User Guide: GPU-Accelerated fcsSOFI GUI

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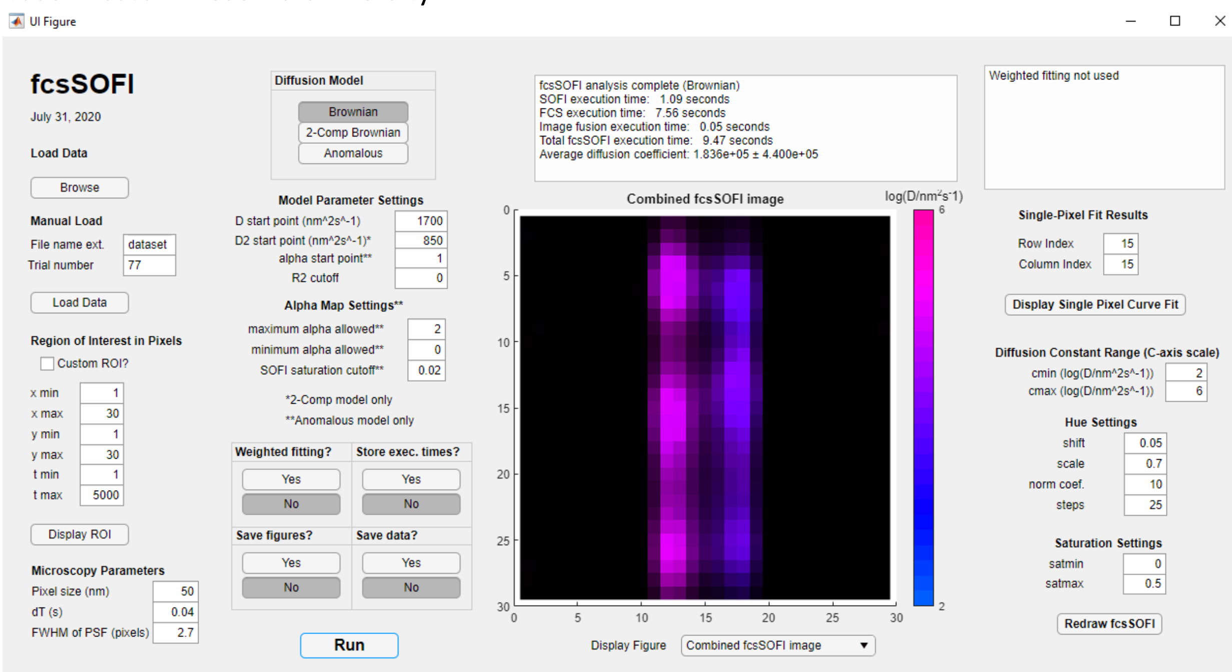


Overview

fcsSOFI combines the correlation techniques of fluorescence correlation spectroscopy (FCS) and super-resolution optical fluctuation imaging (SOFI) to produce super-resolution spatial maps of diffusion coefficients. This has been applied thus far to understand diffusion and spatial properties of porous and complex materials, including agarose and PNIPAM hydrogels, liquid crystals, liquid-liquid phase-separated polymers, and biomolecule condensates. The GPU-accelerated fcsSOFI GUI is designed to make the analysis accessible with a reasonable analysis time so that other scientists can apply fcsSOFI to their data.

More details can be found at: Kisley, L.; Higgins, D.; Weiss, S.; Landes, C.F.; et al. Characterization of Porous Materials by Fluorescence Correlation Spectroscopy Super-resolution Optical Fluctuation Imaging. ACS Nano 2015, 9, 9158–9166. doi:10.1021/acsnano.5b03430. PMID 26235127.

The GPU-accelerated portions of the GUI and script use Gpufit, an open source software library for GPU-parallelized curve fitting developed by Mark Bates, Adrian Przybylski, Björn Thiel, and Jan Keller-Findeisen. The relevant 2017 Scientific Reports publication describing Gpufit can be found here: https://www.nature.com/articles/s41598-017-15313-9#Ack1 and the Gpufit homepage can be found here: https://github.com/gpufit/Gpufit

System Requirements

- NVIDIA graphics card
- CUDA Toolkit version 6.5 or later (https://developer.nvidia.com/cuda-toolkit)
- MATLAB version 2012a or later
- C/C++ compiler

NOTE: CUDA is Nvidia's proprietary GPU-computing development environment and CUDA-based applications, like Gpufit, cannot be run on non-Nvidia hardware (e.g., AMD, Intel graphics cards). A Nvidia brand graphics card is required to run the GPU-parallelized fcsSOFI GUI/script.
At this time, there is no option to operate the fcsSOFI GUI completely serially/on a CPU. A toggle switch between GPU and CPU fitting is a feature that will be added in a future release.

Files and folders maintained on the fcsSOFI GitHub repository:

- README: Description of the fcsSOFI repository (README.md)
- fcsSOFI User Guide - a detailed guide for the fcsSOFI GUI (fcsSOFI_user_guide.pdf, this document!)
- GPU-accelerated fcsSOFI GUI (fcsSOFI_GUI.mlapp)
- GPU-accelerated fcsSOFI script (fcsSOFI_script.m)
- Test data set: simulation of two pores with emitters undergoing Brownian diffusion with D = 10^5 nm^2/s in the left pore and D = 10^4 nm^2/s in the right pore (dataset77.mat, uses default values of GUI, see Figure 1 of the *ACS Nano* fcsSOFI report)
- Colorscale script: Change fcsSOFI colorscale external to GUI (SOFI_ColorScale.m)
- Folder containing external functions called in script (fcsSOFI_External_Functions)
- Customized Gpufit library (Gpufit_build-64-YYYYMMDD)

Instructions for Use

1. Prepare Data for Analysis

- For now, the fcsSOFI GUI only accepts data packaged in .mat files. I recommend manipulating and saving your data in MATLAB and not some other data processing environment (Excel for instance).
- Your data must be in the format of a 3-dimensional single or double array (i.e. 512x512x1000 single). This array should form a "movie" which fcsSOFI can analyze pixel-by-pixel and frame-by-frame over time. The first two dimensions of your array should contain the spatial information of a given frame (the image) and the last dimension should order each frame in time. Each frame should be separated by the same time step dT.

NOTE: To be analyzed by this preliminary GUI, your 3D array must be named 'Data', the default name for an imported array in MATLAB.

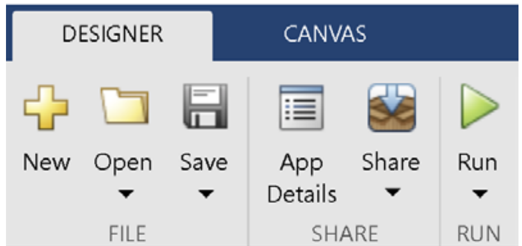
dataset77.mat (MAT-file)

Name	Value
Data	30x30x5000 double

We are working to make the code agnostic to the specific array name, but for now, the array must have the name 'Data'. If this is not the case, redefine your movie as 'Data' in MATLAB and save your .mat file again. Your .mat file can contain other arrays/components/data than just your 'Data' array - the GUI extracts the array 'Data' automatically from any .mat file.

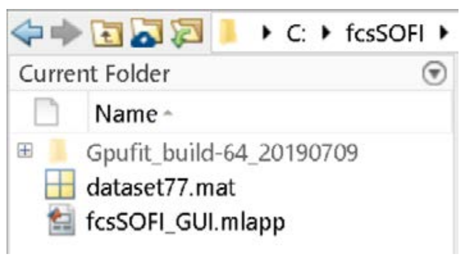
2. Open GUI

- From your file explorer or from the 'Current Folder' side panel in MATLAB, simply double click on the fcsSOFI_GUI MATLAB App file. The GUI will open in one of two ways: immediately ready for use in its own window or via the App Designer editor. If the App Designer editor opens, the GUI can be accessed by pressing the 'Run' button found at the top left of the editor window.



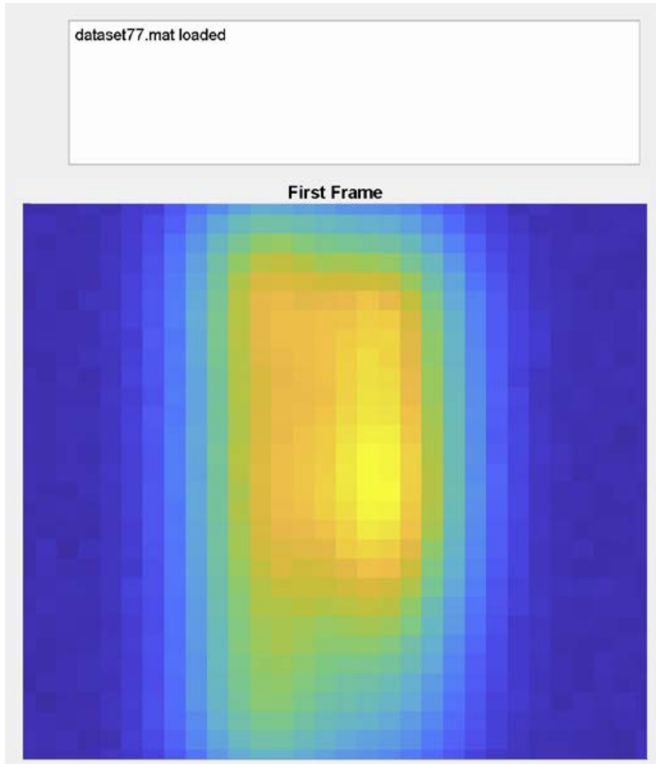
- Alternatively, the GUI can be run by dragging and dropping the fcsSOFI_GUI.mlapp file from the 'Current Folder' side panel into the MATLAB command window, or by using MATLAB's 'run' function within the command window.

