hfoGUIV2 Tutorial

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1 Background

1.1 Introduction

Electrophysiology has allowed people to correlate specific electrical signals to various behaviors/pathologies. Electrophysiologists often characterize signals depending on their frequency, expressed as Hertz (repetitions of the signal per second). An example of a frequency band correlating with a behavior is theta in a mouse. It has been shown that the frequency of the theta signal is directly proportional to the speed of the mouse. In another words, as the animal moves at a faster pace, the frequency of the theta band increases. Hypothetically the animal could have a 5 Hz rhythm at a walking pace, and then a sprint would be reflected in the EEG as maybe an 8 Hz rhythm.

Some examples of what different frequency bands look like are the following:

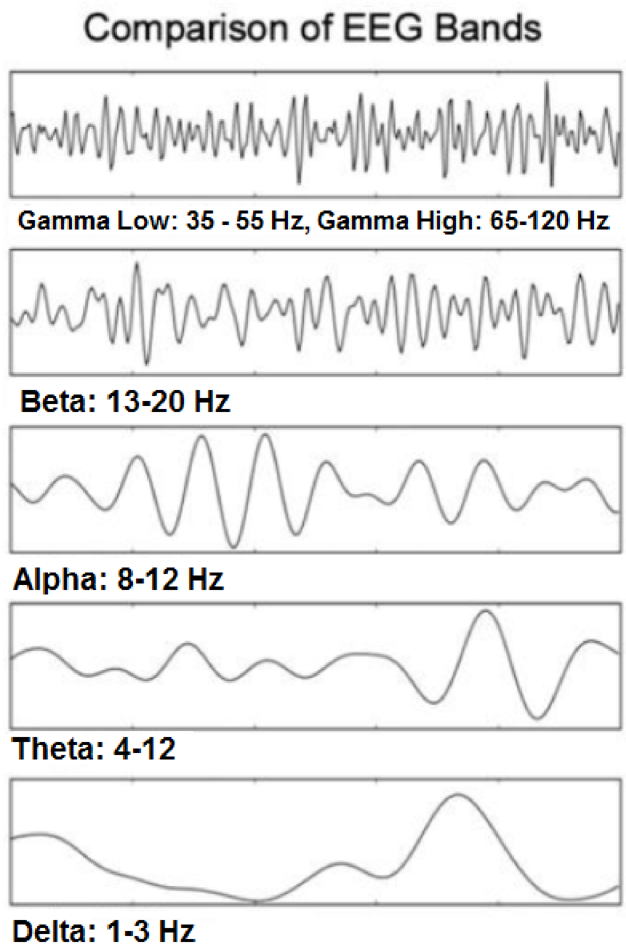
We are interested in **High Frequency Oscillations (HFOs)** (Examples given in the next section). Various labs characterize High Frequency Oscillations differently, however a majority of people tend to describe High Frequency Oscillations as having frequencies between 80 Hz and 500 Hz.

People break down HFO frequency bands into two sub-bands called **Ripples** and **Fast Ripples**. Ripples occur between 80 Hz and 250 Hz, and Fast Ripples occur between 250 Hz and 500 Hz. It is thought that Fast Ripples are related to epileptogenic tissue (tissue that causes seizures in epilepsy). The idea of Fast Ripples when it comes to epilepsy, is that if the recording contains a significant amount of Fast Ripples, the tissue is then responsible for causing seizures, thus the removal of the tissue could potentially mitigate the subject's seizure frequency/severity.

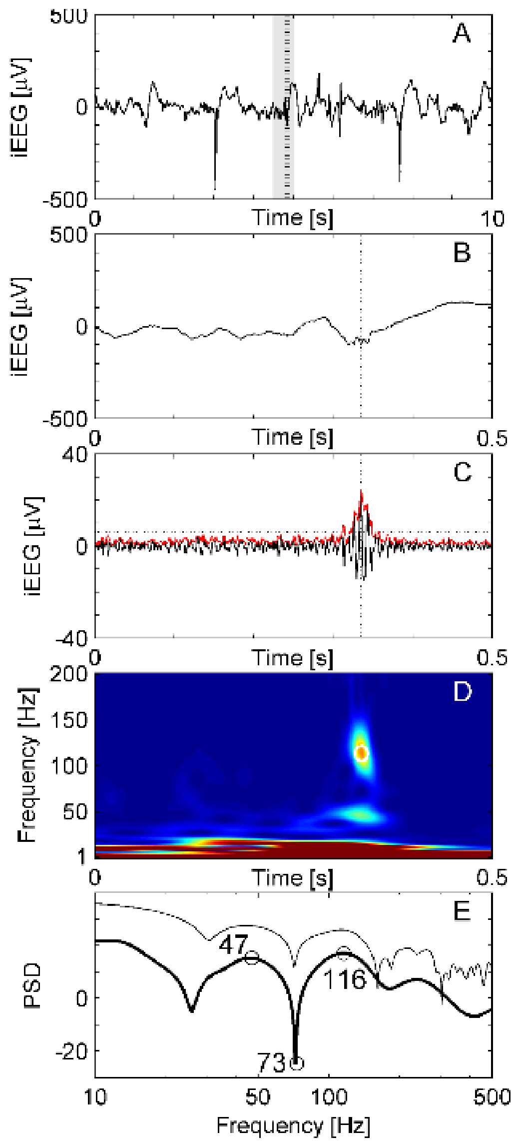
Our lab, being an Alzheimer's related lab, is more interested in Ripples which has been shown to be related to memory. However, some Alzheimer mice models are susceptible to seizures, so we won't completely disregard the Fast Ripples. When relating Ripples to memory we are focusing mainly on Sharp-Wave Ripple events (SPWs) which are Ripples that occur at the depolarization phase of the signal (when a neuron / population of neurons are spiking/activated). Increasing studies show that SPW's occur during memory consolidation/recall. In our case, it has been shown that SPWs occur in place cell replay, were the animal will replay the place cell patterns that were activated during exploration.

1.2 HFO Examples

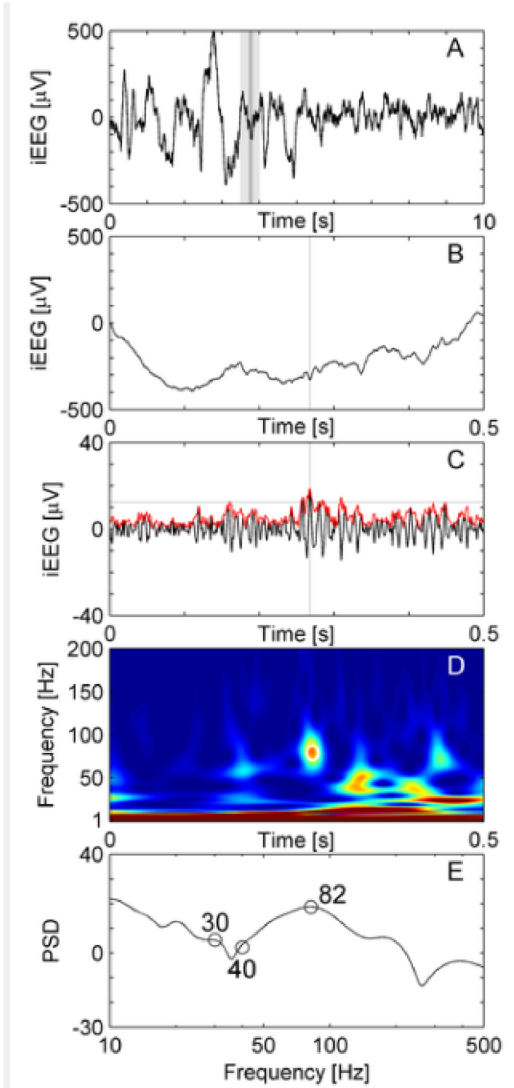
Above is an example of a Ripple provided by Sergey Burnos. By looking at the peak value in the Power Spectral Density (PSD) graph, we notice that the frequency of this ripple is ~116 Hz (the


Comparison of EEG Bands
Gamma Low: 35 - 55 Hz, Gamma High: 65-120 Hz
Beta: 13-20 Hz
Alpha: 8-12 Hz
Theta: 4-12
Delta: 1-3 Hz

