TReCCA Analyser tutorial 2

Smoothing and slope calculation

In this tutorial, we will go through all the parameters that have to be set in the TReCCA Analyser to analyse the time-resolved data available at: www.uni-heidelberg.de/fakultaeten/biowissenschaften/ipmb/biologie/woelfl/Research.html.

This data contains the on-line measurement of dissolved oxygen in the media of the breast cancer cell line MCF-7 when exposed to the anti cancer drug Cisplatin at concentrations ranging from 0 µM to 200 µM, using the commercially available 24-oxygen sensor plate, the OxoDish (PreSens Precision Screening, GmbH). For more details on the experimental procedure and justifications for the analysis, please refer to the corresponding publication: Lochead et al., PLOS ONE, 10(6):e0131233, 2015.

For more information on how to install the program or resolve possible error messages, please refer to the **TReCCA Analyser User Manual (4.0)**, also available on the website cited above.

1- Fast TReCCA Analyser presentation

Visualising the already analysed data using the R-Data and the settings

Open the TReCCA Analyser.

- Click on "**Import/export settings**", "**Load**" and then select the text file: "**MCF-7 and Cisplatin settings2.txt**". All the settings necessary for the data analysis will appear automatically in the "**Data input**", "**Labels & colours**" and "**Analysis options**" tabs.

- Click on "**Import/export R-data**", "**Load**" and then select the text file: "**MCF-7 and Cisplatin2.RData**". All the data will appear automatically in the "**Graph output**" and "**Data output**" tabs.

Analysing data using the settings and .csv files

We will use the data generated in the "Tutorial 1 - Sensor correction, Normalisation, Averaging and IC_{50} determination", "**Medium data.csv**". This file contains the semicolon ";" as separator and the coma "," as decimal separator. The data it contains has been cut after day 5, sensor corrected, medium normalised and the well B3.1 has been excluded.

Open the TReCCA Analyser.

- Click on "**Import/export settings**", "**Load**" and then select the text file: "**MCF-7 and Cisplatin settings2.txt**". All the settings necessary for the data analysis will appear automatically in the "**Data input**", "**Labels & colours**" and "**Analysis options**" tabs.

- In the "**Data input**" tab, click on the file chooser, select the "**Medium data.csv**" file. Click "**Import files**".

- In the "**Labels & colours**" tab, click on "**Load template**" and select the .csv file: "**Template_MCF-7 and Cisplatin_without B3.1.csv**".

- Click on "**Run analysis**". The analysis will be performed and the data will appear automatically in the "**Graph output**" and "**Data output**" tabs.

2- Detailed TReCCA Analyser presentation

Open the TReCCA Analyser. Go through the following tabs and set the corresponding parameters.

Data input

We will use the data generated in the "Tutorial 1 - Sensor correction, Normalisation, Averaging and IC_{50} determination", "**Medium data.csv**". This file contains the semicolon ";" as separator and the coma "," as decimal separator. The data it contains has been cut after day 5, sensor corrected, medium normalised and the well B3.1 has been excluded.

The following parameters therefore have to be entered in the data input.

Data layout

- How many plates do you want to analyse? **1**
- Do you have one document with all the data or one for each plate? **single document**
- Did you already clear all non numerical data? **Yes** *(done in tutorial 1)*
- What did you measure? **PreSens OxoDish**

Cutting borders

- Is a header included in the file(s)? **no** *(excluded in tutorial 1)*

- Are there any columns in the document(s) that are irrelevant for the analysis? **0** columns at the beginning and **0** columns in the end have to be removed.
- How many rows have to be deleted in the end of the document(s)? **0**

File import

- Separator: **;** Decimal separator: **,**
- *Click on the file chooser and insert the file:* "**Medium data.csv**" Wells: **47** *(24 x 2 – well B3.1)*

Click "**Import Files**".

Labels & colours

Click "**Load template**" *and load the file* "**Template_MCF-7 and Cisplatin_ without B3.1.csv**".

Analysis options

Analysis selection

Select "**calculate average**" *(opposite "Raw data") and* "**data smoothing**" *and* "**slope**".

Basic data formatting

- What is the input time unit of your measurement? **day** *(Converted by tutorial 1)*

- Which should be the output unit of the timescale? If you want a timescale different from the ones displayed, please use the options below. **day**

- Reformat the timescale and the measurement unit of the **Raw data**.
- Remove the data before time point t1 = **2.2** and after t2 = **5** (in given output unit).
- Transform the time scale using the formula t' = m * t + b, with m = **1** and b = **0** (applied on data given output unit).
- Use the following factor for the conversion of the measurement unit: **1**

Average
Please select according to which attribute the average should be calculated. **name**
Select "**calculate standard deviation**"

Data smoothing
Smooth the selected datasets by averaging a neighbourhood of **11** points. (Needs to be an odd number!)
Calculate smoothing for: **averaged raw data**

Numerical slope
Smooth the curves prior to calculation by averaging a neighbourhood of **11** points (needs to be an odd number).
Use **15** (smoothed) points on either side of the centre for a linear fit to determine the slope.
Calculate slope for: **averaged raw data**

Graph options

General
Title for all graphs: *Unselect* "**Show title**"
Subtitles for specific graphs: *Select* "**Show subtitles**"
Raw data: **Medium corrected data**
Unformatted raw data: **Unformatted medium corrected data** *(only appears once the analysis is run once)*
Average raw data: **Averaged data**
Smoothed average raw data: **Smoothed averaged data**
Slope of average raw data: **Slope of the smoothed averaged data**

Axes
X-axis label: **Time [day]**
X-axis limits: min = 1**0000** max = **10000** (enter 10000 for automatic limits)

Y-axis label : **Oxygen [% air saturation]**
Y-axis limits : min = **10000** max = **10000** (enter 10000 for automatic limits)

Run analysis

Choose a folder to save the results in and press "**Continue**".

Graph output

Point size: **10.0**
Legend columns: **5**
Legend position: **no legend / below the plot area** *(For the average curve)*
Line width: **1**
Grid colour intensity: **50**
Error colour intensity: **15**
White space: **0.51**

For the average curve, smoothed average data and average slope: select "**Show standard deviation**".

Click on "**Export displayed graph***", give a file name ending with .png for example.*
10 x **7** and then click "**Continue**".

The following graphs should be exported in the results folder:
- Averaged data
- Medium corrected data
- Slope of the smoothed averaged data
- Smoothed averaged data
- Unformatted medium corrected data

Data output

Separator: **;** Decimal separator: **,**

*Select "***export to file***"* (*the names have to end with* .csv) for:

- Raw data: **Medium corrected data.csv**
- Unformatted raw data: **Unformatted medium corrected data.csv**
- Average raw data: **Averaged data.csv**
- Smoothed average raw data: **Smoothed averaged data.csv**
- Slope average raw data: **Slope of the smoothed averaged data.csv**
- Deviation slope average raw data: **Deviation of the slope of the smoothed averaged data.csv**
- Raw data standard deviation: **Standard deviation medium corrected data.csv**

*Click "***Export Files***", they should appear in the results folder.*