"TrackUSF"

A software for analysis of rodents' Ultra-Sonic vocalizations

"TrackUSFs" is a software that analyze ultrasonic vocalization activity in audio clips. It is doing so by detecting ultra-sonic fragments of 6 ms duration within each clip and comparing these fragments between a collection of clips that were collected during an experiment. It is also useful for separating non-vocal strong sounds fragments (such as noise from scratches of the cage floor) from the ultrasonic vocal ones. For more details, please refer to the paper:

Netser S., Nahardiya G., Weiss-Dicker G., Dadush R., Yizhaq Goussha, Harony-Nicolas H., Lior Cohen, Crammer K., and Wagner S. *TrackUSF, a novel methodology for fully automated analysis of ultrasonic vocalizations, reveals modified social communication in a rat model of autism.*

The software was written in Matlab (2017a) and embedded the algorithm described in the paper above in a user-friendly graphical user interface (GUI). It was tested using both a standard HP-i7 computer (i7-4790 CPU @ 3.60 GHz, 8.0GB RAM, windows 7) and an HP workstation (HP Z6 G4 workstation, Xeon® Silver 4108 CPU @ 1.80GHz, 32GB RAM, windows 10).

Getting ready for using TrackUSFs:

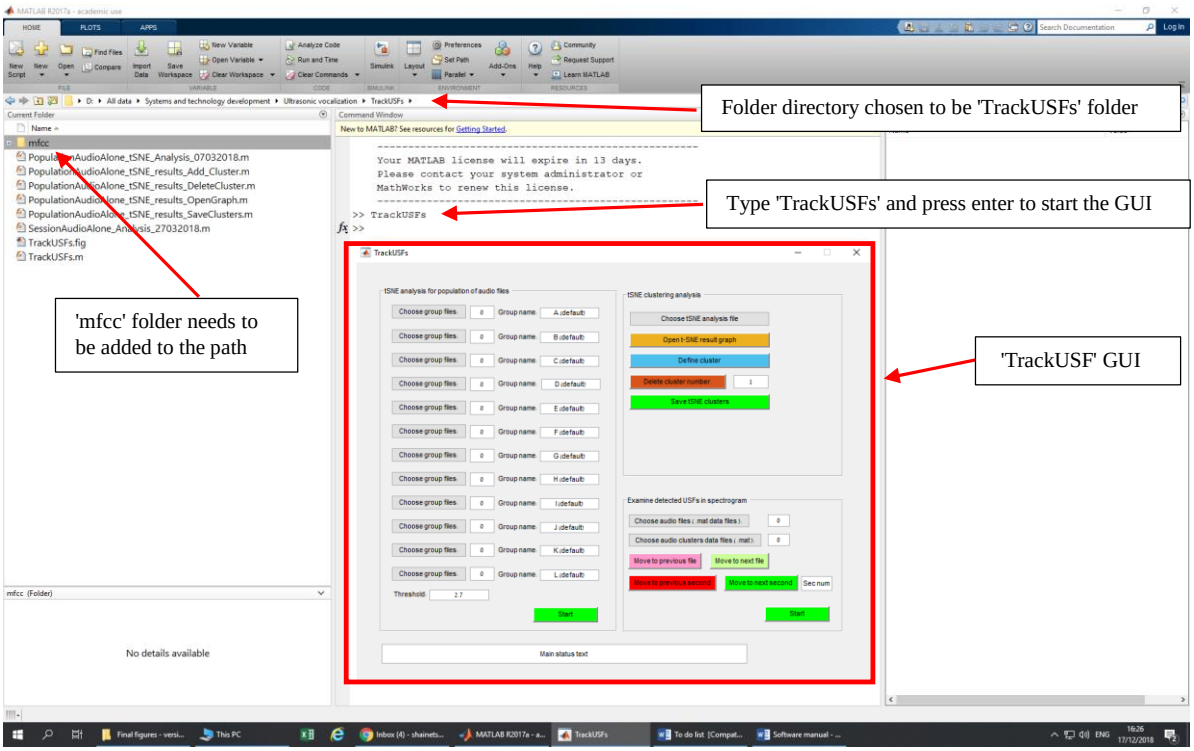
This manual assumes that you have already installed Matlab (2017a or later) on your computer.

