TReCCA Analyser tutorial 1

Sensor correction, Normalisation, Averaging and IC_{50} determination

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 settings.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 Cisplatin.RData**". All the data will appear automatically in the "**Graph output**" and "**Data output**" tabs.

Analysing data using the settings and .csv files

The excel files generated by the experimental procedure (obtained from the SDR software (PreSens Precision Screening GmbH)) are saved as .csv files, the semicolon ";" being used as separator and the coma "," as decimal separator. The first line of the files is deleted as it contains hidden reports, and the well B3 of the second plate as it is an outlier condition. The files are called:

- MCF-7 and cisplatin plate 1_Oxygen.csv
- MCF-7 and cisplatin plate 2_Oxygen.csv

Open the TReCCA Analyser.

- Click on "**Import/export settings**", "**Load**" and then select the text file: "**MCF-7 and Cisplatin settings.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 "**MCF-7 and cisplatin plate 1_Oxygen.csv**" file for the first position and the "**MCF-7 and cisplatin plate 2_Oxygen.csv**" file for the second position.
  Click "**Import files**".

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

- Click on "**Run analysis**". The analysis will be perfomed 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

The excel files generated by the experimental procedure (obtained from the SDR software (PreSens Precision Screening GmbH)) are saved as .csv files, the semicolon ";" being used as separator and the coma "," as decimal separator. The first line of the files is deleted as it contains hidden reports, and the well B3 of the second plate as it is an outlier condition. The files are called:

- MCF-7 and cisplatin plate 1_Oxygen.csv
- MCF-7 and cisplatin plate 2_Oxygen.csv

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

Data layout

- How many plates do you want to analyse? **2**
  (*Press "Enter" for the interface in "File import" to change*)

- Do you have one document with all the data or one for each plate?
  Multiple documents

- Did you already clear all non numerical data? **No**
- What did you measure? **PreSens OxoDish**

Cutting borders

- Is a header included in the file(s)? **yes**
- How many rows of the file(s) are header(s), and what should be done?
  9 Remove header(s).

- Are there any columns in the document(s) that are irrelevant for the analysis?
  1 columns at the beginning and **3** 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: **,**
- #1: *Click on the file chooser and insert the file:*
  "**MCF-7 and cisplatin plate 1_Oxygen.csv**" Wells: **24**
  #2: *Click on the file chooser and insert the file:*
  "**MCF-7 and cisplatin plate 2_Oxygen.csv**" Wells: **23**

Click "**Import Files**".

Labels & colours

Click "**Load template**" *and load the file* "**Template_MCF-7 and Cisplatin.csv**".

To make your own template, it would have been possible to click on "**Autofill labels**"*and* "**Save template**". *Then you could fill up the template in a classical spread sheet application and load it again using the* "**Load template**" *button.*

Analysis options

Analysis selection

Select "**Sensor correction**", "**Medium normalisation**", "**calculate average**" *(opposite "Medium normalisation") and* "**IC50**".

Basic data formatting

- What is the input time unit of your measurement? **sec**
- 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 = **0** 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**"

Medium normalisation

Please enter the name of the wells only containing medium. **Medium**
What is the target value for the medium normalisation (after optional rescaling of the data)? **91.3**

IC50

Please select the data set to be used for the IC50 calculation. **Medium data**
Calculate IC50 from time = **216359** to time = **409892** in given input unit
Calculate IC50 only for every **1**. timepoint.
Please select which function to fit to your data. **4 parameter log-logistic function**
Please enter a name for the X-axis on the IC50 graphics. **Cisplatin [microM]**

Please enter the concentration (without units) for each well name.
To exclude well names from the IC50 calculation put -1.
10 microM: **10**
100 microM: **100**
20 microM: **20**
200 microM: **200**
30 microM: **30**
300 microM: **300**
40 microM: **40**
50 microM: **50**
60 microM: **60**
70 microM: **70**
80 microM: **80**
90 microM: **90**
Exclude: **-1**
Medium: **-1**
Non-treated: **0.1**

Sensor correction
Use **10** time points where the last included one is at t = **8169** in the given input unit.
What is the target value for the sensor correction in % air saturation? **91.3**
Span: **2406** Plateau: **-225.2**

Graph options

General
Title for all graphs: *Unselect* "**Show title**"
Subtitles for specific graphs: *Select* "**Show subtitles**"
Raw data: **Raw data**
Unformatted raw data: **Unformatted raw data** *(only appears once the analysis is run once)*
OxoDish calibration: **Sensor corrected data**
Medium normalisation: **Medium normalised data**
Average medium normalisation: **Averaged data**
Time resolved IC50: **Time-resolved IC50**
Single IC50 fits: **IC50 fitting**

Axes
X-axis label: **Time [day]**
X-axis limits: min = **0** max = **5** (enter 10000 for automatic limits)

Y-axis label : **Oxygen [% air saturation]**
Y-axis limits : min = **10** max = **100** (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: 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:
- Raw data
- Unformatted raw data *(X-axis limits: max = 10 000, press* "**Refresh options**"*)*
- Time-resolved IC50 *(X-axis limits: min = 10000 max = 10000, press* "**Refresh options**"*)*
- IC50 fittings

(*Calculate IC50 only for every* **55**. *time point, Y-axis limits: min:* **10000** *max:* **10000**)

Data output

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

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

- Raw data
- Unformatted raw data
- Medium data
- Average medium data
- Medium data standard deviation
- IC50 values and standard errors
- OxoDish data

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