Manual

FEMTO 2.0

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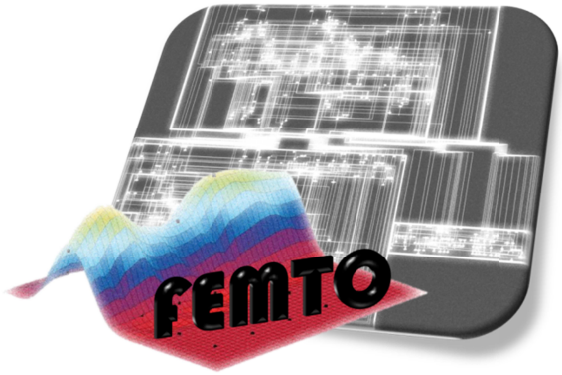
Functional Evaluation of Metabolic Time series Observations



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1. FEMTO 2.0: a Matlab®[1]-based graphical user interface

A functional connection and interpretation of metabolic time series data in context of metabolic networks represents one of the central research topics in systems biology. The motivation for the development of FEMTO (**Fig. 1**) was to provide an intuitive graphical user interface enabling the efficient integration of experimental time series data and metabolic network information. **Compared to version 1.0, the user interface FEMTO 2.0 has changed particularly with respect to the graphical representation and possibility to directly export a MATLAB® Figure File. The calculation algorithm and theoretical background is the same as in version 1.0.**

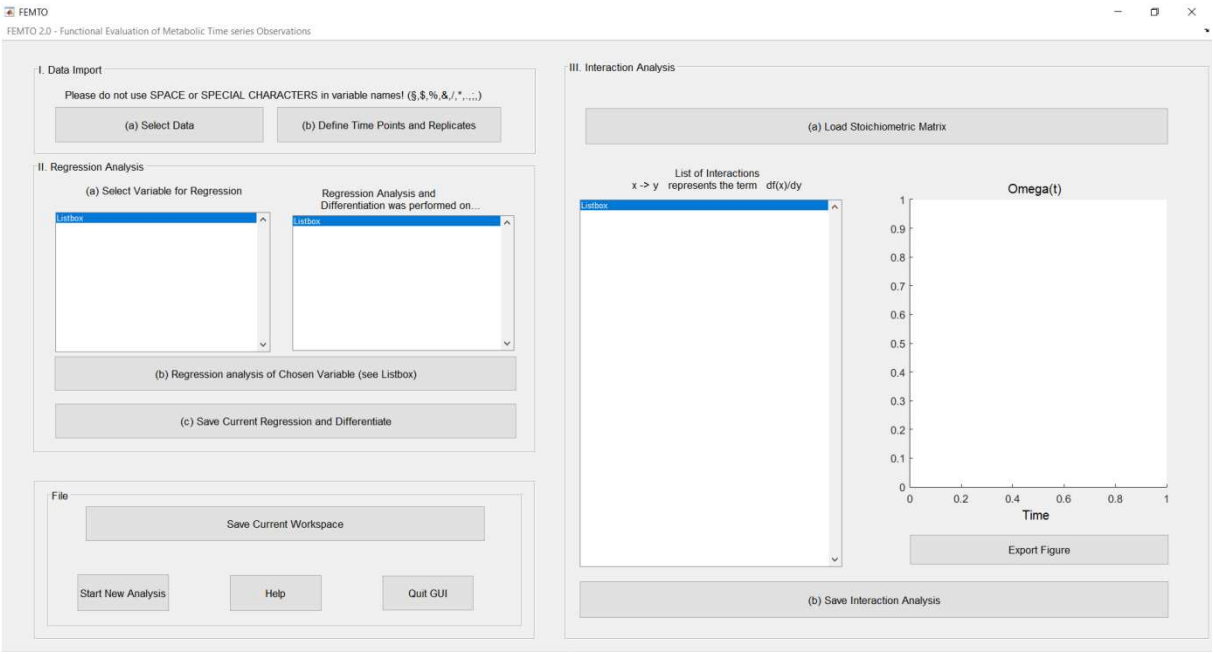

Figure 1: The graphical user interface FEMTO 2.0

This manual is intended to guide the user through the evaluation process of time series data. The main steps which have to be performed during data import, regression analysis and evaluation are graphically comprised by panels and indicated by a sequence of Roman numerals (**Steps I. – III.**; **Fig. 1**). During each of those main steps, several minor steps have to be performed which are indicated by lowercase letters (see **Fig. 1**). To assist the user, buttons will change their colour from grey to cyan as soon as they are tapped.

