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 (Tm) 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.

Installation and user's guide

1. Download the TSA-Tm.m and TSA-Tm.fig files from
   https://github.com/tripplab/TSA-Tm

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

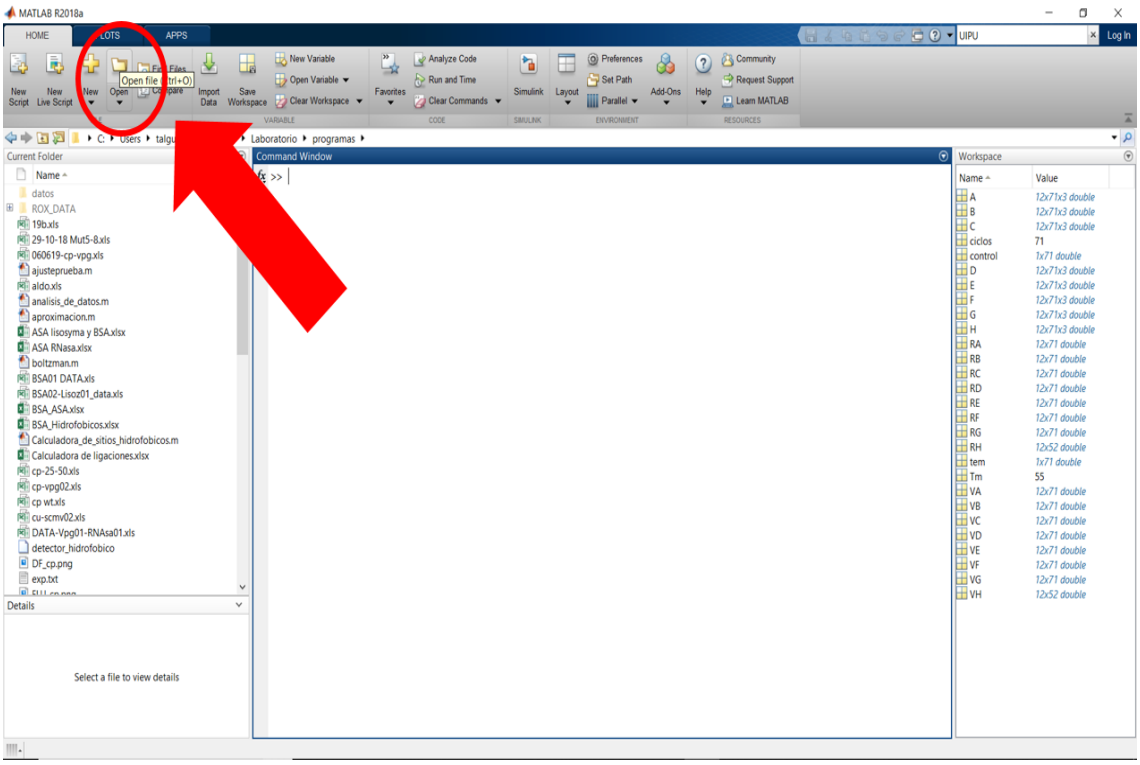

