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EyE Movement Extraction Pipeline Manual
Extracting Eye Movement from EEG Data Using a MATLAB-Based Pipeline and EEGLAB Plugins

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Preface

Dear reader,

This manual details how to analyze eye movements using EEG data recorded with fMRI-compatible EEG caps from BrainProducts. Normally, eye movements are measured with EOG, but due to incompatibility in this study, an alternative method is used. The pipeline runs in MATLAB and uses EEGLAB plugins.

Currently, data is stored in Excel files for easy access, export, and retrieval with MATLAB or other analysis software. Alternative storage options can be considered. Data availability or variability might affect the results, so some variables might need adjustments.

This algorithm was developed in the context of the study:

Simultaneous EEG-fMRI study in healthy humans during induction of propofol anesthesia to investigate the dynamics of thalamocortical functional connectivity in the alpha frequency.

Directed by:

Prof. Dr. med. Gerhard Schneider, PD Dr. Afra Wohlschläger, Dr. med. Svenja Letz

Methods used:

Anesthesia induced with propofol (low, mid, high), EEG (electroencephalogram), functional magnetic resonance imaging (fMRI), magnetic resonance spectroscopy (MR Spectroscopy), and structural magnetic resonance imaging (MRI).

Contents

Prerequisites:

- Matlab (version 2019b or higher)

- EEGLAB plugin (provided within the package)

- Minimum RAM of 32 GB recommended

- Storage space for .eeg files.

- Acces to Webdisk (Klifoanae) recommend

This pipeline package includes:

- EyE Movement Extraction Pipeline Manual

- Software and plugins necessary to run the pipeline

- EyE_pipeline_RS.mat

- EyE_pipeline_FLCK.mat

- condition_order_excell_file_creator.mat

- excell_combiner_interval_data.mat

- general_data_analysis.mat

- flicker_analysis.mat

- within_subj_flicker_analysis.mat

- combined_eye_stim_freq_example.xlsx

- flicker_data_example.xlsx

- resting_state_data_example.xlsx

- subject_condition_data_example.xlsx

This package includes 1 manual PDF file and 3 folders. Within the folder Matlab plugins, you will find the necessary software(CWRegrTool and eeglab2023.0). Within the MATLAB scripts folder, you will find all .mat files, as described above. In the folder Excel file examples, you will find the Excel workbooks as described above.

Before Running the Pipeline

We recommend for analysis of the resting state data to run the script EyE_pipeline_RS.mat and for analysis of the flicker data the EyE_pipeline_FLCK.mat. These are both adjusted to the different data types accordingly.

In both scripts, if something has to be adjusted or is specific to the user/desired outcome of the algorithm, there will be a '!' in front of that line. Therefore, before trying to run anything, simply use the find function and search for '!' to see which lines have to be adjusted or (un-)commented.

The folder structure should be as follows, the main path should be the main folder. Within the main folder, the different subject folders should be present with the .eeg files to be analyzed in those folders Within the main folder is also the subject_information.mat file with the subject data (if you want to remove bad channels) and the average_eye_kernel.mat should be present to convolve the resulting eye traces. In figure 1, you can see an example of the folder structure for running the pipeline.

If you have adjusted all lines that require adjusting and have all files and plugins needed in the appropriate structure, then it is time to run the pipeline. In the following section, there will be an elaborate explanation of what it exactly does.
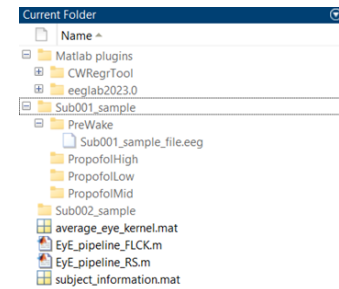

Figure 1 Example Folder structure.

What Happens within the Pipeline

In this section, an elaborate explanation on what happens within the pipelines will be included, though the comments next to it should make it clear enough, this is more if some parts of the pipeline are unclear and to give a bit of an overview. They overlap, but as you will notice, there are some distinctions. For instance, with running EyE_pipeline_RS.mat, there will only be 1 file as output, whereas with EyE_pipeline_FLCK.mat, there will be multiple with different formats.

