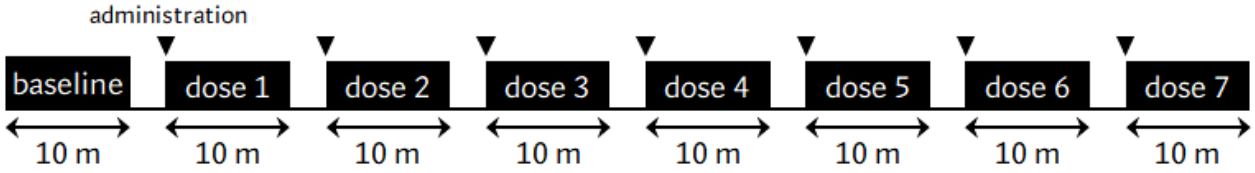
MED64 Peakmap Manual

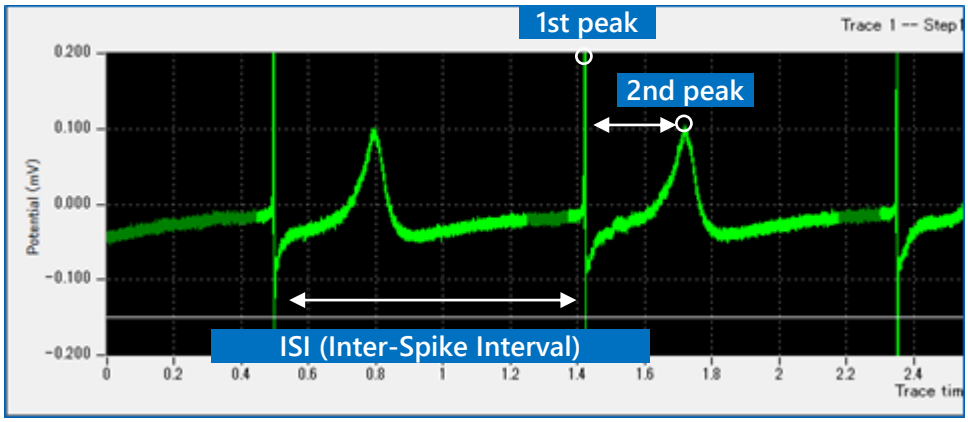
1. Introduction

The MED64 Peakmap is an offline data analysis tool that automatically calculates the FPD of the last 30 beats from data files obtained from FPD (Field Potential Duration) assays on a monolayer sheet-like cardiomyocyte culture. It is designed for assay protocols evaluating dose-dependent effects of compounds applied in up to eight escalating doses, and it enables one-click batch processing to obtain results.



Assumed assay protocol. First, the baseline activity is measured for 10 minutes (data acquisition). Then, the process of adding a dose and recording 10 minutes of data is repeated seven times. The data will be a maximum of 8 files in total.

A monolayer sheet-like cardiomyocytes culture shows the unique extracellular potential waveform with each beat, as shown in the figure below. The FPD is the interval (time width) between peaks, defined as the 1st and 2nd peaks. The FPD is considered to be equivalent to the APD (Action Potential Duration) and drug-induced FPD prolongation is considered a surrogate marker for predicting the proarrhythmic effects of a drug in a clinical condition (since the FPD is positively correlated with the preceding beat-to-beat interval, a value corrected for that beat-to-beat interval is used). In Peakmap, a simple algorithm is used to calculate the FPD and all of the analysis results are displayed in a result table. The total data length of the analyzed interval is displayed in a chart, and the 1st and 2nd peak positions are indicated by editable cursor lines. By using the seek bar, you can select the time periods to display, which makes it easier to examine the timing of EAD waveform appearances.



Extracellular potential of monolayer sheet-like cardiomyocytes culture.

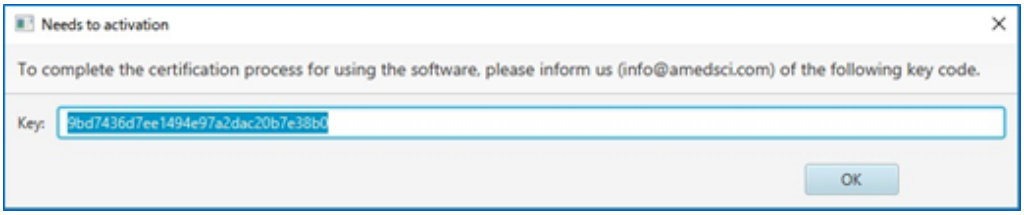
Please note that the analysis does not necessarily require eight data files with eight escalating doses. It is possible to select any 1 through 8 data files with different data recording timings for the batch processing.

The recommended computer operating environment is as follows.

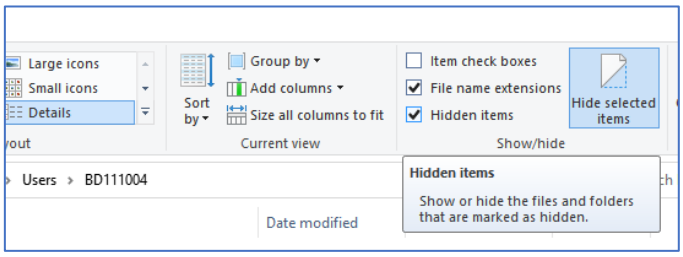
OS	**: Windows 10 64 bit (32-bit version is not supported.)**
CPU	**: Intel Core i7 equivalent or better**
RAM	**: 16 GB**
Space required	**: 64 MB**
Display resolution	**: 1920 x 1080**

2. Installation

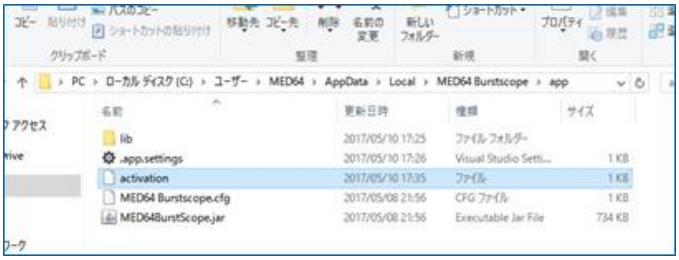
2-1. After double-clicking the installer to install the software, you can use the trial version for 30 days. To continue using the application, you will need to activate it, so please provide us with the Key ID unique to your PC that appears in the pop-up window, and we will send you an activation file that matches the Key ID (for Mobius QT users or Peakmap users only).



2-2. Move the activation file to the following folder under the C drive.
C:¥Users¥User name¥AppData¥Local¥MED64 Peakmap¥app

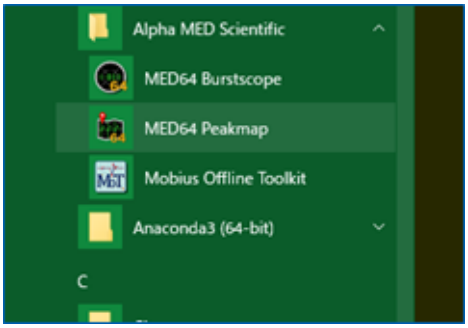


AppData is a hidden item and must be displayed by checking the "Hidden items" checkbox in the "View" tab of the folder settings.



