Clot_or_HaloCL

Notes for analysing clotting fibrinogen or plasma or fibrinolysis by the halo method

The main window opens with a series of Tabs to help the user perform different tasks. The *Help* tab in the app summarises the main features of Clot_or_HaloCL version 0.3 and above. More details are presented below for each Tab.

The *Plots* tab

Data Entry

The app opens with a set of supplied data, which can be used to get a feel of the options. The next step is to load your own data for plotting and analysis using *select dataset* and *Browse*. The app accepts csv, txt and xlsx files, and the format is detected automatically. There is also an option to specify the Excel sheet number, if more than one sheet is available in the Excel file.

Time course data should be formated as a single column of time in column 1 followed by columns of absorbance data. The program detects the length and width of the data so there is no need to specify these dimensions. Data can be read as csv, csv2 (using , instead of . for the decimal point), txt or Excel files. These options are specified using the radio buttons in the left hand panel. It is also necessary to specify if the columns have header text ("Time", well names "A1", "A2"... etc), which is recommended.

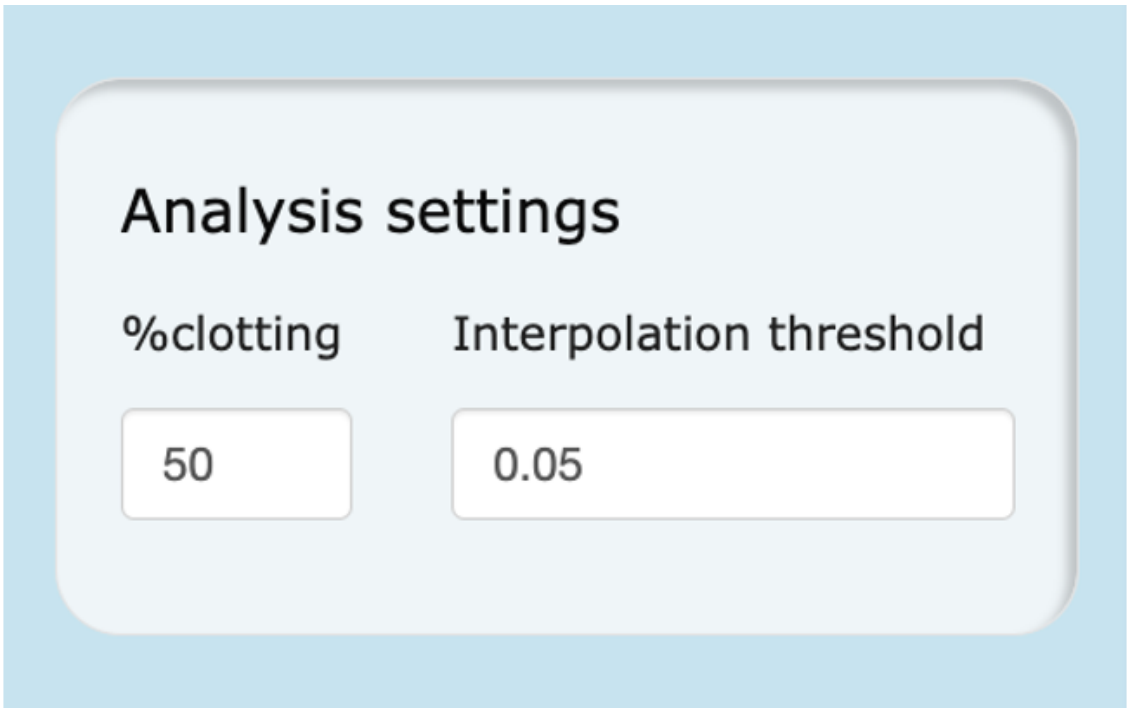
Note: *it is important not to leave empty cells, incomplete columns or rows, or spaces in names of column headers in any data files. Gaps and spaces are the main reasons the program fails to read data or complete an analysis.*

Below is an example of a few rows of data to show how it should be formatted (and how it should appear in the *Raw data* tab).