NOTE: Make sure the GUI (fcsSOFI_GUI.mlapp), the custom Gpufit library folder (Gpufit_build-64_20190709), and your data exist in the same directory, and make that you are working in that directory in MATLAB.



3. Load Data

- Data can be loaded in two ways: either with the "Browse" button or the "Manual Load" text-input boxes. The Browse button opens your file explorer, from which you can select a .mat data file. If you know the file name (and the file is in the same directory as the fcsSOFI GUI), you can manually input that name as a combination of the "File name ext." and "Trial number" inputs. For example, the inputs File name ext. = 'dataset' Trial number = 4 Loads the file 'dataset4.mat'
- After you load the data with either 'Browse' or 'Load Data' buttons, the first frame of your 'Data' array should appear on the GUI axis.



- From here you can choose a specific region of interest (ROI) with the input boxes under 'Region of Interest in Pixels.' The x and y inputs crop the image on a specific range of pixels, and the t inputs select specific frames. Click 'Display ROI' to display the ROI on the first frame.
- The 32x32x5000 simulated data file 'dataset77.mat' is included in this repository. The default inputs to 'File name ext.' and 'Trial number' are already set to load 'dataset77.mat'. As long as your MATLAB path is set to the extracted zip folder for the fcsSOFI repository, you can just press 'Run' right away and the GUI will load and analyze the array contained in 'dataset77.mat'.

4. Run fcsSOFI analysis

- Once your data is loaded, simply press the 'Run' button and the fcsSOFI analysis will commence. Once complete, the fcsSOFI GUI will print execution times in the top white panel and the combined fcsSOFI image will display on the GUI axes. Toggle through the figures with the 'Display Figure' dropdown menu below the axes.

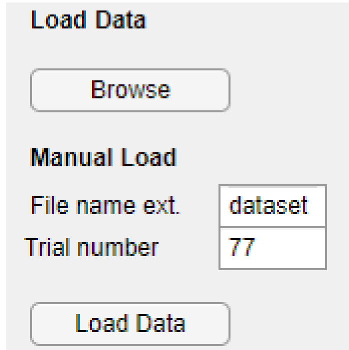
Description of GUI Inputs and Components

The inputs and UI components of the fcsSOFI GUI are listed and described below.

Pre-Processing User Inputs and Components for Running fcsSOFI

Load Data and Manual Load

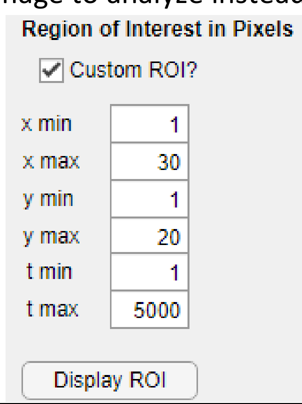
Allows user to load data in one of two ways: either "Browse" button or the "Manual Load" text-input boxes that converts file name ext. and trial number into a convolved string.



GUI Input/Component	Description	Default
Browse (button)	Opens file explorer and allows user to select a .mat data file	N/A
File name ext.	Specifies generic file name common to your data files (i.e dataset)	dataset
Trial number	Specifies data file corresponding to specific trial number	77
Load Data (button)	Manually load data file based on File name ext. and Trial number inputs	N/A

Region of Interest in Pixels

Allows user to select a custom area of image to analyze instead of the full image.

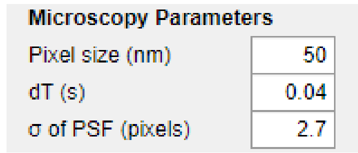


GUI Input/Component	Description	Default
xmin	Minimum horizontal position/pixel of ROI	1
xmax	Maximum horizontal position/pixel of ROI	30
ymin	Maximum vertical position/pixel of ROI	1
ymax	Minimum vertical position/pixel of ROI	30

tmin	First frame to analyze	1
tmax	Last frame to analyze	5000

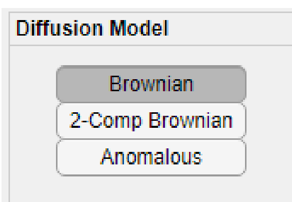
Microscopy Parameters

Physical values from the collected data. Accurate values of pixel size and dT are needed to correctly calculate the diffusion coefficient, while the PSF is used in deconvolution of the super-resolution image.



GUI Input/Component	Description	Default
Pixel size	Size of single pixel in image data in nm	50 nm
dT	Time interval between each frame in seconds	0.04 s
σ of PSF	σ of microscope point spread function in pixels	2.7 pixels

Diffusion Model



Brownian model: $G_2(r,\tau) = a(r)\frac{1}{1+\left(\frac{\tau}{\tau_D}\right)} + b$

Two-component Brownian model:

$$G_2(r,\tau) = a_1(r)\frac{1}{1+\frac{\tau}{\tau_{D_1}}} + a_2(r)\frac{1}{1+\frac{\tau}{\tau_{D_2}}} + b$$

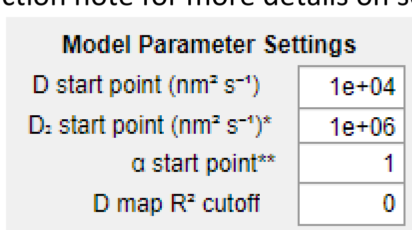
Anomalous model: $G_2(r,\tau) = a(r)\frac{1}{1+\left(\frac{\tau}{\tau_D}\right)^{\alpha}} + b$

Description	Default
Select diffusion curve-fitting model. Toggle between Brownian diffusion, 2-component Brownian diffusion, and Anomalous (Levy flight) diffusion.	Brownian diffusion model

NOTE: The script version of fcsSOFI has the additional option of removing the offset variable “b” in each of the models, since correlation data commonly decays to 0. Fitting with b=0 reduces the number of variables. We have observed this reduces analysis time and, at times, improves accuracy. This will be added as an option in the GUI in a future release.

Model Parameter Settings

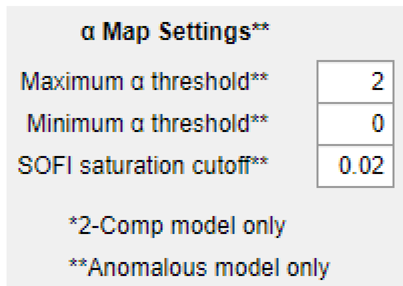
See “Error and Troubleshooting” section note for more details on selecting start points.



GUI Input/Component	Description	Default
D start point	Choose start point for the average diffusion coefficient in nm^2/s	1e4 nm^2/s

D_2 start point	If using the 2-component model, choose start point for the average diffusion coefficient of the second Brownian component	1e6 nm^2/s
α start point	If using the anomalous model, choose start point for the anomalous exponent	1
D map R^2 cutoff	D values from pixels where the fit has an R^2 is lower than the R^2 cutoff are thrown out/not shown in the image. Used as a way to filter poor fits from background pixels where correlation is just noise.	0

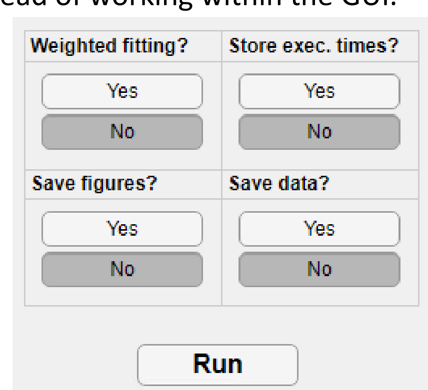
Alpha Map Settings



GUI Input/Component	Description	Default
Maximum α allowed	Maximum value of α to be displayed on the α map (values returned by fitter exceeding this threshold will be zeroed-out)	2
Minimum α allowed	Minimum value of α to be displayed on the α map (values returned by fitter below this threshold will be zeroed-out)	0
SOFI saturation cutoff	Minimum pixel saturation on super-resolution map required to display corresponding pixel in α map. Used as a way to filter background pixels.	0.02

Additional Button Groups and Run Button

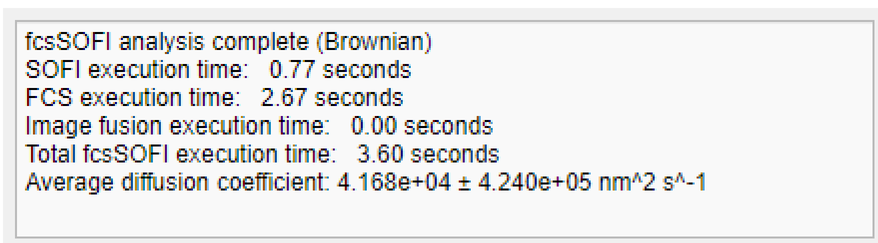
Saved data will be stored in your current directory. We highly recommend saving figures and data for any post-processing of images instead of working within the GUI.



