Kairosight 3.0 Instruction Manual

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Installing Anaconda and Launching KairoSight 3.0

1. First you will need to install Anaconda, which can be found here or if you have an older operating system here.
2. Select "Just Me (Recommended)" when prompted.
3. Clone or download the repository (save the contents to a location that is easy to remember/navigate to).
   a. To find, scroll to the top of this page > locate "Code" (in green) > select the drop-down > locate "Download ZIP" > select "Download ZIP"
   b. Note: If you download the repository, unzip the contents of the zip file (your repository is the folder 'KairoSight-3.0-main')
4. Navigate to your computers search bar and type "Anaconda Prompt"
5. Select the Anaconda Prompt
6. Type 'cd', press the space bar, and then type the directory where you cloned/downloaded the repository (e.g., "OneDrive\Documents\GitHub\KairoSight-3.0-main")
   ** Be sure to include the file name at the end of the directory file path **
   a. Note: you can copy the file path by finding the directory in your folders, right clicking, and pasting the file path into the anaconda prompt
7. Press 'Enter' on your keyboard
8. Enter the following command: conda env create -f kairosight_env.yml
9. Press 'Enter' on your keyboard
10. When Anaconda has finished installing the environment it should instruct you to run step 11
11. Type 'conda activate kairosight_3-0'
12. Press 'Enter' on your keyboard
13. When anaconda has finished this step, close the 'Anaconda Prompt'
14. Navigate to your computers search bar and type 'Anaconda Navigator'
15. When the application finishes opening, navigate to the drop-down next to 'Application'
    a. Note: the drop-down will most likely say 'base(root)'
16. Switch the drop-down to 'kairosight_3-0'

17. In the save 'Anaconda Navigator' window, find the "Spyder" application
18. Launch the "Spyder" application
19. In the top menu select: Tools -> Preferences
20. Select "IPython Console" on the left hand menu
21. Select the "Graphics" tab and make sure the 'Graphics backend' is set to Qt5
22. Select "Apply" to save any changes, and select "OK" to close the window
23. Navigate to: File -> Open -> 'location of KairoSight-3.0-main' -> src (inside your KairoSight-3.0-main folder)
24. Select the 'kairosight_retro.py' file
25. When loaded in spyder, select the green play button (in the top menu)
26. KairoSight should now be up and running

Running KairoSight

1. Press “Select Directory” and choose folder with desired files
2. Select desired folder and press “Select Folder”
3. Select desired file from list and press “Load”
4. Input frame rate
5. Select image type, either “Voltage” or “Calcium”
    a. If a dual image, select the type you want to analyze
6. Check “Crop” if cropping is desired
7. Select “Save Properties”
    a. If you wish to update the cropped area select “Update Properties” and repeat steps 6-7.
8. Select draw mask area and click around the heart to remove the background.
9. Press “Enter” on your keyboard
    a. If the draw mask command causes the program to freeze/stop responding:
        i. Tools ➔ Preferences ➔ iPython console ➔ Graphics ➔ Graphics Backend ➔ Switch Graphics Backend to Automatic
10. Process your image to your specifications, select “Prepare”
    a. See processing section for more information on these settings
11. Move the slider, underneath the image, to see activation through the heart

12. Click “Select Signal” and select first point of activation
13. Click “Select Signal” again and select where the heart is activating a few frames after the initial activation
14. Click “Select Signal” and select the last point of activation
15. To analyze other images, repeat steps, starting at step 3
16. If you wish to redo your signal selection, select “Reset Signal” and repeats steps 9-13.

Running KairoSight with EC Coupling

Follow the steps below if your voltage and calcium images are two separate files:

- If the desired object for imaging is shifted between the calcium and voltage images, follow step 1, otherwise skip to step 2.

1. Register your calcium and voltage images in ImageJ
2. Press “Select Directory” and choose directory with desired calcium file
3. Select desired calcium file and press “Load”
4. Input frame rate
5. Set image type to “Calcium”
6. Check “EC Coupling”
7. Crop image, and keep a note of the size of the cropped image
8. Select draw mask area and click around the heart to remove the background.
9. Press “Enter” on your keyboard
    a. If the draw mask command causes the program to freeze/stop responding:
        i. Tools ➔ Preferences ➔ iPython console ➔ Graphics ➔ Graphics Backend ➔ Switch Graphics Backend to Automatic
10. Process your image to your specifications, select “Prepare”
    a. See processing section for more information on these settings
    b. Note: This automatically saves a .npy file of the calcium data to the scr folder in KairoSight
11. Navigate back to your selected directory and select desired voltage file and press “Load”
12. Input frame rate, should be the same value used for calcium

