

TSA-Tm v1.0 (July 2019)

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In a Thermal Shift Assay experiment, the raw data produced by the readings of the qPCR machine are the fluorescence intensity as a function of cycles or temperature per well. Depending on the plate size and the number of optical channels, 48, 96, or more experiments per run can be made with information from several wavelength readings in a high-throughput fashion. Usually, the qPCR machine control software can export this raw data set. However, further analysis to obtain the melting temperature (T_m) can be a cumbersome process.

Here, we present the development of a Matlab program to calculate such values. In addition to facilitate the analysis by reading and ordering the exported qPCR data in the XLSX formatted file, it provides a graphical interface where the user can choose the optical channel and set of wells to analyze, calculate the average and standard deviation of the fluorescence vs. temperature profile if more than one repetition was included in the experimental design, and numerically calculate dF/dT . The source code and instructions to use the software can be accessed at <https://github.com/tripplab/TSA-Tm>.

Installation and user's guide

1. Download the TSAA.m and TSAA.fig files from

2. Open MATLAB® and press on "Open file".

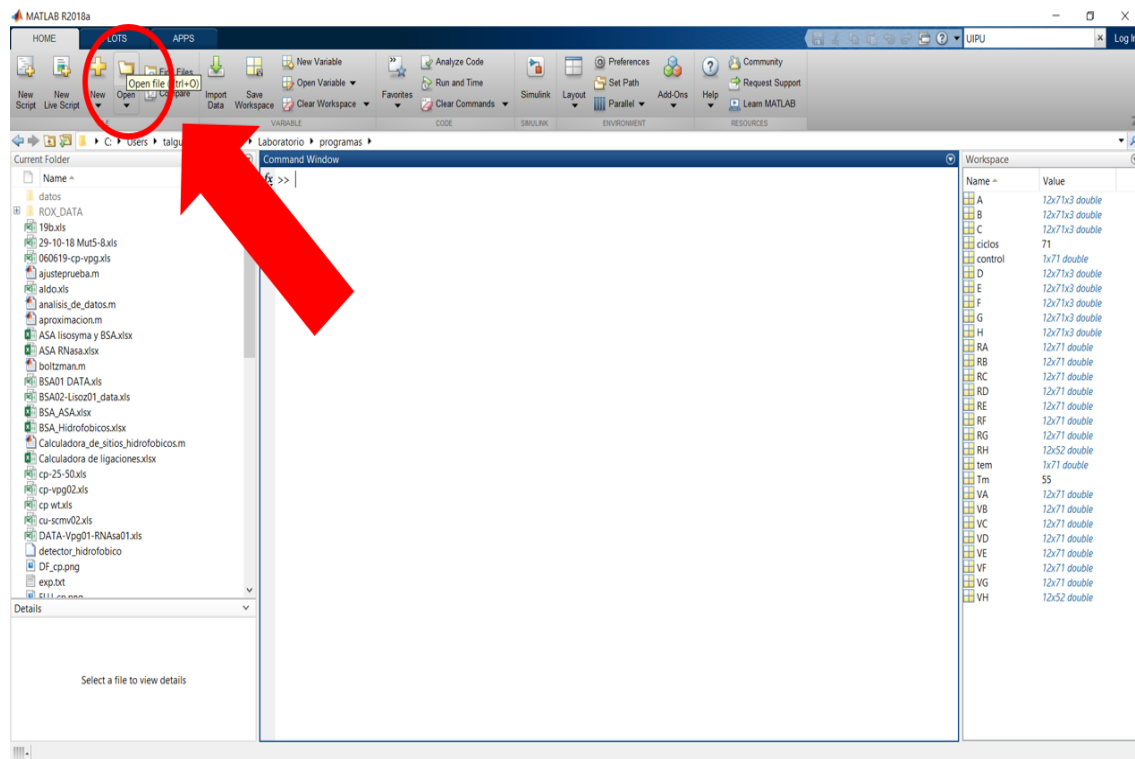


Figure 1: MATLAB®Panel

3. Search the TSAA.m file and press "Open".

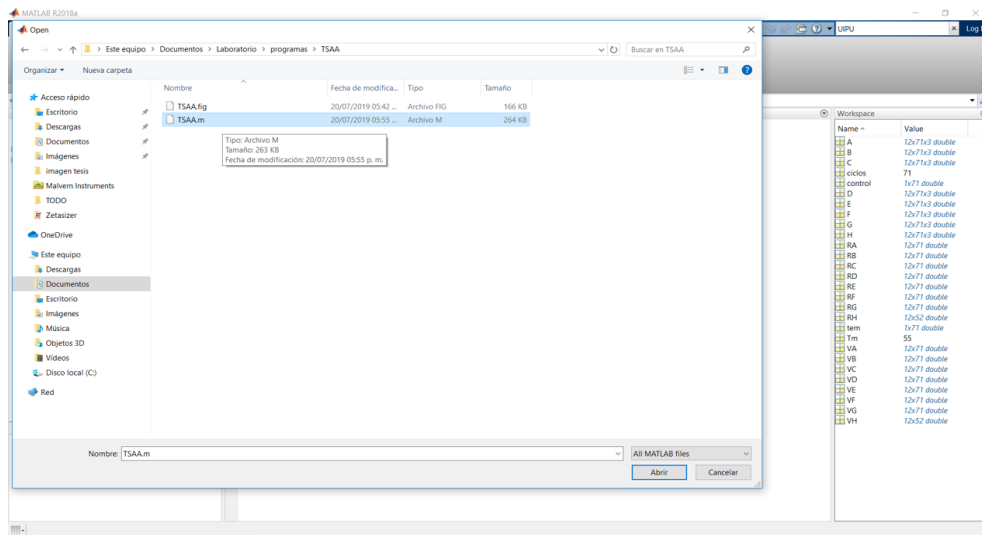


Figure 2: "File explorer" window

4. Then press "run" in MATLAB® or press "F5" key.

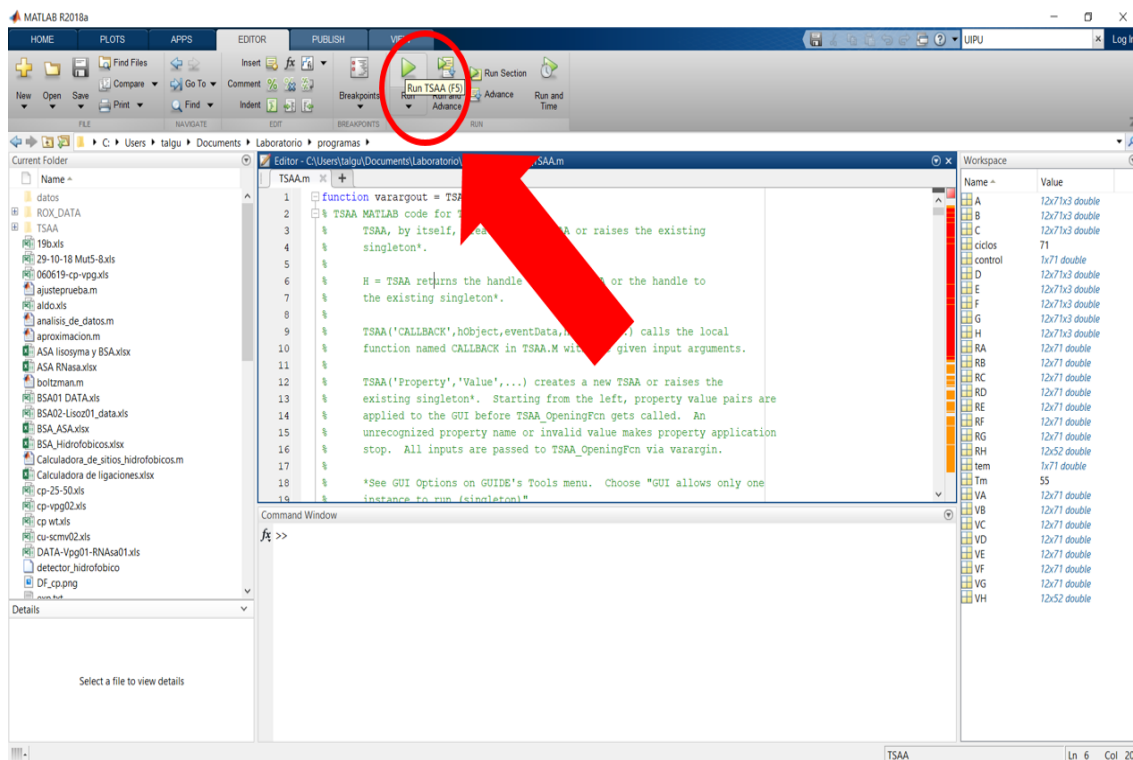


Figure 3: MATLAB®Panel

5. When the TSAA window appears, you can: select the plate that you used, the initial temperature "T0" and the increments "Step". If you check "AUTO", the program will consider "T0 = 25°C" and "Step = 1°C".

