FCS Analysis

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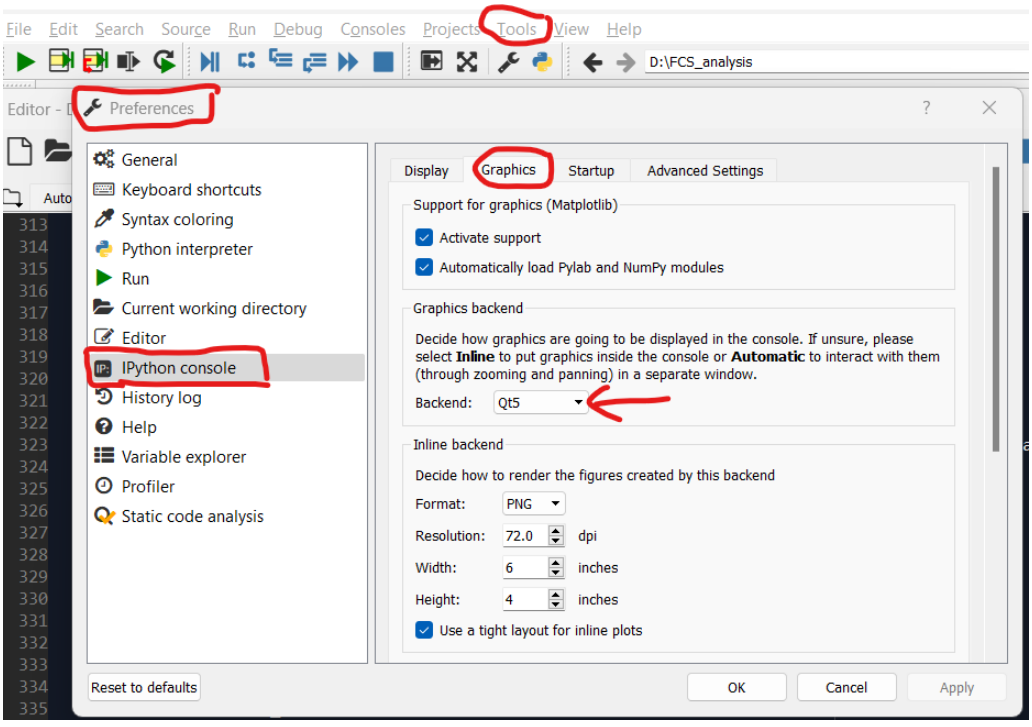
This document provides a detailed guide with step-by-step instructions for performing FCS analysis using a Python-based GUI.

Open **Spyder** in the lab computer and then open python script '**FCS_data_analysis.py**' that is in drive D (highlighted red in Image 1). Now, run the python script by clicking the highlighted yellow button shown in **Image 1**.

*The python script can be downloaded from GitHub: https://github.com/ShivaniSemwal/FCS_analysis/blob/main/FCS_data_analysis.py and modify it according to your requirements.

****Important:** Python IDE used is Spyder and the script is compatible with Python version 3.11. Tkinter is used for developing GUI, make sure tkinter is installed properly on your computer/laptop.

Go to Tools → Preferences→IPython console→Graphics, change **Backend to Qt5** (image below) and apply changes. This will plot graphs in a separate window and not in the terminal.



