

# Peaker Analysis Toolbox

Disclaimer

&

Brief mEPSC analysis tutorial

# Disclaimer

The matlab files included in this analysis toolbox I have written on-and-off over the years for the purposes of facilitating the analysis of my own (and my lab's) electrophysiology data, mainly spontaneous and evoked synaptic currents or potentials. As and when I had a specific analysis task I would write a new script or function and attempt to bolt them on to one another to create a modular analysis toolbox. Some of the code is good but some of it is really ugly, and if I were to do it all again (which I am not!), I certainly would have done it differently. That said I still use much of it and am providing it to embrace open access without responsibility for it's use (or misuse). The toolbox does not have a GUI and is not user friendly (especially if you are not that familiar with Matlab) and it lacks tutorials or a comprehensive manual. That said, I have tried to write extensive help information for each script/function in this toolbox. Researchers I know who have learnt to use this analysis toolbox like it and still use it today. I will attempt to keep up with requests for help and for bug fixes but I do not plan to add much more in the way of functionality.

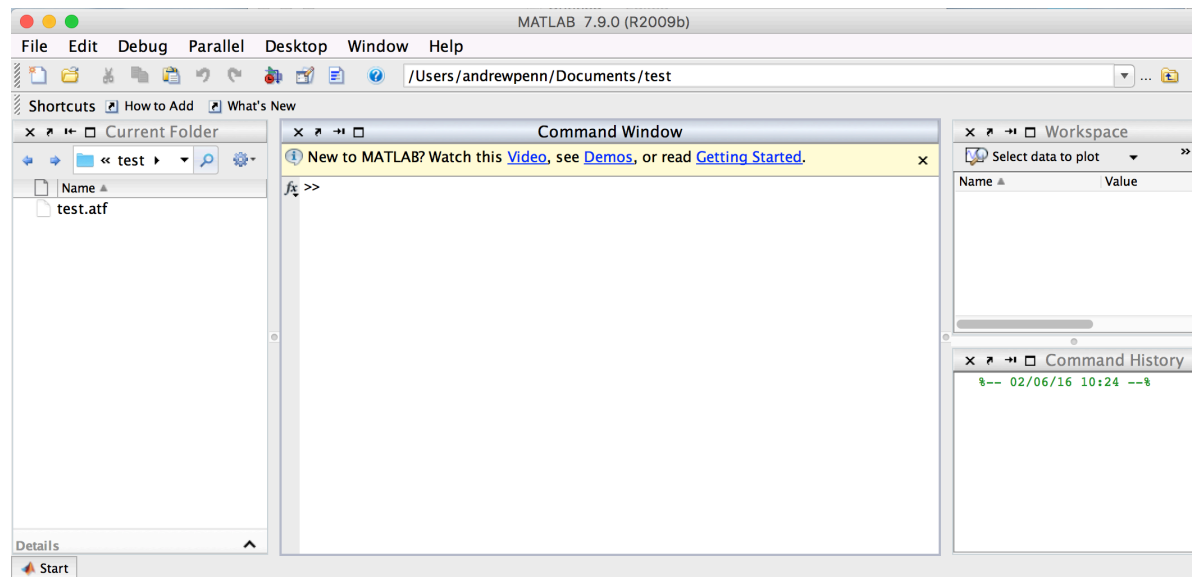
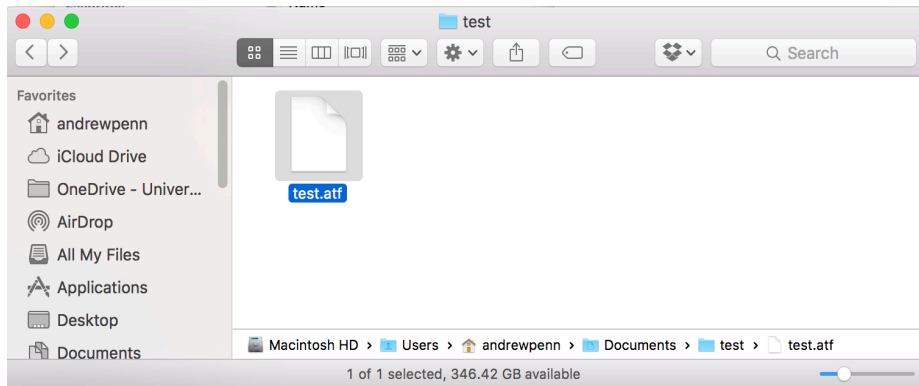
[A.C.Penn@sussex.ac.uk](mailto:A.C.Penn@sussex.ac.uk)

Sussex Neuroscience

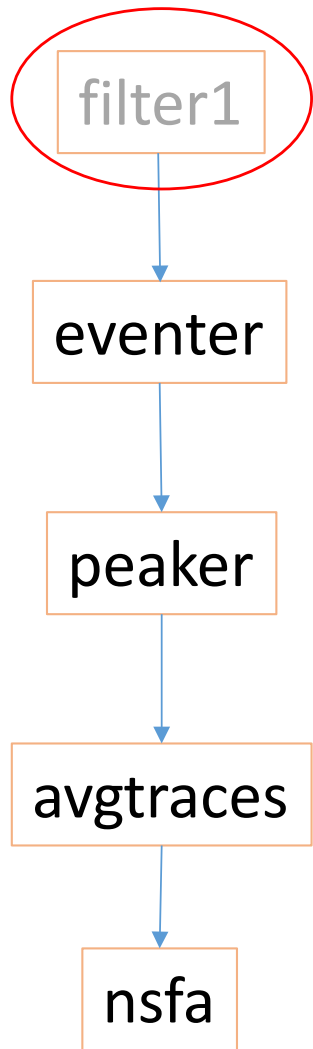
University of Sussex

# Preparation

Create a folder named after the file you wish to analyse and place the file in the folder. For example, I want to analyse traces in a recording called 'test.atf'. Create a folder called test, copy the file into this folder and then move into this directory within Matlab.