EEG Bands



Ripple Example

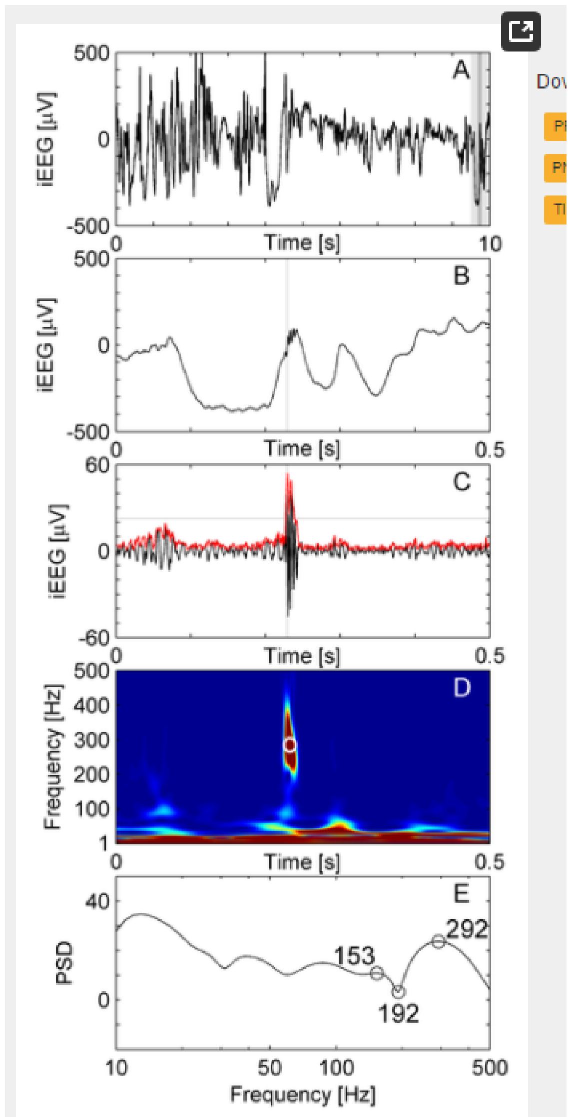


Ripple Example

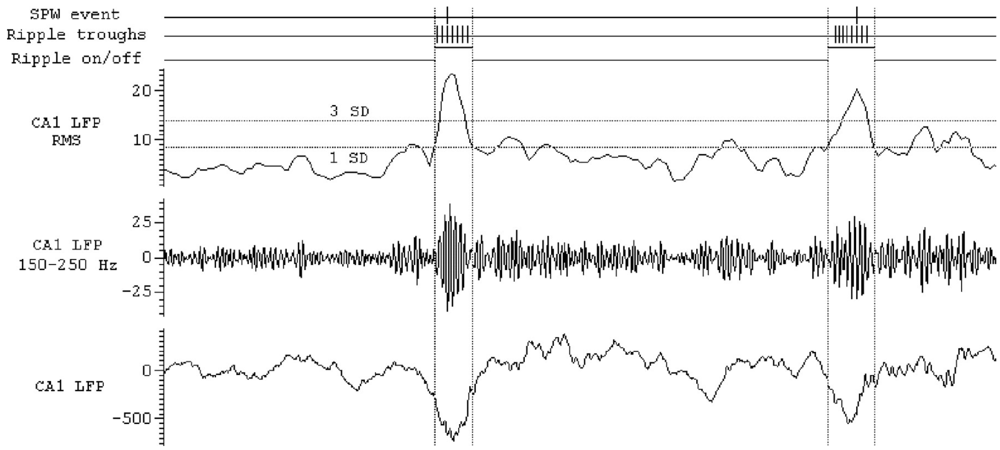
max of the PSD waveform) which places it in the Ripple category. You can see that the ripple event contains oscillations that are significantly increased when comparing it to the surrounding baseline oscillations. The envelope (the red line in the 3rd graph from the top) of the Ripple follows a somewhat Gaussian distribution. These are all properties of a proper HFO. The Stockwell Transform graph (the colorful heat-map located in the fourth position from the top), shows the frequency vs time relationship. At the time point where the Ripple occurs you see a red-ish circle centralized at ~116 Hz. The colors that are closer to the red part of the visible spectrum represent frequencies that are dominant in the signal, and those that are closer to the blue part of the visible spectrum represent frequencies that are not part of (or comprise a small portion of) the waveform.

This is another example of a Ripple provided by Sergey Burnos. As you can see in the PSD and the Stockwell Transform, this Ripple has a lower frequency of about 82 Hz. A slower frequency than the previous example, and it is not as clean as the previous example.

This is a Fast Ripple example provided by Sergey Burnos. As you can see in the PSD and the Stockwell Transform, this event is localized around the 292 Hz mark. Since it is above 250 Hz,



Fast Ripple Example



Sharp Wave Ripple Example

we will consider this a Fast Ripple. The morphology is similar to that of a Ripple, but at a faster frequency.

This is a Sharp-Wave Ripple event. The top graph represents the root mean square (RMS) of the signal, which is what this author uses to automatically detect the event (we will disregard this signal). This middle is the signal filtered between 150 Hz and 250 Hz, which is a portion of the Ripple band. This ensures that all the rest of the signals are removed, and that only the Ripples will remain (making it much easier to detect Ripples). The bottom graph is the raw signal. The difference here is that the Ripple is aligned with a trough (the depolarization phase of the signal) which makes it an SPW instead of just a Ripple. **Note: our recording software flips the y-axis, so our SPWs will occur at the PEAK of the raw signal, and NOT the TROUGH**

This is a second Sharp-Wave Ripple event. Looking at the Stockwell Transform you can see that the peak is localized at about 100 Hz, making it a Ripple. However this Ripple occurs at the Trough of the signal (again in our case it will occur at the peak of the signal), which means that it occurs at the depolarization phase of the signal.

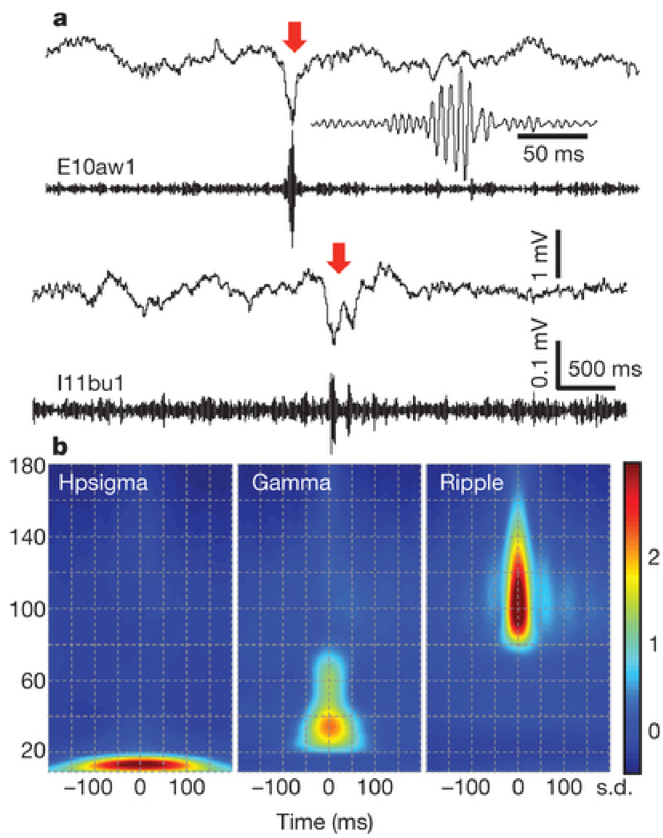
1.3 Papers to Read

Human Intracranial High Frequency Oscillations (HFOs) Detected by Automatic Time-Frequency Analysis by Sergey Burnos et al: **Sergey Burnos has argued for an automatic detection algorithm for Fast Ripples in order to determine if Fast Ripples is indeed an indication of the Seizure Onset Zone (SOZ) in epilepsy patients. Has great images for you to look at for Stockwell Transformations, as well as the iEEG waveforms.**

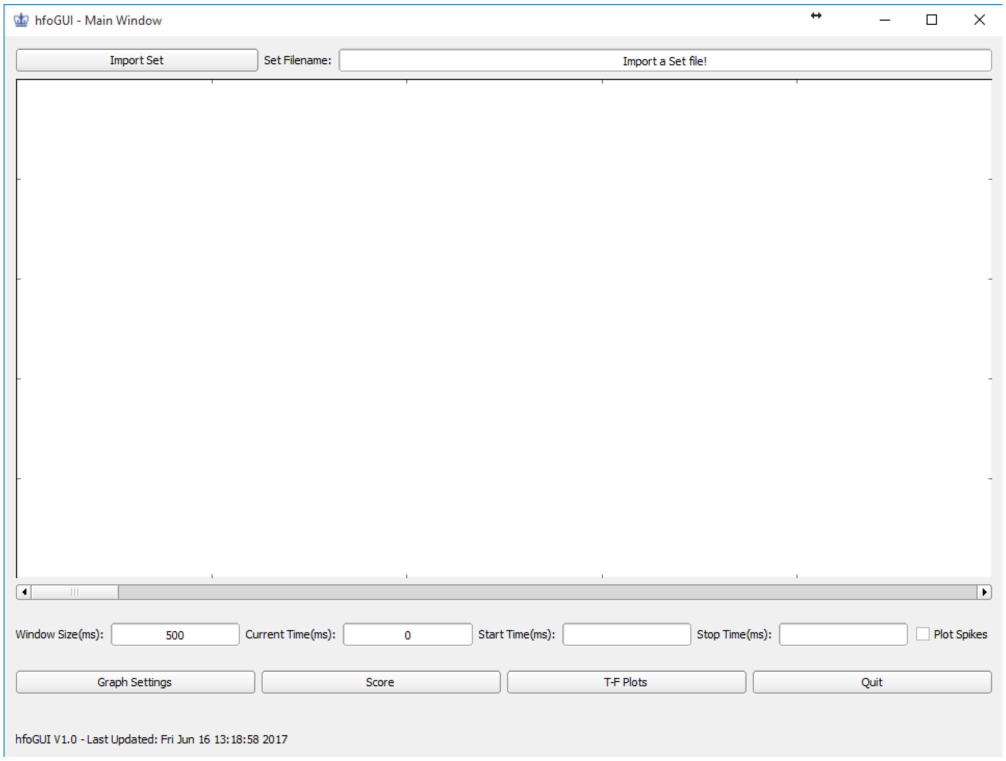
Hippocampal–cortical interaction during periods of subcortical silence by N. K. Logothetis et al: **Suggests that sharp wave ripple events are associated with memory recall in rats and humans.**

Rhythms of the hippocampal network by Laura Lee Colgin et al: **Discusses the various Hippocampal LTP rhythms (theta, gamma, and SPW). It is stated in this paper that theta rhythms are thought to allow the brain to take in and to learn new information, and sharp wave–ripples are thought to be responsible for stabilizing and consolidating memories, and that there is less agreement in the field about the functional significance of gamma rhythms**

Hippocampal Sharp Wave-Ripples Linked to Slow Oscillations in Rat Slow-Wave Sleep by Matthias Mölle et al: **Here they investigated whether the grouping influence of slow oscillations extends to hippocampal sharp wave-ripple (SPW) activity thought to underlie memory replay processes**



Sharp Wave Ripple Example 2



Main Window

during Slow Wave Sleep (SWS)

2 hfoGUIV2 Software

When the GUI is launched, you will see the following window:

2.1 Choose Data File

2.1.1 Type in the Filepath to the .set file that you want to analyze.

The first option is you can type the **full** filepath to the .set file that you want to analyze as shown below.

