User guide for kICS confinement GUI

This guide details how to run installation and usage of kICS GUI. To start, please install the latest available version of Matlab, with image and signal processing as well as fitting toolboxes. Next download the folder 'kICS GUI' and put on the local Matlab folder. It should have all the contents as shown in figure 1:

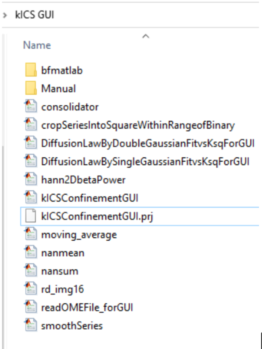

Figure 1.

Next, open Matlab and go to 'Home' tab and click on 'Set Path'. Next click on 'Add with subfolders' and select the 'kICS GUI' folder you unpacked. Click 'Save' and 'Close'. This ensures all the functions within the 'kICS GUI' folder will be visible when GUI is being used.

We will proceed with installing the GUI. Open the 'kICSConfinementGUI.prj' as shown below:

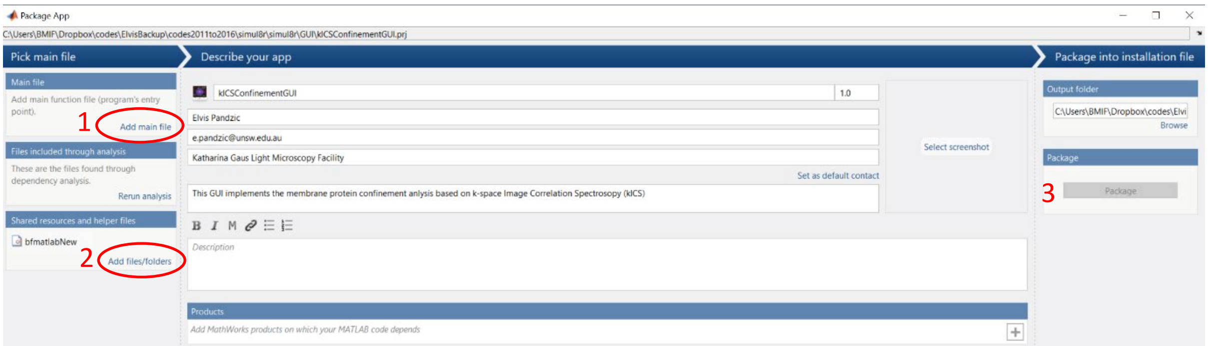

Figure 2.

Click on 'Add main file' as shown in 1 of Figure 2 and select the 'kICSConfinementGUI.mlapp'. Click on 2 and select 'bfmatlab' folder shown in Figure 1. Next, click on 3 (Figure 2) to package the GUI (Figure 3 left). This will create a new file 'kICSConfinementGUI.mlappinstall'. Click on it to display as shown in figure 3 centre. Click on 'Install' to add the GUI to 'Apps' tab.

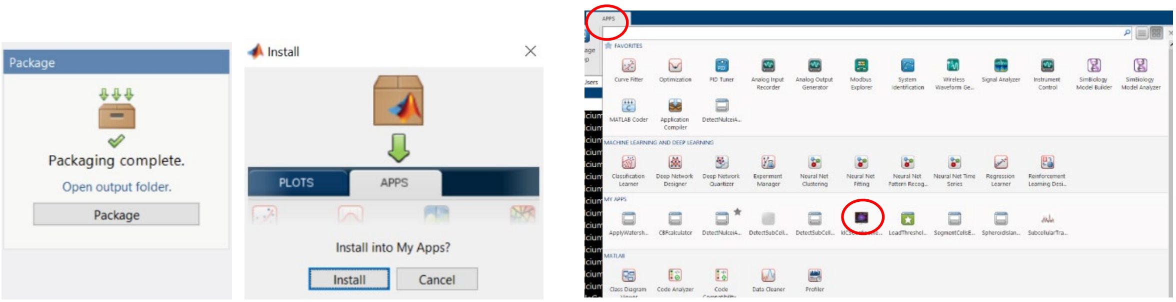

Figure 3.

Once installed, the GUI will appear in the 'Apps' tab (Figure 3 right). Note: before you click on the GUI icon to open the kICS GUI, ensure that Matlab is not in the folde that contains 'kICSConfinementGUI.mlapp' file.

