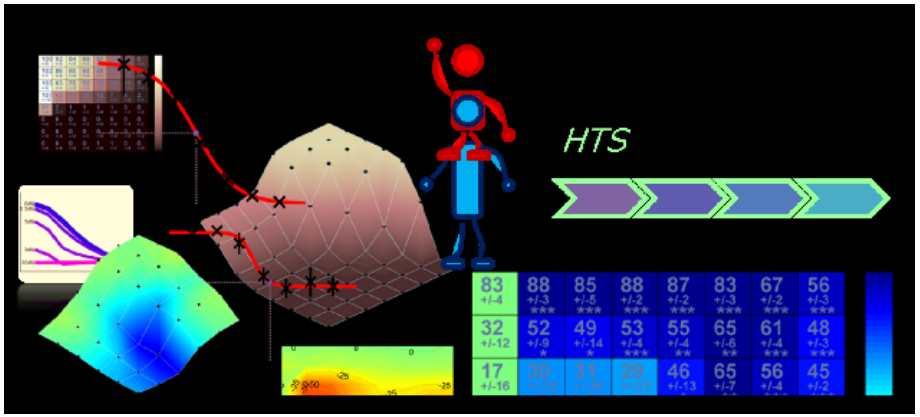
UNIVERSITY OF CAMBRIDGE

CANCER RESEARCH UK | CAMBRIDGE INSTITUTE



Combenefit 2.021

- Quick start user's guide -

April 2016 – C. Fornari/G.Di Veroli

About Combenefit

Combenefit is a free software that enables the analysis, advanced visualization and quantification of drug and other agents combinations.

Combenefit performs combination analyses using the standard Loewe, Bliss and HSA methods. In the next release our new SANE model will also available.

The purpose of this quick guide is to address the basics of how to set up Combenefit and run synergy analyses with the software; and you will see that installing and using Combenefit is quite straightforward. Note that, this user guide does not provide instructions about how to design experiments and interpret results, and it does not explain how drug combinations and mathematical models work.

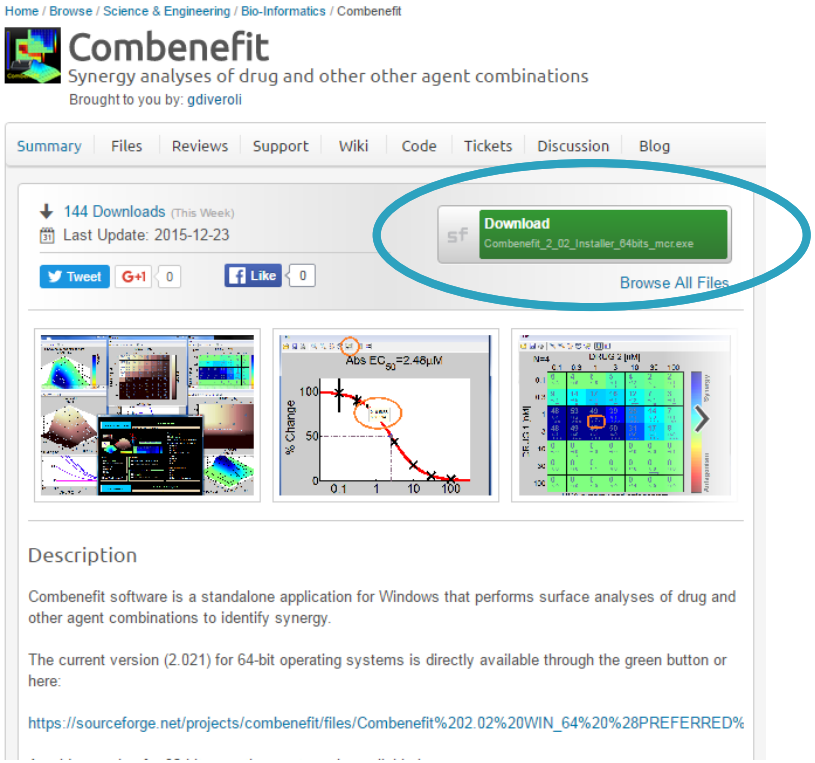
Additional information and citable reference will be published as "*Combenefit: an interactive platform for the analysis and visualisation of drug combinations*"

TABLE OF CONTENT

DOWNLOAD

Download the Combenefit_2_02_Installer_64bits_mcr.exe file from the hosting website:

https://sourceforge.net/projects/combenefit/



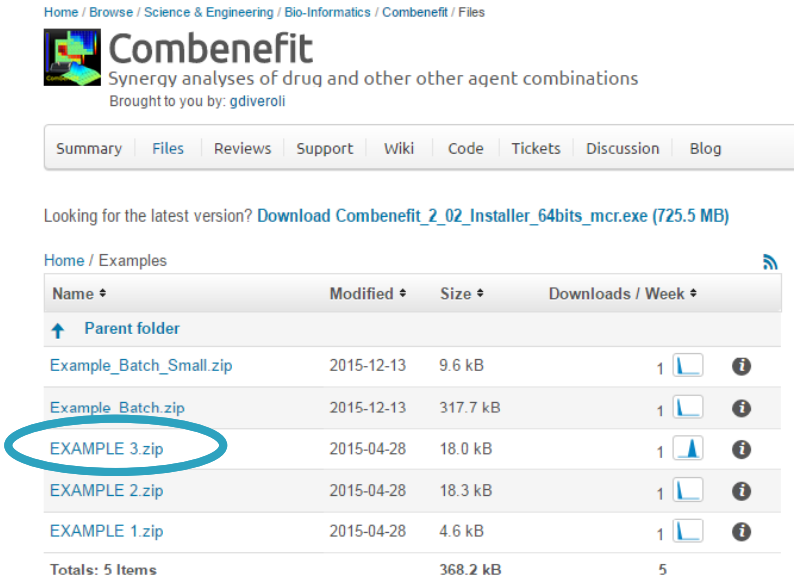
INSTALLATION

Run the installer (notice that you may need the administrator rights) and do the following steps:

1. Click *Next* in the Introduction page;
2. Set the software location (or leave the one suggested by the software) and click *Next*;
3. Install the MATLAB Compiler Runtime clicking *Next*. Notice that you do not need Matlab to run Dr Fit, but you need to have this specific version of the compiler installed. It is free and comes as a package with Combenefit.
4. Check that you accept the terms of the MATLAB Compiler Runtime license agreement and, then, click *Next*.
5. Verify the location where the software is going to be installed and click *Install*. Notice that installing Combenefit and the compiler might take up to a few minutes.
6. Check the log produced by the installation and click *Finish* to close the window.

DATA FORMAT (1/5)

Few examples to run Combenefit are provided on the hosting website, and they illustrate which data formats can be used in the software.