13. Set image type to “Voltage”
14. Check “EC Coupling”
15. Crop image to have the same height and width used for calcium
16. Select “Use Saved Mask” to use the same mask that was used for calcium
17. Press “Enter” on your keyboard
18. Process your image to the same specifications as calcium, select “Prepare”
    a. See processing section for more information on these settings
    b. Note: This automatically saves a .npy file of the voltage data to the scr folder in KairoSight
19. Click “Select Signal” and select first point of activation, identified using the slider
20. Click “Select Signal” and select last point of activation
21. Navigate to the **EC Coupling Analysis** section of the manual

Follow the steps below if your voltage and calcium images are one file:

1. Press “Select Directory” and choose directory with desired file
2. Select desired calcium file and press “Load”
3. Input frame rate
4. Set image type to “Calcium”
5. Check “EC Coupling”
6. Crop image, cutting the y-axis in half and keeping only the calcium side of the image.
    a. Keep a note of the height of the cropped image
7. Select draw mask area and click around the heart to remove the background.
8. Press “Enter” on your keyboard
    a. If the draw mask command causes the program to freeze/stop responding:
        i. Tools ➔ Preferences ➔ iPython console ➔ Graphics ➔ Graphics Backend ➔ Switch Graphics Backend to Automatic
9. Process your image to your specifications, select “Prepare”
    a. See processing section for more information on these settings
    b. Note: This automatically saves a .npy file of the calcium data to the scr folder in KairoSight

10. Select “Update Image Properties”
11. Set image type to “Voltage”
12. Check “EC Coupling”
13. Crop image using the other half of the y-axis, keeping only the voltage side of the image.
    a. Use the same height as your calcium image.
14. Press “Enter” on your keyboard
    a. If the draw mask command causes the program to freeze/stop responding:
        i. Tools ➔ Preferences ➔ iPython console ➔ Graphics ➔ Graphics Backend ➔ Switch Graphics Backend to Automatic
15. Process your image to the same specifications as calcium, select “Prepare”
    a. See processing section for more information on these settings
    b. Note: This automatically saves a .npy file of the calcium data to the scr folder in KairoSight
16. Click “Select Signal” and select first point of activation, identified using the slider
17. Click “Select Signal” and select last point of activation
18. Navigate to the **EC Coupling Analysis** section of the manual

Processing

Binning

Binning the image averages the pixels around each other, reducing the number of pixels in the whole image. Accordingly, follow these guidelines:

1. Alternans: No binning
2. Activation/Conduction Velocity: Any binning

Filtering

Filtering the image reduces the amount of noise from the desired signal. Accordingly, follow these guidelines:

1. Start at ~100 and adjust from there. A smaller filtering value results in more filtering.

Drift

Removing drift from the image removes any upward or downward sloping trend. Accordingly, follow these guidelines.

1. Increasing the order helps level the signal more.
2. 1st order is typically enough to remove any upward or downward drift.
3. Use higher orders if the smaller order doesn't remove all desired drift.

Normalizing

Normalizing the signal sets the min and max from 0 to 1.

Interpolation

Interpolating the image averages points on the signal, allowing the program to find various parts of a noisy signal easily.

1. Note: Always use the least amount of interpolation necessary.

Standard Analysis Options

Activation

To map the activation time across the heart, follow these steps:

1. In the drop-down menu under **Analysis**, select “Activation”
2. In “Start Time”, input the time a beat begins, based on the first activation point
3. In “End Time”, input the time the same desired beat ends, based on the last activation point
4. Select “Map”
5. If the bounds of the default map should be change
    1. In the **Min** and **Max** text boxes, next to “Modify Colorbar”, enter bounds for the colorbar (if the default is not a valid representation of the data)
    2. Select “Modify Colorbar”

APD/CaD

To map the action potential duration or calcium transient duration across the heart, follow these steps:

1. In the drop-down menu under **Analysis**, select “APD” or “CaD”
2. In “Start Time”, input the time the desired beat begins, based on the first activation point
3. In “End Time”, input the time the same desired beat ends, based on the last activation point
4. In “% APD_1" or “% CaD_1”, input the duration percentage desired
5. Select “Map”
6. If there are holes in the maps, adjust the interpolation and repeat step 5.
7. If the bounds of the default map should be change
    1. In the **Min** and **Max** text boxes, next to “Modify Colorbar”, enter bounds for the colorbar (if the default is not a valid representation of the data)
    2. Select “Modify Colorbar”

