section, you will learn how to make your LTP workflow using the *"Single_pulse_recording"* workflow template. Both baseline and LTP will be recorded with this workflow. After recording of the baseline, you will apply theta burst with another workflow, and then come back to this LTP workflow for LTP recording.

1. Open the *"Single_pulse_recording"* workflow template, and modify the experimental parameters. Keep in mind the following important points:

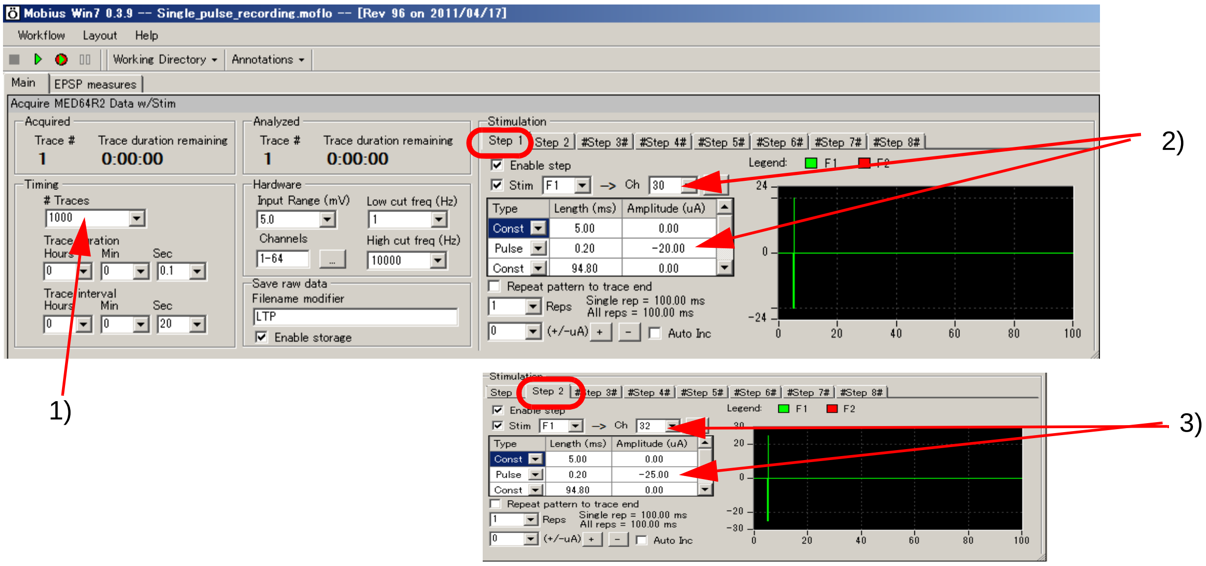
   1) # of traces: set the number so that both the Baseline and LTP recordings will run with this workflow. For example, inputting 300 for the # of traces and 20 seconds for the trace interval will result in a recording 100 minutes long (20 sec x 300 times).

   2) Step 1 stimulation: change the stimulus channel to that selected for the experimental pathway, and amplitude to that identified by the I/O curve for the experimental pathway. In Figure 2-2.3, the stimulus amplitude was changed to -20 µA and stimulus channel was changed to 30ch.
      - Make sure that F2 stimulator is NOT enabled in Step1.

   3) Step 2 stimulation: change the stimulus channel to that selected for the control pathway, and amplitude to that identified by the I/O curve for the control pathway. In the example, the stimulus amplitude was changed to -25 µA and stimulus channel was changed to 32ch. Make sure that F2 stimulator is NOT enabled in Step 2.

4) Make sure Steps 3-8 are NOT enabled.

- In this example, Step1 is selected for the experimental pathway through which the theta burst will be applied after recording of the baseline. Step2 is selected for the control pathway.



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Acquire MED64R2 Data w/Stim

Acquired: Trace # 1; Trace duration remaining 0:00:00

Analyzed: Trace # 1; Trace duration remaining 0:00:00

Timing: # Traces 1000; Trace duration Hours 0, Min 0, Sec 0.1; Trace interval Hours 0, Min 0, Sec 20

Hardware: Input Range (mV) 5.0; Low cut freq (Hz) 1; Channels 1-64; High cut freq (Hz) 10000

Save raw data: Filename modifier LTP; ☑ Enable storage

Stimulation — Step 1: ☑ Enable step; ☑ Stim F1 -> Ch 30

Type	Length (ms)	Amplitude (uA)
Const	5.00	0.00
Pulse	0.20	-20.00
Const	94.80	0.00

☐ Repeat pattern to trace end; 1 Reps; Single rep = 100.00 ms; All reps = 100.00 ms; 0 (+/-uA); ☐ Auto Inc

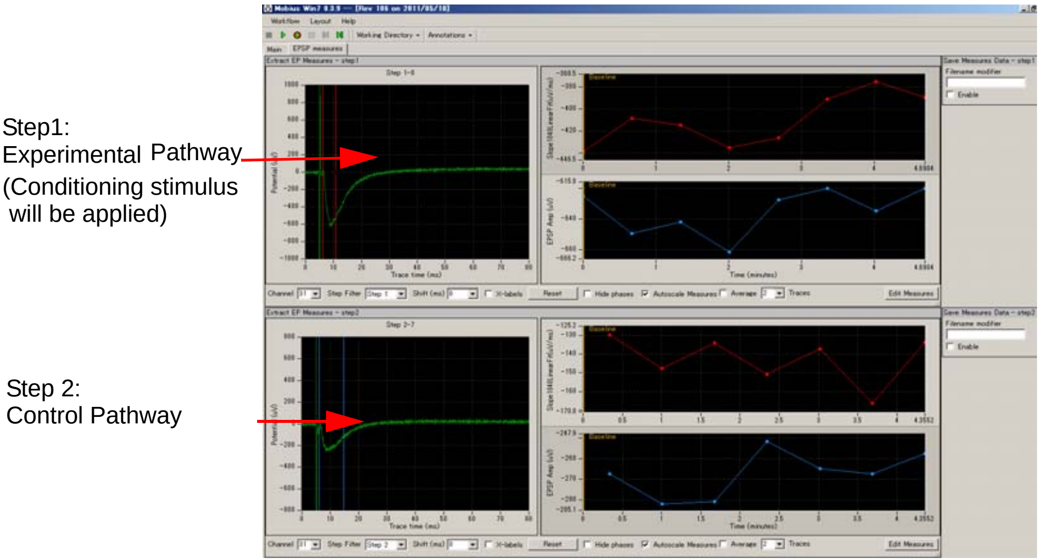
Stimulation — Step 2: ☑ Enable step; ☑ Stim F1 -> Ch 32

Type	Length (ms)	Amplitude (uA)
Const	5.00	0.00
Pulse	0.20	-25.00
Const	94.80	0.00

☐ Repeat pattern to trace end; 1 Reps; Single rep = 100.00 ms; All reps = 100.00 ms; 0 (+/-uA); ☐ Auto Inc

Figure 2-2.3. Recording parameters for LTP recording.

2. Open the EPSP measures tab and modify the analysis parameters.
   1) Change the channel to that identified for on-line monitoring in the previous step.
   2) Change the measurements (analysis) as necessary.
      - The default settings enable Slope1040 Liner Fit and Amplitude Minimum.
   3) Run a few traces with the Green button. Once waveforms appear, set the cursors for the measurements.
      - Refer to section 2-4.5. Analysis of fEPSP signals on page 45 for detail.



Step1:
Experimental Pathway
(Conditioning stimulus
will be applied)

Step 2:
Control Pathway

Figure 2-2.4. Running the LTP workflow.

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

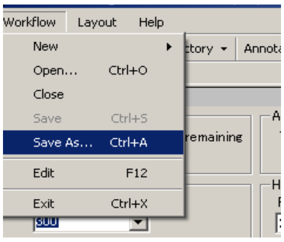
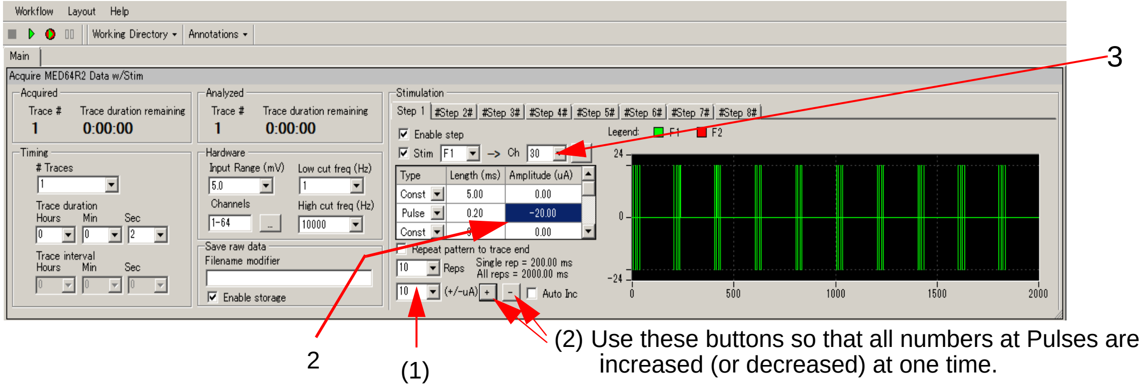


Figure 2-2.4. Saving the LTP workflow.

4. Making a workflow for LTP conditioning stimulus (e.g. Theta burst)

In an LTP experiment, you will need to apply a conditioning stimulus such as a theta burst to the experimental pathway 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 MED64 PC. In this section, you will make a workflow for the theta burst stimulation using the workflow template.

1. Open the *“Theta_burst”* workflow template.
2. Change the stimulus amplitude at the [Pulse] to the value determined for the experimental pathway.
    - Use the +/- button to change the amplitude. For example, in order to change to -20 µA from -10 µA, select 10 µA (Figure 2-2.5, (1)), then click the + button once (Figure 2-2.5, (2))so that amplitudes for all 4 "pulses" are changed to -20 µA. 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 experimental pathway. (Same to that selected for Step1 at your LTP workflow).
4. Make sure steps 2-8 are NOT enabled
5. Make sure F2 stimulator is NOT enabled at Step1.
6. Save as your own Theta Burst workflow



Acquired: Trace # 1, Trace duration remaining 0:00:00

Analyzed: Trace # 1, Trace duration remaining 0:00:00

Timing: # Traces 1; Trace duration Hours 0, Min 0, Sec 2; Trace interval Hours 0, Min 0, Sec 0

Hardware: Input Range (mV) 5.0; Low cut freq (Hz) 1; Channels 1-64; High cut freq (Hz) 10000

Save raw data: Filename modifier; ☑ Enable storage

☑ Enable step

☑ Stim F1 -> Ch 30

Type	Length (ms)	Amplitude (uA)
Const	5.00	0.00
Pulse	0.20	-20.00
Const		0.00

☐ Repeat pattern to trace end

10 Reps; Single rep = 200.00 ms; All reps = 2000.00 ms

10 (+/-uA) + - ☐ Auto Inc

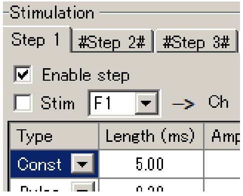
(2) Use these buttons so that all numbers at Pulses are increased (or decreased) at one time.

Figure 2-2.5. Example of a theta burst conditioning stimulus protocol.

7. Uncheck the Stim box for F1, 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 prevent you from applying conditioning stimuli quickly.

     It is recommended to run this workflow with the stimulator disabled (with the “Stim” check box unchecked, Figure 2-2.6) before you start your experiment so that conditioning stimuli is applied right after the baseline recording.



☑ Enable step

☐ Stim F1 --> Ch

Const 5.00

Figure 2-2.6. Disable the stimulator.

8. Check the Stim box for F1 so that the F1 stimulator is enabled again. Leave it open on your MED64 PC.

5. 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-A64HE1 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.

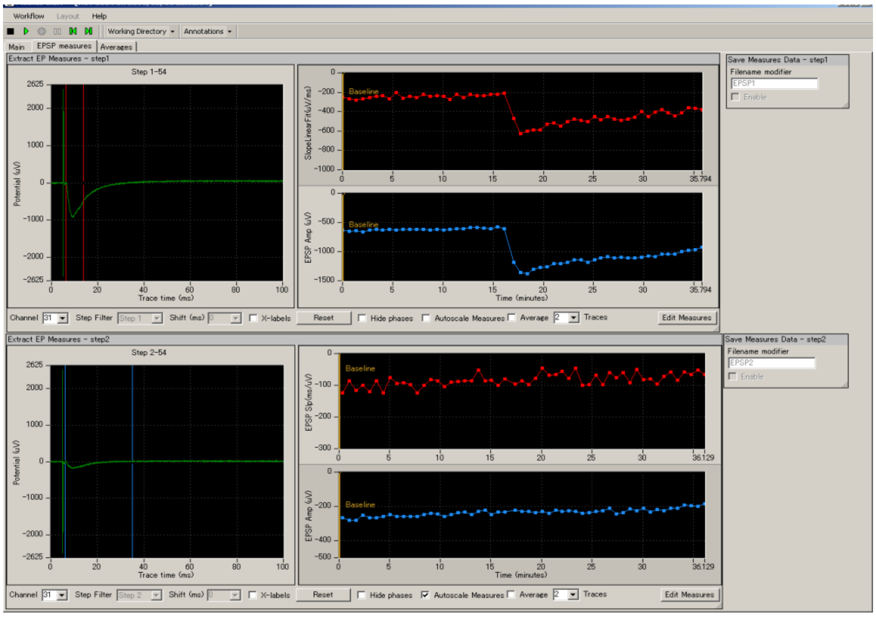


Figure 2-2.7. LTP experiment.

2-3. Drug testing

Mobius' [Compute Measure Averages] module calculates averages and standard deviations for the measurements (waveform analysis) in all experimental phases. This is a useful tool for making a dose-response curve or comparing the results from different phases during drug application. This section will show you how to make a dose-response curve using this module.

Since this module is at the end of the analysis chain, it can slow down the data processing and causes Mobius to stop during your experiment if you perform these analyses online. Thus, it is recommended not to use this module during recording unless you are very familiar with your experiment and preparations. All analyses introduced in this section can be performed later off-line.

One alternative is just making bars (phase-annotations) at the beginning of phases (e.g. drug1) without on-line analysis. The location of the phase bars are saved to the analysis workflow. To do this, skip steps 1-3 and start with step 4.

1. Open the *"Single_pulse_recording"* workflow template.

2. Add modules [Compute Measure Averages] and [Save Measure Averages] to this workflow template.
   1) Make a new tab. Click [Layout] > [Add Tab], then type in the name for the new tab. (Figure 2-3.1)
   2) Open the Editor in the new tab. (Click [Workflow] > [Edit]) (Figure 2-3.2)

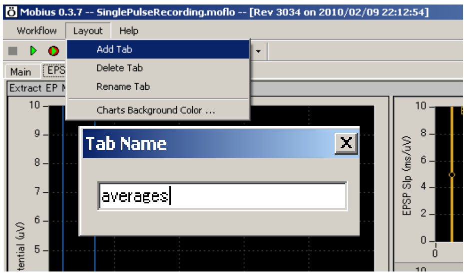


Figure 2-3.1. Making a new tab.

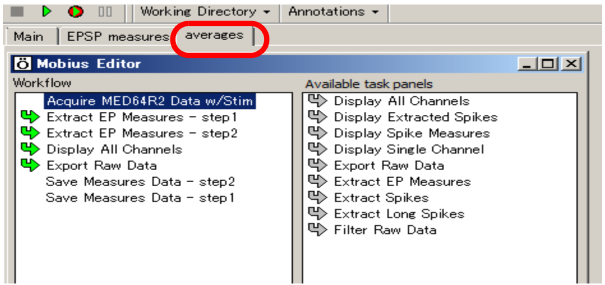


Figure 2-3.2. Editor is opened in the new tab.

   3) Click the [Extract EP Measures -step1-], on the left "Workflow" box, and then double-click the [Compute Measure Averages] on the right Available task panels. (Figure 2-3.3).

      You will see the [Compute Measure Averages] module shift to the left box, under the [Extract EP Measures -step1-] (Figure 2-3.4). The panel for this module will appear in the new tab.

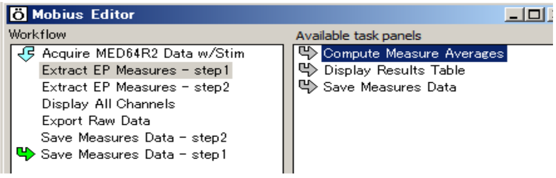


Figure 2-3.3.

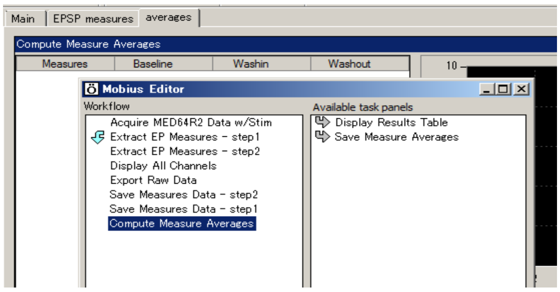


Figure 2-3.4.

4) Click the [Compute Measure Averages] module on the left “Workflow” box, then double-click the [Save Measure Averages] on the right “Available task panels" so that the [Save Measure Averages] module is shifted to the “Workflow” box under the [Compute Measure Averages].

Averages and standard deviations for the [Extract EP Measures -step1-] (top chart in the EPSP measures tab) will be computed and displayed. If online analysis for the other [Extract EP Measurement-step2-](bottom chart) is necessary, add the same modules under the [Extract EP Measures -step2-].

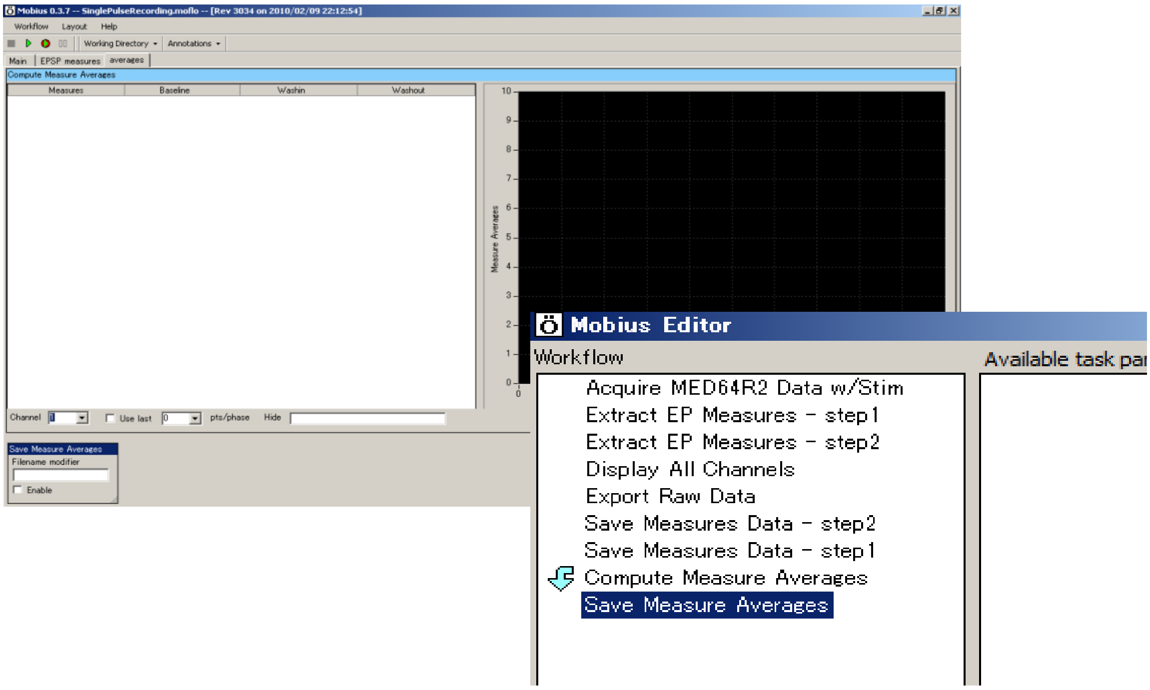
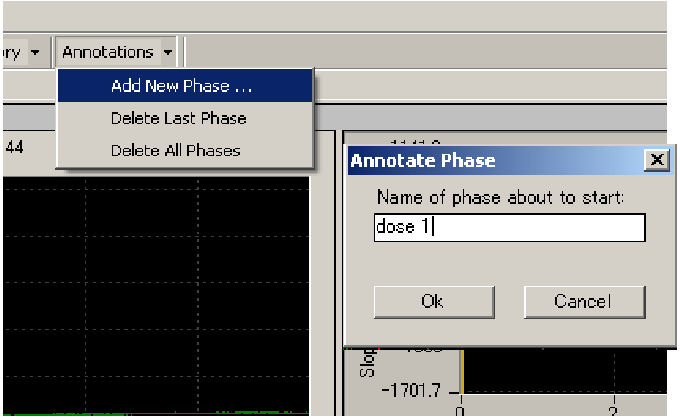


Figure 2-3.5. Control panels for the [Compute Measure Averages] and [Save Measure Averages] modules (left) and the modules in the completed new workflow (right).

3. Open the [Extract EP Measures] module (in the EP measures tab), select measurements for analysis, and set the cursors (refer to section 2-4.5.Analysis of fEPSP signals on page 45).
4. Save it as your own workflow.
5. Run Mobius with the GREEN-RED button. The first phase (baseline) will start from time 0.

6. Pause Mobius with the YELLOW button when the first phase or baseline recording is completed. Click [Annotations] > [Add New phase]. Type the name for the 2nd phase. (e.g. dose 1, Figure 2-3.6)

   Do NOT stop with the Black button. If it is stopped, the experiment will end and new phases will NOT be added.



Name of phase about to start: dose 1

Figure 2-3.6. Making a new phase.

7. When the OK button is clicked, a yellow bar with the chosen name appears on the measurement chart. (Figure 2-3.7)

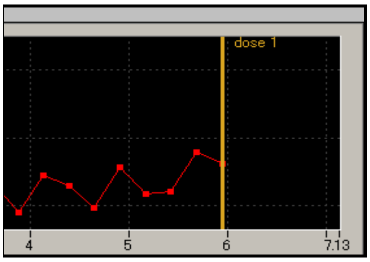


Figure 2-3.7. Adding a new phase.

8. Re-start Mobius by clicking the GREEN-RED button.

   Time in the measurement chart will continue running even during the pause.

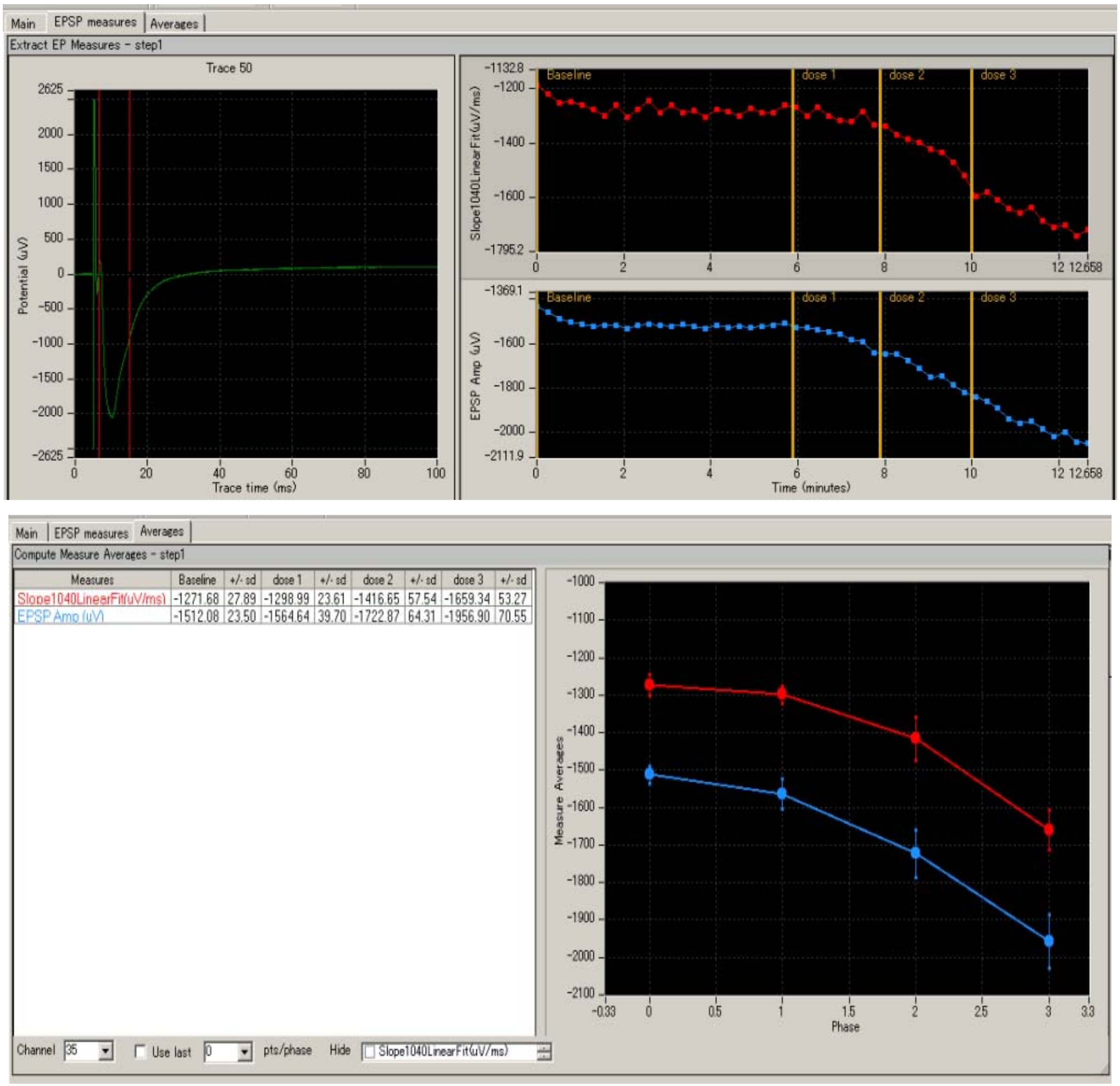
9. Pause Mobius with the YELLOW button when the second phase (e.g. dose 1) is completed. Click [Annotations] > [Add New Phase] again.

10. Type in the name for the 3rd phase. (e.g. dose 2) When the OK button is clicked, the yellow bar with the name appears on the measurement chart.

11. Start Mobius again with the GREEN-RED button. Add subsequent phases using the same procedure.

12. You can see the table and graph of averages for each phase on the [Compute Measure averages] module (in the “Averages” tab) (Refer to Figure 2-3.8)

    - The phase annotations (yellow bars) can be shifted by clicking and dragging during off-line analysis. When data is replayed after the phase annotations are shifted, averages and standard deviations are computed again based on the new phase annotations location.



Measures	Baseline	+/- sd	dose 1	+/- sd	dose 2	+/- sd	dose 3	+/- sd
Slope1040LinearFit(uV/ms)	-1271.68	27.89	-1298.99	23.61	-1416.65	57.54	-1659.34	53.27
EPSP Amp (uV)	-1512.08	23.50	-1564.64	39.70	-1722.87	64.31	-1956.90	70.55

Channel 35 ☐ Use last 0 pts/phase Hide ☐ Slope1040LinearFit(uV/ms)

Figure 2-3.8. [Extract EP Measures] module with phase bars (top) and [Compute Measure Averages] module (bottom).

Input fields in the [Compute Measure Averages] module

1. Channel: Selects the channel to be displayed. The selection cannot be changed during recording.

2. Use last: If this box is checked, the selected (or typed) number of data points are used for computation. This is mostly for off-line analysis.

3. Hide: You can hide the measurement which is checked from the chart.

   Note: the chart can be zoomed up or down by clicking and changing numbers of the maximum and/or minimum scale for both X and Y axis.

2-4. Replay and analysis of acquired data

Mobius data files (.modat) are opened and replayed with the [Replay Raw Data File] module and analyzed using the [Extract EP measures] module. In order to run and analyze the acquired data, you need to create and run an analysis workflow. The quickest way to replay and analyze the acquired fEPSP data is using the *“Single_pulse_analysis”* workflow template (which will be introduced in this chapter).

The analysis workflow including the analysis and saving modules you selected for your recording and [Replay Raw Data File] instead of [Acquire MED64 Data w/Stim] are automatically saved to the same directory where your recording workflow is saved. (Refer to page 17) The other quick way is to use those workflows. The analysis workflow is opened by clicking [Workflow] > [Open] and selecting the workflow (.moflo) file.

2-4.1. Replaying acquired data

1. Click [New] > [From Template]. Select either of [64MD1_1280x1024] or [64MD1_1920x1080] depending on the size of your display monitor. Select [EPs] > [EP_analysis] folders, and then *“Single_pulse_analysis”* workflow template.

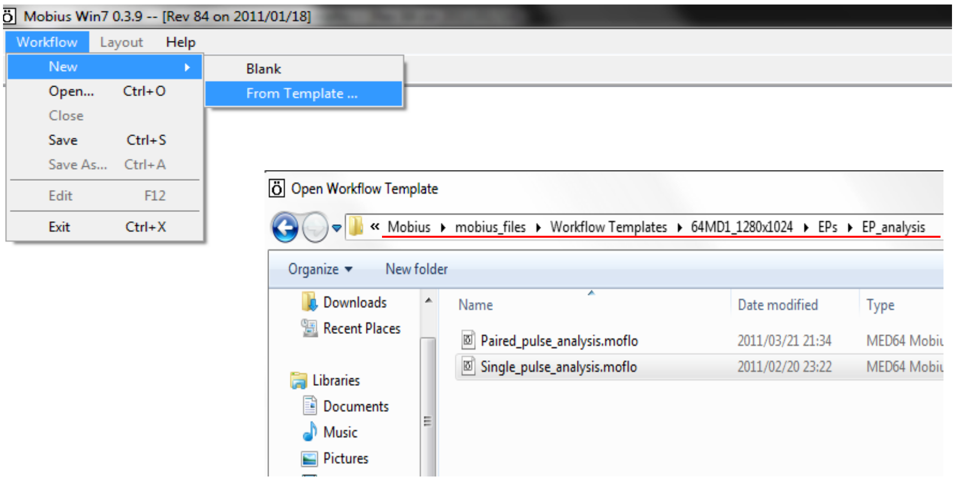
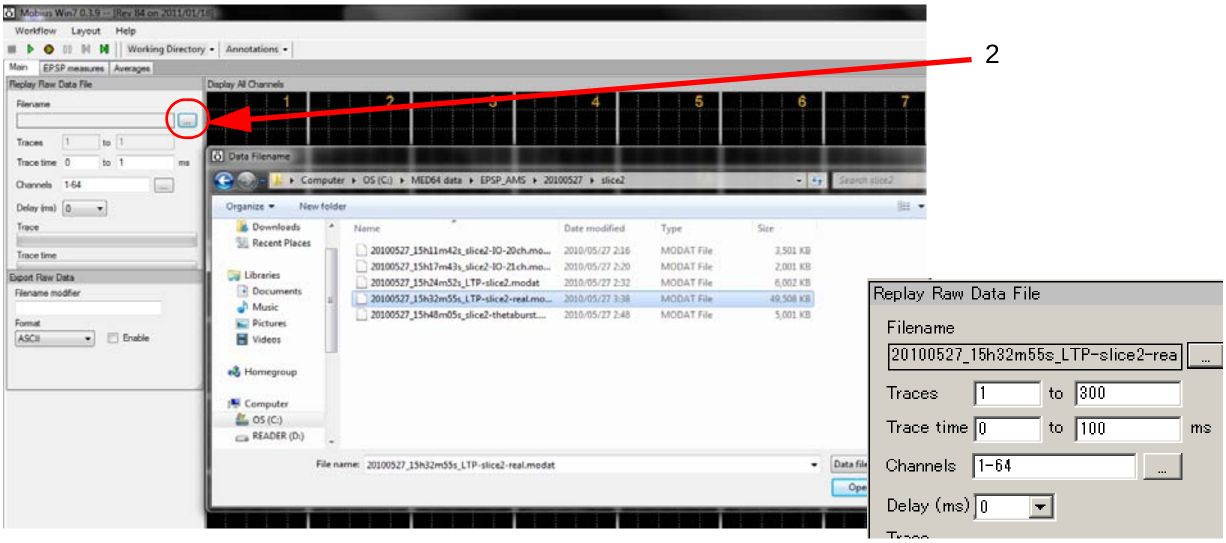


Figure 2-4.1. Opening the analysis workflow template.

2. Open the acquired data file (.modat) by clicking the box next to the [Filename] and selecting the desired file. When the file appears on the Filename box, the data is ready to be replayed by clicking the Green button or Green-Red button. (Figure 2-4.2)

3. When the Green button is clicked, the acquired data is just replayed. Clicking the Green-Red button executes the enabled Exports or Saves.



Computer ▸ OS (C:) ▸ MED64 data ▸ EPSP_AMS ▸ 20100527 ▸ slice2

Name	Date modified	Type	Size
20100527_15h11m42s_slice2-IO-20ch.mo...	2010/05/27 2:16	MODAT File	3,501 KB
20100527_15h17m43s_slice2-IO-21ch.mo...	2010/05/27 2:20	MODAT File	2,001 KB
20100527_15h24m52s_LTP-slice2.modat	2010/05/27 2:32	MODAT File	6,002 KB
20100527_15h32m55s_LTP-slice2-real.mo...	2010/05/27 3:38	MODAT File	49,508 KB
20100527_15h48m05s_slice2-thetaburst....	2010/05/27 2:48	MODAT File	5,001 KB

File name: 20100527_15h32m55s_LTP-slice2-real.modat

Replay Raw Data File

Filename: 20100527_15h32m55s_LTP-slice2-rea

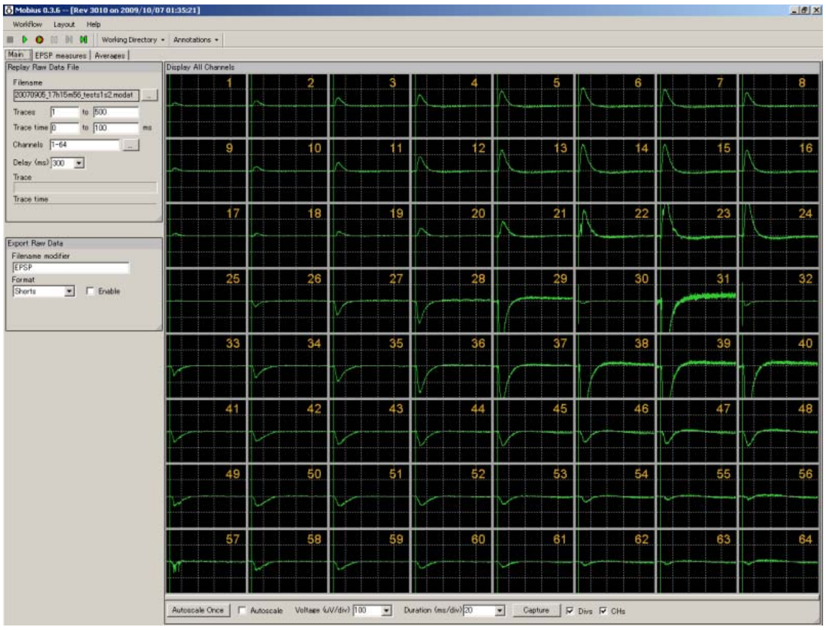
Traces: 1 to 300

Trace time: 0 to 100 ms

Channels: 1-64

Delay (ms): 0

Figure 2-4.2. Opening the data file: When the file name appears in the Filename box, it is ready to be replayed.



Replay Raw Data File

Filename: 20070905_17h15m56_tests1s2.modat

Traces: 1 to 500

Trace time: 0 to 100 ms

Channels: 1-64

Delay (ms): 300

Export Raw Data

Filename modifier: EPSP

Format: Shorts ☐ Enable

☐ Autoscale Voltage (uV/div) 100 Duration (ms/div) 20 ☑ Divs ☑ CHs

Figure 2-4.3. Replaying fEPSP data.

Input fields for the [Replay Raw Data File] module

1. Traces: Selects the trace(s) for processing.
2. Trace time: Selects the trace time for processing.
3. Channels: Selects the channels for processing. Click the box next to the "Channels" to open the channel selector. Channels in green will be enabled.
4. Delay: Set this value to a value greater than zero to insert a delay between data "blocks" sent out by this module. It is useful for slowing down data processing.

2-4.2. Overview of the *“Single_ pulse_analysis”* workflow template

The *"Single_pulse_analysis"* workflow template consists of following modules. (located in the 3 Tabs).

Tab	Modules
Main	Replay Raw Data File/ Display All Channels / Export Raw Data
EPSP measures	Extract EP Measures (2) / Save Meausres Data (2)
Averages	Compute Measure Averages (2) / Save Measuure Averages (2)

In general, the replayed data is sent to the [Extract EP Measures] module, where evoked signals are extracted and their amplitude, slope, area, and time are measured and graphed. Phase measurement averages are computed and graphed accordingly when phase annotations are added. The raw data can be exported in either binary or ASCII ("CSV" -comma separated values- text file). The measurements and averages chart can be saved by "CSV" formatted text file.

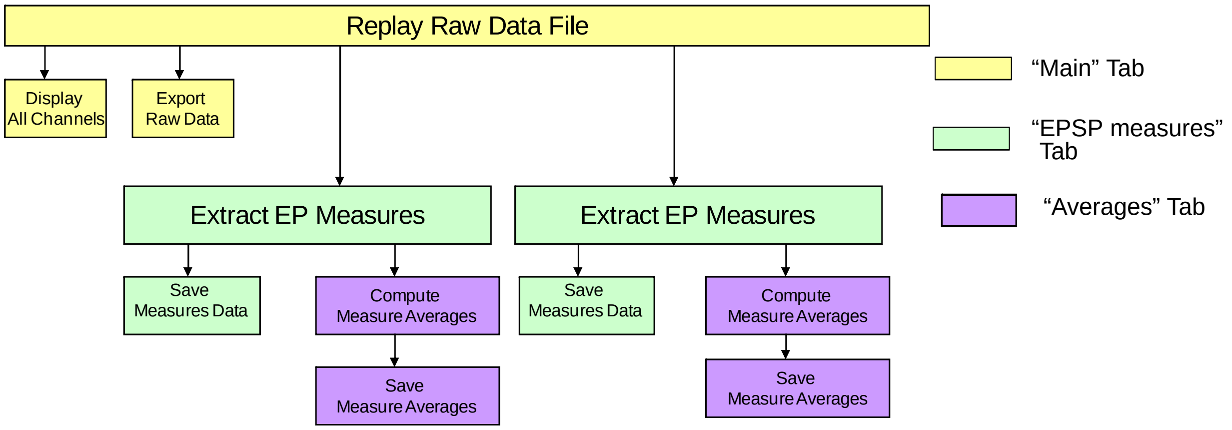


Figure 2-4.4. Module configuration for the *“Single_pulse_analysis”* workflow template.

2-4.3. Analysis of fEPSP signals

Amplitude, slope, area, and time of the extracted waveforms are measured (analyzed) in the [Extract EP measures] module. The extracted evoked potential waveforms are displayed at the left-most chart and time course of the selected measurements are graphed on the right charts.

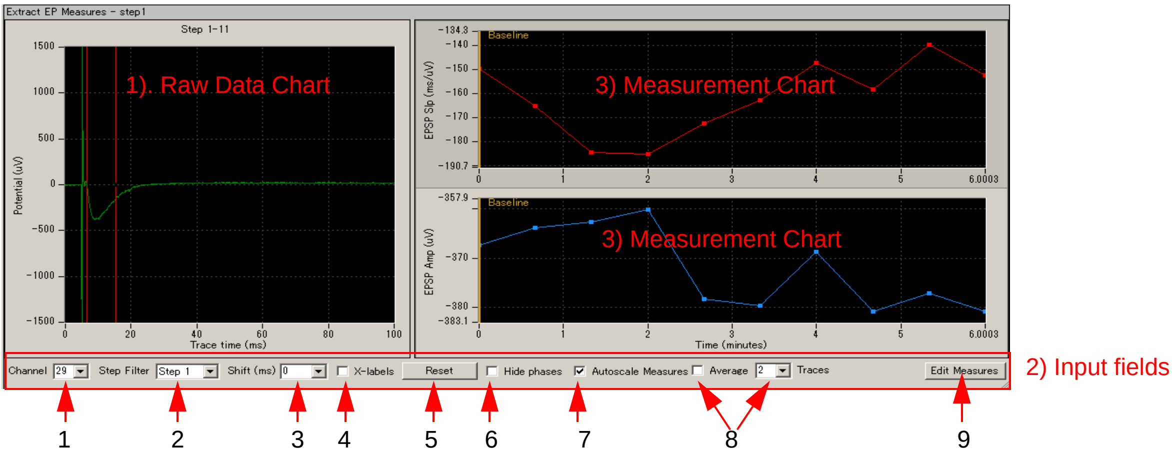


Figure 2-4.5. Extract EP Measures module.

1) Raw data chart

1. The raw data chart on the left most shows the extracted evoked responses waveforms. When a measurement chart is clicked, the corresponding cursor appears on the raw data chart. The cursors for the measurements are shifted by left-clicking and dragging.

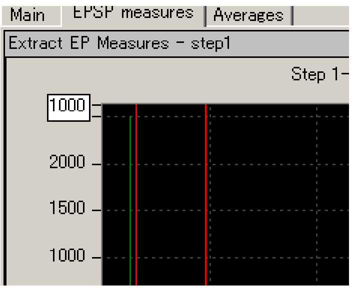


Figure 2-4.6.

2. You can zoom in on the raw data chart by
   1) left-clicking and dragging while pressing the shift button.
   2) changing the maximum (and/or minimum) numbers for both X (time) and Y (amplitude) axes. Click, then, type in the new value(s) for the maximum and/or minimum. (Figure 2-4.6)

   * Double-clicking anywhere on the chart to return to the original axis range.

3. The cursor locations can be copied to all or specific channels by right-clicking and selecting the [Apply Cursors to All Channels] or [Copy Cursors]. Select [Apply Cursors to All Measures] to copy the cursor locations to all measures. (Figure 2-4.7)

   Selecting [Copy Data] allows you to copy the waveform data to an excel work sheet.

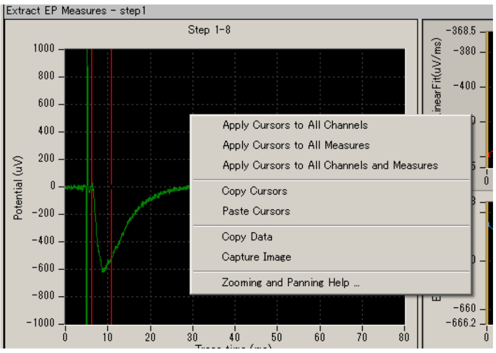
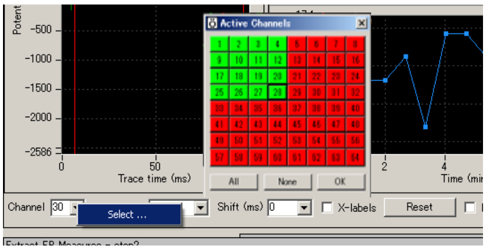


Figure 2-4.7.

2) Input fields

1. Channel No:
   Selects the channel(s) for display by scrolling or typing the numbers directly. These can be changed even during recording or replaying.

   When the channel selector is right-clicked and [Select] is clicked, the Channel selector box appears. The channels of interest are selected here as well. (Figure 2-4.8) This is useful for running the 4-sample system by allowing users to save charts to 4 different CSV files.



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

Channel 30

Shift (ms) 0

☐ X-labels

Figure 2-4.8.

2. Step Filter:
   Selects the Step to be analyzed here. Step1 is selected for the top chart and Step 2 is selected for the bottom in the default settings. Useful for analyzing acquired LTP data with the *"Single_ pulse_recording"* workflow template.

   - In order to analyze Conductor data, ALL must be selected.

3. Shift (ms):
   For analysis of paired pulse experiments. When a number greater than 0 is selected here, the cursors and chart for the 2nd pulse appear.

4. X Labels:
   When this check box is clicked, the time point at which the waveform crosses the cursor is displayed on the Raw data chart.

5. Reset: Resets the shifted cursors to the original positions.

6. Hide phases:
   The phase bar (yellow bars with characters on the measurement chart) is hidden when this button is clicked. Please refer to the "Computing averages for phases" on page 48 for details.

7. Autoscale:
   When this check-box is checked, the scale for the measurement chart is automatically adjusted. You can zoom in and out in the measurement chart by typing the number directly in the maximal values for both the X and Y axes when the Autoscale button is NOT checked.

8. Average:
   When this box is checked, the averaged measurements are computed and graphed. Set the number of traces to be averaged with the selector right to the check box. (e.g. if 2 is input and the box is checked, every two traces (1st and 2nd, 2nd and 3d, 3rd and 4th.) are averaged and graphed.)

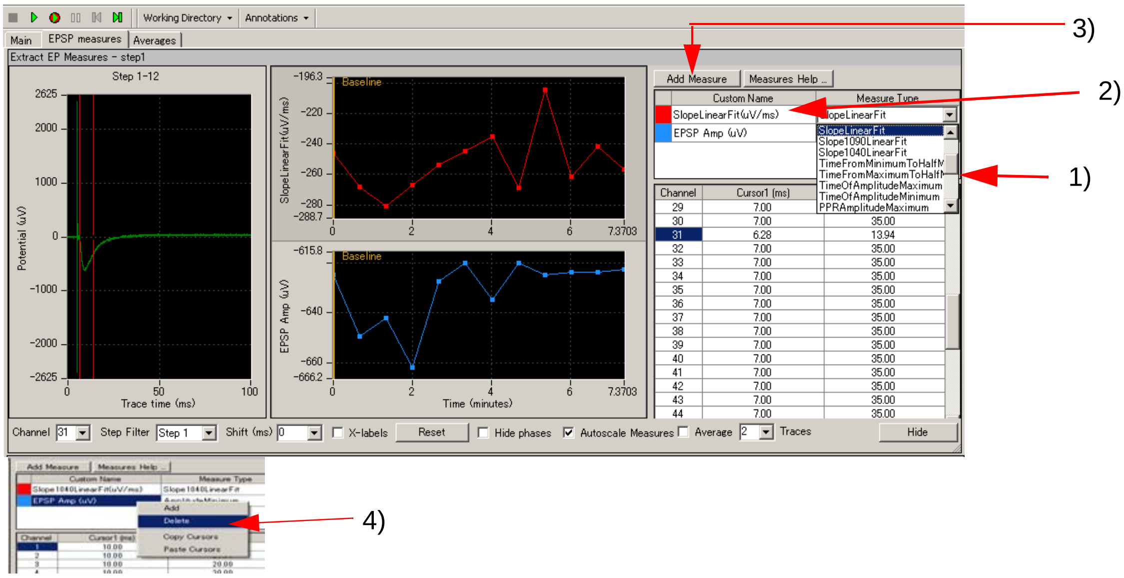
9. Edit Measures: Click this button to select or change the measurement menu.
   1) Select the analysis (measurements) you desire from the [Measure Type] box to change the default measures.
   2) You can name the selected measures by double-clicking and typing directly into the [Custom Name] box.

3) In order to have more measurements, click [Add Measures] button so another row on the table and new measurement chart appear.

4) You can delete unnecessary measurements by right-clicking the custom name, and selecting the [Delete]. (Bottom figure in the Figure 2-4.9)

- Click [Measures Help] button for detailed information on measurements.

5) Click the [Hide] button to close this editor.



	Custom Name	Measure Type
🟥	SlopeLinearFit(uV/ms)	SlopeLinearFit
🟦	EPSP Amp (uV)	

Channel	Cursor1 (ms)	
29	7.00	
30	7.00	35.00
31	6.28	13.94
32	7.00	35.00
33	7.00	35.00
34	7.00	35.00
35	7.00	35.00
36	7.00	35.00
37	7.00	35.00
38	7.00	35.00
39	7.00	35.00
40	7.00	35.00
41	7.00	35.00
42	7.00	35.00
43	7.00	35.00
44	7.00	35.00

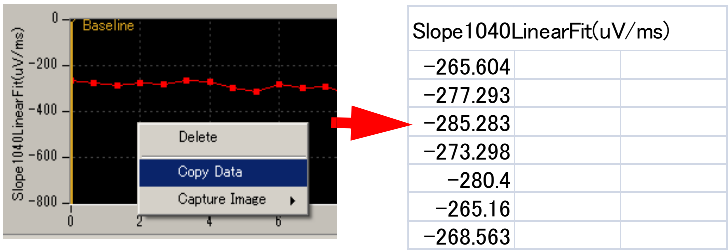
Figure 2-4.9.

3) Measurement chart

The time course for the selected measurements (analysis) are graphed here. Zoom in or out by changing the maximum (and/or minimum) values for the X and/or Y axis. Click and type the new number directly.

Right clicking anywhere in the chart and selecting [Copy Data] allows you to copy the measurement data (only for currently selected channel) into an excel file. (Figure 2-4.10)

Selecting [Capture Image] allows you to copy the chart image.



Slope1040LinearFit(uV/ms)	
-265.604	
-277.293	
-285.283	
-273.298	
-280.4	
-265.16	
-268.563	

Figure 2-4.10. Copying measurement data.

Measurement chart output

After you select the analysis parameters, you probably will want to output a measurement chart. When the check box for [Save Measures Data] is checked and the analysis workflow is run with the Green-Red button, the charts are saved as a "CSV" text file.

The *"Single_pulse_analysis"* workflow template is designed so that the charts for Step1 and Step2 are exported as separate file in the default setting.

Please refer to section 2-4.5 Data output (pages 50-52) for detail.

2-4.4. Computing averages according to phases

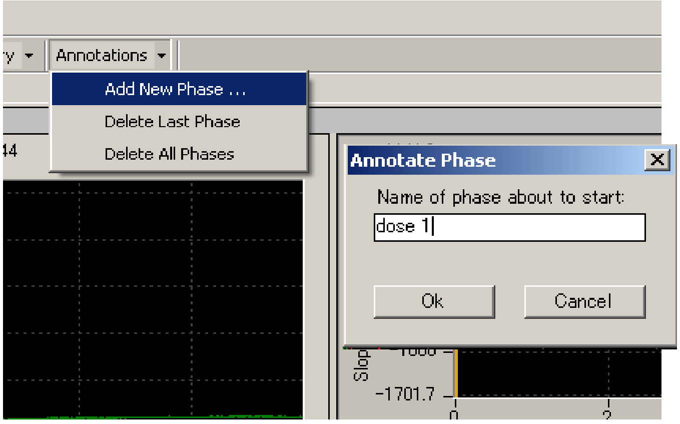
Mobius' [Compute Measure Averages] module calculates averages and standard deviations for the selected measurements (analysis) according to phases, and graph. This is useful for making a dose-response-curve or comparing the measurements between phases such as baseline and LTP.

The *"Single_pulse_analysis"* workflow template includes the [Compute Measure Averages] module. This section will show you how to make a dose-response curve by using this module in the workflow template.

If you would like to add this module to your own analysis workflow, please refer to the step1-2 at the section 3-2. Drug testing on page 38.

1. Open the [Extract EP Measures] module and replay the data by clicking on the Green button. The first phase (baseline) will start from 0.
2. When the first phase (baseline) is finished, Pause Mobius with the YELLOW button. Click [Annotations] > [Add New phase]. (Figure 2-4.11)
3. Type in the name for the 2nd phase. (e.g. dose 1). When the OK button is clicked, the yellow bar with its name appears on the measurement chart in the [Extract EP Measures] module. (Figure 2-4.12)

   NOTICE: If Mobius is stopped with the Black button, replaying is terminated and a phase bar can not be added.



Name of phase about to start: dose 1

Figure 2-4.11.