We hope that the following chapters are helpful in context of data evaluation and toolbox application. However, in case of open questions, problems with version compatibility or suggestions for improvement, please do not hesitate to contact us using the contact information provided on the title page of this document.

The FEMTO developer-Team

[1] www.mathworks.com

2. Requirements

FEMTO 2.0 is a MATLAB®-based graphical user interface and was developed on MATLAB® R2015a (8.5.0) 64-bit(win64). The MathWorks® Curve Fitting Toolbox™ is needed for the regression analysis which is part of the FEMTO workflow.

3. Execution of FEMTO 2.0

1. Save and unpack the FEMTO package to a location on your hard drive
2. Open folder \...**FEMTO** in the *Current Folder* of your MATLAB® window.
3. Type 'FEMTO' in the *Command Window* of MATLAB®. Alternatively, you can start FEMTO 2.0 by choosing 'FEMTO.m' with the left mouse-button and tapping F9 on your keyboard.
4. Remark: to save the path of the folder 'FEMTO' to your MATLAB® search path, use the MATLAB® *pathtool*.

4. Application of FEMTO 2.0

Data Import: Panel I

Clicking on the button **'(a) Select Data'** allows the selection of a file containing time series data. As soon as the data file has been chosen, an import wizard opens which enables the user to specify the data import.

IMPORTANT: Data have to be organized row-wise (rows -> variables; columns -> replicates for each time point). Please have a look at the provided example data set for correct data organization. If the data are not organized correctly, the evaluation will fail!

In the next step **'(b) Define Time Points and Replicates'** the user is asked to provide the time points and number of replicates at each of these time points. Time points have to be entered in the following format: **[1 2 3 …n]** (see **Fig. 2**).

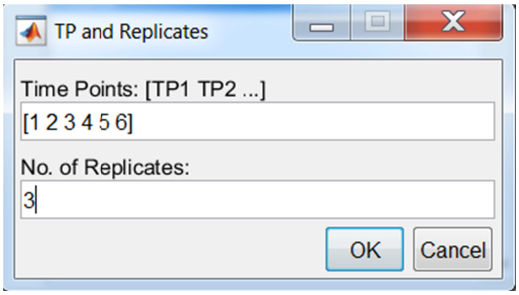

Figure 2: Prompt for definition of time points and number of replicates. In this example, the time points are 1,2,3,4,5,6 (arbitrary time units) and the number of replicates is 3.

Regression Analysis and Differentiation: Panel II

To select a variable for regression, click on listbox **'(a) Select Variable for Regression'**. All variables which have been imported should become visible in the listbox. Select one variable from the list and click on **'(b) Regression analysis of Chosen Variable (see Listbox)'**.

The window of the MathWorks® Curve Fitting Toolbox™ opens. Choose '**timepoints**' for X data, the selected variable for Y data and '**Selected_Weights**' for Weights. The regression method can be chosen by the user. We recommend the '**Smoothing Spline**' with default settings (**Fig. 3**).