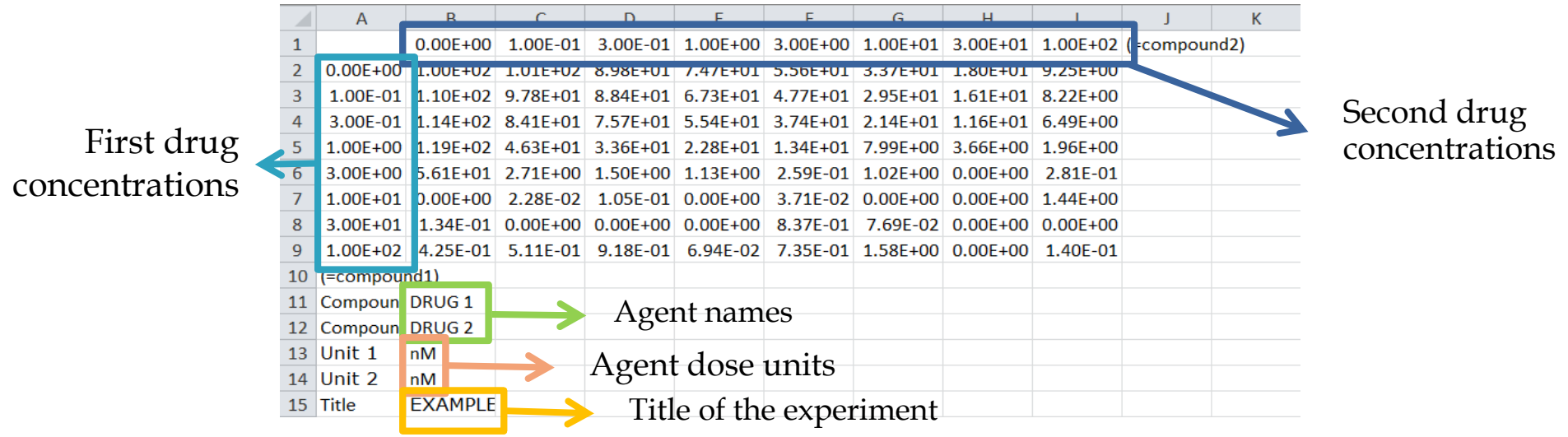


Download, for instance, *Example 3.zip* and unzip it somewhere on your computer. This folder contains four .xls files, one for each replicate of a drug combination experiment.

DATA FORMAT (2/5)

Open the file *REPL1.xls*.

It contains the dose response information from the first replicate of the combination experiments.



	A	B	C	D	E	F	G	H	I	J	K
1		0.00E+00	1.00E-01	3.00E-01	1.00E+00	3.00E+00	1.00E+01	3.00E+01	1.00E+02	(=compound2)	
2	0.00E+00	1.00E+02	1.01E+02	8.98E+01	7.47E+01	5.56E+01	3.37E+01	1.80E+01	9.25E+00		
3	1.00E-01	1.10E+02	9.78E+01	8.84E+01	6.73E+01	4.77E+01	2.95E+01	1.61E+01	8.22E+00		
4	3.00E-01	1.14E+02	8.41E+01	7.57E+01	5.54E+01	3.74E+01	2.14E+01	1.16E+01	6.49E+00		
5	1.00E+00	1.19E+02	4.63E+01	3.36E+01	2.28E+01	1.34E+01	7.99E+00	3.66E+00	1.96E+00		
6	3.00E+00	5.61E+01	2.71E+00	1.50E+00	1.13E+00	2.59E-01	1.02E+00	0.00E+00	2.81E-01		
7	1.00E+01	0.00E+00	2.28E-02	1.05E-01	0.00E+00	3.71E-02	0.00E+00	0.00E+00	1.44E+00		
8	3.00E+01	1.34E-01	0.00E+00	0.00E+00	0.00E+00	8.37E-01	7.69E-02	0.00E+00	0.00E+00		
9	1.00E+02	4.25E-01	5.11E-01	9.18E-01	6.94E-02	7.35E-01	1.58E+00	0.00E+00	1.40E-01		
10	(=compound1)										
11	Compoun	DRUG 1									
12	Compoun	DRUG 2									
13	Unit 1	nM									
14	Unit 2	nM									
15	Title	EXAMPLE									

8 concentrations were tested for both Agent 1 (light blue), and Agent 2 (blue) for a total of 64 combinations.

DATA FORMAT (3/5)

The dose response surface (pink matrix) shows the combination effects.

Note that the first column (green column) and the first row (yellow row) of the surface matrix show the dose response curves for the first and second agent, respectively.

	A	B	C	D	E	F	G	H	I	J	K
1		0.00E+00	1.00E-01	3.00E-01	1.00E+00	3.00E+00	1.00E+01	3.00E+01	1.00E+02	(=compound2)	
2	0.00E+00	1.00E+02	1.01E+02	8.98E+01	7.47E+01	5.56E+01	3.37E+01	1.80E+01	9.25E+00		
3	1.00E-01	1.10E+02	9.78E+01	8.84E+01	6.73E+01	4.77E+01	2.95E+01	1.61E+01	8.22E+00		
4	3.00E-01	1.14E+02	8.41E+01	7.57E+01	5.54E+01	3.74E+01	2.14E+01	1.16E+01	6.49E+00		
5	1.00E+00	1.19E+02	4.63E+01	3.36E+01	2.28E+01	1.34E+01	7.99E+00	3.66E+00	1.96E+00		
6	3.00E+00	5.61E+01	2.71E+00	1.50E+00	1.13E+00	2.59E-01	1.02E+00	0.00E+00	2.81E-01		
7	1.00E+01	0.00E+00	2.28E-02	1.05E-01	0.00E+00	3.71E-02	0.00E+00	0.00E+00	1.44E+00		
8	3.00E+01	1.34E-01	0.00E+00	0.00E+00	0.00E+00	8.37E-01	7.69E-02	0.00E+00	0.00E+00		
9	1.00E+02	4.25E-01	5.11E-01	9.18E-01	6.94E-02	7.35E-01	1.58E+00	0.00E+00	1.40E-01		
10	(=compound1)										
11	Compound	DRUG 1									
12	Compound	DRUG 2									
13	Unit 1	nM									
14	Unit 2	nM									
15	Title	EXAMPLE									

Drug 1 dose response

Drug 2 dose response

Dose response Surface

DATA FORMAT (4/5)

Measured effects must be normalized to control conditions, i.e. no drugs, and values must be tabulated in percentage. Hence, the first cell (blue rectangle) of the surface matrix is 100 and the other cells of the matrix show positive values between 0 and 100.

In case of cell growth assay, measured effects are the surviving fractions.

