Spike sorting software installation

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1. "Extract" is a standalone C program, copy file folder to hard disk.
2. Matlab files will run with Matlab 5, updated with MATLAB_5.2.1_Updater, running in System 9 or Mac classic mode. The main need for System 9 is for compiled .mex files. To install, copy Matlab file folders to the hard disk and set the Matlab path to all folders and subfolders.

Spike sorting for a continuous wave recording

SAB 7/04

Overview:
The overall process is
1. Start with raw data file and set thresholds using Matlab program **Autosetthresh**.
2. Extract spikes using C program **Extract**.
3. Sort spikes using Matlab program **GroupCW**.

1. Set the thresholds for spike extraction.
    Raw data files should be in Analog Input Block (AIB) format and have names ending in .bin.
    In Matlab, choose >File>Open, select the raw data file folder, then hit [Cancel].
    Type **Autosetthresh.**
    Select threshold multiplier (k). Threshold is set to k*median(abs(v)), where v is the voltage. Between 4 and 5 is a reasonable value. Choose the threshold wisely at this point, setting it too low will mean the software will run much more slowly later.
    Enter an output Threshold file name.
    Select raw data file.
    Select channels, default is 2:63.
    Autosetthresh then writes the thresholds to the output file.
    You can continue to calculate the thresholds for additional data files. A better practice is to keep the thresholds the same for all data files. To do this, open the Threshold file, cut and paste the thresholds for each different data file, and then change the name of the data file at the beginning of each threshold list. Also, check the threshold for the event pulse channel (typically ch.2). Raise it to only select event pulses, 500 works well.
2. Extract is a standalone C program that currently accepts several input files. See example files for precise syntax.
    The input is 'extract.in', a text file, organized as follows, with example filenames:

% Relative path for input (":" = this directory, "::" = up one directory, et
:

% Relative path for output (":" = this directory, "::" = up one directory, etc.)
:

% Relative path & filename for file containing filenames & channels
:selfiles.txt

% Relative path & filename for the goodScans file (.sat file) (including extension)
:stimtimes.sat

%Relative path for threshold file created with ***Autosetthresh*** *(see above)*

:1.thresh

% is a comment character

Several other text files are used:

Selfiles.txt:

This file contains the list of filenames (minus the '.bin') and channel numbers from which snippets will be extracted.

Stimtimes.sat:

This file contains a list of filenames, as well as start and end scan #s. For example if filename 'File1.bin' is 30 s long at 10 kHz, it should be listed in the stimtimes.sat file as: File1 {1 300000}.

Stimtimes.vlv:

This is left over from a previous version/use of the software. An update should remove it. However, one must place the list of filenames with empty brackets {}.

To Run extract, launch the program, and choose 'extract.in' as the input file. This extracts spike snippets.

Next, to extract random snippets by changing the last line of extract.in to 5000, for the number of random snippets per channel. This only need be done with one data file, so comment out (%) all but one data file in selfiles.txt. Then launch extract again.

3. Sort spikes in Matlab.

In Matlab, choose >File>Open, select the raw data file folder, then hit [Cancel].

Start **GroupCW** with the following command**:**

```
groupcw ('<sortoutputfile.mat>',{'<datafile1>','<datafile2>', ...}, ...
        {'<.ssnp file1>','<.ssnp file2>', ...},{'.rsnp file'})
```