Figure 1: MATLAB®Panel

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

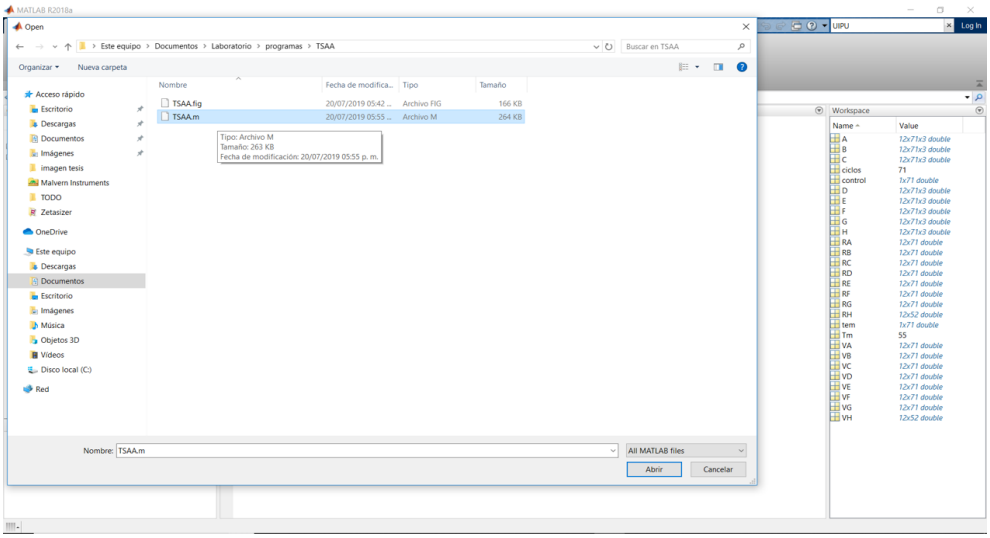

Figure 2: "File explorer" window

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

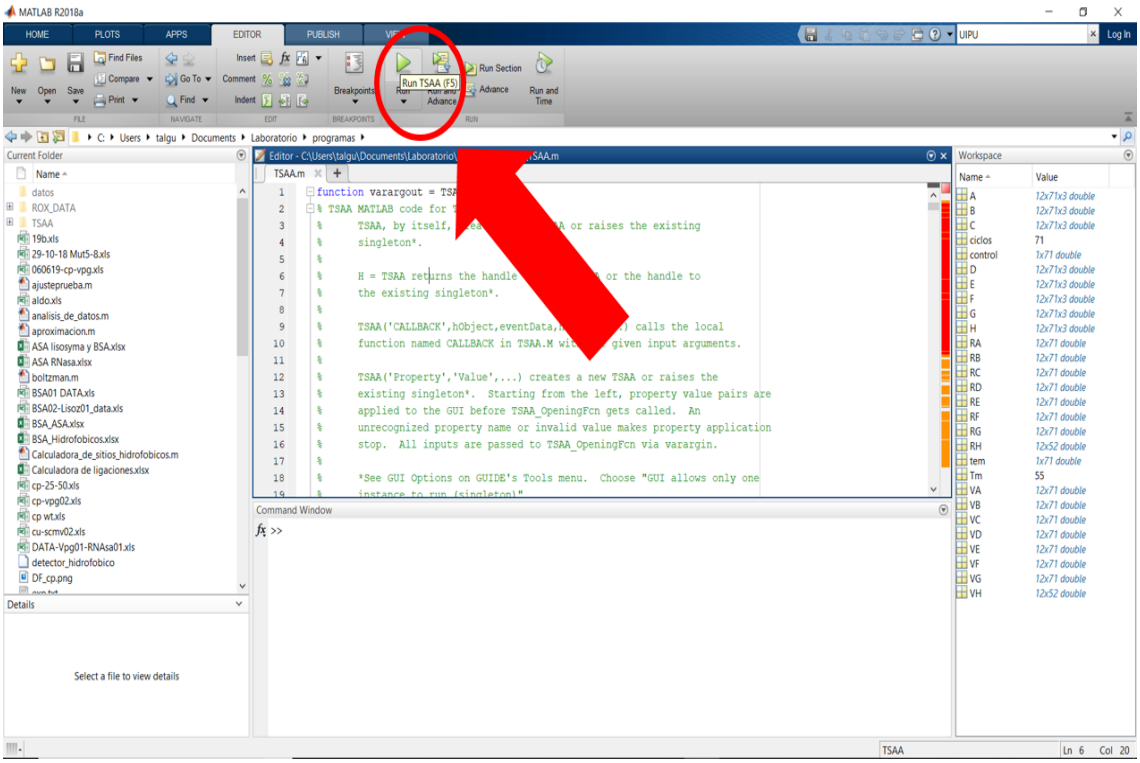

Figure 3: MATLAB®Panel

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 ".