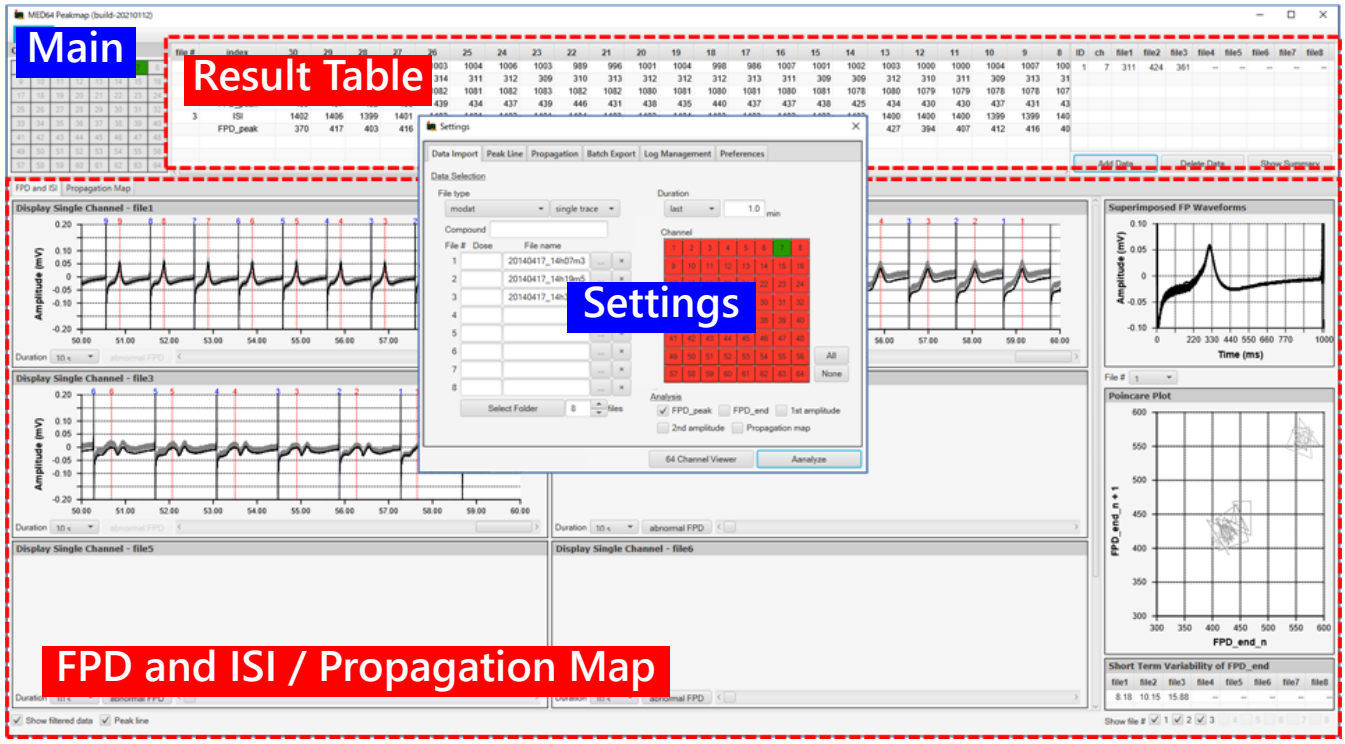
If the activation file is sent as an attached file, it will be named "activation.dat" with the extension.
You can not activate the Peakmap as it is, so please delete the extension ".dat" and move it to the app folder.

2-3. Peakmap can be launched by selecting "MED64 Peakmap" from the "Alpha MED Scientific" folder in the "Start" menu.



3. Screen Configuration

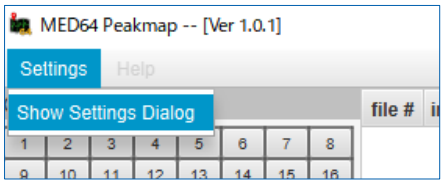
When Peakmap is launched, two windows, Settings and Main, appear as shown in the figure below. In the Settings window, you can enter the analysis conditions, such as the peak search range and threshold values for the FPD analysis, and then click the "Analyze" button to execute the series of processes. The Main window displays the results of the analysis.



The Settings window consists of the following six tabs.

Data Import	Setting of data files, channels, etc., to be analyzed (p. 4).
Peak Line	Setting of an peak search range, its pre-processing filters, etc., (p. 5).
Propagation	Setting of an excitation propagation diagram (p. 10).
Batch Export	Batch output of results obtained from right-clicking on each panel (p. 11).
Log Management	Saving and recalling the analysis settings (p. 11).
Preferences	Customizing settings for a chart display (p. 12).

The Main window consists of three areas: a selector for a display channel, a tally table of analysis results, and a switching tab for displaying the results of the FPD and ISI/Propagation Map analyses. The basic operation of Peakmap is to set the analysis conditions in the Settings window, execute the process, and check the results in the Main window. The Settings window is automatically closed after the analysis, but it can be recalled from the menu bar of the Main window. It is also possible to enter the analysis conditions again to generate the analysis results.



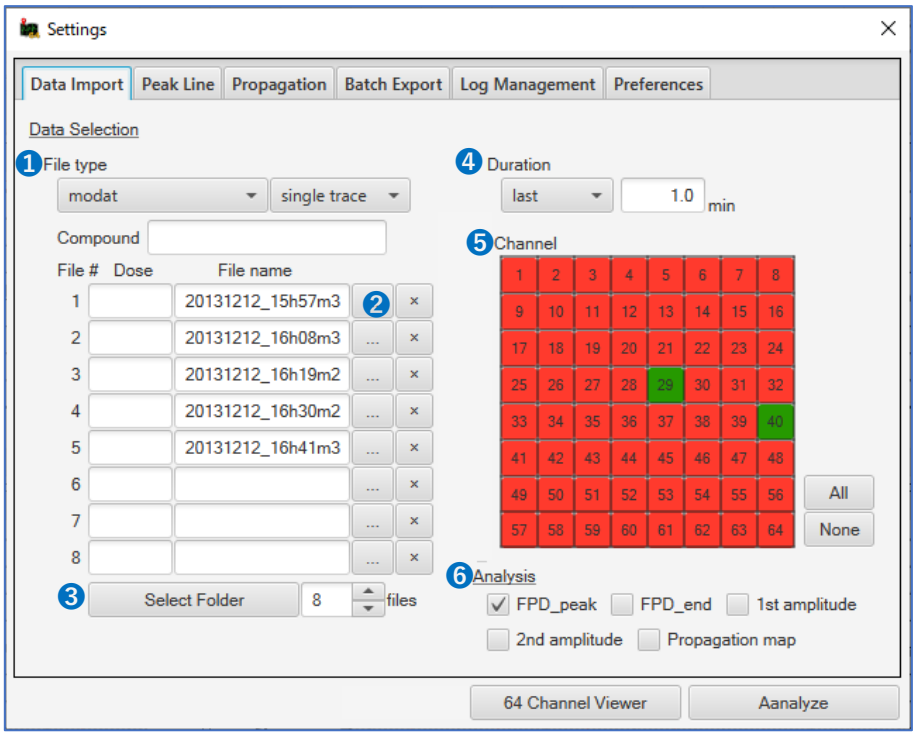
Bring up the Settings window from the Settings → Show Settings Dialog.

