

# Wave clus

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## Automatic spike detection and sorting using wavelets and super-paramagnetic clustering.

### Overview:

A large amount of research in neurophysiology is based on the analysis of extracellular potentials recorded with microwires that capture the action potentials (spikes) of neurons in their surroundings. For many applications it is crucial to know which spike correspond to which neuron, namely -spike sorting- and since recent acquisition systems allows the simultaneous recording of hundreds of channels, it is also important to do this automatically (or semi-automatically) and fast. Wave\_clus is a fast and unsupervised algorithm for spike detection and sorting. Although it gives a first unsupervised solution, this can be further modified according to the experimenters' preference (semi-automatic sorting).

### Distribution and reference:

Wave\_clus is free (and therefore without any warranty) for any non commercial applications. For any commercial application please contact the author ([rqqg1@le.ac.uk](mailto:rqqg1@le.ac.uk)). You can refer to this algorithm just by citing the paper where it is described:

**Unsupervised spike detection and sorting with wavelets and superparamagnetic clustering.**

R. Quian Quiroga, Z. Nadasdy and Y. Ben-Shaul

Neural Computation 16, 1661-1687; 2004.

For a non technical reference about spike sorting see:

R. Quian Quiroga

**Scholarpedia** 2 (12): 3583. 2007

This software was developed by Rodrigo Quian Quiroga. Matias Ison, Juan Martinez-Gomez and Carlos Pedreira contributed to some of the features of the new version.

## Requirements:

Wave\_clus runs under windows, linux and Mac. It requires **matlab 6.5 (R13)** or higher. It uses the function cluster.exe, provided by [Eytan Domani](#), which is an executable that does the super-paramagnetic clustering of the data. In the new release, the wavelet and the signal processing toolboxes are not necessary anymore.

## Brief description of the code and parameters:

Each DataType has an associated **set\_parameters** file in wave\_clus/Parameters\_files, or at the beginning of the batch files. Parameters that can be set by the user appear in red.

i) Spike detection: it first filters the continuous data between **fmin** and **fmax** (as set in the parameters file). Then, it calculates an optimal amplitude threshold, which is set as **stdmin** times the estimated standard deviation of the noise (see the paper for details and advantages of this estimation). It also calculates a maximum threshold, **stdmax**, to avoid high amplitude artifacts. The parameter **detection** sets the threshold to be either positive, negative, or both. For each spike, **w\_pre** datapoints before and **w\_post** datapoints after the spike peak are stored. Spike alignment is done after interpolation of the spike shape with cubic splines. The interpolation is set with the parameters **interpolation** and **int\_factor**. To avoid double detections, spikes should be separated at least a certain number of datapoints, given by **ref**. The sampling rate is set with the parameter **sr**. The parameter **segments** is for reading the data in pieces in order to avoid memory overloads. The code reads **tmax** seconds of data starting from **tmin**.

ii) Feature extraction: it uses a selection of wavelet coefficients chosen with a Kolmogorov Smirnov test of Normality. The parameter **inputs** sets the total number of coefficients and **scales** is the number of scales for the wavelet decomposition. Alternatively, the code allows the use of the first 3 principal components as the spike features instead of wavelets with the parameter **features** (see the paper for comparisons of the different features). In some cases this will be the first step of the analysis, since many systems already give the detected spikes and not the continuous data.

iii) Clustering: uses super-paramagnetic clustering (SPC), an automatic clustering algorithm based on ideas from statistical mechanics developed by the group of [Eytan Domani](#). See the paper for a description of its advantages for spike sorting, and papers by the group of Eytan Domani for other applications. The main variable to change with super-paramagnetic clustering is the ‘temperature’ (see paper). At low temperatures, all spikes will be assigned to a single cluster and at the high temperature limit; each spike will form a single cluster. The optimal temperature for clustering lies in between this two extremes (in the super-paramagnetic regime). In practice, the optimal temperature is set as the largest temperature for which a cluster with at least **min\_clus** members appears. The code actually calculates results for all temperatures between **mintemp** and **maxtemp** in steps of **tempstep**. Changing the temperature is usually the main supervised correction done after clustering, but since results are already calculated for all temperatures in this range, it only implies loading and saving a different set of results.