Time	A2	A3	A4	A5	A6	A7	A8	A9
0	0.045	0.045	0.044	0.043	0.045	0.044	0.043	0.043
20	0.046	0.047	0.046	0.044	0.046	0.047	0.045	0.044
40	0.048	0.05	0.049	0.046	0.049	0.05	0.048	0.046
60	0.052	0.057	0.054	0.05	0.053	0.056	0.054	0.05
80	0.058	0.067	0.062	0.059	0.062	0.065	0.064	0.056
100	0.065	0.081	0.072	0.071	0.075	0.076	0.078	0.064
120	0.072	0.097	0.083	0.086	0.091	0.087	0.094	0.073
140	0.081	0.114	0.095	0.103	0.108	0.101	0.112	0.083

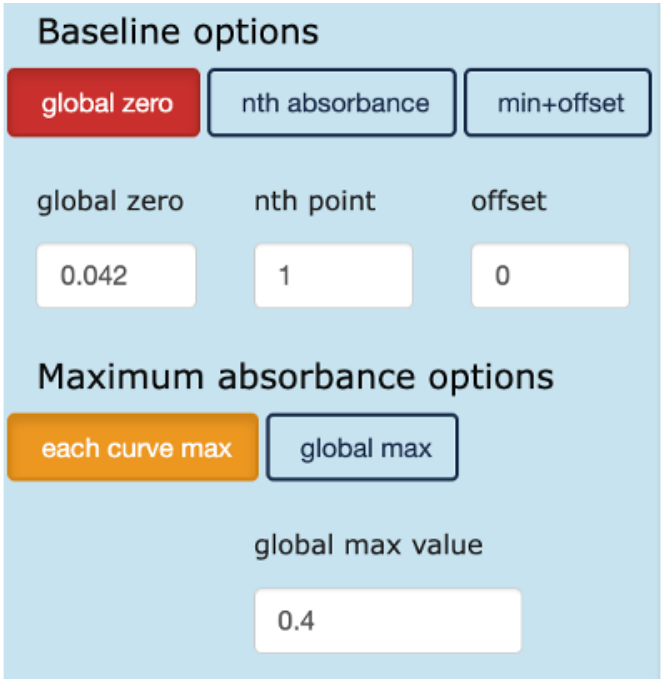
Analysis settings

The default setting for curve analysis is 50% clotting, but this can be changed. The interpolation threshold defines the lower absorbance limit for curve analysis where interpolation of values between time points will be calculated. Below this threshold only the closest time point to the selected % lysis will be used. If you have good quality data, leave this default setting as it is. If you have noisy data, or empty wells, the interpolation algorithm may fail to analyse the data. However, should it be necessary to increase the threshold value, accuracy can be improved by adding extra points using the spline fitting options below.



Baseline and maximum absorbance options

There are several ways to set the baseline.



The baseline options are set using the red selection buttons with numeric inputs from the corresponding numeric input boxes below. The zero value selected will be the absorbance that equates the starting value before clotting (or lysis in the halo method). The first option is for a global zero, which is specified the numerical input box below *global zero*, and may be the starting absorbance for example, if this is consistent. In this case all curves will have the same zero absorbance value.

A second option provided is to set zero for each curves at a particular time point. The default value for the *nth absorbance* is 1, i.e. the first absorbance reading of each curve. Later points can be chosen by increasing the value in the *nth point* input box. With this option, individual curves may have different zero absorbance values but they will all be selected at the same time point. The selected absorbance will be subtracted from each curve.

The third option is to use the minimum absorbance value of each curve with the possibility to add an offset value. This is useful if there is drift or noise and some manual adjustment is required. In this case the zero absorbance can vary for each curve and will likely come from a different time point along the curve. The minimum value and offset will be subtracted from each curve.

The way zero has been set is recorded in the table in the *Settings* tab. Some care is needed when using these options as the chosen zero affects several of the calculated results. Recording the way the baseline is set facilitates reproducible data analysis.

Maximum absorbance options

There are two ways to set the maximum absorbance values for the curves using the orange selection buttons.

each curve max identifies the maximum absorbance for each curve and sets this as a maximum. In this case all the curves may have different maximima.

