EVILFIT

Step-by-Step Tutorial

by Inna Gorshkova

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\

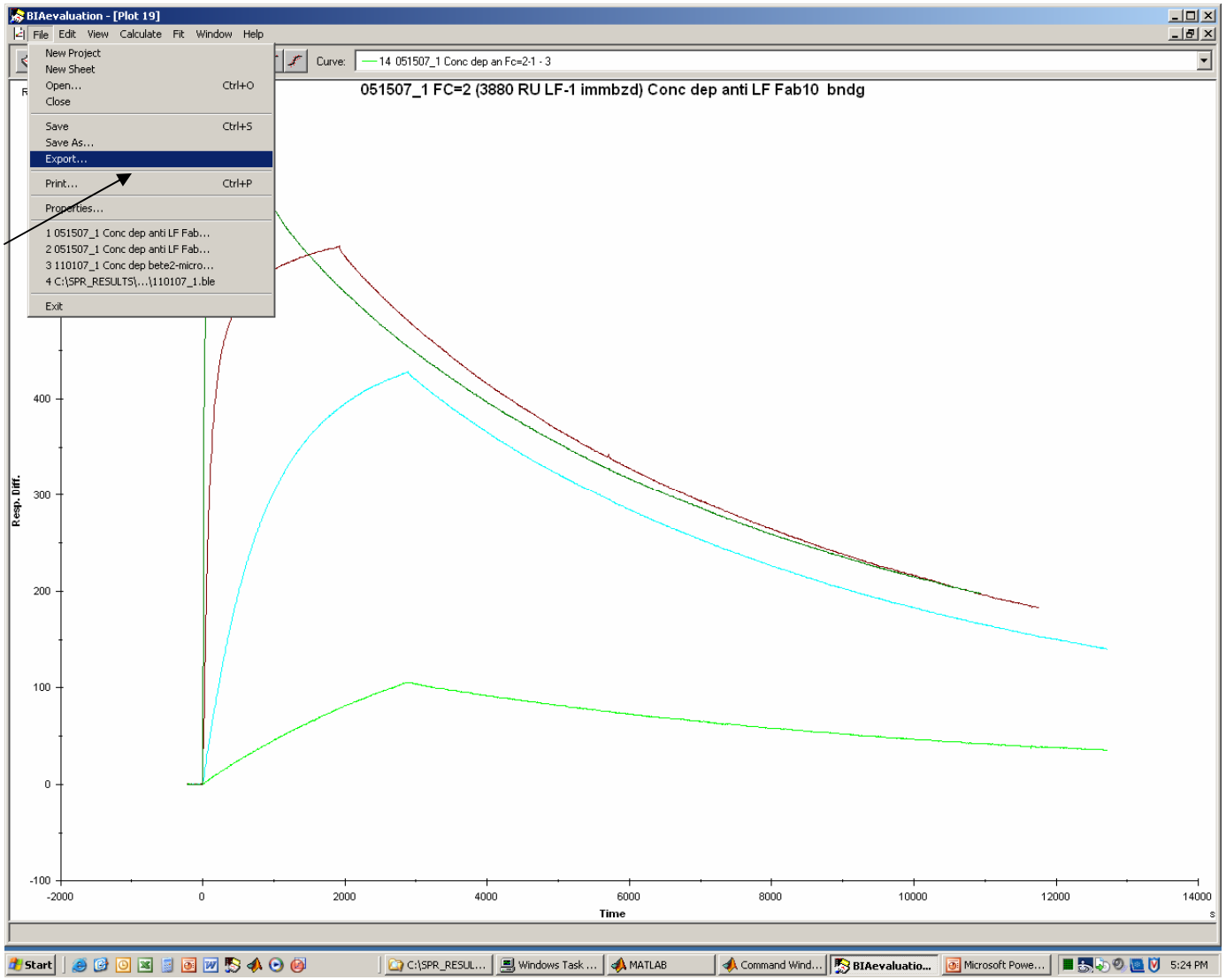
  Choose All Files (*.*) and open the txt.file

  Choose Delimited file type, click <Next>

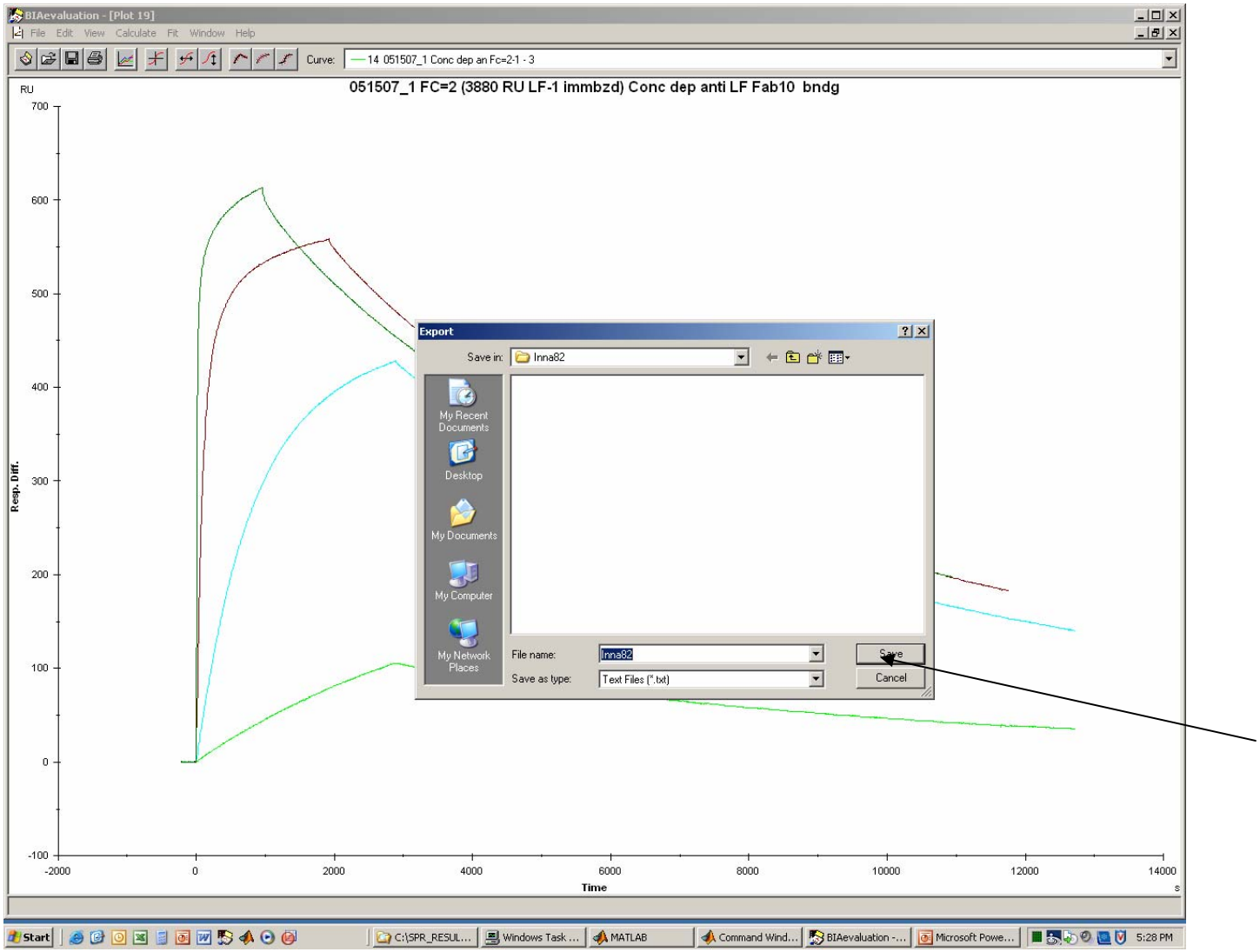
  Choose Tab as a delimiter, click <Next> and then <Finish>

  Data will appear in the Microsoft Excel window

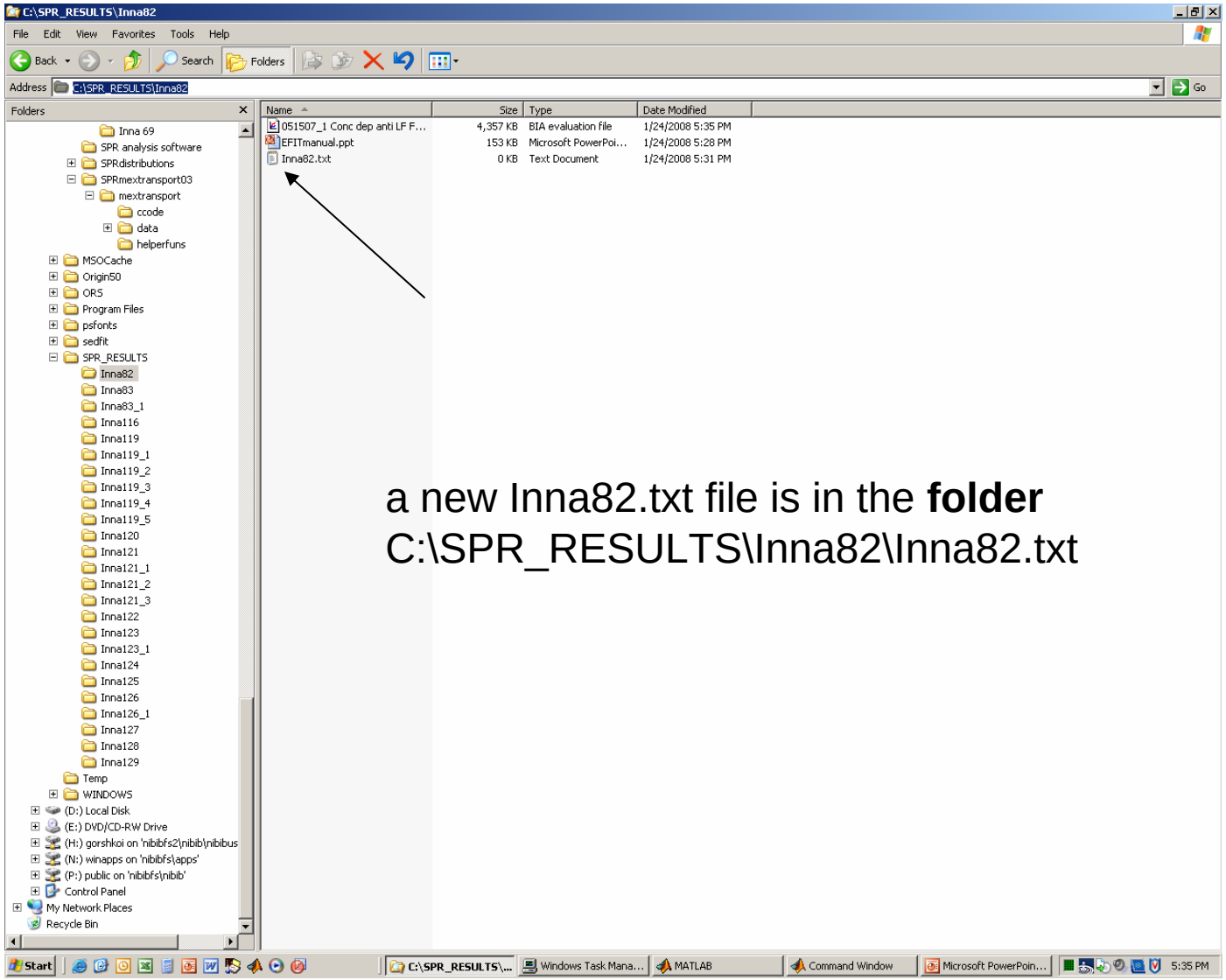
  Save file in the (*.xls) format under the same name; close



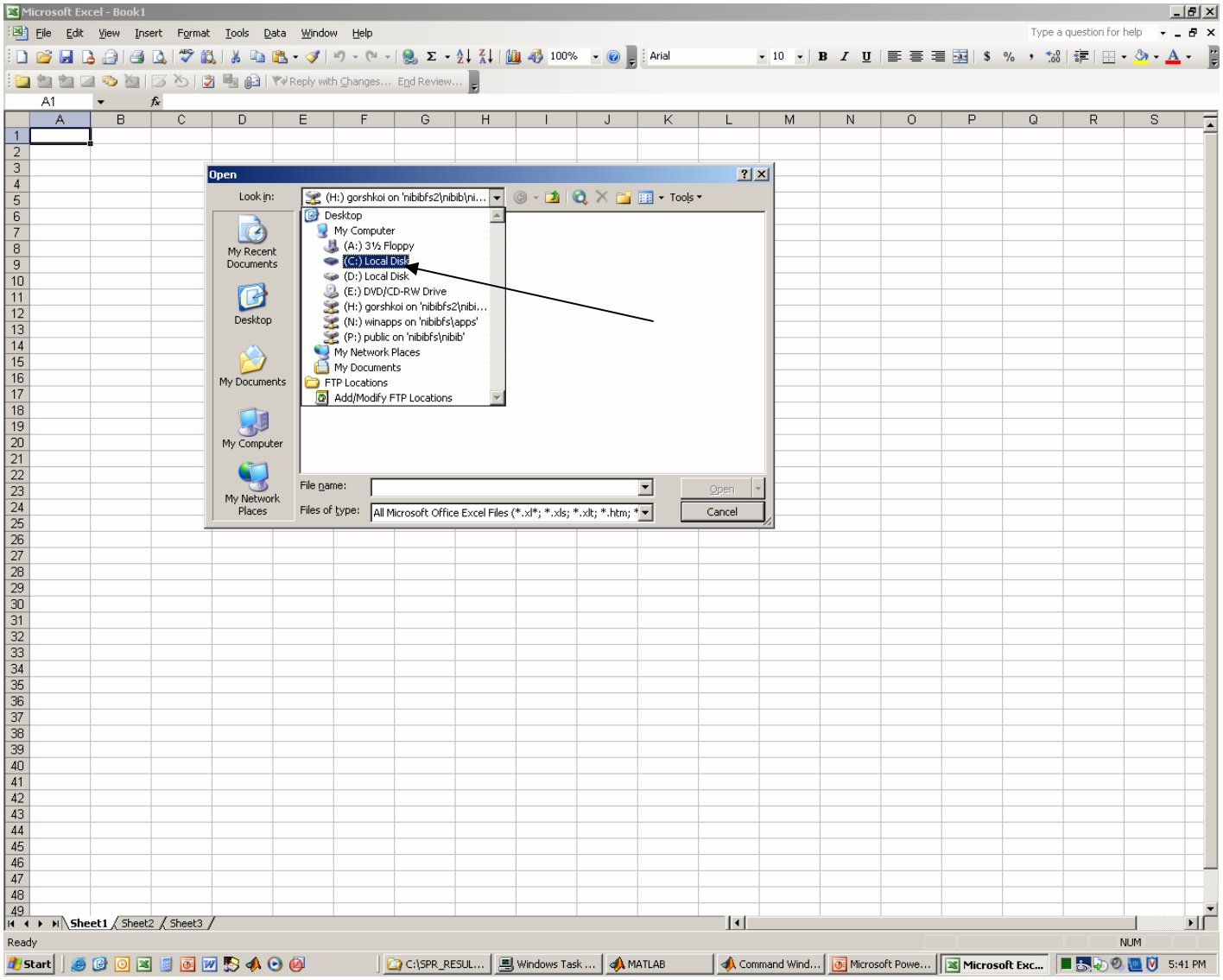
Data are aligned at time of injection = 0, and baseline corrected.
Create a **folder** on C-drive (in this case we call it C:\SPR_RESULTS\Inna82; use any name you like)
Copy BiaEvaluation file into the new **folder**, open it. Export data.



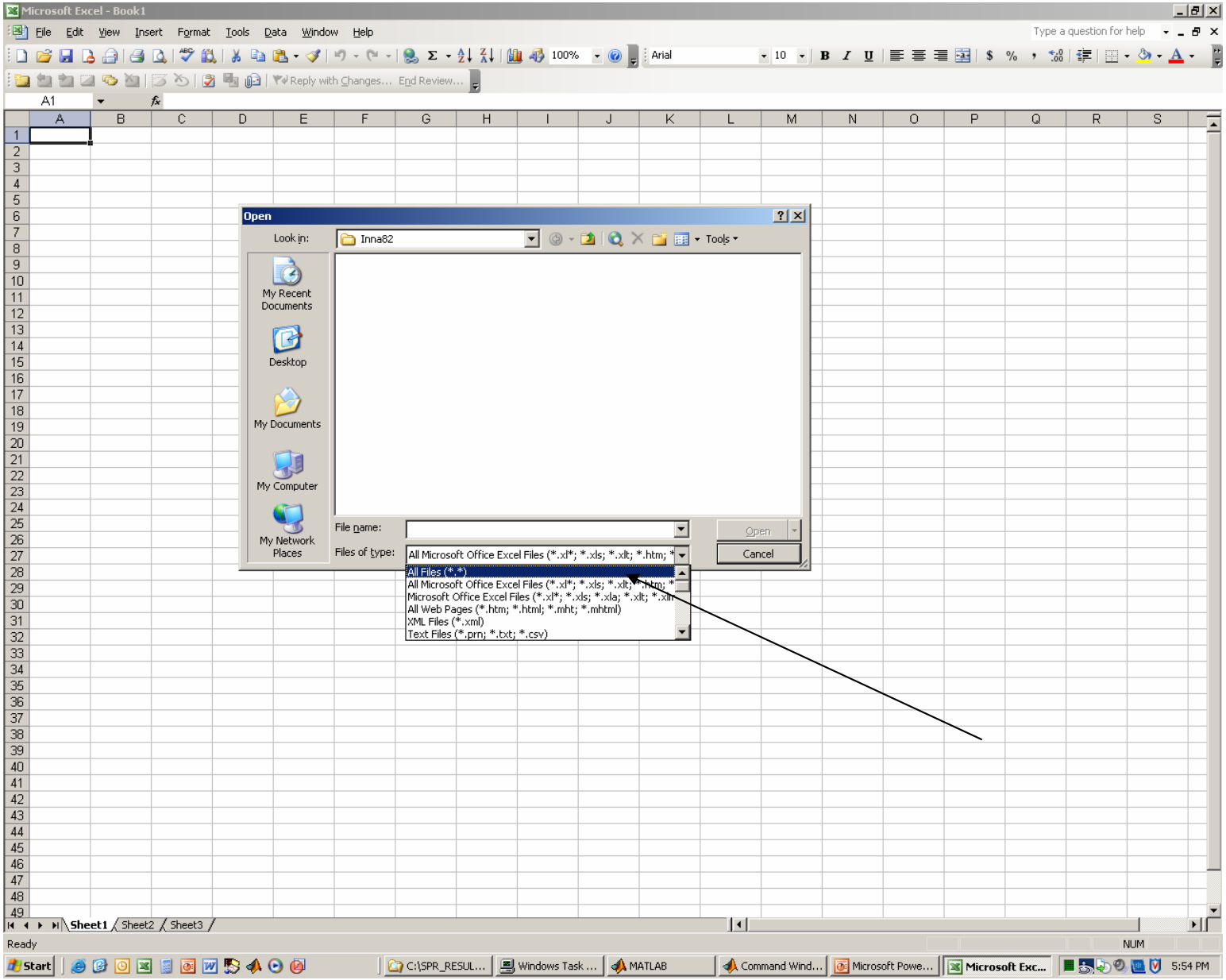
Export data into the same **folder** and save under a new name Inna82.
Close BiaEvaluation file;



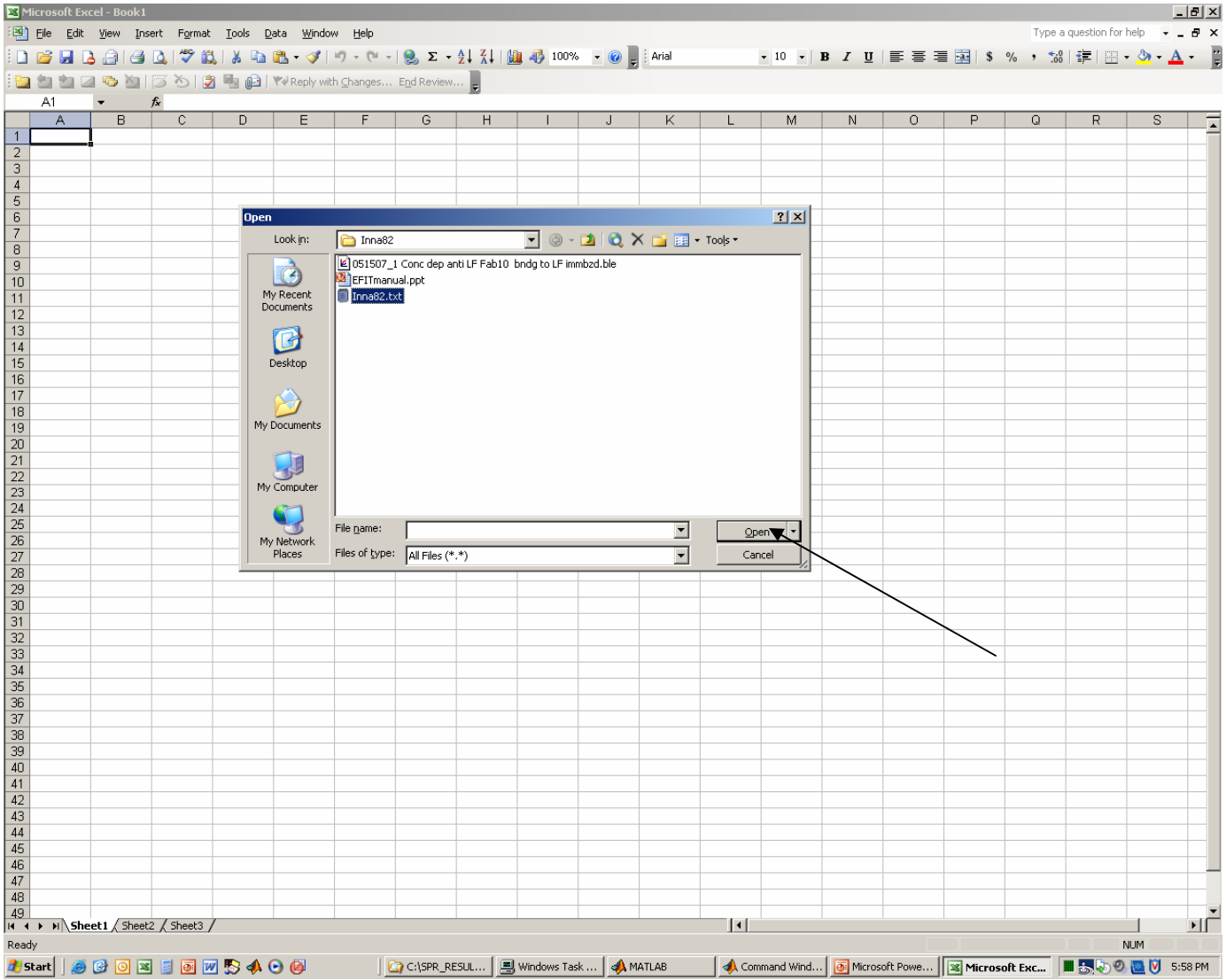
a new Inna82.txt file is in the **folder**
C:\SPR_RESULTS\Inna82\Inna82.txt



