Flux: Reference Manual

Contents

1 Getting Started

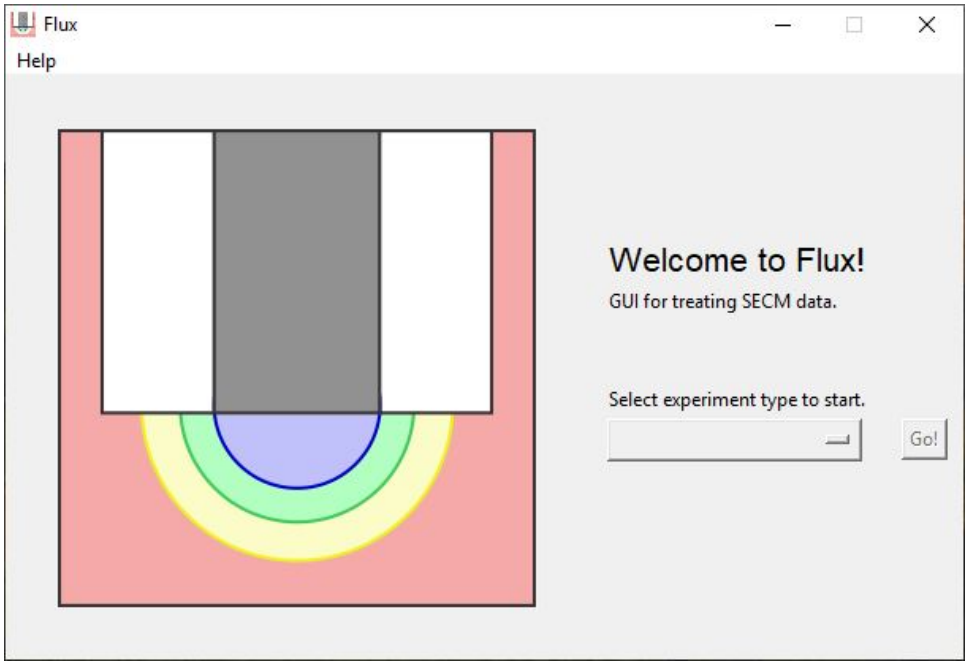
1.1 Installing Flux

Flux is available in two forms: a self-contained .exe that requires no further configuration, or a .py script file that requires a separate Python installation. In both cases, the folder of supporting files must be present in the same folder as the main program file (.exe or .py) or Flux will fail to open.

If using the script in combination with a separate Python installation, the following packages are required:

- numpy v1.16.2
- pandas v0.24.1
- scipy v1.2.1
- scikit-image v0.14.2
- matplotlib v3.0.3
- pyqt v5.12