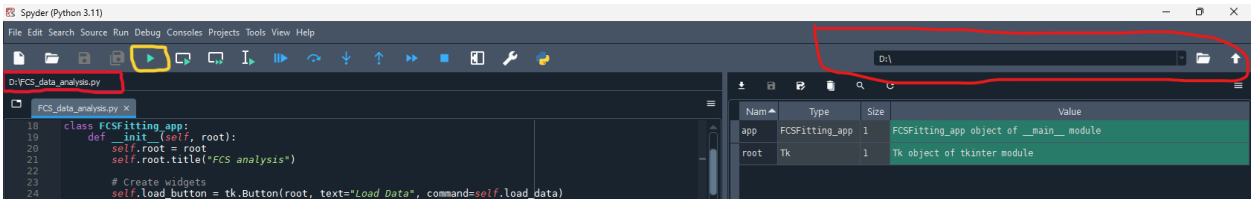


Image 1

After running the script, you will see a GUI shown in **Image 2.**

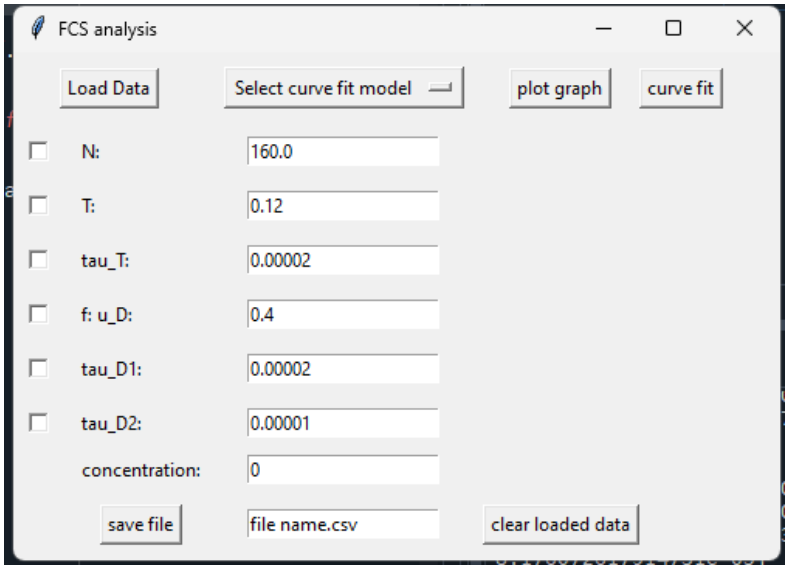


Image 2

Each feature of the GUI is discussed below:

1. **Load Data:** This feature loads the file to be analysed. It can load single as well as multiple files together (**Image 3**).