EyE_pipeline_RS.mat

lines 1-39

- Clearing workspace.

- Allocating correct paths.

- Loading necessary files.

- Selecting subjects and propofol level files to analyze.

- Adding necessary plugins and functions to the path.

lines 41-80

- Loop for going through all selected files and subjects.

- Starting EEGLAB.

- Loading file which will be analyzed.

lines 81-94

- Preprocessing of which:

- Removal of cardio ballistic artifact.

- fMRI gradient artifact.

- (Commented out as most files analyzed were already preprocessed.)

lines 96-102

- Removal of 1st 5 seconds before 1st fMRI volume.

- Removal of coil wires and ECG channels.

lines 104-129

- Optional removal of bad channels which could corrupt ICA.

- First, the filenames are cleaned to extract the corresponding data for the subject and session. (104-113)

- Then, the channels are removed from the data. (119-129)

lines 131-135

- Resampling to milliseconds.

- Changing variable names to avoid confusion later on.

- Applying HPF to get rid of slow drifts.

lines 137-141

- Optional lines for storage of interim datafile.

- (For inspection after preprocessing)

lines 143-150

- Running ICA.

- Running automatic labeling of the components.

- Getting source components.

lines 152-158

- Extraction of eye components.

- Uses threshold high probability for being an eye component.

lines 166-187

- If the score/probability for an eye is higher than 0.20, it will continue with the rest of the pipeline. Otherwise, we will assume there is no eye movement.

- Filtering eye components accounting for slow drifts and high-frequency noise.

lines 188-229

- Per component analysis on the different aspects of the eye movements traces.

- Convolving with the average eye kernel to ensure the component is representable for eye movement.

- Rescaling component for further analyses.

lines 232-277

- Initializing arrays for data storage.

- Finds peaks per interval, also counts them as 1 if they are too close, find the amplitude change of the peaks. (239-262)

- Stores the number of peaks, their intensity (abs change in amplitude), and a combinatory measure in the data array. (263-269)

- Stores the final metrics into values.

lines 279-285

- Prepares the final metric for storage, i.e., eye movement per minute, amplitude changes, etc.

lines 293-326

- Saves variables into an Excel file, checks if this is already present in the folder, if yes it builds upon this, otherwise a new Excel file is created.

EyE_pipeline_FLCK.mat

lines 1-39

- Clearing workspace.

- Allocating correct paths.

- Loading necessary files.

- Selecting subjects and propofol level files to analyze.

- Adding necessary plugins and functions to the path.

lines 41-80

- Loop for going through all selected files and subjects.

- Starting EEGLAB.

- Loading file, which will be analyzed.

lines 82-94

- Preprocessing of which:

- Removal of cardio ballistic artifact.

- fMRI gradient artifact.

- (Commented out as most files analyzed were already preprocessed.)

lines 97-136

- Finding exact times when stimulation frequencies change (finding correct intervals).

- Looks for when triggering changes.

- Interpolates for if there are no triggers (baseline).

- Saves trigger time onsets in MATLAB variable eeg_trigger.mat.

- Generally, the frequency stimulation protocols are changed every 20 seconds. However, there is a slight lag, so this extra code is added to accurately extract the times at the millisecond level when the stimulation frequency changes.

lines 138-139

- Removal of coil wires and ECG channels.

lines 142-166

- Optional removal of bad channels which could corrupt ICA.

- First, the filenames are cleaned to extract the corresponding data for the subject and session. (141-144)

- Then, the channels are removed from the data. (146-166)

lines 167-168

- Resampling to milliseconds.

lines 170-177

- Finds corresponding fMRI volume to the changing frequency stimulation.

- Stores in MATLAB variable mri_volumes.mat.

- Removal of 1st seconds before 1st fMRI volume.

lines 179-181

- Changing variable names to avoid confusion later on.

- Applying HPF to get rid of slow drifts.

lines 182-187

- Optional lines for storage of interim datafile.

