Cardiac Imaging Data Analysis

The functions of BV Workbench corresponding to the data processing function and analysis function described in the following papers are described.

Laughner JI, Ng FS, Sulkin MS, Arthur MA, Efimov IR.
Processing and Analysis of Cardiac Optical Mapping Data Obtained with Potentiometric Dyes.
Am J Physiol Heart Circ Physiol. 2012 Oct 1;303(7):H753-65.

1. Pre-processing of data

0. Undo filters
1. Invert polarity
2. Data Masking
3. Spatial filter (mean filter)
4. FIR filter
5. Drift removal
6. Normalization

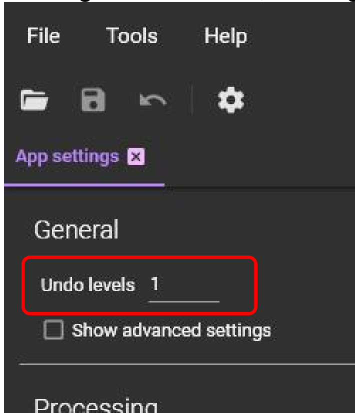
2. Data anlaysis

1. Activation time map / APD (action potential duration) map / Repolarization time map
2. Conduction velocity map
3. Phase map
4. Dominant frequency map
5. Conduction velocity on straight line
6. Save image

1-0. Undo filter

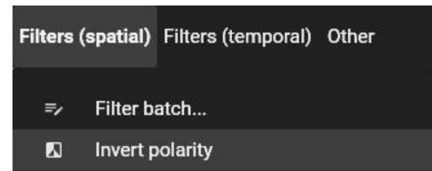
You can undo the last processed filter and undo data by clicking the Undo icon on the toolbar.

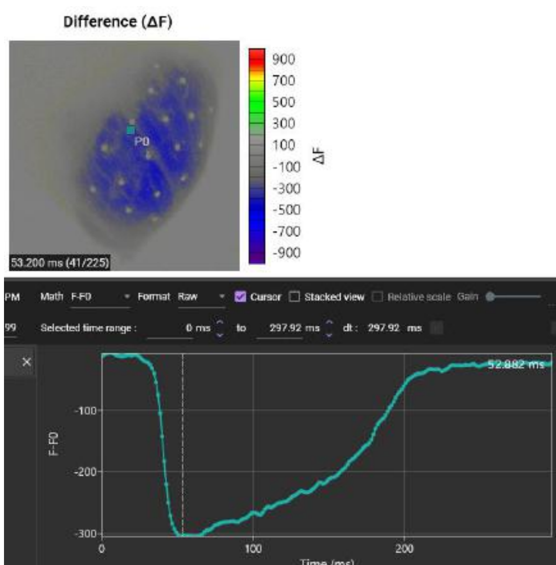
Number of undos that can be undone is set in [Undo levels] on the [App settings] screen. You can set 1 to 10, but the larger the number, the larger the memory usage of the PC. The recommended value is 1.



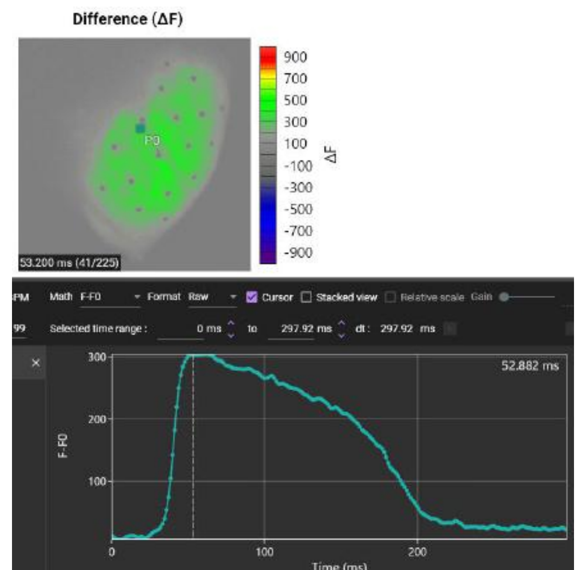
1-1. Invert polarity

When [Filters (spatial)]-[Invert polarity] is executed, polarity of change of F-F(0) is inverted while maintaining brightness value of background image.





Invert polarity



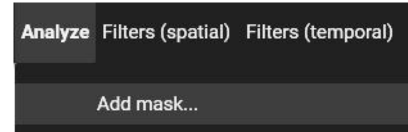
1-2. Data masking

There are two methods, “add mask” and “ROI (polygon)”.

1-2-1. Specifying data analysis area by adding a mask layer (add mask)

To specify data analysis area, you can use a ROI (rectangle or polygon) or add a mask layer. Specify data analysis area on the mask layer.

Select [Analyze]-[Add mask..] to display the following screen.



Specify brightness threshold and set pixels above threshold as data analysis area.

Data analysis area is displayed in pink.

All other pixels are masked and excluded from data analysis area.

Reference layer

Display or input brightness threshold

Invert mask area

Reference layer Background image

Threshold 284 Invert

Edit Size 20 px CLEAR

IMPORT EXPORT OK CANCEL

Delete selection

Specify size of the pen tool and eraser tool.

Pen tool:
Select data analysis area by dragging mouse on image.

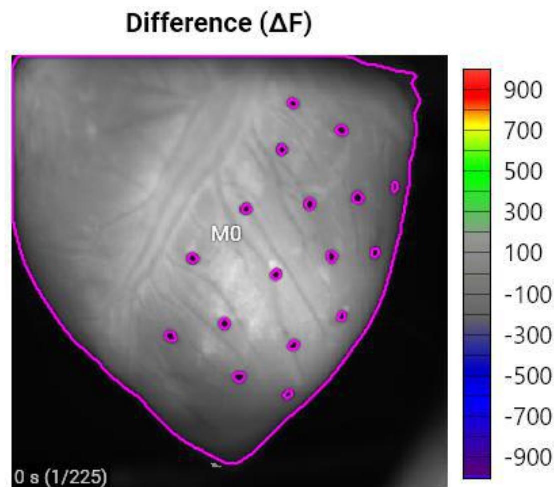
Eraser tool:
Delete data analysis area by dragging mouse on image.