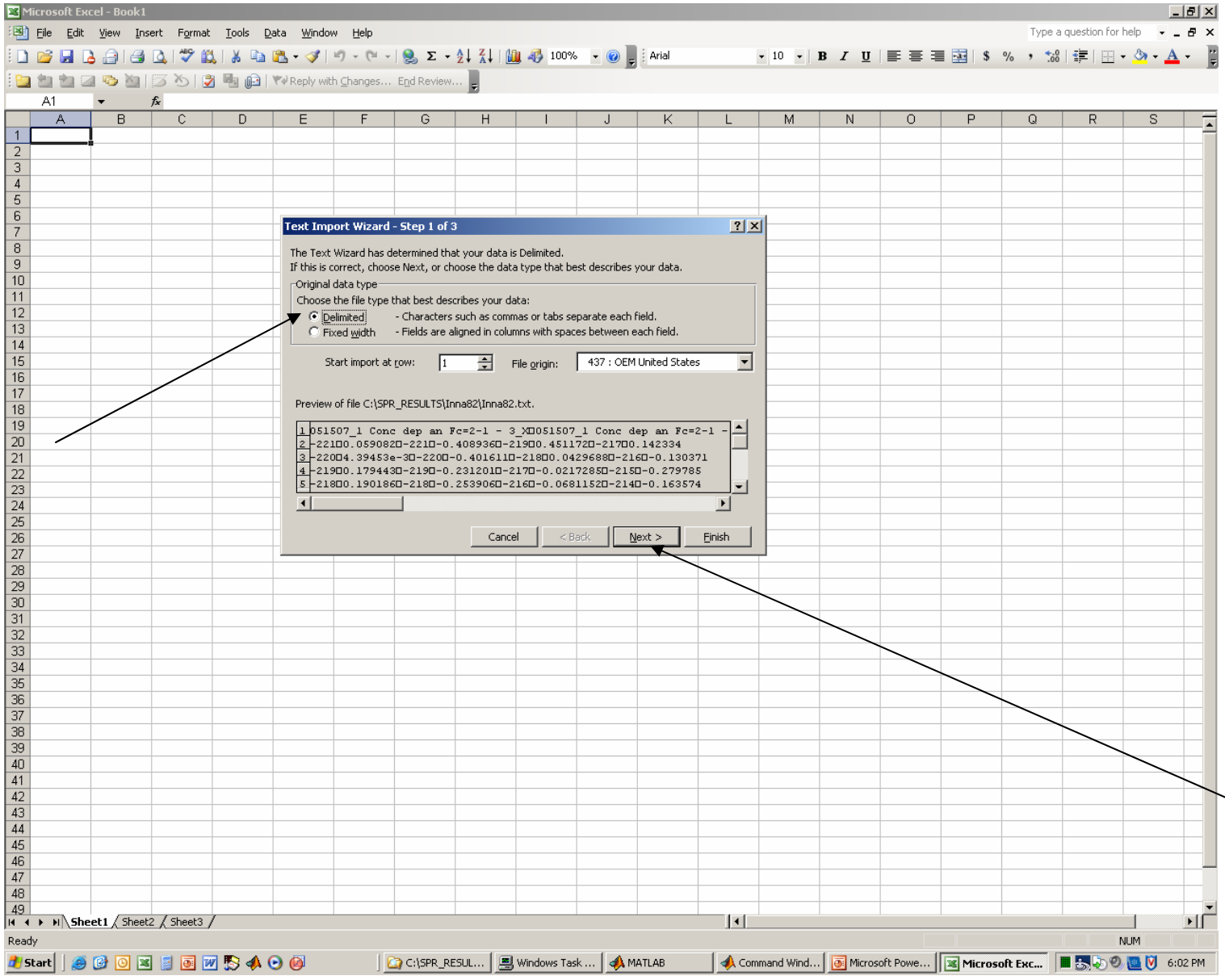
Open Microsoft Office Excel:
File/Open
In the Selection window browse for the txt.file that is in the **folder** C:\SPR_RESULTS\Inna82\



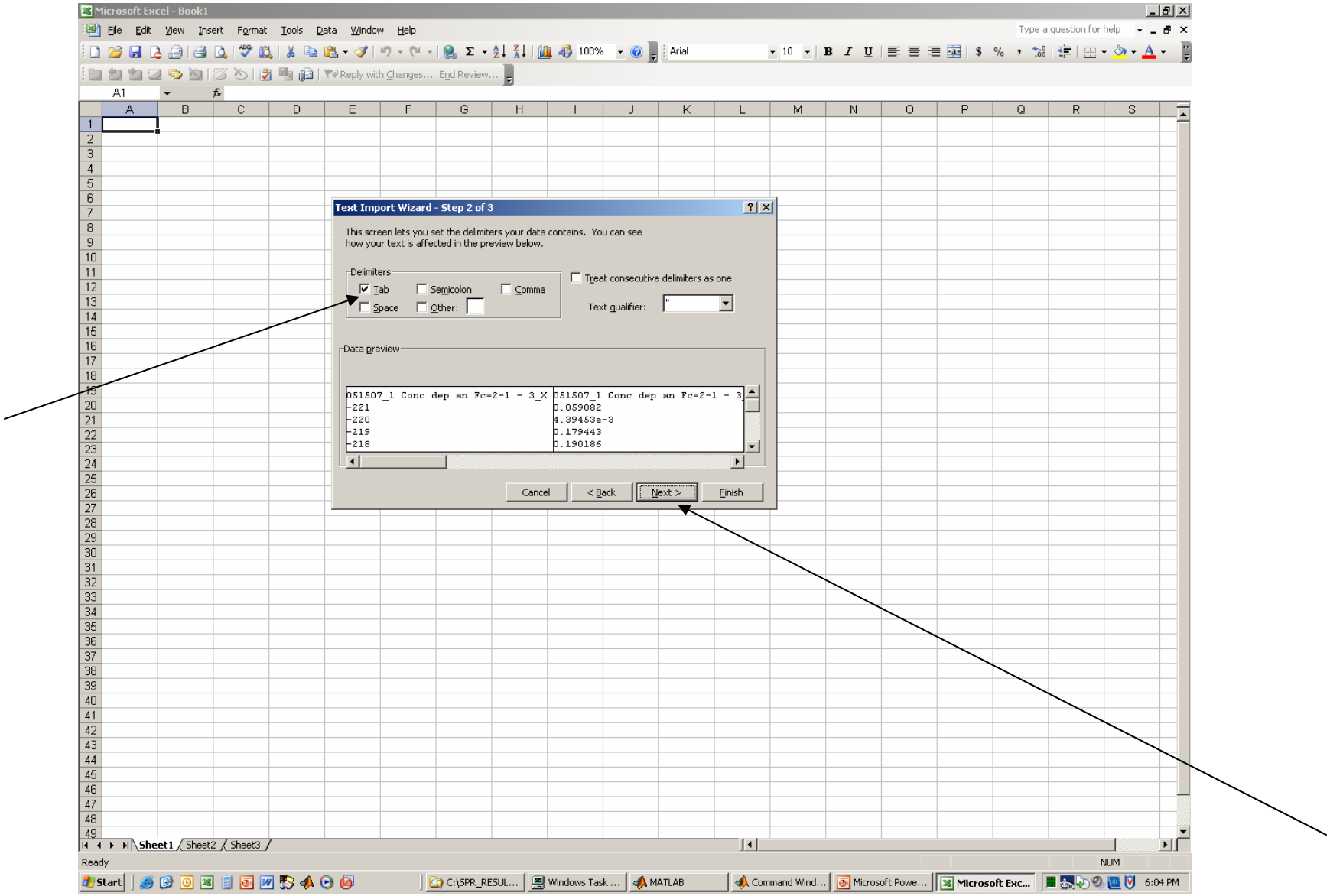
In the Files of type choose All Files (*.*)



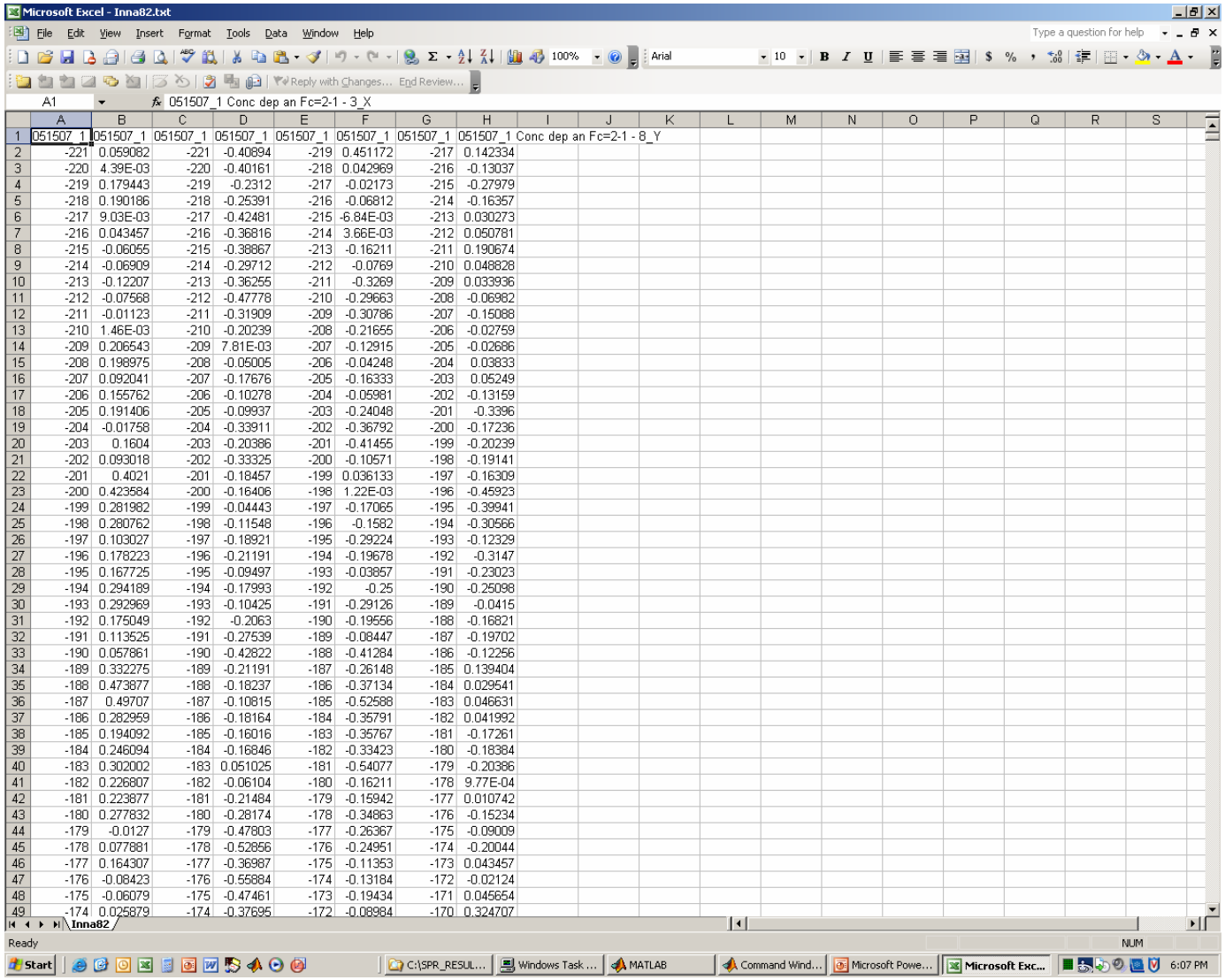
Highlight and open the txt.file



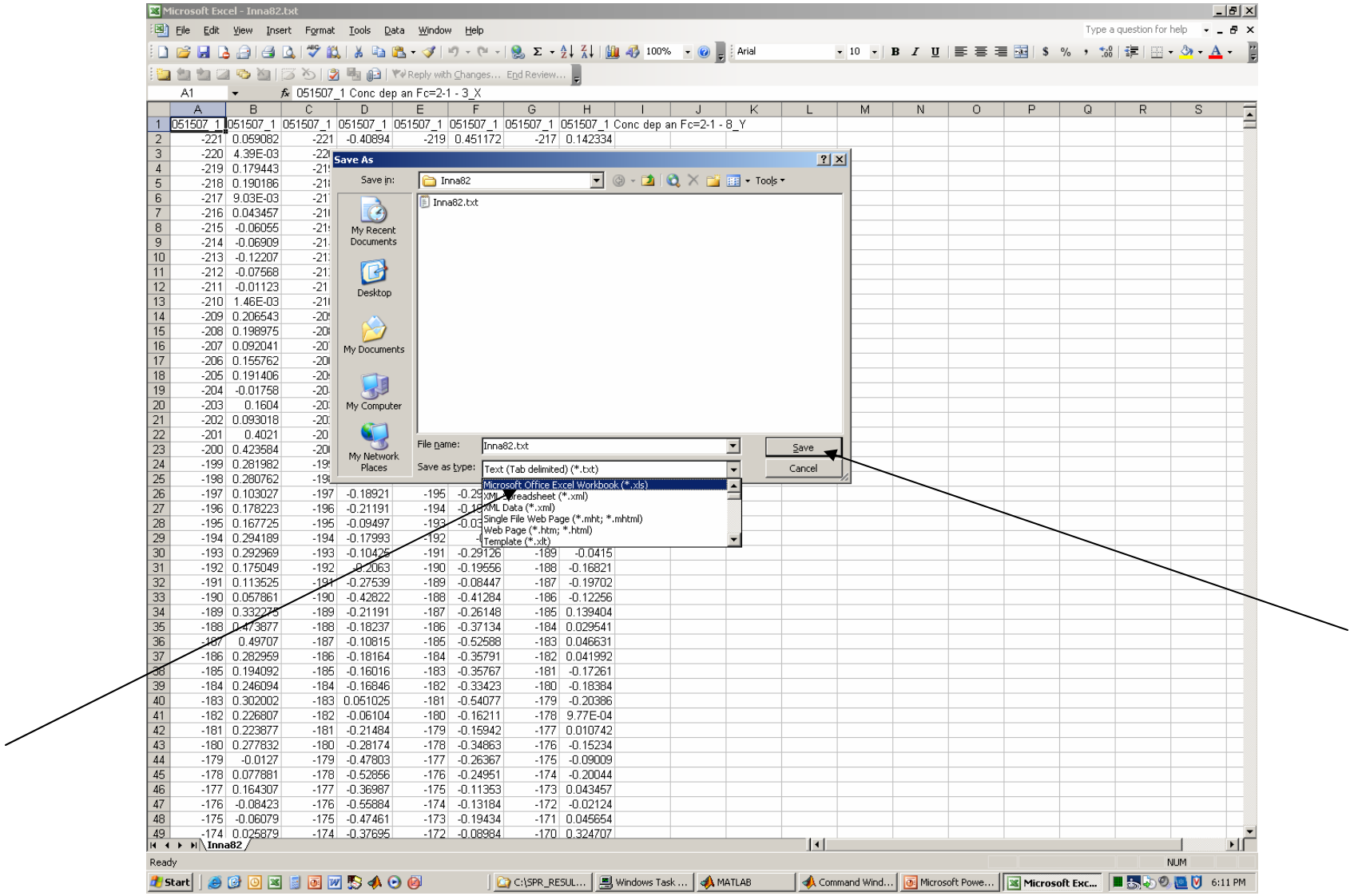
Choose Delimited file type, click <Next>



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



Data will appear in the Microsoft Excel window



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

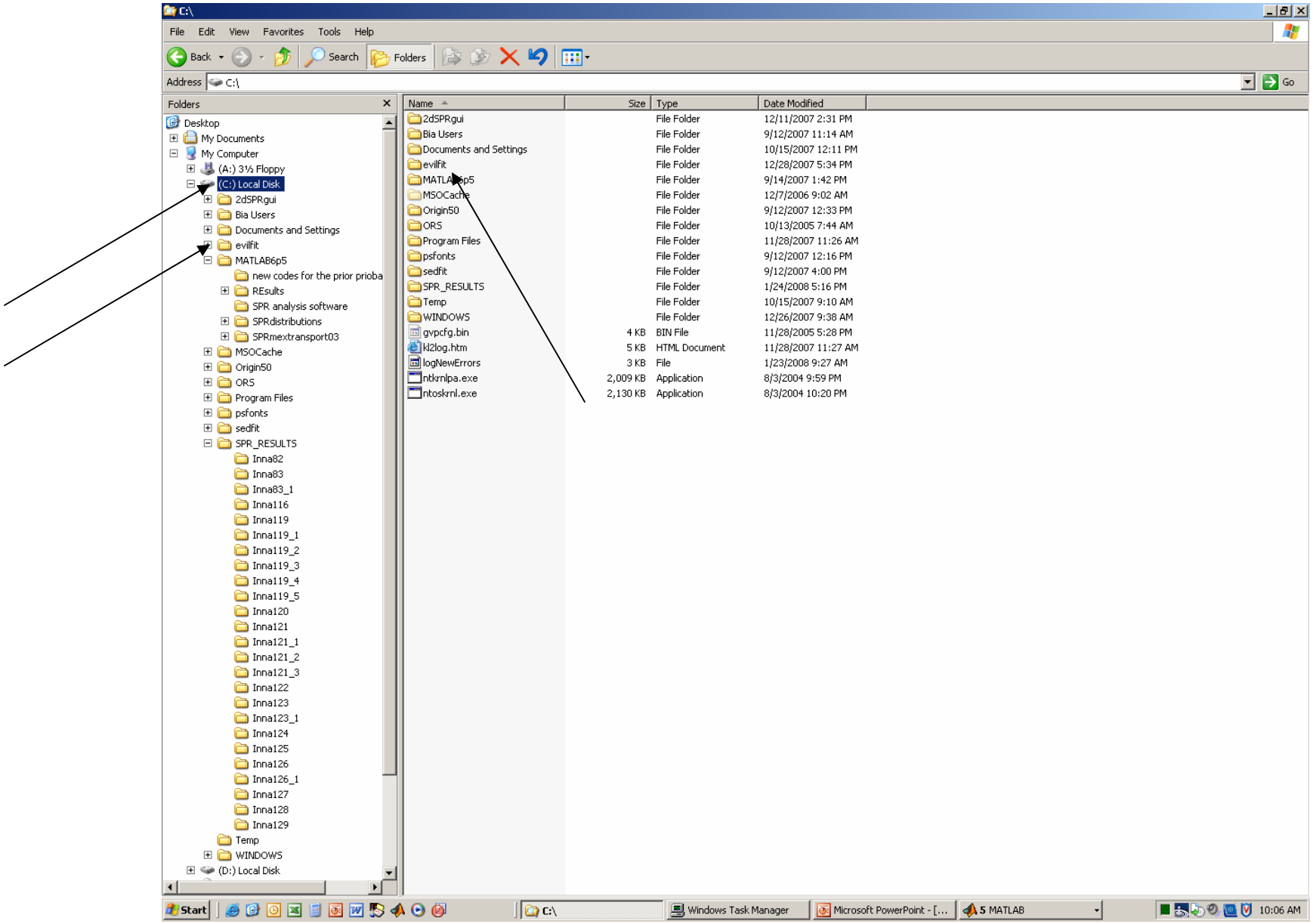
2) Installation, Starting of EVILFIT, and Loading of Files