- (For inspection after preprocessing)

lines 188-191

- Adjusting of trigger time values for further use in the pipeline.

lines 192-201

- Running ICA.

- Running automatic labeling of the components.

- Getting source components.

lines 202-215

- Extraction of eye components.

- Uses threshold high probability for being an eye component.

lines 217-234

- If the score/probability for an eye is higher than 0.20, it will continue with the rest of the pipeline. Otherwise, we will assume there is no eye movement.

- Filtering eye components accounting for slow drifts and high-frequency noise.

lines 239-257

- Per component analysis on the different aspects of the eye movements traces.

- Convolving with the average eye kernel to ensure the component is representable for eye movement.

- Rescaling component for further analyses.

lines 259-322

- Initializing arrays for data storage.

- Finds peaks per predefined interval, also counts them as 1 if they are too close, find the amplitude change of the peaks. (289-315)

- Stores the number of peaks, their intensity (abs change in amplitude), and a combinatory measure in the data array. (316-321)

- Stores the final metrics into values.

lines 332-229

- Prepares the final metric for storage, i.e., eye movement per minute, amplitude changes, etc.

lines 342-375

- Saves general variables into an Excel file, and checks if this is already present in the folder; if yes, it builds upon this; otherwise, a new Excel file is created.

lines 377-459

- Creates a new Excel file, one for each subject and propofol level, and stores the different variables (number of eye movements, average amplitude change, and combinatory measure) per 20-second interval.

- Is stored in the corresponding subject and session folder.

Results of Running the Pipelines

EyE_pipeline_RS.mat

1. Excel file with the corresponding data for each subject and propofol level. This will be stored in a predefined folder or in the main path folder. In our example named: 'resting_state_data.xlsx'. The following variables are stored in the Excel file:

- Filename.

- Total number of eye movements.

- Average eye movements per minute.

- Average amplitude change.

- Combinatory measure 1 and 2, 1 accumulated, 1 averaged per minute.

- Maximum eye score from ICLabel.

EyE_pipeline_FLCK.mat

1. Excel file with for each subject and propofol level the corresponding data. This will be stored in a predefined folder or in the main path folder. In our example, this is named 'flicker_data.xlsx'. The following variables are stored in the Excel file:

- Filename.

- Total number of eye movements.

- Average eye movements per minute.

- Average amplitude change.

- Combinatory measure 1 and 2, 1 accumulated, 1 averaged per minute.

- Maximum eye score from ICLabel.

2. For each subject and run, an additional excel file will be stored, in the example case this will be named eye_mov_per_interval.xlsx. This will be stored in the respective subject and propofol-level folder. These files could not be saved in a similar way as the previous way because sometimes the duration of the recording can differ. In every Excel file you will find the following data:

- The filename of the file you just ran your analyses on.

- A row for each variable 'sum_nr_eye_movements', 'sum_avg_abs_amplitude_change', and 'sum_combined_measure_interval'.

- Columns for each 20-second interval with the corresponding values.

If you want to combine the data from all these files into 1 excel file. , the is a short Matlab script for this provided named excell_combiner_interval_data.mat

3. eeg_times.mat, variable with the times in millisecond precision for when the stimulation frequency changed.
4. mri_volumes.mat, variable with the corresponding MRI volume number at which the stimulation frequency changed.

Additional Code

condition_order_excell_file_creator.mat

This file is provided to create an Excel file of the stimulation frequency protocols for each subject and propofol level.[1] This data is stored separately for each subject and condition on the web disk at: *klifoanae/propofolstudy/Experiments*. When running this .mat file, adjust for which subjects and propofol levels you want to include the protocols. This will create the following file: subject_condition_data.xlsx.

excell_combiner_interval_data.mat

This file is provided to combine the data per 20-second interval of all subjects. This will output an Excel file named: combined_eye_stim_freq.xlsx.