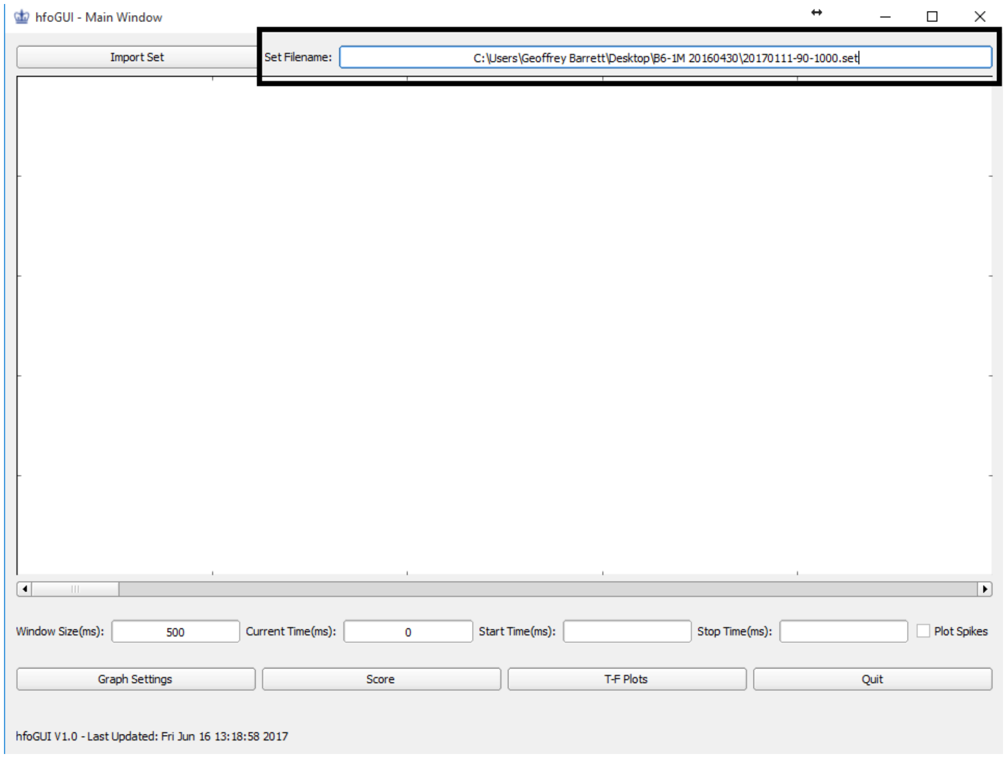
2.1.2 Use the Open File Dialogue from the 'Import Set' button

The other option is to choose a file manually within the GUI by pressing the 'Import Set' button in the upper left corner as shown.

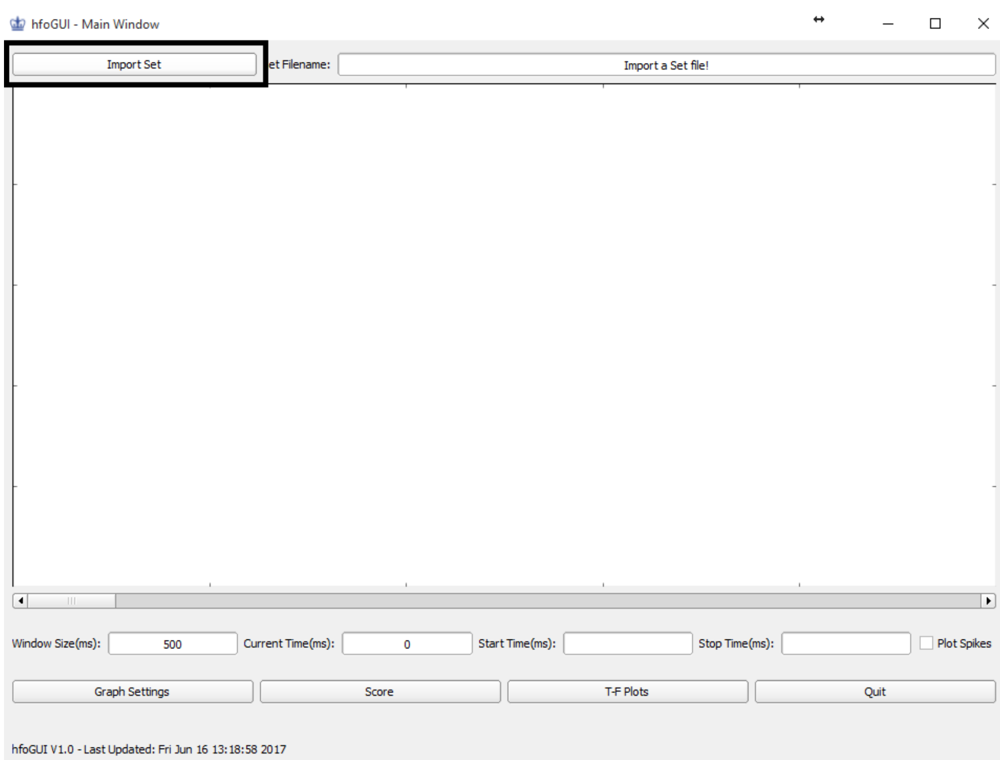
A new window will now be opened where you will have the option to choose a .set file, apply the chosen .set file, or cancel.

If you click the 'Choose a .Set file' button, in the window pictured above, a window will be opened that will allow you to choose a .set file. Navigate to the .set file you would like to analyze, and then press Open, as pictured below.

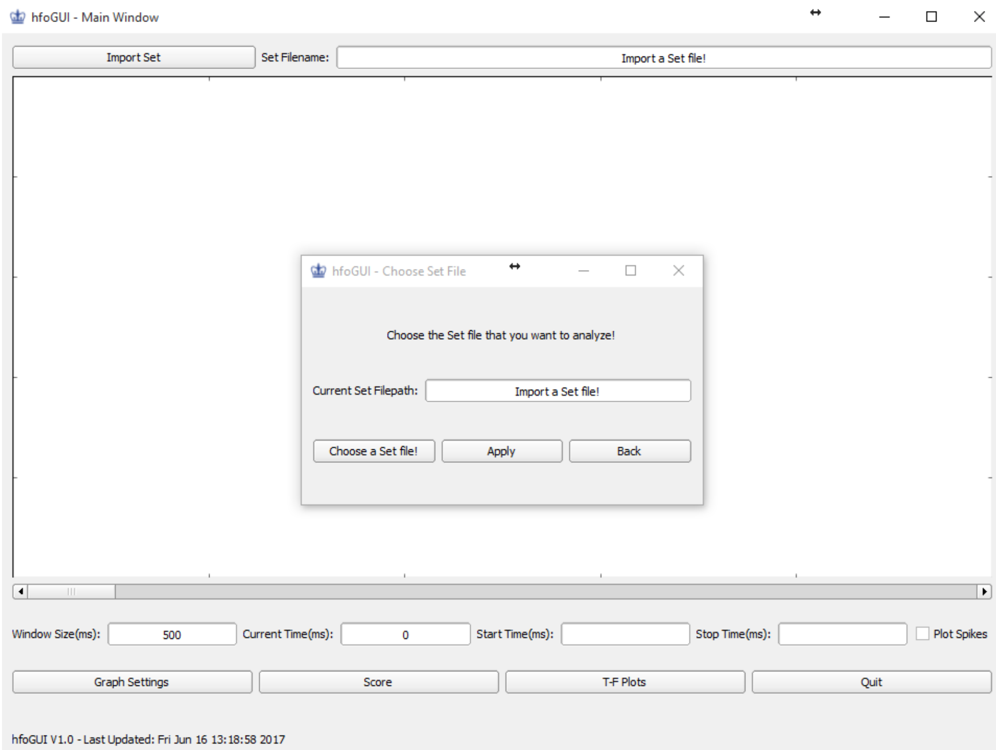
Now if you press apply on the Choose .Set File window, the data can now be loaded. You will see that the Set Filename field will be updated with the current filename.