- 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>


C:\
File Edit View Favorites Tools Help
Back Search Folders
Address C:\
Go
Folders
Desktop
My Documents
My Computer
(A:) 3½ Floppy
(C:) Local Disk
2dSPRgui
Bia Users
Documents and Settings
evilfit
MATLAB6p5
new codes for the prior prioba
REsults
SPR analysis software
SPRdistributions
SPRmextransport03
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
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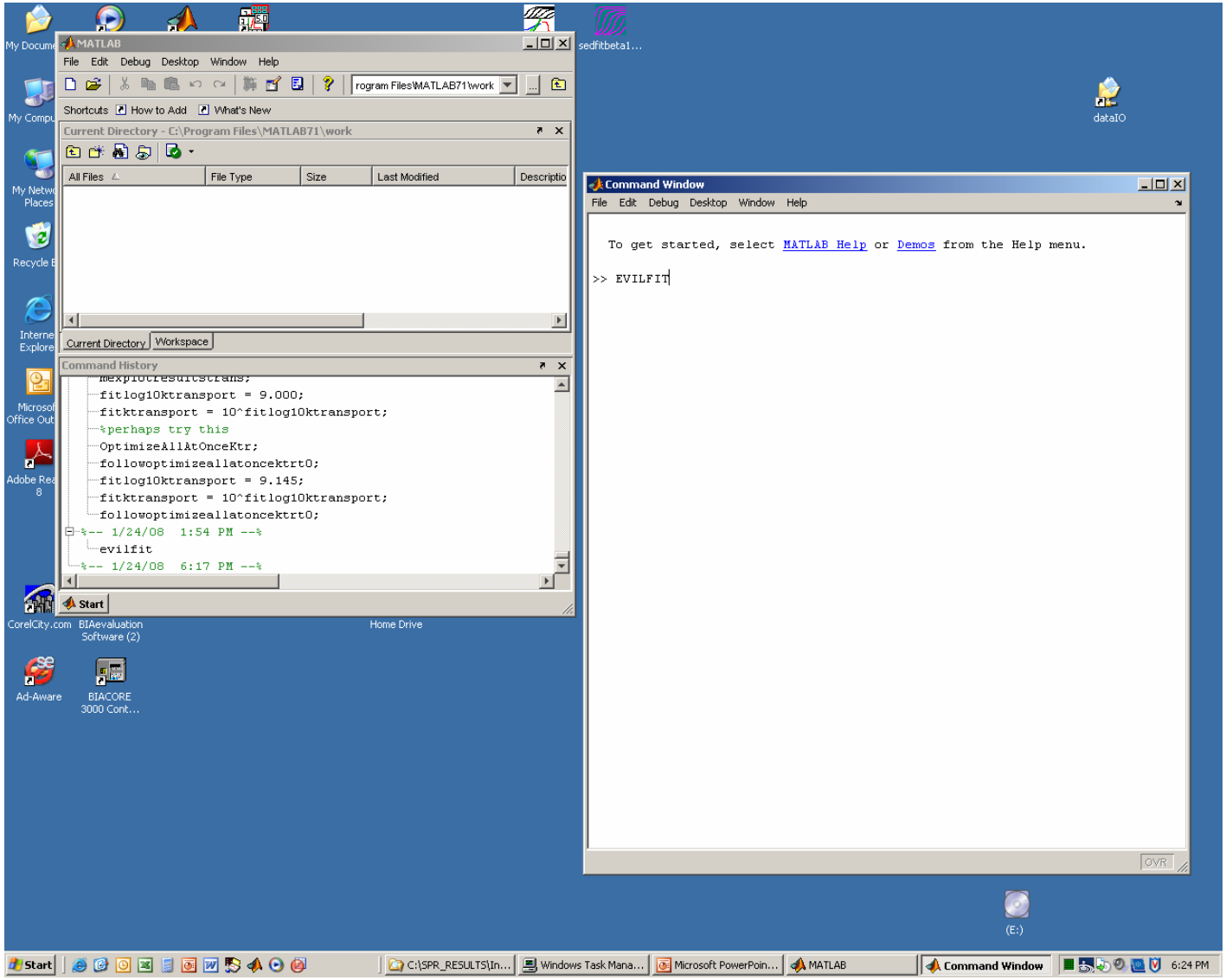
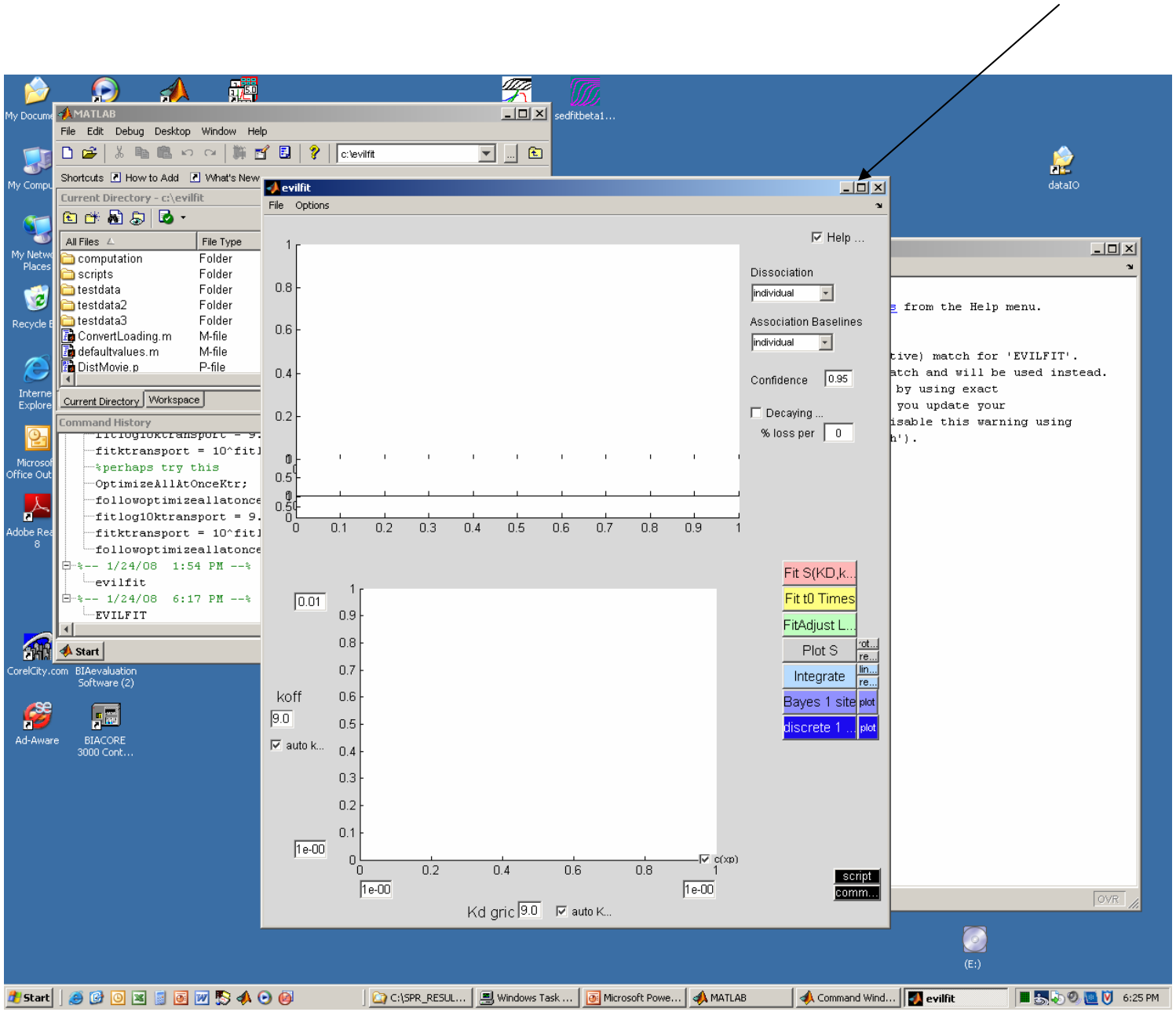
Inna123_1
Inna124
Inna125
Inna126
Inna126_1
Inna127
Inna128
Inna129
Temp
WINDOWS
(D:) Local Disk
Name Size Type Date Modified
2dSPRgui File Folder 12/11/2007 2:31 PM
Bia Users File Folder 9/12/2007 11:14 AM
Documents and Settings File Folder 10/15/2007 12:11 PM
evilfit File Folder 12/28/2007 5:34 PM
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
Start
C:\
Windows Task Manager
Microsoft PowerPoint - [...
5 MATLAB
10:06 AM

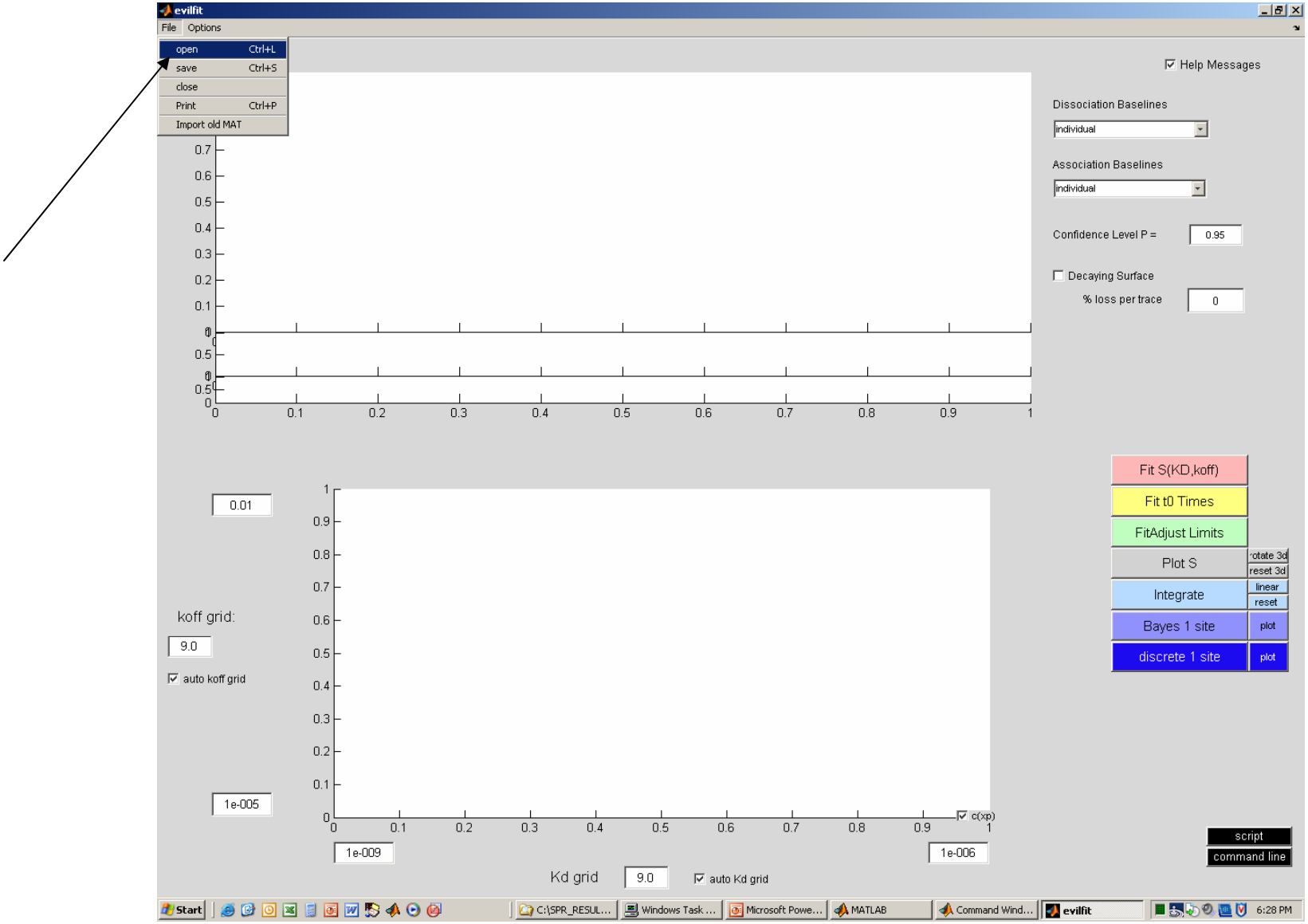
Set path slides,
Add description



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



•EVILFIT will appear on the screen. Maximize it


evilfit
File
Options
open Ctrl+L
save Ctrl+S
close
Print Ctrl+P
Import old MAT
Help Messages
Dissociation Baselines
individual
Association Baselines
individual
Confidence Level P =
0.95
Decaying Surface
% loss per trace
0
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
0.01
koff grid:
9.0
auto koff grid
1e-005
c(xp)
1e-009
1e-006
Kd grid
9.0
auto Kd grid
script
command line
Start
C:\SPR_RESUL...
Windows Task ...
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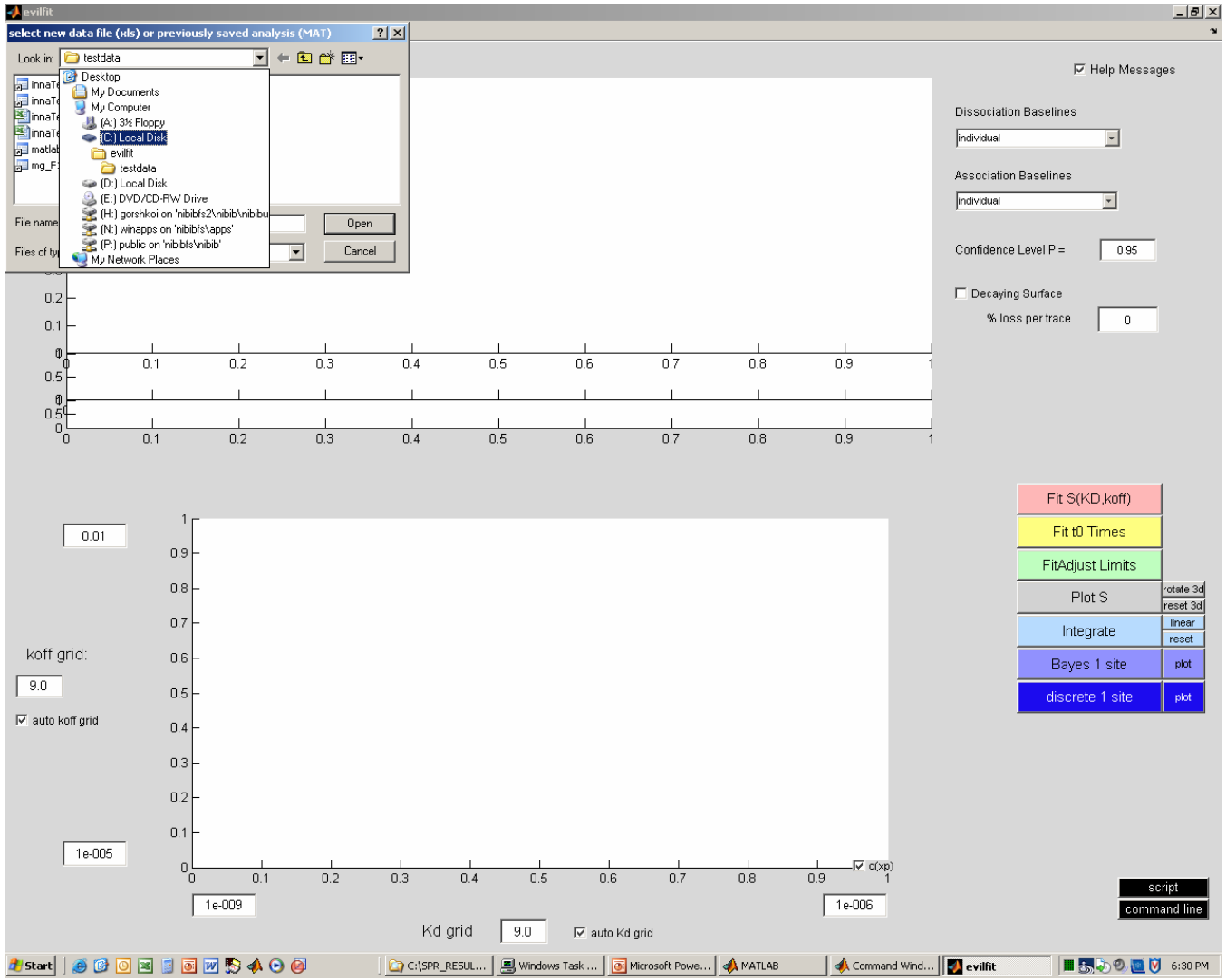
MATLAB
Command Wind...
evilfit
6:28 PM