# Event analysis command workflow



Optional step for traces that are noisy or have ‘wobbly’ background. Function to apply low-pass or low pass filtering. Type ‘help filter1’ for more info. Run in ‘-file’ mode.

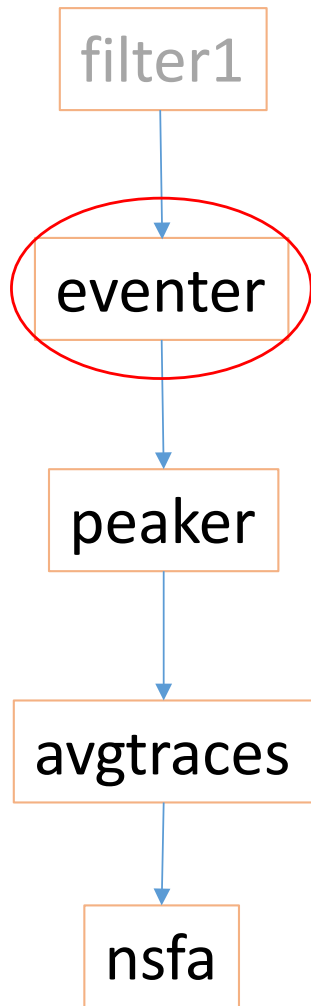
Function to detect events. Type ‘help eventer’ for more info.

Script to measure event parameters. Type ‘help peaker’ for more info.

Script to fine alignment events. Type ‘help avgtraces’ for more info.

Script for non-stationary fluctuation analysis. Type ‘help nsfa’ for more info.

# Event analysis command workflow



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# eventer function command line example

```
>> eventer('test.atf',[0.00045 0.003],'-',4.4,'exclude',[0 0.2],'lpf',300,'wave',1)
```

```
-----EVENTER-----
```

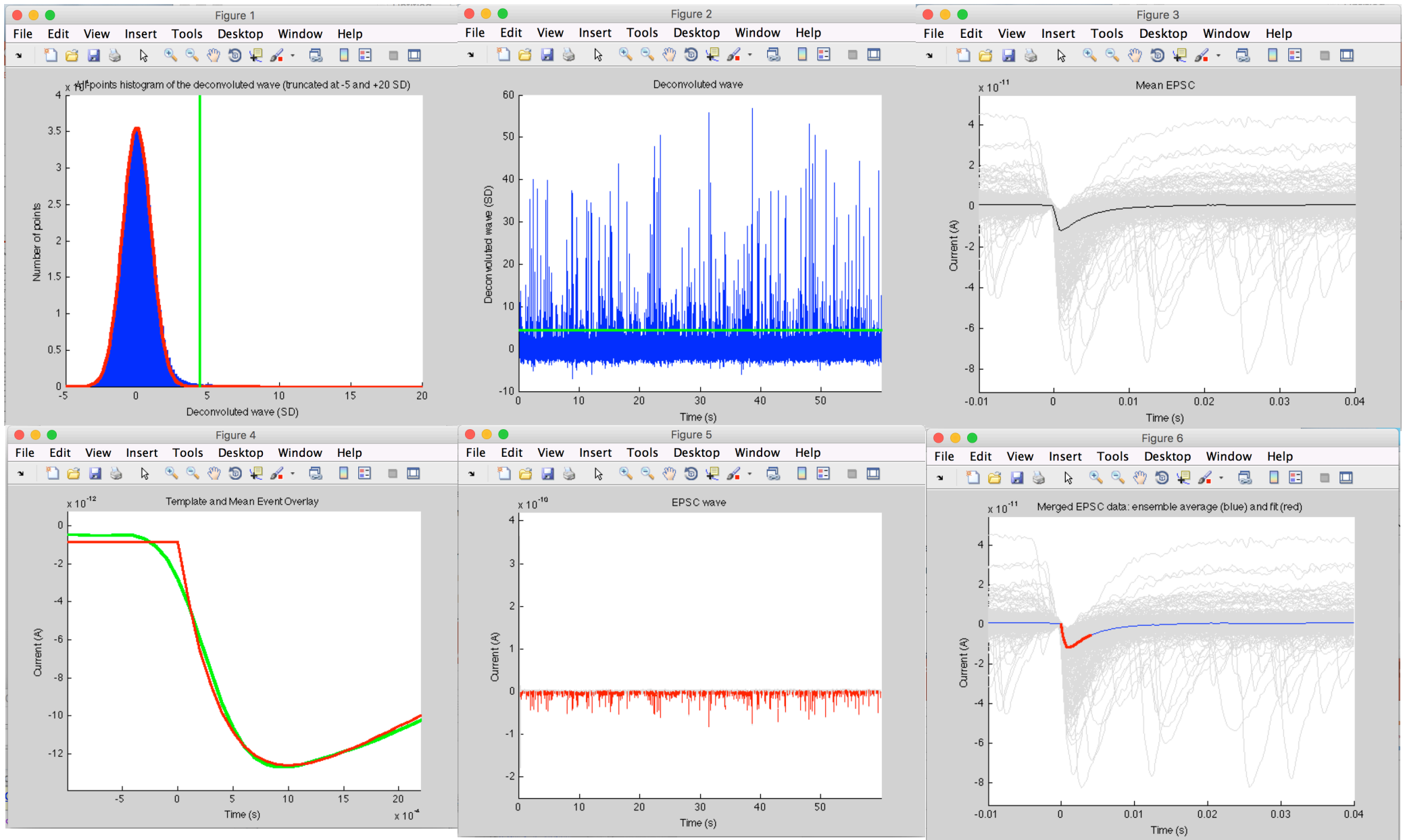
Automatic PSC/PSP detection using FFT-based deconvolution  
and event peak analysis by least-squares template fitting  
Version v1.0 Copyright 2014 Andrew Charles Penn

