**Maximum Entropy Method Instructions**

Dr. Sudipta Maiti created a program to analyze FCS data and create a distribution of size population. The x axis being diffusivity and the y axis being intensity. This program only runs on Windows XP, there is an XP laptop in Dr. Leach’s lab that already has the MEMFCS software on it, as well as sample data.

Before using this software you must contact Dr. S. Maiti and request permission to use his software. They should send a declaration form for using MEMFCS.

<http://www.tifr.res.in/~dcs/index.php?option=com_content&view=article&id=19&Itemid=126>

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**Basics:**

To run this program, open a windows explorer window that only contains 3 files: the MEMFCS program, the ‘data’ file, and the ‘DATMEMFC’ file.

The program will only run if the name of the ‘data’ file matches the name you input within the DATMEMFC file. The ‘data’ file name cannot have spaces and is limited to 8 characters. The DATMEFC file must have this exact name in order to run. You double click the MEMFCS program to run the program. You will have to hit Enter when it prompts you to start. You will have to hit Enter to exit the program.

**Data file:**

Open using Notepad.

There needs to be 3 columns that you obtain from collecting FCS data in software such as Vista Vision.

* The first column is your Gtau (y-axis of your correlation function).
* The second column is your tau (x-axis of your correlation function).
* The third column is simply the weight of each point, keep it at 1 for all points.

When you save your Notepad document, **save file type as “All Files”** and put **quotes around your file name** ex. “PEG2”. This prevents any file extensions from being added to the file and is required to run in the MEMFCS program. The name cannot have spaces and is limited to 8 characters.

**DATMEMFC file:**

Open using Notepad.

The top 13 lines are the code going into the program. Below the Comments line explains what each number in each line is. The most important are the first 5 lines. Below are important explanations.

* **Number of data points in sample** – input the number of rows in your data file.
* **Channel number of the end of FCS data** – matches the number of data points in sample.
* **Optimization range** – the number you input is the number of points the program will skip starting from the first row in your data file. This is useful when you have a lot of noise at the low tau values. Try adjusting this number one at a time and see how this helps your model fit.
* **File name of the FCS data** – this name cannot have spaces and is limited to 8 characters. It must match the name you give your ‘data’ file.
* **Value to be added to I** – look at your correlation function, if the right tail of the data dips below zero, look at the average value below. Input this number as a negative (ex. -0.005). This will adjust your data to remain above zero for the model fit.
* **Nmol Number of molecules in fcs volume** – The confocal volume is equal to: V=pi^(3/4)\*wo^(2)\*zo where wo is the characteristic radial dimension and zo is the characteristic axial dimension (e.g. wo=0.2um; zo=1.67um with Dr. Boukari at DSU). Knowing your concentration of molecules in the sample, you can use Avogadro’s number to determine the number of molecules in the confocal volume.
* **Ads factor-ratio of short to long axis of the ‘elliptical’ FCS spot** – this is wo/zo.
* **Ftrip-Fraction of triplet** – to obtain this value you have to model your data with a 3D Triplet Gaussian model using your software such as Vista Vision. Additional two species models are below to manual input if needed. Fix knowns such as wo and zo and start other values close to what you expect but do not fix. Start the model, if the chi-squared is good then use the fraction of triplet (T) in your DATMEMFC file.
* **Tau\_trip triplet lifetime in msec** – This information is also obtained from your 3D Triplet Gaussian model, this is also known as the relaxation time. Be sure to put it in msec.
* **Pl note lifetime in msec** – this is the triplet lifetime of your flurophore which you should be able to find online. Alexa 488 is about 4.1 ns therefore is 0.0000041 msec.
* **Initial value of flat distribution** – Looking at your correlation function, look to the far left and determine the average value of Gtau for the initial flat peak.
* **Lower value tau** – this is where you want your graph to start on the x-axis.
* **Upper value tau** – this is where you want your graph to end on the x-axis.

When you save your Notepad document, **save file type as “All Files”** and put **quotes around your file name** ex. “PEG2 DATMEMFC”. This prevents any file extensions from being added to the file and is required to run in the MEMFCS program. It is convenient to title this file with the ‘data’ file name, however, you will need to delete the identifier before running the program.