	A	B	C	D	E	F	G	H	I	J	K
1		0.00E+00	1.00E-01	3.00E-01	1.00E+00	3.00E+00	1.00E+01	3.00E+01	1.00E+02	(=compound2)	
2	0.00E+00	1.00E+02	1.01E+02	8.98E+01	7.47E+01	5.56E+01	3.37E+01	1.80E+01	9.25E+00		
3	1.00E-01	1.10E+02	9.78E+01	8.84E+01	6.73E+01	4.77E+01	2.95E+01	1.61E+01	8.22E+00		
4	3.00E-01	1.14E+02	8.41E+01	7.57E+01	5.54E+01	3.74E+01	2.14E+01	1.16E+01	6.49E+00		
5	1.00E+00	1.19E+02	4.63E+01	3.36E+01	2.28E+01	1.34E+01	7.99E+00	3.66E+00	1.96E+00		
6	3.00E+00	5.61E+01	2.71E+00	1.50E+00	1.13E+00	2.59E-01	1.02E+00	0.00E+00	2.81E-01		
7	1.00E+01	0.00E+00	2.28E-02	1.05E-01	0.00E+00	3.71E-02	0.00E+00	0.00E+00	1.44E+00		
8	3.00E+01	1.34E-01	0.00E+00	0.00E+00	0.00E+00	8.37E-01	7.69E-02	0.00E+00	0.00E+00		
9	1.00E+02	4.25E-01	5.11E-01	9.18E-01	6.94E-02	7.35E-01	1.58E+00	0.00E+00	1.40E-01		
10	(=compound1)										
11	Compound	DRUG 1									
12	Compound	DRUG 2									
13	Unit 1	nM									
14	Unit 2	nM									
15	Title	EXAMPLE									

Control condition

DATA FORMAT (5/5)

- A template for data files can be downloaded from the hosting website https://sourceforge.net/projects/combenefit/files/?source=navbar .
- You can add and/or remove both lines and columns from the matrix to obtain the format that is appropriate for your experimental design. However, notice that the minimum number of concentrations is 3 for each agent (i.e. 3x3 matrix), and we would advise to use at least 4 concentrations.
- Greek letter µ for micro is displayed using the LaTeX command *mu* in the unit cell. Other Greek letters and/or symbols can be used as well.
- Please note that text cells (e.g. title) can not contain any of /, |, and \ symbols.
- Files must be saved in the .xls format (i.e. Excel 97-2003 Workbook) and not the .xlsx, or any other formats (e.g. .csv, .txt).

RUNNING COMBENEFIT (1/6)

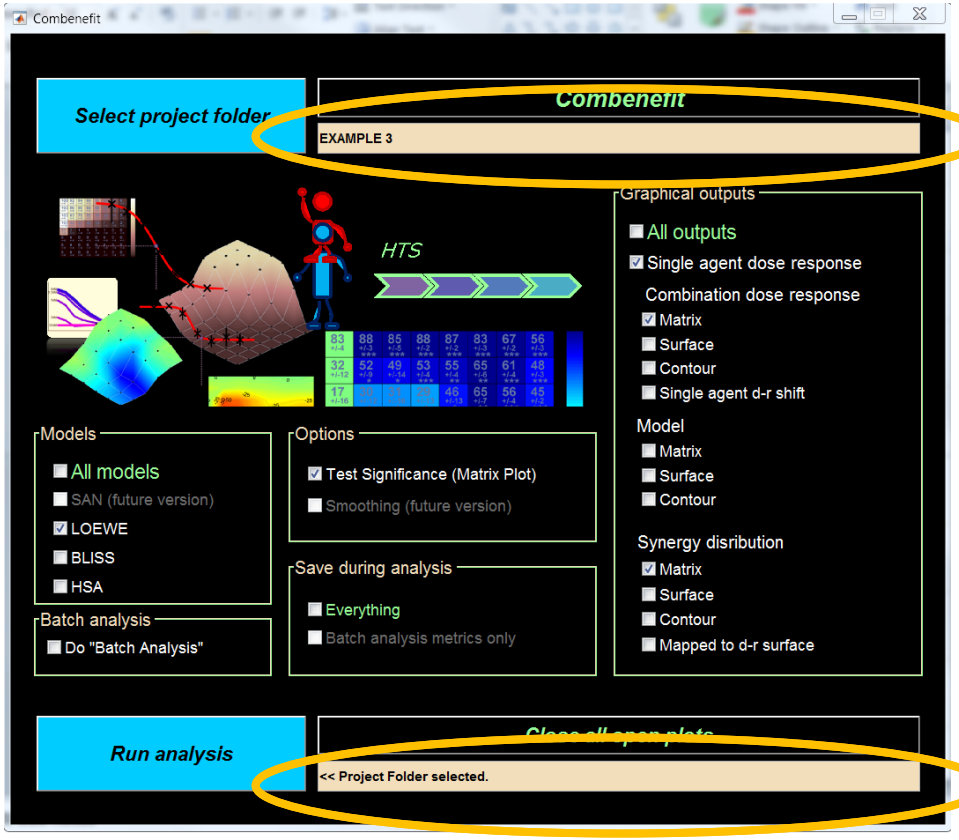
Start Combenefit via the Windows Start Menu or via the shortcut on your desktop. Note that it may take few minutes for the interface to open, depending on your machine.

Once the interface is displayed, use the *Select project folder* button to upload your combination data.

Notice that you have to select the folder where your .xls files are saved, not the actual files in the folder.

Select, for instance, the folder Example 3 containing the combination .xls files we described earlier.

RUNNING COMBENEFIT (2/6)



Once your project folder has been selected, Combenefit displays the folder name in the top light brown display.

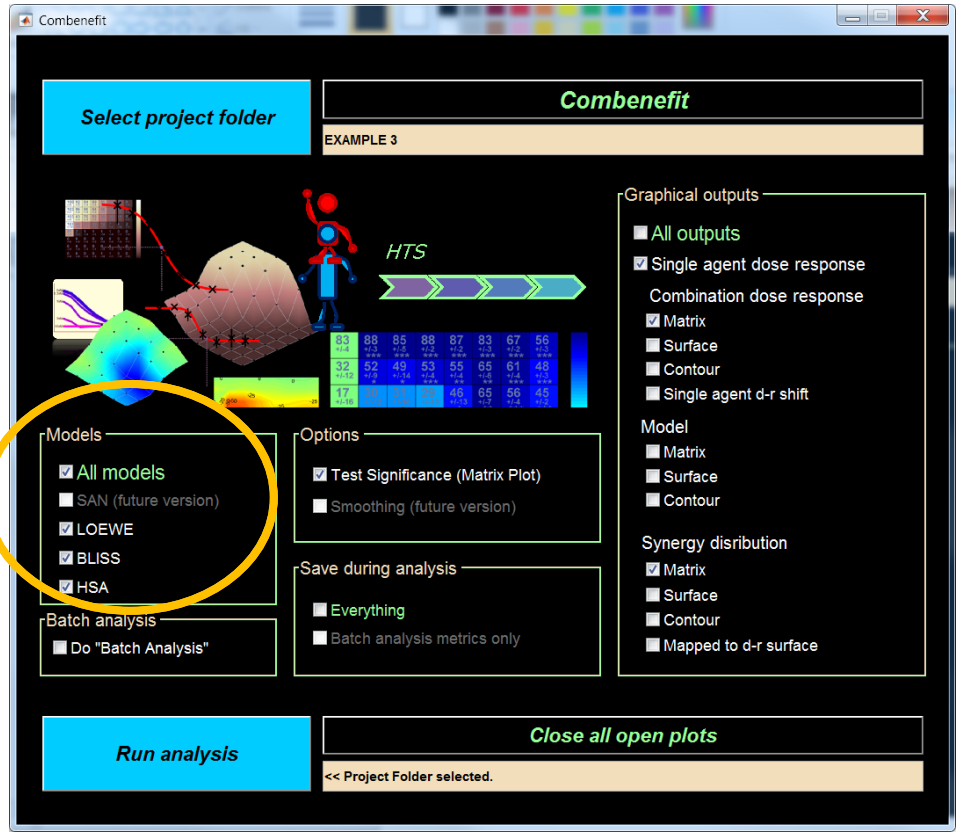
The output notifies that the project folder has been selected.