Filename: test  
Total number of events detected: 625  
Duration of recording analysed (in s): 59.8  
Mean event amplitude (pA): 11.8  
Event frequency (in Hz): 10.5  
High-pass filter cut-off on deconvoluted wave (at -3 dB, in Hz): 1  
Low-pass filter cut-off on deconvoluted wave (at -3 dB, in Hz): 300  
Vector of model template time constants (in s): [0.00045,0.003]  
Standard deviation of the noise of the deconvoluted wave (a.u.): 9.3e-14  
Scale factor of noise standard deviations for threshold setting: 4.4  
False positive event rate (in Hz): 0.0541  
Sign of the event peaks: -  
Number of decay time constants used in the template fit: 0.4  
Minimum acceptable correlation coefficient for the template fit: 0.4  
Episodic data window limits centred around each event: [-0.01,0.04]  
Sample rate of the recording (in kHz): 10  
Isqfit exitflag for fitting the noise peak: 2  
Exclusion zones:  
    0    0.2

```
-----
```

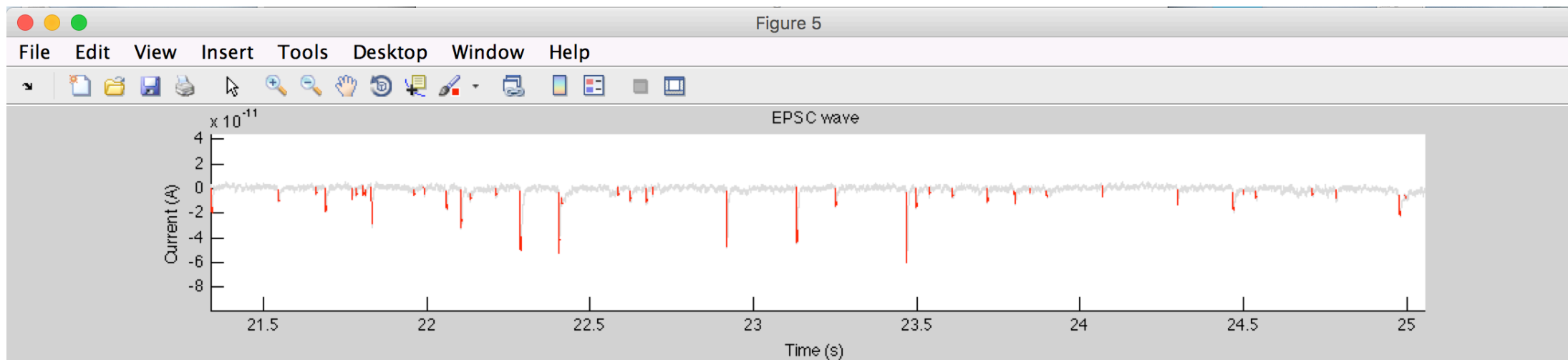
Number of analyses merged: 1  
Total recording time analysed (in s): 59.8  
Total number of events: 624  
Event frequency (in Hz): 10.4  
Ensemble average: mean  
Amplitude of the model EPSC fit (pA): 11.7  
Integral (charge) of the model EPSC fit (fC): 52.89  
Rise time constant of the model EPSC fit (ms): 0.462  
Decay time constant of the model EPSC fit (ms): 3.28  
Isqfit exitflag for fitting the ensemble average event: 2  
Standard deviation of the noise of the deconvoluted waves (a.u.): 9.3e-14

