NATIONAL UNIVERSITY OF SINGAPORE

FCS Plotter

An application for plotting correlation functions

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Instructions manual created by Rutuparna Kulkarni.
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1 Overview

FCS Plotter is a desktop application for viewing the correlation functions and fit-parameter maps produced by Imaging FCS. It reads the .xlsx files that Imaging FCS exports and lets you plot correlation curves, overlay their fits, normalize them for comparison, and generate colour-coded maps of fit parameters such as the number of particles (N) or the diffusion coefficient (D).

FCS Plotter does not calculate correlations or perform fitting - that is done in the ImFCS plugin in Fiji (more details at https://www.dbs.nus.edu.sg/lab/BFL/imfcs_image_j_plugin.html). FCS Plotter is a viewer and figure-preparation tool for data that has already been analysed.

A version for Apple computers is available separately, named FCS Plotter_for_Apple Macbook.

Running the application: To run FCS Plotter, first copy the application file to a local folder on your computer (for example, to the Desktop or Program Files), then open it from there.

2 Loading data

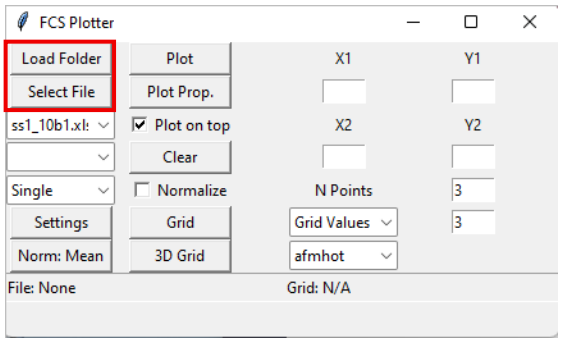

Figure 1. *The FCS Plotter control panel. Data is loaded with the Load Folder and Select File buttons at the upper left; the plotting controls occupy the centre and right of the panel; the status line at the bottom shows the current file.*

1. **Load Folder** - choose the folder containing your .xlsx files. The program scans the folder and lists the files that contain correlation data.
2. **Select File** - then choose a file from the dropdown that appears below the buttons.

The status line at the bottom of the panel displays the loaded file name once a file has been read successfully.

When scanning a folder, FCS Plotter identifies usable files by looking for the lag-time data; folders with no such files produce the message “No compatible files found.” Files produced by ImFCS batch processing normally contain all the required sheets, so batch-processed files can be used directly.

3 Choosing what to plot

Two dropdown menus control the content of a plot.

3.1 Data type (sheet)

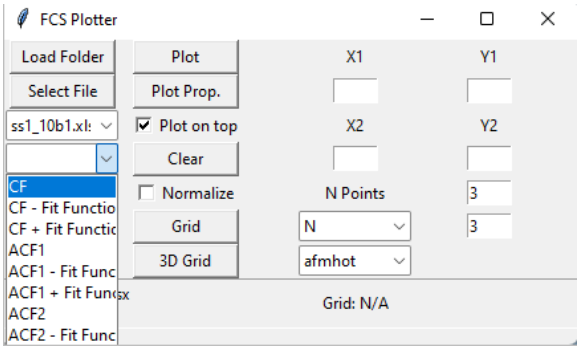

Figure 2. *The data-type dropdown, listing CF, ACF1, ACF2 and their fit-function variants.*

The first dropdown selects which correlation to display:

- **CF** - the raw correlation function.
- **CF - Fit Functions** - only the fitted curves.
- **CF + Fit Functions** - the data and the fit overlaid, drawn in two colours.
- **ACF1**, **ACF2**, and their fit variants - the two autocorrelation channels, with the same three options each.

3.2 Mode (how many pixels)

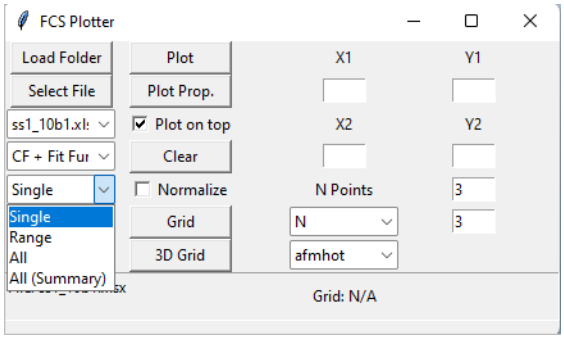

Figure 3. *The mode dropdown, offering Single, Range, All and All (Summary).*

- **Single** - a single pixel. Enter its column and row in the X1 and Y1 boxes.
- **Range** - a rectangular region. Enter one corner in X1, Y1 and the opposite in X2, Y2.
- **All** - every valid (successfully fitted) pixel.
- **All (Summary)** - the mean curve with a shaded band showing the minimum and maximum at each lag time.