Cancel area specification and close the screen

Read location information of selected area from file

Output location information of selected area to file

Confirm the area specification and close the screen

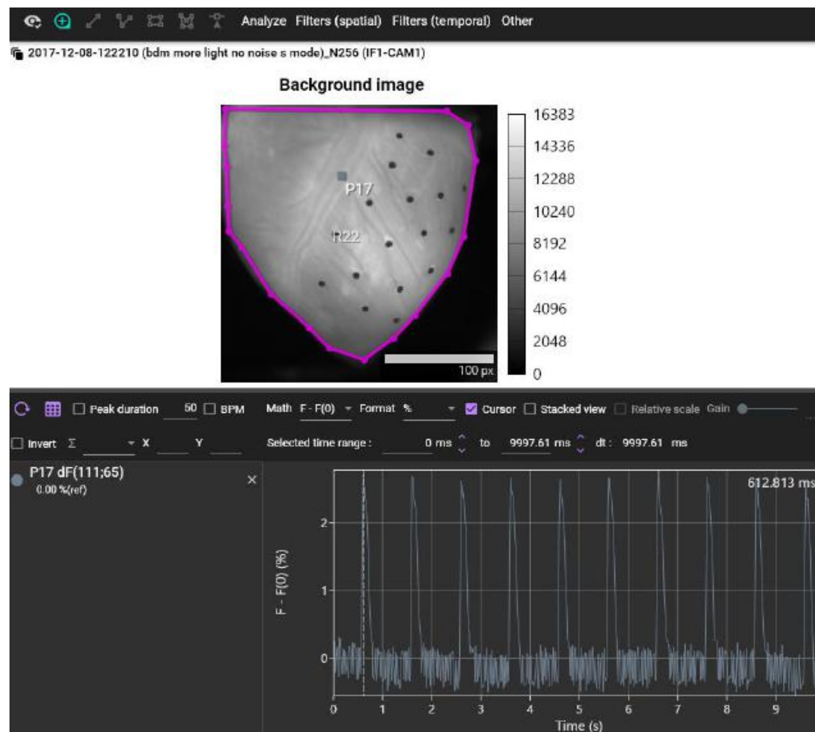


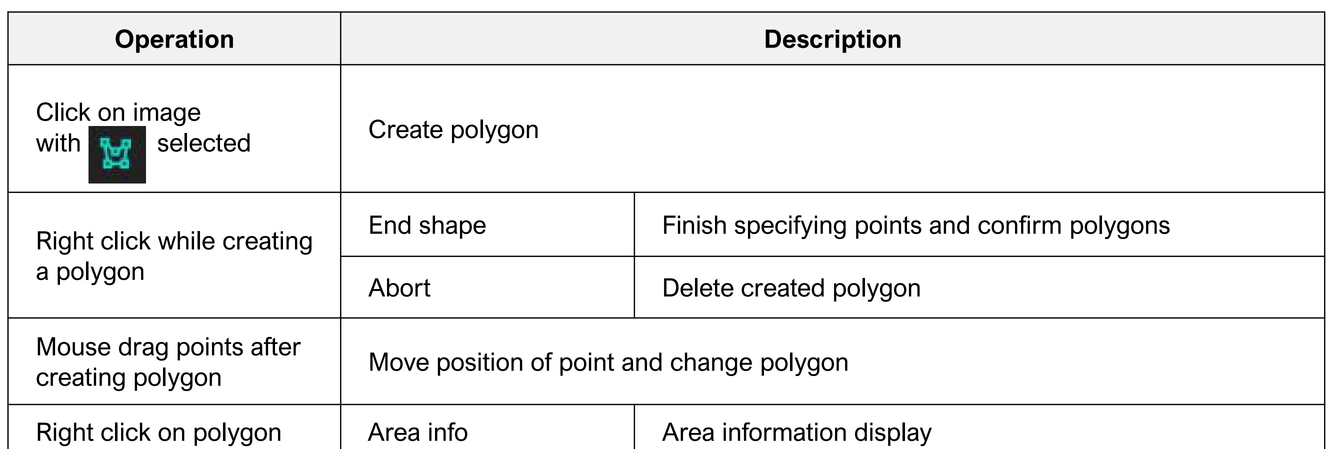
Range specification by mask layer

1-2-2. Specifying data analysis area by ROI (polygon)

With "Add polygon" selected, click on image and specify polygon. A polygon is completed when start point and end point are specified to be the same.

The specified polygon becomes ROI (Region of Interest) and is used for target range of various data analysis and display range of pseudo color.

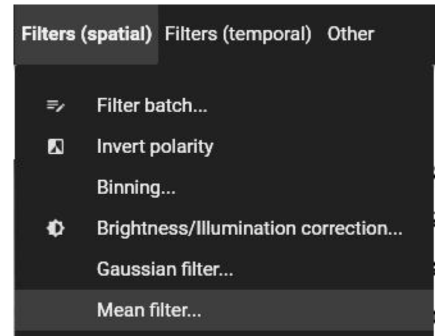




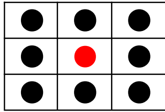
Operation	Description	
Click on image with [icon] selected	Create polygon	
Right click while creating a polygon	End shape	Finish specifying points and confirm polygons
	Abort	Delete created polygon
Mouse drag points after creating polygon	Move position of point and change polygon	
Right click on polygon	Area info	Area information display

1-3. Spatial filter (mean filter)

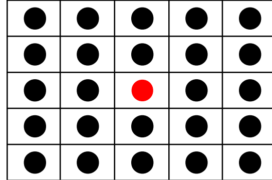
When [Filters (spatial)]-[Mean filter] is executed, the following screen is displayed. Mean filter smooths image and removes noise. Let pixel value be D(t,x,y), and if it is indicated by ●, set average value of data values in the proximity of the PxP range to D(t,x, y).

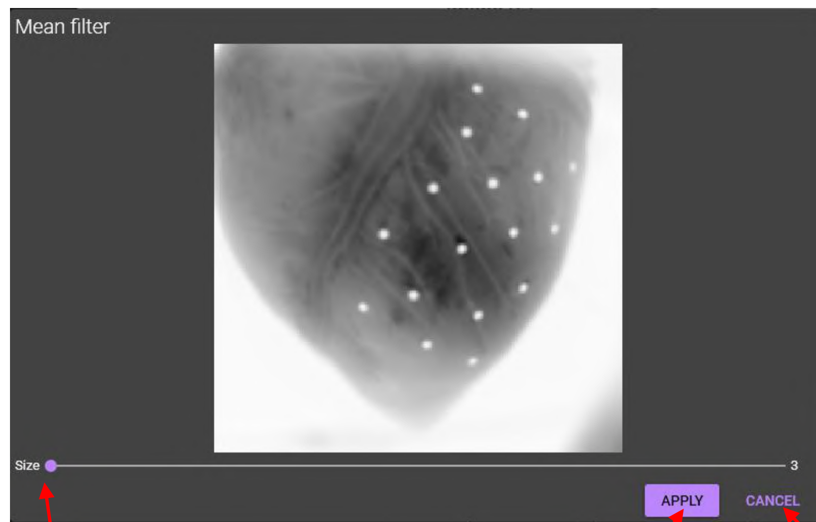


When P=3



When P=5

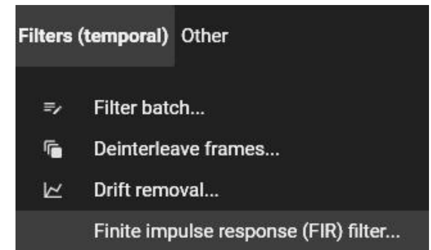




1-4. FIR filter

Click [Filters (temporal)]-[Finite impulse response (FIR) filter...] to display the following screen, which uses a FIR (finite impulse response) filter to remove noise.

If you click on an image, original waveform at that point will be gray, and FIR filtered waveform will be green.



Cutoff frequency 1

Cutoff frequency 2
(Can be input only when Filter type=Band-pass or Band-stop)

Sampling rate during image acquisition

Select filter type
・Low-pass
・High-pass
・Band-pass
・Band-stop

Select window function
・Blackman-Harris
・Hamming
・Hann

Increase value to make frequency response sharper. Output delay is proportional to this value.

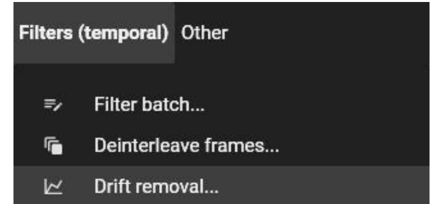
Click to remove the filter delay of (N-1)/2 samples.

Execution

Cancel

1-5. Drift removal

When you click [Filters (temporal)]-[Drift removal], the following screen is displayed and rise/fall (drift) of waveform baseline due to fading of fluorescent dye and change in brightness of light source is corrected.



When you click on an image, original waveform at that point is displayed in gray and waveform after drift removal processing is displayed in green.

Enter integers for [Fitting polynomial degree] and [Downsampling]. Keep in mind that the larger the [Fitting polynomial degree] value, the smaller the signal change, so choose a value that is neither too small nor too large.

Enter optimum values to remove unnatural undulations of waveform and click [APPLY] to start drift removal process. The process may take some time. To cancel drift removal process, click [CANCEL].

Drift removal

Fitting polynomial degree 3 Downsampling 10

Enter polynomial fitting order

Downsampling (Enter an integer of 1 or more to shorten fitting time)

Preview :

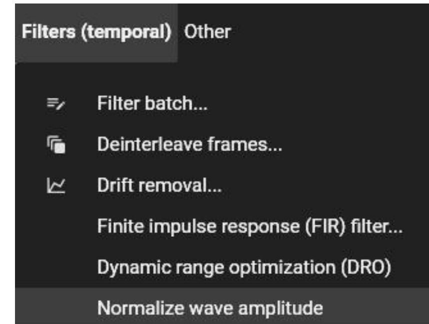
Time (s)

APPLY CANCEL

Execution

Cancel

1-6. Normalization



Correct difference in amplitude of brightness value between each pixel and calculate so that brightness values of all pixels have the same amplitude (0 to 65,535).