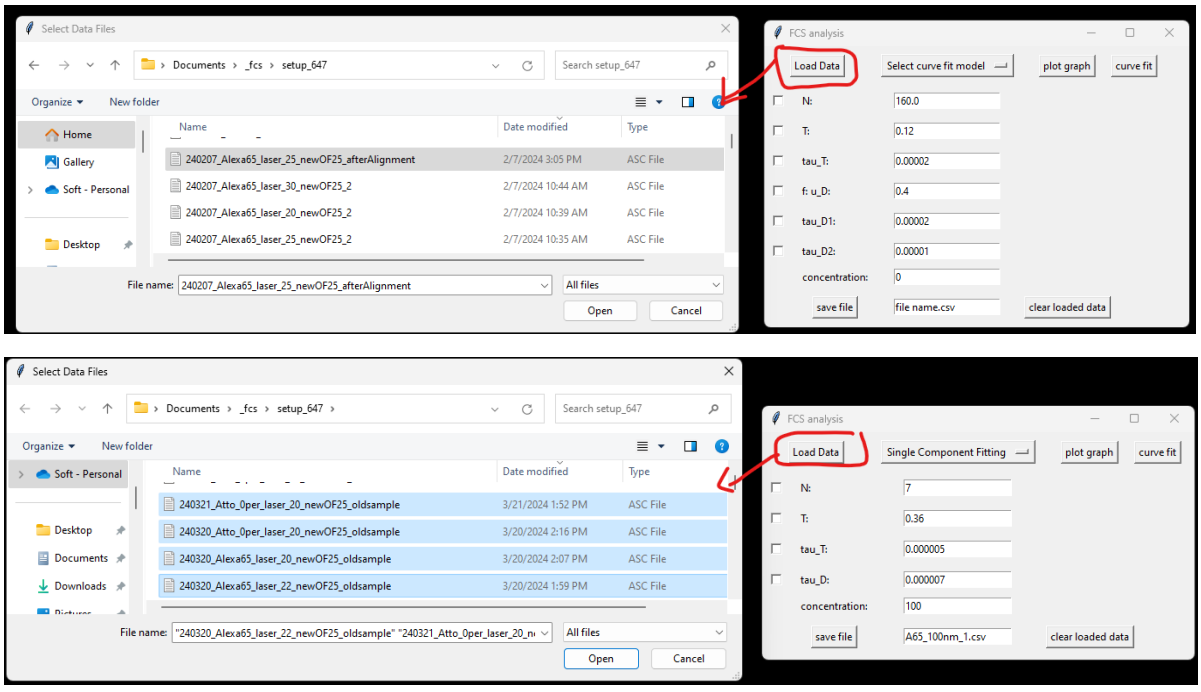


Image 3

2. **Plot Graph:** If you just want to visualize the data (after experiments), first load data (**Image 3**) and then click **Plot Graph.** You will see a plot of selected data (**Image 4**)

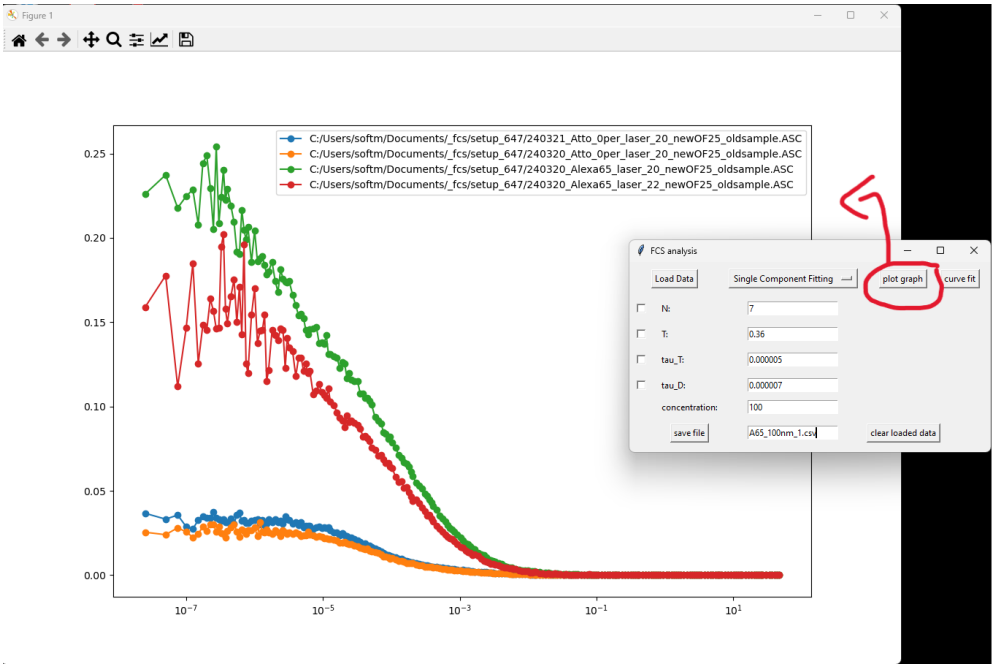


Image 4

Note: a. To plot different data, close the plot and **load data** again >> **plot graph**. All the stored variables or files are cleared after graphs are plotted. **b.** Updated version of the script plots normalized data, check comment in **line#448, 563, 601**.

3. **Select Model for curve fitting:** Load the data, and then select the appropriate fitting model as shown in **Image 5 (left)**. Default initial guess for fitting parameters is already set that can be changed depending on your experimental data (**Image 6**).