After making these selections, click **Plot**. Use **Plot on top** to overlay the new curves on the existing figure, or **Clear** to remove all curves from it.

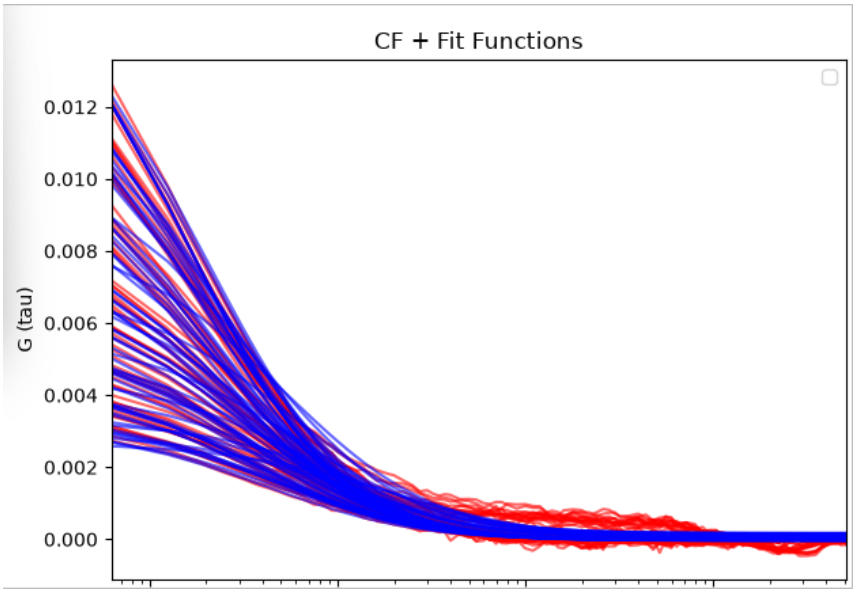

Figure 4. *All mode with CF + Fit Functions selected: every fitted pixel is drawn, data in red and fits in blue. The y-axis shows the correlation amplitude and the x-axis the lag time in seconds.*

4 Normalizing curves

Normalization rescales each curve so that curves of different amplitude can be compared by shape. Tick the **Normalize** box, then use the **Norm** button at the lower left to choose the method. The button toggles between two options:

Method	Divides each curve by	Result
Norm: Mean	the average of its first few points	every curve starts at 1
Norm: G(0)	the fitted zero-lag amplitude (1/N, triplet-corrected)	curves start near 1