Button Group Name	Description	Default
Weighted fitting?	Select “Yes” to weight each single pixel fit by the amplitude of the raw autocorrelation data	No
Store exec. times?	Select “Yes” to create a text file to remotely store execution times entitled [fcsSOFI_excecution_times.txt]	No
Save figures?	Select “Yes” to save figures as .fig files in a new subfolder titled [filename_figures_MM-DD-YYYY_HH-MM]. This allows for figure editing outside the GUI environment	No
Save data?	Select “Yes” to save analyzed data in a .mat file titled [filename_analyzed_MM-DD-YYYY_HH-MM.mat].	No

Post-Processing Inputs and Components for Display and Manipulation of Completed Analysis

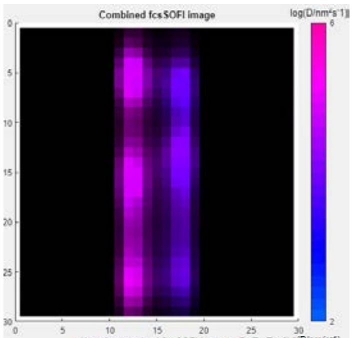
Text Box Area

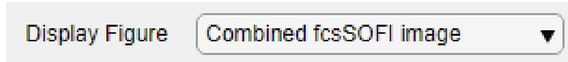


GUI Input/Component	Description	Default
Top text area	Prints completed actions, curve fitting progress from 25% to 100% and a summary of execution times and average diffusion coefficient and anomalous power	N/A
Axes	Displays figures	N/A
Top right text area	Displays curve fit diffusion coefficient estimates, relevant parameters, and goodness of fit indicators	N/A

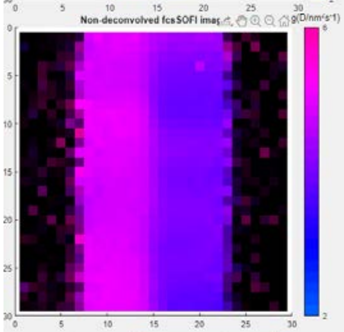
Axes and Display Figure

Axes shows image selected from the dropdown menu to change the displayed figure. (Images shown below for dataset77.mat)

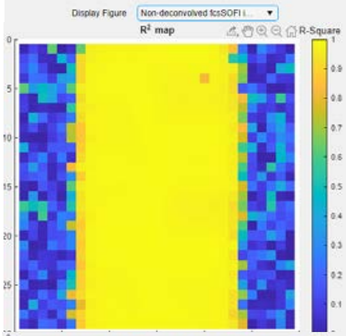




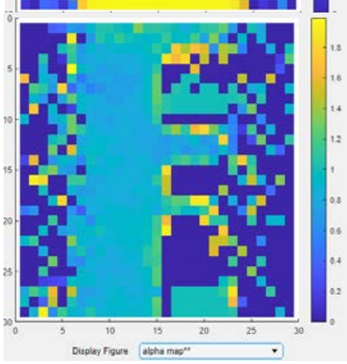
Combined fcsSOFI image – default image with combined fcs hue and SOFI saturation. Includes deconvolution of the microscope point spread function by deconvlucy. Results in resolution improvement of ~2. If two-component Brownian diffusion model is selected, the combined fcsSOFI image is for the first D, while the second D map is combined fcsSOFI image for the second component.



Non-deconvolved fcsSOFI image – does not deconvolve the microscope point spread function. May be useful for data that has feature sizes above the diffraction limit where the deconvolution can introduce artifacts. Results in resolution improvement ~sqrt(2).



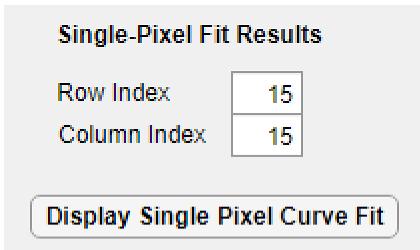
R^2 map – shows goodness of fit metric R^2. Useful to evaluate if there are areas of the image that have incorrect results due to poor curve fits. Areas where diffusing emitters are present should have higher values, while typical values for the background where there is only correlation of noise are <0.5.

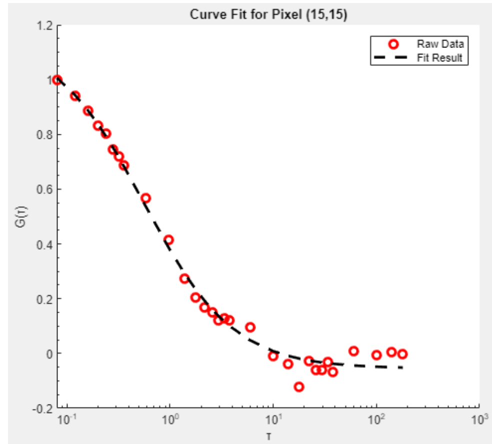


Alpha map – for anomalous diffusion model only. Shows resulting alpha exponent. Diffraction-limited result, but could be combined with SOFI image to produce a super-resolution map of alpha values.

Single Pixel Fit Results

The single pixel fit results allows the user to inspect the data at an individual point in the image. This is useful to compare the curve decay in different areas of the image that may have different diffusion and also to inspect the curve fitting for goodness of fit. The resulting plot shows the normalized correlation versus the lag time in seconds.

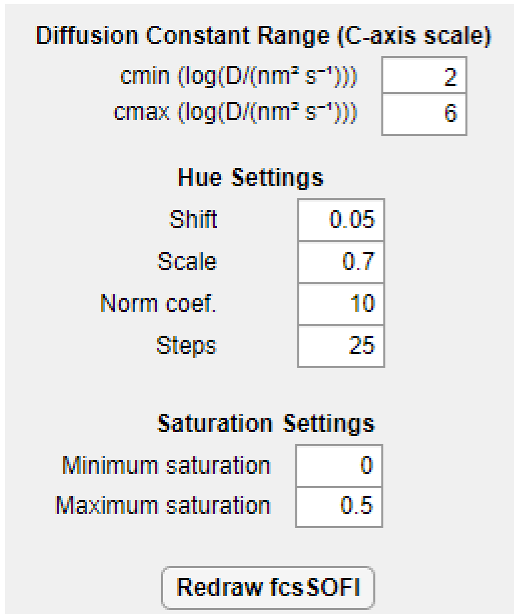




GUI Input/Component	Description	Default
Row index	Select the row index of the desired pixel	15
Column index	Select the column index of the desired pixel	15
Display Single Pixel Curve Fit button	Display visual curve fit results on GUI axis and display curve-fit diffusion coefficient estimates, relevant parameters, and goodness of fit indicators in the top right text area	N/A

Redraw fcsSOFI: Color and Saturation Scaling

fcsSOFI uses a custom script for constructing the colormap so that saturation and hue indicate physical values related to the curve shape magnitude and diffusion coefficient, respectively. Therefore, values in shift and scale may result in drastically different colorscales. The GUI allows for quick changes and inspection, but we recommend editing colormaps with saved data outside of the GUI environment for working with large datasets or preparing images for publications.



GUI Input/Component	Description	Default
cmin	Minimum log(D) displayed on the fcsSOFI image	2
cmax	Maximum log(D) displayed on the fcsSOFI image	6
Shift	Shift the section of the color bar used	0.05
Scale	Scale factor for range of colors used in color bar	0.7
Norm coef.	Normalization coefficient for log(D) data	10
Steps	Number of steps between the minimum and maximum	25
Minimum saturation	Minimum SOFI saturation included	0
Maximum saturation	Maximum SOFI saturation included (lowering this value will highlight low intensity data, raising it to 1 will show the entire range of data)	0.5

Common Issues and Troubleshooting

Error: Any error with “removed from MATLAB’s search path” indicates that you are using the GUI without having the folder it is in as MATLAB’s current path.

```
Error using fcsSOFI_GUI_WS20200720/BrowseButtonPushed
Method 'BrowseButtonPushed' is not defined for class 'fcsSOFI_GUI_WS20200720' or is removed from MATLAB's
search path.

Error using matlab.ui.control.internal.controller.ComponentController/executeUserCallback (line 335)
Error while evaluating Button PrivateButtonPushedFcn.
```

```
Error using fcsSOFI_GUI_WS20200720/LoadDataButtonPushed
Method 'LoadDataButtonPushed' is not defined for class 'fcsSOFI_GUI_WS20200720' or is removed from MATLAB's
search path.

Error using matlab.ui.control.internal.controller.ComponentController/executeUserCallback (line 335)
Error while evaluating Button PrivateButtonPushedFcn.
```

Solution: Either add the folder containing the GUI files to your current search path with the MATLAB function “addpath” or change your Current Folder to

Error: Messages that have to do with array bounds/index sizes are due to challenges of the GUI analyzing datasets of different sizes.

```
Index in position 2 exceeds array bounds (must not exceed 86).

Error in fcsSOFI_GUI_WS20200720/RunButtonPushed (line 526)
          app.DataCombined=app.DataCombined(app.ymin:app.ymax,app.xmin:app.xmax,app.tmin:app.tmax);

Error using matlab.ui.control.internal.controller.ComponentController/executeUserCallback (line 335)
Error while evaluating Button PrivateButtonPushedFcn.
```

Solution: Close the GUI and reopen it again to load a new dataset of a different size.

Error: GUI is unable to load the data you are trying to load.

```
Unrecognized function or variable 'Data'.

Error in fcsSOFI_GUI_WS20200720/BrowseButtonPushed (line
1648)
          app.DataCombined=Data;

Error using matlab.ui.control.internal.controller.ComponentController/executeUserCallback (line 335)
Error while evaluating Button PrivateButtonPushedFcn.
```

Solution: Change the name of your 3D data matrix to “Data” and resave your file as a new .mat file to load

Error: Displaying that an index is out of bounds

```
Index in position 1 exceeds array bounds (must not exceed 50).

Error in fcsSOFI_GUI/RunButtonPushed (line 533)
          app.DataCombined=app.DataCombined(app.ymin:app.ymax,app.xmin:app.xmax,app.tmin:app.tmax);
Error using matlab.ui.control.internal.controller.ComponentController/executeUserCallback (line 309)
Error while evaluating Button PrivateButtonPushedFcn.
```

Solution: Ensure that xmax/xmin/ymax/ymin are not larger than the images. Also, make sure that the “Custom ROI?” box is checked.

