User Manual for ETA

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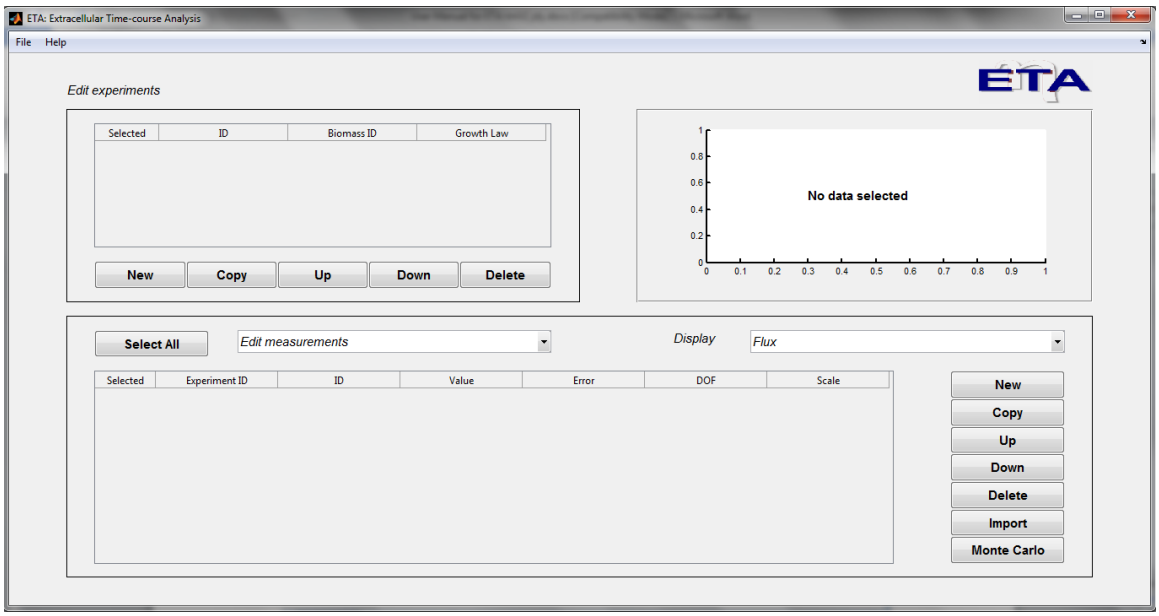
Program Start

Begin a new MATLAB session. Change the MATLAB working directory to the ETA root directory. Type “eta” at the MATLAB command prompt and hit enter. This will launch ETA and bring up the ETA home screen shown below.

Home Screen



On the ETA home screen there are three windows. The upper-left window is where a new experiment (i.e., time course) is created. The upper-right window is where figures are plotted. The bottom window is where individual data sets are edited within experiments.

Data Input

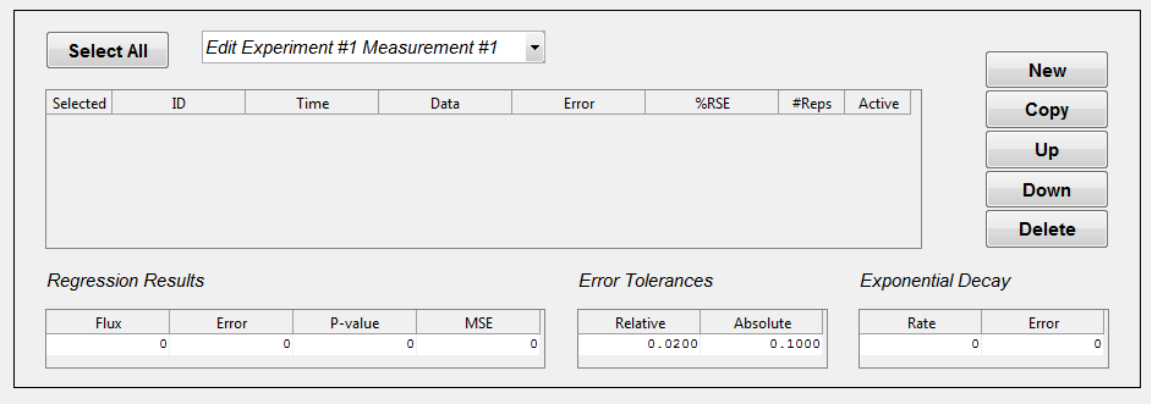
To Make a New Experiment
Each experiment represents a separate time course. If another cell line or condition is studied, a new experiment has to be created. Multiple time courses cannot be analyzed under a single experiment.