4. Data File Selection (Data Import Tab)

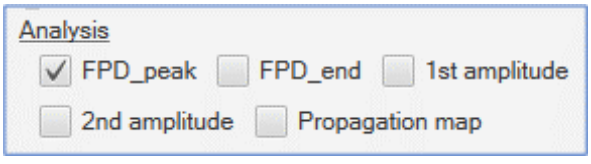
The data file formats that can be processed for analysis are as follows.

modat	Original data format of the MED64 system (MED64 Mobius).
modat (paced)	Electric pacing data of the above.
modax	Original data format of MED64-Presto (MED64 Symphony).
modax (paced)	Electric pacing data of the above.

Select (limit) the files to be analyzed by clicking ❶ Data Type in the figure below, then click ❷ to select the files individually. If the files to be batch processed are all in one folder, it is also possible to batch select the files in the folder by name. In this case, click ❸ to select the appropriate folder. You do not necessarily need to enter a value in the text box for the name of the compound or dose to be applied (this is to provide a means of capturing labeling information, but as of December 2018, this function has not been implemented).

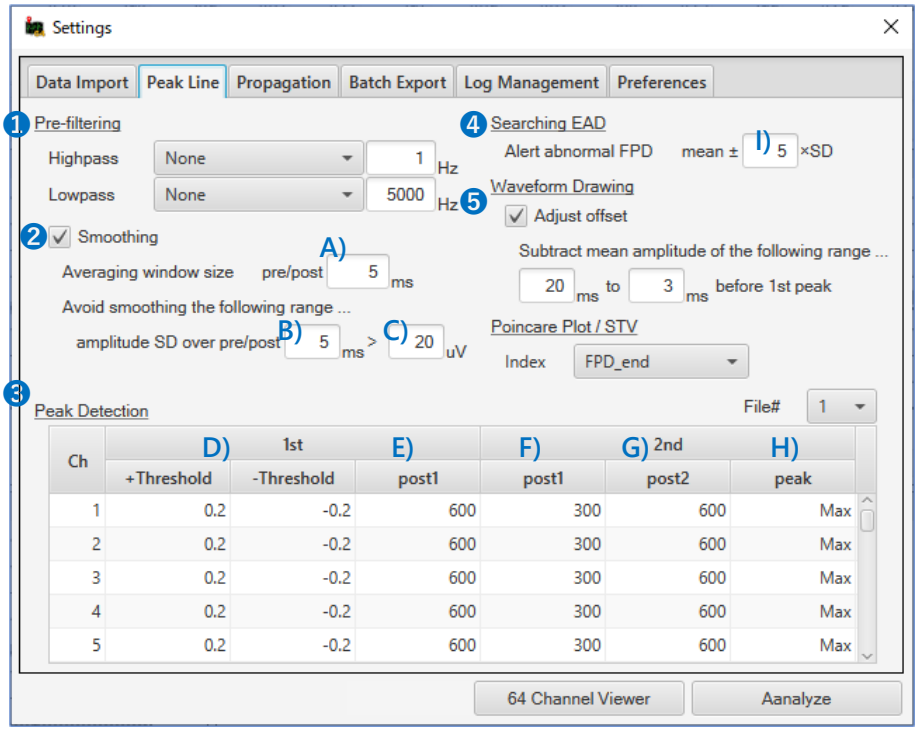


If you want to analyze only the last minute of a stable response after a compound dosage, for example instead of the entire file length, limit the analysis ramge with ❹ Duration (the same condition applies to all files processed in batch). If you do not want to analyze all 64 channels obtained in MED64-Quad II, use ❺ Channel to select the target channels (green: channels to analyze, red: channels not to analyze). Select the measurement to be calculated by analysis in ❻ Analysis. ISI is always calculated, but FPD (FPD_peak; 1st peak to 2nd peak interval), FPD_end (FPD_end; 1st peak to 2nd peak end interval), 1st peak amplitude (1st amplitude), 2nd peak amplitude (2nd amplitude), and propagation delay map (Propagation map) can be selected individually. Unchecking the unnecessary items will reduce the processing time required to obtain the analysis results.

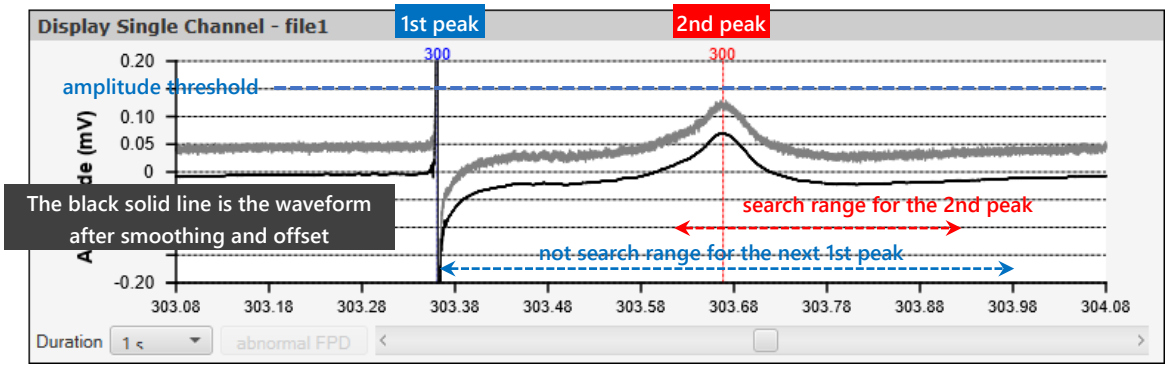


A similar selection and input are required for the other tabs, but since the default values have been entered beforehand, the analysis process can be executed by clicking the "Analyze" button after the file is selected. Peakmap has a "last state preservation specification," which means that all the information you selected and entered is saved when you close the program and is recalled when you start the program again.