Error: Displaying that PSF size exceeds image size

```
Error using deconvlucy>parse_inputs (line 286)
In function deconvlucy, size of the PSF must not exceed
the image size in any nonsingleton dimension.

Error in deconvlucy (line 105)
    parse_inputs(varargin{:});

Error in fcsSOFI_GUI/RunButtonPushed (line 585)
            app.filtim=deconvlucy(app.im,PSF); % Based on
            Geissbuehler bSOFI paper

Error using matlab.ui.control.internal.controller.ComponentController/executeUserCallback (line 309)
Error while evaluating Button PrivateButtonPushedFcn.
```

Solution: The size of the PSF is 20px by 20px, so the minimum ROI size is also 20px by 20px. A simple black border can be applied to datasets smaller than that.

Error: One pixel is a completely different color that doesn't appear on the color scale

Solution: This is almost always due to a failed single-pixel fit rather than a physical phenomenon. This can usually be fixed by adjusting initial guesses for D and/or α. See note on the importance of picking good initial guesses.

Error: Lots/most of the pixels seem to be a color not on the color scale

Solution 1: The R-squared cutoff is too high, and most of the fits have R-squared values below the threshold. Lowering the R-squared cutoff in the GUI should solve this.

Solution 2: Most of the single-pixel fits are failing; reexamine and adjust the initial guesses for D and/or α. See note on the importance of picking good initial guesses.

Note on the importance of picking good initial guesses:
As it stands now, fcsSOFI is fitting two, three, and four variables (for the Brownian, anomalous, and two-component models, respectively) with the Levenberg-Marquadt algorithm. Picking initial guesses that are as accurate as possible will give the fitter the best chance at converging on parameters that accurately represent the autocorrelation data. For D, being on the right order of magnitude is often enough. For α, having an estimate within 0.1 of the true value is usually enough. Of course, if the fits still don't converge, it's often useful to look at single-pixel fits, and choose new initial guesses from there. Users should be aware that the algorithm has sensitivities to input and the output should not be blindly accepted without critical thought.

Appendix: Adding new fitting equations to fcsSOFI MATLAB script using VisualStudio and CMake

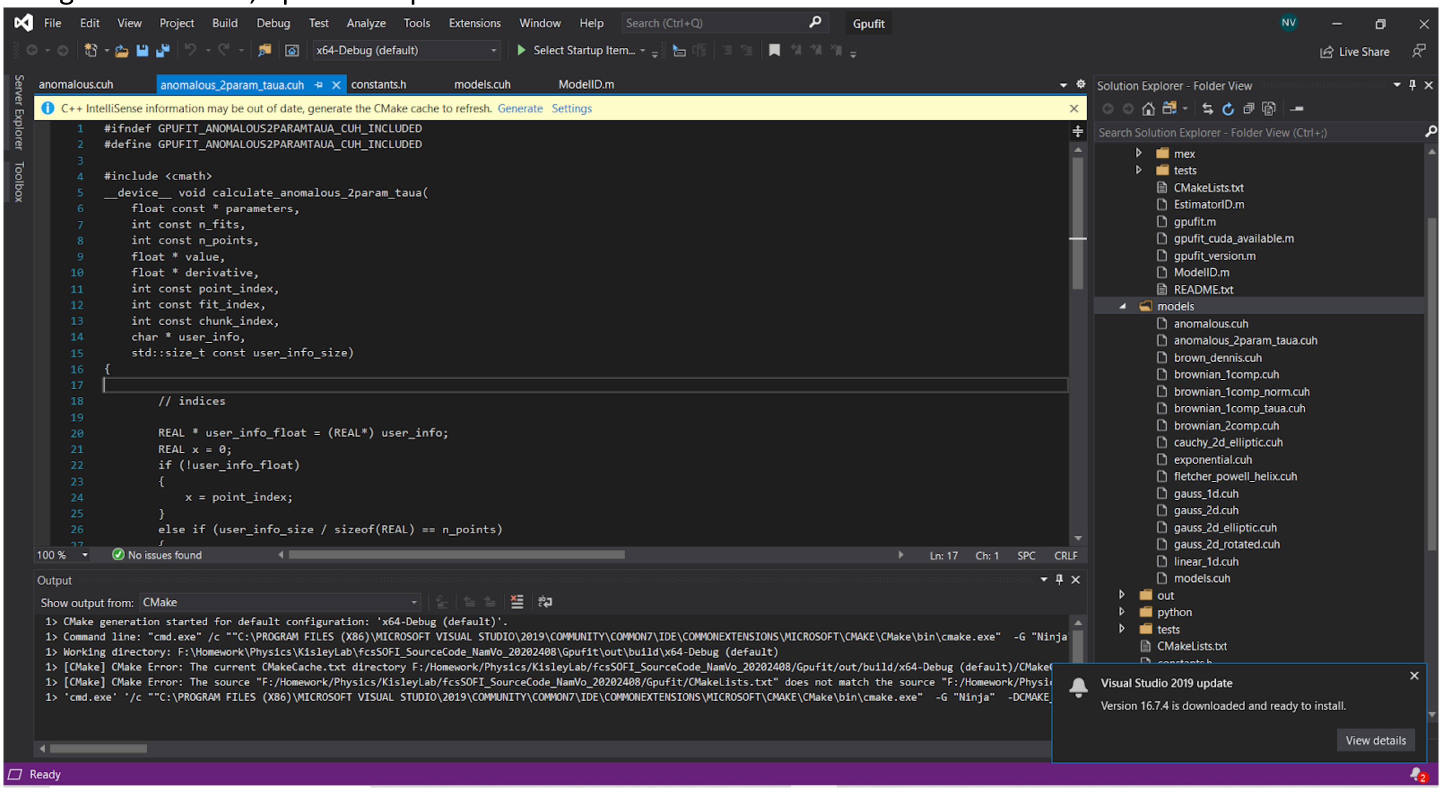
This appendix provides a walk through for adding additional fitting equations for the fcs portion of fcsSOFI to accommodate different diffusion equations. Please refer to the Gpufit homepage for additional information.

Step 1) Download the source code to ensure you are working with a functional version of Gpufit

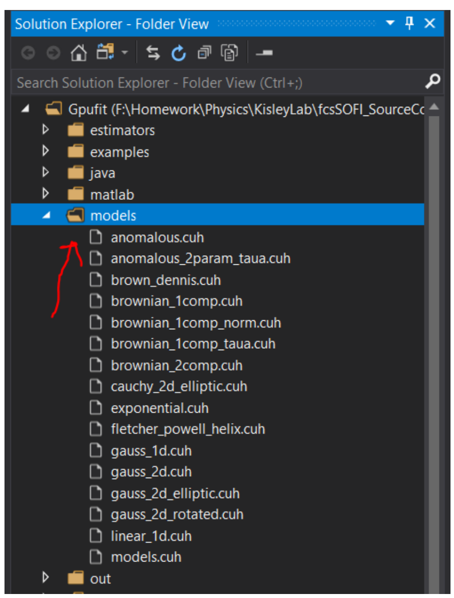
Clean source code from Gpufit: Here

Step 2) Add new equation in Visual Studio

Using Visual Studio, open the Gpufit folder in source code

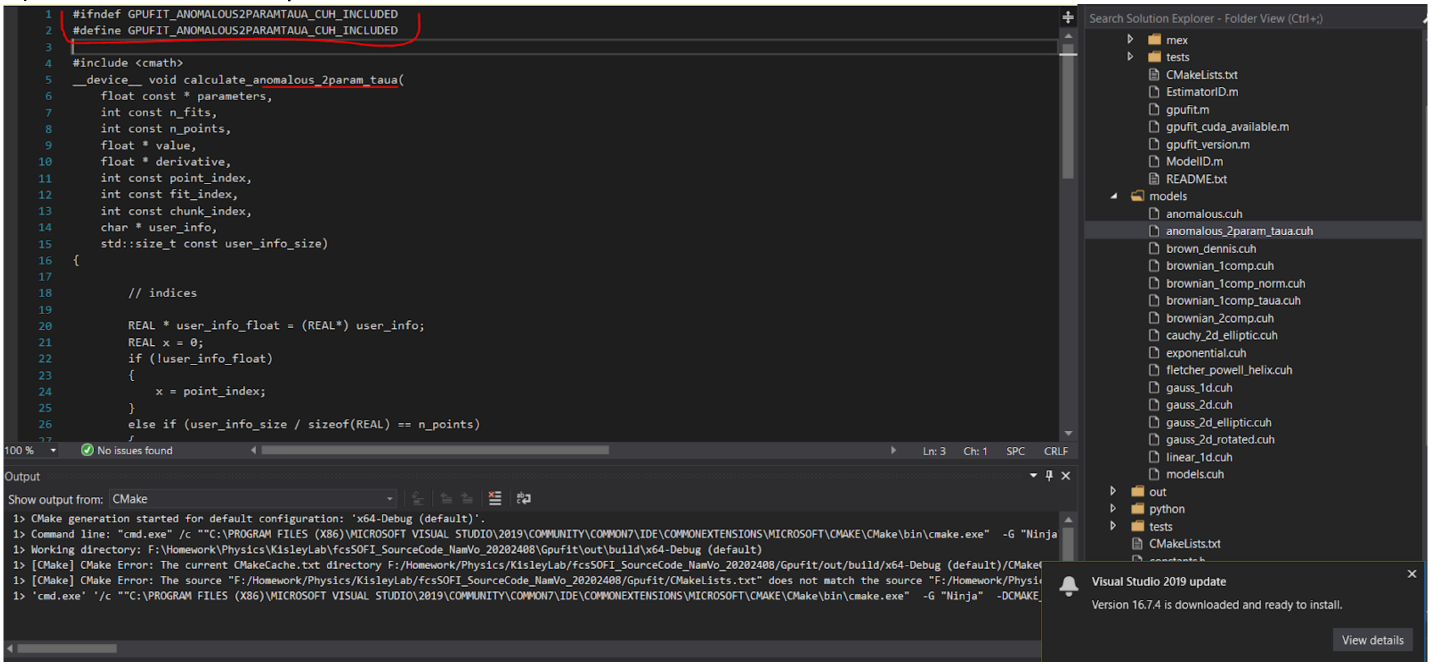


Create a new file in folder "models" with the name XX.cuh, that will be the file which you will include the fitting equation.