1. Download the software folder from GitHub (https://github.com/shainetser/TrackUSF) to your computer.
2. Start Matlab and choose the folder directory to be the one you downloaded TrackUSFs software to.
3. Add path to the "mfcc" folder located inside the main software folder: In the "Current Folder" window, right click on the "mfcc" folder, then "Add to path→Selected folders and subfolders" (This step should be done every time you start Matlab, unless you set the path permanently. For more instructions, please refer to Matlab help).

Using TrackUSF:

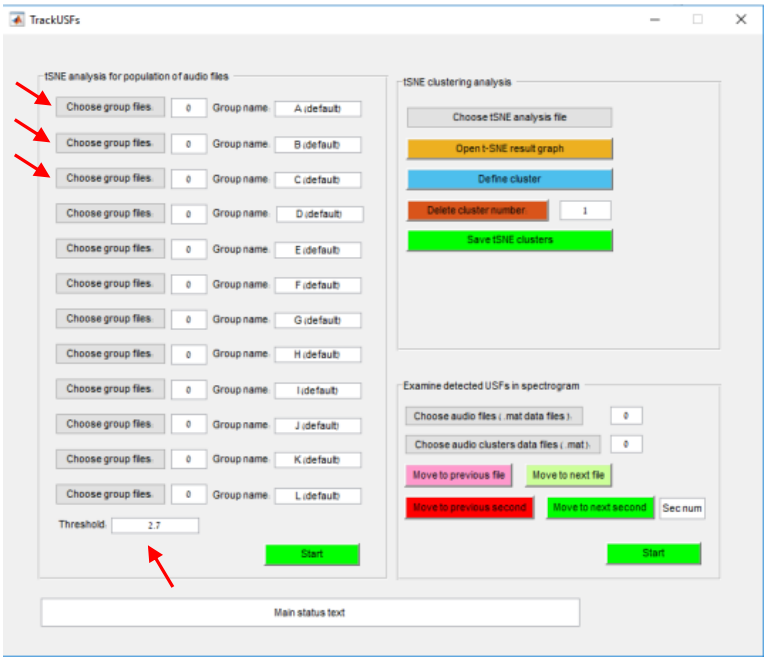
- Type in the command window "TrackUSFs" and press enter.

  The Graphical User Interface (GUI) will open:



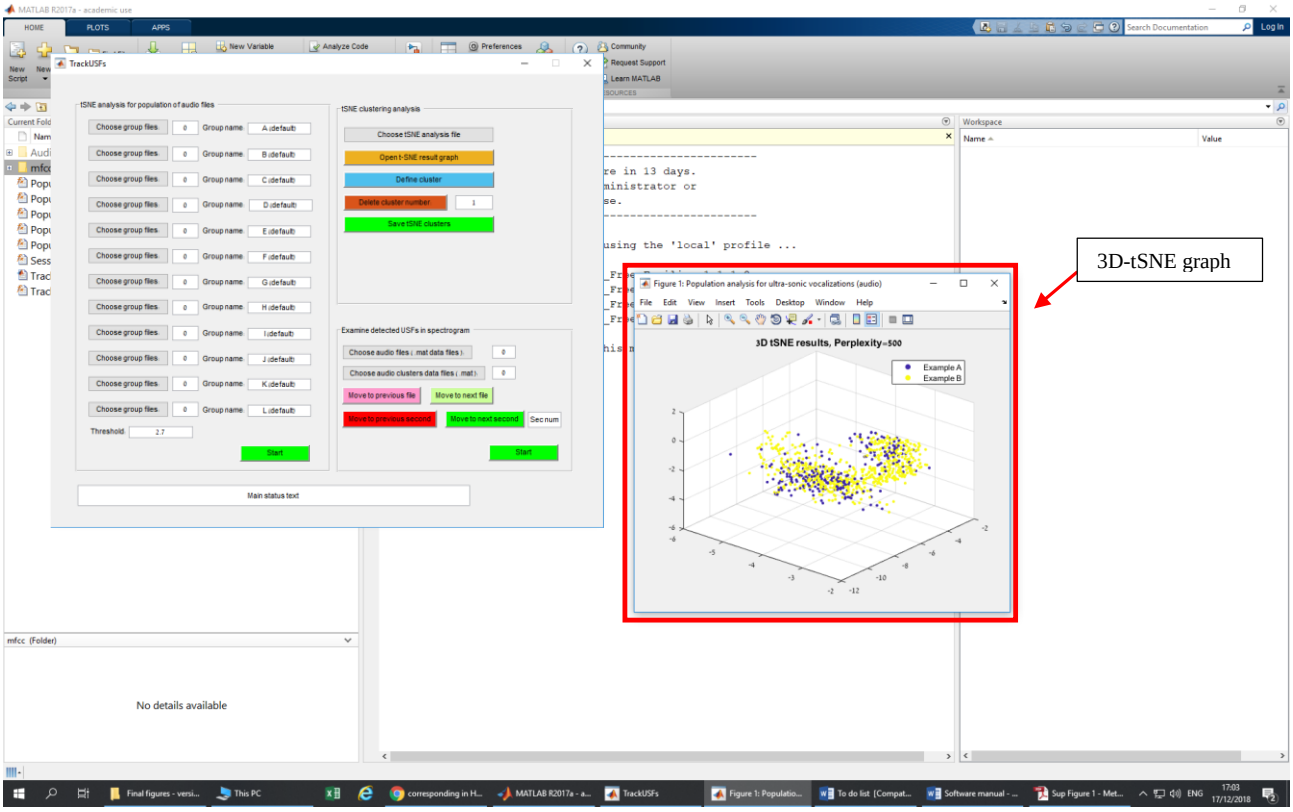
- In the "tSNE analysis for population of audio files" panel upload all audio ('WAV' format) files you would like to analyze for each group separately and name it accordingly.