# eventer function example output figures



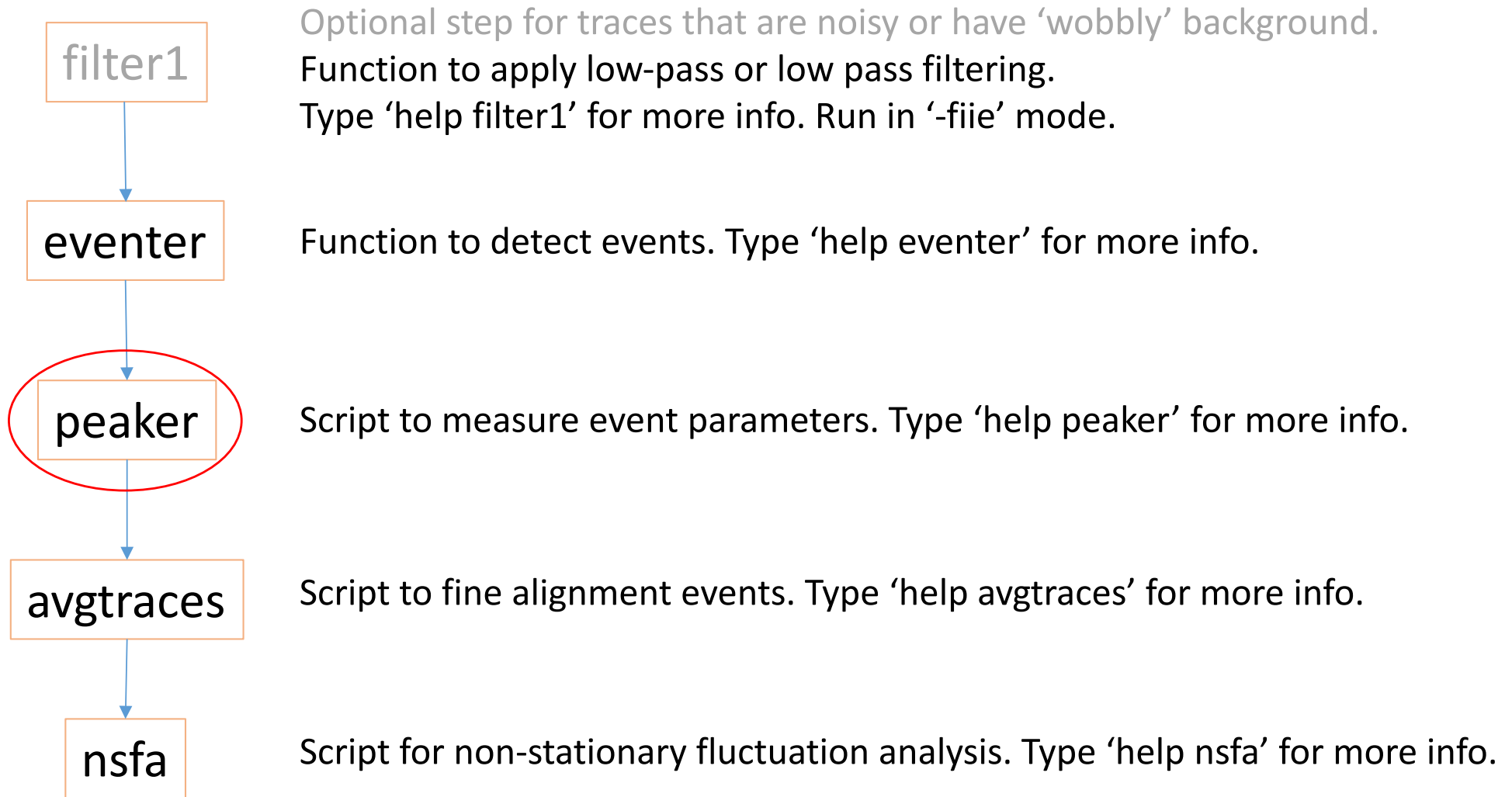
# eventer function example output figures

Expanded view of a section of the trace in figure 5





# Event analysis command workflow



# peaker script command line example

MATLAB 7.9.0 (R2009b)

File Edit Debug Parallel Desktop Window Help

/Users/andrewpenn/Documents/test/eventer.output/ALL\_events

Shortcuts How to Add What's New

Current Folder

Name

- img
- txt
- \_parameters
- ensemble\_average.mat
- ensemble\_average.txt
- event\_data.mat
- fit.txt
- residuals.txt
- summary.txt

Command Window

New to MATLAB? Watch this [Video](#), see [Demos](#), or read [Getting Started](#).

```
>> pwd
ans =
/Users/andrewpenn/Documents/test

>> cd eventer.output/All_events
>> pwd
ans =
/Users/andrewpenn/Documents/test/eventer.output/ALL_events

>> peaker

Data matrix filename (including extension): event_data.mat
Number of traces in this file:
    624

Select the trace number for this analysis (default is all):
Trace number:
    1

The sampling frequency of the recording (in kHz):
    10

Are the recordings voltage (0) or current (1) clamp? (default is 0):
Input the cut-off frequency of the binomial smoothing filter (-3 dB, in kHz): 1
Are the peaks positive (1) or negative (-1) deflections? (default is auto): -1
Input the number of peaks expected per trace: 1
Is the input data baseline subtracted? (1=yes, 0=no, default is yes):
Input the fractional valley threshold (default is 0.5):
fx Set event feature scanning time window (in ms): 3|
```

