



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	n : 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).

E Needs to activation	×
To complete the certification process for using the software, please inform us (info@amedsci.com) of the following key code	e.
Key: 9bd7436d7ee1494e97a2dac20b7e38b0	
ОК	

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.

JK-	100000 - K3032-	사회는 가는는 Mi	8 680 RT	新しい 2オルター	4 5 4 5 1 5 1 5 1 5 1 5 1 5 1 5 1 5 1 5	70/(71	·端集 現在	88 a
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· · · · ·	iib		2017/05/1	0 17:25	ファイル・フォルダー			
rive	App.settings		2017/05/1	0 17:26	Visual Studio Set	11	T.KB	
	activation		2017/05/1	0 17:35	7761		1 KB	
	MED64 Burstscope.c	fg	2017/05/0	8 21:56	CFG 77436		1 KB	
7-7	🕢 MED648unstScope.ju	r.	2017/05/0	8 21:55	Executable Jar Fi	4 3	734 KB	

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.

ia I	MED6	4 Pea	kmap	[V	er 1.0	.1]			
Se	ltings	Н							
Sho	ow Se	ttings	Dial	og				file #	i
1	2	3	4	5	6	7	8		
0	10	11	12	12	14	15	18		

Bring up the Settings window from the Settings \rightarrow 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 ① Data Type in the figure below, then click ② 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 ③ 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).

ata Import Pe	ak Line Pi	ropagation	Batch	Export	Log M	Mana	geme	ent	Pref	erend	es			
ata Selection														
File type					4 D	uratio	n							
modat		 single tr 	ace r	•		last		*		1.	0 m	in		
Compound					6	Chan	nel							
File # Dose	File	name				1	2	3	4	5	6	7	8	
1	201312	12_15h57m3	2	×		9	10	11	12	13	14	15	16	
2	201312	12_16h08m3		×		17	18	19	20	21	22	23	24	
3	201312	12_16h19m2		×		25	26	27	28	29	30	31	32	
4	201312	12_16h30m2		×		33	34	35	36	37	38	39	40	
5	201312	12_16h41m3		×		41	42	43	44	45	46	47	48	
6				×		49	50	51	52	53	54	55	56	All
7	1			×		57	58	59	60	61	62	63	64	None
8	1			×	• -									
3 Se	lect Folder	8	-	iles	6 Ana	ilysis / FP	D_pe	eak	F	PD_	end		1st ar	nplitude
						2n	d am	plitud	le	Pr	opag	ation	n map	

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 ^(a) 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 ^(a) Channel to select the target channels (green: channels to analyze, red: channels not to analyze). Select the measurement to be calculated by analysis in ^(a) 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.

Δ



5. Peak Detection -Peak Line Tab-

ata Impo	ort Peak Line	Propagation	Batch Export	.og Management	Preferences	
re-filterin	9			A Searching EAD		
Highpas	None		• 1 Hz	Alert abnorma	al FPD mear	n ± 1) 5 ×SD
Lowpass	None		· 5000	Naveform Drav	ving	
			HZ HZ	Adjust off	set	
✓ Smo	othing	A)	Subtract me	ean amplitude o	f the following rang
Averag	ing window si	ze pre/post	5 ms	20	to 3 I	before 1st peak
						eelele let peak
Avoid s	smoothing the	following range		Deinenen Diet //	ms ·	
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Avoid ampl	smoothing the itude SD over	following range pre/post 5	ms > C) 20 uV	Poincare Plot / Index FPI	STV D_end	•
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Avoid : ampl	smoothing the itude SD over <u>ction</u>	following range pre/post) 5	E)	Poincare Plot /: Index FPI	STV D_end G) 2nd	▼ File# 1 ·
Avoid : ampl eak Dete Ch	smoothing the itude SD over ction C +Threshold	following range pre/post 5 5	E) post1	Poincare Plot /: Index FPI	STV D_end G) 2nd post2	▼ File# 1 · H) peak
Avoid : ampl eak Dete Ch	smoothing the itude SD over ction C +Threshold 0.	following range pre/post) 5) 1st -Threshold 2 -(E) post1 0.2 60	Poincare Plot /: Index FPI F) post1 0 300	STV D_end G) 2nd post2 600	File# 1 H) peak 0 Max
Avoid : ampl eak Dete Ch 1 2	smoothing the itude SD over ction +Threshold 0. 0.	following range pre/post b 5	E) 1 post1 0.2 60 0.2 60	Poincare Plot /: Index FPI F) post1 0 300 0 300	STV D_end G) 2nd post2 600 600	 File# 1 H) peak 0 Max 0 Max
Avoid : ampl eak Dete Ch 1 2 3	smoothing the itude SD over ction +Threshold 0. 0. 0.	following range pre/post b 5	E) post1 0.2 60 0.2 60 0.2 60	Poincare Plot /: Index FPI post1 0 0 300 0 300 0 300	STV D_end G) 2nd post2 600 600 600	File# 1 H) peak Max Max Max
Avoid : ampl eak Dete Ch 1 2 3 4	smoothing the itude SD over ction +Threshold 0. 0. 0. 0. 0.	following range pre/post 5 5	E) post1 0.2 60 0.2 60 0.2 60 0.2 60 0.2 60	Poincare Plot /: Index FPI 0 300 0 300 0 300 0 300 0 300 0 300	G) 2nd post2 600 600 600 600 600 600	File# 1 H) peak Max Max Max Max Max

① 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. **②** 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 μ V is considered to be the 1st peak range. **③** 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.

