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| Scripting with LIMO EEG | June 14  2013 | |
| I illustrate how to script with the LIMO EEG, keeping all the functionality of the toolbox. Rather than calling low level functions, I show which information are needed to create a LIMO.mat and call high level functions (or wrappers) which do all the hard work0 | |  |

**LIMO toolbox: LInear Modeling of EEG data.**

**‘Standard’ hierarchical modeling using**

**SCRIPTING for mass univariate approach.**

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The LIMO toolbox is a set of Matlab functions primarily designed for hierarchical linear modeling, i.e. analyzes rely on statistical testing using the general linear model The 1st level analysis (per subject) relies on Ordinary Least Squares and 2nd level (group) analysis relies both on robust estimators like trimmed means to deal with outliers and on resampling techniques to ensure the validity of statistics even under non normality and heteroscedasticity of the parameter estimates, whilst keeping the type I error rate at the nominal level. [For now only 2nd level t-tests rely on trimmed means – other designs are being validated and will be available in version 1.5].

Contents

[Before starting 3](#_Toc359009964)

[1st level analysis (for each subject) 4](#_Toc359009965)

[A. Prepare the data 4](#_Toc359009966)

[B. Create the design matrix 4](#_Toc359009967)

[C. Compute parameter estimates 5](#_Toc359009968)

[D. Get the results maps saved automatically 5](#_Toc359009969)

[2nd level analysis (across subjects) 6](#_Toc359009970)

[One-sample t-test 6](#_Toc359009971)

[Two-samples t-test 6](#_Toc359009972)

[Paired t-tests 7](#_Toc359009973)

[Regression 8](#_Toc359009974)

[ANOVA/ANCOVA 9](#_Toc359009975)

[Repeated Measures ANOVA 10](#_Toc359009976)

[Appendix 11](#_Toc359009977)

[Appendix A. Create the design matrix 11](#_Toc359009978)

[Appendix B. Low Level functions used in 1st level analysis 11](#_Toc359009979)

[Appendix C. Low Level functions used in 2st level analysis 11](#_Toc359009980)

# Before starting

LIMO Batch has been created to run your 1st level analysis effortlessly: it asks iteratively subjects folder, .set and variables and then run the analysis for all subjects. Even better, if the .set is in the subject folder and you use the same names across subjects for your categorical and continuous variables then you will be ask only once to select files. Now if you are not happy with limo\_batch.m, you can also script.

Most of the LIMO functions take multiple inputs and often in 2 forms like (data, LIMO) or (data, parameter 1, parameter2, etc). The easiest way to scrip is (1) to create a LIMO.mat file containing the right information, which in turn will allow to call those functions easily and also to use the GUI at any time like e.g. to display results and (2) to use wrappers. Wrappers are meta-functions which call others. For instance, if you want, for 1 subject, to run a glm + bootstrap + tfce you could call each function (limo\_glm1.m, limo\_glm1\_boot.m, limo\_tfce.m), and each time create files to be saved on the disk. Alternatively you can create a LIMO.mat and call limo\_eeg(4) which will do all of this. Below I describe how I script, i.e. using wrappers.

# 1st level analysis (for each subject)

## A. Prepare the data

Create a structure LIMO

LIMO.Level = 1

LIMO.dir = directory for the analysis

LIMO.data.data\_dir = directory where to read them

LIMO.data.data = file name

LIMO.data.chanlocs = import channel location information

LIMO.data.start = when to start the analysis

LIMO.data.end = when to stop the analysis

LIMO.data.sampling\_rate = sampliong rate of the data

LIMO.data.Cat = Categorical variable(s)

LIMO.data.Cont = Continuous variable(s)

LIMO.data.neighbouring\_matrix = matrix describing which electrodes

are neighbourghs (not necessary

unless bootstrap is used)

LIMO.design.fullfactorial = 0/1 specify if interaction should

be included

LIMO.design.zscore = 0/1 zscoring of continuous regressors

LIMO.design.method = 'OLS'

LIMO.design.type\_of\_analysis = ‘Mass-univariate’

LIMO.design.bootstrap = 0/1 indicates if bootstrap should be

performed or not (by default 0 for

group studies)

LIMO.design.tfce = 0/1 indicates to compute TFCE or not

By convention, we distinguish between two types of variables: the categorical variable and the continuous variables. The *categorical variables* are defined by a vector or matrix identifying groups of trials. Each column is considered as a factor whilst each value in each column is considered as a specific condition. The *continuous variables* are defined by a vector or matrix such as each column contains values characterizing each trials. The software handles from 1 to (N trials - Number of categorical + continuous - 1) predictors. Above this limit, there are more columns in X (design matrix) than rows in Y (trials) and no solution can be found.