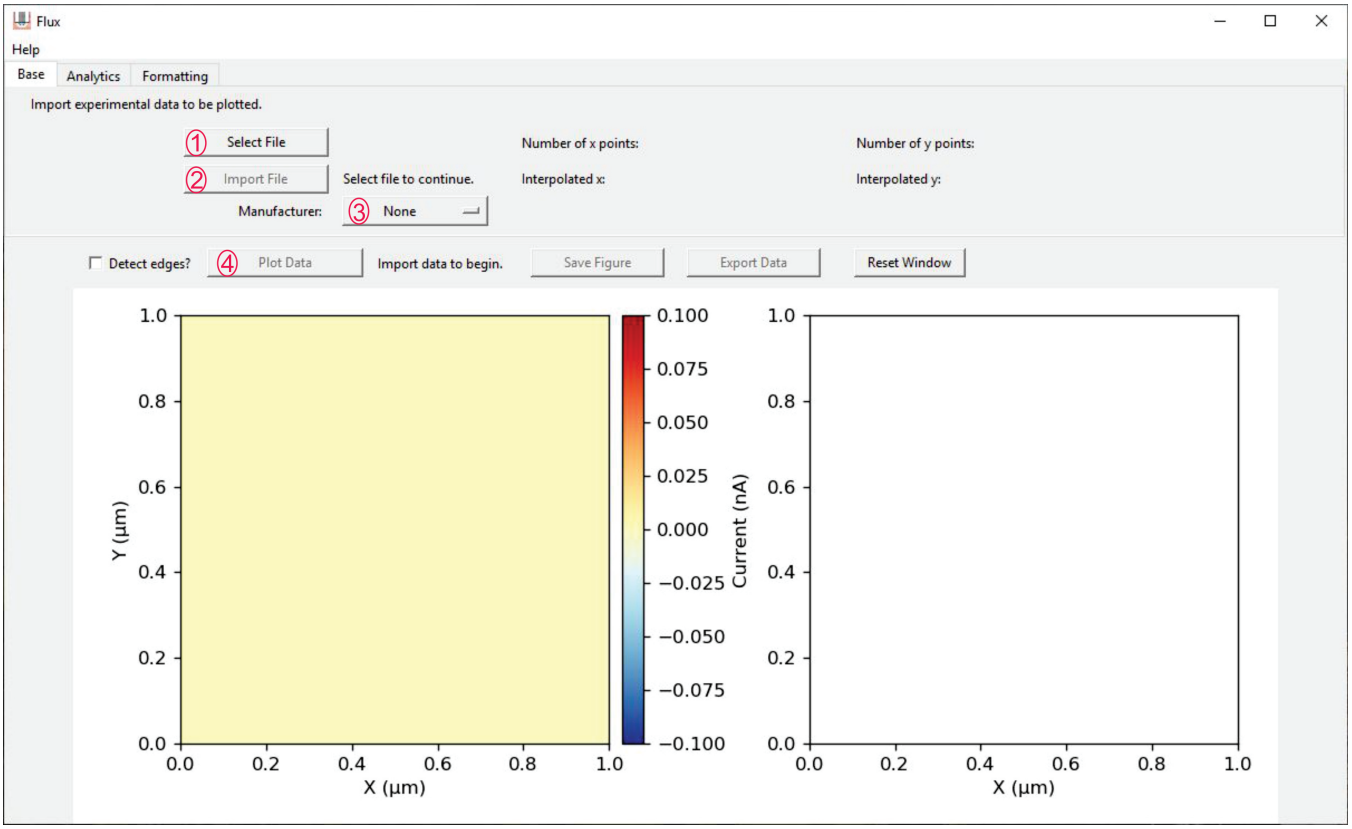
If all of the required files are present, Flux will open on a splash screen with a dropdown menu. Select the relevant experimental type and press **Go!** in order to open a new window.



1.2 Treating your first experiment

1.2.1 Importing data

Each window within Flux is structured the same way. There are three tabs to control different elements of the data treatment process. On the Base tab, first use the **Select File** button to navigate to the file containing your experimental data. Second, select **Import File** to import the data in that file. Depending on the file type, it may be necessary to select your manufacturer to ensure a successful import. Finally, select **Plot Data** to display your data set.



1.2.2 Manipulating data

Additional options for data manipulation are available on the Analytics tab. In the majority of cases, currents can be normalized according to an experimental value (established via prior microelectrode characterization) or theoretical value (estimated using the equation for the steady state current of a disk microelectrode). The specific options available depend on the type of experiment and are discussed in more detail in the relevant sections.

1.3 Customizing formatting and exporting

Additional options for customizing the appearance of the graph are available on the Formatting tab. The axis limits and units of all quantities can be modified based on a predefined list of options. Whenever a formatting change is requested, the **Plot Data** button needs to be selected again to update the appearance of the graph based on the new settings.

When you are satisfied with the appearance of the graph, it can be exported in two forms. Select **Save Figure** to export the graph(s) as they appear on screen as a 400 DPI .png format. Select **Export Data** to export the processed data as a .txt file, which can be imported in another program for replotting based on specific style guidelines or additional analysis.