GroupCW can be restarted using:

```
groupcw ('<sortoutputfile.mat>')
```

File Formats

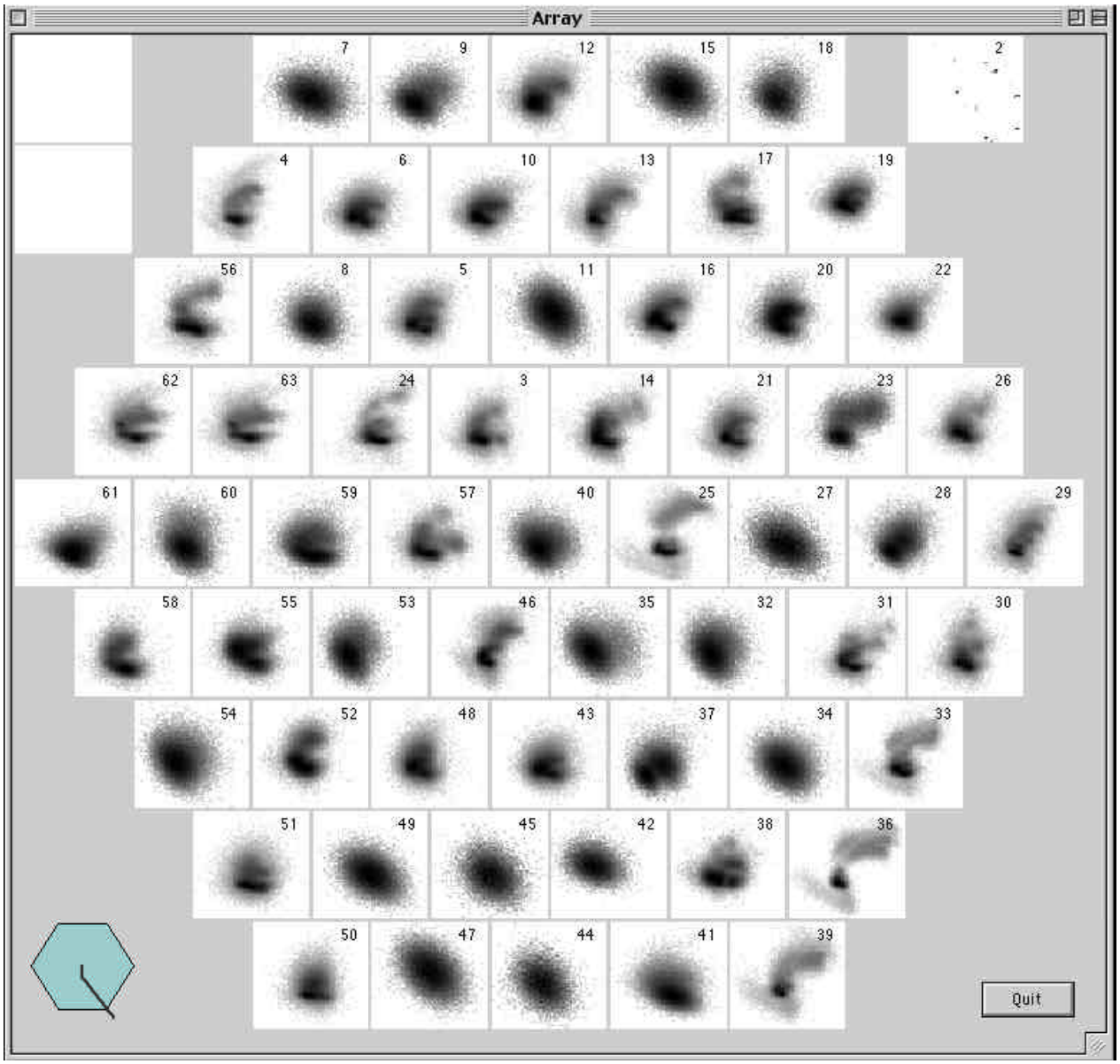
Data files:

Analog Input Block format, described in the Matlab file ReadAIBHeader.m.