iv) Force membership: In many cases, points far from any cluster or in between two clusters are not assigned to any cluster. The function **force\_membership**, (button **Force** in the GUI) will assign

these points to the closest cluster unless they are too far. The algorithms in the Force files subdir were developed in collaboration with [Casimir Wierzynski](#) at Caltech.

v) Template matching: Wave\_clus also has the option of doing template matching after **max\_spk** number of spikes, if the option **match** is set to 'y'. That means that wave\_clus will do SPC clustering for the first **max\_spk** spikes and the rest of the spikes (if any) will be assigned via template matching. The principle of template matching is the same as the one used for **Force\_membership**. The spikes within max\_spk define the templates and the remaining spikes should be assigned with the Force button. Note that template matching can save a lot of time if there are too many spikes to be clustered. In particular, SPC starts taking too long for more than ~30000 spikes.

vi) Fix buttons: This option is for fixing a template before changing the temperature or the force button. It is useful if a selection of clusters at different temperatures is needed. For example, you may want to keep, say, cluster 2 at temperature 0.5 and clusters 1 and 2 at temperature 1.0. Then you click on the **fix button** for cluster 2 at temperature 0.5 and then change the temperature to 1.0. The cluster you fixed will appear as a new cluster 3 at temperature 1.0. You may also don't want to use the force option for all clusters. Then, fix those clusters you want to keep and click the force button.

vii) Undo: This button restores the previous wave\_clus state.

viii) Merge: Merges two or more clusters. The clusters to be merged are selected with the fix buttons.

ix) Polytrodes: Wave\_clus is also able to do spike sorting from polytrodes (including tetrodes), concatenating the spikes from different channels and taking them as a single shape for clustering purposes. First, write the name of the files you want to process as polytrode (in ascii format) in a text file named as polytrode1.txt. The spike detection is done by typing in the matlab command window the batch file Get\_spikes\_pol(1) which will produce the output file polytrode1\_spikes.mat containing the spike times and shapes. Then execute Do\_clustering\_pol(1) which produces the file times\_polytrode1.mat. In case you may want to process for example 2 polytrodes you just have to type Get\_spikes\_pol(1:2) and Do\_clustering\_pol(1:2). You can visualize and change the clustering results with GUI by loading times\_polytrode1.mat using the menu option ASCII (pre-clustered). Using the Plot\_polytrodes button you will be able to visualize the clusters in each channel. The Plot all projections gives the peak amplitudes of the spikes for each channel against the others. In case of CSC data, write the name of the files including the extension (e.g. CH1.CSC) in a text file named also as polytorde1.txt and follow the same steps described to process ascii data.

## Data Input and Output:

Data input is according to each of the **DataType** options. In each case, the parameters used are set in the corresponding `set_parameters` file in the subdir `wave_clus/Parameters_files`. The **CSC** and **Sc** options are for data collected with Neuralynx systems. An exemplary file is given in `wave_clus/Sample_data/UCLA_data/CSC4.Ncs`. The output of `wave_clus` (obtained either using the **Save clusters** button in the GUI or the one given automatically by the batch files) is `times_[filename].mat`, which is a matlab file containing the following variables: **par** (parameters used for clustering), **spikes** (a matrix with the spike shapes), **inspk** (a matrix with the features of the spike shapes) and **cluster\_class** (a matrix with the clustering results). The variable `cluster_class` has 2 columns and *nspk* rows (*nspk* is the number of spikes). The first column is the cluster class, with integers denoting the clusters membership and a value of 0 for those spikes not assigned to any cluster. The second column is the spike times in ms.