Post Analysis Code

The following code will assume that the pipelines have already run and the following files are created:

- resting_state_data.xlsx

- flicker_data.xlsx

- subject_condition_data.xlsx

- combined_eye_stim_freq.xlsx

general_data_analysis.mat

This file has two sections, both performing within-subjects repeated measures of Friedman's test and posthoc Dunn test to check for differences in eye movement between the different propofol levels. The first section analyzes the resting state data, while the second focuses on the flicker frequency data. The division is necessary because the resting state data varies in duration, being recorded in multiple sessions (3-minute and 7-minute sessions), and requires adjustments for this. In contrast, the flicker data sessions have similar durations, so no correction is needed.

The output is a figure with the distributions of the analyzed eye movement measures. There are three measures: the number of eye movements, their average intensity, and a combinatory measure. These are analyzed separately with the corresponding variable names: NrOfEyeMovements, AverageIntensity, and mean_CombinedMeasure. [2] For the resting state data, the number of eye movements is analyzed; for the flicker data, their average intensity is analyzed.

The results should resemble the graphs found in figure 2.
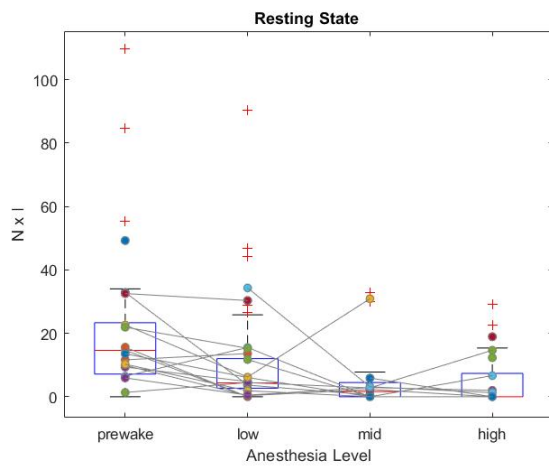
flicker_analysis.mat

This MATLAB file is designed for visualizing preliminary results of eye movement data. It generates scatter plots showing how different eye movement measures vary with frequency. The plots account for the frequency's occurrence in the protocols but do not consider interpersonal differences. These visualizations provide a rough estimate of expected results.

The script begins by loading data and fixing a bug. It then has two sections for plotting the results of different eye movement measures across propofol levels and stimulation frequencies. The first section combines all graphs into a single plot within one figure, while the second section creates multiple subplots within one figure.
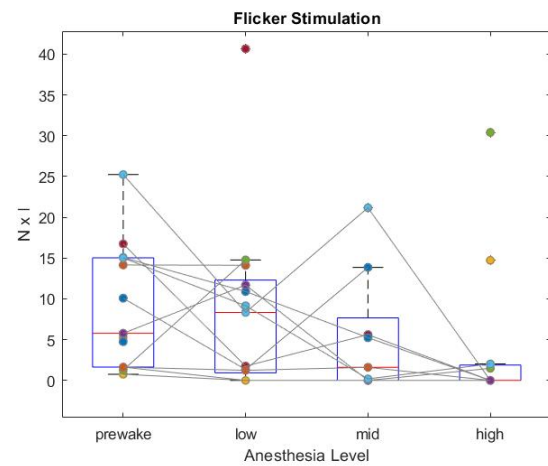
The results should resemble the graph shown in figure 3.

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[1]As the first 15 seconds of the data were removed, the first column of this file should also be removed to align the data.

[2]Change this measure to your liking simply with ctrl+f and replace.

(a) Combinatory Measure in Resting State

(b) Combinatory Measure in Visual Flicker Stimulation

Figure 2 Example results of running script general_data_analysis.mat on general Combintory Measure data from Resting State and Visual Flicker.
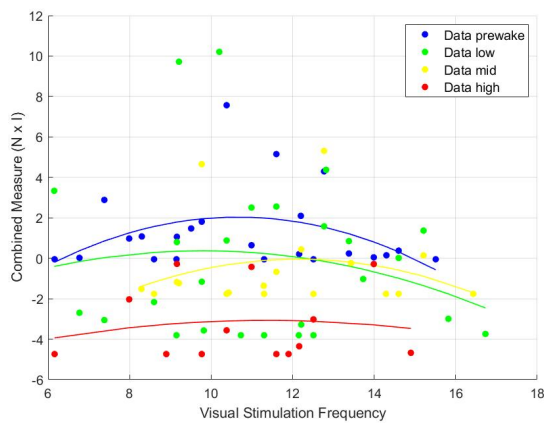