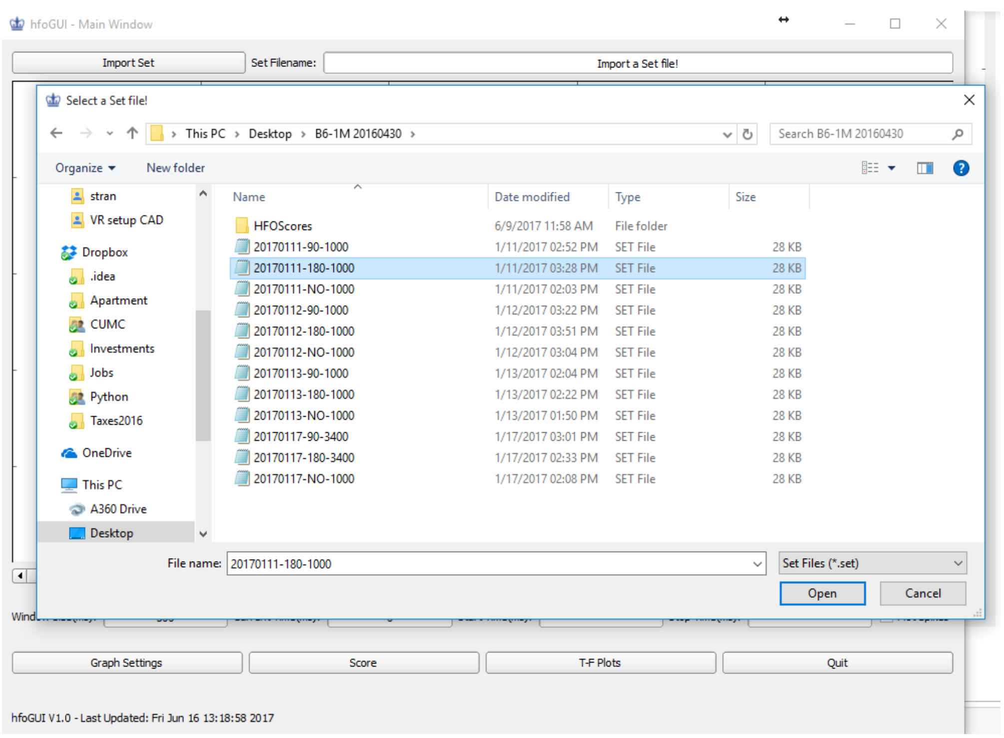
Type Filepath



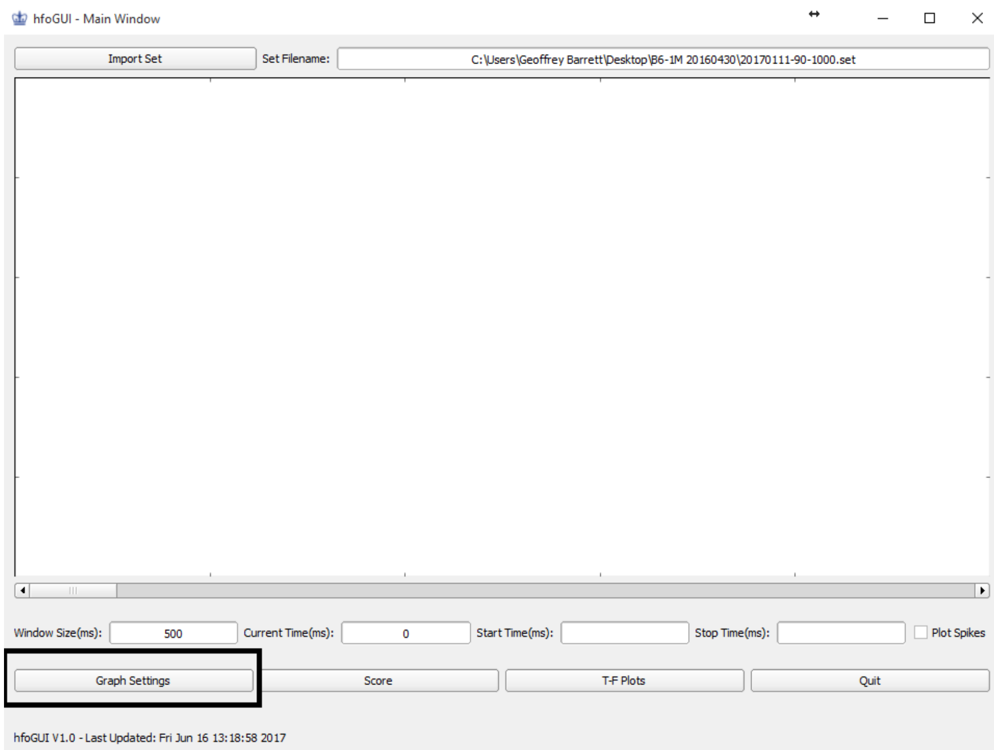
Import Set Button



Import Set Window



Choose Set



Graph Settings Button

2.2 Graph Data

Once the .set file has been chosen we can load/graph the data. All you have to do now is press the "Graph Settings" button down in the lower left corner (pictured below), and add the graphs/load a graph profile.

Now the following window has been opened (pictured below).

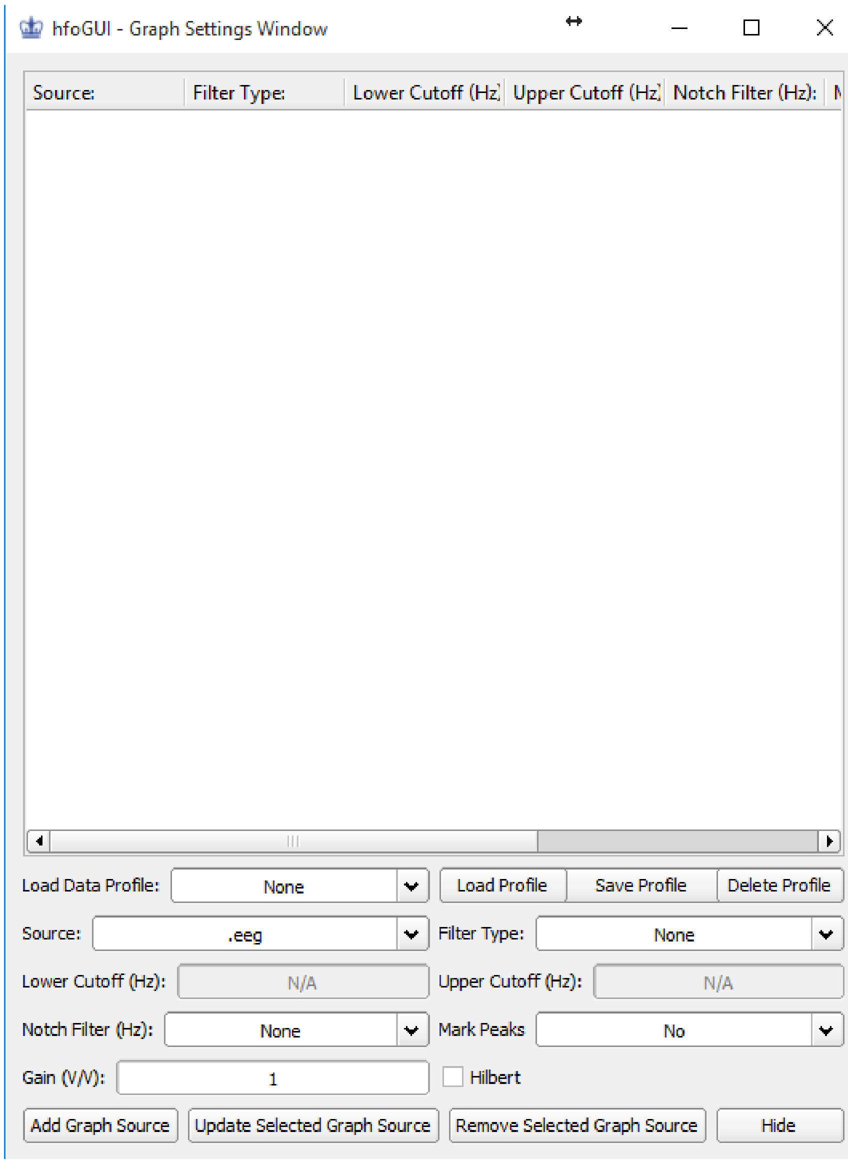
2.2.1 Load Graph Profile

For the most part you will probably be loading the HFO profile from the Load Data Profile dropdown menu. To do this you select HFO from the dropdown menu, and then press the 'Load Profile' button (to the right of the dropdown menu).

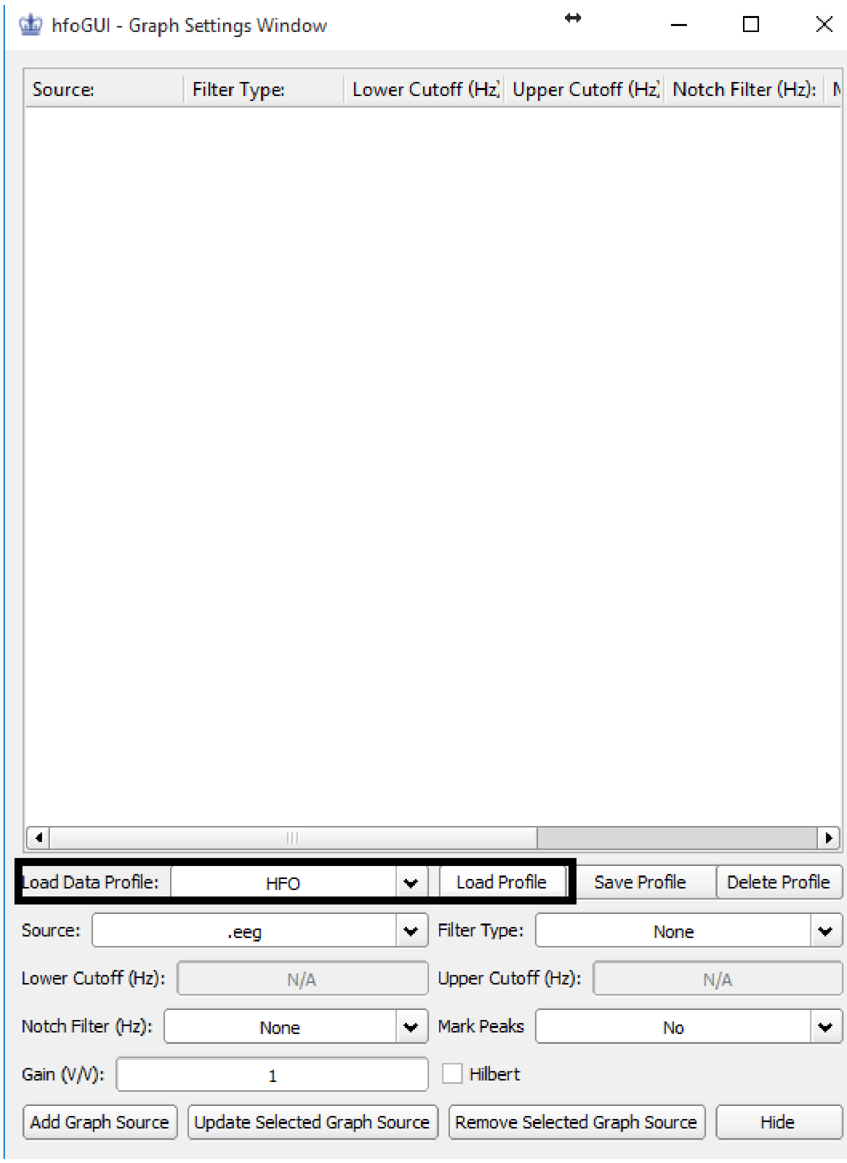
2.2.2 Manually Add Graphs

You can also manually add graphs if you feel as though the current profiles don't fit your needs, and you prefer another layout. You can add multiple waveforms at the same time, the waveforms will be added from the bottom upward. So if you want a waveform to appear at the top, make sure to add it last.