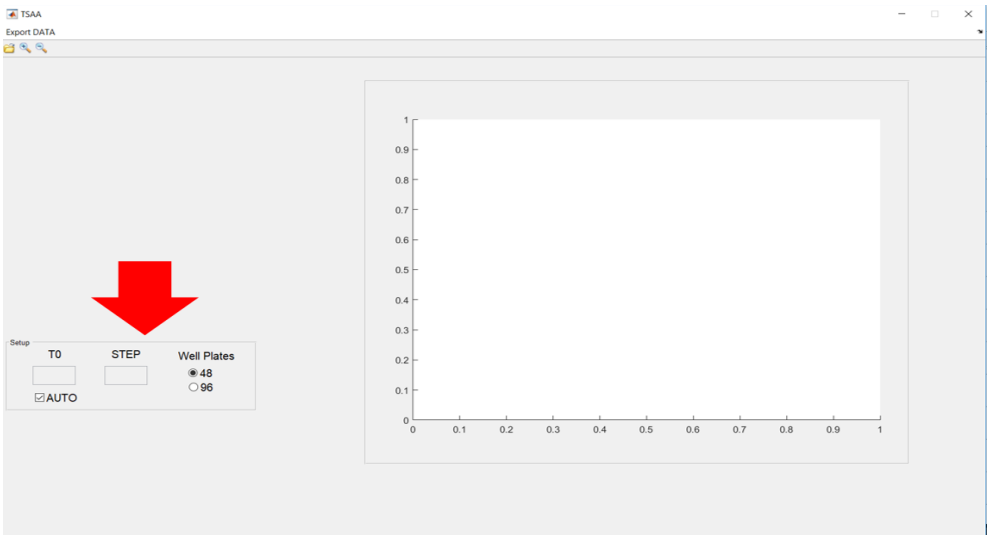

Figure 4: TSAA window : Settings

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

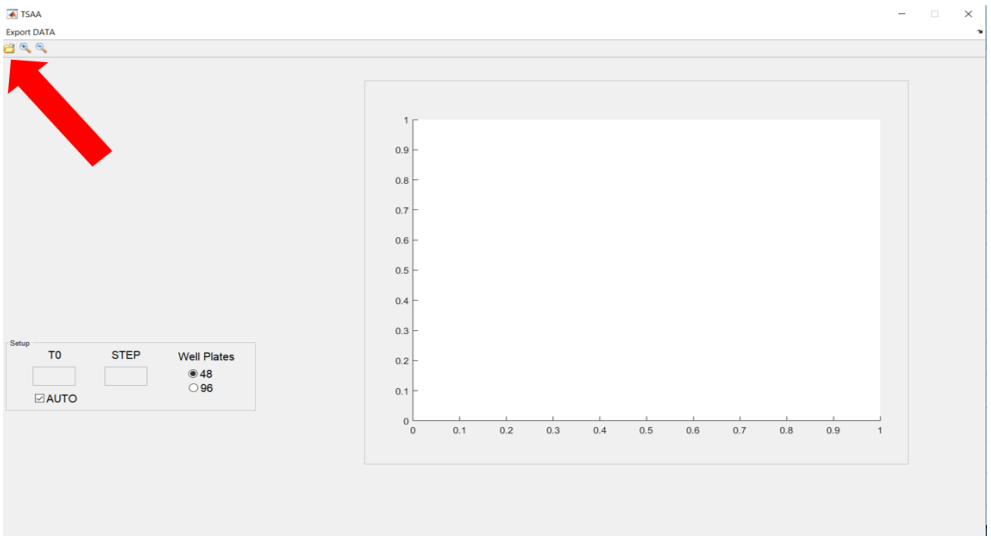

Figure 5: TSAA window : Open files

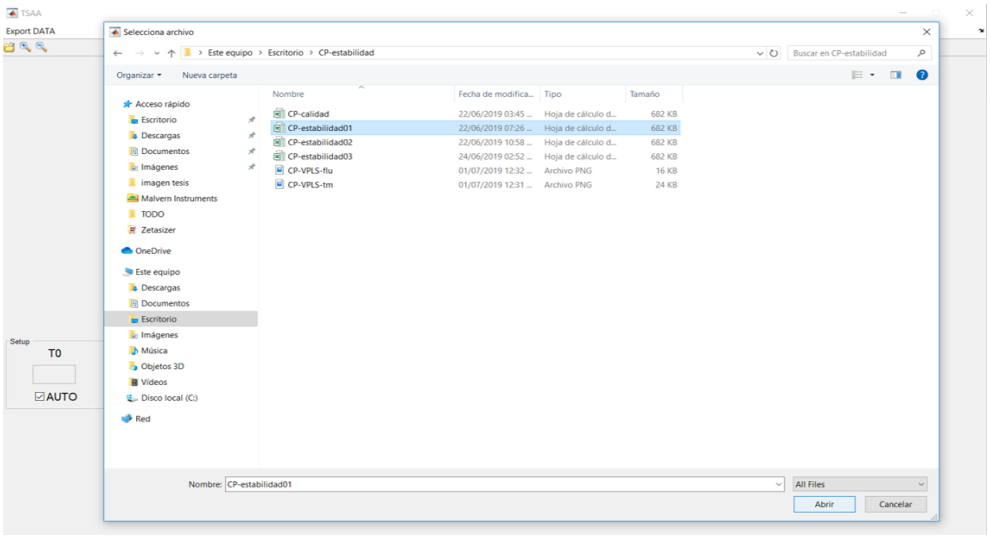

Figure 6: File Explorer

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

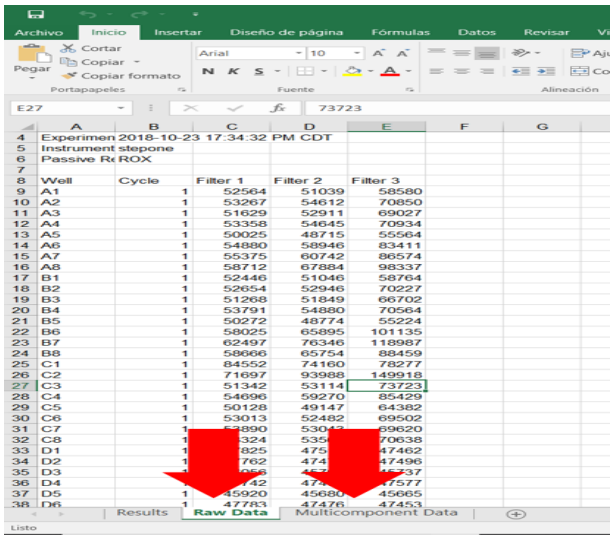

Figure 7: Excel file: Raw Data & Multicomponent Data

7. 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.

