Instruction Manual

Improving real-time in vivo molecular monitoring: multi-Gaussian with Laplacian voltammogram fitting enhances the precision of electrochemical aptamer-based sensors

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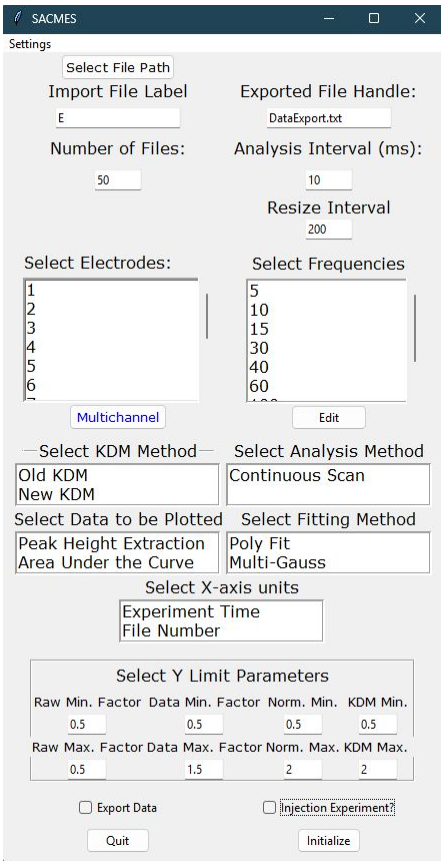
Downloading and Installing SACMES.py

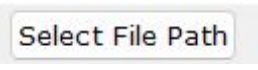
The software can be downloaded from the online GitHub repository which also includes detailed instructions: https://github.com/PlaxcoLab/SACMES_Public

To download the required packages, the program requires a python environment manager, and the instructions are written assuming a conda environment manager. For users employing a different environment manager, the required packages are included in the env.yml file.

Interface of the Input Frame of SACMES.py

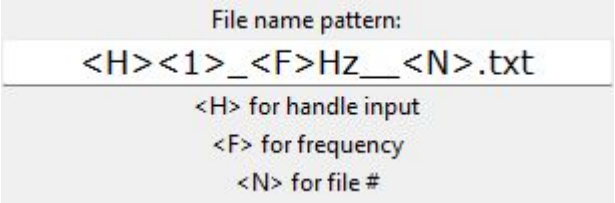


Shown on the left is a screenshot of the input panel of the program, and on the right are example data files for Windows OS.



- Allows for selection of the path to the data folder that contains the voltammograms.

- Text box for specifying the voltammogram file handle, which is “E” here. The file naming pattern follows the following pattern:



- The input file format is as follows:

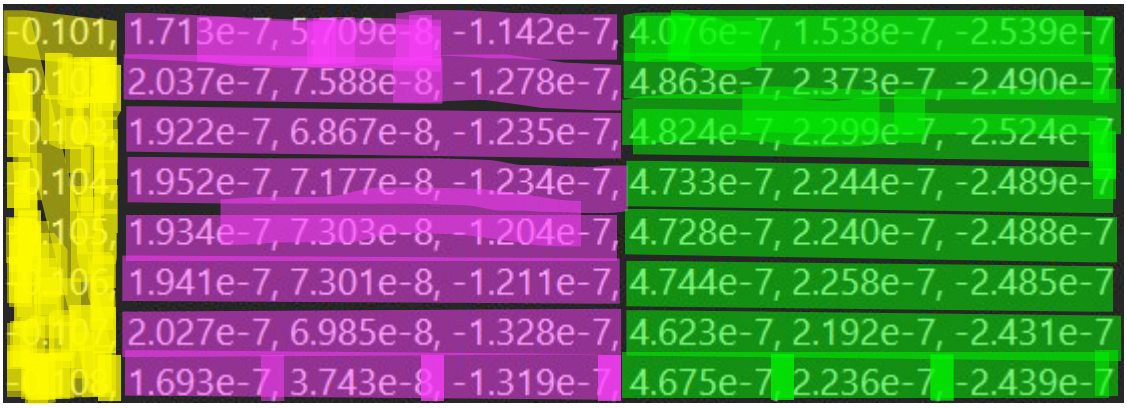
  Column 1: Voltage

  Column 2: Differential Current

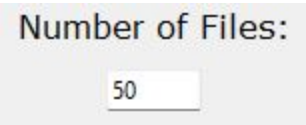
  Column 3: Forward Current

  Column 4: Reverse Current

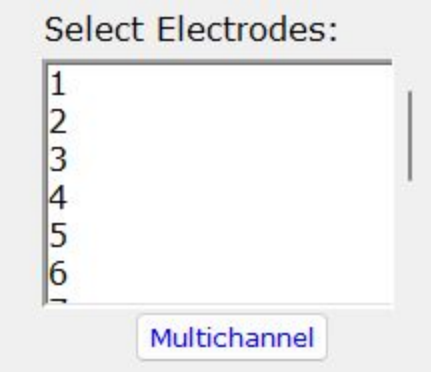
  Columns 2-4 repeat for each additional Electrode.



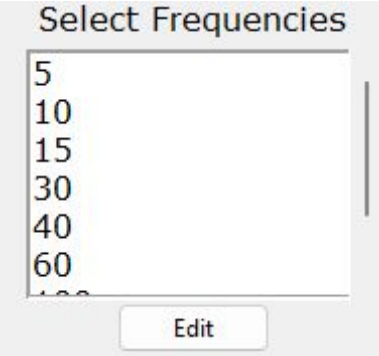
Voltage Currents for Electrode 1 Currents for Electrode 2



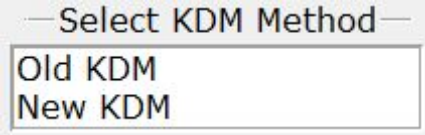
- Text box for specifying the total number of input files.



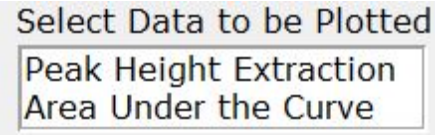
- Selection box for specifying which electrodes are to be analyzed.



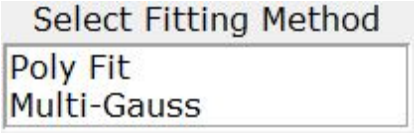
- Selection box for specifying the frequencies to be analyzed. If the frequency is not already on the list, it can be added using the "Edit" button.



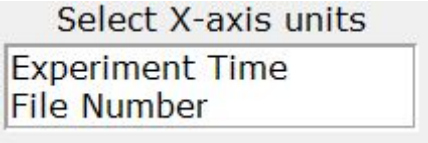
- Selection box for specifying the KDM method to be used (1, 2).



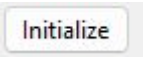
- Selection box for specifying which data are to be plotted in the data visualization frame.
    - Peak Height Extraction: The difference between the maximum value and the minimum value of the fitted voltammogram using the selected fitting method.
    - Area Under Curve: The Riemann Sum with the number of rectangles equaling the number of current points within the fitted voltammogram.



- Selection box for specifying the fitting method to be used.
    - Poly Fit: The original SACMES method, in which the data is first smoothed using Savitzky-Golay filtering and then fitted using polynomial regression of degree 15 (20).
    - Gauss: Multi-Gaussian with Laplacian method, as described in this paper.



- Selection box for specifying the units on the x-axis.
    - Experiment Time: Uses the last modification time of the voltammogram file to determine the time passed since the first file was created.
    - File Number: denotes the x-axis in file numbers.