## B. Create the design matrix

To create your own design matrix see Appendix A, otherwise simply call limo\_design\_matrix to (1) create the design (2) update the LIMO.mat structure and (3) create new files and reorganize the data

[LIMO.design.X, LIMO.design.nb\_conditions, LIMO.design.nb\_interactions, LIMO.design.nb\_continuous] = limo\_design\_matrix(Y, LIMO, 0);

At this stage LIMO is updated with

LIMO.design.X = design matrix

LIMO.design.nb\_conditions = vector that returns the number of

conditions per factor e.g. [2 2 2]

LIMO.design.nb\_interactions = vector that returns the number of

conditions perinteraction e.g. [4 4 4]

LIMO.design.nb\_continuous = scalar that returns the number of

continuous variables e.g. [3]

Also update manually LIMO with

LIMO.design.status = 'to do'

LIMO.design.name = name of the design (as you want)

Save LIMO.mat

## C. Compute parameter estimates

Being in the right directory, simply call *limo\_eeg(4)*

Analyzes are carried out such as the effect of experimental variables on the EEG time are tested for each electrode and time frames independently (in practice however the full time course is analyzed simultaneously electrode by electrode). Files previously created are updated. Depending on the design, files of the statistical results are also saved on the disk: Condition\_effect\_X (refers to a factor effect), Interaction\_effect\_X (refers to an interaction between factors), Covariate\_effect\_X (refers to the effect of a continuous regressor).

The LIMO structure is automatically updated

LIMO.design.weights = matrix of trial weights (ones for OLS)

LIMO.model = information about the statistics

LIMO.model.conditions\_df = df [effect, error]

LIMO.model.interactions\_df = df [effect, error]

LIMO.model.continuous\_df = df [effect, error]

LIMO.design.status = 'done'

If you set LIMO.design.bootstrap =1, the bootstrap will also be computed

If LIMO.design.tfce = 1, TFCE will also be computed.

Note that if you already analyzed the data without bootstrap/tfce, you can simply update those fields by setting the relevant fields to 1 in the LIMO structure and recalling limo(4), as it will skip whatever has already been computed. For more details on the stats function called see Appendix B.

## D. Get the results maps saved automatically

Simply call limo\_eeg(5)

# 2nd level analysis (across subjects)

As for the 1st level, we have two steps to follow: (1) prepare the data and a LIMO.mat (i.e. do the job of *limo\_random\_select.m*) and (2) call the wrapper *limo\_random\_robust.m* (see Appendix C for low level functions used in this wrapper)

## One-sample t-test

Organize the data with dimension electrode \* time frames \* subjects and save as Yr.mat. Prepare and save a LIMO.mat with that information:

LIMO.dir: where is the LIMO.mat and Yr

LIMO.data.chanlocs: chanel locations from the expected electrode file

LIMO.data.neighbouring\_matrix: binary matrix of neighbourhood

LIMO.data.data: cells with the full name of the Betas or con files

LIMO.data.data\_dir: cells with directories of the Betas or con files

LIMO.data.sampling\_rate: should be the same across subjects

LIMO.data.trim1: 1st data point to analyze

LIMO.data.start: 1st data point to analyze in sec

LIMO.data.trim2: last data point to analyze

LIMO.data.end: last data point to analyze in sec

LIMO.design.bootstrap: nb of bootstrap to perform (0 if none)

LIMO.design.tfce: 0 or 1

LIMO.design.name: 'one sample t-test all electrodes'

LIMO.design.electrode: [] (or 1value or set of values for optimized electrode analysis)

LIMO.design.X: []

LIMO.Level = 2;

Then call limo\_random\_robust(1,Yr,parameter number,nboot,tfce)

*with*

1 = a one-sample t-test

Yr = data (dim electrodes, frames, subjects)

parameter number = describe which parameters is analysed (e.g. 1 – used for naming only)

nboot = nb of resamples

tfce = set to 1 to compute TFCE

## Two-samples t-test

Organize the data as two separate matrices with dimension electrode \* time frames \* subjects and save as Y1r.mat and Y2r.mat. Prepare and save a LIMO.mat with that information:

LIMO.dir: where is the LIMO.mat and Yr

LIMO.data.chanlocs: chanel locations from the expected electrode file

LIMO.data.neighbouring\_matrix: binary matrix of neighbourhood

LIMO.data.data: 2 sets of cells e.g. {{1x10 cell} {1x8 cell}} with the full name of the Betas or con files

LIMO.data.data\_dir: cells with directories of the Betas or con files

LIMO.data.sampling\_rate: should be the same across subjects

LIMO.data.trim1: 1st data point to analyze

LIMO.data.start: 1st data point to analyze in sec

LIMO.data.trim2: last data point to analyze

LIMO.data.end: last data point to analyze in sec

LIMO.design.bootstrap: nb of bootstrap to perform (0 if none)