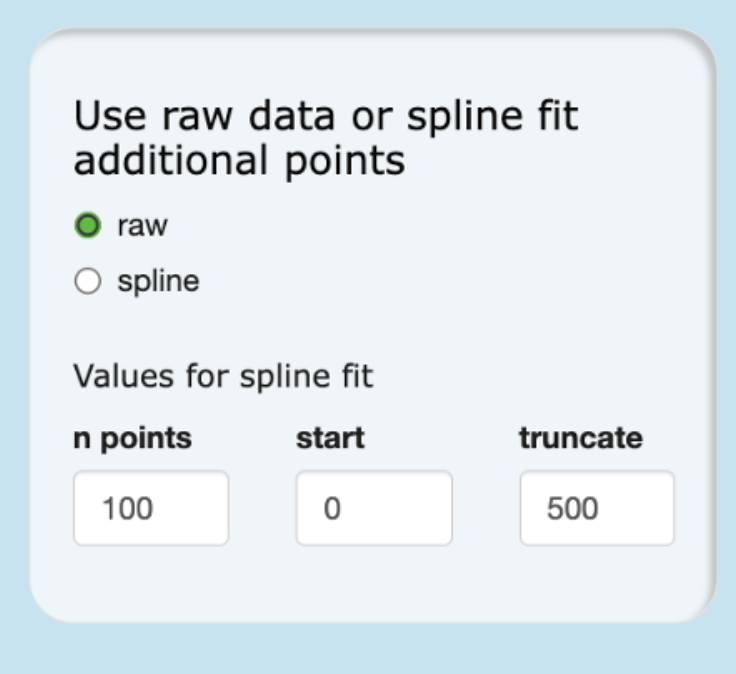
global max sets a value that applies to all the curves and the value is provided in the numerical input box. This may be useful if you expect a particular maximum value but your curves have not all reached the maximum during the run. This value is also useful for halo assays analysis that include some non-clotted blood in the sample wells, corresponding to complete lysis.

Raw or spline fitted data

The next series of options affect specify whether raw data or spline fitted data are used for analysis. All things being equal, it is better to use raw data. However, if data points are sparse or the *threshold value* has been increased and results are not derived by interpolation between data points, accuracy may be improved be selecting the *spline* fitting option. In this case, the number of points to generate, fitting start and truncation of the time course can all be selected using the numerical input boxes.

The newly generated data points are shown in the graphical output sections in the *Curve* tab, and also in the *Raw data* tab.

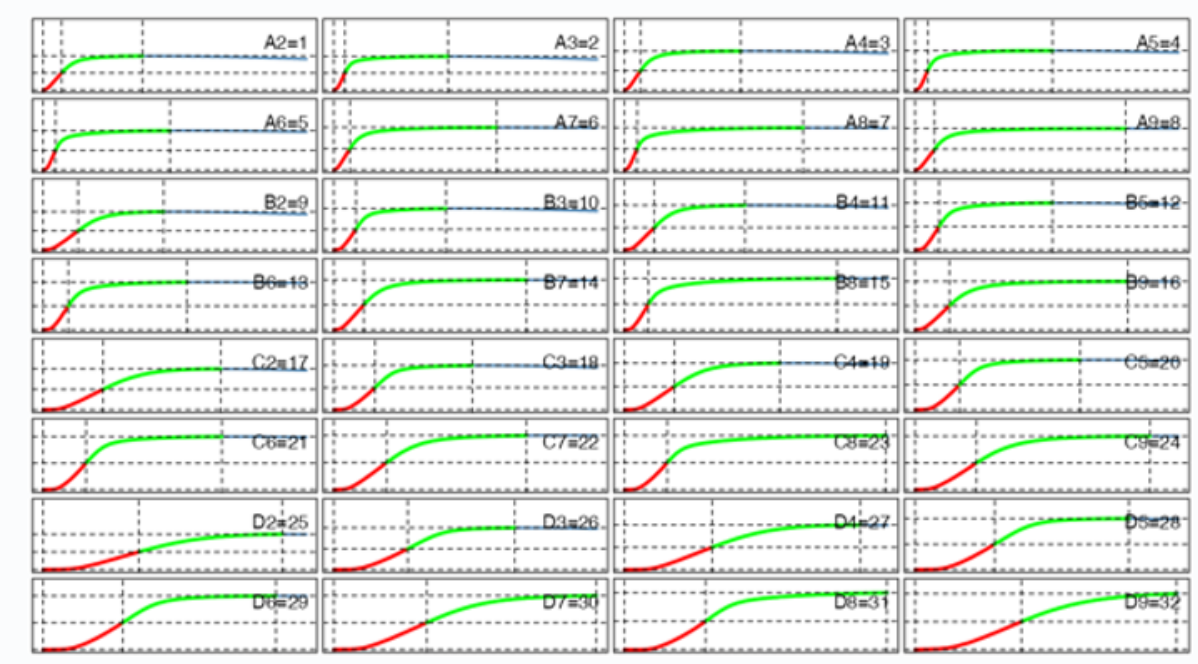
Once again, the default settings of low threshold and raw data are preferable if curve analysis is successful.



