

EVILFIT

Step-by-Step Tutorial

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Draft Version 0.1

1) In Preparation for EVILFIT analysis: Converting BiaEvaluation File into *.xls File

- Create a **folder** on C-drive, for example I use C:\SPR_RESULTS\Inna82
- Copy BiaEvaluation file into the new **folder**, open it
- Export data into the same **folder** and save under a new name, for example, Inna82
- Close BiaEvaluation file;
- a new Inna82.txt file is in the **folder** (C:\SPR_RESULTS\Inna82\Inna82.txt)
- Open Microsoft Office Excel:

File/Open

In the Selection window browse for the txt.file that is in the **folder**

C:\SPR_RESULTS\Inna82\

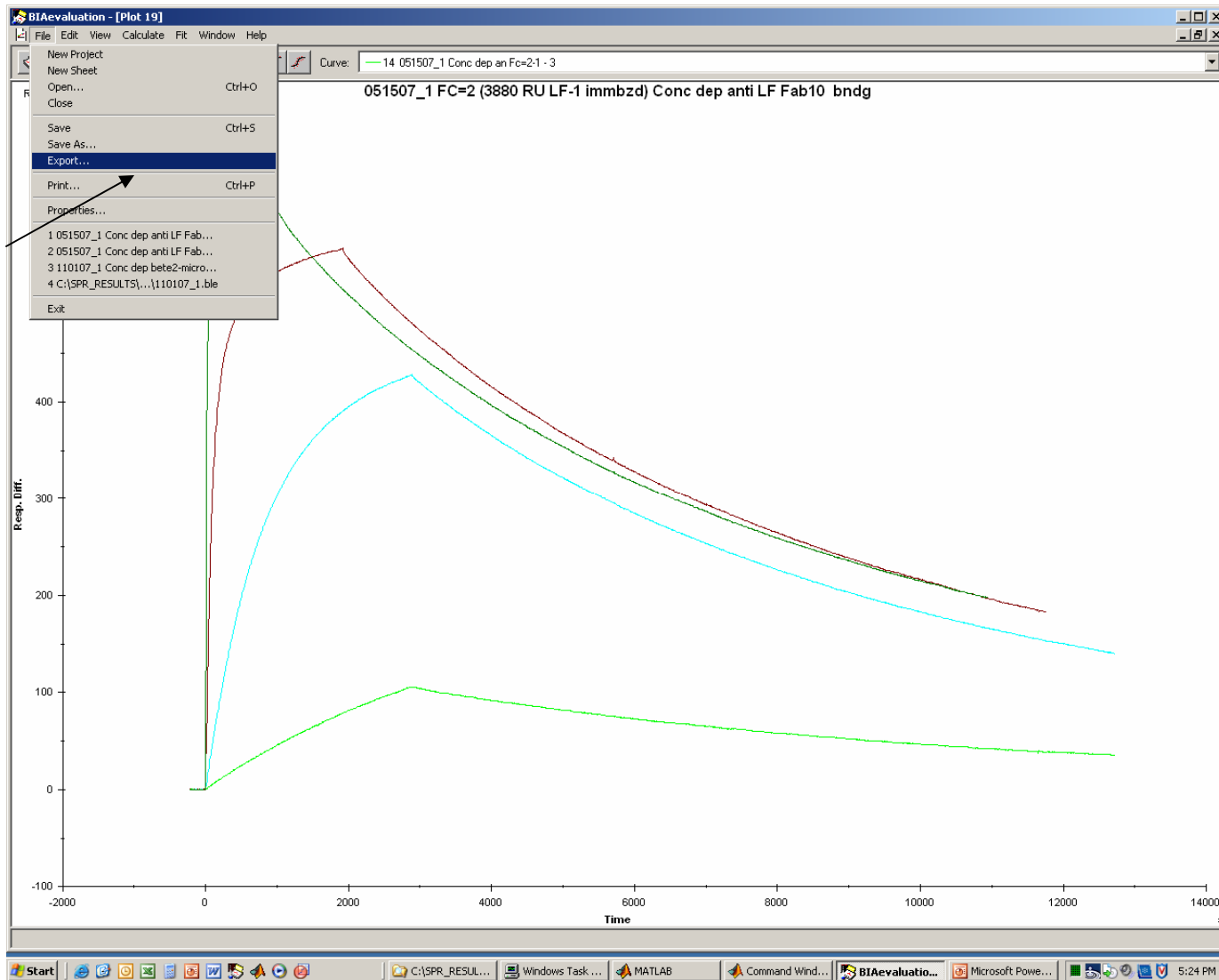
Choose All Files (*.*) and open the txt.file

Choose Delimited file type, click <Next>

Choose Tab as a delimiter, click <Next> and then <Finish>

Data will appear in the Microsoft Excel window

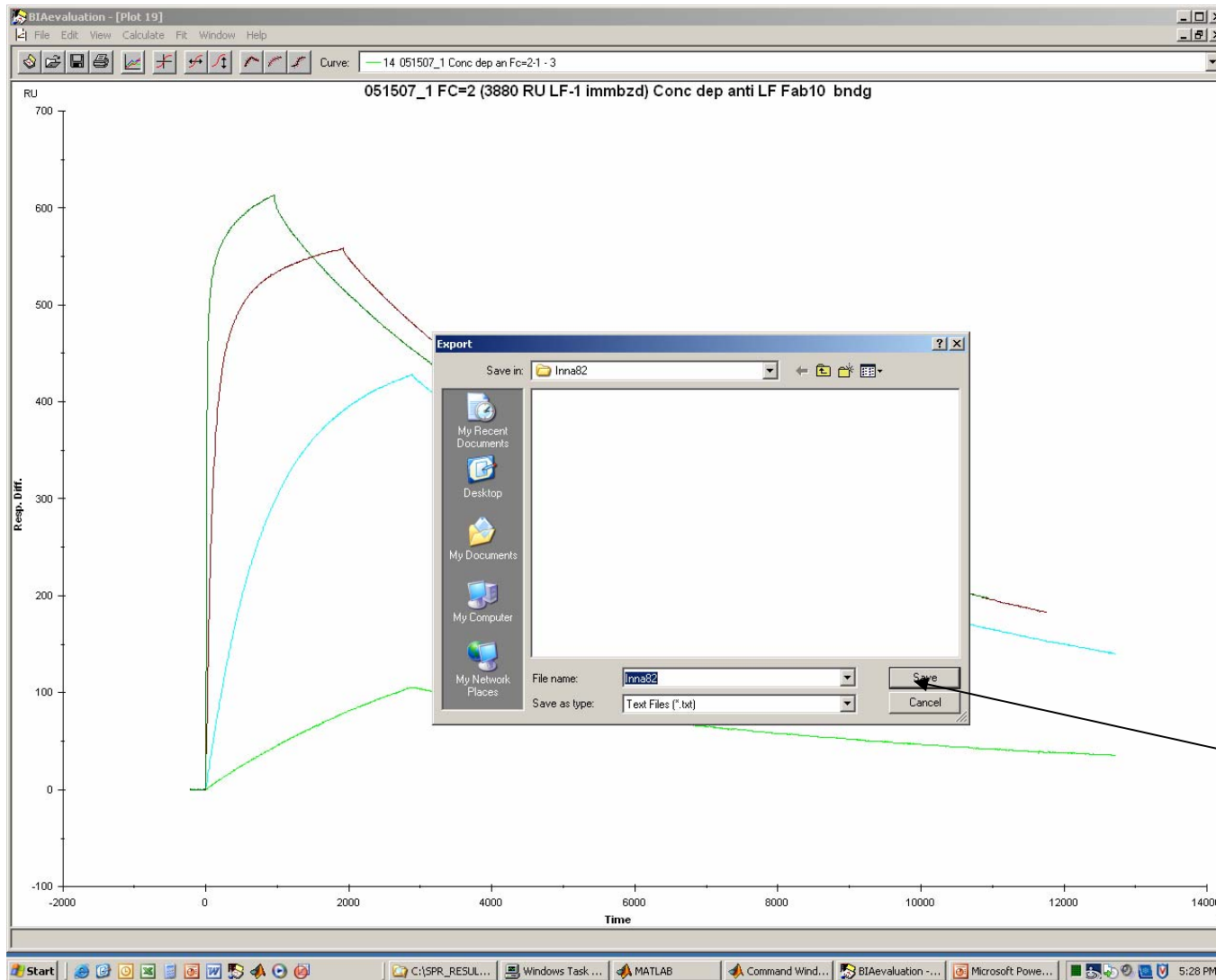
Save file in the (*.xls) format under the same name; close



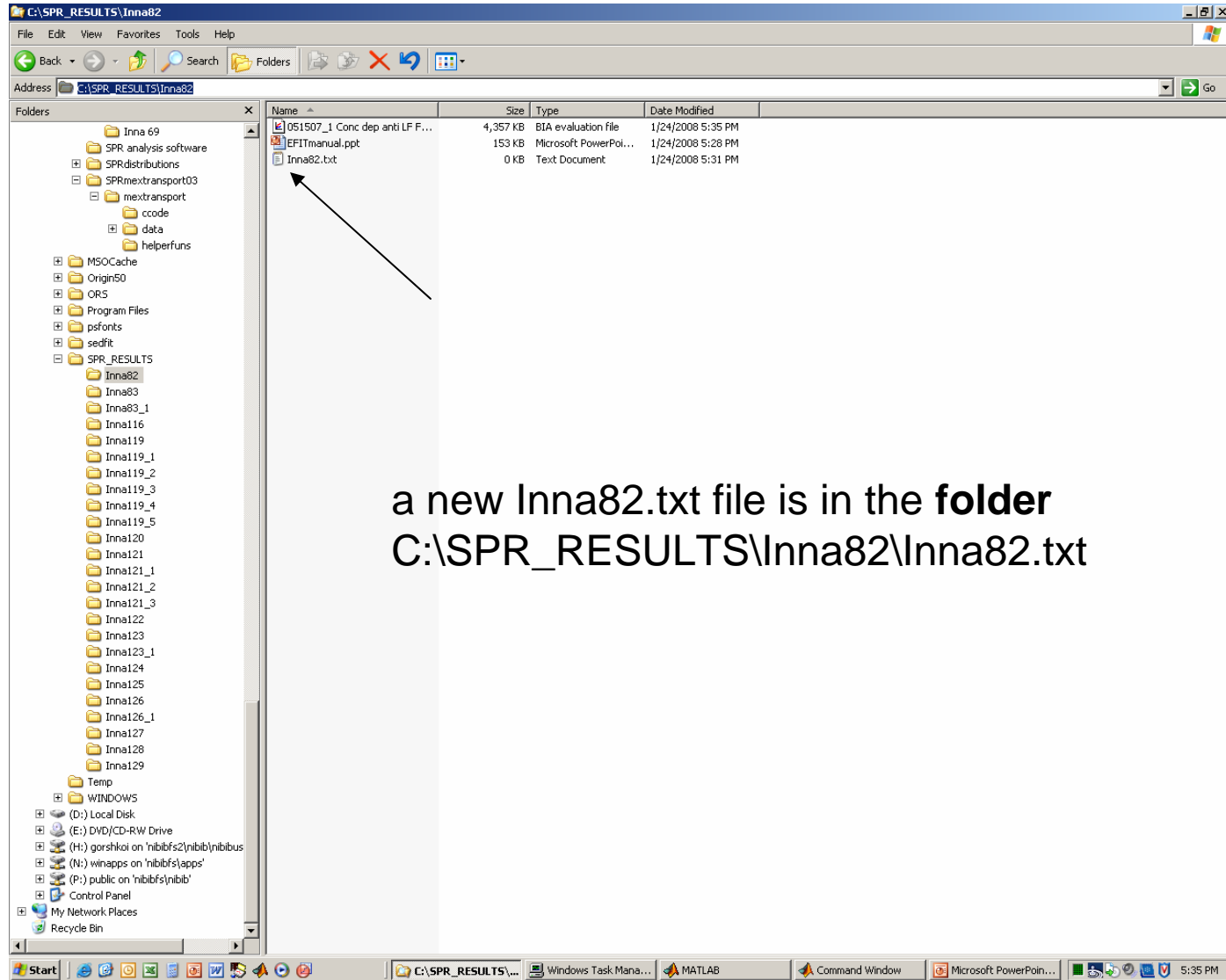
Data are aligned at time of injection = 0, and baseline corrected.

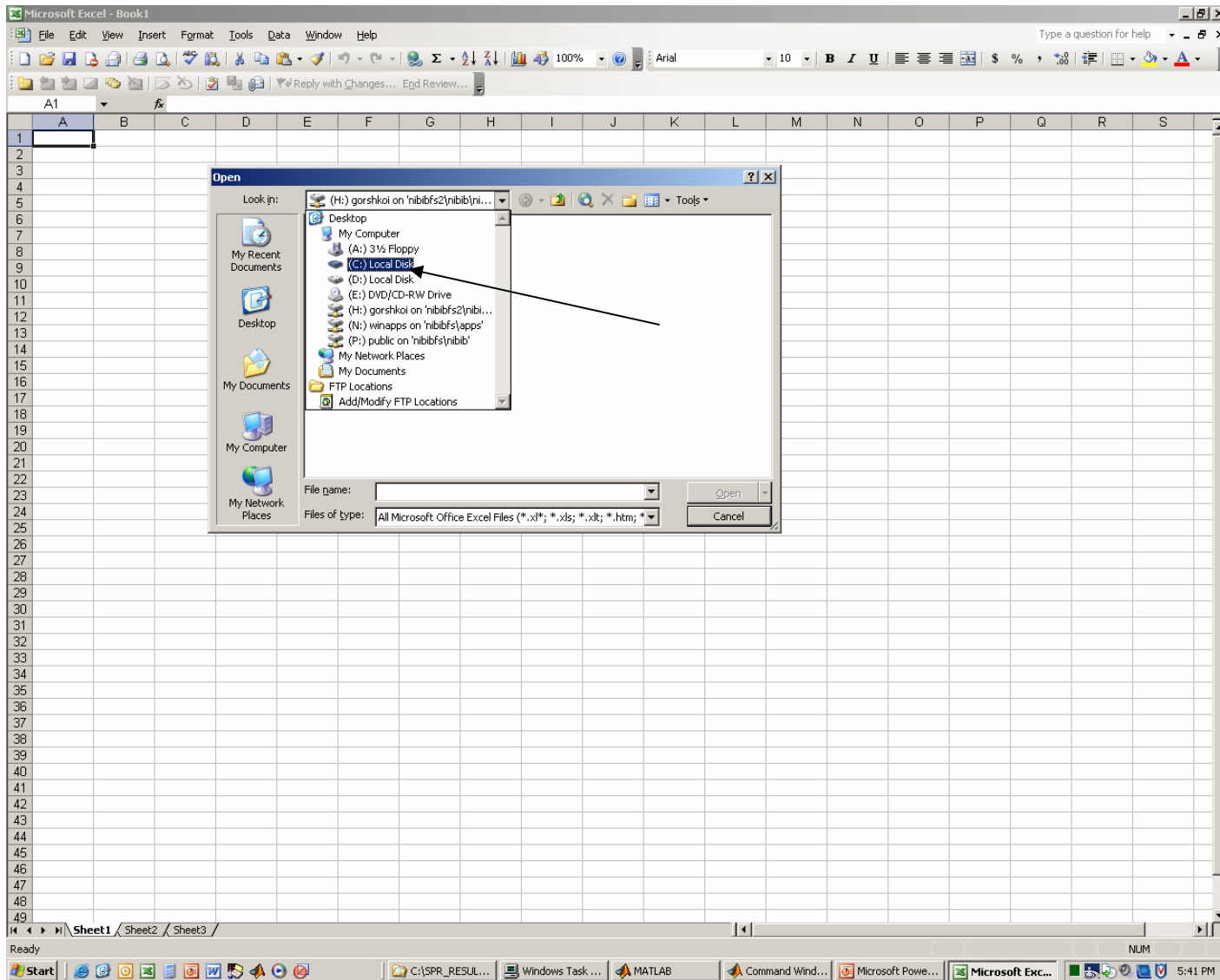
Create a **folder** on C-drive (in this case we call it C:\SPR_RESULTS\Inna82; use any name you like)

Copy BiaEvaluation file into the new **folder**, open it. Export data.



Export data into the same **folder** and save under a new name Inna82.
Close BiaEvaluation file;

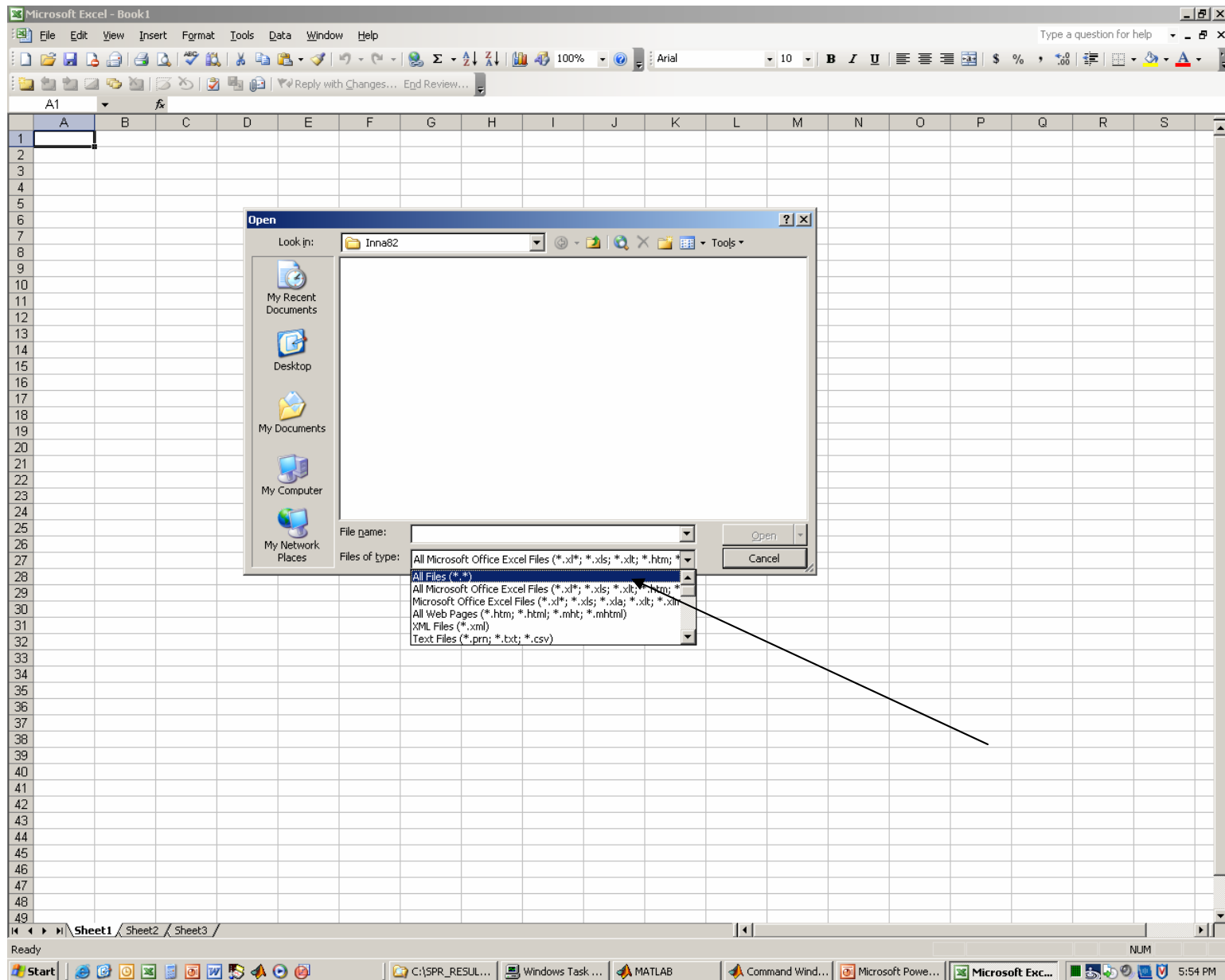




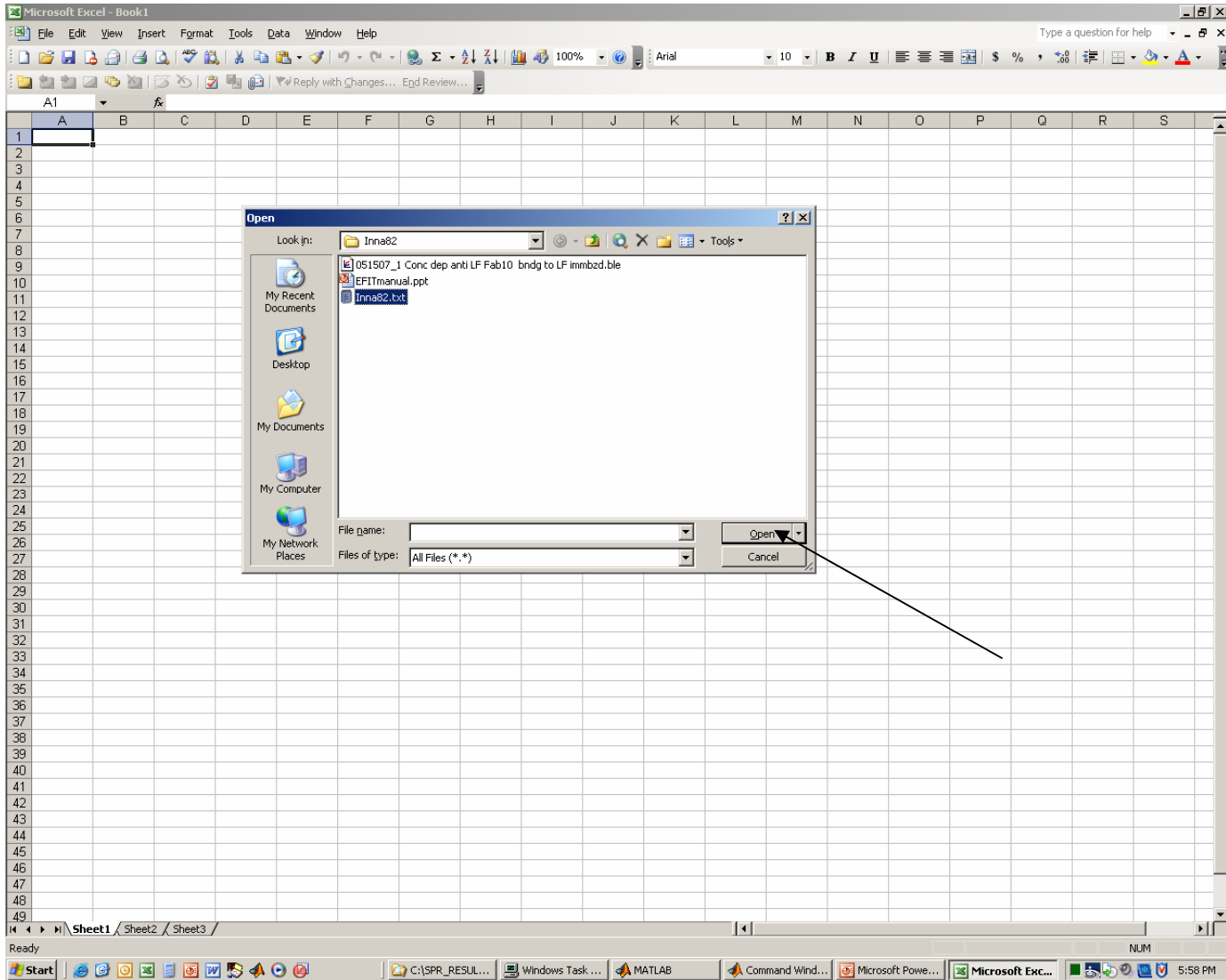
Open Microsoft Office Excel:

File/Open

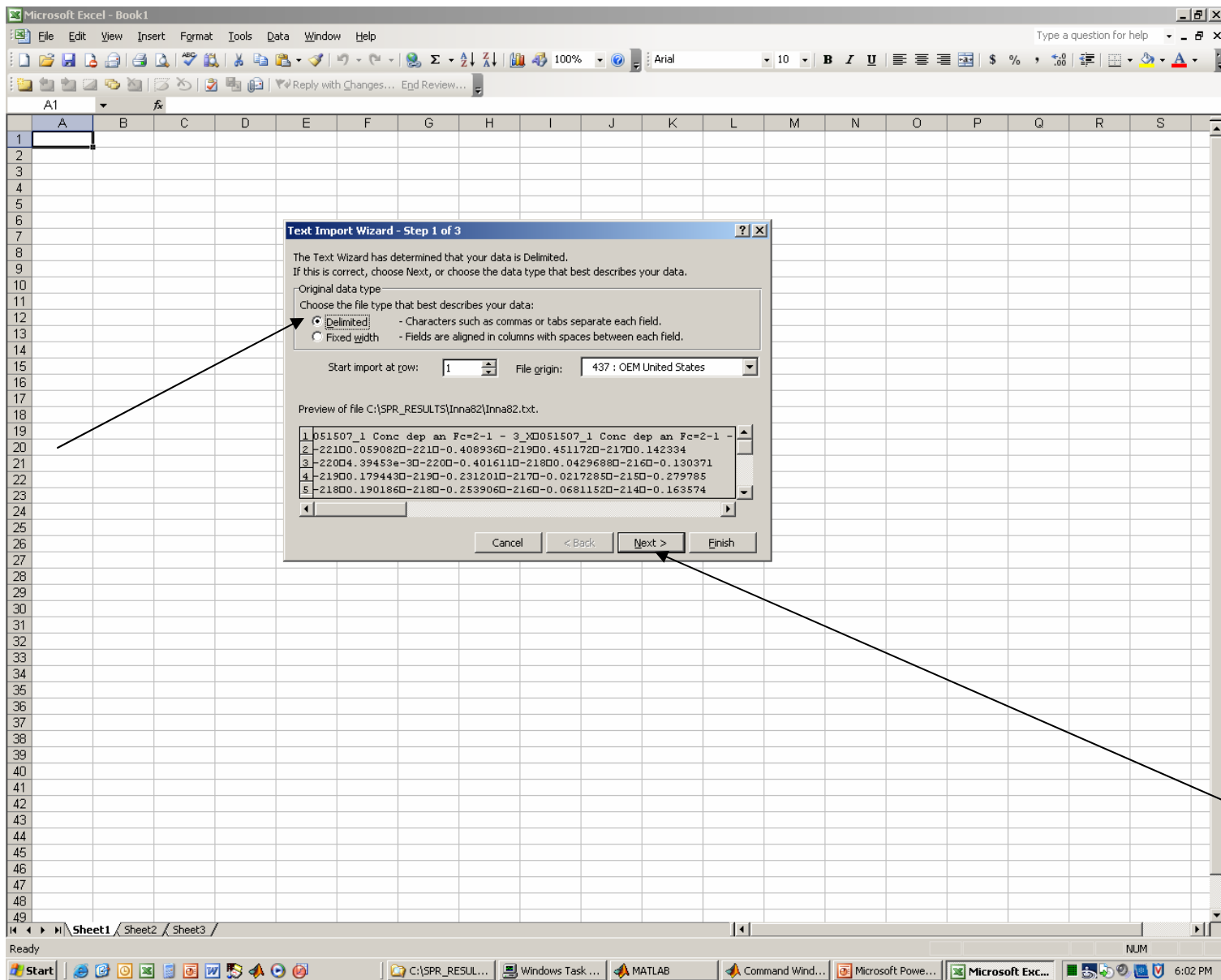
In the Selection window browse for the txt.file that is in the
folder C:\SPR_RESULTS\Inna82



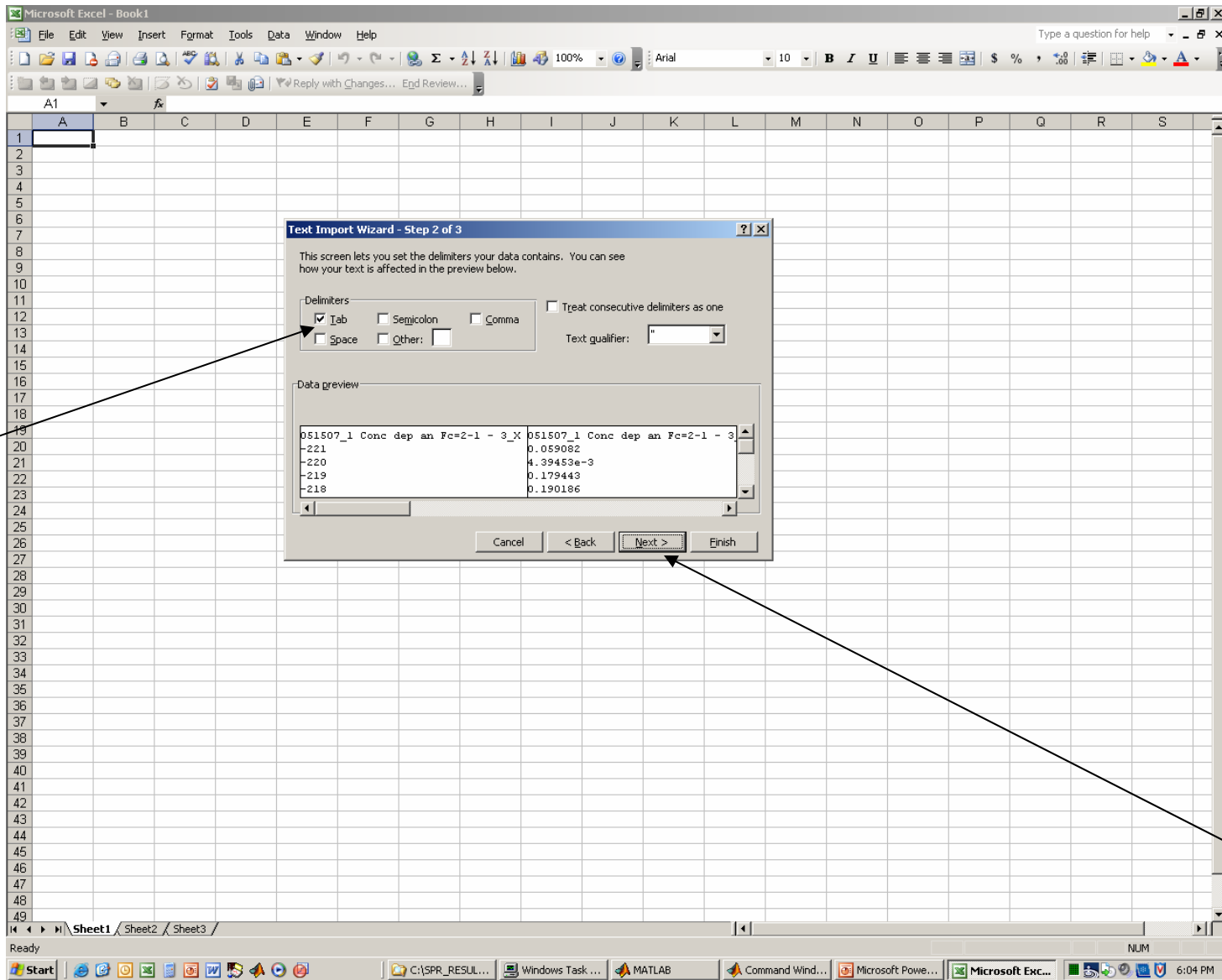
In the Files of type choose All Files (*.*)



Highlight and open the txt.file



Choose Delimited file type, click <Next>



Choose Tab as a delimiter, click <Next> and then <Finish>

Microsoft Excel - Inna82.txt

File Edit View Insert Format Tools Data Window Help

Type a question for help

100% Arial

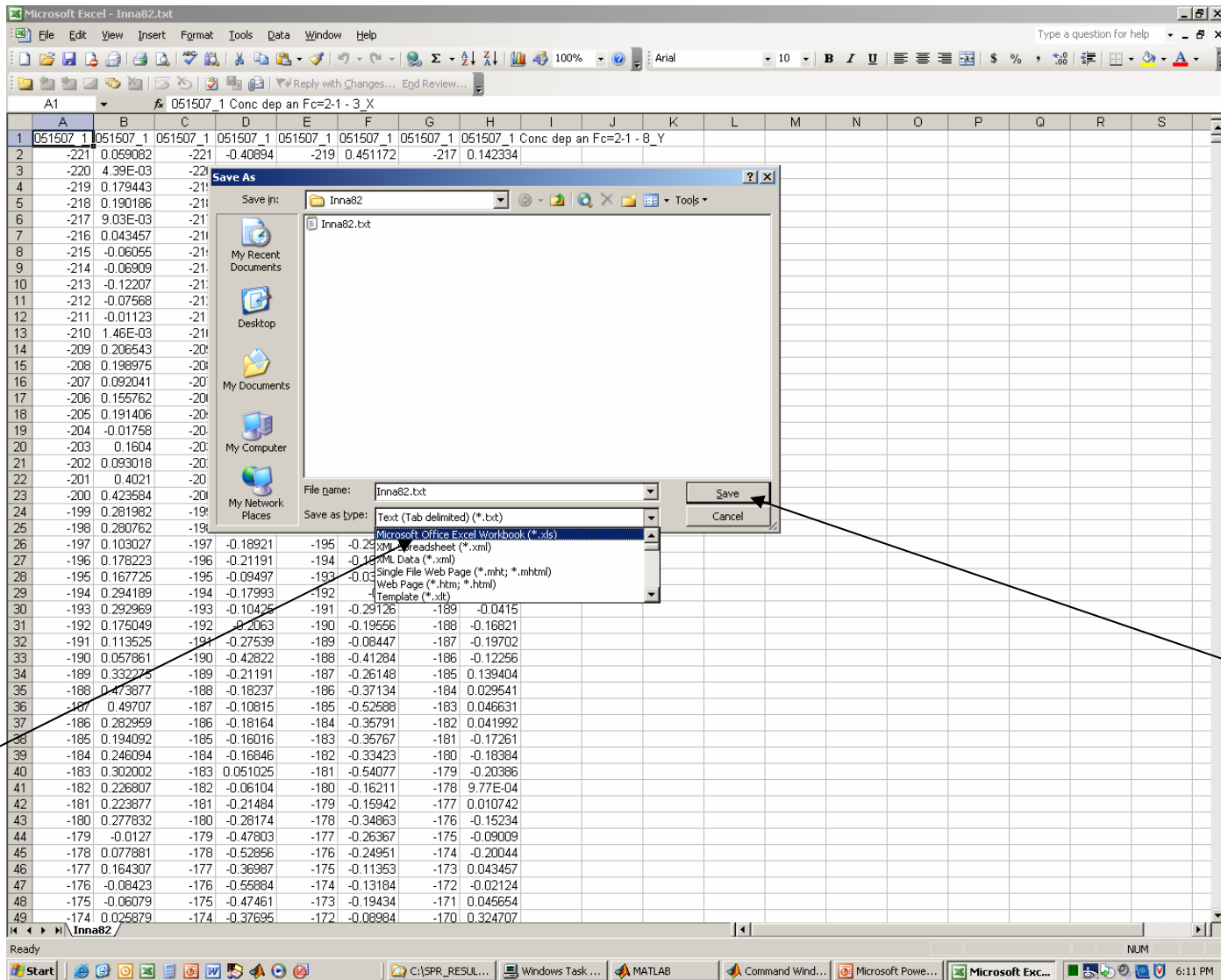
A1 = O51507_1 Conc dep an Fc=2-1 - 3_X

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	O51507_1	O51507_1	O51507_1	O51507_1	O51507_1	O51507_1	O51507_1	O51507_1	Conc dep an Fc=2-1 - 8_Y										
2	-221	0.059082	-221	-0.40894	-219	0.451172	-217	0.142334											
3	-220	4.39E-03	-220	-0.40161	-218	0.042969	-216	-0.13037											
4	-219	0.179443	-219	-0.2312	-217	-0.02173	-215	-0.27979											
5	-218	0.190186	-218	-0.25391	-216	-0.06812	-214	-0.16357											
6	-217	9.03E-03	-217	-0.42481	-215	-6.84E-03	-213	0.030273											
7	-216	0.043457	-216	-0.36816	-214	3.66E-03	-212	0.050781											
8	-215	-0.06055	-215	-0.38867	-213	-0.16211	-211	0.190674											
9	-214	-0.06909	-214	-0.29712	-212	-0.0769	-210	0.048628											
10	-213	-0.12207	-213	-0.36255	-211	-0.3269	-209	0.033936											
11	-212	-0.07568	-212	-0.47778	-210	-0.29663	-208	-0.06982											
12	-211	-0.01123	-211	-0.31909	-209	-0.30786	-207	-0.15088											
13	-210	1.46E-03	-210	-0.20239	-208	-0.21655	-206	-0.02759											
14	-209	0.206543	-209	7.81E-03	-207	-0.12915	-205	-0.02686											
15	-208	0.198975	-208	-0.05005	-206	-0.04248	-204	0.03833											
16	-207	0.092041	-207	-0.17676	-205	-0.16333	-203	0.05249											
17	-206	0.155762	-206	-0.10278	-204	-0.05981	-202	-0.13159											
18	-205	0.191406	-205	-0.09937	-203	-0.24048	-201	-0.3396											
19	-204	-0.01758	-204	-0.33911	-202	-0.36792	-200	-0.17236											
20	-203	0.1604	-203	-0.20386	-201	-0.41455	-199	-0.20239											
21	-202	0.093018	-202	-0.33325	-200	-0.10571	-198	-0.19141											
22	-201	0.4021	-201	-0.18457	-199	0.036133	-197	-0.16309											
23	-200	0.423584	-200	-0.16406	-198	1.22E-03	-196	-0.45823											
24	-199	0.281982	-199	-0.04443	-197	-0.17065	-195	-0.39941											
25	-198	0.280762	-198	-0.11548	-196	-0.1582	-194	-0.30566											
26	-197	0.103027	-197	-0.18921	-195	-0.29224	-193	-0.12329											
27	-196	0.178223	-196	-0.21191	-194	-0.19678	-192	-0.3147											
28	-195	0.167725	-195	-0.09497	-193	-0.03857	-191	-0.23023											
29	-194	0.294189	-194	-0.17993	-192	-0.25	-190	-0.25098											
30	-193	0.292969	-193	-0.10425	-191	-0.29126	-189	-0.0415											
31	-192	0.175049	-192	-0.2063	-190	-0.19556	-188	-0.16821											
32	-191	0.113525	-191	-0.27539	-189	-0.08447	-187	-0.19702											
33	-190	0.057861	-190	-0.42822	-188	-0.41284	-186	-0.12256											
34	-189	0.332275	-189	-0.21191	-187	-0.26148	-185	0.139404											
35	-188	0.473877	-188	-0.18237	-186	-0.37134	-184	0.029541											
36	-187	0.49707	-187	-0.10815	-185	-0.52588	-183	0.046631											
37	-186	0.282959	-186	-0.18164	-184	-0.35791	-182	0.041992											
38	-185	0.194092	-185	-0.16016	-183	-0.35767	-181	-0.17261											
39	-184	0.246094	-184	-0.16846	-182	-0.33423	-180	-0.18384											
40	-183	0.302002	-183	0.051025	-181	-0.54077	-179	-0.20386											
41	-182	0.226807	-182	-0.06104	-180	-0.16211	-178	9.77E-04											
42	-181	0.223877	-181	-0.21484	-179	-0.15942	-177	0.010742											
43	-180	0.277832	-180	-0.28174	-178	-0.34863	-176	-0.15234											
44	-179	-0.0127	-179	-0.47803	-177	-0.26367	-175	-0.09009											
45	-178	0.077881	-178	-0.52856	-176	-0.24951	-174	-0.20044											
46	-177	0.164307	-177	-0.36987	-175	-0.11363	-173	0.043457											
47	-176	-0.08423	-176	-0.55884	-174	-0.13184	-172	-0.02124											
48	-175	-0.06079	-175	-0.47461	-173	-0.19434	-171	0.045654											
49	-174	0.025879	-174	-0.37695	-172	-0.08984	-170	0.324707											

Ready NUM

Start | C:\SPR_RESUL... | Windows Task... | MATLAB | Command Wind... | Microsoft Powe... | Microsoft Exc... | 6:07 PM

Data will appear in the Microsoft Excel window



Save file in the (*.xls) format under the same name; close it

2) Installation, Starting of EVILFIT, and Loading of Files

- Install EVILFIT on (C:) local disk, as described in the file 'installation notes.txt'
- Open MATLAB
- In the Command Window Set Path to EVILFIT. Save Path. (You only need to do this the first time you start EVILFIT.)
- In the Command Window type EVILFIT and click <enter>
- EVILFIT will appear on the screen. Maximize it to full screen view.
- File/open
 - In the Selection window browse for the xls.file that is in the folder C:\SPR_RESULTS\Inna82\Inna82.xls; <open>

The screenshot shows a Windows Explorer window with the following details:

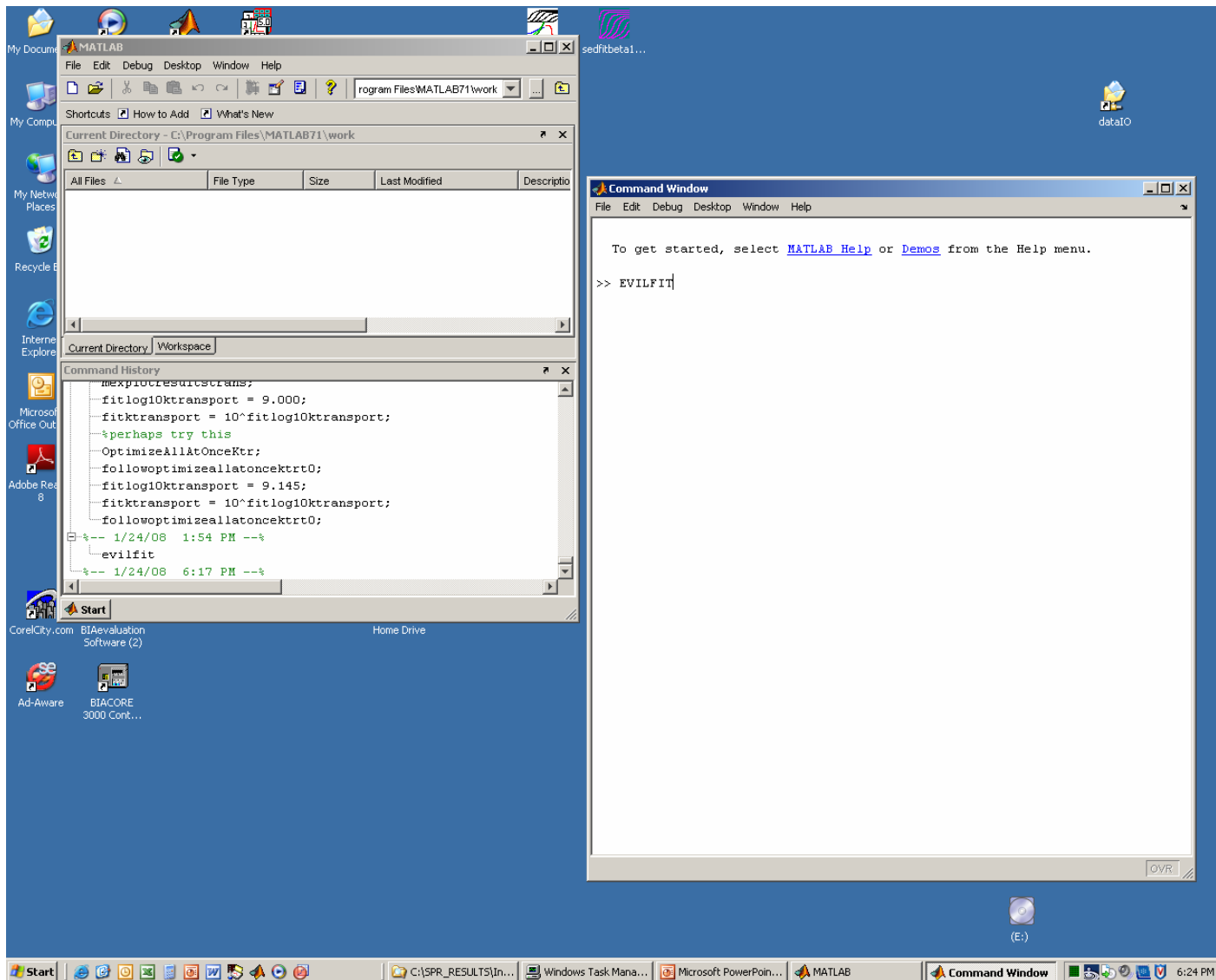
- Address Bar:** C:\
- Left Pane (Folders):** A tree view showing the directory structure. The '2d5SPRgui' folder is selected. Other visible folders include Desktop, My Documents, My Computer, (A:) 3 1/2 Floppy, (C:) Local Disk, 2d5SPRgui, Bia Users, Documents and Settings, evilfit, MATLAB6p5, new codes for the prior proba, RESULTS, SPR analysis software, SPRdistributions, SPRmextranport03, MSOCache, Origin50, ORS, Program Files, psfonts, sedfit, SPR_RESULTS, Inna82, Inna83, Inna83_1, Inna116, Inna119, Inna119_1, Inna119_2, Inna119_3, Inna119_4, Inna119_5, Inna120, Inna121, Inna121_1, Inna121_2, Inna121_3, Inna122, Inna123, Inna123_1, Inna124, Inna125, Inna126, Inna126_1, Inna127, Inna128, Inna129, Temp, WINDOWS, and (D:) Local Disk.
- Right Pane (File List):** A table listing files and folders with columns for Name, Size, Type, and Date Modified.

Name	Size	Type	Date Modified
2d5SPRgui		File Folder	12/11/2007 2:31 PM
Bia Users		File Folder	9/12/2007 11:14 AM
Documents and Settings		File Folder	10/15/2007 12:11 PM
evilfit		File Folder	12/28/2007 5:34 PM
MATLAB6p5		File Folder	9/14/2007 1:42 PM
MSOCache		File Folder	12/7/2006 9:02 AM
Origin50		File Folder	9/12/2007 12:33 PM
ORS		File Folder	10/13/2005 7:44 AM
Program Files		File Folder	11/28/2007 11:26 AM
psfonts		File Folder	9/12/2007 12:16 PM
sedfit		File Folder	9/12/2007 4:00 PM
SPR_RESULTS		File Folder	1/24/2008 5:16 PM
Temp		File Folder	10/15/2007 9:10 AM
WINDOWS		File Folder	12/26/2007 9:38 AM
gvpcfg.bin	4 KB	BIN File	11/28/2005 5:28 PM
kl2log.htm	5 KB	HTML Document	11/28/2007 11:27 AM
logNewErrors	3 KB	File	1/23/2008 9:27 AM
ntkrnlpa.exe	2,009 KB	Application	8/3/2004 9:59 PM
ntoskrnl.exe	2,130 KB	Application	8/3/2004 10:20 PM

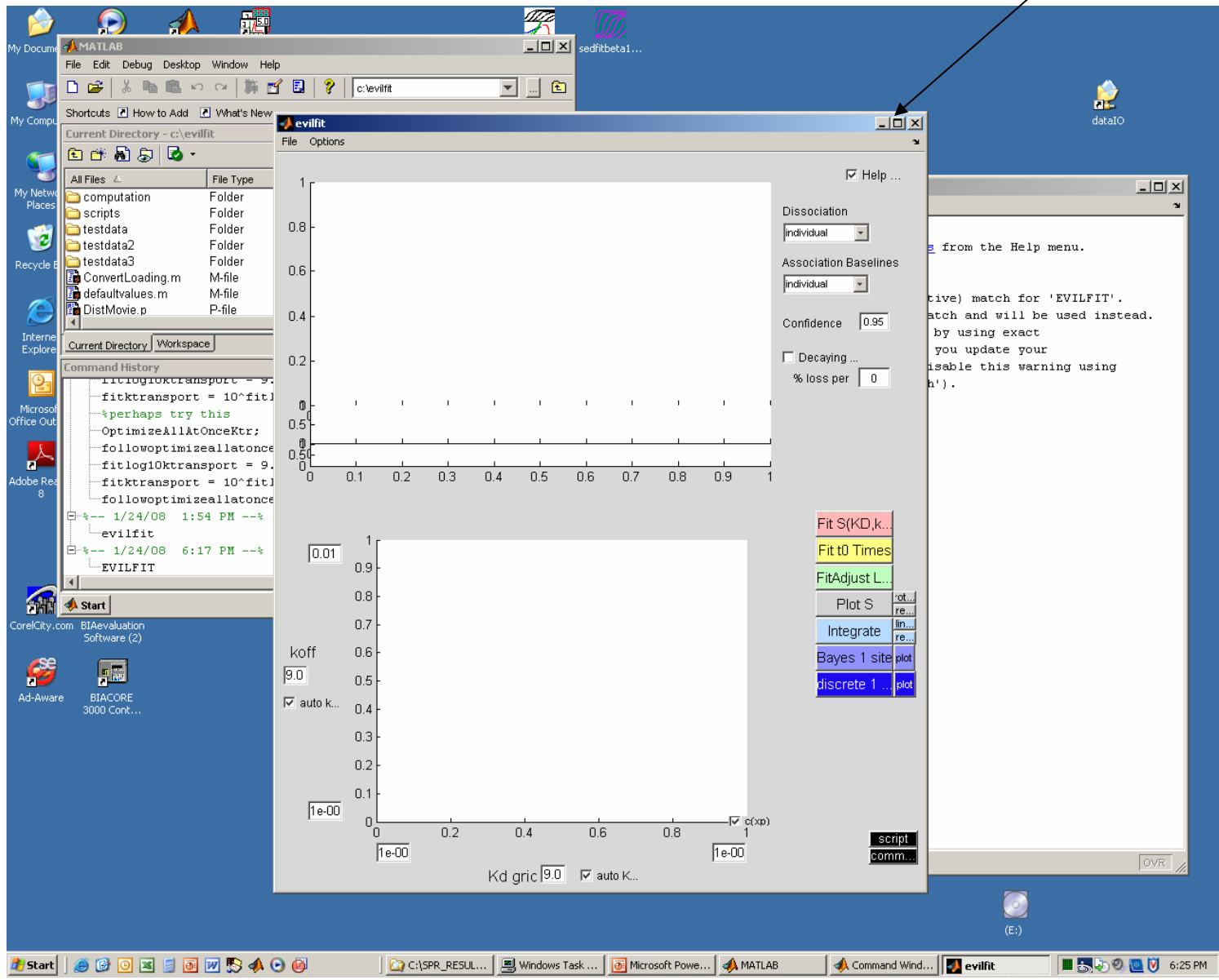
Arrows in the image indicate the following actions:

- One arrow points from the '2d5SPRgui' folder in the left pane to the '2d5SPRgui' folder in the right pane.
- Another arrow points from the 'evilfit' folder in the left pane to the 'evilfit' folder in the right pane.

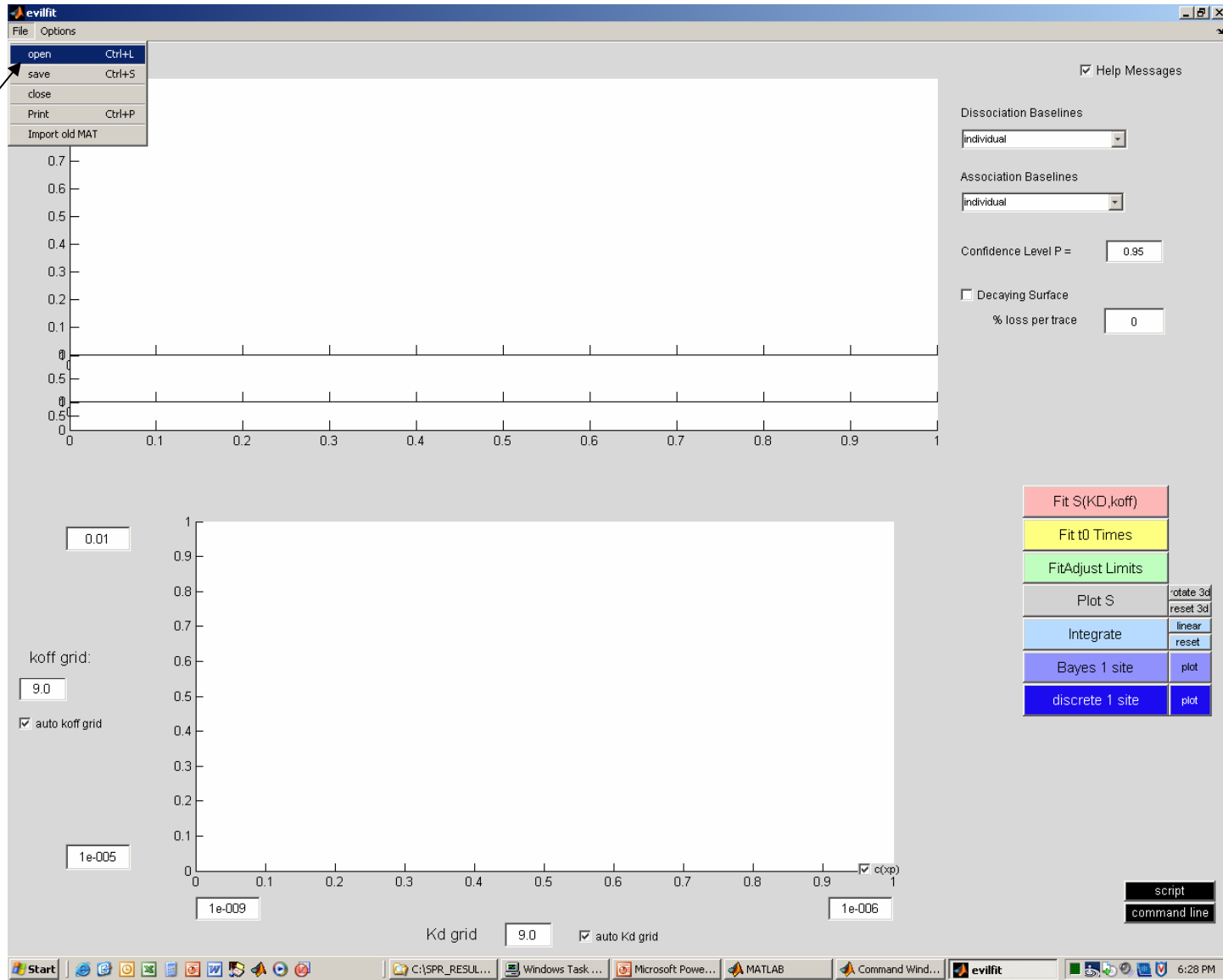
Set path slides,
Add description



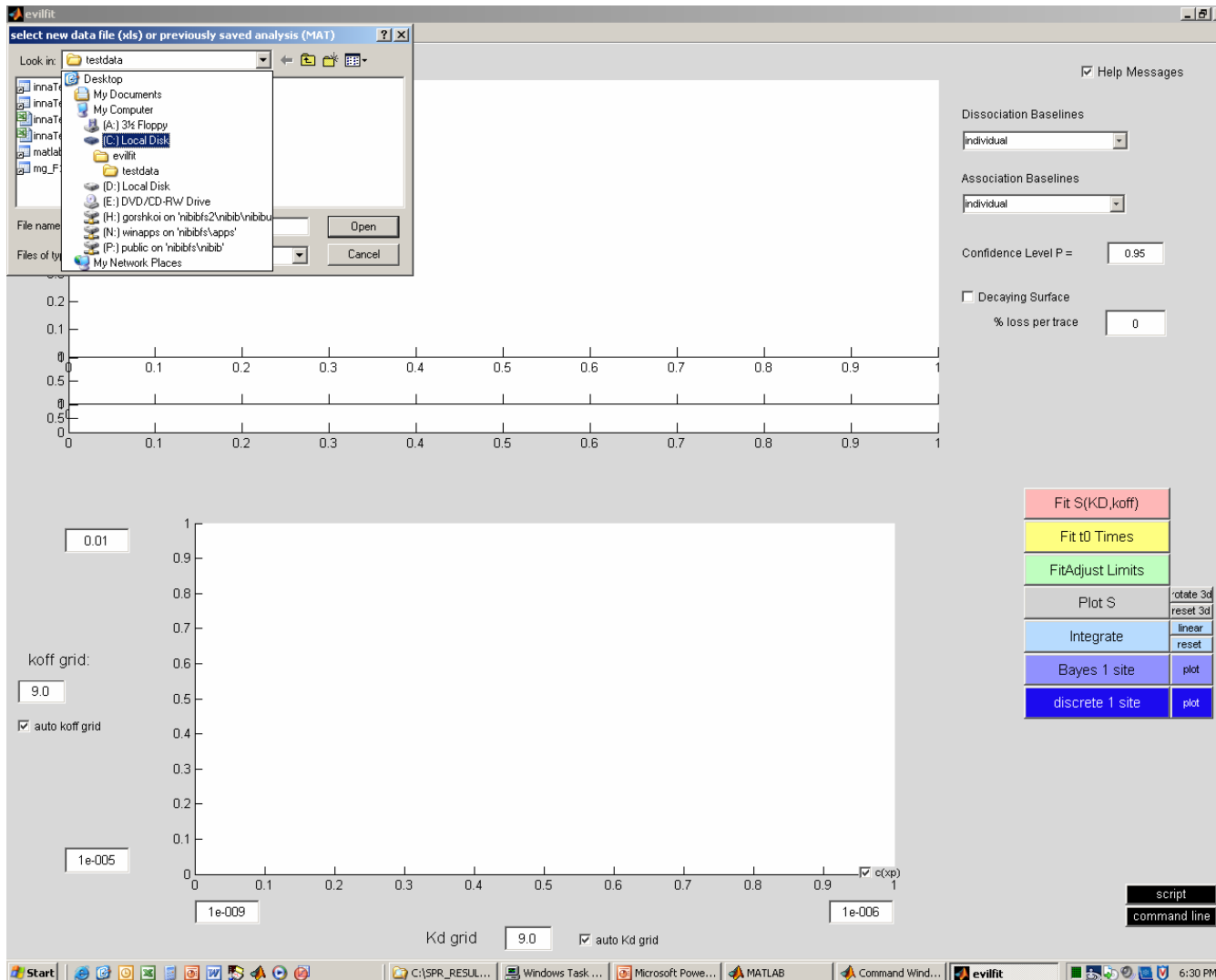
- In the Command Window type EVILFIT and click <enter>



- EVILFIT will appear on the screen. Maximize it



File/open



In the Selection window browse for the xls.file that is in the folder

C:\SPR_RESULTS\Inna82\Inna82.xls;

Note, a xls file with the same data is located in c:\evilfit\testdata

!

evilfit

select new data file (.xls) or previously saved analysis (MAT) ? X

Look in: SPR_RESULTS

- Inna82
- Inna83
- Inna83_1
- Inna116
- Inna119
- Inna119_1
- Inna119_2
- Inna119_3
- Inna119_4
- Inna119_5
- Inna120
- Inna121
- Inna121_1
- Inna121_2
- Inna121_3
- Inna122
- Inna123
- Inna123_1
- Inna124
- Inna125
- Inna126
- Inna126_1
- Inna127
- Inna128

File name:

Files of type: *.xls;*.mat

Open Cancel

Help Messages

Dissociation Baselines: individual

Association Baselines: individual

Confidence Level P = 0.95

Decaying Surface

% loss per trace: 0

0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

0.5
0
-0.5
-1
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

0.01

koff grid: 9.0

auto koff grid

1e-005

1e-009

Kd grid: 9.0 auto Kd grid

1e-006

Fit S(KD,koff)

Fit t0 Times

FitAdjust Limits

Plot S rotate 3d
reset 3d

Integrate linear
reset

Bayes 1 site plot

discrete 1 site plot

script

command line

Start

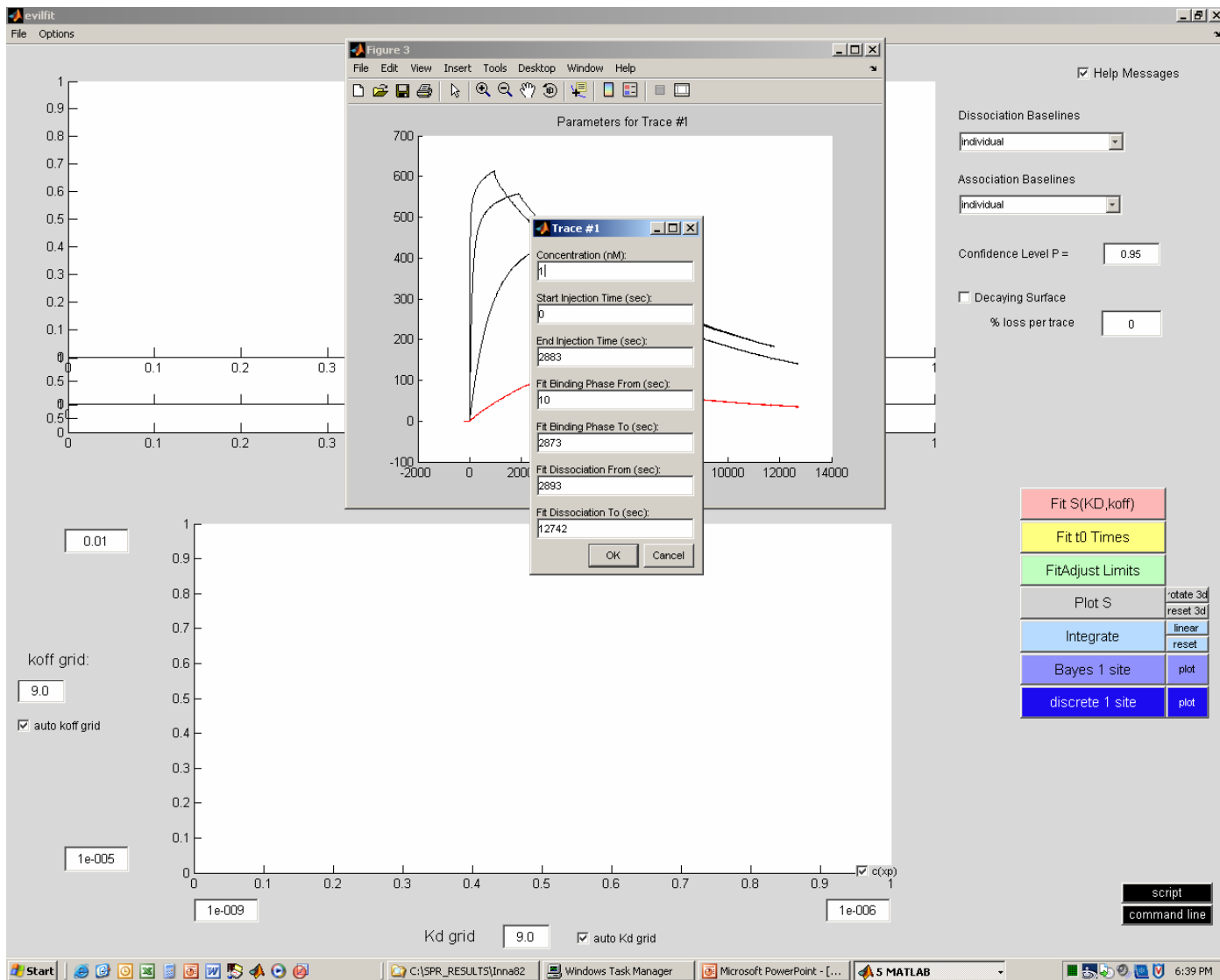
C:\SPR_RESUL... Windows Task... Microsoft Powe... MATLAB Command Wind... evilfit 6:35 PM

The screenshot displays the 'evilfit' software interface. A file selection dialog box is open in the foreground, titled 'select new data file (.xls) or previously saved analysis (.MAT)'. The dialog shows the 'Look in' directory as 'Inna82' and the selected file as 'Inna82.xls'. The 'File name' field contains 'Inna82.xls' and the 'Files of type' dropdown is set to '*.xls;*.mat'. The 'Open' button is highlighted. Two arrows point to the 'Inna82.xls' file and the 'Open' button.