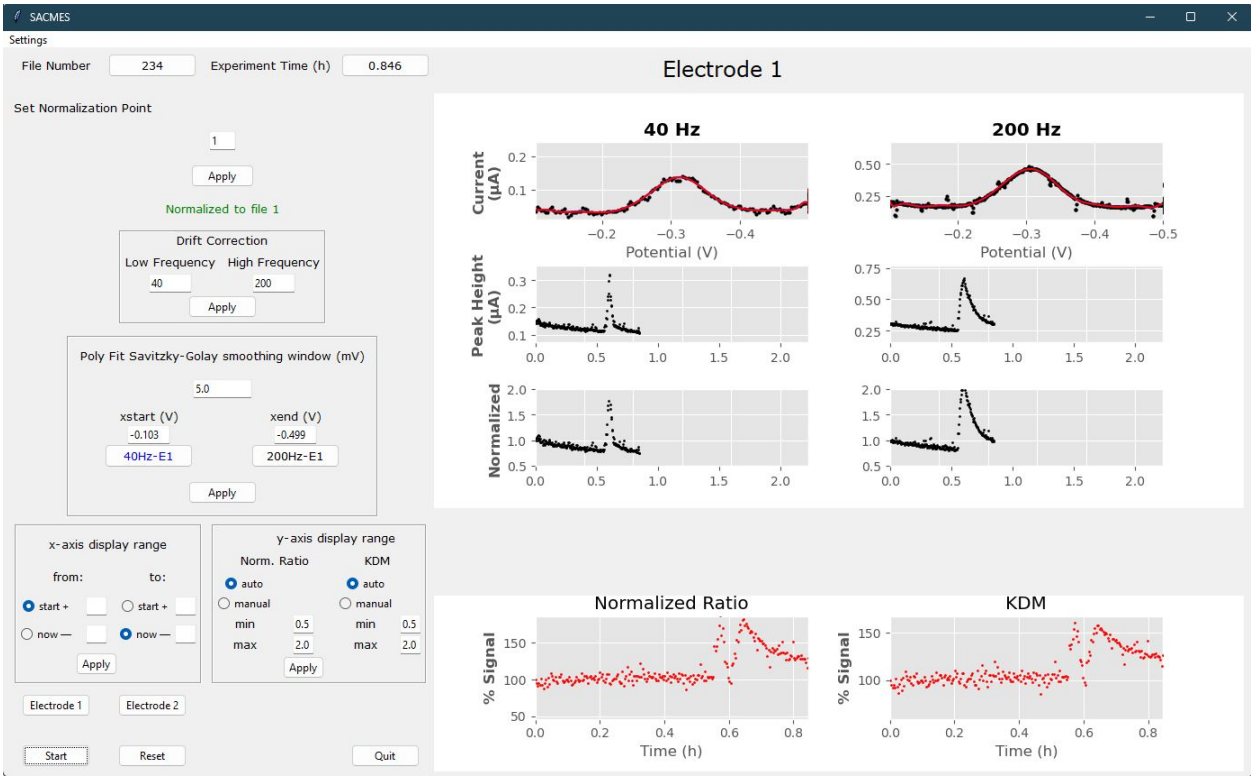


- Initailizes the parameters entered and starts the data manupilation and visualization frame.

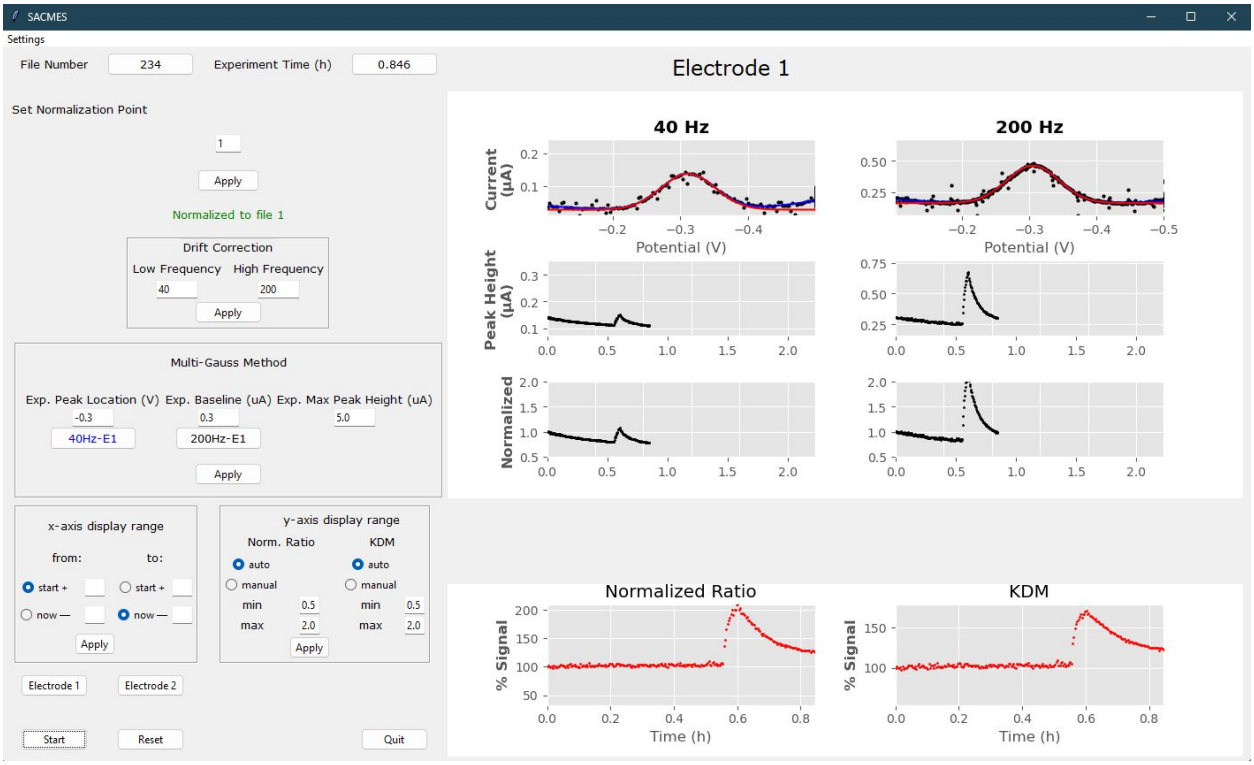
Data Manipulation and Visualization Frame of SACMES.py