## Getting started:

i) Copy the `wave_clus.zip` file into your hard drive and unzip it. This will create a `wave_clus` directory with corresponding subdirs. If you want also to get the simulated data files (the ones used in the Neural Computation paper), download the file `Simulator.zip` in the `wave_clus/Sample_data` subdirectory and unzip it there. This will create the subdir `Simulator` with all the files inside it. If you want to get the real human recording, download `UCLA_data.zip` in `wave_clus/Sample_data` and unzip it there. This will create the subdir `UCLA_data` with a 30' recording inside it. Delete the .zip files once uncompressed. In matlab go to the menu **File/Set Path** and add the directory `wave_clus` with subfolders to the matlab path. Copy the font in the subdir `wave_clus/wave_clus_font` into your windows/fonts/ directory. Now you are ready to use `wave_clus`.

ii) For starting the GUI just type **wave\_clus** in your matlab command line. You can start playing with the simulated data used in the Neural Computation paper by choosing the option **Simulator** in the **DataType** menu. Now you can load the simulated data (with the **Load Data** button) from the subdir `wave_clus/Sample_Data/Simulator` and compare the results with the ones of the paper. You won't necessarily get exactly the same results, since the SPC clustering is an stochastic (i.e. not deterministic) method. Moreover, if you run the same clustering twice you can get slightly different results (but in general is very robust). Check the file **set\_parameters\_simulation** in the subdir `wave_clus/Parameters_files` for the parameters settings for these data.

iii) The file `CSC4` is an example of a 30' multiunit recording from a human epileptic patient. This data was collected at the lab of Itzhak Fried at UCLA, using a Neuralynx system (Tucson, Arizona). If you have a Neuralynx system, you can directly use the **DataType** options **CSC**, **CSC (pre-clustered)**, **Sc** and **Sc (pre-clustered)** with your data. To cluster this data use the option **CSC** in **DataType** and load the data. Alternatively, you can load already clustered data with the option **CSC (pre-clustered)** in **DataType**. This is one nice feature of `wave_clus`, that you can save all possible clustering options and then you just restore different options without recalculating everything (see how to use the batch files below). If you use the option **Plot\_average** instead of **Plot\_all**, results are restored much faster since plotting all the spikes may take too long. It may

well happen that there is not a unique temperature for getting all the clusters (e.g. 1 cluster appears at temp=0.6 and another one at temp=0.7). If this is the case, you can easily obtain all the clusters by fixing cluster 1 at temp=0.6 and then go to the second temperature.

iv) The **ASCII** options in the **DataType** menu are for loading matlab files. These are the ones that you will most likely be using for your data. The option **ASCII** is for continuous data, the option **ASCII spikes** is for clustering spike shapes that have already been detected (e.g. detected on-line by the acquisition system). In both cases there is also an option **(pre-clustered)** to load results previously calculated with the batch files (the GUI saves the output but doesn't save the clustering results for all temperatures). The input data should be a matlab file (extension .mat). It should be either a vector named **data** for **ASCII** files or a matrix named **spikes** (nr. of spikes x length(spike shapes)) plus a vector **index** with the spike times in ms for the **ASCII spikes** option. Before starting don't forget to set the proper sampling rate **sr** in the file **set\_parameters\_ascii**. In the subdir **wave\_clus/Sample\_data** there is a **test.mat** file with a short segment of simulated data that can be loaded using the **ASCII** option and **test1\_spikes.mat** that can be loaded with the **ASCII spikes** option.