LIMO.design.tfce: 0 or 1

LIMO.design.name: 'one 'two samples t-test all electrodes'

LIMO.design.electrode: [] (or 1value or set of values for optimized electrode analysis)

LIMO.design.X: []

LIMO.Level = 2;

Then call limo\_random\_robust(2,y1,y2,parameter number,nboot,tfce)

*with*

2 = two samples t-test

y1 = data (dim electrodes, frames, subjects)

y2 = data (dim electrodes, frames, subjects)

parameter number = describe which parameters is analysed (e.g. 33 – used for naming only)

nboot = nb of resamples

tfce = set to 1 to compute TFCE

## Paired t-tests

Organize the data as two separate matrices with dimension electrode \* time frames \* subjects and save as Y1r.mat and Y2r.mat. Prepare and save a LIMO.mat with that information:

LIMO.dir: where is the LIMO.mat and Yr

LIMO.data.chanlocs: chanel locations from the expected electrode file

LIMO.data.neighbouring\_matrix: binary matrix of neighbourhood

LIMO.data.data: cells with the full name of the Betas or con files

LIMO.data.data\_dir: cells with directories of the Betas or con files

LIMO.data.sampling\_rate: should be the same across subjects

LIMO.data.trim1: 1st data point to analyze

LIMO.data.start: 1st data point to analyze in sec

LIMO.data.trim2: last data point to analyze

LIMO.data.end: last data point to analyze in sec

LIMO.design.bootstrap: nb of bootstrap to perform (0 if none)

LIMO.design.tfce: 0 or 1

LIMO.design.name: 'paired t-test all electrodes'

LIMO.design.electrode: [] (or 1value or set of values for optimized electrode analysis)

LIMO.design.X: []

LIMO.Level = 2;

Then call limo\_random\_robust(3,y1,y2,parameter number,nboot,tfce)

*with*

3 = paired t-test

y1 = data (dim electrodes, frames, subjects)

y2 = data (dim electrodes, frames, subjects)

parameter number = describe which parameters is analysed (e.g. 12 – used for naming only)

nboot = nb of resamples

tfce = set to 1 to compute TFCE

## Regression

Prepare a LIMO.mat file

LIMO.dir: where is the LIMO.mat and Yr

LIMO.data.chanlocs: chanel locations from the expected electrode file

LIMO.data.neighbouring\_matrix: binary matrix of neighbourhood

LIMO.data.data: cells with the full name of the Betas or con files

LIMO.data.data\_dir: cells with directories of the Betas or con files

LIMO.data.sampling\_rate: should be the same across subjects

LIMO.data.trim1: 1st data point to analyze

LIMO.data.start: 1st data point to analyze in sec

LIMO.data.trim2: last data point to analyze

LIMO.data.end: last data point to analyze in sec

LIMO.design.bootstrap: nb of bootstrap to perform (0 if none)

LIMO.design.tfce: 0 or 1

LIMO.design.name: 'regression analysis all electrodes'

LIMO.design.electrode: [] (or 1value or set of values for optimized electrode analysis)

LIMO.design.X: []

LIMO.Level = 2;

Then call limo\_random\_robust(4,y,X,parameter number,nboot,tfce)

*with*

4 = regression analysis

y = data (dim electrodes, frames, subjects)

X = continuous regressor(s) (i.e. a matrix of continuous values)

Alternatively, to avoid some extra questions related to zscoring and running the analysis given the design matrix you can update the LIMO.mat file as follow

LIMO.design.type\_of\_analysis = 'Mass-univariate';

LIMO.data.Cat = 0;

LIMO.data.Cont = X;

LIMO.data.data\_dir = pwd;

LIMO.design.fullfactorial = 0;

LIMO.design.zscore = 1 (default) to zscore otherwise 0;

LIMO.design.status = 'to do';

LIMO.design.method = 'OLS';

Then update and create the design matrix

[LIMO.design.X, LIMO.design.nb\_conditions, LIMO.design.nb\_interactions,LIMO.design.nb\_continuous] = limo\_design\_matrix(data, LIMO,1);

And call limo\_eeg(4)

## ANOVA/ANCOVA

Prepare the data with dimension electrodes \* time frames \* subjects and set a LIMO.mat file

LIMO.dir: where is the LIMO.mat and Yr

LIMO.data.chanlocs: chanel locations from the expected electrode file

LIMO.data.neighbouring\_matrix: binary matrix of neighbourhood

LIMO.data.data: cells with the full name of the Betas or con files

LIMO.data.data\_dir: N sets of cells with directories of the Betas or con files for the N groups of subjects

LIMO.data.sampling\_rate: should be the same across subjects

LIMO.data.trim1: 1st data point to analyze

LIMO.data.start: 1st data point to analyze in sec

LIMO.data.trim2: last data point to analyze

LIMO.data.end: last data point to analyze in sec

LIMO.design.bootstrap: nb of bootstrap to perform (0 if none)