  Set the threshold – if you are not sure, please look for more details in Figure 2A of the paper mentioned above. It is recommended to start with a high threshold of more than 3 (arbitrary units) and then lower it according to the computer performances.



When you are done, press "start" and you will be asked to choose a folder for saving the results. Choose a folder name and location to save it and press "save".

When the analysis is finished the software will create a 3D-tSNE graph of the results. For example:



You will also get a folder containing:

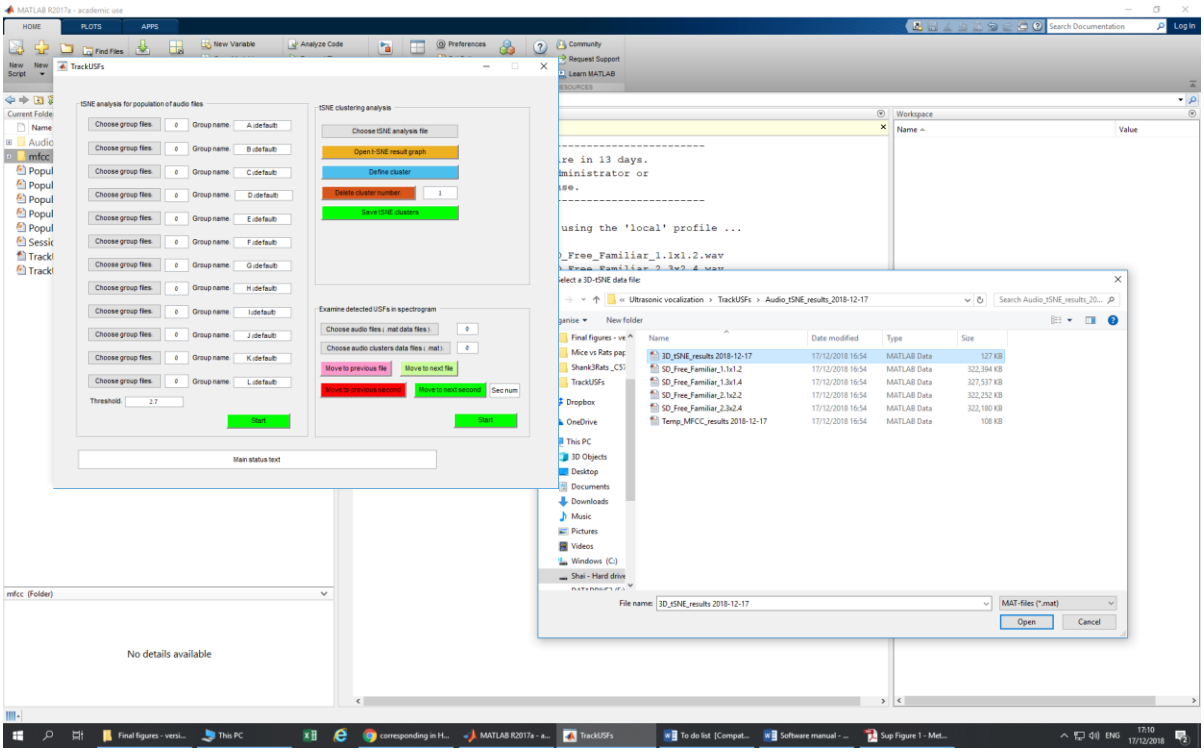
1) The tSNE result file (for example: '3D_tSNE_results 2018-12-17', '.mat' format).