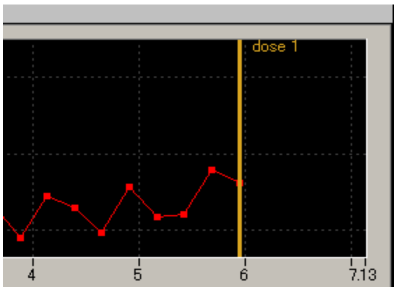
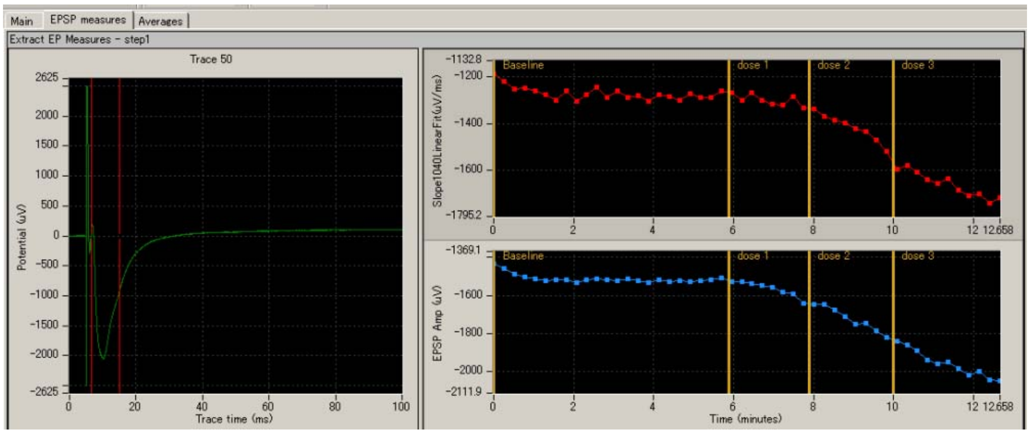
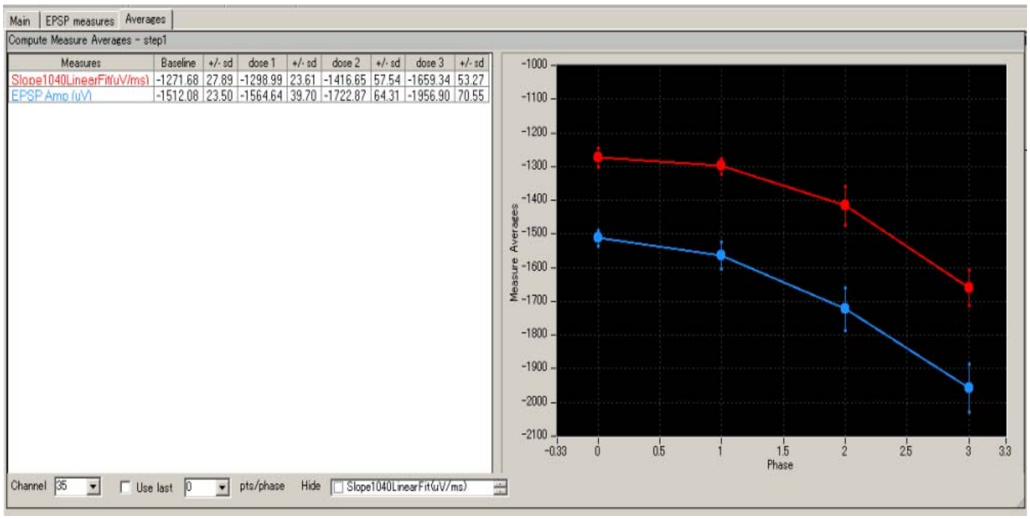


Figure 2-4.12.

4. Start Mobius with the Green button again.
5. Pause Mobius with the YELLOW button when the second phase (e.g. dose 1) is finished. Click [Annotations] > [Add New phase] again.
6. Type the name for the 3rd phase (e.g. dose 2). When the OK button is clicked, the yellow bar for the 3rd phase with its name appears on the measurement chart.
7. Restart Mobius with the Green button. Add subsequent phases with same procedure and replay the data to the end.
8. You will see the values and graphs for each phase in the [Compute Measure Averages] module. (Figure 2-4.13, bottom)
9. The phase annotations (yellow bars) can be shifted by left-clicking and dragging. When the data is replayed after the phase annotations are shifted, averages and standard deviations are recalculated based on the new phase annotation locations.
10. If you are satisfied with the location of the phase bars, save it as your own analysis workflow.

11. In order to save the average chart to a "CSV" file, check the check-box for the [Save Measures Averages] module, then run Mobius with the Green-Red button.





Compute Measure Averages - step1

Measures	Baseline	+/- sd	dose 1	+/- sd	dose 2	+/- sd	dose 3	+/- sd
Slope1040LinearFit(uV/ms)	-1271.68	27.89	-1298.99	23.61	-1416.65	57.54	-1659.34	53.27
EPSP Amp (uV)	-1512.08	23.50	-1564.64	39.70	-1722.87	64.31	-1956.90	70.55

Channel: 35

☐ Use last: 0 pts/phase

Hide: ☐ Slope1040LinearFit(uV/ms)

Figure 2-4.13. [Extract EP Measures] module with phase bars (top), and the table and charts at the [Compute Measure Averages] module (bottom).

Input fields for [Compute Measure Averages] module

1. Channel: Selects the channel to be displayed. The selection can not be changed during replaying.

2. Use last: When this box is checked, the selected (or typed) number of data points are used for computation. For example, if 10 is selected, average and standard deviation are measured for the last 10 data points in each phase.

3. Hide: hides the measurement checked in the chart.

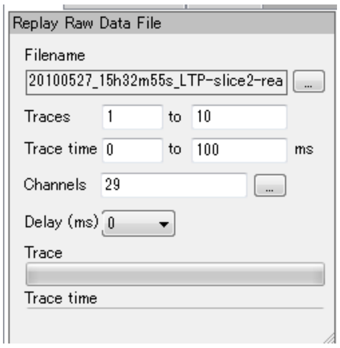
   - Note: the chart can be zoomed up or down by clicking and changing numbers of the maximum and/or minimum scale(s) for both X and/or Y axis.

2-5. Data output

Mobius allows you to output folowing data/analysis of fEPSPs signals:

1. Raw data (waveforms)
2. Measurement chart
3. Average chart

All outputs are executed when Mobius is run by clickikng the Green-Red button with the check boxes for the Save or/and Export modules are checked. (The analysis workflow needs to be saved to execute the output.) Channels for output can be selected with channel selector in the [Replay Raw Data File] module.


Replay Raw Data File

Filename: 20100527_15h32m55s_LTP-slice2-rea

Traces: 1 to 10

Trace time: 0 to 100 ms

Channels: 29

Delay (ms): 0

Trace

Trace time

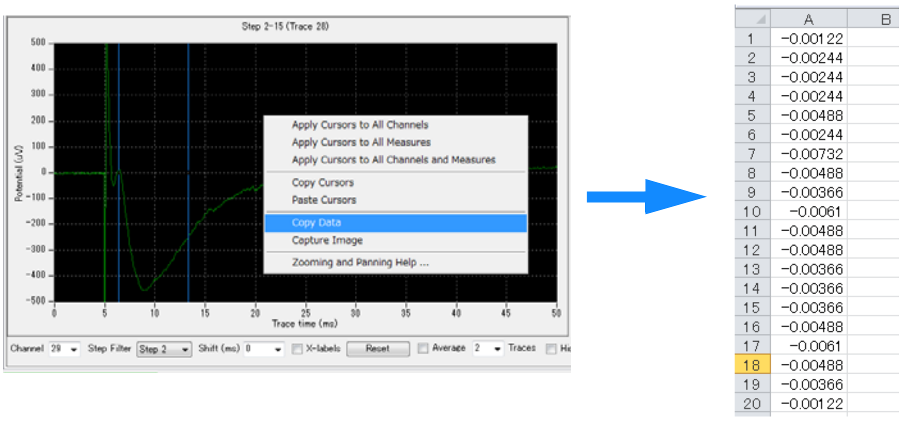
Figure 2-5.1. The [Replay raw Data File] ready for expoert. Data for trace No 1-10 for channel 29 will be output

2-5.1. Export raw data

Please refer to 1-5. Exporting raw data (page 20-21) to export raw data.

Data for a waveform displayed in the [Extract EP Measurement] can be quickly copied to Excel with following procedure:

1. Select the waveform you would like to save in the [Extract EP Measurement]
2. Click anywhere in the Trace chart.
3. Select [Copy Data]
4. Open a new file in the excel, and then paste the data.



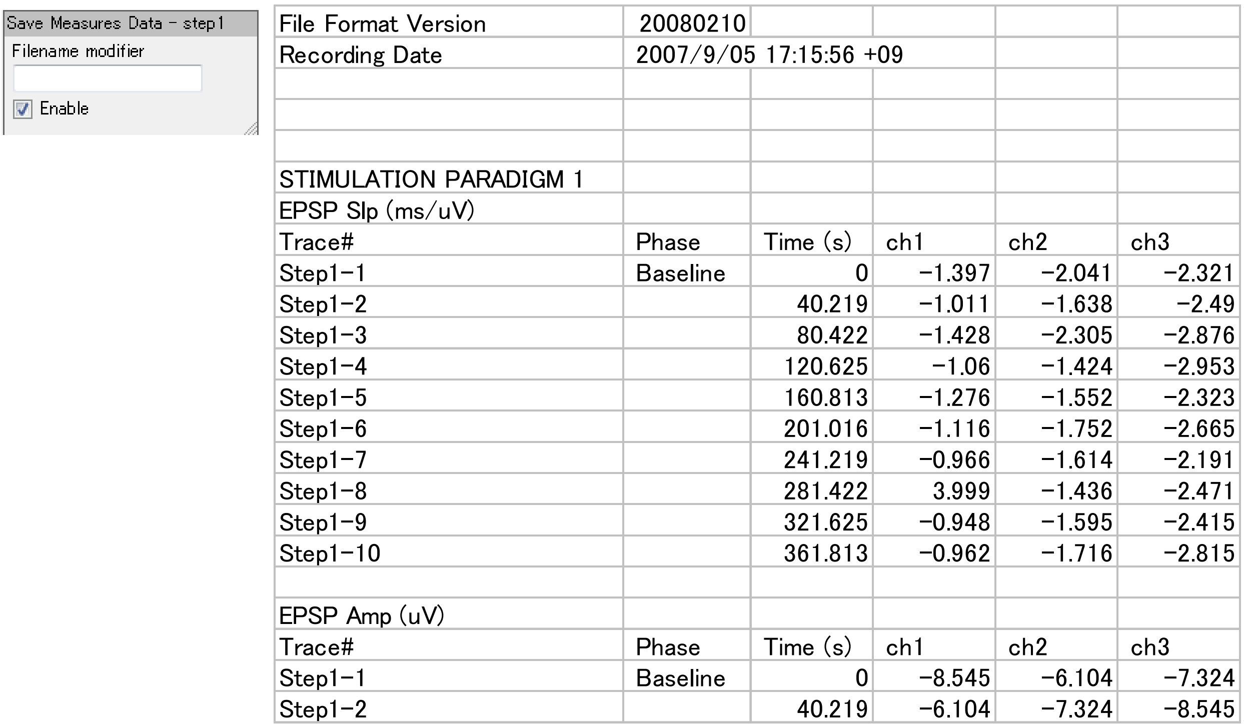
Channel 29 Step Filter Step 2 Shift (ms) 0 ☐ X-labels ☐ Average 2 Traces

	A	B
1	−0.00122	
2	−0.00244	
3	−0.00244	
4	−0.00244	
5	−0.00488	
6	−0.00244	
7	−0.00732	
8	−0.00488	
9	−0.00366	
10	−0.0061	
11	−0.00488	
12	−0.00488	
13	−0.00366	
14	−0.00366	
15	−0.00366	
16	−0.00488	
17	−0.0061	
18	−0.00488	
19	−0.00366	
20	−0.00122	

Figure 2-5.2. Copying the waveform data to excel. Waveform data for Trace 28, ch 29 was copied to excel.

2-5.2. Save the measurement chart

Enable the [Save Measures Data] module. The measurements values are saved as a "CSV" formatted text file.


Save Measures Data - step1

Filename modifier

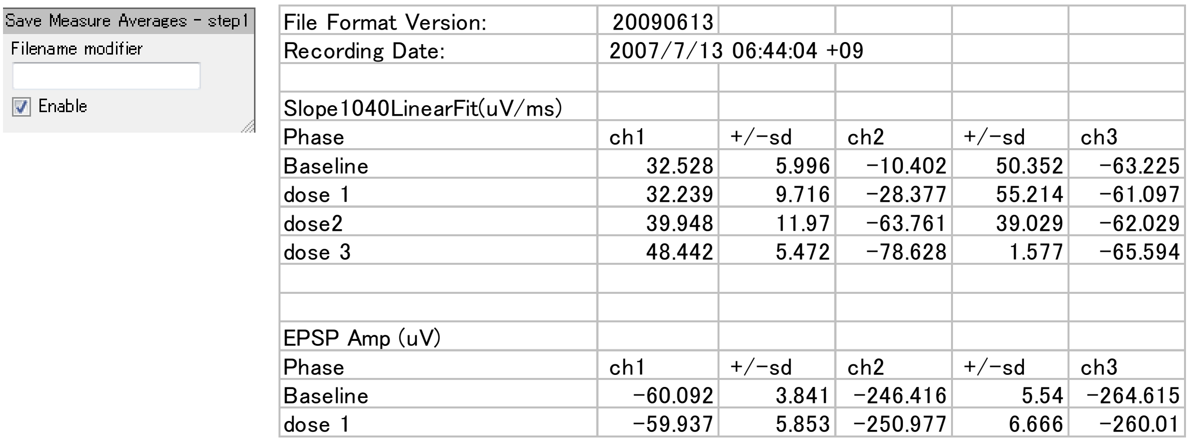
☑ Enable

File Format Version	20080210				
Recording Date	2007/9/05 17:15:56 +09				
STIMULATION PARADIGM 1					
EPSP Slp (ms/uV)					
Trace#	Phase	Time (s)	ch1	ch2	ch3
Step1−1	Baseline	0	−1.397	−2.041	−2.321
Step1−2		40.219	−1.011	−1.638	−2.49
Step1−3		80.422	−1.428	−2.305	−2.876
Step1−4		120.625	−1.06	−1.424	−2.953
Step1−5		160.813	−1.276	−1.552	−2.323
Step1−6		201.016	−1.116	−1.752	−2.665
Step1−7		241.219	−0.966	−1.614	−2.191
Step1−8		281.422	3.999	−1.436	−2.471
Step1−9		321.625	−0.948	−1.595	−2.415
Step1−10		361.813	−0.962	−1.716	−2.815
EPSP Amp (uV)					
Trace#	Phase	Time (s)	ch1	ch2	ch3
Step1−1	Baseline	0	−8.545	−6.104	−7.324
Step1−2		40.219	−6.104	−7.324	−8.545

Figure 2-5.3. [Save Measure Data] module/control pannel (left) and example of the measurment chart saved as an Excel compatible CSV file (right). The chart of slope linerfit and minimum amplitude for Step 1 are saved.

2-5.3. Save the average chart

Enable the [Save Measure Averages] module. The values for the averages and standard deviations are saved as a "CSV" formatted text file.



Save Measure Averages - step1

Filename modifier

☑ Enable

File Format Version:	20090613				
Recording Date:	2007/7/13 06:44:04 +09				
Slope1040LinearFit(uV/ms)					
Phase	ch1	+/-sd	ch2	+/-sd	ch3
Baseline	32.528	5.996	-10.402	50.352	-63.225
dose 1	32.239	9.716	-28.377	55.214	-61.097
dose2	39.948	11.97	-63.761	39.029	-62.029
dose 3	48.442	5.472	-78.628	1.577	-65.594
EPSP Amp (uV)					
Phase	ch1	+/-sd	ch2	+/-sd	ch3
Baseline	-60.092	3.841	-246.416	5.54	-264.615
dose 1	-59.937	5.853	-250.977	6.666	-260.01

Figure 2-5.4. [Save Measure Averages] module/control pannel (left) and example of the measurment chart saved as an Excel compatible CSV file (right).

Chapter 3 Spike Sorter

This chapter will teach you how to record and analyze spontaneous neuronal spikes (single unit activities) either from neuron cultures or brain slices using available workflow templates. In this chapter you will learn:

1. How to record spontaneous neuronal spikes (single unit activities).
2. How to test drug effects.
3. How to replay and analyze spontaneous neuron spikes (single unit activities).
4. How to export analyzed data.

3-1. Recording spontaneous neuronal spikes (single unit activities)

There are 5 workflow templates available for recording spontaneous neuronal spikes (single unit):

1. *Spike_recording* (Recording with extracting spikes and their frequency analysis).
2. *Spike_recording_filter* (1 + filter).
3. *Spike_recording_cluster* (Recording with extracting, clustering spikes, and their frequency analyses).
4. *Spike_recording_filter_cluster* (2+filter).
5. *Spontaneous_recording* (Recording WITHOUT any real-time analysis).

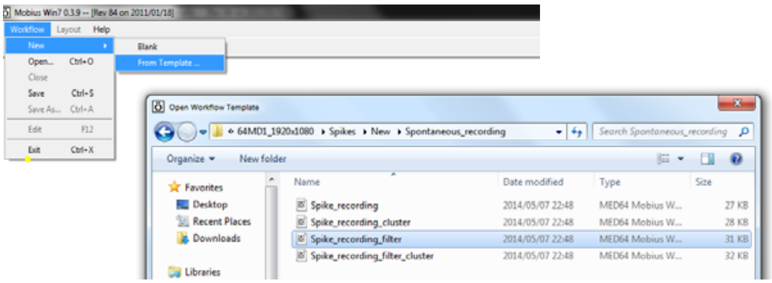
Opening the workflow template

1. Click [Workflow] > [New] > [From Template]. (Figure 3-1.1)

2. Select either of [64MD1_1280x1024] or [64MD1_1920x1080] folder, depending on the size of your display monitor. Select [Spikes] > [New] >[Spontaneous_recording] folders. Select the workflow template.

    - Go to [Basic_recording] folder for the *“Spontaneous_recording”* workflow template.
    - Workflow for the old spike sorter modules are located in the [Archives] folder.

NOTICE:
Connect your PC to the MED64 amplifiers and turn on both amplifiers to open the acquisition workflows.



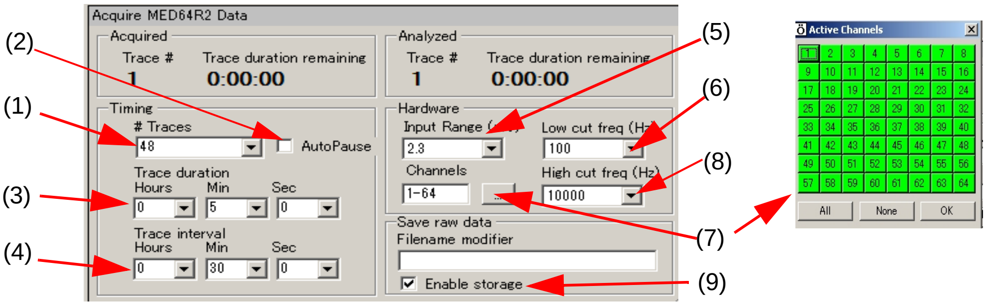
Name	Date modified	Type	Size
Spike_recording	2014/05/07 22:48	MED64 Mobius W...	27 KB
Spike_recording_cluster	2014/05/07 22:48	MED64 Mobius W...	28 KB
Spike_recording_filter	2014/05/07 22:48	MED64 Mobius W...	31 KB
Spike_recording_filter_cluster	2014/05/07 22:48	MED64 Mobius W...	32 KB

Figure 3-1.1. Opening the workflow template.

3-1.1. Setting the acquisition parameters

All the acquisition workflow templates include the [Acquire MED64R2 Data] module. Acquisition parameters are set in this module.

NOTICE:
Connect your PC to the MED64 amplifiers and turn on both amplifiers to open this module.



Acquired: Trace # 1, Trace duration remaining 0:00:00

Analyzed: Trace # 1, Trace duration remaining 0:00:00

Timing
\# Traces: 48
☐ AutoPause
Trace duration: Hours 0, Min 5, Sec 0
Trace interval: Hours 0, Min 30, Sec 0

Hardware
Input Range (mV): 2.3
Low cut freq (Hz): 100
Channels: 1-64
High cut freq (Hz): 10000

Save raw data
Filename modifier:
☑ Enable storage

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

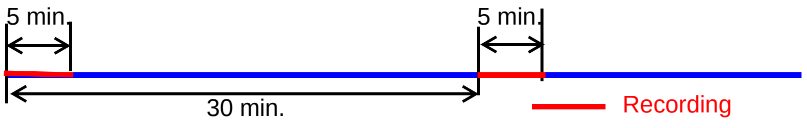
Figure 3-1.2. [Acquire MED64R2 Data] module.

1. #Traces: Number of Traces (sweeps).
2. Auto Pause:

   Mobius will pause automatically when the current trace is finished if the box is checked. The trace interval set below becomes invalid when this box is checked. Mobius re-starts by clicking either of Green button or Green-Red button.
3. Trace duration: Sampling (Recording) time for each trace.
4. Trace interval:

   Interval between traces. (from the beginning of the first trace to the beginning of the next trace.)

   - In the default setting, signals are recorded for 5 minutes every 30 minutes (as seen in the following figure), which is repeated 48 times. Mobius will run for 24 hours in total.
   - The trance interval becomes invalid when AutoPause is checked.



5. Input Range (mV):

   Maximum input signal level. This setting should be adjusted according to the signal amplitude to achieve the maximal dynamic range. Select 2.3 mV, 2.9 mV, 5 mV, 12.5 mV, or 25 mV. Larger number for the Input Range creates lower voltage resolution. 2.3 mV is usually good enough for recording of neuronal spikes.

6. Low cut freq (Hz):

   High pass filter for the amplifier. Select 0.1 Hz, 1.0 Hz, 10 Hz or 100 Hz. If 1.0 Hz is set, the signals with frequencies lower than 1 Hz are filtered out. 100 Hz is recommended for the recording of neuronal spikes.

7. Channels:

   Select the channels for acquisition here. When the square box is clicked, the channel selector pops up. The enabled recording channels are colored green.

8. High cut freq (Hz):

   Low pass filter for the amplifier. Select 1000 Hz, 2000 Hz, 2500 Hz, 5000 Hz, 7500 Hz, or 10000 Hz. If 5000 Hz is selected, signals with frequencies higher than 5000 Hz are filtered out. 10000 Hz is recommended for the recording of neuronal spikes.

9. Save raw data:

   When Mobius is started by clicking the Green-Red recording button while the [Enable storage] is checked, the raw data is saved. The file name can be modified.

3-1.2. Execution buttons

Acquisition is initiated by running an acquisition workflow. Clicking the Green button runs the acquisition workflow without saving data while clicking the Green-red button runs the acquisition workflow while saving data to disc.

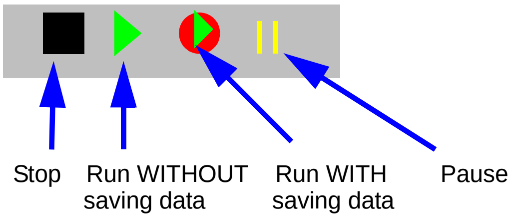
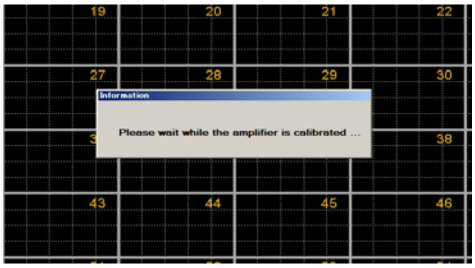


Figure 3-1.3. How the execution buttons work for acquisition.

3-1.3. Before starting your experiment

When an acquisition workflow is run with Green or Green-Red button for the first time after it is opened (or made), Mobius needs several seconds for the calibration (as seen in the pop-up message). Acquisition starts automatically after the calibration.

If you would like to start acquisition immediately after clicking the Green-Red button, run the workflow with the Green button for several seconds, and then stop it before starting your experiment. When the workflow is run for the next time, it will not require calibration and acquisition will start immediately.



Please wait while the amplifier is calibrated ...

Figure 3-1.4. The pop-up message for the calibration.

CAUTION:

- Make sure that the hard drive has enough free space when you start a long-term recording. Acquisition with all 64 channels for 1 hour creates a 9.216GB file.
  (9.216GB is represented as 9,000,000KB, 8,739.06MB, or 8.58GB in the Windows.)

- Avoid saving data to the drive where the OS is installed (usually C drive). Save it to a separate hard drive in the following cases:
  1) When trace duration is set to longer than 30 minutes.
  2) When trace duration is set to longer than 5 minutes and # Traces is greater than 12.

- Extracting and/or clustering spikes can slow down Mobius' data processing and appear to freeze when it extracts more spikes than it can analyze. This most often happens when non-biological signals cross the spike extraction thresholds on all channels at once. (e.g. Unexpected noises cross the thresholds or the baseline fluctuates due to vibration.)

- It is recommended you start your new experiments WITHOUT spike extraction or clustering (using the Spontaneous Recording template or disabling spike detection). Perform your experiments with real-time analysis when you are familiar with your preparation and experiments. When recording is done with extraction, clustering, and filtering, please follow the instructions in section 1-2. Important notice on pages 8-10.

3-1.4. Recording with extracting spikes and their frequency analysis

-Using the *“Spike_recording(_ filter)”* template-

Overview of the *“Spike_recording(_filter)”* workflow templates

The *“Spike_recording(_filter)”* template consists of the following modules.

Tabs	Modules
Main	Acquire MED64R2 Data / Display All Channels / Export Raw Data
Extraction	Extract Spikes Advanced
Spike	Compute Spike Freqs / Save Spikes / Save Spike Freqs / Display Spike Measures
Filter*	Filter Raw Data (2) / Display Single Channel (2)

* “Filter” tab is NOT available with the *“Spike_recording”* workflow template.

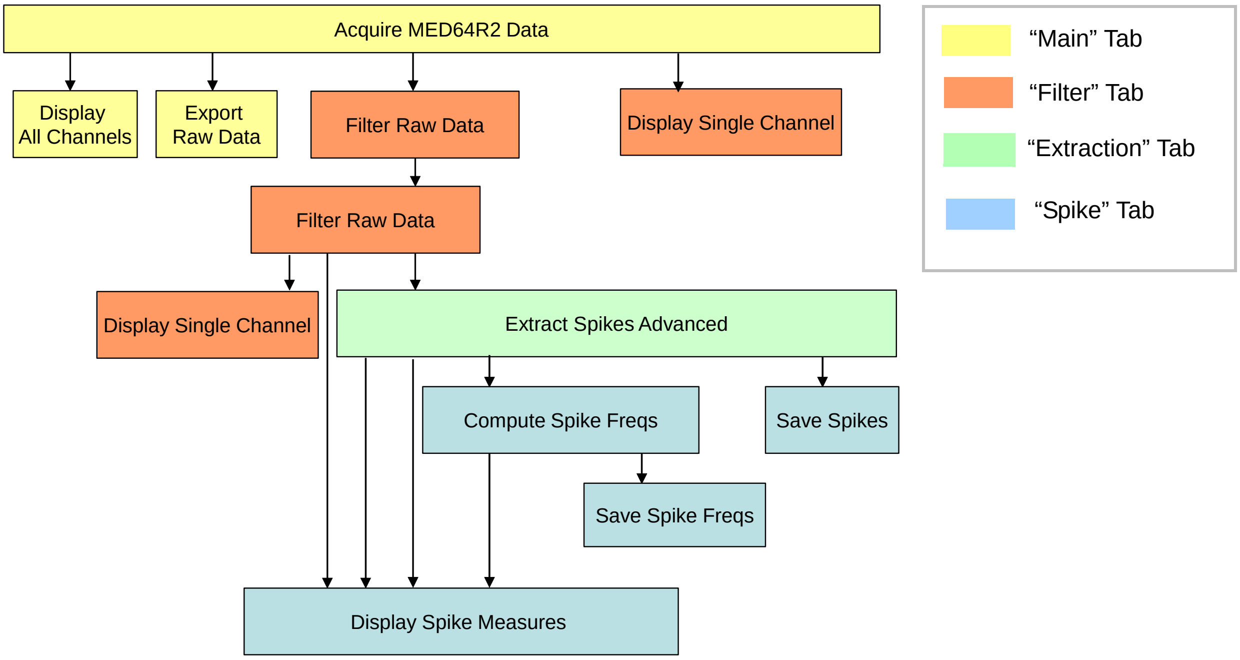
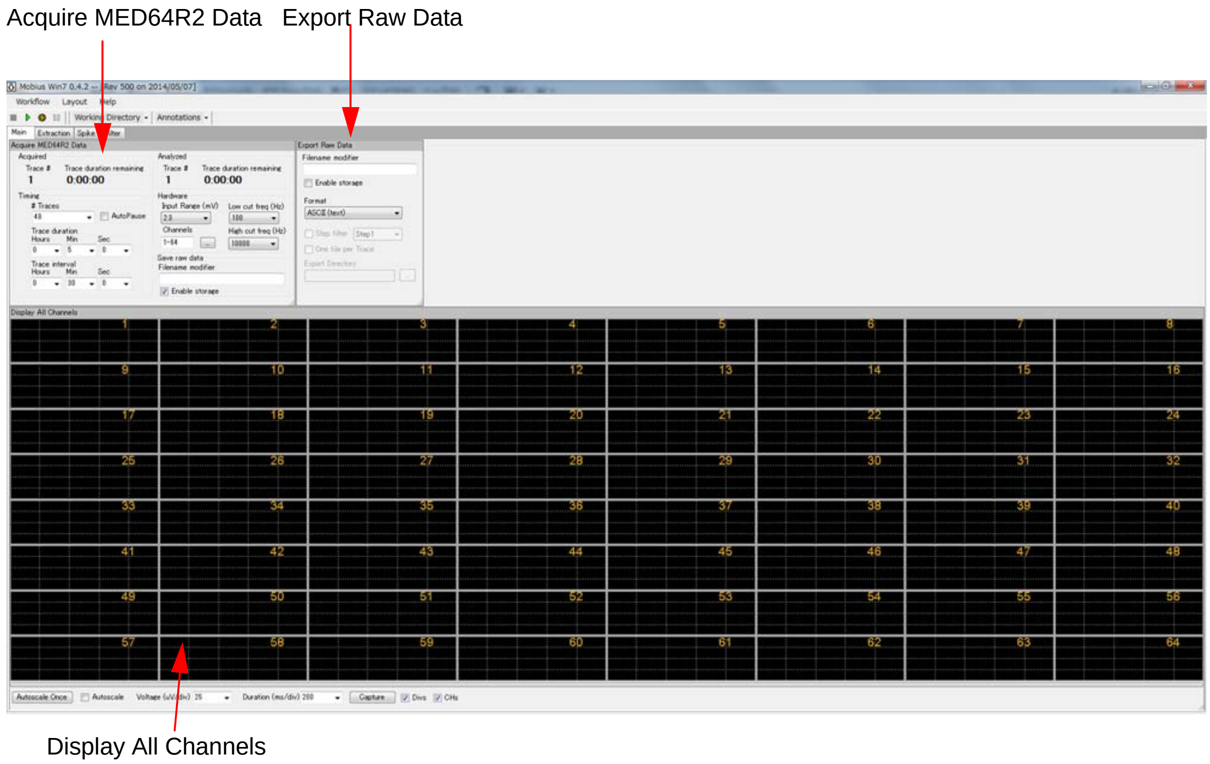


Figure 3-1.5. Module configuration for the *“Spike_recording_filter”* workflow template. The *“Spike_recording”* does nos include modules in the “Filter” Tab.

Thresholds are set using the [Extract Spikes Advanced] module and spikes crossing the set thresholds are extracted. Spike frequency of all extracted spikes is computed and graphed during acquisition. You can monitor 1) spikes are extracted, 2) the extracted spikes’ waveforms, and 3) the spike frequency chart.

2 sets of [Filter Raw Data] modules are added in the “Filter” Tab. Acquired data can be filtered with selected filter menu before sent to the [Extract Spikes Advanced] module. In the “Filter” tab, the top [Display Single Channel] module shows the original non-filtered data while the bottom module shows filtered data. (Refer to the bottom figure on the Figure 3-1-7).



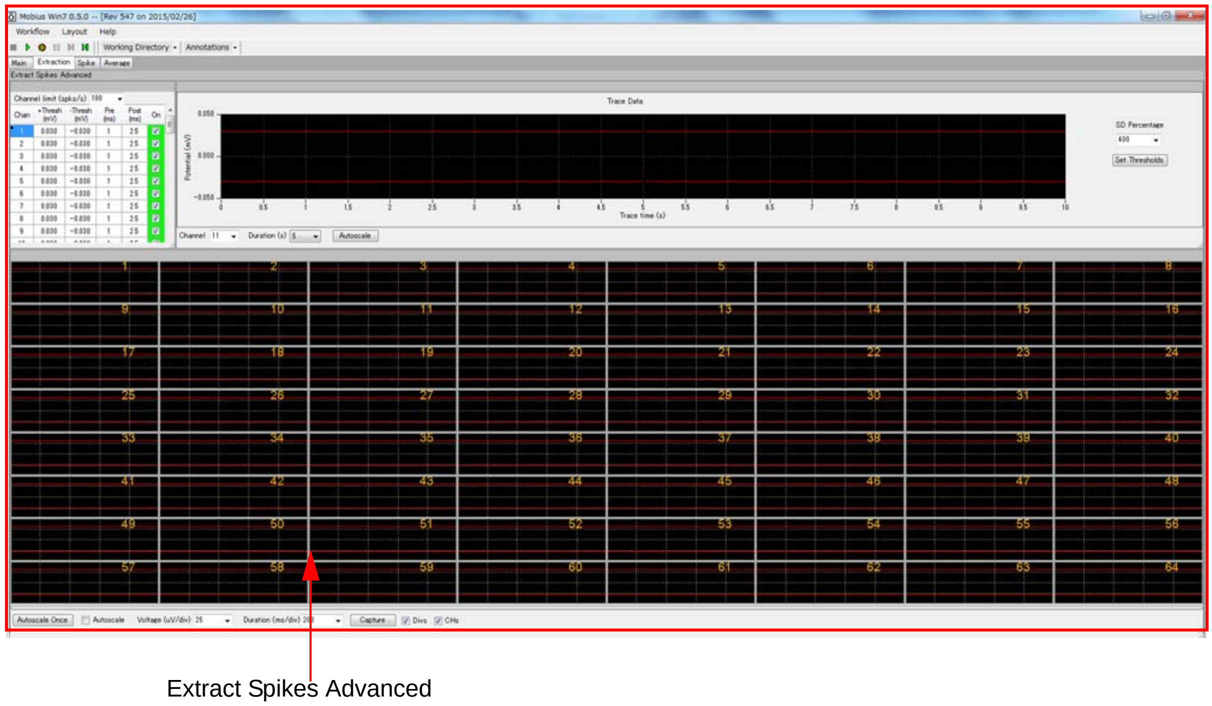
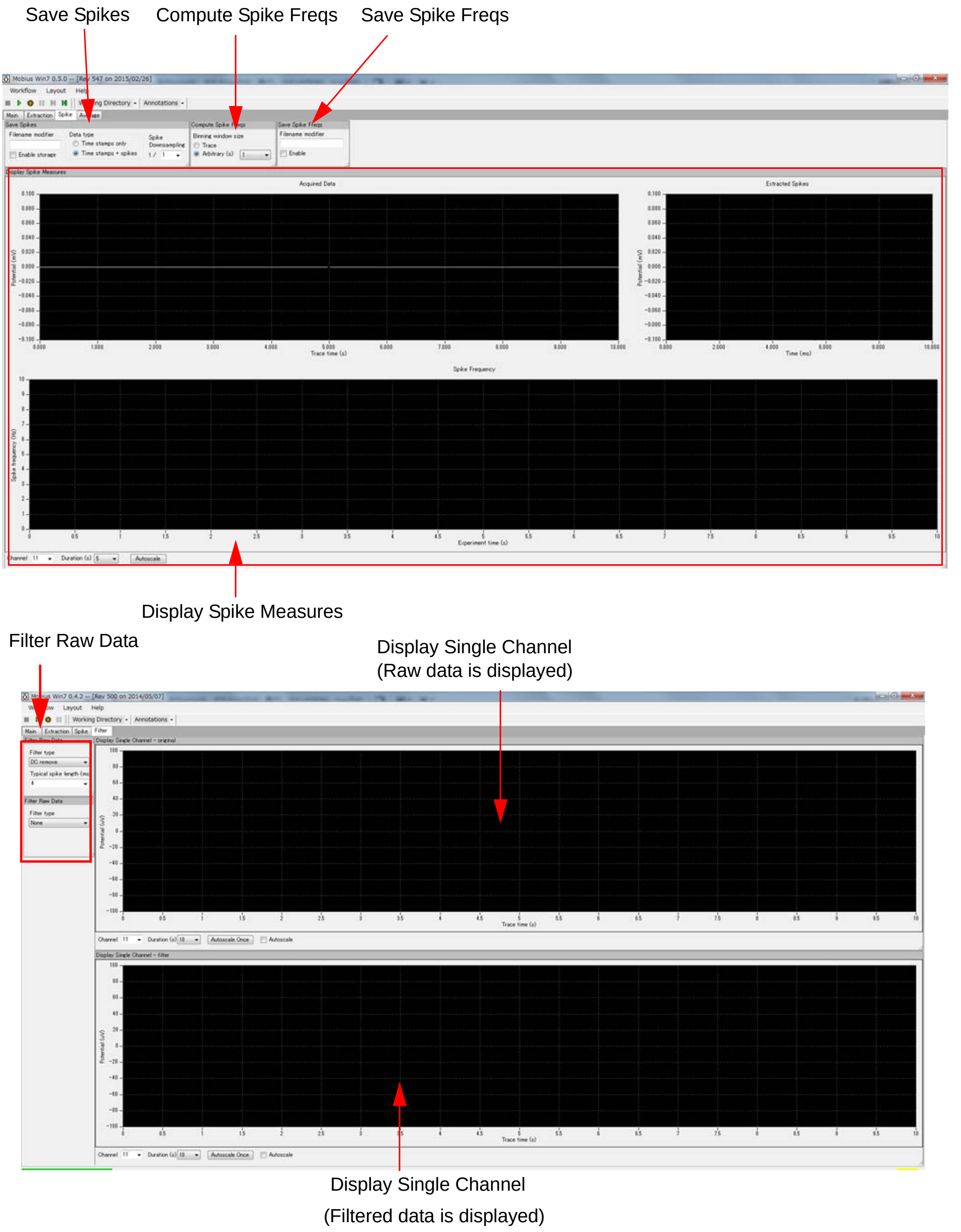


Figure 3-1.6. Control panel for the *“Spike_recording_filter”* workflow template. ”Main” tab (top), and “Extraction” tab (bottom).



Save Spikes: Filename modifier; ☐ Enable storage; Data type: ○ Time stamps only, ● Time stamps + spikes; Spike Downsampling 1 / 1

Compute Spike Freqs: Binning window size: ○ Trace, ● Arbitrary (s) 1

Save Spike Freqs: Filename modifier; ☐ Enable

Channel 11; Duration (s) 5

Filter type: DC remove; Typical spike length (ms): 4

Filter Raw Data: Filter type: None

Channel 11; Duration (s) 10; ☐ Autoscale

Figure 3-1.7. Control panel for the “*Spike_recording_filter*” workflow template. “Spike” tab (top) and “Filter” tab (bottom). The *“Spike_recording”* does not have the “Filter” tab.

Recording spontaneous neuronal spikes with spike extracting and their frequency analysis

Extracting spikes can slow down data processing and Mobius can even freeze when it extracts more spikes than it can process. Refer to CAUTION on the page 56. Please follow the instructions below to avoid stopping during acquisition when this workflow template is used (or this module is used for acquisition.)

- Select 100Hz for the low-cut filter. (Accidental low-frequency noise might cause to stop Mobius).
- Avoid enabling any [Export] or [Save] modules when raw data is saved. Once raw data is saved, all exporting can be performed post-acquisition.
- Set the trace duration (recording time) to less than 10 minutes and the trace interval to longer than “the trace duration x 2” so that the analysis can catch up with the acquisition during the trace interval. Particularly, keep the trace duration short for long-term recording lasting days, weeks, or months.
- Decrease the number of recording channels.

1. Open the *“Spike_recording(_filter)”* workflow template. (Refer to page 53)

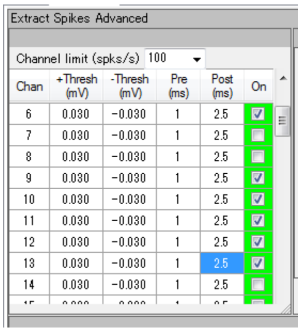
   Change the acquisition parameters in the [Acquire MED64R2 Data].
   (Refer to section 3-1.1. Setting acquisition parameters on page 54-55.)

2. Start Mobius with the Green button and run the workflow template for a few minutes to see signals, and then stop.

3. Select the channels for recording. Click the box next to “Channel” in the [Acquire MED64R2 Data] module to have channel selector.

4. Select the channels for spike extraction in the chart of [Extract Spike Advanced]. (Analysis is not monitored during acquisition for the channels that are un-checked although data is saved.)

   - A selection is applied to all channels easily by 1) right-clicking the check box and 2) selecting [Apply to All].



Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
6	0.030	−0.030	1	2.5	☑
7	0.030	−0.030	1	2.5	☐
8	0.030	−0.030	1	2.5	☐
9	0.030	−0.030	1	2.5	☑
10	0.030	−0.030	1	2.5	☑
11	0.030	−0.030	1	2.5	☑
12	0.030	−0.030	1	2.5	☑
13	0.030	−0.030	1	2.5	☑
14	0.030	−0.030	1	2.5	☐

Figure 3-1.8. Enabling/disenabling the spike extraction.

5. Make sure that [Export Raw Data] is NOT checked (Un check the check-box).

   - Once raw data is saved, this can be done later.

6. Set the analysis parameters.

    1) Change the filter menu depending on the acquired signals.
    (If the *“Spike_recording”* template is used, this is not necessary.)

    2) Set the thresholds.

    - Refer to section 3-3.2 Analyzing the spike rate on pages 74-82 for detail.

    3) Change the parameters for [Compute Spike Freqs].

    4) Make sure [Save Spikes] and [Save Spike Freqs] are NOT enabled.

    - It is not recommended to enable these modules during acquisition. These can be done later once raw data is saved.

7. Run Mobius with the Green button again, then make sure that the thresholds you set are OK.

    - Thresholds can be changed during acquisition. But it is not recommended to do so.

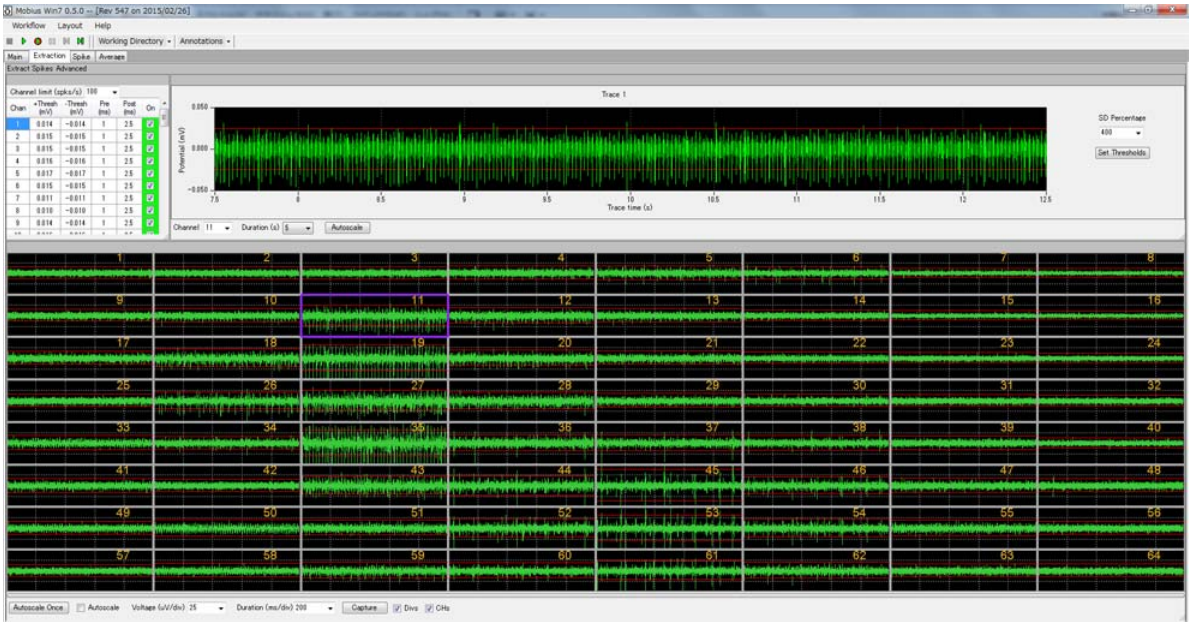
8. Save as your own acquisition workflow. (Refer to page 16) The workflow will be saved as moflo.file.

    - The data file (.modat file) and the Analysis workflow (including all analysis modules and parameters selected for the acquisition workflow and [Replay Raw Data File] module) will be saved in the same folder. (Refer to pages 17)

9. Make sure that the Save raw data in the [Acquire MED64R2 Data] module is checked, then run your workflow with the Green-Red button.

    - It is recommended to start acquisition without spike extraction when you are not familiar with your experiments. **It is strongly recommended to start recording WITHOUT extractions for long term recording over several days or weeks.**

    - Mobius will require you to save the workflow as your own if you try to start Mobius with the Green-Red button without having it already.



Mobius Win7 0.5.0 -- [Rev 547 on 2015/02/26]

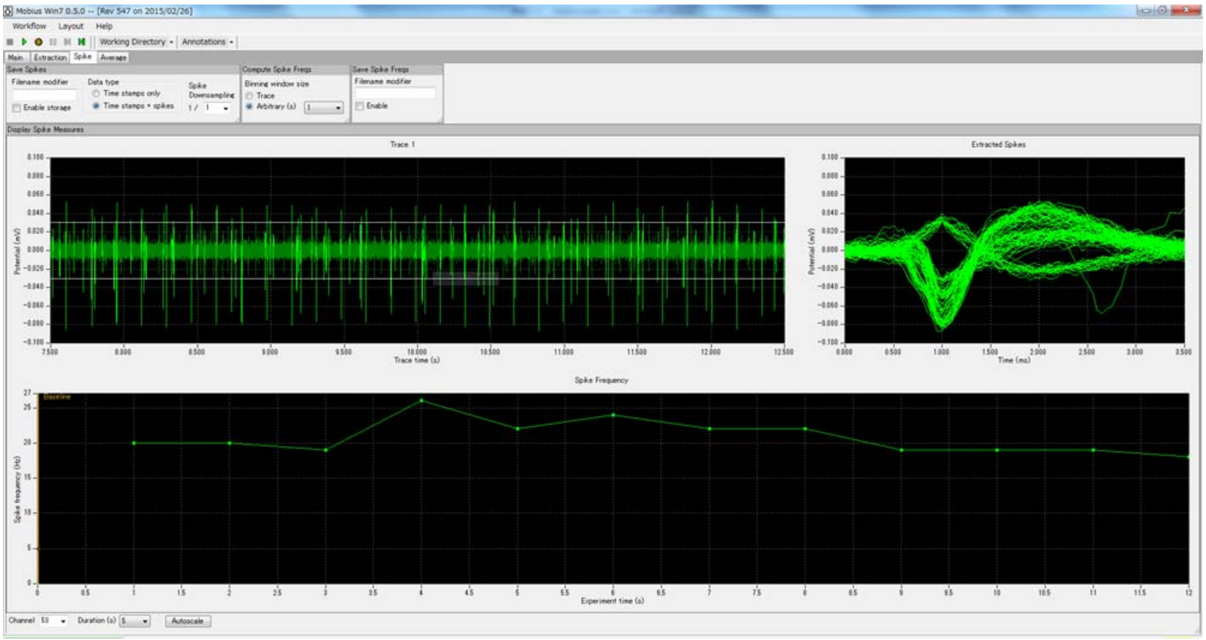
Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
1	0.014	−0.014	1	2.5	☑
2	0.015	−0.015	1	2.5	☑
3	0.015	−0.015	1	2.5	☑
4	0.016	−0.016	1	2.5	☑
5	0.017	−0.017	1	2.5	☑
6	0.015	−0.015	1	2.5	☑
7	0.011	−0.011	1	2.5	☑
8	0.010	−0.010	1	2.5	☑
9	0.014	−0.014	1	2.5	☑

SD Percentage 400

Channel 11 Duration (s) 5

☐ Autoscale Voltage (uV/div) 25 Duration (ms/div) 200 ☑ Divs ☑ CHs



Mobius Win7 0.5.0 -- [Rev 547 on 2015/02/26]

Save Spikes: Filename modifier ☐ Enable storage; Data type ○ Time stamps only ● Time stamps + spikes; Spike Downsampling 1 / 1

Compute Spike Freqs: Binning window size ○ Trace ● Arbitrary (s) 1

Save Spike Freqs: Filename modifier ☐ Enable

Channel 53 Duration (s) 5

Figure 3-1.9. Spike extraction (top) and spike frequency analysis (bottom).

3-1.5. Recording with spike sorting

-Using the *“Spike_recording(_filter)_ cluster* template-

Overview of the *“Spike_recording(_filter)_cluster”* workflow templates

The *“Spike_recording(_filter)_cluster”* workflow template consists of following modules.

Tabs	Modules
Main	Acquire MED64R2 Data / Display All Channels / Export Raw Data
Extraction	Extract Spikes Advanced
Spike	Cluster Spikes / Compute Spike Freqs / Save Spikes / Save Spike Freqs / Display Spike Measures
Filter*	Filter Raw Data (2) / Display Single Channel (2)

* ”Filter” Tab is NOT available with *the “Spike_recording_cluster”* workflow template.

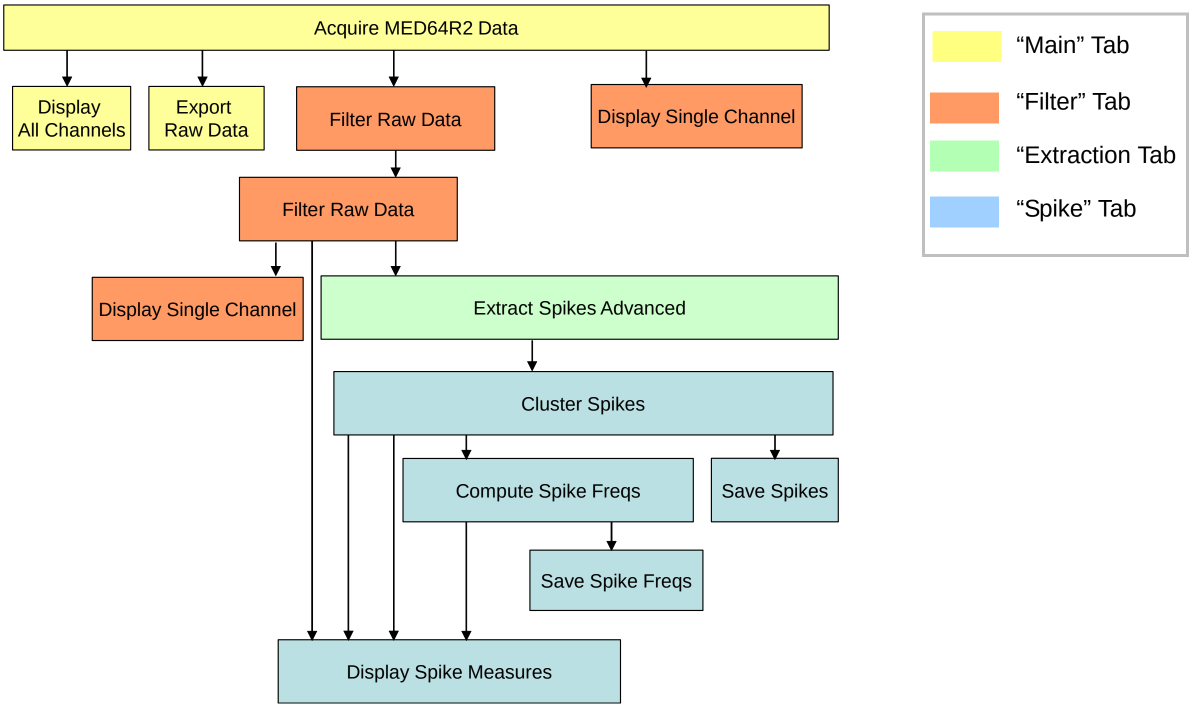


Figure 3-1.10. Module configuration for the *“Spike_recording_cluster_filter”* workflow template. The “*Spike_recording_cluster”* does not include the modules in the “Filter” tab.