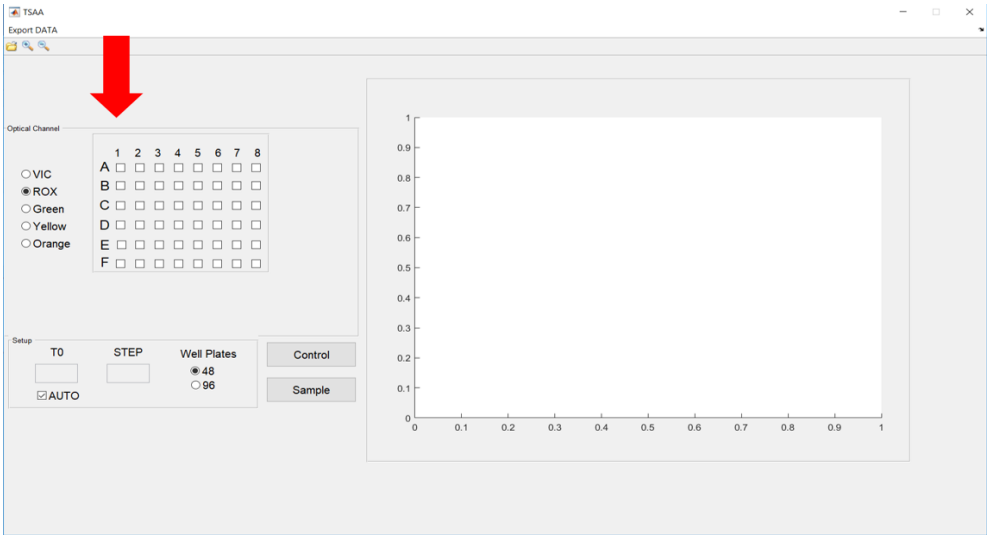

Figure 8: TSAA Panel: 48 well plate panel control

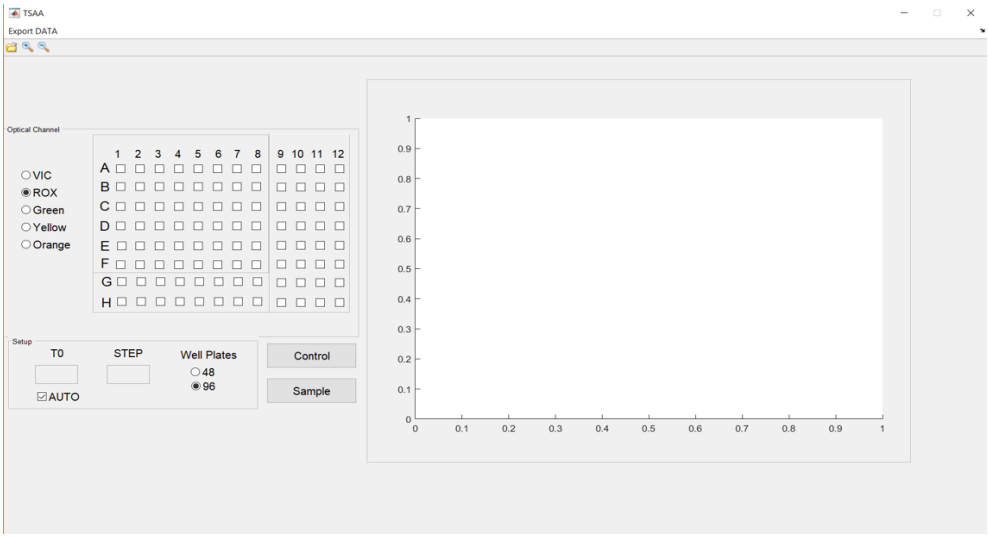

Figure 9: TSAA Panel: 96 well plate panel control

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).

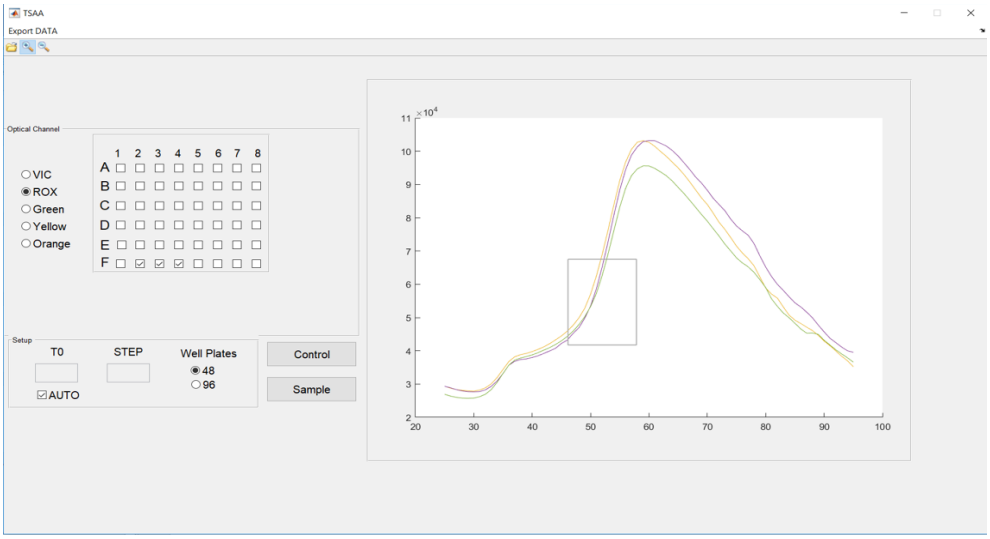

Figure 10: TSAA Panel: Preview Panel

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 Tm are the points where the peaks are higher.