2) Audio data files corresponding for each file loaded before. This files contains the audio data in a '.mat' format and will be used later on for visualization of the results of each file.

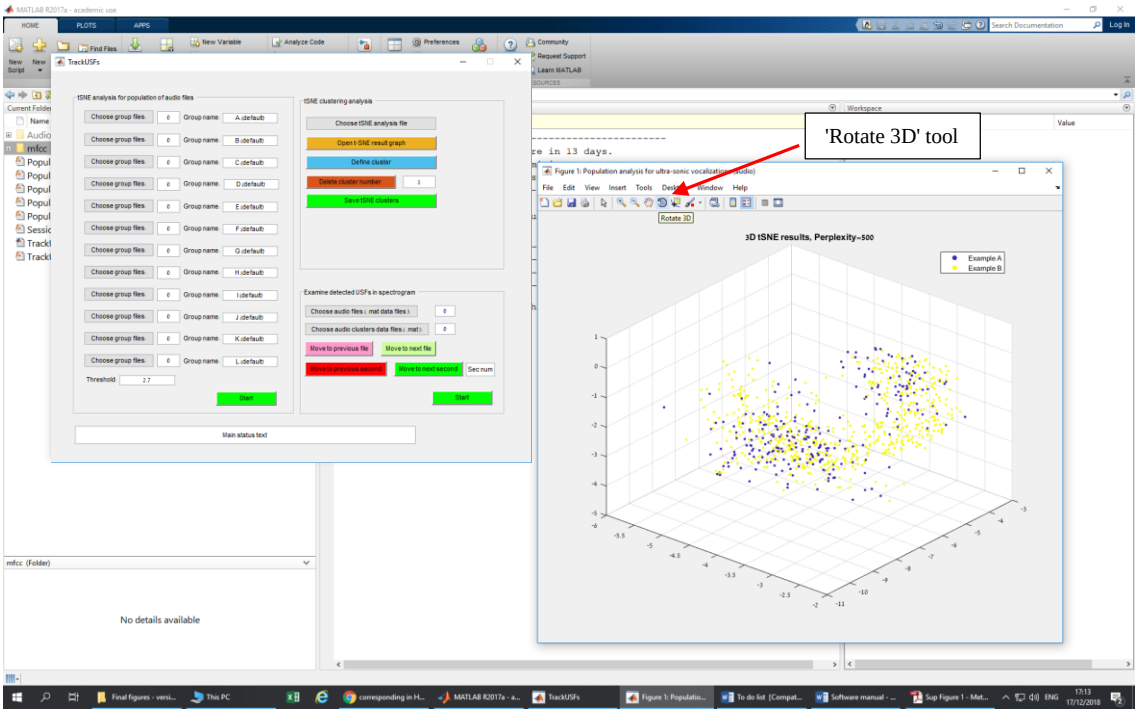
3) An MFCC data file ('.mat' file).

At this point it is recommended to close the result figure obtained.

- In order to register the fragments obtained in the 3D-tSNE figure into different clusters, move to the "tSNE clustering analysis" panel and press the "choose tSNE analysis file".
  Choose the tSNE result file saved before:

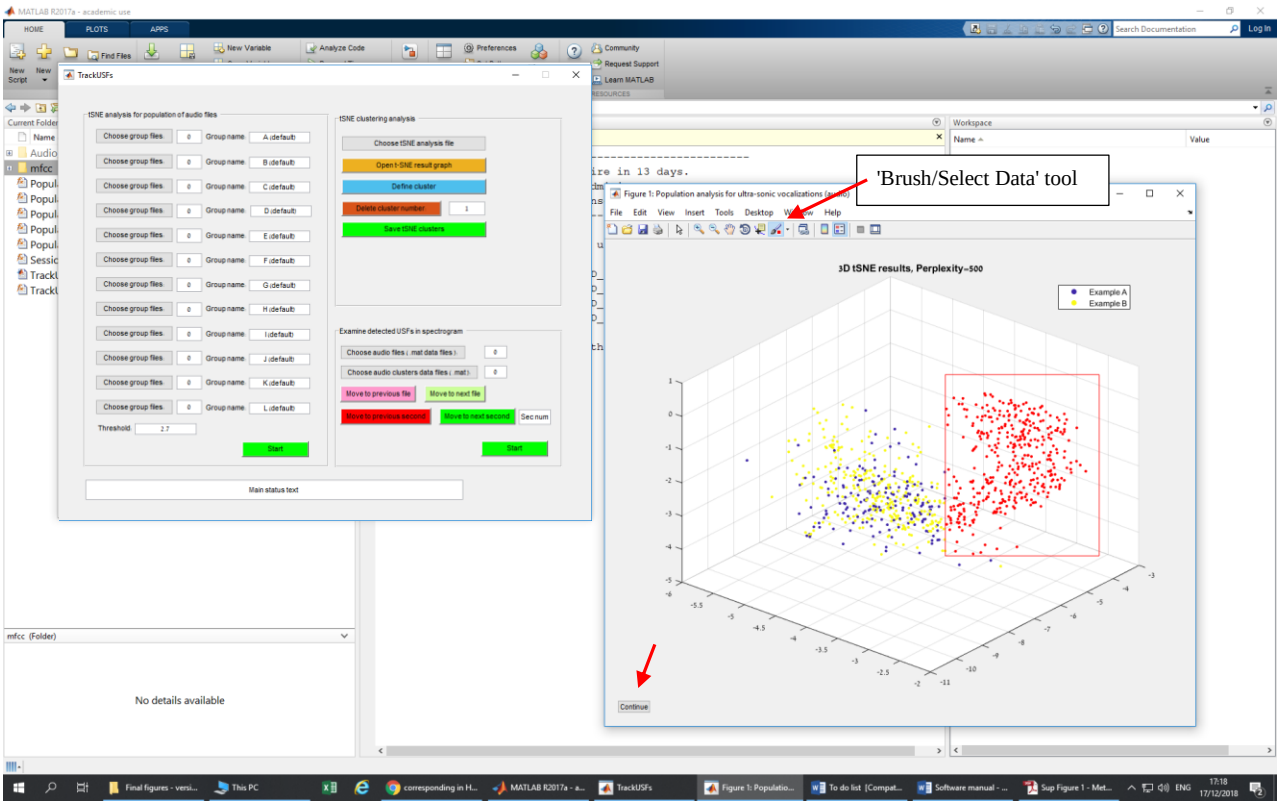


Now, press "open tSNE result graph" and the tSNE result figure will open (identical to the one obtained by the analysis done before). At this point you can rotate the 3D graph using Matlab "Rotate 3D" tool:



Try to visualize the optional clusters.

When you finish and would like to register a specific group of fragments (dots) into a specific cluster, press the "Define cluster" button and the "Brush/Select Data" tool of Matlab will start. At this point you can use it to mark a cluster of fragments.