Once opened, GUI will have following window:

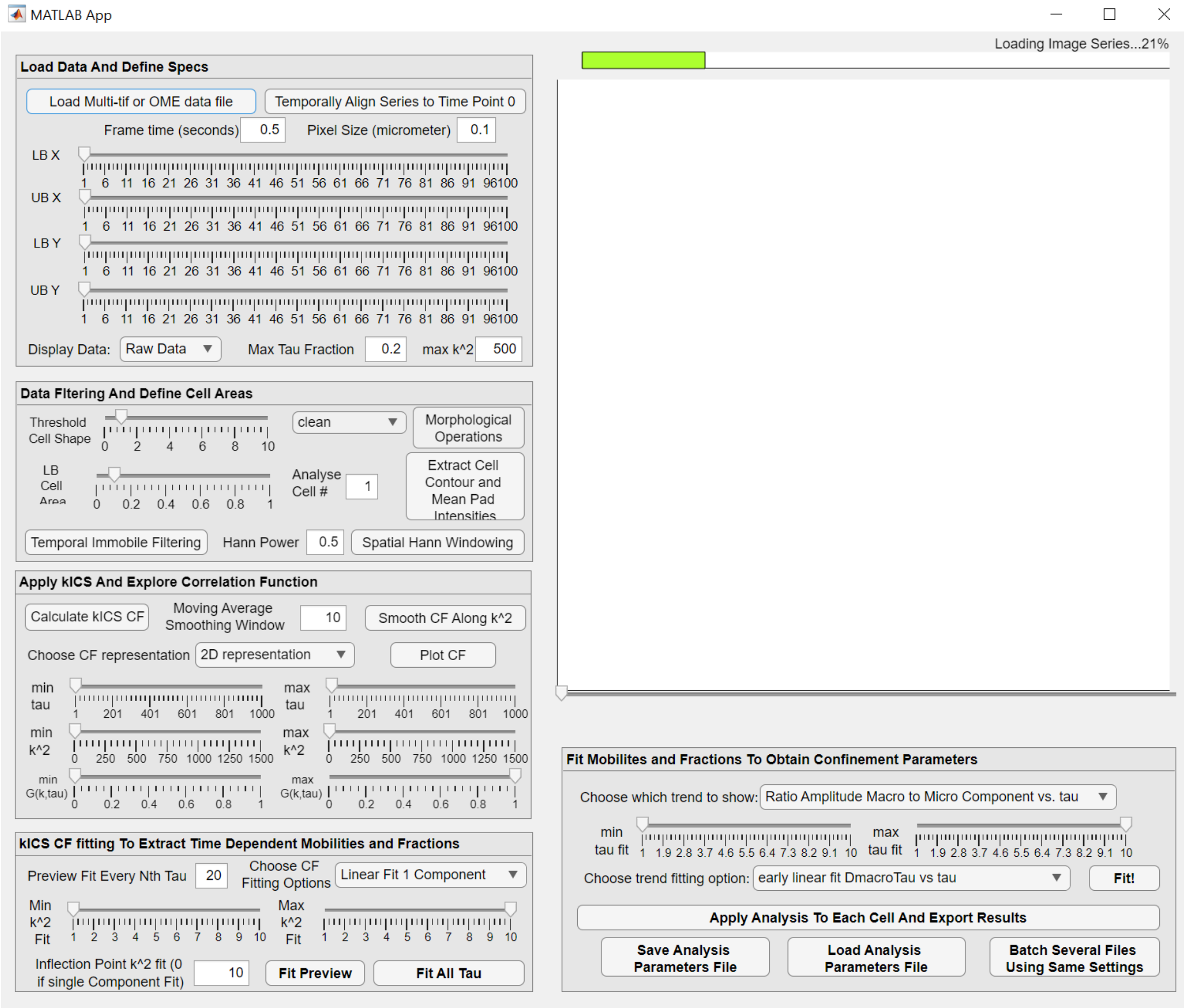

Figure 4.

Click on 'Load Multi-tif or OME data file' to select and open data. Standard OME files czi, nd2, lif, oir will be compatible or a multi-tif file. If metadata is present in the file, it will read in the pixel size and frame time and add them as displayed in the GUI. If values are not accurate, please correct those values before proceeding with the analysis. If data displays a spatial drift between frames, you can use 'Temporally Align Series to Time Point 0' to align data. Use LBX, UBX, LBY and UBY sliders to crop the data in x and y in order to include a single cell inside the field of view, as shown in figure 5 right. You could have multiple cells in the FOV as well, but these sliders will allow you to crop out the areas of the image that are empty. After data is loaded it will display the data as shown in image below.

Use slider underneath to explore other frames in the series.