The data manipulation and visualization frame are created by clicking on the Initialize button of the input frame. It consists of two parts:

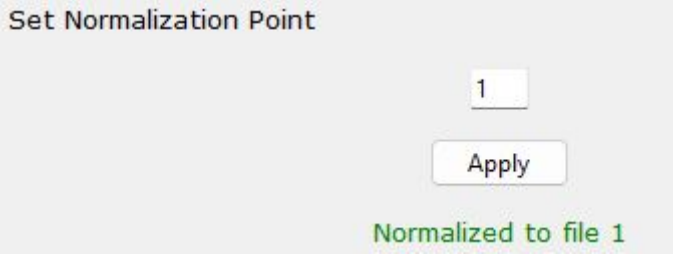
- Data manipulation: On the left side of the frame the user can adjust the parameters associated with data processing.
- Visualization: On the right side of the frame the raw voltammograms are presented, along with the fitted voltammograms, the extracted peak heights, the normalized peak heights, the normalized ratios, and the KDM values.



Shown is the Data Manipulation and Visualization Frame when Poly Fit is selected.

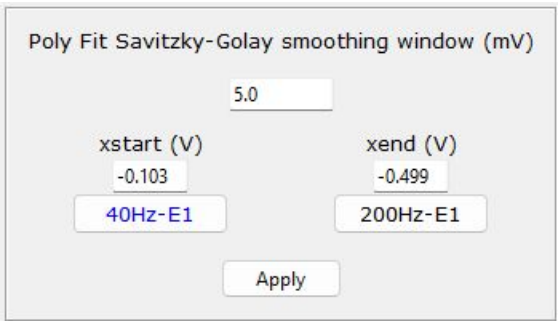


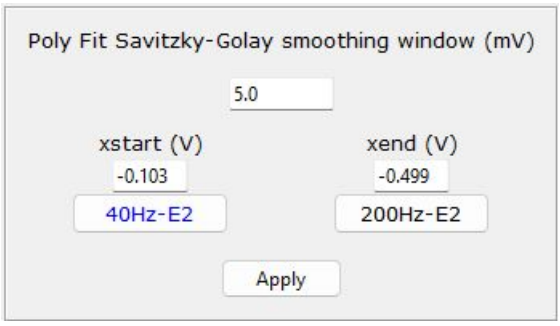
Shown is the Data Manipulation and Visualization Frame when the Multi-Gauss fit is selected.



- Text box for specifying the voltammogram used to generate normalized peak heights (i.e., normalized peak heights are calculated relative to the height of this peak). It is "1" by default, but can be changed by the user.