5. Peak Detection -Peak Line Tab-



❶ Peakmap can also apply filtering as a preprocessing step for FPD analysis, but there is a limit of 5000 Hz only for the upper limit of the low-pass filter. ❷ Smoothing can be applied to remove high-frequency noise components from the waveform, which results in a clearer waveform. By default, the waveform is averaged over A) 5 ms before and after the data point (5 ms / 0.05 ms sampling period = 100 points, 201 points in total) and the moving average is plotted. In this case, if the 1st peak component is smoothed, its shape will be corrupted from the original waveform, making accurate detection of the 1st peak difficult. Therefore, smoothing is limited to the range that excludes the 1st peak component. In the default setting, the SD (standard deviation) is calculated in the range that is B) 5 ms before and after the data point. In the converted chart, the time range exceeding the amplitude threshold of C) 20 μV is considered to be the 1st peak range. ❸ Peakmap searches for a pair of peaks, as shown in the figure below, and the difference between them is the FPD.



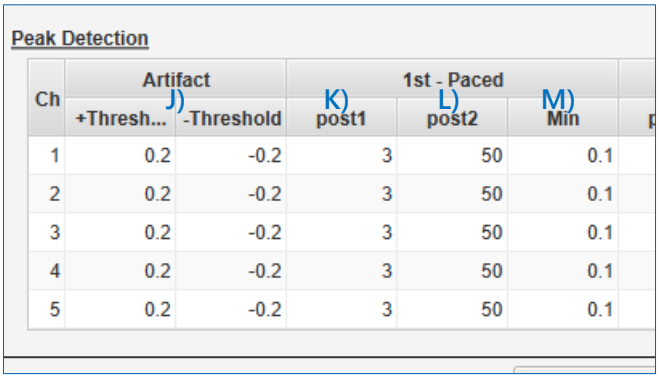
Phase 1: Search for the 1st peak

The peak that appears immediately after the intersection with D) the amplitude threshold (+ for +, – for –) is detected as the 1st peak. The next peak is not searched for until the range specified in E) post1.

Phase 2: Seach for the 2nd peak

Starting from the 1st peak, H) the maximum value in the range from F) post1 to G) post2.

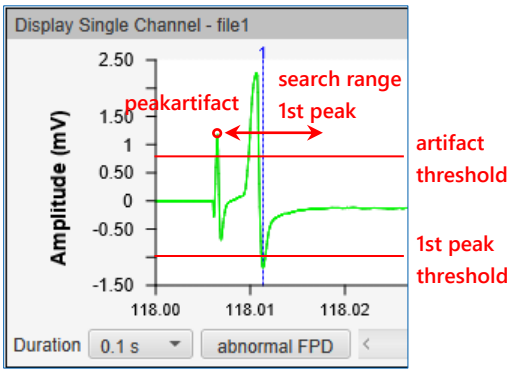
When searching as pacing data, the search algorithm is different because the stimulus artifact exists before the 1st peak.



Peak Detection

Ch	Artifact		1st - Paced			
	J) +Thresh...	-Threshold	K) post1	L) post2	M) Min	
1	0.2	-0.2	3	50	0.1	
2	0.2	-0.2	3	50	0.1	
3	0.2	-0.2	3	50	0.1	
4	0.2	-0.2	3	50	0.1	
5	0.2	-0.2	3	50	0.1	

Condition table when paced data is selected.



Artifacts in paced response.

Phase 1: Search for the stimulus artifact peak

The peak that appears immediately after the intersection with J) the amplitude threshold (+ for +, – for –) is searched for as the artifact peak.

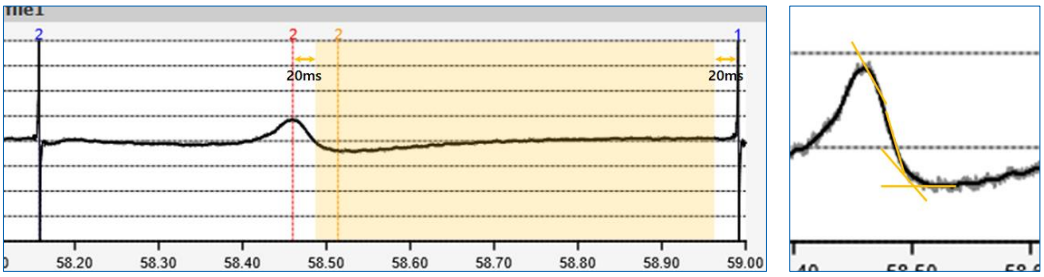
Phase 2: Search for the 1st peak

Starting from the artifact peak, – peaks are searched for in the range from K) post1 to L) post2. If it is deeper (smaller) than M) Min, it is identified as the 1st peak. (When creating the propagation diagram, priority is given to the – peak, which is relatively easy to obtain for all electrodes.) If it is shallower (larger), the + peak between K) post1 and L) post2 is identified as the 1st peak.

Phase 3: Search for the 2nd peak

Starting from the 1st peak, it is H) the maximum value in the range from F) post1 to G) post2.

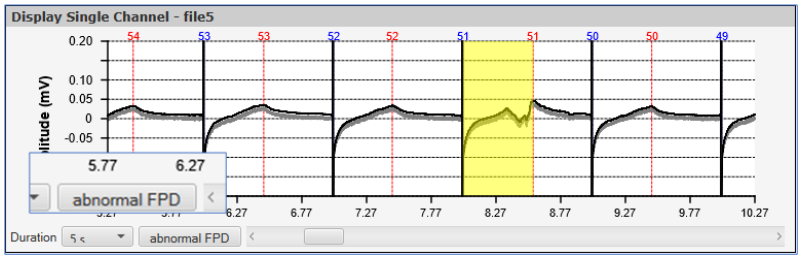
For FPD_end, which was added in version 210112_3 or later, the following algorithm is used to search for the end of the 2nd peak.



Step 1: For the interval from 20 ms after the 2nd peak to 20 ms before the next 1st peak, the slope with the data point 5 ms ahead is calculated by 1 pt in a progressive manner.

Step 2: The first point that is greater than or equal to 0 is the end of the 2nd peak.

❹ The FPDs exceeding I) χ times SD over the average of the FPD of all beats in the data file are highlighted as abnormal FPDs.