Thresholds are set at the [Extract Spikes Advanced Spikes] and spikes crossing over the thresholds are extracted. The extracted spikes are sorted (clustered) based on the similarity of waveform shapes. The spike frequencies of each spike cluster are computed and graphed. You can monitor 1) that spikes are extracted, 2) different spike clusters’ waveforms grouped by clusters, and 3) cumulated spike frequencies of each spike clusters.

2 sets of [Filter Raw Data] modules are added in the “Filter” Tab. The acquired data can be filtered with selected filter menu before sent to the [Extract Spikes Advanced] module. In the “Filter” tab, the top [Display Single Channel] module shows the original non-filtered data while the bottom module shows filtered data. (Refer to the bottom figure on the Figure 3-1-7).

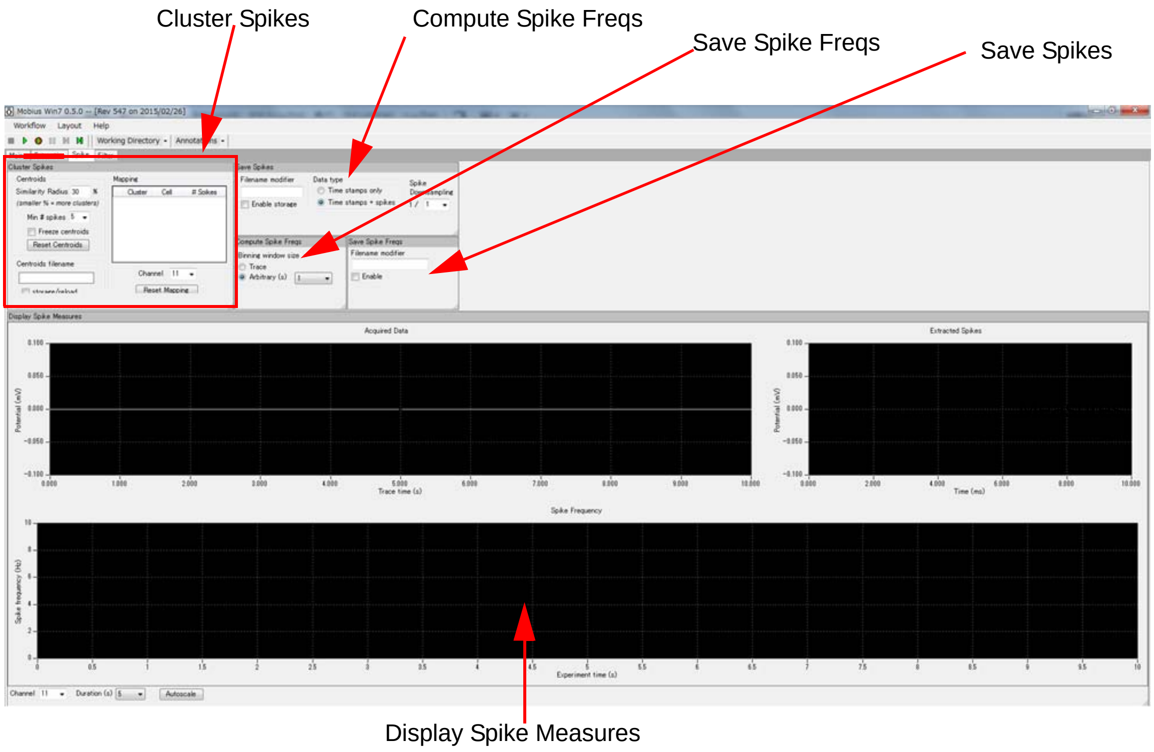


Figure 3-1.11. Control panels in the “Spikes” Tab for the “*Spike_recording(_filter)_cluster*” workflow template. The “Main” and “Filter” tabs are same as “*Spike_recording_ filter”*(Refer to Figure 3-1.5).

Recording spontaneous activities with spike sorting

The purpose of clustering spikes is to identify and separate signals referred from multiple cells acquired on a single channel (electrode) based on their waveforms similarities. The extracted spikes are treated as vector inputs to define centroids (center of mass vector). However, the centroids can not be defined unless “frozen” (ignoring any incoming spikes to alter centroids) and will change depending on the incoming spikes. Thus, on-line spike clustering is limited to give you an idea how many different kinds of spikes you have on each channel (electrode).

More importantly, clustering can slow down Mobius’ data processing and appear to stop it when the analyzing can not keep up with data processing. Thus, it is recommended to:

1) Use this workflow template to get an idea of how many kinds of spikes you have before starting acquisition.

2) Start acquisition **without the spike extraction** or sorting. **It is strongly recommended to do so when recording is performed for long term.**

When on-line spike sorting (clustering) is performed, please follow the instructions below;

- Select 100Hz for the low-cut filter. (Accidental low-frequency noise might Mobius to stop.)

- Do NOT enable any [Export] or [Save] module when raw data is saved.
  (Exporting can be done later off-line.)

- Set the Trace duration (recording time) to less than 10 minutes and the Trace intervals for longer than the trace duration so that the analysis catches up with the acquisition during the trace interval. Particularly, make the trace duration even shorter for long-term recording lasting days, weeks, or months.

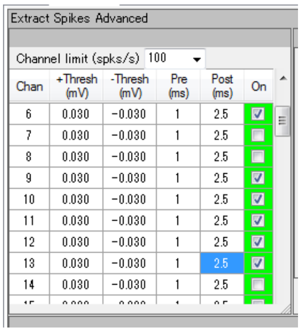
- Decrease the number of recording channels.

1. Open the *“Spike_recording(_filter)_cluster”* workflow template. (Refer to page 53)
   Start Mobius with the Green button and run the workflow template for a few minutes to see signals, and then stop.

2. Select the channels for recording. Click the box next to “Channel” in the [Acquire MED64R2 Data] module to have channel selector.

3. Select the channels for spikes’ extractions in the chart of [Extract Spike Advanced].

   - All analyses are not performed during the acquisition for the channels that are un-checked although data is saved.
   - The selection is applied to all channels easily by 1) right-clicking the check box and 2) selecting [Apply to All].



Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
6	0.030	−0.030	1	2.5	☑
7	0.030	−0.030	1	2.5	☐
8	0.030	−0.030	1	2.5	☐
9	0.030	−0.030	1	2.5	☑
10	0.030	−0.030	1	2.5	☑
11	0.030	−0.030	1	2.5	☑
12	0.030	−0.030	1	2.5	☑
13	0.030	−0.030	1	2.5	☑
14	0.030	−0.030	1	2.5	☐

Figure 3-1.12. Enabling/disenabling the spike extraction.

4. Make sure that [Export Raw Data] is NOT checked (Uncheck the check-box).

   - After raw data is saved, this can be done post acquisition.

5. Set the analysis parameters.

   1) Change the filter menu depending on the acquired signals.
   (If the *“Spike_recording”* template is used, this is not necessary.)

   2) Set the thresholds.
   - Refer to section 3-3.2 Analyzing the spike rate on pages 74-81 for detail.

   3) Modify the parameters for clustering spikes (in the [Cluster Spikes] module).
   - Refer to section 3-3.3 Spike Sorting (pages 83-87) for detail.

   4) Make sure [Save Spikes] and [Save Spike Freqs] are NOT enabled.

   - It is not recommended to enable these modules during acquisition with extracting spikes. These can be done post-acquisition once raw data is saved.

6. Run Mobius with the Green button. Make sure that the thresholds you set are OK.
   - Although thresholds can be changed during acquisition, it is not recommended to do so during acquisition.
7. Save as your own acquisition workflow. (Refer to page 54) The workflow will be saved as moflo.file.
   - The data file (.modat file) and the Analysis workflow (including all analysis module selected for the acquisition workflow and [Replay Raw Data File] module) will be saved in the same folder. (Refer to page 17)
8. Make sure that the Save raw data in the [Acquire MED64R2 Data] module is checked, then run your workflow with the Green-Red button.
   - It is recommended to start acquisition without spike extraction when you are not familiar with your experiments. **It is strongly recommended to start recording WITHOUT extractions during long term recording over several days or weeks.**
   - Mobius will require you to save the workflow as your own if you try to start Mobius with the Green-Red button without having it already.

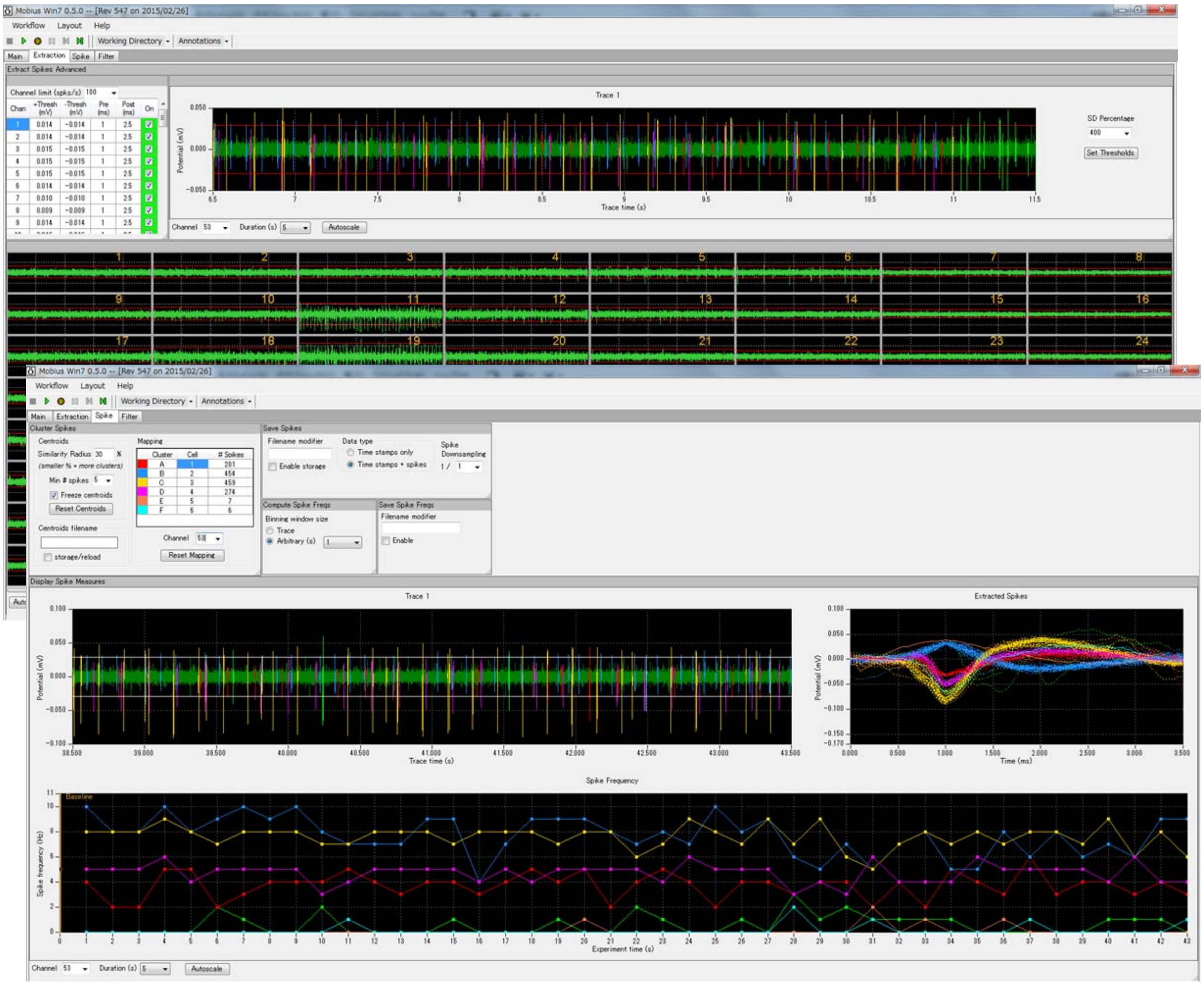


Figure 3-1-13. Spike sorting.

3-1.6. Recording without analysis

-Using *“Spontaneous_recording”* workflow template-

There is workflow template for “recording without any analysis” available in the [Basic_recording] folder. The spike extractions can slow-down Mobius’ data processing and cause it to stop when it extracts more spikes than it can analyze. It is recommended to record signals using this template until you are familiar with your preparation and experiment. Analysis and exporting can be done post-acquisition.

The *“Spontaneous_recording”* workflow template consists of following modules located in the 2 Tabs of “Main” and “Monitor”. Acquired signals are simply displayed at all 64 channels and an any selected channel. The data can be exported to binary or ASCII (“CSV” formatted text file).

Tabs	Modules
Main	Acquire MED64R2 Data / Display All Channels / Export Raw Data
Monitor	Display Single Channel

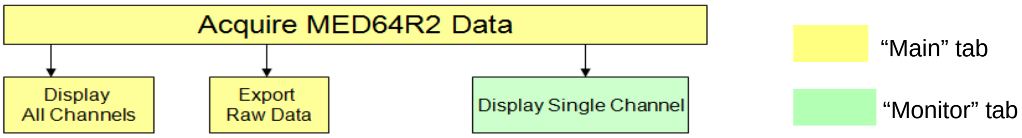


Figure 3-1.14. Module configuration for the *“Spontaneous_recording”* workflow template.

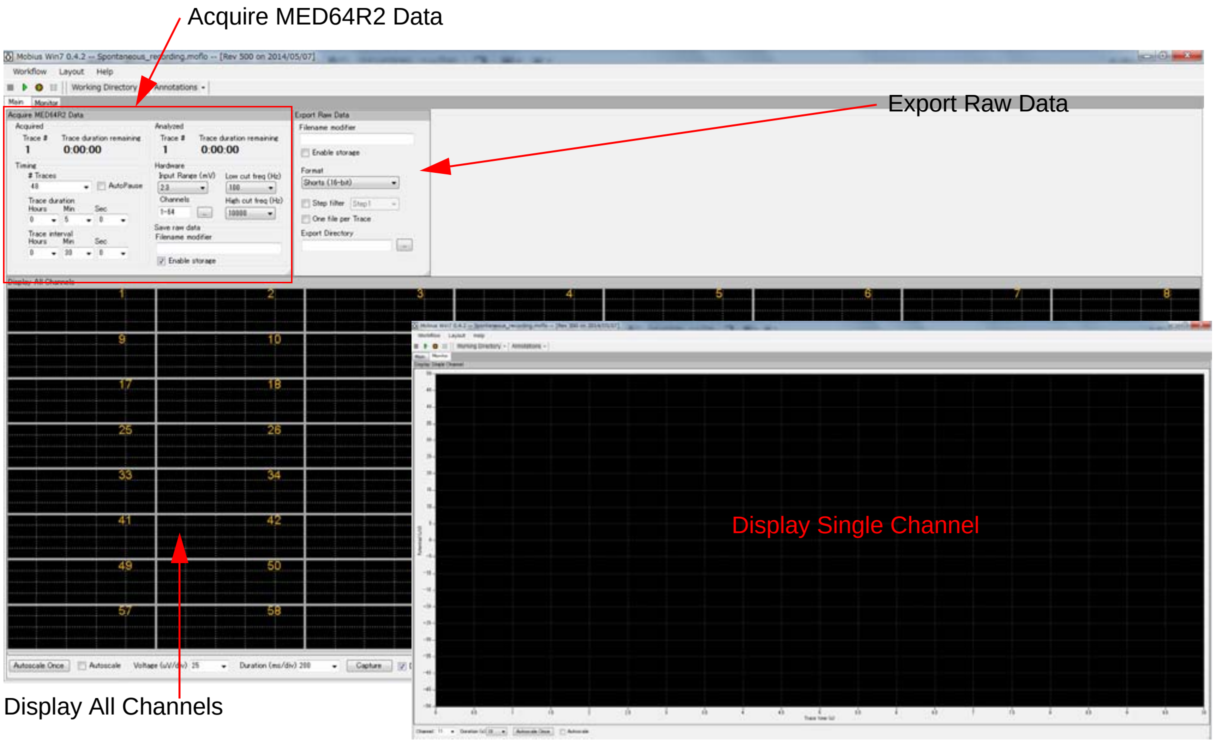


Figure 3-1.15. Control panels in the *“Spontaneous_recording”* workflow template.

3-2. Drug testing

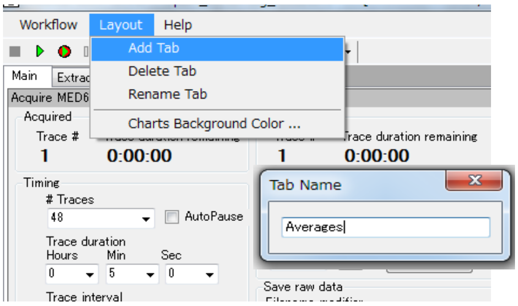
The [Compute Measure Averages] module calculates averages and standard deviations for the spike frequencies for all the experiment's phases. This is a useful tool for making a dose-response curves or comparing phases before, during and after drug applications. This section will demonstrate how to make a dose-response curve for spike frequencies using this module.

Since this module is at the end of the analysis chain, it can slow down the data processing and cause Mobius to stop during your experiment if you perform these analyses during acquisition. So, it is recommended not to use this module during recording unless you are familiar with your preparations and experiment. All analysis introduced in this section can be done post-acquisition.

An alternate solution to avoid stopping is to just set the phase-annotations (yellow bars) without the [Compute Measure Averages], and then analyze later. (The location of the phase bars will be saved in your analysis workflow.) To do this, skip following steps 1-2 and start with Step 3.

1. Open the "*Spike_recording(_filter)*" (or any other) acquisition workflow.

2. Add the [Compute Measure Averages] and [Save Measure Averages] to this workflow template.
    1) Make a new tab. Click [Layout] > [Add Tab], then type in the new tab's name. (Figure 3-2.1).
    2) Open the Editor in the new tab. (Click [Workflow] > [Edit]) (Figure 3-2.2)



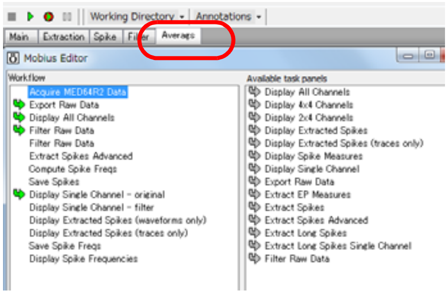


Figure 3-2.1 (left). Making a new tab.
Figure 3-2.2 (right). Opening the Editor in the new tab.

3) Click the [Compute Spike Freqs] in the left "Workflow" box, then double-click [Compute Measure Averages] in the right "Available task panels". (Figure 3-2.3, left)

You will see the [Compute Measure Averages] module shift to the left box, under the [Compute Spike Freqs] module (Figure 3-2.3, right). The control panel for this module appears in the new tab.

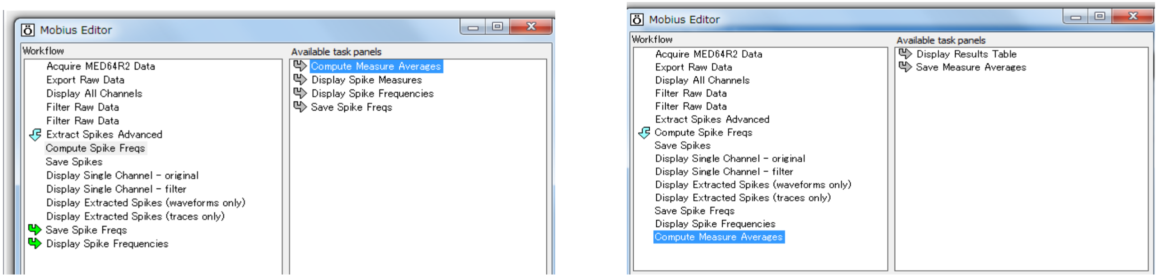


Figure 3-2.3. Adding the [Compute Measure Averages] to the workflow.

4) Click the [Compute Measure Averages] module in the left “Workflow box”, then double-click on [Save Measure Averages] in the right “Available task panels” so that the [Save Measure Averages] is moved to the “Workflow box”, under the [Compute Measure Averages] and the control panel for this module appears in the [Average] tab.

Averages and standard deviations for the spike frequencies will be measured and graphed in the [Compute Measure Averages] control panel.

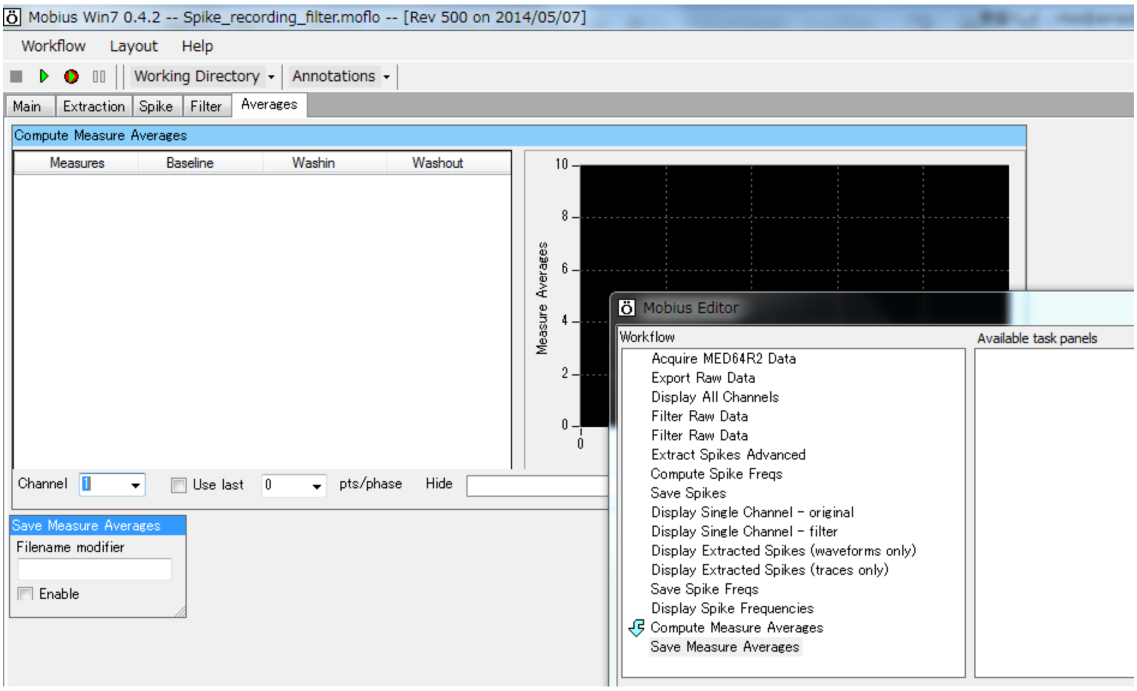


Figure 3-2.4. Control panels for the [Compute Measure Averages] and [Save Measure Averages] modules (top-left) and all modules in the completed new workflow (bottom-right).

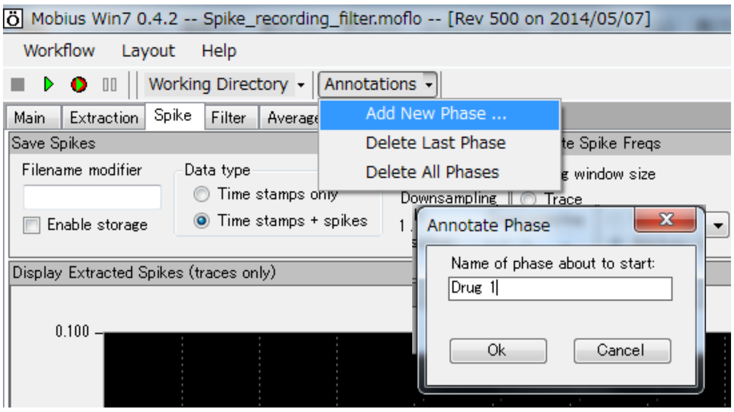
3. Set all acquisition and analysis parameters, and then start Mobius by clicking the Green button. (Refer to page 54-55,77-80)

4. When you are satisfied with the new thresholds settings and other analysis parameters, stop and save it as your own workflow. (Refer to page 54)

5. Run Mobius by clicking the Green-Red button. The first phase (baseline) will start from time 0.

6. Pause Mobius by clicking YELLOW button when the first phase (baseline) is finished. Click [Annotations] > [Add New Phase]. Type in the name for the 2nd phase. (e.g. Drug 1.) (Figure 3-2.5).

Do NOT stop with the Black button. If it is stopped, the experiment will end and new phases will NOT be added.



Name of phase about to start: Drug 1

Figure 3-2.5. Making a new phase.

7. When OK button is clicked, the yellow bar with the chosen name appears in the [Display Spike Frequencies] chart. (Figure 3-2.6)

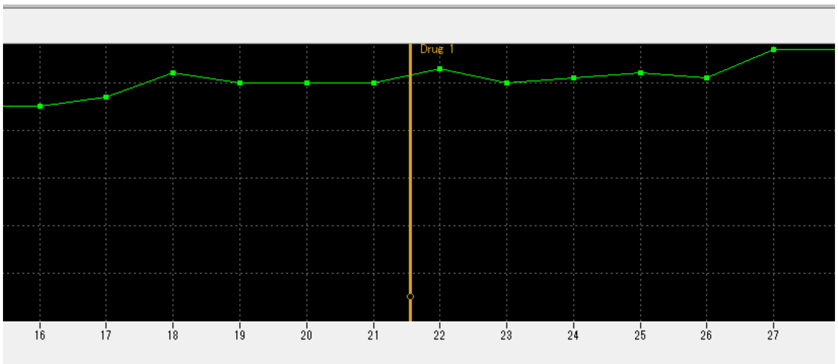


Figure 3-2.6. a new phase is added in the [Display Spike Frequencies].

8. Re-start Mobius with the Green-Red button.

   Time continues running while paused in the measurement charts.

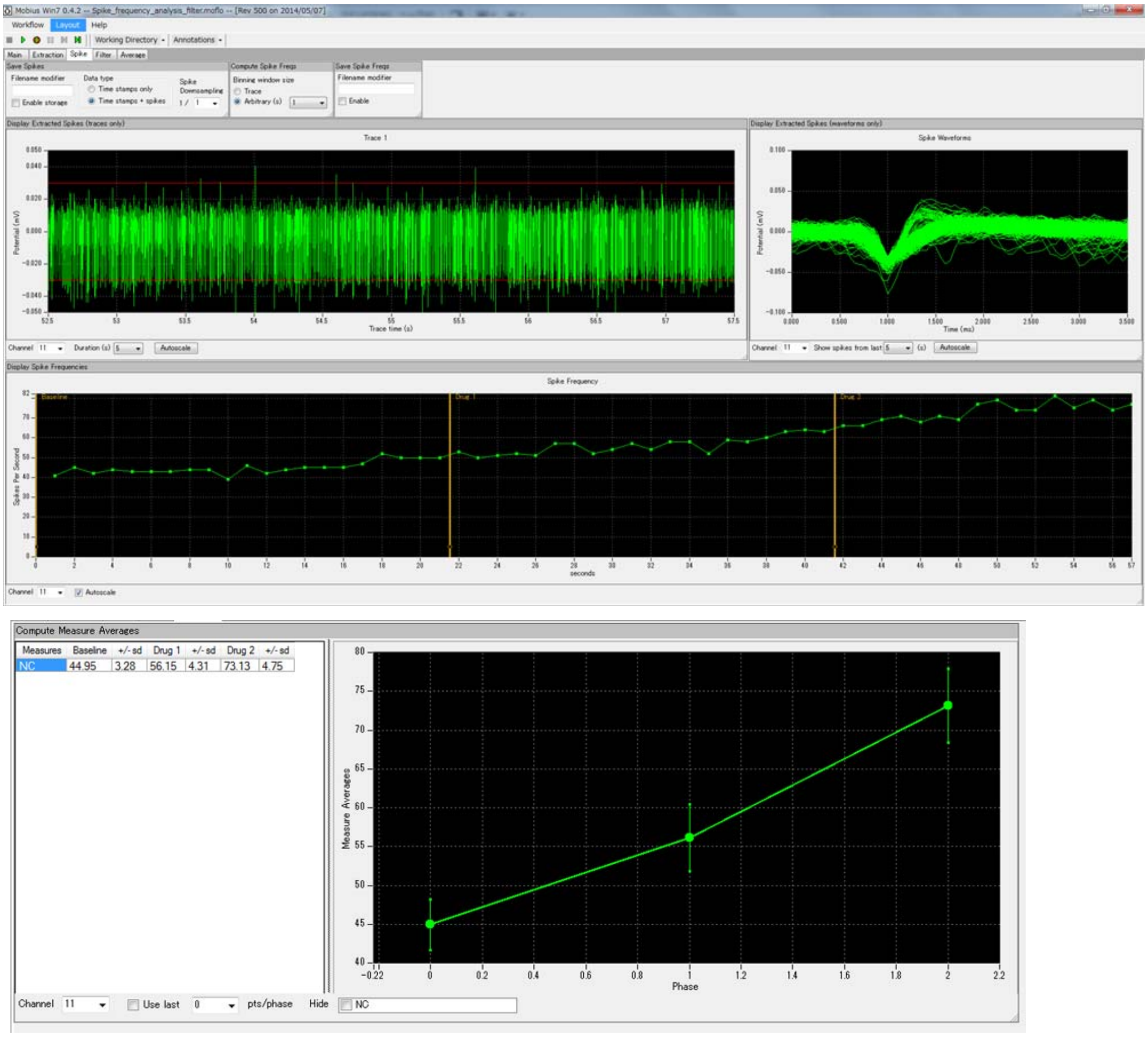
9. When the second phase (e.g. Drug 1) is finished, pause Mobius with the YELLOW button. Click on [Annotations] > [Add New phase].

10. Type in the 3rd phase name (e.g. Drug 2). The yellow bar with the name appears in the spike frequency chart when the OK button is clicked.

11. Start Mobius again with the Green-Red button. Add subsequent phases following the same procedure and run Mobius until the end of experiment.

12. You can see the averaged values for each phase on the table and their graph in the [Compute Measure Averages] (in the “Averages” tab) (Refer to Fig. 3-2.7)

    - The phase annotations (yellow bars) can be shifted by left-click and dragging when the data is analyzed later. Averages and standard deviations will be calculated based on the new phase annotations when the data is replayed.



Measures	Baseline	+/-sd	Drug 1	+/-sd	Drug 2	+/-sd
NC	44.95	3.28	56.15	4.31	73.13	4.75

Channel 11 ☐ Use last 0 pts/phase Hide ☐ NC

Figure 3-2.7. Computed measure averages. [Display Spike Frequencies] module with phase bars (top) and [Compute Measure Averages] module (bottom).

Input fields in the [Compute Measure Averages] module

1. Channel: Selects the channel to be displayed. The selection can not be changed while running workflow.

2. Use last: When this box is checked, the selected (or typed) number of data points are used for computations. This is mostly used for post-acquisition analysis (Refer to page 82).

3. Hide: hides the measurement checked in the chart.

   - Note: the chart can be zoomed up or down by clicking and changing numbers of the maximum and/or scale(s) for both X and Y axis.

3-3. Replay and analysis of acquired data

The Mobius data files (.modat) are opened and replayed with the [Replay Raw Data File] module, and analyzed using modules such as [Extract Spikes Advanced], [Cluster Spikes], and [Compute Spike Freqs]. You will design your Analysis workflow by combining those modules for replay and analysis of the acquired data. The quickest way to replay and analyze the spike data is using the Analysis workflow template, which will be introduced in this chapter.

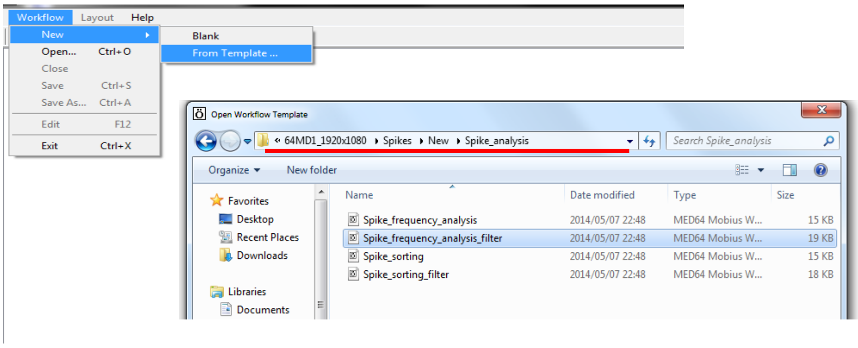
The Analysis workflow with the [Replay Raw Data File] and the analysis modules you selected for your acquisition will be automatically saved to the same directory when you run acquisition workflow with the Green-Red button. These analysis workflows can also be used for quickly performing analysis.

3-3.1. Opening and replaying acquired data

1. Open the analysis workflow template. Click [New] > [From Template]. Select either of [64MD1_1280x1024] or [64MD1_1920x1080] folder, depending on the size of your display monitor.

   Select [New] > [Spikes] > [Spike_analysis] folders, then select an analysis workflow template. Four templates are available for analyzing spontaneous neuron spikes (single unit activities).

   1) ***“Spike_frequency_analysis”***: Spikes are extracted and spike frequency is computed and graphed.
   2) ***”Spike_frequency_analysis_filter”***: 1) + filter
   3) ***“Spike_sorting”***: Spikes are extracted and sorted (clustered). Spike frequencies of clustered spikes are computed and graphed.
   4) ***“Spike_sorting_fiter”***: 3) + filter



Name	Date modified	Type	Size
Spike_frequency_analysis	2014/05/07 22:48	MED64 Mobius W...	15 KB
Spike_frequency_analysis_filter	2014/05/07 22:48	MED64 Mobius W...	19 KB
Spike_sorting	2014/05/07 22:48	MED64 Mobius W...	15 KB
Spike_sorting_filter	2014/05/07 22:48	MED64 Mobius W...	18 KB

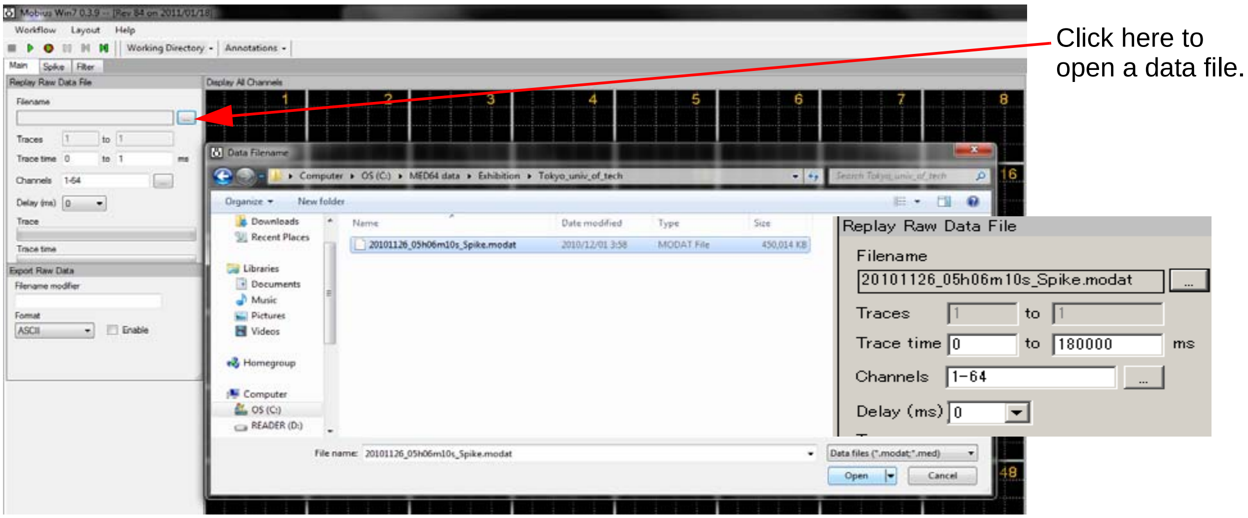
Figure 3-3.1. Opening analysis workflow template.

- In order to open your own analysis workflow, click [Workflow] > [Open], and then select the workflow file (.moflo).

5. Open the data file by clicking the box next to the “Filename” and select the desired .modat file. When the file name appears in the Filename box as seen on the right figure of the Figure 3-3.2, the data is ready to be replayed by clicking the Green button or Green-Red button.

   - Opening large data files can take several minutes. (e.g. Opening 10GB data takes over 1 minute.)

6. The acquired data is just replayed when the Green button is clicked. Clicking the Green-Red button while checking the [Enable] check-box executes the selected Export or Saves.


Click here to open a data file.

Replay Raw Data File
Filename: 20101126_05h06m10s_Spike.modat
Traces: 1 to 1
Trace time: 0 to 180000 ms
Channels: 1-64
Delay (ms): 0

Figure 3-3.2. Opening a data file. When the file name appears in the Filename box, it is ready to be replayed.

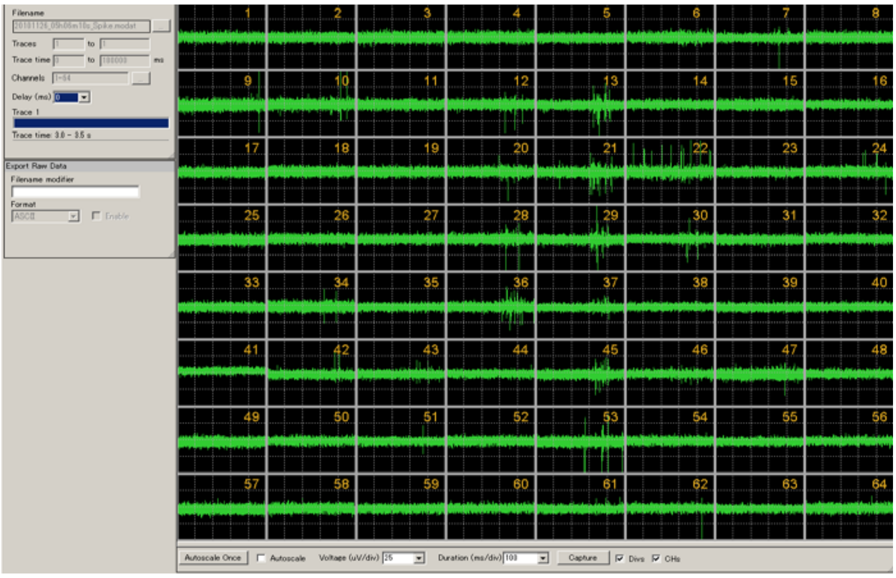


Figure 3-3.3. Replaying spike data.

Input fields for the [Replay Raw Data File] module

1. Traces: Sects the trace(s) for processing.

2. Trace time: Selects the trace time for processing.

3. Channels: Selects the channels for processing. Click the box next to the “Channels” to open the channel selector. Channels in green will be enabled.

4. Delay: Set this value to a value greater than zero to insert a delay between data “blocks” sent out by this module. It is useful for slowing down data processing.

3-3.2. Analyzing the spike frequency

This section describes how to analyze the spike rate with the "*Spike_frequency_analysis_filter* (or, *Spike_frequency_analysis)*" workflow template. These Analysis templates consist of following modules located in the Tabs of "Main", "Extraction", "Spike", "Filter", and "Averages".

Tabs	Modules
Main	Replay Raw Data / Display All Channels / Export Raw Data
Extraction	Extract Spikes Advanced
Spike	Compute Spike Freqs / Save Spikes / Save Spike Freqs / Display Spike Measures
Filter*	Filter Raw Data (2) / Display Single Channel (2)
Averages	Compute Measure Averages / Save Measure Averages

* "Filter" tab is not available with the *"Spike_friquency_analysis"* workflow template.

The raw data is filtered by the selected filter menu. Thresholds are set and spikes crossing the pre-defined thresholds are extracted in the [Extract Spike Advanced] module. Spike frequencies of all extracted spikes are computed and graphed. Raw data can be exported as a binary or ASCII ("CSV" formatted text) file. Waveforms of extracted spikes, time stamp, and spike frequency charts can be saved as "CSV" formatted text file.

The [Compute Measure Averages] module calculates and graphs the spike frequency averages for the different phases. In the "*Spike_frequency_analysis*" template, the replayed data is sent to [Extract Spikes] module without filtering.

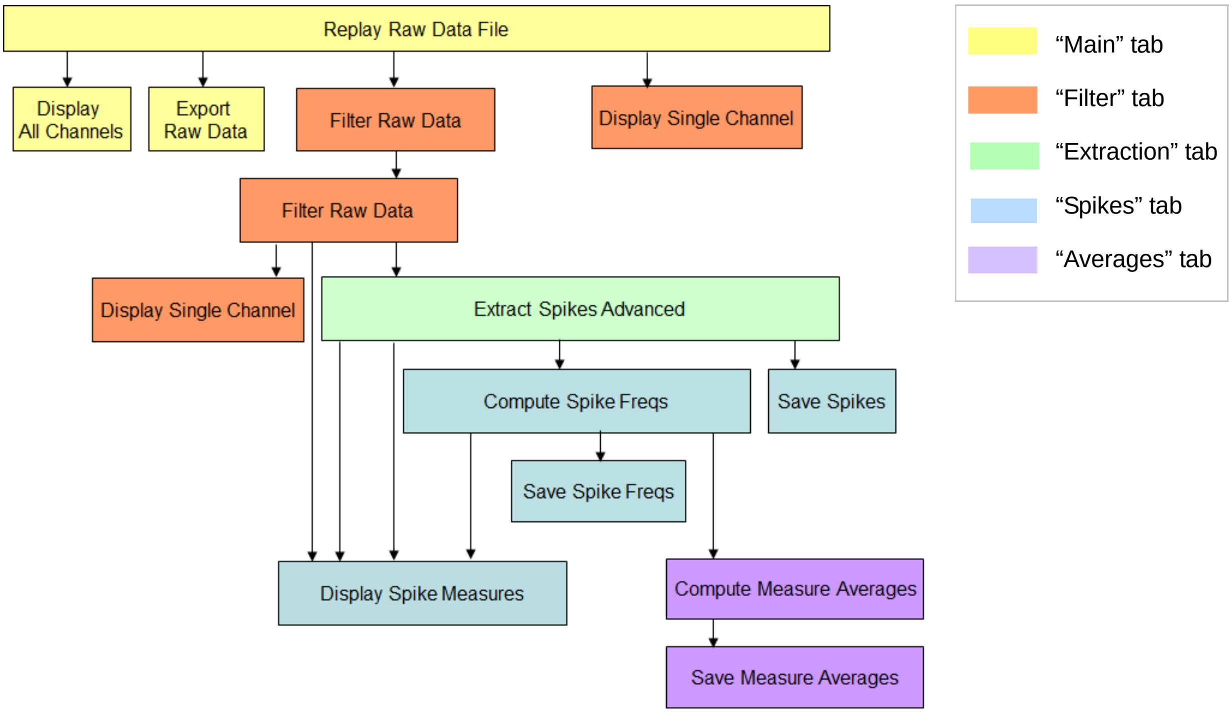
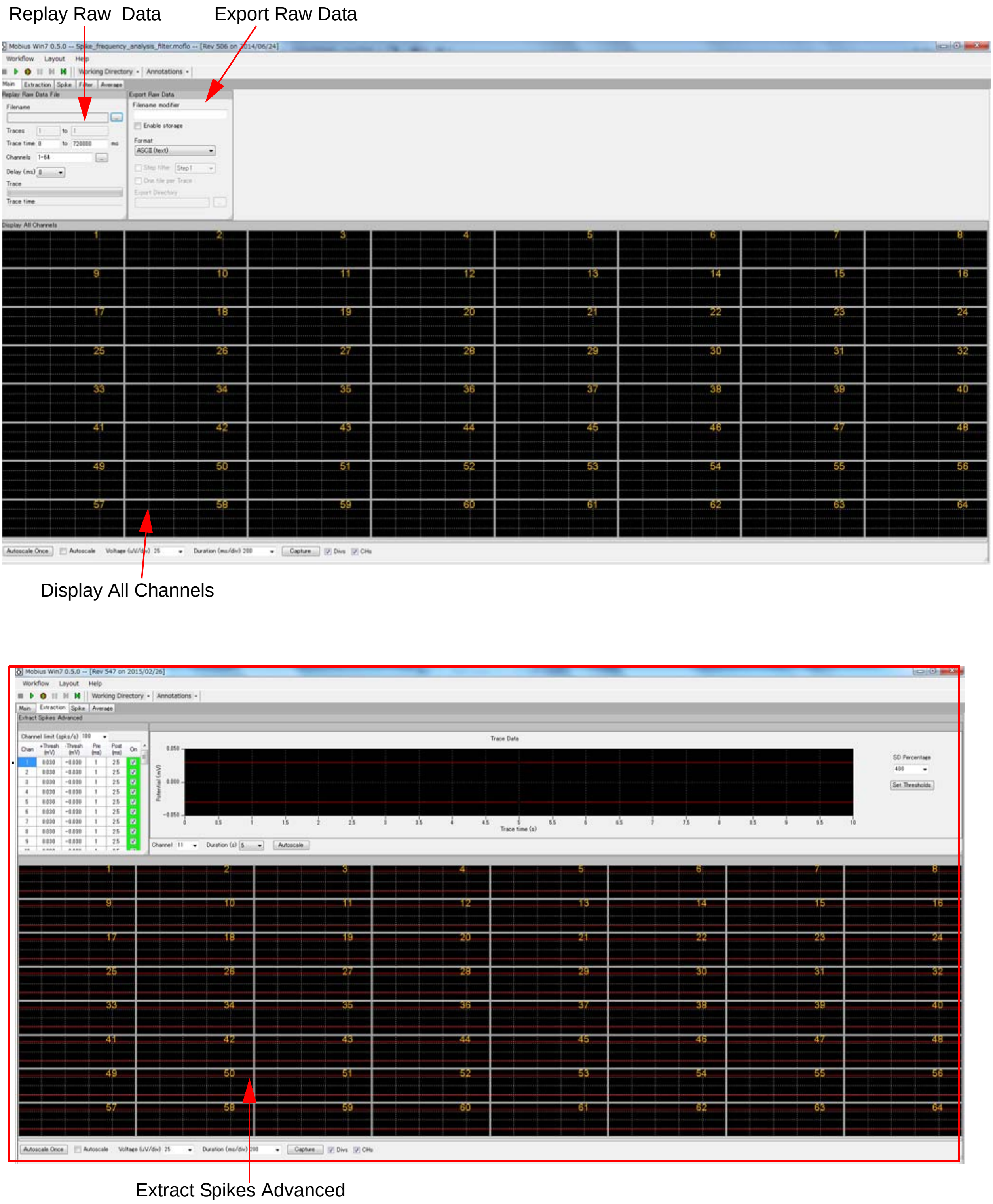


Figure 3-3.4. Module configuration for the *"Spike_frequency_analysis_filter"* workflow template.



Filename

Traces 1 to 1

Trace time 0 to 720000 ms

Channels 1-64

Delay (ms) 0

Trace

Trace time

Filename modifier

☐ Enable storage

Format ASCII (text)

☐ Step filter Step1

☐ One file per Trace

Export Directory

☐ Autoscale Voltage (uV/div) 25 Duration (ms/div) 200 ☑ Divs ☑ CHs

Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
1	0.030	-0.030	1	2.5	☑
2	0.030	-0.030	1	2.5	☑
3	0.030	-0.030	1	2.5	☑
4	0.030	-0.030	1	2.5	☑
5	0.030	-0.030	1	2.5	☑
6	0.030	-0.030	1	2.5	☑
7	0.030	-0.030	1	2.5	☑
8	0.030	-0.030	1	2.5	☑
9	0.030	-0.030	1	2.5	☑

SD Percentage 400

Channel 11 Duration (s) 5

☐ Autoscale Voltage (uV/div) 25 Duration (ms/div) 200 ☑ Divs ☑ CHs

Figure 3-3.5. Control panel for the *"Spike_frequency_analysis_filter"* workflow template. "Main" tab (top), and "Extraction" tab (bottom).

Save Spikes: Filename modifier; ☐ Enable storage; Data type: ○ Time stamps only ● Time stamps + spikes; Spike Downsampling 1 / 1

Compute Spike Freqs: Binning window size: ○ Trace ● Arbitrary (s) 1

Save Spike Freqs: Filename modifier; ☐ Enable

Channel 11; Duration (s) 5

Filter Raw Data: Filter type DC remove; Typical spike length (ms) 4

Filter Raw Data: Filter type None

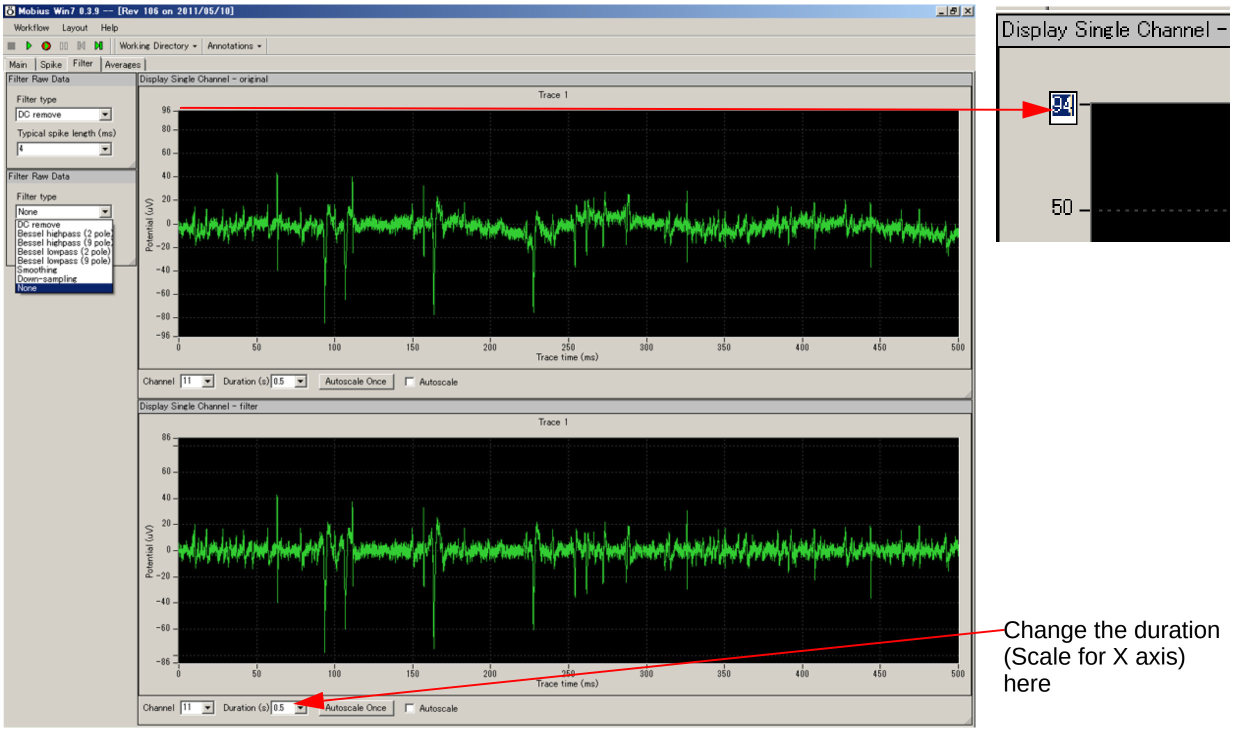
Channel 11; Duration (s) 10; ☐ Autoscale

Figure 3-3.6. Control panels in the *"Spike_frequency_analysis_filter"* workflow template. "Spike" tab (top) and "Filter" tab (bottom). The *"Spike_frequency_analysis"* does not have the "Filter" tab.