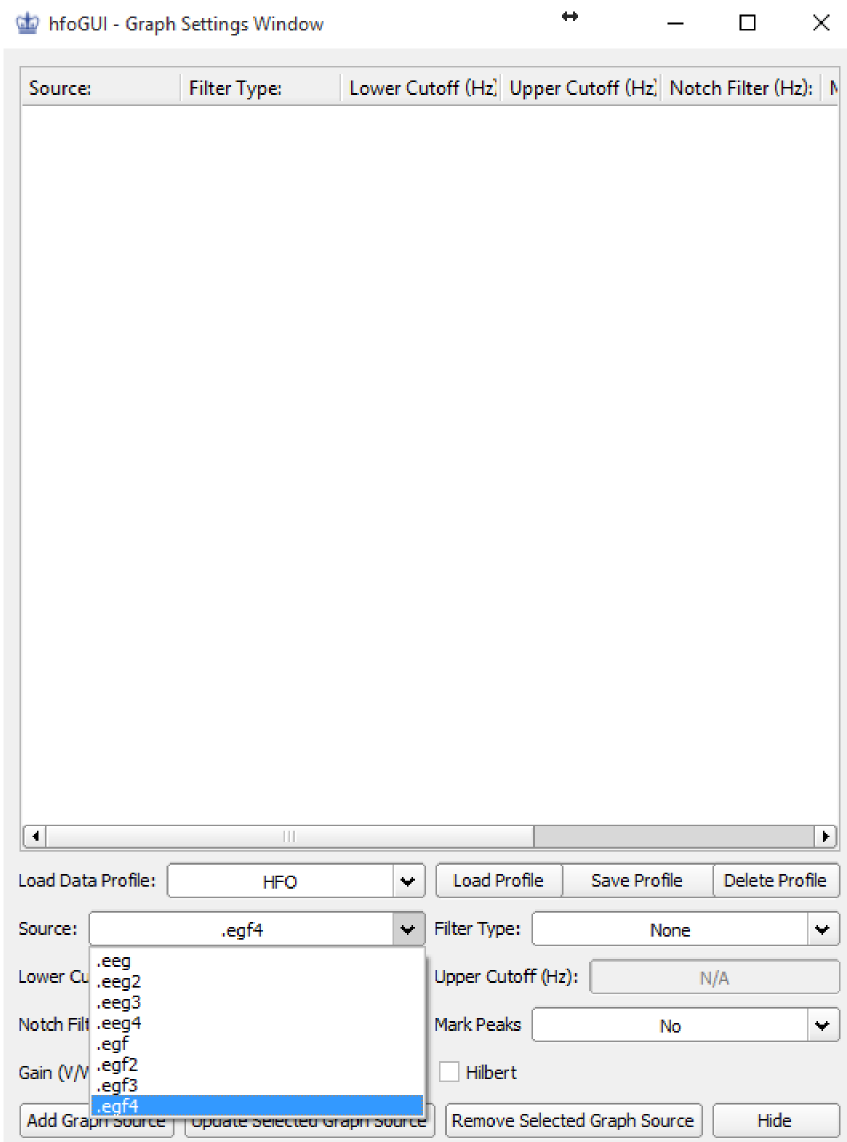
First select the source that you want to choose from the Source dropdown menu (either .eegX, or egfX files). If you want to observe frequencies above 125 Hz (any HFOs) you will need to choose .egf Files as they are recorded at a sampling frequency of 4.8 kHz, while the .eeg files only record at 250 Hz. The difference between the different egf's are their gains. We chose .egf4 because they tend to have lower gains.



Graph Settings Window



Load Profile



Choose Source

The next step, is if you want to filter you need to choose a filter type between the following:

1. None: no filtering will occur.
2. Lowpass: any value above the Upper Cutoff Frequency value will be removed from the signal.
3. Highpass: any value below the Lower Cutoff Frequency value will be removed from the signal.
4. Bandpass: any value below the Lower Cutoff Frequency value and above the Upper Cutoff Frequency will be removed from the signal.

If a filtering option other than "None" was chosen, you will need to set the Upper and/or Lower Cutoff Frequencies.

Next is to notch filter the data. We will most likely implement this notch filter. It removes the 60 Hz (or 50 Hz if not in the United States) noise caused by the electrical hum from the wall outlets. Choose the frequency you want to perform a notch filter on.

Mark Peaks will put a vertical line where the peaks of the signal are. This could be used to visually align the sharp wave peaks with the ripples to detect a Sharp Wave Ripple Event. An example of what this looks like is pictured below.

You can also change the gain. This is a scalar value that will be multiplied by all the data points in the waveform you select. Thus if you choose the .egf file as the source. With a Gain of 2 V/V, the .egf file's data will will be multiplied by 2 and then plotted. The default is a gain of 1 V/V.

Also if you would like to view the Hilbert transformation of the graph, ensure to check that checkbox in the bottom right. This is useful for filtered signals to view Ripples/Fast Ripples as the envelope (produced from the Hilbert transformation) of the Ripple/Fast Ripples should look similar to a Gaussian distribution.

2.2.3 Updating Graphs

If you select a graph from the list, it will re-populate the parameters with the parameters of the selected graph. You can then make changes to any of the parameters as desired, and press the Update Selected Graph Source button, and the GUI will re-graph with the updated settings.

2.2.4 Removing Graph

Waveforms can also get removed, by selecting an item from the list and selecting the Remove Select Graph Source button.

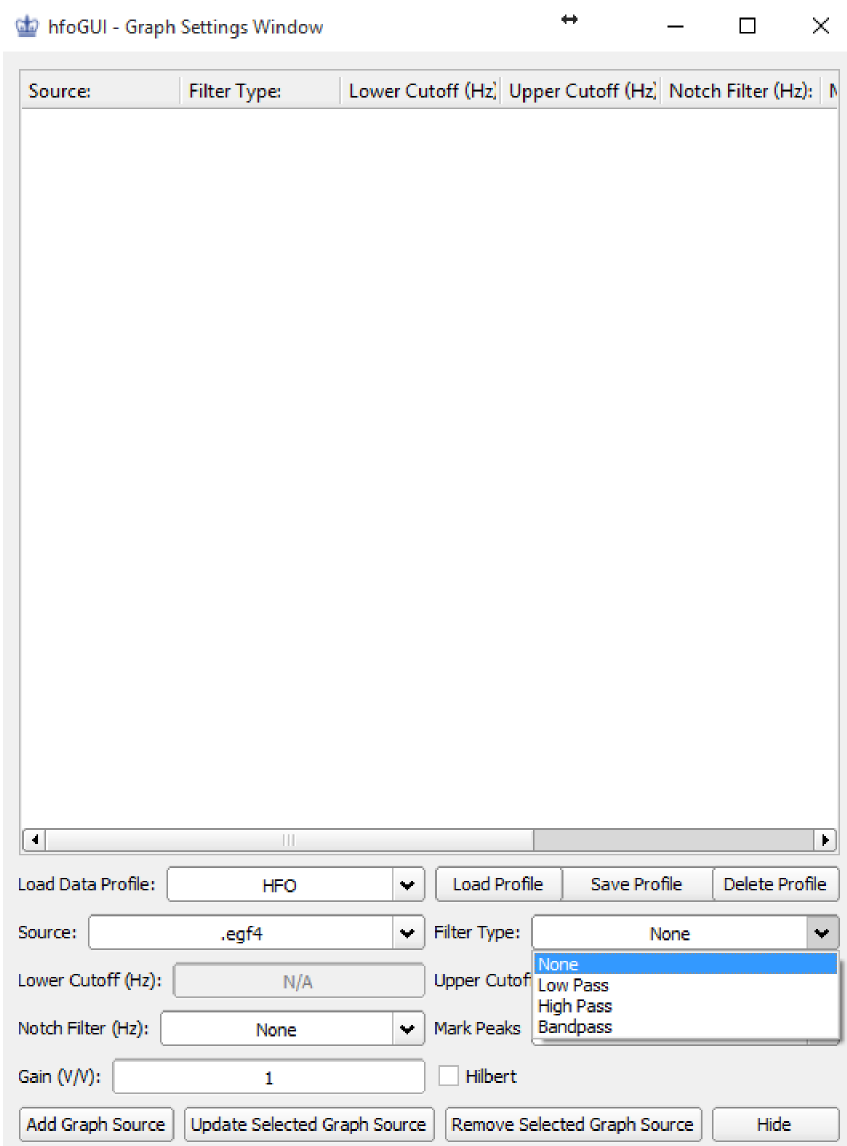
2.3 Using Main GUI

Now that the data is graphed, it is time to learn how to navigate through the data. The main way to look through the data would be to use the scrollbar.