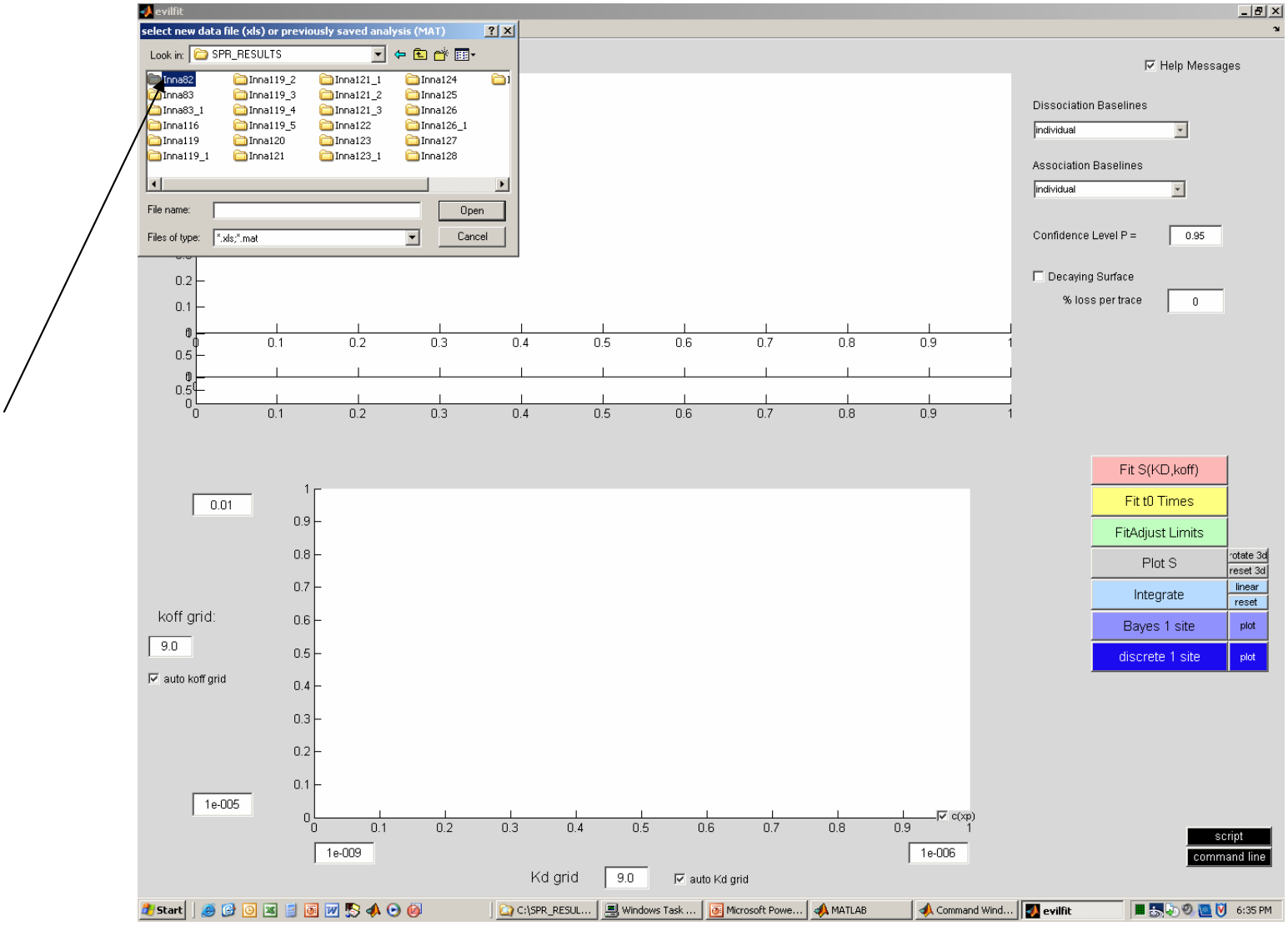
File/open

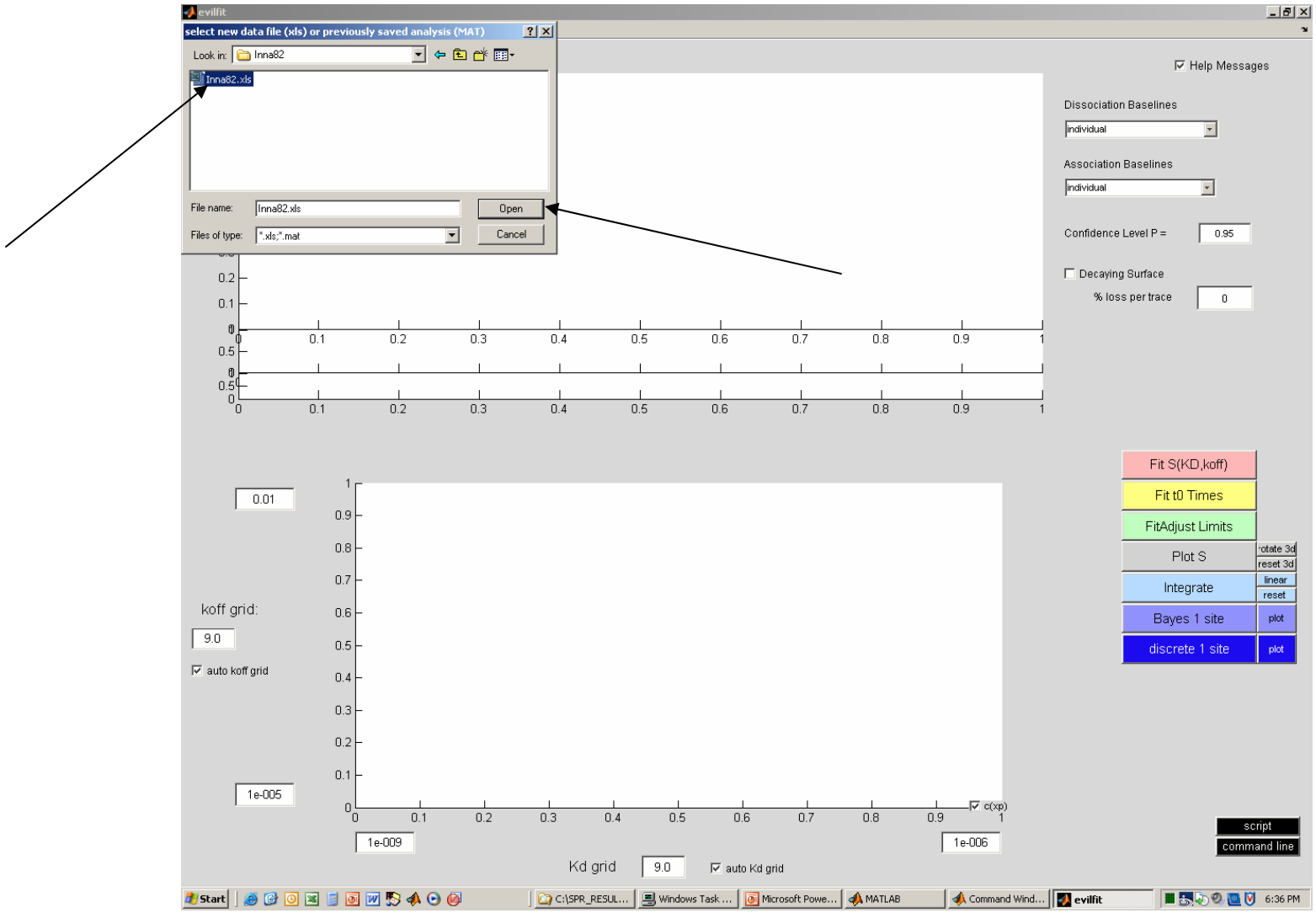


In the Selection window browse for the xls.file that is in the folder

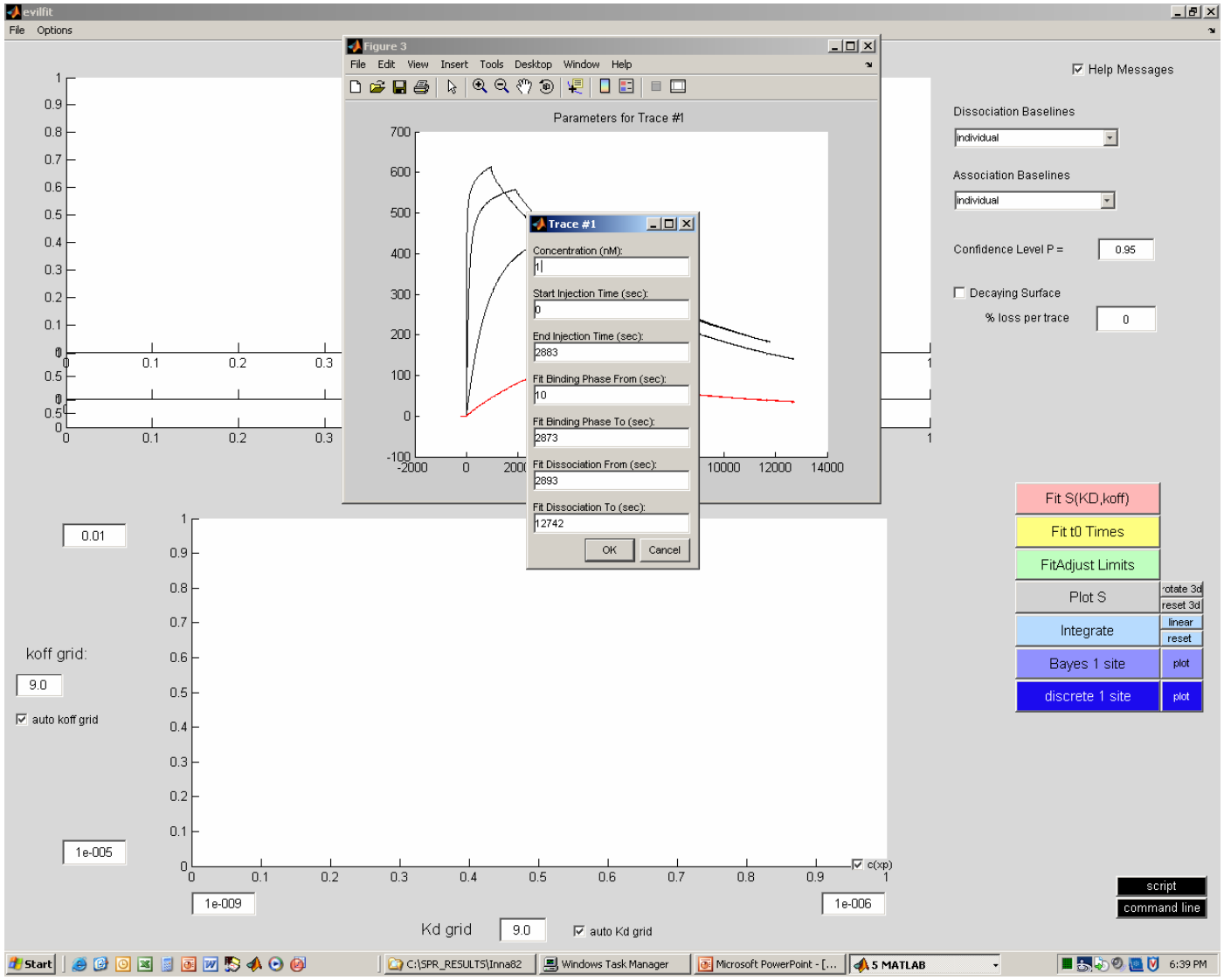
C:\SPR_RESULTS\Inna82\Inna82.xls;

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

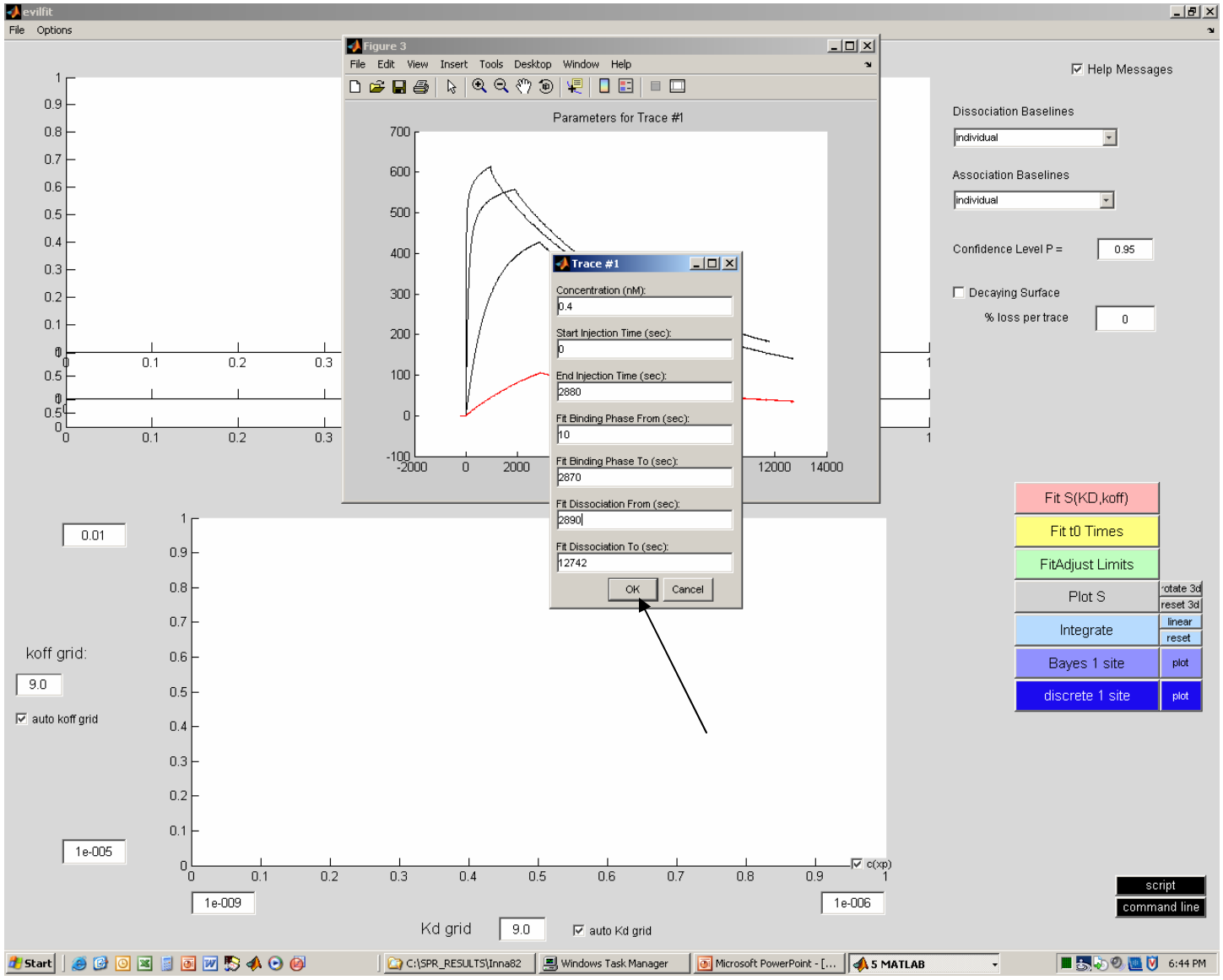

evilfit
select new data file (xls) or previously saved analysis (MAT)
Look in:
SPR_RESULTS
Inna82
Inna83
Inna83_1
Inna116
Inna119
Inna119_1
Inna119_2
Inna119_3
Inna119_4
Inna119_5
Inna120
Inna121
Inna121_1
Inna121_2
Inna121_3
Inna122
Inna123
Inna123_1
Inna124
Inna125
Inna126
Inna126_1
Inna127
Inna128
File name:
Open
Files of type:
.xls;.mat
Cancel
Help Messages
Dissociation Baselines
individual
Association Baselines
individual
Confidence Level P =
0.95
Decaying Surface
% loss per trace
0
0.01
koff grid:
9.0
auto koff grid
1e-005
1e-009
1e-006
c(xp)
Kd grid
9.0
auto Kd grid
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


evilfit
select new data file (xls) or previously saved analysis (MAT)
Look in: Inna82
Inna82.xls
File name: Inna82.xls
Open
Files of type: *.xls;*.mat
Cancel
Help Messages
Dissociation Baselines
individual
Association Baselines
individual
Confidence Level P = 0.95
Decaying Surface
% loss per trace 0
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
0.01
koff grid:
9.0
auto koff grid
1e-005
c(xp)
1e-009
1e-006
Kd grid
9.0
auto Kd grid
script
command line
Start
C:\SPR_RESUL...
Windows Task ...
Microsoft Powe...
MATLAB
Command Wind...
evilfit
6:36 PM

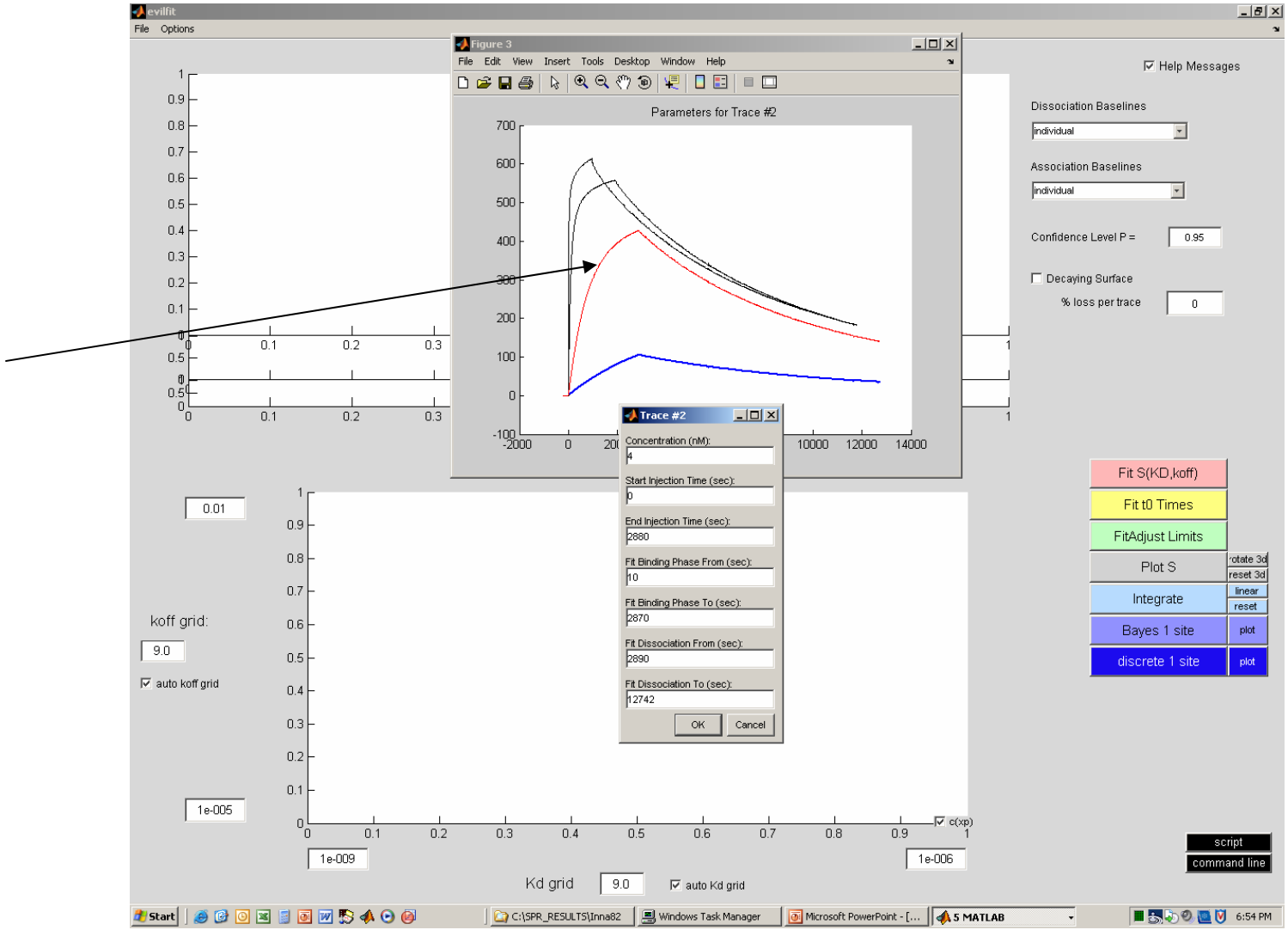
<open>



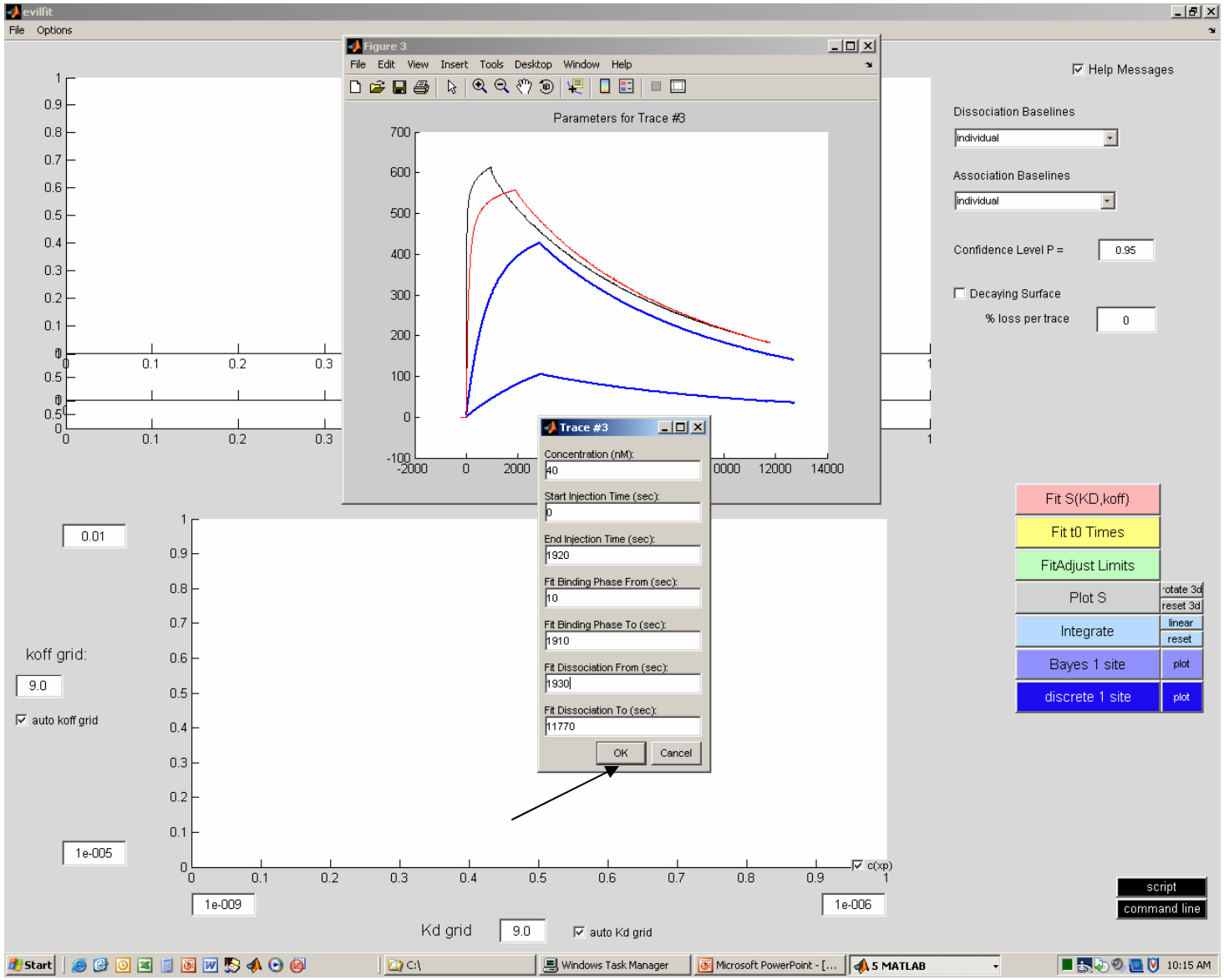
Window with experimental data is open; data for the trace#1 are highlighted in red. Parameters for Trace #1 can be introduced



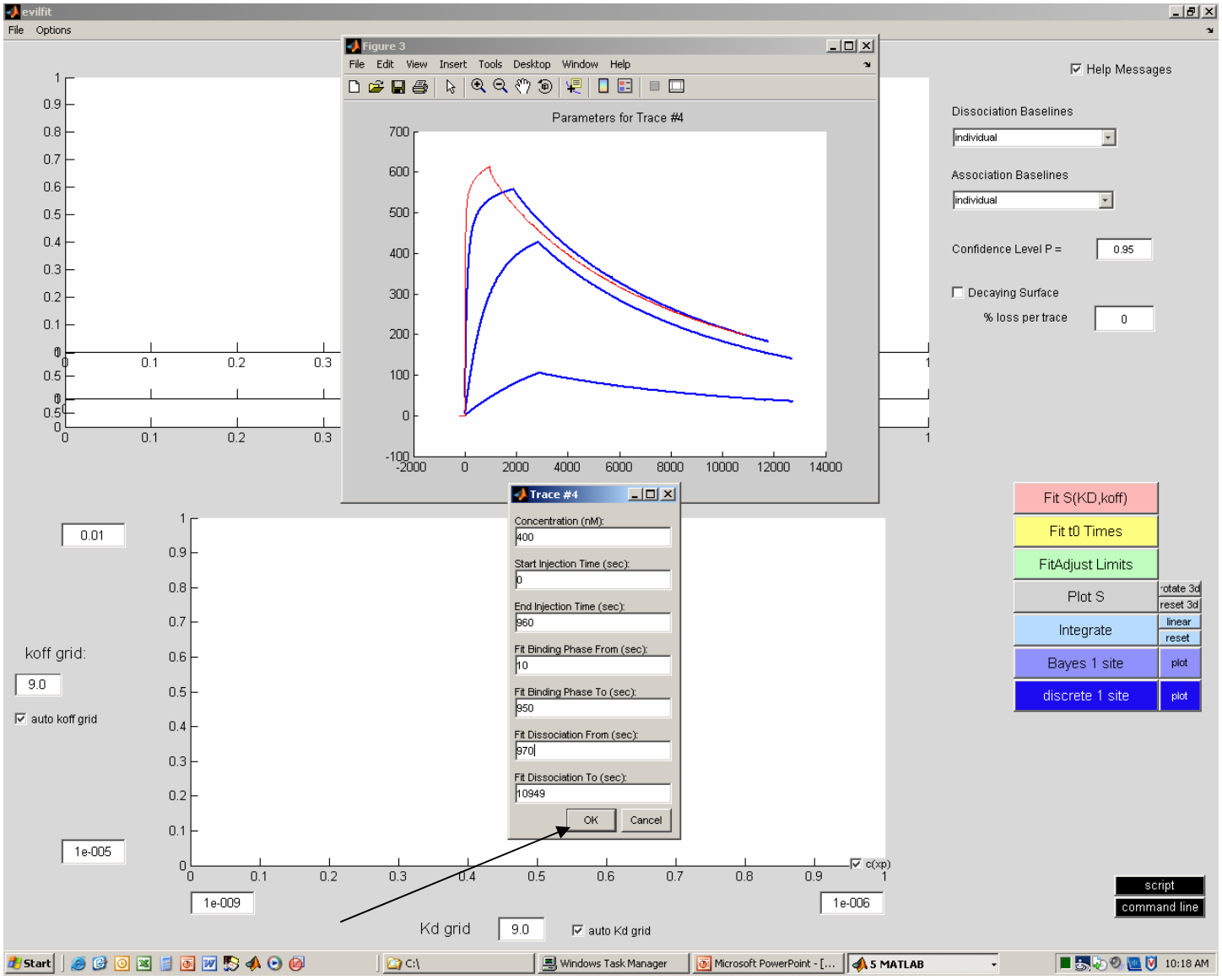
Parameters for Trace #1are as shown; click <OK> to move to Parameters for Trace #2