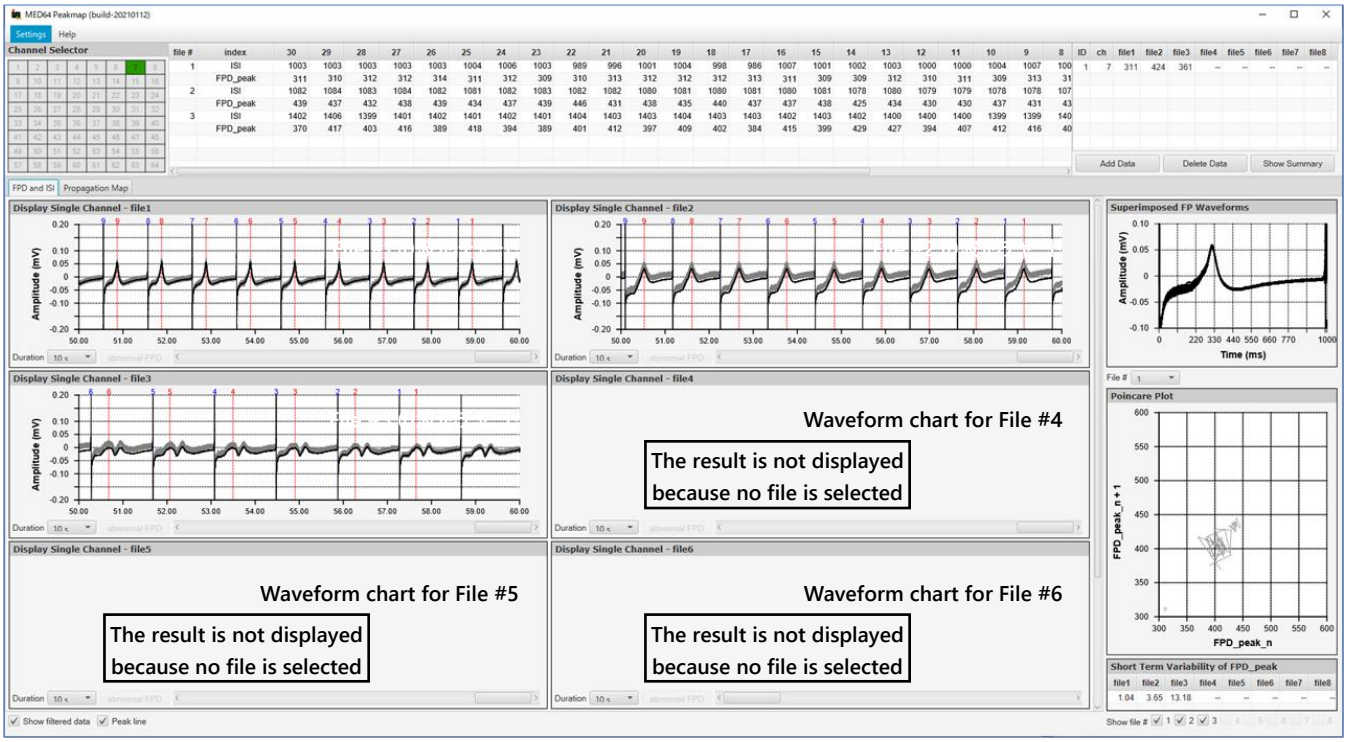


Abnormal FPD waveforms serve as a guide when searching for EAD waveforms. For data files containing abnormal FPDs, the abnormal FPD button becomes active and each click moves the seek bar to the corresponding range.

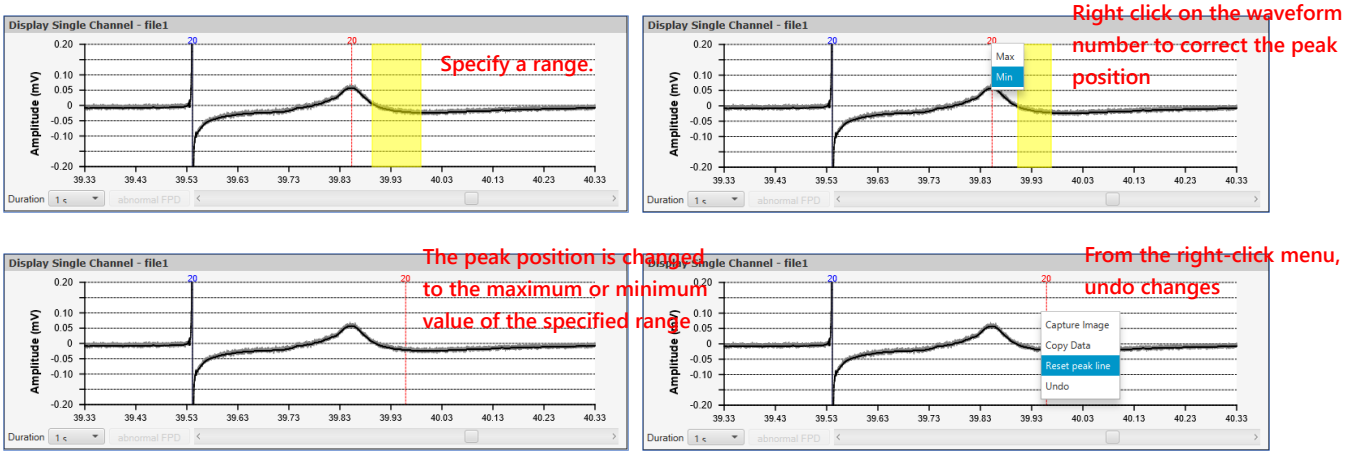
❺ If the FP waveform is displayed out of range due to baseline fluctuations, the average value calculated for each beat in the specified range immediately before the 1st peak is divided from the FP waveform for that beat (offset (zero point) correction per beat) and displayed.

6. Main Window

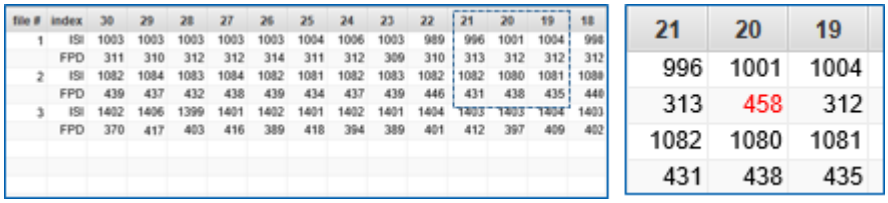
6-1. FPD and ISI Tab



Cursor lines indicating the 1st and 2nd peak positions are shown on the waveform chart. The numbers are assigned in descending order, with the last FP waveform in the data file being 1 (strictly, the 2nd beat from the last). The result table displays the FPD and ISI for the 30th to 1st beats from the last.



Left-clicking and dragging a number moves the cursor line indicating the peak, and the peak position can be modified accordingly. After specifying the range by left-clicking and dragging, the cursor line (peak) can be adjusted to the maximum or minimum value of the specified range by right-clicking on that number. After modifying, right-click on the peak line and select "Reset peak line" to undo all modifications or "Undo" to return to the previous modification. The changes are immediately reflected in the result table and the changes are highlighted in red as history.