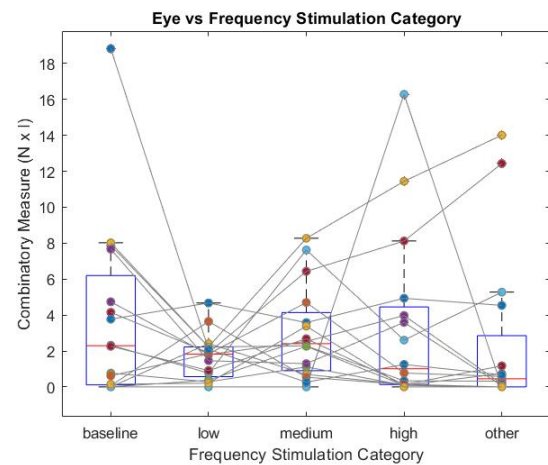**Figure 3** Example results of running script flicker_analysis.mat on Combinatory Measure data.

Figure 4 Example of running within_subj_flicker_analysis.mat on Combinatory Measure data.

within_subj_flicker_analysis.mat

This code analyzes the effect of stimulation frequency on eye movement across different propofol levels using a within-subjects repeated measures Friedman test and a posthoc Dunn test.

First, data is loaded, and a bug is resolved. Note that the 'other' frequency refers to Prewake IAF, and the 'medium' frequency refers to within-condition IAF, which are often similar with only four data points per subject per propofol level.

In the initial sections, a Friedman test is conducted on the general data. The final parts of the script examine the effect of a specific frequency category on eye movement at different propofol levels. This is a proof of concept; some intermediate steps must be completed in Excel due to MATLAB limitations.

The results should resemble the plot in figure 4.

Acknowledgements

External Sources

For enquiries about functions and their functionality in Eeglab please consult the eeglab website and wiki [1] [7]. For particular inquiries about Matlab, please contact the MathWorks helpdesk or website for answers [4] [5]. For more work on visual hallucinations and eye movements, please take a look at work from Manni, Pachetti, and Jiang [6, 3, 2].

References

[1] *Eeglab*. nl-NL. URL: https://www.youtube.com/channel/UCK8x1bdiHWmT0yCNEjTcEvA (visited on 07/29/2024).

[2] Yinyin Jiang et al. "Minor hallucinations in Parkinson's disease with probable rapid eye movement sleep behavior disorder". In: *Frontiers in Neuroscience* 17 (Aug. 2023), p. 1205439. ISSN: 1662-453X. DOI: 10.3389/fnins.2023.1205439. URL: https://www.frontiersin.org/articles/10.3389/fnins.2023.1205439/full (visited on 07/30/2024).

[3] Raffaele Manni. "Rapid eye movement sleep, non-rapid eye movement sleep, dreams, and hallucinations". en. In: *Current Psychiatry Reports* 7.3 (May 2005), pp. 196–200. ISSN: 1535-1645. DOI: 10.1007/s11920-005-0053-0. URL: https://doi.org/10.1007/s11920-005-0053-0 (visited on 07/30/2024).

[4] *Matlab*. en. URL: https://nl.mathworks.com/products/matlab.html (visited on 07/30/2024).

[5] *Matlab answers - matlab central*. URL: https://nl.mathworks.com/matlabcentral/answers/index (visited on 07/30/2024).

[6] Claudio Pacchetti et al. "Relationship between hallucinations, delusions, and rapid eye movement sleep behavior disorder in Parkinson's disease". en. In: *Movement Disorders* 20.11 (Nov. 2005), pp. 1439–1448. ISSN: 0885-3185, 1531-8257. DOI: 10.1002/mds.20582. URL: https://movementdisorders.onlinelibrary.wiley.com/doi/10.1002/mds.20582 (visited on 07/30/2024).

[7] *Welcome to the eeglab wiki*. en-US. URL: https://eeglab.org/ (visited on 07/29/2024).