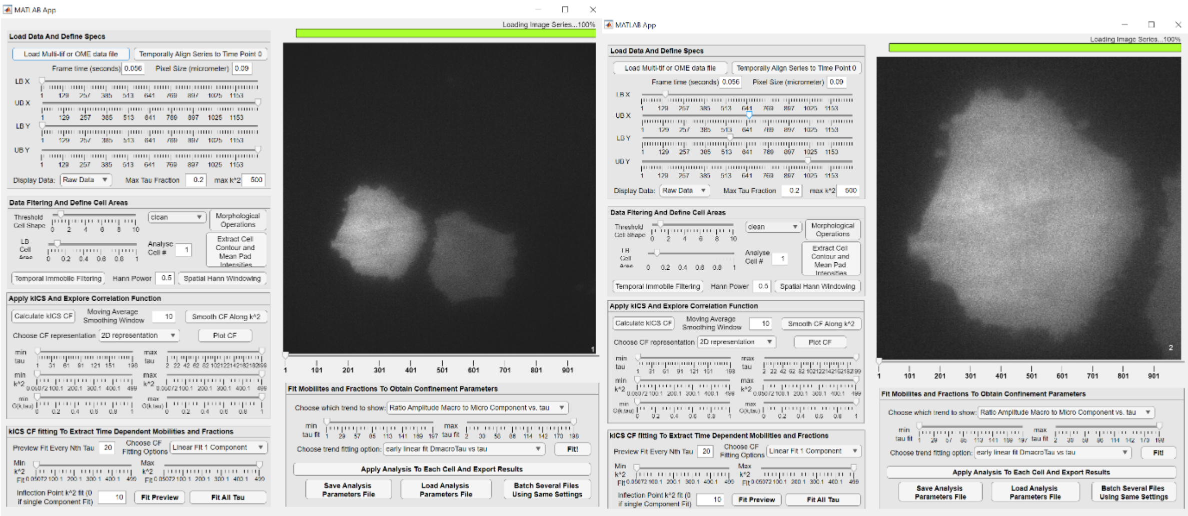

Figure 5.

Adjust max tau fraction to other value if you want to calculate more temoral lags for kICS function. Value 0.2 will be multiplied to the maximum time point of the image series to determine the maximum tau to be considered. If there are 1000 frames, then maximum tau calculated will be 200. Adjust maximum k^2 if you want different threshold in Fourier frequency. Section 'Define Filtering And Define Cell Areas' is used to define masks that define cellular area. First, adjust the threshold slider. This will produce the mask as shown in figure 6 left.

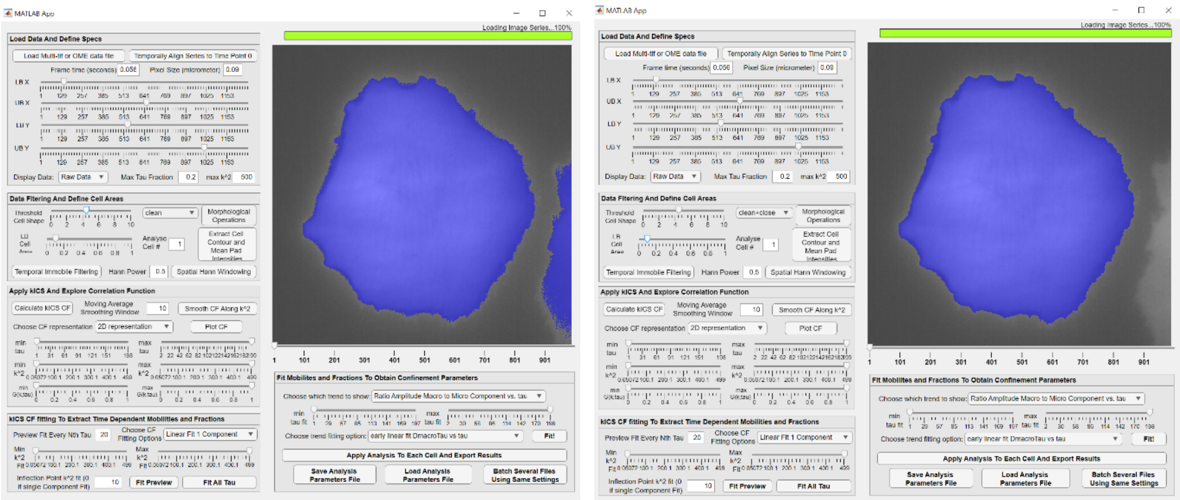

Figure 6.

Filter the mask by applying morhological operations. 'Lb cell area' slider cuts out small debris areas and smaller cell sections as shown in Figure 6 right. If there are multipe cells that are within the field of view, you can enter which cell you want to display and process (Analyse cell #) Make sure that you click on 'Extract Cell Contour…' If you want to remove the obvious immobil component form your time series, click on the 'Temporal immobile filtering'. This might affect the 'slow' or confined component of the series. Next, click on 'Spatial Hann windowing' to apply the Hann window to remove the effect of the edge of images. The default power in Hann is 0.5. Making it bigger will result in more data being cut out and ess effect from image edges. While temporal immobile filtering is not essential for confinement analysis, it is important to apply Hann windowing. Figure 7 left shows masked and right shows the Hann windowed data.