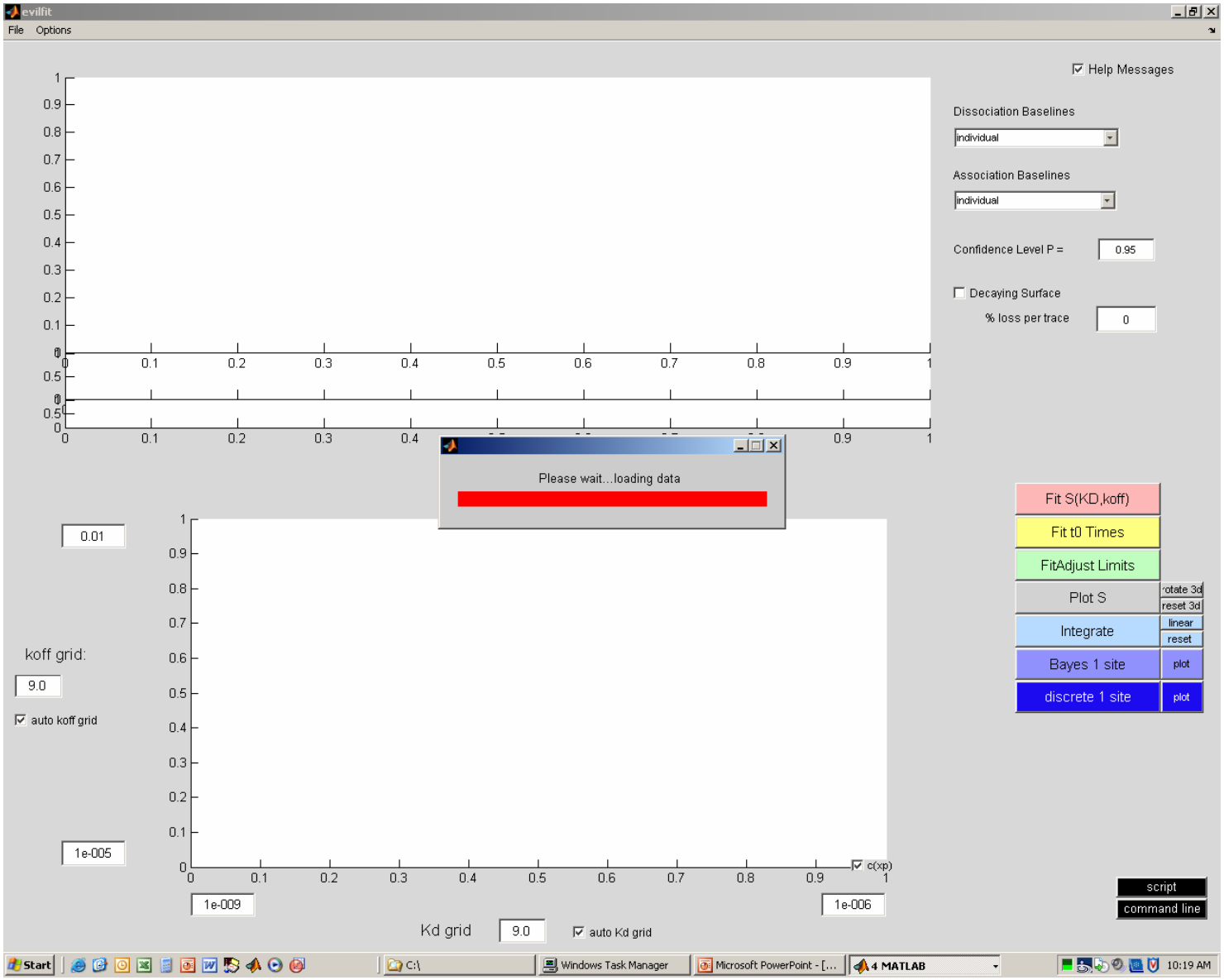
Data for the trace#2 are highlighted in red. Parameters for Trace #2 are as shown; click <OK> to move to Parameters for Trace #3



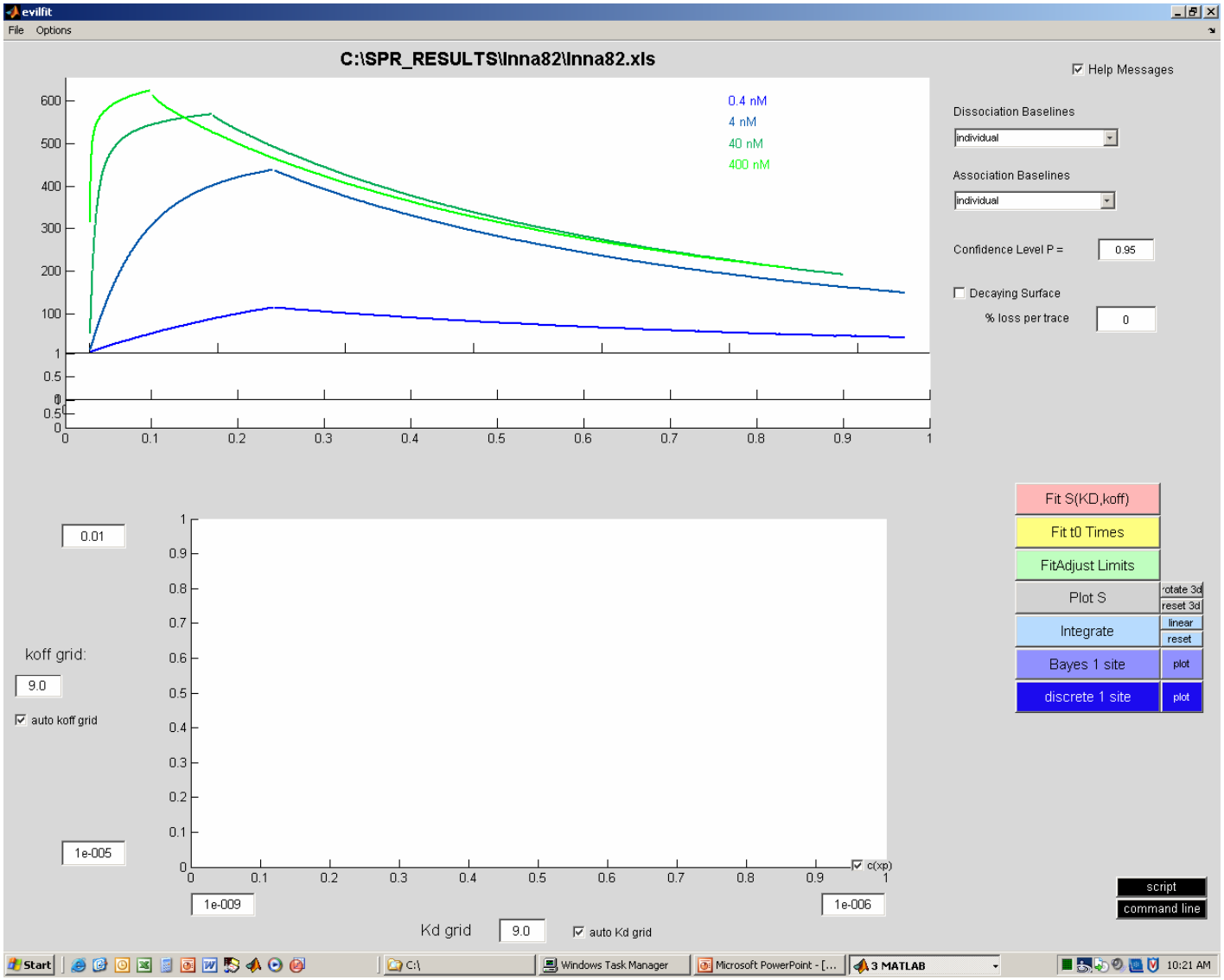
Data for the trace#3 are highlighted in red. Parameters for Trace #3 are as shown; click <OK> to move to Parameters for Trace #4



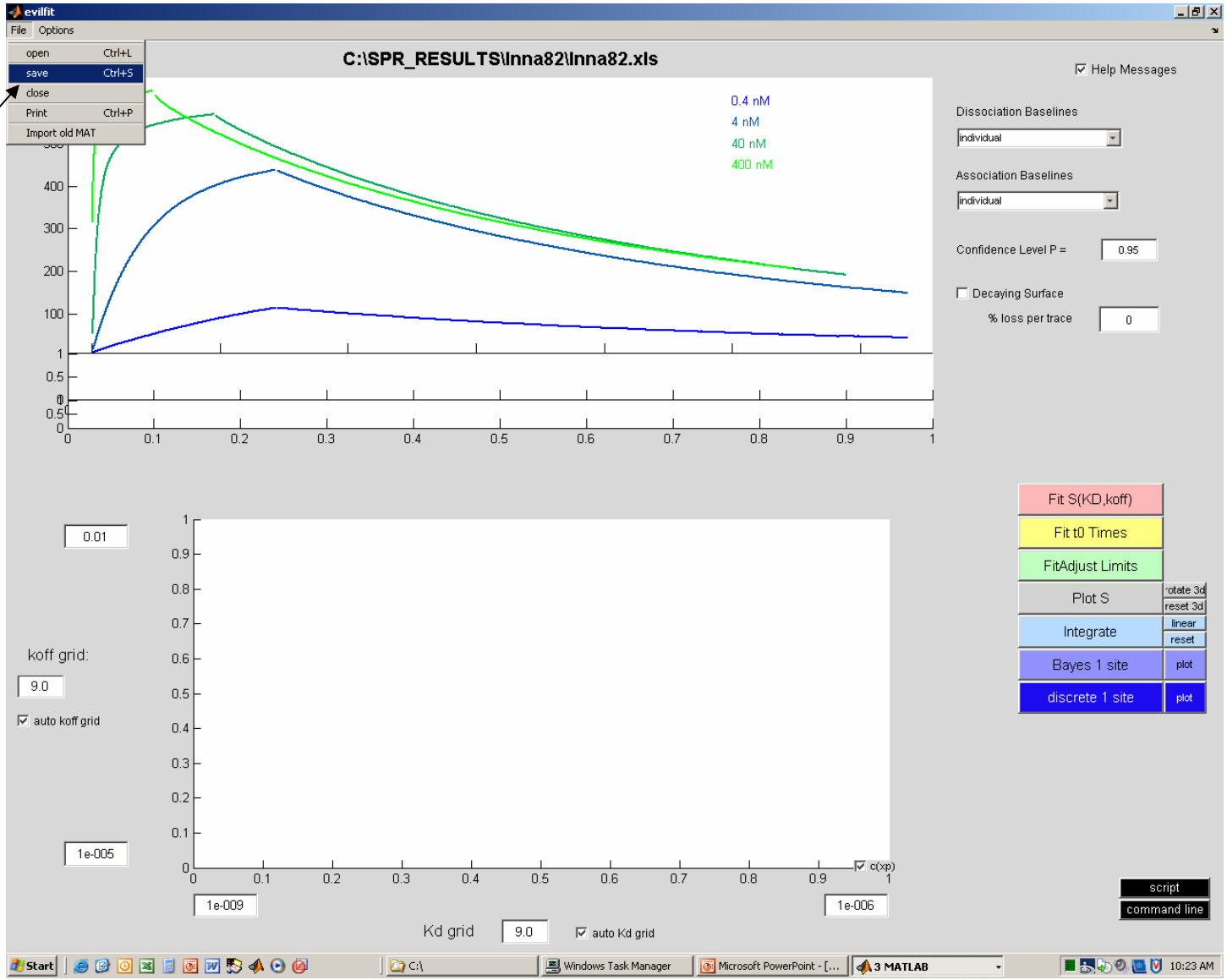
Data for the trace#4 are highlighted in red. Parameters for Trace #4 are as shown; click <OK> to complete the procedure



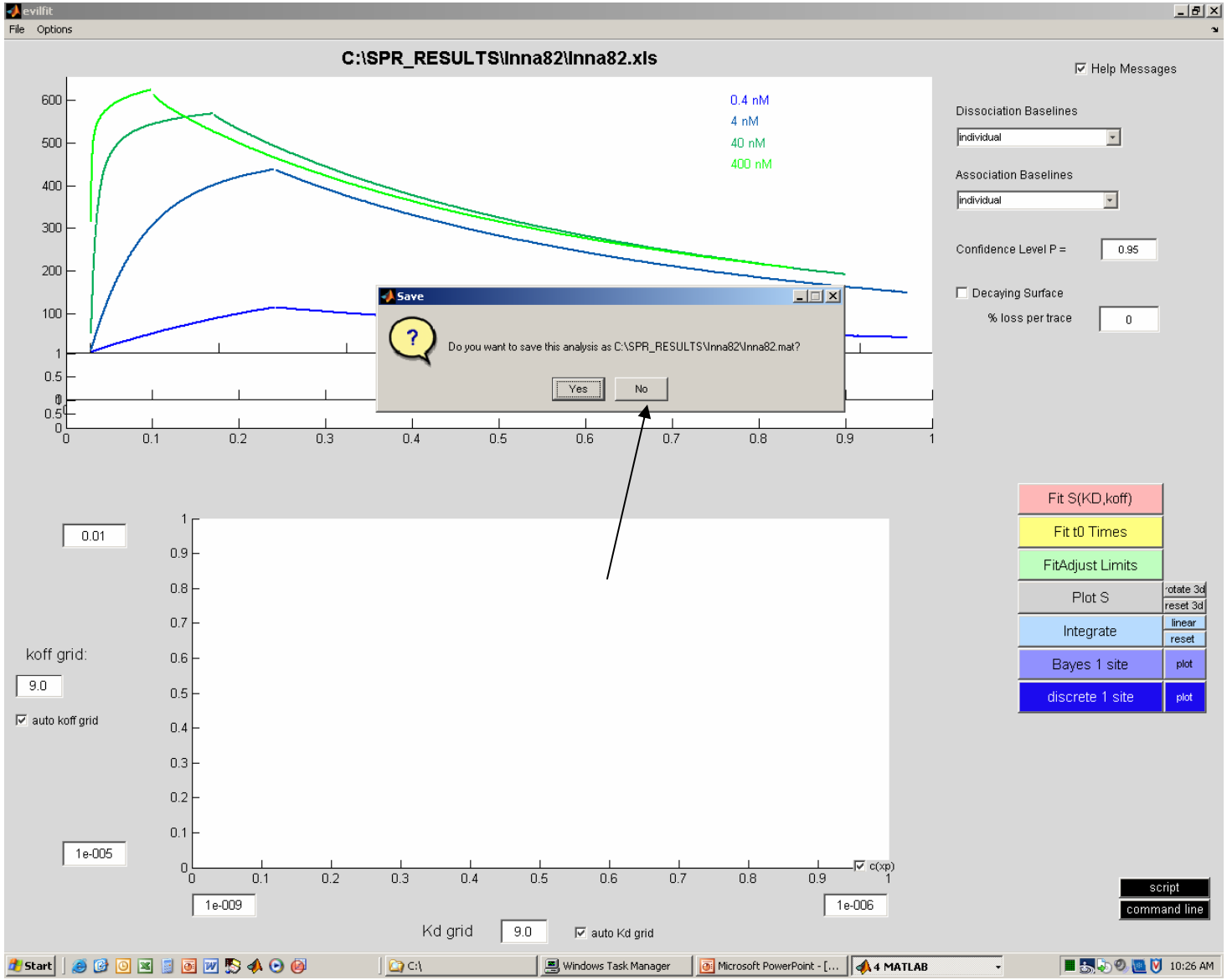
Data are loading…



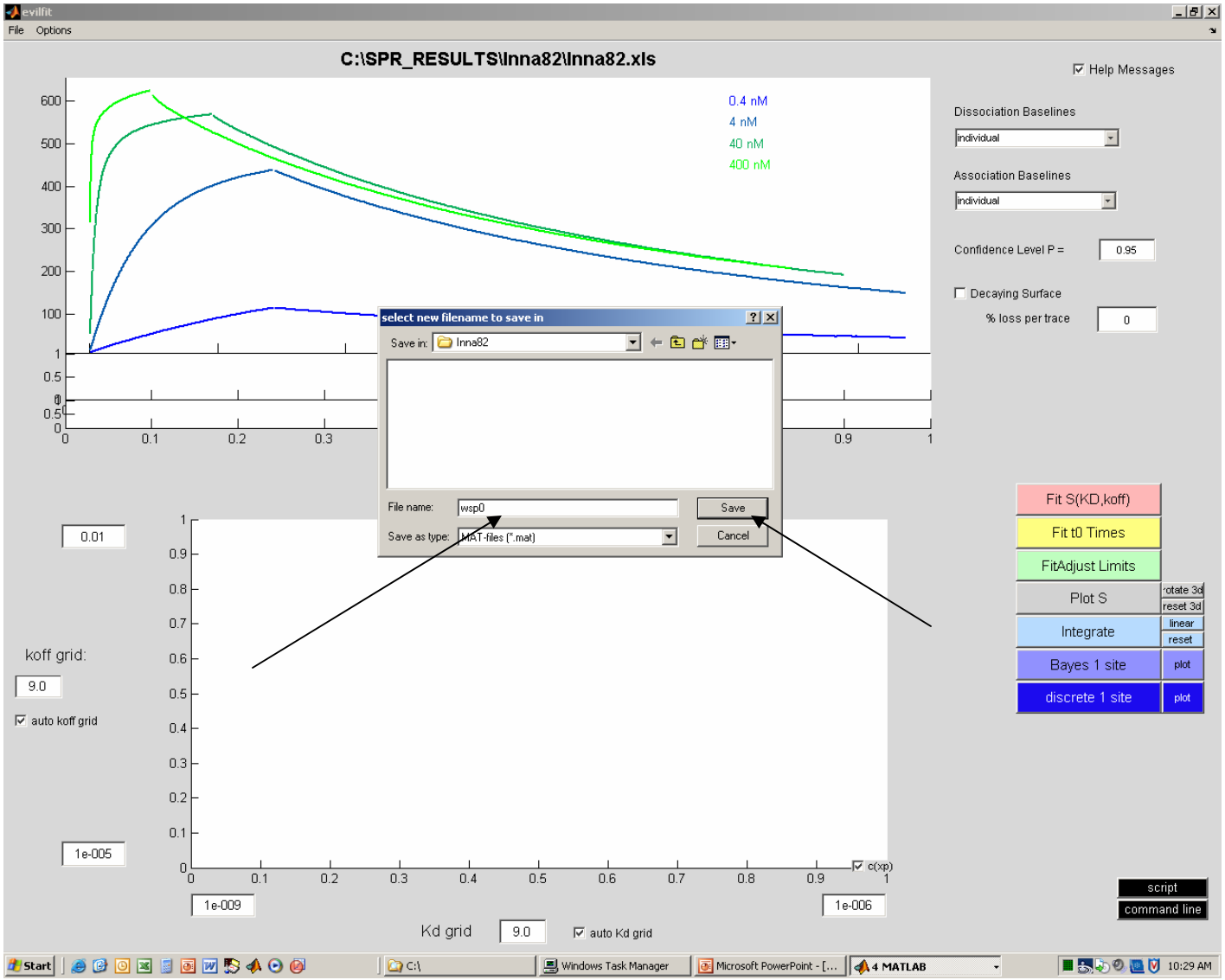
Loading is completed



Save work space as wsp0: File/Save

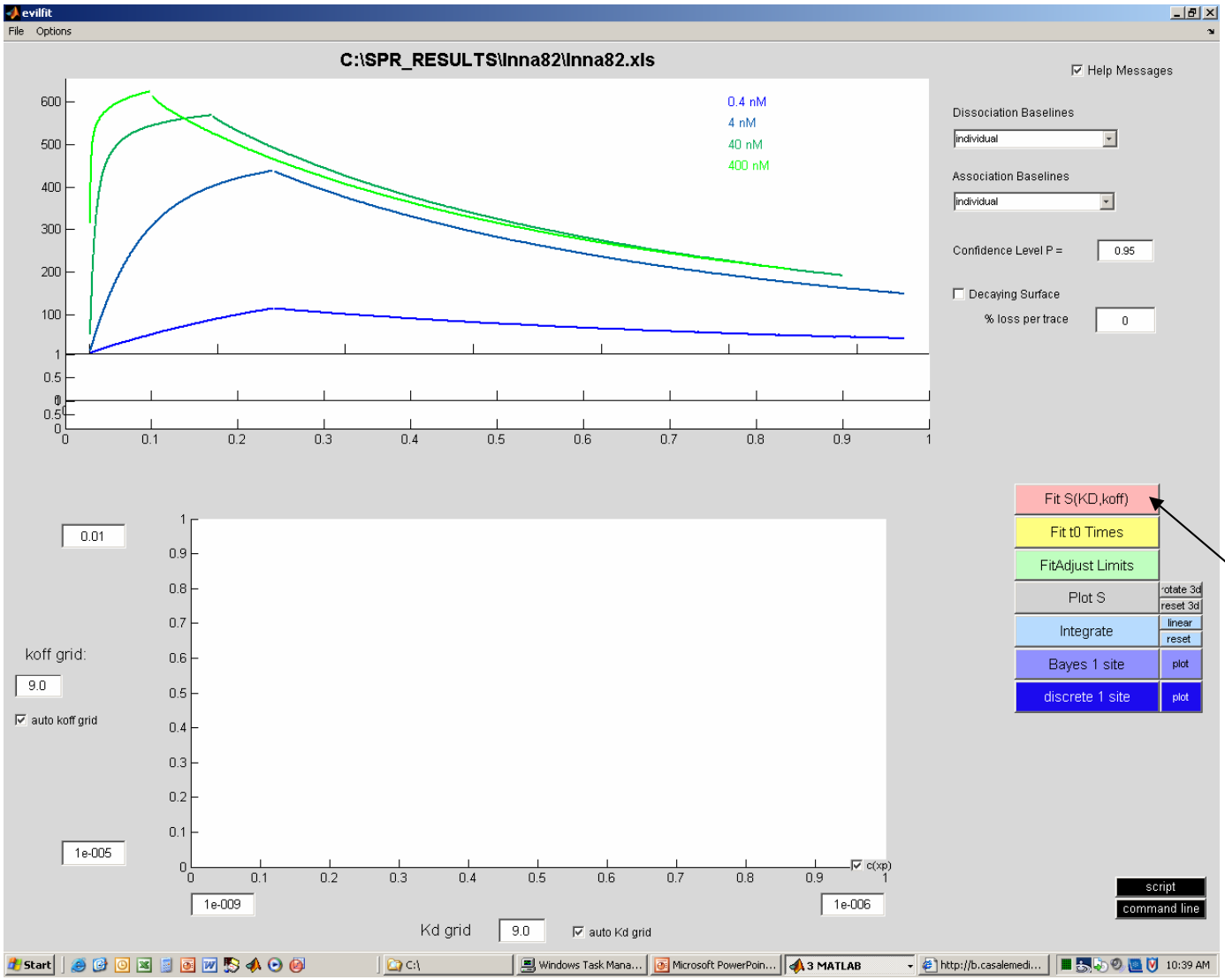


I like to use a different system for saving the workspaces (we will be generating several of them as the analysis comes along). Click “NO” to the question