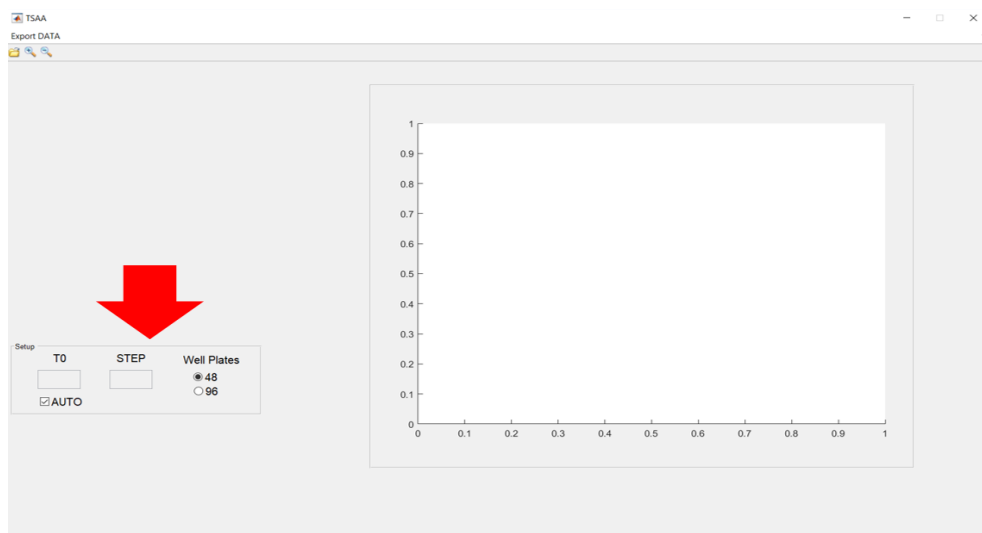


Figure 4: TSAA window : Settings

6. After settings, press "open file" icon, search your excel file and press open.

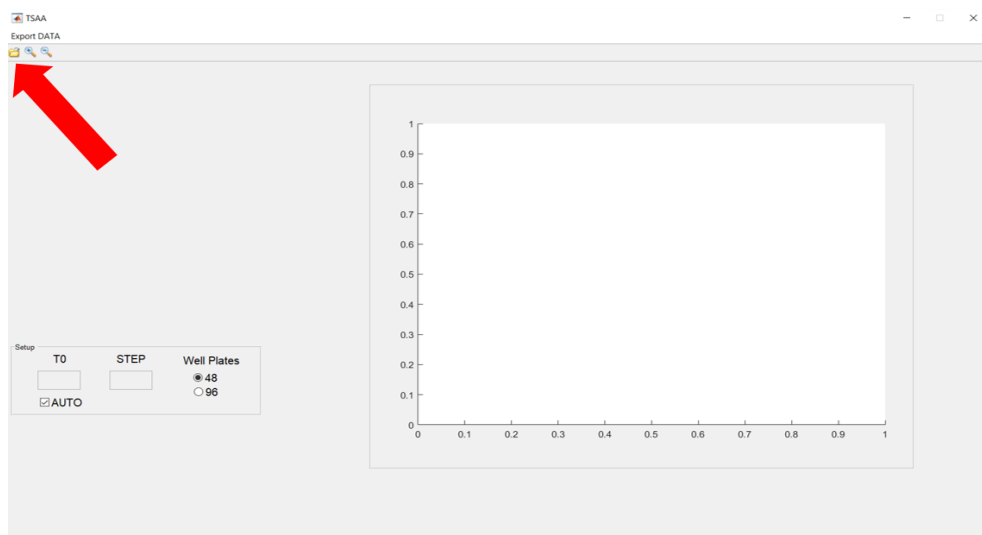


Figure 5: TSAA window : Open files

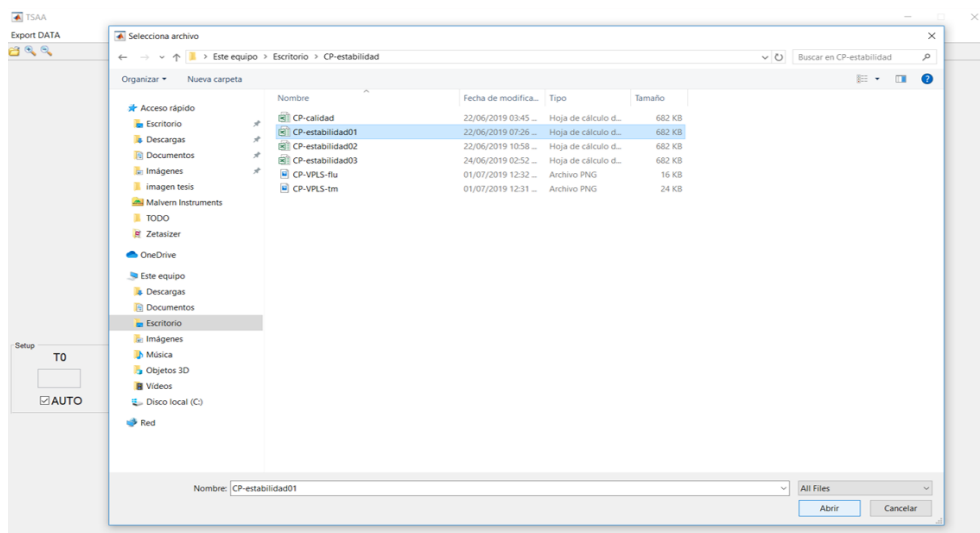


Figure 6: File Explorer

NOTE: You need to be sure that your file contain "Raw Data" and "Multicomponent Data" (Figure 7).

Well	Cycle	Filter 1	Filter 2	Filter 3
A1	1	52564	51039	58580
A2	1	53267	54612	70850
A3	1	51629	52911	69027
A4	1	53358	54645	70934
A5	1	50025	48715	55564
A6	1	54880	58946	83411
A7	1	55375	60742	86574
A8	1	58712	67884	98337
B1	1	52446	51046	58764
B2	1	52854	52946	70227
B3	1	51268	51849	66702
B4	1	53791	54880	70564
B5	1	50272	48774	55224
B6	1	58025	65895	101135
B7	1	62497	76346	118987
B8	1	58066	65754	88459
C1	1	84552	74160	78277
C2	1	71697	93988	149918
C3	1	51342	53114	73723
C4	1	54096	59270	85429
C5	1	50128	49147	64382
C6	1	53013	52482	69602
C7	1	63990	53046	69620
C8	1	6324	535	70638
D1	1	625	475	47462
D2	1	762	474	47496
D3	1	656	453	46537
D4	1	4742	474	47577
D5	1	45920	45680	45605
D6	1	47783	47476	47453

Figure 7: Excel file: Raw Data & Multicomponent Data

- When the program finish to load the data, a control panel will appear(Figure 8, 9), the number of wells that appear depends of the plate that you select.