### Batch files:

Since **wave\_clus** gives an unsupervised clustering of the data, it is possible to run batch processes that will go through several files and save the results. You can later restore the results in the GUI and change them, if needed, loading the data with the **(pre-clustered)** option. The nice thing is that you don't need to calculate everything again. **Wave\_clus** saves the results for all possible temperatures, so, changing the temperature (which is the main parameter to be changed, if needed) means just loading another set of results. There are mainly 2 batch files: **Get\_spikes** (which does the spike detection) and **Do\_clustering** (which does the spike sorting). **Get\_spikes** reads the continuous data and outputs a file **[filename]\_spikes** with the variables **index** (the spike times in ms) and **spikes** with the spike\_shapes. If you already have the spike shapes, you don't have to run **Get\_spikes**, but you should have the same structure as the one saved by **Get\_spikes**. **Do\_clustering** inputs the **[filename]\_spikes** files and does the clustering automatically. In this case, for getting the best out of **wave\_clus** spikes should be aligned having the maximum (minimum) of all them in the same sample. In case spikes have been detected with others method and are not, the function **spike\_alignment\_batch** produces a file **[filename]\_align.mat** with the aligned spikes. The results are saved in the file **times\_[filename].mat**, which has the same structure as the one saved with the GUI. Although it will save and print results with the automatically chosen temperature, it stores results for all temperatures, so that it can be easily changed later with the GUI. Both **Do\_clustering** and **Get\_spikes** go through all the files specified in **files.txt**.

### Comments and updates:

If you have any comments please send them to me at [rqqg1@le.ac.uk](mailto:rqqg1@le.ac.uk). I really hope this algorithm will be useful for you. If it does, or if for some reason is not adequate for your data please let me know. I can't promise that I can introduce suggestions immediately, but I'll try to do it in a

reasonable time. Also let me know by email if you want to keep updated on the release of any new version, related paper, etc.

### **New features in version 2.0:**

- ‘Undo’ button.
- ‘Merge’ button.
- Spike sorting for tetrodes and Polytrodes.
- More auxiliary windows that allow the analysis of up to 33 clusters.
- Cluster marking in the temperature diagram that allow to track the origin of the selected clusters.
- Wavelet toolbox and signal processing toolbox no longer necessary.
- Runs under Windows, Linux , UNIX or Mac.
- Variable random options: Allows specifying the random seed of stochastic algorithm or takes seed from the computers internal clock (default)
- ‘Plot all projections’ button: Plot the projections of the 10 Wavelet Coefficients with larger deviation from normality.
- Fix buttons allow taking clusters at different temperatures.
- Many minor bugs fixed.

## FAQs:

### - What should I change if I don't like the automatic results?

The main parameter to change is the **temperature**. You can also use the **force** button to add some of the non-clustered spikes to the already defined clusters.

### - How can I run wave\_clus faster?

There are few tricks to run faster. First, if you have too many spikes, you can do clustering on a first segment that will define the clusters, and assign the remaining spikes via **template matching** (the templates being the clusters defined in the first segment). This is set with the parameter **max\_spk**. You can also use the option **plot\_average** instead of **plot\_all**, since plotting all spike shapes may take too long. If you just want to check a short segment of the file you can do this using the parameter **tmax**.

### - How can I avoid high temperatures?

Sometimes wave\_clus chooses high temperatures that tend to overcluster the data. To avoid picking up high temperatures, you can set the parameter **min\_clus** to a larger number.

### - What are the Accept / Reject?

Accept saves the clusters by assigning a corresponding integer (say 1 for the first cluster, etc.). Reject gives the cluster a value of 0; i.e. it will merge it with the non-clustered spikes.

### - What are the numbers appearing at the top and bottom of each cluster?

The top number is the number of spikes for the cluster. The bottom one is the number of spikes with an inter-spike-interval (ISI) of less than 3ms. A relatively large number of spikes within 3ms ISI is a sign of a multiunit cluster.

### - What happens if I have more than 3 clusters?

In this case as many extra figures as necessary will be generated (starting from Figure 10).

### - Why the spike features do not show the clusters well separated?

Wave\_clus is plotting only the first 2 wavelet coefficients. It may well happen that other coefficients will separate the clusters even better. You can actually plot 45 projections, corresponding to 10 wavelet coefficients, with the Plot all projections button.

### - Can I edit the plots?

Yes, you can edit, zoom, copy and paste, etc each plot using the Tools menu at the top of the GUI.

### - What is the set\_parameters button?

So far it has no function... Sorry.

### - I get a memory overflow error when loading the data, what can I do?

Just use more **segments** for loading the data in smaller pieces.