

Q. what steps you usually take (in what order) to analyze a traces

Typically, the steps that I take to get the data out of a trace are:

1. Open a file
2. Adjust sampling rate and gain if necessary (Filters/Scales button in the topmost panel)
3. Choose the cutoffs and the detection threshold for the analysis
3. Smooth/Filter a trace (more on this below)
4. Select a segment without spikes, hit Bkg button (more on this below)
5. Hit 'Analyze trace'
6. Then, I usually go through all individual spikes to make sure they look appropriate, nothing is missing and there are no recordings of refrigerator activity.
7. Hit 'Stats' button and then copy and paste the datatable in Excel

Q. the macro seems to miss a lot of spikes (notably, some of the larger ones)...

There might be several reasons for this.

1. The background noise is set improperly. If by some means, the noise on the trace segment selected as background is much higher than on the rest of the recording, obviously the real spikes will fall below the detection threshold. Try choosing a larger segment for background or reduce the threshold (under 'Analyze Trace' button on the main window).
2. The program uses not the trace itself, but its 1st derivative for spike detection. Therefore, spikes that are large (by I_{max}) but slow (by risetime) might be missed. Either try to reduce the detection threshold or apply more filtering. Beware though, that if you have a mixed population of slow and fast spikes, extra filtering will affect faster ones more severely than the others.

If nothing helps, you can always add a spike manually...

Q. Is there a set way in which you go about choosing which filter and which frequency to use?

To have a rough estimate of the filters for a particular recording, hit 'Estimate filters' under 'Filters/scales' menu and the cutoffs will be written in the command window. You can read the details of how this and other things in the program are done in the supplementary materials of Mosharov & Sulzer 2005, Nature Methods paper.