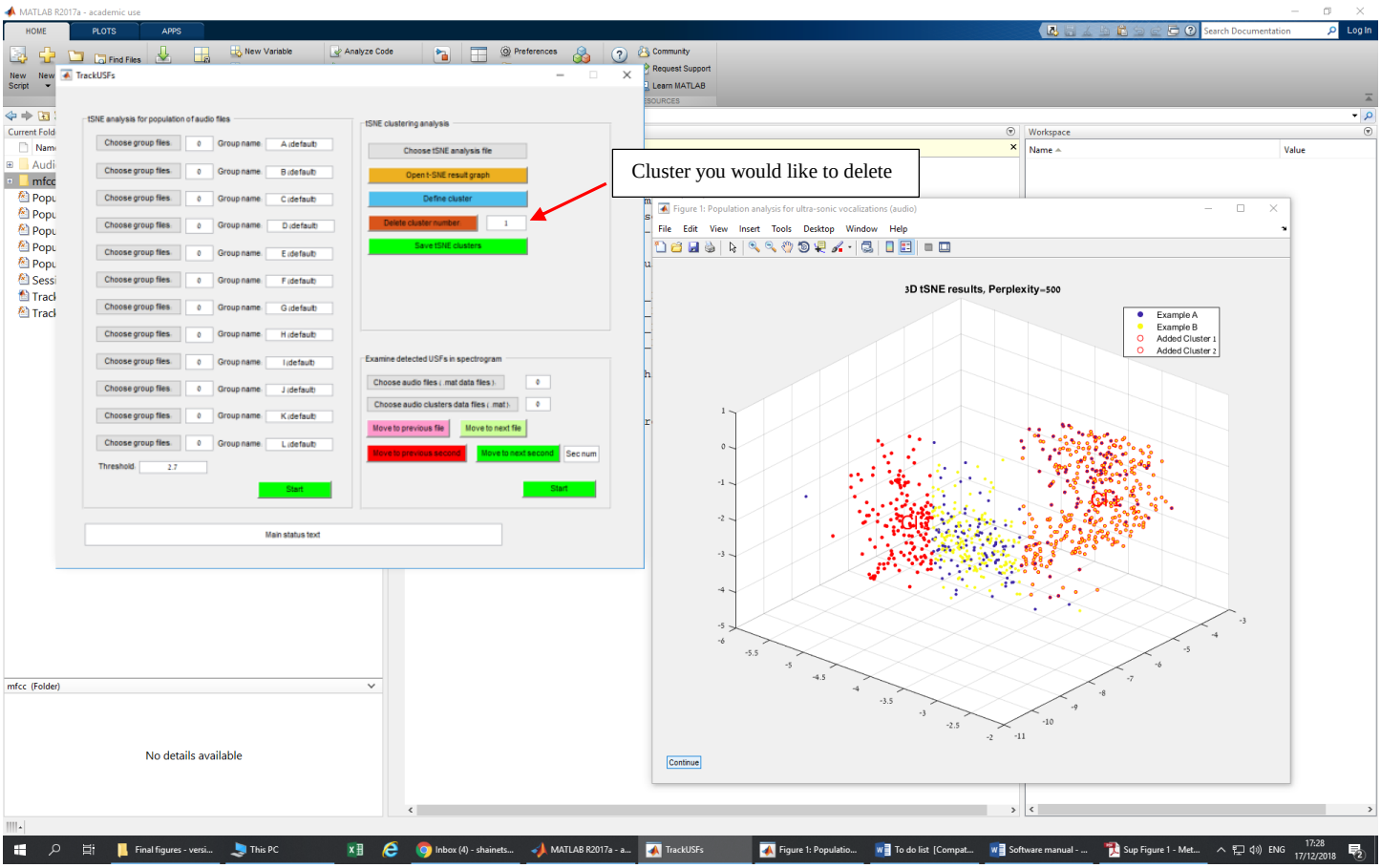
When done, press the "Continue" Button at the lower left corner of the figure.

The software then asks you if you would like to save this cluster. Press 'Yes' or 'No'.

Then you will be asked if you would like to define an additional cluster. If you press 'Yes', you can immediately start marking the additional cluster, as the 'Brush/Select Data' tool is immediately active again. If you press 'No', you can later on press the 'Define cluster' button again to mark more clusters.

It is important to note that you can always switch from the '3D Rotate' tool to the 'Brush/Select Data' tool to get a better visualization of the cluster you would like to define, and only then define it.

In case you would like to delete a cluster, enter the cluster number you would like to delete and press 'Delete cluster number' button.



When finished, press the 'Save tSNE vocalization groups' button and save the results under a specific folder. This folder will contain:

1) The tSNE figure with the defined fragments on it (according to the clusters they are registered to).

2) A clusters data file ('.mat' format) for each original file loaded. You will use them in the next section.