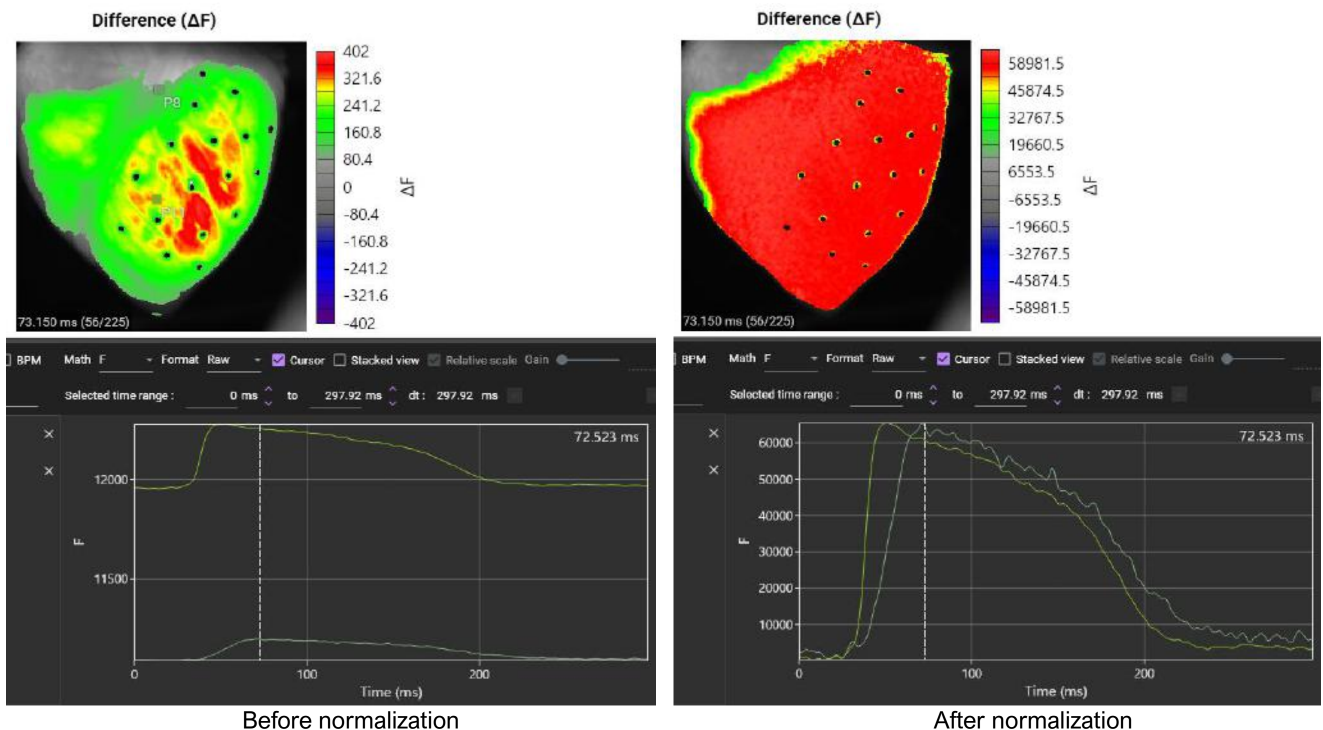
Even with a uniform tissue sample such as an isolated heart, fluorescence intensity may vary depending on location due to factors such as uneven irradiation of excitation light, uneven staining, and tissue thickness. In such cases, using Normalize eliminates influence of external factors that cause differences in amplitude, and makes the signal intensities (waveforms) of all pixels the same as when recording with electrodes.

Correlation of signal intensity between pixels is lost, so it is not suitable for samples such as neuro samples where the signal intensity differs depending on the site.

Click [Filters (temporal)]-[Normalize] to execute.

The algorithm is as follows.

1. For each pixel, check all frames to find the maximum and minimum values.
2. Calculate gain and offset so that the minimum value is 0 and the maximum value is 65535.
3. Apply gain of 2 and offset to all frames

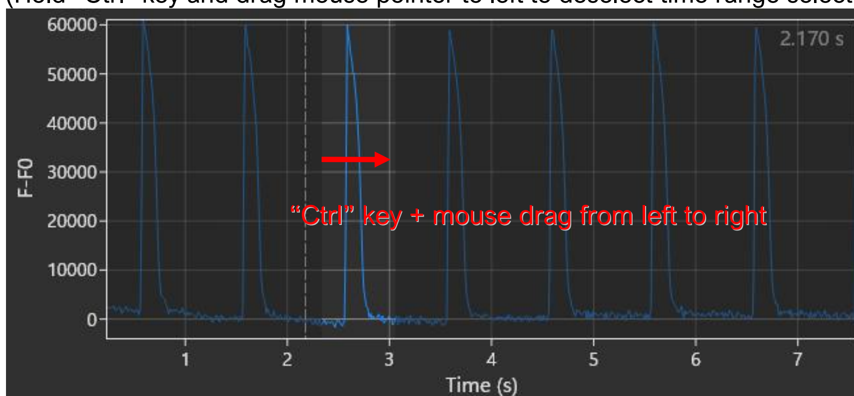


Before normalization　　　After normalization

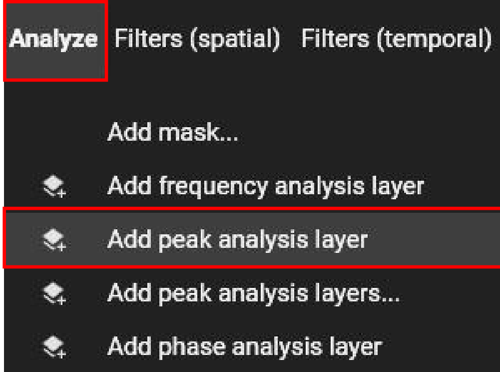
2-1. Activation time map / APD (action potential duration) map / Repolarization time map /

After completing 1-1 to 1.6, It is possible to create various maps.

Hold down "Ctrl" key and drag mouse from left to right on waveform to select waveform range.
(Hold "Ctrl" key and drag mouse pointer to left to deselect time range selection for waveform and select all ranges.)

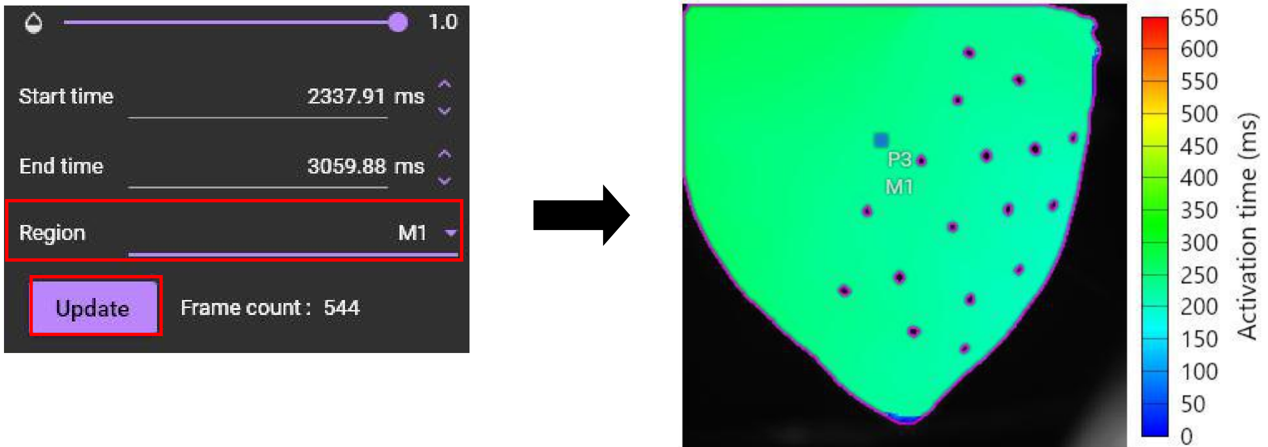


Click [Analyze]-[Add peak analysis layer].



The [peak analysis] layer is added to the list on the left and the settings are displayed

Select mask name or ROI name in [Region] and then, click [Update] button. Color is displayed only within the specified range.



Select "Peak property (map type) ", activation time and set repolarization %.

Peak property: Activation time

Activation: Half rise (Fmax/2)

Repolarization: 50 %

Select map type

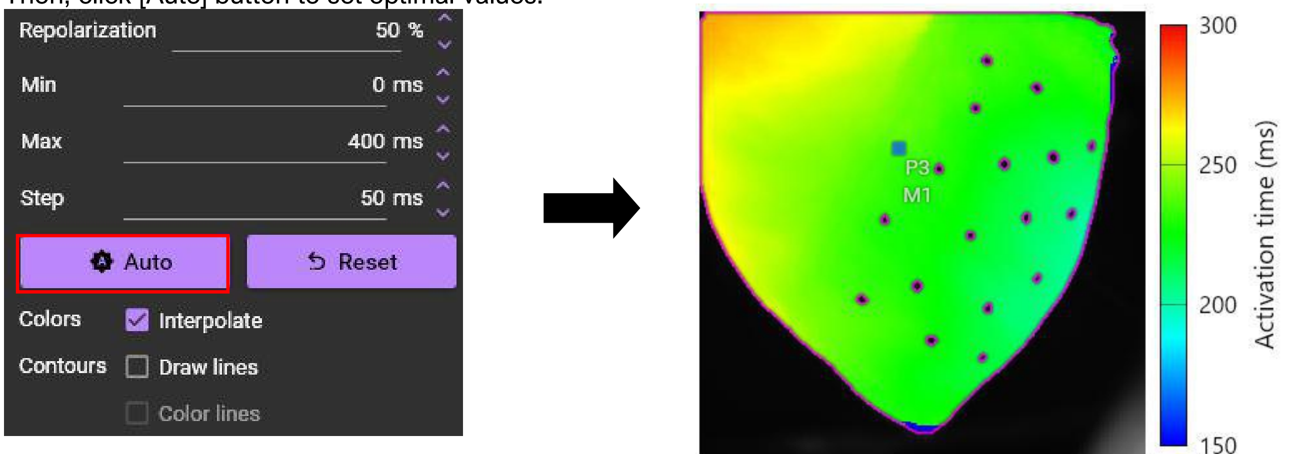
- Activation time
- Repolarization time
- Duration (APDXX)
- Activation to Peak time
- Peak to Repolarization time
- Peak time
- Peak amplitude