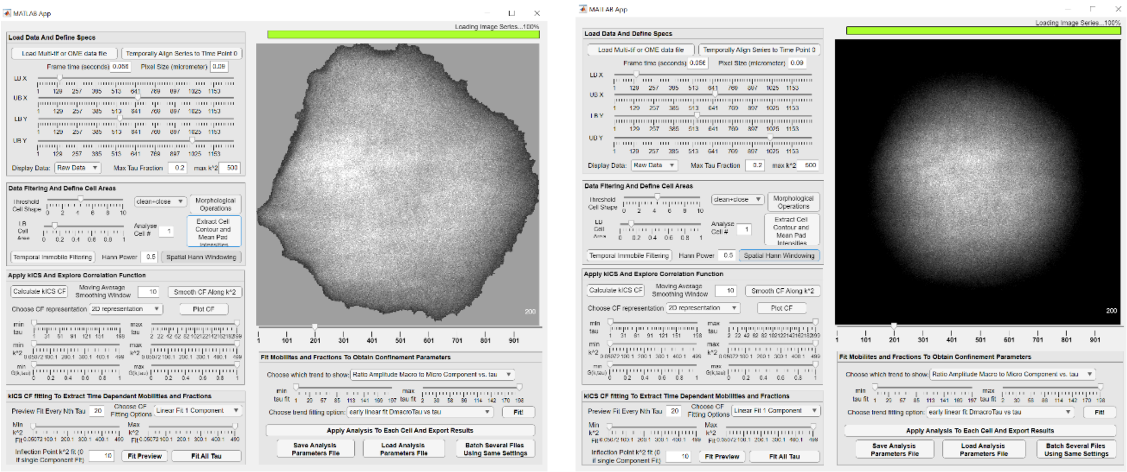

Figure 7.

‘Apply kICS and explore correlation function’ allows you to ‘calculate kICS CF’ and to visualise it in different vays as shown in the dropdown menu of the Figure 8.

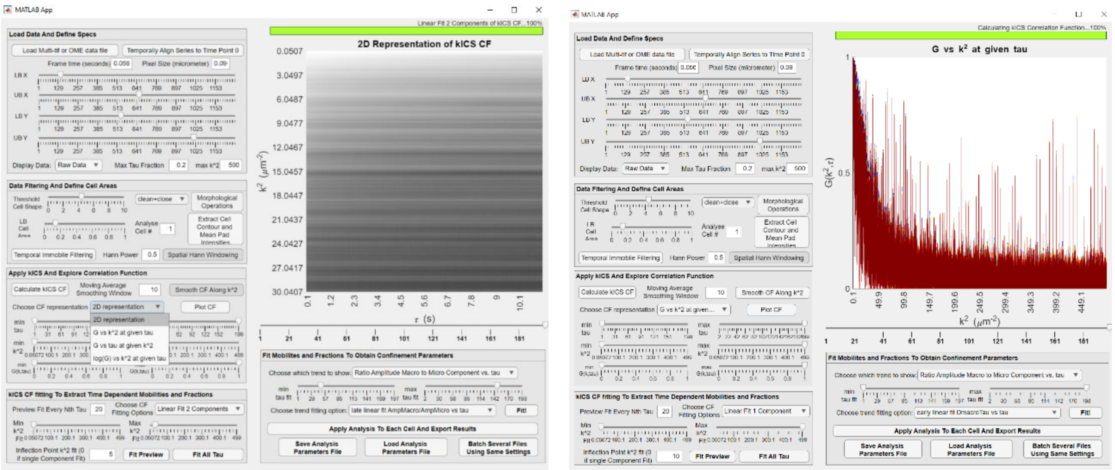

Figure 8.

Figure 8 left is the ‘2D representation’ of kICS CF while right is the ‘G vs k^2 at given tau’ representation. Latter one is showing CF as function of Fourier frequency squared , k^2, where blue o red increases tau. A zoom in to the k^2 range by min and max k^2 sliders as shown in figure 9 left.