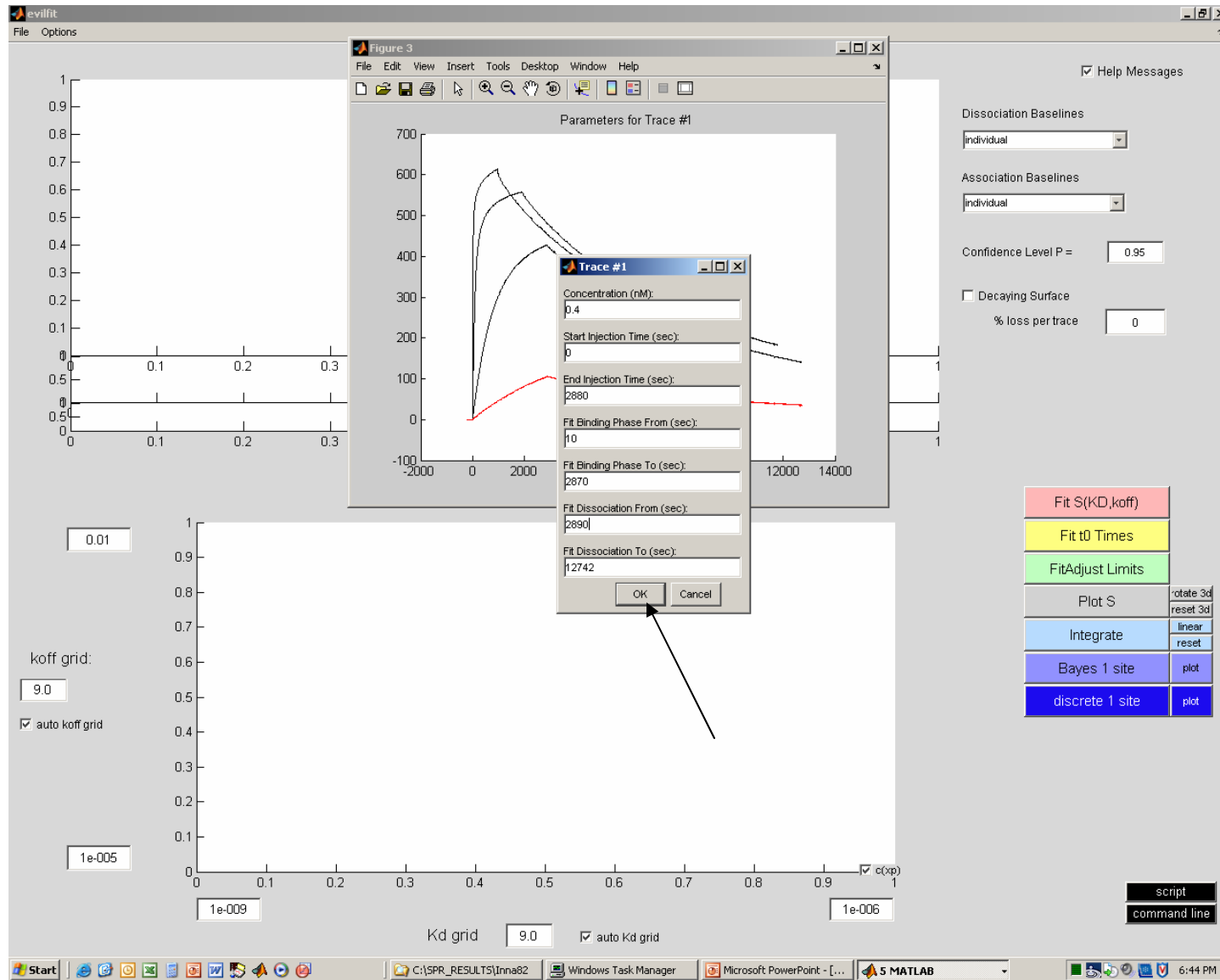
The background interface includes several control panels and plots:

- Help Messages:** A checked checkbox.
- Dissociation Baselines:** A dropdown menu set to 'individual'.
- Association Baselines:** A dropdown menu set to 'individual'.
- Confidence Level P =:** A text box containing '0.95'.
- Decaying Surface:** An unchecked checkbox.
- % loss per trace:** A text box containing '0'.
- Control Buttons:** A vertical stack of buttons: 'Fit S(KD,koff)' (red), 'Fit t0 Times' (yellow), 'FitAdjust Limits' (green), 'Plot S' (grey) with sub-buttons 'rotate 3d' and 'reset 3d', 'Integrate' (light blue) with sub-buttons 'linear' and 'reset', 'Bayes 1 site' (blue) with sub-button 'plot', and 'discrete 1 site' (dark blue) with sub-button 'plot'.
- Scripting:** 'script' and 'command line' buttons.
- Grids:** 'koff grid' with a text box '0.01' and '1e-005', and 'Kd grid' with a text box '9.0' and '1e-009'.
- Plots:** Two empty coordinate systems are visible. The top one has x-axis from 0 to 1 and y-axis from 0 to 0.2. The bottom one has x-axis from 0 to 1 and y-axis from 0 to 1.
- Taskbar:** Shows the Windows taskbar with the Start button and several open applications including 'C:\SPR_RESUL...', 'Windows Task...', 'Microsoft Powe...', 'MATLAB', 'Command Wind...', and 'evilfit'. The system clock shows '6:36 PM'.

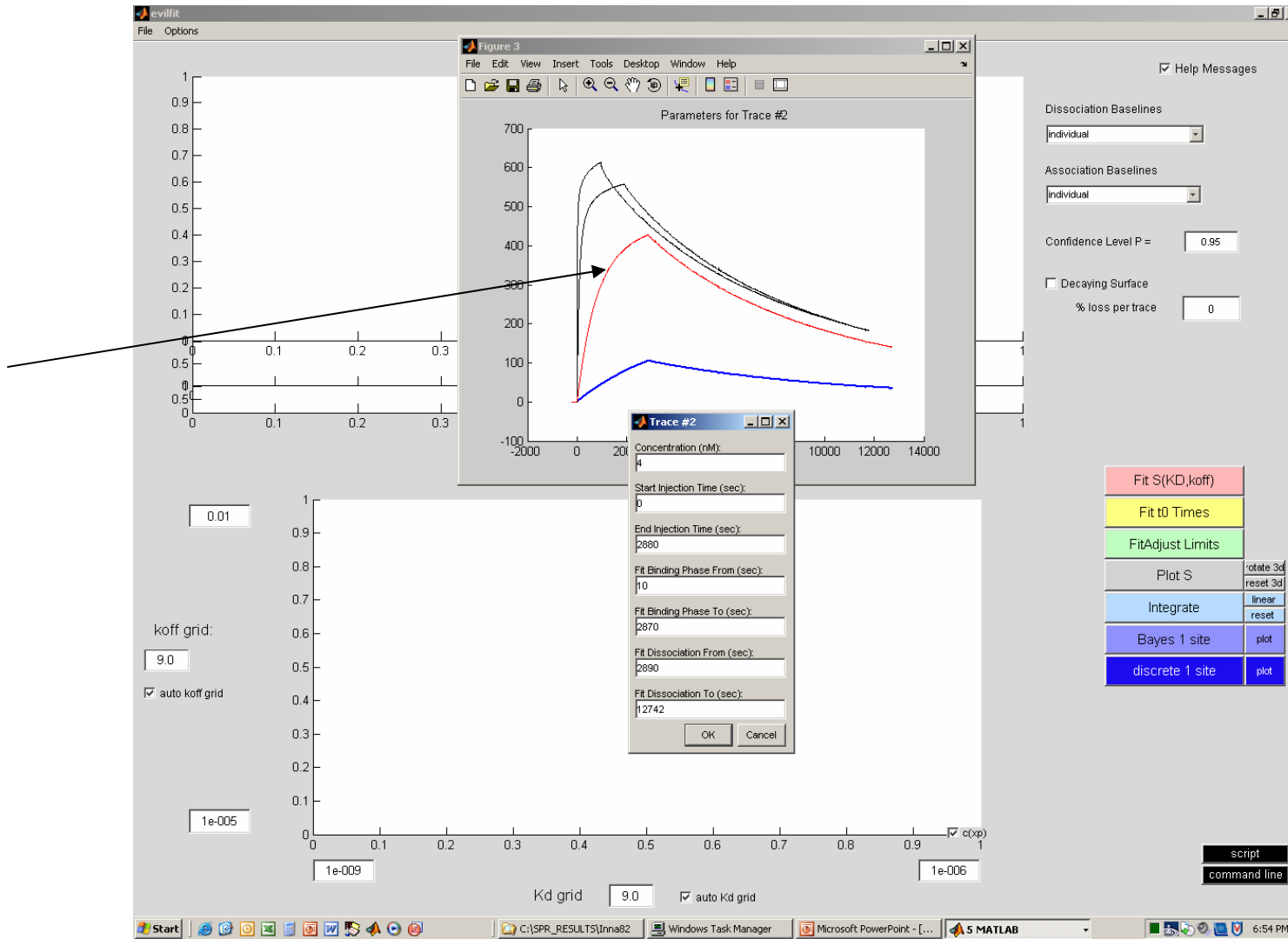
<open>



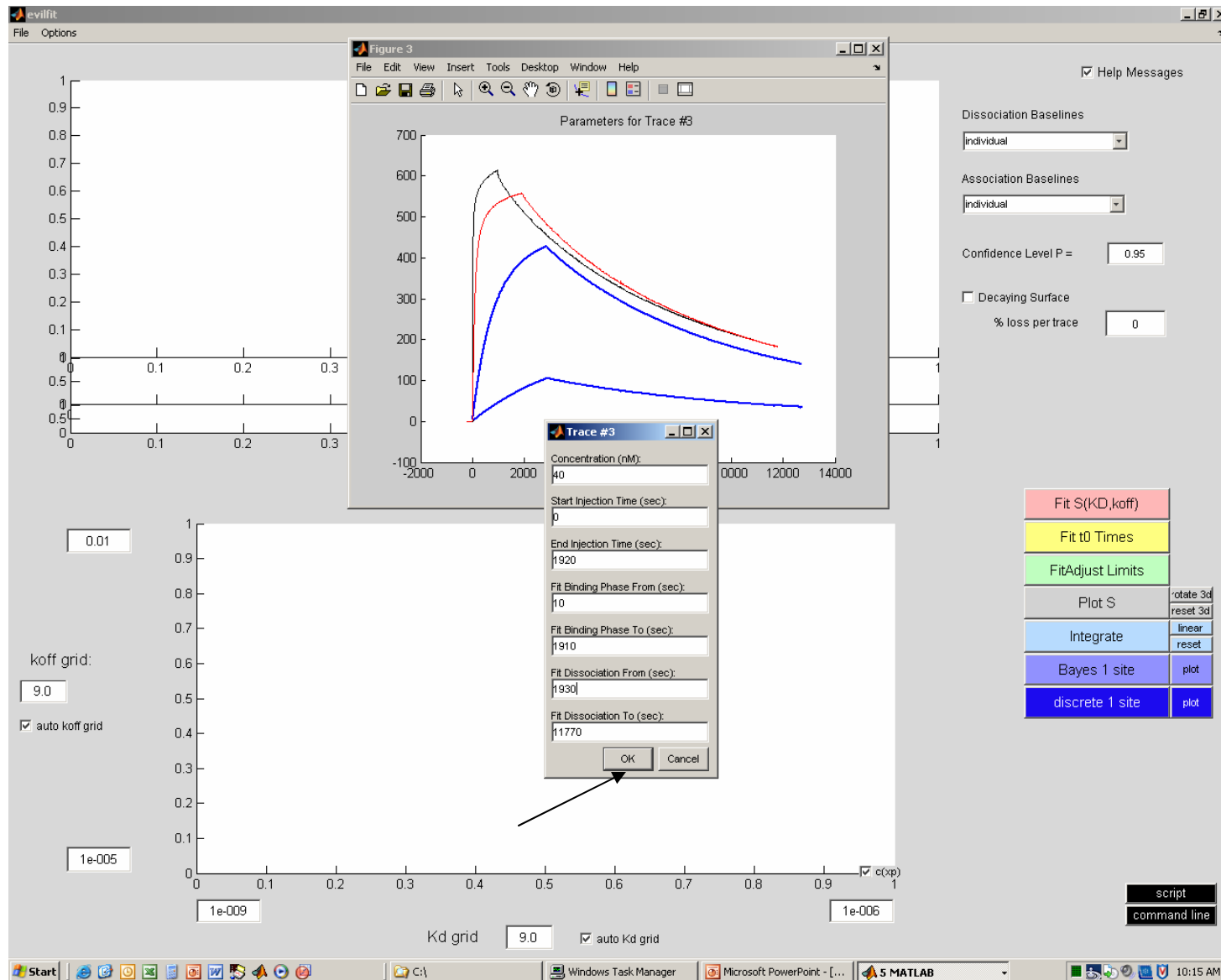
Window with experimental data is open; data for the trace#1 are highlighted in red. Parameters for Trace #1 can be introduced



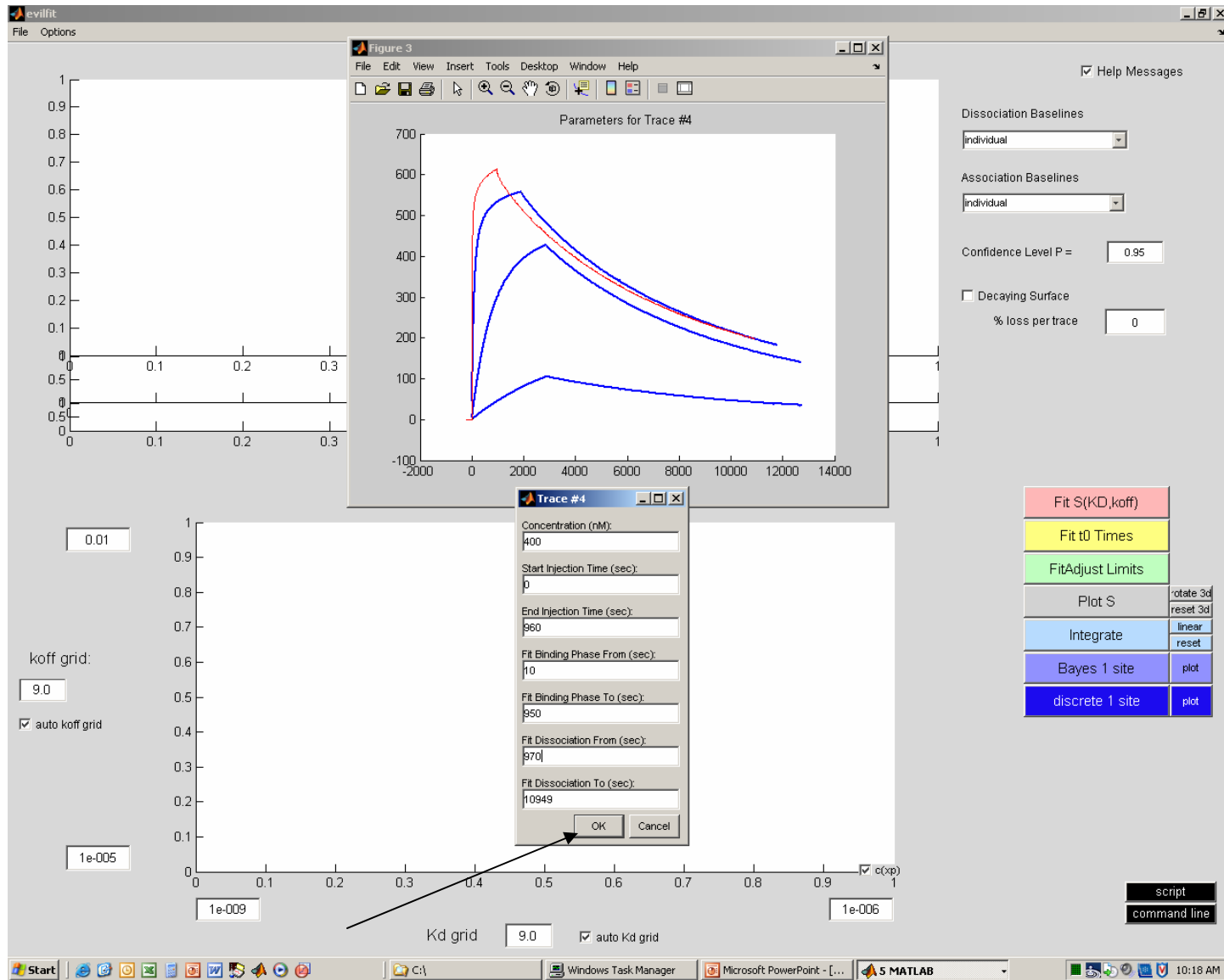
Parameters for Trace #1 are as shown; click <OK> to move to Parameters for Trace #2



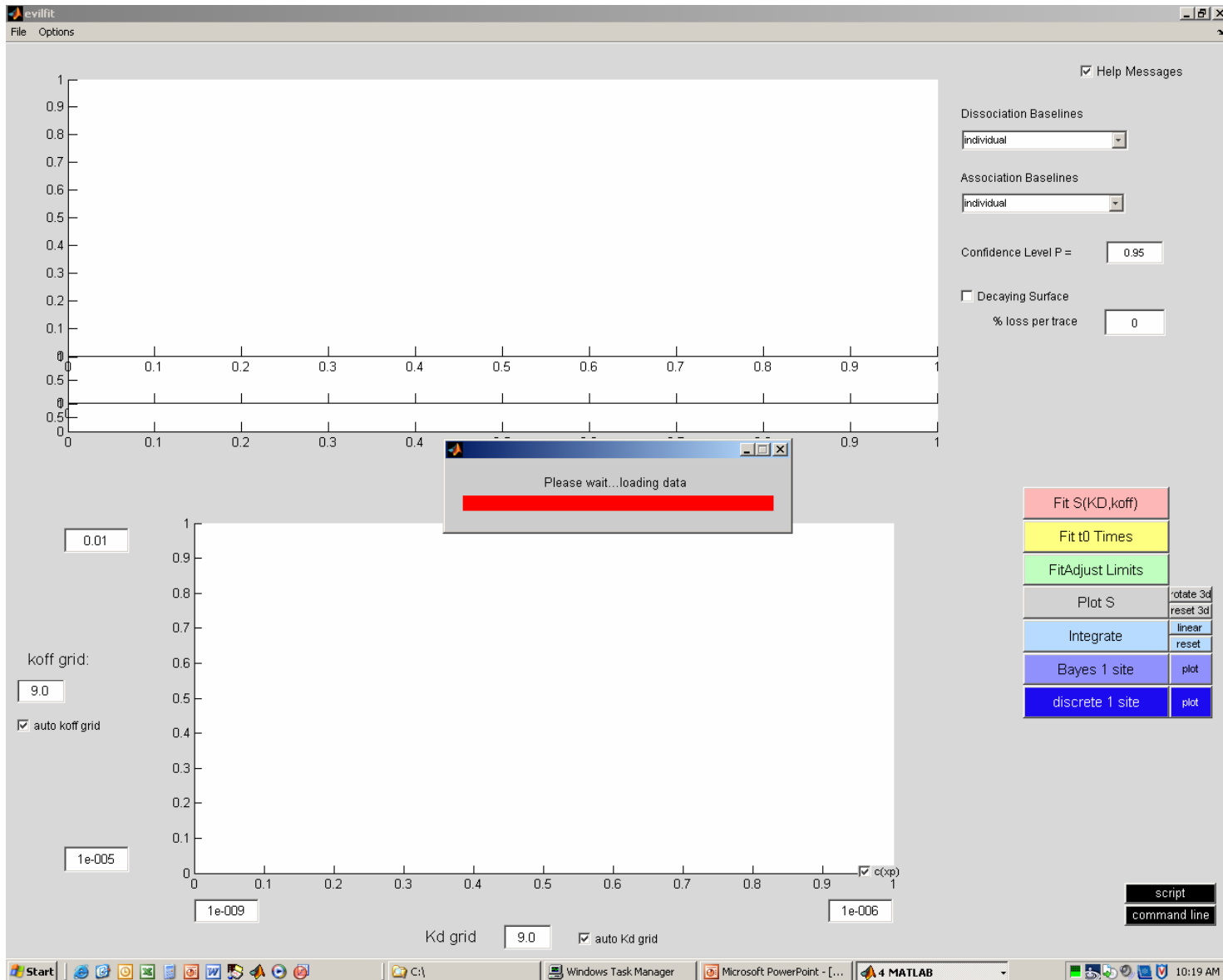
Data for the trace#2 are highlighted in red. Parameters for Trace #2 are as shown; click <OK> to move to Parameters for Trace #3