Setting the filter menu

Raw data can be filtered using the [Filter Raw Data] module (in the Filter tab) before spikes are extracted. Therefore, spikes are extracted from filtered data rather than raw data.

1. Replay data by clicking the Green button to have signal in the Display Single Channel.
2. The workflow template allows you to set 2 different types of filters. Select the type of filter in the “Filter Type” drop down menu and the cut-off freqs (or time) scale in the bottom drop down menu below.
3. The top chart displays the raw data and the bottom chart displays the filtered data. The scale for amplitude (Y axis) can be modified by clicking and typing directly in the maximum and /or minimum amplitude values (Figure 3-3.7).
4. The time (X axis) can be changed by selecting numbers in the “Duration” drop down menu. The maximum duration is 10 sec (Figure 3-3.7).


Mobius Win7 0.3.9 -- [Rev 106 on 2011/05/10]

Filter Raw Data
Filter type: DC remove
Typical spike length (ms): 4

Filter Raw Data
Filter type: None

Channel 11 Duration (s) 0.5

Change the duration (Scale for X axis) here

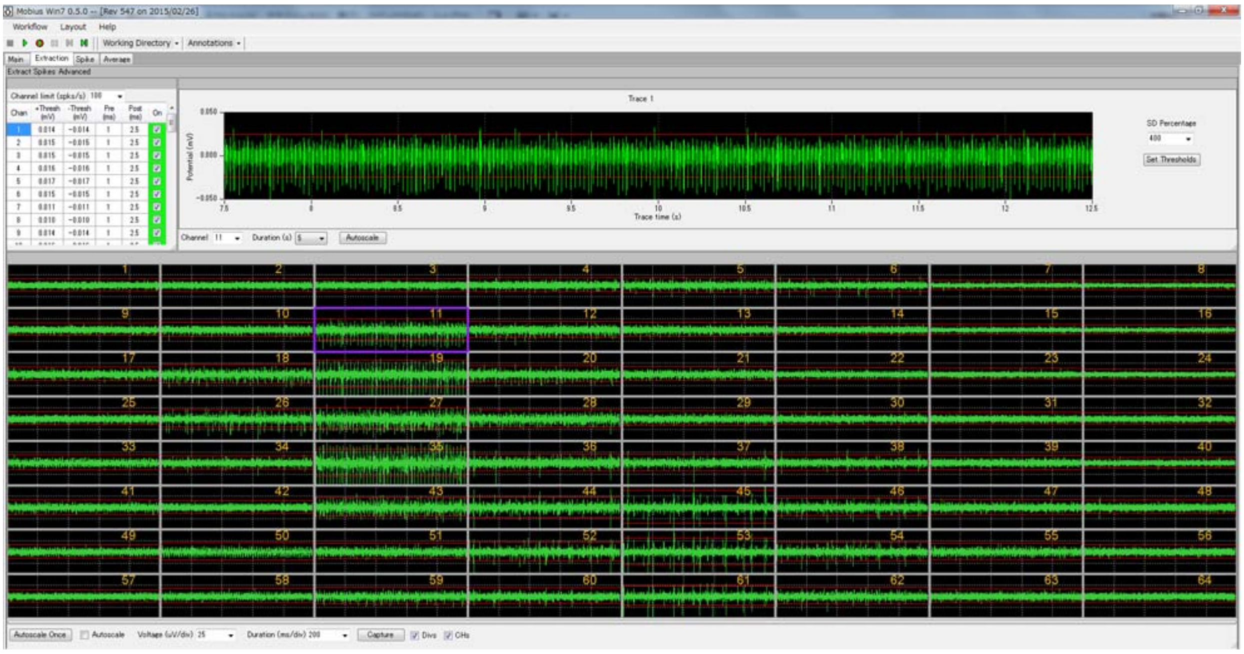
Figure 3-3.7. Setting the filter menu in the Filter tab. The top display shows the raw data and the bottom display shows the filtered data.

Setting the thresholds

Set the thresholds in the [Extract Spike Advanced] module (in the [Extraction] tab). The module has 4 options to set the thresholds:

1. Typing the values in the chart.
2. Moving the bars in the Trace chart.
3. Moving the bars in the 64ch display.
4. Setting the Standard Deviation (SD) percentage of signal amplitude.

Extracted spikes are highlighted with light-green at both single and all channels display.



Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
1	0.014	−0.014	1	2.5	☑
2	0.015	−0.015	1	2.5	☑
3	0.015	−0.015	1	2.5	☑
4	0.016	−0.016	1	2.5	☑
5	0.017	−0.017	1	2.5	☑
6	0.015	−0.015	1	2.5	☑
7	0.011	−0.011	1	2.5	☑
8	0.010	−0.010	1	2.5	☑
9	0.014	−0.014	1	2.5	☑

Channel 11

Duration (s) 5

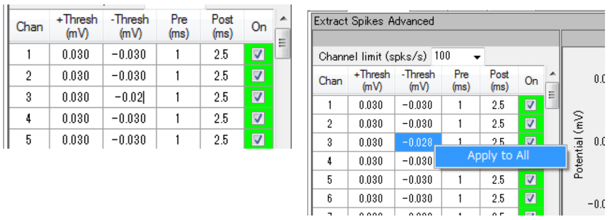
Autoscale

Voltage (uV/div) 25

Duration (ms/div) 200

Figure 3-3.8. [Extract Spikes Advanced] module. Thresholds are set in this module.

The top-left chart allows you to set the values for thresholds as well as pre/post times. Double-click and type in new values directly to change them. A value can be applied to all other channels by right-clicking the number and selecting “Apply to ALL”. (Figure 3-3.9)



Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
1	0.030	−0.030	1	2.5	☑
2	0.030	−0.030	1	2.5	☑
3	0.030	−0.02	1	2.5	☑
4	0.030	−0.030	1	2.5	☑
5	0.030	−0.030	1	2.5	☑

Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
1	0.030	−0.030	1	2.5	☑
2	0.030	−0.030	1	2.5	☑
3	0.030	−0.028	1	2.5	☑
4	0.030	−0.030			
5	0.030	−0.030	1	2.5	☑
6	0.030	−0.030	1	2.5	☑

Figure 3-3.9. Changing the thresholds in the chart (left). The value can be applied to all channels at once (right).

The extractions are on or off using the “On” check box. The selection here is also applied to all channels by right-clicking the check box and selecting “Apply to All”.

- Please refer to Mobius User Guide (5.2.4. Extract Spikes) for more information on thresholds and pre/post times.
- Channel limit selector: the maximum numbers of spikes to be extracted per 1 second per channel is set here. This is useful for preventing Mobius crash due to unexpected high-frequency noise during acquisition.

Thresholds can be changed by clicking and dragging the red threshold bar both in the single channel display or within the all channels display chart. Simply click the red bar, and the drag it to desired location.

Thresholds can be set based on “RMS percentage of the baseline noise”. Select the value for percentage and click the [Set thresholds] button.

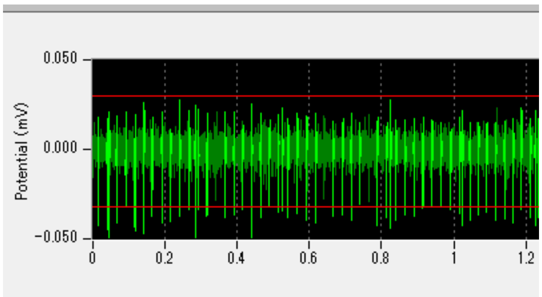


Figure 3-3.10. Moving threshold bars (red). Click and drag them to desired location to set thresholds.

[Display Spike Measures] charts

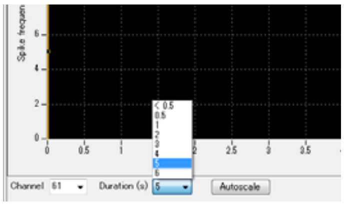
The [Display Spike Measures] has 3 charts and show you the analysis in a selected channel. A channel for displayed is selected in the channel selector in the bottom-left.

1) Trace 1 chart (top-left):
   Spike extractions are displayed. Extracted spikes are high-lighted in light green color.

2) Extracted Spikes chart (top-right):
   Extracted spikes’ waveforms are displayed.

3) Spike Frequency chart (bottom):
   Time course of spike frequency are graphed here.

The duration of the display in the 1) and 2) can be changed in the “Duration” drop down menu (Figure 3-3.11). The time (X) and amplitude (Y) scale can be changed with by clicking the minimum and/or maximum value and typing a new number (Figure 3-3.12). (Scales in the Spike Frequency Chart are unchangeable.



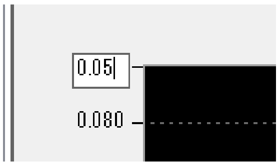
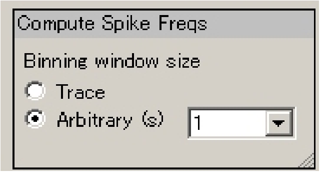


Figure 3-3.11. [Left] Channel selector and Duration drop down menu.
Figure 3-3.12. [Right] Changing the scale for the Time (x) by clicking the maximum value and typing a new number

Setting the parameters for computing spike frequency

Spike frequencies for all extracted spikes are computed with the [Compute Spike Freqs] module. Mobius gives **instantaneous spike frequency for each second at 1 second resolution.** The default setting has 1 second selected for the Binning window size. Extracted spikes are counted every 1 second. Selecting “Trace” instead of “Arbitrary (s)” means the spikes are counted for the whole trace.



Compute Spike Freqs

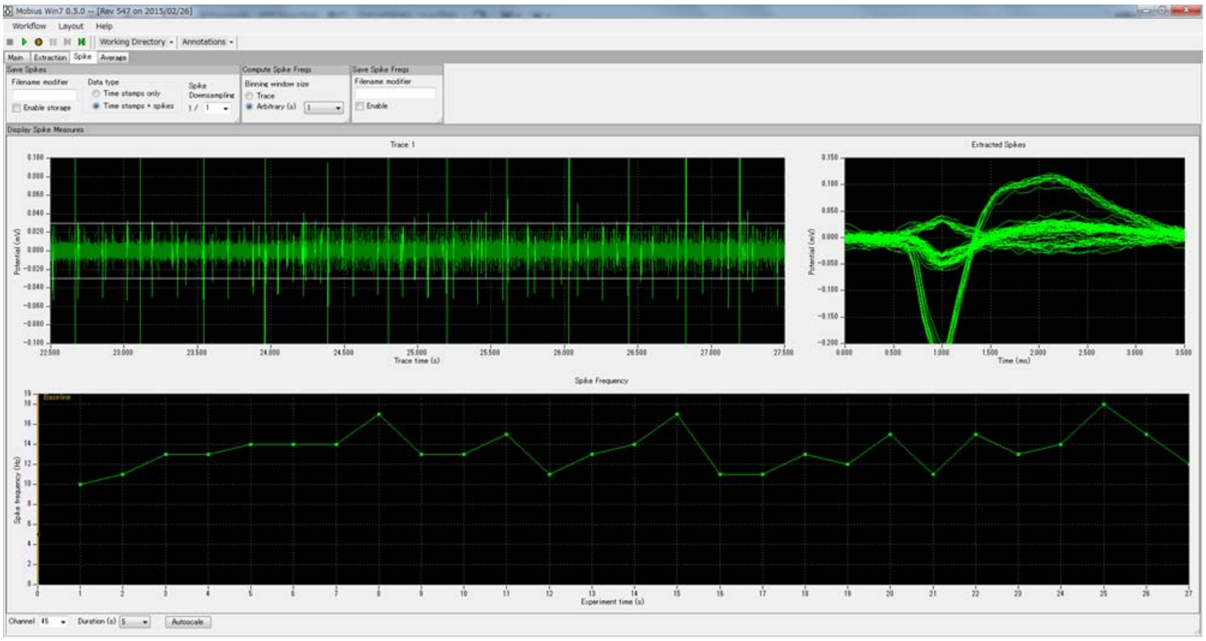
Binning window size

○ Trace

● Arbitrary (s) 1

Figure 3-3.13. [Compute Spike Freqs] control panel.

After setting all analysis parameters, you are now ready to output the data. Please refer to Data Output (for page 86) for exporting the analysis.



Mobius Win7 0.5.0 -- [Rev 547 on 2015/02/26]

Save Spikes: Filename modifier; ☐ Enable storage; Data type: ○ Time stamps only, ● Time stamps + spikes; Spike Downsampling 1 / 1

Compute Spike Freqs: Binning window size: ○ Trace, ● Arbitrary (s) 1

Save Spike Freqs: Filename modifier; ☐ Enable

Channel 45 Duration (s) 5

Figure 3-3.14. Spikes extractions and their frequencies analysis.

NOTE:

The 3 charts in the [Display Spike Measures] are available as independent modules/control panels of [Display Extracted Spikes (traces only)], [Display Extracted Spikes (waveforms only)], and [Display Spike Frequencies] for more flexibility.

3-3.3. Computing spike frequency averages according to phase

-Making the dose-response-curve-

The [Compute Measure Averages] module calculates spike frequency averages and standard deviations for all experimental phases. This is a useful tool for making a dose-response curve. This section will walk you through making a dose-response curve for spike frequency using the *“Spike_ frequency_analysis(_filter)”* workflow template in which this module is included.

If you would like to add this module to your own analysis workflow, please refer to the step1-2 at the section 3-2. Drug testing on pages 66-67. (If you added phase bars while recording, the location of the phase bars were saved to your analysis workflow.)

1. Set the all analysis parameters according to the previous section 3-3.2 Analyzing the spike frequency (Pages 74-81).

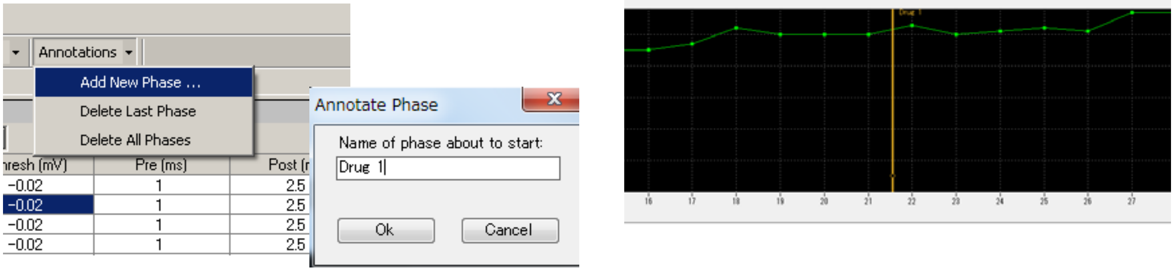
2. Open the “Spike” tab and replay data by clicking the Green button. The first phase (baseline) will start from 0.

   When the first phase is finished, pause Mobius with the YELLOW button. Click [Annotations] > [Add New phase]. Type in the name for the 2nd phase. (Figure 3-3.15, left)

   When the OK button is clicked, the yellow bar with its name appears on the Spike Frequency chart (Figure 3-3.15, right).

   NOTE:

   If Mobius is stopped with the Black button, replaying is terminated and a phase bar can not be added.



Name of phase about to start: Drug 1

resh (mV)	Pre (ms)	Post (r
-0.02	1	2.5
-0.02	1	2.5
-0.02	1	2.5
-0.02	1	2.5

Figure 3-3.15. Adding a new phase bar (left) and the new phase bar added on the spike frequency chart (right).

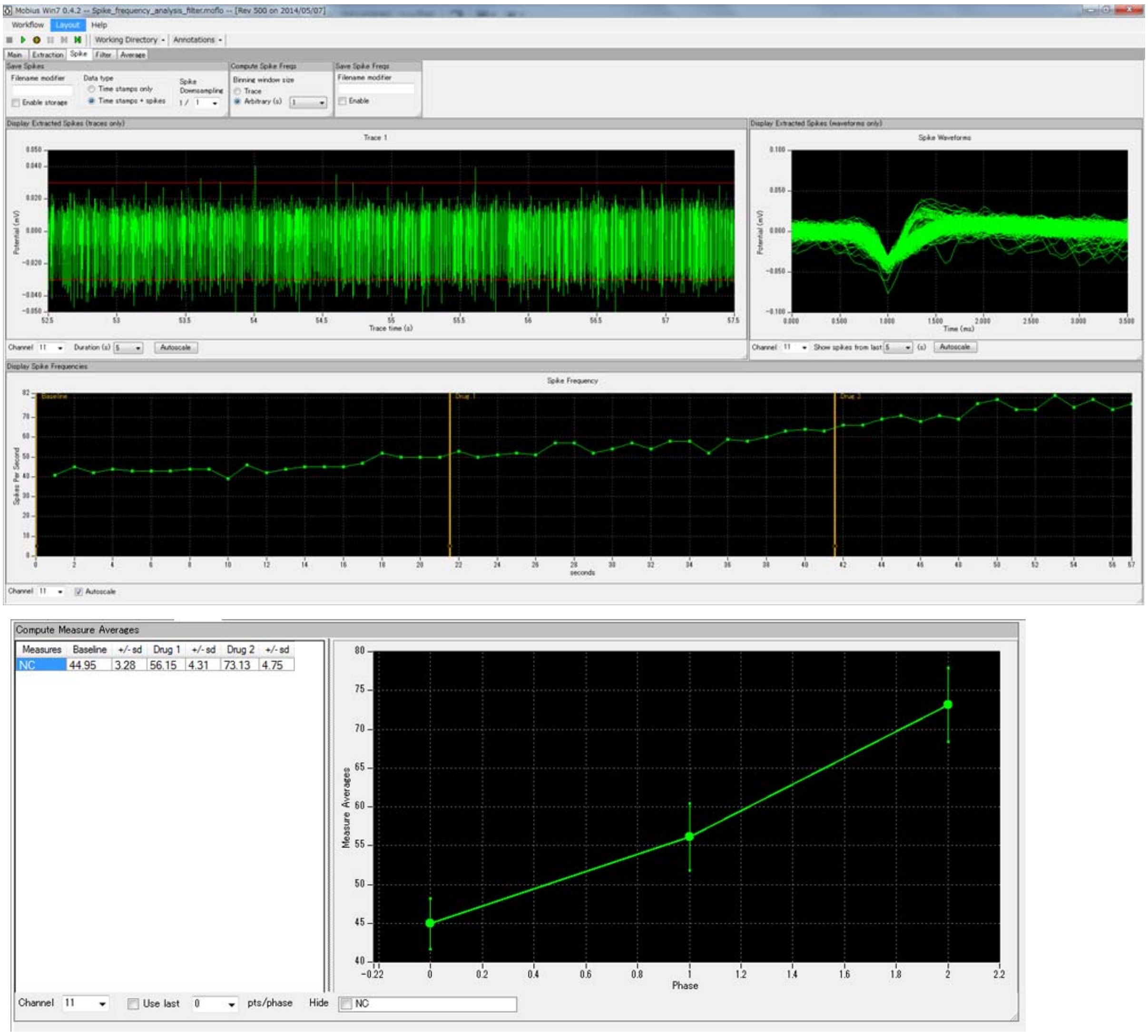
3. Start Mobius by clicking the Green button again. When the 2nd phase is finished, pause Mobius with the YELLOW button and make a new phase.

4. Replay the data to the end. Averages and standard deviations are computed and graphed for each phase (dose) in the [Compute Measure Averages] module in the “Average” tab (Figure 3-3.16).

5. The phase annotations (yellow bars) can be shifted by clicking and dragging. The averages and standard deviations are recalculated once Mobius is run with the Green or Green-Red button after changing the locations of phase bars.

6. If you are satisfied with the location of the phase bar, save it as your own analysis workflow. (Refer to page 54)

7. In order to save the average chart to a "CSV" file, check the check-box for the [Save Measures Averages] module, then run Mobius with the GREEN-RED button.



Measures	Baseline	+/- sd	Drug 1	+/- sd	Drug 2	+/- sd
NC	44.95	3.28	56.15	4.31	73.13	4.75

Channel 11 ☐ Use last 0 pts/phase Hide ☐ NC

Figure 3-3.16. [Display Spike Frequencies] module with phase annotation bars (top) and the graphs for averages and standard deviations in the [Compute Measure Averages] (bottom).

Input fields for [Compute Measure Averages] module

1. Channel: The channel to be displayed is selected here. Channels can NOT be changed during replay.

2. Use last: When this box is checked, the selected (or typed) number of data points are used for computations. For example, if 10 is selected, average and standard deviation are computed for the last 10 traces in each phase.

3. Hide: hides the measurement checked in the chart.

   - Note: the chart can be zoomed up or down by clicking and changing numbers of the maximum and/or minimum scale(s) for both X and/or Y axis.

3-3.4. Spike sorting (clustering)

The purpose of sorting (clustering) spikes is to identity and separate signals referred from multiple cells acquired on a single channel (electrode) based on their waveform similarity.
This section will teach you how to sort (cluster) spikes and analyze the spike frequency using the *"Spike_sorting(_filter)"* workflow template. This template include following modules in the tabs of "Main", "Spike", and "Filter"

Tabs	Modules
Main	Replay Raw Data / Display All Channels / Export Raw Data
Extraction	Extract Spikes Advanced
Spike	Cluster Spikes / Compute Spike Freqs / Save Spikes / Save Spike Freqs / Display Spike Measures
Filter*	Filter Raw Data (2) / Display Single Channel (2)

The raw data is filtered by the selected filter menu. Thresholds are set and spikes that cross the pre-defined threshold are extracted in the [Extract Spike Advanced]. The extracted spikes are sorted based on the waveform shape similarity. Clustered spike frequency is computed and graphed. Raw data can be exported as binary or ASCII ("CSV" format text) file. Extracted spike waveforms, time stamp, spike centroids and spike frequency charts can be saved as "CSV" format text file.

With the *"Spike_sorting"* workflow template, the replayed data is not filtered but sent directly to the [Extract Spikes Advanced].

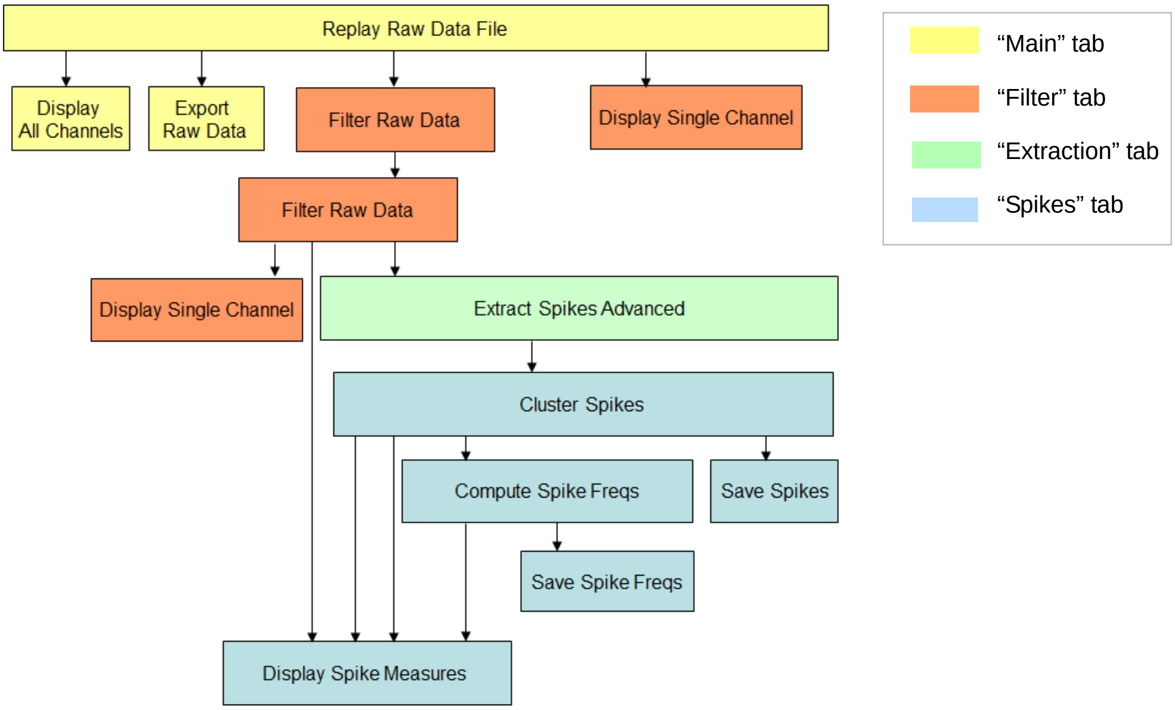
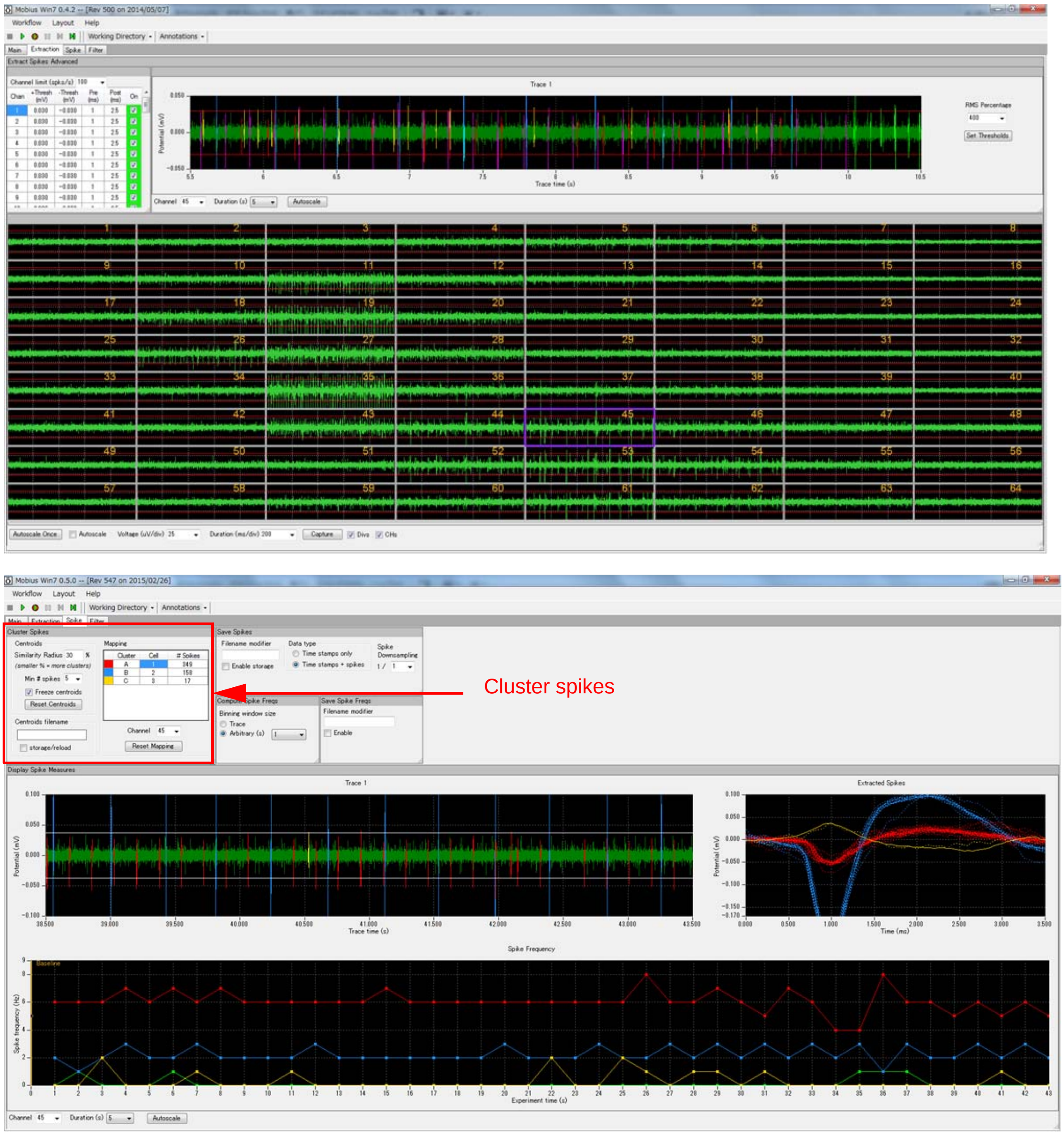


Figure 3-3.17. Module configuration for the *"Spike_sorting_filter"* workflow template.



Cluster spikes

Figure 3-3.18. “*Spike_sorting_filter*” workflow template. [Extraction] tab (top) and [Spikes] tab (bottom). Spikes are color-coded at all display panels.

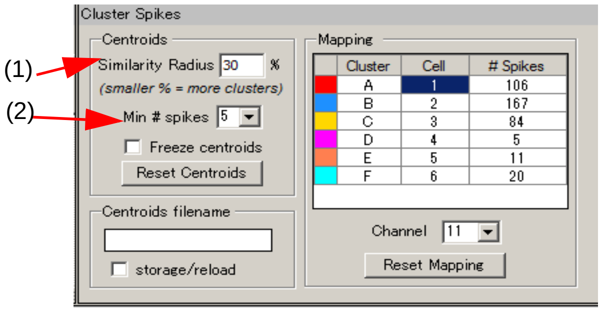
Set the filter menu and thresholds with the same procedure described in the page 77-81.

Setting the menu for spike sorting (clustering)

As mentioned above, the purpose of sorting (clustering) spikes is to identify and separate signals referred from multiple cells acquired on a single electrode based on their waveforms.
The sensitivity of the clustering process is changed using (1) Similarity Radius and (2) Min # spikes parameters.

The default settings are 30% for the Similarity Radius and 5 is for Min # spikes. This means: when spikes with similar waveforms occurs 5 times, a valid cluster is identified and its centroid (an “average” spike shape) is determined. Spikes not assigned to any cluster are not color coded. They will remain green until there are 5 spikes with similar waveforms.

Extracted spikes that come sequentially are assigned to a Cluster (A,B,C, etc.) if their similarity to the Cluster’s centroid is higher than 30%. Spikes with less than 30% similarity to the Centroid will remain green. Un-assigned spikes will be marked as NC in the saved data.



Similarity Radius 30 %
(smaller % = more clusters)
Min # spikes 5
☐ Freeze centroids
☐ storage/reload
Channel 11

	Cluster	Cell	# Spikes
🟥	A	1	106
🟦	B	2	167
🟨	C	3	84
🟪	D	4	5
🟧	E	5	11
🟦	F	6	20

Figure 3-3.19. Cluster Spikes module.

Each Cluster (A,B,C, etc in the Mapping chart) has a different color code (e.g. A with red) and its waveforms and frequencies are displayed with the same color code on the charts in all display modules that come under the [Cluster Spikes] module.

The Parameters to establish valid sets of clusters can be modified as followings:

(1) Similarity

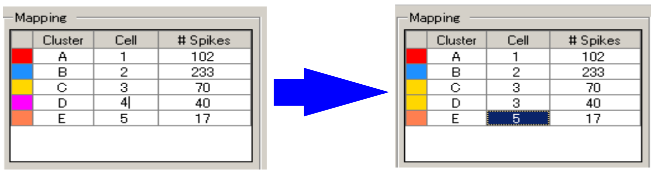
This parameter determines how sensitive the sorting (clustering) is to variations in spike waveforms.

Larger numbers > more sensitive to spike shape variations > produce FEWER clusters.
Smaller numbers > less sensitive to spike shape variations > produce MORE clusters

(2) Min # spikes

This determines how many spikes need to come to be recognized as a Cluster.
Increasing this value means FEWER clusters.

After replaying data to determine centroids, you may see that different clusters (e.g. C and D) actually represent the same cell and you may want to merge them. Change the Cell number for Cluster D to 3 by double-clicking and directly typing in this number so that the Cluster D is merged to the Cluster C (Figure 3-3.20).



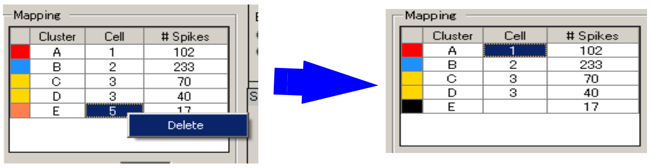
	Cluster	Cell	# Spikes
🟥	A	1	102
🟦	B	2	233
🟨	C	3	70
🟪	D	4	40
🟧	E	5	17

	Cluster	Cell	# Spikes
🟥	A	1	102
🟦	B	2	233
🟨	C	3	70
🟨	D	3	40
🟧	E	5	17

Figure 3-3.20. Merging Cluster D and C.

For another example, you may see that Cluster E is probably just noise and you want to remove it from your analysis. In this case, you can delete this cluster by deleting the number at the Cell column. (Right click the Cell number, then select Delete.) (Figure 3-3.21)

Those changes can be reset by clicking [Reset Mapping] button.



	Cluster	Cell	# Spikes
🟥	A	1	102
🟦	B	2	233
🟨	C	3	70
🟨	D	3	40
🟧	E	5	17

	Cluster	Cell	# Spikes
🟥	A	1	102
🟦	B	2	233
🟨	C	3	70
🟨	D	3	40
⬛	E		17

Figure 3-3.21. Deleting a Cluster.

Once you have a valid set of Clusters that you believe represent different cells, you can freeze them by checking the [Freeze centroids] check-box. This means that incoming spikes will NOT alter the definition of the existing clusters (their centroids), nor cause new clusters to form. Centroids must be frozen to be definitely established. Otherwise, centroids are changing whenever new spike is assigned to a cluster.

CAUTION:

Freeze Centroid to export all analysis data with accurate clustering.

Display of sorted spikes

Spikes' extractions are displayed in the "Trace 1" chart while the waveforms for extracted spikes are displayed in the "Extracted Spikes" chart in the [Display Spike Measures]. Be noted that **extracted spikes are color coded** according to cluster since these modules are under [Cluster Spikes] in the *"Spike_sorting(_filter)"* workflow template. Spikes NOT assigned to any Cluster are displayed in green.

In the "Extracted Spikes" chart, you will see waveforms with solid lines and ones with dot lines. The waveforms with solid lines are the centroid waveforms and the dotted lines are extracts spikes' waveforms (Figure 3-3.22). (Refer to page 79 for further information and modifications for these charts.)

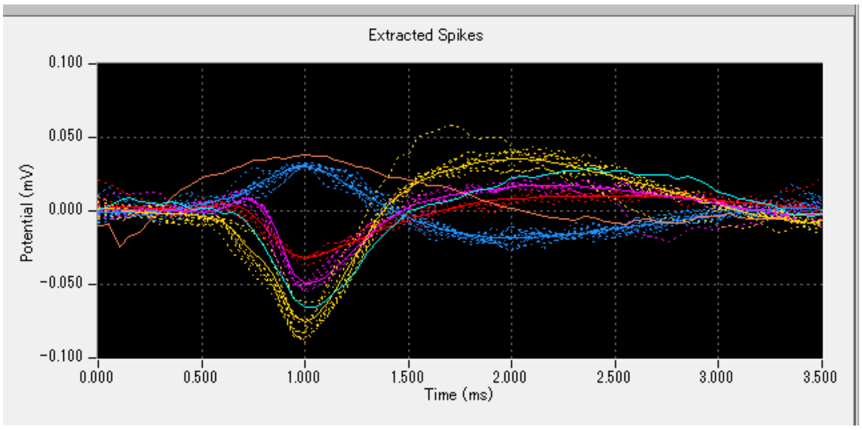


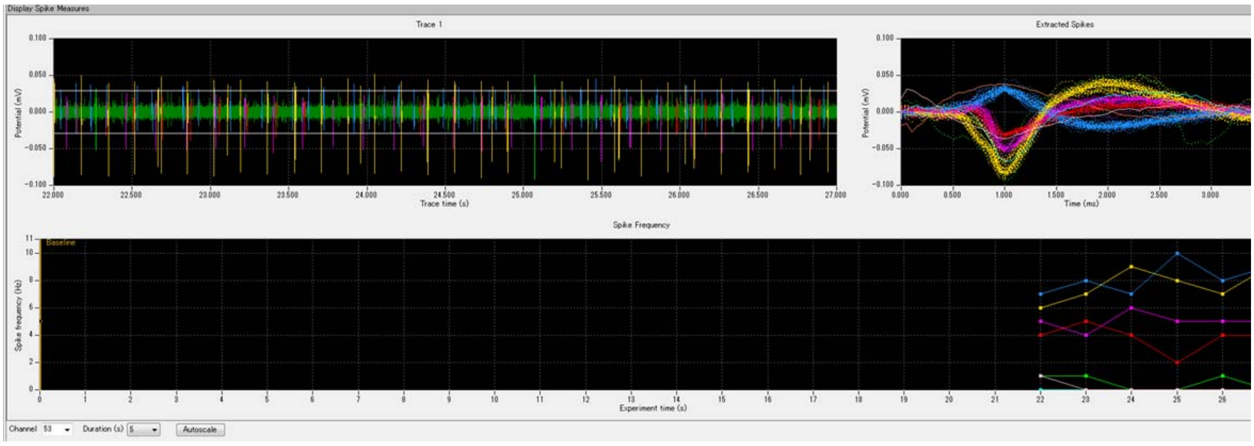
Figure 3-3.22. "Extracted Spikes" chart in the *"Spike_sorting_(filter)"* template. Solid lines show centroid waveforms while dot lines extracted spikes' waveforms.

Computing spike frequencies for sorted spikes

In the *"Spike_sorting(_filter)"* workflow template, [Compute Spike Freqs] is under the [Cluster Spikes] module. Spike frequencies are computed independently for clusters and color-coded in the [Display Spike Frequencies] control panel.

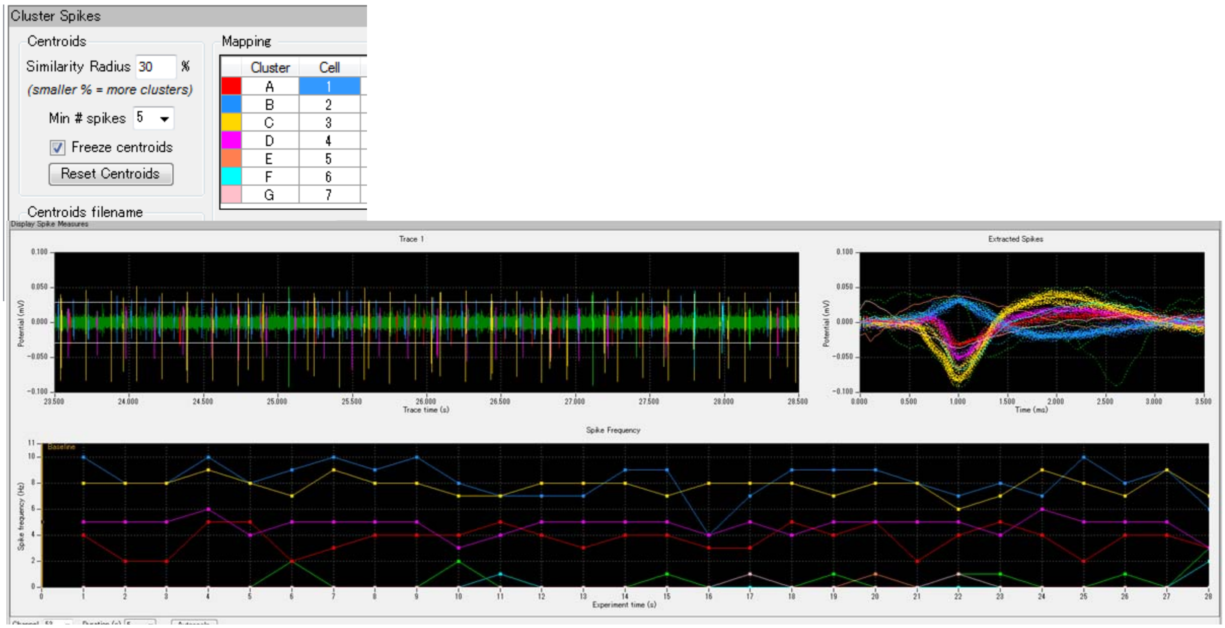
Note that the frequency calculations are reset when a new cluster is formed during replay (or acquisition). When the check box for [Freeze Centroids] is checked, the numbers of clusters are fixed and frequency calculations are not reset anymore.

After replaying all data and modifying the clustering, **check the [Freeze Centroids] to have the spike frequencies analyzed properly.**



Channel 53
Duration (s) 5

Figure 3-3.23. Computing for spike frequencies of clustered spikes. The [Display Spike Frequencies] panel is reset whenever a new cluster is formed.



Cluster Spikes

Centroids

Similarity Radius 30 %

(smaller % = more clusters)

Min # spikes 5

☑ Freeze centroids

Centroids filename

Mapping

	Cluster	Cell
🟥	A	1
🟦	B	2
🟨	C	3
🟪	D	4
🟧	E	5
🟦	F	6
▨	G	7

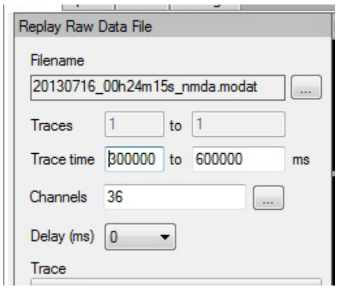
Figure 3-3.24. Spike frequencies are now displayed properly with the Centroid frozen.

After setting all analysis parameters, you are now ready to output the data. Please refer to Data Output (for page 88-91) for exporting analyzed data.

3-4. Data Output

Now, you are ready to output your analyzed data. All analysis results can be saved as “CSV” formatted text file with following procedures.

1. Check the check-box for the [Save] module (e.g. Save Spikes, Save Spike Freqs), that you would like to output the data (Refer to page 89-91). File names can be modified by directly typing them into the Filename modifier box.
2. Select channels, Trace No, trace time for the Saving/Exporting to export in the [Replay Raw Data] module. Channels shown with green will be enabled. (Figure 3-4.1)



Filename: 20130716_00h24m15s_nmda.modat

Traces: 1 to 1

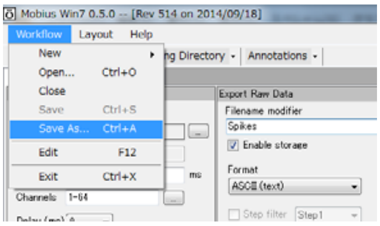
Trace time: 300000 to 600000 ms

Channels: 36

Delay (ms): 0

Figure 3-4.1. [The Replay Raw Data File] ready for export. Data for 300,000-600,000 msec at channel 36 will be saved.

3. After setting all parameters, save the analysis workflow as your own. (Figure 3-4.2)



Filename modifier: Spikes

☑ Enable storage

Format: ASCII (text)

☐ Step filter: Step1

Channels: 1-64

Figure 3-4.2. Saving analysis workflow.

4. Run the analysis workflow with the Green-Red button. The output data will be saved in the same folder where the analysis workflow is saved.

***NOTE*:**

In the *“Spike_sorting(_filter)* “ workflow template, all Save module is under [Cluster Spikes]. All saved data include “Cluster ID”.

CAUTION:

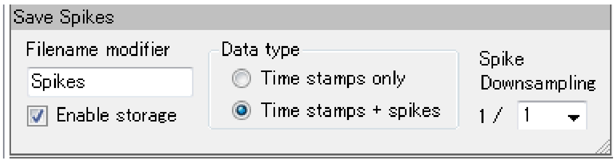
The Centroids MUST be frozen for the all analyzed data saved correctly when clustered spikes are exported.

Please refer to 1-5. Exporting raw data (page 20-21) for exporting raw data.

3-4.1. Saving extracted spikes and time stamps

Check the “Enable storage” in the [Save Spikes], and then run the analysis workflow with the Green-Red button to save the analysis below as a “CSV” formatted text file.

1) Time stamp only, or
2) Time Stamps, and waveforms of all extracted spikes



Save Spikes

Filename modifier: Spikes

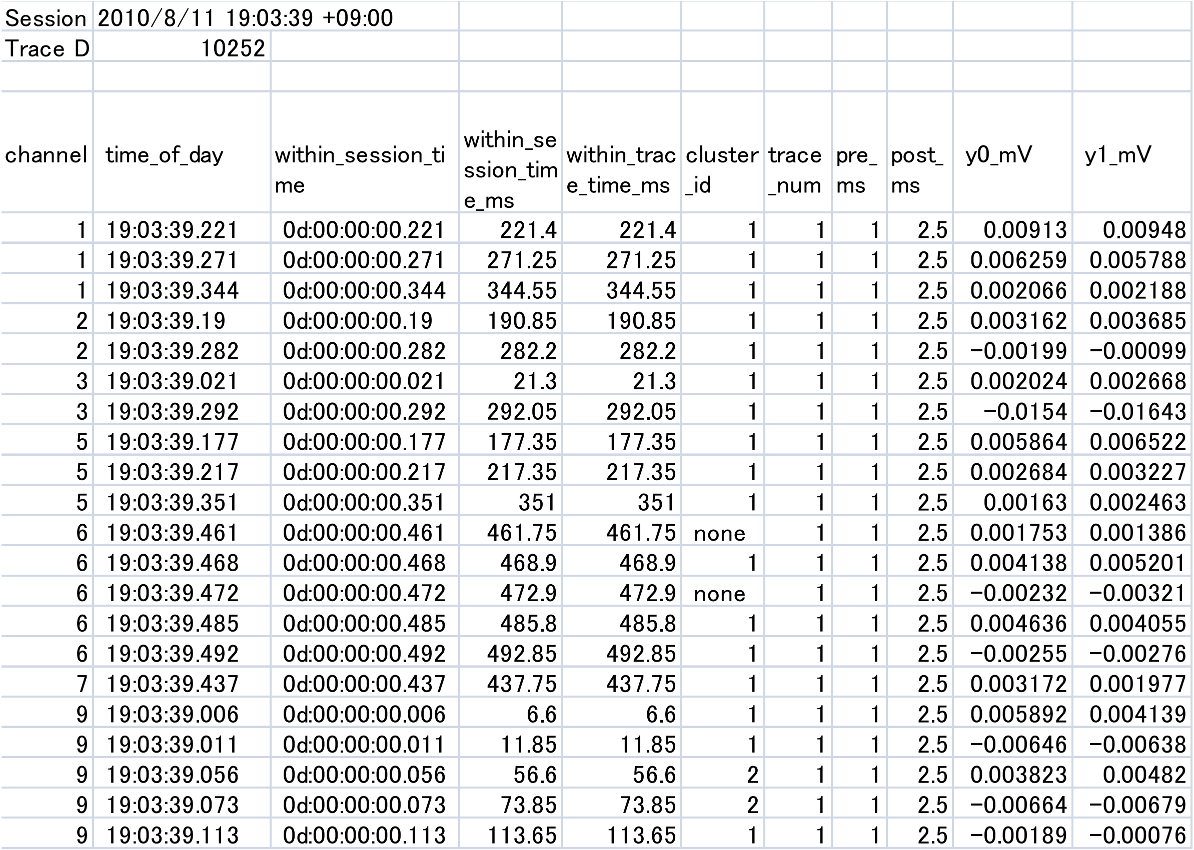
☑ Enable storage

Data type
○ Time stamps only
● Time stamps + spikes

Spike Downsampling 1 / 1

Figure 3-4.3. [Save Spikes] module/control panel. Time stamps and spikes waveforms will be saved.

Figure 3-4.5 is an example of the time stamps and waveforms for clustered spikes saved as a “CSV” file displayed in Excel. Be noted that each Time Stamp and waveforms have cluster IDs since the “*Spike_sorting(_filter)*” workflow template is used. If non-clustered spikes are exported (using “*Spike_frequency_analysis*), the column for cluster_id has all NONE.

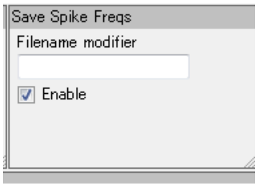


Session	2010/8/11 19:03:39 +09:00									
Trace D	10252									
channel	time_of_day	within_session_time	within_session_time_ms	within_trace_time_ms	cluster_id	trace_num	pre_ms	post_ms	y0_mV	y1_mV
1	19:03:39.221	0d:00:00:00.221	221.4	221.4	1	1	1	2.5	0.00913	0.00948
1	19:03:39.271	0d:00:00:00.271	271.25	271.25	1	1	1	2.5	0.006259	0.005788
1	19:03:39.344	0d:00:00:00.344	344.55	344.55	1	1	1	2.5	0.002066	0.002188
2	19:03:39.19	0d:00:00:00.19	190.85	190.85	1	1	1	2.5	0.003162	0.003685
2	19:03:39.282	0d:00:00:00.282	282.2	282.2	1	1	1	2.5	−0.00199	−0.00099
3	19:03:39.021	0d:00:00:00.021	21.3	21.3	1	1	1	2.5	0.002024	0.002668
3	19:03:39.292	0d:00:00:00.292	292.05	292.05	1	1	1	2.5	−0.0154	−0.01643
5	19:03:39.177	0d:00:00:00.177	177.35	177.35	1	1	1	2.5	0.005864	0.006522
5	19:03:39.217	0d:00:00:00.217	217.35	217.35	1	1	1	2.5	0.002684	0.003227
5	19:03:39.351	0d:00:00:00.351	351	351	1	1	1	2.5	0.00163	0.002463
6	19:03:39.461	0d:00:00:00.461	461.75	461.75	none	1	1	2.5	0.001753	0.001386
6	19:03:39.468	0d:00:00:00.468	468.9	468.9	1	1	1	2.5	0.004138	0.005201
6	19:03:39.472	0d:00:00:00.472	472.9	472.9	none	1	1	2.5	−0.00232	−0.00321
6	19:03:39.485	0d:00:00:00.485	485.8	485.8	1	1	1	2.5	0.004636	0.004055
6	19:03:39.492	0d:00:00:00.492	492.85	492.85	1	1	1	2.5	−0.00255	−0.00276
7	19:03:39.437	0d:00:00:00.437	437.75	437.75	1	1	1	2.5	0.003172	0.001977
9	19:03:39.006	0d:00:00:00.006	6.6	6.6	1	1	1	2.5	0.005892	0.004139
9	19:03:39.011	0d:00:00:00.011	11.85	11.85	1	1	1	2.5	−0.00646	−0.00638
9	19:03:39.056	0d:00:00:00.056	56.6	56.6	2	1	1	2.5	0.003823	0.00482
9	19:03:39.073	0d:00:00:00.073	73.85	73.85	2	1	1	2.5	−0.00664	−0.00679
9	19:03:39.113	0d:00:00:00.113	113.65	113.65	1	1	1	2.5	−0.00189	−0.00076

Figure 3-4.5. Time stamps and waveforms for clustered spikes saved as a “CSV” file displayed in Excel.

3-4.2. Saving the spike frequency data

Check the “Enable” in the [Save Spike Freqs] and run the analysis workflow with the Green-Red button to save the frequency data as a “CSV” formatted text file.



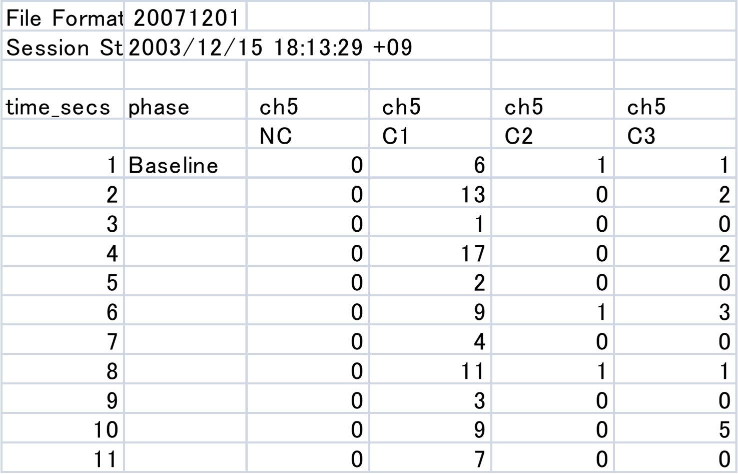
Save Spike Freqs

Filename modifier

☑ Enable

Figure 3-4.6. [Save Spike Freqs] module.

The Figure 3-4.7 is an example of the spike frequency data for clustered spikes saved (only for channel 5) as a “CSV” file displayed in Excel. The data include cluster ID (NC, C1, C2) since the “*Spike_sorting(_filter)*” workflow template is used..