Spike files:

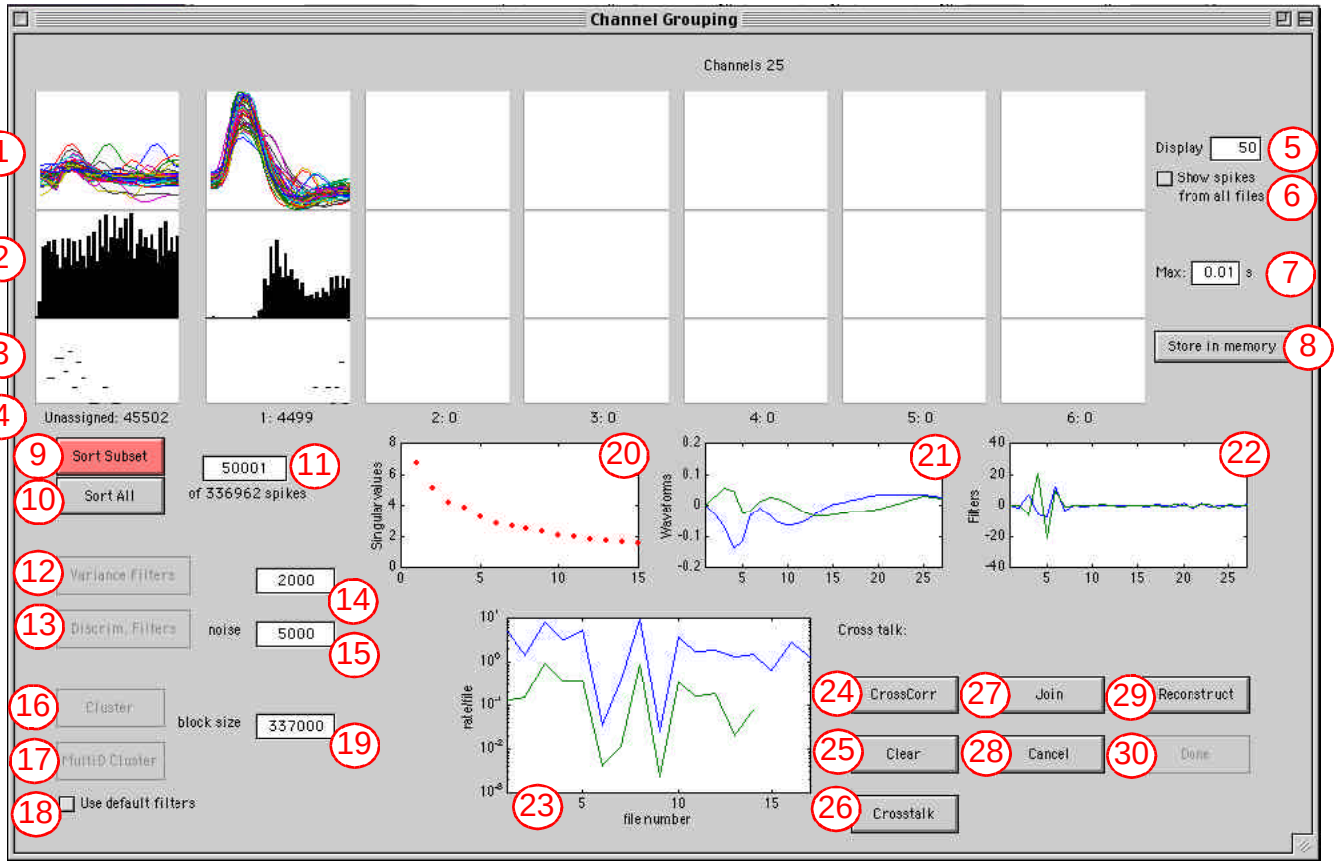
Called "Snippet files", described in the Matlab file ReadSnipHeader.m.

Array window



In the main array window, click on a channel to sort it. First, any previously clustered spikes or crosstalk spikes are removed from that channel, and then the remaining spikes are sorted in the channel grouping window.
Array is displayed in the orientation of the stimulus monitor, (top is top of the stimulus monitor, etc.), with the stimulus monitor to the right of the preparation. Icon at lower left shows the orientation of the array, center electrode trace is indicated.