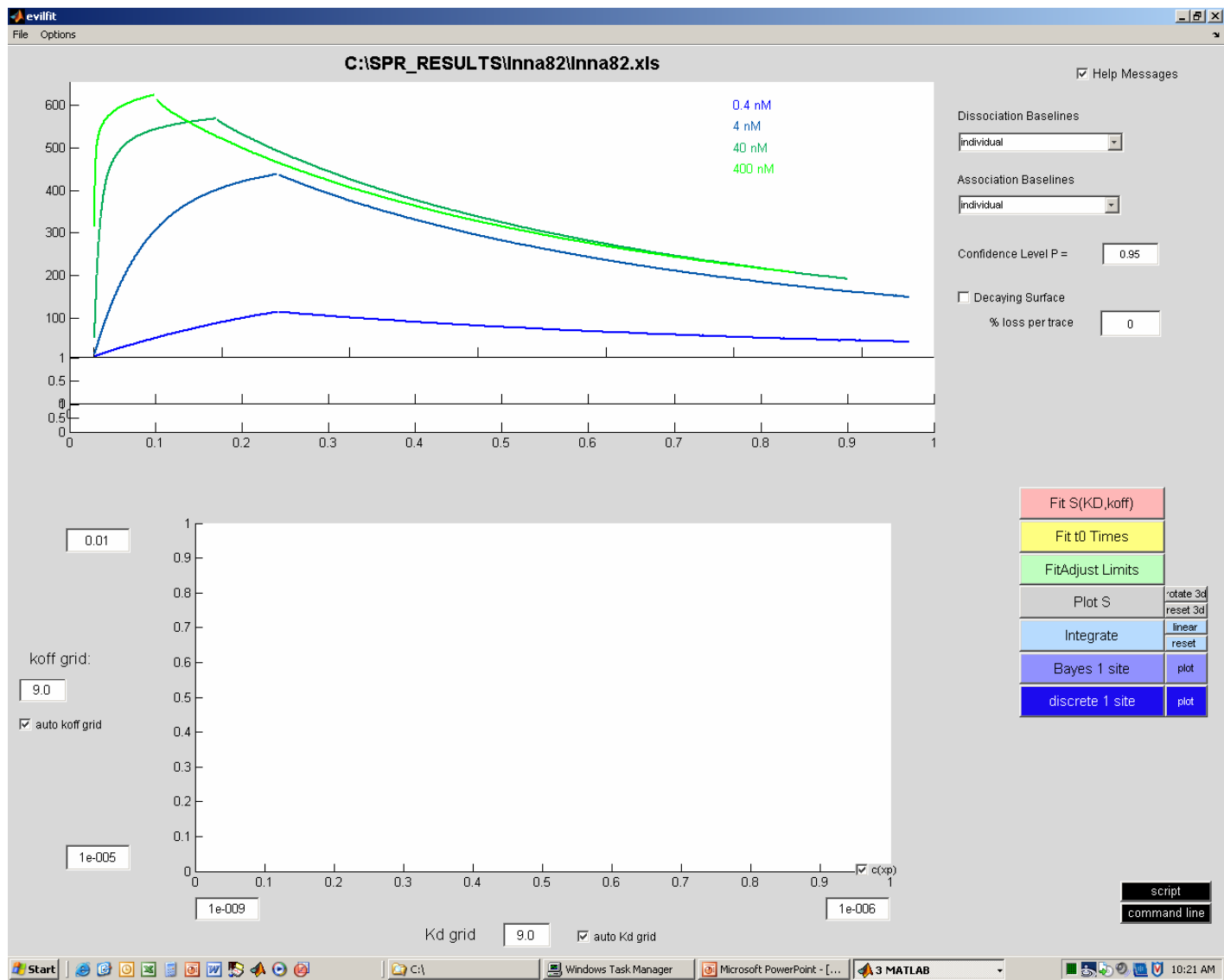
Data for the trace#3 are highlighted in red. Parameters for Trace #3 are as shown; click <OK> to move to Parameters for Trace #4



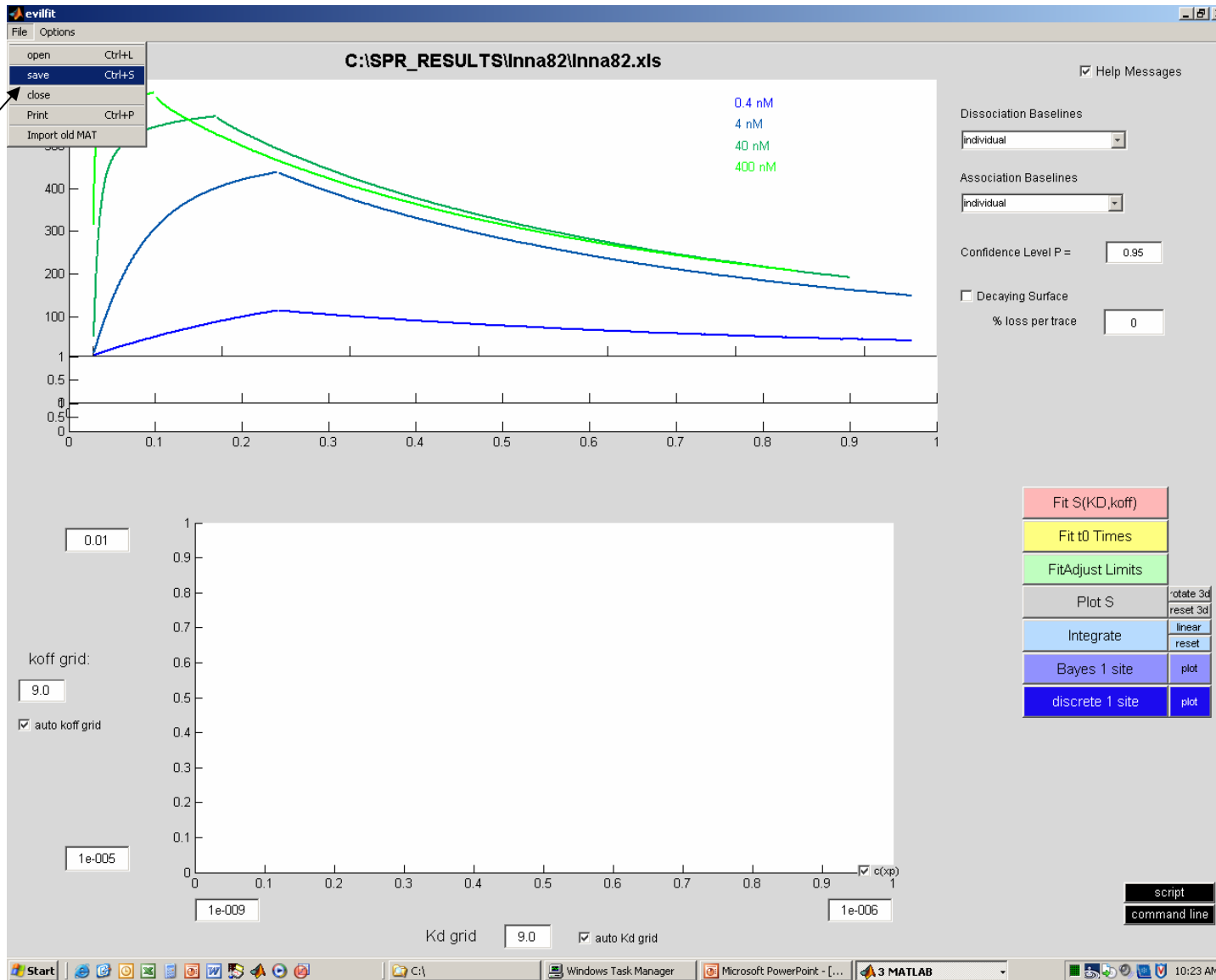
Data for the trace#4 are highlighted in red. Parameters for Trace #4 are as shown; click <OK> to complete the procedure



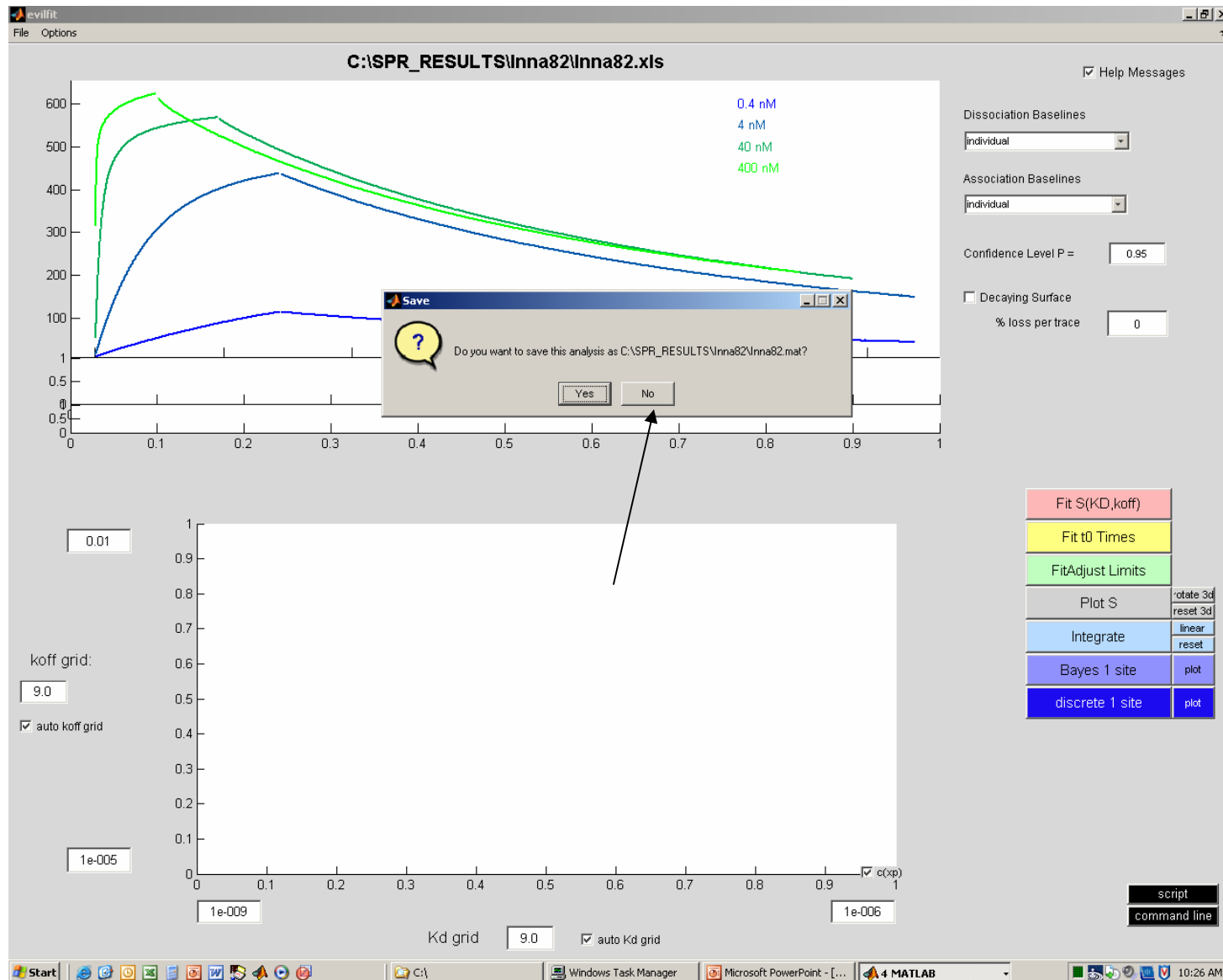
Data are loading...



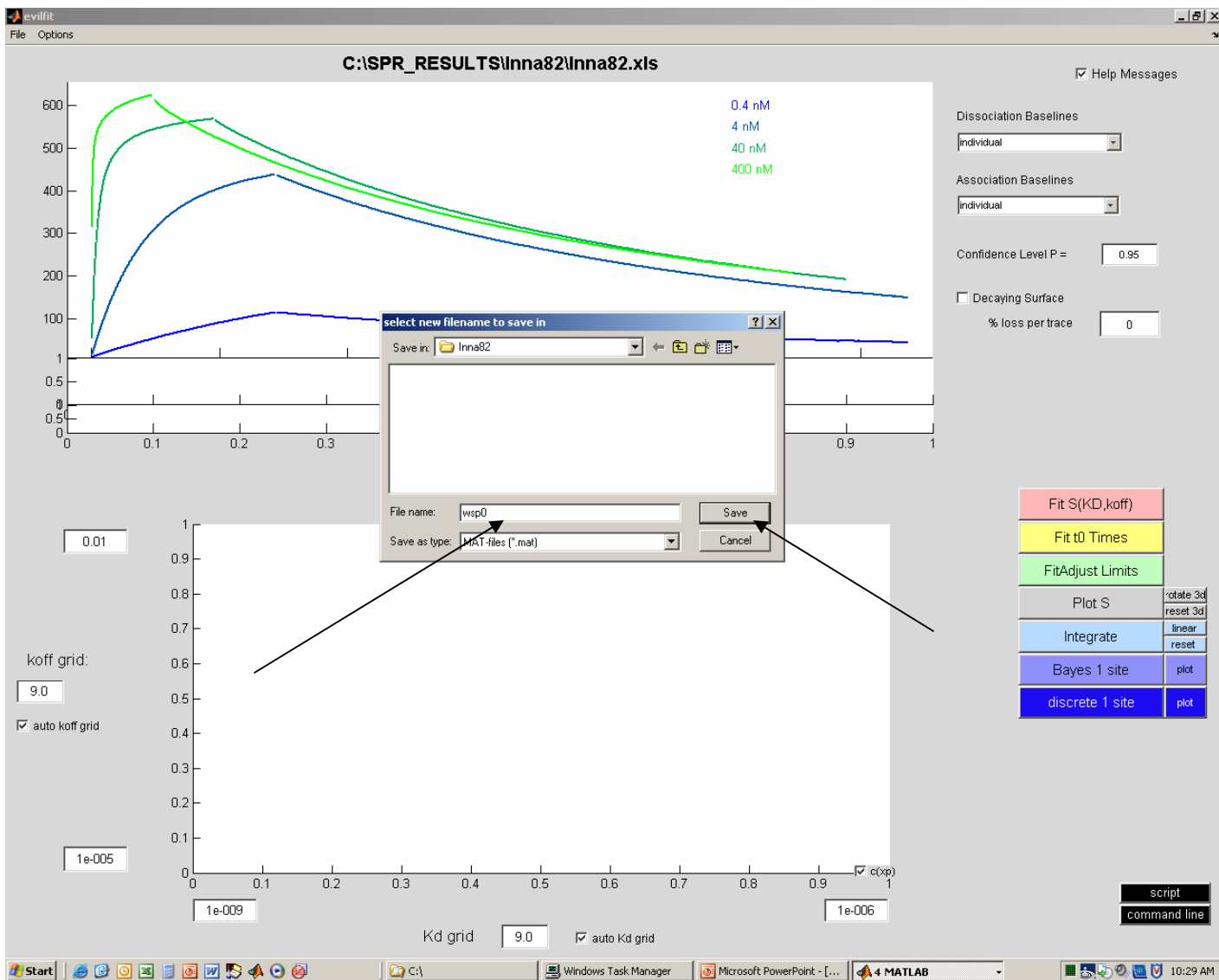
Loading is completed



Save work space as wsp0: File/Save

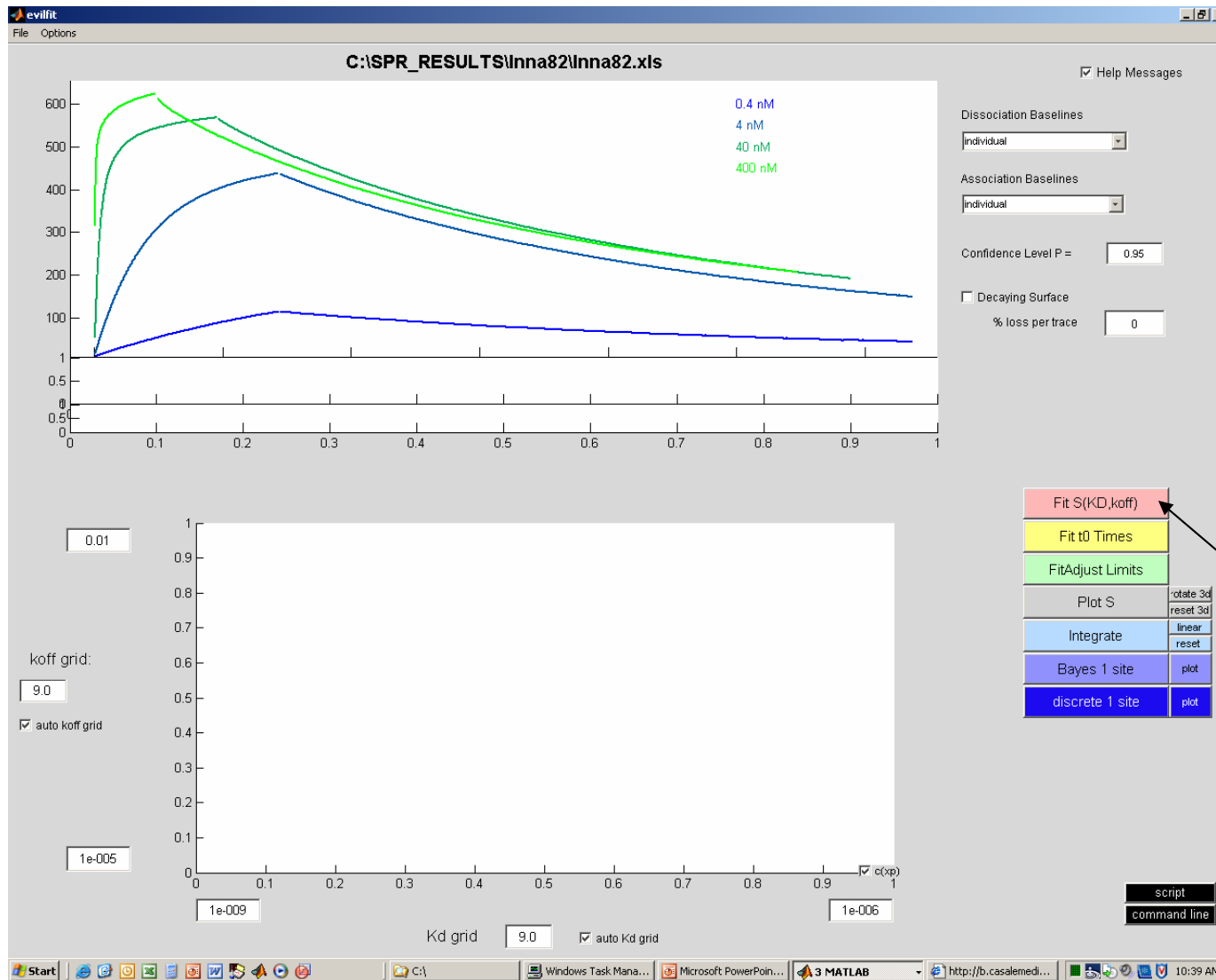


I like to use a different system for saving the workspaces (we will be generating several of them as the analysis comes along). Click “NO” to the question