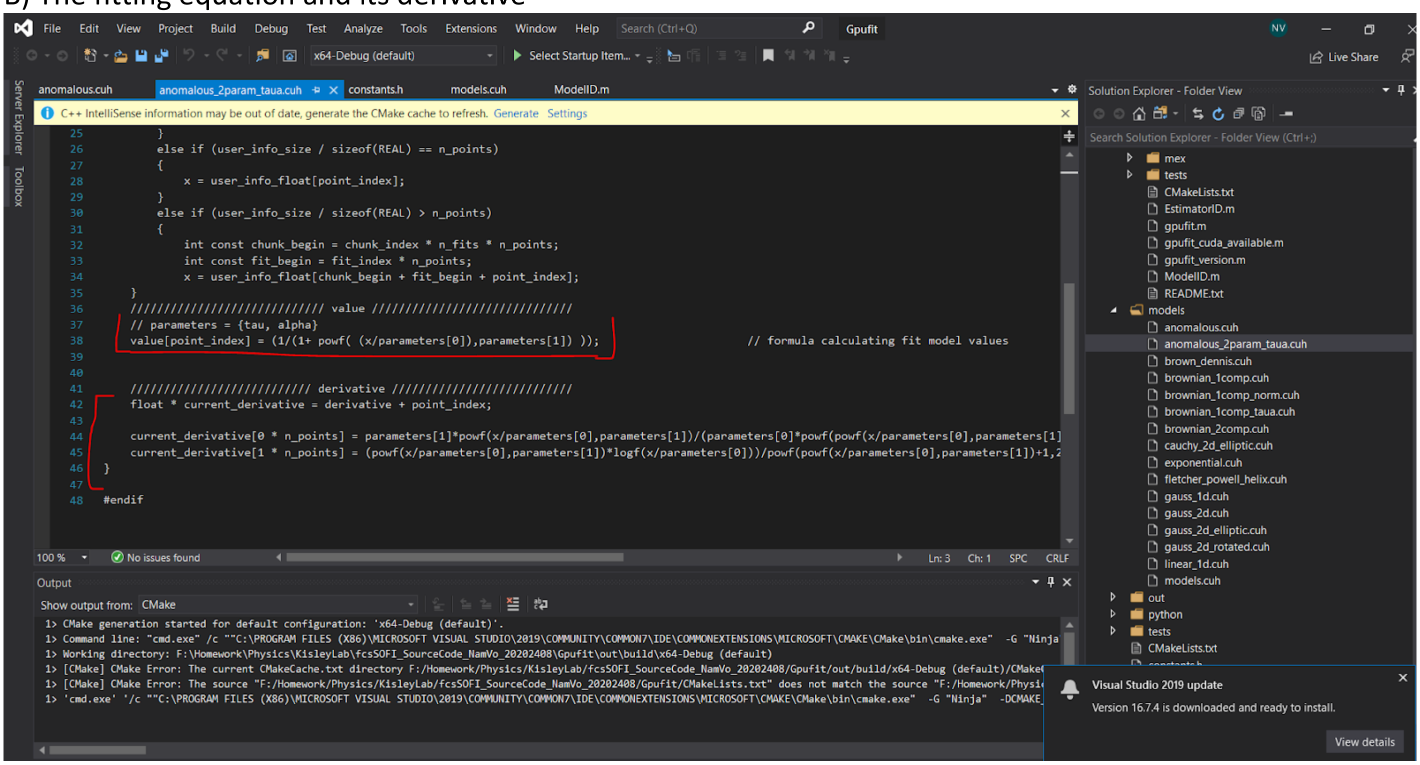


You can copy and paste from previous .cuh files, but remember to change the following (in red):

A) The name of the equation

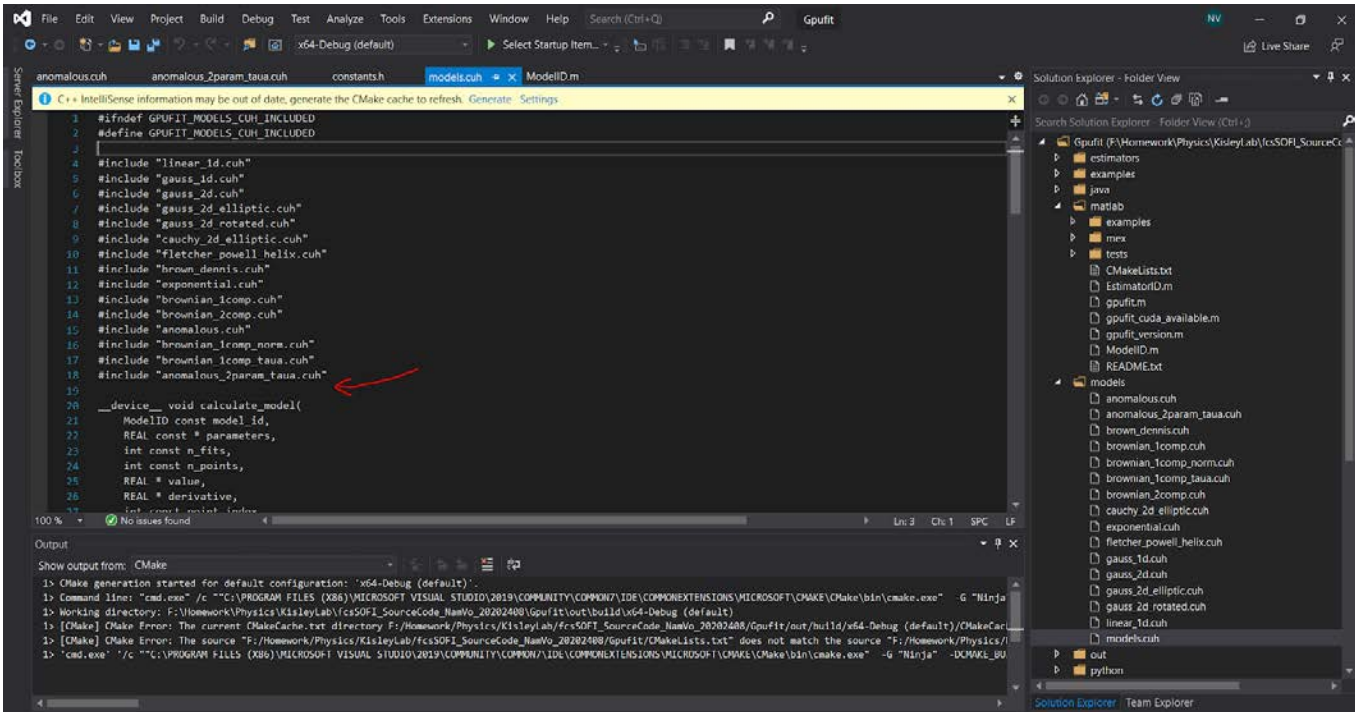


B) The fitting equation and its derivative

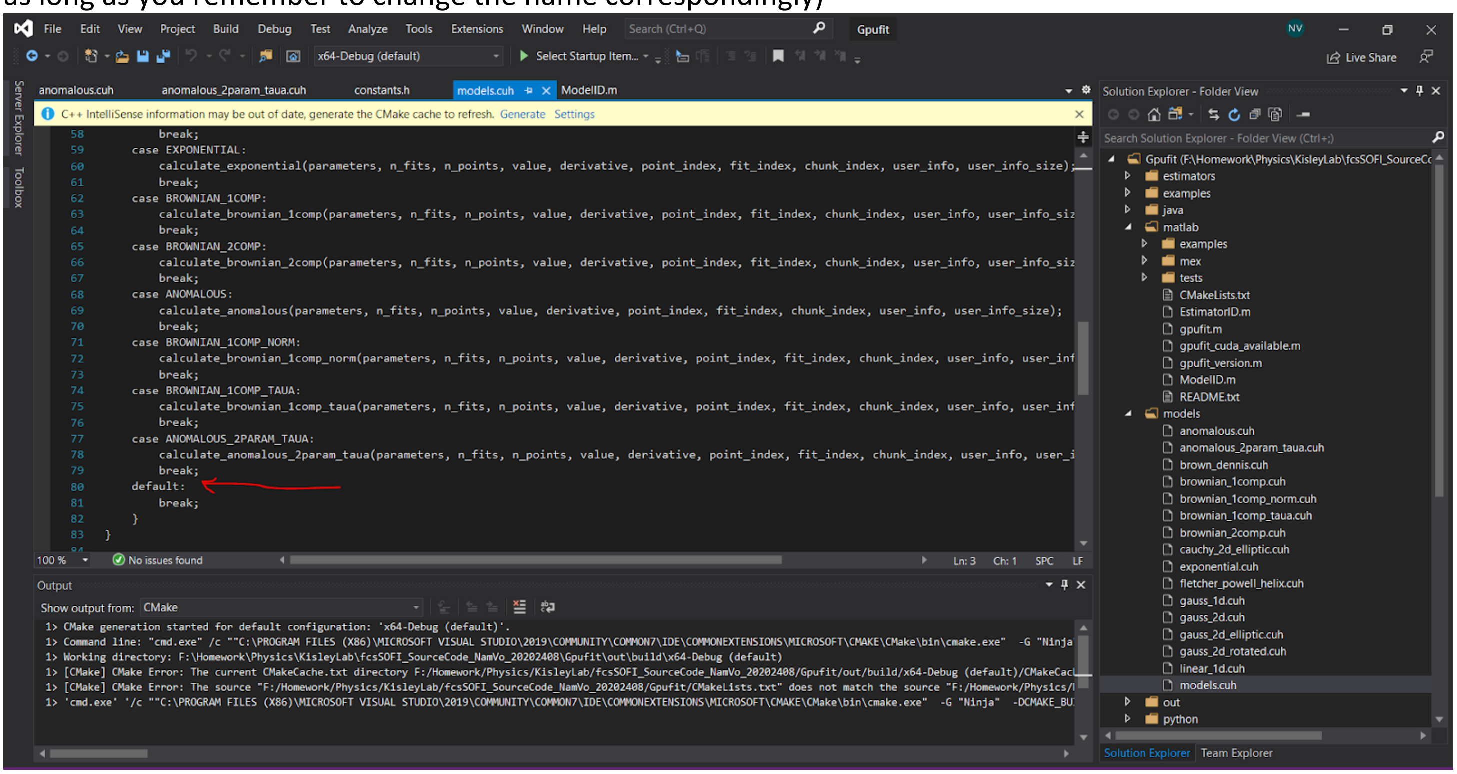


Then remember to update the models.cuh

C) Include the fitting file “XX.cuh” at the start of models.cuh file (as shown where the red arrow is) to “import” your fitting file here:

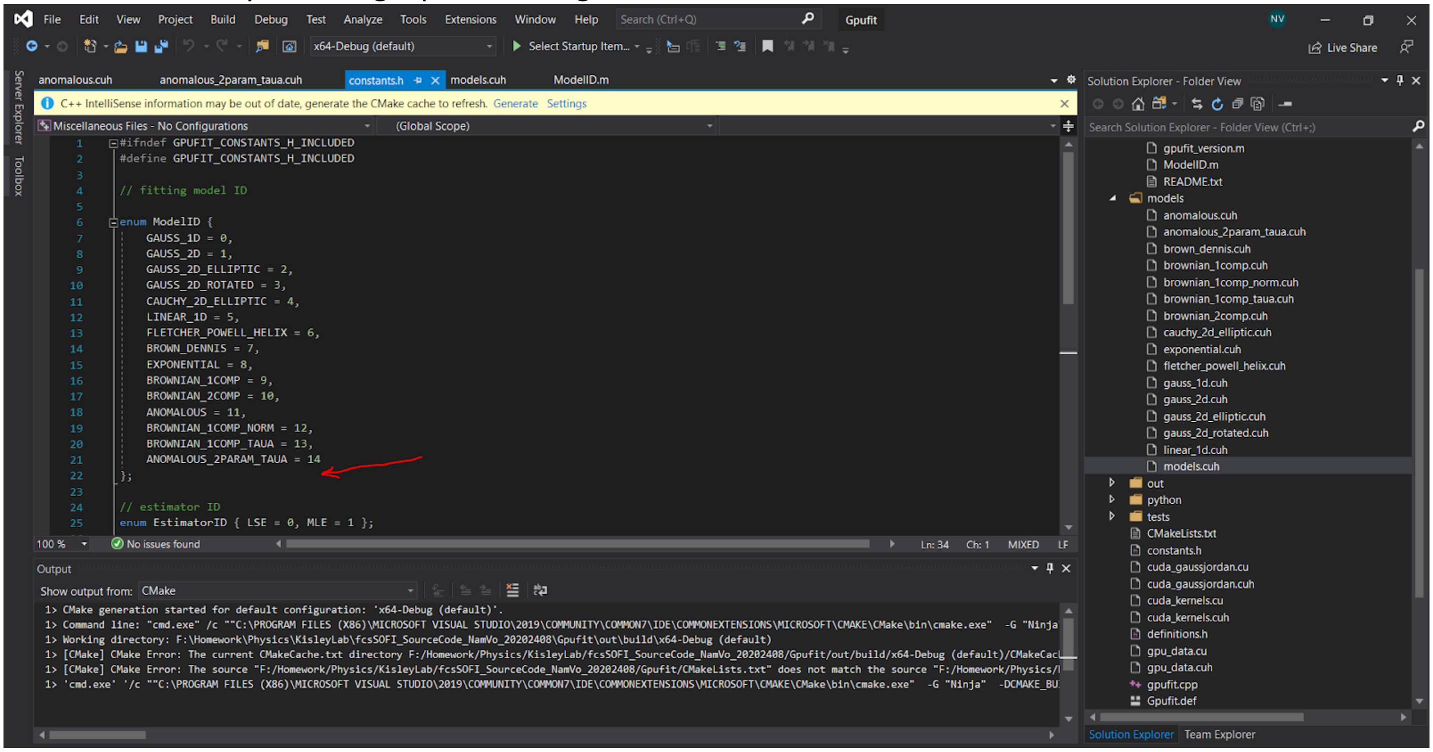


D) Include the new fitting equation where the red arrow is at (you can just copy and paste from previous line, as long as you remember to change the name correspondingly)

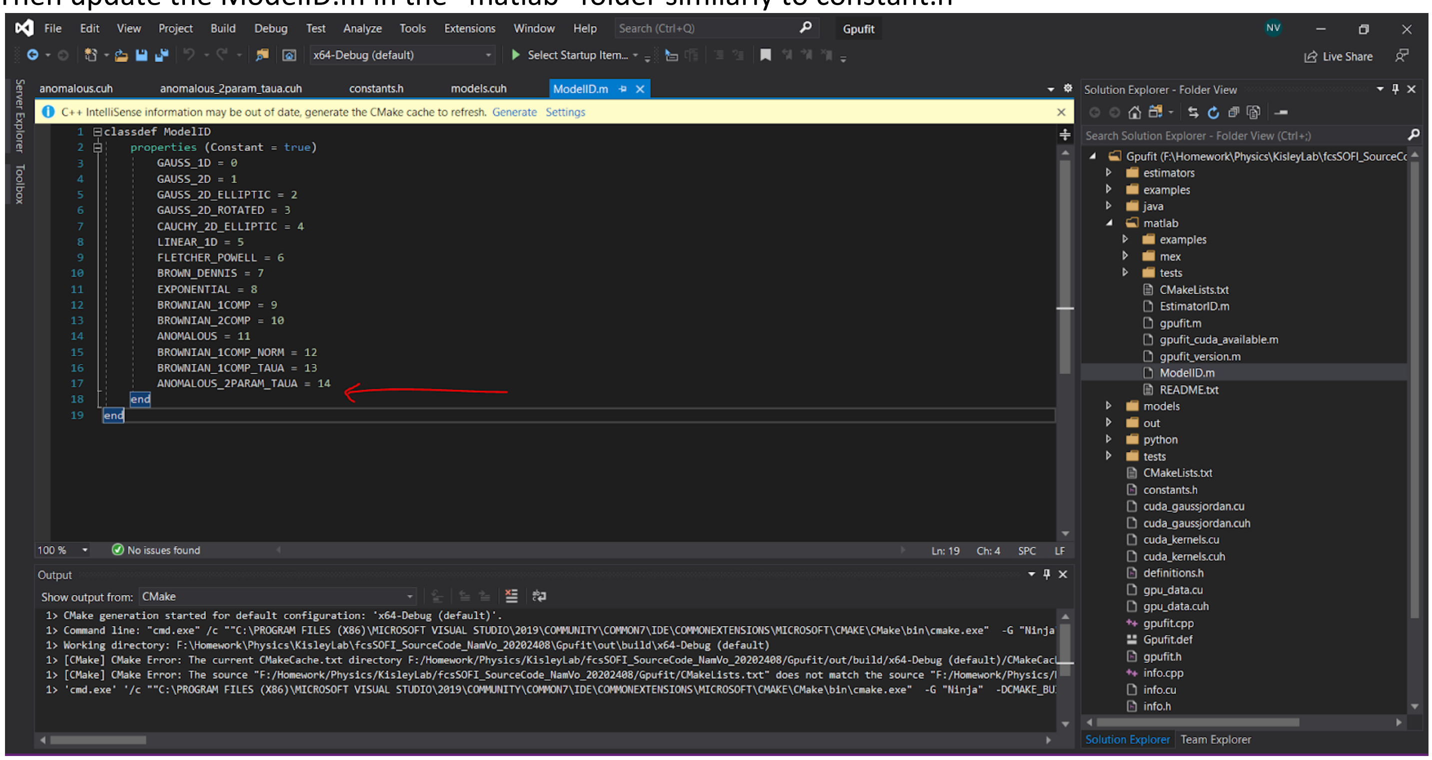


Then update constant.h in folder “tests”:

Write the name of your fitting equation and give it a number.

