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Downloading Anaconda and Opening Spyder

1. First you will need to install Anaconda, which can be found **here**.
2. Clone or download the repository
3. On a Windows PC follow steps 4-5, for Mac skip to steps 6-9.
4. Open the Anaconda Prompt and navigate to the directory where you cloned/downloaded the repository (e.g., "cd OneDrive\Documents\GitHub\kairosight-2.0")
5. Enter the following command to setup the Anaconda environment: conda env create -f kairosight_env.yml
6. Open terminal
7. Enter the following command: 'conda create -n kairosight_env.yml'
8. Proceed with 'y'
9. Enter the following command: 'conda activate kairosight_env.yml'
10. Close the Anaconda Prompt
11. Launch Anaconda Navigator and switch to the newly created environment
12. Launch Spyder
13. In the top menu select: Tools -> Preferences
14. Select IPython console on the left-hand menu
15. Select the Graphics tab and make sure the Graphics backend is set to Qt5
16. Open kairosight_retro.py and hit the play button

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”

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 “% APD_1” or “% CaD_1”, input the duration percentage desired
7. In “% APD_2” or “% CaD_2”, input a peak coefficient of 4
8. Select “Map”
9. 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.
10. 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”

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.