Underneath the upper-left window select the button labeled 'New'. Replace the text in the 'ID' column with an identifier of your choice. The 'Biomass ID' defaults to 'Growth'. This box should have the same ID as the cell density measurement specified in the lower panel. It can be named anything, as long as there is a matching ID in the lower panel. You can specify the 'Growth Law' to be either 'Exponential' or 'Linear' (see Appendix for details).

To Add Measurements to an Experiment
Select the corresponding box under the column heading 'Selected' in the upper-left window. In the bottom window, select the button labeled 'New'. A new data set is created with the columns 'Selected', 'Experiment ID', 'ID', 'Value', 'Error', and 'Scale'. The column 'Experiment ID' matches the ID of the selected experiment and cannot be changed unless the experiment ID is changed in the upper-left panel. The 'ID' entry can be set to any text, but at least one of the entries has to match the 'Biomass ID' in the upper-left panel (e.g., 'Growth'). 'Value' and 'Error', which display the estimated flux values and standard errors, respectively, are program outputs that are not directly editable. However, the 'Scale' column is editable and is used to convert the flux and error values to the desired units.

To Adjust the Units using the Scale Column
The flux units will be determined by the units of cell density (X/V), concentration (C=M/V), and time (t) entered. The units of flux (v) are given by [v] = [C] / [X/V] / [t]. For example, if cell density is entered as 10^6 cells / mL and concentration in mmol/L and time units are in hours, the flux units will be mmol*mL/L/10^6 cells/h. You need to set the scale factor to '0.001' (i.e., 1 L/1000 mL) to convert the units to mmol/10^6 cells/h. If cell density is not normalized to volume, then the scale factor should be multiplied by V to obtain the appropriate units. You can also include a negative sign in the scale factor to convert consumption fluxes to a positive value.

To Add Data to Measurement
There are two methods to add data to a specific measurement. The first is to select the measurement of interest from the dropdown list that says 'Edit Measurements'. This will change the lower panel to the following.



Selecting the button labeled ‘New’ will create a new row with the column headings of ‘Selected’, ‘ID’, ‘Time’, ‘Data’, ‘Error’, ‘%RSE, ‘#Reps’, and ‘Active’. ‘ID’ is a unique text identifier that is used to designate each specific time point. ‘Time’ is where you indicate when the data point was taken. ‘Data’ is the value that was measured. ‘Error’ is the standard error of the measurement. ‘%RSE’ is the relative standard error calculated by dividing the ‘Error’ value by the ‘Data’ value, and is not directly editable. ‘#Reps’ is the number of replicates of that measurement. Under the ‘Active’ column you can select whether each individual data point is included in the regression.

The other method for data entry is to import the data from Excel. After selecting the ‘Edit Measurements’ option on the dropdown menu, there will be a button on the far right labeled ‘Import’. Select a single measurement and press ‘Import’ to bring up a dialog box that will describe what to do next. The method of import is platform-dependent, as described in the next section.

Importing: Mac vs. Windows
For both Mac and Windows operating systems, the data has to be formatted into four columns: ‘Time’, ‘Data’, ‘Error’, ‘Replicates’. For Windows, the data can be on the same worksheet and you will interactively select the area to import. For Macs, each measurement has to be on a separate worksheet within the Excel file. The dialog box will prompt you to enter the name of the file and worksheet to be imported.

Moving Data Around
The experiments in the upper-left panel and the measurements in the lower panel can be copied, moved up or down in the list, or deleted by using the corresponding buttons within each panel. If multiple experiments are selected in the upper-left panel and ‘Edit Measurements’ is displayed in the lower dropdown menu, these buttons will be temporarily disabled. Select a single experiment in the upper-left panel to re-activate the buttons.

Saving and Opening ETA files
Selecting ‘Save As…’ from the ‘File’ menu will open a dialog box to save the data inputs as a MATLAB .mat file. The data can be loaded from this file by selecting ‘Open…’ from the ‘File’ menu and specifying the location of the saved file.