Specify XX% repolarization time

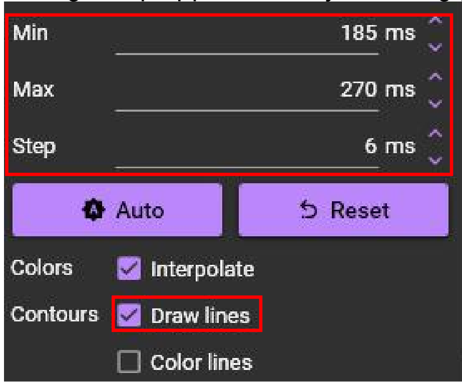
Select activation time

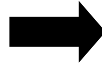
- Half rise (Fmax/2)
- Maximum (Fmax)
- Derivative max (dF/dt))

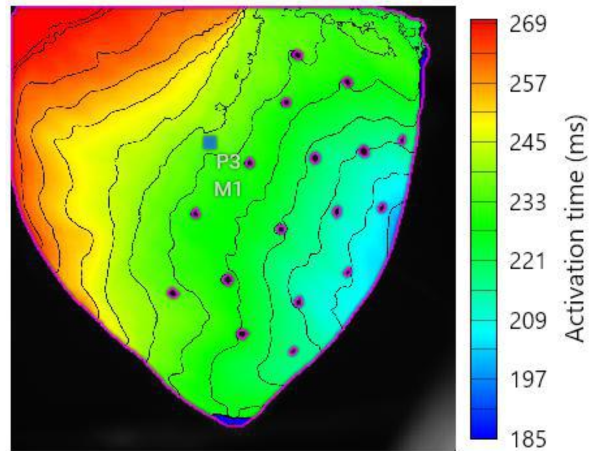
Then, click [Auto] button to set optimal values.



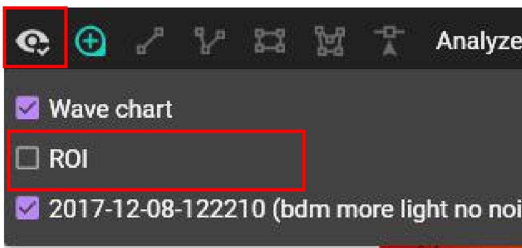
Change map appearance by checking [Draw lines] and adjusting [Min], [Max], [Step].

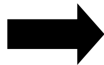


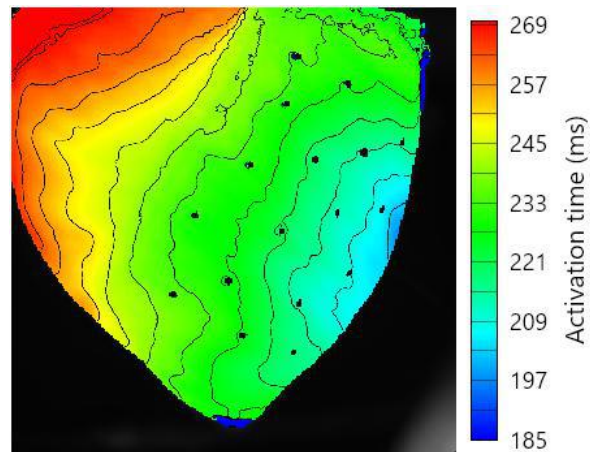




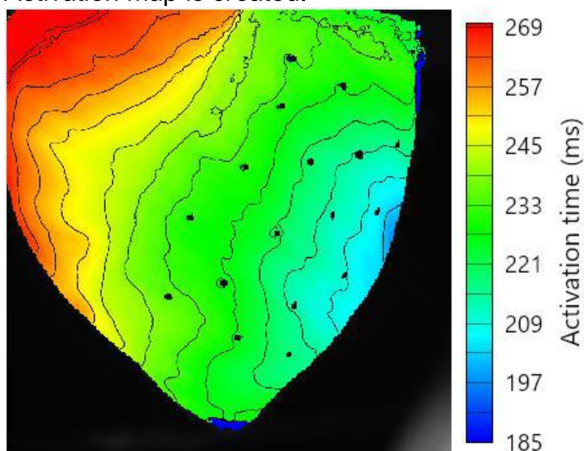
Click the icon and turn off “ROI” to remove the border





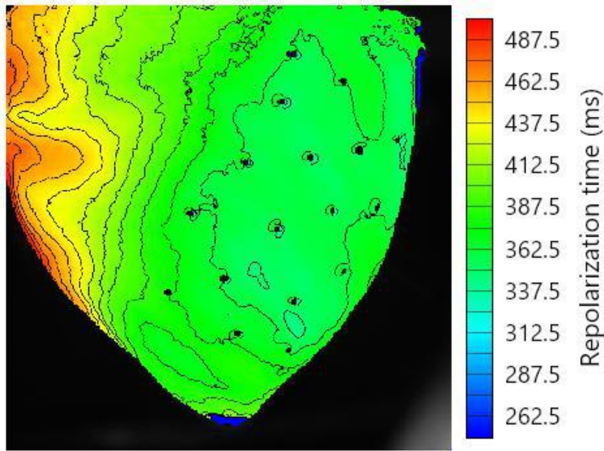


Activation map is created.

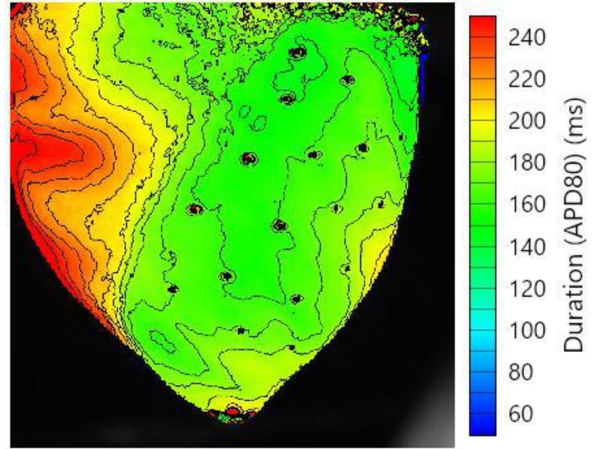


Activation map

You can create a repolarization map, ADP map and other maps in the same way



Repolarization map

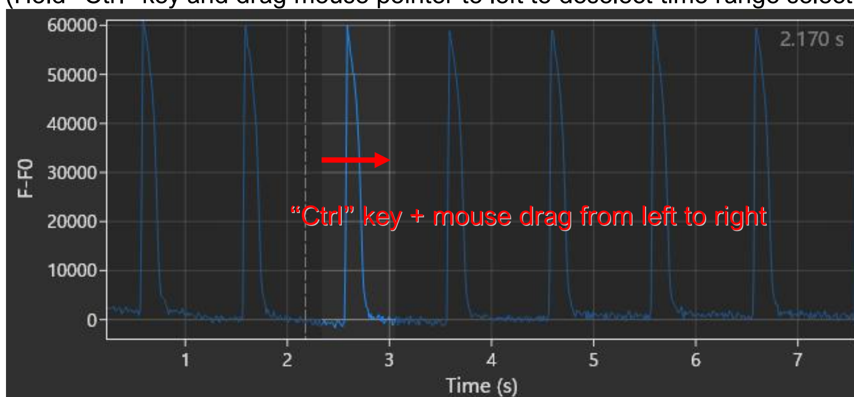


APD80 map

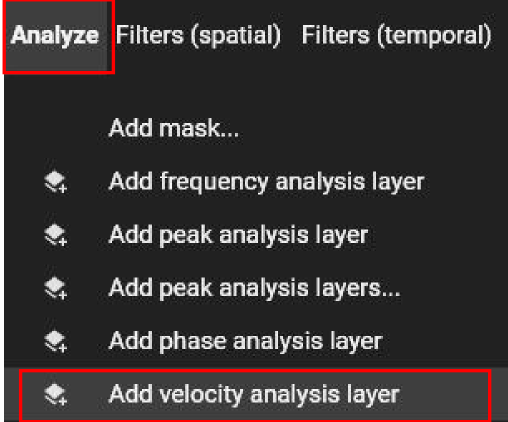
2-2. Conduction velocity map

After completing 1-1 to 1.6, It is possible to create coduction velocity map.