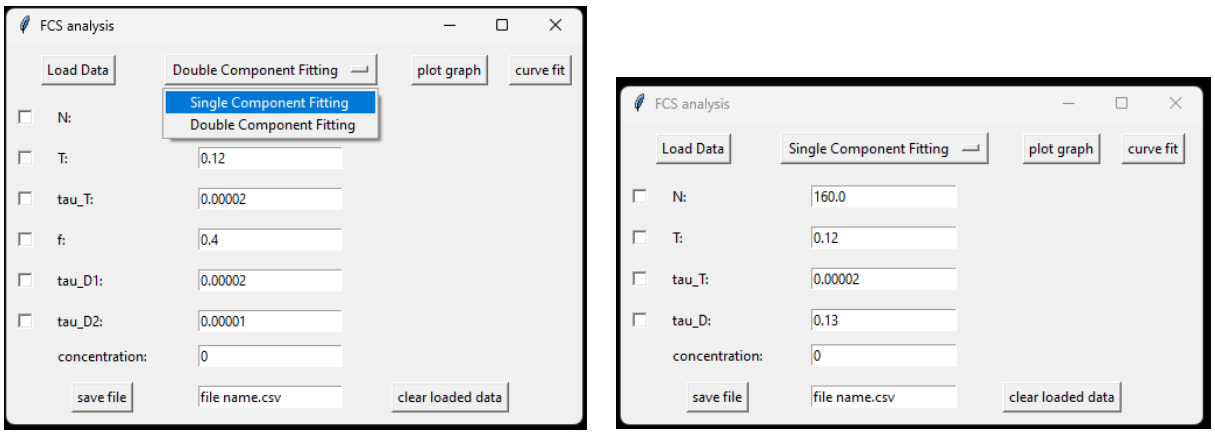


Image 5

Single Component Fitting:

$$(1+\left(\frac{T}{1-T}\right))e^{-\frac{\tau}{\tau_T}} * \frac{1}{N}\left[\left(\frac{1}{1+\frac{\tau}{\tau_D}}\right)\left(\sqrt{\frac{1}{1+\frac{\tau}{R^2\tau_D}}}\right)\right]$$

Double Component Fitting:

$$(1+\left(\frac{T}{1-T}\right)e^{-\frac{\tau}{\tau_T}}) * \frac{1}{N}\left[f * \left(\frac{1}{1+\frac{\tau}{\tau_{D1}}}\right)\left(\sqrt{\frac{1}{1+\frac{\tau}{R^2\tau_{D1}}}}\right) + (1-f) * \left(\frac{1}{1+\frac{\tau}{\tau_{D2}}}\right)\left(\sqrt{\frac{1}{1+\frac{\tau}{R^2\tau_{D2}}}}\right)\right]$$

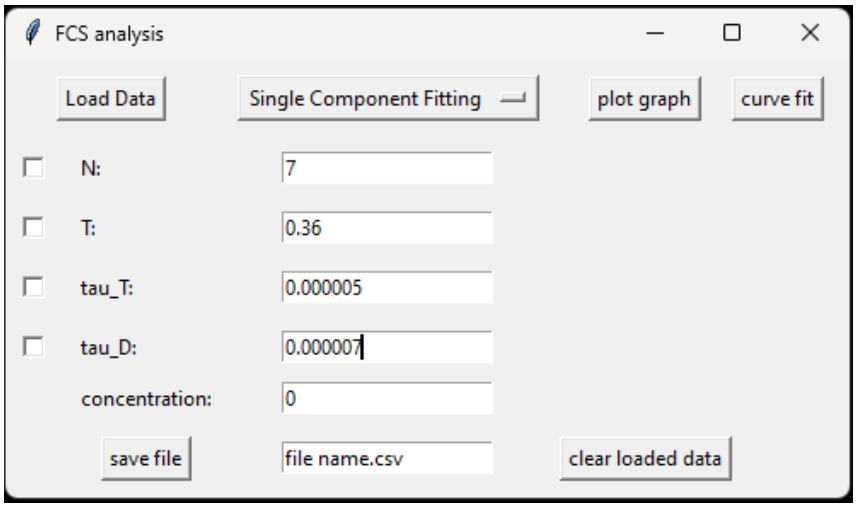
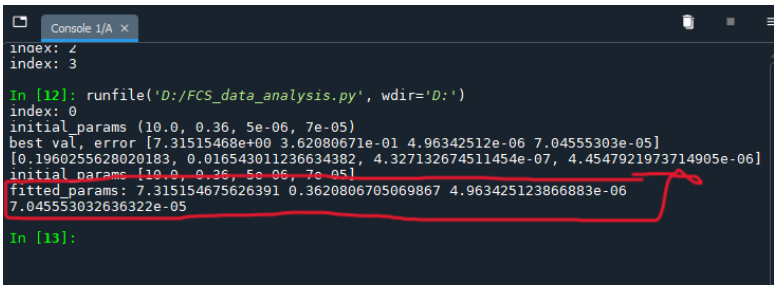


Image 6

4. **Curve Fit:** After loading data, selecting curve-fit model, and setting initial guesses for curve fit parameters, click **curve fit (Image 7)** to see how good the curve fitting looks. If you are happy with the curve fit and fitting parameters, save the graph (**Image 8, Left**) and save results (**Image 10**). Fitting parameters can be viewed at python terminal (**highlighted in Red, image below**).