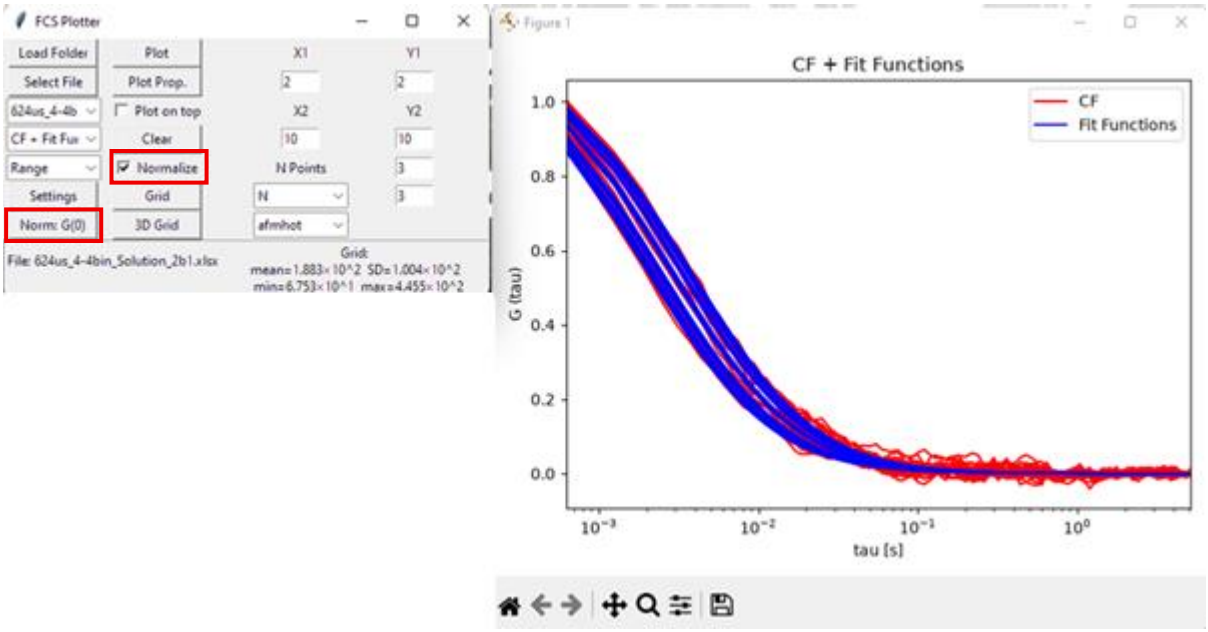

Figure 5. *The same range with Normalize switched on and the Norm: G(0) method selected. Each curve is scaled by its fitted amplitude, so the curves are brought to a common height of about one.*

The **N Points** boxes set how many points are averaged at the start of the curve and at its baseline.

The two methods answer different questions. **Mean** forces every curve to begin at exactly 1, which is convenient for comparing shapes directly. **G(0)** instead shows each curve relative to its fitted amplitude: a curve that begins below 1 indicates that its measured signal is lower than the fit predicted. Curves under the G(0) method therefore do not all begin at exactly 1, and this spread is meaningful - it reflects the agreement between each measured curve and its fit. Pixels that were not fitted are left unnormalized under the G(0) method.

5 Summary view

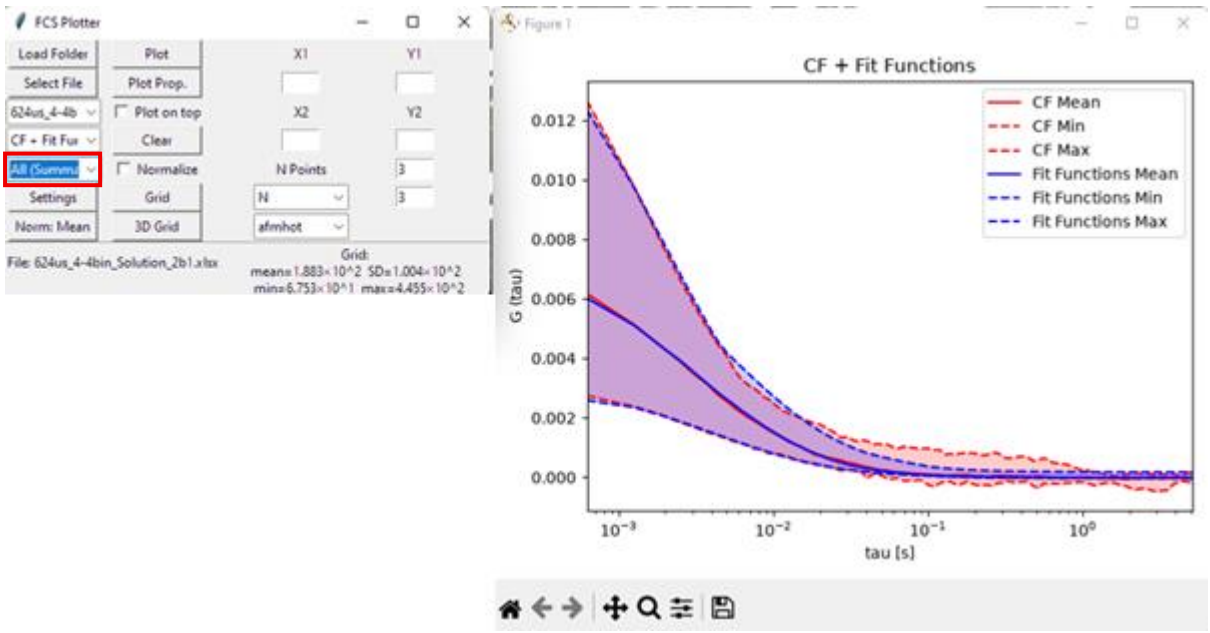

Figure 6. *All (Summary) mode. For each of the data and the fit, a mean curve is drawn together with a shaded band spanning the minimum and maximum value at every lag time.*

For a region or whole image, **All (Summary)** is usually clearer than plotting every curve. It draws the mean curve together with a shaded band spanning the minimum and maximum value at each lag time, shown separately for the data and for the fit functions.