RUNNING COMBENEFIT (3/6)

From the MODELS panel, select the synergy model to be used for the analysis. Several models can be selected together.

Options are: Loewe, Bliss and HSA model. We are also developing a new model to be incorporated in a future version of Combenefit.

Notice that, the results of a combination analysis, i.e. synergy/antagonism levels and distributions can be strongly model dependent. We advise consistency across analyses by always using a specific model or subset of models.

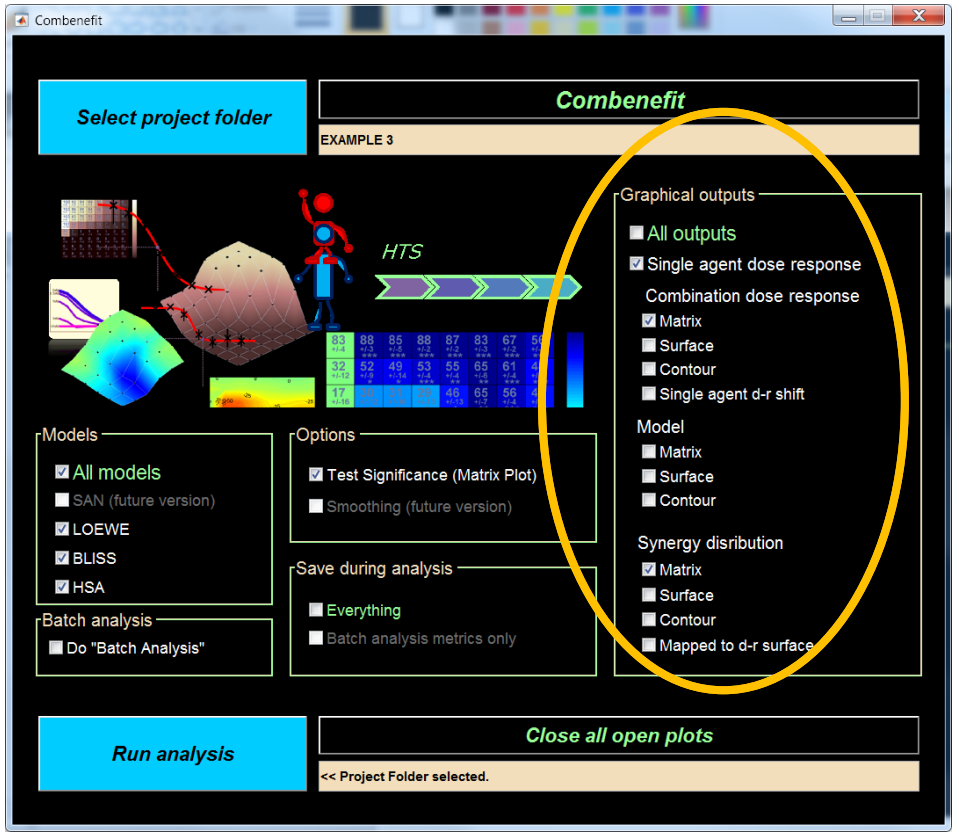


Here, we selected *All models* for illustrative purposes.

RUNNING COMBENEFIT (4/6)

Select which GRAPHICAL OUTPUTS you are interested in.
Combenefit preselects the display of: (i) single agent dose response curves, (ii) combination dose response surface in matrix display, and (iii) synergy distribution in matrix display.
You can remove and/or add other graphical outputs based on your needs.

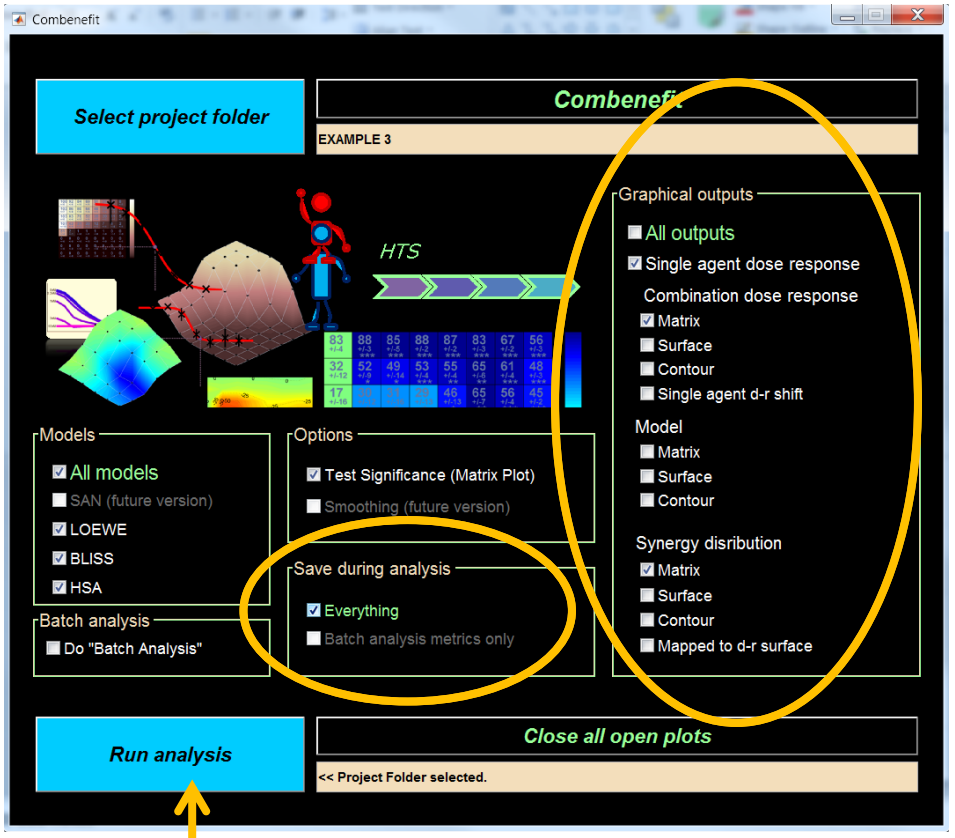
We do not select additional displays for now.



RUNNING COMBENEFIT (5/6)

In the OPTIONS box, select *Test Significance (Matrix Plot)* if you want to test for significant levels of synergy and/or antagonism in the matrix display.
Note that for this option you need to have replicates.

In the SAVE DURING ANALYSIS box, select *Everything* to automatically save Combenefit outputs during the running time.



Click the RUN ANALYSIS button when your set up is completed.

