The FlashAnalysis application

Application Note

FlashAnalysis is a tool that displays LI-600, LI-6400/XT, and LI-6800 flash data. The main goal of the software is to optimize the flash protocol. The program also provides tools to summarize and export data of interest.

Compatibility and installer files

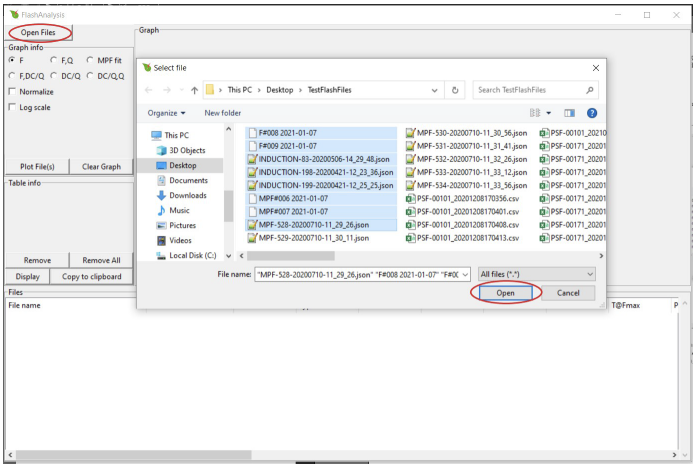
FlashAnalysis was built on Windows 10 and macOS Catalina 10.15.6 with Python 3.8. There has been limited testing on other Mac and Windows versions so there is no guarantee that it will work unless using one of the two versions mentioned earlier. There is a known bug with using the `tkinter` package on Mojave 10.14.6 that causes the computer to restart.

The installers are available for download at the links below.

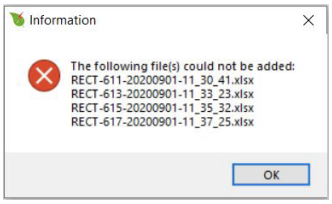
- FlashAnalysis for Windows (FlashAnalysis.-v1.0.exe): licor.com/documents/xt3r1mjsfisnvtrwz3d0nhnb2cmjea3a
- FlashAnalysis for MacOS (FlashAnalysis.-v1.0.dmg): licor.com/documents/b9w74uemg6ayzhye9kyjjs7ph79jh843

Importing files that are saved on your computer

Import data by clicking **Open Files**. Select one or more files then press **Open**. The default file type is 'All' but the user can select to only view the LI-6800 (.json) or LI-600 (.csv) file format from the drop down menu (Windows operating systems only).

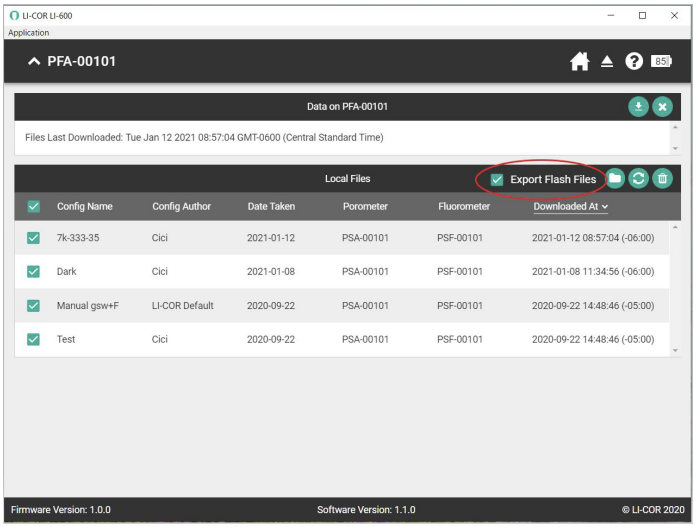


If a file is of a different extension or does not contain flash data, you will see an error message. The error message box shows a list of the files that the program was not able to open. This error could also arise with a flash file collected without a leaf in the chamber and where F_{min} (or F_{max}) is rounded to 0.



Importing data from the LI-600

To load LI-600 files, first download the flash files from the instrument to your computer. Make sure the **Export Flash Files** check box is enabled when downloading.



After the data is downloaded to the computer, extract the files from the .zip file to make them available to the **FlashAnalysis** program.

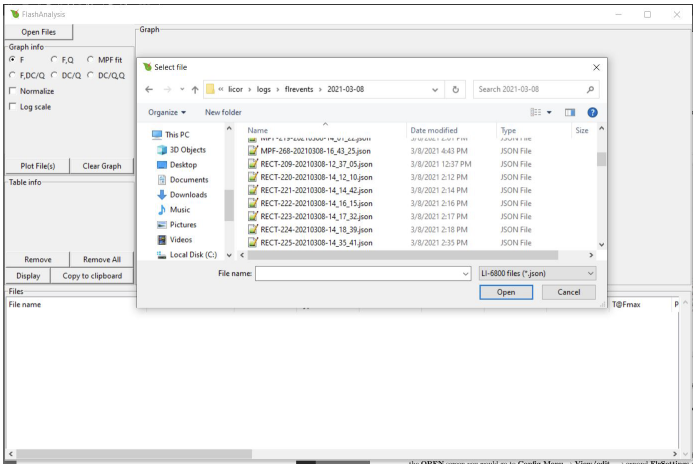
Importing data from the LI-6800

Using Windows Explorer

FlashAnalysis can import data directly from the LI-6800 if the LI-6800 is connected to the same network as your computer. To open the files this way, click **Open Files** and in the file path box type two backslashes followed by your LI-6800 console's

serial number: **\\68C-XXXXXX**. If this does not work, try **\\68C-XXXXXX.local.**.