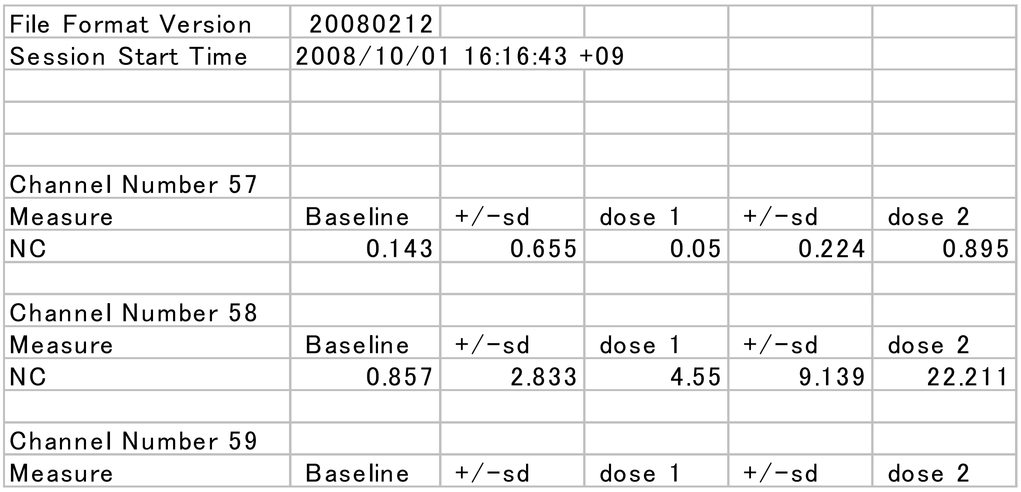


File Format	20071201				
Session St	2003/12/15 18:13:29 +09				
time_secs	phase	ch5	ch5	ch5	ch5
		NC	C1	C2	C3
1	Baseline	0	6	1	1
2		0	13	0	2
3		0	1	0	0
4		0	17	0	2
5		0	2	0	0
6		0	9	1	3
7		0	4	0	0
8		0	11	1	1
9		0	3	0	0
10		0	9	0	5
11		0	7	0	0

Figure 3-4.7.

3-4.3. Saving the average chart

Check the “Enable” in the [Save Measure Averages] and run the analysis workflow with the Green-Red button to save the averages and standard deviations as a “CSV” formatted text file. (Refer to section 3-3.3. Compute averages of spike frequency by phases.)

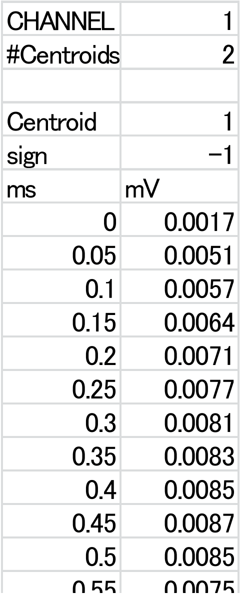


File Format Version	20080212				
Session Start Time	2008/10/01 16:16:43 +09				
Channel Number 57					
Measure	Baseline	+/-sd	dose 1	+/-sd	dose 2
NC	0.143	0.655	0.05	0.224	0.895
Channel Number 58					
Measure	Baseline	+/-sd	dose 1	+/-sd	dose 2
NC	0.857	2.833	4.55	9.139	22.211
Channel Number 59					
Measure	Baseline	+/-sd	dose 1	+/-sd	dose 2

Figure 3-4.8. Example of the average chart saved as a “CSV” file diplayed in Excel. (Only channel 57, 58, and 59 are exported.)

3-4.4. Saving Centroids

The centroid waveforms (shown with solid lines at the [Display Extracted Spike (waveforms only)] are saved as a “CSV” formatted text file by checking the storage/reload in the [Cluster Spikes] and running the workflow with the Green-Red button.



CHANNEL	1
#Centroids	2
Centroid	1
sign	-1
ms	mV
0	0.0017
0.05	0.0051
0.1	0.0057
0.15	0.0064
0.2	0.0071
0.25	0.0077
0.3	0.0081
0.35	0.0083
0.4	0.0085
0.45	0.0087
0.5	0.0085
0.55	0.0075

Figure 3-4.9. Example of the centroids saved as a “CSV” file displayed in excel.

Chapter 4 Mobius QT

This chapter describes how to record and analyze signals from cardiac myocyte cultures, acute heart tissue, or stem cell derived cardiac myocytes using available workflow templates.

4-1. Recording of spontaneous myocardial signals

There are 3 workflow templates available for recording spontaneous myocardial signals.

1. *Simple_recording*:
   For recording myocardial signals (long spikes) WITHOUT any analysis.

2. *Beat_recording*:
   For recordings with extracting myocardial signals (long-spikes), and measuring their frequencies and inter-spike intervals.

3. *QT_recording*:
   For recordings with extracting myocardial signals (long-spikes), measuring their frequencies and analyzing extracted waveforms. Use this workflow template for the Field Potential Duration (FPD) analysis.

After reading this section you should be able to record spontaneous myocardial signals using any of the above workflow templates.

4-1.1. Overview of the acquisition workflow templates

"*QT_recording*" workflow template

The "*QT_recoding*" workflow template consists of following modules located in the "Main", "Detect beating", and "Measurement" tabs.

Tab	Modules
Main	Acquire MED64R2 Data / Display All Channels
Detect beating	Extract Long Spikes /Display Extracted Spikes / Compute Interspike Intervals / Display Interspike Intervals / Save Interspike Intervals
Measurement	Filter Spike Data (2) / Extract Spike Measures / Save Measures Data / Display Results Table

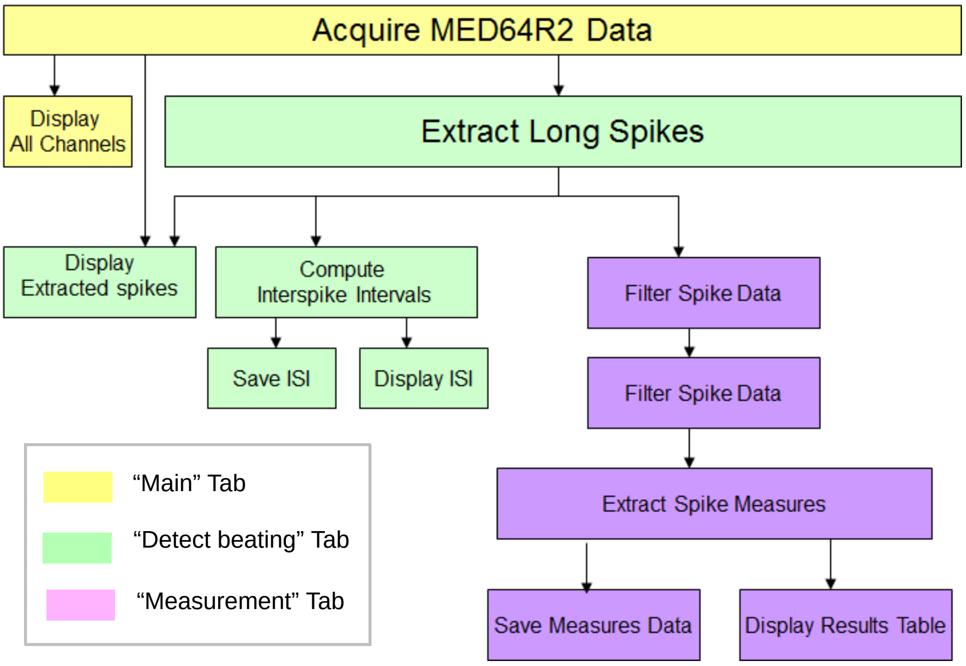


Figure 4-1.1. Module configuration for the "*QT_recording*" workflow template.

With this workflow template, spikes (myocardial signals) crossing pre-determined thresholds are extracted. The extracted long spikes are filtered according to the selected drop down filter options in the [Filter Spike Data] module, and then sent to the [Extract Spike Measures] module, where a variety of waveform analyses can be performed and graphed (e.g., amplitude, slope, area, and peak to peak time etc.). The inter-spike intervals for the extracted long spikes are also computed and graphed. Use this workflow template if your interest is waveform analysis including field potential duration (FPD) for studying QT prolongation.

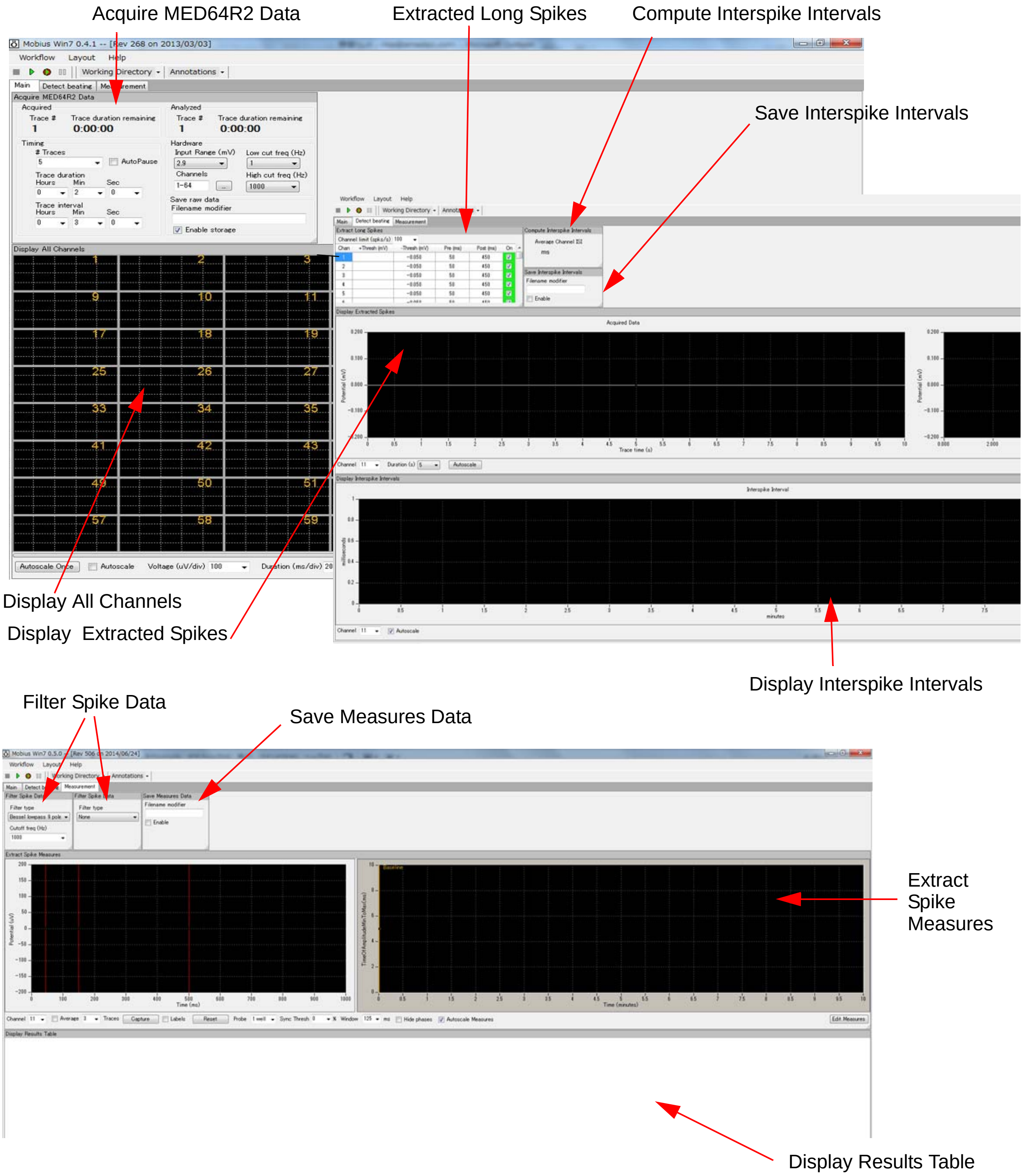


Figure 4-1.2. Control panels for the “*QT_recording*” workflow template. “Main” tab (top-left), “Detect beating” tab, (top-right), and “Measurement” tab (bottom).

“*Beat_recording*” workflow template

The “*Beat_recording*” workflow template consists of following modules located in the “Main” and “Detect beating” tabs.

Tab	Modules
Main	Acquire MED64R2 Data / Display All Channels
Detect beating	Extract Long Spikes / Compute Beats per Minute / Save Beats pre Minute / Display Beats per Minute / Compute Interspike Intervals / Save Interspike Intervals / Display Interspike Intervals / Display Extracted Spikes

With this workflow template, long spikes (myocardial signals) crossing pre-determined thresholds are extracted, and their beat frequencies (beats per minute) and interspike intervals are measured and graphed. Use this template if your interest is beat frequency.

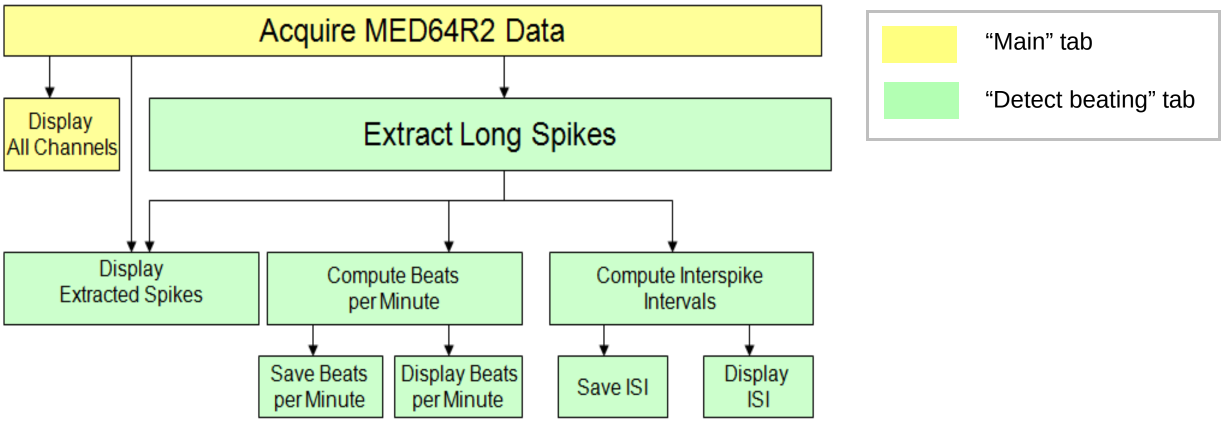


Figure 4-1.3. Module configuration for the “*Beat_recording*” workflow template.

“*Simple_recording*” workflow template

Signals are acquired, and the acquired signals are displayed at all 64 channels.

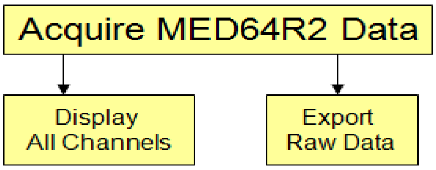


Figure 4-1-4. Module configuration for the “*Simple_recording*” workflow template.

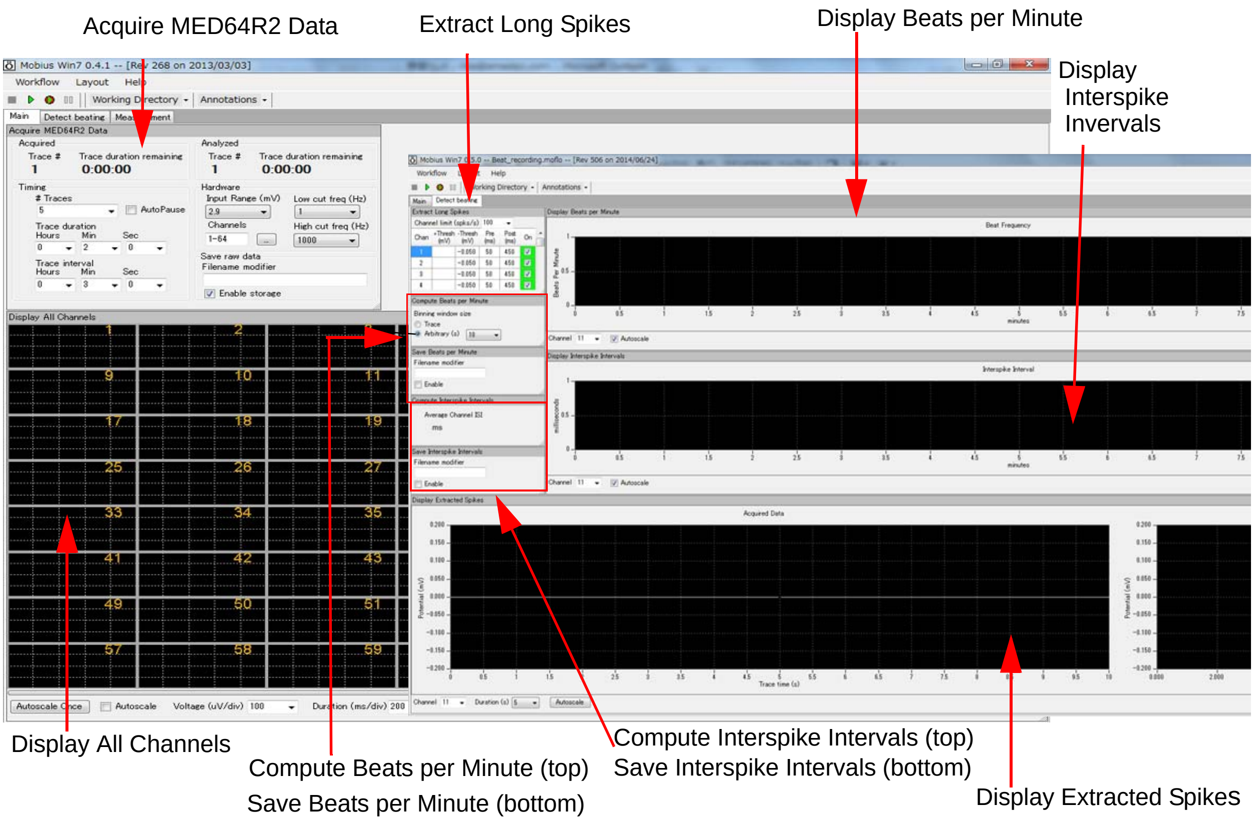


Figure 4-1.5. Control panels for the "*Beat_recording*" workflow template. "Main" tab (left) and "Detect beating" tab (right).

Opening the workflow template

1. Click [Workflow] > [New] > [From Template]. (Figure 4-1.6)
2. Select [64MD1_1280x1024] or [64MD_1920x1080] folder, depending on the size of your display monitor.
3. Select [QT] > [Spontaneous_recording] folder, and then any of the recording workflows.

NOTICE:
Connect your PC to the MED64 amplifiers and turn on both amplifiers to open the recording worfklows.

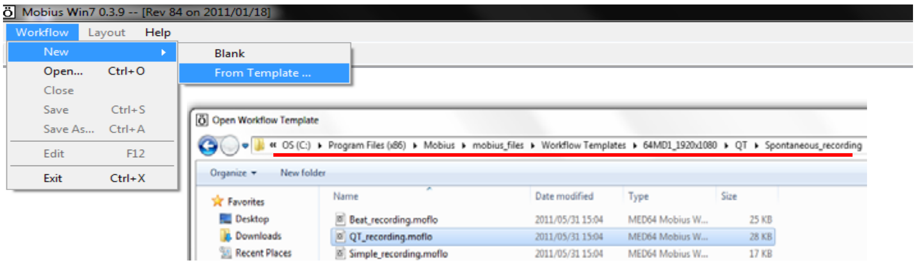
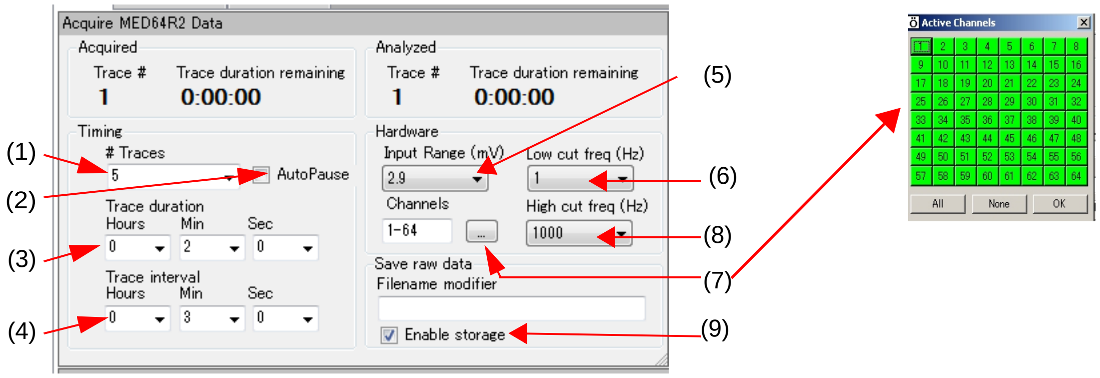


Figure 4-1.6. Opening workflow template.

4-1.2. Setting the acquisition parameters

All the acquisition workflow templates include the [Acquire MED64R2 Data] module. Acquisition parameters are set in this module.

NOTICE:
Connect your PC to the MED64 amplifiers and turn on both amplifiers to open this module.



Acquire MED64R2 Data

Acquired
Trace # 1
Trace duration remaining 0:00:00

Analyzed
Trace # 1
Trace duration remaining 0:00:00

Timing
Traces 5
☐ AutoPause
Trace duration: Hours 0, Min 2, Sec 0
Trace interval: Hours 0, Min 3, Sec 0

Hardware
Input Range (mV) 2.9
Low cut freq (Hz) 1
Channels 1-64
High cut freq (Hz) 1000
Save raw data
Filename modifier
☑ Enable storage

Active Channels

Figure 4-1.7. [Acquire MED64R2 Data] module.

(1) # Traces: Number of Traces (sweeps).

(2) Auto Pause:

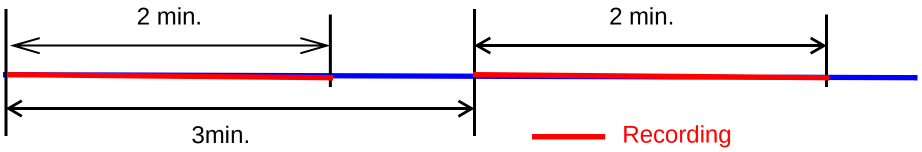
Mobius will pause automatically when the current trace is finished if this box is checked. The trace interval set below becomes invalid when this box is checked. Mobius re-starts by clicking either of Green button or Green-Red button.

(3) Trace duration: Sampling (Recording) time for each trace.

(4) Trace interval:

Interval between traces. (from the beginning of the first trace to the beginning of the next trace.)

- Signals are recorded for 2 minutes every 3 minutes 5 times in the default setting as seen in the following figure. Mobius will run for 15 minutes total.



- The trace interval becomes invalid when Auto Pause is checked.

(5) Input Range (mV):

Maximum input signal level. This setting should be adjusted according to the signal amplitude to achieve the maximal dynamic range. Select 2.3 mV, 2.9 mV, 5 mV, 12.5 mV, or 25 mV. Larger number for the Input Range creates lower voltage resolution.

2.9 mV is set in the default. This is the Input Range recommended for the field potential duration analysis combined with 1 kHz for the “High cut freq” (low-pass filter) to achieve the stability for the repolarization (K^+ efflux) peak time. Change it depending on the size of your signals, purpose of your analysis, and/or the parameter selected for “High cut freq”. It is highly recommended that you change it to 5.0 mV (or greater) when 10 kHz is selected for the “High cut freq”.

(6) Low cut freq (Hz):

High pass filter for the amplifier. Select 0.1 Hz, 1.0 Hz, 10 Hz or 100 Hz. If 1.0 Hz is set, the signals with frequencies lower than 1.0 Hz are filtered out. 1.0 Hz is typically recommended for the recording of myocardial signals.

(7) Channels:

Select the channels for recording here. When the square box is clicked, the channel selector pops up. The enabled recording channels are colored green.

(8) High cut freq (Hz):

Low pass filter for the amplifier. Select 1000 Hz, 2,000 Hz, 2500 Hz, 5000 Hz, 7500 Hz, or 10000 Hz. If 5000 Hz is selected, signals with frequencies higher than 5000 Hz are filtered out.

1000 Hz is typically recommended for the field Potential duration analysis to achieve the stability for the repolarization (K^+ efflux) peak time. Change it depending on the analysis you would like to perform.

(9) Save raw data:

When Mobius is started by clicking the Green-Red recording button while the [Enable storage] is checked, the raw data is saved. The file name can be modified.

4-1.3. Execution button

Acquisition is initiated by running an acquisition workflow. Clicking the Green button runs the acquisition workflow without saving data while clicking the Green-red button runs the acquisition workflow while saving data to disc.

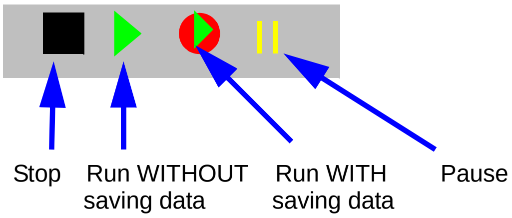
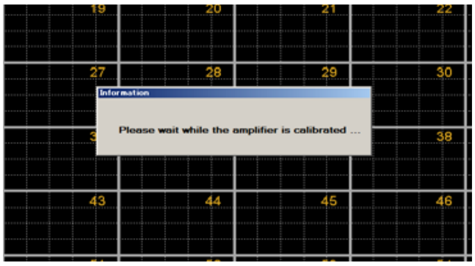


Figure 4-1.8. How the execution buttons work for acquisition.

4-1.4. Before starting your experiment

When a recording workflow is run with Green or Green-Red button for the first time after it is opened (or made), Mobius needs several seconds for the calibration (as seen in the pop-up message). Acquisition starts automatically after the calibration.

If you would like to start acquisition immediately after clicking the Green-Red button, run the workflow with the Green button for several seconds, and then stop it before starting your experiment. When the workflow is run for the next time, it will not require calibration and acquisition will start immediately.



Please wait while the amplifier is calibrated ...

Figure 4-1.9. The pop-up message for the calibration.

CAUTION:

- Make sure that the hard disc drive has enough free space when you start long-term recording. Recording with all 64 channels for 1 hour creates a 9.216GB file.
  (9.216GB is 9,000,000KB, 8,739.06MB, or 8.58GB in Windows.
- Avoid saving data to the drive where the OS is installed (usually C drive). Save it to a separate hard drive, that is internal or eSATA-supported.

4-1.5. Recording spontaneous cardiomyocyte signals

Extraction of long spikes and online analysis require powerful computation and needs to be used carefully during acquisition. Thus, it is recommended to record signal WITHOUT analysis (by disabling the spikes' extractions) until you are familiar with your experiments. When acquisition with analysis is made, please follow the cautions below:

CAUTION:

- Minimize the channels for spikes' extractions.
- Avoid enabling any [Export] or [Save] modules when raw data is saved. Once raw data is saved, you can export post-acquisition.
- Set the trace duration (recording time) to less than 10 minutes and the trace interval to longer than "the trace duration x 2" so that the analysis can catch up with the acquisition during the trace interval. Particularly, keep the trace duration short for long-term recording lasting days, weeks, or months.
- Set the thresholds as low as possible for negative signals (high for positive signals) so that other components of the signals or noise are NOT extracted as spikes.
- Make sure to pause Mobius with the yellow button when a drug is applied.

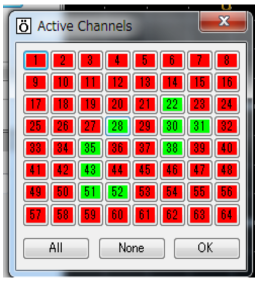
1. Modify the #Traces, Trace duration, and Trace interval in the [Acquire MED64R2 Data] module. (Refer to page 98, section 4-1.2. Setting the acquisition parameters.)

2. Start running the workflow with the Green button to have signals on the screen, and then stop.

3. Select the channel for acquisition.

   1) Click the box next to the “Channels” in the [Acquire MED64R2 Data] module to open the channel selector. (Figure 4-1.11)

   2) The recording channels are colored green and non-recording channels are colored red.



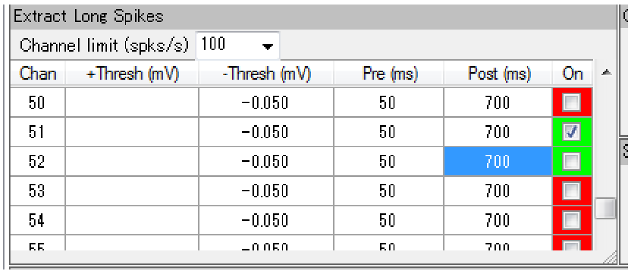
1 🟥	2 🟥	3 🟥	4 🟥	5 🟥	6 🟥	7 🟥	8 🟥
9 🟥	10 🟥	11 🟥	12 🟥	13 🟥	14 🟥	15 🟥	16 🟥
17 🟥	18 🟥	19 🟥	20 🟥	21 🟥	22 🟩	23 🟥	24 🟥
25 🟥	26 🟥	27 🟥	28 🟩	29 🟥	30 🟩	31 🟩	32 🟥
33 🟥	34 🟥	35 🟩	36 🟥	37 🟥	38 🟩	39 🟥	40 🟥
41 🟥	42 🟥	43 🟩	44 🟥	45 🟥	46 🟥	47 🟥	48 🟥
49 🟥	50 🟥	51 🟩	52 🟩	53 🟥	54 🟥	55 🟥	56 🟥
57 🟥	58 🟥	59 🟥	60 🟥	61 🟥	62 🟥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

Figure 4-1.10. Selecting acquisition channels

4. Open the “Detect beating” tab. Select a channel for spikes’ extractions. The default has all channels with “checked”. Click the check box to diable them. The setting can be applied to all other channels by right-clicking and selecting [Apply to All].

   - It is recommended to select only one channel for spike extraction and analysis during acquisition although it is possible to select more than 1. Once data is saved, all analysis can be performed post-acquisition. Particularly, select only one channel when acquisition is made with [Extract Spike Measures] analysis from non-synchronized signals. Otherwise, the waveform cannot be captured on the [Extract Spike Measures].

5. Set the thresholds by typing the number in the chart of Extract Long Spikes (Refer to page 114).



Extract Long Spikes

Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
50		−0.050	50	700	☐ 🟥
51		−0.050	50	700	☑ 🟩
52		−0.050	50	700	☐ 🟩
53		−0.050	50	700	☐ 🟥
54		−0.050	50	700	☐ 🟥
55		−0.050	50	700	☐ 🟥

Figure 4-1.11. Extract Long Spikes module. Channel 51 is selected for extraction. For the Ch 52, data is saved but spikes are not extracted (analysis is not observed.)

6. Set the analysis parameters for the analysis module (Refer to page 113-125).

7. Open the “Main” tab. Make sure “Enable storage” is checked in the “Save raw data” on the [Acquire MED64R2 Data] module.

8. Save it as your own acquisition workflow by clicking [Workflow] > [Save as]. (Figure 4-1.12)

9. Start Mobius with Green-Red button.

   - Mobius will require you to save the workflow as your own if you try to start Mobius with the Green-Red button without having saved it already.

   - The data file (.modat file) and the Analysis workflow (including all analysis module selected for the Acquisition workflow and [Replay Raw Data File] module will be saved in the same folder.

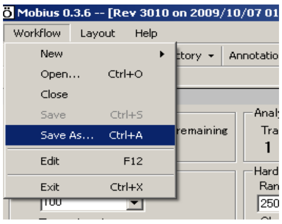


Figure 4-1.12.

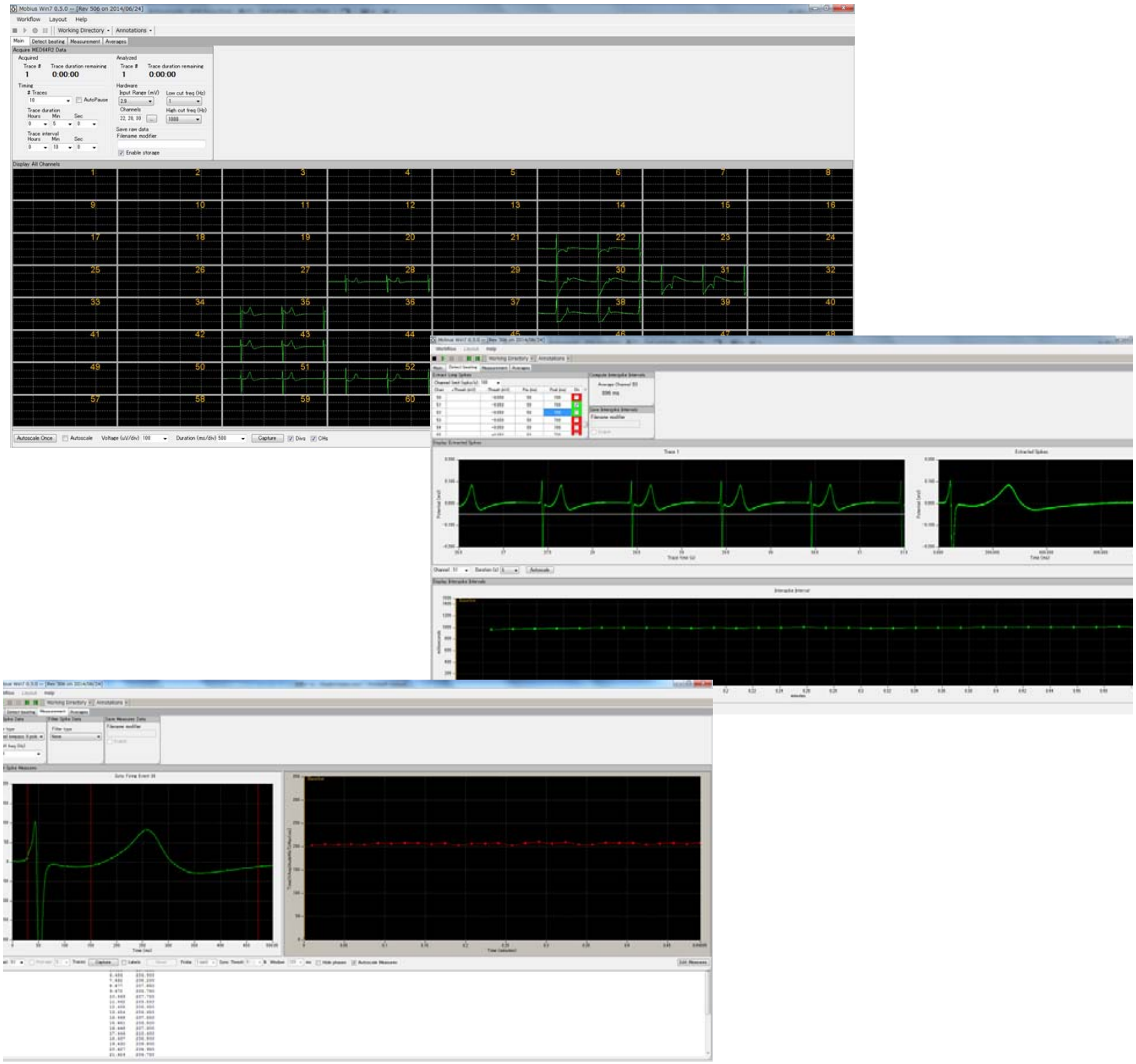


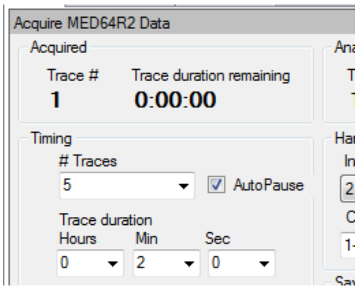
Figure 4-1.13. Recording cardiomyocyte signals with analysis .

4-2. Drug testing

The [Compute Measure Averages] module calculates averages and standard deviations for the waveform analysis (Extract Spike Measures), beat frequencies, and interspike intervals for all the experiment's phases. This is a useful tool for making a dose-response curve or comparing phases before, during and after drug application. This section will demonstrate how to add "phase bars" so as to make a dose-response curve easily and quickly post-acquisition.

1. Open the “*QT_recording*” or “*Beat_recording*” workflow template.

2. Set the acquisition parameter and check the “Auto Pause” check box. (Figure 4-2.1) Acquisition will be PAUSED automatically after each trace recording is finished.



Acquire MED64R2 Data

Acquired

Trace # 1

Trace duration remaining 0:00:00

Timing

Traces 5

☑ AutoPause

Trace duration

Hours 0

Min 2

Sec 0

Figure 4-2.1. Checking the “Auto Pause”.

3. Set the analysis parameters (refer to page 113-). Save it as your own workflow. ([Workflow] > [Save as])

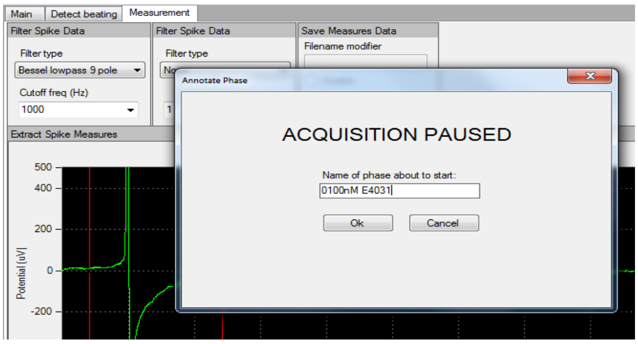
4. Open the Measurement tab. Start recording in Mobius with clicking Green-Red button. The first phase (baseline) will start from time 0.

5. After the first phase is completed, Mobius will pause automatically and the “ACQUISITION PAUSED” pops up.

   Apply the drug and type the name for the 2nd phase in the box. (e.g. 100 nM E4031) (Figure 4-2.2)

   - Do NOT stop Mobius with the Black button. If it is stopped, the experiment will end and new phases will not be added.

   When you are ready for the next phase, click the OK button to re-start Mobius. A yellow bar with chosen name appears on the charts in the [Extract Spike Measures] and [Display beat freq]. (Figure 4-2.3)



Annotate Phase

ACQUISITION PAUSED

Name of phase about to start: 0100nM E4031

Filter Spike Data

Filter type: Bessel lowpass 9 pole

Cutoff freq (Hz): 1000

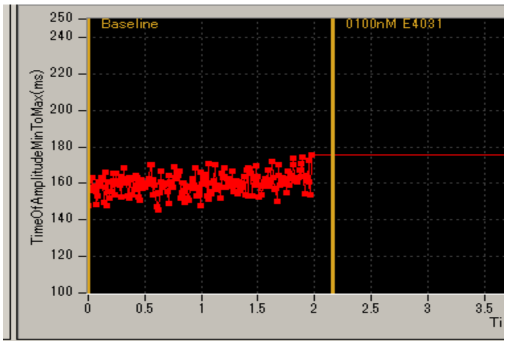


Figure 4-2.2. (Left) Adding a new phase.
Figure 4-2.3. (Right) A new phase added on the [Extract Spikes Measures].

If the Auto-pause is not checked, go forward to the next phases with following procedure:

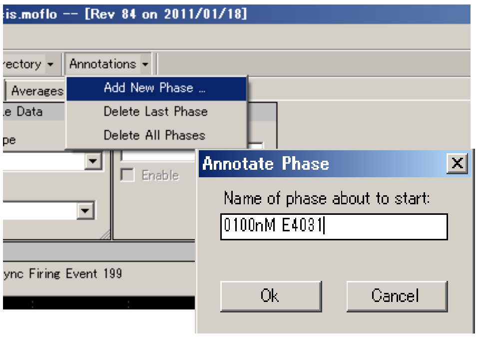
1) Pause Mobius with YELLOW button.

- Do NOT stop Mobius with the Black button.

2) Click [Annotations] > [Add New phase]. Type the name for the 2nd phase. (Figure 4-2.4)

3) When the OK button is clicked, a yellow bar with the chosen name will appear. (Figure 4-2.3)

4) Click the Green-Red button to re-start Mobius.



Name of phase about to start: 0100nM E4031

Figure 4-2.4

6. When the 2nd phase is finished, Mobius pauses automatically again and the pop-up menu comes up.

7. Apply drug, and then type the name for the third phase. (e.g. 1000 nM E4031)

8. Click OK button to re-start Mobius. The Figure 4-2.5 shows the [Extract Spike Measures] module with added phase bars.

9. The analysis workflow including all analysis parameters and phase bars you set are automatically made in the same directory as the acquisition workflow. Use this analysis workflow to make a dose-response-curve later. (Refer to page 124-126 for detailed instructions on how to make a dose-response-curve.)

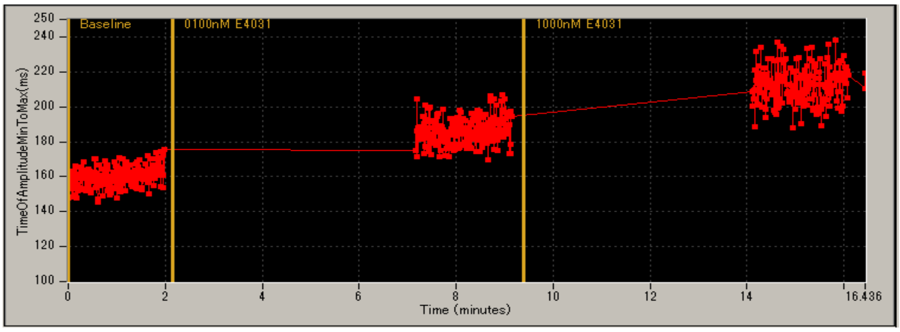


Figure 4-2.5. The [Extract Spike Measures] module with phase bars.

- The phase bars can be added and saved to the analysis workflow even without extracting long spikes. (Signals and analysis are not seen during recording in this case.)

4-3. Replay and analysis of acquired spontaneous data

Mobius data files (.modat) are opened and replayed with the [Replay Raw Data File] module, and analyzed using modules such as [Compute Beat Frequency], [Compute Interspike Intervals], and [Extract Spike Measures]. You will design your analysis workflow by combining those modules for replay and analysis of the acquired data. The quickest way to replay and analyze the data is using the analysis workflow templates, which will be introduced in this chapter.

The following analysis workflow templates are available for analysis of spontaneous myocardial data:

1. *QT_analysis*:
   Workflow for waveform analysis including field potential duration analysis (measurement).

2. *Beat_frequency_analysis*:
   Workflow for signal frequency analysis.

3. *Export_for_propagation_analysis_spontaneous*:
   Workflow for making a propagation map.

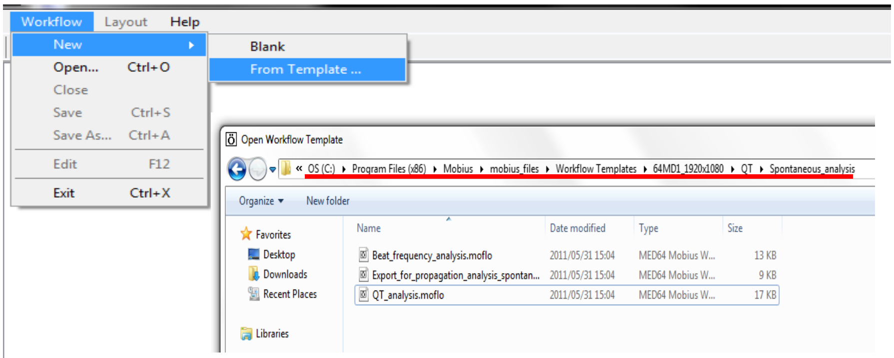
The Analysis workflow with the [Replay Raw Data File] and the analysis modules you selected for your acquisition will be automatically saved to the same directory when you record with the Green-Red button. These workflow templates can also be used for quickly performing analysis.

4-3.1. Opening and replaying acquired data

1. Open the analysis workflow.

   1) Click [Workflow] > [New] > [From Template].
   2) Select [64MD1_1280x1024] or [64MD1_1920x1080], depending on the size of your display monitor.
   3) Select [QT] > [Spontaneous_analysis] folders, then select a workflow template.

   - In order to open your own analysis workflow, click [Workflow] > [Open]. Select the analysis workflow file (.moflo file).



« OS (C:) ▸ Program Files (x86) ▸ Mobius ▸ mobius_files ▸ Workflow Templates ▸ 64MD1_1920x1080 ▸ QT ▸ Spontaneous_analysis

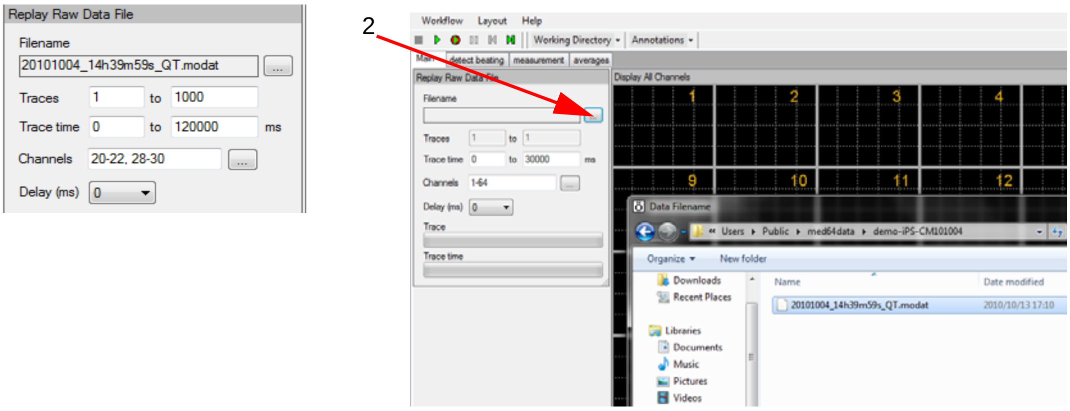
Name	Date modified	Type	Size
Beat_frequency_analysis.moflo	2011/05/31 15:04	MED64 Mobius W...	13 KB
Export_for_propagation_analysis_spontan...	2011/05/31 15:04	MED64 Mobius W...	9 KB
QT_analysis.moflo	2011/05/31 15:04	MED64 Mobius W...	17 KB

Figure 4-3.1. Opening analysis workflow template.

2. Click the box next to the “Filename” and select the .modat file. When the file name appears in the box (as seen on the left figure of the Figure 4-3.2), the data is ready to be replayed by clicking the Green or Green-Red button.

   - It can take several minutes to open large data sets. (e.g. Opening 10GB data can take over 1 minute.)

3. When the Green button is clicked, the acquired data is just replayed. Clicking the Green-Red button while checking the “Enable” check-box executes the Export or Saves.



Replay Raw Data File

Filename 20101004_14h39m59s_QT.modat

Traces 1 to 1000

Trace time 0 to 120000 ms

Channels 20-22, 28-30

Delay (ms) 0

Figure 4-3.2. Opening a Mobius data file (.modat file).

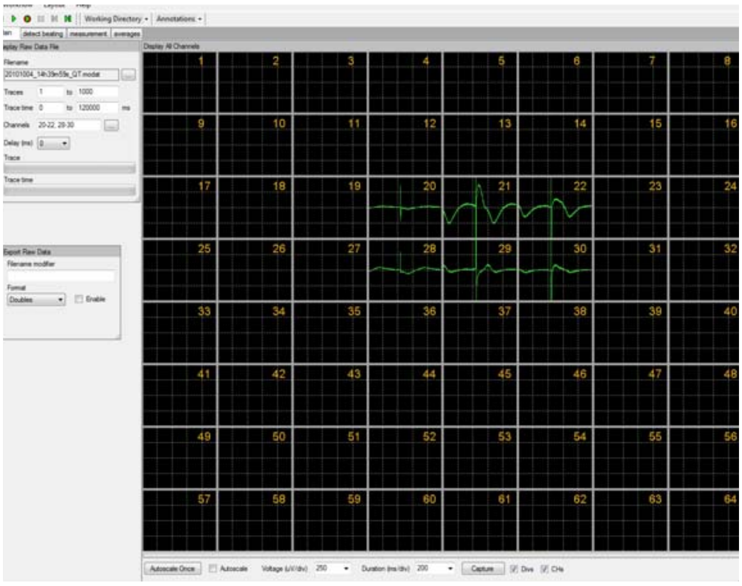


Figure 4-3.3. Replayed spontaneous myocardial data.

Input fields for the [Replay Raw Data File] module

1. Traces: Selects the trace(s) for processing.
2. Trace time: Selects the trace time for processing.
3. Channels: Selects the channels for processing. Click the box next to the “Channels” to open the channel selector. Channels in green will be enabled.
4. Delay: Set this value to a value greater than zero to insert a delay between data “blocks” sent out by this module. It is useful for slowing down data processing.

4-3.2. Overview of the analysis workflow template

This section provides an overview of the workflow templates available for analysis of spontaneous myocardial signals.

“*QT_analysis*” workflow template

Use this workflow template for waveform analysis of the extracted myocardial signals (long-spikes) including field potential duration analysis. This workflow template consists of the following modules located in the “Main”, “Detect beating”, “Measurement”, and “Averages” tabs. Please refer to section 4-3.2 on field potential duration analysis (page 113-121) for instructions on how to use this workflow template.

Tab	Modules
Main	Replay Raw Data File / Display All Channels / Export Raw Data
Detect beating	Extract Long Spikes / Display Extracted Spikes / Compute Interspike Intervals / Save Interspike Intervals / Display Interspike Intervals
Measurement	Filter Spike Data (2) / Extract Spike Measures / Save Measures Data / Display Results Table (for Eextract Spikes Meausres)
Averages	Compute Measure Averages (for Extract Spike Measures) / Save Measure Averages (For ESM)

The replayed raw data is sent to the [Extract Long Spikes] module where long spikes crossing pre-defined thresholds are extracted. The extracted long spikes are filtered, and then sent to the [Extract Spike Measures] module where their amplitude, slope, time, or area is measured and graphed. The [Display Results Table] shows the values for the analyses performed with the [Extracted Spike Measures].

The [Compute Measure Averages] module computes and graphs the averages of the waveform analysis. The extracted long spikes are sent to the [Compute Interspike Intervals] module for their interspike intervals to be analyzed as well. Raw data can be exported as a binary or ASCII ("CSV" formatted text file). The measurement chart and average chart can be saved as "CSV" formatted text file.

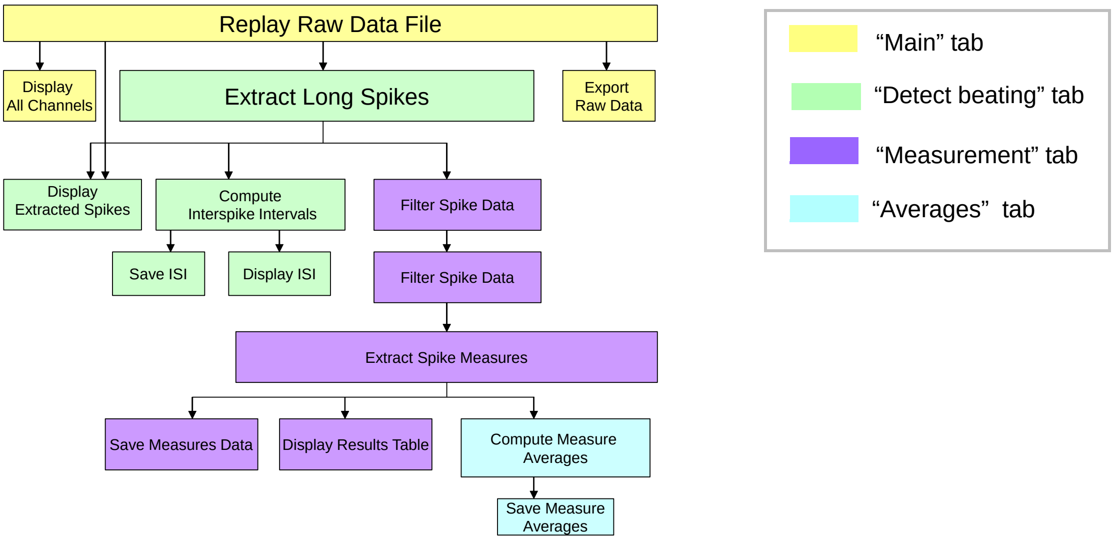


Figure 4-3.4. Module configuration for the “*QT_analysis*” workflow template.