APD/CaD Ensemble

To obtain the values for multiple APD or CaD traces, follow these steps:

1. In the drop-down menu under **Analysis,** select “APD Ensemble” or “CaD Ensemble”
2. In “Start Time”, input the time the desired beat begins, based on the first activation point
3. In “End Time”, input the time the last desired beat ends, based on the last activation point
4. In “Max Amp”, input the maximum amplitude threshold desired.
    a. Note, it should be just below the shortest APD/CaD amplitude.
5. In “% APD_1" or “% CaD_1”, input the duration percentage desired
6. In “% APD_2" or “% CaD_2”, input the second duration percentage desired
7. Select “Map”
8. When the “Save File” is opened, navigate to the desired folder, and give the file its desired name in “File Name”
9. Select “Save”
10. Open file from saved location to evaluate saved parameters

Dynamic Alternan

1. In the drop-down menu under **Analysis**, select “Dynamic Alternan”
2. Identify two consecutive beats that make up an alternan
3. In “Start Time”, input the time the desired alternan begins, based on the first activation point
4. In “End Time”, input the time the same desired alternan ends, based on the last activation point
5. In “Peak Coeff.”, input a peak coefficient of 4
6. Select “Map”
7. If there are holes in the maps, adjust the interpolation and the peak coefficient then select “Map” again.
    a. When interpolation increases, peak coefficient should also increase, and vice versa.

8. This returns a map of the AP or CaT alternan coefficient
9. If the bounds of the default map should be change
    a. In the **Min** and **Max** text boxes, next to “Modify Colorbar”, enter bounds for the colorbar (if the default is not a valid representation of the data)
    b. Select “Modify Colorbar”

Manual Alternan

1. In the drop-down menu under **Analysis**, select “Manual Alternan”
2. Identify two consecutive beats that make up an alternan
3. In “Start Time”, input the time the desired alternan begins, based on the first activation point
4. In “End Time”, input the time the desired alternan ends, based on the last activation point
5. In “Peak Coefficient”, input a peak coefficient of 4
6. If there are holes in the maps, adjust the interpolation and the peak coefficient and map again. When interpolation increases, peak coefficient should also increase, and vice versa.
7. Use the map to determine the optimal level of repolarization from a local minima map (use it to determine the Z value for fixed alternan analysis).
    a. To do this “click” on the area of the image with the highest value in the color bar.
    b. The Z value from this selection will be populated in the command window of Spyder.
        i. Note: This Z value can then be used for the %APD/CaD when the “Fixed Alternan” option is selected from the drop down.
        ii. Also Note: “Z” is the percent repolarization or percent reuptake
8. If the bounds of the default map should be change
    a. In the **Min** and **Max** text boxes, next to “Modify Colorbar”, enter bounds for the colorbar (if the default is not a valid representation of the data)
    b. Select “Modify Colorbar”

Fixed APD Alternan

1. In the drop-down menu under **Analysis**, select “Fixed APD Alternan”
2. Identify two consecutive beats that make up an alternan
3. In “Alt. 1 Start Time”, input the time the first beat of the alternan begins
4. In “Alt. 1 End Time”, input the time the first beat of the alternan ends
5. In “Alt. 2 Start Time”, input the time the second beat of the alternan begins
6. In “Alt. 2 End Time”, input the time the second beat of the alternan ends
7. In “% APD_1” either:
    a. Input the Z value found with manual alternan. This will find the optimal percent repolarization or reuptake for each beat in the alternan.
        i. Allowing you to find the optimal %APD duration.
    b. Input 50
        i. Allowing you to find %APD at 50% repolarization.
8. Select “Map”
9. If the bounds of the default map should be change
    a. In the **Min** and **Max** text boxes, next to “Modify Colorbar”, enter bounds for the colorbar (if the default is not a valid representation of the data)
    b. Select “Modify Colorbar”

Conduction Velocity

1. Follow steps under the **Activation** section
2. Under **Conduction Velocity**, in the text box next to “Scale (px/cm)” enter the scale in terms of pixels/cm
3. Under **Single Vector**, in the text box next to “No of Vectors” input the number of vectors desired
4. Select “Plot”
5. Click the area of the heart that is first activated, as denoted by the colormap
6. Click the area of the heart that is activation last
7. For each additional vector, repeat steps 5-6
8. When finished selecting vectors, press enter (on the keyboard)
9. The conduction velocity will be shown next to its corresponding vector