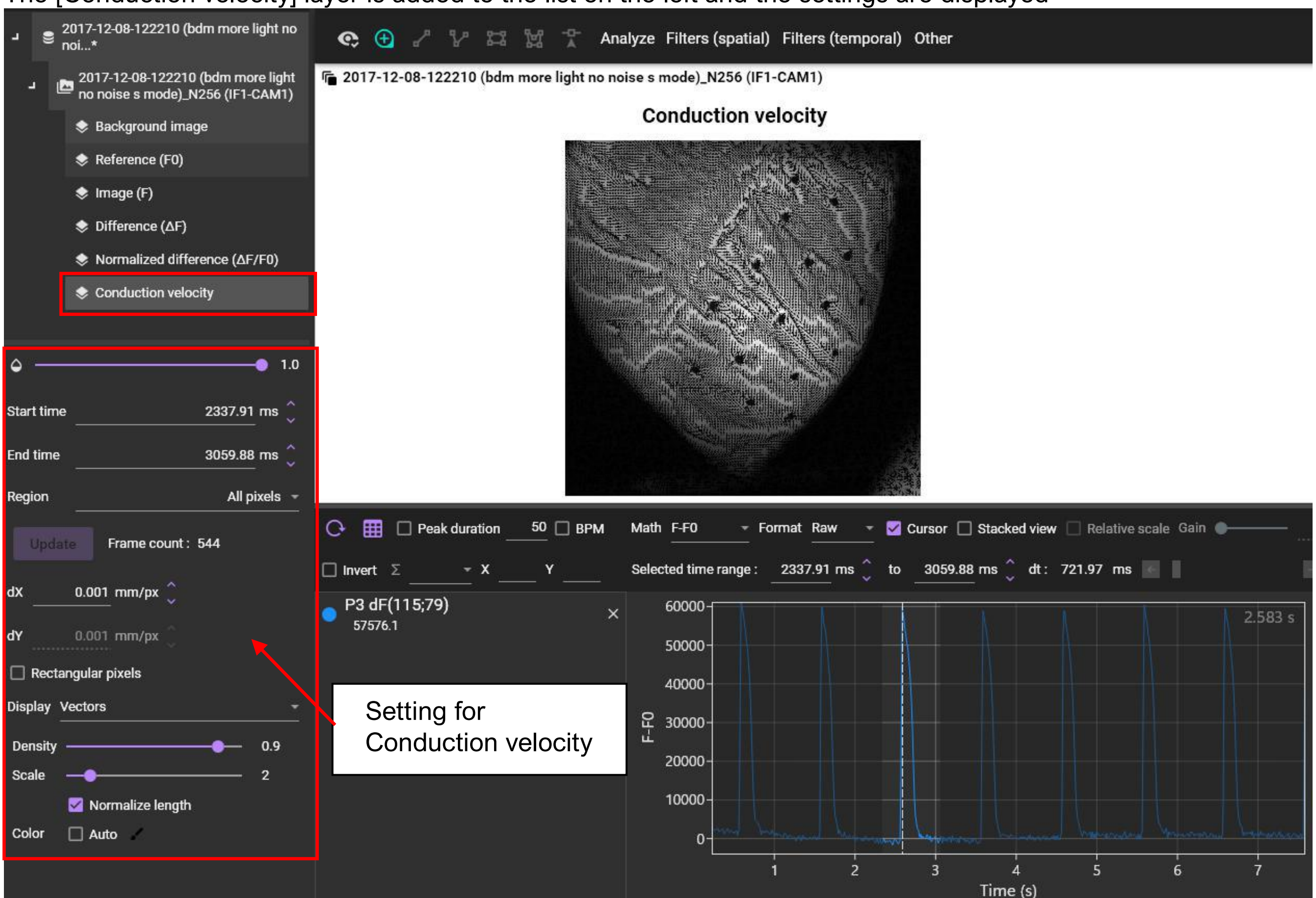
Hold down “Ctrl” key and drag mouse from left to right on waveform to select waveform range.
(Hold “Ctrl” key and drag mouse pointer to left to deselect time range selection for waveform and select all ranges.)



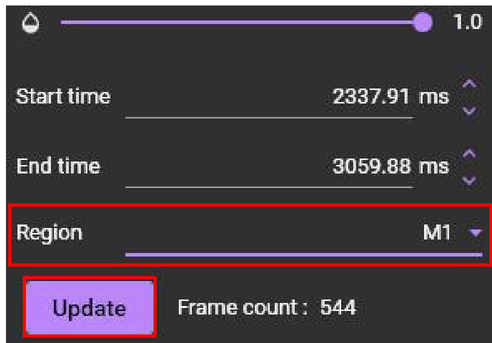
Click [Analyze]-[Add velocity analysis layer].

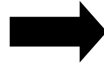


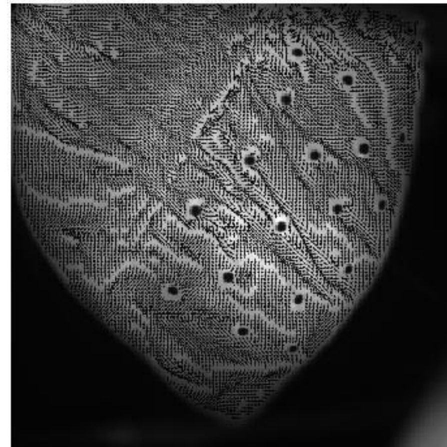
The [Conduction velocity] layer is added to the list on the left and the settings are displayed



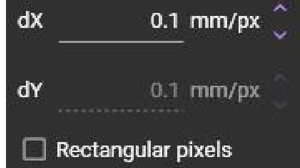
Select mask name or ROI name in [Region] and then, click [Update] button. Conduction velocity is displayed only within the specified range.



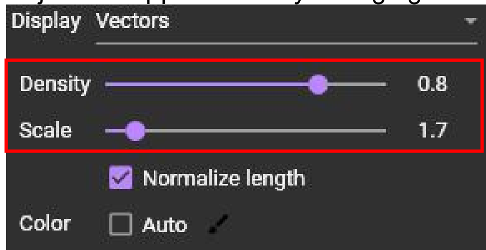


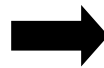


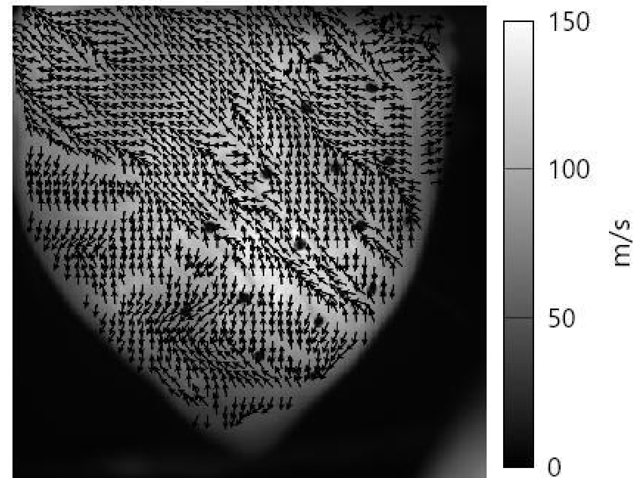
Specify size per pixel in mm.

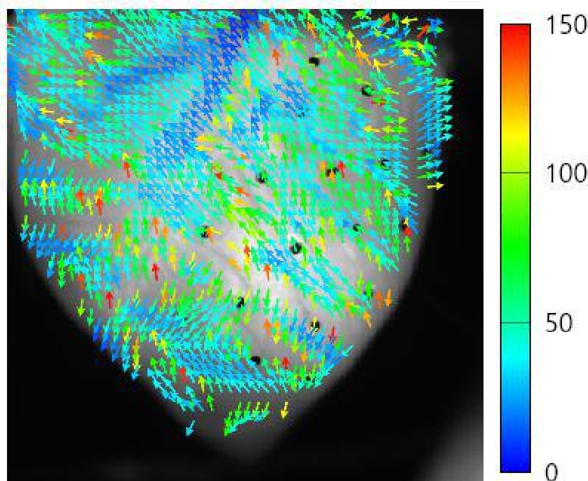


Adjust the appearance by changing the density and scale.