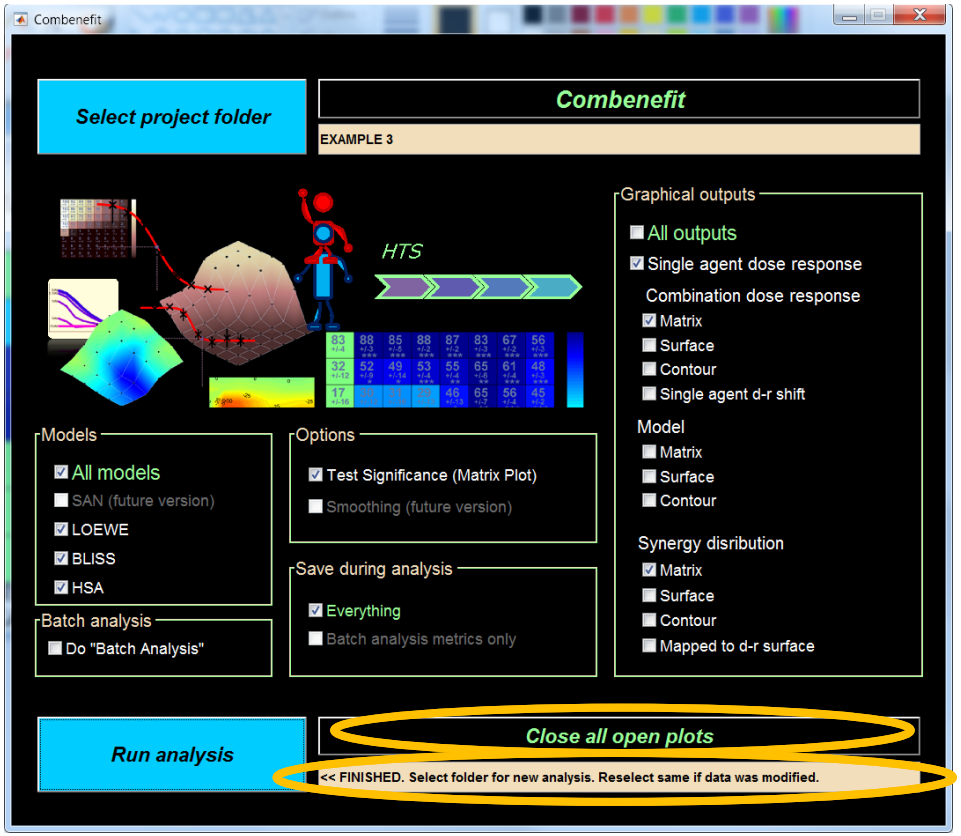
RUNNING COMBENEFIT (6/6)

Once you hit the RUN ANALYSIS button, Combenefit starts to analyse your data. The output window at the bottom of the interface displays various messages showing the steps the software is going through.

Last message will be the one showed in this slide.

During the analysis Combenefit shows the graphics that have been selected by the user. If the *Save* option has been selected, graphics flash after being displayed, showing that they are being saved on running time.

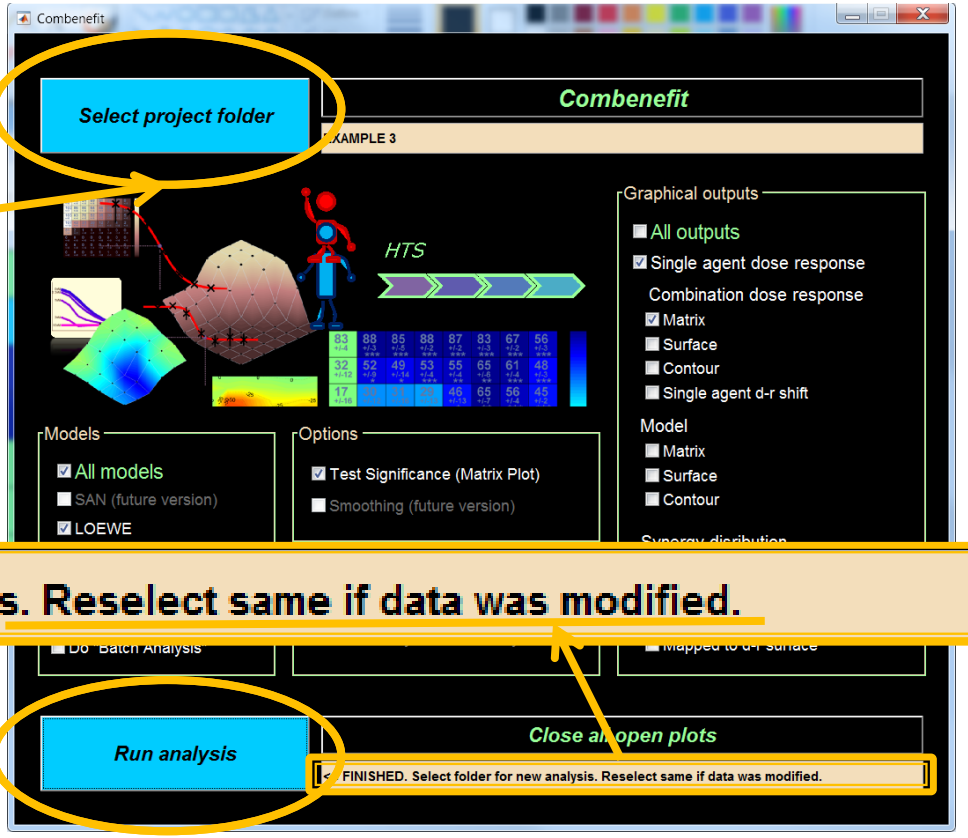
You can clear all plots *Close all open plots* button.



Additional Analyses

You can re-run Combenefit for the same combination with different options (e.g. model, display etc.) without uploading the data again.

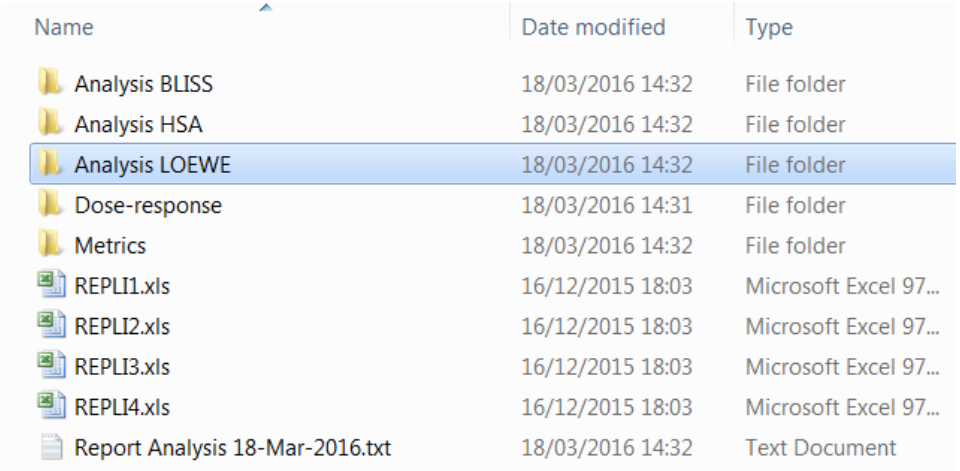
However, if the data (i.e. the .xls file) is manually modified, the same folder needs to be uploaded again by simply re-selecting the folder.