6 Adjusting a plot's appearance

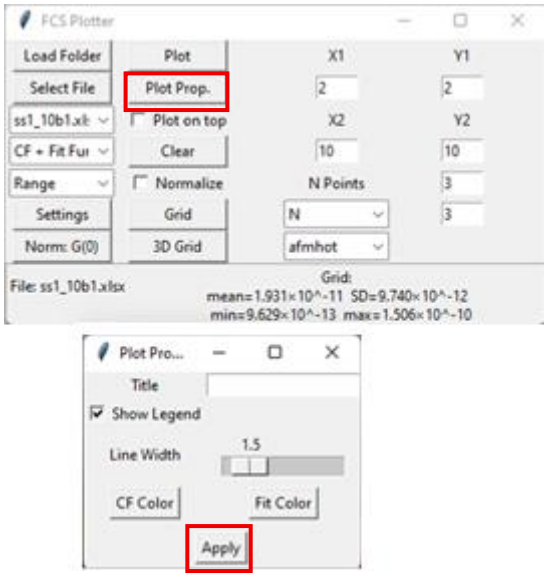

Figure 7. *The Plot Properties window, reached with the Plot Prop. button.*

Click **Plot Prop.** to open the plot-properties window, where you can set the title, show or hide the legend, adjust the line width, and choose the colours used for the data and fit curves. Click **Apply** to redraw the figure with the new settings.

7 Reading the axes

- **x-axis** - lag time τ in seconds on a logarithmic scale.
- **y-axis** - the correlation amplitude G(τ), shown as decimal values.

8 Working within a plot window

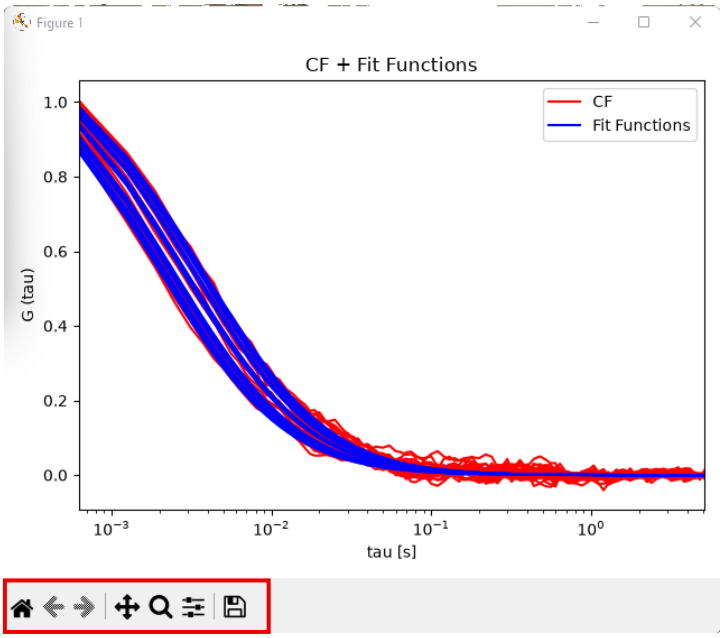

***Figure 8**. The toolbar on the graph shows a set of small icons.*

Each plot opens in its own window with a toolbar of controls along the bottom. These operate on the figure itself and are useful for inspecting the data closely:

- **Zoom** - click the magnifier icon, then left-click and drag a rectangle over the region you want to enlarge; the plot zooms to that area.
- **Pan / Move** - click the move icon (the crossed arrows), then left-click and drag to slide the view around while keeping the zoom level.
- **Back** - returns to the previous view; useful for stepping back after zooming or panning.
- **Forward** - moves to the next view after using Back.
- **Home** - resets the plot to its original, full view.
- **Save** - saves the current figure as an image file. A dialog lets you choose the location, file name, and image format; several formats are available, including PNG, PDF, SVG, and JPEG.

These controls do not change the underlying data; they only change how the figure is displayed.

9 Parameter maps

FCS Plotter can display any fitted parameter as a spatial map across the imaged region.

1. Choose the parameter from the metric dropdown (for example N, D, G, vx, or F2).
2. Click **Grid** for a two-dimensional heat map, or **3D Grid** for a surface plot. A colour scheme can be selected from the colour dropdown (for example afmhot).