C.L	Arti	fact	1st - Paced							
Cn	+Thresh	-Threshold	post1	post2	Min					
1	0.2	-0.2	3	50	0.					
2	0.2	-0.2	3	50	0.					
3	0.2	-0.2	3	50	0.					
4	0.2	-0.2	3	50	0.					
5	0.2	-0.2	3	50	0.					



Condition table when paced data is selected.



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.

4 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.

ALPHA ME



• 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.

ALPHA ME



file #	index	30	29	28	27	26	25	24	23	22	21	20	19	18	21	20	10
1	151	1003	1003	1003	1003	1003	1004	1006	1003	989	996	1001	1004	998	21	20	15
	FPD	311	310	312	312	314	311	312	309	310	313	312	312	312	006	1001	1004
2	131	1082	1084	1083	1084	1082	1081	1082	1083	1082	1082	1080	1081	1080	990	1001	1004
	FPD	439	437	432	438	439	434	437	439	446	431	438	435	440	212	459	212
3	1SI	1402	1406	1399	1401	1402	1401	1402	1401	1404	1403	1403	1404	1403	515	430	312
	FPD	370	417	403	416	389	418	394	389	401	412	397	409	402	1082	1080	1081
															1002	1000	1001
															431	438	435
																100	100

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).

file #	index	30	29	28	27	26	25	24	23
1	ISI	835	835	835	836	835	834	829	83
	FPD_peak	306	306	312	305	303	306	303	30
2	ISI	874						873	87
	FPD_peak	337	Copy to	clipboard				334	33
3	ISI	922	Copy to	clipboard	for MED6	64 Compo	ser forma	t 918	92
	FPD_peak	384	Copy da	ta of all d	etected b	eats to cli	pboard	380	38
4	ISI	983	Contura	Imaga				986	98
	FPD_peak	458	Capture	image				468	46
<u>к</u>	101	1607	Delete s	elected ro	W			1625	163

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.

2	1	ID	ch	file1	file2	file3	file4	file5	file6	file7	file8
963	963 ^	1	6	358	391	446	443	390			-
345	339	2	17	348	396	428	470	391			-
986	984										
397	396										
1019	1019										
436	424										
675	812										
306	483										
171/	1712 ~		Ac	ld Data		De	lete Da	ta	Sho	ow Sum	mary

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).









	2	/					
		Velocity					
	file1	file2	file3	file4	file5	file	
	168	3	158				
	169	3	159				
	167	3	168				
	172	3	160				
	169	3	154				
	170	3	157				
	157	3	153				
	168	3	151				
	161	2	154				

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.

Export Folder		
Batch Export	C:\Users\BD1110	
Summary Table	he location to output	
All data (csv		
FPDcF table		
FPDcF table		

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.





Setting L	<u>og</u>	
	File name	
201312	12_15h57m35s_39-DMSO.modat	smoothingなし、0.1Hz
201404	17_14h07m30s_0uM_E4031.modat	smoothingあり、オフセッ

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.

🧑 Settings						
Data Import Peak	Line Propaga	ation Batch Export	Log Managemen	t Preferences		
Display Single Channel		Poincare Plot	% change of FPDcF			
Line color	📕 Gray 🛛 🔻	File1 color	#d2 👻	Line color	🔳 Black 👻	
Filtered line color	📕 Black 👻	File2 color	₩b4 ▼	Background color	r 🗌 Whi 👻	
Line width	1.0 👻	File3 color	#96 🔻	Grid line color	Black 👻	
Background color	Whi 👻	File4 color	#78 🔻	Typical Example of FP Waveform		
Peak line width	1.0 👻	File5 color	🔳 #5a 💌	File1 color	# 00 -	
Grid line width	1.0 -	File6 color	■ #3c 💌	File2 color	Red T	
Grid line color	Black 👻	File7 color	📕 #1e 🔻	File3 color	#ed •	
Propagation		File8 color	Black 👻	File4 color	#aa *	
		Background color	🗌 Whi 👻	File5 color	Blue 🔻	
Biue		Grid line color	Black 🔻	File6 color	#ba	
				File7 color	#8a 👻	
Red	•			File8 color	#90	
- neu						
			R	estore Classic	Restore Default	
			64 Channe	l Viewer	Aanalyze	

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 \rightarrow Xmx16000m for 12 \rightarrow 16 GB) and save it to change the memory used.

[JVMOptions] -Xmx12000m



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