2 Using Flux

2.1 ImageApp

2.1.1 Importing

The import file functionality currently supports a range of file types:

- .asc (HEKA) files formatted as a series of consecutive line scans, where each line scan contains three columns in the following order: index, distance (m), current (A).
- .mat (HEKA) files formatted as a series of traces (one trace = one line scan), where each trace contains two columns in the following order: distance (m), current (A).
- .txt (Biologic or CH Instruments) files containing a series of three columns in the following order: X (x-position in μm), Y (y-position in μm), Z (current in A). The data does not need to be present at the beginning of the data set (header lines or X-Y table present earlier in the file will be skipped.)
- .dat (Sensolytics) files containing a series of 7 columns in the following order: X (um), X rel (μm), Y (μm), Yrel (μm), Z (μm), Zrel (μm), Ch1 (nA), Ch2 (nA). The data does not need to be present at the beginning of the data set. The number of points will be determined from lines 6 and 7 of the header. Only the currents in the Ch1 column will be plotted.
- .csv (Princeton Applied Research / PAR) files formatted as a matrix of current values (in μA) beginning on line 8. Header line 7 contains the x-positions sampled (in mm),

and the last column of the matrix the y-positions sampled (in mm).

2.1.2 Sign of steady state current

The sign of the theoretical steady state current is calculated assuming the redox mediator is oxidized at the microelectrode. If a reduction process takes place instead, enter a negative concentration to calculate the correct steady state current for normalization.

2.1.3 Number of points

The number of points in the x and y dimensions is related to the sampling density of the original scan. The edge detection algorithm requires square pixels (equal sampling density in each direction). If the data set has an equal number of x and y points initially, no interpolation will be performed and edge detection will be performed on the original image. If the data set has asymmetric sampling, an interpolation algorithm will be applied at the scale of 1 point/μm and edge detection will be performed on the interpolated image. Edge detection on interpolated images may be poor for large scale (mm) SECM images.

2.1.4 Slope correction

If slope correction is enabled, the currents along the specified edge will be fit to a straight line and the slope used to correct all parallel lines. This method works best when one edge of the image is free of any reactive/topographical features of note, and thus any differences in the current are due to a tilt in the substrate.

2.1.5 Plotting

The live graph can be customized to normalize the currents, slope correct the raw images, and perform edge detection. Whenever making a change to data treatment procedure, the 'Plot Data' button needs to be clicked again to regenerate the graph with the new settings.

2.1.6 Saving figures

When requested, the most recent plot of the processed results will be saved as a 400 DPI .png file. Alternatively, the processed data can be exported as a .txt file for replotting in other programs. If the checkbox for edge detection is not enabled, only the SECM image will be saved, regardless of whether or not detected edges from a previous analysis are still displayed on screen.

2.2 CVApp

2.2.1 Importing

The import file functionality currently supports a range of file types:

- .asc (HEKA) files formatted as a series of consecutive sweeps, where each sweep corresponds to a single cycle of the CV. The import functionality assumes each sweep is composed of five columns in the following order: index, time (s), current (A), time (s), potential vs. reference (V).

  The scan rate for this file type is calculated from the potential/time traces over the first 25% of the first cycle. If the potential waveform possesses a peak in this location, the returned value may be inaccurate.

- .txt (Biologic or CH Instruments) files formatted as a series of two columns in the following order: Potential (V), Current (A). Individual cycles do not need to be separated; the number of total cycles is estimated as the number of times the potential hits its maximum value, and the data will be split up into cycles accordingly.

  The scan rate for .txt/CH Instruments files will be pulled from header line 13 of the input file.

- .dat (Sensolytics) files formatted as a series of two columns in the following order: Potential (V), Current (A). Individual cycles do not need to be separated; the number of total cycles is estimated from the input file as the number of times the potential hits its maximum value, and the data will be split up into cycles accordingly.

  The scan rate for this file type will be pulled from header line 19 of the input file.

- .mat (HEKA) files formatted as a series of traces, with two traces per cycle that follow this naming convention: Trace A-B-CycleNo-1 contains time (s) and current (A); Trace A-B-CycleNo-2 contains time (s) and potential (V).

