

ASCAM Instructions (for Mac)

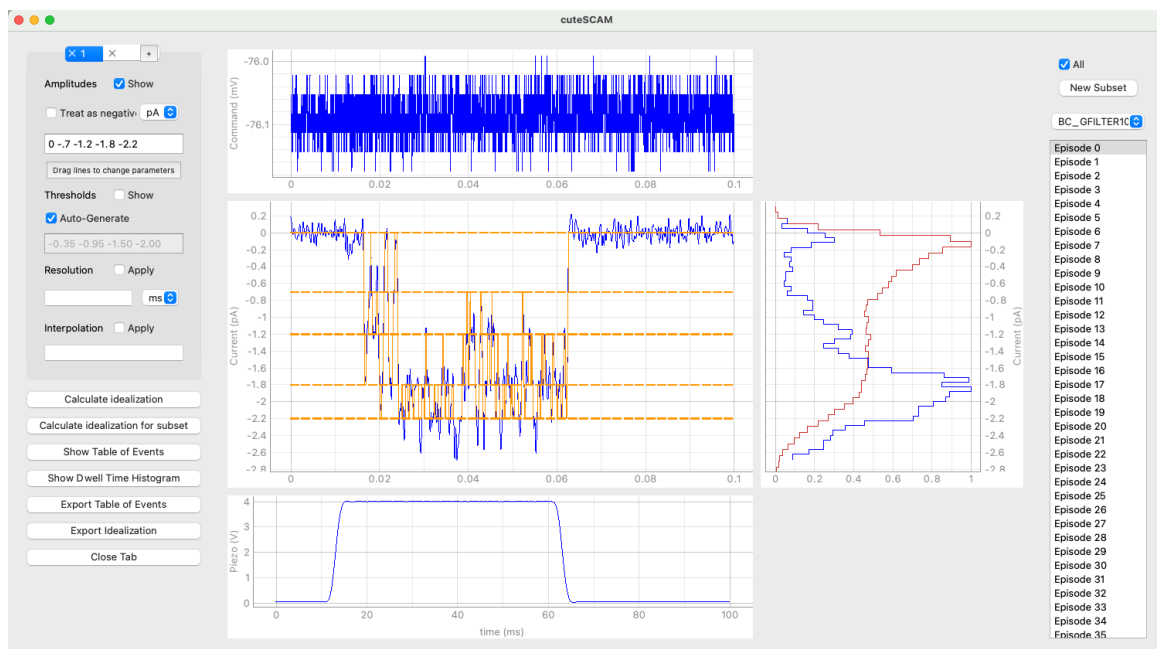
Full installation instructions are in the readme.md file and are not replicated here.

When you launch ASCAM from the Terminal, you will first see the “Start of a new ASCAM session” and then a bunch of package names and the version (commit hash) you are using.

If nothing more happens, don’t forget “export QT_MAC_WANTS_LAYER=1”

The window with a couple of empty graphs (“cuteSCAM”) should open.

To see the features of ASCAM automatically, you can launch with “ascam -t” and the test data will be automatically loaded, processed and ready to be idealized.



ASCAM after opening with the -t (test) flag. Plots of voltage and piezo were enabled, and “calculate idealization” clicked.

Load a file

Go to the File menu and click “Open File”. If you have a data directory that you are working with a lot, it could help to put it into the sidebar.

You can load .mat files or .axgd files. If you don’t have any data to look at, you can use the sample data file of an AMPA receptor single channel recording in the /data directory. This recording was sampled at 40 kHz.

A dialog pops up asking for the sample rate (you have to know it) and what units to use. Normally, also long as you have the correct sample rate, there is nothing more to change.

You can only open one file at a time. The filename and path is given at the top of the window.

After a pause you will see the first record (“Episode 0”) in the data file. The episodes are listed and you can select between them.

The selection box has only one entry, “raw_” because this is the raw, unprocessed data.

On the right, you will see the all points histogram for the displayed data (blue) and for the whole data file (red). It is aligned with the current trace.

If you have voltage profiles and/or piezo jump traces, these can also be displayed (go to the “Plots” menu to enable them).

Filter

- will be applied to all traces in the file.
- This action generates a new “datakey” with the operation summarised. The datakey is added to the box above the list of episodes.

Baseline correction

- will be applied to all traces in the file (each gets it’s own unique background correction).
- there are some options, the defaults are usually fine. You can chose a polynomial (best is likely to be “degree 1” - that is, a straight line with a slope and intercept). You can instead select just an offset that you specify.
- the region that is assumed to be baseline is (by default) the trace outside the region of the piezo switch. You can alternatively use the “active” region of the piezo, if needed.
- If you don’t have this piezo trace in your data file, or want to specify a different region, you can provide time intervals. The syntax is a bit tricky, you write the start and end of each interval in square brackets: [0.01, 0.02]. If you want multiple intervals, write them as a comma separated list: [0.01, 0.02], [0.03,0.04]
- This action generates a new “datakey” with the operation summarised. The datakey is added to the box above the list of episodes.

Subsets

Once the basic processing has been done, you probably want to select some of the episodes for further processing. This is done using theSelect episodes using the “subset” function. Click “New Subset” and you get a dialog. Name the subset (single words only) and nominate a hotkey for marking episode. If you select an episode, you can quickly flip through records with the up/down arrow keys, and select them for the named subset using the hotkey that you define. The episodes selected will be marked in the list with the hotkey. To deselect an episode, type the same hotkey again. Episodes can belong to multiple overlapping subsets if you want to try something out.