Plotting the data

The graphical output in the main panel of the opening page is organised by number of rows specified, using the *Plot number of rows* numerical input box. The plots area is headed by the name of the file being analysed.

The supplied data give an output as shown below for 50% clot formation (also corresponding to 50% clot lysis in the halo fibrinolysis method). Dotted guide lines show the absorbances and time for the selected % clotting and the selected minimum and maximum vaues. Each well also includes the well name and number in the top right corner.



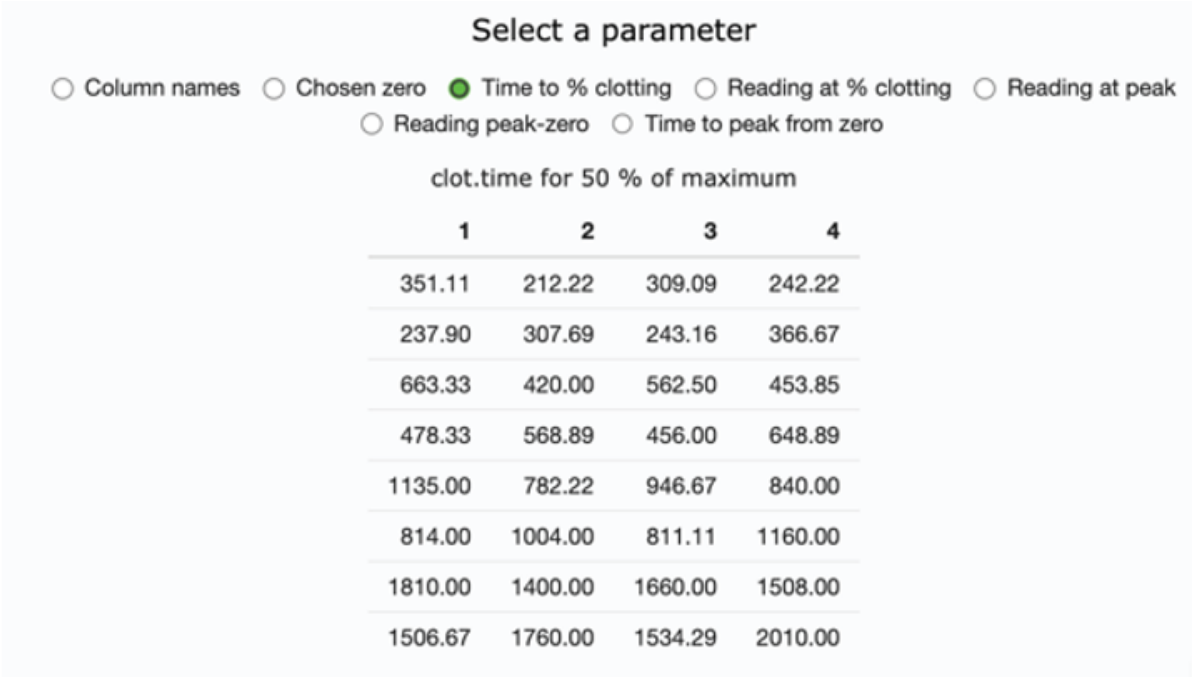
The plots can be expanded to a full screen view by clicking on the expansion button in the bottom right corner of this page.

Results table

The results table (also shown in the figure above), corresponds to the graphical layout and displays the results selected using the radio buttons in the left panel, summarised below.

- **Column names:** Displays the header text in the data file in the specified arrangement
- **Chosen zero:** Displays whatever zero value has been selected for each curve
- **Time to % change:** Time to chosen % clotting or lysis from the start
- **Reading at % clotting:** Absorbance reading at your chosen % change
- **Reading at peak:** Absorbance reading at peak
- **Reading peak-zero:** Absorbance reading at peak with baseline subtracted
- **Time to Peak from zero:** Time to maximum absorbance from the start