Analyse the .mat file of the event data

Workspace

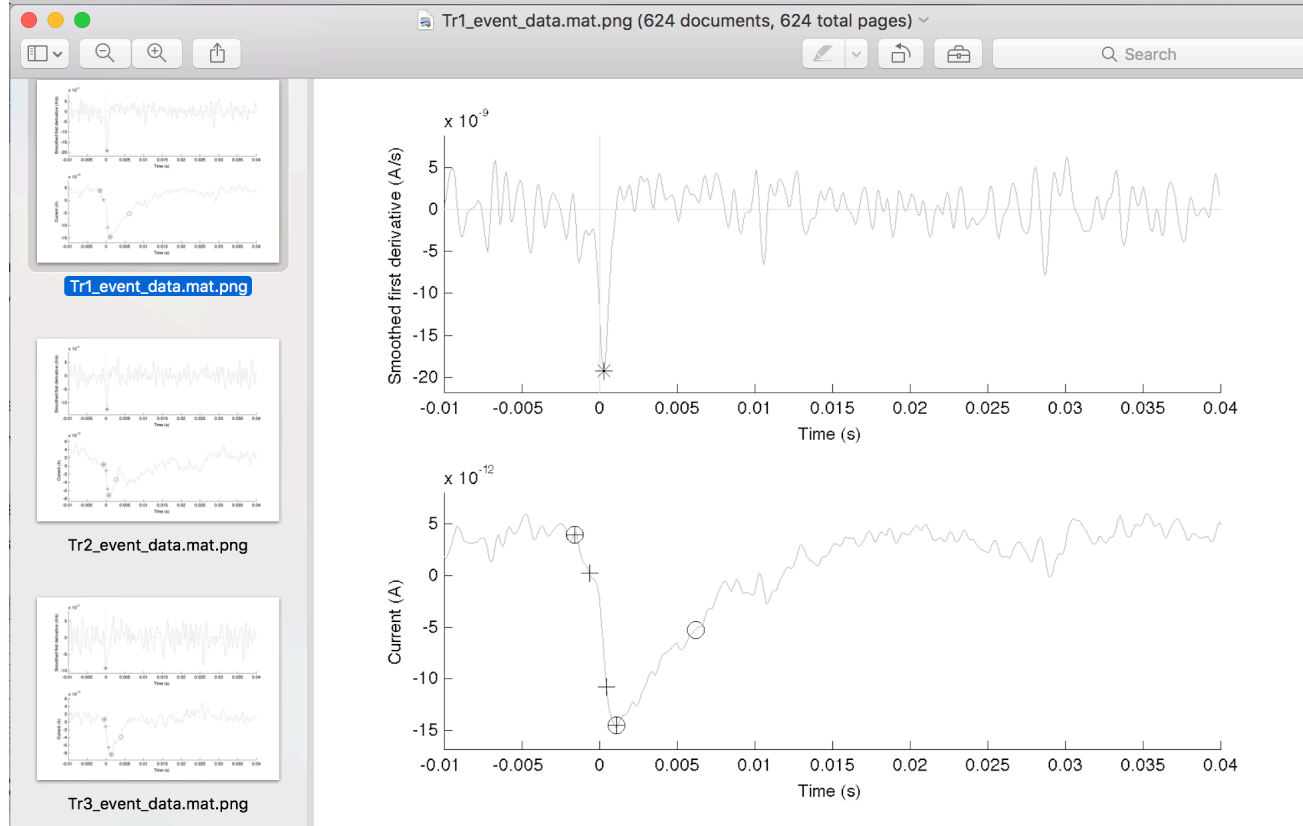
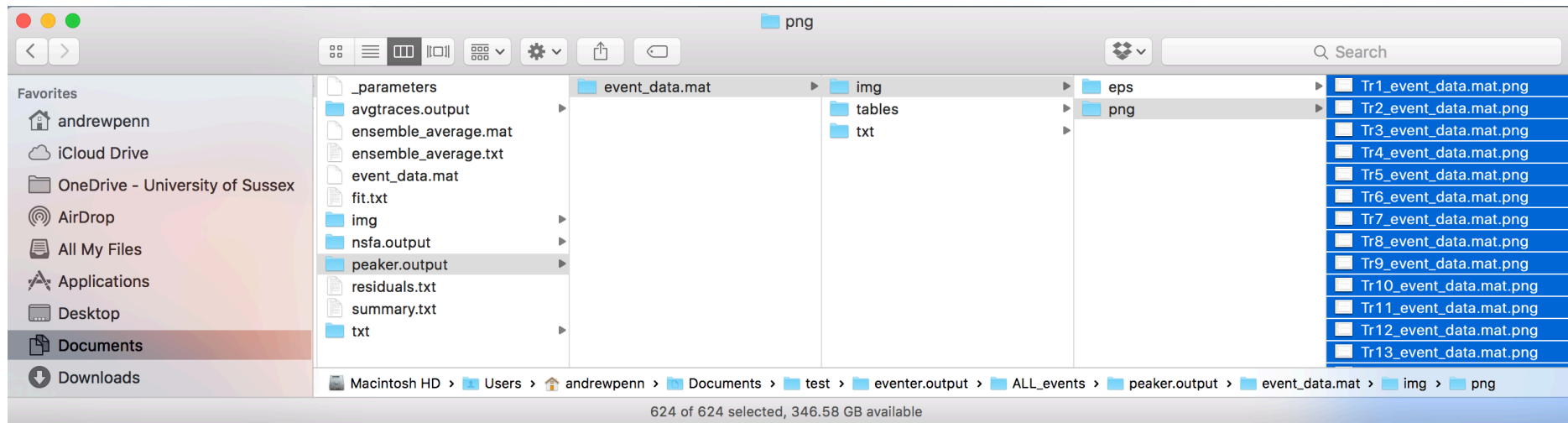
Name	Value
ans	'/Users/andrewpenn/...