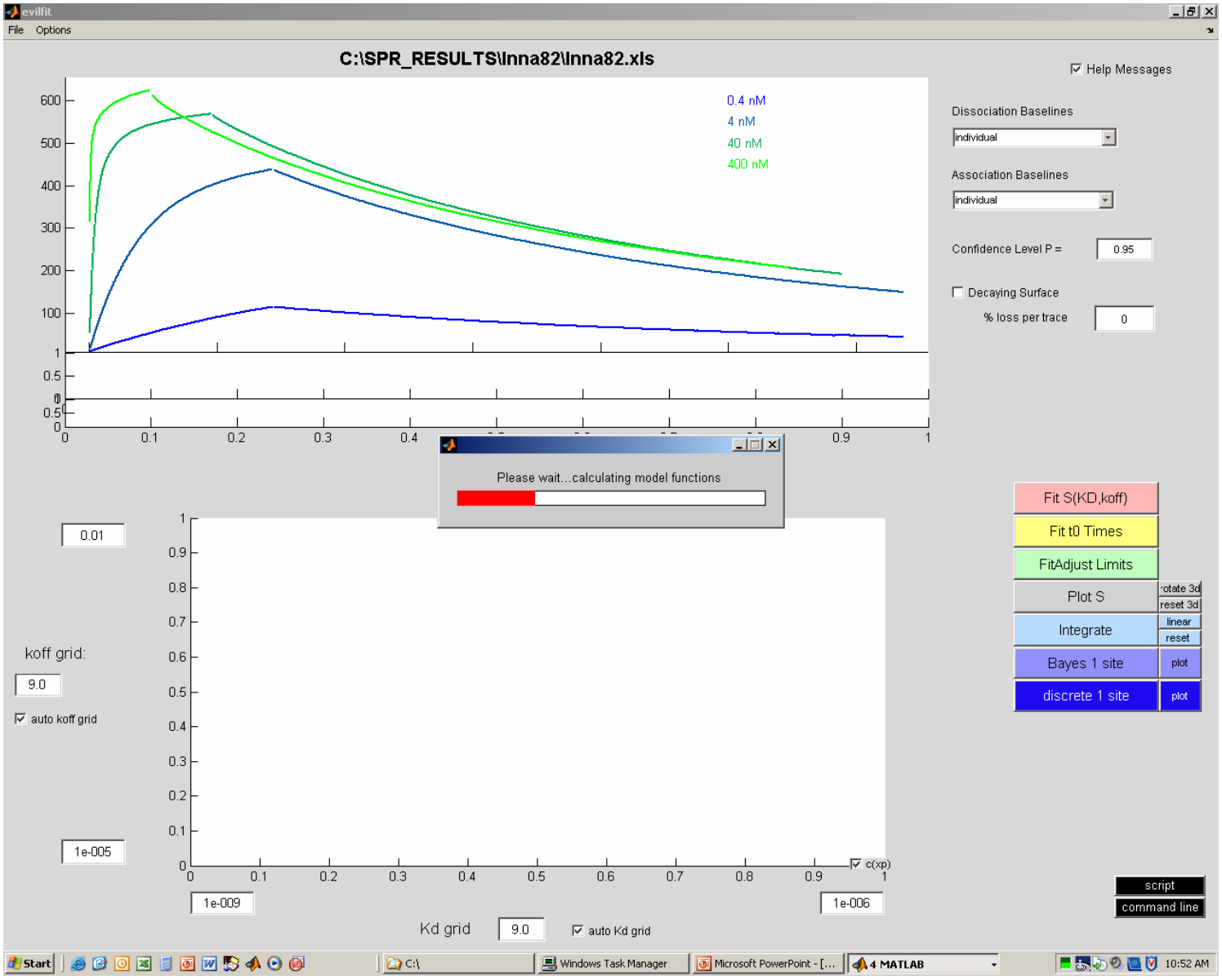


Type File name ‘wsp0’ and click <save>

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


evilfit
File Options
C:\SPR_RESULTS\lnna82\lnna82.xls
0.4 nM
4 nM
40 nM
400 nM
Help Messages
Dissociation Baselines
individual
Association Baselines
individual
Confidence Level P =
0.95
Decaying Surface
% loss per trace
0
Please wait...calculating model functions
0.01
koff grid:
9.0
auto koff grid
1e-005
1e-009
1e-006
c(xp)
Kd grid
9.0
auto Kd grid
Fit S(KD,koff)
Fit t0 Times
FitAdjust Limits
Plot S
rotate 3d
reset 3d
Integrate
linear
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plot
discrete 1 site
plot
script
command line
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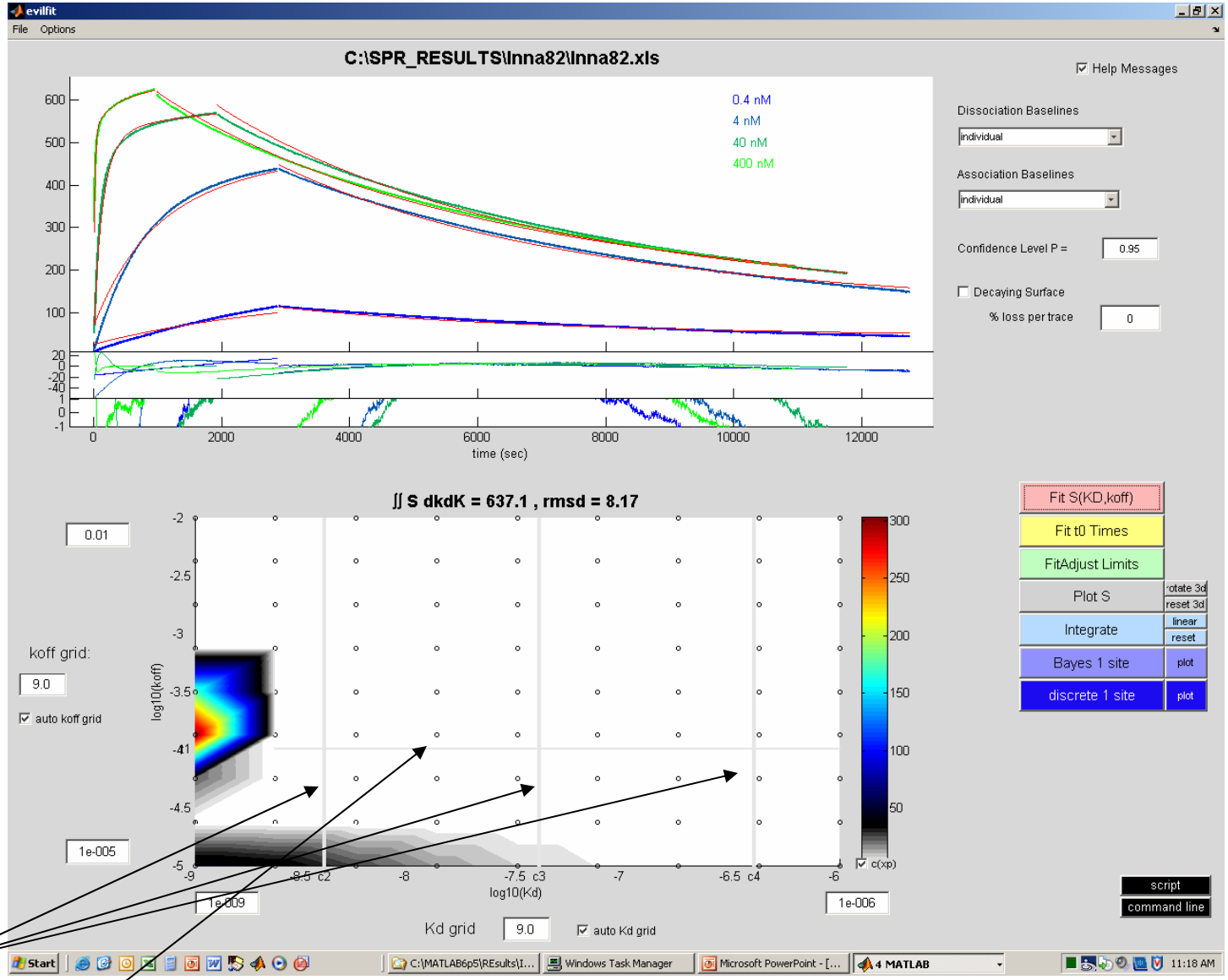
C:\
Windows Task Manager
Microsoft PowerPoint - [...
4 MATLAB
10:52 AM

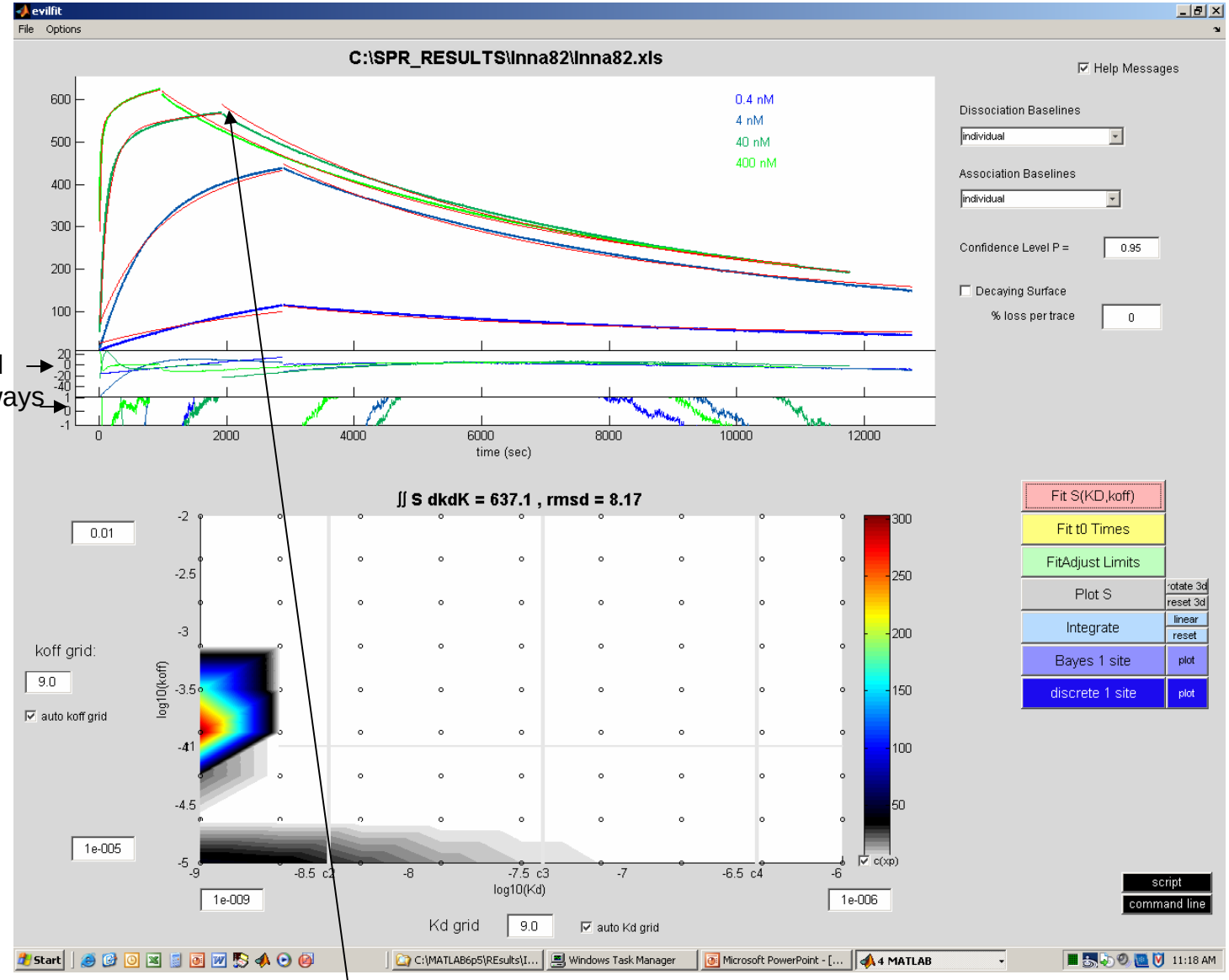
wait



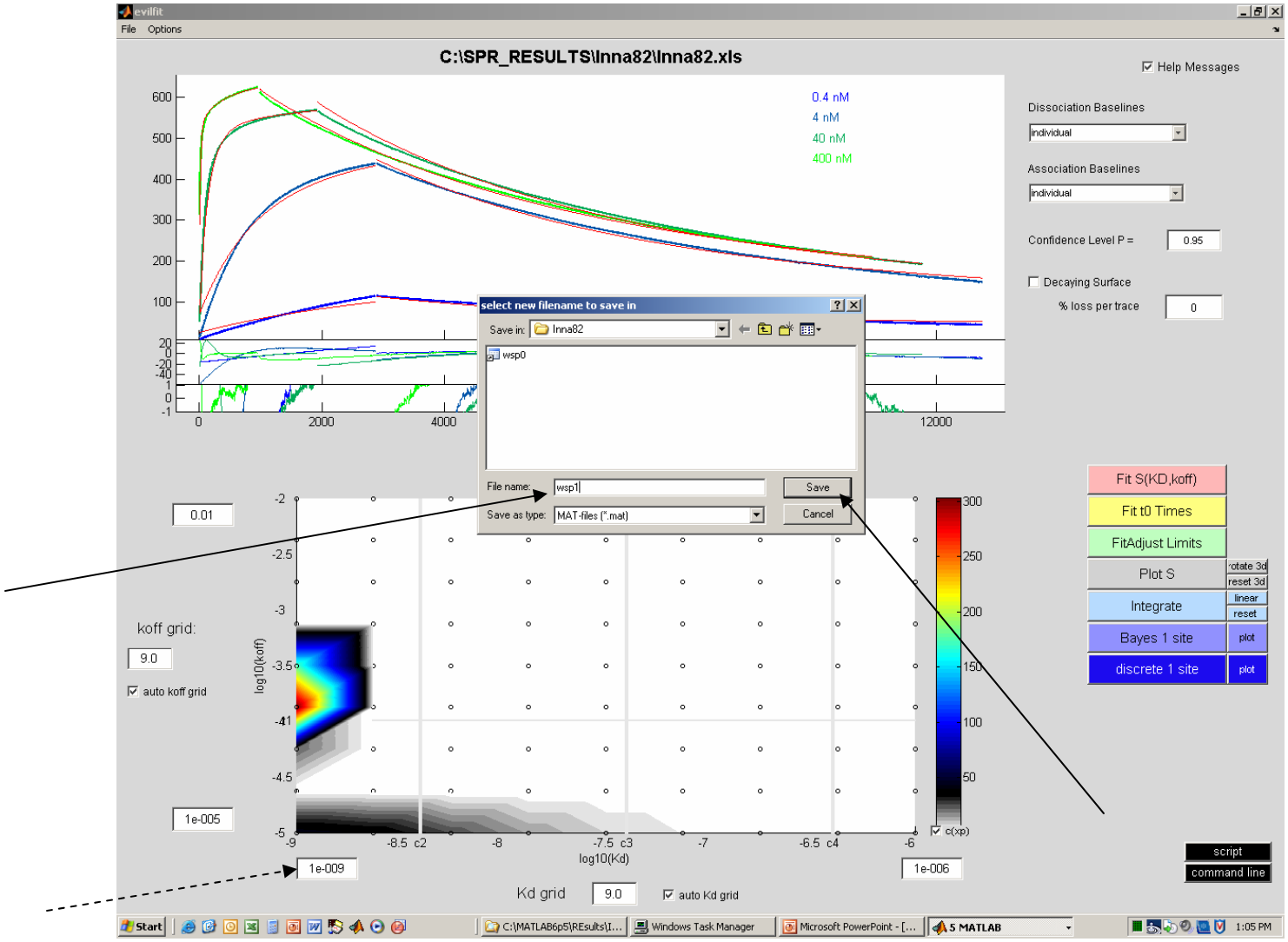
Note the grey vertical lines are at the experimental concentrations. The distribution peak optimally should not be far away from the experimental concentrations (there should be experimental concentrations higher than the peak, for the Kd value to be well-determined).

The horizontal line is at the koff 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 koff-value of the peak to be well-determined by the expeirmental data.