Analysis Results (1/2)

Open the combination folder which contains your .xls files. Several new subfolders have been created.

- The *Analysis Model_Name* folders contain graphical displays, data supporting the model, and the synergy analysis values.
- The *Dose-response* folder contains graphical displays and data corresponding to both dose response curves and surface, i.e. measured effects for the two drugs used as single agents and in combination.
- The *Metrics* folder contain metrics which capture information about the synergy distribution (e.g. maximum, minimum synergy, etc.)
- The *Report Analysis date* file contains the date of the analysis and information about the software version used.



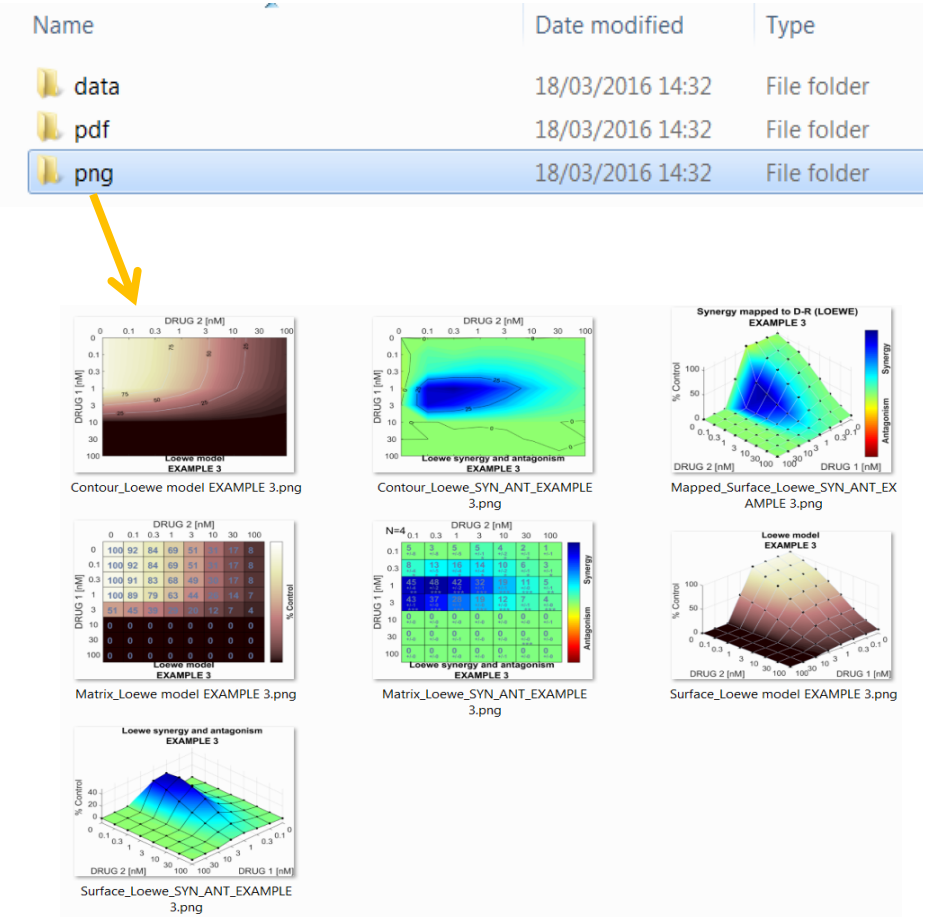
Analysis Results (2/2)

Open the *Analysis LOEWE* folder, which contains the 3 following subfolders:

- *data* contains the numeric data corresponding to the Loewe model analysis, i.e. predicted values and synergy levels;
- *pdf* contains graphical outputs in a .pdf format;
- *png* contains graphical outputs in a .png format.

If the user is interested in display further graphical outputs, a new analysis can be run via the *Run analysis* button, having preselected the additional plots.

These graphics will be automatically saved in the appropriate model folders.



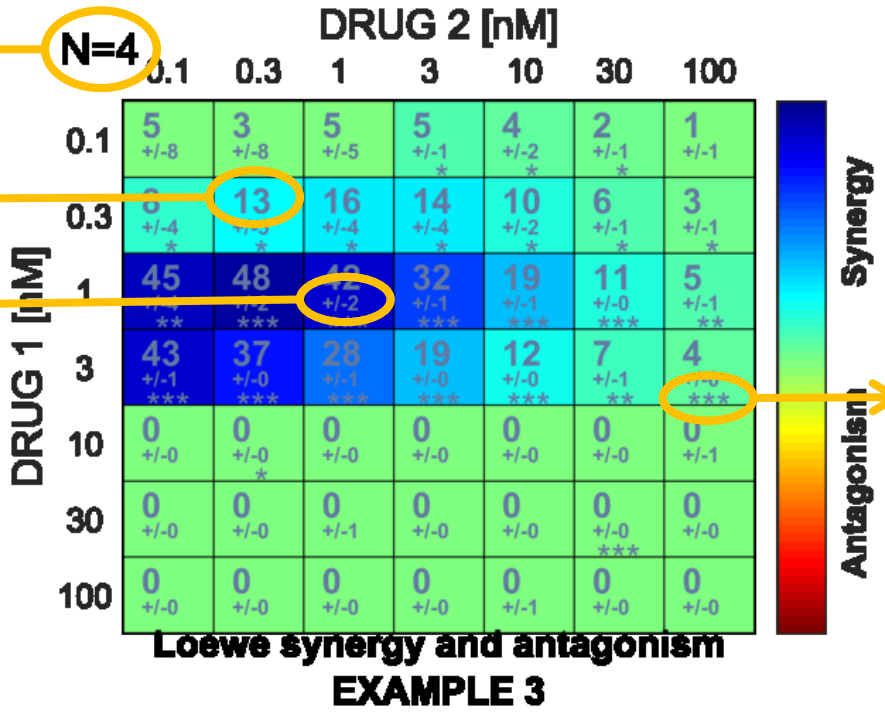
MATRIX SYNERGY PLOT

It shows the synergy/antagonism score for each combination and its statistical significance, if the *Test Significance* option was selected before the analysis.
Stars indicate the level of significance, and only significant combinations are coloured.
If the Test Significance option was not selected and/or only one replicate was available, no stars are displayed and all cells are coloured.
The number of replicates is reported in the top left corner