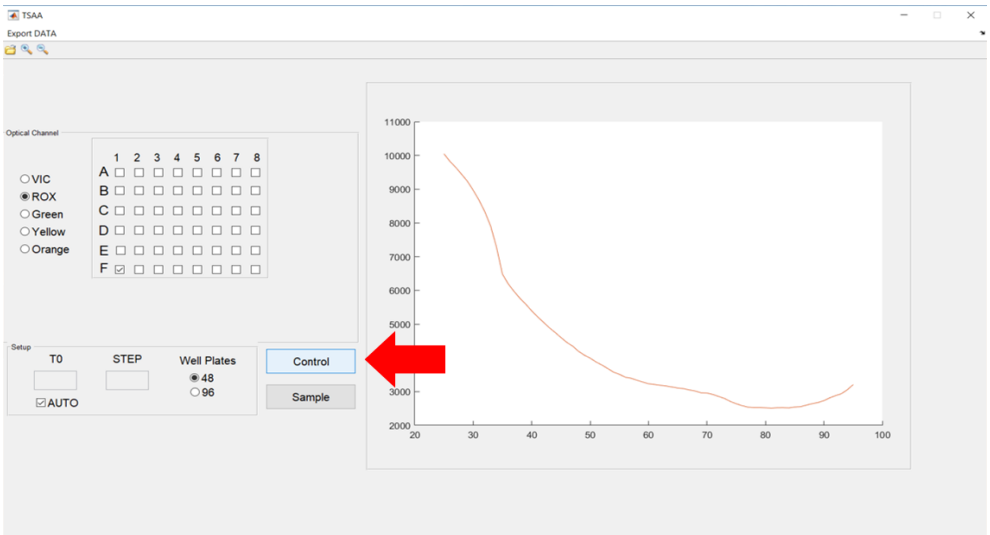

Figure 11: TSAA Panel: Control Sample

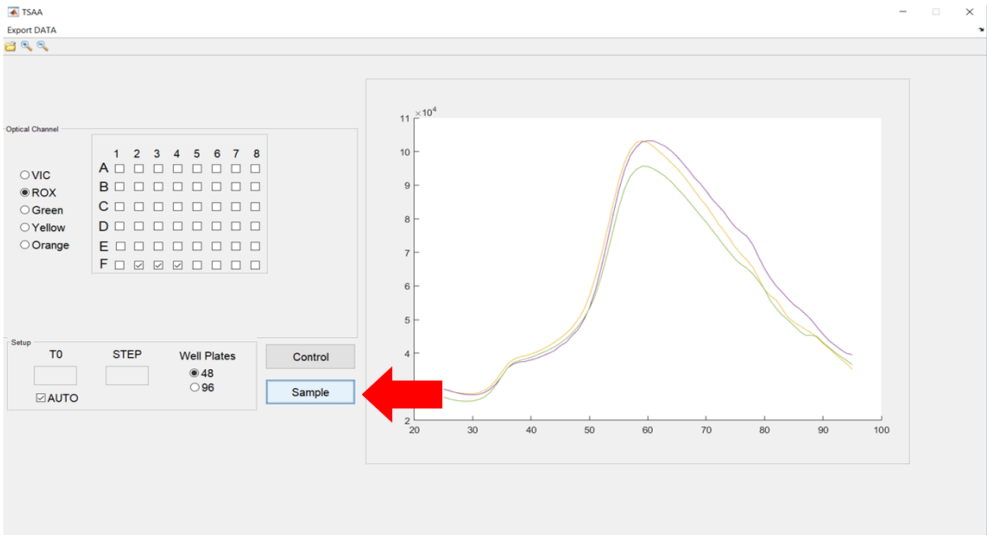

Figure 12: TSAA Panel: Samples

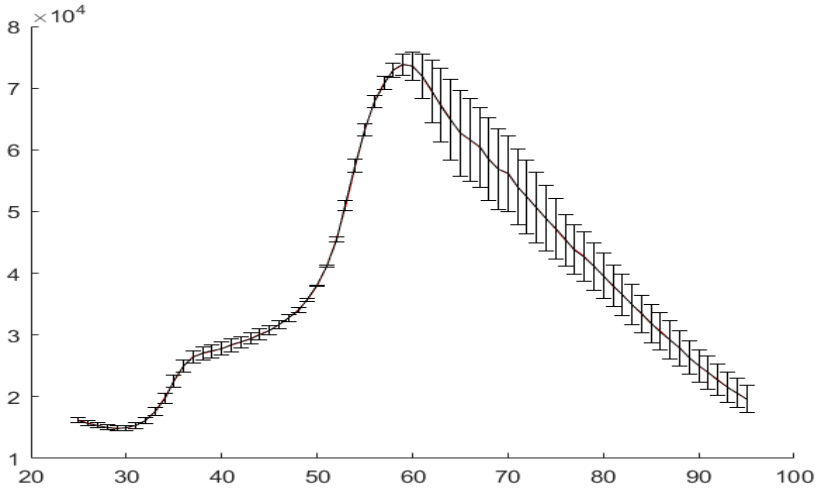