Your flash files will be located in `licor\logs\flrevents\`.



Using USB

You can also export flash files from the LI-6800 using a USB drive. Insert the USB into your LI-6800 and go to **Tools > Manage Files > USB** and select **Copy files to USB**.

Importing data from the LI-6400/XT

With the LI-6400/XT you need to configure the instrument to save the raw flash data in a file upon log. From the **OPEN** screen you would go to **Config Menu > View/edit**... expand **FlrSettings** and under **FlrSettings** expand **Flash** and enable **autosave.**

After you have collected your data, connect the LI-6400/XT to your computer and transfer files using the LI-6400XTerm software. The flash files will be located in the `user/flr/` directory.

Using the FlashAnalysis application

Changing displayed variables

Change or add displayed columns by clicking the **Display** button located under **Table info.** A new window will open up allowing the user to check and clear variables they want displayed. Click **Apply** to save the changes.

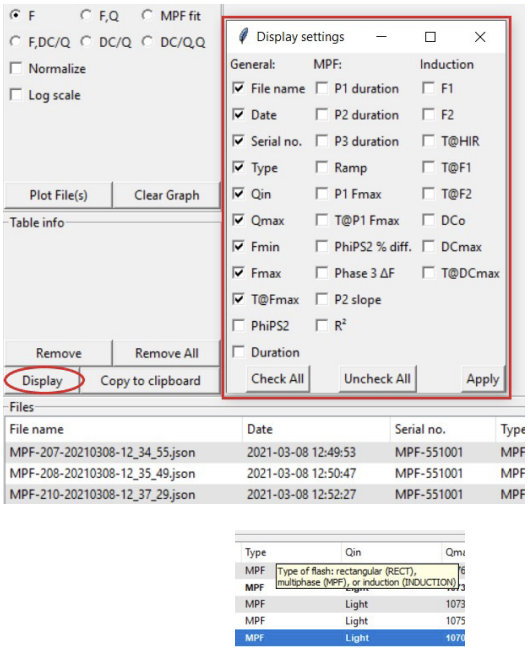


Figure 1. Windows only: Right clicking on the column header allows you to see more detailed information. Right or left click anywhere in the table to remove the pop-up window.

Variable definitions are found in *FlashAnalysis Definitions* on page 4.

Plotting data

Files can be plotted either by double clicking on the file in the table or by highlighting one or several rows then clicking **Plot File(s)** under **Graph info.** Rows in bold font indicate that the file is plotted on the graph. A double click on a bold font row removes the file from the plot. Clear the graph by clicking **Clear Graph.**

Graphing options

You can modify what is graphed by using the radio buttons under **Graph info.** Note that any file that does not have the relevant information will be hidden, e.g. plotted rectangular flash data will be hidden for the MPF fit.

- **F**: plots demodulated fluorescence (*F*) vs. time
- **F,Q**: plots demodulated fluorescence (*F*) vs. time on the left y-axis and actinic light (*Q*) vs. time on the right y-axis
- **MPF fit**: plots demodulated phase 2 fluorescence (*F*) vs. $10^4/Q$
- **F,DC/Q**: plots demodulated fluorescence (*F*) vs. time on the left y-axis and raw fluorescence per quanta of actinic light (DC/*Q*) vs. time on the right y-axis

- **DC/Q**: plots raw fluorescence per quanta of actinic light (DC/*Q*) vs. time
- **DC/Q,Q**: plots raw fluorescence per quanta of actinic light (DC/*Q*) vs. time on the left y-axis and actinic light (*Q*) vs. time on the left y-axis

Below the radio buttons, there are two check buttons that allow you to modify how the data is being displayed on the graphs (not available when **MPF fit** is selected):

- **Normalize**: normalizes the data to its maximal value. Useful for comparing flash data of different magnitudes (e.g. LI-600 vs. LI-6800 files)
- **Log scale**: changes the x-axis to log scale. Useful for plotting induction flashes.

The graph itself comes with some useful tools:

Use zoom (🔍) to zoom in, use home (🏠) to reset the view, and click save (💾) to save the graph as a .png.

Removing data

Click **Remove** to remove highlighted file(s). Click **Remove All** to remove all files.