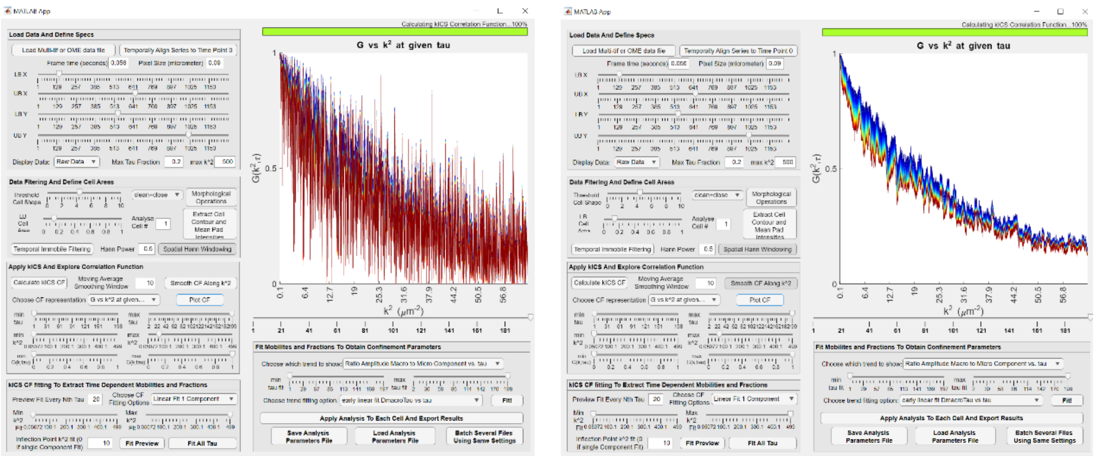

Figure 9.

Note that 'Smooth Cf along k^2' allows you to apply the moving average smoothing of each CF, which improves them visually but can also imrove the fitting. Example of smoothed CF is shown in figure 9 right compared to before smoothing on left. You can also choose the 'log G(k^2,tau) vs k^2 at given tau' representation as shown in figure 10 left.

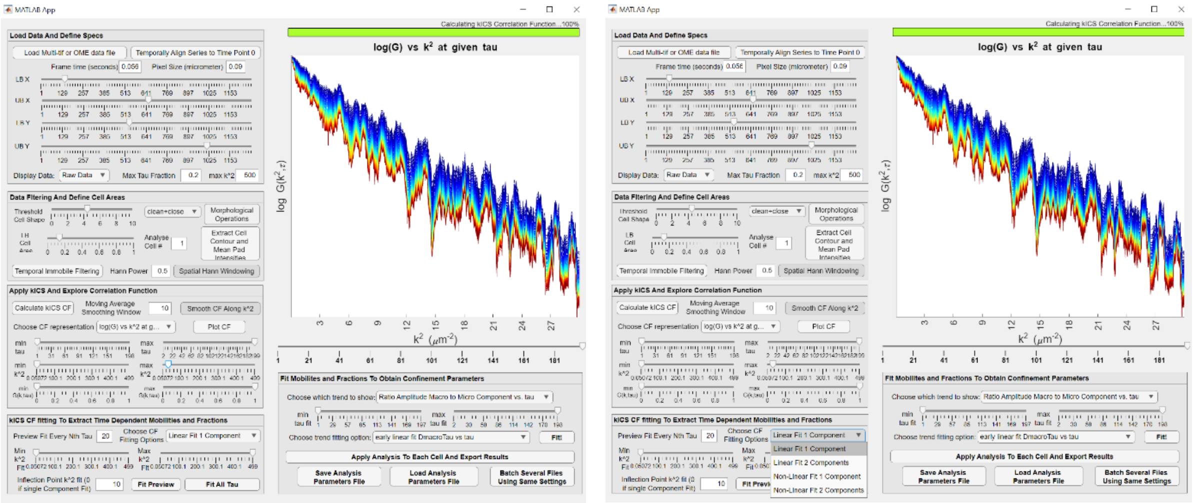

Figure 10.

Section 'kICS Cf fitting to extract time dependent mobilities and fractions' allows you to fit kICS fit with one of the approaches shown in the dropdown menu in the figure 10 right. In case you have a single component kICS CF, like in the case of a single freely diffusing population, It is preferrable to fit with either linearly or non-linearly fit 1 component. Otherwise, for case of kICS CF exhibiting two dynamic components, as emergent with confined particles dynamics, select one of the 2 component fit choices. Adjust min and max k^2 sliders in the fitting section, according to what you found was the right range to fit. Above example, we defined min k^2 to 0 and max to ~30. Set the inflection point to a value where you see the inflection in the CF, especially when log plot as shown in Figure 10. Value of k^2~5 seems to be where inflection is in this example. It is just the intial value, but the algorithm will adjust it. Clicking on the 'Fit Preview' will fit only few taus to allow you to see if the parameters worked, or if you need to adjust the limits and inflection points. Figure 11 left shows preview fit.

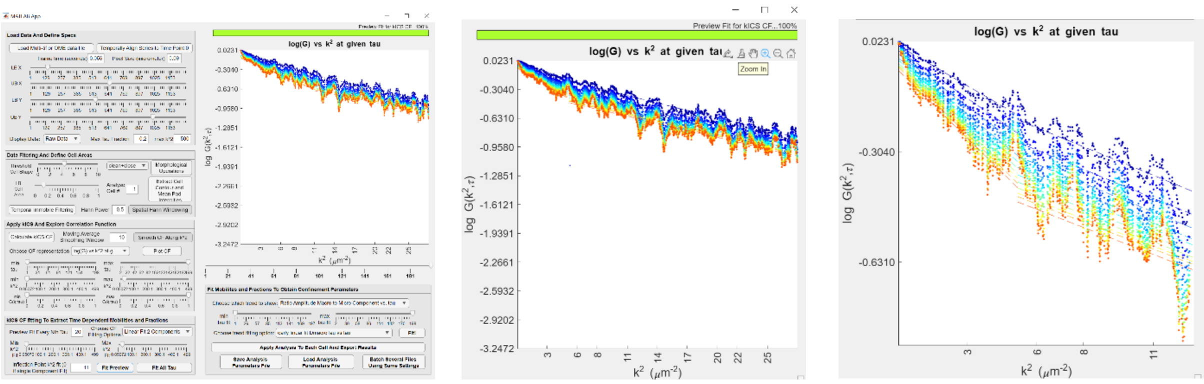

Figure 11.