Figure 13: Fluorescent average graph

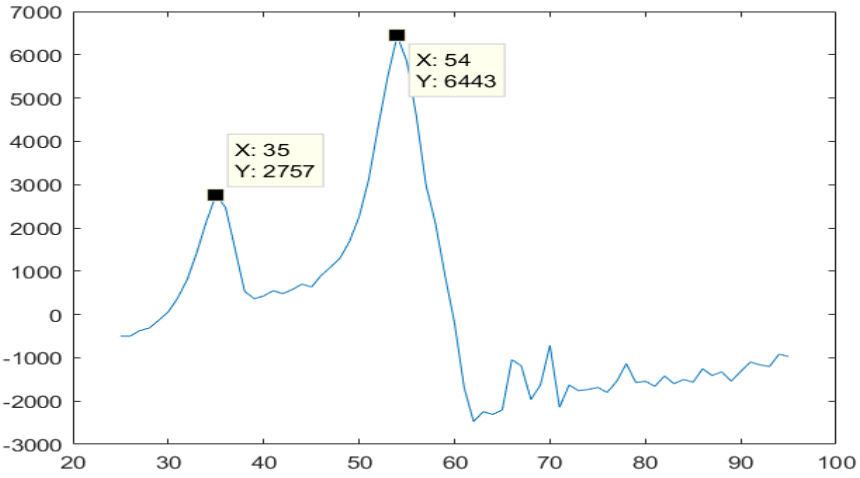

Figure 14: -(dF/dT) graph

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).