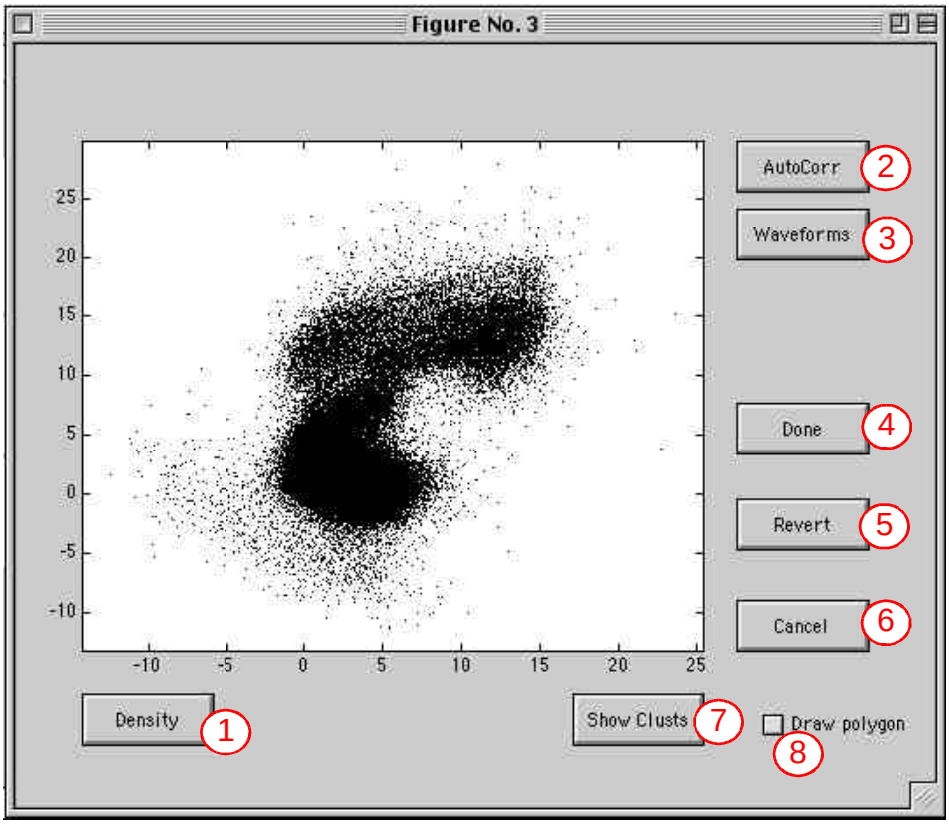
Channel grouping window



1. Sample spike waveforms for unassigned spikes and each defined cell 1-6.
2. Autocorrelation of each defined cell.
3. Amplitude histogram for each defined cell.
4. Number of spikes in each defined cell.
5. Number of sample spike waveforms to display for each cell.
6. Checkbox to display sample waveforms from all file or just one file.
7. Entry box to set maximum time of autocorrelation.
8. When sorting coincident spikes on more than one channel, stores spikes from other channels in memory.
9. Sort a subset of spikes, either the number entered in entry box (11), or the spikes on selected channels.
10. Sort all spikes.
11. Entry box to select the subset of spikes to sort.
12. Create filters from two largest principal components of the selected set of spikes.
13. Create filters to discriminate between selected clusters (Fisher discriminant).
14. Number of spikes used to create principal components filters.
15. Number of random selected waveform segments used to create principal components filters.
16. Cluster selected spikes using current filters, or default filters if checkbox 18 is selected.
17. Cluster spikes using coincident spikes from multiple channels.
18. Use default filters when clustering spikes.
19. Number of spikes to cluster at a time.
20. Singular values for all principal component filters.