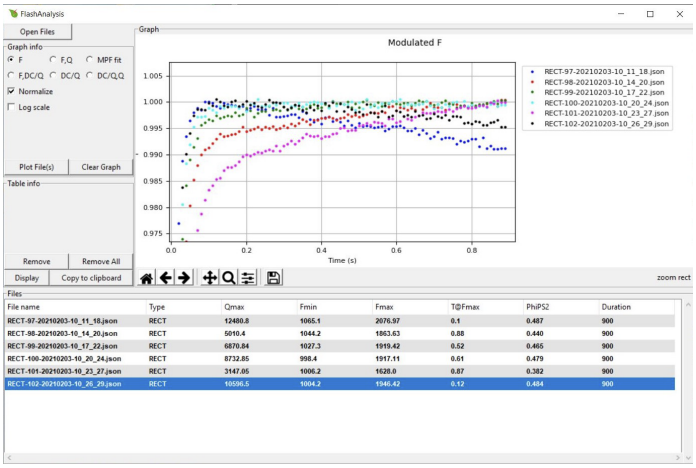
Exporting data

To export the variables you are interested in, make sure they are selected as columns, then highlight the rows (files) you want to export and click **Copy to clipboard**.

Example analysis

Section 27-40, **Using the MultiPhase Flash > Making Measurements** in the LI-6400/XT manual and a section in the LI-6800 manual has details on what to look for in both a rectangular and MPF flash to collect the best data.

In the following example we show six rectangular flashes collected with the LI-6800. To make easier comparisons between the flashes we have normalized the data and zoomed in on the top 2.5%:



The plot above leads to the following conclusions:

1. The highest flash intensity (*Qmax*) yields the highest *Fmax* (RECT-97). One might increase the flash intensity to see if it would cause a higher *Fmax*, but since fluorescence is decreasing over time (dark blue dots) indicating flash-induced quenching, one might consider implementing an MPF.
2. The two lowest flash intensities (RECT-101 and RECT-98), never reach saturation. We can tell this from the continuous rise in fluorescence and the two flashes' *T@Fmax* parameters, which both are close to the total duration. This is not simply a duration issue, since higher intensities (e.g., RECT-99 and RECT-100) do flatten out (fully close PSII reactions centers).
3. RECT-100 at a Qmax of 8,700 µmol m^{-2} s^{-1} gave the most steady fluorescence. It reached saturation quickly and stayed there throughout the flash. *Fmax* occurred at about 0.6 s (*T@Fmax*). A shorter flash could therefor be used, somewhere around 650 to 700 ms would be sufficient.
4. These data suggest an MPF with a flash intensity of about 8,500 µmol m^{-2} s^{-1}. Since fluorescence stayed steady under the flash, a starting phase 1 duration of 300 ms should be fine.
5. If using an MPF, further optimization might be needed to find optimal phase 2 and 3 durations.

FlashAnalysis Definitions

Table 1 below shows the variables that are measured by each instrument.

Table 1. Colored dots indicate whether the variable exists for the specific instrument: LI-600 (†), LI-6800 (‡), and LI-6400/XT (§).

Variable Name	Description	Instrument
	General	
File name	Name of the flash file	† ‡ §
Date	Date (YYYY-MM-DD) and time (HH:MM:SS)	† ‡ §
Serial no.	Fluorometer serial number	† ‡ §
Type	Flash type: rectangular (RECT), multiphase (MPF), induction (INDUCTION)	† ‡ §
Qin	Actinic light (µmol/m^2/s) if available, otherwise: light or dark adapted	† ‡ §
Qmax	Maximum light intensity during the flash (µmol/m^2/s)	† ‡ §
Fmin	Minimal fluorescence (Fo or Fs)	† ‡
Fmax	Maximal fluorescence (Fm or Fm')	† ‡ §
T@Fmax	Time at Fmax (s)	† ‡ §
PhiPS2	Quantum efficiency of photosystem 2 (Fv/Fm or PhiPS2)	† ‡
Duration	Total flash duration (ms)	† ‡ §
	MPF	
P1 duration	Duration of phase 1 (ms)	† ‡ §
P2 duration	Duration of phase 2 (ms)	† ‡ §
P3 duration	Duration of phase 3 (ms)	† ‡ §
Ramp	Decrease in flash intensity from the red target during phase 2 (%)	† ‡ §
P1 Fmax	Maximal fluorescence during phase 1	† ‡ §
T@P1 Fmax	Time of P1 Fmax (s)	† ‡ §
PhiPS2 % diff.	Difference between using Fmax and P1 Fmax for calculating PhiPS2	† ‡ §
Phase 3 ΔF	Measured phase 3 Fmax minus predicted phase 3 Fmax	† ‡ §
P2 slope	Phase 2 regression slope	† ‡ §
R^2	Phase 2 regression R^2	† ‡ §
	Induction	
F1	Fluorescence at 1st inflection point	‡
F2	Fluorescence at 2nd inflection point	‡
T@HIR	Time of "half initial rise" (s)	‡
T@F1	Time of F1 (s)	‡
T@F2	Time of F2 (s)	‡
DCo	Extrapolated pre-flash value for DC/Q fluorescence	‡
DCmax	Max value of DC/Q fluorescence	‡
T@DCmax	Time of DC/Q max value (s)	‡

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