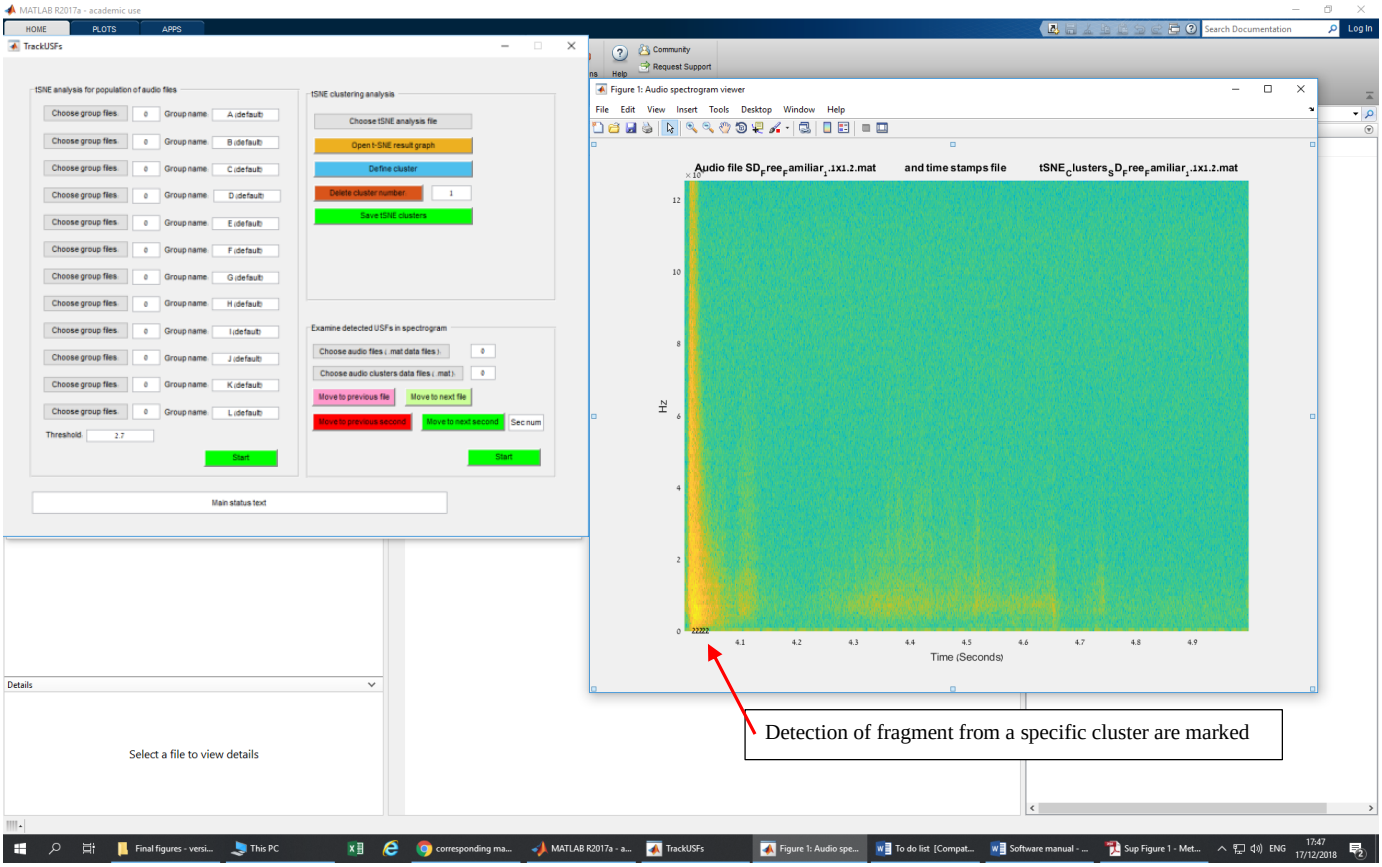
   This files contain the time points of detected fragments (from the beginning of the recorded file), according to the clusters they were registered to. This can be used for further analysis and statistics.

- Finally, for visualization of the detected fragments and their categorization to the different clusters use the 'Examine detected USFs in spectrogram' panel.

  Press the 'Choose audio files (.mat data files)' button and choose one or several files generated in the first part of the analysis (obtained when running the 3D-tSNE analysis in the 'tSNE analysis for population of audio files' panel). Next, press the 'Choose audio clusters data files (.mat)' button and choose one or several files generated in the second part of the analysis (obtained when defining the clusters in the 'tSNE clustering analysis' panel).

  Important: The two lists of files chosen above must be chosen in a corresponding manner, such that the audio data files correspond to the clusters data files.

Pressing 'Start' will result with the opening of an additional figure with a spectrogram of the first second of the first file in the list (In the example below detections of cluster 2 fragments are marked at the bottom of the fifth second):



You can estimate the detection by moving between files and seconds of the recordings using the buttons: 'Move to next file', 'move to previous file', 'move to next second' or 'move to previous second'. You can also select a specific second by typing its number and pressing enter.

All variations of the software are deposited in GitHub under the following links:
https://github.com/shainetser/TrackUSF