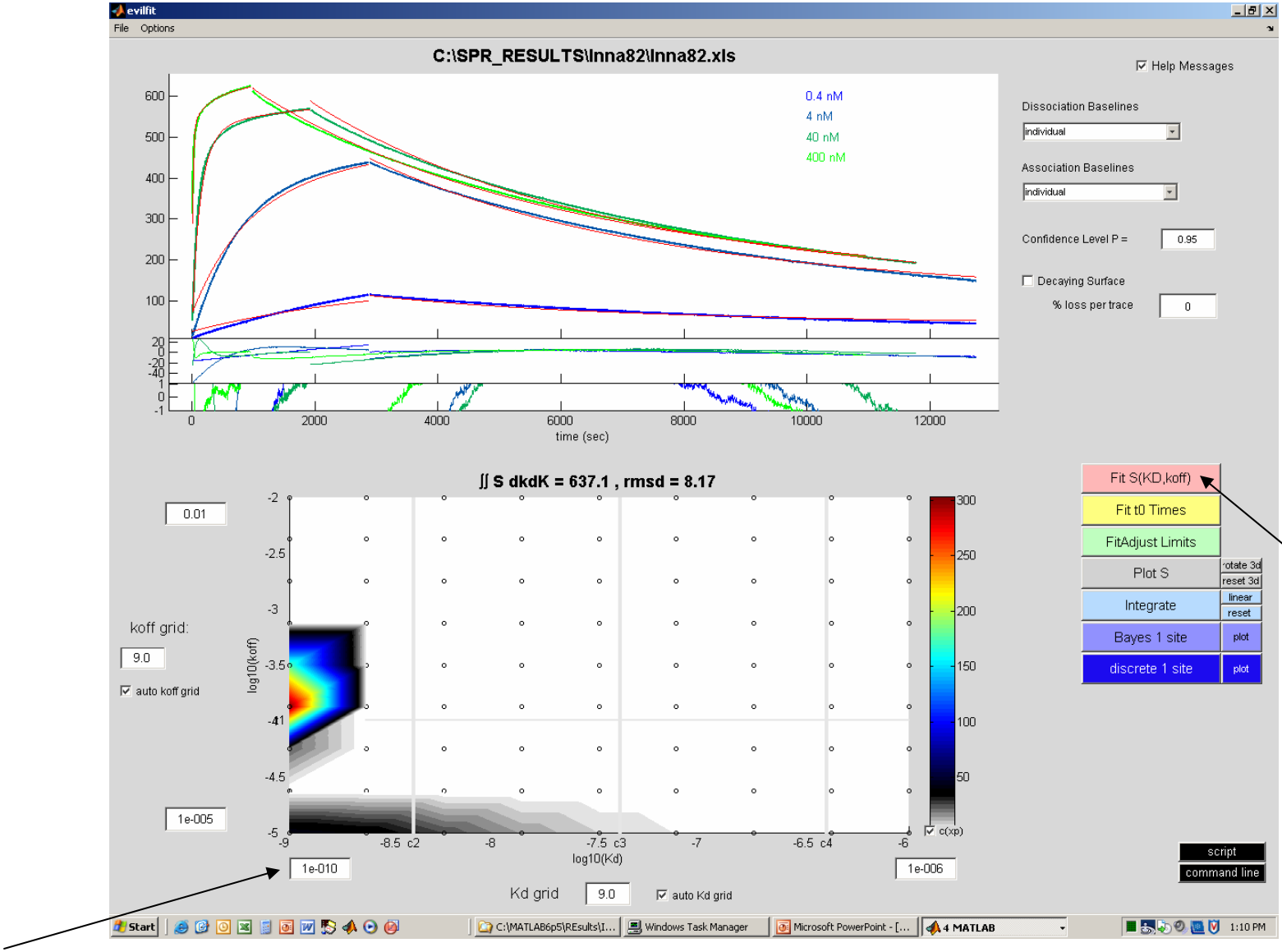
Residuals scaled
in two different ways



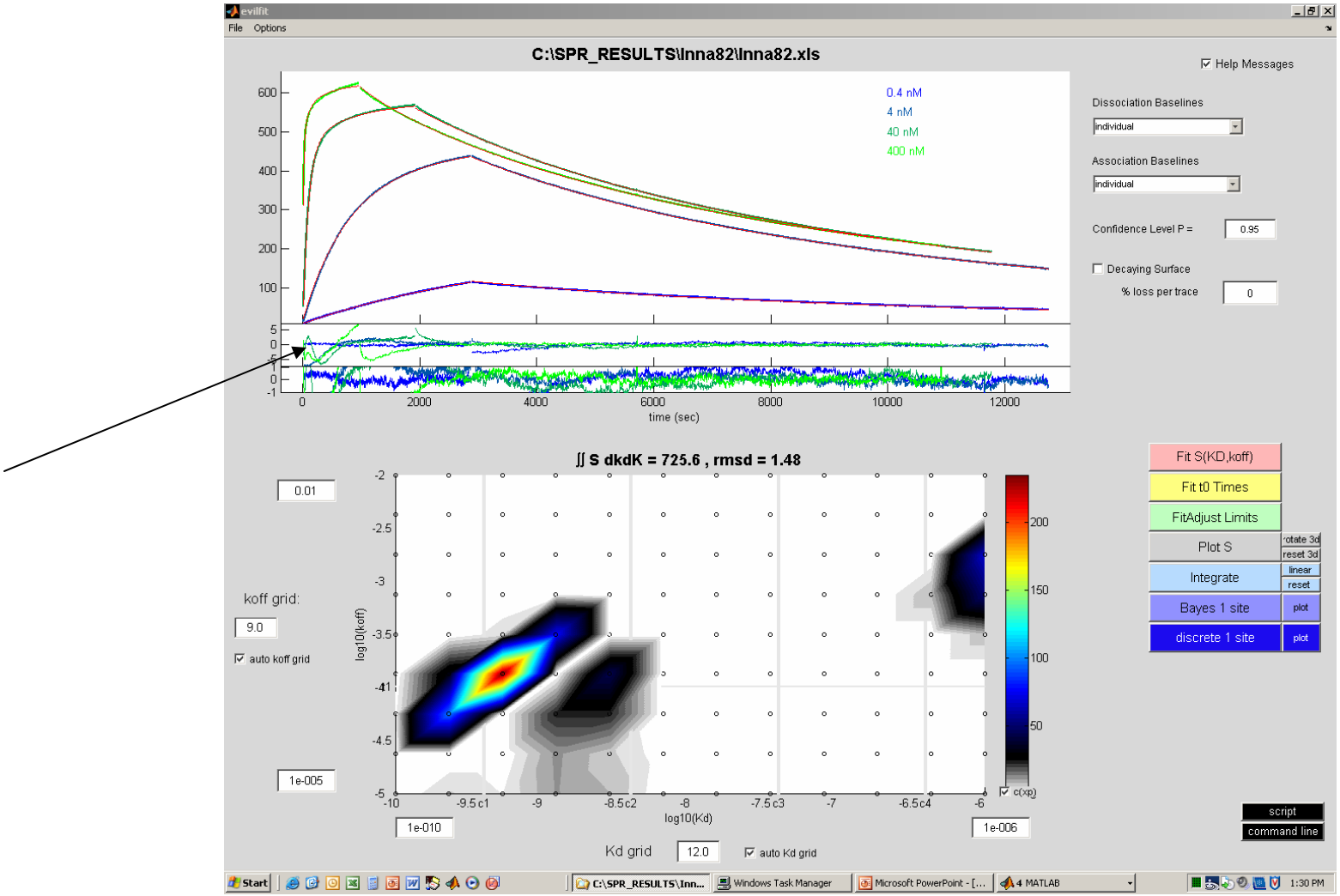
Fit is bad: calculated (red curves) and experimental data do not coincide; rmsd is big. Save work space as wsp1 (using the routine shown for wsp0)



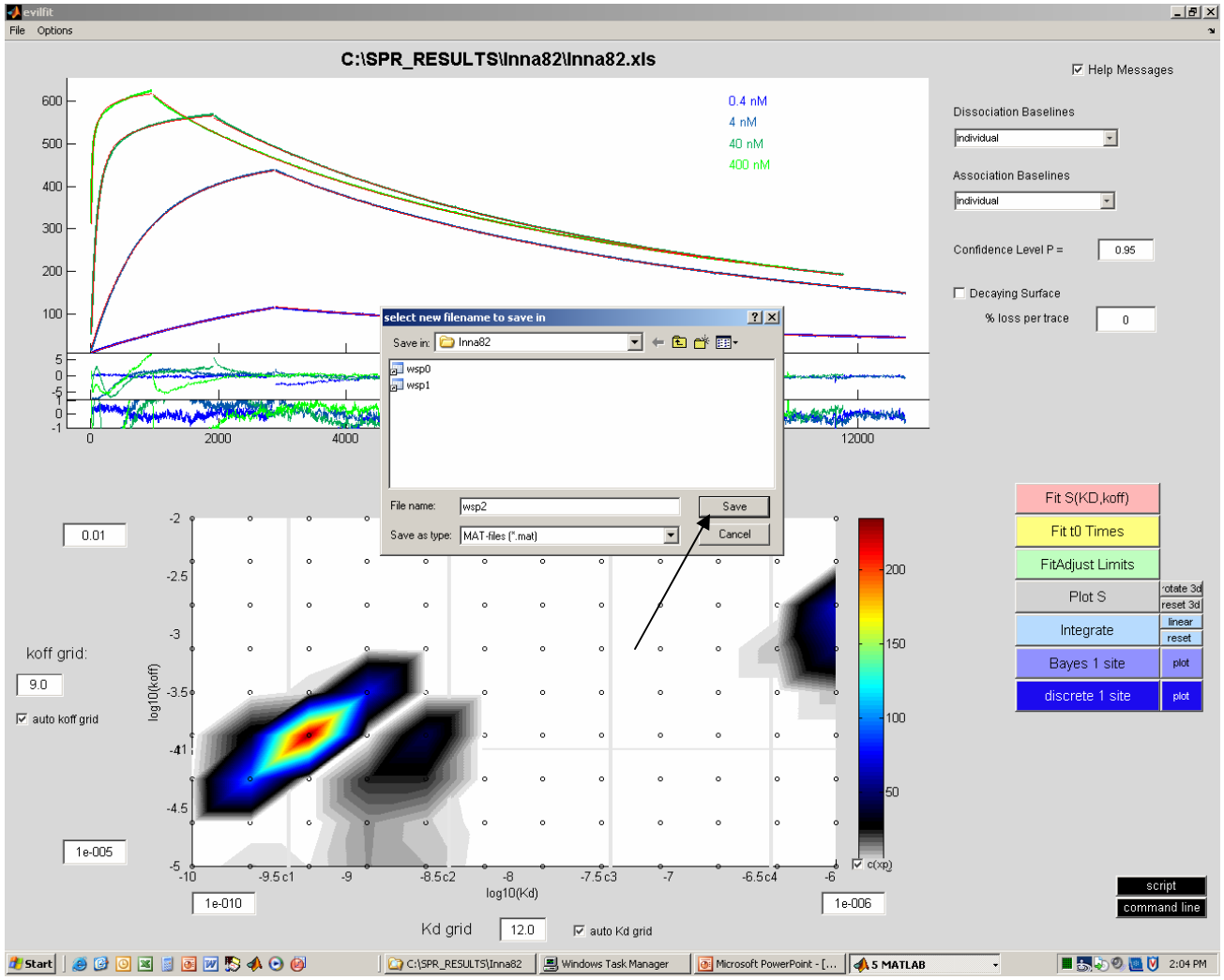
Type file name ‘wsp1’ and click <save> . The obvious next thing to do is to extend limits of parameters: decrease KDmin from 1e-9 to 1e-10



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



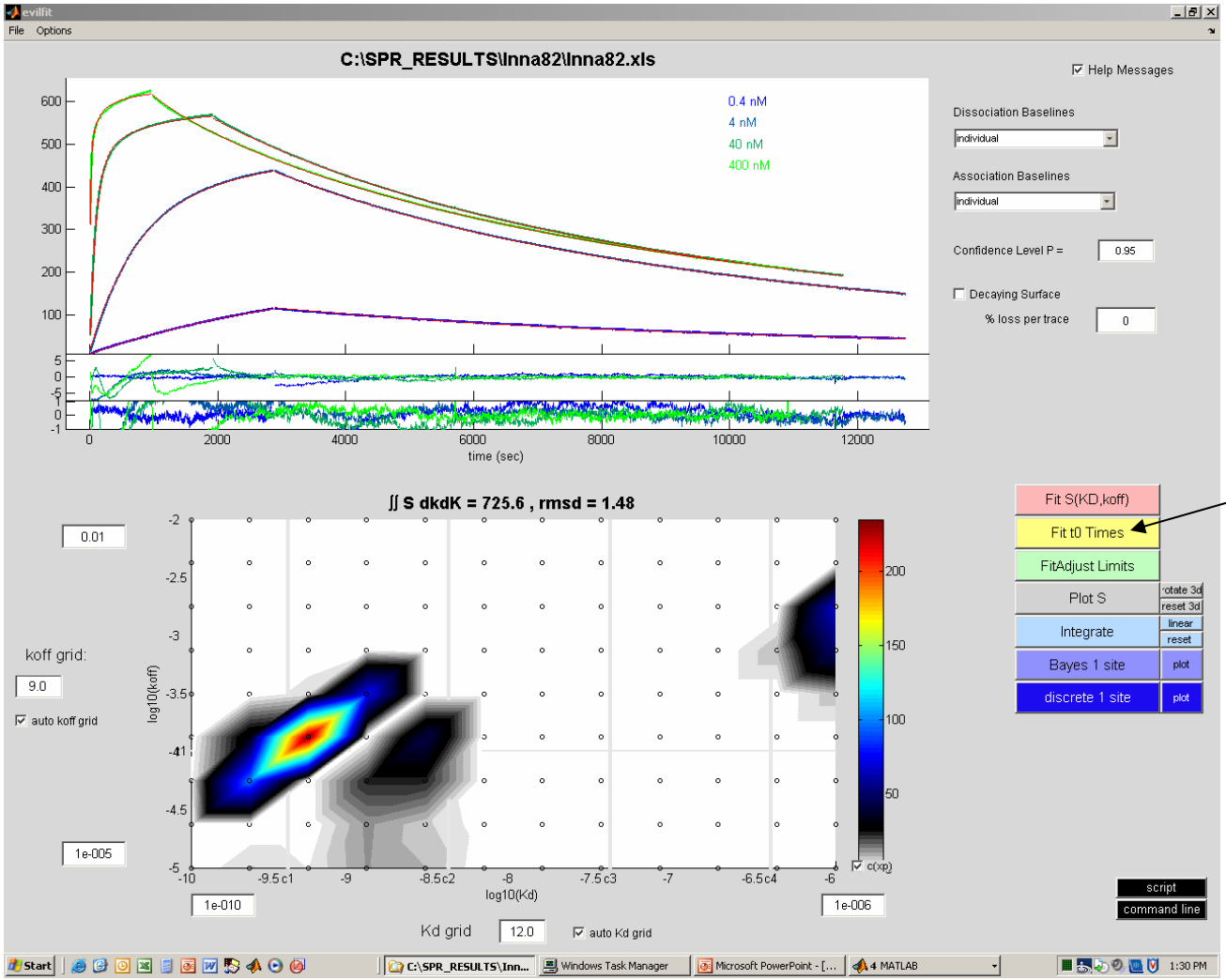
Fit is much better: rmsd is reduced significantly, but residuals are not randomly distributed, especially at start of injections.



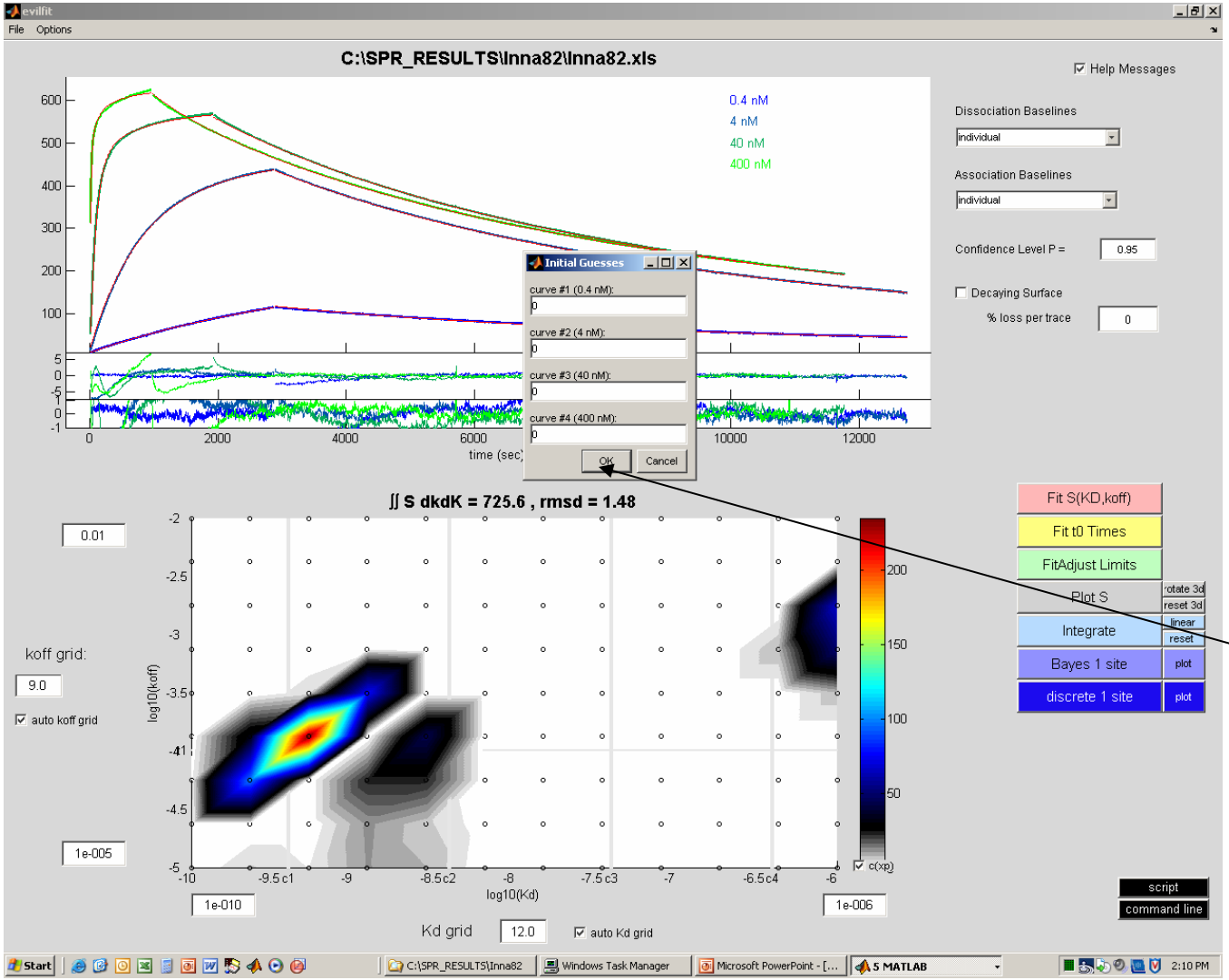
Save current work space as wsp2

3) Refining the Fit more

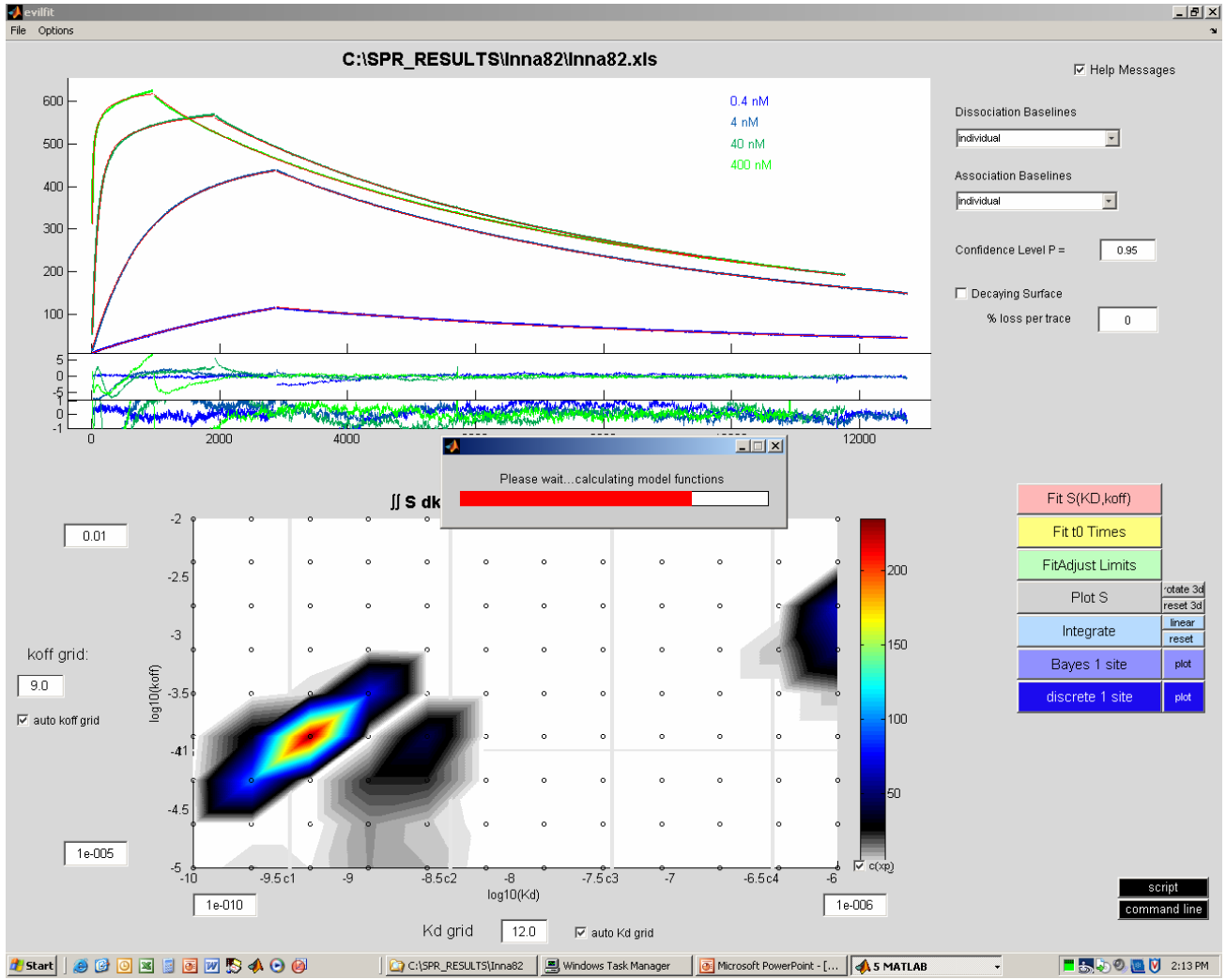
- Fitting t0 times
- Later: Adjusting the range some more