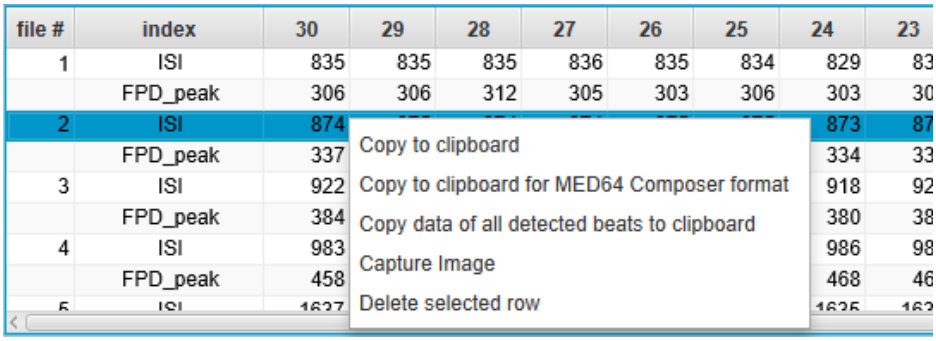


file #	index	30	29	28	27	26	25	24	23	22	21	20	19	18
1	ISI	1003	1003	1003	1003	1003	1004	1006	1003	989	996	1001	1004	998
	FPD	311	310	312	312	314	311	312	309	310	313	312	312	312
2	ISI	1082	1084	1083	1084	1082	1081	1082	1083	1082	1082	1080	1081	1088
	FPD	439	437	432	438	439	434	437	439	446	431	438	435	448
3	ISI	1402	1406	1399	1401	1402	1401	1402	1401	1404	1403	1403	1404	1403
	FPD	370	417	403	416	389	418	394	389	401	412	397	409	402

21	20	19
996	1001	1004
313	458	312
1082	1080	1081
431	438	435

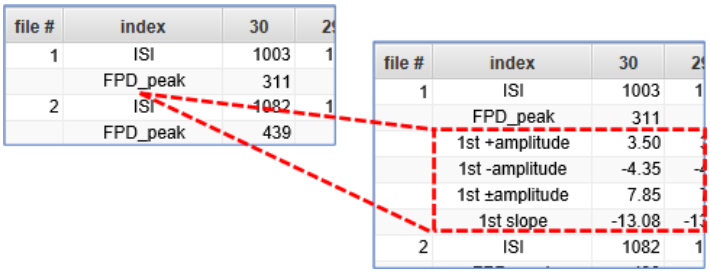
6-2. Result Table

It displays all of the FPD and the ISI (the interval between the 1st peak and the 1st peak of the previous beat) for the last 30 beats of the data file. This table can be saved to the clipboard from the right-click menu and pasted into Excel or another spreadsheet. There is also the option to save the result values in the "MED64 Composer" format (*no longer distributed).

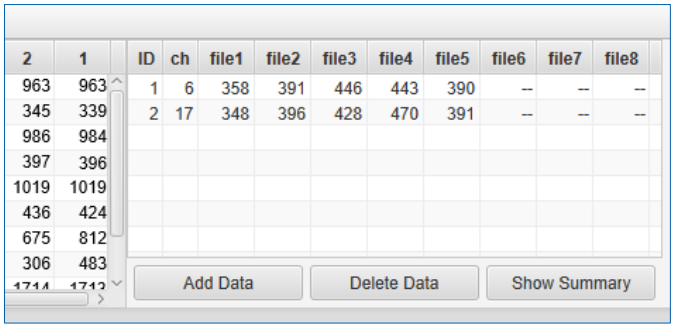


Result Table.

If you also select the 1st peak amplitude as an analysis item in the Settings window and run it, the result will be inserted into the result table as shown in the figure below. Note that the search range of the peak for which the amplitude is calculated is defined internally by the program and is not a setting item (in the case of spontaneous activity, when the 1st peak is detected as the +/– peak, the search range of the –/+ peak is ±10 ms of the +/– peak. For the pacing data, the range is ±1 ms).

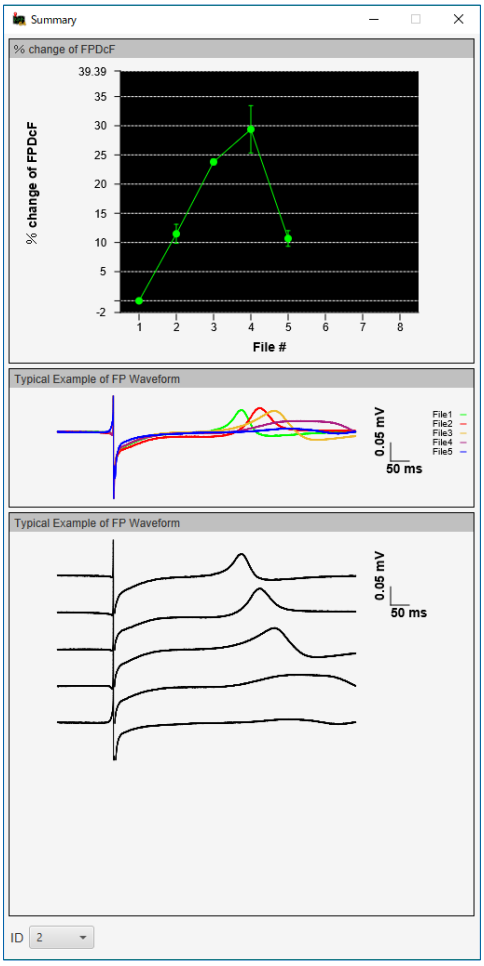


Click "Add Data" under the summary table on the right of the result table to calculate the corrected FPD (FPDcF) using the Fridericia correction formula from the FPD and the ISI of the last 30 beats, and add it to the summary table one row at a time. By clicking "Delete Data," the last row of data added to the table can be deleted. The data added to the summary table will remain when the data file is updated for a reanalysis.



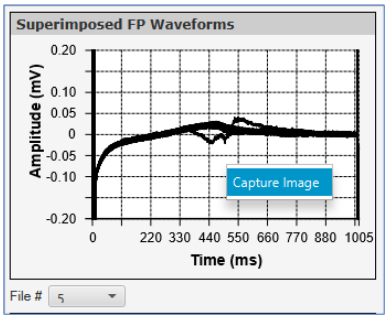
Summary Table.

Clicking "Show Summary" displays a graph of the mean±SEM of the FPDcF displayed in the summary table, and the averaged FP waveform for the last 30 beats. Both of these graphs and typical waveform examples can be saved as images (Capture Image) or values (Copy Data) using the right-click menu.