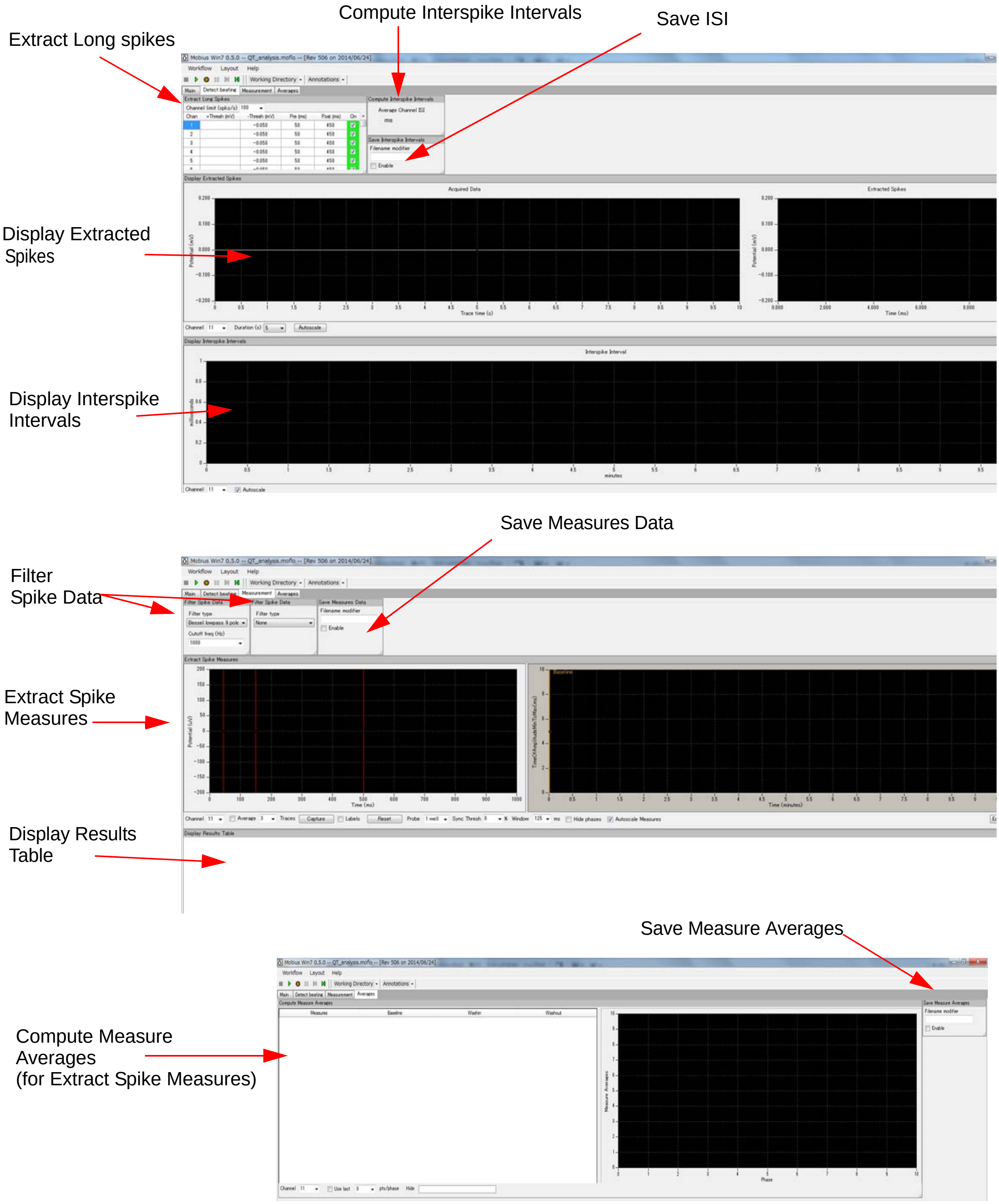


Figure 4-3.5. Control panels for the “*QT_analysis*” workflow template. “Detect beating” tab (top), “Measurement” tab (middle), and “Averages” tab (bottom).

“*Beat_frequency_analysis*” workflow template

Use this workflow template if your interest is beat frequency analysis. The *"Beat_frequency_analysis* " workflow template consists of following modules located in the “Main”, “Detect beating”, and “Averages” tabs. Please refer to section 4-3.3, Beat frequency analysis (page 122-123) for instructions on how to use this workflow template.

Tab	Modules
Main	Replay Raw Data File / Display All Channels / Export Raw Data
Detect beating	Extract Long Spikes / Display Extracted Spikes / Compute Beats per Minute / Save Beats per Minute / Display Beats per Minute / Compute Interspike Intervals / Save Interspike intervals / Display Interspike Intervals
Averages	Compute Measure Averages (for ISI) / Save Measures Averages (For ISI)

The replayed raw data is sent to the [Extract Long Spikes] module in which long spikes crossing pre-defined thresholds are extracted. The extracted long spikes are sent to the [Compute Beats per Minute] module for measuring their frequencies as well as [Compute Interspike Intervals] module for measuring interspike intervals. The [Compute Measure Averages] module computes and graphs the averages of the interspike intervals.

The raw data can be exported as Binary or ASCII ("CSV" formatted text file). The beat frequency chart, inter-spike-interval chart, and averages chart can be saved as a "CSV" formatted text file.

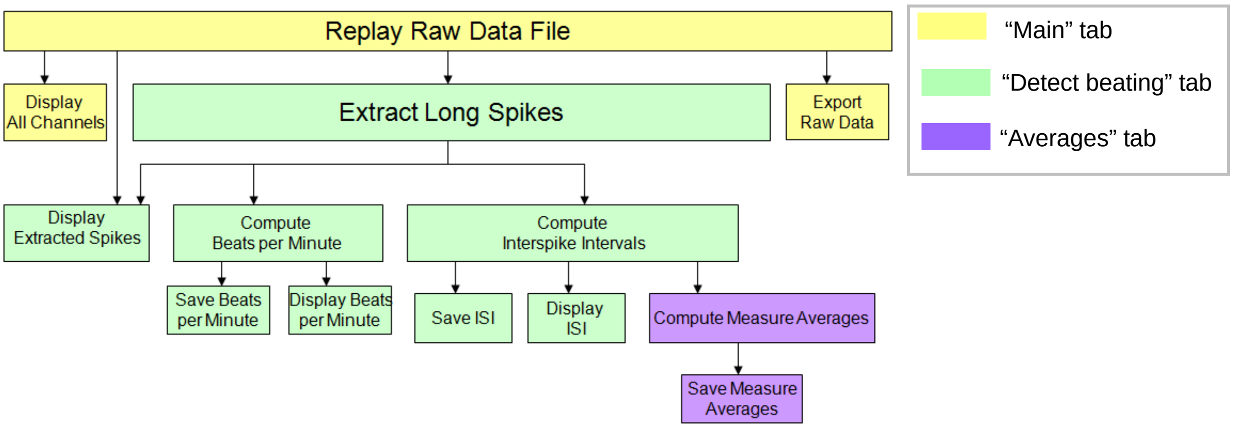
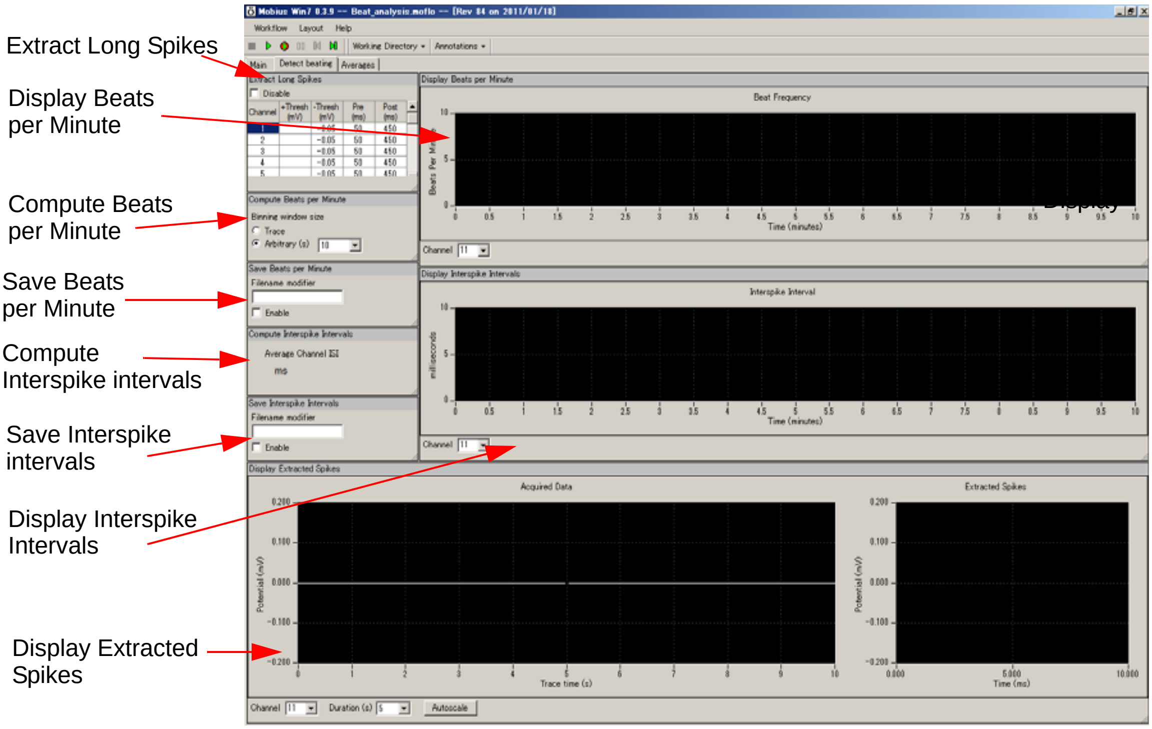


Figure 4-3.6. Module configurations for the “*Beat_frequency_analysis*” workflow template.


Extract Long Spikes

Display Beats per Minute

Compute Beats per Minute

Save Beats per Minute

Compute Interspike intervals

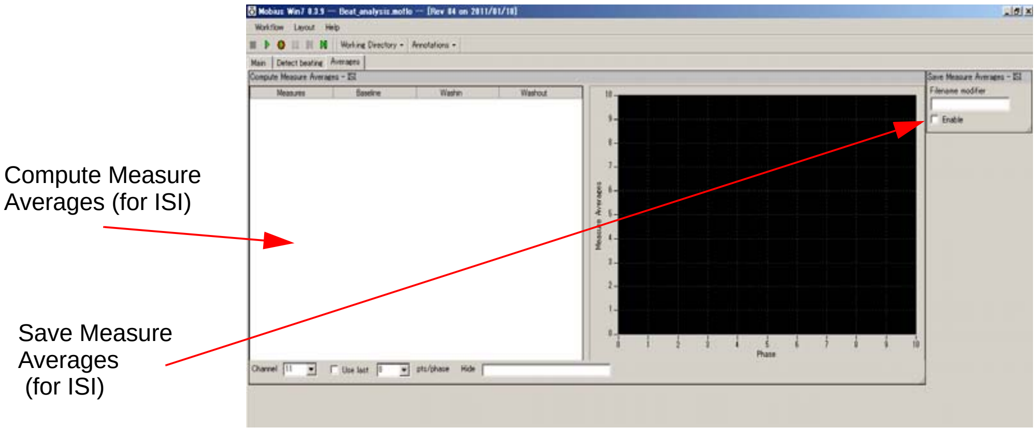
Save Interspike intervals

Display Interspike Intervals

Display Extracted Spikes

Mobius Win7 0.3.9 -- Beat_analysis.moflo -- [Rev 84 on 2011/01/18]

Channel	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)
1		−0.05	50	450
2		−0.05	50	450
3		−0.05	50	450
4		−0.05	50	450
5		−0.05	50	450


Compute Measure Averages (for ISI)

Save Measure Averages (for ISI)

Figure 4-3.7. Control panels for the “*Beat_frequency_analysis*” workflow template. “Detect beating” tab (top) and “Averages” tab (bottom).

“*Export_for_propagation_ analysis_spontaneous*” workflow template

This workflow template allows you to export the time stamps of the extracted long spikes with a “CSV” file. This is useful for creating a propagation map. It consists of following modules located at 3 Tabs. Please refer to section 4-3.6. Propagation analysis for spontaneous data (page 127-129) for instruction on how to use it.

Tab	Modules
Main	Replay Raw Data File / Display All Channels
Filter	Filter Raw Data (2) / Display All Channels
Detect beating	Extract Long Spikes / Save Spikes / Extract Spike Measures

The replayed raw data are filtered, and then sent to the [Extract Long Spikes] module, in which long spikes crossing pre-defined thresholds are extracted. The [Save Spikes] module allows you to save the Time Stamps of the extracted long spikes as a “CSV” file.

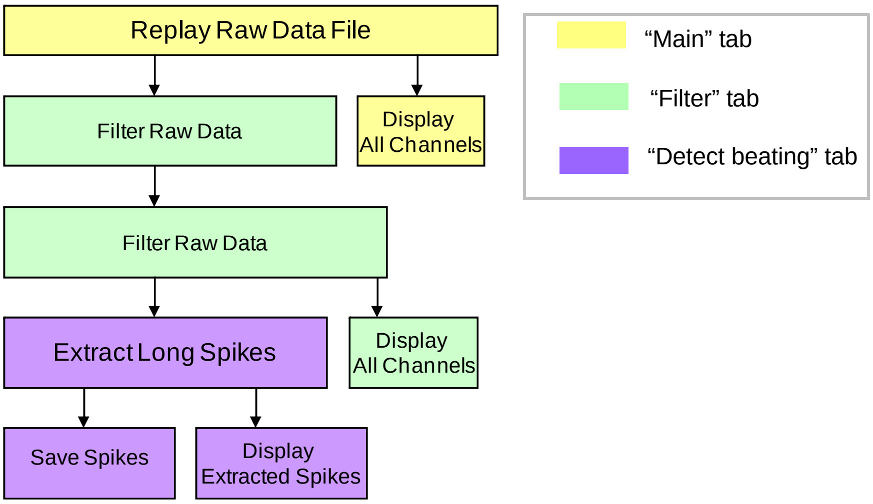
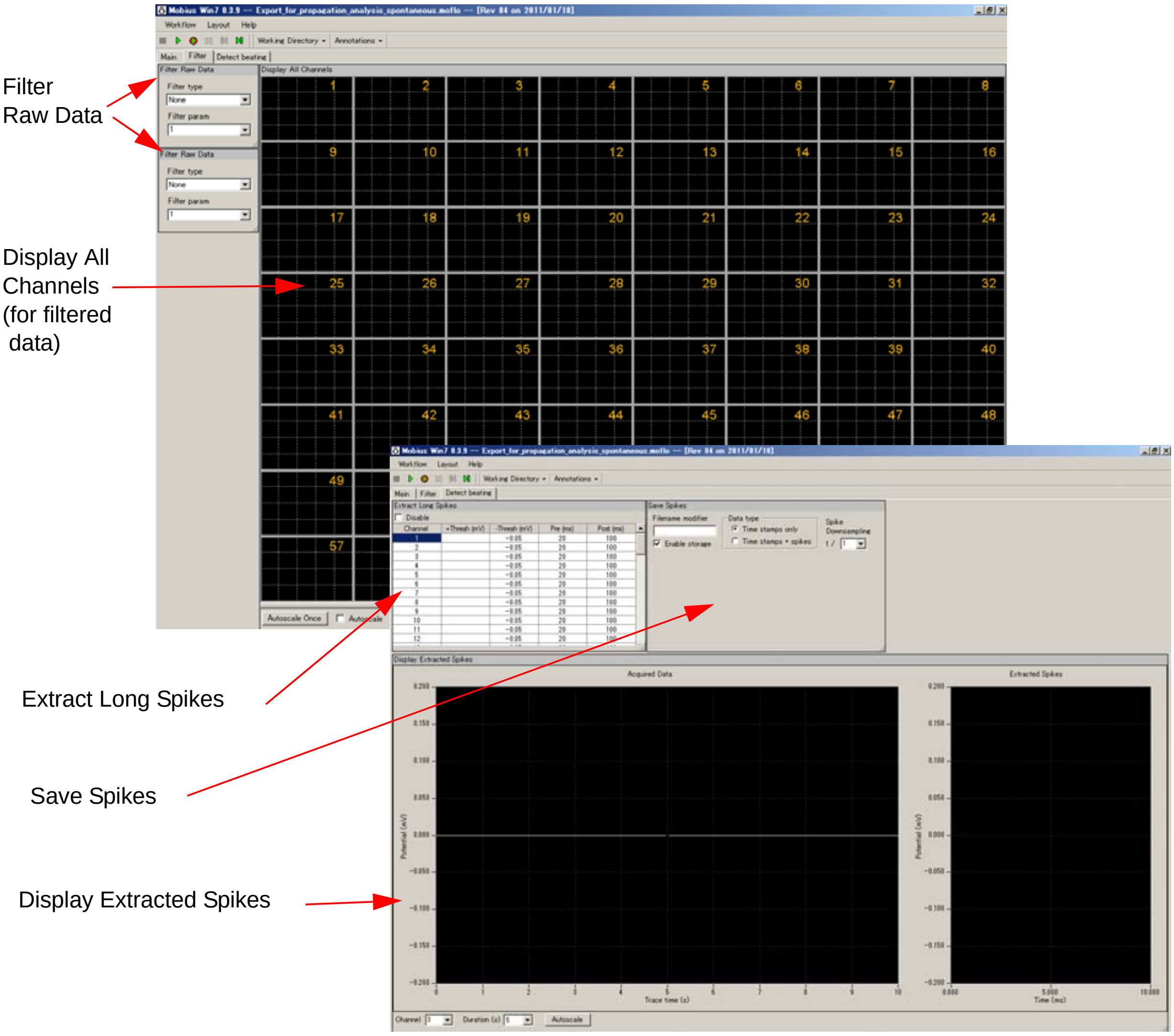


Figure 4-3.8. Module configurations for “*Export_for_propagation_analysis_spontaneous*” workflow template.



Filter type: None
Filter param: 1

☐ Disable

Channel	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)
1		−0.05	20	100
2		−0.05	20	100
3		−0.05	20	100
4		−0.05	20	100
5		−0.05	20	100
6		−0.05	20	100
7		−0.05	20	100
8		−0.05	20	100
9		−0.05	20	100
10		−0.05	20	100
11		−0.05	20	100
12		−0.05	20	100

Filename modifier
☑ Enable storage
Data type: ● Time stamps only ○ Time stamps + spikes
Spike Downsampling 1 / 1

Channel 1 Duration (s) 5

Figure 4-3.9. Control panels for the “*Export_for_propagation_analysis_spontaneous*” workflow. “Filter” tab (left) and “Detect beating” tab (right).

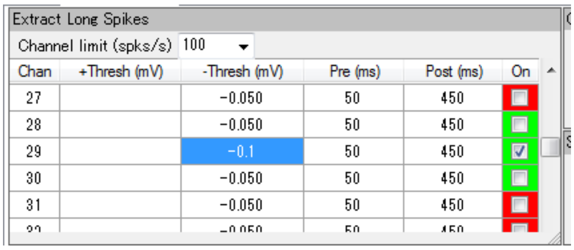
4-3.3. Field potential duration analysis

-Analysis for extracted long spikes' waveforms with the "*QT_analysis*" workflow template-

1. Open the "*QT_analysis*" workflow template, and then the data file. (Refer to page 107)

2. Replay the data by clicking the Green button, and then stop. Select a channel for analysis. Check "On" box for the selected channel in the [Extract Long Spikes] module.

   Or, channel(s) for analysis can be selected by the channel selector in the [Replay Raw Data]. (The channel selector pops up when the box next to channels on the [Reply Raw Data File] module is clicked.)



Extract Long Spikes

Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
27		-0.050	50	450	☐
28		-0.050	50	450	☐
29		-0.1	50	450	☑
30		-0.050	50	450	☐
31		-0.050	50	450	☐

Figure 4-3.10. Selecting analysis channel with the "On" check box in the [Extract Long Spikes].

Notice:

- *It is recommended to select only one channel to speed up your analysis although it is possible to select more than 1 channel. (Extraction of long spikes and following analysis require powerful computation.)*
- *Select one channel particularly when signals are NOT synchronized. Otherwise, the waveforms cannot be captured in the [Extract Spike Measures].*

3. Open the "Detect beating" tab, and and set thresholds.

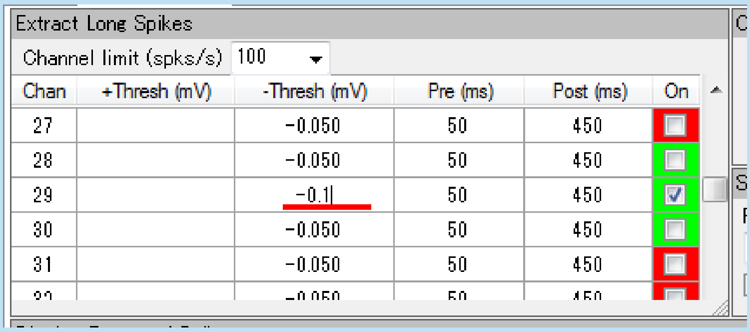
How to set thresholds

- Check the [ON] check box to activate spikes' extractions.
  -Green shows data is acquired (or processed for replay) while Red does not.

- The numbers for Thresholds and Pre/Post times can be changed by double-clicking and typing numbers directly on the chart (Figure 4-3.11).

- Pre/Post times must be identical for all 64 channels. Different numbers can be selected for thresholds.

- The number changed for threshold can be applied to all other channels by right-clicking the number and selecting Apply to All (Figure 4-3.12).

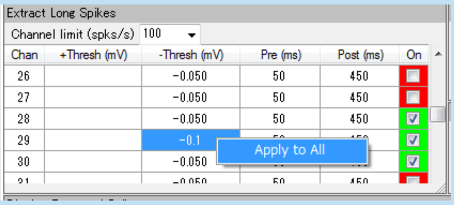


Extract Long Spikes

Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
27		−0.050	50	450	☐
28		−0.050	50	450	☐
29		−0.1	50	450	☑
30		−0.050	50	450	☐
31		−0.050	50	450	☐

Figure 4-3.11. Setting the thresholds for channel 29 by typing the number in the chart.



Extract Long Spikes

Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
26		−0.050	50	450	☐
27		−0.050	50	450	☐
28		−0.050	50	450	☑
29		−0.1			☑
30		−0.050			☑

Figure 4-3.12. Apply to ALL button.

Notice:

- It is not recommended to set thresholds both for + and -. Select either one for detecting long spikes.

- Set the thresholds as low as possible for negative signals (high for positive signals) so that other components of the signals are NOT extracted as spikes. (Figure 4-3.13)

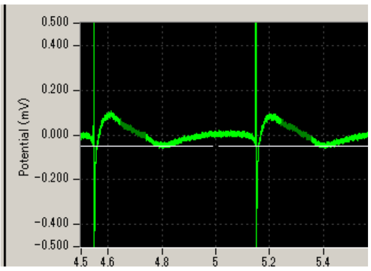


Figure 4-3.13. Bad example for spikes' extrication.

4. Replay data with Green button. The long spikes extracted are high-lighted in light green on the left chart of [Display Extracted Spikes] and waveforms of extracted long spikes are displayed on the right chart. (Figure 4-3.14)

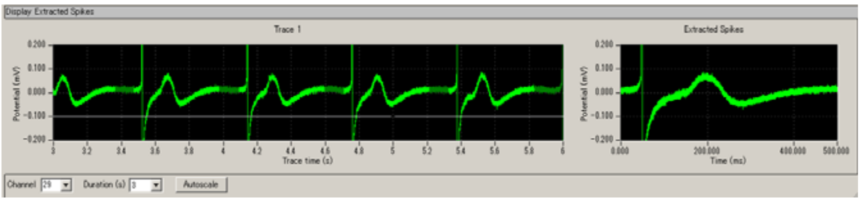


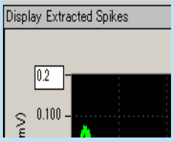
Figure 4-3.14. Long spikes are extracted (high-lighted with light green color).

How to modify the charts in the [Display Extracted Spikes]

[Trace] chart (left):

- The scale for amplitude (Y axis) can be changed by clicking and typing in the desired minimum and/or maximum values (Figure 4-3.15).

- The time duration (X axis) can be changed in the "Duration" drop down menu. (Figure 4-3.16)



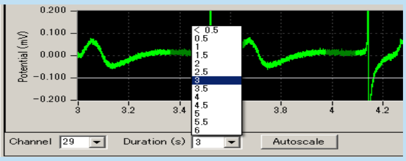


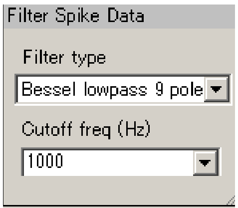
Figure 4-3.15. (Left) Changing the scale for amplifier.
Figure 4-3.16. (Right) Chancing the time duration.

[Extracted Spikes] chart (right):

- The scale for amplitude and time can be changed by clicking and typing in the desired minimum and/or maximum values. (Figure 4-3.15)

- The scale can be adjusted automatically by clicking [Autoscale] button or double-clicking anywhere on the chart.

5. Open the “Measurement” tab by clicking the tab name. The extracted long spikes are filtered before they are sent to the [Extract Spike Measures] module. In the default setting, the “Bessel low pass 9 pole with 1000 Hz” and “None” are selected. Modify the filter type and cut-off frequencies depending on your signals. (Figure 4-13.17)


Filter Spike Data

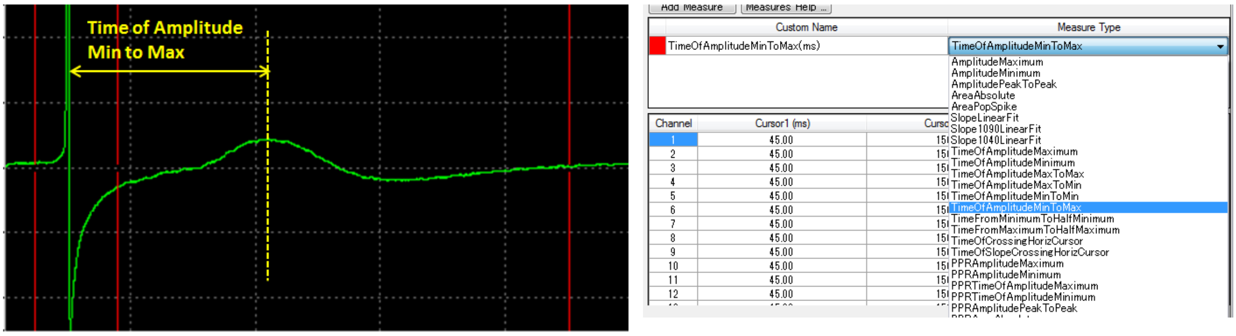
Filter type: Bessel lowpass 9 pole

Cutoff freq (Hz): 1000

Figure 4-3.17.

6. Set the waveform analysis parameters in the [Extract Spike Measures] module.

   The **"Time of Amplitude Min To Max"** is the default setting. This measures the “duration” from the time point for signal’s minimum amplitude between the 2 cursors on the left to the time point for signal’s maximum amplitude between 2 cursors on the right (Figure 4-3.18). Set the cursors by left-clicking and dragging them. Or, change the analysis menu. Similar analysis includes: “Time of Amplitude Max to Max, Max to Min, Min to Mim”.



Custom Name	Measure Type
TimeOfAmplitudeMinToMax(ms)	TimeOfAmplitudeMinToMax

Channel	Cursor1 (ms)
1	45.00
2	45.00
3	45.00
4	45.00
5	45.00
6	45.00
7	45.00
8	45.00
9	45.00
10	45.00
11	45.00
12	45.00

Figure 4-3.18. Definition of the “Time of Amplitude Min to Max” (left) and how to select the measurement type (right).

7. In order to change the measurement type, click [Edit Measures] button, and then select different analysis (measurement) with the drop-down menu for the “Measure Type”. (Figure 4-3.18)

   - Please refer to page 119-121 for detail about the [Extract Spike Measure] module.

8. Replay data with the Green button. When you are satisfied with your analysis parameters, save this as your analysis workflow. (Figure 4-3.19) The measurement chart can be saved as a “CSV” text file when the workflow is run with Green-Red button while checking the “Enable” check box for [Save Measure Data] module is checked. (Refer to section 4-7. Data output on pages 151-154 for details.

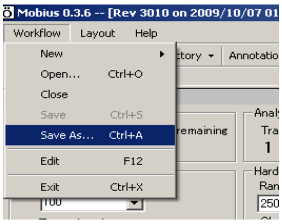
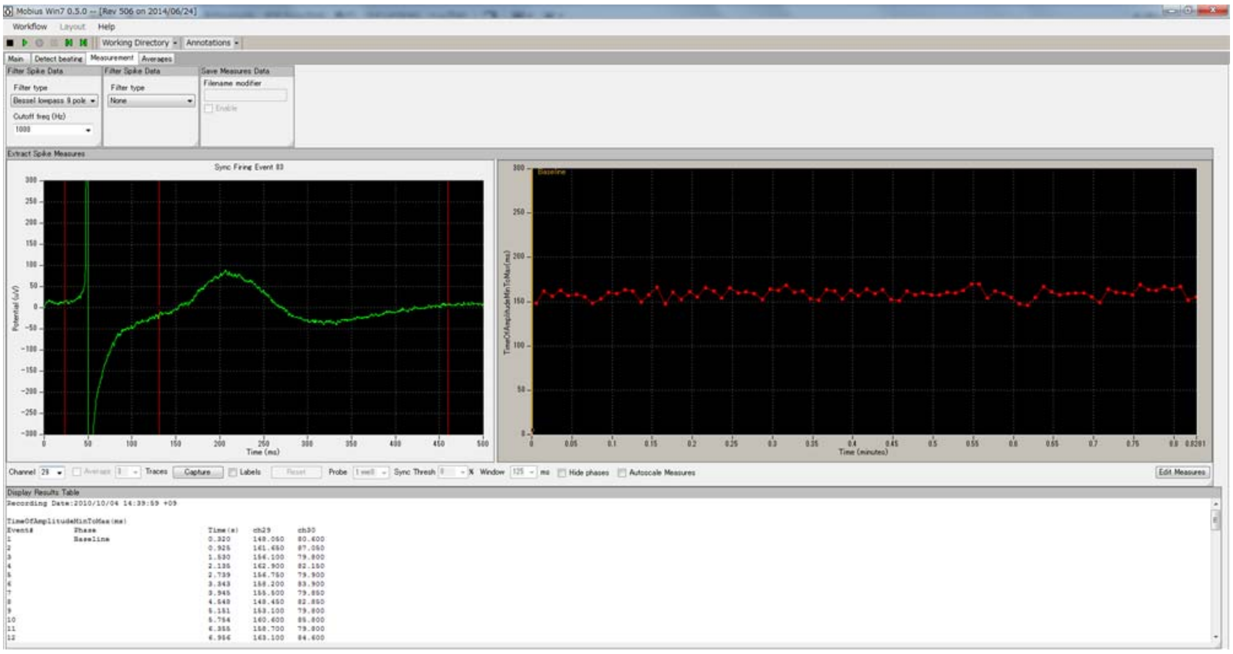


Figure 4-3.19.

   - The “*QT_analysis*” workflow template includes the modules for inter spike intervals. Please refer to section 4-3.4. Beat frequency analysis on pages 122-123 for details.



```
Recording Date:2010/10/04 14:39:59 +09

TimeOfAmplitudeMinToMax(ms)
Event#          Phase                        Time(s)    ch29       ch30
1               Baseline                     0.320      148.050    80.600
2                                            0.925      161.650    87.050
3                                            1.530      156.100    79.800
4                                            2.135      162.900    82.150
5                                            2.739      156.750    79.900
6                                            3.343      158.200    83.900
7                                            3.945      155.500    79.850
8                                            4.548      148.450    82.850
9                                            5.151      153.100    79.800
10                                           5.754      160.600    85.800
11                                           6.355      158.700    79.800
12                                           6.956      163.100    84.600
```

Figure 4-3.20. Field potential duration analysis. The time between peaks are measured with the “Time of Amplitude Max to Max” protocol and graphed (middle-right). The numbers are shown on the [Display Results Table] module (bottom).

Other available analysis (Measures) for analyzing field potential duration

Time of Crossing Horizontal Cursor

- Computes the “time” from the left-most vertical cursor (“Cursor 1”) to the first intersection of the extracted spikes with the horizontal cursor AFTER the right-most vertical cursor (“Cursor 2”). (Figure 4-3.21)

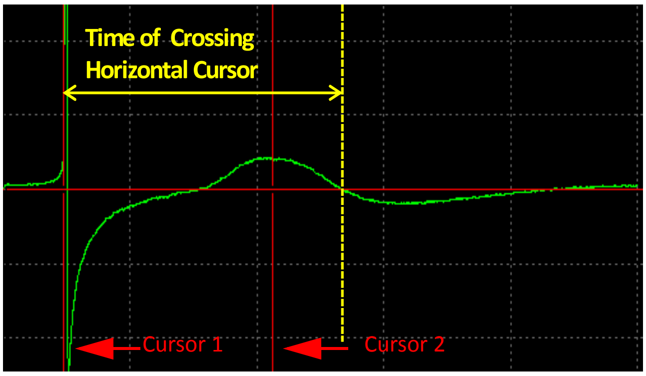


Figure 4-3.21.

Time of Slope Crossing Horizontal Cursor

- This measure uses four cursors as shown in Figure 4-3.22 - three vertical and one horizontal. It computes the “time” from the left-most vertical cursor (“Cursor 1”) to the intersection of the linear fit slope line computed on the data between the second and the third vertical cursor (“Cursor 2” and “Cursor 3”) with the horizontal cursor. (Figure 4-3.22)

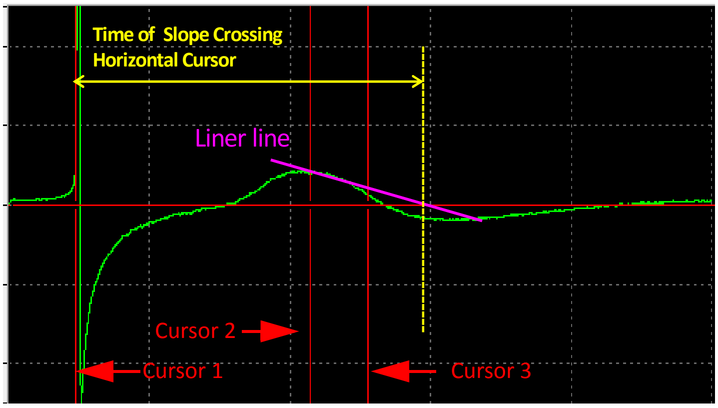
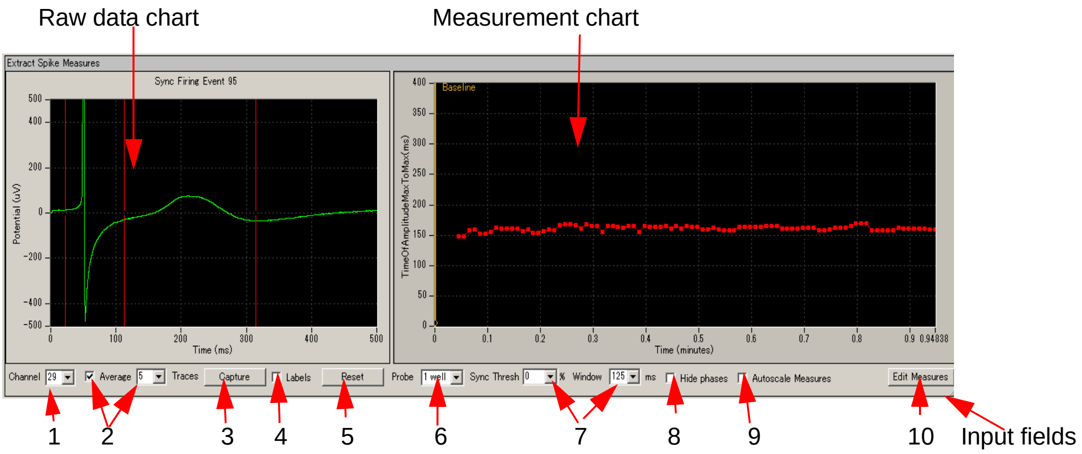


Figure 4-3.22.

[Extract Spike Measures] module

This module allows you to perform waveform analysis for extracted long spikes. Time, amplitude, slope, and area can be measured and graphed.



Channel 29
☑ Average 5 Traces
Probe 1 well
Sync Thresh 0 %
Window 125 ms

Figure 4-3-23. [Extract Spike Measures] module.

Raw data chart

The raw data chart on the left shows the extracted long spikes' waveforms. The cursors for the measurements are shifted by left-clicking and dragging.

You can zoom in on the Raw data chart by:

- Left-clicking and dragging while pressing the shift button.
- Changing the maximum (and/or minimum) numbers for both X (time) and Y (amplitude) axis. Click, and then, type in the new value(s) for the maximum and/or minimum. (Figure 4-3.24)
  - The scale can be adjusted automatically by double clicking anywhere on this chart.

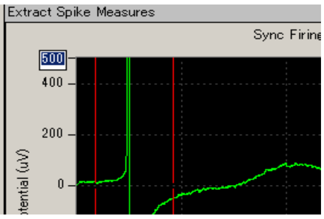


Figure 4-3.24.

The cursor locations can be copied to all or specific channels by right-clicking and selecting the [Apply Cursors to All Channels] or [Copy Cursors]. Select [Apply Cursors to All Measures] to copy the cursor locations to all measures. (Figure 4-3.25)

Selecting [Copy Data] allows you to copy the waveform data for the selected channel and paste to an excel worksheet. (Figure 4-3.25)

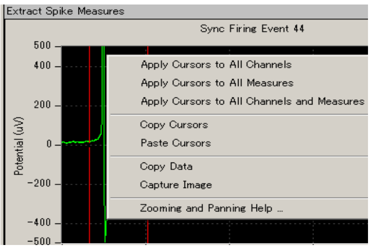
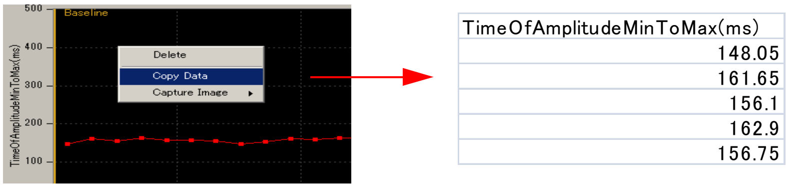


Figure 4-3.25.

Measurement chart

The time course for the selected measurements is graphed here. Zoom in or out by changing the maximum (and/or minimum) values for the X and/or Y axis. Click and type in the new values directly.

Right-clicking anywhere in the chart and selecting [Copy Data] allows you to copy the measurement data for selected channel(s) and paste to an excel worksheet (Figure 4-3.27). Selecting [Capture Image] allows to copy the chart image.



TimeOfAmplitudeMinToMax(ms)
148.05
161.65
156.1
162.9
156.75

Figure 4-3.26. Copy data.

Input fields for the [Extract Spike Measures] module

1. Channel No: Selects the channel for display.

2. “Average” check box + “Traces” dropdown:
   Check this box to average extracted spike waveforms. Set the number of traces to determine how many traces will be averaged (e.g. If 3 is input and the box is checked, every three traces (-1st, 2nd, and 3rd / 2nd, 3rd, and 4th / 3rd, 4th, and 5th) are averaged and plotted.

3. “Capture” button:
   Click the Capture button on three different waveforms to capture each of them on the screen. (Figure 4-3.27) Additional clicking will drop and replace the oldest capture with a new one. Right-click on the [Capture] button to expose a menu of options for clearing captured waveforms. Useful for comparing the waveforms in different phases.

4. “X-labels” checkbox:
   Check this box to display the X-axis positions of the cursors in the left Raw data chart.

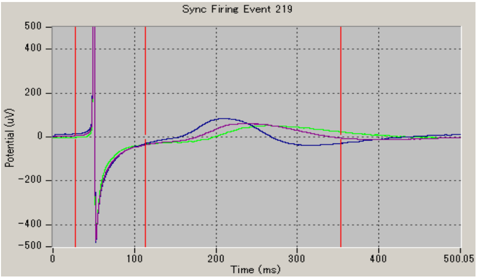


Figure 4-3.27

5. “Reset Cursors” button:
   If zooming operations have caused the cursors in the left Raw data chart to move out-of-sight, click this button to bring them back into view.

6. “Probe” dropdown:
   This input field will be fully enabled in future upgrade.

7. “Sync Thresh % dropdown” + “Windows ms” dropdown:
   This feature is helpful only when more than one channel is analyzed at once. (Selecting more than 1 channel is NOT recommended in current version of Mobius.) You can detect long spikes and filter out unexpected noise by requiring that a certain percentage of active channels (“Sync Thres”) produce long spikes within a certain window of time (“Window”). It is useful to view the output of this panel in [Display Results Table] when adjusting these settings, because you can quickly see which channels are not producing synchronized responses, and deactivate them.

8. “Hide Phases” check box:
   The phase bars (yellow bars with characters on the measurement chart) are hidden when this button is clicked.

9. “Autoscale Measures” check box:
   When this box is checked, the scale for the measurement chart is automatically adjusted. You can zoom in and out in the measurement chart by typing values in the X and Y axes' limits when the Autoscale button is NOT checked.

10. Edit Measures: Click this button to select or change the analysis (measurement) menu.

    1) Select the analysis (measurements) you desire from the [Measure Type] box to change the default measures. (Figure 4-3.28)

    2) You can name the selected measures by double-clicking and typing the desired name directly into the [Custom name] box.

    3) To perform more than one analysis (Measure), click [Add Measures] button so another row on the table and new measurement chart appears. (It is NOT recommended to select more than 1 measure during acquisition because it can slow down analysis significantly.)

    4) You can delete unnecessary measurements by right-clicking the [Custom Name], and selecting [Delete].

    - Click [Measures Help] button for detailed information on measurements.

    5) The table shows the cursor values. Cursors can also be adjusted by changing numbers in this table.

    6) Click the [Hide] button to close the editor.

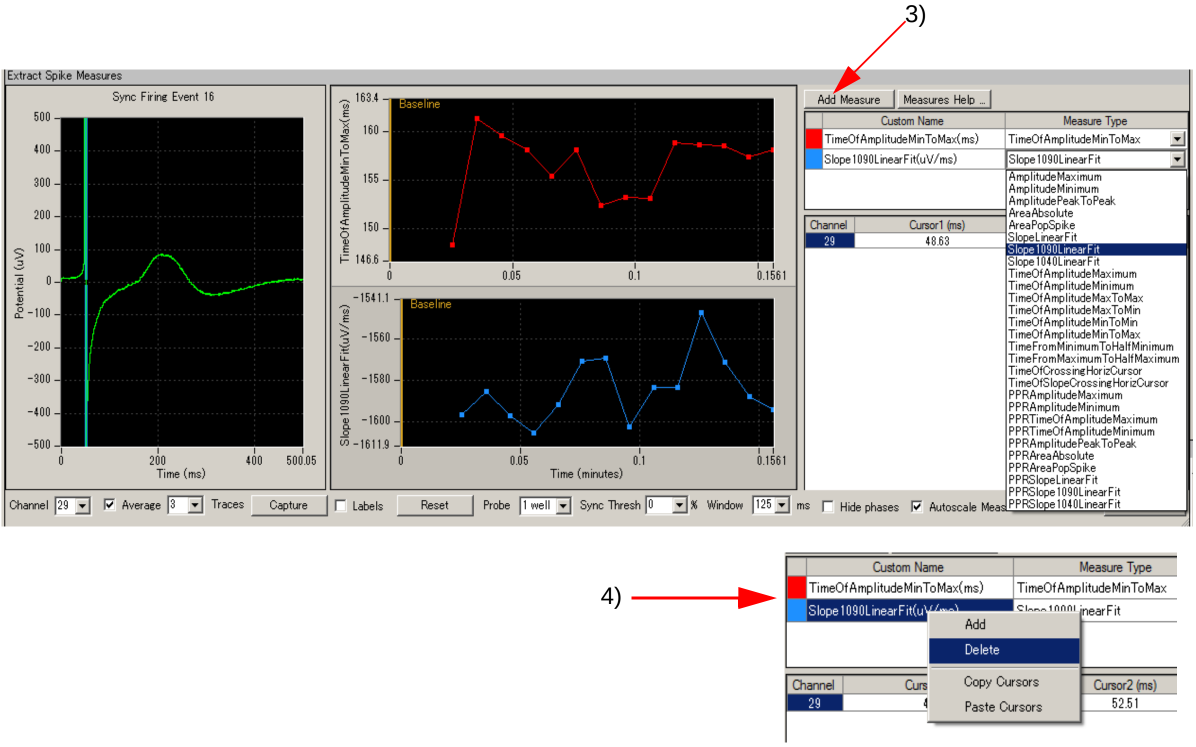


Figure 4-3.28. Edit Measures.

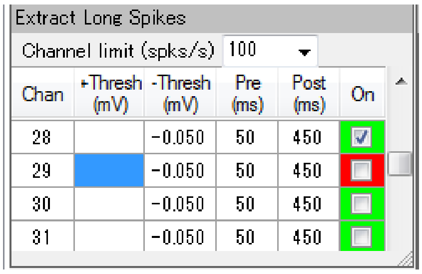
4-3.4. Beat frequency analysis

The [Compute Beats per Minute] and [Compute Interspike intervals] modules in the "*Beat_frequency_analysis*" workflow template are used.

1. Open the analysis workflow template and data file. (Refer to page 105)
2. Replay the data by clicking the Green button, and then stop.
3. Open the “Detect beating” tab. Select channels for analysis. Check “On” box for the selected channel in the [Extract Long Spikes] module.

   Or, channel(s) for analysis can be selected by the channel selector in the [Replay Raw Data]. (The channel selector pops up when the box next to channels on the [Reply Raw Data File] module is clicked.)

   Set the thresholds in the [Extract Long Spikes] module by following instructions described at How to set thresholds at page 114.



Extract Long Spikes

Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
28		-0.050	50	450	☑
29		-0.050	50	450	☐
30		-0.050	50	450	☐
31		-0.050	50	450	☐

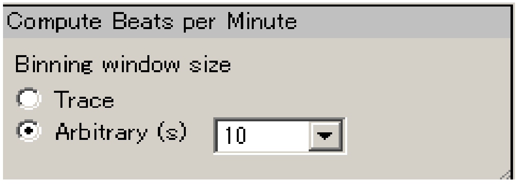
Figure 4-3.29. Selecting analysis channel with the “On” checkbox in the Extract Long Spikes.

4. The [Compute Beats per Minute] module builds a cumulative record of beat frequency changes. The computations are made independently on all channels and displayed on the [Display Beats per Minute] control panel.

   The default setting has 10 seconds selected for the Binning window size. Extracted long spikes are counted every 10 seconds. Selecting "Trace" instead of "Arbitrary (s)" means that spikes are counted for the whole trace (Figure 4-3.30).

5. The [Compute Interspike intervals] measures the time between each successive spike and creates a cumulative record of the changes. The computations are made independently on all channels and displayed on the [Display Interspike Intervals] module.

   The "Average Channel ISI" function in the [Compute Interspike Intervals] module measures the average time between the two most recent spikes across all active channels (Figure 4-3.31).

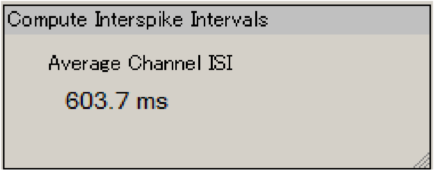


Compute Beats per Minute

Binning window size

○ Trace

● Arbitrary (s) 10



Compute Interspike Intervals

Average Channel ISI

603.7 ms

Figure 4-3.30. (Left) Compute Beats pre Minute module.
Figure 4-3.31. (Right) Compute Interspike Intervals.

- The chart for the [Display Interspike Intervals] and [Display beats per minute] can be zoomed in or out by changing the maximum (and/or minimum) values for the X and/or Y axis. Click and type in the new values directly. (Figure 4-3.32)

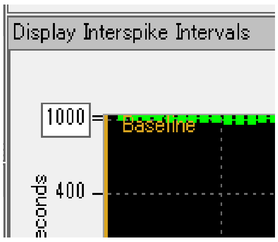


Figure 4-3.32.

6. Replay data with Green button. When you are satisfied with your analysis parameters, save this as your analysis workflow. (by clicking [Workflow] > [Save As]) (Figure 4-3.33)

7. In order to save these charts as "CSV" text files, check the “Enable” box on the [Save Beats per Minute] (and/or [Save Interspike Intervals]), then replay data with GREEN-RED button. (Refer to section 4-7. "Data output on pages 151-154).

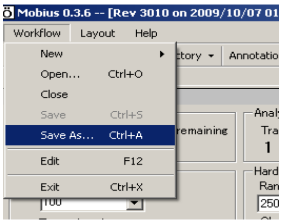


Figure 4-3.33.

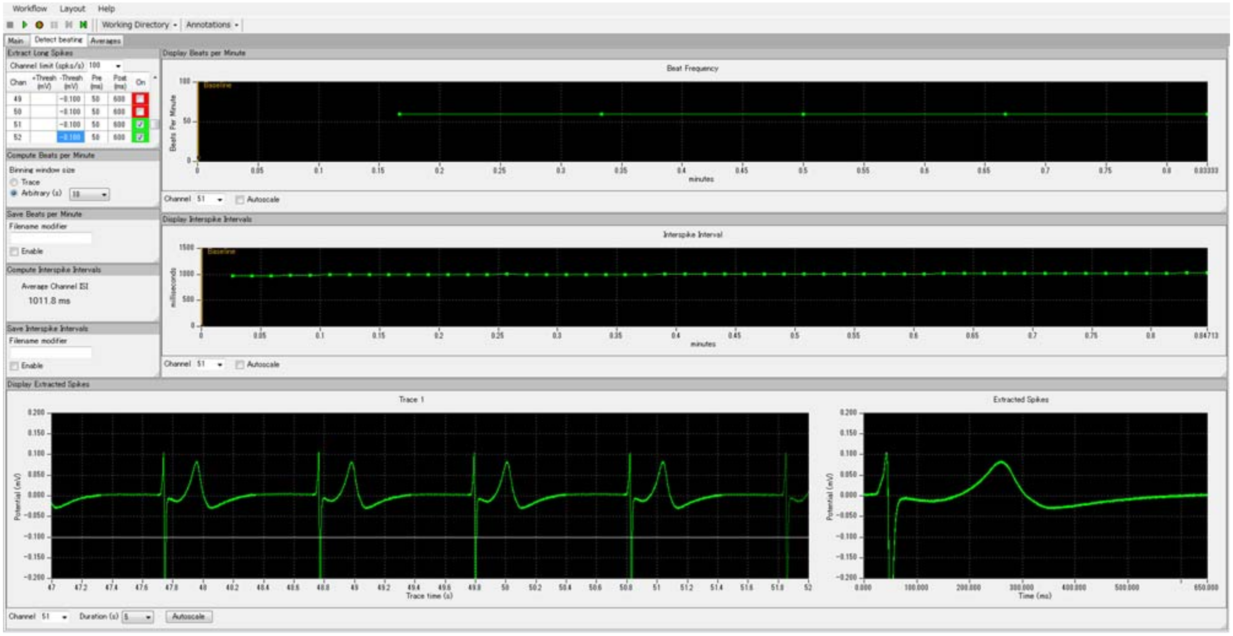


Figure 4-3.34. Analysis performed with the “*Beat_frequency_analysis*” workflow template. Beats (long spikes) are counted by every 10 seconds and displayed on the [Display Beats per MInute] module. (top-graph) Interspike intervals are measured and graphed in the [Display Interspike Intervals] module (center graph). The bottom graphs show long spikes that are extracted over time (high-lighted with light-green) (left) and each extracted long spike (right).

4-3.5. Making a dose-response-curve

This section will walk you through making a dose-response curve with the field potential duration from the acquired data. The Mobius' [Compute Measure Averages] module computes averages and standard deviations of:

1) waveform analysis selected at the [Extract Spike Measures].
2) beat frequencies.
3) interspike intervals.