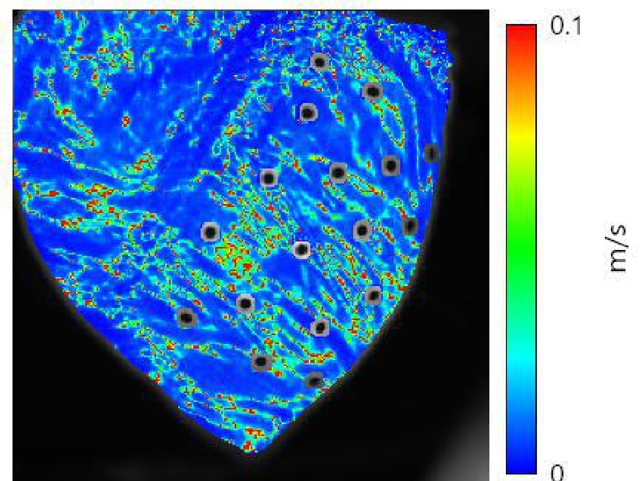






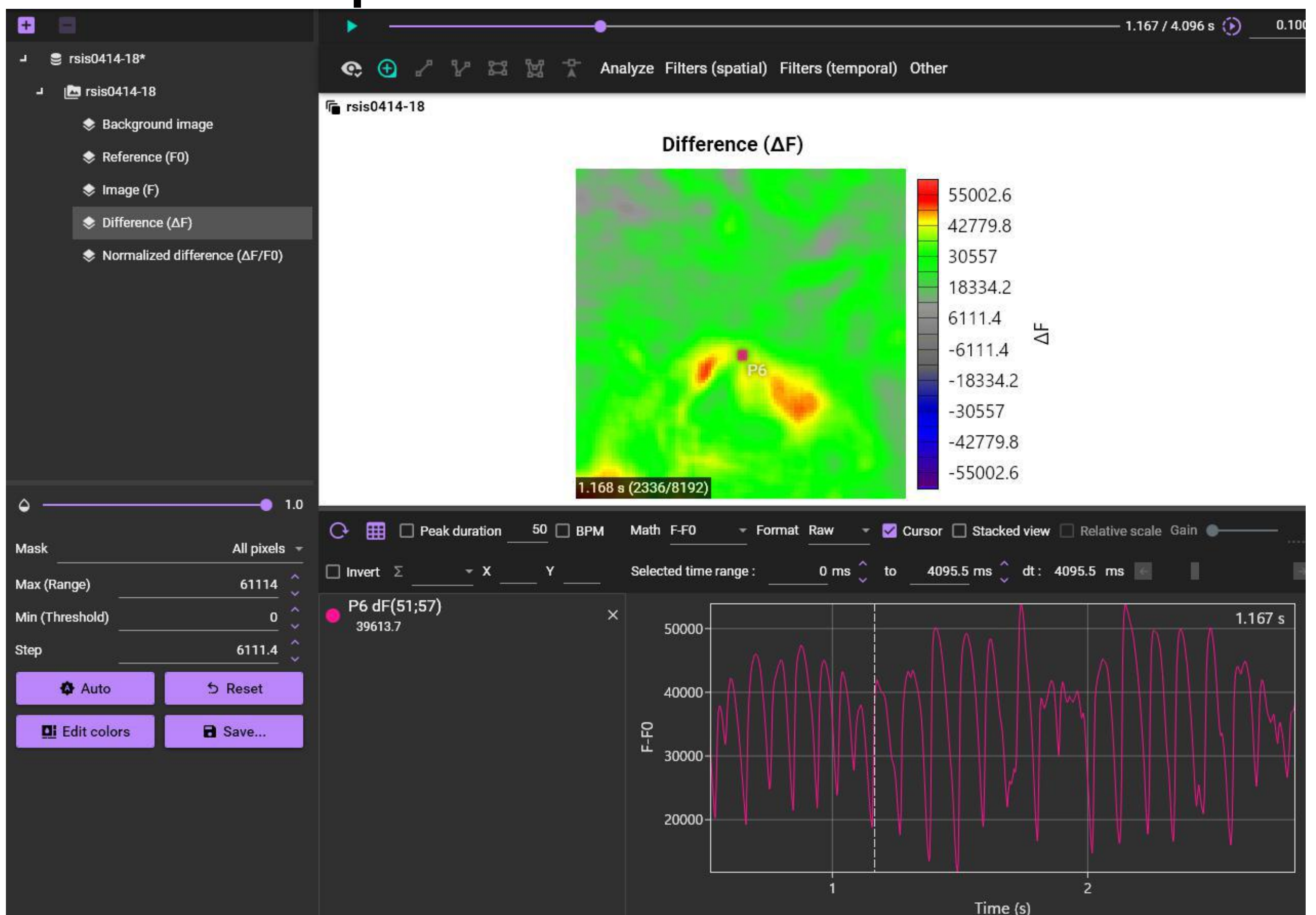


Display with colored arrows

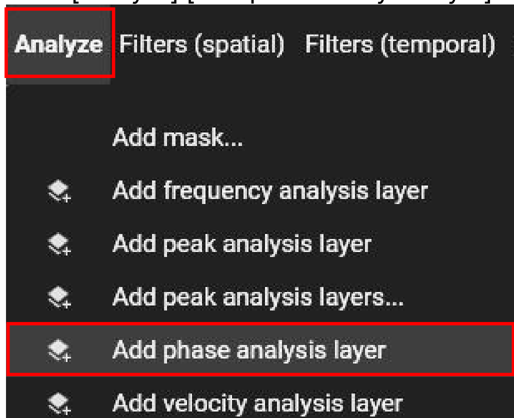


Color display of velocity

2-3. Phase map



Click [Analyze]-[Add phase analysis layer].



The [Phase] layer is added to the list on the left and the settings are displayed when "Phase" is clicked.

Setting for Phase map

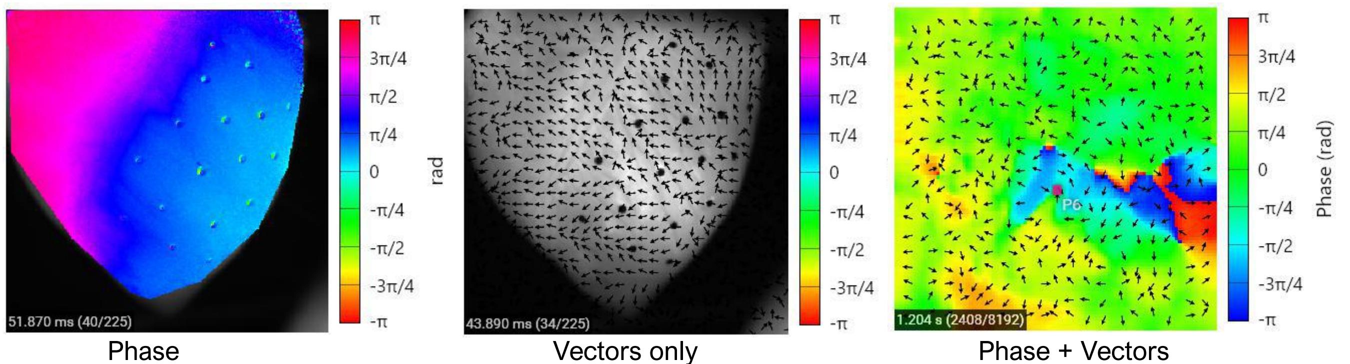
Adjust the appearance by changing the following settings.