S1-S2

1. In the drop-down menu under **Analysis**, select “S1-S2”
2. In “Alt. 1 Start Time”, input the time the beat before the second to last S1 beat begins
3. In “Alt. 1 End Time”, input the time the beat before the second to last S1 beat ends
4. In “Alt. 2 Start Time”, input the time the last S1 beat begins
5. In “Alt. 2 End Time”, input the time the S2 beat ends
    a. Note, your alt. 2 start and end time should encompass two beats (the last S1 beat and the following S2 beat).
6. In “Max Amp”, input the maximum amplitude threshold desired.
    a. Note, it should be just below the shortest APD/CaD amplitude.
7. In “% APD_1” or “% CaD_1”, input the duration percentage desired
8. In “% APD_2” or “% CaD_2”, input a peak coefficient of 4
9. Select “Map”
10. If there are holes in the maps, adjust the interpolation and the peak coefficient then select “Map” again.
    a. When interpolation increases, peak coefficient should also increase, and vice versa.
11. If the bounds of the default map should be change
    a. In the **Min** and **Max** text boxes, next to “Modify Colorbar”, enter bounds for the colorbar (if the default is not a valid representation of the data)
    b. Select “Modify Colorbar”

Signal to Noise Ratio

1. In the drop-down menu under **Analysis**, select “SNR”
2. In “Start Time”, input the time a beat begins, based on the first activation point
3. In “End Time”, input the time a beat’s activation is complete
4. Select “Map”
5. If the bounds of the default map should be change

1. In the **Min** and **Max** text boxes, next to “Modify Colorbar”, enter bounds for the colorbar (if the default is not a valid representation of the data)
2. Select “Modify Colorbar”

EC Coupling Analysis Options

Max Slope EC Coupling

1. In the drop-down menu under **Analysis**, select “Activation EC”
2. In “Start Time”, input the time a beat begins, based on the first activation point
3. In “End Time”, input the time a beat’s activation is complete
4. Select “Map”
5. If the bounds of the default map should be change
    1. In the **Min** and **Max** text boxes, next to “Modify Colorbar”, enter bounds for the colorbar (if the default is not a valid representation of the data)
    2. Select “Modify Colorbar”

Repolarization EC Coupling

1. In the drop-down menu under **Analysis**, select “Repolarization …. EC Latency”
2. In “Start Time”, input the time a beat begins, based on the first activation point
3. In “End Time”, input the time a beat end, based on the last activation point
4. In “% APD_1”, input the percentage of repolarization you are interested in
5. Select “Map”
6. If the bounds of the default map should be change
    1. In the **Min** and **Max** text boxes, next to “Modify Colorbar”, enter bounds for the colorbar (if the default is not a valid representation of the data)
    2. Select “Modify Colorbar”

Region of Interest Analysis

ROI for APD/CAD

1. Generate an SNR map following the instructions under the SNR section.
2. Generate the map analysis that you want to complete the regional analysis on (ex. Activation, APD, S1S2…)
    1. All .csv files of the maps generated will be automatically saved into “Saved Data Maps" in the designated file name. This can be found in the ‘src’ folder in kairosight.
        1. NOTE: If you are analyzing multiple images in one big file, and wish to save all the map data, make sure to make a copy and either put it into a folder there or move to a separate location once you have completed the ROI analysis of the data. You have to move it because once you generate the next SNR and other analysis maps they will overwrite the ones currently saved there.
3. Select the “Region of Interest Analysis” Button
4. Input how many regions you want to analyze and click ok
    1. NOTE: The SNR image will be generated on a scale of 0 - 70
5. Start selecting the areas you wish to analyze.
    1. Couple of important notes
        1. Make sure to have your first point and last point of a region be are the same spot or close enough to each other to enclose the area
        2. If you are doing multiple regions in the analysis, make sure to click enter after completing one region or else it will not register.
        3. To get good results, avoid any noise shown in the SNR and leave a good distance in between the noise and the region areas.
6. When you are finished with the last region and press enter
    1. NOTE: Once all the regions are selected, they will automatically be saved in the ‘Saved Region Maps' under the file name in the src folder in kairosight.
7. When you press enter on the last region, a window will appear with the saved .csv files from the “Saved Data Maps” folder. Select the .csv file of the map you wish to analyze and click open