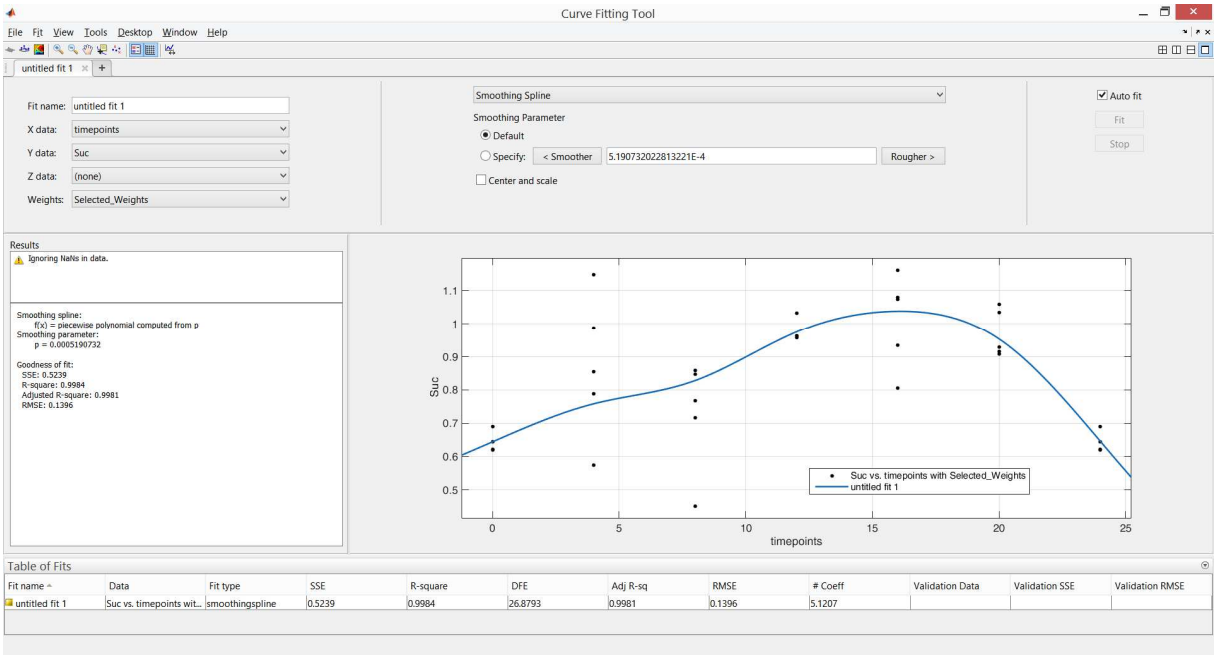

Figure 3: Settings for the regression analysis in the MathWorks® Curve Fitting Toolbox™.

Save the fitting results by clicking on '**Fit -> Save to Workspace…**'. Please use the default settings for saving the fitting results (**Fig. 4**)! Having saved the results to the workspace, go back to the FEMTO main window and click on **'(c) Save Current Regression and Differentiate**' – this makes the results applicable in FEMTO.

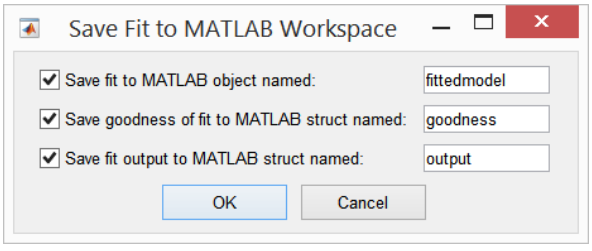

Figure 4: Default settings for saving the regression results.

The above described procedure has to be repeated for all variables. To provide a better overview of successfully fitted and saved variables, all analysed and saved variables are displayed in the listbox **'Regression Analysis and Differentiation was performed on…'**. Simultaneously, differentiation of regression output is performed being necessary for the following interaction analysis.

IMPORTANT: For starting a new regression analysis, it is necessary that the user performs all steps again. Entries in the Curve Fitting Toolbox™ **are not automatically updated**, i.e. to load the correct weights for the regression analysis, the user has to choose '**Selected Weights**' for each individual regression (**Fig. 5**)!