Command History

```
%-- 02/06/16 10:24 --%
```

Start Waiting for input

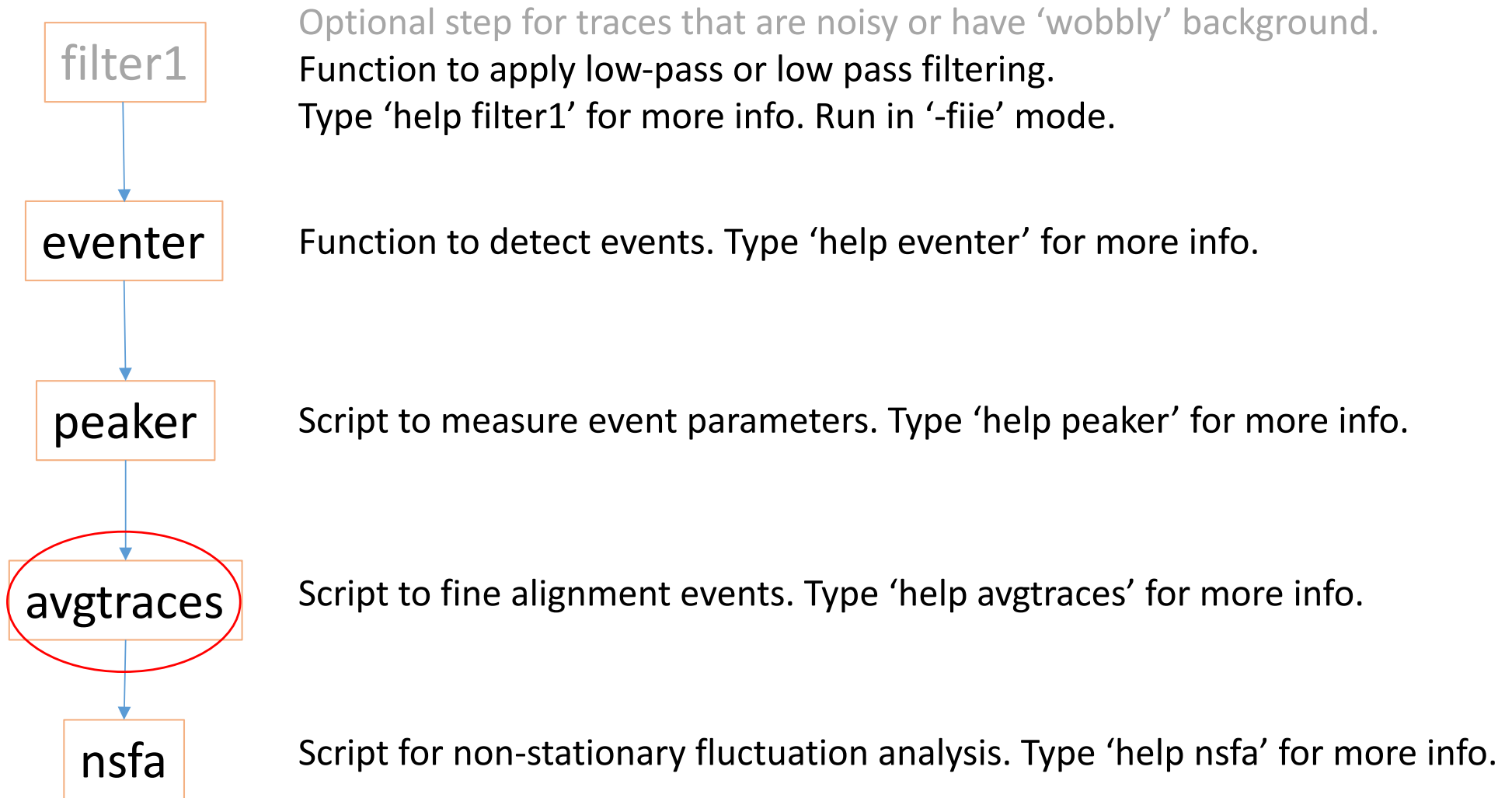
# peaker script command line example



Review png images  
and then run peaker  
again on selected traces  
where measurements  
were either not correct  
or were duplicated.

Note that overlapping  
events can also be  
discarded in later when  
running nsfa.m

# Event analysis command workflow



# avgtraces script command line example

>> avgtraces

Select upto which trace number you wish to analyse (default is all):

Would you like to plot the average trace (1) or not (0, default is yes)?:

Would you like to perform alignment (1) or not (0, default is yes)?:

Would you like to fit an exponential to the decays (1=yes, 0=no, default is no)?: 1

Enter the fraction of the peak to fit exponential from (default is 1): 0.9

Enter the time period after peak to fit the exponential to (in ms): 20

Exiting: Maximum number of function evaluations has been exceeded  
- increase MaxFunEvals option.  
Current function value: 240.010614

Exiting: Maximum number of function evaluations has been exceeded  
- increase MaxFunEvals option.  
Current function value: 266.439015

Enter optional cut-off for 20-80 percent event rise-time (in ms, default is auto): inf

Enter optional cut-off for event decay tau (in ms, default is auto): inf

Traces that could not be fit:

1  
2...

Number of successful traces:

426

Median relative peak amplitude (in pA):

-9.7487

Coefficient of variation of the relative event amplitude:

0.84889

Median peak integral (in fC):

-39.219

Median 20-80 percent risetime (in ms):

0.54272

Median initial rising slope (in nA/ms):

0.012768

Median half-amplitude decay time (in ms):

2.2

Median half-width (in ms):

2.9786

Median decay time constant (in ms):

3.2551

Median instantaneous event frequency (per second):