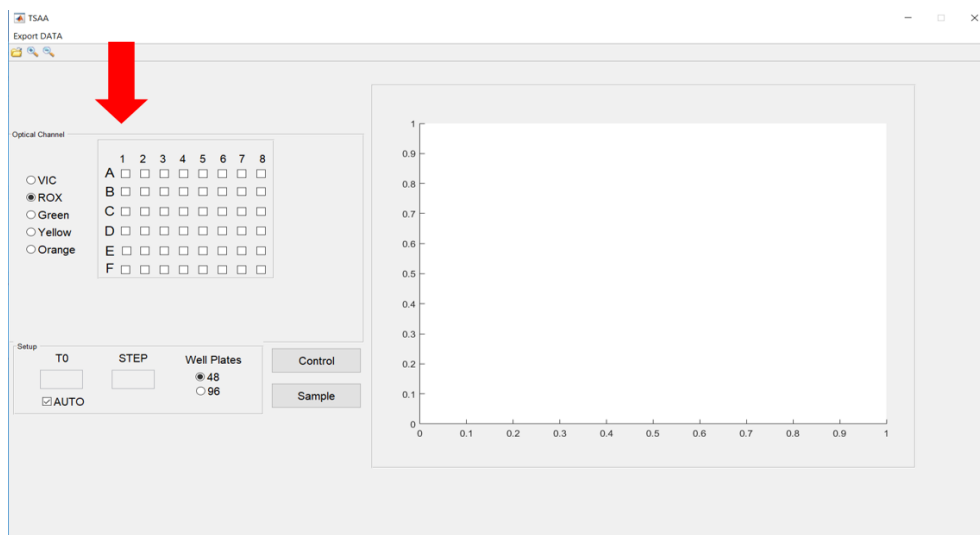


Figure 8: TSAA Panel: 48 well plate panel control

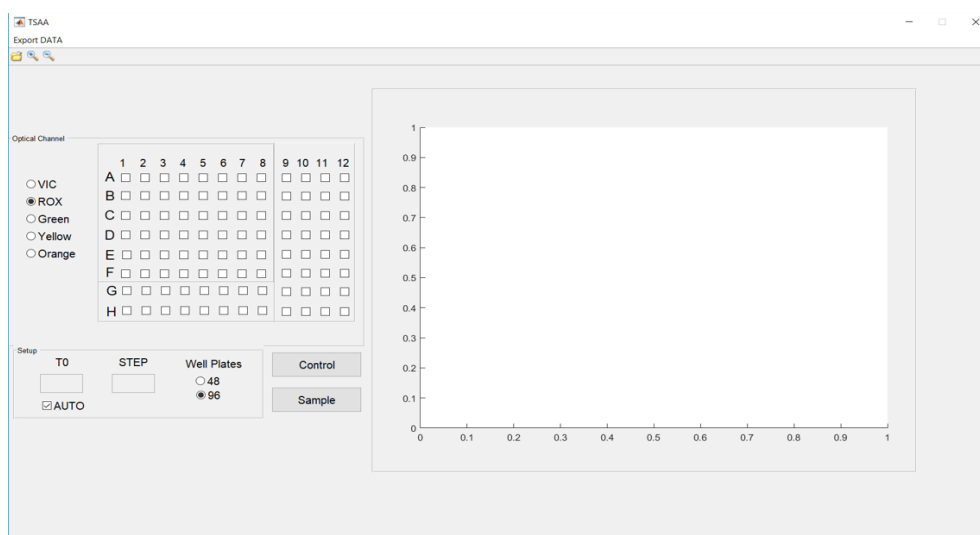


Figure 9: TSAA Panel: 96 well plate panel control

in this panel you can "check" the wells of your samples and the Optical channel then the graphs appear in the preview panel.

8. If you want to graph the samples, you need to check the control well and then press "Control" button(Figure 11).

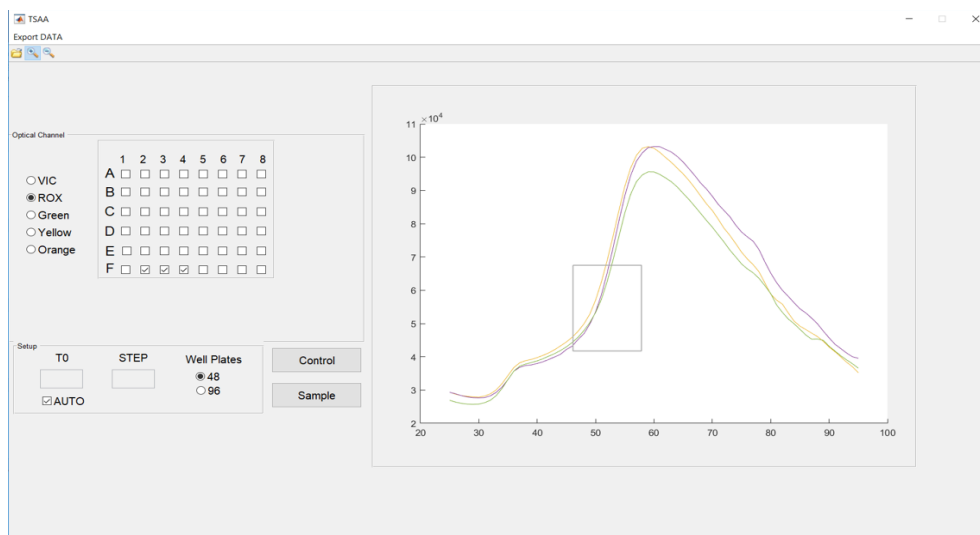


Figure 10: TSAA Panel: Preview Panel

then you need to "check" all of your samples well and press "Sample" button(Figure 12), twice windows will appear, one is the fluorescent average with error bars, and the other one, is the $-(dF/dT)$, the T_m are the points where the peaks are higher.