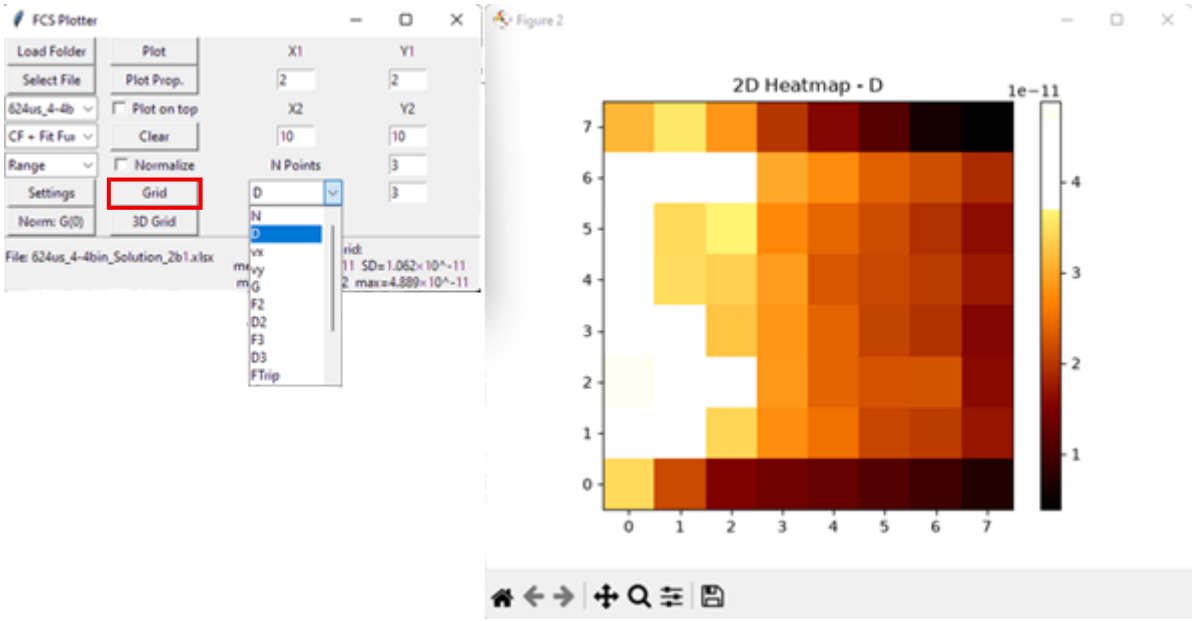

Figure 9. *A two-dimensional heat map of the particle number N. Each cell is one analysed pixel, coloured by its fitted value; the colour bar at the right gives the value scale.*

The map is sized automatically from the pixel coordinates in the file, so it always matches the actual region of interest, whether square or rectangular.

The colour bar is scaled automatically between the smallest and largest value present in the map, and the status area reports the exact mean, standard deviation, minimum, and maximum. Because the scale is recalculated for each map, the same colour represents different values in different maps; always read the numerical values rather than comparing colours between plots.

9.1 Three-dimensional maps (3D Grid)

The 3D Grid button shows the same parameter as a surface instead of a flat map. It uses the same grid as the 2D heat map - one analysed pixel per cell, sized automatically to the region of interest - but plots each value as a height, so the shape of the parameter across the sample is easier to see. The height and the colour both represent the selected parameter, and the colour scheme is the one chosen in the colour dropdown.