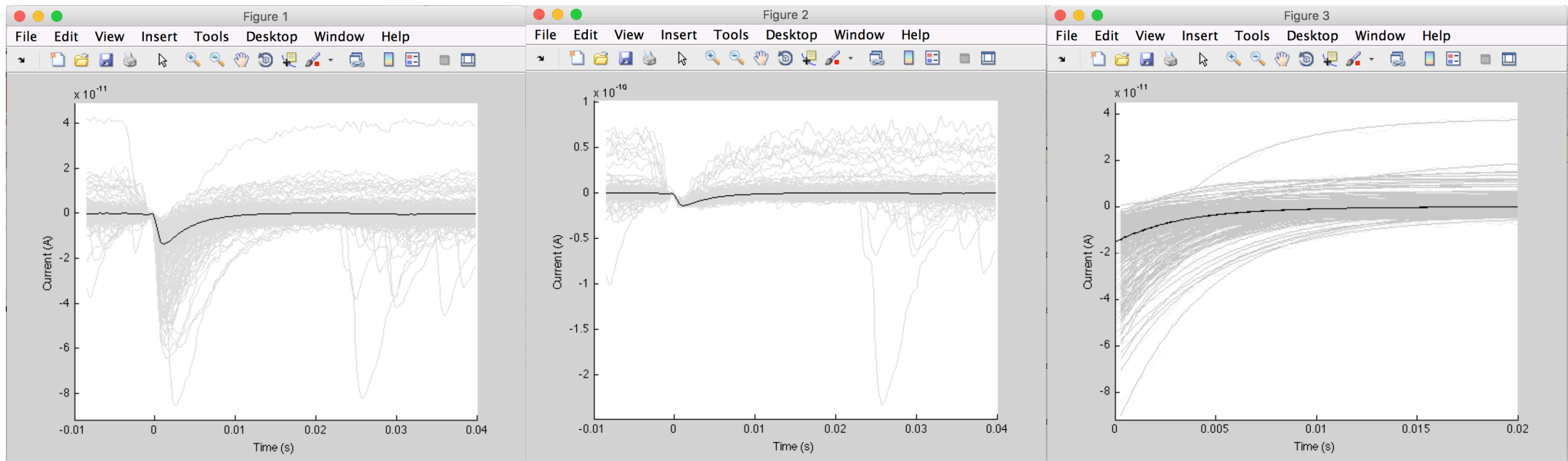
10.11

1) Risetime  
2) Peak  
3) Steepest rise

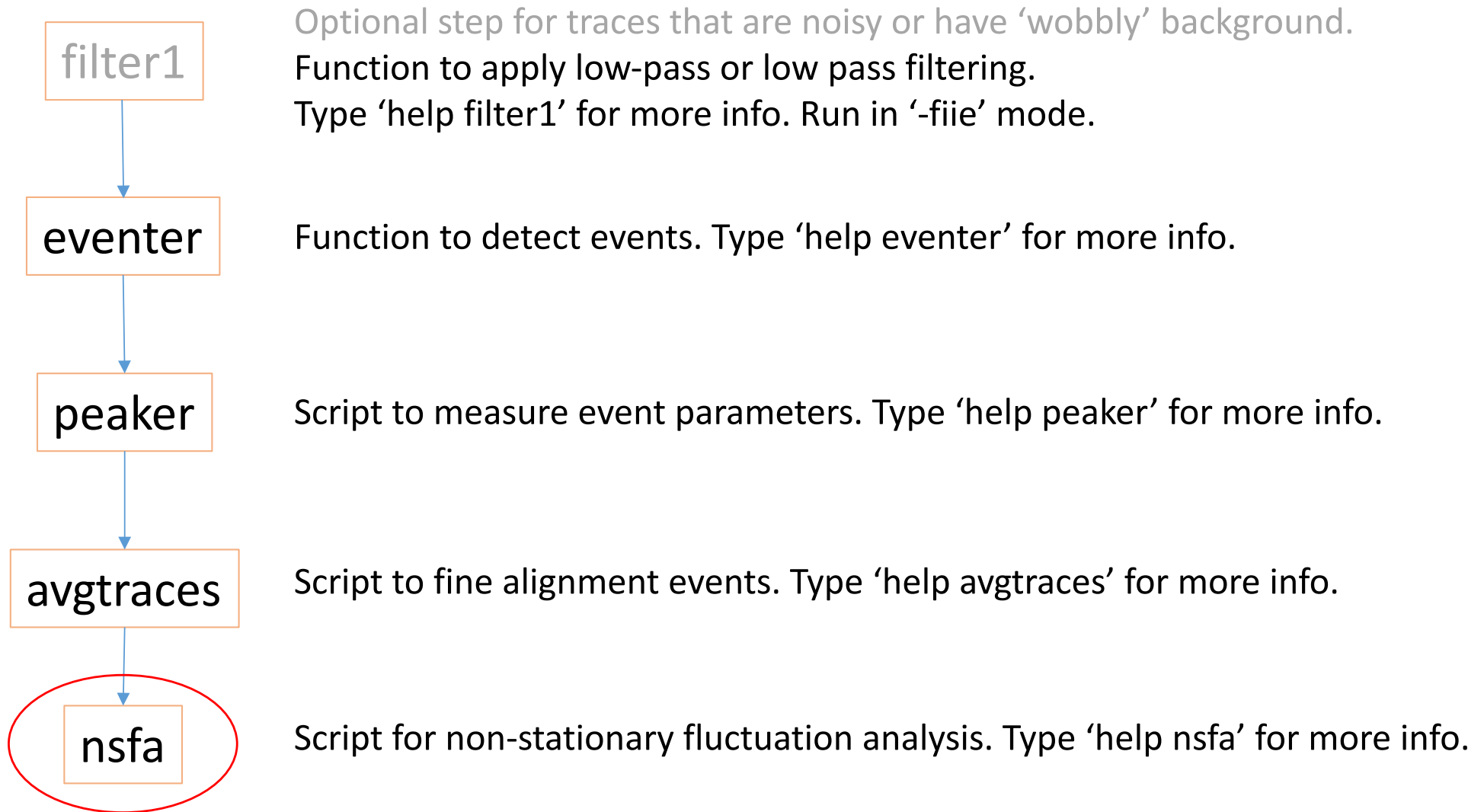
Select which reference point you want to align the events to (default is 1): 1

Remember to clear variables from the workspace before analysing other files

# avgtraces script example output figures



# Event analysis command workflow



# nsfa script command line example

>> nsfa

(1) Conventional

(2) Peak-scaled

Select which analysis to perform (default is 1): 2

Set the -3 dB cut-off of the binomial filter (in kHz, default is none): 1

Number of events discarded for missing data:

1

Enter the number of decay half-times to study peak fluctuations (default is 7):

Number of overlapping events discarded:

0

(1) Perform manual inspection of traces

(2) Load previous manual inspection results

(3) Skip this step

Select an option (default is 1): 1

Number of manually discarded traces:

123

Number of noisy traces discarded:

9

Input a threshold for the maximum event rise time (in ms, default is inf): 0.5

Number of slow rising events discarded:

185

Total number of successful traces:

108

Correlation statistics for peak amplitudes:

Very Weak correlation

Spearman Rank Correlation Coefficient (r):