Display Phase

Colors ☑ Interpolate

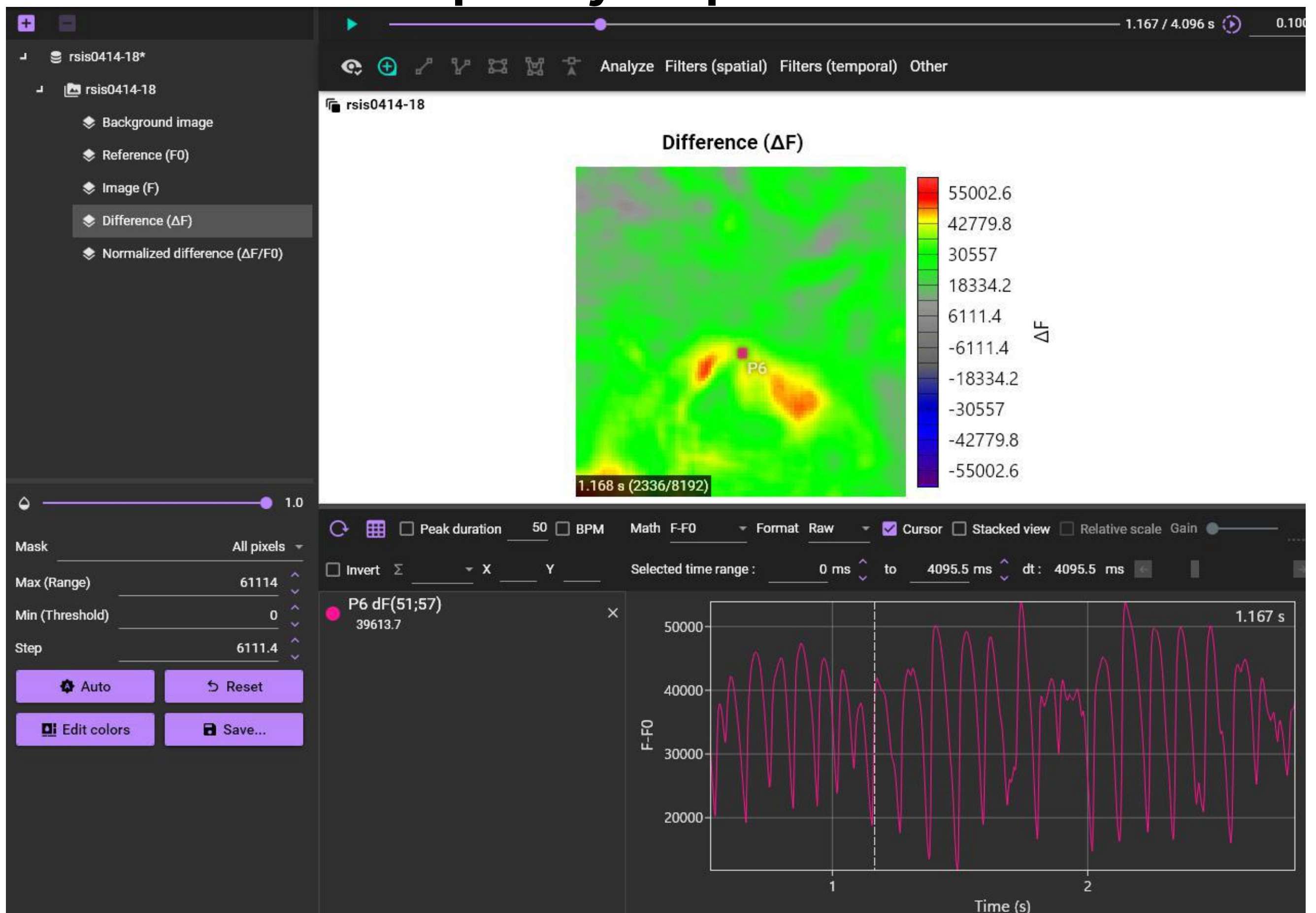
Contours ☐ Draw lines

☐ Color lines

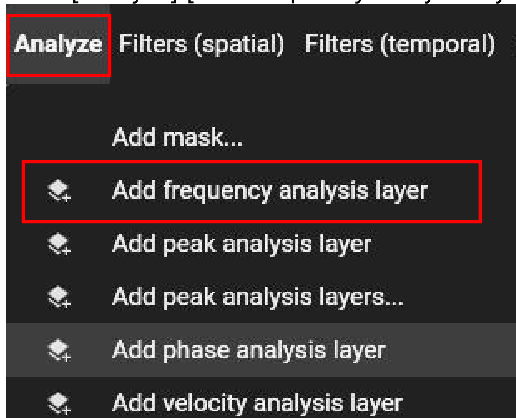


Phase Vectors only Phase + Vectors

2-4. Dominant frequency map



Click [Analyze]-[Add frequency analysis layer].



The [Peak frequency] layer is added to the list on the left and the settings are displayed, when "Peak frequency" is clicked.

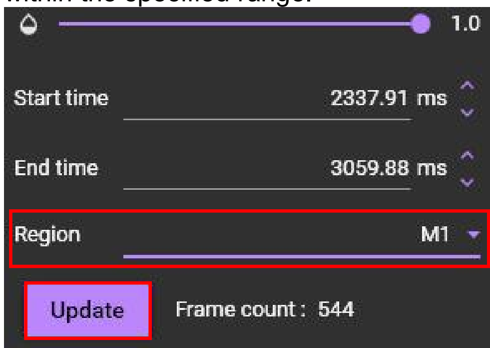
Setting for Peak frequency

Right-click on the color bar and select [Edit colors] to change the color map.

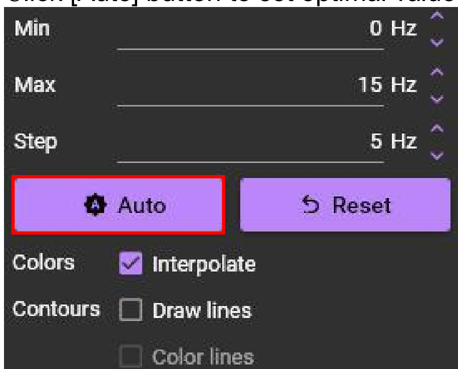
Right click

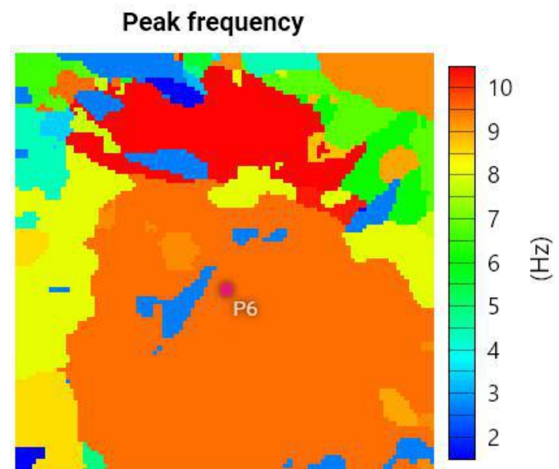
Edit colors...

Select mask name or ROI name in [Region] and then, click [Update] button. Peak frequency map is displayed only within the specified range.

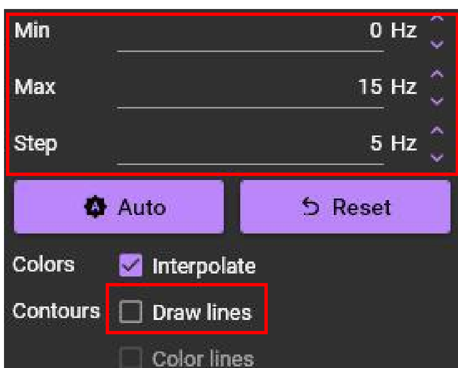


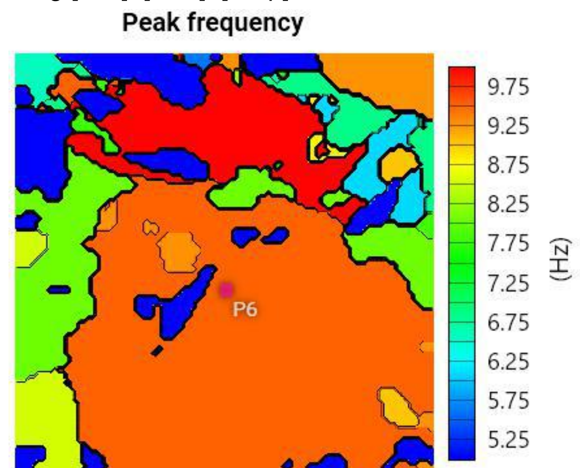
Click [Auto] button to set optimal values.





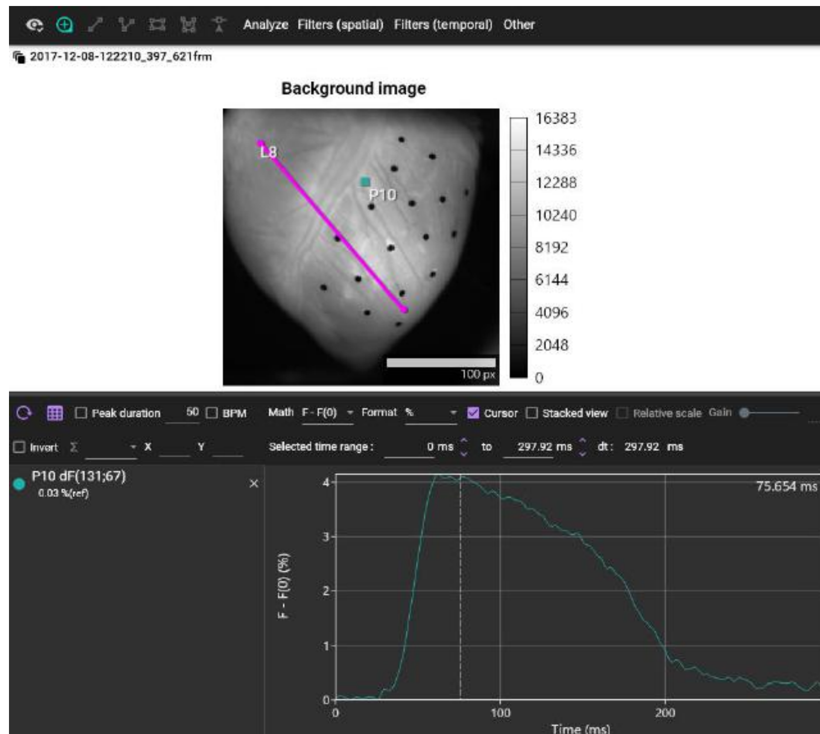
Change map appearance by checking [Draw lines] and adjusting [Min], [Max], [Step].