number of replicates

score

standard deviation

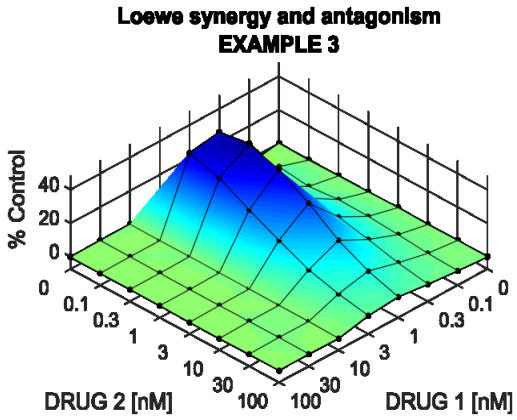


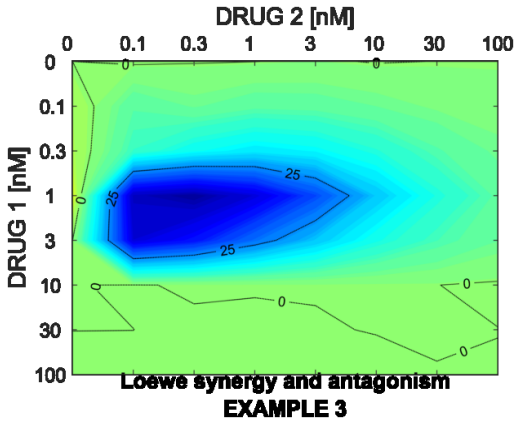
statistical significance

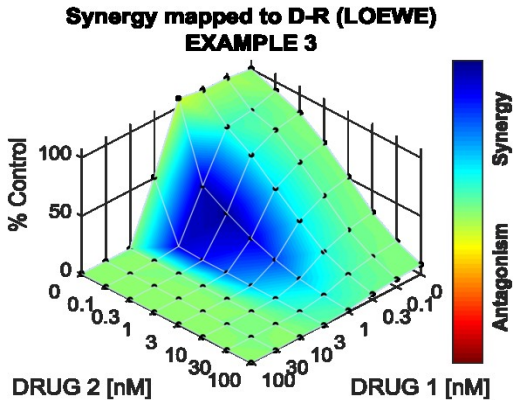
Statistical Significance legend
* $P<5\times 10^{-2}$;
** $P<10^{-3}$;
*** $P<10^{-4}$;

OTHER PLOTS (1/2)

Synergy scores can be displayed in other format such as *Surface* and *Contour plot*, and Synergy distribution can also be *Mapped to d-r surface*.





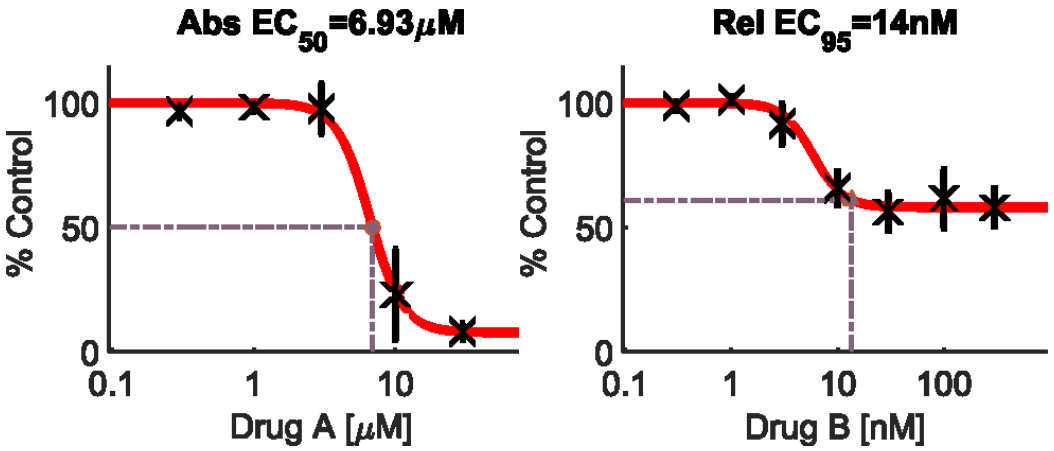


Same types of output displays are available for the Combination dose response (i.e. the experimental data) and model values (i.e. predicted values).

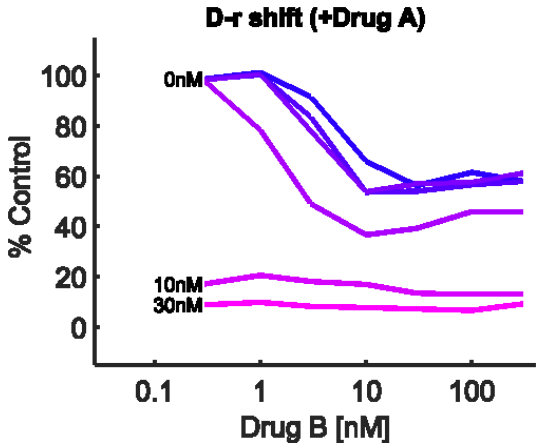
When plots are displayed by Combenefit, the user can also manually edit them (e.g. change the orientation of the 3D surface, zoom in/out, highlight specific values) using the edit commands in the figure window.

OTHER PLOTS (2/2)

Single agent dose response curves are saved in the *Dose-response* folder, together with other useful plots.



The *Single agent dose-response shift* can also be informative:

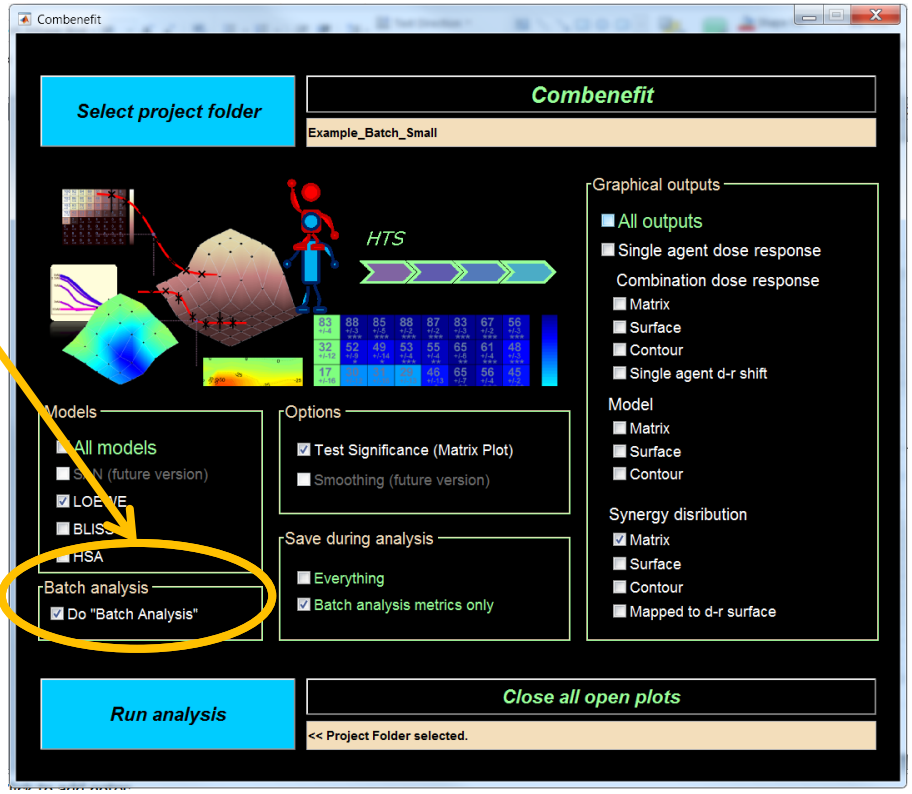


BATCH ANALYSIS (1/3)

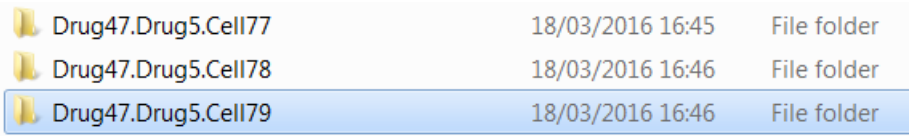
During High Throughput Screens (HTSs), large volume of combinations and cell lines are investigated using automated technology.