Middle of figure 11 shows the howering with the mouse cursor over top right corner of the plot will show extra tools allowing you to zoom in the specific parts of the plot, as shown in the figure 11 right.

‘Fit all tau’ will proceed with fitting kICS CF over all taus, using same setting as defined in preview fit (figure 12 left).

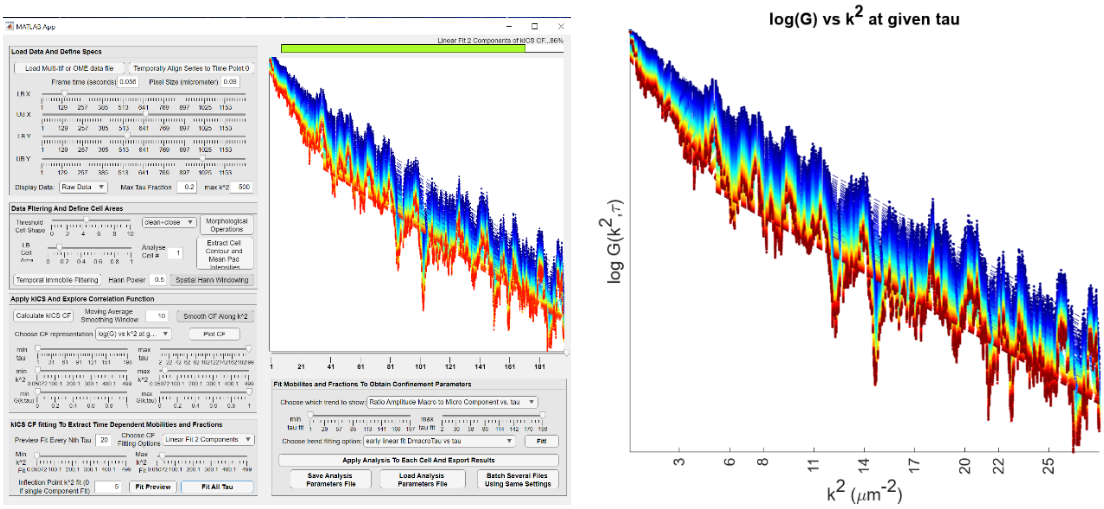

Figure 13.

Section ‘Fit modalities and fractions to obtain confinement parameters’ is used further to characterise the amplitudes and exponents that vary with tau, which resulted from previous fit. These can be further fitted to extract various confinement components. Figure 14 left shown the drop down menu for which trend is to be shown in the axis.

Figure 14.

In the figure 14 left you can see the tau evolution of Dtau Macro vs tau. To characterise it, we will do linear fits for either early or later tau lags range. Early tau range will produce Dmacro. Select ‘late linear fit DmacroTau vs tau’ as shown in figure 14 right, to fit early tau lags of DmacroTau vs tau. Adjust the range of tau using the sliders in the menu. Click on ‘Fit’ to proceed with fit. It will produce and save the plot as shown in figure 15 left. If you want to characterise the DmacroTau over whole tau range, you can apply Anomalous fit by selecting ‘Anomalous fit…’ as shown in figure 14 right.