If fitting is not good, set different initial guesses and click **curve fit**. Keep repeating it till you get the satisfactory results. Once you get the good results, close the graph, click **clear loaded data** (bottom right of the GUI) and re-**load** the same data again and then **curve fit**. Now, you can save the plot and fitting parameters.

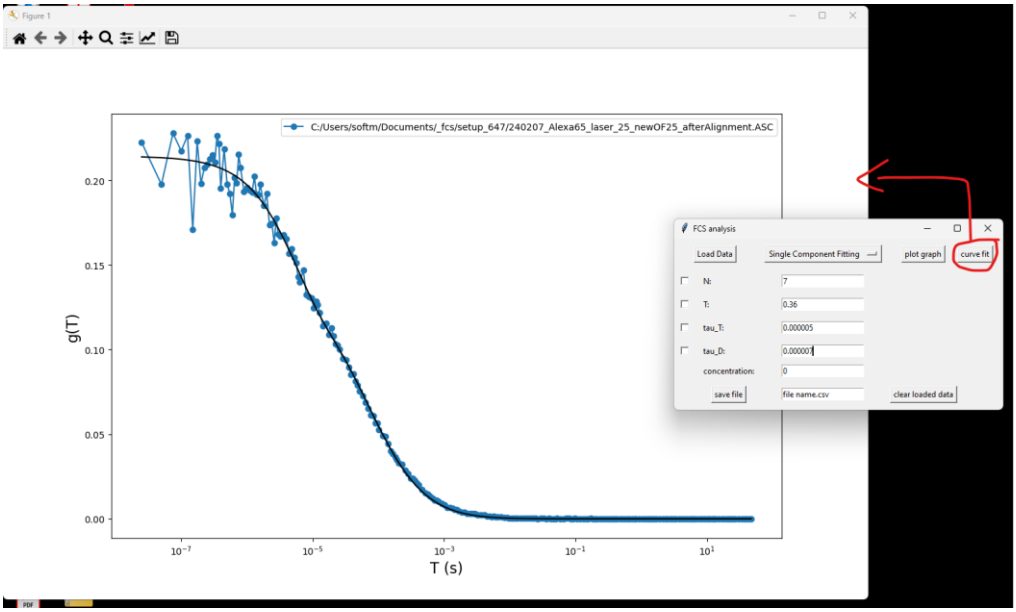
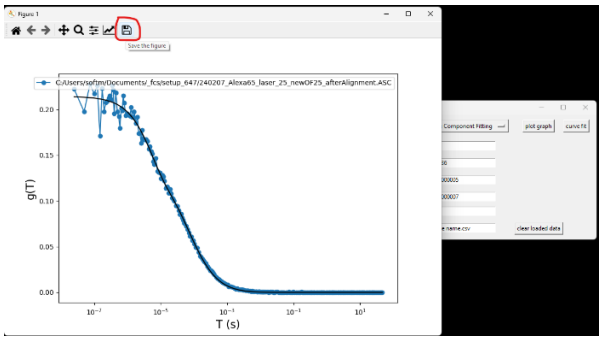


Image 7

Note: Always close the plot and click **clear loaded data** before loading a different or new dataset for curve-fit analysis.