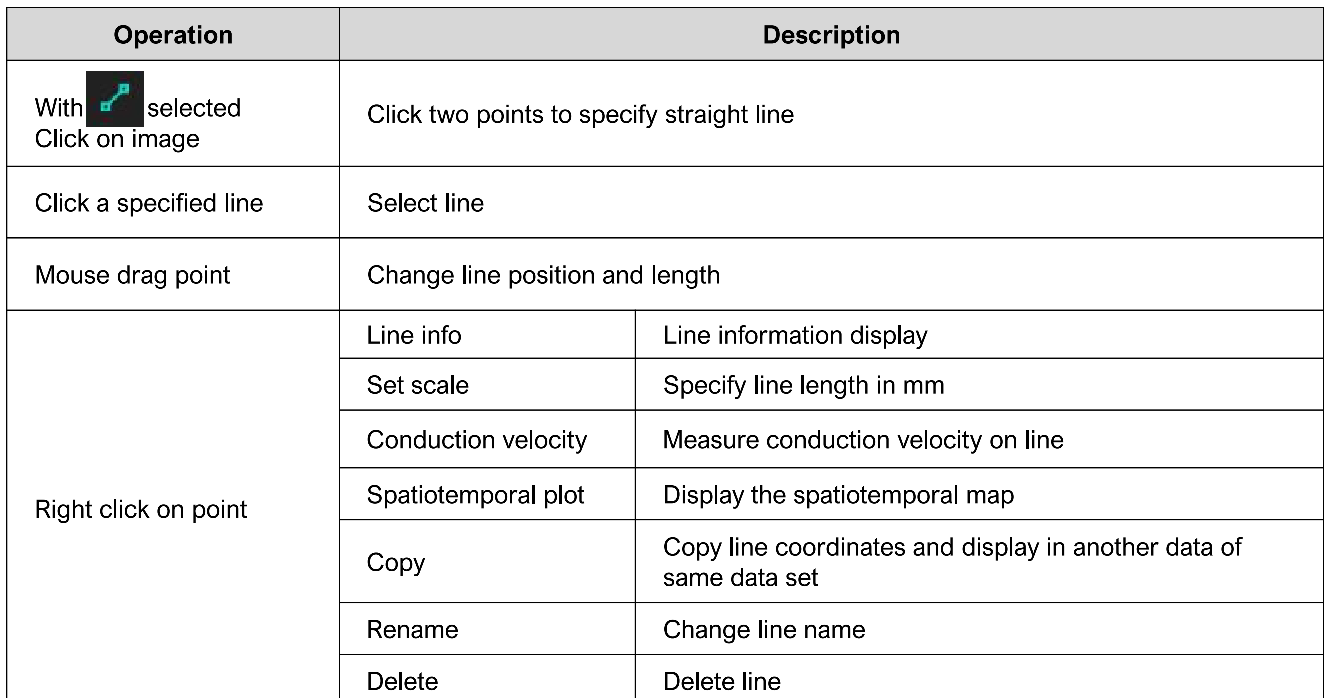




2-5. Conduction velocity on straight line

With "Add line" selected, click two points on image and specify straight line.

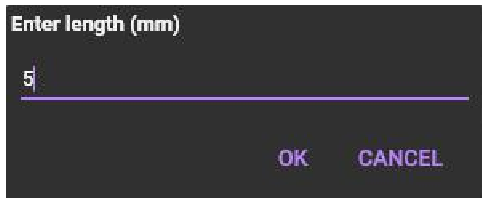




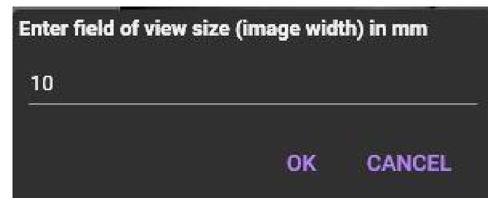
Operation	Description	
With [icon] selected Click on image	Click two points to specify straight line	
Click a specified line	Select line	
Mouse drag point	Change line position and length	
Right click on point	Line info	Line information display
	Set scale	Specify line length in mm
	Conduction velocity	Measure conduction velocity on line
	Spatiotemporal plot	Display the spatiotemporal map
	Copy	Copy line coordinates and display in another data of same data set
	Rename	Change line name
	Delete	Delete line

(a) The scale must be set in advance. There are two ways.

(1) Right-click on line and click [Set scale]. Enter length of straight line in mm.
(2) Right-click on image and click [Image scale]-[Set scale]. Enter horizontal length of image in mm

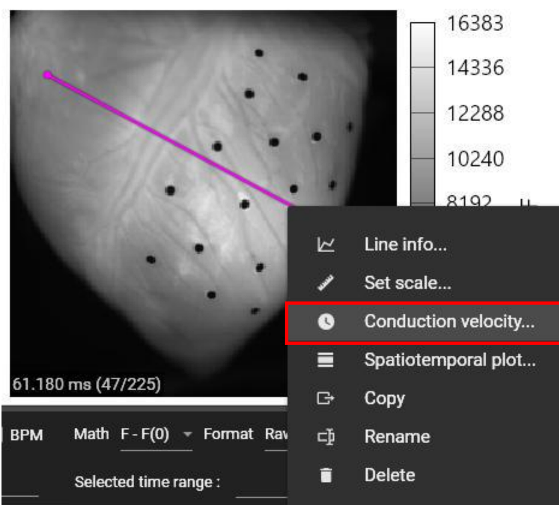


(1) Enter length of straight line in mm.

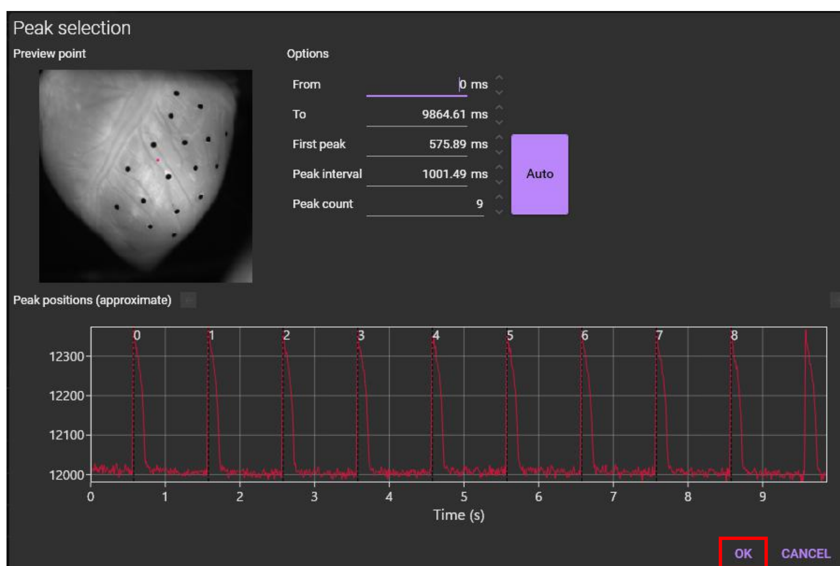


(2) Enter image width in mm.

(b) Right-click on line and click [Conduction velocity].



(c) The [Peak selection] screen is displayed. Peaks are automatically detected and each peak is numbered. Check the displayed settings and click the [OK] button.



(d) The conduction velocities of multiple peaks (action potentials) are calculated and displayed.

Coordinates of start point

Coordinates of end point

Conduction velocity from P1(207,155) to P2(19,27)

Distance : 88.8429 mm

Distance between two points

Average velocity : 1.42397 m/s

Average speed of multiple peaks

	Time 1 (ms)	Time 2 (ms)	Time difference (ms)	Conduction velocity (m/s)
AP0	940.183	1002.38	62.1933	1.4285
AP1	2941.57	3004.57	62.998	1.41025
AP2	4941.71	5003.66	61.9546	1.434
AP3	6942.63	7005.94	63.308	1.40334
AP4	8942.92	9005.1	62.1775	1.42886
AP5	10941.1	11003.3	62.2155	1.42799
AP6	12942.8	13003.9	61.0565	1.45509
AP7	14941.9	15004.2	62.3286	1.4254
AP8	16942.6	17006	63.3566	1.40227

SAVE... CLOSE

Peak number assigned on the Peak selection screen

Peak time of start point

Peak time of end point

Time difference between peak of start point and peak of the end point

Conduction velocity

Output values to CSV file

Close screen

Example of CSV file

Date created	2020/07/07 15:39:57			
Source data nar	2017-12-08-122210 (bdm more light no noise s mode)_N256 (IF1-CAM1)			
Source data pat				
P1	X:207 Y:155			
P2	X:19 Y:27			
Distance (mm)	88.8429311			
Average velocity	1.42396125			
Name	Time 1 (ms)	Time 2 (ms)	Time difference (ms)	CV (m/s)
AP0	940.1833637	1002.376699	62.19333534	1.42849601
AP1	2941.56714	3004.565097	62.99795658	1.410251
AP2	4941.710159	5003.664777	61.95461738	1.434000016
AP3	6942.633474	7005.941476	63.30800204	1.40334442
AP4	8942.920289	9005.097791	62.17750201	1.42885977
AP5	10941.11235	11003.32786	62.21550201	1.42798705
AP6	12942.82892	13003.88542	61.05650197	1.4550937
AP7	14941.88548	15004.21412	62.32863837	1.42539503
AP8	16942.6378	17005.99439	63.3565982	1.40226801

2-6. Save image

You can save selected frame in image format. The supported image formats are PNG, BMP and JPEG.
Right-click on each layer image and select "Export figure" from the displayed menu.