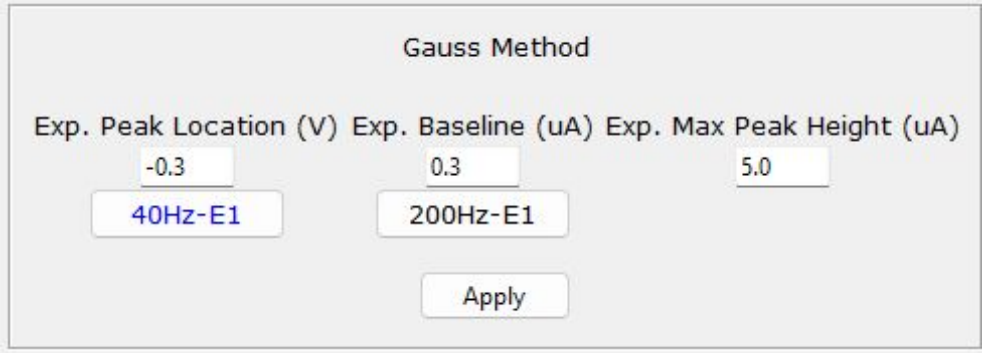
- Shown is the drift correction box, in which the parameters of the KDM (1, 2) and ratiometric (3) analyses (which are used correct baseline drift) are entered. The low and high frequencies can be adjusted by the user. If a frequency that has not been defined is entered a red warning label appears.



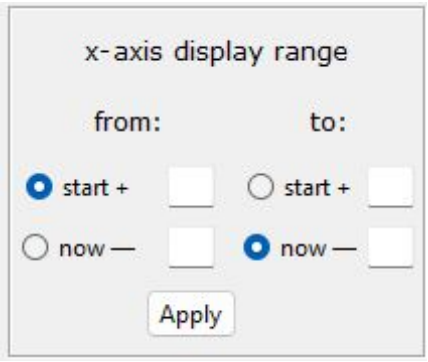


- Shown is the polynomial fit parameter adjustment box. The adjustments are applied after pressing the "Apply" button.
    - The user can specify the voltage window over which Savitzky-Golay smoothing is performed (e.g., in above pictures, at 5 mV intervals). This value must be an odd number.
    - The user can specify the beginning and end of the voltage window over which the fitting is applied. These parameters can be adjusted for each frequency/electrode

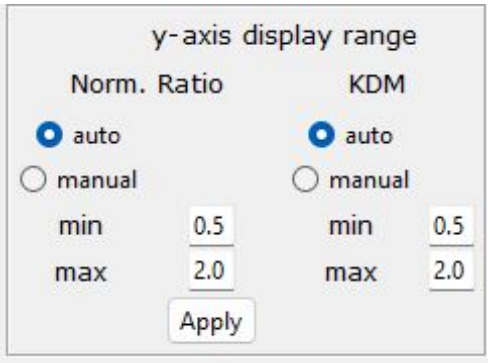
pair by pressing the corresponding button. For electrode switching, see Electrode selection part. The frequency being modified is highlighted in blue.



- Shown is the multiple-Gaussian with Laplacian fit parameter adjustment box. The user can specify the expected peak location (the range is +/- 0.05 V of the specified peak location), the expected baseline (the range is +/- 0.5 µA of the specified baseline) and the expected maximum peak height of the middle Gaussian. These parameters can be adjusted for each frequency-electrode pair by pressing the corresponding button.



- Shown is the adjustment box controlling the width of the x-axis for the ratiometric and KDM display panels.

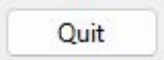


- Shown is the adjustment box controlling the width of the y-axis for the ratiometric and KDM display panels. The “auto” option adjusts the range depending on the data. The user can change the range by pressing the “manual” option and entering the “min” and “max” values.

- Selection boxes for specifying the electrode that is to be the subject of data manipulation and visualization. The number of electrodes depends on the user input on the Input frame.

- Starts the program.

- Resets the program and presents the Input frame.