2.2.2 Plotting

The live graph can be customized to include theoretical/derived values for the data set, alternative reference electrodes, and more. Whenever making a change to the appearance of the graph, the 'Plot Data' button needs to be clicked again to regenerate the graph with the new settings.

2.2.3 Reference electrode

The entry field for reference electrode is used to update the x-axis label of the graph. Only the name of the reference electrode in your preferred format (e.g. 'Li', 'Fc.') should be given in this field. If no entry is given, a default value of 'Ag/AgCl' will be used. Note: Updating this field will only update the label, the numerical values will not be converted.

2.2.4 Formal potential

The formal potential is calculated from the first derivative of the current trace during Cycle 1. The two potentials where this derivative is at a min and max are averaged and reported as the formal potential. Graphically speaking, this corresponds to the inflection point on the curve. This procedure is not recommended if more than one redox couple is visible in the CV.

2.2.5 Experimental steady state current

The experimental steady state current is calculated by looking for the smallest absolute value in the derivative current in the range between the anodic and cathodic peaks (see section on formal potential). Essentially, this function will return the value where the current is changing minimally in the middle 50

2.2.6 Sign of theoretical steady state current

The sign of the theoretical steady state current is calculated assuming the redox mediator is oxidized at the microelectrode. If a reduction process takes place instead, enter a negative concentration to calculate the correct steady state current for normalization.

2.2.7 Saving figures

When requested, the most recent plot of the updated results will be saved as a 400 DPI .png file. Alternatively, the processed data can be exported as a .txt file for replotting in other programs. The current settings (units, additional calculated variables) will be indicated in this text file.

2.3 CAApp

2.3.1 Importing

The import file functionality currently supports a range of file types:

- .asc (HEKA) files containing a single chronoamp. The import file functionality assumes this data set is composed of five columns in the following order: index, time (s), current (A), time (s), potential vs. reference (V).
- .txt (Biologic or CH Instruments) files containing a single experiment composed of one or more steps. The import file functionality assumes this data set is composed of two columns in the following order: time (s), current (A).If multiple steps are present, the data will be plotted as one continuous set. Only the initial potential value will be reported in the 'Potential (V vs. ref)' field.
- .dat (Sensolytics) files containing a single experiment composed of one or more steps. The import file functionality will use the number of channels and method (headerlines 2 and 3) to determine the column order; mono or bipotentiostat experiments with constant or pulsed potential are supported.

2.3.2 Sign of steady state current

The sign of the theoretical steady state current is calculated assuming the redox mediator is oxidized at the microelectrode. If a reduction process takes place instead, enter a negative concentration to calculate the correct steady state current for normalization.

2.3.3 Plotting

The live graph can be customized to include lines for the theoretical or experimental steady state currents, as well as the response time. Whenever making a change to the appearance of the graph, the 'Plot Data' button needs to be clicked again to regenerate the graph with the new settings.

2.3.4 Data treatment

The experimental steady state current is calculated as the average of the last 50 points in the data set. The response time is calculated as the time it takes the current to drop to 110% of its experimental steady state value. The algorithm for this examines the data set against this threshold in reverse order, and may be subject to error in the case of spikes or extremely noisy data.

2.3.5 Response time

The response time is calculated as the time it takes the current to decay to 110% of its steady state value. The algorithm searches in reverse time order, from the last data point

backwards, and returns the first time point where the current meets this criteria.

2.3.6 Saving

When requested, the most recent plot of the updated results will be saved as a 400 DPI .png file. Alternatively, the processed data can be exported as a .txt file for replotting in other programs. The current settings (units, additional calculated variables) will be indicated in this text file.