**Manual Input Models:**

**3D Triplet Gaussian 1 Species Model**

1/N (1 + (f exp(-x/t))/(1 - f)) (1/(w^2 + 4 a x) sqrt(1/(z^2 + 4 a x)))

(1/N)\*(1+(f\*exp(-x/t))/(1-f))\*(1/(w0^2+4\*d\*x)\*sqrt(1/(z0^2+4\*d\*x)))

**d/(df)=** (sqrt(1/(4 a x + z^2)) ((f e^(-x/t))/(1 - f)^2 + e^(-x/t)/(1 - f)))/(N (4 a x + w^2))

(sqrt(1/(4\*d\*x+z0^2))\*((f\*exp^(-x/t))/(1-f)^2+exp^(-x/t)/(1-f)))/(N(4\*d\*x+w0^2))

**d/(dt)=** (f x e^(-x/t) sqrt(1/(4 a x + z^2)))/((1 - f) N t^2 (4 a x + w^2))

(f\*x\*exp^(-x/t)\*sqrt(1/(4\*d\*x+z0^2)))/((1-f)\*N\*t^2\*(4\*d\*x+w0^2))

**d/(da)=** -(2 x (1/(4 a x + z^2))^(3/2) ((f e^(-x/t))/(1 - f) + 1))/(N (4 a x + w^2)) - (4 x sqrt(1/(4 a x + z^2)) ((f e^(-x/t))/(1 - f) + 1))/(N (4 a x + w^2)^2)

-(2\*x\*(1/(4\*d\*x+z0^2))^(3/2)\*((f\*exp^(-x/t))/(1-f)+1))/(N\*(4\*d\*x+w0^2))-(4\*x\*sqrt(1/(4\*d\*x+z0^2))\*((f\*exp^(-x/t))/(1-f)+1))/(N\*(4\*d\*x+w0^2)^2)

**d/(dw)=** -(2 w sqrt(1/(4 a x + z^2)) ((f e^(-x/t))/(1 - f) + 1))/(N (4 a x + w^2)^2)

-(2\*w0\*sqrt(1/(4\*d\*x+z0^2))\*((f\*exp^(-x/t))/(1-f)+1))/(N\*(4\*d\*x+w0^2)^2)

**d/(dz)=** -(z (1/(4 a x + z^2))^(3/2) ((f e^(-x/t))/(1 - f) + 1))/(N (4 a x + w^2))

-(z0\*(1/(4\*d\*x+z0^2))^(3/2)\*((f\*exp^(-x/t))/(1-f)+1))/(N\*(4\*d\*x+w0^2))

**d/(dN)=** -(sqrt(1/(4 a x + z^2)) ((f e^(-x/t))/(1 - f) + 1))/(N^2 (4 a x + w^2))

-(sqrt(1/(4\*d\*x+z0^2))\*((f\*exp^(-x/t))/(1-f)+1))/(N^2\*(4\*d\*x+w0^2))

**3D Single Triplet Gaussian 2 Distinct Species:**

(1 - f + f e^(-x/t))/(1 - f) (g/(w^2 + 4 a x) sqrt(1/(z^2 + 4 a x)) + h/(w^2 + 4 c x) sqrt(1/(z^2 + 4 c x)))

G(x) =

G(x)= ((1-f+f\*e^((-x)/t))/(1-f))\*((g1/(w0^2+4\*d1\*x)\*(1/(z0^2+4\*d1\*x))^(1/2))+(g2/( w0^2+4\*d2\*x)\*(1/(z0^2+4\*d2\*x))^(1/2)))

WolframAlpha code:

((1-f+f\*exp((-x)/t))/(1-f))\*((g/(w^2+4\*a\*x)\*(1/(z^2+4\*a\*x))^(1/2))+(h/( w^2+4\*c\*x)\*(1/(z^2+4\*c\*x))^(1/2)))

f = f1 t = t1 g = g1 h = g2 a = d1 c = d2

**d/df**=((f e^(-x/t) - f + 1) ((g sqrt(1/(4 a x + z^2)))/(4 a x + w^2) + (h sqrt(1/(4 c x + z^2)))/(4 c x + w^2)))/(1 - f)^2 + ((e^(-x/t) - 1) ((g sqrt(1/(4 a x + z^2)))/(4 a x + w^2) + (h sqrt(1/(4 c x + z^2)))/(4 c x + w^2)))/(1 - f)