Idealization

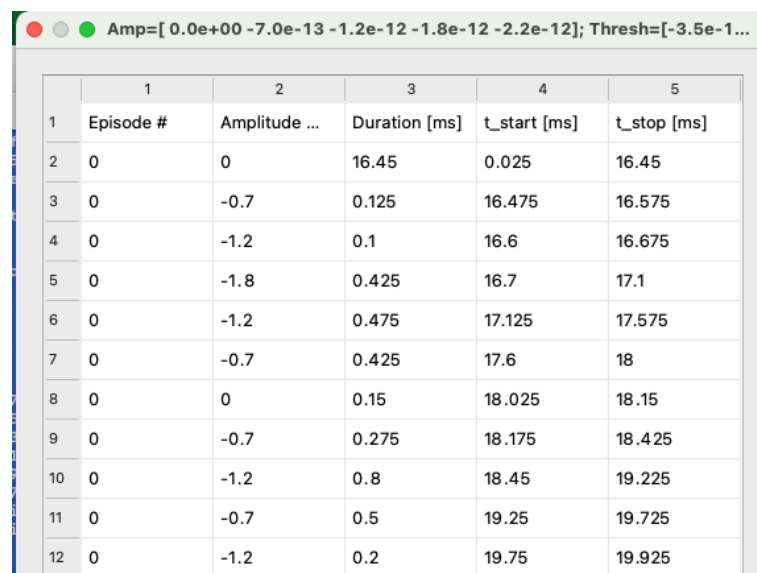
If the episodes you want to work on are part of a subset, you are ready to extract some dwell times. Go to the “Analysis” window and select “Idealize”.

- tabbed idealisation panel will appear on the left hand side
- enter the expected **amplitudes** - if you check “show” they will be drawn on the record. If openings are negative, you can click the “treat as negative” checkbox and write them as positive numbers. By default the units are “pA” but you can change this in the selection box.
- the **thresholds** used for idealisation can be auto-generated, or you can specify them. If you have a lot of open levels (e.g. AMPA, 4 levels) you might want to move the first threshold away from the baseline a bit.
- **resolution** is the briefest event you think you can see reliably. By default it is in units of “ms” but you can change this with the selection box.
- **interpolation** uses the local data points to the threshold crossing to estimate the exact crossing with subsample accuracy.
- use the **subset** function to idealise only some of the events.
- the tabs at the top let you do multiple idealisations (one per tab) and compare them.
- to do the idealisation on a subset only, click the “Calculate idealisation for subset” button. The Terminal gives some feedback on what episode is being processed.

First activation

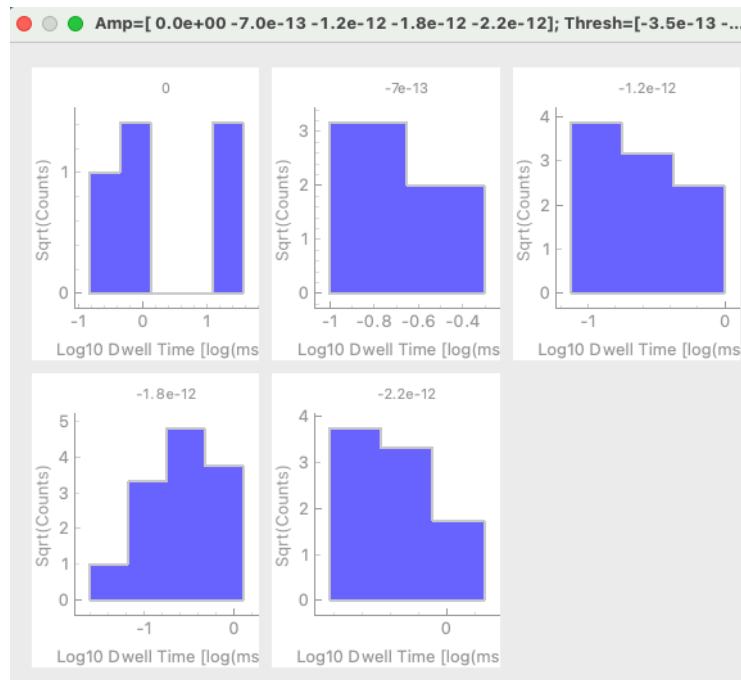
only works on all records at the moment, not subsets.

You can **show** a rudimentary **table of events**



	1	2	3	4	5
	Episode #	Amplitude ...	Duration [ms]	t_start [ms]	t_stop [ms]
2	0	0	16.45	0.025	16.45
3	0	-0.7	0.125	16.475	16.575
4	0	-1.2	0.1	16.6	16.675
5	0	-1.8	0.425	16.7	17.1
6	0	-1.2	0.475	17.125	17.575
7	0	-0.7	0.425	17.6	18
8	0	0	0.15	18.025	18.15
9	0	-0.7	0.275	18.175	18.425
10	0	-1.2	0.8	18.45	19.225
11	0	-0.7	0.5	19.25	19.725
12	0	-1.2	0.2	19.75	19.925

You can also look at **dwelt time histograms** for the events captured by the idealization. These are presented in the Sigworth-Sine form (ordinate in square root vs. abscissa in log time). There are very few events in the example below!



Export table of events

- only events from records that you have idealised will be exported
- only the idealisation from the current tab is exported

You can also **export the idealised trace** itself.

Export of records is not working reliably at the moment.

Close ASCAM by clicking the red radio button at the left hand top corner of the window. If you Quit from the terminal (for example, with Ctrl-z), you will have to “force-quit” the Python icon in the dock (Right-Click -> Force Quit), or use the alt-Cmd-Esc combination to get to the “Force Quit Applications” dialog, in order to get rid of the GUI window.