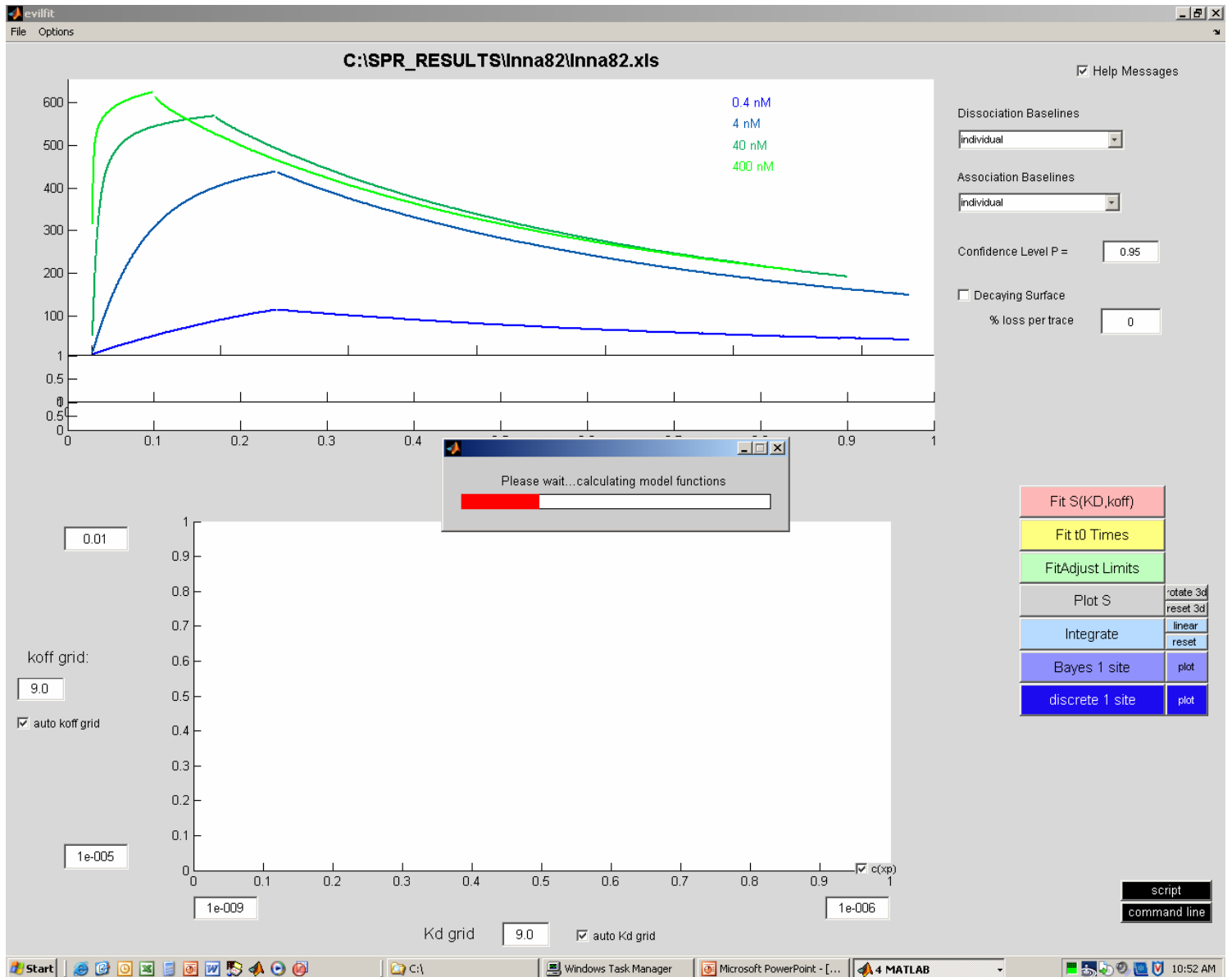


Type File name 'wsp0' and click <save>

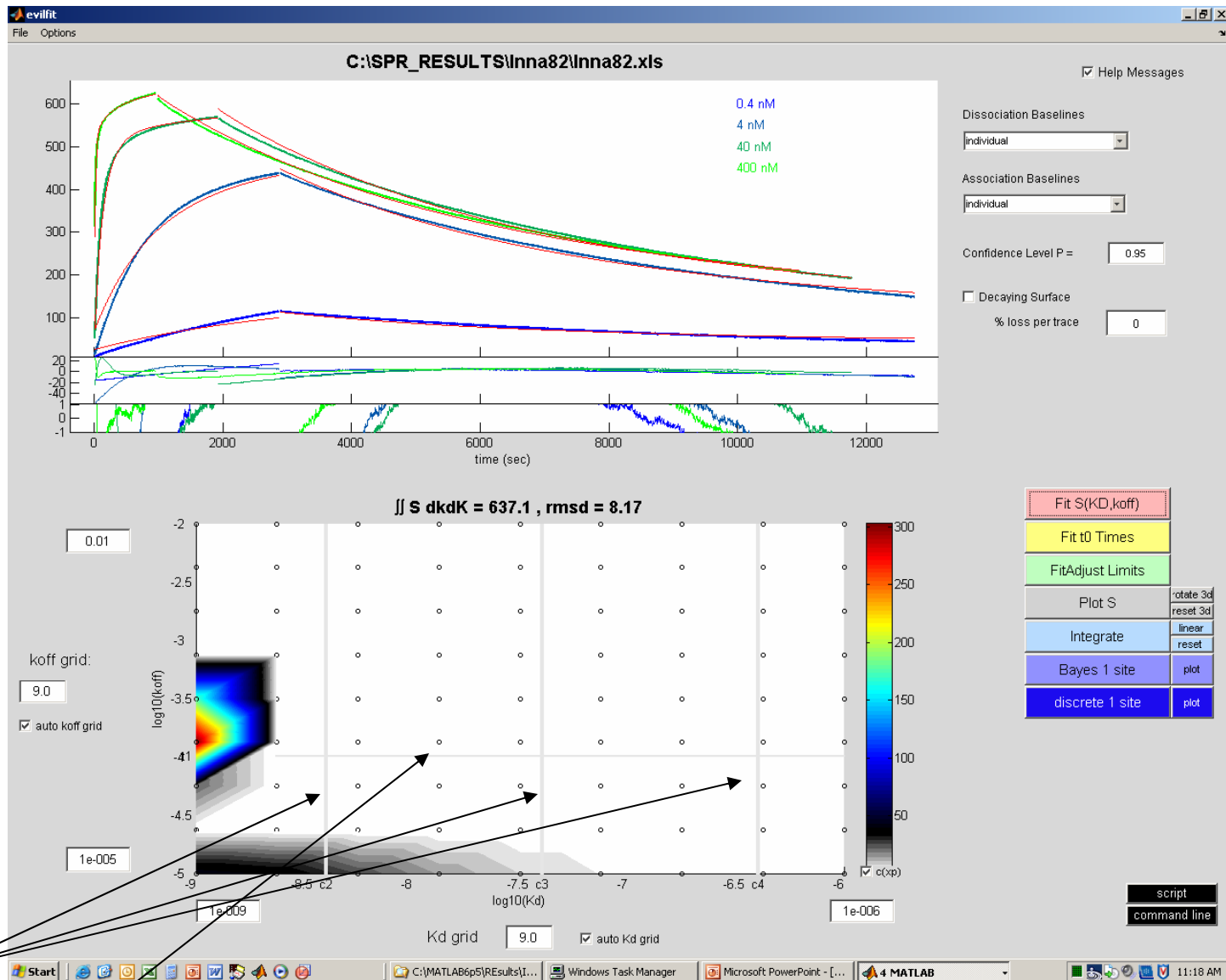
3) Calculating a First Fit



If you know the distribution range, enter it in the boxes at the ends of the axes.
 As a start, we're using here just the default values.
 Click pink button <FitS(KD,koff)> to start distribution analysis



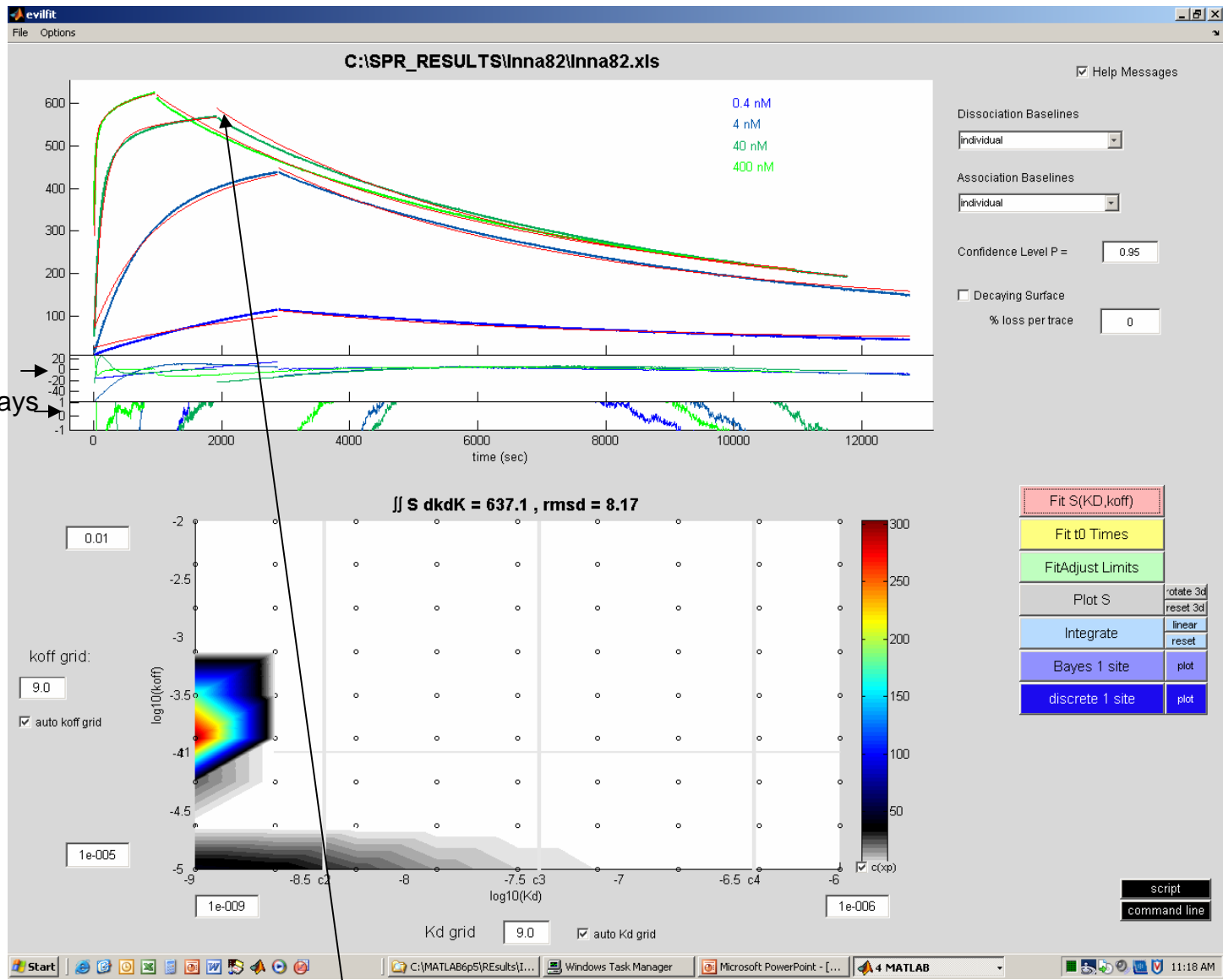
wait



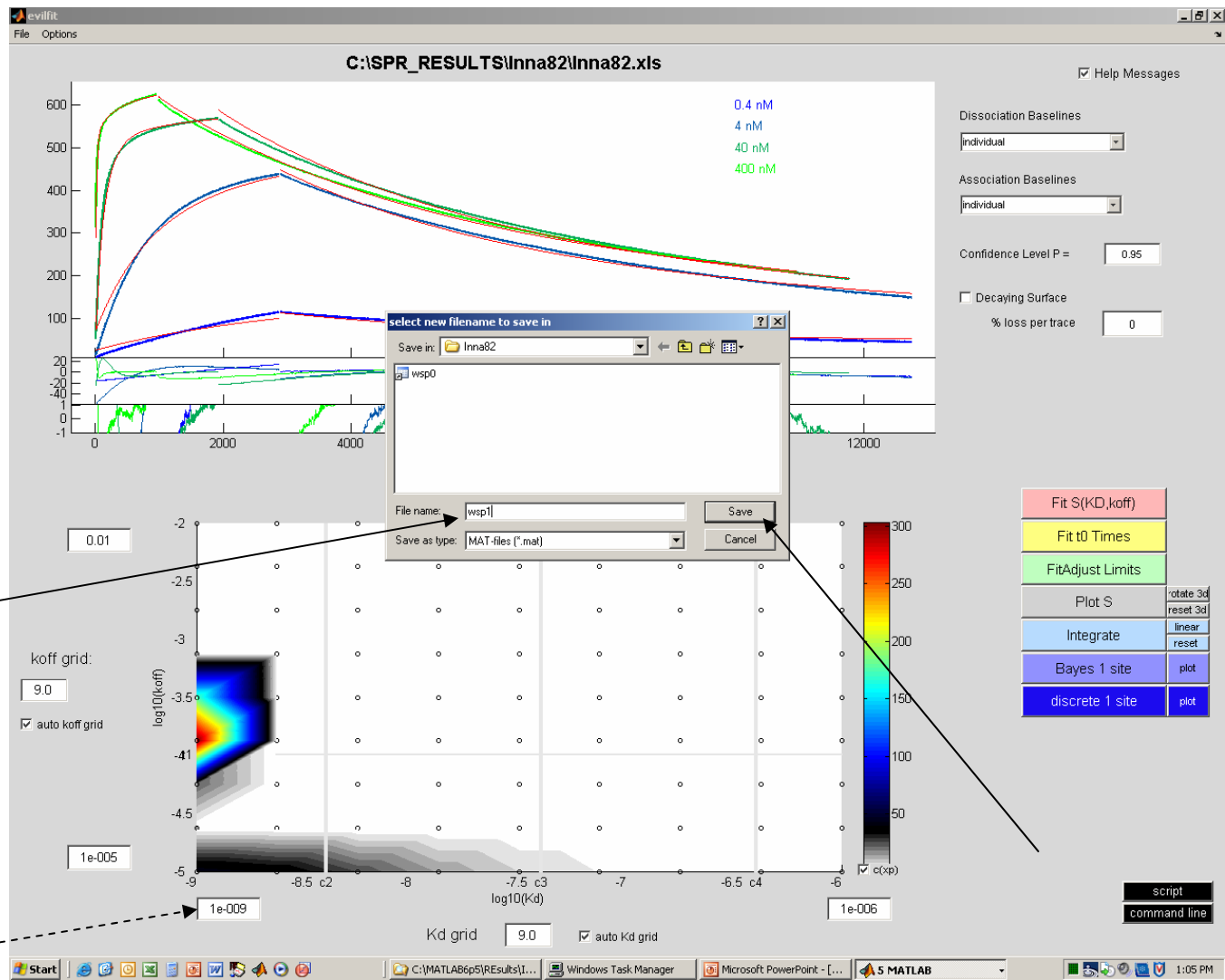
Note the grey vertical lines are at the experimental concentrations. The distribution peak optimally should not be far away from the experimental concentrations (there should be experimental concentrations higher than the peak, for the K_d value to be well-determined).

The horizontal line is at the k_{off} value for which the (longest) experimental observation time in the dissociation phase corresponds to one lifetime. The peak should not be too far below this line in order for the k_{off} -value of the peak to be well-determined by the experimental data.

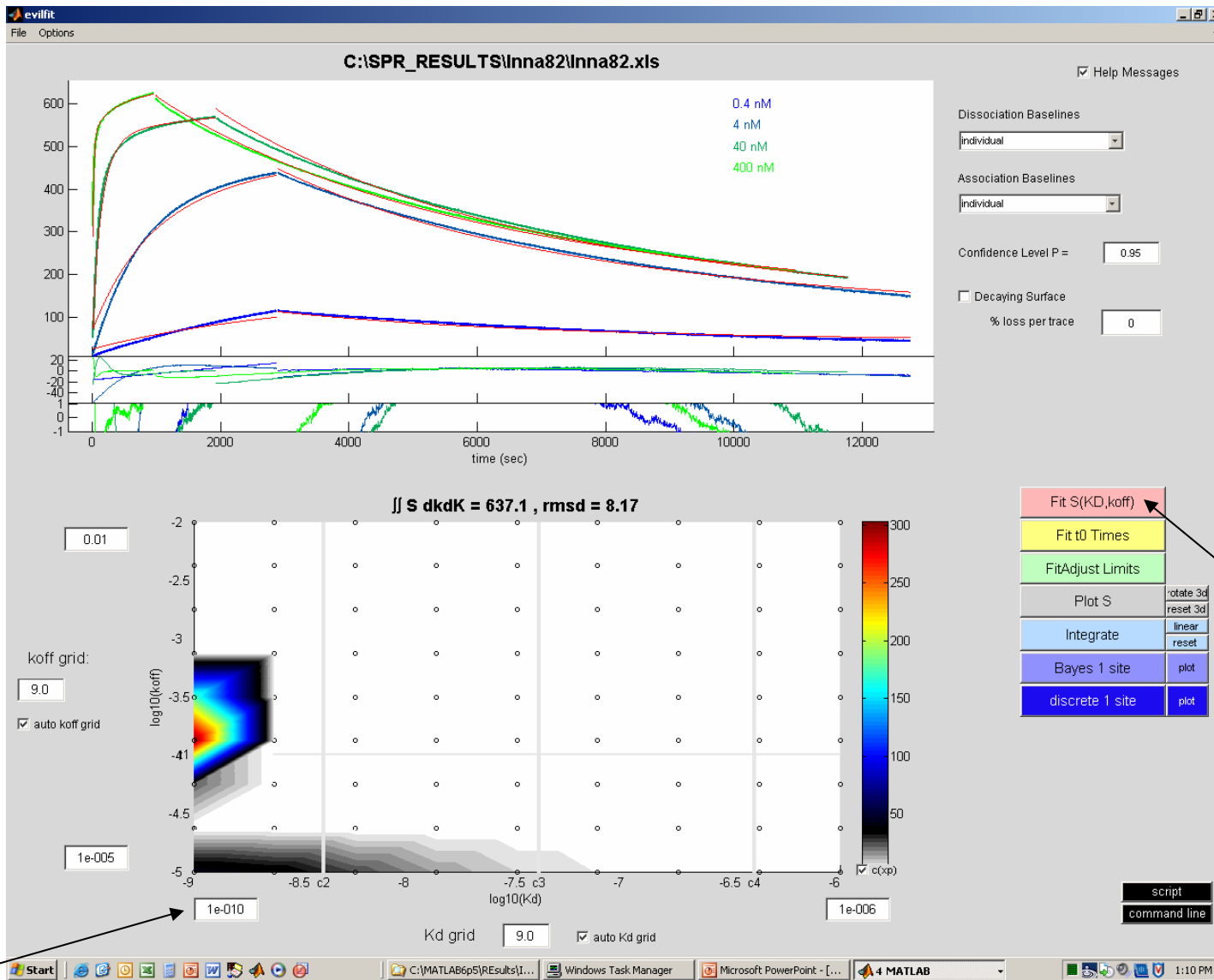
Residuals scaled
in two different ways



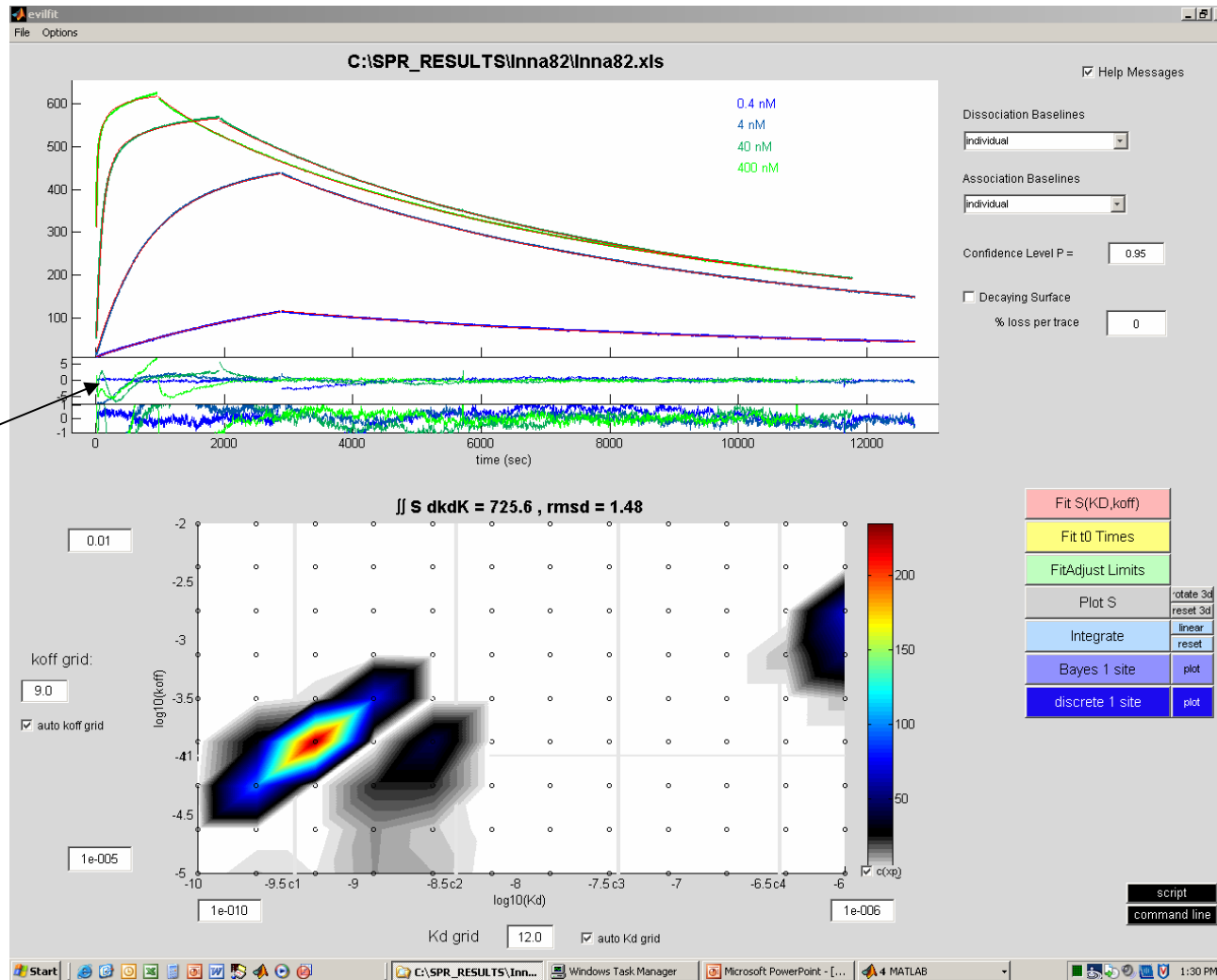
Fit is bad: calculated (red curves) and experimental data do not coincide; rmsd is big. Save work space as wsp1 (using the routine shown for wsp0)