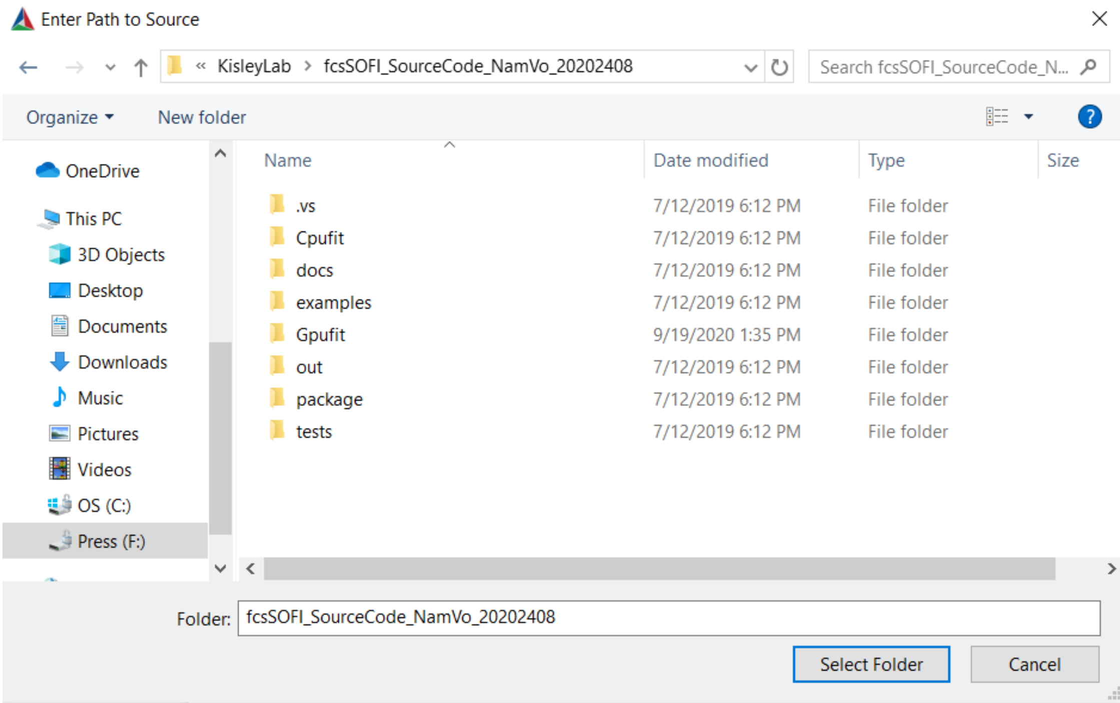


Then update the ModelID.m in the “matlab” folder similarly to constant.h

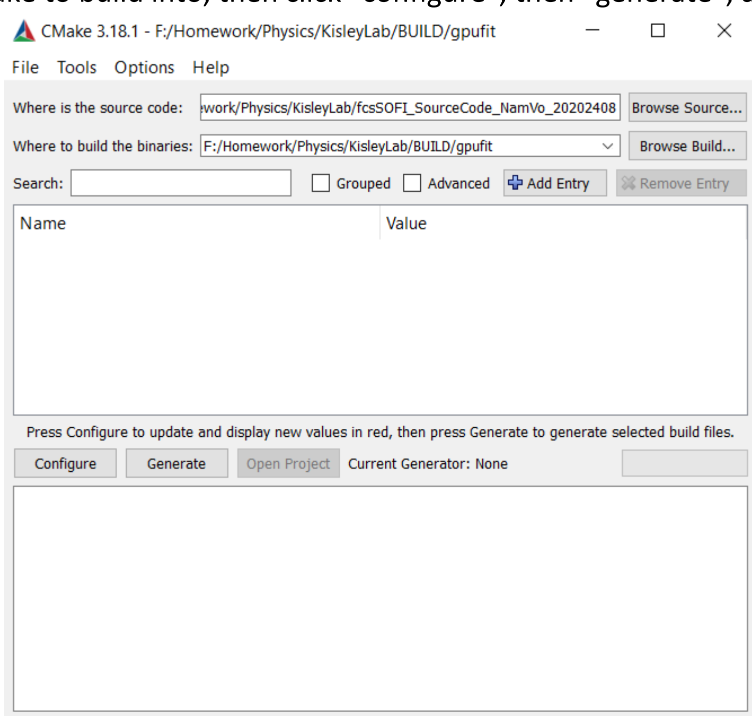


Step 3) Use CMake to configure and build from the Source Code

When you choose the source code, choose from the "outermost", or the folder that has everything (NOT Gpufit, nor Cpufit, ...)

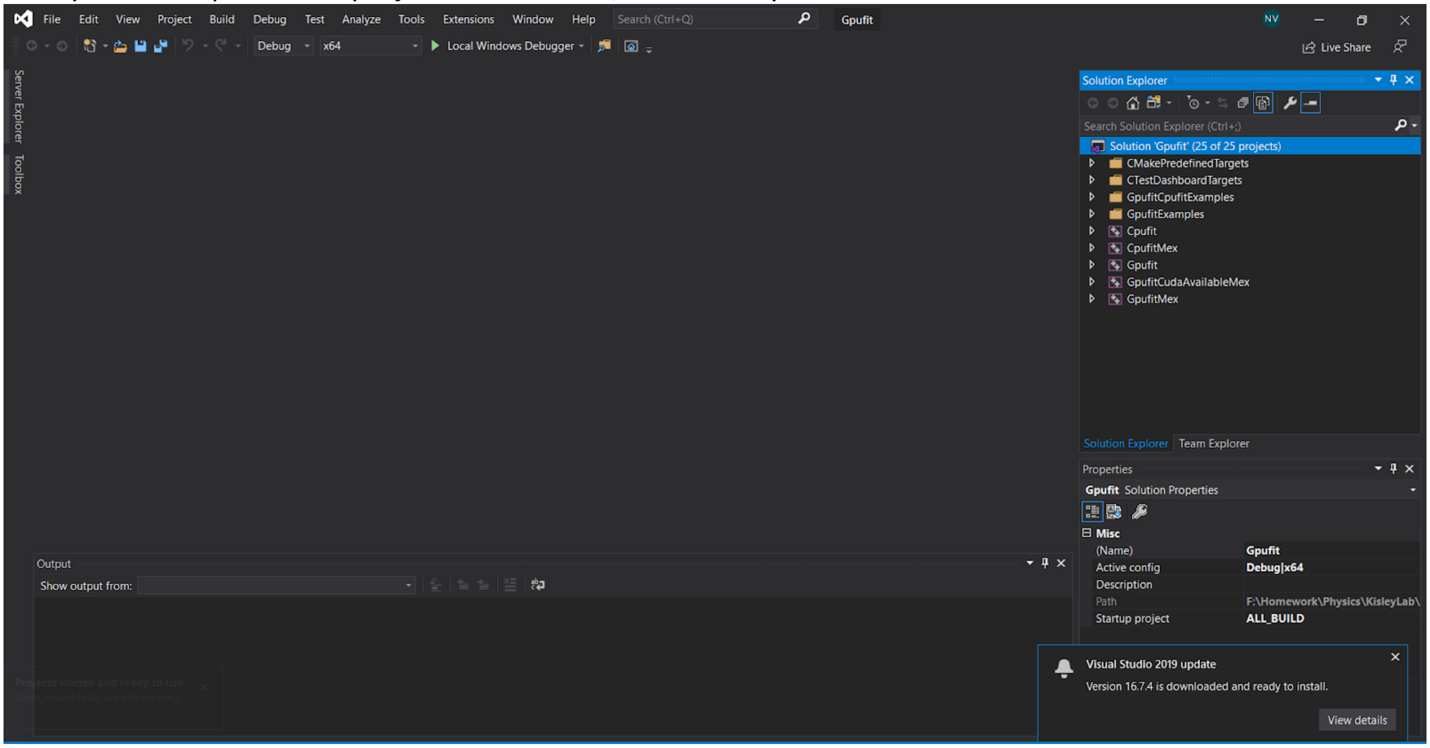


Choose the folder for CMake to build into, then click "configure", then "generate", and finally "open project"