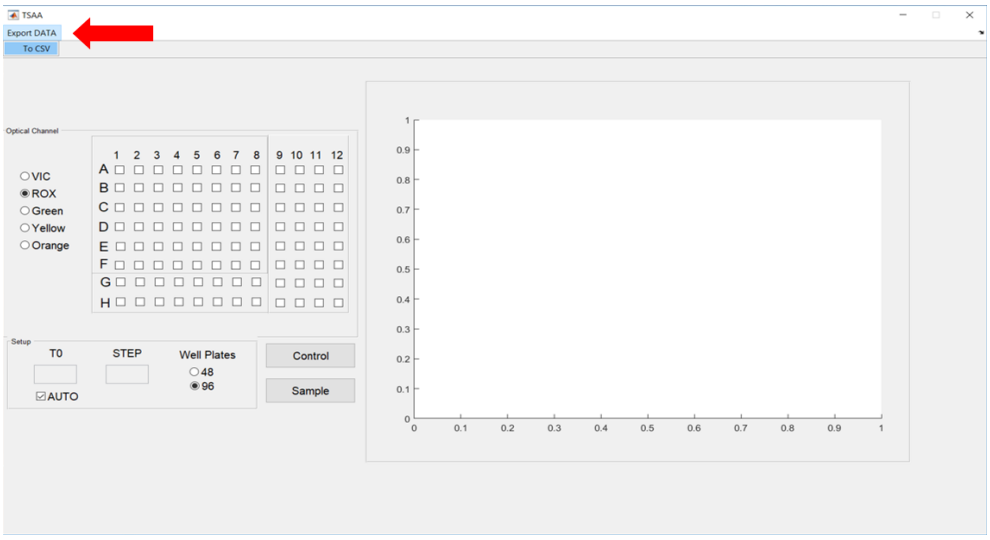

Figure 15: TSAA Panel: Export Samples

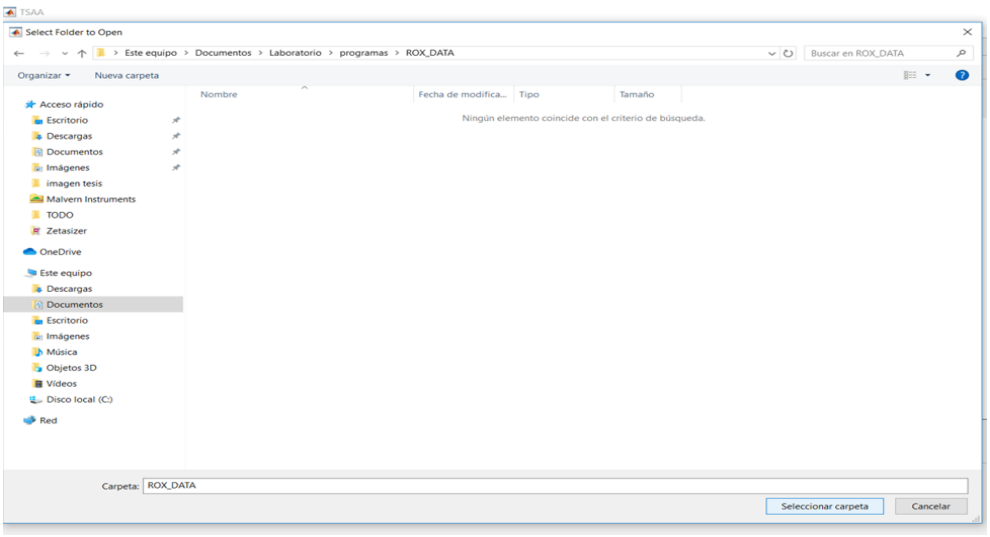

Figure 16: File Explorer

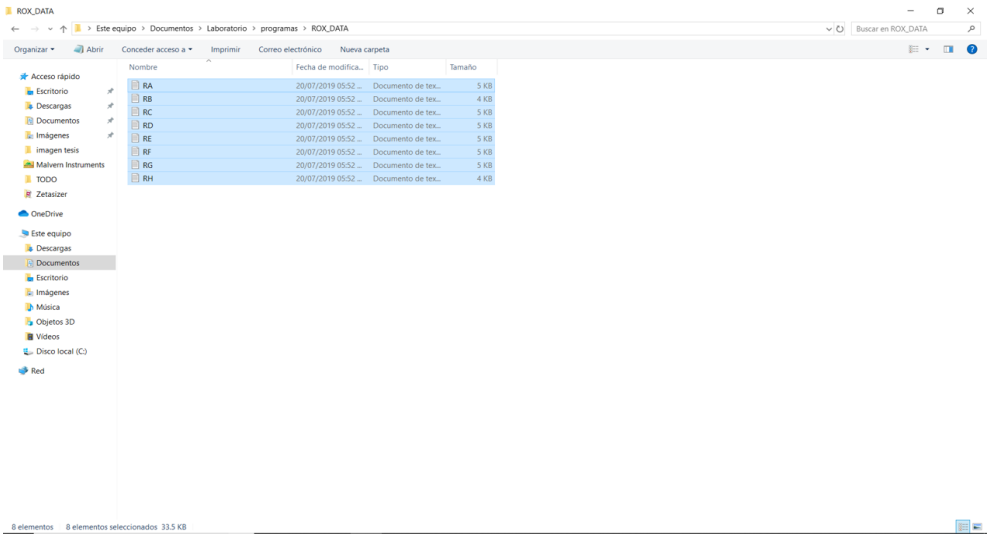

Figure 17: File Explorer: Saved Data