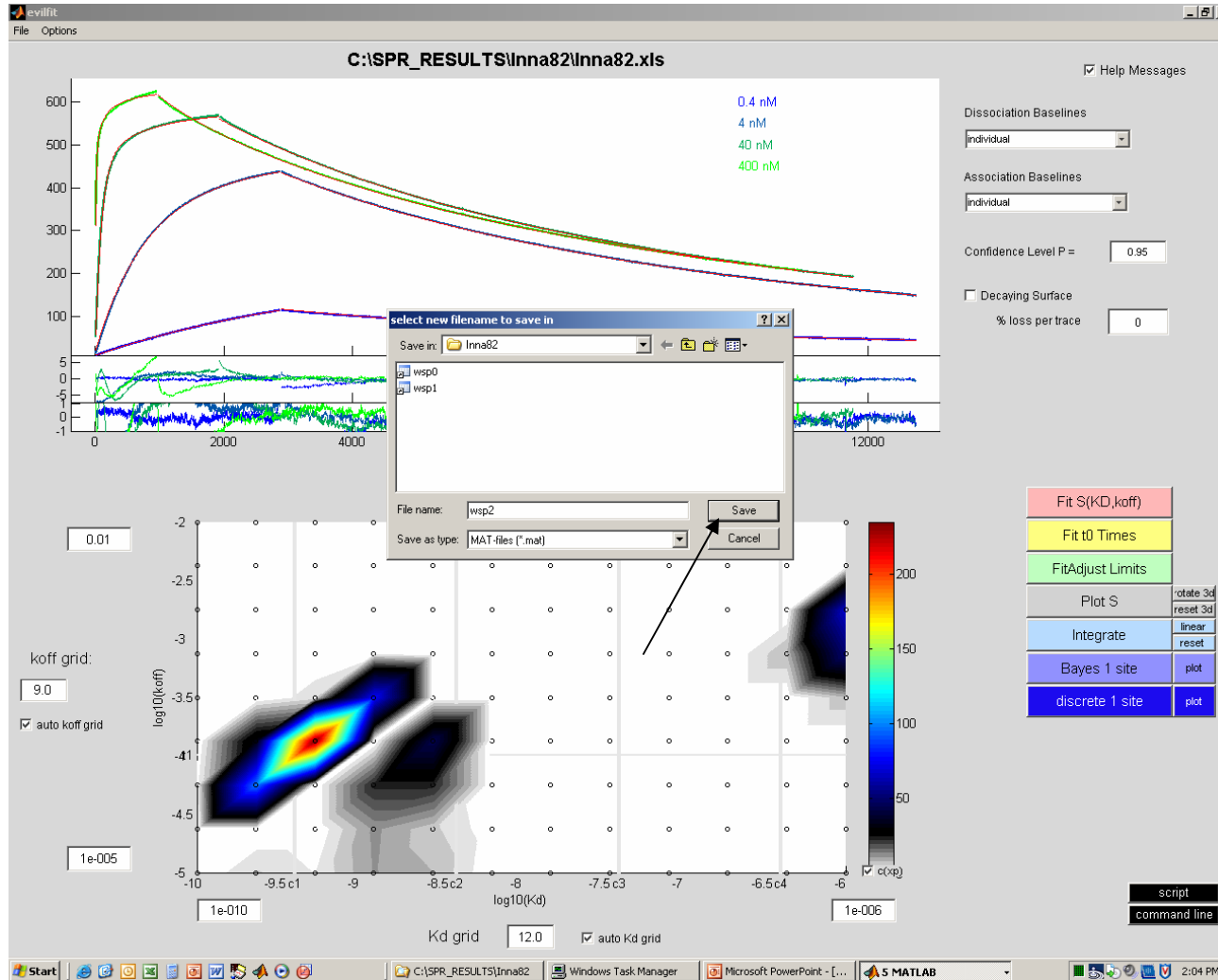
Type file name 'wsp1' and click <save> . The obvious next thing to do is to extend limits of parameters: decrease KD_{min} from $1e-9$ to $1e-10$



Type 1e-10 instead of 1e-9 and click on pink button <FitS(KD,koff)> to start new analysis



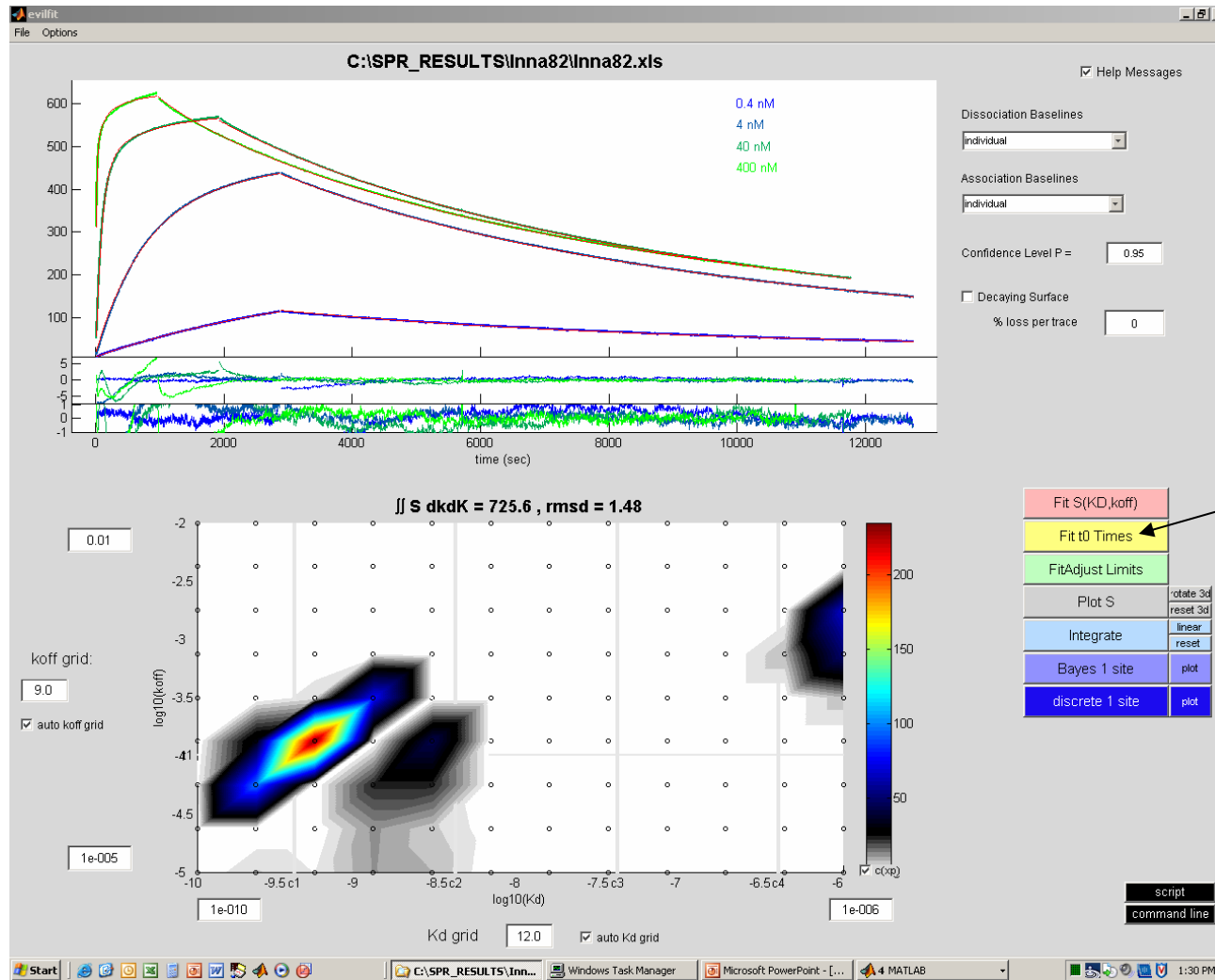
Fit is much better: rmsd is reduced significantly, but residuals are not randomly distributed, especially at start of injections.



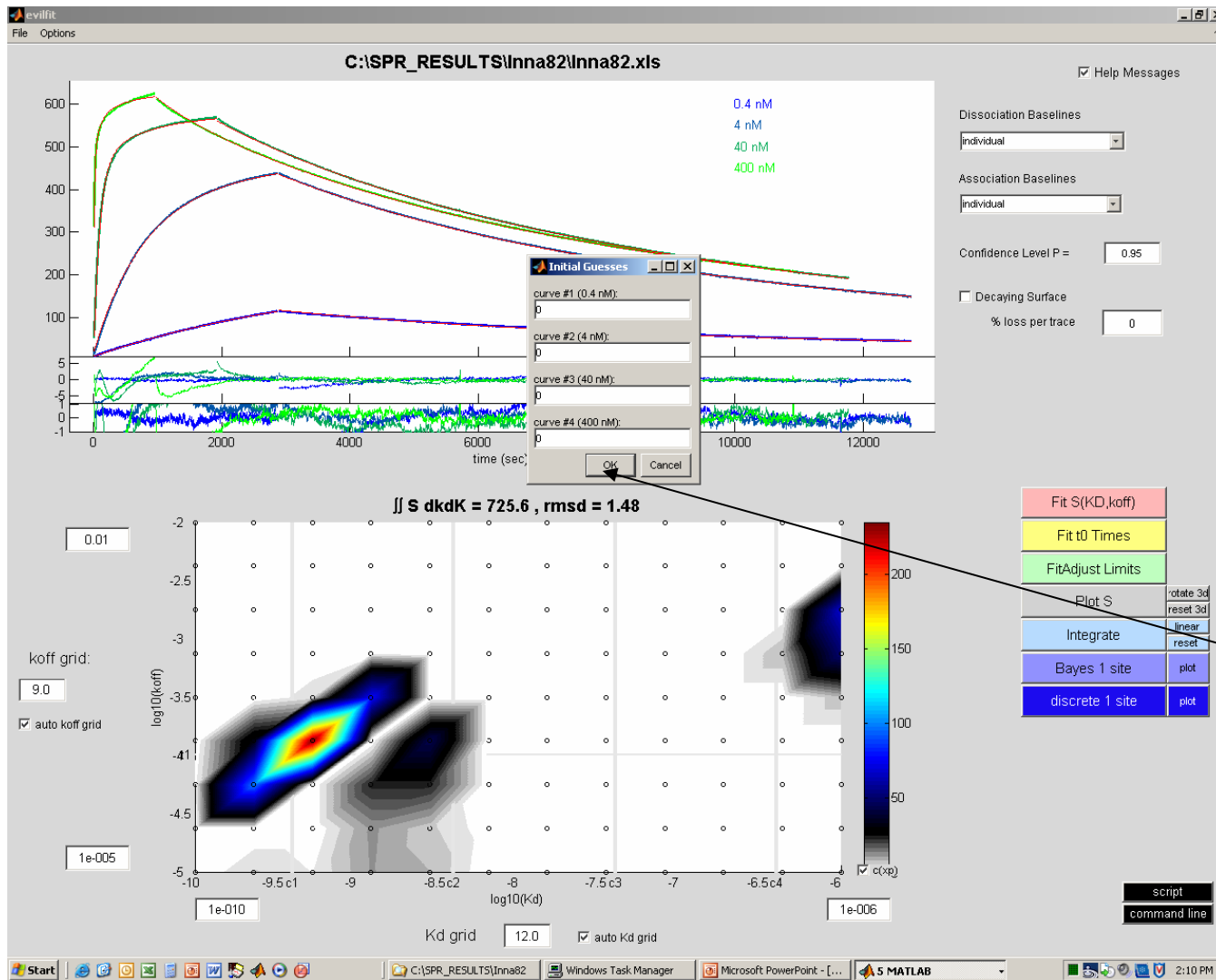
Save current work space as wsp2

3) Refining the Fit more

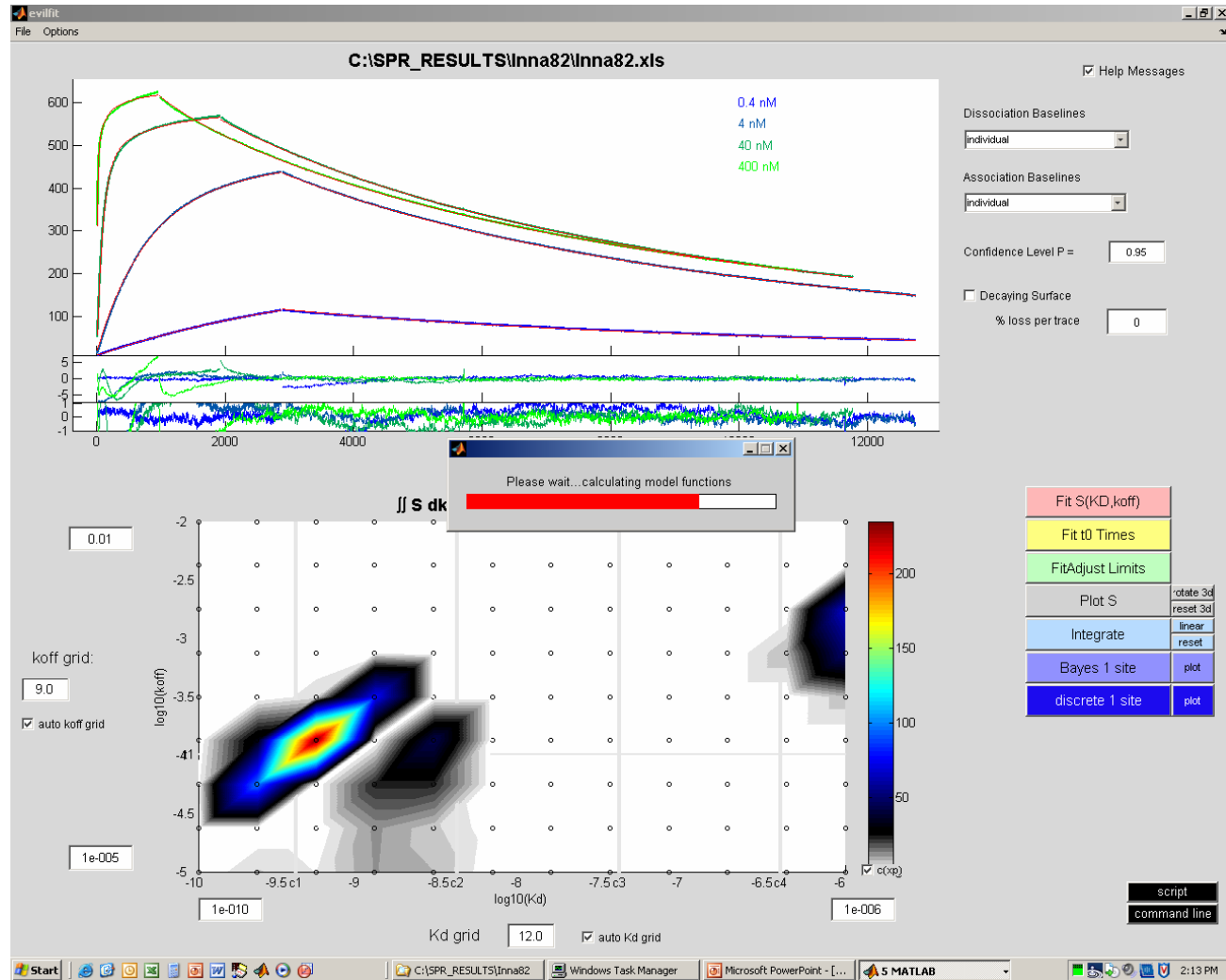
- Fitting t_0 times
- Later: Adjusting the range some more



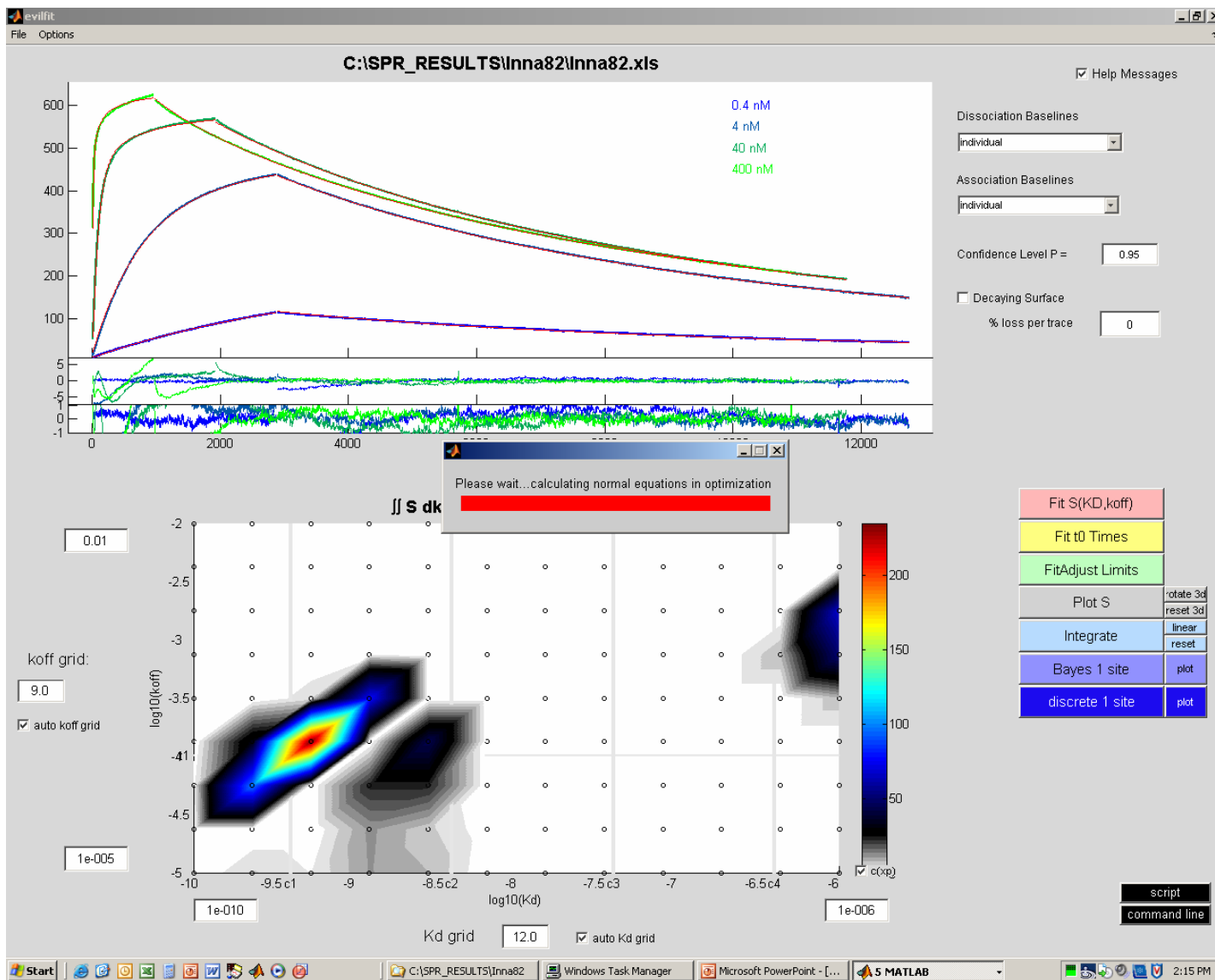
To optimize 't0's, click on yellow button <Fit t0 Times>