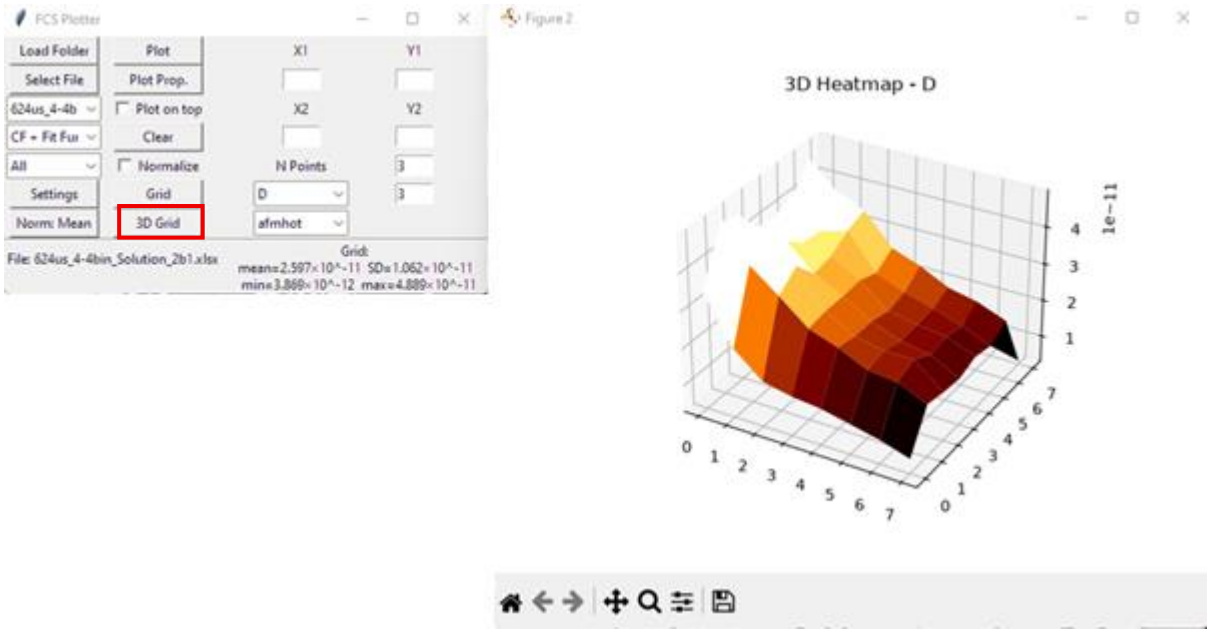

Figure 10. *A three-dimensional surface map of the diffusion coefficient D for the same region shown in Figure 9. Height and colour both represent the fitted value at each pixel.*

The surface can be rotated: click and drag inside the plot to view it from a different angle, which helps in judging gradients and outliers. As with the 2D map, the height scale is set automatically from the data, so surfaces from different measurements are not directly comparable by height alone - read the value scale.

10 Limiting the lag-time range

FCS Plotter can restrict the plot to a chosen range of lag times, so that only part of the correlation curve is shown - for example the early decay, or a window that excludes the noisy

long-time tail. The range is set with the **tau from / to (s)** checkbox and the two boxes beneath it.

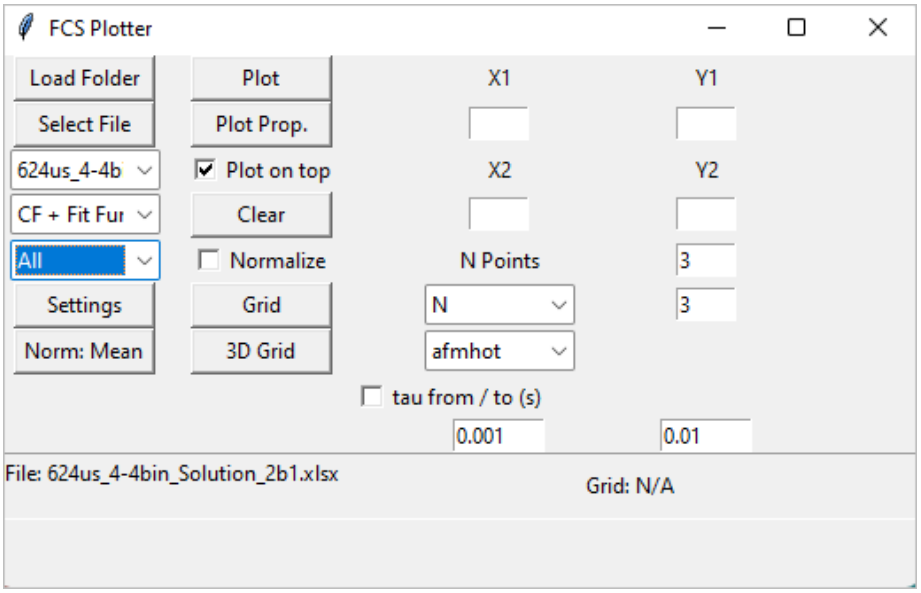

Figure 11. *The lag-time range controls. The tau from / to (s) checkbox enables the limit; the two boxes below set the start and end lag times in seconds.*

Tick the **tau from / to (s)** checkbox, then type the start and end lag times, in seconds, into the two boxes below it (for example 0.001 and 0.01). Either box may be left blank for an open end: an empty first box starts from the beginning of the curve, and an empty second box continues to the end.

The limit is applied when the plot is drawn, so after ticking the checkbox or changing the values you must click **Plot** again to update the graph. To return to the full curve, clear the checkbox and click **Plot** once more. The limit applies to every plot mode and to all curve types, including the fit functions.

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