21. Waveforms selected by current filters.
22. Current filters.
23. Number of spikes in each file. Each defined cell is a different color.
24. Calculated cross correlation between each pair of defined cells.
25. Clear all defined cells.
26. Calculate crosstalk between the selected spikes on this channel and all other channels.
27. Join two defined cells together.
28. Cancel sorting this channel.
29. Reconstruct recording showing each defined cells. Currently only implemented for one channel, not coincident channels.
30. Finish sorting.

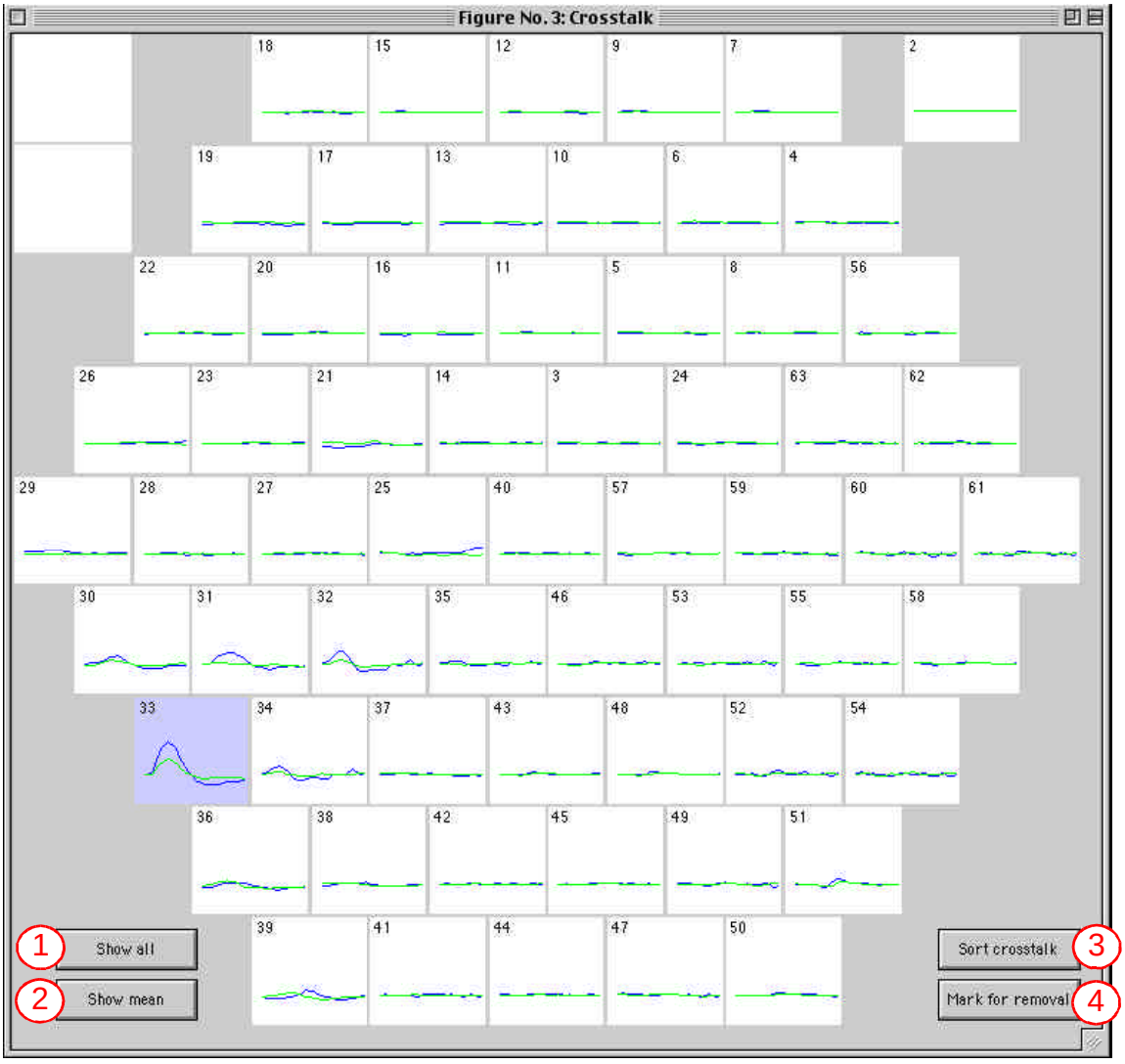
Clustering window



In the clustering window, boxes or polygons are drawn around the spikes fo assign them clusters. After 'Done' (4) is selected, clusters are displayed as defined cells in the channel grouping window.