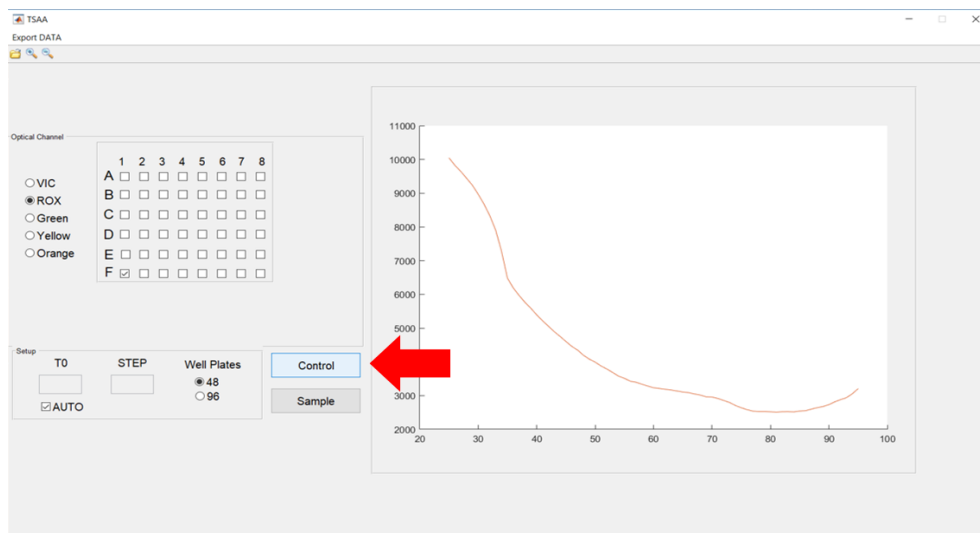


Figure 11: TSAA Panel: Control Sample

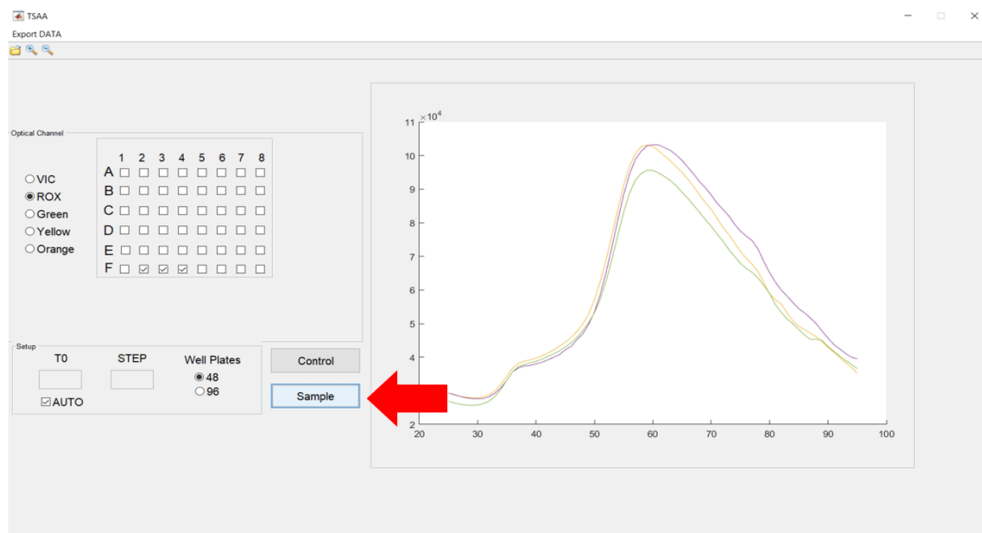


Figure 12: TSAA Panel: Samples

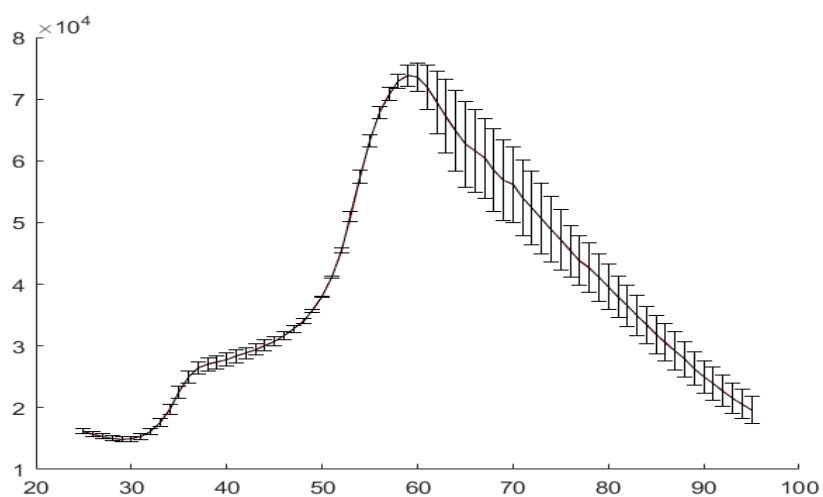


Figure 13: Fluorescent average graph

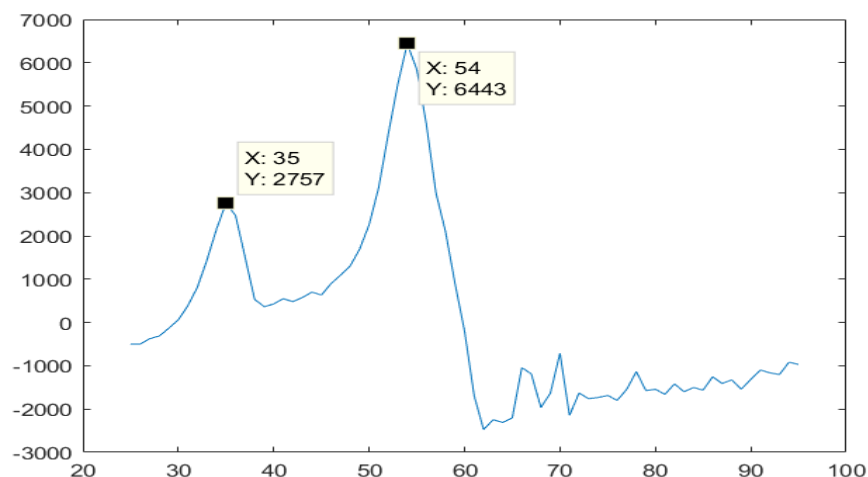


Figure 14: $-(dF/dT)$ graph

Also you can export the fluorescent data to a .txt file in cvs format, you need to select the Optical channel(ROX,VIC,GREEN,YELLOW,ORANGE) and then press on "Export Data" → "To CSV" (Figure 15), then select a folder and press "Select folder" (Figure 16), the .txt files have a matrix of (Well)X(Steps), if you used a 48 well plate then "well = 8", if you used a 96 well plate then "well = 12". Each row of this matrix represent a well from 1 to "8 or 12" (Figure 17).