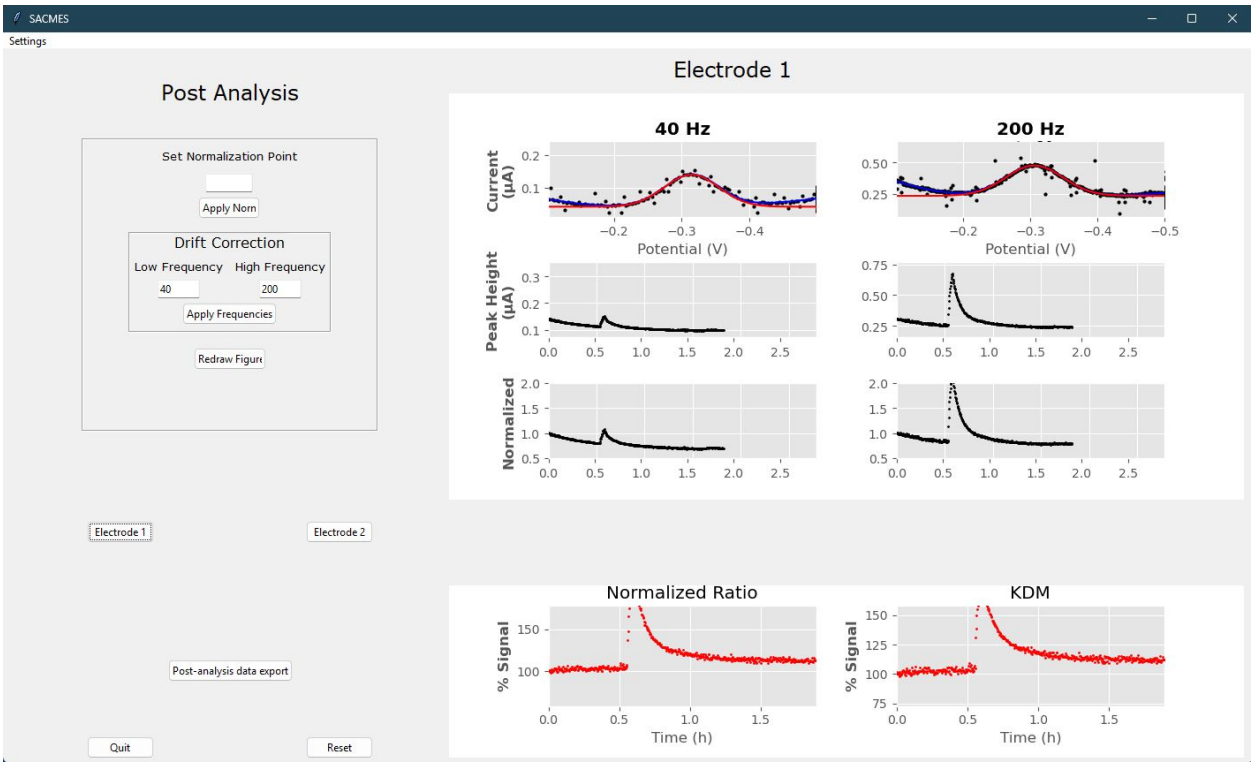


- Ends and closes the program.

Post Analysis Frame of SACMES.py

The post analysis frame appears when the number of analyzed files reaches the user specified input defined in “Input Frame.” This frame does not show up if the user enters a value greater than the actual number of files (e.g., in a real-time experiment in which the number of files that will be generated is uncertain).

On the left side of the frame is the post analysis parameter adjustment section, and on the right is the post analysis visualization frame. The user can readjust the normalization point and the low and high frequencies used for KDM (1, 2) and ratiometric (3) analyses without needing to rerun the program.



Shown is the Post Analysis Frame.

References

1. Ferguson, B. S.; Hoggarth, D. A.; Maliniak, D.; Ploense, K.; White, R. J.; Woodward, N.; Hsieh, K.; Bonham, A. J.; Eisenstein, M.; Kippin, T.; Plaxco K. W.; Soh, H. T. Real-Time, aptamer-based tracking of circulating therapeutic agents in living animals. *Science Trans. Med.* **2013**, 5, 213ra165.
2. Arroyo-Curras, N.; Somerson, J.; Vieira, P.; Ploense, K.; Kippin, T.; Plaxco, K. W. Real-time measurement of small molecules in awake, ambulatory animals. *Proc. Natl. Acad. Sci. USA* **2017**, 114, 645–650.
3. Verrinder, E.; Gerson, H.; Leung, K. K.; Kippin, T. E.; Plaxco, K. W. Dual-frequency, ratiometric approaches to EAB sensor interrogation support the calibration-free measurement of specific molecules in vivo. *ACS Sensors* **2024**, 9, 3205-3211.