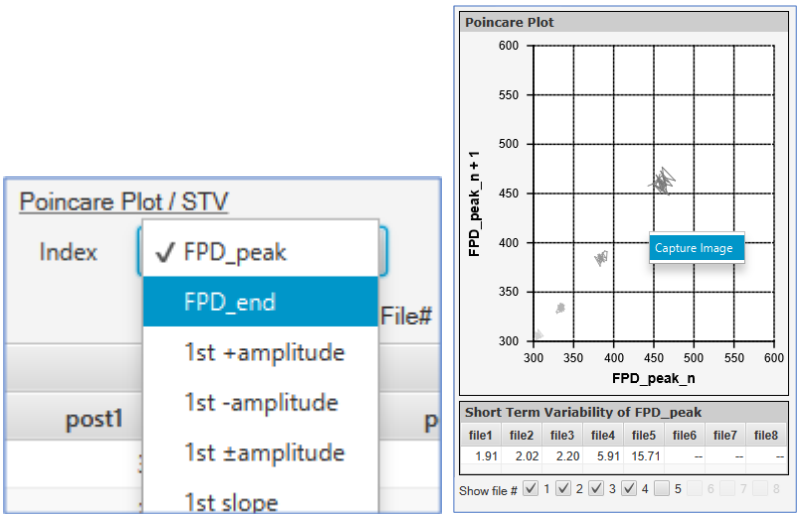
6-3. Superimposed FP Waveforms Panel

This is a chart showing all detected FP waveforms superimposed in each data file. Please refer to this chart as a guide to determine if there are any waveform corruptions or appearances of EAD waveforms before the last 30 beats in the analyzed data file. Right-click to save the image to the clipboard.



6-4. Poincare Plot Panel

The plot is based on the last 30 beats of each data file on the horizontal axis, and the values of the beats immediately following the last 30 beats on the vertical axis, for the measurement specified in the "Peak Line" tab of the Settings window. Any variation in the per-beat value will result in a spread in the plot of the corresponding data file. By default, all data files are displayed in the same color. The color can be changed for each data file in the "Preferences" tab of the Settings window. You can also uncheck the checkbox at the bottom to hide data files, such as those that contain EAD waveforms or that show anomalous values. Right-click to save the image to the clipboard.



6-5. Short Term Variability of FPD Table

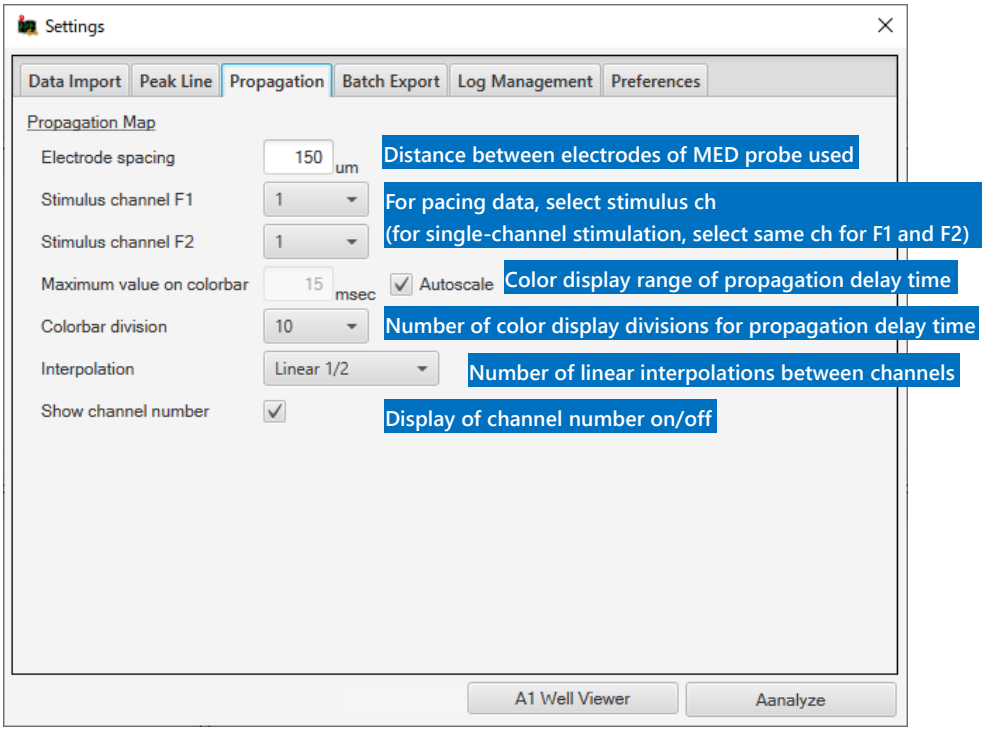
For the last 30 beats of each data file, this is an index that quantifies the variability of FPD per beat using the formula shown below.

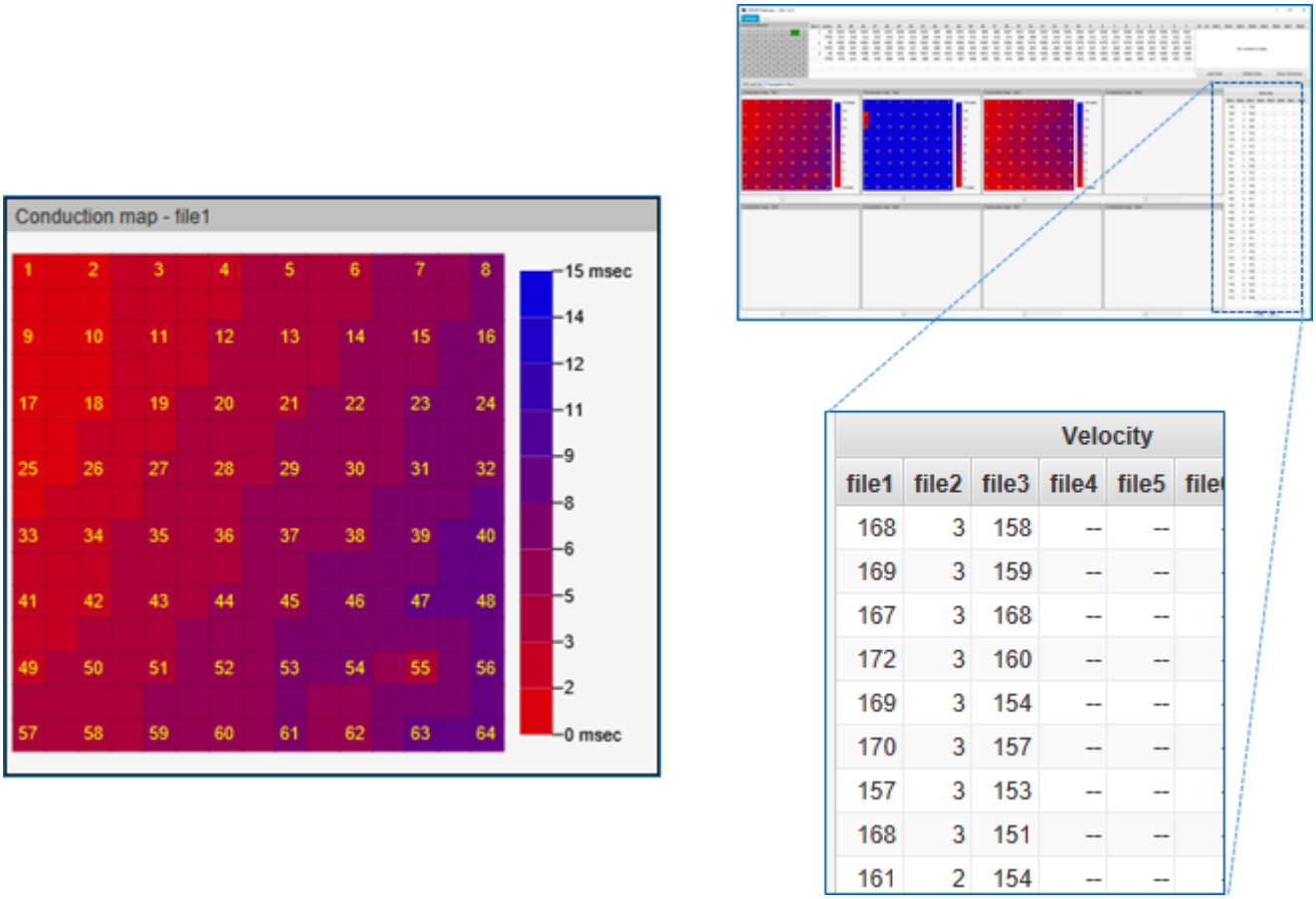
$$STV_{FPD} = \frac{\sum |FPD_{n+1} - FPD_n|}{30 \times \sqrt{2}}$$