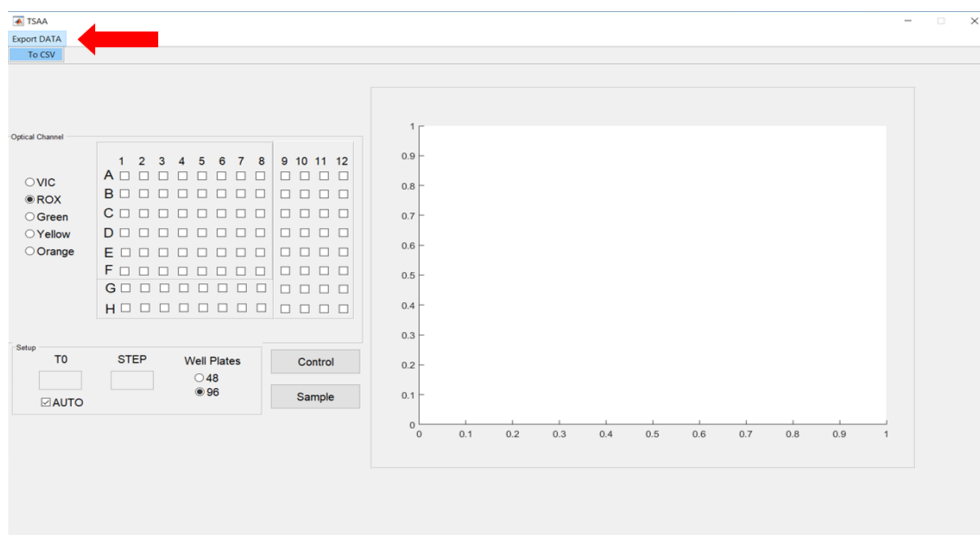


Figure 15: TSAA Panel: Export Samples

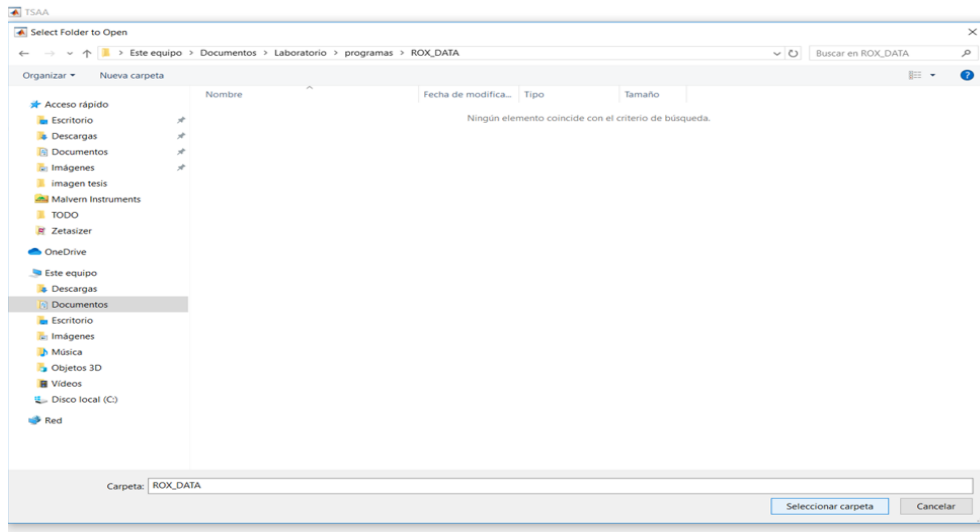


Figure 16: File Explorer

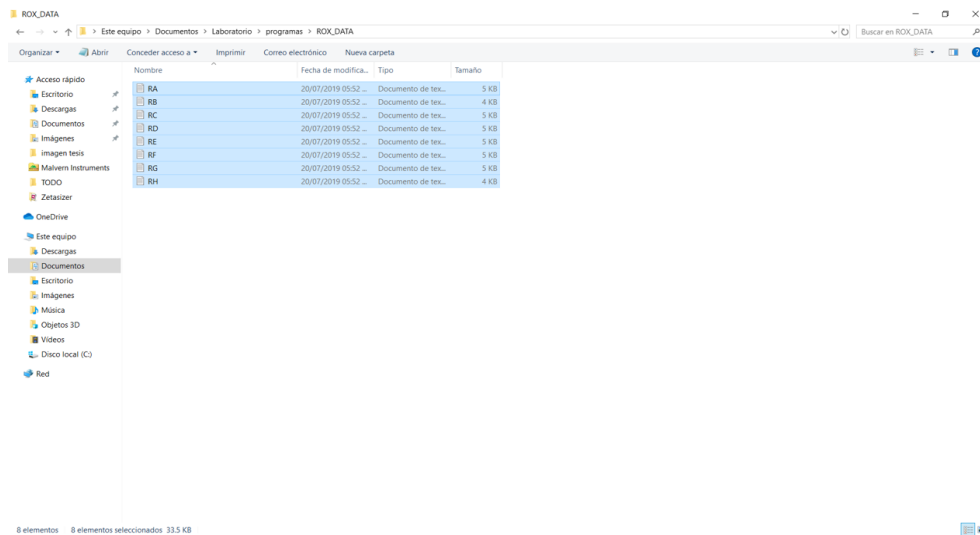


Figure 17: File Explorer: Saved Data