Combenefit can be used to analyse the large amount of data derived with these screens, using the *Do Batch Analysis* option.

Download the Example_Batch_Small.zip file from the hosting website (https://sourceforge.net/projects/combenefit/files/Examples/) and proceed as for the Example 3.zip. A larger example (100 combinations) is also provided in the hosting website.

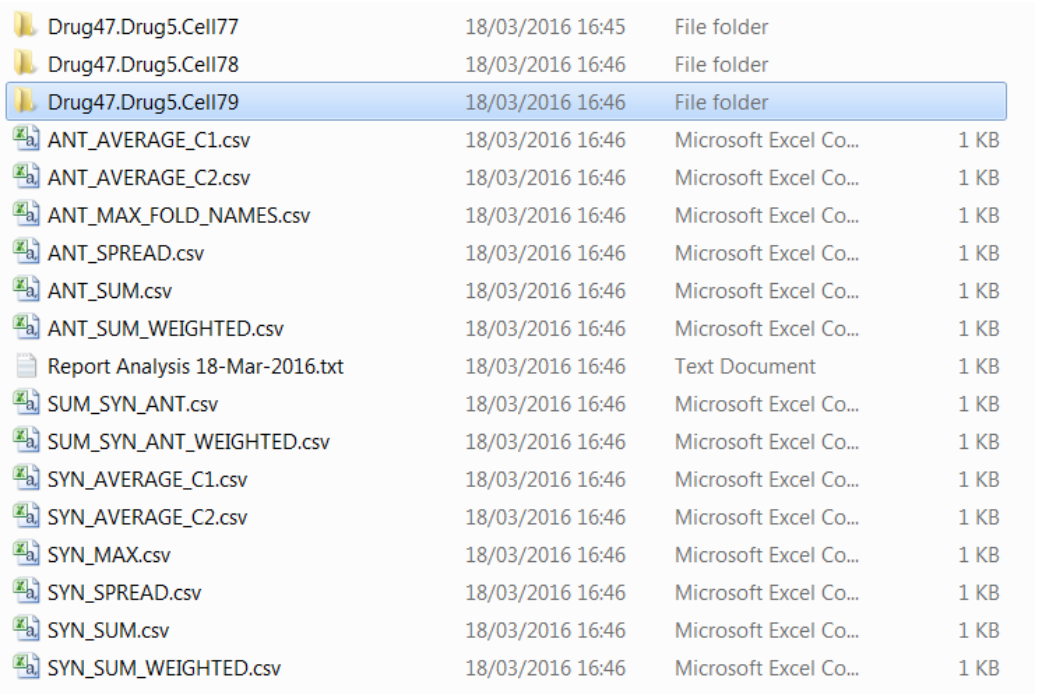


Example_Batch_Samall contains combination experiments of 2 agents, performed in 3 different cell lines.



BATCH ANALYSIS (2/3)

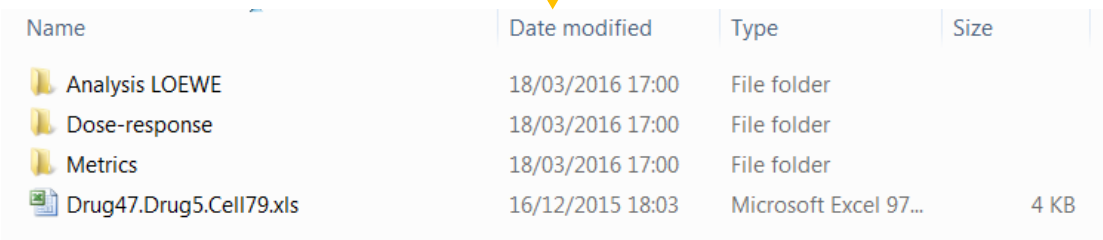
After the Batch Analysis, several .csv files containing analysis results are added to the *Example_Batch_Samall* folder.



Drug47.Drug5.Cell77	18/03/2016 16:45	File folder	
Drug47.Drug5.Cell78	18/03/2016 16:46	File folder	
Drug47.Drug5.Cell79	18/03/2016 16:46	File folder	
ANT_AVERAGE_C1.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
ANT_AVERAGE_C2.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
ANT_MAX_FOLD_NAMES.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
ANT_SPREAD.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
ANT_SUM.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
ANT_SUM_WEIGHTED.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
Report Analysis 18-Mar-2016.txt	18/03/2016 16:46	Text Document	1 KB
SUM_SYN_ANT.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
SUM_SYN_ANT_WEIGHTED.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
SYN_AVERAGE_C1.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
SYN_AVERAGE_C2.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
SYN_MAX.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
SYN_SPREAD.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
SYN_SUM.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB
SYN_SUM_WEIGHTED.csv	18/03/2016 16:46	Microsoft Excel Co...	1 KB

Analysis results are summarized in tables containing all experiments in rows and metrics in columns.

If the *Everything* option was selected among the *Save during analysis* options, graphical outputs are organized within each experiment folder, as for the normal analysis illustrated before.



Name	Date modified	Type	Size
Analysis LOEWE	18/03/2016 17:00	File folder	
Dose-response	18/03/2016 17:00	File folder	
Metrics	18/03/2016 17:00	File folder	
Drug47.Drug5.Cell79.xls	16/12/2015 18:03	Microsoft Excel 97...	4 KB

BATCH ANALYSIS (3/3)

We advise to avoid graphics during batch analyses (uncheck the "*Everything*" option in the saving panel) because it significantly slow-down running time for large screens.

In the context of HTS, we recommend to run the analysis by saving only metrics. Graphics can then be generated at the post-processing levels based on global metrics. Here, Combenefit can be ran in single mode for each one of the identified combinations, by selecting the desired options and graphical displays.

LICENCE & FUTURE VERSIONS

Combenefit is licensed under the MIT Licence and is therefore freely available for everyone, but please acknowledge appropriately.

We hope that this new platform will enable a large number of scientists to robustly and efficiently analyse their data with minimum effort.

Combenefit is constantly being improved and new functionalities are being built in. Please check the official web page where material is being provided for updates https://sourceforge.net/p/combenefit/