5. **Save and Close the plot:** Plots can be saved directly from the toolbar as shown in **Image 8 (Left)** and closed by clicking "X" at the top right of the figure **Image 8(Right).**



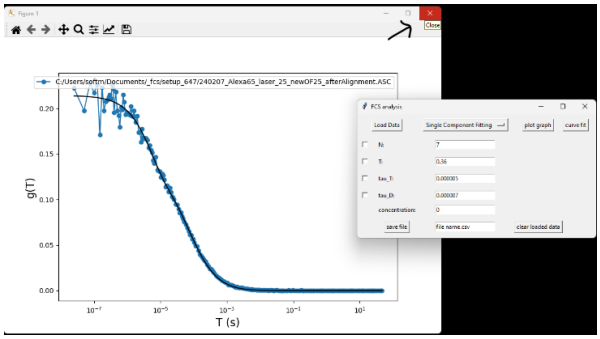


Image 8

6. **Save Results to one file:** If you are saving results from single dataset, enter the concentration of your sample. For example, I have entered 100 because concentration of my sample is 100nM. Let say, you want to save results from 4 datasets having same concentration (100nM), then either enter 100,100,100,100 or 1,2,3,4 in the editable **concentration** field of the GUI (**Image 9**).

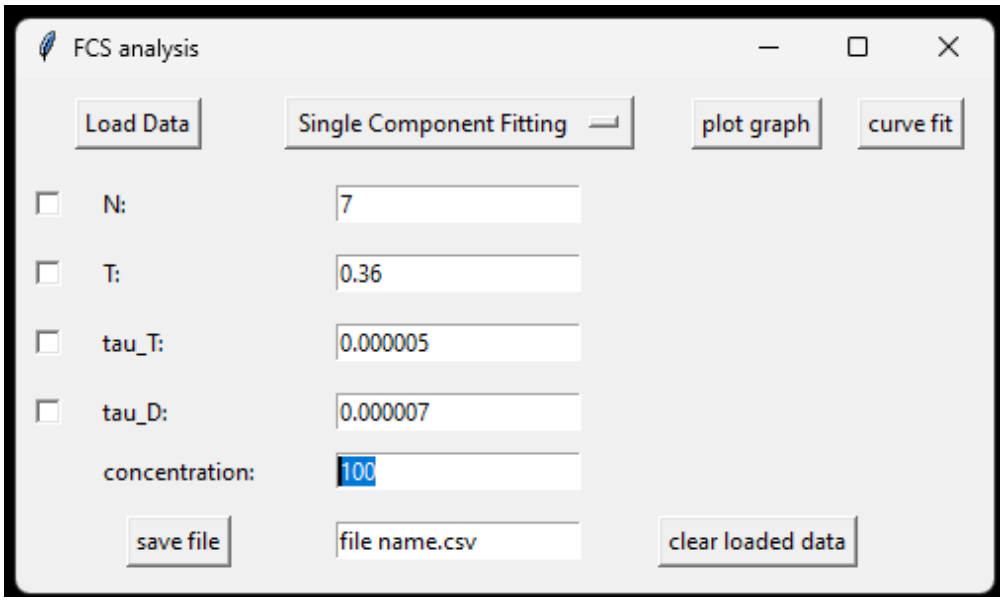
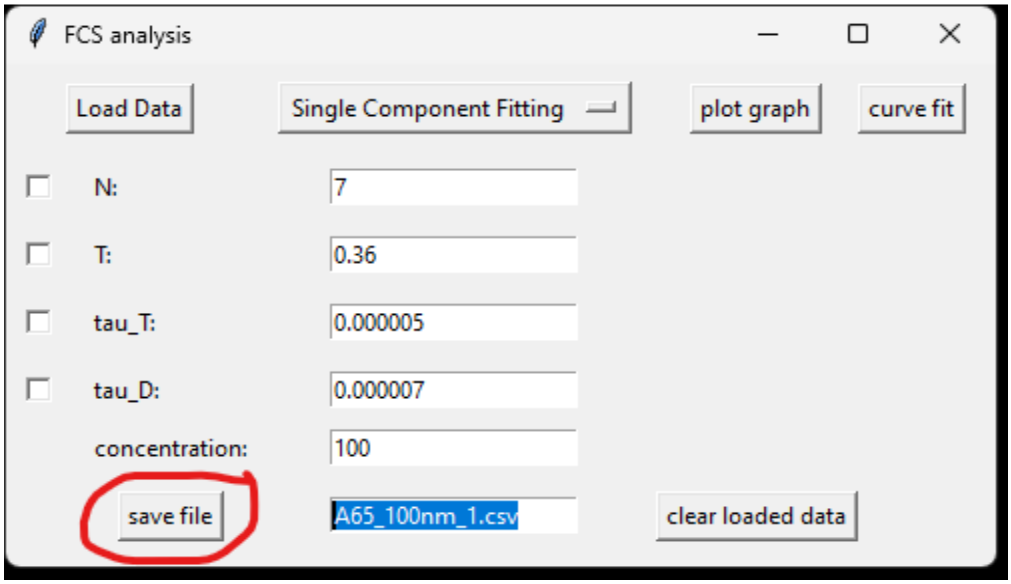