8. It will open another window where you can input the name of the file if you wish to change it from its original name. If you do not, just select “ok” and it will generate the mean, median, sd, n values of the regions.
    1. NOTE: The results will then be automatically saved into “ROI Analysis” under its file name. The results will be displayed under a folder will the map analysis separated into the regions selected and another .csv folder called “all_results”
        1. For example, if APD 30,50,70 was analyzed then there would be three folders named “apd 30.0, apd 50.0, and apd 70.0” with those specific map results of the regions as well as a .csv file called “all_results” that as all the results together
9. When repeating the ROI when another image from the same file, a window will appear asking if you wish to use the same regions or wish to redo them.
    1. If you select “yes”, it will ask how many regions did you want to use from the saved regions
        1. If you are using all the regions saved, just input the numbers of the regions you selected and continue from step 8
            1. If you have four regions saved, type out 1,2,3,4
                1. If you are only wanting one or two of the regions to be analyzed, input the number of the regions (1,3,4) and click okay then continue from step 8.
            2. The regions are numbered in order as they were created (i.e. the first region selected is 1, and the third region selected is 3…).
    2. If you select “no”, you will repeat the procedure from step 4.
10. When finished with the data you had wanted to analyze, if you only want to have the mean, median, sd, or n values saved then click “Individual ROI Results”
    1. Enter the name of the result you wish to have and click ok
    2. The results will be saved in an .csv file in its designated file name folder under the ROI Analysis folder in src.
    3. The .csv file will be named “(result selected) _individual_results”

1. EX) if you only wanted the mean, the .csv file would be named "mean_individual_results"

ROI for EC Coupling

1. First start by loading the voltage signals and generating an SNR file on 15x15 bin with scale from 0 – 70.
2. Go to Kairosight -> Src -> Saved Dated Maps -> Name of file -> Copy the "snr.csv" and move to another folder and name it "snrAP".
3. Then load the calcium signals and generate an SNR file using the same mask from the voltage on 15x15 bin with scale 0 – 70.
4. Go to Kairosight -> src -> Saved Data Maps -> Name of file -> Copy the "snr.csv" move to the same folder where "snrAP" was saved and name it "snrca"
5. Next open a new Console and open "ECcouplingSNR.py".
6. Copy the pathway to the folder that contains "snrAP" and "snrca" and run the file.
    1. A new file named "snr.csv" will be automatically saved to the folder. It is a combined SNR of voltage and calcium.
7. Follow the steps listed under "**Running KairoSight with EC Coupling**" to load the Voltage/Calcium signals using the same mask and crop from the SNR
8. Following the "**Repolarization EC Coupling"** steps, generate Repolarization30, 50, and 70 on a scale of [-5 , 20].
9. Go to Kairosight -> src -> Saved Data Maps -> Name of File -> Copy "Repolarization_ACC30", "Repolarization_ACC50", "Repolarization_ACC70" and paste into the same folder where the snr.csv files are saved.
10. Open "map_post_analysis.py" from the src folder in kairosight.
11. Copy and paste the pathway to the file containing the .csv files generated.
12. Input the File_Id and PCL of the file
    1. Make sure before running the program that there are no existing regions file in the src folder in kairosight or else it will not work as shown in the video.
13. Click run and the snr.csv map will pop up, select four regions wanted.
    1. Couple of important notes
        - Make sure to have your first point and last point of a region be are the same spot or close enough to each other to enclose the area.
        - If you are doing multiple regions in the analysis, make sure to click enter after completing one region or else it will not register.
        - To get good results, avoid any noise shown in the SNR and leave a good distance between the noise and the regional areas.

14. When you press enter on the last region, the four regions and the results will be automatically saved into the src folder in kairosight.
    1. The results can be found in a .csv file called “results”
15. If you are doing the analysis for another PCL just change the PCL number and click run.
16. If you are changing the file; go to the src folder in kairosight, delete the four regions generated (if you wish to save them just copy and move to another folder then delete them from the src folder), and repeat the steps from Step 9.

Troubleshooting

1. If the draw mask command causes the program to freeze/stop responding:
    1. Tools → Preferences → iPython console → Graphics → Graphics Backend → Switch Graphics Backend to Automatic

Notes:

- At high pacing (beats are fused or tend to be fused) --- SNR will be inaccurate, only do SNR with slower pacing
- To increase SNR, increase binning; can be increased to 15x15
- If the dynamic alternan functionality is returning an error, ensure the file used has alternans and try increasing the processing settings on the image.
- You can update the properties to analyze a different part of the image without having to reload the image, you can update properties and change the crop and mask to analyze the desired section of the image.