0.024198

Statistically not significant

Two-tailed P-value from Studentized r coefficient:

0.80368

Correlation statistics for peak rise times:

Very Weak correlation

Spearman Rank Correlation Coefficient (r):

0.15255

Statistically not significant

Two-tailed P-value from Studentized r coefficient:

0.11499

Correlation statistics for peak half-decay times:

Very Weak correlation

Spearman Rank Correlation Coefficient (r):

0.020524

Statistically not significant

Two-tailed P-value from Studentized r coefficient:

0.83302

Manually inspect traces

'x': next trace

'z': previous trace

'SPACE': toggle

delete or keep trace

'ESC': finish inspection

BEWARE that depending on  
your computer platform, you  
may need to change the  
reference numbers for the  
button output variable of ginput  
in the file nsfa.m



Half-decay time (+/- SD, in ms):

2.15 +/- 0.72

Enter the number of bins: 15

Variance of the pre-event baseline noise (in pA<sup>2</sup>):

0.348

Number of excluded bins below the baseline noise level:

1

Enter up to what fraction of the relationship to fit (default is 1): 1

$f(x) = -0.041004 + 1.0132 \cdot x - 0.07562 \cdot x.^2$

Coefficient of determination (R-sq) of the fit:

0.942

Fitted baseline variance (+/- SD, in pA<sup>2</sup>):

-0.041 +/- 0.15

Weighted-mean single channel current (+/- SD, in pA):

-1.01 +/- 0.053

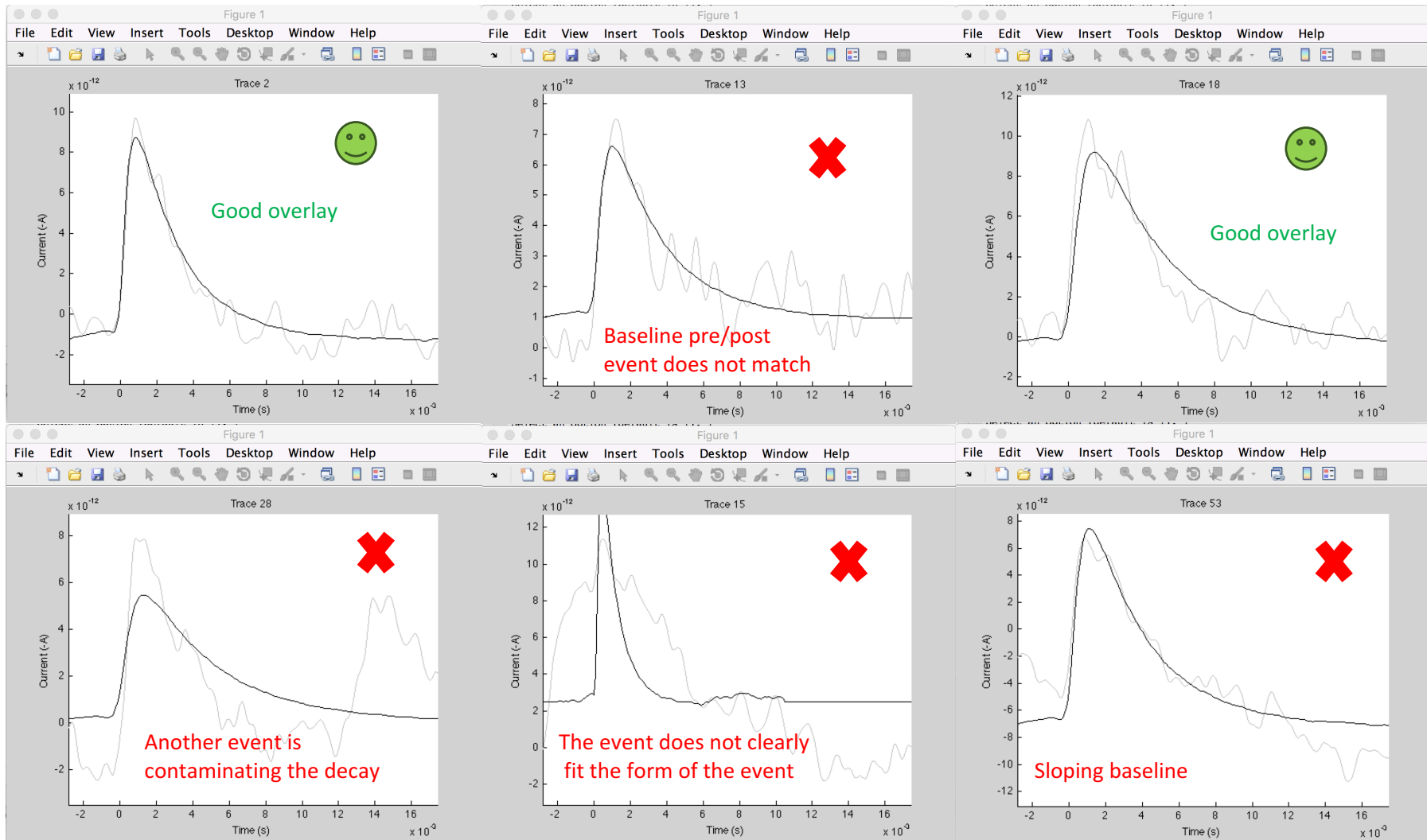
Average number of channels open at peak (+/- SD):

13.2 +/- 0.83

Remember to clear variables from the workspace before analysing other files

# nsfa script: manual event inspection examples

Before performing calculating fluctuations from the mean ensemble event it is necessary to remove traces that could introduce artifacts. Manual inspection of a timecourse-matched template (derived from the ensemble mean) helps the user to review the events



# nsfa script example output figures