Image 9

Now, enter the appropriate filename in the **save file** editable field for saving the results and then click **save file** (**Image 10, Top**). File will be saved in a .csv format in the selected directory (**highlighted red, Image 1**) and saved file can be viewed in excel or python. It saves all the fitting parameters including concentration, total countrate from both the channels and uncertainties (**Image 10, Bottom**).



conc(nM)	Total_countrate (KHz)	N	T	tau_T	tau_D	errN	errT	errtau_T	errtau_D
100	62.99	7.3	0.36	4.96e-06	7.05e-05	0.2	0.02	4.33e-07	4.45e-06

Image 10

7. **Hold values:** If you want to fix the value of a particular fitting parameter, checkmark the square boxes (highlighted red, **Image 11**). In the image below, T is held to a value 0.36. Multiple parameters can be held to a value by checking the square boxes next to the parameters.

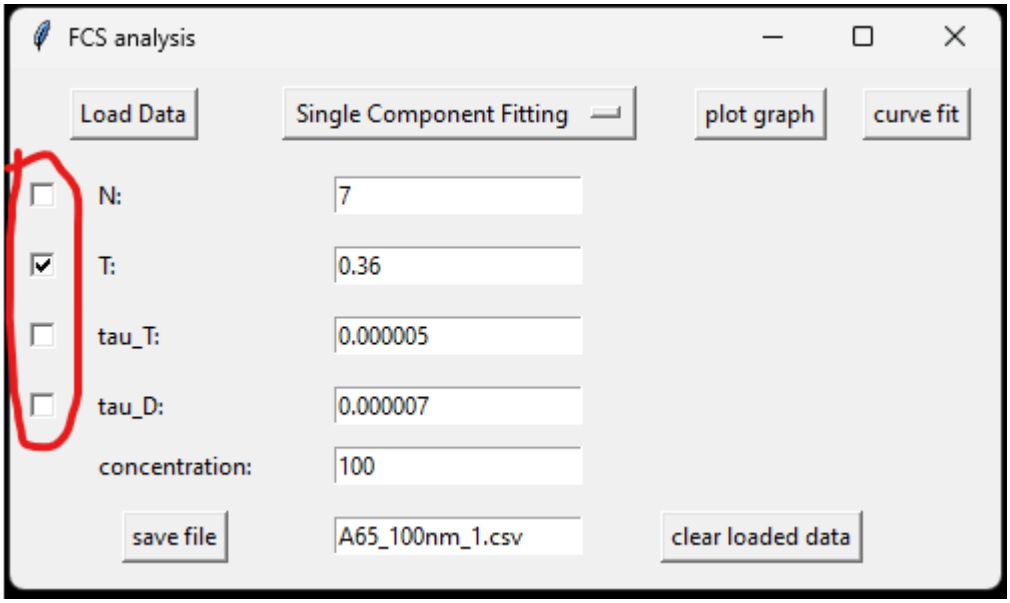


Image 11

Note: To edit **Start** and **End** points of the datasets, make changes in the lines# shown below,

For .ASC files: Line#571, Start index#0 and End is 500. Modify it according to your data.

```
    self.start_end_point.append([0,500]) # set start and end point of the dataset to be analysed;
```

For .SIN files: Line#603, Start index#85 and End is 450. Modify it according to your data.

```
self.start_end_point.append([85,450])  # set start and end point of the dataset to be analysed;
```