LIMO.design.tfce: 0 or 1

LIMO.design.name: N-ways ANOVA all electrodes'

LIMO.design.electrode: [] (or 1value or set of values for optimized electrode analysis)

LIMO.design.X: []

LIMO.Level = 2;

Then call limo\_random\_robust(5,y,cat,cont,nboot,tfce)

with

5 = N-way ANOVA/ANCOVA

y = data (dim electrodes, frames, subjects)

cat = categorical variable(s)

cont = continuous regressors (covariates)

nboot = nb of resamples

tfce = 1 run tcfe

Alternatively, to avoid an extra question related to running the analysis given the design matrix you can update the LIMO.mat file as follow

LIMO.design.type\_of\_analysis = 'Mass-univariate';

LIMO.data.Cat = cat;

LIMO.data.Cont = cont;

LIMO.data.data\_dir = pwd;

LIMO.design.fullfactorial = 0 or 1;

LIMO.design.zscore = 0 or 1;

LIMO.design.status = 'to do';

LIMO.design.method = 'OLS';

Then update and create the design matrix

[LIMO.design.X, LIMO.design.nb\_conditions, LIMO.design.nb\_interactions, LIMO.design.nb\_continuous] = limo\_design\_matrix(data, LIMO,1);

And call limo\_eeg(4)

## Repeated Measures ANOVA

Prepare the data with dimension electrodes \* time frames \* subjects \* repeated measures and set a LIMO.mat file

LIMO.dir: where is the LIMO.mat and Yr

LIMO.data.chanlocs: chanel locations from the expected electrode file

LIMO.data.neighbouring\_matrix: binary matrix of neighbourhood

LIMO.data.data: cells with the full name of the Betas or con files

LIMO.data.data\_dir: cells with directories of the Betas or con files

LIMO.data.sampling\_rate: should be the same across subjects

LIMO.data.trim1: 1st data point to analyze

LIMO.data.start: 1st data point to analyze in sec

LIMO.data.trim2: last data point to analyze

LIMO.data.end: last data point to analyze in sec

LIMO.design.bootstrap: nb of bootstrap to perform (0 if none)

LIMO.design.tfce: 0 or 1

LIMO.design.name: ' Repeated measures ANOVA all electrodes'

LIMO.design.electrode: [] (or 1value or set of values for optimized electrode analysis)

LIMO.design.X: []

LIMO.Level = 2;

Then call limo\_random\_robust(6,y,gp,factor\_levels,nboot,tfce)

with

6 = Repeated measures ANOVA

y = data (dim electrodes, frames, subjects, measures)

gp = a vector defining gps like [111111222222333333]

factor\_levels = a vector specifying the levels of each repeated measures factor like [3 2]

nboot = nb of resamples

tfce = 1 run tcfe

# Appendix

## Appendix A. Create the design matrix

For some complicated types of design you might want to create your own design matrix. For this design matrix to be used with limo functions, a few things need to set.

Categorical variables are always on the left hand side of the matrix and are coded in LIMO.design.nb\_conditions using a vector. For instance [2] means 2 columns, but [2 2] means 2 factors of 2 columns. The design matrix has thus 4 columns in this case, allowing testing the two main effects. LIMO.design.nb\_interactions would be set to 0. However, it can also be set to 4 adding the interaction term. The design matrix has thus 8 columns (2 columns factor 1, 2 columns factor 2, 4 columns for interaction). See *limo\_make\_interactions.m* to automatically generate interaction columns from the main factors.

Continuous variables are on the right hand side of the design matrix and are coded as a scalar in LIMO.design.nb\_continuous. For instance 3 means 3 variables (columns).

The constant term (ones) is coded in the last column.

## Appendix B. Low Level functions used in 1st level analysis

*limo\_design\_matrix*: create design matrix and return outputs for limo\_glm1

*limo\_make\_interaction*: from the data and a set of factors, create the interaction terms (used in limo\_design\_matrix)

*limo\_glm1*: core of the stat computation

*limo\_glm1\_boot*: re-run the GLM under H0

*limo\_contrast*: post-hoc tests from limo\_glm1.m, allows

*limo\_tfce* compute tfce scores for observed and bootstrapped data

## Appendix C. Low Level functions used in 2st level analysis

*limo\_trimci* one sample t-test using trimmed mean

*limo\_yuen\_ttest*: 2 samples t-test using trimmed means

*limo\_yuend\_ttest*: paired t-test using trimmed means

*limo\_glm1* core of the stat computation for regression/anova/ancova – called via limo\_eeg(4)

*limo\_glm1\_boot*: re-run the GLM under H0 – called via limo\_eeg(4)

*limo\_rep\_anova* Hotelling test for repeated measures ANOVA and gp x repeated measures