How to add [Compute Measure Averages] module to your analysis workflow

1. Open the analysis workflow which was automatically made when you acquired the data. (Go to [Workflow] > [Open].)

2. Add the [Compute Measure Averages] module to this analysis workflow.

   1) Create a new tab by clicking [Layout] > [Add Tab] and typing the name for this tab. (Refer to Figure 4-3.35)

   2) Open the new tab, and then open the “Mobius Editor” by clicking [Workflow] > [Edit]. (Figure 4-3.36, left figure)

   3) Click the [Extract Spike Measures] on the left “Workflow” box, and then double-click the [Compute Measure Averages] on the right “Available task panels” box. Now you will see that [Compute Measure Averages] module shift to the left “Workflow” box and the control panel for this modules appears on the new tab. (Figure 4-3.36).

   4) Click the [Compute Measure Averages] on the left "Workflow” box, and then double-click the [Save Measure Averages] on the right "Available task panels" box. The control panel for the [Save Measures Averages] module will be displayed on your screen.

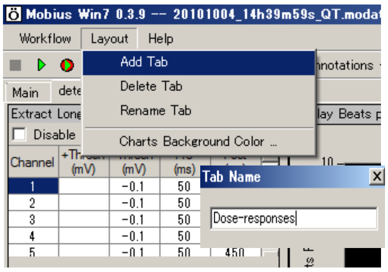


Figure 4-3.35. Adding a new tab.

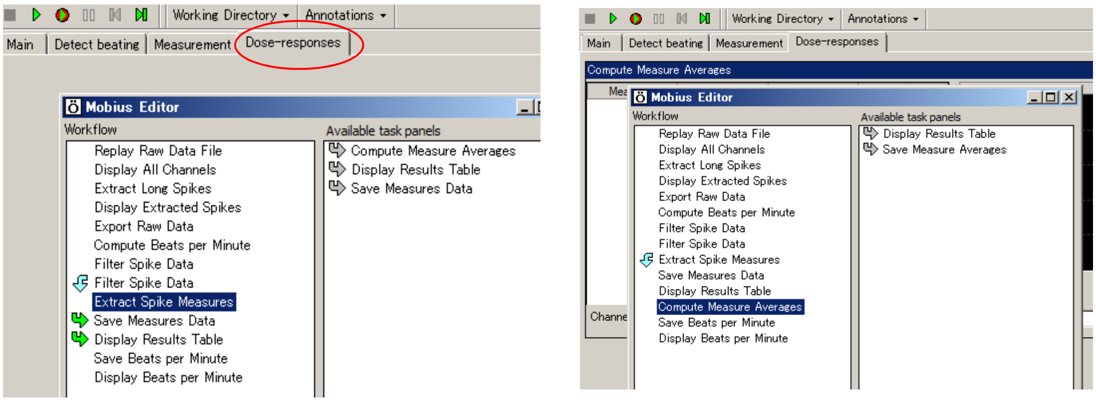


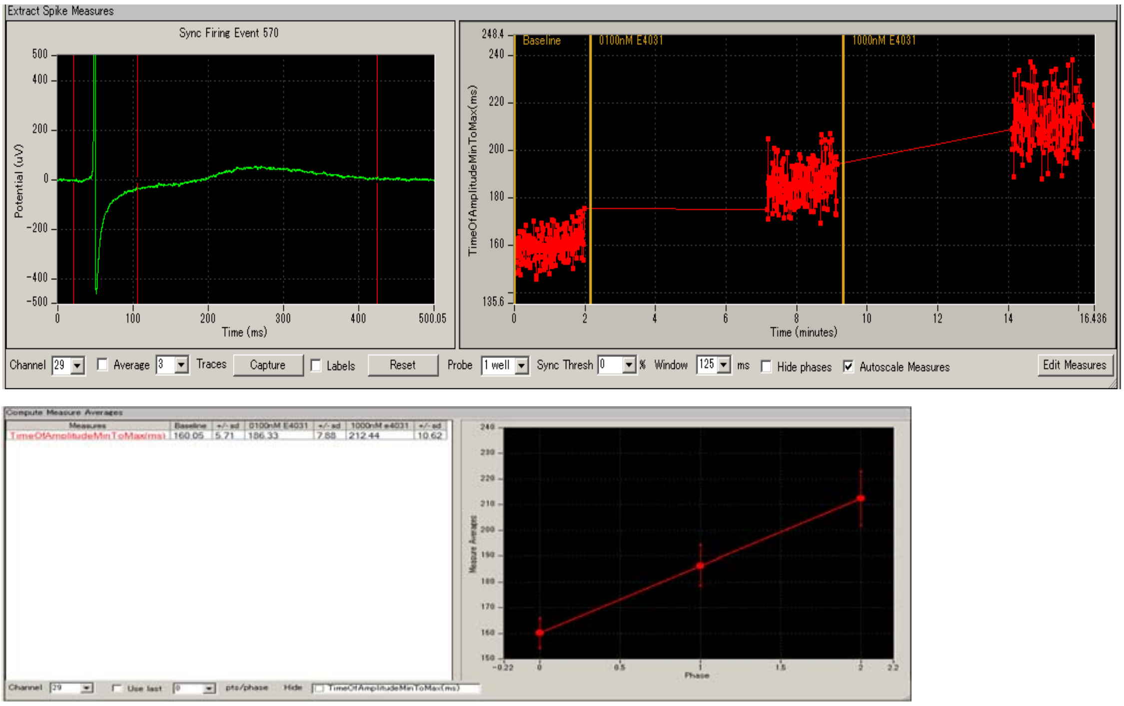
Figure 4-3.36. Mobius Editor was opened in a new tab and the [Extract Spike Measures] is clicked (left). The [Compute Measures Averages] was double-clicked and shifted to the left “Workflow” box. The control panel for this module now appears (right).

3. Set the analysis parameters according to the instructions in 4-3.2. Field potential duration analysis (pages 113-121).

4. Run Mobius with Green button to replay the data to the end. You will see the phase bars you set during your acquisition in the [Extract Spike Measures] module, and averages and standard deviations in the [Compute Measures Average] control panel. (Refer to Figure 4-3.37)

5. The phase bars are shifted by clicking and dragging. The averages and standard deviations are recalculated once Mobius is run with Green or Green-Red button after changing the locations of phase bars.

- If you are working with analysis workflow template or did not add phase bars during recording, add phase bars according to the instructions on the next section, “How to add phase bars”.



Channel 29 ☐ Average 3 Traces ☐ Labels Probe 1 well Sync Thresh 0 % Window 125 ms ☐ Hide phases ☑ Autoscale Measures

Measures	Baseline	+/- sd	0100nM E4031	+/- sd	1000nM e4031	+/- sd
TimeOfAmplitudeMinToMax(ms)	160.05	5.71	186.33	7.88	212.44	10.62

Channel 29 ☐ Use last 0 pts/phase Hide ☐ TimeOfAmplitudeMinToMax(ms)

Figure 4-3.37. The results of “Time of Amplitude Max to Max” with phase bars in the [Extract Spike Measure] (top) and the averages and standard deviations for each phase displayed in the [Compute Measure Averages] control panel.

6. In order to save the average chart, save it as your own analysis workflow first. ([Workflow] > [Save as]) Check the “Enable” check-box on the [Save Measure Averages] module, then run Mobius with the Green-Red button. (Refer to section 4-7. Data output on pages 151-154 for details.)

How to add phase bars

Even if phase bars are not added during recording, they can be added later off-line using the following procedure.

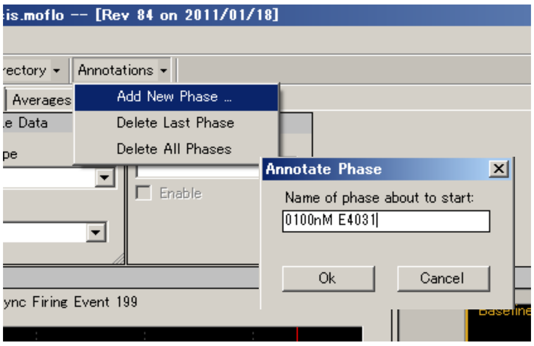
1. Open the “*QT_analysis*” workflow template (or your own analysis workflow), and then open the data file. (Page 105-106)

2. Set the analysis parameters according to the instructions in the 4-3.2. Field potential duration analysis (page 113-122).

3. Open the “Measurement” tab, and then replay the data with the Green button. You will see the first phase, Baseline (yellow bar) is already at time 0.

4. When the first phase is finished, pause Mobius with YELLOW button, and make a new phase.

   - Select [Annotators] > [Add New phase], then, type the name for the new phase. (Figure 4-3.38) Now you will see the new bar at the right chart of the [Extracted Spike Measures]. (Figure 4-3.39).
   - Do NOT stop Mobius with Black button. You will not be able to add more phases.



Name of phase about to start: 0100nM E4031

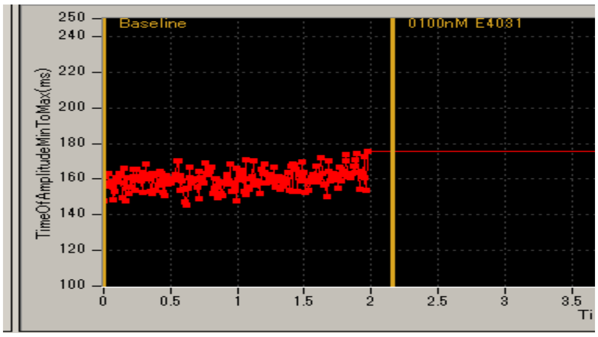


Figure 4-3.38. (Left) Adding a new phase bar.
Figure 4-3.39. (Right) A phase bar added on the [Extract Spike Measures].

5. When the second phase finished, pause Mobius again and make a new phase.
   *The location of the phase annotator (yellow bar) can be shifted later.

6. Replay the data to the end. Averages and standard deviations are computed and graphed for each phase (dose) in the Average tab. (Refer to Figure 4-3.37)

7. In order to save the average chart, save it as your own analysis workflow first. Check the “Enable” check-box on the [Save Measure Average] module, and run Mobius with Green-Red button. (Refer to section 4-7. Data output on pages 151-154 for detail.)

Input fields for [Compute Measure Averages] module

1. Channel:
   The channel to be displayed is selected here. Channels can NOT be changed while running an experiment.

2. Use last:
   If this box is checked, the selected (or typed) number of data points are used for computation. For example, if 10 is selected, average and standard deviation are measured for the last 10 data points in each phase.

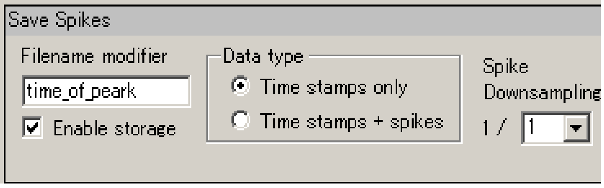
4-3.6. Propagation analysis for spontaneous data

This section describes how to generate a propagation map (distribution of the peak times) using the "*Export_ for_propagation_analysis_spontaneous*" workflow template and excel. You will save the "time for the amplitude peak" as a "CSV" file using this workflow template, and then generate a propagation map using an excel file. The excel file ("Propagation_analysis_spontaneous") is available from the Support page on the MED64 website. www. med64.com/Support.

CAUTION:

- All 64 channels must have signals for the excel file to work.

1. Open the "*Export_ for_propagation_ analysis_spontaneous*" workflow template (Refer to page 111).
2. Open the data file, and replay the data by clicking the Green button. (Refer to section 4-3.1. Opening and replaying acquired data on page 105)
3. When the peak points in the original raw data are not clear enough, modify the filter menu so that peak points are seen clearly.
4. Set the thresholds for the all channels. (Refer to page 114)
5. Replay data again to make sure you are satisfied with the new thresholds.
6. Make sure that "Enable storage" is checked and "Time stamps only" is selected in the [Save Spikes] module (Figure 4-3.40).
7. Save it as your own analysis workflow by clicking [Workflow] > [Save as].
8. Click the Green-Red button so that the time stamps for the peaks are saved as a "CSV" file.



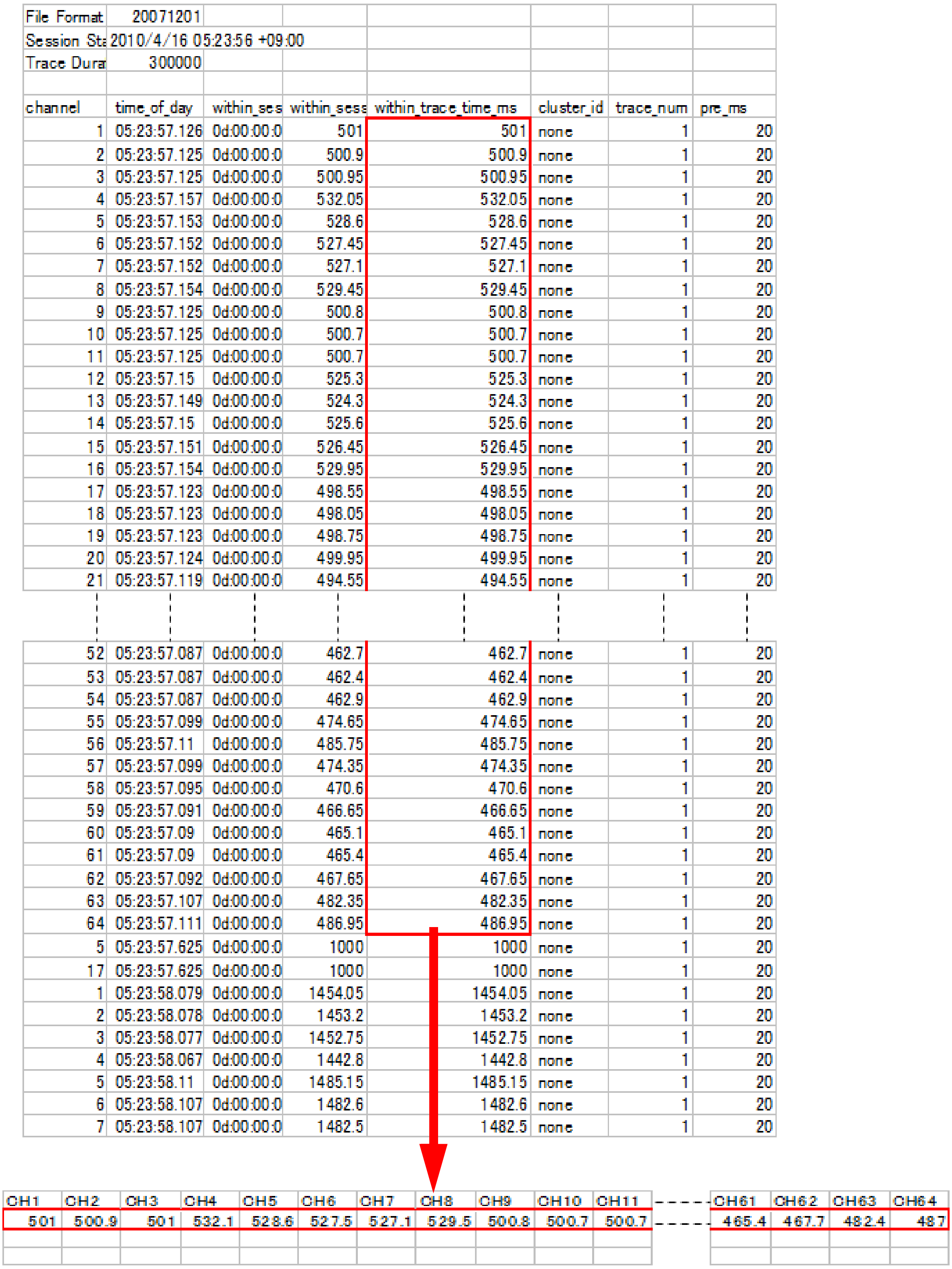
time_of_peark
☑ Enable storage
● Time stamps only
○ Time stamps + spikes
1 / 1

Figure. 4-3.40. [Save Spikes] module.

9. Open the "CSV" file with excel. (Figure 4-3.41 shows an example of the CSV file.)
10. Open the excel file, "Propagation_analysis_spontaneous". This file consists of 4 sheets of [data], [analysis], [map_data], and [map]. You will copy some data to the [data] sheet. Propagation map will be displayed in the [map] sheet.
11. Copy the data in the "within_trace_time_ms" for channel 1-64 (marked with red in the Figure 4-3.41) on your "CSV" file, and paste them in the second row on the [data] sheet in the "Propagation_analysis_spontaneous" excel file.

    Transpose Raw and Line.

12. A propagation map is generated in the "map" sheet of the "Propagation_analysis_spontaneous" excel file (Figure 4-3.42).



File Format	20071201						
Session St	2010/4/16 05:23:56 +09:00						
Trace Dura	300000						
channel	time_of_day	within_ses	within_sess	within_trace_time_ms	cluster_id	trace_num	pre_ms
1	05:23:57.126	0d:00:00:0	501	501	none	1	20
2	05:23:57.125	0d:00:00:0	500.9	500.9	none	1	20
3	05:23:57.125	0d:00:00:0	500.95	500.95	none	1	20
4	05:23:57.157	0d:00:00:0	532.05	532.05	none	1	20
5	05:23:57.153	0d:00:00:0	528.6	528.6	none	1	20
6	05:23:57.152	0d:00:00:0	527.45	527.45	none	1	20
7	05:23:57.152	0d:00:00:0	527.1	527.1	none	1	20
8	05:23:57.154	0d:00:00:0	529.45	529.45	none	1	20
9	05:23:57.125	0d:00:00:0	500.8	500.8	none	1	20
10	05:23:57.125	0d:00:00:0	500.7	500.7	none	1	20
11	05:23:57.125	0d:00:00:0	500.7	500.7	none	1	20
12	05:23:57.15	0d:00:00:0	525.3	525.3	none	1	20
13	05:23:57.149	0d:00:00:0	524.3	524.3	none	1	20
14	05:23:57.15	0d:00:00:0	525.6	525.6	none	1	20
15	05:23:57.151	0d:00:00:0	526.45	526.45	none	1	20
16	05:23:57.154	0d:00:00:0	529.95	529.95	none	1	20
17	05:23:57.123	0d:00:00:0	498.55	498.55	none	1	20
18	05:23:57.123	0d:00:00:0	498.05	498.05	none	1	20
19	05:23:57.123	0d:00:00:0	498.75	498.75	none	1	20
20	05:23:57.124	0d:00:00:0	499.95	499.95	none	1	20
21	05:23:57.119	0d:00:00:0	494.55	494.55	none	1	20

52	05:23:57.087	0d:00:00:0	462.7	462.7	none	1	20
53	05:23:57.087	0d:00:00:0	462.4	462.4	none	1	20
54	05:23:57.087	0d:00:00:0	462.9	462.9	none	1	20
55	05:23:57.099	0d:00:00:0	474.65	474.65	none	1	20
56	05:23:57.11	0d:00:00:0	485.75	485.75	none	1	20
57	05:23:57.099	0d:00:00:0	474.35	474.35	none	1	20
58	05:23:57.095	0d:00:00:0	470.6	470.6	none	1	20
59	05:23:57.091	0d:00:00:0	466.65	466.65	none	1	20
60	05:23:57.09	0d:00:00:0	465.1	465.1	none	1	20
61	05:23:57.09	0d:00:00:0	465.4	465.4	none	1	20
62	05:23:57.092	0d:00:00:0	467.65	467.65	none	1	20
63	05:23:57.107	0d:00:00:0	482.35	482.35	none	1	20
64	05:23:57.111	0d:00:00:0	486.95	486.95	none	1	20
5	05:23:57.625	0d:00:00:0	1000	1000	none	1	20
17	05:23:57.625	0d:00:00:0	1000	1000	none	1	20
1	05:23:58.079	0d:00:00:0	1454.05	1454.05	none	1	20
2	05:23:58.078	0d:00:00:0	1453.2	1453.2	none	1	20
3	05:23:58.077	0d:00:00:0	1452.75	1452.75	none	1	20
4	05:23:58.067	0d:00:00:0	1442.8	1442.8	none	1	20
5	05:23:58.11	0d:00:00:0	1485.15	1485.15	none	1	20
6	05:23:58.107	0d:00:00:0	1482.6	1482.6	none	1	20
7	05:23:58.107	0d:00:00:0	1482.5	1482.5	none	1	20

CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10	CH11	CH61	CH62	CH63	CH64
501	500.9	501	532.1	528.6	527.5	527.1	529.5	500.8	500.7	500.7	465.4	467.7	482.4	487

Figure 4-3.41. Top shows an example of data for saved time stamps displayed in excel. The data in the “Within_trace_time_ms” (marked with red) is copied and pasted to the second row in the [data] sheet of “Propatation_analysis_spontaneous” excel file

--

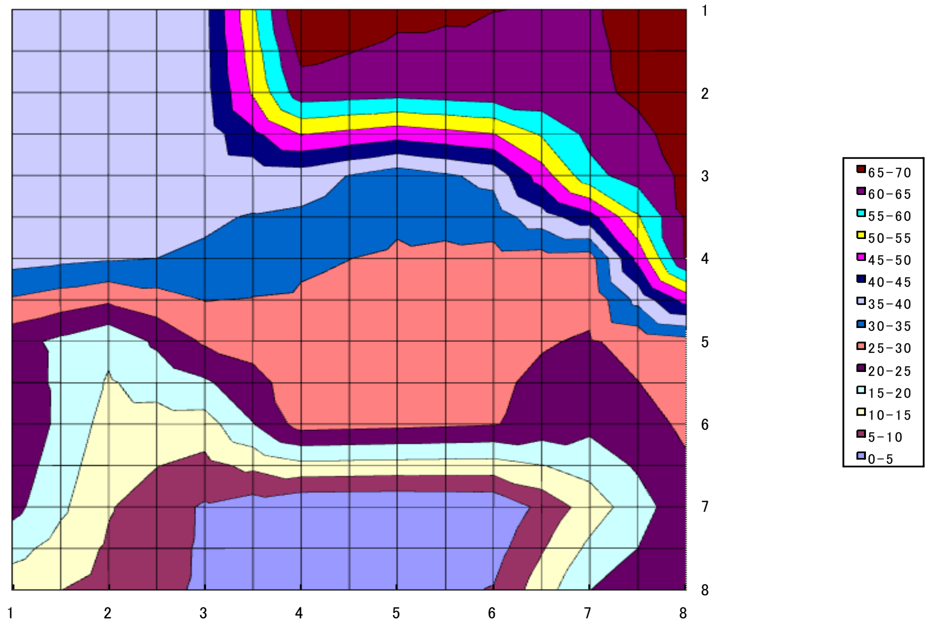


Figure 4-3.42. A propagation map generated in the [map] sheet of the “Propagation_analysis spontaneous file.

4-4. Recording of myocardial signals with pacing

The "*Pacing_recording*" workflow template is available for recording of myocardial signals with electrical stimulation (pacing).

4-4.1. Overview of the "*Pacing_recording*" workflow template

The "*Pacing_recording*" workflow template consists of following modules located in the "Main" and "Pacing measures" tabs.

Tab	Modules
Main	Acquire MED64R2 Data w/Stim / Display All Channels / Export Raw Data
Pacing measures	Extract Long Spikes, Filter Spike Data (2), Extract Spike Measures, Save Measures Data, Display Extracted Spikes

Acquisition and stimulation parameters are set in the [Acquire MED64R2 Data w/Stim] module. Signals are extracted with the [Extract Long Spikes] module. The extracted signals are sent to the [Extract Spike Measures] module, where extracted signals' waveform analysis are performed (amplitude, slope, time, and area). The raw data can be exported either as binary or ASCII data while the measurement chart is saved as a "CSV" formatted text file.

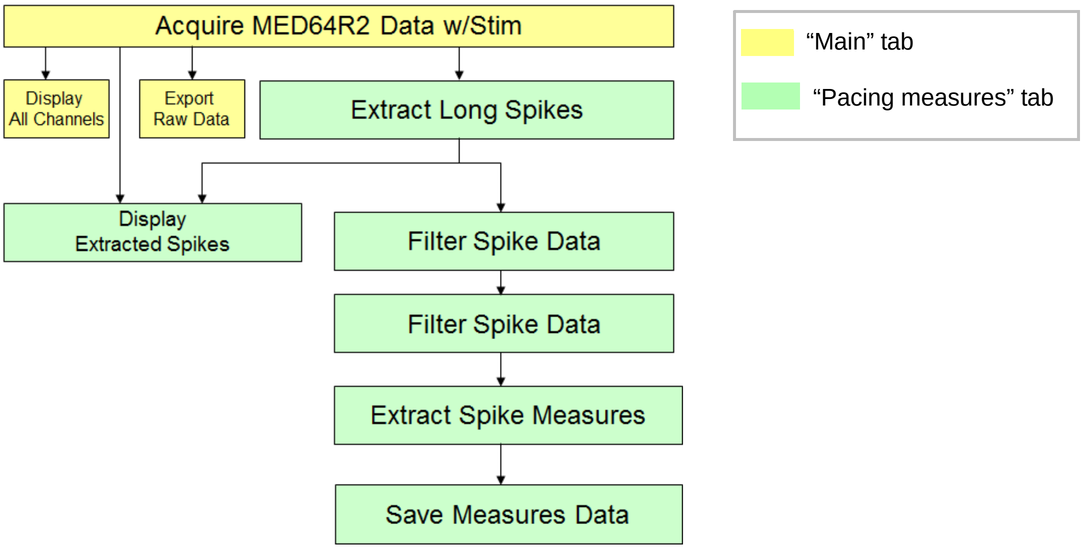


Figure 4-4.1. Module configuration for "*Pacing_analysis*" workflow template.

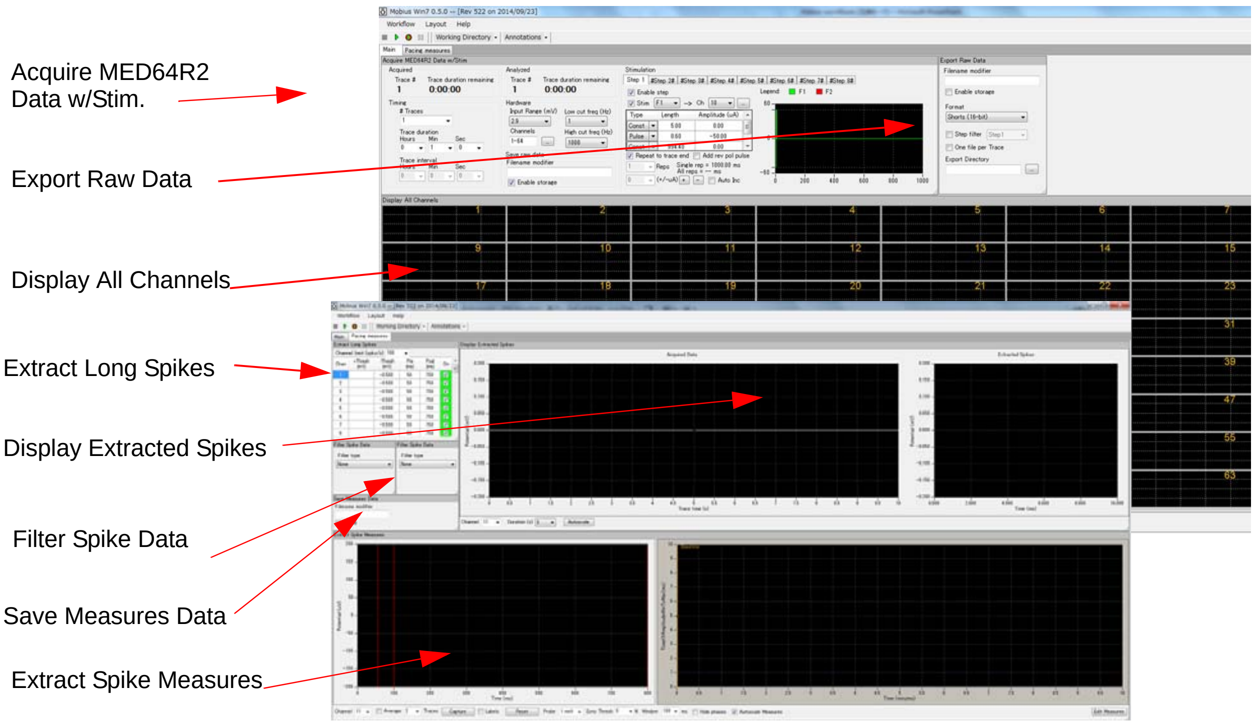


Figure 4-4.2. Control panels for “*Pacing_recording*” workflow template. ”Main” and “Pacing measures” tab.

Opening the workflow template

1. Click [Workflow] > [New] > [From Template].
2. Select [64MD1_1280x1024] or [64MD1_1920x1080] folder, depending of the size of your display monitor. Select [QT] > [Pacing_recording] folder, and then the “*Pacing_recording*” workflow template.

NOTICE:
Connect your PC to the MED64 amplifiers and turn on both amplifiers to open the recording workflow.

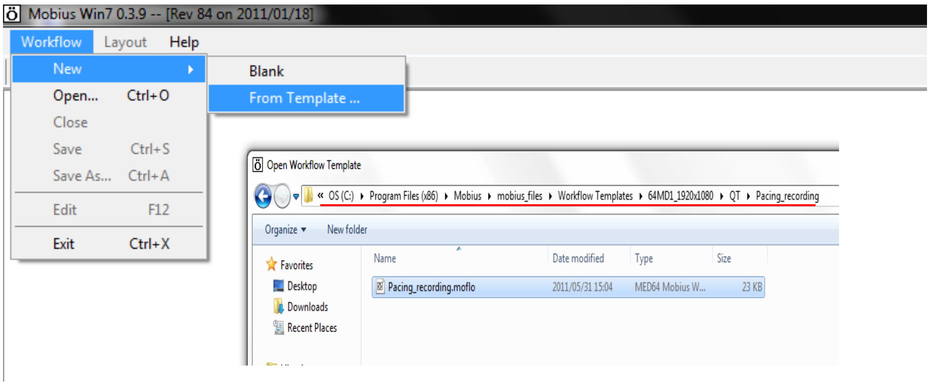
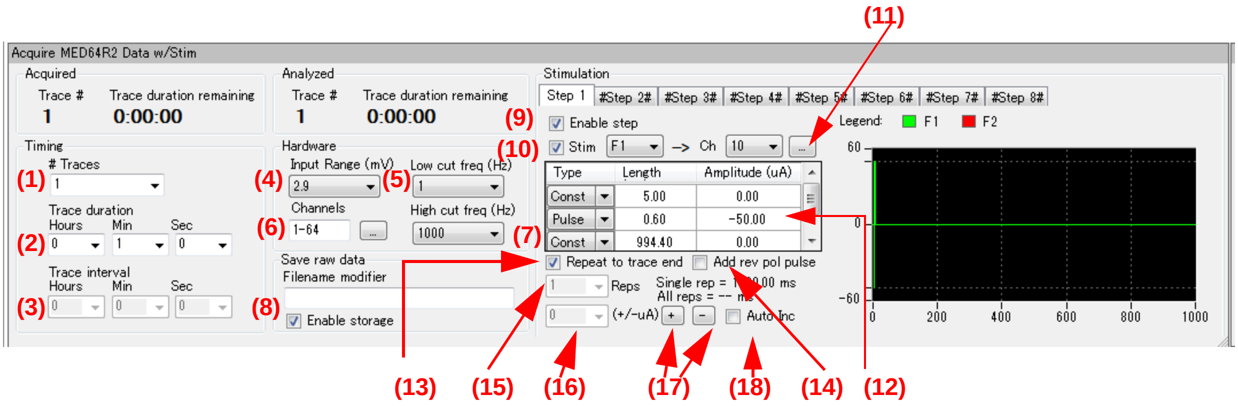


Figure 4-4.3. Opening workflow template.

4-4.2. Setting the acquisition and stimulation parameters

Acquisition and stimulation parameters are set in the [Acquire MED64R2 Data w/Stim] module. The default setting will allow you to stimulate with intervals of 1 second for one minute using the function “Repeat pattern to trace end”. Change the parameters depending on your experimental plan.

NOTICE:
Connect your PC to the MED64 amplifiers and turn on both amplifiers to open this module.



Acquire MED64R2 Data w/Stim

Acquired: Trace # 1, Trace duration remaining 0:00:00

Analyzed: Trace # 1, Trace duration remaining 0:00:00

Timing: # Traces 1; Trace duration Hours 0, Min 1, Sec 0; Trace interval Hours 0, Min 0, Sec 0

Hardware: Input Range (mV) 2.9; Low cut freq (Hz) 1; Channels 1-64; High cut freq (Hz) 1000

Save raw data: Filename modifier; ☑ Enable storage

Stimulation: Step 1 | #Step 2# | #Step 3# | #Step 4# | #Step 5# | #Step 6# | #Step 7# | #Step 8#

☑ Enable step

☑ Stim F1 -> Ch 10

Type	Length	Amplitude (uA)
Const	5.00	0.00
Pulse	0.60	-50.00
Const	994.40	0.00

☑ Repeat to trace end ☐ Add rev pol pulse

1 Reps Single rep = 1000.00 ms All reps = -- ms

0 (+/-uA) + - ☐ Auto Inc

Figure 4-4.4. [Acquire MED64R2 Data w/Stim] module.

Acquisition

1. # Traces: Number of Traces (Sweeps).

2. Trace duration: Sampling (Recording) time for each trace.

3. Trace interval:

   Interval between traces. (From the beginning of the first trace to the beginning of the next trace) In the default setting, the Trace interval is invalid because the #Trace is set for 1. Select any number greater than 1 for # Trace to activate it.

4. Input Range (mV):

   Maximum input signals level. This setting should be adjusted according to the signal amplitude to achieve the maximal dynamic range. Select 2.3 mV, 2.9 mV, 5.0 mV, 12.5 mV, or 25 mV. Larger number for the Input Range creates lower voltage resolution.

   2.9 mV is set in the default. This is the Input Range recommended for the Field Potential Duration analysis combined with 1 kHz for the “High cut freq” (Low-pass filter) to achieve the stability for the Repolarization (K^+ efflux) peak time. Change it depending on the size of your signals and setting for the parameter selected for “High cut freq”. Particularly change it to 5.0 mV (or greater) when 10 kHz is selected for the “High cut freq”.

5. Low cut freq (Hz):

   High pass filter. Select from 0.1, 1.0, 10 and 100 Hz. If 1 is set, signals with frequencies lower than 1 Hz are filtered out. The recommended filter setting for recording myocardial signals is 1.0 Hz.

6. Channels:

   Select the channels for recording here. When the square box is clicked, the channel selector pops up. Green shows enabled channels.

7. High cut freq (Hz):

   Low pass filter. Select 1000 Hz, 2,000 Hz, 2500 Hz, 5000 Hz, 7500 Hz, or 10000 Hz. If 5000 Hz is selected, signals with frequencies higher than 5000 Hz are filtered out. Typically, 1000 Hz is recommended for the Field Potential Duration analysis to achieve the stability for the repolarization peak time. Change it depending on the analysis you would like to perform.

8. Save raw data:

   When Mobius is started with Green-Red button while the [Enable storage] is checked, the raw data is saved. Enter a descriptive file name of your choice.

Stimulation

Each Step is independent and can be programmed with different type of stimulation protocols. Enabled steps will be applied in the order (1 to 8) at the intervals set in the Trace interval.

9. Enable step:

   When the box to the left of [Enable Step] is checked, the Step becomes valid. The default settings have Step1 enabled and Steps 2-8 disabled (shown with # next to each Step).

   - For example, if both Step 1 and Step 2 are enabled, the Step 1 and Step 2 are applied alternatively at the interval set with the Trace interval.

10. Stimulator selector and check box:

    Select the MED64 amplifier's built-in stimulator (F1 or F2) here. Checking the box on the right of "Stim" activates the selected stimulator. The default settings have F1 enabled for Step 1.

    - In order to stimulate through both stimulators simultaneously, enable both F1 and F2 stimulator in Step 1.

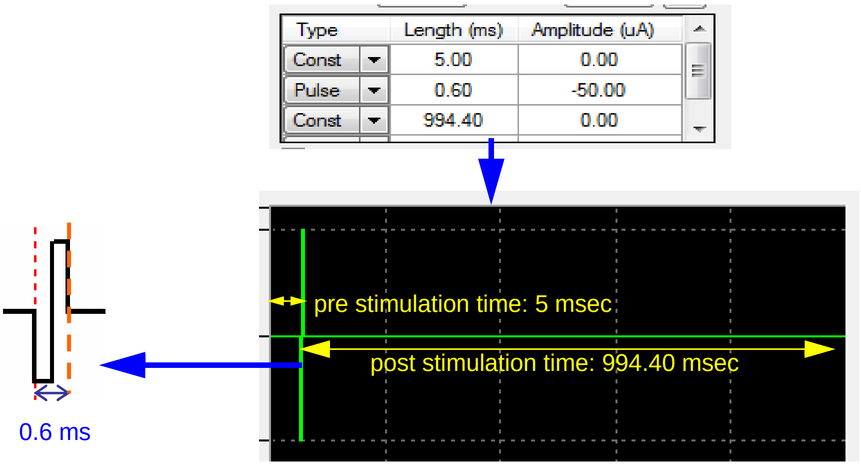
11. Stimulus channel selector:

    Select stimulus channel here. The stimulus channel can be changed either by the drop-down or the pop-up which appears by clicking the square box. The green shows the stimulus channel.

    Mobius needs to be stopped (with Black button) or paused (with Yellow button) for the stimulus channel to be changed.

12. Stimulus waveform editor:

    Make your stimulus protocol here by selecting either [Const] or [Pulse] and typing in the desired [Length] and [Amplitude]. Refer to Figure 4-4.5 for a schematic representation of a stimulus protocol example.



Type	Length (ms)	Amplitude (uA)
Const	5.00	0.00
Pulse	0.60	-50.00
Const	994.40	0.00

Figure 4-4.5. Example of stimulus protocol and its schematic diagram.

- Make sure that the STIMULUS CURRENT selection on the MED64 Head Amplifier (MED-A64HE1S) is set to [NORMAL] to deliver the stimulus current amplitude set in Mobius. Selecting [x2] doubles the output stimulus current amplitude set in Mobius.

  (e.g. When 10 µA is selected by Mobius with [x2] selected, the output stimulus current is 20 µA. When -10 µA is set in the Mobius with NORMAL selected, the output stimulus current amplitude is 10 µA)

- The maximum stimulus amplitude set by Mobius is 100 µA. Set the STIMULUS CURRENT selector for x2 when stimulus amplitude with greater than 100 µA is applied.

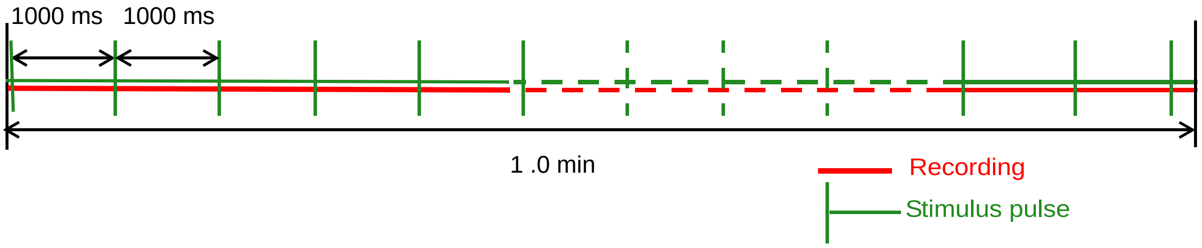
- The maximum number of stimulus pulses to be programmed here at a Step is **21**.

- Do NOT select [Ramp] for the stimulation with the MED64. Only bi-phasic stimulation is recommended.

13. Repeat pattern to trace end check box:

    When this box is checked, the stimulus protocol programed with the Stimulus waveform editor will be repeated to the end of the trace.

    In the default setting where the box is checked, stimulus pulses are delivered with interval of 1000 ms for 1 minute.



14. Add rev pol pulse check button:

    When this box is checked, the other stimulator is automatically activated and creates the stimulus waveform which has identical amplitude (uA) and length (ms) with REVERSED polarity to the given stimulator.

For example, if the box is checked at the default setting, F2 (that is not activated as default) is activated and has stimulus waveform with 50 uA and same duration to the F1

15. Reps selector:

    The stimulus protocol set at the “Stimulus waveform editor” (11) is repeated the number of times set here. Useful for multiple pulse stimulation.

    In the default setting, this selector is invalid because the “Repeat pattern to trace end” is checked. Uncheck the ”Repeat pattern to trace end” to activate this selector.

16. Stimulus current amplitude increment button:

    When the + button is clicked, the absolute value of the stimulus current amplitude for the [Pulse] is increment ed by the value set in the left box. (15*) For example, if 2 is set in the box (15) and the + button is clicked once, the stimulus amplitude will change to -12.00 µA from -10.00 µA. This is useful for changing the stimulus amplitude for multiple pulse stimulations with just one click. The absolute value of the stimulus amplitude can be decreased sequentially using the same procedure when - button is clicked.

    - The stimulus current amplitude can be changed even during recording by using this button. However, the changes are activated on the 2nd trace after the change since Mobius uses the next trace to reset itself.
    - In the default setting, this button is invalid because the “Repeat pattern to trace end” is checked. Uncheck the ”Repeat pattern to trace end” to activate this selector.

17. See #15.

18. Auto Inc check box:

    When this box is checked, the stimulus current amplitude will automatically increase adding the value (in µA) set in the left box (15) to each preceding stimulation pulse. This is useful for making I/O curves.

    - In the default setting, this check box is invalid because the “Repeat pattern to trace end” is checked. Uncheck the ”Repeat pattern to trace end” to activate this selector.

4-4.3. Execution button

Acquisition is initiated by running an acquisition workflow. Clicking the Green button runs the acquisition workflow without saving data while clicking the Green-red button runs the acquisition workflow while saving data to disc.

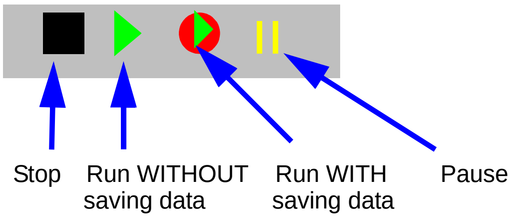
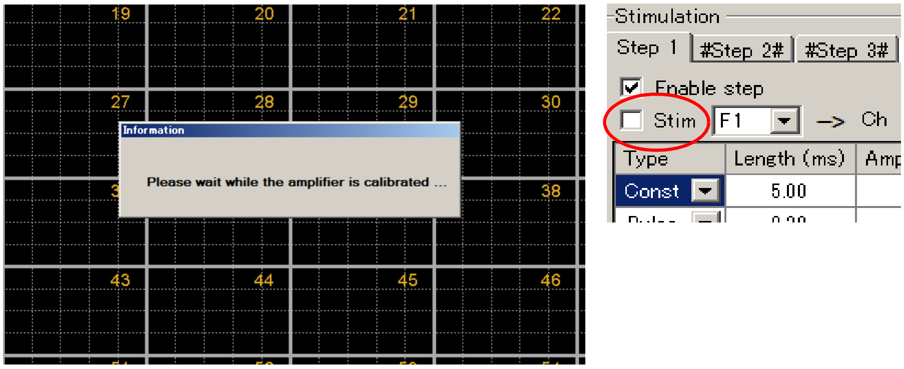


Figure 4-4.6. Execution buttons

4-4.4. Before starting your experiment

When a recording workflow is run with Green or Green-Red button for the first time after it is opened (or made), Mobius needs several seconds for its calibration (as seen in the pop-up message). Acquisition starts automatically after the calibration.

If you would like to start acquisition immediately after clicking the Green-Red button, run the workflow with the green button for a few traces with both stimulators un-enabled, and then stop before starting your experiment. When the workflow is run the next time, Mobius does not require calibration but acquisition will start immediately.



Information

Please wait while the amplifier is calibrated ...

☑ Enable step

☐ Stim F1 -> Ch

Type	Length (ms)	Amp
Const	5.00	

Figure 4-4.7. The left shows pop-up mesage for the calibration. Acquisition always starts after this message dissaears. In order to start recording immediately after clicking the Green-Red button, run Mobius with the Green button for a few traces with stimulator unenabled (right Figure) BEFORE starting your experiment.

4-4.5. Recording of cardiomyocyte signals with pacing

CAUTION:

- Make sure that the hard disc drive has enough free space when you start long-term recording. Recording with all 64 channels for 1 hour creates a 9.216GB file.
  (9.216GB is 9,000,000KB, 8,739.06MB, or 8.58GB in Windows.)

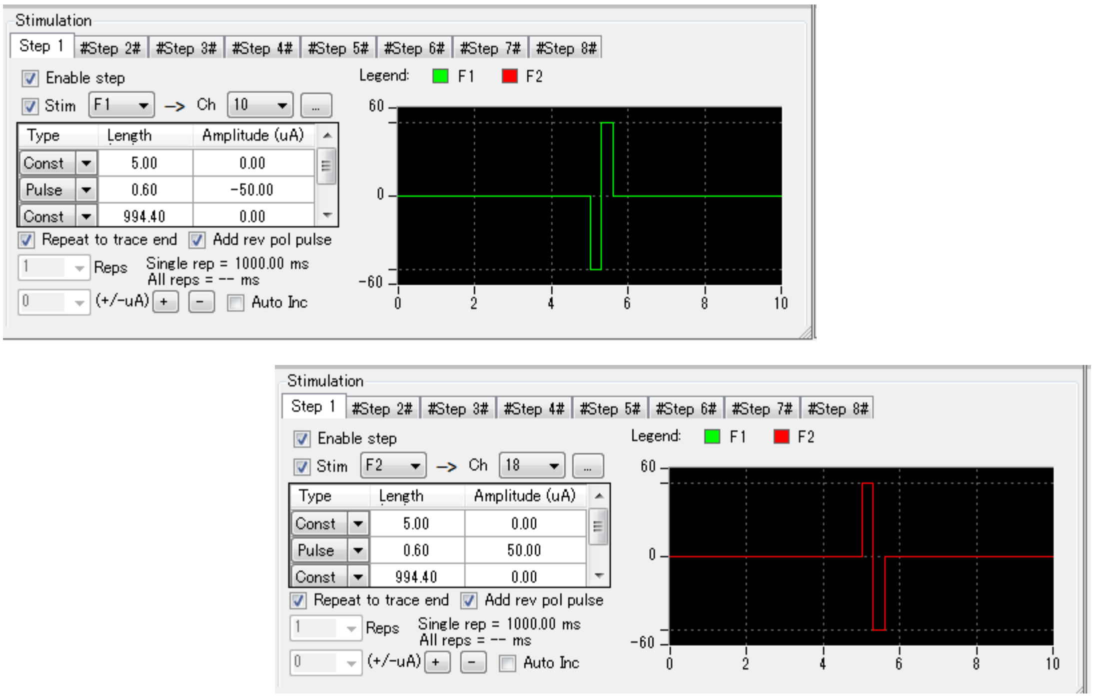
- Avoid saving data to the drive where the OS is installed (usually C drive). Save it to a separate hard drive, that is internal or eSATA-supported.

- Extraction of long spikes and following analysis require powerful computation and need to be used carefully during acquisition. Mobius can appear to crash when it extracts more spikes than it can analyze/display. This most often happens when there is noise or baseline fluctuation that crosses the spike extraction thresholds on all channels at once. (e.g. unexpected noises cross the thresholds the baseline fluctuates due to vibration.)

- It is strongly recommended you start your new experiments WITHOUT on-line (real- time) analysis (by disabling the spike detection).

For pacing to cardiomyocyte, **stimulating 2 adjacent electrodes simultaneously** is recommended to induce pacing activity more effectively. Make sure that **the stimulus waveforms have identical stimulus current amplitude and length but reversed polarities**.

1. Set the stimulus waveform for the F1 stimulator, and then click the [Add rev pol pulse] button. F2 stimulator will have an identical stimulus waveform with reversed polarity to the F1 stimulator.



☑ Enable step

☑ Stim F1 -> Ch 10

Type	Length	Amplitude (uA)
Const	5.00	0.00
Pulse	0.60	-50.00
Const	994.40	0.00

☑ Repeat to trace end ☑ Add rev pol pulse

1 Reps Single rep = 1000.00 ms
All reps = -- ms

0 (+/-uA) + − ☐ Auto Inc

☑ Enable step

☑ Stim F2 -> Ch 18

Type	Length	Amplitude (uA)
Const	5.00	0.00
Pulse	0.60	50.00
Const	994.40	0.00

☑ Repeat to trace end ☑ Add rev pol pulse

1 Reps Single rep = 1000.00 ms
All reps = -- ms

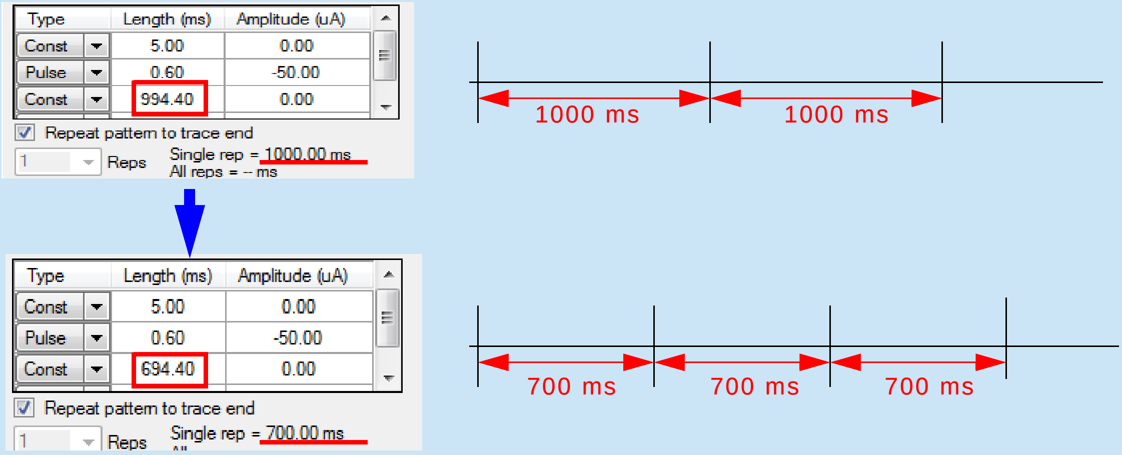
0 (+/-uA) + − ☐ Auto Inc

Figure 4-4.8. Examples of the parameter for bipolar stimulation to adjacent electrodes. Both F1 and F2 are activated and their waveforms are identical except their polarities are reversed. Adjacent channels (ch 10 for F1 and ch 18 for F2) are selected for stimulus channels.

2. Change other parameters on the Acquire MED64R2 Data w/Stim module.
(Refer to section 4-4.2. Setting the acquisition and stimulation parameters on pages 133-136)

How to change the stimulus pulse intervals

The default parameters will allow you to stimulate with the interval of 1000 msec for one minute. In order to change the pulse intervals, change the post-stimulation time (994.80 ms for default) .



Type	Length (ms)	Amplitude (uA)
Const	5.00	0.00
Pulse	0.60	-50.00
Const	994.40	0.00

☑ Repeat pattern to trace end

1 Reps Single rep = 1000.00 ms
All reps = -- ms

Type	Length (ms)	Amplitude (uA)
Const	5.00	0.00
Pulse	0.60	-50.00
Const	694.40	0.00

☑ Repeat pattern to trace end

1 Reps Single rep = 700.00 ms

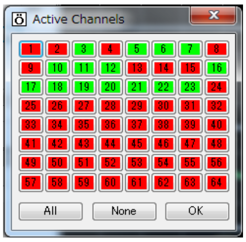
Figure 4-4.9. Changing the stimulus pulse intervals. Change the number for post-stimulus time. The pulse intervals are shown as the “Single rep”.

3. Run Mobius with the Green button.

4. Select the channels for recording.

   1) Click the box next to the “Channels” in the [Acquire MED64R2 Data] module to open the channel selector. (Figure 4-4.10)

   2) The recording channels are colored green and non-recording channels are colored red.