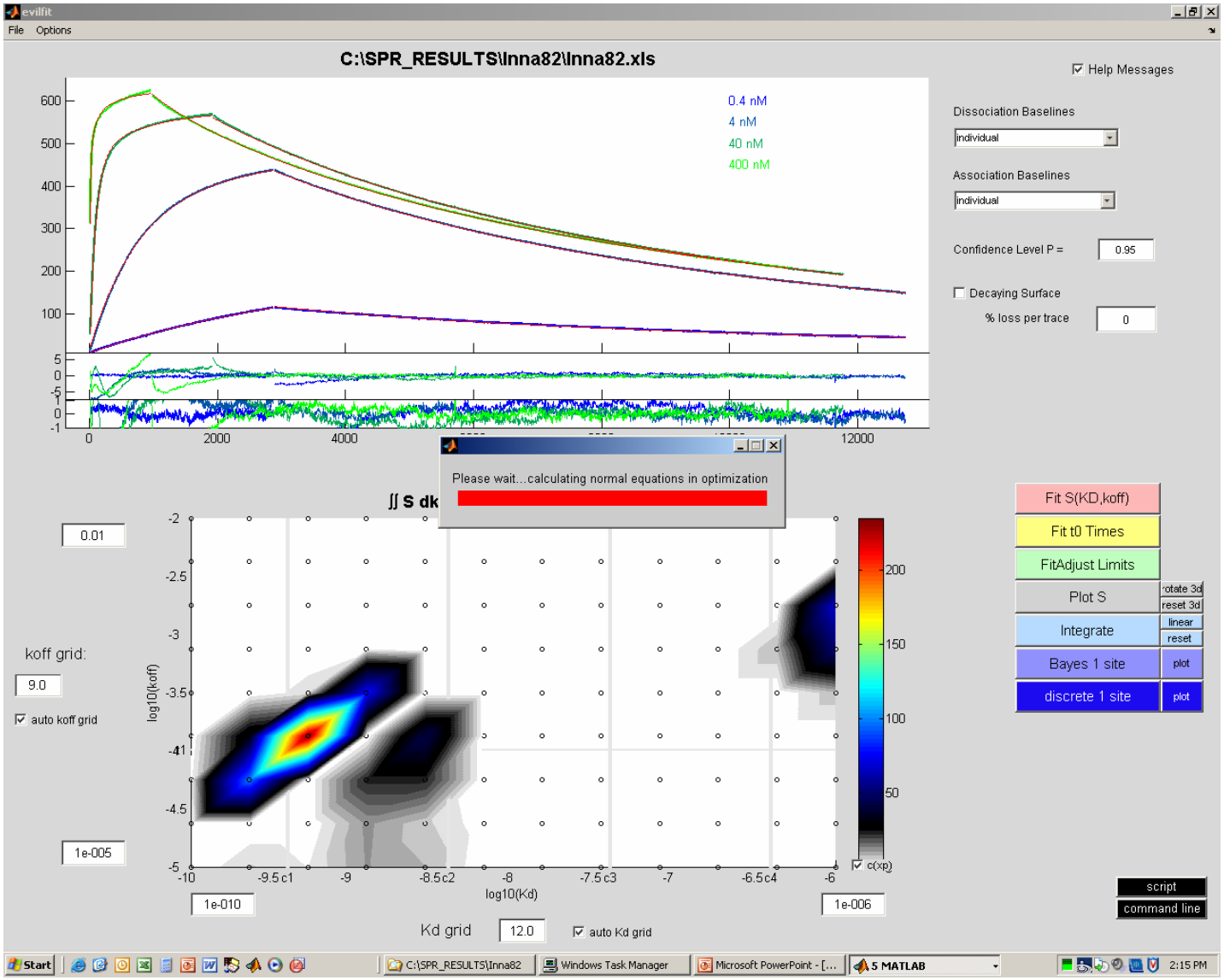
To optimize ‘t0’s, click on yellow button <Fit t0 Times>



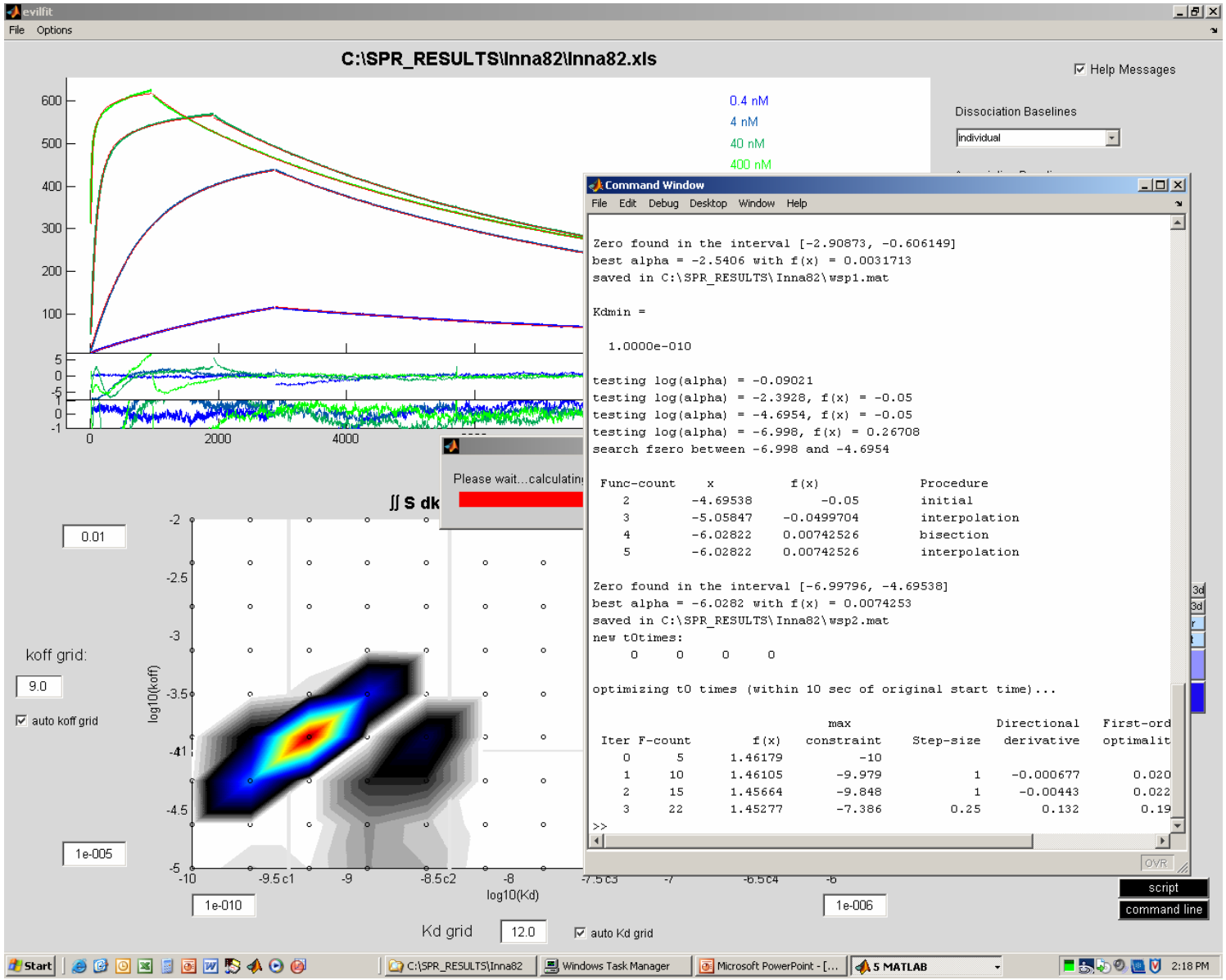
One can start from initial guesses all=0, or manually enter estimates derived from visual inspection; Click <OK>



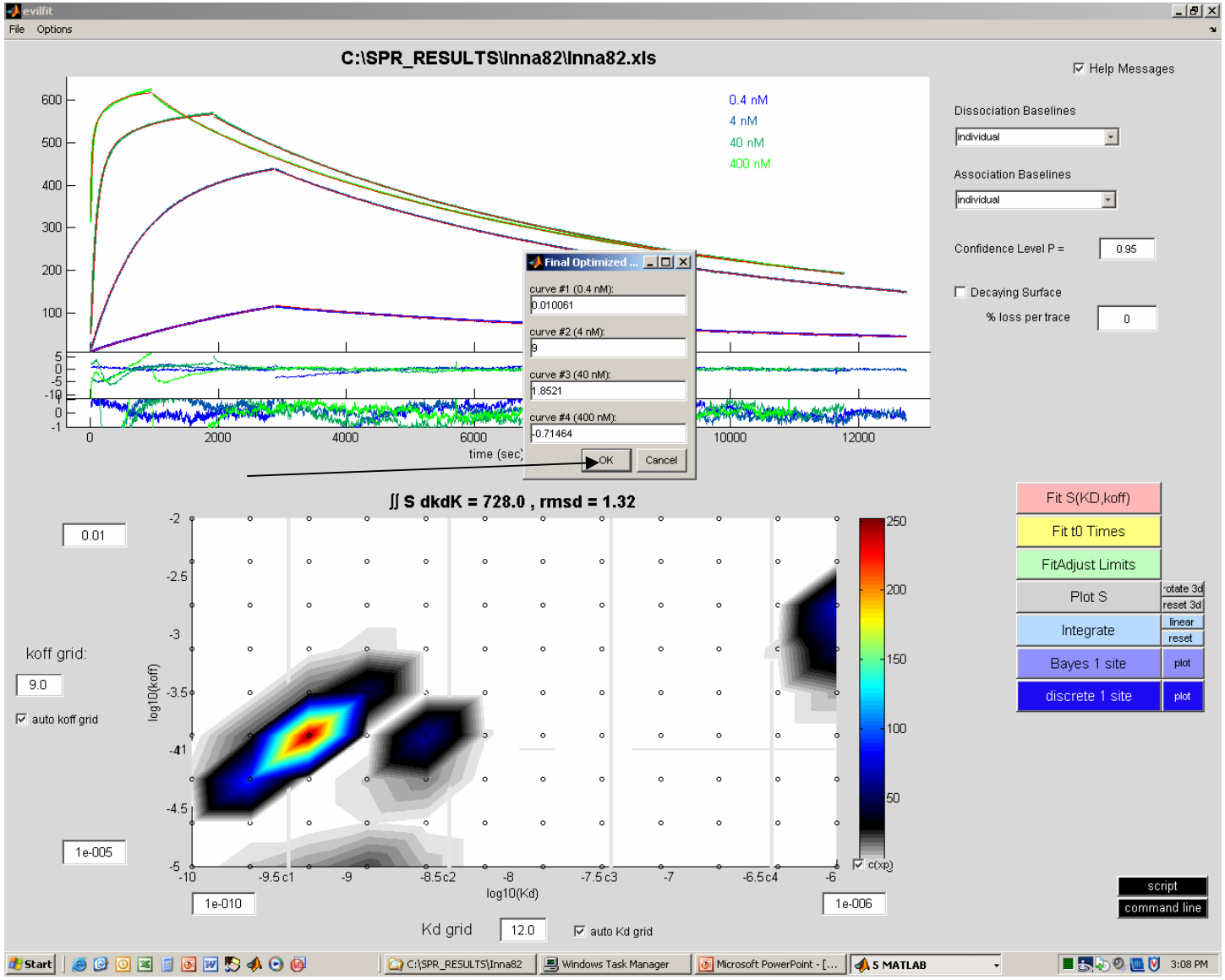
Start optimization of t0s



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

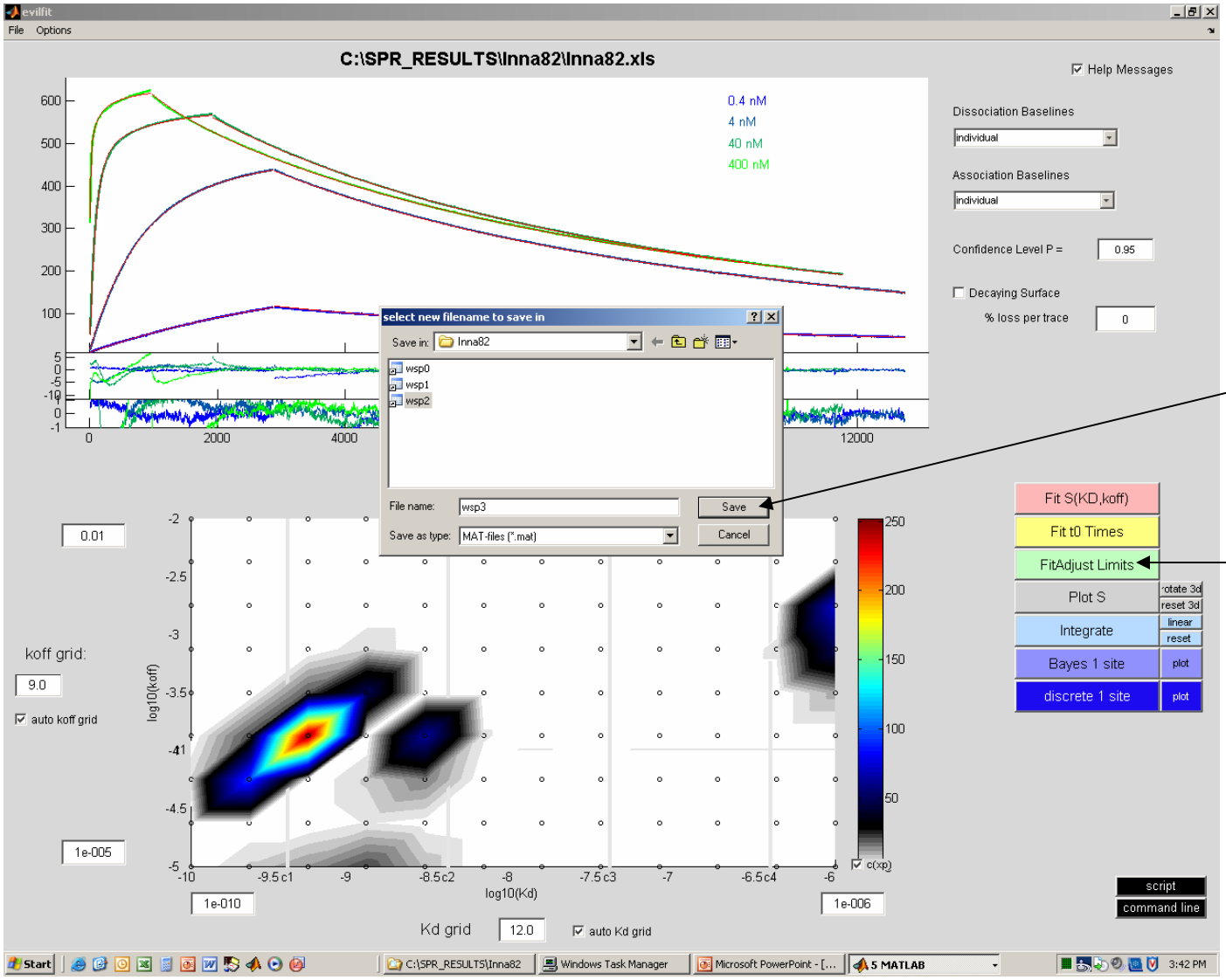


One can follow the progress in Command window



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…



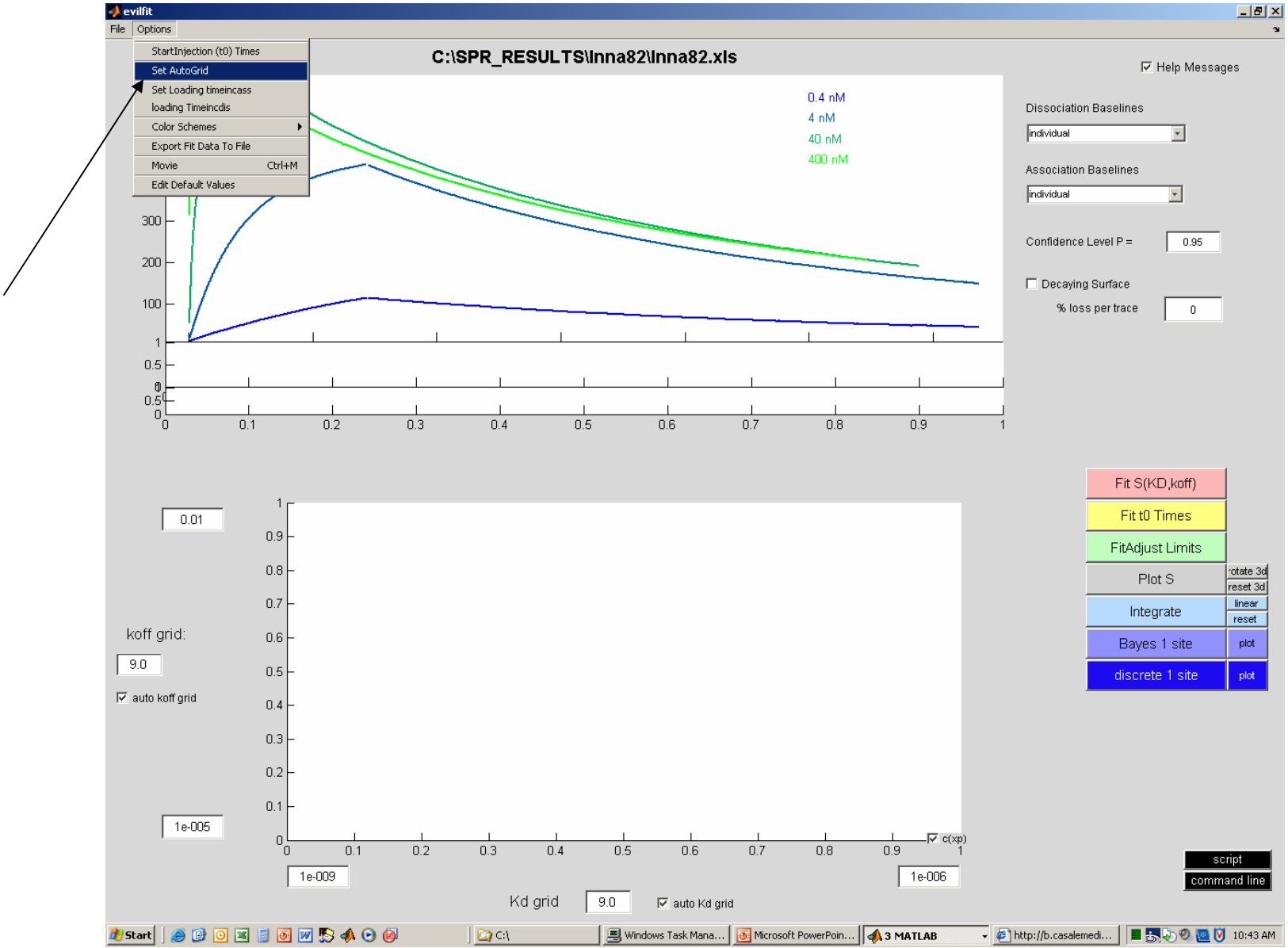
Save current work space as **wsp3** and then Fit Adjust Limits

Description of the Options Settings

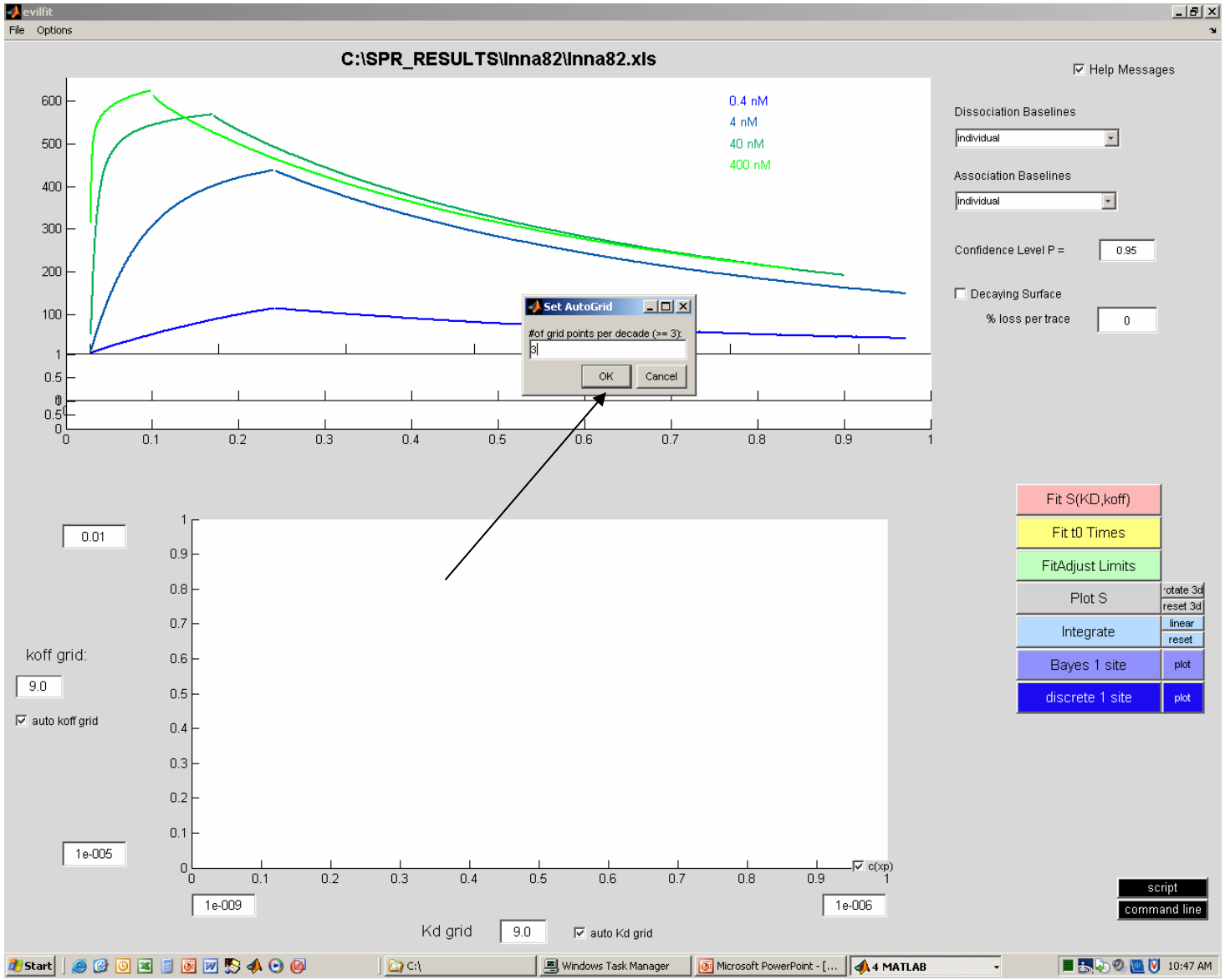
- Options for the default density of grid points per decade

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>