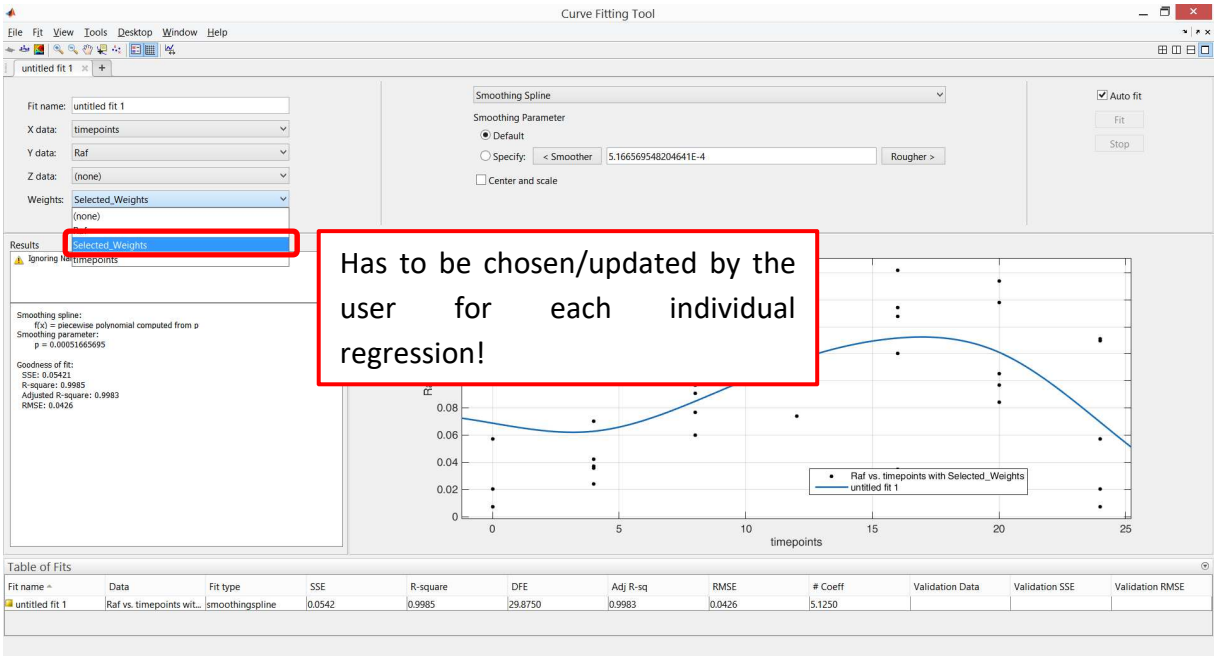

Figure 5: It is important to choose the 'Selected_Weights' for each individual regression separately. The Curve Fitting Toolbox™ will not automatically update the weight information!

Interaction Analysis: Panel III

Clicking on the button **'(a) Load Stoichiometric Matrix'** loads the stoichiometric matrix of the metabolic network. An import wizard window opens which allows the user to choose the stoichiometric matrix information. Following the loading process, the user can select a combination of variables from the **'List of Interactions'** to be displayed in the plot window (entitled **'Omega (t)'**). All combinations which can be chosen from both listboxes are derived from the network information.

IMPORTANT: The **stoichiometric matrix** of a metabolic reaction network is organized in the following format: **variables in rows, reactions in columns**! Variable names, i.e. metabolites, in the stoichiometric matrix have to be named according to the names in the regression

analysis! **Variable names are compared in a case-sensitive manner**, i.e. lower-case and capital letters are distinguished! If variables are not named equivalently, interaction analysis is not possible!

To export the figure, please click on **'Export Figure'** and a new window will open which allows you to save and further modify the plot results.

To save the results of data analysis in a .csv or .xlsx – file, please click on **'(b) Save Interaction Analysis'**. In addition, this will automatically write and save all results in the current MATLAB® workspace (data in 'Calculated_Interactions'; headers of data matrix in 'Labels_Interactions'; **Fig. 6**).

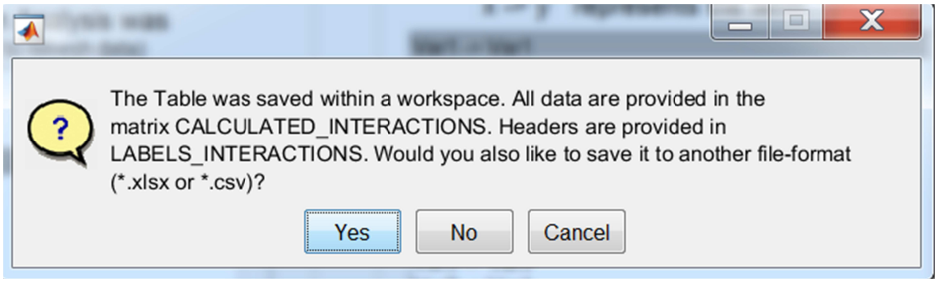

Figure 6: Saving results in csv- or xlsx-format. This dialogue window opens after clicking on '(c) Save Interaction Analysis'.

The File Panel

At each point of of data evaluation, results can be saved to a MATLAB® workspace using the button **'Save Current Workspace'**. The workspace can be used for further data analysis in MATLAB®. A restart of FEMTO 2.0 is possible by clicking on the button 'Start New Analysis'.

IMPORTANT: a restart irreversibly deletes all information and evaluation results from the current analysis!

Example Data

A data set is provided to test the workflow of FEMTO 2.0 ('Example_Data.xlsx'). The data set contains experimental data (3 replicates, 6 time points, 9 variables) and a stoichiometric matrix which can be loaded and applied.