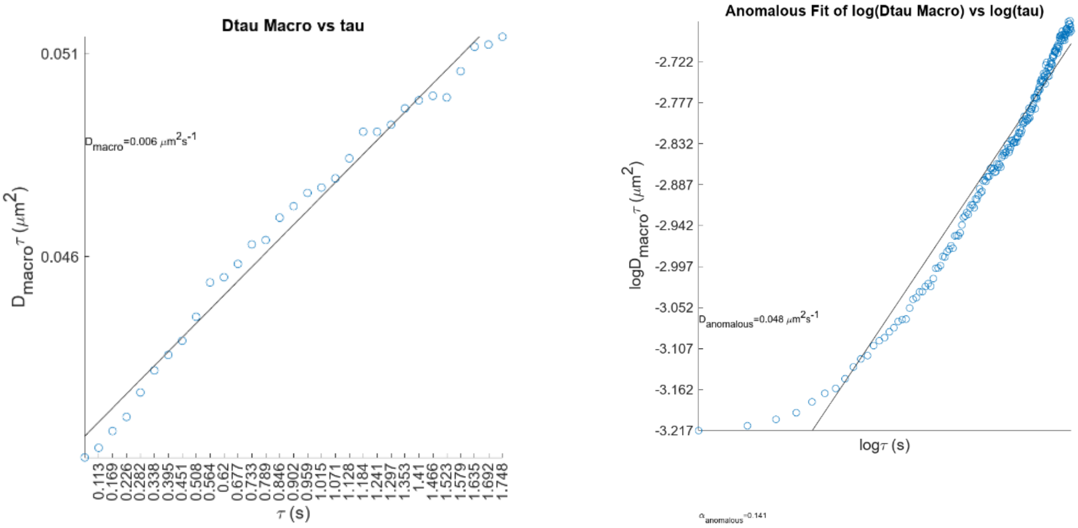

Figure 15.

The resilting fit will be saved and properties such as anomalous exponent and Danomalous will be saved (figure 15 right). You can characterise the DmicroTau in early and late taus regimes as well as ratio of amplitudes of macro and micro components to obtain other confinement parameters (figure 16).

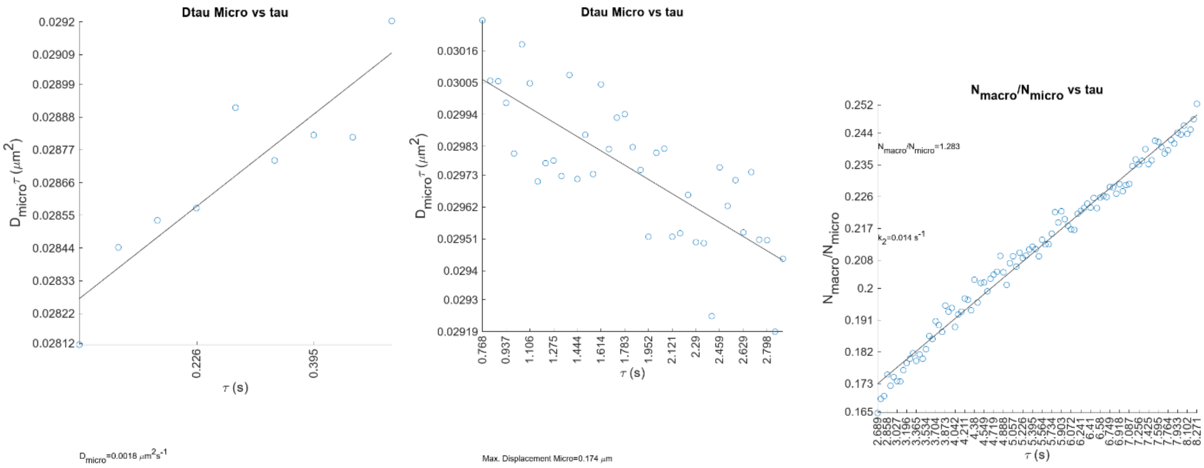

Figure 16.

Finally, you can apply the analysis to all cells within the field of view by clicking ‘Apply analysis to each cell and export results’. This will create an excel file with all the parameters being exported.

Figure 17 shows example of excel sheet that was exported, with first sheet exporting the amplitudes and exponents for both micro (AmpS and DtauF) and macro (AmpF and DtauF) as well as ratio of amplitudes. Second sheet is a summary of confinement parameters extracted from fitting.