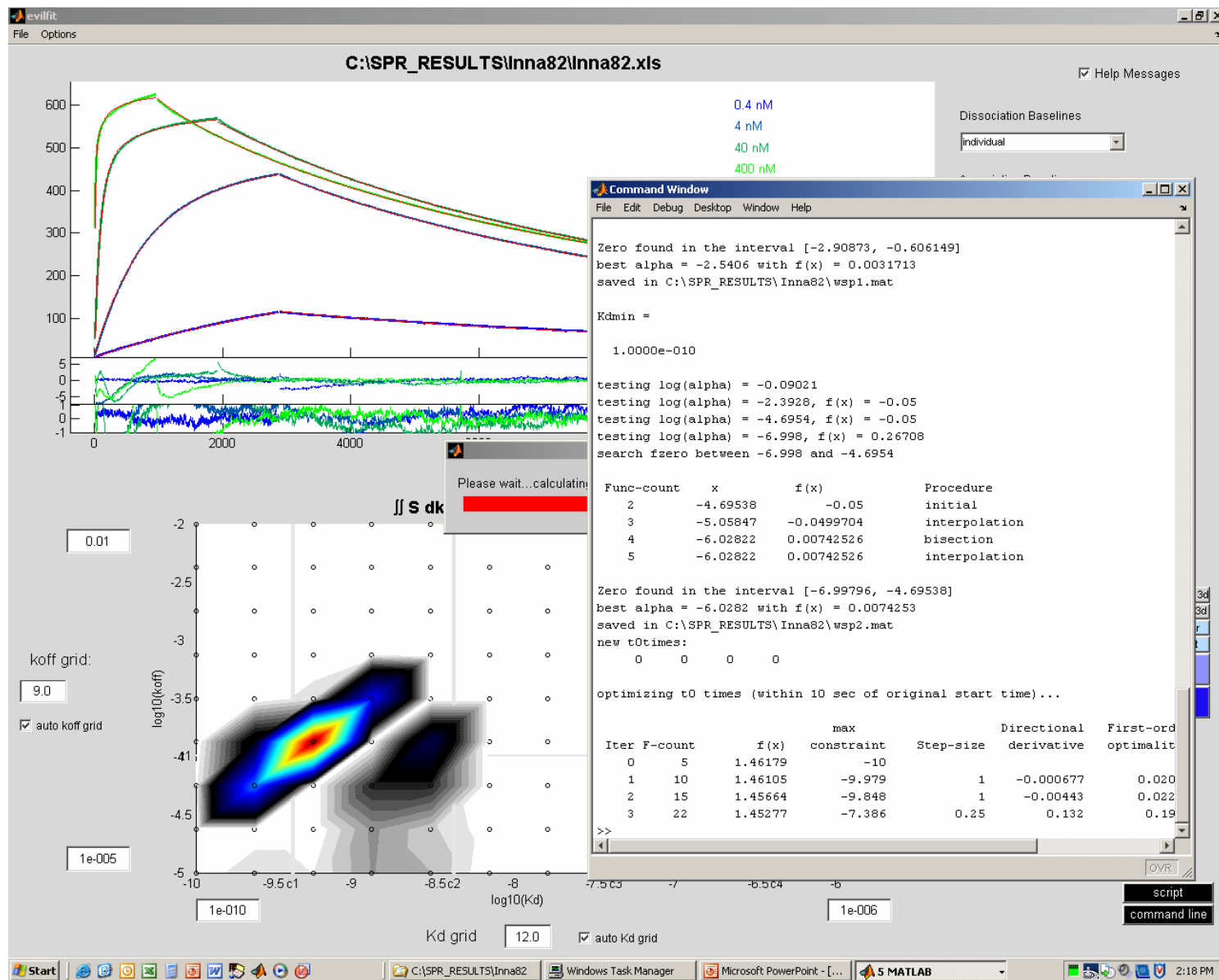
One can start from initial guesses all=0, or manually enter estimates derived from visual inspection; Click <OK>



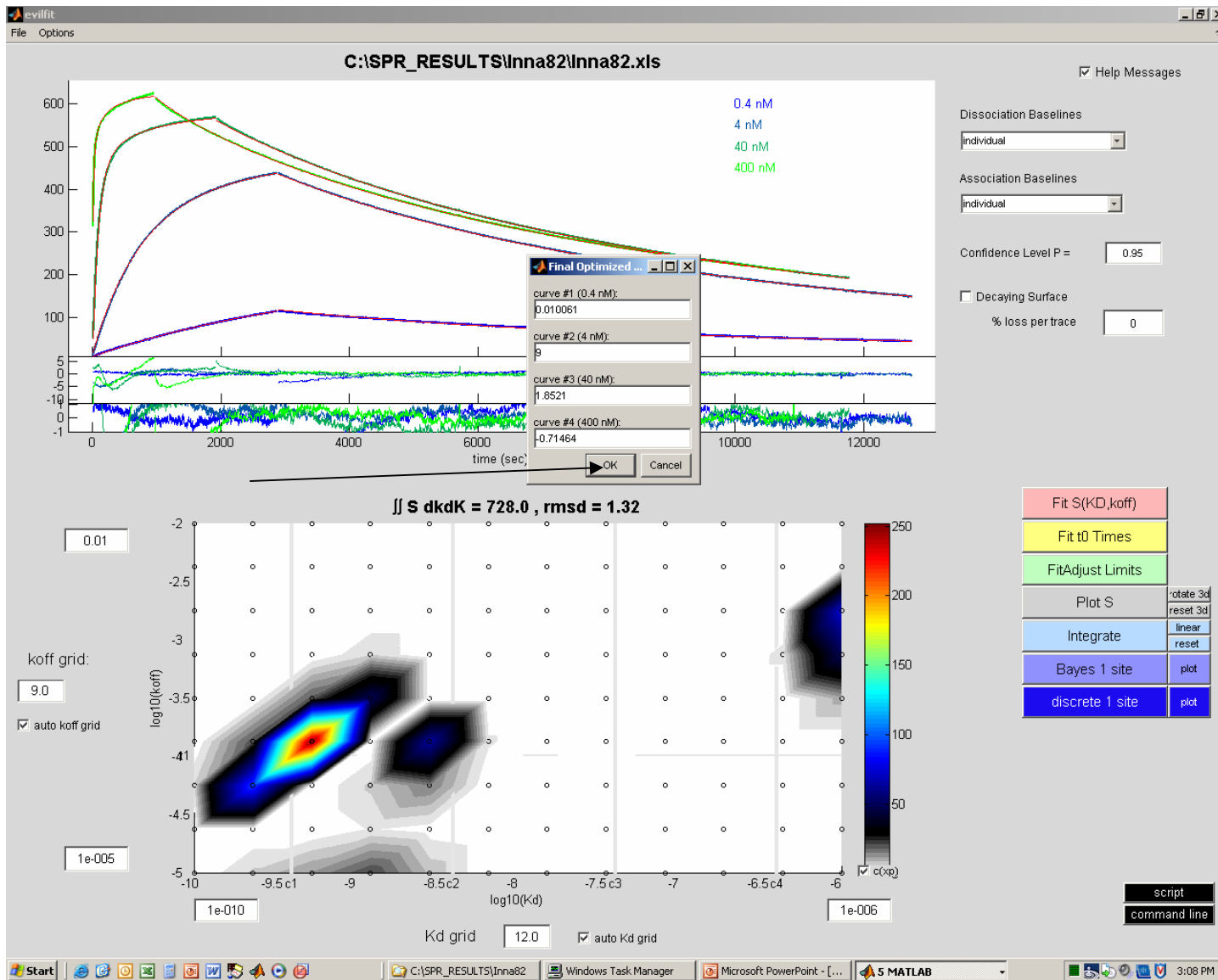
Start optimization of t_0 s



Takes time (dependent on computer, sometimes 1-2 hours). All other programs should be switched off.

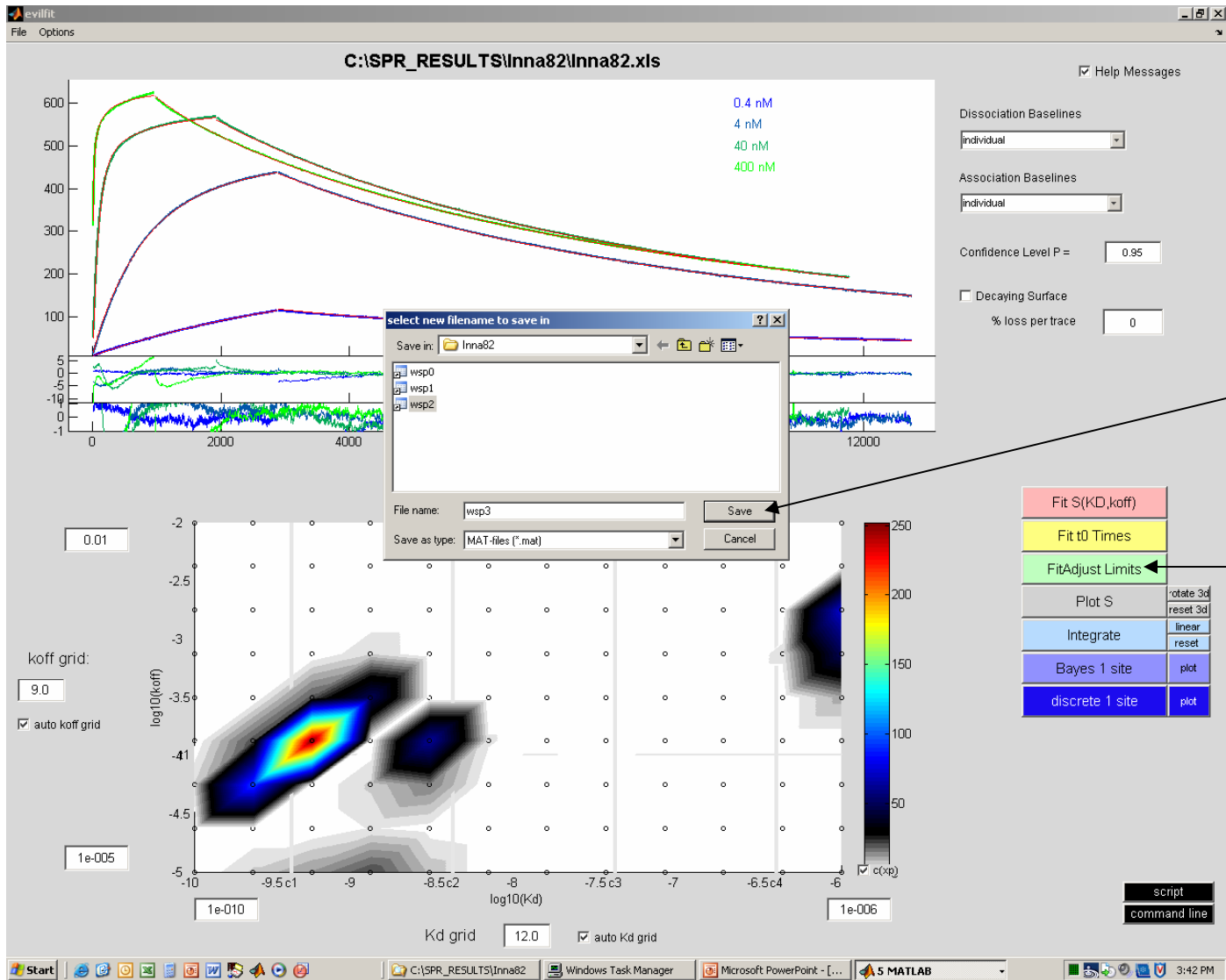


One can follow the progress in Command window



Fit is improved, rmsd has decreased from 1.48 to 1.32. New t0 times are acceptable*. Click <OK>

* t0 for the Trace#1 (0.4 nM) might be improved...



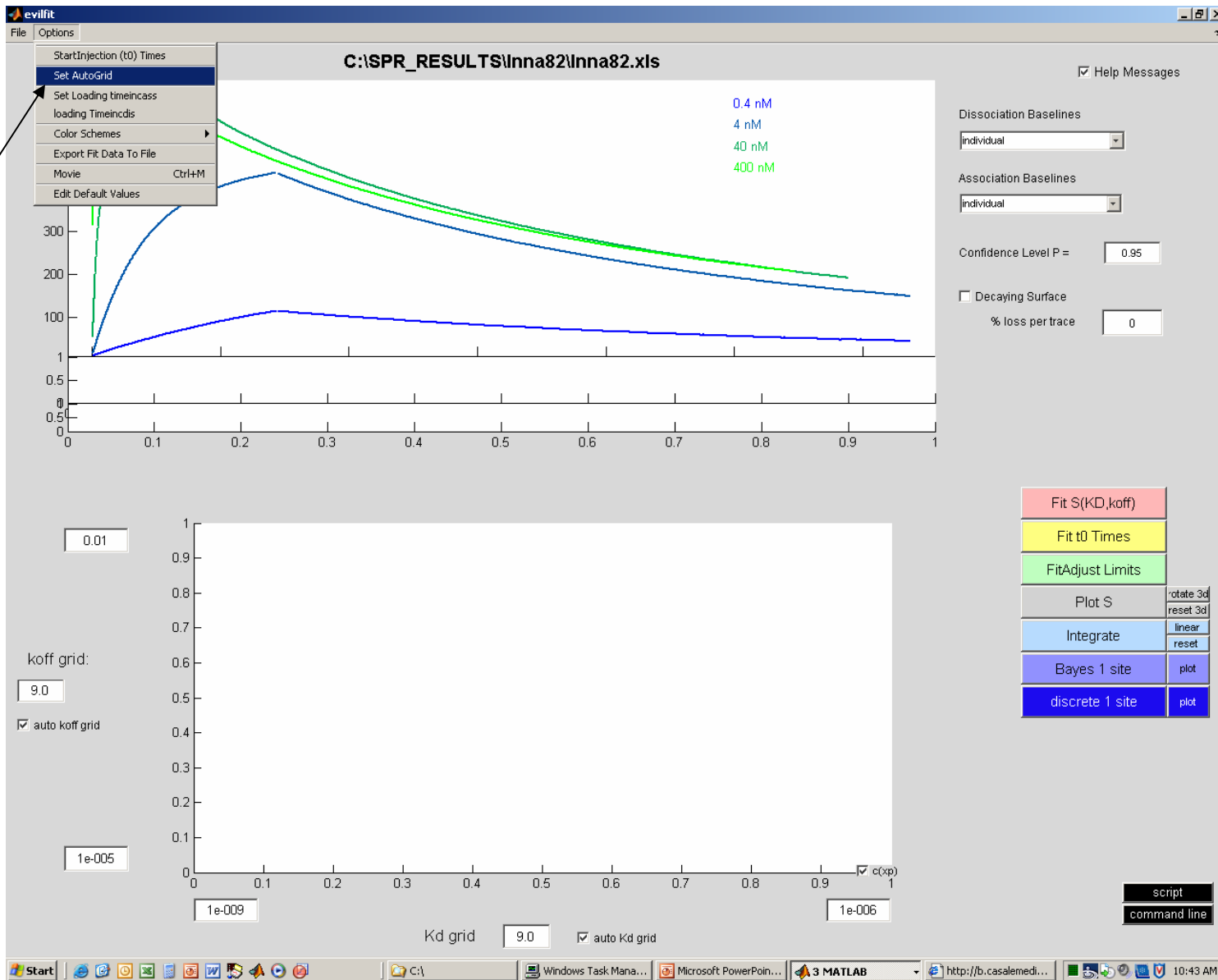
Save current work space as **wsp3** and then Fit Adjust Limits

Description of the Options Settings

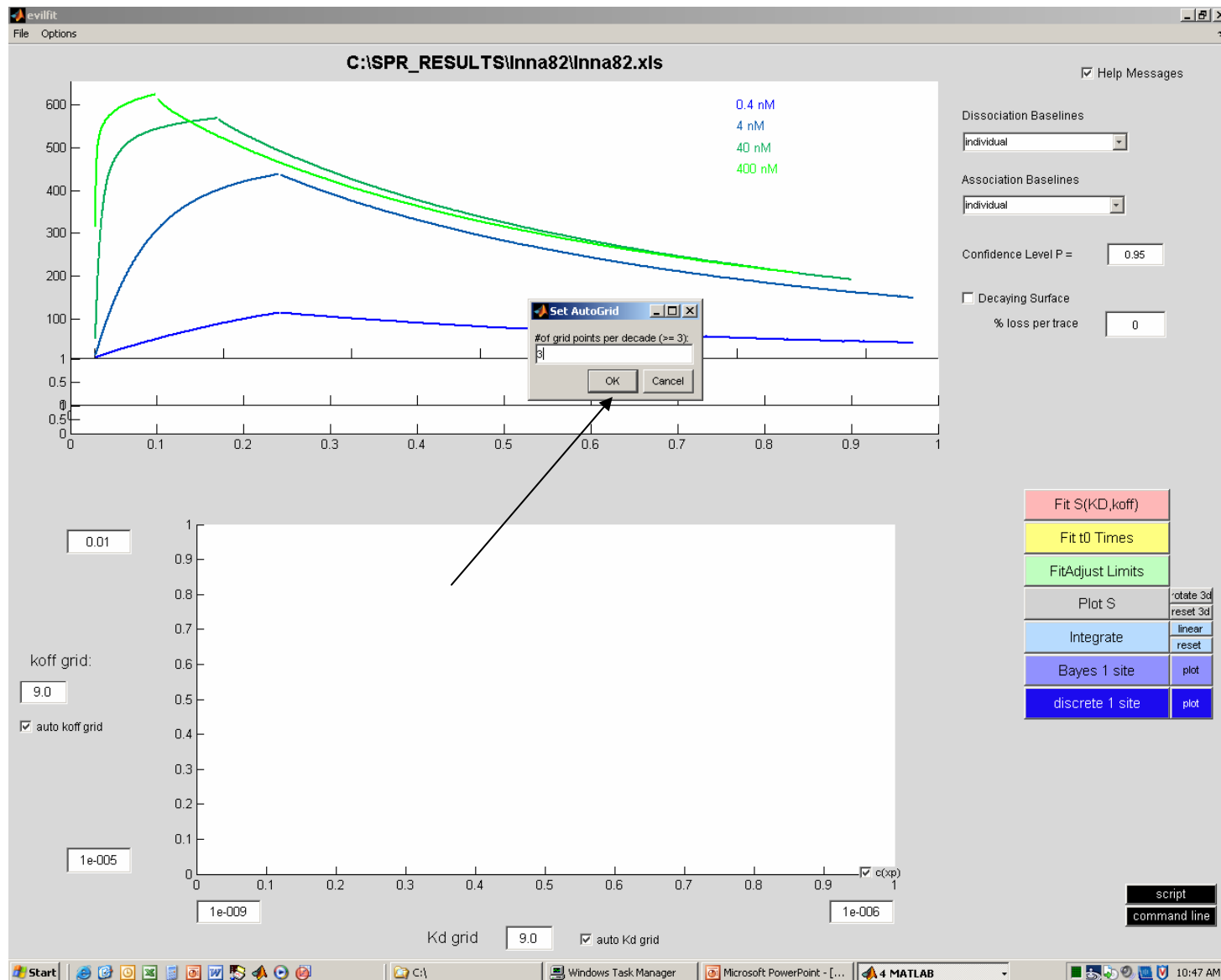
- Options for the default density of grid points per decade

To be included later:

- Setting loading time increments (for other pre-averaging parameters of data when loading)
- Exporting fit data, and changing the appearance of the distribution
- Description of the default values and how to edit them



Select grid: Options/SetAutoGrid



Click <OK> to 3 per decade, that is usually sufficient, or type the desirable value and then click <OK>