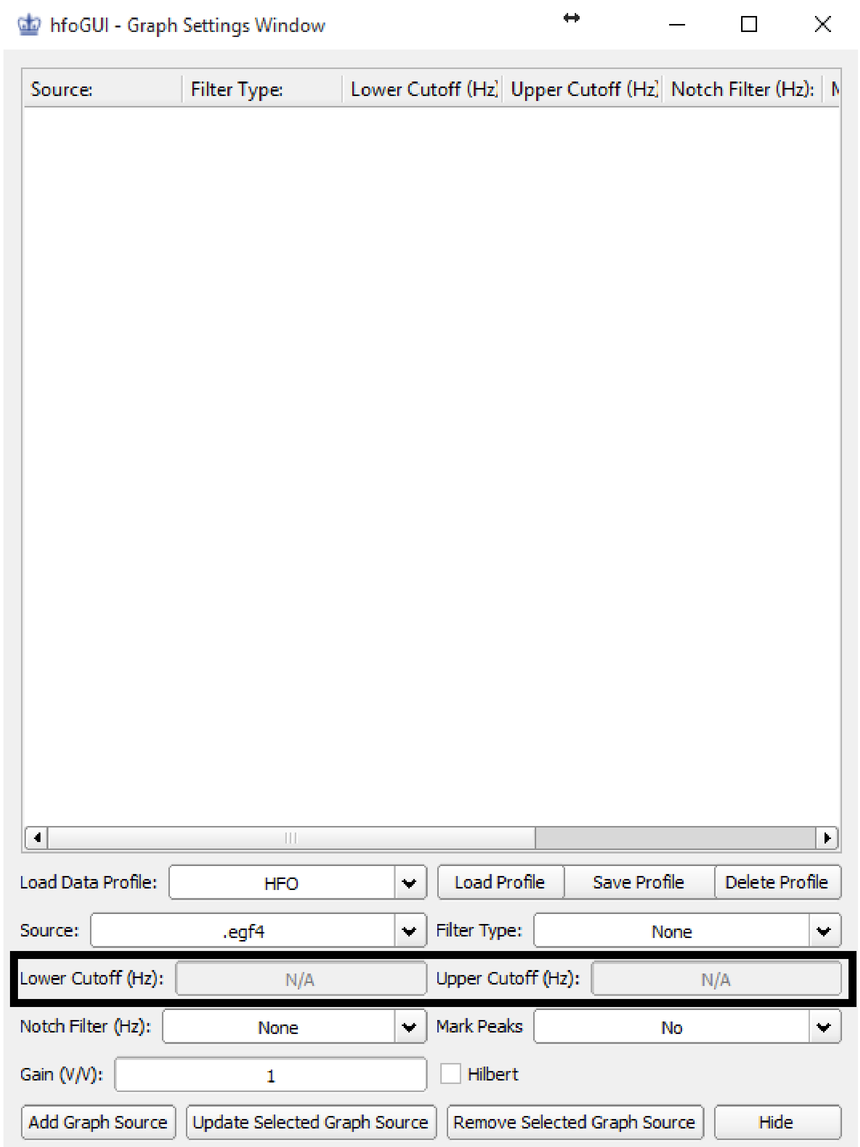
At the bottom of the Main window of the GUI you'll see a few editable fields that list the following parameters:

Window Size: the length of time that the graph will be shown. I usually recommend 500 ms, maybe about 1 second (1000 ms) maximum.

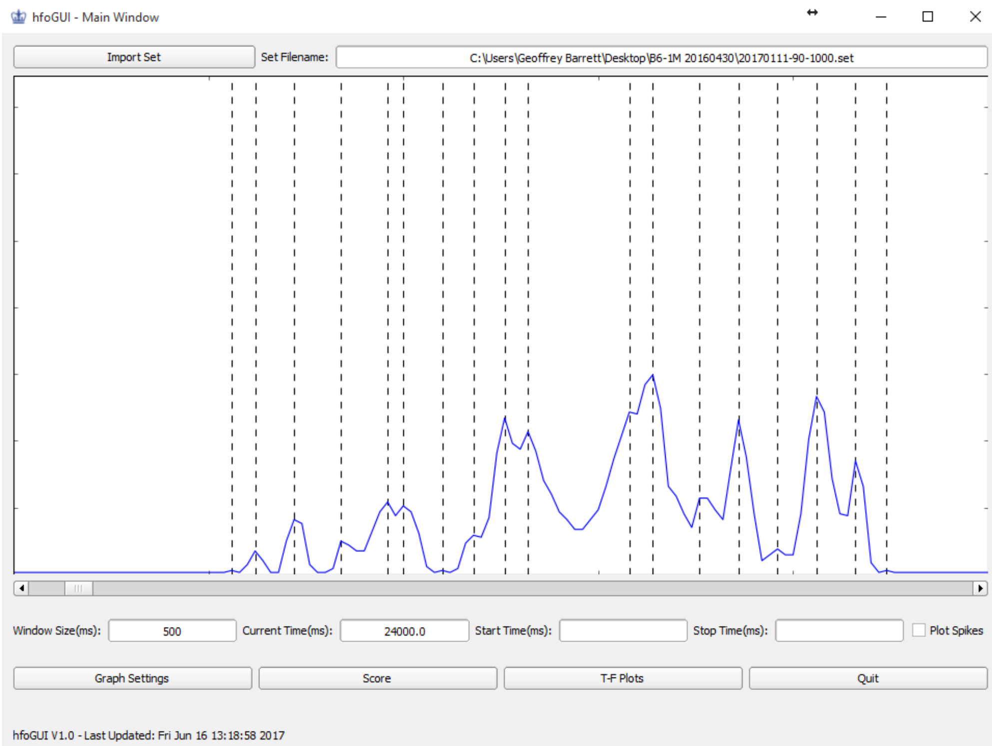
Current Time: the window shows data from this time, and stops at the current time value + the window size value. This could be a secondary way to navigate through the data. If you know certain time points you want to look at, it is much easier to type it as a current time instead of scrolling.



Choose Filter



Choose Frequencies



Mark Peaks

Start Time: the start time of a selected event. It will plot as a vertical red dashed line.
Stop Time: the stop time of a selected event. It will plot as a vertical green dashed line.
These parameters are shown below.

Another parameter that exists is the "Plot Spikes" checkbox. This will plot a vertical line (raster plot) for every spike on every cell so that you can see if there is a spike occurring at that time point. This is not as useful for finding HFO's so I would leave this off.

2.4 Scoring

To open up the window that will allow you to score this file, simply press the "Score" button on the main window and a Score window will pop up that looks like the following:

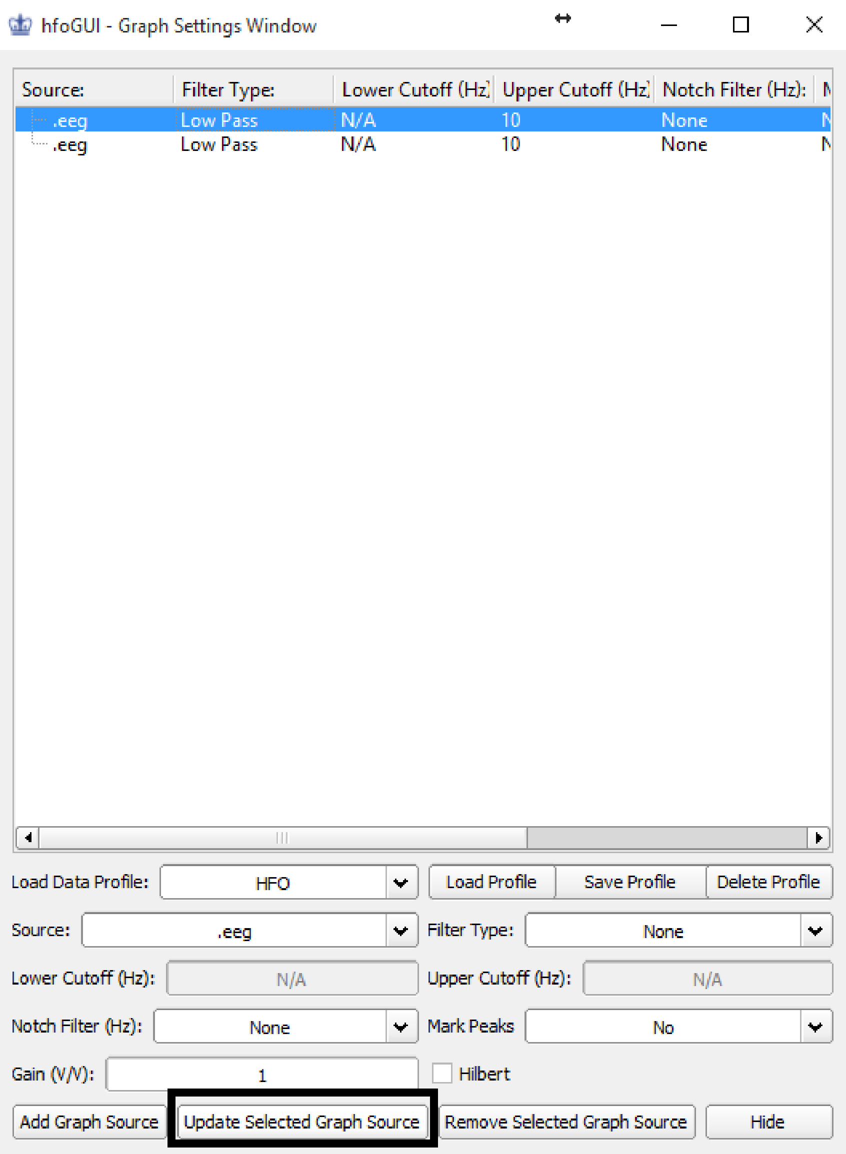
At the top you'll see a dropdown menu labeled "Source" this will contain a list of the source that are currently being plotted. If you are scoring HFOs you'll probably have only one source plotted, in which case you will not need to change this value.