tau	DtauF	AmpF	DtauS	AmpS	RatAmp
0.056390116	0.041050577	0.989967001	0.029202786	0.918419865	1.077902426
0.112780233	0.041287772	0.985324638	0.029518381	0.912303203	1.080040753
0.169170349	0.041700977	0.984233918	0.029679062	0.90856765	1.08328083
0.225560466	0.042050655	0.983038153	0.029700854	0.904615892	1.086691226
0.281950582	0.04265357	0.982953531	0.030039715	0.903556235	1.087872002
0.338340699	0.043197146	0.98239252	0.029914243	0.897737675	1.094297976
0.394730815	0.043578139	0.981717105	0.030118131	0.897324596	1.094049032
0.451120932	0.043928242	0.981410015	0.029869569	0.890150711	1.102521182
0.507511048	0.044518784	0.980941476	0.030158137	0.891116223	1.100800828
0.563901165	0.045365552	0.980686044	0.030083556	0.886975567	1.1056517
0.620291281	0.045494167	0.97959042	0.029954404	0.883012584	1.109373114
0.676681398	0.045822545	0.978902959	0.029984691	0.880895757	1.111258569
0.733071514	0.046303744	0.978667632	0.030012745	0.877349956	1.115481486
0.789461631	0.046410674	0.977990534	0.030265195	0.878229731	1.113593061
0.845851747	0.046962684	0.977220717	0.030056896	0.873736337	1.11843891
0.902241864	0.047245198	0.976433067	0.030054908	0.870855173	1.121234733
0.95863198	0.047573239	0.976827724	0.029979946	0.867837541	1.125588233
1.015022097	0.047705347	0.975818274	0.029805023	0.865473792	1.127496041
1.071412213	0.047932175	0.974694234	0.030182234	0.866367194	1.125035944
1.12780233	0.048426314	0.97487736	0.030047608	0.862562038	1.130211297
1.184192446	0.049082037	0.974455621	0.029708482	0.858656544	1.134860763
1.240582563	0.049081825	0.974229564	0.029771473	0.856199299	1.137853728
1.296972679	0.04925424	0.973195536	0.029780596	0.853628606	1.140069029
1.353362795	0.049657574	0.972673952	0.029727427	0.851835839	1.141856103
1.409752912	0.049856257	0.971731031	0.030076819	0.853023508	1.139160904
1.466143028	0.049969246	0.971513873	0.029716696	0.848244514	1.145322907
1.522533145	0.049927728	0.970700102	0.029869444	0.847473032	1.145405299
1.578923261	0.050576167	0.970711153	0.029733942	0.843518186	1.150788648
1.635313378	0.051176953	0.970700767	0.03004424	0.846306059	1.146985487
1.691703494	0.051231535	0.969486575	0.029820319	0.842117503	1.151248574
1.748093611	0.051423454	0.968807227	0.029928871	0.841671651	1.151051274
1.804483727	0.051952073	0.969307721	0.029941133	0.83908318	1.155198608
1.860873844	0.051896652	0.968438527	0.02982689	0.83673299	1.157404499
1.91726396	0.05191907	0.967481044	0.029748202	0.835179745	1.158410569
1.973654077	0.052398157	0.967739947	0.029517496	0.831381318	1.164014545
2.030044193	0.052758857	0.967177289	0.029807533	0.832817706	1.161331324
2.08643431	0.05273567	0.966695901	0.02982173	0.831321719	1.162842109
2.142824426	0.053467879	0.966662609	0.029517264	0.82754537	1.168108292
2.199214543	0.053475194	0.966105758	0.029529424	0.825667552	1.170090499
2.255604659	0.053448352	0.965003562	0.029662086	0.824273531	1.170732197
2.311994776	0.053634975	0.964838713	0.029498647	0.822364877	1.173248931
2.368384892	0.054314811	0.964887726	0.029495463	0.82065302	1.175756016
2.424775009	0.054565251	0.963917679	0.029242683	0.817158479	1.179596986
2.481165125	0.054669126	0.963869317	0.029757256	0.820643704	1.174528376
2.537555242	0.055127435	0.963545999	0.029623814	0.817895879	1.178079049
2.593945358	0.054934043	0.962730994	0.029713126	0.818401913	1.17635477
2.650335475	0.055382008	0.963011964	0.029532435	0.814941286	1.181694903
2.706725591	0.055520481	0.962182061	0.02974073	0.81610648	1.178990835
2.763115707	0.055676091	0.961278151	0.029507233	0.812074248	1.183731849
2.819505824	0.055449916	0.960205148	0.029505475	0.810539459	1.184649479
2.87589594	0.056096214	0.961112687	0.029189299	0.806384107	1.191879502
2.932286057	0.056183204	0.96049564	0.029441359	0.808408198	1.188131989
2.988676173	0.056380544	0.959842734	0.029330895	0.805877291	1.19105321
3.04506629	0.056911313	0.960011873	0.029544673	0.80691171	1.189735954
3.101456406	0.056715656	0.959289601	0.029550223	0.806403978	1.189589371
3.157846523	0.056717554	0.958963958	0.029471558	0.803559197	1.193395535
3.214236639	0.057755712	0.959158978	0.02944192	0.802115147	1.195787141
3.270626756	0.057470364	0.958203356	0.029334833	0.800326681	1.197265289
3.327016872	0.05733665	0.958347145	0.029276358	0.799181507	1.199160811
3.383406989	0.057755612	0.957360073	0.02949575	0.800101405	1.19654842
3.439797105	0.057646773	0.95675439	0.029368933	0.798146889	1.198719688
3.496187222	0.057882837	0.956887966	0.029451898	0.799229704	1.197262765
3.552577338	0.058439595	0.956587828	0.029405453	0.796822942	1.200502367

MobAndAmpCell1 | ConfParamsCell1 | +

A	B	C	D	E	F	G	H	I	J
Dmacro	Deff	MaxDispMacro	Dmicro	DmicroLate	MaxDispMicro	k2	ratConc	alphaAnomalou:	Danomalous
0.0062	0	0.227609265	0.0018	-0.0002918	0.174026419	0	1.2834	0.142363562	0.04910748