((f\*e^(-x/t)-f+1)\*((g1\*sqrt(1/(4\*d1\*x+z0^2)))/(4\*d1\*x+w0^2)+(g2\*sqrt(1/(4\*d2\*x+z0^2)))/(4\*d2\*x+w0^2)))/(1-f)^2+((e^(-x/t)-1)\*((g1\*sqrt(1/(4\*d1\*x+z0^2)))/(4\*d1\*x+w0^2)+(g2\*sqrt(1/(4\*d2\*x+z0^2)))/(4\*d2\*x+w0^2)))/(1-f)

**d/dt**=(f x e^(-x/t) ((g sqrt(1/(4 a x + z^2)))/(4 a x + w^2) + (h sqrt(1/(4 c x + z^2)))/(4 c x + w^2)))/((1 - f) t^2)

(f\*x\*e^(-x/t)\*((g1\*sqrt(1/(4\*d1\*x+z0^2)))/(4\*d1\*x+w0^2)+(g2\*sqrt(1/(4\*d2\*x+z0^2)))/(4\*d2\*x+w0^2)))/((1-f)\*t^2)

**d/dg**=(sqrt(1/(4 a x + z^2)) (f e^(-x/t) - f + 1))/((1 - f) (4 a x + w^2))

(sqrt(1/(4\*d1\*x+z0^2))\*(f\*e^(-x/t)-f+1))/((1-f)\*(4\*d1\*x+w0^2))

**d/dh**=(sqrt(1/(4 c x + z^2)) (f e^(-x/t) - f + 1))/((1 - f) (4 c x + w^2))

(sqrt(1/(4\*d2\*x+z0^2))\*(f\*e^(-x/t)-f+1))/((1-f)\*(4\*d2\*x+w0^2))

**d/da**= ((f e^(-x/t) - f + 1) (-(2 g x (1/(4 a x + z^2))^(3/2))/(4 a x + w^2) - (4 g x sqrt(1/(4 a x + z^2)))/(4 a x + w^2)^2))/(1 - f)

((f\*e^(-x/t)-f+1)\*(-(2\*g1\*x\*(1/(4\*d1\*x+z0^2))^(3/2))/(4\*d1\*x+w0^2)-(4\*g1\*x\*sqrt(1/(4\*d1\*x+z0^2)))/(4\*d1\*x+w0^2)^2))/(1-f)

**d/dc**= ((f e^(-x/t) - f + 1) (-(2 h x (1/(4 c x + z^2))^(3/2))/(4 c x + w^2) - (4 h x sqrt(1/(4 c x + z^2)))/(4 c x + w^2)^2))/(1 - f)

((f\*e^(-x/t)-f+1)\*(-(2\*g2\*x\*(1/(4\*d2\*x+z0^2))^(3/2))/(4\*d2\*x+w0^2)-(4\*g2\*x\*sqrt(1/(4\*d2\*x+z0^2)))/(4\*d2\*x+w0^2)^2))/(1-f)

**d/dw**= ((f e^(-x/t) - f + 1) (-(2 g w sqrt(1/(4 a x + z^2)))/(4 a x + w^2)^2 - (2 h w sqrt(1/(4 c x + z^2)))/(4 c x + w^2)^2))/(1 - f)

((f\*e^(-x/t)-f+1)\*(-(2\*g1\*w0\*sqrt(1/(4\*d1\*x+z0^2)))/(4\*d1\*x+w0^2)^2-(2\*g2\*w0\*sqrt(1/(4\*d2\*x+z0^2)))/(4\*d2\*x+w0^2)^2))/(1-f)

**d/dz**= ((f e^(-x/t) - f + 1) (-(g z (1/(4 a x + z^2))^(3/2))/(4 a x + w^2) - (h z (1/(4 c x + z^2))^(3/2))/(4 c x + w^2)))/(1 - f)

((f\*e^(-x/t)-f+1)\*(-(g1\*z0\*(1/(4\*d1\*x+z0^2))^(3/2))/(4\*d1\*x+w0^2)-(g2\*z0\*(1/(4\*d2\*x+z0^2))^(3/2))/(4\*d2\*x+w0^2)))/(1-f)