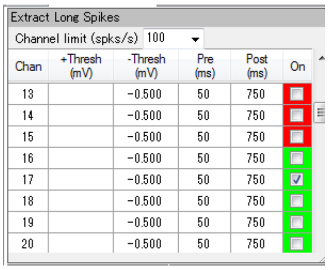


🟥 1	🟥 2	🟩 3	🟥 4	🟩 5	🟩 6	🟩 7	🟥 8
🟥 9	🟩 10	🟩 11	🟩 12	🟥 13	🟥 14	🟥 15	🟩 16
🟩 17	🟩 18	🟩 19	🟩 20	🟩 21	🟩 22	🟩 23	🟥 24
🟥 25	🟥 26	🟥 27	🟥 28	🟥 29	🟥 30	🟥 31	🟥 32
🟥 33	🟥 34	🟥 35	🟥 36	🟥 37	🟥 38	🟥 39	🟥 40
🟥 41	🟥 42	🟥 43	🟥 44	🟥 45	🟥 46	🟥 47	🟥 48
🟥 49	🟥 50	🟥 51	🟥 52	🟥 53	🟥 54	🟥 55	🟥 56
🟥 57	🟥 58	🟥 59	🟥 60	🟥 61	🟥 62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

Figure. 4-4.10.

5. Open the “Pacing measures” tab. Select a channel for spikes’ extractions. The default has all channels with “checked”. Click the check box to disable them. The setting can be applied to all other channels by right-clicking and selecting [Apply to All].

   - It is recommended to select only one channel for spike extraction and analysis during acquisition although it is possible to select more than 1. Once data is saved, all analysis can be performed post-acquisition. Set the thresholds by typing the number in the chart of Extract Long Spikes.



Extract Long Spikes

Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
13		−0.500	50	750	☐
14		−0.500	50	750	☐
15		−0.500	50	750	☐
16		−0.500	50	750	☐
17		−0.500	50	750	☑
18		−0.500	50	750	☐
19		−0.500	50	750	☐
20		−0.500	50	750	☐

Figure 4-1.11. Extract Long Spikes module. Channel 17 is selected for extraction. For the Ch 16,18-20, data is saved while spikes are not extracted (analysis is not observed.)

6. Set the analysis parameters and cursors.
   (Refer to section 4-6.2. Analysis of the evoked potential data on pages 143-147)

7. Save it as your acquisition workflow by clicking [Workflow] >[Save as]. (Figure 4-4.12)

8. Make sure the “Save raw data” in the [Acquire MED64R2 Data w/Stim] module is checked.

9. Run Mobius with the Green-Red button.

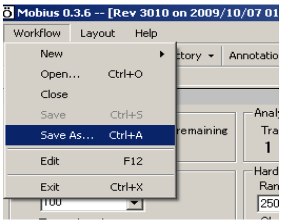


Figure 4-4.12.

- It is recommended without online analysis (by checking the Disable button) unless you are familiar with your experiment and cells. (When the Disable button is checked, no long spikes’ extractions or analysis can be displayed during recording.)

- Mobius will require you to save the workflow as your own if you try to start Mobius with the Green-Red button without having saved it already.

- The data file (.modat file) and the Analysis workflow (including all analysis module selected for the Recording workflow and [Replay Raw Data File] module) will be saved in the same folder. (Refer to pages 17-18)

Figure 4-4.13. Recording of cardiomyocyte signals with pacing. The FPD analysis is performed using the [Extract Spike Measures] module (right).

4-5. Drug testing

The [Compute Measure Averages] module calculates averages and standard deviations for the waveform analysis (Extract Spike Measures). This is a useful tool for analyzing prolongation for the Field Potential Duration with multiple doses. This section will demonstrate how to add “phase bars” so as to make a dose-response curve easily and quickly later off-line.

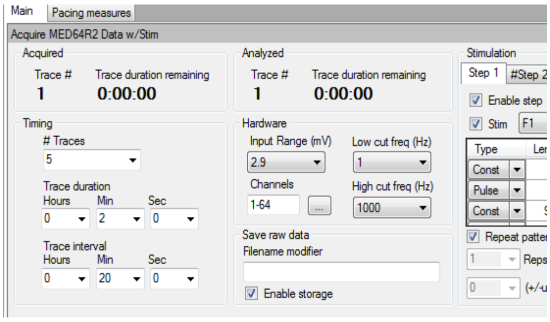
1. Open the *“Pacing_recording”* workflow template.

2. Change the #Traces to the number for doses (or more).

3. Set a large number (e.g. 20 min) for the Trace intervals which allows enough time to apply durg.

4. Modify other acquisition and stimulation parameters as you like.

5. Set the analysis parameters in the Pacing measures tab. (refer to page 143-147). save it as your own workflow. ([Workflow] > [Save as]).



Acquired: Trace # 1; Trace duration remaining 0:00:00
Analyzed: Trace # 1; Trace duration remaining 0:00:00
Traces: 5
Trace duration: Hours 0, Min 2, Sec 0
Trace interval: Hours 0, Min 20, Sec 0
Input Range (mV): 2.9
Low cut freq (Hz): 1
Channels: 1-64
High cut freq (Hz): 1000
Save raw data
Filename modifier:
☑ Enable storage
☑ Enable step
☑ Stim F1
☑ Repeat patte
1 Reps
0 (+/-u

Figure 4-5.1. Example of parameters for drug testing.

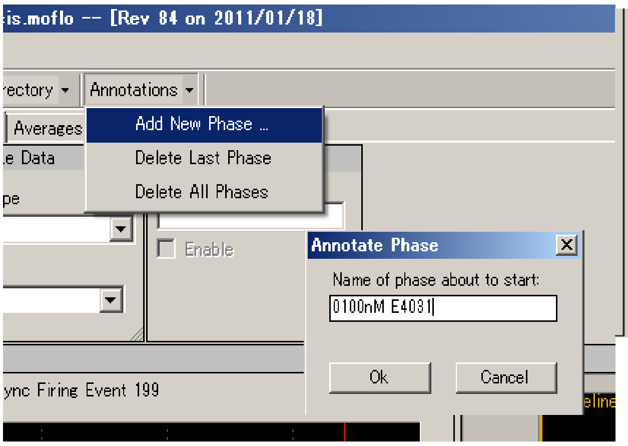
6. Start recording in Mobius with clicking the GREEN-RED button. The first phase is started as “baseline” from time of 0.

7. When the first trace is finished, Mobius will pause automatically. Once it is paused, click YELLOW button so that the next trace will NOT start without clicking the Green-Red button.

    - Do NOT stop with Black button. If it is stopped, the experiment will end and new phases will not be added.

8. Apply the drug and add the 2nd phase.

    Click [Annotations] > [Add New phase]. Type the name for the 2nd phase. (e.g. 100 nM E4031) (Figure 4-5.2) When the OK button is clicked, a yellow bar with the chosen name appears in the [Extract Spike Measures] (Figure 4-5.3)



Name of phase about to start: 0100nM E4031

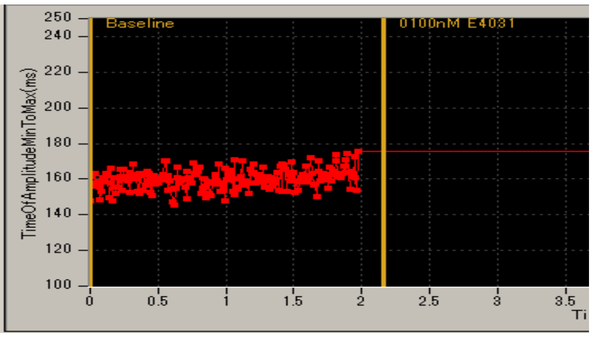


Figure 4-5.2. (left) Adding the 2nd phase.
Figure 4-5.3. (right) Phase bar on the [Extract Spike Measures].

9. When you are ready for the second trace (phase), click GREEN-RED button to re-start Mobius. Using this technique, you can start next phases without waiting for the end of trace interval.

10. When the 2nd trace phase is finished and Mobius pauses automatically, click YELLOW button again not to re-start Mobius automatically.

11. Click [Annotations] > [Add New Phase], then type the name for the third phase. (e.g. 1000 nM E4031). Apply drug again.

12. Click the GREEN-RED button to re-start Mobius to the end of experiment. (or add subsequent phases with same procedure and run Mobius to the end of experiment.)

13. The Figure 4-5.4 shows the [Extract Spike Measures] module with added phase bars.

14. The analysis workflow including all analysis parameters and phase bars you set is automatically made in the same directory as the acquisition workflow. Use this analysis workflow to make a dose-response-curve later off-line. (Refer to page 148-150 for detailed instructions on how to make a dose-response-curve.)

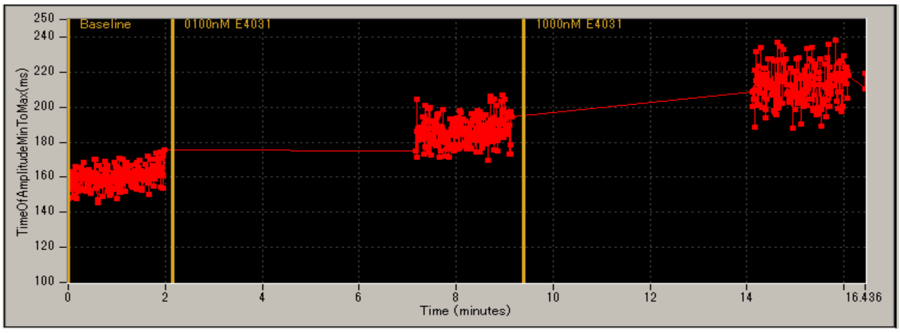


Figure 4-5.4. The [Extract Spike Measures] module with phase bars.

The phase bars can be added and saved to the analysis workflow even without extracting long spikes. (Signals and analysis are not seen during recording in this case.) It is still recommended to perform experiments without spike extracting unless you are very familiar with your experiment and preparations.

4-6. Replay and analysis of acquired paced data

A Mobius data file (.modat) can be opened and replayed with the [Replay Raw Data File] module and analyzed using various analysis modules. You will design your Analysis workflow by combining those modules for replay and analysis of the acquired data.

The quickest way to replay and analyze the paced data is using the analysis workflow which was automatically generated when you run recording workflow with the Green-Red button. The analysis workflow includes the [Replay Raw Data File] and the analysis modules you selected for your recording.

The "*Pacing_analysis*" workflow template is also available to analyze the Field Potential Duration of acquired paced data. This section will introduce you how to perform the FPD analysis using the workflow template.

4-6.1. Replaying acquired data

1. Open the analysis workflow template.

    1) Click [Workflow] > [New] > [From Template].
    2) Select [64MD1_1280x1024] or [64MD1_1920x1080], depending on the size of your display monitor.
    3) Select [QT] > [Pacing_analysis] folders, then select the “*Pacing_analysis*” workflow template.

    - In order to open your own analysis workflow, click [Workflow] > [Open]. Select the analysis workflow file (.moflo file).

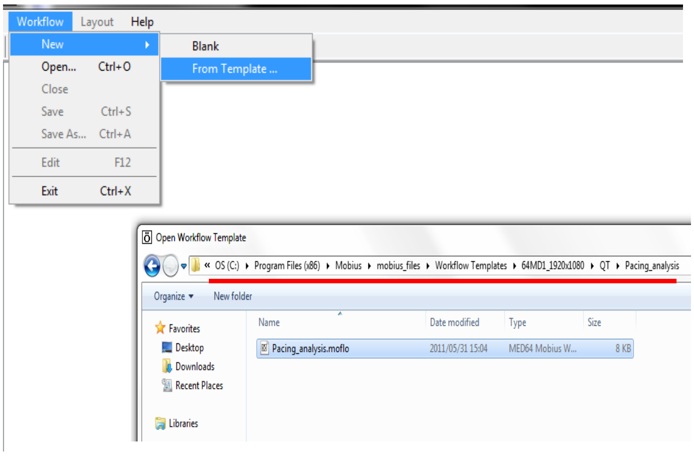
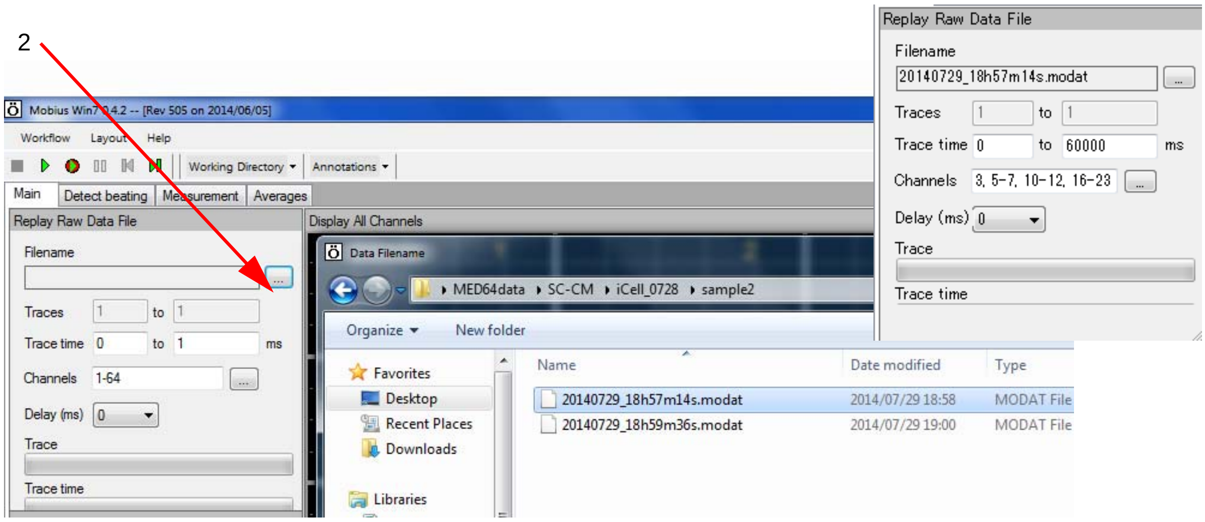


Figure 4-6.1. Opening analysis workflow template.

2. Click the box next to the “Filename” and select the .modat file. When the file name appears on the box as seen on the right figure of the Figure 4-6.2, the data is ready to be replayed by clicking Green button or Green-Red button.

3. When the Green button is clicked, the acquired data is just replayed. Clicking the Green-Red button execute the Exports or Saves which “Enable” check-box is checked.



Replay Raw Data File

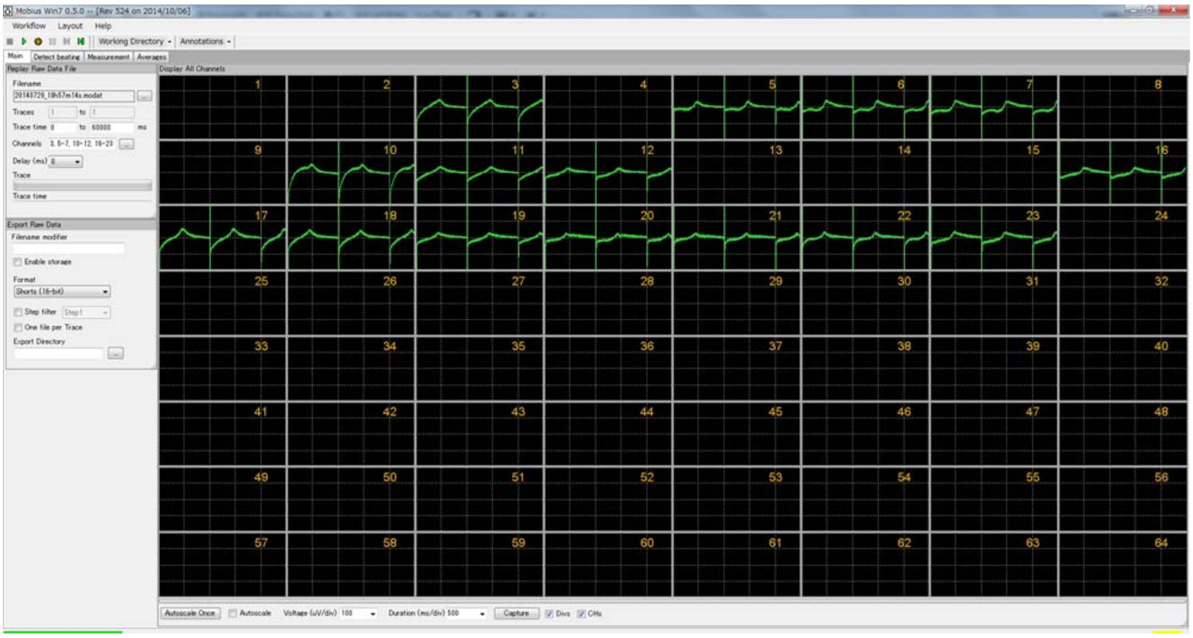
Filename: (empty)
Traces: 1 to 1
Trace time: 0 to 1 ms
Channels: 1-64
Delay (ms): 0

Name	Date modified	Type
20140729_18h57m14s.modat	2014/07/29 18:58	MODAT File
20140729_18h59m36s.modat	2014/07/29 19:00	MODAT File

Replay Raw Data File

Filename: 20140729_18h57m14s.modat
Traces: 1 to 1
Trace time: 0 to 60000 ms
Channels: 3, 5-7, 10-12, 16-23
Delay (ms): 0

Figure 4-6.2. Opening the Mobius data file (.modat file).



Figire 4-6.3. Replayed paced data**.**

Input fields for the [Replay Raw Data File] module

1. Traces: Selects the trace(s) for processing.
2. Trace time: Selects the trace time for processing.
3. Channels: Selects the channels you would like to process. Click the box next to the “Channels” to open the channel selector. Green channels will be processed.
4. Delay: Set this value to a value greater than zero to insert a delay between data "blocks" sent out by this module. It is useful for delaying processing.

4-6.2. Field potential duration analysis for paced cardiomyocyte signals

This section describes how to perform Field Potential Duration (FPD) analysis for paced cardiomyocyte signals using "*Pacing_analysis*" workflow template. The workflow is available for the analysis of the data acquired with the *"Pacing_recording"* workflow template.

Overview of the "*Pacing_analysis*" workflow template

The "*Pacing_analysis*" workflow template consists of following modules located in the "Main" and "Pacing measures" tabs.

Tab	Modules
Main	Replay Raw Data File / Display All Channels / Export Raw Data
Pacing measures	Extract Long Spikes / Filter Spike Data (2) / Extract Spike Measures / Save Measures Data / Display Extracted Spikes

The replayed data is sent to the [Extract Long Spikes] module, where signals which go over pre-determined thresholds are extracted. The extracted signals are sent to the [Extract Spike Measures] module, where amplitude, slope, time, and area are analyzed. (The extracted signals can be filtered out using [Filter Spike Data] module before sent to the [Extract Spike Measures].

The raw data can be exported either as binary or ASCII data while the measurement chart is saved as a "CSV" formatted text file.

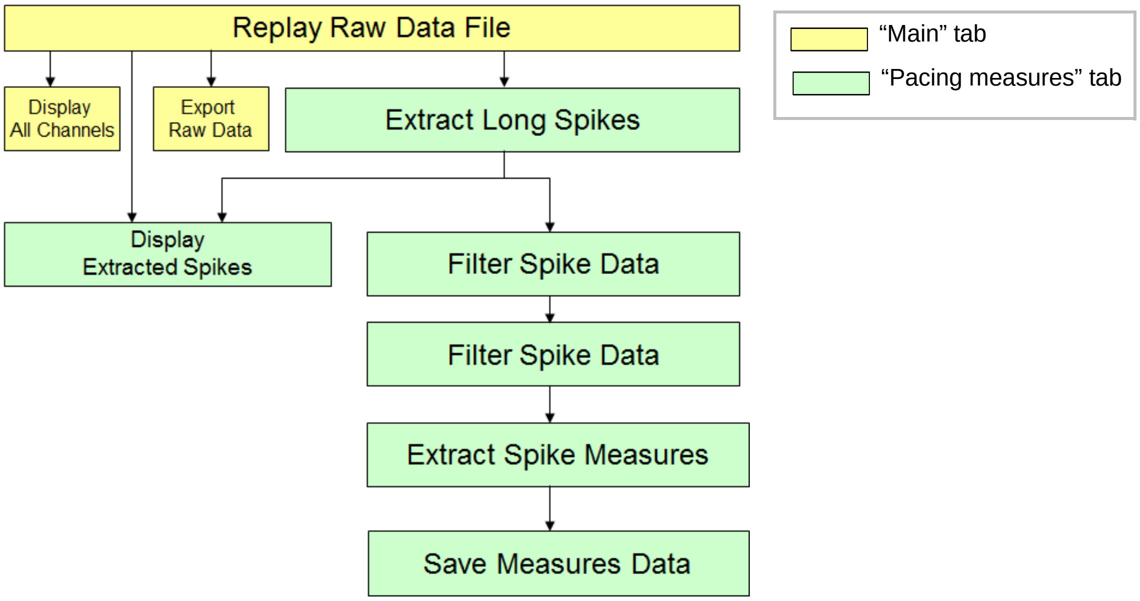


Figure 4-6.4. Module configurations for "*Pacing_analysis"* workflow template.

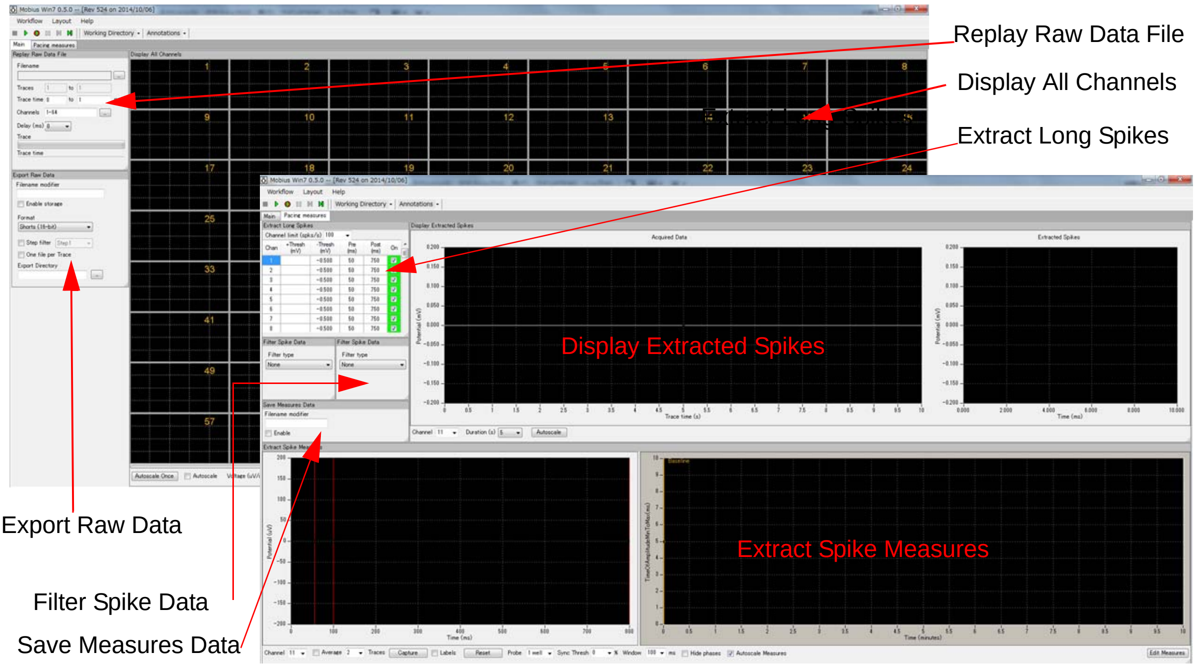


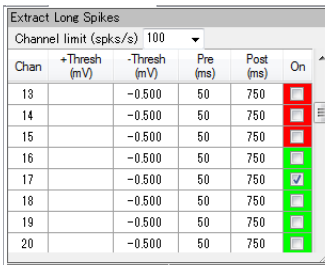
Figure 4-6.5. Control panels for the "*Pacing_analysis*" workflow template. "Main" tab (left) and "Pacing measures "tab (right).

Field potential duration analysis for paced cardiomyocyte signals

1. Open the *"Pacing_analysis"* workflow template (or your own analysis workflow), and then the data file. (Refer to page 105-106)Replay the data by clicking the Green button, and then stop.

2. Open the "Pacing measures" tab. Select a channel for spikes' extractions. The default has all channels with "checked". Click the check box to disable them. The setting can be applied to all other channels by right-clicking and selecting [Apply to All].

   - It is recommended to select only one channel for spike extraction and analysis during acquisition although it is possible to select more than 1 to perform your analysis quickly and stably.



Extract Long Spikes

Channel limit (spks/s) 100

Chan	+Thresh (mV)	-Thresh (mV)	Pre (ms)	Post (ms)	On
13		−0.500	50	750	☐
14		−0.500	50	750	☐
15		−0.500	50	750	☐
16		−0.500	50	750	☐
17		−0.500	50	750	☑
18		−0.500	50	750	☐
19		−0.500	50	750	☐
20		−0.500	50	750	☐

Figure 4-6.6. Extract Long Spikes module. Channel 17 is selected for extraction.

3. Set the thresholds by typing the number in the chart of Extract Long Spikes. Set the thresholds to the location lower than the depolarization spikes but higher than peak of stimulus artifacts so that signals are extracted based on the stimulus artifacts. (Figure. 4-6.7)

4. Perform analysis according to the instruction in the 4-3.3. Field potential duration analysis (page 113-121).
   - Make sure to set the first cursor between stimulus artifact and depolarization spikes for analysis of FPD. (Refer to Figure 4-6.8)

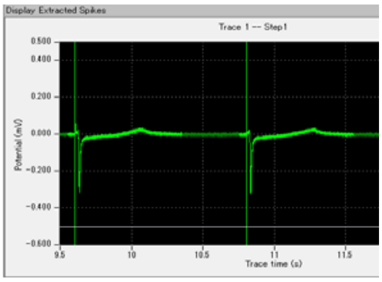
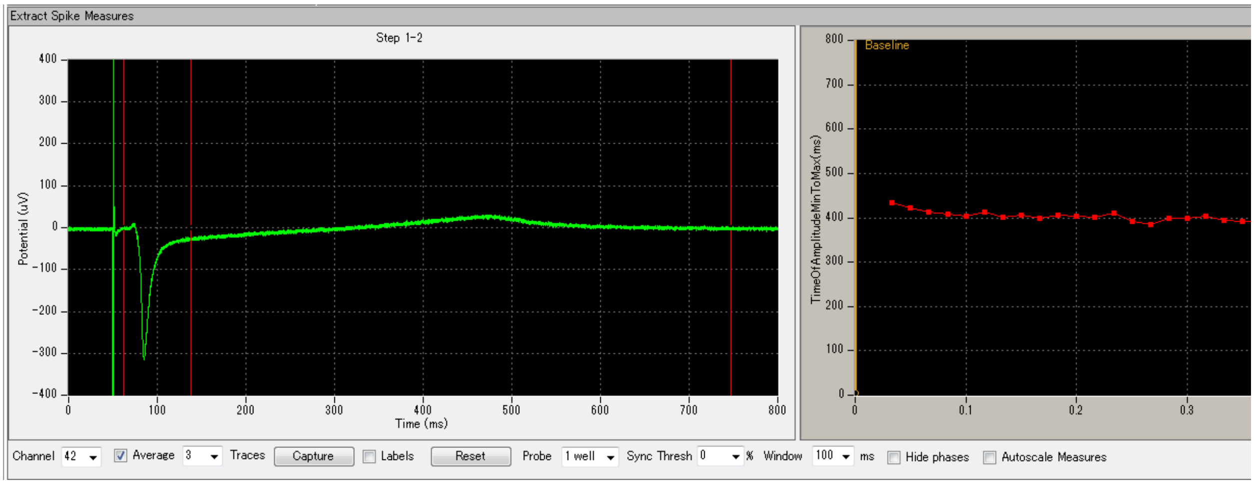


Figure 4-6.7. Setting thresholds for paced data,



Channel 42 ☑ Average 3 Traces ☐ Labels Probe 1 well Sync Thresh 0 % Window 100 ms ☐ Hide phases ☐ Autoscale Measures

Figure 4-6.8. FPD analysis with the “Time of Amplitude Min to Max”. Noted that the first cursor is located after the stimulus artifact and before the depolarization spikes.

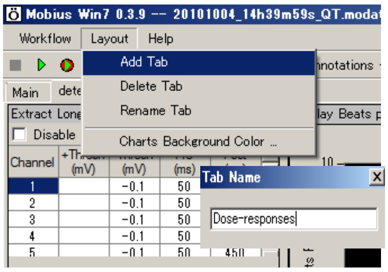
4-6.3. Making a dose-response curve

This section will walk you through making a dose-response curve with the FPD from the acquired pacing data. The Mobius' [Compute Measure Averages] module computes averages and standard deviations of waveform analysis selected at the [Extract Spike Measures].

How to add [Compute Measure Averages] module to your workflow

1. Open the analysis workflow which was automatically made when you acquired the data. (Go to [Workflow] > [Open].)

2. Add the [Compute Measure Averages] module to the analysis workflow.



Dose-responses

Figure 4-6.9. Adding a new tab.

   1) Create a new tab by clicking [Layout] > [Add Tab] and typing the name for this tab. (Refer to Figure 4-6.9)

   2) Open the new tab, and then open the “Mobius Editor” by clicking [Workflow] > [Edit]. (Figure 4-6.10, left figure)

   3) Click the [Extract Spike Measures] on the left “Workflow” box, and then double-click the [Compute Measure Averages] on the right “Available task panels” box. Now you will see that [Compute Measure Averages] module shift to the left “Workflow” box and the control panel for this modules appears on the new tab. (Figure 4-6.10, right figure)

   4) Click the [Compute Measure Averages] on the left "Workflow” box, and then double-click the [Save Measure Averages] on the right "Available task panels" box. The [Compute Measure Averages] is shifted to the left [Workflow] box, and the control panel for the [Save Measures Averages] module appears on the screen.

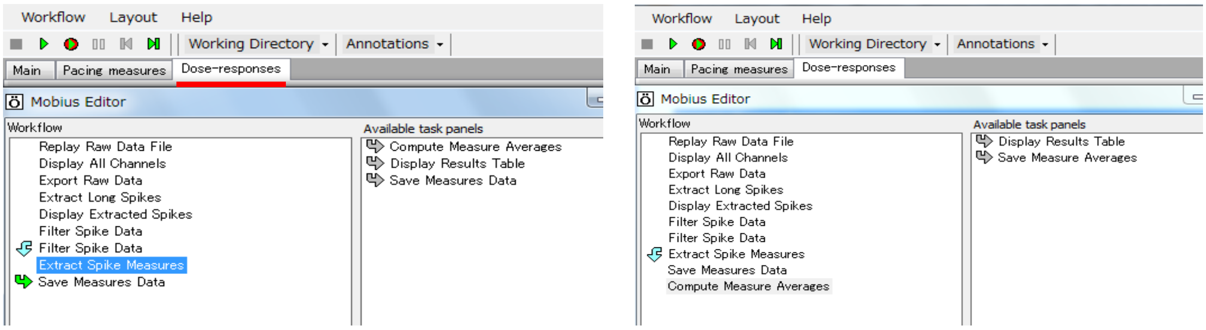


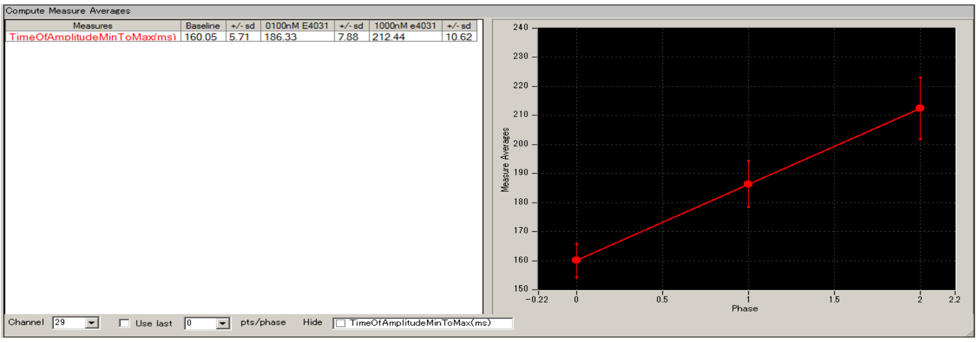
Figure 4-6.10. Mobius Editor was opened in a new tab and the [Extract Spike Measures] is clicked (left). The [Compute Measures Averages] was double-clicked and shifted to the left “Workflow” box (right). The control panel for this module now appears.

3. Set the analysis parameters according to the instructions in 4-3.3. Field potential duration analysis (pages 113-121).

4. Run Mobius with Green button to replay the data to the end. You will see the phase bars you set during your acquisition in the [Extract Spike Measures] module, and averages and standard deviations in the [Compute Measures Average] control panel. (Refer to Figure 4-3.37)

If you work with “pacing_analysis” workflow template or did not add phase bars during recording, add phase bars according to the instruction in the next section, “How to add phase bars”.

5. The phase bars are shifted by clicking and dragging. The averages and standard deviations are recalculated once Mobius is run with Green or Green-Red button after changing the locations of phase bars.



Compute Measure Averages

Measures	Baseline	+/- sd	0100nM E4031	+/- sd	1000nM e4031	+/- sd
TimeOfAmplitudeMinToMax(ms)	160.05	5.71	186.33	7.88	212.44	10.62

Channel 29 ☐ Use last 0 pts/phase Hide ☐ TimeOfAmplitudeMinToMax(ms)

Figure 4-6.11. The results of “Time of Amplitude Min to Max” with phase bars in the [Extract Spike Measure] (top) and the averages and standard deviations for each phase displayed in the [Compute Measure Averages] control panel.

6. In order to save the average chart, save it as your own analysis workflow first. ([Workflow] > [Save as]) Check the “Enable” check-box on the [Save Measure Averages] module, then run Mobius with the Green-Red button. (Refer to section 4-7. Data output on pages 151-154 for details)

How to add phase bars

Even if phase bars are not added during recording, they can be added later off-line with following procedure.

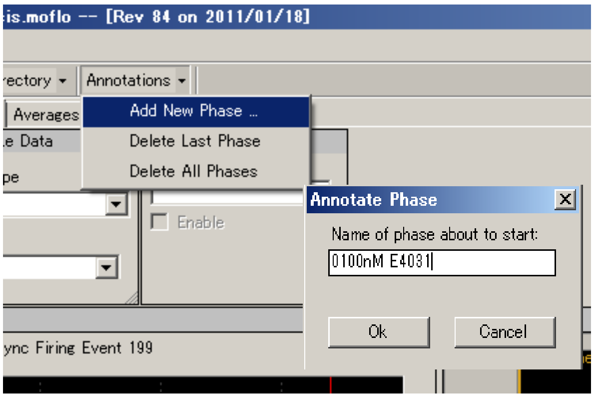
1. Set the analysis parameters according to the instructions in the 4-3.3. Field potential duration analysis (page 113-121).

2. Open the “Measurement” tab, and then replay the data with the Green button. You will see the first phase, Baseline (yellow bar) is already at time 0.

3. When the first phase is finished, pause Mobius with YELLOW button, and make a new phase.

   - Select [Annotators] > [Add New phase], then, type the name for the new phase. (Figure 4-6.12)
     Now you will see the new bar at the right chart of the [Extracted Spike Measures]. (Figure 4-6.13)

   - Do NOT stop Mobius with Black button. You will not be able to add more phases.



Name of phase about to start: 0100nM E4031

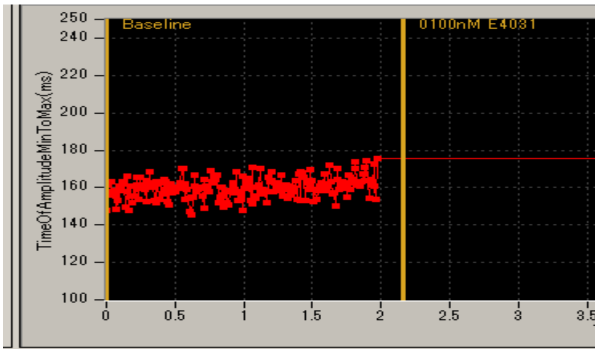


Figure 4-6.12. (left) Adding a new phase bar.
Figure 4-6.13. (Right) A new phase bar on the [Extract Spike Measures].

4. When the second phase finished, pause Mobius again and make a new phase.
   *The location of the phase annotator (yellow bar) can be shifted later.

5. Replay the data to the end. Averages and standard deviations are computed and graphed for each phase (dose) in the Average tab. (Refer to Figure 4-3.37)

6. In order to save the average chart, save it as your own analysis workflow first. Check the “Enable” check-box on the [Save Measure Average] module, and run Mobius with Green-Red button. (Refer to section 4-3.7. Data output on pages 151-154 for detail.)

Input fields for [Compute Measure Averages] module

1. Channel:
   The channel to be displayed is selected here. Channels can NOT be changed while running an experiment.

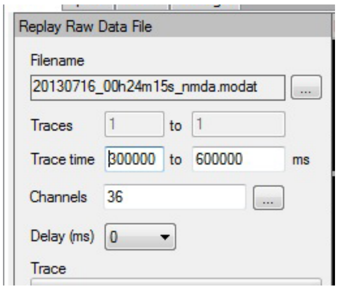
2. Use last:
   If this box is checked, the selected (or typed) number of data points are used for computation. For example, if 10 is selected, average and standard deviation are measured for the last 10 data points in each phase.

4-7. Data output

Mobius has several modules for data output of cardiomyocyte signals and their analysis. All analysis results can be saved as “CSV” formatted text file with following procedures.

1. Check the check-box for the [Save] module (Refer to page 151-152). File names can be modified by directly typing them into the Filename modifier box.

2. Select channels, Trace No, trace time for the saving in the [Replay Raw Data] module. Channels shown with green will be enabled. (Figure 4-7.1)



Replay Raw Data File

Filename 20130716_00h24m15s_nmda.modat

Traces 1 to 1

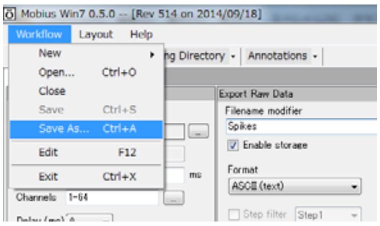
Trace time 300000 to 600000 ms

Channels 36

Delay (ms) 0

Figure 4-7.1. [The Replay Raw Data File] ready for export. Data for 300,000-600,000 msec at channel 36 will be saved.

3. After setting all parameters, save the analysis workflow as your own. (Figure 4-7.2)



Mobius Win7 0.5.0 -- [Rev 514 on 2014/09/18]

Export Raw Data

Filename modifier Spikes

☑ Enable storage

Format ASCII (text)

☐ Step filter Step1

Channels 1-64

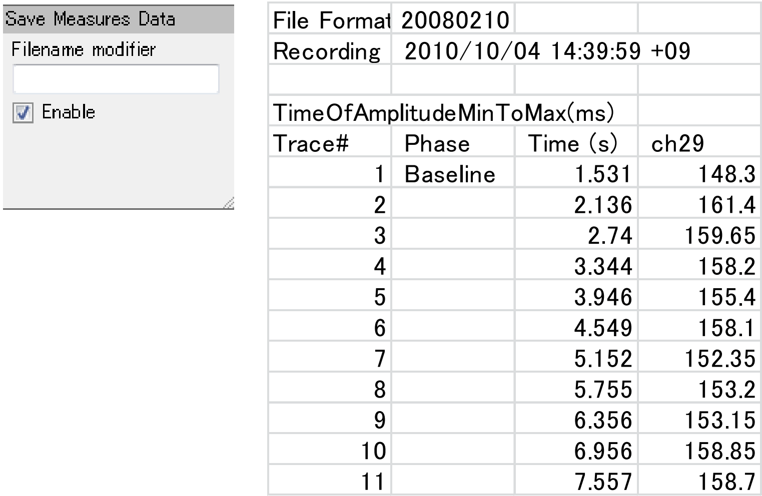
Figure 4-7.2. Saving analysis workflow.

4. Run the analysis workflow with the Green-Red button. The output data will be saved in the same folder where the analysis workflow is saved.

- Refer to page 20-21 for exporting raw data.

4-7.1. Save the measurement chart

Check the [Save Measures Data]. The measurement values in the [Extract Spike Measures] module are saved as a “CSV” formatted text file.



Save Measures Data

Filename modifier

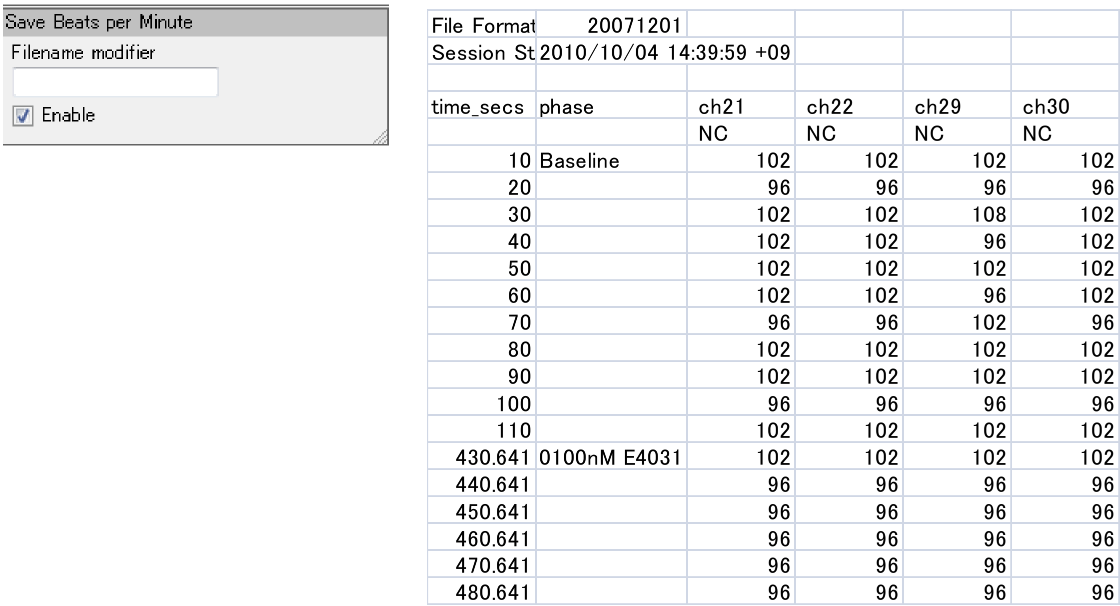
☑ Enable

File Format	20080210		
Recording	2010/10/04 14:39:59 +09		
TimeOfAmplitudeMinToMax(ms)			
Trace#	Phase	Time (s)	ch29
1	Baseline	1.531	148.3
2		2.136	161.4
3		2.74	159.65
4		3.344	158.2
5		3.946	155.4
6		4.549	158.1
7		5.152	152.35
8		5.755	153.2
9		6.356	153.15
10		6.956	158.85
11		7.557	158.7

Figure 4-7.3. [Save Measures Data] module/control panel (left) and example of the “Time Of Amplitude Min to Max (ms)” for channel 29 saved as a “CSV” text file and displayed in excel (right).

4-7.2. Save the beat frequency data

Check the [Save Beats per Minute]. The beat frequency data is saved as a “CSV” formatted text file.



Save Beats per Minute

Filename modifier

☑ Enable

File Format	20071201				
Session St	2010/10/04 14:39:59 +09				
time_secs	phase	ch21	ch22	ch29	ch30
		NC	NC	NC	NC
10	Baseline	102	102	102	102
20		96	96	96	96
30		102	102	108	102
40		102	102	96	102
50		102	102	102	102
60		102	102	96	102
70		96	96	102	96
80		102	102	102	102
90		102	102	102	102
100		96	96	96	96
110		102	102	102	102
430.641	0100nM E4031	102	102	102	102
440.641		96	96	96	96
450.641		96	96	96	96
460.641		96	96	96	96
470.641		96	96	96	96
480.641		96	96	96	96

Figure 4-7.4. [Save Beats per Minute] module/control panel (left), and example of the beat frequency data for channel 21,22, 29, and 30 saved as a “CSV” text file and displayed in excel (right).

4-7.3. Save the interspike interval data

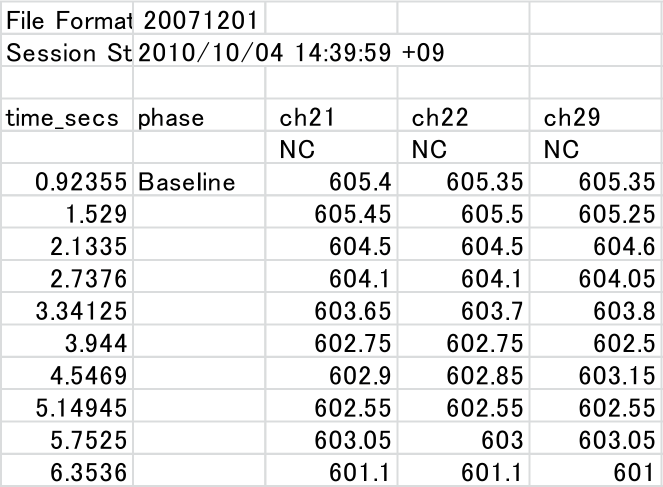
Check the [Save interspike intervals] module. The inter-spike-interval data is saved as a “CSV formatted text file.

- Select SINGLE channel to save ISI data which signals are NOT synchronized for all channels. Otherwise, data will not be saved correctly.

Save Interspike Intervals

Filename modifier

☑ Enable

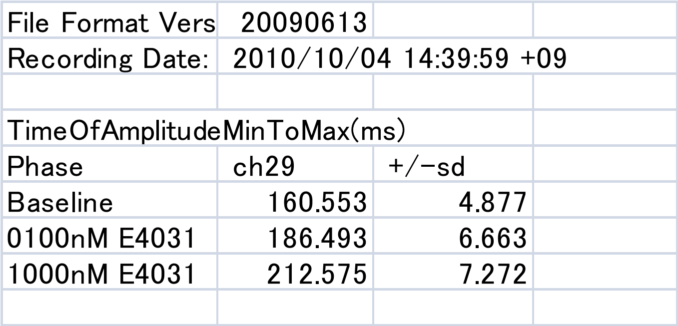


File Format	20071201			
Session St	2010/10/04 14:39:59 +09			
time_secs	phase	ch21	ch22	ch29
		NC	NC	NC
0.92355	Baseline	605.4	605.35	605.35
1.529		605.45	605.5	605.25
2.1335		604.5	604.5	604.6
2.7376		604.1	604.1	604.05
3.34125		603.65	603.7	603.8
3.944		602.75	602.75	602.5
4.5469		602.9	602.85	603.15
5.14945		602.55	602.55	602.55
5.7525		603.05	603	603.05
6.3536		601.1	601.1	601

Figure 4-7-5. [Save Interspike Intervals] module/control panel (left) and the inter-spike-interval data for channel 21, 22, and 29 saved as a “CSV” file displayed in excel (right).

4-7.4. Save the average chart

Check the [Save Measures Averages] module. The averages and standard deviations are saved as a “CSV” formatted text file.



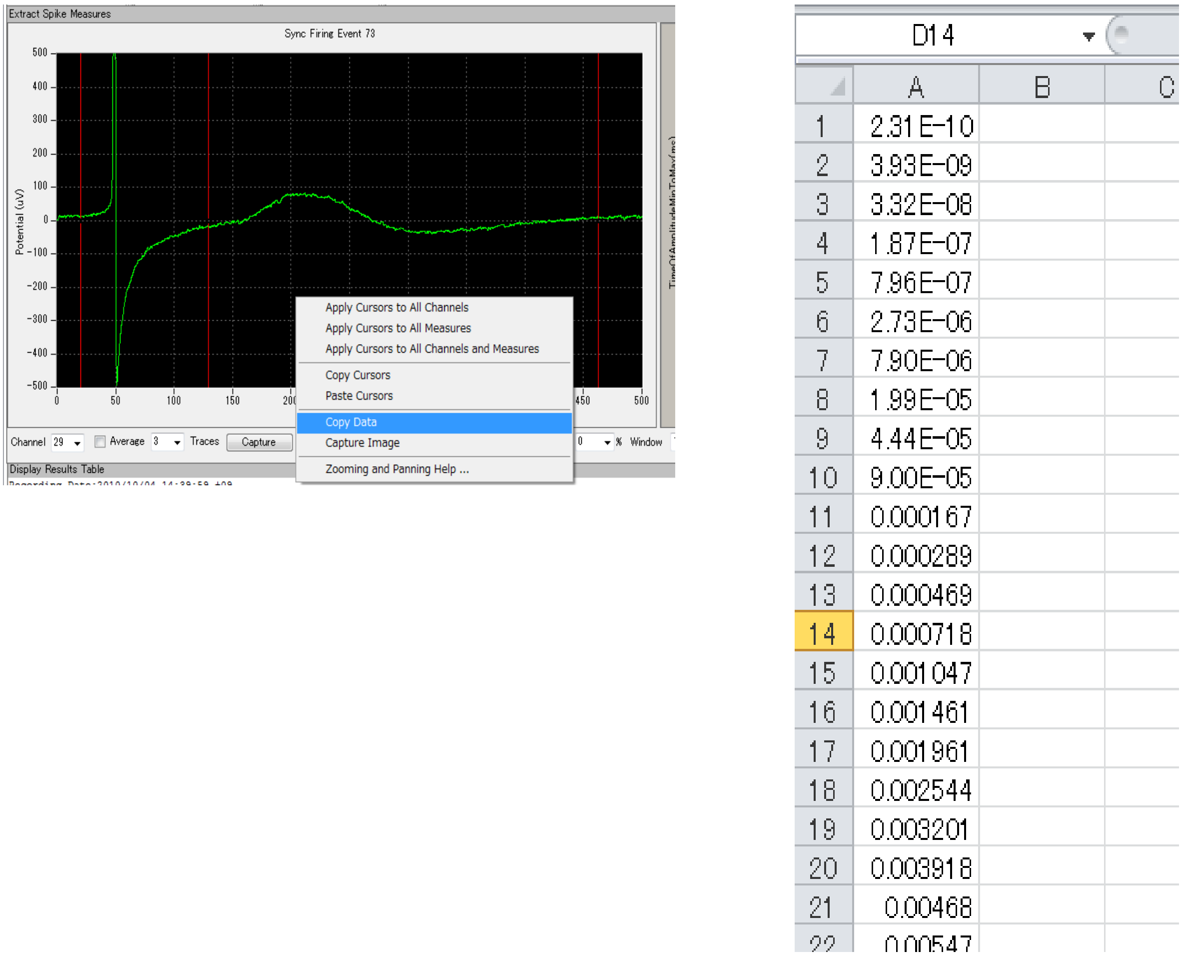
File Format Vers	20090613		
Recording Date:	2010/10/04 14:39:59 +09		
TimeOfAmplitudeMinToMax(ms)			
Phase	ch29	+/-sd	
Baseline	160.553	4.877	
0100nM E4031	186.493	6.663	
1000nM E4031	212.575	7.272	

Figure 4-7.6. [Save the average chart] module/control panel (left) and the averages and standard deviations of the “Time of Amplitude Min to Max” for channel 29 is saved as a “CSV file and displayed in excel (right).

4-7.5. Save extracted long spikes’ waveforms

The waveform for extracted long spikes at selected channel and trace is quickly saved by:

1. Right click anywhere in the Raw data chart and select Copy.
2. Paste it to the Excel sheet.



	A	B	C
1	2.31E-10		
2	3.93E-09		
3	3.32E-08		
4	1.87E-07		
5	7.96E-07		
6	2.73E-06		
7	7.90E-06		
8	1.99E-05		
9	4.44E-05		
10	9.00E-05		
11	0.000167		
12	0.000289		
13	0.000469		
14	0.000718		
15	0.001047		
16	0.001461		
17	0.001961		
18	0.002544		
19	0.003201		
20	0.003918		
21	0.00468		
22	0.00547		

Figure 4-7.7. Copying the extracted long spikes using Mobius (left) and the data pasted in an excel sheet (right).

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