2.4 PACApp

2.4.1 Importing

The import file functionality currently supports various file types:

- .asc (HEKA) files containing a single approach curve. The import file functionality assumes this data set is composed of three or five columns in the following order: index, distance (m), current (A), **distance (m), **current (A). (** = Optional columns)
- .txt (Biologic or CH Instruments) files containing a single approach curve. The import file functionality assumes this data set is composed of two columns in the following order: tip-substrate distance (m), current (A).
- .dat (Sensolytics) files containing a single approach curve. The import file functionality assumes this data set is composed of three columns in the following order: distance (um), relative distance (um), current (nA).
- .mat (HEKA) files formatted as a single trace, which contains two columns in the following order: distance (m), current (A).
- .csv (Princeton Applied Research / PAR) files formatted as a series of three data columns beginning on line 5. Columns are assumed to be in the following order: time (s), distance (mm), current (uA).

2.4.2 Sign of steady state current

The sign of the theoretical steady state current is calculated assuming the redox mediator is oxidized at the microelectrode. If a reduction process takes place instead, enter a negative concentration to calculate the correct steady state current for normalization.

2.4.3 Zero tip-substrate distance

Three options are available for determining the point of zero tip-substrate distance (contact with surface) that all other distances will be calibrated against. Based on the option selected, data points prior to this point (d ¡ 0 after calibration) will be discarded from further analysis. The number of data points remaining after this calibration is given in the 'No. of pts (post-processing)' field.

- If 'First point with data' is selected, the first value in the data set with a non-NaN current value will be treated as d = 0. This is the default option for an asc/HEKA, txt/CH Instruments, or dat/Biologic file.
- If 'First derivative analysis' is selected, the point where the derivative of the current is at a maximum will treated as d = 0. This may be able to correct for a kink in the approach curve where the electrode made contact with the surface and began to bend.
- If 'No calibration' is selected, the distances in the input file will be treated as the true tip-substrate distances and no further calibration will be applied. This is the default option for a txt/Biologic file.

2.4.4 Plotting

The live graph can be customized to include theoretical lines for pure positive/negative feedback and more. Whenever making a change to the appearance of the graph, the 'Plot Data' button needs to be clicked again to regenerate the graph with the new settings.

2.4.5 Fitting data

If 'Fit Rg' is selected, an estimated Rg will be calculated by performing a nonlinear curve fit on the normalized experimental curve against the analytical approximation for negative feedback, in which Rg is a parameter. This is done using the Levenberg-Marquardt curve fitting algorithm with an initial guess of 1, no bounds provided, and default tolerances.

Based on the conditions under which these equations are valid, any points where L ¡ 0.1 will not be included in this analysis. For this function to work, **the checkboxes for normalization, experimental iss, and fit Rg must be selected, and a value for both the electrode radius and experimental iss provided**. This procedure for estimating Rg is only valid when the approach curve displays pure negative feedback behaviour.

If 'Fit Kappa' is selected, an estimated kappa will be calculated in a similar method to the Rg

fitting method described above. The normalized experimental curve will be compared to the analytical approximation for mixed kinetics. For this function to work, **the checkboxes for normalization, experimental iss, and fit Kappa must be selected, and a value for the electrode radius, Rg, and experimental iss provided**. For a non-normalized value of the rate constant (k) to be calculated, a diffusion coefficient must be provided as well. This procedure for estimating kappa is only valid for Rg ¡ 20 and where it is valid to assume the electrode sees an uniformly reactve substrate (small electrode, large features).

2.4.6 Pure feedback settings

If 'show pure feedback cases' is selected, theoretical lines for the pure positive and negative feedback cases will be added to the graph for comparison. This requires the 'normalized currents' checkbox to be selected, and a value to be entered for both the radius and Rg of the electrode. Additionally, it requires either an experimental steady state current or concentration/diffusion coefficient of the redox mediator to be entered in order to calculate a steady state current for normalization.

2.4.7 Saving

When requested, the most recent plot of the updated results will be saved as a 400 DPI .png file. Either of the two subplots individually or the entire figure may be saved. Alternatively, the processed data can be exported as a .txt file for replotting in other programs. The current settings (units, additional calculated variables) will be indicated in this text file. Only the data sets included on the most recent graph will be included in this file.