Regression analysis

Flux Calculation
Once the time course measurements have been entered, the algorithm will automatically calculate a flux rate for the selected experiments based on all data points marked 'Active'. In the case of cell density measurements, the flux value represents the specific growth rate of the culture. In the case of extracellular metabolites, the flux value represents the cell specific rate of product formation (negative in the case of substrate consumption).

Exponential Decay
If it is known that the metabolite of interest is spontaneously decaying or accumulating according to a first-order rate law (i.e., independent of cell metabolism), this adjustment can be made by selecting the corresponding measurement from the 'Edit Measurements' dropdown menu. Enter the value of the exponential decay rate and standard error in the table marked 'Exponential Decay'. Input a negative number for first-order accumulation.

Error Tolerances
Because each measurement data point is weighted by the inverse of its associated standard error, unrealistically low values can bias the regression analysis. Therefore, tolerances can be set to protect against this behavior. Both a relative tolerance (*RelTol*) and an absolute tolerance (*AbsTol*) can be entered. If the standard error of any data point is less than *AbsTol* or *RelTol**(measurement value), then the greater of these two will be used in place of the user-supplied standard error when performing the regression.

Goodness-of-fit
In addition to visualizing the linear fit in the graphical display window, ETA uses an F-test to assess lack-of-fit and reports the mean-squared-error (MSE) and p-value of the measurement selected in the 'Edit Measurements' dropdown menu. The closer the p-value is to 1, the better the data fit the balanced growth model. P-values less than 0.001 should be considered an indicator of poor fit.

Data Visualization

To View Multiple Datasets in the Graph
Select 'Edit Measurements' in the dropdown menu and then select multiple measurements in the lower window to create a bar graph in the upper-right window. If multiple selected measurements have the same ID, those bar graphs will be grouped together. Selecting multiple experiments in the upper-left window will allow the fluxes determined from several experimental time courses to be compared side-by-side.

Copy Plot from Figure Window
Right-clicking on the figure window will reveal a menu with the option 'Copy'. Left-clicking on this option will create a duplicate figure outside of the ETA user interface, which can then be edited using MATLAB's built-in commands and features.

Display (Yields)
It is possible to calculate yield values using the software without any additional data manipulation. When the 'Edit Measurements' option is selected from the dropdown menu, another dropdown bar is available on the right side of the software under the graphical display. Here, all of the yield options are available. The estimated yield values and standard errors are reported when these options are selected.

Monte-Carlo Estimation
Choosing the button labeled 'Monte Carlo' will initiate the calculation of the Monte-Carlo estimate of the error for all measurements in the currently selected experiment(s). This provides a more rigorous (yet, more time-consuming) method for assessing standard errors of the regressed parameters. The Monte Carlo estimates will temporarily overwrite the previous estimates.

Example

An example Excel file is provided in the 'Demo' folder to illustrate the format required for data import. This file is appropriate for either Mac or Windows import. Cell density (cells/mL) is recorded on the first worksheet. Glucose and lactate concentrations (mM) are on the second and third worksheets, respectively. The time unit is hours. The ETA .mat file associated with this data set is also provided. A scale factor of -1E9 for glucose and 1E9 for lactate was used to convert the flux units to nmol/million cells/hour.

Appendix

Balance equations for cell growth
The exponential growth equation is

$$X = X_0 e^{\mu t},$$

where μ is the net specific growth rate, X is the cell density (i.e., cell mass or number per unit volume of culture medium), t is time, and X_0 is the initial cell density at the onset of exponential growth.

The linear growth equation is

$$X = X_0 + \mu t.$$

Balance equations for substrate uptake and product formation
The general balance equation that relates changes in medium composition to extracellular metabolic fluxes under batch growth conditions is

$$\frac{dC}{dt} = -kC + vX,$$

where C is metabolite concentration, k is the first-order degradation rate constant, v is the specific metabolite production rate, and X is the cell density.

Changelog

Version 1.0
Creation date: 2012/9/4

Version 2.0
Creation date: 2012/11/28
Major updates: Introduced linear growth option in addition to exponential growth option