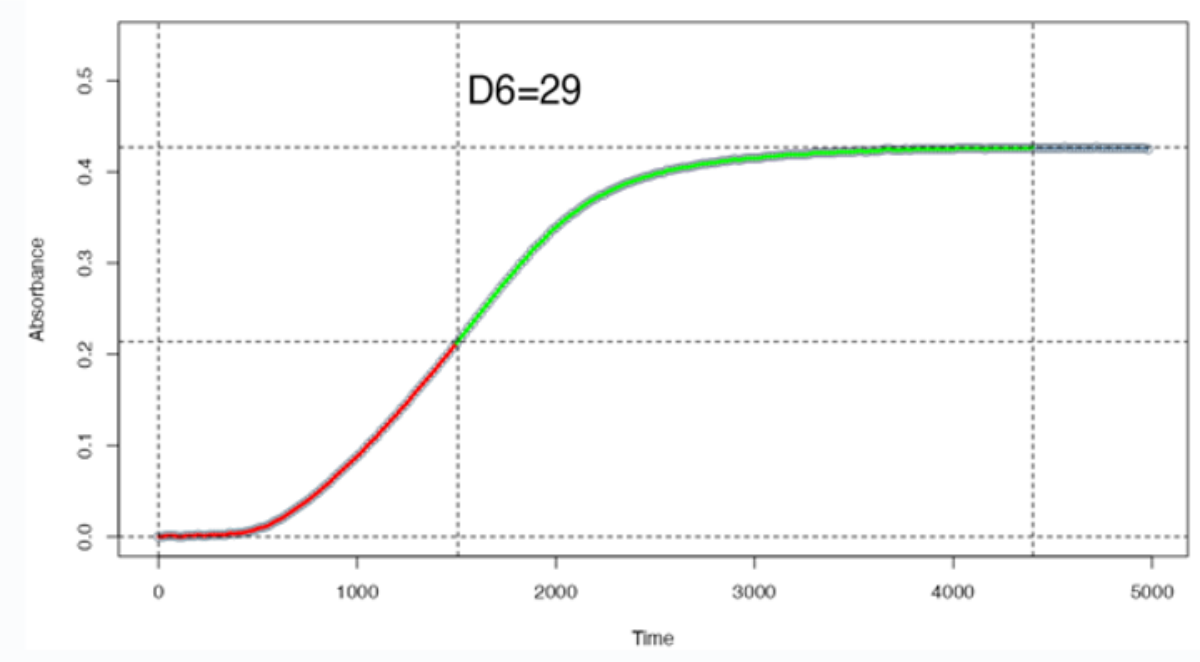
Graphs, results table and setting table can be copied to the clipboard by highlighting and right-clicking using the mouse for pasting into an electronic notebook or OneNote or Excel, for example.



1	2	3	4
351.11	212.22	309.09	242.22
237.90	307.69	243.16	366.67
663.33	420.00	562.50	453.85
478.33	568.89	456.00	648.89
1135.00	782.22	946.67	840.00
814.00	1004.00	811.11	1160.00
1810.00	1400.00	1660.00	1508.00
1506.67	1760.00	1534.29	2010.00

The *Curve* tab

The curve tab allows the user to focus on a single curve, which is selected from the drop-down box in the upper left corner. The plot includes lines corresponding to various analysis selections available. The radio buttons under **Selected Results** specify what is shown in the table below the graph - all results, or results from the first derivative plots of time at maximum increase or time at sign change. As mentioned above, these results are susceptible to noise and should be checked by careful visual inspection alongside the curves.



The *All Results* tab

Here there is a table of all the values from each well for each parameter available. It is possible to click through the data using boxes at the foot of the page and to show data from start to end or in reverse.

The *Raw Data* tab

On this tab the name of the data file loaded is shown and the time and absorbance data. If fitted curves have been generated, the new data will be displayed with additional time points.

The *Explore* tab

This tab provides a simple opportunity to explore your results graphically. The default plot is a heatmap, so you can see patterns and extreme values. If a scatter plot is selected you can select what should be plotted on the x and y axis to investigate relationships between various parameters. Points can be identified by hovering over them with the mouse.

The *Settings* tab

Here a table of settings is provided summarising the settings used in the analysis, which can be copied for future reference to aid reproducibility.

The *Help* tab

The *Help* tab summarises these help notes and provides citation details.

Notes on Halo assays

Settings on the app allow it to be used for halo assay data analysis [Bonnard et al, Sci Rep 7, 2346]. Typically in this assay wells are included that give estimates for baseline and global maximum, which can be input into the app. The use of high absorbance wells for the global maximum value may cause problems for interpolation, so it may be necessary to adjust the threshold and/or use spline fitting when analysing halo assay data.

Code and other apps

R code, data and help files are available on github

More information on Shiny apps and links may be found at Shiny-clots