2.4.1 Score Tab

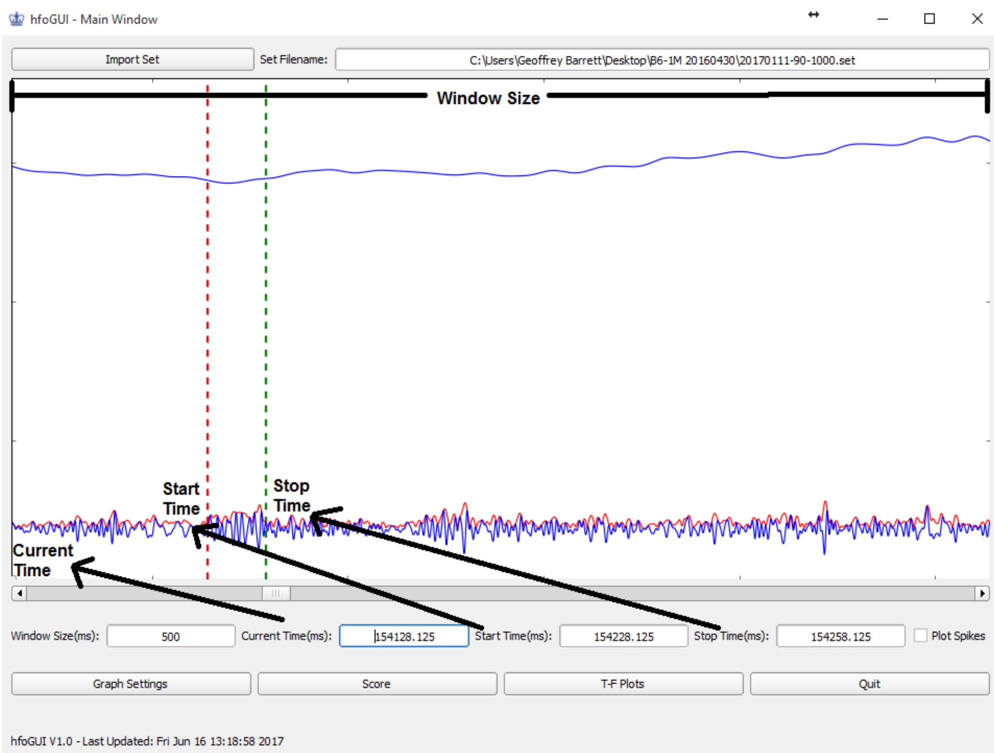
The Scoring Window will default on the tab named "Score".

At the top of this tab you will see two buttons, one for loading a score file, and one for saving a score file. Below this file will be a filename in a text field that has an automatically generated filename based off of the .set filename and the source.

Below the text field, there will exist an empty list containing the three following columns: Score, Start Time, and Stop Time. This empty list will eventually contain all of our added scores.



Update Graph



Main Window Parameters

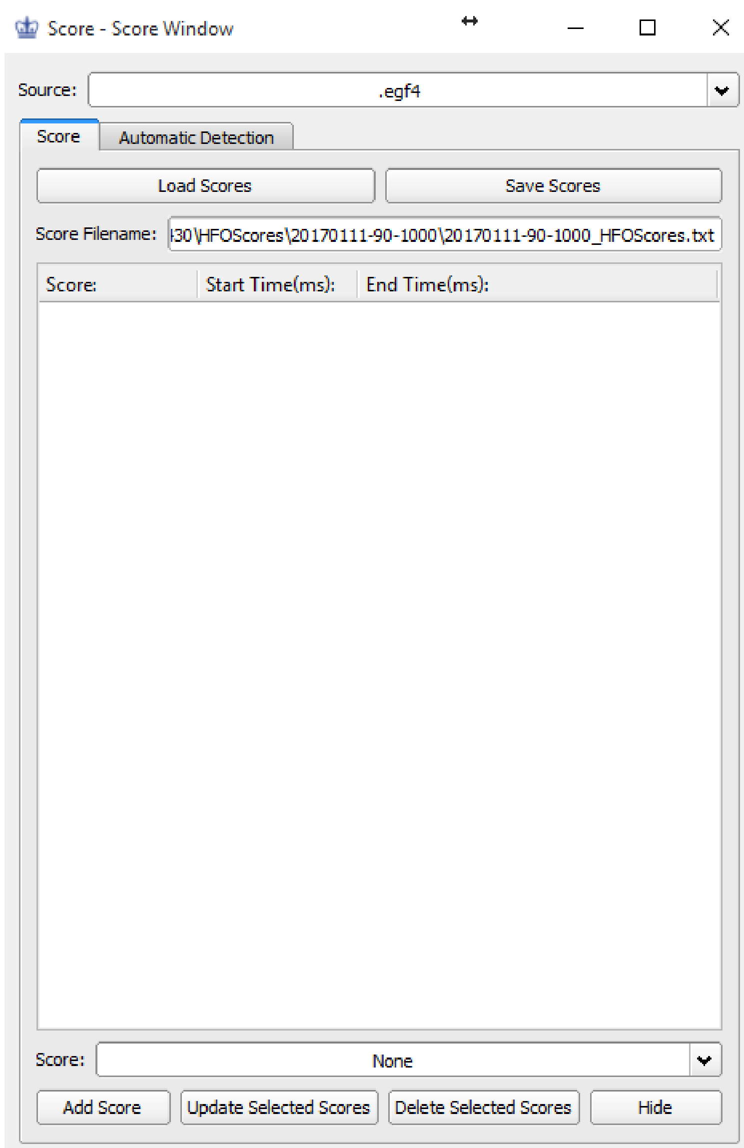
Load Score To load a score, simply press the Load score button. Make sure that the filename in the "Score Filename" text field is what you want to load, as it will look for that text file and simply populate the scores in the List below.

Save Score Simply press the Save Score button and the filename will be taken from the "Score Filename" field and all the values will be saved as a tab delimited text file with the scores, start times, and stop times. You can modify the "Score Filename" text field if you desire, just make sure you know what you called it so you can load that file in at a later time.

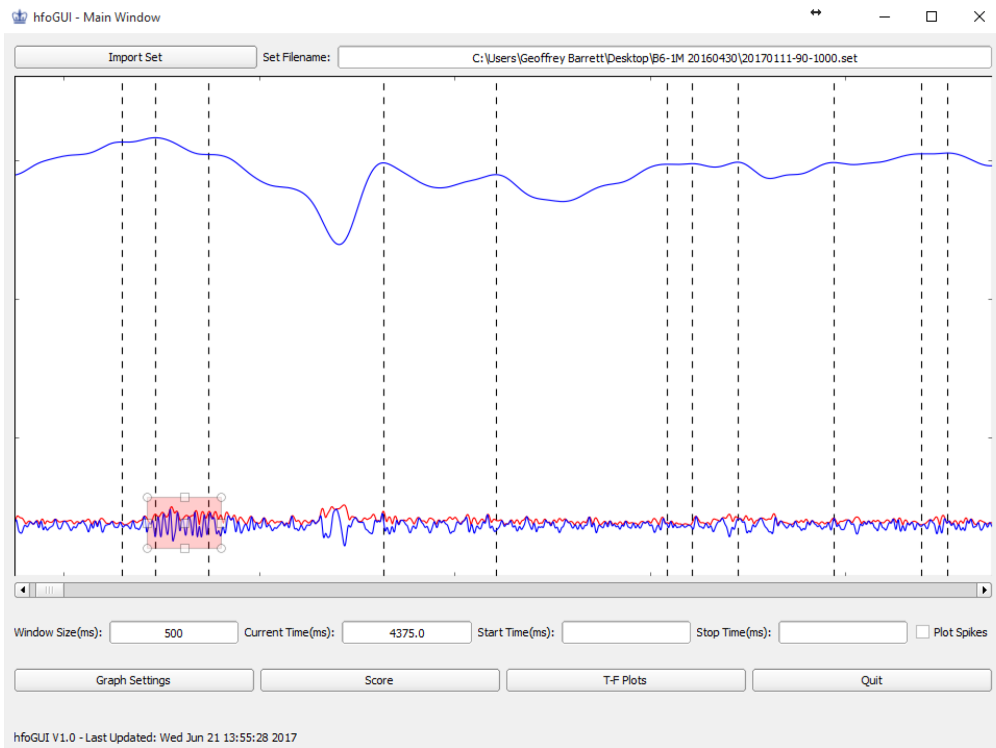
Add Score At the bottom of the Scoring window is a dropdown menu named "Score" which will contain all the possible scores that you can utilize in this GUI (more can be added, and some can be removed if necessary). To manually add a score you will click and drag a box around the segment of the waveform on the Main Window that you want to score. An image of this is shown below.

Now once you have gotten the rectangle appropriately highlighting the event you want to add, press the "Add Score" button in the Scoring Window. This will take the position information of that rectangle, and convert it into the stop and start time of the event you covered with the rectangle. You will see this new score added to the score list in the Scoring Window.

If you select any of these scores that you added from the list of scores, this event will be centered in your Main window and you will see the start and stop time highlighted with red and green dashed lines.



Score Button



Add Score

Update Existing Score If you want to change the score value, you can select the Score from the list on the Scoring window, select the new score from the "Score" dropdown menu, and then click the "Update Selected Scores" button. The new score will now be reflected in the list for the selected score

Delete Score A score can be removed by selecting an score from the list in the Scoring Window, and pressing the "Delete Selected Scores" button.

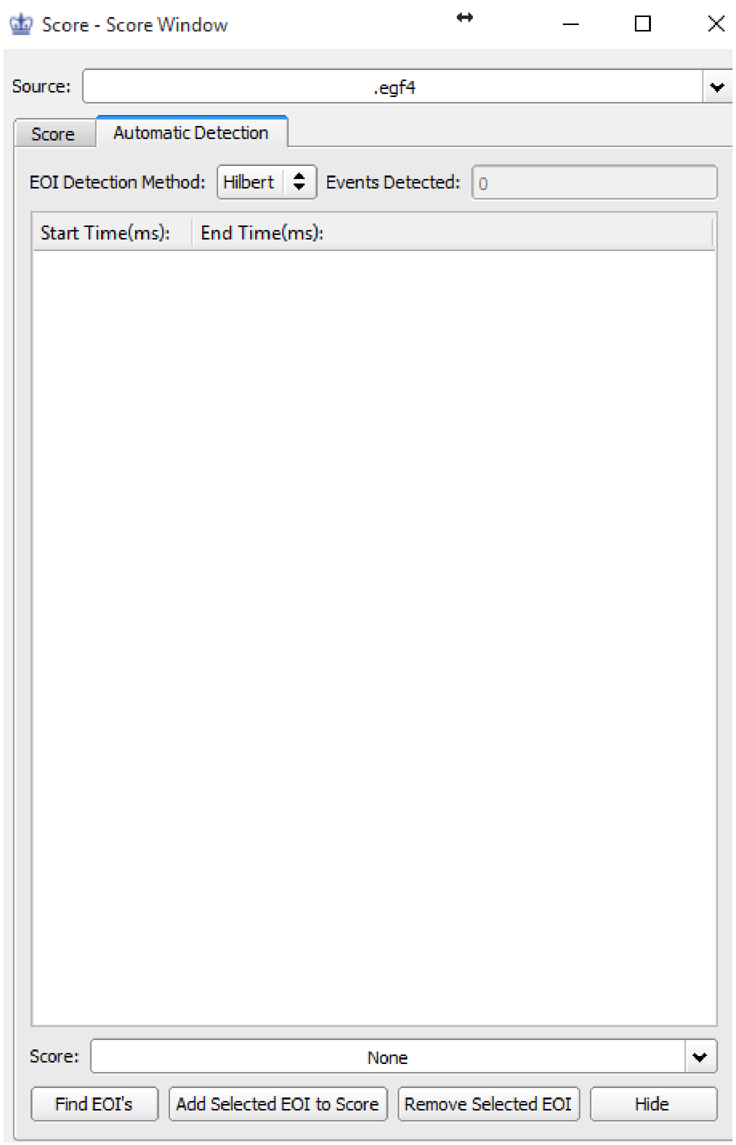
2.4.2 Automatic Detection

Manually scoring these files is quite time consuming, so automatic detection algorithms have been developed in order to save your time. To access these automatic detection algorithms, click the "Automatic Detection" tab on the Scoring window. You will see a similar layout to that of the "Score" tab (image down below).