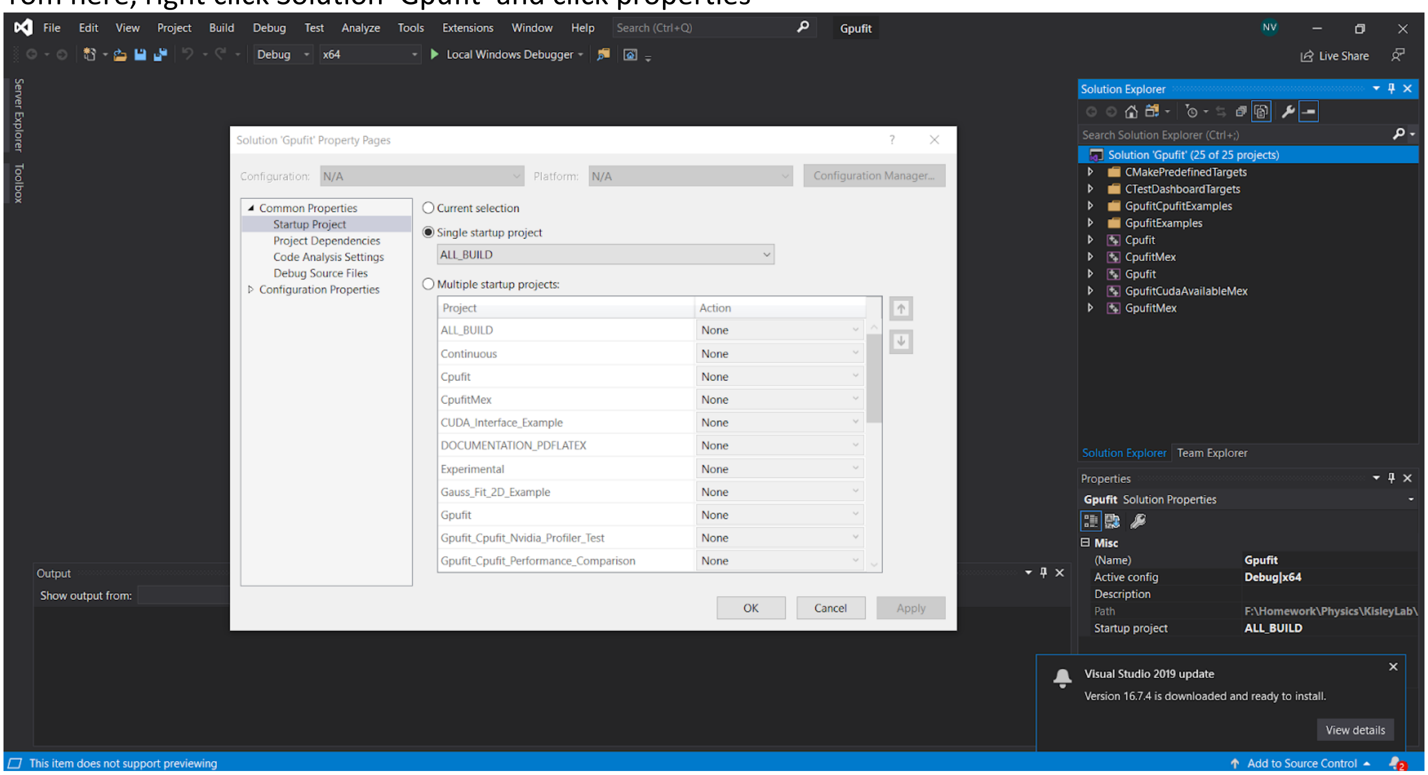


Step 4) Build with Visual Studio

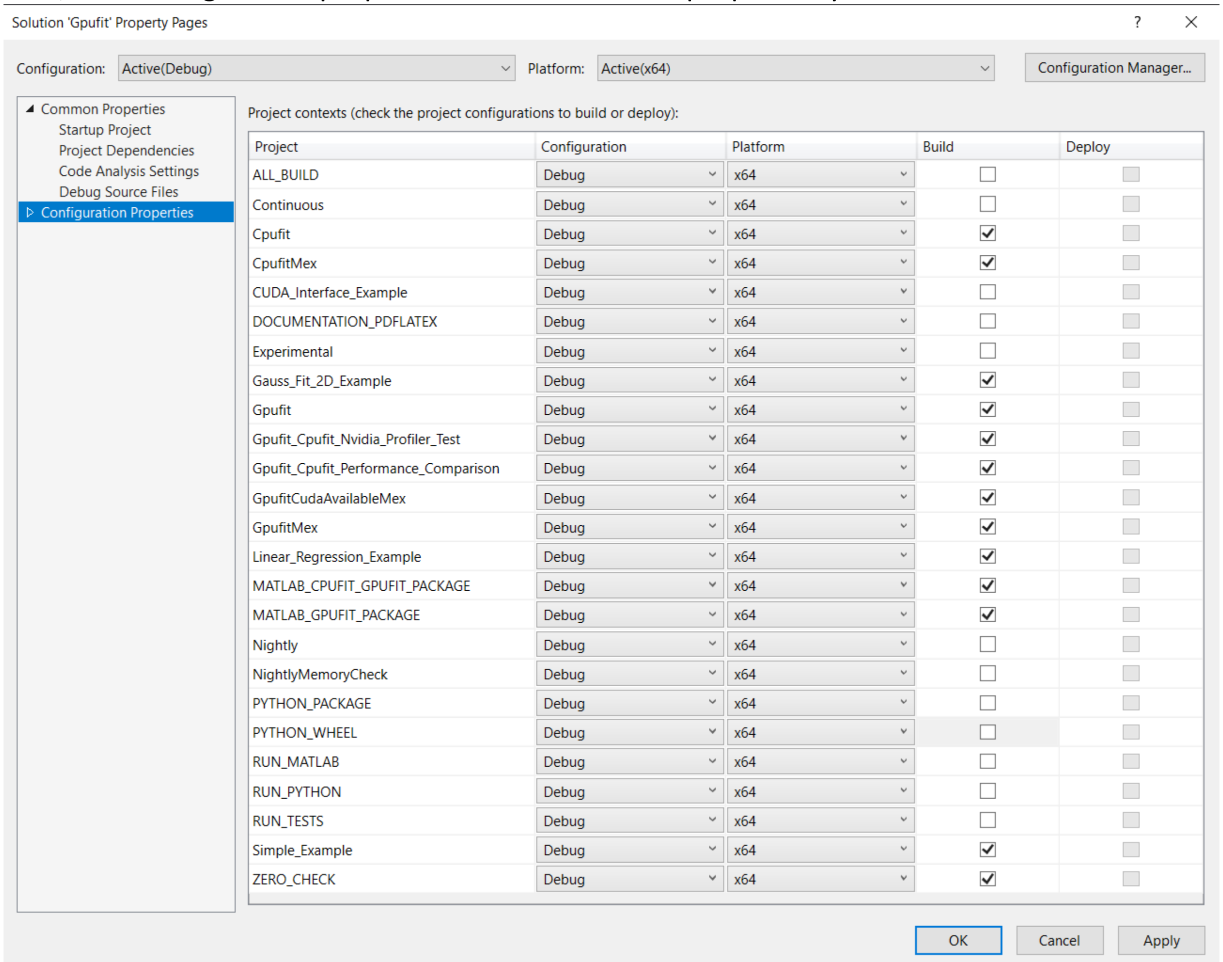
After you have opened the project from CMake, it will take you to Visual Code.



From here, right click Solution 'Gpufit' and click properties



In properties, click configuration properties and select which properties you want to build. Here's what I use.



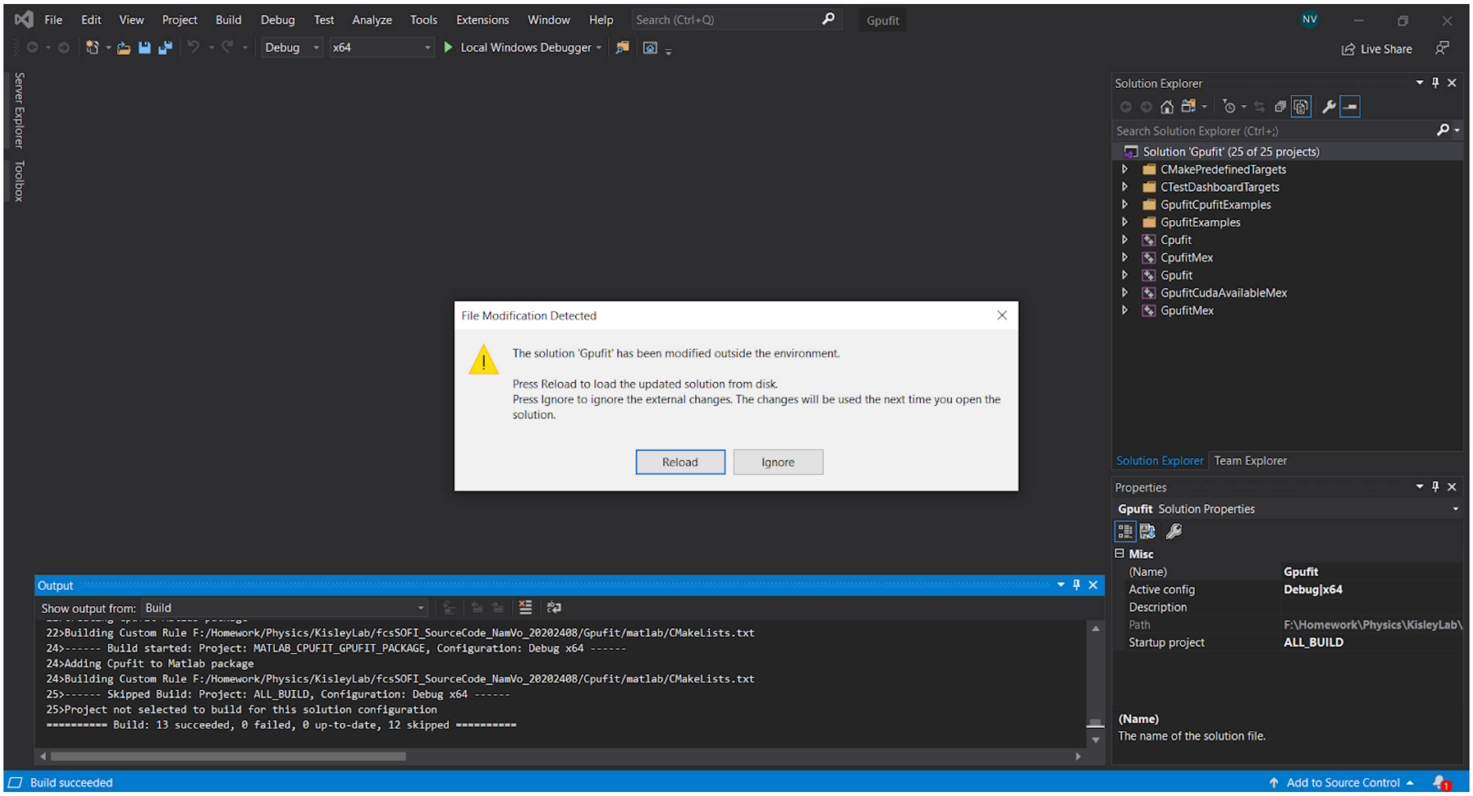
NOTE: Click everything related to Gpufit, Mex, and Matlab (except RUN_MATLAB, which caused errors)

DO NOT: click CUDA_Interface_Example (it's just an example, but requires other computers to have CUDA, which add a layer of requirements but does nothing for the fit)

Click "apply", then "OK", and start building the gpufit folder (just right click Solution 'Gpufit' and click "Build", or just press Ctrl + Shift + B)

Wait for a bit…

If you encounter this "File Modification Detected", clicking "Ignore" will not break the build folder.



After you finish building, it may give you some errors, read to see if there are any important errors that will render the build broken. For example:
If the problem is about broken links, then rebuild it (ctrl + shift + B again).
If rebuilding still gives you too many errors, check your source code again.

Bring the build folder to where the fcsSOFI script is.

Also remember to update the gofStats.m in “fcsSOFI_external_functions” to have the appropriate math to calculate your goodness of fit for your new fitting function.