Numerical information can be saved to the clipboard by right-clicking on the table.

7. Propagation Delay Map -Propagation Tab-

Peakmap can calculate the time difference between 64 channels based on the time information of the detected 1st peak, and it can draw a propagation delay 2D map based on this information. When pacing data is selected, the propagation delay map is created based on the delay time of the 1st peak from the stimulus artifact (as of August 2018, the function for creating propagation delay map for modax data has not been implemented).



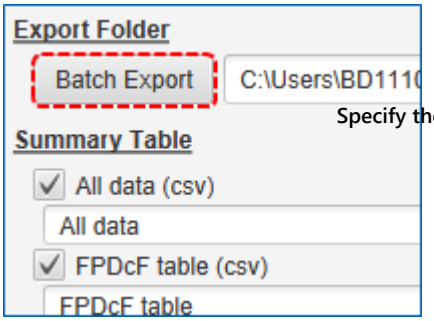


From the top, the final 30th beat, followed by 29th, 28th, ・・・ 1st.

The "Propagation" tab, which shows the results, displays a propagation delay map for each data file, and to the right, the propagation velocity for each of the detected beats. The propagation velocity is calculated based on the time difference and distance between the two channels, referring to the earliest and latest channels of the appearance time of the 1st peak. When pacing data is selected, the propagation velocity is calculated based on the latest channels between the artifact peak and the 1st peak.

8. Batch Export of Results -Batch Export Tab-

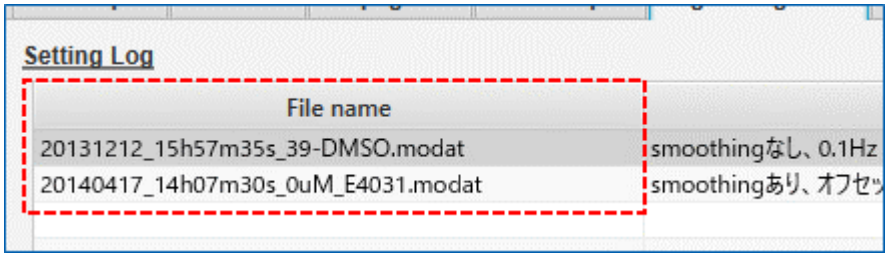
This function batch outputs the numerical data and the image data that are obtained from the right-click menu on each panel of a result table, waveform chart, etc. The items to be output can be selected individually using the check boxes, and the output file name can be specified in a text box. By clicking "Batch Export," the files for each item are individually exported to a specified folder.



Specify the location to output

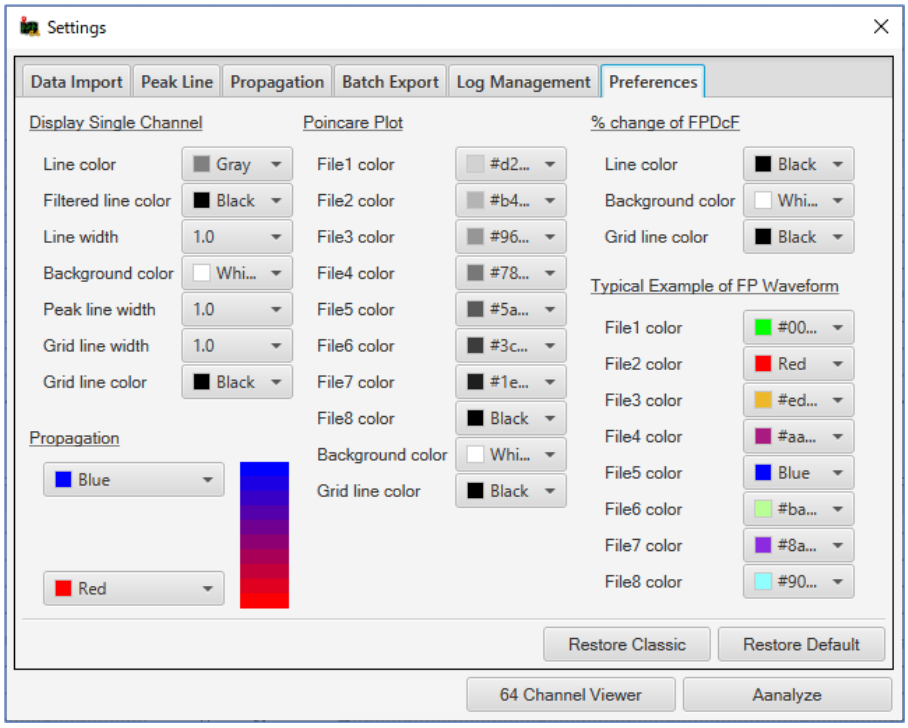
9. Saving and Recalling Analysis Logs -Log Management Tab-

Filter settings and other information obtained during analysis can be saved and recalled (results cannot be saved or recalled). In this case, only the first file of data used in the analysis is saved. Any text can be entered in the memo field.



10. Display Customization -Preferences Tab-

The appearance of the chart, such as its line thickness and background color, can be customized to your liking from the "Preferences" tab of the Settings window.



11. Change Allocation of Memory Used

MED64 Peakmap is configured by default to use up to 10 GB of memory (assuming a 12 GB PC, leaving 4 GB free for other processing). If you have a PC with sufficient memory size, you can increase the limit to improve processing speed.

Open the MED64 Peakmap.cfg file in a text editor at

C/user/user name/AppData/Local/MED64 Peakmap/app

Correct the below (e.g., Xmx12000m → Xmx16000m for 12 → 16 GB) and save it to change the memory used.

[JVMOptions]
-Xmx12000m

Version: 190704

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