To use this tab, select the detection method that you desire from the "EOI Detection Method" (EOI, meaning Event of Interest) dropdown list (there might only be Hilbert as of now as it is simple and widely used). Now pressing the "Find EOI's" button at the bottom of the list will search for potential HFO's. It might take some time so just sit back and relax.

Hilbert Detection The Hilbert detection will apply a Hilbert transform in order to create an envelope traversing the peaks of the signal that will allow us to detect the Gaussian shaped events of the HFOs and use simple thresholding in order to capture events that have amplitudes that are significantly increased from baseline.

Parameters to provide:



Automatic Detection

1. Min/Max Frequency: first the signal will be bandpassed using the min and max frequency values. The default will be the entire HFO frequency range (80 Hz - 500 Hz). This signal will then undergo a Hilbert Transform to create an envelope of the signal.
2. Epoch: the data will be binned into epochs with this width of time in order to calculate the mean and standard deviation of for the thresholding
3. Threshold: if the envelope passes above this threshold (the mean of the epoch + X standard deviations of the epoch), it will be further analyzed in order to determine if it is an EOI. The algorithm will find the points where the envelope crosses 1/2 of the threshold and consider that to be the start and stop points of the EOI.
4. Minimum Time: the EOI must have this minimum time in order to be considered any further, otherwise it is rejected.

Adding EOI to Score Window These EOI's that were found using automatic detection need to be moved to the Score tab's list in order for it to be saved. If you find an item in the list that you believe to be a desired event (in this case, Ripple or Fast Ripple), you can select the item from the list, score the event with the Score dropdown menu, and then press the "Add Selected EOI to Score" button to move it to the score list. You will see that it is removed from the Automatic Detection tab and moved to the Score tab with the selected score you chose (Default: None).

Removing EOI from Automatic Detection Tab If you believe that the event on the list is not an event we are interested in, then simply select it on the list, and press the "Remove Selected EOI" button.

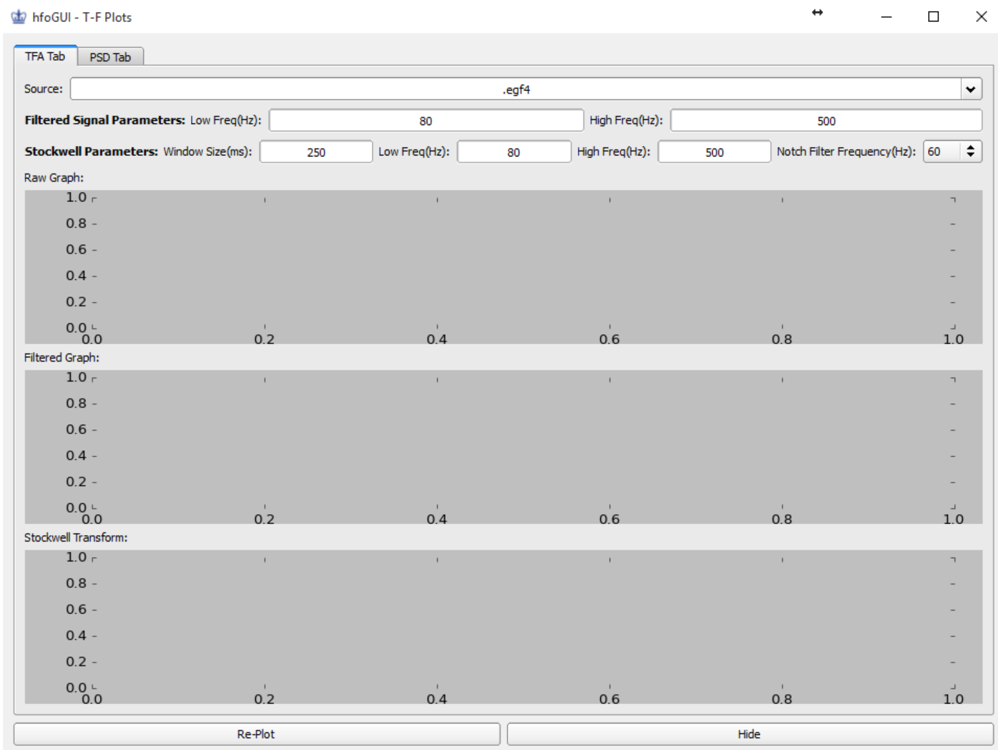
2.4.3 Time Frequency Window

Since the frequencies are so important to our scoring, we will have to determine the frequencies of the graph using various Time-Frequency Analysis techniques (such as the Stockwell Transform). If you click the "T-F Plots" button of the Main window, a Time Frequency window will pop up similar to what is shown below.

TFA Tab The Time Frequency Analysis (TFA) tab is the default tab. It will hold the following graphs of the source in the Source dropdown menu:

1. Raw Graph (Top)
2. Filtered Graph (Middle): this will use the filter settings selected from the Filtered Signal Parameters row. I like to keep it bandpassed from 80-500 Hz, however we might experiment with 100 - 250 Hz, 100 - 400 Hz, etc.
3. Stockwell Graph (Bottom): this will use the settings in the Stockwell Parameter row. We keep this at 80 - 500 Hz as default because in the data I've seen there is a strong *relatively* low frequency base signal that washes out the higher amplitude signal, so we filter that out so we can see a nicer looking Stockwell graph.

If these graphs are empty, this means that you have not selected a point to perform the Time Frequency Analysis on. You must go to the Main window, and click on the graph where you'd like the analysis to be centered around. I suggest using the peak (or center) of the event that you want to look at. A vertical black line will be plotted on the Main window indicating the point that was clicked, and then the three graphs listed above will be populated.



Time Frequency Window

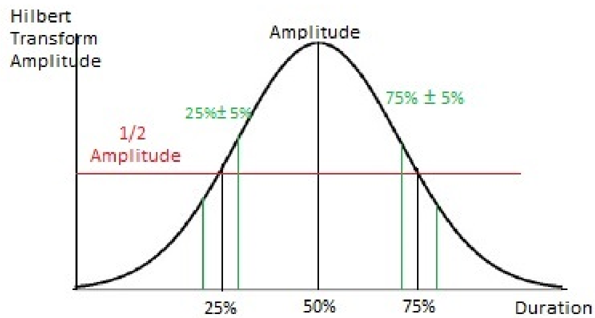
PSD Tab The Stockwell Transform shows a broader view of the relationship between frequency and time where as the Power Spectral Density (PSD) shows a narrower view. It will essentially take a slice of the Stockwell Transform data and plot a Power vs Frequency graph. The frequency with the largest power is the frequency that is dominant in the signal. The PSD will include data at the time point that was selected on the Main window that produced the TFA graphs (the black solid line).

2.4.4 Assessing an Event

In the background section I briefly discussed what a Ripple, Fast Ripple, and Sharp Wave Ripple (SPW) looks like. However I will reiterate it in this section.

1. You will want to see that the peak amplitude (max height) is significantly increased from baseline. For this you will simply look at the signal and see if it is significantly different (higher) than the surrounding signals. If you would like to visually see some horizontal lines with a threshold that you can change to aid you in determining if it is significantly different from the mean, I can add one.

2. The envelope (red line if you clicked the Hilbert Transform option on the Graph Settings window), should resemble a Gaussian distribution. Meaning at roughly the middle of the event should reside the maximum point. At 25% and 75% of the event time, the amplitude should be at about half of that amplitude value (image seen below). **Note: This point doesn't need to be exact, but the closer it looks to a Gaussian distribution, the better**



Gaussian Distribution

3. These Events are short in nature, therefore they should be about 8-250 ms long (a majority will be on the short end of this scale).

4. Analyze the Stockwell transformation of the event to determine the frequency of the signal. A Stockwell transformation essentially takes the waveform and allows us to view the frequency of the signal over time. A signal can be a sum of signals with multiple frequencies, but this will allow you to determine what is the most dominant frequency. Remember the closer to the red part of the visible spectrum, the more dominant the frequency is. Most likely there will be a red-ish circular blob (seen in the examples shown in the beginning of this PDF) at the time of the event. The center if this circular shape will represent the frequency of this event. If the frequency is from 80-250 it is a Ripple, and if it is from 250+ it is a Fast Ripple. Most likely the top graph on the HFO profile will be of the Raw data or a low-pass filtered data below 50 Hz. If there is a Ripple that lines up with the peak of the Raw / low-pass filtered signal. Then it can be scored as a Sharp Wave Ripple (SPW) and not a Ripple.

5. The Power Spectral Density (PSD) can also be used to determine the frequency. It will generally be the maximum value of these graphs.