1.Switch between scatter plot and grayscale plot.
2.Show autocorrelation of currently selected cluster.
3.Open a window to show individual spike waveforms by clicking on each point.
4. Finish drawing clusters.
5. Undo drawn clusters.
6. Quit clustering.
7. Show cluster membership.
8. Checkbox to switch between drawing a box and drawing a polygon.

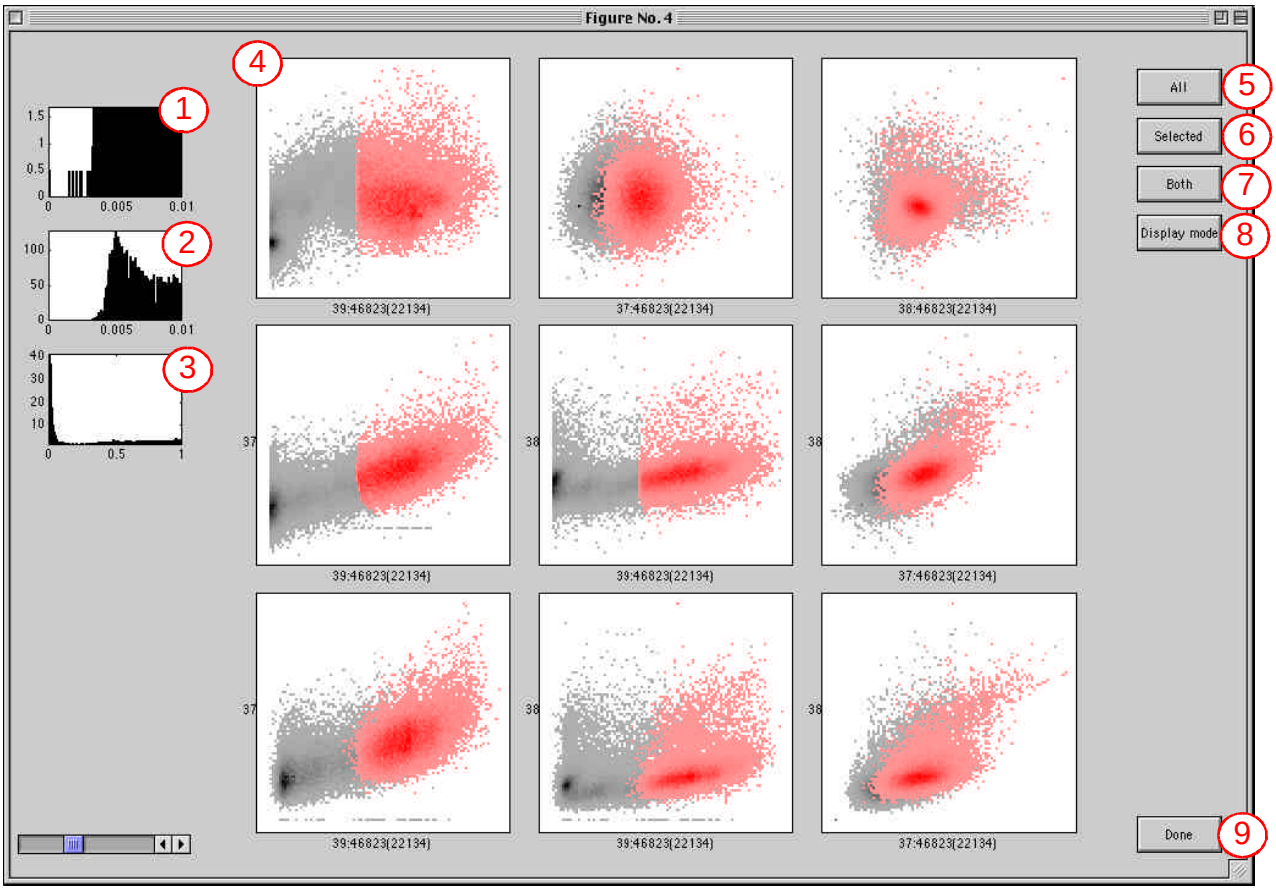
Crosstalk window



In the Channel Grouping Window, spikes are being sorted on one main channel. For the selected spikes on that channel, this window shows spikes coincident in time on all other channels. Different colors are for different cells defined in the channel grouping window. After viewing these waveforms, other channels can be selected by clicking on them. Then sorting proceeds on the Channel Grouping Window using the coincident waveforms (crosstalk) on the selected other channels.
In addition, when sorting in the Channel Grouping Window is finished, coincident waveforms resulting from the same cell should be removed from other channels. To do this, other channels are selected here, then the coincident spikes on these channels are marked for removal (4).

1. Show a sample group of waveforms on all other channels.
2. Show the average waveform on all other channels at the time of a spike on the main channel.
3. Continue sorting in the Channel Grouping Window using the selected channels.
4. Mark coincident spikes on selected channels for removal.

Multidimensional Clustering Window



1. Autocorrelation of selected spikes, 0-0.01 s. Maximum y-value is the average firing firing rate of the selected spikes.
2. Autocorrelation, 0-0.01 s. Maximum y-value is the maximum firing rate.
3. Autocorrelation, 0-1 s.
4. Windows showing spikes projected onto different dimensions. Top row is each individual cell, Filter 2 vs Filter 1.Half of remaining windows are Cell A, Filter 1 vs Cell B Filter 2. Remaining windows are Cell A, peak vs Cell B peak.
5. Show all spikes.
6. Show selected spikes only.
7. Show all spikes with selected spikes colored.
8. Change display mode, scatter plot or grayscale.
9. Finish clustering.

GroupCW file hierarchy

Files in:

parenthesis () are internal functions

brackets [ ] are callback functions

angle brackets <> are compiled mex functions

quotes " indicated cases

} means function calls other functions

```
groupcw
    GetSnipNums
        ReadSnipHeader
            ReadAIHeader
                readChConfig
    setup
        Readfromallchannels
            ReadSnipHeader}
            LoadSnip
                ReadSnipHeader}
        ChooseWaveforms
            [ChooseWfmsCallback]
                'SelectRegion'
                    GetSelRect
                    (GetYData)
                'SelectLine'
                'Delete'
                    (UpdateNumLeft)
                'ThreshStart'
                'ThreshMove'
                'ThreshStop'
                    (GetYData)
                    (UpdateNumLeft)
                'WidthStart'
                'WidthMove'
                'WidthStop'
                'Cancel'
                'Done'
        Build2Filters
            calcFiltFromSnips
                (Subtractmean)
                gsvd
        (calcproj)
            <LoadIndexSnip>
                GetSnipNums}
        makearraywindow
            GetPosition
        GetSnipNums}
        loadproj
        Hist2dcalc
            hist2d
                <hist2dfast>
```

```
arrayplot
    [startsort]}
        Domultichannel
            ReadSnipHeader}
            Domultichanfunctions
                'SetCAxProp'
                'SelectCell'
                'Unselect'
                'SortSubset'
                    getsubset
                'SortAll'
                'Storeinmem'
                    MultiLoadIndexCTMF
                        ReadSnipHeader}
                        <LoadIndexSnip>
                        <loadaibdata>
                    ReadSnipHeader}
                'BuildFilters'
                    (GetSelClust)
                    multiBFI
                        GetSnipNums}
                        BuildRangeMF
                        BuildIndexMF
                        MultiLoadIndexSnippetsMF
                            ReadSnipHeader}
                            <LoadIndexSnip>
                            getsnipsfrommem
                            <loadaibdata>
                        MultiChooseWaveforms
                            [ChooseWfmsCallback]}
                        Build2Filters}
                'DiscrimFilters'
                    (GetSelClust)
                        MultiLoadIndexSnippetsMF}
                        MaxSep
                'Cluster'
                    (GetSelClust)
                     GroupDefaultProj
                        BuildRangeMF
                        BuildIndexMF
                        loadproj
                        (ClusterDefaultProj)
                        ClusterFunctions
                            'DoPolygon'
                                (DeleteClusts)
                                (DisableClusterSelCb)
                                (GetNextClust)
                                GetSelPolygon
                                    'go'
                                    'start'
                                    'firstmove'
                                        (Stayinbounds)
                                    'firstfinish'
```

```
                    'continuing'
                    'delete'
                (Plotpolygons)
                (EnableClusterSelCb)
            'DoBox'
            'SelectCluster'
            'ScatterPlot'
            'DensityPlot'
            'Replot'
            'ShowMembership'
                (ClusterMembers)
            'Clear'
            'Revert'
            'Cancel'
            'Done'
            'KeyTrap'
        ComputeMembership
            <pointsinpolygon>
    GroupMultiChannel
        BuildRangeMF
        BuildIndexMF
        MultiLoadIndexSnippetsMF}
        ClusterSpikeWfms
            Cluster
                ClusterFunctions
            [ClustSWCallback]
            [ClustSPCallback]
        ComputeMembership}
    RebuildUnassigned
'MultiCluster'
    (GetSelClust)
    mgroup
        GetSnipNums}
        loadprojindexed
        getsnipsfrommem
        <loadaibdata>
        addborder
        (densplot)
        [multicluster]
        multiclusterfunctions
            'DoPolygon'
                (DisableClusterSelCb)
                GetSelPolygon}
                (EnableClusterSelCb)
            'SelectCluster'
                (unselect)
                (select)
            'ScatterPlot'
                (plotpolygons)
            'DensityPlot'
                 hist2d}
                 (plotpolygons)
            'Replot'
```

'ShowMembership'
(ClusterMembers)
'Clear'
'grayscale'
'displayselected'
(makecolormap)
'displayall'
'displayboth'
(plotselected)
'displaymode'
'showselected'
(plotac)
'Revert'
'Cancel'
'Done'
RebuildUnassigned
'NumSnips'
'AutoCorrTime'
'UpdateDisplay'
MultiLoadIndexSnippetsMF}
AutoCorrRec
<autocorr>
'DefFiltBox'
'dispsnipsbox'
'CrossCorr'
Crosscorrall
CrossCorrRec
<CrossCorr>
'Clear'
'Delete'
RebuildUnassigned
'Join'
'Recon'
<LoadIndexSnip>
LoadSnipTimes
ReadSnipheader}
ViewReconstruction
<loadaibdata>
SliderWindow
[SliderWindowCB]
'PlotTop'
'Slide'
'Move'
'Stop'
'Select'
'ZoomIn'
'ZoomOut'
'Crosstalk'
getsubset
crosstalkfunctions
ReadSnipheader}
'calculate'
'CTselect'

'showall'
'showmean'
'sortcrosstalk'
'remove'
'Cancel'
'Done'
RemoveCrosstalk
loadsniptimes}
removetimes
(CrossCorrRecRow1)
<CrossCorr>
loadsniptimes}
loadprojindexed
Hist2dcalc}
arrayplot
'DoneUnassigned'
loadsniptimes}
'KeyTrap'
makearraywindow}
arrayplot}
removetimes